Eur J Med Chem (1996) 31, 725–729

Short communication

Synthesis and pharmacological properties of 4(5)-(2-ethyl-2,3-dihydro-2-silainden-2-yl)imidazole, a silicon analogue of atipamezole

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(Received 13 November 1995: accepted 4 March 1996)

Summary — 4(5)-(2-Ethyl-2,3-dihydro-2-silainden-2-yl)imidazole **2a**, 4(5)-(2-methyl-2,3-dihydro-2-silainden-2-yl)imidazole **2b** and 4(5)-(2-ethyl-2,3-dihydro-2-silainden-2-yl)-2-methylimidazole **2c**, C2 silicon analogues of the commercially available α_2-adrenergic receptor antagonist atipamezole **1**, were prepared and their pharmacological properties were evaluated. The results show that **2a** retained significant affinity for the receptors, and the in vivo pharmacokinetic properties of **2a** were comparable to those of **1**.

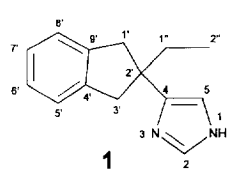
atipamezole / silaindane / Grignard reaction / semi-empirical calculation / adrenergic receptor

Introduction

Atipamezole **1** is a patented drug [1] used extensively in veterinary care, and also currently studied for human medical purposes. As an attempt to prolong the duration of atipamezole action, we now report on the synthesis and pharmacological properties of its C2 silicon analogue 4(5)-(2-ethyl-2,3-dihydro-2-silainden-2-yl)imidazole **2a** and two closely related compounds, 4(5)-(2-methyl-2,3-dihydro-2-silainden-2-yl)-imidazole **2b** and 4(5)-(2-ethyl-2,3-dihydro-2-silainden-2-yl)-2-methylimidazole **2c**. Selection of **2a** as a viable candidate for this purpose was based on (i) observations that replacing a carbon atom with silicon in some cases improves the pharmacokinetic behaviour of drug molecules [2]; and (ii) on semiempirical quantum mechanical calculations. The latter showed that neither the geometry nor the electrostatic surface potential of **2a** differ markedly from those of **1**, but the Si2 atom is more electron deficient than its carbon counterpart, while the electron density of carbon atoms directly bound to silicon is increased. These facts were interpreted to suggest that replacing C2 with silicon would not markedly lower the affinity to receptors, but might shield the neighbouring atoms against nucleophiles, and hence retard the biodegradation. The results showed that **2a** retained significant affinity for the receptors, and the in vivo pharmacokinetic properties of **2a** were comparable to those of **1**.

Chemistry

The structure and electron distribution of **1** and **2a** were studied by semiempirical quantum-chemical calculations based on the PM3 program [3]. Figure 1 shows the minimized structures. The root mean square fit of the molecules using all non-hydrogen atoms was 0.2 Å, indicating that the replacement of C2 with silicon has only a minor effect on the molecular geometry. The Mulliken partial charges obtained are listed in table I. One difference in the electron distribution is obvious: Si2 is more electron deficient than C2, while the carbon atoms bonded directly to Si2 (ie, indan C1, C3; imidazole C4; ethyl C1) are electron rich compared to the corresponding atoms of **1**. This difference



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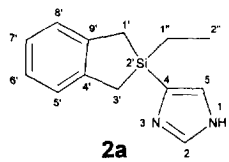


2a

in electron distribution remains similar on protonating the imidazole nitrogen. One may speculate that the increased electron density at the carbon atoms bonded to silicon retards nucleophilic reactions at these sites, and hence possibly stabilizes the molecule against biodegradation. In contrast, the electron density at imidazole nitrogens appears not to be sensitive to replacing C2 with silicon, and therefore no change in the pK_a of this group was expected. On visual comparison no marked differences were observed between the electrostatic surfaces of **1** and **2a**, suggesting that the receptor binding ability might not be significantly altered.

The synthesis of **2a–c** was based on the known reactivity of chloro and methoxy silanes towards Grignard reagents. Imidazol-4-yl anion from *N*-protected 4-iodoimidazole was generated by exchange reaction with ethyl magnesium bromide or methyl magnesium iodide in dichloromethane solution [4], and then slightly less than 1 equiv 2,2-dichloro-2,3-dihydro-2-silaindene or 2,3-dihydro-2,2-dimethoxy-2-silaindene was added. After the reaction was completed, the alkyl group was introduced by another Grignard reaction in the same reaction vessel (scheme 1). This method allowed variation of substituents at both the imidazole ring and silaindane moiety. Finally, the *N*-protection was removed.

2,2-Dichloro-2,3-dihydro-2-silaindene and 2,3-dihydro-2,2-dimethoxy-2-silaindene are easily prepared by methods described in the literature [5, 6]. The use of dichloromethane as a solvent in the Grignard reaction is rather uncommon. In this case the choice of

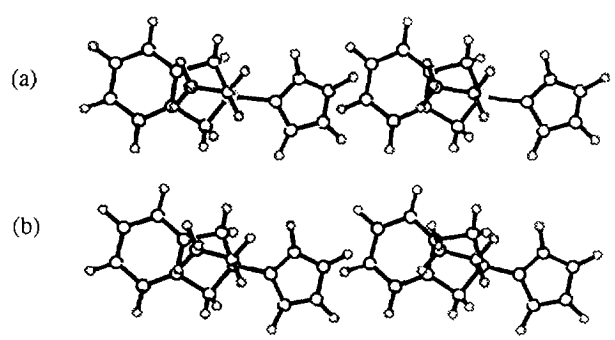
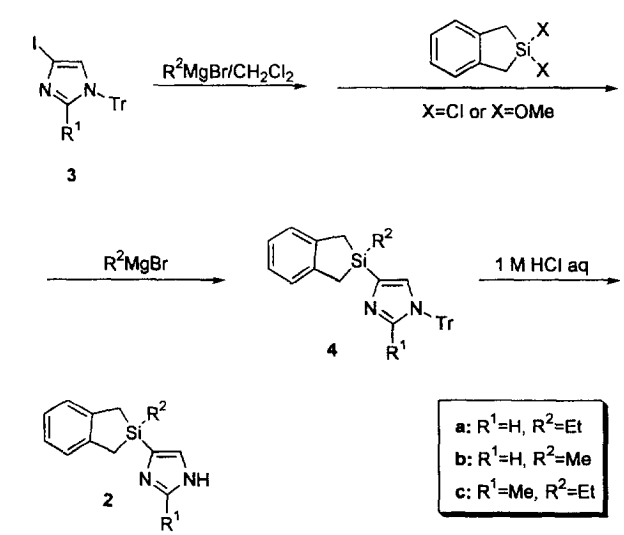


Fig 1. Minimized structures of **2a** (a) and **1** (b).

Table I. Mulliken partial charges[a] of the heavy atoms of atipamezole **1** and 4(5)-(2-ethyl-2,3-dihydro-2-silainden-2-yl)-imidazole **2a**, and their N_1-monoprotonated forms **1H**$^+$, **2aH**$^+$.

Moiety	*Atom*	*1*	*2a*	*1H*$^+$	*2aH*$^+$
Imidazole	N1	–0.14	–0.16	+0.50	+0.49
	C2	–0.34	–0.36	–0.60	–0.59
	N3	+0.31	+0.35	+0.50	+0.54
	C4	–0.34	–0.57	–0.26	–0.57
	C5	–0.23	–0.21	–0.40	–0.38
Indan	C1	–0.18	–0.6	–0.17	–0.60
	C2/Si2	+0.04	+1.18	+0.01	+1.27
	C3	–0.20	–0.62	–0.19	–0.62
	C4	–0.11	–0.07	–0.12	–0.08
	C5	–0.17	–0.18	–0.16	–0.17
	C6	–0.19	–0.20	–0.17	–0.18
	C7	–0.19	–0.20	–0.17	–0.18
	C8	–0.17	–0.18	–0.16	–0.17
	C9	–0.10	–0.07	–0.12	–0.08
Ethyl	C1	–0.23	–0.44	–0.23	–0.44
	C2	–0.32	–0.29	–0.32	–0.29

[a]Calculated using the semi–empirical program PM3.



Scheme 1.

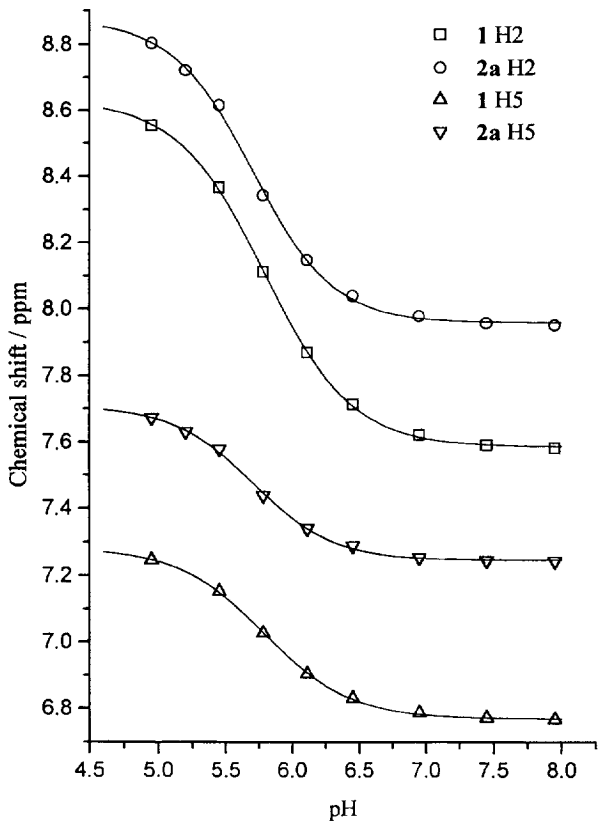


Fig 2. NMR spectroscopic titration curves for **1** and **2a** in 80% Me_2SO/D_2O (v/v).

solvent presumably has significance, since Grignard reagents prepared from 4-haloimidazoles readily form 2-substituted products in etheral solutions [4]. A noncomplexing solvent, such as dichloromethane, enhances the covalent character of the organomagnesium intermediate and hence retards conversion to the thermodynamically-favoured 2-substituted imidazole. The reactions described here did not produce detectable amounts of 2-substituted imidazoles. The trityl protecting group was removed with 1 M aq hydrogen chloride, yielding the hydrochloric salt of **2a–c**.

The pK_a values of **1** and **2a** were determined by NMR spectroscopic titration in 80% Me_2SO/D_2O (v/v). When chemical shifts of H2 and HS of the imidazole moiety were presented as a function of pH of the buffer solution, well-defined titration curves were obtained (fig 2). The inflection points of the fitted curves gave the apparent pK_a values of the compounds: 5.79 ± 0.02 for **1** and 5.70 ± 0.03. The results clearly indicate that replacing the C2 atom of **1** with a more electropositive silicon atom has only a negligible effect on the pK_a value of the imidazole moiety, consistent with the prediction of semiempirical quantum-chemical calculations.

No correlation was observed between the Mulliken partial charges and the ^{13}C NMR chemical shifts. Some qualitative concordence may be noted when comparing relative changes in corresponding atom values in atipamezole and the silicon analogue **2a**.

Pharmacological results and discussion

The affinities of atipamezole **1** and its silicon analogues **2a–c** to the three human α_2 adrenoceptor subtypes α-2A, α-2B and α-2C were determined by binding competition assays with [^{3}H]rauwolscine. The biological material for these experiments consisted of membranes from Shionogi S115 cells stably transfected with a given (one of the three) human α-2 subtype [7, 8]. The results are shown in table II. It is seen that replacing the C2 atom of **1** with silicon lowers the affinity to all the receptor subtypes studied by one order of magnitude. Introduction of a 2-methyl substituent to the imidazole moiety further weakens the binding by a factor of 100.

The potency of **1** and **2a** to block α_2-adrenergic receptors was evaluated in two ways: (i) by determining their dose-dependent ability to reverse detomidine-induced mydriasis in rats [9]; and (ii) by their blocking effects on presynaptic α-2 adrenergic receptors in vitro in a field-stimulated prostatic portion of rat vas deferens [10]. Both approaches proved **2a** to be pharmacologically less effective than **1**. The doses needed to reverse 50% of detomidine-induced mydriasis were 11.6 g·kg^{-1} and 240 g·kg^{-1} with **1** and **2a** respectively (fig 3). Similarly, **2a** opposed the inhibitory effect of detomidine on field-stimulated prostatic portion of rat vas deferens less effectively than **1**, the pA_2-values being 6.5 and 8.3 respectively [11] (fig 4).

Evidently **2a** is pharmacokinetically similar to **1**. With a dose of 3 mg/kg bodyweight, a concentration of 660 ng/mL was measured in rat serum at 0.5 h, indicating an apparent volume of distribution of <5 L/kg bodyweight. The drug seemed to be cleared from serum relatively rapidly, the half-life being around 2 h.

Experimental protocols

Chemistry

Molecular modelling

The Sybyl version 6.1 molecular-modelling package was used. First the molecular structures were built and made energetically reasonable using molecular-mechanics minimization and the Tripos force-field. Based on literature data, the carbon-silicon

bond length parameter was set to 1.87 Å [12]. Subsequently, SCF optimization was performed and Mulliken atom partial charges were calculated using the semi-empirical molecular orbital method PM3 (Mopac version 6.0) [3], in which the valence-shell electrons are considered explicitly. The resulting carbon–silicon bond lengths were 1.88 Å for the imidazole carbon, 1.85 Å for the ethyl carbon and 1.60 Å for the benzylic carbons.

Syntheses

Dichloromethane was dried by refluxing on CaH_2, and then distilled. Diethyl ether was dried over sodium wire. Commercial 3 M ethyl magnesium bromide in diethyl ether (Aldrich) was used. Methyl magnesium iodide was prepared from methyl iodide and magnesium in dry diethyl ether. ^1H and ^{13}C NMR spectra were obtained on Jeol Alpha and Jeol Lambda spectrometers, operating at 500.162 and 399.782 MHz for ^1H and at 125.78 and 100.533 MHz for ^{13}C. Chemical shifts are given as ppm using tetramethylsilane as an internal reference.

4-Iodo-1-triphenylmethylimidazole ***3a***

Compound **3a** was prepared from 4-iodoimidazole as described in the literature [13]. However, pyridine was used as a solvent instead of DMF.

4-Iodo-2-methyl-1-triphenylmethylimidazole ***3b***

Compound **3b** was prepared from 2-methylimidazole as described for **3a**.

4(5)-(2-Ethyl-2,3-dihydro-2-silainden-2-yl)-1-triphenylmethylimidazole ***4a***

Method A. To a solution of **3a** (1.2 g, 2.7 mmol) in dry dichloromethane (11 mL), 1 mL of 3 M solution of ethyl magnesium bromide (2.8 mmol) in diethyl ether was added. After 30 min, 2,2-dichloro-2,3-dihydro-2-silaindene [5, 6] (404 µL, 2.5 mmol) was added and the reaction mixture was left to stand. After 24 h, 1 mL of 3 M solution of ethyl magnesium bromide solution in diethyl ether (2.8 mmol) was added. The next day the reaction mixture was poured into aqueous ammonium chloride and extracted three times with dichloromethane. The combined dichloromethane extracts were dried over Na_2SO_4 and evaporated to dryness. Purification by silica-gel chromatography yielded **4a** 90 mg, 7.7 %.

Method B. After addition of 2,2-dimethoxy-2,3-dihydro-2-silaindene [6] (1 g, 5.2 mmol) to the reaction mixture, prepared from 2.7 g (6.2 mmol) of **3a** in dichloromethane (25 mL) and 2.1 mL of 3 M solution of ethyl magnesium bromide (6.3 mmol) in diethyl ether, the reaction mixture was left to stand for 2–3 h, and another portion of ethyl magnesium bromide (2.1 mL of 3 M solution in diethyl ether, 6.3 mmol) was added. After 2 h, the reaction mixture was worked-up as in *Method A*. Yield of **4a** was 380 mg (16%). MSM (EI+): 470 (M^+); ^1H NMR ($CDCl_3$): 0.88–1.00 (m, 5H), 2.25 (m, 4H), 6.96 (s, 1H), 7.0–7.4 (m, 19H), 7.58 (s, 1H); ^{13}C NMR ($CDCl_3$): 4.9, 7.6, 18.7, 75.3, 125.6, 128.0, 128.1, 129.2, 129.3, 129.7, 136.1, 141.6, 142.1, 142.5. Found: C 82.2, H 6.4, N 5.8%. $C_{32}H_{30}N_2Si$ requires C 81.7, H 6.4, N 6.0%.

Table II. Affinity of **1** and **2a–c** to human α_2-adrenoceptor subtypes, expressed as K_i values (nM)[a].

Compound	*α-2A*	*α-2B*	*α-2C*
1	3.0 ± 1.0	1.8 ± 0.4	2.5 ± 0.2
2a	22 ± 1.2	20 ± 0.3	20 ± 0.8
2b	24 ± 2.0	25 ± 2.0	29 ± 0.9
2c	2000	1100	900

[a]Data are given as means ± SEM of three or four separate experiments (**1** and **2a, b**) or the mean of two experiments (**2c**), each done in duplicate.

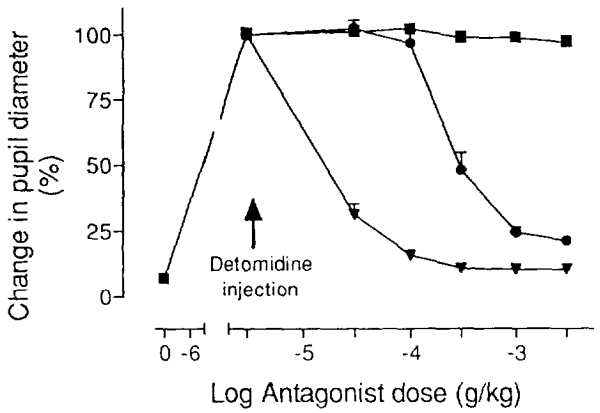


Fig 3. The dose-dependent ability of **1** (▼) and **2a** (•) to reverse detomidine-induced mydriasis in rats. Squares refer to NaCl.

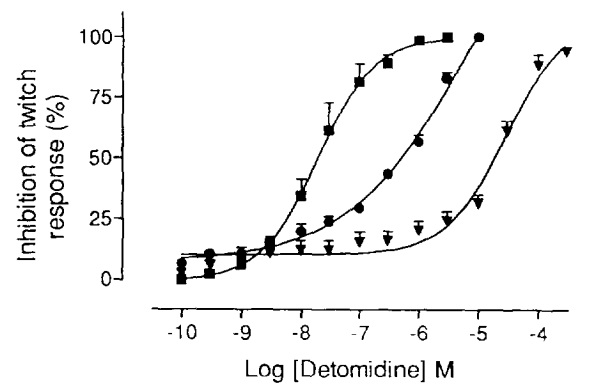


Fig 4. The blocking effects of **1** (▼) and **2a** (•) at 50 µM on synaptic α_2-adrenergic receptors in vitro in a field-stimulated portion of rat vas deferens. Squares show the response in the absence of **1** and **2a**.

4(5)-(2-Methyl-2,3-dihydro-2-silainden-2-yl)-1-triphenylmethylimidazole ***4b***

Compound **4b** was prepared from **3a** as described for **4a** (*Method B*), with the exception that ethyl magnesium bromide was replaced with methyl magnesium iodide. The yield of **4b** was 27%. ^1H NMR ($CDCl_3$): 0.45 (s, 3H), 2.18–2.38 (m, 4H),

6.95 (s, 1H), 7.08 (m, 2H), 7.10 (m, 6H), 7.20 (m, 2H), 7.30 (m, 9H), 7.58 (s, 1H); ^{13}C NMR ($CDCl_3$): 3.9, 19.8, 75.3, 125.7, 128.0, 128.1, 129.3, 129.5, 129.9, 137.1, 141.8, 142.1, 142.6.

4(5)-(2-Ethyl-2,3-dihydro-2-silainden-2-yl)-2-methyl-1-triphenylmethylimidazol ***4c***

Compound **4c** was prepared from **3b** using *Method B*. The yield of **4c** was 14%. ^{1}H NMR ($CDCl_3$): 0.80–1.00 (m, 5H), 1.68 (s, 3H), 2.18–2.30 (m, 4H), 6.85 (s, 1H), 7.04 (m, 2H), 7.09 (m, 6H), 7.21 (m, 2H), 7.32 (m, 9H); ^{13}C NMR ($CDCl_3$): 5.0, 7.6, 17.4, 18.8, 75.0, 125.5, 127.8, 128.0, 129.2, 130.0, 130.1, 132.9, 142.4, 142.5, 149.6. Found: C 82.5, H 6.9, N 4.8%. $C_{33}H_{32}N_2Si$ requires: C 81.8,H 6.7, N 5.8%.

4(5)-(2-Ethyl-2,3-dihydro-2-silainden-2-yl)imidazole hydrochloride ***2a***

Compound **4a** (57 mg, 0.12 mmol) was refluxed for 30 min in aqueous 1 M HCl solution. The solution was cooled and evaporated to dryness. Water and dichloromethane were added. The organic phase was extracted twice with water, and the combined water extracts were evaporated and dried under vacuum. The yield of **2a** was 26 mg (82%). Mp (from water) 164–167 °C; MS (EI+): 228 (M^+ – HCl), 199 (M^+ – HCl – CH_2CH_2); ^{1}H NMR (DMSO-d_6): 0.96 (t, 3H), 1.08 (q, 2H), 2.40 (m, 4H), 7.10 (m, 2H), 725 (m, 2H), 7.90 (s, 1H), 9.3 (s, 1H), 14.7 (s, 2H); ^{13}C NMR (DMSO-d_6): 3.9, 7.1, 17.5, 125.8, 127.0, 127.5, 128.9, 137.2, 140.5. Found: C 57.1, H 6.9, N 11.5%. $C_{13}H_{17}N_2SiCl$ requires C 59.0, H 6.4, N 10.6%.

4(5)-(2-Methyl-2,3-dihydro-2-silainden-2-yl)imidazole hydrochloride ***2b***

Compound **2b** was deprotected as **2a.** The yield of **2b** was 95%. MS (EI+): 214 (M^+ – HCl); ^{1}H NMR (DMSO-d_6): 0.59 (s, 3H), 2.30 (m, 4H), 7.10 (m, 2H), 7.26 (m, 2H), 7.87 (s, 1H), 9.27 (s, 1H), 14.6 (s, 2H); ^{13}C NMR (DMSO-d_6): –4.2, 19.4, 125.9, 127.3, 128.0, 128.9, 137.2, 140.5. Found: C 57.6, H 6.0, N 11.1%. $C_{12}H_{15}N_2SiCl$ requires: C 57.5, H 6.0, N 11.2%.

4(5)-(2-Ethyl-2,3-dihydro-2-silainden-2-yl)-2-methylimidazole hydrochloride ***2c***

Compound **2c** was deprotected as **2a**. The yield of **2c** was 97%. MS (EI+): 242 (M^+ – HCl)$^+$; ^{1}H NMR (DMSO-d_6): 0.92–1.05 (m, 5H), 2.30 (m, 4H), 2.56 (s, 3H), 7.10 (m, 2H), 7.25 (m, 2H), 7.71 (s, 1H), 14.1 (s, 2H); ^{13}C NMR: 3.8, 7.1, 11.0, 17.5, 125.9, 126.2, 127.0, 128.9, 140.5, 147.2.

pK_a measurements

The NMR spectra for pK_a measurements were recorded on a Jeol Alpha 500 MHz spectrometer at 25 °C. A capillary tube, which contained tetramethylsilane in carbon tetrachloride, was used as external reference. Buffer solutions were prepared from *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) and its sodium salt in 80% (v/v) Me_2SO/D_2O SO that the total buffer concentration and ionic strength were 0.214 M. The ionic strength was adjusted with tetramethylammonium chloride. The sample concentration was 0.0107 M in each in NMR measurement. The previously reported pK_a of imidazolium ion in this solvent mixture (5.71) [14] was used to determine the pK_a value of the buffer acid, and hence the pH of each buffer solution.

Pharmacology

Affinity to human α-2 receptor subtypes

Membranes from Shionogi S115 cells stably transfected with one of three human α-2 subtypes [7, 8] and [^{3}H]rauwolscine at a concentration corresponding to its K_D for the respective α-2 adrenoceptor subtype (0.4–1.5 nM) were incubated in 50 mM KH_2PO_4, pH 7.5, with 12 concentrations of test compounds ranging from 1 mM to 300 μM. Each concentration was run in duplicate. Nonspecific binding was defined by 100 μM oxymetazoline. After 30 min at 25 °C, incubations were terminated by rapid vacuum filtration through a glass fibre filter. Filters were washed three times with 5 mL ice-cold incubation buffer, dried and counted for radioactivity in a scintillation counter. Analysis of experiments was carried out using ligand, a nonlinear least-squares curve fitting progran [15]. Experiments were repeated three times.

Mydriasis reversal in rats

Male Sprague–Dawley rats (B&K, Sollentuna, Sweden) weighing 190–300 g were used. The ability of **1** and **2a** to block central α-2 adrenoceptors were studied in vivo as the ability to reverse detomidine (100 μg/kg iv)-induced pupil dilation in rats. Ten minutes after detomidine-induced maximal pupil dilatation, test compounds were administered iv in a cumulative manner at 5 min intervals. The measurement of pupil diameter was performed as described previously [9].

Field stimulated prostatic portion of rat vas deferens

The antagonist effects of **1** and **2a** on presynaptic α-2 adrenoceptors were studied in vitro in a field-stimulated prostatic portion of rat vas deferens as described earlier [10]. The α-2 adrenoceptor blocking potency is expressed as a pA_2-value, calculated as described in the literature [11].

Pharmacokinetics. Estimation of elimination rate

Compound **2a** (3 mg/kg bodyweight) was administered subcutaneously to three adult male Sprague–Dawley rats. At seven predefined times, a blood sample was withdrawn from each rat's tail vein and serum separated by centrifugation. Sera from the same sampling time were combined and their **2a** content analysed by reversed-phase HPLC using fluorescence detection. Concentrations were plotted against sampling time and an estimate of the elimination half-life was obtained graphically.

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