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N^2-Benzyl-N^1-(1-(1-naphthyl)ethyl)-3-phenylpropane-1,2-diamines and conformationally restrained indole analogues: development of calindol as a new calcimimetic acting at the calcium sensing receptor

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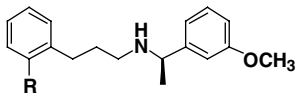
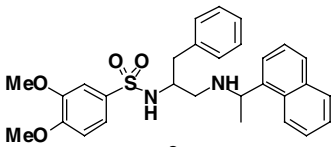
Abstract—The synthesis and calcimimetic activities of two new families of compounds are described. The most active derivatives of the first family, N^2-(2-chloro-(or 4-fluoro-)benzyl)-N^1-(1-(1-naphthyl)ethyl)-3-phenylpropane-1,2-diamine (**4b** and **4d**, respectively, tested at 10 μM) produced 98 ± 6% and 95 ± 4%, respectively, of the maximal stimulation of [^{3}H]inositol phosphates production obtained by 10 mM Ca^{2+} in CHO cells expressing the rat calcium sensing receptor (CaSR). The second family of calcimimetics was obtained by conformationally restraining the compounds of type **4** to provide the 2-aminomethyl derivatives **5**. One of these compounds, (*R*)-2-[*N*-(1-(1-naphthyl)ethyl)aminomethyl]indole ((*R*)-**5a**, calindol), displayed improved calcimimetic activity compared to **4b** and **4d** as well as stereoselectivity. In the presence of 2 mM Ca^{2+}, calindol stimulated [^{3}H]inositol phosphates accumulation with an EC_{50} of 1.0 ± 0.1 or 0.31 ± 0.05 μM in cells expressing the rat or the human CaSR, respectively. The calcimimetic activities of these novel compounds were shown to be due to a specific interaction with the CaSR.

The extracellular calcium sensing receptor (CaSR) is a G protein-coupled receptor (GPCR), which senses extracellular calcium $[Ca^{2+}]_e$ and regulates calcium ion homeostasis.[1] The CaSR possesses a long amino terminal tail typical of family 3 of GPCRs, which includes the metabotropic glutamate receptors, the γ-aminobutyric acid B receptor as well as certain pheromone and taste receptors.[1,2] CaSR cDNA has been cloned from such tissues as the parathyroid, thyroid, kidney and brain.[3–5] In the particular case of the parathyroid cell, the CaSR at the surface detects and responds to small changes of circulating $[Ca^{2+}]_e$, thereby regulating parathyroid



1 : R = Cl **NPS 568**
2 : R = H **NPS 467**



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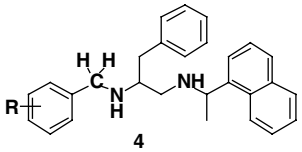

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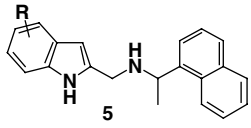

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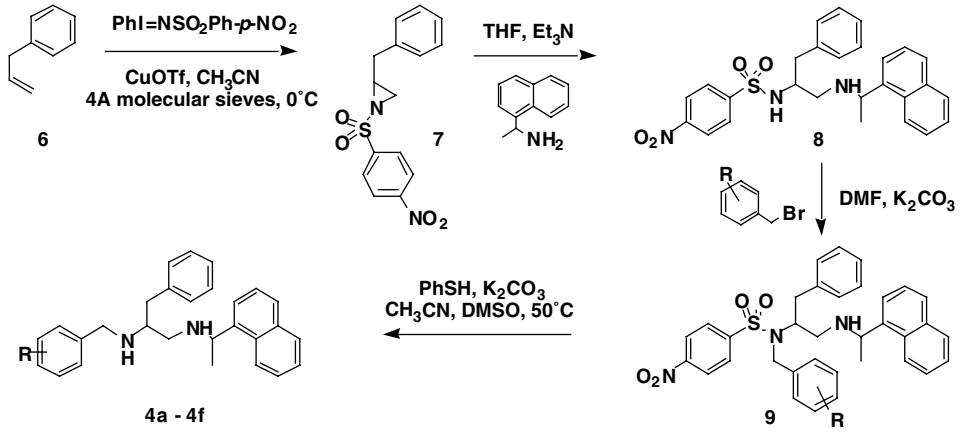
hormone (PTH) secretion.[1] Inactivating mutations of the CaSR gene have been identified as the cause of hypocalciuric hypercalcemia while activating mutations lead to hypocalcemia.[6]

In addition to calcium, cloned CaSR is activated by other divalent cations such as magnesium and by polyamines such as spermine, spermidine, aminoglycosides (e.g., neomycin) and by β-amyloid peptides.[7–11] In the search for more selective CaSR ligands, the synthetic phenylalkylamines, NPS 568 (**1**), and NPS 467 (**2**) have been found to allosterically mimic or potentiate the action of $[Ca^{2+}]_e$[8,12,13] and to interact at the level of the transmembrane domain.[14] For instance, such calcimimetics are able to increase the concentration of cytoplasmic calcium ($[Ca^{2+}]_i$) in bovine parathyroid cells and inhibit PTH secretion.[15,16]

The therapeutic potential of NPS 568 was suggested by experiments in rats in which this compound reduced or eliminated osteitis fibrosa[17] and led to an important reduction of cell proliferation in the parathyroid gland in a model reproducing chronic renal deficiency.[18] PTH levels were also decreased by NPS 568 in patients displaying this same disorder[19] as well as in menopaused women exhibiting primary hyperparathyroidism.[20] With the aim of developing calcimimetics displaying improved activities, tissue specificity and/or pharmacodynamic properties, we recently described[21] the synthesis of a new family of compounds, the N^2-arylsulfonyl-N^1-(1-(1-naphthyl)ethyl)-3-phenylpropane-1,2-diamines, exemplified by the 3,4-dimethoxy derivative **3** whose calcimimetic activities were comparable to that of NPS 568. In this communication, we describe the use of CaSR ligands of type **3** as a starting point for the development of two further families of calcimimetics, their *N*-benzyl analogues of general structure **4** and the 2-substituted indole derivatives **5**, which may be considered as rigid analogues of **3** and of NPS 568 (**1**).

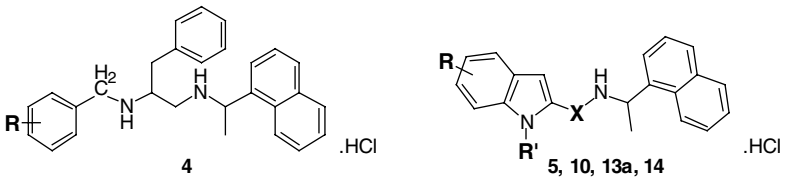
We first compared the effect on calcimimetic activity of replacing the polar arylsulfonyl function of the more active analogues of type **3** by a more lipophilic benzyl group (i.e., $ArSO_2 \rightarrow ArCH_2$). Such a transformation could have important repercussions on tissue distribution, metabolism, activity and selectivity. For the preparation of these compounds, we chose as starting material the *N*-(*p*-nitrophenylsulfonyl)aziridine **7** easily synthesized, as shown in Scheme 1, by reaction of allylbenzene **6** in acetonitrile with 1 equiv of [(*N*-*p*-nitrophenylsulfonyl)imino]phenyliodane in the presence of copper(I) triflate as catalyst.[22–24] The *N*-(*p*-nitrophenylsulfonyl) (*N*-nosyl) group presents the advantages of providing highly reactive aziridines, of being easily removed after aziridine ring opening[25] and, in our particular case, of allowing selective alkylation of the *N*-sulfonyl nitrogen. Thus, compound **7** reacted smoothly with 1-(1-naphthyl)ethylamine in THF containing 0.1 equiv of triethylamine to give 60% of diamine **8**. The sulfonamide nitrogen could then be selectively alkylated by 1.2–1.5 equiv of the appropriate benzyl bromide in DMF using potassium carbonate as base, providing compounds **9**. Finally, removal of the *N*-(*p*-nitrophenylsulfonyl) group of each of the latter compounds was effected by treatment with 3 equiv of thiophenol and potassium carbonate at 50 °C in acetonitrile containing 2% DMSO.

The resulting compounds **4a**–**f**, in the form of their hydrochloride salts, were then evaluated for their calcimimetic activity in Chinese hamster ovarian (CHO) cells transfected [CHO(CaSR)] or not [CHO(WT*)] with an expression vector containing cloned CaSR from rat brain[7] previously shown to be useful for characterizing calcimimetics.[8,21] Activation of the CaSR by Ca^{2+} as well as by NPS 568 has been shown to stimulate PLC activity and accumulation of tritiated inositol phosphate ([^{3}H]IP) in CHO(CaSR) cells prelabelled with myo-[^{3}H]inositol.[7] The [^{3}H]IP accumulation produced by compounds **4a**–**f** (10 μM) in the presence of 2 mM $[Ca^{2+}]_e$ was measured and compared to the effect produced by NPS 568 as well as to the effect produced by 10 mM $[Ca^{2+}]_e$ (a concentration resulting in maximal CaSR activation). As shown in Table 1 and Figure 1, all the *N*-benzyl derivatives **4** prepared demonstrated calcimimetic activity, producing at 10 μM concentration between approximately 70% and 100% of the maximal response obtained by 10 mM Ca^{2+}. The effects of these



Scheme 1.

Table 1. Accumulation of [^{3}H]IP induced by the test compounds in CHO(CaSR) cells expressing rat cloned CaSR



Compound no. (10 μM)	R	R′	X	% [^{3}H]IP accumulation[a]
1 (NPS 568)	—	—	—	105 ± 10
4a	H	—	—	80 ± 8 (78 ± 7)[b]
4b	2-Cl	—	—	98 ± 6 (79 ± 7)
4c	3-Cl	—	—	78 ± 6 (83 ± 3)
4d	4-F	—	—	95 ± 4 (62 ± 6)
4e	4-OMe	—	—	80 ± 6 (80 ± 8)
4f	3,4-Di-OMe	—	—	74 ± 17 (97 ± 11)
(*R*,*S*)-**5a**	H	H	CH_2	103 ± 6
(*R*)-**5a**	H	H	CH_2	117 ± 5
(*S*)-**5a**	H	H	CH_2	32 ± 5
5b	5-F	H	CH_2	87 ± 7
5c	5-Cl	H	CH_2	60 ± 1
5d	5-OMe	H	CH_2	55 ± 5
5e	5,6-Di-OMe	H	CH_2	34 ± 1
10	H	SO_2Ph(3,4-OMe)	CH_2	54 ± 5
13a	H	H	C=O	2 ± 3
14	H	CH_3	CH_2	79 ± 13

[a] CHO(CaSR) cells expressed rat Ca^{2+} sensing receptor cloned from rat brain.[5] These cells were cultured in basal Ham's F-12 medium (0.3 mM Ca^{2+}, 0.6 mM Mg^{2+}) as previously described.[7] Cells, in 24-well plates, were cultured overnight in their growth medium containing 0.5 μCi/mL myo-[^{3}H]inositol (Amersham Corp.). Drugs were dissolved at 10 mM in ethanol and then diluted in basal medium. Cells were incubated with drugs (10 μM) at 37 °C and activation of the phospholipase C enzyme was estimated after quantification of [^{3}H]inositol phosphates ([^{3}H]IP) accumulation that was performed as described.[7] Results are expressed as percentage of the maximal response obtained by 10 mM Ca^{2+} and are means ± SEM of two to five independent experiments performed in triplicate. Results varied by less than 5% in any given experiment.

[b] Values in parentheses refer to the % [^{3}H]IP accumulation for the equivalent sulfonamides at the same concentration; from Ref. 21.

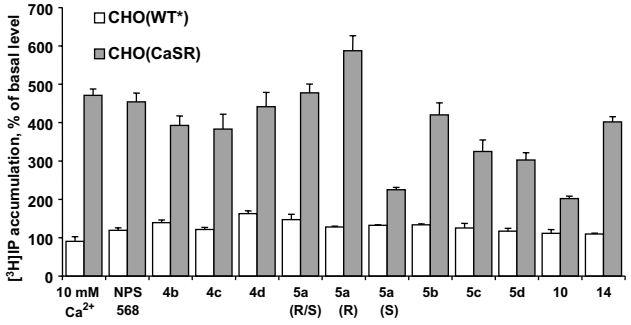
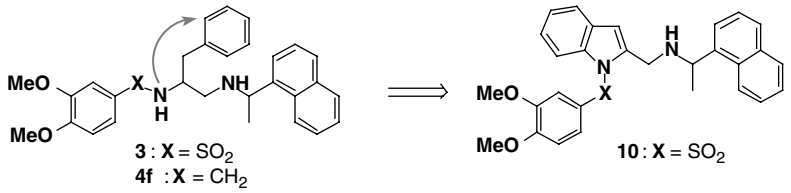


Figure 1. Effects of test compounds of general structure **4** and **5** and of NPS 568 in CHO(CaSR) cells expressing rat cloned CaSR or in CHO(WT*) cells. CHO(CaSR) or CHO(WT*) cells were prelabeled with myo-[^{3}H]inositol in basal Ham's F-12 medium as described in legend to Table 1. [^{3}H]IP accumulation was determined for each test compound and for NPS 568 at 10 μM and in presence of 2 mM $[Ca^{2+}]_e$. The effect of 10 mM Ca^{2+} is presented for comparison. Results are expressed as % of [^{3}H]IP basal level and are means ± SEM of three to five independent experiments performed in triplicate. In CHO(WT*) cells, test compounds and NPS 568 have limited effects on [^{3}H]IP basal level (less than 40% of basal value) whereas they produced robust accumulation of [^{3}H]IP in CHO(CaSR) cells suggesting that these compounds stimulate phospholipase C activity by interacting specifically with the CaSR.

compounds were, moreover, of the same order of magnitude as the *N*-sulfonyl analogues **3**[21] having the same pattern of substitution on the phenyl ring. Thus, the unsubstituted (**4a**), 3-chloro (**4c**) and 4-methoxy derivatives (**4e**) all stimulated [^{3}H]IP accumulation by about 80%, while both the 2-chlorobenzyl (**4b**) and 4-fluorobenzyl (**4d**) derivatives were slightly more active (98 ± 6% and 95 ± 4% stimulation, respectively). On the other hand, the 3,4-dimethoxy substituents, shown to provide the optimal calcimimetic activity in the *N*-sulfonyl series[21] produced a slight diminution of activity in the *N*-benzyl series (74 ± 17% stimulation for **4f** vs 97 ± 11% for **3**). The calcimimetic activities of the most active of these *N*-benzyl derivatives (**4b**,**d**) were comparable, though slightly inferior, to that of NPS 568 at the same concentration of 10 μM (105 ± 10% stimulation). When compounds **4b–4d** were tested in CHO(WT*) cells at the same concentration (10 μM) (Fig. 1), no significant increase of [^{3}H]IP accumulation was measured thereby indicating the specificity of the observed response in CHO(CaSR) cells with this new series of calcimimetics as was the case with **3**.[21]

While in vivo the *N*-benzyl derivatives may present pharmacokinetic and/or pharmacodynamic advantages over their *N*-sulfonyl analogues, it still seemed evident that it would be advantageous to obtain calcimimetic-type compounds demonstrating higher intrinsic activities with respect to the CaSR. In an effort to achieve this, we turned our attention to the study of conformationally restricted analogues of **3** and **4**. Though there are many ways in which a highly flexible molecule such as **3** can be made more rigid, an indole derivative of type **10**,



Scheme 2.

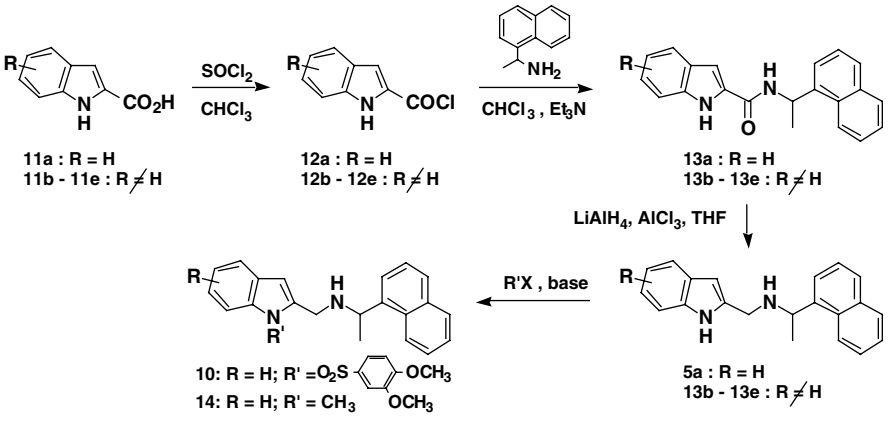
in which the nitrogen atom of the *N*-sulfonyl group is covalently bonded to the 3-phenyl ring, seemed particularly attractive since the resulting 2-substituted indole derivative (Scheme 2) should be easily accessible, thus allowing rapid validation of the working hypothesis.

Compound **10** was thus synthesized in straightforward fashion as shown in Scheme 3. Treatment of indole-2-carboxylic acid **11a** with thionyl chloride in chloroform afforded the acid chloride **12a**. The latter was reacted with 1-(1-naphthyl)ethylamine in chloroform in the presence of triethylamine to give the carboxamide **13a**, which was reduced to the amine **5a** with lithium aluminum hydride/aluminum chloride in THF. Treatment of this compound with 3,4-dimethoxybenzenesulfonyl chloride then gave **10**.

Evaluation of compound **10** for calcimimetic activity in CHO(CaSR) cells showed, however, that it was considerably less active than the unconstrained analogue **3** ($54 \pm 5\%$ stimulation of IP production for **10** vs $97 \pm 11\%$ for **3** at 10 μM concentration) (Table 1). Interestingly, investigation of the precursor of compound **10** having the N-unsubstituted indole moiety (compound **5a**) showed that it was a considerably more efficient stimulator of the IP response ($103 \pm 6\%$ at 10 μM). Other analogues of **5a** having substituents on the indole ring were subsequently prepared in the same manner (compounds **5b**–**e**), and tested for their calcimimetic properties. Substitution of the C-5 indole position by halogens (F, Cl) or by an alkoxy group (MeO) led to loss of activity relative to **5a** as did introduction of 5,6-dimethoxy groups (Table 1). Indole N-methylation was also found to be detrimental to activity (**14**: $79 \pm 13\%$ stimulation) while the precursor carboxamide **13a** was inactive.

The *R* and *S* enantiomers of the most active indole derivative, racemic **5a**, were then prepared separately from the corresponding (*R*)- and (*S*)-aminoethylnaphthalenes and their calcimimetic activities were compared in CHO(CaSR) cells. As shown in Table 1, (*R*)-**5a** (calindol) was shown to be considerably more active than (*S*)-**5a** ($117 \pm 5\%$ vs $32 \pm 5\%$ stimulation, respectively) and as active as NPS 568 ($105 \pm 10\%$ stimulation, Table 1 and Fig. 1). Increasing concentrations of (*R*,*S*)-**5a**, (*R*)-**5a** or (*S*)-**5a** led to a dose-dependent increase of [^{3}H]IP accumulation in CHO(CaSR) cells (Fig. 2A), confirming that (*R*)-**5a** is slightly more effective than the racemic compound but at least 20-fold more-so than (*S*)-**5a**. Both compounds gave a maximal stimulation representing 130% of the 10 mM Ca^{2+} activation. We then determined the potency of the more active compound in eliciting the inositol phosphates response in HEK293 cells transiently expressing the human CaSR. Analysis of the dose response curve (Fig. 2B) gave an EC_{50} for (*R*)-**5a** of 0.31 ± 0.05 μM with a maximal stimulation comparable to that obtained with 10 mM Ca^{2+}. Moreover, when these indolic calcimimetics (**5a**–**d**, **10**, **14**) were tested on CHO(WT*) cells, [^{3}H]IP accumulation was very limited indicating that the CaSR was responsible for the observed response in CHO(CaSR) cells (Fig. 1). These data indicate that calindol is a potent and stereoselective calcimimetic of both the rat and the human CaSR.

In conclusion, this study describes the discovery of two new types of CaSR ligands demonstrating calcimimetic activity. Both series of compounds were derived from the previously described *N*-arylsulfonyl family of calcimimetics, the most active of which was the dimethoxy analogue **3**. In the first group of compounds, replacement of the *N*-arylsulfonyl group by an *N*-benzyl group



Scheme 3.

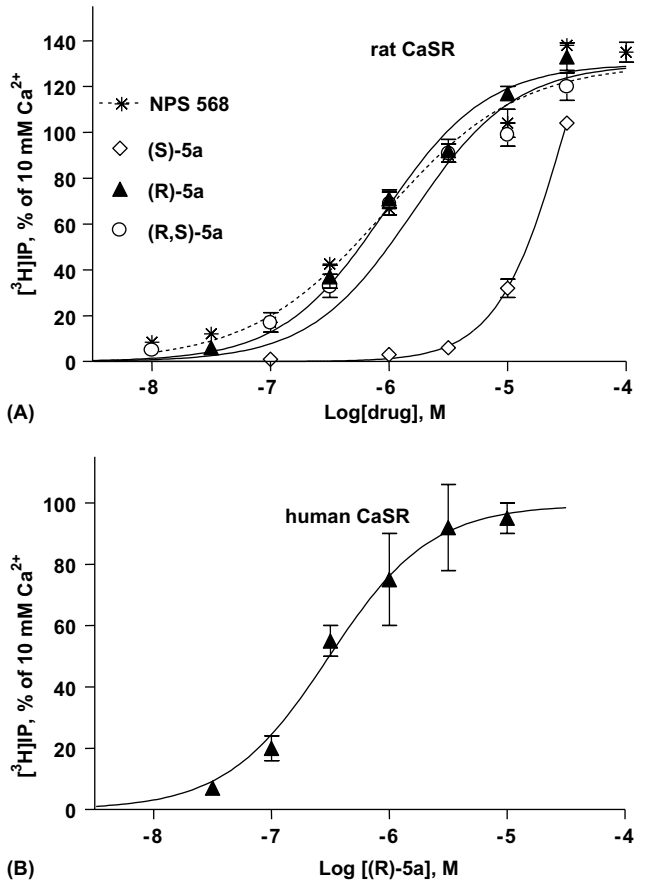


Figure 2. Potency of **5a** compounds and NPS 568 in stimulating accumulation of [^{3}H]IP in (A) CHO(CaSR) cells stably expressing rat CaSR or (B) HEK293 cells transiently transfected[26] with the human CaSR. Cells were prelabeled with myo-[^{3}H]inositol in basal Ham's F-12 medium as described in legend to Table 1. [^{3}H]IP accumulation induced by racemic (*R*,*S*)-**5a** or the (*R*)-**5a** and (*S*)-**5a** enantiomers was determined in presence of 2 mM $[Ca^{2+}]_e$ as indicated. For NPS 568, the determination was made in the presence of 2.3 mM Ca^{2+}. Results are expressed as % of 10 mM Ca^{2+} response and are representative of three to five independent experiments performed in triplicate. In CHO (CaSR), the (*R*)-**5a** (calindol) and (*R*,*S*)-**5a** compounds display an EC_{50} of 1.0 ± 0.1 and of 1.3 ± 0.2 μM, respectively, and produced a similar maximum stimulation representing 130% of the 10 mM Ca^{2+} activation. In HEK293 cells transfected with the human CaSR, (*R*)-**5a** (calindol) displays an EC_{50} of 0.31 ± 0.05 μM.

provided equally active calcimimetics (**4b**,**d**). An interesting observation was that the pattern of substitution of the N-2 aryl ring assuring maximal activity of the sulfonyl derivatives (e.g., the 3,4-dimethoxy derivative **3**) was not the same as for the *N*-benzyl derivatives (e.g., the 2-chloro derivative **4b**). Moreover, in an effort to further improve the efficacy of these compounds, rigid analogues in the form of the 2-aminomethyl indole derivatives **5** were prepared. One of these, calindol ((*R*)-**5a**), evaluated in both rat and human CaSR, was found to be one of the most active calcimimetic compounds known to date. In view of the very restricted number of such compounds so far described, both series of ligands offer new perspectives for the development of clinically useful calcimimetics acting specifically at the CaSR pending a thorough study of their structure–activity relationships and their effects in vivo.

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