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2-Phenyl-4-quinolinecarboxamides: A Novel Class of Potent and Selective Non-Peptide Competitive Antagonists for the Human Neurokinin-3 Receptor

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Pharmacological and molecular biological studies indicate the existence of at least three human tachykinin receptor subtypes, designated neurokinin-1 (NK-1), neurokinin-2 (NK-2), and neurokinin-3 (NK-3),[1–3] which belong to the superfamily of G-protein-coupled receptors possessing seven transmembrane domains.[4] The endogenous ligands for these receptors constitute a family of small neuropeptides, named tachykinins or neurokinins, which share the common carboxy-terminal region Phe-X-Gly-Leu-MetNH$_2$. The main mammalian tachykinins, substance P, neurokinin A (NKA), and neurokinin B (NKB), interact with all three tachykinin receptors, although there is a defined agonist rank order of potency for NK-1, NK-2, and NK-3 receptors, respectively; for example, for the NK-3 receptor the rank potency order is NKB > NKA > substance P.

Over the past few years potent and selective peptide and non-peptide antagonists for the NK-1 and NK-2 receptors have been identified.[5–8] These pharmacological tools accelerated the clarification of physiological and pathophysiological roles of these receptors[9] and the

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potential therapeutic indications for NK-1 and NK-2 receptor antagonists.[10–13] In contrast to the NK-1 and NK-2 receptor research area, there is limited information on the biology and potential pathophysiological significance of the NK-3 receptor. This was, to a large extent, due to the lack of sufficient potency and selectivity of the peptide NK-3 antagonists described thus far[14] and to the absence of non-peptide NK-3 receptor ligands until recently. However, the recent disclosure of the "peptoid" NK-3 antagonist PD 157672[15] and the peptide-derived PD 161182[16] has provided improved reagents for these studies and stimulated the search for more potent and metabolically stable non-peptide NK-3 receptor antagonists.

The human NK-3 (hNK-3) receptor mRNA has been detected, using polymerase chain reaction (PCR), to various regions in the central nervous system (CNS) and also, albeit to a lesser extent, in some peripheral tissues, including kidney, placenta, lung, and colon.[17] Activation of NK-3 receptors modulates the release of various transmitters in the CNS and periphery,[18–20] suggesting that they may have a neuromodulatory role.

Recently, (*S*)-(+)-*N*-{{3-[1-benzoyl-3-(3,4-dichlorophenyl)piperidin-3-yl]prop-1-yl}-4-phenylpiperidin-4-yl}-*N*-methylacetamide, SR 142801 (**1,** Chart 1), was reported as the first potent non-peptide NK-3 receptor antagonist.[21,22] Chemically, SR 142801 derives from a constrained analog of SR 48968, a potent NK-2 receptor antagonist (K_i = 0.51 nM, for displacement of [^{125}I]-NKA) in rat duodenum membranes,[7] which has moderate affinity for NK-3 receptors (IC$_{50}$ = 320 nM, for displacement of [^{3}H]senktide {succinyl-[Asp^{9}MePhe^8]-SP(6–13)}) in guinea pig cerebral cortex membranes.[23]
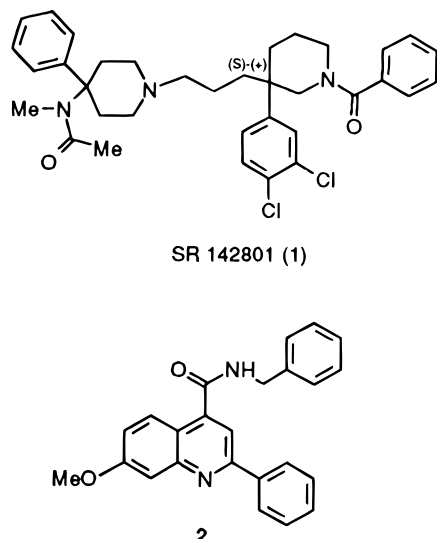
We now report on the discovery of a novel class of potent and selective non-peptide NK-3 receptor antagonists—structurally unrelated to the piperidine derivatives SR 142801 and SR 48968—which are based on the 2-phenylquinoline backbone.[24] Chemical synthesis (Scheme 1), radioligand binding affinities for the cloned human neurokinin receptors stably expressed in CHO cell lines (hNKs-CHO),[17,25,26] *in vitro* functional activity in the rabbit isolated iris sphincter muscle preparation (antagonism of senktide-induced contraction),[27] and structure–activity relationships (SARs) of the novel

Chart 1

SR 142801 (1)

2

Scheme 1.[a] Synthesis of Compounds of General Formula **6a**–**c** and **7a**–**c**

4a, R$_1$ = H	**5a**, R$_1$ = H
4b, R$_1$ = Me	**5b**, R$_1$ = Me
4c, R$_1$ = OMe	**5c**, R$_1$ = OH

6a, (R,S)
6b, (R)
6c, (S)

7a, R$_1$ = H
7b, R$_1$ = Me
7c, R$_1$ = OH

[a] Reagents: (a) KOH, EtOH, reflux; (b) R$_1$ = OMe, 48% HBr, reflux; (c) methyl phenylglycinate hydrochloride, DCC, HOBT, Et$_3$N, THF/MeCN, room temperature; (d) (*S*)-1-phenylpropyl-amine, DCC, HOBT, THF/MeCN, room temperature.

potent NK-3 receptor antagonists **6a**–**c** and **7a**–**c** (Table 1) will be summarized; a comparison to SR 142801 will be presented. For compound **7c**, functional activity in HEK 293 cells expressing the hNK-3 receptor (NKB-induced Ca^{2+} mobilization)[22] will be described.

Results and Discussion. The 4-quinolinecarboxa-mide derivative **2**, specifically designed to arrange the phenyl ring in position 2 and the benzene ring of the carboxamide side chain in a relative space position suitable for a potential interaction with NK (particularly NK-1) receptors,[28] was shown to possess no affinity for hNK-1 receptors (K_i > 10 μM, for displacement of [^{3}H]-substance P binding from hNK-1-CHO membranes) and moderate affinity for the hNK-3 receptor (K_i = 533 nM, for displacement of [^{125}I]MePhe^7-NKB binding from hNK-3-CHO membranes). Thus, compound **2** was uti-lized as a structural lead, suitable for chemical modi-fications aimed at optimizing the NK-3 receptor binding affinity of the compound, by variation of the carboxa-mide side chain in position 4 and of the substitution pattern on the quinoline ring system.

The synthesis of compounds **6a**–**c** and **7a**–**c** was accomplished as described in Scheme 1. Isatin **3** was submitted to the Pfitzinger reaction, by refluxing with phenyl ketones **4a**–**c** in the presence of an excess of potassium hydroxide to obtain the 2-phenylquinoline-4-carboxylic acids **5a,b** in high yields; 3-hydroxy-2-phenylquinoline-4-carboxylic acid (**5c**) was obtained by refluxing the corresponding methoxy intermediate in 48% hydrobromic acid. Secondary carboxamides **6a**–**c** and **7a**–**c** were obtained by coupling the carboxylic acids **5a**–**c** with the appropriate primary amines in a 7:3 mixture of THF and MeCN in the presence of dicyclo-hexylcarbodiimide (DCC) and 1-hydroxybenzotriazole (HOBT) to maximize yields and avoid racemization of the resulting compounds.

Incorporation of the methoxycarbonyl substituent at the benzyl position of compound **2** and removal of the 7-methoxy group produced a 17-fold increase in hNK-3 receptor binding affinity (Table 1, cf. **6a**, hNK-3-CHO binding, K_i = 30.7 nM, with compound **2**, hNK-3-CHO binding, K_i = 533 nM). Further studies with enantio-merically pure stereoisomers **6b** and **6c** revealed that hNK-3 receptor binding affinity and functional activity in rabbit isolated iris sphincter muscle (antagonism of
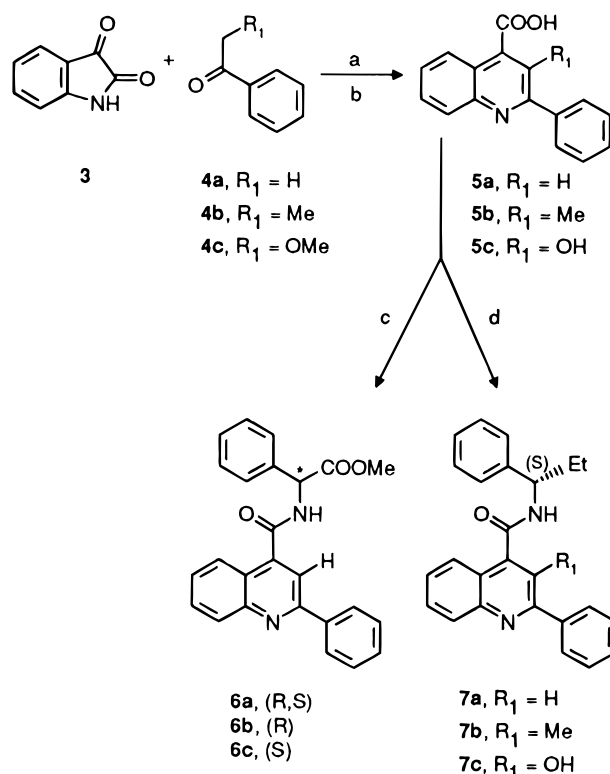
senktide-induced contractions) are enantioselective prop-erties of this chemical series, the (*R*)-stereoisomer **6b** (hNK-3-CHO binding, K_i = 13.3 nM) being about 2-fold more potent than racemic **6a** and almost 2 orders of magnitude more potent than the (*S*)-enantiomer **6c** (hNK-3-CHO binding, K_i = 925 nM) as shown in Table 1. Neurokinin receptor selectivity studies using radio-ligand competition binding with hNK-2 and hNK-1 receptors expressed in CHO cell membranes demon-strated that compound **6b** has an exquisite selectivity for hNK-3 with respect to the hNK-1 receptor (hNK-1/hNK-3 K_i ratio >7500) and a good selectivity versus the hNK-2 receptor (hNK-2/hNK-3 K_i ratio = 91).

Further chemical efforts were aimed at (a) replacing the ester functionality to circumvent potential metabolic instability problems and (b) optimizing the NK-3 recep-tor binding affinity and selectivity. Incorporation of a variety of substituents at the benzyl position (including alkyl, aralkyl, aryl, aminoalkyl, hydroxyalkyl, alkoxy-alkyl, acyl, acid, and amide groups) revealed that linear two or three carbon atom chains maintained high and enantioselective NK-3 binding affinity and *in vitro* functional antagonist activity (data not shown). The ethyl derivative **7a** (hNK-3-CHO binding, K_i = 18.0 nM) was therefore selected to investigate the effect of the quinoline substitution pattern on biological activities. Whereas substitution at position 5, 6, 7, and 8 of the quinoline ring system produced compounds with poten-cies lower or similar to that of the unsubstituted analog, incorporation of alkyl groups at position 3 of the quinoline ring resulted, in general, in an increased binding affinity for the hNK-3 receptor. For example, incorporation of a methyl group, as in compound **7b** (R

Table 1. Chemical Structures, Binding Affinities at Cloned Human Neurokinin Receptors (hNK-1, hNK-2, and hNK-3) Expressed in CHO Cells, and *in Vitro* Functional Activities (Antagonism of Senktide-Induced Contractions) in Rabbit Isolated Iris Sphincter Muscle (RISM) Preparation for Compounds **6a−c**, **7a−c**, and SR 142801
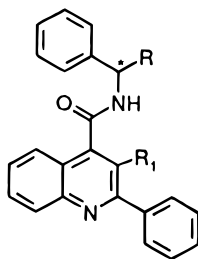
compound	R	R_1	config (*)	binding affinities, K_i mean $\pm$ SEM (nM)[a]			antagonism of senktide-induced contractions in RISM,[b] K_b[f] mean $\pm$ SEM (nM)[a]
				hNK-3[c]	hNK-2[d]	hNK-1[e]	
6a	COOMe	H	(*R,S*)	30.7 ± 3.8	2947 ± 585	>100000	42 ± 12
6b	COOMe	H	(*R*)	13.3 ± 2.7	1221 ± 189	>100000	43 ± 6.2
6c	COOMe	H	(*S*)	925 ± 250	8333 ± 926	>100000	3982 (2)
7a	Et	H	(*S*)	18.0 ± 1.0	237 (2)	>100000	9.7 ± 1.7
7b	Et	Me	(*S*)	4.2 ± 0.6	277 ± 57	>100000	7.7 ± 1.0
7c	Et	OH	(*S*)	1.0 ± 0.1	144 ± 22	>100000	5.4 ± 3.4
SR 142801 (**1**)[g]				1.2 ± 0.2	40.3 ± 5.5	1300 (2)	2.0 ± 0.5

[a] Average of three to eight independent determinations ($n = 3$−8), unless otherwise indicated in parentheses. [b] According to ref 27. [c] Inhibition of [^{125}I]MePhe^7-NKB binding in hNK-3-CHO cell membranes, according to ref 17. [d] Inhibition of [^{125}I]NKA binding in hNK-2-CHO cell membranes, according to ref 25. [e] Inhibition of [^{3}H]substance P binding in hNK-1-CHO cell membranes, according to ref 26. [f] The equilibrium-dissociation constant K_b for the antagonist−NK-3 receptor complex was calculated from the equation: $K_b = [B]/CR - 1$ where CR is the concentration ratio of agonist used in the presence and absence of antagonist B. [g] In-house data.

= Et, R_1 = Me), produced a 4-fold increase of the hNK-3 receptor binding affinity (hNK-3-CHO, K_i = 4.2 nM), possibly due to a limitation of the conformational space of the 4-carboxamide side chain.

This finding led to a comprehensive investigation of the optimal substituent at position 3, aimed at improving NK-3 binding affinity, *in vitro* functional antagonist activity, and selectivity for the hNK-3 versus hNK-2 receptors (a feature apparently reduced in the α-Et series, cf. compound **7a** with **6b**). (*S*)-(−)-*N*-(α-Ethylbenzyl)-3-hydroxy-2-phenylquinoline-4-carboxamide (**7c**, SB 223412), featuring a hydroxy group at position 3 of the quinoline nucleus, possessed the best overall profile identified among compounds synthesized in this class thus far. Compound **7c** is, in fact, as potent (hNK-3-CHO binding, K_i = 1.0 nM; antagonism of senktide-induced contraction in rabbit isolated iris sphincter muscle, K_b = 5.4 nM) as the reference compound SR 142801 (hNK-3-CHO binding, K_i = 1.2 nM; antagonism of senktide-induced contraction in rabbit isolated iris sphincter muscle, K_b = 2.0 nM), but is more selective for hNK-3 versus the other tachykinin receptors: it has a hNK-2/hNK-3 K_i ratio of 144 (vs 13 determined for the 3-H parent, **7a**, and 34 determined for SR 142801) and a hNK-1/hNK-3 K_i ratio > 100 000 (vs 1000 for SR 142801).

Moreover, compound **7c** (at concentrations of 1 or 10 μM) was without effect in a multitude of assays for various receptors, ion channels, and enzymes (data not shown), including sodium channel (site 2) and opioid (μ, κ, δ) receptors for which SR 142801 was described to possess binding affinities in the 0.1 to 1 μM concentration range.[21]

The cellular functional activity of compound **7c** was also evaluated utilizing HEK 293 cells expressing the hNK-3 receptor; compound **7c**, at 10−1000 nM concentration range, produced a concentration dependent antagonism of NKB-induced Ca^{2+} mobilization with a K_b of 3.0 nM. Schild plot analysis of these data revealed

a slope of the regression line of 1.2, consistent with competitive antagonism. Unlike SR 142801,[29] compound **7c** displayed no time dependence for activity and its effects were rapidly reversed by washing, providing further evidence that it is acting as a reversible, competitive antagonist.

In summary, through the use of cloned human neurokinin receptor assays, we have discovered a novel chemical class of potent and selective non-peptide competitive antagonists for the hNK-3 receptor based on the quinoline structure; the most prominent member is compound **7c**, which has equivalent potency and greater selectivity for the hNK-3 versus the hNK-2 and hNK-1 receptors than SR 142801 (**1**), the only potent non-peptide NK-3 receptor antagonist described to date. We have also shown that these novel NK-3 receptor antagonists have potent functional activity in the rabbit isolated iris sphincter muscle preparation. Compounds **7b** and **7c** should prove to be extremely useful pharmacological tools for anatomical localization and *in vitro* and *in vivo* functional characterization of hNK-3 receptors. Information gained from such studies will help to elucidate the most appropriate therapeutic indications for selective NK-3 receptor antagonists.

A more detailed description of our investigations within this chemical class, including SARs for the ester and the 2-phenyl ring replacements, and for the quinoline ring substitution will be reported in subsequent manuscripts, along with the results of our studies aimed at the replacement of the quinoline ring by other (hetero)aromatic systems.

Supporting Information Available: Detailed synthetic procedures and spectroscopic and analytical data for compounds **6a**−**c** and **7a**−**c** (5 pages). Ordering information is given on any current masthead page.

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