Articles

Discovery of a New Series of Potent and Selective Linear Tachykinin NK_2 Receptor Antagonists

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Starting from **1** (MEN14268), a selective tachykinin NK_2 receptor antagonist with an interesting in vitro pharmacological profile, a family of numerous antagonists was obtained through an optimization process focused on iterated structural modifications. The effects of the introduction of a wide variety of substituents on the lipophilic aromatic part of the molecule and the modulation of the structural constraint through the insertion of different achiral α,α-dialkylamino acids were investigated. In particular, aromatic and benzofused heteroaromatic moieties were introduced at the pseudo-N-terminal residue to replace the 2-benzothiophene moiety, and a systematic investigation of the best positioning of substituents onto the aromatic platform was reported for the benzothiophene core. Studies on the modulation of the length and the rigidity of the hydrophilic pseudo-C-terminal pendant are presented. Many heteroaliphatic groups are well tolerated by the receptor in this part of the ligand. The product **48f** (MEN15596), bearing a methyl substituent on the benzothiophene and a tetrahydropyranylmethylpiperidine pendant, was finally selected for its good in vivo activity after intravenous, intraduodenal, and oral administration in guinea pigs.

Introduction

In the tachykinin neurotransmitters family, neurokinin A (NKA) is the one that preferentially activates the NK_2 receptor in both central and peripheral nervous systems.[1] The human tachykinin NK_2 receptor is reported to be involved in several pathological conditions regarding the respiratory, gastrointestinal, and genitourinary tracts, as well as the central nervous system (CNS[a]),[2] and selective antagonists are considered potential candidates for the treatment of asthma, irritable bowel syndrome, cystitis, and depression.[3] Two different tachykinin NK_2 receptor antagonists are presently undergoing advanced clinical studies, the non-peptide saredutant (Sanofi-Aventis)[4] for depression and the bicyclic hexapeptide nepadutant (Menarini)[5] for the treatment of gastrointestinal diseases.

The past work performed in our laboratories has allowed the identification of bicyclic and monocyclic peptides as potent and selective tachykinin NK_2 receptor antagonists. This led to the selection of the sugar-containing bicyclic peptide nepadutant and, later on, of simplified pseudopeptide molecules sharing a common 14-member cycle, 3 aromatic residues, and a basic pendant.[6] We present now the synthesis and the structure−activity relationship (SAR) study of a series of new potent antagonists derived from a lead molecule previously obtained by the screening of original libraries (whose design and preparation with a parallel synthesis approach have been described elsewhere).[7] Library screening allowed us to identify the small molecule linear compound **1** (benzo[*b*]thiophene-2-carboxylic acid {1-[1-(*R*)-(3-morpholin-4-ylpropylcarbamoyl)-2-phenylethylcarbamoyl]cyclopentyl}-amide, MEN14268)[8] (Figure 1), a simple and versatile structure with nanomolar binding affinity for the human NK_2 receptor. During the following lead optimization we decided, with the aim to keep complexity to a minimum, to consider only compounds with no more than a single chiral center and two aromatic rings. Two of the general features of **1**, the α,α-dialkylamino acid as a conformational constraint and the basic nitrogen, were maintained.

Even though from a disconnective point of view **1** is ideally made of four components (Figure 1), most of the manipulations were focused on the external blocks: the aromatic pseudo-N-terminal group and the basic pendant. A series of differently disubstituted amino acids that are known to induce different folding[9] were introduced in place of the cyclopentane derivative. These preliminary investigations confirmed that the cyclopentane could be maintained. The SAR for the replacement of the D-phenylalanine moiety with different natural and nonnatural amino acids will be described elsewhere.

The first series of substitutions was conducted in parallel on the two external components, with the aim of selecting suitable modifications independently from one another. The best substitution on the C-terminal (*C*-[1-(tetrahydropyran-4-ylmethyl)-piperidin-4-yl]methylamine) was then introduced into the reference structure, giving rise to an evolved lead compound (**30b**). This last one was used for further optimization of the pseudo-N-terminal aromatic moiety, confirming in many cases the additivity of two singularly advantageous modifications. The synthetic work culminated with the identification of **48f** (MEN

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[a] Abbreviations: APCI, atmospheric pressure chemical ionization; Boc, *tert*-butoxycarbonyl; BSA, *N*,*O*-bis(trimethylsilyl)acetamide; DBAD, di-*tert*-butyl azodicarboxylate; DBU, 1,8-diazabicyclo[5.4.0]undec-7-ene; DIPEA, *N*,*N*-diisopropylethylamine; DME, 1,2-dimethoxyethane; DMF, *N*,*N*-dimethylformamide; DMSO, dimethyl sulfoxide; CNS, central nervous system; EDC·HCl, *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride; ESI, electrospray; EtOAc, ethyl acetate; GPC, guinea pig isolated proximal colon; HOBt, 1-hydroxybenzotriazole; MeOH, methanol; P_{app}, apparent permeability coefficient; •-PPh_2, resin-bound triphenylphosphine; PSA, polar surface area; SAR, structure−activity relationship; Su, succinimidyl; TFA, trifluoroacetic acid; THF, tetrahydrofuran; TMSCl, trimethylsilyl chloride; Z, benzyloxycarbonyl.

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Figure 1. Schematic representation of chemical blocks contained in the starting lead **1**.

15596)[10] whose pharmacological profile was excellent both in vitro and in vivo. The X-ray crystal structure of the optimized product **48f** pointed out that this compound, as well as the previously reported cyclic antagonists,[11] showed the typical type I β-turn adjustment.[12] Furthermore, site-directed mutagenesis studies confirmed a partial overlapping in the binding site of the different classes of molecules.[8]

Chemistry

The initial work was dedicated to a brief investigation about the conformational constraint. Single syntheses were performed for this series of products according to the generic pathway depicted in Scheme 1. Boc-D-Phe-OSu (**2**) was coupled with 3-morpholinopropylamine in THF at room temperature to obtain **3.** This was treated with hydrochloric acid in dioxane to obtain the deprotected amine that was subsequently coupled with the desired Boc protected amino acid (**4a**−**d**) in the presence of EDC·HCl, HOBt, and DIPEA in DMF to give **5a**−**d**. In a very similar way *N*-Boc-*N*-methylglycine (**4e**) was reacted to give **5e**, the N-methylated analogue of compound **5a**. In order to obtain the corresponding N-methylated analogues of the cyclopentane and cyclohexane derivatives **5f**,**g**, a peculiar synthetic strategy was followed using as the key step a Mitsunobu−Fukuyama[13] alkylation on the *o*-nitrobenzenesulfonyl derivatives **8a**,**b** (Scheme 1), which was preferred to other alkylation methods to completely avoid double methylation and quaternarization of the morpholine. The 1-aminocyclopentanecarboxylic acid and the cyclohexane analogues **6a**,**b** were coupled with the sulfonyl chloride through preactivation with BSA (*N*,*O*-bis(trimethylsilyl)acetamide−trimethylchlorosilane mixture) in dichloromethane. The corresponding hydrolyzed sulfonamidoacids **7a**,**b** were directly coupled with deprotected **3** in presence of EDC·HCl and HOBt to give **8a**,**b**. Treatment with resin-supported triphenylphosphine, methanol, and di-*tert*-butyl azodicarboxylate (DBAD) and subsequent deprotection with potassium carbonate and thiophenol (or 2-mercaptoethanol) gave the desired methylated substrates **5f**,**g**. In order to obtain the final products, **5a**−**e** were Boc-deprotected under the usual acidic conditions, while **5f**,**g** were reacted without modification. Despite the low reactivity of the hindered amino group, the amide formation was in all cases successfully conducted by reaction with the acyl chloride **9** and triethylamine in dichloromethane, giving products **10a**−**g**.

Scheme 2 shows the general synthesis of derivatives containing amides, ureas, and thioureas (see also Table 2) as the pseudo-N-terminal aromatic moiety. The common intermediate **11** was obtained in large quantity with the methodology described in Scheme 1 and stored as a dihydrochloride salt. Starting from the appropriate commercially available acyl chlorides, three benzamides (**12a**−**c**) were obtained. A parallel synthetic approach was adopted for ureas and thioureas: a freshly prepared solution of the free amine **11** in dichloromethane was divided volumetrically into reaction vials, and various commercially available aromatic isocyanates and isothiocyanates were added. The final products **13a**−**g** were sometimes obtained pure after crystallization from the solutions through addition of hexane but more generally were purified through preparative HPLC. Further commercially available acyl chlorides were coupled with the amine **11** to give products **14a**−**h**.

The synthetic work was then focused on the elaboration of potential replacements of the morpholine pendant. We used a parallel approach based on some common intermediates onto which we performed the final functionalizations. As depicted in Scheme 3, we chose for the construction of the functionalizable intermediates a [2 + 2] parallel synthetic strategy having one component (**16**) in common. This one was obtained according to the following multistep synthesis: the amino acid **6a** was at the same time protected on the acid functionality and activated on the amine by reaction with *N*,*O*-bis-trimethylsilylacetamide (BSA) in dichloromethane and then acylated with the acyl chloride **9** giving after hydrolytic workup the acid **15**. This was successfully transformed into the oxazolone **16** by simple treatment with EDC·HCl and triethylamine in THF on the scale of grams. The oxazolone **16** was elected as a suitable common fragment because of its reactivity together with its on-bench stability for months. The first derived platform bore an aldehyde function and was obtained by reaction of the activated Z-protected D-phenylalanine **17** with 1-amino-3-propanol in DME, deprotection under hydrogenation conditions to give **18**, and subsequent reaction with the oxazolone **16** in DMF and diisopropylethylamine to give the alcohol **19** that was oxidized to the corresponding aldehyde **20** with Dess−Martin reagent.[14] Reductive amination with appropriate amines gave final products **21a**−**h** shown in Table 3.

A very similar approach was used to obtain two functionalizable carboxylic intermediates: amino acid **1** was coupled with γ-aminobutyric acid (**22a**) or β-alanine (**22b**) in an acetonitrile/water mixture and triethylamine to give, after deprotection, the amino acids **23a**,**b** suitable for the reaction with the same oxazolone **16** under the conditions described above in the text. The two intermediates **24a**,**b** were transformed into the final products **25a**−**f** (see Table 3) by classical peptide coupling with the desired amines using HOBt, EDC, dichloromethane. In order to introduce a piperidine moiety into the basic pendant, Z-D-Phe-OH **26** was coupled under the usual conditions with the Boc protected piperidines bearing a supplementary free amino functionality in position 4 (**27a**−**c**) and then hydrogenated in the presence of Pd/C to give **28a**−**c**. Further reaction with the oxazolone **16** and subsequent Boc deprotection gave amines **29a**−**c**. These materials were reacted with the desired aldehydes or ketones under reductive conditions to give final products **30a**−**e**.

In order to investigate the best positioning of substituents onto the benzothiophene residue, a chlorine atom was substituted at every hydrogen of the heteroaromatic moiety using a common synthetic strategy (except for the 3-chloro derivative that was commercially available) (see Scheme 4 and Table 4) starting from the chlorosubstituted *o*-fluoro- or *o*-nitrobenzaldehydes. As previously reported in the literature,[15] **31a**−**d** (Table 4) underwent aromatic nucleophilic substitution by the methyl thioglycolate **32** in the presence of a base (K_2CO_3 in DMF or triethylamine in DMSO) to give directly the corresponding cyclized methyl esters **33a**−**d**. These and the commercially available 3-chloro derivative were easily converted into the acyl chlorides through alkaline hydrolysis and subsequent treatment

Scheme 1[a]

[a] Methods: (a) 3-morpholinopropylamine, THF, room temp, 1 h (quantitative); (b) 4 M HCl, dioxane, room temp, 3 h (quantitative); (c) DMF, EDC·HCl, HOBt, DIPEA (quantitative); (d) BSA (+5% TMSCl), 2-nitrobenzenesulfonyl chloride, CH_2Cl_2 (81%), (e) •-PPh$_2$, DBAD, CH_2Cl_2, MeOH, 16 h, room temp (85%); (f) DBU, thiophenol or 2-mercaptoethanol, DMF (70%); (g) NEt_3, CH_2Cl_2, room temp, 12 h (30−60%).

Scheme 2[a]

[a] Methods: (a) Ar′COCl, NEt_3, CH_2Cl_2, room temp, 12 h, (30−60%); (b) Ar″NCX (X = O or S), 40 °C, 3 h → rt overnight (40−60%). For Ar′ and Ar″, see Table 2.

with oxalyl chloride together with a catalytic amount of DMF in dichloromethane. Once again, the common intermediate **11** was used for the parallel final acylating step using the freshly prepared acyl chlorides of **33a−d** with triethylamine in dichloromethane, giving **34a−e**.

Further synthetic efforts were made to obtain derivatives variably substituted at position 6 of the benzothiophene ring (Scheme 5 and Table 5) using, every time possible, a methodology similar to that already described for products of Table 4. Starting from aldehydes **35a−e**, the corresponding methyl esters **36a−e** were recovered with very variable yields after treatment with methyl thioglycolate (**32**). Two exceptions to this very general methodology were necessary: for the cyano derivative the starting 2-nitroterephthalonitrile **37** was treated in the previously reported conditions giving the amino derivative **38** that was subsequently deaminated to the desired ester **39** by using *tert*-butylnitrite in DMF at 50 °C;[15b] for the diethylamino derivative the starting dinitrobenzaldehyde **40** was cyclized and the resulting 6-nitrobenzothiophenyl-2-carboxylic ester **41** was first treated with hydrogen-reduced iron in a solution of hydrochloric acid in water/methanol and then doubly alkylated with ethyl iodide and potassium carbonate in acetonitrile to give

Table 1. Structures of Compounds **10a−g**, Affinities for the Tachykinin NK_2 Receptor Evaluated in Binding Experiments (pK_i Values), Apparent Permeability (P_{app}) Coefficients, and Polar Surface Areas (PSA)

Entry	R R	R'	NK_2 affinity[a] pK_i[c] (hNK_2)	P_{app}[b]	PSA
1	cyclopentane	H	9.18 ± 0.04	9	100.6
10a	H, H	H	< 6.0		103.4
10b	Me, Me	H	7.68 ± 0.07	4.7	101.7
10c	cyclopropane	H	7.02 ± 0.12		102.6
10d	cyclopent-3,4-ene	H	8.26 ± 0.03		101.5
10e	H, H	Me	6.0 ± 0.1		92.8
10f	cyclopentane	Me	7.61 ± 0.02	29.5	93.4
10g	cyclohexane	Me	7.97 ± 0.02	32.2	92.7

[a] Each value is the mean ± SEM of three determinations. [b] Values are expressed in 10^{-6} cm/s; see ref 20. [c] $pK_i = -\log K_i$. Affinities for the human NK_2 receptor estimated against [^{125}I]neurokinin A in radioligand binding experiments.

Table 2. Structures of Compounds **12a−c**, **13a−g**, and **14a−h**, in Vitro Activity Evaluated in Binding Experiments (pK_i Values) and Functional Experiments (pK_B Values), Apparent Permeability Coefficients (P_{app}), and Polar Surface Areas (PSA)

Entry	R^2	NK_2 activity[a] pK_i[c] (hNK_2)	NK_2 activity[a] pK_B[d] (GPC)	P_{app}[b]	PSA
1		9.18 ± 0.04	7.6 ± 0.1	9	100.6
12a		6.91 ± 0.03			99.3
12b		7.04 ± 0.13			142.9
12c		7.83 ± 0.06			100.6
13a		7.90 ± 0.02			113.0
13b		8.38 ± 0.09	6.5 ± 0.2		112.4
13c		8.04 ± 0.01	6.4 ± 0.2		113.0
13d		7.14 ± 0.03			115.5
13e		8.42 ± 0.03	6.9 ± 0.2		111.6
13f		7.55 ± 0.04			97.0
13g		7.96 ± 0.04	< 5		101.4
14a		8.31 ± 0.07	6.3 ± 0.3	6	111.4
14b		8.64 ± 0.02	7.1 ± 0.3		117.9
14c		8.62 ± 0.01	7.0 ± 0.3	10.6	107.5
14d		8.67 ± 0.01	7.0 ± 0.2	5.4	103.2
14e		7.59 ± 0.02		<1	122.8
14f		8.8 ± 0.2	6.6 ± 0.3	18	97.6
14g		9.12 ± 0.03	7.5 ± 0.2	11.7	103.2
14h		8.03 ±0.06			100.1

[a] Each value is the mean ± SEM of three determinations. [b] Values are expressed in 10^{-6} cm/s; see ref 20. [c] $pK_i = -\log K_i$. Affinities for the human NK_2 receptor estimated against [^{125}I]neurokinin A in radioligand binding experiments. [d] $pK_B = -\log K_B$. Antagonist affinities of test compounds for guinea pig NK_2 receptor estimated toward [βAla^8]NKA(4−10) induced contractions of the guinea pig isolated colon (GPC) in the presence of the NK_1 receptor selective antagonist SR140333.

the ester **42**. A new common intermediate **47**, containing the evolved basic pendant of product **30b**, was built. Commercially available methyl tetrahydro-2*H*-pyran-4-carboxylate **43** was hydrolyzed to the corresponding carboxylic acid by treatment with sodium hydroxide (1 M) and immediately transformed into the acyl chloride **44** with oxalyl chloride and catalytic DMF in dichloromethane. Reaction of **44** with isonipecotamide gave the diamide **45** that was successfully reduced to the corresponding diamine **46** using lithium aluminum hydride in refluxing THF. With obvious transformations similar to that used for the obtainment of **5a−g** the corresponding cyclopentane derivative **47** was obtained bearing a functionalizable free amino group. This was acylated with the benzothiophene carbonyl chlorides derived from the esters **33c**, **36a−e**, **39**, and **42** in dichloromethane and triethylamine to give final products **48a−h**.

Results and Discussion

The binding affinities at the human tachykinin NK_2 receptor were evaluated for all the derivatives and are reported in Tables 1−5. For many of the derivatives the functional antagonist potency in guinea pig isolated proximal colon (GPC) is also reported. Because the final target of this work was to identify a compound endowed with good oral bioavailability together with high antagonist activity, preliminary calculation of the polar surface area (PSA) for any of the designed products was done using Sybyl (Tripos, Inc.). According to the literature, a significant correlation exists between the theoretical value of PSA and some experimental measures as the fraction of drug absorbed after oral administration (FA)[16] or the more indirect but easier to determine parameter of the intestinal permeability obtained in the in vitro model of Caco-2 cells as the apparent permeability coefficient (P_{app}).[17] Usually PSA ≥ 140 is considered as an indication of poorly absorbed compounds and corresponds to low ($<5 \times 10^{-6}$ cm/s) values of P_{app}. We verified that the majority of our structures showed acceptable PSA values (see Tables 1−5). Permeability tests were performed on selected compounds to obtain general suggestions for further structural elaborations. In addition, we used P_{app} as a "second level" selection parameter, for example, to choose between two products with similar in vitro pharmacological profiles. Analysis of the correlation between available P_{app} values and the calculated PSA was done a posteriori (see Figure 2) and is discussed later in the text.

In vivo tests were also performed during the screening phase on selected compounds by the intravenous route and by the intraduodenal route as a model for oral administration. Results of these tests for key compounds are reported in Table 7. These gave us the most important feedback on the suitability of the optimization process. The description of the methods is reported in the Experimental section.

In Table 1 the starting lead compound **1** (see Introduction) is compared with products containing various achiral amino acids in the place of 1-aminocyclopentanecarboxylic acid. The loss of the conformational constraint in **10a**, bearing the glycine residue, resulted in a dramatic loss of activity. On the other

Scheme 3[a]

[a] Methods: (a) BSA, CH_2Cl_2, room temp, 16 h (85%); (b) EDC·HCl, NEt_3, THF, room temp, 13 h (88%); (c) 1-amino-3-propanol, NEt_3, DME, room temp, 2 h, (90%); (d) H_2, Pd/C (10%), aqueous HCl, MeOH, room temp, 20 h (quantitative); (e) DMF, DIPEA, room temp, 24 h, (83%); (f) Dess−Martin periodinane, CH_2Cl_2/THF, room temp, 2 h (99%); (g) amine, glacial acetic acid, $Na(CN)BH_3$, MeOH, room temp, 4 h (20−45%); (h) NEt_3, CH_3CN, room temp, 30 min (73%); (i) 4 N HCl in dioxane, CH_2Cl_2/CH_3CN, room temp, 4 h (quantitative); (j) DIPEA, DMF, room temp, 12 h (85%); (k) suitable amine, EDC·HCl, HOBt, NEt_3, DMF, room temp, 16 h (60−70%); (l) EDC·HCl, HOBt, NEt_3, DMF, room temp, 16 h (65−70%); (m) NEt_3, DMF, room temp, 12 h; (n) TFA, CH_2Cl_2, room temp, 1 h (76%); (o) aldehyde, glacial acetic acid, $Na(CN)BH_3$, MeOH, room temp, 1 h (35−55%).

hand, the acyclic dimethyl derivative, known to design a narrower fork between the amino and the acyl groups in comparison to the cyclopentane,[9] gave rise to compound **10b**, still less active than **1**. Two other cyclic structures were introduced (**10c** and **10d**), obtaining in both cases less active compounds. The introduction of a methyl group onto the amide nitrogen was also performed in order to suppress a potential hydrogen-bond donor (**10e**−**g**) and to increase lipophilicity, thus making easier the penetration through the intestinal membrane. In fact, in **10f**,**g** this modification strongly increased the permeability, although negatively affecting the NK$_2$ receptor affinity probably by changing the conformation of the molecule in an unfavorable way for the receptor. These early results were considered sufficient to fix the cyclopentane moiety as the conformational constraint in the proceeding work.

Table 2 reports the initial efforts to find potential substitutions to the benzothiophene as aromatic acyl caps of the pseudo-N-terminal functionality. Three derivatives of monosubstituted benzoic acids (**12a**−**c**) showed reduced activity, and with a very heavy and bulky substituent (**12c**) only, the pK_i was weakly higher. Variously substituted ureidic (**13a**−**e**) and thioureidic (**13f**,**g**) monocyclic groups also showed reduced activity. Benzofused amides showed improved results: benzofuran (**14a**), indole (**14b**), *N*-methylindole (**14c**), and naphthalene (**14d**) gave rise to higher activity both in the binding test, with pK_i > 8.0, and in the functional experiments, with p$K_B \approx 7.0$. The values of pK_i and pK_B were usually comparable to those measured for the reference compound **1**. An exception is represented by the basic quinoline derivative **14e**, which showed lower affinity in the binding test. Finally, by use of the commercially available 2-carboxy biphenyl, 5-phenyl-thiophenyl, and cynnamic derivatives, **14f**, **14g**, **14h** were synthesized and tested giving, in the binding assay, pK_i values slightly inferior to that of the reference compound together with interesting values of P_{app}. In summary, many benzofused or phenylaryl surrogates of benzothiophene were found during this initial SAR study, but no substantial

Table 3. Structures of Compounds **21a−h**, **25a−f**, and **30a−e**, in Vitro Activity Evaluated in Binding Experiments (pK_i Values) and Functional Experiments (pK_B Values), Apparent Permeability Coefficients (P_{app}), and Polar Surface Areas (PSA)

Entry	Basic pendant	NK$_2$ activity[a] pK_i[c] (hNK$_2$)	NK$_2$ activity[a] pK_B[d] (GPC)	P$_{app}$[b]	PSA
1		9.18 ± 0.04	7.6 ± 0.1	9	100.6
21a		9.37 ± 0.05	8.0 ± 0.3	nd	90.4
21b		9.09 ± 0.04	7.6 ± 0.1	1.1	108.6
21c		8.50 ± 0.02	6.4 ± 0.3	1.4	108.9
21d	N−SO$_2$NH$_2$	9.88 ± 0.02	8.1 ± 0.3	<1	144.9
21e		8.69 ± 0.07	6.8 ± 0.3		106.8
21f		8.42 ± 0.02		<1	108.1
21g		8.07 ± 0.04			140.1
21h		8.31 ± 0.01			125.7
25a		8.33 ± 0.15	6.4 ± 0.4	1.7	120.2
25b		9.12 ± 0.06	7.9 ± 0.1	<1	121.1
25c		8.97 ± 0.03	7.7 ± 0.1	2.0	111.6
25d		9.93 ± 0.08	8.3 ± 0.1	<2	122.3
25e		9.05 ± 0.02	8.2 ± 0.2	2.7	119.0
25f		8.93 ± 0.06	8.3 ± 0.1	1.3	118.9
30a		8.71 ± 0.04	7.8 ± 0.1	8.5	98.7
30b		9.65 ± 0.03	8.4 ± 0.1	6.5	97.8
30c		8.71 ± 0.04	7.2 ± 0.2	3.9	100.4
30d		9.26 ± 0.05	7.9 ± 0.2	1.6	100.3
30e		9.07± 0.03	8.5 ± 0.1	<2	100.0

[a] Each value is the mean ± SEM of three determinations. [b] Values are expressed in 10^{-6} cm/s; see ref 20. [c] pK_i = −log K_i. Affinities for the human NK$_2$ receptor estimated against [^{125}I]neurokinin A in radioligand binding experiments. [d] pK_B = −log K_B. Antagonist affinities of test compounds for guinea pig NK$_2$ receptor estimated toward [βAla^8]NKA(4−10) induced contractions of the guinea pig isolated colon (GPC) in the presence of the NK$_1$ receptor selective antagonist SR140333.

Scheme 4[a]

2-Cl **31a**, 3-Cl **31b**, 4-Cl **31c**, 5-Cl **31d** → (HS-CH$_2$-COOMe, **32**; (a) or (b)) → 4-Cl **33a**, 5-Cl **33b**, 6-Cl **33c**, 7-Cl **33d**

33a-d → (c), (d) → [Cl-benzothiophene-COCl] → **11**, (e) → **34a-d**

[a] Methods: (a) X = NO$_2$, K$_2$CO$_3$, DMF, 0 °C → room temp, 5−40 h (70−75%); (b) X = F, NEt$_3$, DMSO, 80 °C, 2 h (75%); (c) K$_2$CO$_3$, H$_2$O, MeOH, room temp, 24 h; (d) (COCl)$_2$, DMF catalyst, CH$_2$Cl$_2$, room temp, 20 h; (e) NEt$_3$, CH$_2$Cl$_2$, room temp, 5 h (30−50%).

advantages were seen for any of the substituents. Simultaneously, a parallel study was conducted for the optimization of

Table 4. Structures of Compounds **34a−e** and in Vitro Activity Evaluated in Binding Experiments (pK_i Values) and Functional Experiments (pK_B Values), Apparent Permeability Coefficients (P_{app}), and Polar Surface Area (PSA) Values

Entry	Cl position	Synthetic method[a]	NK$_2$ activity[b] pK_i[d] (hNK$_2$)	NK$_2$ activity[b] pK_B[e] (GPC)	P$_{app}$[c]	PSA
1	-	-	9.18 ± 0.04	7.6 ± 0.1	9	100.6
34a	4	a	8.54 ± 0.02	7.1 ± 0.1		97.6
34b	5	a	8.82 ± 0.04	7.6 ± 0.1	5.9	102.0
34c	6	b	9.55 ± 0.05	8.0 ± 0.2		100.9
34d	7	b	9.43 ± 0.04	8.0 ± 0.1		100.9
34e	3	[f]	7.52 ± 0.01		15.1	101.9

[a] See Scheme 4. [b] Each value is the mean ± SEM of three determinations. [c] Values are expressed in 10^{-6} cm/s; see ref 20. [d] pK_i = −log K_i. Affinities for the human NK$_2$ receptor estimated against [^{125}I]neurokinin A in radioligand binding experiments. [e] pK_B = −log K_B. Antagonist affinities of test compounds for guinea pig NK$_2$ receptor estimated toward [βAla^8]NKA(4−10) induced contractions of the guinea pig isolated colon (GPC) in the presence of the NK$_1$ receptor selective antagonist SR140333. [f] From commercially available 3-chlorobenzothiophene derivative.

the basic pendant replacing the aminopropylmorpholine moiety of **1** with other aliphatic amines (Table 3). The replacement of the oxygen atom of the morpholine ring with a sulfur atom gave a very active product (**21a**). However, solubility of **21a** was so low that it was impossible to perform the permeability test (standard conditions require solutions at 10−100 μM). The 2,6-piperazinyl derivative **21b** was as active as the reference compound **1** in both assays, but the permeability was very low. The formal extrusion of the oxygen atom to obtain the hydroxyl derivative **21c** was detrimental both for the activity and for the permeability, while the introduction of a sulfonyl urea (**21d**), in analogy to the optimized monocyclic antagonist previously described by our group,[6] was confirmed as favorable for receptor affinity but at the same time critical for the permeability. The 4-morpholinepiperidine group was another pendant previously selected;[6] unfortunately, the corresponding linear compound **21e** was not equally interesting, giving worse results in both the binding assay and functional test. Three additional derivatives containing the simple propylenic spacer were obtained by capping with 4-aminotetrahydropyran (**21f**) and two dihydroxylated amines, one primary and the other secondary (**21g** and **21h**, respectively), but none of these were comparable with the reference compound **1**. The γ-aminobutyric acid and β-alanine (**25a**,**b**) were then introduced as possible slightly less flexible linkers to hold the same "old-fashioned" 4-morpholinepiperidine basic tail as in **21e**. It was immediately clear that the latter and shorter one was better, giving a product (**25b**) as potent as **1**. A small subset of highly potent derivatives with quite different features in terms of basicity, bulkiness, and polarity was built. The *N*-methylpiperazine derivative **25c**, the 2-pyridylpiperazine (**25d**), and the two tetrahydropyran derivatives (**25e**, **25f**) were at least equipotent to the reference compound in the binding test but tended to be more active in the functional assay. Unfortunately the corresponding permeability values were very low. Some products retaining the two six-membered rings but with a smaller numbers of heteroatoms were built. Piperidine linkers and tetrahydropyran pendant were introduced, modulating the total length through the insertion of methylene spacers between the D-Phe and the piperidine or between the two aliphatic cycles. Compounds **30a−c** all showed very interesting

Scheme 5[a]

[a] Methods: (a) **32**, NEt_3, DMSO, 80 °C, 2 h (70−80%) or Cs_2CO_3, DMSO, room temp; (b) **32**, NEt_3, DMSO, 110 °C, 2 h (90%); (c) *t*-$BuNO_2$, DMF, 50−60 °C, 30′ (25%); (d) Fe, H_2O, HCl, MeOH, 60 °C, 5 h, (34%); (e) EtI, K_2CO_3, CH_3CN, reflux 1 week (50%); (f) NaOH, H_2O, room temp, 30′ (80%); (g) $(COCl)_2$, DMF catalyst, CH_2Cl_2, room temp, 1.5 h (quantitative); (h) isonipecotamide, NEt_3, DMF, CH_2Cl_2, room temp, 12 h (70%); (i) $LiAlH_4$, THF, reflux, 4 h (98%); (j) Boc-D-Phe-OSu, THF, 0 °C → room temp, 36 h (83%); (k) HCl, dioxane, room temp, 20 h; (l) *N*-Boc-aminocyclopentanecarboxylic acid, EDC·HCl, HOBT, DIPEA, room temp, DMF, 16 h (87%); (m) NEt_3, CH_2Cl_2, room temp, 7 h, (60−80%).

biological profiles together with an evident trend of increasing activity. **30b** showed subnanomolar activity (pK_i = 9.65), the highest response in the functional test (pK_B = 8.4) ever observed, and a medium value of the P_{app} (6.5). This evidence, together with a preliminary in vivo evaluation (see below), which also showed activity after intraduodenal administration, was sufficient to select **30b** as the evolved lead. The last two products of Table 3, the shorter **30d** and the corresponding asymmetrically spaced **30e**, were once again interesting for their pharmacological activity, being in second position in comparison to **30b** but showing lower permeability values.

A small study was conducted simultaneously on the benzothiophene of **1** to determine the best positioning of substituents. Chlorine (commonly present in other NK$_2$ antagonists) was selected as representative to evaluate the effect of the introduction of a substituent on the heteroaromatic moiety, realizing five monohalogenated derivatives. Results are presented in Table 4 in comparison to **1**. Chlorine in positions 4 (**34a**) and 5 (**34b**) gave quite potent compounds but was slightly less active than the unsubstituted derivative. Position 6 (**34c**) and position 7 (**34d**) seemed to be more sensitive to the substitution for activity enhancement, giving in both tests better results when compared to the reference compound. Finally, the introduction of the halogen in position 3 as in **34e** was detrimental for the affinity, giving pK_i = 7.52 versus pK_i = 9.18 for the reference compound. In order to validate the single fragment optimization process that we performed, the merging of the best results was realized in product **48a**, bearing the selected basic pendant (see discussion for products in Table 3) together with the 6-chlorobenzothiophene acyl group (Table 5). The additivity of the two modifications was immediately evident, as **48a** shows a subnanomolar binding value and the highest functional potency, also confirmed by a functional test on human urinary bladder (pK_B = 9.1). Many other substituents were introduced in position 6 in the place of chlorine: halogens (**48b**,**c**), CF_3 (**48d**), OCH_3 (**48e**), CH_3 (**48f**), CN (**48g**), and diethylamino (**48h**). All of them showed at least nanomolar binding affinities, with the bromine (**48c**), trifluoromethyl (**48d**), methyl (**48f**), and diethylamino (**48h**) derivatives going further and reaching pK_i > 10. The subnanomolar affinity for the human NK$_2$ receptor as measured in the binding test matched well the nanomolar functional potency measured on guinea pig and human isolated tissues. At least three compounds (**48d**, **48f**, **48h**) showed medium/high permeability coefficients. In particular, the 6-methylbenzothiophene derivative **48f** performed well in all tests, reaching the best values for in vitro activity, especially at the human receptor, while maintaining a medium level of permeability.

Table 5. Structures of Compounds **48a**−**h**, in Vitro Activity Evaluated in Binding Experiments (pK_i Values) and Functional Experiments (pK_B Values on Guinea Pig Colon (GPC) and Human Urinary Bladder (HUB)), Apparent Permeability Coefficients (P_{app}), and Polar Surface Areas (PSA)

Entry	R	NK$_2$ activity[a]			P_{app}[b]	PSA
		pK_i[c] (hNK$_2$)	pK_B[d] (GPC)	pK_B[e] (HUB)		
48a	Cl	10.28 ± 0.10	9.0 ± 0.1	9.1 ±0.1	nd	97.9
48b	F	9.63 ± 0.03	8.6 ± 0.3		0.3	97.9
48c	Br	10.24 ± 0.11	8.7 ± 0.1	8.5 ± 0.1	0.8	100.3
48d	CF_3	10.22 ± 0.06	8.5 ± 0.1		6.4	100.4
48e	OCH_3	9.85 ± 0.10	8.5 ± 0.3		1.1	107.7
48f	CH_3	10.12 ± 0.03	9.3 ± 0.1	9.2 ± 0.2	5.1	100.4
48g	CN	8.86 ± 0.05				128.4
48h	$(CH_3CH_2)_2N$	10.01 ± 0.08	8.4 ± 0.2	9.1 ± 0.2	10.1	104.7

[a] Each value is the mean ± SEM of three determinations. [b] Values are expressed in 10^{-6} cm/s; see ref 20. [c] pK_i = −log K_i. Affinities for the human NK$_2$ receptor estimated against [^{125}I]neurokinin A in radioligand binding experiments. [d] pK_B = −log K_B. Antagonist affinities of test compounds for guinea pig NK$_2$ receptor estimated toward [βAla^8]NKA(4−10) induced contractions of the guinea pig isolated colon (GPC) in the presence of the NK$_1$ receptor selective antagonist SR140333. [e] pK_B = −log K_B. Antagonist affinities of test compounds for human NK2 receptor estimated against neurokinin A contractile response in the human isolated urinary bladder (HUB).

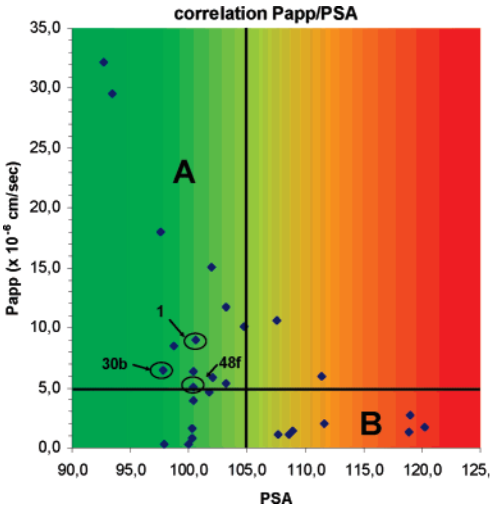

Figure 2. Correlation between calculated PSA and experimental values of P_{app} coefficients.

Table 6. Selectivity of **1** and **48f**: In Vitro Activity versus the Tachykinin NK$_1$ and NK$_3$ Receptors in Guinea Pig Isolated Ileum

compd	pK_B NK$_1$ guinea pig ileum	pK_B NK$_2$ guinea pig colon	pK_B NK$_3$ guinea pig ileum
1[a]	<6	7.6	<6
48f[b]	≤5	9.3	6.0 ± 0.2

[a] Reference 8. [b] Reference 10.

Available P_{app} coefficients were graphed against the calculated PSA (Figure 2) in order to verify whether a lower PSA could be associated with a better permeability coefficient. Even with the small set of data we have collected, it appears possible to define two clusters of products separated by a threshold value of PSA around 105: the first (cluster A, PSA < 105) containing mainly products with acceptable values of permeability (P_{app} ≥ 5), the second (cluster B, PSA > 105) composed of scarcely permeable products except for two cases. It is notable that the starting lead (**1**), the intermediate selected product **30b**, and the final optimized product **48f** are located in the upper left side (A) of Figure 2.

Because one of our goals is the selectivity of these compounds against the NK$_2$ receptor, functional tests were performed to evaluate the antagonist activity of the lead **1** and of the selected compound **48f** for the other tachykinin receptors of the family (NK$_1$ and NK$_3$). Results have been already published[8,10] and are summarized in Table 6. Both compounds showed at least 100:1 selectivity for the NK$_2$ receptor versus the NK$_1$ and NK$_3$ receptors.

In vivo studies were performed in order to compare the activity of **48f** with the intermediate lead **30b** and the initial reference compound **1**. The potency in inhibiting colonic contractions induced by the selective tachykinin NK$_2$ receptor agonist [βAla^8]NKA(4−10) in guinea pig was evaluated after intravenous administration at a dose level of 1 or 3 μmol/kg and after intraduodenal administration at a dose level of 10 μmol/kg. The results, shown in Table 7, are expressed both as maximal inhibitory effect (% change of the control response) and as $\sum$i%max, that is, the sum of the % inhibition, in comparison to the basal colon contraction to [βAla^8]NKA(4−10), at the nine times of observation (5, 30, 60, 90, 120, 150, 180, 210, and 240 min) after iv and id administration of the antagonist and further calculated as the percentage of the sum of theoretical maximal responses:

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

$$\equiv \text{mean \% inhibition over the entire experiment}$$

This parameter, giving a measure of the activity during the entire experimental period, allows evaluation of not only the intensity but also the duration of the antagonist effect: the maximal inhibition obtainable corresponds to $\sum$i%max = 100, while the absence of effect is $\sum$i%max = 0.

As indicated in Table 7, the initial reference compound **1** showed very limited in vivo inhibitory activity, if any. For the intermediate lead **30b**, after iv administration at 3 μmol/kg the maximal effect was high (90 ± 6%) while the duration was limited ($\sum$i%max = 32%). Interestingly **30b** also showed a significant effect after id administration at 10 μmol/kg (maximal effect 53 ± 6% and $\sum$i%max = 36%). Thus, the modification inserted in the basic pendant of the lead compound to give **30b** was able to increase the affinity without preventing the capability of permeating the intestine membrane. Compound **48f** showed a potent, long-lasting, and dose-related inhibitory effect after iv and id administration. Subsequent in vivo investigations[10] confirmed the good results obtained with **48f** (MEN15596): in guinea pigs, **48f** inhibited in a dose-related and persistent manner colon contractions induced by the selective tachykinin NK$_2$ receptor agonist [βAla^8]NKA(4−10), after either intravenous (ED_{50} = 0.18 μmol/kg) or intraduodenal administration (ED_{50} = 3.16 μmol/kg). More interestingly, the compound was also active in inhibiting NK$_2$ mediated colon contractions in guinea pig after oral administration (10−30 μmol/kg).

Conclusions

Novel tachykinin NK$_2$ receptor antagonists with good pharmacological and pharmacokinetic properties were built starting from a lead molecule with good potential of druglikeness. The functionalization of the benzothiophene moiety gave interesting results especially if associated with an increased rigidity of the

Table 7. In Vivo Evaluation of Selected Leads: Inhibition of Colonic Contractions Induced by [βAla[8]]NKA(4−10) in Guinea Pig after Intravenous Administration at 3 and 1 μmol/kg and Intraduodenal Administration at 10 μmol/kg Dose of Compounds

	3 μmol/kg dose, iv		1 μmol/kg dose, iv		10 μmol/kg dose, id	
compd	maximal inhibitory effect (%i) (% change of the control response)	$\sum$i%max [a]	maximal inhibitory effect (%i) (% change of the control response)	$\sum$i%max [a]	maximal inhibitory effect (%i) (% change of the control response)	$\sum$i%max [a]
control	0	0			0	0
1			4 ± 11	0.5		
30b	90 ± 6	32			53 ± 9	36
48f[b]	99 ± 1	92	87 ± 7	66	83 ± 4	74

[a] $\sum i\%max = \sum(\%i)/\sum(\%i_{max}) \times 100 \equiv$ mean % inhibition over the entire experiment, that is, the sum of the % inhibition, in comparison to the basal 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 administration of the antagonist and further calculated as the percentage of the theoretical maximal response [$\sum(\%i_{max}) = 900\%$]. [b] Reference 10.

basic pendant. Through simple modifications of the structure of the starting lead compound **1**, we were able to transform a potent antagonist with poor in vivo activity into an optimized compound **48f** with high affinity, good antagonist activity in both guinea pig and human tissues, and good in vivo activity after intravenous and oral administration.

Experimental Section

Materials and Methods. All reagents and solvents were used without further purification or drying. All reagents were purchased from Sigma-Aldrich (Milan, Italy) unless otherwise specified. Commercial grade anhydrous solvents were purchased from J. T. Baker (Deventer, The Netherlands). Reactions were performed under an atmosphere of nitrogen unless otherwise specified. ^{1}H NMR spectra were acquired on a Varian 200 MHz or Bruker 400, 500, and 600 MHz instruments and recorded in parts per million (ppm) δ values relative to $CHCl_3$ (δ 7.27) or DMSO (δ 2.50) as the internal standard. The data were transferred to an Apple Macintosh computer and processed using the program SwaN-MR.[18] The LC−MS system was a Thermofinnigan LCQ mass spectrometer interfaced with an Agilent series 1100 liquid chromatograph with binary pump, well-plate sampler, column thermostat, and diode array UV detector. Either electrospray (ESI) or atmospheric pressure chemical ionization (APCI) was used as the ionization technique. Ion polarity was positive unless otherwise specified. The samples were analyzed by MS and data-dependent tandem MS, using an isolation width of 2 Th, an activation q of 0.25, and a relative normalized collision energy of 30−35%. Chromatographic separation was achieved using a Luna C8-2 3 μm column (length of 7.5 cm, internal diameter of 4.6 mm). The mobile phases A and B were 0.1% TFA in water and 0.1% TFA in acetonitrile, respectively. A gradient elution was performed from 5% to 95% B in 3 min, followed by a 2 min isocratic step at 95% B at a flow rate of 1 mL/min. Two UV signals, at wavelenghts 220 and 270 nm, were also monitored. HPLC data were recorded using the following methods. Method A parameters are as follows: HP-1100 analytic HPLC system, platinum column RP-18, 3 μm, 100 Å, 33 mm × 7 mm, H_2O + 0.1% TFA/MeCN + 0.1% TFA, 95/5 → 5/95 in 6.5 min + 1 min isocratic, flow rate of 3 mL/min, λ = 220, 270 nm. Method B parameters are as follows: Waters 2695 analytic HPLC system Jupiter column C_{18}, 5 μm, 300 Å, 250 mm × 4.6 mm, H_2O + 0.1% TFA/MeCN + 0.1% TFA, 85/15 → 5/95 in 20 min, flow rate of 1 mL/min, λ = 220, 270 nm. Method C parameters are as follows: HP-1100 analytic HPLC system, Zorbax column SB-18, 3.5 μm, 100 Å (4.6 mm × 50 mm), H_2O + 0.1% TFA/MeCN + 0.1% TFA, 95/5 → 5/95 in 6.5 min + 1 min isocratic, flow rate 3 mL/min, λ = 220, 270 nm. Method D paramters are as follows: Waters 2695 analytic HPLC system, Symmetry column 300 C_{18}, 5 μm, 250 mm × 4.6 mm, 100 Å, H_2O + 0.1% TFA/MeCN + 0.1% TFA, 85/15 → 5/95 in 20 min, flow rate of 1 mL/min, λ = 210, 254 nm. The HPLC purities of the described intermediates, observed at 220 nm, were at least 90% unless otherwise specified.

Binding Experiments. All compounds were tested for their ability to displace the [^{125}I]neurokinin A bound to the human recombinant tachykinin NK_2 receptor in a cell membrane preparations as reported previously.[6] 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 equation

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

Organ Bath Experiments. The experiments were performed on guinea pig (Dunkin Hartley, Charles River, Italy) isolated proximal colon circular smooth muscle preparations (GPC) and on human urinary bladder muscle strips (HUB) obtained from four patients (ages 53−69, both sexes) undergoing cystectomy for bladder base carcinoma.[19] All the experiments were performed in oxygenated (96% O_2 and 4% CO_2) Krebs−Henseleit solution. The preparations were set up according to the methods previously described.[10] The activity of test compounds at tachykinin NK_2 receptors in GPC was assessed against the selective NK_2 receptor agonist [βAla[8]]-NKA(4−10) in the presence of the NK_1 receptor-selective antagonist SR140333 (1 μM). The activity of selected compounds in human urinary bladder at tachykinin NK_2 receptors was evaluated against neurokinin A contractile response. Cumulative concentration−response curves to the agonists were obtained in all preparations, each concentration being added when the effect of the preceding one had reached a steady state. The antagonist affinity of all test compounds (15 min incubation period) was expressed as pK_B (negative logarithm of K_B, the antagonist dissociation constant), which was estimated as the mean of the individual values obtained with the equation

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

In Vitro Intestinal Permeability in Caco2 Cells. The intestinal absorption potential of newly synthesized compounds was evaluated in vitro using the human adenocarcinoma cell line Caco-2 as a model. Caco-2 cells were allowed to differentiate, and the intestinal permeability of the compounds was determined from the transport rates across cell monolayers in the apical-to-basal direction and quantified in terms of the apparent permeability coefficient (P_{app}), as described elsewhere.[20] The drugs were first dissolved in DMSO at 0.1 M and then in Hank's balanced salt solution containing 25 mM HEPES at pH 7.4. The final concentration of compound used in the test was 10−100 μM. All the experiments were carried out in the presence of markers of the integrity of the cell monolayer and of the transport systems. The amount of compound transported as a function of time in Caco-2 media was quantified by HPLC−MS. The P_{app} was obtained by standard methods from the linear regression analysis of the appearance rate of the compound in the receiver compartment and is expressed as 10^{-6} cm/s. The mass balance of compounds, calculated as the ratio of total detected quantities of analytes at the end of the Caco-2 experiment to that originally introduced in the transwell, was around 1, indicating good recovery.

In Vivo Experiments. All the experiments were performed in accordance with the Declaration of Helsinki, with the principles and guidelines of the European Union regulations and the local ethical committee. Male albino guinea pigs (Dunkin Hartley,

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 at a respiration volume of 10 mL/kg. The body temperature was kept constant a 36 °C by a thermoregulated lamp. The abdomen was opened, and a latex balloon, which was 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 hexamethonium bromide (13.8 μmol/kg, iv) as bolus followed by continuous infusion of the same solution at the rate of 300 μL/h to prevent reflex cholinergic responses. The compounds or their vehicle (DMSO) was administered iv (1−3 μmol/kg) in a volume of 100 μL/kg. For intraduodenal administration (id), the NK_2 tachykinin receptor antagonists (10 μmol/kg) were injected in a volume of 1 mL/kg into the proximal duodenum by a 26G needle syringe, at about 2 cm from the pyloric sphincter. [βAla^8]NKA(4−10) (3 nmol/kg, iv) was administered two or three times before the antagonist or the vehicle administration 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.

General Procedure for the Obtainment of 5a-e. [1-*R*-(3-Morpholin-4-yl-propylcarbamoyl)-2-phenylethyl]carbamic Acid *tert*-Butyl Ester (3). Boc-D-Phe-OSu (**2**) (10.42 g, 29 mmol) was added to a solution of 3-morpholine-4-ylpropylamine (4.14 g, 29 mmol) in THF (230 mL), and the resulting solution was stirred vigorously for 1 h at room temperature. Then it was left without stirring overnight. The solvent was evaporated, and the residue was dissolved in EtOAc and washed with saturated $NaHCO_3$. The organic phase was dried over sodium sulfate, then filtered and evaporated to dryness to give **3** as a white solid (11 g, 29 mmol, quantitative yield). ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm): 7.36−7.13 (m, 5H), 6.92 (broad s, 1H), 5.14−4.95 (m, 1H), 4.32−4.15 (m, 1H), 3.7−3.52 (m, 4H), 3.36−3.2 (m, 2H), 3.2−2.9 (m, 2H), 2.38−2.2 (m, 6H), 1.6−1.42 (m, 2H), 1.2 (s, 9H).

{[1-*R*-(3-Morpholin-4-yl-propylcarbamoyl)-2-phenylethylcarbamoyl]methyl}carbamic Acid *tert*-Butyl Ester (5a). A solution of HCl (4 M, 50 mL) in dioxane was added under nitrogen to **3** (11 g, 29 mmol), and the suspension was sonicated for 15 min and then magnetically stirred for 3 h at room temperature. The resulting suspension of the amine dihydrochloride was evaporated to dryness, and the resulting residue was treated with diethyl ether. After the dissolution−evaporation process with ethanol, the hygroscopic filtrate was dried under high vacuum, stored in a $CaCl_2$ capped flask, and used directly for the next reaction. To a solution of the amine dihydrochloride (0.38 g, 1 mmol) and Boc-Gly-OH (0.20 g, 1 mmol) in DMF (8 mL), EDC•HCl (0.22 g, 1.3 mmol), HOBt (0.2 g, 1.5 mmol), and after 5 min DIPEA (0.55 mL, 3 mmol) were added under magnetic stirring. After 2 days at room temperature the solvent was removed under vacuum and the residue was taken up in ethyl acetate and washed six times with $NaHCO_3$ saturated solution. The organic phase was then extracted three times with citric acid (10% aqueous solution), and the extract was slowly poured onto solid K_2CO_3 excess. The resulting alkaline solution was finally extracted three times with ethyl acetate, and the combined organic phase was dried over Na_2SO_4 and evaporated to give **5a** (0.254 g, 0.56 mmol). ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm): 7.49−7.07 (m, 7H), 5.87−5.57 (m, 1H), 4.70−4.46 (m, 1H), 3.89−3.72 (m, 2H), 3.73−3.50 (m, 4H), 3.33−3.12 (m, 2H), 3.12−2.96 (m, 2H), 2.50−2.08 (m, 6H), 1.63−1.47 (m, 2H), 1.42 (s, 9H).

In a similar way, intermediates **5b−e** were obtained.

General Procedure for the Obtainment of 8a,b. 1-(2-Nitrobenzenesulfonylamino)cyclopentanecarboxylic Acid (7a). 1-Aminocyclopentanecarboxylic acid (**6a**) (0.5 g, 3.8 mmol) was suspended in dichloromethane (15 mL), and BSA (containing 5% TMSCl, 1.8 mL, 7.2 mmol) was added. After 1 h of stirring, the solution was clear and a solution of 2-nitrobenzenesulfonyl chloride (0.84, 3.8 mmol) in dichloromethane was added dropwise. After the mixture was stirred for 1 night, the solvent was evaporated under vacuum and the residue treated with $NaHCO_3$ (5% aqueous solution, 25 mL) for 30 min. The aqueous phase was first extracted with ethyl acetate to eliminate the unreacted sulfonyl chloride and then acidified with 1 M HCl to precipitate the desired product. This was solubilized in ethyl acetate, washed with brine, and dried. After solvent removal, **7a** was obtained as a white solid (0.97 g, 3.01 mmol, 81% yield). HPLC: (A) 3.04 min. MS (ESI) *m/z:* 314.8 (MH^+). ^{1}H NMR (200 MHz, DMSO-d_6): δ (ppm) 8.41 (s, 1H), 7.79−8.10 (m, 4H), 2.05−1.75 (m, 4H), 1.65−1.30 (m, 4H).

1-(2-Nitrobenzenesulfonylamino)cyclopentanecarboxylic Acid [1(*R*)-Benzyl-2-(3-morpholin-4-ylpropylamino)ethyl]amide (8a). A solution of **7a** (0.905 g, 2.88 mmol) in DMF (6 mL) was treated with HOBT (1.16 g, 8.6 mmol) and EDC•HCl (0.61 mg, 3.2 mmol). After the mixture was stirred for 10 min, a solution of the amine dihydrochloride obtained from **3** (1.04 g, 2.87 mmol) in DMF (5 mL), as described in the first step of **5a**, and triethylamine (0.85 mL, 5.7 mmol) were added, and the resulting mixture was stirred for 16 h. After that time the solvent was evaporated. The residue was treated with aqueous $NaHCO_3$ (5%) and extracted with EtOAc. The organic phase was washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo to give **8a** (1.59 g, 94% yield). HPLC: (A) 3.81 min. MS (ESI) *m/z* 588.3 (MH^+). ^{1}H NMR (200 MHz, DMSO-d_6): δ (ppm) 10.4 (broad s, 0.5H), 8.5−7.6 (m, 8H), 7.4−7.05 (m, 5H), 4.6−4.3 (m, 1H), 4.1−3.5 (m, 4H), 3.3−2.7 (m, 10H), 2.1−0.8 (m, 10H).

In a similar way, the intermediate **8b** was obtained.

1-Methylaminocyclopentanecarboxylic Acid [1-(*R*)-(3-Morpholin-4-ylpropylcarbamoyl)-2-phenylethyl]amide (5f). Polymer-bound triphenylphosphine (2.24 g, 6.7 mmol, loading of 3 mmol/g) was suspended in dichloromethane (10 mL) and stirred for 0.5 h and then treated with a solution of compound **8a** (1.59 g, 2.7 mmol) in dichloromethane (7 mL). Finally, MeOH (0.27 mL, 6.7 mmol) and a solution of di-*tert*-butyl azodicarboxylate (1.2 g, 5.2 mmol) in dichloromethane (8 mL) were added. The mixture was stirred for 16 h. The reaction mixture was filtered and the resin washed with dichloromethane. The solution was treated with TFA (10 mL) and stirred for 2 h. After that time the solvent was evaporated. The residue was treated with K_2CO_3 (5%) to alkaline pH and then extracted with ethyl acetate. The organic phase was washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo to give the corresponding methylated product (1.39 g, 85% yield; HPLC, (A) 3.37 min, 89.4% purity; MS (ESI) *m/z*, 602.3 (MH^+)) that was directly dissolved in DMF (25 mL) and DBU (1.05 mL, 6.9 mmol). 2-Mercaptoethanol (0.47 mL, 6.9 mmol) was added to this solution. After being stirred for 2 h at room temperature, the reaction mixture was concentrated in vacuo, the residue was dissolved in EtOAc, and the crude product was extracted with 1 N HCl. The acid solution was treated with aqueous K_2CO_3 (5%) to alkaline pH, saturated with NaCl, and then extracted twice with EtOAc. The organic phase was dried over Na_2SO_4, filtered, and concentrated in vacuo to give **5f** (0.77 g, 68% yield). HPLC: (A) 2.3 min. MS (ESI) *m/z*: 417.4 (MH^+).

In a similar way, intermediate **5g** was obtained.

General Procedure for the Formation of Terminal Amide for the Obtainment of 10a−g, 12a−c, 14a−h. Benzo[*b*]thiophene-2-carboxylic Acid {[1-(*R*)-(3-Morpholin-4-ylpropylcarbamoyl)-2-phenylethylcarbamoyl]methyl}amide (10a). **5a** was deprotected with 2 mL of 4 M HCl in dioxane. After 2 h under magnetic stirring at room temperature, diethyl ether was added and the precipitate filtered. The solid was repeatedly treated with ethanol and evaporated, giving after drying 0.225 g (0.56 mmol, quantitative yield) of the deprotected compound (MS (APCI) *m/z*, (MH^+) 349.2). This (0.225 g, 0.56 mmol, considered as dihydrochloride) was suspended in dichloromethane (15 mL), and DIPEA (0.8 mL) was added. Benzo[*b*]thiophene-2-carbonyl chloride **9** (0.11 g, 0.56 mmol) was added, and the mixture was kept at room temperature

under stirring for 12 h. The precipitate was filtered and washed with dichloromethane, giving a first crop of **10a** as the free base (0.09 g, 0.17 mmol, 30% yield). HPLC: (B) 10.8 min, 99%. MS (ESI) *m/z*: 509.3 (MH^+). ^{1}H NMR (500 MHz, DMSO-d_6): δ (ppm) 9.02 (t, J = 5.8 Hz, 1H), 8.23 (d, J = 8.3 Hz, 1H), 8.10 (s, 1H), 8.02 (d, J = 7.5 Hz, 1H), 8.00 (d, J = 7.1 Hz, 1H), 7.92 (t, J = 5.6 Hz, 1H), 7.48−7.42 (m, J = 7.5 Hz, 2H), 7.25−7.15 (m, 5H), 4.46 (m, 1H), 4.19 (m, 4H), 3.93 (dd, J = 16.3/6.0 Hz, 1H), 3.80 (dd, J = 16.3/5.8 Hz, 1H), 3.18−3.01 (m, 2H), 2.98 (dd, J = 13.7−5.4 Hz, 1H), 2.81 (dd, J = 13.7−8.9 Hz, 1H), 2.28 (m, 4H), 2.20 (t, 7.2 Hz, 1H), 1.50 (m, 2H).

In a similar way, **10b**−**e** were obtained. Starting from **5f**,**g** by simple coupling with **9**, **10f**,**g** were obtained.

Products **12a**−**c** and **14a**−**h** were similarly obtained from the common precursor **11** (see Scheme 2).

General Procedure for the Obtainment of Ureas 13a−g. 1-[3-(4-Bromophenyl)ureido]cyclopentanecarboxylic Acid [1-(*R*)-(3-Morpholin-4-yl-propylcarbamoyl)-2-phenylethyl]amide Trifluoroacetate (13a). To a freshly prepared solution of 1-aminocyclopentanecarboxylic acid [1-(3-morpholin-4-ylpropylcarbamoyl)-2-phenylethyl]amide (**11**) (0.189 mmol) (obtained with the same methodology used for **5a** using 1-aminocyclopentanecarboxylic acid in dichloromethane), 4-bromophenyl isocyanate (37 mg, 0.187 mmol) was added, and the solution was heated at 40 °C for 3 h and then left at room temperature overnight. The solvent was then removed under reduced pressure, and the crude was purified through preparative HPLC (Jupiter column C_{18}, 300 Å, 250 mm × 21.20 mm, 15 μm, H_2O + 0.1% TFA, CH_3CN + 0.1% TFA, gradient 20−80% CH_3CN in 40 min), obtaining **13a** as a trifluoroacetate (52.5 mg, 0.07 mmol, 40% yield). HPLC: (B) 12.2 min, 99%. MS (ESI) *m/z*: 603.3 pattern for 1 Br (MH^+). ^{1}H NMR (500 MHz, DMSO-d_6): δ (ppm) 9.52 (broad s, 1H), 8.88 (s, 1H), 7.96 (d, J = 8.4 Hz, 1H), 7.89 (t, J = 5.6 Hz, 1H), 7.44 (d, J = 8.8 Hz, 2H), 7.36 (d, J = 8.8 Hz, 2H), 7.23−7.14 (m, 5H), 6.77 (s, 1H), 4.39 (m, 1H), 3.96 (d, J = 12.3 Hz, 2H), 3.62 (t, J = 12.3 Hz, 2H), 3.24 (m, 1H), 3.19 (dd, J = 13.9, 3.9 Hz, 1H), 3.1−2.95 (m, 3H), 2.91 (dd, J = 13.9, 10.9 Hz, 1H), 2.14 (m, 1H), 1.79 (m, 2H), 1.67−1.50 (m, 7H).

In a similar way, products **13b**−**g** were obtained.

General Procedure for the Obtainment of 21a−h. 1-[(Benzo-[*b*]thiophene-2-carbonyl)amino]cyclopentanecarboxylic Acid (15). To a stirred suspension of **6a** (10 g, 77.5 mmol) in dichloromethane (500 mL), BSA (35 mL, 140 mmol) was added. After 1.5 h the solution was clear and a solution of **9** in dichloromethane (200 mL) was added dropwise in 1.5 h. The resulting opalescent solution was kept at room temperature overnight and then evaporated to dryness. The residue was treated with K_2CO_3 (5% aqueous) and extracted with ethyl acetate. The aqueous phase was acidified with 10% HCl until complete precipitation of the carboxylic acid, which was filtered and recrystallized from acetonitrile to obtain yellowish crystals of **15** (17 g + 2 g (second crop), 66 mmol, 85% yield). HPLC: (C) 3.51 min. ^{1}H NMR (200 MHz, DMSO-d_6): δ (ppm) 12.3 (s, 1H), 8.81 (s, 1H), 8.22 (s, 1H), 8.05−7.9 (m, 2H), 7.50−7.37 (m, 2H), 2.23−1.97 (m, 4H), 1.82−1.61 (m, 4H). ^{13}C NMR (200 MHz, DMSO-d_6): δ (ppm) 175 (s, 1C), 161.45 (s, 1C), 140.1 (s, 1C), 139.7 (s, 1C), 139.1 (s, 1C), 126.1 (d, 1C), 125.3 (d, 1C), 125.0 (d, 1C), 124.8 (d, 1C), 122.65 (d, 1C), 65.6 (s, 1C), 36.3 (t, 2C), 24.1 (t, 2C).

2-Benzo[*b*]thiophen-2-yl-3-oxa-1-azaspiro[4.4]non-1-en-4-one (16). A partial suspension of **15** (16 g, 55 mmol) in THF (500 mL) was treated with EDC·HCl (12.7 g, 66 mmol) and 30 mL of DIPEA. After the mixture was stirred for 2 h at room temperature, 10 mmol of EDC·HCl and DIPEA were added. After an additional 3 h, the solvent was evaporated and the residue was dissolved in 1 L of ethyl acetate and washed with 10% aqueous citric acid solution and water. The organic phase was dried over Na_2SO_4, filtered, and evaporated to dryness. The formed white needles were suspended in a small amount of ethyl acetate and filtered to give 13 g of **16** (48 mmol, 88% yield), another crop was obtained by a second filtration from the mother liquor (1 g). HPLC: (C) 4.97 min, 95% purity, presence of **15** (4%). ^{1}H NMR (200 MHz, DMSO-d_6): δ (ppm) 8.14 (s, 1H), 8.11−7.97 (m, 2H), 7.59−7.42 (m, 2H), 2.18−1.68 (m, 8H). ^{13}C NMR (200 MHz, DMSO-d_6): δ (ppm) 182.6 (s, 1C), 157 (s, 1C), 142.2 (s, 1C), 140.4 (s, 1C), 131.1 (d, 1H), 129.9 (s, 1C), 129.0 (D, 1C), 127.4 (d, 1C), 127.2 (d, 1C), 124.7 (d, 1C), 76.5 (s, 1C), 39.8 (t, 2C), 27.1 (t, 2C).

2-(*R*)-Amino-*N*-(3-hydroxypropyl)-3-phenylpropionamide (18). Z-D-Phe-OSu **17** (5 g, 13 mmol) was dissolved in dimethoxyethane (DME, 50 mL), and the solution was treated with 1-amino-3-propanol (0.76 mL, 13 mmol) in DME (10 mL) and triethylamine (1.74 mL, 0.14 mmol). The turbid mixture was stirred for 2 h and then was diluted with water (100 mL), and the solution was extracted with ethyl acetate (2 × 50 mL). The combined extracts were dried over Na_2SO_4, and the solvent wasremoved under reduced pressure to give a waxy solid, which was further washed with cyclohexane. Drying by suction gave 4.05 g (11.7 mmol, 90% yield) of a white solid. HPLC: (A) 3.48 min. MS (ESI) *m/z*: 357.2 (MH^+). ^{1}H NMR (200 MHz, DMSO-d_6): δ (ppm) 7.94 (broad t, J = 5.3 Hz, 1H), 7.46 (broad d, J = 8.6 Hz, 1H), 7.35−7.10 (m, 10H), 4.93 (s, 2H), 4.4 (t, J = 5.3 Hz, 1H), 4.16 (ddd, H =, 10, 8.6, 4.7 Hz, 1H), 3.4−3.3 (m, 2H), 3.14−3.00 (m, 2H), 3.92 (dd, J = 13.6, 4.7 Hz, 1H), 2.72 (dd, 13.5, 8.6 Hz, 1H), 1.6−1.44 (m, 2H). The Z-protected derivative (4.18 g, 11.7 mmol) was dissolved in methanol (50 mL), and 1 N HCl (15 mL, 15 mmol) was added. The substrate was then hydrogenated in the presence of 10% Pd/C (0.452 g) under normal pressure. After 2 h, a check by TLC (ethyl acetate/hexane, 80/20) indicated that some precursor still remained as a higher running spot, so additional catalyst (0.22 g) was added and the hydrogenation continued for another 2 h, with the reaction then left to stand overnight. The mixture was then filtered off through a pad of Celite, and the filtrate was evaporated down under reduced pressure. The residue was treated with saturated sodium bicarbonate solution (100 mL) and lyophilized. The lyophilized product was taken up in methanol and the suspension filtered off to remove insoluble sodium salts. The resulting filtrate was evaporated down, and the remaining solid residue was washed with diethyl ether. This gave **18** (2.91 g, quantitative yield) as a white solid. ^{1}H NMR (200 MHz, DMSO-d_6): δ (ppm) 7.9−7.73 (m, 1H), 7.35−7.07 (m, 1H), 4.50−4.30 (m, 1H, CHα), 3.47−3.21 (m, 2H), 3.18−3.04 (m, 2H), 2.90 (component of ABX spin system, dd, J_{app} = 13.3, 5.2 Hz, 1H), 2.60 (component of ABX spin system, dd, J_{app} = 13.3, 8.1 Hz, 1H), 1.73 (broad s, 2H), 1.57−1.42 (m, 2H).

Benzo[*b*]thiophene-2-carboxylic Acid {1-[1-(3-Hydroxypropylcarbamoyl)-2-(*R*)-phenylethylcarbamoyl]cyclopentyl}-amide (19). The oxazolone **16** (1 g, 3.7 mmol) was dissolved in DMF (40 mL), and **18** (1.11 g, 5.6 mmol) added. The mixture was stirred at room temperature for 24 h. After this time the mixture was diluted with ethyl acetate (150 mL) and the solution washed with water (3 × 50 mL) and saturated sodium bicarbonate (2 × 50 mL). The organic extract was dried over sodium carbonate to give 1.51 g (83% yield) of material (**19**) as a foamy solid. HPLC: (C) 3.73 min, 94% purity. MS (ESI) *m/z*: 492.1 (MH^+). ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm) 8.88 (s, 1H), 8.30 (s, 1H), 8.04 (m, 1H), 7.98 (m, 1H), 7.83 (d, J = 8.3 Hz, 1H), 7.51 (t, J = 5.6 Hz, 1H), 7.46 (m, 2H), 7.11 − 7.19 (m, 5H), 4.44 (m, 1H), 4.39 (t, J = 5.2 Hz, 1H), 3.43 (m, 2H), 3.2−3.0 (m, 3H), 2.83 (dd, J = 13.9, 10.6 Hz, 1H), 2.22 (m, 1H), 1.9−1.4 (m, 9H).

4-[2-({1-[(Benzo[*b*]thiophene-2-carbonyl)amino]cyclopentanecarbonyl}amino)-3-(*R*)-phenylpropionylamino]propanal (20). The alcohol **19** (2.10 g, 4.26 mmol) was partially dissolved in dichloromethane/THF (40 mL/30 mL), and Dess−Martin periodinane (1.99 g, 4.72 mmol) in dichloromethane (15 mL) was added at room temperature. The turbid reaction mixture was left to stir for 2 h at room temperature, after which the mixture was poured into a saturated solution of sodium bicarbonate (50 mL) in which sodium thiosulphate (10.6 g) had been dissolved. The phases were shaken and separated. The aqueous fraction was then extracted further with chloroform (50 mL), and the combined organic extracts were dried over sodium sulfate. The solvent was removed under reduced pressure to give an oil, which was triturated with diethyl ether. The resulting solid was filtered off by suction to give 2.07 g (99% yield) of **20** as a pale-yellow amorphous solid. HPLC: (C)

3.9 min, 96% purity. MS (ESI) *m*/*z*: 492.1 (MH^+). ^1H NMR (200 MHz, DMSO-d_6): δ (ppm) 9.66 (s, 1H), 8.92 (s, 1H), 8.31 (s, 1H), 8.12−7.38 (m, 6H), 7.29−7.05 (m, 5H), 4.51−4.36 (m, 1H), 3.19−3.10 (m, 1H), 2.87−2.74 (m, 1H), 2.66−2.5 (m, 2H), 2.3−1.4 (m, 10H).

General Procedure for the Introduction of the Amine Functionality (21a−h). Benzo[*b*]thiophene-2-carboxylic Acid {1-[2-Phenyl-(*R*)-1-(3-thiomorpholin-4-ylpropylcarbamoyl)-ethylcarbamoyl]cyclopentyl}amide (21a). The aldehyde **20** (0.4 g, 0.8 mmol) was dissolved in methanol (10 mL), and the thiomorpholine (0.091 g, 4 mmol) was added together with glacial acetic acid (0.8 mL). The solution was stirred for 15 min, and then sodium cyanoborohydride (0.1 g, 0.9 mmol) was added. After the mixture was stirred for at least 4 h, the methanol was stripped off under reduced pressure. The residue was dissolved in 1 M HCl (50 mL) and the solution stirred for 10 min to destroy excess reducing reagent. The aqueous fraction was extracted with ethyl acetate (25 mL). Drying of this organic extract over sodium sulfate and removal of the solvent under reduced pressure gave the crude material that was purified by preparative HPLC (column Combi HT, SB C18, 5 mm, 100 Å, 21 mm × 50 mm), eluting according to the following parameters: H_2O + 0.1% TFA/CH_3CN + 0.1% TFA, 95/5 → 5/95 in 10 min, flow rate 40 mL/min, λ = 220, 270 nm. This gave after lyophilization 80 mg (17% yield) of **21a**. HPLC: (C) 3.63 min, 97%. MS (ESI) *m*/*z*: 579.3 (MH^+). ^1H NMR (500 MHz, DMSO-d_6): δ (ppm) 8.89 (s, 1H), 8.30 (s, 1H), 8.03 (m, 1H), 7.98 (m, 1H), 7.84 (d, *J* = 8.6 Hz, 1H), 7.45−7.50 (m, 3H), 7.12−7.20 (m, 5H), 4.44 (m, 1H), 3.18 (dd, *J* = 13.9−4.1 Hz, 1H), 3.07 (m, 2H), 2.83 (dd, *J* = 13.9, 10.7 Hz, 1H), 2.57 (m, 8H), 2.30 (t, *J* = 7.2 Hz, 4H), 2.24 (dt, *J* = 13.2, 8.1 Hz, 1H), 1.91 (m, 1H), 1.79 (m, 1H), 1.71−1.44 (m, 7H).

In a similar way, products **21b−h** were obtained.

General Procedure for the Obtainment of 25a−f. 4-(2-(*R*)-Amino-3-phenylpropionylamino)butyric Acid (23a). γ-Aminobutyric acid (GABA) (**23a**) (1.42 g, 13.8 mmol) was suspended in acetonitrile (75 mL), and triethylamine (1.92 mL, 13.8 mmol) was added. The mixture was stirred until a turbid solution was formed. Then a solution of Boc-D-Phe-OSu (**1**) (5 g, 13.8 mmol) in acetonitrile (50 mL) was added. The mixture was stirred for 30 min. Then water (50 mL) was added and the stirring was prolonged for further 30 min. Then the mixture was evaporated down under reduced pressure to remove acetonitrile and the aqueous residue neutralized to pH 6−7 with 1 N HCl and extracted with ethyl acetate (2 × 100 mL). The organic extract was dried over Na_2SO_4 and evaporated to dryness. The resulting white solid was then washed with diethyl ether and dried by suction to give 3.53 g (10 mmol, 73% yield) of material as a white powder. HPLC: (A) 3.19 min. MS (APCI) *m*/*z*: 350.9 (MH^+). The Boc protected product (3.5 g, 10 mmol) was dissolved in dichloromethane (125 mL) with addition of acetonitrile (40 mL) as a cosolvent to complete the dissolution of the substrate. Then 4 N HCl in dioxane (28.6 mL, 114 mmol) was added. After 4 h at room temperature the suspension was evaporated to dryness, the residue taken up in ethyl acetate, and the supernatant decanted. The decanting process was repeated with diethyl ether. The residue was taken up in water (120 mL) and the solution lyophilized, giving 2.92 g of **23a** as a hydrochloride (10 mmol, quantitative yield) as a resinous white solid. MS (APCI) *m*/*z*: 251.1 (MH^+). ^1H NMR (200 MHz, $CDCl_3$): δ (ppm) 8.59 (broad t, *J* = 5.1 Hz, 1H), 8.4 (broad s, 3H), 7.34−7.18 (m, 5H), 3.96 (m, 1H), 3.18−2.9 (m, 4H), 2.20−2.02 (m, 2H), 1.61−1.45 (m, 2H).

In a similar way, replacing GABA with β-alanine, product **23b** was obtained with similar yield.

4-[2-(*R*)-({1-[(Benzo[*b*]thiophene-2-carbonyl)amino]cyclopentanecarbonyl}amino)-3-phenylpropionylamino]butyric Acid (24a). Oxazolone **16** (1 g, 3.86 mmol) was dissolved in DMF (15 mL), and **23a** (1.55 g, 5.4 mmol) was added with DIPEA (1.2 mL). The mixture was stirred at room temperature overnight and then was diluted with ethyl acetate (50 mL), and the solution was washed with water (50 mL) and $KHSO_4$ (5% aqueous solution, 50 mL). Drying of the organic fraction with Na_2SO_4 and removal of the solvent under reduced pressure gave 1.72 g (3.3 mmol, 85% yield) of **24a** as a glass. HPLC: (A) 3.98 min. MS (APCI) *m*/*z*: 522.0 (MH^+). ^1H NMR (200 MHz, DMSO-d_6): δ (ppm) 12.04 (broad s, 1H), 8.9 (broad s, 1H), 8.31 (s, 1H), 8.08−7.96 (m, 2H), 7.85 (broad d, *J* = 8.5 Hz, 1H), 7.57 (broad t, *J* = 5.2 Hz, 1H), 7.50−7.40 (m, 2H), 7.25−7.08 (m, 5H), 4.56−4.34 (m, 1H), 3.25−3.01 (m, 3H), 2.8−2.5 (m, 1H), 2.36−2.16 (m, 2H), 2−1.4 (m, 10H).

In a similar way, replacing GABA with β-alanine, product **24b** (4-[2-(*R*)-({1-[(benzo[*b*]thiophene-2-carbonyl)amino]-cyclopentanecarbonyl}amino)-3-phenylpropionylamino]propionic acid) was obtained in similar yield.

General Procedure for the Formation of the Terminal Amide Bond (25a−f). Benzo[*b*]thiophene-2-carboxylic Acid (1-{1-(*R*)-[4-(4-Morpholin-4-ylpiperidin-1-yl)-4-oxobutylcarbamoyl]-2-phenylethylcarbamoyl}cyclopentyl)amide (25a). The carboxylic acid **24a** (0.1 g, 0.192 mmol) was dissolved in DMF (5 mL), and HOBt (0.03 g, 0.22 mmol) and EDC·HCl (0.096 g, 0.50 mmol) were added. The solution was stirred for 15 min, and then 4-piperidin-4-ylmorpholine dihydrochloride (46 mg, 0.18 mmol) and triethylamine (0.053 mL) were added. After being stirred at room temperature for 4 h, the mixture was diluted with EtOAc (25 mL) and the solution washed with saturated aqueous $NaHCO_3$ (20 mL) and water (20 mL). The extract was dried over sodium sulfate and the solvent removed under reduced pressure. The resulting residue was triturated with diethyl ether to facilitate the solidification. After filtration the product **25a** (86 mg, 67% yield) was reanalyzed by HPLC and was found to be sufficiently pure (>90%) such that any further chromatography was not performed. HPLC: (C) 3.33 min. MS (ESI) *m*/*z*: 674.2 (MH^+). ^1H NMR (500 MHz, DMSO-d_6): δ (ppm) 8.90 (s, 1H), 8.30 (s, 1H), 8.03 (m, 1H), 7.98 (m, 1H), 7.85 (d, *J* = 8.5 Hz, 1H), 7.55 (m, 1H), 7.46 (m, 1H), 7.20−7.11 (m, 5H), 4.42 (m, 1H), 4.36 (m, 1H), 3.81 (m, 1H), 3.51 (m, 4H), 3.17 (hidden dd, 1H), 3.16 (m, 1H), 3.05 (m, 1H), 2.90 (m, 1H), 2.85 (dd, *J* = 13.9, 10.7 Hz, 1H), 2.41−2.15 (m, 7H), 2.2 (m, 1H), 1.91 (m, 1H), 1.80 (m, 1H), 1.73−1.45 (m, 11H), 1.21 (m, 2H).

Products **25b−f** were obtained with a parallel and similar approach.

General Procedure for the Obtainment of Products 30a−e. 4-(2-(*R*)-Amino-3-phenylpropionylamino)piperidine-1-carboxylic Acid *tert*-Butyl Ester (28a). Z-Phe-OH (**26**) (3.28 g, 11 mmol) was dissolved in DMF (30 mL), and subsequently HOBt (4.4 g, 33 mmol), EDC·HCl (2.3 g, 12 mmol), 1-Boc-4-aminopiperidine hydrochloride (**27a**) (2.6 g, 11 mmol), and triethylamine (1.6 mL, 11 mmol) were added. The resulting mixture was allowed to stand overnight at room temperature. Then another portion of EDC·HCl (1.15 g, 6 mmol) was added to complete the conversion. The solvent was removed under reduced pressure and the residue treated with 5% $NaHCO_3$ and extracted with ethyl acetate. The organic phase was washed with brine, 5% $KHSO_4$, and brine and then dried over anhydrous sodium sulfate. The filtrate was evaporated under reduced pressure to dryness to give 5 g (94% yield) of a white solid that was directly deprotected. HPLC: (A) 4.46 min. MS (ESI) *m*/*z*: 481.9 (MH^+). ^1H NMR (200 MHz, DMSO-d_6): δ (ppm) 7.91 (d, *J* = 7.6 Hz, 1H), 7.46 (d, J = 8.5 Hz, 1H), 7.4−7.1 (m, 10H), 4.94 (s, 2H), 4.3−4.1 (m, 1H), 3.9−3.6 (m, 3H), 3.0−2.6 (m, 4H), 1.75−1.5 (m, 2H), 1.39 (s, 9H), 1.3−1.0 (m, 2H). An amount of 2.59 g (5.38 mmol) of the product was dissolved in MeOH (80 mL), and a solution of 10% Pd/C (0.25 g) in MeOH/H_2O (9:1 v/v) was added under nitrogen atmosphere. Then the substrate was maintained under hydrogen atmosphere at normal pressure for 2 h, after which the volume of the absorbed hydrogen reached the theoretical quantity (120 mL). The solution was filtered and washed with MeOH/H_2O (9:1 v/v) and evaporated under reduced pressure giving **28a** (1.69 g, 4.87 mmol, 90% yield) as a solid. HPLC: (A) 3.22 min. MS (ESI) *m*/*z*: 348.0 (MH^+). ^1H NMR (200 MHz, DMSO-d_6): δ (ppm) 7.68 (d, *J* = 7.8 Hz, 1H), 7.4−7.1 (m, 5H), 3.9−3.6 (m, 3H), 3.5−3.1 (m, 3H), 2.95−2.55 (m, 4H), 1.75−1.5 (m, 2H), 1.39 (s, 9H), 1.3−1.0 (m, 2H).

Benzo[*b*]thiophene-2-carboxylic Acid {1-[2-Phenyl-1-(*R*)-(piperidin-4-ylcarbamoyl)ethylcarbamoyl]cyclopentyl}amide (29a).

The amino derivative **28a** (1.69 g, 4.9 mmol) and the oxazolone **16** (1.29 g, 4.76 mmol) were dissolved in DMF (35 mL), and triethylamine (0.7 mL, 5 mmol) was added. The resulting mixture was stirred for 1 h at room temperature and then was left to stand overnight. The solvent was removed under reduced pressure and the residue treated with 5% $NaHCO_3$ solution and ethyl acetate, maintaining the emulsion under vigorous stirring for 30 min. The organic phase was then washed with brine and evaporated to dryness to give, in quantitative yield, the Boc-protected adduct that was directly dissolved in dichloromethane (75 mL) to perform the deprotection step. To the resulting solution was added TFA (25 mL), and stirring was continued for 1 h. The solvent was evaporated, and the residue was taken up with 5% $NaHCO_3$ and solid $NaHCO_3$ and extracted with ethyl acetate (600 mL) and $CHCl_3$ (1000 mL). The chloroform extract was washed with brine and dried. Then the solvent was removed to give 1.9 g (3.6 mmol, 76% yield) of **29a** that was directly used for the final step. A small amount was purified by preparative HPLC (Jupiter column C_{18}, 300 Å, 250 mm × 21.20 mm, 15 μm, H_2O + 0.1% TFA, CH_3CN + 0.1% TFA, gradient 20−80% CH_3CN in 40 min, flow rate of 20 mL/min) to give the corresponding trifluoroacetate that was fully characterized. HPLC: (C) 3.29 min. MS (ESI) *m/z*: 519.2 (MH^+). ^1H NMR (500 MHz, DMSO-d_6): δ (ppm) 8.89 (s, 1H), 8.51 (broad s, 1H), 8.28 (broad s, 1H), 8.31 (s, 1H), 8.00 (m, 2H), 7.85 (d, *J* = 8.3 Hz, 1H), 7.56 (d, *J* = 7.3 Hz, 1H, NH), 7.48 (m, 2H), 7.20−7.10 (m, 5H), 4.41 (m, 1H), 3.85 (m, 1H), 3.29 (broad hidden m, 1H), 3.16 (dd, *J* = 13.9, 4.2 Hz, 1H), 3.02 (broad m, 2H), 2.83 (dd, *J* = 13.9, 10.5 Hz, 1H), 2.22 (m, 1H), 1.95−1.72. (m, 5H), 1.71−1.45 (m, 6H).

In a similar way, starting from 1-Boc-4-aminomethyl-piperidine (**27b**) and 1-Boc-4-aminoethylpiperidine (**27c**), the corresponding intermediates **29b**,**c** were obtained.

Benzo[*b*]thiophene-2-carboxylic Acid (1-{2-Phenyl-1-(*R*)-[1-(tetrahydropyran-4-ylmethyl)piperidin-4-ylcarbamoyl]ethylcarbamoyl}cyclopentyl)amide (30a). To a solution of **29a** (0.2 g, 0.4 mmol) in anhydrous MeOH (10 mL) were added glacial acetic acid (0.15 mL), tetrahydropyranyl-4-carboxyaldehyde (0.057 g, 0.5 mol), and finally $NaCNBH_3$ (0.032 g, 0.5 mmol). The mixture was stirred for 1 h and then allowed to stand overnight. HCl, 1 M (1 mL), was added, and after the mixture was stirred for 30 min, the methanol was evaporated. The residue was diluted to 5 mL with acetonitrile and directly purified by preparative HPLC (Luna, C-8(2), 15 μm, 250 mm × 21.2 mm, H_2O + 0.1% TFA, CH_3CN + 0.1% TFA, gradient 30−60% CH_3CN in 30 min, flow rate of 20 mL/min). After lyophilization of the collected pure fractions, 80 mg (0.13 mmol, 35% yield) of **30a** were obtained. HPLC (C) = 3.44 min (99%). MS (ESI) *m/z*: 617.3 (MH^+). ^1H NMR (400 MHz, DMSO-d_6): δ (ppm) 8.84 (s, 1H), 8.30 (s, 1H), 7.98 (m, 1H), 7.97 (m, 1H), 7.82 (d, *J* = 8.5 Hz, 1H), 7.47 (m, 1H), 7.36 (d, *J* = 7.7, 1H), 7.20−7.10 (m, 5H), 4.43 (m, 1H), 3.84 (m, 2H), 3.49 (m, 1H), 3.17 (dd, 13.9, 4.1 Hz, 1H), 2.84 (dd, *J* = 13.9, 10.6 Hz, 1H), 2.78 (broad s, 2H), 2.22 (dt, *J* = 13.3, 8.0 Hz, 1H), 1.95−1.45 (m, 17H), 1.13 (m, 2H).

With an analogous procedure and the corresponding carbonyl compounds, products **30b**−**e** were obtained.

General Procedures for the Obtainment of Methyl Esters 33a−d and 36a−e. 4-Chlorobenzo[*b*]thiophene-2-carboxylic Acid Methyl Ester (33a). To a solution of 6-nitro-2-chlorobenzaldehyde (**31a**) (2.78 g, 15 mmol) and anhydrous K_2CO_3 (2.5 g, 18.1 mmol) in anhydrous DMF (30 mL), mercaptoacetic acid methyl ester **32** (1.36 mL, 15 mmol) was added slowly at 0 °C. The reaction mixture was stirred at 0 °C for further 30 min and then was maintained at room temperature (without stirring overnight) for 40 h. Then the mixture was poured into ice/water (400 mL) under vigorous stirring. The resulting solid was filtered, washed with water, and allowed to dry in air. The crude solid was finally crystallized from ethanol (96%) to give pure **33a** (2.43 g, 71% yield). HPLC: (B) 19.2 min. ^1H NMR (200 MHz, DMSO-d_6): δ (ppm) 8.12−8.05 (m, 2H), 7.63−7.51 (m, 2H), 3.92 (s, 3H).

With the same method, intermediate **33b** was obtained.

6-Chlorobenzo[*b*]thiophene-2-carboxylic Acid Methyl Ester (33c). To solution of 4-chloro-2-fluorobenzaldehyde (**32c**) (2.31 g, 14.6 mmol) in DMSO (20 mL), mercaptoacetic acid methyl ester (1.5 mL, 16.5 mmol) and triethylamine (6 mL) were added. The resulting mixture was stirred under nitrogen atmosphere at 80 °C for 2 h and then allowed to cool to room temperature and poured into 800 mL of ice/water under vigorous stirring. After 1 h of digestion the formed solid was filtered, washed with water, and dried by suction. Crystallization from ethanol gave pure **33c** (2.45 g, 10.8 mmol, 74% yield). ^1H NMR (200 MHz, DMSO-d_6): δ (ppm) 8.28−8.21 (m, 2H), 8.08−8.02 (m, 1H), 7.56−7.50 (m, 1H), 3.90 (s, 3H).

By analogy, the regioisomer **33d** was obtained. Methyl esters **36a**−**e** were also obtained according to this methodology.

General Procedure for the Obtainment of Products 34a−e. 4-Chlorobenzo[*b*]thiophene-2-carboxylic Acid {1-[1-(*R*)-(3-Morpholin-4-yl-propylcarbamoyl)-2-phenylethylcarbamoyl]cyclopentyl}amide (34a). 4-Chlorobenzo[*b*]thiophene-2-carboxylic acid methyl ester (**33a**) (1.4 g, 6.2 mmol) was dissolved in MeOH (200 mL), and K_2CO_3 aqueous solution (2.78 g in 200 mL) was added rapidly. The suspension was stirred for 5 h and allowed to stand overnight. After an additional 6 h under vigorous agitation, the resulting clear solution (HPLC (B) = 14.6 min) was acidified with concentrated HCl and the precipitate filtered, washed with water, and dried. A portion of the obtained carboxylic acid (0.21 g, 1 mmol) was suspended in anhydrous dichloromethane (12 mL) and $(COCl)_2$ (0.1 mL, 1.15 mmol), and 1 drop of DMF was added. After the mixture was stirred for 8 h and stood for one night, the solvent was removed under reduced pressure and the residue dried under high vacuum and then directly added (0.093 g) to a stirred solution of the amine **11** (0.14 g, 0.3 mmol) and triethylamine (0.2 mL) in anhydrous dichloromethane (10 mL). The resulting mixture was stirred at room temperature for 5 h, and then the solvent was removed under reduced pressure. Ethyl acetate was added to the residue, and the organic solution was treated repeatedly with 10% aqueous K_2CO_3, dried over Na_2SO_4, and evaporated to dryness. The crude (0.158 g) was purified by FCC (eluent, 100 mL of ethyl acetate, then ethyl acetate/MeOH, 6/1, 300 mL), giving **34a**. HPLC: (B) 13.79 min (96%). MS (ESI): *m/z*: 597.3 (MH^+). ^1H NMR (500 MHz, DMSO-d_6): δ (ppm) 9.09 (s, 1H), 8.47 (s, 1H), 8.03 (d, *J* = 8.1 Hz, 1H), 7.89 (d, *J* = 8.6, 1H), 7.56 (d, *J* = 7.7 Hz, 1H), 7.48 (t, *J* = 7.9 Hz, 1H), 7.44 (t, *J* = 5.6 Hz, 1H), 7.20−7.11 (m, 5H), 4.44 (m, 1H), 3.52 (t, *J* = 4.6 Hz, 4H), 3.18 (dd, *J* = 13.9, 4.1 Hz, 1H), 3.09 (m, 2H), 2.83 (dd, *J* = 13.9, 10.8 Hz, 1H), 2.30−2.20 (m, 5H), 2.25 (t, *J* = 7.3 Hz, 2H), 1.92 (m, 1H), 1.79 (m, 1H), 1.70−1.46 (m, 7H).

In a similar way, products **34b**−**d** were obtained and purified. Starting from the commercially available 3-chlorobenzothiophene-2-carboxy derivative, product **34e** was similarly obtained.

3-Amino-6-cyanobenzo[*b*]thiophene-2-carboxylic Acid Methyl Ester (38). 2-Nitroterephthalonitrile (**37**, 1.73 g, 10 mmol) was dissolved in DMSO (20 mL), and triethylamine (5 mL) and mercaptoacetic acid methyl ester (1 mL, 10 mmol) were subsequently added. The resulting mixture was warmed to 110 °C for 2 h and then was left to cool to room temperature and poured into water (350 mL) under vigorous stirring. The formed suspension was stirred for another 30 min and then filtered, washed with water, and dried. **38** (2.1 g, 90% yield) was obtained as a greenish solid. ^1H NMR (200 MHz, DMSO-d_6): δ (ppm) 8.48 (broad s, 1H), 8.31 (d, *J* = 8.4 Hz, 1H), 7.79 (broad d, *J* = 8.5 Hz, 1H), 3.81 (s, 3H).

6-Cyanobenzo[*b*]thiophene-2-carboxylic Acid Methyl Ester (39). To a stirred solution of *tert*-butyl nitrite (1.60 mL, 12 mmol) in DMF (20 mL) kept at 50−57 °C under nitrogen atmosphere, a solution of **38** (2.06 g, 8.9 mmol) in DMF (27 mL) was slowly added (30 min). The reaction time was prolonged for another 30 min at 50 °C, and then the solution was cooled and diluted with 300 mL of diethyl ether. The organic phase was washed with HCl (20%) and brine and then dried over magnesium sulfate and evaporated to dryness to give a pitchy solid. Crystallization from ethanol and flash chromatography (cyclohexane/dichloromethane, 1:1) gave 0.49 g (25% yield) of **39** as a white solid. ^1H NMR (200

MHz, $CDCl_3$): δ (ppm) 8.20 (broad s, 1H), 8.10 (s, 1H), 7.97 (d, J = 8.3 Hz, 1H), 7.63 (dd, J = 8.1, 1.2 Hz, 1H), 3.98 (s, 3H).

6-Nitrobenzo[*b*]thiophene-2-carboxylic Acid Methyl Ester (41). To a solution of 2,4-dinitrobenzaldehyde (**40**) (11.67 g, 59 mmol) in 70 mL of DMSO, an amount of 20 mL (144 mmol) of triethylamine and mercaptoacetic acid methyl ester (5.5 mL, 60 mmol) was subsequently added (exothermic reaction). After the mixture was stirred for 4 h at room temperature, a precipitate was observed. After 64 h, the mixture was warmed to 110 °C for 1 h. The precipitated yellow solid (**41**), accumulated by cooling, was filtered, washed with DMSO and water, and dried (6.07 g, 25.6 mmol, 43% yield). ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 8.81 (d, J = 1.9 Hz, 1H), 8.27 (dd, J = 8.8, 2.0 Hz, 1H), 8.14 (s, 1H), 8.0 (d, J = 8.8 Hz, 1H), 3.99 (s, 3H).

6-Diethylaminobenzo[*b*]thiophene-2-carboxylic Acid Methyl Ester (42). 41 (2.37 g, 10 mmol) was added to a suspension of hydrogen-reduced iron (2.25 g, 40 mmol) in 45 mL of H_2O at 60 °C together with 4 mL of hydrochloric acid (37%). MeOH (50 mL) was added. After 5 h at reflux, the suspension was filtered and the solid was treated with 85 mL of diluted HCl (0.5 M) at reflux. The pH of the hot filtered solution was then adjusted to about 6. The resulting suspension was filtered and the solid washed with water and dried on the bench overnight. The solid was treated with dichloromethane in an ultrasound bath twice and filtered. The collected organic solution was dried over a phase separator and evaporated giving 705 mg (34% yield) of the 6-aminobenzo[*b*]-thiophene-2-carboxylic acid methyl ester (HPLC (D) = 7.8 min) that was directly used for the following step. A portion of the amino derivative (0.28 g, 1.35 mmol) was suspended in acetonitrile (80 mL), and ethyl iodide (2.35 mL, 29 mmol) and potassium carbonate (1.01 g, 7.3 mmol) were added during about 50 h at reflux. The solvent was evaporated and the residue was dissolved in ethyl acetate, washed with saturated $NaHCO_3$, dried, and evaporated to give **42** as an oil (177 mg, 50% yield). HPLC: (D) 9.5 min ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 7.87 (s, 1H), 7.62 (d, J = 8.9 Hz, 1H), 6.95 (d, J = 2.25 Hz, 1H), 6.81 (dd, J = 8.9, 2.3 Hz, 1H), 3.89 (s, 3H), 3.40 (q, J = 7.1 Hz, 4H), 1.19 (t, J = 7.1 Hz, 6H).

Tetrahydropyran-4-carbonyl Chloride (44). The ester **43** (10 mL, 74 mmol) was rapidly dropped into a 1 N sodium hydroxide solution (60 mL, 60 mmol), and NaOH was added until the pH was permanently alkaline. The solution was kept at room temperature for 30 min more and extracted three times with dichloromethane using IST columns. The aqueous phase was then acidified with concentrated HCl (37%) until pH 2 was attained and repeatedly extracted with dichloromethane (5 × 40 mL) using the same separation method. The collected organic phase was directly evaporated to dryness, yielding the carboxylic acid as a white solid (7.83 g, 60 mmol, 80% yield). To a stirred solution of the carboxilic acid (2 g, 15.4 mmol) in dichloromethane, oxalyl chloride (2 mL, 23.6 mmol) and a catalytic amount of DMF (2 drops) were added subsequently. The mixture, outgassing with regularity, was kept at room temperature for 90 min and then evaporated to dryness under reduced pressure to give **44** as a whitish residue. ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 4.18−3.80 (m, 2H), 3.66−3.22 (m, 2H), 3.15−2.76 (m, 1H), 2.25−1.57 (m, 4H). ^{13}C NMR (200 MHz, $CDCl_3$): δ 175.6 (s, 1C), 66.5 (t, 2C), 51.8 (d, 1C), 28.9 (t, 2C).

1-(Tetrahydropyran-4-carbonyl)piperidine-4-carboxylic Acid Amide (45). Acyl chloride **44** (2.30 g, 15.4 mmol) in 20 mL of dichloromethane was added dropwise to a solution of isonipecotamide (1.97 g, 15.4 mmol) in DMF (20 mL) and triethylamine (5 mL). The reaction mixture was left at room temperature without stirring during the night and then evaporated under reduced pressure. The slurry residue was treated with 10 mL of sodium hydroxide (1 M) and extracted with dichloromethane (18 × 20 mL). The collected extracts gave after evaporation 2.6 g (10.8 mmol, 70% yield) of the diamide **45** as a whitish solid. ^{1}H NMR (200 MHz, DMSO-d_6): δ (ppm) 7.52−7.13 (broad s, 1H), 7.03−6.62 (broad s, 1H), 4.54−4.19 (m, 1H), 4.10−3.67 (m, 3H), 3.53−3.21 (m, 2H), 3.15−2.72 (m, 2H), 2.69−2.43 (m, 1H), 2.43−2.21 (m, 1H), 1.89−1.11 (m, 8H).

***C*-[1-(Tetrahydropyran-4-ylmethyl)piperidin-4-yl]methylamine (46).** The diamide **45** (0.5 g, 2 mmol) was dissolved in THF (20 mL), and lithium aluminum hydride (1 M in THF, 6.6 mL, 6.6 mmol) was added via syringe. After 10 min the mixture was warmed and kept at reflux for 4 h. After cooling down to room temperature, the mixture was quenched at 0 °C by sequential addition of water (0.25 mL), 15% NaOH (0.25 mL), and then water (0.73 mL). The obtained suspension was filtered by suction and the solid washed with more THF. The organic filtrates were combined and evaporated under reduced pressure, yielding the free diamine **46** (0.43 g, 2.05 mmol, 98% yield) as a viscous oil from which crystals separated in air. MS (ESI) *m/z*: 213.1 (MH^+). ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 3.83−4.01 (m, 2H), 3.22−3.45 (m, 2H), 2.73−2.93 (m, 2H), 2.44−2.61 (m, 2H), 2.04−2.21 (m, 2H), 1.49−1.95 (m, 6H), 0.98−1.36 (m, 6H).

1-Aminocyclopentanecarboxylic Acid (2-Phenyl-1-(*R*)-{[1-(tetrahydropyran-4-ylmethyl)piperidin-4-ylmethyl]carbamoyl}-ethyl)amide (47). The diamine **46** (9.35 g, 44 mmol) was dissolved in anhydrous THF (300 mL) and the resulting solution cooled to 0 °C. Boc-D-Phe-OSu (16 g, 44 mmol) was added in portions. The mixture was then brought up to room temperature. After 36 h, potassium carbonate solution (10% w/w in water, 100 mL) was added, and after the mixture was stirred for 1 h, the organic phase was diluted with ethyl acetate, separated, and washed with more carbonate (five times) and brine. Filtration of the organic phase through an IST phase separator column and its evaporation gave 19.9 g of crude product that was then recrystallized from boiling ethyl acetate (160 mL) to yield, after filtration by suction, the Boc-protected adduct (16.9 g, 36.8 mmol, 83% yield) as a white solid. HPLC: (D) 10.3 min, >99% purity. ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 7.04−7.46 (m, 5H), 5.59−5.99 (m, 1H), 4.84−5.25 (broad s, 1H), 4.15−4.36 (m, 1H), 3.73−4.14 (m, 2H), 3.20−3.51 (m, 2H), 2.86−3.20 (m, 4H), 2.71−2.86 (m, 2H), 1.96−2.29 (m, 2H), 1.40 (s, 9H), 0.97−1.91 (m, 12H). Hydrogen chloride in dioxane (18 mL, 4 M, 72 mmol) was rapidly added to a solution of the obtained product (3.56 g, 7.7 mmol) in dioxane (190 mL) under magnetic stirring. During 20 h at room temperature under stirring, a crystalline solid appeared in the suspension. The suspension was diluted with diethyl ether (250 mL) and stirred for 3 h. The solid was filtered by suction and allowed to dry in air. The obtained amine dihydrochloride (3.86 g, quantitative yield) was stored as a nonhygroscopic white solid or used directly as follows. To a solution of 1-*tert*-butoxycarbonylaminocyclopentanecarboxylic acid (2.06 g, 8.98 mmol) in 26 mL of dry DMF under nitrogen, EDC·HCl (1.78 g, 9.28 mmol) and HOBt (1.23 g, 9.10 mmol) were added. The mixture was stirred for 40 min at room temperature. Afterward, the diamine dihydrochloride (3.35 g, 7.75 mmol) and DIPEA (1.5 mL, 8.83 mmol) were added. After 5 h of being stirred, the mixture was left without stirring for 16 h and then concentrated under reduced pressure. The residue was treated with ethyl acetate and $NaHCO_3$ saturated in water. The organic phase was separated, washed several times with $NaHCO_3$ saturated in water, and then extracted with 10% citric acid in water. The combined aqueous extracts were added to an excess of solid $NaHCO_3$ with caution. The resulting basic solution was extracted with ethyl acetate, and the organic extracts were dried on Na_2SO_4 anhydrous for 2 days. Afterward, the mixture was filtered and the solvent was evaporated to yield 3.85 g of a foamy solid (87% yield) that was directly dissolved in 32 mL of dioxane under nitrogen. An amount of 15 mL of 4 M HCl in dioxane was added under magnetic stirring. The mixture was stirred for 5 h, and then diethyl ether (220 mL) was added. The mixture was stirred for 6 h again and the white solid was filtered by suction, washed with diethyl ether, and dried in air to give **47** as a dihydrochloride (4.15 g, quantitative yield). ^{1}H NMR (200 MHz, DMSO-d_6): δ (ppm) 10.45−10.06 (broad s, 1H), 8.71−8.54 (m, 1H), 8.52−8.37 (m, 1H), 8.39−8.2 (broad s, 3H), 7.41−7.08 (m, 5H), 5.31−4.9 (m, 2H), 4.65−4.39 (m, 1H), 3.91−3.71 (m, 2H), 3.52−2.65 (m, 10H), 2.29−1.95 (m, 2H), 1.93−1.39 (m, 14H), 1.35−1.01 (m, 2H).

General Procedure for the Obtainment of the Final Products 48a−h. 6-Chlorobenzo[*b*]thiophene-2-carboxylic Acid [1-(2-

Phenyl-1(*R*)-{[1-(tetrahydropyran-4-ylmethyl)piperidin-4-ylmethyl]carbamoyl}ethylcarbamoyl)ciclopentyl]amide (48a). The dihydrochloride of the amine **47** (0.17 g, 0.3 mmol) was dissolved in dichloromethane under nitrogen, and triethylamine (0.3 mL) was added. A solution of acyl chloride (**33c**, 0.05 mmol) obtained from the methyl ester **32c** as previously described was added, and the solution was stirred for 7 h at room temperature. The solvent was removed under reduced pressure, and the residue was treated with ethyl acetate and K_2CO_3 solution (10%) for 3 h under vigorous stirring. A solid separated at the interface and was filtered. The organic phase was then washed with additional K_2CO_3 solution (10%) and dried with Na_2SO_4. The filtrate was evaporated to dryness and purified by flash chromatography (eluent, ethyl acetate, 130 mL, then ethyl acetate methanol, 3/1, 350 mL), while the insoluble solid was dissolved in dichloromethane and washed with K_2CO_3 solution (10%), dried over anhydrous Na_2SO_4, filtered, and evaporated to dryness. The obtained pure material was added to that obtained by flash chromatography to give **48a** (0.135 g, 67% yield). HPLC: (D) 13.3 min (97%). MS (ESI) *m/z*: 665.4 (MH$^+$). ^{1}H NMR (600 MHz, DMSO-d_6): δ (ppm) 8.93 (s, 1H), 8.28 (s, 1H), 8.20 (d, J = 2.0 Hz, 1H), 8.00 (d, J = 8.6 Hz, 1H), 7.85 (d, J = 8.6 Hz, 1H), 7.49 (dd, J = 8.6, 2.0 Hz, 2H), 7.44 (t, J = 5.9 Hz, 1H), 7.20−7.12 (m, 5H), 4.45 (m, 1H), 3.80 (m, 2H), 3.25 (m, 2H), 3.17 (dd, J = 13.9, 4.3 Hz, 1H), 2.93 (m, 2H), 2.83 (dd, J = 13.9, 10.6 Hz, 1H), 2.68 (m, 2H), 2.23 (dt, J = 13.3, 8.1 Hz, 1H), 2.00 (d, J = 7.3 Hz, 2H), 1.90 (m, 1H), 1.78 (m, 2H), 1.73−1.45 (m, 5H), 1.70 (t, J = 11.7 Hz, 1H), 1.65 (m, 4H), ∼1.6 (m, 2H), 1.54 (m, 5H), 1.47 (m, 1H), 1.34 (m, 1H), 1.05 (m, 4H).

In a similar way products **48b-h** were obtained.

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Supporting Information Available: Tables containing complete NMR assignments, MS analysis results, and HPLC data (t_R + purity) for all the final products; spectroscopic characterization of intermediates that are not explicitly described in the Experimental Section. This material is available free of charge via the Internet at http://pubs.acs.org.

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