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# Serendipity in drug-discovery: A new series of 2-(benzyloxy)benzamides as TRPM8 antagonists

Alan Brown [a,*], David Ellis [a], David A. Favor [b,†], Tony Kirkup [c,†], Wolfgang Klute [d,†], Malcolm MacKenny [e,†], Gordon McMurray [f], Adam Stennett [g,†]

[a] Worldwide Medicinal Chemistry, Pfizer Neusentis, Granta Park, Great Abington, Cambridge CB21 6GS, UK
[b] Shanghai ChemPartner, Shanghai City, China
[c] St Anslem's RC Secondary School, Canterbury, Kent, UK
[d] Astra Zeneca, Vastra Gotaland County, Sweden
[e] Peakdale Molecular, Sandwich, Kent, UK
[f] Pfizer Neusentis, Granta Park, Great Abington, Cambridge CB21 6GS, UK
[g] The Kings School, Canterbury, Kent, UK

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ABSTRACT

A new series of 2-(benzyloxy)benzamides are presented that are potent functional antagonists of TRPM8 and possess improved LipE and LE compared to the original lead. They were discovered through a series of compound libraries and we present a powerful visualization method for the chemical space explored with each library. Remarkably this new series originated from the highest risk design strategy where compounds were synthesised with the least degree of similarity to the lead structure.



TRP (transient receptor potential) channels belong to a large family of nonselective cation channels that function in a variety of processes, including temperature sensation.[1] Members of the TRP family have been shown to be multifunctional receptors that can be activated by physical (heat, cold, voltage, and stress), chemical (osmolality, pH) and specific ligand challenges. Our interest in TRPM8[2] stems from its presence in bladder urothelium and sensory nerves and the potential that modulation of it will have a beneficial effect in the treatment of urgency and frequency associated with overactive bladder.[3]

Although TRPM8 has no known endogenous ligand, there are a number of reported small molecules that modulate the activity of this ion channel. These can either activate (agonise) or deactivate (antagonize) the ion channel with respect to an external stimuli, such as cold. A naturally occurring compound that activates the TRPM8 ion channel is menthol.[4] A number of other agonists have also been described (Fig. 1). These include the menthol derived WS-12 and icilin.[1b,5]

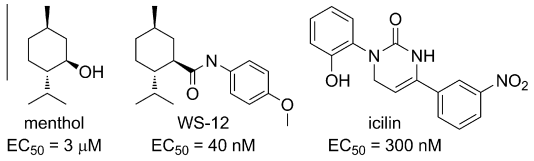


**Figure 1.** Agonists of TRPM8.

Upon initiating our work in this area Bayer had reported on the discovery of potent small molecular antagonists of TRPM8 (Fig. 2).[6] Subsequently, Janssen, Amgen and others[7] also disclosed additional classes of antagonists. Researchers at GlaxoSmithKline recently reported the in vivo activity in rats of a 2-(benzyloxy)benzamide based TRPM8 antagonist (AMTB, **3**, described in a Bayer patent) for overactive bladder and painful bladder syndrome.[8] It is this series, and more specifically analogue **4**, that served as the starting point for the work described in this communication.

At the outset of this work our key goals were to improve the overall ligand efficiency (LE)[9] and lipophilic efficiency (LipE)[10] of compound **4**. We were also keen to replace the electron rich thiophene and the diamine moiety in **4**, since the potential metabolic instability of the thiophene group and the possibility for internal

* Corresponding author.
*E-mail addresses:* Alan.D.Brown@pfizer.com (A. Brown), dellis44781@gmail.com (D. Ellis).
† Current address.

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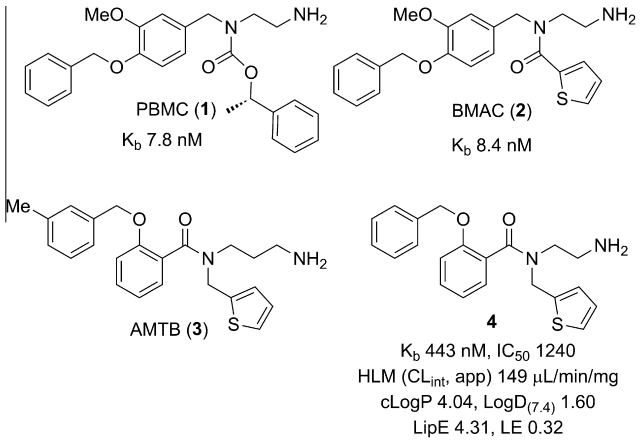
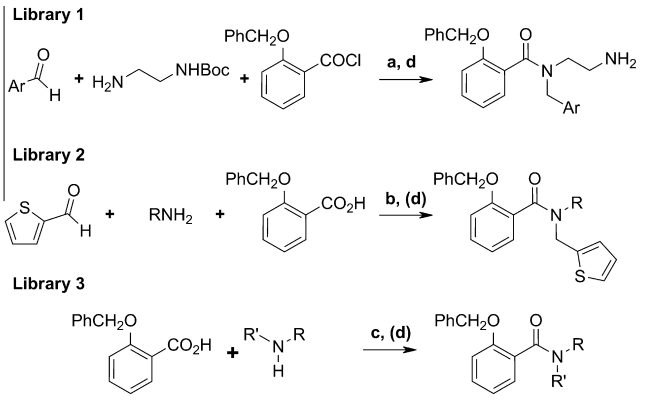



**Figure 2.** Reported TRPM8 antagonist (Pfizer generated data).




**Scheme 1.** Reagents and conditions: (a) MeOH, 30 °C, 3 h; $NaBH_4$, 30 °C, 16 h, then $CH_2Cl_2$, $NEt_3$, 30 °C, 16 h; (b) $NaHCO_3$, MeOH, 50 °C; $NaBH_4$, rt 16 h, then HATU, DIEA DMA, rt, 16 h; (c) HATU, $NEt_3$, DMF, 60 °C, 16 h or HOBt, EDC, $NEt_3$, DMF, 30 °C, 16 h; (d) TFA, $CH_2Cl_2$, 30 °C, 1 h (if applicable).

rearrangement of the ethylene diamine were seen as additional liabilities in this lead series.[11]

Initially we aimed to fully develop the SAR around the basic side chain and the thiophene residue of compound **4**. We hoped to make rapid progress towards compounds with improved properties within a close similarity space around **4**; however owing to the complexity of the issues identified, we also decided to engage in a more risk-taking lead-hopping exercise with more speculative structural changes.

Accordingly we synthesised compound libraries evolving from three design strategies with descending degrees of similarity towards salicylamide **4** (Fig. 3).

In library 1 the thiophene moiety was varied with more polar residues derived from aromatic and heteroaromatic aldehydes (Scheme 1). A total of 130 compounds were synthesized using predominantly *c*log*P* cut-offs (*c*log*P* range from 1.5–5.0).

Library 2 replaced the ethylamine unit with a wider selection of 216 cyclic and acyclic residues, using primary amines as a resourceful source of monomers. The majority of R groups contained an additional basic center; however we also investigated whether low molecular weight (MW) polar and neutral side chains would be tolerated.

Unlike the previous examples, library 3 adopted a lead-hopping approach, which we hoped would lead us into a new, structurally distinct series. The entire amide N-substituent (thiophene and ethylamine) was replaced with a wide range of structurally diverse polar and basic residues, derived from primary and secondary amines. Since compounds from library 3 showed much lower similarity to **4** than the previous strategies, we decided to maximize our chances of success by increasing the size of the library to 436 members. Library compounds were selected from a very large set of commercial and proprietary amines, applying simple cut-offs of *c*log*P* <5 and MW <500, and favoring the presence of a basic side chain.

The chemistry to synthesize these libraries was straightforward and employed well established amide bond formations, reductive aminations and deprotections of *tert*-butoxycarbonyl (Boc) groups (Scheme 1).

In order to visualize a similarity distribution of all library compounds in 2D, we generated a distance matrix for all compounds based on their structural similarity, followed by a multi-dimensional scaling (MDS) analysis as a dimension reducing technique (Fig. 4a).[12] MDS proved to be an ideal tool to represent the chemical space occupied by libraries following different hypotheses, with compounds from 'close-in' library 1 appearing within the proximity of the probe and those from 'lead-hopping' libraries 2 and 3 occupying distal areas within the MDS space.

Screening results of these libraries are displayed in Figure 4b using identical MDS coordinates.[13] Since the ECFP-4 fingerprints[12] used for the similarity calculations have been previously suggested to correlate with biological activity, one would expect compounds occurring within close proximity on the MDS plot to have a high likelihood of similar biological activity.[14] Indeed we found that compounds from library 1, with relatively high similarities

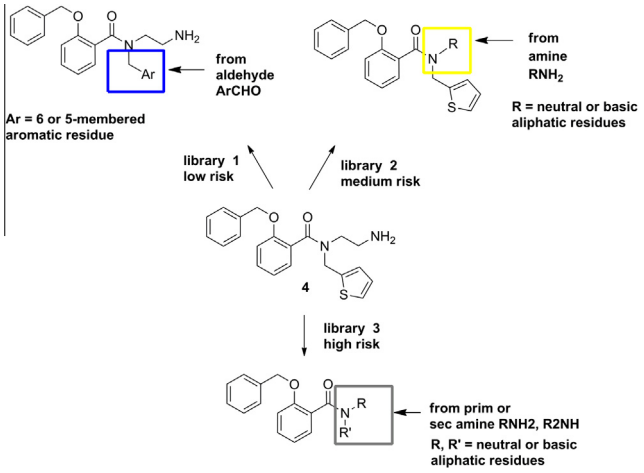



**Figure 3.** High, medium and low risk library strategies around **4**.

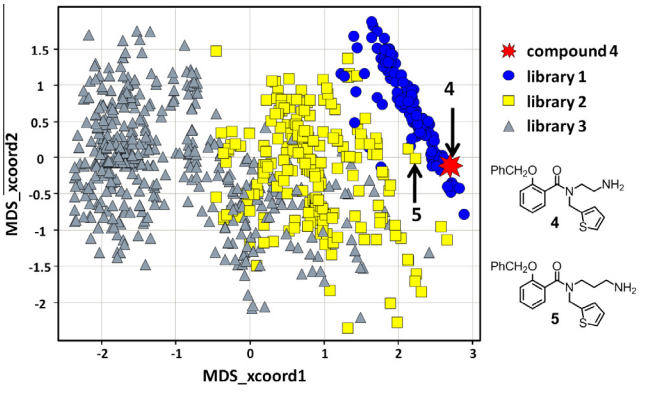



**Figure 4a.** 2D MDS derived similarity distribution of libraries according to design strategy.

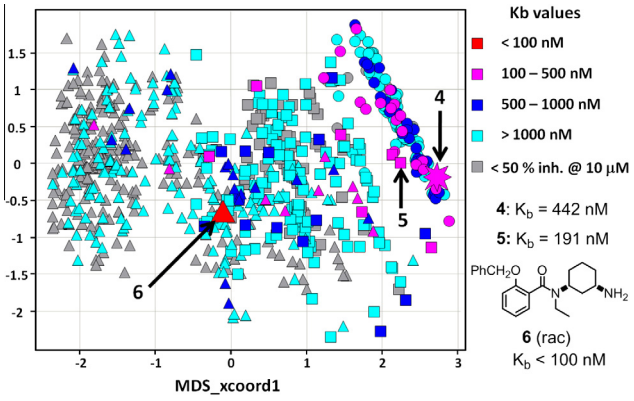



**Figure 4b.** 2D MDS derived similarity distribution of libraries and TRPM8 activity.

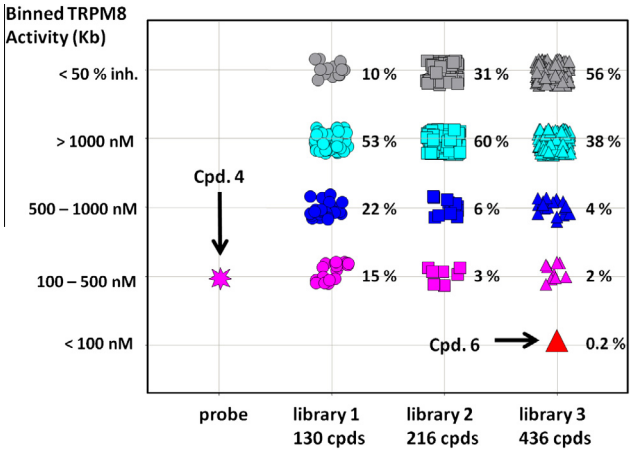
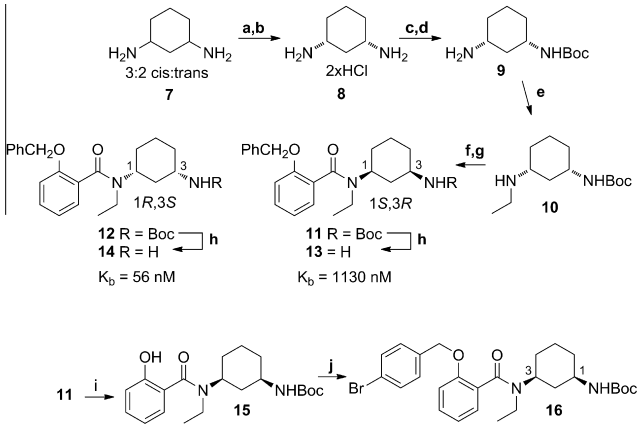



**Figure 4c.** Percentage distribution of TRPM8 activity by design strategy.




**Scheme 2.** Reagents and conditions: (a) $NiCl_2$:$6H_2O$, 82%; (b) HCl, 49%; (c) 1 equiv NaOH, 65%; (d) $Boc_2O$, 65%; (e) $NaBH_4$, AcOH, $CHCl_3$, 33 °C, 64%; (f) *o*-benzyloxybenzoyl chloride, DIEA, $CH_2Cl_2$, 76%; (g) HPLC separation; (h) TFA, $CH_2Cl_2$, 97%; (i) $H_2$/Pd/C, dihydrotoluene EtOH, quant.; (j) *p*-bromobenzyl bromide, $K_2CO_3$, DMF, rt, 70%.

**Figure 5.** Asymmetric unit of the **16** (ethyl acetate) crystal structure.

towards **4**, on average showed the highest likelihood of activity (also see Fig. 4c, as measured by the number of compounds with activities <1 μM). However, the level of potencies never significantly exceeded that of probe compound **4** or the AMTB analogue **5**. A sharp drop-off of average activities was observed for compounds from library 2.

Compounds derived from lead hopping library 3 on average showed very poor activities, as one would expect based on their low similarity to lead compound **4**. However there was one notable exception: Compound **6** derived from *N*-ethyl-*cis*-1,3-diaminocyclohexane displayed significantly improved potency ($K_b$ <100 nM)[15] versus **4**. Since it was also lacking the undesirable thiophene and ethylene diamine moieties, further efforts focused on this new series.

In order to confirm the activity of **6**, the individual enantiomers were synthesised starting from commercially available *cis*/*trans* 1,3-diaminocyclohexane **7** (Scheme 2). The *cis* isomer **8** was isolated by means of its insoluble nickel complex, formed from $NiCl_2$:$6H_2O$ in water.[16]

Selective mono-protection of **8** with Boc group was achieved using a variation of a literature procedure,[17] where the di-HCl salt was treated with 1 equivalent of NaOH to afford the mono HCl salt in situ. Mono-ethylation of **9** was achieved via a little used method from the aminoglycoside field.[18,19] Acylation of **10** with *o*-benzyloxybenzoyl chloride afforded the 2-(benzyloxy)benzamide in racemic form. At this point the individual enantiomers **11** and **12** were separated via chiral HPLC. Removal of the Boc group with TFA yielded **13** and **14**.[20]

To determine the absolute configuration of the active cyclohexane diamine, a *p*-brombenzylether analog of **11** (from the less active series) was prepared and crystallized in two steps from **11** (Scheme 2). The absolute configuration of **16** was established as 1*R*,3*S* by single crystal X-ray crystallography (Fig. 5; please note that the numbering priority changes when the primary amine is protected with a Boc group). By inference the configuration of the dicyclohexyl amine of the series derived from compound **12** is the opposite to that of **11**.

The individual enantiomers (**13** and **14**) showed a ~20fold difference in affinity for TRPM8, with the (1*R*,3*S*)-3-aminocyclohexyl enantiomer having the higher affinity. Compound **14** demonstrated an improved LE (0.38 from 0.31) compared to **4** and a significantly improved LipE (4.31–6.16). The racemic *trans* isomer **17** was >25fold less potent than the more potent enantiomer **14**. None of the other close-in modifications of the (1*R*,3*S*)-3-aminocyclohexyl moiety afforded compounds with an affinity of <2 μM (Fig. 6).

The SAR around the *N*-ethyl group was investigated next. Initially a small range of *rac*-*N*-alkyl-*cis*-1,3-diaminocyclohexanes were synthesised via N-alkylation of the racemic diamine **9**. However due to poor yields obtained, we used an alternative method where **31**[21] was reacted in a reductive amination with primary amines to form the *cis*-isomer with good selectivity.[22] In a typical example the reaction with cyclopropylamine afforded a 9:1 mixture of *cis*/*trans* isomers; the *cis*-isomer **32** (Scheme 3) could

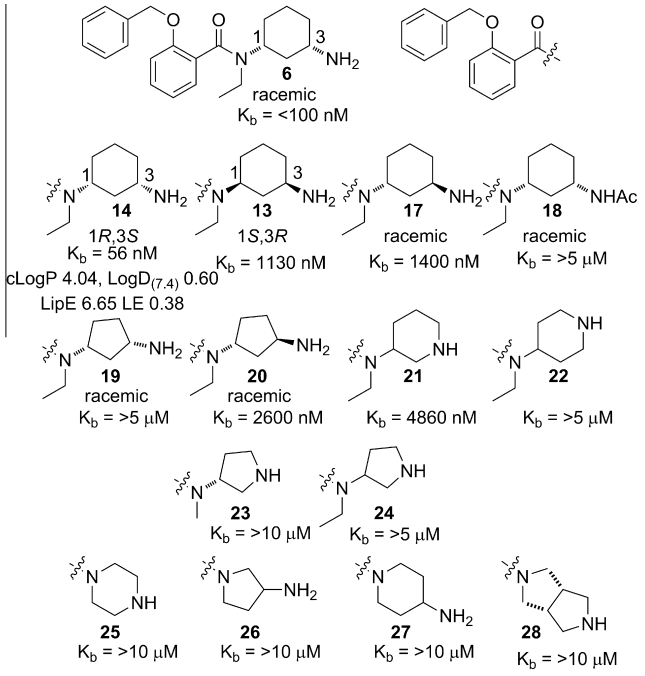
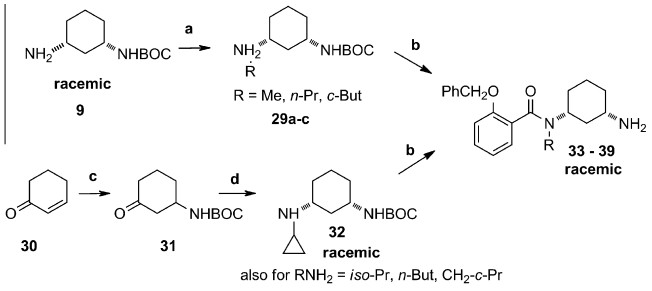
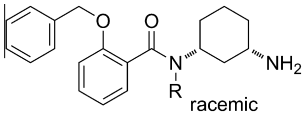



**Figure 6.** SAR of *N*-ethyl-*cis*-1,3-diaminocyclohexane based analogs.




**Scheme 3.** Reagents and conditions: (a) **29a,b**: alkyliodide, CsOH, DMF, **29c**: cyclobutanone, $NaBH(OAc)_3$, THF, AcOH; (b) *o*-benzyloxybenzoyl chloride, DIEA, $CH_2Cl_2$, then TFA, $CH_2Cl_2$; (c) *t*-$BuOCONH_2$, $Bi(NO_3)_3$, $CH_2Cl_2$, 55%; (d) (1) cyclopropylamine, MeOH, rt, (2) LiBH4, −78 °C, (3) oxalic acid, recrystallisation MeOH/EtOAc 1:2, 37%.

**Table 1**
SAR of *N*-ethyl substitution of *cis*-1,3-diamino-cyclohexane based analogs




| Compound | R | TRPM8:$K_b$ (nM) | TRPM8:$IC_{50}$ (nM) |
|---|---|---|---|
| **33** | Me | 3670 | 3150 |
| **34** | Pr | 1430 | 1230 |
| **35** | *c*-Butyl | 794 | 1360 |
| **36** | *c*-Propyl | 2250 | 2010 |
| **37** | *iso*-Propyl | 5130 | 8780 |
| **38** | *n*-Bu | 1560 | 1400 |
| **39** | $CH_2$-*c*-Pr | 665 | 593 |

subsequently be obtained in 98% purity after re-crystallisation of its oxalate salt.

The SAR with respect to the *N*-ethyl group again proved to be tight with the best (**39**) being ~10fold less potent than **14** (Table 1).

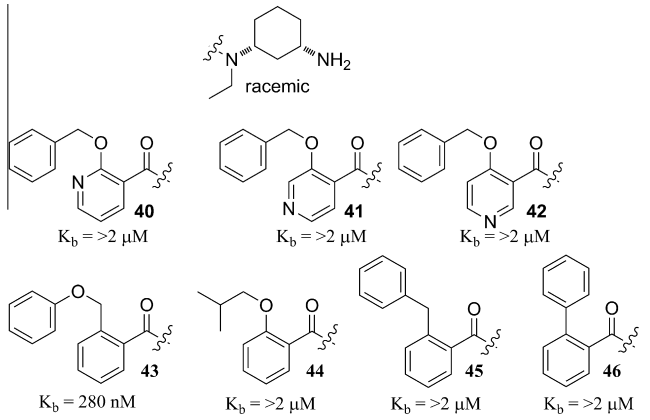
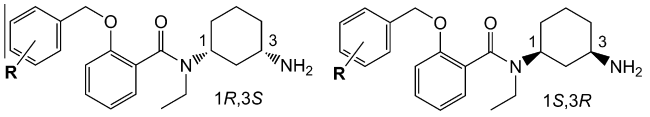



**Figure 7.** SAR of 2-(benzyloxy)benzamide moiety.

**Table 2**
SAR of benzyl substitutions




| Compound | R | Chirality | TRPM8:$K_b$ (nM) | TRPM8:$IC_{50}$ (nM) |
|---|---|---|---|---|
| **14** | H | 1*R*,3*S* | 56.3 | 76.2 |
| **47** | *o*-Me | 1*R*,3*S* | 12.7 | 15.1 |
| **48** | *o*-Me | 1*S*,3*R* | 246 | 291 |
| **50** | *o*-F | 1*R*,3*S* | 17.8 | 21.1 |
| **51** | *o*-F | 1*S*,3*R* | 367 | 435 |
| **52** | *o*-Cl | 1*R*,3*S* | 13 | 29.9 |
| **53** | *o*-Cl | 1*S*,3*R* | 72 | 85.4 |
| **54** | *m*-Cl | Racemic | 96.6 | 96.9 |
| **55** | *p*-Cl | 1*R*,3*S* | 16.1 | 19.3 |
| **56** | *p*-Cl | 1*S*,3*R* | 214 | 254 |
| **57** | *o*-CN | Racemic | 1250 | 1850 |
| **58** | *m*-CN | Racemic | 491 | 493 |
| **59** | *p*-CN | Racemic | 4590 | 6780 |

SAR of the 2-(benzyloxy)benzamide was explored with a structurally diverse set of carboxylic acids. A representative set of some of the analogs made are shown in Figure 7. Of all the compounds made, only the isomeric analog **43** had potency of <2 μM.

Substitution of the benzyl group was the only modification investigated that either retained or improved the potency of the series. Selected compounds are shown in Table 2, with *o*-methyl, *o*-fluoro, *o*-chloro and *p*-chloro substituents on the more potent core affording compounds (**47**, **50**, **52**, **55**) with 3–4fold improved potencies over **14**. Again, the opposite enantiomers displayed significantly weaker activities. Cyano substitutions resulted in a significant loss of potency.

As detailed in summary Table 3, the most potent compounds displayed significantly improved LipE over **4** and very low intrinsic clearance in a human liver microsome assay (HLM Clint app). Com-

**Table 3**
Properties of the most potent compounds

| Compound | **4** | **14** | **50** | **55** |
|---|---|---|---|---|
| TRPM8 $IC_{50}$ (nM) | 1240 | 76.2 | 21.1 | 19.3 |
| TRPM8 $K_b$ (nM) | 443 | 56.0 | 17.8 | 16.1 |
| Log$D_{(7.4)}$ | 1.60 | 0.60 | 0.70 | 1.30 |
| LipE | 4.31 | 6.65 | 6.98 | 6.41 |
| LE | 0.32 | 0.38 | 0.40 | 0.40 |
| HLM CLint, app (μL/min/mg) | 149 | <8 | <8 | <10 |

LipE and LE were calculated based on $IC_{50}$ values.

pound **14** was also screened in a CEREP bioprint screen (against a wide range [>80] of receptors, enzymes and ion channels) and only rat calcium channel L-site diltiazem had inhibition of >60% (61%) at 10 μM.

In conclusion, we have demonstrated how a lead-hopping design strategy allowed us to replace an unattractive side-chain in **4** with a novel 1,3-diamino cyclohexane residue. Compounds from this series are potent functional antagonists of TRPM8 and have improved LiPE and intrinsic clearance compared to the original lead. Clearly the success of this strategy was highly dependent on employing a quality monomer selection, allowing us to cover a wide area of chemical space in our lead-hopping library.

## Acknowledgments

We thank Mark Gardner for bringing to our attention 2D MDS plots as a method for visualizing the chemical space of libraries with different design hypotheses. We acknowledge Cheryl Doherty for solving the X-ray structure and determining the absolute configuration of **16** and Laure Hitzel for carrying out the HPLC separation of **11** and **12**.

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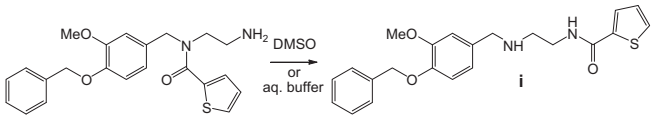
11. The conversion of BMAC (**2**) to **i** was observed in DMSO and aqueous buffer at a range of pH's with a half life of <2 h

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12. Similarities were calculated using ECFP-4 fingerprints, MDS *x*-coordinates were calculated in Scitegic Pipeline Pilot™ using a 'classical' CMDS approach based on *R* statistics components available in Pipeline Pilot™. For more information see: Rodgers, D.; Hahn, M. *J. Chem. Inf. Model.* **2010**, *50*, 742.
13. All TRPM8 data was generated in a HEK cell line transfected with a human TRPM8 receptor construct. TRPM8 inhibitory activity was measured by monitoring WS12 induced elevation of $[Ca^{2+}]$ using FLIPR® detection. The geometric mean of the agonist $EC_{50}$ and slope were used to generate modified $K_b$ values in addition to $IC_{50}$ values. Experiments were carried out in the presence of the activating agonist WS12 at a final concentration of 350 nM. All reported TRPM8 data is based on a minimum of three independent experiments.
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15. Original sample prepared via a library was not tested at low enough concentrations to determine an accurate $K_b$. A few other compounds from library three showed activities of <500 nM, however due to their inferior LE they were not pursued further.
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