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N′,2-Diphenylquinoline-4-carbohydrazide based NK_3 receptor antagonists

Jason M. Elliott,[a,*] Robert W. Carling,[a] Mark Chambers,[a] Gary G. Chicchi,[c] Peter H. Hutson,[b] A. Brian Jones,[a] Angus MacLeod,[a] Rose Marwood,[b] Georgina Meneses-Lorente,[a] Elena Mezzogori,[a] Fraser Murray,[b] Michael Rigby,[b] Inmaculada Royo,[d] Michael G. N. Russell,[a] Bindi Sohal,[a] Kwei Lan Tsao[c] and Brian Williams[a]

[a]*Department of Medicinal Chemistry, Merck Sharp & Dohme Research Laboratories, The Neuroscience Research Centre, Terlings Park, Eastwick Road, Harlow, Essex CM20 2QR, UK*
[b]*Department of In Vivo Neuroscience, Merck Sharp & Dohme Research Laboratories, The Neuroscience Research Centre, Terlings Park, Eastwick Road, Harlow, Essex CM20 2QR, UK*
[c]*Department of Immunology, Merck Research Laboratories, Rahway, NJ 07065, USA*
[d]*CIBE-Merck Research Laboratories, Merck, Sharp and Dohme de España, c/ Josefa Valcárcel, 38 Madrid 28027, Spain*

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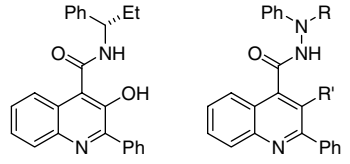
Abstract—A new class of potent NK_3R antagonists based on the *N*′,2-diphenylquinoline-4-carbohydrazide core is described. In an ex vivo assay in gerbil, the lead compound **2g** occupies receptors within the CNS following oral dosing (Occ_{90} 30 mg/kg po; plasma Occ_{90} 0.95 μM) and has good selectivity and promising PK properties.

The mammalian tachykinins and their receptors have been extensively studied,[1] with the majority of drug discovery work focused on substance P and its preferred receptor NK_1 and, to a lesser extent, the NKA/NK_2R system. However, attention has recently turned to NKB and the NK_3 receptor, which have now been implicated in a range of conditions, including nociception, inflammation, cough and schizophrenia.[2] This paper describes our development of a novel series of orally active non-peptide NK_3R antagonists which are able to occupy receptors within the CNS.



1 Talnetant **2**

A number of non-peptide based hNK_3R antagonists have been reported, including a series based on quinoline cores, of which Talnetant[3] (**1**, hNK_3R IC_{50} 2.4 ± 0.8 nM)[4] is a leading example. The SAR leading to the (*S*)-*N*-(1-phenylpropyl)carboxamide group at the 4-position of this series has been described.[3] However, the possibility that this group could be replaced by an achiral phenylhydrazide (i.e., **2**) has not previously been considered.

Quinoline-4-carboxylic acids were easily prepared by Pfitzinger reactions of appropriately substituted, commercially available isatins with aryl ketones under either acid or base catalysis (Scheme 1).[6] Coupling to phenylhydrazines could be carried out under a variety of peptide coupling conditions. As expected, coupling occurs exclusively to the more reactive hydrazine 2-nitrogen. Compounds with electron-withdrawing acyl or carbamate groups on the hydrazine 1-nitrogen were easily

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* Corresponding author. Tel.: +44 1279 440000; fax: +44 1279 440390; e-mail: jason@elliottj25.fsnet.co.uk

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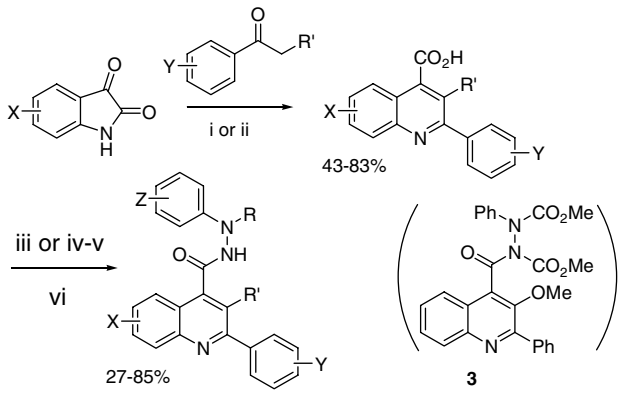
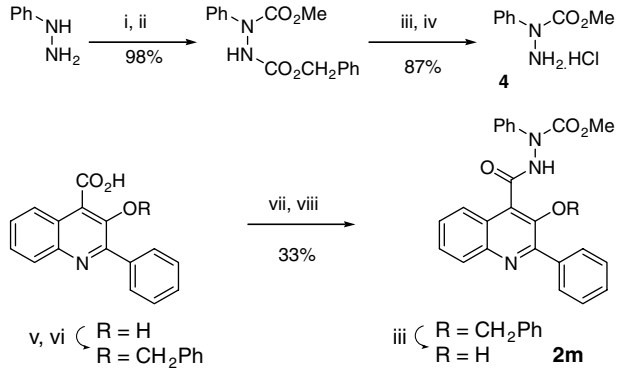


Scheme 1. Reagents and conditions: (i) HCl–H_2O (Concd), AcOH, reflux; (ii) KOH, EtOH–H_2O, reflux; (iii) $PhN(R)NH_2$, EDC, HOBT, Et_3N; (iv) $(COCl)_2$, DMF, CH_2Cl_2; (v) $PhNHNH_2$, Et_3N, CH_2Cl_2; (vi) RCOCl, PhMe, reflux.

prepared by first coupling the quinoline carboxylic acid to phenylhydrazine followed by treatment with an appropriate acyl chloride or chloroformate. This gave acylation exclusively on the hydrazine 1-nitrogen provided the reaction was carried out under neutral conditions (toluene, reflux); addition of base resulted in variable amounts of a second, unwanted acylation of the hydrazine 2-nitrogen (e.g., **3**). An alternative approach was the preparation of 1,1-disubstituted hydrazine **4** and coupling to give the target molecules in a single step (Scheme 2). In practice, **4** was less reactive than phenyl(alkyl)hydrazines, resulting in poor yields under mild peptide bond formation conditions, but reaction occurred smoothly with preformed quinoline-4-carbonyl chlorides. An example of this approach was the synthesis of the 3-hydroxyquinoline **2m** via its *O*-benzyl-protected analog.

Our initial studies focused on optimizing the hydrazide N-substituent. With the quinoline C-3 substituent fixed as methoxy, a series of analogs were prepared (Table 1, **2a**–**2i**). We were gratified to find that these showed promising hNK_3R affinity, with considerable tolerance



Scheme 2. Reagents and conditions: (i) $PhCH_2OCOCl$, $NaHCO_3$, K_2CO_3, EtOAc, H_2O, 0–5 °C; (ii) MeOCOCl, PhMe 100 °C; (iii) H_2 (40 psi), Pd–C, MeOH; (iv) HCl, MeOH; (v) $PhCH_2Br$, NaI, THF, reflux; (vi) NaOH, MeOH, H_2O; (vii) $(COCl)_2$, DMF, CH_2Cl_2; (viii) compound **4**, Et_3N, CH_2Cl_2.

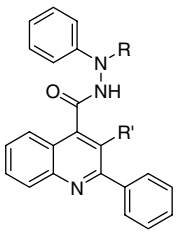
for alkyl, aryl, and carbonyl containing N-substituents. In particular, the introduction of a second phenyl group gave very high affinity (**2d**, hNK_3R IC_{50} 0.85 nM). However, further exploration of this compound showed that it had poor pharmacokinetic stability (incubation with rat liver microsomes: **2d**, 65% turnover after 15 min.) which made it unattractive as a lead. It seemed possible that this liability was a consequence of the two electron-rich *N*-phenyl rings; indeed, replacing one ring with an electron-withdrawing group improved metabolic stability. In particular, carbomethoxy substitution gave an optimal combination of good affinity and metabolic stability (**2g**, hNK_3R IC_{50} 8.8 nM; incubation with rat liver microsomes: 21% turnover after 15 min.). Larger carbamate groups led to reductions in affinity (**2h**–**2i**).

With the hydrazine N-substituent fixed as carbomethoxy, we next explored the effect of the substituent at C-3 (Table 1, **2j**–**2m**). Groups at this position improved affinity, but it is interesting to note that methoxy (**2g**), methyl (**2k**), and amino (**2l**) were preferred over hydroxyl (**2m**) in this series, in contrast to the series which led to Talnetant (**1**).[3]

The effect of aromatic substitution was next explored by introduction of a single fluorine at all available positions (Table 2). In many cases, this led to losses in affinity relative to the unsubstituted compound **2g**, although fluorination at the *meta*-position of the *N*-phenyl ring (**4b**) or the quinoline 5-position (**4g**) was tolerated. Only fluorination at the quinoline 8-position (**4j**) offered a marginal improvement in affinity, but introduction of larger substituents (**4k**–**4m**) was not tolerated.

The ability of compounds to occupy NK_3 receptors within the CNS is critical for any potential drug for the treatment of psychiatric conditions such as schizophrenia. We therefore required an assay to assess this property for our lead compounds. We decided to target a biochemical (rather than behavioral) assay to directly measure receptor occupancy. As with other NK receptors, species differences in NK_3R pharmacology make rats or mice inappropriate for study.[1] However, the gerbil NK_3 receptor is close to the human receptor, showing very similar antagonist affinities (e.g., **2g**, NK_3R IC_{50}s: human, 8.8 nM; gerbil, 4.5 nM; rat, 66 nM). We surveyed a range of NK_3R radioligands for a combination of affinity, low non-specific binding, and high brain penetration but found none suitable for an in vivo binding assay. Instead, we developed an ex vivo binding assay in which occupancy of novel ligands was measured in gerbil cortex and striatum using [^{3}H]-senktide, a peptidic agonist (derivatized fragment 6–11 of substance P) as the radioligand. Routinely, compounds were dosed orally and occupancy was measured 45 min. post dosing with collection of plasma for drug level determination.[8] In this assay, Talnetant (**1**) had an Occ_{90} dose of 75 mg/kg po and a plasma Occ_{90} of 3.8 μM. Under the same conditions, the high affinity antagonist **2d** achieved 70% occupancy at 10 mg/kg po (plasma concentration 0.04 μM). This was an encouraging result as it demonstrated that compounds in this class were active in vivo. However, while the low plasma exposure needed for

Table 1.



Compound	R	R′	hNK_3 IC_{50}[a] (nM)	hPXR Response[b] (%Rif. at 10 μM)
1	Talnetant		2.4 ± 0.8	62
2a	H	OMe	32 ± 4	
2b	Me	OMe	57 ± 2	
2c	Et	OMe	16 ± 7	
2d	Ph	OMe	0.85 ± 0.24	68
2e	COMe	OMe	210 ± 25	
2f	COEt	OMe	22 ± 3	
2g	CO_2Me	OMe	8.8 ± 5.1	49
2h	CO_2Et	OMe	60 ± 22	
2i	CO_2iBu	OMe	190 ± 20	
2j	CO_2Me	H	91 ± 18	44
2k	CO_2Me	Me	8.4 ± 0.4	56
2l	CO_2Me	NH_2	10 ± 2	64
2m	CO_2Me	OH	39 ± 4	

[a] Displacement of [^{125}I] labeled neurokinin B from the cloned hNK_3 receptor expressed in CHO cells. Data are means ± SD ($n = 3$ or more).[4]

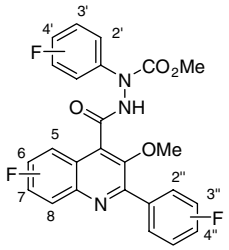
[b] Increase in hPXR activation in HepG2 cells transiently transfected with hPXR (% of 10 μM rifampicin positive control).[7]

occupancy was a reflection of very good affinity and availability to the receptor, the dose required was indicative of the problems of poor metabolic stability and hence bioavailability for **2d**. A more interesting lead was the less labile carbamate **2g** which was orally active with an Occ_{90} of 30 mg/kg po and a plasma Occ_{90} of 0.95 μM.

In addition to good stability in vitro in microsomal incubations, **2g** had promising PK properties (rat: F, 43%; $t_{1/2}$, 4.6 h; dog: F, 36%; $t_{1/2}$, 3 h). We felt it was important to verify at an early stage that the presence of an acylated hydrazide in **2g** did not lead to the formation of potentially toxic hydrazine metabolites. Both in vitro and in vivo we found no evidence of oxidation or cleavage of the hydrazine N–N bond, or release of a hydrazine. Moreover, Ames testing[9] of **2g** was negative in both the presence and absence of liver microsomal enzymes.

Cellular functional NK_3R antagonist activity of **2g** was measured in inositol phosphate generation studies using CHO/hNK_3R cells in response to eledoisin or senktide stimulation.[10] Eledoisin caused a concentration-dependent increase in inositol phosphate generation in these cells with an EC_{50} of 5.7 ± 0.1 nM ($n = 2$). In a Schild analysis of its antagonist behavior, **2g** caused a concentration-dependent rightward shift in the EC_{50} of eledoisin with no diminution of the maximal agonist response (K_b of 11 nM, slope of 1.0), indicative of competitive antagonism. When titrated versus an approximate EC_{50} concentration (3 nM) of senktide, **2g** inhibited inositol phosphate generation with an IC_{50} of 28 ± 6 nM ($n = 2$). Similarly, **2g** (2.8 μM) was able to completely block senktide-induced (30 nM) Ca^{2+} mobilization in CHO/hNK_3R cells. Compound **2g** demonstrated no measurable agonist activity for inositol phosphate

Table 2.



Compound	Substitution	hNK_3 IC_{50}[a] (nM)	hPXR Response[b] (%Rif. at 10 μM)
4a	2′-F	53 ± 26	—
4b	3′-F	7.4 ± 4.8	54
4c	4′-F	33 ± 33	—
4d	2″-F	110 ± 48	—
4e	3″-F	22 ± 6	69
4f	4″-F	24 ± 5	54
4g	5-F	11 ± 3	55
4h	6-F	38 ± 4	62
4i	7-F	28 ± 5	62
4j	8-F	4.1 ± 0.3	75
4k	8-Br	430 ± 93	88
4l	8-CN	35% ± 11% inh. at 1 μM	87
4m	8-CF_3	27% ± 15% inh. at 1 μM	87

[a] Displacement of [^{125}I] labeled neurokinin B from the cloned hNK_3 receptor expressed in CHO cells. Data are means ± SD ($n = 3$ or more).[4]

[b] Increase in hPXR activation in HepG2 cells transiently transfected with hPXR (% of 10 μM rifampicin positive control).[7]

generation or Ca^{2+} mobilization at concentrations up to 10 μM.

Further profiling of **2g** showed that it had good selectivity over hNK_1R and hNK_2R (IC_{50}s >1 μM) as well as a panel of other receptors and ion channels, including the hERG ion channel (K_i > 8 μM), blockade of which can lead to QT interval prolongation and severe side effects. **2g** showed low levels of CYP450 inhibition (human liver microsomes, IC_{50}s: 2C9, 2D6, >30 μM; 3A4, 12 μM), but caused CYP3A4 induction in human hepatocytes at high concentrations (77% of positive control at 20 μM),[13] suggesting a risk for drug–drug interactions. A key step in the main pathway for CYP3A4 induction is activation of the hPXR nuclear receptor;[7] **2g** was found to activate hPXR in vitro (HepG2 cells transiently transfected with hPXR; 49% of 10 μM rifampicin positive control). The substitutions reported here were shown to have no effect on this activation (Tables 1 and 2); this was a critical issue which required resolution.

In summary, we have shown that 2-phenyl quinolines substituted at C-4 with a phenylhydrazide are potent hNK_3R antagonists which are capable of occupying receptors within the CNS following oral dosing. The lead compound **2g** showed good selectivity and promising PK properties. The further development of this series to improve the in vivo profile while addressing the remaining issues of CYP induction and weak CYP3A4 inhibition will be reported in the following paper.

Acknowledgment

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4. NK_3 binding studies were conducted as a modification of a previously reported protocol[5] incubating 3×10^4 CHO cells stably expressing human NK_3 receptors with 30 pM [^{125}I] labeled neurokinin B for 1 h. at 25 °C followed by filtration over GF/C filter plates pre-soaked in 0.5% BSA.
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8. Test compounds were administered po as suspensions in 0.5% methyl cellulose. After 45 min, animals were humanely culled and trunk blood collected into EDTA-coated tubes. Plasma was obtained by centrifugation of whole blood at 3000 rpm, at room temp. Cortex and striatum were removed, then homogenized in 20 volumes 50 mM Tris, pH 7.4, containing 4 mM $MnCl_2$, 100 μg/ml bacitracin, 1 μM phosphoramidon, and 1 mM EDTA. 1.8 ml of the resulting homogenate was incubated with 200 μl [^{3}H]-senktide (3 nM final) for 30 min. Samples were harvested over GF/C filters presoaked in 0.1% PEI/0.5% Triton, using 50 mM Tris, pH 7.4, as a wash buffer. Filters were each dissolved in 10 ml Ecoscint overnight. Radioactivity present in samples was determined on a Beckman LS6500 counter. Percent occupancy was calculated as follows:- 100 − (sample dpm − mean non-specific dpm/mean total dpm minus mean non-specific dpm). Plasma drug levels were determined by LC–MS/MS following protein precipitation.
9. No 2-fold or greater increases in revertants relative to solvent control in a microbial mutation assay using 5 *Salmonella typhimurium* and *Escherichia coli* test strains either with or without metabolic activation by liver microsomal enzymes from rats pretreated with xenobiotics.
10. Agonist-stimulated inositol phosphate generation in CHO/hNK3R cells was measured as a modification of a previously reported protocol.[11] All steps were conducted in a 37 °C incubator with 5% CO_2 unless otherwise noted. Cells (5×10^4/well) were plated overnight in 150 μl of growth medium in 96-well plates. The following day, the wells were washed once with loading medium (inositol-free medium (ICN #1642954) supplemented with 0.02% bovine serum albumin, 2 mM L-glutamine, 70 mM Hepes, pH 7.5, and 1× HT supplement (100×, Gibco #11067-030)), followed by the addition of 1 μCi of [^{3}H]-myo-inositol (NEN #NET114A) in 150 μl loading medium. Inositol phosphate generation was measured the following day as follows. LiCl (10 mM) was added to each well for 15 min. The monolayers were then pre-incubated with antagonists for 30 min. followed by a 30 min. challenge with agonist (eledoisin or senktide) after which the medium was removed and the cells were lysed for 1 h in 60 μl of 10 mM formic acid at 25 °C. A 25 μl aliquot of each lysate was incubated with 1 mg RNA Binding Y Si (2–5 μm) SPA beads (Amersham #RPNQ0013) in Optiplates for 2 h. with shaking at 25 °C. The resulting [^{3}H]-inositol phosphates were quantitated using a Packard Topcount. NK_3 receptor-mediated Ca^{2+} mobilization was measured by FLIPR (Fluorescence Imaging Plate Reader) analysis.[12]
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13. Increase in human CYP3A4 mRNA in primary cultured human hepatocytes measured by TaqMan PCR. Data are expressed as percentage of the positive control, rifampicin, which was used at a concentration of 10 μM. Meneses-Lorente, G.; Pattison, C.; Guyomard, C.; Chesne, C.; Heavens, R. P.; Watt, A. P.; Sohal, B. *Drug Metabolism and Disposition*, submitted for publication.