Journal of Medicinal Chemistry

Volume 43, Number 5 March 9, 2000

Communications to the Editor

α_2 Adrenoceptor Agonists as Potential Analgesic Agents. 2. Discovery of 4-(4-Imidazo)-1,3-dimethyl-6,7-dihydro-thianaphthene as a High-Affinity Ligand for the α_{2D} Adrenergic Receptor

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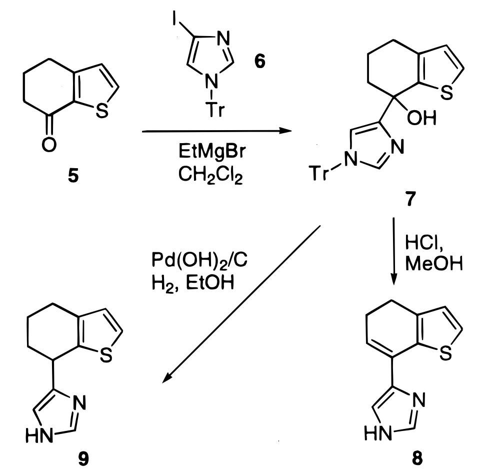
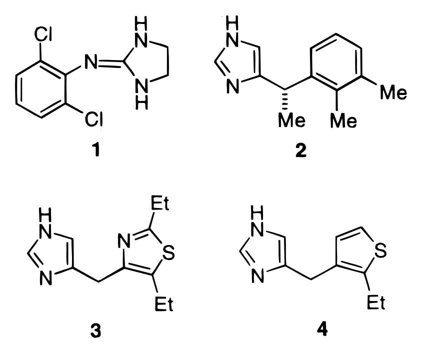
Received November 15, 1999

Introduction. The treatment of pain continues to be the subject of considerable pharmaceutical and clinical research.[1] α_2 Adrenoceptor agonists are intriguing because they produce clinically effective analgesia that is devoid of opioid-related side effects.[2] The α_2 adrenergic agonist clonidine (**1**) is prescribed for the epidural treatment of severe pain, and the α_2 agonist dexmedetomidine (**2**) is used in veterinary practice and for humans in several countries outside the United States.[3] The primary site of antinociceptive action for α_2 adrenergic analgesia is in the dorsal horn of the spinal cord.[2a] α_2 Adrenergic receptors are localized largely in the presynaptic membrane, and activation of these receptors inhibits the release of pain-inducing neurotransmitters such as substance P, glutamic acid, and CGRP. We have recently reported the potent α_2 adrenergic receptor affinity and analgesic activity associated with (imidazomethyl)oxazoles and -thiazoles, such as **3** (RWJ-37210).[4] In addition, we have investigated a large number of (imidazomethyl)thiophenes, such as **4,** that have a similar biological profile.[5] Analyzing the preferred conformations of **4** by molecular modeling revealed significant torsional freedom for rotation of the two single bonds between the imidazole and thiophene.

Scheme 1





To restrict the rotation of this molecule, we extended and combined the 2-ethyl substituent with the bridging carbon atom to obtain the (imidazo)thianaphthene chemical series (e.g. **8** and **9**, Scheme 1). These compounds are potent α_2 adrenergic receptor ligands, and certain members of the series are very active in animal models of antinociception. 4-(4-Imidazo)-6,7-dihydro-thianaphthene (**14**) has a favorable biological profile in vivo and is a prototype for this class of analgesic agents.

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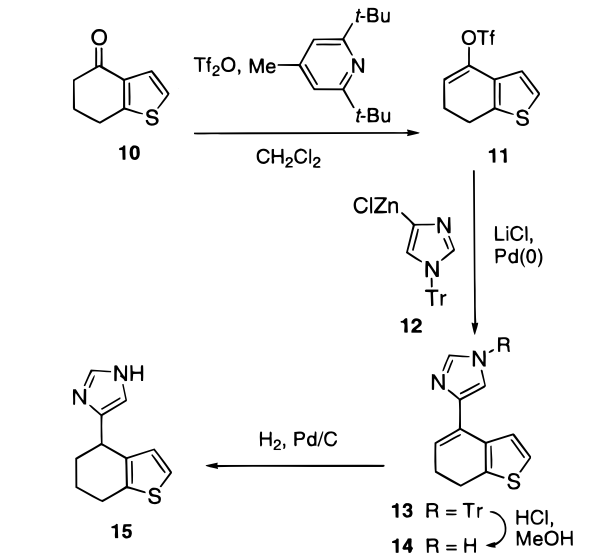
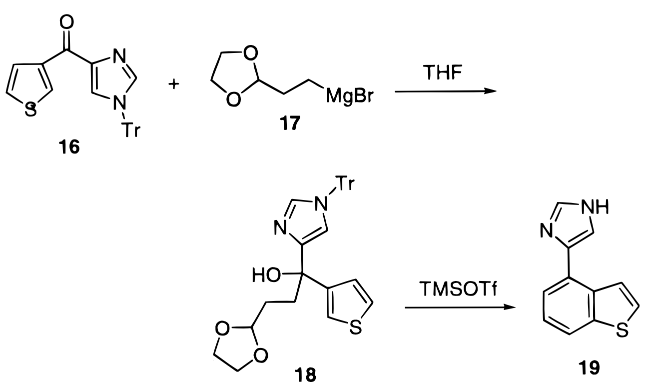
10.1021/jm990569e CCC: $19.00 © 2000 American Chemical Society
Published on Web 02/17/2000

Scheme 2



Scheme 3

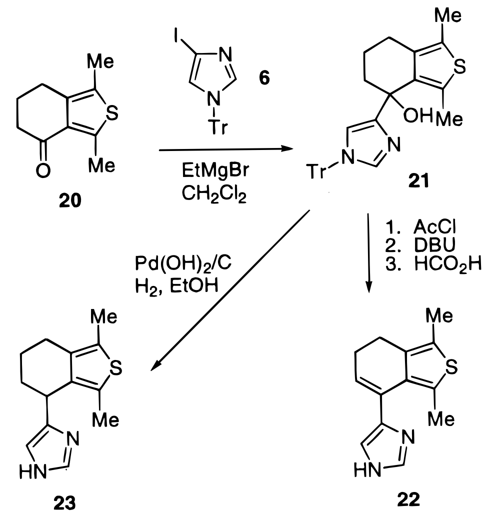


Chemistry. Several complementary methods were used to prepare the (imidazo)thianaphthenes. Reaction of thienyl ketone **5**[6] with the Grignard reagent[7] derived from 4-iodo-1-tritylimidazole (**6**) gave alcohol **7** (84%; Scheme 1). This material was either treated with HCl in MeOH to yield unsaturated derivative **8** (20%) or reduced to fully saturated **9** by hydrogenation over palladium on carbon (29%). Although this method was used for the preparation of **14** and **15**, we also developed a strategy involving vinyl triflate coupling as shown in Scheme 2. Thienyl ketone **10** was converted to vinyl triflate **11** in 90% yield by using triflic anhydride and a hindered pyridine base. Coupling of either the 4-imidazo zinc reagent **12** or the 4-(tributylstannyl)imidazole (not shown) gave alkene **13** (78% when using zincate **12**).[8] Cleavage of the trityl group with HCl in MeOH provided target **14**, which was further hydrogenated reducing the alkene to afford saturated congener **15** (88%).

Parent unsaturated thianaphthene **19** was prepared starting with condensation of thienyl ketone **16** and (dioxanyl)ethyl Grignard reagent **17** (Scheme 3).[9] Resulting alcohol **18** was obtained in 90% yield. Treatment with trimethylsilyl triflate promoted a Friedel–Crafts reaction and dehydration to give target **19** (40%).

To complete the series, so that all possible positions of thiophene ring fusion were represented, we used thiophene **20** as a starting ketone (Scheme 4). The 2,5-methyl groups were present originally only as a synthetic convenience in the preparation of **20** in order to direct the position of keto ring fusion.[6] Ketone **20** was condensed with the Grignard reagent derived from **6** to yield **21** (94% yield). Dehydration of **21** was accomplished by preparing the acetate and elimination of the acetoxy group with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU). The trityl group was then removed under the mild conditions of formic acid at room temperature[10] producing target **22** (50% overall yield). Compound **21** was also reduced using catalytic hydrogenation with palladium hydroxide to give saturated derivative **23** (39%).

Scheme 4



Pharmacology. The α_2 adrenergic receptors are members of the seven-transmembrane G-protein-coupled (GPCR) family and are unusual because they have a very large third intracellular loop.[11] There are three α_2 adrenoceptor subtypes that have been identified and cloned in humans: α_{2A}, α_{2B}, and α_{2C}. The pharmacologically defined α_{2D} adrenergic receptor is the rat (and mouse) molecular ortholog of the human α_{2A} subtype and is often referred to as rat α_{2A}. Because α_{2D} and α_{2A} can be differentiated pharmacologically, there is value in testing both to better understand the predictive value of rodent models for efficacy in the clinic. Pharmacological ranking of α_2 adrenergic agents comparing receptor subtype affinity with in vivo activity in rats and mice, as well as studies with knock-out mice in which the α_{2A} adrenergic receptor subtype has been inactivated, suggests that the rodent α_{2A} (α_{2D}) adrenergic receptor is the one primarily responsible for antinociception.[12] The functional roles of the α_{2B} and α_{2C} receptors are not presently well-established.

Compounds **8**, **9**, **14**, **15**, **19**, **22**, and **23** were tested for in vitro receptor binding and in vivo biological activity in animal models predictive of analgesic activity in humans (Table 1). The α_{2D} adrenergic receptor binding assay from rat was the primary in vitro test, using *p*-aminoclonidine as the displaceable ligand.[13] The compounds were also evaluated at the rat α_1 adrenergic receptor[13] and in cells containing native α_{2A}, α_{2B}, and α_{2C} adrenergic receptors using RX 821002 as the ligand.[14]

The compounds were tested for potential antinociceptive activity initially at a screening dose of 30 mg/

Table 1. Biological Activity of (Imidazo)benzothiophene Derivatives and Reference Compounds

compd	adrenergic receptor subtype affinity (K_i values, nM)[a]						in vivo antinociceptive testing[b]	
	α_{2D}	α_{2A}	α_{2B}	α_{2C}	α_1	α_1/α_{2D}	MAIT, % inhib at 30 mg/kg po	RAIT, ED_{50} (mg/kg, po)
8	3.0	224	940	ND	399	133	60	ND
9	0.35	87%[c]	ND	54%[c]	91	260	73	ND
14	1.5	254	621	ND	443	295	100	15.1 [7.2, 40.3]
15	0.56	27	99	172	110	196	100	0.9 [0.54, 1.53]
19	2.6	277	663	ND	343	132	60	ND
22	0.0086	25	100	26	110	11627	40	ND
23	2.9	68	141	205	48	17	93	7.9 [3.9, 13.5]
1	0.39	853		160	116	297	0.05 mg/kg[d] [0.09, 0.01]	0.10 [0.0012, 0.5]
2	0.015				5	333	0.09 mg/kg[d] [0.04, 0.17]	0.12 [0.06, 0.21]

[a] Receptor binding K_i's were determined using 5−8 concentrations in triplicate. [b] MAIT, mouse abdominal irritant test; RAIT, rat abdominal irritant test; 95% confidence limits shown in brackets. Screening in the MAIT at 30 mg/kg po was conducted on groups of 15 animals. [c] Percent inhibition of binding at 1 μM. [d] ED_{50} values with 95% confidence limits in brackets.

Table 2. Profile of Compound **14** (RWJ-52353)

α_{2D} adrenergic receptor	1.5 nM K_i
α_1 adrenergic receptor	443 nM K_i
mouse abdominal irritant	11.6 mg/kg po[a] [6.8, 20.0]
rat abdominal irritant	15.1 mg/kg po [7.2, 40.3]
mouse 48 °C hot plate	25.9 mg/kg po [18.6, 38.2]
mouse tail flick	100.2 mg/kg po [48.0, 223.6]

[a] ED_{50} value [95% confidence limits].

kg po in the mouse abdominal irritant test (MAIT) using acetylcholine bromide as the irritant.[15] If a compound showed >75% inhibition of the response, it was tested further in the rat air-induced abdominal irritant test (RAIT).[16] Compound **14** has been examined in a variety of additional animal models including the mouse hot-plate[17] and tail-flick assays (Table 2).[18]

Results and Discussion. All of the compounds tested showed excellent receptor affinity for the α_{2D} adrenergic receptor subtype. Saturated derivatives **9** (0.35 nM K_i) and **15** (0.56 nM K_i) were ca. 3−10 times more potent than their singly unsaturated counterparts **8** and **14**. Fully aromatic thianaphthene **19** also had good affinity (2.6 nM K_i). Surprisingly, alkene **22** was exquisitely active at the α_{2D} adrenergic receptor, showing a very high affinity of 0.0086 nM K_i. In this case, the saturated derivative (**23**) was *less* active (2.9 nM K_i). Alkenes **8**, **14**, and **22** are chemically stable over prolonged periods (>3 months) at room temperature, showing no signs of air oxidation to their aromatic counterparts such as **19**. The very high affinity of **22** may be due in part to a butressing effect imparted by the methyl groups on the thiophene ring.

The compounds of the present study were tested in cell lines natively expressing other α_2 adrenergic receptor subtypes. The compounds displayed higher affinity for the α_{2A} subtype, relative to the α_{2B}, by a factor of 2−4-fold, and **15** and **23** had lower affinity for the α_{2C} adrenergic subtype relative to the α_{2B}. Alkene **22**, however, had equal affinity at α_{2A} and α_{2C} and less at α_{2B}. In general, there was only modest selectivity for the α_2 adrenergic subtypes found in humans.

These compounds bound the rat α_1 less avidly than the α_{2D} adrenergic receptors. The ratio of α_1/α_{2D} K_i values varied between 17 (for **23**) and >10 000 (for **22**). As binding to the α_1 adrenoceptor may result in unwanted cardiovascular effects, we have tried to minimize this interaction. Typical α_1/α_{2D} K_i values for these compounds range between 130 and 300.

All of the compounds show at least some activity in the MAIT at the oral screening dose of 30 mg/kg. The most potent compounds in the MAIT (**14**, **15**, and **23**) were then studied further in the RAIT. Unsaturated derivative **14** had an ED_{50} of 15.1 mg/kg po in the RAIT, and alkenes **15** and **23** were more potent (ED_{50} = 0.9 and 7.9 mg/kg).

We chose 4-(4-imidazo)-6,7-dihydrothianaphthene **14** for further in vivo evaluation. It is a potent α_{2D} adrenergic receptor ligand (K_i = 1.5 nM) with an α_1/α_{2D} K_i ratio of 295. Further, **14** is achiral, obviating the need for the resolution of enantiomers or chiral synthesis. A listing of the analgesic testing of **14** (RWJ-52353) is given in Table 2. In addition to activity in the MAIT and RAIT upon oral administration, it is also active in more stringent tests such as the mouse hot-plate and tail-flick tests, albeit at higher doses. There is no clear correlation in the series between α_{2D} receptor affinity and analgesic activity. For example, **22** with high α_{2D} adrenergic receptor affinity shows relatively little analgesic activity, with **15** showing the greatest degree of analgesia and being the third most potent at the α_{2D} adrenergic receptor. This type of discrepancy between binding affinity at an in vitro receptor and biological activity upon oral administration is often due to differential pharmacokinetics of the compounds being evaluated. In addition, the relative agonist activity of the compounds was inferred from in vivo and functional testing, so it is possible that some are not full α_{2D} adrenergic agonists.

Conclusion. The (imidazo)thianaphthenes are a new class of α_2 adrenergic agents that display potent receptor binding affinity and are active in animal models of analgesia. 4-(4-Imidazo)-1,3-dimethyl-6,7-dihydrothianaphthene (**22**) is an exceedingly potent ligand for the α_{2D} adrenergic receptor (K_i = 0.0086 nM) and is >10 000-fold selective relative to the α_1 adrenergic receptor. This compound is more potent than dexmedetomidine at α_{2D} and is a useful pharmacological tool drug for studies involving the α_{2D} adrenergic receptor.

Acknowledgment. We thank Drs. Ellen W. Baxter, John R. Carson, and David A. Demeter for helpful discussions.

Supporting Information Available: Experimental details. This information is available free of charge via the Internet at http://pubs.acs.org.

Note Added after ASAP Posting. The version of this paper that appears in the printed March 9 issue is missing Scheme 4 and Tables 1 and 2. The ASAP posting on February 17, 2000, and the current web version are correct. An Addition and Correction for the print version is available as manuscript number JM000128R.

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