N^1-Arylsulfonyl-N^2-(1-(1-naphthyl)ethyl)-1,2-diaminocyclohexanes: A New Class of Calcilytic Agents Acting at the Calcium-Sensing Receptor

Albane Kessler,[a] Hélène Faure,[b] Marie Cécile Roussanne,[b] Sandrine Ferry,[b] Martial Ruat,[b] Philippe Dauban,*[a] and Robert H. Dodd*[a]

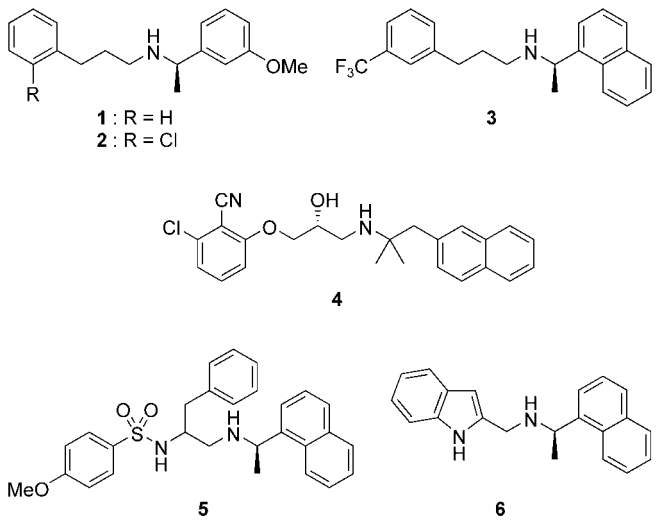
The calcium-sensing receptor (CaSR) is a G-protein-coupled receptor (GPCR) that senses extracellular calcium $[Ca^{2+}]_e$, thereby maintaining calcium homeostasis in the organism. It belongs to the heptahelical family of receptors, which includes the metabotropic glutamate receptors (mGluR), the B-type γ-aminobutyric acid receptor ($GABA_B$-R) as well as certain pheromone and taste receptors.[1,2] As shown by cloning of its cDNA, the CaSR is present in many tissues, including the brain and the parathyroid gland.[3–5] At the surface of the latter, the CaSR detects and responds to small changes of circulating $[Ca^{2+}]_e$, thereby regulating parathyroid hormone (PTH) secretion. There exists a negative feedback relationship between $[Ca^{2+}]_e$ levels and PTH secretion. Thus, high levels of $[Ca^{2+}]_e$ activate the CaSR, thereby inhibiting PTH secretion, whereas low $[Ca^{2+}]_e$ levels diminish CaSR activation, stimulating PTH secretion. These observations led to the hypothesis that compounds that could activate this receptor (CaSR "agonists") should lead to decreased levels of circulating PTH. Such "agonists" could be of therapeutic benefit in diseases such as hyperparathyroidism.[6–8] Alternatively, compounds that could block the action of the CaSR (CaSR "antagonists") should theoretically lead to increased plasma levels of PTH. Because increased PTH levels are associated with bone formation,[9] CaSR antagonists could provide a novel approach to the treatment of osteoporosis. However, to be clinically useful, such compounds would also have to provide only short-term elevations in PTH levels since chronically high PTH levels are known to lead to bone loss.[10]

The first small organic molecules reported to interact specifically and with good affinities with the CaSR were NPS *R*-467 (**1**) and NPS *R*-568 (**2**, Scheme 1).[8,11] These compounds were shown to increase the concentration of cytoplasmic calcium ($[Ca^{2+}]_i$) in bovine parathyroid cells and, most significantly, to inhibit PTH secretion.[11,12] Since these compounds, referred to

[a] *Dr. A. Kessler, Dr. P. Dauban, Dr. R. H. Dodd*
Institut de Chimie des Substances Naturelles
Centre National de la Recherche Scientifique
91198 Gif-sur-Yvette Cedex (France)
Fax: (+33)-1-69-07-72-47
E-mail: robert.dodd@icsn.cnrs-gif.fr

[b] *Dr. H. Faure, Dr. M. C. Roussanne, Dr. S. Ferry, Dr. M. Ruat*
Institut de Neurobiologie Alfred Fessard, IFR 2118 CNRS
Laboratoire de Neurobiologie Cellulaire et Moléculaire UPR 9040 CNRS
91198 Gif-sur-Yvette Cedex (France)

 DOI: 10.1002/cbic.200400049



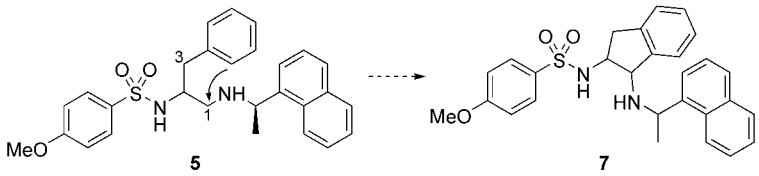
Scheme 1. *Structural formulae of calcium-sensing receptor calcimimetics (**1**–**3**, **5**, **6**) and a calcilytic (**4**).*

as calcimimetics, produce their effects only in the presence of $[Ca^{2+}]_e$, it can be hypothesized that they interact with the CaSR in an allosteric fashion, leading to this receptor's overall increased sensitivity to calcium ions. An analogue of **1** and **2**, Cinacalcet (**3**), is presently undergoing clinical trials for the treatment of hyperparathyroidism.[13]

While these calcimimetics demonstrated that PTH levels could be effectively lowered by activation of the CaSR, the corollary, that is, that inhibition of the CaSR increases PTH levels, could not be demonstrated because of the lack of CaSR antagonists or calcilytics. Recently, however, such a compound (NPS 2143, **4**) has been reported.[14,15] In HEK293 cells that artificially express the human parathyroid CaSR, this compound was shown to inhibit the increase in intracellular Ca^{2+} concentrations produced by increasing $[Ca^{2+}]_e$ (IC_{50} = 43 nM) as well as that produced by the calcimimetic NPS *R*-467 (**1**). Moreover, NPS 2143 significantly increased PTH secretion in primary cultures of bovine parathyroid cells. While in vivo, NPS 2143 effectively produced a four- to fivefold increase in plasma PTH levels in both healthy rats and in ovariectomized rats (the classical animal model of osteoporosis), these increased levels were too sustained over time to cause a net increase in bone density, a chronic elevation of PTH levels being known to lead to bone loss.[16] While these results were partially disappointing, they had the merit of offering a proof-of-concept in that it was demonstrated that a calcilytic agent can effectively increase PTH secretion in vivo.

We recently reported the discovery of two new families of calcimimetic compounds. The first of these, represented by the N^1-(4-methoxybenzenesulfonyl)-1,2-diaminopropane derivative **5** (Scheme 1), is a direct analogue of NPS *R*-568 (**2**), differing mainly by the addition of an arylsulfonamide side-chain.[17] Compound **5** (10 μM) produced over 80% of the maximal stimulation of inositol phosphate ([^{3}H]IP) production obtained by 10 mM Ca^{2+} in Chinese hamster ovary (CHO) cells that express the CaSR. However, in an effort to obtain more potent compounds and/or obtain new calcilytic agents, we proceeded to prepare rigid analogues of compound **5**. The 2-aminomethylindole derivative **6** (Calindol) represents just such a conformationally restrained analogue. This compound displayed calcimimetic activity now superior to that of **5** and comparable to that of NPS *R*-568 (117% stimulation at 10 μM concentration).[18] Thus, rigidification of the highly flexible compound **5** did effectively lead to more potent calcimimetics. However, the development of new calcilytic derivatives still eluded us.

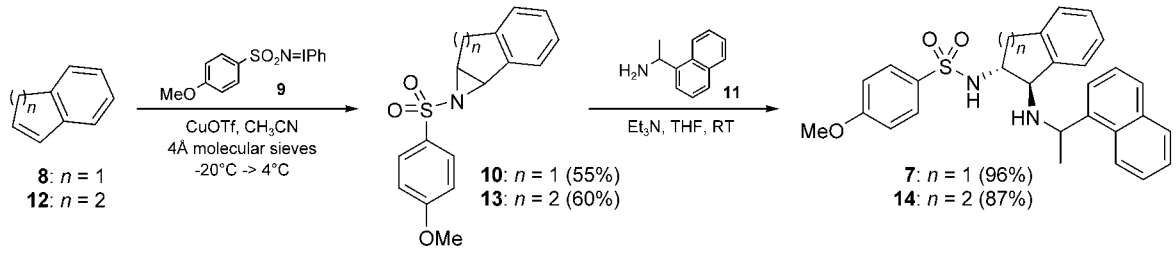
We thus proceeded to prepare rigid analogues of **5** in an alternative fashion. While there are many different ways of doing so, we believed a molecule such as **7** (Scheme 2), in which the



Scheme 2. *Proposed conformationally restrained derivative of the calcimimetic **5**.*

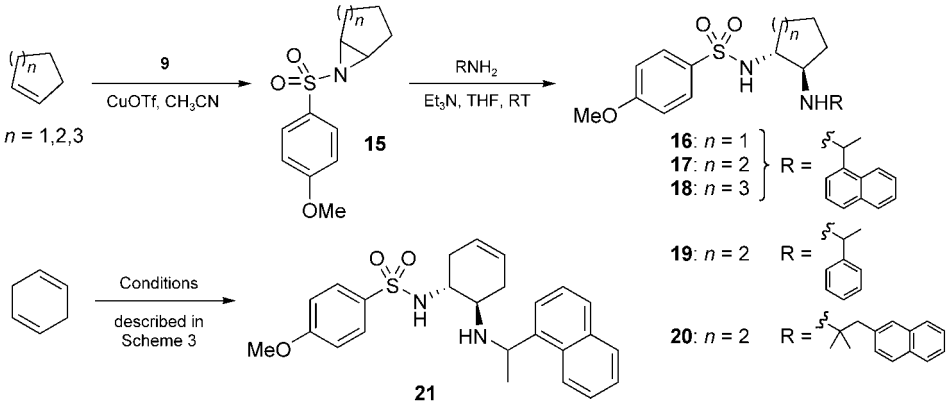
phenyl group at C-3 is covalently bound to C-1 would be particularly interesting, since this would lock the two amino functions into very precise conformations. Moreover, such compounds can be easily prepared from indene by using the copper-catalyzed olefin aziridination procedure previously employed for the synthesis of **5**.[17,19,20]

Thus, treatment of a slight excess (1.67 equiv) of indene **8** in acetonitrile at 0 °C with 1 equivalent of ((*N*-4-methoxybenzenesulfonyl)imino)phenyliodane **9**[21] in the presence of 10 mol% copper(I) triflate and molecular sieves provided aziridine **10** in 55% yield (Scheme 3). The latter was treated with (*R,S*)-1-(1-naphthyl)ethylamine (**11**), which attacked uniquely the benzylic



Scheme 3. *Synthesis of rigid analogues **7** and **14**.*

position to afford the desired compound **7** in high yield. Compound **7** was then evaluated for calcilytic activity in CHO cells that express the cloned rat CaSR [CHO(CaSR)] by measuring its ability to inhibit the stimulation in [^{3}H]IP production induced by 9 mM Ca^{2+}.[22] However, this compound (10 μM) did not block the action of calcium (data not shown), so it cannot be considered to be a calcilytic agent. By comparison, the calcilytic NPS 2143 effectively inhibited [^{3}H]IP production by 79±19% at a tenfold lower concentration. Replacement of the 5-membered ring of **7** by a 6-membered ring was also easily accomplished by using 1,2-dihydronaphthalene (**12**) as starting material for the preparation of the intermediate aziridine **13**, but again, the product (**14**) displayed no calcilytic activity.



Scheme 4. *Cycloalkyl ring and arylalkylamine modifications.*

The fused phenyl rings of **7** and **14** were then eliminated by using cyclopentene and cyclohexene for the aziridination reaction, providing, by way of the aziridine intermediates of general structure **15**, compounds **16** and **17**, respectively (Scheme 4). While again, the first of these compounds (**16**) did not display a calcimimetic profile, the cyclohexyl derivative **17** now clearly demonstrated calcilytic properties. Thus, **17** (10 μM) was able to block 33±4% of the Ca^{2+} (4 mM) stimulated [^{3}H]IP production in CHO(CaSR) cells and 62±9% of the IP response induced by 9–10 mM Ca^{2+} in the same cells (Figure 1A and Table 1). We then evaluated the effect of **17** (10 μM) in inhibiting the IP response to Ca^{2+} in AtT-20 (mouse pituitary) cells that constitutively express the CaSR.[12] AtT-20 cells responded to Ca^{2+} and induced a twofold increase of IP response at 2 mM Ca^{2+} compared to the basal level observed at 0.3 mM Ca^{2+}, which corresponds to a high-affinity binding site as previously reported in these cells.[12] AtT-20 cells also showed a 12-fold maximal increase of IP response at 8 mM Ca^{2+}. Compound **17** did not affect the response to 2 mM Ca^{2+}; this indicates that this Ca^{2+} response was not sensitive to **17** in these cells. However, **17** reduced the response to 8 mM Ca^{2+} by more than 60% (Figure 1B); this indicates that it also displayed calcilytic properties towards the CaSR constitutively expressed in At-T20 cells. We then determined the potency of **17** in inhibiting the IP response elicited by 9 mM Ca^{2+} in CHO-(CaSR) cells. Analysis of the dose-response curve (Figure 2) gave an IC_{50} for **17** of 5±2 μM. Moreover, when **17** (10 μM) was tested on wild-type CHO cells [CHO(WT*)], that is, not expressing CaSR, [^{3}H]IP accumulation was very limited (Figure 1A, C). In addition, the effects of ATP (300 μM) acting on a purinergic receptor, another G-protein-coupled receptor linked to PLC activation, was not inhibited in either CHO(WT*) or CHO(CaSR) cells (Figure 1C). These data indicate that **17** displays calcilytic properties and selectivity for both the cloned rat and mouse CaSR.

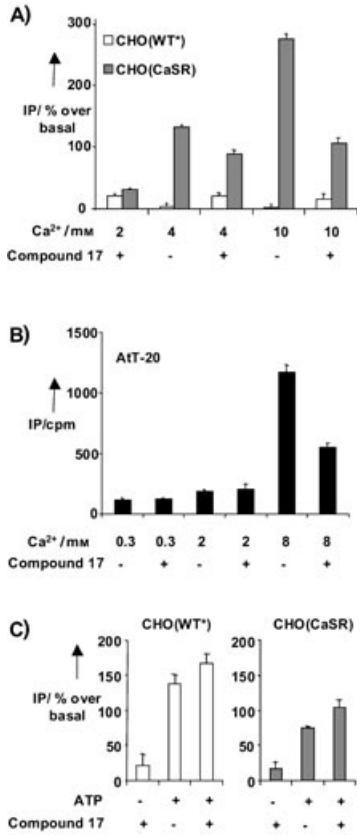
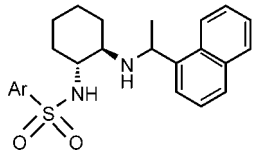
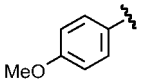
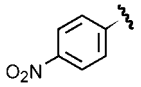
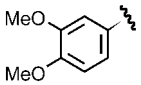
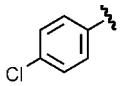
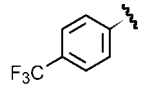
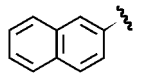
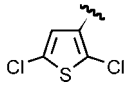


Figure 1. *Pharmacological properties of compound **17** on IP response in CHO(WT*), CHO(CaSR) and AtT20 cells. CHO(WT*), CHO(CaSR) (A, C) or AtT-20 (B) cells were prelabelled overnight with myo-[^{3}H]inositol, washed twice with basal Ham's F-12 medium supplemented with 10 mM LiCl and incubated at the indicated extracellular Ca^{2+} concentration alone, or in presence of 10 μM compound **17** (A, B). In (C), cells were incubated in presence of 2 mM extracellular Ca^{2+} and ATP (300 μM) or compound **17** (10 μM), or both. IP levels were measured as described in the Experimental Section. Data are expressed as percentage of IP response over basal level (IP/% over basal) determined in presence of 2 mM extracellular Ca^{2+} in (A) and (C), or in counts per minute per well in (B), and are means±S.E.M. of triplicates from a typical experiment representative of 2–3 experiments. Compound **17** reduces the IP response induced by 4 or 10 mM Ca^{2+} in CHO(CaSR) cells (A) or by 8 mM Ca^{2+} in AtT-20 cells (B) and has no or limited effects on basal IP levels in CHO(WT*) cells at any Ca^{2+} concentration tested (A); this indicates its specificity. It does not inhibit the IP response induced by ATP acting through another G-protein-coupled receptor expressed constitutively in CHO(WT*) or CHO(CaSR) cells (C).*

Table 1. *Inhibition of the [^{3}H]IP accumulation produced by Ca^{2+} (9 mM) in CHO cells expressing rat cloned CaSR by the test compounds* ***17***, ***21***, ***24–31*** *(as hydrochloride salts).*



Compound	Ar	% inhibition of [^{3}H]IP accumulation [10^{-5} M]
17		62 ± 9
21[a]		24 ± 8
24		58 ± 4
25		45 ± 4
26		37 ± 5
27		73 ± 4
28		27 ± 2
29		75 ± 7
30		62 ± 7
31		59 ± 8

[a] Has a double bond at the 4,5-position of the cyclohexyl ring.

This calcilytic activity was lost when the cyclohexyl ring of **17** was replaced by a cycloheptyl ring (**18**). Interestingly, replacement of the naphthyl group of **17** by a phenyl group (**19**) or by a 2-methyl-1-naphthylprop-2-yl moiety identical to that found in NPS 2341 (**20**) led to inactive compounds, while introduction of a double bond in the cyclohexane ring of **17** (i.e., **21**) led to substantial loss of activity (24 ± 8% inhibition). The cyclohexyl and 1-(1-naphthyl)ethyl groups thus seem to be minimum structural requisites to assure activity in this new family of 1,2-diamino calcilytics.

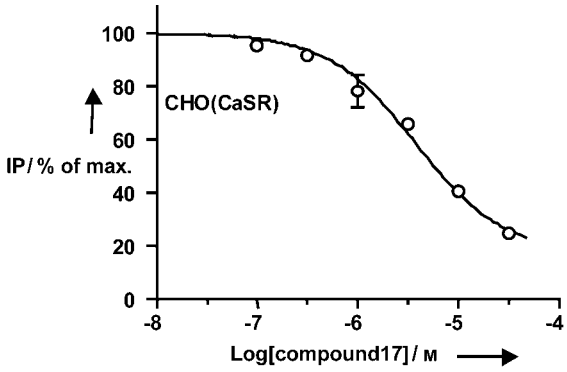
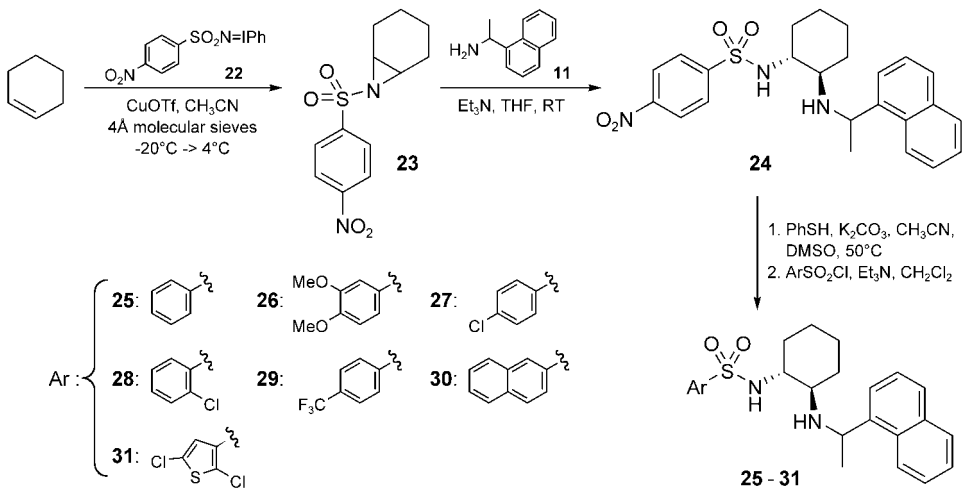


Figure 2. *Potency of compound* ***17*** *in inhibiting Ca^{2+}-induced [^{3}H]IP accumulation by the rat CaSR expressed in CHO(CaSR) cells. Concentration-dependent inhibition of Ca^{2+}-stimulated (9 mM) increases in the IP response by compound* ***17****. Data are expressed as percentage of maximal Ca^{2+} (9 mM) IP response (IP/% of max) and are the means ± S.E.M. of triplicates from a typical experiment out of three experiments.*

The cyclohexyl α-methylnaphthylamino derivative **17** was then used as a template for the further study of the structure–activity relationships in this new class of calcilytic agents, emphasis being given to variation of the substituents on the arylsulfonyl moiety. These compounds were prepared as shown in Scheme 5. Thus, reaction of cyclohexene with, this time, ((*N*-4-nitrobenzenesulfonyl)imino)phenyliodane (**22**)[21] in the presence of copper(I) triflate gave the *N*-nosyl-protected aziridine **23**. The latter was smoothly opened by (*R,S*)-1-(1-naphthyl)-ethylamine to afford diamine **24**, and the nosyl group was removed by the action of the thiophenol anion.[23] The resulting primary amine could then be sulfonylated by various arylsulfonyl chlorides in the presence of base to give compounds **25**–**31**.

As shown in Table 1, all the substituted *N*-(arylsulfonyl)amino cyclohexane derivatives exhibited calcilytic actions to a greater or lesser extent, depending on the type and the position of the aryl substituent. Thus, with compound **17** as the standard, both removal of the 4-methoxy group (**25**) and addition of a



Scheme 5. *Arylsulfonyl modifications.*

3-methoxy group (**26**) led to some loss of calcilytic activity (45 ± 4% and 37 ± 5% inhibition, respectively, at 10^{-5} M). Replacement of the electron-donating methoxy group of **17** by a strongly electron-withdrawing nitro group (**24**) had little effect on the measured activity (58 ± 4%), while replacement by a chlorine atom (**27**) produced a slight increase in activity (73 ± 4%), which was lost when the chloro atom was placed in the 2-position (**28**, 27 ± 2%). The 4-trifluoromethyl derivative (**29**) was found to be equipotent (75 ± 7% inhibition) with the 4-chloro analogue **27**. Finally, the use of aromatic rings other than phenyl also furnished active calcilytics. Thus, the 2-naphthylsulfonamide **30** and the 3-(2,2-dichlorothiophene)sulfonamide **31** were only slightly less active than **17** (62 ± 7% and 59 ± 8% inhibition, respectively).

Thus, by the simple tactic of constructing rigid analogues of the highly flexible calcimimetic derivative **5**, we have developed a new family of CaSR calcilytics having, as an essential feature, a 1,2-diaminocyclohexane backbone of general structure **17**. The rigidification imparted to these compounds by a cycloalkyl ring is indeed important for calcilytic activity. However, simple constraint is not a sufficient condition for imparting such activity since, all else being identical, the cyclopentyl and cycloheptyl analogues as well as the cyclohexene derivative (**16**, **18** and **21**, respectively) are inactive. These diamino cyclohexyl derivatives provide a large number of positions where structural variations can be introduced in an effort to increase their activity, as this preliminary study demonstrates, and, most importantly, modulate their pharmacokinetic properties. Work in this direction is currently underway.

Experimental Section

Preparation of compound 23: A mixture of cyclohexene (4.87 mL, 48 mmol), copper triflate (569 mg, 1.6 mmol) and 4 Å molecular sieves in acetonitrile (32 mL) was cooled to −20 °C, and solid ((4-nitrobenzenesulfonyl)imino)phenyliodane (**22**, 6.49 g, 16 mmol) was added in portions with stirring. Stirring was maintained for 6 h at −20 °C after completion of the addition and then for 16 h at 4 °C. The reaction mixture was filtered through celite, the filtrate was concentrated under vacuum, and the residue was purified by column chromatography on silica gel (heptane/ethyl acetate 8:2) to afford aziridine **23** as a pale yellow solid (57%). M.p. 134–136 °C; ^{1}H NMR (250 MHz, $CDCl_3$) δ = 1.24–1.44 (m, 4H; H-3, H-4), 1.82 (m, 4H; H-2, H-5), 3.13 (m, 2H; H-1, H-6), 8.15 (d, 2H; $J_{2',3'}$ = 11.3 Hz, H-2′), 8.39 (d, 2H; $J_{3',2'}$ = 11.3 Hz, H-3′); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ = 19.2, 22.7, 41.0, 124.2, 128.8, 145.0, 151.0; IR (film) $\tilde{\nu}$ = 1172, 1347, 1540 cm^{-1}; ESMS *m/z*: 282 $[M+H]^+$; elemental analysis calcd (%) for $C_{12}H_{14}N_2O_4S$: C 51.05, H 5.00, N 9.92, S 11.36; found: C 50.94, H 5.02, N 10.04, S 11.07.

Preparation of compound 24: A solution of aziridine **23** (1 equiv), (*R*,*S*)-1-(1-naphthyl)ethylamine (**11**, 2 equiv) and triethylamine (0.7 equiv) in anhydrous THF (c = 0.25 M) was stirred for 16 h at room temperature. The solvent was removed under vacuum, and the residue was purified by column chromatography on silica gel (heptane/ethyl acetate 8:2, then 7:3). A first diastereomer of compound **24** (less active) was eluted and was isolated as a yellow solid (50%). M.p. 159–161 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ = 0.83–1.01 (m, 1H; H-4a or H-5a), 1.16–1.34 (m, 3H; H-4, H-5b or H-4a, H-5), 1.44 (d, 3H; J = 6.0 Hz, CHCH_3), 1.62–1.78 (m, 2H; H-3 or H-6), 2.14–2.26 (m, 2H; H-6 or H-3), 2.47 (dt, 1H; J = 3.0 and 9.0 Hz, H-1), 2.65 (dt, 1H; J = 3.0 and 9.0 Hz, H-2), 4.67 (q, 1H; J = 6.0 Hz, C*H*CH$_3$), 7.43–7.50 (m, 4H; ArH), 7.69–7.76 (m, 1H; ArH), 7.87–7.92 (m, 3H; ArH), 8.00–8.35 (m, 3H; ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 22.9, 24.1, 24.7, 31.7, 32.6, 49.6, 57.8, 58.4, 122.4, 122.8, 123.9, 124.4, 125.5, 125.6, 126.0, 127.7, 128.3, 129.2, 130.5, 134.0, 140.5, 146.0, 149.8; IR (KBr) $\tilde{\nu}$ = 1165, 1348, 1529, 3303 cm^{-1}; ESMS *m/z*: 454 $[M+H]^+$.

Continued elution of the chromatography column provided the second diastereomer of compound **24** (more active) (50%): ^{1}H NMR (300 MHz, $CDCl_3$) δ = 0.80–1.27 (m, 4H; H-4, H-5), 1.41 (d, 3H; J = 6.0 Hz, CHCH_3), 1.46–1.60 (m, 2H; H-3 or H-6), 1.92–2.00 (m, 2H; H-6 or H-3), 2.19–2.22 (m, 1H; H-1), 2.80 (dt, 1H; J = 6.0 and 12.0 Hz, H-2); 4.70 (q, 1H; J = 6.0 Hz, C*H*CH$_3$), 7.45–7.50 (m, 4H; ArH), 7.74–7.77 (m, 1H; ArH), 7.86–7.90 (m, 3H; ArH), 8.20–8.27 (m, 3H; ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 22.5, 24.2, 24.6, 31.7, 32.4, 49.9, 57.7, 58.5, 122.4, 122.9, 124.2, 125.7, 126.2, 127.9, 128.3, 129.3, 131.3, 134.3, 141.4, 147.1, 149.8; IR (KBr) $\tilde{\nu}$ = 1165, 1348, 1529, 3303 cm^{-1}; ESMS *m/z*: 454 $[M+H]^+$; elemental analysis calcd (%) for $C_{24}H_{27}N_3O_4S$: C 63.55, H 6.00, N 9.26, S 7.07; found: C 63.45, H 5.97, N 9.41, S 6.99.

General procedure for the preparation of sulfonamides 25–31: A solution of compound **24** (1 equiv of the mixture of diastereomers) in acetonitrile/DMSO (96:4, concentration 0.165 mmol in 2 mL) was treated with solid potassium carbonate (4 equiv) and thiophenol (3 equiv). The reaction mixture was stirred for 6 h at 50 °C, then cooled, and the solvents were evaporated under reduced pressure. Purification of the residue by column chromatography on silica gel (ethyl acetate/methanol 2:1, then 1:1) afforded the free primary amine (1:1 mixture of diastereomers) as a pale yellow oil in quantitative yield. ^{1}H NMR (250 MHz, $CDCl_3$) δ = 0.84–1.03 (m, 3H; $H_{cyclohex}$), 1.05–1.26 (m, 3H; $H_{cyclohex}$), 1.47 (d, 1.5H; J = 6.0 Hz, CHCH_3 of diastereomer 1), 1.48 (d, 1.5H; J = 6.0 Hz, CHCH_3 of diastereomer 2), 1.56–1.67 (m, 1H; $H_{cyclohex}$), 1.85–1.98 (m, 1H; $H_{cyclohex}$), 2.06–2.18 (m, 1H; H-2), 2.29 (dt, 0.5H; J = 5.5 and 11.5 Hz, H-1 of diastereomer 1), 2.37 (dt, 0.5H; J = 5.5 and 11.5 Hz, H-1 of diastereomer 2), 4.77 (q, 0.5H; J = 6.0 Hz, C*H*CH$_3$ of diastereomer 1), 4.88 (q, 0.5H; J = 6.0 Hz, C*H*CH$_3$ of diastereomer 2), 7.42–7.52 (m, 3H; ArH), 7.65–7.74 (m, 2H; ArH), 7.84–7.87 (m, 1H; ArH), 8.18–8.23 (m, 1H; ArH); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ = 22.9, 24.8, 24.9, 25.0, 25.3, 31.4, 32.1, 34.5, 35.4, 50.4, 50.5, 55.6, 55.7, 60.1, 61.2, 122.7, 122.9, 123.1, 123.3, 125.2, 125.5, 125.6, 125.7, 127.0, 127.1, 128.9, 130.6, 133.8, 134.6, 141.3, 142.9; ESMS *m/z*: 268 $[M+H]^+$.

A solution of this amine (1 equiv) in dichloromethane (c = 0.5 mmol in 6 mL) was treated with triethylamine (1 equiv) at room temperature and, after 10 min, with the appropriate arylsulfonyl chloride (1 equiv). The reaction mixture was stirred for 15 h, the solvent was removed under reduced pressure, and the residue was purified by column chromatography on silica gel (heptane/ethyl acetate 8:2, then 7:3). The more slowly moving component, corresponding to the more active sulfonamide diastereomer, was isolated.

Cell culture: CHO(CaSR) and CHO(WT*) are Chinese hamster ovary cells transfected or not, respectively, with the rat calcium-sensing receptor and have been described.[22] These cells and AtT-20 cells were cultured in basal Ham's F-12 medium (0.3 mM Ca^{2+}, 0.6 mM Mg^{2+}) as previously described.[12,22]

[^{3}H]Inositol phosphate ([^{3}H]IP) formation: Cells were cultured overnight in their growth medium containing myo-[^{3}H]inositol (0.5 μCi mL^{-1}, Amersham Biosciences) in 24-well plates. Calcilytics were dissolved at 10 mM in ethanol and then diluted in basal

medium. The activation of phospholipase C was estimated after quantification of [^{3}H]IP accumulation performed as described.[22] The [^{3}H]IP responses of CHO(CaSR) and CHO(WT*) cells and AtT-20 cells were determined at the indicated calcium concentration in the presence or not of ATP and compound **17**, as indicated. Otherwise, calcilytics were evaluated in presence of Ca^{2+} (9 mM). In all cases, the calcilytics were incubated for 30 min in the presence of the calculated final concentration of Ca^{2+}. Under these experimental conditions, no precipitation of material in the medium was observed after addition of Ca^{2+}.

Acknowledgements

We thank the Institut de Chimie des Substances Naturelles for a fellowship (A.K.).

Keywords: calcium · ligand design · receptors · structure–activity relationships · sulfonamides

[1] E. M. Brown, R. J. MacLeod, *Physiol. Rev.* **2001**, *81*, 239 – 297.
[2] J.-P. Pin, T. Galvez, L. Prézeau, *Pharmacol. Ther.* **2003**, *98*, 325 – 354.
[3] E. M. Brown, G. Gamba, D. Riccardi, M. Lombardi, R. Butters, O. Kifor, A. Sun, M. Hediger, J. Lytton, S. C. Hebert, *Nature* **1993**, *366*, 575 – 580.
[4] D. Riccardi, J. Park, W. S. Lee, G. Gamba, E. M. Brown, S. C. Hebert, *Proc. Natl. Acad. Sci. USA* **1995**, *92*, 131 – 135.
[5] M. Ruat, M. E. Molliver, A. M. Snowman, S. H. Snyder, *Proc. Natl. Acad. Sci. USA* **1995**, *92*, 3161 – 3165.
[6] E. M. Brown, M. Pollak, S. C. Hebert, *Annu. Rev. Med.* **1998**, *49*, 15 – 29.
[7] E. F. Nemeth in *Principles of Bone Biology* (Eds.: J. P. Belezekian, L. G. Raisz, G. Rodan) **1996**, Academic Press, San Diego, pp. 1019 – 1035.
[8] E. F. Nemeth, J. Fox, *Trends Endocrinol. Metab.* **1999**, *10*, 66 – 71.
[9] a) N. E. Lane, S. Sanchez, G. W. Modin, H. K. Genant, E. Pierini, C. D. Arnaud, *J. Clin. Invest.* **1998**, *102*, 1627 – 1633; b) T. Fujita, T. Inoue, H. Morii, H. Norimatsu, H. Orimo, H. E. Takahashi, K. Yamamoto, M. Fukunaga, *Osteoporosis Int.* **1999**, *9*, 296 – 306.
[10] S. J. Silverberg, F. Gartenberg, T. P. Jacobs, E. Shane, E. Siris, R. B. Staron, D. J. McMahon, J. P. Bilezekian, *J. Clin. Endocrinol. Metab.* **1995**, *80*, 729 – 734.
[11] E. F. Nemeth, M. E. Steffey, L. G. Hammerland, B. C. P. Hung, B. C. Van Wagenen, E. G. DelMar, M. F. Balandrin, *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 4040 – 4045.
[12] S. Ferry, B. Chatel, R. H. Dodd, C. Lair, D. Gully, J.-P. Maffrand, M. Ruat, *Biochem. Biophys. Res. Commun.* **1997**, *238*, 866 – 873.
[13] L. A. Sorbera, R. M. Castañer, M. Bayés, *Drugs Future* **2002**, *27*, 831 – 836.
[14] M. Gowen, G. B. Stroup, R. A. Dodds, I. E. James, B. J. Votta, B. R. Smith, P. K. Bhatnagar, A. M. Lago, J. F. Callahan, E. G. DelMar, M. A. Miller, E. F. Nemeth, J. Fox, *J. Clin. Invest.* **2000**, *105*, 1595 – 1604.
[15] E. F. Nemeth, E. G. DelMar, W. L. Heaton, M. A. Miller, L. D. Lambert, R. L. Conklin, M. Gowen, J. G. Gleason, P. K. Bhatnagar, J. Fox, *J. Pharmacol. Exp. Ther.* **2001**, *299*, 323 – 331.
[16] D. P. Rutella, *Drug Discovery Dev.* **2002**, *5*, 477 – 486.
[17] P. Dauban, S. Ferry, H. Faure, M. Ruat, R. H. Dodd, *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2001 – 2004.
[18] A. Kessler, H. Faure, M. Ruat, P. Dauban, R. H. Dodd, *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3345 – 3349.
[19] D. A. Evans, M. M. Faul, M. T. Bilodeau, *J. Am. Chem. Soc.* **1994**, *116*, 2742 – 2753.
[20] For a review, see: P. Dauban, R. H. Dodd, *Synlett* **2003**, 1571 – 1586.
[21] M. J. Sodergren, D. A. Alonso, A. V. Bedekar, P. G. Andersson, *Tetrahedron Lett.* **1997**, *38*, 6897 – 6900.
[22] M. Ruat, A. M. Snowman, L. D. Hester, S. H. Snyder, *J. Biol. Chem.* **1996**, *271*, 5972 – 5975.
[23] T. Fukuyama, C.-K. Jow, M. Cheung, *Tetrahedron Lett.* **1995**, *36*, 6373 – 6374.

Received: February 18, 2004

