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IDENTIFICATION AND CHARACTERIZATION OF m4 SELECTIVE MUSCARINIC ANTAGONISTS

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Abstract: Our interest in the area of m4 muscarinic antagonists has led us to study a series of benzoxazine isoquinolines. One of the most potent and selective compounds of this series is example **1** with an IC_{50} value of 90.7nM at m4 receptors, and 72-fold (m1), 38-fold (m2), 10-fold (m3), and 82-fold (m5) more selective compared to the other receptors. The synthesis and receptor binding affinity of analogs of **1** are reported.

The characterization of the five distinct muscarinic receptor subtypes (m1–m5) has made it possible to target specifically the blockade of one muscarinic receptor subtype.[1] Therefore, identification of selective ligands for the muscarinic receptor subtypes, m1–m5, may be useful as tools in the study and treatment of various central nervous system disorders. The m1, m2, and m4 receptors in the brain have been identified in the striatum using immunocytochemical and in situ hybridization techniques, with the highest level of m4 receptors occurring in the striatum.[1] These receptors are believed to be involved in the control of motor function, and therefore, selective m4 muscarinic antagonists may be useful in the treatment of movement disorders such as Parkinson's Disease (PD). Two commonly known "m4" muscarinic antagonists are Artane and himbacine. Artane, an m1/m4 muscarinic antagonist, has been used clinically to suppress tremor and relieve rigidity associated with the early stages of PD.[2] However, these motor benefits could potentially be accompanied by cognitive dulling, since m1 receptors, found predominately in the hippocampus and neocortex, are intimately involved in cognitive function. Therefore, an antagonist specific for m4 over m1 receptors could be efficacious against PD motor symptoms without causing deleterious cognitive side effects. Himbacine, an alkaloid that has been isolated from the bark of *Galbulimima* species of trees found in North Queensland and New Guinea,[3] binds to both m2 and m4 receptors, and has been claimed as a relatively potent and selective antagonist for cardiac receptors, which are predominantly m2.[4]

Presented here is a series of benzoxazine isoquinolines that have been synthesized and characterized as potential m4 selective muscarinic antagonists. The affinity of these compounds for the five human receptor subtypes (m1-m5) was determined by [^{3}H]-NMS[5] binding using membranes from transfected Chinese Hamster Ovarian (CHO) cells.[1a,6] The structural type, represented by the examples illustrated in Table 1, was identified as m4 selective antagonists via mass screening. From the series of compounds synthesized and tested, the most potent analog identified was example **1**, which demonstrated a 72-fold selectivity at the m4 receptors over m1 receptors. To date, no truly m4 selective synthetic muscarinic antagonist is known.[7] Therefore, these results clearly illustrate that **1** is the first m4 selective synthetic muscarinic antagonist to be identified.

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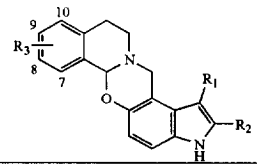
TABLE 1.



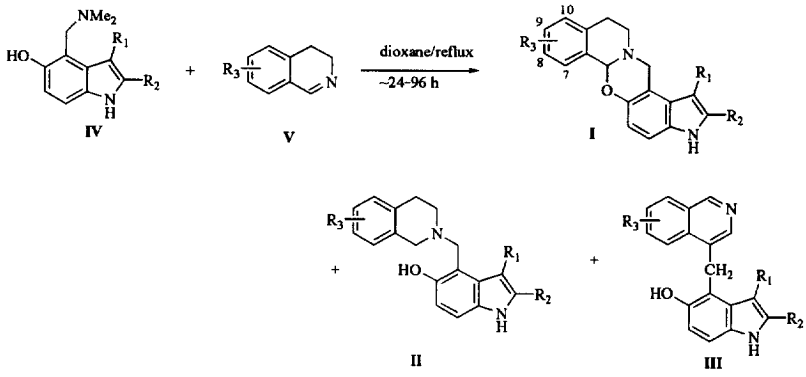
Example	R_1	R_2	R_3	IC_{50} (nM)[a] m1	m2	m3	m4	m5
———	Artane[8a]	———		1.3	18.6	26.3	6.7	10.3
1	**$CO_2CH_2CH_3$**	**CH_3**	**9-OMe**	**6558.7**	**3440.7**	**950.0**	**90.7**	**7411.7**
2	CO_2CH_3	CH_3	9-OMe	3788.0	13138.0	3394.0	501.0	4093.0
3	$CO_2CH_2CH_3$	H	9-OMe	5616.8	4563.5	3704.2	333.5	5999.2
4	H	CH_3	9-OMe	10766.6	1151.5	15293.8	9735.2	17980.7
5	$CO_2(CH_2)_2CH_3$	CH_3	9-OMe	1961.9	1905.1	1482.3	359.6	3807.6
6	$CO_2CH(CH_3)_2$	CH_3	9-OMe	4058.0	2643.0	2817.0	2050.0	4737.0
7	$CO_2CH_2CH_3$	CH_3	8-OMe	9017.8	3002.9	10804.1	6027.8	8211.3
8	CO_2CH_3	CH_3	H	7121.2	4662.4	12612.2	9927.5	5760.1
9	$CO_2CH_2CH_3$	CH_3	8,9-di-OMe	12903.4	9341.4	1616.4	301.1	26644.8
10	$CO_2CH_2CH_3$	H	8,9-di-OMe	8221.3	7703.7	10315.1	1498.3	47361.5
11	H	CH_3	8,9-di-OMe	32653.5	14677.7	32589.6	4741.3	43462.7
12	$CO_2CH_2CH_3$	CH_3	9-Me	3277.5	9524.7	2720.9	464.8	3884.2
13	$CO_2CH_2CH_3$	CH_3	9-OCH_2CH_3	4250.0	2565.0	2629.0	1624.0	8624.0
14	$CO_2CH_2CH_3$	CH_3	9-OC_6H_5	1959.3	1646.0	3067.8	2633.7	2033.9
15	$CO_2CH_2CH_3$	CH_3	7-Me	6109.7	<1000	2827.7	1474.8	1731.8
16	$CO_2CH_2CH_3$	CH_3	H	5009.3	2680.7	5493.9	3713.8	4187.1
———	Himbacine[8b]	———		548.2	17.5	333.6	114.4	1016.0

[a] The affinity of these compounds for the five human muscarinic receptor subtypes (m1-m5) was determined by the displacement of [^{3}H]-NMS binding using membranes from transfected CHO cells. All compounds were tested two to four times with duplicate tubes (SEM is $\leq$ 10% in all cases). Complete protocol is described by F. Dorje et al.[1a] and N.J. Buckley et al.[6]

Chemistry

The series of benzoxazine isoquinoline analogs (**I**) illustrated in Table 1 were synthesized by coupling substituted indoles (**IV**) with various dihydroisoquinolines (**V**) as shown in Equation 1.[9]

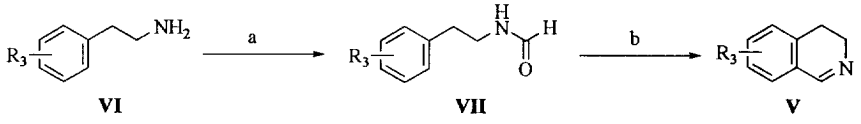
Equation 1



The synthesis of analogs of **1** yielded a mixture of benzoxazine isoquinoline **I**, phenol **II**, and rearranged isoquinoline **III**. The desired isoquinoline **I** was isolated in various yields. In general, compounds with an ester functionality at R_1 and 8,9-diOMe at R_3 (**9** and **10**) afforded the highest yields (23%) of **I**. Isoquinoline **I** was isolated and characterized as the major product (5–20%) for **1**, **2**, **4**, **5**, and **9–16**, whereas isoquinoline **III** (10–30%) was the major product for **3** and **6–8**, and the minor product for **13**, **14**, and **15**. The phenol **II** was detected in low yields (<10%) for **1**, **2**, **8**, **9**, **12**, **13**, and **16**.

Synthesis of dihydroisoquinolines (**V**) involved a two-step procedure (Eq 2).[10,11] Treatment of the corresponding phenethylamine (**VI**) with formic acid, followed by ring closure of **VII** with phosphorous oxychloride gave dihydroisoquinoline **V** (**1–7** and **9–10**). For **8** and **16**, phosphorous pentoxide was used for ring closure of **VII**.

Equation 2



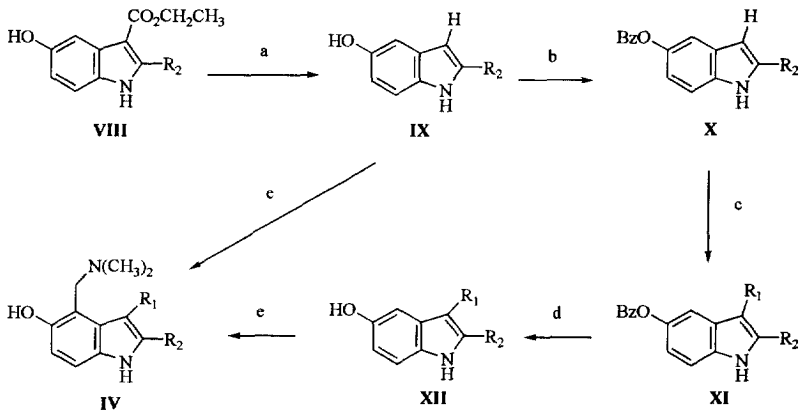
VI VII V

(a) 95-97% HCO_2H; (b) $POCl_3$ or P_2O_5, Tetralin

Scheme 1 illustrates the synthetic route utilized for the majority of indoles **IV**. For **5** and **6**, decarboxylation of ethyl-5-hydroxy-2-methylindole-3-carboxylate **VIII**, in the presence of sodium hydroxide, gave intermediate **IX**.[12] Protection of phenol **IX** with benzyl chloride in the presence of potassium carbonate yielded **X**.[13] Treatment of **X** with ethyl magnesium bromide and the appropriate chloroformate gave the

corresponding ester **XI**. Deprotection of **XI** using standard conditions yielded the 5-hydroxy substituted indole **XII**.[14] Treatment of **XII** with dimethylamine and paraformaldehyde in methanol gave the desired indoles (**IV**). For **4** and **11** ($R_2 = CH_3$) and **3** and **10** ($R_2 = H$), deprotection of **X** and **XI**, respectively, yielded intermediate **XII**, which was required to give indole **IV**. For **1**, **7**, **9**, and **12–16**, ethyl 5-hydroxy-2-methylindole-3-carboxylate (**VIII**, $R_2 = CH_3$) was treated directly with dimethylamine and paraformaldehyde to give indole **IV**.

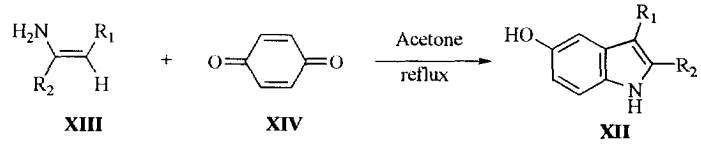
Scheme 1



(a) sol. NaOH, 2 N NaOH; (b) $PhCH_2Cl$, K_2CO_3 CH_3CN; (c) EtMgBr, R_1Cl, Et_2O; (d) H_2/20% Pd-C, EtOH/THF; (e) $(CH_3)_2NH$, $(HCHO)_n$, MeOH

The synthetic route of the required indoles (**IV**) for **2** and **8** is illustrated in Equation 3.[15] Condensation of ethyl-3-methylaminocrotonate (**XIII**) and 1,4-benzoquinone (**XIV**) yielded **XII**, which then could be converted to the desired indoles **IV** as shown in Scheme 1.

Equation 3



Results and Discussion

A series of benzoxazine isoquinolines have been synthesized and studied as potential selective m4 muscarinic antagonists. From this group of compounds, example **1**, the most selective synthetic m4 antagonist reported to date, was identified. Example **1** is as potent as himbacine at the m4 receptor (IC_{50} = 90.7 nM vs. 114.4 nM, respectively), but is about 13x less potent than Artane (IC_{50} = 90.7nM vs. 6.7 nM, respectively). However, example **1** is extremely more selective at the other receptors than himbacine or Artane (Table 2).

TABLE 2. Selectivity of **1**, Artane, and himbacine versus m4 Receptors.

	Hm1	Hm2	Hm3	Hm5
Example 1:	72-fold	38-fold	10-fold	82-fold
Artane:	<1-fold	3-fold	4-fold	2-fold
Himbacine:	5-fold	<1-fold	3-fold	9-fold

In an effort to improve the affinity of **1** at the m4 receptor and maintain its selectivities at the other receptors, various modifications at R_1, R_2, and R_3 were examined. Replacement of the methyl group at R_2, the ethyl ester group at R_1, or the methoxy group at R_3 of **1** with hydrogen (**3**, **4**, and **16**, respectively) decreased affinity at the m4 receptor (4- to 100-fold), and either decreased (**3**) or eliminated (**4** and **16**) selectivities at the other receptors. Different ester groups (R_1) showed a 4- to 22-fold decrease in m4 affinity and either illustrated a decrease (**2** and **5**) or almost completely eliminated selectivity (**6**) at the other receptors. When R_3 of **2** was replaced with a hydrogen (**8**), the m4 affinity decreased an additional 20-fold, and no selectivity was observed. An additional methoxy substituent on the isoquinoline ring (**9–11**) yielded a 3- to 50-fold decrease in affinity at the m4 receptor and a decrease in selectivity at the other receptors. Various substituents (R_3) on the isoquinoline ring yielded a decrease in affinity (5- to 66-fold) at the m4 receptor and no selectivity (**7**, **13–15**) or decreased (**12**) selectivity at all other receptors. Many phenols **II** and rearranged isoquinolines **III** were also tested against all five muscarinic subtypes.[16] However, all analogs were inactive against all subtypes, which may indicate that this pentacyclic ring system of **I** is an important pharmacophore for muscarinic activity. The methyl iodide salt of **1** was also synthesized and its binding results illustrated no activity at any receptor.[17] Apparently, a basic nitrogen within the molecule is necessary for good binding affinity and selectivity at all receptors. To confirm the antagonist properties of **1**, functional data was obtained.[18a] In Hm1, Hm3, and Hm5 CHO cells, **1** prevented the stimulation of phosphatidylinositol (PI) hydrolysis produced by carbachol (m1: IC_{50} = 10,000 nM; m3: IC_{50} = 880 nM; m5: IC_{50} = 25,200 nM). In Hm2 and Hm4 CHO cells, it blocked carbachol-induced inhibition of forskolin-stimulated cAMP accumulation (m2: IC_{50} = 3.8 nM; m4: IC_{50} = 0.4 nM). No agonist activity was observed at any of the receptor subtypes.[18b]

In conclusion, a series of benzoxazine isoquinolines have been synthesized and characterized as selective m4 muscarinic antagonists. The most potent and selective compound of this series, example **1** ($R_1 = CO_2Et$, $R_2 = CH_3$, R_3 = 9-OMe), has been identified as the most selective synthetic m4 antagonist known to date. The results have determined that, apparently, the binding site of the m4 receptor prefers the substituents of **1**, and various R_1, R_2, and R_3 groups are not well-tolerated, as illustrated by the overall decrease in affinity at the m4 receptor and the substantial decrease in selectivities at all receptors (Table 1).

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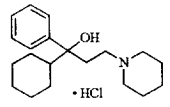
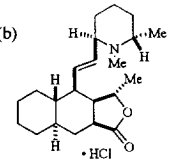
References and Notes

1. (a) Dorje, F.; Wess, J.; Lambrecht, G.; Tacke, R.; Mutschler, E.; Brann, M. R. *J. Pharmacol. Exp. Ther.* **1991**, *256*, 727; (b) Bolden, C.; Cusack, B.; Richelson, E. *J. Pharmacol. Exp. Ther.* **1992**, *260*, 576.
2. Adamson, D. W.; Wilkenson, S. U.S. Patent 2 682 543, 1954; *Chem. Abstr.* **1955**, *49*, 9039.
3. Taylor, W. C.; Ritchie, E. In *The Alkaloids*; Manske, R. H. F., Ed.; Academic: New York, 1967; Vol. 9, pp 529–543.
4. (a) Miller, J. H.; Aagaard, P. J.; Gibson, V. A.; McKinney, M. *J. Pharmacol. Exp. Ther.* **1992**, *263*, 663.; (b) Himbacine analogs, such as dihydrohimandravine, himandravine, N-methylhimandravine, dihydrohimbeline, N-methyldihydrohimandravine, and dihrohumabcine, which were obtained from W.C Taylor at the University of Sydney, showed no activity against m1–m5.
5. NMS, N-methylscopolamine, is a nonselective muscarinic antagonist. Its binding results are: m1: IC_{50} = 1.8 nM; m2: IC_{50} = 4.3 nM; m3: IC_{50} = 1.1 nM; m4: IC_{50} = 2.3 nM; m5: IC_{50} = 1.9 nM.
6. Buckley, N. J.; Bonner, T. I.; Buckley, C. M.; Brann, M. R. *Mol. Pharmacology* **1989**, *35*, 469.
7. It has been reported (Adem, A; Karlsson, E. *Life Sciences* **1997**, *60*, 1069) that muscarinic neurotoxins, which are isolated from Mamba snake venoms, are most selective for m1 and m4 receptors.
8. (a)  (b) 
9. von Strandtmann, M.; Cohen, M. P.; Shavel, Jr., J. U.S. Patent 3 649 626, 1972; *Chem. Abstr.* **1972**, *76*, 140844.
10. Gulland, J. M.; Virden, C. J. *J. Chem. Soc.* **1929**, 36, 1791.
11. Witkop, B. *Gelsemine* **1948**, 70, 1424.
12. Beer, J. S.; Clarke, K.; Davenport, J. F.; Robertson, A. *J. Chem. Soc.* **1951**, 2029.
13. Vyas, G. N.; Shah, N. M. In *Org. Syn. Coll. Vol. IV*; Rabjohn, N., Ed.; John Wiley and Sons: New York, 1963; pp 836–838.
14. Marchelli, R.; Hutzinger, O.; Heacock, R.A. *Canadian J. Chem.* **1969**, 47, 4376.
15. Steck, E. A. U.S. Patent 2 852 527, 1958; *Chem. Abstr.* **1959**, *53*, 8163.
16. Phenol analogs (**II**) of **1**, **4**, **8**, and **10** and rearranged isoquinoline (**III**) of all examples shown in Table 1 except of **4**, **9**, and **11** were tested.
17. Binding data for methyl iodide salt of **1**:

%Inhibition	Hm1	Hm2	Hm3	Hm4	Hm5
100 nM	12.7	14.7	0.0	0.0	0.9
1000 nM	24.5	29.9	0.0	19.9	1.6

18. (a) Schwarz, R. D.; Nelson, C. B.; Augelli-Szafran, C. E.; Penvose-Yi, J. R.; Jaen, J. C.; Wiley, J.; Frey, K. A. In: Book of Abstracts and Proceedings, The Seventh International Symposium on Subtypes of Muscarinic Receptors, Vienna, Virginia, **1996**. Detailed pharmacological data of **1** will be described by Schwarz, R. D., et. al., in a future communication; (b) The selectivity between subtypes appear slightly different than the binding results and reflect the use of two separate assays (PI hydrolysis and cAMP accumulation).