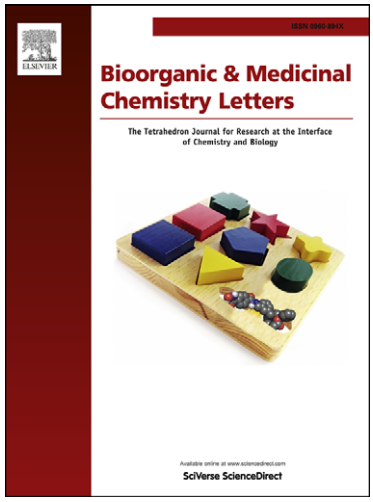

New Potent Calcimimetics: I. Discovery of a series of novel trisubstituted ureas

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New Potent Calcimimetics: I. Discovery of a series of novel trisubstituted ureas

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Abstract— Starting from Fendiline and R-568, we identified a novel series of urea compounds as positive allosteric modulators of the calcium sensing receptor (CaSR), as part of a program to identify novel therapeutics for secondary hyperparathyroidism. Initially identified disubstituted ureas were converted to trisubstituted urea lead **20e**, which was further modified to increase *in vivo* potency. Replacing a carbomethoxy substituent by various bioisosteres led to compound **46** which exhibited potent *in vitro* and *in vivo* activity after oral administration.

Hyperparathyroidism is characterized by high circulating levels of parathyroid hormone (PTH) due to its increased secretion by parathyroid glands.[1,2] The GPCR Class C calcium-sensing receptor (CaSR)[3] is expressed on the surface of parathyroid cells and up regulates PTH secretion in response to low extracellular calcium concentration. The CaSR proved to be an attractive target for treatment of secondary hyperparathyroidism, in patients with chronic renal failure on dialysis, with the discovery of Cinacalcet (AMG-073, Sensipar®) as a positive allosteric modulator (calcimimetic, Figure 1).[4]

Most of the potent calcimimetics reported so far belong to the arylalkylamine family.[5-7] In this paper, we describe the identification of a new trisubstituted urea series which is structurally distinct from the known arylalkylamines positive allosteric modulators. *In vitro* and *in vivo* data of optimized compound **46** are presented.

Based on the literature data available at the beginning of the program, we started from known arylalkylamine allosteric modulator R-568 and the weakly active Fendiline (Figure 1).[8] The *gem* diphenyl moiety, which is present in Fendiline and is known to be a privileged structure fragment for GPCRs,[9] was chosen as a key feature for designing new calcimimetics. We discovered that we were able to replace the arylalkylamine with a trisubstituted urea leading to compounds with *in vitro* activity below 100 nM and oral *in vivo* activity.

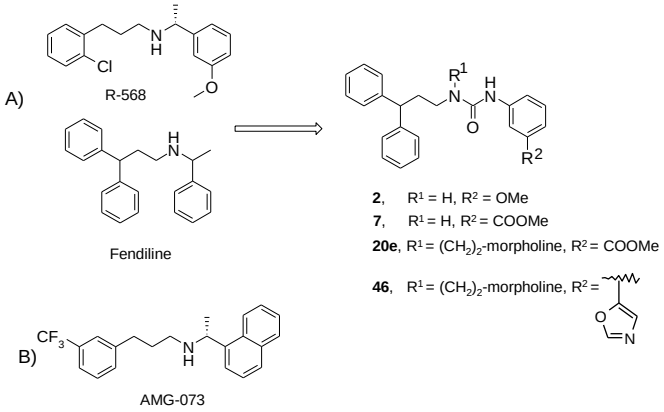


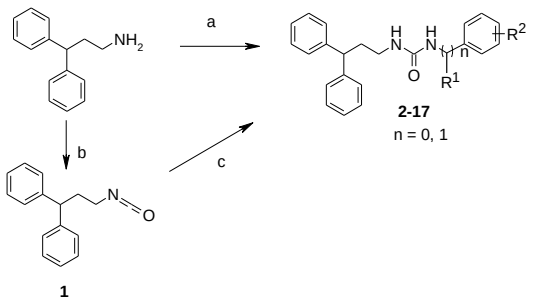
Figure 1. A) From R-568 and Fendiline to urea based positive CaSR allosteric modulators. B) Cinacalcet (AMG-073)

Disubstituted ureas **2-17** were obtained by coupling commercially available aryl isocyanates and 3,3-diphenyl-propylamine in dichloromethane at room temperature. Alternatively, isocyanate **1**, which was prepared from 3,3-diphenyl-propylamine, was coupled with different aromatic amines to provide the desired

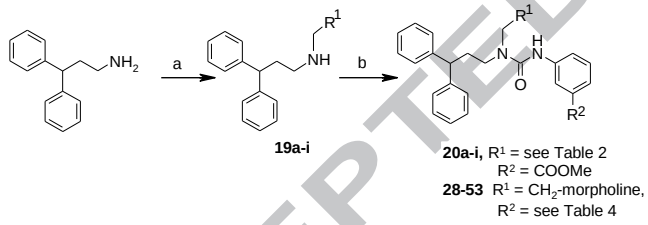
ureas (Scheme 1).

N-Substituted ureas **20a-20i** were prepared in two steps from 3,3-diphenyl-propylamine (Scheme 2). This amine was first alkylated with various alkyl halides under basic conditions (K_2CO_3).[10,11] The secondary amines **19a-19i** were then converted to ureas **20a-20i** by addition of commercially available *m*-substituted methyl ester phenyl isocyanate in dichloromethane, or converted to ureas **28-53** with the corresponding isocyanate.[11]

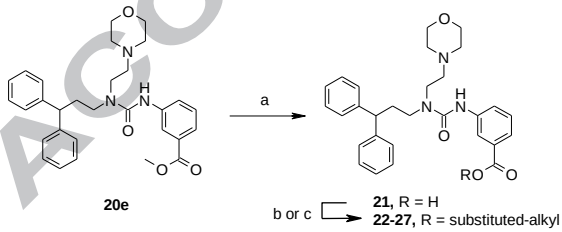
Esters **22-27** were prepared either by alkylation of carboxylic acid **21** (obtained by saponification of ester **20e**) with various alkyl halides under basic conditions (K_2CO_3), or by activation of acid **21** with DCC and DMAP and addition of various alcohols in dichloromethane (Scheme 3).



Scheme 1. Reagents and conditions: (a) $Ar(CH)_nR^1$-N=C=O, CH_2Cl_2, rt, 60-85%; (b) Diphosgene, charcoal, toluene, 120°C; (c) Aryl-NH_2/ BenzylNH_2 , CH_2Cl_2, rt, 48% (for 2 steps).



Scheme 2. Reagents and conditions: (a) R^1-CH_2-Br, K_2CO_3, CH_3CN, reflux 60-70%; (b) R^2-Ph-N=C=O, CH_2Cl_2, rt, 75-85%.



Scheme 3. Reagents and conditions: (a) NaOH, MeOH, rt, 92%; (b) R-X, K_2CO_3, DMF, reflux, 70-98%; (c) ROH, DCC, DMAP, CH_2Cl_2, rt, 75-80%.

The compounds were evaluated in CHO cells transfected with the hCaSR and a 6xTRE luciferase reporter system.[12] Compounds were tested in dose response, with increasing calcium concentration. The increasing concentration of a positive allosteric modulator induces a dose proportional leftward shift of the hCaSR calcium responses. The values indicated in this paper correspond to an EC_{50} at 2 mM of calcium. The most active compounds were then tested *in vivo* for their ability to decrease PTH levels in normal rats. Our two starting points, R-568 and Fendiline, were active at 80 and 1000 nM respectively, and led to compound **46**, active at 60 nM. Cinacalcet was found at 80 nM in this assay.

Disubstituted ureas **2-17** were examined for calcimimetic activity using the luciferase assay with the results shown in Table 1. Replacement of the α-methyl arylalkyl amino group of R-568 by aryl and benzyl-substituted ureas was first explored, leading to compounds with activity ranging from 2-15 µM. *Meta*-substituted methyl ester **7** was found to be the most active, but at 2 µM, 85-fold less active than R-568 (EC_{50} = 80 nM). Interestingly, *meta*-substituted carboxylic acid **14** was found significantly less active, indicating that the nature of the *meta*-substituent is important for the resulting potency. Benzylic derivatives **15-17** were also prepared and exhibited some positive CaSR allosteric modulation, but were less active than compound **7**.

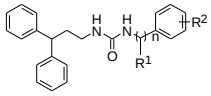
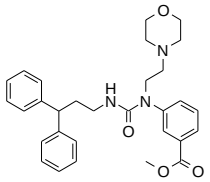


Table 1. Calcimimetic activity of disubstituted ureas **2-17** [a]

Compound	n	R^1	R^2	EC_{50}, µM
2	0		*m*-OMe	8
3	0		*p*-OMe	15
4	0		*o*-Cl	6
5	0		*p*-Cl	4
6	0		*m*-Cl	4
7	0		*m*-COOMe	2
8	0		*p*-COOEt	3
9	0		*p*-Me	4
10	0		*p*-Ph	4
11	0		*m*-Ph	15
12	0		*m*-COOMe, *p*-Cl	2
13	0		*m*-CF_3, *p*-Cl	3
14	0		*m*-COOH	20
15	1	(*R*)-Me	-H	6
16	1	H	*p*-OMe	15
17	1	H	*o*-Cl	4

[a] EC_{50} values in µM (n ≥ 1) have been determined by Luciferase assay.

Further substitution of the urea led to the observation that blocking the aniline NH resulted in significantly reduced activity (compound **18**, EC_{50} = 10 µM, Figure 2). Substitution of the other NH gave rise to the compounds shown in Table 2.



18

Figure 2. Substitution on the aniline NH.

Compound **20a**, with a nitrogen bearing an isobutyl group, was as active as the non-substituted urea **7** (EC_{50} = 1.5 µM), indicating that it is possible to modulate the physicochemical properties of the series by modifying the nitrogen substituent on the urea. Taking into account the low solubility and high lipophilicity of compound **20a**, solubilising groups were then introduced at this position. Among them, the morpholinoethyl group not only improved solubility in acidic media but also increased potency up to 100 nM (compound **20e**), at a level comparable to R-568 (80 nM) in our assay. The fact that the corresponding cyclohexylethyl compound **20d** is 100-fold less active indicated that additional H bonding with morpholine was critical for improved potency.

We explored the side chain length on the nitrogen as well as other substitutions. We identified morpholine **20f** and piperazines **20g** and **20h** which all have a 3 carbon atom linker as potent compounds.

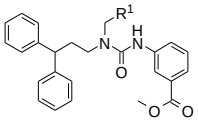
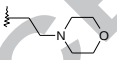
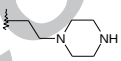
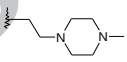
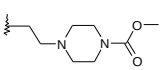


Table 2. Calcimimetic activity of trisubstituted urea **20a-i** [a]

Compound	R^1	EC_{50}, µM
20a	-^{i}Pr	1.5
20b	-Ph	2
20c	-COO^{t}Bu	0.8
20d		10
20e		0.1
20f		0.15
20g		0.06
20h		0.1
20i		0.4

[a] EC_{50} values in µM (n ≥ 1) have been determined by Luciferase assay.

Compound **20e** was evaluated *in vivo* (30 mg/kg) for its ability to decrease PTH levels in normal Sprague Dawley rats following oral administration.[13] **20e** lowered PTH levels (-60%) 30 minutes post dose, but became weakly active after 2 hours. We postulated that the weak effect was related to rapid hydrolysis of the ester group to the corresponding acid **21,** which was less active (20 fold-less active *in vitro* on the CaSR (EC_{50} = 2 µM)). This was confirmed by *in vivo* stability measurements of the ester **20e** after oral administration in rat, indicating that only the corresponding acid could be detected after 30 minutes in plasma. In order to address this issue, other esters (**22-27**) were prepared from carboxylic acid **21** (Scheme 3) and the *in vitro* rat plasma stability was measured after 30 and 60 minutes (Table 3). Most of the esters were in the same range of activity as methyl ester **20e**. However, *tert*-butyl ester **25** and isopropyl ester **22** were much more stable in plasma after 1 h (50 and 95% remaining, respectively) and thus proved to be the best compromise between potency and ester stability. These two esters were then evaluated *in vivo* in normal rats but did not result in better decrease in PTH levels, likely due to too low exposure after oral administration.

Another approach was then investigated to increase *in vivo* potency of compound **20e**. The methyl ester of **20e** was replaced by various known ester bioisosteres[14] and other substituents. The activities of these compounds listed in Table 4 ranged from 60 nM to 1 µM. *N*-(Me)-substituted tetrazole was an especially potent surrogate of the methyl ester. Compound **52,** 2-*N*-(Me) substituted-tetrazole (EC_{50} = 60 nM) was 2-fold more active than ester **20e**. The corresponding *N*H-tetrazole **51** was only weakly active (EC_{50} = 1 µM) comparable to the carboxylic acid **21**. Ethyl ketone **28** was also a good surrogate of the methyl ester (EC_{50} = 150 nM). Another potent compound also identified was the oxazole **46** (EC_{50}= 60 nM). Also of interest were *meta* substituted-phenyls. Substitution with 5-membered rings (furans **44**, **45** and oxazole **46**) led to compounds with potency similar to ester **20e**.

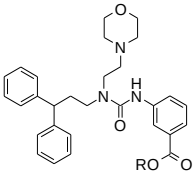


Table 3. Potency and plasma stability of esters **20e-27** [a]

Compound	R	EC_{50} µM	Plasma stability [b] 30 min	Plasma stability[c] 60 min
20e	-Me	0.1	18	1
21	-H	2	nd	nd
22	-^{i}Pr	0.15	75	50
23	-CH_2Ph	0.2	5	1
24	-$CH(CH_3)C(CH_3)_3$	1.0	80	80
25[d]	-^{t}Bu	0.3	95	95
26	-$(CH_2)_2OH$	0.15	nd	nd
27	-$(CH_2)_3CF_2CF_3$	0.4	nd	nd

[a] EC_{50} values in µM (n = 2) have been determined by Luciferase assay.
[b] Percentage of compound remaining in rat plasma (*in vitro*) after 30 minutes.
[c] Percentage of compound remaining in rat plasma (*in vitro*) after 60 minutes. [d] Compound **25** was obtained by addition of triphosgene and diisopropylethylamine to commercially available 3-aminobenzoic acid tertbutyl ester in CH_2Cl_2 followed by addition of amine **19e**.

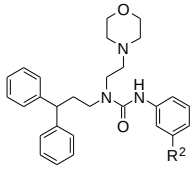


Table 4. Calcimimetic activity of analogues **28-53** [a]

Compound	R^2	EC_{50}, μM
28	-$COCH_2CH_3$	0.15
29	-COMe	0.55
30	-CH_2OH	0.8
31	-CH_2OMe	0.4
32	-SMe	0.13
33	-SCF_3	0.35
34	-SOMe	0.8
35	-SO_2Me	1
36	-SO_2CF_3	0.4
37	-OCF_3	0.2
38	-CN	0.8
39	-CF_3	0.4
40	-OMe	0.3
41	-NMe_2	0.3
42	- phenyl	0.2
43	-3-pyridinyl	0.2
44	-2-Furanyl	0.15
45	-1-Furanyl	0.1
46	-5-oxazolyl	0.06
47	-5-(3-Me)-isoxazolyl	0.15
48	-2-thiazolyl	0.3
49	-2-*N*H-benzimidazolyl	0.6
50	-2-benzothiazolyl	0.15
51	-5-*N*H-tetrazolyl	1
52	-5-(2-*N*Me)- tetrazolyl	0.06
53	-5-(1-*N*Me)- tetrazolyl	0.2

[a] EC_{50} values in nM ($n \geq 2$) have been determined by Luciferase assay.

Three potent compounds tetrazole **52**, oxazole **46** and ketone **28** were evaluated *in vivo* in normal rats at 30 mg/kg po and PTH levels measured over time.[13] As anticipated, these three compounds exhibited much longer duration of action *in vivo* than the methyl ester analogue **20e** (Figure 3). Oxazole **46** decreased PTH levels almost completely (-95 %) for at least 2 hours post dose (30mg/kg given orally).[15]

Based on its promising *in vitro* and *in vivo* profile, further characterization of oxazole **46** was carried out. Overall, oxazole **46** had an acceptable safety (Ames) and selectivity profile (Cerep screen, <50% inhibition at 10 μM on a panel of 30 receptors/ions channels/enzymes) but resulted in hERG inhibition (-73% at 1 μM in the patch clamp assay) which prevented its further development.

In conclusion, a novel series of potent trisubstituted urea derivatives, as positive allosteric modulators of the calcium sensing receptor, exemplified by compound **46**, was developed. Starting from known Fendiline and R-568, we discovered that we were able to replace the alkylamine with an aryl urea leading to compounds having good *in vitro* potency. Substitution of the NH urea by the morpholinoethyl group not only improved the physicochemical properties but resulted in compounds with good *in vitro* potency. In particular, we were pleased to see that compound **20e**, (100 nM), substituted with a methyl ester on the aniline ring, was also efficacious *in vivo* and was able to decrease PTH levels up to 60% 10 minutes post dose. An SAR optimization around the *meta* substitution on the aniline ring, to replace the labile ester group, was performed to improve *in vivo* efficacy. In particular, replacing the ester group by bioisosteres led to oxazole **46**, which showed good *in vitro* potency and *in vivo* efficacy after oral administration.

These data paved the way for the identification of other compounds around the trisubstitued urea series with comparable *in vitro/in vivo* potency and improved ADMET properties. These will be described in the following paper.[16]

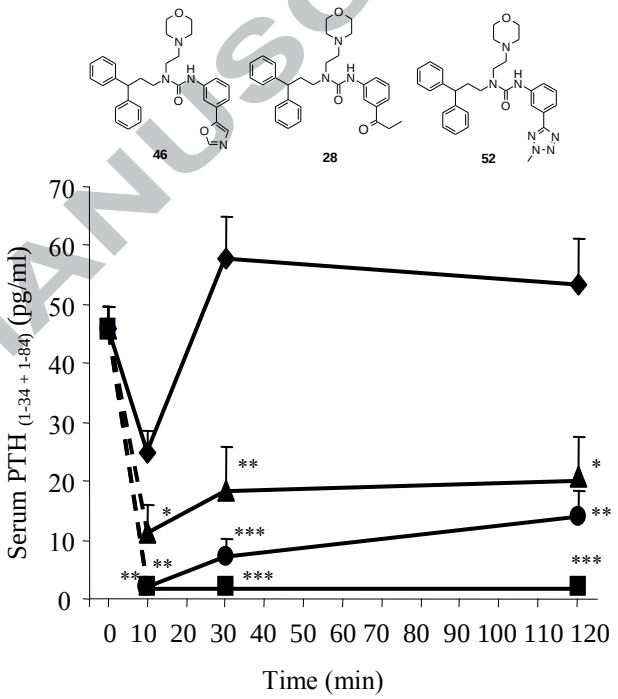


Figure 3. *In vivo serum PTH 10, 30 and 120 minutes after oral administrat*ion of compounds (30 mg/kg) in male Sprague-Dawley rats. (Control vehicle (♦), cpd **46** (■), cpd **28** (▲), cpd **52** (●)).
Values are means of two experiments (n = 7-15). *p<0.05, **p<0.01, ***p<0.0001 significantly different from 'control vehicle' group (Anova, t-test)

Acknowledgments

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12. The human parathyroid cell Ca^{2+} receptor cDNA was subcloned into the mammalian expression vector PECE (Ref. 1). The luciferase reporter was subcloned into the mammalian expression vector pGL3basic (Promega). Resistance to neomycin (pSV2-neo) and resistance to puromycin (pSG5-puro) were used as selection markers. All these plasmids were simultaneously transfected into CHO cells by calcium phosphate precipitation. Transfected cells were grown in F12 medium containing 7.5% foetal bovine serum, 100 U/ml penicillin and 100 µg/ml streptomycin (as 1% Pen-Strep, BioWithaker), neomycin (750 µg/ml) and puromycin (5 µg/ml). Neomycin and puromycin resistant colonies were subcloned and assayed for activation against a range of calcium concentration. Clone 8-5-5 was used to assess the effects of compounds on $[Ca^{2+}]i$. This stably transfected cell line is termed ET8-5-5.

For measurements of $[Ca^{2+}]i$, the cells were recovered from tissue culture flasks by brief treatment with Trypsin-EDTA (Invitrogen; containing 0.53 mM EDTA•4Na in HBSS) and then seeded in Culture treated 96-well plates (Greiner) at 50K cells per well in the growth media (same as above, except neomycin 400 µg/ml). Cells were grown in 37°C TC incubator for 24 hours. The culture medium was then removed and replaced with F12 medium, 1% Pen-Strep for an overnight foetal bovine serum starvation in 37°C TC incubator. Then the starvation medium was removed and replaced with a test buffer (20 mM HEPES pH 7.4, 125 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 5.5 mM Glucose, 2 g/L lysosyme and 0.3 mM $CaCl_2$) supplemented with a range of test compound concentrations crossed against a superadded range of $CaCl_2$ concentrations. The cells were incubated with the test compounds for 5 hours in 37°C TC incubator. Then the test buffer was discarded, and cells were added with the substrate for Luciferase from SteadyLite Kit (Perkin-Elmer).The luminescence was recorded.

13. *in vivo mesurements* : Overnight- fasted male Sprague-Dawley rats (200-220g BW, CERJ, France) were orally dosed by gavage with calcimimetics (30 mg/kg) or vehicle at a volume of 5 ml/kg. Compounds were formulated in methylcellulose 0.5%. Vehicle-treated controls rats received the same volume of vehicle. Rats were sacrificed by decapitation at various times after dosing (0, 10, 30, 120 minutes). Blood samples were collected in Sarstedt Z tubes, allowed to clot for 20 minutes and centrifuged (3000 rpm, Jouan CR422) at 4°C. Serums were removed and stored at -20°C until assayed. Serum PTH was quantified according to the provider's instructions using a rat IRMA kit (Immutopics, 50-2000).

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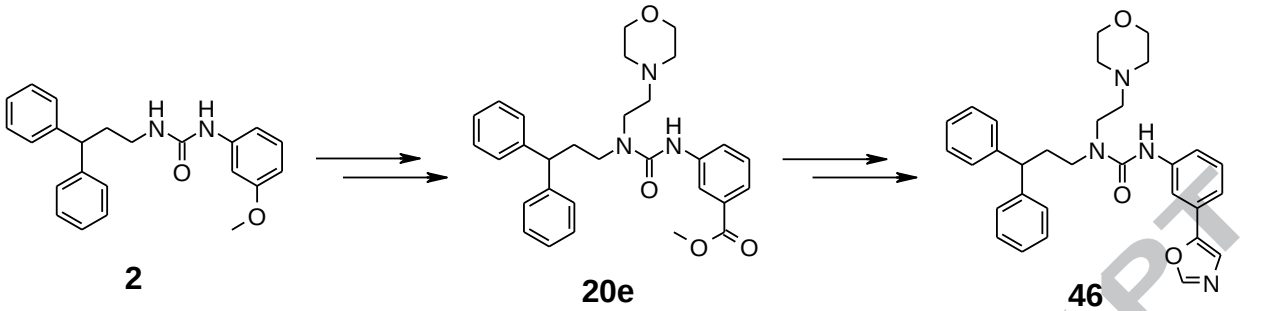
15. The effect starts to be significant at 10 minutes post dose, even with a drop in the control group at 10 minutes possibly due to the effect of stress on PTH level after oral gavage. At 30 minutes and 2 hours, the vehicle group is back to normal range and PTH level remains very low in the treated group with compound **46**.

16. Deprez, P.; Temal, T.; Jary, H.; Auberval, M.; Lively, S.; Guédin, D.; Vevert, J.-P. *Bioorg. Med. Chem. Lett*. **2013**, *23*, submitted.



2

CaSR EC_{50} = 8 µM

20e

CaSR EC_{50} = 0.1 µM
low oral activity in rats

46

CaSR EC_{50} = 0.06 µM
potent oral activity in rats