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PHARMACOLOGICAL EVALUATION OF UK-14,304 ANALOGS AT CLONED HUMAN α ADRENERGIC RECEPTORS

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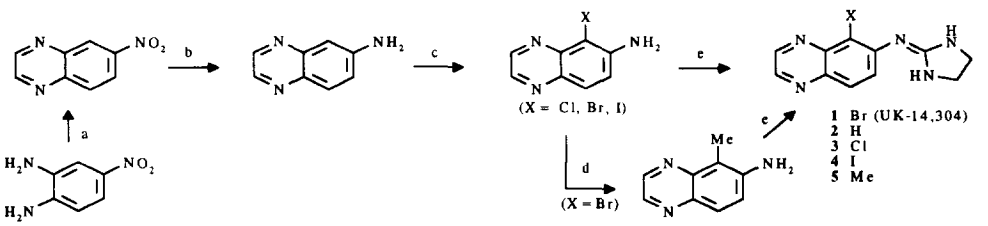
Abstract: As a part of a program directed at the identification of subtype selective α_2 agonists , a series of analogs of UK-14,304 (**1**) were synthesized. Binding affinities and functional efficacies were measured at cloned human α adrenergic receptors. A number of analogs showed high binding affinity and good selectivity for the human α_{2A} receptor subtype.

α Adrenergic receptors are plasma membrane receptors that mediate the physiological actions of the endogenous catecholamines epinephrine and norepinephrine. They can be subdivided into two major classes (α_1 and α_2), with each class representing a group of related receptor subtypes. Pharmacological and molecular biological studies indicate that the α_2 adrenergic receptor consists of at least three subtypes: α_{2A}, α_{2B} and α_{2C}.[1] Although an additional subtype (α_{2D}) was proposed to exist in the rat genome, it was later demonstrated to be a species homolog of the human α_{2A} subtype.[2] In general, α_2 adrenergic receptors have been considered to be useful targets for the treatment of a variety of conditions such as pain, depression, ischemia, hypertension, glaucoma, obesity and diabetes.[3] However, the precise physiological role for each subtype has not been determined.

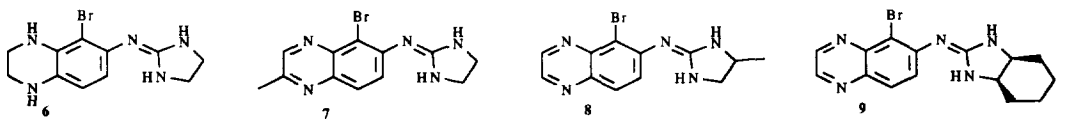
UK-14,304 (**1**) has been known to be a potent and selective α_2 adrenergic receptor agonist for over two decades.[4] It has been used in studies of vasoconstriction,[5] hypertension[6] and, more recently, lowering intraocular pressure.[7] In addition, a number of its analogs such as the tetrahydroquinoxaline derivative AGN 190851[8] have been prepared and evaluated in *in vitro* and *in vivo* animal studies.[9, 10] However, at the time of this study, UK-14,304 and its analogs had not been evaluated at cloned human α adrenergic receptors. Our aim was to prepare several key analogs of UK-14,304 and evaluate their pharmacological profiles at cloned human α adrenergic receptors.

Halogen- and methyl-substituted quinoxalines **1-5** were synthesized according to the procedures described in the literature starting from 4-nitrophenylenediamine (Scheme 1).[4] AGN 190851 (**6**) was prepared from compound **1** in >95% yield (H_2, PtO_2).[8] Treatment of 6-nitroquinoxaline with MeLi provided 2-methyl-6-nitro-quinoxaline in 61% yield after chromatography. This compound was converted to the 2-methyl analog **7** using

Scheme 1

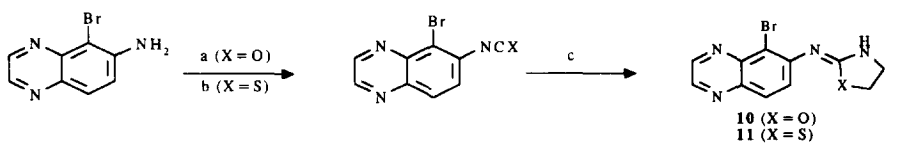


Reagents a) Glyoxal sodium bisulfate (91%)[4]; b) H_2, Pd-C (> 95%); c) X_2 (X = Cl, Br, I), AcOH (> 95%); d) Me_4Sn, $Cl_2Pd(PPh_3)_2$ (95%); e) 2-Imidazolin-2-ylsulfonic acid[12](46-67%);



the procedure illustrated in Scheme 1. The regiochemistry of the product was confirmed by comparison with an authentic sample.[11] We were also able to prepare quinoxalines substituted on the imidazoline ring (**8** and **9**) employing a reaction sequence involving preparation of the intermediate isothiocyanate of 5-bromo-6-amino-quinoxaline[4] followed by reaction with 2-methyl- and *cis*-cyclohexylethylenediamine, respectively. The corresponding oxazoline (**10**) and thiazoline (**11**) analogs of UK-14,304 were synthesized as illustrated in Scheme 2.

Scheme 2



Reagents a) Phosgene, $CHCl_3$ (86%); b) Thiophosgene, $CHCl_3$ (64%); c) Chloroethylamine, MeOH (60-85%)

The binding and functional assays were performed using stably transfected human α_1[13] and α_2[14] adrenergic receptors. Equilibrium competition binding assays, using [^{3}H]prazosin for α_1 receptors and [^{3}H]rauwolscine for α_2 receptors, were performed with membrane preparations from cultured LM(tk$^-$) cells stably transfected with the cloned human adrenoceptor subtypes except for α_{2B}, which was expressed in Y-1 cells. Estimates of equilibrium inhibition constants are shown as pKi and were determined by non-linear regression analysis. The agonist potency (expressed as pEC_{50}) was measured as a function of its ability to inhibit the forskolin-stimulated synthesis of cyclic adenosine monophosphate. The intrinsic activity (IA) is the measurement of the fraction of the maximum inhibition produced by a compound relative to norepinephrine. Each compound was tested a minimum of 3 times in the assays and the margin of error was within 7% of the mean. The binding and functional data of compounds **1-11**

Table 1. Binding and Functional Activities at Cloned Human α Adrenergic Receptors

Compound	α_2						α_1[15]		
	A		B		C		A	B	D
	pKi	pEC_{50} (IA)	pKi	pEC_{50} (IA)	pKi	pEC_{50} (IA)	pKi	pKi	pKi
1, UK-14,304	8.17	9.21 (1.0)	7.46	6.55 (1.0)	7.08	8.02 (1.0)	5.55	5.27	6.03
2	6.51	NA	6.66	NA	5.85	NA	4.74	5.49	4.91
3	7.43	8.63 (1.0)	7.16	6.76 (1.0)	6.71	7.51 (1.0)	5.29	4.98	5.62
4	8.17	7.94 (1.0)	7.37	NA	6.39	7.26 (1.0)	6.01	5.72	6.55
5	8.14	8.92 (1.0)	8.05	7.11 (1.0)	7.55	7.85 (1.0)	5.83	5.48	6.25
6	8.19	8.55 (1.0)	7.31	6.05 (1.0)	6.67	6.98 (1.0)	5.95	5.36	5.57
7	7.13	6.07 (1.0)	6.49	NA	6.19	6.08 (0.8)	<4	<4	5.30
8	5.42	4.80 (0.8)	5.49	NA	5.13	NA	4.69	4.17	4.90
9	4.83	NA	4.74	NA	4.94	NA	4.85	3.98	4.38
10	4.58	NA	4.07	NA	4.31	NA	4.04	3.85	3.99
11	4.99	5.56 (0.9)	4.76	NA	4.55	NA	4.67	4.22	4.56

[NA; not active at the highest concentration (100 μM) tested]

at the cloned human α_1 and α_2 adrenergic receptors are presented in Table 1. The data show that the α adrenergic activity is sensitive to substituent effects. The compounds with C-6 substituents (**1** and **3-5**) have similar binding affinities and, except for the α_{1B} receptor, have higher affinities than unsubstituted analog **2**. With regard to functional activity, compounds **1, 3** and **5** are potent, full agonists at all α_2 receptor subtypes while the iodo compound **4** exhibits decreased potency at α_{2A} and α_{2C} and is without functional activity at the α_{2B} receptor. On the other hand, the unsubstituted analog **2** shows only moderate binding affinity and no functional activity at the α_2 receptor subtypes. The substituent effects observed in this study seem to result from steric rather than electronic factors.[10] For instance, electronegative substituents such as Br (**1**) and Cl (**3**) provide almost identical pharmacological profiles compared to a methyl substituent (**5**) while substitution with the bulkier substituent I (**4**) results in a greater than 10 fold decrease in functional efficacy at the α_{2A} receptor. In general, most compounds show moderate to excellent selectivity for α_2 relative to α_1 receptors, with good α_{2A} subtype selectivity typically being observed in both binding and functional assays. In particular, AGN 190851 (**6**) possesses good α_{2A} subtype selectivity by virtue of its decreased α_{2C} binding and functional activity profile relative to UK-14,304. On the other hand, substitutions on the C-2 position of the quinoxaline ring (**7**) as well as on the imidazoline ring (**8** and **9**) decrease binding and functional activity. Replacement of the imidazoline NH with O or S (**9** vs. **10** and **11**) also resulted in decreased activity.

In conclusion, a series of UK-14,304 analogs were synthesized and evaluated at cloned human α adrenergic receptors. UK-14,304 and the tetrahydroquinoxaline analog AGN 190851 were selective for the human α_{2A} subtype. These observations support the premise that the α_{2A} receptor may play a significant role in mediating the biological effects of these compounds.[16]

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16. This work was presented in poster form: Jeon, Y.; Luo, C.; Gluchowski, C.; Forray, C.; Vaysse, P.J.-J.; Branchek, T.A.; Weinshank, R.L.; Hartig, P.R. 206th American Chemical Society National Meeting, Chicago, IL, August 22-27, **1993**, *MEDI #52*.

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