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Discovery and structure–activity relationships of 2-benzylpyrrolidine-substituted aryloxypropanols as calcium-sensing receptor antagonists

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Abstract—A structure–activity relationship study of the amine portion of the calcilytic compound NPS-2143 resulted in the discovery of substituted 2-benzylpyrrolidines as replacements for the 1,1-dimethyl-2-naphthalen-2-yl-ethylamine. When compared to NPS-2143, a newly discovered compound, **3h**, exhibited similar potency as a calcium-sensing receptor (CaR) antagonist and a superior human ether-a-go-go related gene (hERG) profile.
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Osteoporosis is a major and growing disease that affects over 28 million people, especially postmenopausal women. Currently, available therapies include anti-resorptive agents such as estrogen, bisphosphonates, calcitonin and selective estrogen receptor modulators (SERMs). These anti-resorptive agents can prevent bone loss but have limited effects on new bone formation and each has been associated with efficacy, safety, tolerability and/or cost limitations. The only FDA-approved anabolic agent, teriparatide, a synthetic 1-34 amino acid peptide fragment of human parathyroid hormone (PTH), has been used to treat severe osteoporosis as a daily subcutaneous injection.[1] As an alternative to daily PTH injections, one could envision stimulating the release of endogenous PTH from the parathyroid glands. Evidence suggests that physiologically relevant plasma levels of endogenous PTH are sufficient to elicit a bone anabolic response.[2] It is well established that PTH secretion is inversely related to plasma calcium concentration.[3] The cloning of a calcium-sensing receptor (CaR) from parathyroid tissue has recently elucidated the molecular basis of this control mechanism.[4] A small molecule CaR antagonist and/or a negative allosteric modulator could act as an oral anabolic agent. NPS-2143 is the first reported orally active low molecular weight calcilytic that stimulates the secretion of endogenous PTH by antagonizing the CaR on the surface of parathyroid cells.[5] The resulting increase in endogenous circulating PTH results in the stimulation of new bone growth. In animal studies, when co-administered with the anti-resorptive agent 17β-estradiol, NPS-2143 increased trabecular bone volume and bone mineral density, and the results were comparable to those obtained by daily PTH injection.

In vitro profiling shows that NPS-2143 has potential liabilities such as potent hERG inhibition. Cardiac hERG channel blockage is an important mechanism for drug-induced QT prolongation associated with torsades de pointes and sudden death[6] and in a patch-clamp assay, NPS-2143 inhibited the hERG K^+ channel with an IC_{50} of 194 nM. In an attempt to modulate this activity, we embarked on a search for a replacement of the secondary *gem*-dimethylamine in NPS-2143, a common pharmacophore in most of the compounds disclosed in

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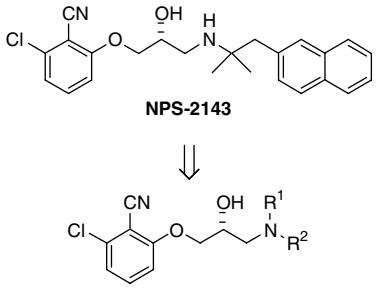


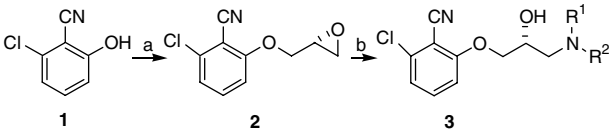
Figure 1.

the patent literature (Fig. 1).[7] A solution phase library approach was undertaken where a structurally diverse set of amines was employed.

The synthesis of the solution phase library was carried out in a 96-well mini-reactor (Scheme 1). 3-Chloro-2-cyanophenol **1** was treated with an excess of (*R*)-epichlorohydrin in the presence of Cs_2CO_3 in refluxing CH_3CN overnight. Upon completion of the reaction, the solids were filtered and the excess reagent was removed by rotary evaporation. The resulting crude epoxide **2** was then redissolved in *i*-PrOH and aliquoted into a set of 93 primary and secondary amines. The reaction mixtures were heated in capped vials at 80 °C overnight. The excess primary amines were trapped with formylated polystyrene resin and the excess secondary amines were removed with an isocyanate resin. The crude products were purified by loading onto a cation exchange resin (SCX) column, which was first washed with MeOH to remove the nonbasic impurities and then with NH_3/MeOH to afford the final products **3** in 8–100% yields (average 80%) after preparative HPLC purification.

The products from the above library were screened in a fluorescence based intracellular Ca^{2+} mobilization assay in HEK cells containing over-expressed recombinant human calcium-sensing receptor. Inhibition by test compounds of intracellular Ca^{2+} release stimulated by extracellular Ca^{2+} was measured using FLIPR technology as previously described.[8] 4-Benzylpiperidine **3a** was identified from this assay as a lead CaR antagonist with an IC_{50} of 2.2 μM (Fig. 2).

A brief SAR study of compound **3a** was carried out focusing on different substituted cyclic amines. Table 1 summarizes the antagonist activities of exemplary piperidine and pyrrolidine analogues of compound **3a**. *meta*-Substitution of the 4-benzyl group with a methoxy group (**3c**) improved the potency by 3-fold, while *para*-substitution (**3b**) resulted in a dramatic loss of activity.



Scheme 1. Reagents and conditions: (a) (*R*)-epichlorohydrin, Cs_2CO_3, CH_3CN, reflux overnight, 84%; (b) *i*-PrOH, NHR^1R^2, 80 °C, overnight, 8–100%.

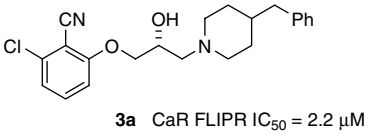
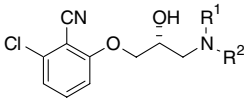
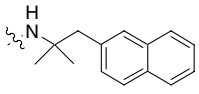
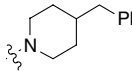
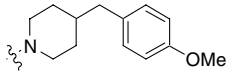
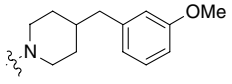
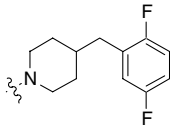
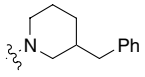
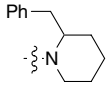
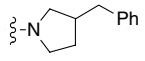


Figure 2. Hit from library.

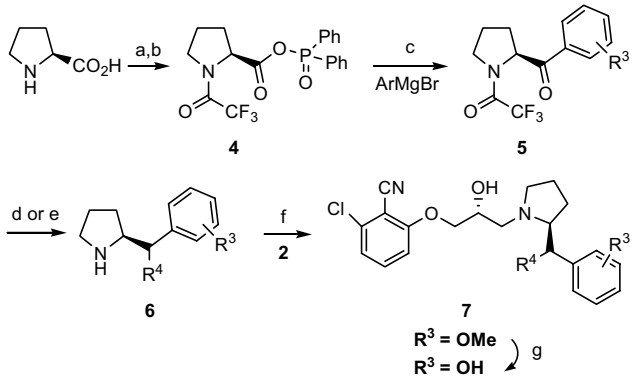
The 2,5-difluoro-substituted compound **3d** showed similar activity to **3a**. Moving the benzyl group from the 4-position to the 3- or 2-positions on the piperidine ring (**3e** and **3f**) led to greatly reduced activity. However, when the ring size was reduced from the six-membered ring piperidine to a five-membered ring pyrrolidine, an improvement in activity was observed. While the 3-benzylpyrrolidine **3g** showed an IC_{50} of 10 μM, the

Table 1. Inhibition of intracelluar Ca^{2+} release by substituted piperidines and pyrrolidines as measured by FLIPR™



Compd	NR^1R^2	FLIPR IC_{50} (μM)[a]
NPS-2143		0.08 (±0.03)
3a		2.2 (±0.04)
3b		>30
3c		0.64 (±0.04)
3d		2.1 (±0.34)
3e		>30
3f		>30
3g		10 (±0.37)
3h		0.35 (±0.004)
(*S*)-**3h**		0.12 (±0.006)
(*R*)-**3h**		>30

[a] Values are means of two experiments, standard deviation is given in parentheses.



Scheme 2. Reagents and conditions: (a) CF_3CO_2Et, 1,1,3,3-tetramethylguanidine, 82%; (b) $ClP(O)Ph_2$, NEt_3, 79%; (c) THF, −70 °C to rt, 17–60%; (d) H_2/Pd/C, EtOH, HCl, 48 h, 100%; (e) (i) $BF_3{\cdot}OEt_2$, Et_3SiH, 41–55%; (ii) 12.5 N HCl, *i*-PrOH reflux, 100%; (f) EtOH, 110 °C, 16 h, 47–55%; (g) BBr_3, CH_2Cl_2, −70 °C to rt, 80%.

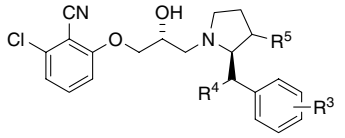
2-benzylpyrrolidine **3h** was over 6-fold more potent than **3a**, with an IC_{50} of 0.35 μM.

The two diastereomers composing **3h** were separated by chiral preparative HPLC.[9] The fast eluting diastereomer A was found to have an IC_{50} of 120 nM while the slow eluting diastereomer B was inactive at concentrations up to 30 μM. The absolute stereochemistry at the 2-benzyl position of diastereomer A was subsequently confirmed to be *S* after an independent asymmetric synthesis of diastereomer A starting from chiral (*S*)-2-benzylpyrrolidine prepared by the procedure of Nordlander et al.[10]

An alternate route was utilized for the synthesis of other substituted 2-benzylpyrrolidine analogues **7a**–**j** as illustrated in Scheme 2.[11] D-proline was protected with a trifluoroacetyl group and converted to the mixed anhydride **4**. Treatment of **4** with various Grignard reagents provided the ketones **5**, which were converted to benzylpyrrolidines **6** (R^4 = H) either in one step by hydrogenolysis in acidic EtOH, or in a stepwise fashion by reduction with $BF_3{\cdot}OEt_2/Et_3SiH$ followed by deprotection with refluxing HCl in *i*-PrOH. In some cases, the partially reduced hydroxyl intermediates **6** (R^4 = OH) were also isolated. Coupling of pyrrolidines **6** with epoxide **2** provided the final compounds **7**. In cases where the R^3 substituent was a methoxy group, the compound could be demethylated to the corresponding phenol by BBr_3. The diastereomeric purity of **7** was determined by chiral analytical HPLC and was found to be generally over 95% except for **7a**, where epimerization had occurred at the 2-position of the pyrrolidine during the synthesis and a 2:3 mixture of diastereomers was obtained.

Table 2 summarizes the SAR around the 2-benzylpyrrolidine of (*S*)-**3h**. Substitution of a methoxy group at the *meta*- or *para*-position of the phenyl ring, as well as an electron-withdrawing 3-F substitution was tolerated (compounds **7b**, **7c** and **7g**). Moving the methoxy group to the *ortho*-position (**7a**) resulted in a dramatic loss of activity. Disubstitution in compound **7d** led to a 3-fold decrease in potency. When the methoxy groups

Table 2. Inhibition of intracelluar Ca^{2+} release by substituted 2-benzylpyrrolidines as measured by FLIPR™



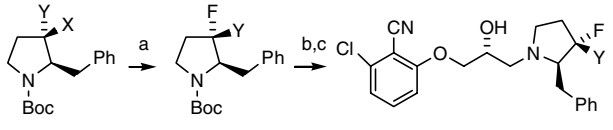
Compd	R^3	R^4	R^5	FLIPR IC_{50} (μM)[a]
(*S*)-**3h**	H	H	H	0.12 (±0.006)
7a	2-MeO	H	H	>30
7b	3-MeO	H	H	0.13 (±0.006)
7c	4-MeO	H	H	0.20 (±0.006)
7d	3,4-Di-MeO	H	H	0.41 (±0.02)
7e	2-OH	H	H	0.10 (±0.007)
7f	4-OH	H	H	>30
7g	3-F	H	H	0.15 (±0.005)
7h	4-Me_2N	H	H	1.9 (±0.16)
7i	3-MeO	OH	H	>30
7j	3-F	OH	H	2.3 (±0.19)
7k	H	H	OH(*R*)	1.4 (±0.22)
7l	H	H	F(*S*)	2.8 (±0.29)
7m	H	H	Di-F	>30

[a] Values are means of two experiments, standard deviation is given in parentheses.

in these compounds were demethylated, a surprising pattern was observed. The 4-hydroxy compound **7f** was inactive, while the 2-hydroxyl compound **7e** showed potent inhibition with an IC_{50} of 100 nM. A basic dimethylamino group (**7h**) led to a >10-fold decrease in activity. Hydroxyl substitution at the benzylic position (**7i** and **7j**), as well as hydroxylation on the pyrrolidine ring (**7k**), proved unfavourable for the antagonistic activity. Attempts to reduce the basicity of the pyrrolidine nitrogen through the addition of β-fluorines (**7l** and **7m**) led to a stepwise reduction of activity as the number of fluorines increased.

The synthesis of the fluorinated analogues is shown in Scheme 3 starting from the known compounds 2-benzylpyrrolidin-3-ol or 2-benzylpyrrolidin-3-one.[12] Fluorination of the alcohol or ketone with bis(2-methoxyethyl)aminosulfur trifluoride (BAST)[13] followed by TFA deprotection provided the mono- or di-fluorinated pyrrolidines, which were coupled with epoxide **2** in the absence of solvent to provide the final compounds **7l** and **7m**.

Compound (*S*)-**3h** was tested in a K^+ patch-clamp assay for the hERG inhibitory activity and was found to show reduced potency (IC_{50} = 1.24 μM) compared with NPS-2143 (IC_{50} = 0.19 μM). The decreased hERG



8a (X = OH, Y = H) **8b (X = Y = O)** **9a (Y = H)** **9b (Y = F)** **7l (Y = H)** **7m (Y = F)**

Scheme 3. Reagents and conditions: (a) BAST, CH_2Cl_2, −78 °C to rt, overnight, 23% (Y = H), 72% (Y = F); (b) TFA, CH_2Cl_2; (c) **2**, 90 °C, overnight, two steps 37% (Y = H), 74% (Y = F).

activity of (*S*)-**3h** suggests it is possible to maintain the CaR antagonist activity while reducing the hERG liability for this series of compounds.

In conclusion, a solution phase library approach was undertaken to find a novel series of calcium-sensing receptor antagonists as exemplified by (*S*)-**3h**. This new compound showed comparable potency to NPS-2143 as a calcium-sensing receptor antagonist but possessed reduced hERG blocking activity.

Acknowledgements

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References and notes

1. (a) Lopez, F. J. *Curr. Opin. Chem. Biol.* **2000**, *4*, 383; (b) Rotella, D. P. *Curr. Opin. Drug Discov. Dev.* **2002**, *5*, 477.
2. Fox, J.; Miller, M. A.; Stroup, G. B.; Nemeth, E. F.; Miller, S. C. *Bone* **1997**, *21*, 163.
3. Brown, E. M.; MacLeod, R. J. *Physiol. Rev.* **2001**, *81*, 239.
4. Brown, E. M.; Gamba, G.; Riccardi, D.; Lombardi, M.; Butters, R.; Kifor, O.; Sun, A.; Hediger, M. A.; Lytton, J.; Hebert, S. C. *Nature* **1993**, *366*, 575.
5. (a) Gowen, M.; Stroup, G. B.; Dodds, R. A.; James, I. E.; Votta, B. J.; Smith, B. R.; Bhatnagar, P. K.; Lago, A. M.; Callahan, J. F.; Delmar, E. G.; Miller, M. A.; Nemeth, E. F.; Fox, J. J. *Clin. Invest.* **2000**, *105*, 1595; (b) Nemeth, E. F. *J. Mol. Endocrinol.* **2002**, *29*, 15.
6. For reviews, see: (a) Crumb, W.; Cavero, I. *Pharm. Sci. Technol. Today* **1999**, *2*, 270; (b) Vandenberg, J. I.; Walker, B. D.; Campbell, T. J. *Trends Pharmacol. Sci.* **2001**, *22*, 240.
7. (a) Lago, A. M.; Callahan, J. F.; Bhatnagar, P. K.; Del Mar, E. G.; Bryan, W. M; Burgess, J. L. WO 0153254, 2001; (b) Lago, A. M. WO 0045816, 2000; (c) Bhatnagar, P. K; Callahan, J. F.; Del Mar, E. G.; Lago, A. M. WO 9951241, 1999.
8. Schroeder, K. S.; Neagle, B. D. *J. Biomol. Screen.* **1996**, *1*, 75.
9. Chiral preparative HPLC conditions: CHIRALPAK® OD column, 5×50 cm, with 20% isopropanol/hexane containing 0.1% triethylamine as the eluent at a flow rate of 50 mL/min.
10. Nordlander, J. E.; Njoroge, F. G.; Payne, M. J.; Warman, D. *J. Org. Chem.* **1985**, *50*, 3481.
11. (a) Fisher, L. E.; Rosenkranz, R. P.; Clark, R. D.; Muchowski, J. M.; McClelland, D. L.; Michel, A.; Caroon, J. M.; Galeazzi, E.; Eglen, R.; Whiting, R. L. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 2371; (b) Fisher, L. E.; Muchowski, J. M.; Galeazzi, E.; Rosenkranz, R. P.; McClelland, D. L. US Patent 5100912, 1992.
12. (a) Peet, N. P.; Kim, H.-O.; Marquart, A. L.; Angelastro, M. R.; Nieduzak, T. R.; White, J.; Friedrich, D.; Flynn, G.; Webster, M. E.; Vaz, R.; Linnik, M. D.; Koehl, J. R.; Mehdi, S.; Bey, P.; Emary, B.; Hwang, K.-K. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2365; (b) Courcambeck, J.; Bihel, F.; Michelis, C. D.; Quelever, G.; Kraus, J. L. *J. Chem. Soc., Perkin Trans. 1* **2001**, 1421.
13. Lal, G. S.; Pez, G. P.; Pesaresi, R. J.; Prozonic, F. M.; Cheng, H. *J. Org. Chem.* **1999**, *64*, 7048.