Synthesis and evaluation of halogenated 20-HETE formation inhibitors

by

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20-hydroxyecosatetraenoic acid (20-HETE) and epoxygenesatetraenoic acids (EETs) are metabolites of arachidonic acid (AA) formed via CYP hydroxylases and CYP epoxygenases. 20-HETE is a potent vasoconstrictor while EETs show opposite vasodilation activity. A number of studies suggest neuroprotective effects of 20-HETE formation inhibition in animal models of subarachnoid hemorrhage (SAH) and ischemic stroke. These results indicate that 20-HETE formation inhibition is a promising approach for the treatment of brain injury after stroke. Several compounds, such as, 1-ABT, 17-ODYA, 10-SUYS, DDMS and DBDD show 20-HETE formation inhibition. However, their use in treatment has been impeded by shortcomings such as weak potency, low selectivity and poor BBB penetration. 20-HETE formation inhibitors HET0016 and TS-011 exhibit high potency and selectivity but are plagued by poor pharmacokinetic properties. Compound 24 is another 20-HETE formation inhibitor where the labile formamidine moiety seen in HET0016 and TS-011 is replaced with its isostere pyrazole. Unfortunately, compound 24 still suffers from poor metabolic stability. Novel leads UPMP00010 and UPMP00019 were synthesized in the McDermott lab with higher metabolic stability than 24. Their derivatives with simple non-halogen substitutions on the phenyl moiety show improved potency. This work was focused on the synthesis of compounds with halogen substitutions on the UPMP00010/UPMP00019 scaffold with the aim to evaluate their biological activities. 20 compounds with CNS drug likeness characteristics were synthesized. Available data for these compounds shows that compound 16a has very high potency against 20-HETE formation in human and rat microsomal preparations.
(IC_{50}=49.5 \text{nM} \text{ in HLM, IC}_{50}=337 \text{nM} \text{ in RLM}) \text{ and high metabolic stability (100\% of original}
\text{compound remaining after 30 minutes incubation in HLM). Compound 17a also shows high}
\text{potency and metabolic stability (100\% @30 min incubation in HLM) as well as high CNS}
\text{permeability potential (P_{app,A-B}=41.8\times10^{-6} \text{ cm/s}, efflux ratio=0.672). Compounds 20a and 21a are}
\text{also attractive according to their activities in both human and rat microsomes.}

\textbf{Key words:} 20-HETE, EETs, SAH, ischemic stroke, brain injury.
Table of Contents

1.0 Introduction .................................................................................................................................................. 1

1.1 Stroke .......................................................................................................................................................... 1

1.1.1 Subarachnoid hemorrhagic stroke ................................................................................................. 1

1.1.2 Ischemic stroke .................................................................................................................................. 4

1.2 Metabolism of arachidonic acid and biological functions of metabolites ......................................... 5

1.2.1 Metabolic pathway of arachidonic acid ......................................................................................... 5

1.2.2 Formation and biological function of 20-HETE ......................................................................... 7

1.2.3 Formation and biological function of EETs ................................................................................. 8

1.2.4 Effects of 20-HETE in stroke ......................................................................................................... 9

1.2.4.1 20-HETE and SAH ............................................................................................................... 9

1.2.4.2 20-HETE and ischemic stroke .............................................................................................. 10

1.3 20-HETE formation inhibitors ............................................................................................................. 10

1.4 Lead compounds UPMP00010 and UPMP00019 ............................................................................. 13

1.5 Lipinski’s rule of five and CNS drug likeness ..................................................................................... 15

1.6 Derivative optimization paradigm and identification of simple potent 20-HETE formation inhibitors with non-halogen substitutions ......................................................................................... 16

2.0 Chemistry ..................................................................................................................................................... 19

3.0 Results and discussion ............................................................................................................................. 21

3.1 Inhibition of 20-HETE formation in HLM, RLM, RKM and rCYP4F2 ........................................ 21

3.2 Kinetic solubility ...................................................................................................................................... 25

3.3 HLM stability and MDR1-MDCK permeability of selected compounds ........................................ 26
4.0 Conclusion and future directions ................................................................. 29

5.0 Experimental section ................................................................................. 30

  5.1 Instrumentation and reagents ................................................................. 30

  5.2 General procedure I: Synthesis of N-Boc-4-aryl-dyhydropyridines............... 30

  5.3 General procedure II: Synthesis of 4-aryl-piperidines .............................. 31

  5.4 General procedure III: THP protection of iodopyrazoles............................ 31

  5.5 General procedure IV: Synthesis of 4-aryl-1-(1H-pyrazol-4-yl, 3-yl)piperidines .. 32

  5.6 Data for each analog................................................................................. 32

Appendix A ........................................................................................................ 57

Appendix B ........................................................................................................ 60

Bibliography ...................................................................................................... 116
List of Tables

Table 1. Selected UPMP00010 and UPMP00019 derivatives with simple non-halogen substituents ................................................................. 18

Table 2. Key properties and activities, available to this point, for halogenated derivatives 13a-15a, 13b-15b ................................................................. 22

Table 3. Key properties and activities, available to this point, for halogenated derivatives 16a-21a, 16b-21b ................................................................. 23

Table 4. Key properties and activities, available to this point, for halogenated derivatives 22a, 22b ............................................................................. 25

Table 5. MDR1-MDCK permeability of compounds 17a, 20a and 22a ......................................................................................... 27

Table 6. Metabolic stability results for compounds 16a, 17a ................................................................. 28
List of Figures

Figure 1. Metabolic pathways of arachidonic acid ................................................................. 6

Figure 2. 20-HETE formation inhibitors .................................................................................... 11

Figure 3. Physicochemical properties and activities of compound 24, UPMP00010 and UPMP00019 ......................................................................................................................... 14

Figure 4. Structure of two possible tautomers of HET0016, UPMP00010 and UPMP00019..... 15
List of Schemes

Scheme 1. Synthesis of compounds 13a-22a, 13b-22b
Preface

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1.0 Introduction

1.1 Stroke

Stroke is the fifth leading cause of death in the US\(^1\). Despite declining mortality rates over the past decade, stroke still kills around 140,000 Americans and costs the United States around $40 billion each year\(^1\). According to the Stroke Council of the American Heart Association (AHA)/American Stroke Association (ASA), stroke is generally defined as a neurological dysfunction as a result of an acute focal or global injury in the central nervous system (CNS)\(^2\). A transient ischemic attack (TIA) is classically characterized as an episode of focal neurological dysfunction lasting less than 24 hours\(^3\). In TIA, though stroke-like symptoms occur, irreversible injury (infarction) does not present as in stroke. Based on clinical features, stroke can be divided into two types: hemorrhagic stroke and ischemic stroke\(^4\).

1.1.1 Subarachnoid hemorrhagic stroke

Hemorrhagic stroke occurs when cerebral vasculature ruptures and extravasated blood accumulates in either the brain or the subarachnoid space, and thus can be sub-divided into two subtypes: intracerebral hemorrhage (ICH) and subarachnoid hemorrhage (SAH), accounting for around 10% and 3% of all strokes respectively\(^1\).

Subarachnoid hemorrhage is one of the most devastating and dangerous type of strokes though it appears to be less frequent than ischemic stroke and intracerebral hemorrhage\(^5\). The average age of a patient that suffers SAH is 55\(^6\). There is a 12% mortality rate in patients prior to
receiving medical intervention and 40% mortality rate within 30 days of initial resuscitation and a 50% disability rate among survivors. SAH can be caused by aneurysm, arteriovenous malformation and traumatic brain injury. Rupture of aneurysm accounts for 85% cases of the SAH.

There are two phases of SAH: early brain injury (EBI) and delayed cerebral ischemia (DCI).

Early brain injury occurs within the first 72 hours after the initial event and is mediated by a combined effects of transient cerebral ischemia and extravasated blood. Brain is one of the most energy demanding organs. Although it consists only 2% of a person’s body weight the brain consumes around 20% of required daily-intake of energy. Glucose is the preferred fuel for the brain and ketone bodies serve as a supplemental source of energy in periods of starvation. Unlike other organs such as liver and skeletal muscle for example, the brain does not store glucose in the form of glycogen, and therefore the normal operation of brain largely depends on stable blood flow and glucose supply. To maintain cerebral blood flow (CBF), cerebral perfusion pressure (CPP) is kept within a narrow range around 60mmHg and is fundamentally supported by the intrinsic myogenicity of cerebral vascular smooth muscle. Upon the rupture of aneurysm, patients experience a sudden increase of intracerebral pressure (ICP). This increase often leads to downstream complications such as cell apoptosis and cerebral ischemia as well as loss of consciousness. The precise mechanism of increased ICP remains unclear and can be multifactorial. Increased ICP is hypothesized to be caused by effect from extravasated blood, vasoconstriction and occlusion of cerebrospinal fluid (CSF) circulation. Increased ICP directly causes reduced CPP and resultant reduced cerebral blood flow (CBF). Mean artery pressure (MAP) increases under the effects of activated sympathetic nervous system to attenuate the reduced CPP. Under the systemic effect of activated sympathetic nervous system, in addition to circulatory syndromes such as
tachycardia and tachypnoea, cerebral vessel constriction is also enhanced, decreasing CPP and further aggregating CBF insufficiency. Ostrowski et al. established a rat SAH model and recorded that ICP was 7.3±1.18 mm Hg in the control group prior to SAH and reached 75.18±14.83 mm Hg in the SAH group, while the CPP dropped from 107.38±3.02 mm Hg to 38.71±10.76 mm Hg in SAH group\textsuperscript{12}. CBF also dropped dramatically within 1 minute of the attack, to 26.26%±2.23% of the baseline value\textsuperscript{12}. Impairment of the blood brain barrier (BBB) caused by apoptotic death of cerebral endothelium cells increases the passage of fluid into the brain and causes cerebral edema which further aggregates the ischemia in the brain\textsuperscript{13}. Park et al. has shown that most apoptosis was observed in endothelium cells and administration of z-VAD-FMK, a pan-caspase inhibitor, in a rat SAH model caused decreased level of apoptosis markers and was followed by reduced vasospasm and edema as well as improved neurological outcomes\textsuperscript{13}. Currently, there is no specific treatment for early brain injury in SAH. Endovascular coiling and neurosurgical clip significantly improved the outcome of SAH patient\textsuperscript{14}, possibly by preventing re-bleed of aneurysms\textsuperscript{6}.

After EBI, SAH survivors may deteriorate as a result of delayed cerebral ischemia (DCI). DCI, which occurs 3-14 days after ictus, causes poor outcomes and death in 30% of SAH patients\textsuperscript{5}. DCI shares similarities in pathology with EBI and even EBI contributes to DCI. The pathological mechanism of DCI remains unclear and is hypothesized to be associated with vasospasm, arteriolar constriction, micro-thrombosis and cortical spreading ischemia\textsuperscript{10, 15}. Given the multifactorial nature of DCI, a number of interventions have been considered. Application of statins, anticoagulant therapy, and magnesium, though showed some improvement over certain symptoms like vasospasm or thrombosis on patients, failed to present any significant effect on relevant outcomes\textsuperscript{16-18}. So far nimodipine, an L-type calcium channel antagonist, remains the only medication to improve outcomes on patients with SAH\textsuperscript{19, 20}. 

3
1.1.2 Ischemic stroke

Ischemic stroke is the most common type of stroke. It is caused when CBF is blocked or partially reduced. CBF blockade can be caused by either thrombus that is formed within cerebral vasculature or a blood clot that traveled to the brain from another site of the body, and therefore, ischemic stroke can be further divided into two subtypes: thrombotic stroke and embolic stroke respectively. Brain injury from ischemic stroke is the result of initial ischemia and consequent cascades, that involve energy depletion, malfunction of ion gradients, release of excitatory neurotransmitters, production of oxygen free radicals and apoptosis. After the ictus, reduced oxygen and glucose supply in the brain directly leads to focal energy depletion. Dysfunctional ion pumps on the surface of neurons and glia fail to maintain the balance of ions like Na+, K+ across the cellular membrane. Ion imbalance causes an increased permeation of water into the cell and thus cellular swelling and damage present. Excessive release of excitatory neurotransmitters, glutamate and aspartate, aggravate energy insufficiency by increasing cellular activity. This contributes to the formation of free radicals and apoptosis. All these factors in addition to the over-expressed proinflammatory genes, lead to post-ischemic inflammation and increase the size of irreversible neurological injury.

Intravenous thrombolysis and mechanical thrombectomy are two major medical interventions for ischemic stroke. Ischemic tissue can be divided into two layers. The inner core, which is perfused below 10-25% of normal level, is at risk of dying within minutes or displays irreversible necrosis. The outer layer, known as the penumbra, is less hypo-perfused and is much easier to salvage. Early recovery of blood flow is highly important in this setting. The absolute incidence rate difference (ARD) for favored outcomes is decreased by 6% per hour delay of CBF recovery. So far, intravenous injection of recombinant tissue-type plasminogen activator (IVrt-
PA) is the only drug approved by FDA and has shown significant reduction on poor outcomes after ischemic stroke. However, application of IVrt-PA is limited due to its narrow therapeutic window. This treatment must be applied within 3 hours of stroke onset\textsuperscript{27}. Mechanical thrombectomy is also applicable if the patient is opposed to IVrt-PA\textsuperscript{28}. Two mechanical devices, “mechanical embolus removal in cerebral ischemia (MERCI) device” and “Penumbra Stroke system” were approve by FDA in 2004 and 2008 respectively for treatment of ischemic stroke in addition to IVrt-PA\textsuperscript{27}.

1.2 Metabolism of arachidonic acid and biological functions of metabolites

1.2.1 Metabolic pathway of arachidonic acid

Arachidonic acid, a polyunsaturated omega-6 fatty acid (C20:4 n-6; AA), is an essential component of the cell membrane, rendering membrane fluidity and flexibility\textsuperscript{29}. Metabolites of AA exhibit a variety of biological functions. They play an important role in the regulation of renal and pulmonary function, vascular tone and inflammation\textsuperscript{30}. Free AA is metabolized via three major enzymatic pathways: the cyclooxygenase (COX), the lipoxygenase (LOX) and the cytochrome P450 (CYP 450) pathways (Figure. 1).
Cyclooxygenase enzymes show dual functions, acting as cyclooxygenase and peroxidase. Free AA is essentially converted into prostaglandin PGG2 via cyclo-oxygenation reaction, followed by a peroxidase reaction, reducing PGG2 to prostaglandin PGH2. Prostaglandin PGH2 is then converted to prostaglandin PGD2, PGE2, PGF2; prostacyclin PGI2 and thromboxane TXA2 and TXB2 under various synthases.

Lipoxygenase LOX-5, LOX12 and LOX-15 catalyze the oxygenation of the 5-, 12-, and 15-carbon atoms of AA respectively to form corresponding 5-, 12- and 15-hydroperoxyeicosatetraenoic acid (5-, 12 and 15-HPETE), which are converted subsequently to 5-, 12- and 15-hydroxyeicosatetraenoic acid (5-, 12- and 15-HETE). 5-LOX activated by FLAP (5-lipoxygenase-activating protein) further dehydrates 5-HPETE to produce leukotriene LTA4 which is then converted into leukotriene LTC4, LTD4, LTE4 and LTB4.
In addition to these two classic pathways, free AA can be alternatively metabolized by membrane bound and heme-containing cytochrome P450 enzymes in a NADPH-dependent manner to produce 20-hydroxyeicosatetraenoic acid (20-HETE) and epoxyeicosatrienoic acids (ETTs) via CYP hydroxylases and CYP epoxygenases respectively.\(^36\)

1.2.2 Formation and biological function of 20-HETE

20-HETE is produced in the liver, kidney, lung, heart and brain in a paracrine manner. The formation of 20-HETE is mainly catalyzed by the CYP4A and the CYP4F families.\(^37\)\(\text{-}\)42 Experimental results demonstrated the predominant role of CYP4F2 and CYP4A11 in both human kidney and liver to produce 20-HETE.\(^39\), 43 In rats, the corresponding isoforms are CYP4A1, CYP4A2, CYP4A8, CYP4F1 and CYP4F4.\(^{44, 45}\) In the brain, CYP4A enzymes play a major role in the formation of 20-HETE.\(^37\), 42 CYP hydroxylases also catalyze the formation of AA to 16-, 17-, 18- and 19-HETE but the relative ratios of these products vary with enzyme isoforms.\(^46, 47\)

20-HETE is a potent vasoconstrictor in a number of tissues including liver, kidney, heart, lung and the cerebral vasculature.\(^41, 48, 49\) Gebremedhin et al. observed that 20-HETE decreased the internal diameter of cerebral arteries in rat in a dose related manner. The maximum effect of 20-HETE was achieved at 10\(^{-6}\) mol/L, leading to 25.3% reduction of artery diameter. The vessel constricting effects of 20-HETE are mediated by the inhibition of large conductance calcium activated potassium channels (\(K_{Ca}\)) and the activation of L-type \(Ca^{2+}\) channels on vascular smooth muscle (VSM).\(^50\) Harder et al. proved that \(K_{Ca}\) channels on cat cerebral micro-vessels were inhibited by 20-HETE and the inhibition was alleviated by 17-octadecynoic acid (17-ODYA), a compound that inhibits 20-HETE formation.\(^37\) Gebremedhin et al. further proved that 20-HETE activates L-type \(Ca^{2+}\) channels on cat cerebral VSM and treatment with nifedipine, an L-type \(Ca^{2+}\)
channel inhibitor, was followed by a reduction in vessel constriction\textsuperscript{38}. The precise signaling pathways involved in vasoconstriction remain unclear but protein kinase C (PKC) appears to play an important role\textsuperscript{41, 45}. Lange et al. demonstrated that 20-HETE decreased the diameter of cat middle cerebral arteries in a dose-dependent manner and this reduction could be alleviated by a PKC inhibitor\textsuperscript{51}.

20-HETE, produced by VSM, vascular endothelium and astrocytes\textsuperscript{36, 41, 48, 50}, plays a central role in the autoregulation of cerebral blood flow in the brain\textsuperscript{50}. Gebremedin et al. showed that the concentration of 20-HETE in cerebral arteries increased six fold when transmural pressure changed from 20 to 140 mm Hg and the followed pressure-induced vasoconstriction could be blocked by various 20-HETE inhibitors\textsuperscript{50}. In addition to CBF autoregulation, 20-HETE was proven to promote angiogenesis, vascular remodeling and vascular inflammation under pathological conditions\textsuperscript{52-54}.

1.2.3 Formation and biological function of EETs

Four regioisomers of epoxyeicosatrienoic acid (EET) 5,6-, 8,9-, 11,12- and 14,15-EET are produced via the CYP epoxygenase pathway\textsuperscript{55}. Generally, the production of EETs is catalyzed predominantly by the CYP2C and the CYP2J families in both humans and rats\textsuperscript{47}. In human and rat heart, EETs are predominantly produced by CYP2J\textsuperscript{256}. In the kidney and liver of both humans and rats, CYP2C8 and CYP2C9 are mainly responsible for the production of EETs\textsuperscript{57}. CYP epoxygenases produce EET isomers in different ratios. The ratio of 14,15-EET and 11,12-EET produced by CYP2C8 is 1.25:1, while 8,9-EET, 11,12-EET and 14,15-EET are produced by CYP2C9 in a ratio of 1:2:4.6\textsuperscript{58}. CYP2J2 produces equal amount of 4 EET isomers\textsuperscript{59}.
EETs are mainly produced in the liver, kidney and brain, and show opposite function than 20-HETE. In the brain, ETTs are mainly produced in cerebral VSM cells and astrocytes\textsuperscript{41,48}. EETs activate the large conductance calcium activated potassium channels (K\textsubscript{Ca}) and inhibit the L-type calcium channels, causing hyperpolarization and vasodilation\textsuperscript{60}. Promotion of angiogenesis and vascular remodeling is the only effect of EETs shared with 20-HETE\textsuperscript{61}. EETs also exhibit anti-inflammatory properties and inhibit platelet aggregation\textsuperscript{62}.

1.2.4 Effects of 20-HETE in stroke

Stroke is the fifth leading cause of death in the US and few effective treatments are available. 20-HETE is a potent vasoconstrictor with pro-inflammation and pro-angiogenesis activity, and numerous preclinical studies suggest that inhibition of its formation may be beneficial for neuroprotection after stroke.

1.2.4.1 20-HETE and SAH

A number of animal studies have implicated 20-HETE in cerebral vasospasm and the decrease of CBF after SAH. For example, Kehl et al. using a rat SAH model showed that after injury the regional cerebral blood flow (rCBF) decreased by 30\%, and that reduction correlated with elevation of the 20-HETE concentration in the cerebrospinal fluid (CSF) from 12 to 199 ng/ml\textsuperscript{63}. He further showed that the acute fall of rCBF was mitigated and returned to a normal level, 1 hour after initial ictus, in animals pretreated with two 20-HETE formation inhibitors: 17-ectadecynoic acid (17-ODYA, 1.5 nmol intrathecally) and N-hydroxy-N\'-(4-butyl-2-methylphenyl)formamidine (HET0016, 10mg/kg intravenously)\textsuperscript{63}. In a dual-hemorrhage model of SAH in rats, Takeuchi et al. confirmed the effects of another 20-HETE inhibitor, N-(3-chloro-4-
morpholin-4-yl)phenyl-N’-hydroxyimido formamide (TS-011, 0.1 mg/kg intravenously), and showed that it dilated the cerebral arteries and recovered the CBF to a normal level\textsuperscript{64}. Hacein-Bey et al. further showed that TS-011 can attenuate vasoconstriction and vasospasm in a dual hemorrhage model of SAH in dogs\textsuperscript{65}.

Clinical research showed that the level of 20-HETE in CSF of patients with acute aneurysmal subarachnoid hemorrhage (aSAH) was significantly associated with the presence of DCl\textsuperscript{66}. Donnelly et al. further demonstrated that patients with a high or moderate level of 20-HETE in CSF showed 2-3 fold higher rate of mortality and had poor outcomes\textsuperscript{67}.

1.2.4.2 20-HETE and ischemic stroke

Various studies demonstrated the role of 20-HETE in ischemic stroke. Miyata et al. found that direct injection of 20-HETE to the carotid artery of rats induced ischemic infarct similar to the infarct presented in a rat transient occlusion of the middle cerebral artery (MCAO) ischemic stroke model\textsuperscript{68}. In the MCAO stroke model of rats, HET0016 (10mg/kg, intraperitoneally once daily from day 1 to day 6) and TS-011 (0.01-1.0 mg/kg) significantly reduced the infarct size\textsuperscript{69, 70}. Clinical studies also demonstrated the plasma level of 20-HETE as a strong predictor of neurological deterioration in patients with acute minor ischemic stroke\textsuperscript{71}.

1.3 20-HETE formation inhibitors

20-HETE plays an important role in the cerebral vasoconstriction after stroke, and efforts to find 20-HETE formation inhibitors over the years led to a variety of 20-HETE formation inhibitors.
1-Aminobenzotriazole (1-ABT), a compound that was originally synthesized in 1960, is the first identified 20-HETE formation inhibitor\textsuperscript{72}. 1-ABT is not selective, however, and inhibits both 20-HETE and EETs formation\textsuperscript{73}. It inhibits potently all CYP isoforms in a suicide manner by the generation of benzyne within the active site of the CYP enzymes that reacts with their heme moiety\textsuperscript{74}.

Derivatives of long chain fatty acids represent another group of 20-HETE formation inhibitors. Inhibition is achieved in either suicide or competitive manner with these compounds. Suicide inhibitors include 17-octadecynoic acid (17-ODYA) and sodium 10-undecynyl sulfate (10-SUYS)\textsuperscript{75, 76}. 17-ODYA inhibits 20-HETE and EETs equally, with IC\textsubscript{50} values of 7 μM and 5 μM respectively in rat kidney\textsuperscript{77, 78}. The IC\textsubscript{50} of 10-SUYS toward 20-HETE formation in rats is 10.1±2.6 μM\textsuperscript{79}. 10-SUYS does not affect epoxigenases up to a maximal tested concentration of 50
μM\textsuperscript{79}. Competitive inhibitors, \textit{N}-methylsulfonyl-12,12-dibromododec-11-enamide (DDMS) and 12,12-dibromododec-11-enoic acid (DBDD) exhibit higher potency and selectivity, with the same IC\textsubscript{50} value of 2 μM towards omega-hydroxylation and IC\textsubscript{50} value of 60 and 51μM against epoxidation respectively\textsuperscript{78}. However, micromolar IC\textsubscript{50} and 25-30 fold selectivity are far from enough and they also suffer from common deficits of fatty acid analogues, including high protein binding affinity and poor BBB penetration.

\textit{N}-(4-Butyl-2-methylphenyl)-\textit{N}'-hydroxyformamidine (HET0016) is a potent and selective 20-HETE formation inhibitor discovered by Taisho Pharmaceuticals through high-throughput screening\textsuperscript{80}. The IC\textsubscript{50} value of HET0016 against 20-HETE formation in rat renal microsomes and human renal microsomes is 35±4 nM and 8.9±2.7 nM respectively, whereas the IC\textsubscript{50} for inhibition of EETs in rat renal microsomes is 100 fold higher (IC\textsubscript{50}=2800±300 nM)\textsuperscript{81}. HET0016 also exhibits high selectivity over other of CYP isoforms. It has an IC\textsubscript{50} value of 3300, 83900 and 71000 nM against CYP2C9, CYP2D6 and CYP3A4 respectively\textsuperscript{81}. Unfortunately, despite its good activity, the pharmaceutical application of HET0016 is limited. It shows low solubility and poor stability in acidic environment possibly due to its \textit{N}-hydroxyformamidine moiety\textsuperscript{82}. Additionally, HET0016 has a short half-life, around 40 minutes in rats\textsuperscript{70}.

\textit{N}-(3-Chloro-4-morpholin-4-yl)phenyl-\textit{N}'-hydroxyimido formamide (TS-011) is a derivative of HET0016. This compound maintains potency and selectivity and has improved solubility over its parent (HET0016). The IC\textsubscript{50} of TS-011 against the formation of 20-HETE in human renal microsomes, recombinant CYP4A and CYP4F ranges from 10 nM to 50 nM\textsuperscript{68}. Unfortunately, TS-011, like its parent, suffers from poor pharmacokinetic properties, and an even shorter half-life (less than 10 minutes) in rats\textsuperscript{68, 69}.
In order to improve the stability of compounds with the formamidine group, isosteric replacements of the formamidine have been explored. Among the compounds synthesized, compound 24 (number corresponds to compound number in ref. 82) showed good potency (IC$_{50}$=26.2 nM against 20-HETE formation in human renal microsomes) and selectivity when compared with that of HET0016. However, assessment of compound 24 for metabolic stability in the laboratory of Dr. Samuel Poloyac at the University of Pittsburgh showed that this compound suffers from poor metabolic stability as well. When incubated with human liver microsomes for half an hour, only 35% of the original compound remained.

1.4 Lead compounds UPMP00010 and UPMP00019

Compound 24, despite its metabolic shortcomings, appeared to be a good starting point for new lead development in the McDermott lab. The studies describing the exploration of isosteres for the formamidine group suggest that the pyrazole moiety in compound 24 affords good potency and selectivity for 20-HETE formation inhibition when compared to non-pyrazole formamidine isosteres seen in other derivatives of compound 24.

Scaffold-hopping is a key strategy in medicinal chemistry for designing novel compounds. Through scaffold-hopping, compound UPMP00010 was designed. In this compound, the pyrazole-phenyl moiety seen in compound 24 was replaced by a pyrazole-piperidine motif. UPMP00010 was designed under the assumption that the pyrazole-piperidine moiety has a lower degree of planarity than the pyrazole-phenyl motif. The expectation was that this would lead to an increase in aqueous solubility. Disruption of molecular planarity is an important strategy employed in medicinal chemistry to increase the aqueous solubility of small molecules, because it can disrupt
their molecular stacking\textsuperscript{87}. Additionally, it was thought the electron donating piperidine moiety might further increase solubility by increasing the pKa of the pyrazole moiety.

![Chemical structures](image)

<table>
<thead>
<tr>
<th>Cmpd</th>
<th>LogP</th>
<th>PSA</th>
<th>Acidic pK\textsubscript{a}</th>
<th>IC\textsubscript{50} of 20-HETE formation in HLM (nM)</th>
<th>IC\textsubscript{50} of EETs formation in HLM (nM)</th>
<th>Kinetic solubility limit (\textmu M)</th>
<th>HLM stability (% remaining after 30 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>3.0</td>
<td>37.9</td>
<td>13.6</td>
<td>10</td>
<td>/</td>
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<td>35%</td>
</tr>
<tr>
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<td>31.9</td>
<td>13.9</td>
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<td>&gt;50000</td>
<td>&gt;600</td>
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<tr>
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<td>31.9</td>
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<td>187</td>
<td>&gt;10000</td>
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</tbody>
</table>

**Figure 3. Physicochemical properties and activities of compound 24, UPMP00010 and UPMP00019**

In order to pursue higher potency, UPMP00019 was designed. Crystal structures reveal that the formamidine group presents in a cis configuration\textsuperscript{88}. This, in turn, suggests two possible tautomers for HET0016 as shown in figure 4. As such, the 4-pyrazole moiety of UPMP00019, where two nitrogen atoms are positioned symmetrically, better mimics the two possible tautomers of the formamidine group seen in HET0016.

UPMP00010 and UPMP00019 when tested for 20-HETE formation inhibition in Dr. Poloyac’s lab were found to be potent and highly selective. Both compounds had middle to low nM potency against 20-HETE formation inhibition as shown in figure 3. Both compounds showed
no inhibition on the formation of EETs in human liver microsomes up to highest tested concentrations of 50 µM and 10 µM respectively. Additionally, both compounds were much more stable in human liver microsomes when compared to compound 24. These attributes made UPMP00010 and UPMP00019 particularly attractive candidates for further optimization in the McDermott lab.

![Figure 4. Structure of two possible tautomers of HET0016, UPMP00010 and UPMP00019](image)

1.5 Lipinski’s rule of five and CNS drug likeness

Lipinski’s rule of 5 is a guideline often used in drug discovery for the evaluation of the drug-likeness of compounds. This rule is only applied to drugs whose permeation is achieved through passive diffusion. According to Lipinski’s rule of 5, a compound is predicted to have good
oral absorption or membrane permeability if it has less than 5 hydrogen bond donors (HBD), 10 hydrogen bond acceptors (HBA), molecular weight (MW) less than 500 and the calculated logP (clogP) less than 5\textsuperscript{89}. An additional rule proposed by Veber recommends that the number of rotatable bonds (NRB) should also be also less than 10 in order to achieve good bioavailability\textsuperscript{90}. Since the target of our compounds is in the brain, it is very important that compounds have suitable physicochemical properties to cross the BBB. In 2005, Pajouhesh et al. summarized studies done on physicochemical properties of CNS drugs\textsuperscript{91}. These studies suggest that CNS drugs usually have narrower property ranges than those described by the rule of 5. Successful CNS drugs have MW less than 450, clogP less than 3.5, HBD less than 3, HBA less than 7, NRB less than 8, and polar surface area (PSA) less than 70 Å\textsuperscript{2} \textsuperscript{91}.

1.6 Derivative optimization paradigm and identification of simple potent 20-HETE formation inhibitors with non-halogen substitutions

The aim of the McDermott lab is to optimize UPMP00010 and UPMP00019 as neuroprotectants after ischemic events.

As the objective is inhibition of 20-HETE formation in the brain, prior to synthesis, potential derivatives are assessed against the physicochemical properties of successful CNS drugs and only derivatives with suitable properties are synthesized. After synthesis, compounds are evaluated for potency at a single point titration at 500nM in human liver microsomes (HLM), rat liver microsomes (RLM), rat kidney microsomes (RKM) and recombinant CYP4F2 (rCYP4F2) in the lab or our collaborator Dr. Samuel Poloyac at the University of Pittsburgh. The objective of examining the potency of compounds in human and rat microsomes is to better understand the
interspecies potency differences and to ensure the preclinical suitability of compounds for study in animal models. Compounds that show greater than 50% inhibition at 500nM in HLM are assessed further for IC$_{50}$ of 20-HETE formation inhibition, kinetic solubility and metabolic stability.

Metabolic stability is assessed by the percentage of original compound remaining after incubation in HLM for 30 minutes, as this is a good preliminary indicator of intrinsic hepatic clearance$^{92}$. In general, compounds which show a percentage of original compound remaining greater than 80% are regarded to have low hepatic clearance. Based on potency and/or other attributes compounds may also be selected for MDR1-MDCK permeability assay. MDR1-MDCK cells are Madin Darby canine kidney cells transfected with MDR1 gene, which encodes for drug efflux protein, P-glycoprotein (P-gp). MDR1-MDCK permeability assay is an important method to examine the BBB penetration potential of CNS drugs$^{93}$. Drugs that can passively cross BBB have high values of absorptive permeability coefficients (Papp, A-B), ranging from 3.4×10$^{-6}$ to 20.2×10$^{-6}$ cm/s. The values of Papp (A-B) for CNS negative drugs are much lower, from 0.03×10$^{-6}$ to 0.83×10$^{-6}$ cm/s$^{93}$. 
Previous efforts from our lab showed that substitutions on the benzene ring of UPMP00010 and UPMP00019 with simple non-halogen groups (table 1) can lead to derivatives that have improved activity, excellent 20-HETE/EETs inhibition selectivity and good BBB permeability potential (UPMP00031, \( P_{\text{app}} (A-B) = 32.0 \times 10^{-6} \text{ cm/s} \), \( P_{\text{app}} (B-A) = 25.7 \times 10^{-6} \text{ cm/s} \)).

This work is focused on the design and synthesis of UPMP00010 and UPMP00019 derivatives with halogen substitutions with the objective to ascertain and understand their biological activities.

---

Table 1. Selected UPMP00010 and UPMP00019 derivatives with simple non-halogen substituents

<table>
<thead>
<tr>
<th>Compd</th>
<th>Structure</th>
<th>logP</th>
<th>PSA</th>
<th>Solub</th>
<th>HLM%</th>
<th>RLM%</th>
<th>RKM%</th>
<th>rCYP4F2%</th>
<th>IC_{50} (20-HETE)</th>
<th>EETs inhibition</th>
</tr>
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<tr>
<td>UPMP00031</td>
<td><img src="image1.png" alt="structure" /></td>
<td>2.5</td>
<td>41.1</td>
<td>35.0</td>
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<td>25.9</td>
<td>4.9</td>
<td>67.7</td>
<td>73</td>
<td>3.1% @50000</td>
</tr>
<tr>
<td>UPMP00027</td>
<td><img src="image2.png" alt="structure" /></td>
<td>2.6</td>
<td>41.1</td>
<td>106.0</td>
<td>72.3</td>
<td>31.8</td>
<td>0</td>
<td>41.4</td>
<td>142</td>
<td>&gt;2500</td>
</tr>
<tr>
<td>UPMP00029</td>
<td><img src="image3.png" alt="structure" /></td>
<td>2.5</td>
<td>41.1</td>
<td>111.0</td>
<td>62.7</td>
<td>45.6</td>
<td>0</td>
<td>48.9</td>
<td>169</td>
<td>&gt;50000</td>
</tr>
<tr>
<td>UPMP00028</td>
<td><img src="image4.png" alt="structure" /></td>
<td>2.6</td>
<td>41.1</td>
<td>494.0</td>
<td>51.7</td>
<td>42.3</td>
<td>0</td>
<td>6.5</td>
<td>104</td>
<td>2.6% @50000</td>
</tr>
</tbody>
</table>

LogP: partition coefficient; PSA: polar surface area; Solub: kinetic solubility (µM); HLM%/RLM%/RKM%/rCYP4F2%: inhibition% of 20-HETE formation in HLM/RLM/RKM/rCYP4F2; IC_{50} (20-HETE): IC_{50} of 20-HETE formation in HLM (nM); ND: not done.
2.0 Chemistry

Halogenated derivatives of compounds UPMP00010 and UPMP00019 shown in tables 2, 3 and 4 were synthesized as shown in scheme 1.

![Scheme 1: Synthesis of compounds 13a-22a, 13b-22b](image)

Ar=

<p>| | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
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<tr>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>7</td>
<td>8</td>
<td>9</td>
<td>10</td>
<td>11</td>
</tr>
</tbody>
</table>

Scheme 1. Synthesis of compounds 13a-22a, 13b-22b. Reagents and conditions: (a) 1 equiv of 1, 1 equiv of Ar-Br, 3 equiv of K$_2$CO$_3$, 0.05 equiv of PdCl$_2$dpff, Dioxane/H$_2$O (10:1), 95°C, sealed flask; (b) 0.1 equiv of 10% Pd/C or 0.05 equiv of PtO$_2$, R.T, H$_2$, sealed flask; (c) HCl in dioxane saturated solution, R.T, sealed flask; (d) 1 equiv of 12a or 12b, 2 equiv of piperidine (2b-11b), 0.2 equiv of Cul, 0.4 equiv of proline, 3 equiv of K$_2$CO$_3$, DMSO, 95°C, sealed tube.
At first, the synthesis of 4-aryl-piperidines 2b-11b was pursued. Synthesis of these piperidines was accomplished in three steps, that included a Suzuki coupling of commercially available boronate 1 and desired arylbromides 2-11 to yield intermediate coupling products 2a-11a\textsuperscript{94} followed by hydrogenation\textsuperscript{95} and then Boc deprotection. THP-protected iodopyrazoles 12a and 12b were prepared by reacting dihydropyran with 4- and 3-iodopyrazoles respectively in DCM using p-toluolosulfonic acid as catalyst\textsuperscript{96}. The synthesis of final products 13a-22a and 13b-22b was the result of a CuI/proline-catalyzed coupling of THP-protected iodopyrazoles 12a, 12b with the corresponding 4-aryl-piperidines 2b-11b\textsuperscript{97}, followed by a THP deprotection to free the pyrazole NH.
3.0 Results and discussion

Halogenated derivatives 13a-15a and 13b-15b (table 2) were prepared for ascertaining the effect of mono-halogen substitution on the phenyl group of UPMP00010 and UPMP00019 while derivatives 16a-22a and 16b-22b (tables 2 and 3) were made for ascertaining the effect of halogen substitution on potent compounds that had previously prepared in our lab. The in vitro activities of these novel compounds were and are currently being assessed in Dr. Samuel Poloyac’s lab at the University of Pittsburgh. The kinetic solubilities of compounds were measured at University of Pittsburgh Drug Discovery Institute by Dr. Larry Vernetti.

3.1 Inhibition of 20-HETE formation in HLM, RLM, RKM and rCYP4F2

Although our data at this point is not complete, it suggests that simple F substitution is well tolerated with respect to potency. For example, derivatives 13a, 13b, 14a and 14b either maintained or had improved potency against the 20-HETE formation in human liver microsomes as shown in table 2.
The data available so far also appears to suggest that overall, F substitution increases the potency of compounds with a simple methoxy group substituent on the benzene moiety. Specifically, when compared to their parent (UPMP00031 and UPMP00027) compounds 16a, 17a, 16b and 17b overall have improved potency against 20-HETE formation. For the fluorinated derivatives only, the data appears to suggest that for compounds with F substitution on the ortho-position, relative to the piperidine ring, have improved potency against 20-HETE formation in RLM over their meta counterparts. For example, compounds 14a, 14b, 17a and 17b appear to have higher potency in RLM when compared to 13a, 13b, 16a and 16b.
Table 3. Key properties and activities, available to this point, for halogenated derivatives 16a-21a, 16b-21b

<table>
<thead>
<tr>
<th>Compd</th>
<th>Structure</th>
<th>PSA</th>
<th>logP</th>
<th>Solub</th>
<th>HLM%</th>
<th>RLM%</th>
<th>RKM%</th>
<th>rCYP4F2%</th>
<th>IC$_{50}$</th>
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<td>UPMP00031</td>
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<td>73.1</td>
<td>25.9</td>
<td>4.9</td>
<td>67.7</td>
<td>73.0</td>
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<td>UPMP00027</td>
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<td>2.6</td>
<td>106.0</td>
<td>72.3</td>
<td>31.8</td>
<td>0</td>
<td>41.4</td>
<td>142.0</td>
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<td>111.0</td>
<td>62.7</td>
<td>45.5</td>
<td>0</td>
<td>48.9</td>
<td>169.0</td>
</tr>
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<td>494.0</td>
<td>51.7</td>
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<td>0</td>
<td>6.5</td>
<td>194.0</td>
</tr>
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<td>16a</td>
<td></td>
<td>41.2</td>
<td>2.6</td>
<td>35.4</td>
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<td>21.0</td>
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<tr>
<td>16b</td>
<td></td>
<td>41.2</td>
<td>2.7</td>
<td>137.1</td>
<td>82.0</td>
<td>17.0</td>
<td>31.0</td>
<td>79.0</td>
<td>TBD</td>
</tr>
<tr>
<td>17a</td>
<td></td>
<td>41.2</td>
<td>2.6</td>
<td>39.6</td>
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<td>41.2</td>
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</tr>
<tr>
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<td>2.6</td>
<td>234.0</td>
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<td>TBD</td>
<td>TBD</td>
<td>TBD</td>
<td>TBD</td>
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<tr>
<td>18b</td>
<td></td>
<td>41.2</td>
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<td>TBD</td>
<td>TBD</td>
<td>TBD</td>
<td>TBD</td>
</tr>
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<td>19a</td>
<td></td>
<td>41.2</td>
<td>2.6</td>
<td>127.0</td>
<td>TBD</td>
<td>TBD</td>
<td>TBD</td>
<td>TBD</td>
<td>TBD</td>
</tr>
<tr>
<td>19b</td>
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<td>41.2</td>
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<td>201.0</td>
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<td>TBD</td>
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<td>20a</td>
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<td>19.5</td>
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<td>85.8</td>
<td>TBD</td>
</tr>
<tr>
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<td>41.2</td>
<td>3.7</td>
<td>204.9</td>
<td>83.2</td>
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<td>TBD</td>
<td>52.1</td>
<td>TBD</td>
</tr>
<tr>
<td>21a</td>
<td></td>
<td>41.2</td>
<td>3.1</td>
<td>18.7</td>
<td>81.3</td>
<td>44.3</td>
<td>TBD</td>
<td>79.7</td>
<td>TBD</td>
</tr>
<tr>
<td>21b</td>
<td></td>
<td>41.2</td>
<td>3.7</td>
<td>133.0</td>
<td>69.0</td>
<td>7.3</td>
<td>TBD</td>
<td>72.7</td>
<td>TBD</td>
</tr>
</tbody>
</table>

PSA: polar surface area; logP: calculated partition coefficient; Solub: kinetic solubility (μM); HLM%/RLM%/RKM%/rCYP4F2%: inhibition% of 20-HETE formation in HLM/RLM/ RKM/rCYP4F2 at 500nM; IC$_{50}$/IC$_{50}$: IC$_{50}$ against 20-HETE formation in HLM (nM); TBD: to be determined; ND: not done.
For derivatives that contain a chlorine substituent, the data available appears to suggest that Cl substitution is not detrimental for potency. Comparison of compounds 20a, 20b, 21a and 21b to their parent compounds UPMP00031 and UPMP00027 suggests that these chlorinated derivatives have either similar or better potency than their parents (table 3). A comparison of the available potency data for the chloro-derivatives also seems to suggest that Cl substitution on the meta-position of benzene ring, relative to the piperidine, is preferred. For instance, compounds 20a and 20b are more potent in HLM and RLM than their counterparts 21a and 21b. Unlike F-substituted compounds, derivatives with Cl substituents appear to be more potent in RLM when the Cl substituent is meta-positioned. For instance, compounds 20a and 20b exhibited higher activity in RLM than compounds 21a and 21b.

Though our activity data is not yet complete, it seems to suggest that F substitution does not negatively impact the activity of previously made compounds with a para-amide group on the benzene ring as shown in table 4.

The available data points to a general trend. 4-Pyrazole analogs overall are more potent than 3-pyrazole analogs. This pattern can be explained by the fact that the symmetric 4-pyrazole better mimics the formamidine moiety of HET0016 and is consistent with previous lab experience.
Table 4. Key properties and activities, available to this point, for halogenated derivatives 22a, 22b

<table>
<thead>
<tr>
<th>Compd</th>
<th>Structure</th>
<th>PSA</th>
<th>logP</th>
<th>Solub</th>
<th>HLM%</th>
<th>RLM%</th>
<th>RKM%</th>
<th>rCYP4F2%</th>
<th>IC50</th>
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</thead>
<tbody>
<tr>
<td>UPMP00060</td>
<td><img src="image" alt="Structure" /></td>
<td>52.2</td>
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<td>&gt;600</td>
<td>86.0</td>
<td>52.0</td>
<td>43.0</td>
<td>58.0</td>
<td>NYA</td>
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<tr>
<td>UPMP00061</td>
<td><img src="image" alt="Structure" /></td>
<td>52.2</td>
<td>2.9</td>
<td>&gt;600</td>
<td>20.0</td>
<td>8.0</td>
<td>0</td>
<td>14.0</td>
<td>ND</td>
</tr>
<tr>
<td>22a</td>
<td><img src="image" alt="Structure" /></td>
<td>52.2</td>
<td>1.9</td>
<td>234.0</td>
<td>81.6</td>
<td>TBD</td>
<td>TBD</td>
<td>63.6</td>
<td>TBD</td>
</tr>
<tr>
<td>22b</td>
<td><img src="image" alt="Structure" /></td>
<td>52.2</td>
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<td>&gt;600</td>
<td>82.3</td>
<td>TBD</td>
<td>TBD</td>
<td>52.2</td>
<td>TBD</td>
</tr>
</tbody>
</table>

PSA: polar surface area; logP: calculated partition coefficient; Solub: kinetic solubility (µM); HLM%/RLM%/RKM%/rCYP4F2%: inhibition% of 20-HETE formation in HLM/RLM/RKM/rCYP4F2 at 500nM; IC50/IC50: IC50 against 20-HETE formation in HLM (nM); TBD: to be determined; NYA: not yet available; ND: not done

At this time, within the available dataset, compound 16a, with IC50 of 49.5 nM against 20-HETE formation in HLM, is the most potent compound in this series of halogenated derivatives. Compound 16a also demonstrates good inhibition of 20-HETE formation in RLM. This is highly desirable because key in vivo brain injury models use rat as the species of choice.

### 3.2 Kinetic solubility

All halogenated derivatives have been tested for their kinetic solubility. Kinetic solubility is defined as the concentration of a solute when its induced precipitate first appears in the solution\(^{98}\). The solubility results for the halogenated derivatives described in this work are available in tables 2, 3 and 4 and suggests that 3-pyrazole analogs are usually more soluble than the 4-pyrazole analogs. This trend is consistent with previous experience in the lab. Generally, halogen substituents appear to have no beneficial effect on the solubility of compounds. All compounds,
except 22b, have lower solubility than that of compound 24 and UPMP00010. Half of the derivatives prepared have similar or slightly higher solubility than that of UPMP00019.

3.3 HLM stability and MDR1-MDCK permeability of selected compounds

Derivatives 17a, 20a and 22a were assessed for BBB penetration potential in an MDR1-MDCK permeability assay at AMRI, a contract research organization.

Derivative selection was informed by assay cost, structural diversity, available potency and other MDR1-MDCK data in the broader class for comparison.

The results of MDR1-MDCK permeability assay (table 5) suggests high BBB penetration potential for halogenated compounds 17a, 20a and 22a. All three compounds exhibit a $P_{app}$ (A to B) value larger than 3.0 and an efflux ratio value less than 3.0. The results further indicate that for di-substituted compounds, F or Cl group on the benzene moiety are well tolerated with respect to BBB permeability potential. Compounds 17a and 20a have similar efflux ratios to their non-halogenated parent compound UPMP00031. The efflux ratio of fluoroamide derivative 22a is also close to that of its parent compound UPMP00060.
Table 5. MDR1-MDCK permeability of compounds 17a, 20a and 22a

<table>
<thead>
<tr>
<th>Compd</th>
<th>Direction</th>
<th>$P_{app}(10^{-6} \text{ cm/s})$</th>
<th>Efflux ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>UPMP00031</td>
<td>A to B</td>
<td>32.0</td>
<td>0.803</td>
</tr>
<tr>
<td></td>
<td>B to A</td>
<td>25.7</td>
<td></td>
</tr>
<tr>
<td>UPMP00060</td>
<td>A to B</td>
<td>18.4</td>
<td>2.185</td>
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<td></td>
<td>B to A</td>
<td>40.2</td>
<td></td>
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<tr>
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<td>34.8</td>
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</tr>
<tr>
<td>22a</td>
<td>A to B</td>
<td>22.3</td>
<td>1.99</td>
</tr>
<tr>
<td></td>
<td>B to A</td>
<td>44.4</td>
<td></td>
</tr>
</tbody>
</table>

Assessment of the metabolic stability of halogenated derivatives is still ongoing in Dr. Poloyac’s lab. At the time of this writing there is data available for the fluoro-derivatives 16a and 17a. The results demonstrate that the metabolic stability of these compounds is excellent and similar to that of leads UPMP00010 and UPMP00019 and their parent compound UPMP00031 (table 6).
Table 6. Metabolic stability results for compounds 16a, 17a

<table>
<thead>
<tr>
<th>Compd</th>
<th>Remaining after HLM @30min</th>
</tr>
</thead>
<tbody>
<tr>
<td>UPMP00010</td>
<td>91%</td>
</tr>
<tr>
<td>UPMP00019</td>
<td>100%</td>
</tr>
<tr>
<td>UPMP00031</td>
<td>92%</td>
</tr>
<tr>
<td>16a</td>
<td>100%</td>
</tr>
<tr>
<td>17a</td>
<td>100%</td>
</tr>
</tbody>
</table>
4.0 Conclusion and future directions

In summary, 20-HETE formation inhibition is a promising approach for neuroprotection after stroke. In this study, compounds were synthesized to explore the effects of halogen substitution on the UPMP00010/UPMP00019 leads and on some of their derivatives with respect to activity, solubility, metabolic stability and BBB penetration. The available data suggests that F substitutions or Cl substitutions are well tolerated and/or lead to potency increase. As for solubility, nearly all compounds prepared show decreased solubility when compared to compound 24 and UPMP00010. Half of the derivatives synthesized have similar or slightly better solubility to that of UPMP00019 while for the remaining half their solubility is worse. With respect to BBB permeability and microsomal stability, the available data appears to suggest that the high BBB penetration potential and high metabolic stability seen in lead compounds and non-halogenated derivatives successfully translate to their halogenated counterparts.

Future studies that involve the completion of the activity and selectivity assessment of this set of halogenated derivatives is an important task. It would complete the structure activity relationships (SAR) and help in new derivative design. Increasing the solubility of potent halogenated compounds is another important task. For example, replacement of the methoxy present in halogenated derivatives with small amino-containing or hydroxy-containing side chains to explore the possibility of increasing activity and solubility at the same time would be of interest. Alternatively, substitution of the benzene moiety in halogenated derivatives by more hydrophilic heterocycles, such as pyridine ring, would also be an interesting avenue to improve aqueous solubility and expand the SAR.
5.0 Experimental section

5.1 Instrumentation and reagents

\(^1\)H NMR spectra of compounds were acquired with a 300, 400 or 600 MHz Bruker Advance Spectrometer. Applied Biosystems 2000 MS mass spectrometer was applied to obtain the MS spectra of compounds. Infrared spectra were obtained with a Bruker Alpha attenuated total reflectance (ATR) instrument. Column chromatography was performed with a Teledyne Isco Combiflash Rx instrument. Chemical reagents were purchased from Sigma Aldrich or Fisher Scientific.

5.2 General procedure I: Synthesis of N-Boc-4-aryl-dihydropyridines

A mixture of commercially available tert-butyl 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3,6-dihydropyridine-1(2H)-carboxylate (1 eq), desired arylbromide (1 eq), potassium carbonate (3 eq) and PdCl\(_2\)dpnf (0.05 eq) as catalyst in dioxane/H\(_2\)O (10:1) under Argon was stirred and heated up to 95°C until TCL showed the consumption of starting material. The reaction mixture was then filtered over celite and washed with DCM. The combined organic layer was dried over sodium sulfate and concentrated to a residue which was purified by silica gel column chromatography to acquire compounds (3a-11a).
5.3 General procedure II: Synthesis of 4-aryl-piperidines

A mixture of N-Boc-4-aryl-dyhydropyridines of interest and 10% Pd/C (0.1 eq) or PtO$_2$ (0.05 eq) in EtOH was stirred under hydrogen at atmospheric pressure until TLC or LC-MS showed the consumption of starting material. The mixture was filtered over celite and washed with EtOH. The combined organic layer was then concentrated to acquire N-Boc-4-aryl-piperidines which was then dissolved in 4N HCl in dioxane and stirred at room temperature until TLC showed that the reaction was over. The reaction mixture was evaporated to remove extra HCl and dioxane. The residue was then partitioned between DCM and saturated sodium sulfate solution. The aqueous layer was extracted with DCM until TLC showed that there is no UV absorption in the extract. The combined organic layer was dried over sodium sulfate and concentrated to acquire compounds 4-aryl-piperidines (3b-11b).

5.4 General procedure III: THP protection of iodopyrazoles

A mixture of pyrazole (1 eq), dihydropyran (1.1 eq) and p-toluolosulfonic acid monohydrate (0.1 eq) was dissolved in DCM and stirred at room temperature until TLC showed that the consumption of starting material. The reaction mixture was partitioned between DCM and saturated sodium carbonate solution. The aqueous layer was then extracted with DCM until no TLC indicated no UV absorption in the organic extract. The combined organic layer was dried with sodium sulfate and concentrated to a residue which was purified by silica gel column chromatography to acquire compounds (12a and 12b).
5.5 General procedure IV: Synthesis of 4-aryl-1-(1H-pyrazol-4-yl, 3-yl)piperidines

A mixture of desired 4-aryl-piperidine (2b-11b) (2 eq), THP protected iodopyrazole (12a and 12b) (1 eq) and potassium carbonate (3eq) in DMSO was followed with an addition of CuI (0.2 eq) and proline (0.4 eq). The mixture was stirred and heated up to 95°C until TLC showed the consumption of starting material. Reaction mixture was cooled down and partitioned between DCM and dilute aqueous ammonia. The aqueous layer was extracted with DCM until no UV absorption in the extract. The combined organic layer was dried over sodium sulfate and concentrated to a residue which was purified silica gel column chromatography to acquire the corresponding THP-protected intermediate. The intermediate was dissolved in 4N HCl in dioxane at room temperature and stirred until TLC showed the consumption of starting material. The reaction was quenched with EtOAc and saturated sodium carbonate to achieve PH=11 in the aqueous layer. The aqueous layer was extracted with DCM until no UV in the extract. The combined organic layer was then dried over sodium sulfate and concentrated to a residue which was purified by silica gel column chromatography to acquire product (13a-22a, 13b-22b).

5.6 Data for each analog

Synthesis of tert-butyl 4-(2-fluorophenyl)-3,6-dihydropyridine-1(2H)-carboxylate (3a)

Compounds was synthesized from the coupling of 2-fluoro-bromobenzene and tert-butyl 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3,6-dihydropyridine-1(2H)-carboxylate according to general procedure I. The crude residue was purified by silica gel column
chromatography and 0-30% EtOAc in hexane gradient to acquire the product as white solid (94% yield).

$^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 1.50 (s, 9H), 2.51 (apparent bs, 2H), 3.62 (t, J=5.6 Hz, 2H), 4.07 (apparent s, 2H), 5.93 (bs, 1H), 7.01-7.06 (m, 1H), 7.08-7.12 (m, 1H), 7.20-7.24 (m, 2H).

ATR IR (cm$^{-1}$) 3005, 2980, 2906, 2879, 2839, 1684, 1656, 1611, 1573, 1533, 1483, 1441, 1424, 1389, 1364, 1342, 1309, 1278, 1251, 1237, 1203, 1163, 1111, 1057, 1033, 1022, 983, 967, 938, 863, 825, 810, 766, 747, 727, 695, 628.

LC-MS (ESI) m/z for C$_{16}$H$_{20}$FNO$_2$ calculated: 277.15, observed [M+H]: 278.4.

**Synthesis of tert-butyl 4-(4-chlorophenyl)-3,6-dihydropyridine-1(2H)-carboxylate (4a)**

Compounds was synthesized from the coupling of 4-chloro-bromobenzene and tert-butyl 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3,6-dihydropyridine-1(2H)-carboxylate according to general procedure I. The crude residue was purified by silica gel column chromatography and 0-20% EtOAc in hexane gradient to acquire the product as pale colorless syrup. (86% yield).

$^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 1.49 (s, 9H), 2.48 (apparent bs, 2H), 3.63 (t, J=5.6 Hz, 2H), 4.04-4.08 (m, 2H), 6.02 (bs, 1H), 7.30 (s, 4H).

ATR IR (cm$^{-1}$) 3004, 2975, 2928, 2899, 2865, 2836, 2248, 1687, 1593, 1492, 1476, 1448, 1414, 1392, 1364, 1338, 1235, 1162, 1112, 1059, 1011, 986, 970, 934, 919, 909, 863, 840, 805, 769, 730, 647.

LC-MS-(ESI) m/z for C$_{16}$H$_{20}$ClNO$_2$ calculated: 293.12, observed [M+H]: 294.4.
Synthesis of tert-butyl 4-(3-fluoro-4-methoxyphenyl)-3,6-dihydropyridine-1(2H)-

carboxylate (5a)

Compounds was synthesized from the coupling of 4-bromo-2-fluoro-1-methoxybenzene
and tert-butyl 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3,6-dihydropyridine-1(2H)-
carboxylate according to general procedure I. The crude residue was purified by silica gel column
chromatography and 0-15% EtOAc in hexane gradient to acquire the product as colorless syrup.
(52% yield).

$^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 1.48 (s, 9H), 2.46 (s, 2H), 3.62 (t, $J$=5.6 Hz, 2H), 3.89 (s, 
3H), 4.04-4.07 (m, 2H), 5.96(bs, 2H), 6.91 (apparent t, $J$=8.8 Hz,1H), 7.06-7.14 (m, 2H).

ATR IR (cm$^{-1}$) 3003, 2972, 2931, 2840, 1689, 1619, 1578, 1518, 1415, 1364, 1338, 1319,
1266, 1235, 1161, 1132, 1111, 1058, 1027, 1000, 979, 955, 869, 858, 827, 802, 759, 673, 618.

LC-MS (ESI) m/z for C$_{17}$H$_{22}$FNO$_3$ Calculated: 307.16, Observed [M+H]: 308.6.

Synthesis of tert-butyl 4-(2-fluoro-4-methoxyphenyl)-3,6-dihydropyridine-1(2H)-
carboxylate (6a)

Compounds was synthesized from the coupling of 4-bromo-3-fluoro-1-methoxybenzene
and tert-butyl 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3,6-dihydropyridine-1(2H)-
carboxylate according to general procedure I. The crude residue was purified by silica gel column
chromatography and 0-10% EtOAc in hexane gradient to acquire the product as colorless syrup.
(89% yield).

$^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 1.49 (s, 9H), 2.48 (apparent bs, 2H), 3.60 (t, $J$=5.6 Hz, 2H),
3.80 (s, 3H), 4.04-4.06 (m, 2H), 5.86 (bs, 1H), 6.60 (dd, $J$=12.8, 2.4 Hz, 1H), 6.66 (dd, $J$= 8.0, 2.4 
Hz, 1H), 7.15(apparent t, $J$=8.4, 1H).
ATR IR (cm<sup>-1</sup>) 2973, 2931, 2837, 1690, 1620, 1572, 1505, 1415, 1364, 1337, 1319, 1288, 1154, 1110, 1058, 1034, 986, 973, 952, 928, 832, 803, 768, 730, 711, 675, 627.

LC-MS (ESI) m/z for C<sub>17</sub>H<sub>22</sub>FN<sub>3</sub> calculated: 307.16, observed [M+H]: 308.4.

**Synthesis of tert-butyl 4-(3-fluoro-5-methoxyphenyl)-3,6-dihydropyridine-1(2H)-carboxylate (7a)**

Compounds was synthesized from the coupling of 1-bromo-3-fluoro-5-methoxybenzene and tert-butyl 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3,6-dihydropyridine-1(2H)-carboxylate according to general procedure I. The crude residue was purified by silica gel column chromatography and 0-20% EtOAc in hexane gradient to acquire the product as yellowish liquid (88% yield).

<sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>) δ 1.48 (s, 9H), 2.47 (bs, 2H), 3.62 (bs, 2H), 3.80 (s, 3H), 4.06 (bs, 2H), 6.02 (apparent s, 1H), 6.51 (apparent dt, J=4.2, 2.4 Hz, 1H), 6.66-8.68 (m, 2H).

ATR IR (cm<sup>-1</sup>) 3003, 2974, 2932, 2864, 2839, 1690, 1610, 1585, 1453, 1419, 1363, 1333, 1281, 1238, 1197, 1162, 1135, 1112, 1054, 1016, 982, 961, 863, 838.

**Synthesis of tert-butyl 4-(4-fluoro-3-methoxyphenyl)-3,6-dihydropyridine-1(2H)-carboxylate (8a)**

Compounds was synthesized from the coupling of 4-bromo-1-fluoro-2-methoxybenzene and tert-butyl 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3,6-dihydropyridine-1(2H)-carboxylate according to general procedure I. The crude residue was purified by silica gel column
chromatography and 0-15% EtOAc in hexane gradient to acquire the product as white solid. (88% yield).

\[^1\text{H} \text{NMR (600 MHz, CDCl}_3\] \(\delta\) 1.49 (s, 1H), 2.49 (bs, 2H), 3.63 (t, \(J=5.4\) Hz, 2H), 3.90 (s, 3H), 4.06 (bs, 2H), 5.96 (bs, 1H), 6.86-6.89 (m, 1H), 6.95 (dd, \(J=8.4, 2.4\) Hz, 1H), 7.00-7.04 (m, 1H).

ATR IR (cm\(^{-1}\)) 3002, 2974, 2936, 2917, 2897, 2861, 2843, 1679, 1643, 1603, 1519, 1482, 1467, 1445, 1419, 1391, 1363, 1341, 1329,1292,1273,1245,1196, 1164, 1116, 1063, 1032, 997, 978, 953, 870, 851, 809, 780, 763, 670, 633, 620.

**Synthesis of tert-butyl 4-(3-chloro-4-methoxyphenyl)-3,6-dihydropyridine-1(2H)-carboxylate (9a)**

Compounds was synthesized from the coupling of 4-bromo-2-chloro-1-methoxybenzene and tert-butyl 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3,6-dihydropyridine-1(2H)-carboxylate according to general procedure I. The crude residue was purified by silica gel column chromatography and 0-10% EtOAc in hexane gradient to acquire the product as colorless syrup. (78% yield).

\[^1\text{H} \text{NMR (400 MHz, CDCl}_3\] \(\delta\) 1.49 (s, 9H), 2.46 (apparent bs, 2H), 3.62 (t, \(J=5.6\), 2H), 3.90 (s, 3H), 4.04-4.07 (m, 2H), 5.96 (bs, 1H), 6.89 (d, \(J=8.4\) Hz, 1H), 7.23 (dd, \(J=8.4, 2.4\), 1H), 7.39 (d, \(J=2.4\) Hz, 1H).

ATR IR (cm\(^{-1}\)) 3003, 2973, 2930, 2838, 1687, 1601, 1562, 1504, 1477, 1454, 1414, 1364, 1337, 1286, 1254, 1234, 1161, 1111, 1063, 1020, 991, 977, 948, 880, 863, 802, 767, 710, 666, 618, 603.
Synthesis of tert-butyl 4-(2-chloro-4-methoxyphenyl)-3,6-dihydropyridine-1(2H)-carboxylate (10a)

Compounds was synthesized from the coupling of 4-bromo-3-chloro-1-methoxybenzene and tert-butyl 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3,6-dihydropyridine-1(2H)-carboxylate according to general procedure I. The crude residue was purified by silica gel column chromatography and 0-10% EtOAc in hexane gradient to acquire the product as yellowish syrup (68% yield).

\[ {^1}H \text{ NMR (400 MHz, CDCl}_3 \delta 1.50 \text{ (s, 9H), 2.41 (bs, 2H), 3.60-3.62 (m, 2H), 3.79 (s, 3H), 4.03 (bs, 2H), 5.62 (bs, 1H), 6.77 (dd, J=8.8, 2.8 Hz, 1H), 6.91 (d, J=2.8 Hz, 1H), 7.08 (d, J=8.4 Hz, 1H).} \]

ATR IR (cm\(^{-1}\)) 3003, 2973, 2931, 2836, 1689, 1602, 1562, 1547, 1493, 1478, 1454, 1412, 1364, 1336, 1284, 1235, 1204, 1163, 1109, 1062, 1037, 973, 940, 863, 840, 809, 768, 718, 689.

LC-MS (ESI) m/z for C\(_{17}\)H\(_{22}\)ClNO\(_3\) calculated: 323.13, observed [M+H]: 324.5.

Synthesis of tert-butyl 4-(2-fluoro-4-(N-methylacetamido)phenyl)-3,6-dihydropyridine-1(2H)-carboxylate (11a)

Compounds was synthesized from the coupling of N-(4-bromo-3-fluorophenyl)-N-methylacetamide and tert-butyl 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3,6-dihydropyridine-1(2H)-carboxylate according to general procedure I. N-(4-bromo-3-fluorophenyl)-N-methylacetamide was prepared according to Org. Biomol., 2018, 16, 3881 and Chem. Asian J. 2010, 5, 2336-2340. The crude residue was purified by silica gel column chromatography and 0-50\% EtOAc in hexane gradient to acquire the product as yellowish solid (88% yield).
\[ ^1\text{H NMR (600 MHz, CDCl}_3 \] \( \delta \) 1.51 (s, 9H), 1.93 (s, 3H), 2.52 (bs, 2H), 3.27 (s, 3H), 3.64 (bs, 2H), 4.10 (bs, 2H), 5.99 (bs, 1H), 6.92 (d, \( J=11.4 \) Hz, 1H), 6.97 (d, \( J=7.8 \) Hz, 1H), 7.29-7.31 (m, 1H).

ATR IR (cm\(^{-1}\)) 3055, 2976, 2930, 2870, 2852, 2829, 1686, 1665, 1610, 1562, 1502, 1477, 1447, 1417, 1363, 1340, 1312, 1290, 1261, 1239, 1159, 1114, 1078, 1053, 1032, 981, 974, 937, 903, 882, 860, 849, 830, 817, 766, 730, 705, 657, 626.

LC-MS (ESI) m/z for C\(_{19}\)H\(_{25}\)FN\(_2\)O\(_3\) calculated: 348.18, observed [M+H]: 349.7.

**Synthesis of 4-(2-fluorophenyl)piperidine (3b)**

Synthesized from compound 4 by according to general procedure II. The product was acquired as yellowish syrup (99% yield).

\[ ^1\text{H NMR (400 MHz, CDCl}_3 \] \( \delta \) 1.66 (qd, \( J=12.4, 4.0 \) Hz, 2H), 1.78-1.85 (m, 2H), 2.78 (td, \( J=12.0, 2.4 \) Hz, 2H), 2.99 (tt, \( J=12.0, 3.6 \) Hz, 1H), 3.16-3.22 (m, 2H), 6.97-7.04 (m, 1H), 7.07-7.12 (m, 1H), 7.13-7.20 (m, 1H), 7.21-7.26 (m, 1H).

ATR IR (cm\(^{-1}\)) 3277, 3082, 3063, 3039, 2935, 2920, 2849, 2807, 2734, 2683, 2629, 1673, 1614, 1582, 1489, 1449, 1431, 1387, 1368, 1318, 1286, 1252, 1221, 1139, 1120, 1094, 1036, 1021, 977, 956, 937, 872, 860, 798, 751, 623.

LC-MS (ESI) m/z for C\(_{11}\)H\(_{14}\)FN calculated: 179.11, observed [M+H]: 179.9.

**Synthesis of 4-(4-chlorophenyl)piperidine (4b)**

Synthesized from compound 5 according to general procedure II. The intermediate before deprotection was purified by silica gel column chromatography and 0-20% Et\(_2\)O in hexanes gradient. The product was acquired as colorless syrup (53% yield).
\(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 1.55-1.67 (m, 2H), 1.81 (apparent d, \(J=9.2\) Hz, 2H), 2.59 (tt, \(J=12.0\), 3.6 Hz, 1H), 2.74 (apparent t, \(J=12.0\) Hz, 2H), 3.20 (apparent broad based d, \(J=11.6\) Hz, 2H), 7.13 (d, \(J=8.4\) Hz, 2H), 7.27 (d, \(J=8.8\) Hz, 2H).

ATR IR (cm\(^{-1}\)) 3267, 3042, 3025, 2917, 2847, 2808, 2734, 1643, 1491, 1466, 1443, 1429, 1409, 1387, 1364, 1318, 1296, 1270, 1244, 1229, 1196, 1140, 1096, 1011, 977, 956, 866, 821, 765, 716, 689, 632.

LC-MS (ESI) m/z for C\(_{11}\)H\(_{14}\)ClN calculated: 195.08, observed [M+H]: 196.1.

**Synthesis of 4-(3-fluoro-4-methoxyphenyl)piperidine (5b)**

Synthesized from compound 6 according to general procedure II. The product was acquired as colorless liquid (75% yield).

\(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 1.51-1.63 (m, 4H), 1.77-1.84 (m, 2H), 2.55 (apparent tt, \(J=12.0\), 3.6 Hz, 1H), 2.72(apparent td, \(J=12.0\), 2.4 Hz, 2H), 3.16-3.19 (m, 2H), 3.87 (s, 3H), 6.87-6.97 (m, 3H).

ATR IR (cm\(^{-1}\)) 3342, 2951, 2935, 2918, 2841, 2821, 2798, 2742, 2687, 2637, 1619, 1582, 1518, 1463, 1445, 1426, 1385, 1363, 1323, 1293, 1268, 1243, 1217, 1183, 1159, 1141, 1122, 1104, 1050, 1019, 981, 952, 911, 900, 877, 841, 800, 755, 624.

LC-MS (ESI) m/z for C\(_{12}\)H\(_{16}\)FNO calculated: 209.12, Observed [M+H]: 210.1.

**Synthesis of 4-(2-fluoro-4-methoxyphenyl)piperidine (6b)**

Prepared from compound 7 according to general procedure II. The product was acquired as colorless liquid (59% yield).
$^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 1.62 (qd, $J$=12.4, 4.0 Hz, 2H), 1.74-1.81 (m, 2H), 2.75 (td, $J$=12.4, 2.8 Hz, 2H), 2.90 (tt, $J$=12.4, 3.6 Hz, 1H), 3.15-3.18 (m, 2H), 3.77 (s, 3H), 6.58 (dd, $J$=12.0, 2.4, 1H), 6.65 (apparent dd, $J$=8.8, 2.4 Hz, 1H), 7.13 (t, $J$= 8.8 Hz, 1H).

ATR IR (cm$^{-1}$) 3270, 3001, 2933, 2838, 2808, 2733, 1622, 1582, 1505, 1464, 1443, 1368, 1317, 1303, 1283, 1245, 1190, 1151, 1118, 1096, 1035, 1020, 978, 948, 845, 831, 805, 759, 728, 626.

LC-MS (ESI) m/z for C$_{12}$H$_{16}$FNO Calculated: 209.12, observed [M+H]: 210.2.

**Synthesis of 4-(3-fluoro-5-methoxyphenyl)piperidine (7b)**

Prepared from compound 8 according to general procedure II. The product was acquired as yellowish waxy solid (67% yield).

$^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 1.65 (qd, $J$=12.6, 3.6 Hz, 2H), 1.85 (apparent distorted d, $J$=12.0 Hz, 2H), 2.34 (bs, 1H), 2.60 (tt, $J$=18.0, 3.9 Hz, 1H), 2.76 (apparent t, $J$=11.7 Hz, 2H), 3.23 (apparent d, $J$=12.0 Hz, 2H), 3.80 (s, 3H), 6.45-6.58(m, 3H).

ATR IR (cm$^{-1}$) 3282, 3002, 2932, 2843, 2808, 2734, 2682, 1612, 1589, 1456, 1432, 1387, 1364, 1332, 1318, 1300, 1273, 1250, 1228, 1053, 1032, 1009, 998, 973, 942, 911, 839, 801, 779, 688.

LC-MS (ESI) m/z for C$_{12}$H$_{16}$FNO calculated: 209.12, observed [M+H]: 210.0.

**Synthesis of 4-(4-fluoro-3-methoxyphenyl)piperidine (8b)**

Prepared from compound 9 according to general procedure II. The product was acquired as white waxy solid (60% yield).

40
\[ ^1H \text{NMR (300 MHz, CDCl}_3 \] \delta 1.59 (qd, J=11.7, 3.9, 2H), 2.42 (bs, 1H), 2.52 (tt, J=12.0, 3.6 Hz, 1H), 2.69 (td, J=12.1, 2.1 Hz, 2H), 3.16 (apparent d, J=11.7 Hz, 2H), 3.81 (s, 3H), 6.63-6.69 (m, 1H), 6.76 (dd, J=8.1, 2.1 Hz, 1H), 6.92 (dd, J=11.4, 9.6 Hz, 1H).

ATR IR (cm\(^{-1}\)) 3330, 3041, 2931, 2845, 2733, 1608, 1516, 1464, 1449, 1417, 1368, 1362, 1270, 1212, 1189, 1153, 1188, 1083, 1031, 979, 959, 938, 888, 851, 809, 787, 774, 741, 635.

LC-MS (ESI) m/z for C\(_{12}\)H\(_{16}\)FNO Calculated: 209.12, observed [M+H]: 210.3.

**Synthesis of 4-(3-chloro-4-methoxyphenyl)piperidine (9b)**

Synthesized from compound 10 according to general procedure II. The hydrogenated product before deprotection was purified with silica gel column chromatography and 0-20% Et\(_2\)O in hexanes gradient. The product was acquired as colorless syrup (48% yield).

\[ ^1H \text{NMR (400 MHz, CDCl}_3 \] \delta 1.50-1.62 (m, 2H), 1.80 (apparent d, J=13.6 Hz, 2H), 2.54 (tt, J=12.0, 3.6 Hz, 1H), 2.72 (td, J=12.0, 2.4 Hz, 2H), 3.15-3.19 (m, 2H), 3.88 (s, 3H), 6.86 (d, J=8.4 Hz, 1H), 7.07 (ddd, J=8.4, 2.4, 0.4 Hz, 1H), 7.22 (d, J=2.4 Hz, 1H).

ATR IR (cm\(^{-1}\)) 3268, 3002, 2927, 2839, 2809, 2734, 1603, 1568, 1501, 1461, 1441, 1410, 1386, 1362, 1318, 1284, 1255, 1195, 1182, 1140, 1105, 1062, 1021, 977, 955, 876, 805, 787, 752, 717, 693, 610.

LC-MS (ESI) m/z for C\(_{12}\)H\(_{16}\)ClNO Calculated: 225.09, observed [M+H]: 226.3.

**Synthesis of 4-(2-chloro-4-methoxyphenyl)piperidine (10b)**

Synthesized from compound 11 according to general procedure II. The hydrogenated intermediate before Boc-deprotection was purified with silica gel column chromatography and 0-20% Et\(_2\)O in hexanes gradient. The product was acquired as colorless syrup (40 % yield).
$^1$H NMR (600 MHz, CDCl$_3$) $\delta$ 1.52-1.61 (m, 4H), 1.83 (apparent d, $J$=11.4 Hz, 2H), 2.79 (td, $J$=12.0, 2.4 Hz, 2H), 3.20 (d, $J$=11.4 Hz, 1H), 3.79 (s, 3H), 6.82 (dd, $J$=8.4, 2.4 Hz, 1H), 6.92 (d, $J$=3.0 Hz, 1H), 7.20 (d, $J$=9.0 Hz, 1H).

ATR IR (cm$^{-1}$) 3269, 3069, 3001, 2932, 2835, 2814, 2736, 2684, 2630, 1604, 1565, 1493, 1462, 1440, 1387, 1366, 1318, 1284, 1268, 1249, 1229, 1193, 1138, 1085, 1038, 1018, 977, 955, 857, 839, 808, 776, 768, 713, 690, 601.

LC-MS (ESI) m/z for C$_{12}$H$_{16}$ClNO Calculated: 225.09, Observed [M+H]: 226.4.

**Synthesis of N-(3-fluoro-4-(piperidin-4-yl)phenyl)-N-methylacetamide (11b)**

Synthesized from compound 12 according to general procedure II. The product was isolated as yellowish waxy solid syrup (96% yield).

$^1$H NMR (600 MHz, CDCl$_3$) $\delta$ 1.69 (qd, $J$=12.0, 4.2 Hz, 2H), 1.84 (apparent d, $J$=10.8 Hz, 2H), 1.91 (s, 3H), 2.80 (td, $J$=12.0, 2.4 Hz, 2H), 2.97-3.03 (m, 1H), 3.21-3.24 (m, 2H), 3.25 (s, 3H), 6.88 (d, $J$=10.2 Hz, 1H), 6.95 (d, $J$=7.8 Hz, 1H), 7.26-7.31 (m, 1H).

ATR IR (cm$^{-1}$) 3308, 3262, 3051, 2979, 2930, 2852, 1661, 1615, 1568, 1504, 1469, 1444, 1413, 1366, 1305, 1256, 1243, 1207, 1190, 1178, 1137, 1096, 1077, 1037, 1025, 1006, 981, 963, 955, 910, 868, 835, 795, 752, 737, 691, 661.

LC-MS (ESI) m/z for C$_{14}$H$_{19}$FN2O calculated: 250.15, observed [M+H]: 251.4.

**Synthesis of 4-iodo-1-(tetrahydro-2H-pyran-2-yl)-1H-pyrazole (12a)**

Compound was prepared according to general procedure III. Crude product was chromatographed using a 0-20% EtOAc in hexane. Compounds isolated as a colorless viscous syrup. (45% yield).
Synthesis of 3-iodo-1-(tetrahydro-2H-pyran-2-yl)-1H-pyrazole (12b)

Compound was prepared according to general procedure III. Crude product was chromatographed using a 0-20% EtOAc in hexane. Compounds isolated as a white solid. (42% yield).

1H NMR (400 MHz, CDCl$_3$) δ 1.56-1.70 (m, 3H), 1.99-2.09 (m, 3H), 3.64-3.70 (m, 1H), 4.02 – 4.07 (m, 1H), 5.34-5.37(m, 1H), 6.45 (d, J=2.4 Hz, 1H), 7.43 (d, J=2.4, 1H).

Synthesis of 4-(3-fluorophenyl)-1-(1H-pyrazol-4-yl)piperidine (13a)

Compound was prepared from coupling of 4-iodo-1-(tetrahydro-2H-pyran-2-yl)-1H-pyrazole and commercially available 4-(3-fluorophenyl)piperidine according to the general procedure IV. Before THP deprotection, the intermediate was purified with silica gel column chromatography and 0-100% EtOAc in hexane gradient. The THP-deprotected product was purified with silica gel column chromatography and 0-100% EtOAc in hexane gradient to acquire the final product as off-white solid (22% yield).

1H NMR (400 MHz, CDCl$_3$) δ 1.92-2.03 (m, 4H), 2.58-2.67 (m, 1H), 2.67-2.76 (m, 2H), 3.51 (apparent d, J= 12.0 Hz, 2H), 6.80-6.98 (m, 2H), 7.03 (d, J= 7.6 Hz, 1H), 7.25-7.31 (m, 1H), 7.32 (s, 2H).

1H NMR (400 MHz, DMSO-$d_6$) δ 1.72-1.82 (m, 4H), 2.58-2.67 (m, 1H), 3.42 (apparent d, J=11.6 Hz, 1H), 7.02 (ddd, J=10.8, 8.8, 1.6 Hz, 1H), 7.10-7.14 (m, 2H), 7.26 (s, 2H), 7.31-7.37 (m, 1H), 12.26 (bs, 1H).
ATR IR (cm\(^{-1}\)) 3146, 3122, 3077, 2940, 2919, 2874, 2844, 2811, 2674, 1613, 1579, 1525, 1489, 1463, 1445, 1389, 1360, 1338, 1299, 1245, 1224, 1137, 1107, 1073, 1028, 995, 943, 892, 863, 839, 780, 762, 750, 694, 660, 650.

LC-MS (ESI) m/z calculated for C\(_{14}\)H\(_{16}\)FN\(_3\): 245.13, observed [M+H]: 246.3.

**Synthesis of 4-(3-fluorophenyl)-1-(1H-pyrazol-3-yl)piperidine (13b)**

Compound was prepared from coupling of 3-iodo-1-(tetrahydro-2H-pyran-2-yl)-1H-pyrazole and commercially available 4-(3-fluorophenyl)piperidine according to the general procedure IV. Before THP deprotection, the intermediate was purified with silica gel column chromatography and 0-100% EtOAc in hexane gradient. The THP-deprotected product was purified with silica gel column chromatography and 0-100% EtOAc in hexane gradient to acquire the final product as off-white solid (27% yield).

\(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 1.80-1.97 (m, 4H), 2.66 (tt, \(J=12.0, 4.0\) Hz, 1H), 2.86 (td, \(J=12.0, 2.8\) Hz, 2H), 3.87 (apparent d, \(J=12.4\) Hz, 2H), 5.79 (d, \(J=2.4\) Hz, 1H), 6.86-6.97 (m, 2H), 7.02 (d, \(J=7.6\) Hz, 1H), 7.23-7.30 (m, 1H), 7.42 (d, \(J=2.4\) Hz, 1H).

\(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) 1.72 (apparent qd, \(J=12.0, 4.4\) Hz, 2H), 1.81 (distorted apparent d, \(J=11.6\) Hz, 2H), 2.63-2.70 (m, 3H), 3.75 (d, \(J=12.0\) Hz, 2H), 5.71 (s, 1H), 6.98-7.04 (m, 1H), 7.08-7.13 (m,2H), 7.31-7.37 (m, 1H), 7.45 (s, 1H), 11.79 (bs, 1H).

ATR IR (cm\(^{-1}\)) 3254, 3074, 3055, 2967, 2939, 2912, 2843, 2819, 2756, 1610, 1584, 1544, 1478, 1462, 1447, 1386, 1312, 1279, 1260, 1244, 1230, 1194, 1140, 1113, 1102, 1066, 1041, 1020, 990, 943, 927, 892, 873, 850, 833, 795, 755, 699, 682.

LC-MS (ESI) m/z calculated for C\(_{14}\)H\(_{16}\)FN\(_3\): 245.13, observed [M+H]: 246.5.
Synthesis of 4-(2-fluorophenyl)-1-(1H-pyrazol-4-yl)piperidine (14a)

Compound was prepared from coupling of 4-iodo-1-(tetrahydro-2H-pyran-2-yl)-1H-pyrazole and compound 4a according to the general procedure IV. Before THP deprotection, the intermediate was purified with silica gel column chromatography and 0-100% EtOAc in hexane gradient. The THP-deprotected product was purified with silica gel column chromatography and 0-100% EtOAc in hexane gradient to acquire the final product as off-white solid (13% yield).

$^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 1.90-2.05 (m, 4H), 2.74 (td, $J$=11.6, 2.8 Hz, 2H), 2.99 (apparent tt $J$=12.0, 4.0 Hz, 1H), 3.51 (d, $J$= 11.6 Hz, 2H), 7.00-7.06 (m, 1H), 7.09-7.14 (m, 1H), 7.16-7.23 (m, 1H), 7.27-7.32 (m and s overlapping, 3H).

$^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 1.75-1.90 (m, 4H), 2.54-2.58 (m, 2H), 2.83-2.29 (m, 1H), 3.44 (apparent d, $J$=11.6 Hz, 2H), 7.12-7.19 (m, 2H), 7.23-7.31 (m, 3H), 7.35-7.39 (m, 1H), 12.25 (bs, 1H).

ATR IR (cm$^{-1}$) 3146, 3123, 3074, 2946, 2921, 2872, 2845, 2808, 2757, 2669, 1614, 1575, 1522, 1448, 1452, 1388, 1360, 1325, 1268, 1247, 1221, 1186, 1143, 1132, 1115, 1089, 1078, 1036, 992, 947, 905, 870, 845, 836, 819, 792, 751, 698, 652.

LC-MS (ESI) m/z calculated for C$_{14}$H$_{16}$FN$_3$: 245.13, observed [M+H]: 246.3.

Synthesis of 4-(2-fluorophenyl)-1-(1H-pyrazol-3-yl)piperidine (14b)

Compound was prepared from coupling of 3-iodo-1-(tetrahydro-2H-pyran-2-yl)-1H-pyrazole and compound 4a according to the general procedure IV. Before THP deprotection, the intermediate was purified with silica gel column chromatography and 0-100% EtOAc in hexane gradient. The THP-deprotected product was purified with silica gel column chromatography and 0-100% EtOAc in hexane gradient to acquire the final product as white solid (13% yield).
\( ^1\text{H NMR} \) (400 MHz, DMSO-\( d_6 \)) \( \delta \) 1.75-1.82 (m, 4H), 2.66-2.72 (m, 2H), 2.87-2.97 (m, 1H), 3.77 (d, \( J = 12.0 \text{ Hz} \), 2H), 5.72 (s, 1H), 7.11-7.19 (m, 2H), 7.23-7.30 (m, 1H), 7.31-7.38 (m, 1H), 7.45 (s, 1H), 11.78 (s, 1H).

ATR IR (cm\(^{-1}\)) 3171, 3081, 3039, 2935, 2849, 2811, 2753, 2706, 1579, 1540, 1510, 1488, 1462, 1453, 1384, 1316, 1282, 1272, 1255, 1240, 1221, 1178, 1154, 1102, 1079, 1066, 1038, 1019, 983, 926, 905, 869, 812, 791, 749.

LC-MS (ESI) m/z calculated for C\(_{14}\)H\(_{16}\)FN\(_3\): 245.13, observed \([\text{M+H}]^+\): 246.5.

**Synthesis of 4-(4-chlorophenyl)-1-(1H-pyrazol-4-yl)piperidine (15a)**

Compound was prepared from coupling of 4-iodo-1-(tetrahydro-2H-pyran-2-yl)-1H-pyrazole and compound 5a according to the general procedure IV. Before THP deprotection, the intermediate was purified with silica gel column chromatography and 0-60% EtOAc in hexane gradient. The THP-deprotected product was purified with silica gel column chromatography and 0-100% EtOAc in hexane gradient to acquire the final product as off-white solid (23% yield).

\( ^1\text{H NMR} \) (400 MHz, CDCl\(_3\)) \( \delta \) 1.86-1.93 (m, 4H), 2.53-2.63 (m, 1H), 2.63-2.72 (m, 2H), 3.45-3.51 (m, 2H), 7.18 (apparent d, \( J = 8.0 \text{ Hz} \), 2H), 7.28 (apparent d, \( J = 8.4 \text{ Hz} \), 2H).

1H NMR (400 MHz, DMSO-\( d_6 \)) \( \delta \) 1.68-1.82 (m, 4H), 2.50-2.57 (m, 2H), 2.57-2.64 (m, 1H), 3.42 (apparent d, \( J = 11.6 \text{ Hz} \), 2H), 7.26 (s, 2H), 7.29-7.37 (m, 4H), 12.24 (s, 1H).

ATR IR (cm\(^{-1}\)) 3145, 3121, 2940, 2919, 2850, 2809, 2759, 2670, 1576, 1527, 1491, 1462, 1444, 1407, 1386, 1359, 1338, 1318, 1294, 1267, 1246, 1230, 1197, 1176, 1134, 1113, 1089, 1040, 1025, 1011, 990, 951, 905, 860, 837, 822, 790, 768, 720, 677, 653.

LC-MS (ESI) m/z calculated for C\(_{14}\)H\(_{16}\)ClN\(_3\): 261.10, observed \([\text{M+H}]^+\): 262.5.
**Synthesis of 4-(4-chlorophenyl)-1-(1H-pyrazol-3-yl)piperidine (15b)**

Compound was prepared from coupling of 3-iodo-1-(tetrahydro-2H-pyran-2-yl)-1H-pyrazole and compound 5a according to the general procedure IV. Before THP deprotection, the intermediate was purified with silica gel column chromatography and 0-40% EtOAc in hexane gradient. The THP-deprotected product was purified with silica gel column chromatography and 0-100% EtOAc in hexane gradient to acquire the final product as off-white solid (18% yield).

$^1$H NMR (400 MHz, CDCl$_3$) δ 1.79-1.93 (m, 4H), 2.63 (apparent tt, $J=11.6$, 4.0 Hz, 1H), 2.83 (td, $J= 12.4$, 3.6 Hz, 2H), 3.83-3.89 (m, 2H), 5.80 (d, $J=2.4$ Hz, 1H), 7.18 (apparent d, $J=8.4$ Hz, 2H), 7.28 (apparent d, $J=8.4$ Hz, 2H), 7.42 (d, $J= 2.4$ Hz, 1H).

ATR IR (cm$^{-1}$) 3153, 3070, 3027, 2942, 2917, 2815, 2752, 1537, 1490, 1477, 1459, 1440, 1411, 1382, 1340, 1312, 1292, 1271, 1254, 1236, 1198, 1175, 1155, 1088, 1062, 1038, 1011, 983, 927, 906, 864, 820, 745, 715, 697, 670.

LC-MS (ESI) m/z calculated for C$_{14}$H$_{16}$ClN$_3$: 261.10, observed [M+H]: 262.5.

**Synthesis of 4-(3-fluoro-4-methoxyphenyl)-1-(1H-pyrazol-4-yl)piperidine (16a)**

Compound was prepared from coupling of 4-iodo-1-(tetrahydro-2H-pyran-2-yl)-1H-pyrazole and compound 6a according to the general procedure IV. Before THP deprotection, the intermediate was purified with silica gel column chromatography and 0-60% EtOAc in hexane gradient. The THP-deprotected product was purified with silica gel column chromatography and 0-10% MeOH in DCM gradient to acquire the final product as off-white solid (14% yield).

$^1$H NMR (400 MHz, CDCl$_3$) δ 1.84-1.92 (m, 4H), 2.50-2.58 (m, 1H), 2.66 (apparent td, $J=11.6$, 4.0 Hz, 2H), 3.45-3.50 (m, 2H), 3.88 (s, 3H), 6.87-7.00 (m, 3H), 7.27 (s, 2H), 9.71 (broad, 1H).
\( ^1H \) NMR (400 MHz, DMSO-\( d_6 \)) \( \delta \) 1.66-1.82 (m, 4H), 2.49-2.59 (m, 3H), 3.40 (apparent d, \( J=11.6 \) Hz, 2H), 3.80 (s, 3H), 7.01-7.15 (m, 3H), 7.25 (s, 2H), 12.24 (s, 1H).

ATR IR (cm\(^{-1}\)) 3135, 3118, 3078, 2960, 2929, 2840, 2817, 2763, 1619, 1574, 1515, 1461, 1444, 1423, 1388, 1360, 1322, 1294, 1258, 1215, 1183, 1124, 1102, 1080, 1026, 995, 956, 860, 811, 756, 696, 654.

LC-MS (ESI) m/z calculated for C\(_{15}\)H\(_{18}\)FN\(_3\)O: 275.14, observed [M+H]: 276.4.

**Synthesis of 4-(3-fluoro-4-methoxyphenyl)-1-(1H-pyrazol-3-yl)piperidine (16b)**

Compound was prepared from coupling of 3-iodo-1-(tetrahydro-2H-pyran-2-yl)-1H-pyrazole and compound 6a according to the general procedure IV. Before THP deprotection, the intermediate was purified with silica gel column chromatography and 0-30% EtOAc in hexane gradient. The THP-deprotected product was purified with silica gel column chromatography and 0-100% EtOAc in hexane gradient to acquire the final product as off-white solid (19% yield).

\( ^1H \) NMR (400 MHz, CDCl\(_3\)) \( \delta \) 1.81 (qd, \( J=12.0, \) 4.0 Hz, 2H), 1.91 (distorted apparent d, \( J=12.8 \) Hz, 2H), 2.58 (tt, \( J=11.6, \) 4.0 Hz, 1H), 2.83 (td, \( J=12.4, \) 2.8 Hz, 2H), 3.82-3.88 (s overlap with apparent d, 5H), 5.79 (d, \( J=2.4 \) Hz, 1H), 6.85-7.01 (m, 3H), 7.41 (d, \( J=2.4 \), 1H).

ATR IR (cm\(^{-1}\)) 3264, 3144, 3001, 2985, 2933, 2853, 2841, 2822, 2747, 1701, 1622, 1583, 1542, 1583, 1542, 1518, 1506, 1480, 1464, 1442, 1426, 1381, 1341, 1325, 1314, 1292, 1260, 1246, 1217, 1190, 1180, 1149, 1124, 1106, 1097, 1061, 1039, 1019, 985, 949, 936, 923, 894, 936, 923, 894, 881, 856, 840, 755, 680.

LC-MS (ESI) m/z for C\(_{15}\)H\(_{18}\)FN\(_3\)O calculated: 275.14, observed [M+H]: 276.3.

**Synthesis of 4-(2-fluoro-4-methoxyphenyl)-1-(1H-pyrazol-4-yl)piperidine (17a)**
Compound was prepared from coupling of 4-iodo-1-(tetrahydro-2H-pyran-2-yl)-1H-pyrazole and compound 7a according to the general procedure IV. Before THP deprotection, the intermediate was purified with silica gel column chromatography and 0-100% EtOAc in hexane gradient. The THP-deprotected product was purified with silica gel column chromatography and 0-100% EtOAc in hexane gradient to acquire the final product as off-white solid (17% yield).

$^1$H NMR (400 MHz, CDCl$_3$) δ 1.86-1.98 (m, 4H), 2.64-2.74 (m, 2H), 2.84-2.93 (m, 1H), 3.48 (apparent d, $J$=11.6 Hz, 2H), 3.78 (s, 3H), 6.61 (dd, $J$=12.4, 2.4 Hz, 1H), 6.67 (dd, $J$=8.8, 2.4 Hz, 1H), 7.15 (t, $J$=8.4 Hz, 1H), 7.27 (s, 2H) 9.74 (broad s, 1H).

ATR IR (cm$^{-1}$) 3153, 3074, 2943, 2971, 2837, 2810, 2760, 2670, 1623, 1579, 1506, 1470, 1460, 1441, 1420, 1391, 1355, 1341, 1351, 1341, 1300, 1257, 1231, 1202, 1187, 1153, 1130, 1114, 1104, 1076, 1032, 992, 947, 903, 857, 822, 795, 774, 709, 695, 646.

LC-MS (ESI) m/z calculated for C$_{13}$H$_{15}$FN$_3$O: 275.14, observed [M+H]: 276.4.

**Synthesis of 4-(2-fluoro-4-methoxyphenyl)-1-(1H-pyrazol-3-yl)piperidine (17b)**

Compound was prepared from coupling of 3-iodo-1-(tetrahydro-2H-pyran-2-yl)-1H-pyrazole and compound 7a according to the general procedure IV. Before THP deprotection, the intermediate was purified with silica gel column chromatography and 0-100% EtOAc in hexane gradient. The THP-deprotected product was purified with silica gel column chromatography and 0-100% EtOAc in hexane gradient to acquire the final product as off-white solid (9% yield).

$^1$H NMR (400 MHz, DMSO-$d_6$) δ 1.71-1.81 (m, 4H), 2.45-2.73 (m, 2H), 2.79-2.86 (m, 1H), 3.73-3.80 (s overlapping with d, 5H), 5.71 (d, $J$=2.4 Hz, 1H), 6.72-6.80 (m, 2H), 7.23 (t, $J$=8.8 Hz, 1H), 7.46 (d, $J$=2.0 Hz, 1H), 11.86 (broad s, 1H).
ATR IR (cm⁻¹) 3237, 3154, 3069, 3029, 2950, 2922, 2837, 2811, 2755, 2707, 2676, 1622, 1582, 1542, 1474, 1461, 1444, 1384, 1341, 1329, 1301, 1270, 1257, 1240, 1194, 1152, 1113, 1100, 1078, 1058, 1036, 1015, 988, 980, 946, 925, 900, 859, 832, 801, 769, 729, 712, 686.

LC-MS (ESI) m/z calculated for C₁₅H₁₈FN₃O: 275.14, observed [M+H]: 276.4.

**Synthesis of 4-(3-fluoro-5-methoxyphenyl)-1-(1H-pyrazol-4-yl)piperidine (18a)**

Compound was prepared from coupling of 4-iodo-1-(tetrahydro-2H-pyran-2-yl)-1H-pyrazole and compound 8a according to the general procedure IV. Before THP deprotection, the intermediate was purified with silica gel column chromatography and 0-45% EtOAc in hexane gradient. The THP-deprotected product was purified with silica gel column chromatography and 0-100% EtOAc in hexane gradient to acquire the final product as yellowish solid (23% yield).

¹H NMR (300 MHz, CDCl₃) δ 1.91-2.03 (m, 4H), 2.53-2.64 (m, 1H), 2.67-2.76 (m, 2H), 3.47-3.52 (m, 2H), 3.80 (s, 3H), 6.48 (dt, J=10.5, 2.4 Hz, 1H), 6.54-6.60 (m, 2H), 7.33 (s, 2H).

ATR IR (cm⁻¹) 3139, 3118, 3096, 2964, 2935, 2875, 2842, 2815, 2785, 1612, 1598, 1573, 1522, 1461, 1447, 1434, 1387, 1360, 1329, 1299, 1284, 1258, 1245, 1222, 1196, 1151, 1131, 1107, 1056, 1036, 1002, 968, 946, 890, 867, 841, 769, 729, 692, 669, 650, 617.

LC-MS (ESI) m/z calculated for C₁₅H₁₈FN₃O: 275.14, observed [M+H]: 276.5.

**Synthesis of 4-(3-fluoro-5-methoxyphenyl)-1-(1H-pyrazol-3-yl)piperidine (18b)**

Compound was prepared from coupling of 3-iodo-1-(tetrahydro-2H-pyran-2-yl)-1H-pyrazole and compound 8a according to the general procedure IV. Before THP deprotection, the intermediate was purified with silica gel column chromatography and 0-35% EtOAc in hexane gradient. The THP-deprotected product was purified with silica gel column chromatography and
0-100% EtOAc in hexane gradient to acquire the final product as tan solid (38% yield, compound by NMR contains ~4% of unknown impurity).

\[^1\text{H NMR (300 MHz, CDCl}_3\text{)} \delta 1.76-1.95 \text{ (m, 4H), 2.62 (apparent tt, } J=11.7, 3.9 \text{ Hz, 1H), 2.87 (td, } J=12.4, 3.0 \text{ Hz, 2H), 3.78 (s, 3H), 3.83-3.89 \text{ (m, 2H), 5.78 (d, } J=2.4 \text{ Hz, 1H), 6.47 (apparent dt, } J=10.5, 2.4 \text{ Hz, 1H), 6.53-6.59 \text{ (m, 2H), 6.84 (bs, 1H), 7.43 (d, } J=2.4 \text{ Hz, 1H).}

\]

ATR IR (cm\(^{-1}\)) 3257, 3138, 3114, 3010, 2990, 2967, 2942, 2918, 2928, 2811, 2758, 1619, 1589, 1541, 1478, 1458, 1435, 1387, 1372, 1332, 1315, 1301, 1291, 1277, 1260, 1248, 1226, 1189, 1145, 1132, 1096, 1065, 1050, 1025, 999, 992, 973, 943, 932, 897, 875, 851, 821, 752, 724, 703, 686, 657, 619.

LC-MS (ESI) m/z calculated for C\(_{15}\)H\(_{18}\)FN\(_3\)O: 275.14, observed [M+H]: 276.4.

**Synthesis of 4-(4-fluoro-3-methoxyphenyl)-1-(1H-pyrazol-4-yl)piperidine (19a)**

Compound was prepared from coupling of 4-iodo-1-(tetrahydro-2H-pyran-2-yl)-1H-pyrazole and compound 9a according to the general procedure IV. Before THP deprotection, the intermediate was purified with silica gel column chromatography and 0-50% EtOAc in hexane gradient. The THP-deprotected product was purified with silica gel column chromatography and 0-10% MeOH in DCM gradient to acquire the final product as white solid (25% yield).

\[^1\text{H NMR (300 MHz, CDCl}_3\text{)} \delta 1.90-2.02 \text{ (m, 4H), 2.53-2.63 \text{ (m, 1H), 2.66-2.75 \text{ (m, 2H), 3.47-3.52 \text{ (m, 2H), 3.89 (s, 3H), 6.73-6.79 \text{ (m, 1H), 6.84 (dd, } J=8.4, 2.1 \text{ Hz, 1H), 7.01 (dd, } J=11.1, 8.1 \text{ Hz, 1H), 7.31 (s, 2H).}

\]

ATR IR (cm\(^{-1}\)) 3235, 3116, 3065, 3010, 2939, 2916, 2880, 2844, 2809, 2751, 2672, 1608, 1569, 1516, 1467, 1444, 1419, 1387, 1359, 1324, 1297, 1283, 1272, 1261, 1252, 1208, 1190, 1154, 1138, 1121, 1104, 1033, 993, 933, 889, 852, 816, 764, 703, 666, 637, 604.
LC-MS (ESI) m/z calculated for C_{15}H_{18}FN_{3}O: 275.14, observed [M+H]: 276.4.

**Synthesis of 4-(4-fluoro-3-methoxyphenyl)-1-(1H-pyrazol-3-yl)piperidine (19b)**

Compound was prepared from coupling of 3-iodo-1-(tetrahydro-2H-pyran-2-yl)-1H-pyrazole and compound 9a according to the general procedure IV. Before THP deprotection, the intermediate was purified with silica gel column chromatography and 0-50% EtOAc in hexane gradient. The THP-deprotected product was purified with silica gel column chromatography and 0-100% EtOAc in hexane gradient to acquire the final product as white solid (24% yield).

$^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 1.66-1.83 (m, 4H), 2.57-2.72 (m, 3H), 3.76 (apparent d, $J$=12.0 Hz, 2H), 3.84 (s, 3H), 5.72 (d, $J$=2.4 Hz, 1H), 6.78-6.84 (m, 1H), 7.04-7.14 (m, 2H), 7.45 (d, $J$=2.1 Hz, 1H), 11.79 (bs, 1H).

ATR IR (cm$^{-1}$) 3255, 3129, 3070, 3012, 2961, 2942, 2919, 2818, 2749, 2700, 1608, 1544, 1514, 1480, 1461, 1419, 1380, 1312, 1296, 1275, 1260, 1247, 1208, 1194, 1153, 1122, 1103, 1065, 1029, 1018, 990, 984, 939, 924, 893, 854, 815, 783, 750, 723, 683, 637, 608.

LC-MS (ESI) m/z calculated for C_{15}H_{18}FN_{3}O: 275.14, observed [M+H]: 276.5.

**Synthesis of 4-(3-chloro-4-methoxyphenyl)-1-(1H-pyrazol-4-yl)piperidine (20a)**

Compound was prepared from coupling of 4-iodo-1-(tetrahydro-2H-pyran-2-yl)-1H-pyrazole and compound 10a according to the general procedure IV. Before THP deprotection, the intermediate was purified with silica gel column chromatography and 0-35% EtOAc in hexane gradient. The THP-deprotected product was purified with silica gel column chromatography and 0-100% EtOAc in hexane gradient to acquire the final product as white solid (16% yield).
\(^1\)H NMR (600 MHz, CD\(_3\)OD) \(\delta\) 1.80-1.91 (m, 4H), 2.58 (apparent tt, \(J=12.0, 3.6\) Hz, 1H), 2.66 (td, \(J=12.0, 2.4\) Hz, 2H), 3.49 (apparent d, \(J=11.4\) Hz, 2H), 3.85 (s, 3H), 7.00 (d, \(J=8.4\) Hz, 1H), 7.16 (dd, \(J=12.0, 1.8\) Hz, 1H), 7.26 (d, \(J=2.4\) Hz, 1H), 7.37 (s, 2H).

\(^1\)H NMR (600 MHz, CDCl\(_3\)) \(\delta\) 1.86-1.92 (m, 4H), 2.51-2.57 (m, 1H), 2.67 (td, \(J=10.8, 4.2\) Hz, 2H), 3.48 (d, \(J=11.4\) Hz, 1H), 3.89 (s, 3H), 6.89 (d, \(J=8.4\) Hz, 1H), 7.10 (dd, \(J=8.4, 2.4\) Hz, 2H).

ATR IR (cm\(^{-1}\)) 3165, 3144, 3122, 3105, 3073, 2979, 2947, 2917, 2880, 2836, 1664, 1604, 1574, 1527, 1502, 1463, 1443, 1405, 1383, 1360, 1333, 1315, 1286, 1260, 1231, 1198, 1185, 1134, 1107, 1063, 1040, 1022, 993, 936, 919, 875, 855, 816, 785, 769, 716, 662, 612.

LC-MS (ESI) m/z calculated for C\(_{15}\)H\(_{18}\)ClN\(_3\)O: 291.11, observed [M+H]: 292.6

**Synthesis of 4-(3-chloro-4-methoxyphenyl)-1-(1H-pyrazol-3-yl)piperidine (20b)**

Compound was prepared from coupling of 3-iodo-1-(tetrahydro-2H-pyran-2-yl)-1H-pyrazole and compound 10a according to the general procedure IV. Before THP deprotection, the intermediate was purified with silica gel column chromatography and 0-40% EtOAc in hexane gradient. The THP-deprotected product was purified with silica gel column chromatography and 0-100% EtOAc in hexane gradient to acquire the final product as white solid (18% yield, compound by NMR contains 9% of impurity).

\(^1\)H NMR (600 MHz, CDCl\(_3\)) \(\delta\) 1.82 (apparent qd, \(J=11.4, 4.2\) Hz, 2H), 1.90 (apparent distorted d, \(J=13.2\) Hz, 2H), 2.59 (tt, \(J=12.6, 3.6\) Hz, 1H), 2.83 (td, \(J=12.0, 3.0\) Hz, 2H), 3.83-3.86 (m, 2H), 3.89 (s, 3H), 5.80 (d, \(J=1.8\) Hz, 1H), 6.88 (d, \(J=9.0\) Hz, 1H), 7.10 (dd, \(J=8.4, 2.4\) Hz, 1H), 7.25 (d, \(J=2.4\) Hz, 1H), 7.42 (d, \(J=2.4\) Hz, 1H).
ATR IR (cm⁻¹) 3244, 3137, 3119, 3003, 2957, 2931, 2827, 2756, 1605, 1541, 1505, 1476, 1455, 1439, 1400, 1382, 1342, 1314, 1295, 1277, 1257, 1234, 1185, 1167, 1110, 1099, 1062, 1016, 984, 945, 912, 886, 876, 834, 815, 766, 712, 696, 679, 611.

LC-MS (ESI) m/z calculated for C₁₅H₁₈ClN₃O: 291.11, observed [M+H]: 292.6

Synthesis of 4-(2-chloro-4-methoxyphenyl)-1-(1H-pyrazol-4-yl)piperidine (21a)

Compound was prepared from coupling of 4-iodo-1-(tetrahydro-2H-pyran-2-yl)-1H-pyrazole and compound 11a according to the general procedure IV. Before THP deprotection, the intermediate was purified with silica gel column chromatography and 0-35% EtOAc in hexane gradient. The THP-deprotected product was purified with silica gel column chromatography and 0-100% EtOAc in hexane gradient to acquire the final product as white solid (13% yield).

¹H NMR (300 MHz, DMSO-d₆) δ 1.73-1.79 (m, 4H), 2.54-2.59 (m, 2H), 2.87-2.97 (m, 1H), 3.45 (apparent d, J=11.7 Hz, 2H), 3.76 (s, 3H), 6.92 (dd, J=8.7, 2.7 Hz, 1H), 7.02 (d, J=2.7, 1H), 7.28 (s, 2H), 7.32 (d, J=8.7 Hz, 1H), 12.14 (bs, 1H).

ATR IR (cm⁻¹) 3268, 3112, 3074, 2996, 2979, 2959, 2938, 2921, 2892, 2853, 2838, 2810, 2751, 2672, 1600, 1570, 1489, 1463, 1448, 1437, 1407, 1385, 1358, 1330, 1317, 1285, 1260, 1228, 1192, 1182, 1140, 1131, 1097, 1073, 1039, 989,934, 909, 874, 854, 818, 788, 773, 744, 698, 689, 660.

LC-MS (ESI) m/z calculated for C₁₅H₁₈ClN₃O: 291.11, observed [M+H]: 292.6

Synthesis of 4-(2-chloro-4-methoxyphenyl)-1-(1H-pyrazol-3-yl)piperidine (21b)

Compound was prepared from coupling of 3-iodo-1-(tetrahydro-2H-pyran-2-yl)-1H-pyrazole and compound 11a according to the general procedure IV. Before THP deprotection, the
intermediate was purified with silica gel column chromatography and 0-30% EtOAc in hexane gradient. The THP-deprotected product was purified with silica gel column chromatography and 0-100% EtOAc in hexane gradient to acquire the final product as white solid (16% yield, compound contains ~3% impurity as judged by NMR).

$^1$H NMR (600 MHz, CDCl$_3$) $\delta$ 1.81 (apparent qd, $J$=12.0, 3.6 Hz, 2H), 1.92-1.94 (m, 2H), 3.11 (tt, $J$=12.0, 3.6, 1H), 2.90 (td, $J$=12.0, 2.4, 2H), 3.80 (s, 3H), 3.86-3.90 (m, 2H), 5.82 (s, 1H), 6.82 (dd, $J$=9.0, 3.0 Hz, 1H), 6.94 (d, $J$=2.4 Hz, 1H), 7.20 (d, $J$=8.4 Hz, 1H), 7.43 (d, $J$=2.4 Hz, 1H), 9.25 (bs, 1H).

ATR IR (cm$^{-1}$) 3248, 3161, 2965, 2942, 2921, 2909, 2833, 2807, 2751, 1603, 1570, 1542, 1497, 1475, 1459, 1443, 1400, 1383, 1340, 1306, 1288, 1278, 1268, 1233, 1198, 1186, 1148, 1098, 1059, 1034, 1013, 987, 980, 925, 906, 879, 842, 826, 790, 763, 726, 717, 686.

LC-MS (ESI) m/z calculated for C$_{15}$H$_{18}$ClN$_3$O: 291.11, observed [M+H]: 292.5.

**Synthesis of N-(4-(1-(1H-pyrazol-4-yl)piperidin-4-yl)-3-fluorophenyl)-N-methylacetamide (22a)**

Compound was prepared from coupling of 4-iodo-1-(tetrahydro-2H-pyran-2-yl)-1H-pyrazole and compound 12a according to the general procedure IV. Before THP deprotection, the intermediate was purified with silica gel column chromatography and 0-100% EtOAc in hexane gradient. The THP-deprotected product was purified with silica gel column chromatography and 0-5% MeOH in DCM gradient to acquire the final product as white solid (9% yield).

$^1$H NMR (600 MHz, CD$_3$OD) $\delta$ 1.88-2.01 (m overlap with s, 7H), 2.72-2.76 (m, 2H), 3.00-3.04 (m, 1H), 3.23 (s, 3H), 3.54 (d, $J$=11.4 Hz, 2H), 7.12 (apparent d, $J$=9.0 Hz, 2H), 7.40-7.45 (m overlap with s, 3H).
ATR IR (cm\(^{-1}\)) 3191, 3106, 3059, 2980, 2940, 2849, 2809, 2757, 1630, 1610, 1573, 1504, 1461, 1444, 1421, 1387, 1351, 1325, 1308, 1284, 1244, 1230, 1205, 1180, 1139, 1092, 1077, 1037, 989, 930, 906, 890, 870, 843, 799, 733, 688, 664, 657, 628.

LC-MS (ESI) m/z calculated for C\(_{17}H_{21}FN_4O\): 316.17, observed [M+H]: 317.6.

**Synthesis of 4-(2-chloro-4-methoxyphenyl)-1-(1H-pyrazol-3-yl)piperidine (22b)**

Compound was prepared from coupling of 3-iodo-1-(tetrahydro-2H-pyran-2-yl)-1H-pyrazole and compound 12a according to the general procedure IV. Before THP deprotection, the intermediate was purified with silica gel column chromatography and 0-100% EtOAc in hexane gradient. The THP-deprotected product was purified with silica gel column chromatography and 0-100% EtOAc in hexane gradient to acquire the final product as white solid (22% yield).

\(^1\)H NMR (300 MHz, DMSO-\(d_6\)) \(\delta\) 1.78-1.83 (m, 7H), 2.66-2.77 (m, 2H), 2.88-2.99 (m, 1H), 3.16 (s, 3H), 3.78 (apparent d, \(J=12.3\) Hz, 2H), 5.73 (d, \(J=2.1\) Hz, 1H), 7.16 (apparent d, \(J=7.8\) Hz, 1H), 7.26 (apparent d, \(J=11.7\) Hz, 1H), 7.41 (apparent t, \(J=8.4\) Hz, 1H), 7.47 (d, \(J=1.8\) Hz, 1H), 11.81 (bs, 1H).

ATR IR (cm\(^{-1}\)) 3912, 3066, 3026, 2956, 2928, 2879, 2855, 2807, 2752, 1637, 1610, 1574, 1538, 1501, 1475, 1465, 1442, 1421, 1383, 1356, 1311, 1276, 1264, 1238, 1226, 1202, 1185, 1142, 1122, 1109, 1094, 1069, 1041, 1019, 982, 922, 906, 878, 850, 777, 743, 699, 678, 662, 628.

LC-MS (ESI) m/z calculated for C\(_{17}H_{21}FN_4O\): 316.17, observed [M+H]: 317.6.
Appendix A

Abbreviations

AA: arachidonic acid
BBB: blood brain barrier
Boc: tert-butyl carbonate
COX: cyclooxygenase
CYP: cytochrome P450 enzymes
CBF: cerebral blood flow
CSF: cerebrospinal fluid
DCI: delayed cerebral ischemia
DBDD: 12,12-dibromododec-11-enoic acid
DDMS: N-methylsulfonyl-12,12-dibromododec-11-enamide
DMSO: dimethyl sulfoxide
DMF: dimethylformamide
DCM: dichloromethane
EET: epoxyecosatetraenoic acid
EBI: early brain injury
ESI: electron spray ionization
EtOAc: ethyl acetate
Et3N: triethylamine
HBD: hydrogen bond donor
HBA: hydrogen bond acceptor
HCl: hydrochloric acid
HLM: human liver microsomes
ICP: intracranial pressure
IR: infrared spectroscopy
LT: leukotriene
LOX: lipoxygenase
MCAO: middle cerebral artery occlusion
MeOH: methanol
NRB: number of rotatable bonds
NMR nuclear magnetic resonance spectroscopy
PdCl$_2$(dppf): [1,1’-Bis(Diphenylphosphino)ferrocene]dichloropalladium (II)
PSA: polar surface area
PG: prostaglandin
PKC: protein kinase C
RLM: rat liver microsomes
RKM: rat kidney microsomes
R.T.: room or ambient temperature
rCYP4F2: recombinant CYP4F2
SAR: structure activity relationship
SAH: subarachnoid hemorrhage
TXA: thromboxane
THP: tetrahydropyran
20-HETE: 20-hydroxyecosatetraenoic acid

1-ABT: 1-aminobenzotraizole

10-SUYS: sodium 10-undecynyl sulfate

17-ODYA: 17-octadecynoic acid
Appendix B

Experimental spectra
[Chemical structures and spectra are shown, with annotations and data parameters.]
**Shimadzu LC Controller Detector**

From Sample 1 (YPZ-I-43-001) of YPZ-I-43-SET1

Max. 2.954

![Graph showing chromatogram](image1.png)

**D1: Exp 1, 0.622 min from Sample 1 (YPZ-I-43-001) of YPZ-I-43-SET1 with Turbo Spray**

Max. 8.866 cps.

![Mass spectrum](image2.png)


