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Structure−Activity Relationships of 6-Methyl-benzo[*b*]thiophene-2-carboxylic Acid (1-{(*S*)-1-Benzyl-4-[4-(tetrahydropyran-4-ylmethyl)piperazin-1-yl]butylcarbamoyl}cyclopentyl)amide, Potent Antagonist of the Neurokinin-2 Receptor

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As part of a project aimed at the identification of a series of small, orally available antagonists for the hNK_2 receptor, starting from one of our capped dipeptide libraries, we succeeded in the chemical optimization of the first identified leads, finally producing a class of molecules with significant activity in our animal model after iv administration. We herein report the results of further chemical modifications made to reduce the overall peptide character of this series and the consequent improvement of their in vivo antagonist activity. The present work identified 6-methylbenzo[*b*]thiophene-2-carboxylic acid (1-{(*S*)-1-benzyl-4-[4-(tetrahydropyran-4-ylmethyl)piperazin-1-yl]butylcarbamoyl}cyclopentyl)amide (**10i**), endowed with subnanomolar potency in all the in vitro tests and being highly potent and of long duration upon in vivo testing after both iv and id dosing.

Introduction

Three mammalian neurokinin (tachykinin) receptors have been cloned and expressed. They are classified as neurokinin-1 (NK_1), neurokinin-2 (NK_2), and neurokinin-3 (NK_3) and are activated by three endogenous peptides (tachykinins) widely distributed in the mammalian peripheral and central nervous systems. Although each of these tachykinins is the preferred ligand for one of the receptors, NK_1 for substance P (SP[a]), NK_2 for neurokinin A (NKA), and NK_3 for neurokinin B (NKB), all three are capable of full agonist activity at each of the receptors, albeit with reduced affinity.

The human NK_2 receptor (hNK_2) has been identified and supported as a suitable target for development of novel drugs to be used for the treatment of a number of diseases in the respiratory, gastrointestinal, and genitourinary tracts and in the CNS. As part of a project aimed at the identification of a series of small, orally available antagonists for the hNK_2, starting from one of our capped dipeptide libraries, we were able to identify a number of molecules with subnanomolar binding affinity for the hNK_2 receptor.[1] All the molecules were characterized by a rigid core structure containing an α,α-cyclopentaneglycine fragment. These first initial structures were further elaborated and led to compounds with low nanomolar potency on an in vitro functional test and significant NK_2 antagonist activity on guinea pig after iv administration at a dose of 3 μmol/kg. One of the best compounds reported was (*R*)-4′-methyl-*N*-(1-(1-oxo-3-phenyl-1-((1-((tetrahydro-2*H*-pyran-4-yl)methyl)piperidin-4-yl)methylamino)propan-2-ylcarbamoyl)cyclopentyl)biphenyl-4-carboxamide ($pK_i = 9.5$, Figure 1).[2]

These molecules showed a general common topological profile: a rigid and essentially hydrophobic portion (A), containing the dipeptide α,α-cyclopentaneglycine-D-Phe capped with a planar hydrophobic acyl group at the N-terminus, and a flexible hydrophilic portion (B) as carboxy terminal capping group.

Portion A seems to be strongly sensitive to even very small chemical modifications, whereas portion B, which gives indeed a large contribution to the binding energy, is very tolerant to modifications. The two portions A and B are joined by an amide bond. In addition to this, X-ray crystallography and in solution NMR both are consistent with a bioactive where the two aromatic groups of the portion A point toward opposite directions in 3D space.[2]

We herein report the results of the chemical modifications made to further improve the in vivo antagonist activity of this class of molecules.

We started with the idea to reduce the overall peptide character through elimination of one amide linkage. From previous work it was known that both the amide bonds formed by the α,α-cyclopentaneglycine were essential for the binding affinity. Every attempt to alkylate, eliminate, or substitute them with classical isosteres resulted in a dramatic drop in pK_i. As a consequence, our effort was concentrated on the elimination of the amide linkage between portions A and B, with accompanying modifications of portion B.

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[a] Abbreviations: AcOH, acetic acid; AcCN, acetonitrile; BSA, bistrimethylsilylacetamide; CNS, central nervous system; DCC dicyclohexylcarbodiimide, DIPEA, diisopropylethylamine; DCM, dichloromethane; DME, 1,2-dimethoxyethane; DMF, dimethylformamide; DMSO, dimethyl sulfoxide; EDCA, *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide; EtOAc, ethyl acetate; Et_2O, diethyl ether; EtOH, ethanol; GPC, guinea pig colon; HOAt, 1-hydroxy-7-azabenzotriazole; HOBt, 1-hydroxybenzotriazole; HUB, human urinary bladder; IBS, irritable bowel syndrome; MeOH, methanol; NK, neurokinin; SP, susbtance P; TFA, trifluoroacetic acid; THF, tetrahydrofuran.

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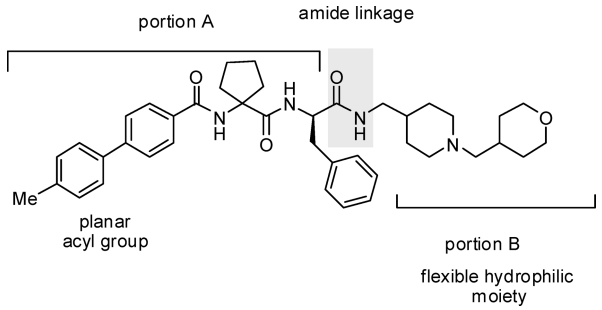
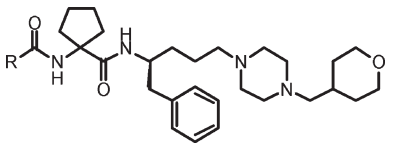
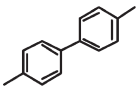
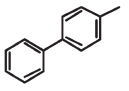
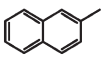
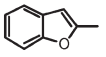
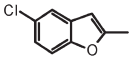
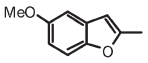
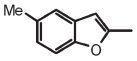
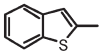
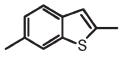


Figure 1. General structure of our NK_2 antagonists represented by (*R*)-4′-methyl-*N*-(1-(1-oxo-3-phenyl-1-((1-((tetrahydro-2*H*-pyran-4-yl)methyl)piperidin-4-yl)methylamino)propan-2-ylcarbamoyl)-cyclopentyl)biphenyl-4-carboxamide.[2]

Table 1. Binding Affinity for the hNK_2 Receptor, in Vitro Functional Activity, and Permeability of Compounds of General Formula **10**



10

Compound	R	pKi (hNK2)±SEM[a]	pK_B (GPC)±SEM[b]	Papp[c] (10^{-6} cm/s)
10a		9.2±0.04	7.8±0.10	7.10
10b		9.3±0.04	8.7±0.12	13.6
10c		9.5±0.05	8.2±0.03	14.6
10d		9.2±0.04	8.2±0.12	20.2
10e		9.6±0.12	7.6±0.15	13.7
10f		9.2±0.03	7.4±0.11	8.60
10g		9.4±0.008	8.7±0.09	30.3
10h		9.6±0.07	8.2±0.10	11.0
10i		10.3±0.08	8.6±031	11.2

[a] pK_i for inhibition of specific binding of [^{125}I]neurokinin A to recombinant hNK_2 receptor in a cell membrane preparation. [b] pK_B functional activity in guinea pig isolated proximal colon. For details see the Experimental Section. [c] Caco-2 permeability. For details see the Experimental Section.

Once prepared, the compounds were submitted to binding affinity evaluation on the hNK_2 receptor and the results are reported in Tables 1−3. Since our in vivo test was going to be performed on guinea pig, the most active molecules ($pK_i \geq 8.5$) were evaluated for functional antagonist potency in guinea pig isolated proximal colon (GPC pK_B). Finally, for a selected panel of antagonists, an in vivo evaluation was performed (iv dosing).

Chemistry

Wishing to remove the aforesaid amide bond, we decided that the first modification to be realized was its substitution with an ethylene group combined with the introduction of a second nitrogen atom onto the piperidine ring (i.e., a piperazine) in order to avoid an excessive increase of local lipophilic character, as shown in Figure 2.

A number of planar, relatively lipophilic aromatic systems were used as N-terminal capping group R according to the general synthetic sequence reported in Scheme 1. α,β-Unsaturated ester **1**[3] was saturated through catalytic hydrogenation (**2**), submitted to basic hydrolysis (**3**), and finally coupled, under standard conditions (EDCA, HOBt, DIPEA), with amine **5** to afford amide **6**. Removal of the Boc group (**7**) followed by $LiAlH_4$ reduction gave amine **9**. The introduction of portion A to produce the final molecules (**10a**−**i**) was achieved in a single step by reaction of the free amine **9** with oxazolones **15a**−**i** in DMF at room temperature. These oxazolones were obtained in a two-step synthetic sequence from acyl chlorides of general formula **13** that were reacted with 1-amino-1-cyclopentanecarboxylic acid in the presence of BSA. BSA here has the double role of activating the amino group on one side and preventing the free carboxylic acid to form a mixed anhydride on the other, hence avoiding the need to protect and deprotect the amino acid. The intramolecular closure of the so obtained N-capped amino acid **14** with EDCA completed the sequence.

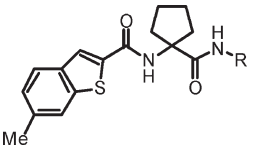
Through the application of the same synthetic pathway but using the L isomer **11** as starting material, we prepared **12**, the enantiomer of **10i**. The reaction of the intermediate **7** with oxazolone **15i** was repeated for the synthesis of **8**, an analogue of **10i** where an amide group was reintroduced. However, since in this case the amide is tertiary, the overall balance still resulted in a net elimination of a hydrogen bond group donor compared to the starting motif.

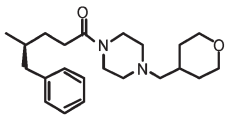
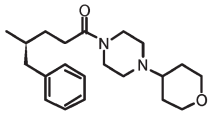
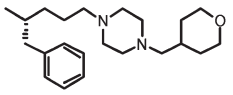
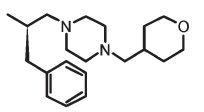
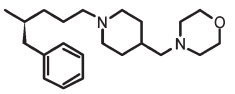
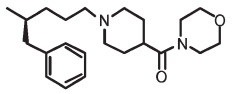
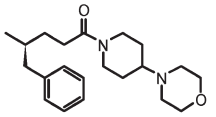
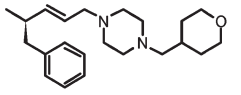
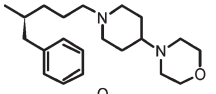
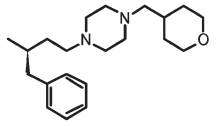
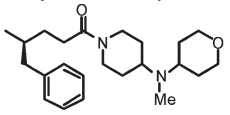
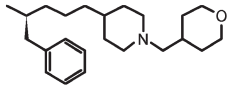
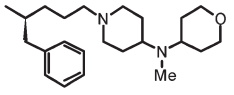
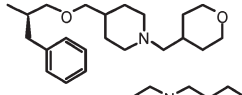
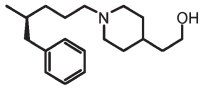
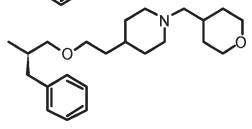
When possible, final compounds were simply crystallized; otherwise, they were purified by preparative HPLC to obtain the corresponding trifluoroacetate salts.

Pushed by the intention to explore further the motif of the amide/ethylene substitution with slight modifications on the flexible hydrophilic moiety, we coupled either acid **3** or its *N*-succinimidyl activated form **4** (Scheme 2)[4] to a series of piperidine and piperazine derivatives. This led, after Boc deprotection, to amine hydrochlorides **16**−**21**. An exhaustive reduction of the carbonyl groups with $LiAlH_4$ in THF at refluxing temperature, prior to the coupling with oxazolone **15i**, allowed us to obtain the fully reduced compounds **22**, **24**, **26**, **27**, and **28** (Scheme 2). The direct reaction of the intermediate amines **17**, **18**, and **21** with oxazolone **15i** led to compound **23**, **25**, and **29**, giving the opportunity to further explore the properties of the tertiary amides.

The increased flexibility of the C-terminal portion due to the amide/ethylene replacement encouraged us to prepare and test also the shorter homologues of **10i**. The coupling reaction of the commercially available acid **38** with amine **5**, followed by Boc deprotection, exhaustive reduction with $LiAlH_4$, and final reaction with oxazolone **15i** gave **40**, the one methylene unit shorter homologue of **10i** (Scheme 3). The two methylene units shorter homologue **32** was prepared from reductive amination of the commercially available Boc-protected aldehyde of

Table 2. Binding Affinity for the hNK_2 Receptor and in Vitro Functional Activity[a]



Compound	R	Ki (hNK2)±SEM	pK_B (GPC)±SEM	Compound	R	Ki (hNK2)±SEM	pK_B (GPC)±SEM
7		10.1±0.1	7.9±0.15	**29**		10.1±0.10	8.2±0.07
12		9.2±0.04	7.7±0.06	**32**		8.7±0.03	7.6±0.08
22		9.0±0.08	7.8±0.008	**35**		9.1±0.07	7.8±0.10
23		8.7±0.09	7.8±0.08	**37**		9.3±0.06	8.2±0.13
24		9.3±0.04	7.8±0.04	**40**		7.8±0.06	––
25		9.5±0.04	7.8±0.07	**47**		9.9±0.15	7.9±0.21
26		8.9±0.05	7.8±0.08	**55a**		9.2±0.03	7.6±0.15
27		8.7±0.04	8.1±0.08	**55b**		9.3±0.05	6.9±0.07

[a] pK_i for inhibition of specific binding of [^{125}I]neurokinin A to recombinant hNK_2 receptor in a cell membrane preparation; for details see the Experimental Section. pK_B gunctional activity in guinea pig isolated proximal colon; for details see the Experimental Section.

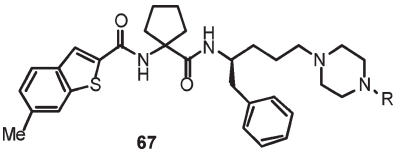
D-phenylalanine (**30**) with amine **5** in presence of $Na(AcO)_3BH$[5] as reducing agent, followed by Boc deprotection in acidic media and subsequent reaction with oxazolone **15i**. We also reduced the newly created flexibility by synthesizing the trans-olefin as amide geometrical isoster. Wittig reaction of aldehyde **30** with (formylmethylene)triphenylphosphorane gave the unsaturated aldehyde **33**, which was submitted to reductive amination with amine **5** in the Magid conditions to give olefin **36**. Boc deprotection of the amine and then reaction with oxazolone **15i** led to the planned analogue **37**. The reductive amination of **33** with morpholine(piperidin-4-yl)methanone, followed by the three-step sequence catalytic hydrogenation/acidic Boc-deprotection/oxazolone reaction, afforded compound **35**, where the methylene joining the two C-terminal six-membered rings is replaced by a carbonyl, with an overall reduction of the system rotational freedom.

Compound **47**, where an additional nitrogen atom has been eliminated, was obtained from aldehyde **41**.[6] This aldehyde was submitted to a Wittig reaction with the ylide generated in situ from phosphonium salt **50**, which was in turn prepared according to the sequence reported in Scheme 4, and afforded **42**. Subsequent hydrogenation of **42** followed by selective removal of the trityl group in mild acidic media and reaction with oxazolone **15i** resulted in intermediate **45**. Removal of the Boc group in strong acidic medium and then reductive amination with 4-tetrahydropyranylaldehyde gave **47** (Scheme 4).

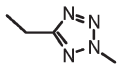
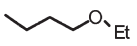
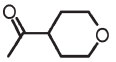
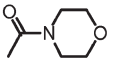
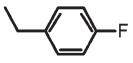
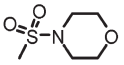
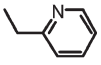
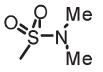
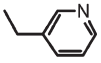
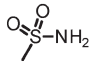
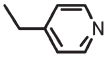
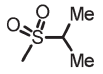
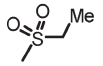
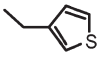
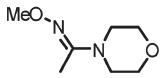
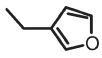
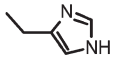
The two compounds containing an ether junction between portions A and B were both prepared from D-phenylalaninol **51**. Alkylation with mesylate **57** or iodide **49** of the alcoholate of **51** formed in situ gave ethers **52a** and **52b**, respectively. Reaction with oxazolone **15i** followed by Boc cleavage in acidic media and reductive amination with 4-tetrahydropyranylaldehyde yielded products **55a** and **55b** (Scheme 5).

The exploration was completed by the preparation of a number of molecules differing from **10i** only at the substituent on the terminal piperazine nitrogen, which were prepared according to two major routes, A and B, reported in Scheme 6.

Table 3. Binding Affinity for the hNK_2 Receptor and in Vitro Functional Activity of Compounds of General Formula **67**



67

Compound	R	Ki (hNK2)±SEM[a]	pK_B (GPC)±SEM[b]	Compound	R	Ki (hNK2)±SEM[a]	pK_B (GPC)±SEM[b]
7		10.1±0.1	7.9±0.15	**67l**		9.5±0.08	7.9±0.21
10i		10.3±0.08	8.6±0.31				
47		9.9±0.15	7.9±0.21	**67m**		9.5±0.05	7.2±0.07
55a		9.2±0.03	7.6±0.15	**67n**		10±0.09	8.1±0.19
67a		9.6±0.09	8.1±0.09	**67o**		9.4±0.09	8.2±0.12
67b		9.2±0.03	7.8±0.11	**67p**		8.7±0.03	7.8±0.16
67c		9.1±0.04	8.0±0.24	**67q**		9.4±0.06	8.3±0.08
67d		9.9±0.20	8.2±0.26	**67r**		9.3±0.05	8.4±0.12
67e		9.8±0.13	7.9±0.15	**67s**		9.5±0.06	7.9±0.22
67f		9.5±0.10	8.2±0.11	**67t**		9.2±0.07	7.8±0.34
67g		9.6±0.09	8.4±0.15	**67u**		9.3±0.06	7.9±0.06
67h		9.8±0.13	7.7±0.35	**67v**		9.2±0.04	8.0±0.21
67i		9.8±0.11	8.2±0.12	**67w**		9.1±0.08	8.0±0.18
67j		9.8±0.12	8.2±0.26				
67k		9.0±0.06	8.0±0.07				

[a] pK_i for inhibition of specific binding of [^{125}I]neurokinin A to recombinant hNK_2 receptor in a cell membrane preparation. For details see the Experimental Section. [b] pK_B functional activity in guinea pig isolated proximal colon. For details see the Experimental Section.

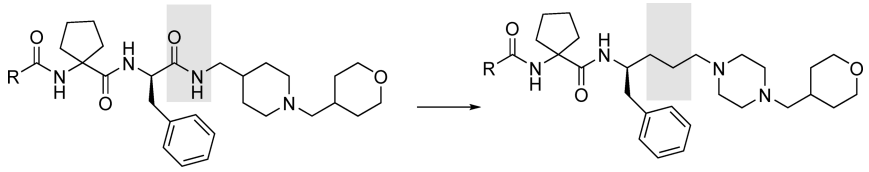
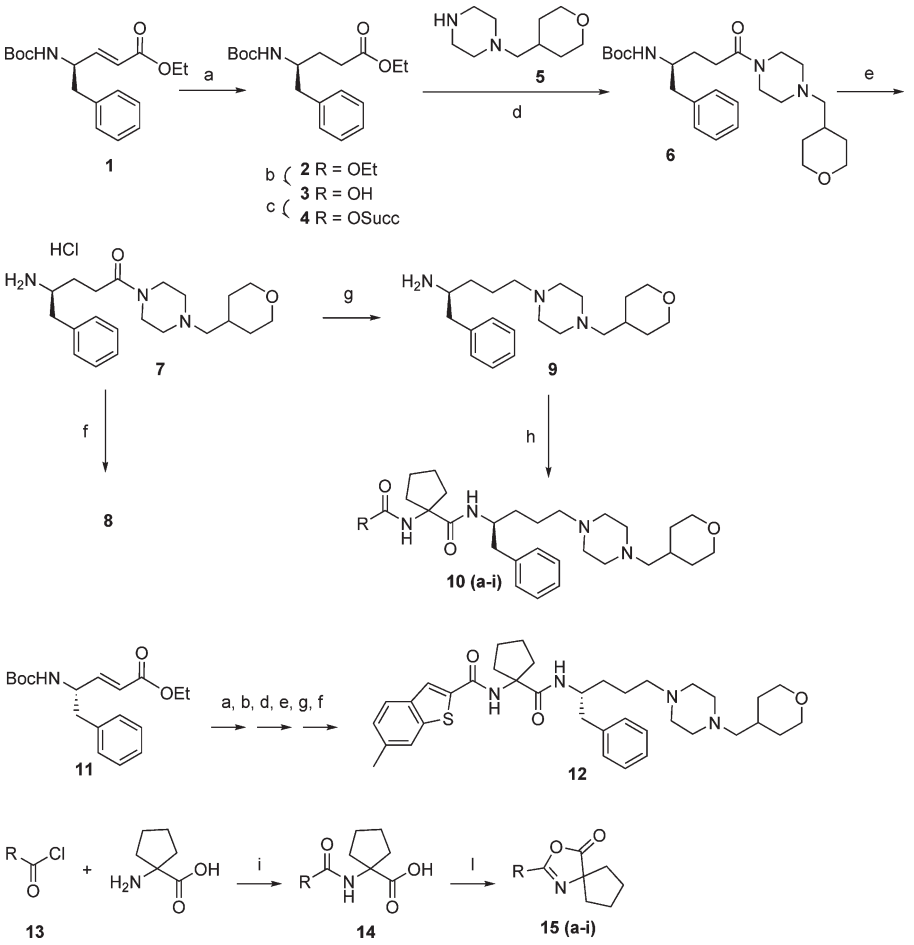


Figure 2. Working strategy: removal of the amide bond linking portions A and B.

In the first one the cleavage of the Boc group of ester **2** afforded **58**, which was reacted with oxazolone **15i** to obtain ester **59**. Basic hydrolysis of the ester, followed by coupling with *N*,*O*-dimethylhydroxylamine to prepare the Weinreb amide and reduction with $LiAlH_4$ afforded aldehyde **62**. This was reductively aminated in presence of $Na(OAc)_3BH$ with variously functionalized piperidines **69** to produce the main part of the derivatives **67**.

Alternatively, reductive amination of aldehyde **33** with Cbz-piperazine afforded **63**, which was sequentially deprotected in acidic media, coupled with oxazolone **15i**, and submitted to hydrogenation in the presence of ammonium

Scheme 1[a]



[a] Reagents: (a) 10% Pd−C, H_2, EtOH; (b) 1 M NaOH, MeOH/H_2O, 1:1; (c) DCC, *N*-hydroxysuccinimide, THF; (d) EDCA, HOBt, DIPEA, DMF, room temp; (e) 4 N HCl in dioxane, room temp; (f) **15i**, DMF, room temp; (g) $LiAlH_4$, THF, room temp; (h) **15a**−**i**, DMF, room temp; (i) BSA, DCM, room temp; (l) EDCA, DIPEA, DME, room temp.

formate. Through this latest step the double bond was saturated and the Cbz group was removed to produce **66**. Reductive amination with a variety of aldehydes gave the remaining compounds **67**. Formyl derivative **67p** was obtained as a side product of the homogeneous hydrogenation reaction and isolated through preparative HPLC purification.

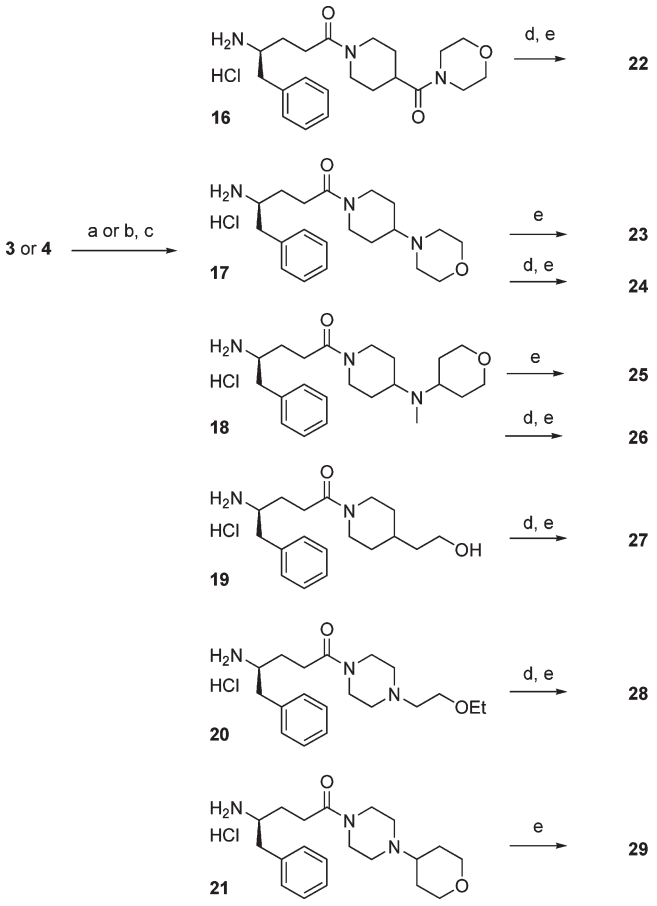
Results and Discussion

In the first set of compounds (Table 1) of general formula **10**, portion B was maintained constant while varying the N-terminal capping group. Binding affinities were always in the subnanomolar range, reaching the low picomolar value with the methylbenzothiophene derivative **10i**. The results from the functional activity test on guinea pig isolated proximal colon were also quite good, with values always in the low nanomolar range. The permeability evaluated in the Caco-2 cell system was also generally good (Table 1). We found the high values of permeability for the benzofurane derivatives **10d** (20.2) and **10g** (30.3) intriguing when compared with those of the corresponding benzothiophenes **10h** (11.0) and **10i** (11.1). Further experimental work with the Caco-2 cells excluded that the observed difference was due to active transport. A search in the Cambridge Crystallographic Database for benzothiophene and benzofurane-1-carboxylic acid amides showed an interesting feature: all the benzofurans seemed to have the ring oxygen in the anti configuration with respect to the amide carbonyl (B, Figure 3), while for all the benzothiophenes the situation is inverted (**A**, Figure 3).

We might suppose that the proximity of the heterocyclic oxygen helps somewhat the reduction of the hydrogen bond potential of the amide bond. This would facilitate the desolvation of the solute, necessary to enter the hydrophobic, non-hydrogen bonding interior of the membrane. In fact, desolvation is considered the rate limiting step in transport across the cell membrane.[7] These conformational preferences appeared to further confirm our general observation, formulated for very close chemical series, that the SARs relative to compounds bearing 6-substituted benzothiophenes as acyl capping groups paralleled well with those of compounds capped with the corresponding 5-substituted benzofuranes and vice versa.

Considering the data reported in Table 1, we decided that the best balance between binding affinity to the human receptor and functional activity was that given by the 6-methylbenzothiophene capping group, and therefore, we used this moiety for the study of further modifications in the flexible hydrophobic portion. Our previous work had shown a clear preference for the phenyl alanine in the D configuration over the L one. However, we wondered if the increase in flexibility due to the elimination of the amide could reverse the situation. Compound **12**, an enantiomer of **10i**, demonstrated that this was not the case. In fact both the binding affinity and the functional activity of **12** were almost 1 log unit lower

Scheme 2[a]



[a] Reagents: (a) EDCA, HOBt, DIPEA, amine, DMF, room temp; (b) DIPEA, amine, THF; (c) 4 N HCl in dioxane; (d) $LiAlH_4$, THF, reflux; (e) **15i**, DMF, room temp.

(9.2 vs 10.3 for pK_i and 7.7 vs 8.6 for pK_B, respectively) than those of **10i**.

Analogous results were obtained by introducing a trans double bond as a geometrical mimic of the anti amide linkage (compound **37**). Shortening the aliphatic chain connecting the hydrophobic portion by one (**40**) or two (**32**) methylenes was also deleterious for binding affinity, with a drop of 1 and 2 log units, respectively (Table 2).

The reintroduction of a carbonyl group near the piperazine ring (**7**), the displacement of the nitrogen atom from the first ring to the second one (**22**), the deletion of a nitrogen atom on the first ring through substitution with one carbon unit (**47**), and the introduction of an oxygen atom on the flexible C-terminal chain (**55a** and **55b**) seemed to have no major effect on the binding but a considerable impact on functional activity, which drops below 8. The same was true for many analogues with the exception of the derivative where the pyran ring was opened to form an alcohol (**27**). In this case a significant fall in binding affinity was also observed.

In the subsequent panel of derivatives, the C-terminal piperazine was maintained whereas the molecular properties were modulated through the piperazine N substituent (Table 3). Also in this case, in spite of the different chemical nature of the groups introduced, like basic aromatic heterocycles, hydrophilic heterocycles, sulfonamides, sulfamides, and guanidines, the binding affinities were all subnanomolar, confirming that very likely the rigid lipophilic portion plays the role of an anchoring section for the receptor according to the Verkhivker definition.[8] The results concerning the in vitro functional activity reported in Tables 1−3 show that the pK_B is generally 1 log unit or more lower than the pK_i and this is not dependent on the hydrophylicity/lipophlicity balance. The fact that the antagonist potency at the guinea pig receptor is within 1 log unit lower than the affinity for the human receptor is a general trend for this series of compounds, already observed in the past, and seems not to be dependent on the species but rather on the different experimental conditions used to estimate the affinity (radioligand binding) and the antagonist potency (smooth muscle contractility assay in the GPC). In addition, because of the different incubation times of the experiments and considering the high values of binding affinities, we cannot exclude effects due to differences in residence time.[9] Very likely, when such differences are higher than 1 log unit, in addition to the different testing conditions, they are also related to the differences between human and GP receptors. Indeed this was the case for the few compounds within this project that were tested for functional activity on isolated urinary bladder (HUB), where the difference remained within 1 log unit.

In Vivo Pharmacology. As anticipated, selected compounds were tested in our animal model.[10] The potency in inhibiting the colonic contractions induced by the selective tachykinin NK_2 receptor agonists [βAla^8]neurokinin A(4−10) ([βAla^8]neurokinin A(4−10), 3 nmol kg^{-1} iv) in guinea pig was evaluated after iv administration at a cutoff dose of 3 μmol kg^{-1}. The results, obtained after iv administration of the antagonist, are expressed both as maximal inhibitory effect reached (%i_{max}) and as $\sum$i%max (Table 4). The latter value is expressed as the sum of the percent inhibition vs the basal response of the colon contractions induced by the selective agonist at nine time-point observations (5, 30, 60, 90, 120, 150, 180, 210, and 240 min) after iv administration of the antagonist and further calculated as a percentage of the theoretical maximal inhibitory response ($\sum$(%$i_{max\text{-}th}$)), which is a constant and equal to 900. The mean percent inhibition over the entire experiment is expressed as

$$\sum \text{i\%max} = \frac{\sum(\%\text{i})}{\sum(\%\text{i}_{\text{max-th}})} \times 100$$

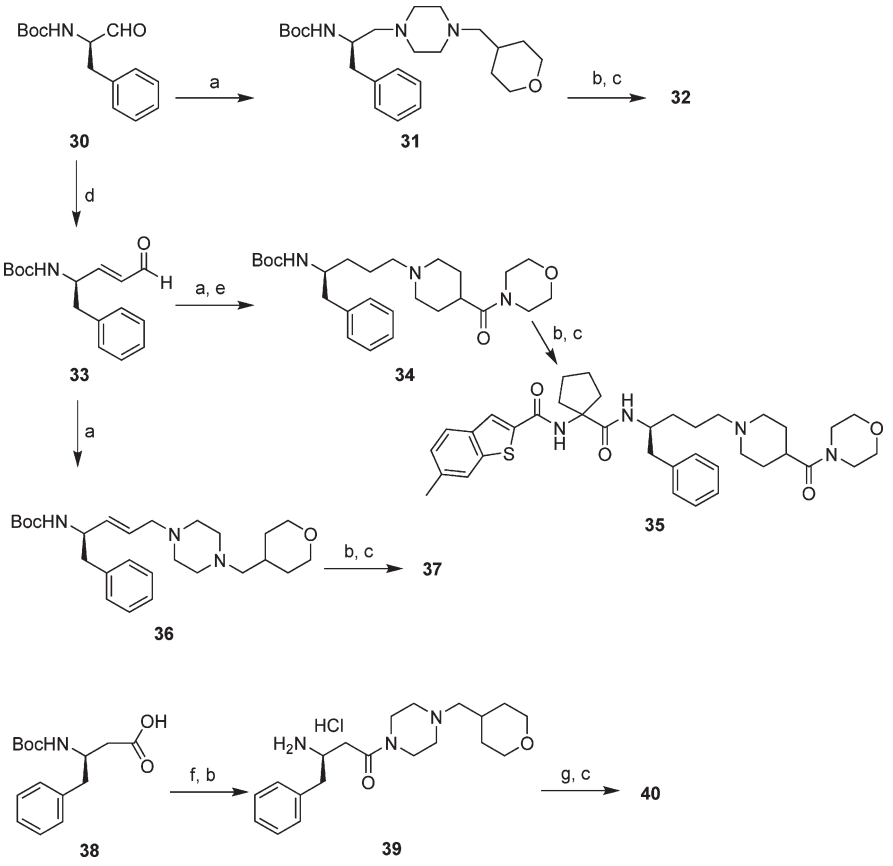
This parameter gives a measure of the activity during the entire experimental period therefore allowing the evaluation of both the intensity and the duration of the antagonist effect. The maximal inhibition corresponds to $\Sigma i\%$ max = 100, while the absence of effect results in $\Sigma i\%$max = 0.

In this case, as expected, activities were very different for the different compounds, reflecting the complexity of the multiparametric nature of the interaction of a small molecule with a living organism. The interplay between tissues distribution, metabolic degradation, protein binding, etc. resulted in almost no in vivo activity for **22** to the full antagonist potency of **10i**, in spite of the two compounds differing only for the position of one nitrogen atom. In fact, while in **22** the nitrogen atom is in the second ring of the flexible portion B, in **10i** it is in the first heterocyclic ring of the same molecular portion.

Because of its very good activity, **10i** was further profiled. It was tested for functional activity on human bladder (HUB) and found to have pA_2 = 9.7. Administration via id at a dose of 10 μmol/kg showed a significant systemic activity (Figure 4).

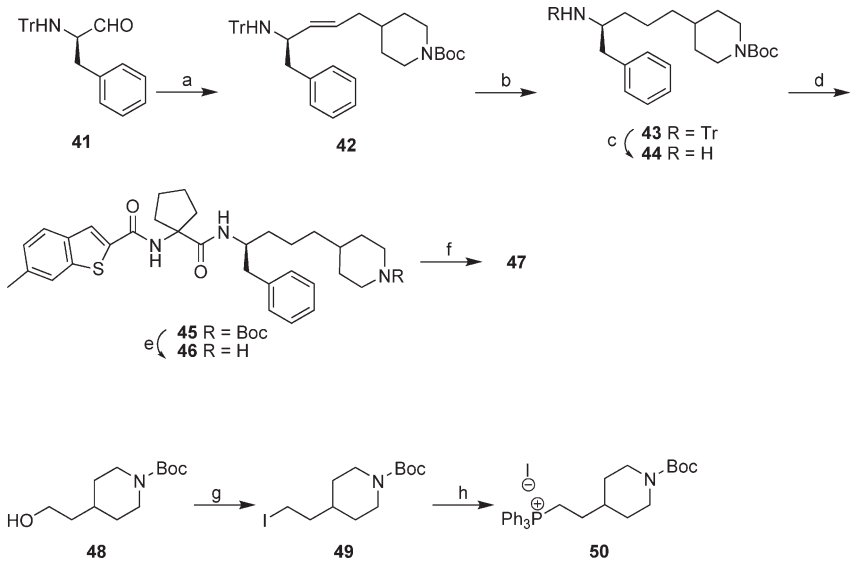
In conclusion a work that started with libraries of capped dipeptides and then optimized separately at the various

Scheme 3[a]



[a] Reagents: (a) **5** or morpholine(piperidin-4-yl)methanone, $Na(AcO)_3BH$, THF, 0 °C; (b) 4 N HCl in 1,4-dioxane, room temp; (c) **15i**, DMF, room temp; (d) (formylmethylene)triphenylphosphorane, DCM, room temp; (e) 10% Pd/C, EtOH; (f) **5**, EDCA, HOAt, DIPEA, DMF, room temp; (g) $LiAlH_4$, THF.

Scheme 4[a]



[a] Reagents: (a) NaHMDS 1M, THF, **50**; (b) 10% Pd−C, H_2, EtOH; (c) 1% TFA, DCM; (d) **15i**, DIPEA, DMF; (e) 4 N HCl in 1,4-dioxane; (f) tetrahydropyranecarboxaldehyde, $Na(OAc)_3BH$, DCM; (g) PPh_3, imidazole, I_2, DCM; (h) PPh_3, CH_3CN.

components while continuing to reduce the peptidic character led to the identification of compound **10i** endowed with subnanomolar potency in all the in vitro tests and that was highly potent and of long duration upon in vivo testing, after both iv and id dosing.

Experimental Section

(**A**) **Chemistry.** Commercial chemicals and solvents were of reagent grade and used without further purification. Merck silica gel (Kieselgel 60) was used for analytical thin-layer chromatography (TLC, F_{254} plates) and flash chromatography (230−400 mesh).

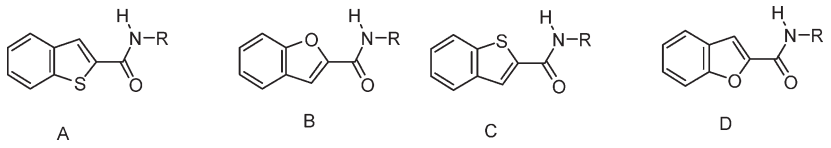
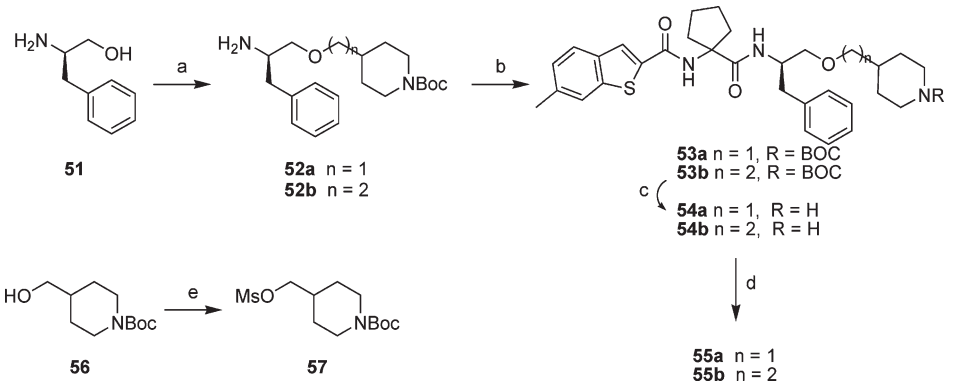


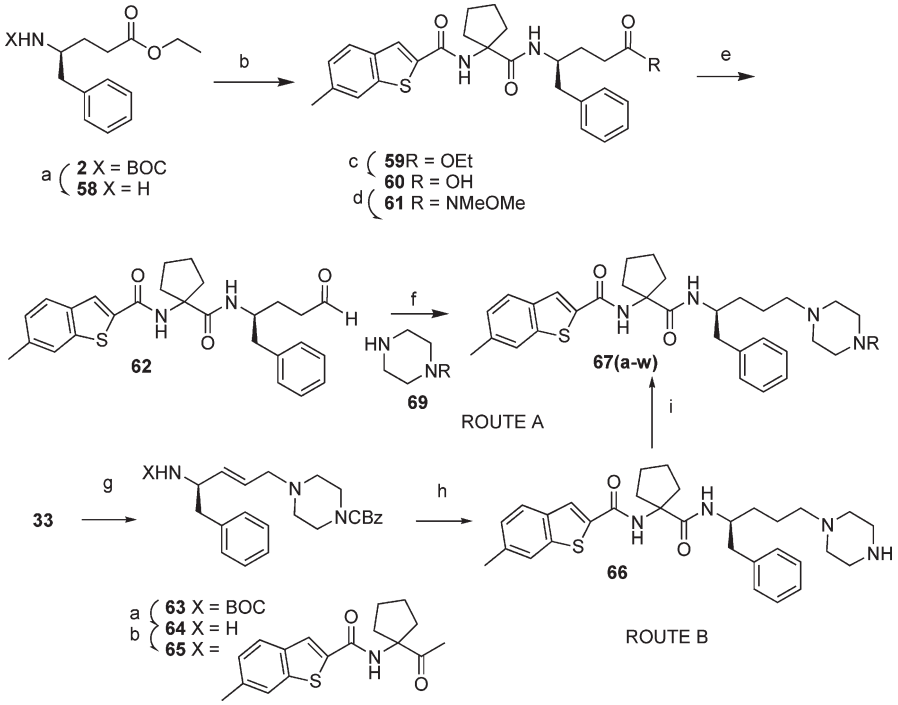
Figure 3. Possible planar conformations for benzothiophene- and benzofurane-2-carboxamides. Conformations **A** and **B** are represented in the Cambridge Crystallographic Database, while conformations **C** and **D** are not represented.

Scheme 5[a]



[a] Reagents: (a) KH, THF, **57** or **49**; (b) **15i**, DMF, room temp; (c) 4 N HCl in dioxane, room temp; (d) 4-tetrahydropyranylaldehyde, $Na(AcO)_3BH$, DCM, room temp; (e) MsCl, DCM, DMAP.

Scheme 6[a]



[a] Reagents: (a) 4 N HCl in 1,4-dioxane; (b) **15i**, DIPEA, DMF, room temp; (c) 1 M NaOH, H_2O/MeOH; (d) EDAC, HOBt, *N*,*O*-dimethylhydroxylamine HCl, DIPEA, DMF; (e) $LiAlH_4$, THF; (f) **A**, $Na(OAc)_3BH$, DCM; (g) Cbz-piperazine, $Na(OAc)_3BH$, DCM; (h) ammonium formate, 10% Pd/C, EtOH; (i) RCHO, $Na(OAc)_3BH$, DCM.

Purity was evaluated by analytical HPLC using a 600 E Waters pump coupled to a Jasco 875 UV detector and a Merck-Hitachi D-2500 integrator or a system comprising a Jasco PU-980 pump, LG-980-02 gradient unit, a UV-975 UV/vis detector, and a Merck-Hitachi D-2500 integrator or a Beckman System Gold apparatus or an Agilent 1100 analytic HPLC system. The solvents were (A) water + 0.1% TFA and (B) AcCN + 0.1% TFA with flow rate of 1 mL/min. System A consisted of the following: Symmetry RP-C18 column, 3.5 μm, 4.6 mm × 100 mm; $\lambda = 214$ nm; 1 min 10% solvent B, then from 10% B to 80% B in 10 min; flow of 1.0 mL/min. System B consisted of the following: Vydac pept and prot C18 column, 5 μm, 4.6 mm × 250 mm; $\lambda = 223$ nm; gradient from 20% B to 100% B in 20 min. All the tested compounds had purities of ≥95%.

Preparative reverse phase HPLC was performed on a Waters 600E apparatus with a Jasco 874 UV detector or on a Waters

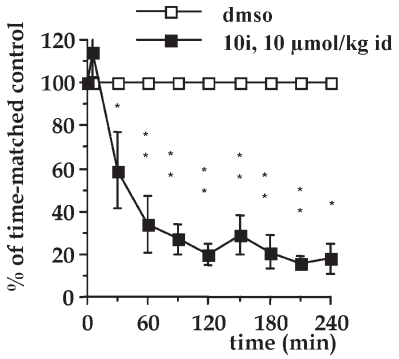


Figure 4. In vivo activity of compound **10i** after id administration at a dose of 10 μmol/kg in inhibiting the colonic contractions induced by the NK_2 receptor agonist [βAla^8]neurokinin A(4−10). The vehicle is DMSO.

Table 4. In Vivo Evaluation of Selected Compounds[a]

compd	$\sum$i%max[b]	%i$_{max}$ ± SEM[c]	compd	$\sum$i%max[b]	%i$_{max}$ ± SEM[c]
7	65	99 ± 1	**67c**	45	63 ± 5
10a	44	60 ± 7	**67d**	63	66 ± 4
10b	66	86 ± 6	**67e**	72	90 ± 4
10d	55	65 ± 5	**67f**	71	84 ± 9
10e	88	100 ± 0	**67g**	55	86 ± 7
10f	80	98 ± 1	**67h**	50	83 ± 8
10g	91	100 ± 0	**67i**	83	88 ± 9
10h	67	82 ± 9	**67j**	47	67 ± 13
10i	94	100 ± 0	**67k**	22	83 ± 8
22	8	62 ± 9	**67l**	35	72 ± 9
25	60	90 ± 6	**67n**	79	97 ± 2
27	11	47 ± 11	**67o**	71	100 ± 0
29	59	99 ± 1	**67q**	39	85 ± 5
35	26	81 ± 8	**67r**	62	97 ± 2
37	42	72 ± 14	**67s**	48	80 ± 9
47	41	67 ± 11	**67t**	43	62 ± 5
55a	23	46 ± 3	**67u**	19	85 ± 7
67a	75	100 ± 0	**67v**	29	77 ± 5
67b	60	76 ± 7	**67y**	47	100 ± 0

[a] Inhibition of colonic contractions induced by [βAla^8]NKA(4−10) in guinea pig after iv administration at 3 μmol/kg. [b] $\sum$i%max = $\sum$(%i)/$\sum$(%i$_{max\text{-}th}$) × 100 = mean % inhibition over the entire experiment, that is, the sum of the % inhibition, vs the basal response of the colon contraction to [βAla^8]neurokinin A(4−10) at the nine times of observation (5, 30, 60, 90, 120, 150, 180, 210, and 240 min) after iv administration of the antagonist and further calculated as the percentage of the theoretical maximal inhibitory response [$\sum$(%i$_{max\text{-}th}$) = 900%]. [c] Maximal inhibitory effect as % inhibition of the basal response.

Delta-Prep 3000 apparatus. The mobile phases were the same as for the analytical systems. Gradient elution was employed. The columns used were a SymmetryPrep C18, 7 μm, 19 mm × 300 mm, a Hibar Lichrosorb RP-18, 7 μm, 25 mm × 250 mm, a Vydac C18, 10 μm, 22 mm × 250 mm, or Jupiter, 15 μm, 250 mm × 21.2 mm. Peak detection was at 220 and 254 nm. Chemical yields are not optimized. NMR experiments were recorded on a Varian Gemini 200 model J200 HC, a Varian 300 MHz spectrometer (equipped with a 5 mm inverse probe), a Bruker Avance 400 MHz, or a Bruker Avance 600 MHz machine and are referenced to residual solvent signals: $CDCl_3$ (δ 7.26) or DMSO-d_6 (δ 2.49). Chemical shifts are reported in δ units (parts per million) and are assigned as singlet (s), doublet (d), doublet of doublets (dd), triplet (t), multiplet (m), broad signal (br), or very broad signal (vbr). Coupling constants (J) are reported in hertz (Hz).

Mass spectra were recorded using a Waters Alliance 2795 HPLC system fitted with a UV-PDS 996 diode array detector, a ZMD mass spectrometer, and a GL Science Inertsil ODS-3 column (50 mm × 3 mm, 3 μm) or a ThermoFinnigan LCQ equipped with APCI or ESI source.

4-*tert*-Butoxycarbonylamino-5-phenylpentanoic Acid (3). A solution of **1** (9.39 g, 29.4 mmol) in EtOH (180 mL) was stirred with 10% Pd/C (939 mg) under hydrogen atmosphere for 5 h. The catalyst was filtered off and the solvent was removed in vacuo affording **2** as a white solid (9.00 g, 95%). Ester **2** (2.00 g, 6.22 mmol) was dissolved in 1 M NaOH and 1:1 MeOH/H_2O. The hydrolysis was immediate. Then 1 M HCl was added dropwise until complete precipitation of the crude acid, which was collected by filtration, washed with water, and finally dried in vacuo to obtain crude **3** (1.66 g, 91%), used as such without further purification. ^{1}H NMR (δ, DMSO-d_6, 300 MHz): 11.99 (bs, 1H), 7.29−7.16 (m, 5H), 6.72 (d, 1H, J = 6.7 Hz), 3.60 (m, 1H), 2.65 (m, 2H), 2.20 (m, 2H), 1.70−1.62 (m, 1H), 1.54−1.46 (m, 1H), 1.32 (s, 9H).

{1-Benzyl-4-oxo-4-[4-(tetrahydropyran-4-ylmethyl)piperazin-1-yl]butyl}carbamic Acid *tert*-Butyl Ester (6). Acid **3** (1.00 g, 3.40 mmol) was dissolved in dry DMF (10 mL). Amine **5** (690 mg, 4.08 mmol), HOBt (551 mg, 4.08 mmol), EDCA (783 mg, 4.08 mmol), and DIPEA (0.59 mL, 3.40 mmol) were added. The mixture was stirred at room temperature. At the end of the reaction (HPLC control), $NaHCO_3$ aqueous saturated solution (30 mL) was added and stirring was continued for an additional 30 min. Then EtOAc was added (50 mL). The two phases were separated, and the organic one was washed with $NaHCO_3$ saturated aqueous solution (×3), 1 N NaOH, and brine until neutral pH was obtained. Then it was dried over Na_2SO_4 and concentrated under reduced pressure to obtain crude **6** (1.29 g, 82.3%). ^{1}H NMR (δ, DMSO-d_6, 300 MHz): 7.35−7.10 (m, 5H), 6.70 (d, 1H), 3.95 (m, 1H), 3.90−3.75 (m, 2H), 3.65−3.50 (m, 2H), 3.30−3.20 (m, 4H), 2.70−2.60 (m, 2H), 2.40−2.20 (m, 6H), 2.20−2.05 (m, 2H), 1.85−1.45 (m, 5H), 1.45−1.20 (m, 9H), 1.20−1.0 (m, 2H). MS m/z calcd for $C_{26}H_{41}N_3O_4$: 459.62. Found 460.3 $[M + 1]^+$. HPLC purity: system A, t_R = 7.265 min.

4-Amino-5-phenyl-1-[4-(tetrahydropyran-4-ylmethyl)piperazin-1-yl]pentan-1-one Dihydrochloride (7). HCl, 4 M in dioxane (20 mL), was added to a solution of **6** (1.29 g, 2.80 mmol) in dioxane (5 mL), and the resulting mixture was stirred at room temperature for 1 h. The solvent was removed in vacuo. Et_2O was added and removed (×3) in order to totally eliminate the residue HCl. The resulting solid was finally triturated with Et_2O and collected by filtration to afford **7** as a yellow solid (1.20 g, quantitative). MS m/z calcd for $C_{21}H_{33}N_3O_2$: 359.51. Found 360.1 $[M + 1]^+$. HPLC purity: system A, t_R = 4.138 min.

1-Benzyl-4-[4-(tetrahydropyran-4-ylmethyl)piperazin-1-yl]-butylamine Hydrochloride (9). The hydrochloride salt of amine **7** (1.10 g, 2.08 mmol) was suspended in dry THF (30 mL). The suspension was cooled in an ice bath under nitrogen atmosphere, and a 1 M THF solution of $LiAlH_4$ (20.8 mmol) was added. The mixture was then stirred at room temperature for 3 h. LC−MS control showed the total consumption of the starting material and the formation of the product. The reaction mixture was cooled to 3−4 °C. Then the excess of $LiAlH_4$ was quenched by slow and cautious addition of ice. When the hydride was completely quenched (no more H_2 evolution was observed), NaOH pellets was added and the resulting suspension was stirred at room temperature for 1 h. The inorganic salts were filtered off throughout Celite washing with DCM. The biphasic system obtained was saturated with NaOH. Then it was transferred into a separation funnel and the phases were separated. The organic phase was dried over Na_2SO_4, filtered, and the solvent was removed under reduced pressure. The desired product was obtained as a pale-yellow oil used in the next reaction without any further purification. MS m/z calcd for $C_{21}H_{35}N_3O$: 345.52. Found 346.2 $[M + 1]^+$.

General Procedure for the Synthesis of Oxazolones 15a−i. To a suspension of the 1-amino-1-cyclopentanecarboxylic acid (5.70 g, 97%, 44 mmol) in dry DCM (200 mL), BSA (22 mL, 90 mmol) was rapidly added under inert atmosphere. The mixture was kept under magnetic stirring at room temperature until the solution became completely clear. A solution of the selected acyl chloride **13** (37 mmol) in DMC (100 mL) was added

dropwise. After one night at room temperature the solvent was removed under reduced pressure and the residue treated with 5% aqueous K_2CO_3 (300 mL) and extracted with EtOAc (×3). The aqueous phase was acidified with 37% HCl to the complete precipitation of intermediate **14** as a solid which was collected by filtration and dried under vacuum. Intermediate **14** (9.9 mmol) was dissolved in DME (48 mL). Then EDCA (2.08 g, 10.8 mmol) and DIPEA (1.69 mL, 9.9 mmol) were added. If necessary, DMF was added in order to obtain a clear solution. The reaction mixture was stirred at room temperature overnight and then concentrated under reduce pressure. The residue was partitioned between EtOAc and 5% $NaHSO_4$. The phases were separated and the organic one was washed with 5% $NaHCO_3$ and brine, dried over Na_2SO_4, filtered, and concentrated to give oxazolones **15a**−**i** in quantitative yield.

General Procedure for the Reaction of Oxazolones 15 with Amines 7 and 9. To a stirred solution of the amine (0.87 mmol) in DMF (5 mL), the selected oxazolone **15** (1.21 mmol) was added. The resulting mixture was stirred at room temperature for 15 h. HPLC showed total consumption of the amine. EtOAc (20 mL) and 10% $NaHCO_3$ (20 mL) were added, and the two phases were separated. The organic layer was washed with brine (10 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting crude product was purified by flash chromatography (silica, $CHCl_3$/MeOH various proportions) or by preparative HPLC.

6-Methylbenzo[*b*]thiophene-2-carboxylic Acid (1-{(*S*)-1-Benzyl-4-oxo-4-[4-(tetrahydropyran-4-ylmethyl)piperazin-1-yl]-butylcarbamoyl}cyclopentyl)amide Trifluoroacetate Salt (8). Amine **7** was reacted with oxazolone **15i** according to the general procedure. The crude product was purified by preparative HPLC. ^{1}H NMR (δ, DMSO-d_6, 400 MHz): 9.11 (brs, 1H), 8.3 (s, 1H), 8.05 (s, 1H), 8.03 (d, 1H), 7.85 (s, 1H), 7.69−7.38 (m, 3H), 7.32−7.00 (m, 4H), 4.02−3.98 (m, 1H), 3.72−3.50 (m, 4H), 3.44−3.36 (m, 3H), 2.80−2.65 (m, 3H), 2.43 (s, 3H), 2.52−2.33 (m, 4H), 2.09−1.81 (m, 7H), 1.69−1.51 (m, 6H), 1.45−1.28 (m, 6H). MS *m*/*z* calcd for $C_{37}H_{48}N_4O_4S$: 644.3. Found 645.3 $[M + 1]^+$.

4′-Methylbiphenyl-4-carboxylic Acid (1-{(*S*)-1-Benzyl-4-[4-(tetrahydropyran-4-ylmethyl)piperazin-1-yl]butylcarbamoyl}-cyclopentyl)amide Trifluoroacetate Salt (10a). Amine **9** was reacted with oxazolone **15a** according to the general procedure. The crude product was purified by preparative HPLC. ^{1}H NMR (δ, DMSO-d_6, 400 MHz): 9.15 (brs, 1H), 8.40 (s, 1H), 7.96 (d, 2H, *J* = 8.4 Hz), 7.75 (d, 2H, *J* = 8.4 Hz), 7.62 (d, 2H, *J* = 8.4 Hz), 7.40 (d, 1H), 7.30 (d, 2H, *J* = 8.4 Hz), 7.20 (m, 5H), 4.00 (m, 1H), 3.80 (m, 2H), 3.25 (m, 2H), 3.15−2.70 (m, 10H), 2.40 (m, 2H), 2.35 (s, 3H), 2.25 (m, 2H), 2.00 (m, 1H), 1.85 (m, 1H), 1.80−1.35 (m, 13H). MS *m*/*z* calcd for $C_{41}H_{54}N_4O_3$: 650.4 Found 651.5 $[M + 1]^+$. HPLC purity: system A, t_R = 8.265 min.

Biphenyl-4-carboxylic Acid (1-{(*S*)-1-Benzyl-4-[4-(tetrahydropyran-4-ylmethyl)piperazin-1-yl]butylcarbamoyl}cyclopentyl)-amide Trifluoroacetate Salt (10b). Amine **9** was reacted with oxazolone **15b** according to the general procedure. The crude product was purified by preparative HPLC. ^{1}H NMR (δ, DMSO-d_6, 400 MHz): 9.15 (brs, 1H), 8.40 (s, 1H), 8.00 (dd, 2H), 7.75 (m, 4H), 7.50 (m, 2H), 7.30 (m, 1H), 7.25 (m, 1H), 7.20 (m, 5H), 4.00 (m, 1H), 3.80 (m, 2H), 3.25 (m, 2H), 3.15−2.70 (m, 10H), 2.40 (m, 2H), 2.25 (m, 2H), 2.00 (m, 1H), 1.85 (m, 1H), 1.80−1.35 (m, 13H), 1.15 (m, 2H). MS *m*/*z* calcd for $C_{40}H_{52}N_4O_3$: 636.9. Found 637.5 $[M + 1]^+$. HPLC purity: system B, t_R = 9.46 min.

Naphthalene-2-carboxylic Acid (1-{(*S*)-1-Benzyl-4-[4-(tetrahydropyran-4-ylmethyl)piperazin-1-yl]butylcarbamoyl}cyclopentyl)amide Trifluoroacetate Salt (10c). Amine **9** was reacted with oxazolone **15c** according to the general procedure. The crude product was purified by preparative HPLC. ^{1}H NMR (δ, DMSO-d_6, 400 MHz): 9.15 (brs, 1H), 8.50 (m, 2H), 8.00 (m, 4H), 7.60 (m, 2H), 7.45 (m, 1H), 7.20 (m, 5H), 4.00 (m, 1H), 3.80 (m, 2H), 3.30 (m, 2H), 3.20−2.60 (m, 10H), 2.40 (m, 2H), 2.30 (m, 2H), 2.00 (m, 1H), 1.90 (m, 1H), 1.80−1.35 (m, 13H), 1.10 (m, 2H). MS *m*/*z* calcd for $C_{38}H_{50}N_4O_3$: 610.3. Found 611.5 $[M + 1]^+$. HPLC purity: system B, t_R = 8.53 min.

Benzofuran-2-carboxylic Acid (1-{(*S*)-1-Benzyl-4-[4-(tetrahydropyran-4-ylmethyl)piperazin-1-yl]butylcarbamoyl}cyclopentyl)-amide Trifluoroacetate Salt (10d). Amine **9** was reacted with oxazolone **15d** according to the general procedure. The crude product was purified by preparative HPLC. ^{1}H NMR (δ, DMSO-d_6, 400 MHz): 9.25 (brs, 1H), 8.50 (s, 1H), 7.80 (d, 1H), 7.70 (d, 1H), 7.65 (s, 1H), 7.50 (m, 2H), 7.35 (dd, 1H), 7.20 (m, 5H), 4.05 (m, 1H), 3.85 (m, 2H), 3.35 (m, 2H), 3.20−2.80 (m, 8H), 2.70 (m, 2H), 2.40 (m, 2H), 2.20 (m, 2H), 2.00 (m, 1H), 1.85 (m, 1H), 1.80−1.35 (m, 13H), 1.15 (m, 2H). MS *m*/*z* calcd for $C_{36}H_{48}N_4O_4$: 600.4. Found 601.5 $[M + 1]^+$. HPLC purity: system B, t_R = 8.25 min.

5-Chlorobenzofuran-2-carboxylic Acid (1-{(*S*)-1-Benzyl-4-[4-(tetrahydropyran-4-ylmethyl)piperazin-1-yl]butylcarbamoyl}-cyclopentyl)amide Trifluoroacetate Salt (10e). Amine **9** was reacted with oxazolone **15e** according to the general procedure. The crude product was purified by preparative HPLC. ^{1}H NMR (δ, DMSO-d_6, 400 MHz): 8.60 (s, 1H), 7.93 (d, 1H), 7.74 (d, 1H), 7.63 (s, 1H), 7.50 (m, 2H), 7.20 (m, 5H), 4.10−2.90 (m, 17H), 2.72 (m, 2H), 2.20 (m, 1H), 2.00−1.05 (m, 16H). MS *m*/*z* calcd for $C_{36}H_{47}ClN_4O_4$: 634.3. Found 635.4 $[M + 1]^+$. HPLC purity: system B, t_R = 8.84 min.

5-Methoxybenzofuran-2-carboxylic Acid (1-{(*S*)-1-Benzyl-4-[4-(tetrahydropyran-4-ylmethyl)piperazin-1-yl]butylcarbamoyl}-cyclopentyl)amide Trifluoroacetate Salt (10f). Amine **9** was reacted with oxazolone **15f** according to the general procedure. The crude product was purified by preparative HPLC. ^{1}H NMR (δ, DMSO-d_6, 400 MHz): 8.50 (s, 1H), 7.60 (d, 1H), 7.58 (s, 1H), 7.50 (d, 1H), 7.30−7.07 (m, 7H), 4.11−2.50 (m, 18H), 3.85 (s, 3H), 2.20 (m, 1H), 2.00−1.05 (m, 17H). MS *m*/*z* calcd for $C_{37}H_{50}N_4O_5$: 630.4. Found 631.4 $[M + 1]^+$. HPLC purity: system B, t_R = 8.84 min.

5-Methylbenzofuran-2-carboxylic Acid (1-{(*S*)-1-Benzyl-4-[4-(tetrahydropyran-4-ylmethyl)piperazin-1-yl]butylcarbamoyl}-cyclopentyl)amide Trifluoroacetate Salt (10g). Amine **9** was reacted with oxazolone **15g** according to the general procedure. The crude product was purified by preparative HPLC. ^{1}H NMR (δ, DMSO-d_6, 400 MHz): 8.45 (s, 1H), 7.55 (3H, m), 7.45 (m, 1H), 7.30 (d, 1H), 7.20 (m, 5H), 4.00 (m, 1H), 3.90−2.80 (m, 16H), 2.70 (m, 2H), 2.40 (s, 3H), 2.20 (m, 1H), 2.00 (m, 1H), 1.90−1.05 (m, 15H). MS *m*/*z* calcd for $C_{37}H_{50}N_4O_4$: 614.8. Found 615.4 $[M + 1]^+$. HPLC purity: system A, t_R = 7.43 min.

Benzo[*b*]thiophene-2-carboxylic Acid (1-{(*S*)-1-Benzyl-4-[4-(tetrahydropyran-4-ylmethyl)piperazin-1-yl]butylcarbamoyl}-cyclopentyl)amide Trifluoroacetate Salt (10h). Amine **9** was reacted with oxazolone **15h** according to the general procedure. The crude product was purified by preparative HPLC. ^{1}H NMR (δ, DMSO-d_6, 400 MHz): 9.25 (brs, 1H), 8.60 (s, 1H), 8.25 (s, 1H), 8.00 (dd, 2H), 7.45 (m, 3H), 7.20 (m, 5H), 4.00 (m, 1H), 3.95−2.80 (m, 16H), 2.73 (m, 2H), 2.20 (m, 1H), 2.00 (m, 1H), 1.95−1.25 (m, 13H), 1.15 (m, 2H). MS *m*/*z* calcd for $C_{36}H_{48}N_4O_3S$: 616.3. Found 617.5 $[M + 1]^+$. HPLC purity: system B, t_R = 8.40 min.

6-Methylbenzo[*b*]thiophene-2-carboxylic Acid (1-{(*S*)-1-Benzyl-4-[4-(tetrahydropyran-4-ylmethyl)piperazin-1-yl]butylcarbamoyl}cyclopentyl)amide (10i). Amine **9** was reacted with oxazolone **15i** according to the general procedure. The crude product was purified by flash chromatography (silica, $CHCl_3$/MeOH 95:5). ^{1}H NMR (δ, DMSO-d_6, 400 MHz): 8.34 (s, 1H), 8.15 (s, 1H), 7.83−7.80 (m, 2H), 7.28 (d, 1H, *J* = 8.4 Hz), 7.23−7.12 (m, 6H), 4.00−3.94 (m, 1H), 3.83−3.79 (m, 2H), 3.29−3.16 (m, 3H), 2.73−2.61 (m, 2H), 2.45 (s, 3H), 2.25−2.08 (m, 10H), 2.08−1.83 (m, 6H), 1.67−1.51 (m, 6H), 1.46−1.22 (m, 5H), 1.21−1.00 (m, 2H). MS *m*/*z* calcd for $C_{37}H_{50}N_4O_3S$: 630.3. Found 631.2 $[M + 1]^+$. HPLC purity: system A, t_R = 7.488 min.

6-Methylbenzo[*b*]thiophene-2-carboxylic Acid {1-[(*S*)-1-Benzyl-4-(4-morpholin-4-ylmethylpiperidin-1-yl)butylcarbamoyl]-cyclopentyl}amide (22). A solution of acid **3** (250 mg, 0.85 mmol), EDCA (180 mg, 0.94 mmol), and HOBt (128 mg, 0.93 mmol) in DMF (7 mL) was stirred at room temperature for 1 h. A solution of 4-(morpholine-4-carbonyl)piperidinium chloride (200 mg, 0.85 mmol) and DIPEA (242 mg, 1.87 mmol) in DMF (2 mL) was added, and the resulting mixture was stirred overnight. It was then partitioned between 2 N NaOH and EtOAc, and the resulting phases were separated. The organic phase was washed with water (×3), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was dissolved in 4 N HCl in dioxane and stirred for 1 h. The solvent was removed in vacuo to afford **16** (280 mg, 80%) that was used as such without further purification. Compound **16** (250 mg, 0.61 mmol) was treated with a 1 M $LiAlH_4$ solution in THF (10 mL) at refluxing temperature for 3 h. The LC−MS control showed the total consumption of the starting material and the formation of the product. The reaction mixture was cooled to 3−4 °C. Then the excess of $LiAlH_4$ was quenched by slow and cautious addition of ice. When the hydride was completely quenched (no more H_2 evolution was observed), NaOH pellets were added and the resulting suspension was stirred at room temperature for 1 h. The inorganic salts were filtered off throughout Celite washing with DCM. The biphasic system obtained was saturated with NaOH. Then it was transferred into a separatory funnel and the phases were separated. The organic phase was dried over Na_2SO_4 and filtered, and the solvent was removed under reduced pressure. The desired product was obtained as a pale-yellow oil (110 mg, 52%) and used in the next reaction without any further purification. The crude amine was reacted with oxazolone **15i** according to the general procedure. The resulting product was purified by flash chromatography (silica, from $CHCl_3$/MeOH, 99:1 to 95:5, with final elution with EtOH) to obtain 70 mg (37%) of **22**. ^{1}H NMR (δ, DMSO-d_6, 400 MHz): 8.40 (s, 1H), 8.18 (s, 1H), 7.80 (m, 2H), 7.28 (m, 2H), 7.20 (m, 5H), 4.00 (m, 1H), 3.58 (m, 4H), 2.70 (m, 4H), 2.45 (s, 3H), 2.30 (m, 4H), 2.20 (m, 2H), 2.10 (m, 2H), 2.00 (m, 1H), 1.90 (m, 3H), 1.75−1.20 (m, 13H), 0.95 (m, 2H). MS *m*/*z* calcd for $C_{37}H_{50}N_4O_3S$: 630.8. Found 631.3 $[M + 1]^+$. HPLC purity: system A, $t_R = 7.482$ min.

6-Methylbenzo[*b*]thiophene-2-carboxylic Acid {1-[(*S*)-1-Benzyl-4-(4-morpholin-4-ylpiperidin-1-yl)-4-oxobutylcarbamoyl]-cyclopentyl}amide (23). A solution of acid **3** (203 mg, 0.69 mmol), EDCA (146 mg, 0.76 mmol), and HOBt (104 mg, 0.76 mmol) in 1:1 THF/DMF (6 mL) was stirred at room temperature for 1 h. 4-Morpholinopiperidine (129 mg, 0.76 mmol) was added, and the mixture was stirred for an additional 10 h. The mixture was then partitioned between EtOAc and 10% $NaHCO_3$. The two phases were separated and the organic one was washed with water, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting solid was triturated with Et_2O and collected by filtration. It was then treated with 4 N HCl in dioxane for 1 h. The solvent was removed under reduced pressure to obtain **17** (218.9 mg, 83%), which was used as such without further purification. Crude amine **17** was reacted with oxazolone **15i** according to the general procedure. The resulting crude was purified by flash chromatography ($CHCl_3$/MeOH, 95:5, 70 mg). ^{1}H NMR (δ, DMSO-d_6, 400 MHz): 8.40 (s, 1H), 8.18 (s, 1H), 7.81 (m, 2H), 7.37 (m, 1H), 7.28 (d, 1H), 7.20 (m, 5H), 4.28 (m, 1H), 3.95 (m, 1H), 3.75 (m, 1H), 3.55 (m, 4H), 2.9−2.68 (m, 3H), 2.50−2.00 (m, 8H), 2.45 (s, 3H), 2.00−1.45 (m, 12H), 1.15 (m, 2H). MS *m*/*z* calcd for $C_{36}H_{46}N_4O_4S$: 630.8. Found 631.3 $[M + 1]^+$. HPLC purity: system A, $t_R = 8.144$ min.

6-Methylbenzo[*b*]thiophene-2-carboxylic Acid {1-[(*S*)-1-Benzyl-4-(4-morpholin-4-ylpiperidin-1-yl)butylcarbamoyl]cyclopentyl}-amide Trifluoroacetate Salt (24). Crude amine **17** (110 mg) was treated with a 1 M solution of $LiAlH_4$ in THF at reflux for 4 h. The solution was cooled to room temperature and quenched by cautious addition of ice and NaOH pellets. The resulting mixture was stirred for an additional hour. Then the inorganic salts were filtered off through Celite washing with DCM. The biphasic resulting solution was separated into its components and the organic one was dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give a colorless oil (110 mg) that was reacted with oxazolone **15i** (94 mg) according to the general procedure. The resulting crude was purified by preparative HPLC. ^{1}H NMR (δ, DMSO-d_6, 400 MHz): 10.3 (brs, 1H), 9.37 (brs, 1H), 8.58 (s, 1H), 8.25 (s, 1H), 7.90 (d, 1H), 7.80 (s, 1H), 7.45 (m, 1H), 7.30 (d, 1H), 7.20 (m, 5H), 4.20−2.85 (m, 16H), 2.75 (m, 2H), 2.45 (s, 3H), 2.40−1.30 (m, 16H). MS *m*/*z* calcd for $C_{36}H_{48}N_4O_3S$: 616.8. Found 631.3 $[M + 1]^+$. HPLC purity: system A, $t_R = 7.299$ min.

6-Methylbenzo[*b*]thiophene-2-carboxylic Acid [1-((*S*)-1-Benzyl-4-{4-[methyl(tetrahydropyran-4-yl)amino]piperidin-1-yl}-4-oxobutylcarbamoyl)cyclopentyl]amide (25). A solution of acid **3** (400 mg, 1.36 mmol), EDCA (287 mg, 1.49 mmol), and HOBt (203 mg, 1.49 mmol) in THF (10 mL) was stirred at room temperature for 1 h. *N*-Methyl-*N*-(tetrahydro-2*H*-pyran-4-yl)piperidin-4-amine dihydrochloride salt (370 mg, 1.36 mmol) and DIPEA (579 mg, 4.49 mmol) were then added, and the resulting mixture was stirred at room temperature until the total consumption of the starting materials. EtOAc and 0.1 N HCl were added, and the phases were separated. The organic phase was washed with 10% $NaHCO_3$, water, and brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The so obtained crude product was dissolved in 4 N HCl in dioxane (10 mL) and stirred at room temperature. At the end of the reaction (HPLC control) the solvent was distilled off and the resulting solid was triturated with Et_2O to afford crude **18** (346 mg, 0.77 mmol) which was used as such without further purification. Crude amine **18** was reacted with oxazolone **15i** according to the general procedure. The crude mixture was purified by flash chromatography (from 99:1 to 95:5 $CHCl_3$/MeOH). ^{1}H NMR (δ, DMSO-d_6, 400 MHz): 8.45 (s, 1H), 8.20 (s, 1H), 7.90 (d, 1H), 8.80 (dd, 1H), 7.32 (m, 2H), 7.20 (m, 5H), 4.35 (m, 1H), 4.00 (m, 1H), 3.85 (m, 3H), 3.30 (m, 4H), 2.90−2.60 (m, 5H), 2.48 (s, 3H), 2.48−2.05 (m, 2H), 2.10 (s, 3H), 2.00−1.15 (m, 17H). MS *m*/*z* calcd for $C_{38}H_{50}N_4O_4S$: 658.9. Found 659.3 $[M + 1]^+$. HPLC purity: system A, $t_R = 8.173$ min.

6-Methylbenzo[*b*]thiophene-2-carboxylic Acid [1-((*S*)-1-Benzyl-4-{4-[methyl(tetrahydropyran-4-yl)amino]piperidin-1-yl}-butylcarbamoyl)cyclopentyl]amide Trifluoroacetate Salt (26). Crude **18** (226 mg, 0.506 mmol) was dissolved in dry THF (10 mL) under nitrogen atmosphere, and $LiAlH_4$ (115 mg, 3.03 mmol) was added. The resulting mixture was refluxed overnight. It was then cooled in an ice bath and solid ice followed by careful addition of 1 N NaOH. The resulting system was stirred for an additional 1 h. The white precipitate formed was removed by filtration through a Celite pad, washing with Et_2O, DCM, and water. NaOH pellets were added to the biphasic filtrate. The phases were separated, and the organic one was washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure to obtain the intermediate amine as a solid, which was reacted as such with oxazolone **15i** according to the general procedure. Crude **24** was purified by preparative HPLC. ^{1}H NMR (δ, DMSO-d_6, 400 MHz): 9.70 (brs, 1H), 9.30 (brs, 1H), 8.55 (s, 1H), 8.25 (s, 1H), 7.90 (d, 1H), 7.80 (s, 1H), 7.50 (d, 1H), 7.30 (d, 1H), 7.20 (m, 5H), 4.10 (m, 1H), 3.7−3.2 (m, 4H), 3.20−2.90 (m, 4H), 2.73 (m, 5H), 2.50 (s, 3H), 2.25 (m, 3H), 2.00 (s, 3H), 2.00−1.30 (m, 17H). MS *m*/*z* calcd for $C_{38}H_{52}N_4O_3S$: 644.9. Found 645.1 $[M + 1]^+$. HPLC purity: system A, $t_R = 7.357$ min.

6-Methylbenzo[*b*]thiophene-2-carboxylic Acid (1-{(*S*)-1-Benzyl-4-[4-(2-hydroxyethyl)piperidin-1-yl]butylcarbamoyl}cyclopentyl)amide (27). A solution of **4** (500 mg, 1.28 mmol) and 4-ethanolpiperidine (165 mg, 1.28 mmol) in THF (10 mL) was stirred at room temperature. At the end of the reaction the mixture was partitioned between EtOAc and 10% $NaHCO_3$ and the two resulting phases were separated. The organic layer was washed with water and brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give crude Boc-protected **19** as an oil. This oil was dissolved in 4 N HCl in dioxane and stirred for 1 h

at room temperature. The solvent was removed under reduced pressure, and the resulting **19** yellow oil was used in the next reaction without any further purification. Crude **19** was dissolved in dry THF (10 mL) and treated with $LiAlH_4$ (5 mL) at refluxing temperature. After 20 h MeOH (1 mL) and then water (10 mL) were sequentially added. The resulting system was stirred at room temperature for an additional hour. The inorganic salts were filtered off through a pad of Celite, washing with water and Et_2O. The solvents were removed under reduced pressure, and the residue was treated with 4 N HCl. The solvents were eliminated under reduced pressure and the solid obtained was dried to obtain the intermediate amine as the hydrochloride salt (127 mg, 0.35 mmol). This was reacted with oxazolone **15i** (100 mg, 0.35 mmol) according to the general procedure. ^{1}H NMR (δ, DMSO-d_6, 400 MHz): 8.30 (s, 1H), 8.05 (s, 1H), 8.02 (d, 1H), 7.93 (m, 1H), 7.66−7.40 (m, 4H), 7.32−7.11(m, 3H), 4.01−3.97 (m, 1H), 3.65 (s, 1H), 3.47 (m, 2H), 2.81−2.51 (m, 4H), 2.43 (s, 3H), 2.47−2.40 (m, 4H), 2.10−1.81 (m, 4H), 1.60−1.40 (m, 11H), 1.39−1.29 (m, 4H). MS m/z calcd for $C_{34}H_{45}N_3O_3S$: 575.8. Found 576.2 $[M + 1]^+$. HPLC purity: system A, $t_R = 8.342$ min.

6-Methylbenzo[*b*]thiophene-2-carboxylic Acid (1-{(*S*)-1-Benzyl-4-[4-(3-ethoxypropyl)piperazin-1-yl]butylcarbamoyl}cyclopentyl)amide Trifluoroacetate Salt (28). A solution of acid **3** (190 mg, 0.782 mmol), EDCA (165 mg, 0.86 mmol), and HOBt (117 mg, 0.86 mmol) in DMF/THF, 1:1 (6 mL), was stirred at room temperature for 1 h. 1-(2-Ethoxyethyl)piperazine-1,4-diium chloride (190 mg, 0.78 mmol) and DIPEA (252 mg, 1.955 mmol) were added, and stirring was continued for an additional 4 h. The solution was then partitioned between EtOAc and 0.1 N HCl. The two phases were separated, and the organic one was washed with 10% $NaHCO_3$ and brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude residue was dissolved in 4 N HCl in dioxane and stirred at room temperature for 2 h. The solvent was distilled off under reduced pressure and the residue was triturated with Et_2O, collected by filtration, and dried to obtain crude **20** (210 mg, 0.56 mmol) that was used as such without further purification. The amide **20** was treated with a 1 M $LiAlH_4$ solution in THF (10 mL) at refluxing temperature for 3 h. The LC−MS control showed the total consumption of the starting material and the formation of the product. The reaction mixture was cooled to 3−4 °C. Then the excess of $LiAlH_4$ was quenched by slow and cautious addition of ice. When the hydride was completely quenched (no more H_2 evolution was observed), NaOH pellets was added and the resulting suspension was stirred at room temperature for 1 h. The inorganic salts were filtered off throughout Celite, washing with DCM. The biphasic system obtained was saturated with NaOH. Then it was transferred into a separatory funnel and the phases were separated. The organic phase was dried over Na_2SO_4 and filtered, and the solvent was removed under reduced pressure. The desired product was obtained as a pale -yellow oil (115 mg, 64%) and used in the next reaction without any further purification. A solution of oxazolone (81 mg, 0.28 mmol) and the so obtained amine (100 mg, 0.31 mmol) in DMF (5 mL) was stirred at room temperature for 3 h. The HPLC showed the total consumption of the oxazolone and the formation of the product. NaOH, 2 N (7 mL), and EtOAc (10 mL) were then added and the phases separated. The organic phase was washed with water, brine, dried over Na_2SO_4, and filtered, and the solvent was removed under reduced pressure. The crude reaction was purified by preparative HPLC to give a white solid (62 mg, 36%). ^{1}H NMR (δ, DMSO-d_6, 400 MHz): 8.50 (s, 1H), 8.20 (s, 1H), 7.88 (d, 1H), 7.80 (s, 1H), 7.40 (brs, 1H), 7.30 (d, 1H), 7.20 (m, 5H), 4.00 (m, 1H), 3.40 (m, 6H), 2.80 (m, 8H), 2.75 (m, 3H), 2.45 (s, 3H), 2.20 (m, 1H), 2.00−1.35 (m, 14H), 1.15 (t, 3H). MS m/z calcd for $C_{36}H_{50}N_4O_3S$: 618.9. Found 619.3 $[M + 1]^+$. HPLC purity: system A, $t_R = 7.844$ min.

6-Methylbenzo[*b*]thiophene-2-carboxylic Acid (1-{(*S*)-1-Benzyl-4-oxo-4-[4-(tetrahydropyran-4-yl)piperazin-1-yl]butylcarbamoyl}cyclopentyl)amide (29). A solution of acid **3** (1.40 g, 4.77 mmol) in THF (25 mL) was cooled in an ice bath. *N*-Hydroxysuccinimide (0.55 g, 4.77 mmol) and DCC (1.08 g, 5.24 mmol) were added in three portions during 10 min. The resulting mixture was stirred at room temperature for 12 h. Then it was filtered and concentrated under reduced pressure to obtain **4** (1.53 g, 88%) as a white solid. A solution of compound **4** (350 mg, 0.90 mmol), 4-(tetrahydro-2*H*-pyran-4-yl)piperazin-1-ium chloride (218 mg, 0.90 mmol), and DIPEA (255 mg, 1.98 mmol) in THF (10 mL) was stirred at room temperature. At the end of the reaction (HPLC control) the mixture was partitioned between EtOAc and 10% $NaHCO_3$. The two phases were separated, and the organic one was further washed with water, dried over Na_2SO_4, and concentrated under reduced pressure to give crude *N*-Boc derivative of **21** (401 mg, 75%) which was used as such without further purification. This crude was dissolved in 4 N HCl in dioxane and stirred at room temperature. At the end of the reaction the solvent was distilled off under reduced pressure and the resulting solid was collected and triturated with Et_2O to afford crude **21** (258 mg, 94%). This product was reacted with oxazolone **15i** according to the general procedure to obtain **29** as a white solid after flash chromatographic purification (silica, MeOH in $CHCl_3$ from 1% to 5%, 90 mg, 35% yield). ^{1}H NMR (δ, DMSO-d_6, 400 MHz): 8.45 (s, 1H), 8.13 (s, 1H), 7.82 (dd, 2H), 7.40 (d, 1H), 7.32 (d, 1H), 7.18 (m, 5H), 3.95 (m, 1H), 3.82 (m, 2H), 3.43−3.14 (m, 6H), 2.73 (m, 2H), 2.43 (s, 3H), 2.39−2.09 (m, 7H), 2.04 (m, 1H), 1.93 (m, 1H), 1.88−1.41 (m, 10H), 1.27 (m, 2H). MS m/z calcd for $C_{36}H_{46}N_4O_4S$: 630.8. Found 631.2 $[M + 1]^+$. HPLC purity: system A, $t_R = 8.141$ min.

6-Methylbenzo[*b*]thiophene-2-carboxylic Acid (1-{(*R*)-2-Phenyl-1-[4-(tetrahydropyran-4-ylmethyl)piperazin-1-ylmethyl]-ethylcarbamoyl}cyclopentyl)amide Trifluoroacetate Salt (32). A mixture of **30** (72 mg, 0.28 mmol), 1-[(tetrahydro-2*H*-pyran-4-yl)methyl]piperazine dihydrochloride salt (75 mg, 0.29 mmol) and $Na(AcO)_3BH$ (42 mg, 0.28 mmol) in DCM (5 mL) was stirred at room temperature. At the end of the reaction 10% $NaHCO_3$ aqueous solution was added and the resulting phases were separated. The aqueous one was back-extracted with DCM (×2). The combined organic phase was washed with water, brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure to afford crude **31**. This crude was dissolved in 4 N HCl in dioxane (4 mL) and stirred at room temperature for 1 h. At the end of the reaction the solvent was distilled off under reduced pressure. The resulting solid was triturated with Et_2O and then collected by filtration to give the intermediate amine (75 mg, 75% yield of two steps), which was reacted without further purification with oxazolone **15i** (70 mg, 0.24 mmol) according to the general procedure. The crude product was purified by preparative HPLC to obtain **32** as the trifluoroacetate salt (95 mg, 63%). ^{1}H NMR (δ, DMSO-d_6, 400 MHz): 8.90 (brs, 1H), 8.30 (brs, 1H), 7.85 (m, 2H), 7.70 (brs, 1H), 7.25 (m, 6H), 4.10 (m, 1H), 3.85 (m, 4H), 3.50−2.60 (m, 13H), 2.45 (s, 3H), 2.30 (m, 1H), 2.00−1.45 (m, 11H), 1.20 (m, 2H). MS m/z calcd for $C_{35}H_{46}N_4O_3S$: 602.8. Found 603.3 $[M + 1]^+$. HPLC purity: system A, $t_R = 13.63$ min.

(*R*,*E*)-*tert*-Butyl 5-Oxo-1-phenylpent-3-en-2-ylcarbamate (33). A mixture of **30** (600 mg, 2.41 mmol) and (formylmethylene)-triphenylphosphorane (805 mg, 2.64 mmol) in DCM (40 mL) was stirred at room temperature overnight. At the end of the reaction (TLC control, silica, $CHCl_3$/MeOH, 9:1) the solvent was distilled off in vacuo at low temperature and the residue, because of stability concerns, was used as such, calculating the title aldehyde **33** from the ^{1}H NMR spectrum. ^{1}H NMR (δ, DMSO-d_6, 400 MHz): 9.51 (d, 1H, $J = 7.8$ Hz), 7.32−7.20 (m, 5H), 7.00 (dd,, 1H, $J = 5.4$ and 15.6 Hz), 7.00 (dd, 1H, $J = 5.4$ and 13.5 Hz), 4.45 (m, 1H), 2.92 (dd, 1H, $J = 5.3$, 13.6 Hz), 2.77 (dd, 1H, $J = 9.3$, 13.6 Hz), 1.31 (s, 9H). HPLC purity: system A, $t_R = 10.26$ min.

6-Methylbenzo[*b*]thiophene-2-carboxylic Acid (1-{(*S*)-1-Benzyl-4-[4-(morpholine-4-carbonyl)piperidin-1-yl]butylcarbamoyl}-cyclopentyl)amide Trifluoroacetate Salt (35). A mixture of **33** (379 mg, 1.36 mmol), morpholino(piperidin-4-yl)methanone (213 mg, 0.906 mmol), and $Na(AcO)_3BH$ (576 mg, 2.72 mmol) in DCM (5 mL) was stirred at room temperature. At the end of

the reaction 2 M NaOH was added, the resulting phases were separated, and the aqueous one was back-extracted with DCM (×3). The combined organic phase was washed with water, brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. This crude mixture was purified by flash chromatography (silica, $CHCl_3$/MeOH, 99:1 and then 95:5) to obtain the allylic amine in 40% yield. A portion of this amine was submitted to catalytic hydrogenation (EtOH, 5% Pd/C). The catalyst was filtered off over a pad of Celite, and the solvent was removed under reduced pressure to give crude **34**. This was treated with 4 N HCl in dioxane (5 mL) and EtOAc (5 mL) for 1 h. The solvents were distilled off under reduced pressure, and the so obtained amine hydrochloride (120 mg, 0.277 mmol) was reacted with oxazolone **15i** according to standard conditions. The crude product was purified by preparative HPLC to obtain **35** as the trifluoroacetate salt. ^{1}H NMR (δ, DMSO-d_6, 400 MHz): 8.95 (brs, 1H), 8.50 (d, 1H), 8.20 (d, 1H), 7.80 (m, 2H), 7.45 (m, 1H), 7.25 (m, 6H), 4.10 (m, 1H), 3.50 (m, 10H), 3.10 (m, 1H), 2.90 (m, 4H), 2.75 (m, 2H), 2.45 (s, 3H), 2.15 (m, 1H), 2.00−1.30 (m, 15H). MS m/z calcd for $C_{37}H_{48}N_4O_4S$: 644.8. Found 645.3 $[M + 1]^+$. HPLC purity: system A, $t_R = 8.337$ min.

6-Methylbenzo[*b*]thiophene-2-carboxylic Acid (1-{(*E*)-(*R*)-1-Benzyl-4-[4-(tetrahydropyran-4-ylmethyl)piperazin-1-yl]but-2-enylcarbamoyl}cyclopentyl)amide Trifluoroacetate Salt (37). A solution of **33** (100 mg, 0.36 mmol), amine **5** (66 mg, 0.36 mmol), and $Na(OAc)_3BH$ (228 mg, 1.07 mmol) in THF (2 mL) was stirred at 0 °C for 1 h. At the end of the reaction (HPLC control) the mixture was partitioned between EtOAc and 5% $NaHCO_3$ aqueous solution and the phases were separated. The organic one was washed with water, brine, dried over Na_2SO_4, filtered, and concentrated to give crude **36** which was used as such without further purification. This crude was treated with 4 N HCl in dioxane for 1 h. Then the solvent was distilled off in vacuo and the residue was reacted as such with oxazolone **15i** according to the general procedure. Crude **37** was purified by preparative HPLC. ^{1}H NMR (δ, DMSO-d_6, 400 MHz): 9.40 (brs, 1H), 8.50 (s, 1H), 8.20 (s, 1H), 7.90 (d, 1H), 7.80 (s, 1H), 7.70 (m, 1H), 7.30 (d, 1H), 7.20 (m, 5H), 5.90 (m, 1H), 5.60 (m, 1H), 4.60 (m, 1H), 3.80 (brm, 6H), 3.30 (m, 4H), 3.20 (brm, 6H), 2.80 (m, 2H), 2.45 (s, 3H), 2.20 (m, 1H), 2.00−1.45 (m, 10H), 1.15 (m, 2H). MS m/z calcd for $C_{37}H_{48}N_4O_3S$: 628.9. Found 629.1 $[M + 1]^+$. HPLC purity: system A, $t_R = 7.624$ min.

6-Methylbenzo[*b*]thiophene-2-carboxylic Acid (1-{(*R*)-1-Benzyl-3-[4-(tetrahydropyran-4-ylmethyl)piperazin-1-yl]propylcarbamoyl}cyclopentyl)amide Trifluoroacetate Salt (40). A solution of Boc-β-Homophe-OH (85 mg, 0.3 mmol), EDCA (60 mg, 0.3 mmol), and HOAt (40 mg, 0.3 mmol) in DMF (5 mL) was stirred at room temperature for 1 h. *N*-Methyl-*N*-(tetrahydro-2*H*-pyran-4-yl)piperidin-4-amine dihydrochloride salt (75 mg, 0.3 mmol) and DIPEA (113 mg, 0.90 mmol) were then added, and the resulting mixture was stirred at room temperature. At the end of the reaction, DMF was distilled off under reduced pressure and the oily residue was partitioned between EtOAc and 0.1 N HCl. The phases were separated and the organic one was washed with 10% $NaHCO_3$ aqueous solution, water, and brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The so obtained crude product was dissolved in 4 N HCl in dioxane (4 mL) and stirred at room temperature. At the end of the reaction (HPLC control) the solvent was distilled off and the resulting solid was triturated with Et_2O and collected by filtration to afford the intermediate amine hydrochloride (91 mg, 80% yield of two steps). The amine was dissolved in dry THF (10 mL) and treated with 1 M $LiAlH_4$ in THF (1 mL) at room temperature. After 1 h MeOH (1 mL) and water (10 mL) were sequentially added and the mixture was left stirring at room temperature for an additional hour. Inorganic salts were removed by filtration through a pad of Celite, washing with water and Et_2O. The solvents were removed under reduced pressure and the residue was treated with 4 N HCl for 1 h. The solvent was removed under reduced pressure to obtain the intermediate amine hydrochloride (80 mg, 87%). This was reacted with oxazolone **15i** (63 mg, 0.22 mmol) according to the general procedure. The crude product was purified by preparative HPLC to obtain **40** as trifluoroacetate salt (110 mg, 71%). ^{1}H NMR (δ, DMSO-d_6, 400 MHz): 8.70 (s, 1H), 8.25 (s, 1H), 7.85 (d, 1H), 7.75 (s, 1H), 7.60 (d, 1H), 7.25 (m, 6H), 4.05 (m, 1H), 3.85 (m, 4H), 3.60−3.00 (m, 10H), 2.80 (m, 2H), 2.70 (m, 2H), 2.45 (s, 3H), 2.20 (m, 1H), 2.00−1.45 (m, 12H), 1.20 (m, 2H). MS m/z calcd for $C_{36}H_{48}N_4O_3S$: 616.8. Found 617.4 $[M + 1]^+$. HPLC purity: system A, $t_R = 13.57$ min.

4-[5-Phenyl-4-(tritylamino)pent-2-enyl]piperidine-1-carboxylic Acid *tert*-Butyl Ester (42). A suspension of **50** (0.614 g, 1.02 mmol) in dry THF (3.0 mL) was stirred under nitrogen at room temperature. A 1 M solution of NaHMDS in THF (1.0 mL, 1.02 mmol) was added dropwise. After about 10 min the salt was completely dissolved and the solution was bright-orange. The mixture was then cooled to −40 °C, and a solution of **41** (0.600 g, 1.53 mmol) in dry THF (3 mL) precooled at −40 °C was added dropwise. The resulting mixture was stirred under nitrogen while allowing it to warm to room temperature. Et_2O (10 mL) and brine (10 mL) were added, and the resulting phases were separated. The organic layer was further washed with brine (10 mL), dried on Na_2SO_4, filtered, and concentrated under reduced pressure. The so obtained crude product was purified by flash chromatography (silica, petroleum ether/EtOAc from 99:1 to 95:5) to obtain pure **42** (0.200 g, 0.34 mmol) as a colorless oil. ^{1}H NMR (δ, DMSO-d_6, 300 MHz): 7.53−7.42 (m, 9H), 7.31−7.22 (m, 9H), 7.22−7.03 (m, 6H), 6.78 (d, 2H, $J = 7.2$ Hz), 5.29 (t, 1H, $J = 10.4$ Hz), 4.97 (m, 1H), 3.72 (m, 2H), 3.30 (m, 1H), 2.41 (m, 2H), 2.18−2.04 (m, 2H), 1.38 (s, 9H), 1.30−0.36 (m, 2H).

Benzo[*b*]thiophene-2-carboxylic Acid (1-{(*S*)-1-Benzyl-4-[1-(tetrahydropyran-4-ylmethyl)piperidin-4-yl]butylcarbamoyl}cyclopentyl) Trifluoroacetate Salt (47). Compound **42** (200 mg, 0.34 mmol) was dissoled in EtOH (10 mL) and hydrogenated in the presence of 5% Pd/C. At the end of the reaction the catalyst was filtered off throughout Celite and the solvents were concentrated under reduced pressure to give crude **43** (0.140 g, 0.23 mmol) that was treated for 15 min with a 1% solution of TFA in DCM (5 mL). Two drops of water were added, and stirring was continued for additional 10 min. Then 2 M NaOH (5 mL) was added, and the resulting phases were separated. The aqueous one was extracted with DCM (2 × 10 mL), and the combined organic phase was dried over Na_2SO_4, filtered, and concentrated under reduced pressure to obtain crude **44** which was used as such in the next reaction. A solution of amine **44** (100 mg, 0.288 mmol) and oxazolone **15i** (82 mg, 0.288 mmol) in DMF (4 mL) was stirred at room temperature overnight. It was then partitioned between EtOAc and 5% aqueous Na_2HCO_3, and the phases were separated. The organic one was washed with water, brine, dried over Na_2SO_4, filtered, and concentrated to afford crude **45**. This crude was treated with 4 N HCl in dioxane for 20 min. The solvent was removed under reduced pressure. Et_2O was added and distilled off several times to obtain **46** as a solid residue (70 mg) which was used as such without further purification. $Na(AcO)_3BH$ (78 mg, 0.37 mmol) was added to a stirred solution of **46** (70 mg, 0.123 mmol) and tetrahydro-2*H*-pyran-4-carbaldehyde (42 mg, 0.37 mmol) in DCM (5 mL), and the resulting mixture was stirred at room temperature overnight. A few drops of 1 N NaOH were added, and the crude mixture was concentrated and finally purified by preparative HPLC. ^{1}H NMR (δ, DMSO-d_6, 400 MHz): 8.65 (brs, 1H), 8.40 (s, 1H), 8.15 (s, 1H), 7.80 (m, 2H), 7.30 (d, 1H), 7.15 (m, 6H), 4.00 (m, 1H), 3.75 (m, 2H), 3.40 (m, 4H), 2.90 (m, 2H), 2.75 (m, 4H), 2.50 (s, 3H), 2.20 (m, 1H), 2.10−1.00 (m, 23H). MS m/z calcd for $C_{38}H_{51}N_3O_3S$: 629.9. Found 630.3 $[M + 1]^+$. HPLC purity: system A, $t_R = 9.069$ min.

4-(2-Iodoethyl)piperidine-1-carboxylic Acid *tert*-Butyl Ester (49). A solution of PPh_3 (1.20 g, 4.60 mmol) and imidazole (0.32 g, 4.60 mmol) in DCM (30 mL) was treated with I_2 (1.17 g, 4.60 mmol) for 30 min at room temperature. A solution of **48** (0.81 g, 3.54 mmol) in DCM (5 mL) was added, and the resulting mixture was stirred at room temperature overnight. Et_2O (100 mL) and water (50 mL)

were added. The biphasic mixture was transferred into a separatory funnel, and the phases were separated. The organic phase was washed with a saturated solution of $NaHCO_3$ (100 mL), a 5% aqueous solution of Na_2SO_3 (100 mL), and brine (100 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude product was purified by flash chromatography (silica, petroleum ether/EtOAc, 9:1) to obtain **49** (1.08 g, 90%) as a colorless oil. ^{1}H NMR (δ, DMSO-d_6, 300 MHz): 3.91 (m, 2H), 3.30 (m, 2H), 2.68 (m, 2H), 1.72 (q, 2H, J = 6.9 Hz), 1.62 (d, 2H, J = 14.1 Hz), 1.46−1.56 (m, 1H), 1.39 (s, 9H), 1.04 (dq, 2H, J = 4.3, 11.5 Hz).

[2-(1-*tert*-Butoxycarbonylpiperdin-4-yl)ethyl]triphenylphosphonium Iodide (50). A solution of iodide **49** (0.696 g, 2.05 mmol) and PPh_3 (0.592 g, 2.25 mmol) in acetonitrile (5 mL) was refluxed under stirring for 2 days. The solvent was then distilled off under reduced pressure to obtain a white solid mixture of **50** and excess PPh_3. Et_2O (10 mL) was added and the resulting suspension was stirred for 10 min in order to dissolve the PPh_3. Crude **50** was then collected by filtration as a white solid (0.94 g, 74%). ^{1}H NMR (δ, DMSO-d_6, 300 MHz): 7.91−7.84 (m, 3H), 7.83−7.78 (m, 12H), 3.94 (m, 2H), 3.50−3.65 (m, 2H), 2.66 (m, 2H), 1.73 (m, 3H), 1.60−1.45 (m, 2H), 1.38 (s, 9H), 1.10−0.96 (m, 2H).

6-Methylbenzo[*b*]thiophene-2-carboxylic Acid (1-{(*R*)-2-Phenyl-1-[1-(tetrahydropyran-4-ylmethyl)piperidin-4-ylmethoxymethyl]-ethylcarbamoyl}cyclopentyl)amide (55a). KH (102 mg, 2.56 mmol) was added to a solution of D-phenylalaninol (386 mg, 2.56 mmol) in dry THF (24 mL). The resulting suspension was stirred under nitrogen for 2 h at room temperature. Then a solution of *tert*-butyl 4-((methylsulfonyloxy)methyl)piperidine-1-carboxylate (750 mg, 2.56 mmol) in dry THF was added dropwise and the resulting mixture was stirred for further 12 h. The solvent was partially distilled off in vacuo. The residual solution was diluted with EtOAc, then washed with 10% $NaHCO_3$ and brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude product was purified by flash chromatography (silica, $CHCl_3$/MeOH, 95:5) to obtain **52a** (125 mg). Amine **52a** (125 mg, 0.36 mmol) was reacted with oxazolone **15i** (102 mg, 0.36 mmol) according to the standard procedure, and the crude product was treated with 4 N HCl in dioxane to produce crude **54a**. Crude **54a** (89 mg, 0.16 mmol) and 4-tetrahydropyranylaldehyde (54 mg, 0.47 mmol) were dissolved in DCM (3 mL) and treated with $Na(AcO)_3BH$ (100 mg, 0.47 mmol) at room temperature. At the end of the reaction 2 N NaOH was added and the resulting phases were separated. The aqueous one was back-extracted with DCM (×2). The combined organic phase was washed with water, brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. This crude mixture was purified by flash chromatography (DCM/MeOH, 95:5) to afford **55a** (50.4 mg, 51% yield). ^{1}H NMR (δ, DMSO-d_6, 400 MHz): 8.50 (s, 1H), 8.20 (s, 1H), 7.85 (m, 2H), 7.25 (m, 1H), 7.20 (m, 6H), 4.05 (m, 3H), 3.80 (m, 2H), 3.20 (m, 10H), 2.75 (m, 2H), 2.45 (s, 3H), 2.20−1.00 (m, 18H). MS m/z calcd for $C_{37}H_{49}N_3O_4S$: 631.9. Found 632.4 $[M + 1]^+$. HPLC purity: system A, t_R = 8.710 min.

6-Methylbenzo[*b*]thiophene-2-carboxylic Acid [1-((*R*)-2-Phenyl-1-{2-[1-(tetrahydropyran-4-ylmethyl)piperidin-4-yl]ethoxymethyl}ethylcarbamoyl)cyclopentyl]amide (55b). KH (530 mg, 13.2 mmol) was added to a solution of D-phenylalaninol (2.00 g, 13.24 mmol) in dry THF (100 mL). The resulting suspension was stirred under nitrogen for 2 h at room temperature. Then a solution of 4-(2-iodoethyl)piperidine-1-carbamic acid *tert*-butyl ester (4.50 g, 13.24 mmol) in dry THF (50 mL) was added dropwise and the resulting mixture was stirred for a further 12 h. The solvent was partially distilled off in vacuo. The residual solution was diluted with EtOAc, then washed with 2 N NaOH, brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude product was purified by flash chromatography (silica, $CHCl_3$/MeOH, 95:5) to obtain **52b** (420 mg). A solution of amine **52b** (0.25 g, 0.69 mmol) in DMF (5 mL) was added to a stirred solution of oxazolone **15i** (0.197 g, 0.69 mmol) in DMF (10 mL). The resulting solution was stirred at room temperature for an additional 12 h. It was then diluted with EtOAc, washed with 10% $NaHCO_3$ and brine, dried over Na_2SO_4, filtered, and the solvent was removed under reduced pressure. The crude product was purified by flash chromatography (silica, $CHCl_2$/MeOH, 98:2) to obtain **53b** (0.40 g, 0.62 mmol). ^{1}H NMR (δ, DMSO-d_6, 400 MHz): 8.45 (s, 1H), 8.20 (s, 1H), 7.85 (d, 1H), 7.80 (s, 1H), 7.30 (d, 1H), 7.25 (dd, 1H), 7.20−7.10 (d, 1H), 4.05 (m, 1H), 3.85 (m, 2H), 3.40−3.20 (m, 4H), 2.80−2.60 (m, 4H), 2.45 (s, 3H), 2.15 (m, 1H), 2.00−1.20 (m, 21H), 0.90 (m, 2H). A solution of **53b** (0.40 g, 0.62 mmol) in 4 N HCl in dioxane (5 mL) was stirred at room temperature for 30 min. The solvent was distilled off in vacuo, and to the gummy residue Et_2O was added and removed until the formation of a solid occurred. The solid was triturated with Et_2O and collected by filtration to obtain **54b** (0.33 g, 91%) as a hydrochloride salt. A solution of **54b** (100 mg, 0.17 mmol) and 4-tetrahydropyranylaldehyde (58 mg, 0.51 mmol) in dichloromethane (20 mL) was stirred at room temperature under nitrogen. Sodium triacetoxyborohydride (110 mg, 0.51 mmol) was added once, and stirring was continued for 2 h. The solvent was distilled off under reduced pressure, and the residue was dissolved in EtOAc. The resulting solution was washed with 10% $NaHCO_3$, brine, dried over Na_2SO_4, filtered, and the solvent was removed under reduced pressure. The resulting crude product was purified by flash chromatography (silica, $CHCl_3$/MeOH, 95:5) to obtain **55b** (70 mg, 64%). ^{1}H NMR (δ, DMSO-d_6, 400 MHz): 8.45 (s, 1H), 8.20 (s, 1H), 7.85 (d, 1H), 7.80 (s, 1H), 7.30 (d, 1H), 7.25 (dd, 1H), 7.20−7.10 (m, 5H), 4.05 (m, 1H), 3.85 (m, 2H), 3.40−3.20 (m, 6H), 2.80−2.65 (m, 4H), 2.45 (s, 3H), 2.15 (m, 1H), 2.00−1.85 (m, 5H), 1.70−1.00 (m, 18H). MS m/z calcd for $C_{38}H_{51}N_3O_4S$: 645.9. Found 646.3 $[M + 1]^+$. HPLC purity: system A, t_R = 8.947 min.

(*S*)-5-Ethoxy-5-oxo-1-phenylpentan-2-aminium Chloride (58). Compound **2** (4.50 g, 14.0 mmol) was dissolved in dry dioxane (17 mL) and cooled to 0 °C. A 4 M solution of HCl in dioxane (70 mL) was added, and stirring was continued for 20 min, letting the mixture reach room temperature. At the end of the reaction (HPLC control) the solvent was distilled off in vacuo to obtain **58** as a white solid (3.50 g, 97%). ^{1}H NMR (δ, DMSO-d_6, 300 MHz): 8.15 (s, 3H), 7.40−7.20 (m, 5H), 4.00 (q, 2H), 3.40 (m, 1H), 3.00 (dd, 1H), 2.80 (dd, 1H), 2.60−2.30 (m, 2H), 1.75 (m, 2H), 1.15 (t, 3H). MS m/z calcd for $C_{13}H_{19}NO_2$: 221.3. Found 222.1 $[M + 1]^+$. HPLC purity: system B, t_R = 5.29 min.

(*S*)-4-(1-(6-Methylbenzo[*b*]thiophene-2-carboxamido)cyclopentanecarboxamido)-5-phenylpentanoic Acid (60). Amine **58** (1.99 g, 7.74 mmol) was dissolved in dry DMF (32 mL). Oxazolone **15i** (2.00 g, 7.74 mmol) and DIPEA (501 mg, 7.74 mmol) were sequentially added, and the mixture was stirred at room temperature. At the end of the reaction the solution was diluted with EtOAc and washed with 2% $NaHSO_4$ (×2) and brine, dried over Na_2SO_4, filtered, and concentrated. The residue was triturated with Et_2O to obtain **59** as a white solid (2.50 g, 4.90 mmol, 64%) used as such without further purification.

This crude was dissolved in MeOH (20 mL) and THF (15 mL). Then 4 N NaOH (20 mL) was added under stirring. At the end of the reaction (HPLC control) 6 N HCl was added dropwise until complete precipitation of the product. The precipitate was collected by filtration and dried to afford crude **60** (2.34 g, quantitative yield) which was used as such without further purification. ^{1}H NMR (δ, DMSO-d_6, 400 MHz): 8.40 (s, 1H), 8.20 (s, 1H), 7.83 (m, 2H), 7.30 (m, 2H), 7.15 (m, 5H), 4.00 (m, 1H), 2.70 (m, 2H), 2.45 (s, 3H), 2.38−1.40 (m, 12H). MS m/z calcd for $C_{27}H_{30}N_2O_4S$: 478.2. Found 479.3 $[M + 1]^+$.

(*S*)-*N*-(1-(5-(Methoxy(methyl)amino)-5-oxo-1-phenylpentan-2-ylcarbamoyl)cyclopentyl)-6-methylbenzo[*b*]thiophene-2-carboxamide (61). Acid **60** (2.24 g, 4.68 mmol) was dissolved in dry DMF (22 mL) and cooled down to 0 °C. EDCA (1.07 g, 5.60 mmol), HOBt (756 mg, 5.60 mmol), *N*,*O*-dimethylhydroxylamine (546.3 mg, 5.60 mmol), and DIPEA (2.40 mL, 14.0 mmol) were

sequentially added, and stirring was continued at room temperature. At the end of the reaction (HPLC control) the mixture was diluted with EtOAc and saturated aqueous solution of $NaHCO_3$ while stirring. The two phases were separated, and the organic one was washed with saturated aqueous solution of $NaHCO_3$ (×3), 5% $NaHSO_4$ (×2), and brine, dried over Na_2SO_4, filtered, and concentrated to obtain crude **61** (2.39 g, 98%). ^{1}H NMR (δ, DMSO-d_6, 400 MHz): 8.40 (s, 1H), 8.20 (s, 1H), 7.90−7.80 (m, 2H), 7.40−7.25 (m, 2H), 7.25−7.10 (m, 5H), 4.10−3.90 (m, 1H), 3.60 (s, 3H), 3.05 (s, 3H), 2.80−2.65 (m, 2H), 2.45 (s, 3H), 2.40−1.40 (m, 12H). MS m/z calcd for $C_{29}H_{35}N_3O_4S$: 521.7. Found 522.3 $[M + 1]^+$. HPLC purity: system B, $t_R = 12.98$ min.

(*S*)-6-Methyl-*N*-(1-(5-oxo-1-phenylpentan-2-ylcarbamoyl)cyclopentyl)benzo[*b*]thiophene-2-carboxamide (62). Compound **61** (1.19 g, 2.29 mmol) was dissolved in dry THF (20 mL) and cooled to −30 °C under nitrogen atmosphere. A 1 M solution of $LiAlH_4$ in THF (2.98 mL, 2.98 mmol) was added dropwise while stirring. At the end of the reaction (TLC control, silica, petroleum ether/EtOAc, 1:1) 0.1 N HCl was added while stirring at −30 °C until complete elimination of residual $LiAlH_4$. The mixture was partitioned between water and EtOAc. The organic phase was then separated, washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure to obtain crude **62** (1.08 g, quantitative yield). ^{1}H NMR (δ, DMSO-d_6, 400 MHz): 9.60 (s, 1H), 8.40 (s, 1H), 8.20 (s, 1H), 7.85−7.75 (m, 2H), 7.35−7.20 (m, 2H), 7.20−7.05 (m, 5H), 3.95 (m, 1H), 2.75−2.60 (m, 2H), 2.45 (s, 3H), 2.25−1.40 (m, 12H). MS m/z calcd for $C_{27}H_{30}N_2O_3S$: 462.6. Found 463.2 $[M + 1]^+$. HPLC purity: system B, $t_R = 12.99$ min.

Route A. General Procedure To Obtain Compounds 35 through Reductive Amination on Aldehyde 62. Aldehyde **62** (90 mg, 0.19 mmol) was dissolved in dry THF (5 mL). Compound **63** (0.21 mmol) was added, followed by $NaB(OAc)_3H$ (62 mg, 0.29 mmol) and some drops of acetic acid to reach a pH of around 5. Stirring was continued at room temperature until the end of the reaction. The mixture was then diluted with a saturated aqueous solution of $NaHCO_3$ and extracted with EtOAc. The organic phase was washed with a saturated aqueous solution of $NaHCO_3$ and brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure to obtain crude **35** that was purified by preparative HPLC.

(*R*,*E*)-Benzyl 4-(4-(*tert*-butoxycarbonylamino)-5-phenylpent-2-enyl)piperazine 1-Carboxylate (63). To a solution of aldehyde **33** (4.534 g, 16.48 mmol) in THF (160 mL), Cbz-piperazine (3.63 g, 16.5 mmol), $NaB(OAc)_3H$ (4.887 g, 23.06 mmol), and AcOH (1.046 mL, 18.29 mmol) were added. At the end of the reaction, the mixture was concentrated, diluted with EtOAc, and washed with 5% $NaHCO_3$ aqueous solution and H_2O. The organic phase was dried over Na_2SO_4, filtered, and concentrated to obtain a crude containing product **63**, some byproduct, and triphenylphosphine oxide (which contaminated the starting material **33**). This crude material was treated with hexane, affording a supernatant solution and an oily residue enriched with triphenylphosphine oxide. The solution was separated, and the oily residue was further treated with hexane. The combined supernatant solution was dried over $MgSO_4$, filtered, and evaporated under vacuum to give **63** (5.60 g). Purification of this crude by flash chromatography (silica, hexane/EtOAc/Et_3N from 85:13:2 to 65:33:2) afforded compound **63** of sufficient purity to be used as such in the subsequent reactions. MS m/z calcd for $C_{28}H_{37}N_3O_3$: 463.2. Found 464.2 $[M + 1]^+$.

(*S*)-6-Methyl-*N*-(1-(1-phenyl-5-(piperazin-1-yl)pentan-2-ylcarbamoyl)cyclopentyl)benzo[*b*]thiophene-2-carboxamide (66). Boc-protected amine **63** (350 mg, 0.73 mmol) was dissolved in 4 N HCl in dioxane (8 mL) while cooling at 0 °C. At the end of the reaction (HPLC control) the solvent was distilled off under reduced pressure. The residue was triturated with Et_2O, collected by filtration, and dried to obtain crude **64** (200 mg, 0.42 mmol) which was directly used in the reaction with oxazolone for the preparation of **65** following the general procedure. Crude compound **65** (1.23 g, 1.86 mmol) was dissolved in absolute EtOH (100 mL) and hydrogenated in the presence of 10% Pd/C (1.20 g) and ammonium formate (938 mg, 14.9 mmol). At the end of the reaction (HPLC control) the catalyst was filtered off on a Celite pad and partitioned between DCM and 0.1 N NaOH. The phases were separated, and then the organic one was washed with brine, dried over Na_2SO_4, filtered, and concentrated to obtain crude **66** (780 mg, 79%), which was used as such without further purification. It contained around 9% of the *N*-formyl derivative **67p**. MS m/z calcd for $C_{31}H_{40}N_4O_2S$: 532.7. Found 533.2 $[M + 1]^+$.

6-Methylbenzo[*b*]thiophene-2-carboxylic Acid (1-{(*S*)-1-Benzyl-4-[4-(tetrahydropyran-4-yl)piperazin-1-yl]butylcarbamoyl}cyclopentyl)amide Trifluoroacetate Salt (67a). ^{1}H NMR (δ, DMSO-d_6, 400 MHz): 9.10 (brs, 1H), 8.55 (brs, 1H), 8.22 (s, 1H), 7.82 (m, 2H), 7.41 (m, 1H), 7.30 (m, 1H), 7.22 (m, 5H), 4.00 (m, 1H), 3.90 (m, 2H), 3.50−2.65 (m, 13H), 2.70 (m, 4H), 2.20 (m, 1H), 2.00−1.20 (m, 16H). MS m/z calcd for $C_{36}H_{48}N_4O_3S$: 616.9. Found 617.3 $[M + 1]^+$. HPLC purity: system A, $t_R = 7.422$ min.

6-Methylbenzo[*b*]thiophene-2-carboxylic Acid {1-[(*S*)-1-Benzyl-4-(4-pyridin-2-ylpiperazin-1-yl)butylcarbamoyl]cyclopentyl}-amide Trifluoroacetate Salt (67b). ^{1}H NMR (δ, DMSO-d_6, 400 MHz): 9.75 (brs, 1H), 8,60 (s, 1H), 8.30 (s, 1H), 8.20 (d, 1H), 7.90 (d, 1H), 7.80 (s, 1H), 7.65 (m, 1H), 7.50 (d, 1H), 7.35 (d, 1H), 7.20 (m, 5H), 6.90 (d, 1H), 6.80 (d, 1H), 4.30 (m, 2H), 4.10 (m, 1H), 3.50 (m, 2H), 3.20 (m, 3H), 3.15 (m, 3H), 2.75 (m, 2H), 2.45 (s, 3H), 2.20 (m, 1H), 1.95 (m, 1H), 1.90−1.20 (m, 10H). MS m/z calcd for $C_{36}H_{43}N_5O_2S$: 609.8. Found 610.3 $[M + 1]^+$. HPLC purity: system A, $t_R = 7.605$ min.

6-Methylbenzo[*b*]thiophene-2-carboxylic Acid {1-[(*S*)-1-Benzyl-4-(4-pyrimidin-2-ylpiperazin-1-yl)butylcarbamoyl]cyclopentyl}-amide Trifluoroacetate Salt (67c). ^{1}H NMR (δ, DMSO-d_6, 400 MHz): 9.55 (brs, 1H), 8.55 (s, 1H), 8.45 (m, 2H), 8.22 (s, 1H), 7.85 (d, 1H), 7.75 (s, 1H), 7.45 (d, 1H), 7.30 (d, 1H), 7.20 (m, 5H), 6.80 (m, 1H), 4.70 (m, 1H), 4.15 (m, 1H), 3.60−2.90 (m, 8H), 2.80 (2H, m), 2.45 (s, 3H), 2.20 (s, 1H), 1.95 (s, 1H), 1.90−1.30 (m, 10H). MS m/z calcd for $C_{35}H_{42}N_6O_2S$: 610.8. Found 611.3 $[M + 1]^+$. HPLC purity: system A, $t_R = 8.997$ min.

6-Methylbenzo[*b*]thiophene-2-carboxylic Acid (1-{(*S*)-1-Benzyl-4-[4-(4-fluorobenzyl)piperazin-1-yl]butylcarbamoyl}cyclopentyl)amide (67d). ^{1}H NMR (δ, DMSO-d_6, 400 MHz): 9.10 (brs, 1H), 8.50 (s, 1H), 8.20 (s, 1H), 7.85 (d, 1H), 7.70 (s, 1H), 7.40 (m, 4H), 7.30 (d, 1H), 7.30 (m, 5H), 4.05 (m, 1H), 3.65−2.30 (m, 12H), 2.45 (s, 3H), 2.20 (m, 1H), 1.95 (m, 1H), 1.85−1.30 (m, 12H). MS m/z calcd for $C_{38}H_{45}FN_4O_2S$: 640.8. Found 641.4 $[M + 1]^+$. HPLC purity: system A, $t_R = 8.406$ min.

6-Methylbenzo[*b*]thiophene-2-carboxylic Acid {1-[(*S*)-1-Benzyl-4-(4-pyridin-2-ylmethylpiperazin-1-yl)butylcarbamoyl]cyclopentyl}amide Trifluoroacetate Salt (67e). ^{1}H NMR (δ, DMSO-d_6, 400 MHz): 9.21 (brs, 1H), 8.50 (d, 1H), 8.27 (s, 1H), 8.07 (s, 1H), 8.03 (d, 1H), 7.85 (s, 1H), 7.71−7.37 (m, 4H), 7.33−7.06 (m, 6H), 4.02−3.96 (m, 1H), 3.94 (s, 2H), 2.80−2.65 (m, 2H), 2.45 (s, 3H), 2.51−2.30 (m, 10H), 2.10−1.84 (m, 4H), 1.56−1.51 (m, 4H), 1.46−1.30 (m, 4H). MS m/z calcd for $C_{37}H_{45}N_5O_2S$: 623.8. Found 624.3 $[M + 1]^+$. HPLC purity: system A, $t_R = 7.606$ min.

6-Methylbenzo[*b*]thiophene-2-carboxylic Acid {1-[(*S*)-1-Benzyl-4-(4-pyridin-3-ylmethylpiperazin-1-yl)butylcarbamoyl]cyclopentyl}amide Trifluoroacetate Salt (67f). ^{1}H NMR (δ, DMSO-d_6, 400 MHz): 9.15 (brs, 1H), 8.65 (m, 2H), 8.52 (s, 1H), 8.20 (s, 1H), 7.95 (m, 1H), 7.85 (d, 1H), 7.77 (s, 1H), 7.58 (m, 1H), 7.40 (m, 1H), 7.22 (m, 6H), 4.05 (m, 1H), 4.70 (m, 2H), 3.42 (m, 2H), 3.15−2.85 (m, 6H), 2.73 (m, 2H), 2.45 (s, 3H), 2.40 (m, 2H), 2.20 (m, 1H), 1.90 (m, 1H), 1.85−1.03 (m, 10H). MS m/z calcd for $C_{37}H_{45}N_5O_2S$: 623.8. Found 624.3 $[M + 1]^+$. HPLC purity: system A, $t_R = 8.406$ min.

6-Methylbenzo[*b*]thiophene-2-carboxylic Acid {1-[(*S*)-1-Benzyl-4-(4-pyridin-4-ylmethylpiperazin-1-yl)butylcarbamoyl]cyclopentyl}amide Trifluoroacetate Salt (67g). ^{1}H NMR (δ, DMSO-d_6, 400 MHz): 9.30 (brs, 1H), 8.75 (m, 2H), 8.54 (s, 1H), 8.20 (s, 1H), 7.83 (d, 1H), 7.70 (m, 3H), 7.42 (d, 1H), 7.20 (m, 6H), 4.05 (m, 1H), 3.80 (m, 2H), 3.45 (m, 2H), 3.20−2.80 (m, 6H), 2.70 (m, 2H), 2.45 (s, 3H), 2.40 (m, 2H), 2.20 (m, 1H),

1.95 (m, 1H), 1.80−1.30 (m, 10H). MS *m*/*z* calcd for $C_{37}H_{45}N_5O_2S$: 623.8. Found 624.3 $[M + 1]^+$. HPLC purity: system A, $t_R = 8.406$ min.

6-Methylbenzo[*b*]thiophene-2-carboxylic Acid {1-[(*S*)-1-Benzyl-4-(4-thiophen-2-ylmethylpiperazin-1-yl)butylcarbamoyl]cyclopentyl}amide Trifluoroacetate Salt (67h). ^{1}H NMR (δ, DMSO-d_6, 400 MHz): 9.20 (brs, 1H), 8.52 (s, 1H), 8.20 (s, 1H), 7.85 (d, 1H), 7.75 (s, 1H), 7.55 (d, 1H), 7.40 (dd, 1H), 7.30 (d, 1H), 7.20 (m, 5H), 7.10 (m, 2H), 4.10 (m, 1H), 3.95 (m, 2H), 3.45 (m, 2H), 3.00 (m, 6H), 2.75 (m, 2H), 2.45 (s, 3H), 2.40 (m, 2H), 2.20 (m, 1H), 1.95 (m, 1H), 1.90−1.30 (m, 10H). MS *m*/*z* calcd for $C_{36}H_{44}N_4O_2S_2$: 628.9. Found 629.3 $[M + 1]^+$. HPLC purity: system A, $t_R = 8.455$ min.

6-Methylbenzo[*b*]thiophene-2-carboxylic Acid {1-[(*S*)-1-Benzyl-4-(4-thiophen-3-ylmethylpiperazin-1-yl)butylcarbamoyl]cyclopentyl}amide Trifluoroacetate Salt (67i). ^{1}H NMR (δ, DMSO-d_6, 400 MHz): 9.45 (brs, 1H), 8.55 (s, 1H), 8.20 (s, 1H), 7.80 (d, 1H), 7.75 (s, 1H), 7.60 (d, 1H), 7.50 (s, 1H), 7.40 (d, 1H), 7.30 (d, 1H), 7.20 (m, 6H), 4.00 (m, 1H), 3.90 (m, 2H), 3.50 (m, 2H), 3.00 (m, 6H), 2.70 (m, 2H), 2.65 (m, 2H), 2.45 (s, 3H), 0.20 (m, 1H), 1.95 (m, 1H), 1.85−1.30 (m, 10H). MS *m*/*z* calcd for $C_{36}H_{44}N_4O_2S_2$: 628.9. Found 629.3 $[M + 1]^+$. HPLC purity: system A, $t_R = 8.119$ min.

6-Methyl-benzo[*b*]thiophene-2-carboxylic Acid {1-[(*S*)-1-Benzyl-4-(4-furan-3-ylmethylpiperazin-1-yl)butylcarbamoyl]cyclopentyl}amide Trifluoroacetate Salt (67j). ^{1}H NMR (δ, DMSO-d_6, 400 MHz): 9.15 (brs, 1H), 8.32 (s, 1H), 8.05 (s, 1H), 8.03 (d, 1H), 7.88 (s, 1H), 7.70−7.40 (m, 3H), 7.30−7.05 (m, 6H), 6.10 (d, 1H), 4.00−3.95 (m, 1H), 3.60 (s, 2H), 2.80−2.65 (m, 2H), 2.43 (s, 3H), 2.50−2.35 (m, 10H), 2.09−1.85 (m, 4H), 1.56−1.51 (m, 4H), 1.46−1.30 (m, 4H). MS *m*/*z* calcd for $C_{36}H_{44}N_4O_3S$: 612.8. Found 613.3 $[M + 1]^+$. HPLC purity: system A, $t_R = 7.922$ min.

6-Methylbenzo[*b*]thiophene-2-carboxylic Acid (1-{(*S*)-1-Benzyl-4-[4-(3*H*-imidazol-4-ylmethyl)piperazin-1-yl]butylcarbamoyl}-cyclopentyl)amide Trifluoroacetate Salt (67k). ^{1}H NMR (δ, DMSO-d_6, 400 MHz): 9.20 (brs, 1H), 9.10 (s, 1H), 8.55 (s, 1H), 8.25 (s, 1H), 7.85 (dd, 1H), 7.75 (s, 1H), 7.60 (s, 1H), 7.45 (m, 1H), 7.30 (m, 1H), 7.20 (m, 5H), 4.05 (m, 1H), 3.70 (brs, 2H), 3.48 (m, 2H), 3.10 (m, 1H), 2.95 (m, 5H), 2.70 (m, 2H), 2.45 (s, 3H), 2.45 (m, 2H), 2.25 (m, 1H), 1.95 (m, 1H), 2.85−1.30 (m, 10H). MS *m*/*z* calcd for $C_{35}H_{44}N_6O_2S$: 612.8. Found 613.3 $[M + 1]^+$. HPLC purity: system A, $t_R = 7.922$ min.

6-Methylbenzo[*b*]thiophene-2-carboxylic Acid (1-{(*S*)-1-Benzyl-4-[4-(2-methyl-2*H*-tetrazol-5-ylmethyl)piperazin-1-yl]butylcarbamoyl}cyclopentyl)amide Trifluoroacetate Salt (67l). ^{1}H NMR (δ, DMSO-d_6, 400 MHz): 9.10 (brs, 1H), 8.55 (s, 1H), 8.20 (s, 1H), 7.85 (m, 2H), 7.45 (d, 1H), 7.30 (d, 1H), 7.20 (m, 5H), 7.35 (s, 3H), 4.00 (m, 1H), 3.85 (s, 2H), 3.70 (m, 2H), 3.45 (m, 2H), 3.00 (m, 6H), 2.70 (m, 2H), 2.45 (s, 3H), 2.20 (m, 1H), 1.95 (m, 1H), 1.85−1.30 (m, 10H). MS *m*/*z* calcd for $C_{34}H_{44}N_8O_2S$: 628.8. Found 629.4 $[M + 1]^+$. HPLC purity: system A, $t_R = 8.329$ min.

6-Methylbenzo[*b*]thiophene-2-carboxylic Acid (1-{(*S*)-1-Benzyl-4-[4-(3-ethoxypropyl)piperazin-1-yl]butylcarbamoyl}cyclopentyl)amide Trifluoroacetate Salt (67m). ^{1}H NMR (δ, DMSO-d_6, 400 MHz): 8.50 (brs, 1H), 8.20 (s, 1H), 7.85 (d, 1H), 7.80 (s, 1H), 7.40 (brs, 1H), 7.30 (d, 1H), 7.20 (M, 5H), 4.05 (m, 1H), 3.40 (m, 6H), 3,00 (m, 10H), 2.75 (m, 2H), 2.50 (s, 3H), 2.20 (m, 1H), 2.00−1.30 (m, 13H), 1.10 (t, 3H). MS *m*/*z* calcd for $C_{36}H_{50}N_4O_3S$: 618.9. Found 619.3 $[M + 1]^+$. HPLC purity: system A, $t_R = 7.844$ min.

6-Methylbenzo[*b*]thiophene-2-carboxylic Acid (1-{(*S*)-1-Benzyl-4-[4-(tetrahydropyran-4-carbonyl)piperazin-1-yl]butylcarbamoyl}cyclopentyl)amide Trifluoroacetate Salt (67n). ^{1}H NMR (δ, DMSO-d_6, 400 MHz): 9.55 (brs, 1H), 8.55 (s, 1H), 8.25 (s, 1H), 7.85 (d, 1H), 7.80 (s, 1H), 7.45 (d, 1H), 7.30 (d, 1H), 7.20 (m, 5H), 4.40−4.00 (m, 3H), 2.85 (m, 2H), 3.45 (m, 5H), 3.00 (m, 6H), 2.75 (m, 2H), 2.45 (s, 3H), 2.15 (m, 1H), 2.00−1.30 (m, 15H). MS *m*/*z* calcd for $C_{37}H_{48}N_4O_4S$: 644.8. Found 645.2 $[M + 1]^+$. HPLC purity: system A, $t_R = 8.302$ min.

6-Methylbenzo[*b*]thiophene-2-carboxylic Acid (1-{(*S*)-1-bBenzyl-4-[4-(1-methyl-1*H*-pyrrole-2-carbonyl)piperazin-1-yl]butylcarbamoyl}cyclopentyl)amide Trifluoroacetate Salt (67o). ^{1}H NMR (δ, DMSO-d_6, 400 MHz): 9.60 (brs, 1H), 8.55 (s, 1H), 8.20 (s, 1H), 8.50 (m, 2H), 8.72 (s, 1H), 7.45 (d, 1H), 7.20 (m, 6H), 7.00 (s, 1H), 6. 45 (s, 1H), 6.10 (s, 1H), 4.40 (m, 1H), 4.00 (m, 1H), 3.70 (s, 3H), 3.10 (m, 6H), 2.75 (m, 2H), 2.45 (s, 3H), 2.15 (m, 1H), 1.95 (m, 1H), 1.90−1.30 (m, 11H). MS *m*/*z* calcd for $C_{37}H_{45}N_5O_3S$: 639.8. Found 640.3 $[M + 1]^+$. HPLC purity: system A, $t_R = 9.056$ min.

6-Methylbenzo[*b*]thiophene-2-carboxylic Acid {1-[(*S*)-1-Benzyl-4-(4-formylpiperazin-1-yl)butylcarbamoyl]cyclopentyl}amide (67p). ^{1}H NMR (δ, DMSO-d_6, 400 MHz): 8.40 (s, 1H), 8.15 (s, 1H), 7.90 (s, 1H), 7.80 (m, 2H), 7.30 (m, 2H), 7.15 (m, 5H), 4.00 (m, 1H), 3.25 (m, 2H), 3.20 (m, 2H), 2.70 (m, 2H), 2.42 (s, 3H), 2.20 (m, 6H), 1.90 (m, 3H), 1.75−1.30 (m, 9H). MS *m*/*z* calcd for $C_{32}H_{40}N_4O_3S$: 560.75. Found 561.5 $[M + 1]^+$. HPLC purity: system B, $t_R = 9.62$ min.

6-Methylbenzo[*b*]thiophene-2-carboxylic Acid (1-{(*S*)-1-Benzyl-4-[4-(morpholine-4-carbonyl)piperazin-1-yl]butylcarbamoyl}-cyclopentyl)amide Trifluoroacetate Salt (67q). ^{1}H NMR (δ, DMSO-d_6, 400 MHz): 9.40 (brs, 1H), 8.58 (brs, 1H), 8.25 (s, 1H), 7.88 (d, 1H), 7.80 (s, 1H), 7.45 (d, 1H), 7.20 (m, 6H), 4.25 (m, 1H), 3.50 (m, 12H), 3.10 (m, 6H), 2.75 (m, 2H), 2.45 (s, 3H), 2.15 (m, 1H), 2.00−1.30 (m, 11H). MS *m*/*z* calcd for $C_{36}H_{47}N_5O_4S$: 645.85. Found 646.3 $[M + 1]^+$. HPLC purity: system A, $t_R = 8.37$ min.

6-Methylbenzo[*b*]thiophene-2-carboxylic Acid (1-{(*S*)-1-Benzyl-4-[4-(morpholine-4-sulfonyl)piperazin-1-yl]butylcarbamoyl}-cyclopentyl)amide Trifluoroacetate Salt (67r). ^{1}H NMR (δ, DMSO-d_6, 400 MHz): 9.50 (brs, 1H), 8.55 (s, 1H), 8.25 (s, 1H), 7.85 (m, 1H), 7.80 (s, 1H), 7.45 (m, 1H), 7.20 (m, 6H), 4.05 (m, 1H), 3.60 (M, 8H), 3.15 (m, 10H), 2.75 (m, 2H), 2.45 (s, 3H), 2.20 (m, 1H), 1.90−1.30 (m, 11H). MS *m*/*z* calcd for $C_{35}H_{47}N_5O_4S_2$: 681.9. Found 682.1 $[M + 1]^+$. HPLC purity: >98%, system B, t_R = 11.14 min.

6-Methylbenzo[*b*]thiophene-2-carboxylic Acid {1-[(*S*)-1-Benzyl-4-(4-dimethylsulfamoylpiperazin-1-yl)butylcarbamoyl]cyclopentyl}amide Trifluoroacetate Salt (35s). ^{1}H NMR (δ, DMSO-d_6, 400 MHz): 9.50 (brs, 1H), 8.55 (s, 1H), 8.25 (s, 1H), 7.90 (m, 1H), 7.80 (s, 1H), 7.45 (m, 1H), 7.25 (m, 6H), 4.05 (m, 1H), 3.60 (m, 4H), 3.15 (m, 6H), 2.80 (s, 6H), 2.75 (m, 2H), 2.45 (s, 3H), 2.20 (m, 1H), 1.95 (m, 1H), 1.90−1.30 (m, 10H). MS *m*/*z* calcd for $C_{33}H_{45}N_5O_4S_2$: 639.9. Found 641.0 $[M + 1]^+$. HPLC purity: system B, $t_R = 9.046$ min.

6-Methylbenzo[*b*]thiophene-2-carboxylic Acid {1-[(*S*)-1-Benzyl-4-(4-methylsulfamoylpiperazin-1-yl)butylcarbamoyl]cyclopentyl}amide Trifluoroacetate Salt (67t). ^{1}H NMR (δ, DMSO-d_6, 400 MHz): 9.40 (brs, 1H), 8.50 (s, 1H), 8.20 (s, 1H), 7.85 (d, 1H), 7.75 (s, 1H), 7.45 (d, 2H), 7.20 (m, 5H), 4.00 (m, 1H), 3.70 (m, 4H), 3.10 (m, 6H), 2.75 (m, 2H), 2.60 (s, 3H), 2.45 (s, 3H), 2.15 (m, 1H), 1.95 (m, 1H), 1.90−1.30 (m, 11H). MS *m*/*z* calcd for $C_{32}H_{43}N_5O_4S_2$: 625.8. Found 626.4 $[M + 1]^+$. HPLC purity: system B, $t_R = 10.63$ min.

6-Methylbenzo[*b*]thiophene-2-carboxylic Acid (1-{(*S*)-1-Benzyl-4-[4-(propane-2-sulfonyl)piperazin-1-yl]butylcarbamoyl}cyclopentyl)amide Trifluoroacetate Salt (67u). A solution of **66** (100 mg, 0.188 mmol) in dry DCM (5 mL) was stirred at room temperature. *i*-$PrSO_2Cl$ (32.1 mg, 0.22 mmol) and DIPEA (33 μL, 0.188 mmol) were added. At the end of the reaction (HPLC control) the mixture was diluted with DMC and washed with 0.1 N NaOH and brine. The resulting organic phase was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude product was purified by preparative HPLC to obtain pure **35u** (22 mg, 18%). ^{1}H NMR (δ, DMSO-d_6, 400 MHz): 9.45 (brs, 1H), 8.55 (brs, 1H), 8.20 (s, 1H), 7.90 (d, 1H), 7.80 (s, 1H), 7.45 (m, 1H), 7.20 (m, 6H), 4.00 (m, 1H), 3.70 (m, 1H), 3.60−3.00 (m, 10H), 2.70 (m, 2H), 2.45 (s, 3H), 2.20 (m, 1H), 2.00−1.30 (m, 11H), 1.20 (d, 6H). MS *m*/*z* calcd for $C_{34}H_{46}N_4O_4S_2$: 638.9. Found 639.4 $[M + 1]^+$. HPLC purity: system B, $t_R = 11.44$ min.

6-Methylbenzo[*b*]thiophene-2-carboxylic Acid {1-[(*S*)-1-Benzyl-4-(4-ethanesulfonylpiperazin-1-yl)butylcarbamoyl]cyclopentyl}amide (67v). A solution of **66** (90 mg, 0.17 mmol) in dry DCM (5 mL) was stirred at room temperature. $EtSO_2Cl$ (24 mg, 0.186 mmol) and DIPEA (until pH 7−8) were added. At the end of the reaction (HPLC control) the solution was partitioned between DMC and 0.1 M NaOH, and the organic phase was separated, washed with brine, and concentrated. The crude product was purified by flash chromatography (silica, $CHCl_3$/MeOH, 99:1 to 97:3) to obtain **35v** (56 mg, 53%). ^{1}H NMR (δ, DMSO-d_6, 400 MHz): 8.40 (s, 1H), 8.15 (s, 1H), 7.85 (m, 2H), 7.25 (m, 2H), 7.15 (m, 5H), 3.95 (m, 1H), 2.95 (m, 6H), 2.70 (m, 2H), 2.45 (s, 3H), 2.25 (m, 2H), 2.20 (m, 4H), 1.90 (m, 3H), 1.75−1.20 (m, 9H), 1.15 (t, 3H). MS *m*/*z* calcd for $C_{33}H_{44}N_4O_4S_2$: 624.8. Found 625.5 $[M + 1]^+$. HPLC purity: system B, t_R = 11.06 min.

6-Methylbenzo[*b*]thiophene-2-carboxylic Acid [1-((*S*)-1-Benzyl-4-{4-[[(*E*)-ethylimino](tetrahydropyran-4-yl)methyl]piperazin-1-yl}butylcarbamoyl)cyclopentyl]amide Trifluoroacetate Salt (67w). ^{1}H NMR (δ, DMSO-d_6, 400 MHz): 8.60 (brs, 1H), 8.30 (s, 1H), 8.15 (s, 1H), 7.85 (d, 1H), 7.80 (s, 1H), 7.20 (m, 7H), 4.05 (m, 1H), 3.09 (m, 2H), 3.80 (m, 4H), 3.60−2.90 (m, 8H), 2.70 (m, 2H), 2.45 (s, 3H), 2.20 (m, 1H), 2.00−1.35 (m, 18H), 1.25 (t, 3H). MS *m*/*z* calcd for $C_{39}H_{53}N_5O_3S$: 671.9. Found 672.3 $[M + 1]^+$. HPLC purity: system A, t_R = 7.687 min.

6-Methylbenzo[*b*]thiophene-2-carboxylic Acid {1-[(*S*)-1-Benzyl-4-(4-{[(*E*)-isopropylimino]morpholin-4-ylmethyl}piperazin-1-yl)butylcarbamoyl]cyclopentyl}amide Trifluoroacetate Salt (67x). ^{1}H NMR (δ, DMSO-d_6, 400 MHz): 10.00 (brs, 1H), 8.60 (s, 1H), 8.20 (s, 1H), 7.95 (d, 1H), 7.85 (d, 1H), 7.75 (s, 1H), 7.45 (m, 1H), 7.30 (d, 1H), 7.20 (m, 5H), 4.05 (m, 1H), 3.90−3.00 (m, 17H), 2.75 (m, 2H), 2.45 (s, 3H), 2.15 (m, 1H), 1.95 (m, 1H), 1.90−1.35 (m, 11H), 1.20 (d, 6H). MS *m*/*z* calcd for $C_{39}H_{54}N_6O_3S$: 686.9. Found 687.4 $[M + 1]^+$. HPLC purity: system A, t_R = 7.97 min.

6-Methylbenzo[*b*]thiophene-2-carboxylic Acid {1-[(*S*)-1-Benzyl-4-(4-{[(*Z*)-methoxyimino]morpholin-4-ylmethyl}piperazin-1-yl)butylcarbamoyl]cyclopentyl}amide Trifluoroacetate Salt (67y). ^{1}H NMR (δ, DMSO-d_6, 400 MHz): 9.80 (brs, 1H), 8.60 (s, 1H), 8.25 (s, 1H), 7.85 (d, 1H), 7.80 (s, 1H), 7.50 (d, 1H), 7.30 (d, 1H), 7.20 (m, 5H), 4.05 (m, 1H), 3.65 (s, 3H), 3.50 (m, 8H), 3.40−3.00 (m, 10H), 2.75 (m, 2H), 2.45 (s, 3H), 2.20 (m, 1H), 2.00 (m, 1H), 1.90−1.40 (m, 10H). MS *m*/*z* calcd for $C_{37}H_{50}N_6O_4S$: 674.9. Found 675.3 $[M + 1]^+$. HPLC purity: system A, t_R = 7.79 min.

(B) In Vitro Intestinal Permeability. The intestinal absorption potential of the synthesized compounds was evaluated in vitro using the human adenocarcinoma cell line Caco-2, as previously described.[11]

Compounds were first dissolved in DMSO at 0.1 M, then in Hank's balanced salt solution, 25 mM HEPES, pH 7.4, at 100 μM and applied on the apical side of differentiated epithelial monolayers. The amount of compound transported from the apical to the basolateral compartment was quantified by HPLC−MS in order to determine the apparent permeability coefficient (P_{app}) in the absorption direction. The P_{app} was obtained from the linear regression analysis of the appearance rate of the compound in the receiver compartment and is expressed as 10^{-6} cm/s. All the experiments were carried out in the presence of markers of both passive and active transport systems, and the integrity of the monolayer before and after compound addition was also verified.

(C) Binding Experiments. All compounds were tested for their ability to displace the [^{125}I]neurokinin A specifically bound to the recombinant hNK_2 receptor in a cell membrane preparation as reported previously.[12] The radioligand was from Amersham Biosciences (Buckinghamshire, U.K.). Nonspecific binding was determined in the presence of unlabeled neurokinin A (1 μM). The affinity of test compounds was expressed in terms of pK_i (−log K_i), derived from the following equation:[13]

$$K_i = \frac{IC_{50}}{1 + \frac{[\text{radioligand}]}{K_d}}$$

(D) Organ Bath Experiments. The experiments were performed on guinea pig (Dunkin Hartley, Charles River, Italy) isolated proximal colon circular smooth muscle preparation (GPC). All experiments were performed in oxygenated (96% O_2 and 4% CO_2) Krebs−Henseleit solution. The preparations were set up according to methods previously described.[12] The activity of test compounds at tachykinin NK_2 receptors in GPC was assessed against selective NK_2 receptor agonist [βAla^8]-NKA(4−10) in the presence of the NK_1 receptor selective antagonist SR 140333 (1 μM). The antagonist affinity of all test compounds (15 min of incubation period) was expressed as pK_B (−log K_B, the antagonist dissociation constant), which was estimated as the mean of the individual values obtained with the following equation:

$$pK_B = \log[(CR) - 1] - \log(\text{antagonist concentration})$$

where CR is the ratio of equieffective concentrations (EC_{50}) of agonist in the presence and in absence of the antagonist.

(E) Functional Experiments on Human Urinary Bladder. Mucosa-free strips of detrusor muscle were excised from the urinary bladder dome of patients undergoing cystectomy because of carcinoma of the bladder base, as described previously.[14] The strips were placed in 5 mL organ baths filled with oxygenated Krebs−Henseleit solution at 37 °C, under a resting tension of 10 mN. Mechanical activity developed by the preparations was recorded isometrically. The test compounds were tested for their ability to block neurokinin A-induced contractions. Antagonist affinity was expressed as pK_B; pK_B = log(CR − 1) − log-(antagonist concentration).

(F) In vivo Experiments. All the experiments were performed in accordance with the Declaration of Helsinki, with the principles and the guidelines of the European Union regulations and the local ethical committee. Briefly, male Dunkin−Hartley guinea pigs (Charles River, Italy) weighing 350−400 g were anesthetized with urethane (1.5 g/kg sc) and a polyethylene catheter was inserted into the left jugular vein for intravenous (iv) administration of drugs. Guinea pigs were mechanically ventilated with a ventilation pump at a rate of 50 strokes/min and a respiration volume of 10 mL/kg. The body temperature was kept constant at 36 °C by a thermoregulated lamp. The abdomen was opened, and a latex balloon, which was obtained by a condom head, was connected to a PE90 polyethylene catheter, inserted into the proximal colon at about 2−3 cm from the cecum, and filled with 0.5 mL of saline. The intracolonic balloon was connected to a pressure transducer (Transpac IV, Abbott, Italy) for intraluminal pressure recording by means of a MacLab/8S ML 780 data acquisition system (ADInstruments, U.K.). Five minutes before starting the experiments, guinea pigs were treated with the ganglionic blocker hexamethonium bromide (13.8 μmol/kg iv) followed by continuous infusion of the same solution at a rate of 300 μL/h to prevent reflex cholinergic responses. The compounds of their vehicle (DMSO) were administered iv (0.3−3 μmol/kg) in a volume of 100 μL/kg. The selective NK_2 receptor agonist [βAla8]NKA(4−10) (3 nmol/kg, iv) was administered two or three times before the antagonist of the vehicle in order to stabilize the colon contractile responses, and the challenge was repeated at 5, 30, and then every 30 min until 4 h after antagonist administration.

References

(1) Sisto, A.; Altamura, M.; Cardinali, F.; D'Andrea, P.; Rossi, C.; Fattori, D. α,α-Cyclic aminoacids as useful scaffolds for the preparation of hNK_2 receptor antagonists. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 4841–4844.

(2) Porcelloni, M.; D'Andrea, P.; Rossi, C.; Sisto, A.; Ettorre, A.; Madami, A.; Altamura, M.; Giuliani, S.; Meini, S.; Fattori, D. α,α-Cyclopentaneclycine dipeptides capped with biaryls as tachykinin NK2 receptor antagonists. *ChemMedChem* **2008**, 1048–1060.
(3) Sakaitani, M.; Ohfune, Y. Syntheses and reactions of silyl carbamates. 2. A new mode of cyclic carbamate formation from *tert*-butyldimethylsilyl carbamates. *J. Am. Chem. Soc.* **1990**, *112*, 1150–1158.
(4) Bodanszky, M.; Bodanszky, A. *The Practice of Peptide Synthesis*; Springer Verlag: Berlin, 1994; p 104.
(5) Abdel-Magid, A. F.; Carson, K. G.; Harris, D. B.; Maryanoff, C. A.; Shah, R. D. Reductive amination of aldehydes and ketones with sodium triacetoxyborohydride. Studies on direct and indirect reductive amination procedure. *J. Org. Chem.* **1996**, *61*, 3849–3862.
(6) Amnon, A.; Persky, R. Improved stereocontrolled synthesis of threo peptidyl epoxides. *J. Org. Chem.* **1994**, *3*, 653–657.
(7) Conradi, R. A.; Hilgers, A. R.; Ho, N. F. H.; Burton, P. S. The influence of peptide structure on transport across Caco-2 cells. II. Peptide bond modification which results in improved permeability. *Pharm. Res.* **1992**, *9*, 435–439.
(8) Rejito, P. A.; Verkhivker, G. M. Unraveling principles of lead discovery: from unfrustrated energy landscapes to novel molecular anchors. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 8945–8950.
(9) Zhang, R.; Monsma, F. The importance of drug−target residence time. *Curr. Opin. Drug Discovery Dev.* **2009**, *12*, 488–496.
(10) Fedi, V.; Altamura, M.; Catalioto, R.-M.; Giannotti, D.; Giolitti, A.; Giuliani, S.; Guidi, A.; Harmat, N. J. S.; Lecci, A.; Meini, S.; Nannicini, R.; Pasqui, F.; Tramontana, M.; Triolo, A.; Maggi, C. A. Discovery of a new series of potent and selective linear tachykinin NK2 receptor antagonists. *J. Med. Chem.* **2007**, *50*, 4793–4807.
(11) Larger, P.; Altamura, M.; Catalioto, R.-M.; Giuliani, S.; Maggi, C. A.; Valenti, C.; Triolo, A. Simultaneous LC−MS/MS determination of reference pharmaceuticals as a method for the characterization of the Caco-2 cell monolayer absorption properties. *Anal. Chem.* **2002**, *74*, 5273–5281.
(12) Fedi, V.; Altamura, M.; Balacco, G.; Canfarini, M.; Criscuoli, M.; Giannotti, D.; Giolitti, A.; Giuliani, S.; Guidi, A.; Harmat, N. J. S.; Nannicini, R.; Pasqui, F.; Patacchini, R.; Perrotta, E.; Tramontana, A; Triolo, A.; Maggi, C. A. Insertion of an aspartic acid moiety into cyclic pseudopeptides: synthesis and biological characterization of potent antagonists for the human tachykinin NK-2 receptor. *J. Med. Chem.* **2004**, *47*, 6935–6947.
(13) Cheng, Y.; Prusoff, W. H. Relationship between the inhibition constant (K1) and the concentration of inhibitor which causes 50% inhibition (I50) of an enzymatic reaction. *Biochem. Pharmacol.* **1973**, *22*, 3099–3108.
(14) Maggi, C. A.; Santicioli, P.; Patacchini, R.; Cellerini, M.; Turini, D.; Barbanti, G.; Beneforti, P.; Rovero, P.; Meli, A. Contractile response of the human isolated urinary bladder to neurokinins: involvement of NK-2 receptors. *Eur. J. Pharmacol.* **1988**, *145*, 335–340.