Muscarinic Analgesics with Potent and Selective Effects on the Gastrointestinal Tract: Potential Application for the Treatment of Irritable Bowel Syndrome

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Irritable bowel syndrome (IBS) is a pathopysiolocal condition characterized by abnormal bowel habits that are frequently accompanied by abdominal pain. Current therapy based on reducing high-amplitude GI contractions with nonselective muscarinic antagonists is limited in efficacy due to typical muscarinic side effects and provides no pain relief. We have previously found potent antinociceptive agents acting through muscarinic receptors. In the present work, new 1,2,5-thiadiazole-based structures with muscarinic activity have been evaluated both for activity as analgesics in the mouse withing assay and for activity in normalizing spontaneous cluster contractions in ferret jejunum as a model of IBS in humans. (5*R*,6*R*)-*exo*-6-[4-[(4,4,4-Trifluorobutyl)thio]-1,2,5-thiadiazol-3-yl]-1-azabicyclo[3.2.1]octane (**35**, LY316108/NNC11-2192) was found to offer an exceptional profile combining analgesic potency in mouse writhing (ED_{50} = 0.1 mg/kg) along with potency for normalization of GI motility (ED_{50} = 0.17 mg/kg). This combination of GI and analgesic potency suggests **35** as an excellent candidate for evaluation as a potential treatment of IBS.

Introduction

Irritable bowel syndrome (IBS) is a pathophysiological condition of the gastrointestinal tract, defined by a characteristic symptomatology of altered bowel habits, such as diarrhea, constipation, or alternating episodes of both, frequently accompanied by abdominal pain.[1] Approximately 3% of the United States population has been diagnosed with symptoms of IBS, comprising almost 3.5 million physician visits yearly.[2] In addition, it is estimated that as much as one-third of the United States population experiences IBS-like symptoms without consulting a physcian.[3] This syndrome clearly has a major impact on health care resources and the economy due to lost productivity.

Recent evidence suggests that the major symptoms of IBS are due, at least in part, to disturbed gastrointestinal motility, characterized by hypercontractility.[4−6] In patients with IBS the relationship between abnormal gastrointestinal motility and the cause of abdominal pain is unresolved. Although the etiology and pathophysiology of IBS are unknown, it is well established that neurons within the enteric nervous system of the gut wall contain acetylcholine and that muscarinic receptors are located on these enteric neurons as well as smooth muscle cells and mucosal enterocytes.[7−10] The relative importance of cholinergic neurotransmission in the regulation of gastrointestinal function under physiological and pathophysiological conditions is unknown; however, muscarinic agonists and antagonists profoundly influence gastrointestinal tract function.[11,12] In humans, muscarinic agonists such as bethanechol are used to prevent gastroesophageal reflux because they increase pressure within the lower esophageal sphincter.[13] Furthermore, atropine-like muscarinic antagonists act as antispasmodics by reducing gastrointestinal hypermotility in patients with IBS; however, the efficacy of currently available muscarinic compounds is limited because of typical anticholinergic side effects which result from a lack of muscarinic receptor selectivity.[14−16]

IBS patients have been observed to have discrete clusters of jejunal contractility concurrent with the sensation of abdominal pain.[5] A remarkably similiar pattern of spontaneous jejunal motility has been found in the anesthetized ferret.[17] On the basis of this similarity, the ferret has been used here as a preclinical model of IBS for the testing of new compounds based on their ability to inhibit the pattern of spontaneous cluster contractions.

Abdominal pain is perhaps the most troublesome symptom of IBS. However, currently available analgesic therapy does not provide relief because of the undesirable side effect profiles of opioid or nonsteroidal antiinflammatory drugs.[18,19] Increasing evidence is accumulating to suggest that muscarinic agents can possess analgesic activity.[20] We have previously found muscarinic ligands based on the azacycle 1,2,5-thiadiazole moiety to possess potent antinociceptive activity with a wide safety margin relative to typical muscarinic side effects.[21,22] These muscarinic analgesics would appear to provide a good starting point for the search for compounds that could treat both pain and gastrointestinal hypermotility. In the present study our goal was to discover a potent nonopioid analgesic that would inhibit gastrointestinal hypercontractility without major side effects.

Chemistry

We have previously disclosed the usefulness of 1,2,5-thiadiazoles and pyrazines in the design of new mus-

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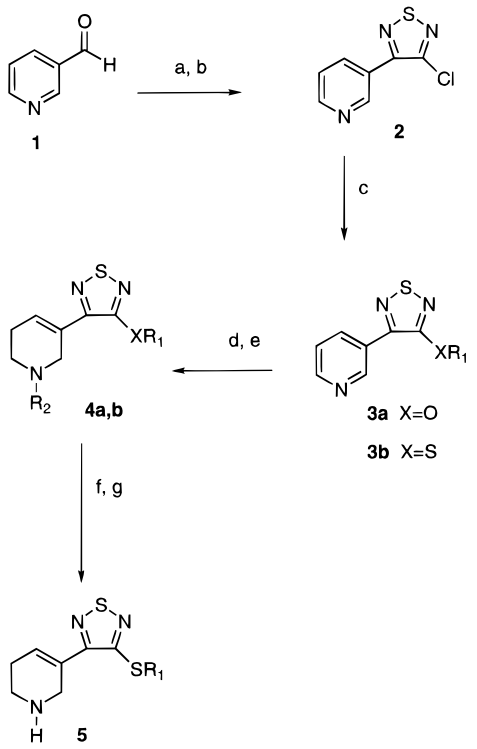
Scheme 1[a]



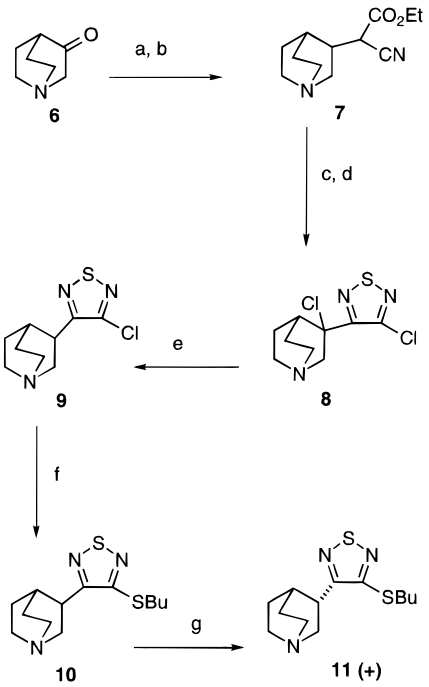
[a] (a) KCN, NH_4Cl; (b) S_2Cl_2; (c) NaOR; or Na_2S, RBr; (d) MeI or EtI; (e) $NaBH_4$; (f) vinyl chloroformate; (g) HCl.

carinic agonists.[22−24] The SAR disclosed herein is based on varying the azacycle attached to these useful heterocycles. Tetrahydropyridyl, quinuclidyl, and 1-azabicyclo[3.2.1]octyl groups have been found to be particularly effective in our labs and by others.[25−27] The alternative use of the 1-azabicyclo[2.2.1]heptyl group has been previously reported to result in extremely potent but nonselective muscarinic drugs.[21]

Tetrahydropyridines were prepared by the method outlined in Scheme 1.[22] Strecker reaction with pyridinecarboxaldehyde (**1**) afforded an intermediate aminonitrile which was cyclized to the 1,2,5-thiadiazole **2** by treatment with S_2Cl_2. Alkoxy side chains were substituted onto the 1,2,5-thiadiazole by displacement with the corresponding sodium or potassium alkoxide. Alternatively, alkylthio side chains were incorporated by a two-step, one-pot process involving initial displacement with Na_2S followed by alkylation with the corresponding alkyl bromide. Pyridine **3a** or **3b** was then alkylated with MeI or EtI and reduced with $NaBH_4$ to give the desired tetrahydropyridines **4a** or **4b**. In some cases the *N*-methyltetrahydropyridines were further transformed by N-demethylation with vinyl chloroformate followed by acid hydrolysis to give *N*-hydridotetrahydropyridine **5**.

Preparation of thiadiazole-substitued quinuclidine **11** is shown in Scheme 2.[22] Knoevenagel condensation of quinuclidinone (**6**) and ethyl cyanoacetate afforded an unsaturated cyanoester intermediate which was hydrogenated to **7**. Heterocyclization of **7** to the α-chlorochlorothiadiazole **8** was achieved through reaction with isoamyl nitrite to give an intermediate cyano oxime followed by treatment with S_2Cl_2. Atmospheric hydrogenation with Pd/C resulted in selective hydrogenolysis of the quinuclidinyl chloride to give **9**. Displacement of the thiadiazole chloride with Na_2S followed by alkylation with bromobutane gave **10**, which was resolved crystallographically with D-tartaric acid to give (+)-**11**.

Scheme 2[a]



[a] (a) Ethyl cyanoacetate, $AcONH_4$, AcOH; (b) H_2, Pd/C; (c) isoamyl nitrite, Na; (d) S_2Cl_2; (e) H_2, Pd/C; (f) Na_2S, BrBu; (g) D-tartaric acid.

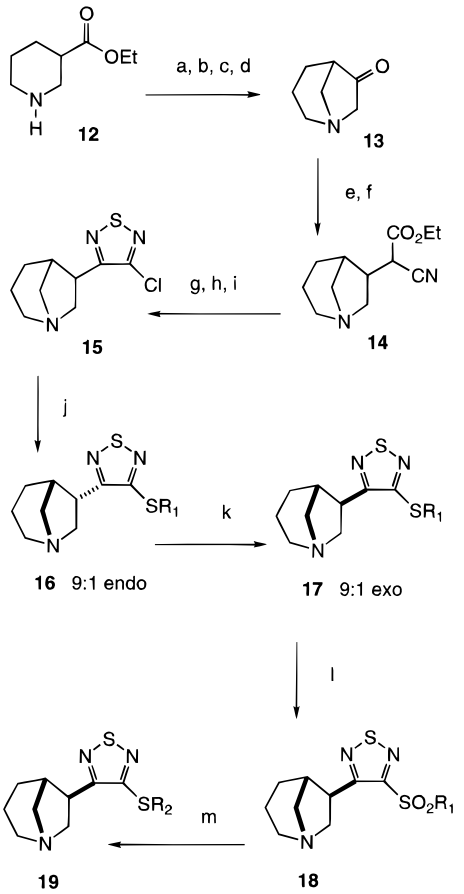
Preparation of the azabicyclo[3.2.1]octanes commenced with ethyl nipecotate (**12**)[29] (Scheme 3). Alkylation with ethyl bromoacetate followed by Claisen condensation and decarboxylation afforded racemic **13**. Resolved material was prepared at this stage by crystallization with D- or L-tartaric acid. Knoevenagel condensation with ethyl cyanoacetate and hydrogenation of the unsaturated intermediate gave cyano ester **14**. Conversion to an intermediate oxime with isoamyl nitrite, heterocyclization with S_2Cl_2, and hydrogenolysis of the resulting α-chloro group on the azabicyclo[3.2.1]-octane ring afforded **15**. Thioetherification with Na_2S and alkyl bromide gave **16**, typically as a 9:1 mixture favoring the endo diastereomer. Equilibration of **16** with KO-*t*-Bu resulted in conversion to a 9:1 mixture now favoring the exo isomer **17**.

The preparation of trifluoro-substituted side chains necessitated a modification of the above route due to instability to the basic conditions employed for diastereomer equilibration. As an alternative, **17** (R_1 = butyl), was oxidized with oxone to the sulfone **18**. The sulfone was then displaced with Na_2S and followed by alkylation with the desired trifluoroalkyl bromide to afford **19**.

Pharmacology

Discrete clusters of jejunal contractility separated by periods of quiescence have been observed in IBS patients concurrently with the sensation of abdominal pain.[5] The anesthetized ferret displays a similar pattern of spontaneous jejunal motility.[17] Given the close resemblence in jejunal activity to the clinical disease state, the anesthetized ferret has been used in the present work as an *in vivo* model for IBS. Test compounds were evaluated for their ability to inhibit this characteristic pattern of spontaneous jejunal motil-

Scheme 3[a]



[a] (a) $BrCH_2CO_2Et$; (b) KOtBu; (c) HCl; (d) resolution with D- or L-tartaric acid; (e) ethyl cyanoacetate; (f) H_2, Pd/C; (g) isoamyl nitrite, NaOEt; (h) S_2Cl_2; (i) H_2, Pd/C; (j) Na_2S, RBr; (k) NaOEt; (1) $KHSO_5$; (m) Na_2S, RBr.

ity. Transmural potential difference (PD) between lumen and serosa in the jejunum was measured as an on-line marker of intestinal secretory activity.[10,30,31] While the exact relationship between PD and diarrhea or constipation remains speculative, minimizing the effect on PD was considered desirable to reduce the liklihood of unwanted gastrointestinal side effects.[32] In order to maximize the therapeutic−safety ratio, compounds were sought with the widest possible separation between the dose required for inhibition of spontaneous jejunal motility and the dose producing an increase in PD.

Full details of the anesthetized ferret assay have been published elsewhere.[9,10] Briefly, intestinal motility was measured manometrically using catheters inserted into the jejunum. Potential difference was measured using a pair of agar-KCl electrodes, with one placed in the lumen, the other in contact with serosal fluid, and both connected via calomel half cells to an electrometer.

Analgesic activity was assessed *in vivo* using the abdominal constriction response in mice to the administration of 0.5% acetic acid, ip, the so-called mouse writhing assay.[33] Compounds with a potency equal to or better than morphine were desired. Under the test conditions used here, morphine had an ED_{50} of 0.3 mg/kg, sc.

The *in vivo* SAR in the ferret model was developed based on the threshold dose required to inhibit spontanous jejunal motility. A select number of compounds were evaluated to determine a dose of a compound that inhibited spontaneous motility by 50% (ED_{50}). Throughout the SAR, transmural potential difference was expressed as the dose of compound that stimulated an increase in transmural potential difference of greater than 1 mV (ED_{1mV}).

Standard compounds were evaluated for their effect in the above animal models. Atropine is a muscarinic antagonist with well-characterized antispasmodic activity, including inhibition of spontaneous clusters of gastrointestinal motility in the ferret. In the ferret model above, atropine had no effect on transmural potential difference. Atropine was devoid of antinociceptive activity in the mouse writhing assay. In the ferret, muscarinic agonists such as acetylcholine increased intestinal motility and increased transmural potential difference in the direction of lumen negativity.

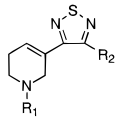
Affinity for muscarinic receptors was determined using [^{3}H] oxotremorine-M in radioligand displacement experiments in rat cortex. The use of oxotremorine-M for receptor binding studies is considered representative of binding to the agonist conformational state of muscarinic receptors without regard to selectivity for subtypes of muscarinic receptors.[34]

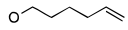
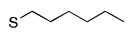
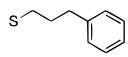
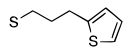
Results and Discussion

On the basis of the similarity of the pattern of discrete clusters of jejunal motility between IBS patients and the anesthetized ferret, we sought to find compounds that would potently inhibit this response. We also wanted the compounds to show a wide separation of dose for these desired effects relative to doses causing an increase in transmural potential difference across the lumen of the intestinal wall, as a marker of undesired intestinal secretory activity. We also desired the compounds to be potent analgesics in the mouse writhing assay in order to treat the abdominal pain associated with abnormal contractility in IBS patients Compounds acting at muscarinic receptors would appear to offer a novel method for simultaneously treating both the intestinal hypercontractility and pain associated with this disorder with a single pharmacologic agent. Compounds were selected for evaluation in the ferret model primarily based on affinity for muscarinity receptor binding and activity in the mouse writhing assay. However, the pharmacological diversity of compounds tested was broadened to include some with good affinity for muscarinic receptors, as measured by displacement of [^{3}H] oxotremorine-M, even though lacking analgesic activity. Results are shown in Table 1.

The structure activity profile of these compounds clearly varies between the different assays. We have previously reported on the muscarinic analgesic activity of a series of azacycle-substitued 1,2,5-thiadiazoles acting at muscarinic receptors.[21,22] A notable structure−activity feature of that series is the optimal analgesic activity that was observed for alkoxy and thioether side chains of three and four carbons in chain length. This optimum analgesic activity residing at chain lenghts of three and four carbons was also found for the present series of compounds reported in Table 1. Among the tetrahydropyridines, **20** and **21** are reasonably potent analgesics, but each of these compounds lacked separation between effects on motility and transmural potential difference. Also, **20** was 10-fold more potent than

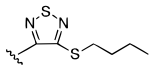
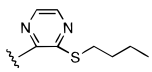
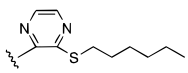
Table 1. Tetrahydropyridine Analogs



Compound	R_1	R_2	Receptor Binding [^{3}H]-Oxo-M Ki (±SEM) nM	Analgesia Mouse Writhing ED_{50} (±SEM) mg/kg, sc	GI Activity Inhibition Motility Threshold Dose[a] μg/kg, iv (n)[b]	GI Side-Effects Potential Difference $ED_{(1\ mV)}$[c] μg/kg, iv (n)[b]
20[d]	CH_3		1.4±0.12	0.41±0.05	3 (2)	7±4 (2)
21	H		3.5±0.5	0.14±0.01	30 (2)	30 (2)
22	CH_3		3±0.5	0.9±0.1	30 (1)	3 (1)
23	CH_2CH_3		18±2.9	>10	>30 (2)	>30 (2)
24	CH_3		1.0±0.15	>10	10 (2)	30 (2)
25	CH_3		10±1.7	>10	30 (3)	30 (3)

[a] Minimum effective dose to inhibit jejunal motility. [b] Number of determinations given in parentheses; SEM reported when replicate values differed from each other. [c] Dose required to produce a 1 mV increase in transmural potential difference. [d] See ref 23 for synthesis.

Table 2. Quinuclidine Analogs

Compound	R	Receptor Binding [^{3}H]-Oxo-M Ki (±SEM) nM	Analgesia Mouse Writhing ED_{50} (±SEM) mg/kg, sc	GI Activity Inhibition Motility Threshold Dose[a] μg/kg, iv (n)[b]	GI Side-Effects Potential Difference $ED_{(1\ mV)}$[c] μg/kg, iv (n)[b]
26		0.40±0.05	0.19±0.02	3 (2)	10 (2)
27[d]		0.5±0.11	3.2±0.01	3 (2)	30 (2)
28[d]		4±0.4	2.3±0.2	>30 (3)	>30 (3)

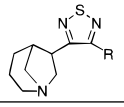
[a] Minimum effective dose to inhibit jejunal motility. [b] Number of determinations given in parentheses; SEM reported when replicate values differed from each other. [c] Dose required to produce a 1 mV increase in transmural potential difference. [d] See ref 26 for synthesis.

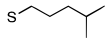
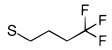
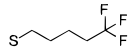
21 at inhibiting motility while being 3-fold less potent in the mouse writhing assay. Compound **22** is a very potent and functionally selective m1 agonist; however it was only moderately potent in the mouse writhing assay, consistent with previous resports that analgesia is not mediated by m1 receptors.[35,36] Compound **22** also had very poor activity for inhibiting motility in the ferret jejunum. Compound **23** showed less affinity for muscarinic receptor binding in the oxotremorine-M assay than any other compound reported in this paper and was inactive in mouse writhing and in the ferret jejunum at the highest doses tested (10 mg/kg, sc in the mouse, and 30 μg/kg, iv in the ferret). In line with observations about optimal chain length, compounds **24** and **25** with propylphenyl and propylthiophene side chains, respectively, were of only limited activity in both the mouse writhing and ferret models. No tetrahydropyridyl compounds were found with both good analgesic potency and good separation between effects on motility and potential difference.

The first compound in the series with some separation between motility and potential difference effects, along with potent analgesic activity, was found by combining a quinuclidine with a thiobutylthiadiazole, as in **26** (see Table 2). We, and others, have previously reported the bioisosteric replacement of the pyrazine heterocycle for the 1,2,5-thiadiazole system with favorable results.[24−26] The thiobutylpyrazine substituted with a quinuclidine, **27**, showed 10-fold separation between motility and potential difference effects, but unfortunately the compound was only weakly active as an analgesic. Increasing the length of the pyrazine side chain to thiohexyl, as in compound **28**, resulted in completely abolishing both motility and potential difference effects and also diminished the compound's affinity for binding muscarinic receptors.

Most of the azabicyclo[3.2.1]octane compounds examined were quite potent, both as analgesics and as inhibiters of motility (see Table 3). For example, compound **29** was the most potent analgesic in the series and was also very potent at inhibition of motility. However, **29** was also equipotent at increasing transmural potential difference. The resolved (+)-enantiomer of this compound, **30**, was also tested and found to be

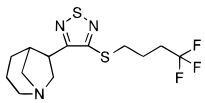
Table 3. 1-Azabicyclo[3.2.1]octane Analogs



Compound		R	Receptor Binding [^{3}H]-Oxo-M Ki (±SEM) nM	Analgesia Mouse Writhing ED_{50} (±SEM) mg/kg, sc	GI Activity Inhibition Motility Threshold Dose[a] µg/kg, iv (n)[b]	GI Side-Effects Potential Difference $ED_{(1\ mV)}$[c] µg/kg, iv (n)[b]
29	(±) exo		0.36±0.06	0.03±0.003	1 (2)	1 (2)
30	(5S, 6S) exo		0.45±0.08	0.19±0.02	1 (2)	1 (2)
31	(±) exo		0.91±0.14	0.95±0.01	30 (3)	3 (3)
32	(5S, 6S) exo		0.44±0.02	0.10±0.001	3 (2)	10 (2)
33	(±) exo		0.40±0.04	0.10±0.03	1 (6)	30 (6)
34	(5S, 6S) exo		0.24±0.01	0.07±0.002	1 (2)	10 (2)

[a] Minimum effective dose to inhibit jejunal motility. [b] Number of determinations given in parentheses; SEM reported when replicate values differed from each other. [c] Dose required to produce a 1 mV increase in transmural potential difference.

Table 4. Resolved Isomers



Compound	config.	Receptor Binding [^{3}H]-Oxo-M Ki (±SEM) nM	Analgesia Mouse Writhing ED_{50} (±SEM) mg/kg, sc	GI Activity Inhibition Motility ED_{50}[a] (±SEM) µg/kg, iv	GI Side-Effects Potential Difference $ED_{(1\ mV)}$[b] (±SEM) µg/kg, iv
35	(5R, 6R)- exo	0.42±0.11	0.1±0.01	0.17±0.04	15±5
36	(5S, 6S) exo	0.33±0.06	0.35±0.008	17±5.6	2±1
37	5R, 6S) endo	25±4	>10	4.7±1.3	>30
38	(5S, 6R) endo	9.6±1.1	1.8±0.3	5.3±0.8	>30
morphine			0.3		
atropine		0.9±0.2	>30	0.73±0.17	>30

[a] Dose to produce a 50% reduction in spontaneous motility. [b] Dose required to produce a 1 mV increase in transmural potential difference.

equivalent in GI activity to its racemate, **29**. The one exception to the analgesic potency of the [3.2.1]azabicyclics was **31**, substituted with a thioisohexyl side chain, resulting in diminished analgesic potency and a reversal of the desired selectivity, with greater potency for increasing transmural potential difference than for inhibition of motility.

Incorporation of a trifluoro functionality at the end of the thiadiazole side chain afforded compounds with the desired profile of analgesic potency combined with selectivity for inhibition of motility versus increasing transmural potential difference. In particular, **33** with a 1-thio-4,4,4-trifluorobutyl side chain showed 30-fold separation between motility and potential difference effects along with very potent activity in the mouse writhing assay. The analogous compounds with trifluoropropyl and trifluoropentyl side chains, **32** and **34**, respectively, were similarly potent as analgesics but were somewhat less selective between motility inhibition and potential difference effects.

On the basis of the superior attributes of **33** in meeting the desired pharmacological profile, the compound was resolved into its constituent enantiomers (see Table 4). For comparison purposes, the corresponding isomers with endo configuration were also evaluated, **37** and **38**. Each of the endo isomers is much less active than the associated exo enantiomers in both mouse writhing and ferret GI assays. Between the enantiomers with exo stereochemistry, the (5*R*,6*R*) isomer, **35** (LY316108/NNC 11-2192), was clearly superior. Compound **35** was 3-fold more potent as an analgesic than its enantiomer, **36**. Also, **35** was 88-fold more selective for inhibition of motility in comparison to its effect on transmural potential difference. In contrast, **36** was actually more selective for increasing the potential difference than for inhibition of motility.

The trifluoro functionality also imparted enhanced bioavailability characteristics. In rats, plasma concentrations of **35** were nearly 40-fold higher than those seen with a comparable dose of **30**, the congener to **35**

differing only in the absence of the terminal trifluoro substituents on the side chain. Mean plasma concentrations of 805 and 21 ng/mL were found for **35** and **30**, respectively, measured 1 h following an oral dose of 30 mg/kg of each. Similiarly, for oral administration in mice, area under the curve (AUC) of parent plasma concentration plotted against time was over 40-fold higher for **35** than for **30**, after normalizing for dose (AUC/dose: 193 for **35** vs 4.5 for **30**).

Hepatic metabolism studies in isolated perfused rat liver suggest that the increased plasma levels of **35** may be due to enhanced metabolic stability for **35** relative to **30**. Analysis of rat liver perfusate for parent and putative metabolites was carried out using HPLC/mass spectrometry. It was found that nonfluorinated aliphatic side chains, as in **30**, underwent extensive oxidation to hydroxy- and keto-substituted side chains. In contrast, no side chain oxidation was found for **35**, demonstrating that the terminal trifluoro group effectively blocked side chain oxidation by the liver in this model. The substantially higher plasma concentrations of parent **35** observed in vivo may be a result of reduced first pass hepatic metabolism imparted by the trifluoro substituents.

Compound **35** showed an outstanding pharmacological profile with very high affinity for muscarinic receptors as measured by displacement of [^{3}H]oxotremorine-M and is a highly potent analgesic in the mouse writhing assay. Compound **35** also potently inhibited the characteristic discrete clusters of jejunal contractions in the ferret. Much higher doses of compound were required before an increase in transmural potential difference was observed, indicating minimal effect on fluid and electrolyte secretion. It is hoped that this high potency for analgesia and motility inhibition will result in a compound with unique therapeutic efficacy for the treatment of IBS. Similarly, it is hoped that the low potency for increasing transmural potential difference will be predictive of a lack of GI side effects. On oral administration, compound **35** also showed enhanced plasma levels of parent relative to those found for the nonfluorinated analog **30**. This suggests a highly desirable profile of biological activity for **35**, suggesting its potential for clinical evaluation in the treatment of IBS.

Experimental Section

All compounds were prepared as racemates except were specifically indicated otherwise.

Melting points were determined with a Mel-Temp apparatus and are uncorrected. Proton and carbon magnetic resonance spectra were recorded on a GE QE-300 spectrometer at 300 and 75 MHz, respectively, and are reported in ppm on a δ scale from internal tetramethylsilane. Microanalyses, mass spectral measurements, and X-ray crystal structures were determined by the Structural and Organic Chemistry Research Department of the Lilly Research Laboratories. Optical rotations were obtained on a Perkin-Elmer Model 248 automatic polarimeter. Gas chromotagraphy was performed on a Hewlett-Packard 5890 instrument with an HP-1 megabore capillary GC column and flame-ionization detection. Preparative chromatography was performed on a Waters Prep 2000 system.

When necessary, solvents and reagents were dried prior to use. Diethyl ether and tetrahydrofuran were distilled from sodium metal/benzophenone ketyl. All other reagents were used as received from Aldrich Chemical Co., Milwaukee, WI.

3-[4-(Butylthio)-1,2,5-thiadiazol-3-yl]-1,2,5,6-tetrahydropyridine Oxalate Salt (21). To a solution of 3-[4-(butylthio)-1,2,5-thiadiazol-3-yl]-1,5,6-tetrahydro-1-methylpyridine[22] (0.86 g, 2.4 mmol) in 1,2-dichloroethane (20 mL) was added a solution of 1-chloroethyl chloroformate (0.35 g, 2.4 mmol) in 1,2-dichloroethane at 0 °C. The reaction mixture was heated to 40 °C for 2 h and evaporated. The residue was dissolved in methanol, heated to reflux for 1 h, and evaporated. The residue was dissolved in diluted sodium hydroxide and extracted with ether. The combined ether phases were dried and evaporated. Crystallization as the oxalate salt from acetone gave **21**: mp 157−159 °C. Anal. ($C_{13}H_{19}N_3O_4S_2$) C, H, N.

3-[4-(5-Hexenyloxy)-1,2,5-thiadiazol-3-yl]-1,2,5,6-tetrahydro-1-methylpyridine Maleate Salt (22). To a solution of 5-hexen-1-ol (900 mg, 9 mmol) and sodium hydride (310 mg, 13 mmol) in dry tetrahydrofuran was added a solution of 3-(4-chloro-1,2,5-thiadiazol-3-yl)pyridine[23] (590 mg, 3 mmol) in dry tetrahydrofuran. The reaction mixture was stirred at room temperature for 1 h. Water was added, and the mixture was extracted with ether. The ether phase was dried and evaporated. The residue was taken up in 5 mL of acetone, methyl iodide (0.5 mL, 7.5 mmol) was added, and the mixture was stirred at room temperature for 18 h. The resulting precipitate was collected by filtration and then suspended in ethanol (20 mL). Sodium borohydride (150 mg, 4 mmol) was added to this solution and the reaction mixture was stirred at −10 °C for 0.5 h. After evaporation, the residue was dissolved in water and extracted with ethyl acetate. The dried organic phases were evaporated, and the residue was purified by column chromatography (SiO_2, eluent: ethyl acetate/methanol (4:1)). Compound **22** was crystallized as the maleate salt from acetone to yield 250 mg. Anal. ($C_{18}H_{25}N_3O_5S$) C, H, N.

3-[4-(Hexylthio)-1,2,5-thiadiazol-3-yl]-1,2,5,6-tetrahydro-1-ethylpyridine Oxalate (23). A solution of 3-[4-(hexylthio)-1,2,5-thiadiazol-3-yl]pyridine [23] (1.2 g, 4.3 mmol) and ethyl iodide (2.0g, 13 mmol) in acetone (4 mL) was stirred at 40 °C for 16 h. The precipitate was collected by filtration and suspended in ethanol (10 mL). Sodium borohydride (410 mg, 10.8 mmol) was added to the solution, and the mixture was stirred for 1 h at 0 °C. Water was added, and the mixture was extracted with ethyl acetate. After drying, the ethyl acetate phase was evaporated and the residue purified by column chromatography (eluent: ethyl acetate/methanol (4:1)). Crystallization with oxalic acid from acetone gave **23**; mp 134−135 °C. Anal. ($C_{17}H_{27}N_3O_4S_2$) C, H, N.

3-[4-[(3-Phenylpropyl)thio]-1,2,5-thiadiazol-3-yl]-1,2,5,6-tetrahydro-1-methylpyridine Oxalate (24). Sodium hydrogen sulfide monohydrate (0.25 g, 3.3 mmol) was added to a solution of 3-(3-chloro-1,2,5-thiadiazol-4-yl)pyridine[23] (0.59 g, 3.0 mmol) in DMF (20 mL) at room temperature, and the reaction mixture was stirred for 1 h. Potassium carbonate (1.24 g, 9 mmol) and 1-bromo-3-phenylpropane (0.90 g, 4.5 mmol) were added, and the reaction mixture was stirred for an additional 24 h. Water (50 mL) was added and the mixture extracted with ether. The combined ether phases were dried and evaporated. The residue was taken up in acetone (5 mL), and methyl iodide was added. The reaction was stirred at room temperature for 20 h, and then evaporated. The residue was taken up in ethanol (20 mL), sodium borohydride (290 mg, 7.5 mmol) was added, and the reaction mixture was stirred at −10 °C for 1 h. After evaporation the residue was dissolved in water and extracted with ethyl acetate. The dried organic phases were evaporated, and the residue was purified by column chromatography (SiO_2, eluent: ethyl acetate/methanol (4:1)). Compound **24** was crystallized as the oxalate salt from acetone: mp: 110−110.5 °C. Anal. ($C_{19}H_{23}N_3O_4S_2$) C, H, N.

3-[4-[(3-(2-Thienyl)-1-propyl)thio]-1,2,5-thiadiazol-3-yl]-1,2,5,6-tetrahydro-1-methylpyridine Oxalate (25). A solution of 3-(4-chloro-1,2,5-thiadiazol-3-yl)pyridine[23] (2.0 g, 10.1 mmol) in DMF (10 mL) was cooled to 5 °C, and potassium carbonate (2.8 g, 20.2 mmol) and sodium hydrosulfide monohydrate (1.5 g, 20.2 mmol) were added to the reaction. After the mixture was stirred for 1 h, additional potassium carbonate (1.4 g, 10.1 mmol) and a solution of 3-(2-thienyl)-1-chloropropane (1.8 g, 11.2 mmol) in DMF (5 mL) were added, and the solution was stirred for 1 h at room temperature. The reaction was quenched with water, and then the mixture was extracted with CH_2Cl_2 (3 × 75 mL). The organic phase was dried over

NaCl/Na_2SO_4 and then evaporated under vacuum. The residue was purified by flash chromatography, eluting with 1:1 ethyl acetate/hexane. The resulting oil was taken up in acetone (30 mL) and methyl iodide (2.0 g, 14 mmol) added. The reaction mixture was stirred for 16 h at room temperature and evaporated under vacuum. The residue was suspended in ethanol (15 mL) and $NaBH_4$ (0.53 g, 14 mmol) added. After being stirred for 0.5 h, the solution was evaporated under vaccum and the residue was dissolved in water and extracted with ethyl acetate. The organic phases were dried over NaCl/Na_2SO_4 and evaporated under vacuum. The residue was purified by flash chromotagraphy, eluting with 5% EtOH, 0.5% NH_4OH, and $CHCl_3$. Crystallization as the oxalate salt gave 350 mg of **25**: mp 98−100 °C. Anal. ($C_{17}H_{21}N_3O_4S_3$) C, H, N.

(*S*)-3-[4-(Butylthio)-1,2,5-thiadiazol-3-yl]-1-azabicyclo-[2.2.2]octane Tartrate (26). A mixture of 3-quinuclidinone hydrochloride, **7** (100 g, 620 mmol), ethyl cyanoacetate (140 g, 1240 mmol) and triethylamine (93.5 g, 930 mmol) was heated at 80 °C for 2 h. After the mixture was cooled to room temperature, toluene (1.5 L) was added. The organic phase was washed with H_2O (2 × 100 mL), and concentrated HCl was added to pH = 4.0. After azeotropic removal of H_2O, the precipitated crystals were filtered and washed with cold acetone. The crystals were suspended in absolute EtOH (1000 mL) and hydrogentated over Pd/C (3 g, 10%) at room temperature and atmospheric pressure. The catalyst was filtered and the filtrate evaporated to half volume. Upon addition of Et_2O and cooling, a solid precipitate separated. The precipitate was added to a solution of Na metal (11.5 g, 500 mmol) dissolved in an EtOH/MeOH mixture (1:1, 500 mL), and the reaction mixture was stirred at room temperature for 30 min. The reaction mixture was cooled to 5 °C, and isoamyl nitrite (64.45 g, 650 mmol) was added at such a rate that the temperature was kept below 10 °C. The reaction mixture was then evaporated at reduced pressure. Traces of alcohols were removed by azeotropic evaporation with toluene. The residue was dissolved in DMF (250 mL) and slowly added to a solution of S_2Cl_2 (203 g, 1500 mmol) in DMF (75 mL). The temperature of the reaction mixture was kept just below 0 °C during the addition. After addition, the reaction mixture was allowed to warm to room temperature and stirred at this temperature for 42 h. Ice water (500 mL) was carefully added, and the suspension was heated to 70 °C to redissolve precipitated compound. Sulfur was filtered off and the filtrate made alkaline with 4 N NaOH. The alkaline suspension was extracted with toluene (2 × 500 mL). The organic extracts were dried over $MgSO_4$ and concentrated under vacuum. The crude compound was redissolved in EtOH (250 mL) and then precipitated with concentrated HCl. This compound was taken up in absolute EtOH (500 mL) and hydrogenated over Pd/C (8 g, 5%) at room temperature and atmospheric pressure. The catalyst was filtered off and the filtrate evaporated at reduced pressure to $^1/_{10}$ volume. The residue was suspended in Et_2O and made alkaline with 4 N NaOH, followed by extraction with Et_2O (2 × 500 mL) and evaporation under vacuum of the combined ethereal extracts. To a solution of this residue in DMF (500 mL) were added K_2CO_3 (38 g, 274 mmol) and $NaHS{\cdot}H_2O$ (22.3 g, 301 mmol). The reaction mixture was stirred at rooom temperature for 2 h and then cooled to 0 °C. 1-Bromobutane (41.3 g, 301mmol) was added. The reaction mixture was stirred overnight, diluted with H_2O (2700 mL), and extracted with toluene (2 × 800 mL). The combined toluene extracts were dried over $MgSO_4$ and evaporated under vacuum (81.9 g). The residue was taken up in absolute EtOH (250 mL), D-tartaric acid (43.4 g, 290 mmol) in THF (280 mL) was added. The mixture was heated at 50 °C until a homogeneous solution was obtained. The mixture was slowly cooled to 5 °C over 2−3 h. The precipitated crystals were filtered, washed with THF (25 mL), and dried. The crude material was recrystallized from a 1:1 mixture of EtOH and THF giving 42.1 g of **26** as the (+)-tartrate salt: mp 106−108 °C; $[\alpha]_D$ free base = +14.94° (*c* = 4.09, MeOH). Anal. for tartrate salt ($C_{17}H_{27}N_3O_6S_2$) C, H, N.

(+)-*exo*-6-[4-(Butylthio)-1,2,5-thiadiazol-3-yl]-1-azabicyclo[3.2.1]octane Oxalate (29). A solution of 1-azabicyclo-[3.2.1]octane-6-one, **13**[29] (41.25 g, 0.33 mol), acetic acid (2 mL), ammonium acetate (1.25 g), and ethyl cyanoacetate (37 g, 0.33 mol) in toluene (500 mL) was refluxed with a Dean−Stark trap for 40 h. The water phase was made basic with 28% ammonium hydroxide solution and extracted with ether (4 × 200 mL). The organic phases were dried over magnesium sulfate and evaporated. The residue was purified by column chromatography (eluent CH_2Cl_2/MeOH (9:1)). The resulting product (41 g, 0.19 mol) in absolute ethanol (500 mL) was treated with 10% Pd/C (5 g) and hydrogen in a Parr shaker at 30 psi for 5 h. Filtration and evaporation yielded **14**, 36 g (0.16 mol). The crude product was taken up in absolute ethanol (100 mL) and added to a solution of sodium (4 g, 0.21 mol) in absolute ethanol (100 mL). Isoamyl nitrite (25 mL, 0.19 mol) was added over 0.5 h, and the mixture was heated at 50 °C for 4 h. Evaporation of the reaction mixture gave a crude sodium salt, which was used without further purification. A solution of the crude salt in DMF (150 mL) was added to a solution of S_2Cl_2 (50 mL, 0.68 mol) in DMF (100 mL) at 0 °C over 1 h. The reaction mixture was stirred overnight, and ice water (500 mL) was added. The mixture was filtered and the filter cake washed with 1 N HCl (3 × 100 mL). The water solution was extracted with ether (2 × 200 mL) and then basified with a 28% NH_4OH solution and extracted with ether (4 × 200 mL). The combined ether extracts from the last extraction were dried and evaporated. The residue was purified by column chromatography (eluent: CH_2Cl_2/MeOH (9:1)) to give 11 g of product. A solution of the product (1.3 g, 5 mmol) in absolute ethanol (100 mL) was treated with 10% Pd/C (300 mg) in a Parr shaker at 20 psi for 4 h. The solution was filtered and evaporated. The residue was purified by column chromatography (CH_2Cl_2/MeOH/TEA (9:1:0.25)). The first fractions contained the exo isomer, and the later fractions contained the endo isomer. To a solution of the exo isomer (229 mg, 1.0 mmol) in DMF (20 mL) was added sodium hydrogen sulfide monohydrate (230 mg, 3.0 mmol). The reaction mixture was stirred at room temperature for 1 h. Potassium carbonate (1.38 g, 10 mmol) and 1-butyl bromide (343 mg, 2.5 mmol) were added, and the mixture was stirred for 1 h. A solution of 1 N HCl was added and the mixture extracted with ether (2 × 50 mL). The aqueous solution was basified with a 28% NH_4OH and extracted with ether (2 × 50 mL). The ether phase was dried and evaporated. The residue was crystallized as the oxalate salt from acetone/ether to give 200 mg of **29**: mp 143−145 °C. Anal. ($C_{15}H_{23}N_3O_4S_2$) C, H, N.

***exo*-(5*S*,6*S*)-6-[4-(Butylthio)-1,2,5-thiadiazol-3-yl]-1-azabicyclo[3.2.1]octane (−)-(D)-Tartrate (30).** To a solution of (+)-**29** free base (5.5 g, 19.43 mmol) in ethanol (50 mL) was added a solution of (−)-D-tartaric acid (2.9 g, 19.4 mmol) in water (10 mL). Ether (200 mL) was added to the solution to give a slightly cloudy solution. Compound **30** was precipitated overnight, and the crystals were collected by filtration (3.05 g). Recrystallizaiton twice from elthanol/ether gave 1.90 g of **30**: mp 106−108 °C; (Free base) [α] = +14.94° (*c* = 4.09/MeOH). Anal. as the tartrate salt ($C_{17}H_{27}N_3O_6S_2$) C, H, N.

(+)-*exo*-6-[4-[4-Methylpentyl)thio]-1,2,5-thiadiazol-3-yl]-1-azabicyclo[3.2.1]octane Oxalate (31). The same procedure as for compound **29** was used, but with 1-bromo-4-methylpentane as the alkyl bromide. Anal. as the oxalate salt ($C_{17}H_{27}N_3O_4S_2$) C, H, N.

(5*S*,6*S*)-*exo*-6-[4-[3,3,3-Trifluoropropyl)thio]-1,2,5-thiadiazol-3-yl]-1-azabicyclo[3.2.1]octane Oxalate (32). To a solution of compound **30** (2.8 g, 10 mmol), 1 N HCl (10 mL), and water (23 mL) at 0 °C was added Oxone (9.2 g, 15 mmol) in water (45 mL). The reaction was stirred overnight at room termperature. The pH of the reaction mixture was adjusted to above 9, and then the reaction mixture was extracted with $CHCl_3$ (3 × 100 mL). The organic extracts were dried over NaCl/Na_2SO_4 and then evaporated. To a solution of the resulting sulfone (0.5 g, 1.6 mmol) in DMF (10 mL) was added $Na_2S(H_2O)_9$ (0.5 g, 2 mmol). The reaction was stirred at 100 °C for 3 h, whereupon 1-iodo-3,3,3-trifluoropropane (448 mg, 2 mmol) in DMF (5 mL) was added to the reaction. After 30 min, heating was discontinued, and the reaction mixture was stirred at room temperature for 16 h. The reaction mixture was quenched with water (20 mL) and then extracted with ethyl acetate (3 × 50 mL). The organic extracts were dried

over $NaCl/Na_2SO_4$ then evaporated. The resulting oil was purified by flash chromatography over silica gel (96.7% $CHCl_3$, 3% EtOH, 0.3% NH_4OH) to yield **32** (210 mg, 0.65 mmol). Crystallization with oxalic acid in acetone gave a salt with mp 93−96 °C. Anal. ($C_{14}H_{18}N_3O_4S_2F_3$) C, H, N.

(+)-*exo*-6-[4-[4,4,4-Trifluorobutyl)thio]-1,2,5-thiadiazol-3-yl]-1-azabicyclo[3.2.1]octane Oxalate (33). The same procedure as for compound **29** was used, but with 1-bromo-4,4,4-trifluorobutane as the alkyl bromide: mp 101−105 °C. Anal. as the oxalate salt ($C_{15}H_{20}N_3O_4S_2F_3$) C, H, N.

(5*S*,6*S*)-*exo*-6-[4-[(5,5,5-Trifluoropentyl)thio]-1,2,5-thiadiazol-3-yl]-1-azabicyclo[3.2.1]octane Oxalate (34). The same procedure as for compound **32** was used, but with 1-bromo-5,5,5-trifluoropentane as the alkyl halide: mp 125−127 °C. Anal. as the oxalate salt ($C_{16}H_{22}N_3O_4S_2F_3$) C, H, N.

(5*R*,6*R*)-*exo*-6-[4-[(4,4,4-Trifluorobutyl)thio]-1,2,5-thiadiazol-3-yl]-1-azabicyclo[3.2.1]octane Oxalate (35) and (5*R*,6*S*)-*endo*-6-[4-[(4,4,4-Trifluorobutyl)thio]-1,2,5-thiadiazol-3-yl]-1-azabicyclo[3.2.1]octane Oxalate (37). To a solution of (+)-1-azabicyclo[3.2.1]octan-6-one[29] (124 g, 1000 mmol) in EtOH (100 mL) was added a solution of (−)-camphorsulfonic acid (232 g, 1000 mmol) in EtOH (200 mL). The mixture was heated to 70 °C and slowly cooled over 2 h to 5 °C. The precipitated crystals were collected by filtration and washed with cold EtOH (3 × 40 mL). The crude compound was crystallized from EtOH (150 mL) (57.3 g). The resolved material was then converted to **35** and **37** by the same procedure as for **29**, using 1-bromo-4,4,4-trifluorobutane as the alkyl halide. Crystallization with oxalic acid in acetone of the exo and endo fractions gave [5*R*-(*exo*)]-6-[4-[(4,4,4-Trifluorobutyl)thio]-1,2,5-thiadiazol-3-yl]-1-azabicyclo[3.2.1]octane oxalate (**35**) (mp 153−154 °C) and [5*R*-(*endo*)]-6-[4-[(4,4,4-trifluorobutyl)thio]-1,2,5-thiadiazol-3-yl]-1-azabicyclo[3.2.1]-octane oxalate (**37**) (mp 70 °C). Anal. for **35** ($C_{15}H_{20}F_3N_3O_4S_2$) C, H, N. Anal. for **37** ($C_{15}H_{20}F_3N_3O_4S_2$) C, H, N.

(5*S*,6*S*)-*exo*-6-[4-[(4,4,4-Trifluorobutyl)thio]-1,2,5-thiadiazol-3-yl]-1-azabicyclo[3.2.1]octane Oxalate (36). The same procedure as for compound **32** was used, but with 1-bromo-4,4,4-trifluorobutane as the alkyl halide: mp 147−151 °C. Anal. ($C_{15}H_{20}F_3N_3O_4S_2$) C, H, N.

(5*S*,6*R*)-*endo*-6-[4-[(4,4,4-Trifluorobutyl)thio]-1,2,5-thiadiazol-3-yl]-1-azabicyclo[3.2.1]octane Oxalate (38). To a solution of (+)-1-azabicyclo[3.2.1]octan-6-one[29] (124 g, 1000 mmol) in EtOH (100 mL) was added a solution of (+)-camphorsulfonic acid (232 g, 1000 mmol) in EtOH (200 mL). The mixture was heated to 70 °C and slowly cooled over 2 h to 5 °C. The precipitated crystals wre collected by filtration and washed with cold EtOH (3 × 40 mL). The crude compound was crystallized from EtOH (150 mL) (57.3 g). The resolved material was then converted to **38** by the same procedure as for **29**, using 1-bromo-4,4,4-trifluorobutane as the alkyl halide. Crystallization with oxalic acid in acetone gave [5*S*-(*endo*)]-6-[4-[(4,4,4-trifluorobutyl)thio]-1,2,5-thiadiazol-3-yl]-1-azabicyclo-[3.2.1]octane ethanedioate **38**: mp 102−105 °C. Anal. ($C_{15}H_{20}F_3N_3O_4S_2$) C, H, N.

Mouse Writhing Antinociceptive Assay. Separate groups of 5−10 mice (male Cr1: CF1, Charles River, Portage, MI) each were administered vehicle or a dose of a test compound subcutaneously (sc), followed 25 min later by an intraperitoneal (ip) injection of 0.5% acetic acid. Each mouse was then placed in an individual clear plastic observation, chamber and the total number of writhes made by each mouse was counted between 5 and 10 min after acetic acid administration (30−35 min after vehicle or test compound). Data are expressed as the mean number of writhes during the 5 min observation period. ED_{50} values were determined by standard linear regression techniques.

Ferret Jejunal Motility and Potential Difference Assay. Full details of this assay have been described elsewhere.[9,10] Briefly, the experiments were performed on male ferrets weighing between 800 and 1200 g that had been fasted for 24 h prior to surgery. Ferrets were anesthetized with a single intraperitoneal injection of 50% urethane and then were surgically prepared for drug testing by cannulating the left jugular vein for drug delivery and the left carotid artery for monitoring blood pressure and heart rate and then inserting intraluminal manometry catheters into the jejunum for motility measurements. Transmural potential difference was determined using calomel half-cells connected by salt bridges; one salt bridge was inserted into the lumenal fluid of the jejunum and the other via a wick electrode into the serosal cavity fluid. Each animal served as its own control for assessment of drug effect.

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