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Modulation on C- and N-Terminal Moieties of a Series of Potent and Selective Linear Tachykinin NK_2 Receptor Antagonists

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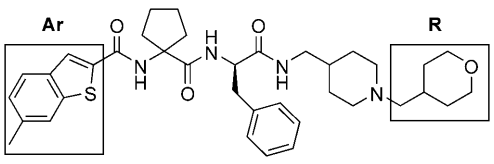
Herein we describe the synthesis of a series of new potent tachykinin NK_2 receptor antagonists by the modulation of the C- and N-terminal moieties of ibodutant (MEN 15596, **1**). The N-terminal benzo[*b*]thiophene ring was replaced by different substituted naphthalenes and benzofurans, while further modifications were evaluated at the C-terminal tetrahydropyran moiety. Most compounds demonstrated a high affinity for the human NK_2 receptor and high in vitro antagonist potency, indicating that a wide range of substituents at both termini can be incorporated in the molecule without detrimental effects on the interactions with the NK_2 receptor. Selected compounds were tested in vivo confirming their activity as NK_2 antagonists. In particular, after both iv and id administration to guinea pig, compound **61 b** was able to antagonize NK_2-induced colonic contractions with a potency and duration-of-action fully comparable to the reference compound **1** (MEN 15596, ibodutant).

Introduction

The natural tachykinins, substance P (SP), neurokinin A (NKA) and B (NKB) are small peptides widely distributed in the central and peripheral nervous systems where they have been shown to act as neurotransmitters. Each of them binds preferentially to one of the three G-protein coupled receptors termed NK_1, NK_2 and NK_3, respectively.[1] NKA, in particular, preferentially activates the NK_2 receptor, known to be distributed in both central and peripheral structures of human body. In humans, NK_2 receptors are highly expressed in muscle layers and neurons of the gastrointestinal tract where they can affect functions such as motility, secretion and visceral nociception.[2] Thus tachykinin NK_2 receptor antagonists can be considered as potential candidates for the treatment of gastrointestinal diseases characterized by disturbance of intestinal motility and altered perception of pain, such as irritable bowel syndrome or postoperative ileus.[3]

We have recently reported the synthesis and structure–activity relationship study of a series of new potent and selective tachykinin NK_2 receptor antagonists, leading to the identification of ibodutant (MEN 15596, **1**; Figure 1) endowed with subnanomolar affinity and antagonist potency for the human NK_2 receptor, and long lasting NK_2 receptor blocking activity in in vivo animal models.[4] The compound is presently in clinical development for the treatment of irritable bowel syndrome.



ibodutant (MEN 15596, **1**)

Figure 1. Reference compound ibodutant (MEN 15596, **1**) and main modification sites.

In our previous work, we demonstrated that modifications to the structure of our compounds are possible, within limits, without detrimental effects on receptor affinity. In fact, a pharmacophore model was proposed in the past by our group[5] for interaction of these compounds with the human NK_2 receptor: three hydrophobic and one positively charged group were considered as the main features. This hypothesis was first confirmed by the X-ray structure of **1**[6] and recently acquired further support from site-directed mutagenesis studies,[7] which evaluated the affinity of compound **1** for some mutated human NK_2 receptors. A docking mode was proposed for **1** in which, in addition to the previously defined interactions, contribution of the positive charge of the piperidinyl group that could find a hydrogen bond counterpart in the hydroxy group of Tyr 289, and a lipophilic interaction between the same

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Tyr 289 and the tetrahydropyranyl group of ibodutant, would further contribute to ligand binding stabilization.

Our next goal was to further explore the space for chemical modifications, in order to identify further lead compounds in case any unexpected drawbacks should be encountered in the development of **1**. Herein, we describe the synthesis of ibodutant analogues through modifications to the two terminal groups, namely the tetrahydropyran moiety[8] (R, Figure 1) and the aromatic moiety (Ar, Figure 1).[9] While retaining high receptor affinity and in vivo potency was our primary requirement, protection of sites possibly sensitive to oxidative metabolism, such as the sulfur atom on the benzothiophene ring or the junction between the piperidine and the tetrahydropyranyl ring in the R moiety was also desirable.

Results and Discussion

Synthesis

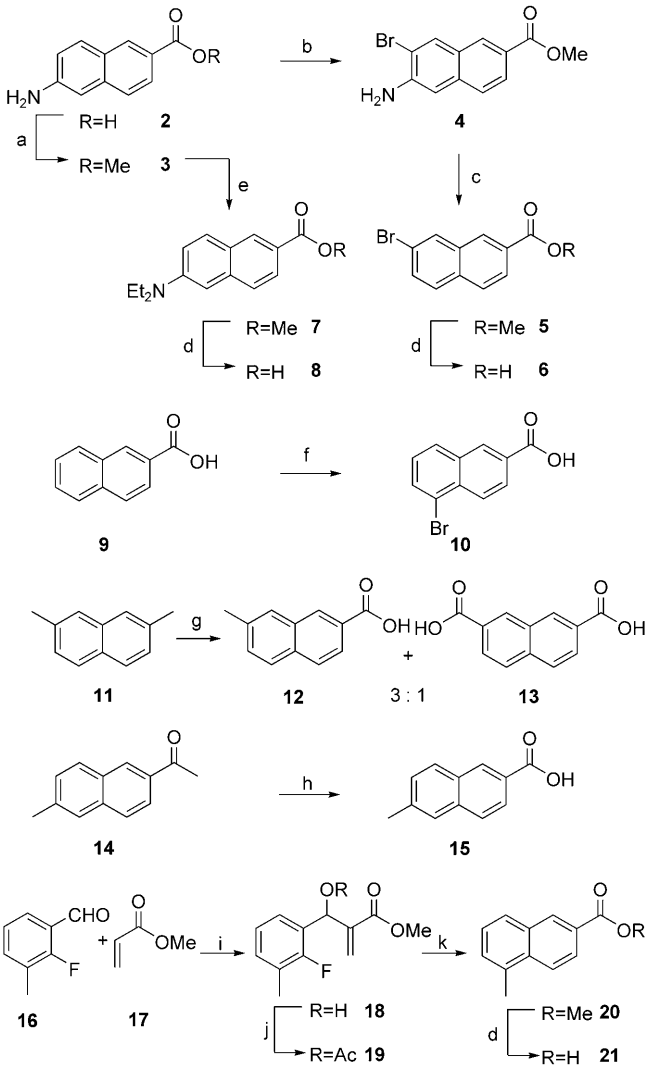
In the initial work, we decided to modulate the aromatic N-terminal moiety of **1** by the introduction of substituted benzofuran and naphthalene rings. The synthesis of non-commercially available naphthalene-2-carboxylic acids is reported in Scheme 1.

The 7-bromo derivative **4** was synthesized in a one-pot reaction from the commercially available 6-amino derivative **2** by double bromination at positions 5 and 7, followed by selective debromination at position 5 using HBr and Na_2SO_3 in acetic acid[10] and subsequent re-esterification to give the bromo derivative **4** in 90% yield. Deamination of **4** was achieved by treatment with *tert*-butyl nitrite (80% yield), followed by hydrolysis of the methyl ester to yield the carboxylic acid **6**. The diethylamino derivative **8** was obtained from methyl ester **3** using EtI and K_2CO_3 in acetonitrile, followed by treatment with NaOH. Bromination of naphthalene-2-carboxylic acid **9** with bromine in hot glacial acetic acid gave a mixture of monobromo derivatives from which the 5-bromo-naphthalene-2-carboxylic acid **10** was isolated by crystallization.[11]

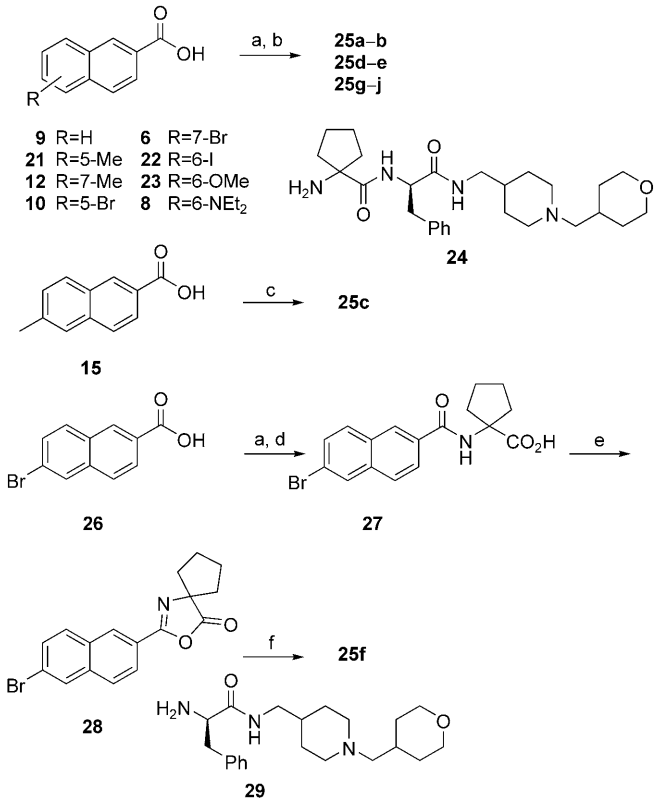
2,7-Dimethylnaphthalene **11** was oxidized using excess potassium permanganate to give carboxylic acids **12** and **13** in a molar ratio of 3:1. The mixture was used directly in the next coupling and the corresponding derivatives were separated by flash chromatography. The commercially available 2-acetyl-6-methyl-naphthalene **14** underwent the haloform reaction in the presence of bromine and NaOH and yielded the carboxylic acid **15** in quantitative yield. The 5-methyl derivative **21** was synthesized using a different strategy; benzannulation of the Baylis–Hillman adduct **19**, obtained from 2-fluoro-3-methyl benzaldehyde (**16**) and methyl acrylate **17** by treatment with 1,4-diazabicyclo[2.2.2]octane (DABCO), followed by addition of AcCl and pyridine.[12] The original conditions reported by Kim et al.[12] were modified in order to use nitromethane as the nucleophile in the synthesis of methyl ester **20** and avoid substitution at position 4, changing the base/solvent system from K_2CO_3/DMF to Cs_2CO_3/DMSO.

Carboxylic acids **6**, **8**, **9**, **10**, **12**, **21**, **22** and **23** were transformed into acyl chlorides by treatment with oxalyl chloride and catalytic DMF and coupled with amine **24**[4a] to give the final compounds **25a**,**b**,**d**,**e**,**g**–**j** described in Scheme 2. Carboxylic acid **15** was condensed with amine **24** using *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC·HCl), 1-hydroxybenzo-triazole (HOBt) and *N*,*N*-diisopropylethylamine (DIPEA) to yield final compound **25c**. For the synthesis of compound **25f**, we first formed oxazolone **28** by treatment of adduct **27** with EDC·HCl and triethylamine, followed by addition of amine **29.**[4a]

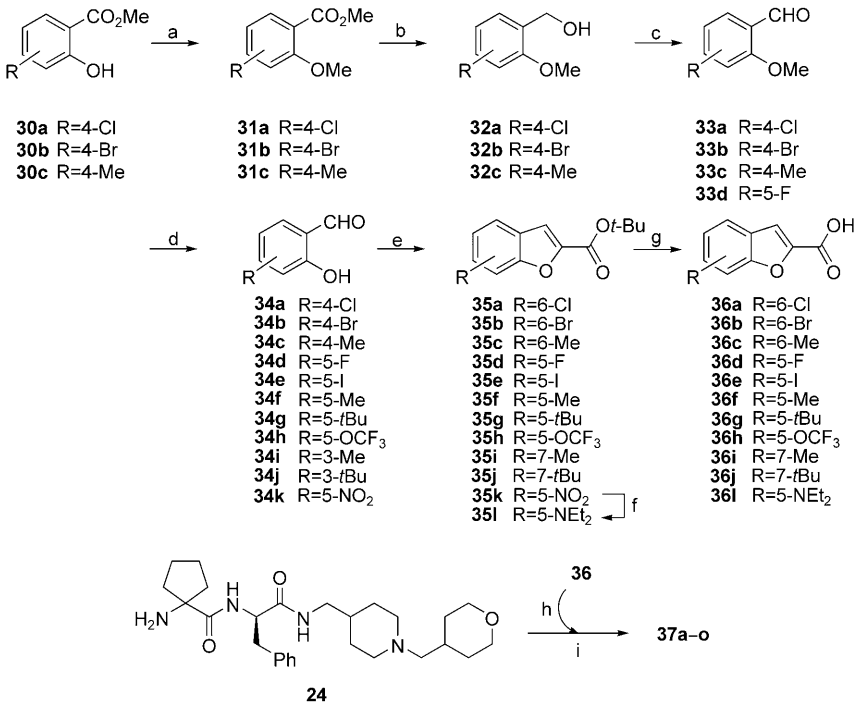
The synthesis of non-commercially available substituted benzo[*b*]furan-2-carboxylic acid *tert*-butyl esters **35a**–**k**, starting from 2-hydroxybenzaldehydes **34a**–**k** and *tert*-butyl bromoacetate, using cesium carbonate is shown in Scheme 3.[13] Compounds **34**, where not commercially available, were synthesized from methyl esters **30a**–**c** or 2-methoxybenzaldehyde



Scheme 1. *Reagents and conditions*: a) $SOCl_2$, CH_3OH, reflux, 3 h; b) 1) Br_2, AcONa, AcOH, 50 °C, 3 h; 2) HBr, Na_2SO_3, AcOH, reflux, 30 min; 3) $SOCl_2$, CH_3OH, reflux, 3 h; c) *tert*-BuONO, DMF, 50 °C, 1 h; d) NaOH 1 M, EtOH, RT, 5 h; e) EtI, K_2CO_3, CH_3CN, reflux, 7 h; f) Br_2, AcOH, reflux, 1.5 h; g) $KMnO_4$, Py, H_2O, reflux, 3 h; h) Br_2, NaOH 5 M, dioxane, RT, 2.5 h; i) DABCO, RT, 15 h; j) AcCl, Py, toluene, RT, 3 h; k) $MeNO_2$, Cs_2CO_3, DMSO, 50 °C, 10 h.



Scheme 2. *Reagents and conditions*: a) $(COCl)_2$, DMF_{cat}, CH_2Cl_2, RT, 1 h; b) **24**, Et_3N, CH_2Cl_2, RT, 16 h; c) **24**, EDC·HCl, HOBt, DIPEA, DMF, RT, 16 h; d) 1-amine-cyclopentanecarboxylic acid, *N*,*O*-bis(trimethylsilyl)acetamide, CH_2Cl_2, RT, 16 h; e) EDC·HCl, DIPEA, THF, RT, 18 h; f) **29**, Et_3N, DMF, 55 °C, 2 d.



Scheme 3. *Reagents and conditions*: a) 1) KOH, CH_3OH, RT, 1 h; 2) MeI, THF, RT, 2 d; b) $LiAlH_4$, THF, 0 °C, 2 h; c) MnO_2, $CHCl_3$, reflux, 2 h; d) $AlCl_3$, CH_2Cl_2, RT, 10 h; e) $BrCH_2CO_2tertBu$, Cs_2CO_3, DMSO, 100 °C, 2.5 h; f) 1) H_2, Pd/C 10%, EtOH, RT, 6 h; 2) K_2CO_3, EtI, CH_3CN, reflux, 18 h; g) TFA, CH_2Cl_2, 0 °C, 3 h; h) $(COCl)_2$, CH_2Cl_2, DMF, RT, 1 h; i) Et_3N, CH_2Cl_2, RT, 16 h.

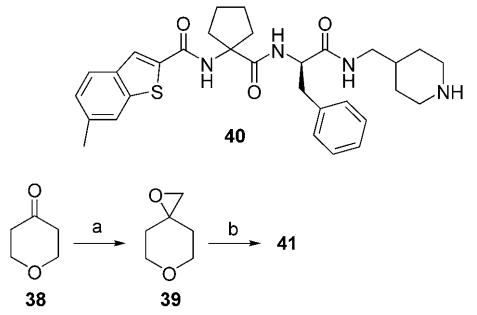
33 d (Scheme 3). Treatment of **30 a**–**c** with KOH and MeI gave the methoxy derivatives **31 a**–**c**, which underwent reduction by $LiAlH_4$ to yield benzyl alcohols **32 a**–**c**. The latter were oxidized to benzaldehydes **33 a**–**c** by treatment with MnO_2. Carboxylic acids **36 a**–**j**,**l** were obtained from the *tert*-butyl esters **35 a**–**j**,**l** by treatment with TFA. Final compounds **37 a**–**o** were synthesized from the corresponding benzo[*b*]furan-2-carboxylic acids **36** and amine **24** via acyl chloride formation.

Next, we turned our attention to modification of the tetrahydropyran moiety. We attempted to introduce a substituent in position 4 in order to increase diversity without increasing the complexity of the structure through the generation of new stereocenters. Additionally, we modified the methylene spacer through the introduction of a *gem*-dimethyl group, a cyclopropyl group or an amidine functionality.

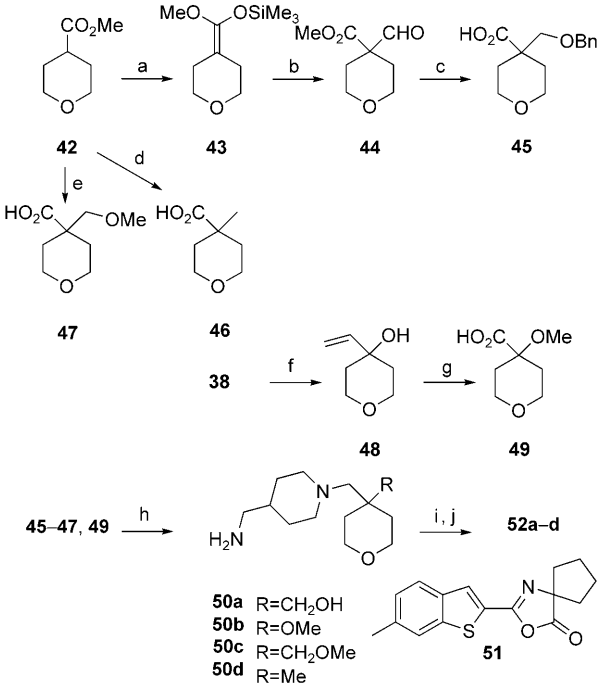
Starting from tetrahydropyranone **38**, the final compound **41** was synthesized via ring opening of epoxide **39**[14] in the presence of amine **40**[4a] (Scheme 4).[15]

A series of derivatives was generated from the methyl ester **42**, which was converted into the trimethylsilyl ketene acetal **43** by treatment with lithium diisopropylamide and trimethylsilyl chloride, to the methyl derivative **46** by treatment with LDA and MeI, and to the methoxymethyl derivative **47** by treatment with LDA and methyl bromoacetate in THF (Scheme 5). The silyl ketene acetal **43** was further treated with PCl_5 to give the aldehyde **44**,[16] which was reduced to the corresponding alcohol using $NaBH_4$ and finally protected as the benzyl ether **45**. Tetrahydropyranone **38** was alkylated with vinyl magnesium bromide to yield alcohol **48**, which was methylated and oxidized to carboxylic acid **49**. Compounds **45**, **46**, **47** and **49** were converted into diamines **50 a**–**d** by treatment with isonipecotamide and Et_3N in dichloromethane, followed by reduction with $LiAlH_4$ in THF. Final compounds **52 a**–**d** were obtained from fragments **50 a**–**d** and oxazolone **51**.[4a]

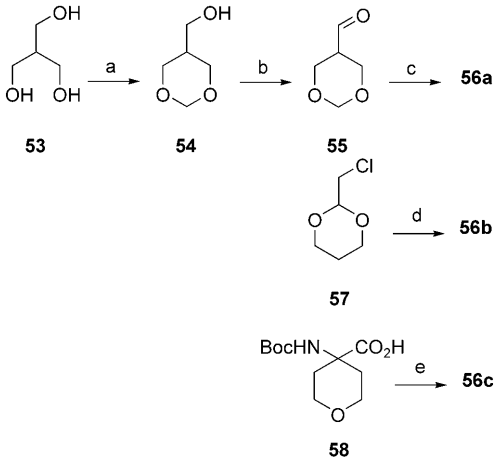
The [1,3]-dioxolane derivative **56 a** was synthesized from triol **53**, which was treated with $CH_2(OMe)_2$, *p*-toluenesulfonic acid and LiBr in dichloromethane to give **54**. Subsequent oxidation to the aldehyde **55** was achieved using the Dess–Martin periodinane reagent (Scheme 6). The final [1,3]-dioxolane compound **56 a** was obtained by reductive amination of amine **40** with aldehyde **55** using $NaCNBH_3$. Compound **56 b** was synthesized by direct alkylation of amine **40** with dioxolane **57**, while compound **56 c** was formed through condensation of



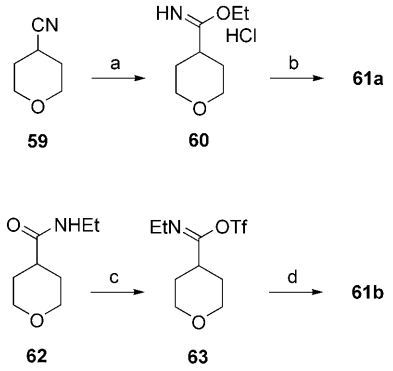
Scheme 4. *Reagents and conditions*: a) $Me_3S(I)O$, NaH, THF, reflux, 5 h; b) **40**, $LiN(SO_2CF_3)_2$, CH_3CN, RT, 7 d.



Scheme 5. *Reagents and conditions*: a) 1) LDA, THF, 0 °C, 30 min; 1) TMSCl, RT, 30 min; b) DMF, PCl_5, CH_2Cl_2, RT, 20 h; c) 1) $NaBH_4$, CH_3OH, H_2O, 0 °C→RT, 1 h; 2) NaH, BnBr, THF, RT, 16 h; 3) NaOH 1 M, RT, 1 d; d) 1) LDA, MeI, THF, 0 °C→RT, 16 h; 2) NaOH 1 M, RT, 1 d; e) 1) LDA, $BrCH_2OMe$, THF, 0 °C→RT, 16 h; 2) NaOH 1 M, RT, 1 d; f) vinylMgBr, THF, RT, 1 h; g) 1) MeI, NaH, THF, RT, 16 h; 2) $NaIO_4$, $RuCl_3$, CCl_4/CH_3CN, RT, 3 h; h) 1) $(COCl)_2$, DMF, $CHCl_3$, RT, 3 h; 2) isonipecotamide, Et_3N, CH_2Cl_2, RT, 4 h; 3) Pd/C 10%, H_2 (**50a** only), RT, 8 h; 4) $LiAlH_4$ 1 M, THF, reflux, 4 h; i) 1) Boc-D-Phe-OSu, DMF, RT, 16 h; 2) TFA, CH_2Cl_2, RT, 30 min; m) DMF, RT, 18 h.



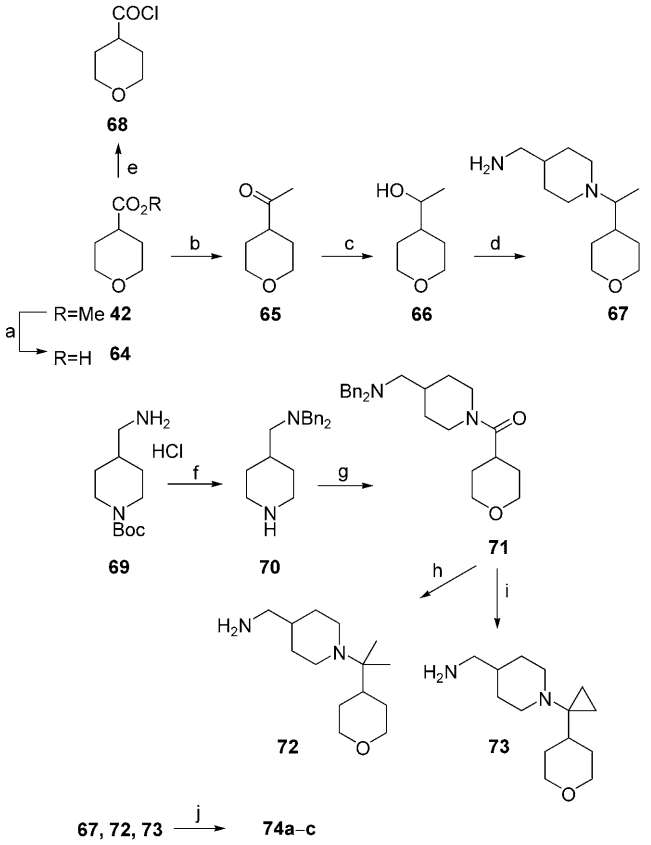
Scheme 6. *Reagents and conditions*: a) $CH_2(OMe)_2$, TsOH, LiBr, CH_2Cl_2, RT, 16 h; b) Dess–Martin periodinane, CH_2Cl_2, RT, 2 h; c) **40**, AcOH, $NaCNBH_3$, CH_3OH, RT, 16 h; d) **40**, KI, Cs_2CO_3, DMF, 85 °C, 4 h; e) 1) **40**, EDC·HCl, HOBt, RT, 16 h; 2) TFA, CH_2Cl_2, RT, 2 h.



Scheme 7. *Reagents and conditions*: a) HCl, EtOH, dioxane, 4 °C, 2 d; b) **40**, Et_3N, EtOH, 80 °C, 3 d; c) Tf_2O, Py, CH_2Cl_2, −40 °C, 2 h; d) **40**, CH_2Cl_2, RT, 18 h.

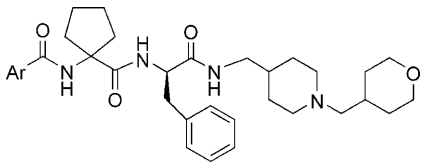
α,α-dialkylamino acid **58** and amine **40** in the presence of EDC·HCl and HOBt, followed by Boc deprotection. Amidine derivatives **61a** and **61b** were obtained respectively from imidates **60** and **63** by direct treatment with amine **40** (Scheme 7). Nitrile derivative **59** was converted into imidate **60** by treatment with HCl in EtOH, while *N*-ethylimidate **63** was synthesized from *N*-ethylamide **62** by treatment with triflic anhydride and pyridine.

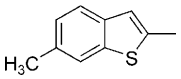
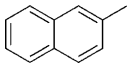
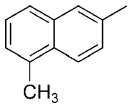
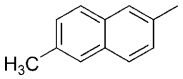
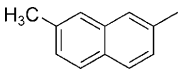
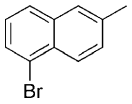
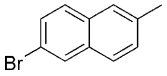
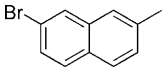
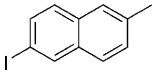
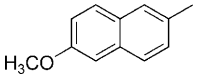
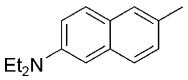
The substitution on the methylene spacer between the tetrahydropyran and piperidine rings was achieved as described in Scheme 8. Carboxylic acid **64** was alkylated with methyl lithium to give methyl ketone **65**,[17] which was reduced by $NaBH_4$ to the racemic secondary alcohol **66**. The latter was converted into the triflate and underwent nucleophilic substitution with isonipecotamide to give the correspondent diamide, which was reduced with $LiAlH_4$. Amide **71** was synthesized starting from amine hydrochloride **69**, which was first protected as the *N*,*N*-dibenzylamine, then deprotected on the piperidine ring and alkylated with acyl chloride **68**. Amide **71** was treated with $MeMgBr{\cdot}Et_2O$ in the presence of $POCl_3$, then the benzyl groups were removed by catalytic hydrogenation to yield amine **72**. Conversely, titanium-mediated cyclopropanation of amide **71** ($Ti(OiPr)_4$, EtMgBr in THF)[18] gave the cyclopropylamine derivative **73** after reductive deprotection. The alkylated derivatives **67**, **72**, **73** underwent peptide coupling with Boc-D-phenylalanine *N*-hydroxysuccinimide (Boc-D-Phe-OSu), then, after Boc deprotection, were condensed with oxazolone **51** to give final compounds **74a–c** (compound **74a** was obtained as a ~1:1 diastereomeric mixture, determined by ^1H NMR analysis).



Scheme 8. *Reagents and conditions*: a) NaOH 1 M, RT, 6 h; b) 1) MeLi, THF, 0 °C, 2 h; 2) TMSCl, 0 °C→RT, 1 h; c) $NaBH_4$, CH_3OH, RT, 2 h; d) 1) Tf_2O, Py, CH_2Cl_2, −30 °C→0 °C, 1 h; 2) isonipecotamide, DMF, RT, 16 h; 3) $LiAlH_4$, THF, 0 °C→reflux, 1 h; e) $(COCl)_2$, $CHCl_3$, RT, 18 h; f) 1) BnBr, Et_3N, CH_3CN, 100 °C, 3 h; 2) TFA, CH_2Cl_2, RT, 1.5 h; g) **68**, Et_3N, CH_2Cl_2, RT, 3 h; h) 1) $POCl_3$, 100 °C, 2 h; 2) MeMgBr·Et_2O, THF, RT, 16 h; 3) HCO_2NH_4, Pd/C, *i*PrOH, 60 °C, 1 h; i) 1) $Ti(OiPr)_4$, EtMgBr, THF, RT, 16 h; 2) HCO_2NH_4, Pd/C, *i*PrOH, 60 °C, 2 h; j) 1) Boc-D-Phe-OSu, DMF, RT, 16 h; 2) TFA, CH_2Cl_2, RT, 30 min; 3) **51**, DMF, RT, 18 h.

Table 1. Compounds **25 a–j**: structures, affinity and antagonist potency values for the tachykinin NK_2 receptor evaluated in binding experiments (pK_i) and functional experiments (pK_B).



Entry	Ar	NK_2 assay[a] pK_i[b] (hNK_2)	pK_B[c] (GPC)
1		10.10 ± 0.13	9.30 ± 0.07
25 a		9.81 ± 0.08	8.47 ± 0.18
25 b		9.87 ± 0.09	8.44 ± 0.17
25 c		9.90 ± 0.22	8.13 ± 0.18
25 d		9.89 ± 0.05	7.87 ± 0.34
25 e		9.84 ± 0.12	8.52 ± 0.13
25 f		10.00 ± 0.09	8.84 ± 0.21
25 g		9.82 ± 0.16	8.50 ± 0.20
25 h		10.26 ± 0.08	9.00 ± 0.17
25 i		9.51 ± 0.04	8.45 ± 0.20
25 j		9.25 ± 0.04	7.10 ± 0.27

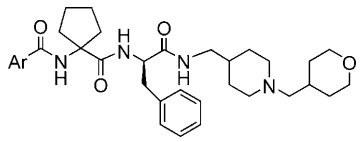
[a] Values are the mean ± SEM ($n = 3$). [b] $pKi = -\log K_i$. Affinity values for the human NK_2 receptor were estimated against [^{125}I]neurokinin A in radioligand binding experiments. [c] $pK_B = -\log K_B$. Antagonist potency values of test compounds for guinea pig NK_2 receptor estimated toward [βAla^8]NKA(4-10) induced contractions of the isolated colon smooth muscle (GPC) in the presence of the NK_1 receptor-selective antagonist SR140333.

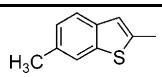
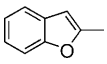
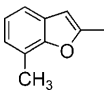
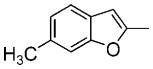
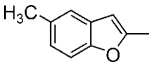
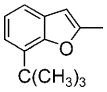
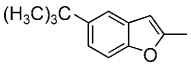
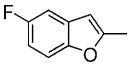
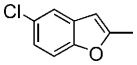
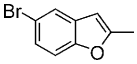
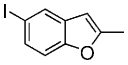
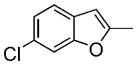
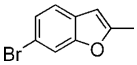
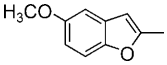
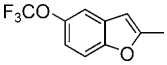
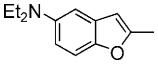
In vitro pharmacology

All the derivatives were evaluated for their binding affinity at the human tachykinin NK_2 receptor and functional antagonist potency in guinea pig isolated proximal colon (GPC) (see Tables 1–3). Table 1 gives the results of these assays for derivatives **25 a–j** containing a substituted naphthalene ring; compound **1** was also tested for comparison. On the basis of the results of our previous work,[4] we chose to insert substituents on the "external" benzo-fused ring, as substitution on the inner ring, closer to the carbonyl group, was shown to be detrimental to receptor affinity. All compounds in Table 1 gave very high affinities for the human NK_2 receptor, with pK_i values in the sub-nanomolar range and close to that of **1**, thus indicating that the presence of even bulky substituents, such as bromine or iodine, could be well tolerated by the receptor.

However, methyl substitution in position 5 (**25 b**), 6 (**25 c**) and 7 (**25 d**) of the naphthalene ring corresponded to a net decrease in antagonist potency (pK_B) with respect to both the reference 6-methylbenzothiophene compound **1**, and, although to a lesser extent, to the unsubstituted naphthalene **25 a**. Bromine substitution in the same positions, 5 (**25 e**), 6 (**25 f**) and 7 (**25 g**), gave better pK_B values than the unsubstituted **25 a**, although not as good as **1**. The best results were obtained with the 6-iodo derivative **25 h**, which was the only compound to exhibit a higher NK_2 receptor affinity than **1** and comparable functional potency. Following this result, other 6-substituted compounds were evaluated, however neither the 6-methoxy (**25 i**) nor the 6-diethylamino (**25 j**) derivatives led to an increase in potency, which was instead very low for the latter. Similar structure–activity relationships can be observed from the results of the benzofuran derivatives **37 a–o** (see Table 2).

Table 2. Compounds **37a–o**: structures, affinity and antagonist potency values for the tachykinin NK_2 receptor evaluated in binding experiments (pK_i) and functional experiments (pK_B).



Entry	Ar	NK_2 assay[a] pK_i[b] (hNK_2)	pK_B[c] (GPC)
1		10.10 ± 0.13	9.30 ± 0.07
37a		9.03 ± 0.02	7.92 ± 0.02
37b		9.10 ± 0.04	7.80 ± 0.15
37c		9.60 ± 0.07	8.32 ± 0.08
37d		9.81 ± 0.09	8.75 ± 0.12
37e		10.02 ± 0.08	7.62 ± 0.02
37f		9.88 ± 0.06	8.22 ± 0.22
37g		9.25 ± 0.04	8.33 ± 0.14
37h		9.97 ± 0.07	8.53 ± 0.12
37i		10.07 ± 0.07	9.02 ± 0.08
37j		10.18 ± 0.11	7.73 ± 0.09
37k		9.52 ± 0.07	8.35 ± 0.20
37l		9.63 ± 0.11	8.52 ± 0.10
37m		9.36 ± 0.05	7.63 ± 0.13
37n		9.30 ± 0.03	7.40 ± 0.15
37o		9.22 ± 0.02	7.48 ± 0.13

[a] Values are the mean ± SEM ($n = 3$). [b] $pK_i = -\log K_i$. Affinity values for the human NK_2 receptor were estimated against [^{125}I]neurokinin A in radioligand binding experiments. [c] $pK_B = -\log K_B$. Antagonist potency values of test compounds for guinea pig NK_2 receptor estimated toward [βAla^8]NKA(4-10) induced contractions of the isolated colon smooth muscle (GPC) in the presence of the NK_1 receptor selective antagonist SR140333.

Again, all compounds in Table 2 showed sub-nanomolar affinity at the human NK_2 receptor, thus confirming the tolerance of a broad range of different substituents on the "external" benzofused ring. However, the test for functional activity allowed us to differentiate further between the substituents and positions. Irrespective of size, the insertion of alkyl moieties in positions 5 (methyl, **37d** and *tert*-butyl, **37f**) and 6 (methyl, **37c**) gave compounds with greater functional potency compared with compounds with alkyl moieties in position 7 (methyl, **37b** and *tert*-butyl, **37e**). In the halogen series, in contrast to the trends observed for the naphthalene derivatives, the 5-iodo derivative **37j** gave the worst results in the functional potency assay, while the 5-fluoro (**37g**), 5- and 6-chloro (**37h**, **37k**) derivatives were all better than the unsubstituted benzofuran **37a**. The best results were obtained with the 5-bromo derivative **37i**, while the 5-methoxy (**37m**), 5-trifloromethoxy (**37n**) and diethylamino (**37o**) derivatives led to a net decrease in functional potency, the latter confirming the results of the naphthalene series.

With respect to the compounds with modifications on the tetrahydropyranyl moiety, the insertion of polar groups in position 4 of the tetrahydropyran ring, such as in the hydroxy (**41**), hydroxymethyl (**52a**), methoxy (**52b**) and methoxymethyl (**52c**) derivatives, did not significantly alter the receptor affinity, but the antagonist potency decreased up to 10 times (**52c**) compared with the reference compound **1** (see Table 3).

Interestingly, the simple methyl derivative **52d** gave results quite comparable to **1**, both for receptor affinity and antagonist potency. Changes from a tetrahydropyran to a [1,3]-dioxolane moiety (**56a** and **56b**) led to reduced functional activity. The introduction of basic groups in this part of the molecule, such as the amine **56c** bearing a second α,α-dialkylamino acid and the amidines **61a** and **61b**, was well tolerated by the receptor. In particular, the *N*-ethylamidine **61b** gave a pK_B value comparable to compound **1**. Finally, the monomethylated diastereomeric mixture **74a**, the *gem*-dimethyl **74b** and the cyclopropyl **74c** derivatives were tested but showed no substantial advantages over the parent compound.

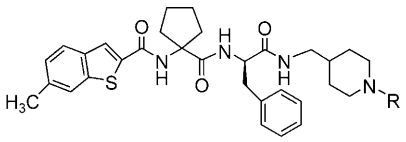
In vivo pharmacology

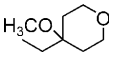
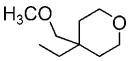
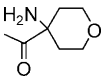
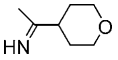
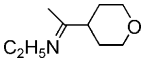
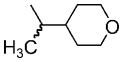
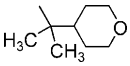
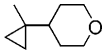
Some of the best compounds obtained were also tested in vivo to evaluate their true value in comparison with the reference compound **1**. The potency of the compounds in inhibiting colonic contractions induced by the selective tachykinin NK_2 receptor agonist [βAla^8]NKA(4-10) (3 nmol kg^{-1} iv) was evaluated after intravenous (iv) administration at the dose of 3 μmol kg^{-1} and after intraduodenal (id) administration at the dose of 10 μmol kg^{-1} in guinea pig. The results, shown in Table 4, are expressed as both the maximal inhibition ($i\%_{max}$; percent change in comparison with the basal colon contraction caused by [βAla^8]NKA(4-10)) and as the $\Sigma i\%_{max}$, that is the sum of the percent inhibition values (taken at 5/30/60/90/120/150/180/210/240 min after iv and id administration of the antagonist) calculated as percentage of the sum of theoretical maximal responses, see Equation (1):

$$\sum i\%_{max} = \left[\frac{\sum (\%\, i)}{\sum (\%\, i_{max} - \text{th})}\right] \times 100 \quad (1)$$

≡ mean percent inhibition over the entire experiment

Table 3. Compounds **41**, **52a–d**, **56a–c**, **61a–b**, **74a–c**: structures, affinity and antagonist potency values for the tachykinin NK_2 receptor evaluated in binding experiments (pK_i) and functional experiments (pK_B).



Entry	Ar	NK_2 assay[a] pK_i[b] (hNK_2)	pK_B[c] (GPC)
1		10.10 ± 0.13	9.30 ± 0.07
41		9.99 ± 0.09	8.78 ± 0.20
52a		10.10 ± 0.08	8.55 ± 0.06
52b		10.26 ± 0.07	8.27 ± 0.24
52c		9.70 ± 0.07	8.23 ± 0.14
52d		9.86 ± 0.09	9.17 ± 0.06
56a		9.93 ± 0.06	8.10 ± 0.11
56b		9.81 ± 0.06	8.20 ± 0.25
56c		9.81 ± 0.03	8.58 ± 0.11
61a		9.77 ± 0.09	7.63 ± 0.09
61b		10.00 ± 0.14	8.94 ± 0.23
74a		9.61 ± 0.06	8.25 ± 0.15
74b		9.54 ± 0.02	7.72 ± 0.15
74c		9.15 ± 0.01	8.35 ± 0.05

[a] Values are the mean ± SEM ($n = 3$). [b] $pK_i = -\log K_i$. Affinity values for the human NK_2 receptor were estimated against [^{125}I]neurokinin A in radioligand binding experiments. [c] $pK_B = -\log K_B$. Antagonist potency values of test compounds for guinea pig NK_2 receptor estimated toward [βAla^8]NKA(4-10) induced contractions of the isolated colon smooth muscle (GPC) in the presence of the NK_1 receptor selective antagonist SR140333.

This last parameter gives a measure of the activity during the entire experimental period, and allows us to evaluate not only the intensity but also the duration of the antagonist effect. The maximal inhibition obtainable corresponds to $\Sigma i\%_{max} = 100$, while the absence of effect is $\Sigma i\%_{max} = 0$.[4]

Of those compounds studied in vitro, four derivatives (**25h**, **37i**, **52d**, **61b**) were studied in vivo and their results are reported in Table 4. The naphthalene compound **25h** and the modified tetrahydropyran derivative **52d** showed a slightly lower maximal inhibitory effect ($i\%_{max} = 94 \pm 4\%$ and 90 ± 4, respectively) than **1** after iv administration, but a dramatic decrease in the effect after id administration ($i\%_{max} = 38 \pm 10$ and 29 ± 20; $\Sigma i\%_{max} = 29$ and 15%, respectively). The benzofuran derivative **37i** maintained a potent inhibitory effect after iv ($i\%_{max} = 99 \pm 1$, $\Sigma i\%_{max} = 79\%$) and id ($i\%_{max} = 90 \pm 2$, $\Sigma i\%_{max} = 73\%$) administration, comparable to the reference compound **1**, but showed poorer duration-of-action in the given time. In compound **61b**, in which the amine functionality of **1** was replaced by an amidine also giving a modified junction between the piperidine and tetrahydropyran groups, we observed the same effect as for reference compound **1**, both after iv ($i\%_{max} = 100 \pm 0$) and id ($i\%_{max} = 82 \pm 7$) administration. The duration of the antagonist effect for **61b** was also comparable to **1** both after iv and id administration, thus indicating that **61b** should be investigated further.

Table 4. In vivo evaluation of selected leads: inhibition of colonic contractions induced by [βAla^8]NKA(4-10) in guinea pig.[a]

Compd	$i\%_{max}$[b] (iv)	$\Sigma i\%_{max}$[c]	$i\%_{max}$[b] (id)	$\Sigma i\%_{max}$[c]
control	0	0	0	0
1	99 ± 1	92	83 ± 4	74
25h	94 ± 4	67	38 ± 10	29
37i	99 ± 1	79	90 ± 2	73
52d	90 ± 4	75	29 ± 20	15
61b	100 ± 0	97	82 ± 7	69

[a] Intravenous (iv) administration: dose = 3 μmol kg^{-1}. Intraduodenal (id) administration: dose = 10 μmol kg^{-1}. [b] $i\%_{max}$ is the maximal percent inhibition; $\Sigma i\%_{max}$ is the sum of the percent inhibition at the nine times of observation. [c] see Equation (1) in the text.

Conclusions

Three series of potent tachykinin NK_2 receptor antagonists were identified by modulation of the tetrahydropyran moiety (R) on the basic side and of the benzothiophene moiety (Ar) on the aromatic side of ibodutant (**1**). Most compounds demonstrated a high affinity for the human NK_2 receptor and high in vitro antagonist potency, indicating that the two termini of the molecule can accommodate a wide range of substituents without loss of interactions with the NK_2 receptor. Selected compounds were tested in vivo confirming their activity as NK_2 antagonists. In particular, after both iv and id administration in guinea pig, compound **61b** was able to antagonize NK_2-induced colonic contractions with a potency and duration-of-action fully comparable to the reference compound **1**, thus warranting further evaluation in pharmacological and toxicological studies.

Experimental Section

Chemistry

All reagents and solvents were used as purchased (from Sigma–Aldrich (Milan, Italy) unless otherwise specified). Commercial grade anhydrous solvents were purchased from J. T. Baker (Deventer, Holland). Naphthalene-2-carboxylic acids **9**, **22**, **23**, **26**, **34e–k**, benzo[*b*]furan-2-carboxylic acid, 5-chloro-, 5-bromo- and 5-methoxybenzo[*b*]furan-2-carboxylic acid were commercially available. Reactions were performed under an atmosphere of nitrogen, unless

otherwise specified. Silica gel (Merck, Kieselgel 60) was used for analytical TLC (F_{254} plates) and flash chromatography (230–400 mesh). Melting points were measured with a digital elecrothermal apparatus Büchi B-540 and are uncorrected. ^{1}H NMR spectra were acquired on a Varian 200 MHz and 600 MHz instruments. δ values are give in parts per million (ppm) relative to the residual solvent peak. The LC–MS system was a Thermo Finnigan LCQ mass spectrometer, interfaced with an Agilent series 1100 liquid chromatograph and a diode array UV detector. Either electrospray (ESI+) or atmospheric pressure chemical ionization (APCI) were used as the ionization techniques. The HRMS system was a Micromass Q-ToF spectrometer, equipped with an electrospray ion source with a LockSpray nebulizer, interfaced with an Agilent series 1200 liquid chromatograph and variable wavelength UV detector. More complete details of instrumentation and parameters are given in the Supporting Information.

For the synthesis of compounds **5**, **7**, **10**, **12**, **15**, **19** and **20**, see Supporting Information.

General procedure for the synthesis of compounds 25 a–b, 25 d–e, 25 g–j (GP 1)

Oxalyl chloride (1.40–1.50 equiv) and DMF (2 drops) were added to a solution of carboxylic acid (1.00 equiv, 0.45 mmol) in CH_2Cl_2 (3 mL) at RT. The reaction mixture was stirred for 1 h, then the solvent was removed in vacuo. The residue was redissolved in CH_2Cl_2 (15 mL) and treated with Et_3N (0.2 mL). The reaction mixture was stirred for 5 min, then was treated with amine **24** (0.8 equiv) and stirred overnight. The solvent was removed in vacuo, the residue treated with CH_3OH (15 mL) and stirred for 4 h. The solvent was removed in vacuo, the crude was treated with EtOAc (50 mL) and 10% aq K_2CO_3 (35 mL) and the phases separated. The organic phase was washed with 10% aq K_2CO_3 (3×30 mL) and dried (Na_2SO_4). After removal of the solvent in vacuo the residue was purified by column chromatography or preparative (prep) HPLC.

Naphthalene-2-carboxylic acid [1-(2-phenyl-1(*R*)-{[1-(tetrahydropyran-4-ylmethyl)piperidin-4-ylmethyl]carbamoyl}ethylcarbamoyl)cyclopentyl]amide (25 a): Amide **25 a** was obtained according to GP 1: HPLC (B): t_R=12.3 min, 97% purity; ^{1}H NMR (600 MHz, [D$_6$]DMSO): δ=8.78 (s, 1H), 8.53 (br s, 1H), 8.03–7.97 (m, 4H), 7.80 (d, J=8.6 Hz, 1H), 7.65–7.60 (m, 2H), 7.52 (br t, J=5.9 Hz, 1H), 7.20–7.11 (m, 5H), 4.46 (ddd, J=10.5, 8.7, 4.3 Hz, 1H), 3.81–3.77 (m, 2H), 3.27–3.20 (m, 2H), 3.18 (dd, J=13.4, 8.0 Hz, 1H), 3.00–2.95 (m, 1H), 2.90–2.84 (m, 2H), 2.60–2.56 (m, 2H), 2.29 (dt, J=13.4, 8.1 Hz, 1H), 2.00–1.92 (m, 3H), 1.82–1.77 (m, 1H), 1.72–1.45 (m, 12H), 1.32–1.25 (m, 1H), 1.06–0.95 ppm (m, 4H); MS (ESI+): *m/z*: 625.5 [*M*+H]$^+$.

5-Methylnaphthalene-2-carboxylic acid [1-(2-phenyl-1(*R*)-{[1-(tetrahydropyran-4-ylmethyl)piperidin-4-ylmethyl]carbamoyl}ethylcarbamoyl)cyclopentyl]amide (25 b): Amide **25 b** was obtained according to GP 1 as a white solid after purification (CH_2Cl_2/CH_3OH, 6:1): HPLC (B): t_R=13.0 min, 98% purity; mp: 170–172 °C; ^{1}H NMR (600 MHz, [D$_6$]DMSO): δ=8.78 (s, 1H), 8.52 (s, 1H), 8.02 (d, J=8.8 Hz, 1H), 8.02 (dd, J=8.8, 1.7 Hz, 1H), 7.87 (d, J=7.6 Hz, 1H), 7.79 (d, J=8.6 Hz, 1H), 7.52–7.46 (m, 3H), 7.20–7.11 (m, 5H), 4.47 (ddd, J=10.4, 8.8, 4.2 Hz, 1H), 3.81–3.77 (m, 2H), 3.26–3.13 (m, 2H), 2.99–2.94 (m, 1H), 2.90–2.83 (m, 2H), 2.69 (s, 3H), 2.59–2.53 (m, 2H), 2.30 (dt, J=13.2, 8.1 Hz, 1H), 1.99–1.89 (m, 3H), 1.81–1.77 (m, 1H), 1.71–1.46 (m, 12H), 1.31–1.23 (m, 2H), 1.96–0.94 ppm (m, 4H); MS (ESI+): *m/z*: 639.4 [*M*+H]$^+$; HRMS: *m/z* [*M*+H]$^+$ calcd for $C_{39}H_{51}N_4O_4$: 639.3910, found: 639.3904.

6-Methylnaphthalene-2-carboxylic acid [1-(2-phenyl-1(*R*)-{[1-(tetrahydropyran-4-ylmethyl)piperidin-4-ylmethyl]carbamoyl}ethylcarbamoyl)cyclopentyl]amide trifluoroacetate (25 c): EDC·HCl (104 mg, 0.54 mmol) and HOBt (74 mg, 0.54 mmol) were added to a solution of **15** (94 mg, 0.51 mmol) in DMF (5 mL) at RT. The reaction mixture was stirred for 50 min, then amine **24** (165 mg, 0.30 mmol) and DIPEA (0.25 mL, 1.10 mmol) were added and stirring continued overnight. The solvent was removed in vacuo and the residue treated with CH_2Cl_2 (25 mL) and 10% aq K_2CO_3 (20 mL). The organic phase was separated, washed with 10% aq K_2CO_3 (3× 20 mL) and dried over a phase separator. After removal of the solvent, the residue was purified by prep HPLC (Jupiter column C_{18}, 300 Å, 250 mm × 21.20 mm, 15 μm; H_2O+0.1% TFA/CH_3CN+0.1% TFA, 15→95% CH_3CN over 40 min) and compound **25 c** was obtained as a white solid (123 mg, 54%): HPLC (B): t_R=13.1 min, 95% purity; mp: 82–83 °C; ^{1}H NMR (600 MHz, [D$_6$]DMSO): δ=8.79 (s, 1H), 8.48 (br s, 1H), 7.97–7.89 (m, 3H), 7.83 (d, J=8.5 Hz, 1H), 7.65 (br t, J=5.9 Hz, 1H), 7.47 (d, J=8.6 Hz, 1H), 7.21–7.12 (m, 5H), 4.45 (ddd, J=10.6, 8.5, 4.3 Hz, 1H), 3.86–3.83 (m, 2H), 3.31–3.27 (m, 2H), 3.18 (dd, J=14.0, 4.1 Hz, 1H), 3.14–3.11 (m, 1H), 3.06–2.99 (m, 2H), 2.88 (dd, J=13.9, 10.6 Hz, 1H), 2.96–2.91 (m, 1H), 2.82 (br t, J=6.2 Hz, 2H), 2.71–2.64 (m, 1H), 2.62–2.53 (m, 1H), 2.51 (s, 3H), 2.29 (dt, J=13.3, 8.1 Hz, 1H), 2.00–1.90 (m, 2H), 1.82–1.48 (m, 11H), 1.38–1.28 (m, 2H), 1.21–1.14 ppm (m, 2H); MS (ESI+): *m/z*: 639.5 [*M*+H]$^+$; HRMS: *m/z* [*M*+H]$^+$ calcd for $C_{39}H_{51}N_4O_4$: 639.3910, found: 639.3930.

For characterization data of compounds **25 d**, **25 e**, **25 g–j**, see Supporting Information.

2-(6-Bromonaphthalen-2-yl)-3-oxa-1-azaspiro[4.4]non-1-en-4-one (28): Oxalyl chloride (4.15 mL, 47.75 mmol) and DMF (few drops) were added to a mixture of **26** (8.00 g, 31.86 mmol) in CH_2Cl_2 (300 mL). The resulting solution was stirred at RT for 4 h, then the solvent was removed in vacuo to give the acyl chloride as a white solid. *N*,*O*-Bis(trimethylsilyl)acetamide (15.30 mL, 62.4 mmol) was added to a suspension of 1-aminecyclopentanecarboxylic acid (4.03 g, 31.20 mmol) in CH_2Cl_2 (300 mL) and stirred for 1 h. A solution of the acyl chloride of acid **26** (8.39 g, 31.20 mmol) in CH_2Cl_2 (300 mL) was added to the solution dropwise. The resulting solution was stirred at RT for 2 h, then the solvent was removed in vacuo. The residue was diluted with 5% aq K_2CO_3 (100 mL) and the aqueous layer was extracted with EtOAc (100 mL), acidified with 37% aq HCl to pH<2 and extracted with EtOAc (3×100 mL). The organic phase was dried (Na_2SO_4), filtered and concentrated in vacuo to give **27** (HPLC (A): t_R=4.0 min, 90% purity). A solution of **27** (11.30 g, 31.20 mmol), EDC·HCl (9.00 g, 46.80 mmol) and DIPEA (20.0 mL, 11.23 mmol) in THF (300 mL) was stirred at RT overnight. The solvent was removed in vacuo and the residue was dissolved in EtOAc (150 mL). The organic phase was washed with 10% aq Na_2CO_3 (100 mL), 10% aq citric acid (100 mL), H_2O (100 mL). The organic phase was dried (Na_2SO_4), filtered and concentrated in vacuo to give oxazolone **28** as a white solid (7.10 g, 65% total yield): HPLC (A): t_R=5.6 min, 96% purity.

6-Bromonaphthalene-2-carboxylic acid [1-(2-phenyl-1(*R*)-{[1-(tetrahydropyran-4-ylmethyl)piperidin-4-ylmethyl]carbamoyl}ethylcarbamoyl)cyclopentyl]amide trifluoroacetate (25 f): A mixture of oxazolone **28** (0.20 g, 0.58 mmol) and amine **29** (0.21 g, 0.58 mmol) in Et_3N (0.2 mL, 1.50 mmol) and DMF (10 mL) was heated at 55 °C for 2 d, then the solvent was removed in vacuo and the residue purified by prep HPLC (Luna column C8(2), 300 Å, 250 mm × 21.20 mm, 15 μm; H_2O+0.1% TFA/CH_3CN+0.1% TFA, 5→95% CH_3CN over 40 min): HPLC (A): t_R=3.9 min, 98% purity; mp: 79–80 °C; ^{1}H NMR (600 MHz, [D$_6$]DMSO): δ=8.85 (s, 1H), 8.67 (br s, 1H,

NH^+), 8.54 (s, 1H), 8.33–8.31 (s, 1H), 8.04–8.00 (m, 3H), 7.84 (d, J= 8.5 Hz, 1H, NH), 7.75 (dd, J=8.7, 1.9 Hz, 1H, NH), 7.64 (brt, J= 5.8 Hz, 1H, NH), 7.20–7.13 (m, 5H), 4.46–4.42 (m, 1H), 3.86–3.83 (m, 2H), 3.39–3.36 (m, 2H), 3.32–3.37 (m, 2H), 3.17 (dd, J=4.2, 14.0 Hz, 1H), 3.06–2.99 (m, 1H), 2.96–2.91 (m, 1H), 2.87 (dd, J=10.6, 14.0 Hz, 1H), 2.82 (brt, J=6.2 Hz, 2H), 2.72–2.66 (m, 1H), 2.62–2.56 (m, 1H), 2.30–2.24 (m, 1H), 1.98–1.91 (m, 2H), 1.82–1.48 (m, 11H), 1.36–1.28 (m, 2H), 1.21–1.15 ppm (m, 2H); MS (ESI+): *m/z*: 703.3 $[M+H]^+$; HRMS: *m/z* $[M+H]^+$ calcd for $C_{38}H_{48}BrN_4O_4$: 703.2859, found: 703.2831.

General procedure for the synthesis of methoxy derivatives 31a–c (GP 2)

4-Methyl-2-methoxybenzoic acid methyl ester (31c): KOH (2.15 g, 38.30 mmol) was added to a solution of **30c** (5.35 g, 32.20 mmol) in CH_3OH (200 mL) and the mixture was stirred at RT for 1 h. The solvent was removed, the residue was dissolved in THF (200 mL) and treated with CH_3I (12 mL, 0.19 mol). The reaction mixture was stirred for 2 d, treated with Et_2NH (30 mL) and stirred for a further 3 h. The solvent was removed in vacuo, the residue portioned between EtOAc (100 mL) and H_2O (50 mL). The organic phase was separated, washed with 3 M aq HCl (3×50 mL), brine (50 mL), saturated aq $NaHCO_3$ (3×50 mL) and dried (Na_2SO_4). After removal of the solvent compound **31c** (5.53 g, 95%) was obtained as an oil: ^{1}H NMR (200 MHz, $CDCl_3$): δ=7.72 (d, J=8.2 Hz, 1H), 6.80–6.75 (m, 2H), 3.89 (s, 3H), 3.86 (s, 3H), 2.38 ppm (s, 3H).

In a similar way, intermediates **31a**–**b** were obtained.

General procedure for the synthesis of benzyl alcohols 32a–c (GP 3)

4-Methyl-2-methoxybenzyl alcohol (32c): A solution of **31c** (5.53 g, 30.72 mmol) in THF (26 mL) was added to a 1 M $LiAlH_4$ suspension in THF (32.30 mmol, 32.3 mL) at 0 °C. The reaction mixture was stirred at the same temperature for 2 h, then Et_2O (30 mL) was added, followed by the careful addition of H_2O (1.25 mL), 15% aq NaOH (1.25 mL) and H_2O (3.7 mL). The solid was removed by filtration and rinsed with Et_2O, the filtrate was concentrated to dryness to give benzyl alcohol **32c** as a colorless oil (4.28 g, 92%): ^{1}H NMR (200 MHz, $CDCl_3$): δ=7.12 (d, J=7.5 Hz, 1H), 6.73 (d, J=7.5 Hz, 1H), 6.68 (s, 1H), 4.61 (s, 2H), 3.81 (s, 3H), 2.34 ppm (s, 3H).

In a similar way, intermediates **32a**–**b** were obtained (see the Supporting Information for characterization data).

General procedure for the synthesis of benzaldehydes 33a–c (GP 4)

4-Chloro-2-methoxybenzaldehyde (33a): A solution of benzyl alcohol **32a** (4.28 g, 24.88 mmol) in $CHCl_3$ (50 mL) was added to a mixture of MnO_2 (25.10 g, 0.29 mol) in $CHCl_3$ (50 mL). The reaction was heated at reflux for 2 h, then filtered through a Na_2SO_4 and Celite pad and washed several times with $CHCl_3$. The filtrate was concentrated in vacuo to give aldehyde **33a** as a yellow solid (4.01 g, 95%): ^{1}H NMR (200 MHz, $CDCl_3$): δ=10.40 (s, 1H), 7.73 (d, J=7.8 Hz, 1H), 6.84 (d, J=7.9 Hz, 1H), 6.78 (s, 1H), 3.92 ppm (s, 3H).

In a similar way, intermediates **33b**–**c** were obtained (see the Supporting Information for characterization data).

General procedure for the synthesis of phenol derivatives 34a–d (GP 5)

4-Methyl-2-hydroxybenzaldehyde (34c): $AlCl_3$ (20.7 g, 0.15 mol) was added to a solution of aldehyde **33c** (4.00 g, 26.66 mmol) in CH_2Cl_2 (160 mL) and the resulting mixture was stirred at RT for 10 h. Then it was poured into a mixture of 37% aq HCl (200 mL) and ice (400 g) and the organic solvent was removed. The aqueous solution was heated at 90 °C for 30 min, then cooled in an ice bath. The product was extracted with CH_2Cl_2 (3×100 mL), the organic phase was washed with brine and dried (Na_2SO_4). After removal of the solvent, aldehyde **34c** (3.43 g, 95%) was obtained as a solid: ^{1}H NMR (200 MHz, $CDCl_3$): δ=11.04 (s, 1H), 9.82 (s, 1H), 7.43 (d, J=7.8 Hz, 1H), 6.85–6.79 (m, 2H), 2.38 ppm (s, 3H).

In a similar way, intermediates **34a**–**b**,**d** were obtained (see the Supporting Information for characterization data).

General procedure for the synthesis of benzo[*b*]furan-2-carboxylic acid *tert*-butyl ester synthesis 35a–k (GP 6)

***tert*-Butyl-6-methylbenzo[*b*]furan-2-carboxylate (35c)**: Cs_2CO_3 (25.30 g, 77.66 mmol) was added to a solution of aldehyde **34c** (3.42 g, 25.11 mmol) in DMSO (40 mL), the mixture was heated to 100 °C then treated with *tert*-butyl bromoacetate (3.9 mL, 26.38 mmol) and heated for 2.5 h. The reaction mixture was cooled and poured into H_2O (400 mL) and EtOAc (200 mL). The organic phase was separated, washed several times with 10% aq K_2CO_3 and dried (Na_2SO_4). After removal of the solvent, **35c** (3.90 g, 67%) was obtained as a brown oil: ^{1}H NMR (200 MHz, $CDCl_3$): δ=7.51 (d, J=7.9 Hz, 1H), 7.37–7.35 (m, 2H), 7.10 (d, J=8.3 Hz, 1H), 2.48 (s, 3H), 1.62 ppm (s, 9H).

In a similar way, intermediates **35a**–**b**,**d**–**k** were obtained (see the Supporting Information for characterization data).

***tert*-Butyl-6-dimethylaminobenzo[*b*]furan-2-carboxylate (35l)**: A mixture of **35k** (2.63 g, 10.00 mmol) and Pd/C 10% (500 mg) in EtOH (200 mL) was stirred under H_2 for 12 h. The catalyst was filtered off and the solvent was removed in vacuo to give the amino derivative as a solid. The crude solid (0.54 g, 2.31 mmol) was redissolved in CH_3CN (25 mL) and treated with K_2CO_3 (0.75 g, 5.43 mmol) and EtI (0.50 mL, 6.22 mmol). The reaction mixture was heated at reflux and stirred for 18 h, then the solvent was removed in vacuo and the residue diluted with EtOAc (25 mL). The organic phase was washed with saturated aq $NaHCO_3$ (3×15 mL) and dried (Na_2SO_4). After removal of the solvent compound **35l** was obtained as an oil (618 mg, 92%): ^{1}H NMR (200 MHz, $CDCl_3$): δ=7.40 (d, J= 9.1 Hz, 1H), 7.31–7.29 (m, 1H), 6.93 (dd, J=9.1, 2.6 Hz, 1H), 6.84–6.82 (m, 1H), 3.35 (q, J=7.1 Hz, 4H), 1.62 (s, 9H), 1.15 ppm (t, J= 7.1 Hz, 6H).

General procedure for the synthesis of benzo[*b*]furan-2-carboxylic acids 36a–j,l (GP 7)

6-Chlorobenzo[*b*]furan-2-carboxylic acid (36a): TFA (30 mL) was added to an ice-cold solution of **35a** (1.44 g, 5.70 mmol) in CH_2Cl_2 (30 mL). The violet solution was stirred at 0 °C for 3 h, then the solvent was removed and carboxylic acid **36a** was obtained as a grey solid (1.10 g, quant): ^{1}H NMR (200 MHz, $[D_6]$DMSO): δ=7.92 (s, 1H), 7.81 (d, J=8.5 Hz, 1H), 7.70–7.68 (m, 1H), 7.41 ppm (dd, J=8.4, 1.8 Hz, 1H).

In a similar way, intermediates **36b**–**j**,**l** were obtained (see the Supporting Information for characterization data).

Final compounds **37a–o** were synthesized according to GP 1 (see above).

Benzo[*b*]furan-2-carboxylic acid [1-(2-phenyl-1(*R*)-{[1-(tetrahydropyran-4-ylmethyl)piperidin-4-ylmethyl]carbamoyl}ethylcarbamoyl)cyclopentyl]amide (37a): HPLC (B): t_R = 11.9 min, 97% purity; ^{1}H NMR (600 MHz, [D$_6$]DMSO): δ = 8.80 (s, 1H), 7.84 (d, J = 8.6 Hz, 1H), 7.80 (d, J = 7.7 Hz, 1H), 7.67 (dd, J = 8.4, 0.8 Hz, 1H), 7.62 (d, J = 0.9 Hz, 1H), 7.51–7.47 (m, 2H), 7.38–7.34 (m, 1H), 7.20–7.11 (m, 5H), 4.45 (ddd, J = 10.5, 8.6, 4.3 Hz, 1H), 3.82–3.78 (m, 2H), 3.28–3.22 (m, 2H), 3.15 (dd, J = 13.8, 4.3 Hz, 1H), 3.00–2.95 (m, 1H), 2.92–2.87 (m, 1H), 2.83 (dd, J = 13.9, 10.5 Hz, 1H), 2.69–2.66 (m, 2H), 2.25 (dt, J = 13.4, 8.1 Hz, 1H), 2.02–2.00 (m, 2H), 1.97–1.92 (m, 1H), 1.81–1.76 (m, 1H), 1.72–1.51 (m, 12H), 1.38–1.32 (m, 1H), 1.10–1.01 ppm (m, 4H); MS (ESI+): m/z: 615.3 $[M+H]^+$.

7-Methylbenzo[*b*]furan-2-carboxylic acid [1-(2-phenyl-1(*R*)-{[1-(tetrahydropyran-4-ylmethyl)piperidin-4-ylmethyl]carbamoyl}ethylcarbamoyl)cyclopentyl]amide (37b): Compound **37b** was synthesized from carboxylic acid **36i** and purified by column chromatography (CH_2Cl_2/CH_3OH, 10:1): HPLC (B): t_R = 13.2 min, 99% purity; mp: 121–124 °C; ^{1}H NMR (600 MHz, [D$_6$]DMSO): δ = 8.69 (s, 1H), 7.85 (d, J = 8.6 Hz, 1H), 7.63 (s, 1H), 7.60 (d, J = 7.7 Hz, 1H), 7.52 (br t, J = 5.8 Hz, 1H), 7.30–7.22 (m, 2H), 7.20–7.11 (m, 5H), 4.46 (ddd, J = 10.5, 8.7, 4.3 Hz, 1H), 3.81–3.78 (m, 2H), 3.27–3.21 (m, 2H), 3.17 (dd, J = 13.9, 4.2 Hz, 1H), 3.01–2.97 (m, 1H), 2.92–2.87 (m, 1H), 2.84 (dd, J = 13.9, 10.6 Hz, 1H), 2.69–2.61 (m, 2H), 2.55 (s, 3H), 2.27 (dt, J = 13.3, 8.0 Hz, 1H), 2.00–1.98 (m, 2H), 1.96–1.90 (m, 1H), 1.80–1.75 (m, 1H), 1.70–1.43 (m, 12H), 1.38–1.31 (m, 1H), 1.09–1.01 ppm (m, 4H); MS (ESI+): m/z: 629.4 $[M+H]^+$; HRMS: m/z $[M+H]^+$ calcd for $C_{37}H_{49}N_4O_5$: 629.3703, found: 629.3719.

For characterization data of compounds **37c–o**, see Supporting Information.

1,6-Dioxaspiro[2.5]octane (39): NaH (2.19 g, 57.00 mmol, 60% dispersion in mineral oil pre-washed with pet ether) was added to a suspension of trimethylsulfonoxonium iodide (12.62 g, 57.00 mmol) in THF (95 mL). The reaction mixture was heated at reflux for 3 h, then ketone **38** (4.90 g, 49.00 mmol) was added, and the mixture was held at reflux for a further 2 h. The mixture was filtered and the filtrate was concentrated in vacuo. The residue was redissolved in CH_2Cl_2 (90 mL) and any solid material removed by filtration. The filtrate was concentrated in vacuo to give the epoxide **39** as a liquid (4.11 g, 73%): ^{1}H NMR (200 MHz, $CDCl_3$): δ = 3.79–3.71 (m, 4H), 2.61 (s, 2H), 1.85–1.71 (m, 2H), 1.50–1.34 ppm (m, 2H).

6-Methylbenzo[*b*]thiophene-2-carboxylic acid [1-(2-phenyl-1(*R*)-{[1-(4-hydroxytetrahydropyran-4-ylmethyl)piperidin-4-ylmethyl]-carbamoyl}ethylcarbamoyl)cyclopentyl]amide (41): A mixture of epoxide **39** (9.92 mmol), amine **40** (1.00 g, 1.83 mmol) and LiN(SO_2CF_3)$_2$ (525 mg, 1.83 mmol) in CH_3CN (50 mL) was stirred at RT for 7 d. The reaction mixture was concentrated in vacuo and the residue diluted with CH_2Cl_2 (50 mL). The organic phase was washed with 5% aq $NaHCO_3$ (5 mL) and dried (Na_2SO_4). After removal of the solvent in vacuo, the residue was triturated with Et_2O and filtered off to give compound **41** (1.18 g, 98%) as a yellow solid: HPLC (A): t_R = 3.6 min, 97% purity; mp: 101–105 °C; ^{1}H NMR (600 MHz, [D$_6$]DMSO): δ = 8.80 (s, 1H), 8.22 (s, 1H), 7.86–7.80 (m, 3H), 7.49 (br t, J = 5.7 Hz, 1H), 7.28 (dd, J = 8.2, 0.9 Hz, 1H), 7.20–7.11 (m, 5H), 4.47–4.42 (m, 1H), 4.03 (s, 1H), 3.62–3.52 (m, 4H), 3.17 (dd, J = 13.9, 4.1 Hz, 1H), 2.98–2.78 (m, 5H), 2.45 (s, 3H), 2.22 (dt, J = 13.7, 8.2 Hz, 1H), 2.16 (s, 2H), 2.05–1.98 (m, 2H), 1.92–1.88 (m, 1H), 1.80–1.75 (m, 1H), 1.70–1.44 (m, 9H), 1.39–1.27 (m, 3H), 1.14–1.07 ppm (m, 2H); MS (ESI+): m/z: 661.4 $[M+H]^+$; HRMS: m/z $[M+H]^+$ calcd for $C_{37}H_{49}N_4O_5S$: 661.3424, found: 661.3423.

[Methoxy-(2*H*-pyran-4(4*H*,5*H*,6*H*)-ylidene)methoxy]trimethylsilane (43): Methyl ester **42** (10.00 g, 69.44 mmol) was added to a freshly prepared LDA solution (75.87 mmol, 28.1 mL of 2.7 M solution of *n*BuLi in heptane and 76.27 mmol, 10.69 mL of *i*Pr$_2$NH) in THF (65 mL) at 0 °C. The yellow mixture was stirred at 0 °C for 30 min, then TMSCl (22 mL, 0.17 mol) was added and the mixture warmed to RT. After 30 min stirring, Et_2O (150 mL) was added and the solid was removed by filtration. The filtrate was concentrated in vacuo and the residue treated with Et_2O in order to precipitate more lithium salts, which were again removed by filtration. Silyl ketene acetal **43** was obtained as a yellow liquid (13.55 g, 90%): ^{1}H NMR (200 MHz, $CDCl_3$): δ = 3.61–3.55 (m, 4H), 3.49 (s, 3H), 2.23–2.09 (m, 4H), 0.19 ppm (s, 9H).

4-Formyltetrahydro-2*H*-pyran-4-carboxylic acid methyl ester (44): A solution of dry DMF (0.31 mol) in dry CH_2Cl_2 (40 mL) was added to a solution of PCl_5 (11.98 g, 77.80 mmol) in dry CH_2Cl_2 (22 mL) at 0 °C. The colorless solution was stirred for 30 min, then **43** (8.40 g, 38.89 mmol) was added. The reaction mixture was stirred at RT for 20 h, then diluted with CH_2Cl_2 (100 mL). The organic phase was washed with saturated aq $NaHCO_3$ (2×100 mL), H_2O (2×100 mL), brine (100 mL) and dried (Na_2SO_4). After removal of the solvent, ester **44** was obtained as an orange oil (5.64 g, 84%): R_f = 0.56 (EtOAc/hexane, 1:1); ^{1}H NMR (200 MHz, $CDCl_3$): δ = 9.50 (s, 1H), 3.73 (s, 3H), 3.64–3.59 (m, 4H), 2.15–1.89 ppm (m, 4H).

4-(Benzyloxymethyl)tetrahydro-2*H*-pyran-4-carboxylic acid (45): $NaBH_4$ (0.39 g, 9.21 mmol) was added portionwise to a solution of **44** (5.64 g, 32.79 mmol) in CH_3OH (65 mL) and H_2O (13 mL) at 0 °C. After the addition was complete, the reaction mixture was stirred at RT for 30 min. Then 1 M aq HCl (4.8 mL) was added and CH_3OH removed in vacuo. The residue was diluted with $CHCl_3$ (80 mL), the phases were separated and the organic phase was washed with H_2O (20 mL), brine (20 mL) and dried (Na_2SO_4). Removal of the solvent in vacuo gave a yellow oil (4.64 g, 81%): R_f = 0.47 (EtOAc/hexane 4:1). The residue was dissolved in THF (60 mL) and added to a mixture of NaH (60% dispersion in silicone oil, 2.34 g, 58.50 mmol, pre-washed with pet ether) in THF (36 mL) at 0 °C. The resulting mixture was stirred at RT for 30 min, then a solution of benzyl bromide (4.48 g, 26.00 mmol) in THF (20 mL) was added and the reaction mixture stirred at RT overnight. Et_2O (100 mL) was added and the solid filtered off. The filtrated was concentrated in vacuo to give the correspondent benzyloxymethyl ester (7.10 g, 91%) as a yellow oil: R_f = 0.78 (EtOAc/hexane 1:1). The latter was treated with aq 1 M NaOH and the resulting mixture was stirred at RT for 1 d. The aqueous phase was extracted with EtOAc (55 mL), then acidified to pH 1 using 37% aq HCl and extracted with EtOAc. The combined organic fractions were dried (Na_2SO_4) and the solvent was removed in vacuo to give carboxylic acid **45** (3.08 g, 46%) as a solid: HPLC (A): t_R = 3.1 min; R_f = 0.64 (EtOAc/hexane 1:1); ^{1}H NMR (200 MHz, $CDCl_3$): δ = 7.37–7.24 (m, 5H), 4.49 (s, 2H), 3.82–3.68 (m, 2H), 3.55–3.38 (m, 2H), 3.47 (s, 2H), 2.12–2.05 (m, 2H), 1.64–1.51 ppm (m, 2H).

4-Methyltetrahydro-2*H*-pyran-4-carboxylic acid (46): Prepared in the same way as compound **47** (see below): ^{1}H NMR (200 MHz, $CDCl_3$): δ = 3.81 (dt, J = 4.2, 11.9 Hz, 2H), 3.60–3.47 (m, 2H), 2.14–2.04 (m, 2H), 1.52 (ddd, J = 14.1, 10.0, 4.1 Hz, 2H), 1.29 ppm (s, 3H).

4-Methoxymethyltetrahydro-2*H*-pyran-4-carboxylic acid (47): Methyl ester **42** (5.00 g, 34.72 mmol) was added to a freshly prepared LDA solution (23.6 mL of 2.7 M solution of *n*BuLi in heptane and 5.35 mL of *i*Pr$_2$NH) in THF (35 mL) at 0 °C. The reaction mixture was stirred at the same temperature for 30 min, then bromomethyl methyl ether (7.72 g, 59.00 mmol) was added and the resulting

mixture was stirred at RT overnight. Saturated aq NH_4Cl (25 mL) was added and the organic solvent removed in vacuo. The aqueous layer was extracted with Et_2O (2×75 mL), the combined fractions were washed with 10% aq $Na_2S_2O_3$ (25 mL) and dried (Na_2SO_4). Removal of the solvent in vacuo gave the methyl ester, which was hydrolyzed by treatment with 1 M aq NaOH, as described for compound **45**, to yield carboxylic acid **47** (4.53 g, 75%) as a yellow oil: ^{1}H NMR (200 MHz, $CDCl_3$): δ = 3.88–3.81 (m, 2H), 3.64–3.53 (m, 2H), 3.46 (s, 2H), 3.35 (s, 3H), 2.11–2.04 (m, 2H), 1.72–1.52 ppm (m, 2H).

4-Hydroxy-4-vinyltetrahydro-2*H*-pyran (48): A solution of **38** (5.00 g, 50.00 mmol) in THF (10 mL) was added to vinylmagnesium bromide (75.00 mmol, 75.0 mL of a 1 M solution in THF). The reaction mixture was stirred at RT for 1 h, then cooled in an ice bath and treated with saturated aq NH_4Cl (10 mL). The mixture was allowed to warm to RT and H_2O (50 mL) was added. The aqueous phase was extracted with EtOAc (50 mL), the organic phase was washed with brine and dried (Na_2SO_4). After removal of the solvent in vacuo, vinyl derivative **48** (5.79 g, 90%) was obtained as a yellow oil: R_f = 0.31 (Et_2O/hexane 7:3); ^{1}H NMR (200 MHz, $CDCl_3$): δ = 5.93 (dd, J = 17.3, 10.7 Hz, 1H), 5.28–5.04 (m, 2H), 3.86–3.65 (m, 3H), 1.87–1.72 (m, 4H), 1.52–1.45 ppm (m, 2H).

4-Methoxytetrahydro-2*H*-pyran-4-carboxylic acid (49): A solution of **48** (3.23 g, 25.23 mmol) in THF (50 mL) was added dropwise to a mixture of NaH (60% dispersion in silicone oil, 2.24 g, 56.00 mmol, pre-washed with pet ether) in THF (35 mL) at 0 °C. The reaction mixture was stirred for 30 min, then CH_3I (8.65 g, 2.50 mmol) was added and stirring was continued at RT overnight. Et_2O (85 mL) was added and the solid filtered off. The filtrated was concentrated in vacuo to give the methoxy derivative (2.61 g, 73%). The crude was redissolved in a mixture of CCl_4 (37 mL), CH_3CN (37 mL), H_2O (55 mL) and treated with sodium metaperiodate (16.10 g, 75.44 mmol) and $RuCl_3{\cdot}3H_2O$ (2.5% mol, 120 mg, 0.46 mmol). The reaction mixture was stirred at RT for 3 h, then solid was removed by filtration and rinsed with $CHCl_3$ (3×50 mL). The two phases of filtrate were separated, the aqueous layer extracted with $CHCl_3$ (2×50 mL) and the combined organic phases dried (Na_2SO_4). After removal of the solvent carboxylic acid **49** (2.36 g, 58%) was obtained as a brown oil: ^{1}H NMR (200 MHz, $CDCl_3$): δ = 9.53 (br s, 1H), 3.80–3.70 (m, 4H), 3.32 (s, 3H), 2.17–2.00 (m, 2H), 1.89–1.82 ppm (m, 2H).

General procedure for the synthesis of amines 50a–d (GP 8)

***C*-[1-(4-Hydroxymethyltetrahydropyran-4-ylmethyl)piperidin-4-yl]methylamine (50a)**: Oxalyl chloride (1.62 mL, 19.10 mmol) and DMF (2 drops) were added to a solution of **45** (1.50 g, 6.00 mmol) in $CHCl_3$ (60 mL). The reaction mixture was stirred at RT for 3 h, then the solvent was removed in vacuo to give the crude acyl chloride. A solution of acyl chloride in CH_2Cl_2 (25 mL) was added to a suspension of isonipecotamide (0.75 g, 6.00 mmol) and Et_3N (0.87 mL, 6.08 mmol) in CH_2Cl_2 (33 mL). The reaction mixture was stirred at RT for 4 h, then diluted with CH_2Cl_2 (50 mL) and washed with H_2O (50 mL), brine and dried (Na_2SO_4). After removal of the solvent in vacuo the diamide (1.90 g, 91%) was obtained as a yellow oil, which was then redissolved in CH_3OH (50 mL), treated with Pd/C 10% (170 mg) and stirred under H_2 for 8 h. The reaction was filtered through Celite and concentrated in vacuo to give the hydroxymethyl derivative, which was redissolved in THF (50 mL), treated with a 1 M $LiAlH_4$ solution in THF (9.50 mmol, 9.53 mL) and heated at reflux for 4 h. The reaction mixture was cooled to 0 °C and treated with H_2O (1 mL), 1 M aq NaOH (0.5 mL) and H_2O (7.3 mL). The solid impurity was filtered off and rinsed with THF. The filtrate was concentrated in vacuo and the residue diluted with $CHCl_3$ (100 mL). The organic phase was separated and dried (Na_2SO_4). After removal of the solvent in vacuo the diamine **50a** (610 mg, 42%) was obtained as an oil: ^{1}H NMR (200 MHz, $CDCl_3$): δ = 3.81–3.45 (m, 6H), 2.98–2.92 (m, 1H), 2.56–2.43 (m, 3H), 2.23–2.13 (m, 1H), 1.70–1.16 ppm (m, 12H).

In a similar way, intermediates **50b–d** were obtained (see the Supporting Information for characterization data).

General Procedure for the Synthesis of 52a–d (GP 9)

Boc-D-Phe-OSu (1.00 equiv) was added to a solution of amine (1.00 equiv, 0.66 mmol) in DMF (10 mL) and the reaction mixture was stirred at RT overnight. The solvent was removed in vacuo and the residue was treated with 5% aq $NaHCO_3$. The aqueous phase was extracted with CH_2Cl_2 (3×20 mL), the combined organic fractions were washed with brine and dried (Na_2SO_4). After removal of the solvent, the residue was redissolved in CH_2Cl_2 (6 mL) and treated with TFA (2 mL). The reaction mixture was stirred 30 min, then diluted with toluene (10 mL) and the solvent removed in vacuo. The residue was treated with 5% aq $NaHCO_3$ and the free base extracted with CH_2Cl_2 (3×10 mL). The organic solvent was removed in vacuo and the residue redissolved in DMF (10 mL). Oxazolone **51** (1.00 equiv) was added and the reaction mixture stirred overnight. The solvent was removed in vacuo, the residue was treated with 5% aq $NaHCO_3$ and the aqueous phase was extracted with EtOAc (3×10 mL). The combined organic fractions were washed with brine and dried (Na_2SO_4). After removal of the solvent in vacuo the crude was purified by flash chromatography or prep HPLC.

6-Methylbenzo[*b*]thiophene-2-carboxylic acid [1-(2-phenyl-1(*R*)-{[1-(4-hydroxymethyltetrahydropyran-4-ylmethyl)piperidin-4-ylmethyl]carbamoyl}ethylcarbamoyl)cyclopentyl]amide trifluoroacetate (52a): Amide **52a** was obtained according to GP 9 as a white freeze-dried material after prep HPLC (Symmetry C_{18}, 300 Å, 300 mm × 19 mm, 7 μm, H_2O + 0.1% TFA/CH_3CN + 0.1% TFA, 30→60% CH_3CN over 40 min, flow rate = 20 mL min^{-1}): HPLC (A): t_R = 3.7 min, 97% purity; ^{1}H NMR (600 MHz, [D$_6$]DMSO): δ = 8.86 (s, 1H), 8.26 (s, 1H), 7.90–7.87 (m, 2H), 7.82 (s, 1H), 7.60 (br t, J = 6.2 Hz, 1H), 7.31 (d, J = 8.2 Hz, 1H), 7.23–7.14 (m, 5H), 5.45 (br s, 1H), 4.47–4.43 (m, 1H), 3.63–3.52 (m, 6H), 3.19 (dd, J = 13.9, 4.2 Hz, 1H), 3.07–2.85 (m, 8H), 2.47 (s, 3H), 2.27–2.21 (m, 1H), 1.95–1.90 (m, 1H), 1.81–1.50 ppm (m, 16H); MS (ESI+) m/z: 675.5 $[M+H]^+$.

For characterization data of compounds **52a–d**, see Supporting Information

(1,3-Dioxan-5-yl)methanol (54): A mixture of triol **53** (2.29 g, 21.60 mmol), $CH_2(OMe)_2$ (8 mL, 86.40 mmol), LiBr (0.40 g, 4.30 mmol) and *p*-toluenesulfonic acid (0.36 g, 2.16 mmol) in CH_2Cl_2 (20 mL) was stirred at RT overnight. The solvent was removed in vacuo and the crude purified by column chromatography (CH_2Cl_2/CH_3OH 9:1) to give **54** (800 mg, 31%) as an oil: R_f = 0.5 (CH_2Cl_2/CH_3OH 9:1); ^{1}H NMR (200 MHz, $CDCl_3$): δ = 4.83 (q, J = 7.1 Hz, 2H), 4.00 (dd, J = 11.6, 3.8 Hz, 2H), 3.83–3.71 (m, 4H), 2.03–1.86 ppm (m, 1H).

1,3-Dioxan-5-carbaldehyde (55): Dess–Martin periodinane (20 mL, 7.00 mmol) was added to a solution of **54** (800 mg, 6.77 mmol) in CH_2Cl_2 (50 mL), the reaction mixture was stirred for 2 h, then saturated aq $NaHCO_3$ (100 mL) and $Na_2S_2O_3$ (25 g) were added. After 30 min stirring, the phases were separated, the aqueous layer extracted with CH_2Cl_2 (2×50 mL) and the combined organic phases were dried (Na_2SO_4), filtered and concentrated to give aldehyde **55** as a white solid (700 mg, 90%): ^{1}H NMR (200 MHz, $CDCl_3$): δ = 9.88

(s, 1H), 4.92–4.80 (m, 2H), 4.30 (dd, J=12.0, 4.2 Hz, 2H), 4.08 (dd, J=12.0, 3.5 Hz, 2H), 2.50–2.41 ppm (m, 1H).

6-Methylbenzo[*b*]thiophene-2-carboxylic acid [1-(2-phenyl-1(*R*)-{[1-([1,3]dioxan-5-ylmethyl)piperidin-4-ylmethyl]carbamoyl}ethylcarbamoyl)cyclopentyl]amide (56a): A mixture of **40** (200 mg, 0.36 mmol) and **55** (700 mg, 6.00 mmol) in CH_3OH (5 mL) was treated with AcOH (0.1 mL) and $NaCNBH_3$ (100 mg, 1.50 mmol), then stirred at RT overnight. The reaction was treated with 1 M aq HCl (to pH 3) and stirred for a further 30 min, then 5% aq K_2CO_3 was added until the solution reached pH 10. The aqueous phase was extracted with $CHCl_3$ (3×50 mL) and the combined organic phases were dried (Na_2SO_4). The crude material was concentrated in vacuo and purified by flash column chromatography (EtOAc/CH_3OH, 9:1): HPLC (A): t_R=3.7 min, 97% purity; mp: 95–98 °C; ^{1}H NMR (600 MHz, [D$_6$]DMSO): δ=8.80 (s, 1H), 8.22 (s, 1H), 7.86–7.83 (m, 2H), 7.82 (s, 1H), 7.46 (br t, J=5.8 Hz, 1H), 7.28 (dd, J=8.2, 0.7 Hz, 1H), 7.20–7.11 (m, 5H), 4.81 (d, J=6.0 Hz, 1H), 4.63 (d, J=6.0 Hz, 1H), 4.45 (ddd, J=10.5, 8.8, 4.3 Hz, 1H), 3.90–3.86 (m, 2H), 3.46–3.42 (m, 2H), 3.18–3.15 (m, 1H), 2.94 (t, J=6.4 Hz, 2H), 2.84 (dd, J=13.9, 10.6 Hz, 1H), 2.70–2.68 (m, 2H), 2.45 (s, 3H), 2.23 (dt, J=13.4, 8.2 Hz, 1H), 2.09–2.05 (m, 2H), 1.94–1.88 (m, 2H), 1.80–1.33 (m, 11H), 1.09–1.02 ppm (m, 2H); MS (ESI+): m/z: 647.3 $[M+H]^+$; HRMS: m/z $[M+H]^+$ calcd for $C_{36}H_{47}N_4O_5S$: 647.3267, found: 647.3250.

For the synthesis of compounds **56b**, **56c**, **60**, **61a** and **61b**, see Supporting Information.

1-(Tetrahydro-2*H*-pyran-4-yl)ethanol (66): A 1.6 M CH_3Li solution in Et_2O (15.6 mL, 25.00 mmol) was added quickly to a solution of **64** (1.30 g, 10.00 mmol) in THF (60 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 2 h, then TMSCl (21 mL) was added and the reaction was warmed to RT. The reaction was neutralized with aq 1 M HCl and extracted with Et_2O (3×60 mL). The combined organic fractions were dried (Na_2SO_4), filtered and concentrated in vacuo to give ketone **65** as an oil. The crude ketone was redissolved in CH_3OH (20 mL), treated with $NaBH_4$ (220 mg, 5.70 mmol) and stirred for 2 h. The reaction was then treated with 1 M aq HCl (10 mL) and the organic solvent removed in vacuo. The residue was diluted with saturated aq $NaHCO_3$ (20 mL) and CH_2Cl_2 (20 mL), the organic phase was separated and dried (Na_2SO_4). After removal of the solvent alcohol **66** (405 mg, 31%) was obtained as an oil: ^{1}H NMR (200 MHz, $CDCl_3$): δ=4.08–3.97 (m, 2H), 3.44–3.31 (m, 2H), 1.80–1.26 (m, 6H), 1.18 ppm (s, 3H).

{1-[1-(Tetrahydro-2*H*-pyran-4-yl)ethyl]piperidin-4-yl}methanamide (67): Triflic anhydride (0.26 mL, 1.67 mmol) was added to a solution of **66** (218 mg, 1.67 mmol) and pyridine (0.13 mL, 1.67 mmol) in CH_2Cl_2 (8 mL) at −30 °C. The reaction was stirred at 0 °C for 30 min then warmed to RT and stirred for a further 30 min. The reaction was then diluted with CH_2Cl_2 (20 mL), and the organic phase was separated, washed with 5% aq $NaHCO_3$ and dried (Na_2SO_4). The solvent volume was reduced in vacuo to 3 mL and the residue was added to a solution of isonipecotamide (256 mg, 2.00 mmol) in DMF (3 mL). The reaction was stirred for 16 h, then the solvent was removed in vacuo and the residue diluted with CH_2Cl_2 (10 mL) and 5% aq K_2CO_3 (10 mL). The organic solvent was separated, dried (Na_2SO_4), filtered and concentrated to give the amide as a white solid. The crude amide was redissolved in THF (10 mL) and treated with a 1 M $LiAlH_4$ solution in THF (1.20 mmol, 1.2 mL) at 0 °C. The reaction mixture was heated at reflux for 1 h, then was cooled to 0 °C and treated with H_2O (0.1 mL), 1 M aq NaOH (0.3 mL) and stirred for 30 min. The precipitate was filtered off and rinsed with THF. Diamine **67** was obtained as an oil: ^{1}H NMR (200 MHz, $CDCl_3$): δ=3.97 (dd, J=11.6, 3.7 Hz, 2H), 3.35 (t, J=11.8 Hz, 2H), 2.70–2.36 (m, 5H), 2.23 (dd, J=8.8, 6.6 Hz, 1H), 2.09–1.47 (m, 6H), 1.33–1.09 (m, 4H), 0.88 ppm (d, J=6.6 Hz, 3H); MS (ESI+): m/z: 227.3 $[M+H]^+$.

***N*,*N*-Dibenzyl-1-(piperidin-4-yl)methanamine (70)**: Et_3N (3.2 mL, 23.08 mmol) and benzyl bromide (1.85 mL, 15.55 mmol) were added to a suspension of **69** (1.90 g, 7.60 mmol) in CH_3CN (20 mL). The resulting mixture was stirred at 100 °C for 6 h and at RT overnight. The solvent was removed in vacuo and the crude oil was purified by column chromatography ($CHCl_3$/CH_3OH, 25:1) to give the *N*,*N*-dibenzylamino derivative, which was then redissolved in CH_2Cl_2 (25 mL), treated with TFA (25 mL) and the solution stirred at RT for 1.5 h. The reaction mixture was diluted with toluene and the solvent was removed in vacuo. The residue was dissolved in CH_2Cl_2 (25 mL), treated with 5% aq K_2CO_3 and the phases were separated. The organic phase was dried (Na_2SO_4), filtered and concentrated to give amine **70** as an oil (1.4 g, 63%): ^{1}H NMR (200 MHz, $CDCl_3$): δ=7.38–7.18 (m, 10H), 3.51 (s, 4H), 3.05–2.90 (m, 2H), 2.53 (dt, J=12.1, 2.5 Hz, 2H), 2.30–2.14 (m, 2H), 1.81–1.60 (m, 4H), 1.03–0.83 (m, 2H); MS (ESI+): m/z: 295.1 $[M+H]^+$.

4-{[(Dibenzylamino)methyl]piperidin-1-yl}(tetrahydro-2*H*-pyran-4-yl)methanone (71): A solution of **68** (1.10 g, 7.43 mmol) in CH_2Cl_2 (10 mL) was added to a solution of **70** (1.40 g, 4.76 mmol) and Et_3N (2 mL, 14.30 mmol) in CH_2Cl_2 (20 mL). The reaction was stirred at RT for 3 h, then 5% aq K_2CO_3 (10 mL) was added, the organic phase was separated and dried (Na_2SO_4). After removal of the solvent the crude was purified by column chromatography (CH_2Cl_2/CH_3OH, 50:1) to give amide **71** (1.60 g, 76%) as a solid: R_f=0.45 ($CHCl_3$/CH_3OH 20:1); ^{1}H NMR (600 MHz, [D$_6$]DMSO): δ=7.34–7.30 (m, 8H), 7.25–7.21 (m, 2H), 4.27 (d, J=12.6 Hz, 1H), 3.87 (d, J=13.2 Hz, 1H), 3.82–3.79 (m, 2H), 3.50 (q, J=13.4 Hz, 4H), 3.35 (dt, J=11.7, 2.2 Hz, 2H), 2.95 (t, J=12.3 Hz, 1H), 2.80 (tt, J=11.2, 3.9 Hz, 1H), 2.52–2.46 (m, 1H), 2.19 (d, J=7.2 Hz, 2H), 1.90–1.82 (m, 1H), 1.78 (t, J=11.3 Hz, 2H), 1.57–1.39 (m, 4H), 0.85–0.78 (m, 1H), 0.72–0.65 ppm (m, 1H).

{1-[2-(Tetrahydro-2*H*-pyran-4-yl)propan-2-yl]piperidin-4-yl}methanamine (72): A mixture of **71** (250 mg, 0.60 mmol) and $POCl_3$ (1 mL) was heated at 100 °C for 2 h, then cooled down and concentrated in vacuo. The residue was dissolved in THF (2.5 mL) and added to CH_3MgBr (1 mL, 3 M in Et_2O) at −10 °C. The reaction mixture was stirred at RT overnight, then H_2O was added and the product extracted with EtOAc (10 mL). The organic phase was washed with 5% aq $NaHCO_3$ and H_2O and dried (Na_2SO_4). The crude was purified by column chromatography (CH_2Cl_2/CH_3OH, 20:1) to give amine **72** (60.0 mg, 42%): R_f=0.30 ($CHCl_3$/CH_3OH 20:1), which was subsequently debenzylated by hydrogenation in *i*PrOH (2 mL) in the presence of Pd/C 10% (50.0 mg) and NH_4COOH (50 mg) at 60 °C for 1 h: ^{1}H NMR (200 MHz, $CDCl_3$): δ=4.00 (dd, J=11.0, 4.2 Hz, 2H), 3.33 (dt, J=11.5, 2.0 Hz, 2H), 3.00–2.93 (m, 2H), 2.56 (d, J=6.3 Hz, 2H), 2.33–2.30 (m, 2H), 2.03 (t, J=10.8 Hz, 2H) 1.74–1.05 (m, 8H), 0.88 ppm (s, 6H).

{1-[2-(Tetrahydro-2*H*-pyran-4-yl)cyclopropyl]piperidin-4-yl}methanamine (73): EtMgBr (1.65 mmol, 0.55 mL, 3 M in Et_2O) was added to a solution of **71** (320 mg, 0.78 mmol) and Ti(O*i*Pr)$_4$ (0.84 mmol, 0.25 mL) in THF (5 mL) at RT. The reaction mixture was stirred overnight, then poured into H_2O (20 mL) and stirred for a further 30 min. EtOAc was added, the organic phase was separated and dried (Na_2SO_4). After removal of the solvent the crude was purified by column chromatography ($CHCl_3$, 100%) to give the cyclopropyl derivative **73** protected as the *N*,*N*-dibenzylamine (62.0 mg, 33%): R_f=0.60 ($CHCl_3$/CH_3OH 9:1); ^{1}H NMR (200 MHz, $CDCl_3$): δ=

7.42–7.17 (m, 10H), 4.04–3.90 (m, 2H), 3.50 (s, 6H), 3.50–3.29 (m, 2H), 2.74–2.66 (m, 2H), 2.23 (dd, $J=10.8$, 7.0 Hz, 2H) 1.91–1.31 (m, 8H), 1.06–0.81 (m, 2H), 0.58–0.52 (m, 2H), 0.40–0.34 ppm (m, 2H). The compound was deprotected by hydrogenation in *i*PrOH (2 mL) in the presence of Pd/C 10% (50 mg) and NH_4COOH (50 mg) at 60 °C for 2 h to give amine **73**: MS (ESI+): *m/z*: 239.1 $[M+H]^+$.

Products **74a–c** were obtained in a similar way as described in GP 9 (see above).

6-Methylbenzo[*b*]thiophene-2-carboxylic acid [1-(2-(*R*)-phenyl-1-{[1-(1(RS)-tetrahydropyran-4-ylethyl)piperidin-4-ylmethyl]carbamoyl}ethylcarbamoyl)cyclopentyl]amide trifluoroacetate (74a): Amide **74a** was obtained as a white freeze-dried material after prep HPLC (Symmetry C_{18}, 300 Å, 300 mm × 19 mm, 7 μm, H_2O + 0.1% TFA/CH_3CN + 0.1% TFA, gradient 30 to 70% CH_3CN in 40 min, flow rate 20 mL min^{-1}): HPLC (A): $t_R=3.9$ min, 99% purity; mp: 69–72 °C; ^{1}H NMR (600 MHz, $[D_6]$DMSO): $\delta=8.87$ (s, 1H), 8.26 (s, 1H), 7.89 (d, $J=9.0$ Hz, 1H), 7.87 (d, $J=8.2$ Hz, 1H), 7.81 (s, 1H), 7.62 (br t, $J=5.9$ Hz, 1H), 7.29 (d, $J=8.2$ Hz, 1H), 7.22–7.13 (m, 5H), 4.48–4.43 (m, 1H), 3.89–3.85 (m, 2H), 3.40–3.28 (m, 4H), 3.19 (dd, $J=13.9$, 3.9 Hz, 1H), 3.14–3.08 (m, 2H), 3.03–2.87 (m, 3H), 2.85 (dd, $J=13.9$, 10.9 Hz, 1H), 2.45 (s, 3H), 2.24 (dt, $J=13.4$, 8.1 Hz, 1H), 2.07–2.02 (m, 1H), 1.94–1.27 (m, 15H), 1.18 ppm (d, $J=6.9$ Hz, 3H); MS (ESI+): *m/z*: 659.3 $[M+H]^+$; HRMS: *m/z* $[M+H]^+$ calcd for $C_{38}H_{51}N_4O_4S$: 659.3631, found: 659.3607.

For characterization data of compounds **74b**,**c**, see Supporting Information.

Biology

[^{125}I]Neurokinin A was purchased from Amersham Biosciences (Buckinghamshire, UK). The IC_{50} value is given as the ligand concentration at which 50% radioligand-specific binding is inhibited. Male adult albino guinea pigs (Dunkin Hartley) were from Charles River, Italy.

All experiments involving animals were performed in accordance with the Declaration of Helsinki, with the principles and guidelines of the European Union regulations and the local ethical committee.

Binding experiments

All compounds (1 pM–10 μM) were tested for their ability to inhibit [^{125}I]neurokinin A (0.15 nM) binding to membranes of CHO expressing the human tachykinin NK_2 receptor under known assay conditions.[5] Nonspecific binding was determined in the presence of unlabeled neurokinin A (1 μM). Affinity is expressed as the pK_i ($-\log K_i$), obtained by normalizing the measured IC_{50} value by the radioligand dissociation constant (K_d) and its concentration ([L*]), according to the Cheng–Prusoff[19] relationship.

Organ bath experiments

The experiments were performed on guinea pig isolated proximal colon circular smooth muscle preparations (GPC). 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.[4b] 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). Antagonists were pre-incubated for 15 min before performing the agonist concentration–response measurements, and their potency was expressed as the pK_B value calculated from the equation:

$$pK_B = \log[CR-1] - \log[\text{antagonist}] \quad (2)$$

where CR is the ratio of equieffective concentrations (EC_{50}) of agonist in the presence and absence of antagonist.[20]

In vivo experiments

Male albino guinea pigs (350–400 g) were anesthetized with urethane (1.5 g kg^{-1}, sc) and a polyethylene catheter was inserted into the left jugular vein for iv administration of drugs. Guinea pigs were mechanically ventilated with a ventilation pump at a rate of 50 strokes min^{-1} and at a respiration volume of 10 mL kg^{-1}. The body temperature was kept constant at 36 °C by a thermoregulated lamp. The abdomen was opened and a latex balloon, obtained from 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, Chalgrove, UK). Five minutes before starting the experiments, guinea pigs were treated with hexamethonium bromide (13.8 μmol kg^{-1}, iv) as bolus followed by continuous infusion of the same solution at a rate of 300 μL h^{-1} to prevent reflex cholinergic responses. The compounds or their vehicle (DMSO) were administered iv (3 μmol kg^{-1}) in a volume of 100 μL kg^{-1}. For id administration, the NK_2 tachykinin receptor antagonists (10 μmol kg^{-1}) were injected in a volume of 1 mL kg^{-1} into the proximal duodenum by a 26G needle syringe, at ~2 cm from the pyloric sphincter. [βAla]NKA(4-10) (3 nmol kg^{-1}, 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.

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