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Analogues of (3*R*)-7-Hydroxy-*N*-[(1*S*)-1-{[(3*R*,4*R*)-4-(3-hydroxyphenyl)-3,4-dimethyl-1-piperidinyl]methyl}-2-methylpropyl)-1,2,3,4-tetrahydro-3-isoquinolinecarboxamide (JDTic). Synthesis and in Vitro and in Vivo Opioid Receptor Antagonist Activity

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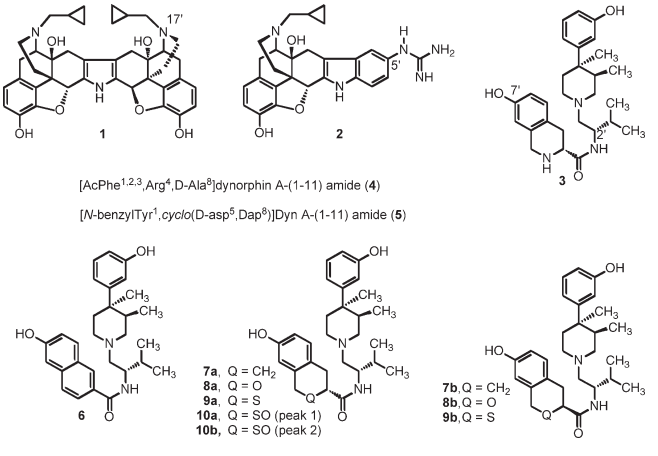
The synthesis of compounds **6**, **7a**,**b**, **8a**,**b**, **9a**,**b**, and **10a**,**b** where the amino −NH− group of JDTic (**3**) was replaced with an aromatic =CH−, CH_2, O, S, or SO group was accomplished and used to further characterize the SAR of the compound **3** class of κ opioid receptor antagonists. All of the compounds showed subnanomolar to low nanomolar K_e values at the κ opioid receptor. The most potent compound was **7a**, where the amino −NH− group of **3** was replaced by a methylene (−CH_2−) group. This compound had a K_e = 0.18 nM and was 37- and 248-fold selective for the κ relative to the μ and δ opioid receptors, respectively. Similar to compound **3**, compound **7a** antagonized selective κ agonist U50,488-induced diuresis after sc administration in rats. In contrast to **3**, where κ antagonist activity lasted for three weeks, compound **7a** did not show any κ antagonist activity after one week.

Introduction

Kappa opioid receptor selective antagonists are of considerable interest as potential pharmacotherapies for addiction (cocaine, opiate, alcohol, nicotine, and possibly others),[1−7] depression,[1,8−10] anxiety disorders,[11] obesity,[12−14] and psychosis disorders.[15] Opioid antagonists with varying degrees of receptor potency and selectivity have been developed for the κ opioid receptor. The first highly selective and potent κ opioid receptor antagonist was norBNI (**1**).[16,17] Continued studies identified 5′-GNTI (**2**) as a more potent and selective κ opioid antagonist.[18−20] In 2001, JDTic (**3**), an *N*-substituted *trans*-(3*R*,4*R*)-dimethyl-4-(3-hydroxyphenyl)piperidine analogue, was developed as the first highly potent and selective κ opioid receptor antagonist from this class of opioid antagonists.[21] More recently, the dynorphin analogues zyklophin and arodyn (**4** and **5**, respectively) were developed as selective κ opioid receptor antagonists.[22,23]

SAR[a] studies previously conducted on **3** identified the 7′-phenolic and (*S*)-2′-isopropyl groups as features important for the κ selectivity and suggested that the nitrogen of the hydroxy-D-Tic group might also be uniquely required for the κ selectivity.[24−26] To gain additional information as to the role that the amine nitrogen of the hydroxy-D-Tic group plays in determining the κ opioid in vitro antagonist efficacy and selectivity, the JDTic analogues **6**, **7a**,**b**, and **8a**,**b**, **9a**,**b**, and **10a**,**b**, where the amino −NH− group was replaced with an aromatic =CH−, CH_2, O, S, or SO group, were synthesized and evaluated for their ability to antagonize μ, δ, and κ opioid agonists in a [^{35}S]GTPγS in vitro antagonist efficacy test. The stereochemical assignments for the position α to the carbonyl group for compounds **7a**,**b**, **8a**,**b**, and **9a**,**b** are arbitrary. The isomer with the lowest K_e value for the κ opioid receptor in each pair was given the **a** designation. Compounds **10a**,**b** are the two possible sulfoxide diastereoisomers derived from **9a**.

Analogue **7a**, with a K_e = 0.18 nM at the κ opioid receptor and 37- and 248-fold selectivity for the κ receptor relative to the μ and δ opioid receptors, was the most potent and selective analogue tested. The potency and duration of action of **7a** to antagonize κ agonist U50,488-induced diuresis in rats was compared to that of **3**.



Chemistry

Analogues **6**, **7a**,**b**, **8a**,**b**, **9a**,**b**, and **10a**,**b** of **3** were synthesized by the methods depicted in Schemes 1−3. Coupling of

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[a] Abbreviations: GPCRs, G-protein-coupled receptors; cDNAs, cDNA; SAR, structure−activity relationship; [^{35}S]GTPγS, sulfur-35 guanosine-5′-*O*-(3-thio)triphosphate; DAMGO, [D-Ala^2,MePhe^4,Gly-ol^5]enkephalin; DPDPE, [D-Pen^2,D-Pen^5]enkephalin; U69,593, (5α,7α,8β)-(−)-*N*-methyl-*N*-[7-(1-pyrrolidinyl)-1-oxaspiro[4,5]dec-8-yl]benzeneacetamide; CHO, Chinese hamster ovary; GDP, guanosine diphosphate; BOP, benzotriazole-1-yloxy-tris(dimethylamino)phosphonium hexafluorophosphate; HBTU, *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate; HMPA, hexamethylphosphoramide; LOA, lithium diisopropylamide; Tic, tetrahydroisoquinolinecarboxylic acid; SAR, structure−activity relationship.

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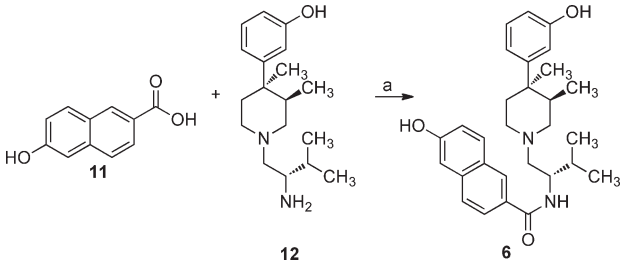
6-hydroxy-2-naphthoic acid (**11**) with 3-{1-[(2*S*)-amino-3-methylbutyl]-(3*R*,4*R*)-dimethyl-4-piperidinyl}phenol (**12**)[27] using benzotriazole-1-yloxy-tris(dimethylamino)phosphonium hexafluorophorphole (BOP) in tetrahydrofuran containing triethylamine at 25 °C gave the desired analogue **6** (Scheme 1). Our initial plan for the synthesis of **7a** and **7b** was to start with (+)- and (−)-**13**. Since all attempts to resolve (±)-**13** directly into its optical isomers were unsuccessful, we used the strategy outlined in Scheme 2 to prepare (+)- and (−)-**13**, which were then converted to **7a** and **7b**. This strategy was based on a report from Fox et al.,[28] who showed that the tetrahydro-2*H*-indeno[1,2*d*]-2-ones serve as a readily cleavable chiral auxiliary suitable for large-scale normal phase separation of isomers. Thus, treatment of (3a*R*-*cis*)-3,3a,8,8a-tetrahydro-2*H*-indeno[1,2*d*]oxazol-2-one (**14**) with ethyl lithium provided the lithium salt, which was acetylated with the acid chloride prepared by treating (±)-**13**[29] with thionyl chloride in toluene to give **15a** and **15b**, which were separated by standard silica chromatography. Hydrolysis of **15b** using lithium hydroxide in tetrahydrofuran containing 30% hydrogen peroxide yielded (+)-**13**.[30] Coupling of (+)-**13** to **12**[27] using BOP in tetrahydrofuran containing triethylamine at 25 °C provided **16a**. Treatment of **16a** with boron tribromide in methylene chloride at −78 °C afforded **7a**. Subjecting **15a** to the same set of reaction conditions provided (−)-**13**, **15b**, and **7b**.

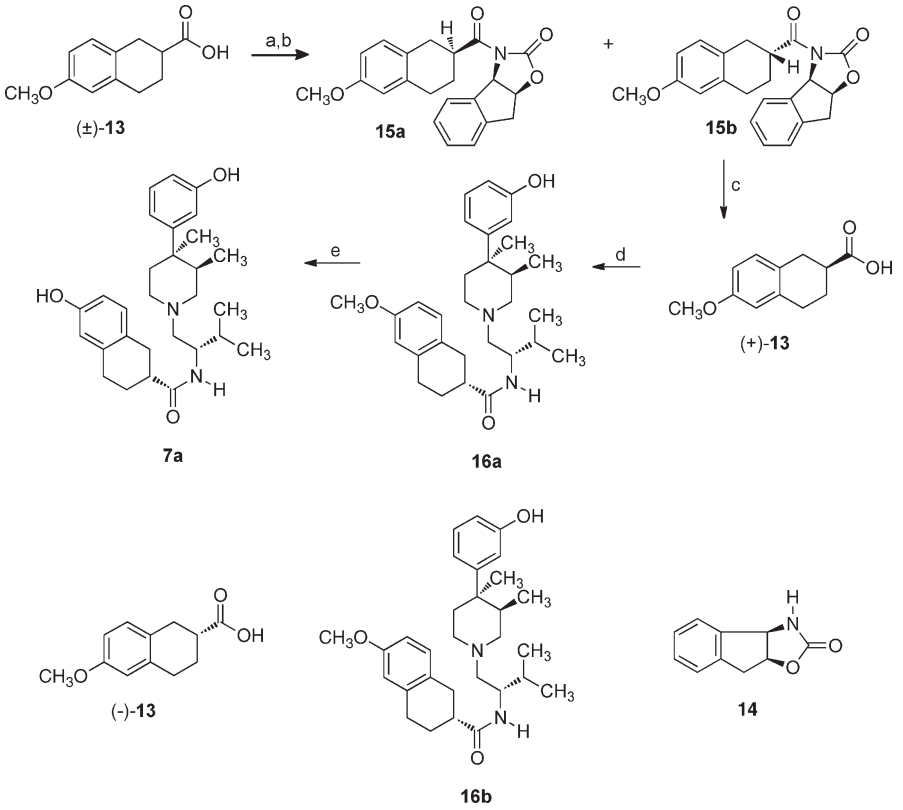
On the basis of the ease of separating **7a**,**b** using chiral auxiliary **14**, a similar synthetic strategy was applied to the synthesis of **9a**,**b** and **10a**,**b**. In this case, the synthesis of the 7-methoxy-isothiochroman-3-carboxylic acid [(±)-**20**] had to be developed. Scheme 3 outlines the synthesis used to prepare (+)- and (−)-**20** and subsequent conversion to **9a** and **9b**. Condensation of 7-methoxy-isothiochroman-4-one (**17**)[31] with methyl cyanoformate using lithium diisopropylamide (LDA) as the base in tetrahydrofuran containing hexamethylphosphoramide (HMPA) gives keto ester (**18**).[32] Reduction of **18** using triethylsilane in trifluoroacetic acid effected removal of the 4-keto group to give **19**.[33] Hydrolysis of **19** using potassium hydroxide in methanol provided (±)-**20**. The lithium salt of **14** was acylated with the acid chloride prepared by treating (±)-**20** with oxalyl chloride in methylene chloride to give **21a** and **21b**, which were separated by normal phase silica gel chromatography. Hydrolysis of **21a** using lithium hydroxide in aqueous tetrahydrofuran afforded (+)-**20**. It should be noted that hydrolysis of the auxiliary was equally effective without the presence of peroxide in the basic media. Coupling of (+)-**20** with **12**[27] using BOP in tetrahydrofuran containing triethylamine at 25 °C provided **22a**. Treatment of **22a** with boron tribromide in methylene chloride at −78 °C afforded **9a**. Subjecting **21b** to a set of reaction conditions

Scheme 1[a]



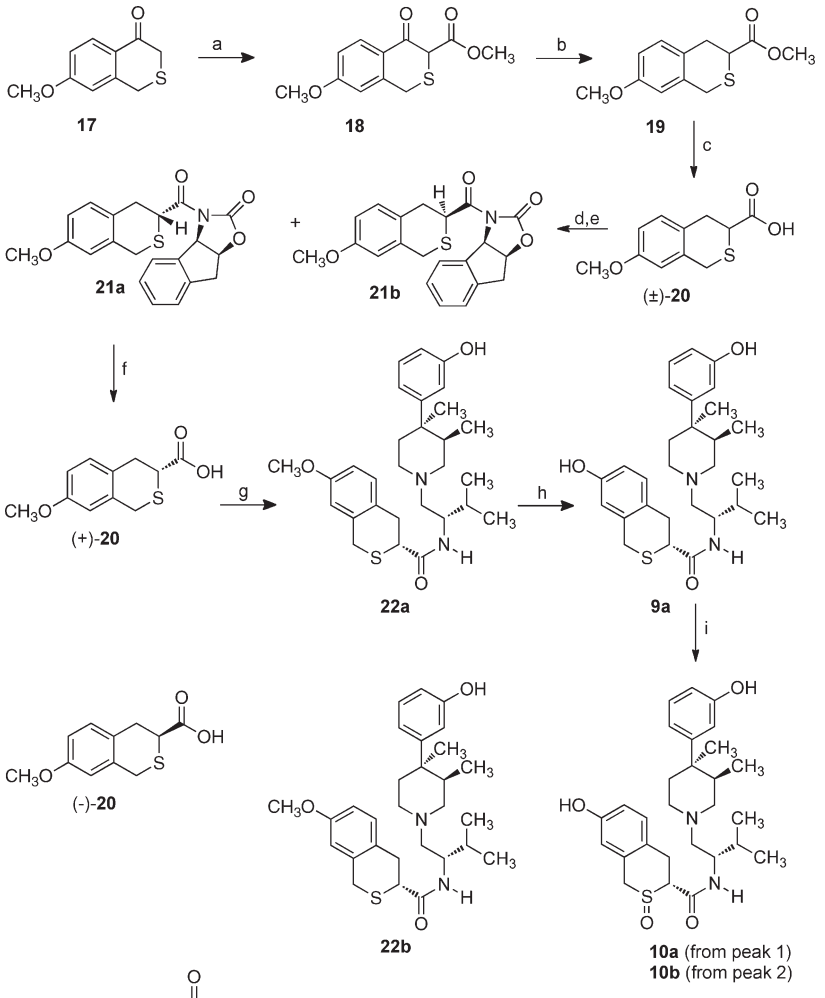
[a] Reagents and conditions: (a) BOP, TEA, THF, 25 °C.

Scheme 2[a]



[a] Reagents and conditions: (a) thionyl chloride, toluene; (b) EtLi, (3a*R*-*cis*)-3,3a,8,8a-tetrahydro-2-hindeno[1,2*d*]oxazol-2-one (**14**); (c) 30% H_2O_2, LiOH, 3:1 THF/H_2O; (d) **12**, BOP, TEA, THF 25 °C; (e) BBr_3, CH_2Cl_2 −78 °C.

Scheme 3[a]



[a]Reagents: (a) CH_3OCCN, LDA, HMPA, THF; (b) Et_3SiH, TFA; (c) KOH, CH_3OH, 0 °C; (d) $(COCl)_2$, CH_2Cl_2; (e) **14**, EtLi, THF; (f) LiOH, THF/H_2O; (g) **12**, BOP, TEA, THF, 25 °C; (h) BBr_3, CH_2Cl_2, -78 °C; (i) MCPBA, CH_2Cl_2, 0 °C.

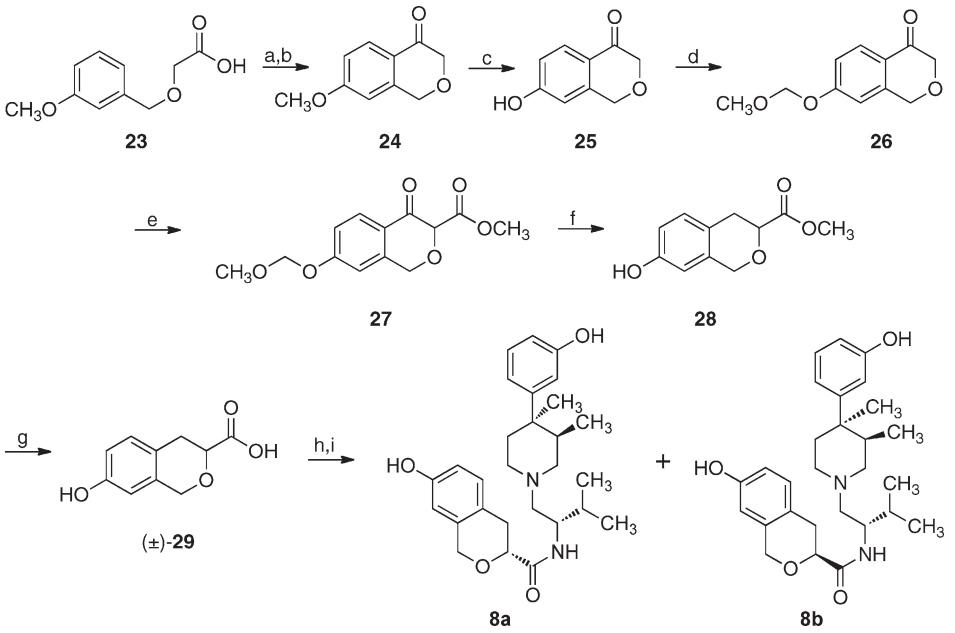
analogous to those used for **21a** provided (−)-**20**, **22b**, and **9b**. Oxidation of **9a** using 3-chloroperoxybenzoic acid in methylene chloride provided sulfoxide diastereomers **10a** and **10b** that were separated using normal phase silica gel chromatography.

The synthesis of compounds **8a** and **8b** was more complex than that of **7a**,**b** and **9a**,**b** due to the requirement of phenolic protecting groups in the presence of a benzylic ether (see Scheme 4). Cyclization of 3-methoxybenzyloxyacetic acid (**23**) using oxalyl chloride followed by tin(IV) chloride in chlorobenzene yielded chromanone **24**.[34] Demethylation of **24** to the phenol **25** was accomplished using sodium ethanethiolate in dimethylformamide.[35] The phenol group in **25** was reprotected as the methoxymethyl ether **26** using chloromethylmethyl ether and diisopropylethylamine in methylene chloride. Condensation of **26** with methyl cyanoformate using lithium diisopropylethylamine as the base in tetrahydrofuran containing hexamethylphosphoramide (HMPA) afforded the keto ester **27**.[32] Treatment of **27** with triethylsilane in trifluoroacetic acid afforded both removal of the 4-keto group and the methoxylmethyl protecting group to give **28**.[33] Hydrolysis of **28** using lithium hydroxide in a water, tetrahydrofuran, and ethanol mixture provided (±)-**29**. Coupling of (±)-**29** to **12** using BOP in tetrahydrofuran containing triethylamine at 25 °C yielded a mixture of (+)-**8** that were separated using reverse phase HPLC to provide **8a**,**b**.

Pharmacology

Compounds **6**, **7a**,**b**, **8a**,**b**, **9a**,**b**, and **10a**,**b** were first evaluated at 10 μM for intrinsic activity in the [^{35}S]GTPγS binding assay at all three opioid receptors. As none of these compounds displayed measurable intrinsic activity at this concentration, they and the reference compounds **1** and **3** were evaluated for antagonist efficacy and selectivity at the opioid receptors. These data were obtained by monitoring the ability of test compounds to inhibit stimulated [^{35}S]GTPγS binding produced by the selective agonists DAMGO (μ), DPDPE (δ), or U69,593 (κ) using cloned human opioid receptors expressed in CHO cells.[36] Agonist dose−response curves were run in the presence or absence of a single concentration of test compound. The K_e values were calculated using the formula: $K_e = [L]/DR - 1$, where [L] is the concentration of test compound and DR is the ratio of agonist EC_{50} value in the presence or absence of test compound, respectively. At least two different concentrations of test compound were used to calculate the K_e, and the concentrations were chosen such that the agonist EC_{50} exhibited at least a 4-fold shift to the right and there was a clear upper asymptote to the agonist + compound concentration−response curve. The K_e values along with those for the reference compound **1** are shown in Table 1.

Scheme 4[a]



[a] Reagents and conditions: (a) oxalyl chloride, CH_2Cl_2; (b) $SnCl_4$, chlorobenzene, 0 °C; (c) NaSEt, DMF, reflux; (d) MOMCl, *i*-Pr_2EtN; (e) methyl cyanoformate, LDA HMPA, THF; (f) TFA, Et_2SiH; (g) LiOH, THF/MeOH/H_2O, (1:1:1) 0 °C; (h) **12**, BOP, TEA, THF, 25 °C; (i) preparative HPLC.

Table 1. Comparison of Inhibition of Agonist-Stimulated [^{35}S]GTPγS Binding in Cloned Human μ, δ, and κ Opioid Receptors for **6**, **7a**,**b**, **8a**,**b**, **9a**,**b**, and **10a**,**b** to **3** and **1**[a,b]

compd	μ, DAMGO K_e (nM)	δ, DPDPE K_e (nM)	κ, U69,593 K_e (nM)	μ/κ	δ/κ
1	26.7 ± 7[c]	29 ± 8[c]	0.05 ± 0.02[c]	520	580
3	25.1 ± 3.5[c]	76.4 ± 2.7[c]	0.02 ± 0.01[c]	1255	3830
6	2.53 ± 0.34	219 ± 41	3.93 ± 1.2	0.7	56
7a	6.67 ± 1.2	44.8 ± 7.7	0.18 ± 0.03	37	248
7b	11.2 ± 2.4	205 ± 59	1.37 ± 0.36	8.2	150
8a	7.4 ± 2.6	197 ± 64	0.71 ± 0.16	10	278
8b	5.2 ± 1.6	134 ± 26	5.7 ± 1.6	0.9	24
9a	5.57 ± 1.27	395 ± 122	0.99 ± 0.25	6	399
9b	13.4 ± 2.9	182 ± 48	1.58 ± 0.07	8.5	115
10a	7.9 ± 2.2	208 ± 55	2.7 ± 2.2	2.9	77
10b	1.8 ± 0.3	61 ± 11	0.55 ± 0.12	3.3	111

[a] Data represent means ± SE from at least three independent experiments. [b] The average percent stimulation and agonist EC_{50} values for the μ, κ, and δ [^{35}S]GTPγS binding assays were 200% and 120 nM, 220% and 380 nM, and 50% and 6 nM, respectively. K_e values were calculated from experiments where the antagonist produced at least a 4-fold shift in the agonist ED_{50}. [c] The K_e values for **3** supplied by the NIDA Opioid Treatment Discovery Program (OTDP) were 3.41, 79.3, and 0.01 nM for the μ, δ, and κ receptors, respectively (ref 24).

Compound **7a** was evaluated for its ability to antagonize U50,488-induced diuresis in male Sprague−Dawley rats (Charles River Laboratories, Raleigh, NC).

Results and Discussion

Compounds **6**, **7a**,**b**, **8a**,**b**, **9a**,**b**, and **10a**,**b** were evaluated for their μ, δ, and κ agonist or antagonist activity in the [^{35}S]GTPγS antagonist efficacy binding assay. The data are shown in Table 1 compared to **1** and **3**. None of the compounds had any μ, δ, or κ intrinsic activity at 10 μM. As expected, all compounds were antagonists of the DAMGO (μ), DPDPE (δ), or U69,593 (κ) stimulated binding. Similar to **1** and **3**, the compounds were more potent at the μ and κ than at the δ opioid receptor. Compounds **1** and **3** with K_e values of 0.05 and 0.02 nM, respectively, are highly potent antagonists for the κ receptor. In addition, both compounds are highly selective for the κ relative to the μ and δ opioid receptors. The compounds **6**, **7a**,**b**, **8a**,**b**, **9a**,**b**, and **10a**,**b**, where the amino −NH− groups have been replaced by an aromatic =CH−, $-CH_2-$, O, S, or SO group, showed K_e values ranging from 0.18 to 5.7 nM for κ, 1.8 to 13.4 nM for μ, and 44.8 to 395 nM for δ and thus are potent κ and μ opioid receptor antagonists. In the cases of **7**, **8**, **9**, and **10**, one isomer was more potent than the other isomer. The differences in potencies were 7.6-, 8-, 1.6-, and 4.9-fold for **7a**,**b**, **8a**,**b**, **9a**,**b**, and **10a**,**b**, respectively. The K_e values at the κ opioid receptor for the more potent **7a**, **8a**, and **9a** are 0.18, 0.77, and 0.99 nM, respectively. The diastereomeric sulfoxides **10a** and **10b** prepared from **9a** had K_e values of 2.7 and 0.55 nM at the κ opioid receptor, respectively. The more potent isomers **7a**, **8a**, **9a**, and **10b** are 9-, 36-, 50-, and 28-times less potent than **3** as a κ opioid antagonist. This data shows that replacing the amino −NH− group of **3** with a $-CH_2-$ was better tolerated at the κ opioid receptor than replacement by an −O− or −S−. Compound **7a** with K_e values of 6.67, 44.8, and 0.18 nM at the μ, δ, and κ opioid receptors was the most potent and selective κ opioid receptor antagonist of the compounds evaluated. The low antagonist efficacy of compounds **6**, **7a**,**b**, **8a**,**b**, **9a**,**b**, and **10a**,**b** relative to **3** strongly suggests that the −NH group of **3** is critical to its high in vitro antagonist efficacy at the κ opioid receptor.

With the exception of **8b**, all of the compounds were less potent at the μ opioid receptor than the κ opioid receptor. However, with the exception of **7a**, which had a 37-fold selectivity for κ over μ, the potencies of the μ and κ opioid receptor were similar. The K_e values at the μ opioid receptor ranged from 1.8 to 11.2 nM. The sulfoxide **10b** with a K_e = 1.8 nM was the most potent μ opioid antagonist. All of the compounds had relatively low antagonist efficacies for the δ opioid receptor. Compound **7a** with a K_e = 44.8 shows the highest potency at the δ opioid receptor.

Structure−activity relationship (SAR) studies of **1** and **2** and analogues led Portoghese to propose that the 17′-amino group of **1** and the 5′-guanidinyl amino moiety of **2** as the primary structural features leading to the κ opioid selectivity of these two compounds (see Metcalf and Coop[37] and Aldrich[38] for reviews of the SAR studies). Portoghese and co-workers explained the κ selectivity of **1** and **2** using the message-address concept developed by Schwyzer[39] for peptide hormones. In this concept, molecular features common to a series of compounds recognized by a family of receptors are defined as the message. The "address" portion of this concept is a specific structural feature of the ligand that affords subtype selectivity through interaction with a site or specific residue(s) unique to a receptor subtype. In the case of **1** and **2**, Portoghese used molecular modeling studies[40] coupled with site-directed mutagenesis to provide additional evidence that the 17′-amino group of **1** and the 5′-guanidinium amino group of **2** serve as the address by forming an ion pair with Glu297 at the top of the TM6 in the κ opioid receptor.[19,41−44]

To determine if **3** could be interacting with the κ opioid receptor in a way similar to that proposed for **2** and **1**, structures of **3** and **2** were constructed and compared. Low-energy conformations of **3** which provide an overlay of the primary pharmacophoric groups of **3** and **2** (as represented by the centroid of the phenolic rings of **3** and **2** and the centroid of the Tic aromatic ring of **3** and the guanidinium group of **2**) were identified via conformational analysis (Figure 1). Although the geometry and dimensions of **3** conformations do not permit an atom-for-atom overlay of the 7-hydroxy-D-Tic-group hydrogen-bonding substituents of **3** with the guanidinium nitrogens of **2**, both the 7-hydroxy-D-Tic secondary amine NH group and phenol oxygen are projected into the same general region and presumably could participate in some type of interactions in the κ-address locus proposed for **1** and **2** of the κ-opioid receptor (Figure 1).

Compounds **6**, **7a**,**b**, **8a**,**b**, **9a**,**b**, and **10a**,**b** all have a phenol group in a location similar to the 7-hydroxy-D-Tic phenol group of **3** but lack the −NH group present in **3**. Therefore, the lower κ opioid antagonist efficacy of each to these compounds seems due largely to the absence of the −NH group present in **3**. Previous studies have shown that the 7-hydroxy-D-Tic phenol oxygen is essential for the high κ opioid receptor efficacy of **3**.[25,26] Thus, these results suggest that the highly potent κ opioid receptor efficacy of **3** is due to strong interactions at the κ opioid receptor by the 7-hydroxy-D-Tic secondary amine −NH group and the phenol oxygen as suggested by the molecular modeling studies (Figure 1). However, these results do not rule out the possibility that **3** is interacting with the κ opioid receptor in a way different from that of **1** and **2**.

To gain information concerning the in vivo activity, the effect of **7a** to antagonize κ agonist U50,488-induced diuresis was compared to that of **3**. The results from the study are summarized in Figure 2. At week 0 (first 5 h after dosing sc),

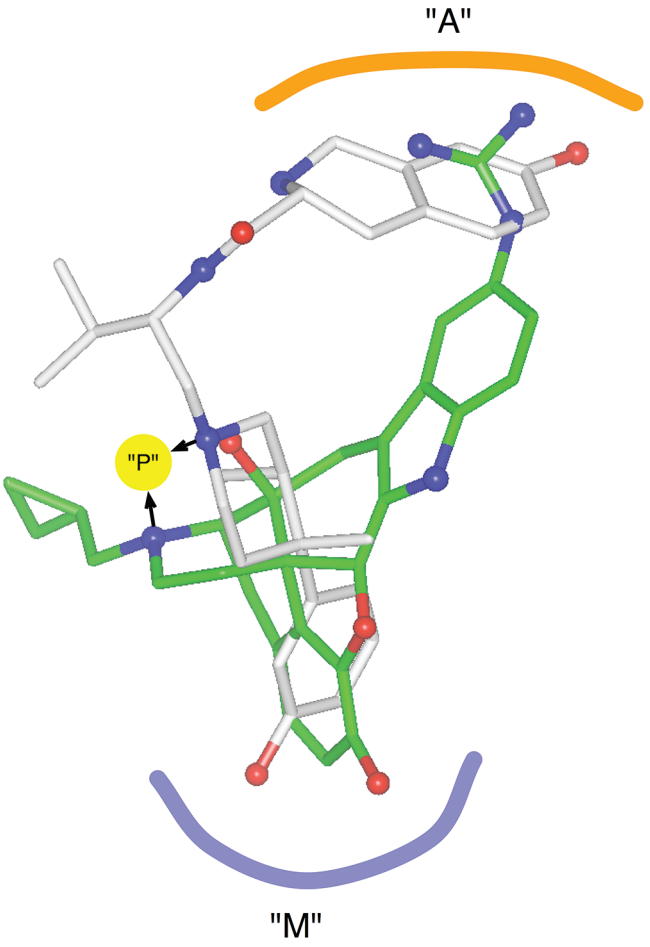


Figure 1. Alignment of a local-energy minimum conformation of **3** (gray) and **2** (green) with heteroatoms indicated by CPK-colored spheres (blue for nitrogen and red for oxygen). Possible shared-pharmacophore regions are labeled "A" for the opioid address region, "M" for the opioid message region, and "P" for a polar interaction site adjacent to the protonated nitrogens.

compound **3** showed dose-related antagonism of urine output relative to vehicle (Figure 2A). At week 1 (no further dosing with **3**), **3** continued to show dose-related antagonism of U50,488-induced diuresis. In keeping with our previous findings with **3**,[45] the level of antagonism at week 1 was the same as or greater than at week 0.

At week 0 (first 5 h after dosing sc), **7a** showed dose-related antagonism of U50,488-induced diuresis but was less potent than **3**, which is consistent with its 9-fold lower in vitro antagonist potency at the κ opioid receptor compared to **3** in the [^{35}S]GTPγS in vitro antagonist efficacy test (Figure 2B). Repeated measures ANOVA (StatView v5; SAS Institute Inc., Cary, NC) with factors of Dose and Week revealed a marginally significant effect of Dose ($F_{(3,12)}$ = 2.74; $P < 0.1$) but a significant Dose × Week interaction ($F_{(1,12)}$ = 106.8; $P < 0.0001$). Examining these data within Week indicated the ability of **7a** to antagonize U50,488-mediated diuresis was dose dependent and confined to Week 0. The effect of individual doses of **7a** on urine output was compared to U50,488 using Dunnett's test. At Week 0, ANOVA indicated there was a significant overall effect of Dose ($F_{(3,12)}$ = 7.53; $P < 0.005$) to lower urine output, with the 10 and 30 mg/kg doses causing significant antagonism of the diuretic effects of U50,488. However, at Week 1 (no further dosing with **7a**), antagonism of U50,488 could no longer be detected (ANOVA; $F_{(1,12)}$ = 0.69; NS).

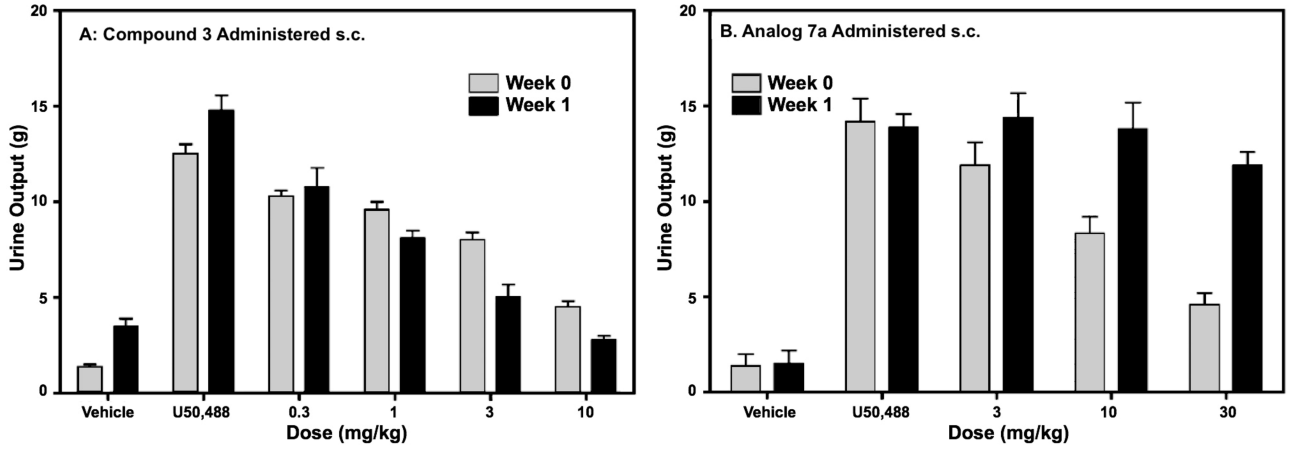


Figure 2. Comparison of **7a** to **3** on U50,488-induced diuresis in rats. (A) Antagonism of U50,488-induced urine output in various sc doses (mg/kg) of **3**. (B) Antagonism of U50,488-induced urine output by various sc doses (mg/kg) of **7a**, and compound **3** and **7a** were only administered once at week 0.

Thus, the activity of **7a** lasted less than 1 week, which contrasts sharply with the activity of **3** as well as other κ opioid receptor antagonists such as **1**, which can persist for up to 3 weeks.[45] The shorter duration of action of **7a** compared to **3** and **1** showed that selective, high affinity κ opioid receptor antagonists can be identified with shorter durations of activity.

Conclusions

In summary, replacement of the amino $-$NH$-$ group of **3** with an aromatic $=$CH$-$, $-CH_2-$, O, or S provided compounds with reduced κ opioid receptor in vitro antagonist potency and reduced κ selectivity relative to the μ and δ opioid receptors. These results confirm our earlier results showing that the amino $-$NH$-$ group of **3** is highly important to its high κ opioid receptor in vitro antagonist potency as well as κ selectivity. However, molecular modeling studies show that the amino $-$NH$-$ group of **3** and the amino group in GNTI are not likely to be interacting with the κ opioid receptor in the same way. Interestingly, compound **7a**, where the amino $-$NH$-$ of **3** has been replaced by a methylene ($-CH_2-$) group, showed subnanomolar κ potency and reasonable selectivity for the κ relative to the μ and δ opioid receptors. Compound **7a** antagonized selective κ agonist U50,488-induced diuresis with a potency that parallels its κ efficacy in the [^{35}S]GTPγS antagonist assay. In contrast to **3**, whose activity as an antagonist of U50,488-induced diuresis lasted for 3 weeks, the activity of **7a** lasted less than 1 week.

Experimental Section

^{1}H NMR spectra were determined on a Bruker 300 spectrometer using tetramethylsilane as an internal standard. Mass spectral data were obtained using a Finnegan LCQ electrospray mass spectrometer in positive ion mode at atmospheric pressure. Silica gel 60 (230$-$400 mesh) was used for column chromatography. All reactions were followed by thin-layer chromatography using Whatman silica gel 60 TLC plates and were visualized by UV. Optical rotations were measured on an Auto Pol III polarimeter. All solvents were reagent grade. HCl in dry diethyl ether was purchased from Aldrich Chemical Co. and used while fresh before discoloration. CMA-80 is a mixture of 80% chloroform, 18% methanol, and 2% concentrated ammonium hydroxide. CMA-80 is a mixture of 80% methylene chloride, 18% methanol, and 2% concentrated ammonium hydroxide. Purity of compounds (>95%) was established by elemental analysis except for **8a** and **8b**. HPLC analysis was used to establish their purity. Elemental analysis was performed by Atlantic Microlab, Inc., Norcross, GA.

HPLC-grade solvents were purchased from Burdick & Jackson (Muskegon, MI). Preparative HPLC was carried out utilizing a Varian Prostar HPLC system (Walnut Creek, CA) equipped with Prostar 210 pumps, Prostar 701 fraction collector, and a Prostar 335 photodiode array detector (PDA), with data collected and analyzed using Galaxie Chromatography Workstation software (version 1.9.3.2). Analytical HPLC was carried out utilizing a Varian Prostar HPLC system (Walnut Creek, CA) equipped with Prostar 210 pumps and a Prostar 330 photodiode array detector (PDA), with data collected and analyzed using Star Chromatography Workstation software (version 6.41). For preparative HPLC, a Gemini-NX C18 (5 μM; 250 mm $\times$ 21.2 mm) column was used with a 19 mL/min flow rate, while for analytical HPLC, a Gemini-NX C18 (5 μm; 250 mm $\times$ 4.6 mm) column was used with a 1 mL/min flow rate (both from Phenomenex, Torrance, CA). For analytical HPLC, a MetaTherm HPLC column temperature controller (Varian) maintained the column at 30 °C.

6-Hydroxynaphthalene-2-carboxylic Acid-{1-[4-(3-hydroxyphenyl)-(3*R*,4*R*)-*trans*-dimethyl-piperidinylmethyl]-(2*S*)-methylpropyl}amide Hydrochloride (6). 6-Hydroxy-2-naphthoic acid (100 mg, 0.53 mmol) was added to a solution of **12**[27] (154 mg, 0.53 mmol) and BOP reagent (235 mg, 0.53 mmol) in THF (40 mL) and was allowed to stir under nitrogen for 15 min. Triethylamine (1.18 g, 0.012 mol) in THF (10 mL) was added, and the reaction mixture was allowed to stir at room temperature for 3 h. $NaHCO_3$ (50 mL) and Et_2O (50 mL) were added to the reaction mixture, and the organic layer was separated, washed with brine, dried, (Na_2SO_4), and concentrated under reduced pressure to afford 0.32 g of an amorphous solid. The solid was purified using medium pressure silica gel chromatography, eluting with EtOAc$-$CMA80 (1:1) to afford 0.095 g of a white amorphous solid. ^{1}H NMR ($CDCl_3$) δ 8.20 (s, 1H), 7.75 (m, 2H), 7.62 (d, J = 9.0 Hz, 1H), 7.10 (m, 4H), 6.71 (m, 2H), 6.60 (d, 6.0 Hz, 1H), 4.42 (m, 1H), 2.32$-$2.92 (bm, 7H), 2.25 (bt, 1H), 2.05 (m, 1H), 1.92 (bs, 1H), 1.57 (bd, 1H), 1.27 (s, 3H), 1.06 (dd, J = 4.8, 2.1 Hz, 6H), 0.55 (d, J = 6.9 Hz, 3H). The resulting solid was dissolved in CH_2Cl_2-MeOH (1:1) and acidified with 1 M ethereal HCl. The mixture was concentrated in vacuo, then dried to yield 0.065 g (23%) of **6** as a white solid: mp 207$-$211 °C. Anal. ($C_{29}H_{37}ClN_2O_3\cdot 2H_2O$) C,H, N.

6-Hydroxy-1,2,3,4-tetrahydro-naphthalene-2(+)-carboxylic Acid-{1-[4-(3-hydroxyphenyl)-(3*R*,4*R*)-*trans*-dimethyl-piperidinylmethyl]-(2*S*)-methylpropyl}amide (7a) Hydrochloride. A 1.0 M solution of BBr_3 (8.2 mL. 8.2 mmol) in CH_2Cl_2 was added at $-$78 °C under N_2 to **16b** (0.39 g, 0.82 mmol) in CH_2Cl_2 (25 mL). The dark-brown solution was allowed to stir at $-$78 °C for 0.5 h

and allowed to warm to room temperature. A saturated solution of $NaHCO_3$ (50 mL) was cautiously added, and the biphasic mixture was extracted with EtOAc (3 × 100 mL). The organic extracts were combined, dried ($MgSO_4$), and concentrated under reduced pressure to provide a brown oil. The oil was purified using medium pressure column chromatography on silica using $CHCl_3$−MeOH−NH_4OH (8:1.8:0.2) as the eluent to provide 0.30 g (77%) of **7a** as a colorless oil. The hydrochloride salt was prepared by adding a 1.0 M solution of HCl in Et_2O to the free base in MeOH. The solution was concentrated under reduced pressure and the resulting solid recrystallized from EtOH−Et_2O to provide **7a**·HCl as white plates: mp 189−191 °C; $[\alpha]^{22}_D$ +113.7 °C (c = 0.18, MeOH). ^{1}H NMR (CD_3OH) δ 0.74 (d, J = 6.78 Hz, 3 H), 0.90 (d, J = 6.78 Hz, 3 H), 0.93 (d, J = 6.78 Hz, 3 H), 1.27 (s, 3 H), 1.55 (d, J = 12.81 Hz, 1 H), 1.68−1.89 (m, 2 H), 1.95 (m, 2 H), 2.36−2.81 (m, 12H), 4.02 (ddd, J = 9.61, 5.09, 4.90 Hz, 1 H), 6.50 (d, J = 2.26 Hz, 1 H), 6.57 (ddd, J = 15.26, 8.10, 2.26 Hz, 2 H), 6.70−6.80 (m, 2 H), 6.85 (d, J = 8.29 Hz, 1 H), 7.10 (t, J = 8.10 Hz, 1 H), 7.81 (br s, 1 H). Anal. ($C_{29}H_{41}ClN_2O_3 \cdot 0.75H_2O$) C, H, N.

6-Hydroxy-1,2,3,4-tetrahydro-naphthalene-2(−)-carboxylic Acid-{1-[4-(3-hydroxyphenyl)-(3*R*,4*R*)-*trans*-dimethyl-piperidinyl-methyl]-(2*S*)-methylpropyl}amide (7b) Hydrochloride. A 1.0 M solution of BBr_3 (8.2 mL, 8.2 mmol) in CH_2Cl_2 was added at −78 °C under N_2 to **16a** (0.70 g, 1.45 mmol) in CH_2Cl_2 (50 mL). The dark-brown solution was allowed to stir at −78 °C for 0.5 h and allowed to warm to room temperature. A saturated solution of $NaHCO_3$ (100 mL) was cautiously added, and the biphasic mixture was extracted with EtOAc (3 × 150 mL). The organic extracts were combined, dried ($MgSO_4$), and concentrated under reduced pressure to provide a brown oil. The oil was purified using medium pressure column chromatography on using silica $CHCl_3$−MeOH−NH_4OH (8:1.8:0.2) as the eluent to provide 0.057 g (83%) of **7b** as a colorless oil. The hydrochloride salt was prepared by adding a 1.0 M solution of HCl in Et_2O to **7b** in MeOH. The solution was concentrated under reduced pressure and recrystallized from EtOH−Et_2O to provide **7b**·HCl as tan cubes: mp 193−195 °C; $[\alpha]^{22}_D$ + 67.0 °C (c = 0.22, MeOH). ^{1}H NMR (CD_3OD) δ 0.76 (d, J = 7.32 Hz, 3 H), 0.91 (d, J = 6.84 Hz, 3 H), 0.95 (d, J = 6.84 Hz, 3 H), 1.27−1.30 (s, 3 H), 1.57 (d, J = 11.23 Hz, 1 H), 1.75−1.86 (m, 2 H), 1.95−2.03 (m, 2 H), 2.29 (td, J = 12.57, 4.15 Hz, 1 H), 2.34−2.41 (m, 1 H), 2.42−2.87 (m, 10 H), 4.02 (dt, J = 9.77, 4.88 Hz, 1 H), 6.49 (m, 1 H), 6.52 (dd, J = 8.30, 2.44 Hz, 1 H), 6.58 (dd, J = 7.81, 1.95 Hz, 1 H), 6.74 (m, 1 H), 6.77 (d, J = 7.81 Hz, 1 H), 6.82 (d, J = 8.30 Hz, 1 H), 7.10 (t, J = 8.06 Hz, 1H). Anal. ($C_{29}H_{41}ClN_2O_3 \cdot 1.5H_2O$) C, H, N.

7-Hydroxy-3,4-dihydro-1*H*-isochromene-3-carboxylic Acid-{1-[4-(3-hydroxyphenyl)-(3*R*,4*R*)-*trans*-dimethyl-piperidinyl-methyl]-(2*S*)-methylpropyl}amide (8a,b). 7-Hydroxy-3,4-dihydro-1*H*-isochromene-3-carboxylic acid [(±)-**29**, 66 mg, 0.34 mmol] was added to a solution of **12**[27] (98 mg, 0.34 mmol) and BOP reagent (150 mg, 0.34 mmol) in THF (20 mL) and was allowed to stir under nitrogen for 15 min. Triethylamine (69 mg, 0.68 mmol) in THF (10 mL) was added, and the reaction was allowed to stir at room temperature for 3 h. To the reaction mixture were added $NaHCO_3$ (30 mL) and Et_2O (30 mL), and the organic layer was separated, washed with brine, dried, (Na_2SO_4), and concentrated under reduced pressure to afford an oil. The oil was purified by medium pressure silica gel chromatography, eluting with $CHCl_3$−CMA80 (1:1) to afford 0.129 g of a pale-yellow oil as a mixture of **8a** and **8b**. The diastereomers were purified using preparative HPLC. A sample of 41 mg of **8a,b** in DMSO (200 μL) was purified via reversed phase HPLC using an isocratic MeOH−H_2O (0.1% DEA) solvent system at 19 mL/min for 35 min, and 19 mL fractions were collected. Fractions were combined to obtain two pools. The first pool contained 21.6 mg of 100% pure compound **8a** (the first compound to elute), and the second pool contained 16 mg of **8b**, the second peak determined to be 87% pure. The second peak was repurified to afford 9.2 mg of **8b** at 98.7% purity. The purity of isolates was determined via analytical HPLC using an isocratic solvent system at MeOH−H_2O (70:30) (0.1% DEA) for 15 min. Chromatograms were observed at 220 nm, and the samples were dissolved in MeOH.

7-Hydroxy-3,4-dihydro-1*H*-isochromene-3-carboxylic Acid-{1-[4-(3-hydroxyphenyl)-(3*R*,4*R*)-*trans*-dimethyl-piperidinyl-methyl]-(2*S*)-methylpropyl}amide (8a, peak 1). $[\alpha]^{22}_D$ +108.6 °C, (c = 0.11, MeOH). ^{1}H NMR ($CDCl_3$) δ 0.71 (d, J = 6 Hz, 3H), 0.93 (m, 6H), 1.28 (m, 4H), 1.56 (d, J = 12 Hz, 1H), 1.87 (m, 1H), 1.95 (m, 1H), 2.26 (ddd, 1H), 2.41−2.55 (m, 4H), 2.64−2.80 (m, 2H), 2.90−2.96 (dd, J = 3, 18 Hz, 1H), 3.99 (m, 1H), 4.14 (dd, J = 6, 12 Hz, 1H), 4.84 (d, 1H), 4.87 (d, J = 12 Hz, 1H), 6.45 (s, 1H), 6.58 (m, 2H), 6.71 (m, 2H), 6.91 (d, J = 9 Hz, 1H), 7.07 (dd, J = 9, 9 Hz, 1H). HRMS m/z 467.2908 $(M + H)^+$, predicted 467.2910.

7-Hydroxy-3,4-dihydro-1*H*-isochromene-3-carboxylic Acid-{1-[4-(3-hydroxyphenyl)-(3*R*,4*R*)-*trans*-dimethyl-piperidinyl-methyl]-(2*S*)-methylpropyl}amide (8b, peak 2). $[\alpha]^{22}_D$ −16.3 °C (c = 0.08, MeOH). ^{1}H NMR ($CDCl_3$) δ 0.71 (d, J = 6 Hz, 3H), 0.93 (m, 6H), 1.28 (m, 4H), 1.56 (d, J = 12 Hz, 1H), 1.87 (m, 1H), 1.95 (m, 1H), 2.26 (ddd, 1H), 2.41−2.55 (m, 4H), 2.64−2.80 (m, 2H), 2.90−2.96 (dd, J = 3, 18 Hz, 1H), 3.99 (m, 1H), 4.14 (dd, J = 6, 12 Hz, 1H), 4.84 (d, 1H), 4.87 (d, J = 12 Hz, 1H), 6.45 (s, 1H), 6.58 (m, 2H), 6.71 (m, 2H), 6.91 (d, J = 9 Hz, 1H), 7.07 (dd, J = 9, 9 Hz, 1H). HRMS m/z 467.2905 $(M + H)^+$, predicted 467.2910.

7-Hydroxyisothiochroman-3(+)-carboxylic Acid-{1-[4-(3-hydroxyphenyl)-(3*R*,4*R*)-*trans*-dimethyl-piperidinylmethyl]-(2*S*)-methylpropyl}amide (9a). A 1.0 M solution of BBr_3 (9.1 mL, 9.1 mmol) in CH_2Cl_2 was added at −78 °C under N_2 to **22a** (0.45 g, 0.91 mmol) in CH_2Cl_2 (100 mL). The dark-brown solution was allowed to stir at −78 °C for 0.5 h and allowed to warm to 0 °C for 2 h. A saturated solution of $NaHCO_3$ (100 mL) was cautiously added, and the biphasic mixture was extracted with EtOAc (3 × 150 mL). The organic extracts were combined, dried ($MgSO_4$), and concentrated under reduced pressure to provide a brown oil. The oil was purified using medium pressure column chromatography on silica gel using $CHCl_3$−MeOH−NH_4OH (8:1.8:0.2) as an eluent to provide a 0.43 g (98%) of **9a** tan semisolid. The solid was recrystallized from acetone−petroleum ether to afford **9a** as white needles: mp 133−135 °C; $[\alpha]^{22}_D$ +108.7 °C, (c = 0.20, MeOH). ^{1}H NMR (CD_3OD) δ 0.69−0.74 (m, 9H), 0.89−0.98 (m, 1H), 1.28 (s, 3H), 1.52−1.59 (d, J = 12.9 Hz, 1H), 1.64−1.68 (m, 1H), 1.94−1.96 (m, 1H), 2.17−2.47 (m, 4H), 2.58−2.62 (d, J = 11.3 Hz, 1H), 2.72−2.75 (d, J = 11.3 Hz, 1H), 2.89−2.96 (dd, J = 5.3, 15 Hz, 1H), 3.11−3.18 (dd, J = 7.54, 14.3 Hz, 1H), 3.62−3.77 (m, 3H), 3.83−3.90 (m, 1H), 6.54−6.65 (m, 3H), 6.71−6.76 (m, 2H), 6.91−6.94 (d, J = 8.2 Hz, 1H), 7.06−7.11 (t, J = 7.9 Hz, 1H). Anal. ($C_{28}H_{38}N_2O_3S \cdot 0.25H_2O$) C, H, N.

7-Hydroxyisothiochroman-3(−)-carboxylic Acid-{1-[4-(3-hydroxyphenyl)-(3*R*,4*R*)-*trans*-dimethyl-piperidinylmethyl]-(2*S*)-methylpropyl}amide Hydrochloride (9b). A 1.0 M solution of BBr_3 (9.0 mL, 9.0 mmol) in CH_2Cl_2 was added at −78 °C under N_2 to **22b** (0.44 g, 0.90 mmol) in CH_2Cl_2 (100 mL). The dark-brown solution was allowed to stir at −78 °C for 0.5 h and allowed to warm to 0 °C for 2 h. A saturated solution of $NaHCO_3$ (100 mL) was cautiously added, and the biphasic mixture was extracted with EtOAc (3 × 150 mL). The organic extracts were combined, dried ($MgSO_4$), and concentrated under reduced pressure to provide a brown oil. The oil was purified using medium pressure column chromatography on silica gel using $CHCl_3$−MeOH−NH_4OH (8:1.8:0.2) as an eluent to provide 0.40 g (93%) of **9b** as a tan semisolid. ^{1}H NMR (CD_3OD) δ 0.71−0.74 (d, J = 6.8 Hz, 3H), 0.83−0.85 (d, J = 6.8 Hz, 3H), 0.88−0.90 (d, J = 6.8 Hz, 3H), 1.06−1.13 (m, 1H), 1.26 (s, 3H), 1.50−1.54 (d, J = 12.4 Hz, 1H), 1.79−1.94 (m, 2H), 2.15−2.39 (m, 4H), 2.48 (brs, 1H), 2.71−2.75 (d, J = 11 Hz, 1H), 2.97−3.10 (m, 2H), 3.58−3.78 (m, 3H), 3.85−3.91 (m, 1H), 6.57−6.60 (d, J = 7.9 Hz, 1H), 6.63 (m, 2H), 6.73 (m, 2H), 6.95−6.98 (d, J = 8.2 Hz, 1H), 7.06−7.12 (t, J = 7.9 Hz, 1H). The hydrochloride salt was prepared by adding a 1.0 M solution of HCl in Et_2O to **9b** in MeOH. The solution was concentrated under reduced pressure and recrystallized

from EtOH−Et_2O to provide **9b**·HCl as white cubes: mp 224−227 °C (191−194 °C softens), $[\alpha]^{22}_D$ +62.1 °C, (c = 0.38, MeOH). Anal. C, H, N.

7-Hydroxy-2-oxo-2-isothiochroman-3(+)-carboxylic Acid-{1-[4-(3-hydroxyphenyl)-(3*R*,4*R*)-*trans*-dimethyl-piperidinylmethyl]-(2*S*)-methylpropyl}amide (10a,b) Resorcylate. 3-Chloroperoxybenzoic acid (0.94 g, 0.41 mmol) was added to an ice-cold solution of **9a** (0.20 g, 0.41 mmol) in CH_2Cl_2 (20 mL). The solution was allowed to stir at 0 °C for 30 min and was quenched by the addition of saturated sodium bicarbonate. The slurry was extracted with CH_2Cl_2 (3 × 30 mL), dried ($MgSO_4$), and concentrated under reduced pressure to afford a mixture of diastereomers as a pale-yellow oil. The diastereomers were separated by silica gel chromatography using $CHCl_3$−MeOH−NH_4OH (8:1.8:0.2) as the eluent to provide **10a** (first to elute, 28 mg, 14%) and **10b** (second to elute, 21 mg, 10%) as pale-yellow oils. Additional product was collected as a mixture of diastereomers.

7-Hydroxy-2-oxo-2-isothiochroman-3(+)-carboxylic Acid-{1-[4-(3-hydroxyphenyl)-(3*R*,4*R*)-*trans*-dimethyl-piperidinylmethyl]-(2*S*)-methylpropyl}amide (10a) Resorcylate. The first spot to elute off the column was converted to the resorcylate salt by dissolving 3,5-dihydroxybenzoic acid (1.1 equiv) in acetone and adding the amine in acetone. The solution was concentrated, and the solid was recrystallized from EtOAc and hexane to provide 17 mg of **10a** as a white solid: mp 170−173 °C; $[\alpha]^{22}_D$ +4.7 °C, (c = 0.11, MeOH). ^{1}H NMR (CD_3OD) δ 0.80 (d, J = 6.8 Hz, 3H), 1.01 (m, 6H), 1.27−1.40 (m, 4H), 1.72 (d, 1H), 1.85 (m, 1H), 2.13 (m, 1H), 2.41 (m, 1H), 2.79−2.86 (m, 3H), 3.0−3.08 (m, 3H), 3.18−3.23 (m, 1H), 3.62 (dd, J = 6, 9 Hz, 1H), 4.10−4.16 (m, 3H), 6.38 (dd, 1H), 6.59−6.79 (m, 5H), 6.91 (dd, 1H), 6.96−6.99 (d, J = 9 Hz, 1H), 7.10−7.15 (m, 2H). Anal. ($C_{35}H_{44}N_2O_8S\cdot 1.5H_2O$) C, H, N.

7-Hydroxy-2-oxo-2-isothiochroman-3(+)-carboxylic acid-{1-[4-(3-hydroxyphenyl)-(3*R*,4*R*)-*trans*-dimethyl-piperidinylmethyl]-(2*S*)-methylpropyl}amide (10b) Resorcylate. The second spot to elute off the column was converted to the resorcylate salt by dissolving 3,5-dihydroxybenzoic acid (1.1 equiv) in acetone and adding the amine in acetone. The solution was concentrated, and the solid was recrystallized from EtOAc and hexane to provide 12 mg of **10b** resorcylate as a white solid: mp 186−189 °C; $[\alpha]^{22}_D$ +102.0 °C, (c = 0.15, MeOH). ^{1}H NMR (CD_3OD) δ 0.82 (dd, J = 6, 3 Hz, 3H), 0.97 (m, 6H), 1.26 (dd, J = 4, 12 Hz 1H), 1.36 (s, 3H), 1.72−1.76 (d, 8 Hz, 1H), 1.90 (m, 1H), 2.18 (m, 1H), 2.46 (m, 1H), 2.91−3.32 (m, 6H), 3.51 (m, 1H), 3.72−3.87 (m, 1H), 4.0−4.33 (m, 3H), 6.37 (dd, J = 6, 6 Hz, 1H), 6.60−6.78 (m, 5H), 6.91 (d, J = 3 Hz, 2H), 7.01−7.15 (m, 2H). Anal. ($C_{35}H_{44}N_2O_8S\cdot 1.25H_2O$) C, H, N.

2(+)-6-Methoxy-1,2,3,4-tetrahydronaphthalene-2-carboxylic Acid [(+)-13]. A 30% solution of hydrogen peroxide (6.96 mmol, 0.24 mL) in H_2O was added at 0 °C to a solution of **15a** (0.42 g, 1.16 mmol) in 3:1 THF−H_2O (25 mL). Lithium hydroxide hydrate (0.098 g, 2.32 mmol) was added to the solution in portions. The suspension was allowed to stir for 0.5 h at 0 °C and for 2 h at room temperature. A 1.5 N solution of Na_2SO_3 (15 mL) was added in a dropwise manner, and the biphasic solution was basified (pH ≈ 9) with saturated sodium bicarbonate solution. The solution was extracted (2 × 50 mL) with EtOAc, made acidic to pH 3 with HCl (10 M solution), and extracted (3 × 100 mL) with CH_2Cl_2. The organic extracts were combined, dried ($MgSO_4$), and concentrated under reduced pressure to provide a white solid. The solid was recrystallized from EtOAc−petroleum ether to provide 0.22 g (92%) of (+)-**13** as white needles: mp 129−130 °C; $[\alpha]^{22}_D$ +57.27 °C, (c = 0.22, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ 1.87−1.90 (m, 1H), 2.20−2.25 (m, 1H), 2.74−2.98 (m, 5H), 3.77 (s, 3H), 6.63 (s, 1H), 6.68−6.72 (dd, J = 2.7, 8.4 Hz, 1H), 7.0−7.03 (d, J = 8.4 Hz, 1H).

2(−)-6-Methoxy-1,2,3,4-tetrahydronaphthalene-2-carboxylic Acid [(−)-13]. A 30% solution of hydrogen peroxide (3.3 mmol, 0.11 mL) in H_2O was added at 0 °C to a solution of **15b** (0.20 g, 0.55 mmol) in THF−H_2O (3:1) (15 mL). Lithium hydroxide hydrate (0.046 g, 1.10 mmol) was added to the solution in portions. The suspension was allowed to stir for 0.5 h at 0 °C and for 2 h at room temperature. A 1.5 N solution of Na_2SO_3 (10 mL) was added in a dropwise manner, and the biphasic solution was basified (pH ≈ 10) with saturated sodium bicarbonate solution. The solution was extracted (2 × 50 mL) with EtOAc, made acidic to pH 3 with HCl (10 M solution), and extracted (3 × 100 mL) with CH_2Cl_2. The organic extracts were combined, dried ($MgSO_4$), and concentrated under reduced pressure to provide a white solid. The solid was recrystallized from EtOAc−petroleum ether to provide 0.102 g (90%) of (−)-**13** as white needles: mp 121−122 °C; $[\alpha]^{22}_D$ −56.9 °C (c = 0.25, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ 1.87−1.90 (m, 1H), 2.20−2.25 (m, 1H), 2.74−2.98 (m, 5H), 3.77 (s, 3H), 6.63 (s, 1H), 6.68−6.72 (dd, J = 2.7, 8.4 Hz, 1H), 7.0−7.03 (d, J = 8.4 Hz, 1H).

(3a*R*-*cis*)-3-(6-Methoxy-1,2,3,4-tetrahydronaphthalene-2(+ and −)-carbonyl)-3,3a,8,8a-tetrahydro-2*H*-indeno[1,2-*d*]oxazol-2-one (15a,b). A 2.0 M solution of thionyl chloride (7.25 mL, 14.3 mmol) in CH_2Cl_2 was added to a solution of (±)-**13**[29] (0.29 g, 1.43 mmol) in toluene (20 mL). The solution was heated at reflux for 8 h, cooled to room temperature, and concentrated under reduced pressure to provide 6-methoxy-1,2,3,4-tetrahydronaphthalene-2-carbonyl chloride as a tan solid. In a separate flask, a 0.50 M solution of ethyl lithium (3.0 mL, 1.50 mL) in benzene−yclohexane (90:10) was added to a solution of (3a*R*-*cis*)-3,3a,8,8a-tetrahydro-2*H*-indeno[1,2-*d*]oxazol-2-one (0.25 g, 1.43 mmol) in THF (20 mL) at 0 °C under N_2. The suspension was allowed to stir at 0 °C for 0.5 h and was cooled to −78 °C. A solution of 6-methoxy-1,2,3,4-tetrahydronaphthalene-2-carbonyl chloride (0.29 g, 1.43 mmol) in THF (10 mL) was then added in a dropwise manner to the −78 °C slurry. The resulting slurry was allowed to warm to room temperature over 2 h, and water (100 mL) was added. The suspension was extracted with CH_2Cl_2 (3 × 100 mL). The organic extracts were combined, dried ($MgSO_4$), and concentrated under reduced pressure to provide a mixture of **15a** and **15b** as a tan solid. The two compounds were separated using silica gel medium pressure column chromatography petroleum ether−Et_2O (70:30) to provide each of the diastereomers in approximately 50% theoretical yield. The yield improves with additional chromatography. The less polar spot was later identified as the (+) isomer while the more polar was (−).

(3a*R*-*cis*)-3-(6-Methoxy-1,2,3,4-tetrahydronaphthalene-2(+)-carbonyl)-3,3a,8,8a-tetrahydro-2*H*-indeno[1,2-*d*]oxazol-2-one (15a). The solid was recrystallized from EtOAc−petroleum ether to provide 0.12 g (46%) of **15a** as a white solid: mp 168−169 °C. ^{1}H NMR ($CDCl_3$) δ 1.80−1.85 (m, 1H), 2.10−2.21 (m, 1H), 2.71−3.13 (m, 4H), 3.38 (d, J = 3.3 Hz, 2H), 3.76 (s, 3H), 3.84 (m, 1H), 5.27 (m, 1H), 5.96−5.99 (d, J = 9 Hz, 1H), 6.62 (s, 1H), 6.70−6.71 (dd, J = 2.4, 8.1 Hz, 1H), 6.99−7.04 (dd, J = 3.6, 8.4 Hz, 1H), 7.24−7.32 (m, 3H), 7.57−7.60 (d, J = 7.5 Hz, 1H).

(3a*R*-*cis*)-3-(6-Methoxy-1,2,3,4-tetrahydronaphthalene-2(−)-carbonyl)-3,3a,8,8a-tetrahydro-2*H*-indeno[1,2-*d*]oxazol-2-one (15b). The solid was recrystallized from EtOAc−petroleum ether to provide 0.13 g (50%) of **15b** as a white solid: mp 162−164 °C. ^{1}H NMR ($CDCl_3$) δ 1.85−1.98 (m, 1H), 2.12−2.18 (m, 1H), 2.84−2.95 (m, 4H), 3.40−3.41 (d, J = 3.3 Hz, 2H), 3.77 (s, 3H), 3.85−3.95 (m, 1H), 5.28−5.33 (m, 1H), 5.97−5.99 (d, J = 6.9 Hz, 1H), 6.57−6.69 (m, 2H), 6.95−6.98 (d, J = 8.4 Hz, 1H), 7.26−7.42 (m, 3H), 7.60−7.62 (d, J = 7.5 Hz, 1H).

6-Methoxy-1,2,3,4-tetrahydro-naphthalene-2(+)-carboxylic Acid-{1-[4-(3-hydroxyphenyl)-(3*R*,4*R*)-*trans*-dimethyl-piperidinyl-methyl]-(2*S*)-methylpropyl}amide (16a). 2(+)-6-Methoxy-1,2,3,4-tetrahydronaphthalene-2-carboxylic acid [(+)-**13**, 0.22 g, 1.07 mmol] was added under N_2 to a solution of BOP (0.47 g, 1.07 mmol), TEA (0.23 g, 2.35 mmol), and **12**[27] (0.31 g, 1.07 mmol) in anhydrous THF (50 mL). The solution was allowed to stir at room temperature for 6 h, and a saturated $NaHCO_3$ solution (100 mL) was added. The biphasic mixture was extracted with EtOAc (3 × 100 mL). The organic extracts were combined, dried ($MgSO_4$), and concentrated under reduced pressure to provide an oil. The oil was purified using medium pressure column chromatography on silica gel using $CHCl_3$−MeOH−NH_4OH (9:0.8:0.2) at the eluent

to provide 0.39 g (77%) of **16a** as a colorless oil. ^{1}H NMR ($CDCl_3$) δ ppm 0.72 (d, J = 6.78 Hz, 3 H), 0.82−0.96 (m, 6 H), 1.26 (s, 3 H), 1.55 (d, J = 12.43 Hz, 1 H), 1.78−2.07 (m, 4 H), 2.18−2.88 (m, 12 H), 3.73 (s, 3 H), 4.00−4.16 (m, 1 H), 6.05 (d, J = 7.54 Hz, 1 H), 6.57 (d, J = 2.64 Hz, 1 H), 6.62−6.77 (m, 3 H), 6.84 (m, 1 H), 6.93 (d, J = 8.67 Hz, 1 H), 7.11 (t, J = 7.91 Hz, 1 H).

6-Methoxy-1,2,3,4-tetrahydro-naphthalene-2(−)-carboxylic Acid-{1-[4-(3-hydroxyphenyl)-(3*R*,4*R*)-*trans*-dimethyl-piperidinyl-methyl]-(2*S*)-methylpropyl}amide (16b). 2(−)-6-Methoxy-1,2,3,4-tetrahydronaphthalene-2-carboxylic acid [(−)-**13**, 0.31 g, 1.48 mmol] was added under N_2 to a solution of BOP (0.65 g, 1.48 mmol), TEA (0.33 g, 3.26 mmol), and **12** (0.43 g, 1.48 mmol) in anhydrous THF (65 mL). The solution was allowed to stir at room temperature for 6 h, and a saturated $NaHCO_3$ solution (100 mL) was added. The biphasic mixture was extracted with EtOAc (3 × 100 mL). The organic extracts were combined, dried ($MgSO_4$), and concentrated under reduced pressure to provide an oil. The oil was purified using medium pressure column chromatography on silica gel using $CHCl_3$−MeOH−NH_4OH (9:0.8:0.2) as the eluent to provide 0.70 g (98%) of **16b** as a colorless oil. ^{1}H NMR ($CDCl_3$) δ ppm 0.66−0.78 (d, J = 6.9 Hz, 3 H), 0.83−0.97 (m, 6 H), 1.25 (s, 3 H), 1.53 (d, J = 12.43 Hz, 1 H), 1.78−2.10 (m, 4 H), 2.20−2.97 (m, 12 H), 3.73 (s, 3 H), 4.03 (m, 1 H), 6.03 (d, J = 7.54 Hz, 1 H), 6.57 (d, J = 2.26 Hz, 1 H), 6.61−6.75 (m, 3 H), 6.82 (m, 1H), 6.90 (d, J = 8.29 Hz, 1 H), 7.10 (t, J = 7.72 Hz, 1 H).

7-Methoxy-isothiochroman-4-one-3-carboxylic Acid Methyl Ester (18). A 2.0 M solution of LDA in heptane−THF−ethylbenzene (1.61 mL, 3.21 mmol) was added in a dropwise manner to a solution of 7-methoxyisothiochroman-4-one (**17**)[31] (0.50 g, 2.57 mmol) in THF (50 mL) at −78 °C under N_2. After 30 min at −78 °C, HMPA (0.46 g, 2.57 mmol) and methyl cyanoformate (0.27 g, 3.21 mmol) were added, and the yellow solution was allowed to stir at −78 °C for 30 min. The solution was then allowed to warm to room temperature, and a saturated solution of NH_4Cl (100 mL) was added. The slurry was extracted with EtOAc (3 × 75 mL), and the organic extracts were combined, dried ($MgSO_4$), and concentrated under reduced pressure to provide a bright-yellow oil. The oil was purified on silica gel medium pressure chromatography using petroleum ether−EtOAc (9:1) as the eluent to provide 0.51 g (78%) of **18** as a bright-yellow oil. ^{1}H NMR ($CDCl_3$) δ 3.73 (s, 2H), 3.83 (s, 3H), 3.85 (s, 3H), 6.67 (d, J = 3 Hz, 1H), 6.45 (dd, J = 3, 8.7 Hz, 1H), 7.80 (d, J = 8.7 Hz, 1H), 12.52 (s, 1H).

7-Methoxy-isothiochroman-3-carboxylic Acid Methyl Ester (19). Triethylsilane (8.08 mmol, 0.94 g) was added to a solution of **18** (0.51 g, 2.02 mmol) in trifluoroacetic acid (15 mL) at room temperature under N_2. The reaction mixture was allowed to stir at room temperature for 2 h and was concentrated under reduced pressure. The resulting oil was dissolved in EtOAc (100 mL) and washed with a saturated $NaHCO_3$ solution (3 × 75 mL). The organic extracts were combined, dried ($MgSO_4$), and concentrated to provide an oil. The oil was purified by silica gel medium pressure chromatography using petroleum ether−EtOAc (9:1) as the eluent to provide 0.34 g, (70%) of **19** as a pale-yellow oil. ^{1}H NMR ($CDCl_3$) δ 3.14 (m, 2H), 3.58−3.63 (d, J = 15 Hz, 1H), 3.73−3.86 (m, 8H), 6.70 (d, J = 3 Hz, 1H), 6.75 (dd, J = 3, 9 Hz, 1H), 7.10 (d, J = 9 Hz, 1H).

7-Methoxy-isothiochroman-3-carboxylic Acid [(±)-20]. Potassium hydroxide (0.80 g, 14.3 mmol) was added to a solution of **19** (0.34 g, 1.43 mmol) in MeOH (50 mL). The solution was heated at 60 °C for 2 h, cooled to room temperature, and diluted with H_2O (100 mL). The solution was made acidic with 6 N HCl and extracted with EtOAc (3 × 100 mL). The organic extracts were combined, dried ($MgSO_4$), and concentrated to give 0.28 g (88%) of (±)-**20** as a pale-yellow solid. The solid was used in the next step without further purification.

3(+)-7-Methoxy-isothiochroman-3-carboxylic Acid [(+)-20]. Lithium hydroxide hydrate (0.093 g, 2.2 mmol) was added at 0 °C to a solution of **21a** (0.42 g, 1.10 mmol) in THF−H_2O (3:1) (25 mL). The suspension was allowed to stir for 0.5 h at 0 °C. The reaction was made basic (pH ≈ 9) with saturated sodium bicarbonate solution, and the solution was extracted with Et_2O (1 × 100 mL), made acidic to pH 3 with HCl (6 N solution), and extracted with EtOAc (3 × 100 mL). The organic extracts were combined, dried ($MgSO_4$), and concentrated under reduced pressure to provide 0.25 g (100%) of (+)-**20** as a white solid. The solid was recrystallized from toluene−petroleum ether to provide (+)-**20** as tan cubes: mp 117−118 °C; $[\alpha]^{22}_D$ +98.3 °C (c = 0.24, MeOH). ^{1}H NMR (CD_3OD) δ 2.97−3.01 (dd, J = 9.3, 15 Hz, 1H), 3.10−3.17 (dd, J = 5.1, 15.3, 1H), 3.63−3.88 (m, 6H), 6.75−6.77 (m, 2H), 7.08−7.11 (d, J = 8.2 Hz, 1H).

3(−)-7-Methoxy-isothiochroman-3-carboxylic Acid [(−)-20]. Lithium hydroxide hydrate (0.055 g, 1.32 mmol) was added at 0 °C to a solution of **21b** (0.25 g, 0.66 mmol) in THF−H_2O (3:1) (15 mL). The suspension was allowed to stir for 0.5 h at 0 °C. The reaction was made basic (pH ≈ 9) with saturated sodium bicarbonate solution, and the solution was extracted with Et_2O (1 × 100 mL), made acidic to pH 3 with 6 N HCl, and extracted with EtOAc (3 × 100 mL). The organic extracts were combined, dried ($MgSO_4$), and concentrated under reduced pressure to provide 0.14 g (92%) of (−)-**20** as a white solid (0.136 g, 92%). The solid was recrystallized from toluene−petroleum ether to provide (−)-**20** as pale-yellow needles: mp 121−122 °C; $[\alpha]^{22}_D$ −100.8 °C (c = 0.26, MeOH). ^{1}H NMR (CD_3OD) δ 2.97−3.01 (dd, J = 9.3, 15 Hz, 1H), 3.10−3.17 (dd, J = 5.1, 15.3, 1H), 3.63−3.88 (m, 6H), 6.75−6.77 (m, 2H), 7.08−7.11 (d, J = 8.2 Hz, 1H).

(3a*R*-*cis*)-3-(7-Methoxy-isothiochroman-3(+ and −)-carbonyl)-3,3a,8,8a-tetrahydro-2*H*-indeno[1,2-*d*]oxazol-2-one (21a,b). A 2.0 M solution of oxalyl chloride (3.57 mL, 7.14 mmol) in CH_2Cl_2 was added under N_2 to a solution of (±)-**20** (0.80 g, 3.57 mmol) and a drop of DMF in CH_2Cl_2 (100 mL). The solution was allowed to stir at room temperature for 3 h and was concentrated under reduced pressure to provide 7-methoxy-isothiochroman-3-carbonyl chloride as a tan oil. 7-Methoxy-isothiochroman-3-carbonyl chloride was used in the next step without further purification. In a separate flask, a 0.50 M solution of ethyl lithium (8.6 mL, 4.28 mmol) in benzene−cyclohexane 90:10 was added to a solution of (3a*R*-*cis*)-3,3a,8,8a-tetrahydro-2*H*-indeno[1,2-*d*]-oxazol-2-one (**14**, 0.75 g, 4.28 mmol) in THF (100 mL) at 0 °C under N_2. The suspension was allowed to stir at 0 °C for 0.5 h and was then cooled to −78 °C. A solution of 7-methoxy-isothiochroman-3-carbonyl chloride (0.86 g, 3.57 mmol) in THF (10 mL) was added in a dropwise manner to the −78 °C slurry. The resulting slurry was allowed to warm to room temperature over 2 h, and water (150 mL) was then added. The suspension was extracted with CH_2Cl_2 (3 × 150 mL). The organic extracts were combined, dried ($MgSO_4$), and concentrated under reduced pressure to provide a mixture of **21a** and **21b** as a tan solid. The mixture was separated by silica gel medium pressure column chromatography using petroleum ether−Et_2O (60:40) as the eluent to provide each of the diastereomers in 62% [**21a**, (+)-isomer] and 37% [**21b**, (−)-isomer] theoretical yield. The yield improves with additional chromatography. The higher R_f spot on TLC was identified as the (+)-isomer, while the more polar spot was the (−)-isomer.

(3a*R*)-*cis*)-3-(7-Methoxy-isothiochroman-3(+)-carbonyl)-3,3a,8,8a-tetrahydro-2*H*-indeno[1,2-*d*]oxazol-2-one (21a). The solid was recrystallized from EtOAc−petroleum ether to provide 0.42 g (62%) of **21a** as a white solid: mp 146−147 °C. ^{1}H NMR ($CDCl_3$) δ 3.09−3.16 (dd, J = 6, 15.6 Hz, 1H), 3.20−3.28 (dd, J = 7.2, 15.3 Hz, 1H), 3.38−3.39 (d, J = 3.6 Hz, 2H), 3.59−3.64 (d, J = 15 Hz, 1H), 3.79 (s, 3H), 3.85−3.90 (d, J = 15 Hz, 1H), 4.97−5.01 (m, 1H), 5.30−5.35 (m, 1H), 5.92−5.95 (d, J = 7 Hz, 1H), 6.71−6.72 (d, J = 2.7 Hz, 1H), 6.75−6.79 (dd, J = 2.4, 8.4 Hz, 1H), 7.06−7.09 (d, J = 8.4 Hz, 1H), 7.23−7.38 (m, 3H), 7.58−7.61 (d, J = 7.5 Hz, 1H).

(3a*R*-*cis*)-3-(7-Methoxy-isothiochroman-3(−)-carbonyl)-3,3a,8,8a-tetrahydro-2*H*-indeno[1,2-*d*]oxazol-2-one (21b). The solid was recrystallized from EtOAc−petroleum ether to provide 0.25 g (37%) of **21b** as a white solid: mp 176−178 °C. ^{1}H NMR ($CDCl_3$) δ 3.14−3.19

(dd, J = 6, 12.6 Hz, 1H), 3.24−3.29 (dd, J = 7.5, 15.3 Hz, 1H), 3.39−3.40 (d, J = 3.6 Hz, 2H), 3.48−3.55 (d, J = 15 Hz, 1H), 3.79 (s, 3H), 3.83−3.88 (d, J = 15 Hz, 1H), 4.91−4.95 (m, 1H), 5.29−5.33 (m, 1H), 5.96−5.99 (d, J = 7 Hz, 1H), 6.71−6.72 (d, J = 2.7 Hz, 1H), 6.76−6.80 (dd, J = 2.4, 8.4 Hz, 1H), 7.10−7.12 (d, J = 8.4 Hz, 1H), 7.26−7.38 (m, 3H), 7.58−7.61 (d, J = 7.5 Hz, 1H).

7-Methoxy-isothiochroman-3(+)-carboxylic Acid-{1-[4-(3-hydroxyphenyl)-(3*R*)-(4*R*)-*trans*-dimethyl-piperidinylmethyl]-(2*S*)-methylpropyl}amide (22a). 3(+)-7-Methoxyisothiochroman-3-carboxylic acid [(+)-**20**, (0.25 g, 1.12 mmol)] was added under N_2 to a solution of BOP (0.50 g, 1.12 mmol), TEA (0.23 g, 2.24 mmol), and **12**[27] (0.33 g, 1.12 mmol) in anhydrous THF (50 mL). The solution was allowed to stir at room temperature for 6 h, and a saturated $NaHCO_3$ solution (100 mL) was added. The biphasic mixture was extracted with EtOAc (3 × 100 mL). The organic extracts were combined, dried ($MgSO_4$), and concentrated under reduced pressure to provide an oil. The oil was purified using medium pressure column chromatography using $CHCl_3$−MeOH−NH_4OH (9:0.8:0.2) to provide 0.5 g (81%) of **22a** as a pale-yellow semisolid. ^{1}H NMR ($CDCl_3$) δ 0.49−0.55 (m, 6H), 0.67−0.69 (d, J = 6 Hz, 3H), 1.24 (s, 3H), 1.48−1.52 (d, J = 12 Hz, 1H), 1.62−1.70 (m, 1H), 1.86−1.88 (m, 1H), 2.14−2.52 (m, 6H), 2.61−2.71 (m, 2H), 2.89−2.95 (dd, J = 5.1, 14.4 Hz, 1H), 3.31−3.38 (dd, J = 5.4, 14.4 Hz, 1H), 3.57−3.62 (d, J = 13.8 Hz, 1H), 3.65−3.69 (d, J = 13.8 Hz, 1H), 3.74 (s, 3H), 3.84−3.87 (m, 1H), 6.70−6.72 (m, 3H), 6.84−6.89 (m, 2H), 7.03−7.12 (m, 2H).

7-Methoxy-isothiochroman-3(−)-carboxylic Acid-{1-[4-(3-hydroxyphenyl)-(3*R*,4*R*)-*trans*-dimethyl-piperidinylmethyl]-(2*S*)-methylpropyl}amide (22b). 3(−)-7-Methoxy-isothiochroman-3-carboxylic acid [(−)-**20**, 0.24 g, 1.07 mmol] was added under N_2 to a solution of BOP (0.47 g, 1.07 mmol), TEA (0.21 g, 2.14 mmol), and **12**[27] (0.31 g, 1.07 mmol) in anhydrous THF (50 mL). The solution was allowed to stir at room temperature for 6 h, and a saturated $NaHCO_3$ solution (100 mL) was added. The biphasic mixture was extracted with EtOAc (3 × 100 mL). The organic extracts were combined, dried ($MgSO_4$), and concentrated under reduced pressure to provide an oil. The oil was purified by silica gel medium pressure column chromatography using $CHCl_3$−MeOH−NH_4OH (9:0.8:0.2) as the eluent to provide 0.44 g (84%) of **22b** as a pale yellow semisolid. ^{1}H NMR ($CDCl_3$) δ 0.65−0.68 (d, J = 6.9 Hz, 3H), 0.77−0.79 (d, J = 4.2 Hz, 3H), 0.84−0.86 (d, J = 6.6 Hz, 3H), 1.27 (s, 3H), 1.47−1.51 (d, J = 12.3 Hz, 1H), 1.80−2.70 (m, 11H), 3.03−3.09 (dd, J = 5.4, 14.7 Hz, 1H), 3.17−3.24 (dd, J = 6.3, 14.4 Hz, 1H), 3.60−3.65 (d, J = 14.1 Hz, 1H), 3.67−3.72 (d, J = 14.1 Hz, 1H), 3.77 (s, 3H), 3.83−3.87 (m, 1H), 6.59−6.83 (m, 5H), 7.05−7.16 (m, 2H).

7-Methoxy-1*H*-isochromen-4(3*H*)-one (24). Oxalyl chloride (92.5 mL, 2.0 M in CH_2Cl_2, 185 mmol) was added dropwise to a solution of (3-methoxybenzyloxy)acetic acid (**23**)[34] (23.2 g, 0.12 mol) in CH_2Cl_2 (850 mL) at 0 °C under N_2, and a few drops of DMF were added to initiate the reaction. The reaction mixture was stirred for 2 h at room temperature and was concentrated to give a brown oil. Chlorobenzene (500 mL) was then added, and the mixture was allowed to stir using a mechanical stirrer. The mixture was cooled to 0 °C in an ice bath, $SnCl_4$ (29 mL, 246.8 mmol) was added slowly, and the reaction mixture was allowed to stir at 0 °C under N_2 for 1 h. The reaction mixture was quenched with saturated $NaHCO_3$ (150 mL) and H_2O (150 mL) and then extracted with CH_2Cl_2 (3 × 250 mL). The combined organics were dried over $NaSO_4$, and the solvent was removed under reduced pressure to afford crude compound which was purified by silica gel medium pressure column chromatography using 10−40% EtOAc in hexane to give 10.9 g (52%) of **24** as a white solid. ^{1}H NMR ($CDCl_3$) δ 8.02 (d, J = 8.6 Hz, 1H), 6.92 (d, J = 7.9 Hz, 1H), 6.67 (s, 1H), 4.85 (s, 2H), 4.33 (s, 2H), 3.88 (s, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 193.1, 164.6, 144.6, 129.4, 123.6, 114.5, 109.1, 73.7, 68.4, 56.0. LCMS (APCI) m/z $(M + H)^+$ 179.2.

7-Hydroxy-1*H*-isochromen-4(3*H*)-one (25). 7-Methoxy-1*H*-isochromen-4(3*H*)-one (**24**, 11.0 g, 0.062 mol) was dissolved in DMF (320 mL), and EtSNa (7.8 g, 0.093 mol) was added. The reaction mixture was heated at reflux for 3 h, H_2O (450 mL) was added, and the mixture was extracted with EtOAc (300 mL). The aqueous layer was cooled, acidified to pH ≈ 4 with 1 M HCl, and then extracted with EtOAc (3 × 300 mL). The combined organics were washed with bleach (200 mL) and dried over sodium sulfate, and the solvent was removed under reduced pressure to afford crude product, which was purified by silica gel medium pressure column chromatography using 10−50% EtOAc in hexane as the eluent to give 7.2 g (71%) of **25** as a colorless solid. ^{1}H NMR (CD_3OD) δ 7.85−7.80 (m, 1H), 6.77 (d, J = 6.4 Hz, 1 H), 6.6 (s, 1H), 4.76 (s, 3H), 4.22 (s, 2H). ^{13}C NMR (CD_3OD, 75 MHz) δ 195.5, 165.2, 147.0, 130.3, 123.3, 116.8, 111.7, 74.4, 69.2. LCMS (ESI) m/z 165.3 $(M + H)^+$; 163.4 $(M - H)^+$.

7-(Methoxymethoxy)-1*H*-isochromen-4(3*H*)-one (26). 7-Hydroxy-1*H*-isochromen-4(3*H*)-one (**25**, 7.0 g, 0.43 mol) was dissolved in CH_2Cl_2 (350 mL), and the solution was cooled to 0 °C under N_2. *i*-Pr_2EtN (8.3 g, 0.064 mol) was added, and then MOMCl (5.15 g, 0.064 mol) was added slowly. The reaction mixture was stirred for 8 h at room temperature, and saturated $NaHCO_3$ (200 mL) was added. The suspension was extracted with EtOAc (3 × 250 mL), the combined organics were dried over sodium sulfate, and the solvent was removed under reduced pressure. The solid was purified by silica gel medium pressure column chromatography using 10−40% EtOAc in hexane as the eluent to give 7.8 g (88%) of **26** as a colorless solid. ^{1}H NMR ($CDCl_3$) δ 8.02 (d, J = 8.6 Hz, 1H), 7.03 (d, J = 8.6 Hz, 1H), 6.84 (s, 1H), 5.24 (s, 2H), 4.85 (s, 2H), 4.33 (s, 2H), 3.49 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 192.8, 161.8, 144.2, 128.9, 124.0, 116.1, 110.7, 94.1, 73.4, 68.1, 56.4. LCMS (APCI) m/z 209.3 $(M + H)^+$.

Methyl 7-(Methoxymethoxy)-4-oxo-3,4-dihydro-1*H*-isochromen-3-carboxylate (27). LDA (31 mL, 2.0 M in THF−heptane−ethylbenzene, 62.0 mmol) was then added to a solution of **26** (6.6 g, 0.032 mol) in THF (400 mL) at −78 °C. The mixture was stirred at −78 °C for 30 min, and HMPA (5.74 g, 5.6 mL, 0.032 mol) was added followed by methylcyanoformate (5.4 g, 0.064 mol). The reaction mixture was stirred at −78 °C for 2 h and warmed to room temperature. The reaction mixture was quenched with saturated NH_4Cl (250 mL) and then extracted with EtOAc (3 × 300 mL). The combined organics were dried over sodium sulfate, and the solvent was removed under reduced pressure to afford crude compound which was purified by silica gel medium pressure column chromatography using 10−30% EtOAc in hexane as eluent to give 4.35 g (52%) of **27** as a colorless solid. NMR shows a mixture of ketone and enol tautomers. ^{1}H NMR ($CDCl_3$) δ 8.02 (d, J = 8.7 Hz, 0.7 H), 7.61 (d, J = 8.5 Hz, 0.2H), 7.03 (d, J = 8.7 Hz, 1H), 6.81 (s, 1H), 5.24 (s, 1H), 5.23 (s, 2H), 4.97−4.85 (m, 2H), 3.91 (s, 0.7H), 3.84 (s, 2.5H), 3.48 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 187.1, 166.9, 162.3, 143.5, 129.6, 124.4, 116.5, 110.5, 94.2, 80.5, 65.9, 56.4, 52.8. LCMS (ESI) m/z 267.1 $(M + H)^+$.

Methyl 7-hydroxy-3,4-dihyro-1*H*-isochromene-3-carboxylate (28). TFA (80 mL) was added to **27** (4.3 g, 0.016 mol) at 0 °C, and Et_3SiH (7.52 g, 0.065 mol) was added. The reaction mixture was stirred at room temperature for 2 h and then was concentrated under reduced pressure. Saturated $NaHCO_3$ (100 mL) was added, and the mixture was extracted with EtOAc (3 × 150 mL). The combined organics were dried (Na_2SO_4), and the solvent was removed under reduced pressure to afford crude compound which was purified by silica gel medium pressure column chromatography using 10−40% EtOAc in hexane as eluent to give 2.22 g (66%) of **28** as a colorless solid. ^{1}H NMR ($CDCl_3$) δ 6.99 (d, J = 8.0 Hz, 1H), 6.70 (d, J = 8.1 Hz, 1H), 6.48 (s, 1H), 5.76 (s, 1H), 4.91 (d, J = 15.0 Hz, 1H), 4.78 (d, J = 15.0 Hz, 1H), 4.36 (t, J = 7.0 Hz, 1H), 3.82 (s, 3H), 2.99 (d, J = 6.9 Hz, 2H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 171.9, 154.4, 134.8, 129.9, 123.3, 114.4, 110.7, 73.6, 67.8, 52.4, 30.1. LCMS (ESI) m/z 209.3 $(M + H)^+$; 231.5 $(M + Na)^+$.

7-Hydroxy-3,4-dihydro-1*H*-isochromene-3-carboxylic Acid (29). Lithium hydroxide hydrate (45 mg, 1.08 mmol) was added to a solution of **28** (0.100 g, 0.48 mmol) in a mixture

of THF−H_2O−MeOH (1:1:1) (25 mL) at 0 °C. The solution was allowed to stir for 1 h and warmed to room temperature. A 1 N solution of HCl was added until the mixture was acidic and was then extracted with $CHCl_3$ (3 × 50 mL). The organic extracts were combined, dried ($MgSO_4$), and concentrated under reduced pressure to provide 0.75 g (82%) of (±)-**29** as a white solid. This solid was used in the next step without further purification. ^{1}H NMR (CD_3OD) δ 2.96, (m, 3H), 4.32 (dd, J = 3, 9 Hz, 1H), 4.74 (m, 2H), 6.46 (s, 1H), 6.63 (d, J = 9 Hz, 1H), 6.95 (d, J = 9 Hz, 1H).

Compounds 3 and 2 Alignment. Three-dimensional structures of **3** and **2** were built using the Sybyl fragment library and optimized using the MMFF94 force-field and charges. A systematic conformational search followed by a multifit flexible structural alignment was performed using **2** as the rigid template (excepting the guanidinium group of **2**, which was allowed to freely rotate during the multifit alignment). Six **2**−**3** atom pairs and a spring constant of 20 kcal/mol were used to align **3** to the **2** template. The atom pairs were the **3** and **2** phenolic ring centroid, **3** atoms 3, 4, 5, 3-methyl, and 4-methyl paired with **2** atoms 14, 13, 15, 10, and 5 (morphine numbering). The centroid of the Tic aromatic ring of **3** was paired with the centroid of the guanidinium group of **2**. After alignment, a final energy minimization was performed using the MMFF94 force-field to allow the structure of **3** to relax to a local minimum energy conformation. This process was repeated for each of the 10 lowest energy conformations of **3** obtained from an initial systematic conformational search in order to identify the lowest energy conformation of **3** that could provide a close alignment to the **2** pharmacophore template.

Evaluation of 7a to Block U50,488-Induced Diuresis. Adult male Sprague−Dawley rates (Charles River Laboratories, Raleigh, NC) were used for these studies. The test compound and U50,488 doses were prepared fresh in distilled deionized water (vehicle) and administered (1 mL/kg body weight) via subcutaneous injection. Six groups of four rats were used to evaluate each test compound: vehicle control, agonist control (10 mg/kg), test compound **7a** at 3, 10, or 30 mg/kg followed by agonist (10 mg/kg), and compound **7a** followed by vehicle (30 mg/kg). Each rat was weighed prior to dosing. One rat from each group was dosed in succession and the pattern repeated to distribute any effects of time of day across all groups. After dosing, each rat was placed into a metabolic chamber and urine output was collected hourly for 5 h. Urine output for each collection period was calculated as (urine + collection tube weight)-collection tube tare weight. The effect of test compound on total urine output was assessed by analysis of variance with repeated measures (subject within Group) and factors of Group and Time and their interaction, or one-way ANOVA, where appropriate. A univariate ANOVA was run only if a significant effect was observed following the multivariate ANOVA. Significance was assumed at $p < 0.05$ for the individual factors and $p < 0.1$ for their interaction.

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Supporting Information Available: Elemental analysis data for compounds **6**, **7a**,**b**, **9a**,**b**, and **10a**,**b** and HPLC traces for **8a** and **8b**. This material is available free of charge via the Internet at http://pubs.acs.org.

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