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N^1-Arylsulfonyl-N^2-(1-aryl)ethyl-3-phenylpropane-1,2-diamines as Novel Calcimimetics Acting on the Calcium Sensing Receptor

Philippe Dauban,[a] Sandrine Ferry,[b] Hélène Faure,[b] Martial Ruat[b] and Robert H. Dodd[a,*]

[a]*Institut de Chimie des Substances Naturelles, Centre National de la Recherche Scientifique, 91198 Gif-sur-Yvette Cedex, France*
[b]*Laboratoire de Neurobiologie Cellulaire et Moléculaire, Junior Group ATIPE, UPR 9040 du CNRS, 91198 Gif-sur-Yvette Cedex, France*

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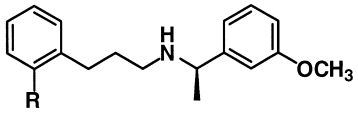
Abstract—The synthesis and calcimimetic properties of N^1-arylsulfonyl-N^2-(1-aryl)ethyl-3-phenylpropane-1,2-diamines are described. The most active compound of the series (**3n**, used at 10 μM) produced 97±11% of the maximal stimulation of [^{3}H]IP production obtained by 10 mM Ca^{2+} in CHO cells expressing the calcium sensing receptor (CaSR). This calcimimetic activity was due to a specific interaction of this compound with the CaSR.

The extracellular calcium sensing receptor (CaSR) is a G-protein coupled receptor which senses extracellular calcium $[Ca^{2+}]_e$ as demonstrated recently by the cloning of its cDNA from various tissues including parathyroid and brain.[1–3] This protein belongs to the heptahelical family of receptors that includes metabotropic glutamate receptors (mGluR)[4] and the γ-aminobutyric acid receptor ($GABA_B$-R)[5] as well as certain pheromone and taste receptors.[6,7]

At the surface of the parathyroid cell, the CaSR detects and responds to small changes of circulating $[Ca^{2+}]_e$ leading to regulation of parathyroid hormone (PTH) secretion.[2,8] Elevated concentrations of $[Ca^{2+}]_e$ decrease PTH secretion such that the CaSR provides the essential component of calcium homeostasis in the mammalian organism. In humans, point mutations in the CaSR structure produce modifications in $[Ca^{2+}]_e$ set point that can result in dramatic physiological effects.[9–11] Expression of CaSR occurs also in other tissues implicated in calcium homeostasis including thyroid and kidney, and in brain where its expression has been observed in neurons and oligodendrocytes.[3,12,13]

Fibroblasts transfected with cloned rat CaSR have allowed initiation of detailed studies of CaSR pharmacology and its associated transduction signals.[14–16] CaSR is positively coupled to phospholipases C and A2 and its activity can be evaluated by the accumulation of inositol phosphates, intracellular calcium mobilization and release of arachidonates.

Besides calcium, cloned CaSR is activated by magnesium and other divalent cations, by charged molecules such as spermine, spermidine and aminoglycosides and possibly by β-amyloid peptides.[1,14,15,17,18] Recently, two small, non-cationic organic molecules (NPS 568 and NPS 467, **1** and **2**, respectively) were reported to interact with cloned CaSR and to mimic or potentiate the effects of $[Ca^{2+}]_e$.[15,16,19] These compounds, referred to as calcimimetics, were shown to increase the concentration of cytoplasmic calcium ($[Ca^{2+}]_i$) in bovine parathyroid cells and inhibit PTH secretion. In the thyroid, CaSR presumably regulates calcitonin secretion and might be preferentially targetted by NPS 467 compared to parathyroid CaSR. This selectivity is possibly related to minor biochemical differences in the core protein, suggesting that tissue-specific calcimimetics can be designed.[8] Since these compounds only produce effects in the presence of $[Ca^{2+}]_e$, it has been hypothesized that they interact allosterically with the CaSR to increase its sensitivity to $[Ca^{2+}]_e$.



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

In rats, NPS 568 reduced or eliminated osteitis fibrosa[20] and allowed 20–50% reduction of cell proliferation

*Corresponding author. Tel.: +33-1-69-82-45-04; fax: +33-1-69-07-72-47; e-mail: robert.dodd@icsn.cnrs-gif.fr

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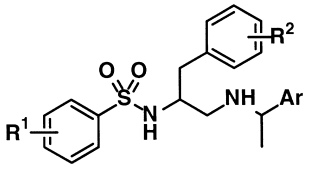
observed in the parathyroid gland in a model reproducing chronic renal deficiency.[21] In humans, NPS 568 reduced PTH concentrations in patients suffering from the same disorder[22] and was, moreover, shown to decrease PTH and $[Ca^{2+}]_e$ levels in menopaused women exhibiting primary hyperthyroidism.[23] Thus, while the clinical value of calcimimetics acting at the CaSR has been clearly demonstrated in syndromes where calcium homeostasis and PTH secretion are perturbed, there is an obvious need for new compounds showing improved activity, tissue specificity and/or pharmacokinetic profiles. As a first step in this direction, we wish to report herein the calcimimetic activities of *N*-arylsulfonyl-1,2-diamines of general structure **3**, a new class of CaSR ligands.



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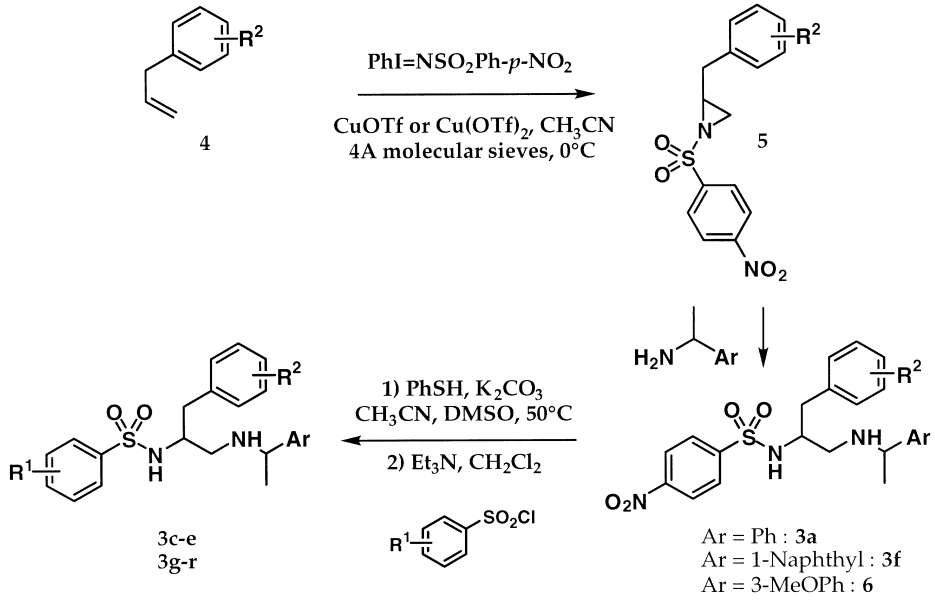
The compounds developed in this study were synthesized as shown in Scheme 1. Thus, commercially-available aryl-substituted allylbenzenes **4** in acetonitrile were treated at 0 °C with 1 equiv of [(*N*-*p*-nitrophenylsulfonyl)imino]phenyliodinane in the presence of catalytic copper (I or II) triflate and molecular sieves to give the corresponding *N*-sulfonylaziridines **5**.[24,25] The latter, upon reaction with a 1-arylethylamine at room temperature, afforded the *p*-nitro derivatives **3a, 3f, 6** in 90–95% yields. Removal of the *N*-*p*-nitrophenylsulfonyl group could be effected by heating the latter compounds at 50 °C in acetonitrile containing 2% DMSO, 3 equiv of thiophenol and an excess potassium carbonate.[26] The resulting primary amines were then reacted in dichloromethane with 1.1 equiv of various substituted arylsulfonyl chlorides in the presence of triethylamine to provide the corresponding mono-*N*-arylsulfonyl derivatives **3c–e, 3g–r**. The hydrochloride salts of these amines were prepared for biological evaluation.

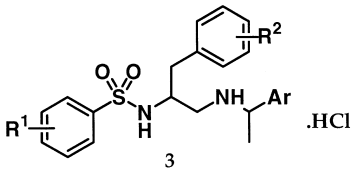
The calcimimetic-like properties of the synthesized compounds **3a–r** were evaluated in a biochemical assay performed on Chinese hamster ovarian (CHO) cells transfected with an expression vector containing cloned CaSR from rat brain [CHO(CaSR)] or not [CHO(WT*)].[14] CHO(CaSR) has been shown to stimulate tritiated inositol phosphate ([^{3}H]IP) accumulation upon activation of the CaSR by Ca^{2+} and other divalent cations and by NPS 568.[14] Thus, [^{3}H]IP accumulation produced by 10 μM of each compound or NPS 568 in the presence of 2 mM $[Ca^{2+}]_e$ was measured and compared to the effect produced by 10 mM $[Ca^{2+}]_e$ (a concentration eliciting maximal CaSR activation). Results are shown in Table 1. For several representative compounds, a comparison of their potency in stimulating [^{3}H]IP accumulation in CHO(CaSR) and CHO(WT*) cells was made in the presence of 2 mM $[Ca^{2+}]_e$. Results are shown in Figure 1.

The reference compound, NPS 568 (**1**), produced 106±15% of the maximal response and thus acts as a calcimimetic in CHO(CaSR) cells. New compounds having an unsubstituted α-methylbenzylamino group were first studied. While both the *p*-nitrophenylsulfonamide **3a** and its reduced amino form **3b** (obtained by catalytic hydrogenation of **3a**) were practically inactive, the *p*-toluenesulfonamide **3c** showed some weak calcimimetic character (16±2%). By analogy with the structure of NPS 568, an *m*-methoxy group was then included on the α-methylbenzyl moiety. This led, in the case of compound **3d**, to a small increase in activity (36±8% stimulation) compared to the de-OMe derivative **3c**. The simultaneous presence of a *p*-methoxy group on the phenylsulfonamide moiety (compound **3e**) had no effect on this activity (34±4% stimulation). The α-methylbenzyl group was then replaced by the 1-(1-naphthyl)ethyl



Scheme 1.

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



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Compound (10 μM)	Ar	R^1	R^2	% [^{3}H]IP Accumulation
NPS 568				106±15
3a	Ph	4-NO_2	H	8±3
3b	Ph	4-NH_2	H	2±2
3c	Ph	4-CH_3	H	16±2
3d	3-CH_3OPh	4-CH_3	H	36±8
3e	3-CH_3OPh	4-CH_3O	H	34±4
3f	1-Naphthyl	4-NO_2	H	28±10
3g	1-Naphthyl	4-CH_3	H	68±8
3h	1-Naphthyl	4-Cl	H	38±15
3i	1-Naphthyl	4-CF_3	H	27±2
3j	1-Naphthyl	H	H	78±7
3k	1-Naphthyl	4-CH_3O	H	80±8
3l	1-Naphthyl	2-Cl	H	79±7
3m	1-Naphthyl	3-Cl	H	83±3
3n	1-Naphthyl	3,4-di-CH_3O	H	97±11
3o	1-Naphthyl	4-CH_3O	2-Cl	60±3
3p	1-Naphthyl	4-CH_3O	2-CH_3O	65±1
3q	1-Naphthyl	4-CH_3O	4-CH_3O	45±1
3r	1-Naphthyl	4-CH_3O	3,4-OCH_2O	47±2

[a]CHO(CaSR) cells expressed rat Ca^{2+} sensing receptor cloned from rat brain.[3] These cells were cultured in basal Ham's F-12 medium (0.3 mM Ca^{2+}, 0.6 mM Mg^{2+}) as previously described.[14] 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.[14] Results are expressed as percentage of the maximal response obtained by 10 mM Ca^{2+} and are means ±S.E.M. of two to five independent experiments performed in triplicate. Results varied by less than 5% in any given experiment.

group. In the case of the *p*-nitrophenylsulfonamide derivative **3f**, this led to a substantial increase in activity (28±10% stimulation) compared to **3a**, while replacement of the nitro function of **3f** by a methyl group (compound **3g**) led to a further 2.5-fold increase in activity (68±8% stimulation). Whereas replacement of the methyl group of **3g** by chloride (compound **3h**) or trifluoromethyl (**3i**) led to significant loss of activity (38±15% and 27±2% stimulation respectively), both the absence of a *para* substituent (**3j**) or the presence of a *p*-methoxy group (**3k**) improved activity (78–80% stimulation). This activity was maintained in the case of the *o*-Cl and *m*-Cl derivatives **3l** and **3m**, respectively. The 3,4-dimethoxy derivative **3n** was subsequently observed to be the most active calcimimetic of this series, providing 97±11% of the stimulation of [^{3}H]IP accumulation produced by 10 mM Ca^{2+}, and thus comparable to NPS 568 in activity.

Attention was also given to substituents on the phenyl ring separated from the secondary amine function by three carbon atoms, as in NPS 568. By analogy with the latter, the *o*-chloro derivative was prepared (**3o**) but this

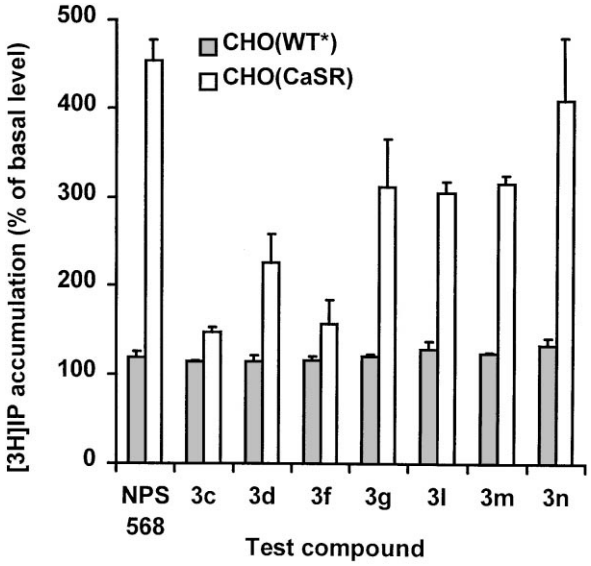

Figure 1. Effects of test compounds of general structure 3 and NPS 568 in CHO(CaSR) cells expressing rat cloned CaSR or in CHO(WT*) cells. CHO(CaSR) or CHO(WT*) cells were prelabeled with myo-[^{3}H]inositol in basal Ham's F-12 medium as described in legend to Table 1. [^{3}H]IP accumulation was determined for each test compound and for NPS 568 at 10 μM and in presence of 2 mM $[Ca^{2+}]_e$. Results are expressed as % of [^{3}H]IP basal level and are means ±S.E.M. 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 more robust accumulation of [^{3}H]IP in CHO(CaSR) cells suggesting that these compounds stimulate phospholipase C activity by interacting specifically with CaSR.

modification was shown to be detrimental to calcimimetic activity (compare to compound **3k**). Other substituents [*o*-methoxy (**3p**), *p*-methoxy (**3q**), 3,4-methylenedioxy (**3r**)] also led to diminished activities.

As shown in Figure 1, NPS 568 had limited effect on [^{3}H]IP accumulation in CHO(WT*) cells (i.e., not expressing CaSR) indicating that the large stimulation in [^{3}H]IP accumulation produced by this compound in CHO(CaSR) cells (460% of basal values) is due to its specific interaction with the CaSR. A similar observation was made with the test compounds. Thus, even the weakly stimulating compounds **3c**, **3d** and **3f**, did so only in the presence of the CaSR, with only negligible effects in CHO(WT*) cells. The selective action was even more evident for the more active compounds **3g**, **3l** and **3m**. For the most active compound **3n**, a profile similar to that of NPS 568 was observed, with a stimulation of [^{3}H]IP accumulation of 446% in CHO(CaSR) cells and again, less than 40% in CHO(WT*) cells. It thus appears that these arylsulfonyl derivatives are stimulating IP production by interacting specifically with the CaSR.

In conclusion, the new N^1-arylsulfonyl-N^2-arylethyl diamines described herein represent the second family of compounds displaying calcimimetic activity via interaction with the CaSR. While these compounds incorporate an NPS-like backbone, the presence of the additional arylsulfonamide moiety allows for a great deal of further structural modifications. Such modifications should

permit improvement of potencies and development of tissue-specific calcimimetics. Studies aimed at further characterizing the in vitro and in vivo calcimimetic properties of these compounds are currently underway.

Acknowledgements

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