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1,3-Biarylureas as Selective Non-peptide Antagonists of the Orexin-1 Receptor

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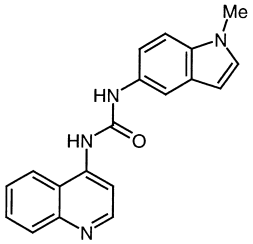
Abstract—This communication reports SARs for the first orexin-1 receptor antagonist series of 1-aryl-3-quinolin-4-yl and 1-aryl-3-naphthyridin-4-yl ureas. One of these compounds, **31** (SB-334867), has excellent selectivity for the orexin-1 receptor, blood–brain barrier permeability and shows in vivo activity following ip dosing. © 2001 Elsevier Science Ltd. All rights reserved.

Introduction

Recently, two novel hypothalamic neuropeptides orexin-A and orexin-B were reported. Both orexin-A, a 33 amino acid peptide, and orexin-B, a 28 amino acid peptide, are derived from the same precursor, prepro-orexin. These two peptides activate two closely related, previously orphan, G-protein coupled receptors, orexin-1 (OX_1) and orexin-2 (OX_2).[1] Orexin-A shows an approximately 10-fold higher functional affinity for the OX_1 receptor than orexin-B, whilst at the OX_2 receptor, orexin-A and orexin-B show similar functional affinity.[2] In the adult rat brain, prepro-orexin mRNA and immunoreactive orexin-A are localised in neurons within and around the lateral hypothalamus. These areas are known to be involved in a range of physiological actions, including regulation of feeding and energy balance. Indeed, orexin-A has been shown to stimulate feeding when injected into the lateral ventricles of male Wistar rats and prepro-orexin mRNA is elevated in rats after withdrawal of food, suggesting a physiological role of orexins in feeding.[3] Recent data also suggest roles for the orexins in regulation of blood pressure,[4] the neuroendocrine system[5] and the sleep–wake cycle.[6]

To explore the physiological role of the OX_1 receptor a programme of work was initiated to identify selective OX_1 receptor antagonists. This communication describes 1,3-biaryl ureas as the first antagonists of the OX_1 receptor and progress in optimising this structural class, leading to a compound, **31**, with excellent selectivity for the OX_1 receptor, demonstrable blood–brain barrier permeability and in vivo activity.

High throughput functional screening of the in-house collection against a CHO cell line expressing the human OX_1 receptor, using a FLIPR based calcium assay,[2] identified the 1-aryl-3-(quinolin-4-yl)urea **1**.



1 OX_1 pKb 7.5, 5-HT_{2B} pKi 8.6, 5-HT_{2C} pKi 7.6

This compound has good functional affinity for the OX_1 receptor[7] and generally good selectivity over other GPCRs, including the OX_2 receptor (pK_b 5.6). However, high binding affinity for both the 5-HT_{2B} and

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5-HT_{2C} receptors was seen. This poor selectivity limited the potential of **1** as a tool compound.

Indole Ring SAR

To develop an understanding of the SAR of **1**, attention was initially directed to the role of the indole ring in determining both functional affinity and selectivity (Table 1). Amongst indole modifications, functional affinity comparable to **1** was seen with the 1, 2-dimethylindole **2**. A large *N*-substituent—as in the *N*-benzyl analogue **3**—was not tolerated while the des-methyl analogue **4** was only marginally active. The indole ring could be replaced by the indoline **5** retaining full OX_1 functional affinity but also high 5-HT_{2C} affinity. Previous studies on substituted 1-(3-pyridylcarbamoyl)-indoline 5-HT_{2C} receptor antagonists, for example **6**, had established that a lipophilic 5,6-disubstituted indoline ring gave high 5-HT_{2C} receptor affinity.[8] It was therefore hypothesised that removal of the lipophilic pyrrolo ring from indole **1** to give substituted phenyl derivatives should reduce 5-HT_{2C} receptor affinity.

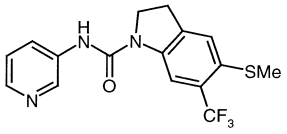

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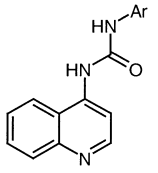
Table 1. 1-Quinolin-4-yl-3-(hetero)arylurea SAR



Compound	Ar	OX_1 pK_b[a]	5-HT_{2B} pK_i[a]	5-HT_{2C} pK_i[a]
1	1-Methylindol-5-yl	7.5	8.6	7.6
2	1,2-Dimethylindol-5-yl	7.3	8.4	7.1
3	1-Benzylindol-5-yl	<6.1		
4	Indol-5-yl	6.2		
5	1-Methylindolin-5-yl	7.6	7.9	7.3
7	C_6H_5	<6.1		
8	3-Cl-C_6H_4	6.4		
9	3-NMe_2-C_6H_4	6.1		
10	3-OMe-C_6H_4	6.6		
11	4-NMe_2-C_6H_4	7.8	7.1	6.9
12	4-Pyrrolidin-1-yl-C_6H_4	6.4		
13	4-NEt_2-C_6H_4	7.4	7.3	7.5
14	4-NH_2-C_6H_4	<6.1		
15	4-CN-C_6H_4	<6.2		
16	4-SMe-C_6H_4	7.9	7.5	7.5
17	4-OMe-C_6H_4	6.8		
18	3-Cl-4-OMe-C_6H_3	7.4	7.8	7.3
19	3-Cl-4-NMe_2-C_6H_3	7.4		
20	3,4-diOMe-C_6H_3	<6.3		
21	3,4-$O(CH_2)_3O$-C_6H_3	7.4		
22	Benzoxazol-6-yl	7.0		
23	2-Methylbenzoxazol-6-yl	7.7	6.2	6.2
24	Benzoxazol-5-yl	<6.2		

[a]Values are means of three experiments standard deviation <0.1.

The unsubstituted phenyl analogue **7** was inactive, no 2-substituted analogue showed significant activity (data not shown) while 3-substituted analogues, for example **8**–**10**, gave only modest OX_1 receptor functional affinity. However, the 4-*N*,*N*-dimethylamino substituted analogue **11** showed excellent functional affinity, 50-fold selectivity over OX_2 (pK_b 6.1) and 8-fold selectivity over the 5-HT_{2C} receptor, consistent with the hypothesis. The pyrrolidine **12** had 20-fold lower affinity suggesting the binding site accommodating this substituent is sterically constrained, although the *N*,*N*-diethyl analogue **13** was only marginally less active. It is likely that a lipophilic interaction is involved as demethylation (**14**) gave a >50-fold drop in OX_1 receptor functional affinity and the polar nitrile **15** was inactive. The lipophilic methylthio analogue **16** retained excellent functional affinity but unlike **11** lacked significant selectivity over 5-HT_{2B} or 5-HT_{2C} receptors.

Although 3-mono-substitution gave only modest affinity, increased affinity could be achieved with 3,4-disubstitution. For example, increased affinity was seen with a 3-chloro-4-methoxy substituent **18** relative to the corresponding modestly active mono-substituted analogues **8** and **17**. This may be due to conformational constraint of the 4-substituent optimising an interaction of the methyl group with a small lipophilic pocket. Disappointingly no such increase was seen for the 3-chloro-4-dimethylamino analogue **19** relative to **11**. 3,4-Dimethoxy substitution (**20**) resulted in loss of activity, but activity was restored when constrained in benzodioxepin **21**.

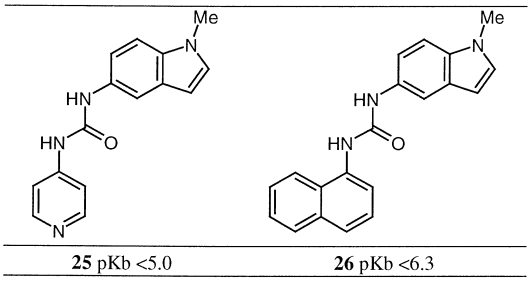
To further develop the hypothesis that reducing lipophilicity of the indole ring 3-position of **1** should increase selectivity over the 5-HT_{2C} receptor, a series of benzoxazole derivatives was prepared. The un-substituted benzoxazole **22** retained moderate OX_1functional affinity. Introduction of a 2-methyl substituent, **23**, gave a 5-fold increase in functional affinity consistent with the presence of a small hydrophobic pocket in the receptor. Furthermore, **23** showed 30-fold selectivity over both 5-HT_{2B} and 5-HT_{2C} receptors. Compared to **1**, **23** shows a 160-fold reduction in binding affinity at the 5-HT_{2B} receptor and 25-fold reduction at the 5-HT_{2C} receptor. The regioisomeric benzoxazole **24** was inactive.

Quinoline Ring SAR

Having demonstrated that good functional affinity and significant selectivity could be achieved by modification of the indole ring of **1**, attention was directed to the quinoline ring (Fig. 1). The lack of activity of the substituted pyridyl (**25**) and naphthyl (**26**) analogues highlighted the importance of both the quinoline benzo-ring and the quinoline ring nitrogen for good functional affinity.

Seeking to decrease lipophilicity and to increase solubility, aza-substitution was investigated (Table 2). The inactivity of the quinazolin-4-yl analogue **27** may reflect

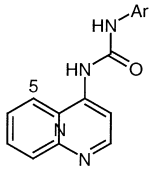
an intramolecular hydrogen bond mediated *EZ* conformation in solution. This contrasts with an extended *ZZ* urea conformation in biaryl ureas lacking intramolecular hydrogen bonding potential.[9–11] Good functional affinity was seen with 1,5-naphthyridines **28**–**31**. Furthermore, the benzoxazole **31** (**SB-334867**) showed 50-fold selectivity over OX_2 (pK_b 5.7), 100-fold or greater selectivity over 5-HT_{2B}, 5-HT_{2C} and approximately 50 other molecular targets, in particular other GPCRs and ion channels. Full details of the in vitro pharmacology of **SB-334867**, including binding data, are described elsewhere.[12] Broadly, aryl ring substituent SAR in the 1,5-naphthyridines followed that seen in the corresponding quinolines although functional affinities were marginally reduced. 1,6- **32** and 1,8-naphthyridines **33** were substantially less active than the 1,5-naphthyridines.



25 pKb <5.0 **26** pKb <6.3

Fig 1. Quinoline modifications.

Table 2. Aza-substituted quinolines



Compound	Ar	N	OX_1 pK_b[a]	5-HT_{2B} pK_i[a]	5-HT_{2C} pK_i[a]
27	1-Methylindol-5-yl	-3-	<6.3		
28	1-Methylindol-5-yl	-5-	7.3		
29	4-$NMe_2C_6H_4$	-5-	7.4		
30	4-$SMeC_6H_4$	-5-	7.5		
31	2-Methylbenzoxazol-6-yl	-5-	7.4	5.4	<5.3
32	2-Methylbenzoxazol-6-yl	-6-	5.9		
33	2-Methylbenzoxazol-6-yl	-8-	6.5		

[a]Values are means of three experiments standard deviation <0.1.

CNS Penetration of OX_1 Receptor Antagonists

Several compounds were assessed for CNS penetration by intravenous infusion of drug to steady state (Table 3). Disappointingly, only low level penetration was achieved with quinoline analogues with the most penetrant being the highly lipophilic, non-selective **13**. In contrast, the more polar naphthyridine **SB-334867** showed CNS penetration as determined by intravenous infusion to steady state and also from a bolus 10 mg/kg ip dose (brain level 2 h post dose 3.8 μM, 4 h <30 nM). The relatively short duration of **SB-334867** in the brain following ip dosing reflected its short half-life ($t_{1/2}$ = 0.4 h). It also showed low oral bioavailability (10%).

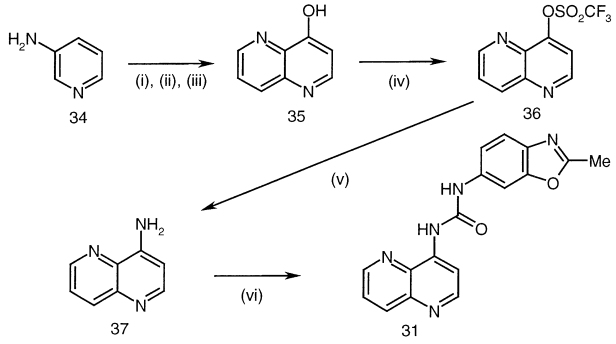
Despite a modest pharmacokinetic profile, **SB-334867** shows a range of in vivo activities mediated by orexin-A following ip dosing. These activities include blockade of orexin-A induced grooming[13] and both orexin-A induced and natural feeding.[14]

Synthesis of SB-334867

SB-334867 was prepared according to Scheme 1. The hydroxy compound **35** was prepared according to literature methods.[15] Conversion to amine **37** was accomplished through a two-step process via triflate **36** and subsequent amination with propylamine hydrochloride in pyridine—a modification of a literature procedure.[16] This route gave moderate yields but products were readily isolated. The final key urea formation involved conversion of 2-methylbenzoxazole-6-carboxylic acid to the acyl azide in situ using diphenyl phosphoryl azide at room temperature, adding the weakly nucleophilic 4-amino-1,5-naphthyridine and heating to reflux in toluene. Under these conditions, a good yield of **SB-334867** was obtained with only small quantities of symmetrical urea by-product.

SB-334867 was isolated as the HCl salt.[17]

Other compounds described were prepared by a similar procedure or by procedures described elsewhere.[18,19]



Scheme 1. Synthesis of **SB-334867**. Conditions: (i) Diethyl ethoxymethylenemalonate, Dowtherm A; (ii) 4% NaOH; (iii) heat, 43% over three steps; (iv) $(CF_3SO_2)O$, 2,6-lutidine, 34%; (v) nPrNH$_2$·HCl 85%; (vi) 2-methylbenzoxazole-6-carboxylic acid, $(PhO)_2P(O)N_3$, 72% (as HCl salt).

Table 3. CNS penetration of selected compounds

Compound	CNS penetration ratio brain/blood[a]	Css (μM)	cLogP[b]
11	0.06:1	4.2	4.0
13	0.16:1	0.7	5.1
16	0.1:1	1.0	4.4
31 (**SB-334867**)	0.4:1	0.49	2.8

[a]Steady state brain–blood ratio following intravenous infusion of 1.8 mg/kg/h free base drug for 12 h.
[b]cLogP version 3 licensed by Daylight CIS Inc. of Mission Viejo, CA, USA and BioByte Corp. of Claremont, CA, USA.

Summary

In this communication, SAR of the high throughput screening hit **1** at the OX_1 receptor has been outlined. The importance of the quinoline nitrogen and the quinoline benzo-ring has been demonstrated. The presence of a small hydrophobic pocket associated with the aryl ring substituent is postulated. The urea is proposed to bind to the receptor in the extended low energy *ZZ* conformation. Selectivity has been enhanced by modifying both the indole and quinoline motifs of **1**. Finally by replacement of the quinoline by a 1,5-naphthyridine, CNS penetration has been increased. The naphthyridine **SB-334867** possesses CNS penetration, excellent selectivity over a range of other receptors and ion channels and in vivo activity following ip dosing.

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7. Screening protocol in brief: CHO cells stably expressing OX_1 receptors (CHO-OX_1), were seeded (20,000 cells/well) into black walled clear-base 96-well plates (Costar, UK) in MEM-Alpha medium and cultured overnight. Cells were loaded with the cytoplasmic calcium indicator, Fluo-3AM (4 μM; Teflabs, Austin, TX, USA) in the presence of 2.5 mM probenecid, and then washed four times with, and finally resuspended in, Tyrode's medium containing 2.5 mM probenecid and 1% gelatine, before being incubated for 30 min at 37 °C with either buffer alone (control) or buffer containing test compound (10 pM–10 μM). The fluorescence ($\lambda_{ex} = 488$ nm, $\lambda_{EM} = 540$ nm) was then monitored using a fluorometric imaging plate reader (FLIPR; Molecular Devices, UK) before and after the addition of orexin-A in the continued presence of the test compound. Data analysis: responses were measured as peak fluorescence intensity (FI) minus basal FI, and curve-fitting and parameter estimation were carried out according to a four parameter logistic model, and results are expressed as a pK_b. Results are the mean of three separate determinations. For further details see refs 2 and 12.
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17. Characterisation of **SB-334867** hydrochloride ^{1}H NMR (250 MHz, DMSO-d_6) δ 2.61 (3H, s), 7.32 (1H, dd, $J = 2$ and 9 Hz), 7.65 (1H, d, $J = 9$ Hz), 8.08 (1H, d, $J = 2$ Hz), 8.17 (1H, dd, $J = 4$ and 9 Hz), 8.67 (1H, dd, $J = 1$ and 10 Hz), 8.77 (1H, d, $J = 7$ Hz), 9.09 (1H, d, $J = 7$ Hz), 9.22 (1H, dd, $J = 1$ and 4 Hz), 10.80 (1H, s), 10.92 (1H, s). LC–MS (ES^+, TFA/MeCN/H_2O variable gradient) calcd for $C_{17}H_{13}N_5O_2 + H$ 320, found 320 (100%).
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