

## SUPPORTING INFORMATION

### The “Cyclopropyl Fragment” is a Versatile Player that Frequently Appears in Preclinical/Clinical Drug Molecules

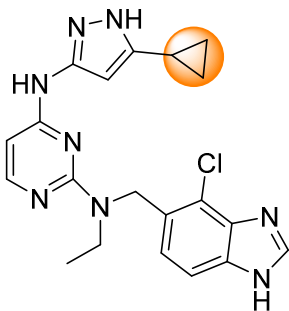
*Tanaji T. Talele*

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## Preclinical/Clinical/Approved Drug Candidates for Cancer Therapy:

### *Kinase Inhibitors –*

**P21 activated kinase (PAK) inhibitor 1:** The p21 activated kinases (PAKs) are a family of enzymes that serve as targets for GTP-binding proteins such as cell division control protein 42 homolog (Cdc42) and Rac. These enzymes are classified as group I (PAK1-3) and group II (PAK4-6) enzymes.<sup>1</sup> It has been shown that group I PAK inhibitors have significant antineoplastic activity against various breast cancer cell lines, thus providing the basis for development of group I selective PAK inhibitors.<sup>2</sup> Subsequently, Crawford and coworkers<sup>3</sup> reported the discovery of the 5-cyclopropylpyrazole containing compound **1**, which had excellent selectivity, potency, and a favorable pharmacokinetic profile as shown in Figure S1. Co-crystal structural studies on a closely related analog of compound **1**-PAK1 showed that the cyclopropyl ring was able to efficiently fill the vacant space available around the gatekeeper residue Met344.<sup>3</sup>



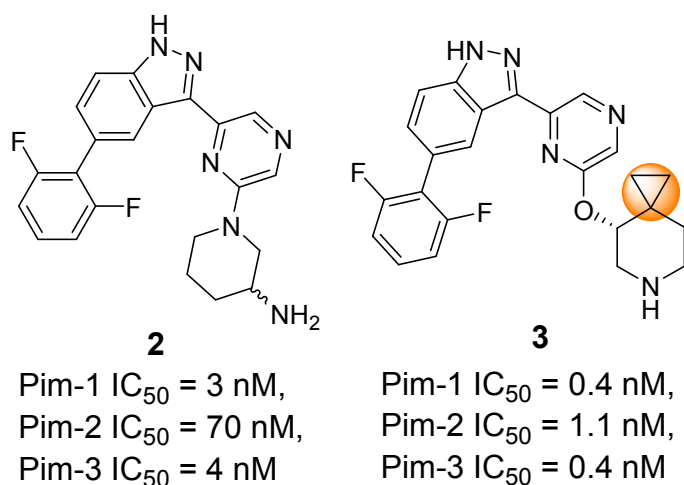
**1**

PAK1  $K_i$  = 5 nM, PAK4  $K_i$  = 547 nM,  
human liver microsomal Cl = 10 mL/min/kg,  
rat liver microsomal Cl = 33 mL/min/kg

**Figure S1.** Structure and *in vitro* potency of PAK1 inhibitor **1**.

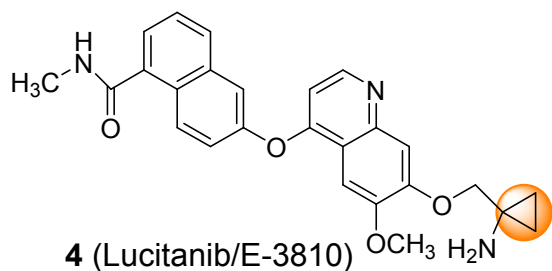
**Pim Kinase Inhibitor 3:** The family of proviral integration site for Moloney murine leukemia virus (Pim) is comprised of three constitutively active serine/threonine kinases (Pim-1, -2 and -3), with overlapping functions have been implicated in numerous hematological malignancies

and solid tumors.<sup>4</sup> Moreover, three of these kinases are known to be redundant in the oncogenesis,<sup>5</sup> thereby supporting the idea to develop pan-Pim kinase inhibitors. Wang and coworkers<sup>6</sup> sought to develop highly potent pan-Pim inhibitor for the treatment of multiple myeloma and hematological malignancies. They started with the HTS hit optimized lead compound **2**. (Figure S2) Subsequently, they utilized the structure-activity relationship (SAR) around the piperidine moiety, yielding the most potent spirocyclopropyl pan-Pim inhibitor, **3**, with an (*R*)-stereochemistry. (Figure S2) The x-ray crystal structure of **3** with Pim-1 kinase (PDB: 4WRS) indicated van der Waals interactions between the spirocyclopropane and the glycine-rich loop residues Leu44, Gly45 and Phe49.<sup>6</sup>



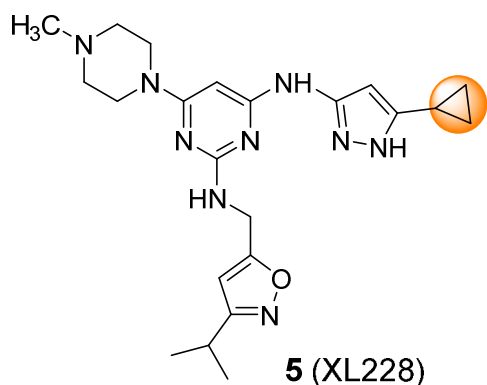
**Figure S2.** Structures and *in vitro* potency of Pim kinase inhibitors **2** and **3**.

**Tyrosine Kinase Inhibitor 4:** Compound **4** (lucitanib/E-3810) a novel cyclopropyl-containing naphthalene carboxamide derivative is found to inhibit VEGFRs-1, -2, and -3 and FGFRs-1 and -2 tyrosine kinases.<sup>7</sup> (Figure S3)



**Figure S3.** Structure of tyrosine kinase inhibitor **4**.

**Multiple Tyrosine Kinase Inhibitor 5:** Compound **5** (XL228) is an inhibitor of multiple tyrosine kinases<sup>8</sup> and a potent inhibitor of the T315I mutant form of the Abl protein as shown in Figure S4.<sup>9</sup> Compound **5** had been evaluated in Phase 1 clinical trials for the treatment of cancer (NCT00464113).

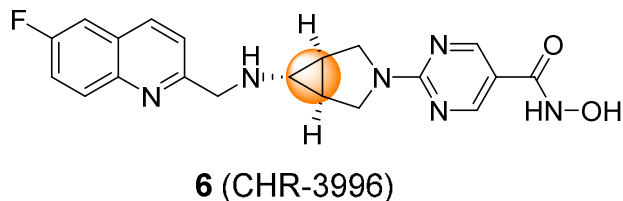


**Figure S4.** Structure of tyrosine kinase inhibitor **5**.

#### *Non-kinase inhibitors –*

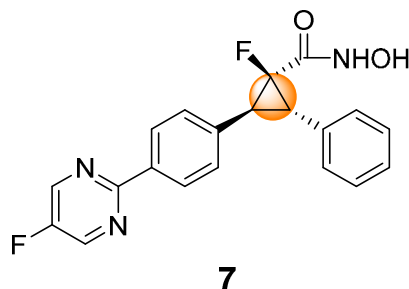
**Histone Deacetylase (HDAC) Inhibitor 6:** Compound **6** (CHR-3996) is an orally bioavailable example of a selective class I HDAC inhibitor with a potential for the treatment of a broad range of human cancers.<sup>10</sup> Discovery of **6** involved the use of a cyclopropyl ring fused across the 3,4-position of the pyrrolidine ring as a novel stereospecific linker for favorable spacing of the

pendant headgroup quinoline ring and the zinc binding pyrimidine hydroxamate moiety.<sup>10</sup>  
(Figure S5)



**Figure S5.** Structure of HDAC inhibitor **6**.

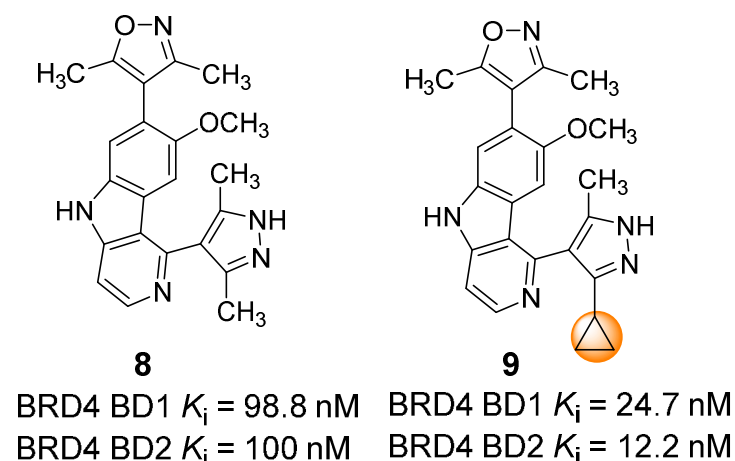
**HDAC Inhibitor 7:** It is a preclinical inhibitor of class IIa HDAC. The cyclopropyl moiety in **7** serves as a chiral scaffold that hold onto three key groups (fluoropyrimidinyl capping group, HDAC IIa active site Zn chelating hydroxamate head group, and selectivity pocket targeting phenyl substituent) in a defined three dimensional space as shown in Figure S6.<sup>11</sup>



**Figure S6.** Structure of HDAC inhibitor **7**.

**Bromodomain Inhibitor 9:** Bromodomain-containing proteins (BRDs) are critical for the epigenetic regulation of gene transcription and they do so by binding to acetylated lysine residues of histone tails.<sup>12</sup> The pharmacological inhibition of BRDs is a valid strategy for the treatment of leukemia.<sup>13</sup> To determine the role of methyl groups on the pyrazole moiety of their lead compound **8**, Ran and coworkers<sup>14</sup> analyzed the binding mode of **8** to the BRD4 domains BD1 and BD2. (Figure S7) This study led to replacing the 3-methyl group on the pyrazole

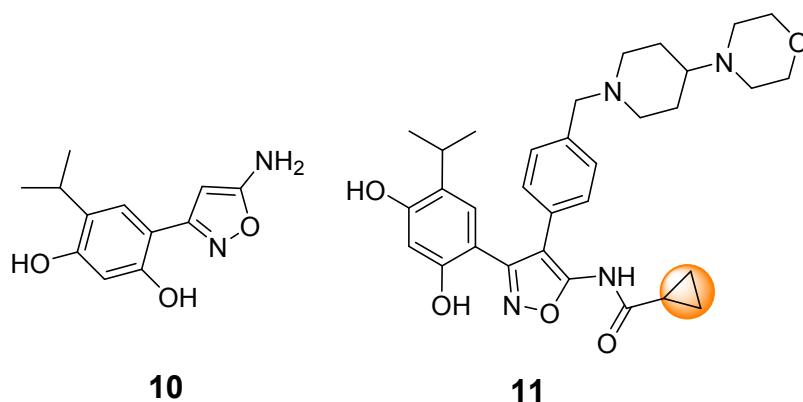
moiety with a larger cyclopropyl group, which should result in extensive hydrophobic contacts with the BD1 and BD2 domains of BRD4. Indeed, the modification of **8** led to discovery of **9**, containing a 3-cyclopropyl-5-methyl-1*H*-pyrazole moiety. Compound **9** was ~4- and ~8-fold more potent than **8** against BD1 and BD2 domains of BRD4, respectively. (Figure S7) The co-crystal structure of **9** with the BRD4 BD2 domain (PDB ID: 4Z93) revealed optimal fit of the cyclopropyl moiety into a hydrophobic pocket lined by residues Trp374, Pro375, His437, Val439, and Met442.<sup>14</sup>



**Figure S7.** Structures and *in vitro* potency of BRD inhibitors **8** and **9**.

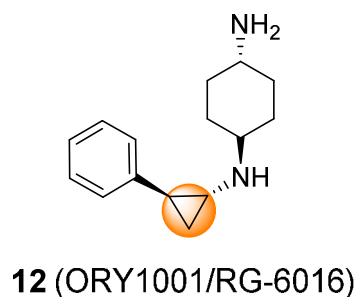
**Heat Shock Protein 90 (HSP90) Inhibitor 11:** HSP90 maintains the conformation, stability and function of several signaling proteins that play a key role in cell proliferation, cell cycle progression, angiogenesis, invasion and metastasis.<sup>15, 16</sup> Furthermore, it exerts chaperone activity on many oncogenic proteins, thus making HSP90 an attractive therapeutic target for anticancer drug development.<sup>17</sup> Chen et al.,<sup>18</sup> identified a very weak binding fragment **10**, comprising an HSP90 inhibitory resorcinol pharmacophore and an isoxazole moiety (no inhibition data reported) as a starting lead. These investigators further optimized fragment **10** at positions 4 and

5 of the isoxazole ring to obtain compound **11**. (Figure S8) Compound **11** had a  $K_i$  of 0.03  $\mu\text{M}$  for HSP90 and inhibited the proliferation of several cancer cell lines, with an average  $\text{GI}_{50}$  value of 88 nM. In addition, compound **11** has drug-like properties ( $t_{1/2}$  = 1.98 h, AUC = 356 ng.h/mL, and oral bioavailability = 9.2%). The cyclopropane carbonyl moiety contributes to the enhanced *in vitro* and *in vivo* efficacy, as well as an acceptable pharmacokinetic (PK) profile.<sup>18</sup>



**Figure S8.** Structures of HSP90 inhibitors **10** and **11**.

**Lysine Specific Demethylase 1 (LSD1) Inhibitor 12:** Addition of the 4-aminocyclohexyl group to the amine group of tranilcypromine resulted in the highly selective LSD1 inhibitor compound **12**/ORY-1001/RG-6016 ( $\text{KDM1A IC}_{50}$  = 6.5 nM and  $\text{KDM1B IC}_{50}$  = 43.5  $\mu\text{M}$ ) as shown in Figure S9.<sup>19,20</sup> Compound **12** is being clinically investigated for treating relapsed or refractory acute leukemia (eudraCT number 2013-002447-29).

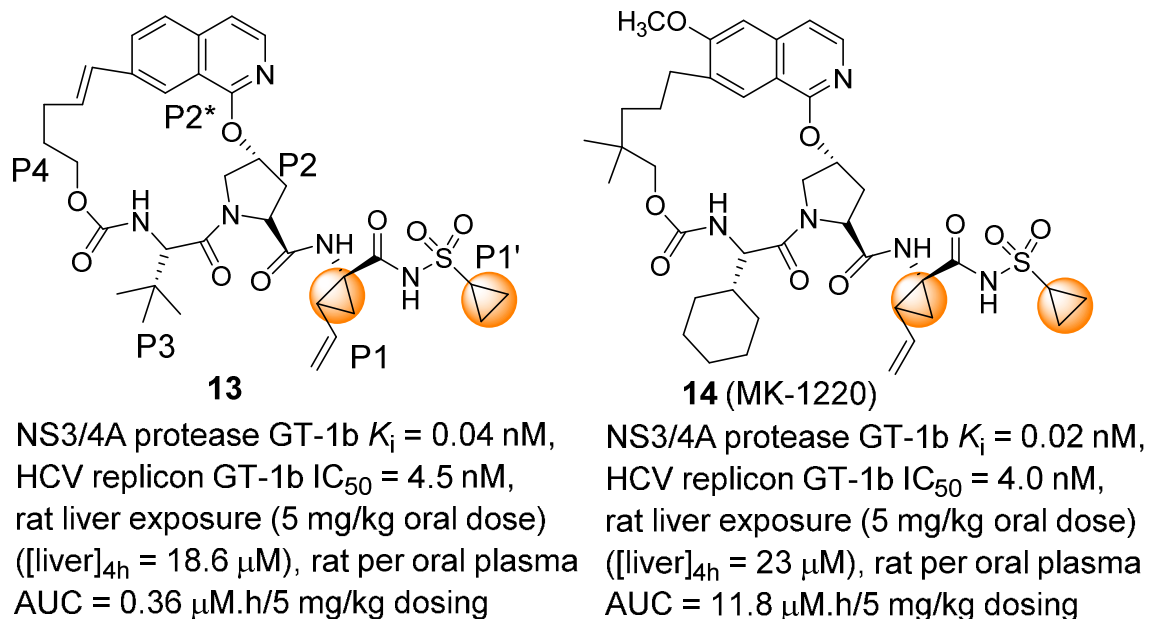


**Figure S9.** Structure of LSD1 inhibitor **12**.

**Preclinical/Clinical/Approved Drug Candidates for Infectious Diseases:**

**Anti-HCV Drug Candidates:**

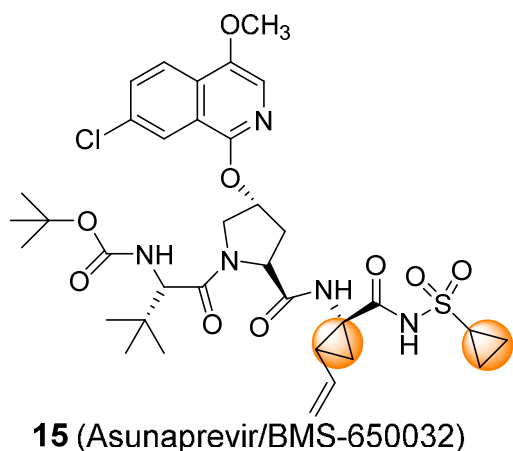
**Compound 14:** Liverton and coworkers<sup>21</sup> previously reported compound **13**, which had a picomolar affinity for HCV NS3/4A protease, appreciable HCV replicon activity, and a remarkable rat liver exposure. (Figure S10) However, it had a suboptimal plasma exposure. To obtain new inhibitors with improved rat liver and plasma exposures, Rudd and coworkers<sup>22</sup> optimized compound **13**. The optimization strategy involved exploration of the substitution pattern on the P2\* isoquinoline moiety and structural variations at the P3 and P4 moieties, which yielded compound **14** (MK-1220),<sup>22</sup> with an excellent overall profile. (Figure S10) The vinylcyclopropyl P1 moiety and cyclopropylsulfonimide P1' moiety in **14** play the same role as that described for other related HCV NS3/4A protease inhibitors.



**Figure S10.** Structures and *in vitro* potency of HCV NS3/4A protease inhibitors **13** and **14**.



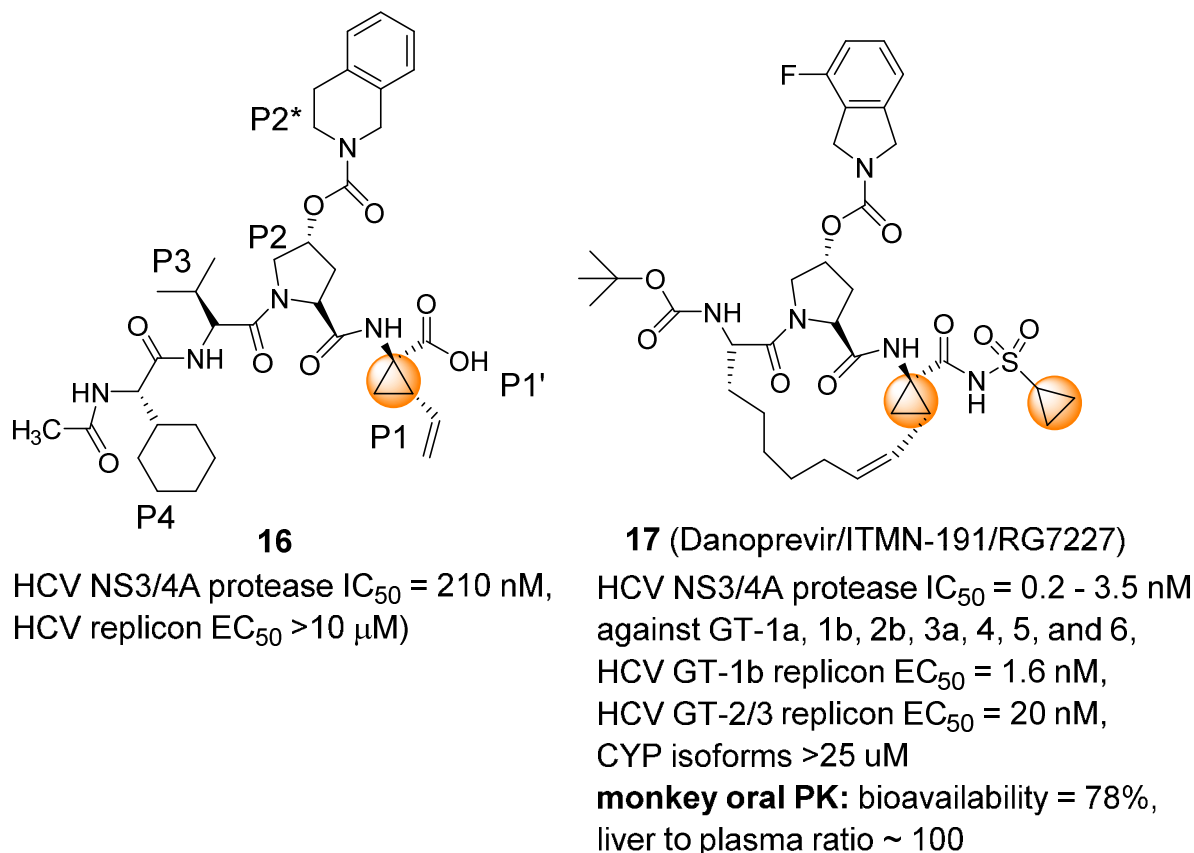
**Compound 15 (Asunaprevir):** Compound **15** (asunaprevir/BMS-650032)<sup>23</sup> is a tripeptidic, acyl cyclopropyl sulfonamide inhibitor of HCV NS3/4A that is structurally related to compound **36** (see manuscript main text for the structure of compound **36**). While clinical trial on **36** was discontinued due to drug-related cardiotoxicity, including altered heart rate and sinus node recovery time, **15** is at present undergoing Phase III clinical trials for the treatment of HCV infections. (Figure S11) Work by Scola and coworkers<sup>23</sup> led to the development of new clinical candidates with a preclinical antiviral and pharmacokinetic profile similar to that of **36**, but with a significantly reduced risk of cardiovascular side effects. This objective was attained through appropriate selection of the substitution pattern on the P2\* isoquinoline ring to reduce cardiotoxicity. Extensive SAR experiments on the P2\* isoquinoline ring led to the discovery of **15**, a compound demonstrating an excellent hepatotropic distribution, *in vivo* efficacy and a safe cardiovascular profile.<sup>23</sup> The carboxyl terminus of this tripeptide contains an acyl cyclopropyl sulfonamide P1' moiety that is well established in HCV NS3/4A protease inhibitor research to confer enhanced potency in the cell-based HCV replicon assays compared to the carboxylic acid progenitor counterpart.<sup>24</sup> On the other hand, the P1 vinylcyclopropyl moiety fit optimally into a shallow S1 pocket and it also contributes to a favorable pharmacokinetic profile, similar to that described for other vinylcyclopropyl-containing HCV protease inhibitors.



**Figure S11.** Structure of HCV NS3/4A protease inhibitor **15**.

**Compound 17 (Danoprevir):** Compound **17** (danoprevir/ITMN-191/RG7227) is a second generation, macrocyclic non-covalent HCV NS3/4A protease inhibitor, with slow off-rate kinetics.<sup>25</sup> At present, this compound is undergoing Phase II clinical evaluation in combination with pegylated interferon- $\alpha$ /ribavirin. In 2014, Jiang and coworkers<sup>26</sup> launched research work aimed at identifying novel second generation HCV NS3/4A protease inhibitors. Although **16** was considered a promising lead based on a high inhibitory potency towards HCV NS3/4A protease, it, however, lacked acceptable cellular HCV replicon-based potency as a result of its high solvent-accessible surface area. (Figure S12) To address this concern, lead **16** was modified by four structural modifications, namely (a) macrocyclization of the P1 and P3 moieties to shield the highly polar surface area; (b) replacement of the polar ionizable carboxyl P1 headgroup with a bioisosteric acyl cyclopropyl sulfonamide, with the expectation that the negative charge on the sulfonamide could be spread over more atoms, which increased cell permeability and ensured high potency due to additional van der Waal's interactions between the cyclopropyl P1' group and the S1' residues; (c) truncation of the peptide *N*-terminus and (d) optimization of the P2\*

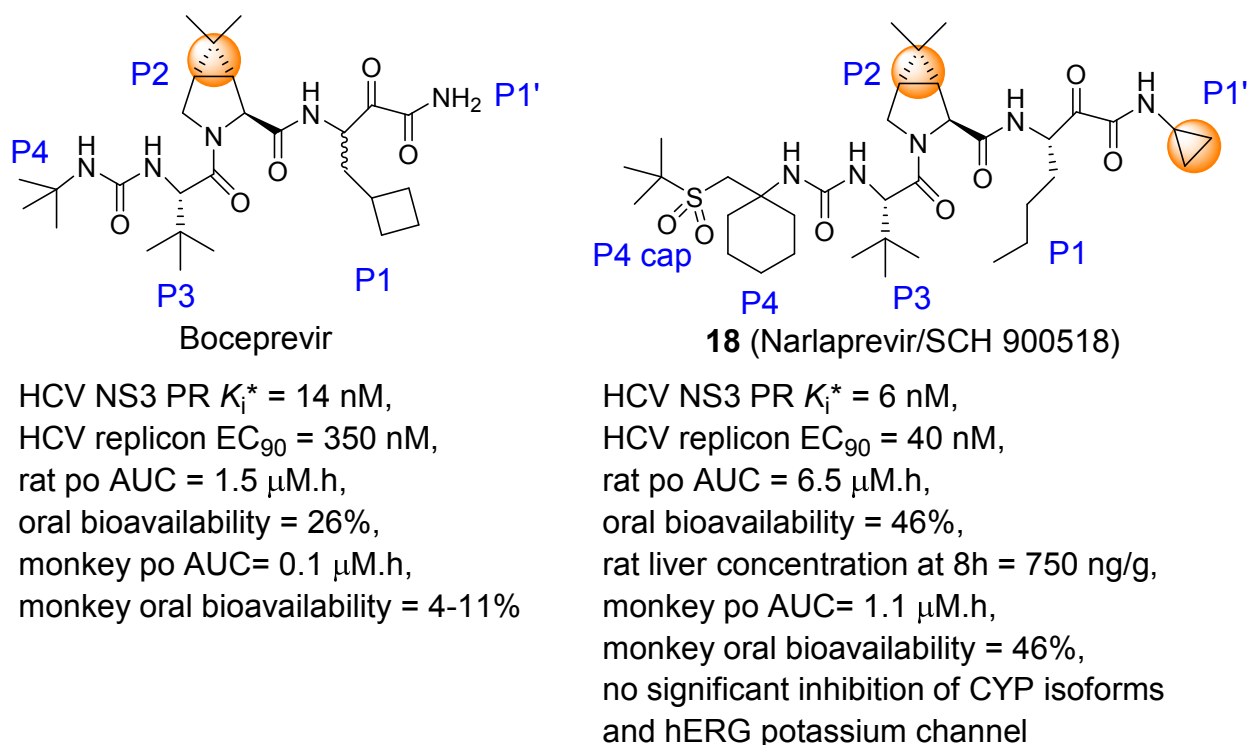
heterocycle. These aforementioned modifications led to the discovery of compound **17** with an excellent overall profile as shown in Figure S12.<sup>26</sup>



**Figure S12.** Structures, *in vitro* potency and PK properties of HCV NS3/4A protease inhibitors **16** and **17**.

**Compound 18 (Narlaprevir):** As an effort to discover second generation HCV NS3/4A protease inhibitors with improved *in vitro* enzymatic/cell-based replicon and *in vivo* potencies, as well as a desirable pharmacokinetic profile with an emphasis on exposure in monkeys, Arasappan and coworkers<sup>27</sup> initiated an extensive structure-based drug design and SAR studies on boceprevir (compound **25** in manuscript main text), a clinically approved drug. (Figure S13) These SAR studies involved (a) the extension of the *N*-terminus in the form of a P4 cyclohexyl

moiety capped with a *tert*-butyl sulfone group to increase contact with the enzyme; (b) replacement of the cyclobutylmethyl P1 side chain with a *n*-butyl group, and (c) insertion of a cyclopropyl moiety on the amino group of the P1'  $\alpha$ -ketoamide. These efforts resulted in discovery of **18** (narlaprevir/SCH 900518), a compound with an excellent PK/PD profile as shown in Figure S13.<sup>27</sup> The SAR and x-ray co-crystal structure data revealed that the cyclopropyl ring was the optimal P1' moiety.<sup>27</sup>

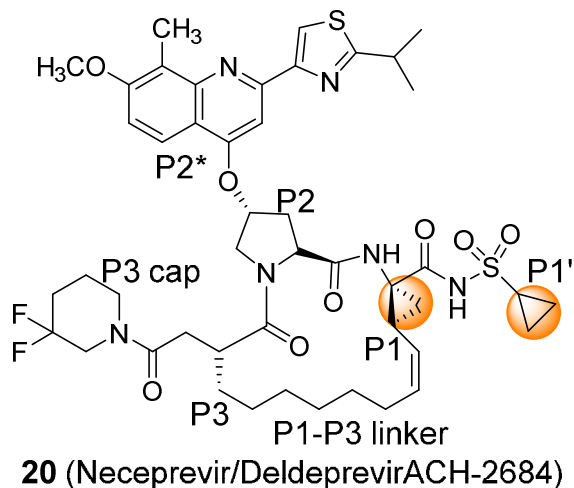


**Figure S13.** Structure, *in vitro* potency and PK properties of HCV NS3/4A protease inhibitor **18**.

**Compound 19 (Vedoprevir):** Compound **19** (vedoprevir/GS-9451) is a tripeptidic HCV NS3/4A protease inhibitor that features a unique P2\* quinoline ring with the following water solubilizing functionalities: a P1 carboxylic acid headgroup and a bicyclohexane (*cis*-cyclopropylpentyl) P3 cap.<sup>28</sup> Sheng and coworkers<sup>28</sup> initiated SAR studies to enhance the interactions of the P3 cap group with S3 residues and to improve water solubility and

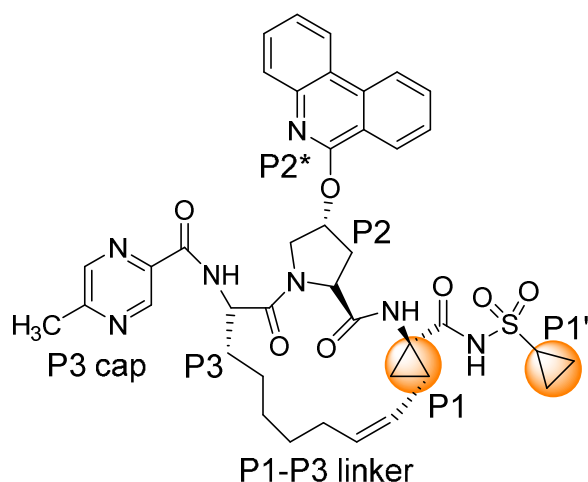


(HCV NS3 protease GT-1a and 1b  $IC_{50} = 100$  pM) with an excellent pharmacokinetic and safety profile as shown in Figure S15.<sup>29</sup>



**Figure S15.** Structure of HCV NS3/4A protease inhibitor **20**.

**Compound 21 (Paritaprevir):** Compound **21** (paritaprevir/ABT-450) a macrocyclic peptidomimetic derivative is another example of a non-covalent inhibitor of HCV NS3/4A proteases from various genotypes of HCV with nanomolar potency as shown in Figure S16.<sup>30</sup> Compound **21**, similar to many NS3/4A protease inhibitors, has a cyclopropylvinyl P1 moiety and acyl cyclopropylsulfonamide P1' moiety that contribute to enhancing potency and to rendering a favorable pharmacokinetic profile.

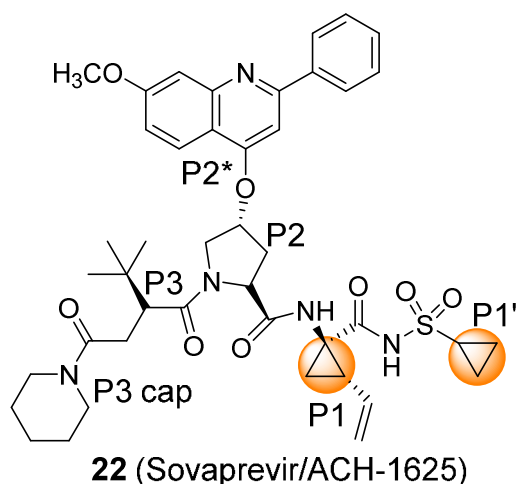


**21** (Paritaprevir/ABT-450)

GT-1a HCV NS3/4A  $EC_{50}$  = 1 nM,  
 GT-1b HCV NS3/4A  $EC_{50}$  = 0.21 nM,  
 GT-2a HCV NS3/4A  $EC_{50}$  = 5.3 nM,  
 GT-3a HCV NS3/4A  $EC_{50}$  = 19 nM,  
 GT-4a HCV NS3/4A  $EC_{50}$  = 0.09 nM,  
 GT-6a HCV NS3/4A  $EC_{50}$  = 0.69 nM)

**Figure S16.** Structure and *in vitro* potency of HCV NS3/4A protease inhibitors **21**.

**Compound 22 (Sovaprevir):** Compound **22** (sovaprevir/ACH-1625) a non-covalent linear peptidomimetic HCV NS3/4A protease inhibitor contains in its structure vinylcyclopropyl P1 and cyclopropylsulfonamide P1' moieties that bind to the S1 and S1' sites of the enzyme, respectively, while providing favorable pharmacodynamic and pharmacokinetic properties as shown in Figure S17.<sup>31</sup>



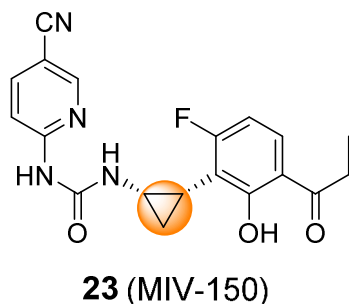
**Figure S17.** Structure of HCV NS3/4A protease inhibitors **22**.

### Anti-HIV compounds

**Compound 23:** HIV-1 reverse transcriptase (HIV-1 RT) is a well-established target for the development of inhibitors for the treatment of HIV infections. Compound **23** (MIV-150)<sup>32</sup> is a third generation non-nucleoside reverse transcriptase inhibitor (NNRTI) that is currently undergoing clinical trials for the prevention of HIV infection in women. (NCT02033109) Compound **23** was discovered in 1999 by Hogberg and colleagues as shown in Figure S18.<sup>32</sup> Compound **23** is a novel, 1*S*,2*S*-*cis*-cyclopropane-containing compound, with an excellent *in vitro* enzyme- and cell-based potencies against wild type, as well as several mutant variants.<sup>32</sup> Compared to the ethyl urea bridge analogs, the *cis*-cyclopropyl urea analogs were significantly more potent. This was reasoned based on the favorable role of a restricted conformation towards efficient binding to both wild type and mutant HIV-1 RT.<sup>32</sup> The x-ray co-crystal structure studies with cyclopropyl urea analogs showed extensive hydrophobic interactions between the cyclopropyl ring and the side chains of Leu100, Val179 and Tyr181.<sup>32</sup> The rate at which **23** induced *in vitro* resistance was significantly slower compared to reference NNRTIs.<sup>32</sup> Compound



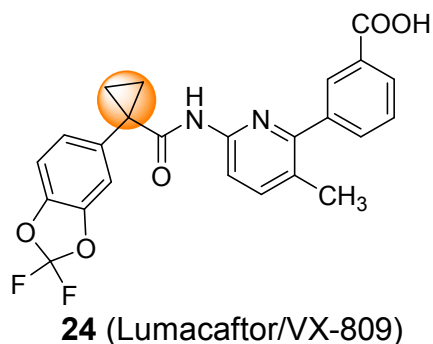
**23** is very poorly absorbed when administered orally, which prompted its clinical development as a vaginal microbicide.<sup>32</sup>



**Figure S18.** Structure of HIV-1 RT inhibitor **23**.

***Respiratory System Drug Candidates:***

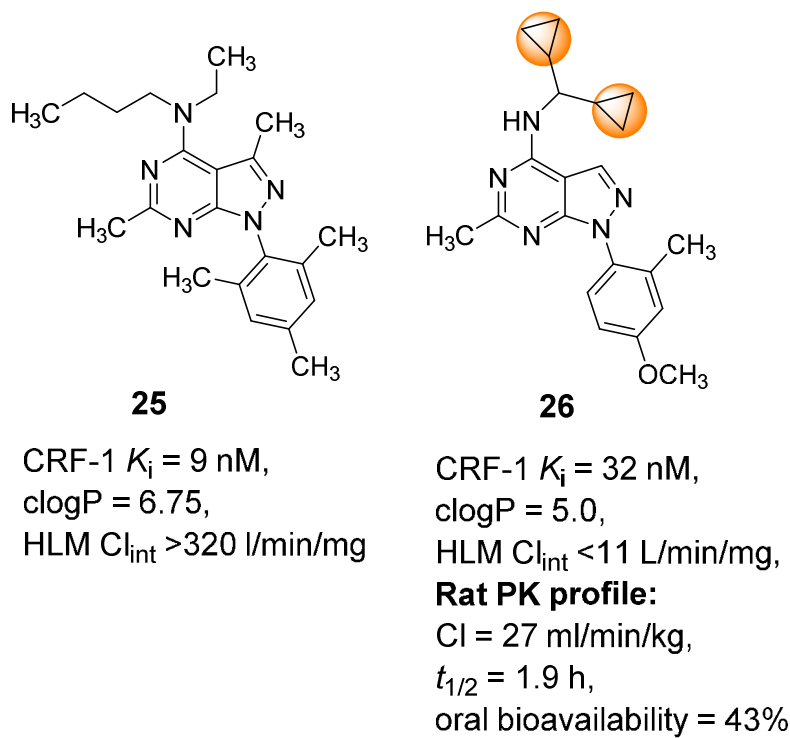
**Lumacaftor:** Cystic fibrosis (CF) is an inherited disease caused by mutations in a gene encoding the CF transmembrane conductance regulator (CFTR), a membrane protein that functions as a chloride channel.<sup>33</sup> The most common mutation, F508ΔCFTR, produces an abnormal protein that disrupts the usual flow of chloride ions and water into and out of cell, with the eventual accumulation of a thick and sticky mucus in the lungs, digestive tract and other body organs.<sup>34</sup> Compound **24** (lumacaftor/VX-809), a cyclopropane carboxamide derivative, referred to as “corrector compound”, was discovered through a high-throughput screening approach and subsequent SAR optimization as shown in Figure S19.<sup>35</sup> Compound **24** is found to partially rescue the functional expression of F508ΔCFTR and forward trafficking to the cell membrane, and has the potential to be used as a treatment for cystic fibrosis. Although the role of the cyclopropyl ring remains to be determined, it is possible that the presence of a spirocyclopropyl moiety may eliminate the metabolically labile unsubstituted benzylic carbon atom.



**Figure S19.** Structure of CF corrector compound **24**.

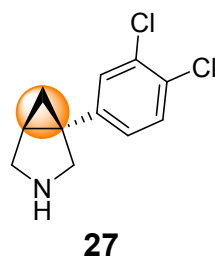
#### **CNS Active Preclinical/Clinical Drug Candidates:**

**CRF-1 Antagonist 26:** Corticotrophin releasing factor-1 (CRF-1) receptor activation by the endogenous 41 amino acid long CRF peptide is responsible for modulation of the hypothalamic–pituitary–adrenal (HPA) axis.<sup>36</sup> CRF-1 antagonists have been tested in models of anxiety, depression<sup>37</sup> and female sexual behavior.<sup>38</sup> CRF-1 antagonists reported in the literature up to 2009 have significantly higher lipophilicity, which impeded their success in the clinic.<sup>39, 40</sup> This prompted Miller and coworkers<sup>41</sup> to initiate a program to develop small molecule CRF-1 antagonists with a drug-like lipophilicity profile. They began with a novel pyrazolopyrimidine scaffold to produce lead compound **25** as shown in Figure S20. However, this compound was associated with high lipophilicity and poor *in vitro* human liver microsomal metabolic stability. Additional SAR studies on lead **25** were initiated to identify novel CRF-1 antagonists with acceptable pharmacokinetic properties. These SAR studies led to discovery of compound **26**, which feature a 4-dicyclopropylmethylamino group as a substitute for 4-*N*-ethyl-*N*-butylamine as shown in Figure S20. *In vivo* rat PK studies indicated that compound **26** has a good PK profile as shown in Figure S20.<sup>41</sup>



**Figure S20.** Structures, *in vitro* potency and PK properties of CRF-1 antagonists **25** and **26**.

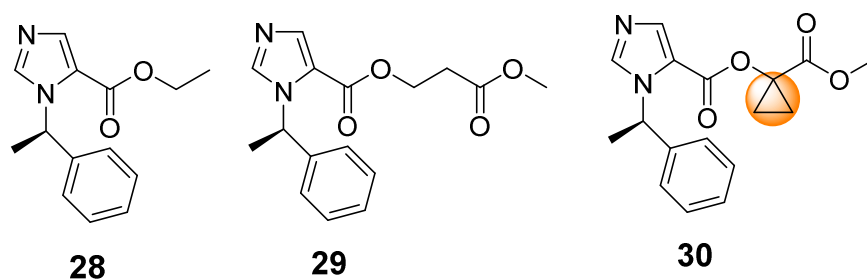
**Compound 27 (Amitifadine):** Compound **27** a triple reuptake (SERT, NET and DAT) inhibitor is currently undergoing phase III clinical trials for the treatment of major depressive disorders.<sup>42</sup> (Figure S21)



**Figure S21.** Structure of triple reuptake inhibitor **27**.

**Compound 28/Etomidate (Amidate) Prodrug:** Compound **28** an imidazole analog is given by the intravenous route as a hypnotic and anesthetic drug.<sup>43</sup> However, it produces a significant

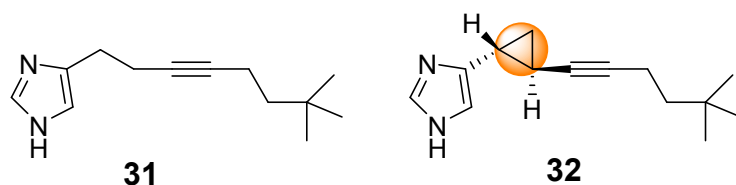
decrease in glucocorticoid synthesis by inhibiting the enzyme  $11\beta$ -hydroxylase.<sup>44, 45</sup> To circumvent this problem, the first generation of a metabolically soft analog of compound **28** such as compound **29** (methoxycarbonyl etomidate) was developed as shown in Figure S22.<sup>46</sup> Unfortunately, this compound was rapidly metabolised and thus less potent, necessitating high dosing.<sup>47</sup> Subsequently, Ge and coworkers<sup>48</sup> developed a second generation soft analog of compound **28**, such as compound **30** (cyclopropyl-methoxycarbonyl etomidate), that had a longer duration of action and a higher potency than first generation soft analog **29**. (Figure S22) These authors noted that the insertion of the cyclopropyl moiety next to the metabolically vulnerable ester group, combined with decreasing the linker length between the two ester groups, would produce a steric hindrance to the enzymatic ester hydrolysis.<sup>48</sup> These molecular modifications led to a longer duration of action and a ~8-fold enhancement of potency for compound **30**.<sup>49</sup>



**Figure S22.** Structures of etomidate and its prodrugs **28-30**.

**Compound 32 (Cipralisant):** Ali and colleagues<sup>50</sup> have undertaken a SAR study focusing on three regions: (a) non-polar acetylene spacer group, (b) conformational restriction of the ethyl bridge located between the imidazole headgroup and the acetylene, and (c) finally hydrophobic tail group. These studies culminated in the synthesis of compound **31** (histamine  $H_3$   $K_i = 0.8$  nM), with sub-nanomolar  $H_3$  receptor binding affinity. In subsequent work, these investigators

restricted the conformation of the ethyl bridge through insertion of a chiral cyclopropane spacer. This conformational restriction facilitated the projection of the pendant pharmacophore groups (the imidazole headgroup and a hydrophobic tail group) by a particular enantiomer into the respective binding pockets of the histamine H<sub>3</sub> receptor. Eventually the more potent (1*R*, 2*R*) clinical compound **32** (histamine H<sub>3</sub>  $K_i$  = 0.12 nM) was developed as shown in Figure S23.<sup>50</sup>



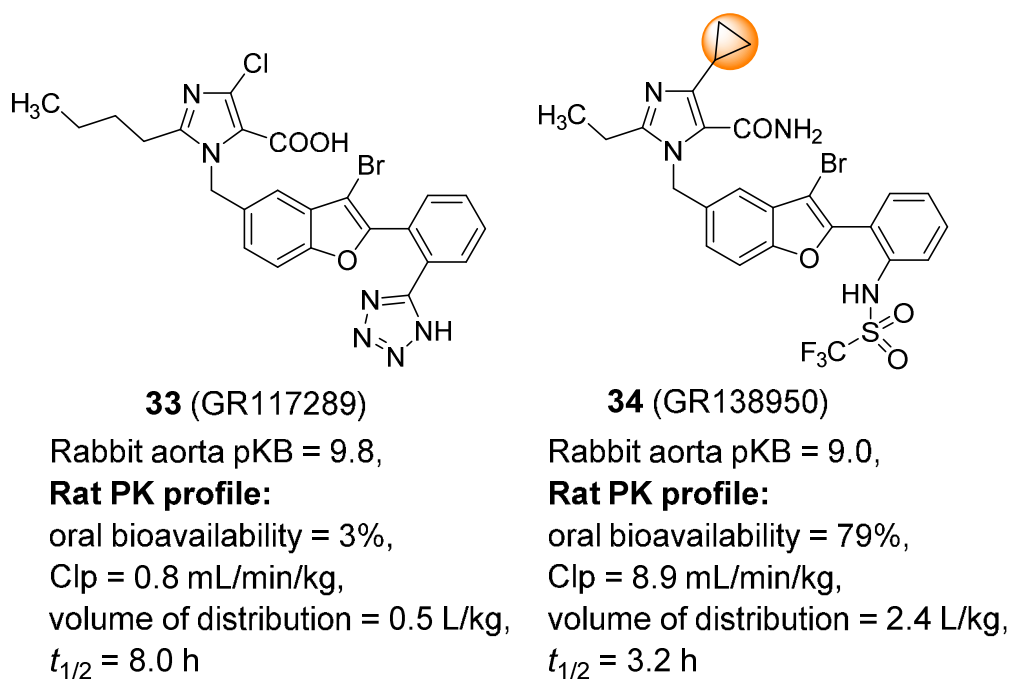
**Figure S23.** Structures of histamine H<sub>3</sub> receptor antagonists **31** and **32**.

***Cardiovascular preclinical/clinical drug candidates:***

**Compound 34 (Saprisartan):** Compound **34** (GR138950) is a potent and selective antagonist of the angiotensin 1 (AT1) receptor, with a characteristic insurmountable/noncompetitive slow dissociation kinetics mechanism.<sup>51</sup>

In the search for non-peptidic angiotensin II antagonists, Middlemiss et al.,<sup>52</sup> discovered compound **33** (zolasartan/GR117289), which, however, had a very poor oral bioavailability. (Figure S24) To overcome this PK concern, Dowle and colleagues<sup>53</sup> modified compound **33** to improve its oral bioavailability. The low oral bioavailability of compound **33** was thought to result from its diacidic nature. This prompted these investigators to conduct further SAR studies, with the intention of developing clinical drug candidates with monoacidic functionalities that are anticipated to have improved oral bioavailability. Early SAR studies produced new compounds, where the 2-butyl, 5-carboxyl and 2-tetrazolyl groups were, respectively, replaced with the potency-enhancing 2-ethyl, oral absorption-enhancing 5-carboxamide and 2-

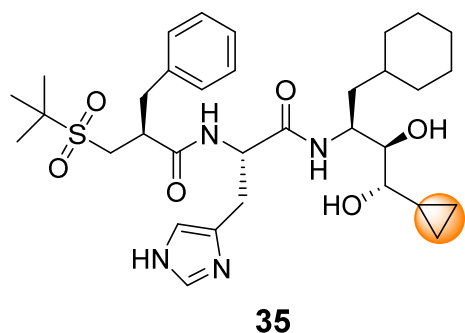
trifluoromethanesulfonamide groups. However, these early lead structures had higher clearances than compound **33**.<sup>53</sup> Subsequently, the replacement of the 4-chloro group on the imidazole ring with the cyclopropyl ring resulted in compound **34**. (Figure S24) Compound **34** not only showed a high *in vitro* potency, but more importantly, increased oral bioavailability and reduced plasma clearance in rats and dogs.<sup>53,54</sup>



**Figure S24.** Structures, *in vitro* potency and PK properties of AT<sub>1</sub> receptor antagonists **33** and **34**.

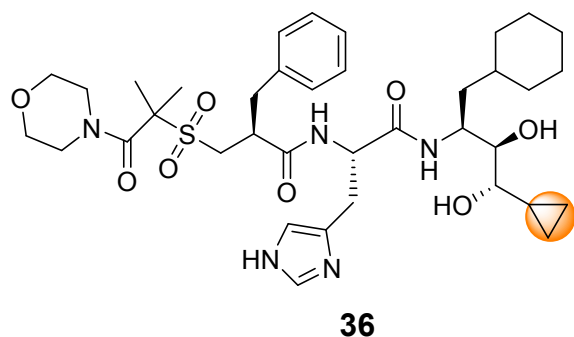
**Compound 35 (Remikiren):** The design and development of compound **35** (remikiren/Ro 42-5892), a second generation transition-state peptidomimetic inhibitor of renin, was inspired by the identification of the aspartyl protease transition-state inhibitor pepstatin and structural modifications in angiotensinogen substrate cleavage site fragments.<sup>55</sup> In compound **35** the Leu(P1)-Val(P1') scissile bond has been bioisosterically replaced with a cyclohexylalanine-CHOHCHOH (vicinal diol)-cyclopropyl group. (Figure S25) The cyclopropyl P1' moiety seem

to optimally fit within the S1' subsite of the renin enzyme. Although compound **35** was the first orally effective and potent human renin inhibitor ( $IC_{50} = 0.7 \text{ nM}$ ), its development was discontinued due to limited oral bioavailability and efficacy.<sup>56, 57</sup>



**Figure S25.** Structure of renin inhibitor **35**.

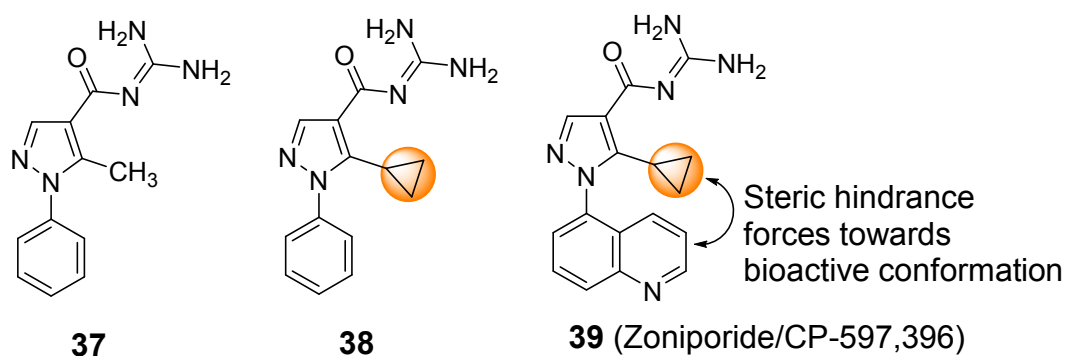
**Compound 36 (Ciprokiren):** Compound **36** (ciprokiren/Ro 44-9375)<sup>58</sup> is structurally analogous to compound **35**, and was designed and developed as a highly specific and potent transition-state inhibitor of renin ( $IC_{50} = 0.07 \text{ nM}$ ) for the treatment of hypertension. (Figure S26) Similar to **35**, development of **36** was also discontinued for unknown reasons.<sup>58</sup>



**Figure S26.** Structure of renin inhibitor **36**.

**Compound 39 (Zoniporide):** The sodium-hydrogen exchanger-1 (NHE-1) transport protein plays an important role in mediating myocardial injury. Consequently, potent and selective

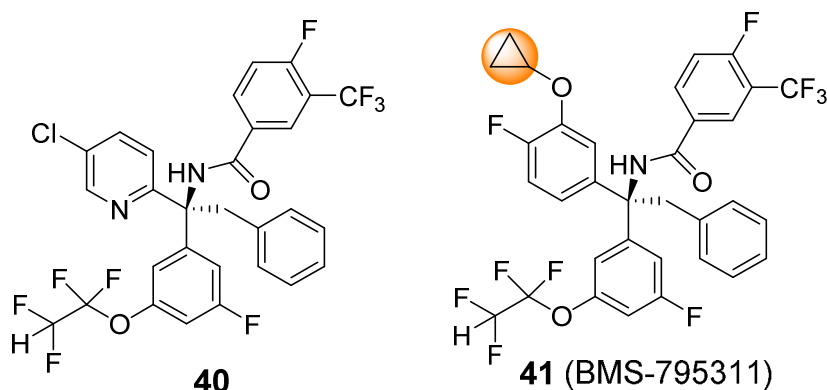
inhibitor of NHE-1 may be useful in reducing myocardial injury that occurs during ischemia/reperfusion.<sup>59</sup> Among the six different isoforms of NHE (NHE-1 through NHE-6),<sup>60</sup> NHE-1 is the only one present in the heart; therefore, it became apparent to develop a highly selective NHE-1 inhibitor<sup>61</sup> with suitable aqueous solubility for intravenous administration. To this end, Guzman-Perez and coworkers<sup>62</sup> carried out structural modifications on the pyrazole lead compound **37** (NHE-1  $IC_{50}$  = 830 nM and NHE-2  $IC_{50}$  = 3900 nM). (Figure S27) The replacement of the 5-methyl group in **37** with the 5-cyclopropyl group led to **38** with enhanced potency and selectivity (NHE-1  $IC_{50}$  = 350 nM and NHE-2  $IC_{50}$  = 12,800 nM). (Figure S27) While keeping the cyclopropyl ring constant, the next structural modification involved the replacement of the phenyl ring with the quinoline ring, resulting in the discovery of a potent and highly selective NHE-1 inhibitor, **39** (zoniporide/CP-597,396) (NHE-1  $IC_{50}$  = 59 nM and NHE-2  $IC_{50}$  = 12,000 nM) as shown in Figure S27, with a desirable pharmacokinetic profile and an aqueous solubility suitable for intravenous administration.<sup>62</sup> These investigators recognized the synergistic contribution of the cyclopropyl and quinoline substituents towards enhanced potency and selectivity. This recognition was further substantiated by molecular modeling studies which showed the existence of an optimum conformation of **39** as a result of steric hindrance between the pendant quinoline and cyclopropyl rings.<sup>62</sup>





**Figure S27.** Structure of sodium-hydrogen exchanger-1 (NHE-1) transport inhibitors **37-39**.

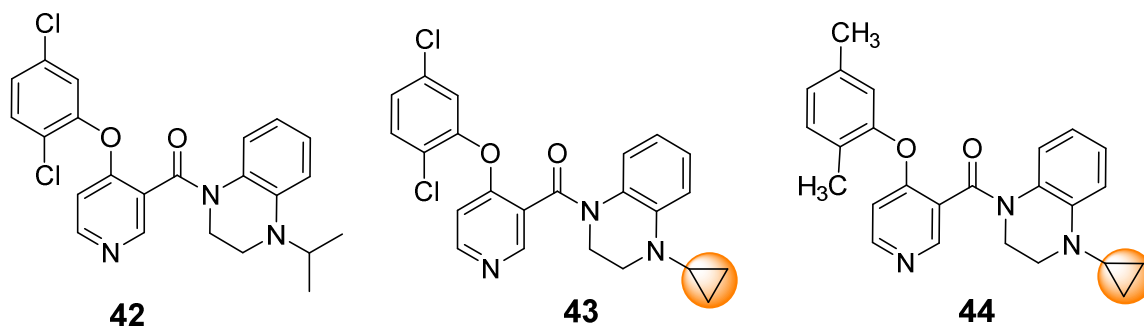
**Cholesteryl Ester Transfer Protein (CETP) Inhibitor 41:** CETP transfers cholesteryl esters from high density lipoprotein (HDL) to very low density lipoprotein (VLDL) and low density lipoprotein (LDL) in exchange for triglycerides, eventually reducing the levels of LDL-cholesterol (the “bad cholesterol”) and elevating the levels of HDL-cholesterol (the “good cholesterol”).<sup>63</sup> Therefore, inhibition of CETP could prevent cardiovascular diseases.<sup>64</sup> Qiao and coworkers<sup>65</sup> synthesized the potent diphenylpyridylethanamine (DPPE) compound **40**<sup>66</sup> that showed a CETP inhibitory activity of 36 nM in an *in vitro*, enzyme-based, scintillation proximity assay (SPA). (Figure S28) One of the problems with compound **40** was that it had a 100-fold higher IC<sub>50</sub> (3.6 μM) value compared to the SPA IC<sub>50</sub> when tested in a human whole plasma assay (hWPA).<sup>66</sup> The structure of compound **40** was optimized to achieve a high plasma exposure upon oral dosing and eliminate off-target blood pressure increases.<sup>65</sup> Extensive SAR studies on compound **40** eventually led to the cyclopropyl ether analog **41** (BMS-795311), which had a good balance of hWPA potency and pharmacokinetic profile (CETP SPA IC<sub>50</sub> = 3.8 nM, CETP hWPA IC<sub>50</sub> = 0.22 μM, human liver microsome (HLM) % remaining during 10 min incubation = 97, mouse liver microsome (MLM) % remaining during 10 min incubation = 100, po AUC<sub>0-8h</sub> = 20.4 μM.h) as shown in Figure S28.<sup>65</sup> The significantly greater *in vivo* efficacy of compound **41** (maximum inhibition of CETP activity at 1 mg/kg oral dose) in a hCETP/apoB-100 dual Tg mice model was attributed to an enhanced plasma exposure.<sup>65</sup> More importantly, compound **41** did not exhibit any significant effect on the mean systolic/diastolic blood pressure, heart rate, or locomotor activity compared to vehicle in telemeterized rats.<sup>65</sup>



**Figure S28.** Structures of CETP inhibitors **40** and **41**.

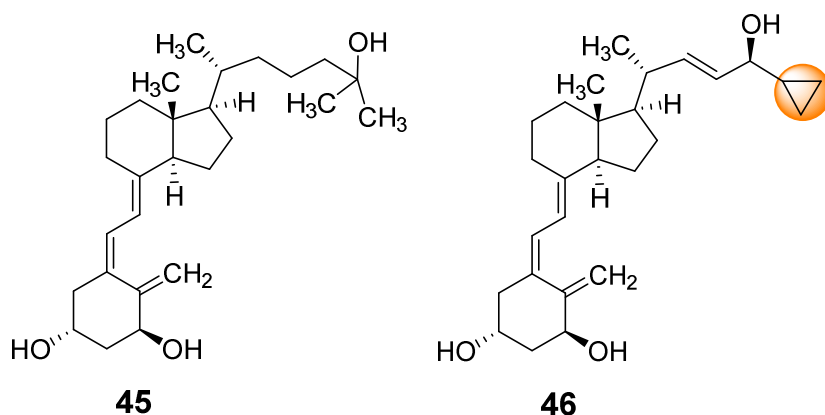
#### Preclinical/Clinical Drug Candidates for the Endocrine System:

**TGR5 Agonist 44:** The bile acids are the endogenous activators of TGR5, a G-protein coupled receptor, which is highly expressed in the gallbladder<sup>67</sup> and moderately in other organs such as intestine.<sup>68</sup> The active TGR5 triggers the secretion of glucagon-like peptide-1 (GLP-1), that modulates glucose homeostasis.<sup>69</sup> Therefore, TGR5 agonists are potentially useful for the treatment of metabolic disorders such as type 2 diabetes mellitus (T2DM). Duan and colleagues<sup>70</sup> sought to develop more potent TGR5 agonists. They quickly synthesized the promising phoxynicotinamide lead compound **42** (hTGR5  $EC_{50}$  = 69 nM). (Figure S29) Subsequent replacement of the *N*-isopropyl group in **42** with an isosteric cyclopropyl group, led to >40-fold potent TGR5 agonist, **43** (hTGR5  $EC_{50}$  = 1.5 nM). (Figure S29) Eventually, the bioisosteric replacement of both the Cl groups on the phenoxy ring with the methyl groups led to the discovery of **44** (hTGR5  $EC_{50}$  = 0.72 nM), with sub-nanomolar potency. (Figure S29) The rationale for these structural changes was to increase the metabolic inactivation of the compound in the gallbladder, where TGR5 activation leads to adverse effects on gallbladder.<sup>70</sup>



**Figure S29.** Structures of TGR5 agonists **42-44**.

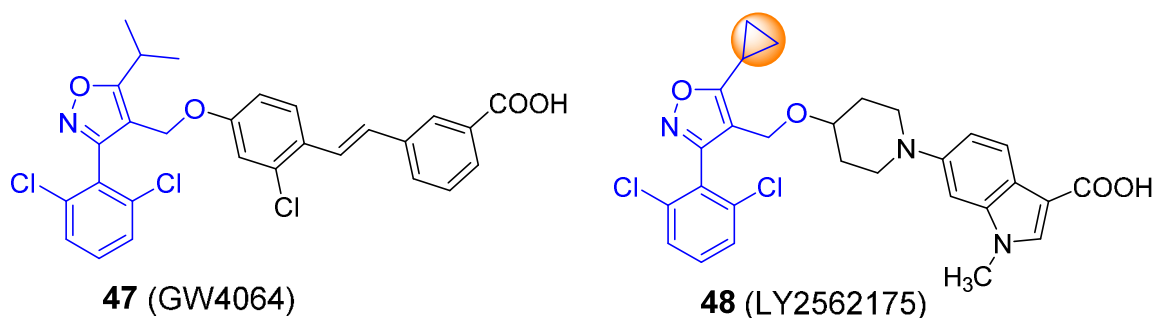
**Calcipotriol (Dovonex):** Compound **45** (calcitriol), a physiologically active form of vitamin D, has antiproliferative actions and it increases the differentiation in basal keratinocytes *in vitro*; however, it has calcium and bone metabolism liability.<sup>71, 72</sup> To eliminate this latter liability, a cyclopropyl ring was added and the side chain was rigidified in compound **45** to obtain the clinically effective, noncalcaemic analog, compound **46** (calcipotriol). (Figure S30) Compound **46**, currently approved for the treatment of psoriasis, produces its therapeutic effects by binding to vitamin D3 receptors on keratinocytes to promote cell differentiation, and the simultaneous reduction of epidermal cell proliferation.<sup>73</sup>



**Figure S30.** Structures of **45** and **46**.

**Preclinical/Clinical Drug Candidates for Metabolic Disorders:**

**Farnesoid X Receptor (FXR) Agonist 48:** FXR, a member of the nuclear receptor subfamily, having bile acids as their endogenous ligands. Therefore, FXR agonists could be used in the treatment of chronic hepatitis, cholestasis, liver fibrosis and atherosclerosis.<sup>74,75</sup> Maloney et al.,<sup>76</sup> were the first ones to report the potent, synthetic FXR agonist **47** (GW4064) to be active *in vivo*. Compound **47** was discovered during the synthesis and testing of a combinatorial library of 9900 compounds and subsequent three-component focused synthesis. The SAR studies indicated the favorable role of position 5 of the isoxazole ring in **47**. For example, the replacement of the methyl group with an isopropyl group yielded ~4-fold increase in activity, a result that suggested that the binding of the 5-substituents to a lipophilic pocket of FXR.<sup>76</sup> Genin and colleagues<sup>77</sup> recently discovered the carboxy indolylpiperidine analog, **48** (LY2562175), which resembles the blue highlighted portion of the **47** structure, except for the isopropyl group, which was exchanged with a cyclopropyl group and the presence of a carboxy indolylpiperidine in place of the original carboxy styrylphenyl moiety. (Figure S31) Based on a superior lipid modulatory activity and PK profile of compound **48**, human testing of this drug advanced to clinical trials for treating dyslipidemia.<sup>77</sup>



**Figure S31.** Structures of FXR agonists **47** and **48**.

## ABBREVIATIONS USED

AUC, area under the curve; BRD, bromodomain-containing protein; CETP, cholesteryl ester transfer protein; CF, cystic fibrosis; CFTR, cystic fibrosis transmembrane conductance regulator; Cl, clearance;  $Cl_{int}$ , intrinsic clearance;  $Cl_p$ , plasma clearance; CRF-1, corticotrophin releasing factor-1 receptor; DAT, dopamine reuptake transporter; FGFR, fibroblast growth factor receptor; FXR, farnesoid X receptor; HDAC, histone deacetylase; hERG, human ether-a-go-go-related gene encoded potassium channel; HLM, human liver microsomes; HSP90, heat shock protein 90; hWPA, human whole plasma assay; KDM, lysine (K) demethylase; LSD1, lysine-specific demethylase 1; MLM, mouse liver microsomes; NET, norepinephrine reuptake transporter; NHE-1 and 2, sodium-hydrogen exchanger-1 and 2; NNRTI, non-nucleoside reverse transcriptase inhibitor; PAK, P21 activated kinase; PK, pharmacokinetic; SAR, structure-activity relationship; SERT, serotonin reuptake transporter; SPA, scintillation proximity assay; VEGFR 1, 2 and 3, vascular endothelial growth factor receptor 1, 2 and 3; VLDL/LDL, very low density and low density lipoproteins

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