Novel 6-substituted-2-morpholino-8-(1-(arylamino or aryloxo)ethyl)-4H-benzo[e][1,3]oxazin-4-ones as Selective PI3K Inhibitors and Anticancer Compounds

Submitted by

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Abbreviations

ADP	adenosine diphosphate
AKT	Protein kinase B (PKB)
Ar	aryl group
ATP	adenosine triphosphate
ATR	attenuated total reflectance
conc.	concentrated
DCM	dichloromethane
DMF	dimethylformamide
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DNA-PK	DNA-dependent protein kinase
DPPP	1,3-Bis(diphenylphosphino)propane
DSBs	double-strand DNA breaks
FTIR	Fourier transform infrared spectroscopy
HR	homologous recombination
HCl	hydrochloric acid
hVps34	human **v**acuolar **p**rotein **s**orting 34
IR	ionizing radiation
K_2CO_3	potassium carbonate
MP	melting point
mTOR	The mammalian target of rapamycin
NaOH	sodium hydroxide
NMR	nuclear magnetic resonance spectroscopy
NHEJ	non-homologous end joining
$Ph_3P(SCN)_2$	triphenyldithiocyanato-λ^5-phosphane
$Pb(SCN)_2$	lead(II) thiocyanate
$PdCl_2(PPh_3)_2$	bis(PPh_3)palladium(II) dichloride
PDE	phosphodiesterase
$Pd(OAc)_2$	palladium(II) acetate
Ph	phenyl
PPh_3	triphenylphosphine

$PPh_3(SCN)_2$	triphenylphosphine thiocyanate
PI3K	phosphatidylinositol 3-kinase
PIKK	phosphatidylinositol 3-kinase-related kinase
ppm	parts per million
PTEN	phosphatase and tensin homolog deleted from chromosome 10
r.t.	room temperature
SAR	structure activity relationship
THF	tetrahydrofuran
TLC	thin layer chromatography

Summary

This thesis contains eight chapters, describing the synthesis and biological activity of some new 1,3-benzoxazin-4-ones.

Chapter 1 presents a general introduction of 1,3-benzoxazin-4-one derivatives and their biological activity, specifically the P13K, DNA-PK and PDE3A inhibition. It also discusses previously reported synthetic procedures of 1,3-benzoxazin-4-ones. Synthesis and biological activity of the derivatives which are structurally similar to 1,3-benzoxazin-4-ones is also compared. The chapter provides a context to the proposed work and the objectives of the work.

Chapter 2 discusses the different methods attempted for the synthesis of 6-methyl-2-morpholino-8-(1-(arylamino or aryloxo)ethyl)-4H-benzo[e][1,3]oxazin-4-ones.

Chapter 3 describes the attempted synthesis of 6-methoxycarbonyl-2-morpholino-8-(1-(arylamino or aryloxo)ethyl)-4H-benzo[e][1,3]oxazin-4-ones.

Chapter 4 and 5 discusses the attempted synthesis of 6-(carbamoyl or dimethylcarbamoyl)-2-morpholino-8-(1-(arylamino or aryloxo)ethyl)-4H-benzo[e][1,3]oxazin-4-ones and the attempted synthesis of 10-aryl-6-methyl-2-morpholino-4H,8H-chromeno[6,7-e][1,3]oxazine-4,8-diones, respectively.

Chapter 6 provides information about the P13K, DNA-PK and PDE3A assay results for some of the newly synthesized compounds. Some of those compounds have shown remarkable activity against PI3Kβ and PI3Kδ.

Chapter 7 contains major conclusions drawn from this work, highlighting the important observations and results of the work, and possible future directions that can be followed from this work.

Chapter 8 specifies the detailed information about the experimental procedures for all the synthesized compounds in addition to the procedures employed for evaluation of biological activity of some selected compounds.

Statement of Authorship

Except where reference is made in the text of the thesis, this thesis contains no material published elsewhere or extracted in whole or in part from a thesis submitted for the award of any other degree or diploma.

No other person's work has been used without due acknowledgment in the main text of the thesis.

This thesis has not been submitted for the award of any degree or diploma in any other tertiary institution.

All PI3K, DNA-PK and PDE3A assays were performed by Reaction Biology Corporation, One Great Valley Parkway, Suite 2 Malvern, PA 19355 USA. X-ray crystallography was performed at LIMS, Bundoora, Victoria, 3083.

Some PI3K assays and High-Resolution Mass Spectrometry were performed at the Monash Institute of Pharmaceutical Sciences, 381 Royal Parade, Parkville, Victoria, 3052.

26/ 08/ 2021

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Ehtesham Mohammed

Publications

1. Ehtesham Mohammed, Jasim Al-Rawi, Philip Thompson, Michael Angove, 'Synthesis and biological evaluation of some 6-methyl-2-morpholino-8-(1-(arylamino or aryloxo)ethyl)-4H-benzo[e][1,3]oxazin-4-ones against PI3K, PDE3A and DNA-PK enzymes'. Manuscript.

Poster Presentation:

1. Ehtesham Mohammed, Dr Jasim Al-Rawi, Prof. Philip Thompson, 'Some 8-(amino/oxo)ethyl)benzooxazin-4-ones as PI3Kβ inhibitors expected to be anticancer', LIMS fellows research symposium, November 2019, Melbourne, Australia.

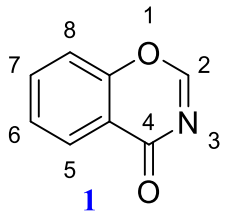
Oral Presentation:

1. Ehtesham Mohammed, 'Synthesis of some new 6,8-substituted-2-morpholino-1,3-benzoxazin-4-ones and their activity against PI3K, DNA-PK and PDE3A' presented in the postgraduate research seminars of the Department of Pharmacy and Biomedical Sciences, La Trobe University (April 2019, April 2020, and August 2021).

CHAPTER 1: INTRODUCTION

1.1 1,3-benzoxazin-4-ones

1,3-benzoxazin-4-ones are heterocyclic organic compounds with oxygen at first position, nitrogen at third position and a carbonyl group at the fourth position. The following structure **1** (Figure 1.1) shows the arrangement of atoms and numbering of atoms in the structure.



1

Figure 1.1: 1,3-benzoxazin-4-one

The rapid spread of cancer has stimulated an unparalleled level of research activity directed towards the search for new lead structures that may be of use in designing new antitumor drugs. In this analysis, some 1,3-benzoxazin-4-one derivatives have been found to be associated with various biological activities such as antibacterial, antiproliferative,[1] antifungal,[2] antihypertensive,[3] cardiovascular activity,[4] analgesic, anti-inflammatory and sedative.[5] Some N-substituted-1,3-benzoxazines **2** have also been reported to be active as antituberculosis agents.[1] Whereas some 1,3-benzoxazine derivatives with amino substitution at 2- position have been reported to show herbicidal and insecticidal activity.[6, 7]

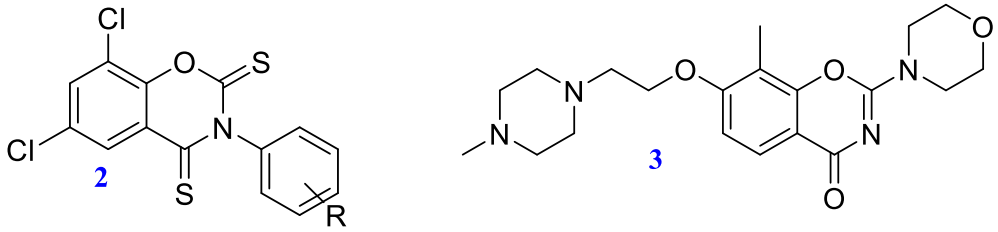


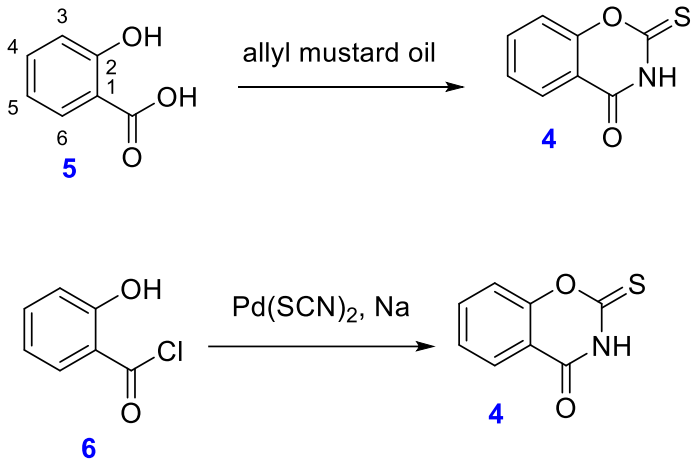
Figure 1.2: Some biologically active benzoxazines

Recently, one 1,3-benzoxazinone with 7-O substitution **3** have been reported to exhibit antiplatelet activity.[8] One the other hand, 1,3-benzoxazines have also been used for non-biological purposes like polymers in composite materials.[9]

However, the focus of this review is to explore the chemistry of 1,3-benzoxazin-4-ones particularly with 2-morpholine substitution as they have shown significant anticancer activity.

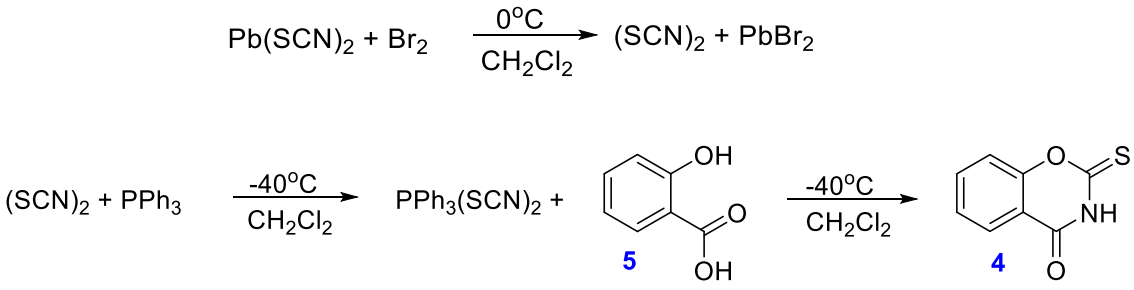
1.2 Synthesis of 1,3- benzoxazine derivatives

The synthesis of 2-thio-1,3-benzoxazinone **4** was first reported in the year 1935.[10] It was synthesized in very low yield by the reaction of salicylic acid **5** with allyl mustard oil. Later the synthesis of same compound was achieved in higher yields (90%) by subjecting salicyloyl chloride **6** to palladium thiocyanate with sodium metal as shown in Scheme 1.1.[11]



Scheme 1.1: Early synthesis of 2-thio-1,3-benzoxazinone

With development in synthetic techniques, new methods of synthesis of compound **4** and its derivatives evolved. The use of triphenylphosphine for synthesis of thiocyanates was first reported by Tamura et al and this thiocyanogen was found to be useful in synthesis of isothiocyanates.[12] Thiocyanogen was produced by adding bromine to lead thiocyanate, and this thiocyanogen was reacted with triphenylphosphine to give triphenylphosphine thiocyanogen, which was reacted with salicylic acid to produce compound **4** as seen in Scheme 1.2**.**

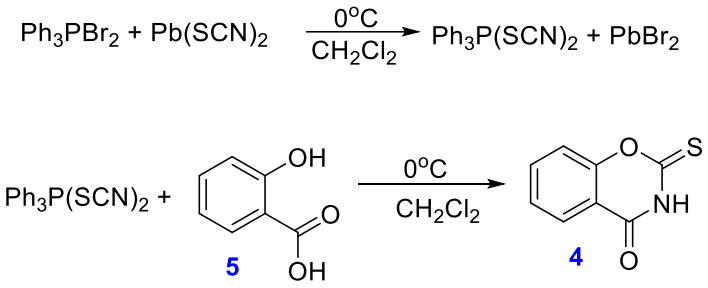


Scheme 1.2: Synthesis of 2-thio-1,3-benzoxazinone by triphenylphosphine thiocyanogen

The above method had some disadvantages like separation of thiocyanogen and its susceptibility to moisture. Later, a modified method was developed which involved one-pot generation of triphenylphosphine thiocyanate.[13] This method involved formation of triphenylphosphine dibromide by addition of bromine to triphenylphosphine and then formation of triphenylphosphine thiocyanate in the same pot by addition of excess ammonium thiocyanate. This method has some drawbacks such as poor solubility of the reactants and the formation of ammonium bromide as by-product which made the isolation of pure product difficult.

1.2.1 One-pot generalized synthesis of 2-thioxo-1,3-benzoxazin-4-ones

Taking cue from the above-mentioned methods, a more efficient method was developed.[14] This method (Scheme 1.3) involves the synthesis of 2-thioxo-1,3-benzoxazin-4-ones by in situ generation of triphenylphosphine thiocyanate from the reaction of lead thiocyanate with freshly prepared triphenylphosphine dibromide.



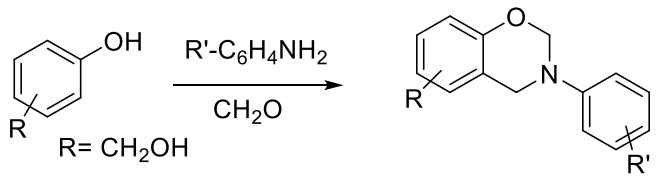
Scheme 1.3: One-pot generalized synthesis of 2-thioxo-1,3-benzoxazin-4-ones

This reaction is not only applicable to salicylic acid but also to substituted 2-hydroxybenzoic acids.[15] This method has a few advantages. Because the reaction is carried out in 'one-pot' the need to isolate intermediates is removed leading to a simpler procedure. Since the reaction is carried out at 0°C the need for liquefied gases or dry ice

does not arise. Moreover, the work-up procedure is relatively uncomplicated as one of the by-products formed is lead bromide which can be removed easily from the reaction mixture by filtration and the Ph_3PO can be removed by the treatment with toluene.[14]

1.2.2 Other methods of 1,3-benzoxazine synthesis

N-substituted 1,3-benzoxazines have also been prepared by various methods. One of the recent procedure (Scheme 1.4) is the use of 4-hydroxybenzyl alcohol **6**, paraformaldehyde, and aniline, which are refluxed in toluene as solvent to produce (3-phenyl-3,4-dihydro-2H-1,3-benzoxazin-6-yl)methanol **7**.

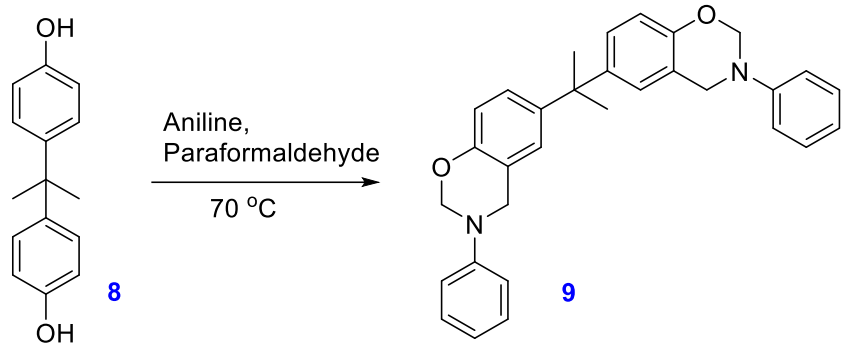


6 **7**

Scheme 1.4: Synthesis of N-substituted 1,3-benzoxazines

There are also some similar methods for synthesis of N-substituted 1,3-benzoxazine, which involve the reaction of aniline with phenol and formaldehyde that have been reported in the literature.[16]

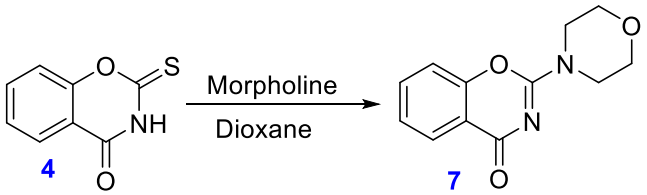
In another method, a nonsolvent procedure for the 3,4-dihydro-2H-3alkyl(aryl)1,3-benzoxazine monomer synthesis was reported.[17] The reaction was conducted in a melt in the absence of solvent, with solid paraformaldehyde used to produce a good yield of benzoxazine (Scheme 1.5).



Scheme 1.5: Synthesis of N-substituted 1,3-benzoxazines

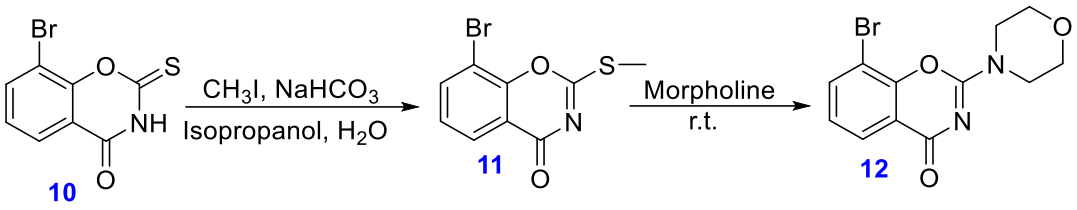
1.2.3 Synthesis of 2-morpholino-1,3-benzoxazin-4-ones

As mentioned in the introductory notes, 2-morpholino-1,3-benzoxazin-4-ones are significantly biologically active. In this purview, two important methods have been developed to substitute morpholine at position 2. One method (Scheme 1.6) is the reaction of compound **4** with morpholine by reflux in dioxane.[18] Though this method is efficient in most cases, some substituents were lost during reflux in dioxane. One of the examples of loss of substituent is debromination from 8- position.



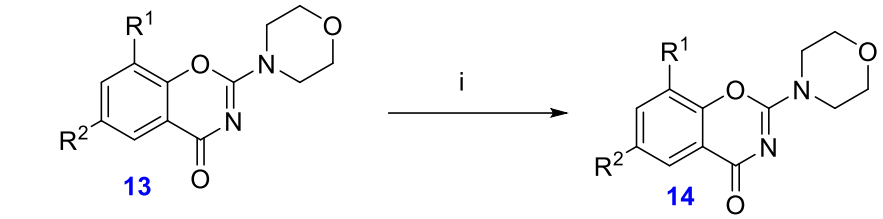
Scheme 1.6: Synthesis of 2-morpholino-1,3-benzoxazin-4-ones by dioxane method

To overcome this problem a recent method of synthesis of this compound was developed. In this method 8-bromo-substituted-2-thioxo-1,3-benzoxazin-4-one **10** was reacted with methyl iodide in a water-isopropanol solvent system containing sodium bicarbonate. This resulted in the formation of 2-methylthio intermediate **11**. Excess of morpholine was then added (Scheme 1.7) to this intermediate to get the desired 2-morpholino-8-bromo-substituted-1,3-benzoxazin-4-one **12**.[19]



Scheme 1.7: Synthesis of 2-morpholino-1,3-benzoxazin-4-ones from 2-methylthio intermediate

To overcome the loss of bromine at position 8 in compound **10**, Suzuki coupling reactions were used. This was done using 3-bromo-2-hydroxybenzoic acid, which was prepared by de-sulphonation of 3-bromo-2-hydroxy-5-sulfobenzoic acid. This was then cyclized with triphenylphosphine reaction and morpholine was substituted at 2- position. Ultimately, the Suzuki coupling reaction was done on 6-or 8-bromo-2-morpholino-1,3-benzoxazin-4-one **13** by using K_2CO_3 in dioxane-water solvent system, aryl-boronic acids and $PdCl_2(PPh_3)_2$ as catalyst to successfully synthesise -aryl, 8-aryl-6-chloro- and 6-aryl-2-morpholino-1,3-benzoxazines **14** (Scheme 1.8).[19]



i = K_2CO_3, $PdCl_2(PPh_3)_2$, $ArB(OH)_2$, 1,4-Dioxane/H_2O (70:30), 50°C, 16 hrs

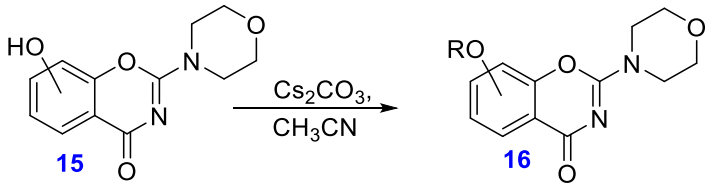
R1 = Br, R2 = H
R1 = Br, R2 = Cl
R1 = H, R2 = Br

R1 = Ar, R2 = H
R1 = Ar, R2 = Cl
R1 = H, R2 = Ar

Ar = dibenzothiophenyl, dibenzofuranyl; naphthalenyl; phenyl; paramethoxyphenyl; parachlorophenyl; parahydroxymethylphenyl; 3-aminophenyl; paraamidophenyl; pyridin-3-yl; thiophen-2-yl; thiophen-3-yl; benzothiophen-2-yl

Scheme 1.8: Suzuki coupling synthesis of 8-aryl, 8-aryl-6-chloro- and 6-aryl-2-morpholino-1,3-benzoxazines using aryl-boronic acids and $PdCl_2(PPh_3)_2$

The synthesis of 5, 6, 7, 8-*O*-Substituted benzoxazines has also been undertaken.[15] In these reactions hydroxy substituted 2-morpholino-1,3-benzoxazin-4-ones **15** were reacted with hydrohalogen salts of 2, 3, and 4-(halomethyl)-pyridines (Scheme 1.9).



R = benzyl; 1-bromo-2-ethyl; pyridin-2-yl; pyridin-3-yl; pyridin-4-yl

Scheme 1.9: Synthesis of 5, 6, 7, 8-*O*-Substituted 2-morpholino benzoxazines

1.3 Biological activity of 1,3 benzoxazine derivatives

The biological activity of 1,3 benzoxazine derivatives is wide ranged. A group of N-substituted 1,3-benzoxazines possess a variety of biological activity. N-cyclohexyl-1,3-benzoxazine analogues **17** have shown to be highly cytotoxic for certain tumours.[20] The substitution of the phenyl groups at N- position (as in **18**) rendered antibacterial and antifungal activity to these compounds.[21-23] The N-phenyl substitution with dichloro on the phenyl ring was particularly active against the bacterium *Staphylococcus aureus*.[21, 22]

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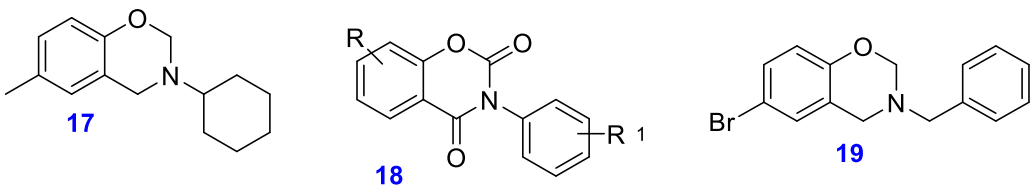


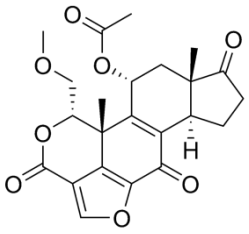
Figure 1.3: Substituted N-phenyl-1,3-benzoxazines

Some other substituted N-phenyl-1,3-benzoxazines (Figure 1.3) have been reported to show antituberculosis activity, anti-algal activity, analgesic activity and antitumor activity.[23-27]

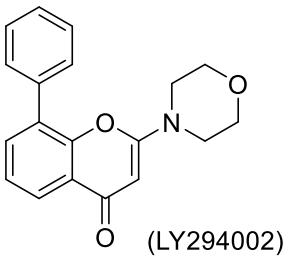
1.4 DNA-PK and its inhibitors

DNA-dependent protein kinase (DNA-PK) is an important protein that is involved in the repair of DNA double-strand breaks that occur because of oxidative damage and exogenous stimuli like ionising radiation treatment.[28] A typical cancer therapy consists of chemotherapeutic drugs alone or in combination with ionizing radiation (IR).[29] These therapies result in the formation of double-strand DNA breaks leading to ultimate cell death. However, the main hurdle in this cancer treatment is the evolution of multiple mechanisms for repairing DNA breaks including homologous recombination (HR),[30] and non-homologous end joining (NHEJ).[31] The NHEJ pathway is more common than HR pathway for DNA repair. DNA-PK is an enzyme that mediates DNA repair specifically through the NHEJ pathway.[32, 33] Hence, it has been hypothesized that targeting this enzyme will enhance the efficacy of current cancer treatments.

One of the first DNA-PK inhibitors was Wortmannin which is a furanosteroid obtained from the fungus *Penicillium funiculosum*. It is a non-competitive inhibitor of PI3Ks and has an IC_{50} in the nanomolar range (IC_{50} of 5 nM for PI3Ks and IC_{50} of ~250 nm for DNA-PK).[34]



20 Wortmannin

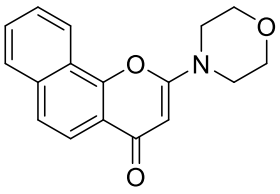


(LY294002)

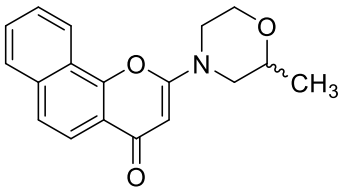
21

Figure 1.4: DNA-PK inhibitors Wortmannin **20** and LY294002 **21**

Another compound called LY294002 **21** was derived from plant flavonoid quercetin was found to be DNA-PK inhibitor.[35] It is a competitive inhibitor of DNA-PK with IC_{50} value of 1.4 µM. [36]



22 (NU7026)



23

Figure 1.5: DNA-PK inhibitors **22** and **23**

Later, compound NU7026 was found to be 50 times more selective for DNA-PK than PI3Ks with an IC_{50} value of 0.23 µM.[37] When a methyl group was added on the morpholine ring, the activity was equipotent **23** (IC_{50} =0.19 µM) (Figure 1.5). Nevertheless, addition of more methyl groups, at the 2- and 6-position of morpholine, or replacement of the morpholine ring by piperidine or piperazine resulted in a loss of activity.[37, 38]

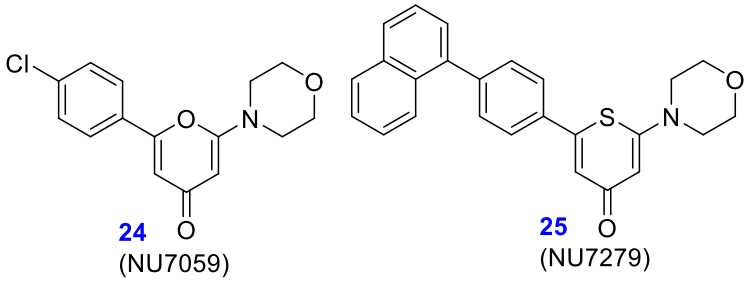


Figure 1.6: DNA-PK inhibitors **24** and **25**

The work was extended to synthesize 6-substituted-2-morpholino-pyran-4-one and 6-substituted-2-morpholinothiopyran-4-one. In this work the compound **24** (IC_{50}=0.18 µM) and **25** (IC_{50}=0.19 µM) were discovered (Figure 1.6) and were found to be 10-fold more potent against DNA-PK than NU7026.[39, 40]

6-, 7-,and 8-aryl substituted chromen-4-ones were synthesized and found to be selectively active against DNA-PK.[41] The dibenzofuranyl group attached to the chromone structure made this a very potent sub-micromolar inhibitor of DNA-PK compound **26** (NU7427) (IC_{50} = 0.04 µM). When this scaffold was appended with a dibenzylthiophenyl moiety, it resulted in a highly potent and selective DNA-PK inhibitor compound 8-dibenzothiophen-4-yl-2-morpholin-4-yl-chromen-4-one **26** (X=S) (NU7441) with DNA-PK inhibitory activity (IC_{50} = 0.02 µM) (Figure 1.7).[37]

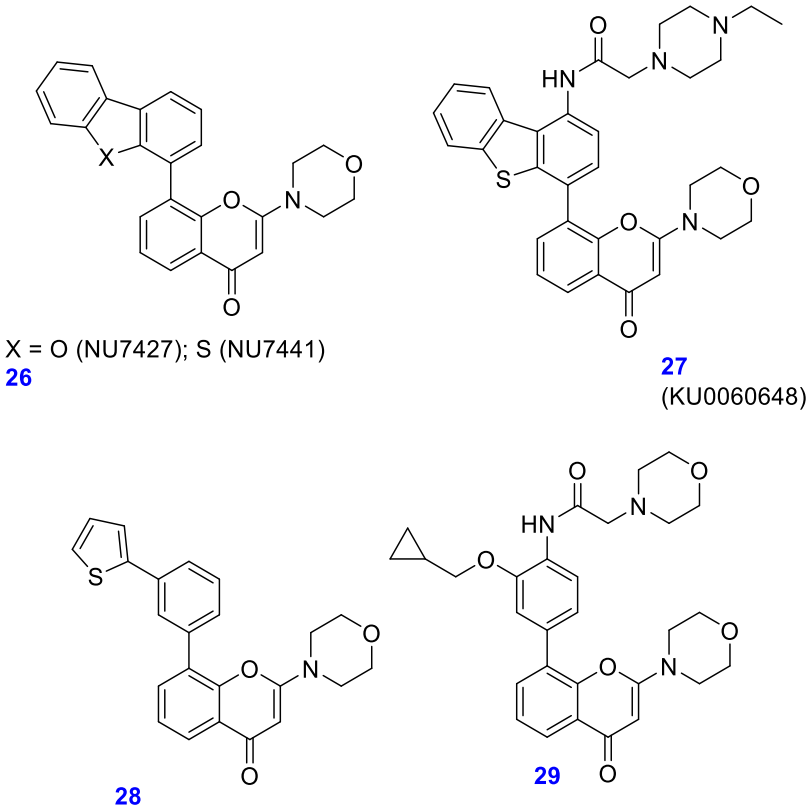


Figure 1.7: Chromen-4-one analogues as DNA-PK inhibitors

Another synthesised compound that was found to exhibit high potency against DNA-PK as well as increase the cytotoxicity of ionizing radiation (IR) *in vitro* 10-fold or more was compound **27** KU0060648 (Figure 1.7); (DNA-PK IC_{50} = 5 nM, IR dose modification ratio = 13). In addition, compound KU0060648 has shown to potentiate DNA-damage inducing TOP2 poisons (doxorubicin, etoposide).[42-44] Two more derivatives of LY294002 have been reported to show enhanced DNA-PK inhibitory activity (i.e., 8-biarylchromenon-4-one **28**, IC_{50} =18 nM and O-alkoxy- phenylchromen-4-one **29**, IC_{50} = 8 nM).[45, 46]

1.5 PI3Ks and their inhibitors

Another group of enzymes called Phosphatidylinositol 3-kinases (PI3Ks) play a major role in cell survival and cell proliferation. These enzymes are lipid kinases, known for their role in the PI3K/AKT/mTOR signalling pathway and act as intermediate signalling molecules.[47, 48] The PI3K signalling pathway is dysregulated frequently in abnormal conditions like cancers, and hence exploited by tumour cells for increased proliferative potential, escape from apoptosis, tissue invasion, and metastasis.[49]

There are three classes of PI3Ks (I-III) in humans, which differ from each other based on the structural characteristics, specificity to substrates, and nature of lipid end-products. Class I of PI3Ks are again separated into 2 subclass i.e. IA and IB. Class IA is frequently associated with cancer.[50, 51] This class of PI3Ks are structurally comprised of catalytic PI3K (α, β, and δ isoforms) and regulatory p85 subunits (p85, p55, and p50 isoforms). Class IB consists of catalytic PI3Kγ and regulatory PI3K.[49] Class II enzymes exist in 3 isoforms (PI3KC2α, PI3KC2β and PI3KC2γ). But these are monomers with high molecular weight which lack regulatory subunits and possess a single catalytic unit. Class III PI3Ks are heterodimer having a catalytic (hVps34) subunit associated with a regulatory (p150) subunit.[52]

PI3Ks enable the phosphorylation of phosphatidylinositol, and this phosphorylated phosphatidylinositol act as secondary messenger which in turn activate multiple effector kinase pathways, including BTK, AKT, PKC, NF-kappa-B, and JNK/SAPK pathways, and ultimately result in survival and growth of normal cells. Growth factors, cytokines, hormones/chemokines, and integrins are four major extracellular signals which activate PI3Ks and which transmit the signals through appropriate pathways to regulator diverse cellular processes such as cell cycle, apoptosis, and cellular metabolism.[53]

Therefore, PI3Ks have been recognized as a worthwhile target for novel anti-cancer therapies. In this purview, several molecules have been developed in recent years as PI3K inhibitors, which are currently in different stages of development.

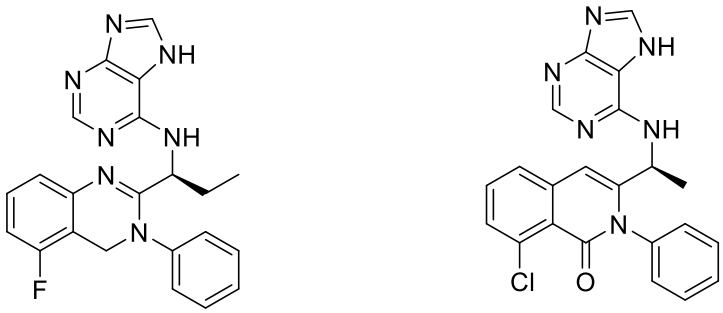


Figure 1.8: Idelalisib **30** and IPI-145 **31**

There are molecules like Idelalisib **30** and IPI-145 **31** (Figure 1.8) that have proved to be active against chronic lymphocytic leukemia/small lymphocytic leukemia, indolent non-Hodgkin's lymphoma (iNHL), and mantle cell lymphoma (MCL). One the other hand, PI3K inhibitors like Buparlisib, GDC-0941, GDC-0032 and BEZ-235 have been reported to target breast tumours, glioblastoma multiforme (GBM) and non-small cell lung (NSCLC) cancer.[54]

1.6 PDE3A and its inhibitors

Phosphodiesterases (PDEs) superfamily consists of 11 PDE gene families (PDE1 to PDE11). They are distinguished based on their primary amino acid sequences, their affinities for cAMP and cGMP, their sensitivities to specific inhibitors, their biochemical

and physical properties and their biological regulatory pathways.[55] The PDE superfamily influences disease pathogenesis and can be new therapeutic targets to treat multiple diseases for example, penile erectile dysfunction (PDE5, targeted by sildenafil),[56] psoriatic arthritis (PDE4, targeted by apremilast)[57] and intermittent claudication (PDE3, targeted by cilostazol).[58] In addition, decreased cAMP and/or cGMP generation have been reported in several cancer pathologies because of overexpression of PDE isoforms.[59] PDE isoforms which increase the levels of intracellular cAMP and/or cGMP, can be selectively inhibited. This may regulate the tumour microenvironment and stimulate apoptosis and cell cycle arrest in a wide range of tumour cells.[60, 61]

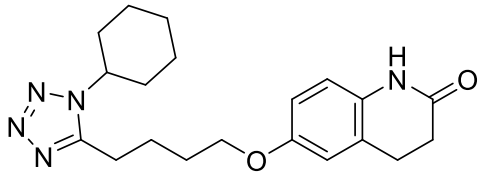
PDE3 and PDE4 are major cAMP-hydrolysis isozymes in cardiovascular tissues.[62] PDE1, PDE3, PDE4, and PDE5 are found in aortic smooth muscle cells.[63, 64] PDE1, PDE2, PDE3, and PDE4 are expressed in the heart,[65] whereas PDE2, PDE3, and PDE5 are found in platelets.[66, 67] PDE3 is the only cAMP-regulating isozyme expressed in all of mentioned tissues. These tissues contribute significantly to the pathogenesis of arteriosclerosis obliterans and restenosis after angioplasty. The inhibition of PDE3 activity in cardiovascular tissues resulted in increasing the levels of cAMP with subsequent decrease in platelet aggregation and smooth muscle cell proliferation *in vitro*, and stimulation of a cardiotonic effect.[67, 68]

Two PDE3 genes have been identified in humans viz. PDE3A and PDE3B, and these genes are found on human chromosomes 11 and 12, respectively.[69] PDE3A is associated with the cardiovascular system, regulating platelet aggregation and vascular smooth muscle cell (VSMC) growth, whereas PDE3B mediates for insulin action in the regulation of lipolysis.[70]

When PDE3A is abnormally activated, it disproportionately decreases cAMP levels in designated compartments to lower PKA phosphorylation of VASP (vasodilator

stimulated phosphoprotein) and RAF1. In that way it enhances VSMC proliferation, as well as MLCK (myosin light-chain kinase), causing VSMC contraction. These variations likely are the reason for the hypertensive and stroke phenotypes characteristic of HTNB (Hypertension with brachydactyly) with lowered PKA activity causing raised parathyroid hormone-related peptide (PTHrP) levels and so contributing to brachydactyly.[71]

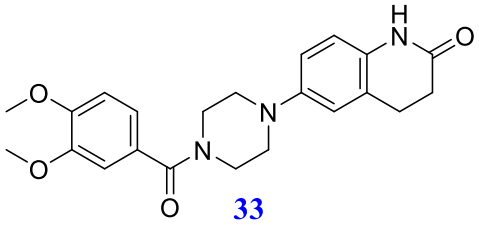
These studies explain therapeutic prospects for the use of selective inhibitors of PDE3 in HTNB. PDE3 inhibitors were developed for the treatment of congestive heart disease.



32

Figure 1.9: Cilostazol **32**

Cilostazol **32** (Figure 1.9) is used to treat vascular claudication which may be an effective therapeutic for HTNB, specially as it has lately shown to be useful in prophylaxis of stroke. Vesnarinon **33** (3,4-dihydro-6-[4-(3,4-dimethoxybenzoyl)-1-piperazinyl]-2(1H)-quinolinone) was reported for treatment of congestive heart failure as it showed positive inotropic effect on the heart.[72]



33

Figure 1.10: PDE3A inhibitor, Vesnarinone **33**

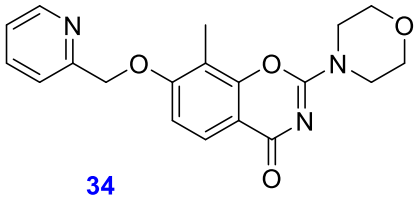
Though many efforts have been made to understand and highlight the PDE3–ligand interactions,[73] the information is still insufficient and based on structure–activity relationship (SAR) data collected from documentation of new molecules that were discovered by trial and error. Some new series of cardiotonic agents were synthesized by modifying the structure of vesnarinone, which was designed using computerized study of PDE3–ligand interaction. The PDE3A and PDE3B inhibitory activities of these compounds were evaluated and the potential cardiotonic activity of the best PDE3A inhibitors was assessed.[74]

The pharmacophore essential for platelet aggregation is the 1,3-benzoxazine skeleton, with a morpholino group at position 2 and substitution at position 8 and/ or O-substitution at position 7. As mentioned earlier, PDE3A is the predominant PDE in platelets, PDE3A inhibition is closely related to antiplatelet activity. Previously several benzoxazine compounds have been tested for their antiplatelet activity and found to be very promising. The newly synthesized 1,3-benzoxazin-4-ones in this work will also be tested for PDE3A inhibition.

1.7 1,3-benzoxazines as selective DNA-PK inhibitors and PI3K inhibitors

Though the DNA-PK and PI3K enzymes are different in nature, these classes of enzymes have related origin and have similar kinase domains. So, the molecules synthesized as inhibitors of these kinases are analogous structurally.[75]

The substitution at 7- and 8- positions to 2-morpholino-1,3-benzoxazines have been reported to enhance DNA-PK (DNA- dependent protein kinase) inhibition. A series of such compounds were prepared and found to exhibit moderate to high DNA-PK inhibitor activity.[19]

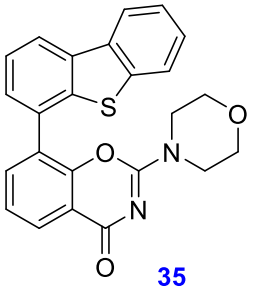


34

Figure 1.11: 7- and 8- substituted benzoxazine as DNA-PK inhibitor

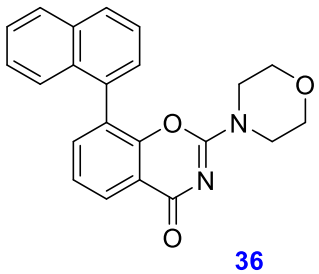
This compound **34** was also found to have radio sensitizing activity on lung and colon cancer cells.[76] This compound promoted apoptosis, and hence, can be used as synergist with radiotherapy. The structural activity relationship showed that indeed the substitution at 7- and 8- positions enhance DNA-PK inhibitor activity whereas substitution at 5- and 6- positions rendered decrease in this activity.[77]

Following this lead, few more similar compounds were synthesized by Morrison et al. Interestingly, 8-aryl substitution to 2-morpholino-1,3-benzoxazines (LTURM34) (Figure 1.12) rendered potent DNA-PK inhibitor activity (IC_{50} of 0.034 µM) with selective potency over class I PI3K enzyme.[19]



35

LTURM34

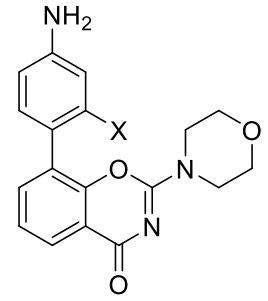


36

LTURM36

Figure 1.12: LTURM34 and LTURM36 (These products are available as reagents in the market)

A 2-morpholino-1,3-benzoxazine derivative LTURM36 has also been reported to be a highly potent selective inhibitor of PI3Kδ isoform of this enzyme.[19]



37 X = H; **38** X = F

Figure 1.13: PI3K isoform selective inhibitors **37** and **38**

Treatment of conditions like autoimmune disorders can require chronic dosing highly PI3K-isoform selective compounds, which are potential drug candidates for further development. Shuttleworth *et al.*[78] investigated PI3K isoform selectivity by introducing functionality to the 8-phenyl group of compounds **37** and **38** (Figure 1.13).

1.8 Synthesis and biological activity of structurally similar compounds

There are some structurally similar compounds to 1,3-benzoxazines that were synthesized and found to have various biological applications. These compounds have been the source of inspiration to synthesize 1,3-benzoxazines. One such group of compounds is chromen-4-ones.

1.8.1 Chromen-4-ones

Some 2-amino substituted chromones rendered antiplatelet activity.[79]

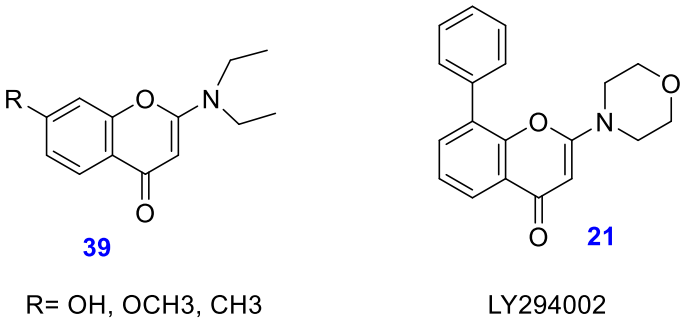
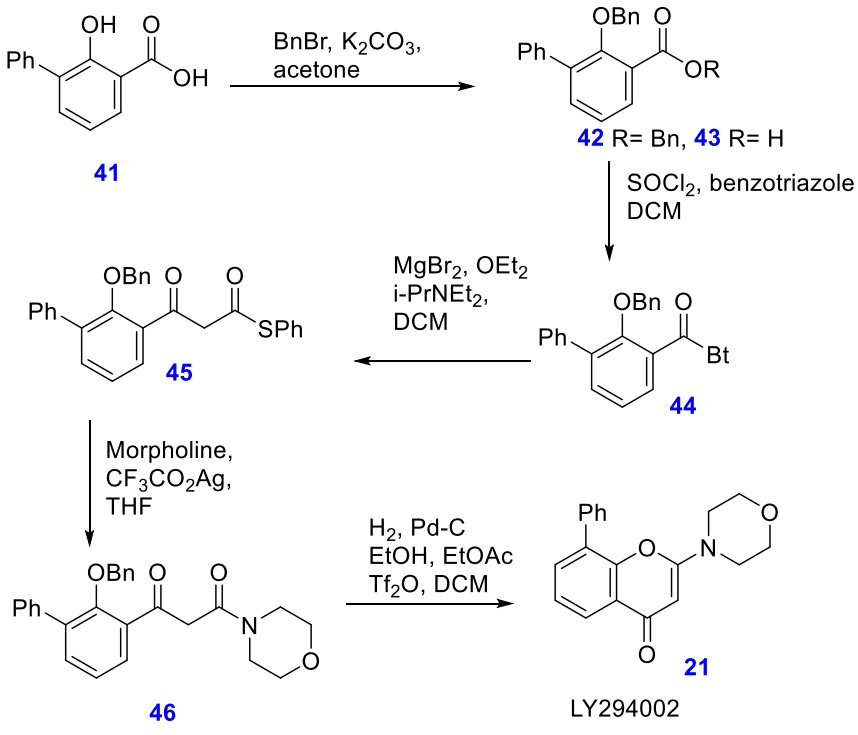


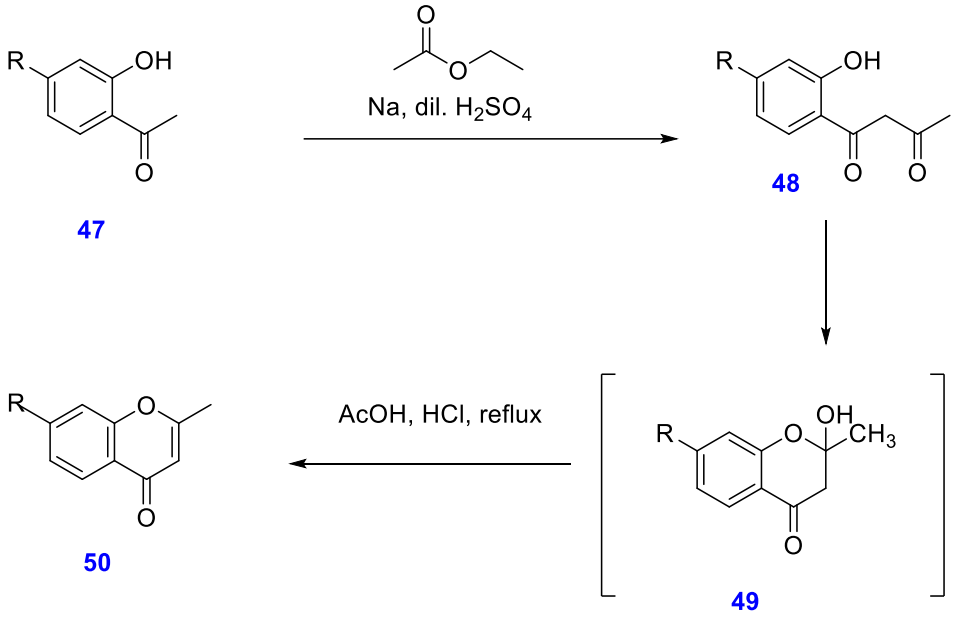
Figure 1.14: 2-amino substituted chromones

From the literature it is found that morpholine substitution at 2- position to chromen-4-ones enhanced its biological activity further.[37] The compound 2-morpholino-8-phenylchromen-4-one (LY294002) (Figure1.14) was non-selectively active against DNA-PK.[35] The study also reinforced the role of morpholine substituent at 2- position, as replacing it with piperazine or thiomorpholine decreased the activity.[28] The synthesis of LY294002 was achieved according to the following Scheme 1.10.[80]



Scheme 1.10: Synthesis of LY294002 **21**

Azimvand J synthesized some new 2-methyl-chromen-4-one derivatives **50** from 2-acetyl phenol and ethyl acetate as reactants, which exhibited moderate antibacterial activity (Scheme 1.11).[81]



Scheme 1.11: Synthesis of 2-methyl-chromen-4-one derivatives

In recent studies, compound **51** (R)-8-(1-(3,5-Difluorophenylamino)ethyl)-N,N-dimethyl-2-morpholino-4-oxo-4H-chromene-6-carboxamide (AZD8186) **51** was found to be highly potent selectively against PI3Kβ and PI3Kδ. In this compound it was also observed that the problem of higher lipophilicity of previously synthesized pyrido[1,2-a]pyrimid-4-one derivatives **52** was overcome, making it more suitable for oral administration (Figure 1.15).[82]

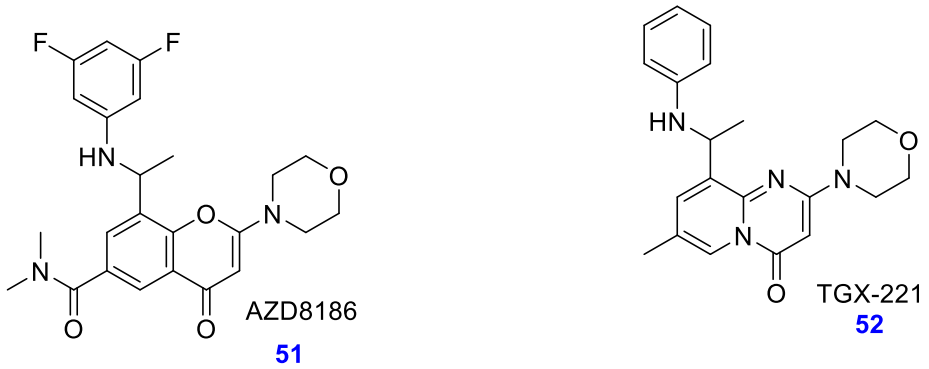
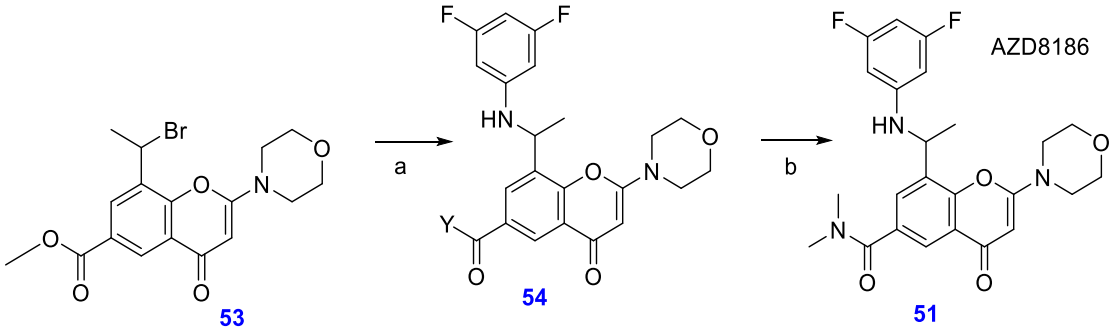


Figure 1.15: Structures of AZD8186 **51** and TGX-221 **52**

This kind of chromeno analogues (AZD8186) were synthesized in different ways depending upon the type of substitution at 6- and 8- positions. The following pathway (Scheme 1.12) was specifically used to synthesize the chromenone derivative **51** (AZD8186).



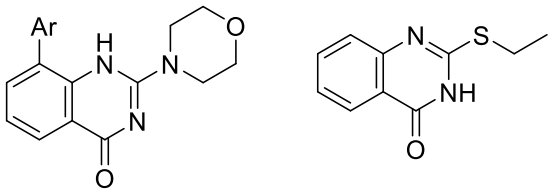
a= 3,5-F-Ph-NH_2, KI, $CHCl_3$-MeOH, rt; b= TSTU, R1R2NH, $NiPr_2Et$, DCM, r.t.

Scheme 1.12: Synthesis of AZD8186 **51**

1.8.2 Quinazolin-4-ones

Another group of similar compounds is quinazolin-4-ones have been synthesized (**55, 56**) by similar methods as 1,3-benzoxazinones syntheses. However, they were found to be not as active against DNA-PK, though they showed some antiplatelet activity.[83] While some other quinazolines have been found to have central nervous depressant

activity, helping in protection against electric shocks and CNS stimulant activity (Figure 1.16).[84]

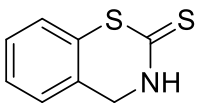


55 **56**

Figure 1.16: Structurally similar quinazolin-4-ones

1.8.3 1,3-benzothiazines

Besides chromones and quinazolines, there is another group of structurally similar compounds called 1,3-benzothiazines that have been found to have biological activities such as analgesic and antimicrobial properties.[85] These compounds have sulphur at 1-position instead of oxygen as in 1,3-benzoxazines.

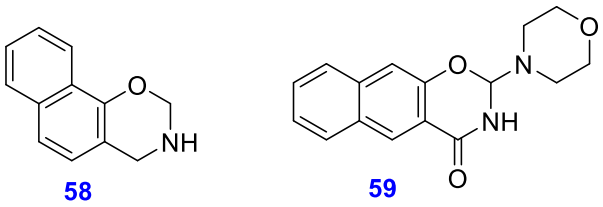


57

Figure 1.17: 1,3-benzothiazine

1.8.4 Naphtho-1,3-oxazines

Finally, another group of structurally similar compounds worth mentioning is naphtho-1,3-oxazines. A one-pot synthesis by reaction of naphthol and various anilines was done in formalin to get some new 3-substituted naphtho-1,3-oxazines. These compounds were found to exhibit substantial antibacterial and antifungal activity.[86, 87]



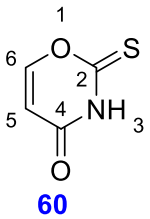
58 59

Figure 1.18: Naphtho-1,3-oxazines

In another study of naphtho-1,3-oxazines, linear 6,7-fused, 5,6-angular fused and 7,8-angular fused-aryl-morpholino-naphtho-1,3-oxazines (**58, 59**) were synthesized from 2-hydroxynaphthoic acids by Morrison et al (Figure 1.18).[88] Some of these compounds were found to be potent selective PI3Kδ inhibitors while the other were found have good antiplatelet activity.

1.9 Synthesis of some 1,3-oxazine derivatives

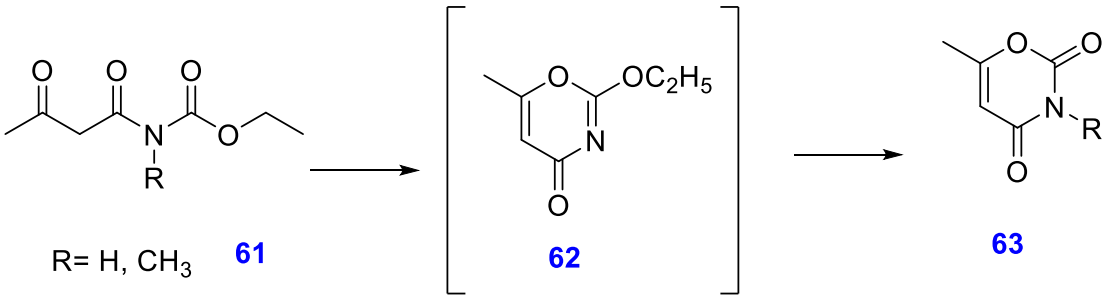
1,3-Oxazines are a group of monocyclic compounds with a six-membered ring containing an oxygen and a nitrogen at 1- and 3- positions respectively. The following structure **60** shows the arrangement of atoms and numbering of atoms in the structure (Figure 1.19).



60

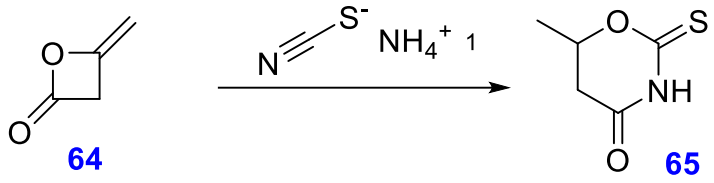
Figure 1.19: 2-thioxo-1,3-oxazin-4-one

One of the earliest synthesis of these compounds was conducted by Warrener et al, by reaction of N-acetylacetyl urethane with concentrated sulphuric acid to give 6-methyl-1,3-oxazine **63**.[89]



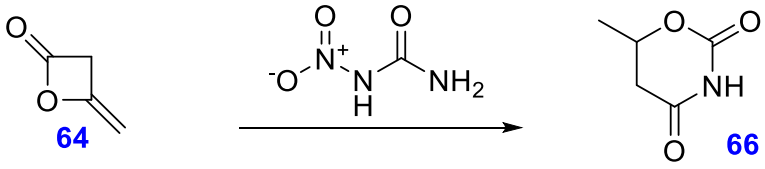
Scheme 1.13: Synthesis of 6-methyl-1,3-oxazin-2,4-dione **63**

Some 1,3-Oxazine derivatives were synthesized from diketene. In this process, diketene **64** was reacted with ammonium thiocyanate in acetone, to give 2-thio-6-methyl-2,3-dihydro-2,4-diketo-l,3-oxazine **65** (Scheme 1.14).[90]



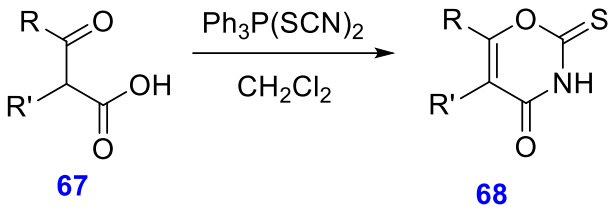
Scheme 1.14: Synthesis of 1,3-oxazinone derivatives from diketene

In another procedure diketene **64** with nitrourea was used to synthesize 1,3-oxazine (Scheme 1.15).



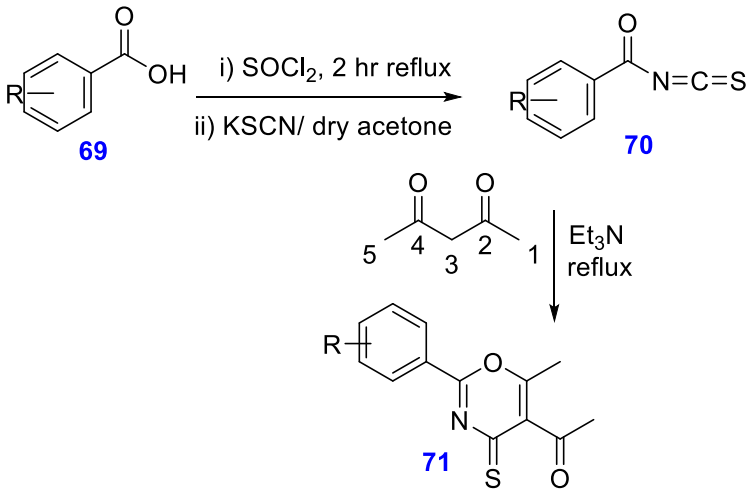
Scheme 1.15: Synthesis of 6-methyl-1,3-oxazin-2,4-dione **66**

One of the most prominent and recent works to synthesize 1,3-oxazines was done by Pritchard et al. In this work, the synthesis of 2-thio-1,3-oxazines **68** was carried out using β-keto acids which were reacted with $Ph_3P(SCN)_2$ to give substituted 2-thio-1,3-oxazines.[91]



Scheme 1.16: Synthesis of 2-thio-1,3-oxazines from β-keto acids

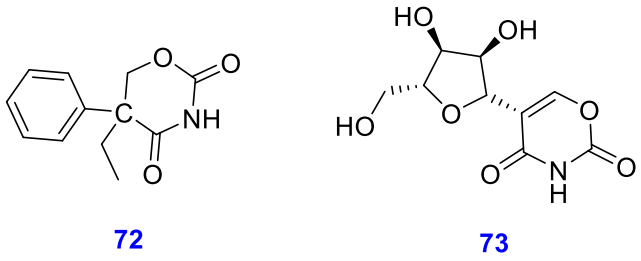
In another study, some 1,3-oxazine derivatives were prepared by one-pot reaction of benzoyl isothiocyanates **70** with acetylacetone in triethylamine (Scheme 1.17).[92]



Scheme 1.17: Synthesis of 1,3-oxazines from benzoyl isothiocyanates and acetylacetone

1.10 Biological activity of oxazine derivatives

The 1,3-oxazine moiety has been reported to have a broad range of biological activities. Some 5,5-disubstitued-1,3-oxazin-2,4-diones **72** have shown potent sedative activity.[93] Further investigations also show that the substitution at 5- position in 1,3-oxazines is important. A nucleoside antibiotic, 5-[3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-1,3-oxazine-2,4-dione **73** (oxazinomycin) was discovered which had substitution at 5-position of 1,3-oxazine structure. This antibiotic has profound activity against bacteria like *S. aureus and E.coli*.[94] Oxazinomycin was also found to have significant activity against certain tumours (Figure 1.20).[94]



72 **73**

Figure 1.20: 5,5-disubstitued-1,3-oxazin-2,4-dione **72** and oxazinomycin **73**

Furthermore, some 2-substituted 1,3-oxazine derivatives **74** had analgesic and antipyretic effects.[95] Another 1,3-oxazine derivative (2-arylaminomethyleneamino-6-aryl-1,3-oxazin-4-one) **75** was reported to show anti-inflammatory activity (Figure 1.21).[96]

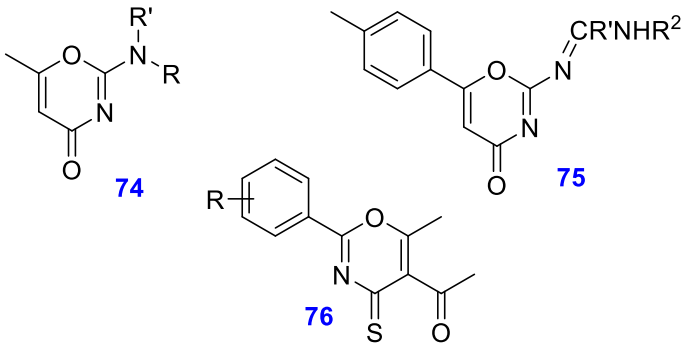


Figure 1.21: Biologically active 1,3-oxazine derivatives

Very recently, Qamar et al discovered that some 1,3-oxazine derivatives **76** were active as carbonic anhydrase inhibitors while some were moderately active as antioxidants (Figure1.21).[92] The substituted phenyl ring at second position in the structure was found to be essential for this carbonic anhydrase inhibitory activity.

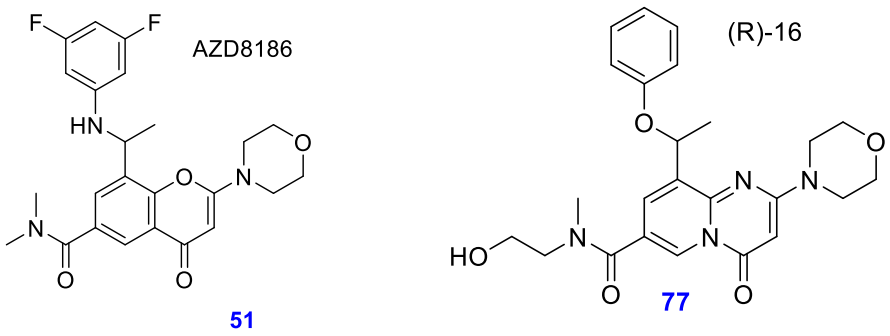
1.11 Proposed work

Novel 6-Substituted-2-morpholino-8-(1-(arylamino or aryloxo)ethyl)-4H-benzo[e][1,3]oxazin-4-ones, here are being hypothesized as selective PI3K inhibitor and anticancer reagents.

When aniline and amide substituents were optimized, it led to discovery of potent PI3Kβ/δ inhibitors with tremendous selectivity against PI3Kβ and PI3Kγ. Greater metabolic stability and suitable physical properties for oral administration was seen through the series of some synthesized chromenones. This was due to the lower lipophilicity of the chromen-4-one core compared to the previously described pyrido[1,2-a]pyrimid-4-one core.[82]

Of those, compound **51** (AZD8186) on oral administration showed greater pharmacodynamic modulation of p-Akt in PTEN-deficient PC3 prostate tumour bearing mice and showed inhibition of tumour growth completely in the mice PTEN-deficient PC3 prostate tumour xenograft model. It was selected as a clinical candidate and has recently entered phase I of clinical trials. Further in vitro and in vivo biological characterization has recently been reported.[82]

In addition, structure-based optimization of pharmacokinetic properties resulted in compound **77** (*R*)-**16**, a novel, orally bioavailable PI3Kβ inhibitor with potent in vivo anti-thrombotic effect.[97]



51 **77**

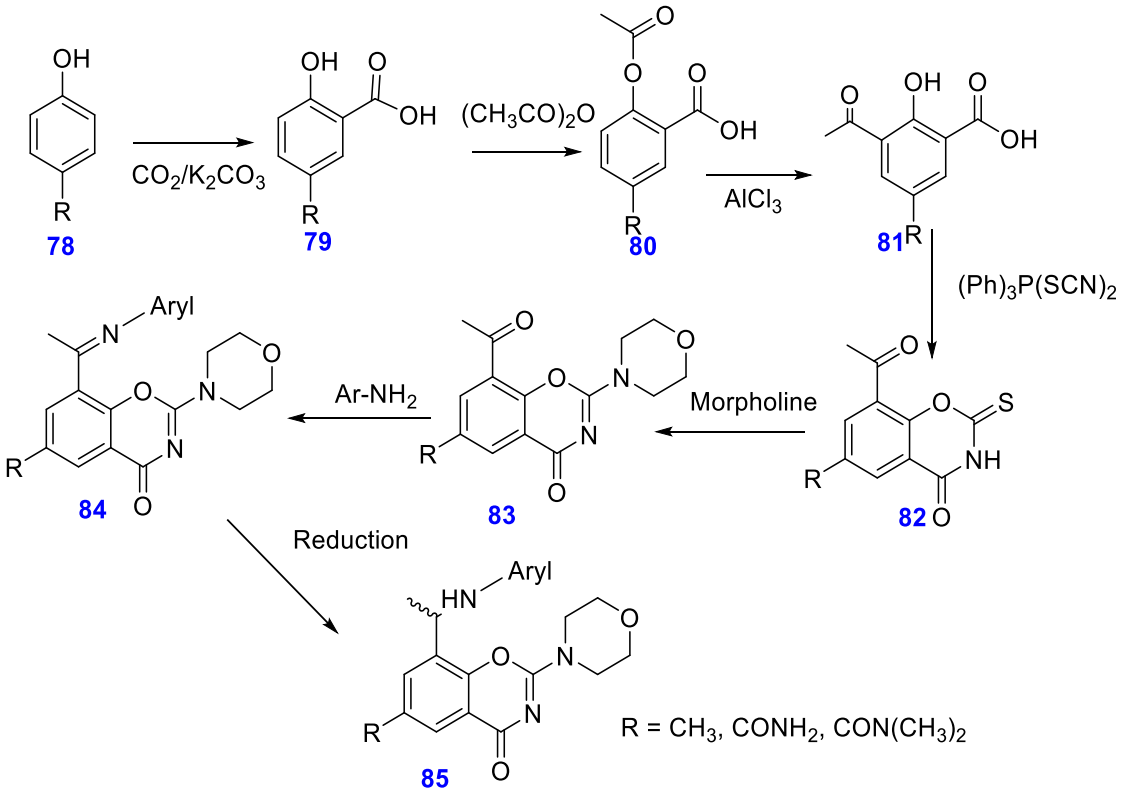
Figure 1.22: AZD8186 **51** and *(R)*-16 **77**

Therefore, it can be established that substitution at 6- position and substituted anilinoethyl at 8- position are potent pharmacophores for selective PI3K inhibitor activity. Moreover, phenoxy-ethyl at 8- position has shown selective PI3K inhibitor activity. The significance of 2-morpholino substituent with 1,3-benzoxazin-4-ones has already been emphasized elsewhere in this review. It is also known that pharmacokinetic properties such as aqueous solubility, rapid clearance from circulation, and bioavailability have been improved in such structural analogues by having a benzoxazinone scaffold.[98] Taking all these into consideration the following section outlines the pathways that were proposed to synthesize some 6-substituted-2-morpholino-8-(1-(arylamino or aryloxo)ethyl)-4H-benzo[e][1,3]oxazin-4-ones.

1.11.1 Schemes for the proposed work

The synthesis process starts with carboxylation of 4-substituted phenols **78** to give 5-substituted salicylic acids **79**. In the second step, the compound **79** will be subjected to

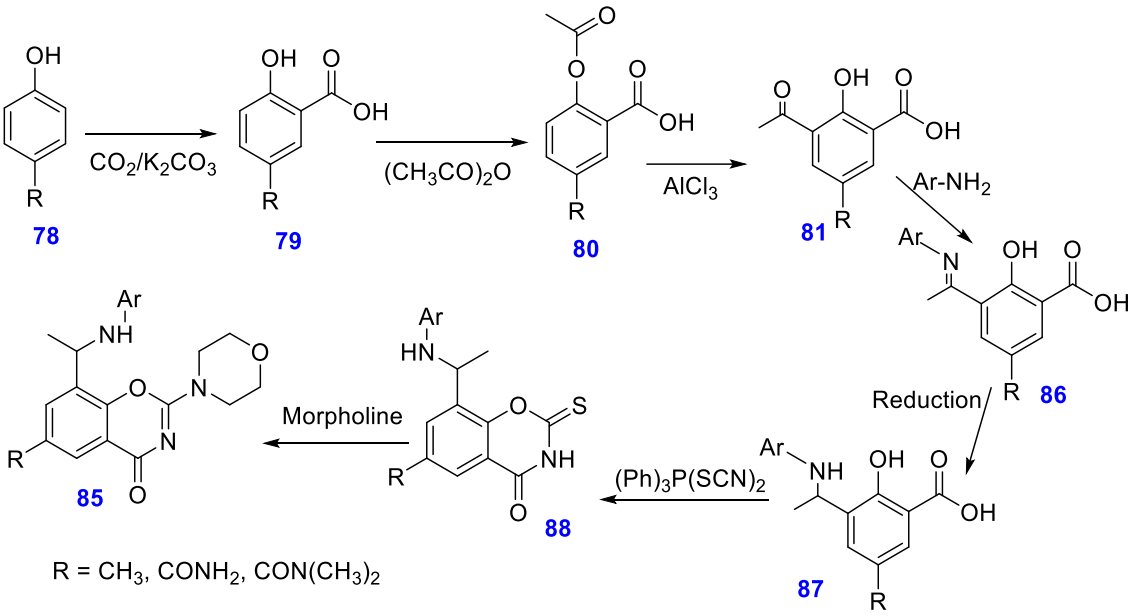
O- acetylation by acetic anhydride to form 2-acetoxy-5-substituted benzoic acid **80**, which is then reacted with aluminium chloride (Fries rearrangement) to give 3-acetyl-2-hydroxy-5-substituted benzoic acid **81**. This will be cyclized using freshly prepared triphenylphosphine thiocyanate to give 6-substituted-2-thioxo-8-acetyl-benzoxazin-4-one **82**. This benzoxazine will be reacted with methyl iodide and then morpholine to give 6-substituted-2-morpholino-8-acetyl-benzoxazin-4-one **83**. The compound **83** will be reacted with aniline to give a Schiff base compound **84** which is then reduced by sodium borohydride to give the proposed 6-substituted-2-morpholino-8-(1-(arylamino)ethyl-4H-benzo[e][1,3]oxazin-4-one **85** (Scheme 1.18).



Scheme 1.18: First proposed synthesis

1.11.2 Alternative pathway- 1

An alternative pathway for the synthesis of the proposed 6-Substituted-2-morpholino-8-(1-(arylamino)ethyl)-4H-benzo[e][1,3]oxazin-4-ones, was designed in case the proposed original scheme is unsuccessful.

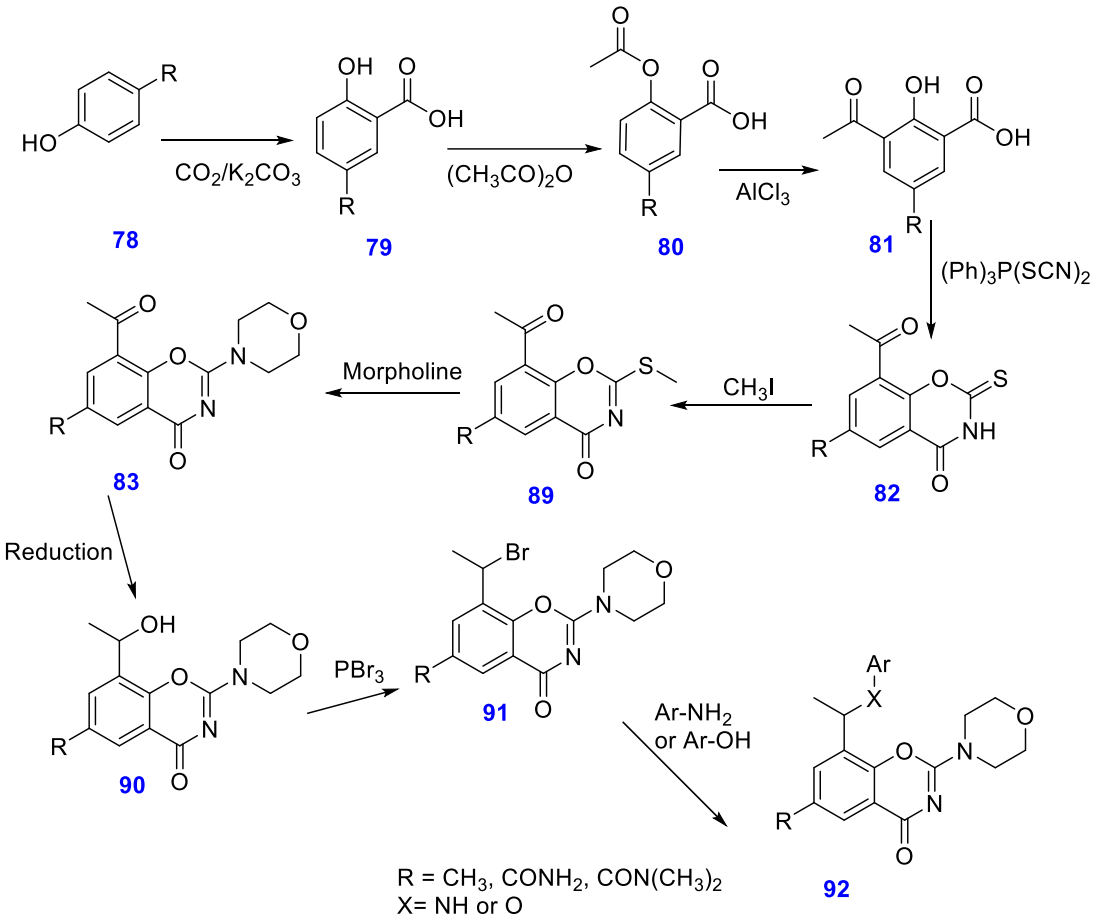


Scheme 1.19: Alternative pathway- 1

In this method of synthesis, the 3-acetyl-2-hydroxy-5-substituted benzoic acid **81** will be reacted with a substituted aniline to give a Schiff base at position 3 **86**. This Schiff base **86** will be hydrogenated to amino compound by sodium borohydride to give 3-anilinoethyl-2-hydroxy-5-substituted benzoic acid **87**. The compound **87** synthesized is cyclized by freshly prepared triphenylphosphine thiocyanate to give 6-substituted-8-(1-arylamino)ethyl-2-thioxo-benzoxazin-4-one, followed by substitution of morpholine at position 2 to give 6-Substituted-2-morpholino-8-(1-(arylamino)ethyl)-4H-benzo[e][1,3]oxazin-4-one **85** (Scheme 1.19).

1.11.3 Alternative pathway- 2

A second alternative pathway was designed in the event of the first two pathways being unsuccessful. This pathway has more steps and intermediates compared to the first two pathways, however, the proposed 6-substituted-2-morpholino-8-(1-(arylamino)ethyl)-4H-benzo[e][1,3]oxazin-4-ones can be synthesized in this way (Scheme 1.20).



Scheme 1.20: Alternative pathway- 2

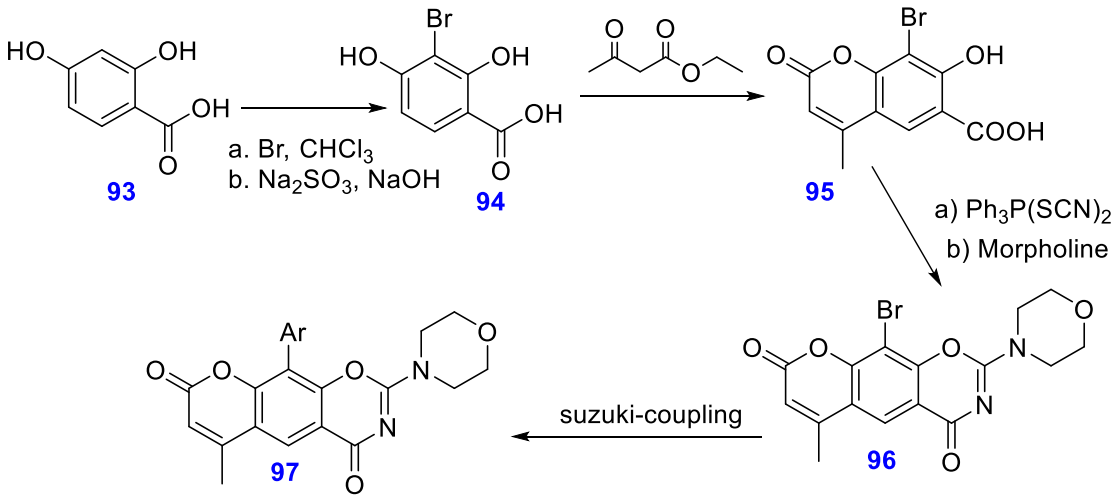
Alternative pathway-2 is similar to the first proposed pathway up to the synthesis of 6-substituted-2-morpholino-8-acetyl-benzoxazin-4-one **83**. Then the compound **83** will be reduced to give 6-substituted-2-morpholino-8-hydroxyethyl-benzoxazin-4-one **90**.

Compound **90** will be brominated to replace alcohol group to form 6-substituted-2-morpholino-8-bromoethyl-benzoxazin-4-one **91**.

Finally, the bromo derivative will be reacted with substituted aniline to synthesize 6-substituted-2-morpholino-8-(1-(arylamino)ethyl)-4H-benzo[e][1,3]oxazin-4-one **92**. This pathway also facilitates the synthesis of 6-Substituted-2-morpholino-8-(1-(aryloxo)ethyl)-4H-benzo[e][1,3]oxazin-4-one **92** by reacting the bromo derivative with a substituted phenol.

1.11.4 Part -2 of proposed work

It was also proposed to synthesise some new 10-aryl-6-methyl-2-morpholino-4H,8H-chromeno[6,7-e][1,3]oxazine-4,8-diones. Since chromeno[1,3]oxazine-4,8-diones have shown to significant DNA-PK inhibitor activity, we expect the same for the proposed structure. The multi-step synthetic pathway was designed which makes use of 2,4-dihydroxy benzoic acid as the starting compound (Scheme 1.21).



Scheme 1.21: Proposed synthesis of 10-aryl-6-methyl-2-morpholino-4H,8H-chromeno[6,7-e][1,3]oxazine-4,8-diones

1.12 Objectives of the work

Thorough review of the available literature revealed that the 1,3-benzoxazin-4-ones have shown wide ranging biological activity. The importance of morpholine ring at second position as potent heterocyclic pharmacophore has also been established. Arylaminoethyl and aryloxoethyl at the 8^{th} position have also been found useful in enhancing the biological activity. The synthesis of proposed compounds will be achieved as described in the synthetic pathways.

In this work, broadly, two types of structural modifications will be achieved i.e. arylaminoethyl, and aryloxoethyl at position 8 with methyl or methyl ester or amide at position 6. The synthesized compounds will be subjected to their structural elucidation by various analytical techniques (NMR, IR, and Mass spectroscopy).

The synthesized compounds will also be evaluated for their DNA-PK, PI3K and PDE3A inhibitory activities, with an aim to discover novel, effective and drug-like molecules. Indeed, the PDE3A inhibition activity was a dimension later added to the proposed work owing to the synthesis of 6-substituted-2-morpholino-8-(1-(aryloxo)ethyl)-4H-benzo[e][1,3]oxazin-4-ones compounds, in addition to the originally proposed 6-substituted-2-morpholino-8-(1-(arylamino)ethyl)-4H-benzo[e][1,3]oxazin-4-ones.

CHAPTER 2: SYNTHESIS OF 6-METHYL-2-MORPHOLINO-8-(1-(ARYLAMINO OR ARYLOXO)ETHYL)-4H-BENZO[E][1,3]OXAZIN-4-ONE

The synthesis of 6-methyl-2-morpholino-8-(1-(arylamino or aryloxo)ethyl)-4H-benzo[e][1,3]oxazin-4-ones was attempted. The pathways that were followed are mentioned in the proposed Scheme of work in Chapter 1.

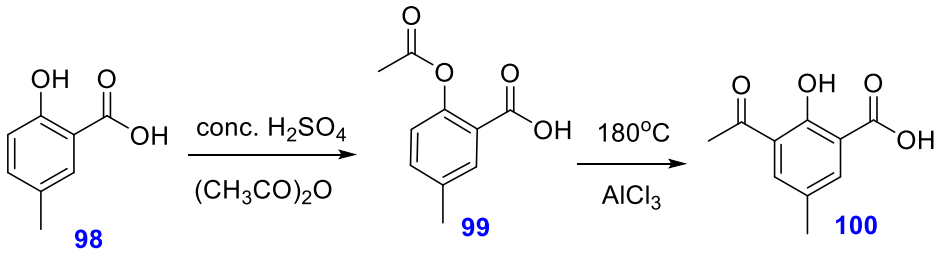
2.1 Synthesis of 8-acetyl-6-methyl-2-morpholino-1,3-benzoxazin-4-one

The first requirement for the synthesis of 8-acetyl-6-methyl-2-morpholinobenzoxazine is the synthesis of 8-acetyl-6-methyl-2-thio-1,3- benzoxazin-4-one (Scheme 2.3). The 2-C=S results in its corresponding 2-marcapto tautomer and react with morpholine to form the desired compound.

2.1.1 Synthesis of 3-acetyl-2-hydroxy-5-methylbenzoic acid

The synthesis of 8-acetyl-6-methyl-2-morpholinobenzoxazine first required preparation of 3-acetyl-2-hydroxy-5-methylbenzoic acid (Scheme 2.1). This was synthesized using a two-step process and 5-methyl salicylic acid **98** was used as starting compound (Scheme 2.1). 5-methyl salicylic acid was o-acetylated using acetic anhydride according to a previously reported procedure[99] to produce 2-acetoxy-5-methyl benzoic acid **99**. 3-

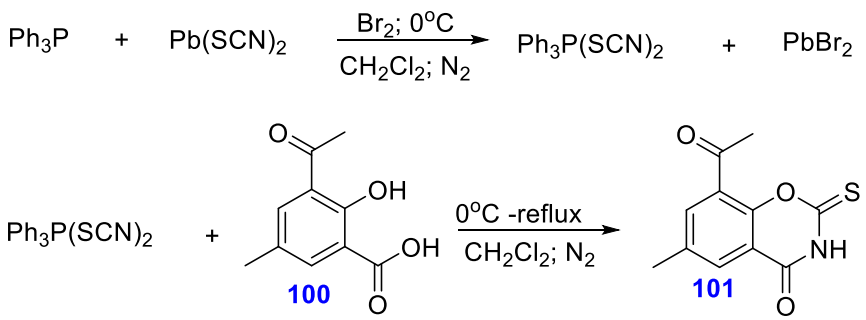
acetyl-2-hydroxy-5-methylbenzoic acid **100** was prepared by using a solventless Fries rearrangement reaction which involves heating a mixture of 2-acetoxy-5-methyl benzoic acid and aluminium chloride at 180°C whilst argon was passed through the reaction vessel[99] (Scheme 2.1). This method is higher yielding compared to other reported synthetic methods for the same compound.[100, 101]



Scheme 2.1.: Synthesis of 3-acetyl-2-hydroxy-5-methylbenzoic acid **100**

2.1.2 Synthesis of 8-acetyl-6-methylbenzoxazinone

A generalized one-pot reaction for the synthesis of 2-thioxo-1,3-benzoxazin-4-one compounds was successfully developed in which $Ph_3P(SCN)_2$ was prepared according to a previously reported procedure.[14] Moreover, *in situ* synthesized Ph_3PBr_2 was used in the synthesis of $Ph_3P(SCN)_2$ which was then used in the cyclization of 3-acetyl-2-hydroxy-5-methylbenzoic acid **100** to its corresponding substituted 2-thioxo-1,3-benzoxazine (Scheme 2.2).



Scheme 2.2.: Synthesis of 8-acetyl-6-methylbenzoxazinone **101**

The $PbBr_2$ precipitate was filtered off after completion of the reaction, and the mother liquor was evaporated to dryness under reduced pressure. The $PbBr_2$ waste was hot filtered using acetone to extract any remaining product and then evaporated under reduced pressure. The combined solid from the above was triturated using toluene.

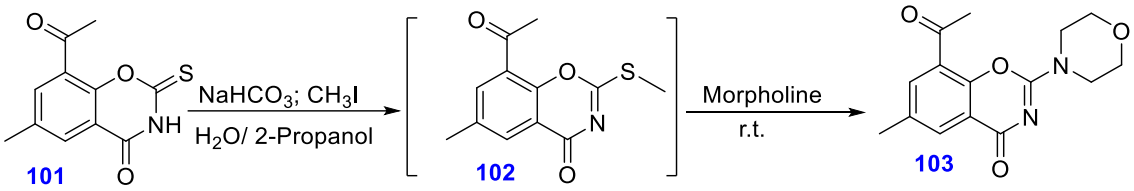
2.1.3 Synthesis of 8-acetyl-6-methyl-2-morpholino-1,3-benzoxazinone

The tautomerisation of the 2-C=S group of the synthesized compound 8-acetyl-6-methylbenzoxazinone (Scheme 2.2) can be effectively utilized for the synthesis of 8-acetyl-6-methyl-2-morpholino-1,3-benzoxazinone (Scheme 2.3). This method has advantages over earlier synthetic methods, like minimizing the number of steps and avoiding the use of hazardous reagents such as cyanogen bromide.[102]

Initially, 8-acetyl-6-methylbenzoxazinone **101** was reacted with morpholine according to a previously reported method.[18] The benzo-1,3-oxazine was dissolved in dry 1,4-dioxane, morpholine (5 times excess) was then added drop-wise with stirring. The reaction mixture was heated to reflux until the evolution of hydrogen sulphide gas had ceased, which was found to take approximately 2 hours. An impure product was obtained in low yield (<50%). Hydrogen sulphide gas was not a concern as the reaction was done in a fume hood.

A better procedure was reported by Ihmaid *et al.*[77] for the preparation of 2-methylsulfanyl-1,3-benzoxazin-4-one intermediates which were successfully replaced with secondary amines, benzylamine and 3-aminopyridine. Morrison *et al.*[19] attempted the reaction using morpholine by adding it dropwise to the reaction mixture 30 minutes after the addition of iodomethane, removing the need to isolate the 2-methylsulfanyl intermediates. This procedure was eventually used with slight modifications to react 8-

acetyl-6-methylbenzoxazinone **101** with iodomethane in a solution of $NaHCO_3$ and water/2-propanol (1:1) to give 2-methylsulfanyl-1,3-benzoxazinone **102 (**which was only once isolated for characterisation). 2-methylsulfanyl-1,3-benzoxazinone was formed as a thick yellow precipitate within the reaction mixture and then morpholine was added dropwise directly to the reaction mixture and allowed to stir at room temperature overnight. The reaction time was only 3 hours in earlier reported procedure but in this investigation, it was needed to prolong it to improve the yield as the 2-methylsulfanyl-1,3-benzoxazinone **102** was slow to react. The reaction mixture was filtered and washed with water to remove any remaining $NaHCO_3$. It was found that the filtrate contained a good fraction of the product and so it was extracted with ethyl acetate and dried. The dried solid and the dried solid from the extract were combined and recrystallized from toluene to obtain 8-acetyl-6-methyl-2-morpholino-1,3-benzoxazinone **103** (Scheme 2.3).



Scheme 2.3: Synthesis of 8-acetyl-6-methyl-2-morpholino-1,3-benzoxazinone **103**

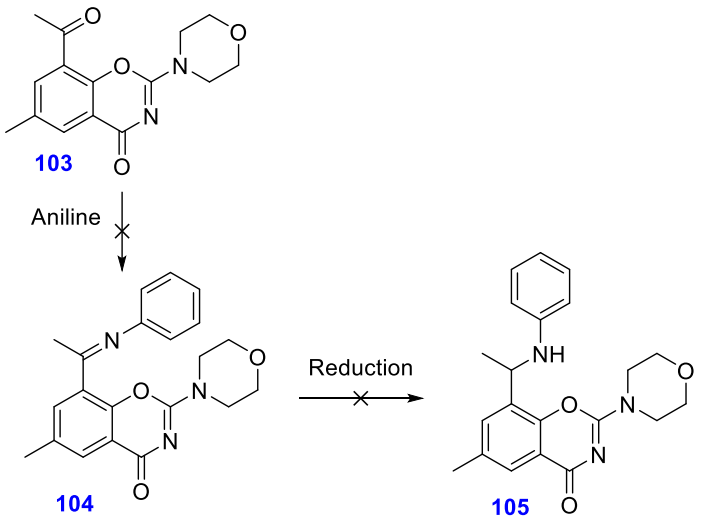
2.2 Attempted reductive amination of 8-acetyl-6-methyl-2-morpholino-1,3-benzoxazinone

A previously reported efficient method of reductive amination[103] was applied here with slight modifications. 8-acetyl-6-methyl-2-morpholino-1,3-benzoxazinone **103** along with aniline and catalytic amount of concentrated HCl were stirred for 15 minutes in

30 ml acetonitrile hoping to form an imine. The solvent was evaporated to give the crude imine, which re-dissolved in 30 ml acetonitrile then subjected to reduction by the portion-wise addition of $NaBH_4$ over 5 minutes. The reaction is then left to stir for 30 minutes. The solvent was then evaporated under reduced pressure to give an oily residue. Water was added to the resulting oil and extracted by chloroform. The analysis of the product showed that it was a mixture of aniline and 8-hydroxyethyl-6-methyl-2-morpholino-1,3-benzoxazinone. Indeed, the reaction was unsuccessful (Scheme 2.4).

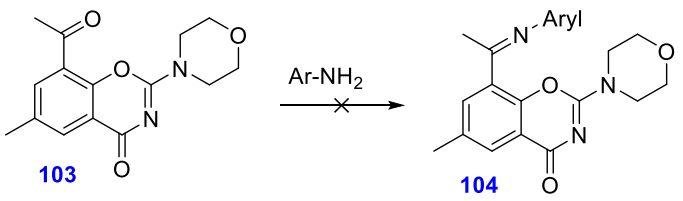
Suspecting the cause of failure of reaction as the solvent, it was changed from acetonitrile to methanol since it has been reported to be the best suitable solvent for reductive amination reactions involving sodium borohydride.[104] Moreover, the reaction time was increased to 24 hours (followed by TLC). The reaction was again unsuccessful as in Scheme 2.4. The process was repeated at least thrice to confirm the results.

Another similar reductive amination reaction which was previously reported for the compound PIK-108[105], was employed in this case with the compound, aniline, glacial acetic acid and sodium cyanoborohydride in a single pot and methanol as solvent, heated to reflux overnight. The compound **103** failed to react.



Scheme 2.4: Attempted reductive amination of 8-acetyl-6-methyl-2-morpholino-1,3-benzoxazinone **103**

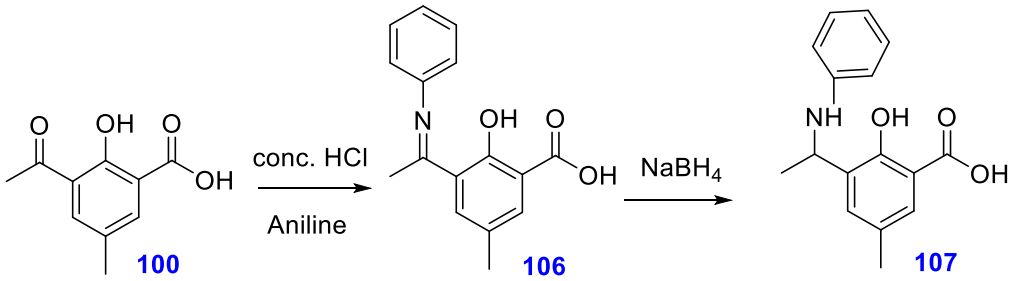
An attempt was made to first try amination of 8-acetyl-6-methyl-2-morpholino-1,3-benzoxazinone **103**. The procedure was similar as in the first step of reductive amination but modified to reflux the reaction mixture in methanol and without concentrated HCl. The reaction was followed by TLC for 2 days and it was inferred that it failed.



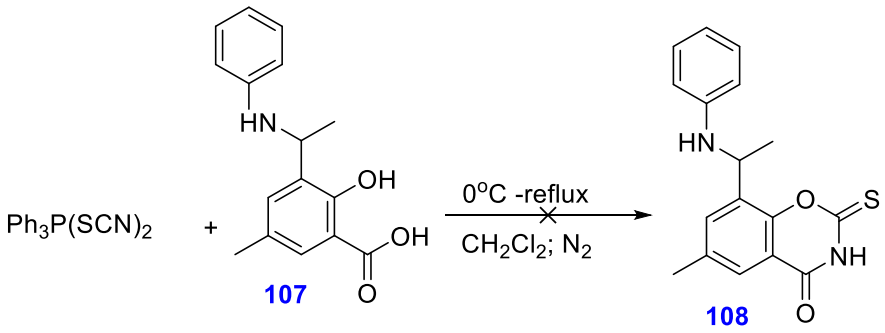
Scheme 2.5: Amination of 8-acetyl-6-methyl-2-morpholino-1,3-benzoxazinone **103**

Another effort was made for the same reaction (Scheme 2.5) but this time employing a different procedure.[106] This process involved the use of dry toluene as solvent with molecular sieves refluxed at 80 ºC. The reaction was followed by TLC for 24 hours and it was found that the compound was unreacted. This procedure was repeated with reflux in this instance and the reaction was allowed for two days (followed by TLC). Again, there was no reaction. Possible steric hindrance, which is not uncommon in carbonyl compounds, is more likely to be the cause for the above reaction to fail.

2.3 Attempted amination and reduction of 3-acetyl-2-hydroxy-5-methylbenzoic acid and subsequent cyclization



Scheme 2.6: Amination and reduction of 3-acetyl-2-hydroxy-5-methylbenzoic acid **100**



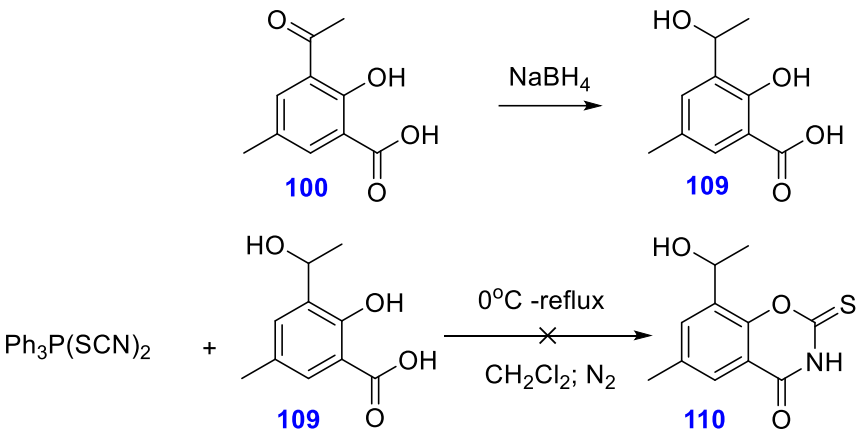
Scheme 2.7: Attempted cyclization of 2-hydroxy-5-methyl-3-(1-(phenylamino)ethyl)benzoic acid **107**

3-acetyl-2-hydroxy-5-methylbenzoic acid **100** was subjected to amination reaction following a previously reported method[103] with slight modifications. The reaction was successful and gave a good yield of 2-hydroxy-5-methyl-3-(1-(phenylimino)ethyl)benzoic acid **106**. This compound **106** was then reduced easily by sodium borohydride to give 2-hydroxy-5-methyl-3-(1-(phenylamino)ethyl)benzoic acid **107** in moderate yields (Scheme 2.6).

This compound **107** was then made to react with freshly prepared triphenylphosphine thiocyanate expecting the cyclization reaction to happen, but it failed to react (Scheme 2.7).

2.4 Attempted cyclization of 2-hydroxy-3-(1-hydroxyethyl)-5-methylbenzoic acid

Another way to get to the desired intermediate 8-hydroxyethyl-6-methyl-2-thioxobenzoxazinone **110** was to first reduce the acetyl group to hydroxy group in the compound 3-acetyl-2-hydroxy-5-methylbenzoic acid **100** and then subject it to cyclization reaction to form a benzoxazine derivative as seen in Scheme 2.8.

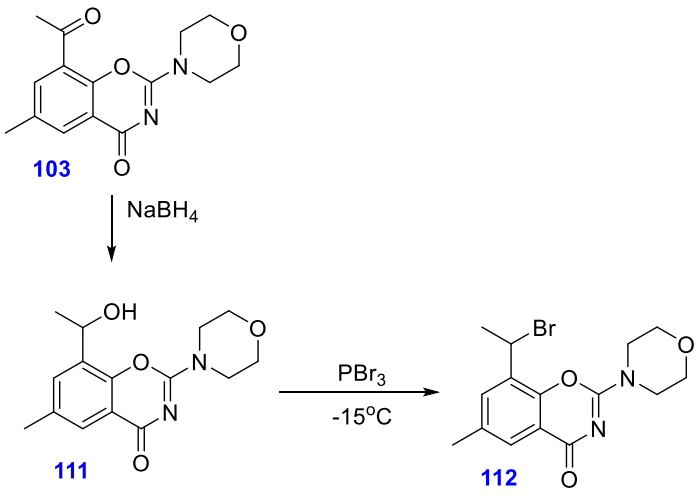


Scheme 2.8: Synthesis of 2-hydroxy-3-(1-hydroxyethyl)-5-methylbenzoic acid **109** and its attempted cyclization

The compound 3-acetyl-2-hydroxy-5-methylbenzoic acid **100** was easily reduced by sodium borohydride to give the product **109** in moderate yield. The reaction of this compound **109** with freshly prepared triphenylphosphine thiocyanate was unsuccessful.

2.5 Synthesis of 8-bromoethyl-6-methyl-2-morpholino-1,3-benzoxazinone

As a result of aforementioned synthetic pathways failing, 8-bromoethyl-6-methyl-2-morpholino-1,3-benzoxazinone **112** was prepared according to a previously reported procedure[82] with some modifications to enhance the yields. The compound 8-acetyl-6-methyl-2-morpholino-1,3-benzoxazinone **103** was first reduced to 8-hydroxyethyl-6-methyl-2-morpholino-1,3-benzoxazinone **111** by using sodium borohydride. The yield was very low as the carbonyl group at position-4 was also reduced. Hence the reaction was carried out at subzero temperature and reaction time was reduced to get better yield.



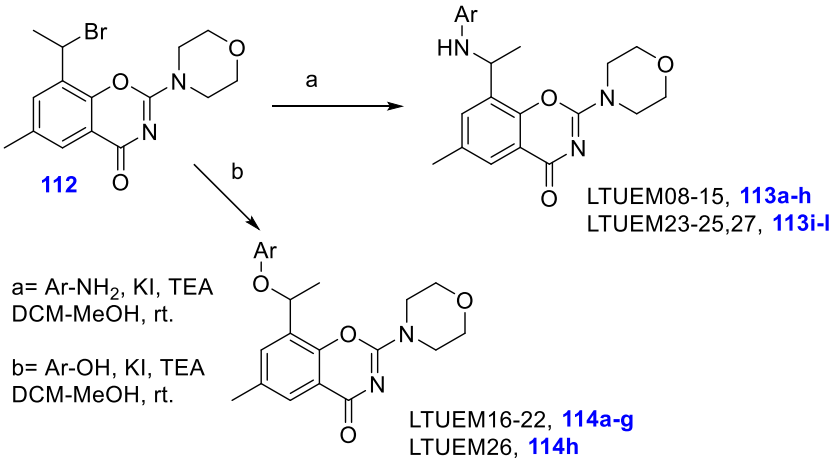
Scheme 2.9: Synthesis of 8-bromoethyl-6-methyl-2-morpholino-1,3-benzoxazinone **112**

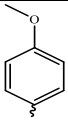
In the next step, the compound was reacted with phosphorus tribromide under anhydrous conditions at 0 °C as reported previously.[82] The bromo derivative **112** was formed although in very low yields. In this reaction corrosive hydrogen bromide is evolved, which is toxic, and reacts violently with water. So, care was taken to trap it with a basic

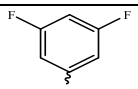
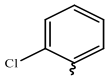
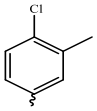
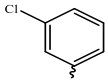
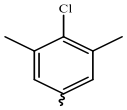
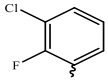
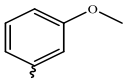
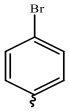
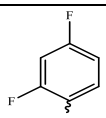
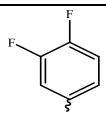
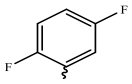
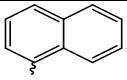
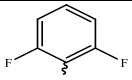
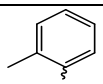
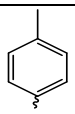
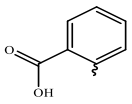
solution. The yield was improved by taking the temperature down to -15 ºC following a more efficient way of bromination of alcohols.[107]

2.6 Synthesis of 6-methyl-2-morpholino-8-(1-(arylamino or aryloxo)ethyl)-4H-benzo[e][1,3]oxazin-4-ones

The ultimate step in the process of synthesis of 6-methyl-2-morpholino-8-(1-(arylamino or aryloxo)ethyl)-4H-benzo[e][1,3]oxazin-4-ones involves an easy method in which 8-bromoethyl-5-methyl-2-morpholino-1,3-benzoxazinone **112** is reacted with a substituted aniline or a substituted phenol (Scheme 2.10).



Compound	***Ar***	***Compound***	***Ar***
LTUEM08 113a		**LTUEM18 114c**	

LTUEM09 113b		**LTUEM19 114d**	
LTUEM10 113c		**LTUEM20 114e**	
LTUEM11 113d		**LTUEM21 114f**	
LTUEM12 113e		**LTUEM22 114g**	
LTUEM13 113f		**LTUEM23 113i**	
LTUEM14 113g		**LTUEM24 113j**	
LTUEM15 113h		**LTUEM25 113k**	
LTUEM16 114a		**LTUEM26 114h**	
LTUEM17 114b		**LTUEM27 113l**	

Scheme 2.10: Synthesis of 6-methyl-2-morpholino-8-(1-(arylamino or aryloxo)ethyl)-4H-benzo[e][1,3]oxazin-4-ones

Potassium iodide was used to make the substitution easier in place of bromine whereas triethylamine sped up the reaction. However, for the compound **LTUEM27 113l** a

different procedure was employed with 8-hydroxyethyl-6-methyl-2-morpholino-1,3-benzoxazinone **111** as the starting material for this reaction as mentioned in the experimental chapter.

2.6.1 Structure elucidation of 6-methyl-2-morpholino-8-(1-(arylamino or aryloxo)ethyl)-4H-benzo[e][1,3]oxazin-4-ones

The structures of the old and newly synthesized were confirmed using FTIR, ^{1}H and ^{13}C NMR spectroscopy. The ^{1}H and ^{13}C NMR spectra give strong support for the proposed structures. Assignment of the C-2′ and C-3′ of the morpholine were made with the help of previously reported 2-morpholino-1,3-benzoxazin-4-ones.[18, 77] ChemDraw professional (V17.1) was also used for simulated ^{1}H and ^{13}C NMR spectra as references to aid the analysis of the new products. No ^{13}C NMR data is reported for some of the previously reported compounds which are reported in the literature. X-ray crystallography was also used to confirm the structure of one of the newly synthesized arylamino derivatives.

2-methylsulfanyl-1,3-benzoxazin-4-one intermediate was confirmed using FTIR, ^{1}H and ^{13}C NMR spectroscopy. The 2-methylsulfanyl substituent appears as a singlet at ~ 2.60 ppm in accordance with the previously reported ^{1}H NMR values. Whilst the ^{13}C NMR spectrum shows the loss of the 2-thiocarbonyl at ~ 181.0 ppm and the presence of a second methyl signal at ~ 13.0 ppm confirming that the reaction had taken place.

2.6.2 Comparison of clogP

The clogP values of the synthesized compounds were predicted using the program Bio-loom, to understand and compare their hydrophilic-lipophilic nature.

Compound	*clogP*	*Compound*	*clogP*
LTUEM08 113a	2.76	**LTUEM18 114c**	3.59
LTUEM09 113b	3.46	**LTUEM19 114d**	4.00
LTUEM10 113c	3.77	**LTUEM20 114e**	4.85
LTUEM11 113d	3.77	**LTUEM21 114f**	5.35
LTUEM12 113e	4.02	**LTUEM22 114g**	3.59
LTUEM13 113f	3.93	**LTUEM23 113i**	3.46
LTUEM14 113g	3.39	**LTUEM24 113j**	3.46
LTUEM15 113h	3.94	**LTUEM25 113k**	3.46
LTUEM16 114a	3.50	**LTUEM26 114h**	4.00
LTUEM17 114b	4.00	**LTUEM27 113l**	3.48
TGX-221	2.1	**PIK-108**	2.9
AZD6482	2.8		

Table 2.1: Predicted clogP of synthesized compounds and comparison with the analogues of different cores.

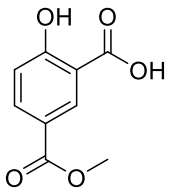
It was observed that, in general, the arylamino derivatives are more hydrophilic compared to the aryloxo derivatives. In both the series the hydrophilicity decreased as the number of substituents increased on the 8-aryl. TGX-221 and PIK-108 have the same exact structure as the prototype compound LTUEM08, except the scaffolds. TGX-221 has a pyrido[1,2-a]pyrimid-4-one core whereas PIK-108 has a chromen-4-one scaffold. The clogP values agree with our hypothesis that the 1,3-benzoxazin-4-one scaffold has better aqueous solubility than the corresponding chromen-4-one, as seen with LTUEM08 (clogP = 2.76) and its corresponding chromen-4-one derivative PIK-108 (clogP = 2.9). Moreover, the compound LTUEM08 has slightly higher lipophilicity compared to its corresponding pyrido[1,2-a]pyrimid-4-one derivative TGX-221 (clogP = 2.1).

CHAPTER 3: ATTEMPTED SYNTHESIS OF 6-METHOXYCARBONYL-2-MORPHOLINO-8-(1-(ARYLAMINO OR ARYLOXO)ETHYL)-4H-BENZO[E][1,3]OXAZIN-4-ONES

3.1 Synthesis of 8-acetyl-6-methoxycarbonyl-2-morpholinobenzoxazinone

To synthesize 8-acetyl-6-methoxycarbonyl-2-morpholinobenzoxazine, the synthesis of 8-acetyl-6-methoxycarbonyl-2-thio-1,3- benzoxazin-4-one was the prerequisite. The 2-C=S results in its corresponding 2-marcapto tautomer and reacts with morpholine to form the required compound.

3.1.1 Synthesis of the starting compound 2-hydroxy-5-(methoxycarbonyl)benzoic acid

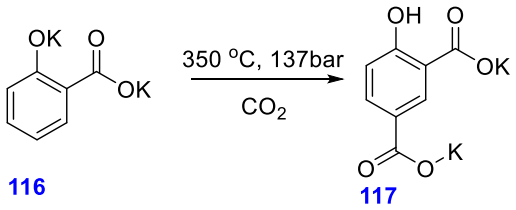


115

Figure 3.1: 2-hydroxy-5-(methoxycarbonyl)benzoic acid **115**

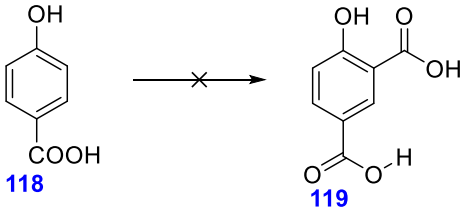
The closest commercially available compound to 2-hydroxy-5-(methoxycarbonyl)benzoic acid **115** was 4-hydroxyisophthalic acid **119**.

4-hydroxyisophthalic acid is formed as a constituent of "brown dust" residue from the commercial synthesis and purification process of salicylic acid from sodium phenoxide.[108] It is also obtained naturally from the roots of *Decalepis hamiltonii* and has been found to possess potent antioxidant, antiproliferative, analgesic and antipyretic properties.[109-112] Because of the expense, it was attempted to synthesize it in the lab. There is only one established method for the synthesis of this compound in which it is synthesized from dipotassium salicylate **116** to give dipotassium 4-hydroxyisophthalate **117** which can be later hydrolysed to give 4-hydroxyisophthalic acid, as seen in the Scheme 3.1. This method involves the use of very high pressure (137 bar) and a very high temperature (350ºC) in a specialized high-pressure resistant reactor.[113]



Scheme 3.1: Synthesis of dipotassium 4-hydroxyisophthalate **117**

Since this method was not feasible in a normal organic chemistry lab, conventional methods of carboxylation were tried in order to synthesize 4-hydroxyisophthalic acid **119**. One such method is a direct carboxylation by using a bicarbonate, water as solvent and passing CO_2 at 90 ºC.[114] However, the reaction failed in this case (Scheme 3.2).



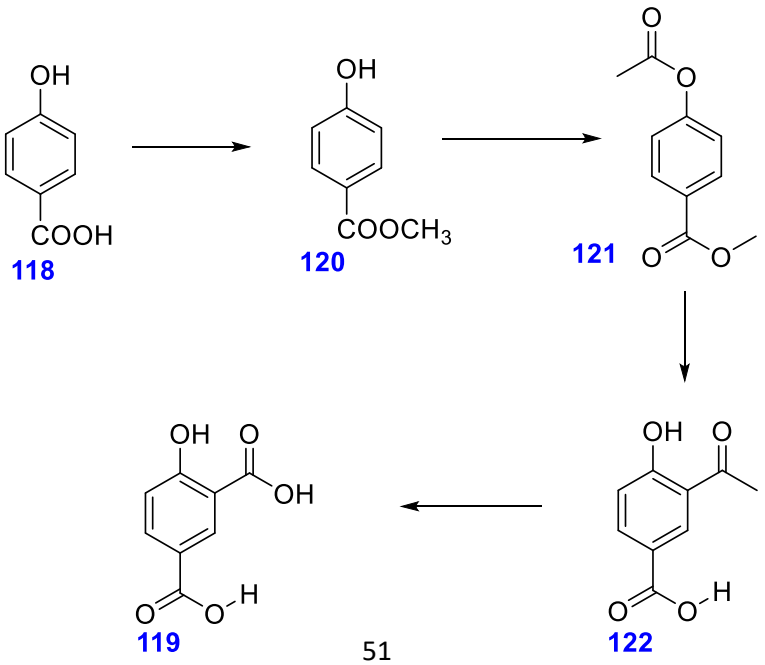
Scheme 3.2: Attempted carboxylation of 4-hydroxybenzoic acid **118**

Another reported method of carboxylation of phenols at atmospheric pressure was attempted. In this procedure, 4-hydroxybenzoic acid **118** was reacted with sodium hydride and 2,4,6-trimethylphenol while passing through CO_2 gas and heating to 100 ^oC.[115] It failed to react and the starting compound was recovered.

In another method, triethylamine, sodium iodide and magnesium chloride were used to react with 4-hydroxybenzoic acid **118** in acetonitrile as solvent while passing CO_2 gas, expecting a carboxylation reaction as reported by Tirpak *et al*.[116] This method did not produce any measurable yield of product.

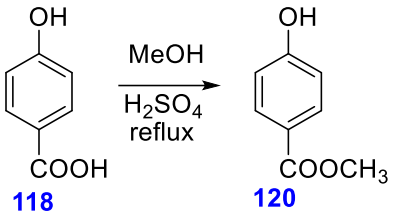
A modified method of carboxylation at high pressure was tried in a Büchi reactor. While passing CO_2, the reactants 4-hydroxybenzoic acid **118**, sodium/potassium carbonate and potassium acetate were heated to 230 ^oC, in glycerol under high pressure (up to 0.6 MPa). The residue was collected as a decomposed product.

Since the above methods failed to produce the desired compound, a new method was investigated to synthesize 4-hydroxyisophthalic acid **119** from 4-hydroxybenzoic acid **118**, a multistep process as seen in the Scheme 3.3.



Scheme 3.3: Synthesis of 4-hydroxyisophthalic acid **119** through acetylation

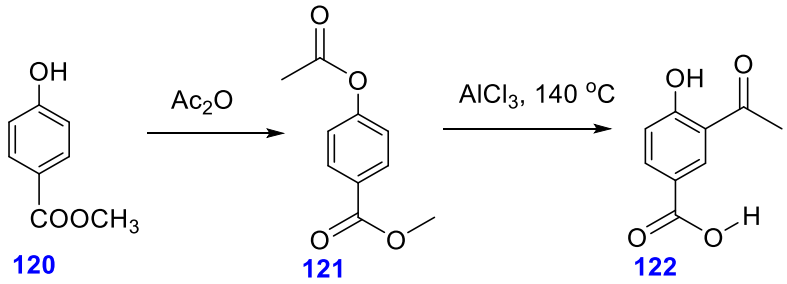
The first step of this process used the starting compound 4-hydroxybenzoic acid **118** which was converted to an ester **120**, in order to make subsequent O-acetylation easier. This was achieved using methanol and concentrated sulphuric acid without difficulty (Scheme 3.4).



118 **120**

Scheme 3.4: Esterification of 4-hydroxybenzoic acid **118**

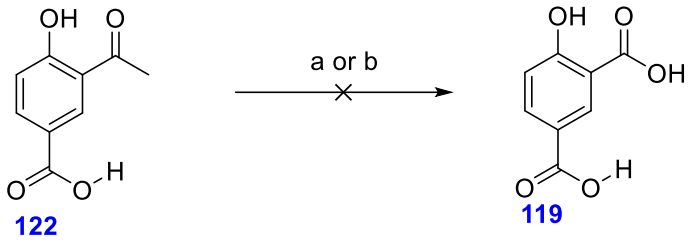
Next, methyl 4-hydroxybenzoate **120** was dissolved in acetic anhydride with addition of 1 drop of concentrated sulfuric acid, at 110 ºC for 1 hour. The O-acetylated product **121** was obtained and taken for a rearrangement reaction by using aluminium chloride. Eventually 3-acetyl-4-hydroxybenzoic acid **122** was obtained, though this is a different product to what is reported in the literature.[117] The methyl ester was not retained and instead a carboxylic acid was collected as the product (Scheme 3.5).



120 **121** **122**

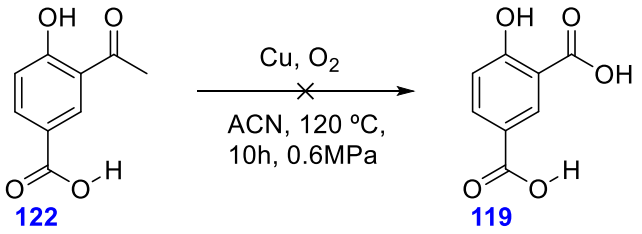
Scheme 3.5: O-acetylation and rearrangement of methyl-4-hydroxybenzoate **120**

The following step in this synthesis process was to oxidize the ketone in 3-acetyl-4-hydroxybenzoic acid **122** to get the compound 4-hydroxyisophthalic acid **119** (Scheme 3.6). Initially, a classic method of oxidation of ketones to carboxylic acids, viz, Haloform reaction was attempted. In a bromoform reaction, the ketone, bromine, and sodium hydroxide were used in the ratio 1:3:8 at ice cool temperature. However, the desired product was not formed and instead a bromination reaction had occurred.



Scheme 3.6: Haloform reactions; reagents and conditions: (a) Bromine, NaOH, 0 ºC; (b) 5% NaOH, potassium iodide-iodine reagent.

An iodoform reaction was undertaken using potassium iodide-iodine reagent following the method from literature.[118] The compound **122** failed to react (Scheme 3.6).



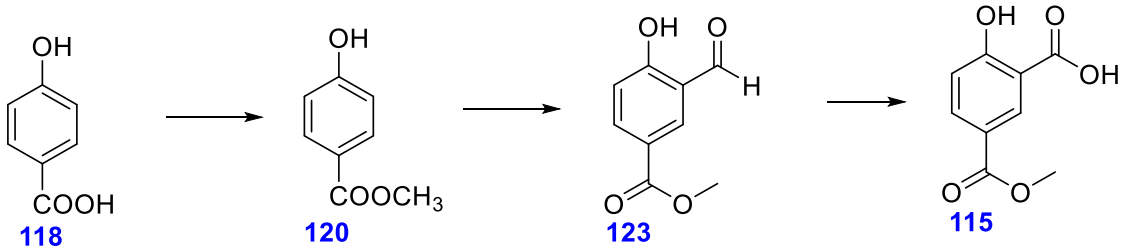
Scheme 3.7: Copper catalysed oxidation

Oxidation by the use of transition metals as catalysts is well-known. One such method from the literature was used to oxidize ketone to carboxylic acid to get the desired product.[119] In this method, $Cu(NO_3)_2.3H_2O$ was used while passing Oxygen gas for 10

hours (Scheme 3.7). In this work the compound 3-acetyl-4-hydroxybenzoic acid **122** did not oxidize contrary to the probability.

Reaction of 3-acetyl-4-hydroxybenzoic acid **122** with other oxidizing agents such as hydrogen peroxide, potassium permanganate and sodium dihydrogen phosphate were tried separately, but with no success.

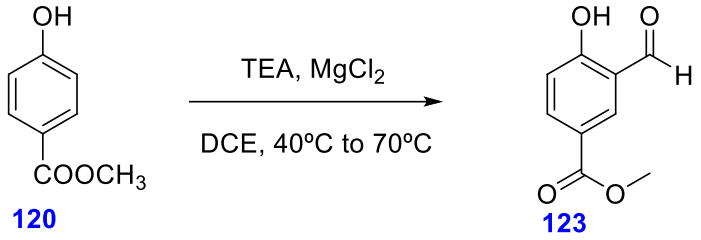
Another multistep process was designed and investigated to synthesize the starting material (2-hydroxy-5-(methoxycarbonyl)benzoic acid) **115** as in Scheme 3.8.



Scheme 3.8: Synthesis of 2-hydroxy-5-(methoxycarbonyl)benzoic acid **115**

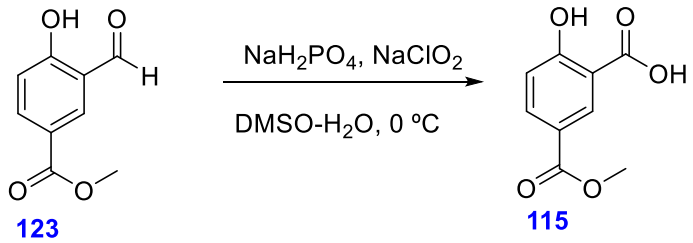
The Scheme indicates that the first step is the esterification of 4-hydroxybenzoic acid **118** as described previously in this chapter. In the second step, methyl-4-hydroxybenzoate **120** is reacted with a formyl group delivering agent such as formaldehyde or paraformaldehyde to give methyl-3-formyl-4-hydroxybenzoate **123**. A few methods were tried to achieve this ranging from the use of solvents such as trifluoroacetic acid,[120, 121] acetonitrile,[122, 123] chloroform,[100] dimethylformamide[124] and tetrahydrofuran to 1,2-dichloroethane. The desired product was formed, but in very low yields. A modified method was developed in which methyl-4-hydroxybenzoate **120** was first mixed with triethylamine and dry magnesium chloride and stirred at 40 ºC in dry 1,2-dichloroethane. Later paraformaldehyde was added and stirred overnight at 70 ºC (Scheme 3.9). This method gave the product **123** in very good yields (~80%). The

rationale to change the solvent to dry 1,2-dichloroethane (a higher boiling point solvent) was to prevent the sublimation of paraformaldehyde while having the reaction mixture heated to 70 ºC.



Scheme 3.9: Efficient method for formylation of methyl-4-hydroxybenzoate **120**

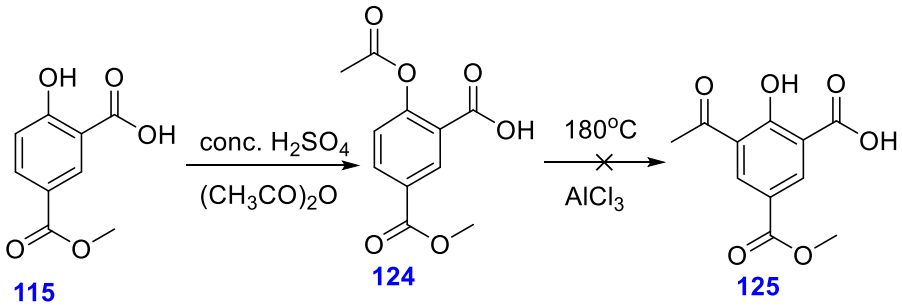
In the next step, methyl-3-formyl-4-hydroxybenzoate **123** was subjected to an oxidation reaction in acetonitrile using sodium dihydrogen phosphate, 30% aqueous hydrogen peroxide solution and aqueous sodium chlorite solution, as reported in the literature.[125] The yield was very low (~15%) and hence another similar method[126] was used, in which a combination of water and dimethylsuphoxide was the solvent system to dissolve compound **123** and sodium dihydrogen phosphate. The mixture was charged with aqueous sodium chlorite solution and was stirred at 0 ºC overnight to give ~66% yield of the desired product that is 2-hydroxy-5-(methoxycarbonyl)benzoic acid **115** (Scheme 3.10).



Scheme 3.10: Synthesis of 2-hydroxy-5-(methoxycarbonyl)benzoic acid **115** by oxidation of methyl-3-formyl-4-hydroxybenzoate **123**

3.1.2 Attempted synthesis of 3-acetyl-2-hydroxy-5-(methoxycarbonyl)benzoic acid

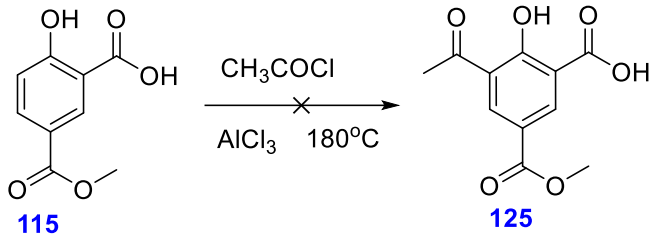
A two step process involving o-acetylation and subsequent rearrangement of the acetyl group seemed to be the best way forward (Scheme 3.11). 2-hydroxy-5-(methoxycarbonyl)benzoic acid **115** was o-acetylated using acetic anhydride according to a previously reported procedure.[99] The product formed i.e. 2-acetoxy-5-(methoxycarbonyl)benzoic acid **124** was taken for a Fries rearrangement reaction, a solventless reaction carried out using anhydrous aluminium chloride whilst passing argon through the reaction flask.[99] However, the reaction did not occur and the product obtained was 4-hydroxyisophthalic acid.



Scheme 3.11: Attempted synthesis of 3-acetyl-2-hydroxy-5-(methoxycarbonyl)benzoic acid **125**

Other methods of rearrangement of the ketone group were tried at temperatures ranging from 120 ºC to 180 ºC but were not successful.[100, 101]

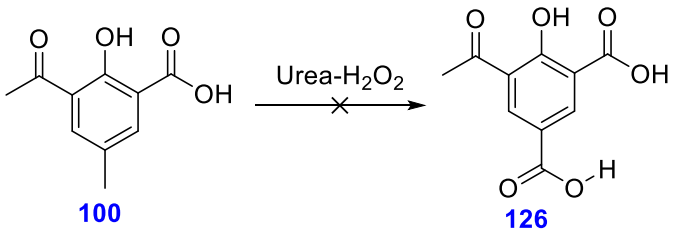
A direct method of C-acetylation, which is a typical Friedel-Crafts acylation was also attempted. In this process, the compound 2-hydroxy-5-(methoxycarbonyl)benzoic acid **115** was suspended in nitrobenzene and reacted with acetyl chloride using a lewis acid i.e. anhydrous aluminium chloride, The product was 4-hydroxyisophthalic acid **119** indicating that desired acetylation reaction did not materialize (Scheme 3.12).



Scheme 3.12: Attempted C-acetylation of 2-hydroxy-5-(methoxycarbonyl)benzoic acid **115**

The same reaction was attempted at temperatures varying from 120 ºC to 180 ºC and also by changing the solvent to toluene, and acetone. However, the result was either no reaction or the formation of 4-hydroxyisophthalic acid **119**.

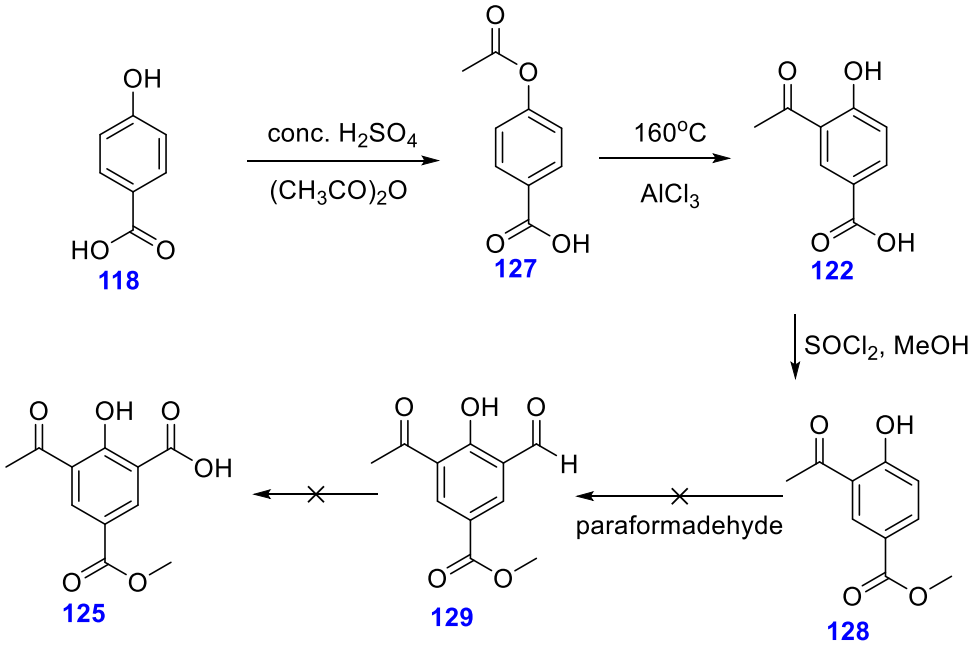
Another approach to synthesize the compound 3-acetyl-2-hydroxy-5-(methoxycarbonyl)benzoic acid **125** was to first oxidize a compound 3-acetyl-2-hydroxy-5-methylbenzoic acid **100** which was synthesized in the previous Chapter, and then selectively esterify the obtained carboxylic acid **126**. This reaction was attempted in a microwave reactor using a freshly prepared urea-hydrogen peroxide catalyst[127] (Scheme 3.13). The compound **100** failed to oxidize to **126** and was recovered as unreacted.



Scheme 3.13: Attempted oxidation of 3-acetyl-2-hydroxy-5-methylbenzoic acid **100**

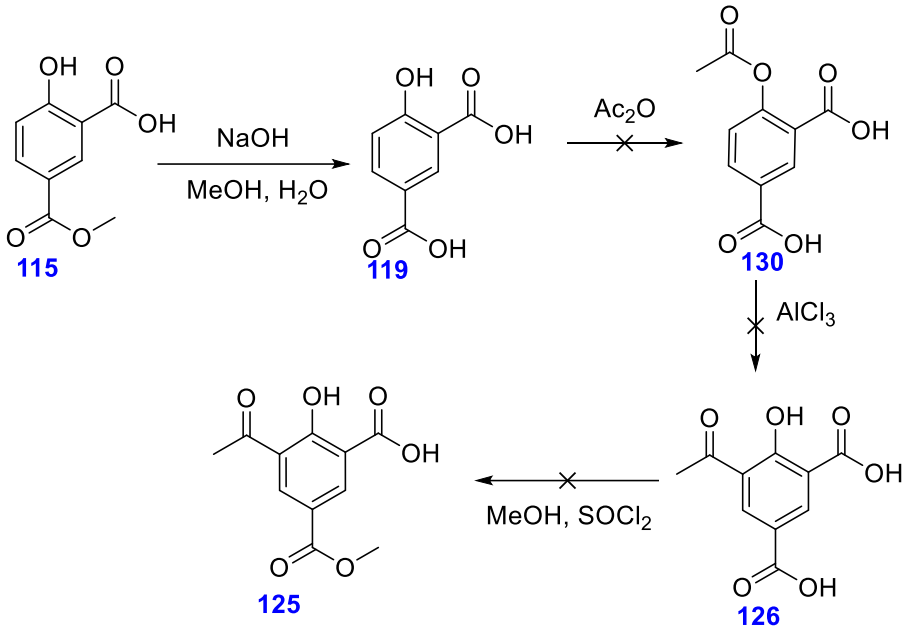
Furthermore, a scheme was designed to synthesize 3-acetyl-2-hydroxy-5-(methoxycarbonyl)benzoic acid **125** by acetylating the compound 4-hydroxybenzoic acid **118** first, followed by formylation and oxidation.

The compound **118** was easily o-acetylated and rearranged to give 3-acetyl-4-hydroxybenzoic acid **122**. Thereafter, the carboxylic acid was converted to ester by a reaction with methanol using thionyl chloride.[128] Subsequently, to have a carboxylic acid group at the ortho position to the hydroxy group, the first step was formylation. This was attempted using paraformaldehyde, triethylamine and magnesium chloride, as established above in the Scheme. However, the compound failed to react, and hence could not be used for the oxidation reaction (Scheme 3.14).



Scheme 3.14: Attempted synthesis of 3-acetyl-2-hydroxy-5-(methoxycarbonyl)benzoic acid **125**

Another method for synthesizing 3-acetyl-2-hydroxy-5-(methoxycarbonyl)benzoic acid **125** was a pathway which starts with the hydrolysis 2-hydroxy-5-(methoxycarbonyl)benzoic acid **115** to 4-hydroxyisophthalic acid **119** and ultimately, selectively esterify the compound 3-acetyl-4- hydroxyisophthalic acid **126** as seen in the Scheme 3.15.



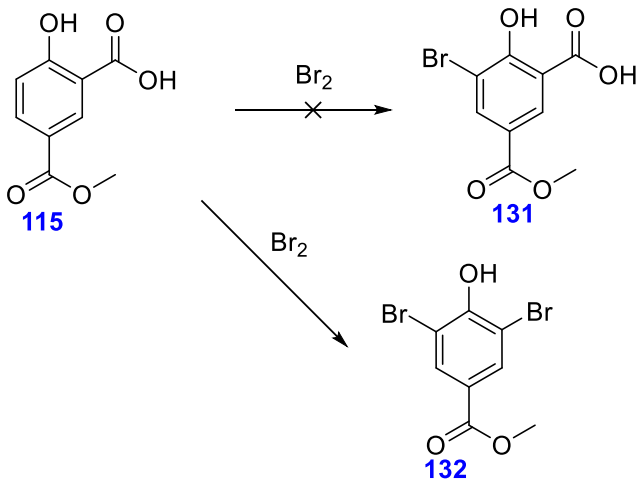
Scheme 3.15: Attempted synthesis of 3-acetyl-2-hydroxy-5-(methoxycarbonyl)benzoic acid through 4-hydroxyisophthalic acid **119**

The compound 2-hydroxy-5-(methoxycarbonyl)benzoic acid **115** was hydrolysed in alkaline conditions to get 4-hydroxyisophthalic acid **119** in good yields, using a previously reported procedure.[129] Afterwards, the compound **119** was subjected to o-acetylation using acetic anhydride. The reaction was attempted at various temperatures (50 ºC to 145 ºC) and followed by TLC. There was no evidence that o-acetylation had occurred.

3.1.3 Synthesis of 3-bromo-2-hydroxy-5-(methoxycarbonyl)benzoic acid

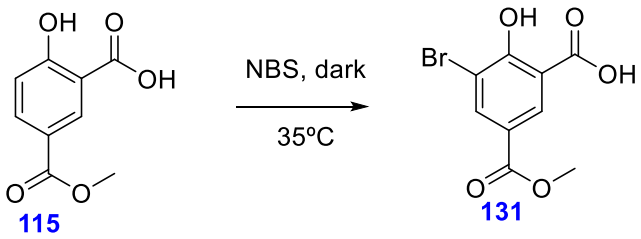
As a consequence of acetylation not occurring, it was decided to substitute 2-hydroxy-5-(methoxycarbonyl)benzoic acid **115** with a bromine at position-3 so that it can later be acetylated using metal complex catalysts. A conventional method of bromination,[114] using liquid bromine dissolved in chloroform and reacting with 2-hydroxy-5-(methoxycarbonyl)benzoic acid **115** was carried out (Scheme 3.16). The desired product

131 was not obtained and instead, the carboxylic acid was lost and a dibromo product **132** was formed.



Scheme 3.16: Bromination by liquid bromine

In another attempt N-bromosuccinimide was used to plant bromine at position-3. This reaction was attempted in dark conditions at a temperature of 35 ºC (Scheme 3.17). It is different from the literature procedures[130, 131] given that the reaction time in this case was significantly reduced to prevent the formation of a dibromo product, and the method proved successful. The crude product was obtained in good yields and was recrystallized to give pure compound **131**.

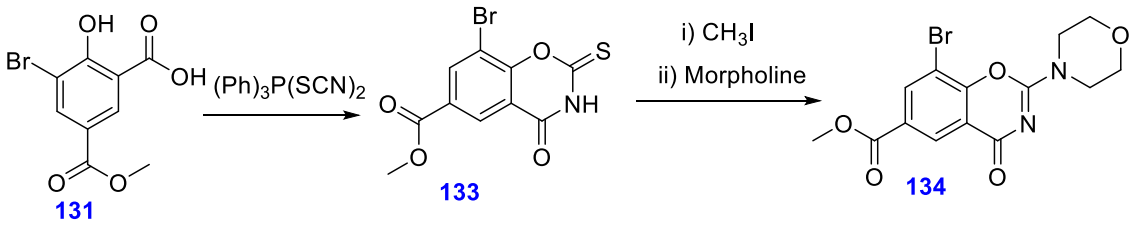


Scheme 3.17: Synthesis of 3-bromo-2-hydroxy-5-(methoxycarbonyl)benzoic acid **131**

3.1.4 Synthesis of 8-bromo-6-methoxycarbonyl-2-morpholino-1,3-benzoxazin-4-one

Firstly, the 2-thioxo-1,3-benzoxazin-4-one derivative was synthesized using a previously established method[14] which involves the use of freshly prepared $Ph_3P(SCN)_2$ which is itself prepared from *in situ* synthesized Ph_3PBr_2. The freshly prepared $Ph_3P(SCN)_2$ was then reacted with 3-bromo-2-hydroxy-5-(methoxycarbonyl)benzoic acid **131** to give 8-bromo-6-methoxycarbonyl-2-thioxobenzoxazinone **133** (Scheme 3.18).

In the next step, an efficient method reported by Morrison *et al,*[19] was used with slight modifications to react the 2-thioxo compound with methyl iodide first and then with morpholine to give 8-bromo-6-methoxycarbonyl-2-morpholino-1,3-benzoxazin-4-one **134**.

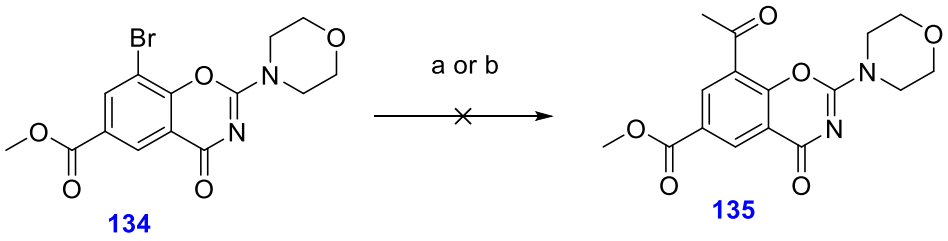


Scheme 3.18: Synthesis of 8-bromo-6-methoxycarbonyl-2-morpholino-1,3-benzoxazin-4-one **134**

3.1.5 Attempted synthesis of 8-acetyl-6-methoxycarbonyl-2-morpholinobenzoxazinone

In the literature very few methods of acetylation of a heterocylic bromine derivative are available, and those methods involve the use of expensive metal complex catalysts. One such method was attempted using butylvinyl ether as acetylating reagent in DMF-water solvent system in alkaline conditions (potassium carbonate). Palladium(II) acetate and

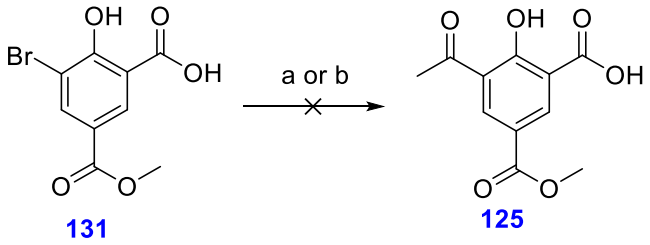
DPPP (1,3-Bis(diphenylphosphino)propane) were added as catalysts followed by an extensive workup procedure.[132] The compound 8-bromo-6-methoxycarbonyl-2-morpholino-1,3-benzoxazin-4-one **134** lost morpholine and acetylation did not occur (Scheme 3.19).



Scheme 3.19: Attempted synthesis of 8-acetyl-6-methoxycarbonyl-2-morpholinobenzoxazinone, reagents and conditions: (a) Butylvinyl ether, DPPP, $Pd(OAc)_2$; (b) tributyl(1-ethoxyvinyl)stannane, $Pd(PPh_3)_2Cl_2$, dioxane, 90 °C

In another attempt, **134** was reacted with tributyl(1-ethoxyvinyl)stannane and using bis(triphenylphosphine)palladium(II) chloride catalyst in dry conditions.[82] Tributyl(1-ethoxyvinyl)stannane was used for its vinylstannane group that would give an acetyl anion equivalent after undergoing hydrolysis. After an extensive workup process the product was obtained and analyzed only to conclude that the compound failed to react (Scheme 3.19).

Similarly, the acetylation reactions with these two methods were carried out separately, to convert 3-bromo-2-hydroxy-5-(methoxycarbonyl)benzoic acid **131** to 3-acetyl-2-hydroxy-5-(methoxycarbonyl)benzoic acid **125** (Scheme 3.20). The compound did not react. Steric hindrance of the ketone functional group is the most likely cause for failure of this reaction.



Scheme 3.20: Attempted synthesis of 3-acetyl-2-hydroxy-5-(methoxycarbonyl)benzoic acid, reagents and conditions: (a) Butylvinyl ether, DPPP, $Pd(OAc)_2$; (b) tributyl(1-ethoxyvinyl)stannane, $Pd(PPh_3)_2Cl_2$, dioxane, 90 °C

3.2 Discussion

There were quite a few failed attempts for the synthesis of 2-hydroxy-5-(methoxycarbonyl)benzoic acid **115**. First, the failure of direct carboxylation of 4-hydroxybenzoic acid **118** by CO_2 occurred and it is more likely because of hindrance from the carboxylic acid. Moreover, very high-pressure (137 bar) conditions can force this reaction, but it was not achievable in our lab. The use of magnesium halide and triethylamine for a direct carboxylation favours carboxylation of ketone rather than carboxylic acids. On the other hand, haloform reactions were attempted for the oxidation of ketone to carboxylic acid for the compound **122.** Perhaps, in these reactions the presence of a phenolic group and a carboxylic acid group has not allowed keto-enol tautomerism for the methyl ketone, which is crucial for haloform reaction to occur. Hence, a different method was developed which involved esterification, formylation, and oxidation reactions to achieve the synthesis of **115** successfully.

Also observed in this chapter is the failure of both Fries rearrangement for the compounds **124** and **130** and an unsuccessful Friedel-Crafts acylation of **115** even after several attempts under anhydrous conditions along with varying conditions of

temperature, solvent, molar ratios, and reaction times. The only explanation for these is that the ring of the compounds in these instances is deactivated for electrophilic aromatic substitution reactions. Such results were unexpected, and so it prompted us to synthesize the compound **131** and take it for further steps.

After these attempts, this part of the work was concluded, as it was not feasible to synthesize the designed molecules i.e. 6-methoxycarbonyl-2-morpholino-8-(1-(arylamino or aryloxo)ethyl)-4H-benzo[e][1,3]oxazin-4-ones through 8-bromo-6-methoxycarbonyl-2-morpholino-1,3-benzoxazin-4-one. The only positive outcome in this chapter was the efficient synthesis of 2-hydroxy-5-(methoxycarbonyl)benzoic acid **115** under normal laboratory conditions.

CHAPTER 4: ATTEMPTED SYNTHESIS OF 6-(CARBAMOYL OR DIMETHYLCARBAMOYL)-2-MORPHOLINO-8-(1-(ARYLAMINO OR ARYLOXO)ETHYL)-4H-BENZO[E][1,3]OXAZIN-4-ONES

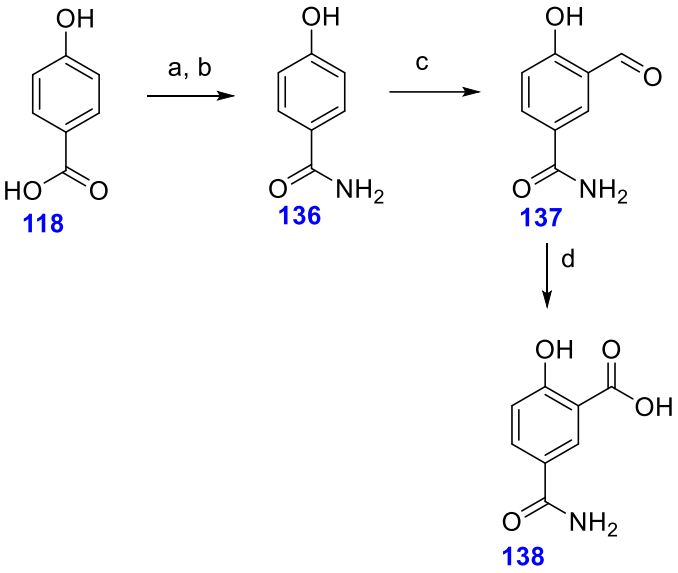
In this chapter, the various attempts to synthesize the designed molecules i.e. 6-(carbamoyl or dimethylcarbamoyl)-2-morpholino-8-(1-(arylamino or aryloxo)ethyl)-4H-benzo[e][1,3]oxazin-4-ones through the starting compounds 5-carbamoyl-2-hydroxy-benzoic acid and 5-(dimethylcarbamoyl)-2-hydroxy-benzoic acid are discussed. As presented in the first chapter, this design, if successfully synthesized, would act as highly potent selective PI3Kβ/δ inhibitors.

4.1 Synthesis of the starting compounds 5-carbamoyl-2-hydroxy-benzoic acid and 5-(dimethylcarbamoyl)-2-hydroxy-benzoic acid

The starting compounds for this synthesis pathway were 5-carbamoyl-2-hydroxy-benzoic acid and 5-(dimethylcarbamoyl)-2-hydroxy-benzoic acid. They were not commercially available at reasonable cost and so it was attempted to synthesize them in the laboratory.

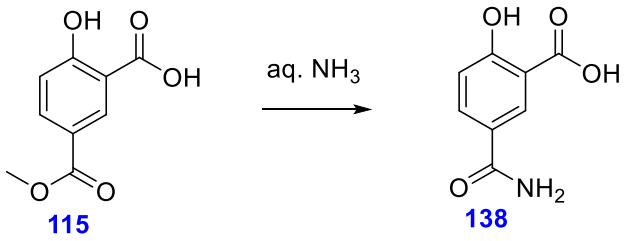
4.1.1 Synthesis of 5-carbamoyl-2-hydroxy-benzoic acid

A pathway was designed to synthesize 5-carbamoyl-2-hydroxy-benzoic acid **138** by first converting 4-hydroxybenzoic acid **118** to 4-hydroxybenzamide **136** followed by its formylation and oxidation to get the product **138**. (Scheme 4.1)



Scheme 4.1: Synthesis of 5-carbamoyl-2-hydroxy-benzoic acid **138** by 4-hydroxybenzoic acid **118**, reagents and conditions: a. thionyl chloride, 85 ºC, b. aqueous ammonia, 0 ºC to room temperature, 3 hours c. paraformaldehyde, magnesium chloride, triethylamine, 70 ºC, overnight. d. sodium chlorite, sodium dihydrogenphosphate 0 ºC to room temperature, overnight

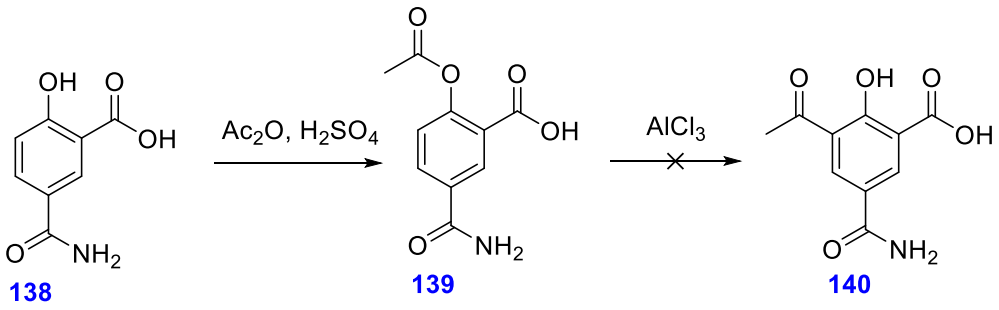
Following a previously reported procedure[75], 4-hydroxybenzoic acid **118** was reacted with thionyl chloride first at 85 ºC for 30 minutes and then with aqueous ammonia at ice cold temperature for 30 minutes followed by stirring at room temperature. After the workup procedure the product was obtained and analysed to conclude that the reaction did not occur.



Scheme 4.2: Synthesis of 5-carbamoyl-2-hydroxy-benzoic acid **138** from 2-hydroxy-5-(methoxycarbonyl)benzoic acid **115**

In another method to synthesize 5-carbamoyl-2-hydroxy-benzoic acid, compound 2-hydroxy-5-(methoxycarbonyl)benzoic acid **115** that was prepared in the previous chapter was used. A previous established method[133] was improvised, and the compound **115** was stirred with excess of aqueous ammonia at room temperature and the reaction was monitored by TLC (Scheme 4.2). The reaction completed successfully in two days and after acidification the product was precipitated. A good yield of the crude product **138** was obtained.

4.1.2 Attempted synthesis of 3-acetyl-5-carbamoyl-2-hydroxybenzoic acid



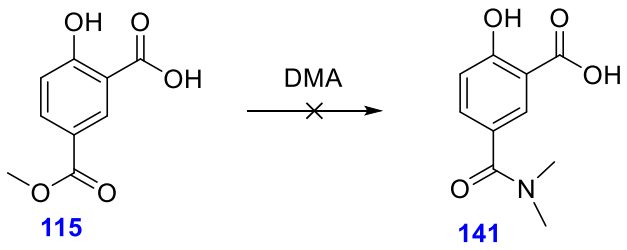
Scheme 4.3: Attempted synthesis of 3-acetyl-5-carbamoyl-2-hydroxybenzoic acid **140**

5-carbamoyl-2-hydroxy-benzoic acid **138** was allowed to react with acetic anhydride to in a classic o-acetylation reaction to give the product 2-acetoxy-5-carbamoyl-benzoic acid **139**. The product was obtained with acetic acid as an impurity, which was removed by thorough evaporation.

Subsequently, the compound 2-acetoxy-5-carbamoyl-benzoic acid **139** was subjected to a Fries rearrangement reaction using anhydrous aluminium chloride (Scheme 4.3). The reaction was attempted at various temperatures (120-180 ºC) but was not successful, and the product obtained was unreacted starting material. Even though the amide is an electron donating group it seems that it is not strong enough to activate the ring to accept a ketone, an electron withdrawing and sterically hindered group.

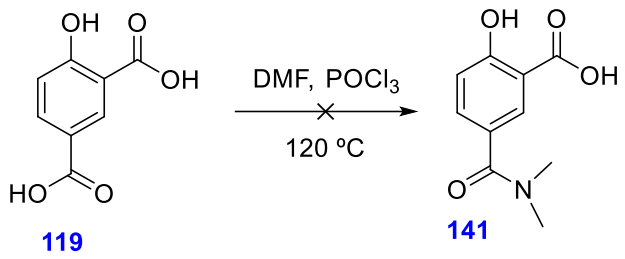
4.1.3 Synthesis of 5-(dimethylcarbamoyl)-2-hydroxy-benzoic acid

Using a similar procedure as the synthesis of 5-carbamoyl-2-hydroxy-benzoic acid **138** from 2-hydroxy-5-(methoxycarbonyl)benzoic acid **115**, the compound 2-hydroxy-5-(methoxycarbonyl)benzoic acid **115** was allowed to react with dimethylamine and the reaction was followed by TLC (Scheme 4.4). An unreacted product was obtained as a precipitate after acidification.



Scheme 4.4: Attempted synthesis of 5-(dimethylcarbamoyl)-2-hydroxy-benzoic acid from 2-hydroxy-5-(methoxycarbonyl)benzoic acid **115**

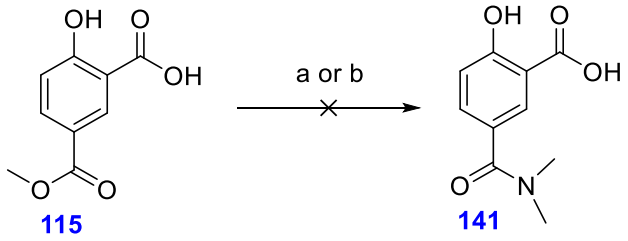
In another attempt, 4-hydroxyisophthalic acid **119** that was synthesized in the previous chapter was used (Scheme 4.5). It was a reacted with excess of N,N-dimethylformamide and equal moles of phosphoryl chloride according to a reported procedure.[134] However, the reaction was not successful and the desired product was not obtained.



119 **141**

Scheme 4.5: Attempted synthesis of 5-(dimethylcarbamoyl)-2-hydroxy-benzoic acid from 4-hydroxyisophthalic acid **119**

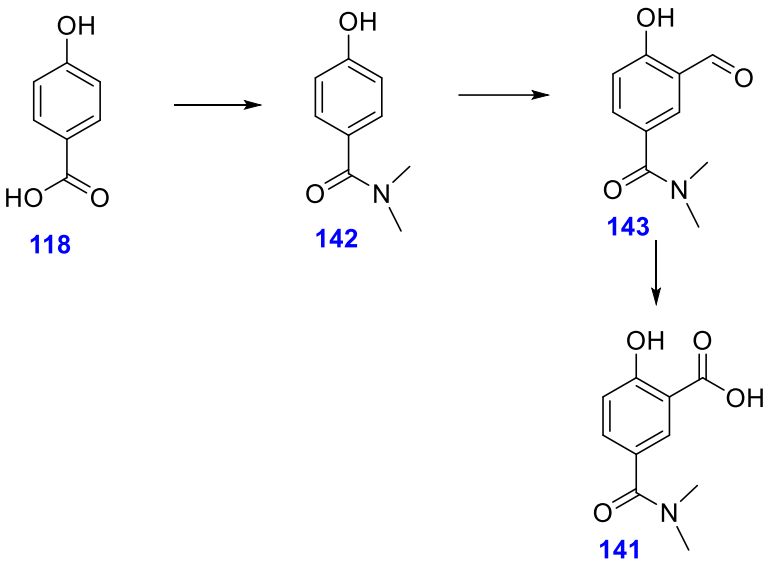
The effort was continued, this time allowing 2-hydroxy-5-(methoxycarbonyl)benzoic acid **115** to react with N,N-dimethylformamide at 120 ºC (Scheme 4.6). The reaction was followed by TLC for one day. The compound failed to react as the 'product' was found to be same as the starting compound after analysis. A similar method was tried again on the compound **115**. In addition to N,N-dimethylformamide, phosphoryl chloride was also used. Nevertheless, the desired product **141** was not obtained and the reaction was not successful.



115 **141**

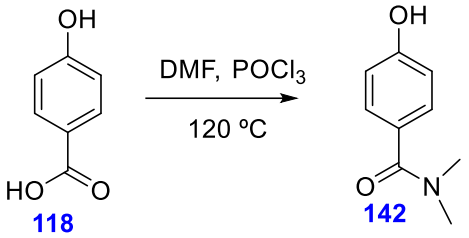
Scheme 4.6: Attempted synthesis of 5-(dimethylcarbamoyl)-2-hydroxy-benzoic acid **141** from 2-hydroxy-5-(methoxycarbonyl)benzoic acid **115** using N,N-dimethylformamide, reaction conditions: a. N,N-dimethylformamide, 120 ºC; b. N,N-dimethylformamide, phosphoryl chloride, 120 ºC

Since the attempts to synthesize 5-(dimethylcarbamoyl)-2-hydroxy-benzoic acid **141** from 2-hydroxy-5-(methoxycarbonyl)benzoic acid **115** and from 4-hydroxyisophthalic acid **119** did not yield desired results, another pathway was attempted (Scheme 4.7). In this, the compound 4-hydroxybenzoic acid **118** would be converted to 4-hydroxy-N,N-dimethylbenzamide **142**, followed by established methods of formylation and oxidation to give 5-(dimethylcarbamoyl)-2-hydroxy-benzoic acid **141**.



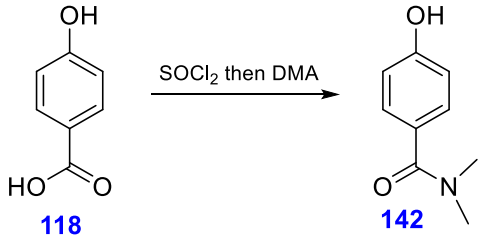
Scheme 4.7: Synthesis of 5-(dimethylcarbamoyl)-2-hydroxy-benzoic acid **141** from 4-hydroxybenzoic acid **118**.

Following this scheme, the compound **118** was first reacted with N,N-dimethylformamide and phosphoryl chloride at 120 ºC (Scheme 4.8). The product **142** was obtained but in very low yields (around 4%). The reaction was retried with modifications in conditions like temperature, reaction time and reaction vessel, but the yield did not improve.



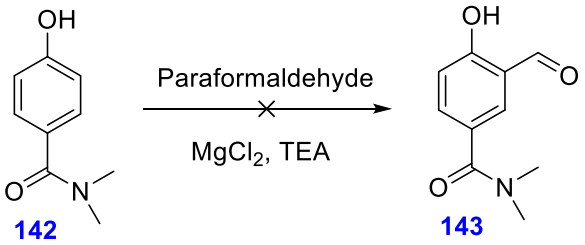
Scheme 4.8: Synthesis of 4-hydroxy-N,N-dimethylbenzamide **142**

Another method for the synthesis of 4-hydroxy-N,N-dimethylbenzamide reported in the literature which is a two-step process.[135] In the first step 4-hydroxybenzoic acid **118** is heated to reflux with thionyl chloride. The resulting residue after evaporation of thionyl chloride was reacted with dimethylamine to give the product **142** in good yields (Scheme 4.9).



Scheme 4.9: Two-step synthesis of 4-hydroxy-N,N-dimethylbenzamide **142**

In the next step for the synthesis of 5-(dimethylcarbamoyl)-2-hydroxy-benzoic acid **141**, the compound 4-hydroxy-N,N-dimethylbenzamide **142** was subjected to a formylation reaction. The procedure is same as the one described in the previous chapter, which makes use of paraformaldehyde, magnesium chloride and triethylamine (Scheme 4.10). However, the desired product **143** was not obtained.



Scheme 4.10: Attempted synthesis of 3-formyl-4-hydroxy-N,N-dimethylbenzamide **143**

There is another way of synthesizing the intermediate compound 3-formyl-4-hydroxy-N,N-dimethylbenzamide **143** which involves the use of ruthenium catalyst. However, because of lack of resources (ruthenium catalyst) this synthesis pathway was aborted.

4.2 Discussion

For the synthesis of compound **136**, the acid chloride method did not prove efficient. This could be because of the formation of ammonium chloride when the acid chloride was reacted with aqueous ammonia. The use of **115** from the previous chapter for the synthesis of **138** proved to be an easier method. Interestingly, this method did not work for the synthesis of **141** with dimethylamine, suggesting that a secondary amine non-reactive with a phenolic acid ester. Anyhow, the compound **138** could not be taken for further steps as its o-acetylated derivative failed to undergo a Fries rearrangement reaction. This was contrary to our expectations. Even though the amide is an electron donating group it seems that it is not strong enough to activate the ring (along with a phenol and carboxylic acid) and to accept a ketone, an electron withdrawing and sterically hindered group.

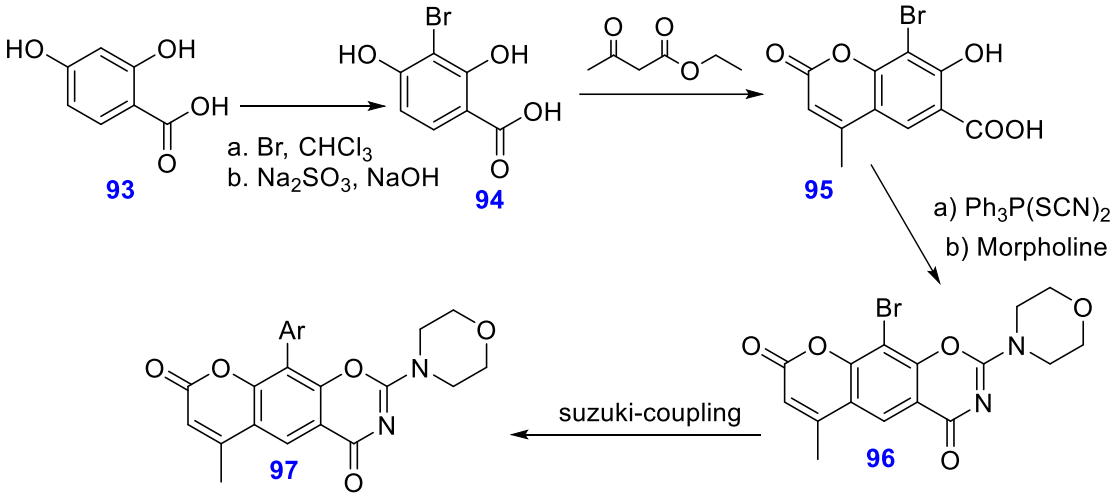
Regarding the synthesis of the compound **141** through **119**, it is likely that a phosphate ester was formed, which did not allow the formation of an amide. Likewise, N,N-

dimethylformamide is less reactive compared to ammonia and so this makes sense. The same seems true for the reaction of compound **118** with N,N-dimethylformamide, which resulted in very low yield of the product **142**. The yield of **142** improved significantly by using a method of first converting **118** to an acid chloride by using thionyl chloride and then reacting it with secondary amine.

Indeed, one of the starting compounds (5-carbamoyl-2-hydroxybenzoic acid **138**) failed to follow the proposed scheme of work (Chapter 1) and the other starting compound (5-(dimethylcarbamoyl)-2-hydroxy-benzoic acid **141**) was not synthesized after the aforementioned attempts. Though it can be attempted to synthesize 5-(dimethylcarbamoyl)-2-hydroxy-benzoic acid **141** (using ruthenium catalyst if feasible), it is more likely that it would not be able to react in the further steps of synthesis as it happened with 5-carbamoyl-2-hydroxybenzoic acid **138**.

CHAPTER 5: ATTEMPTED SYNTHESIS OF 10-ARYL-6-METHYL-2-MORPHOLINO-4H,8H-CHROMENO[6,7-E][1,3]OXAZINE-4,8-DIONES

In quest of discovering a new series of efficient DNA-PK inhibitors, a molecule 10-aryl-6-methyl-2-morpholino-4H,8H-chromeno[6,7-e][1,3]oxazine-4,8-dione and a synthetic pathway to achieve it was designed (Scheme 5.1).

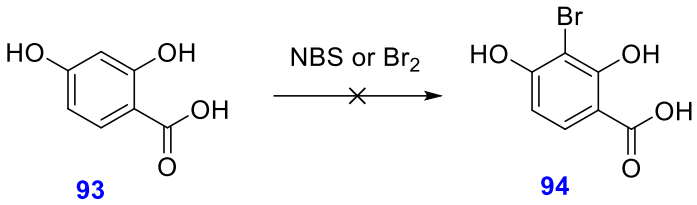


Scheme 5.1: Synthesis of 10-aryl-6-methyl-2-morpholino-4H,8H-chromeno[6,7-e][1,3]oxazine-4,8-diones **97**

The most suitable starting compound in this synthetic pathway was 2,4-dihydroxybenzoic acid **93**. It was commercially available and was purchased as laboratory grade for use without further purification.

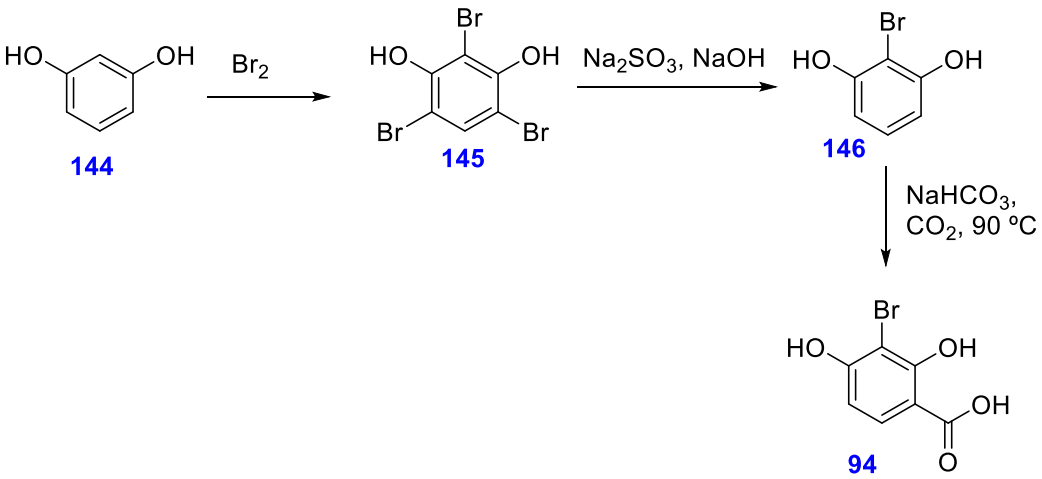
5.1 Synthesis of 3-bromo-2,4-dihydroxybenzoic acid

The compound 2,4-dihydroxybenzoic acid **93** was first reacted with N-bromosuccinimide and the reaction was followed by TLC. The reaction did not occur, so, another method was tried. In this instance, the compound **93** was allowed to react with liquid bromine in chloroform solution (Scheme 5.2). The compound failed to react.



Scheme 5.2: Attempted synthesis of 3-bromo-2,4-dihydroxybenzoic acid **94**

In this pursuit, another procedure was used for the synthesis of 3-bromo-2,4-dihydroxybenzoic acid **94**. This method makes use of resorcinol **144** and involves three steps (Scheme 5.3).

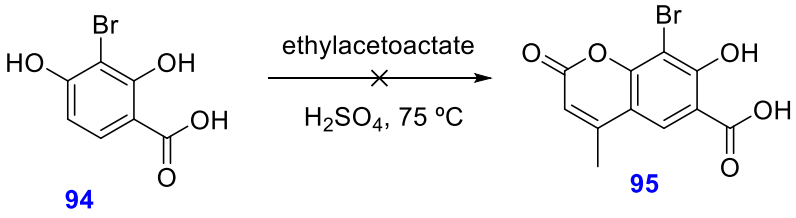


Scheme 5.3: Synthesis of 3-bromo-2,4-dihydroxybenzoic acid **94**

In the first step, resorcinol **144** was brominated by using bromine dissolved in chloroform. The reaction mixture was heated to reflux overnight to give 2,4,6-tribromoresorcinol **145**[114]. In the second step, following a literature procedure[136], 2,4,6-tribromoresorcinol **145** was partially debrominated by making use of aqueous sodium sulphite and sodium hydroxide solution to get 2-bromoresorcinol **146**. In the third step 2-bromoresorcinol **146** was subjected to a carboxylation reaction in which the compound is reacted with aqueous sodium bicarbonate while passing carbon dioxide gas through the reaction mixture for 90 minutes at 90 ºC. Finally, the compound 3-bromo-2,4-dihydroxybenzoic acid **94** was synthesized in modest yields of about 23%.

5.2 Synthesis of 8-bromo-7-hydroxy-4-methyl-2-oxo-2H-chromene-6-carboxylic acid

Following an established method of synthesis of chromene-6-carboxylic acids with β ketonic esters[137], the compound 3-bromo-2,4-dihydroxybenzoic acid **94** was reacted with ethyl acetoacetate with catalytic amounts of concentrated sulphuric acid. The reaction did not occur. The process was repeated with aqueous sulphuric acid (70%). However, the experiment was unsuccessful yet again (Scheme 5.4).



94 **95**

Scheme 5.4: Attempted synthesis of 8-bromo-7-hydroxy-4-methyl-2-oxo-2H-chromene-6-carboxylic acid **95**

The most likely cause of the failure of this reaction is the bromine group in 3-bromo-2,4-dihydroxybenzoic acid which has rendered the ring deactivated by being more electron withdrawing than expected.

5.3 Discussion

First, bromination of the compound 2,4-dihydroxybenzoic acid **93** was attempted using liquid bromine. The reaction failed because a brominated phenol was formed as the carboxylic acid group left the ring as CO_2 and a Br group attached. The use of N-bromosuccinimide for the same reaction instead of liquid bromine did not yield desired result. This can be explained by the para position in compound **93** being blocked by carboxylic acid and that N-bromosuccinimide is para selective. Hence, an alternative method was used to synthesize 3-bromo-2,4- dihydroxybenzoic acid **94**, which was eventually successful.

The reaction of 3-bromo-2,4- dihydroxybenzoic acid **94** with ethylacetoacetate was attempted expecting a typical Pechmann condensation reaction. However, it failed to react possibly because the ring is deactivated by having both bromine and carboxylic acid groups.

Considering the fact that it was not possible to synthesize the chromene-6-carboxylic acid derivative, this design was not pursued further.

CHAPTER 6: BIOLOGICAL EVALUATION OF SYNTHESIZED 1,3-BENZOXAZIN-4-ONES

The synthesized compounds were investigated for their biological activity against class I PI3K group of enzymes, DNA-PK and PDE3A enzymes.

6.1 PI3K inhibition

Phosphatidylinositol 3-kinase (PI3K) is a group of intracellular signal transduction enzymes which are crucially involved physiological process in the cell including cell growth, proliferation, adhesion and survival.[138] The process of cell proliferation can be decreased by inhibition of PI3K signalling, which can also promote cell death. Consequently, these group of enzymes are desirable targets for cancer therapeutics.

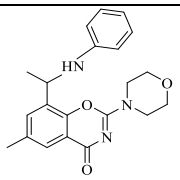
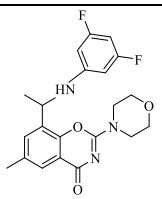
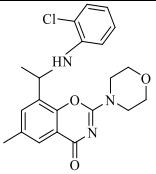
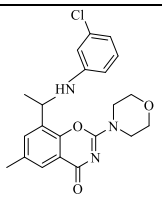
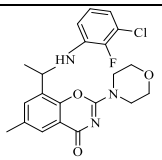
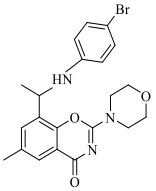
In the past, numerous PI3K pathway inhibitors have been developed and are being evaluated in preclinical and clinical trials.[78, 132] The PI3K inhibitors are of two types- isoform-specific inhibitors and pan-PI3K inhibitors. Pan-PI3K inhibitors can target all class IA PI3K in the cancer serum half-life.[139-141] These drugs bind to and inhibit a broad range of kinase isoforms and complexes with low specificity and lead to harmful side effects. For example, the α isoform of PI3K has been associated with a variety of human cancers and also it is selectively required in angiogenesis to control the endothelial cell migration.[142] The δ isoform has been implicated in a number of diseases and biological processes which express primarily in hematopoietic cells including leukocytes such as T-cells, dendritic cells, neutrophils, mast cells, β-cells, and macrophages. Additionally, the γ isoform contributes in leukocyte signalling and has been implicated in inflammation, rheumatoid arthritis, and autoimmune diseases such as lupus. PI3K β is

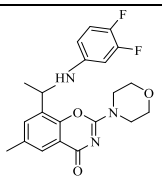
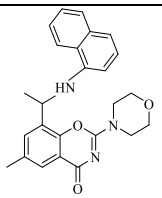
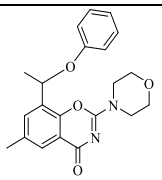
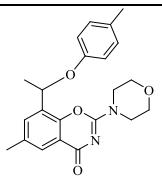
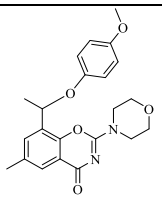
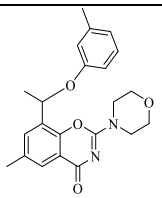
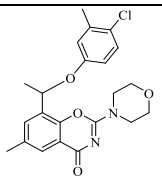
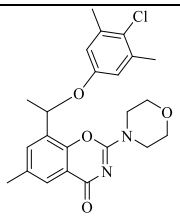
associated with various types of cancer including PTEN-negative cancer and HER2-overexpressing cancer like breast cancer and ovarian cancer.[143] Therefore it is important to offer an alternative approach that efficiently targets disease-related pathways, and limits undesirable side effects.

A recently discovered inhibitor of both DNA-PK and PI3K was the chromen-4-one based compound NU7441 **26**.[41] Another chromen-4-one compound is AZD8186 **51** which was found active against PI3Kβ and PI3Kδ and is under clinical trials for treatment of PTEN-deficient cancers.[82] Particularly interesting is that several structurally similar 8-aryl-2-morpholino-1,3-benxoxazin-4-ones have also been found to exhibit inhibition activity against DNA-PK and PI3K. Considering this fact, the 8- and 6- substituted 2-morpholino benzoxazines prepared in this project were evaluated for their inhibition of PI3K. Importantly, the activity of 8-(1-((3,5-difluorophenyl)amino)ethyl)-6-methyl-2-morpholino-4H-benzo[e][1,3]oxazin-4-one (LTUEM09 **113b**) was examined to provide a direct comparison with the activity of the potent chromen-4-one analogue AZD8186 **51**.

Out of the 20 synthesized compounds, 14 compounds were evaluated for their activity against PI3Kβ, whereas two prototypes (one phenylamine and one phenoxy derivative) were evaluated for their activity against PI3Kγ and PI3Kδ. Most of the analysed compounds showed very good activity against PI3Kβ with compounds LTUEM08 **113a**, LTUEM09 **113b**, LTUEM11 **113d**, LTUEM14 **113g**, and LTUEM15 **113h** showing 100% inhibition. The compounds LTUEM10 **113c**, LTUEM12 **113e**, LTUEM13 **113f**, LTUEM16 **114a**, LTUEM20 **114e**, and LTUEM21 **114f** showed more than 90% inhibition against PI3Kβ. The remaining compounds showed low activity against PI3Kβ.

Both the analysed compounds showed low activity against PI3Kγ (41.54% and 12.39% PI3Kγ inhibition for LTUEM08 **113a** and LTUEM16 **114a** respectively). However, the compound LTUEM08 **113a** showed very good activity against PI3Kδ (87.39% inhibition).

Compound	Structure	% inhibition of PI3Kα	% inhibition of PI3Kβ (Monash)	% inhibition of PI3Kγ	% inhibition of PI3Kδ
LTUEM08 **113a**		60.82	102.18 (60)	41.54	87.39
LTUEM09 **113b**		-	100.48 (80)	-	-
LTUEM10 **113c**		-	96.39 (97)	-	-
LTUEM11 **113d**		-	99.64 (82)	-	-
LTUEM12 **113e**		-	95.99 (63)	-	-
LTUEM13 **113f**		-	91.73 (27)	-	-

LTUEM14 **113g**		-	100.21	-	-
LTUEM15 **113h**		-	102.34	-	-
LTUEM16 **114a**		48.77	91.69	12.39	72.26
LTUEM17 **114b**		-	37.97	-	-
LTUEM18 **114c**		-	27.56	-	-
LTUEM19 **114d**		-	85.97	-	-
LTUEM20 **114e**		-	91.35	-	-
LTUEM21 **114f**		-	91.35	-	-

PI3K α,β, γ and δ percentage inhibition at 10 μM

Control compound was PI-103

Table 6.1: PI3K inhibition activities of some synthesized compounds

The potent activities of the compounds against PI3Kβ and PI3Kδ can be attributed to the aniline pharmacophore (in compounds LTUEM08-15 **113a- 113h**) which bonds well at the binding site with its polar hydrogen.

6.2 DNA PK inhibition

DNA-dependent protein kinase (DNA-PK) is a multicomponent component serine/threonine protein kinase. This enzyme plays an important role not only in proliferation of the cell but also in the repair of mammalian DNA double strand breaks (DSBs) which is the main cytotoxic lesion produced by IR and chemotherapies.[34, 132] It is noteworthy that human cell lines with DNA-PK malfunction are hypersensitive to agents that elicit DNA DSBs. Thus, DNA-PK is an attractive therapeutic target for the modulation of DNA DSB repair in cancer therapy and selective DNA-PK inhibitors have application as radio- and chemo-potentiators in the treatment of cancer.[144-147]

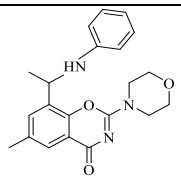
The PI-3K inhibitor LY294002 **21** (2-morpholino-8-phenyl-4H-chromen-4one) also exhibits ATP-competitive inhibition of DNA-PK (=6 μM) though not very potent.[34] So, it can be of interest to study the DNA-PK inhibitory activity for the present work too. Extensive structure-activity relationship (SAR) studies done on LY294002 **21** were aimed at the development of potent and selective DNA-PK inhibitors. In this analogue, the 2-morpholino-4H-chromen-4-one moiety is connected at the 8-position to an aryl or hetero-aryl ring, which may be substituted or unsubstituted, with a dibenzofuran-4-yl or dibenzothiophen-4yl group proving especially favourable.[37, 39] These studies lead to the

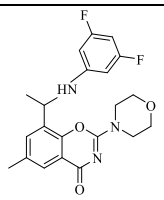
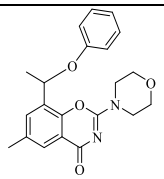
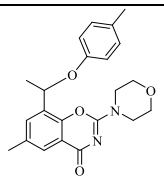
discovery of NU7441 **26**, which combined potent DNA-PK inhibition (IC50 =42 ± 2 nM) with good selectivity over other PIKKs as well as PI-3K family members.[37] The compound NU7441 **26** also sensitized human tumour cell lines to IR and etoposide *in vitro* and *in vivo*,[148] although further biological studies were hindered by pharmaceutical problems resulting from low aqueous solubility of the chromenone derivative.

A homology model of the ATP-binding site of the DNA-PK was used to guide inhibitor design. It was derived from crystal structure of PI3Kγ.[149] The model predicted that groups introduced at the dibenzothiophene 1-position of NU7441 **26** would be directed out of the binding pocket into bulk solvent. The effect of substitution at the 1-position of the dibenzothiophen-4-yl moiety on both potency and physicochemical properties were investigated by synthesis of a library of analogues. Several newly synthesised compounds showed high potency against DNA-PK and potentiated the cytotoxicity of ionizing radiation (IR) *in vitro* 10-fold or more (e.g. (KU0060648 **27**); DNA-PK IC_{50}=5 nM, IR dose modification ratio = 13). Moreover, the compound KU0060648 **27** was shown to enhance not only IR *in vitro*, but also DNA-damage inducing TOP2 poisons (doxorubicin, etoposide) both *in* vitro and *in vivo*. Some compounds were found to be potent mixed DNA-PK and PI3K inhibitors, including compound **27**.[43, 44] The significant biological activity of product KU0060648 **27** was supplemented by better drug-like properties than those of NU7441 **26**, and satisfactory plasma protein binding, combined with weak activity against the hERG ion channel (involved in cardiac repolarisation) and a panel of cytochrome P450 (CYP) drug-metabolising enzymes.[28] Additional derivatives of LY294002 **21** and NU7441 **26** have been reported to have enhanced DNA-PK inhibitory activity (i.e., 8-biarylchromenon-4-one, IC_{50} =18 nM and O-alkoxy-phenylchromen-4-one, IC_{50} = 8nM, Figure 1.13).[45, 46]

In view of these findings, 1,3-benzoxazin-4-ones have been developed from structural analogues of chromen-4-ones as specific inhibitors of the PIKK family to increase the efficacy of chemotherapeutic agents against cancer cells. This effect is instrumented through specific inhibition of DNA-PK and DNA repair mechanisms. 1,3-benzoxazin-4-ones represent a modified scaffold to the corresponding chromone, quinolone and pyridopyrimidinone bicyclic compounds which have also been widely applied to those targets, some of which have undergone extensive clinical and pre-clinical investigation.[19] Indeed, the compounds in this work were prepared specifically for their PI3K inhibition, it was worth seeing their DNA-PK inhibition as the structural analogues have shown this activity.

Four prototypes LTUEM08 **113a**, LTUEM09 **113b**, LTUEM16 **114a**, and LTUEM17 **114b** were selected from the series to be evaluated against DNA-PK. It was found that the compound LTUEM09 **113b** showed good activity (78.38% inhibition of DNA-PK) and was the most active among the four analysed compounds. The compound LTUEM17 **114b** showed the least activity against DNA-PK (12.22%). However, none of the compounds have shown a remarkable activity and that was expected considering the design was directed for a PI3K inhibitory activity.

Compound	Structure	% inhibition DNA-PK
LTUEM08 **113a**		61.43

LTUEM09 **113b**		78.39
LTUEM16 **114a**		28.16
LTUEM17 **114b**		12.22

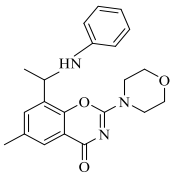
DNA-PK Percentage inhibition at 10 µM

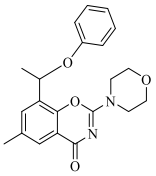
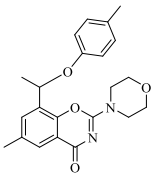
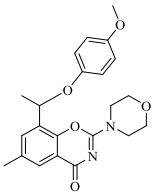
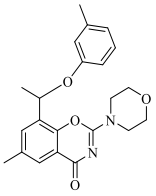
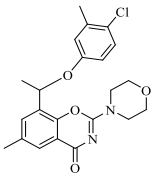
Control compound for DNA-PK assay is PI-103. IC_{50} = 0.0485 µM

Table 6.2: DNA-PK inhibition activities of some synthesized compounds

6.3 PDE3A inhibition

Six compounds were selected to evaluate their activity against PDE3A. The compounds LTUEM16 **114a** and LTUEM19 **114d** showed moderate activity (22.95% and 19.83% inhibition respectively).

Compound	Structure	PDE3A
LTUEM08 **113a**		1.84

LTUEM16 **114a**		22.95
LTUEM17 **114b**		-0.66
LTUEM18 **114c**		13.13
LTUEM19 **114d**		19.83
LTUEM20 **114e**		4.10

PDE3A percentage inhibition at 10 µM

Control compound for PDE3A assay is IBMX. IC_{50} = 7.0 µM

Table 6.3: PDE3A inhibition activity of some synthesized compounds

As expected, the compound LTUEM08 **113a** showed least activity as it is lacking the phenoxy group. It was believed that the compounds with phenoxy moiety would act as potent PDE3A inhibitors, however, the results are contrary.

6.4 Docking studies

A previously reported homology model of catalytic subunit of PI3K was used for this study.[150] Two prototypes LTUEM08 **113a** and LTUEM16 **114a** were selected from the series and were docked in the active site of the homology model using Autodock Vina 1.1.2.[151] The residues were kept rigid for the protein. Figures 6.1 and 6.2 show compounds LTUEM08 **113a** and LTUEM16 **114a** docked respectively with the surface coloured by atom type. The figures also highlight the key residues interacting with these compounds. Hydrogen bonds are shown as green dashed lines. Only the important hydrogen (of amine group) is shown to simplify the view.

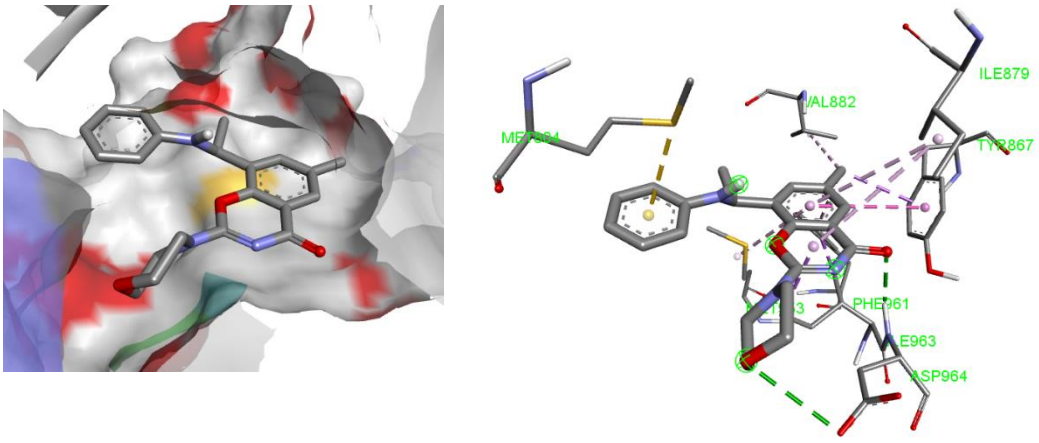


Figure 6.1: Compound LTUEM08 **113a** docked in the binding site of the modelled structure of PI3K catalytic subunit

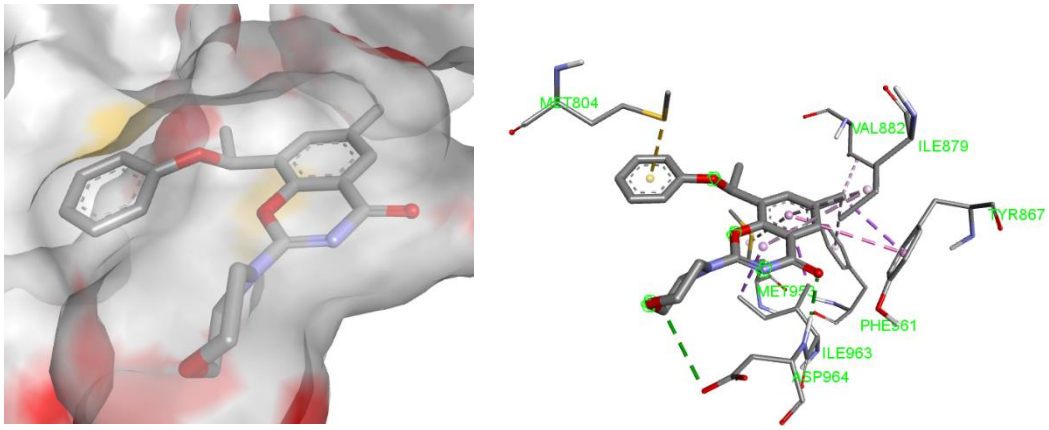


Figure 6.2: Compound LTUEM16 **114a** docked in the binding site of the modelled structure of PI3K catalytic subunit

According to this homology model, the morpholine at the 2-position of the 1,3-benzoxazin-4-one forms a crucial interaction in the kinase hinge-region with Asp964 which is different from other morpholine containing inhibitors of the PIKK family, as seen with the previously reported analogues. Hydrogen bonding between the oxazine carbonyl and the side chain of Asp964 is also constantly observed. The increased potency of **113a** can be explained by the docked poses generated. The 8-substituted anilino group of this compound found to sit in the binding site. Introducing an ether linker in **114a** instead of an amine reduced potency against all PI3K isoforms except PI3Kβ. In addition, the increased activity of **113a** can be attributed to the favourable methyl group sticking into a highly hydrophobic region. Though the role of the methyl on the carbon bearing the aniline for selectivity is unclear, the constraints caused by the presence of this methyl may limit the conformational flexibility of the ligand and favour a T-shape conformation over a flat shaped conformation.

CHAPTER 7: CONCLUSION AND FUTURE DIRECTIONS

The following conclusions can be drawn from the observations and results obtained from experiments.

In continuation to the work done on a library of biologically active 1,3-benzoxazin-4-ones in Al-Rawi lab in La Trobe University, some new 6,8-substituted-2-morpholino-1,3-benzoxazin-4-ones were successfully synthesized. During the course of synthetic process, literature procedures were improvised to give good yields of the intermediates and of final compounds. Specifically, the Fries rearrangement reaction of 2-acetoxy-5-methyl benzoic acid saw significantly improved yields. It was also found that the reductive amination reaction or an amination reaction to form a Schiff base with a ketone and an aniline derivative, did not work owing to more steric hindrance from acetyl group than expected. The yields were also improved in a selective reduction reaction of 8-acetyl-6-methyl-2-morpholino-1,3-benzoxazinone **103** and in a bromination reaction of 8-hydroxyethyl-6-methyl-2-morpholino-1,3-benzoxazinone **111**.

Although the endeavour to synthesize 6-methoxycarbonyl-2-morpholino-8-(1-(arylamino or aryloxo)ethyl)-4H-benzo[e][1,3]oxazin-4-ones failed, the investigations were valuable to understand the reactivity of 2-hydroxy-5-(methoxycarbonyl)benzoic acid which itself is a derivative of 4-hydroxyisophthalic acid **119**, an expensive compound. In the process, 2-hydroxy-5-(methoxycarbonyl)benzoic acid **115** was synthesized in very high yields and this can be converted to 4-hydroxyisophthalic acid **119** with ease, thereby establishing a cost-effective way to obtain it. Acetylation of both 3-bromo-2-hydroxy-5-(methoxycarbonyl)benzoic acid **131** and 8-bromo-6-

methoxycarbonyl-2-morpholino-1,3-benzoxazin-4-one **134** could not be achieved by any means.

Synthesis of 6-(carbamoyl or dimethylcarbamoyl)-2-morpholino-8-(1-(arylamino or aryloxo)ethyl)-4H-benzo[e][1,3]oxazin-4-ones was also attempted. 5-carbamoyl-2-hydroxybenzoic acid **138** was synthesized but could not be taken forward to further steps, confirming that the ring is deactivated for electrophilic aromatic substitutions. 5-(dimethylcarbamoyl)-2-hydroxybenzoic acid **141** could be synthesized in the future to continue the work of finding a lead structure for potent selective P13K inhibition and eventually an anticancer compound.

The newly synthesized 6-methyl-2-morpholino-8-(1-(arylamino or aryloxo)ethyl)-4H-benzo[e][1,3]oxazin-4-ones were evaluated for their biological activity. The selected compounds which were found to be active against PI3Kβ and PI3Kδ should be studied more in vitro for their activity against cancer cell lines. Moreover, since the benzoxazine scaffold provides a better pharmacokinetic profile, the compounds should be taken forward for pharmaceutical studies.

CHAPTER 8: EXPERIMENTAL

TLC silica gel 60 F_{254} on aluminium sheets from Merck Millipore were used for Thin Layer Chromatography (TLC) and the spots were visualized under UV 254 nm light. All the melting point determinations were carried out using a Gallenkamp melting point apparatus and all melting points stand uncorrected. To record Infrared spectra a FTIR Spectrometer fitted with a diamond ATR accessory from Agilent Equinox Cary 630 was used. For ^{1}H NMR and ^{13}C NMR spectra a Bruker Avance 300 NMR spectrometer at 300 MHz for ^{1}H and 75 MHz for ^{13}C was used respectively. Also used was a Bruker 500 MHz Avance III UltraShield Plus and a Bruker 400 MHz Avance III HD Ultrasheild Ascend NMR Spectrometer for some compounds. For a few compounds, Reveleris X2 chromatography system of Grace Discovery Sciences was used for flash chromatography utilizing commercially available Reveleris Silica Cartridges (24g, 12g, etc.). All ^{1}H and ^{13}C NMR spectral results are recorded as chemical shifts (δ). The chemical shifts recorded in solvent $CDCl_3$ are relative to the internal TMS (0 ppm) for ^{1}H spectra and solvent peak (77.1 ppm) for ^{13}C spectra; whereas the chemical shifts recorded in solvent d_6-DMSO are relative to the solvent peak of 2.5 for ^{1}H spectra and 39.5 ppm for ^{13}C spectra. ^{1}H NMR multiplicities are expressed as singlet (s), broad singlet (bs) doublet (d), double doublet (dd), triplet (t), double triplet (dt), quartet (q), multiplet (m) and broad multiplet (bm). HRMS analyses were carried out on an Agilent 6200 series TOF/6500 series Q-TOF B.06.01 (B6172 SP1) Mass Spectrometer coupled to an Agilent 1290 Infinity (Agilent, Palo Alto, CA). All data were acquired and reference mass were corrected via a dual-spray electrospray ionization (ESI) source. A Rigaku oxford diffraction supernova diffractometer was used for X-ray crystallography.

All the solvents used were purchased as laboratory grade and were used without further purification unless otherwise stated. Petroleum spirits was purchased with a boiling point range of 40-60 °C. Dichloromethane (DCM) was dried over and distilled from calcium hydride and stored over type 4A molecular sieves. All other organic solvents were dried and stored over type 4A molecular sieves before use.

Note: Numbering used throughout this chapter does not adhere to IUPAC convention.

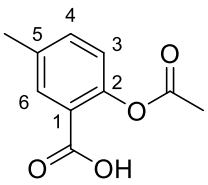
8.1 Synthesis of 6-methyl-2-morpholino-8-(1-(arylamino or aryloxo)ethyl)-4H-benzo[e][1,3]oxazin-4-ones

General procedure A:

To a stirred solution of 8-(1-bromoethyl)-6-methyl-2-morpholino-4H-benzo[e][1,3]oxazin-4-one **112** (0.56 mmol) and substituted aniline (or substituted phenol) (2.24 mmol) in dichloromethane and methanol (4:1), potassium iodide (0.56 mmol) was added. Triethylamine (1.4 mmol) was then added. The mixture was stirred at room temperature for 48 hours. The solvents were evaporated, and the residue was subjected to flash chromatography (hexane-ethyl acetate, and/or ethyl acetate-methanol). Some of the compounds were obtained as oil and were precipitated as solid crystals by adding minimum amount of diethyl ether.

8.1.1 Synthesis of 8-acetyl-6-methyl-2-thioxo-2,3-dihydro-4H-benzo[e][1,3]oxazin-4-one

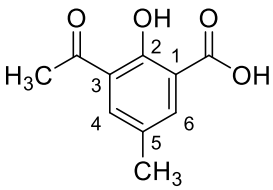
2-acetoxy-5-methyl-benzoic acid 99



5-methyl salicylic acid **98** (2.2 g, 15 mmol) was stirred in an Erlenmeyer flask with acetic anhydride (7 mL). A drop of concentrated sulfuric acid (about 50 µL) was added to this and swirled gently. After 20-30 minutes the mixture became brown coloured and it was poured into cold water with stirring. The product was extracted with ethyl acetate, dried over $MgSO_4$ and evaporated under reduced pressure to obtain white solid 2.4 g (85%).

MP 131-132 °C (Lit. 158-160 °C). ν_{max} (ATR) /cm^{-1} 3000-2500 br (O-H), 1678 s (C=O), 1753 s (C=O carboxylic). **^{1}H NMR** ($CDCl_3$, 300 K) δ 7.89 (s, 1H, H-6), 7.39 (d, 1H, $J_{H3,H4}$=8.1 Hz, H-3), 6.99 (d, 1H, $J_{H4,H3}$=8.1 Hz, H4), 2.38 (s, 3H, $OCOCH_3$), 2.31 (s, 3H, CH_3); **^{13}C NMR** ($CDCl_3$, 300 K) δ 169.3 (COOH), 169.2 (OC=O), 148.3 (C-2), 135.4 (C-5), 134.8 (C-6), 132.1 (C-4), 123.4 (C-1), 123.0 (C-3), 20.37 ($COCH_3$), 20.33 (CH_3).

3-acetyl-2-hydroxy-5-methyl benzoic acid 100

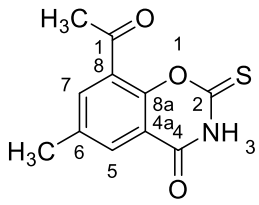


2-acetoxy-5-methyl-benzoic acid **99** (2.4 g, 12.3 mmol) was mixed with Aluminium chloride (4.92 g, 37 mmol) thoroughly and the mixture was heated to 180 °C with Argon inlet in a two-necked round bottomed flask. The mixture was maintained at this temperature for 3 hours with stirring. The resultant solid was cooled to room temperature, crushed to yellow powder, and poured onto 100 g ice with 25 mL HCl. Ethyl acetate (50 mL) was

added to it after the ice melted and was stirred until a suspension was formed, followed by extraction with dichloromethane (x2). The organic extract was dried over $MgSO_4$ and was evaporated under reduced pressure. A cream-colored solid was obtained 1.95 g (81% crude). The product was recrystallized with Dichloromethane/ Hexane (1:1).

MP 128-129 ºC (Lit. >180 ºC) ν_{max} (ATR) /cm^{-1} 3100-2300 br (O-H), 1670 s (C=O), 1642 m (C=O). **^{1}H NMR** ($CDCl_3$, 300 K) δ 8.18 (s, 1H, $J_{H6,H4}$ = 1.8 Hz, H-6), 7.79 (s, 1H, $J_{H4,H6}$ = 1.8 Hz, H-4), 2.69 (s, 3H, $COCH_3$), 2.36 (s, 3H, CH_3); **^{13}C NMR** ($CDCl_3$, 300 K) δ 204.3 (C=O carbonyl), 165.0 (C=O, carboxyl), 158.5 (C-2), 139.9 (C-6), 135.8 (C-4), 128.8 (C-5), 119.7 (C-3), 116.2 (C-1), 26.5 ($COCH_3$), 19.7 (CH_3).

8-acetyl-6-methyl-2-thioxo-2,3-dihydro-4H-benzo[e][1,3]oxazin-4-one 101

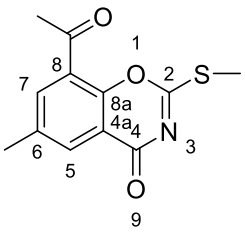


A suspension of 3-acetyl-2-hydroxy-5-methyl benzoic acid **100** (0.89 g, 4.6 mmol) in dichloromethane (20 mL) was added to a reaction mixture freshly prepared $Pb(SCN)_2$ according to the previously reported procedure. The reaction mixture was filtered and $PbBr_2$ filter cake was subjected to hot filtration using acetone (≈ 100 mL) to extract the product. Both the DCM and acetone filtrates were separately evaporated to dryness under reduced pressure and minimal toluene was added to triturate the resulting solid. The solids that precipitated out of the filtrates were collected by filtration, combined, and recrystallized from toluene to give 0.61 g of the title compound (60% yield),

MP 236-239 °C; ν_{max} (ATR) /cm^{-1} 3178 w (NH), 1679 m (C=O), 1596 m (C=C), 1157 s (C=S); **^{1}H NMR** (d_6-DMSO, 340 K) δ 7.91 (s, 1H, H-7), 7.89 (s, 1H, H-5), 2.66 (s, 3H, $COCH_3$), 2.38 (s, 3H, CH_3); **^{13}C NMR** (d_6-DMSO, 340 K) δ 195.6 ($C=OCH_3$), 180.8

(C=S), 157 (CONH), 151.4 (C-8a), 136.1 (C-5), 135.3 (C-6), 130.2 (C-7), 125.9 (C-8), 116 (C-4a), 31.08 (CH_3CO), 19.81 (CH_3). HRMS (ESI): *m/z* calculated for $C_{11}H_9NO_3S$: 236.0376 $[M+H]^+$, found: 236.0382.

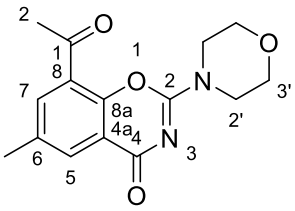
8-acetyl-6-methyl-2-(methylthio)-4H-benzo[e][1,3]oxazin-4-one 102



In a 50 mL beaker containing 20 mL of 1:1 RO water and 2-propanol, sodium bicarbonate (1.0 g, 11.90 mmol) was suspended. The compound **101** (0.47 g, 2.0 mmol) was added, the reaction mixture was heated to 60 °C while stirring and then removed from heat and allowed to cool to room temperature with stirring. Iodomethane (0.5 mL, 8.0 mmol) was added dropwise and allowed to stir at room temperature for 30 minutes or until a thick precipitate had formed. The solid was filtered and washed with water to obtain a creamy white compound (400 mg, 80%).

MP 155-160 °C; ν_{max} (ATR) /cm^{-1} 1703 m (C=O), 1675 s (C=O), 1600 s (C=N), 1555 s (C=C); **^{1}H NMR** ($CDCl_3$, 300 K) δ 8.11 (s, 1H, H-7), 7.94 (s, 1H, H-5), 2.70 (s, 3H, SCH_3), 2.64 (s, 3H, $COCH_3$), 2.45 (s, 3H, CH_3); **^{13}C NMR** ($CDCl_3$, 300 K) δ 194.6 (C=O carbonyl), 173.1 (C-4), 162.2 (C-2), 151.0 (C-8a), 135.9 (C-5), 131.6 (C-7), 131.6 (C-6), 125.5 (4a), 117.3 (C-8), 30.8 ($COCH_3$), 20.1 (CH_3), 13.9 (SCH_3).

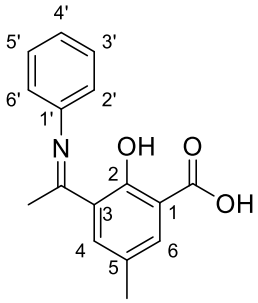
8-acetyl-6-methyl-2-morpholino-4H-benzo[e][1,3]oxazin-4-one 103



In a 50 mL beaker containing 20 mL of 1:1 RO water and 2-propanol, sodium bicarbonate (1.0 g, 11.90 mmol) was suspended. The compound **101** (0.47 g, 2.0 mmol) was added, the reaction mixture was heated to 60 °C while stirring and then removed from heat and allowed to cool to room temperature with stirring. Iodomethane (0.5 mL, 8.0 mmol) was added dropwise and allowed to stir at room temperature for 30 minutes or until a thick precipitate had formed. Morpholine (1.0 mL, 11.48 mmol) was added and the reaction mixture was stirred for an additional 3 hours. The solvent was evaporated, and the product was extracted by dichloromethane and dried over $MgSO_4$ and evaporated under reduced pressure. The crude solid was then recrystallized from toluene to obtain the product (0.52 g, 90%).

MP 175-180 °C; ν_{max} (ATR) /cm^{-1} 2974 w, 2926 w, 2869 w (C-C), 1672 m (C=O), 1612 m (C=N), 1556 s (C=C); **^{1}H NMR** ($CDCl_3$, 300 K) δ 8.12 (s, 1H, H-7), 7.82 (s, 1H, H-5), 3.9 to 3.78 (bm, 8H, morpholine), 2.64 (s, 3H, $COCH_3$), 2.44 (s, 3H, CH_3); **^{13}C NMR** ($CDCl_3$, 300 K) δ 195.4 (C=O carbonyl), 165.7 (C-4), 156.1 (C-2), 149.0 (C-8a), 135.1 (C-5), 134.3 (C-7), 131.7 (C-6), 124.5 (4a), 117.3 (C-8), 65.6 (C-3'), 43.9 (C-2'), 29.0 ($COCH_3$), 20.1 (CH_3). HRMS (ESI): *m/z* calculated for $C_{15}H_{16}N_2O_4$: 289.1183 $[M+H]^+$, found: 289.1194.

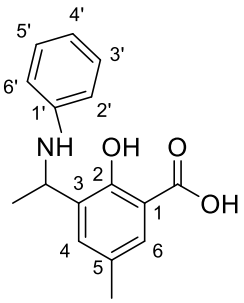
2-hydroxy-5-methyl-3-(1-(phenylimino)ethyl)benzoic acid 106



3-acetyl-2-hydroxy-5-methyl benzoic acid **100** (194 mg, 1 mmol) was dissolved in methanol (15 mL) and to it aniline (186 mg, 2 mmol) was added. Few drops of conc. HCl were added and the reaction mixture was stirred at room temperature for 30 minutes. Solvent was evaporated and the residue was washed with ether to remove excess aniline. The product was filtered and dried to get yellow powder (230 mg, 85%).

MP 184-186 ^{o}C $\boldsymbol{\nu_{max}}$ (ATR) /cm^{-1} 3060 w (O-H), 1685 (C=O). **^{1}H NMR** ($CDCl_3$, 300 K) δ 8.19 (d, 2H, $J_{H6,H4}$ = 2.1 Hz, H-6, H-4), 7.52 (t, 2H, *J*= 7.8, H-3', H-5'), 7.43 (t, 1H, *J*= 7.2, H-4'), 7.22 (d, 2H, *J*= 7.5, H-2', H-6'), 2.57 (s, 3H, CH_3), 2.30 (s, 3H, NC-CH_3); **^{13}C NMR** ($CDCl_3$, 300 K) δ 174 (C=O carbonyl), 170.5 (C=N), 167.6 (C-2), 140.9 (C-1'), 136.6 (C-4), 133.9 (C-6), 129.1 (C-3', 5'), 128.2 (C-5), 127.9 (C-4'), 123.8 (C-6'), 123.7 (C-2'), 118.8 (C-3), 115.3 (C-1), 19.7 (CH_3), 15.9 (NC-CH_3).

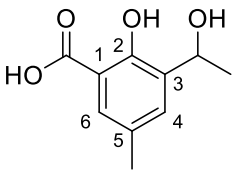
2-hydroxy-5-methyl-3-(1-(phenylamino)ethyl)benzoic acid 107



The compound 2-hydroxy-5-methyl-3-(1-(phenylimino)ethyl)benzoic acid **106** (269 mg, 1 mmol) was dissolved in methanol (15 mL) and sodium borohydride (41.5 mg, 1.1 mmol) was added to it portion-wise. The mixture was stirred at room temperature for 15 minutes. Water (15 mL) was added to the mixture, and the product was extracted with ethyl acetate, dried with magnesium sulphate, evaporated in vacuo and dried to get the product as pale yellow solid (81 mg, 30%).

MP 123-130 °C (decomp.) ν_{max} (ATR) /cm^{-1} 3402 w (NH), 2967 w (O-H), 1655 (C=O). **^{1}H NMR** ($CDCl_3$, 300 K) δ 7.57 (s, 1H, H-6), 7.33 (s, 1H, H-4), 7.11 (t, 2H, *J*= 7.5, H-3', H-5'), 6.71 (t, 1H, *J*= 6.6, H-4'), 6.59 (d, 2H, *J*= 8.1, H-2', H-6'), 4.8 (q, 1H, *J*= 6.9, 2.21 (s, 3H, CH_3), 1.51 (d, 3H, *J*= 6.9, NC-CH_3).

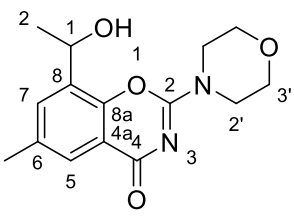
2-hydroxy-3-(1-hydroxyethyl)-5-methylbenzoic acid 109



The compound 3-acetyl-2-hydroxy-5-methyl benzoic acid **100** (194 mg, 1 mmol) was dissolved in dry methanol (15 mL) at 0 °C and sodium borohydride (41.5 mg, 1.1 mmol) was added while stirring. After 15 minutes, the reaction mixture was quenched with water (15 mL) and was extracted thoroughly by ethyl acetate. The extract was dried over magnesium sulphate, filtered, solvent evaporated and the product was dried to get fine powder (98 mg, 50%).

MP 150-152 °C ν_{max} (ATR) /cm^{-1} 3413-2964 br (O-H), 1659 s (C=O). **^{1}H NMR** ($CDCl_3$, 300 K) δ 7.61 (s, 1H, H-6), 7.39 (s, 1H, H-4), 5.13 (q, 1H, CH-OH) 2.29 (s, 3H, CH_3), 1.53 (d, 3H, CH-CH_3).

8-hydroxyethyl-6-methyl-2-morpholino-4H-benzo[e][1,3]oxazin-4-one 111

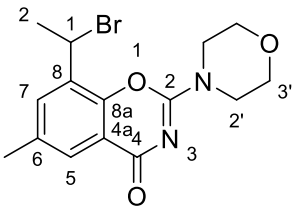


To a solution of 8-acetyl-6-methyl-2-morpholino-4H-benzo[e][1,3]oxazin-4-one **103**, (576 mg, 2 mmol) in methanol (20 mL) and DCM (10 mL), was added Sodium borohydride (82 mg, 2.2 mmol) in an ice bath at 0 °C, and stirred for 5 minutes. The reaction mixture was quenched with water (25 mL), volatiles were evaporated and extracted with DCM

(x 2). The organic layer was dried over $MgSO_4$ and evaporated under reduced pressure to give pale solid (290 mg, 50%).

MP 210-218 °C (decomp.) ν_{max} (ATR) /cm^{-1} 3361 w (OH), 2968 w, 2925 w, 2862 w (C-C), 1655 m (C=O), 1621 m (C=N), 1547 s (C=C); **^{1}H NMR** ($CDCl_3$, 300 K) δ 7.7 (s, 1H, H-7), 7.5 (s, 1H, H-5), 5.21 (q, 1H, *J*=6.6 Hz, CHOH), 3.86, 3.77 (m, 8H, morpholine) 2.36 (s, 3H, CH_3), 1.51 (d, 3H, *J*=5.7 Hz, CH_3-CH); **^{13}C NMR** (d_6-DMSO, 340 K) δ 165.4 (C-4), 156.1 (C-2), 147.6 (C-8a), 134.1 (C-7), 134 (C-6), 131.3 (C-5), 124.3 (C-8), 116.1 (C-4a), 62.1 (CHOH) 65.2 (C-3'), 43.7 (C-2'), 23.9 (CH_3CH), 20.4 (CH_3). HRMS (ESI): *m/z* calculated for $C_{15}H_{18}N_2O_4$: 291.1339 $[M+H]^+$, found: 291.1352.

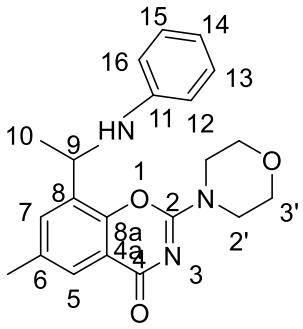
8-(1-bromoethyl)-6-methyl-2-morpholino-4H-benzo[e][1,3]oxazin-4-one 112



8-hydroxyethyl-6-methyl-2-morpholino-4H-benzo[e][1,3]oxazin-4-one **111** (580 mg, 2 mmol) was dissolved in 13 mL of DCM. Phosphorus tribromide (2.2 mL, 2.2 mmol) was added to the above solution in an ice bath under nitrogen purging. This was allowed to stir at room temperature for 24 hours. Then more phosphorus tribromide (0.4 mL, 0.4 mmol) was added under nitrogen purging and stirred at room temperature for further 16 hours. After the reaction, the solvent was evaporated, 20 mL ice water was added. pH was adjusted to 6 by saturated sodium carbonate solution. The solid was filtered and washed with water and then with diethyl ether to give white solid. (543 mg, 77%).

MP 165-166 °C ν_{max} (ATR) /cm^{-1} 2926 w, 2854 w (alkane C-C),1669 m (C=O), 1618 m (C=N), 1554 s (C=C); **^{1}H NMR** ($CDCl_3$, 300 K) δ 7.9 (d, 1H, *J*=1 Hz, H-5), 7.51 (d, 1H, *J*=1.5 Hz, H-7), 5.44 (q, 1H, *J*=7 Hz, CHBr), 3.81 to 3.96 (bm, 8H, morpholine) 2.42 (s, 3H, CH_3), 2.12 (d, 3H, *J*=7.5 Hz, CH_3-CH); **^{13}C NMR** ($CDCl_3$, 300 K) δ 166.8 (C-4), 156.3 (C-8a), 148.7 (C-2), 135.4 (C-7), 131.8 (C-6), 129.3 (C-5), 127.9 (C-8), 117.4 (C-4a), 40.3 (CHBr) 66.2 (C-3'), 44.7 (C-2'), 24.6 (CH_3CH), 21.0 (CH_3). HRMS (ESI): *m/z* calculated for $C_{15}H_{17}BrN_2O_3$: 353.0495 $[M+H]^+$, found: 353.0497.

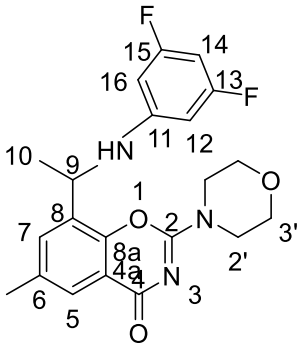
Synthesis of 6-methyl-2-morpholino-8-(1-(phenylamino)ethyl)-4H-benzo[e][1,3]oxazin-4-one (LTUEM08) 113a



The compound synthesized according to General Procedure A by reacting 8-(1-bromoethyl)-6-methyl-2-morpholino-4H-benzo[e][1,3]oxazin-4-one **112** with aniline. The crude product was subjected to flash chromatography and the product was obtained as a cream coloured solid (106 mg, 51%).

MP 95-100 °C ν_{max} (ATR) /cm^{-1} 3339 (NH), 2920 w, 2856 w (alkane C-C),1663 m (C=O), 1618 m (C=N), 1547 s (C=C); **^{1}H NMR** ($CDCl_3$, 300 K) δ 7.83 (s, 1H, H-5), 7.49 (d, 1H, *J*=1.5 Hz, H-7), 7.12 (t, 2H, *J*=8 Hz, (H-13, H-15), 6.7 (t, 1H, *J*=7.5 Hz, (H-14), 6.48 (d, 2H, *J*=8 Hz, (H-12, H-16), 4.83 (q, 1H, *J*=6.5 Hz, CH-NH), 3.72 to 4.0 (bm, 8H, morpholine) 2.34 (s, 3H, CH_3), 1.57 (d, 3H, *J*=6.5 Hz, CH_3-CH); **^{13}C NMR** ($CDCl_3$, 300 K) δ 166.9 (C-4), 155.9 (C-2), 148.3 (C-8a), 145.8 (C-11), 135.0 (C-7), 131.1 (C-6), 130.3 (C-8), 128.6 (C-13, C-15), 125.7 (C-4a), 117.4 (C-14), 116.3 (C-5), 112.4 (C-12, C-16), 46.85 (C-9) 65.6 (C-3'), 43.84 (C-2'), 21.82 (CH_3CH), 20.44 (CH_3).

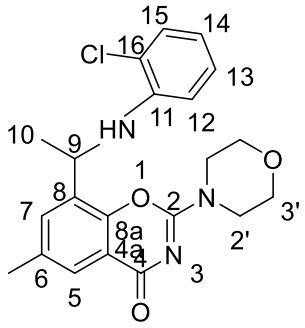
8-(1-((3,5-difluorophenyl)amino)ethyl)-6-methyl-2-morpholino-4H-benzo[e][1,3]oxazin-4-one (LTUEM09) 113b



To a stirred solution of 8-(1-bromoethyl)-6-methyl-2-morpholino-4H-benzo[e][1,3]oxazin-4-one **112** (200 mg. 0.56 mmol) and 3,5-difluoroanilne (289 mg, 2.24 mmol) in dichloromethane and methanol (4:1), potassium iodide (94 mg, 0.56 mmol) was added. Triethylamine (141.6 mg, 1.4 mmol) was then added as described in General procedure A. The product was obtained as a cream coloured solid (113 mg, 50%)

MP 120-130 °C ν_{max} (ATR) /cm^{-1} 3305 (NH), 2967 w, 2921 w, 2859 w (alkane),1666 m (C=O), 1619 m (C=N), 1551 s (C=C); **^{1}H NMR** ($CDCl_3$, 300 K) δ 7.83 (d,1H, *J*=1 Hz, H-5), 7.41 (d, 1H, *J*=1.5 Hz, H-7), 6.12 (t, 1H, *J*=2 Hz (C-H), *J*=5.5 Hz (C-F), (H-14), 6.0 (d, 2H, *J*=1.5 Hz (C-H), *J*=8 Hz (C-F), (H-12, H-16), 4.79 (dq, 1H, *J*=6.5 Hz, CHNH), 4.38 (d, 1H, *J*=5.5 Hz, NH), 3.78 (bm, 8H, morpholine) 2.35 (s, 3H, CH_3), 1.58 (d, 3H, *J*=7 Hz, CH_3-CH); **^{13}C NMR** ($CDCl_3$, 300 K) δ 166.9 (C-4), 165 (d, C-15, *J*=125 Hz, C-F), 163.1 (d, C-13, *J*=126 Hz, C-F), 156.5 (C-2),148.8 (d, C-11, *J*=117 Hz), 148.7 (C-8a), 135.7 (C-7), 131.3 (C-6), 129.9 (C-8), 126.8 (C-5), 117.3 (C-4a), 95.8 (d, C-16, *J*=58 Hz), 95.7 (d, C-12, *J*=58 Hz), 93.0 (t, C-14, *J*=208 Hz), 47.2 (C-9) 66.2 (C-3'), 44.5 (C-2'), 22.2 (CH_3CH), 21 (CH_3).

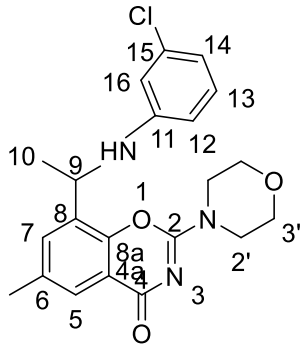
8-(1-((2-chlorophenyl)amino)ethyl)-6-methyl-2-morpholino-4H-benzo[e][1,3]oxazin-4-one (LTUEM10) 113c



8-(1-bromoethyl)-6-methyl-2-morpholino-4H-benzo[e][1,3]oxazin-4-one **112** was reacted with 2-chloroaniline according to General Procedure A. The product was obtained as a cream coloured solid after purification by flash chromatography (95 mg, 42%).

MP 115-120 °C ν_{max} (ATR) /cm^{-1} 3416 (NH), 2960 w, 2920 w, 2866 w (alkane),1672 m (C=O), 1620 m (C=N), 1551 s (C=C); **^{1}H NMR** ($CDCl_3$, 300 K) δ 7.84 (d,1H, *J*=1 Hz, H-5), 7.43 (d, 1H, *J*=1 Hz, H-7), 7.28 (d, 1H, *J*=1.5 Hz, H-15) 7.01 (t, 1H, *J*=1.5 Hz, *J*=6.5 Hz, H-13), 6.63 (dt, 1H, *J*=1 Hz, *J*=7.5 Hz, H-14), 6.33 (d, 1H, *J*=8 Hz, H-12), 4.84 (dq, 1H, *J*=6.5 Hz, CHNH), 4.65 (d, 1H, *J*=5 Hz, NH) 3.77 to 3.93 (bm, 8H, morpholine) 2.35 (s, 3H, CH_3), 1.64 (d, 3H, *J*=6.5 Hz, CH_3-CH); **^{13}C NMR** ($CDCl_3$, 300 K) δ 167.0 (C-4), 156.5 (C-2), 149.0 (C-8a), 142.4 (C-11), 135.7 (C-7), 131.7 (C-8), 130.4 (C-6), 129.2 (C-15), 127.8 (C-5), 126.6 (C-4a), 119.1 (C-13), 117.9 (C-14), 117.3 (C-16), 111.7 (C-12), 47.9 (C-9) 66.1 (C-3'), 44.4 (d, *J*=576, Hz, C-2'), 22.6 (CH_3CH), 21.0 (CH_3).

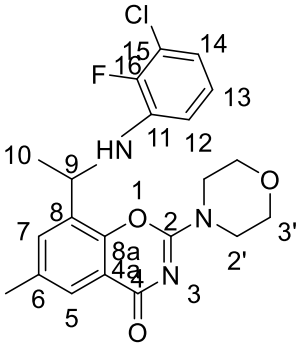
8-(1-((3-chlorophenyl)amino)ethyl)-6-methyl-2-morpholino-4H-benzo[e][1,3]oxazin-4-one (LTUEM11) 113d



The compound was synthesized according to General Procedure A by reacting 8-(1-bromoethyl)-6-methyl-2-morpholino-4H-benzo[e][1,3]oxazin-4-one **112** with 3-chloroaniline. The product was obtained as a cream coloured solid after purification by flash chromatography (105 mg, 46%).

MP 103-106 °C ν_{max} (ATR) /cm^{-1} 3319 (NH), 2963 w, 2920 w, 2858 w (alkane),1665 m (C=O), 1619 m (C=N), 1550 s (C=C); **^{1}H NMR** ($CDCl_3$, 300 K) δ 7.84 (d,1H, *J*=1 Hz, H-5), 7.44 (d, 1H, *J*=1.5 Hz, H-7), 7.02 (t, 1H, *J*=8 Hz, H-13) 6.66 (dd, 1H, *J*=1 Hz, *J*=8 Hz, H-14), 6.49 (s, 1H, H-16), 6.34 (dd, 1H, *J*=2 Hz, *J*=8 Hz, H-12), 4.82 (q, 1H, *J*=6.5 Hz, CHNH), 4.12 (d, 1H, *J*=7 Hz, NH) 3.75 to 3.98 (bm, 8H, morpholine) 2.35 (s, 3H, CH_3), 1.58 (d, 3H, *J*=6.5 Hz, CH_3-CH); **^{13}C NMR** ($CDCl_3$, 300 K) δ 167.0 (C-4), 156.5 (C-2), 149.0 (C-8a), 147.7 (C-11), 135.7 (C-7), 135.0 (C-15), 131.4 (C-6), 130.3 (C-8), (C-5), 126.6 (C-4a), 117.8 (C-14), 117.2 (C-13), 112.6 (C-16), 111.1 (C-12), 47.1 (C-9) 66.2 (C-3'), 44.5 (C-2'), 22.3 (CH_3CH), 21.0 (CH_3).

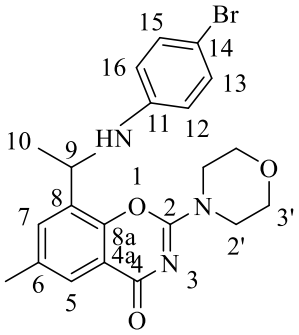
8-(1-((3-chloro-2-fluorophenyl)amino)ethyl)-6-methyl-2-morpholino-4H-benzo[e][1,3]oxazin-4-one (LTUEM12) 113e



The compound was synthesized according to General Procedure A by using 3-chloro-2-fluoroaniline with 8-(1-bromoethyl)-6-methyl-2-morpholino-4H-benzo[e][1,3]oxazin-4-one **112**. The product was obtained as a cream coloured solid after purification by flash chromatography (110 mg, 46%).

MP 146-148 °C ν_{max} (ATR) /cm^{-1} 3291 (NH), 2972 w, 2919 w, 2856 w (alkane),1662 m (C=O), 1612 m (C=N), 1551 s (C=C); **^{1}H NMR** ($CDCl_3$, 300 K) δ 7.84 (d,1H, *J*=1 Hz, H-5), 7.42 (d, 1H, *J*=1.5 Hz, H-7), 6.77 (dt, 1H, *J*=1 Hz, *J*=8 Hz, H-13) 6.68 (dt, 1H, *J*=1 Hz, *J*=8 Hz, H-14), 6.22 (t, 1H, *J*=8 Hz, H-12), 4.82 (dq, 1H, *J*=6.5 Hz, CHNH), 4.34 (bs, 1H, NH) 3.73 to 3.93 (bm, 8H, morpholine) 2.35 (s, 3H, CH_3), 1.63 (d, 3H, *J*=7 Hz, CH_3-CH); **^{13}C NMR** ($CDCl_3$, 300 K) δ 166.9 (C-4), 156.5 (C-2), 148.9 (C-8a), 146.0 (C-16), 136.1 (C-7), 135.8.6 (C-11), 131.6 (C-8), 130.0 (C-6), 126.7 (C-5), 124.6 (C-13), 120.6 (C-4a), 118.2 (C-15), 117.3 (C-14), 110.0 (C-12), 47.7 (C-9) 66.3 (C-3'), 44.8 (C-2'), 22.6 (CH_3CH), 21.0 (CH_3).

8-(1-((4-bromophenyl)amino)ethyl)-6-methyl-2-morpholino-4H-benzo[e][1,3]oxazin-4-one (LTUEM13) 113f

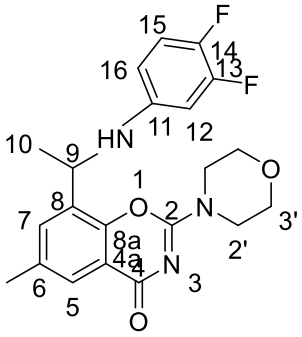


The compound was synthesized according to General Procedure A using 4-bromoaniline with 8-(1-bromoethyl)-6-methyl-2-morpholino-4H-benzo[e][1,3]oxazin-4-one **112**. The product was obtained as a cream coloured solid after purification by flash chromatography (125 mg, 49%).

MP 182-183 °C ν_{max} (ATR) /cm^{-1} 3319 (NH), 2962 w, 2916 w, 2863 w (alkane),1657 m (C=O), 1622 m (C=N), 1544 s (C=C); **^{1}H NMR** ($CDCl_3$, 300 K) δ 7.83 (s,1H, H-5), 7.43 (s, 1H, H-7), 7.19 (d, 2H, *J*=8.5 Hz, H-13, 15) 6.35 (d, 2H, *J*=8.5 Hz, H-12, 16), 4.78 (p, 1H, *J*=6 Hz, CHNH), 4.05 (d, 1H, *J*=4.5 Hz, NH) 3.75 to 3.76 (bm, 8H, morpholine) 2.34 (s, 3H, CH_3), 1.57 (d, 3H, *J*=6.5 Hz, CH_3-CH); **^{13}C NMR** ($CDCl_3$, 300 K) δ 167.0 (C-4), 156.5 (C-2), 148.9 (C-8a), 145.5 (C-11), 135.7 (C-7), 132.0 (C-15, C-13), 131.4

(C-6), 130.4 (C-8), 126.5 (C-5), 117.2 (C-4a), 114.5 (C-12, C-16), 109.6 (C-14), 47.4 (C-9) 66.1 (C-3'), 44.5 (d, *J*=278.5 Hz, C-2'), 22.4 (CH_3CH), 21.0 (CH_3).

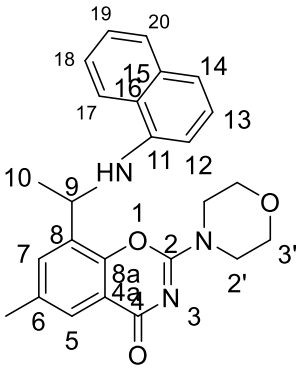
8-(1-((3,4-difluorophenyl)amino)ethyl)-6-methyl-2-morpholino-4H-benzo[e][1,3]oxazin-4-one (LTUEM14) 113g



8-(1-bromoethyl)-6-methyl-2-morpholino-4H-benzo[e][1,3]oxazin-4-one **112** was reacted with 3,4-difluoroaniline according to General Procedure A. The product was obtained as a light grey coloured solid after purification by flash chromatography (120 mg, 53%).

MP 115-125 °C ν_{max} (ATR) /cm^{-1} 3323 (NH), 2972 w, 2925 w, 2860 w (alkane),1666 m (C=O), 1619 m (C=N), 1554 s (C=C); **^{1}H NMR** (d_6-DMSO, 340 K) δ 7.58 (d,1H, *J*=1 Hz, H-5), 7.47 (d, 1H, *J*=2 Hz, H-7), 7.02 (m, 1H, *J*=1.5 Hz (C-H), *J*=9 Hz (C-F), H-15), 6.47 (m, 1H, *J*=2.5 Hz (C-H), *J*=7 Hz (C-F) H-12), 6.30 (m, 1H, *J*=2 Hz (C-H), *J*=3.5 Hz (C-F), (H-16), 6.24 (d, 1H, *J*=6.5 Hz, NH), 4.83 (p, 1H, *J*=6.5 Hz, CHNH), 3.74 (bm, 8H, morpholine) 2.31 (s, 3H, CH_3), 1.49 (d, 3H, *J*=6.5 Hz, CH_3-CH); **^{13}C NMR** (d_6-DMSO, 340 K) δ 165.9 (C-4), 157.0 (C-2), 151.3 (d, C-8a, *J*=53 Hz), 149.4 (C-13), 145.5 (d, C-11, *J*=36 Hz), 142.7 (d, C-14, *J*=52 Hz), 140.9 (C-7), 134.9 (C-6), 132.2 (C-8), 131.7 (C-5), 125.4 (C-4a), 117.7 (t, C-15, *J*=101 Hz), 108.5 (t, C-16, *J*=11 Hz), 101.3 (d, C-12, *J*=81 Hz), 47.1 (C-9) 65.9 (C-3'), 44.7 (C-2'), 22.4 (CH_3CH), 21.0 (CH_3). HRMS (ESI): *m/z* calculated for $C_{21}H_{21}F_2N_3O_3$: 402.1624 $[M+H]^+$, found: 402.1638.

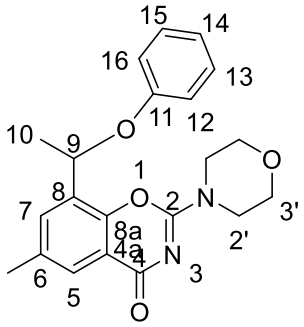
6-methyl-2-morpholino-8-(1-(naphthalen-1-ylamino)ethyl)-4H-benzo[e][1,3]oxazin-4-one (LTUEM15) 113h



The compound was synthesized according to General Procedure A by reacting naphthylamine with 8-(1-bromoethyl)-6-methyl-2-morpholino-4H-benzo[e][1,3]oxazin-4-one **112**. The product was obtained as a cream coloured solid after purification by flash chromatography (95 mg, 40%**).**

MP 165-166 °C ν_{max} (ATR) /cm^{-1} 3349 (NH), 2958 w, 2927 w, 2851 w (alkane C-C),1668 m (C=O), 1619 m (C=N), 1557 s (C=C); **^{1}H NMR** ($CDCl_3$, 300 K) δ 7.89 (dd, 1H, *J*=4 Hz, *J*=6 Hz, H-20), 7.84 (s, 1H, H-5), 7.8 (dd, 1H, *J*=3.5 Hz, *J*=6.5 Hz, H-17), 7.51 (d, 1H, *J*=1.5 Hz, H-7), 7.49 (m, 2H, *J*=3 Hz, H-14, H-19), 7.21 (m, 2H, *J*=8 Hz, H-13, H-18), 6.29 (d, 2H, *J*=7 Hz, H-12,), 5.02 (q, 1H, *J*=6 Hz, CH-NH), 4.68 (s, 1H, NH) 3.65 to 3.95 (bm, 8H, morpholine) 2.30 (s, 3H, CH_3), 1.72 (d, 3H, *J*=6.5 Hz, CH_3-CH); **^{13}C NMR** ($CDCl_3$, 300 K) δ 167.1 (C-4), 156.6 (C-2), 149.1 (C-8a), 141.5 (C-11), 135.7 (C-7), 134.3 (C-15), 131.5 (C-8), 130.7 (C-6), 128.8 (C-5), 126.5 (C-20), 126.3 (C-13), 125.9 (C-19), 125.0 (C-18), 123.1 (C-17), 119.5 (C-16), 118.0 (C-4a), 117.2 (C-14), 105.3 (C-12,), 47.6 (C-9) 66.2 (C-3'), 44.7 (C-2'), 22.5 (CH_3CH), 21.0 (CH_3). HRMS (ESI): *m/z* calculated for $C_{25}H_{25}N_3O_3$: 416.1969 $[M+H]^+$, found: 416.1989.

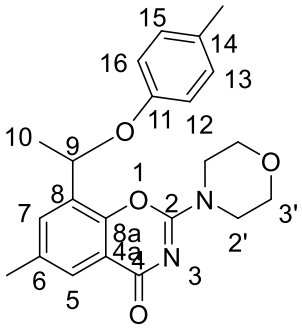
6-methyl-2-morpholino-8-(1-phenoxyethyl)-4H-benzo[e][1,3]oxazin-4-one (LTUEM16) 114a



The compound was synthesized according to General Procedure A by reaction of 8-(1-bromoethyl)-6-methyl-2-morpholino-4H-benzo[e][1,3]oxazin-4-one **112** with phenol. The impure product was obtained initially as an oil, then precipitated as white solid in minimum diethyl ether (80 mg, 38%**).**

MP 150-153 °C ν_{max} (ATR) /cm^{-1} 2922 w, 2865 w (alkane C-C),1669 m (C=O), 1624 m (C=N), 1560 s (C=C); **^{1}H NMR** ($CDCl_3$, 300 K) δ 7.86 (d, 1H, *J*=0.5 Hz, H-5), 7.49 (d, 1H, *J*=1.5 Hz, H-7), 7.23 (t, 2H, *J*=8.5 Hz, H-13, H-15), 6.93 (t, 1H, *J*=7.5 Hz, H-14), 6.82 (d, 2H, *J*=8.5 Hz, H-12, H-16), 5.5 (q, 1H, *J*=6.5 Hz, CH-O), 3.73 to 3.91 (bm, 8H, morpholine) 2.37 (s, 3H, CH_3), 1.71 (d, 3H, *J*=6.5 Hz, CH_3-CH); **^{13}C NMR** ($CDCl_3$, 300 K) δ 166.9 (C-4), 157.5 (C-11), 156.5 (C-2), 148.6 (C-8a), 135.6 (C-7), 132.0 (C-6), 129.6 (C-5, C-13), 129.1 (C-8), 127.1 (C-15), 121.3 (C-4a), 117.2 (C-14), 115.4 (C-12, C-16), 70.6 (C-9) 66.3 (C-3'), 44.5 (C-2'), 21.9 (CH_3CH), 21.0 (CH_3). HRMS (ESI): *m/z* calculated for $C_{21}H_{22}N_2O_4$: 367.1652 $[M+H]^+$, found: 367.167.

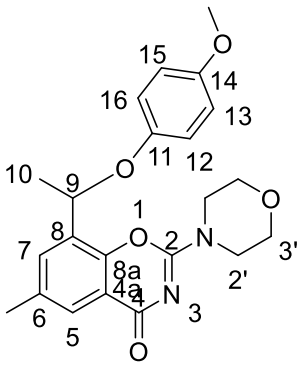
6-methyl-2-morpholino-8-(1-(p-tolyloxy)ethyl)-4H-benzo[e][1,3]oxazin-4-one (LTUEM17) 114b



The compound was synthesized by reacting 8-(1-bromoethyl)-6-methyl-2-morpholino-4H-benzo[e][1,3]oxazin-4-one **112** with 4-methylphenol according to General Procedure A. The crude product was subjected to flash chromatography, obtained as an oil, and then precipitated as white coloured solid by minimum diethyl ether (91 mg, 42%**).**

MP 225-230 °C ν_{max} (ATR) /cm^{-1} 2919 w, 2866 w (alkane C-C),1669 m (C=O), 1623 m (C=N), 1561 s (C=C); **^{1}H NMR** ($CDCl_3$, 300 K) δ 7.85 (s, 1H, H-5), 7.49 (d, 1H, *J*=1.5 Hz, H-7), 7.02 (d, 2H, *J*=8.5 Hz, H-13, H-15), 6.71 (d, 2H, *J*=8.5 Hz, H-12, H-16), 5.53 (q, 1H, *J*=6.5 Hz, CH-O), 3.74 to 3.91 (bm, 8H, morpholine) 2.37 (s, 3H, CH_3), 2.25 (s, 3H, CH_3), 1.69 (d, 3H, *J*=6.5 Hz, CH_3-CH); **^{13}C NMR** ($CDCl_3$, 300 K) δ 167.0 (C-4), 156.5 (C-11), 155.4 (C-2), 148.6 (C-8a), 135.6 (C-7), 132.1 (C-6), 130.6 (C-5), 130.0 (C-13, C-15), 129.3 (C-14), 127.0 (C-8), 117.2 (C-4a), 115.3 (C-12, C-16), 70.8 (C-9) 66.1 (C-3'), 44.1 (C-2'), 22.0 (CH_3CH), 21.0 (phenolic CH_3), 20.4 (CH_3). HRMS (ESI): *m/z* calculated for $C_{22}H_{24}N_2O_4$: 381.1809 $[M+H]^+$, found: 381.1818.

8-(1-(4-methoxyphenoxy)ethyl)-6-methyl-2-morpholino-4H-benzo[e][1,3]oxazin-4-one (LTUEM18) 114c

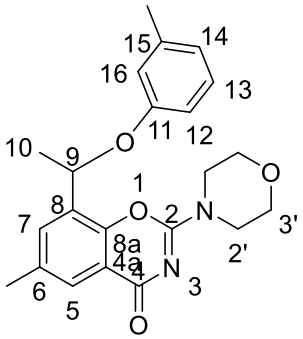


The compound was synthesized by the reaction of 8-(1-bromoethyl)-6-methyl-2-morpholino-4H-benzo[e][1,3]oxazin-4-one **112** with 4-methoxyphenol according to General Procedure A. The crude product was subjected to flash chromatography, obtained as an oil, and then precipitated as white coloured solid by minimum diethyl ether (100 mg, 44%**).**

MP 190-195 °C ν_{max} (ATR) /cm^{-1} 2963 w, 2930 w, 2862 w (alkane C-C),1668 m (C=O), 1622 m (C=N), 1560 s (C=C); **^{1}H NMR** (d_6-DMSO, 340 K) δ 7.63 (d, 1H, *J*=1.5 Hz, H-5), 7.57 (d, 1H, *J*=2 Hz, H-7), 6.86 (dd, 2H, *J*=6.5 Hz, *J*=2 Hz, H-12, H-16), 6.80 (dd, 2H, *J*=6.5 Hz, *J*=2 Hz, H-13, H-15), 5.67 (q, 1H, *J*=6.5 Hz, H-9), 3.73 (bs, 8H, morpholine), 3.67 (s, 3H, OCH_3), 2.35 (s, 3H, CH_3), 1.63 (d, 3H, *J*=6.5 Hz, CH_3-CH);

^{13}C NMR (d_6-DMSO, 340 K) δ 165.7 (C-4), 156.8 (C-2), 154.3 (C-8a), 151.7 (C-14), 148.9 (C-11), 135.1 (C-7), 132.2 (C-6), 130.3 (C-5), 126.1 (C-8), 117.5 (C-4a), 117.4 (C-12, C-16), 115.3 (C-13, C-15), 71.1 (C-9) 65.9 (C-3'), 55.9 (OCH_3) 44.7 (C-2'), 22.0 (CH_3), 20.9 (CH_3CH). HRMS (ESI): *m/z* calculated for $C_{22}H_{24}N_2O_5$: 397.1758 $[M+H]^+$, found: 397.1777.

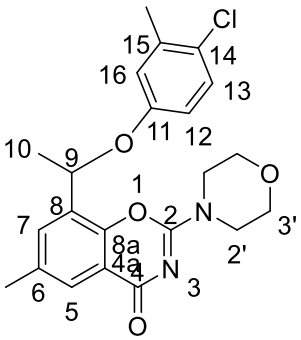
6-methyl-2-morpholino-8-(1-(m-tolyloxy)ethyl)-4H-benzo[e][1,3]oxazin-4-one (LTUEM19) 114d



The compound was synthesized by reacting 8-(1-bromoethyl)-6-methyl-2-morpholino-4H-benzo[e][1,3]oxazin-4-one **112** with 3-methylphenol according to General Procedure A. The crude product was subjected to flash chromatography, obtained as an oil, and then precipitated as white coloured solid in minimum diethyl ether (90 mg, 42%**).**

MP 153-157 °C ν_{max} (ATR) /cm^{-1} 3056 w, 2978 w, 2928 w, 2866 w (alkane C-C),1672 m (C=O), 1624 m (C=N), 1567 s (C=C); **^{1}H NMR** (d_6-DMSO, 340 K) δ 7.63 (d, 1H, *J*=1.5, H-5), 7.57 (d, 1H, *J*=1.5 Hz, H-7), 7.10 (t, 1H, *J*=8 Hz, H-13) 6.77 (s, 1H, H-16), 6.71 (dt, 2H, *J*=8.5 Hz, *J*=2 Hz, H-12, H-14), 5.77 (q, 1H, *J*=6.5 Hz, CH-O), 3.71 to 3.74 (bm, 8H, morpholine) 2.35 (s, 3H, CH_3), 2.24 (s, 3H, CH_3), 1.64 (d, 3H, *J*=6.5 Hz, CH_3-CH); **^{13}C NMR** (d_6-DMSO, 340 K) δ 165.7 (C-4), 157.8 (C-11), 156.8 (C-2), 148.9 (C-8a), 139.5 (C-15), 135.1 (C-7), 132.1 (C-6), 130.1 (C-5), 129.7 (C-8), 126.2 (C-13), 122.2 (C-4a), 117.5 (C-14), 117.0 (C-16), 112.8 (C-12), 70.3 (C-9) 65.9 (C-3'), 44.7 (C-2'), 22.0 (phenolic CH_3), 21.4 (CH_3), 20.9 (CH_3CH). HRMS (ESI): *m/z* calculated for $C_{22}H_{24}N_2O_4$: 381.1809 $[M+H]^+$, found: 381.1823.

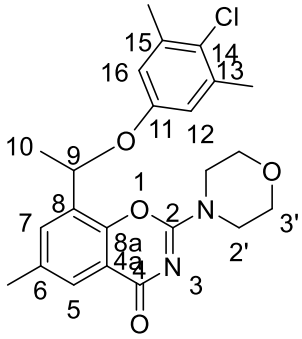
8-(1-(4-chloro-3-methylphenoxy)ethyl)-6-methyl-2-morpholino-4H-benzo[e][1,3]oxazin-4-one (LTUEM20) 114e



The compound was synthesized by reacting 8-(1-bromoethyl)-6-methyl-2-morpholino-4H-benzo[e][1,3]oxazin-4-one **112** with 4-chloro-3-methylphenol according to General Procedure A. The crude product was subjected to flash chromatography, obtained as an oil, and then precipitated as white coloured solid in minimum diethyl ether (100 mg, 42%).

MP 158-160 °C ν_{max} (ATR) /cm^{-1} 2951 w, 2930 w, 2863 w (alkane C-C),1672 m (C=O), 1622 m (C=N), 1562 s (C=C); **^{1}H NMR** (d_6-DMSO, 340 K) δ 7.64 (s, 1H, H-5), 7.56 (d, 1H, *J*=1.5 Hz, H-7), 7.22 (d, 1H, *J*=9 Hz, H-13) 6.95 (d, 1H, *J*=2.5 Hz, H-16), 6.77 (dd, 1H, *J*=9 Hz, *J*=3 Hz, H-12), 5.77 (q, 1H, *J*=6.5 Hz, CH-O), 3.72 to 3.73 (bm, 8H, morpholine) 2.35 (s, 3H, CH_3), 2.26 (s, 3H, CH_3), 1.64 (d, 3H, *J*=6.5 Hz, CH_3-CH); **^{13}C NMR** (d_6-DMSO, 340 K) δ 165.6 (C-4), 156.8 (C-11), 156.5 (C-2), 148.9 (C-8a), 137.0 (C-7), 135.1 (C-13), 132.1 (C-6), 130.0 (C-5), 129.7 (C-14), 126.3 (C-15), 125.6 (C-8), 119.1 (C-4a), 117.6 (C-16), 115.0 (C-12), 70.8 (C-9) 65.9 (C-3'), 44.7 (C-2'), 22.0 (CH_3), 20.9 (phenolic CH_3), 20.0 (CH_3CH). HRMS (ESI): *m/z* calculated for $C_{22}H_{23}ClN_2O_4$: 415.1419 $[M+H]^+$, found: 415.1439.

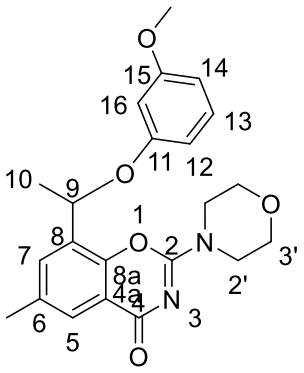
8-(1-(4-chloro-3,5-dimethylphenoxy)ethyl)-6-methyl-2-morpholino-4H-benzo[e][1,3]oxazin-4-one (LTUEM21) 114f



The compound was synthesized by reacting 8-(1-bromoethyl)-6-methyl-2-morpholino-4H-benzo[e][1,3]oxazin-4-one **112** with 4-chloro-3,5-dimethylphenol according to General Procedure A. The crude product was subjected to flash chromatography, obtained as an oil, and then precipitated as white coloured solid in minimum diethyl ether (100 mg, 41%**).**

MP 170-175 °C ν_{max} (ATR) /cm^{-1} 2967 w, 2918 w, 2865 w (alkane C-C),1673 m (C=O), 1622 m (C=N), 1561 s (C=C); **^{1}H NMR** (d_6-DMSO, 340 K) δ 7.64 (s, 1H, H-5), 7.58 (d, 1H, *J*=1.5 Hz, H-7), 6.78 (s, 2H, H-12, H-16), 5.77 (q, 1H, *J*=6.5 Hz, CH-O), 3.71 to 3.73 (bm, 8H, morpholine) 2.36 (s, 3H, CH_3), 2.26 (bs, 6H, 3,5-dimethyl), 1.64 (d, 3H, *J*=6.5 Hz, CH_3-CH); **^{13}C NMR** (d_6-DMSO, 340 K) δ 165.6 (C-4), 156.8 (C-11), 155.8 (C-2), 149.0 (C-8a), 137.1 (C-13, C-15), 135.1 (C-7), 132.2 (C-6), 129.7 (C-5), 126.3 (C-8), 126.0 (C-14), 117.5 (C-4a), 116.3 (C-12, C-16), 70.7 (C-9) 65.9 (C-3'), 44.7 (C-2'), 21.8 (CH_3), 20.9 (CH_3CH), 20.7 (phenolic dimethyl). HRMS (ESI): *m/z* calculated for $C_{23}H_{25}ClN_2O_4$: 429.1576 $[M+H]^+$, found: 429.1593.

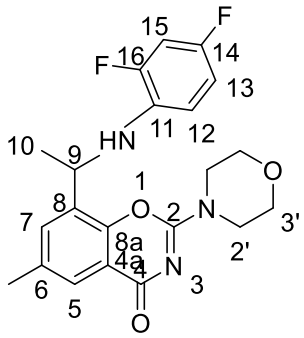
8-(1-(3-methoxyphenoxy)ethyl)-6-methyl-2-morpholino-4H-benzo[e][1,3]oxazin-4-one (LTUEM22) 114g



The compound was synthesized by reacting 8-(1-bromoethyl)-6-methyl-2-morpholino-4H-benzo[e][1,3]oxazin-4-one **112** with 3-methoxyphenol according to General Procedure A. The crude product was subjected to flash chromatography, obtained as an oil, and then precipitated as white coloured solid in minimum diethyl ether (110 mg, 49%**).**

MP 126-128 °C ν_{max} (ATR) /cm^{-1} 2955 w, 2919 w, 2864 w (alkane C-C),1669 m (C=O), 1620 m (C=N), 1561 s (C=C); **^{1}H NMR** (d_6-DMSO, 340 K) δ 7.64 (d, 1H, *J*=1.5 Hz, H-5), 7.58 (d, 1H, *J*=2 Hz, H-7), 7.13 (t, 1H, *J*=8 Hz, H-13), 6.52 (d, 1H, *J*=1.5 Hz, H-16) 6.50 (dd, 2H, *J*=7.5 Hz, *J*=2.5 Hz, H-12, H-14), 5.78 (q, 1H, *J*=6.5 Hz, H-9), 3.72 to 3.74 (bm, 8H, morpholine), 3.70 (s, 3H, OCH_3), 2.36 (s, 3H, CH_3), 1.65 (d, 3H, *J*=6.5 Hz, CH_3-CH); **^{13}C NMR** (d_6-DMSO, 340 K) δ 165.6 (C-4), 161.0 (C-15), 158.9 (C-11), 156.8 (C-2), 148.9 (C-8a), 135.1 (C-7), 132.2 (C-6), 130.4 (C-5), 130.0 (C-13), 126.2 (C-8), 117.5 (C-4a), 108.2 (C-12), 107.3 (C-14), 102.7 (C-16), 70.5 (C-9) 65.9 (C-3'), 55.5 (OCH_3) 44.7 (C-2'), 22.0 (CH_3), 20.9 (CH_3CH). HRMS (ESI): *m/z* calculated for $C_{22}H_{24}N_2O_5$: 397.1758 $[M+H]^+$, found: 397.1771.

8-(1-((2,4-difluorophenyl)amino)ethyl)-6-methyl-2-morpholino-4H-benzo[e][1,3]oxazin-4-one (LTUEM23) 113i



The compound was synthesized by reacting 8-(1-bromoethyl)-6-methyl-2-morpholino-4H-benzo[e][1,3]oxazin-4-one **112** with 2,4-difluoroaniline according to General Procedure A. The product was obtained as a white coloured solid after purification by flash chromatography (110 mg, 48%).

MP 140-142 °C ν_{max} (ATR) /cm^{-1} 3339 (NH), 2961 w, 2932 w, 2865 w (alkane),1659 m (C=O), 1618 m (C=N), 1564 s (C=C); **^{1}H NMR** ($CDCl_3$, 300 K) δ 7.84 (d, 1H, *J*=1 Hz, H-5), 7.43 (d, 1H, *J*=1.5 Hz, H-7), 6.80 (m, 1H, *J*=3 Hz (C-H), *J*=8.5 Hz (C-F), H-12), 6.63 (dt, 1H, *J*=1.5 Hz (C-H), *J*=8 Hz (C-F), H-13), 6.24 (m, 1H, *J*=5.5 Hz (C-H), *J*=9 Hz (C-F), H-15), 4.78 (p, 1H, *J*=6.5 Hz, CHNH), 4.09 (s, 1H, NH), 3.74 to 3.93 (bm, 8H, morpholine) 2.35 (s, 3H, CH_3), 1.61 (d, 3H, *J*=7 Hz, CH_3-CH); **^{13}C NMR** ($CDCl_3$, 300 K) δ 167.0 (C-4), 156.5 (C-2), 153.0 (C-16), 149.8 (C-14), 148.9 (C-8a), 135.8 (C-7), 131.6 (C-8, C-6), 130.4 (C-5), 126.6 (C-11), 117.3 (C-4a), 112.6 (C-12), 110.7 (d, C-13, *J*=101 Hz), 103.6 (t, C-15, *J*=106 Hz), 48.1 (C-9) 66.3 (C-3'), 44.2 (C-2'), 22.7 (CH_3CH), 21.0 (CH_3). HRMS (ESI): *m/z* calculated for $C_{21}H_{21}F_2N_3O_3$: 402.1624 $[M+H]^+$, found: 402.1639.

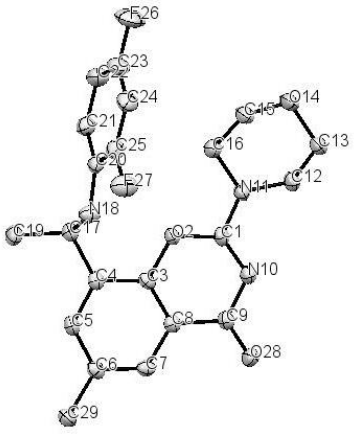
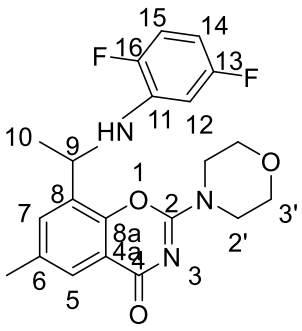


Figure 8.1: X-ray crystallography of (LTUEM23) **113i**

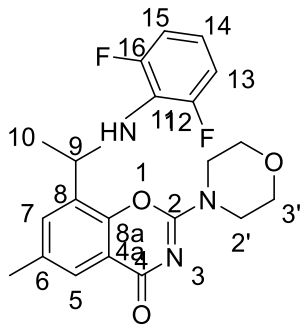
8-(1-((2,5-difluorophenyl)amino)ethyl)-6-methyl-2-morpholino-4H-benzo[e][1,3]oxazin-4-one (LTUEM24) 113j



The compound was synthesized by reacting 8-(1-bromoethyl)-6-methyl-2-morpholino-4H-benzo[e][1,3]oxazin-4-one **112** with 2,5-difluoroaniline according to General Procedure A. The product was obtained as a white coloured solid after purification by flash chromatography (100 mg, 44%).

MP 172-175 °C ν_{max} (ATR) /cm^{-1} 3342 (NH), 2977 w, 2930 w, 2862 w (alkane),1663 m (C=O), 1621 m (C=N), 1551 s (C=C); **^{1}H NMR** ($CDCl_3$, 300 K) δ 7.85 (s, 1H, H-5), 7.42 (d, 1H, *J*=1.5 Hz, H-7), 6.91 (m, 1H, *J*=3 Hz (C-H), *J*=9 Hz (C-F), H-15), 6.27 (m, 1H, *J*=3 Hz (C-H), *J*=8 Hz (C-F), H-12), 6.06 (m, 1H, *J*=3 Hz (C-H), *J*=7 Hz (C-F), *J*=20 Hz (C-F), H-14), 4.78 (p, 1H, *J*=6.5 Hz, CHNH), 4.36 (s, 1H, NH), 3.79 to 3.95 (bm, 8H, morpholine) 2.36 (s, 3H, CH_3), 1.62 (d, 3H, *J*=7 Hz, CH_3-CH); **^{13}C NMR** ($CDCl_3$, 300 K) δ 166.9 (C-4), 160.4 to 158.5 (C-13), 156.5 (C-2), 148.9 (C-8a), 148.3 to 146.4 and 136.1 (t, *J*=54 Hz, C-16) 135.8 (C-7), 131.5 (C-11), 129.8 (C-6, C-8), 126.9 (C-5), 117.4 (C-4a), 114.8 (q, *J*=41 Hz, C-15), 102.5 (q, C-14, *J*=29 Hz), 99.7 (d, C-12, *J*=126 Hz), 47.5 (C-9) 66.3 (C-3'), 44.2 (C-2'), 22.4 (CH_3CH), 21.0 (CH_3). HRMS (ESI): *m/z* calculated for $C_{21}H_{21}F_2N_3O_3$: 402.1624 $[M+H]^+$, found: 402.1637.

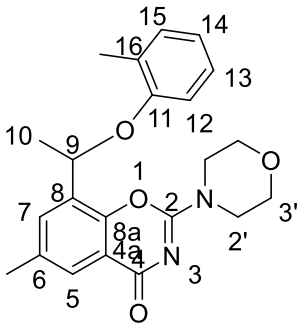
8-(1-((2,6-difluorophenyl)amino)ethyl)-6-methyl-2-morpholino-4H-benzo[e][1,3]oxazin-4-one (LTUEM25) 113k



The compound was synthesized by reacting 8-(1-bromoethyl)-6-methyl-2-morpholino-4H-benzo[e][1,3]oxazin-4-one **112** with 2,6-difluoroaniline according to General Procedure A. The product was obtained as a white coloured solid after purification by flash chromatography (105 mg, 46%).

MP 155 °C ν_{max} (ATR) /cm^{-1} 3325 (NH), 2956 w, 2929 w, 2862 w (alkane),1669 m (C=O), 1620 m (C=N), 1559 s (C=C); **^{1}H NMR** ($CDCl_3$, 300 K) δ 7.81 (d, 1H, *J*=1 Hz, H-5), 7.37 (d, 1H, *J*=1.5 Hz, H-7), 6.75 (dt, 2H, *J*=1 Hz (C-H), *J*=8 Hz (C-F), H-13, H-15), 6.66 (m, 1H, *J*=1.5 Hz (C-H), *J*=6 Hz (C-F), H-14), 5.24 (p, 1H, *J*=7 Hz, CHNH), 3.95 (s, 1H, NH), 3.80 to 3.91 (bm, 8H, morpholine) 2.36 (s, 3H, CH_3), 1.58 (d, 3H, *J*=6.5 Hz, CH_3-CH); **^{13}C NMR** ($CDCl_3$, 300 K) δ 167.1 (C-4), 156.6 (C-2), 154.5 (C-12), 152.5 (C-16), 149.0 (C-8a), 135.4 (C-7), 131.5 (C-8), 131.2 (C-6), 126.5 (C-5), 124.4 (C-14), 118.7 (C-11), 117.1 (C-4a), 111.6 (C-13) 111.5 (C-15), 49.1 (C-9) 66.3 (C-3'), 44.2 (C-2'), 22.8 (CH_3CH), 21.0 (CH_3). HRMS (ESI): *m/z* calculated for $C_{21}H_{21}F_2N_3O_3$: 402.1624 $[M+H]^+$, found: 402.1638.

6-methyl-2-morpholino-8-(1-(o-tolyloxy)ethyl)-4H-benzo[e][1,3]oxazin-4-one (LTUEM26) 114h

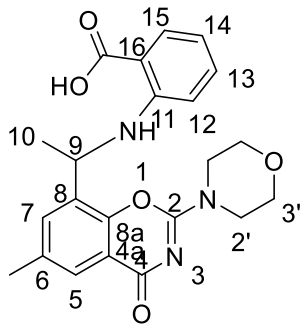


The compound was synthesized by reacting 8-(1-bromoethyl)-6-methyl-2-morpholino-4H-benzo[e][1,3]oxazin-4-one **112** with 2-methylphenol according to General Procedure A. The crude product was

subjected to flash chromatography, obtained as an oil, and then precipitated as white coloured solid in minimum diethyl ether (95 mg, 44%).

MP 132 °C ν_{max} (ATR) /cm^{-1} 2981 w, 2934 w, 2866 w (alkane C-C),1671 m (C=O), 1623 m (C=N), 1558 s (C=C); **^{1}H NMR** ($CDCl_3$, 300 K) δ 7.86 (d, 1H, *J*=1, H-5), 7.50 (d, 1H, *J*=1.5 Hz, H-7), 7.15 (d, 1H, *J*=7.5 Hz, H-12), 7.03 (t, 1H, *J*=7.5 Hz, H-13), 6.85 (t, 1H, *J*=7 Hz, H-14), 6.61 (d, 1H, *J*=8.5 Hz, H-15), 5.57 (q, 1H, *J*=6.5 Hz, CH-O), 3.67 to 3.92 (bm, 8H, morpholine) 2.38 (s, 3H, CH_3), 2.28 (s, 3H, CH_3), 1.71 (d, 3H, *J*=6.5 Hz, CH_3-CH); **^{13}C NMR** ($CDCl_3$, 300 K) δ 167.0 (C-4), 156.5 (C-11), 155.9 (C-2), 148.5 (C-8a), 135.7 (C-7), 131.9 (C-6), 131.0 (C-15), 129.6 (C-5), 127.3 (C-8), 127.0 (C-16), 126.7 (C-13), 121.0 (C-4a), 117.1 (C-14), 112.3 (C-12), 70.6 (C-9) 66.2 (C-3'), 44.7 (C-2'), 22.2 (CH_3), 21.0 (CH_3CH), 16.4 (phenolic CH_3). HRMS (ESI): *m/z* calculated for $C_{22}H_{24}N_2O_4$: 381.1809 $[M+H]^+$, found: 381.1826.

2-((1-(6-methyl-2-morpholino-4-oxo-4H-benzo[e][1,3]oxazin-8-yl)ethyl)amino)benzoic acid (LTUEM27) 113l



8-hydroxyethyl-6-methyl-2-morpholino-4H-benzo[e][1,3]oxazin-4-one **111** (200 mg, 0.69 mmol) was dissolved in 4 mL of DCM. Phosphorus tribromide (0.75 mL, 0.75 mmol) was added to the above solution and stirred at 40 °C 2.5 hours. Then 2-aminobenzoic acid (113 mg, 0.82 mmol) and triethylamine (272 mg, 2.7 mmol) were added and the mixture was stirred at 40 °C overnight. 2 mL RO water was added stirred for 5 minutes and separated, and the organic phase concentrated to 1 mL and stirred at r.t. Then 3.5 mL acetone was added

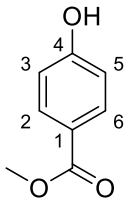
followed by careful addition of 4M HCl (0.8 mL). This was stirred overnight and solid was filtered and purified by flash chromatography. (15 mg, 5%)

MP 190-192 °C ν_{max} (ATR) /cm^{-1} 3176 bw (NH), 2964 m, 2923 m, 2858 m (alkane C-C), 1722 m (C=O), 1663 s (C=O), 1590 m (C=N), 1563 m (C=C); **^{1}H NMR** (d_6-DMSO, 340 K) δ 8.16 (dd, 1H, *J*=1 Hz, *J*=8 Hz, H-15), 7.86 (dt, 1H, *J*=1.5 Hz, *J*=7 Hz, H-13), 7.68 (d, 1H, *J*=8 Hz, H-12), 7.63 (d, 1H, *J*=2 Hz, H-5), 7.56 (dt, 1H, *J*=1 Hz, *J*=7, H-14), 7.55 (s, 1H, H-7), 5.46 (q, 1H, *J*=6.5 Hz, CHNH), 3.34 to 3.60 (bm, 8H, morpholine) 2.41 (s, 3H, CH_3), 1.82 (d, 3H, *J*=7 Hz, CH_3-CH); **^{13}C NMR** (d_6-DMSO, 340 K) δ 162.2 (C-16'), 152.2 (C-4), 151.7 (C-2), 148.9 (C-8a), 143.9 (C-11), 135.8 (C-7), 135.5 (C-13), 134.9 (C-15), 131.3 (C-6), 130.3 (C-8), 127.9 (C-5), 127.7 (C-4a), 127.2 (C-14), 126.3 (C-12), 121.3 (C-16) 66.09 (C-3'), 53.2 (C-9), 44.6 (C-2'), 25.6 (CH_3CH), 20.8 (CH_3).

8.2 Attempted synthesis of 6-methoxycarbonyl-2-morpholino-8-(1-(arylamino or aryloxo)ethyl)-4H-benzo[e][1,3]oxazin-4-ones

8.2.1 Synthesis of 2-hydroxy-5-(methoxycarbonyl)benzoic acid

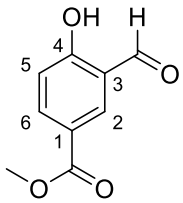
Methyl 4-hydroxybenzoate 120



4-hydroxybenzoic acid **118** (10 g, 72.4 mmol) was dissolved in 130 mL of methanol and concentrated sulphuric acid (1.9 mL, 36.2 mmol) was added. The reaction mixture was heated to reflux for 4 hours while stirring. The solvent was evaporated, diluted with water, extracted with ethyl acetate, washed with brine, dried over magnesium sulphate, and evaporated to dryness to collect the product as white solid (10 g, 91%)

MP 120 °C. **ν_{max}** (ATR) /cm^{-1} 3287 m (O-H), 1676 s (C=O). **^{1}H NMR** ($CDCl_3$, 300 K) δ 7.93 (d, 2H, $J_{H2,H6}$=8.7 Hz, H-2, H-6), 6.84 (d, 2H, $J_{H3,H5}$=8.7 Hz, H-3, H-5), 3.87 (s, 3H, CH_3); **^{13}C NMR** ($CDCl_3$, 300 K) δ 167.3 (C=O), 160.1 (C-OH), 131.9 (C-2,C-6), 122.4 (C-1), 115.2 (C-3, C-5), 52.0 (CH_3).

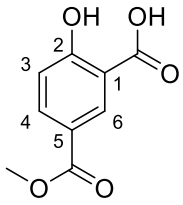
Methyl-3-formyl-4-hydroxybenzoate 123



Methyl 4-hydroxybenzoate **120** (6.08 g, 40 mmol) was dissolved in dry dichloromethane (200 mL) and anhydrous magnesium chloride (18.8 g, 200 mmol) and triethylamine (33 mL, 240 mmol) were added. The mixture was heated to 40 ºC while stirring for 1 hour. Then paraformaldehyde (12 g, 400 mmol) was added and heated to 70 ºC while stirring for overnight. The reaction mixture was cooled to 0 ºC and HCl (1M, 200 mL) was added and stirred for 1 hour. The mixture was filtered and washed with dichloromethane (100 mL). The organic layer was separated and washed with HCl (1M, 100 mL) and then with brine (100 mL). The extract was dried over magnesium sulphate and evaporated to dryness to give the crude product (5.8 g, 80.5%).

MP 80-81 °C. **ν_{max}** (ATR) /cm^{-1} 3096 m (O-H), 2959 (CH_3), 2867 (C-H), 1711 (C=O), 1646 (C=O) **^{1}H NMR** ($CDCl_3$, 300 K) δ 11.3 (s, 1H, OH), 9.9 (s, 1H, CHO), 8.3 (d, 1H, *J*=2.1 Hz, H-2), 8.17 (dd, 1H, $J_{H2,H6}$=2.1 Hz, $J_{H5,H6}$=9 Hz, H-6), 7.02 (d, 1H, $J_{H6,H5}$=8.7 Hz, H-5), 3.9 (s, 3H, CH_3); **^{13}C NMR** ($CDCl_3$, 300 K) δ 196 (CHO), 165.1 (C=O), 164.6 (C-OH), 137.4 (C-6), 135.7 (C-2), 121.8 (C-3), 119.6 (C-1), 117.5 (C-5), 51.8 (CH_3).

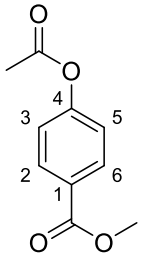
2-hydroxy-5-(methoxycarbonyl)benzoic acid 115



The aldehyde methyl 3-formyl-4-hydroxybenzoate **123** (5.8 g, 32.2 mmol) was reacted with sodium dihydrogen phosphate (9.8 g, 81.9 mmol) and sodium chlorite (7.1 g, 78.5 mmol) according to a previously reported procedure[126] but the reaction was prolonged to overnight to increase the yield. After the reaction completed, saturated sodium carbonate solution (33 mL) was added and stirred well and filtered. Both the filtrate and residue (dissolved in water) were acidified to pH 1 using HCl. The precipitate formed was filtered, washed with water, and dried to give the product as off-white solid (4.2 g, 66%).

MP 175-185 °C. **ν_{max}** (ATR) /cm^{-1} 3066 m (O-H), 2965 (CH_3), 1723 (C=O), 1662 (C=O) **^{1}H NMR** (d_6-DMSO, 340 K) δ 8.36 (d, 1H, *J*=2.4 Hz, H-6), 7.96 (dd, 1H, $J_{H4,H6}$=2.4 Hz, $J_{H3,H4}$=8.7 Hz, H-4), 6.96 (d, 1H, $J_{H4,H3}$=8.7 Hz, H-3), 3.8 (s, 3H, OCH_3); **^{13}C NMR** (d_6-DMSO, 340 K) δ 170.9 (COOH), 165.4 (C=O), 165.1 (C-OH), 135.2 (C-4), 132.2 (C-6), 120.4 (C-5), 117.6 (C-3), 114.2 (C-1), 51.8 (CH_3).

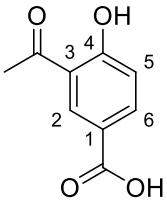
Methyl 4-acetoxybenzoate 121



Methyl 4-hydroxybenzoate **120** (1.52 g, 10 mmol) was dissolved in acetic anhydride (0.96 mL). Upon addition of 1 drop of concentrated sulfuric acid, the mixture was heated to 110 ºC while stirring for 1 h. The mixture was poured into water (10 mL) and extracted with diethyl ether. The organic layer was washed with a saturated NaHCO3 solution, dried over magnesium sulphate, and evaporated under reduced pressure to obtain the product as oil (1.8 g, 92.7 %).

MP 120-125 °C. **ν_{max}** (ATR) /cm^{-1} 2953 (CH_3), 1761 (C=O), 1718 s (C=O). **^{1}H NMR** ($CDCl_3$, 300 K) δ 8.05 (d, 2H, $J_{H2,H6}$=8.7 Hz, H-2, H-6), 7.15 (d, 2H, $J_{H3,H5}$=8.7 Hz, H-3, H-5), 3.89 (s, 3H, OCH_3), 2.30 (s, 3H, CH_3CO) ; **^{13}C NMR** ($CDCl_3$, 300 K) δ 168.2 (C=O ketone), 165.6 (C=O ester), 153.5 (C-4), 130.4 (C-2,C-6), 127.0 (C-1), 120.9 (C-3, C-5), 51.5 (OCH_3). 20.4 (CH_3CO).

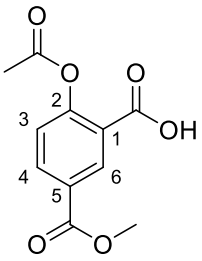
3-acetyl-4-hydroxybenzoic acid 122



The oil methyl 4-acetoxybenzoate **121** (1.8 g, 9.2 mmol) was thoroughly mixed with aluminium chloride (1.71 g, 12.8 mmol) and heated with stirring at 140 °C for 2 hours. The mixture was then poured into water (10 mL), acidified, filtered, and dried to afford methyl 3-acetyl-4-hydroxybenzoic acid **122** (1.5 g, 89% crude).

MP 130-135 °C. **ν_{max}** (ATR) /cm^{-1} 2811 m br, (O-H), 1676 (C=O), 1642 (C=O) **^{1}H NMR** (d_6-DMSO, 340 K) δ 8.34 (d, 1H, *J*=2.1 Hz, H-2), 8.0 (dd, 1H, $J_{H2,H6}$=2.1 Hz, $J_{H5,H6}$=8.7 Hz, H-6), 7.01 (d, 1H, $J_{H5,H6}$=8.7 Hz, H-5), 2.63 (s, 3H, CH_3); **^{13}C NMR** (d_6-DMSO, 340 K) δ 203.1 (C=O ketone), 166.0 (C=O carboxylic), 163.5 (C-4), 136.0 (C-6), 132.6 (C-2), 121.8 (C-3), 120.7 (C-1), 117.6 (C-5), 27.6 (CH_3).

2-acetoxy-5-(methoxycarbonyl)benzoic acid 124

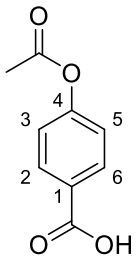


The reaction was carried out according to a previously reported procedure in which the compound 2-hydroxy-5-(methoxycarbonyl)benzoic acid **115** (196 mg, 1 mmol) was reacted with acetic anhydride (0.288 mL, 3 mmol) with a drop of concentrated

sulphuric acid, heated to 110 °C while stirring for one hour. The mixture was quenched with RO water (10 mL), extracted with ethyl acetate, dried over magnesium sulphate, and evaporated to the product (220 mg, 92%).

MP 140 °C. ν_{max} (ATR) /cm^{-1} 2958 m (CH_3), 1764 (C=O carbonyl), 1725 (C=O), 1681 (C=O carboxyl) **^{1}H NMR** ($CDCl_3$, 300 K) δ 8.76 (d, 1H, *J*=2.1 Hz, H-6), 8.25 (dd, 1H, $J_{H4,H6}$=2.1 Hz, $J_{H3,H4}$=8.7 Hz, H-4), 7.2 (d, 1H, $J_{H4,H3}$=8.7 Hz, H-3), 3.93 (s, 3H, OCH_3), 2.34 (s, 3H, CH_3CO).

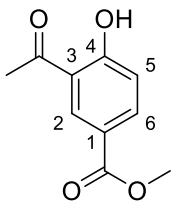
4-acetoxybenzoic acid 127



4-hydroxybenzoic acid **118** (2 g, 14.4 mmol) was reacted with acetic anhydride (8.1 mL, 86 mmol) with addition of a drop of concentrated sulphuric acid. The mixture was stirred and heated to reflux at 130 °C for 4 hours and then cooled to room temperature. RO water (5 mL) was added and the precipitate was filtered, washed with water and dried to give the product (2.44 g, 93.8%).

MP 115-118 °C. ν_{max} (ATR) /cm^{-1} 2550-3066 m br (OH), 1751 (C=O carbonyl), 1677 (C=O carboxyl) **^{1}H NMR** ($CDCl_3$, 300 K) δ 8.13 (d, 2H, *J*=8.4 Hz, H-2, H-6), 7.2 (d, 2H, *J*=8.7 Hz, H-3, H-5), 2.31 (s, 3H, CH_3CO).

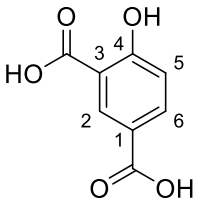
Methyl 3-acetyl-4-hydroxybenzoate 128



3-acetyl-4-hydroxybenzoic acid **122** (1.8 g, 10 mmol) was dissolved in methanol (20 mL) in the presence of thionyl chloride (1.08 mL) and heated to reflux for 3 hours. The solvent was evaporated, residue was suspended in water, filtered and washed aqueous sodium bicarbonate solution to give the product as white solid (1.8 g, 92.7%).

MP 145-148 °C. **ν_{max}** (ATR) /cm^{-1} 2958 (CH_3), 1715 s (C=O), 1637 s (C=O). **^{1}H NMR** ($CDCl_3$, 300 K) δ 8.45 (s, 1H, H-2), 8.1 (d, 1H, $J_{H5,H6}$=8.1 Hz, H-6), 6.98 (d, 1H, $J_{H5,H6}$=8.1 Hz, H-5), 3.89 (s, 3H, OCH_3), 2.67 (s, 3H, CH_3CO).

4-hydroxyisophthalic acid 119

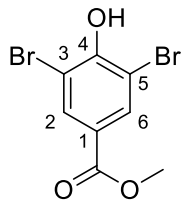


2-hydroxy-5-(methoxycarbonyl)benzoic acid **115** (196 mg, 1 mmol) was dissolved in water-methanol (10 mL) solvent system and was hydrolysed using sodium hydroxide (135 mg, 3.37 mmol) under reflux for 2 hours. The mixture was neutralized to pH 6 using hydrochloric acid, the precipitate filtered and dried to give the product as white solid (149 mg, 82%).

MP 310-315 °C (decomp.). **ν_{max}** (ATR) /cm^{-1} 2554-2932 m (O-H), 1674 (C=O), 1591 (C=C), **^{1}H NMR** (d_6-DMSO, 340 K) δ 8.34 (d, 1H, *J*=2.4 Hz, H-2), 7.84 (dd, 1H, $J_{H2,H6}$=2.4 Hz, $J_{H5,H6}$=8.7 Hz, H-6), 6.79 (d, 1H, $J_{H4,H3}$=8.7 Hz, H-5). **^{13}C NMR** (d_6-DMSO, 340 K) δ 171.5 (COOH), 167.4 (COOH), 167 (C-OH), 135 (C-6), 132.7 (C-2), 120 (C-1), 117.4 (C-5), 116.7 (C-3). HRMS (ESI): *m/z* calculated for $C_8H_6O_5$: 181.0142 [M-H]$^-$, found: 181.015.

8.2.2 Synthesis of methyl 8-bromo-2-morpholino-4-oxo-4H-benzo[e][1,3]oxazine-6-carboxylate

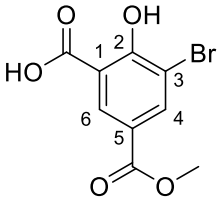
Methyl 3,5-dibromo-4-hydroxybenzoate 132



2-hydroxy-5-(methoxycarbonyl)benzoic acid **115** (196 mg, 1 mmol) was dissolved in RO water (0.4 mL) and liquid bromine (176 mg, 1.1 mmol) was added slowly. The mixture was stirred at room temperature overnight, filtered, washed with RO water and dried to give the product (278 mg, 90%)

MP 188-190 °C (decomp.). **ν_{max}** (ATR) /cm^{-1} 2851, 2956, 3026, 3084 m, 1704 (C=O), **^{1}H NMR** (d_6-DMSO, 300 K) δ 8.03 (s, 2H, H-2, H-6), 3.82 (s, 3H, OCH_3); **^{13}C NMR** (d_6-DMSO, 340 K) δ 164.3 (C=O), 155.6 (C-4), 133.6 (C-2, C-6), 123.8 (C-1), 111.8 (C-3, C-5), 52.8 (OCH_3).

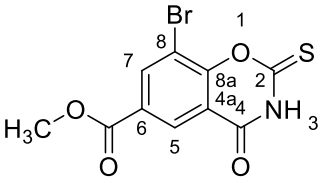
3-bromo-2-hydroxy-5-(methoxycarbonyl)benzoic acid 131



2-hydroxy-5-(methoxycarbonyl)benzoic acid **115** (196 mg, 1 mmol) was dissolved in N,N-dimethylformamide (2.5 mL). N-bromosuccinimide (196 mg, 1.1 mmol) was then added and the mixture was heated to 35 °C with stirring for 45 minutes under dark conditions. The reaction mixture was cooled to room temperature and RO water (2 mL) was added and stirred for 30 minutes. The precipitate was filtered and dried to give crude product which was recrystallized using petroleum spirits- ethyl acetate. (186 mg, 67%).

MP 200-220 °C (decomp.). **ν_{max}** (ATR) /cm^{-1} 3152 m (OH), 2960 (CH_3), 1694 (C=O), 1672 (C=O), **^{1}H NMR** (d_6-DMSO, 300 K) δ 8.34 (d, 1H, *J*=2 Hz, H-4) 8.23 (d, 1H, *J*=1.6 Hz, H-6), 3.84 (s, 3H, OCH_3); **^{13}C NMR** (d_6-DMSO, 300 K) δ 171.3 (C=O carboxylic), 164.7 (C-2), 162.4 (C=O, carbonyl), 138.5 (C-4), 131.4 (C-6), 121.4 (C-5), 115.1 (C-1), 111.2 (C-3), 52.7 (OCH_3).

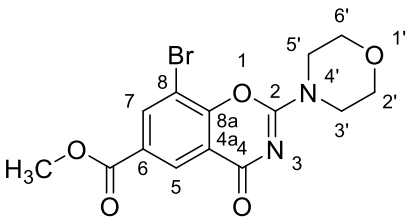
Methyl 8-bromo-4-oxo-2-thioxo-3,4-dihydro-2H-benzo[e][1,3]oxazine-6-carboxylate 133



A suspension of 3-bromo-2-hydroxy-5-(methoxycarbonyl)benzoic acid **131** (550 mg, 2 mmol) in dichloromethane (20 mL) was added to a reaction mixture containing freshly prepared $Pb(SCN)_2$ according to the previously reported procedure. The reaction mixture was filtered and $PbBr_2$ filter cake was subjected to hot filtration using acetone (≈ 100 mL) to extract the product. Both the DCM and acetone filtrates were separately evaporated to dryness under reduced pressure and minimal toluene was added to triturate the resulting solid. The solids that precipitated out of the filtrates were collected by filtration and combined to give 0.47 g of the crude compound (74%).

MP 230-240 °C (decomp.). **ν_{max}** (ATR) /cm^{-1} 3159 m (OH), 2955 (CH_3), 1730 (C=O), 1701 (C=O), **^{1}H NMR** (d_6-DMSO, 300 K) δ 8.46 (d, 1H, *J*=1.6 Hz, H-7) 8.32 (d, 1H, *J*=1.6 Hz, H-5), 3.9 (s, 3H, OCH_3); **^{13}C NMR** (d_6-DMSO, 300 K) δ 181.2 (C=S, C-2) 164.1 (C=O, carbonyl), 157.1 (C=O, C-4), 155.5 (C-8a), 139.1 (C-7), 128.1 (C-5), 127.2 (C-6), 118.2 (C-4a), 109.7 (C-8), 53.3 (OCH_3).

Methyl 8-bromo-2-morpholino-4-oxo-4H-benzo[e][1,3]oxazine-6-carboxylate 134

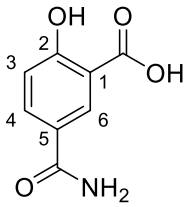


Sodium hydrogen carbonate (0.87 g, 10.3 mmol) was suspended in a 50 mL beaker containing 20 mL of 1:1 RO water and 2- propanol. The compound **133** (0.55 g, 1.74 mmol) was added, the reaction mixture was warmed on a hotplate to 60 °C and then removed from heat and allowed to cool to room temperature with stirring. Iodomethane (0.43 mL, 6.88 mmol) was added drop-wise and allowed to stir at room temperature for 30 min or until a thick precipitation had formed. Morpholine (0.87 mL, 9.98 mmol) was added and the reaction mixture was stirred for overnight. The solvent was evaporated. At the end from the reaction mixture the product was extracted by dichloromethane and dried over $MgSO_4$ and was evaporated under reduced pressure to obtain the crude solid product (0.47 g, 73%).

MP 220-222 °C; ν_{max} (ATR) /cm^{-1} 2988 w, 2956 w, 2866 w (C-C), 1730 m (C=O), 1605 m (C=N), 1561 s (C=C); **^{1}H NMR** (d_6-DMSO, 300 K) δ 8.39 (s, 1H, H-7), 8.35 (s, 1H, H-5), 3.9 (s, 3H, OCH_3), 3.74 to 3.78 (bm, 8H, morpholine); **^{13}C NMR** (d_6-DMSO, 300 K) δ 164.55 (C-4), 164.52 (C=O, carbonyl), 156.2 (C-8a), 153.7 (C-2), 137.2 (C-7), 127.9 (C-5), 127.5 (C-4a), 119 (C-6), 109.9 (C-8), 65.7 (C-2', 6'), 53.2 (OCH_3), 45.0, 44.8 (C-3', 5').

8.3 Attempted synthesis of 6-(carbamoyl or dimethylcarbamoyl)--2-morpholino-8-(1-(arylamino or aryloxo)ethyl)-4H-benzo[e][1,3]oxazin-4-ones

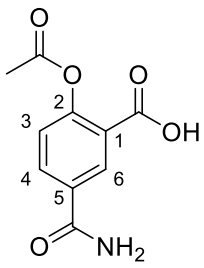
5-carbamoyl-2-hydroxybenzoic acid 138



2-hydroxy-5-(methoxycarbonyl)benzoic acid **115** (196 mg, 1 mmol) was reacted with 25% aqueous ammonia solution (4.48 mL, 60 mmol) by stirring the mixture for 2 days at room temperature, according to a modified procedure.[133] The reaction mixture was acidified with HCl and a precipitate was formed. It was purified by dissolving in sodium bicarbonate solution and reprecipitated by HCl. The pure product was filtered and dried (150 mg, 82%).

MP 286-288 °C, lit 292-296; ν_{max} (ATR) /cm^{-1} 3434 m (NH), 3209 (OH), 1665 (C=O); **^{1}H NMR** (d$_6$-DMSO, 300 K) δ 8.37 (d, 1H, *J*=2.4 Hz, H-6), 8.02 (dd, 1H, *J*=2.4 Hz, *J*=6.4 Hz, H-4), 7.0 (d, 1H, *J*=4.4, Hz, H-3); **^{13}C NMR** (d$_6$-DMSO, 300 K) δ 172 (COOH), 167.1 (C=O amide), 163.7 (C-2), 135 (C-4), 130.8 (C-6), 125.7 (C-5), 117.3 (C-3), 113.2 (C-1).

2-acetoxy-5-carbamoylbenzoic acid 139

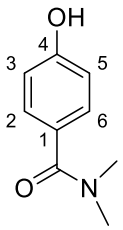


5-carbamoyl-2-hydroxybenzoic acid **138** (980 mg, 5.4 mmol) was mixed with acetic anhydride (1.56 mL, 16.2 mmol) and a drop of concentrated sulphuric acid was added. The reaction mixture was refluxed at 110°C and followed by TLC. After the reaction was

complete, water was added and stirred well. The product was extracted by ethyl acetate, solvent evaporated and dried.

MP 270-275 °C (decomp.); ν_{max} (ATR) /cm^{-1} 3236 m (NH), 1769 (C=O), 1697 (C=O); **^{1}H NMR** (d_6-DMSO, 300 K) δ 8.38 (d, 1H, *J*=2.4 Hz, H-6), 8.03 (dd, 1H, *J*=2.4 Hz, *J*=8.8 Hz, H-4), 7.03 (d, 1H, *J*=8.8, Hz, H-3); **^{13}C NMR** (d_6-DMSO, 300 K) δ 172.4 (COOH), 171.6 (C=O acetyl), 166.7 (C=O amide), 164.8 (C-2), 136.6 (C-4), 132.7 (C-5), 122.1 (C-6), 117.9 (C-1), 113.4 (C-3), 21.4 (CH_3).

4-hydroxy-N,N-dimethylbenzamide 142

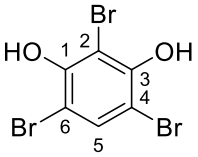


4-hydroxybenzoic acid **118** (276 mg, 2 mmol) was mixed with thionyl chloride (1.5 mL, 20 mmol) and heated to reflux at 85 °C for 1 hour. thionyl chloride was then evaporated leaving behind a white residue, to which dimethylamine solution (8.5 mL, 2M in THF) was added and then heated to reflux at 85 °C for 18 hours, a modification to a previously reported procedure.[135] The reaction mixture was cooled to 0 °C and neutralized with aqueous HCl. Then it was extracted by ethyl acetate, dried with magnesium sulphate and the extract was evaporated to dryness to the compound in moderate yield. (200 mg, 60%).

MP 150-155 °C; ν_{max} (ATR) /cm^{-1} 3014 (OH), 1605 (C=O); **^{1}H NMR** (d_6-DMSO, 300 K) δ 9.86 (s, 1H, OH), 7.31 (d, 2H, *J*=0.8 Hz, H-2, H-6), 6.83 (d, 2H, *J*=0.8, Hz, H-3, H-5), 2.99 (s, 6H, $N(CH_3)_2$); **^{13}C NMR** (d_6-DMSO, 300 K) δ 170.7 (C=O amide), 158.9 (C-4), 129.6 (C-2, C-6), 127.1 (C-1), 115.1 (C-3, C-5), 39.8 ($N(CH_3)_2$).

8.4 Attempted synthesis of 10-aryl-6-methyl-2-morpholino-4H,8H-chromeno[6,7-e][1,3]oxazine-4,8-diones

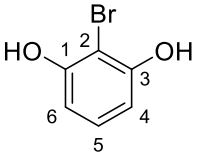
2,4,6-tribromobenzene-1,3-diol 145



Resorcinol **144** (6 gm, 54.5 mmol) was dissolved in chloroform (54 mL) and bromine (27 gm, 8.7 mL, 168 mmol) in 30 mL chloroform was slowly added to it according to a literature procedure.[114] It was refluxed overnight, and the solvent was evaporated to give the solid product (18.7 g, 99%).

MP 112-115 °C; ν_{max} (ATR) /cm^{-1} 3466 bs (O-H), 3079 m. **^{1}H NMR** ($CDCl_3$, 300 K) δ 7.58 (s, 1H, H-5), 5.91 (s, 2H, OH); **^{13}C NMR** ($CDCl_3$, 300 K) δ 149.4 (C-1 & C-3), 132.6 (C-5), 100.0 (C-4 & C-6), 97.9 (C-2).

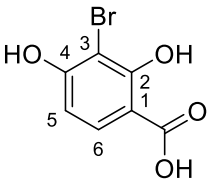
2-bromobenzene-1,3-diol 146



2,4,6-tribromobenzene-1,3-diol **145** (18.9 g, 44.81 mmol) was added to a solution of Na_2SO_3 (13.7 g, 89.62 mmol) and NaOH (4.35 g, 89.62 mmol) in 137 mL water and 27.5 ml methanol following a literature procedure.[136] After 20 minutes, the reaction mixture was acidified with HCl and concentrated to half its volume. Then the solution was extracted with ether (3x20 ml). Ether fraction was dried with $MgSO_4$, filtered, and evaporated *in vacuo* to give the product as solid crystals (10.2 g, 99%).

MP 96-98 °C; ν_{max} (ATR) /cm^{-1} 3316 bm (O-H), 1585, 1462. **^{1}H NMR** ($CDCl_3$, 300 K) δ 7.09 (t, 1H, *J* =8.4 Hz, H-5), 6.59 (d, 2H, *J*=8.4 Hz, H-4 & H-6), 5.35 (s, 2H, OH); **^{13}C NMR** ($CDCl_3$, 300 K) δ 152.6 (C-1 & C-3), 128.7 (C-5), 107.7 (C-4 & C-6), 99.0 (C-2).

3-bromo-2,4-dihydroxybenzoic acid 94



2-bromobenzene-1,3-diol **146** (10 g, 52.91 mmol) was added to a solution of $NaHCO_3$ (13.23 g, 132.27 mmol) in 40 ml water. The reaction was carried out at 90^{o}C for 90 minutes with CO_2 flow. After 90 minutes, the reaction mixture was transferred to a beaker and 150 ml water was added, acidified with HCl, and kept in fridge. A dark coloured solid was precipitated which was filtered and dried in vacuum oven (4.20 g, 34.24%).

MP 180-182^{o}C. ν_{max} (ATR) /cm^{-1} 3454 w (O-H), 1643 m (C=O), 1608 s (C=C). **^{1}H NMR** (d_6-DMSO, 300 K) δ 7.61 (d, 1H, *J* =8.7 Hz, H-6), 6.51 (d, 1H, *J* =8.7 Hz, H-5); **^{13}C NMR** (d_6-DMSO, 300 K) δ 171.8 (COOH), 160.7 (C-2), 160.3 (C-4), 130.1 (C-6), 107.6 (C-5), 105.6 (C-1), 97.4 (C-3).

8.5 DNA PK assay

All the biological assays (DNA-PK, PDE3A and PI3K inhibition) were performed by and at Reaction Biology Corporation, One Great Valley Parkway, Suite 2 Malvern, PA 19355 USA. All compounds were in powder form which were then resuspended in DMSO to make a 10 mM stock solution and evaluated for their human DNA-PK inhibition. The compounds were tested in a single dose duplicate mode at a concentration

of 10 μM. The control compound, PI-103(3-(4-morpholin-4-ylpyrido[2,3]furo[2,4-b]pyrimidin-2-yl)phenol) was used as positive control and DMSO as a negative control. Control compound was tested in 10-dose IC_{50} mode with 3-fold serial dilution starting at 20 μM. Reactions were carried out at 10 μM ATP.

8.6 PDE3A assay

The compounds were dissolved in DMSO to make a 10 mM stock solution. Compounds were tested in a single dose duplicate at a concentration of 10 uM. The control compound IBMX (3-Isobutyl-1-methylxanthine) was tested in a 10-dose IC_{50} with 3-fold serial dilution starting at 100 uM. The reaction was carried out at room temperature for one hour. The reaction product, AMP, was detected by Transcreener, a Fluorescence polarization assay.

8.7 PI3K inhibition assay

The compounds were resuspended to 10 mM stock in DMSO. PI3K enzyme activity was determined using a bioluminescence assay (ADP-Glo Kinase assay) measuring ATP consumption.[152] The compounds were tested in single dose duplicate mode at 10 μM. The control compound, PI-103, was tested in 10-dose IC_{50} with 3-fold serial dilution starting at 1 μM. Reactions were carried out at 10 μM ATP. Curve fits were performed where the enzyme activities at the highest concentration of compounds were less than 65%.

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