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# Design and synthesis of calindol derivatives as potent and selective calcium sensing receptor agonists

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ABSTRACT

We report the first comprehensive structure–activity study of calindol (**4**, (*R*)-*N*-[(1*H*-indol-2-yl)methyl]-1-(1-naphthyl)ethanamine), a positive allosteric modulator, or calcimimetic, of the calcium sensing receptor (CaSR). While replacement of the naphthyl moiety of calindol by other aromatic groups (phenyl, biphenyl) was largely detrimental to calcimimetic activity, incorporation of substituents on the 4, 5 or 7 position of the indole portion of calindol was found to provide either equipotent derivatives compared to calindol (e.g., 4-phenyl, 4-hydroxy, 5-hydroxycalindol **44**, **52**, **53**) or, in the case of 7-nitrocalindol (**51**), a 6-fold more active calcimimetic displaying an $EC_{50}$ of 20 nM. Unlike calindol, the more active CaSR calcimimetics were shown not to act as antagonists of the closely related GPRC6A receptor, suggesting a more selective profile for these new analogues.



## 1. Introduction

G protein-coupled receptors (GPCRs), present exclusively in eukaryotes, are involved in a myriad of diseases and are the target of around half of all modern medicinal drugs.[1] Also referred to as seven transmembrane (7TM) domain receptors, GPCRs are able to sense molecules present outside the cell going from small molecules and peptides to large proteins. These then activate complex signal transduction pathways inside the cell resulting in a variety of cellular responses. Among this GPCR superfamily, the extracellular calcium sensing receptor (CaSR) has come under considerable focus for the discovery of new allosteric modulators with high therapeutic potential.[2]

The CaSR belongs to class C of GPCRs, which includes its close analogue GPRC6A, receptors for pheromones, odorants, taste, the excitatory amino acid glutamate, and the neurotransmitter γ-aminobutyric acid (GABA).[3] This receptor class shares an exceptionally large extra-cellular domain analogous to the bilobed 'Venus flytrap' domain (VFT) of bacterial periplasmic binding proteins.[4] The human CaSR is the unique cation-sensing GPCR and its localization in the parathyroid,[5] kidney[6] and brain[7] has received the most attention.[8] By detecting small changes in extracellular $Ca^{2+}$ levels, the CaSR regulates calcium by controlling parathyroid hormone secretion (PTH) in a feedback process and thus plays a major role in maintaining calcium homeostasis.[9] Although $Ca^{2+}$ is the endogenous ligand, the CaSR is also sensitive to several di- and trivalent cations ($Mg^{2+}$, $Gd^{3+}$) and organic cationic compounds such as spermine, polyamine, poly-L-arginine[10] and amyloid β-peptide.[8]

In the parathyroid gland, positive allosteric modulators of the CaSR, or calcimimetics, diminish PTH secretion.[11] While such modulators do not directly activate the receptor, they potentiate its action by increasing both the potency and efficacy of the endogenous agonists. In this context, primary hyperparathyroidism (1HPT) is an endocrine disorder characterized by excessive secretion of PTH by the parathyroid glands[12] while secondary hyperparathyroidism (2HPT), associated with chronic kidney disease, is associated with an increase in PTH as a compensatory response to reduced calcium levels. Calcimimetics are able to increase the sensitivity of the CaSR to circulating serum calcium and to consequently reduce both PTH and calcium levels.[13] Proof-of-concept was provided by one of the first-described calcimimetics NPS R-568 (**1**)[14] but the poor bioavailability of this compound precluded its clinical use. The structurally analogous derivative cinacalcet (**2**) (Sensipar®), however, provided clear clinical data demonstrating the ability of calcimimetic compounds to lower

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the circulating levels of PTH in patients with 1HPT or 2HPT and was the first calcimimetic to be approved for treatment of 2HPT in patients on dialysis and of hypercalcemia in patients with parathyroid cancer.[15]

The success of cinacalcet provided a powerful stimulus to the development of calcimimetics as therapeutic agents for the treatment of conditions characterized by high circulating PTH levels.[15–17] We ourselves have described several new families of calcimimetics including the acyclic arylsulfonamide derivatives exemplified by compound **3**[18] and conformationally restricted analogues of this family represented by calindol (**4**)[19] and the cyclic arylsulfonamide **5** (Fig. 1).[20] As with calcimimetics **1**–**5**, the α-methylarylamine group is a common structural feature of most of the calcimimetics described to date.[17] However, calcimimetics devoid of such a motif, for example, in which the branched α-methylarylamine is replaced by a linear urea functionality, have also been reported.[17,21,22]

Of these, calindol has proven to be a valuable tool for the study of the localization and mode of action of the CaSR. Thus, calindol was shown to stabilize a conformation of the CaSR's 7TM domain that in turn facilitates the active closed state of the VFT domain thereby increasing the receptor's affinity for $Ca^{2+}$.[23] Calindol has also been used to characterize the CaSR in endothelial cells in both porcine coronary and rat mesenteric arteries.[24] Calindol induced hyperpolarization of the vascular myocytes pointing to a role of CaSR in regulating blood pressure.[25] Calindol was employed to show that compromising the CaSR pathway may contribute to the vascular complications associated with type II diabetes via CaSR-mediated vasodilation.[26] Recently, it was found that coupling calindol with lanthanum chloride inhibited the calcification of vascular smooth muscle cells.[27] Finally, calindol allowed investigation of the function of the closely related GPRC6A receptors.[28,29] The latter are activated by calcium ions but also by basic amino acids such as L-ornithine.[30] While GPRC6A has been linked to inflammation and endocrine functions,[31] little is known concerning its physiological role.[32] We have previously shown, however, that calindol acts as a GPRC6A antagonist, inhibiting L-ornithine-mediated activation of this receptor.[29] These important findings encouraged us to undertake a more thorough structure–activity relationship study of calindol in an effort to optimize its calcimimetic activity and to eventually obtain more selective CaSR ligands with respect to GPRC6A (Fig. 2).

We now report the synthesis and calcimimetic activities of a series of structural analogues of calindol differing in the types and position of substituents incorporated on the indole moiety as well as replacement of the naphthalene unit by various aryl groups leading to the development of a calcimimetic having a 6-fold superior activity compared to calindol and displaying no significant activity with respect to GPRC6A.

## 2. Chemistry

Pharmacomodulation of the indole portion of calindol was first undertaken using a short and efficient methodology. Thus, appropriately substituted indole-2-carboxylic acids (**6**–**18**) (obtained from commercial sources or prepared as described in the Section 5) were coupled to (*R*)-1-(1-naphthyl)ethylamine (**19**)[33] using EDCI/HOBt in the presence of triethylamine (Scheme 1).[34] The resulting amides (**20**–**32**) were then reduced to the corresponding secondary amines (**33**–**45**) by the action of a mixture of lithium aluminium chloride and aluminium trichloride in refluxing THF. The structures and yields of the amides and amines so-prepared are reported in Table 1.

The strong amide reducing conditions could not be applied to preparation of nitrocalindol derivatives without concomitant reduction of the nitro group. Ethyl 5-nitroindole-2-carboxylate **46** and its 7-nitro analogue **47** were thus first reduced to the alcohols **48** and **49** using diisobutylaluminium hydride in THF and dichloromethane at −50 °C (Scheme 2). The alcohols were then coupled to (*R*)-naphthylethylamine (**19**) under standard Mitsunobu conditions to afford the desired 5-nitrocalindol (**50**) and 7-nitrocalindol (**51**).[35]

Some of the calindol analogues so-prepared then served as starting material for the synthesis of additional derivatives. Thus, 4-hydroxy- and 5-hydroxycalindol **52** and **53** were prepared by $BBr_3$-promoted demethylation of 4-methoxycalindol **33** and palladium/carbon catalyzed hydrogenolysis of 5-benzyloxycalindol **39**, respectively (Scheme 3).[36,37]

Calindol (**4**) was originally conceived as a structurally more rigid analogue of the acyclic arylsulfonamide **3**.[19] The gain in potency observed as a result encouraged us to study an alternative rigid calindol derivative, the 1-naphthylethylaminocarbazole **56**, which was easily prepared as shown in Scheme 4. Thus, indole-3-butanoic acid (**54**) was cyclized to give the carbazole-2-one derivative **55** using PPA in toluene[38] and the latter was subjected to reductive amination with (*R*)-1-(1-naphthyl)ethylamine (**19**) in the presence of sodium triacetoxyborohydride[39] providing compound **56** as a diastereomeric mixture with an overall yield of 68%.

Replacement of the naphthyl moiety of calindol by substituted aryl groups was also implemented. Thus, as shown in Scheme 5 and Table 2, the 1-arylethanamines **57**–**66** (obtained from commercial sources or prepared as described in the Section 5) were coupled to 1*H*-indole-2-carboxylic acid using EDCI/HOBt and the resulting amides **67**–**76** were reduced as before by the combined action of $LiAlH_4$ and $AlCl_3$ to provide calindol analogues **77**–**86**. Yields were generally moderate to good for the reduction step except in the case of bromo compounds **83** and **84** (in which partial debromination was observed) and the biphenyl derivative **85**, none of which exceeded 30%.

## 3. Results and discussion

The new calindol analogues synthesized were then evaluated for their calcimimetic activity in Chinese hamster ovarian (CHO) cells stably expressing cloned CaSR from rat brain (CHO(CaSR)) as previously described.[10] In these cells, $Ca^{2+}$ as well as positive allosteric modulators (calcimimetics) stimulate phospholipase C (PLC) activity resulting in accumulation of inositol phosphates (IP). In order to rapidly identify the most active compounds, the IP accumulation produced by first 10 μM and/or 3 μM of each new derivative was measured in the presence of 3 mM $[Ca^{2+}]_e$ and compared to the effect produced by calindol.

The effects of structural variations on the indole portion of calindol were first studied. Thus, as shown in Table 3, while all the indole-modified analogues displayed calcimimetic activity, both the nature and the position of the substituents introduced had significant effects on the level of this activity with respect to calindol. Compared to calindol, which inhibited 83% of IP accumulation at 3 μM, it was immediately apparent that introduction of one or more alkoxy substituents (i.e., methoxy derivatives **33**–**36**, 5-benzyloxy derivative **39**), a 5-methyl (**38**), a 3- or 5-halogen (**41**, **37**) led in all cases to reduced calcimimetic activity as did a 7-hydroxy function (**42**), the electron-withdrawing 5-methylsulfonyl (**40**) and 5-nitro (**50**) groups, IP accumulation inhibition being in the 0–60% range. Interestingly, introduction of a hydroxy function at the 4- or 5-positions (**52**, **53**) instead of the 7-position (**42**) had essentially no effect on the calcimimetic activity of calindol. Concerning the phenyl substituted analogues **43**–**45**, no significant gain or loss in calcimimetic activity was observed for the 5- and 7-phenyl derivatives (**44** and **45**) compared to calindol