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Discovery of novel 1-arylmethyl pyrrolidin-2-yl ethanol amines as calcium-sensing receptor antagonists

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Abstract—A 3D quantitative structure–activity relationship study for inhibition of calcium-sensing receptor in the aryloxypropanolamine series predicted that these molecules adopt a U-shaped conformation with pi-stacking between the two aromatic rings. This hypothesis led to the discovery of novel 1-arylmethyl pyrrolidin-2-yl ethanol amines capable of antagonizing the calcium-sensing receptor with potency comparable to that of NPS-2143.

Osteoporosis is a serious and prevalent disease associated with significant morbidity and mortality. In osteoporotic individuals, there is an imbalance between bone resorption and bone formation resulting in a progressive loss of bone mass and structure. Current therapies involve anti-resorptive agents that impede further loss of bone mass once the disease has manifested and can elicit a modest increase in bone mineral density; however, bone which is lost is not restored.[2] Teriparatide, a synthetic 1–34 amino acid peptide fragment of human parathyroid hormone (PTH), is the only FDA-approved anabolic agent that, upon daily subcutaneous injection, has been shown to increase bone mineral density and reduce fracture rates in humans.[2] In contrast to the anabolic effects observed after intermittent administration, it is well documented that continuous exposure to PTH results in increases in bone turnover with a subsequent loss in bone mass. As a viable alternative to subcutaneous PTH therapy, one can envision stimulating the release of endogenous PTH from the parathyroid glands in order to stimulate bone formation. It is well established that PTH secretion is inversely related to the plasma calcium concentration, which is mediated by the calcium-sensing receptor (CaR) expressed on the surface of parathyroid cells.[3] CaR, a member of the metabotropic glutamate-like G-protein coupled receptor family, is coupled through changes in phosphoinositide (PI) turnover to the release of calcium from intracellular stores. Preclinical proof-of-principle for the CaR approach includes studies with the CaR antagonist (calcilytic), NPS-2143.[4] Intravenous, bolus injection of NPS-2143 to osteopenic ovariectomized rats resulted in a transient increase in serum PTH compatible with the anabolic profile of the hormone. Oral administration of NPS-2143 resulted in a sustained increase in plasma PTH and a concurrent increase in bone turnover without any change in bone mass. The absence of an increase in bone mass is consistent with the observation of the sustained levels of PTH. Indeed, an increase in bone mass was observed in the presence of an anti-resorptive agent due to the uncoupling of bone resorption from the anabolic component. These results indicate that a calcilytic agent with rapid absorption and a short half-life should evoke the desired transient increase in plasma PTH levels, translating into a bone anabolic response.

The recently disclosed calcilytic compound NPS-2143 (**1**) had an IC_{50} of 0.05 μM in a fluorescence-based intracellular calcium mobilization assay in human TT cells containing the endogenous calcium-sensing receptor.[5] The results from a brief SAR study around NPS-2143 are summarized in Table 1.[6] Removal of the hydroxyl group (**4**) led to greatly reduced activity; however, the hydroxyl enantiomers **1** and **2** exhibited similar levels of potency. N-methylation of the secondary amine (**3**)

Keywords: Osteoporosis; Calcium-sensing receptor antagonist.
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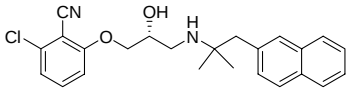
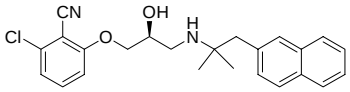
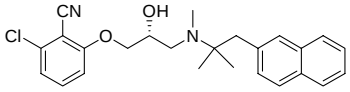
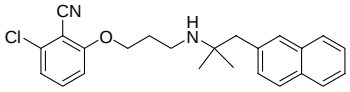
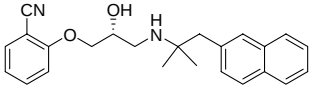
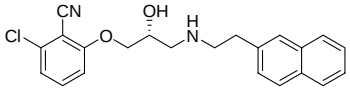
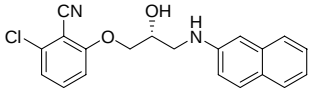
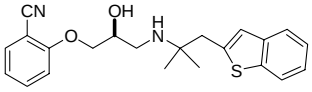
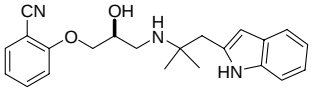
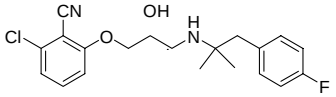
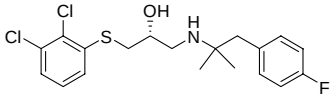
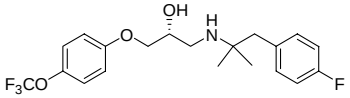
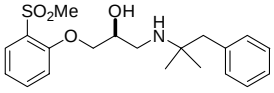
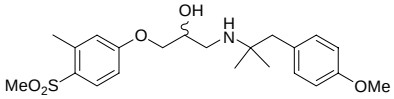
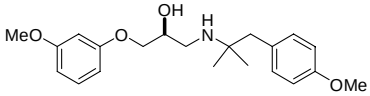
Table 1. Inhibition of intracellular Ca^{2+} release by aryloxypropanolamines in human TT cells as measured by FLIPR™

Compound	Structure	CaR IC_{50}[a] (μM)
1 (NPS-2143)		0.05
2		0.05
3		0.27
4		0.88
5		0.03
6		3.86
7		4.60
8		0.06
9		0.10
10		0.60
11		6.18
12		3.30
13		9.43
14		11.36
15		1.39

[a] Values are means of two experiments.

diminished the potency by 5-fold, while deletion of the gem-dimethyl (**6**) resulted in a dramatic loss of potency. Replacement of naphthalene with other fused bicyclic aromatic rings (**8** and **9**) was tolerated with a modest loss of activity. This information was used to develop a comparative molecular similarity index analysis (CoMSiA) model for three-dimensional quantitative structure–activity relationship in this series. The CoMSiA model is a well-established method for generating bioactive conformation hypotheses.[7] A conformational search of NPS-2143 around the bonds labeled 1 and 2 provided three lowest energy conformations as

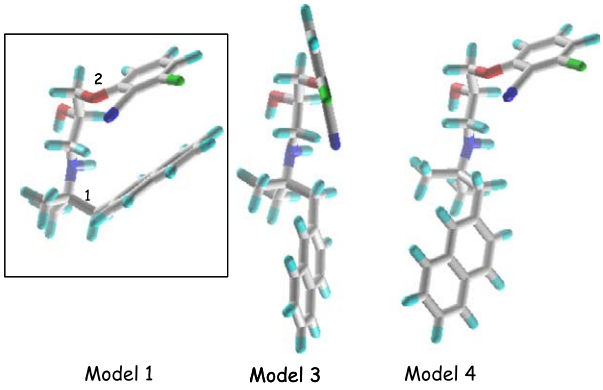


Figure 1. Conformations of NPS-2143 used to construct 3 of the 4 models used with CoMSiA studies. The other conformation used was very similar to that of Model 1 with a H-bond between the hydroxyl hydrogen and the oxygen atom attached to the phenyl ring.

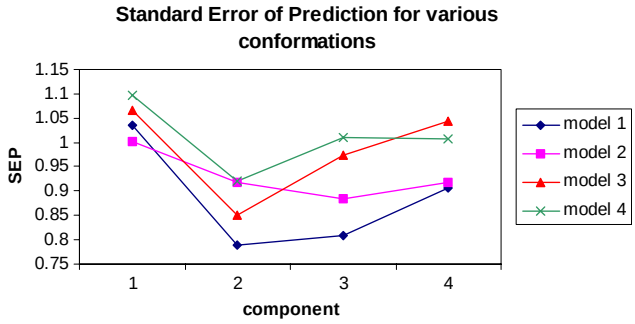


Figure 2. The standard error of prediction for the four CoMSiA models plotted versus the number of components. The lowest standard error of prediction was associated with Model 1.

shown in Figure 1. In addition, another conformation with an internal H-bond between the hydroxyl hydrogen and the oxygen atom attached to the phenyl ring (Model 2) was also evaluated. Each model was subjected to a leave-one-out cross-validation and the corresponding standard error of prediction is plotted versus the component number for the four models (Fig. 2). The CoMSiA studies predicted Model 1 to be the bioactive conformation based on the lowest standard error of prediction. Perhaps, these molecules adopt such a U-shaped bioactive conformation due to an energetically favorable pi-stacking between the aromatic groups. As supported by the data shown in Table 1 (**5** and **6**), the linker containing a gem-dimethyl moiety is also likely to play a pivotal role in the adoptation of this conformation.

One can envisage a further improvement in potency in this series by restricting some degrees of freedom to increase the propensity of the molecule to exist in the bioactive conformation. Out of the various possibilities considered to constrain the degrees of freedom, the 1-arylmethyl pyrrolodin-2-yl ethanolamines allowed the resulting compound to generate the desired U-shaped conformation with pi-stacking between the two aromatic moieties. As depicted in Figure 3, the CoMSiA model predicted that R-configuration is essential at the asymmetric center on the pyrrolidine ring for the compound to adopt a bioactive U-shaped conformation similar to NPS-2143. Indeed, all the S-enantiomers at the pyrrolidine prepared to date have been devoid of CaR inhibitory activity (data not shown). Further support for the CoMSiA model is rendered by the lack of potency of compound **22** (Table 2), which does not prefer the U-shaped conformation (Fig. 3) due to the torsion angle requirements imposed by the amide group.

The initial route utilized for the synthesis of these compounds is illustrated in Scheme 1. Selective N-alkylation of D-proline **16** was followed by conversion of the carboxylic acid to the corresponding chloromethyl ketone **17** via a three-step sequence through a mixed anhydride and diazoketone intermediates.[8] The diastereomeric epoxides **18**, obtained by reduction of the ketone and subsequent intramolecular cyclization, were separated by silica gel chromatography and coupled with amines **19**[6] to provide the corresponding ethanol amines **20**. The diastereomeric purity of the final compounds was determined to be >98% by analytical HPLC.[9] A solution-phase parallel synthesis was developed for the preparation of subsequent analogs (Scheme 2). Carbobenzyloxy-D-proline **23** was converted to a mixture of diastereomeric epoxides **24** that were separated by column chromatography, coupled with the amines **19**, and the protecting group was removed to give the pyrrolidines **25**. Reductive amination of pyrrolidines **25** with aromatic aldehydes in the presence of zinc chloride or titanium isopropoxide provided the desired ethanol amines **20**.[10]

A summary of the SAR around the *N*-benzylpyrrolidine of compound **21** is presented in Table 2. Com-

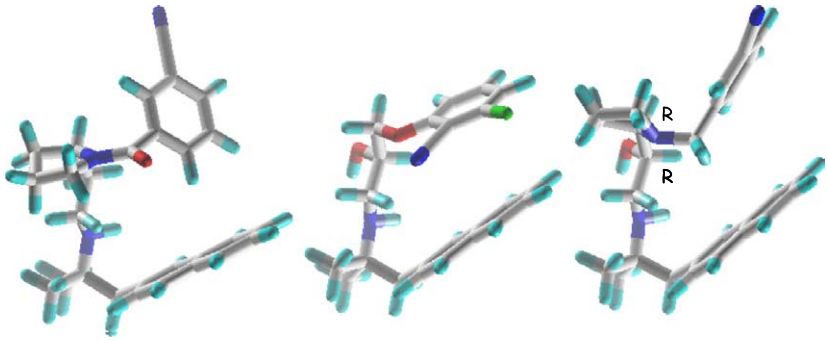
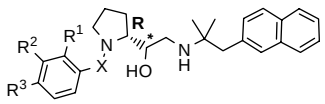


Figure 3. Compounds with R-configuration at the pyrrolidine ring (e.g., **21d** on the right) can exhibit conformations similar to that of NPS-2143 (center structure). Compound **22** on the left is predicted to be inactive.

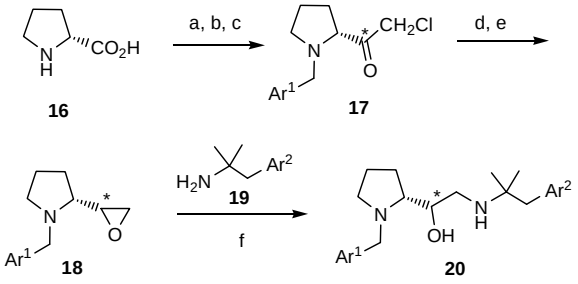
Table 2. Inhibition of intracellular Ca^{2+} release by 1-arylmethyl pyrrolidin-2-yl ethanolamines in human TT cells as measured by FLIPR™



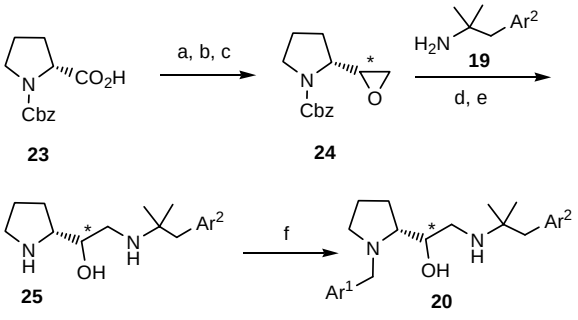
21: X = CH_2
22: X = CO

Compound	R^1	R^2	R^3	CaR IC_{50}[a] (μM)
21a(*S*)	CN	H	H	1.27
21b(*R*)	CN	H	H	0.34
21c(*R*)	H	CN	H	0.19
21d(*R*)	H	H	CN	0.25
21e(*R*)	NO_2	H	H	0.16
21f(*R*)	H	NO_2	H	0.14
21g(*R*)	H	H	NO_2	0.79
21h(*R*)	OH	H	H	0.97
21i(*R*)	H	F	H	0.75
21j(*R*)	H	Br	H	1.57
21k(*R*)	H	Ph	H	2.47
21l(*R*)	H	OMe	H	1.19
21m(*R*)	H	OH	H	1.16
21n(*R*)	H	CO_2H	H	6.84
21o(*R*)	F	F	H	0.36
21p(*R*)	OH	F	H	0.14
21q(*R*)	OH	OMe	H	1.56
21r(*R*)	OH	NO_2	H	0.14
21s(*R*)	CN	Cl	H	0.24
22(*R*)	H	CN	H	8.43

[a] Values are means of two experiments.



Scheme 1. Reagents and conditions: (a) Ar^1CH_2Br, KOH, *i*PrOH, 40 °C; (b) i—*i*BuCOCl, Et_3N, THF, −15 °C to rt; ii—CH_2N_2, Et_2O, 0 °C; (c) HCl, dioxane, 0 °C; (d) $NaBH_4$, THF, MeOH, 0 °C; (e) KOH, EtOH, rt; (f) EtOH, 110 °C.

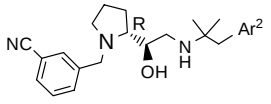


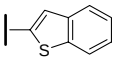
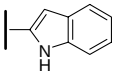
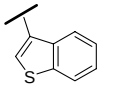
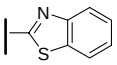
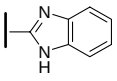
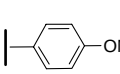
Scheme 2. Reagents and conditions: (a) i—*i*BuCOCl, Et_3N, THF, −15 °C to rt; ii—CH_2N_2, Et_2O, 0 °C; iii—HCl, dioxane, 0 °C; (b) $NaBH_4$, THF, MeOH, 0 °C; (c) KOH, EtOH, rt; (d) EtOH, 110 °C; (e) H_2, Pd/C, EtOH; (f) i—Ar^1CHO, $ZnCl_2$ or $Ti(OiPr)_4$, MeOH/THF; ii—$NaBH_3CN$, THF.

pounds in this series exhibited a modest preference for the R-configuration at the hydroxyl asymmetric center (e.g., **21a** and **21b**). An electron-withdrawing substitution was preferred at the *meta*- or *ortho*-position of the phenyl ring. For example, a *meta*-cyano (**21c**) or nitro (**21f**) substituent provided an order of magnitude increase in in potency over the corresponding *m*-methoxy substituent (**21l**). Moving the substitution to the *para*-position resulted in up to a 5-fold loss of activity (**21f** and **21g**). Increasing the size of this group resulted in a concomitant reduction in CaR inhibitory potency (**21j** and **21i**). Polar functional groups, such as a carboxylic acid (**21n**), proved unfavorable for antagonist activity. Preliminary exploration of 2,3-disubstitution failed to provide any clear trends. Whereas the 2-hydroxy-3-fluoro substitution (**21p**) augmented the potency 5-fold relative to either substitution alone, introduction of a 2-hydroxyl in 3-methoxyphenyl or 3-nitrophenyl compounds (**21q** and **21r**) did not provide any increase in potency.

A brief SAR study was carried out focusing on the naphthalene ring replacements of compound **21c**. Table 3 summarizes the antagonist activities of exemplary analogues prepared in this series. Similar to the observations made in the aryloxypropanolamine series (Table 1), replacement of the naphthalene moiety with a 2-substituted benzothiophene or indole (**26a** and **26b**) resulted in a modest loss of activity. The decrease in CaR inhibitory potency was more dramatic for 3-substituted benzothiophene (**26c**) and 4-methoxyphenyl (**26f**) replacements of the naphthalene functionality. The lower potency of compound **26e** suggests that a H-bond accepting nitrogen is not tolerated in the potentially hydrophobic pocket occu-

Table 3. Inhibition of intracellular Ca^{2+} release by 1-arylmethyl pyrrolidin-2-yl ethanolamines in human TT cells as measured by FLIPR™



Compound	Ar^2	CaR IC_{50}[a] (μM)
26a		0.27
26b		0.44
26c		1.00
26d		1.35
26e		2.84
26f		2.67

[a] Values are means of two experiments.

pied by the bicyclic aromatic ring on the surface of the calcium-sensing receptor.

Compounds **21c** and **21p** have emerged as the lead candidates from this series with CaR antagonist potency that is 3- to 4-fold less than that of NPS-2143. These compounds were evaluated in a functional assay for inhibition of extracellular Ca^{2+}-induced inositol triphosphate (IP) generation in rat MTC cells that constitutively express the calcium-sensing receptor.[11] Compounds **21c** and **21p** inhibited Ca^{2+}-induced IP accumulation with respective IC_{50} values of 2.20 and 2.56 μM, relative to NPS-2143 which exhibited an IC_{50} of 0.53 μM in this assay.

In conclusion, a three-dimensional quantitative structure–activity relationship study for inhibition of calcium-sensing receptor in the aryloxypropanolamine series predicted that these molecules adopt a U-shaped conformation with pi-stacking between the two aromatic rings and this hypothesis led to the discovery of novel 1-arylmethyl pyrrolidin-2-yl ethanol amines capable of antagonizing the human and rat calcium-sensing receptors with potency comparable to that of NPS-2143. These data offer a new avenue for the development of novel anabolic agents for the treatment of osteoporosis.

Acknowledgments

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10. Kim, S.; Oh, C. H.; Ko, J. S.; Ahn, K. H.; Kim, Y. J. *J. Org. Chem.* **1985**, *50*, 1927.
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