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1,3-Dimethyl-7-substituted-1,2,3,4-tetrahydroisoquinolines as Probes for the Binding Orientation of Tetrahydroisoquinoline at the Active Site of Phenylethanolamine *N*-Methyltransferase[1]

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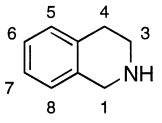
Abstract—In order to determine the function of epinephrine (Epi) in the central nervous system, we have targeted the enzyme that catalyzes the final step in the biosynthesis of Epi, phenylethanolamine *N*-methyltransferase (PNMT; EC 2.1.1.28). 1,2,3,4-Tetrahydroisoquinolines (THIQs) are inhibitors of this enzyme, but also display affinity for the α_2-adrenoceptor. To gain further understanding about how THIQs bind at the PNMT active site and in an attempt to further increase the selectivity of THIQ-type inhibitors versus the α_2-adrenoceptor, a series of *cis*- and *trans*-1,3-dimethyl-7-substituted-THIQs were synthesized. Evaluation of these compounds suggests that THIQs bind in two different orientations at the PNMT active site, based on the lipophilicity of the 7-substituent. However, no significant increases in selectivity versus the α_2-adrenoceptor were observed for these compounds.

Introduction

Epinephrine (Epi) was first discovered in the central nervous system (CNS) in the late 1940s[3] and early 1950s[4] where it was found to constitute around 5% of the total catecholamine content of the CNS.[5] Its presence in the brain was further supported by the finding of the enzyme that catalyzes the final step in the biosynthesis of Epi, phenylethanolamine *N*-methyltransferase (PNMT: EC 2.1.1.28).[6] PNMT and Epi are co-localized in the CNS, being sequestered principally in two specific regions (C1 and C2) of the medulla oblongata.[7,8] Based primarily upon this localization, Epi neurons have been postulated to be involved in (1) the regulation of blood pressure and respiration,[9] (2) the secretion of hormones from the pituitary gland,[10] (3) food and water intake,[8] (4) the regulation of body temperature,[8] (5) control of exercise tolerance,[11] (6) effects on ethanol intoxication,[12] (7) regulation of the α_2-adrenoceptor in the hypothalamus,[13,14] and (8) some of the neurodegeneration seen in Alzheimer's disease.[15,16] Therefore, in order to determine the role of Epi in these processes, our group has sought to selectively block the formation of Epi in the brain by inhibiting PNMT.

1,2,3,4-Tetrahydroisoquinolines (THIQs) have been found to be potent inhibitors of PNMT.[17] However, one of the disadvantages for this class and all classes of PNMT inhibitors is that they display affinity for the α_2-adrenoceptor.[18–21] Unsubstituted THIQ (**1**) is not a very potent inhibitor of PNMT and actually displays more affinity for the α_2-adrenoceptor (Table 1). Previous studies from our laboratory and others have indicated that there are two areas on the THIQ nucleus where substitution could increase both the potency and selectivity for PNMT versus the α_2-adrenoceptor. These areas are at the 3- and 7-positions of THIQ.



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For mono-substituted THIQs, substitution at the 7-position was found to be the most important factor for PNMT potency.[22] For optimal PNMT potency, it was determined that substitution of chlorine atoms at both the 7- and 8-positions of THIQ (SK&F 64139: **2**) was needed.[23] However, **2** displays high affinity for the α_2-adrenoceptor (Table 1).[19] In order to determine other factors that would differentiate the PNMT active site from the α_2-adrenoceptor, a comparative molecular field analysis (CoMFA) study,[24] a type of three-dimensional QSAR analysis, was performed on a set of 30

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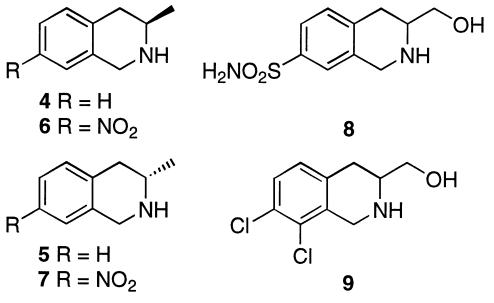
Table 1. In vitro activity of several PNMT inhibitors

Compound		PNMT (A) K_i, μM	α_2 (B) K_i, μM	B/A Selectivity
1	THIQ[a]	9.7	0.35	0.037
2	SK&F 64139[b]	0.22	0.021	0.095
3	SK&F 29661[c]	0.55	100	180
4	*R*-(−)[d]	38	5.7	0.15
5	S-(+)[d]	1.0	0.49	0.49
6	*R*-(−)[d]	1.30	53	41
7	S-(+)[d]	0.25	19	76
8	(±)[d]	0.34	1400	4100
9	(±)[b]	0.38	0.15	0.39

[a] Grunewald, G. L.; Dahanukar, V.H.; Ching, P.; Criscione, K. R. *J. Med. Chem.* **1996**, *39*, 3539–3546.
[b] Ref 28.
[c] Grunewald, G. L.; Dahanukar, V.H.; Caldwell, T. M.; Criscione, K. R. *J. Med. Chem.* **1997**, *40*, 3997–4005.
[d] Unpublished results from Dahanukar, Vilas H., Ph.D. dissertation, University of Kansas, Lawrence, KS, 1994.

7-substituted-THIQs for both the α_2-adrenoceptor and for PNMT.[25] The results of this study indicated that when all of these compounds were aligned in the same manner at both PNMT and the α_2-adrenoceptor, a satisfactory predictive model could not be obtained. It was only when these THIQs were aligned in two different orientations (Fig. 1), a lipophilic (A) or a hydrophilic (B) orientation, with the lone pair on the THIQ nitrogen placed in the same area of space, that a good predictive model could be obtained. The results of this study suggested that THIQs that could bind in lipophilic orientation A (+π) would be found to be 'non-selective' inhibitors of PNMT, whereas THIQs that could bind in the hydrophilic orientation (−π) would be 'selective' for PNMT versus the α_2-adrenoceptor. SK&F 29661 (**3**) is an example of the latter and is one of the more selective inhibitors of PNMT versus the α_2-adrenoceptor known (Table 1). However, this compound has been found to be unable to penetrate the blood brain barrier, presumably due to the high polarity of the 7-aminosulfonyl substituent.[26]

The second area on the THIQ nucleus that was found to effect both potency and selectivity for PNMT was the 3-position. Previous investigations on the effects of steric bulk tolerance at the PNMT active site had been conducted by examining methyl substitution on other areas of the aliphatic portion of THIQ (positions 1, 3, and 4).[27] This study found that methyl substitution at the 3-position increased the potency of THIQ for PNMT, while slightly decreasing its affinity for the α_2-adrenoceptor (PNMT $K_i = 2.13\,\mu M$; α_2 $K_i = 0.76\,\mu M$). The overall rank order of potency for the racemic methyl substituted THIQs at PNMT was (±)-3-methyl-THIQ ($K_i = 2.13\,\mu M$) > (±)-1-methyl-THIQ ($K_i = 33.1\,\mu M$) > (±)-4-methyl-THIQ ($K_i = 70.1\,\mu M$). The area around the 3-position of THIQ was further explored and was found to possess limited steric bulk tolerance, permitting either a (±)-3-methyl ($K_i = 2.13\,\mu M$) or (±)-3-hydroxymethyl group ($K_i = 1.06\,\mu M$).[28] Substitution of larger groups, such as (±)-3-ethyl ($K_i = 23.9\,\mu M$)[28] or (±)-3-methoxymethyl ($K_i = 9.18\,\mu M$),[29] resulted in decreased affinity for PNMT.



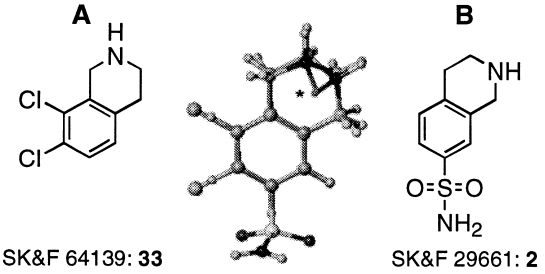
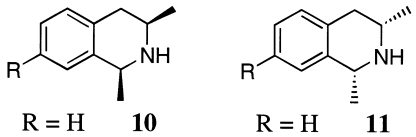


Figure 1. Two possible orientations of 7-substituted-THIQs.[25] Orientation A is proposed for lipophilic (+π) 7-substituents, while hydrophilic (−π) 7-substituents are proposed to bind in orientation B. Between these two structures is a SYBYL-generated view of SK&F 64139 (**2**) in orientation A, superimposed on SK&F 29661 (**3**) in orientation B, showing that the THIQ nitrogen lone pairs can reach the same area. The asterisk marks the area in space where the lone pairs of **2** in orientation A and **3** in orientation B may overlap.

Later studies showed that by combining either a 3-methyl or 3-hydroxymethyl substituent with a hydrophilic electron-withdrawing 7-substituent that multiplicative increases in selectivity could be obtained.[29] Compounds **6**–**8** are examples of this type of synergism and **8** is the most selective inhibitor of PNMT yet reported (Table 1). However, this compound is more polar than SK&F 29661, due to the 3-hydroxymethyl moiety ($\pi = -1.03$), and is even less likely to penetrate into the brain. As expected when a 3-substituent was combined with a lipophilic 7-substituent, such as in the case of the 3-hydroxymethyl derivative of SK&F 64139 (**9**),[28] this compound was found to be a nonselective inhibitor of PNMT (Table 1). This finding was again attributed to the fact that this compound probably binds in the proposed lipophilic orientation A at the α_2-adrenoceptor, thereby increasing its affinity (Fig. 1). In contrast, compounds **6**–**8**, which contain hydrophilic 7-substituents, cannot bind in the lipophilic orientation,

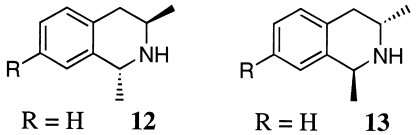
and thus are selective for PNMT. For enantiomers **6** and **7**, it was found that the *S*-enantiomer **7** was more potent at both PNMT and the α_2-adrenoceptor (see Table 1). It was interesting to note that addition of the 7-nitro substituent dramatically increased the potency for both enantiomers of 3-methyl-THIQ for PNMT, while concomitantly decreasing their affinity for the α_2-adrenoceptor (Table 1). As both enantiomers were found to be relatively potent at PNMT, this would suggest that there is a reasonable amount of steric bulk tolerance around the 3-position for compounds binding in the hydrophilic orientation. Since we had previously explored substitution at both the 3- and 7-positions on THIQ, we deemed it necessary to examine other positions on THIQ for substitution in order to further differentiate between binding at the PNMT active site and the α_2-adrenoceptor.

Therefore, in order to clarify the issue of binding orientation of THIQs at the PNMT active site and the α_2-adrenoceptor, and in an attempt to increase the selectivity of these inhibitors for PNMT versus the α_2-adrenoceptor, 1,3-dimethyl-THIQs (**10**–**21**) were proposed for synthesis. The 7-bromo and 7-nitro substituents for these THIQs were chosen for this study for two reasons. First, these substituents possess the appropriate lipophilicity [7-bromo ($\pi = 0.86$), 7-nitro ($\pi = -0.28$)] to allow alignment in the proposed lipophilic and hydrophilic orientations, respectively. Second, these compounds could be easily synthesized from common intermediates. If these compounds bind in the same orientation at both PNMT and the α_2-adrenoceptor, one would expect to observe the same rank order of potency for these compounds. However, if these compounds bind in different orientations based upon the lipophilicity of the 7-substituent, the 1-methyl moiety will be oriented in two different regions of space (Fig. 2). Therefore, the 7-nitro-substituted THIQs (**14**–**17**) and 7-bromo-THIQs (**18**–**21**) might display different rank orders of potency. Also, it should be noted that the addition of a 7-nitro group increases the potency of 3-methyl-THIQs (**6** and **7**) for PNMT while decreasing their potency for the α_2-adrenoceptor. It will be of interest to compare how the addition of a 1-methyl moiety affects the potency of these compounds for PNMT and if these compounds will be able to further differentiate between the PNMT active site and the α_2-adrenoceptor.



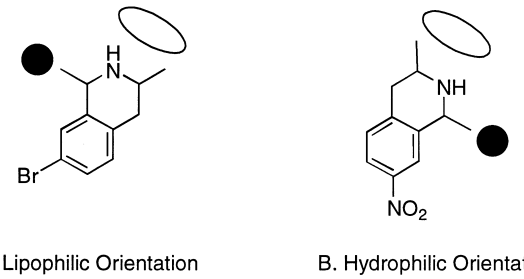
R = H **10**
R = NO_2 **14**
R = Br **18**

R = H **11**
R = NO_2 **15**
R = Br **19**



R = H **12**
R = NO_2 **16**
R = Br **20**

R = H **13**
R = NO_2 **17**
R = Br **21**



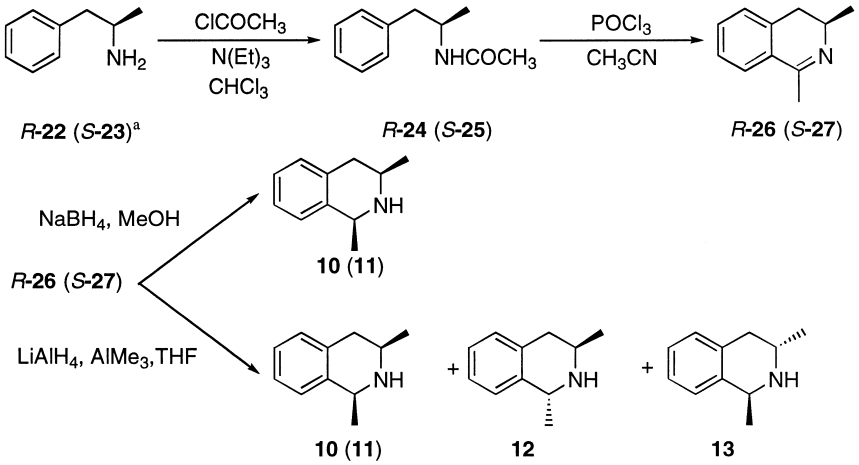
A. Lipophilic Orientation B. Hydrophilic Orientation

Figure 2. 1,3-Dimethyl-7-substituted-THIQs shown in the proposed lipophilic and hydrophilic binding orientation. According to this hypothesis the 1-methyl moiety will occupy different regions in space (depicted by the black circles), which have not been previously examined, depending upon the orientation of the molecule. The white ellipse shows the similar region of space occupied by the 3-methyl moiety in both orientations.

Results and Discussion

Synthesis

The (*cis* and *trans*)-1,3-dimethyl-tetrahydroisoquinoline nucleus is found in a variety of naturally occurring alkaloids (e.g. 1,3-(−)-ancistrocladine and (−)-ancistrobrevine C) which have been previously synthesized.[30] Based upon these syntheses, the following synthesis of **10**–**13** was performed. Beginning with the appropriate phenylpropylamine *R*-**22** (*S*-**23**),[31] acetylation of the primary amine with acetyl chloride forms amide *R*-**24** (*S*-**25**), which was treated with $POCl_3$ (Bischler–Napieralski) to form imine *R*-**26** (*S*-**27**) (Scheme 1). Reduction of the imine with $NaBH_4$ in MeOH forms the *cis*-1,3-dimethyl-THIQ 1*S*,3*R*-**10** (1*R*,3*S*-**11**), while reduction of the imine with $LiAlH_4$ and $AlMe_3$ in THF formed a mixture of both *cis*- and *trans*-1,3-dimethyl-THIQs. The *cis*- and *trans*-diastereomers were separated by flash column chromatography (silica gel). Examination of the *trans*-THIQ product by chiral HPLC indicated that racemization had occurred at the 3-position under the reaction conditions. Bringman et al.[30] had reported in their synthesis of 6,8-dimethoxy-*trans*-1,3-dimethyl-THIQ that racemization had not occurred under these same reduction conditions. We suggest that the reason that racemization did not occur for this particular compound is because it contained an 8-methoxy group which stabilizes the $AlMe_3$-dihydroisoquinoline complex through resonance.[32] Compound **26** does not have an 8-methoxy group to help stabilize this complex, which allows isomerization of the imine to occur leading to a mixture of enantiomers (**12** and **13**). A similar type of cationic imine isomerization has been reported by Takahata et al.[33] However, this type of imine isomerization has been more commonly reported as base catalyzed.[34,35] The enantiomers of *trans*-1,3-dimethyl-THIQ (**12** and **13**) were separated through recrystallization of the diastereomeric salts of (−) or (+)-L-dibenzoyl tartrate monohydrate. From each phenylpropylamine (*R*-**22** or *S*-**23**), there are two possible THIQ diastereomers that can be produced for a total of four 1,3-dimethyl-tetrahydroisoquinolines [two *cis*-(1*S*,3*R*-**10** and 1*R*,3*S*-**11**) and two *trans*-(1*R*,3*R*-**12** and 1*S*,3*S*-**13**)].



Scheme 1. [a]This reaction scheme was repeated for phenylpropylamine (*S*-23) and the products of this synthesis are shown in parentheses.

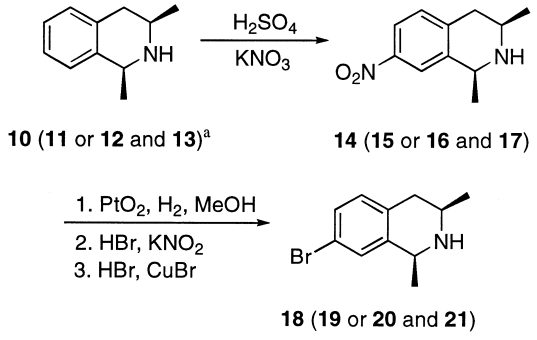
Nitration of tetrahydroisoquinoline **10** occurred chiefly at the 7-position to form **14** (Scheme 2).[36] Reduction of the 7-nitro group to the amine, followed by a Sandmeyer reaction, yielded **18**. This reaction sequence was repeated with **11** and the racemic mixture of **12** and **13**, to yield **15** and the racemic mixture of **16** and **17**, and 7-bromo-THIQs (**19** and the racemic mixture of **20** and **21**). The enantiomers of the **16**, **17** and **20**, **21** were separated by recrystallization of the diastereomeric salts formed with *S*-(+)- and *R*-(−)-mandelic acid. Absolute stereochemistry for the *trans*-THIQs was assigned in the following manner. **21** was crystallized as the *S*-(+)-mandelate salt to give crystals suitable for X-ray diffraction. The stereochemistry of **21** was assigned relative to the known configuration of *S*-(+)-mandelic acid from the X-ray crystal structure (Fig. 3). The stereochemistry for **12**, **13**, **16**, **17**, and **20** was assigned relative to **21**, given that these *trans*-THIQs [(+)-**12** and (−)-**13**, (+)-**16** and (−)-**17**, and (+)-**20** and (−)-**21**] gave the same order of elution during chiral HPLC analysis and displayed the same sign of rotation. Enantiomeric excess (ee) for all final compounds was determined to be greater than 95% by chiral HPLC.

Biochemistry

All compounds were evaluated as their hydrobromide salts for their activity as inhibitors of PNMT and inhibitors of the binding of [^{3}H]clonidine to the α_2-adrenoceptor. Bovine adrenal PNMT was prepared using the methods developed by Connett and Kirshner through the isoelectric precipitation step.[37] The in vitro activity of these compounds was determined using a standard radiochemical assay that has been previously described.[38] Inhibition constants were determined by using three different concentrations of the inhibitor with phenylethanolamine as the substrate.

α_2-Adrenoceptor binding assays were performed using a standard radiochemical assay developed by U'Prichard et al.[39] which uses [^{3}H]clonidine as the radioligand to define specific binding and phentolamine to determine the non-specific binding affinity. This procedure has been used in order to simplify the comparison with previous results.

Direct comparison of the selectivities (α_2 K_i/PNMT K_i) of **6**, **7** (Table 1), and **10**–**21** (Table 2) indicates that the incorporation of the 1-methyl-moiety did not produce any significant increase in selectivity for these compounds.



Scheme 2. [a]This synthesis scheme was repeated for compounds **11** and the racemic mixture of **12** and **13**.

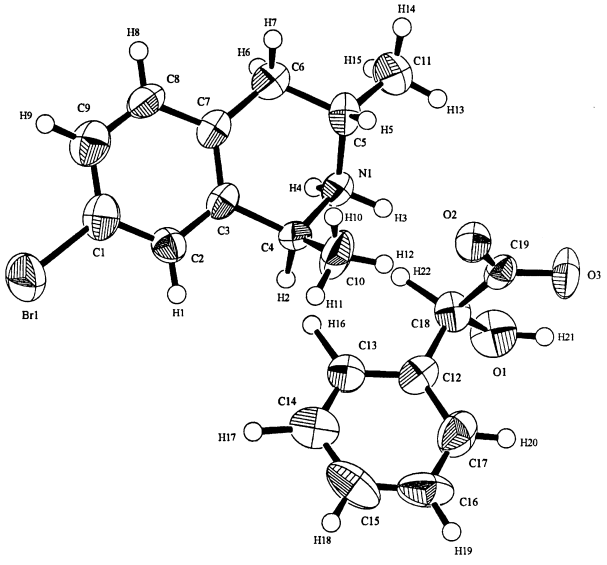


Figure 3. ORTEP diagram of the (*S*)-(+)-mandelate salt of *trans*-(1*S*,3*S*)-**21**. The atom numbering scheme agrees with the tables supplied in the supplemental material and thermal motion ellipsoids are shown at the 50% level.

Table 2. Biochemical evaluation of 1,3-dimethyl-1,2,3,4-tetrahydroisoquinolines

Compound	1,3-Dimethyl configuration	7-Substituted	PNMT K_i, μM	α_2 K_i, μM	α_2/PNMT Selectivity
10	*cis*-1*S*,3*R*	H	340 ± 10	38 ± 1	0.11
11	*cis*-1*R*,3*S*	H	21 ± 1	36 ± 1	1.7
12	*trans*-1*R*,3*R*	H	4.1 ± 0.2	17 ± 1	4.1
13	*trans*-1*S*,3*S*	H	36 ± 2	67 ± 1	1.9
14	*cis*-1*S*,3*R*	NO_2	11 ± 1	82 ± 1	7.4
15	*cis*-1*R*,3*S*	NO_2	6.6 ± 0.4	69 ± 1	10
16	*trans*-1*R*,3*R*	NO_2	0.48 ± 0.07	36 ± 1	75
17	*trans*-1*S*,3*S*	NO_2	20 ± 3	57 ± 2	2.9
18	*cis*-1*S*,3*R*	Br	5.6 ± 0.2	11 ± 1	2.0
19	*cis*-1*R*,3*S*	Br	6.6 ± 0.4	4.0 ± 0.1	0.61
20	*trans*-1*R*,3*R*	Br	0.62 ± 0.04	2.3 ± 0.2	3.7
21	*trans*-1*S*,3*S*	Br	0.60 ± 0.10	5.7 ± 0.4	9.5
26	3*R*	H	710 ± 20	36 ± 1	0.05
27	3*S*	H	670 ± 20	32 ± 1	0.05

However, examination of the rank order of potencies for each set of 7-unsubstituted, 7-nitro, and 7-bromo-THIQs indicates that these compounds do not display the same relative order of affinity for PNMT. The rank order PNMT affinity for the 1,3-dimethyl-7-unsubstituted-THIQs by increasing K_i for PNMT is **12** (*trans*-1*R*,3*R*) < **11** (*cis*-1*R*,3*S*) < **13** (*trans*-1*S*,3*S*) < **10** (*cis*-1*S*,3*R*). For the 7-nitro-THIQs, the rank order of potency by increasing K_i at PNMT was **16** (*trans*-1*R*,3*R*) < **15** (*cis*-1*R*,3*S*) < **14** (*cis*-1*S*,3*R*) < **17** (*trans*-1*S*,3*S*). For the 7-bromo-THIQs, the rank order of potency at PNMT was **21** (*trans*-1*S*,3*S*) = **20** (*trans*-1*R*,3*R*) < **18** (*cis*-1*S*,3*R*) < **19** (*cis*-1*R*,3*S*). The overall rank order of potency for all 12 compounds indicates that they are probably binding in two different orientations (A and B; Fig. 1) at PNMT.

For example, the rank order of potency for the *cis*-enantiomers of 7-bromo- (**18** and **19**) and 7-nitro-1,3-dimethyl-THIQ (**14** and **15**), are directly opposite and indicates that these compounds are binding differently at the PNMT active site. Another example of this change in rank order of potency can be seen by direct comparison of the PNMT activity of **17** (*trans*-1*S*,3*S*), the least potent 'hydrophilic' 7-nitro-THIQ, and **21** (*trans*-1*S*,3*S*), the most potent 'lipophilic' 7-bromo-THIQ. These compounds have the same absolute stereochemistry, but display the direct opposite rank order of potency in their series. This would seem to indicate that **17** and **21** probably bind in orientations B and A (Fig. 1), respectively, at the PNMT active site. These data add support to the hypothesis that THIQs bind differently at PNMT, which may be dependent upon the lipophilicity of the 7-substituent.

Unexpectedly, both enantiomers of *cis*-7-nitro-THIQs (**14** and **15**) and *cis*-7-bromo-THIQs (**18** and **19**) were found to be moderately potent for PNMT. It was anticipated that if these compounds were aligned in either the hydrophilic orientation B (**14** and **15**) or the lipophilic orientation A (**18** and **19**) (Fig. 1) that one of the enantiomers in each case would be much less potent due to the energy required to adopt the 1,3-diaxial conformation. However, there was not much differentiation between the two enantiomers of these compounds. Examination of the energy differences between the diaxial and diequatorial conformations of **10** and **11** (Fig. 4) indicates that these compounds will probably be found in the diequatorial conformation. It is possible in this case that stereochemical factors and not the lipophilicity of the 7-substituent is dominating the binding of these *cis*-THIQs. Therefore, these compounds may bind in a different orientation because of their stereochemistry and not their lipophilicity. This is supported by the switch in order of potency for the *cis*-enantiomers of 7-nitro- (1*S*,3*R*-**14** K_i = 11 μM > 1*R*,3*S*-**15** K_i = 6.6 μM) and 7-bromo-1,3-dimethyl-THIQ (1*S*,3*R*-**18** K_i = 5.6 μM < 1*R*,3*S*-**19** K_i = 6.6 μM), which indicates that PNMT preferentially binds different enantiomers in the hydrophilic and hydrophobic orientations.

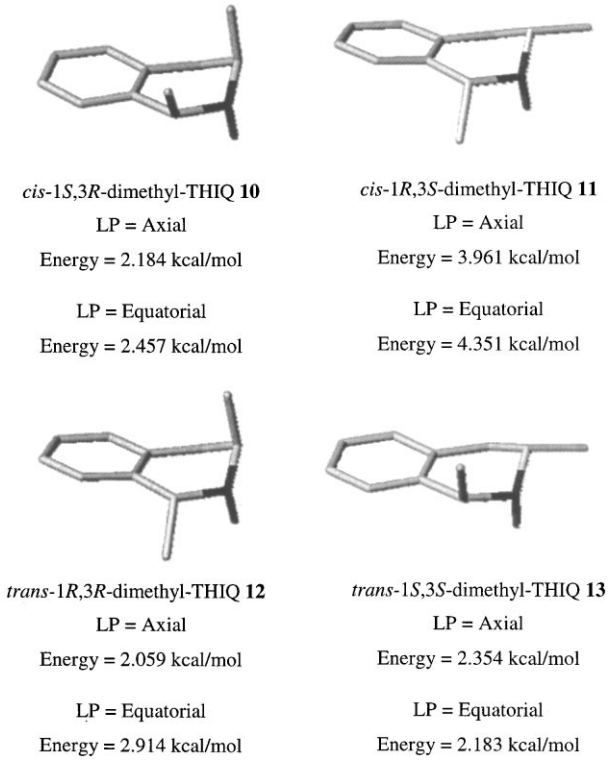


Figure 4. SYBYL-generated conformations and minimized energies for both *cis*- (**10** and **11**) and *trans*-1,3-dimethyl-1,2,3,4-tetrahydroisoquinolines (**12** and **13**). The starting coordinates for *trans*-1,3-dimethyl-THIQ **13** were taken from the X-ray crystal structure of **21** (Fig. 3) and the geometry optimized using the Tripos force field.

The *trans*-1,3-dimethyl-7-nitro-THIQ (**16**) was found to be the most selective inhibitor in this series of 12 *cis* and *trans*-1,3-dimethyl-THIQs, due mostly to its higher affinity for PNMT. The stereochemistry at the 3-position of **16** is *R*, which is opposite that of the most selective 3-methyl-7-nitro-THIQ (**7**), which has *S* stereochemistry. As previously discussed, the 3-methyl group of compounds **6** and **7** occupies an area of steric bulk tolerance at the PNMT active site, which is likely why these compounds do not display much stereoselectivity at PNMT. In contrast, **16** and **17** also possess a 1-methyl moiety. Enantiomer **17** has the same absolute stereochemistry as *trans*-1,3-dimethyl-THIQ (**13**) (Fig. 4), the coordinates of which were taken from the X-ray crystal structure of **21** (Fig. 3). When **16** and **17** are aligned in hydrophilic orientation B (Fig. 1), the 1-methyl group will either be directed into the plane of the THIQ nucleus as in **16** or out of the plane of the THIQ nucleus as in **17** (Fig. 5). For these two enantiomers, **17** was found to be the least active (Table 2), which would indicate that there is an area of steric bulk intolerance at the 1-position ('above' the plane of the THIQ nucleus) at PNMT for THIQs that may bind in the hydrophilic orientation (Fig. 5). A similar trend is found for **14**, which displays decreased affinity relative to **15** and **16** (Table 2). This trend is also found for the 7-unsubstituted-THIQs **10** and **13**, which display decreased affinity relative to **11** and **12**. In contrast, it appears that the PNMT active site exhibits more steric bulk tolerance at the 1-position for THIQs (**20** and **21**) that may bind in the lipophilic orientation, as these compounds were found to be equipotent at PNMT (Table 2). It was interesting to note that the addition of either the 7-nitro or the 7-bromo-substituent to these THIQs increased the potency of these compounds for PNMT by a factor of 2- to 60-fold. Overall, as expected, the 7-nitro-THIQs were found to be more selective than either the 7-bromo- or 7-unsubstituted compounds. Also, imines **26** and **27** were evaluated at both PNMT and the α_2-adrenoceptor and these compounds were found to exhibit almost no affinity for PNMT (Table 2). The lone pair on the nitrogen of dihydroisoquinolines **26** and **27** can only be oriented into a similar region of space as that occupied by the lone pair on the nitrogen of THIQ if it were oriented equatorially. The lack of PNMT activity of compound **26** and **27** seems to indicate that the lone pair on THIQ must be aligned axially in order to interact with the PNMT active site. This result confirms the conclusions of previous studies from this group which indicated that the lone pair on THIQ interacts with PNMT in an axial manner.[27]

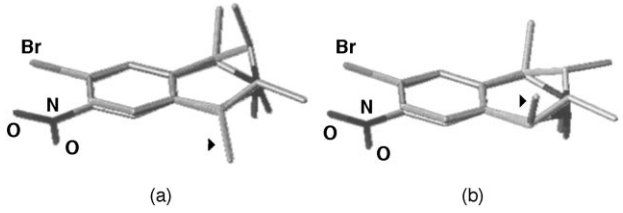


Figure 5. (a) Molecular modeling overlay of **16**, the most potent *trans*-7-nitro-THIQ, and **20**. The arrow indicates the 1-methyl moiety of **16** which shows the methyl group pointing below the plane of the THIQ nucleus. (b) Overlay of **17**, the least potent *trans*-7-nitro-THIQ, and **20**. The arrow indicates the 1-methyl moiety of **17** which shows the methyl group pointing out of the plane of the THIQ nucleus.

Overall for the α_2-adrenoceptor, the addition of the 1-methyl moiety did not cause any major changes in the rank order of affinity of these compounds, which indicates that they are probably binding in a similar manner at the α_2-adrenoceptor. It also appears that the α_2-adrenoceptor is less stereoselective in the binding of these compounds than the PNMT active site (Table 2). The addition of the 7-nitro substituent decreased the affinity of these 1,3-dimethyl-THIQs for the α_2-adrenoceptor with the exception of compound **17**, which displayed a slight increase in affinity. As expected, addition of a hydrophilic electron-withdrawing group at the 7-position decreased the affinity of THIQs for the α_2-adrenoceptor. Also as expected, the addition of a 7-bromo substituent increased the affinity of every 1,3-dimethyl-THIQ for the α_2-adrenoceptor. Unfortunately, the incorporation of a 1-methyl group caused no significant increases in selectivity for these compounds for PNMT versus the α_2-adrenoceptor.

Conclusions

A series of 1,3-dimethyl-THIQs (7-unsubstituted, 7-nitro, and 7-bromo) were synthesized and evaluated for activity at PNMT and the α_2-adrenoceptor. It was found, in this series of compounds, that the rank order of potency at PNMT of THIQs bearing a hydrophilic 7-nitro-substituent was different than that of THIQs bearing a lipophilic 7-bromo-substituent. This difference in rank order of potency indicates that these compounds are probably binding in two different orientations (lipophilic A and hydrophilic B) at the PNMT active site. It also appears that binding of THIQs at PNMT is not only influenced by lipophilicity, but also by stereochemical factors which may outweigh lipophilicity in the binding of ligands. It seems that THIQs, which can bind in the lipophilic orientation, experience more steric bulk tolerance at the 1-position, whereas THIQs that can bind in the hydrophilic orientation find that PNMT only tolerates steric bulk 'below' the plane (Fig. 5). Incorporation of a 1-methyl substituent to increase selectivity (PNMT versus α_2-adrenoceptor) did not have the desired effect. Imines **26** and **27** were found to be poor inhibitors of PNMT, supporting the conclusion that the lone pair on THIQ must be in an axial orientation in order to bind to PNMT. The data afforded by these 1,3-dimethyl-THIQs provide important insight about how the PNMT active site interacts with THIQs, dependent upon the lipophilicity of the 7-substituent, and what stereochemical considerations must be taken into account in the design of new and more selective inhibitors of PNMT.

Experimental

All of the reagents and solvents used were 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 but are otherwise uncorrected. Proton (^{1}H) and carbon (^{13}C) nuclear magnetic resonance spectra were taken on a Varian XL-300, a GE QE-300, or a Bruker DRX-400 spectrophotometer. Proton chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane (TMS, 0.00 ppm) and carbon chemical shifts are reported in ppm relative to $CDCl_3$ (77.0 ppm). For the hydrobromide salts of the THIQs, NMR spectra were recorded in deuterated dimethyl sulfoxide (DMSO-d_6) or deuterated methanol (CD_3OD) and the chemical shifts are reported relative to DMSO-d_6 (2.49 ppm for ^{1}H and 39.7 ppm for ^{13}C) and CD_3OD (3.30 ppm for ^{1}H and 49.0 ppm for ^{13}C). Multiplicity abbreviations: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; bs, broad singlet; e, exchangeable. Infrared spectra were obtained on a Perkin–Elmer 1420 infrared spectrophotometer. Electron-impact mass spectra (EIMS), chemical-ionization mass spectra (CIMS), and high resolution mass spectra (HRMS) were obtained on a Varian Atlas CH-5 or a Ribermag R 10-10 mass spectrophotometer. The intensity of each peak in the mass spectrum relative to the base peak is reported in parentheses. Microanalyses were performed on a Hewlett–Packard Model 185B CHN analyzer at the University of Kansas. High performance liquid chromatography was performed on a Shimadzu LC 6A system, using a *Chiralcel OJ* column (0.46×25 cm), which was purchased from Daicel (USA) Inc., New York. Optical rotations were performed on a Perkin–Elmer 241 polarimeter using the sodium D line as the light source. Note: Due to the small amounts of the *trans*-1,3-dimethyl-THIQs available for use in these experiments and the low observed rotation for these compounds, the error introduced by the accuracy of the polarimeter was considerable (ca. 10–20%). Thus, the purity of these compounds was more accurately reflected by the chiral HPLC experiments.

Solvents were routinely distilled prior to use. All methanol (MeOH) and ethanol (EtOH) used were anhydrous unless stated otherwise and were prepared by distillation over magnesium. Anhydrous tetrahydrofuran (THF) and diethyl ether (Et_2O) were distilled from sodium-benzophenone ketyl. Methylene chloride (CH_2Cl_2) and chloroform ($CHCl_3$) were obtained by distillation from phosphorous pentoxide (P_2O_5). In some cases anhydrous solvents were used directly from Aldrich Sure Seal® bottles. Hexanes refers to the mixture of hexane isomers (bp 40–70 °C) and brine refers to a saturated aqueous solution of NaCl. All reactions that required anhydrous conditions were performed under a positive nitrogen (N_2) flow, and all glassware was either oven-dried or flame-dried before use.

S-Adenosyl-L-methionine used in the radiochemical assays was obtained from Sigma Chemical Co. [^{3}H]-*S*-Adenosyl-L-methionine was purchased from American Radiolabeled Chemicals, St. Louis, MO. [^{3}H]Clonidine was purchased from DuPont New England Nuclear, Boston, MA, for use in the α_2-adrenoceptor assays. Bovine adrenal glands were obtained from Davis Meat Processing, Overbrook, KS.

(*R*)-(−)-*N*-Acetyl-α-methylphenylethylamine (24). To an ice cold solution of (*R*)-α-methylphenylethylamine **22** [**22**·1/2 H_2SO_4: $[\alpha]_D^{20} = -21°$ (*c* 1.0, H_2O), (lit. $[\alpha]_D^{20} = -20°$ (*c* 1.0, H_2O))][31] (1.85 g, 13.7 mmol) and $N(Et)_3$ (3.6 mL) in dry CH_2Cl_2 (50 mL), acetyl chloride (1.1 mL, 14 mmol) was added dropwise over a period of 5 min. The mixture was stirred overnight at room temperature. The reaction solution was washed with 2 N HCl (3×30 mL), dried over anhydrous Na_2SO_4 and the solvent was removed under reduced pressure. The residue was purified by flash chromatography (silica gel) with EtOAc/hexane (1/1) to yield a yellow liquid which was distilled bulb to bulb (120–140 °C, 0.8 mm Hg) to yield a white solid (1.76 g, 74.3%); mp 117–119 °C (lit. 124–125 °C);[40] $[\alpha]_D^{20} = +7.9°$ (*c* 0.80, EtOH); ^{1}H NMR ($CDCl_3$) δ 7.27–7.14 (m, 5H, Ar-H), 5.34 (m, 1H, NH), 4.23 (m, 1H, CH), 2.79–2.65 (m, 2H, CH_2), 1.90 (s, 3H, $COCH_3$), 1.08 (d, J = 6.9 Hz, 3H, CH_3). Anal. calcd for $C_{11}H_{15}NO$: C, 74.54; H, 8.53; N, 7.90. Found: C, 74.26; H, 8.70; N, 8.10.

(*S*)-(+)-*N*-Acetyl-α-methylphenylethylamine (25). Compound **25** was synthesized and purified in the same manner as **24**, using **23** [**23**·1/2 H_2SO_4: $[\alpha]_D^{20} = +21°$ (*c* 1.0, H_2O)] (2.90 g, 21.5 mmol) as the starting material, to yield the title compound as a white solid (3.35 g, 88.2%); mp 117–119 °C (lit. 124–125 °C);[40] $[\alpha]_D^{20} = -8.2°$ (*c* 1.06, EtOH). Anal. calcd for $C_{11}H_{15}NO$: C, 74.54; H, 8.53; N, 7.90. Found: C, 74.18; H, 8.80; N, 7.64.

(3*R*)-(+)-1,3-Dimethyl-3,4-dihydroisoquinoline[40] hydrobromide (26·HBr). To a solution of **24** (1.85 g, 10.4 mmol) in dry CH_3CN (200 mL), $POCl_3$ (5 mL) was added. The reaction mixture was heated to reflux for 16 h. The solvent was removed under reduced pressure. The residue was carefully poured onto ice-water (50 mL) and the aqueous solution was extracted with CH_2Cl_2 (2×30 mL). The aqueous mixture was made basic with $NH_4OH_{(aq)}$ and extracted with CH_2Cl_2 (3×50 mL). The combined organic layers were washed with brine and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure to yield a yellow oil which was distilled bulb to bulb (65–75 °C/0.1 mm Hg) to yield a colorless liquid (970 mg, 58.8%). The hydrobromide salt was formed by the addition of methanolic HBr and recrystallized from EtOH/EtOAc/hexane to yield white needles; mp 175–177 °C; $[\alpha]_D^{20} = +38°$ (*c* 1.05, EtOH); IR (neat) 3380–3100 (broad), 2940, 2900, 1610, 1560, 1440, 1360, 1270, 750 cm^{-1}; ^{1}H NMR ($CDCl_3$, free base) δ 7.42 (d, J = 6.9 Hz, 1H, Ar-H), 7.36–7.18 (m, 2H, Ar-H), 7.10 (d, J = 6.9 Hz, 1H, Ar-H), 3.50 (m, 1H, H-3), 2.75 (m, 1H, H-4), 2.42 (m, 1H, H-4), 2.35 (s, 3H, 1-CH_3), 1.34 (d, J = 6.6 Hz, 3H, 3-CH_3); CIMS *m*/*z* (relative intensity) 160 ($M^+ + 1$, 100), 144 (13), 131 (8). Anal. calcd for $C_{10}H_{13}N$·HBr: C, 55.02; H, 5.88; N, 5.83. Found: C, 55.08; H, 6.16; N, 5.87.

(3*S*)-(−)-1,3-Dimethyl-3,4-dihydroisoquinoline[40] hydrobromide (27·HBr). Dihydroisoquinoline **27** was synthesized and purified in the same manner as **26**, using amide **25** (2.95 g, 1.67 mmol) as the starting material, to yield **27**·HBr as white crystals (1.46 g, 55.1%); mp 175–

177 °C; $[\alpha]_D^{20} = -41°$ (*c* 1.00, EtOH). Anal. calcd for $C_{10}H_{13}N{\cdot}HBr$: C, 55.02; H, 5.88; N, 5.83. Found: C, 54.66; H, 5.80; N, 5.71.

***cis*-(1*S*,3*R*)-(−)-1,3-Dimethyl-1,2,3,4-tetrahydroisoquinoline[40] hydrobromide (10·HBr).** To an ice-cold solution of dihydroisoquinoline **26** (370 mg, 2.33 mmol) in dry MeOH (100 mL), $NaBH_4$ (530 mg, 14.0 mmol) was added. The reaction mixture was stirred for 2 h at room temperature. The solvent was removed under reduced pressure and the residue was made basic (pH > 10) with 1 N NaOH. The resulting solution was extracted with CH_2Cl_2 (4×30 mL) and the organic layers were combined, washed with brine and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure and the residue was purified by flash column chromatography (silica gel) with $CH_2Cl_2/CH_3OH/NH_4OH_{(aq)}$ (100/10/0.5) to give a white solid. The hydrobromide salt of this compound was formed by the addition of methanolic HBr. The solvent was removed and the residue recrystallized from MeOH/EtOAc/hexane to yield white crystals (524 mg, 92.9%); mp 275–277 °C; $[\alpha]_D^{20} = -59°$ (*c* 1.0, MeOH); ^{1}H NMR ($CDCl_3$, free base) δ 7.25–7.10 (m, 4H, Ar-H), 4.60 (m, 1H, H-1), 3.55 (m, 1H, H-3), 3.18 (m, 1H, H-4), 2.92 (m, 1H, H-4), 2.00 (d, J = 6.9 Hz, 3H, 1-CH_3), 1.34 (d, J = 6.6 Hz, 3H, 3-CH_3); CIMS *m*/*z* (relative intensity) 162 ($M^+ + 1$, 100), 146 (80), 117 (25). Anal. calcd for $C_{11}H_{15}N{\cdot}HBr$: C, 54.56; H, 6.66; N, 5.78. Found: C, 54.96; H, 6.92; N, 5.53.

***cis*-(1*R*,3*S*)-(+)-1,3-Dimethyl-1,2,3,4-tetrahydroisoquinoline[40] hydrobromide (11·HBr).** Compound **11** was synthesized and purified in the same manner as **10**·HBr, using dihydroisoquinoline **27** (760 mg, 4.78 mmol) as the starting material to yield **11**·HBr as white needles (567 mg 73.6%); mp = 276–278 °C; $[\alpha]_D^{20} = +61°$ (*c* 1.0, MeOH). Anal. calcd for $C_{11}H_{15}N{\cdot}HBr$: C, 54.56; H, 6.66; N, 5.78. Found: C, 54.72; H, 6.70; N, 5.44.

***trans*-(1*R*,3*R*)-(+)-1,3-Dimethyl-1,2,3,4-tetrahydroisoquinoline[40] hydrobromide (12·HBr).** A suspension of $LiAlH_4$ (800 mg, 21.1 mmol) in THF (100 mL) was stirred at −78 °C. Dihydroisoquinoline **26** (470 mg, 2.95 mmol) in THF (50 mL) was added dropwise to this mixture. A solution of 2 M $Al(CH_3)_3$ in hexane (10.3 mL, 20.6 mmol) was added dropwise to the reaction mixture, which was stirred for 1 h at −78 °C. The solution was stirred for 1 h at −45 °C, 1 h at −20 °C and 1 h at 0 °C, after which THF (50 mL) and NaF (12.3 g, 292 mmol) were added. After stirring for 3 min, water (4.0 mL) was carefully added dropwise and the mixture was stirred for 30 min. The mixture was filtered and the filtrate extracted with CH_2Cl_2 (3×100 mL). The organic extracts were combined, the solvent removed and the residue subjected to flash chromatography (silica gel) with $CH_2Cl_2/MeOH/NH_4OH_{(aq)}$ (10/1/0.05) to isolate both *cis*- and *trans*-1,3-dimethyl-THIQs. Their hydrobromide salts were formed and isolated as described previously. *Trans*: 253 mg (35.4%); *cis*: 203 mg (28%). Chiral HPLC analysis of the *trans*-1,3-dimethyl-THIQ product indicated that the 3-methyl moiety had racemized during the reduction yielding both *trans* enantiomers **12** and **13**. Compound **12** was separated from the racemic mixture of **12** and **13** (69.7 mg, 0.432 mmol) by one recrystallization with (−)-L-dibenzoyl-tartaric acid monohydrate. The tartrate salt was converted to the hydrobromide salt and recrystallized to yield **12**·HBr as white crystals (12.2 mg, 11.7%); mp = 279–281 °C; $[\alpha]_D^{20} = +12°$ (*c* 2.8, MeOH); ^{1}H NMR ($CDCl_3$, free base) δ 7.10–7.02 (m, 4H, ArH), 4.21 (q, J = 6.9 Hz, H-1), 3.30–3.23 (m, 1H, H-3), 2.77 (dd, J = 16.2 z, J = 3.9 Hz, 1H, H-4), 2.43 (dd, J = 16.2 Hz, J = 9.9 Hz, 1H, H-4), 1.44 (d, J = 6.9 Hz, 3H, 1-CH_3), 1.19 (d, J = 6.9 Hz, 3H, 3-CH_3); ^{13}C NMR (CD_3OD, salt) δ 133.2, 130.8, 129.2, 128.2, 127.4, 126.6, 50.9, 45.2, 33.2. 19.8, 17.6; CIMS *m*/*z* (relative intensity) 162 ($M^+ + 1$, 100), 146 (80), 117 (25). Anal. calcd for $C_{11}H_{15}N{\cdot}HBr$: C, 54.56; H, 6.66; N, 5.78. Found: C, 54.45; H, 6.63; N, 5.57.

***trans*-(1*S*,3*S*)-(−)-1,3-Dimethyl-1,2,3,4-tetrahydroisoquinoline[40] hydrobromide (13·HBr).** Compound **13** was isolated by recrystallization of the racemic mixture of *trans* THIQs **12** and **13** (50.8 mg, 0.315 mmol) with (+)-L-dibenzoyl tartaric acid monohydrate. The tartrate salt was converted to the hydrobromide salt and recrystallized as described previously to yield the title compound as white crystals (10.9 mg, 14.3%); mp = 279–281 °C; $[\alpha]_D^{20} = -15°$ (*c* 1.8, MeOH). Anal. calcd for $C_{11}H_{15}N{\cdot}HBr$: C, 54.56; H, 6.66; N, 5.78. Found: C, 54.71; H, 6.54; N, 5.51.

***cis*-(1*S*,3*R*)-(−)-1,3-Dimethyl-7-nitro-1,2,3,4-tetrahydroisoquinoline hydrobromide (14·HBr).** Compound **10** (450 mg, 2.80 mmol) was dissolved in concd H_2SO_4 (12 mL). The solution was cooled in an ice bath and KNO_3 (285 mg, 2.80 mmol) was added. The reaction mixture was stirred overnight at room temperature. The reaction solution was carefully poured onto ice, extracted with CH_2Cl_2 (3×30 mL), the combined organic extracts washed with brine and dried over anhydrous Na_2SO_4. The solvent was removed to yield a brown oil, which was purified on a silica gel column with $CH_2Cl_2/EtOAc/NH_4OH_{(aq)}$ (20/1/0.1) to give a yellow oil, which was converted to the hydrobromide salt by the addition of methanolic HBr. The solvent was removed and the HBr salt was recrystallized from MeOH/EtOAc to yield yellow needle-like crystals (185 mg, 32.1%); mp 290–292 °C; $[\alpha]_D^{20} = -31°$ (*c* 10, MeOH); ^{1}H NMR (CD_3OD) δ 8.28 (s, 1H, H-8), 8.20–8.17 (m, 1H, H-6), 7.54–7.52 (m, 1H, H-5), 4.87–4.79 (m, 1H, H-1), 3.78–3.74 (m, 1H, H-3), 3.36–3.30 (m, 1H, H-4), 3.14–3.06 (m, 1H, H-4), 1.44 (d, J = 6.8 Hz, 3H, 1-CH_3), 1.19 (d, J = 6.5 Hz, 3H, 3-CH_3); ^{13}C NMR (CD_3OD) δ 147.7, 139.6, 134.9, 130.6, 122.8, 121.1, 52.9, 50.2, 33.9, 18.1, 17.7; CIMS *m*/*z* (relative intensity) 207 ($M^+ + 1$, 100), 191 (20). Anal. calcd for $C_{11}H_{14}NO_2{\cdot}HBr$: C, 46.01; H, 5.26; N, 9.76. Found: C, 46.00; H, 5.25; N, 9.61.

***cis*-(1*R*,3*S*)-(+)-1,3-Dimethyl-7-nitro-1,2,3,4-tetrahydroisoquinoline hydrobromide (15·HBr).** Synthesis of the hydrobromide salt of **15** was performed using the same conditions as used in the synthesis of **14**. Compound **11** (480 mg, 1.98 mmol) was used as the starting material to yield **15**·HBr as white needle-like crystals (330 mg,

58.1%); mp 290–291 °C; $[\alpha]_D^{20} = +29°$ (*c* 10, MeOH). Anal. calcd for $C_{11}H_{14}NO_2 \cdot HBr$: C, 46.01; H, 5.26; N, 9.76. Found: C, 45.97; H, 5.22; N, 9.38.

***trans*-(1*R*,3*R*)-(+)-1,3-Dimethyl-7-nitro-1,2,3,4-tetrahydroisoquinoline hydrobromide (16·HBr).** The same reaction conditions used for the synthesis and purification of **14** were employed for the synthesis of **16** using the racemic mixture of THIQs **12** and **13** (350 mg, 2.17 mmol) as the starting material. Compound **16** was isolated from the racemic mixture of **16** and **17** by one recrystallization with *R*-(−)-mandelic acid in EtOH/hexanes. The hydrobromide salt was formed in the manner described previously to yield **16**·HBr as white needles (414 mg, 66.6%); mp 293–294 °C; $[\alpha]_D^{20} = +8.3°$ (*c* 0.10, MeOH); ^{1}H NMR (CD_3OD) δ 8.26 (d, $J = 2.2$ Hz, 1H, ArH-8), 8.18 (dd, $J = 2.2$ Hz, $J = 8.5$ Hz, 1H, ArH-6), 7.53 (d, $J = 8.5$ Hz, 1H, ArH-5), 4.90–4.83 (m, 1H, H-1), 3.91–3.87 (m, 1H, H-3), 3.32 (dd, $J = 4.8$ Hz, $J = 18.1$ Hz, 1H, H-4), 2.96 (dd, $J = 10.2$ Hz, $J = 18.1$ Hz, 1H, H-4), 1.72 (d, $J = 6.9$ Hz, 3H, 1-CH_3), 1.49 (d, $J = 6.6$ Hz, 3H, 3-CH_3). Anal. calcd for $C_{11}H_{14}NO_2 \cdot HBr$: C, 46.01; H, 5.26; N, 9.76. Found: C, 46.06; H, 5.00; N, 9.93.

***trans*-(1*S*,3*S*)-(−)-1,3-Dimethyl-7-nitro-1,2,3,4-tetrahydroisoquinoline hydrobromide (17·HBr).** The same conditions that were used for the isolation of **16** were employed for the isolation of **17** using the racemic mixture of **16** and **17** (290 mg, 1.80 mmol) crystallized with S-(+)-mandelic acid in EtOH/hexanes. Compound **17**·HBr was formed in the manner described previously and isolated as white needles (272 mg, 52.7%); mp 293–294 °C; $[\alpha]_D^{20} = -15°$ (*c* 0.10, MeOH). Anal. calcd for $C_{11}H_{14}NO_2 \cdot HBr$: C, 46.01; H, 5.26; N, 9.76. Found: C, 46.14; H, 5.03; N, 9.65.

***cis*-(1*S*,3*R*)-(−)-1,3-Dimethyl-7-bromo-1,2,3,4-tetrahydroisoquinoline hydrobromide (18·HBr).** In a 250 mL Parr shaker bottle, **14**·HBr (115 mg, 0.40 mmol was dissolved in MeOH (50 mL). PtO_2 (30 mg) was added and the mixture was hydrogenated at 50 psi for 4 h. The reaction mixture was filtered and the solvent removed. The residue was dissolved in ice-cold 48% $HBr_{(aq)}$ (0.2 mL) and water (0.7 mL). To this mixture, a solution of sodium nitrite (30 mg, 0.435 mmol) in water (0.7 mL) was added dropwise. After 30 min, excess HNO_2 was destroyed by the addition of urea (a negative starch-iodide test was obtained at this time). The resultant solution was added to a well stirred mixture of CuBr (172 mg, 1.20 mmol), 48% HBr (0.4 mL) and water (1 mL) at 35 °C. After this addition, the reaction mixture was warmed to 75–80 °C and stirred at this temperature for 1.5 h. The reaction mixture was cooled to room temperature and stirred overnight. The solution was adjusted to pH >10 with 25% $NaOH_{(aq)}$. EtOAc (50 mL) was added to solubilize the product and the blue copper salts were removed by filtration through Celite. The Celite was washed with EtOAc (2×20 mL). The filtrate was extracted with ethyl acetate (3×50 mL), the combined organic extracts dried over anhydrous Na_2SO_4 and evaporated to give a yellowish oil. This crude product was dissolved in CH_2Cl_2 and a methanolic HBr solution was added to form the HBr salt. The solvent was removed and the residue recrystallized from MeOH/EtOAc to yield the title compound as yellow crystals (55 mg, 44.1%); mp = 283–285 °C; $[\alpha]_D^{20} = -66°$ (*c* 10.5, MeOH); ^{1}H NMR (CD_3OD) δ 7.52 (s, 1H, ArH-8), 7.42 (d, $J = 8.2$ Hz, 1H, ArH-6), 7.14 (d, $J = 8.2$ Hz, 1H, ArH-5), 4.62 (q, $J = 6.9$ Hz, 1H, H-1), 3.62–3.56 (m, 1H, H-3), 3.07 (dd, $J = 4.5$ Hz, $J = 17.4$ Hz, 1H, H-4), 2.85 (dd, $J = 11.7$ Hz, $J = 17.4$ Hz, 1H, H-4), 1.72 (d, $J = 6.9$ Hz, 3H, 1-CH_3), 1.49 (d, $J = 6.6$ Hz, 3H, 3-CH_3); ^{13}C NMR (CD_3OD, salt) δ 135.4, 113.3, 131.1, 130.9, 128.7, 121.0, 52.7, 50.4, 33.4, 18.2, 17.8; CIMS *m*/*z* (relative intensity) 242 ($M^+ + 2$, 100), 240 (M^+, 100), 226 (25), 224 (25). Anal. calcd for $C_{11}H_{14}NBr \cdot HBr$: C, 41.15; H, 4.71; N, 4.36. Found: C, 41.48; H, 5.07; N, 4.15.

***cis*-(1*R*,3*S*)-(+)-1,3-Dimethyl-7-bromo-1,2,3,4-tetrahydroisoquinoline hydrobromide (19·HBr).** The synthesis of **19** was performed in the same manner as **18** using the hydrobromide salt of **15** (235 mg, 0.819 mmol) as the starting material. The hydrobromide salt of the title compound was isolated as yellow crystals (151 mg, 57.4%); mp 282–284 °C; $[\alpha]_D^{20} = +64°$ (*c* 10.5, MeOH). Anal. calcd for $C_{11}H_{14}NBr \cdot HBr$: C, 41.15; H, 4.71; N, 4.36. Found: C, 41.14; H, 5.00; N, 4.20.

***trans*-(1*R*,3*R*)-(+)-1,3-Dimethyl-7-bromo-1,2,3,4-tetrahydroisoquinoline hydrobromide (20·HBr).** The synthesis of **20** was performed and purified in the same manner as **18** using the racemic mixture of the hydrobromide salts of **16** and **17** (305 mg, 1.06 mmol) as the starting material. The racemic mixture of **20** and **21** was isolated as their HBr salts to give a yellow crystal (165 mg, 48.5%). Compound **20** was isolated by one crystallization of the racemic mixture of **20** and **21** (34.9 mg, 0.145 mmol) with *R*-(−)-mandelic acid in EtOH/hexanes. The diastereomeric salt was isolated and the free base **20** was reformed. The HBr salt was formed in the manner described previously to yield the title compound **20**·HBr as off-white crystals (14.4 mg, 25.3%); mp 240–242 °C; $[\alpha]_D^{20} = +9.0°$ (*c* 0.10, MeOH); ^{1}H NMR (CD_3OD) δ 7.46 (d, $J = 2.0$ Hz, 1H, ArH-8), 7.40 (dd, $J = 2.0$ Hz, $J = 8.2$ Hz, 1H, ArH-6), 7.12 (d, $J = 8.2$ Hz, 1H, ArH-5), 4.69 (q, $J = 6.9$ Hz, 1H, H-1), 3.90–3.78 (m, 1H, H-3), 3.13 (dd, $J = 4.8$ Hz, $J = 17.5$ Hz, 1H, H-4), 2.78 (dd, $J = 10.5$ Hz, $J = 17.5$ Hz, 1H, H-4), 1.65 (d, $J = 6.9$ Hz, 3H, 1-CH_3), 1.45 (d, $J = 6.3$ Hz, 3H, 3-CH_3); ^{13}C NMR (CD_3OD, salt) δ 135.6, 131.3, 131.1, 130.2, 129.6, 120.7, 50.5, 45.1, 32.8, 19.7, 17.6 Anal. calcd for $C_{11}H_{14}NBr \cdot HBr$: C, 41.15; H, 4.71; N, 4.36. Found: C, 41.14; H, 5.00; N, 4.20.

***trans*-(1*S*,3*S*)-(−)-1,3-Dimethyl-7-bromo-1,2,3,4-tetrahydroisoquinoline hydrobromide (21·HBr).** Compound **21** was isolated using the same conditions that were used for the isolation of **20**, except the racemic mixture of **20** and **21** (67.9 mg, 0.282 mmol) was recrystallized with *S*-(+)-mandelic acid in EtOH/hexanes. The diastereomeric salt was isolated and the free base **21** reformed. The hydrobromide salt of **21** was made in the same manner as described earlier to yield **21**·HBr as off-white crystals (16.3 mg, 18.0%); mp 241–243 °C; $[\alpha]_D^{20} = -12.0$ (*c* 0.22, MeOH). Anal. calcd for $C_{11}H_{14}NBr \cdot HBr$: C, 41.15; H, 4.71; N, 4.36. Found: C, 41.30; H, 4.36; N, 4.24.

Determination of enantiomeric excess

Enantiomeric excess (ee) was determined for compounds **10**–**21** to be greater than 95% and was assessed in the following manner. Using Chiral HPLC analysis eluting with hexanes/isopropyl alcohol/$NH(Et)_2$ (90/10/1), all resolved compounds appeared to be a single distinct peak. However, for the racemic mixtures of these compounds, baseline separation could not be obtained due to tailing caused by the THIQ amine. Several solutions of each isolated enantiomer (2.0 mg in 5 mL isopropyl alcohol) were prepared and mixed in the following proportions, (95/5, 97.5/2.5 and 98.75/1.25). These solutions were analyzed and it was found each enantiomer could easily be detected as a shoulder in the 97.5/2.5 mixture, but not in the 98.75/1.25 mixture, which implied that the ee of each of these compounds was at a minimum greater than 95%.

Radiochemical assay for PNMT activity

The assay used for this study has been described previously.[37] A normal assay mixture consists of 50 μL of 0.5 M phosphate buffer (pH 8.0), 25 μL of 10 mM AdoMet, 5 μL of [^{3}H]AdoMet, that contains 3×10^5 dpm (specific activity approximately 15 mCi/mmol), 25 μL of substrate solution (phenylethanolamine), 25 μL of inhibitor solution, 25 μL of the enzyme preparation, and water to achieve a total volume of 250 μL. The mixture is incubated for 30 min at 37 °C, and quenched with the addition of 250 μL of 0.5 M borate buffer (pH 10) and the mixture extracted with 2 mL of toluene/isoamyl alcohol (7/3). A 1-mL aliquot of the organic layer is removed, transferred to a scintillation vial and diluted with cocktail for counting. The mode of inhibition for all of the inhibitors assayed was determined to be competitive by inspection of the 1/V versus 1/S plots of the data. All assays were run in duplicate with 3 inhibitor concentrations over a five fold range. K_i values were determined by a hyperbolic fit of the data.

α_2-Adrenoceptor radioligand binding assay

The radioligand binding assay was performed using the methods developed by U'Prichard et al.[38] Male Sprague Dawley rats were decapitated and the cortexes removed and homogenized with 20 volumes (w/v) of ice-cold 50 mM Tris/HCl buffer (pH 7.7 at 25 °C). Homogenates were centrifuged three times for 10 min at $50{,}000\times g$ 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 ca. 19.2 mCi/mmol, final concentration 2.0 nM), various concentrations of the inhibitors, and an aliquot of freshly suspended tissue (800 μL) to 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 50 mM Tris/HCl buffer (pH 7.7 at 25 °C). The filters were counted in vials containing premixed scintillation cocktail. Non-specific binding was determined as the concentration of ligand bound in the presence of 2 μM phentolamine. All assays 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 of the Hill coefficients were approximately equal to one.

X-ray crystallography

Crystals of **21**·(*S*)-mandelic acid were recrystallized from EtOH/hexanes. A clear prism crystal of approximate dimensions $0.30\times0.10\times0.40$ mm was used for data collection.

Crystal data. $C_{19}H_{22}O_3NBr$, $M = 392.29$, orthorhombic, $a = 8.920$ (3), $b = 25.015$ (2), $c = 8.255$ (2) Å, $V = 1842.0$ (6) Å^3, space group $P2_12_12_1$ (#19), $Z = 4$, $D_c = 1.415$ g/cm^3, $\mu(CuK\alpha) = 31.75$ cm^{-1}, $F(000) = 808.00$. Cell constants and an orientation matrix for data collection, obtained from a least-squares refinement using the setting angles of 15 carefully centered reflections in the range $33.75 < 2\theta < 35.79°$. The data were collected at a temperature of 230 ± 1 °C using the ω-2θ scan technique to a maximum 2θ value of 120.1°. Omega scans of several intense reflections, made prior to data collection, had an average width at half-height of 0.43 with a take-off angle of 6.0°. Scans of $(1.37 + 0.30 \tan\theta)°$ were made at a speed of 16.0°/min (in ω). The weak reflections ($I < 12.0\sigma(I)$) were rescanned (maximum of 3 scans) and the counts were accumulated to ensure good counting statistics. Stationary background counts were recorded on each side of the reflection. The ratio of peak counting time to background counting time was 2/1. The diameter of the incident beam collimator was 0.5 mm, the crystal to detector distance was 400 mm, and the detector aperture was 6.0×6.0 mm (horizontal×vertical).

Data reduction. Of the 3216 reflections which were collected, 1631 were unique ($R_{int} = 0.086$); equivalent reflections were merged. The intensities of three representative reflection were measured after every 150 reflections. Over the course of data collection, the standards decreased by 3.3%. A linear correction factor was applied to the data to account for this phenomenon.

The linear absorption coefficient, μ, for Cu-Kα radiation is 31.8 cm^{-1}. An empirical absorption correction based on azimuthal scans of several reflections was applied which resulted in transmission factors ranging from 0.89 to 1.00. The data were corrected for Lorentz and polarization effects.

Structure solution and refinement. The structure was solved by direct methods[41] and expanded using Fourier techniques.[42] The non-hydrogen atoms were refined anisotropically. Hydrogen atoms were included but not refined. The final cycle of full-matrix least-squares refinement[43] was based on 2274 observed reflections ($I > 3.00\sigma(I)$) and 217 variable parameters and converged (largest parameter shift was 0.00 times its esd) with unweighted and weighted agreement factors of:

$$R = \Sigma \| F_o | - | F_c \| / \Sigma | F_o | = 0.069$$

$$R_w = [(\Sigma_w(|F_o| - |F_c|)^2/\Sigma_w F_o^2)]^{1/2} = 0.074$$

The standard deviation of an observation of unit weight[44] was 2.84. The weighting scheme was based on counting statistics and included a factor ($p = 0.020$) to downweight the intense reflections. Plots of Σ_w ($|F_o| - |F_c|)^2$ versus $|F_o|$, reflection order in data collection, $\sin\theta/\lambda$ and various classes of indices showed no unusual trends. The maximum and minimum peaks on the final difference Fourier map corresponded to 0.64 and -0.63 $e^-/Å^3$, respectively.

Neutral atom scattering factors were taken from Cromer and Waber.[45] Anomalous dispersion effects were included in F_{calc};[46] the values for $\Delta f'$ and $\Delta f''$ were those of Creagh and McAuley.[47] The values for the mass attenuation coefficients are those of Creagh and Hubbell.[48] All calculations were performed using the teXsan[49] crystallographic software package of Molecular Structure Corporation.

Supplementary material available

Final fractional coordinates and equivalent isotopic temperature factors, bond distances and angles, anisotropic thermal parameters, torsion angles, and selected least-squares planes are reported (6 tables).

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References and Notes

1. This paper is dedicated to the memory of Sir D. H. R. Barton for the fundamental contributions he made in conformation and its importance in chemistry. It was Barton, at a cocktail party following a research seminar at the University of Wisconsin in the 1950s, who discussed with Edward E. Smissman the thoughts Barton had of the importance of conformation to organic chemistry that sparked Smissman's realization of the relevance of conformation to the interaction of drugs with receptors. Smissman embarked on his highly successful research in the 1950s and 1960s to exploit this with the design and synthesis of conformationally-restricted (rigid) analogues of drugs and neurotransmitters. Today the concept of rigid analogues in drug design is taken for granted, and it was Smissman, later reinforced by conversations this author had with Barton, that sparked my own interest in applications of the concept of rigid analogues to an understanding of the molecular mechanism of action of amphetamine, and further to the subject of this paper. Both men were giants in their field, and it is noteworthy that there was a synergism in the recognition of the work of one by the other in ways that neither would have seen alone.
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