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Discovery and hit-to-lead evaluation of piperazine amides as selective, state-dependent Na_v1.7 inhibitors†‡

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Na_v1.7 is a particularly compelling target for the treatment of pain. Herein, we report the discovery and evaluation of a series of piperazine amides that exhibit state-dependent inhibition of Na_v1.7. After demonstrating significant pharmacodynamic activity with early lead compound **14** in a Na_v1.7-dependent behavioural mouse model, we systematically established SAR trends throughout each sector of the scaffold. The information gleaned from this modular analysis was then applied additively to quickly access analogues that encompass an optimal balance of properties, including Na_v1.7 potency, selectivity over Na_v1.5, aqueous solubility, and microsomal stability.

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Introduction

The voltage-gated sodium channel Na_v1.7 plays a key role in the propagation of pain signalling in humans.^{1,2} Na_v1.7 is expressed in high levels in nociceptive sensory neurons within the dorsal root ganglia,³ and mutations of the gene encoding for Na_v1.7, *SCN9A*, are linked to a variety of pain disorders.⁴ Given that current pain treatments suffer from either lack of efficacy or dose-limiting toxicity,⁵ Na_v1.7 is a particularly compelling target for the treatment of pain. However, despite extensive studies by various organizations,⁶ a Na_v1.7-selective inhibitor has yet to reach patients.⁷ One challenge associated with targeting Na_v1.7 for pain is garnering selectivity over other members of the Na_v family of channels (Na_v1.1–Na_v1.9). In particular, inhibition of Na_v1.5, which is

expressed in atrial and ventricular myocytes, has been shown to prolong the cardiac QRS wave in humans, causing a decrease in conduction velocity which may ultimately lead to adverse cardiovascular events.⁸

Recently, we have reported the lead optimization of heteroaryl sulfonamides and biaryl acyl sulfonamides as potent Na_v1.7 inhibitors possessing a high degree of selectivity over other Na_v isoforms.⁹ Despite excellent potency and Na_v1.7 selectivity, the development of these sulfonamides has been hampered by several factors. In addition to PXR activation and inhibition of multiple CYP isoforms, lipophilic acids such as these are frequently subject to transporter-mediated clearance pathways, and wide variance of such clearance routes amongst preclinical species leads to challenges in accurately predicting human dose.^{7c} Accordingly, we pursued the advancement of alternative chemotypes with diverse physicochemical properties, particularly those outside of lipophilic acid chemical space.

Furthermore, we were interested in developing chemical matter that displayed use- or state-dependent inhibition of Na_v1.7.¹⁰ An abnormally hyperactive nociceptive neuron with frequent action potential firing or a depolarized membrane potential significantly increases the proportion of channels in certain inactivated states including fast- and slow-inactivated states.¹¹ A compound that has selective affinity towards Na_v1.7 in these inactive conformations should not only inhibit nociceptive hyperactivity but also possess functional selectivity over other Na_v channels in cells firing at lower rates or with less depolarized membrane potentials. This functional selectivity would potentially garner a larger margin of safety over a comparable inhibitor that lacks state dependence.

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Our interest in a state- or use-dependent, nonacidic $\text{Na}_v1.7$ inhibitor led to the discovery of a novel series of piperazine amides. Herein we report our efforts to understand the structure–activity relationship of the series, which led to the development of potent inhibitors of $\text{Na}_v1.7$ possessing favourable physicochemical and pharmacokinetic properties as well as selectivity over $\text{Na}_v1.5$. We also provide preliminary results of a representative early lead piperazine amide in a histamine-induced mouse pruritus model of $\text{Na}_v1.7$ engagement.⁹

Chemistry

In order to quickly access derivatives of key positions around the scaffold, we employed two distinct synthetic routes (Scheme 1). An Ugi three-component coupling using benzyl isocyanate (**1**), a benzaldehyde **2**, and *N*-Boc-piperazine (**3**) allowed us to access key intermediate **4** in short order (Scheme 1a).¹² Subsequent acid-mediated Boc removal and amide coupling with a benzoic acid derivative **5** afforded racemic final compounds **6**. Analogues containing variations to the piperazine core were also made using this methodology.

While the Ugi three-component protocol worked well to rapidly deliver a variety of analogues, limitations to the isocyanate component scope as well as scalability concerns prompted us to develop alternative methodology (Scheme 1b). AIBN-promoted bromination of an α -toluic acid ester **7** provided bromides **8**.¹³ Bromide displacement using either *N*-Boc-piperazine (**3**) or a suitably pre-functionalized piperazine amide derivative **9** afforded esters **10**. Subsequent ester saponification and amide coupling with a primary amine **11** facilitated the synthesis of analogues **12** directly when **9** was employed and an intermediate similar to **4** when **3** was employed. Chiral separation of late-stage intermediates (e.g., **4**) or final compounds was used to furnish enantio-enriched products.¹⁴ Compounds were generally resistant to racemization/epimerization at the carbonyl α -methine unless exposed to strongly basic conditions for prolonged periods.

Results and discussion

Owing to our interest in developing state-dependent inhibitors of $\text{Na}_v1.7$, we evaluated compounds using two distinct electrophysiological assays run on IonWorks Barracuda (IWB) and PatchXpress (PX) automated electrophysiology platforms. In the IWB assay protocol, a series of high frequency depolarizing pulses at 5 Hz are applied to cells following a four-minute period where cells are unclamped, with a resting membrane potential around -20 mV, to induce a slow-inactivated state. Within this series of pulses, a potency shift between the first tonic pulse and after the entire sequence is indicative of use dependence. In the PX assay protocol, cells are voltage-clamped at a membrane potential resulting in 20–50% channel inactivation. We postulated that compounds exhibiting enhanced potency against $\text{Na}_v1.7$ in the IWB assay protocol when compared to the PX assay protocol would demonstrate increased affinity to a slow-inactivated channel state and/or exhibit use-dependent block.¹⁵

As previously mentioned, selectivity over $\text{Na}_v1.5$ is critical for establishing a safety window. $\text{Na}_v1.5$ is expressed in the heart, where it controls the upstroke of the cardiac action potential.⁸ We determined $\text{Na}_v1.5$ potency using a PX assay, where channels are partially inactivated. The IWB assay voltage parameters are not relevant to normal $\text{Na}_v1.5$ function, as human cardiomyocytes are not exposed to high frequency pulses or prolonged periods of depolarization under physiological conditions. Our decision to use the PX assay to determine selectivity over $\text{Na}_v1.5$ was substantiated through Langendorff isolated rabbit heart studies¹⁶ using several of our piperazine amides; QRS prolongation was more correlated with $\text{Na}_v1.5$ PX potency rather than $\text{Na}_v1.5$ IWB potency.¹⁷

Screening Amgen's compound collection identified compound **13** (Fig. 1) as a potent inhibitor of $\text{Na}_v1.7$.¹⁹ Subsequent analysis revealed that this compound was 35-fold less potent in the $\text{Na}_v1.7$ PX assay compared to the IWB assay, highlighting preferential block of a slow-inactivated state or use-dependent block.²⁰ In addition to its potency, this

Scheme 1 (a) Ugi three-component coupling and (b) α -toluic acid ester routes to piperazine amide analogues. Reagents and conditions: (a) HOAc, TFE; (b) TFA, CH₂Cl₂; (c) T3P, NEt₃, CH₂Cl₂; (d) NBS, AIBN, CCl₄, 85 °C; (e) K₂CO₃, DMF; (f) LiOH, THF, H₂O, MeOH; (g) T3P, NEt₃, CH₂Cl₂.

Fig. 1 Structure and properties of screening hit 13.

compound exhibited good permeability and was not a substrate for Pgp-mediated efflux.²¹ Despite these advantageous properties, 13 exhibited high intrinsic clearance in human and mouse liver microsomes and lacked selectivity over $\text{Na}_v1.5$.

In order to quickly drive a decision whether or not to pursue this series, we sought to evaluate either 13 or a close derivative *in vivo*. In order to address the high microsomal intrinsic clearance of this compound, we initially hypothesized that eliminating the metabolically labile piperazinyl benzylic amine functionality would impart a degree of stability while lowering clogP.²² Indeed, replacement of the benzylic amine with an aryl amide in compound 14 not only lowered microsomal turnover but also increased selectivity over $\text{Na}_v1.5$ while maintaining potency in our $\text{Na}_v1.7$ IWB assay (Fig. 2a). Oral administration at a 100 mg kg^{-1} dose in mice provided sufficient exposure to warrant further study.

Piperazine amide 14 was subsequently evaluated in a mouse histamine-induced pruritus model of $\text{Na}_v1.7$ engagement (Fig. 2b). In humans, a gain-of-function $\text{Na}_v1.7$ mutation has been reported to cause episodes of spontaneous itch.²³ Furthermore, $\text{Na}_v1.7$ knockout mice fail to exhibit scratching behaviour following an intradermal histamine challenge.^{4a} The robustness and reliability of this behaviour, the ease of study execution, and this assay's translational potential to human studies make this model particularly useful for the *in vivo* evaluation of $\text{Na}_v1.7$ inhibition,²⁴ as we have previously reported.^{9a} In the current experiment, 14 (or a vehicle control solution) was administered orally at a dose of 300 mg kg^{-1} to C57BL/6 male mice 60 minutes prior to intra-

dermal injection of histamine to the nape of the neck. The number of scratching bouts was then recorded by an experimenter blinded to compound treatment over a 30 minute period. At this dose, 14 produced a statistically significant (76%) reduction in scratching bouts compared to vehicle. This reduction was similar in magnitude to that produced by a 30 mg kg^{-1} oral dose of diphenhydramine (DPH), an anti-histamine that served as a positive control. The plasma concentration of 14 measured immediately after the study (plasma $C_u = 0.82 \mu\text{M}$, 1.5 h) indicated a 1.8-fold coverage over the mouse IWB IC_{50} . Also noteworthy is the lack of significant effects associated with a 300 mg kg^{-1} oral dose of compound 14 in a mouse open-field assay, which afforded similar exposure as the histamine-induced itch study.²⁵ This indicates that the reduction in scratching behaviour in the histamine pruritus model is unlikely to be the result of nonspecific alterations in locomotor activity.²⁶

Having shown *in vivo* target engagement with a member of this series, we sought to make further improvements to microsomal stability and selectivity over $\text{Na}_v1.5$ via judicious SAR analysis. While we could leverage *in silico* tools to guide our subsequent SAR studies concerning microsomal stability, the lack of structural information concerning the differential binding of 14 to both $\text{Na}_v1.7$ and $\text{Na}_v1.5$ motivated us to simultaneously evaluate a variety of SAR hypotheses in a rapid, efficient manner. With this in mind, we divided the scaffold of 14 into four distinct domains (see Fig. 2a) and leveraged our synthetic methodology to simultaneously explore SAR across all sectors. Moreover, we aspired to establish if SAR, particularly concerning selectivity over $\text{Na}_v1.5$, would be additive across these domains and would allow us to quickly access high quality lead compounds for subsequent *in vivo* analysis.

First, we further explored the SAR of the amide A-ring exemplified by compounds 15–18 (Table 1).²⁷ In line with 14, these amides generally possessed levels of selectivity over $\text{Na}_v1.5$ greater than benzyl piperazine 13 and were significantly more metabolically stable. 3,5- and 2,5-disubstitution provided the optimal balance of $\text{Na}_v1.7$ potency and selectivity over $\text{Na}_v1.5$. This balance is exemplified with analogue 16; accordingly, a 3,5-difluorophenyl A-ring was selected for

Fig. 2 (a) *In vitro* and *in vivo* parameters of 14. Sectors of SAR analysis are labelled in the structure of 14. (b) Reduction of scratching behaviour observed with 14 (300 mg kg^{-1} , p.o.) and diphenhydramine (DPH, 30 mg kg^{-1} , p.o.) in a mouse histamine-induced pruritus model compared to vehicle, with exposure levels of 14. ***, $p < 0.001$; ****, $p < 0.0001$ versus vehicle group (one-way ANOVA followed by Dunnett's tests).

Table 1 SAR of A-ring amide sector

No.	Structure	Na _v 1.7 IWB IC ₅₀ (μM)	LE/LipE ^a	Na _v 1.5 PX IC ₅₀ (μM)	Na _v 1.5/1.7 selectivity	HLM Cl _{int} (μL min ⁻¹ mg ⁻¹)	Solubility at pH 7.4 (μM)	clog <i>P</i>
14		0.37	0.26/1.4	0.90	2.4×	74	96	5.0
15		1.48	0.24/2.0	9.1	6.2×	27	454	3.9
16		0.26	0.27/2.1	1.3	5.0×	95	137	4.4
17		0.62	0.25/2.4	3.1	3.8×	29	248	3.8
18		0.35	0.24/1.5	0.40	1.1×	50	44	5.0

^a LipE = -log(Na_v1.7 IWB IC₅₀) - clog *P*.**Table 2** SAR of the B-ring aryl sector

No.	Structure	Na _v 1.7 IWB IC ₅₀ (μM)	LE/LipE ^a	Na _v 1.5 PX IC ₅₀ (μM)	Na _v 1.5/1.7 selectivity	HLM Cl _{int} (μL min ⁻¹ mg ⁻¹)	Solubility at pH 7.4 (μM)	clog <i>P</i>
19		0.80	0.24/1.7	6.5	8.1×	<14	74	4.4
20		0.20	0.25/2.0	0.50	2.4×	33	30	4.7

^a LipE = -log(Na_v1.7 IWB IC₅₀) - clog *P*.

inclusion in subsequent SAR analysis of other regions along the scaffold. A *meta*-nitrile substituent (**17**) was effective for lowering *clogP* and decreasing microsomal turnover. Insertion of a nitrogen atom to form pyridine **15** resulted in a loss of Na_v1.7 potency but also an increase in selectivity over Na_v1.5 and microsomal stability. While large, lipophilic groups tended to increase Na_v1.7 potency, these groups did not necessarily lead to gains in lipophilic efficiency and frequently led to decreases in both selectivity over Na_v1.5 and aqueous solubility, as illustrated by analogue **18**. We surmised that this region of our scaffold may be residing in a lipophilic pocket of both Na_v1.5 and Na_v1.7; however, it seemed probable that a key polar or π -stacking interaction driven by electron-withdrawing substituents along this ring engendered differential binding in favour of Na_v1.7.

We next turned our attention to modifications of the remainder of the scaffold. After exploring various mono-substitution patterns along the B-ring, we found that 4-cyano substitution (**19**) provided a desirable combination of Na_v1.7 potency and selectivity over Na_v1.5 (Table 2). In addition, this group also blocked a putative site of oxidative metabolism which in combination with its greater polarity led to a lower observed intrinsic clearance in human liver microsomes. Further modifications based upon **19** in attempts to improve

Na_v1.7 potency, such as derivative **20**, led to erosions in selectivity over Na_v1.5 with concomitant increases in microsomal turnover and decreases in aqueous solubility.

The SAR around the benzylic amide C-ring position was extensively explored (Table 3). Methylation of the secondary amide to afford **21** resulted in a significant loss of Na_v1.7 potency, illustrating the importance of this hydrogen-bond donor. We hypothesized that several positions along this region would be particularly prone to oxidative metabolism. Both blocking the *para* position of the aryl ring with a trifluoromethyl group and increasing polarity *via* the use of a pyridine in **22** resulted in an increase in microsomal stability and selectivity over Na_v1.5.

We additionally pursued several strategies for masking the potentially metabolically labile benzylic methylene group attached to the C-ring. Excision of this methylene entirely, resulting in aniline **23**, also led to an increase in microsomal stability and enhanced selectivity over Na_v1.5. Further optimization led to discovery of the 6-(2,2,2-trifluoroethoxy)pyridin-3-amine group, found in **24**, which conferred a marked improvement in potency and microsomal stability. Despite these improvements, the anilines and pyridinamines generally suffered from poor aqueous solubility and significantly higher molecular weight. We therefore targeted small, alkyl

Table 3 SAR of C-ring benzyl amide sector

No.	Structure	Na _v 1.7 IWB IC ₅₀ (μM)	LE/LipE ^a	Na _v 1.5 PX IC ₅₀ (μM)	Na _v 1.5/1.7 selectivity	HLM Cl _{int} (μL min ⁻¹ mg ⁻¹)	Solubility at pH 7.4 (μM)	<i>clogP</i>
21		1.22	0.24/1.3	10	8.4×	306	220	4.6
22		0.21	0.25/2.7	7.9	22×	14	225	4.0
23		0.42	0.24/0.8	9.5	23×	24	0	5.6
24		0.11	0.25/1.7	3.0	27×	<14	19	5.2
25		0.95	0.27/2.5	7.7	8.1×	<14	472	3.5
26		0.31	0.27/2.4	6.7	22×	<14	346	4.1

^a LipE = -log(Na_v1.7 IWB IC₅₀) - *clogP*.

replacements to this benzylic moiety to not only address these shortcomings but also to achieve our general goal of increasing metabolic stability. Cyclopropyl **25** and homologated derivative **26** provided competent replacements to the aryl ring, resulting in compounds with lower molecular weight and microsomal turnover, increased LipE and increased solubility, and adequate Na_v1.7 potency while maintaining existing levels of selectivity over Na_v1.5.

To culminate our SAR explorations around the scaffold, we investigated replacements for the piperazine D-ring (Table 4). We hypothesized that rigidifying this ring would result in increased selectivity over Na_v1.5, presumably by disfavoured conformations that preferentially engage Na_v1.5. In this regard, a particularly effective strategy was the deployment of bridging methylene and ethylene units along the piperazine ring. Greater selectivity over Na_v1.5 was observed with analogues containing bridging elements placed toward the A-ring-connected portion of the piperazine. Both diazabicyclo[3.1.1]heptane **27** and diazabicyclo[3.2.1]octane **28** were significantly more selective over Na_v1.5 than parent piperazine **14** with comparable Na_v1.7 potency. In general, these bridged piperazines suffered from increased microsomal clearance and lower aqueous solubility.

With explorations of each individual sector completed, we were now in a position to test our hypothesis that SAR would be additive once these individual components were aggregated in new compounds (Table 5). In addition to primarily addressing selectivity over Na_v1.5, we also sought to ameliorate any issues associated with specific functionality through prudent combinations of groups across the scaffold. For instance, while the 6-(2,2,2-trifluoroethoxy)pyridin-3-amine C-ring in **24** imparted stability and potency, aqueous solubility worsened;

perhaps targeting analogues that contain particularly solubilizing moieties in other portions of the scaffold would improve solubility while maintaining or enhancing the benefits imparted by this group. Accordingly, analogue **29** was prepared. Through the strategic use of the pyridyl A-ring, this analogue featured increased solubility and selectivity over Na_v1.5 while maintaining high levels of microsomal stability and Na_v1.7 potency. The attachment of a *para*-nitrile to the toluic acid ring in **30** also engendered improved selectivity over Na_v1.5.

We also focused efforts on improving the Na_v1.7 potency of the alkyl-substituted C-ring amides and found that cyclopropylmethyl amides **31** and **32**, both bearing diazabicyclo[3.2.1]octane and *para*-nitrile toluic acid rings, not only possessed increased potency, low microsomal clearance, and high aqueous solubility but also significantly improved levels of selectivity over Na_v1.5 (Table 5). These compounds had particularly high levels of Na_v1.7 lipophilic efficiency and an excellent overall balance of properties. Similar explorations were undertaken with derivatives of cyclopropylethyl amide **26**. 3-Cyano-5-fluorophenyl A-ring amide **33** maintained a similar level of Na_v1.7 potency with a substantial increase in selectivity over Na_v1.5. A significant loss of potency was observed for most derivatives bearing a *para*-nitrile B-ring; however, through the use of the lipophilic 2-chloro-5-fluorophenyl amide, **34** bore only a twofold loss of potency compared to **26** while maintaining >40-fold selectivity over Na_v1.5, good aqueous solubility, and microsomal stability. Owing to their favourable properties, analogues **33** and **34** were subjected to further analysis. Both compounds exhibited low intrinsic clearance in mouse liver microsomes, particularly when compared to **14**. Oral administration of **33** and **34** in mice at 100

Table 4 SAR of piperazine sector

No.	Structure	Na _v 1.7 IWB IC ₅₀ (μM)	LE/LipE ^a	Na _v 1.5 PX IC ₅₀ (μM)	Na _v 1.5/1.7 selectivity	HLM Cl _{int} (μL min ⁻¹ mg ⁻¹)	Solubility at pH 7.4 (μM)	clog P
27		0.35	0.26/1.7	2.4	7.0×	>399	83	4.8
28		0.46	0.25/1.0	4.2	9.0×	270	35	5.3

^a LipE = -log(Na_v1.7 IWB IC₅₀) - clog P.

Table 5 Additive SAR reveals piperazine analogues with good overall balance of properties^a

No.	Structure	Na _v 1.7 IWB IC ₅₀ (μM)	LE/LipE	Na _v 1.5 PX IC ₅₀ (μM)	Na _v 1.5/1.7 selectivity	HLM Cl _{int} (μL min ⁻¹ mg ⁻¹)	Solubility at pH 7.4 (μM)	clog <i>P</i>
29		0.34	0.23/1.8	11	32×	<14	49	4.6
30		0.25	0.22/2.0	9.6	39×	<14	15	4.7
31		0.58	0.25/3.0	>42	>73×	19	322	3.3
32		0.72	0.25/2.9	42	59×	<14	326	3.3
33		0.35	0.26/3.0	14	41×	<14	405	3.5
34		0.64	0.24/2.1	27	42×	<14	154	4.1

^a LipE = -log(Na_v1.7 IWB IC₅₀) - clog *P*.**Table 6** *In vitro* and *in vivo* parameters of **33** and **34**

	33	34
Human Na _v 1.7 IWB IC ₅₀ (μM)	0.35	0.64
Mouse Na _v 1.7 IWQ ²⁸ IC ₅₀ (μM)	0.91	0.60
HLM/MLM Cl _{int} (μL min ⁻¹ mg ⁻¹)	<14/134	<14/59
Mouse plasma <i>f</i> _u	0.13	0.11
MW/clog <i>P</i> /tPSA	470/3.5/76	505/4.1/76
Mouse PK (100 mg kg ⁻¹ P. O. dose)		
<i>C</i> _{max} (μM)	12.8	29.8
<i>C</i> _{max,unbound} (μM)	1.66	3.28

mg kg⁻¹ revealed improved coverage of the mouse IWQ²⁸ IC₅₀: 1.8-fold and 5.4-fold, respectively (Table 6).

Conclusions

Through high-throughput screening efforts, we identified **13**, a novel chemotype that displayed state-dependent inhibition of Na_v1.7. Metabolic liability was mitigated through piperazinyl amide analogue **14**, which also possessed improved selectivity over Na_v1.5. In a histamine-induced pruritus assay in mice, oral dosing of piperazine amide **14** at 300 mg kg⁻¹ resulted in a significant decrease in scratching

bouts, indicative of the effectiveness of this series toward engaging Na_v1.7 in a therapeutically relevant manner. Subsequent SAR explorations of each sector of the scaffold allowed us to quickly access analogues of this compound with an emphasis on balancing Na_v1.7 potency, selectivity over Na_v1.5, and microsomal stability. Enabled by these modular SAR studies, we circumvented the need for a time- and resource-intensive matrix SAR effort and were able to rapidly access highly desirable analogues with enhanced selectivity over Na_v1.5 and a good overall balance of properties, exemplified by compounds 31–34, with 33 and 34 displaying favourable mouse PK. It is noteworthy that we were able to significantly increase selectivity over Na_v1.5 in this series while concurrently maintaining consistent Na_v1.7 LE and improving Na_v1.7 LipE. Our hope is that the unique properties of this series may be leveraged to deliver tools for use by the broader scientific community to further elucidate Na_v1.7 biology and function.

Experimental section

In vitro assays

Protocols for IWB and PX assays are provided in the ESI.†

In vivo studies

Descriptions of mouse P. O. PK studies and the histamine-induced pruritus experiment are provided in the ESI.†

General synthetic methodology

Representative experimental details for the syntheses of 14 and 34 are found below. Further experimental details and spectral data are found in the ESI.†

(R)-N-Benzyl-2-(4-(2-chloro-5-fluorobenzoyl)piperazin-1-yl)-2-phenylacetamide (14). To a DMF (1.0 L) solution of *tert*-butyl piperazine-1-carboxylate (3, 100 g, 538 mmol) was added potassium carbonate (80 g, 58 mmol) and methyl 2-bromo-2-phenylacetate (73.5 g, 320 mmol). The reaction contents were stirred at rt for 12 h. The reaction mixture was then filtered through a bed of celite, and the filtrate was concentrated *in vacuo*. The material was purified by column chromatography using 60–120 mesh silica gel, eluting with 20% EtOAc in heptane, to obtain *tert*-butyl 4-(2-methoxy-2-oxo-1-phenylethyl)piperazine-1-carboxylate (100 g, 67% yield) as a colourless oil. ¹H NMR (400 MHz, CDCl₃) δ 7.44 (d, *J* = 6.0 Hz, 2H), 7.42–7.33 (m, 3H), 4.06 (s, 1H), 3.71 (d, *J* = 1.1 Hz, 3H), 3.48 (s, 4H), 2.58–2.30 (m, 4H), 1.46 (d, *J* = 1.1 Hz, 9H). LCMS (ESI) *m/z*: 335.2 (M + H)⁺.

To a THF (125 mL)/H₂O (62.5 mL) solution of *tert*-butyl 4-(2-methoxy-2-oxo-1-phenylethyl)piperazine-1-carboxylate (25 g, 74.8 mmol, 1.0 equiv.) was added lithium hydroxide (8.9 g, 370 mmol). The reaction mixture was stirred at rt for 12 h. The reaction mixture was then concentrated *in vacuo*. The material was acidified to pH 5 using 0.5 N aq. HCl. The layers were separated, and the aqueous layer was extracted thrice with CH₂Cl₂. The organic extracts were combined, dried over

Na₂SO₄, and concentrated under reduced pressure to provide 2-(4-(*tert*-butoxycarbonyl)piperazin-1-yl)-2-phenylacetic acid (20 g, 84% yield) as a white amorphous solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 7.48–7.33 (m, 5H), 4.22 (s, 1H), 2.56–2.51 (m, 4H), 2.47–2.40 (m, 4H), 1.37 (s, 9H). LCMS (ESI) *m/z*: 321.2 (M + H)⁺.

To a CH₂Cl₂ (105 mL) solution of 2-(4-(*tert*-butoxycarbonyl)piperazin-1-yl)-2-phenylacetic acid (21 g, 66 mmol) and phenylmethanamine (9.13 g, 85.0 mmol) was added triethylamine (27.4 mL, 197 mmol) and propylphosphonic anhydride (31.3 g, 98 mmol). The reaction mixture was stirred at rt for 12 h. The reaction was diluted with CH₂Cl₂ (250 mL) and H₂O (300 mL). The layers were separated, and the organic layer was washed with brine, dried over Na₂SO₄, and concentrated *in vacuo*. The material was purified by column chromatography using 60–120 mesh silica gel, eluting with 20% EtOAc in hexane, to provide *tert*-butyl 4-(2-(benzylamino)-2-oxo-1-phenylethyl)piperazine-1-carboxylate (18 g, 67% yield) as a white amorphous solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 7.47–7.40 (m, 2H), 7.41–7.26 (m, 5H), 7.24–7.20 (m, 1H), 7.18–7.12 (m, 2H), 4.32–4.20 (m, 2H), 3.90 (d, *J* = 2.3 Hz, 1H), 3.32 (d, *J* = 8.6 Hz, 4H), 2.28 (q, *J* = 4.0, 3.2 Hz, 4H), 1.38 (d, *J* = 2.1 Hz, 9H). LCMS (ESI) *m/z*: 410.2 (M + H)⁺.

To a 1,4-dioxane (74 mL) solution of *tert*-butyl 4-(2-(benzylamino)-2-oxo-1-phenylethyl)piperazine-1-carboxylate (14.7 g, 35.9 mmol) was added hydrogen chloride (4.0 M in 1,4-dioxane, 40. mL, 180 mmol), and the reaction mixture was stirred at rt for 12 h. The reaction mixture was then concentrated *in vacuo*. The material was then stirred with Et₂O (50 mL) and filtered to afford racemic *N*-benzyl-2-phenyl-2-(piperazin-1-yl)acetamide hydrochloride as white amorphous solid. This racemate was subjected to preparatory SFC purification using a (S,S) Whelk-O column (5 μm, 5 × 15 cm), eluting with 30% MeOH containing 0.2% HNEt₂ at a flowrate of 350 mL min^{−1} with diode array detection at 225 nm to provide (R)-*N*-benzyl-2-phenyl-2-(piperazin-1-yl)acetamide (5.08 g, 16.4 mmol, 46% yield, >99% ee) as a white amorphous solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 7.69–7.54 (m, 2H), 7.47 (dd, *J* = 5.3, 2.9 Hz, 3H), 7.31–7.18 (m, 3H), 7.16–7.03 (m, 2H), 5.03 (s, 1H), 4.31 (q, *J* = 9.1, 7.2 Hz, 2H), 3.35 (q, *J* = 14.6, 12.9 Hz, 4H), 3.18 (d, *J* = 31.3 Hz, 4H). LCMS (ESI) *m/z*: 310.2 (M + H)⁺.

To a CH₂Cl₂ (5.4 mL) solution of 2-chloro-5-fluorobenzoic acid (0.283 g, 1.62 mmol) and (R)-*N*-benzyl-2-phenyl-2-(piperazin-1-yl)acetamide (0.502 g, 1.62 mmol) was added triethylamine (0.34 mL, 2.4 mmol) and propylphosphonic anhydride (50 wt% in EtOAc, 1.5 mL, 2.4 mmol). The solution was stirred at rt for 16 h. Sat. aq. NaHCO₃ was added. The mixture was stirred at rt for 30 min and partitioned. The aqueous layer was twice with CH₂Cl₂. The combined organic extracts were combined, washed with brine, dried over MgSO₄, filtered, and concentrated *in vacuo*. The material was absorbed onto a plug of silica gel and purified by chromatography through a Biotage Snap Ultra pre-packed silica gel column (25 g), eluting with a gradient of 10% to 70% EtOAc in heptane, to provide 14 (0.69 g, 1.5 mmol, 91% yield) as a white

amorphous solid. ^1H NMR (500 MHz, $\text{DMSO}-d_6$) δ 8.68 (t, J = 6.1 Hz, 1H), 7.58–7.53 (m, 1H), 7.42 (d, J = 7.0 Hz, 2H), 7.36–7.28 (m, 5H), 7.27–7.23 (m, 2H), 7.22–7.17 (m, 1H), 7.14 (d, J = 7.8 Hz, 2H), 4.33–4.21 (m, 2H), 3.95 (s, 1H), 3.73–3.56 (m, 2H), 3.17 (t, J = 4.9 Hz, 2H), 2.48–2.38 (m, 2H), 2.38–2.28 (m, 2H). ^{13}C NMR (101 MHz, $\text{DMSO}-d_6$) δ 169.98, 164.04, 161.94, 159.50, 139.38, 137.40, 137.32, 136.94, 131.42, 131.34, 128.61, 128.18, 127.76, 127.01, 126.68, 124.49, 124.46, 117.64, 117.41, 115.17, 114.93, 79.15, 73.89, 73.87, 50.43, 46.09, 41.95, 41.09, ^{19}F NMR (376 MHz, $\text{DMSO}-d_6$) δ -115.02 (s, 1F). $[\alpha]_D^{25}$ = -65.7° (c 1.35, CHCl_3). HPLC (System A) R_t 1.00 min, >95% purity. HRMS (ESI) m/z : $[\text{M} + \text{H}]^+$ calculated for $\text{C}_{26}\text{H}_{25}\text{ClFN}_3\text{O}_2$: 466.1692, found 466.1697.

(R)-2-(4-(2-chloro-5-fluorobenzoyl)piperazin-1-yl)-2-(4-cyanophenyl)-N-(2-cyclopropyl-2,2-difluoroethyl)acetamide (34). To a CH_2Cl_2 (800 mL) solution of *tert*-butyl 4-(1-(4-cyanophenyl)-2-methoxy-2-oxoethyl)piperazine-1-carboxylate (80.0 g, 223 mmol) was added trifluoroacetic acid (86.0 mL, 1.12 mol). The reaction mixture was stirred at rt for 2 h. The reaction mixture was then concentrated *in vacuo*. The material was dissolved in H_2O and washed twice with MTBE. The aqueous layer was basified with 20% NaHCO_3 (aq.) to adjust to about pH 9 and then extracted twice with 10% MeOH in CH_2Cl_2 . The organic extracts were combined, washed with brine, dried over Na_2SO_4 , filtered, and concentrated *in vacuo* to afford methyl 2-(4-cyanophenyl)-2-(piperazin-1-yl)acetate (50.0 g, 193 mmol, 87% yield) as a colorless syrup. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ = 7.98–7.78 (m, 2H), 7.66–7.56 (m, 2H), 4.45 (s, 1H), 3.64 (s, 3H), 3.56 (s, 1H), 2.90 (t, J = 4.9 Hz, 4H), 2.48–2.41 (m, 4H). LCMS (ESI) m/z : 260.1 ($\text{M} + \text{H}$) $^+$.

To a CH_2Cl_2 (300 mL) solution of methyl 2-(4-cyanophenyl)-2-(piperazin-1-yl)acetate (20.0 g, 77.0 mmol) was added 2-chloro-5-fluorobenzoic acid (8.0 g, 77 mmol). Triethylamine (21.5 mL, 154 mmol) and propylphosphonic anhydride (50 wt% in EtOAc, 49.1 g, 154 mmol) was added to the reaction mixture after cooling to 0 °C. The reaction mixture was then stirred for 16 h while slowly warming to rt. The reaction mixture was then quenched with sat. aq. NaHCO_3 solution and was extracted twice with CH_2Cl_2 . The organic extracts were combined, dried over Na_2SO_4 , filtered, and concentrated *in vacuo*. The material was purified by column chromatography through a Redi-Sep pre-packed silica gel column (120 g), eluting 0% to 50% EtOAc in hexane, to provide methyl 2-(4-(2-chloro-5-fluorobenzoyl)piperazin-1-yl)-2-(4-cyanophenyl)acetate (20.0 g, 48.1 mmol, 62% yield) as a white amorphous solid. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ = 7.96–7.78 (m, 2H), 7.68–7.50 (m, 3H), 7.36–7.22 (m, 2H), 4.46 (d, J = 2.9 Hz, 1H), 3.64 (d, J = 2.1 Hz, 3H), 3.14 (t, J = 4.8 Hz, 4H), 2.47–2.30 (m, 4H). LCMS (ESI) m/z : 416.1 ($\text{M} + \text{H}$) $^+$.

To a THF (100 mL)/MeOH (100 mL)/ H_2O (200 mL) solution of methyl 2-(4-(2-chloro-5-fluorobenzoyl)piperazin-1-yl)-2-(4-cyanophenyl)acetate (20.0 g, 48.1 mmol) was added lithium hydroxide (5.76 g, 240 mmol), and the reaction mixture was stirred at rt for 2 h. Volatiles were then removed *in vacuo*. The concentrate was cooled to 0 °C and acidified with 1.5 N HCl to about pH 6. The aqueous layer was then extracted

thrice with CH_2Cl_2 . The organic extracts were combined, washed with brine, dried over Na_2SO_4 , filtered, and concentrated *in vacuo* to afford racemic 2-(4-(2-chloro-5-fluorobenzoyl)piperazin-1-yl)-2-(4-cyanophenyl)acetic acid (15.0 g, 37.3 mmol, 78% yield) as a white amorphous solid. This racemate was subjected to preparatory SFC purification using a Chiralpak AD-H column (5 μm , 3 $\times$ 25 cm), eluting with 30% MeOH containing 0.5% HNET_2 at a flowrate of 120 mL min^{-1} with diode array detection at 230 nm to provide (R)-2-(4-(2-chloro-5-fluorobenzoyl)piperazin-1-yl)-2-(4-cyanophenyl)acetic acid (4.11 g, 96.7% ee) as a white amorphous solid. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ = 7.70 (d, J = 7.9 Hz, 2H), 7.65–7.49 (m, 3H), 7.31 (dd, J = 8.4, 6.6 Hz, 2H), 3.84–3.56 (m, 4H), 3.22–3.11 (m, 2H), 2.75 (q, J = 7.1 Hz, 1H), 2.43–2.31 (m, 1H), 2.24 (dt, J = 10.6, 5.1 Hz, 1H). LCMS (ESI) m/z : 402.1 ($\text{M} + \text{H}$) $^+$.

To a CH_2Cl_2 (0.6 mL) mixture of 2-cyclopropyl-2,2-difluoroethanamine (0.035 g, 0.25 mmol) and (R)-2-(4-(2-chloro-5-fluorobenzoyl)piperazin-1-yl)-2-(4-cyanophenyl)acetic acid (0.050 g, 0.12 mmol) was added triethylamine (0.035 mL, 0.25 mmol) and propylphosphonic anhydride (50 wt% in EtOAc, 0.16 mL, 0.25 mmol). The mixture was shaken for 16 h at rt. The mixture was filtered and purified *via* high throughput parallel purification to provide 34 (36.1 mg, 0.0715 mmol, 60% yield) as a white amorphous solid. ^1H NMR (500 MHz, $\text{DMSO}-d_6$) δ 8.64 (br s, 1H), 7.82 (br d, J = 8.30 Hz, 2H), 7.61 (br d, J = 7.92 Hz, 2H), 7.56 (br s, 1H), 7.25–7.37 (m, 2H), 4.18 (br s, 1H), 3.53–3.74 (m, 4H), 3.14–3.20 (m, 2H), 2.26–2.47 (m, 4H), 1.27 (br s, 1H), 0.39–0.54 (m, 4H). ^{13}C NMR (101 MHz, $\text{DMSO}-d_6$) δ 169.35, 164.05, 161.95, 159.50, 142.39, 137.33, 137.26, 132.16, 131.44, 131.36, 129.56, 124.48, 124.45, 122.00, 118.67, 117.68, 117.45, 115.19, 114.94, 110.60, 72.72, 50.53, 50.38, 50.01, 49.80, 46.07, 43.38, 43.08, 41.05, 13.92, 13.64, 13.37, 0.82. ^{19}F NMR (376 MHz, $\text{DMSO}-d_6$) δ = -105.61–107.76 (m, 2F), -115.01 (s, 1F). $[\alpha]_D^{25}$ = -47.3° (c 0.63, CHCl_3). HPLC (System A) R_t 1.07 min, >95% purity. HRMS (ESI) m/z : $[\text{M} + \text{H}]^+$ calculated for $\text{C}_{25}\text{H}_{24}\text{ClF}_3\text{N}_4\text{O}_2$: 505.1613, found 505.1621.

Abbreviations

BCRP	Breast cancer resistance protein
$\log P$	Calculated logarithm of the octanol/water partition coefficient
Cl_{int}	Intrinsic clearance
C_{max}	Maximum plasma concentration
f_u	Fraction unbound
HLM	Human liver microsomes
IC_{50}	Half-maximum inhibitory concentration
IWB	IonWorks Barracuda assay
LE	Ligand efficiency
LipE	Lipophilic efficiency
MLM	Mouse liver microsomes
MW	Molecular weight
P-gp	P-glycoprotein
P_{app}	Apparent permeability coefficient

PK	Pharmacokinetics
PX	PatchXpress assay
SAR	Structure–activity relationship
$t_{1/2}$	Half life
tPSA	Topological polar surface area

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