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Exploring the active site of phenylethanolamine *N*-methyltransferase with 1,2,3,4-tetrahydrobenz[*h*] isoquinoline inhibitors☆

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Abstract—1,2,3,4-Tetrahydrobenz[*h*]isoquinoline (THBQ, **11**) is a potent, inhibitor of phenylethanolamine *N*-methyltransferase (PNMT). Docking studies indicated that the enhanced PNMT inhibitory potency of **11** (hPNMT K_i = 0.49 μM) versus 1,2,3,4-tetrahydroisoquinoline (**5**, hPNMT K_i = 5.8 μM) was likely due to hydrophobic interactions with Val53, Met258, Val272, and Val269 in the PNMT active site. These studies also suggested that the addition of substituents to the 7-position of **11** that are capable of forming hydrogen bonds to the enzyme could lead to compounds (**14**–**18**) having enhanced PNMT inhibitory potency. However, these compounds are in fact less potent at PNMT than **11**. Furthermore, 7-bromo-THBQ (**19**, hPNMT K_i = 0.22 mM), which has a lipophilic 7-substituent that cannot hydrogen bond to the enzyme, is twice as potent at PNMT than **11**. This once again illustrates the limitations of docking studies for lead optimization.

1. Introduction

The role of epinephrine as a hormone in the sympathetic nervous system was first described by Walter Cannon in 1914.[2] An acute stress response triggers the release of epinephrine from the adrenal gland, which initiates the physiological changes associated with fight–flight syndrome (FFS). In contrast to the vast understanding of the functions of epinephrine relating to FFS, its role in the mammalian central nervous system (CNS), where it accounts for approximately 5% of the total CNS catecholamine content,[3–5] remains unclear.[6] Primarily on the basis of CNS localization, epinephrine neurons are thought to be involved in the regulation of blood pressure, respiration, and body temperature,[7] the secretion of hormones from the pituitary gland,[8] the regulation of α_2-adrenoceptors in the hypothalamus,[9] and some of the neurodegeneration seen in Alzheimer's disease.[10–12] As one approach to elucidate the role(s) of epinephrine in the CNS, our laboratory has targeted phenylethanolamine *N*-methyltransferase (PNMT; EC 2.1.1.28). This enzyme catalyzes the terminal step in the biosynthesis of epinephrine (Fig. 1), in which a methyl group is transferred from *S*-adenosyl-L-methionine (AdoMet; **3**) to the primary amine of norepinephrine (**1**) to form epinephrine (**2**) and the cofactor product *S*-adenosyl-L-homocysteine (AdoHcy; **4**).

Compounds based on the 1,2,3,4-tetrahydroisoquinoline (THIQ, **5**, Table 1) nucleus have been found to be some of the most potent inhibitors of PNMT reported, particularly those compounds having electron-withdrawing substituents in the 7-position (**6**–**10**).[13] SK&F 64139[14] (**6**) and SK&F 29661[15] (**7**) are two of the most well-studied inhibitors of PNMT, but the former is nonselective

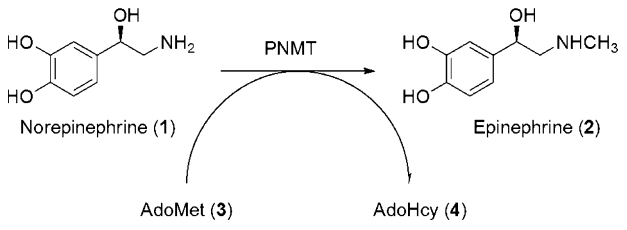

Figure 1. The terminal step in epinephrine (**2**) biosynthesis is the transfer of a methyl group from AdoMet (**3**) to norepinephrine (**1**) to form epinephrine (**2**) and the cofactor product AdoHcy (**4**).

Keywords: Phenylethanolamine *N*-methyltransferase; Enzyme inhibitors; 1,2,3,4-Tetrahydrobenz[*h*]isoquinoline; Structure-based design.
☆ Ref. 1.
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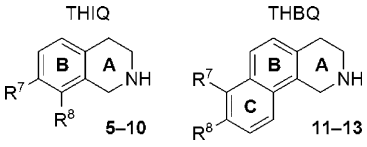
Table 1. In vitro human PNMT (hPNMT) and α_2-adrenoceptor affinity of some PNMT inhibitors



Compound	R^7	R^8	K_i (μM) ± SEM[a]		Selectivity α_2/hPNMT	*C*log *P*[c]
			hPNMT	α_2[b]		
5[d]	H	H	5.8 ± 0.5	0.35 ± 0.11	0.060	1.60
6[e]	Cl	Cl	0.0031 ± 0.0006[f]	0.021 ± 0.005	6.8	2.90
7[g]	SO_2NH_2	H	0.28 ± 0.02[f]	100 ± 10	360	–0.24
8[h]	NO_2	H	0.12 ± 0.01	4.3 ± 0.3	36	1.34
9[h]	CN	H	1.5 ± 0.1	7.3 ± 0.2	4.9	1.03
10[h]	Br	H	0.056 ± 0.003	0.23 ± 0.13	77	2.46
11[i,j]	H	H	0.49 ± 0.05	0.016 ± 0.002	0.033	2.77
12[i,k]	H	OH	1.4 ± 0.1	0.078 ± 0.002	0.056	2.10
13[i,k]	H	OMe	2.3 ± 0.3	0.84 ± 0.03	0.37	2.69

[a] Standard error of the mean.
[b] In vitro activities for the inhibition of [^{3}H]clonidine binding to the α_2-adrenoceptor.
[c] Calculated log *P*.
[d] Ref. 49.
[e] Ref. 14.
[f] Ref. 42.
[g] Ref. 15.
[h] Ref. 13.
[i] The data for these compounds were originally reported for the inhibition of bovine PNMT.
[j] Ref. 25.
[k] Ref. 26.

due to significant affinity for the α_2-adrenoceptor,[16] while the latter is selective for PNMT, but does not cross the blood–brain barrier (BBB),[15] most likely due to the high polarity of the 7-aminosulfonyl substituent. According to an in vitro BBB model,[17,18] the apparent minimum lipophilicity (*C*log *P*) for partial permeability through the BBB is 0.13–0.57 for THIQs.[19] A potent inhibitor of PNMT, which exhibits minimal affinity for the α_2-adrenoceptor and is able to cross the BBB, would be a useful pharmacological tool for defining the role(s) of epinephrine in the CNS.

Analysis of the crystal structure of human PNMT (hPNMT) co-crystallized with inhibitors[20–23] and substrates,[24] coupled with site-directed mutagenesis studies,[22] has helped to identify the key amino acids within the hPNMT active site, some of which are shown in Figures 2 and 3. Examination of the crystal structure of hPNMT in complex with **6** and **4** (hPNMT·**6**·**4**, Fig. 2) indicated that the increased inhibitory potency of **6** versus **5** was due to hydrophobic interactions between the 7-chloro and Val53, the 8-chloro and Val269 and Val272, and both the 7- and 8-chloro with Met258,[22] while a comparable study of hPNMT in complex with **7** and **4** (hPNMT·**7**·**4**, Fig. 3) indicated that the increased inhibitory potency of **7** versus **5** was due to hydrogen bonds between both sulfonamide oxygens of **7** and the side chain of Lys57.[20] Also, water-mediated hydrogen bonds are indicated between one of the sulfonamide oxygens of **7** and the main-chain carbonyl oxygen of Arg44 and the side chains of Tyr126 and Lys57.[20] Additional crystallography studies have also indicated the presence of a hydrogen bond acceptor, the main-chain carbonyl oxygen of Asn39, adjacent to the 7-position of THIQ.[23]

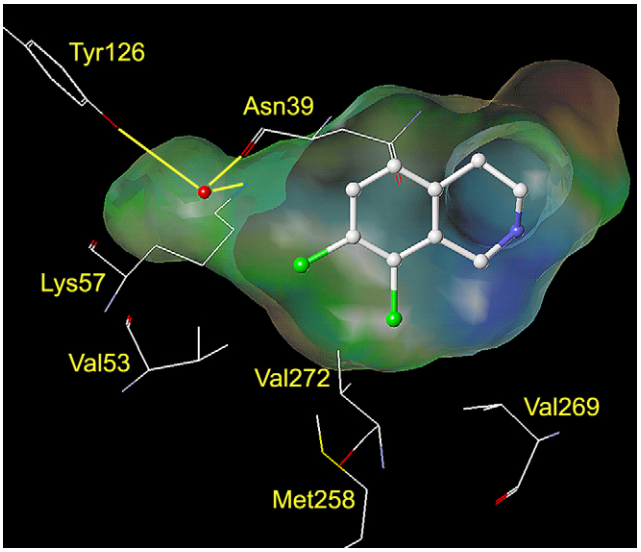


Figure 2. This figure shows the amino acids (carbon is white, nitrogen is blue, oxygen is red, chlorine is green, and sulfur is yellow) interacting with SK&F 64139 (**6**) at the active site of hPNMT.[22,48] A Connolly (solvent accessible) surface exposing **6** is shown and a lipophilic potential is mapped on the Connolly surface whereby the areas shown in green are neutral, blue are hydrophilic, and brown are lipophilic. The brown lipophilic region of the Connolly surface adjacent to Val53, Met258, Val272, and Val269 is not easily observed, as it is perpendicular to the plane of the page. Water mediated hydrogen bonds are shown in yellow. Hydrogens are not shown for clarity.

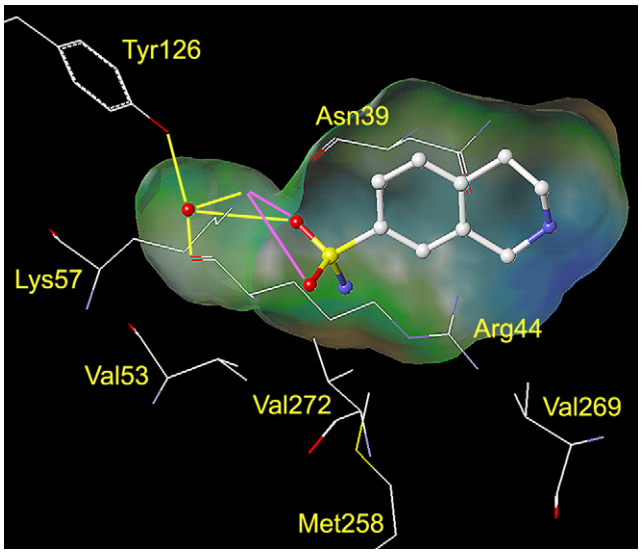


Figure 3. This figure shows the amino acids (carbon is white, nitrogen is blue, oxygen is red, and sulfur is yellow) interacting with SK&F 29661 (**7**) at the active site of hPNMT.[20,48] A Connolly (solvent accessible) surface exposing **7** is shown and indicates the presence of a binding pocket adjacent to the sulfonamide group of **7**. A lipophilic potential is mapped on the Connolly surface whereby the areas shown in green are neutral, blue are hydrophilic, and brown are lipophilic. The brown lipophilic region of the Connolly surface adjacent to Val53, Met258, Val272, and Val269 is not easily observed, as it is perpendicular to the plane of the page. Hydrogen bonds between the sulfonamide oxygens of **7** and Lys57 are shown in magenta. Water mediated hydrogen bonds between one of the sulfonamide oxygens, the side chain of Tyr126, the main-chain carbonyl oxygen of Arg44, and the side chain of Lys57 are shown in yellow. Hydrogens are not shown for clarity.

Prior to obtaining the crystal structure of hPNMT, the 10-fold increase in PNMT inhibitory potency of 1,2,3,4-tetrahydrobenz[*h*]isoquinoline (THBQ, **11**, Table 1) versus **5** was attributed to interactions of the additional aromatic ring (ring C, Table 1), which likely bound in the same area in the active site as the 7- and 8-chloro groups of **6**.[25] On the basis of the PNMT inhibitory potency of lead compound **11**, 8-substituted-THBQs **12** and **13** were prepared and evaluated in order to mimic some of the apparent interactions of the sulfonamide of **7** with PNMT.[26] Compounds **12** and **13** were found to be slightly less potent than parent compound **11** suggesting that they did not form additional favorable interactions with PNMT.

When the X-ray crystal structures of hPNMT·**6**·**4** and hPNMT·**7**·**4** became available, docking studies with **11**–**13** (see Section 4.2) indicated that the 8-position was likely not the ideal site to add substituents that could form hydrogen bonds to the enzyme and that substituents on the 7-position of **11** would be more likely to form hydrogen bonding interactions with the enzyme (see Section 4.3). Thus, a series of 7-substituted-THBQs (**14**–**18**, Section 4.1, Table 2) having functional groups in the 7-position that could act as either hydrogen bond donors or acceptors were prepared and evaluated at hPNMT and the α_2-adrenoceptor. In addition, **19**, which has a lipophilic electron-withdrawing substituent, was included in this series.

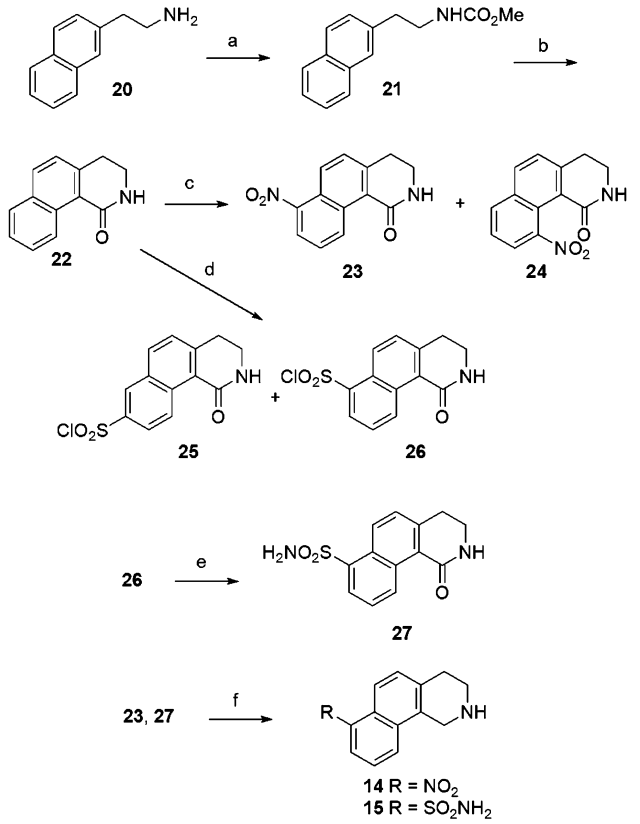
Compound **11** is a nonselective inhibitor of PNMT due to significant affinity for the α_2-adrenoceptor. THIQs having hydrophilic electron-withdrawing 7-substituents (e.g., **7**–**9**) have reduced affinity for the α_2-adrenoceptor.[13] If 7-substituted-THBQs bind to the α_2-adrenoceptor in a similar manner as 7-substituted-THIQs, the proposed THBQs having hydrophilic electron-withdrawing 7-substituents (**14**, **15**, and **18**) would likely show reduced affinity for the α_2-adrenoceptor in comparison to **11**, thus increasing their overall selectivity. In addition, some THIQs having hydrophilic 7-substituents (e.g., **7**) are not able to penetrate the BBB.[19] Since the THBQ scaffold ($C\log P = 2.77$) is more lipophilic than the THIQ scaffold ($C\log P = 1.60$), the proposed THBQs are more likely to cross the BBB than similarly substituted THIQs.

2. Chemistry

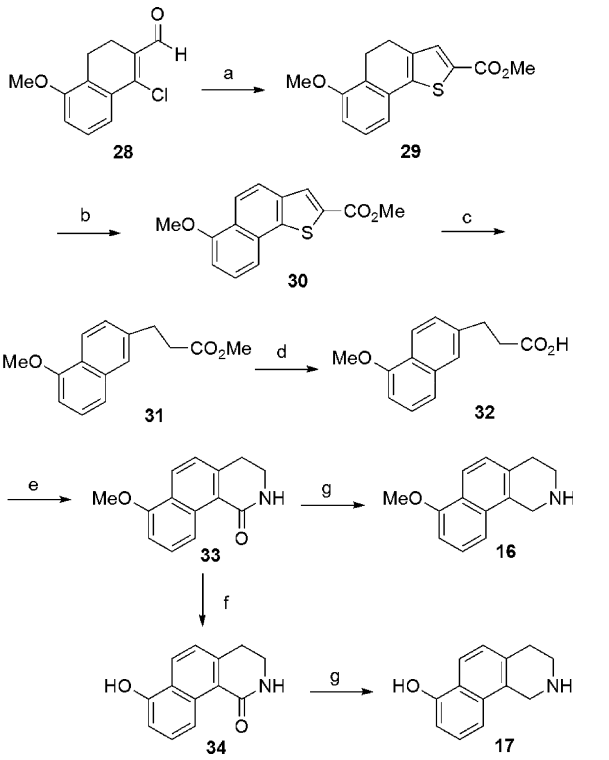
The synthesis of THBQs **14** and **15** (Table 2) is shown in Scheme 1. 2-(Naphthylen-2-yl)ethanamine (**20**) was converted to carbamate **21** with methylchloroformate and pyridine. Cyclization of **21** was accomplished under dehydrating conditions with polyphosphoric acid to form **22**.[27] Treatment of **22** with KNO_3 in H_2SO_4 yielded a mixture of mononitro- and dinitro-3,4-dihydrobenz[*h*]isoquinolin-1(2*H*)-ones. The major monosubstituted products, **23** and **24**, were isolated via flash chromatography. The structures of these two regioisomers were confirmed by X-ray crystallography. Treatment of **22** with chlorosulfonic acid yielded a mixture of monosubstituted regioisomers, from which **25** and **26** were isolated as the major products. The structures of these two regioisomers were also confirmed by X-ray crystallography.[28] Compound **25** was converted to aminosulfonyl **27** with ammonium hydroxide in acetonitrile. Compounds **23** and **27** were reduced with diborane to yield THBQs **14** and **15**, respectively.

The synthesis of THBQs **16** and **17** (Table 2) is shown in Scheme 2. 1-Chloro-5-methoxy-3,4-dihydronaphthalene-2-carboxaldehyde (**28**) was prepared from 5-methoxytetralone according to a known procedure.[29] Treatment of **28** with methyl thioglycolate, TEA, and pyridine, followed by KOH (aq), formed cyclized product **29**.[30] Oxidation of **29** with DDQ yielded **30**.[30] Desulfurization with Raney Nickel[31] afforded ester **31**, which was hydrolyzed with LiOH to form acid **32**. The intermediate isocyanate formed by a Curtius rearrangement of the acid chloride of **32** was cyclized with $AlCl_3$ to yield **33**.[32] Cleavage of the methyl ether of **33** with HBr/AcOH afforded **34**. Lactams **33** and **34** were reduced with diborane to yield THBQs **16** and **17**, respectively.

The synthesis of THBQs **18** and **19** (Table 2) is shown in Scheme 3. Compound **34** was reacted with $PhNTf_2$ and TEA to afford **35**. Aryl triflate **35** was reduced to **36** with diborane. Workup with acetic acid was required because



Scheme 1. Reagents: (a) $ClCO_2Me$, pyridine, CH_2Cl_2; (b) polyphosphoric acid; (c) H_2SO_4, KNO_3; (d) chlorosulfonic acid; (e) NH_4OH, CH_3CN; (f) BH_3·THF.



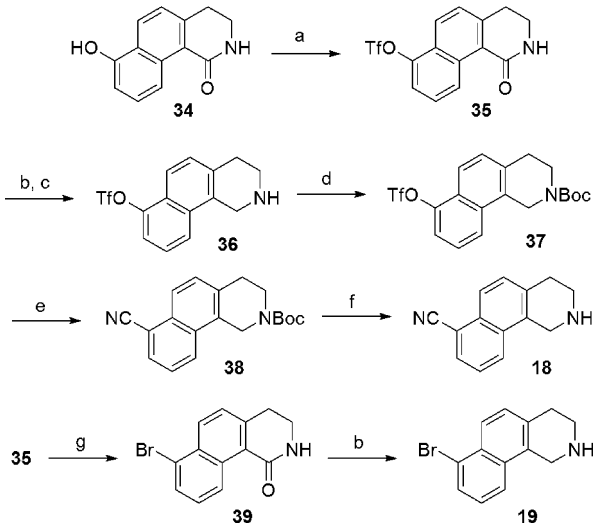
Scheme 2. Reagents and condition: (a) methyl thioglycolate, TEA, pyridine; then KOH; (b) DDQ; (c) Raney Nickel; (d) LiOH; (e) $(COCl)_2$; then NaN_3; then toluene (reflux); then $AlCl_3$; (f) HBr, AcOH; (g) BH_3·THF.

standard diborane workup conditions (methanol/HCl) led to cleavage of the triflate group.[33] Boc protection of **36** followed by microwave-promoted palladium-catalyzed cyanation afforded **38**,[34] which was deprotected with trifluoroacetic acid to afford **18**. Attempts to convert **34** directly to aryl bromide **39** using Ph_3PBr_2[35,36] were unsuccessful, thus an alternative route was employed. Aryl triflate **35** was converted to the trimethyltin intermediate with hexamethylditin[37] and then reacted with NBS[38] to yield **39**, which was subsequently reduced with diborane to afford **19**.

3. Biochemistry

The PNMT inhibitory potency for compounds **11**–**13** was originally determined using bovine PNMT. The hPNMT inhibitory potency for these compounds is now reported for comparison purposes. In the current study, human PNMT (hPNMT) with a C-terminal hexahistidine tag was expressed in *Escherichia coli*.[39,40] The radiochemical assay conditions, previously reported for the bovine enzyme,[41] were modified to account for the high binding affinity of some inhibitors.[39,42] Inhibition constants were determined using four concentrations of phenylethanolamine as the variable substrate, and three concentrations of inhibitor.

α_2-Adrenergic receptor binding assays were performed using cortex obtained from male Sprague–Dawley rats.[43] [^{3}H]Clonidine was used as the radioligand to define the specific binding and phentolamine was used to define the nonspecific binding. Clonidine was used as the ligand to define α_2-adrenergic binding affinity to simplify the comparison with previous results.[44]



Scheme 3. Reagents: (a) $PhNTf_2$, TEA; (b) BH_3·THF; (c) AcOH, H_2O; (d) Boc_2O, TEA; (e) $Zn(CN)_2$, $Pd(Ph_3P)_4$; (f) trifluoroacetic acid; (g) Sn_2Me_6, $Pd(Ph_3P)_4$, LiCl; then NBS.

4. Results and discussion

4.1. Biochemical evaluation of 7-substituted-THBQs

The biochemical data for 7-substituted-THBQs **14**–**19** are shown in Table 2. Contrary to the predictions from docking studies, the addition of a 7-substituent (NO_2, SO_2NH_2, OMe, OH, or CN, **14**–**18**) to THBQ (**11**) that could potentially form hydrogen bonds with the enzyme did not lead to an increase in PNMT inhibitory potency. In comparison to parent compound **11**, THBQs **14** (NO_2) and **17** (OH) are 2-fold less potent at hPNMT, THBQs **16** (OMe) and **18** (CN) are 4-fold less potent, and THBQ **15** (SO_2NH_2) is almost 70-fold less potent. Unexpectedly, 7-bromo-THBQ, which has a lipophilic 7-substituent, is 2-fold more potent as an inhibitor of PNMT than is **11**. As anticipated, THBQs having hydrophilic electron-withdrawing 7-substituents (**14**, **15**, and **18**) show a significant reduction in affinity for the α_2-adrenoceptor (25-, 180-, and 37-fold, respectively) versus **11**. In the following sections, docking studies using the X-ray crystal structures of hPNMT·**6**·**4** and hPNMT·**7**·**4**, and SAR analysis of the α_2-adrenoceptor are used to explain these data.

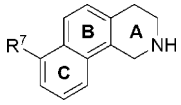
4.2. hPNMT docking studies with 8-substituted-THBQs

Docking studies (AutoDock 3.0)[45] with THBQ **11** (Table 1) indicate that, similarly to **6**, its enhanced inhibitory potency as compared to that of **5** is likely due to favorable hydrophobic interactions with Val53, Met258, Val272, and Val269 (Fig. 4A). Interactions between the amine group of THIQ inhibitors and Glu219 are known to play a key role in their binding to hPNMT.[20–22] The amine group of **11** is predicted to interact with Glu219 in the same manner as that of **6**. According to the docking studies with **11**, it is apparent that, in order for 8-substituted-THBQs to bind in the active site of hPNMT, a significant shift of Val53 away from the 8-substituent or a shift of the inhibitor in the active site would be required. Docking studies with 8-hydroxy-THBQ (**12**, Fig. 4B) illustrate this point. Compound **12** is predicted to twist in the active site relative to **11**, which shifts the C ring of **12** away from Val53, Met258, Val272, and Val269. The reduced ability of the C ring of **12** to participate in hydrophobic interactions with Val53, Met258, Val272, or Val269 is consistent with the observed reduction in potency of **12** (hPNMT K_i = 1.4 μM) versus **11** (hPNMT K_i = 0.49 μM). Similar results were obtained from docking studies with 8-methoxy-THBQ **13** (results not shown, hPNMT K_i = 2.3 μM). Docking studies with **12** (or **13**) indicate that the phenol (or methoxy) group could interact with Lys57 or Asn39 through hydrogen bonds. However, if these interactions are taking place, their benefit is apparently counteracted by the loss of the hydrophobic interactions with Val53, Met258, Val272, or Val269.

4.3. hPNMT docking studies with 7-substituted-THBQs

Docking studies using both hPNMT·**6**·**4** (Fig. 4B) and hPNMT·**7**·**4** (not shown) with 7-substituted-THBQs **14**–**19** (Table 2) showed that the 7-substituents of these compounds could occupy an auxiliary binding pocket. It is predicted that 7-substituted-THBQs would need to twist in the active site relative to **11**, which would shift the C ring of THBQ away from Val53, Met258, Val272, and Val269, but to a lesser degree than the 8-substituted-THBQs (e.g., **12**, Fig. 4B). These docking studies also suggested that 7-substituted-THBQs could maintain these hydrophobic interactions while simultaneously forming hydrogen bonding interactions with Lys57 or Asn39, resulting in compounds having enhanced PNMT inhibitory potency versus **11**. This does not appear to be the case, however, as the THBQs having 7-substituents that could act as either hydrogen bond acceptors (**14**, **16**, and **18**) or as both hydrogen bond donors and acceptors (**15** and **17**) were less potent at PNMT than **11**. Similarly to **12** and **13**, if hydrogen bonding interactions are taking place, their benefit is apparently offset by the loss of the hydrophobic interactions of the THBQ C ring with Val53, Met258, Val272, or Val269. 7-Bromo-THBQ (**19**), which is the only analogue in this series having a lipophilic 7-substituent, is twice as potent at PNMT than **11**. This is possibly due to hydrophobic interactions between the bromine of **19** and the aliphatic portion of the side chain of Arg44. Such favorable hydrophobic interactions would not be expected to occur between Arg44 and the hydrophilic 7-substituents of **14**–**18**.

Table 2. In vitro human PNMT (hPNMT) and α_2-adrenoceptor affinity of some PNMT inhibitors



Compound	R^7	K_i (μM) ± SEM[a]		Selectivity α_2/hPNMT	*C*log *P*[c]
		hPNMT	α_2[b]		
14	NO_2	0.90 ± 0.10	0.41 ± 0.04	0.46	2.51
15	SO_2NH_2	33 ± 3	2.9 ± 0.3	0.088	0.93
16	OMe	2.1 ± 0.2	0.16 ± 0.02	0.76	2.69
17	OH	0.91 ± 0.06	0.011 ± 0.001	0.012	2.10
18	CN	2.3 ± 0.2	0.59 ± 0.06	0.26	2.20
19	Br	0.22 ± 0.03	0.10 ± 0.01	0.45	3.63

[a] Standard error of the mean.
[b] In vitro activities for the inhibition of [^{3}H]clonidine binding to the α_2-adrenoceptor.
[c] Calculated log *P*.

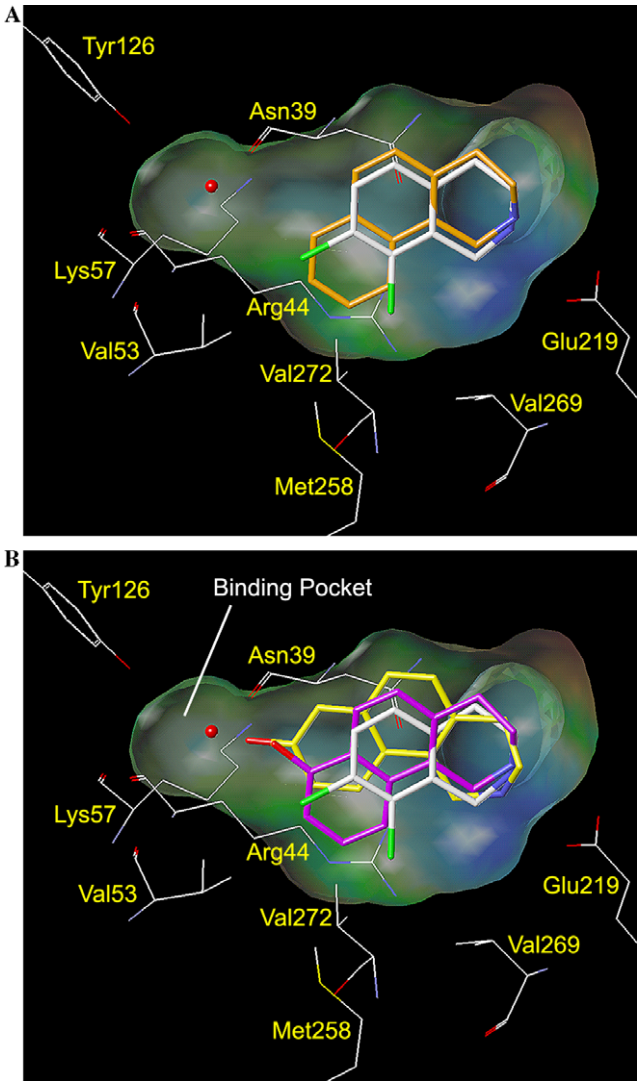
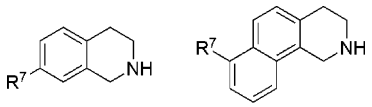


Figure 4. This figure shows the amino acids (carbon is white, nitrogen is blue, oxygen is red, chlorine is green, and sulfur is yellow) interacting with SK&F 64139 (**6**, carbons are shown in white) at the active site of hPNMT.[22,48] A Connolly (solvent accessible) surface exposing **6** is shown and a lipophilic potential is mapped on the Connolly surface whereby the areas shown in green are neutral, blue are hydrophilic, and brown are lipophilic. The brown lipophilic region of the Connolly surface adjacent to Val53, Met258, Val272, and Val269 is not easily observed, as it is perpendicular to the plane of the page. Hydrogens are not shown for clarity. (A, top) shows **11** (carbons are shown in orange) docked into the active site of hPNMT. The C ring of **11** is predicted to occupy the same area in the active site as the 7- and 8-chloro groups of **6**, thus forming favorable hydrophobic interactions with Val53, Met258, Val272, and Val269. (B, bottom) shows **12** (carbons are shown in yellow) and **17** (carbons are shown in purple) docked into the active site of hPNMT. Compounds **12** and **17** are predicted to twist in the active site relative to **11**, which shifts the C rings of **12** and **17** away from Val53, Met258, Val272, and Val269. Additional docking studies showed that the THBQ nucleus of **13** overlays with that of **12** and the THBQ nuclei of **14**–**16**, **18**, and **19** overlay with that of **17**. Thus, the 8-methoxy group of **13** is predicted to occupy the same area in the active site as the 8-hydroxy group of **12**, and the 7-substituents of **14**–**16**, **18**, and **19** are predicted to occupy the same area in the active site as the 7-hydroxy group of **17**.

Table 3. Effects of 7-substituents on THIQ (**5**) and THBQ (**11**) α_2-adrenoceptor affinity



Compound	R^7	$\alpha 2$[a] K_i (μM) ± SEM[b]	Ratio
8	NO_2	4.3 ± 0.3	12[c]
9	CN	7.3 ± 0.2	21[c]
7	SO_2NH_2	100 ± 10	290[c]
10	Br	0.23 ± 0.13	0.66[c]
14	NO_2	0.41 ± 0.04	26[d]
18	CN	0.59 ± 0.06	37[d]
15	SO_2NH_2	2.9 ± 0.3	180[d]
19	Br	0.10 ± 0.01	6.3[d]

[a] In vitro activities for the inhibition of [^{3}H]clonidine binding to the α_2-adrenoceptor.
[b] Standard error of the mean.
[c] Ratio of α_2-adrenoceptor affinity versus the unsubstituted (R^7 = H) parent compound (**5**, α_2 K_i = 0.35).
[d] Ratio of α_2-adrenoceptor affinity versus the unsubstituted (R^7 = H) parent compound (**11**, α_2 K_i = 0.016).

4.4. α_2-Adrenoceptor binding studies

THIQs having hydrophilic electron-withdrawing 7-substituents, such as **7**–**9**, have considerably less affinity for the α_2-adrenoceptor than unsubstituted THIQ **5** (Table 3).[13] Similarly, THBQs having hydrophilic electron-withdrawing 7-substituents (**14**, **15**, and **18**) have less affinity for the α_2-adrenoceptor than **11**. For the nitro, cyano, and aminosulfonyl THIQ and THBQ derivatives, the relative α_2-adrenoceptor affinity versus the parent compounds is very similar. Also, the rank order of affinity (nitro > cyano > aminosulfonyl) is the same. These observations suggest that the 7-substituents of these THBQs are occupying the same area of the α_2-adrenoceptor binding site as 7-substituents of THIQ. THIQs having lipophilic 7-substituents, such as **6** and 7-bromo-THIQ (**10**), have more affinity for the α_2-adrenoceptor than **5**. It was thus expected that **19** would have more affinity for the α_2-adrenoceptor than **11**. However, **19** has 6-fold less affinity for the α_2-adrenoceptor than **11**, indicating that lipophilic 7-substituents of THBQ are likely not occupying the same area of the α_2-adrenoceptor binding site as lipophilic 7-substituents of THIQ. Although **14**, **15**, **18**, and **19** have less affinity for the α_2-adrenoceptor than **11**, they nonetheless maintain sub-micromolar to low micromolar affinity for this target and are thus nonselective for PNMT.

5. Conclusions

Although docking studies suggested that the addition of substituents to the 7-position of **11** that are capable of forming hydrogen bonds to the enzyme could lead to compounds having enhanced PNMT inhibitory potency, these compounds (**14**–**18**) were in fact less potent than **11** at PNMT, illustrating the limitations of docking

studies for the optimization of lead compounds.[46] Compound **19**, which has a lipophilic 7-substituent, is twice as potent as **11** at PNMT, but is nonselective due to significant affinity for the α_2-adrenoceptor. The co-crystallization of one or more of these THBQs with hPNMT would be required to determine the exact nature of their binding interactions.

6. Experimental

6.1. General methods

All reagents and solvents were of reagent grade or were purified by standard methods before use. Melting points were determined in open capillary tubes on a Thomas–Hoover melting point apparatus calibrated with known compounds. Proton (^{1}H NMR) and carbon (^{13}C NMR) nuclear magnetic resonance spectra were taken on a Bruker DRX-400, Bruker AM-500, or Bruker AV-800 spectrophotometer. High-resolution mass spectra (HRMS) were obtained on a Ribermag R 10-10 mass spectrophotometer. Thin-layer chromatography (TLC) was performed on K6F silica gel 60 Å (Whatman) glass-backed plates. Flash chromatography was performed using silica gel 60 (230–400 mesh) supplied by Universal Adsorbents, Atlanta, Georgia.

Anhydrous methanol and ethanol were used unless stated otherwise, and were prepared by distillation over magnesium. Other solvents were routinely distilled prior to use. Anhydrous tetrahydrofuran (THF) and diethyl ether (Et_2O) were distilled from sodium-benzophenone ketyl. Hexanes refer to the mixture of hexane isomers (bp 40–70 °C) and brine refers to a saturated solution of NaCl. All reactions that required anhydrous conditions were performed under a positive nitrogen or argon flow, and all glassware was either oven-dried or flame-dried before use. [methyl-^{3}H]AdoMet and [^{3}H]clonidine were obtained from Perkin-Elmer (Boston, MA).

6.2. Radiochemical assay of PNMT inhibitors

A typical assay mixture consisted of 25 μL of 0.5 M phosphate buffer (pH 8.0), 25 μL of 50 μM unlabeled AdoMet, 5 μL of [methyl-^{3}H]AdoMet, containing approximately 3×10^5 dpm (specific activity approximately 15 Ci/mmol), 25 μL of substrate solution (phenylethanolamine), 25 μL of inhibitor solution, 25 μL of enzyme preparation (containing 30 ng hPNMT and 25 μg of bovine serum albumin), and sufficient water to achieve a final volume of 250 μL. After incubation for 30 min at 37 °C, the reaction mixture was quenched by addition of 250 μL of 0.5 M borate buffer (pH 10.0) and was extracted with 2 mL of toluene/isoamyl alcohol (7:3). A 1 mL portion of the organic layer was removed, transferred to a scintillation vial, and diluted with cocktail for counting. The mode of inhibition was ascertained to be competitive in all cases reported in Tables 1 and 2 by examination of the correlation coefficients (r^2) for the fit routines as calculated in the Enzyme Kinetics module (version 1.1) in SigmaPlot (version 7.0).[32] While all K_i values reported were calculated using competitive kinetics, it should be noted that there was not always a great difference between the r^2 values for the competitive model versus the non-competitive model. All assays were run in duplicate with three inhibitor concentrations over a 5-fold range. K_i values were determined by a hyperbolic fit of the data using the Single Substrate—Single Inhibitor routine in the Enzyme Kinetics module (version 1.1) in SigmaPlot (version 7.0). For inhibitors with apparent IC_{50} values less than 0.1 μM (as determined by a preliminary screen of the compounds to be assayed), the Enzyme Kinetics Tight Binding Inhibition routine was used to calculate the K_i values.

6.3. α_2-Adrenoceptor radioligand binding assay

The radioligand receptor binding assay was performed according to the method of U'Prichard et al.[29] Male Sprague–Dawley rats were decapitated, and the cortexes were dissected out and homogenized in 20 volumes (w/v) of ice-cold 50 mM Tris/HCl buffer (pH 7.7 at 25 °C). Homogenates were centrifuged thrice for 10 min at 50,000*g* with resuspension of the pellet in fresh buffer between spins. The final pellet was homogenized in 200 volumes (w/v) of ice-cold 50 mM Tris/HCl buffer (pH 7.7 at 25 °C). Incubation tubes containing [^{3}H]clonidine (specific activity approximately 55 Ci/mmol, final concentration 2.0 nM), various concentrations of drugs, and an aliquot of freshly resuspended tissue (800 μL) in a final volume of 1 mL were used. Tubes were incubated at 25 °C for 30 min and the incubation was terminated by rapid filtration under vacuum through GF/B glass fiber filters. The filters were rinsed with three 5 mL washes of ice-cold 50 mM Tris buffer (pH 7.7 at 25 °C). The filters were counted in vials containing premixed scintillation cocktail. Non-specific binding was defined as the concentration of bound ligand in the presence of 2 μM phentolamine. All assays were run in quadruplicate with five inhibitor concentrations over a 16-fold range. IC_{50} values were determined by a log-probit analysis of the data and K_i values were determined by the equation $K_i = IC_{50}/(1 + [\text{Clonidine}]/K_D)$, as all Hill coefficients were approximately equal to 1.

6.4. Molecular modeling

Connolly surfaces were generated in SYBYL® on a Silicon Graphics Octane workstation.[47] Docking of the various inhibitors into the PNMT active site was performed using AutoDock 3.0.[45] The default settings for AutoDock 3.0 were used. The compound to be docked was initially overlayed with the co-crystallized ligand and minimized with the Tripos force field.

6.5. Synthesis

6.5.1. Methyl 2-naphthylethylcarbamate (21). 2-Naphthyleneethylamine (**20**, 1.60 g, 9.34 mmol) was dissolved in dry CH_2Cl_2 (50 mL) and pyridine (5 mL), and the solution was stirred at 0 °C. Methyl chloroformate (0.87 mL, 11 mmol) was added dropwise and the solution was stirred overnight at ambient temperature. Ice-cold water (40 mL) was added and the mixture was

stirred for 30 min. The organic phase was removed, washed with 3 N HCl (3× 30 mL) and brine (30 mL), and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure to yield the crude product as a yellow oil, which was purified by flash chromatography eluting with hexanes/EtOAc (4:1) to yield carbamate **21** as a white solid (1.55 g, 6.76 mmol, 72%): mp 95–97 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.85–7.81 (m, 2H), 7.66 (s, 1H), 7.52–7.45 (m, 2H), 7.37–7.34 (m, 1H), 4.72 (b, 1H), 4.26 (b, 1H), 3.68 (s, 3H), 3.59–3.54 (m, 2H), 3.00 (m, J = 6.8 Hz, 2H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 157.0, 136.2, 133.5, 132.3, 128.4, 127.7, 127.5, 127.2, 127.1, 126.2, 125.6, 52.1, 42.1, 36.3; HRMS (ESI$^+$) *m*/*z* calcd for $C_{14}H_{16}NO_2$ (MH^+) 230.1181, obsd 230.1179.

6.5.2. 3,4-Dihydrobenz[*h*]isoquinolin-1(2*H*)-one (22). Polyphosphoric acid (75 g) was heated to 120 °C and carbamate **21** (3.00 g, 15.2 mmol) was added. After stirring for 45 min, the reaction mixture was poured into ice water (100 mL) and stirred vigorously for 5 min. This aqueous mixture was extracted with CH_2Cl_2 (3× 75 mL). The combined organic extracts were washed with brine (50 mL) and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure to yield an off-white solid, which was purified by flash chromatography eluting with EtOAc/hexanes (1:1) to yield lactam **22** as a white solid (1.80 g, 9.13 mmol, 60%): mp 158–160 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.42 (d, J = 8.7 Hz, 1H), 7.94 (d, J = 8.3 Hz, 1H), 7.85 (d, J = 8.1 Hz, 1H), 7.65–7.62 (m, 1H), 7.53–7.50 (m, 1H), 7.33 (d, J = 8.3 Hz, 1H), 7.17 (b, 1H), 3.59 (t, J = 6.6 Hz, 2H), 3.13 (t, J = 6.6 Hz, 2H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 167.4, 140.3, 133.6, 133.2, 132.1, 128.6, 128.3, 127.1, 126.1, 125.8, 124.3, 39.8, 30.9; HRMS (ESI$^+$) *m*/*z* calcd for $C_{13}H_{12}NO$ (MH^+) 198.0919, obsd 198.1924.

6.5.3. 7-Nitro-3,4-dihydrobenz[*h*]isoquinolin-1(2*H*)-one (23) and 10-nitro-3,4-dihydrobenz[*h*]isoquinolin-1(2*H*)-one (24). Lactam **22** (300 mg, 1.52 mmol) was dissolved in concentrated H_2SO_4 (5 mL) and stirred at 0 °C. KNO_3 (115 mg, 1.14 mmol) was added in small portions over the course of 15 min. The reaction continued for 1 h at 0 °C and then was poured slowly onto ice (50 g). The aqueous mixture was extracted with CH_2Cl_2 (4× 40 mL) and the combined organic extracts were washed with brine and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure to yield an off-white solid, which, according to ^{1}H NMR (crude), consisted of unreacted starting material and a mixture of numerous mono- and di-substituted nitration products. Purification by flash chromatography eluting with hexanes/2-propanol (4:1) yielded unreacted starting material (70 mg, 0.35 mmol, 23%, R_f = 0.45 in 4:1 hexanes/2-propanol), the desired product, lactam **23** (R_f = 0.30 in 4:1 hexanes/2-propanol), and the undesired regioisomer, lactam **24** (R_f = 0.25 in 4:1 hexanes/2-propanol), as the major products. Compound **23** was recrystallized from $CHCl_3$/hexanes to yield yellow needles (45 mg, 0.19 mmol, 12%): mp 184–186 °C; ^{1}H NMR (800 MHz, $CDCl_3$) δ 9.82 (d, J = 8.7 Hz, 1H), 8.61 (d, J = 8.8 Hz, 1H), 8.19 (d, J = 7.5 Hz, 1H), 7.70–7.68 (m, 1H), 7.59 (d, J = 8.9 Hz, 1H), 6.30 (b, 1H), 3.64–3.62 (m, 2H), 3.20 (t, J = 6.5 Hz, 2H); ^{13}C NMR (800 MHz, $CDCl_3$) δ 165.9, 147.1, 141.1, 133.2, 132.5, 128.7, 126.0, 124.8, 124.4, 123.3, 39.2, 30.3; HRMS (ESI$^+$) *m*/*z* calcd for $C_{13}H_{11}N_2O_3$ (MH^+) 243.0770, obsd 243.0773. Compound **24** was recrystallized from CH_2Cl_2/hexanes to yield yellow needles (38 mg, 0.16 mmol, 10%): mp dec 297–299 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.14–8.12 (m, 1H), 8.07–8.05 (m, 1H), 7.98 (d, J = 8.4 Hz, 1H), 7.58–7.55 (m, 1H), 7.48 (d, J = 8.4 Hz, 1H), 6.23 (b, 1H), 3.71–3.67 (m, 2H), 3.15 (t, J = 6.2 Hz, 2H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 164.7, 148.4, 142.4, 134.2, 133.2, 131.6, 126.8, 125.0, 124.9, 124.7, 122.1, 39.0, 30.8; HRMS (ESI$^+$) *m*/*z* calcd for $C_{13}H_{11}N_2O_3$ (MH^+) 243.0770, obsd 243.0759. The structures of **23** and **24** were confirmed by X-ray crystallography. See Section 7.

6.5.4. 8-Chlorosulfonyl-3,4-dihydrobenz[*h*]isoquinolin-1(2*H*)-one (25) and 7-chlorosulfonyl-3,4-dihydrobenz[*h*]isoquinolin-1(2*H*)-one (26). Lactam **22** (500 mg, 2.54 mmol) was treated with chlorosulfonic acid (10 mL) with stirring at 0 °C. The reaction mixture was permitted to warm to ambient temperature and the reaction was continued for 30 min. with stirring. The reaction mixture was cautiously added via pipet onto ice (200 mL). A white precipitate formed. The mixture was extracted with CH_2Cl_2 (4× 50 mL). The combined organic extracts were washed with brine and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure to yield a white solid, which consisted of a mixture of monosubstituted sulfonyl chloride regioisomers according to ^{1}H NMR (crude). Hot filtration with $CHCl_3$ yielded a white solid, which was further purified by recrystallization from CH_2Cl_2/hexanes to yield **25** (95 mg, 0.321 mmol, 13%) as white needles: mp 219–221 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.64 (d, J = 9.4 Hz, 1H), 8.49 (d, J = 2.0 Hz, 1H), 8.04–8.01 (m, 2H), 7.48 (d, J = 8.3 Hz, 1H), 7.48–7.43 (m, 2H), 6.35 (b, 1H), 3.57–3.54 (m, 2H), 3.14 (t, J = 6.5 Hz, 2H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 165.7, 144.3, 140.9, 134.5, 134.2, 131.7, 129.6, 128.7, 128.1, 124.4, 123.1, 39.2, 30.6; HRMS (ESI$^+$) *m*/*z* calcd for $C_{13}H_{11}ClNO_3S$ (MH^+) 296.0148, obsd 296.0152. The filtrate from the hot filtration was purified by flash chromatography eluting with hexanes/2-propanol (2:1) to yield the desired regioisomer as a white solid, which was further purified by recrystallization from CH_2Cl_2/hexanes to yield **26** (120 mg, 0.406 mmol, 16%) as white needles: mp dec 215–217 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.94 (d, J = 8.8 Hz, 1H), 8.97 (d, J = 8.8 Hz, 1H), 8.42–8.41 (m, 1H), 7.76–7.73 (m, 1H), 7.69 (d, J = 8.8 Hz, 1H), 6.25 (b, 1H), 3.67–3.63 (m, 2H), 3.24 (t, J = 6.5 Hz, 2H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 165.6, 141.4, 139.7, 135.9, 132.9, 129.2, 128.8, 128.2, 127.3, 125.7, 124.9, 39.2, 30.2; HRMS (ESI$^+$) *m*/*z* calcd for $C_{13}H_{11}ClNO_3S$ (MH^+) 296.0148, obsd 296.0152. The structures of **25** and **26**[28] were confirmed by X-ray crystallography. See Section 7.

6.5.5. 7-Aminosulfonyl-3,4-dihydrobenz[*h*]isoquinolin-1(2*H*)-one (27). Concd NH_4OH (8 mL) was added to a stirred solution of sulfonyl chloride **26** (50 mg,

0.169 mmol) in acetonitrile (4 mL). The reaction mixture was stirred for 16 h at ambient temperature. The solvent was removed under reduced pressure and the resulting residue was purified by flash chromatography eluting with acetone/hexanes (2:1) to yield lactam **27** as a white solid (37 mg, 0.13 mmol, 79%): mp 298–300 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.37 (d, J = 8.8 Hz, 1H), 8.55 (d, J = 8.8 Hz, 1H), 8.03 (br, 1H), 7.91–7.89 (m, 1H), 7.49 (br, 2H), 7.48–7.43 (m, 2H), 3.18–3.14 (m, 2H), 2.86 (t, J = 6.5 Hz, 2H); ^{13}C NMR (500 MHz, DMSO-d_6) δ 164.9, 141.0, 139.6, 131.8, 131.1, 128.6, 127.4, 127.1, 125.7, 125.5, 124.6, 38.1, 29.5; HRMS (ESI$^+$) m/z calcd for $C_{13}H_{13}N_2O_3S$ (MH^+) 277.0647, obsd 277.0648.

6.5.6. Methyl 6-methoxy-4,5-dihydronaphtho[1,2-*b*]thiophene-2-carboxylate (29). To a stirred solution of **28** (11.5 g, 51.8 mmol) and methyl thioglycolate (5.09 mL, 57.0 mmol) in pyridine (60 mL), was added triethylamine (10.5 mL, 75.1 mmol) dropwise. The reaction was continued for 1.5 h at 50 °C and then cooled to ambient temperature. An aqueous solution of 50% (w/w) KOH (15 mL) was added and the reaction mixture was stirred for an additional 20 min. The reaction mixture was poured over crushed ice (100 g) and then partitioned between CH_2Cl_2 (300 mL) and 2 M HCl (200 mL). The organic layer was removed and washed with 2 M HCl (2× 100 mL), 10% NaOH (100 mL), and brine (100 mL). The CH_2Cl_2 solution was dried over anhydrous Na_2SO_4 and the solvent was removed under reduced pressure to yield a yellow oil. This crude product was purified by flash chromatography eluting with hexanes/ether (5:1) to yield a white solid. Recrystallization from ether/hexanes gave **29** as white needles (12.8 g, 46.7 mmol, 90%): mp 107–109 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.59 (s, 1H), 7.21–7.18 (m, 1H), 7.07 (d, J = 7.7 Hz, 1H), 6.83 (d, J = 8.1 Hz, 1H), 3.88 (s, 3H), 3.86 (s, 3H), 2.96 (t, J = 7.9 Hz, 2H), 2.80 (t, J = 7.9 Hz, 2H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 163.0, 156.8, 143.2, 137.8, 133.8, 131.6, 130.0, 127.5, 123.7, 116.3, 110.5, 55.6, 52.1, 23.2, 20.9; HRMS (ESI$^+$) m/z calcd for $C_{15}H_{15}O_3S$ (MH^+) 275.0742, obsd 275.0729.

6.5.7. Methyl 6-methoxynaphtho[1,2-*b*]thiophene-2-carboxylate (30). Compound **29** (12.4 g, 45.2 mmol) and DDQ (11.3 g, 49.7 mmol) were dissolved in dry benzene (150 mL) and heated at reflux for 3.5 h. After cooling to ambient temperature, the resulting solution was purified by flash chromatography eluting with $CHCl_3$/hexanes/EtOAc (5:5:1) to yield **30** as a white solid. (11.8 g, 43.4 mmol, 96%): mp 119–121 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.13 (d, J = 9.2 Hz, 1H), 7.59 (s, 1H), 7.72 (d, J = 9.1 Hz, 1H), 7.64 (d, J = 8.2 Hz, 1H), 7.46–7.43 (m, 1H), 6.87 (d, J = 7.8 Hz, 1H), 3.96 (s, 3H), 3.90 (s, 3H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 163.2, 156.3, 140.9, 137.0, 132.3, 131.6, 129.6, 127.5, 123.4, 121.8, 120.1, 116.1, 105.7, 55.7, 52.5; HRMS (ESI$^+$) m/z calcd for $C_{15}H_{13}O_3S$ (MH^+) 273.0585, obsd 273.0582.

6.5.8. Methyl 3-(5-methoxynaphthalen-2-yl)propanoate (31). Compound **30** (5.80 g, 21.3 mmol) and Raney nickel were refluxed in MeOH (75 mL) for 18 h. The reaction mixture was filtered through Celite, which was washed with MeOH (2× 75 mL). The solvent was removed under reduced pressure and the resulting oil was redissolved in EtOAc (150 mL). This organic solution was washed with saturated $NaHCO_3$ (75 mL) and brine (50 mL), and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure and the crude product was purified by flash chromatography eluting with hexanes/EtOAc (8:1) to yield **31** as a clear oil (4.10 g, 16.8 mmol, 79%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.21 (d, J = 8.6 Hz, 1H), 7.62 (d, J = 0.84 Hz, 1H), 7.39–7.34 (m, 3H), 6.82–6.78 (m, 1H), 4.02 (s, 3H), 3.70 (s, 3H), 3.14 (t, J = 7.8 Hz, 2H), 2.75 (t, J = 7.8 Hz, 2H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 173.4, 155.5, 138.6, 134.7, 126.2, 126.1, 126.1, 124.2, 122.3, 119.9, 103.4, 55.5, 51.7, 35.6, 31.1; HRMS (ESI$^+$) m/z calcd for $C_{15}H_{17}O_3$ (MH^+) 245.1178, obsd 245.1160.

6.5.9. 3-(5-Methoxynaphthalen-2-yl)propanoic acid (32). Compound **31** (3.50 g, 14.3 mmol) was dissolved in THF/H_2O (5:1, 75 mL) and cooled to 0 °C. LiOH·H_2O (3.61 g, 86.0 mmol) was added and the reaction mixture was stirred for 6 h at ambient temperature. The reaction mixture was partitioned between CH_2Cl_2 (250 mL) and brine (150 mL), and was acidified (pH ≈ 1) with 1 M HCl. The organic layer was removed and the aqueous layer was extracted with CH_2Cl_2 (2× 75 mL). The combined organic extracts were washed with brine (25 mL) and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure and the crude product was purified by flash chromatography eluting with $CHCl_3$/EtOAc (2:1) to yield **32** as a white solid (3.15 g, 13.7 mmol, 96%): mp 125–127 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.12 (d, J = 8.6 Hz, 1H), 7.54 (d, J = 0.75 Hz, 1H), 7.30–7.29 (m, 1H), 7.27–7.25 (m, 1H), 6.72–6.69 (m, 1H), 3.92 (s, 3H), 3.05 (t, J = 7.8 Hz, 2H), 2.71 (t, J = 7.8 Hz, 2H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 177.1, 155.5, 138.2, 134.7, 126.2, 126.2, 126.1, 124.3, 122.4, 119.9, 103.5, 55.5, 35.2, 30.7; HRMS (ESI$^+$) m/z calcd for $C_{14}H_{15}O_3$ (MH^+) 231.1021, obsd 231.1039.

6.5.10. 7-Methoxy-3,4-dihydrobenz[*h*]isoquinolin-1(2*H*)-one (33). Compound **32** (400 mg, 1.74 mmol) was treated with oxalyl chloride (2.0 mL) and stirred for 2 h at ambient temperature. The reaction mixture was evaporated to dryness, redissolved in dry benzene (1 mL), and again evaporated to dryness to remove trace amounts of oxalyl chloride. The crude acid chloride was dissolved in acetone (5 mL) and cooled to 0 °C. Sodium azide (250 mg, 3.84 mmol) was dissolved in H_2O (750 μL) and added to this solution. The reaction mixture was stirred at ambient temperature for 1 h and quenched with ice water (25 mL). The resulting solution was made basic (pH ≈ 10) with Na_2CO_3 and extracted with EtOAc (3× 20 mL). The organic layers were pooled and dried over anhydrous Na_2SO_4 and the solvent was removed under reduced pressure. The resulting crude acyl azide (390 mg, 1.53 mmol, 88%) was dissolved in dry toluene (10 mL) and heated at reflux for 3 h. The toluene was removed under reduced pressure and the crude isocyanate was dissolved in DCE (15 mL). $AlCl_3$ (600 mg, 4.50 mmol) was added and the reaction mixture was stir-

red for 16 h at 60 °C. The reaction mixture was cooled to ambient temperature, quenched with 1 M HCl (40 mL), and extracted with CH_2Cl_2 (3× 50 mL). The organic layers were pooled, washed with brine (50 mL), and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure and the crude product was purified by flash chromatography eluting with $CHCl_3$/EtOAc (3:1) to yield **33** as a white solid (190 mg, 0.836 mmol, 48%): mp 182–184 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.87 (d, J = 8.9 Hz, 1H), 8.35 (d, J = 8.6 Hz, 1H), 7.45–7.42 (m, 1H), 7.23 (d, J = 8.6 Hz, 1H), 6.78 (d, J = 7.7 Hz, 1H), 6.14 (b, 1H), 3.93 (s, 3H), 3.50–3.47 (m, 2H), 3.05 (t, J = 6.5 Hz, 2H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 167.0, 155.3, 140.4, 132.8, 128.1, 126.8, 125.4, 124.6, 123.3, 118.9, 103.9, 55.6, 39.5, 30.5; HRMS (ESI$^+$) m/z calcd for $C_{14}H_{14}NO_2$ (MH^+) 228.1024, obsd 228.1014.

6.5.11. 7-Hydroxy-3,4-dihydrobenz[*h*]isoquinolin-1(2*H*)-one (34). Compound **33** (1.25 g, 5.50 mmol) was dissolved in 48% HBr (45 mL) and glacial acetic acid (35 mL), and was heated at reflux (120 °C) for 2 h. The reaction mixture was poured into ice-cold H_2O (300 mL), the pH was adjusted to neutral with $NaHCO_3$, and extracted with EtOAc (3× 100 mL). The organic layers were pooled and extracted with 10% (w/v) NaOH (2× 100 mL). The basic aqueous extracts were combined, the pH was adjusted to neutral with 3 M HCl, and extracted with EtOAc (3× 100 mL). The combined organic extracts were pooled, washed with brine, and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure to yield compound **34** (1.06 g, 4.97 mmol, 90%) as a crystalline off-white solid: mp dec 246–248 °C; ^{1}H NMR (500 MHz, DMSO-d_6 with 5% D_2O) δ 10.1 (s, 1H), 8.77 (d, J = 8.7 Hz, 1H), 8.32 (d, J = 8.5 Hz, 1H), 7.42 (d, J = 8.5 Hz, 1H), 7.39–7.37 (m, 1H), 6.91 (d, J = 7.4 Hz, 1H), 3.40 (t, J = 6.5 Hz, 2H), 3.06 (t, J = 6.5 Hz, 2H); ^{13}C NMR (800 MHz, DMSO-d_6) δ 137.9, 137.6, 130.3, 129.5, 127.3, 126.8 (q, J = 276 Hz), 126.4, 81.8 (d, J = 172 Hz), 53.6, 53.1 (d, J = 18.6 Hz), 31.3 (q, J = 29 Hz), 44.4, 26.2 (d, J = 5.9 Hz), 15.8 (q, J = 3.5 Hz); HRMS (ESI$^+$) m/z calcd for $C_{13}H_{12}NO_2$ (MH^+) 214.0868, obsd 214.0862.

6.5.12. 7-Trifluoromethanesulfonate-3,4-dihydrobenz[*h*]-isoquinolin-1(2*H*)-one (35). Compound **34** (400 mg, 1.88 mmol) and $PhNTf_2$ (804 mg, 2.25 mmol) were dissolved in dry CH_2Cl_2 (50 mL) and stirred at 0 °C. Triethylamine (523 μL, 3.75 mmol) was added dropwise and the reaction mixture was permitted to warm to ambient temperature and stirred for 15 h. The solvent was removed under reduced pressure and the resulting residue was purified by flash chromatography eluting with EtOAc/hexanes (1:1) to yield a white solid. Recrystallization from CH_2Cl_2/hexanes yielded **35** as white needles (590 mg, 1.71 mmol, 91%): mp 144–146 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.42 (d, J = 8.8 Hz, 1H), 8.13 (d, J = 8.6 Hz, 1H), 7.56–7.53 (m, 1H), 7.45–7.40 (m, 2H), 6.09 (b, 1H), 3.54–3.51 (m, 2H), 3.10 (t, J = 6.6 Hz, 2H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 165.9, 145.5, 141.2, 133.2, 127.6, 127.5, 127.1, 126.3, 125.1, 124.2, 118.7 (q, J = 318.3), 117.7, 39.3, 30.4; HRMS (FAB$^+$) m/z calcd for $C_{14}H_{11}F_3NO_4S$ (MH^+) 346.0361, obsd 346.0358.

6.5.13. 7-Trifluoromethanesulfonate-1,2,3,4-tetrahydrobenz[*h*]isoquinoline (36). Compound **35** (120 mg, 0.348 mmol) was dissolved in THF (10 mL). One molar BH_3·THF (1.4 mL, 1.4 mmol) was added dropwise to the solution, which was heated at reflux for 3 h. The reaction mixture was cooled to 0 °C and AcOH (1 mL) was added dropwise. The reaction mixture was permitted to warm to ambient temperature and was stirred for 10 min. H_2O (2 mL) was added and the THF was removed under reduced pressure. MeOH (10 mL) was added and the resulting mixture was heated at reflux for 30 min. The MeOH was removed under reduced pressure and the resulting solution was added to brine (50 mL), made basic (pH ≈ 12) with 1 M NaOH, and extracted with CH_2Cl_2 (3× 50 mL). The combined organic extracts were pooled and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure and the crude product was purified by flash chromatography eluting with CH_2Cl_2/MeOH (6:1) to yield **36** (108 mg, 0.326 mmol, 94%) as a clear oil that solidified on standing: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.91 (d, J = 8.7 Hz, 1H), 7.83 (d, J = 8.4 Hz, 1H), 7.54–7.50 (m, 1H), 7.44 (d, J = 7.6 Hz, 1H), 7.39 (d, J = 8.7 Hz, 1H), 4.46 (s, 2H), 3.26 (t, J = 5.7 Hz, 2H), 3.00 (t, J = 5.5 Hz, 2H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 146.6, 134.5, 132.7, 131.0, 130.4, 125.9, 125.4, 122.9, 119.3, 119.1 (q, J = 320.3), 117.4, 45.8, 43.4, 30.1; HRMS (ESI$^+$) m/z calcd for $C_{14}H_{13}F_3NO_3S$ (MH^+) 332.0568, obsd 332.0560.

6.5.14. *N*-Boc-7-trifluoromethanesulfonate-1,2,3,4-tetrahydrobenz[*h*]isoquinoline (37). To a solution of **36** (95.0 mg, 0.287 mmol) and Boc_2O (81 mg, 0.373 mmol) in CH_2Cl_2 (10 mL) was added triethylamine (60 μL, 0.43 mmol). The reaction mixture was stirred for 5 h at ambient temperature and the solvent was removed under reduced pressure. The resulting oil was purified by flash chromatography eluting with CH_2Cl_2/hexanes (2:1) to yield **37** (123 mg, 0.285 mmol, 99%) as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95–7.93 (m, 2H), 7.59–7.55 (m, 1H), 7.48 (d, J = 7.7 Hz, 1H), 7.43 (d, J = 8.6 Hz, 1H), 5.02 (s, 2H), 3.79 (t, J = 5.6 Hz, 2H), 3.03 (t, J = 5.5 Hz, 2H), 1.54 (s, 9H); HRMS (ESI$^+$) m/z calcd for $C_{19}H_{24}F_3N_2O_5S$ (MNH_4^+) 449.1358, obsd 449.1358.

6.5.15. *N*-Boc-7-cyano-1,2,3,4-tetrahydrobenz[*h*]isoquinoline (38). DMF (7.0 mL), **37** (70 mg, 0.162 mmol), $Zn(CN)_2$ (48 mg, 0.41 mmol), and 15 mol% tetrakis(triphenylphosphine)palladium (28 mg, 0.024 mmol) were added to a 10 mL pressurized vial containing a magnetic stirrer. The vial was sealed and heated (using an Emrys Creator from Personal Chemistry AB, Uppsala, Sweden) at 80 °C for 45 min. The reaction mixture was partitioned between a saturated solution of Na_2CO_3 (50 mL) and CH_2Cl_2 (50 mL). The organic layer was removed and the aqueous phase was extracted with CH_2Cl_2 (2× 25 mL). The combined organic extracts were pooled, washed with brine, and dried over anhydrous Na_2SO_4. The solvent was removed under reduced

pressure and the crude product was purified by flash chromatography eluting with CH_2Cl_2/EtOAc (30:1) to yield **38** (38.5 mg, 0.125 mmol, 77%) as a white solid: mp 154–156 °C; ^{1}H NMR (500 MHz, $CDCl_3$, 45 °C) δ 8.14–8.10 (m, 2H), 7.91 (d, J = 7.4 Hz, 1H), 7.61–7.58 (m, 1H), 7.47 (d, J = 8.7 Hz, 1H), 5.03 (s, 2H), 3.79 (t, J = 5.9 Hz, 2H), 3.04 (t, J = 5.8 Hz, 2H), 1.56 (s, 9H); ^{13}C NMR (500 MHz, $CDCl_3$, 45 °C) δ 154.8, 133.9, 131.6, 131.3, 130.1, 129.1, 126.9, 125.3, 123.6, 117.7, 110.9, 80.1, 43.4, 40.9, 29.5, 28.4; HRMS (FAB$^+$) m/z calcd for $C_{19}H_{21}N_2O_2$ (MH^+) 309.1603, obsd 309.1592.

6.5.16. 7-Cyano-1,2,3,4-tetrahydrobenz[*h*]isoquinoline hydrochloride (18·HCl). To a stirred solution of **38** (50 mg, 0.16 mmol) in CH_2Cl_2 (4 mL) was added TFA (1 mL). The reaction mixture was stirred at ambient temperature for 2 h and then partitioned between basic brine (50 mL) and CH_2Cl_2 (50 mL). The organic layer was removed and the aqueous phase was extracted with CH_2Cl_2 (25 mL). The combined organic extracts were pooled and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure and the crude product was purified by flash chromatography eluting with CH_2Cl_2/MeOH (6:1) to yield **18** as a white solid. This amine was dissolved in CH_2Cl_2 and dry $HCl_{(g)}$ was bubbled through the solution to form the hydrochloride salt, which was recrystallized from EtOH/hexanes to yield **18**·HCl (37 mg, 0.15 mmol, 93%) as white crystals: mp 255–257 °C; ^{1}H NMR (500 MHz, CD_3OD) δ 8.25 (d, J = 8.8 Hz, 1H), 7.92–7.90 (m, 2H), 7.54–7.51 (m, 2H), 4.82 (s, 2H), 3.63 (t, J = 6.3 Hz, 2H), 3.34 (d, J = 6.3 Hz, 1H); ^{13}C NMR (800 MHz, CD_3OD) δ 132.5, 131.6, 131.3, 129.8, 129.6, 127.0, 126.3, 124.5, 124.2, 116.9, 110.6, 42.2, 40.7, 25.3; HRMS (ESI$^+$) m/z calcd for $C_{14}H_{13}N_2$ (MH^+) 209.1079, obsd 209.1077; Anal. Calcd for $C_{14}H_{12}N_2 \cdot 1/8H_2O$: C, 68.08; H, 5.41; N, 11.34. Found: C, 68.10; H, 5.16; N, 11.22.

6.5.17. 7-Bromo-3,4-dihydrobenz[*h*]isoquinolin-1(2*H*)-one (39). To a stirred solution of **35** (100 mg, 0.290 mmol) in dioxane (3 mL) were added lithium chloride (38.6 mg, 0.869 mmol), 5 mol% tetrakis(triphenylphosphine)palladium (16.7 mg, 0.0145 mmol), and hexamethylditin (60 μL, 0.29 mmol). The resulting mixture was stirred at 105 °C for 4 h. The reaction mixture was then cooled to 0 °C and *N*-bromosuccinamide (62 mg, 0.35 mmol) was added. The reaction mixture was stirred for 30 min at 0 °C and then permitted to warm to ambient temperature and stirred for an additional 30 min. The reaction mixture was poured into a saturated solution of $Na_2S_2O_4$/NaCl (1:1, 50 mL) and extracted with CH_2Cl_2 (3× 25 mL). The combined organic extracts were pooled, washed with brine, and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure and the crude product was purified by flash chromatography eluting with CH_2Cl_2/acetonitrile (8:1) to yield **39** (64.0 mg, 0.232 mmol, 80%) as a white solid: mp 199–201 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.19 (d, J = 8.8 Hz, 1H), 8.19 (d, J = 8.6 Hz, 1H), 7.58–7.57 (m, 1H), 7.23–7.21 (m, 2H), 5.89 (b, 1H), 3.37–3.34 (m, 2H), 2.93 (t, J = 6.5 Hz, 2H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 166.3, 140.6, 133.2, 131.8, 131.6, 130.1, 128.1, 126.7, 126.7, 122.9, 39.4, 30.4; HRMS (ESI$^+$) m/z calcd for $C_{13}H_{11}BrNO$ (MH^+) 276.0024, obsd 276.0017.

6.6. General procedure for lactam reduction. Synthesis of 14–17 and 19

The appropriate lactam (**23**, **27**, **33**, **34**, or **39**) (0.1–0.4 mmol) was dissolved in THF (10 mL) and 1 M BH_3·THF (10 equiv) was added. The solution was heated at reflux for 4 h, cooled to ambient temperature, and MeOH (10 mL) was added dropwise. The solvent was removed under reduced pressure and a solution of MeOH (10 mL) and 6 N HCl (10 mL) was added to the residue. The mixture was heated at reflux for 3 h and the MeOH was removed under reduced pressure. Water (25 mL) was added to the mixture, which was then made basic (pH ≈ 10) with 10% NaOH. The basic solution was extracted with CH_2Cl_2 (4× 30 mL) and the combined organic extracts were dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure to yield the free amine, which was purified by flash chromatography eluting with EtOAc. The free amine was dissolved in $CHCl_3$ and dry $HCl_{(g)}$ was bubbled through the solution to form the hydrochloride salt, which was recrystallized from EtOH/hexanes or MeOH/Et_2O to yield THBQs **14**–**17** or **19**.

6.6.1. 7-Nitro-1,2,3,4-tetrahydrobenz[*h*]isoquinoline hydrochloride (14·HCl). Compound **23** (45 mg, 0.186 mmol) was reduced to THBQ **14** according to Section 6.6. The hydrochloride salt was recrystallized from MeOH/ether to yield **14**·HCl (37.0 mg, 0.140 mmol, 75%) as white crystals: mp 236–238 °C; ^{1}H NMR (800 MHz, CD_3OD) δ 8.34 (d, J = 9.0 Hz, 1H), 8.26–8.24 (m, 2H), 7.80–7.78 (m, 1H), 7.64 (d, J = 9.0 Hz, 1H), 4.87 (s, 2H), 3.64 (t, J = 6.3 Hz, 2H), 3.35 (t, J = 6.3 Hz, 2H); ^{13}C NMR (800 MHz, CD_3OD) δ 148.0, 131.4, 131.0, 130.0, 127.3, 125.6, 123.8, 123.7, 122.8, 122.3, 42.5, 40.7, 25.3; HRMS (ESI$^+$) m/z calcd for $C_{13}H_{13}N_2O_2$ (MH^+) 229.0977, obsd 229.0970; Anal. Calcd for $C_{13}H_{12}N_2O_2 \cdot 1/4H_2O$: C, 58.00; H, 5.05; N, 10.41. Found: C, 58.28; H, 4.76; N, 10.24.

6.6.2. 7-Aminosulfonyl-1,2,3,4-tetrahydrobenz[*h*]isoquinoline hydrochloride (15·HCl). Compound **27** (30.0 mg, 0.109 mmol) was reduced to THBQ **15** according to Section 6.6. The hydrochloride salt was recrystallized from MeOH/ether to yield **15**·HCl (24.0 mg, 0.0803 mmol, 74%) as white crystals: mp 231–233 °C; ^{1}H NMR (800 MHz, CD_3OD) δ 8.72 (d, J = 9.0 Hz, 1H), 8.31 (d, J = 7.4 Hz, 1H), 8.14 (d, J = 8.6 Hz, 1H), 7.75–7.71 (m, 1H), 7.57 (d, J = 9.0 Hz, 1H), 4.86 (s, 2H), 3.64 (t, J = 6.3 Hz, 2H), 3.35 (t, J = 6.3 Hz, 2H); ^{13}C NMR (800 MHz, CD_3OD) δ 165.6, 141.4, 139.7, 135.9, 132.9, 129.2, 128.8, 128.2, 127.3, 125.7, 124.9, 39.2, 30.2; HRMS (ESI$^+$) m/z calcd for $C_{13}H_{15}N_2O_2S$ (MH^+) 263.0854, obsd 263.0852; Anal. Calcd for $C_{13}H_{14}N_2O_2S \cdot 1/4H_2O$: C, 51.48; H, 5.15; N, 9.24. Found: C, 51.64; H, 5.16; N, 8.99. The structure of **15** was confirmed by X-ray crystallography. See Section 7.

6.6.3. 7-Methoxy-1,2,3,4-tetrahydrobenz[*h*]isoquinoline hydrochloride (16·HCl). Compound **33** (90.0 mg, 0.396 mmol) was reduced to THBQ **16** according to Section 6.6. The hydrochloride salt was recrystallized from MeOH/ether to yield **16**·HCl (78 mg, 0.31 mmol, 79%) as white crystals: mp 241–243 °C; ^{1}H NMR (500 MHz, CD_3OD) δ 8.23 (d, J = 8.8 Hz, 1H), 7.57–7.54 (m, 1H), 7.40 (d, J = 8.6 Hz, 1H), 7.34 (d, J = 8.8 Hz, 1H), 7.02 (d, J = 7.8 Hz, 1H), 4.75 (s, 2H), 4.03 (s, 3H), 3.60 (t, J = 6.3 Hz, 2H), 3.28 (t, J = 6.2 Hz, 2H); ^{13}C NMR (500 MHz, CD_3OD) δ 156.0, 131.0, 129.7, 127.4, 125.6, 124.2, 122.1, 121.9, 113.2, 104.2, 54.7, 42.6, 40.9, 25.3; HRMS (ESI$^+$) *m/z* calcd for $C_{14}H_{14}NO_2$ (MH^+) 228.1024, obsd 228.1014.HRMS (ESI$^+$) *m/z* calcd for $C_{14}H_{16}NO$ (MH^+) 214.1232, obsd 214.1227; Anal. Calcd for $C_{14}H_{15}NO$·HCl: C, 67.33; H, 6.46; N, 5.61. Found: C, 67.16; H, 6.26; N, 5.45.

6.6.4. 7-Hydroxy-1,2,3,4-tetrahydrobenz[*h*]isoquinoline hydrochloride (17·HCl). Compound **34** (75.0 mg, 0.352 mmol) was reduced to THBQ **17** according to Section 6.6. The hydrochloride salt was recrystallized from MeOH/ether to yield **17**·HCl (61.8 mg, 0.262 mmol, 74%) as white crystals: mp 241–243 °C; ^{1}H NMR (500 MHz, CD_3OD) δ 8.22 (d, J = 8.7 Hz, 1H), 7.44–7.40 (m, 1H), 7.29 (d, J = 8.7 Hz, 1H), 7.28 (d, J = 8.5 Hz, 1H), 6.90 (d, J = 7.6 Hz, 1H), 4.72 (s, 2H), 3.60 (t, J = 6.3 Hz, 2H), 3.27 (t, J = 6.2 Hz, 2H); ^{13}C NMR (500 MHz, CD_3OD) δ 154.1, 131.3, 129.3, 127.5, 124.9, 123.6, 122.4, 121.9, 111.9, 108.1, 42.7, 41.0, 25.3; HRMS (ESI$^+$) *m/z* calcd for $C_{13}H_{13}NO$ (MH^+) 200.1075, obsd 200.1057; Anal. Calcd for $C_{13}H_{14}ClNO$: C, 66.24; H, 5.99; N, 5.94. Found: C, 68.10; H, 5.90; N, 5.91.

6.6.5. 7-Bromo-1,2,3,4-tetrahydrobenz[*h*]isoquinoline hydrochloride (19·HCl). Compound **39** (50.0 mg, 0.181 mmol) was reduced to THBQ **19** according to Section 6.6. The hydrochloride salt was recrystallized from EtOH/hexanes to yield **19**·HCl (39 mg, 0.13 mmol, 72%) as white crystals: mp dec 280–282 °C; ^{1}H NMR (500 MHz, CD_3OD) δ 8.25 (d, J = 8.8 Hz, 1H), 7.92–7.90 (m, 2H), 7.54–7.51 (m, 2H), 4.82 (s, 2H), 3.63 (t, J = 6.3 Hz, 2H), 3.33 (t, J = 6.3 Hz, 2H), ^{13}C NMR (500 MHz, CD_3OD) δ 131.3, 130.7, 130.6, 130.1, 128.2, 127.3, 126.9, 123.3, 123.1, 121.5, 42.4, 40.8, 25.3; HRMS (ESI$^+$) *m/z* calcd for $C_{13}H_{13}NBr$ (MH^+) 262.0231, obsd 262.0227; Anal. Calcd for $C_{13}H_{12}BrClN$: C, 52.29; H, 4.39; N, 4.69. Found: C, 52.13; H, 4.23; N, 4.65.

7. X-ray crystallography

The structures of compounds **23**–**25** and **15** have been deposited in the Cambridge Crystallographic Data Centre, reference numbers 619,681–619,684, respectively.

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