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Pyrrolidine-carboxamides and oxadiazoles as potent hNK_1 antagonists

Jonathan R. Young,[a,*] Ronsar Eid,[a] Cherilyn Turner,[a] Robert J. DeVita,[a] Marc M. Kurtz,[b] Kwei-Lan C. Tsao,[b] Gary G. Chicchi,[b] Alan Wheeldon,[c] Emma Carlson[c] and Sander G. Mills[a]

[a]*Department of Medicinal Chemistry, Merck Research Laboratories, PO 2000, Rahway, NJ 07065-0900, USA*
[b]*Department of Immunology, Merck Research Laboratories, PO 2000, Rahway, NJ 07065-0900, USA*
[c]*Merck Sharpe & Dohme Research Laboratories, Harlow, UK*

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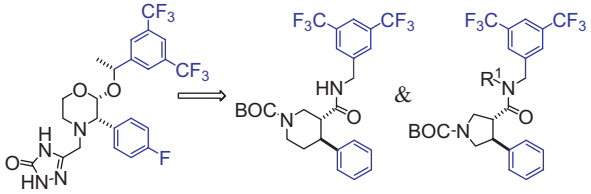
Abstract—The preparation and structure–activity-relationships of novel pyrrolidine-carboxamides and oxadiazoles are described. Compounds in this series were found to be potent hNK_1 antagonists in vitro and efficacious in vivo with minimal interactions with P_{450} liver enzymes. Oxadiazole analog **22** was determined to have excellent hNK_1 binding affinity, functional activity, and a good PD response in vivo.

Neurokinin-1 (NK_1) is a member of the superfamily of G-protein-coupled-receptors whose natural ligand is Substance P. The receptor is highly expressed in both the CNS and also peripheral tissues. Antagonism of the NK_1 receptor has been proposed as a therapeutic strategy for conditions such as cystitis,[1] emesis,[2] and major CNS disorders[3] due to effects of NK_1 agonists in animal models of those disorders.

Previous efforts from these laboratories produced Aprepitant (**1**)[4] which is approved for use in chemotherapy-induced nausea and vomiting and for post-operative nausea and vomiting (Fig. 1). The goals of our backup program focused on achieving sufficient brain penetration and pharmacokinetics consistent with once daily oral dosing, while minimizing the potential for cytochrome P_{450} interactions. This paper will describe the discovery of novel pyrrolidine scaffold **3** and a subsequent article will describe the piperidine scaffold **2**, both of which led to potent and efficacious NK_1 antagonists with insignificant inhibition of the P_{450} class of enzymes.



1: Aprepitant
NK_1 IC_{50} = 0.09 nM
Cyp 3A4 IC_{50} = 46 nM

2 (±):
NK_1 IC_{50} = 450 nM

3a (±): R^1 = H
NK_1 IC_{50} = 142 nM
3b: R^1 = Me
NK_1 IC_{50} = 11 nM

Figure 1. From Aprepitant (**1**) to rationally derived leads **2** and **3**.

An essential structural component to Aprepitant (**1**) and other NK_1antagonists has been the bis-phenyl motif, typically a tethered-bis-CF_3-phenyl moiety adjacent to a phenyl group appended to a central core ring. While maintaining this crucial feature, we were interested in replacing the ether tether found in **1** with an amide side-chain. To generate diversity, a systematic survey of disubstituted pyrrolidine and piperidine scaffolds employing an amide linkage to the bis-CF_3-phenyl was undertaken in which all regio- and stereochemical combinations were prepared and evaluated. Two promising leads emerged from this initial study, specifically piperidine **2** and pyrrolidine **3a** (Fig. 1).

Keywords: NK_1 antagonists; Substance P.
* Corresponding author. Present address: Merck & Co., Inc., 33 Avenue Louis Pasteur, Boston MA 02115, USA. Tel.: +1 6179922331; fax: +1 6179922406; e-mail: jonathan_young@merck.com

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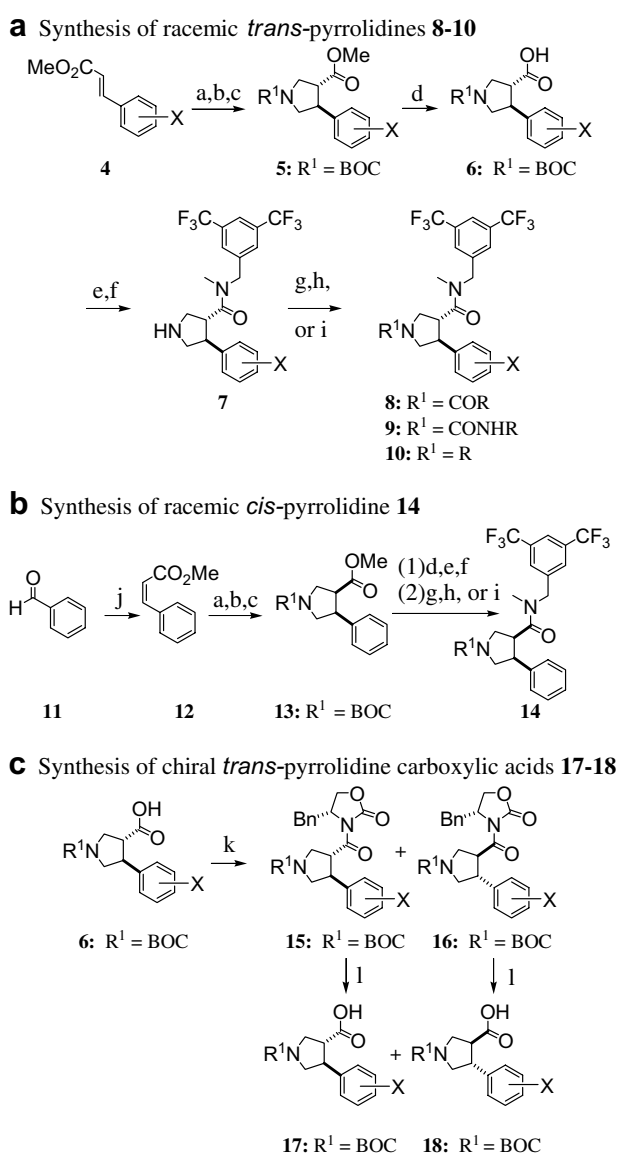


Scheme 1. Reagents and conditions: (a) *N*-(methoxymethyl)-*N*-(trimethylsilylmethyl)benzylamine, TFA, DCM; (b) ACE-Cl, PhMe, 110 °C; MeOH, 80 °C; (c) BOC_2O, DMAP, Et_3N, DCM; (d) NaOH, THF; (e) EDC, HOBT, DMAP, *N*-methyl-3,5-bis-trifluoromethylbenzylamine, DCM; (f) HCl-EtOAc; (g) EDC, DMAP, R_1CO_2H, DCM; (h) 1-piperidinecarbonyl chloride, DCM, Et_3N, DMAP or cyclohexylisocyanate; (i) cyclohexanecarboxaldehyde, $Na(OAc)_3BH$, HOAc, DCE; (j) $(F_3CCH_2O)_2POCH_2CO_2Me$, NaHMDS, 18-C-6, THF; (k) pivoyl chloride, Et_3N, DCM; (*S*)-benzyloxazolidine, LiCl, Et_3N, THF; separate diastereomers; (l) LiOOH, THF.

Azomethine ylide [3+2] cycloadditions afforded convenient access, after protecting group manipulations, to both trans and cis pyrrolidine derivatives **5** and **13** from the corresponding *E* and *Z*[5] cinnamate esters **4** and **12**, respectively, as key synthetic intermediates (Scheme 1).

The racemic analogs in Table 1 required preparation of the common pyrrolidine intermediate **7** which was readily prepared from ester **5** after saponification, amide coupling, and pyrrolidine deprotection. Pyrrolidine **7** was converted to amides **8** (EDC, HOBT, carboxylic acids), ureas **9** (carbamoyl chlorides or isocyanates), or alkylamines **10** (reductive amination) to yield the targeted analogs. *Cis*-pyrrolidine **14** was prepared from ester **13** analogously to the sequence from **5** to **9**.

The pure enantiomers found in Table 2 were prepared from acid **6** by conversion to the separable acyl-oxazolidinone diastereomers **15** and **16**[6] under the conditions reported by Ho and Mathre.[7] Hydrolysis of intermediates **15** and **16** using LiOOH furnished chiral acids **17** and **18** which were transformed to **27**–**29** (see Table 2) according to the procedures described for racemic acid **6**.

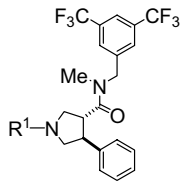
Oxadiazoles **20**, **22**, **25**, and **26** were prepared from amide-oximes **19**, **21**, and **24** by methodology reported from these laboratories,[8] wherein the requisite acids were coupled to the amide-oximes (EDC/HOBT) followed by thermal cyclodehydration (Scheme 2). Amide-oximes **19**, **23**, and **24** in turn were prepared in three steps from acids **17a**, **18a**, and **23**,[9] respectively, by conversion to the primary amides, dehydration to the nitrile, followed by treatment with hydroxylamine. Deprotection of the *tert*-butylcarbamate group under acidic conditions followed by coupling to 1-acetyl-4-piperidinecarboxylic acid furnished targeted amides **20**, **22**, **25**, and **26**.

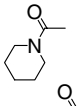
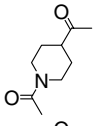
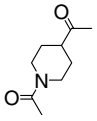
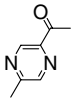
The hNK_1 binding affinities of carboxamides **3** (Fig. 1) were found to be sensitive to amide substitution. For example, *N*-methyl-amide **3b** led to a >10-fold improvement in receptor binding affinity over **3a**. We therefore maintained this potency-enhancing feature in subsequent analogs.

We next focused on *N*-pyrrolidine variations (Table 1) and found that amides (**8**) were generally well tolerated and preferred over amines (**10**). For example, the hNK_1 antagonist activity of cyclohexylamide **8a** was 2-orders of magnitude more potent than the corresponding amine **10a**. Urea **9a** which is isosteric with amide **8a** was substantially less potent than **8a**. The homologated urea **9b** on the other hand was equipotent to **8a**. Incorporation of basic substituents, such as piperidine **8b**, led to decreased receptor binding affinity compared to **8a**. However, acylated derivatives of **8b,** such as amide **8c**, retained the potency of **8a**. The receptor binding affinity of the pyrimidine amides was sensitive to the substitution pattern in which the IC_{50}'s were in the order: **8d** < **8f** ≪ **8g**. Pyrazines **8e** and **8h** and pyrimidine **8d** were the most potent heteroaromatic carboxamide analogs identified.

Intrigued by the potent hNK_1 binding activity of **8c**, we prepared the single enantiomer and determined that the active component was the 3(*R*)-carboxamide-4(*S*)-phenyl isomer **27** (Table 2). As a major indication for NK_1 antagonists is the treatment of chemotherapy-induced emesis, it is beneficial to design NK_1 antagonists that lack the potential to participate in drug-drug interactions. Accordingly, we were gratified to observe that **27** along with all analogs in Table 2 were only weak inhibitors of the P_{450} liver enzymes.

Table 1. Optimization of the nitrogen substituent



Entry[a]	R^1	hNK_1[b] IC_{50} (nM)
3a[c]		142
3b		11
8a		0.68
10a		62
9a		13
9b		0.79
8b		4.1
8c		0.42
14a[d]		3.7
8d		0.84
8e		0.74
8f		3.4
8g		24
8h		0.58

[a] All compounds are racemic and gave satisfactory ^1H NMR and mass spectral data.

[b] Displacement of [^{125}I] SP from the human hNK_1 receptor expressed in CHO cells. All values represent the average of 2–6 independent experiments. Interexperimental variability was typically less than twofold. See Ref. 4 and references cited therein.

[c] **3a** is a secondary amide (see Fig. 1).

[d] **14a** was a *cis*-3,4 pyrrolidine.

Receptor binding affinity was also sensitive to the stereochemistry of the pyrrolidine scaffold. In contrast to the morpholine scaffold present in Aprepitant **1** wherein the 1,2-*cis* relationship was favored (10-fold),[4a] the 1,2-*trans* stereochemistry of **8c** was > 10-fold more potent than that of the *cis*-isomer **14a** in the pyrrolidine class of hNK_1 inhibitors. This finding was consistent with the cyclopentane analogs described from these laboratories.[10]

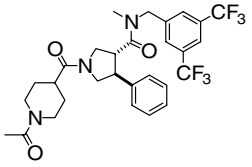
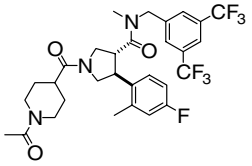
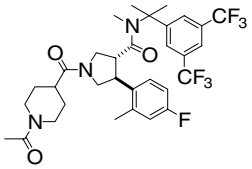
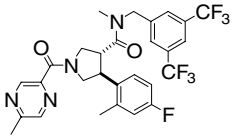
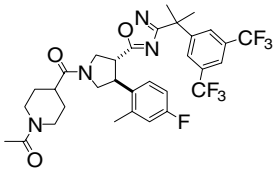
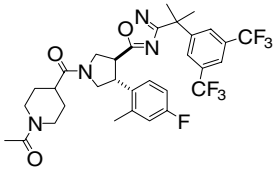
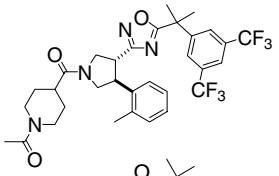
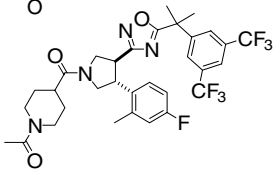
Administration of an NK_1 agonist (icv) has previously been reported to elicit a rapid and robust foot tapping response in gerbils and provides the basis for an important in vivo model of functional antagonist activity.[11] The assay was performed by dosing the test compound by iv infusion, followed by the central administration of the NK_1 agonist at a specified time post-drug infusion and monitoring the ability of the compound to block the agonist effect.

We evaluated **8c** in the in vivo gerbil foot tapping (GFT) model and found that **8c** demonstrated rapid brain penetration and was able to effectively block the agonist-induced foot-tapping response in the gerbil at an early time point ($t = 0$; $ID_{50} = 0.41$ mpk), but showed a poor duration of action (24 h pre-dose prior to agonist administration). This was consistent with the short in vivo half-life determined in rats ($t_{1/2} = 0.7$ h). Amide **8c** showed a 10-fold shift in receptor-antagonism when the binding assay was performed in the presence of 50% human serum, which often correlates with in vivo plasma protein binding, and should have a significant impact on in vivo activity.

Another potential contributing factor to the poor duration of the pharmacodynamic response could be due to a rapid dissociation from the receptor. We have recently developed a functional binding assay (IP-1) that measures the ability of an hNK_1 antagonist (100 nM) to block the biochemical response (inositol phosphate generation) to Substance P (at a concentration of 10 μM) in CHO cells engineered to overexpress hNK_1.[12] Significant suppression of the maximal response can be interpreted as a desirable property for an antagonist, insofar as it implies that the compound can maintain receptor blockade even in the presence of a large excess of agonist. The data are reported as the percent of the Substance P response remaining (% SPRR) after a 30 min incubation time between the drug (100 nM) and hNK_1 cells followed by treatment with the agonist (10 μM). Pyrrolidine **8c** was found to be functionally ineffective (81% SPRR) at blocking the effect of Substance P in this assay.

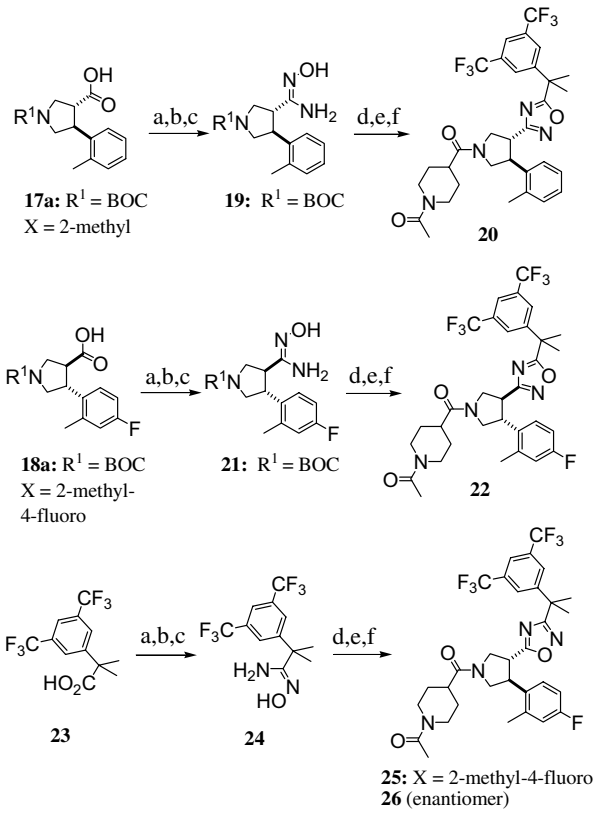
During the course of our optimization studies of the 4-phenylpyrrolidine substituent, a key discovery that led to improved IP-1 functional activity across this series was incorporation of an *ortho*-methyl on the phenyl ring. For example, 2-methyl-4-fluorophenyl analog **28** was able to block 97% (3% SPRR) of the Substance response despite showing only a modest twofold improvement in intrinsic binding activity over analog **27**. Amide **28** showed good in vivo activity for up to 4 h in the GFT

Table 2. SAR for enantiomerically pure pyrrolidine carboxamides and oxadiazles

Compound[a]	Structure[a]	hNK_1[b] IC_{50} (nM)/50% HS	IP-1[12] % SPRR	P_{450} Inh Isozyme: IC_{50} (μM)	Gerbil Foot Tapping[11]
8c		0.42/4.5	81%	3A4: 18 2C9: 26 2D6: 24	ED_{50} = 0.41 mpk at (0 h) 29% inh at 3 mpk (24 h)
27		0.12/ND	87%		
ent-**27**		0.97/ND	ND[c]	ND	ND
28		0.05/0.21	3%	3A4: 11 2C9: 69 2D6: 92	100% inh at 2 mpk (4 h) 24% inh at 6 mpk (24 h)
29		0.05/0.51	6%	3A4: 49 2C9: >100 2D6: 57	100% inh at 2 mpk (4 h) 2% inh at 6 mpk (24 h)
30		0.09/8.0	23%	3A4: 11 2C9: 43 2D6: 73	ED_{50} = 6 mpk at (24 h)
24		0.07/2.8	4%	3A4: 23 2C9: 47 2D6: >100	92% inh at 2 mpk (4 h) 1% inh at 6 mpk (24 h)
25		0.17/20	91%	3A4: 28 2C9: 89 2D6: >100	1% inh at 6 mpk (24 h)
20		0.07/6	66%	3A4: 100 2C9: 9 2D6: 63	ND
22		0.03/0.17	13%	ND	60% inh at 6 mpk (24 h)

[a] All compounds, except **8 c**, are enantiomerically pure and gave satisfactory ^1H NMR and mass spectral data.
[b] Displacement of [^{125}I]SP from the human hNK_1 receptor expressed in CHO cells. All values represent the average of 2–6 independent experiments. Interexperimental variability was typically less than twofold. See Ref. 4 and references cited therein.
[c] ND stands for no data obtained.

model but was only marginally efficacious after 24 h. Analog **29**, bearing a geminal-dimethyl group at the benzylic position, was equipotent to **28** but resulted in no improvement in in vivo activity. The 4-methylpyrazine carboxamide **30** had good intrinsic receptor-binding affinity that was substantially diminished in the



Scheme 2. Reagents and conditions: (a) EDC, HOBT, H_4NCl, *N*-methylmorpholine; (b) 2,4,6-trichlorotriazine, DMF; (c) H_2NOH, EtOH–H_2O, 80 °C; (d) EDC, DMAP, **23**; toluene 110 °C; (e) HCl–EtOAc; (f) EDC, DMAP, 1-acetyl-4-piperidinecarboxylic acid, DCM; (g) EDC, DMAP, **17** or **18** (X = 2-methyl-4-fluoro); toluene 110 °C.

presence of human serum (90-fold), yet **30** had only slightly reduced IP-1 functional potency compared to the acetylpiperidine analogs. Interestingly, compound **30** did provide 24 h efficacy in the gerbil assay. Based on these data, we cannot rule out the possibility that the in vivo activity was driven in part due to an active metabolite.

We next examined amide replacements embedded in the tether to see if improved metabolic stability would translate to longer duration of in vivo activity. We prepared the enantiomeric oxadiazoles **24** and **25**, which were surprisingly equipotent in terms of intrinsic receptor affinity, but only isomer **24** demonstrated substantial functional receptor blockade. Although oxadiazole **24** showed in vivo activity out to 4 h, this modification did not provide the 24 h coverage that was desired. The regioisomeric oxadiazoles **20** and **22** had exquisite intrinsic activity, but only **22** had a low shift in the presence of 50% human serum (sixfold) and good functional activity. An interesting and surprising feature was that **22** possessed the opposite absolute configuration to the other carboxamides described thus far. Oxadiazole **22** showed improved (24 h) in vivo activity compared to amide **28** presumably due to enhanced metabolic stability.

We have described a series of pyrrolidine carboxamides and oxadiazoles that displayed potent antagonism toward the hNK_1 receptor and showed reduced potential for inhibition of CYP_{450} metabolizing enzymes compared to Aprepitant. Acylation of the pyrrolidine core led to increased hNK_1 binding affinity. The *ortho* substituent on the 4-phenyl group was important for IP-1 functional activity. Replacement of the amide tether with an oxadiazole was well-tolerated and equal to the amide in all in vitro respects. Finally, carboxamide **30** and oxadiazole **22** were capable of inhibiting the effect of an agonist 24 h after an initial iv dose in the gerbil foot tapping assay.

Acknowledgments

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9. Acid **23** was prepared in 2 steps from methyl 3,5-bistrifluoromethylphenylacetate: (1) LHMDS (3 eq), THF, MeI (3 eq); (2) NaOH, MeOH.
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12. Briefly, CHO cells (2.5×10^4) stably expressing recombinant human NK_1 receptors were plated in 96-well tissue culture dishes overnight followed by prelabeling overnight with [^{3}H]-myo-inositol (10–25 Ci/mmol, 1 μCi/well) in inositol-free medium with 0.02% BSA at 37 °C. The cells were incubated with 10 mM LiCl, to inhibit inositol monophosphatases, in Hepes-buffered inositol-free (DMEM) medium with 0.02% BSA for 15 min. Antagonists (100 nM) or DMSO were added for 30 min then Substance P (10 μM) was added for another 30 min, all at 37 °C. The reaction was terminated by removal of the medium and addition of 60 μL of 10 mM formic acid for 60 min. at 22 °C. Lysates (15 μL) were incubated with 1 mg RNA Binding Yittrium Silicate (2–5 μm) SPA beads (Amersham) in Optiplates with shaking for 2 h at 22 °C. [^{3}H]-inositol phosphate generation was quantitated on a Packard Topcount and the data were analyzed using Prism (GraphPad).