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ISOARECOLONES AND ARECOLONES : SELECTIVE CENTRAL NICOTINIC AGONISTS THAT CROSS THE BLOOD-BRAIN BARRIER

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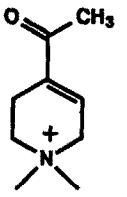
Abstract: Isoarecolones and arecolones bearing different ketone substituents were synthesized and their affinities at central muscarinic and nicotinic receptors determined. The compounds were selective for nicotinic receptors, produced appropriate nicotinic responses, and crossed the blood brain barrier in rats.

Nicotinic cholinergic receptors are widely distributed in the human brain and have been shown to mediate central neurotransmission both directly and indirectly in animals.[1-4] Their contributions to higher functions, including learning and memory, have been the focus of many recent studies.[5-7] Nicotinic receptor dysfunction has also been implicated in a number of central nervous system diseases either by finding the receptors diminished in number, as in the case of several types of dementia and Parkinson's disease,[8-12] or in finding clinical efficacy with nicotine in CNS disorders or smoking addiction.[13,14]

Nicotinic receptors are not homogeneous as demonstrated by both radioligand binding studies and molecular genetics.[15,16] The differences in the neuronal nicotinic receptors and those located at neuromuscular junction are particularly noteworthy and the diversity of subunits and the multicomponent nature of neuronal nicotinic receptors suggests the possibility of developing subtype specific neuronal nicotinic agents. Therefore, the goal of our research program is to develop new, more selective, centrally acting, nicotinic agonists that can be used in neurotransmitter replacement therapy, therapies where nicotine is currently used, and as tools to study the physiological role of nicotinic receptors.



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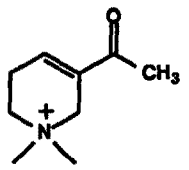

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The *Torpedo* nicotinic receptor affinities and frog rectus abdominis muscle constricting properties of a series of quaternary isoarecolones **1**, arecolones **2**, and derivatives have been studied with many of these compounds showing potent nicotinic agonist activity.[17-20] Moreover, the affinity of many of these compounds for central muscarinic cholinergic receptors was many fold less than their affinities for *Torpedo* nicotinic receptors. The

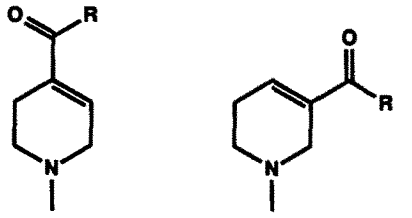
affinities of these compounds for central nicotinic receptors were not investigated, but it is unlikely that any of these compounds would be useful as centrally acting agents because their charged quaternary structure would not permit them to penetrate the blood brain barrier. By contrast, uncharged isoarecolone **3a** has been shown to bind to central nicotinic receptors and appears to cross the blood brain barrier because it produced nicotine-like discriminative effects in rats.[21] Therefore, we chose to study the relative central nicotinic and muscarinic affinities of a series of uncharged isoarecolones and the isomeric arecolones to detect potentially selective, centrally acting nicotinic agonists. In addition, the neurochemical effects of selected compounds were investigated to study their ability to penetrate the blood brain barrier and to provide evidence for function nicotinic agonist activity.

Chemistry: The arecolones and isoarecolones were prepared by our previously reported synthetic routes from N,O-dimethylamides of isoarecaidine and arecaidine, respectively.[22,23]

Radioligand binding: The affinities of the compounds for nicotinic receptor sites in rat cortex were determined using competitive radioligand binding studies with [^{3}H]methyl-carbachol (MCC).[24,25] MCC has been shown to be a nicotinic agonist[4] and affinity for the MCC binding sites has correlated well with psychotropic effects in rats.[26] The affinities of the compounds for muscarinic receptor sites in rat hippocampus were determined using competitive radioligand binding assays employing [^{3}H]oxotremorine-M (Oxo-M).[27] Oxo-M is a potent muscarinic agonist lacking muscarinic subtype selectivity. The affinity of a compound for Oxo-M binding sites has been interpreted as the compounds affinity for the "muscarinic agonist conformational state."[28] The ratio of a compound's K_is for Oxo-M and MCC, respectively, was treated as a measure of selectivity for nicotinic agonist activity (Table I).

Neurochemistry: Nicotine increases dopamine turnover in rat brain as demonstrated by production of increased dopamine metabolite concentrations.[2,29] To determine if compounds from these series were centrally available and had neurochemical effects similar to nicotine, the concentrations of dopamine metabolites dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) in rat striatum and nucleus accumbens were determined after subcutaneous administration of nicotine, **3f**, and **4f** by previously reported methods (Table II).[30]

Table I. Muscarinic and nicotinic receptor affinities of arecolones and isoarecolones.



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R	no.	K_i, nM[a] MCC	K_i, nM[a] Oxo-M	Nicotinic Selectivity K_i Oxo-M/K_i MCC
Me	3a	48.4 ± 2.2	5425 ± 1420	112
	4a	32.7 ± 5.5	4380 ± 260	134
Et	3b	25.7 ± 2.9	>5000	>195
	4b	5.8 ± 1.5	1950 ± 375	336
n-Pr	3c	67.3 ± 14.7	>5000	>74
	4c	13.1 ± 2.7	2730 ± 550	208
n-Bu	3d	148.7 ± 9.9	4345 ± 1100	29
	4d	61.3 ± 4.6	1385 ± 250	23
t-Bu	3e	56.7 ± 15.9	6250 ± 3500	110
1-propynyl	3f	22.0 ± 0.4	2690 ± 620	122
	4f	5.8 ± 1.1	1650 ± 330	284
phenyl	3g	766 ± 42	3750 ± 1050	5
	4g	178 ± 6.3	2450 ± 600	14
benzyl	3h	946 ± 17.3	3500 ±760	4
	4h	123 ± 24.7	1885 ± 550	15
OMe	arecoline	224 ± 14	40 ± 1	0.18
nicotine		8.4 ± 1.5	>5000	>595

[a] Assays were run in triplicate, IC_{50}s determined, and K_is calculated from the formula $K_i = IC_{50}/(1 + F/K_d)$. Maximum concentrations of compounds used were 10 μM. Detailed methods are thosed used in references 25 (MCC) and 27 (Oxo-M).

Results: The affinities of the isoarecolones for nicotinic receptors was highest with compact small substituents attached to the carbonyl group, e.g. **3a,b,c,e,f**, all having K_is below 100 nM. Highest affinities were obtained with propynyl and ethyl substituted **3f** and **3b**, respectively, which had three fold less affinity than nicotine. Larger **R** groups, e.g., *n*-butyl, **3d**, phenyl, **3g**, benzyl, **3h**, produced much lower affinities for nicotinic receptors.

Table II. Effects of nicotine, 3f, and 4f on rat brain dopamine metabolites.

Treatment[a] mg/kg, s.c.	DOPAC nMoles/gm (% control)	HVA nMoles/gm (% control)	DOPAC nMoles/gm (% control)	HVA nMoles/gm (% control)
	STRIATUM		NUCLEUS ACCUMBENS	
Control	1.55 ± 0.13 (100)	0.89 ± 0.05 (100)	1.39 ± 0.12 (100)	0.55 ± 0.06 (100)
(-)-Nicotine-1 mg/kg	2.18 ± 0.08 (140)[b]	1.34 ± 0.07 (151)[b]	2.00 ± 0.11 (144)[b]	0.86 ± 0.08 (157)[b]
3f- 30 mg/kg	1.91 ± 0.08 (123)[b]	1.25 ± 0.03 (140)[b]	1.66 ± 0.05 (120)	0.73 ± 0.03 (132)[b]
4f- 30 mg/kg	1.80 ± 0.08 (115)	1.21 ± 0.06 (135)[b]	1.79 ± 0.12 (129)[b]	0.88 ± 0.04 (159)[b]

[a] Rats were sacrificed 40 minutes after dosing with nicotine and 30 minutes after dosing with controls, **3f** and **4f**.
[b] $p = <0.05$.

The nicotinic receptor affinity-substituent relationship seen with the isoarecolones was mirrored in the arecolones **4**, but the affinities of the arecolones were much higher than that of the similarly substituted isoarecolones. Highest affinities were seen with the ethyl and propynyl substituted **4b** and **4f**, respectively, which had higher affinity for nicotinic receptors than nicotine.

The isoarecolones **3** and arecolones **4** had poor affinities for Oxo-M binding sites with K_is exceeding 1000 nM. Generally, the arecolones had higher affinities for Oxo-M binding sites than similarly substituted isoarecolones. By contrast, the muscarinic agonist arecoline had high affinity for Oxo-M binding sites, almost 50 fold greater than **4b**, the arecolone where a methylene group replaces the oxygen of arecoline.

Compounds **3f** and **4f** were chosen for the neurochemical studies because they showed the highest affinities for nicotinic receptors in their respective series and their similar substitution pattern would permit a direct comparison of the 4- and 3- substitution pattern on the 1,2,5,6-tetrahydropyridine ring, respectively. Significant increases in the dopamine metabolites DOPAC and/or HVA were produced in rat striatum and nucleus accumbens after subcutaneous administration of nicotine (1 mg/kg), **3f** (30 mg/kg), and **4f** (30 mg/kg). Previous studies with nicotine using the same doses in rats have not shown increases in DOPAC and HVA in the striatum.[2,29] This was probably due to methodological differences because dopamine metabolites were increased in other brain regions. Much higher doses of **3f** and **4f** than of nicotine were used to assure detection of a neurochemical effect and to demonstrate that the compounds penetrate the blood brain barrier. Future neurochemical experiments will further define the relative efficacies and potencies of these compounds.

Discussion: Generally, better than 100 fold selectivity for nicotinic receptors over muscarinic receptors is seen with small substituents attached to the ketone in the isoarecolones and arecolones, e.g., **3a,b,c,e,f, 4a,b,c,f,** (Table I). Highest selectivity is seen with ethyl substituted arecolone **4b** which shows greater than 300 fold selectivity for nicotinic receptors, shares with **4f** the highest affinity for nicotinic receptors among the isoarecolones and arecolones tested, and has affinity for nicotinic receptors greater than (-)-nicotine. Compounds with larger substituents, e.g., **3d,g,h, 4d,g,h**, although selective for nicotinic receptors, show much less selectivity due to increases in affinities for muscarinic receptors and decreases in affinities for nicotinic receptors compared to the compounds with smaller substituents. By contrast, arecoline shows almost six fold selectivity for muscarinic receptors over nicotinic receptors because of its much higher affinity for muscarinic receptors. Although exact nicotinic selectivity ratios for nicotine, **3c**, and **3d**, were not obtained because high enough concentrations of these compounds were not tested to obtain K_is for Oxo-M binding, it appears that none of the isoarecolones or arecolones show quite as high a selectivity for nicotinic receptors as nicotine. Future testing in nicotinic and muscarinic functional assays will be required to demonstrate agonist specificity, but the binding selectivity seen using agonist radioligands suggests that some selectivity should be obtained.

Although not comparable in all cases, the arecolones **4** generally show somewhat better selectivity for nicotinic receptors than the isoarecolones **3** with the same **R** substituent attached to the ketone, e.g., compare **3a**, **4a**; **3f**, **4f**. The arecolones also have higher affinity for nicotinic receptors than the isoarecolones, suggesting that the arecolones are a more promising series of nicotinic agonists. Our computation studies show that arecolone **4a** can fit the nicotinic pharmacophore model described by Beers and Reich in energetically feasible conformations, but future modeling studies will be required to investigate **R** substituent effects.[31]

The isoarecolones and arecolones appear to penetrate the blood brain barrier because subcutaneous administration of **3f** or **4f** produces the increases in dopamine metabolites in rat striatum and nucleus accumbens that are seen with nicotine. In addition, **3a** has been shown to produce nicotine-like discrimination in nicotine trained rats.[21] Although not yet conclusively proven, the increases in dopamine metabolites produced by **3f** and **4f** suggests that these compounds are nicotinic agonists.

This study has demonstrated that isoarecolones and arecolones appropriately substituted with small substituents have high affinities and selectivities for nicotinic acetylcholine receptors compared to muscarinic acetylcholine receptors. The affinities for nicotinic receptors of some arecolones, **4b** and **4f**, exceed that of nicotine. Both isoarecolones and arecolones cross the blood brain barrier and produce changes in dopamine metabolites consistent with nicotinic agonist activity. The higher selectivities and affinities of the arecolones for nicotinic receptors compared to isoarecolones suggests that the arecolones are a more promising series of

nicotinic agonists.

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