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Benzazaborinines as Novel Bioisosteric Replacements of Naphthalene: Propranolol as an Example

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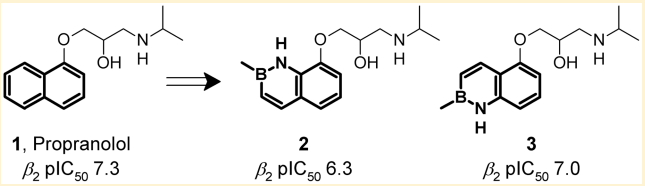
Supporting Information

ABSTRACT: Two benzazaborinine analogues of propranolol were synthesized and extensively profiled *in vitro* and *in vivo*. These analogues showed potency and physicochemical and *in vitro* ADME−tox profiles comparable to propranolol. In addition, both benzazaborinine analogues showed excellent bioavailability and brain penetration following subcutaneous administration in a pharmacokinetic study in rats. These studies unveil the potential of aromatic azaborinines as bioisosteric replacements of naphthalene in drug discovery programs.



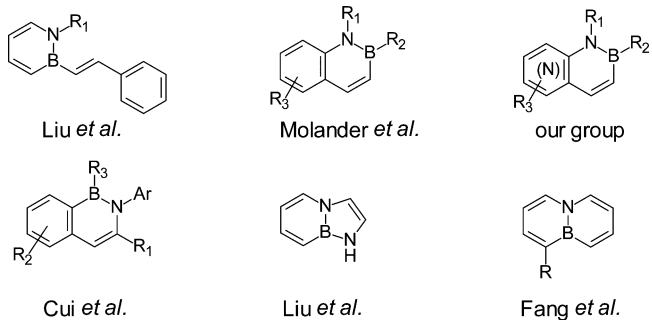
INTRODUCTION

Since the early days of drug discovery, medicinal chemists have sought to replace scaffolds and functional groups in their lead molecules with alternative groups that retain the desired biological activity of the parent compound. These so-called bioisosteric replacements have been widely exploited not only to modulate properties of drugs but also to carve out intellectual property space in highly competitive research areas.[1,2] By now, numerous reports have been published of successful isosteric replacements, with some in a very narrow biological space[3] but others across multiple target classes.[4,5] This field has now matured to the extent that databases exist of bioisosteres[6−8] and several common molecular modeling software packages can automatically generate analogues of leads with bioisosteric groups or even predict novel replacements.[9] A relevant aspect in the discovery of novel bioisosteres is the advancement of synthetic chemistry itself, and the biological evaluation of molecules containing novel chemotypes in a drug discovery context.

The bioisosteric replacement of aromatic and heretoaromatic systems has been extensively studied.[10] Although many bioisosteres of five-membered heteroaromatics bearing one or more O, S, and N atoms have been found, a limited number of bioisosteres for six-membered aromatics has been reported. Thus, isosteres have been limited to nitrogen-containing analogues of benzene.[1,11]

In this context, the recent disclosure of novel synthetic methodology toward the preparation of functionalized azaborinine aromatics has attracted our attention. We hypothesized that these boron-containing scaffolds may provide new bioisosteric replacements for mono- and bicyclic six-membered aromatics.[12,13] Representative examples of novel azaborinine scaffolds published by Liu,[14,15] Molander,[16] Fang,[17] Cui,[18] and our group[19] are shown in Chart 1.

Chart 1. Azaborinine Scaffolds Accessible through Novel Synthetic Methodology



To date, very limited data on the physicochemical characteristics and behavior of azaborinines in biological systems has been published,[20,21] which so far has limited their use by medicinal chemists in drug discovery. Here we report on the first example of the bioisosteric replacement of naphthalene by a benzazaborinine scaffold, presenting a comparative study between the well-known β-blocker drug propranolol (**1**)[22] and the benzazaborinine derivatives **2** and **3** (Chart 2).

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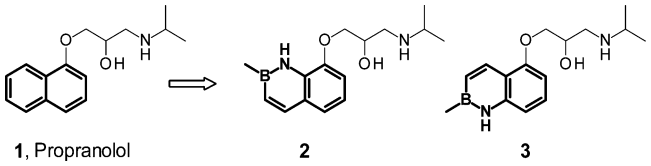
Chart 2. Benzazaborinine Analogues 2 and 3 of Propranolol (1)



CHEMISTRY

Benzazaborinines **2** and **3** were designed to be as similar as possible to propranolol, and the boron substituent was chosen to be a methyl, which we considered the best compromise between synthetic accessibility and similarity to **1**. Compounds **2** and **3** were synthesized following the methodology recently described by Molander,[16] in which benzazaborinines are formed by reaction of 2-aminostyrenes with potassium alkyltrifluoroborates in the presence of $SiCl_4$.

Thus, the synthesis of 8-alkoxy-2-methyl-1,2-dihydro-1,2-benzazaborinine (**2**) started from aldehyde **4**, which was converted to styrene **5** via Wittig olefination (Scheme 1). The nitro group in **5** was then reduced with iron in acetic acid to afford the 2-aminostyrene derivative **6**. For the cyclization of **6** to the corresponding azaborinine **7**, the conditions reported by Molander ($MeBF_3K$, $SiCl_4$, in PhMe−CPME at 60 °C for 2 h) were not successful, and only starting material could be detected. However, when the reaction temperature was lowered to 40 °C and CPME was solely used as solvent, compound **7** could be isolated in 12% yield. Cleavage of the methyl ether in **7** to phenol **8** was achieved with BBr_3. Since **8** was unstable during workup with aqueous sodium bicarbonate, the reaction was quenched with ice−water, and crude **8** was used as such after extraction with DCM. Thus, alkylation of crude **8** with epichlorohydrin and Cs_2CO_3 in DMF afforded the crude epoxide **9**, which was finally transformed into the target compound **2** by reaction with isopropylamine in low overall yield (4%, three steps).[23]

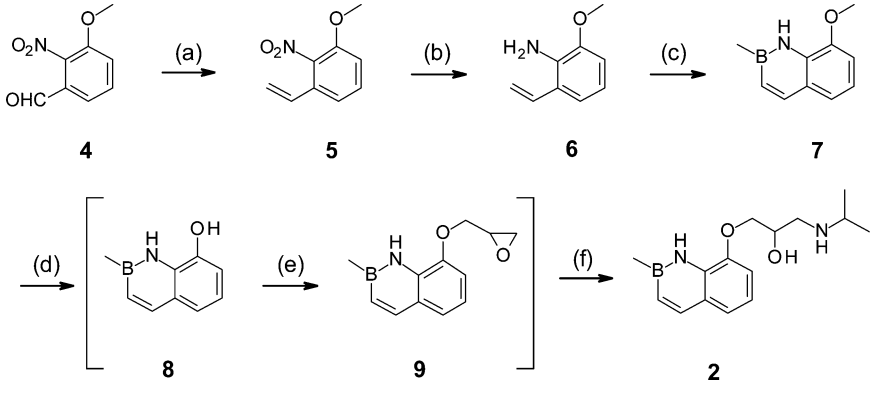
The isomeric 5-alkoxy-2-methyl-1,2-dihydro-1,2-benzazaborinine (**3**) was synthesized similarly, as outlined in Scheme 2. 2-Aminostyrene **12** was obtained from *ortho*-nitro bromobenzene **10** via sequential reduction of the nitro group with iron, followed by Suzuki cross-coupling of the resulting aniline **11** with potassium ethenyltrifluoroborate.[24] In this case, Molander's reaction conditions nicely afforded the desired methyl-2,1-borazaronaphthalene **13** in 79% yield by cyclization of the 2-aminostyrene **12** with potassium methyltrifluoroborate. Compound **13** proved to be more stable toward BBr_3 mediated O-demethylation than **8**, and thus 1,2-benzazaborinin-5-ol **14** was isolated in 58% yield. Further alkylation with epichlorohydrin and subsequent opening of the epoxide with isopropylamine provided 5-alkoxy azaborinine **3** in moderate yield.

RESULTS AND DISCUSSION

In order to confidently profile **2** and **3** across different assays, we first assessed their chemical stability using a battery of LCMS-based experiments under air atmosphere (Table 1). To our delight, **3** was completely stable up to 2 days in phosphate buffer, pH 7.4 (Table 1, entry 1), while minor degradation was observed for **2** under the same conditions. In DMSO at rt, both **2** and **3** were completely stable up to 2 days (Table 1, entry 2). In line with this finding, both **2** and **3** remained stable after 24 days of storage at rt in our compound storage system at 5 mM in DMSO solution (haystack). In 0.1 N HCl, immediate degradation was observed for both **2** and **3**, and no compound remained after 1 day at rt. We hypothesize that this degradation occurs after protonation of the azaborinine nitrogen, which breaks aromaticity of the heterocycle rendering the boron more susceptible to degradation. This may occur via an oxidative mechanism similar to that we have recently postulated for borazaroquinolines.[19] This mechanistic proposal is supported by the addition of hydrogen peroxide (Table 1, entry 4), which accelerates degradation of **2** and **3**. Much slower degradation was observed in 0.1 N NaOH (Table 1, entry 5), with still over 80% of both **2** and **3** remaining after 2 days of incubation at rt. Propranolol (**1**) showed no signs of degradation under the conditions tested.

With confidence of compound integrity during storage in DMSO and assay buffer conditions, **1**, **2**, and **3** were further profiled for both agonistic and antagonistic activity on a panel of 26 receptors to assess primary activity and selectivity. Benzazaborinine derivative **2** was found to be 10-fold less potent (**2** pIC_{50} 6.3) than **1** on the β2 adrenergic receptor; nevertheless the isomeric boron derivative **3** showed comparable activity (**3** pIC_{50} 7.0; **1** pIC_{50} 7.3) and also behaved similar to **1** on β1 and 5HT1A receptors. In addition, pIC_{50} values below 5.3 on all other receptors were found for **1** and **2** (see Supporting Information). These findings illustrate for the

Scheme 1. Preparation of Compound 2[a]



[a]Reagents and conditions: (a) Ph_3PMeBr, LHDMS, THF, 11 h, rt, 61%; (b) Fe, AcOH, EtOH, 90 °C, 6 h, 82%; (c) $MeBF_3K$, $SiCl_4$, CPME, 40 °C, 12 h, 12%; (d) BBr_3, DCM, 0 °C, 1 h; (e) epichlorohydrin, Cs_2CO_3, ACN, 60 °C, 12 h; (f) $^iPrNH_2$, ^iPrOH, 50 °C, 20 h, 4% (3 steps).

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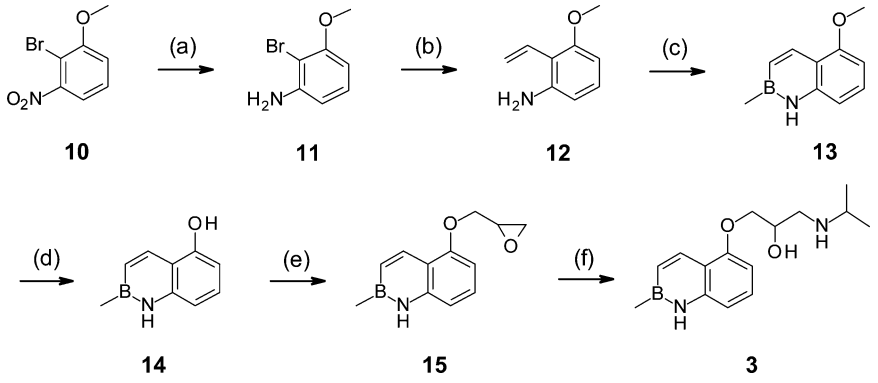
Scheme 2. Preparation of Compound 3[a]



[a]Reagents and conditions: (a) Fe, NH_4Cl, PhMe-water, 100 °C, 9 h, 98%; (b) $H_2C{=}CHBF_3K$, $Pd(OAc)_2$, SPhos, K_3PO_4, ACN−water, 95 °C, 18 h, 39%; (c) $MeBF_3K$, $SiCl_4$, PhMe−CPME, 60 °C, 2 h, 79%; (d) BBr_3, DCM, 0 °C, 1 h, 58%; (e) epichlorohydrin, Cs_2CO_3, ACN, 60 °C, 12 h, 53%; (f) $^{i}PrNH_2$, $^{i}PrOH$, 50 °C, 20 h, 73%.

Table 1. Comparison of Chemical Stability of 2 and 3 in Solution versus Propranolol (1)[a]

entry	additive	days	1 (%)[b]	2 (%)[b]	3 (%)[b]
1	buffer pH 7.4	0	100	96	96
		1	100	93	96
		2	100	91	96
2	DMSO	0	100	98	97
		1	100	96	97
		2	100	96	96
3	haystack[c]	24	100	98	97
4	0.1 N HCl	0	100	61	53
		1	100	0	0
5	0.1 N HCl + 0.5% H_2O_2	0	100	0	0
		1	100	0	*d*
6	0.1 N NaOH	0	100	95	95
		1	100	88	89
		2	100	82	83

[a]LCMS vials containing 50 μL of 10 mg/mL **1**, **2**, or **3** solution in DMSO + 400 μL of additive (HCl, NaOH, phosphate buffer, or DMSO) were analyzed directly after preparing the sample ("0 days"), after 1 day, and after 2 days at rt. [b]Compound remaining (%) in UV trace of LCMS. [c]DMSO (5 mM) solution for screening, stored at rt under air. [d]Not tested.

first time the potential of the benzazaborinine scaffold as a naphthalene bioisostere.

Computational analysis of molecular shape and electrostatic surface properties provides insight into bioisosterism, and we have investigated this for **1**, **2**, and **3**.[1] This comparison is best performed on the binding conformation. The recent boom in GPCR structures has furnished multiple AR examples. However, there is no crystal structure for propranolol binding at the β2 adrenergic receptor. Therefore, crystal structures solved in the presence of structurally similar molecules were used to model the plausible binding mode of **1** and our two azaborinine derivatives, see Supporting Information. The electrostatic surface potentials were calculated by optimizing the molecules to the closest local minima starting from their corresponding proposed binding conformation. Quantum mechanical calculations were performed at the B3LYP/6-311++G** level of theory using the Jaguar v8.8 program (Schrodinger Inc.). Figure 1 reveals that the molecules have very high shape similarity, and importantly the electrostatic surface potentials are also highly comparable. Molecules **1** and

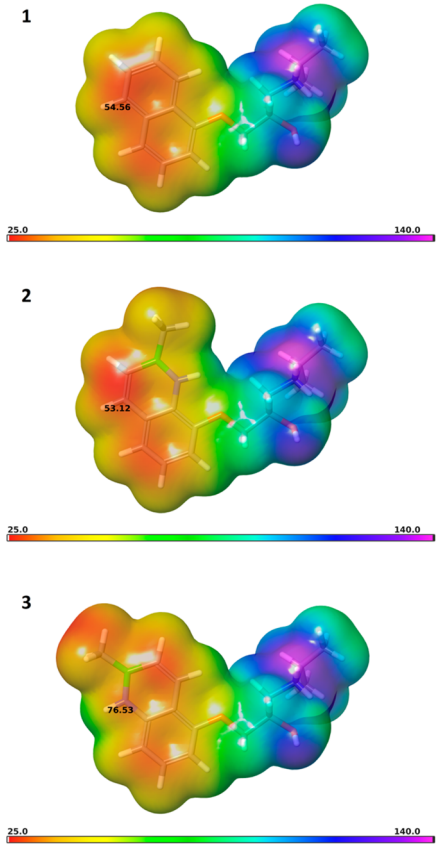


Figure 1. Electrostatic potential surface for molecules **1**, **2**, and **3**. The label shows the maximum contribution of the corresponding hydrogen atom to the positive electrostatic potential surface.

2 are most alike in all aspects, whereas molecule **3** introduces a small region of additional positive charge due to the exposed NH of the azaborinine moiety. Analysis in the context of the β2 AR structures suggests that this group in **3** is presented toward transmembrane α helix 5 and may have a small beneficial effect on its activity; see discussion in the Supporting Information. Overall, these results suggest only modest changes due to the introduction of the azaborinine compared with the naphthalene of propranolol and corroborate the observed bioisosteric activity at the β2 adrenergic receptor.

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Table 2. Physicochemical and *in Vitro* ADME Properties of 2 and 3 versus Propranolol (1)

	1	2	3
β2 pIC_{50}[a]	7.3	6.3	7.0
β1 pIC_{50}[a]	6.6	6.2	6.7
5HT1A pIC_{50}[a]	5.4	<5.3	5.7
23 other receptors pIC_{50} or pEC_{50}	all <5.3	all <5.3	all <5.3
log *D* (pH 7.4)[b]	1.62	2.31	1.81
S + log *P*[c]	2.96	2.07	2.14
S + log *D*[c]	1.17	0.31	0.36
kin. solubility (μmol/L)	764	699	647
CL_{int} h (μL/min/mg)[d]	28.8	8.7	11.0
CL_{int} m (μL/min/mg)[d]	61	>346	175
CL_{int} r (μL/min/mg)[d]	>346	289	>346
CYP (pIC_{50})	3A4 < 5, 2D6 5.3, 1A2 < 5, 2C19 < 5, 2C8 < 5, 2C9 < 5	3A4 < 5, 2D6 6.4, 1A2 5.5, 2C19 < 5, 2C8 < 5, 2C9 < 5	3A4 < 5, 2D6 5.6, 1A2 < 5, 2C19 < 5, 2C8 < 5, 2C9 < 5
P_{app} A→B (nm/s)[e]	35	13	23
B→A/A→B ratio[e]	1.1	2.1	1.7
f_u, brain (r, %)	2.8	0.55	1.9
f_u, plasma (h, %)	42.1	17.9	36.7
f_u, plasma (m, %)	21.2	7.4	21.0
f_u, plasma (r, %)	23.9	9.6	29.0

[a]cAMP quantification in hβ2-CHO cells. [b]Determined chromatographically.[33] [c]Calculated with ADMET Predictor 6.[25] [d]*In vitro* clearance determined by incubation of with human (h), mouse (m), or rat (r) liver microsomes, expressed per milligram of protein. [e]MDCK-MDR1 cell line.

In order to further investigate the utility of benzazaborinines **2** and **3** for medicinal chemistry projects, these were profiled along with **1** in a series of *in vitro* physicochemical and ADME assays. The results obtained are summarized in Table 2. Experimental log *D* values at pH 7.4 indicate a slight increase in lipophilicity for compound **3** compared with **1** (log *D* 1.81 vs 1.62, respectively), whereas isomer **2** was found to be significantly more lipophilic (log *D* 2.31). Interestingly the opposite trend is observed for the *S* + log *P* and *S* + log *D* values calculated with ADMET Predictor (version 6),[25] which seem to overestimate the polarity of the azaborinines. Concomitantly, kinetic solubility at pH 7.4 for **2** (699 μM) and **3** (647 μM) was decreased compared with **1** (764 μM) but still in a comparable range. *In vitro* intrinsic clearance values for **2** and **3** following incubation of 1 μM compound with hLM showed improved metabolic stability versus **1**: 8.7 and 11.0 μL/[min/(mg of protein)] for **2** and **3**, versus 28.8 μL/[min/(mg of protein)] for **1**. In mLM and rLM, all three compounds were highly unstable, with **1** being relatively more stable compared with **2** and **3** in mLM. Another interesting finding was the CYP450 inhibition profile of **1**, **2**, and **3**. Boron derivative **3** showed a similar inhibition profile (CYP2D6 pIC_{50} 5.6) to propranolol **1**, a known monozymic CYP2D6 inhibitor (pIC_{50} 5.3). Isomer **2** was found to be a more potent CYP2D6 inhibitor (pIC_{50} 6.4) showing additional inhibition of the 1A2 isoform with pIC_{50} 5.5. Permeability of **1**−**3** was measured in a MDR/MDCK cell line from the apical-to-basal (A→B) and the basal-to-apical (B→A) compartments. All three compounds showed good permeability and no significant efflux. In rat brain homogenate, which is expected to correlate well with other species,[26] **1** had an unbound fraction (f_u) of 2.8%. Compounds **2** and **3** showed a lower f_u in brain of 0.55% and 1.9%, respectively, which nicely correlates to their above-mentioned log *D* values. Unbound fractions versus human, mouse, and rat plasma proteins were also measured for **1**−**3**. Again, **3** had the most comparable value to **1** with very high plasma free fractions in all three species (h 36.7%, m 21.0%, r 29.0%), whereas values for **2** were significantly lower (h 17.9%, m 7.4%, r 9.6%). With the results from this *in vitro* evaluation, we concluded that **3** is the most comparable benzazaborinine isomer to **1** in terms of pharmacological profile and physicochemical and ADME properties.

One of our initial concerns for the use of azaborinines in medicinal chemistry programs was that, similar to furan, for example,[27] the reduced aromaticity of the ring system could lead to the formation of reactive metabolites via metabolic activation. Therefore, glutathione (GSH) and cyanide conjugation following enzymatic activation studies were conducted for **1**, **2**, and **3** in hLMs fortified with isotopically labeled GSH and KCN. Samples were analyzed by UPLC/HRMS and checked for the presence of GSH and CN trapped metabolites with the result that neither GSH conjugates nor CN adducts were observed for the boron derivatives **2** and **3** or propranolol **1**. The three compounds were also subjected to high content cytotoxicity screening (HCCS) in a human hepatocarcinoma cell line (HepG2 cells).[28,29] EC_{20} values were determined for different observations using multiplex end point analysis (MIAS-2 apparatus). The cell health indicators examined were nuclear count, nuclear size, mitochondrial membrane potential (both its intensity and number of cells), mitochondrial area, and plasma membrane permeability (see Supporting Information for full data set). The lowest concentration at which any of these cellular changes occur is defined as the lowest toxic concentration (LTC). Based on an internal analysis of 99 hepatotoxic and non-hepatotoxic compounds in the HCCS, the LTC for hepatotoxicity was set at 30 μM. The results obtained for **1**−**3** are summarized in Table 3. As expected the marketed drug propranolol (**1**) was completely devoid of cytotoxicity (LTC > 100 μM), whereas **2** was found

Table 3. Cytoxicity and Mutagenicity Profiling of 1, 2, and 3

	1	2	3
cytotoxicity (LTC, EC_{20}, μM)	>100	18.7	59.0
Ames II positive	no	no	no

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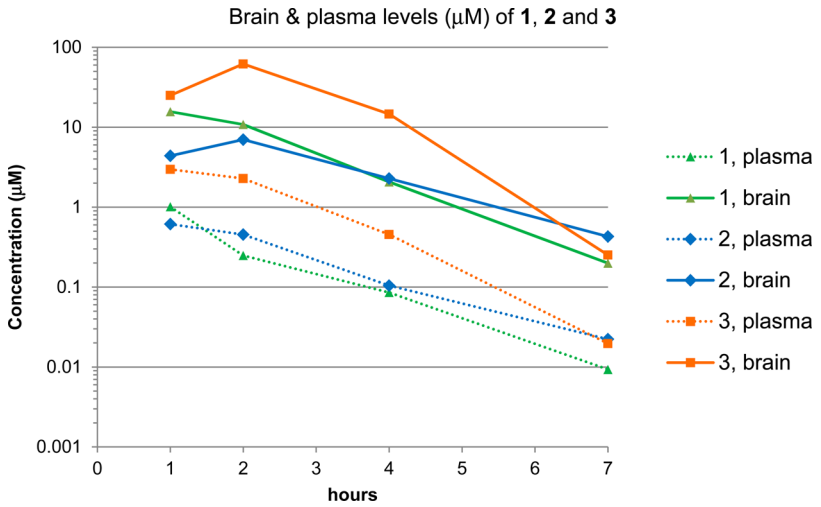

Figure 2. Pharmacokinetic profile of compounds **2** and **3** versus propranolol (**1**). Compounds were formulated with 20% HPβCD(aq) and dosed at 5 mg/kg sc to male Swiss mice (n = 3/time point).

to have a risk for hepatotoxicity (LTC 18.7 μM). Interestingly compound **3** had a higher LTC value of 59.0 μM and was not predicted to be hepatotoxic based on our internal criteria (LTC > 30 μM). All three compounds were also negative in the Ames II mutagenicity assay (Table 3).

Finally, azaborinines **2** and **3** were compared with **1** in a pharmacokinetic study in the mouse.[30] Since all three compounds showed high intrinsic clearance (CL_{int}) in mLM (Table 2) but more importantly **2** and **3** showed instability in acidic media (Table 1), dosage was given sc rather than po. The degradation at low pH was also observed when formulating **2** and **3** with HCl or tartaric acid. Both compounds could however be solubilized at 0.5 mg/mL at neutral pH with 20% (w/v) HPβCD(aq) and were dosed at 5 mg/kg in mice next to propranolol **1** (HCl salt), which for comparison was also formulated with 20% (w/v) HPβCD(aq). The data obtained are summarized in Figure 2; a detailed PK report for **1**–**3** is provided in Table S3 of the SI. Compounds **2** and **3** were both bioavailable following sc administration with C_{max} values in brain of 7 (**2**) and 62 μM (**3**), respectively, compared with 16 μM for **1**. In addition benzazaborinines **2** and **3** readily crossed the blood–brain barrier with a brain/plasma AUC ratio (K_p) of 13 and 19, respectively, compared with a K_p value of 23 for **1**. The unbound brain/unbound plasma AUC ratio ($K_{p,uu}$) was determined from the total brain and plasma AUCs and corrected by the *in vitro* free fraction determined in brain homogenate and plasma. For compounds **2** and **3** $K_{p,uu}$ was estimated to be 0.8 and 1.3, respectively, which would indicate negligible efflux at the blood–brain barrier (BBB), this being consistent with the results obtained in the *in vitro* P-gp assay in Table 2. Compound **1** had an estimated $K_{p,uu}$ of 2.7, which may suggest active uptake at the BBB and is consistent with what has been reported in the literature.[31]

CONCLUSION

In summary, we have prepared two derivatives of propranolol in which the naphthalene scaffold was replaced by a 2-methyl-1,2-dihydro-1,2-benzazaborinine. Both synthesized analogues **2** and **3** were chemically stable at neutral pH and were broadly profiled. Activity profiling across a panel of 26 receptors demonstrated comparable inhibitory potency on the β2 receptor and a similar selectivity profile for propranolol and the benzazaborinine isomer **3**. Shape and electrostatic properties were similar between the naphthyl and azaborinine analogues. In addition, **3** had a very comparable *in vitro* ADME–tox profile to **1** and interestingly showed improved metabolic stability in human liver microsomes. Moreover both **2** and **3** were bioavailable via subcutaneous administration in the mouse and readily crossed the blood–brain barrier. One limitation we have observed for 1,2-benzazaborinines is their acid sensitivity, which poses a challenge for the development of oral drugs containing 1,2-benzazaborinines. However, technologies exist like enteric coated capsules or coformulation with proton pump inhibitors, which should allow the development of 1,2-benzazaborinines as oral drugs.[32] Overall our data support the potential of 1,2-benzazaborinines as bioisosteric replacements for naphthalene in drug discovery programs, including those targeting CNS indications.

EXPERIMENTAL SECTION

log *D*. The log D of **1**–**3** was determined chromatographically at Sirius Analytical Ltd.[33]

Kinetic Solubility. A 50 mM DMSO solution of **1**–**3** was diluted to 1 mM with phosphate buffer at pH 7.4 and shaken for 4 h at room temperature. Insoluble material was separated by centrifugation at 3700 rpm for 10 min, and the concentration of drug in the supernatant was measured by UPLC/UV using a three point reference calibration using stock solutions of **1**–**3** in phosphate buffer at pH 7.4.

Metabolic Stability in Liver Microsomes (CL_{int}). To test for metabolic stability, compounds (1 μM) were incubated at 37 °C with mouse, rat, and human liver microsomes at a protein concentration of 0.5 mg of protein/mL of microsomal protein, 1 mM NADPH, 1 mM $MgCl_2$, and 0.1 M phosphate buffer, pH 7.4. DMSO stock solutions (5 mM) of each compound were diluted with acetonitrile/water (1:1) to provide a working stock solution at 0.1 mM. The total incubation volume was 0.5 mL with a final total solvent content of 0.01% (v/v) DMSO and 0.5% (v/v) acetonitrile. The reaction was initiated by the addition of 100 μL of prewarmed NADPH solution. Sequential aliquots of the incubation mixture were removed at 0, 5, 10, 20, 40, and 60 min and quenched with 200 μL of acetonitrile. Each sample was centrifuged and analyzed using a specific HPLC-MS/MS technique and the percentage of test compound remaining was used to calculate the half-life and subsequent intrinsic clearance (CL_{int}).

CYP450 Inhibition. The potential to reversibly inhibit the major human P450 isoforms (CYP 1A2, 2C8, 2C9, 2C19, 2D6, and 3A4 (midazolam probe)) was determined using a cocktail of specific probe substrates that are known to be selectively metabolized to defined

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metabolites by the isoforms under investigation. Probe substrates for the individual CYP isoforms were prepared separately in assay buffer (phenacetin, tolbutamide, *S*-mephenytoin dextromethorphan) or water (amodiaquine, midazolam). These were combined at the appropriate concentration with human liver microsomes and preincubated for approximately 10 min. The reaction was initiated by the addition of NADPH and incubated for a further 10 min in the presence of NADPH after which time the reaction was quenched. Each sample was injected onto a LC/MS system using a method selective for the simultaneous measurement of all seven probe substrate metabolites (and associated deuterated internal standards). The percentage activity data were plotted against concentration) to determine the IC_{50} values against each P450 isoform.

Plasma Protein Binding. The free fraction in mouse, rat, and human plasma was determined by rapid equilibrium dialysis (RED device, Thermo Fisher Scientific, Geel, Belgium). The RED device consists of a 48 well plate containing disposable inserts bisected by a semipermeable membrane creating two chambers. A 300 μL aliquot of plasma containing test compound at 5 μM was placed one side and 500 μL of phosphate buffered saline (PBS) the other. The plate was sealed and incubated at approximately 37 °C for 4.5 h. After 4.5 h, samples were removed from both the plasma and buffer compartment and analyzed for test compound using a specific LC-MS/MS method to estimate free and bound concentrations.

Nonspecific Binding to Brain Tissue. The *in vitro* nonspecific binding of test compounds to rat brain homogenate was determined using the RED device (see above). Each test compound was diluted with rat brain homogenate, prepared following a 1:10 dilution with PBS, to achieve a final concentration of 5 μM. The plate was incubated at approximately 37 °C for 5 h. After 5 h, samples were removed from both the brain homogenate and buffer compartment and analyzed for test compound using a specific LC-MS/MS method to estimate free and bound concentrations.

***In Vitro* Permeability and P-gp Efflux.** The *in vitro* permeability and potential to be transported by P-glycoprotein (P-gp) was determined using an MDCK cell line transfected with human MDR1 (P-glycoprotein). Each test compound (5 μM) was added to either the apical (A) or basolateral (B) side of a confluent monolayer of MDCK-MDR1 cells, and permeability in the A→B and B→A direction was measured by monitoring the appearance of the test compound on the opposite side of the membrane using a specific LC-MS/MS method. Efflux ratios (B→A/A→B) were calculated to determine whether the test compound was subject to efflux by P-gp.

Glutathione/Cyanide Trapping Experiments. The ability to form reactive intermediates was investigated following incubation of test compound (40 μM) for 1 h at 37 °C with human liver microsomes (1 mg/mL protein) fortified with glutathione (GSH) or cyanide (CN). After 1 h, the reaction was stopped by the addition of 1 mL of methanol and centrifuged. A specific LC-MS/MS method was used to identify any GSH or CN adducts based on MS response.

Chemistry. All final compounds were characterized by ^{1}H NMR and LC/MS. Purity of final compounds was ≥97% according to LC/MS with UV (diode array) detection. ^{1}H nuclear magnetic resonance spectra were recorded on a Bruker 300 MHz spectrometer. For the ^{1}H spectra, all chemical shifts are reported in parts per million (δ) units and are relative to the residual signal at 7.26 and 2.50 ppm for $CDCl_3$ and DMSO, respectively. All the LC/MS analyses were performed using an Agilent G1956A LC/MS quadrupole coupled to an Agilent 1100 series liquid chromatography (LC) system consisting of a binary pump with degasser, autosampler, thermostated column compartment, and diode array detector. The mass spectrometer (MS) was operated with an atmospheric pressure electrospray ionization (API-ES) source in positive ion mode. The capillary voltage was set to 3000 V and the fragmentor voltage to 70 V, and the quadrupole temperature was maintained at 100 °C. The drying gas flow and temperature values were 12.0 L/min and 350 °C, respectively. Nitrogen was used as the nebulizer gas at a pressure of 35 psi. Data acquisition was performed with Agilent Chemstation software. Analyses were carried out on a YMC pack ODS-AQ C18 column (50 mm long × 4.6 mm ID; 3 μm particle size) at 35 °C, with a flow rate of 2.6 mL/min. A gradient elution was performed from 95% (water + 0.1% formic acid)/5% acetonitrile to 5% (water + 0.1% formic acid)/95% acetonitrile in 4.8 min; the resulting composition was held for 1.0 min; from 5% (water + 0.1% formic acid)/95% acetonitrile to 95% (water + 0.1% formic acid)/5% acetonitrile in 0.2 min. The standard injection volume was 2 μL. Acquisition ranges were set to 190−400 nm for the UV-PDA detector and 100−1400 m/z for the MS detector.

*1-Methoxy-2-nitro-3-vinylbenzene (**5**).* A 100 mL, two-necked, round-bottom flask was equipped with a magnetic stirring bar was charged with methyltriphenylphosphonium bromide (7.73 g, 21.64 mmol) and dry THF (50 mL) under nitrogen. The solution was cooled to 0 °C, and lithium hexamethyldisilazide (LHDMS; 21.64 mL, 1 M in THF, 21.64 mmol) was added dropwise over 10 min. The reaction mixture was then stirred for 1 h at 0 °C. To this solution 3-methoxy-2-nitrobenzaldehyde (2.94 g, 16.27 mmol) in dry THF (15 mL) was added dropwise, and the mixture was stirred at rt for 11 h. The reaction was quenched by addition of a saturated solution of NH_4Cl (75 mL). The reaction mixture was extracted with EtOAc (120 mL). The organic layer was washed with brine, dried over $MgSO_4$, and filtered. The volatiles were evaporated *in vacuo*, and the residue thus obtained was purified by flash column chromatography (silica, EtOAc in heptane 0/100 to 40/60). The desired fractions were collected and concentrated *in vacuo* to give **5** (1.78 g, 61%) as colorless needles. Analytical data were in concordance with literature.[34]

*2-Methoxy-6-vinylaniline (**6**).* A mixture of 1-methoxy-2-nitro-3-vinylbenzene (**5**; 2.10 g, 11.72 mmol), powdered iron (2.61 g, 46.88 mmol), and acetic acid (13.43 mL, 234.41 mmol) in ethanol (85 mL) was heated at 90 °C for 6 h. After cooling to rt, the mixture was filtered over a Celite pad. The filtrate was diluted with water and EtOAc. The organic layer was separated and washed with water and brine, dried over $MgSO_4$, filtered, and concentrated to dryness under reduced pressure. The crude product was purified by flash column chromatography (silica; EtOAc in heptane 0/100 to 10/90). The desired fractions were collected and concentrated *in vacuo* to give **6** (1.45 g, 82% yield) as an orange oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.95 (dd, J = 5.6, 3.4 Hz, 1H), 6.79 (dd, J = 17.5, 11.2 Hz, 1H), 6.74−6.68 (m, 2H), 5.64 (dd, J = 17.4, 1.3 Hz, 1H), 5.31 (dd, J = 11.1, 1.3 Hz, 1H), 3.98 (br s, 2H), 3.86 (s, 3H). LC/MS (m/z): 97% pure, R_t = 1.903 min, $[M + H]^+$ 150.

*8-Methoxy-2-methyl-1,2-dihydrobenzo[e][1,2]azaborinine (**7**).* To an oven-dried vial equipped with a stirring bar was added potassium methyltrifluoroborate (0.73 g, 6.03 mmol). The vial was sealed with a Teflon septum, evacuated under vacuum, and purged with nitrogen three times. Dry CPME (40 mL) was added, followed by 2-methoxy-6-vinyl-phenylamine (**6**; 0.90 g, 6.03 mmol). This mixture was degassed with nitrogen for 5 min. Then, $SiCl_4$ (0.69 mL, 6.03 mmol) and triethylamine (1.25 mL, 9.04 mmol) were added at rt, and the mixture was heated at 40 °C for 14 h, after which it was cooled to rt. Saturated $NaHCO_3$ solution was added, and the mixture was extracted with EtOAc. Organic layer was separated, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude product was purified by flash column chromatography (silica; EtOAc in heptane 0/100 to 10/90). The desired fractions were collected and concentrated *in vacuo* to afford **7** (0.12 g, 12%) as colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.37 (s, 1H), 7.91 (d, J = 11.5 Hz, 1H), 7.20 (d, J = 7.9 Hz, 1H), 7.04 (t, J = 7.9 Hz, 1H), 6.90 (d, J = 7.8 Hz, 1H), 6.83 (dd, J = 11.5, 1.7 Hz, 1H), 4.00 (s, 3H), 0.77 (s, 3H). LC/MS (m/z): 99% pure, R_t = 4.274 min, $[M + H]^+$ 174.

*1-Isopropylamino-3-((2-methyl-1,2-dihydro-benzo[e][1,2]-azaborinin-8-yl)oxy)-propan-2-ol (**2**).* To a solution of 8-methoxy-2-methyl-1,2-dihydrobenzo[*e*][1,2]azaborinine (**7**; 0.20 g, 1.15 mmol) in dry DCM (10 mL) cooled with acetone/ice bath was added dropwise boron tribromide (0.43 mL, 4.62 mmol). The reaction solution was stirred at 0 °C for 1 h. The mixture was poured into ice−water, and DCM/MeOH (10/1) was added. The organic layer was separated, dried over $MgSO_4$, filtered over a silica gel pad, and concentrated *in vacuo*. The crude product **8** (0.130 g, 54%) was used in the next reaction step without further purification. To a solution of crude 2-methyl-1,2-dihydrobenzo[*e*][1,2]azaborinin-8-ol (**8**; 0.13 g, 0.81 mmol) in acetonitrile (10 mL), Cs_2CO_3 (0.53 g, 1.63 mmol) and

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epichlorohydrin (0.08 mL, 1.63 mmol) were added under nitrogen atmosphere. The reaction mixture was stirred at 60 °C for 14 h, then cooled to rt and diluted with water and EtOAc. The organic layer was separated, dried over $MgSO_4$, and filtered over a silica gel pad, and the solvents were evaporated *in vacuo*. The crude product **9** (31 mg, 17%) was used without further purification in the next reaction step. A solution of 2-methyl-8-(oxiran-2-ylmethoxy)-1,2-dihydrobenzo[*e*]-[1,2]azaborinine (**9**; 0.03 g, 0.14 mmol) and isopropylamine (0.04 mL, 0.432 mmol) in 2-propanol (1 mL) was stirred in a closed vessel at 50 °C for 20 h. After cooling to rt, the volatiles were evaporated *in vacuo*, and the residue thus obtained was purified by flash column chromatography (silica; DCM/MeOH 100/0 to 90/10). The desired fractions were collected and concentrated *in vacuo* to yield a sticky solid, which was triturated with *n*-pentane−Et_2O to afford **2** (16 mg, 4% yield over three steps) as a beige solid. Mp: 99.5 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.48 (s, 1H), 7.89 (d, J = 11.5 Hz, 1H), 7.21 (d, J = 7.8 Hz, 1H), 7.00 (t, J = 7.9 Hz, 1H), 6.89 (d, J = 7.8 Hz, 1H), 6.82 (d, J = 11.5 Hz, 1H), 4.36−4.23 (m, 1H), 4.18−4.05 (m, 2H), 3.11−2.84 (m, 3H), 1.25−1.15 (m, 6H), 0.78 (s, 3H). LC/MS (m/z): 99% pure, R_t = 2.320 min, $[M + H]^+$ 275. HRMS: calcd for $C_{16}H_{24}BN_2O_2$ $[M + H]^+$ 275.1925, found 275.1935.

*2-Bromo-3-methoxyaniline (**11**).* A mixture of 2-bromo-3-nitro-anisole (5 g, 24.54 mmol), ammonium chloride (4.61 g, 86.19 mmol), and powdered iron (4.81 g, 86.19 mmol) in toluene (85 mL) and water (50 mL) was vigorously stirred at 100 °C for 6 h. TLC (EtOAc in heptane 10:90 v/v) showed complete conversion. The reaction mixture was cooled to rt and filtered over a Celite pad. The filtrate was diluted with EtOAc (100 mL) and water. The organic layer was dried over $MgSO_4$, filtered, and concentrated to dryness under reduced pressure. The crude product was purified by flash column chromatography (silica, 80 g; EtOAc in heptane 0/1 to 1/9). The desired fractions were collected and concentrated *in vacuo* to give **11** (4.3 g, 98% yield) as an orange oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.05 (t, J = 8.1 Hz, 1H), 6.42 (d, J = 8.0 Hz, 1H), 6.31 (d, J = 8.1 Hz, 1H), 4.15 (s, 2H), 3.87 (s, 3H). LC/MS (m/z): 99% pure, R_t = 2.463 min, $[M + H]^+$ 201, 203.

*3-Methoxy-2-vinylaniline (**12**).* A vessel equipped with a magnetic stirring bar was charged with potassium vinyltrifluoroborate (3.71 g, 27.76 mmol), 2-bromo-3-methoxyaniline (**11**; 2.8 g, 13.85 mmol), $Pd(OAc)_2$ (0.093 g, 0.415 mmol, 3 mol %), SPhos (0.34 g, 0.83 mmol, 6 mol %), acetonitrile (30 mL), and water (20 mL). The mixture was purged with nitrogen for 5 min, and the vessel was sealed, after which it was heated at 95 °C for 10 h. The mixture was diluted with water and extracted with EtOAc. The organic layer was separated, dried ($MgSO_4$) and filtered, and the solvents were evaporated *in vacuo*. The crude product was purified by flash column chromatography (silica; EtOAc in heptane 0/100 to 10/90). The desired fractions were collected and concentrated *in vacuo* to afford **12** (0.8 g, 39% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.01 (t, J = 8.1 Hz, 1H), 6.76 (dd, J = 18.2, 11.7 Hz, 1H), 6.36 (d, J = 8.1 Hz, 1H), 6.31 (d, J = 8.2 Hz, 1H), 5.63 (dd, J = 18.2, 2.0 Hz, 1H), 5.55 (dd, J = 11.8, 2.0 Hz, 1H), 3.97 (br s, 2H), 3.80 (s, 3H). LC/MS (m/z): 95% pure, R_t = 1.693 min, $[M + H]^+$ 150.

*5-Methoxy-2-methyl-1,2-dihydro-benzo[e][1,2]azaborinine (**13**).* To an oven-dried closed vial equipped with a stirring bar was added potassium methyltrifluoroborate (0.98 g, 8.04 mmol). The vial was sealed with a Teflon septum, evacuated under vacuum, and purged with nitrogen three times. Dry CPME (20 mL) and dry toluene (20 mL) were added, followed by 3-methoxy-2-vinyl-phenylamine (**12**; 1.20 g, 8.04 mmol). This mixture was degassed with nitrogen for 5 min. Then, silicon tetrachloride (0.92 mL, 8.04 mmol) and triethylamine (1.67 mL, 12.06 mmol) were added at rt. The resulting mixture was heated at 60 °C for 1.5 h and then cooled to rt. Saturated $NaHCO_3$ solution was added, and the mixture was extracted with EtOAc. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude product was purified by flash column chromatography (silica; EtOAc in heptane 0/100 to 10/90). The desired fractions were collected and concentrated *in vacuo* to give **13** (1.10 g, 79% yield) as colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.38 (d, J = 11.8 Hz, 1H), 7.63 (br s, 1H), 7.30 (d, J = 8.1 Hz, 1H), 6.80 (d, J = 8.2 Hz, 1H), 6.75 (dd, J = 11.9, 1.5 Hz, 1H), 6.58 (d, J = 8.0 Hz, 1H), 3.94 (s, 3H), 0.73 (s, 3H). LC/MS (m/z): 99% pure, R_t = 3.884 min, $[M + H]^+$ 174.

*2-Methyl-1,2-dihydro-benzo[e][1,2]azaborinin-5-ol (**14**).* To a solution of 5-methoxy-2-methyl-1,2-dihydro-benzo[*e*][1,2]azaborinine **13** (0.50 g, 2.89 mmol) in dry DCM (10 mL) cooled with acetone/ice bath was added dropwise boron tribromide (0.54 mL, 5.78 mmol) under nitrogen. The reaction solution was stirred at 0 °C for 1 h. The mixture was poured into a mixture of ice and a saturated $NaHCO_3$ solution, and the product was extracted with EtOAc (2×). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue thus obtained was purified by flash column chromatography (silica; EtOAc in heptane 0/100 to 60/40). The desired fractions were collected and concentrated *in vacuo* to afford **14** (0.27 mg, 58% yield) as a white solid. Mp: 101.2 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.29 (d, J = 11.7 Hz, 1H), 7.61 (br s, 1H), 7.19 (t, J = 8.0 Hz, 1H), 6.85−6.72 (m, 2H), 6.51 (d, J = 7.8 Hz, 1H), 5.06 (s, 1H), 0.74 (s, 3H). LC/MS (m/z): 99% pure, R_t = 3.070 min, $[M + H]^+$ 160.

*2-Methyl-5-oxiran-2-ylmethoxy-1,2-dihydro-benzo[e][1,2]-azaborinine (**15**).* To a solution of 2-methyl-1,2-dihydro-benzo[*e*]-[1,2]azaborinin-5-ol (**14**, 0.27 g, 1.69 mmol) in acetonitrile (15 mL), Cs_2CO_3 (1.10 g, 3.39 mmol) and epichlorohydrin (0.16 mL, 1.86 mmol) were added under nitrogen atmosphere. The reaction mixture was stirred at 60 °C for 14 h. After cooling to rt, $NaHCO_3$ and EtOAc were added. The organic layer was separated, dried ($MgSO_4$), and filtered, and the solvents were evaporated *in vacuo*. The crude product was purified by flash column chromatography (silica; EtOAc in heptane 0/100 to 30/70). The desired fractions were collected and concentrated *in vacuo* to yield **15** (0.20 g, 54% yield) as a sticky solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.41 (d, J = 11.8 Hz, 1H), 7.64 (br s, 1H), 7.33−7.19 (m, 1H), 6.83 (d, J = 8.2 Hz, 1H), 6.77 (d, J = 11.8 Hz, 1H), 6.57 (d, J = 8.0 Hz, 1H), 4.32 (dd, J = 11.0, 3.1 Hz, 1H), 4.10 (dd, J = 11.0, 5.5 Hz, 1H), 3.51−3.37 (m, 1H), 2.95 (t, J = 4.5 Hz, 1H), 2.82 (dd, J = 4.7, 2.6 Hz, 1H), 0.73 (s, 3H). LC/MS (m/z): 98% pure, R_t = 3.563 min, $[M + H]^+$ 216.

*1-Isopropylamino-3-((2-methyl-1,2-dihydro-benzo[e][1,2]-azaborinin-5-yl)oxy)-propan-2-ol (**3**).* A solution of 2-methyl-5-oxiranylmethoxy-1,2-dihydro-benzo[*e*][1,2]azaborinine (**15**; 0.20 g, 0.93 mmol) and isopropylamine (0.15 mL, 1.86 mmol) in 2-propanol (3 mL) was stirred in a closed vessel at 50 °C for 20 h. The solvent was evaporated under reduced pressure. The crude product was purified by flash column chromatography (silica; DCM/MeOH 100/0 to 90/10). The desired fractions were collected and concentrated *in vacuo* to yield a sticky solid, which was triturated with *n*-pentane−Et_2O to afford **3** (187 mg, 73% yield) as a white solid. Mp: 131.3 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.36 (d, J = 11.8 Hz, 1H), 7.64 (br s, 1H), 7.31−7.21 (m, 1H), 6.82 (d, J = 8.2 Hz, 1H), 6.75 (d, J = 11.8 Hz, 1H), 6.59 (d, J = 8.0 Hz, 1H), 4.22−3.97 (m, 3H), 3.06−2.92 (m, 1H), 2.93−2.73 (m, 2H), 1.11 (d, J = 6.2 Hz, 6H), 0.73 (s, 3H). LC/MS (m/z): 99% pure, R_t = 1.972 min, $[M + H]^+$ 275. HRMS: calcd for $C_{16}H_{24}BN_2O_2$ $[M + H]^+$ 275.1925, found 275.1933.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jmedchem.5b01088.

Modeling of the binding mode of propranolol, **2**, and **3** at the β2 adrenergic receptor, detailed PK data, 26 receptor panel screening data, and cytotoxicity data for **1**−**3** (PDF)

Molecular strings for compounds (XLS)

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

AR, adrenergic receptor; AUC_{0-inf}, area under the curve until infinite time; AUC_{0-last}, area under the curve until the last time point measured; BBB, blood−brain barrier; cAMP, 3′−5′-cyclic adenosine monophosphate; CHO cells, Chinese hamster ovary cells; CL_{int}, intrinsic clearance; C_{max}, maximum concentration; CPME, cyclopentyl methyl ether; CYP, cytochrome P; f_u brain, unbound fraction in rat brain homogenate; f_u plasma, unbound fraction in plasma; GSH, glutathione; HCCS, high content cytotoxicity screening; HPβCD, (2-hydroxypropyl)-β-cyclodextrin [128446-35-5]; hLM, human liver microsomes; K_p, brain-to-plasma ratio; $K_{p,uu}$, unbound brain to unbound plasma AUC ratio; LTC, lowest toxic concentration; MDCK, Madin−Darby canine kidney cells used for membrane permeability screening; MDR1, multidrug resistance gene 1 (codes for P-gp); mLM, mouse liver microsomes; NADPH, nicotinamide adenine dinucleotide phosphate; P_{app}, apparent permeability; PBS, phosphate buffered saline; rLM, rat liver microsomes; sc, subcutaneous; SPhos, 2-dicyclohexylphosphino-2′,6′-dimethoxybiphenyl

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