# Synthesis and Biological Evaluation of Novel 2-benzoylbenzofurans as Potential Anticancer Agents

A THESIS

SUBMITTED TO THE FACULTY OF THE UNIVERSITY OF MINNESOTA

BY

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Dr. Venkatram Mereddy

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## Abstract

The heterocyclic benzofuran moiety is a privileged chemical entity that has been utilized in the development of novel pharmaceuticals. Benzofurans have been studied for therapeutic uses in cancer, cardiovascular diseases, antimicrobial, psychotic disorders, renal disorders and inflammation. Another privileged structure in drug development is the piperazine unit. The addition of this structure has been shown to greatly improve water solubility making this template well utilized in many clinically successful drugs. Our group's interest in the development of anticancer agents has pushed us to discover the therapeutic potential of piperazine bearing benzofurans. A recent report involving the synthesis and evaluation of an piperazino *N*,*N*-diethyl benzofuran has peaked our interest due to the simplicity of synthesis and good cytotoxicity properties. In this regard, we sought to synthesis and evaluate a series of piperazine substituted 2-benzoylbenzofurans. Through a two-step Rap-Stoermer condensation between salicylaldehydes and phenacyl bromide prior to ipso substitution with piperazine, numerous candidate compounds have been synthesized. These compounds have been evaluated via MTT cell proliferation assay for their cytotoxic properties on six cancer cell lines: 4T1, 67NR, MIA PaCa-2, MCF7, MDA-MB-231, and WiDr. Compounds **2.36** and **2.40** were found to be the most potent with $IC_{50}$ values ranging from ~2-4 μM and ~2-8 μM across all cell lines. The synthesis and *in vitro* evaluation studies are presented in this thesis.

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## List of abbreviations

| | |
|---|---|
| FDA | Food and Drug Administration |
| SSRI | Selective serotonin reuptake inhibitor |
| μM | microMolar, 10-6 moles/L |
| $IC_{50}$ | inhibitory concentration where 50% of biological response is inhibited |
| HIF-1α | hypoxia inducible factor 1-alpha |
| nM | nanoMolar, 10-9 moles/L |
| HDAC | histone deacetylase |
| HDIs | histone deacetylase inhibitors |
| $K_2CO_3$ | potassium carbonate |
| NaOH | sodium hydroxide |
| °C | degrees Celcius |
| BOP | benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate |
| $CH_3CN$ | acetonitrile |
| $NEt_3$ | triethylamine |
| Δ | heat |
| $CF_3COOH$ | trifluoroacetic acide |
| $H_2O$ | water |
| HCl | hydrochloric acid |
| MeOH | methanol |
| CYP19 | aromatase, estrogen synthetase, a member of the cytochrome P450 superfamily |
| DMF | dimethylformamide |
| Mg | magnesium |
| THF | tetrahydrofuran |
| NaH | sodium hydride |
| NaOMe | sodium methoxide |
| $I_2$ | iodine |
| MTT | 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide |
| W | Watts |
| MW | microwave |
| mTOR | mammalian target of rapamycin |
| mTORC1 | mammalian target of rapamycin complex 1 |
| $NaBH_3CN$ | sodium cyanoborohydride |
| TMSCl | trimethylsilyl chloride |
| $BBr_3$ | boron tribromide |
| DCM | dichloromethane |

| | |
|---|---|
| $CH_2Cl_2$ | dichloromethane |
| HCHO | formaldehyde |
| EtOH | ethanol |
| $NaBH_4$ | sodium borohydride |
| MsCl | mesyl chloride |
| MeCN | acetonitrile |
| EtOAC | ethyl acetate |
| NaOAc | sodium acetate |
| AcOH | acetic acid |
| MeI | methyl iodide |
| $ZnCl_2$ | Zinc chloride |
| $AgNO_3$ | silver nitrate |
| $PdCl_2(PPh_3)_2$ | Palladium(II)bis(triphenylphosphine) dichloride |
| CuI | copper(I) iodide |
| Mnk | MAPK interacting kinase, Mitogen-activated protein kinase (MAPK) interacting kinases |
| HATU | 1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate |
| DIEA | Ethyldiisopropylamine, 'Hünig's base' |
| DMSO | dimethyl sulfoxide |
| $(CH_3)_2SO_4$ | dimethyl sulfate |
| CSI | chlorosulfonyl isocyanate |
| 1 N HCl | 1 normal HCl, molar concentration/equivalence factor |
| $CH_3OH$ | methanol |
| $H_2NNH_2$ | hydrazine |
| BOP-Cl | Phosphoric acid bis(2-oxooxazolidide) chloride |
| TvL | Trametes versicolor laccase |
| HPLC | high pressure liquid chromatography |
| HIF-1 pathway | hypoxia inducible factor 1 pathway |
| EDC | N-Ethyl-N′-(3-dimethylaminopropyl)carbodiimide hydrochloride |
| HOBt | hydroxybenzotriazole |
| MEK1 | mitogen-activated protein kinase kinase 1 |
| $TiCl_4$ | Titanium tetrachloride |
| DDQ | 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone |
| $NaH_2PO_4$ | monosodium phosphate |
| $NaClO_2$ | sodium chlorite |
| tBuOH | tert-butanol |
| $LiNH_2$ | lithium amide |
| $^{i}Pr_2NEt$ | N-Ethyl-N-(propan-2-yl)propan-2-amine |

| | |
|---|---|
| TFAA | trifluoroacetic anhydride |
| $Ac_2O$ | acetic anhydride |
| $AlCl_3$ | aluminum trichloride |
| AcCl | Acetyl chloride |
| MOM-Cl | Methyl chloromethyl ether |
| $Cs_2CO_3$ | cesium carbonate |
| MSA | methanesulfonic acid |
| RT | room temperature |
| $Br_2$ | bromine |
| TEA | triethylamine |
| $SOCl_2$ | thionyl chloride |
| KOH | potassium hydroxide |
| $Al_2O_3$ | aluminum oxide |
| $NH_4OAc$ | ammonium acetate |
| CYP450 | cytochrome P450 |
| IR | infrared |
| KF | potassium fluoride |
| KOAc | potassium acetate |
| $Na_2CO_3$ | sodium carbonate |
| $K_3PO_4$ | tripotassium carbonate |
| DABCO | 1,4-diazabicyclo[2.2.2]octane |
| TLC | Thin Layer Chromatography |
| SDS | sodium dodecyl sulfate |
| HER2 | human epidermal growth factor receptor 2 |
| SEM | standard error of the mean |
| SAR | structure activity relationship |
| $^1$H -NMR | Proton ($^1$H) nuclear magnetic resonance spectrum |
| $^{13}$C -NMR | Carbon ($^{13}$C) nuclear magnetic resonance spectrum |
| HRMS | High-resolution mass spectrometry |
| ESI | Electrospray ionization |
| $MgSO_4$ | magnesium sulfate |
| $POCl_3$ | Phosphorus(V) oxide chloride |
| $NaHCO_3$ | sodium bicarbonate |
| FBS | fetal bovine serum |
| α-MEM | alpha Minimal Essential Medium |
| $CO_2$ | carbon dioxide |
| MHz | Mega Hertz |
| δ | delta value in ppm for NMR spectra |
| *J* | coupling constant |

| In NMR characterization | |
|---|---|
| s | singlet |
| d | doublet |
| t | triplet |
| q | quartet |
| m | multiplet |
| dd | doublet of doublets |
| dt | doublet of triplets |
| br. | broad |

## Chapter 1: Benzofurans

### 1.A Benzofuran Structure in Clinically Used Drugs

The heterocyclic benzofuran moiety is a privileged structure with a wide range of pharmaceutical activity. Found in a broad range of natural products, the benzofuran structure is metabolically well tolerated and utilized synthetically in the development of novel pharmaceuticals. Benzofurans have been studied for therapeutic uses in cancer, cardiovascular diseases, antimicrobial, psychotic disorders, renal disorders and inflammation. For example, amiodarone **1.1** is a benzofuran based antiarrhythmic agent, clinically approved in 1974 for the treatment and prevention of ventricular arrhythmias.[1,2] Dronedarone **1.2** is another benzofuran derivative approved in 2009 for the treatment of atrial fibrillation in patients with cardiac arrhythmias.[3] Similarly, saprisartan **1.3** is used as an angiotensin II receptor antagonist for the treatment of hypertension and heart failure.[4] In 2004, the FDA approved the $M_3$ muscarinic acetylcholine receptor antagonist darifenacin **1.4** for the treatment of urinary incontinence.[5] Citalopram **1.5**, a selective serotonin reuptake inhibitor (SSRI) was approved in 1998 for the treatment of major depression.[6,7] In 2011, the FDA approved the use of serotonergic antidepressant vilazodone **1.6** for the treatment of major depressive disorder.[8] A noncompetitive inhibitor of xanthine oxidase benzbromanone **1.7** has been used as an uricosuric agent in the treatment of gout.[9,10]

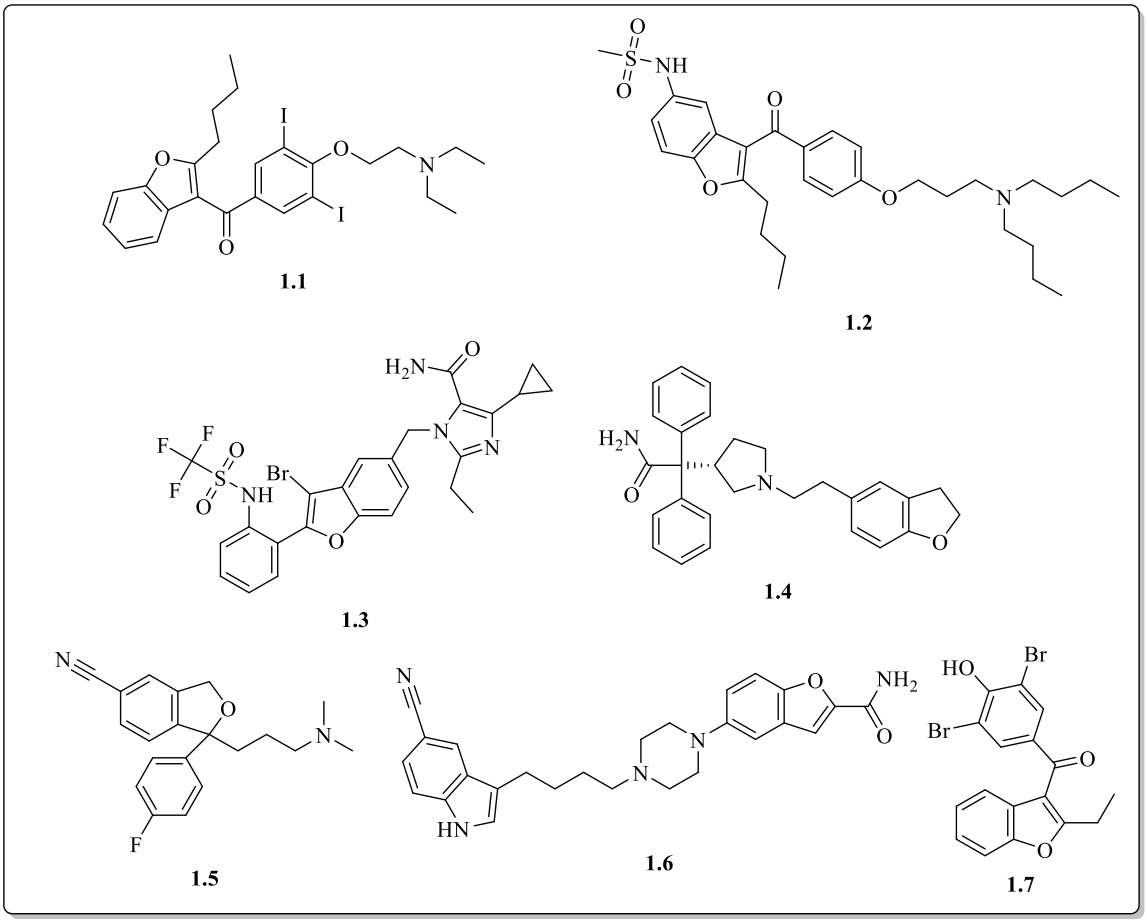



**Figure 1.0**: Representative examples of benzofuran containing clinically used drugs.

### 1.B Benzofuran Structure in Natural Products

Natural products containing benzofuran structures have been utilized as potential agents for the treatment of cancer. One example is rocaglaol **1.8**, found in *Aglaia perviridis*, was shown to have an $IC_{50}$ value of 0.7 μM against a human colon cancer cell line, while having an $IC_{50}$ value >50 μM on noncancerous cells.[11] Compound **1.9** was isolated from *Broussonetia papyrifera* to be used as a potential aromatase inhibitor in the treatment of breast cancers and was shown to inhibit aromatase with $IC_{50}$ value of 0.1 μM.[12] Isolated

from *Morus alba,* compound **1.10** exhibited anticancer properties by inhibition of HIF-1α at ~7 μM.[13]

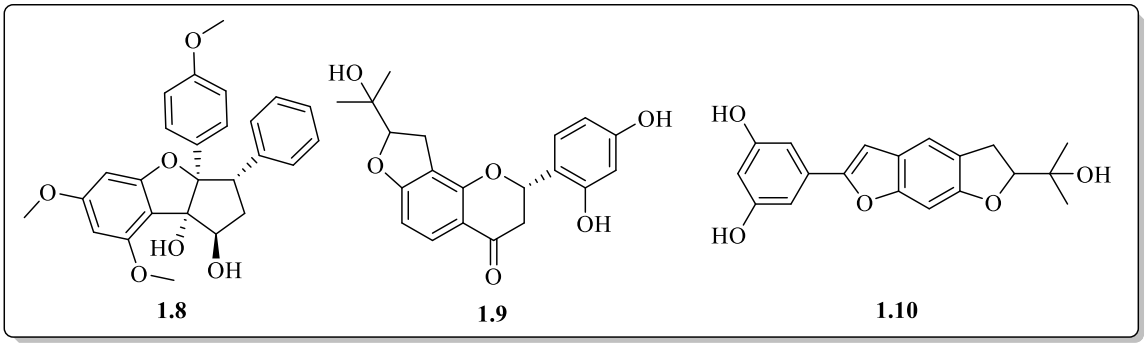



**Figure 1.1**: Benzofuran based natural products with anticancer properties.

### 1.C Benzofuran Derivatives in Clinical Trials

Some benzofuran derivatives have been evaluated for their anticancer properties in humans. Developed at Ohio State University by Griver, silvestrol **1.11** was tested in clinical trials at the stage IIA level. Compound **1.11** was shown to have $IC_{50}$ values of 12-86 nM in a variety of cell lines and has been found to deplete cellular Cyclin D levels.[14,15] Developed by Pharmacyclics, abexinostat **1.12** was in Phase II clinical trials in 2014 as a histone deacetylase inhibitor ($K_i$ of 7 nM) for the treatment of B-cell lymphoma.[16] Developed by Asoh *et. al*, **1.13** was identified as a clinical candidate after showing *in vitro* $IC_{50}$ of 1 nM on squamous cell lung carcinoma cell line QG56 and almost total tumor regression in QG56 xenograft in mice with no noticeable body weight loss.[17] Compound BNC105P **1.14** is currently in Phase II clinical trial in combination with ibrutinib for patients with relapsed/refractory chronic lymphocytic leukemia.[18,19]

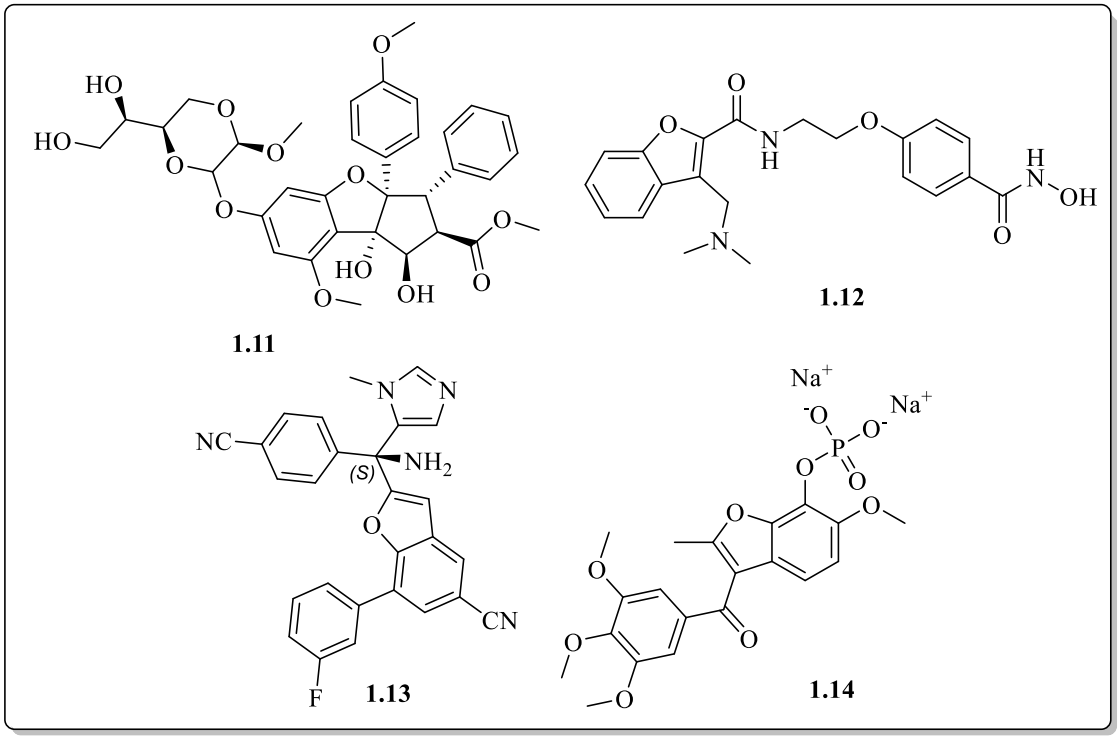



**Figure 1.2:** Benzofuran based anticancer compounds that were/are in clinical trials.

### 1.D Benzofuran Derivatives as Antimicrobial Agents

The benzofuran template is not limited to human based pathologies, as many antimicrobial agents have been synthesized containing the benzofuran template. Compound **1.15** was found to be a potential antifungal agent with N-myristoyl transferase inhibition properties having an enzyme $IC_{50}$ value of 6 nM and an antifungal $IC_{50}$ value of 37 nM found against *Candida albicans.*[20] Compound **1.16** was screened against 5 gram positive strains, 2 gram negative strains, and 1 fungal strain and was found to have zone of inhibition greater than penicillin, chloramphenicol, tetracycline, ampicillin, gentamicin and ketoconazole.[21] Screened against *Staphylococcus aureus* and *Escherichia coli*, derivatives of compound **1.17** was found to have a zone of inhibition similar to that of ampicillin.[22] Compound **1.18** was screened against 5 bacterial and 5 fungal strains showing relative

percent inhibition of 80-93%.[23] Screened against *S. aureus*, *Staphylococcus pyogenes*, *E. coli* and *Pseudomonas aeruginosa* derivatives of compound **1.19** containing the sulphonamide benzofuran moiety showed comparable antibacterial activity to known antibiotic ciprofloxacin.[24]

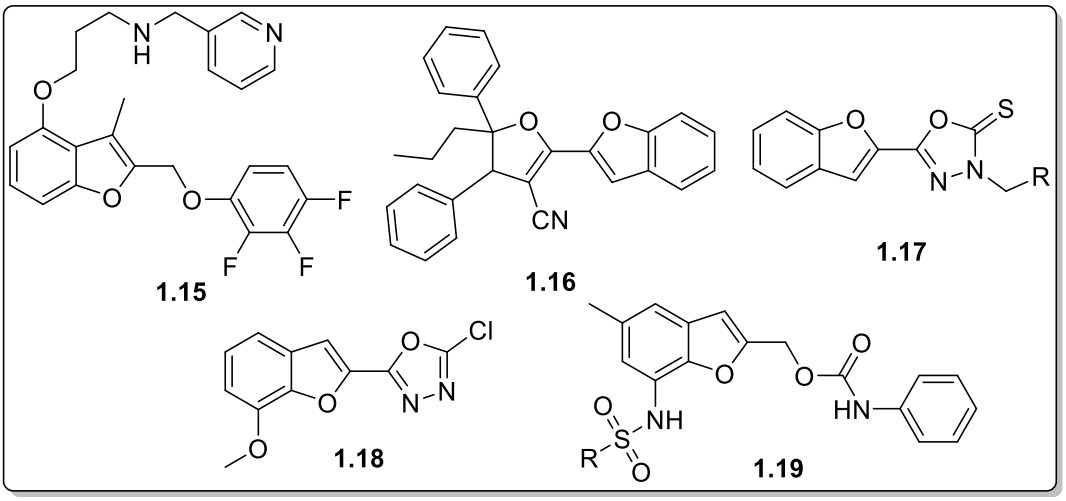
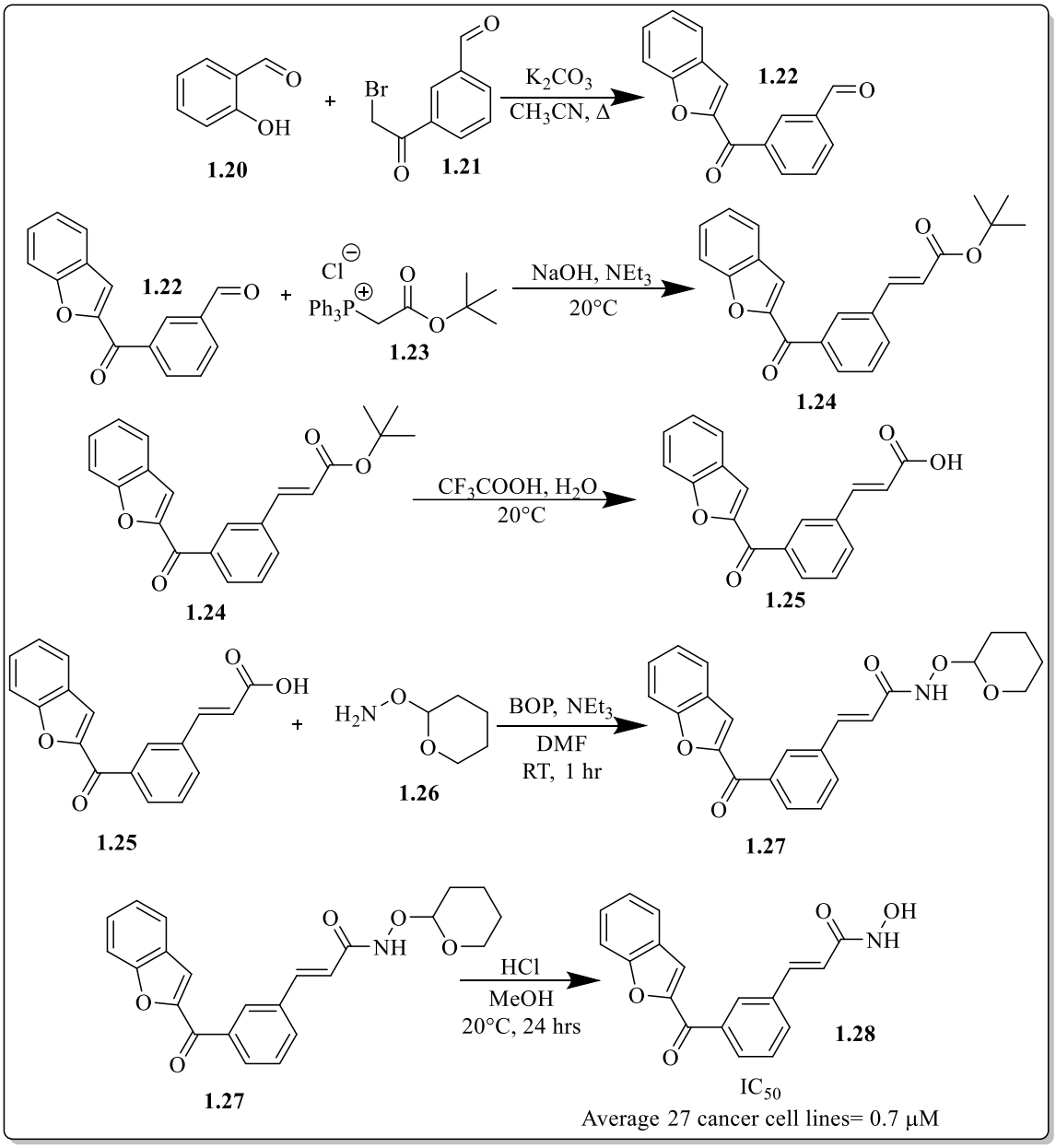



**Figure 1.3:** Benzofuran based candidate compounds as antifungal and antimicrobial agents.

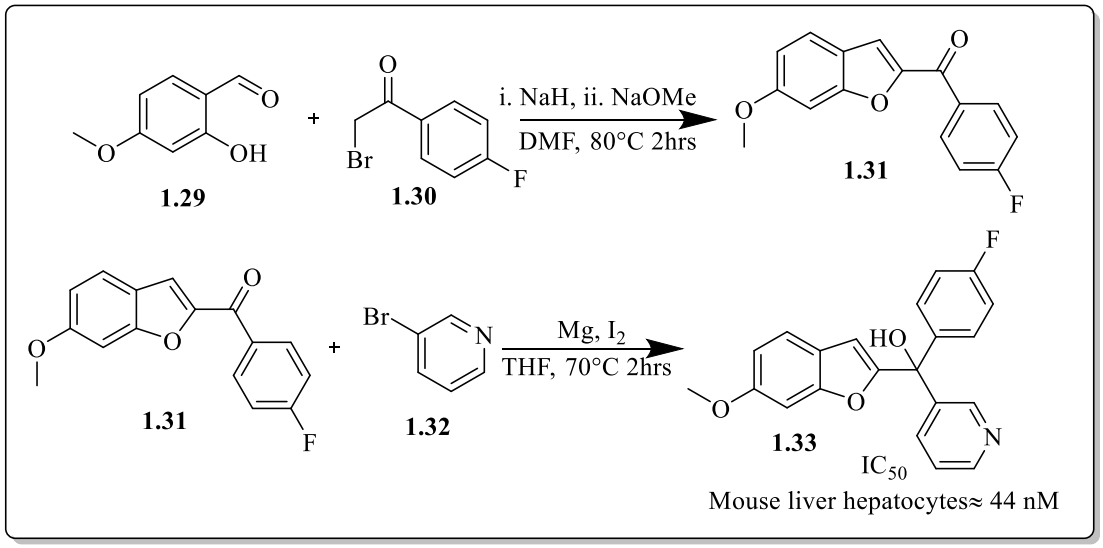
## 1.E Synthesis of Functionalized Benzofurans as Medicinal Agents

Mahboobi *et al.* reported that 2-aroylbenzofurans with N-hydroxyacylamide substructures could be used as histone deacetylase (HDAC) inhibitors (HDIs). Benzofuran **1.28** was found to have an average $IC_{50}$ value of ~0.7 μM across a panel of 27 cancer cell lines.[25] The synthesis involves the Rap-Stoermer condensation of salicylaldehyde **1.20** with **1.21** in the presence of $K_2CO_3$ in refluxing acetonitrile to obtain the aroylbenzofuran **1.22** in 49% yield. The aldehyde in **1.22** was subjected to Wittig olefination with (2-(*tert*-butoxy)-2-oxoethyl)triphenylphosphonium chloride **1.23** in the presence of NaOH and triethylamine at 20°C to obtain the respective acrylic *tert*-butyl ester **1.24**. The *tert*-butyl ester in **1.24** was hydrolyzed at 20°C using trifluoroacetic acid and water to obtain the acrylic acid **1.25**. Amide **1.27** was synthesized via BOP coupling with the acrylic acid **1.25** and *O*-(tetrahydro-2*H*-pyran-2-yl)hydroxylamine **1.26** in the presence of triethylamine base and subsequent deprotection of tetrahydropyranyl ether to obtain N-hydroxyacrylamide derivative **1.28**.[25]



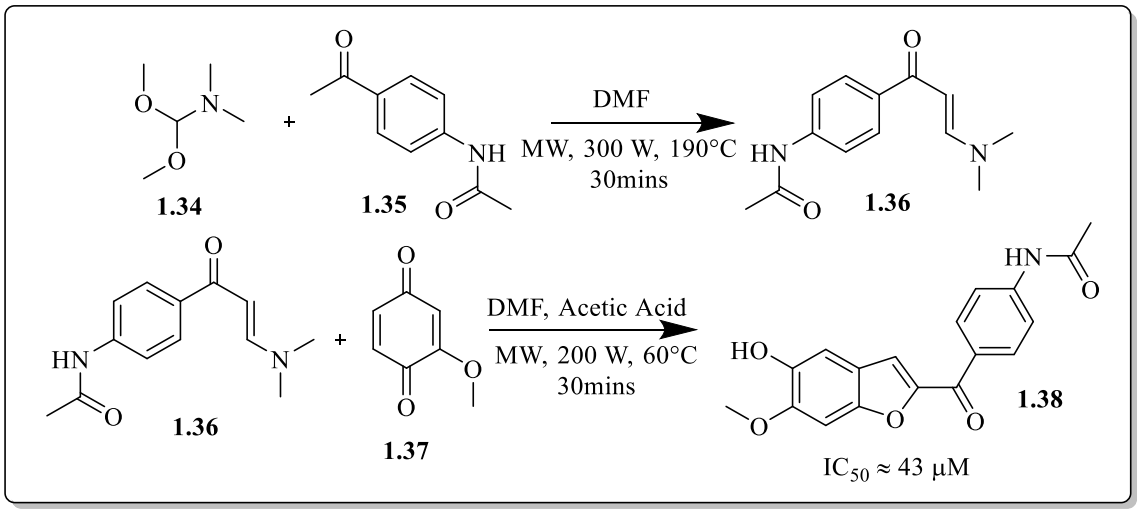

**Scheme 1.0**: Synthesis of benzofuran hydroxamic acid **1.28** as an HDAC inhibitor.

Saberi *et al.* reported that 1-(benzofuran-2-yl)(phenylmethyl)pyridine structures such as **1.33** could be used as CYP19 (aromatase) inhibitors with an $IC_{50}$ of 44 nM on mouse liver hepatocytes. The synthesis of (6-methoxybenzofuran-2-yl)(phenyl)(pyridin-3-yl)methanol **1.33** began with the Rap-Stoermer condensation of salicylaldehyde **1.29** with phenacyl bromide **1.30** in the presence of base at 80°C in DMF. The resulting benzofuran **1.31** was subjected to a Grignard reaction with 3-bromopyridine after *in situ* Grignard reagent formation using Mg and $I_2$ in THF to give **1.33** at 80% yield.[26]



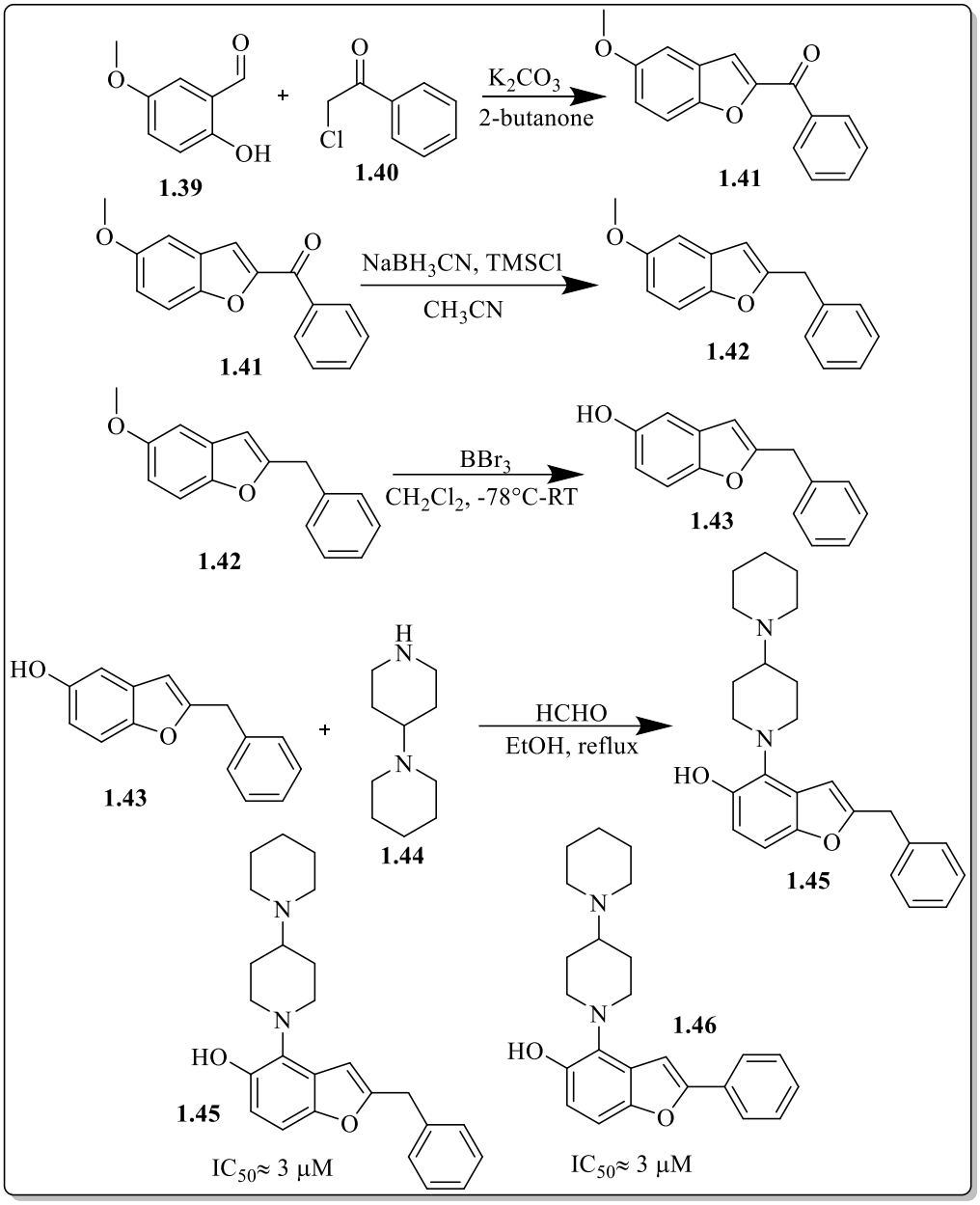

**Scheme 1.1**: Synthesis of benzofuran pyridyl derivative **1.33** as an aromatase inhibitor.

Li *et al.* reported that 3-acyl-5-hydroxybenzofurans could be used as anti-estrogen agents for the treatment of breast cancer. Using a cell proliferation MTT colorimetric assay they found that **1.38** had an $IC_{50}$ value of ~43 μM against human mammary carcinoma cell line MCF7. The synthesis of **1.38** was achieved via a microwave-assisted protocol. First, **1.34** and **1.35** were dissolved in DMF and heated to 190°C and were further subjected to 300 W of microwave irradiation. After the formation of enaminone **1.36**, quinine **1.37** was added followed by acetic acid. This reaction mixture was heated to 60°C and subjected to 200 W of microwave irradiation to yield **1.38** which was purified via column chromatography.[27]



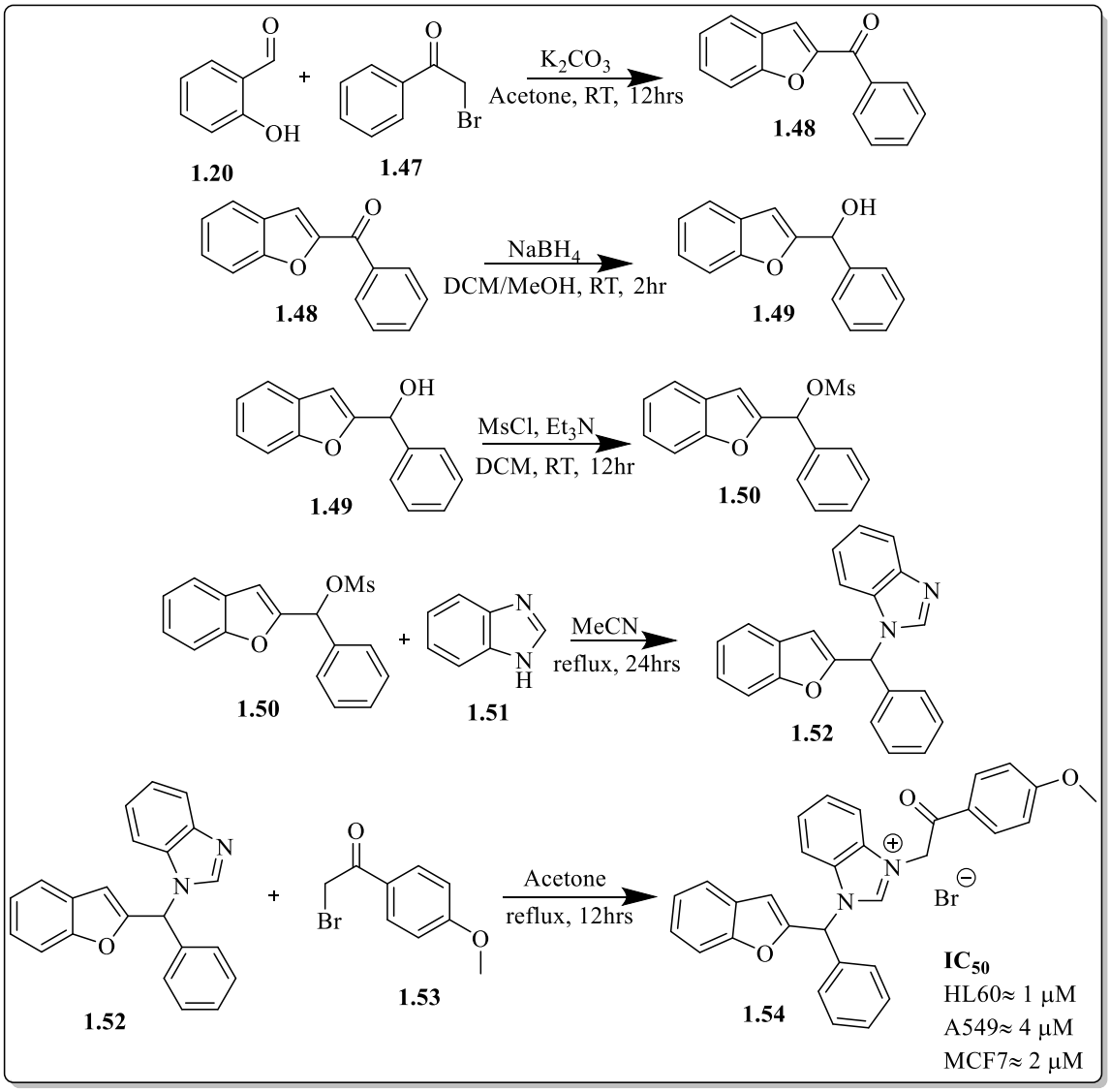

**Scheme 1.2**: Synthesis of benzofuran phenacylacetamide derivative **1.38** as an antiestrogen agent.

Salomé *et al.* reported that 2-benzyl-benzofurans and 2-phenyl-benzofurans could be used as mTOR complex 1 (mTORC1) inhibitors. After screening of their library using a cell proliferation MTT colorimetric assay, they found 2-benzyl-benzofuran **1.45** and 2-phenyl-benzofuran **1.46** to have $IC_{50}$ values of ~3 μM on a laryngeal squamous carcinoma cell line SQ20B. The synthesis of the 2-benzyl-benzofuran derivatives started with the Rap-Stoermer condensation of salicylaldehyde **1.39** and phenacyl chloride **1.40** in the presence of $K_2CO_3$ using 2-butanone as solvent giving **1.41** in 98% yield. The condensation was followed by complete deoxygenation of the ketone using $NaBH_3CN$/TMSCl in acetonitrile to give the resulting 2-benzyl-benzofuran 1.42 (yield= 90%). The 2-benzyl-benzofuran **1.42** underwent O-demethylation using $BBr_3$ in DCM at -78°C to obtain **1.43** in 84% yield.The hydroxy substituted 2-benzyl-benzofuran **1.43** was then subjected to the Mannich reaction using paraformaldehyde and secondary amine **1.44** while refluxing in ethanol to obtain the piperdine derivative **1.45**.[28]



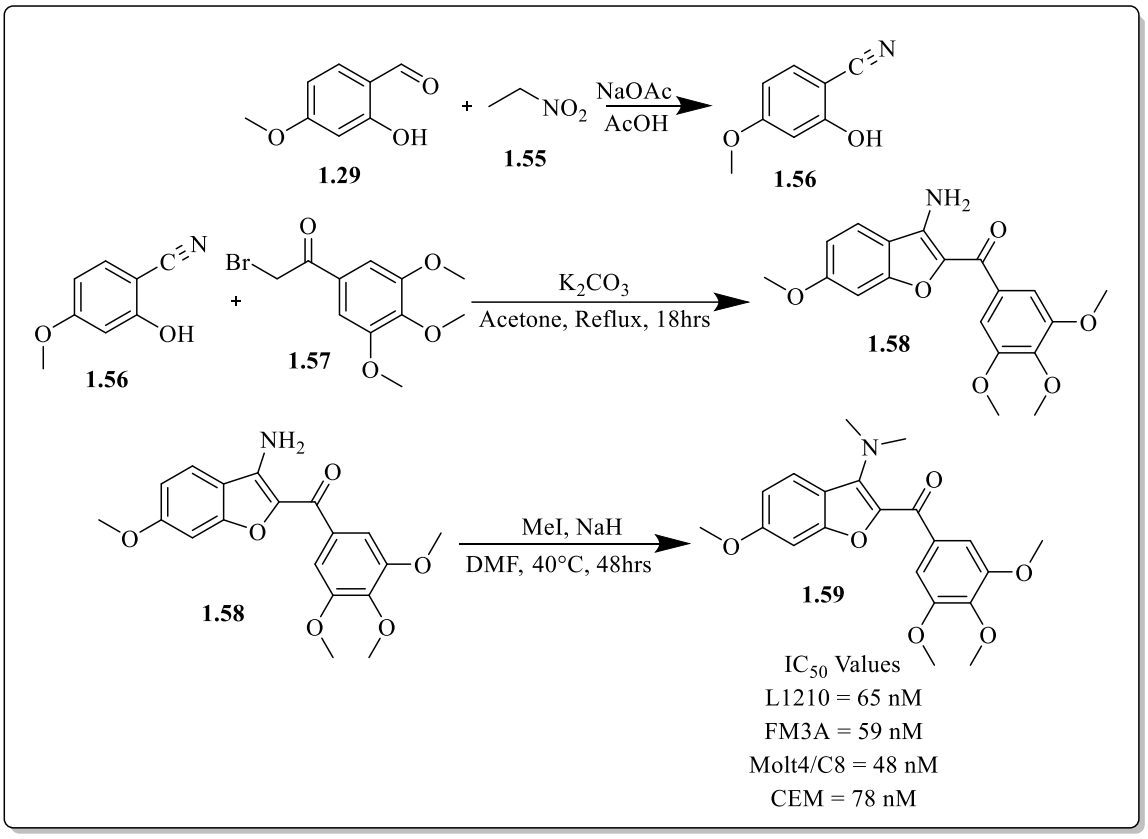

**Scheme 1.3**: Synthesis of benzofuran piperdine derivatives **1.45** and **1.46** as mTORC1 inhibitors.

Wang *et al.* discovered that 2-benzyl-benzofuran based imidazolium salts could be used as potential anticancer agents. Lead compound **1.54** was screened against leukemia (HL-60), human lung adenocarcinoma (A549) and human mammary carcinoma (MCF7) cell lines and $IC_{50}$ values of ~1 μM, ~4 μM, and ~2 μM were obtained. The synthesis of **1.54** was obtained through Rap-Stoermer condensation of salicylaldehyde **1.20** with phenacyl bromide **1.47** in the presence of base in acetone. The resulting benzofuran **1.48** was reduced using $NaBH_4$ followed by generation of the mesylate **1.50** using mesyl chloride in the presence of triethylamine. Compound **1.50** underwent nucleophilic substitution reaction with benzimidazole **1.51** in acetonitrile to give **1.52**. The resulting benzimidazole **1.52** was salted out with **1.53** in acetone to yield the desired 2-benzyl-benzofuran based benzimidazolium salt **1.54** with a yield of 84%.[29]



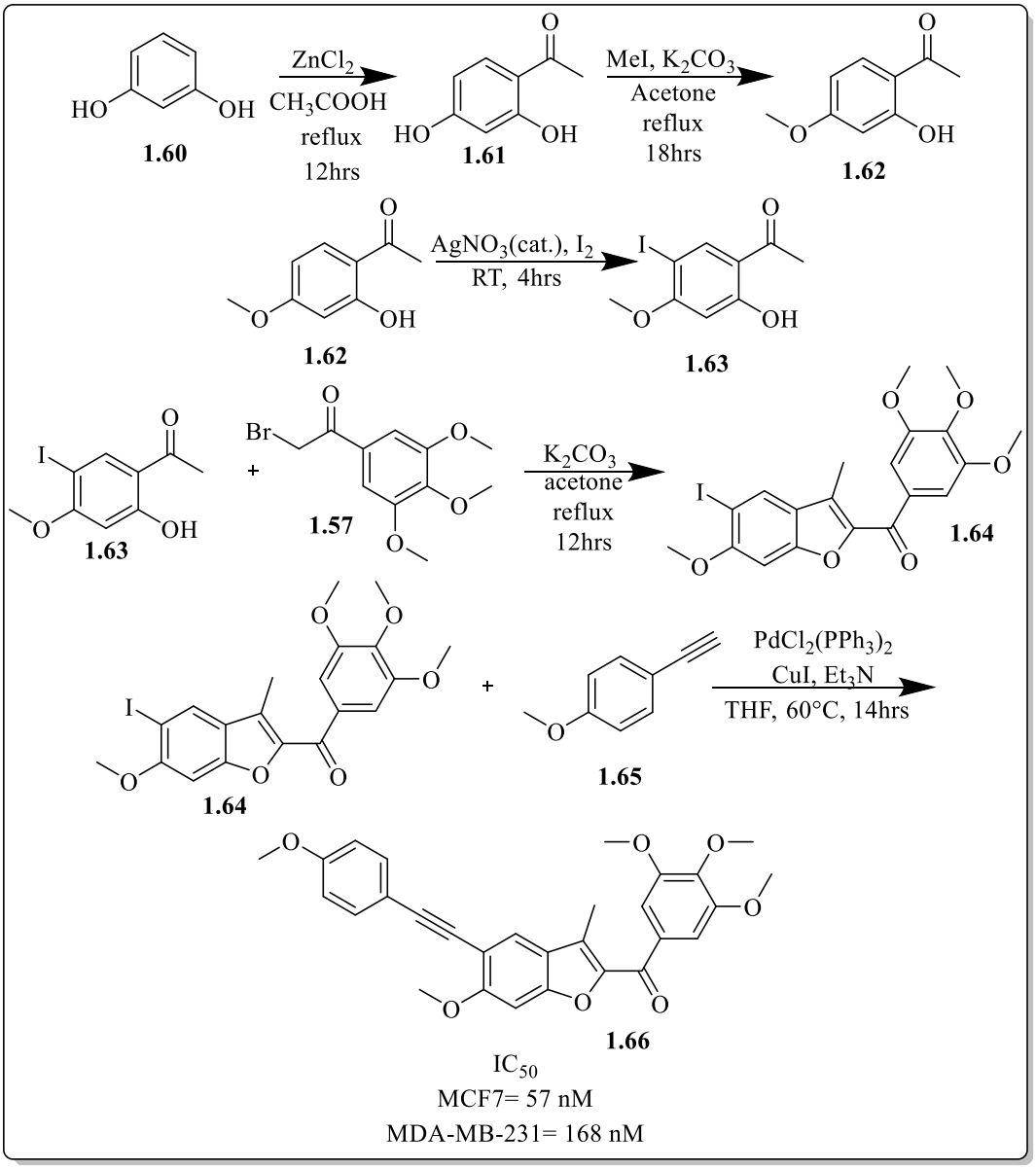

**Scheme 1.4**: Synthesis of benzofuran imidazolium salt **1.54** as an anticancer agent.

The subsect of trimethoxy benzyl *N*,*N*-dimethyl amino benzofuran derivates as tubulin polymerization inhibitors for the treatment of cancer was achieved by Romagnoli *et al.* Compound **1.59** was screened across murine leukemia (L1210), murine mammary carcinoma (FM3A), human acute lymphoblastic leukemia (Molt4/C8), and human childhood acute lymphoblastic leukemia (CEM) cell lines. Compound **1.59** was found to have $IC_{50}$ values of 65 nM, 59 nM, 48 nM, and 78 nM respectively on these cell lines. Synthesis of **1.59** was achieved by refluxing **1.29** in glacial acetic acid using nitroethane **1.55** and sodium acetate. Following nitrile formation, **1.56** underwent Rap-Stoermer condensation conditions with phenacyl bromide **1.57** to yield the desired 3-amino benzofuran derivative **1.58** with a 77% yield after further purification via column chromatography (40% EtOAc/petroleum ether). The resulting free amine in **1.58** was alkylated with methyl iodide in DMF in the presence of base to give **1.59** with 44% yield after column chromatography purification (30% EtOAc/petroleum ether).[30]



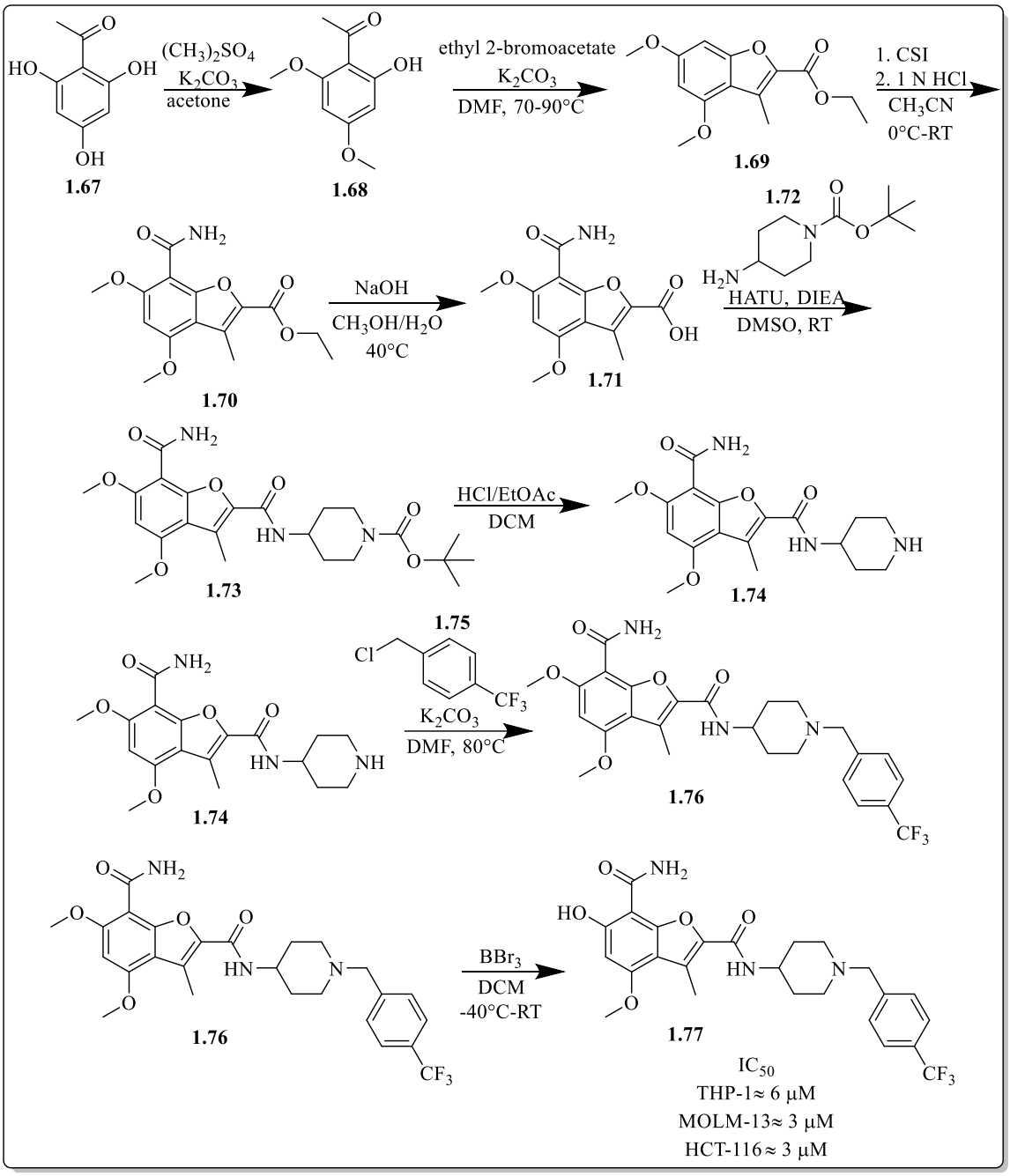

**Scheme 1.5**: Synthesis of benzofuran *N*,*N*-dimethyl amino derivative **1.59** as a tubulin polymerization inhibitor.

Kamal *et al*. synthesized benzofurans that could be used as anticancer agents for the treatment of breast cancer. Lead derivative **1.66** was found to have $IC_{50}$ values of 57 nM and 168 nM on human mammary gland adenocarcinoma cell line MCF7 and triple-negative human mammary gland adenocarcinoma cell line MDA-MB-231 respectively. Resorcinol **1.60** underwent Friedel-Crafts acylation using $ZnCl_2$ and acetic acid to obtain **1.61**. Compound **1.61** was subsequently methylated with methyliodide in the presence of base to give **1.62** at an 85% yield after further purification via column chromatography. Using catalytic amounts of $AgNO_3$ in the presence of $I_2$, compound **1.62** underwent iodination to provide **1.63** at 60% yield. Using Rap-Stoermer condensation conditions, acetophenone **1.63** was reacted with phenacyl bromide **1.57** to obtain the benzofuran **1.64** in 75% yield after further purification via recrystallization in ether. The benzofuran derivative **1.64** was coupled to 1-ethynyl-4-methoxybenzene **1.65** under Sonogashira coupling conditions using $PdCl_2(PPh_3)_2$ catalyst, CuI co-catalyst, and triethylamine base in THF achieving alkyne **1.66**.[31,32]



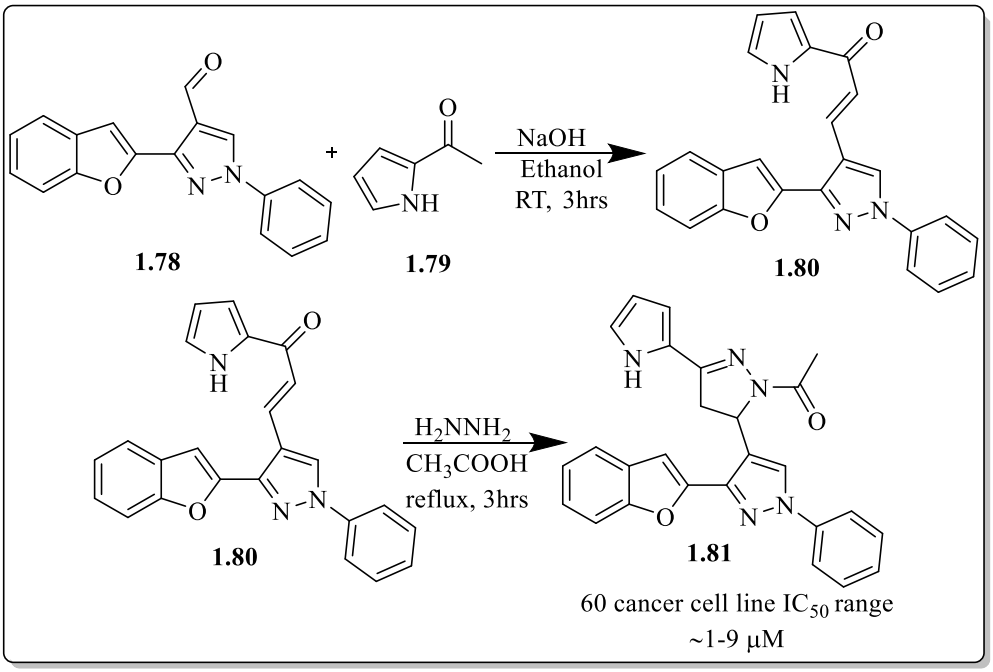

**Scheme 1.6**: Synthesis of benzofuran alkynyl derivative **1.66** as an anticancer agent.

Wang *et al.* found that 6-hydroxy-4-methoxy-3- methylbenzofuran-7-carboxamide derivatives **1.77** could be used as Mnk inhibitors for cancer therapy. Compound **1.77** was screened against a human acute monocytic leukemia cell line (THP-1), a human adult myeloid leukemia cell line (MOLM-13), and a human colorectal carcinoma cell line (HCT-116). The $IC_{50}$ values elicited by **1.77** were ~6 μM, ~3 μM, and ~3 μM for THP-1, MOLM-13, and HCT-116 respectively. The synthesis of lead derivative **1.77** began with dimethylation of **1.67** using dimethylsulfate in the presence of a base in acetone. The dimethylated ortho-hydroxy acetophenone **1.68** then underwent Rap-Stoermer condensation with ethyl bromoacetate in the presence of base to afford benzofuran **1.69** in 34% yield after further purification via column chromatography. The freshly synthesized **1.69** was subjected to an electrophilic aromatic substitution reaction using chlorosulfonyl isocyanate and HCl in acetonitrile to give carbamoyl substituted benzofuran **1.70** in 94% yield. This was followed by hydrolysis of the ethyl ester to acquire **1.71** in 90% yield. The subsequent carboxylic acid **1.71** underwent amide coupling with Boc protected 4-aminopiperdine **1.72** using HATU and DIEA base in DMSO to afford **1.73** in 65% yield. After Boc deprotection, **1.74** was alkylated with 4-(trifluromethyl)benzyl chloride **1.75** in the presence of a base in DMF to obtain **1.76**. The selective demethylated benzofuran derivative **1.77** was obtained by treating **1.76** with $BBr_3$ in DCM at -40°C.[33]



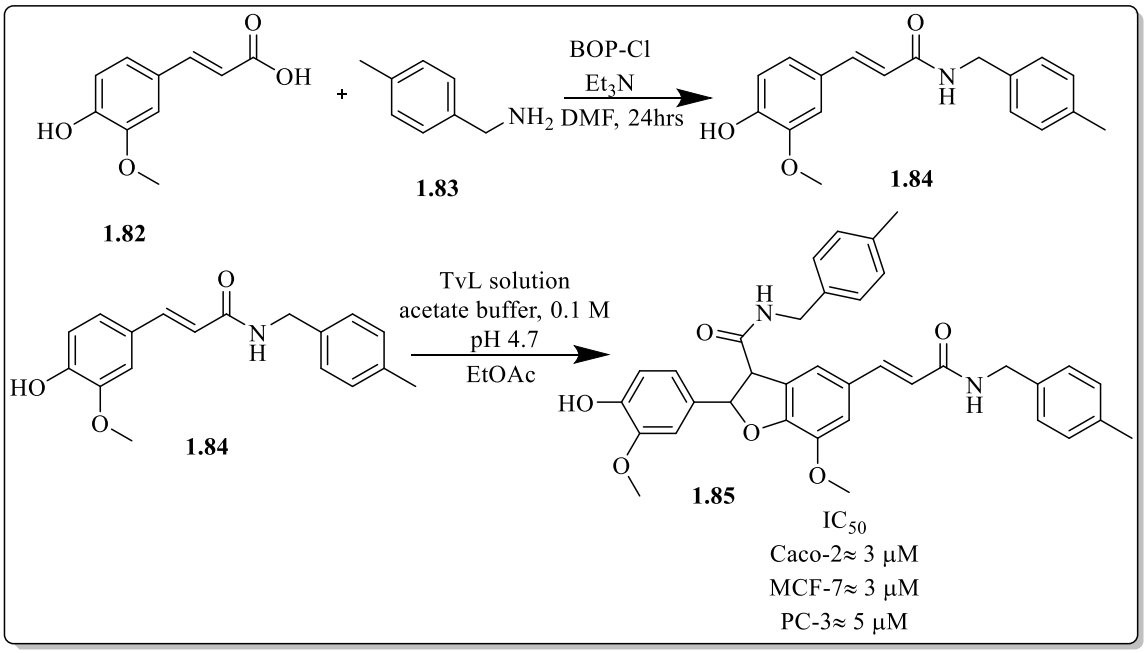

**Scheme 1.7**: Synthesis of benzofuran carboxamide derivative **1.77** as an Mnk inhibitor.

El Karim *et al.* found that benzofuran-pyrazole containing molecules could be used as potential anticancer agents. Compound **1.81** was identified as the lead cytotoxic agent after being screened on over 60 cancer cell lines with $IC_{50}$ values ranging from ~1-9 μM. This panel included leukemia, non-small cell lung cancer, colon cancer, CNS cancer, melanoma, ovarian cancer, renal cancer, prostate cancer, and breast cancer cell lines. Derivatives developed from a previous study were formylated via Vilsmeier Haack conditions to yield **1.78**.[34] Using Claisen–Schmidt condensation conditions starting aldehyde **1.78** was treated with 2-acetylpyrrole **1.79** in the presence of NaOH in ethanol to afford chalcone **1.80** in 88% yield after recrystallization. The resulting chalcone **1.80** underwent condensation with hydrazine to give benzofuran-pyrazole product **1.81** in 66% yield.[35]



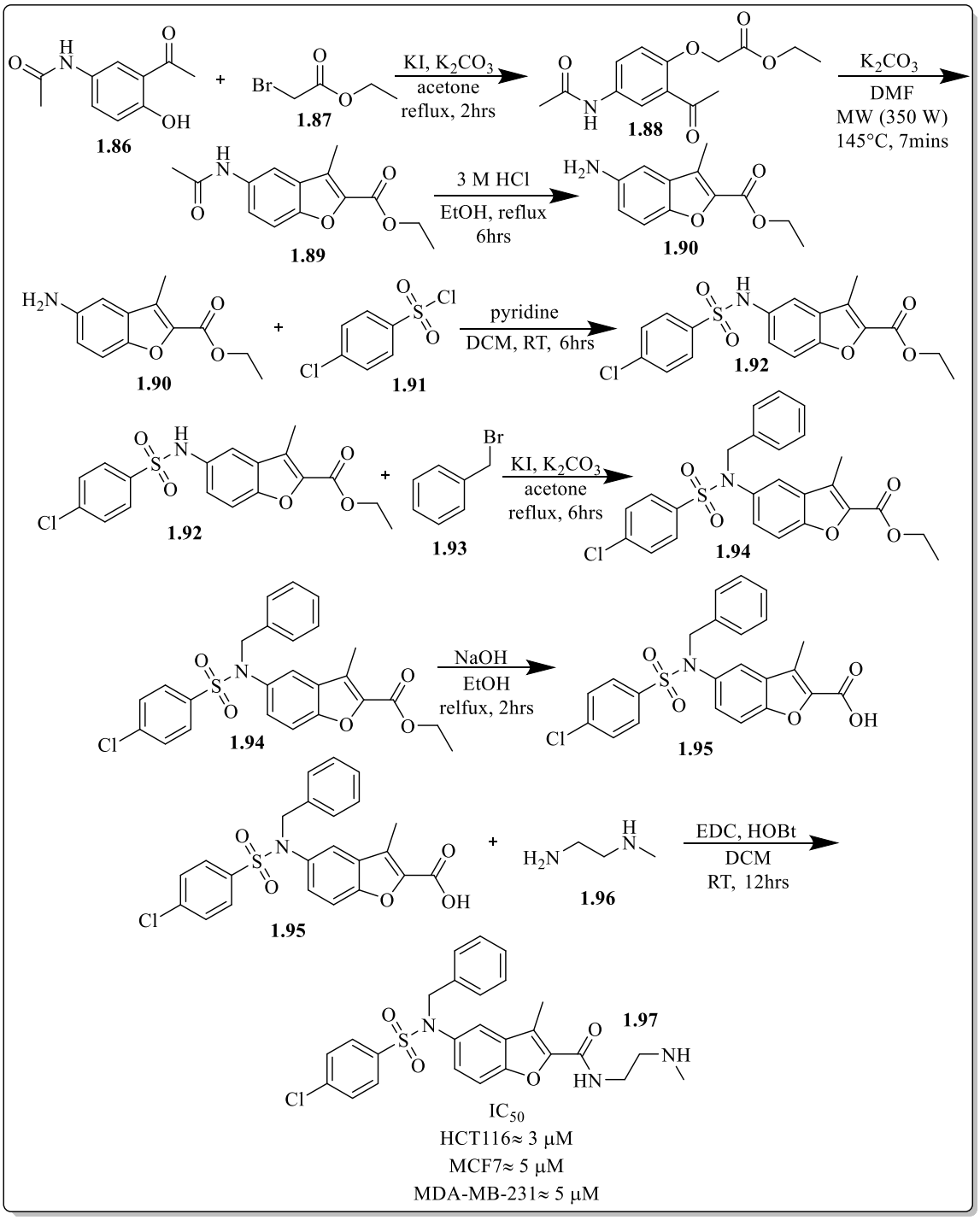

**Scheme 1.8**: Synthesis of benzofuran pyrazole derivative **1.81** as an anticancer agent.

Cardullo *et al*. found that a racemic mixture of dihydrobenzofuran neolignanamide **1.85** could be used as an anticancer agent by inducing cell cycle arrest and apoptosis. Racemic mixture of **1.85** had $IC_{50}$ values of ~3 μM on human colorectal carcinoma cell line (Caco-2), ~3 μM on human mammary gland adenocarcinoma cell line (MCF-7), and ~5 μM on grade IV human prostate adenocarcinoma (PC-3). Synthesis of **1.85** began with amide coupling of ferulic acid **1.82** and 4-methylbenzylamine **1.83** using BOP-Cl coupling agent in the presence of a base using DMF as a solvent to give enamide **1.84** in 82% yield after further purification via column chromatography (5% MeOH/DCM). The resulting amide was stirred in ethyl acetate and treated with a solution of *Trametes versicolor* laccase (TvL, 0.1M, pH=4.7) to yield the racemic neolignanamide **1.85**. The racemate was separated into pure (2R,3R) and (2S,3S) enantiomers via HPLC and screened against the cancer cell lines. However, no significant change in cytotoxicity was observed suggesting that chirality at the C-2 and C-3 position doesn't play a role in the mechanism of action for **1.85**.[36]



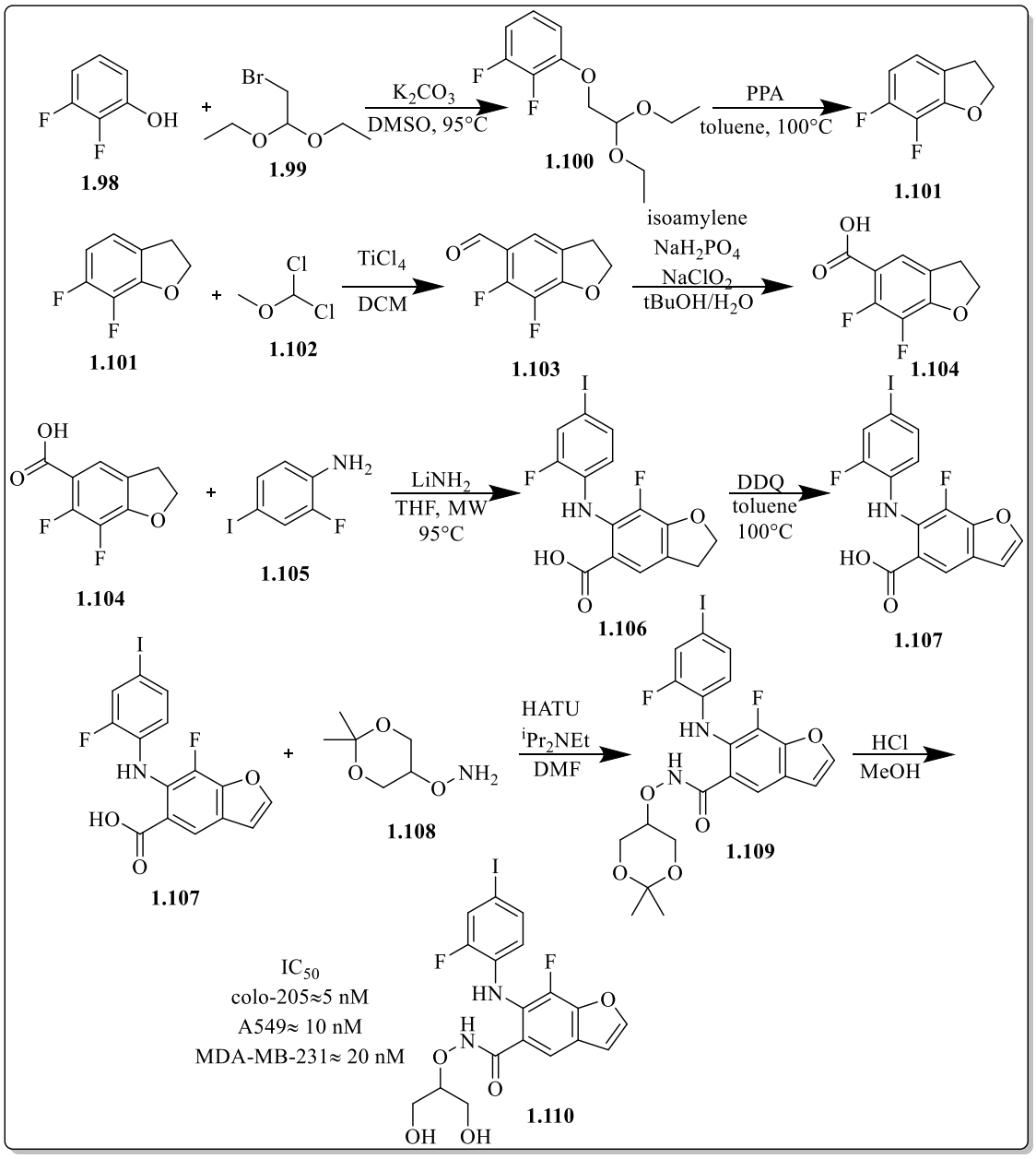

**Scheme 1.9**: Synthesis of dihydrobenzofuran neolignanamide **1.85** as an anticancer agent.

Yang *et al.* found that sulfonamide containing benzofuran derivatives could be used as anticancer agents through inhibition of HIF-1 pathway. Chemical library was screened against human colorectal carcinoma cell line HCT116, human mammary gland adenocarcinoma cell line MCF7 and triple-negative human mammary gland adenocarcinoma cell line MDA-MB-231. Compound **1.97** was identified as the lead with $IC_{50}$ values of ~3 μM, ~5 μM, ~5 μM for HCT116, MCF7, and MDA-MB-231 respectively. Synthesis began with O-alkylation of phenolic acetophenone **1.86** by ethyl bromoacetate **1.87** using KI and $K_2CO_3$ in acetone followed by microwave assisted condensation of **1.88** in the presence of a base to give the benzofuran **1.89** in 59% yield. After acid mediated acetyl deprotection, **1.90** was treated with 4-chlorosulfonyl chloride **1.91** in the presence of pyridine in DCM to afford the sulfonamide **1.92** in 69% yield. Sulfonamide **1.92** was alkylated with benzyl bromide **1.93** using KI and $K_2CO_3$ followed by ester hydrolysis to obtain carboxylic acid **1.95** in 81%. The acid **1.95** was coupled with amine **1.96** using EDC and HOBt in DCM to give the benzofuran amide **1.97** in 44% yield.[37]



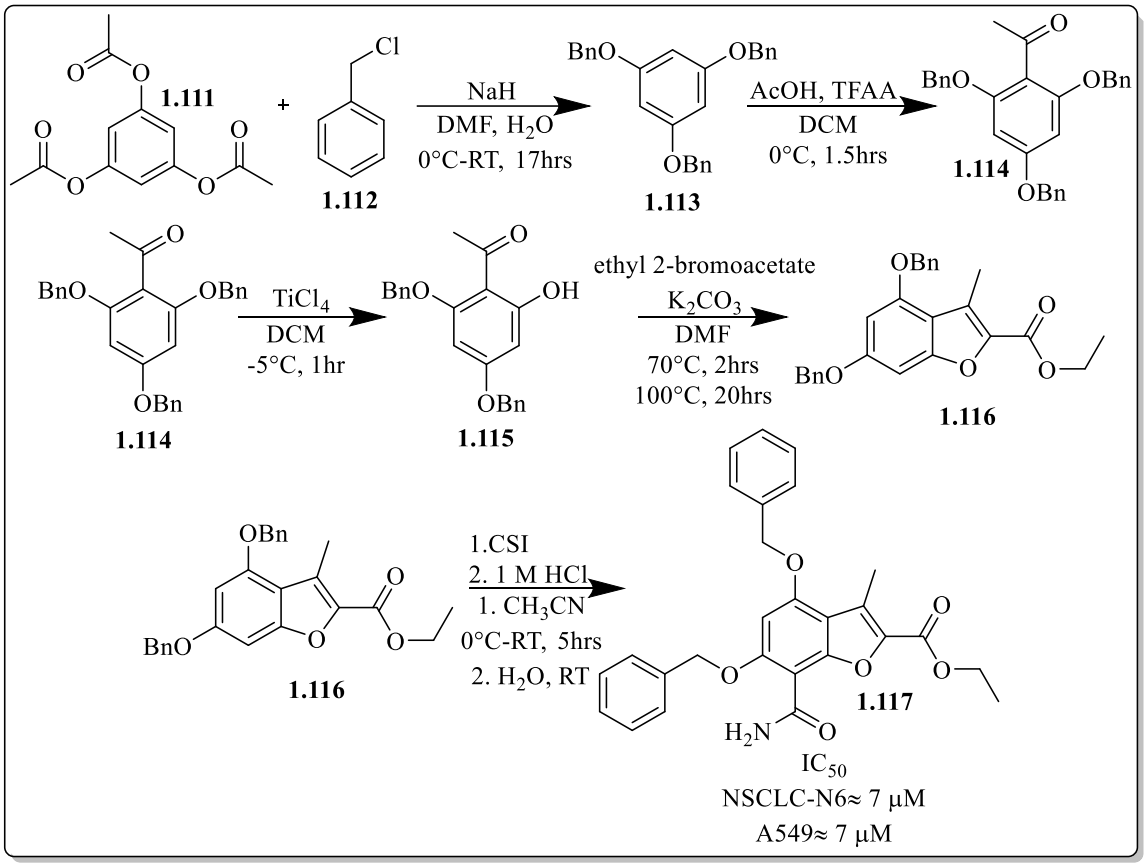

**Scheme 1.10**: Synthesis of benzofuran sulfonamide **1.97** as an HIF-1 pathway inhibitor.

Lu *et al.* discovered that benzodihydrofuran derivatives could be used as MEK1 inhibitors for the treatment of cancer. Screened against colorectal adenocarcinoma cell line COLO-205, human lung adenocarcinoma cell line A549, and triple-negative human mammary gland adenocarcinoma cell line MDA-MB-231, benzofuran **1.110** showed nanomolar $IC_{50}$ values of ~5 nM, ~10 nM, and ~20 nM respectively. Synthesis was achieved by O-alkylation of difluorophenol **1.98** using alkyl bromide **1.99** in the presence of a base in DMSO. This was followed by treatment of resulting ethoxy intermediate **1.100** to polyphosphoric acid in toluene to obtain cyclized dihydrobenzofuran **1.101**. The dihydrobenzofuran **1.101** was formylated using $TiCl_4$ and dichloro(methoxy)methane **1.102** to give the aldehyde **1.103** which was subsequently oxidized to carboxylic acid **1.104** via Pinnick oxidation. Microwave assisted ipso substitution on the dihydrobenzofuran **1.104** was carried out with substituted aniline **1.105** using lithium amide in THF. The dihydrobenzofuran was oxidized using DDQ in toluene to obtain benzofuran **1.107.** The benzofuran acid **1.107** was coupled to amine **1.108** using HATU coupling agent and Hünig's base in DMF. The resulting amide **1.109** was deprotected with acid to give the final dihydropropane benzofuran product **1.110**.[38]



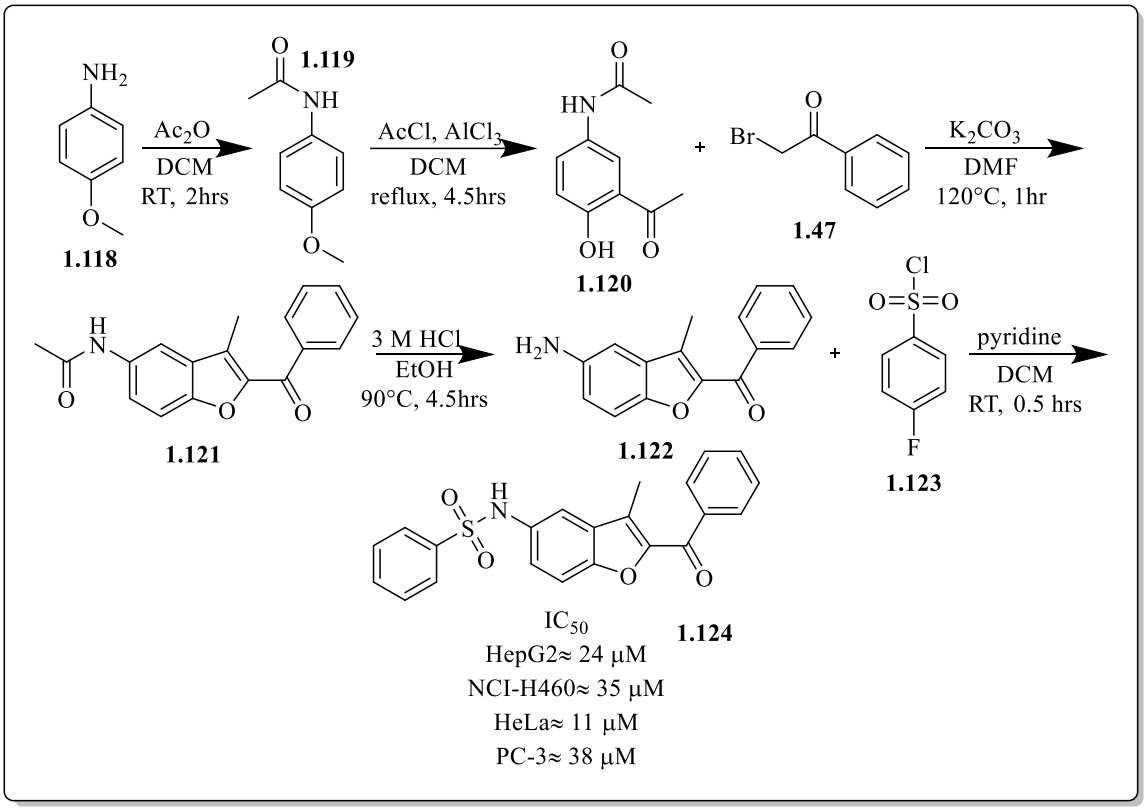

**Scheme 1.11**: Synthesis of benzofuran carboxamide **1.110** as an MEK1 inhibitor.

Bazin *et al.* discovered that benzofuran based analogs of cercosporamide could be used as potential anticancer agents in the treatment of non-small cell lung cancer. Carbamoyl containing benzofuran **1.117** was realized as the most potent derivative after screening against human lung adenocarcinoma cell line A549, and human epidermoid lung carcinoma NSCLC-N6 with $IC_{50}$ values ~7 μM. Triacetoxyphloroglucinol **1.111** was tribenzylated using benzyl chloride **1.112** in the presence of NaH to give **1.113** in 78% yield after recrystallization in methanol. Tribenzyl **1.113** was acylated using trifluoroacetic anhydride and acetic acid in DCM to afford the acetophenone **1.114** in 75% yield after further purification via column chromatography (60% DCM/cyclohexane). O-debenzylation was achieved using $TiCl_4$ to give 2-hydroxy acetophenone **1.115** which underwent subsequent Rap-Stoermer condensation with ethyl bromoacetate **1.87** to obtain benzofuran **1.116** in 52% yield after further purification via column chromatography (20% DCM/hexanes). Benzofuran **1.116** was further subjected to an electrophilic aromatic substitution reaction using chlorosulfonyl isocyanate and HCl in acetonitrile to give the carbamoyl containing benzofuran **1.117** in 87% yield.[39]



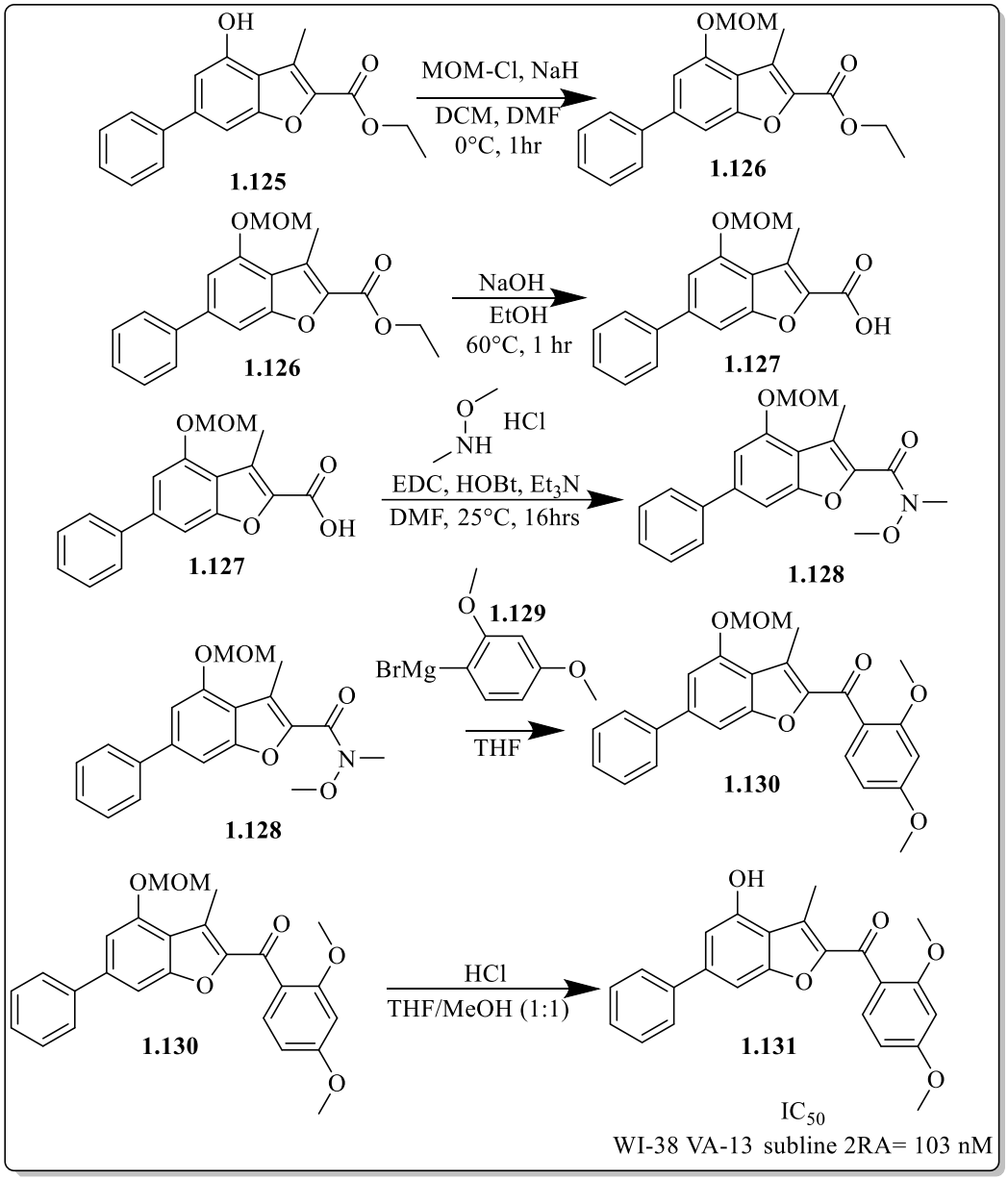

**Scheme 1.12**: Synthesis of dibenzyloxybenzofuran amide **1.117** as an anticancer agent.

Yang *et al.* found that benzofuransulfonamides could be used as potential anticancer agents. Molecular library was screened against human liver hepatocellular carcinoma HepG2 cell line, human large cell lung carcinoma NCI-H640, human cervical adenocarcinoma cell line HeLa, and grade IV human prostate adenocarcinoma PC-3. Lead **1.124** had $IC_{50}$ values of ~24 μM, ~35 μM, ~11 μM, and ~38 μM on the cell lines respectively. Synthesis was achieved from acetylation of *p*-anisidine **1.118** using acetic anhydride in DCM to give amide **1.119** in 30% yield. Acetylated **1.119** underwent Friedel-Crafts acylation which concurrently underwent demethylation to give 2-hydroxy acetophenone derivate **1.120** in 82% yield. Under Rap-Stoermer condensation conditions **1.120** was treated with phenacyl bromide **1.47** to afford benzofuran **1.121** in 72% yield. Following acetyl deprotection, **1.122** was coupled with 4-fluorobenzenesulfonyl chloride **1.123** in the presence of a base to achieve **1.124** in 70% yield.[40]



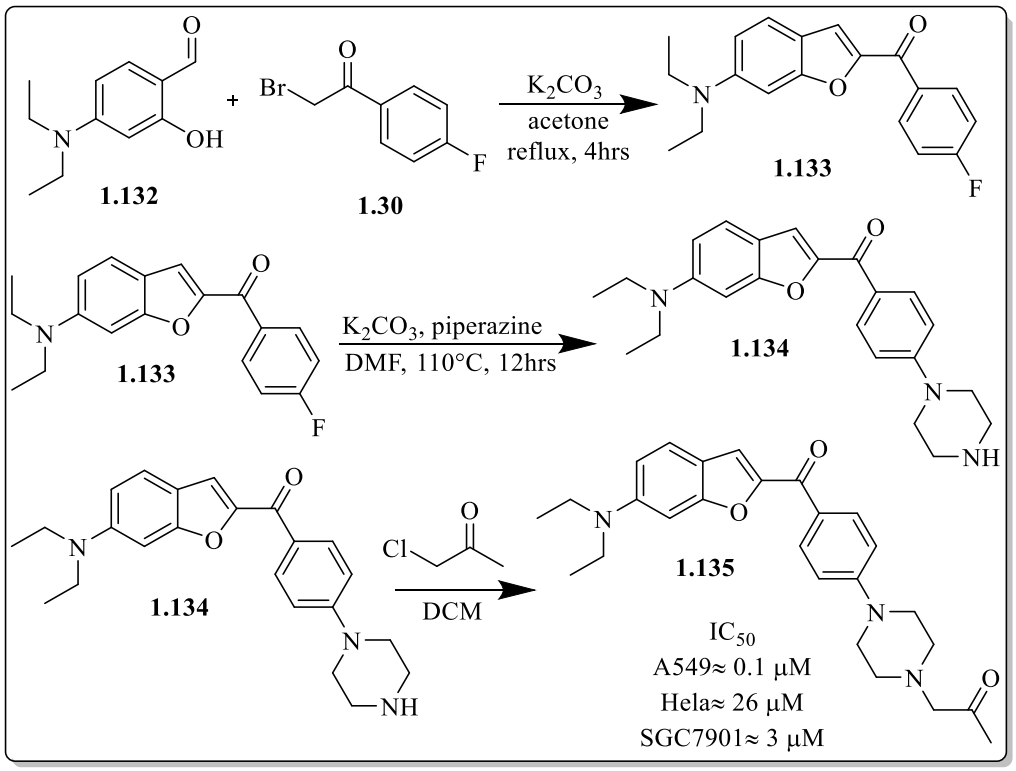

**Scheme 1.13**: Synthesis of benzofuransulfonamide **1.124** as an anticancer agent.

Hayakawa *et al.* discovered that substituted phenylmethanone benzofurans could be used as potential anticancer agents. Lead derivative **1.131** was found to have and $IC_{50}$ of 103 nM against WI-38 VA-13 subline 2RA cell line. Synthesis began with protection of the hydroxy of the phenolic group on benzofuran **1.125** with MOM-Cl followed by ester hydrolysis to give carboxylic acid **1.127**. The acid **1.127** was coupled with *N,O*-dimethylhydroxylamine using EDC and HOBt in the presence of a base. The resulting amide **1.128** underwent a Grignard reaction with **1.129** to obtain the 2-methanone benzofuran **1.130**. Following MOM deprotection, the desired product **1.131** was obtained.[41,42]



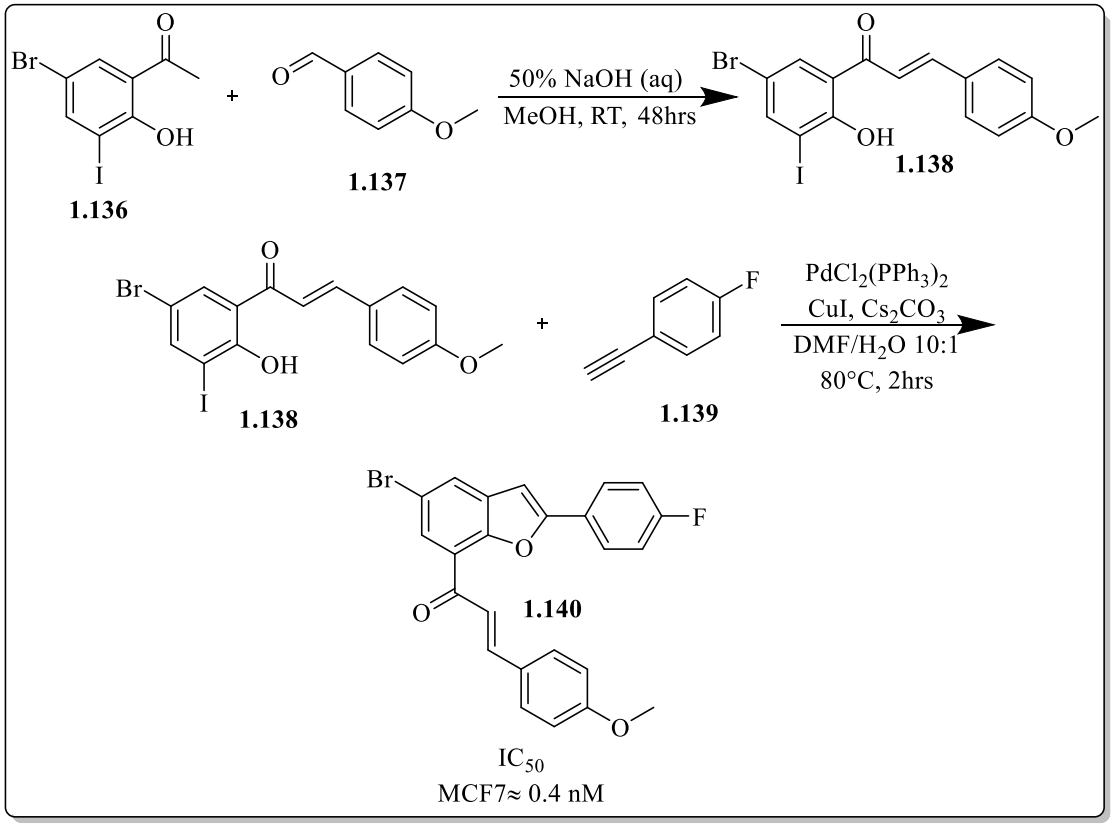

**Scheme 1.14**: Synthesis of benzofuran methanone **1.131** as an anticancer agent.

Ma *et al.* discovered that N-aroyl piperazine benzofurans could be used as potential anticancer agents. Substituted N-aryl piperazine benzofurans were screened against human lung adenocarcinoma cell line A549, human cervical adenocarcinoma cell line HeLa, human gastric carcinoma cell line SGC7901 which identified **1.135** as the lead derivative. Acetone substituted N-aryl piperazine benzofuran **1.135** showed $IC_{50}$ values of ~0.1 μM on A549, ~26 μM on Hela, and ~3 μM on SGC7901 cell lines. Synthesis began with Rap-Stoermer condensation of 4-(diethylamino)salicylaldehyde **1.132** with 4-fluorophenacyl bromide **1.30** to give **1.133** in 84% yield. The resulting **1.133** underwent ipso substitution with piperazine in DMF to afford **1.134** in 82% yield. The piperazine substituted benzofuran derivative **1.134** was alkylated with chloroacetone to obtain **1.135** in 86% yield.[43]



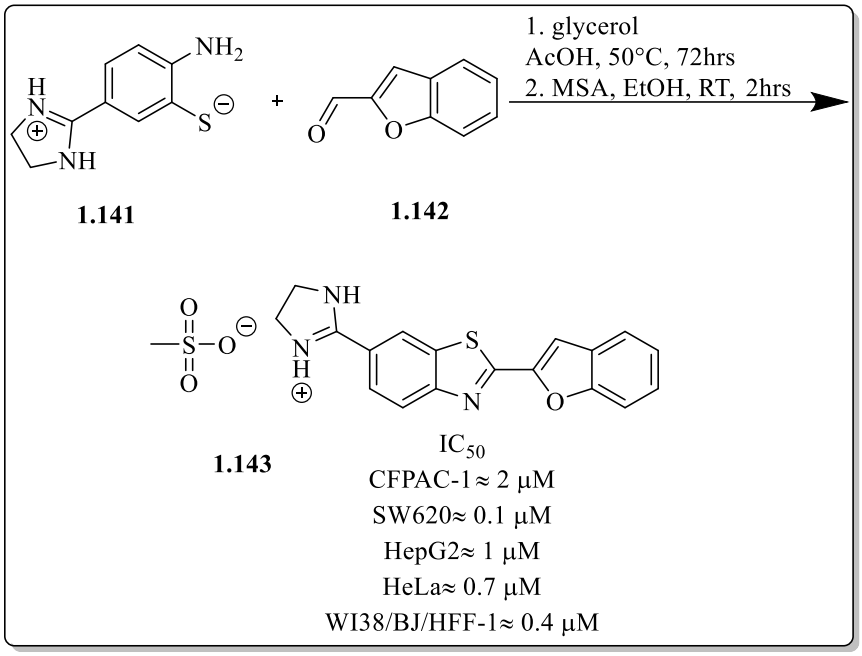

**Scheme 1.15**: Synthesis of *N,N*-dialkylbenzofuran piperazine amine **1.135** as an anticancer agent.

Mphahlele *et al.* developed benzofuran-chalcones that could be used as anticancer agents for the treatment of breast cancer. Library was screened against human mammary carcinoma cell line MCF7 and compound **1.140** was identified as lead showing an $IC_{50}$ value of ~0.4 nM on this cell line. Synthesis began with aldol condensation of hydroxy acetophenone **1.136** with 4-methoxy benzaldehyde **1.137** in the presence of NaOH in methanol. The resulting chalcone **1.138** was subjected to Sonogashira coupling with phenylacetylene **1.139** using palladium (II) catalyst and CuI co-catalyst in DMF/$H_2O$ (10:1) to afford the benzofuran chalcone **1.140**.[44]



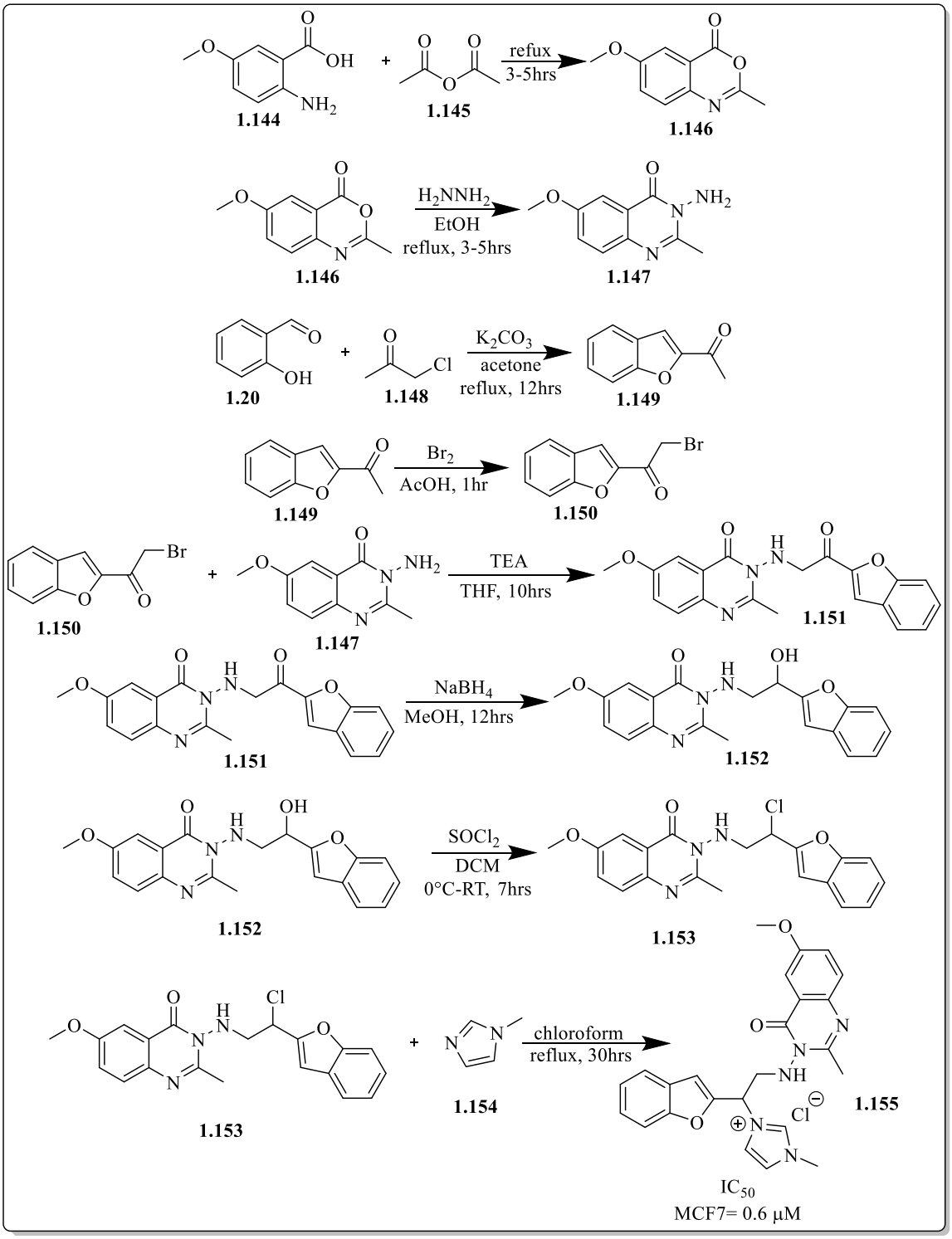

**Scheme 1.16**: Synthesis of benzofuran chalcone **1.140** as an anticancer agent.

Racané *et al.* discovered that 2-heteroaryl substituted 6-(2-imidazolinyl)benzothiazole mesylates could be used as potential anticancer agents. Chemical library was screened against human ductal pancreatic adenocarcinoma cell line CFPAC-1, human metastatic colorectal adenocarcinoma SW620, human liver hepatocellular carcinoma cell line HepG2, human cervical adenocarcinoma cell line HeLa, human lung fibroblasts WI38, and human skin fibroblasts cell lines BJ & HFF-1. Benzofuran **1.143** was identified as lead and had $IC_{50}$ values of ~2 μM on CFPAC-1, ~0.1 μM on SW620, ~1 μM on HepG2, ~0.7 μM on HeLa, and ~0.4 μM across fibroblast cell lines. Zwitterionic compound **1.141** from a previous study[45] was treated with **1.142** in a condensation reaction in acetic acid. Upon addition of methanesulfonic acid the mesylate salt **1.143** was obtained in 53% yield.[46]



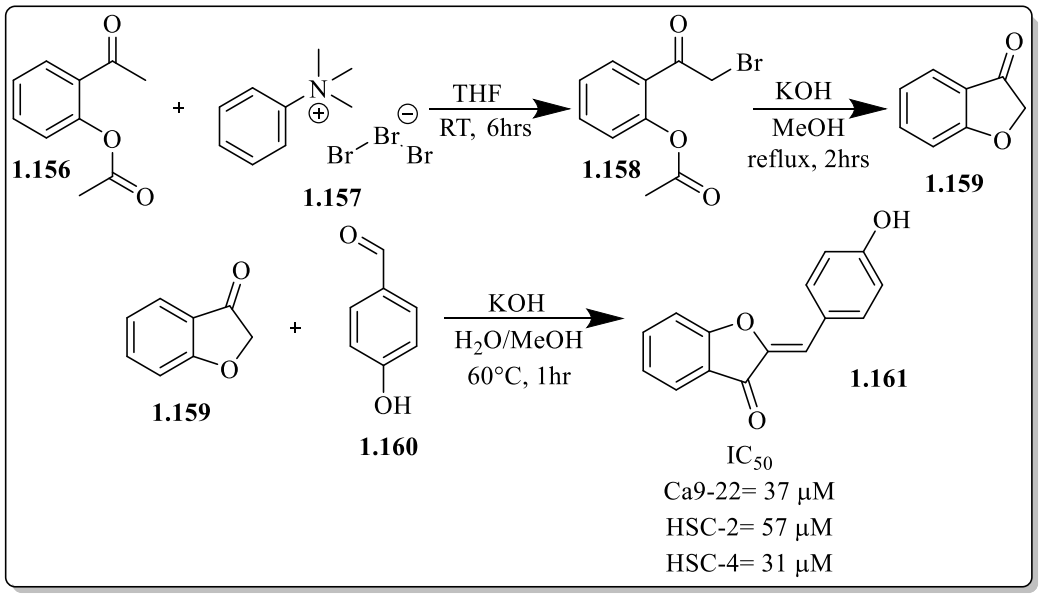

**Scheme 1.17**: Synthesis of benzofuran benzothiazole methanesulfonate **1.143** as a potential anticancer agent.

Asadi *et al.* discovered that compounds containing quinazoline, imidazolium, with benzofuran moieties could be used as potential anticancer agents. Benzofuran imidazolium salt **1.155** was identified as the lead with an $IC_{50}$ value of ~0.6 μM on human mammary carcinoma cell line MCF7. Synthesis of the quinazoline component began with acetylation of **1.144** with acetic anhydride **1.145** followed by dehydration to give the acetanthranil **1.146**. To obtain the free amine quinazoline derivative **1.147**, acetanthranil **1.146** was refluxed in the presence of hydrazine in ethanol. The synthesis of the benzofuran component began with Rap-Stoermer condensation of salicylaldehyde **1.20** with chloroacetone **1.148** to afford **1.149**. The 2-acetyl benzofuran **1.149** was brominated using bromine in acetic acid. In the presence of triethylamine base in THF, quinazoline **1.147** was alkylated with benzofuran acetylbromide **1.150** to obtain **1.151**. Following $NaBH_4$ reduction of **1.151**, the resulting alcohol **1.152** was chlorinated with thionyl chloride in DCM to obtain the chloride **1.153**. The chloro derivative **1.153** was immediately treated with methyl imidazole **1.154** in chloroform to afford the final imidazolium salt **1.155**.[47]



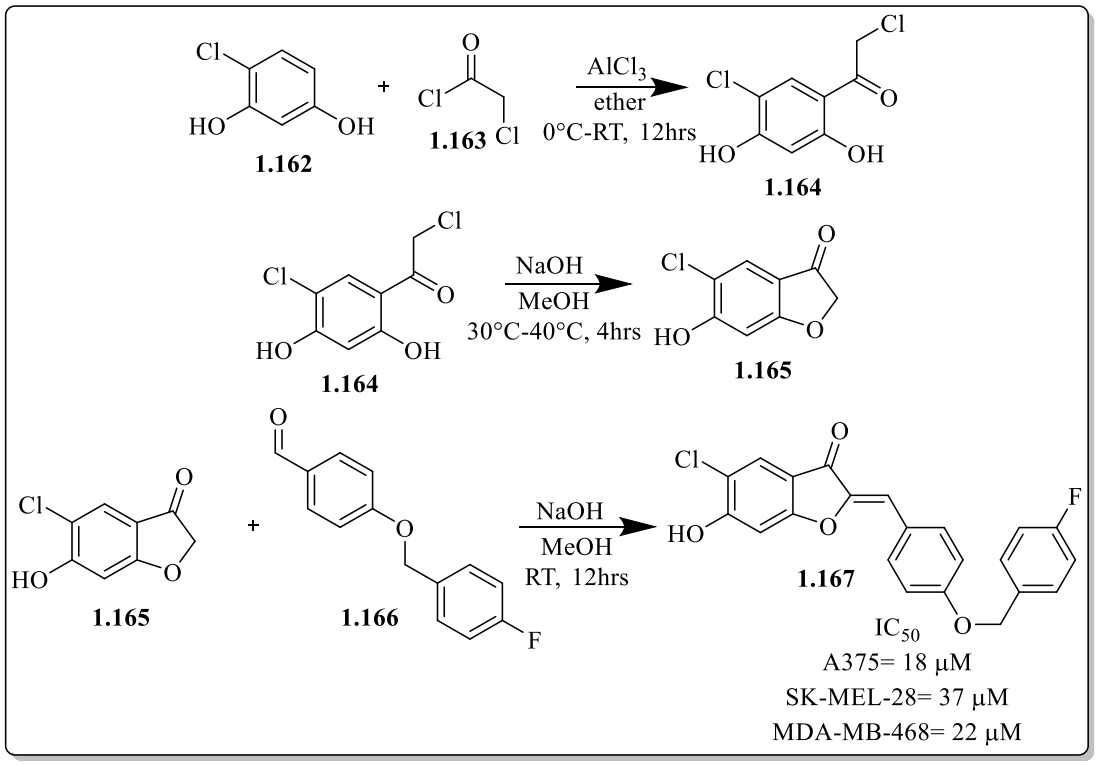

**Scheme 1.18**: Synthesis of quinazoline imidazolium benzofuran salt **1.155** as a potential anticancer agent.

Uesawa *et al.* discovered that aurones could be used as potential anticancer agents in the treatment of oral squamous carcinoma. Compounds were screened against human gingival squamous carcinoma cell line Ca9-22, human oral cavity squamous carcinoma cell line HSC-2, and tongue squamous carcinoma cell line HSC-4. Benzofuran **1.161** was identified as lead with $IC_{50}$ values of 37 μM on Ca9-22, 57 μM on HSC-2, and 31 μM on HSC-4.[48] Synthesis of derivatives began with treating acetylated acetophenone **1.156** with **1.157** in THF to obtain brominated derivative **1.158**. After refluxing in the presence of a base afforded the dihydrobenzofuranone **1.159**. The dihydrobenzofuranone **1.159** underwent an aldol condensation with aldehyde **1.160** in the presence of KOH to give aurone **1.161** in 23% yield.[49]



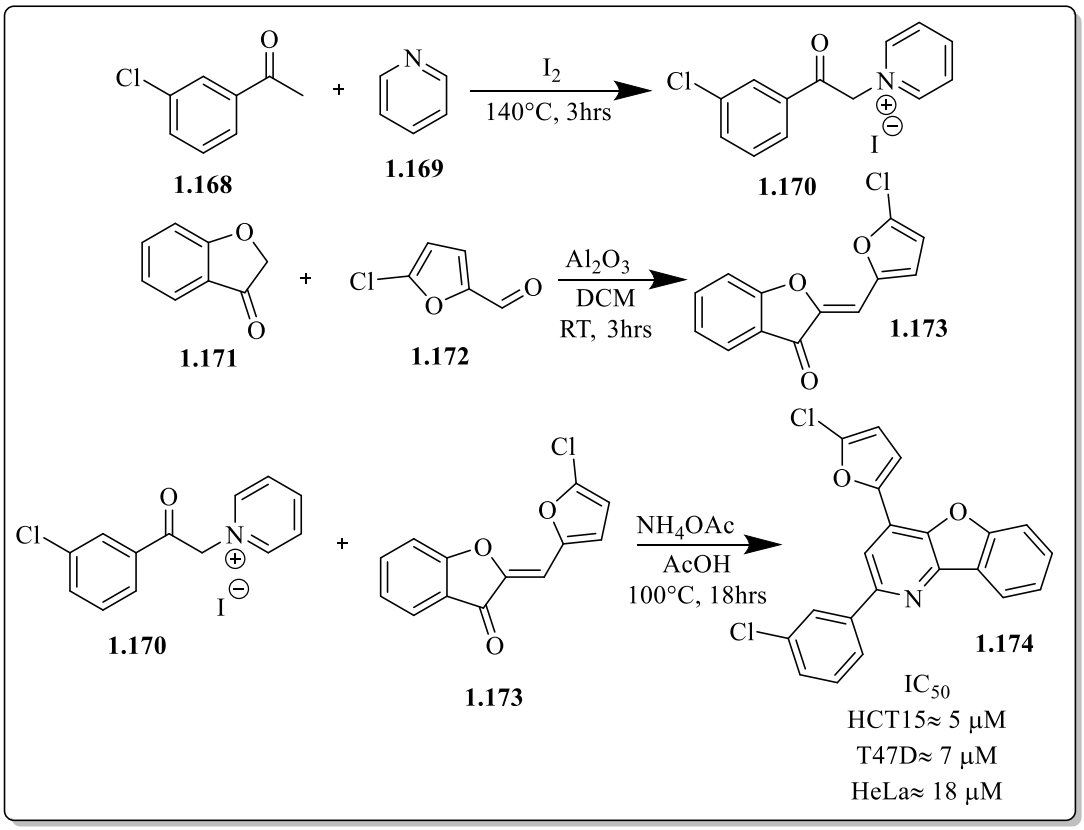

**Scheme 1.19**: Synthesis of benzofuranone **1.161** as an anticancer agent.

Kadasi *et al.* discovered that aurones could be used as potential anticancer agents. Compound **1.167** was identified as the lead derivative after screening against human skin malignant melanoma cell line A375, human skin malignant melanoma cell line SK-MEL-28, and human mammary gland adenocarcinoma cell line MDA-MB-468. Kadasi *et al.* found $IC_{50}$ values for **1.167** to be ~18 μM on A375, ~37 μM on SK-MEL-28, and ~22 μM on MDA-MB-468. Synthesis was achieved through Friedel-Crafts acylation of **1.162** in ether by treating with 2-chloroacetyl chloride **1.163** using $AlCl_3$ catalyst to obtain **1.164**. Acylated product **1.164** was heated in the presence of a base to obtain dihydrobenzofuranone **1.165**. Resulting dihydrobenzofuranone **1.165** was subjected to aldol condensation with **1.166** in the presence of NaOH to obtain aurone **1.167** in 72% yield.[50]




**Scheme 1.20**: Synthesis of substituted benzofuranone **1.167** as an anticancer agent.

Thapa Magar *et al.* discovered that 2-chlorophenyl-substituted benzofuro[3,2-b]pyridines could be used as potential anticancer agents. Chemical library was screened against human colorectal carcinoma cell line HCT15, human mammary gland ductal carcinoma cell line T47D, and human cervical adenocarcinoma cell line HeLa. Compound **1.174** was identified as lead with $IC_{50}$ values of ~5 μM, ~7 μM, and ~18 μM on the cell lines. Synthesis began with the iodization of **1.168** with iodine followed by alkylation to pyridine **1.169** to obtain the iodide salt **1.170**. Additionally, dihydrobenzofuranone **1.171** was treated with **1.172** and underwent an aluminum oxide catalyzed condensation to afford aurone **1.173**. The iodide salt **1.170** and aurone **1.173** were subjected to a Knoevenagel condensation reaction using ammonium acetate as base to obtain the final compound **1.174**.[51]




**Scheme 1.21**: Synthesis of chlorinated benzofuropyridine **1.174** as an anticancer agent.

Owing to our group's longstanding interest in developing novel anticancer agents has prompted us to explore benzofurans as potential therapeutics for cancer treatment. As can be seen above, a wide variety of substituted benzofurans exhibit potent cell proliferation inhibition properties via different molecular mechanisms of action. One particular literature report on the synthesis of piperazine substituted *N*,*N*-diethyl benzoylbenzofuran **1.135** (**Scheme 1.15**) has caught our attention due to the ease of preparation and good cytotoxic properties.[43] These candidate compounds can be readily prepared via Rap-Stoermer condensation, followed by ipso substitution with piperazine. Although benzofurans and piperazine have a track record of high metabolic stability, but the presence of *N*,*N*-diethyl group in **1.135** makes them susceptible for CYP450 enzymatic N-dealkylation and elimination. In this regard, we undertook a project on the development of metabolically stable piperazino benzoylbenzofurans as potential anticancer agents.

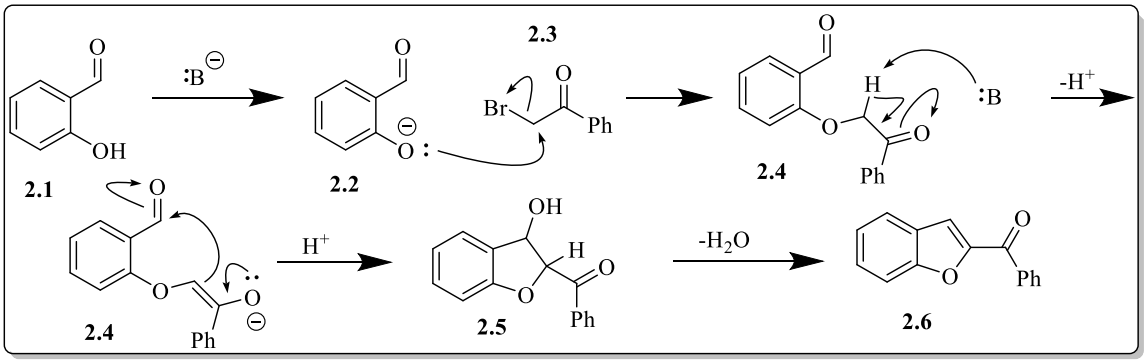
## Chapter 2: Synthesis and Evaluation

### 2.A Rap-Stoermer condensation for synthesis of benzofurans

Rap-Stoermer condensation is a very robust synthetic reaction to obtain 2-aroylbenzofurans through the base catalyzed condensation of salicylaldehydes with phenacyl halides (**Scheme 2.1**). The reaction was first identified in 1895 by Edoardo Rap from the reaction of salicylaldehyde, phenacyl bromide, and KOH in ethanol.[52] The reaction was further characterized in 1900 by Richard Stoermer and has hence forth been termed Rap-Stoermer condensation.[53] The Rap-Stoermer condensation initially was carried out in the presence of caustic bases like KOH and NaOH using ethanol as solvent. These reactions typically had low to moderate yields, and could not be carried out in the presence of base sensitive functional groups.[54,55] Although Rap-Stoermer condensations have classically been carried out in ethanol, the reaction is not limited to only polar protic solvents. Different solvents have been utilized such as water,[56,57] acetonitrile,[25,58,59] dimethylformamide,[26,33,39] tetrahydrofuran,[60] acetone,[29,30,32,43,47] and even phase-transfer reactions in water/dichloromethane[61] to carry out Rap-Stoermer type condensations.

An attempt to discover a more environmentally friendly synthetic strategy for benzofuran production was explored by Yoshizawa et. al. in 2003 using a solvent-free approach. Reactions of salicylaldehyde and phenacyl bromide using $K_2CO_3$ refluxing in ethanol for 3 hours was found to have yields of 70-90% when compared to solvent-free conditions which provided almost quantitative yields. Additionally, this group set out to better characterize the Rap-Stoermer reaction mechanism (**Scheme 2.1**) via IR spectral monitoring in Nujol mulls. Upon addition of $K_2CO_3$ to salicylaldehyde **2.1**, the original C=O absorption band of 1664 $cm^{-1}$ decreased while the formation of a new C=O absorption

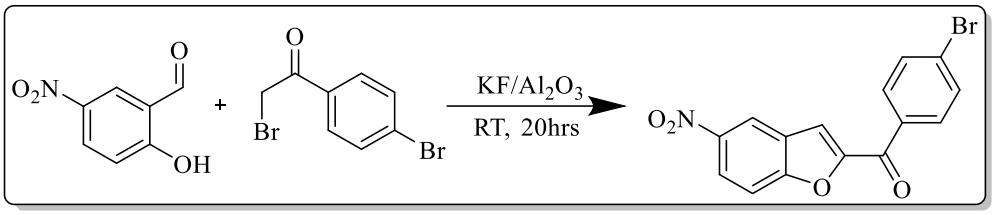
band at 1670 $cm^{-1}$ was observed, attributed to the formation of potassium phenoxy salt **2.2**. After the addition of the phenacyl bromide **2.3**, the 1670 $cm^{-1}$ absorption band decreased with the presence of two new bands appearing at 1696 $cm^{-1}$ and 1641 $cm^{-1}$ respectively suggesting the formation of intermediate **2.4**. After further reaction time it was found that only the 1696 $cm^{-1}$ absorption band decreased, with the presence of a hydroxyl absorption band at 3465 $cm^{-1}$ supporting the formation of the ketoalcohol intermediate **2.5**. The 1641 $cm^{-1}$ absorption band matched the C=O absorption band in the resulting benzofuran intermediate **2.5** and further reaction time resulted in the decrease of the 43ydroxyl absorption band. To verify the ketoalcohol intermediate, the reaction mixture was extracted with ether, characterized via IR and subsequently converted into benzofuran **2.6** under Rap-Stoermer condensation conditions.[62] The general reaction mechanism for Rap-Stoermer condensation is depicted in **Scheme 2.1**.




**Scheme 2.1:** Representative mechanism for base catalyzed Rap-Stoermer condensation characterized via IR spectral monitoring.[62]

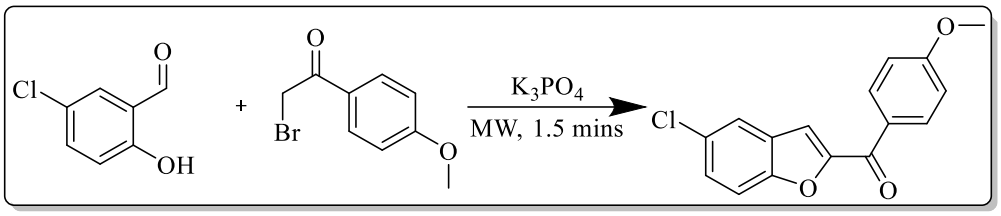
In 2008, Sharifi et. al. discovered a novel solvent-free reaction condition for the synthesis of 2-aroylbenzofurans via Rap-Stoermer condensation using $KF/Al_2O_3$ at room temperature. This approach allowed for high functional group compatibility including

electron donating and electron withdrawing salicylaldehydes to be used to obtain greater than 90% yield.[63]



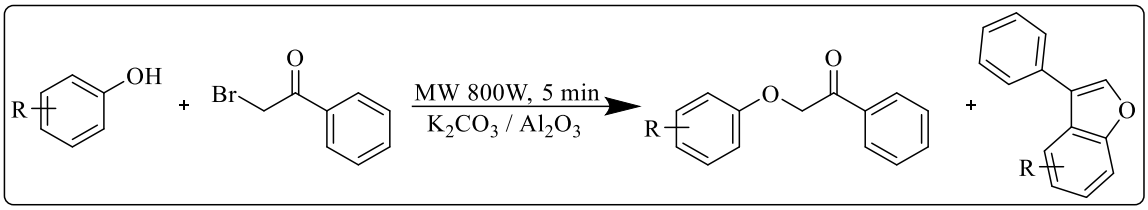

**Scheme 2.2:** Example of solvent-free Rap-Stoermer reaction using $KF/Al_2O_3$ with an electron withdrawing substituent on salicylaldehyde.[63]

The development of solvent-free Rap-Stoermer conditions to synthesize benzofuran derivatives have led to further optimization of new solvent-free conditions. In 2007, Rao et. al. employed the use of solvent-free microwave mediated conditions to rapidly synthesize 2-aroylbenzofurans in minutes. After exploring inorganic bases such as NaOAc, KOAc, $Na_2CO_3$, $K_2CO_3$, and $K_3PO_4$ it was found that $K_3PO_4$ was the most effective at producing benzofurans in high yields. Variation in microwave irradiation power was explored, finding that 600 W was the most consistent at obtaining high yields of benzofurans, but the reaction still occurred at varying powers. This microwave mediated solvent free Rap-Stoermer was carried out with a variety of 3,5 mono- and di-substituted salicylaldehyde derivatives showing its versatility in the generation of 2-aroylbenzofurans.[64]




**Scheme 2.3:** Example of solvent-free microwave-mediated Rap-Stoermer condensation using $K_3PO_4$ as base.[64]

Solvent-free Rap-Stoermer condensation reactions have been further modified by Wang et. al. in 2009. Using microwave-assisted organic synthesis and mineral-supported reagents, the Rap-Stoermer scope has been expanded to utilizing phenols versus salicylaldehydes for the formation of 3-arylbenzofurans. The reaction involves the initial formation of the phenoxy intermediate. After trying a spectrum of inorganic bases, it was found that $Na_2CO_3$ gave the best benzofuran selectivity (70% benzofuran, <1 % phenoxy) whereas $K_2CO_3$ gave the highest overall benzofuran yield (80% benzofuran, 10% phenoxy). When comparing mineral-supported reagents such as neutral $Al_2O_3$, basic $Al_2O_3$, acidic $Al_2O_3$, silica gel, and molecular sieves as catalysts it was found that neutral $Al_2O_3$ gave the best benzofuran yield (80% benzofuran, 10% phenoxy). Under $K_2CO_3$ and neutral $Al_2O_3$ conditions using 800 W microwave irradiation power yielded benzofurans in variable yield (20-80%) from a variety of phenol derivatives.[65] This novel one-pot synthesis of benzofurans from phenols offers a pathway of obtaining diverse 3-arylbenzofurans from simple phenols.




**Scheme 2.4:** Synthesis of 3-arylbenzofurans via microwave-mediated solid state Rap-Stoermer condensation conditions using $K_2CO_3/Al_2O_3$.[65]

Typical Rap-Stoermer procedures require bases that can hinder the potential for benzofuran synthesis with base sensitive functional groups such as esters, nitriles and certain protecting groups. To overcome this problem, Meshram et. al. explored the novel use of organic base 1,4-diazabicyclo[2.2.2]octane (DABCO) to mediate the Rap-Stoermer condensation. It was found that 20 mol% of DABCO in THF at room temperature successfully allowed for the synthesis of a variety of benzofuran derivatives. This new discovery broadens the scope of the Rap-Stoermer condensation by allowing the usage of base sensitive functional groups also.[60]

**2.B Applications of Benzofuran Structural Unit in Medicine**

Benzofurans are biologically privileged structures and can been found in a variety of natural products.[11–13] Because of their biological prevalence, several analogs of benzofurans have been synthesized and used to treat cardiovascular diseases, depression, renal disorders and cancer.[1–8] Additionally, several new derivatives containing the benzofuran moiety have entered clinical trials as anticancer agents showing nM inhibitory concentrations *in vitro* and *in vivo.*[14–19] The benzofuran structure is not limited to human

pathologies as many potent antimicrobial agents have been developed showing greater antimicrobial activity than known antimicrobial agents.[20–24]

## 2.C Applications of Piperazine Structural Unit in Drug Discovery

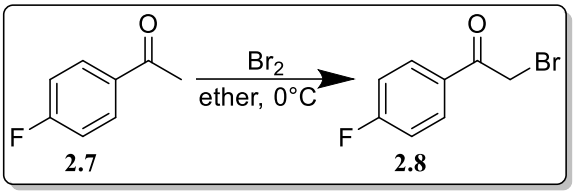
Piperazine is considered as a highly privileged structure in drug discovery. It consists of two nitrogen atoms symmetrically aligned in a six-membered ring. This heterocyclic moiety was first introduced in 1953 as an anthelmintic agent.[66,67] Since then, piperazine has persisted with its use as an anthelmintic agent,[68,69] but has shown to have a variety of pharmacological uses such as: antidepressant,[70–74] anticancer,[75–79] antibacterial,[80–83] antipsychotic,[84–86] antihistamine,[87–91] antianginal.[92,93] The introduction of piperazine on a drug template greatly improves the water solubility, and this property has been well utilized as many clinically successful drugs have the piperazine moiety in them.

## 2.D Synthesis of piperazine containing benzofurans as potential anticancer agents

Because of the above-mentioned biological relevance of benzofurans and piperazines, we envisioned to synthesize novel piperazine containing benzofurans to create water soluble and potent anti-cancer agents. In this regard, we decided to pursue Rap-Stoermer condensation owing to its simplicity and structural diversity in obtaining benzofurans. As discussed in the beginning of the chapter, this reaction involves the condensation of a salicylaldehyde with phenacyl bromide in the presence of a base. The versatility of the Rap-Stoermer condensation reaction allows for structure activity

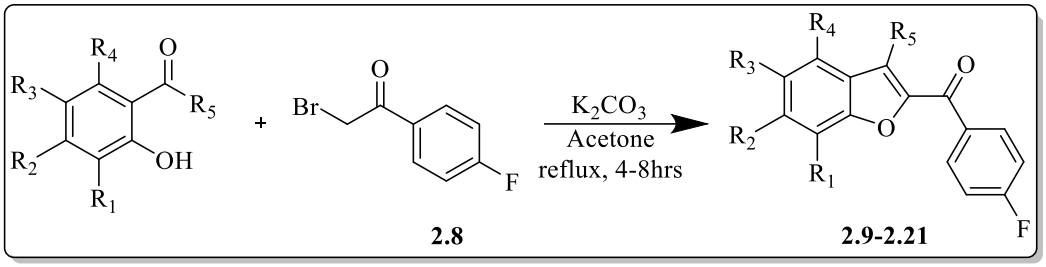
relationship studies evaluating the effect of substituents of the salicylaldehyde on biological activity.

Synthesis of the substituted benzofuran-2-yl(4-fluorophenyl)methanone derivatives **2.9**-**2.21** required the synthesis of 4-fluorophenacyl bromide **2.8**. To obtain the bromide, 4'-fluoroacetophenone **2.7** was dissolved in ether followed by the slow addition of bromine at 0°C. The reaction occurs within 45 minutes and leaving it for further time will lead to undesirable di and tri-brominated species. The monobromide **2.8** was isolated via column chromatography (5% EtOAc/hexanes).




**Scheme 2.5:** Synthesis of 2-bromo-1-(4-fluorophenyl)ethan-1-one **2.8**.

To generate the substituted benzofuran-2-yl(4-fluorophenyl)methanone library, a variety of commercially available salicylaldehydes containing electron donating or electron withdrawing groups on the phenyl ring were utilized. All respective aldehydes were treated with $K_2CO_3$ in acetone, followed by the addition of 4-fluorophenacyl bromide **2.8** and refluxed for 4-12 hours until completion of the reaction. The salicylaldehydes containing electron donating functional groups reacted notably slower with the phenoxy intermediate as observed via TLC, followed by the cyclized product after more time elapsed.




**Scheme 2.6**: Example synthesis of substituted benzofuran-2-yl(4-fluorophenyl)methanone derivatives **2.9**-**2.21**.

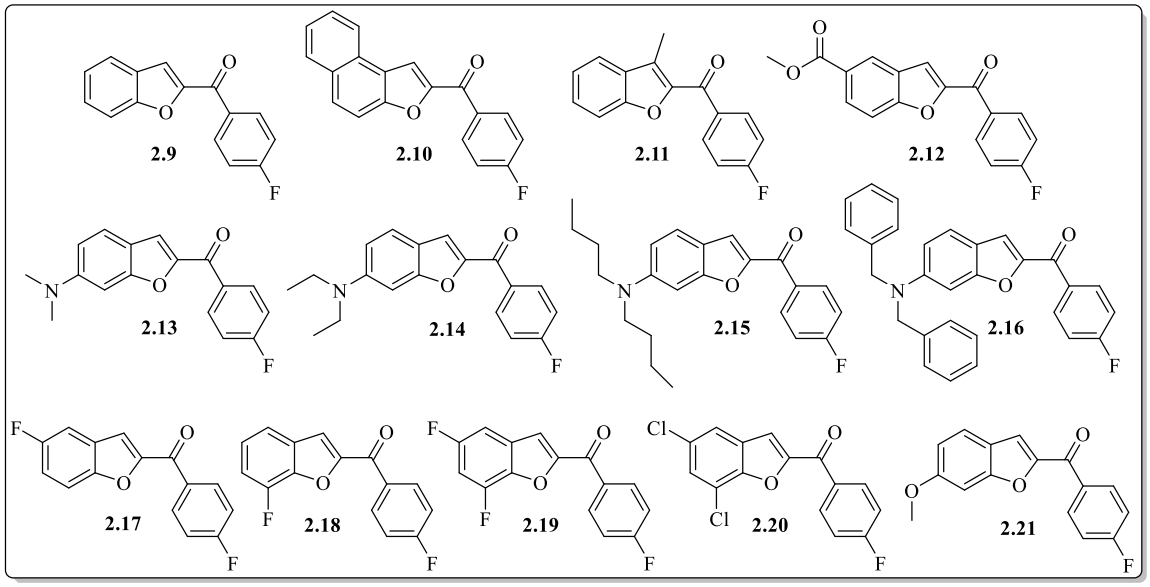
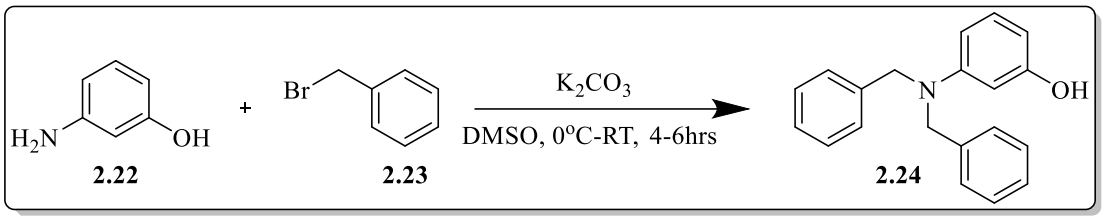


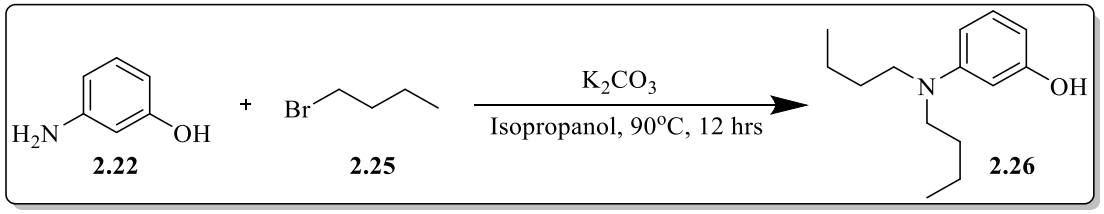

**Figure 2.1:** Substituted benzofuran-2-yl(4-fluorophenyl)methanone derivatives **2.9**-**2.21**.

The salicylaldehydes 4-(dibenzylamino)-2-hydroxybenzaldehyde **2.27** and 4-(dibutylamino)-2-hydroxybenzaldehyde **2.28** used were not commercially available and we synthesized them using known literature procedures. Aldehyde **2.27** was synthesized via 3-aminophenol **2.22** with benzyl bromide **2.23** in the presence of $K_2CO_3$ in DMSO at 10°C.

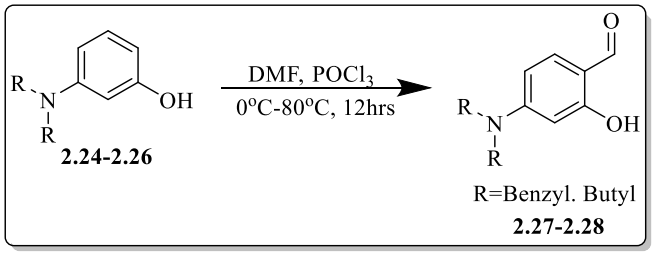



**Scheme 2.7:** Synthesis of 3-(dibenzylamino)phenol **2.24** from 3-aminophenol.

Aldehyde **2.28** was synthesized via alkylation of 3-aminophenol **2.22** with butyl bromide **2.25** in the presence of $K_2CO_3$ in isopropanol at 90°C. The dialkylated aminophenols **2.24** and **2.26** were subjected to Vilsmeier-Haack formylation conditions to yield salicylaldehydes **2.27** and **2.28** (**Scheme 2.9**).

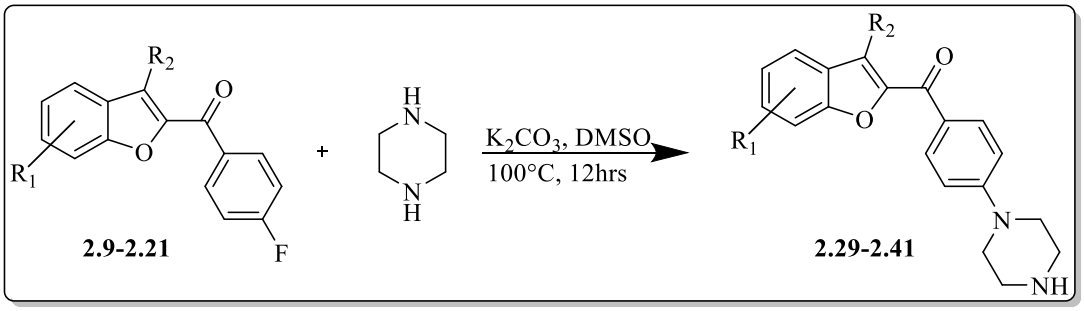



**Scheme 2.8:** Synthesis of 3-(dibutylamino)phenol **2.26** from 3-aminophenol.




**Scheme 2.9:** Synthesis of 4-(dialkylamino)-2-hydroxybenzaldehydes **2.27** and **2.28** from 3-(dialkylamino)phenol.

The synthesize 4-fluorobenzoylbenzofurans **2.9**-**2.21** were further functionalized by the addition of piperazine via IPSO substitution. The flurobenzofurans **2.9**-**2.21** were dissolved in DMSO and treated with piperazine in the presence of $K_2CO_3$ heated at 100°C, to yield the piperazino benzofurans **2.29**-**2.41** (**Scheme 2.10**).




**Scheme 2.10:** Synthesis of substituted benzofuran-2-yl(4-(piperazin-1-yl)phenyl)methanone derivatives via Ipso substitution from substituted benzofuran-2-yl(4-fluorophenyl)methanones.

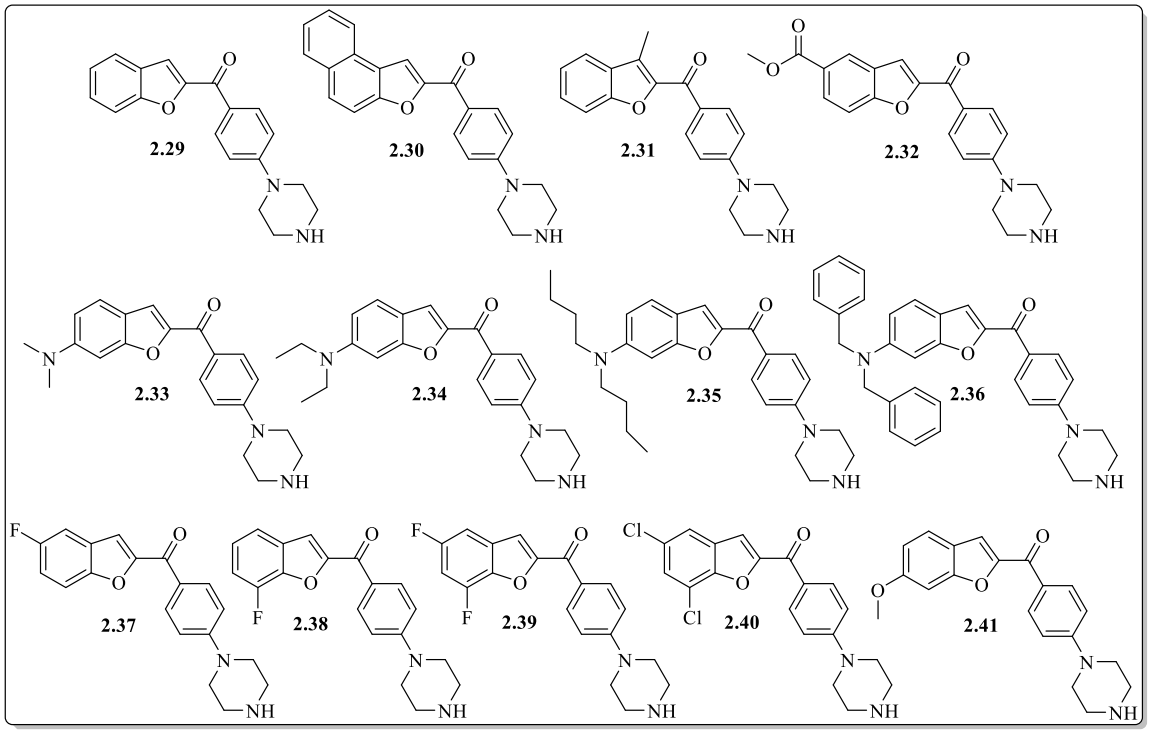
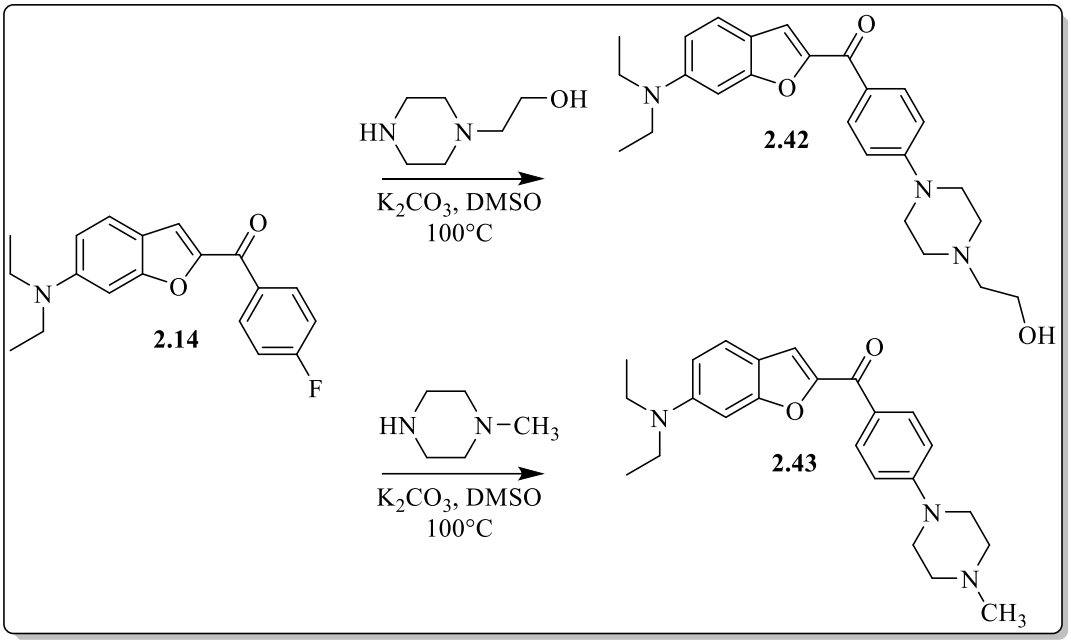


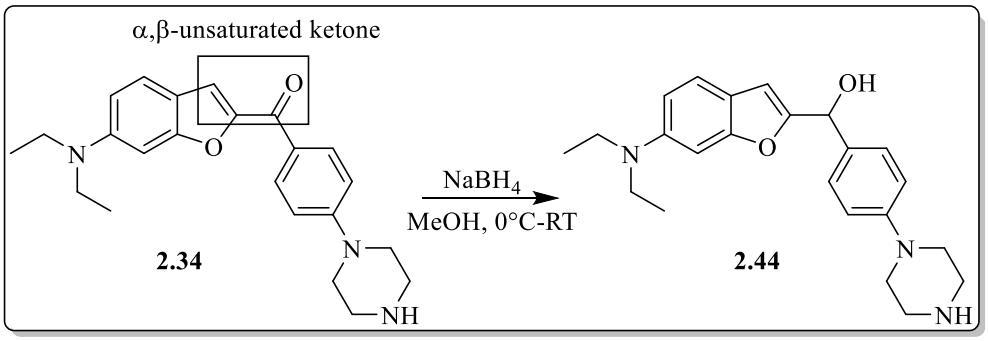

**Figure 2.2:** Substituted benzofuran-2-yl(4-(piperazin-1-yl)phenyl)methanone derivatives **2.29-2.41**.

To further assess the structure activity relationship (SAR) of the piperazine moiety, some piperazine substituted derivatives were synthesized via IPSO substitution. Compound **2.42** was synthesized using 1-(2-hydroxyethyl)piperazine as the secondary amine and was purified via column chromatography (70% EtOAc/hexanes – 10% MeOH/EtOAc). Compound **2.43** was synthesized using 1-methylpiperazine as the secondary amine and was purified via column chromatography (70% EtOAc/hexanes – 10% MeOH/EtOAc).




**Scheme 2.11**: Synthesis of substituted (6-(diethylamino)benzofuran-2-yl)(4-(piperazin-1-yl)phenyl)methanones **2.42** and **2.43**.

We then explored the role of α,β-unsaturated ketone in the benzofuran moiety in providing the biological activity. In this regard, the ketone group was reduced to alcohol using sodium borohydride in methanol to obtain the alcohol **2.44**.




**Scheme 2.12:** Reduction of α,β-unsaturated ketone in **2.34** to alcohol **2.44**.

## 2.E MTT Cell Viability Assay

To evaluate the *in vitro* cell proliferation inhibition properties of the synthesized derivatives **2.29**-**2.41**, they were subjected to the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) based cell proliferation assay. Cells were seeded at $5x10^4$ cells/mL in a 96-well plate, followed by 72 hr incubation with serial diluted test compounds. Living cells reduce MTT to insoluble formazan, which can be dissolved upon cell lyses using SDS, allowing absorbance of formazan at 570 nm to be measured. The test compounds were then evaluated as a percent of the control (vehicle only) group to determine their ability to inhibit cell proliferation. These percentages were plotted against the logarithmic concentration values to generate a sigmoidal percent viability growth curve. From this curve, $IC_{50}$ values were obtained, which represent the concentration for which the test compounds inhibited 50% of cell proliferation compared to the control group.

For all the synthesized compounds **2.29**-**2.41**, $IC_{50}$ values were obtained for 6 cancerous cell lines, which include MDA-MB-231, 4T1, 67NR, MCF7, WiDr, and MIA PaCa-2. The cell line MDA-MB-231 is a human mammary gland epithelial adenocarcinoma derived from a metastatic site. This cell line is also a triple-negative breast cancer meaning it lacks estrogen receptor, progesterone receptor, and human epidermal growth factor receptor 2 (HER2). Triple negative breast cancer is an aggressive cancer with limited treatment options, using MDA-MB-231 cell line serves as a model to assess the cytotoxicity of synthesized compounds. MCF7 is an estrogen and progesterone receptor positive human mammary gland epithelial adenocarcinoma cell line derived from a metastatic site. This cell line represents a model of hormone sensitive breast cancer cell

line which affects approximately 2 out of every 3 patients diagnosed with breast cancer.[94] The 4T1 cell line is a metastatic stage IV murine mammary gland epithelial cell line that serves as a model for highly malignant metastatic breast cancer. 67NR is derived from the same stage IV tumor site as 4T1 only they are less metastatic. The WiDr cell line is a human epithelial colorectal adenocarcinoma used as a model for colorectal cancer. The MIA PaCa-2 cell line is a human epithelial pancreatic carcinoma cell line used as a model for pancreatic cancer.

Against the metastatic breast cancer cell line 4T1, dibenzylic compound **2.36** containing an electron donating substituent on the benzofuran ring, was the most potent with an $IC_{50}$ value of ~3μM (**Figure 2.3**, **D**). When the substituent was switched with an electron withdrawing substituent such as a methyl ester in compound **2.32**, potency was reduced with an $IC_{50}$ value of ~8 μM. The neutral simple benzofuran derivative **2.29** had an $IC_{50}$ value of ~9 μM which was three times less potent than **2.36**, further supporting the importance of an electron donating substituent on the benzofuran ring. Another derivative containing an electron donating *N*,*N*-diethyl group **2.34** had a low $IC_{50}$ value of ~4 μM. Dichlorinated benzofuran compound **2.40** of the electron withdrawing series also had a low $IC_{50}$ value of ~5 μM, which could be attributed from an increased metabolic stability found by the addition of chlorine to benzene rings. It is of note that activity is greatly reduced with increase in the carbon chain of the *N*,*N*-dialkyl derivatives with the dibutyl compound **2.35** having an $IC_{50}$ of ~30 μM.

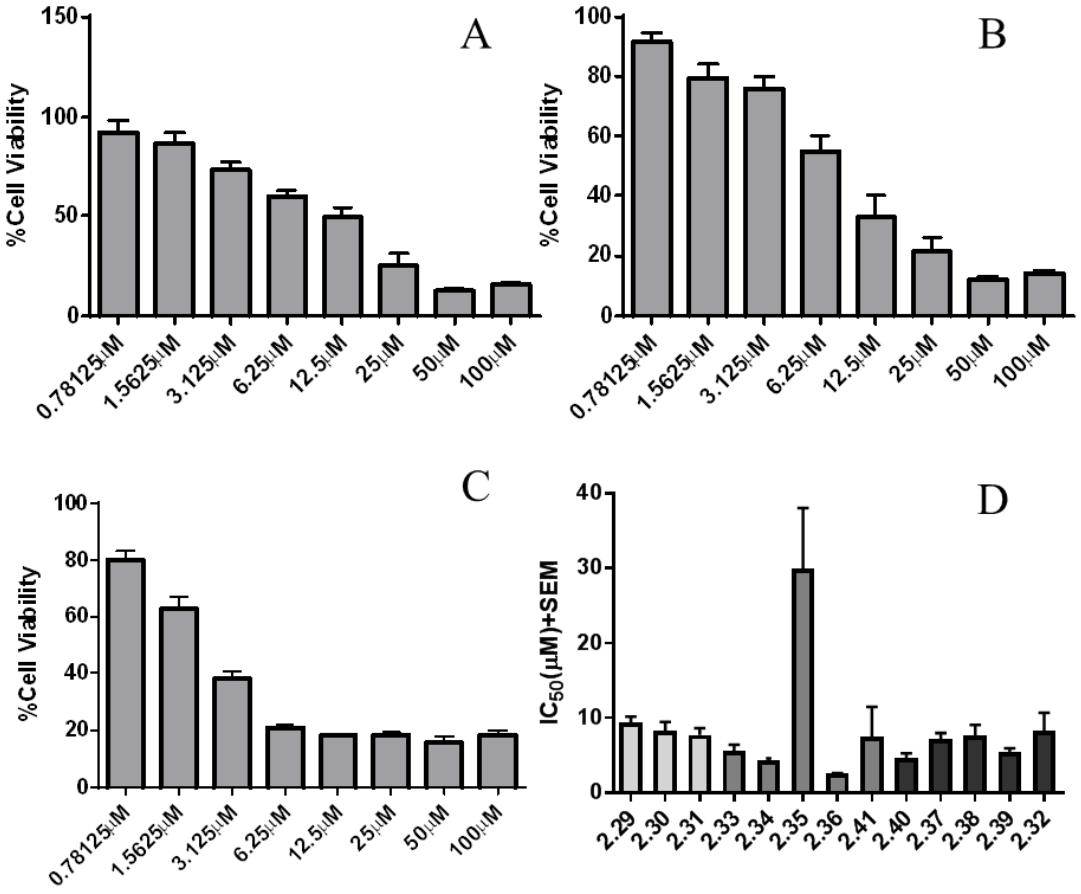



**Figure 2.3:** (A-C) percent cell viability of 4T1 against serial diluted concentrations (100-0.78 μM) of neutral (A), electron withdrawing (B), and electron donating (C) example derivatives **2.29**, **2.32**, and **2.36** respectively. D contains inhibitory concentrations (μM + SEM) of all derivatives **2.29-2.41** grouped as neutral, electron donating, and electron withdrawing.

Against the cell line 67NR, the dibenzylic compound **2.36** was the most potent with an $IC_{50}$ value of ~2 μM (**Figure 2.4**, **D**). Additionally, from the electron donating group containing series, diethyl compound **2.34** had an $IC_{50}$ value of ~5 μM. The simple neutral

benzofuran derivative **2.29** had an $IC_{50}$ value of ~27 μM. Adding an electron withdrawing group decreased potency compared to **2.36** with a larger $IC_{50}$ value of ~13 μM. As with 4T1, the longer alkyl chain of **2.35** reduced potency significantly with an $IC_{50}$ value of ~42 μM.

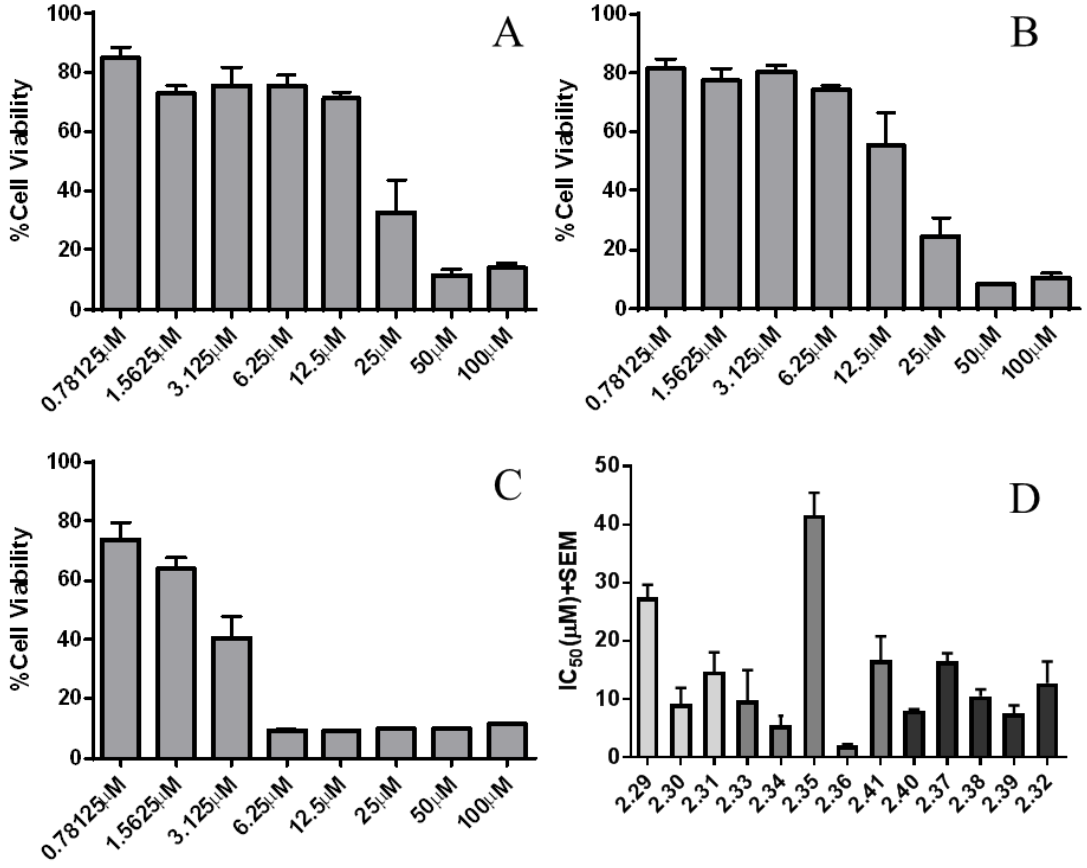



**Figure 2.4:** (A-C) percent cell viability of 67NR against serial diluted concentrations (100-0.78 μM) of neutral (A), electron withdrawing (B), and electron donating (C) example derivatives **2.29**, **2.32**, and **2.36** respectively. D contains inhibitory concentrations (μM + SEM) of all derivatives **2.29-2.41** grouped as neutral, electron donating, and electron withdrawing.

Against the triple negative breast cancer cell line MDA-MB-231, dibenzylic compound **2.36** was the most potent with an $IC_{50}$ value of ~4 μM. The simple benzofuran derivative **2.29** had an $IC_{50}$ value of ~25 μM which is over six times less potent than **2.36**. From the neutral containing derivatives, naphthalene compound **2.30** had an $IC_{50}$ value of ~12 μM which is only slightly higher than the second most potent dichlorinated derivative **2.40** against MDA-MB-231, with an $IC_{50}$ value of ~9 μM respectively. The addition of an electron withdrawing group in the methyl ester **2.32** reduced potency significantly with an $IC_{50}$ value of ~36 μM.

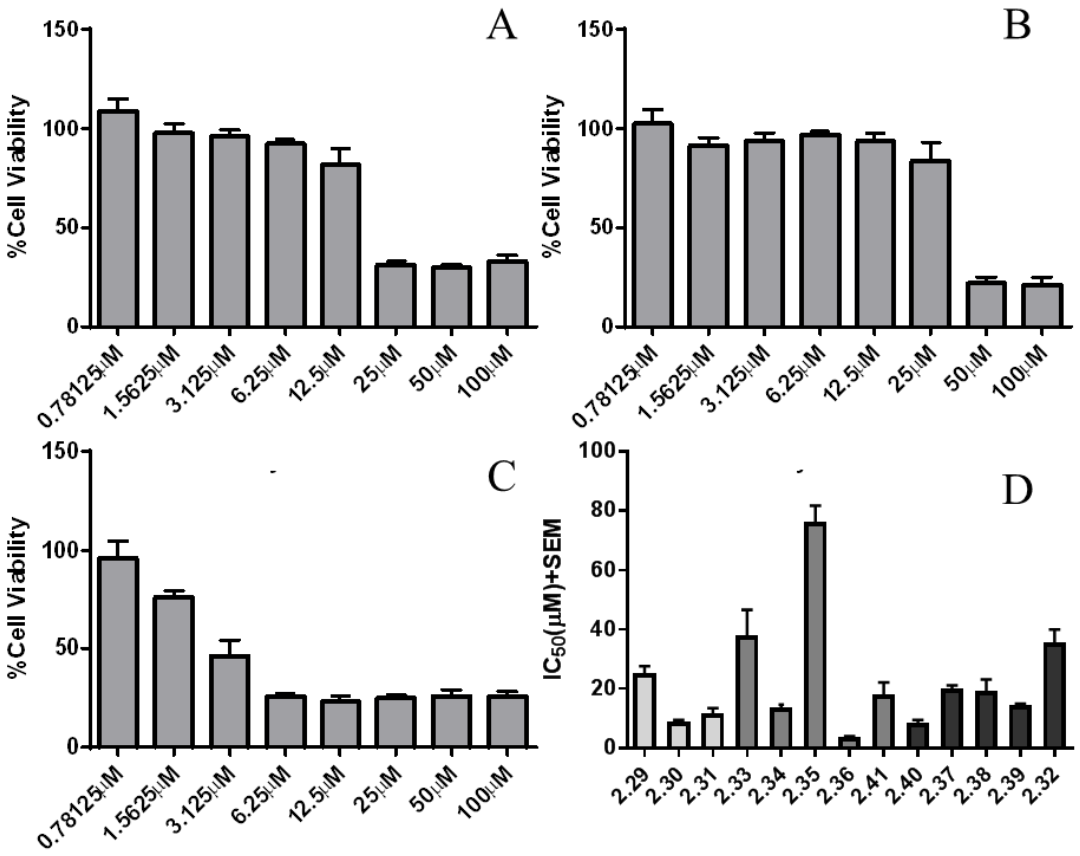



**Figure 2.5:** (A-C) percent cell viability of MDA-MB-231 against serial diluted concentrations (100-0.78 μM) of neutral (A), electron withdrawing (B), and electron donating (C) example derivatives **2.29**, **2.32**, and **2.36** respectively. D contains inhibitory concentrations (μM + SEM) of all derivatives **2.29-2.41** grouped as neutral, electron donating, and electron withdrawing.

Against the estrogen receptor positive breast cancer cell line MCF7, compound **2.36** was the most potent with an $IC_{50}$ value of ~3 μM. The simple neutral benzofuran **2.29** had an $IC_{50}$ value of ~14 μM, approximately four times less potent than the dibenzylic **2.36**. The presence of a strong electron withdrawing group decreased potency significantly, with

an $IC_{50}$ value of ~51 μM for methyl ester **2.32**. However, the weakly electron withdrawing halogen series had significantly more potent $IC_{50}$ values than **2.32** with $IC_{50}$ values for **2.40**, **2.38**, and **2.39** of ~4 μM, ~6 μM, and ~7 μM respectively.

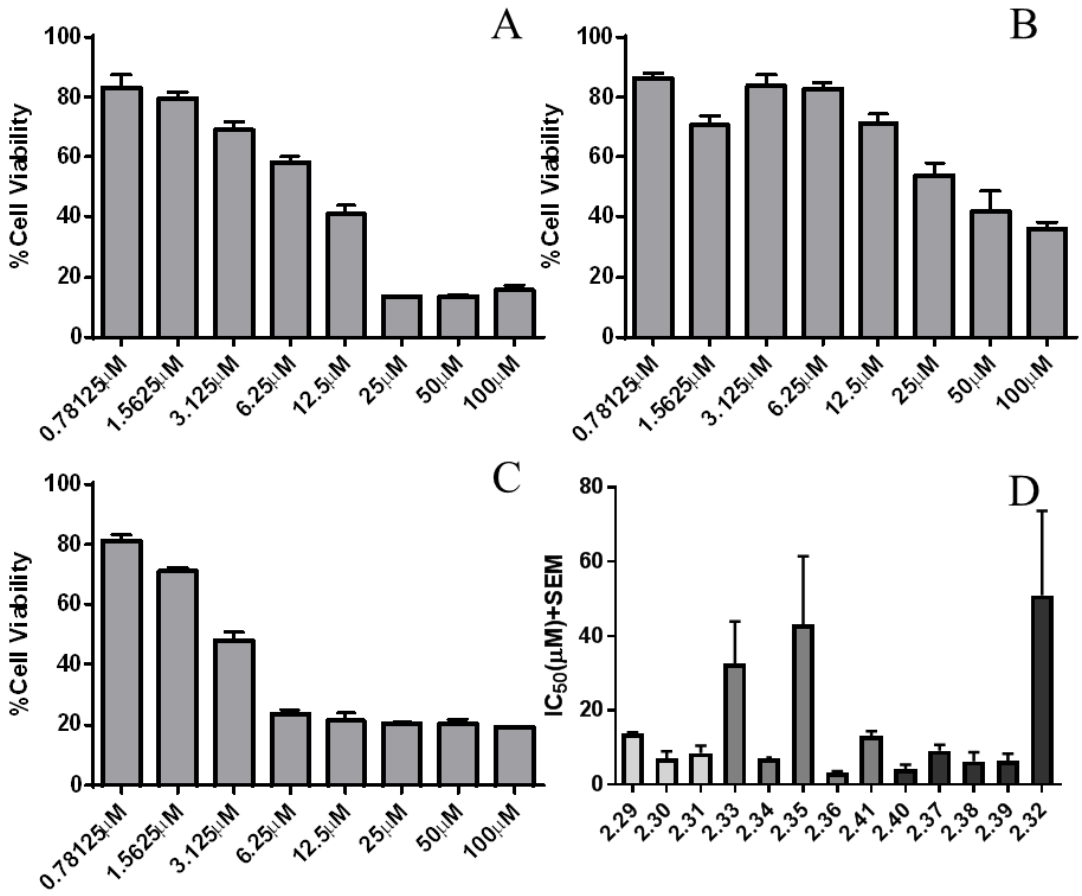



**Figure 2.6:** (A-C) percent cell viability of MCF7 against serial diluted concentrations (100-0.78 μM) of neutral (A), electron withdrawing (B), and electron donating (C) example derivatives **2.29**, **2.32**, and **2.36** respectively. D contains inhibitory concentrations (μM + SEM) of all derivatives **2.29-2.41** grouped as neutral, electron donating, and electron withdrawing.

Against the colorectal cell line WiDr, the dichlorinated benzofuran compound **2.40** was the most potent with an $IC_{50}$ value of ~2 μM. All derivatives were found to have $IC_{50}$ below 10 μM, however the methyl ester **2.32** of the electron withdrawing series had a reduced potency with an $IC_{50}$ value of ~8 μM. The simple neutral benzofuran **2.29** had an $IC_{50}$ value of ~5 μM. Of the electron donating series compounds dimethyl **2.33**, diethyl **2.34**, and dibenzyl **2.36** had similar potencies with $IC_{50}$ values of ~2 μM, ~2 μM, and ~3 μM respectively. The longer alkyl chain derivative **2.35** had a lower $IC_{50}$ value of ~7 μM.

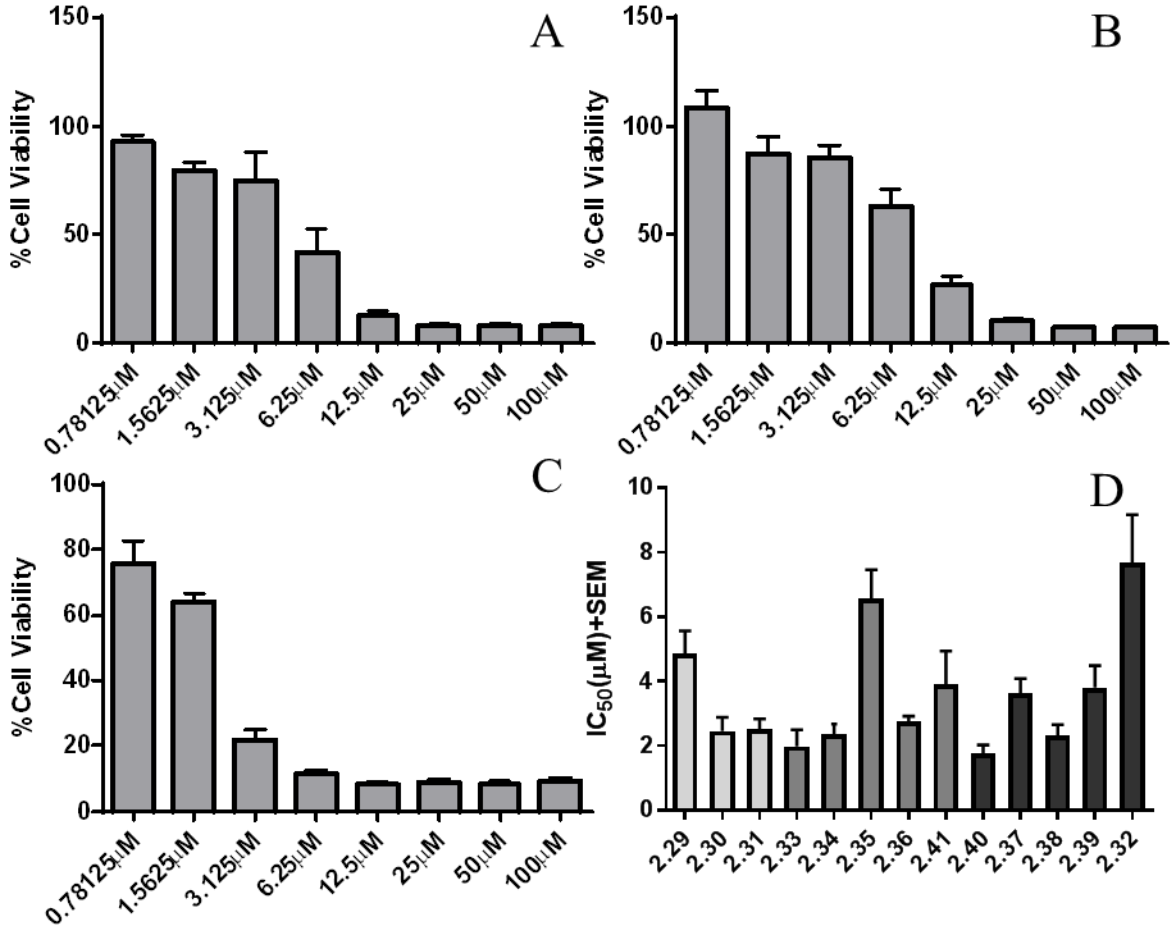



**Figure 2.7:** (A-C) percent cell viability of WiDr against serial diluted concentrations (100-0.78 μM) of neutral (A), electron withdrawing (B), and electron donating (C) example derivatives **2.29**, **2.32**, and **2.40** respectively. D contains inhibitory concentrations (μM + SEM) of all derivatives **2.29**-**2.41** grouped as neutral, electron donating, and electron withdrawing.

Against the pancreatic cancer cell line MIA PaCa-2, compound **2.40** was the most potent with an $IC_{50}$ value of ~3 μM. The electron donating substituent containing derivative

**2.36** had similar potency with an $IC_{50}$ value of ~4 μM. The simple neutral benzofuran **2.29** had an $IC_{50}$ value of ~22 μM, about a five-fold decrease in potency. The presence of a strong electron withdrawing group found in **2.32** similarly reduced potency but to a lesser extent with an $IC_{50}$ value of ~17 μM. Against this cell line, replacing the hydrogen in the 3 position on the benzofuran with a methyl found on **2.31**, there was an increase in potency when compared to **2.29** with an $IC_{50}$ value of ~5 μM.

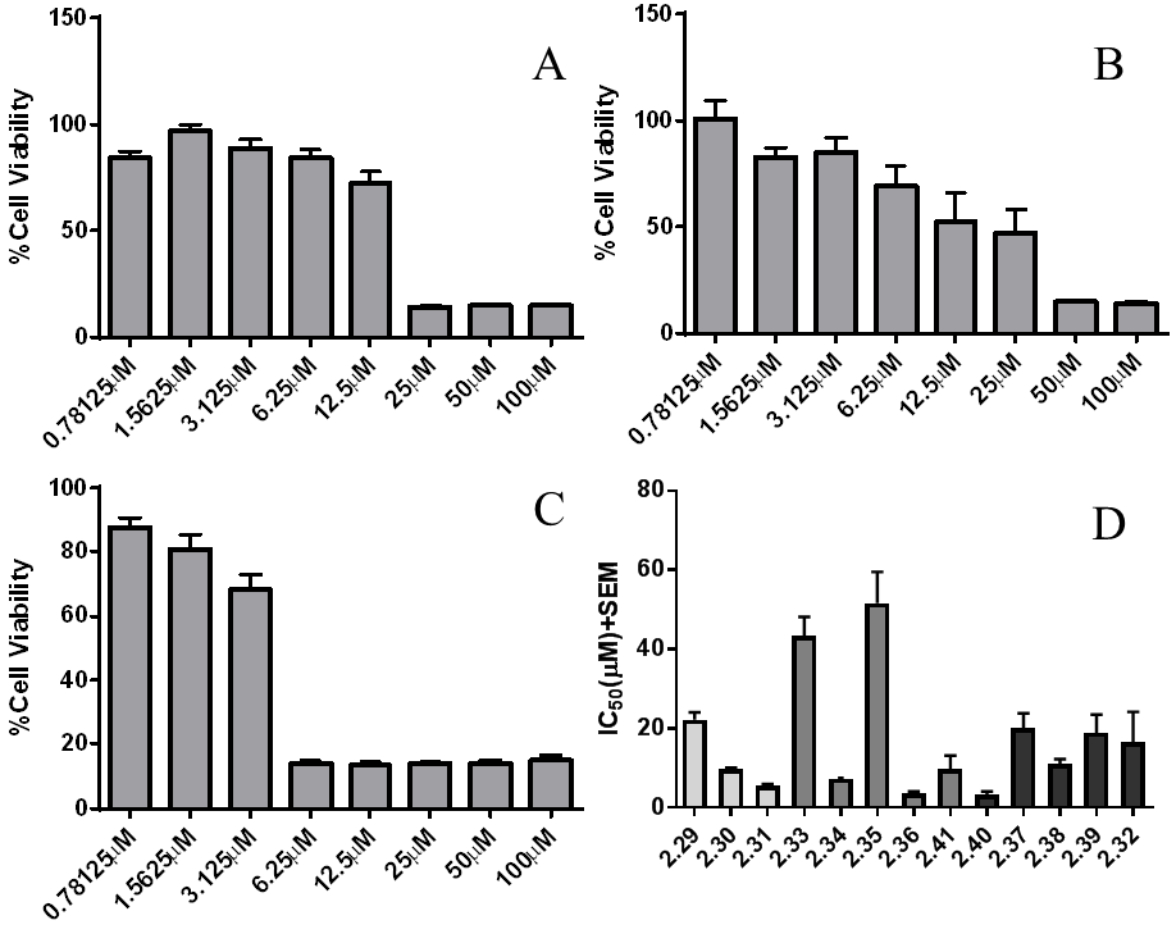



**Figure 2.8:** (A-C) percent cell viability of MIA PaCa-2 against serial diluted concentrations (100-0.78 μM) of neutral (A), electron withdrawing (B), and electron donating (C) example derivatives **2.29**, **2.32**, and **2.40** respectively. D contains inhibitory concentrations (μM + SEM) of all derivatives **2.29-2.41** grouped as neutral, electron donating, and electron withdrawing.

It is important to note that after synthesis of the substituted benzofuran-2-yl(4-fluorophenyl)methanones **2.9**-**2.21**, these compounds were also evaluated against 6 cancer

cell lines via MTT cell proliferation assay. These derivatives all had $IC_{50}$ values >100 μM which is well above a therapeutic concentration and can be generally considered as nontoxic derivatives.

The derivatives **2.29-2.31**, with neutral substitutions on the benzofuran ring were screened for their cell proliferation inhibition properties. Compound **2.29** was screened as a hydrochloride salt for solubility purposes along with its free base counterpart and no significant differences in cell proliferation was observed. Of the neutrally substituted series, compound **2.30** was the most potent at inhibiting cell proliferation in MDA-MB-231, MCF7, 67NR, and WiDr cancer cell lines (**Figure 2.9**). It was determined that compound **2.31** was more potent at inhibiting cell proliferation in 4T1 and MIA PaCa-2 with $IC_{50}$ values of ~8 and ~5 μM respectively. All compounds of this series inhibited cell proliferation in WiDr with $IC_{50}$ values ranging from ~2-5 μM.

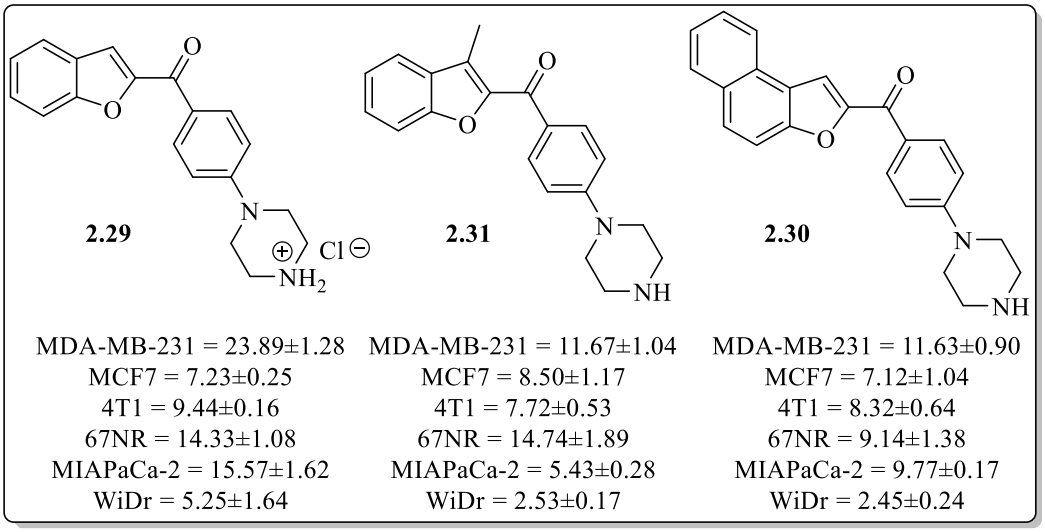



**Figure 2.9:** $IC_{50}$± SEM (μM ) values of compounds **2.29-2.30**. Values were obtained from triplicate MTT cell proliferation assays.

After evaluating the neutral substituted derivatives, the effect of electron-withdrawing substituents was explored. Similarly to compound **2.29**, the hydrochloride salt of **2.38** was used due to solubility issues. The most potent of this series was compound **2.40** with $IC_{50}$ values ranging from ~2-8 μM across the cell lines. Of the compounds in this series, compound **2.40** had almost a two-fold lower $IC_{50}$ (~9 μM) than the other compounds of this series against the MDA-MB-231 cell line. The fluorine containing derivatives **2.37-2.39** all had similar $IC_{50}$ values against the cell lines. Compound **2.32** with the strongest electron-withdrawing group was found to have a significant decrease in cell proliferation inhibition with $IC_{50}$ values of ~8-51 μM against the cell lines.

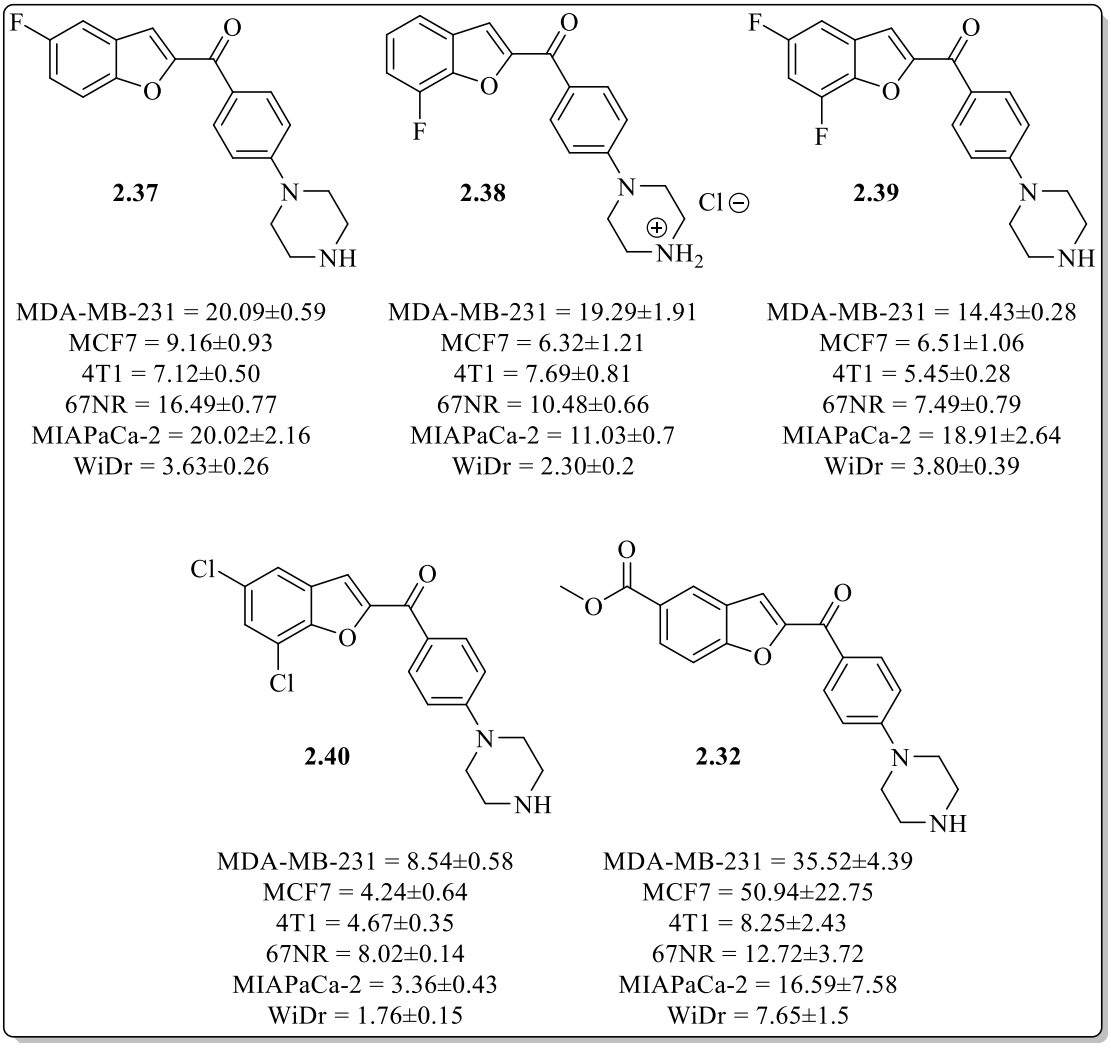



**Figure 2.10:** $IC_{50}$± SEM (μM ) values of compounds **2.37-2.40** , and **2.32**. Values were obtained from triplicate MTT cell proliferation assays.

To assess if electron-donating groups influenced potency, compounds **2.34** and **2.41** were evaluated. It was found that **2.34** was more potent across all cell lines compared with **2.41.** To further explore the SAR of the *N,N*-dialkyl group, derivatives with differing dialkyl chain lengths and a dibenzyl were synthesized and evaluated. It was found that both decreasing and increasing the number of carbons in the chain reduced potency across the

cell lines. The dibenzyl substituted **2.36** was found to be the most potent with $IC_{50}$ values ranging from ~2-4 μM across the cell lines, with **2.34** having comparable $IC_{50}$ values.

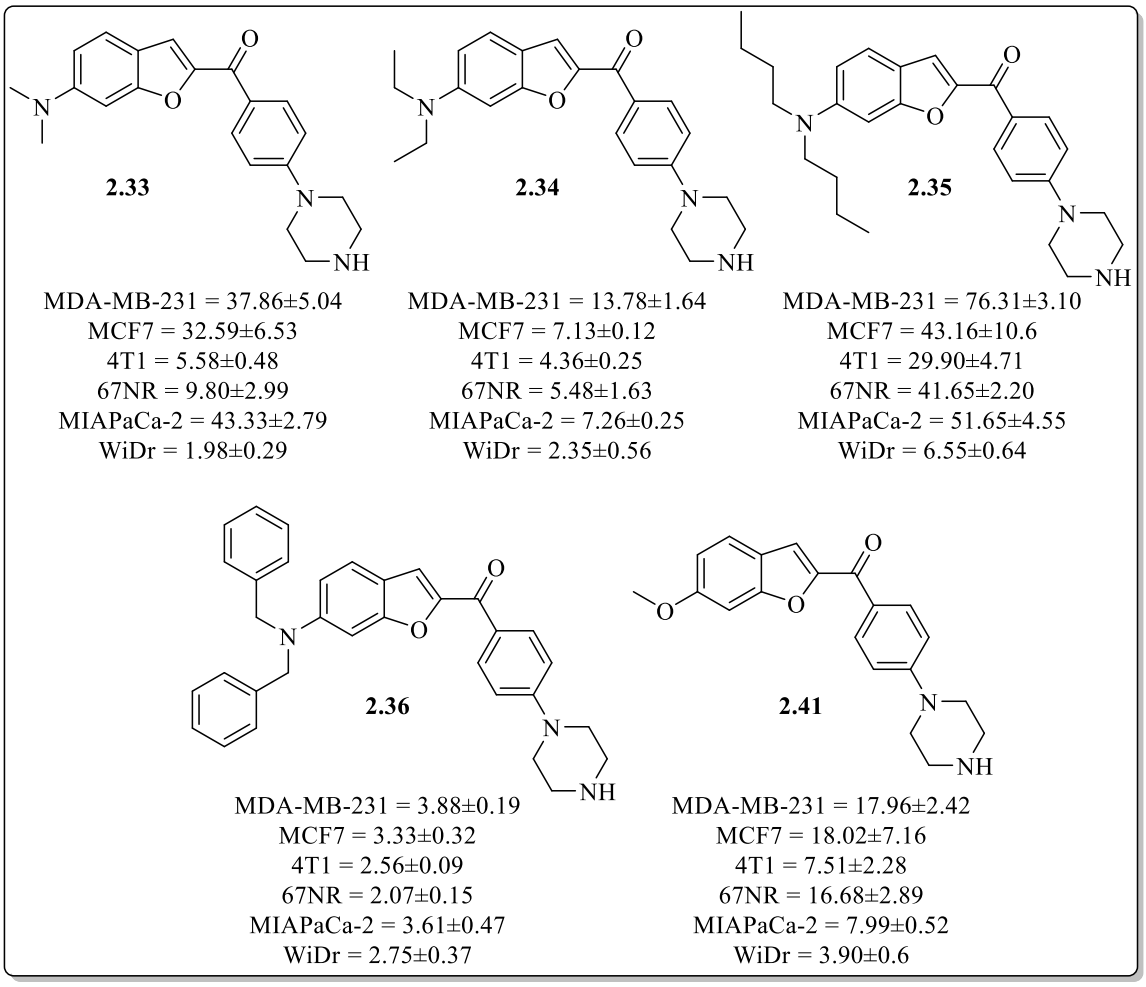



**Figure 2.11:** $IC_{50}$± SEM (μM ) values of compounds **2.33-2.36**, and **2.41**. Values were obtained from triplicate MTT cell proliferation assays.

Exploring the SAR of the NH in **2.34**, **2.42** and **2.43** were synthesized and evaluated. It was determined that substituting the piperazine leads to at least two-fold decrease in potency across all cell lines (**Figure 2.12**). Exploring the SAR of the α,β-unsaturated ketone it was found that reduction of the ketone to an alcohol greatly reduced

potency in all cell lines besides WiDr, suggesting that the α,β-unsaturated ketone is required for potency.

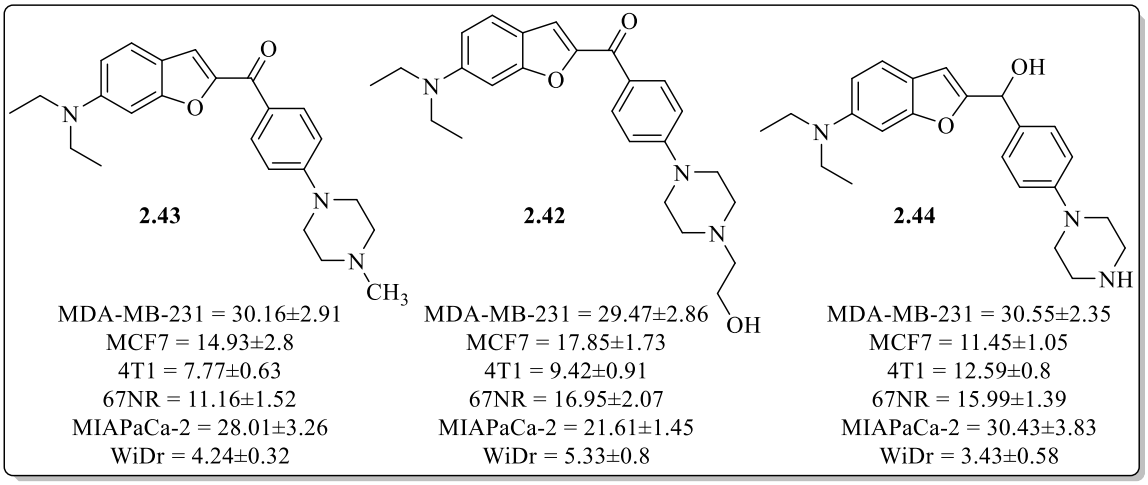



**Figure 2.12:** $IC_{50}$± SEM (μM ) values of compounds **2.42-2.44**. Values were obtained from triplicate MTT cell proliferation assays.

In conclusion, 16 substituted 2-(4-piperazinyl)benzoylbenzofurans were synthesized and evaluated against 6 cancer cell lines, which include 4T1, 67NR, MIA PaCa-2, MCF7, MDA-MB-231, and WiDr. It was found that *N*,*N*-benzyl substituted **2.36** and dichloro substituted **2.40** were the most potent derivatives across all cells with $IC_{50}$ values ranging from ~2-4 μM and ~2-8 μM. A small SAR was done to explore if the role of α,β-unsaturated ketone in the benzofuran moiety in providing the biological activity. After ketone reduction to alcohol, there was a significant decrease in potency, suggesting that the α,β-unsaturated system on the benzofuran is important for potency. Another small SAR was carried out to understand the importance of substituted piperazines. In this regard N-hydroxyethyl **2.42** and N-methyl **2.43** were synthesized. It was found that potency was only partially decreased, leaving the possibility of future SAR studies on the effects of

piperazine substitution. Future studies will involve further functionalizing biologically relevant benzofurans and determination of molecular mechanism of action. Upon identification of a lead, an *in vivo* systemic toxicity study will be carried out to determine the maximum tolerated dose along with a pharmacokinetic and pharmacodynamics profile of the lead will be obtained. Subsequently, *in vivo* tumor xenograft/syngraft efficacy study as a single agent or in combination will be carried out.

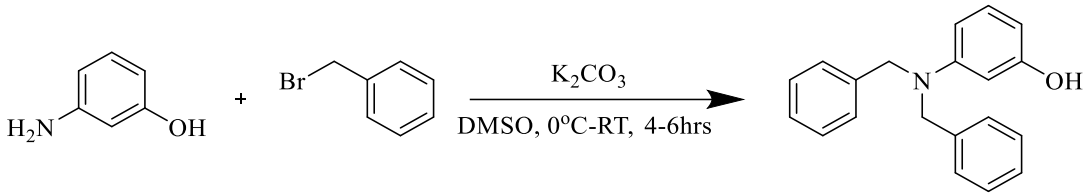
## Chapter 3: Experimental and Characterization

### 3.A Materials and Methods

1-Bromobutane was obtained from VWR International, 4-fluorophenacyl bromide was obtained from AkSci, benzyl bromide, 3-aminophenol, potassium carbonate, phosphorous oxychloride, salicylaldehyde, piperazine, and substituted salicylaldehydes were purchased from Sigma-Aldrich (St. Louis, MO). The $^{1}$H- and $^{13}$C-NMR spectra were plotted on a Varian Oxford-500 spectrometer. High-resolution mass spectrometry (HRMS) was recorded using a Bruker micrOTOF-Q III ESI mass spectrometer. Elemental analysis (CHN) results were obtained from Atlantic Microlab services.

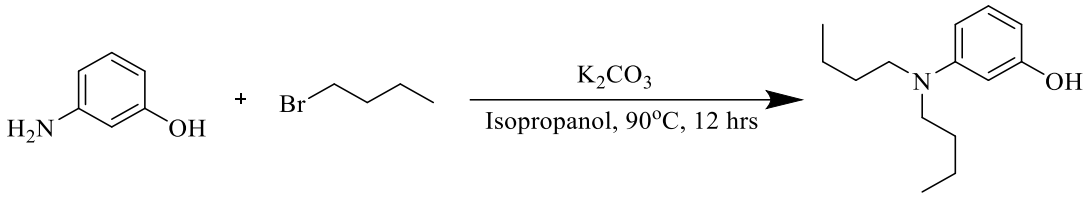
### 3.B Experimental Procedures

*Representative procedure for the synthesis of 3-(dibenzylamino)phenol*



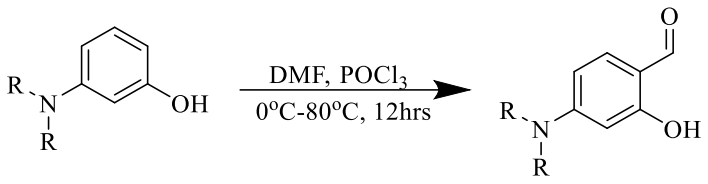

3-aminophenol (20 mmol) was dissolved in DMSO (50 mL) followed by addition of $K_2CO_3$ (50 mmol). The reaction was cooled to 20°C, followed by a dropwise addition of benzyl bromide (20 mmol) over the course of 1 hour. The reaction progress was monitored by TLC (10% EtOAC/hexanes) immediately after addition of benzyl bromide and a TLC was taken every subsequent 10 minutes to monitor the formation of the mono and dialkylated products. Upon completion of the selective dibenzylation of the amine, the reaction was quenched by pouring into dilute HCl at 0°C and extracted with EtOAc (3x100 mL). The organic layer was washed with water (2x100 mL) to remove DMSO. The organic layer was then dried over anhydrous $MgSO_4$ and concentrated using a rotary evaporator to yield the 3-(dibenzylamino)phenol product **2.24**. The crude product was utilized for the next reaction without further purification.

*Representative procedure for the synthesis of 3-(dibutylamino)phenol*




3-aminophenol (10 mmol) was dissolved in isopropanol (50 mL) followed by addition of $K_2CO_3$ (25 mmol). Butyl bromide was added to the reaction and refluxed at 90°C for 12 hours. The reaction progress was monitored by TLC (10% EtOAC/hexanes) to monitor the formation of the mono and dialkylated products. Upon completion of the alkylation, the reaction was quenched by pouring into dilute HCl at 0°C and extracted with EtOAc (3x75 mL). The organic layer was then dried over anhydrous $MgSO_4$ and concentrated using a rotary evaporator to yield the 3-(dibutylamino)phenol product **2.26**. The crude product was utilized for the next reaction without further purification.

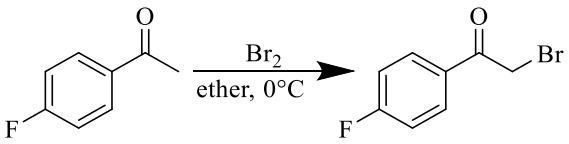
*General procedure for the formylation of 3-(dialkylamino)phenol products under Vilsmeier-Haack conditions*




R= Butyl, Benzyl

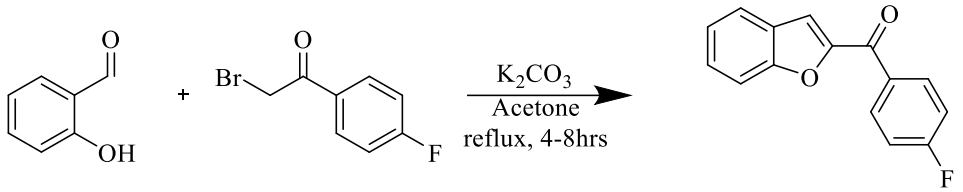
The 3-(dialkylamino)phenol (1mmol) derivatives were dissolved in DMF (15 mL) and cooled to 0°C followed by dropwise addition of $POCl_3$ (1.5 mmol). The reaction was then brought to room temperature and heated at 80°C for 12 hours. The reaction progress was monitored by TLC (10% EtOAC/hexanes). Upon consumption of all starting materials, the reactions were quenched with $NaHCO_3$ (3 mmol) and extracted with ether (3x30 mL). The ether layer was washed with water (3x20 mL) to remove DMF and crude product was dried under rotary evaporator (yield = 70%).

*Representative procedure for the synthesis of 2-bromo-1-(4-fluorophenyl)ethan-1-one*



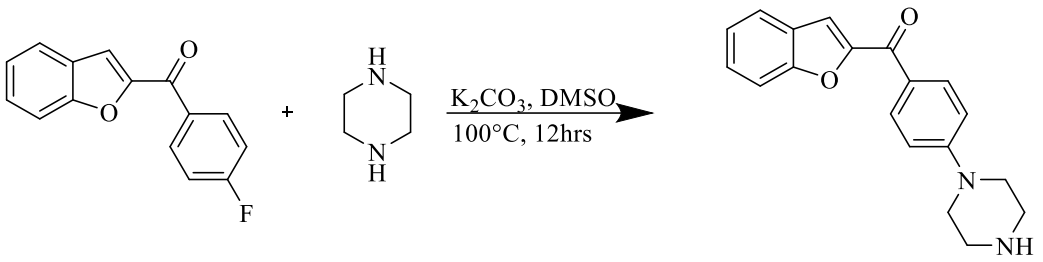

1-(4-fluorophenyl)ethan-1-one (36 mmol) was dissolved in ether (175 mL). The reaction was brought to 0°C followed by the slow addition of $Br_2$ (32 mmol). The reaction was kept at 0°C for 15 minutes then was brought to room temperature. The reaction was monitored via TLC (5% EtOAc/hexanes) for the formation of the mono-brominated species. After 45 minutes, the reaction was quenched by pouring the ether over a saturated $NaHCO_3$ solution at 0°C. The mixture was separated and the aqueous layer was further extracted with ether (2x50 mL). The ether layer was dried with $MgSO_4$ and concentrated using a rotary evaporator to yield crude product. Crude product was purified using column chromatography (5% EtOAc/hexanes) to give pure 2-bromo-1-(4-fluorophenyl)ethan-1-one in 70% yield.

*Representative procedure for the synthesis of substituted benzofuran-2-yl(4-fluorophenyl)methanone derivatives **2.9-2.21** via Rap-Stoermer condensation*



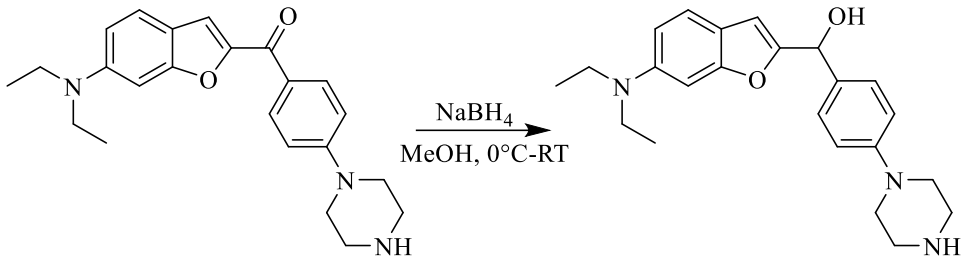

Salicylaldehyde (1 mmol) was stirred in acetone (30 mL) in the presence of $K_2CO_3$ (3 mmol) for 20 minutes. After color change, 4-fluorophenacyl bromide (1.2 mmol) was added and reaction was refluxed at 80°C. Reaction progress was monitored via TLC (30% EtOAc/hexanes) for the consumption of the salicylaldehyde starting material and upon completion of the reaction, the solvent was removed using a rotary evaporator. The reaction mixture was then stirred in water, the resulting precipitate was filtered using a filter paper on a Buchner funnel and subsequently washed with hexanes to remove excess 4-fluorophenacyl bromide.

*Representative procedure for the preparation of substituted benzofuran-2-yl(4-(piperazin-1-yl)phenyl)methanone derivatives* ***2.29-2.41*** *via Ipso substitution*



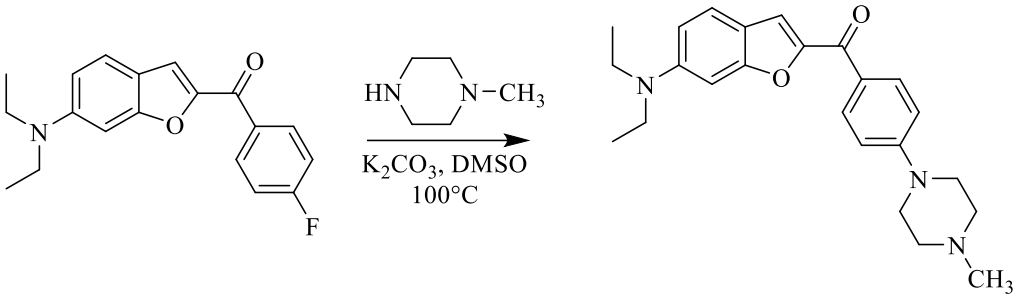

Benzofuran-2-yl(4-fluorophenyl)methanone (1mmol) was dissolved in DMSO (10 mL) followed by the addition of excess piperazine (5 mmol). The reaction was brought to 90°C and allowed to stir for 12 hours. The reaction progress was monitored by TLC (10% EtOAc/hexanes to 40% EtOAc/hexanes) for the consumption of starting materials. After completion of the reaction, the reaction mixture was cooled to room temperature, and poured over ice which resulted in precipitate formation. The resulting precipitate was filtered using a filter paper on a Buchner funnel and products were further purified via column chromatography.

*Synthesis of (6-(diethylamino)benzofuran-2-yl)(4-(piperazin-1-yl)phenyl)methanol via sodium borohydride reduction.*



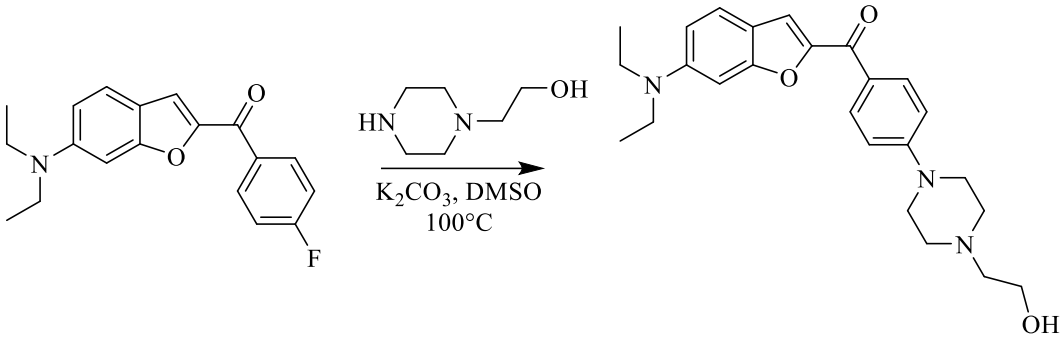

(6-(diethylamino)benzofuran-2-yl)(4-(piperazin-1-yl)phenyl)methanone (2 mmol) was dissolved in MeOH (10 mL) and cooled to 0°C. Sodium borohydride (1 mmol) was added slowly over the course of 10 mins. Reaction was then brought to room temperature and the reaction progress was monitored via TLC (100% EtOAc) for the consumption of starting material. Upon completion, reaction was stirred in dilute NaOH and the resulting product was filtered using a filter paper on a Buchner funnel. The crude product was further purified via silica gel column chromatography (15% MeOH/EtOAc elution) yielding the pure product in 85% yield.

*Synthesis of (6-(diethylamino)benzofuran-2-yl)(4-(4-methylpiperazin-1-yl)phenyl)methanone via Ipso Substitution*



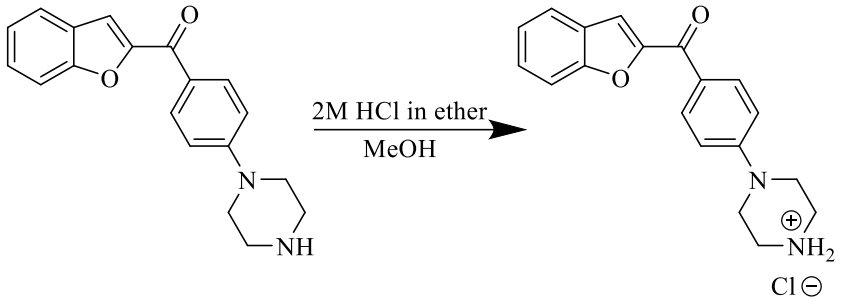

(6-(diethylamino)benzofuran-2-yl)(4-fluorophenyl)methanone (1 mmol) was dissolved in DMSO (10 mL) followed by excess 1-methylpiperazine (5 mmol) in the presence of $K_2CO_3$ (3 mmol). Reaction was then heated at 100°C for 24 hrs and reaction progress was monitored via TLC. Upon completion, reaction mixture was cooled to room temperature and poured over ice to precipitate out the crude product and was subsequently filtered using a filter paper on a Buchner funnel. The crude was further purified via silica gel column chromatography (20% MeOH/EtOAc elution) to obtain product in 90% yield.

*Synthesis of (6-(diethylamino)benzofuran-2-yl)(4-(4-(2-hydroxyethyl)piperazin-1-yl)phenyl)methanone via Ipso Substitution*




(6-(diethylamino)benzofuran-2-yl)(4-fluorophenyl)methanone (1 mmol) was dissolved in DMSO (10mL) followed by excess 2-(piperazin-1-yl)ethan-1-ol (5 mmol) in the presence of $K_2CO_3$ (3 mmol). Reaction was then heated at 100°C for 48 hrs and progress monitored via TLC. Upon completion, reaction mixture was cooled to room temperature and poured over ice to precipitate the crude product and was subsequently filtered using a filter paper on a Buchner funnel. The crude product was further purified via silica gel column chromatography (20% MeOH/EtOAc elution) to obtain the pure product in a yield of 85%.

*Synthesis of 4-(4-(benzofuran-2-carbonyl)phenyl)piperazin-1-ium chloride*




Benzofuran-2-yl(4-(piperazin-1-yl)phenyl)methanone (1 mmol) was dissolved in MeOH followed by the addition of HCl in ether (1.1 mmol). Solution was stirred for 5 hours then concentrated under vacuum to obtain crude. The crude was then stirred in ethyl acetate and filtered to yield pure product in 90% yield.

*Cell Culture*

MDA-MB-231 cells (ATCC) were grown in DMEM supplemented with 10% FBS and penicillin-streptomycin (50 µg/ml). MIA PaCa-2 cells (ATCC) were cultured in DMEM supplemented with 10% FBS, 2.5% horse serum and penicillin-streptomycin (50 µg/ml). 4T1 cells (ATCC) and 67NR (gift from Dr. Jon Holy from the University of Minnesota Duluth) were cultured in RPMI-1640 supplemented with 10% FBS and penicillin-streptomycin (50 µg/ml). MCF7 cells (Masonic Cancer Center) were grown in α-MEM supplemented with 6% FBS, penicillin-streptomycin (50µg/ml), epidermal growth factor (0.0125 µg/mL), hydrocortisone (0.001 mg/mL), 1X NEAA, insulin (0.001 mg/mL), HEPES (12 mM), sodium pyruvate (0.5 mM). WiDr cells (ATCC) were grown in MEM supplemented with 10% FBS and penicillin-streptomycin (50 µg/ml). All cells were incubated at 37°C and 5% $CO_2$.

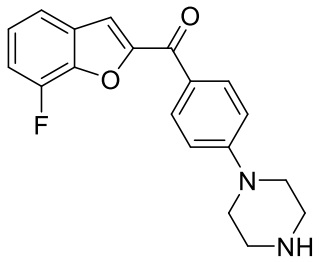
*MTT Cell Proliferation Assay*

Confluent cell cultures were treated with trypsin and resuspended at $5x10^4$ cells/mL. To a 96-well plate, 100 µl of the $5x10^4$ cells/mL solution were added and allowed to incubate at 37°C, 5% $CO_2$ for 24 hours. Compounds were then added and allowed to incubate for 3 days. At this time 10 µL of MTT (5 mg/mL) was added to the 96-well plate and further incubated for 4 hours. Following the 4-hour incubation with MTT, 100 µL of SDS (0.1 g/mL, 0.01N HCl) was added and 96-well plates were allowed to incubate for an additional 4 hours. Absorbance values were then taken at 570 nm using a BioTek Synergy 2 Multimode Microplate Reader.

*Purification of the synthesized compounds*

All compounds purified via column chromatography unless otherwise mentioned. Hydrochloride salts were purified via washings with ethyl acetate or ether, as necessary.

**(7-fluorobenzofuran-2-yl)(4-(piperazin-1-yl)phenyl)methanone**

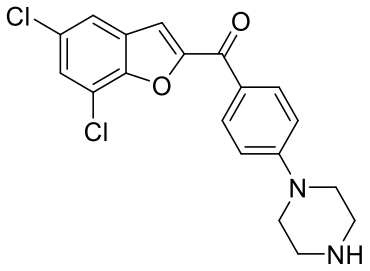



**1H NMR (500 MHz, CHLOROFORM-d):** Shift 8.16 (d, J = 5.0 Hz, 2H), 7.56 (d, J = 3.0 Hz, 1H), 7.49 (d, J = 7.5 Hz, 1H), 7.17 - 7.27 (m, 2H), 6.95 (dd, J = 6.5, 2 Hz, 2H), 3.40-3.38 (m, 4H), 3.06-3.03 (m, 4H)

**13C NMR (126 MHz, CHLOROFORM-d):** Shift 181.4, 154.7, 154.5, 149.3, 147.3, 142.9, 132.2, 130.6, 126.2, 124.3, 124.2, 118.5, 118.4, 114.2, 113.4, 113.3, 48.3, 45.9

**HRMS (ESI) m/z:** calculated for $C_{19}H_{17}FN_2O_2$ $[M+1]^+$: 325.1308, found 325.1437.

**(5,7-dichlorobenzofuran-2-yl)(4-(piperazin-1-yl)phenyl)methanone**



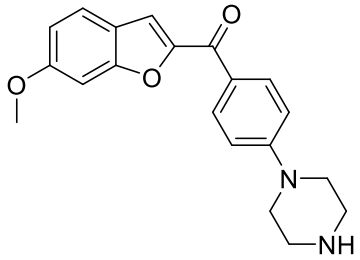

**1H NMR (500 MHz, CHLOROFORM-d):** δ 8.16 (d, J = 8.5 Hz, 2H), 7.59 (s, 1H), 7.49 (s, 1H), 7.45 (s, 1H), 6.94 (d, J = 9.5 Hz, 2H), 3.39 (m, 4H), 3.03 (t, J = 5.00 Hz, 4H)

**13C NMR (126 MHz, CHLOROFORM-d):** δ 180.7, 155.5, 154.8, 150.0, 132.3, 129.5, 129.3, 127.3, 125.8, 120.8, 118.4, 113.5, 113.2, 48.2, 45.8

**HRMS (ESI) m/z:** calculated for $C_{19}H_{16}Cl_2N_2O_2$ $[M]^+$: 375.0622, found 375.0771.

**Anal. Calcd for $C_{19}H_{16}Cl_2N_2O_2$** (375.2): C 60.82, H 4.30, N 7.47 **Found:** C 60.62, H 4.21, N 7.33

**(6-methoxybenzofuran-2-yl)(4-(piperazin-1-yl)phenyl)methanone**

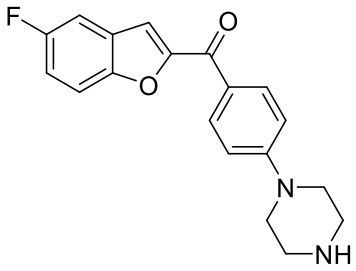



**1H NMR (500 MHz, CHLOROFORM-d):** δ 8.05 (d, J = 8.5 Hz, 2H), 7.57 (d, J = 8 Hz, 1H), 7.44 (s, 1H), 7.10 (s, 1H), 6.95-6.93 (m, 3H), 3.89 (s, 3H), 3.36 (t, J = 5.5 Hz, 4H), 3.04 (m, 4H)

**13C NMR (126 MHz, CHLOROFORM-d):** δ 181.9, 160.7, 157.2, 154.5, 152.7, 131.7, 127.1, 123.3, 120.5, 115.3, 114.0, 113.4, 95.7, 55.7, 48.5, 45.9

**HRMS (ESI) m/z:** calculated for $C_{20}H_{20}N_2O_3$ $[M+1]^+$: 337.1508, found 337.1631.

**(5-fluorobenzofuran-2-yl)(4-(piperazin-1-yl)phenyl)methanone**

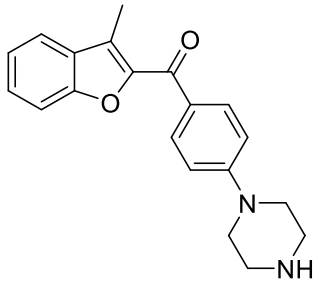



**1H NMR (500 MHz, CHLOROFORM-d):** δ 8.08 (d, J = 9 Hz, 2H), 7.57 (dd, J = 3.5, 9.3 Hz, 1H), 7.46 (s, 1H), 7.36 (d, J = 8.3 Hz, 1H), 7.20 (t, J = 9.5 Hz, 1H), 6.95 (d, J = 8.79 Hz, 2H), 3.39 (t, J = 1.00 Hz, 4H), 3.05 (t, J = 1.00 Hz, 4H)

**13C NMR (126 MHz, CHLOROFORM-d):** δ 181.9, 160.4, 158.5, 154.7, 154.7, 151.9, 132.0, 127.8, 126.4, 115.9, 115.7, 114.3, 114.3, 113.3, 113.3, 113.2, 108.0, 107.8, 48.3, 45.9

**HRMS (ESI) m/z:** calculated for $C_{19}H_{17}FN_2O_2$ $[M+1]^+$: 325.1308, found 325.1438.

**(3-methylbenzofuran-2-yl)(4-(piperazin-1-yl)phenyl)methanone**



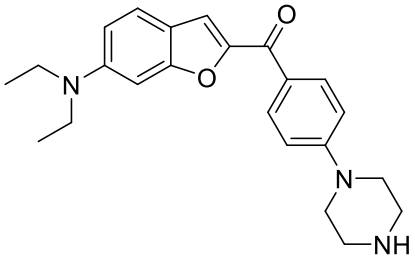

**1H NMR (500 MHz, CHLOROFORM-d):** δ 8.12 (d, J = 8.5 Hz, 2H), 7.68 (d, J = 8 Hz, 1H), 7.54 (d, J = 8 Hz, 1H), 7.46 (t, J = 7.5 Hz, 1H), 7.33 (t, J = 7 Hz, 1H), 6.94 (d, J = 8.5 Hz, 2H), 3.37 (m, 4H), 3.04 (t, J = 5 Hz, 4H), 2.63 (s, 3H)

**13C NMR (126 MHz, CHLOROFORM-d):** δ 183.8, 154.4, 154.0, 149.0, 132.1, 129.4, 127.6, 127.5, 125.2, 123.1, 121.1, 113.3, 112.0, 48.4, 45.9, 9.9

**HRMS (ESI) m/z:** calculated for $C_{20}H_{20}N_2O_2$ $[M+1]^+$: 321.1558, found 321.1669.

**Anal. Calcd for $C_{20}H_{20}N_2O_2$** (320.4): C 74.98, H 6.29, N 8.74 **Found:** C 75.06, H 6.47, N 8.60

**(6-(diethylamino)benzofuran-2-yl)(4-(piperazin-1-yl)phenyl)methanone**

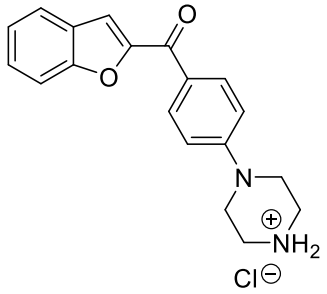



**1H NMR (500 MHz, CHLOROFORM-d):** δ 8.02 (d, J = 8.5 Hz, 2H), 7.46 (d, J = 9 Hz, 1H), 7.38 (s, 1H), 6.93 (d, J = 8.5 Hz, 2H), 6.79 (s, 1H), 6.75 (d, J = 9 Hz, 1H), 3.43 (q, J = 7 Hz, 4H), 3.34 - 3.32 (m, 4H), 3.05 - 3.03 (m, 4H), 1.22 (t, J = 7 Hz, 6H)

**13C NMR (126 MHz, CHLOROFORM-d):** δ 181.8, 158.8, 154.2, 151.1, 149.0, 131.4, 127.9, 123.3, 116.5, 116.4, 113.6, 110.9, 93.1, 48.6, 45.9, 45.0, 12.5

**HRMS (ESI) m/z:** calculated for $C_{23}H_{27}N_3O_2$ $[M+1]^+$: 378.2136, found 378.2268.

**4-(4-(benzofuran-2-carbonyl)phenyl)piperazin-1-ium chloride**



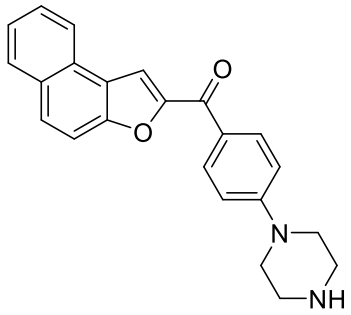
**1H NMR (500 MHz, DMSO-d6):** δ 9.53 (br., 2H), 7.97 (d, J = 9 Hz, 2H), 7.83 (d, J = 8 Hz, 1H), 7.72-7.69 (m, 2H), 7.52 (t, J = 7.5 Hz, 1H), 7.35 (t, J = 7 Hz, 1H), 7.11 (d, J = 8.5 Hz, 2H), 3.64 (br., 4H), 3.19 (br., 4H)

**13C NMR (126 MHz, DMSO-d6):** δ 181.6, 155.4, 153.6, 152.7, 131.9, 128.5, 127.3, 127.1, 124.4, 124.0, 115.6, 114.4, 112.6, 44.2, 42.7

**HRMS (ESI) m/z:** calculated for $C_{19}H_{19}ClN_2O_2$ $[M]^+$: 307.1441, found 307.1569.

**Anal. Calcd for $C_{19}H_{19}ClN_2O_2$** (342.8): C 66.57, H 5.59, N 8.17 **Found:** C 66.32, H 5.43, N 8.08

**naphtho[2,1-b]furan-2-yl(4-(piperazin-1-yl)phenyl)methanone**



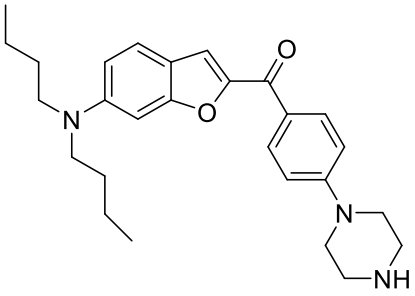

**1H NMR (500 MHz, CHLOROFORM-d):** δ 8.18 (d, J = 8.5 Hz, 1H), 8.14 (d, J = 8.5 Hz, 2H), 8.00 (s, 1H), 7.96 (d, J = 8.5 Hz, 1H), 7.88 (d, J = 9.5 Hz, 1H), 7.74 (d, J = 8.5 Hz, 1H), 7.64 (t, J = 7 Hz, 1H), 7.54 (t, J = 7.5 Hz, 1H), 6.97 (d, J = 8.5 Hz, 2H), 3.40 (t, J = 5 Hz, 4H), 3.07 (t, J = 5 Hz, 4H)

**13C NMR (126 MHz, CHLOROFORM-d):** δ 181.8, 154.5, 154.0, 152.9, 131.9, 130.5, 129.3, 129.0, 128.2, 127.2, 127.0, 125.4, 123.4, 123.0, 113.9, 113.5, 112.9, 48.3, 45.8

**HRMS (ESI) m/z:** calculated for $C_{23}H_{20}N_2O_2$ $[M+1]^+$: 357.1558, found 357.1638.

**Anal. Calcd for $C_{23}H_{20}N_2O_2$** (356.4): C 77.51, H 5.66, N 7.86 **Found:** C 77.25, H 5.75, N 7.93

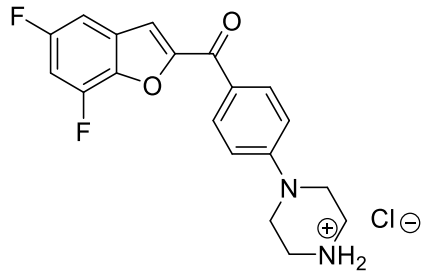
**(6-(dibutylamino)benzofuran-2-yl)(4-(piperazin-1-yl)phenyl)methanone**




**1H NMR (500 MHz, CHLOROFORM-d):** δ 8.14 (s, 1H), 8.02 (d, J = 7 Hz, 2H), 7.46 (d, J = 8.5 Hz, 1H), 7.38 (s, 1H), 6.97 (d, J = 9 Hz, 2H), 6.74 - 6.71 (m, 2H), 3.74 (t, J = 5.00 Hz, 2H), 3.56 (t, J = 5 Hz, 2H), 3.40-3.33 (m, 8H), 1.65-1.59 (m, 4H), 1.38 (sxt, J = 7.5 Hz, 4H), 0.98 (t, J = 7 Hz, 6H)

**13C NMR (126 MHz, CHLOROFORM-d):** δ 181.8, 160.7, 159.0, 153.4, 150.7, 149.5, 131.3, 129.3, 123.3, 117.0, 116.2, 114.7, 111.0, 93.0, 51.4, 48.9, 47.7, 45.1, 39.7, 29.3, 20.3, 14.0

**4-(4-(5,7-difluorobenzofuran-2-carbonyl)phenyl)piperazin-1-ium chloride**

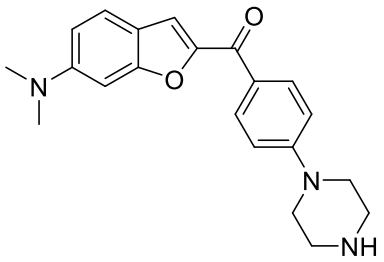



**1H NMR (500 MHz, DMSO-d6):** δ 9.63 (br., 2H), 7.98 (d, J = 8.5 Hz, 2H), 7.77 (s, 1H), 7.56 - 7.50 (m, 2H), 7.13 (d, J = 8.5 Hz, 2H), 3.68 (t, J = 4.5 Hz, 4H), 3.21 (t, J = 4.5 Hz, 4H)

**13C NMR (126 MHz, DMSO-d6):** δ 181.0, 159.5, 157.6, 157.5, 154.7, 153.8, 148.3, 148.2, 146.3, 139.2, 139.1, 132.1, 130.5, 130.4, 126.4, 115.5, 114.3, 105.4, 105.2, 104.1, 103.9, 44.1, 42.7

**HRMS (ESI) m/z:** calculated for **$C_{19}H_{16}F_2N_2O_2$** $[M+1]^+$: 343.1213, found 343.1386.

**(6-(dimethylamino)benzofuran-2-yl)(4-(piperazin-1-yl)phenyl)methanone**

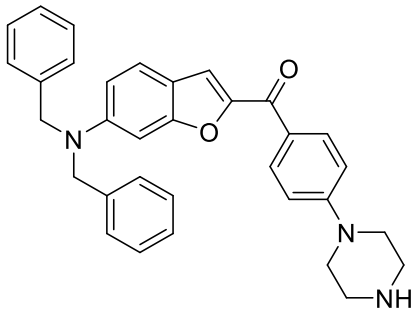



**1H NMR (500 MHz, CHLOROFORM-d):** δ 8.03 (d, J = 8.5 Hz, 2H), 7.50 (d, J = 9 Hz, 1H), 7.41 (s, 1H), 6.94 (d, J = 8.5 Hz, 2H), 6.82-6.79 (m, 2H), 3.35-3.33 (m, 4H), 3.06-3.04 (m, 10H)

**13C NMR (126 MHz, CHLOROFORM-d):** δ 181.8, 158.4, 154.3, 151.5, 151.3, 131.5, 127.8, 123.1, 117.2, 116.3, 113.5, 111.3, 93.9, 48.6, 45.9, 40.8

**HRMS (ESI) m/z:** calculated for $C_{21}H_{23}N_3O_2$ $[M+1]^+$: 350.1823, found 350.1944.

**(6-(dibenzylamino)benzofuran-2-yl)(4-(piperazin-1-yl)phenyl)methanone**

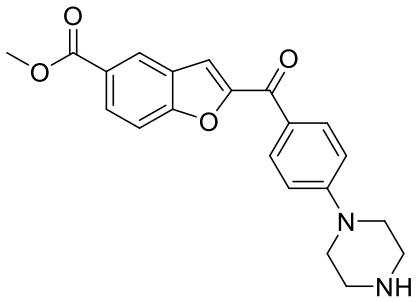



**1H NMR (500 MHz, CHLOROFORM-d):** δ 7.99 (d, J = 8.5 Hz, 2H), 7.44 (d, J = 8.5 Hz, 1H), 7.37 - 7.32 (m, 5H), 7.28 - 7.25 (m, 6H), 6.91 (d, J = 8.5 Hz, 2H), 6.85 (s, 1H), 6.82 (d, J = 8.5 Hz, 1H), 4.75 (s, 4H), 3.32 (t, J = 5 Hz, 4H), 3.03 (t, J = 5 Hz, 4H)

**13C NMR (126 MHz, CHLOROFORM-d):** δ 181.8, 158.3, 154.3, 151.5, 150.2, 137.7, 131.4, 128.8, 127.7, 127.2, 126.5, 123.3, 117.6, 116.1, 113.5, 111.4, 94.6, 55.0, 48.5, 45.9

**HRMS (ESI) m/z:** calculated for $C_{33}H_{31}N_3O_2$ $[M+1]^+$: 502.2450, found 502.2556.

**methyl 2-(4-(piperazin-1-yl)benzoyl)benzofuran-5-carboxylate**

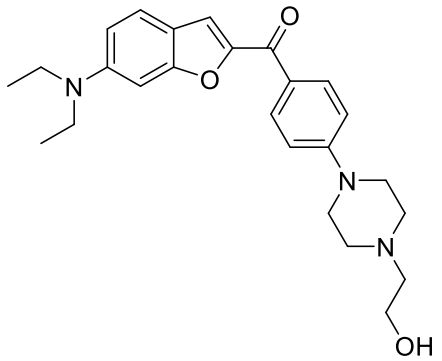



**1H NMR (500 MHz, CHLOROFORM-d):** δ 8.47 (s, 1H), 8.17 (d, J = 9 Hz, 1H), 8.09 (d, J = 9 Hz, 2H), 7.66 (d, J = 8.5 Hz, 1H), 7.55 (s, 1H), 6.95 (d, J = 7.5 Hz, 2H), 3.96 (s, 3H), 3.40-3.38 (m, 4H), 3.05-3.03 (m, 4H)

**13C NMR (126 MHz, CHLOROFORM-d):** δ 181.7, 166.8, 157.9, 154.8, 154.4, 132.0, 128.9, 127.1, 126.3, 126.2, 125.6, 114.6, 113.3, 112.3, 52.3, 48.3, 45.9

**HRMS (ESI) m/z:** calculated for $C_{21}H_{20}N_2O_4$ $[M+1]^+$: 365.1457, found 365.1625.

**(6-(diethylamino)benzofuran-2-yl)(4-(4-(2-hydroxyethyl)piperazin-1-yl)phenyl)methanone**

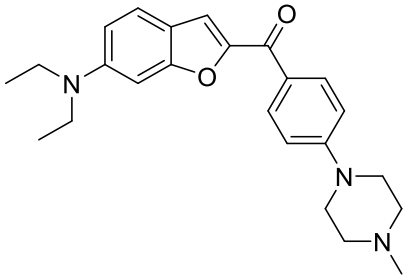



**$^{1}$H NMR (500 MHz, CHLOROFORM-*d*):** δ 8.01 (d, *J*=8.5 Hz, 2 H), 7.47 (d, *J*=9 Hz, 1 H), 7.38 (s, 1 H), 6.94 (d, *J*=8 Hz, 2 H), 6.79-6.74 (m, 2 H), 3.68 (br., 2 H), 3.44-3.39 (m, 8 H), 2.69-2.63 (m, 6 H), 1.22 (t, *J*=6.5 Hz, 6 H)

**$^{13}$C NMR (126 MHz, CHLOROFORM-*d*):** δ 181.8, 158.9, 153.8, 151.0, 149.0, 131.4, 128.1, 123.3, 116.6, 116.4, 113.7, 110.8, 93.1, 59.3, 57.8, 52.6, 47.6, 45.0, 12.5

**HRMS (ESI) m/z:** calculated for $C_{25}H_{31}N_3O_3$ $[M+1]^+$: 422.2399, found 422.2438.

**(6-(diethylamino)benzofuran-2-yl)(4-(4-methylpiperazin-1-yl)phenyl)methanone**

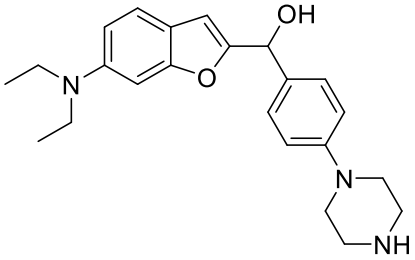


**$^{1}$H NMR (500 MHz, CHLOROFORM-*d*):** δ 8.00 (d, *J*=9 Hz, 2 H), 7.46 (d, *J*=9 Hz, 1 H), 7.38 (s, 1 H), 6.93 (d, *J*=9.5 Hz, 2 H), 6.78 (s, 1 H), 6.75 (d, *J*=8.5 Hz, 1 H), 3.45-3.38 (m, 8 H), 2.57 (t, *J*=5 Hz, 4 H), 2.36 (s, 3 H), 1.21 (t, *J*=7 Hz, 6 H)

**$^{13}$C NMR (126MHz ,CHLOROFORM-d):** δ 181.8, 158.9, 153.8, 151.0, 148.9, 131.4, 128.0, 123.3, 116.6, 116.4, 113.6, 110.8, 93.1, 54.8, 47.5, 46.2, 45.0, 12.5

**HRMS (ESI) m/z:** calculated for $C_{24}H_{29}N_3O_2$ $[M+1]^+$: 392.2293, found 392.2333.

**(6-(diethylamino)benzofuran-2-yl)(4-(piperazin-1-yl)phenyl)methanol**




**$^{1}$H NMR (500 MHz, CHLOROFORM-d):** δ 7.35 (d, *J* = 9 Hz, 2H), 7.28-7.25 (m, 1H), 6.89 (d, *J* = 9 Hz, 2H), 6.74 (s, 1H), 6.65 (d, *J* = 8.5 Hz, 1H), 6.34 (s, 1H), 5.81 (s, 1H), 3.35 (q, *J* = 7 Hz, 4H), 3.11-3.08 (m, 4H), 3.00-2.98 (m, 4H), 1.15 (t, *J* = 7.5 Hz, 6H)

**$^{13}$C NMR (126 MHz, CHLOROFORM-d):** δ 157.3, 156.3, 151.6, 146.4, 132.0, 127.8, 121.0, 117.5, 115.8, 109.8, 103.6, 94.8, 70.3, 50.2, 46.1, 45.1, 12.5

**HRMS (ESI) m/z:** calculated for $C_{22}H_{29}N_3O_2$ $[M+1]^+$: 380 .2293, found 380.2333.

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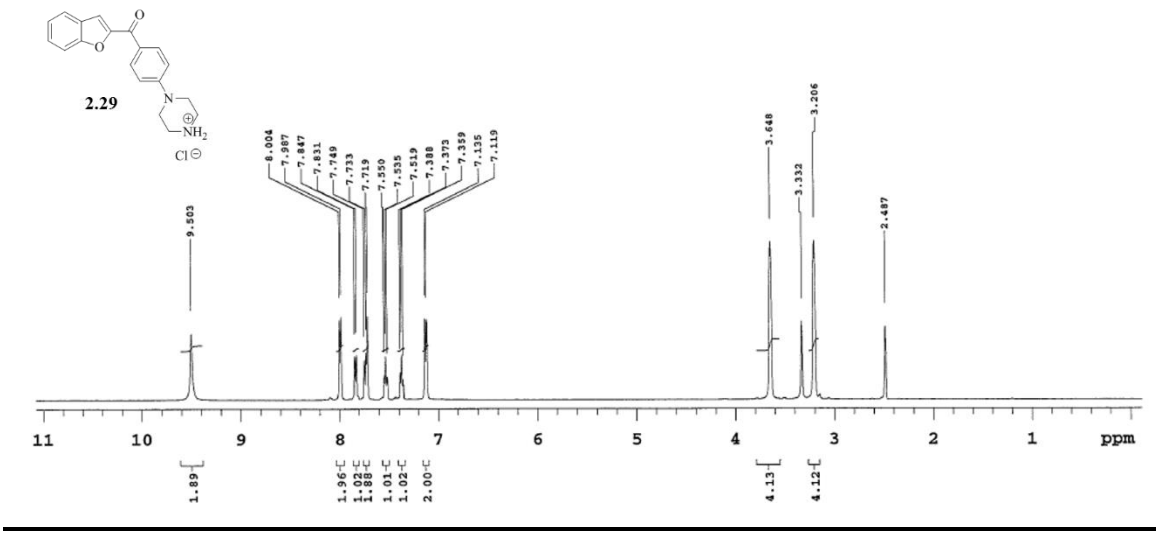
# Appendix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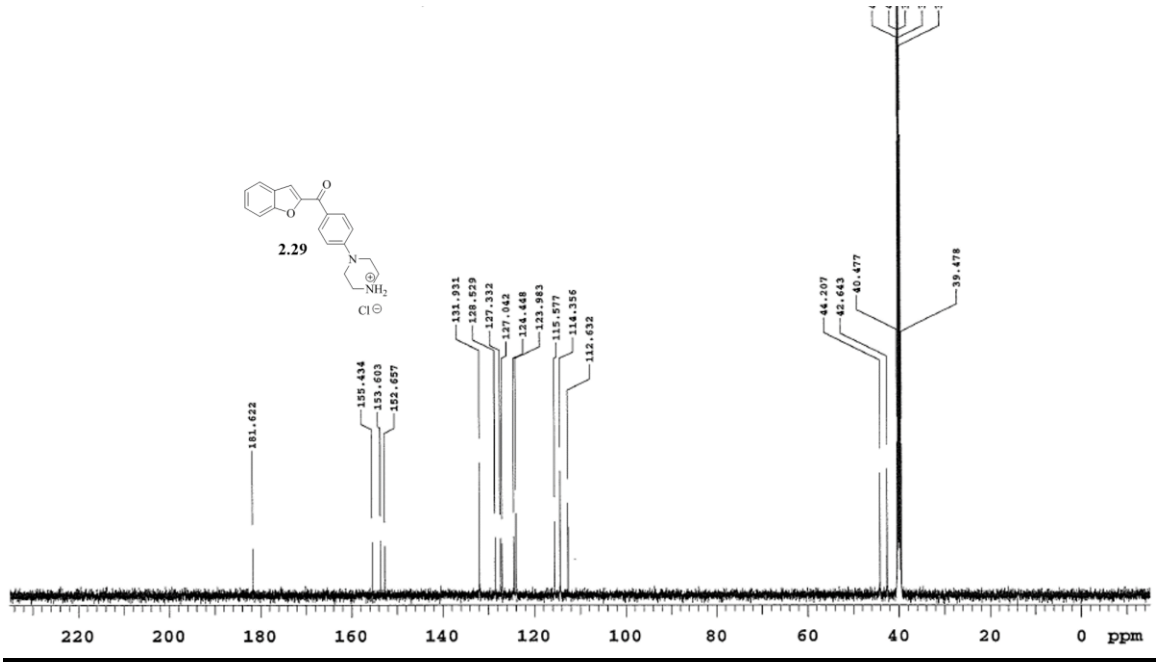
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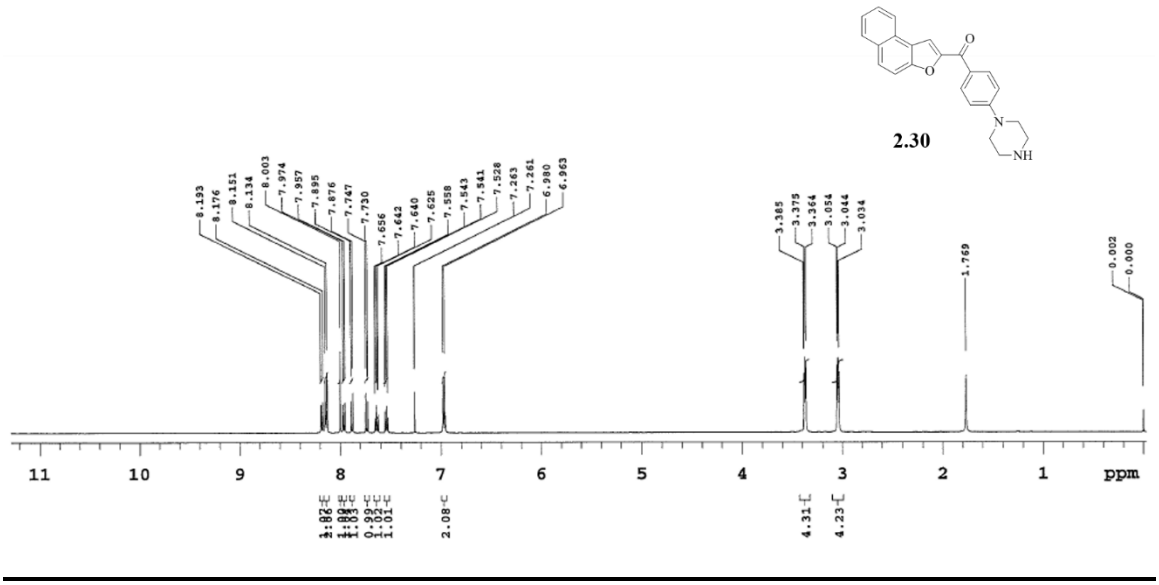
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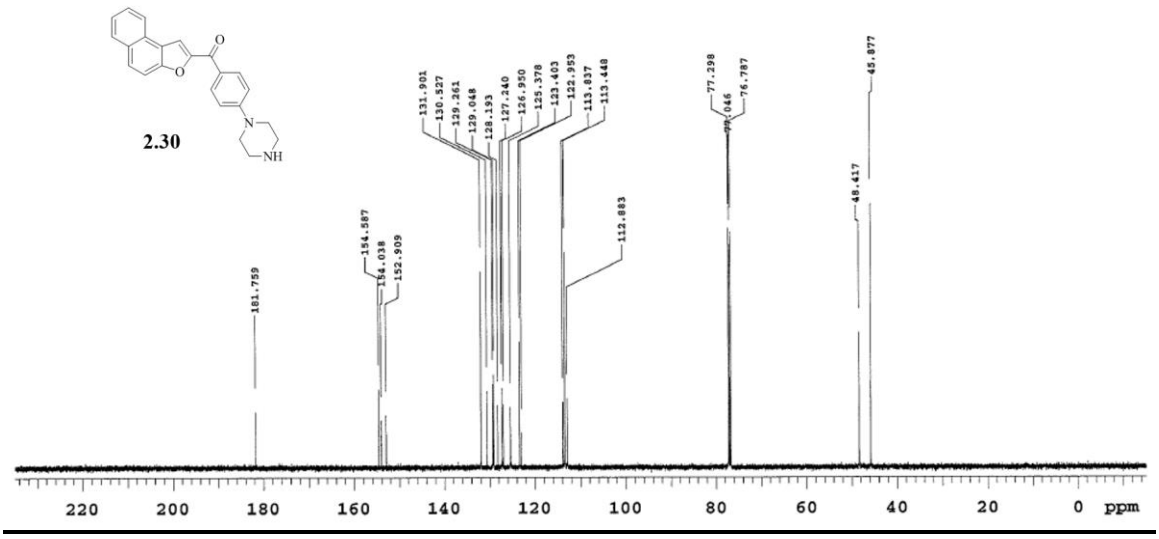
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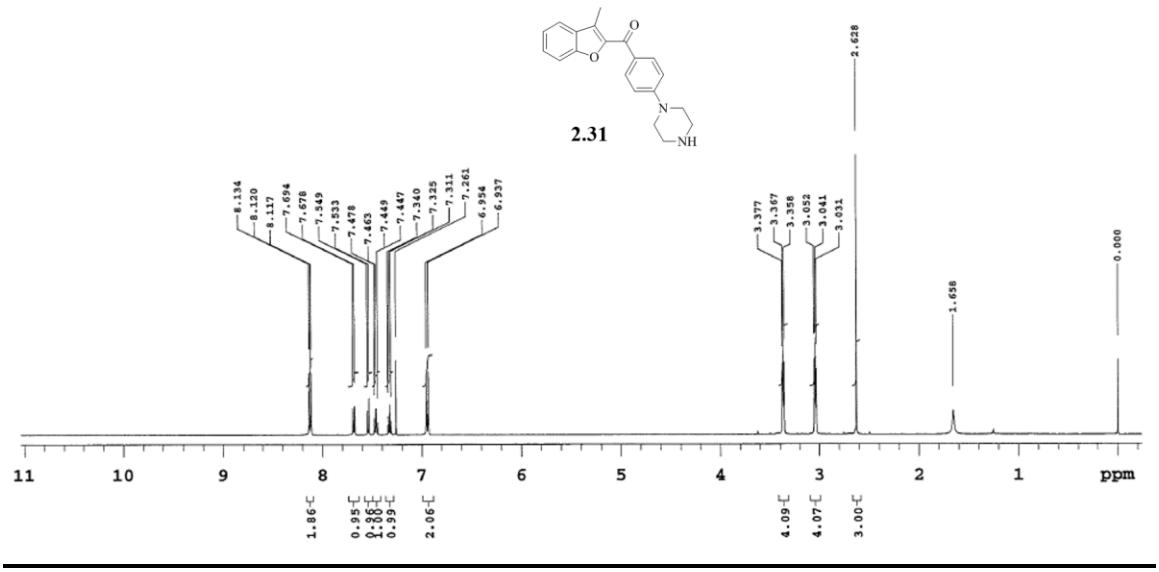
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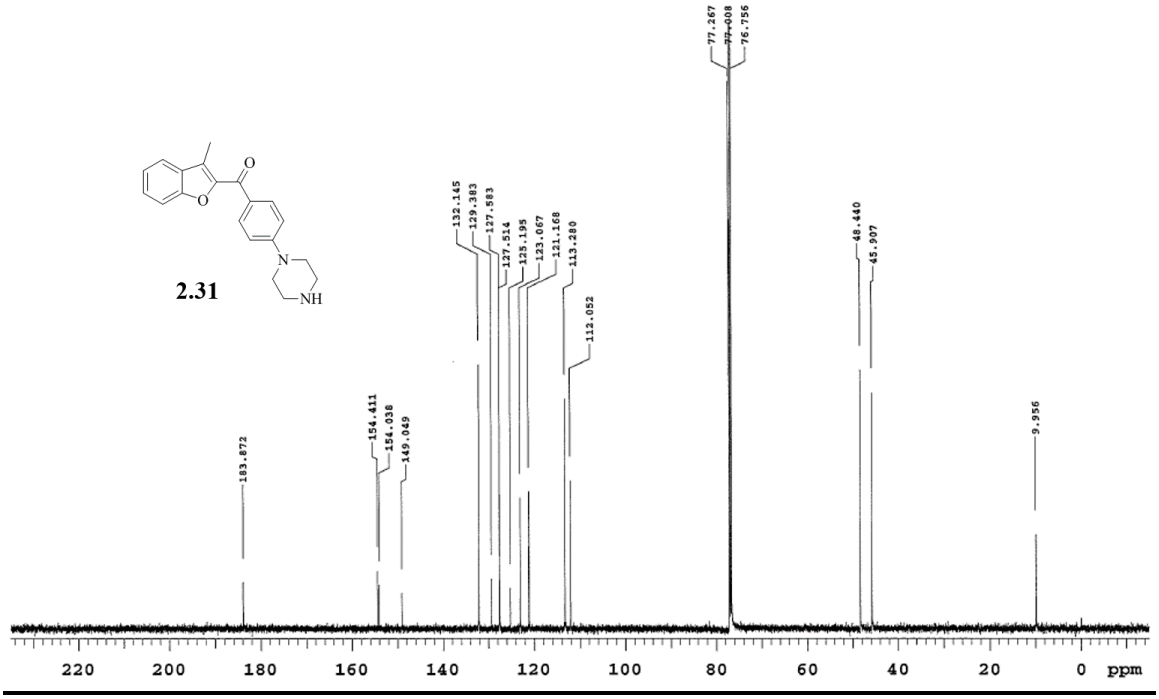
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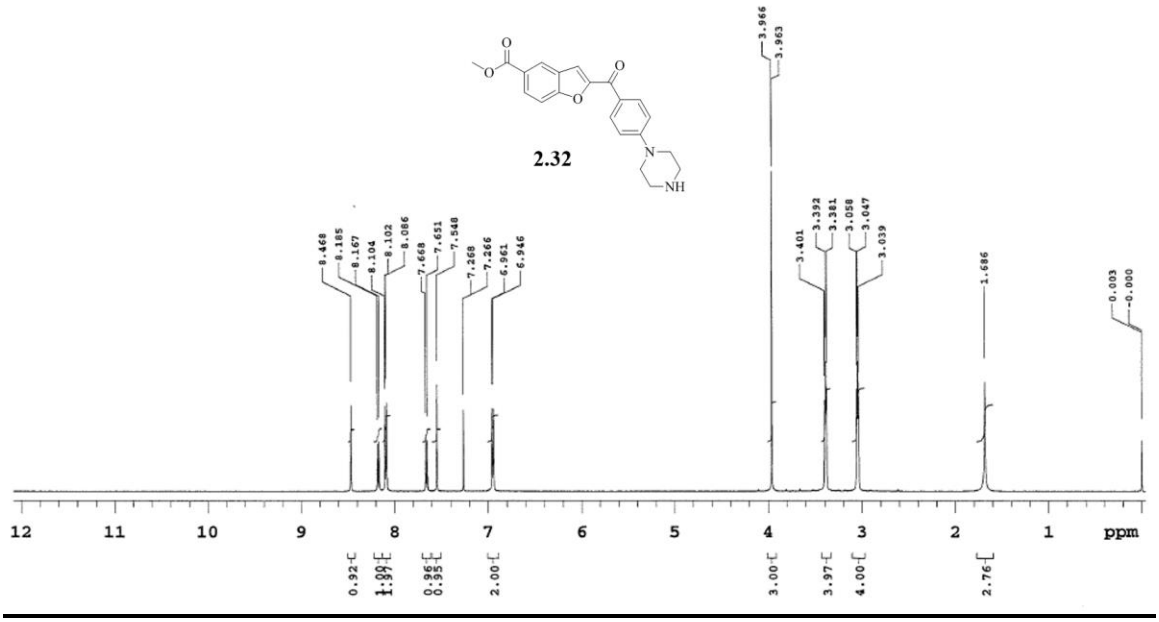
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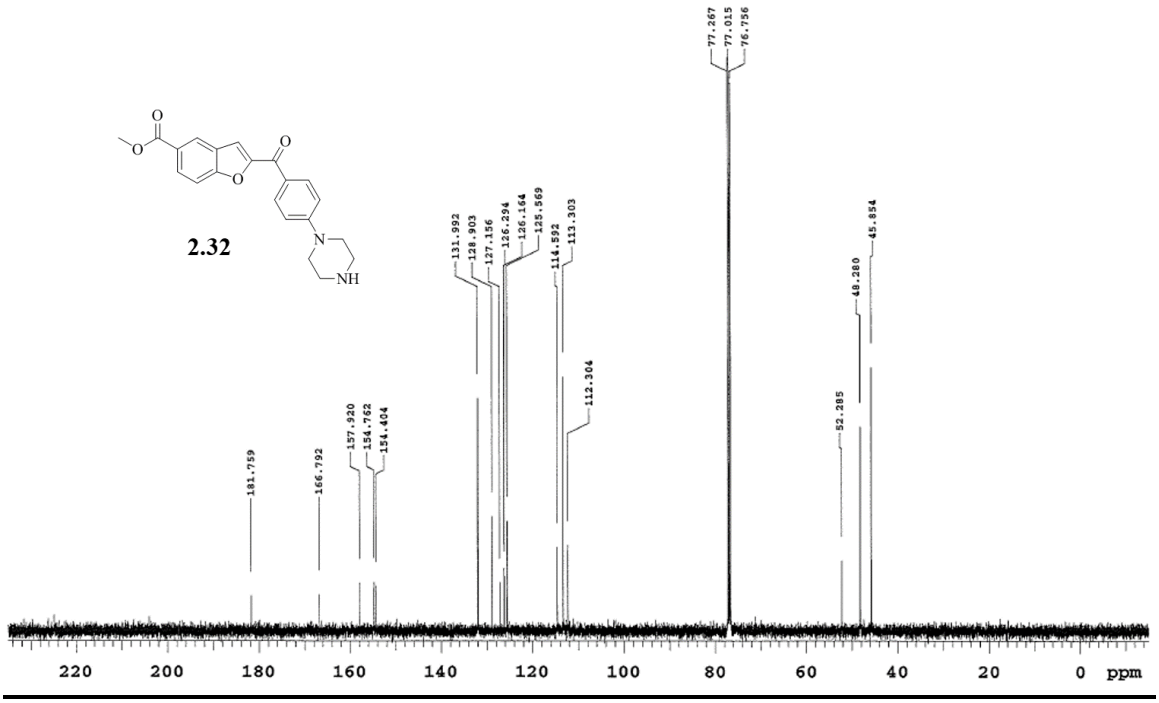
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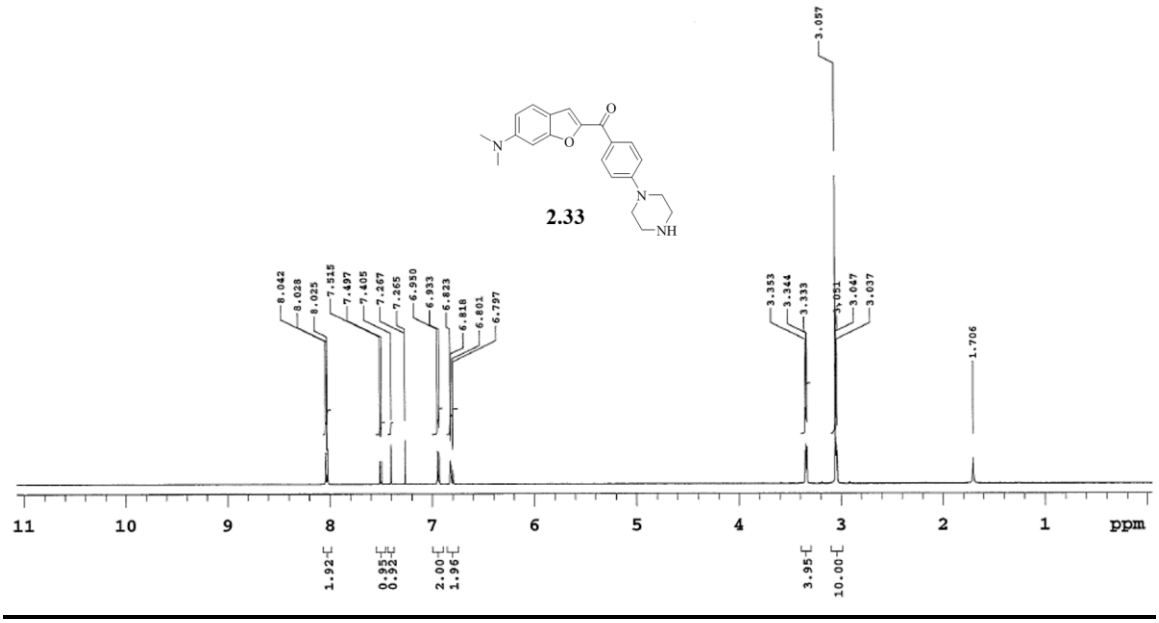
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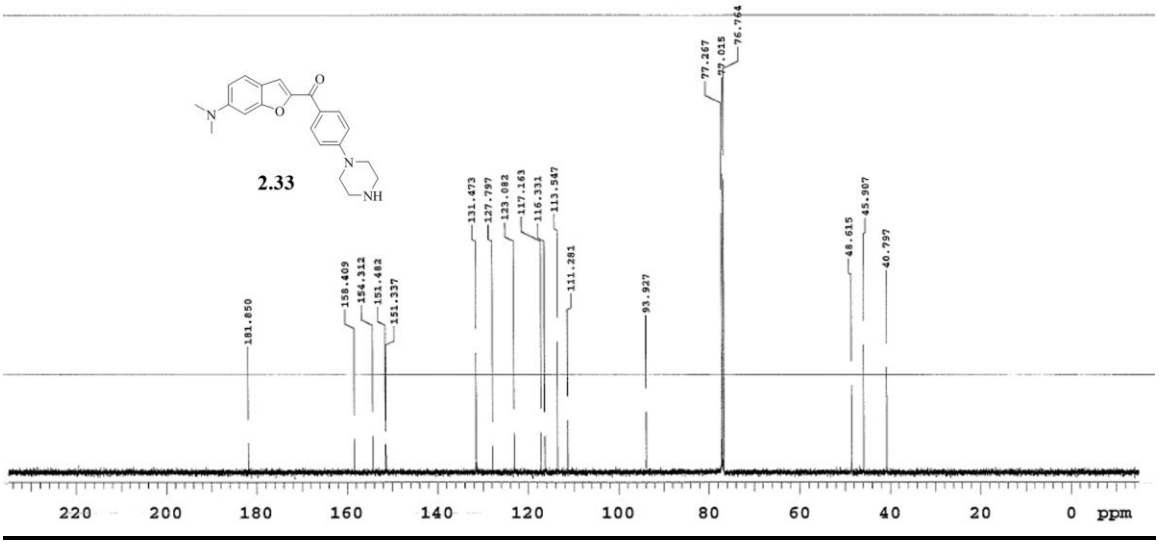
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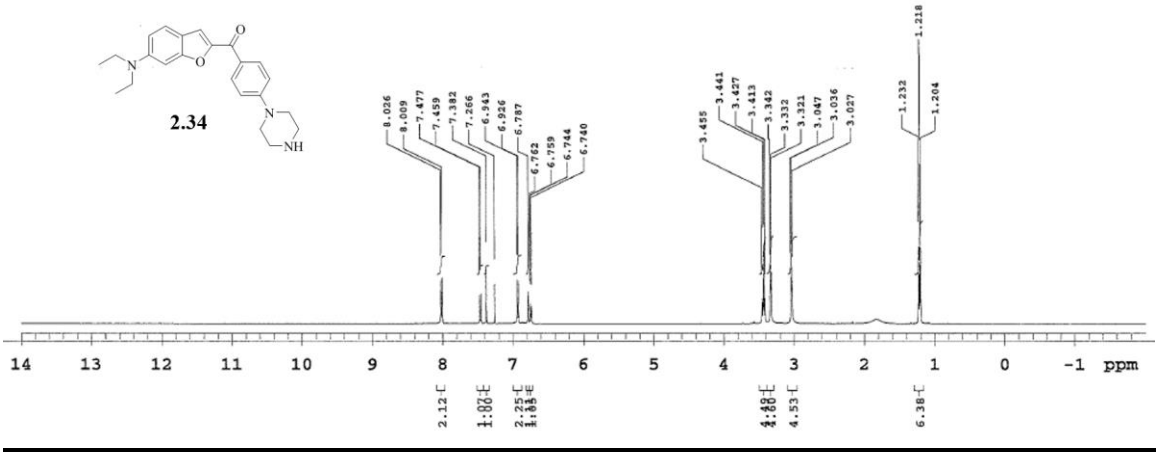
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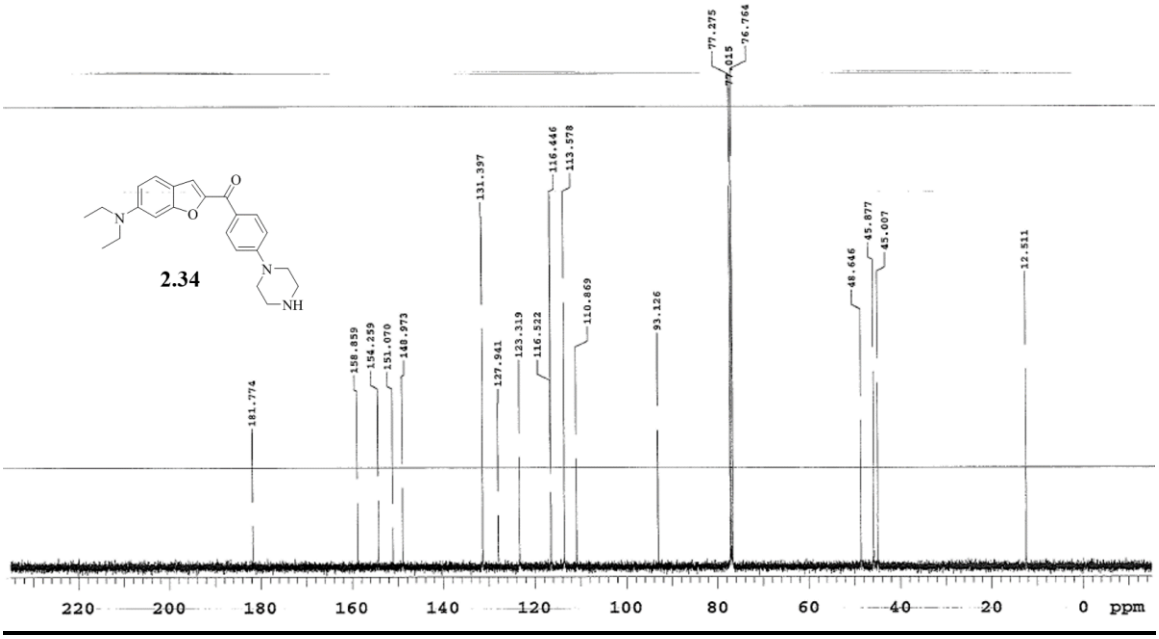
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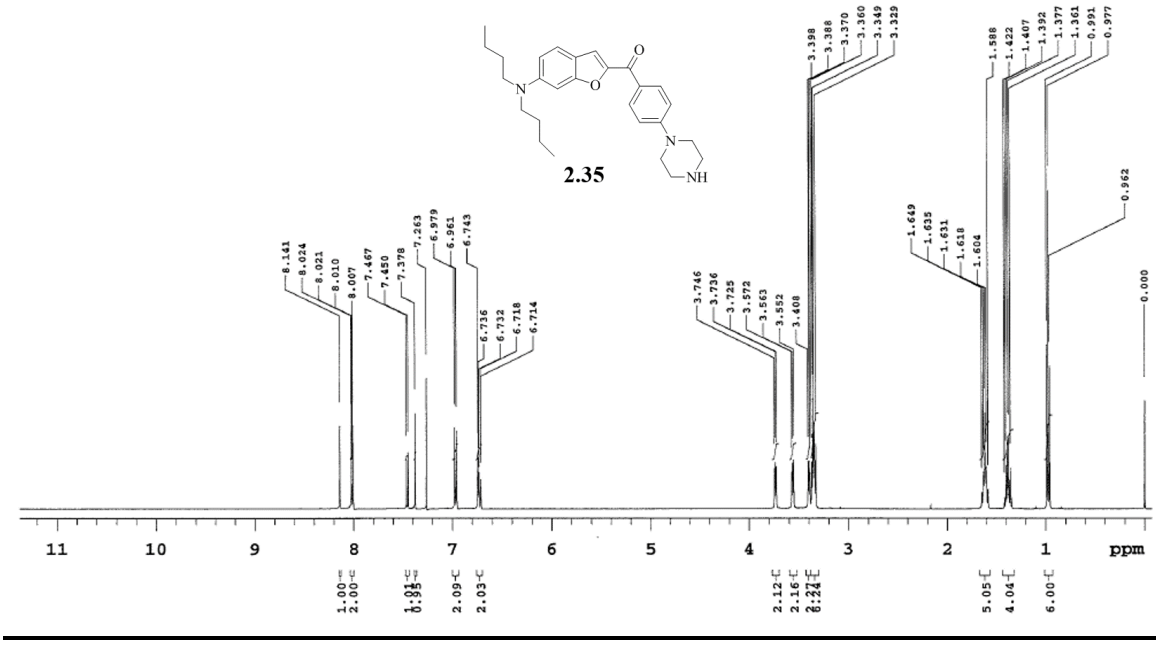
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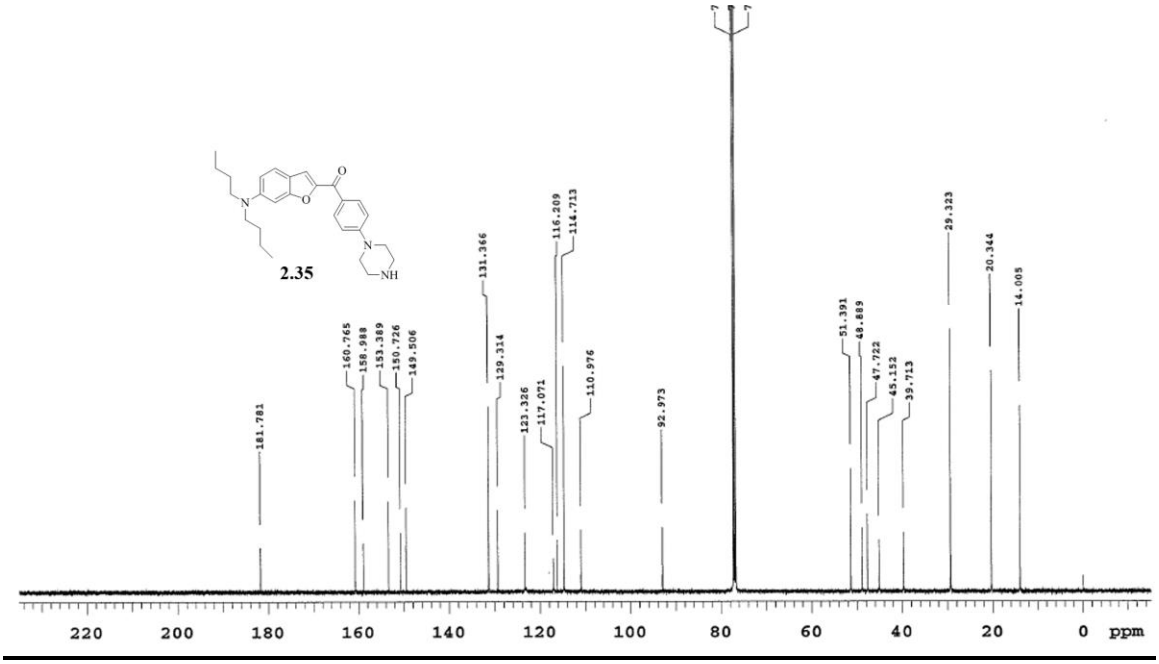
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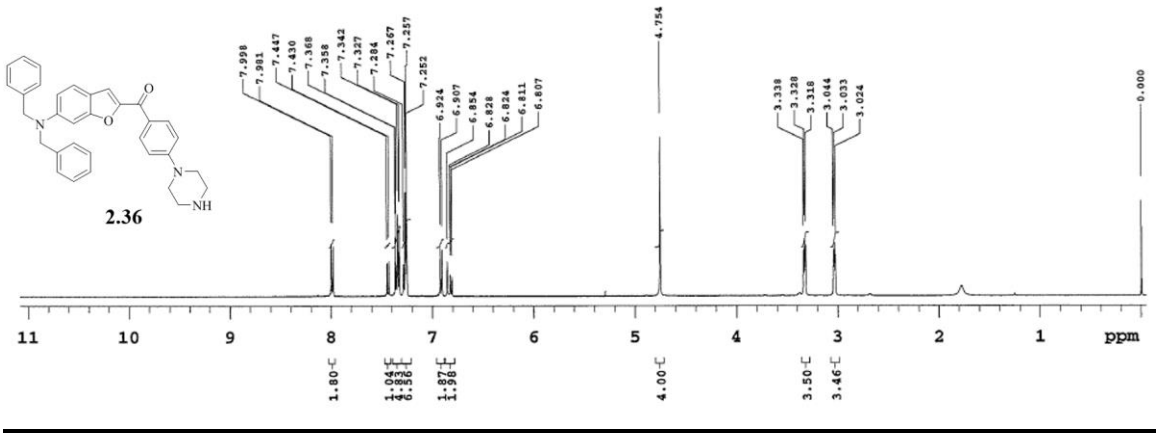
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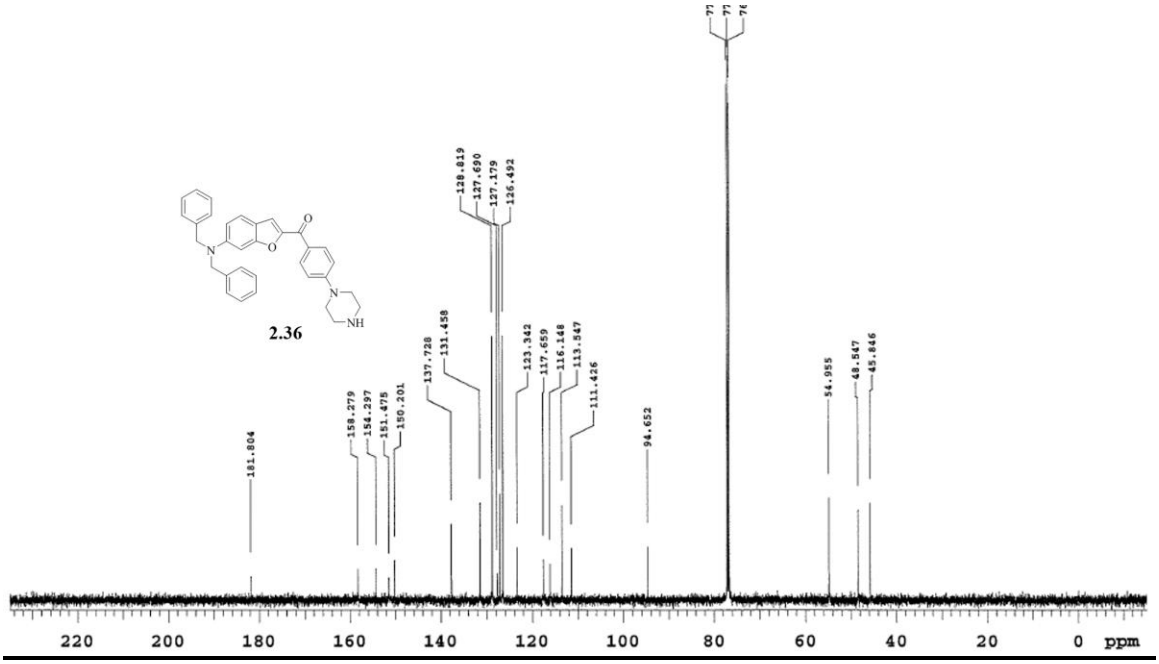
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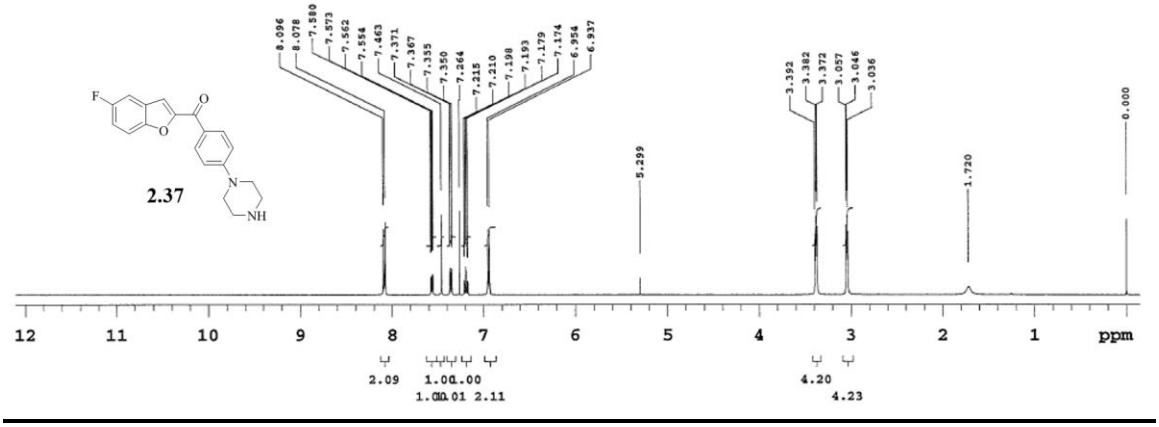
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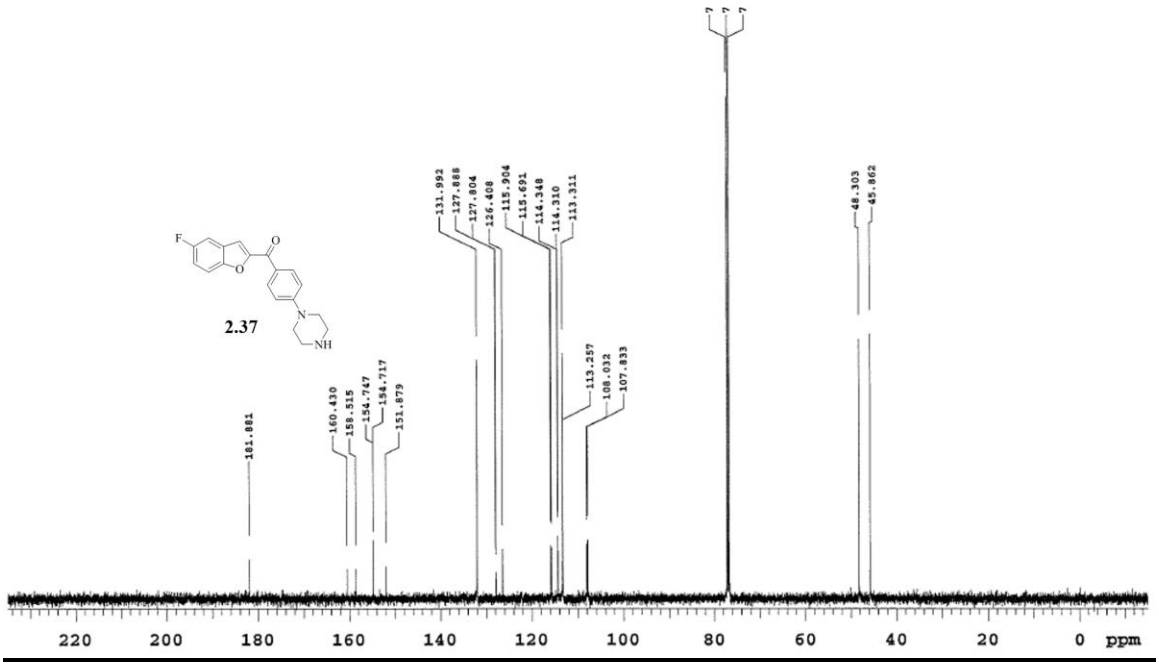
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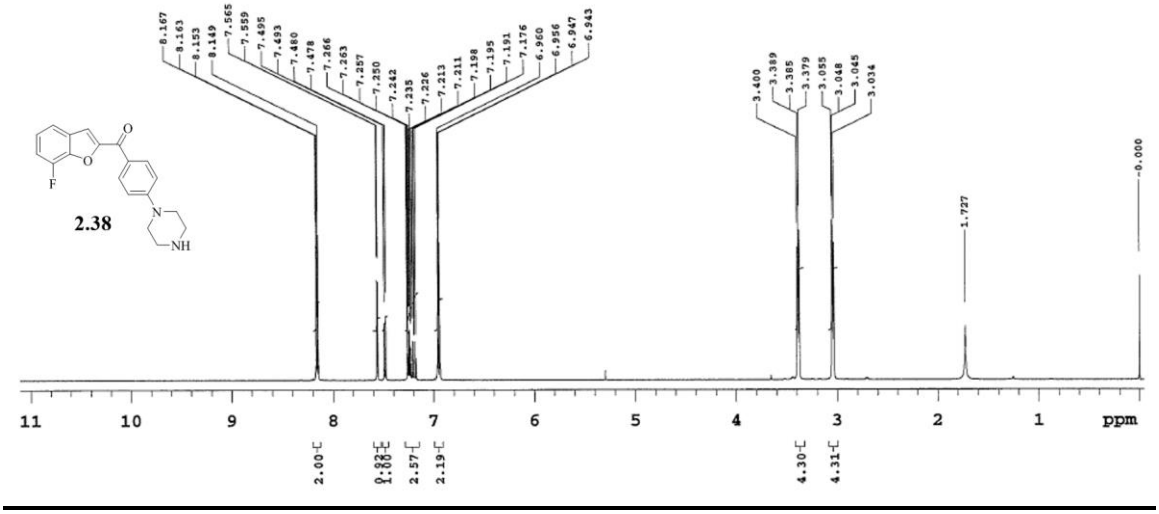
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7.250
7.242
7.235
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7.198
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7.191
7.176
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6.956
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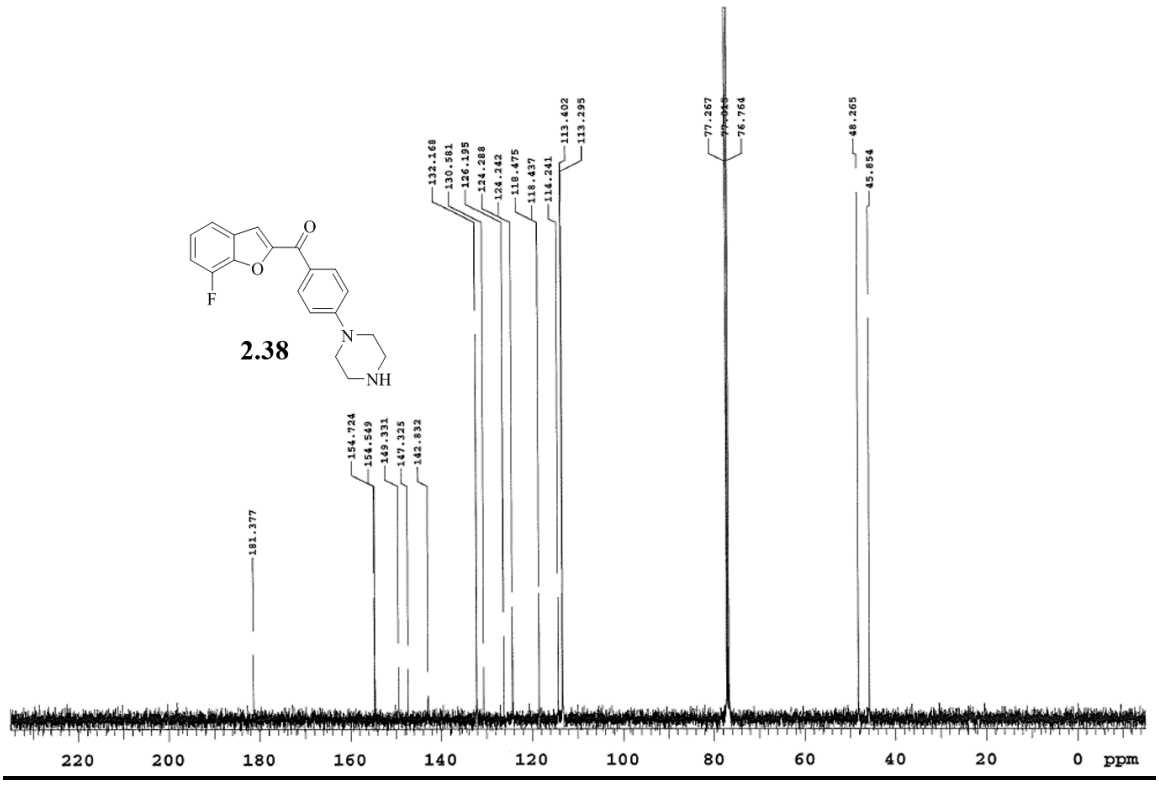
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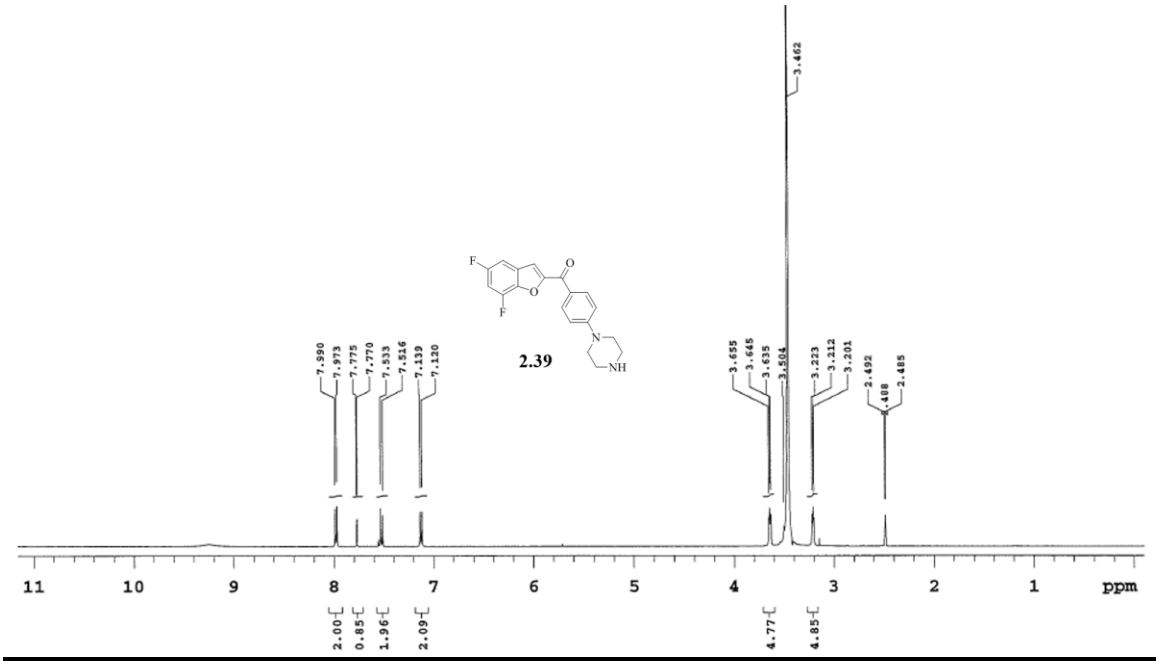
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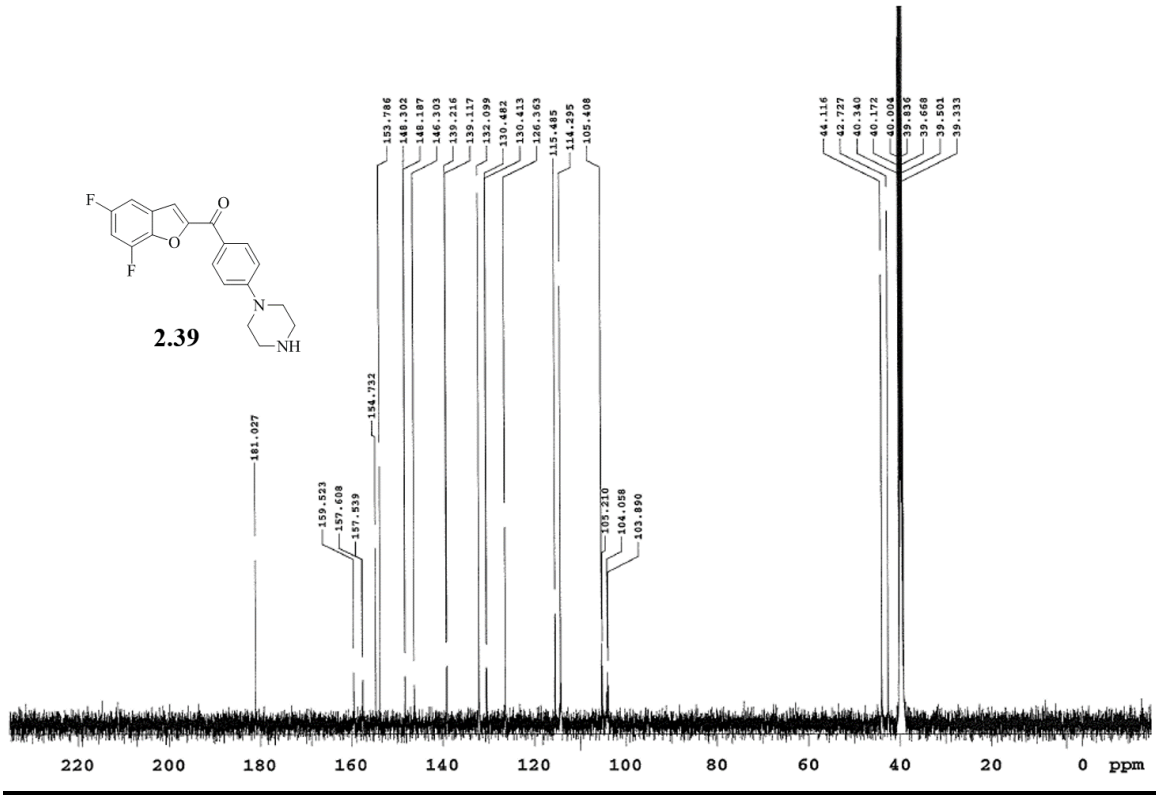
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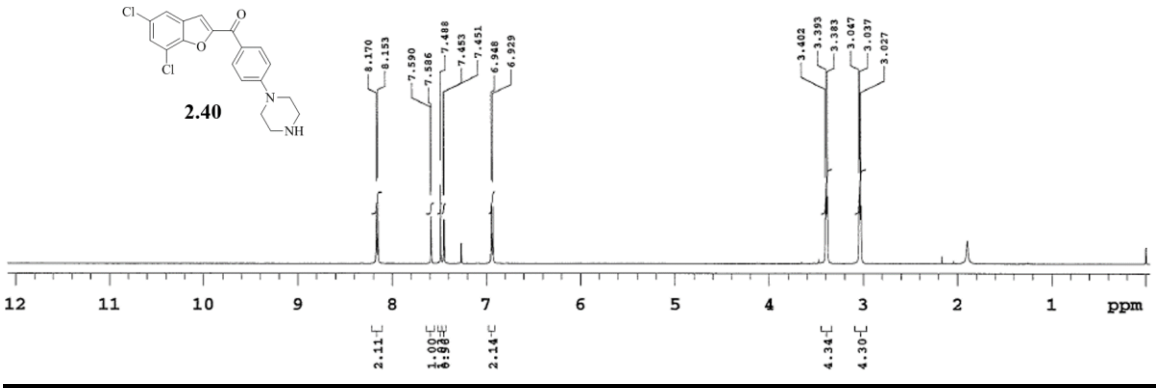
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Cl
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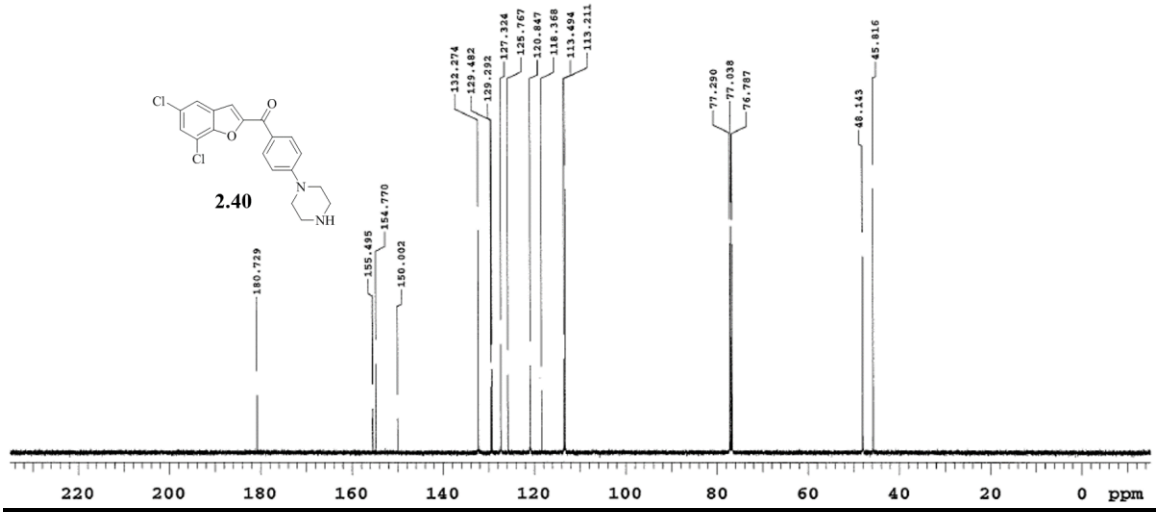
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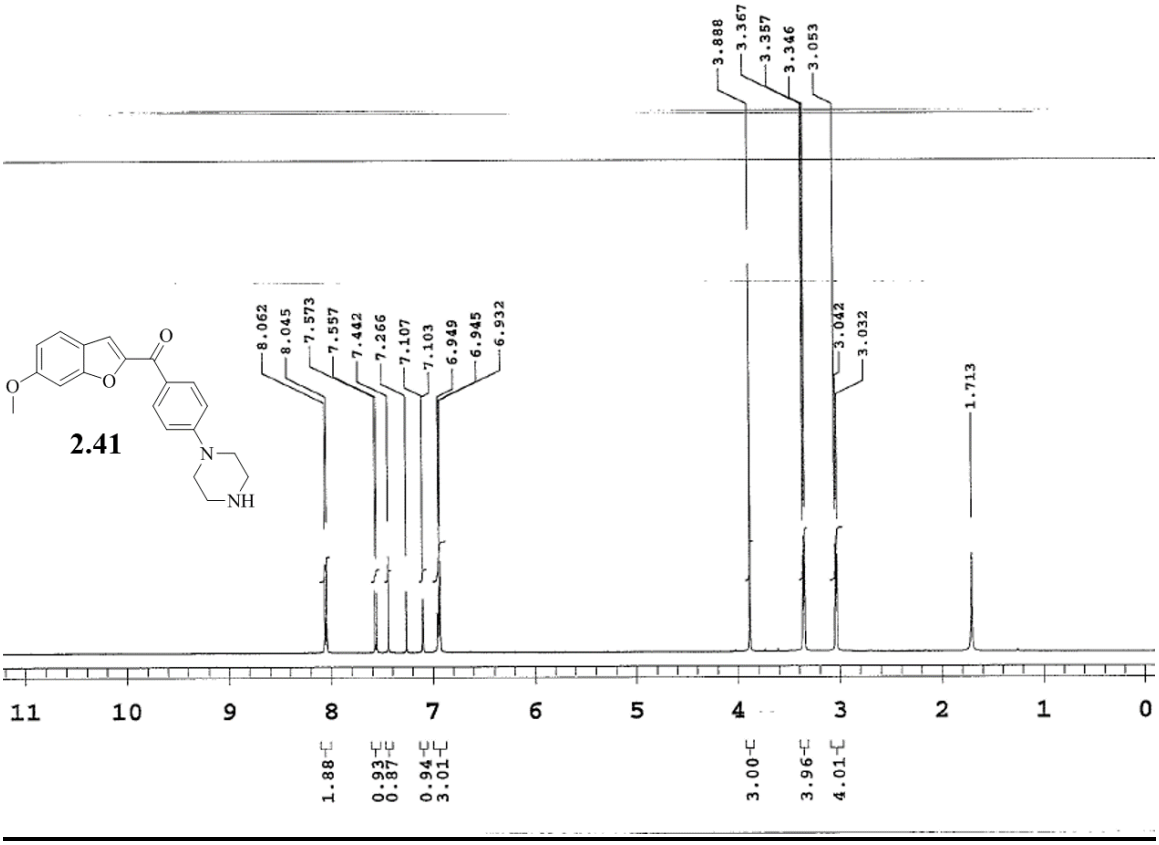
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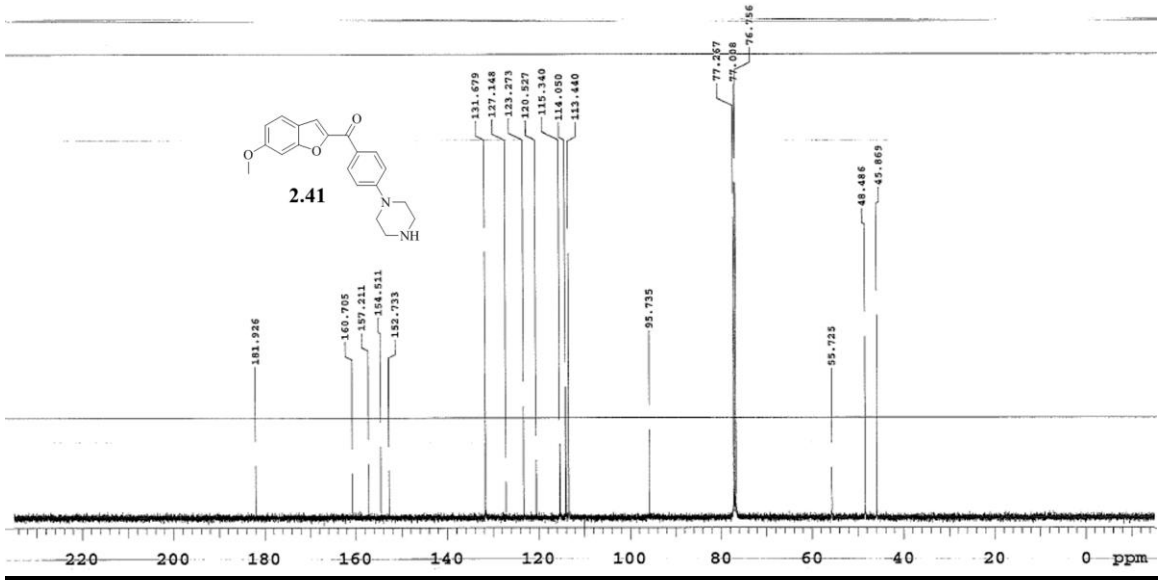
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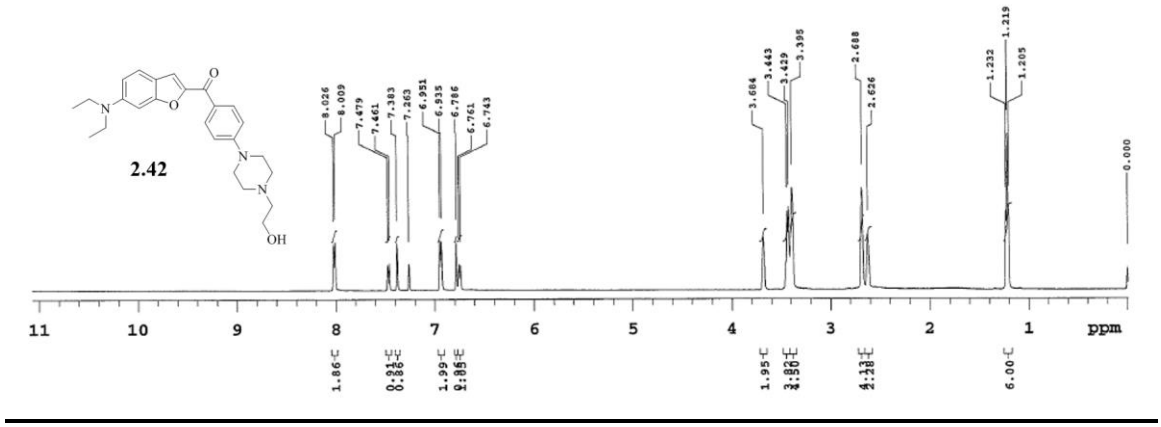
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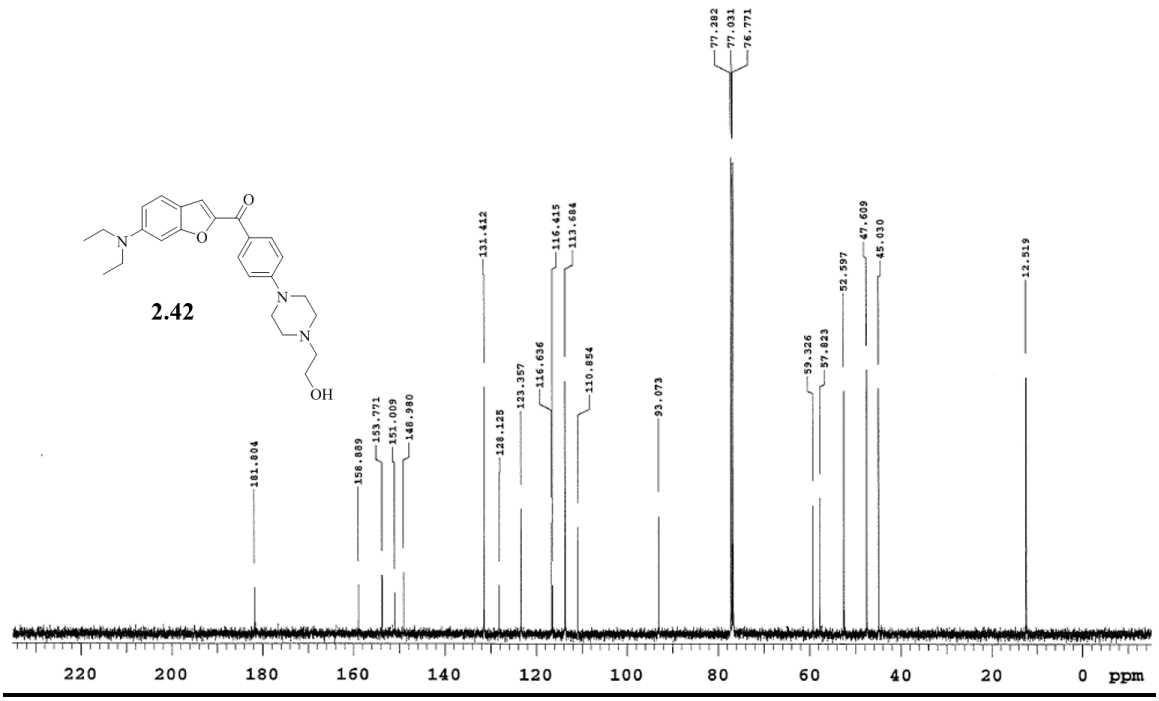
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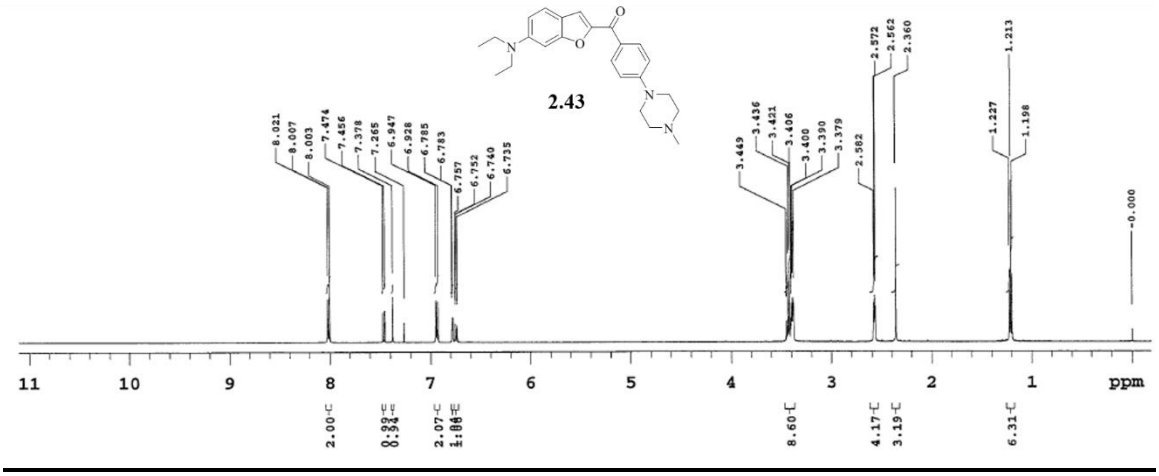
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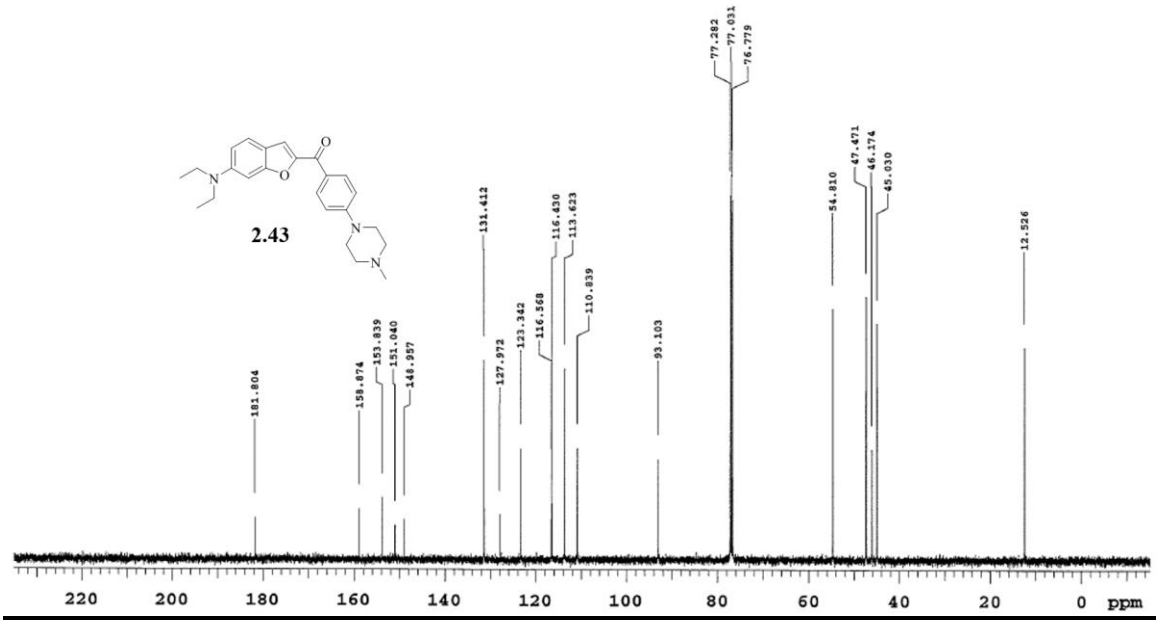
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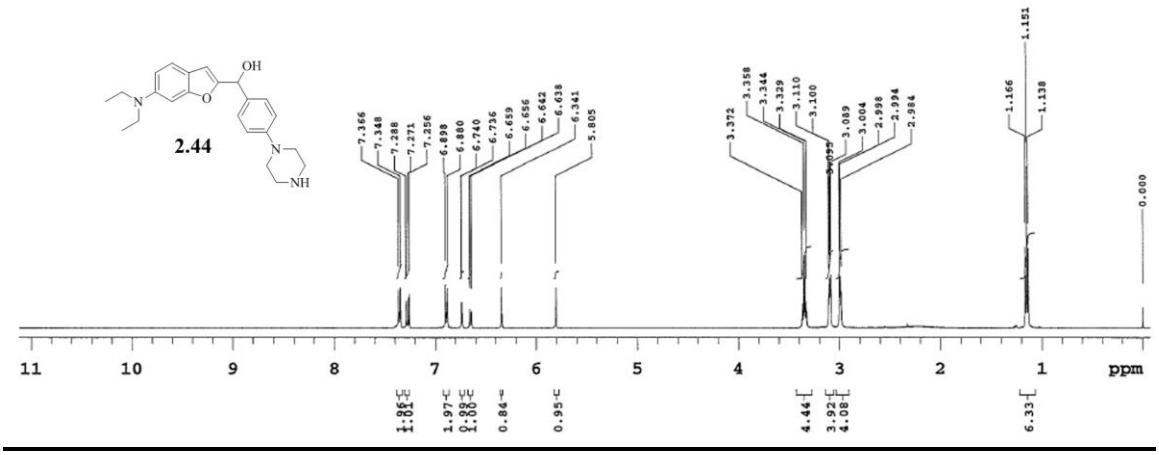
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7
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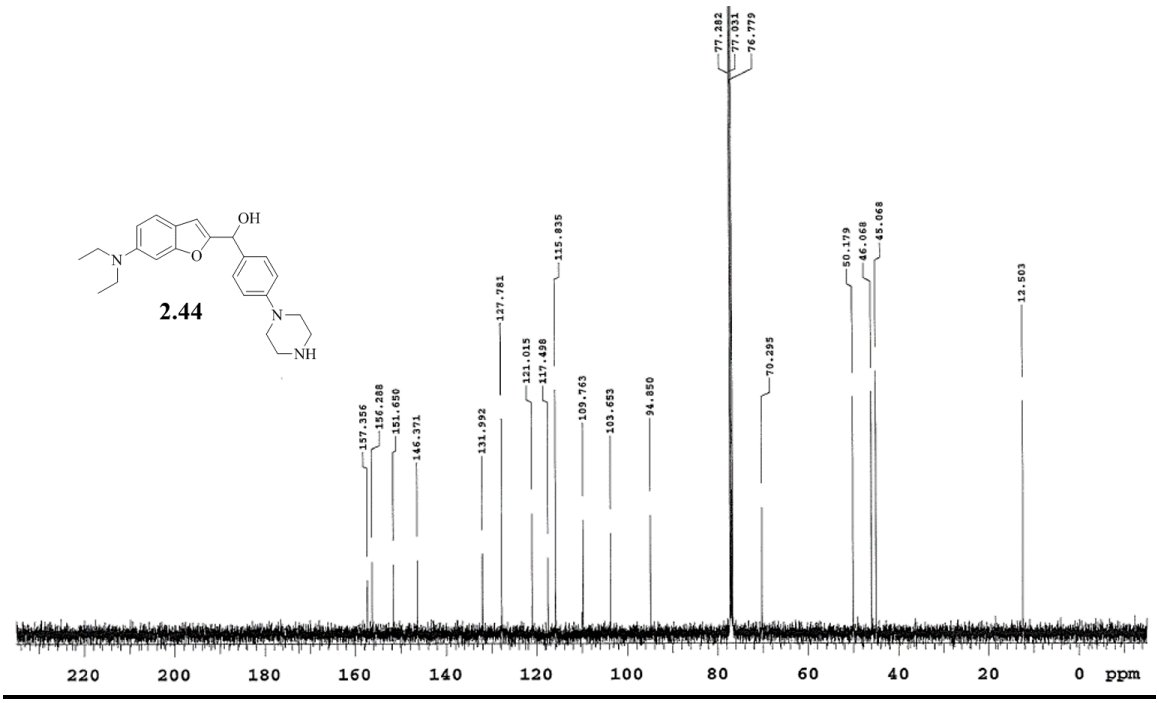
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