Exploring the Structure−Activity Relationship of the Ethylamine Portion of 3-Iodothyronamine for Rat and Mouse Trace Amine-Associated Receptor 1

Edwin S. Tan,[†] Motonori Miyakawa,[‡] James R. Bunzow,[§] David K. Grandy,[§] and Thomas S. Scanlan*[,‡]

Chemistry and Chemical Biology Graduate Program and Departments of Pharmaceutical Chemistry and Cellular & Molecular Pharmacology, University of California at San Francisco, 600 16th Street, San Francisco, California 94143-2280, and Department of Physiology and Pharmacology, Oregon Health and Science University, 3181 Southwest Sam Jackson Park Road, Mail Code L334, Portland, Oregon 97239

Received January 12, 2007

3-Iodothyronamine (**1**, T_1AM) is a naturally occurring derivative of thyroid hormone that can potently activate the orphan G protein-coupled receptor (GPCR) known as the trace amine-associated receptor 1 ($TAAR_1$). We have previously found that modifying the outer ring of the phenoxyphenethylamine core scaffold of **1** can improve potency and provide potent agonists. In this study, we explored the tolerance of rat and mouse $TAAR_1$ ($rTAAR_1$ and $mTAAR_1$) for structural modifications in the ethylamine portion of **1**. We found that incorporating unsaturated hydrocarbon substituents and polar, hydrogen-bond-accepting groups were beneficial for $rTAAR_1$ and $mTAAR_1$, respectively, providing compounds that were equipotent or more potent than **1**. Additionally, we have discovered that a naphthyl group is an excellent isosteric replacement for the iodophenyl ring of **1**.

Introduction

3-Iodothyronamine (**1,** T_1AM;[a] Figure 1), a decarboxylated and deiodinated metabolite of thyroxine (**T_4**; Figure 1**)** and 3,3′,5-triiodothyronine (**T_3**; Figure 1**)**, is an endogenous compound that can potently activate the orphan G protein-coupled receptor (GPCR) known as the trace amine associated receptor 1 ($TAAR_1$).[1,4,5] Compound **1** activates rat and mouse $TAAR_1$ ($rTAAR_1$ and $mTAAR_1$) with effective concentrations for half-maximal stimulation (EC_{50}) of 14 nM and 112 nM, respectively.

Initial pharmacological studies by Bunzow et al.[3] showed that rat $TAAR_1$ can tolerate a diverse set of structural moieties in the alkylamine portion of the ligands. It can be maximally activated by compounds having simple ethylamine groups (i.e., tyramine, phenethylamine, 4-hydroxyamphetamine) or complex, rigid, and/or highly functionalized alkylamines [i.e., dihydroergotamine, agroclavine, *R*-(−)-apomorphine].

Previously we have demonstrated that $rTAAR_1$ and $mTAAR_1$ can tolerate different alkylation states at the charged amine and changes in both the outer and inner rings of the phenoxyphenethylamine core scaffold of **1**.[2] 3-Iodophenyltyramine (EC_{50} = 2.4 nM) and *N*-methyl-4′-fluoro-3-iodofluorophenyltyramine (EC_{50} = 2.7 nM) were found to be the most potent compounds for $rTAAR_1$ and *N*-methyl-*p*-trifluorobenzyltyramine (EC_{50} = 12 nM) for $mTAAR_1$. In this study, we explore the structure−activity relationship (SAR) of the ethylamine portion and expand the SAR of the phenoxyphenethylamine core of **1**. Here we report four distinct scaffolds that are equipotent or more potent than **1**.

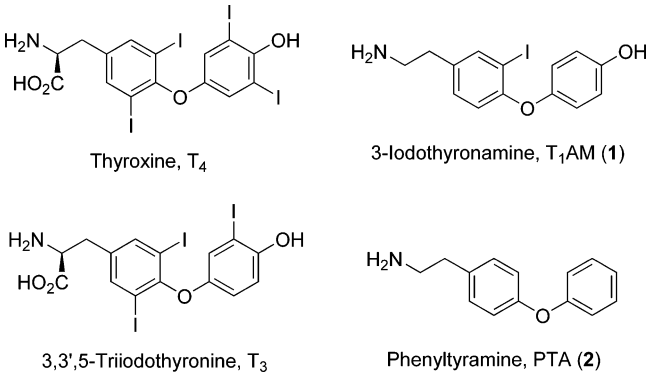


Figure 1. Structures of thyroxine (T_4), 3,3′,5-triiodothyronine (T_3), 3-iodothyronamine (**1**, T_1AM), and phenyltyramine (**2**, PTA).

Results

Synthesis. Since $rTAAR_1$ and $mTAAR_1$ are homologous to the β-adrenergic (βAR) and dopamine receptors, we explored their tolerance for structural features commonly found in existing βAR and dopamine receptor agonists and antagonists. These structural motifs were incorporated into phenyltyramine (**2**, PTA; Figure 1) rather than **1** because omitting the outer-ring hydroxyl and inner-ring iodine significantly simplified the syntheses.

The aryloxypropylamines (Scheme 1) contain an oxymethylene bridge between the aromatic and ethylamine groups as found in the β blockers alprenolol, pindolol, and propranolol (Figure 2). Compounds **3** (ET-1) and **5** (ET-11) were readily synthesized by coupling Boc-3-bromopropylamine (**29**) with *p*- and *m*-phenoxyphenol to provide ethers **32** and **33**, respectively. Boc deprotection with 3 N anhydrous HCl gave the hydrochloride salts of **3** and **5** in good yields. Mono-*N*-methyl **6** (ET-12) was obtained by reacting **32** with NaH and MeI followed by acid deprotection. Di-*N*-methyl **4** (ET-6) was synthesized by Eschweiler−Clarke reaction of the free amine of **3**.[6]

The benzamidoalkylamines (Scheme 2) contained an amide linker between the aromatic and ethylamine portion of the phenoxyphenethylamine scaffold, similar to dopamine receptor antagonists metoclopramide, nafadotride, and sulpiride (Figure 2). The length of the carbon chain connecting the basic and

* To whom correspondence should be addressed: phone (503) 494-9292; fax (503) 494-9275; e-mail scanlant@ohsu.edu.

† Chemistry and Chemical Biology Graduate Program, UCSF.

‡ Departments of Pharmaceutical Chemistry and Cellular & Molecular Pharmacology, UCSF.

§ Oregon Health and Science University.

[a] Abbreviations: T_1AM, 3-iodothyronamine; $TAAR_1$, trace amine-associated receptor 1; $rTAAR_1$, rat trace amine-associated receptor 1; $mTAAR_1$, mouse trace amine-associated receptor 1; $hTAAR_1$, human trace amine-associated receptor 1; SAR, structure−activity relationship; GPCR, G protein-coupled receptor; βAR, β-adrenergic receptor; PTA, phenyltyramine; HEK293, human embryonic kidney 293; cAMP, cyclic adenosine monophosphate; EFC, enzyme fragment complementation.

10.1021/jm0700417 CCC: $37.00
Published on Web 05/12/2007

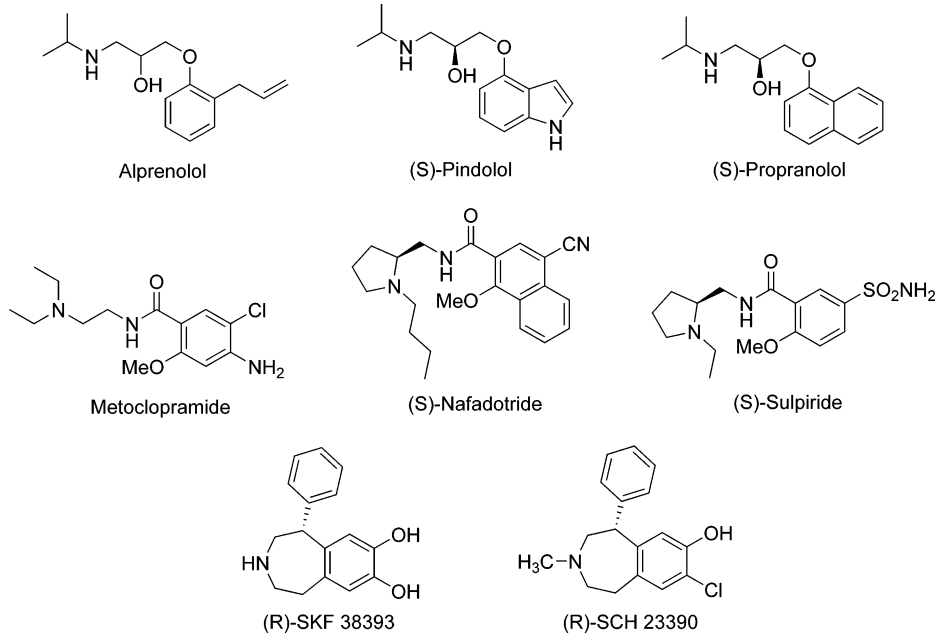
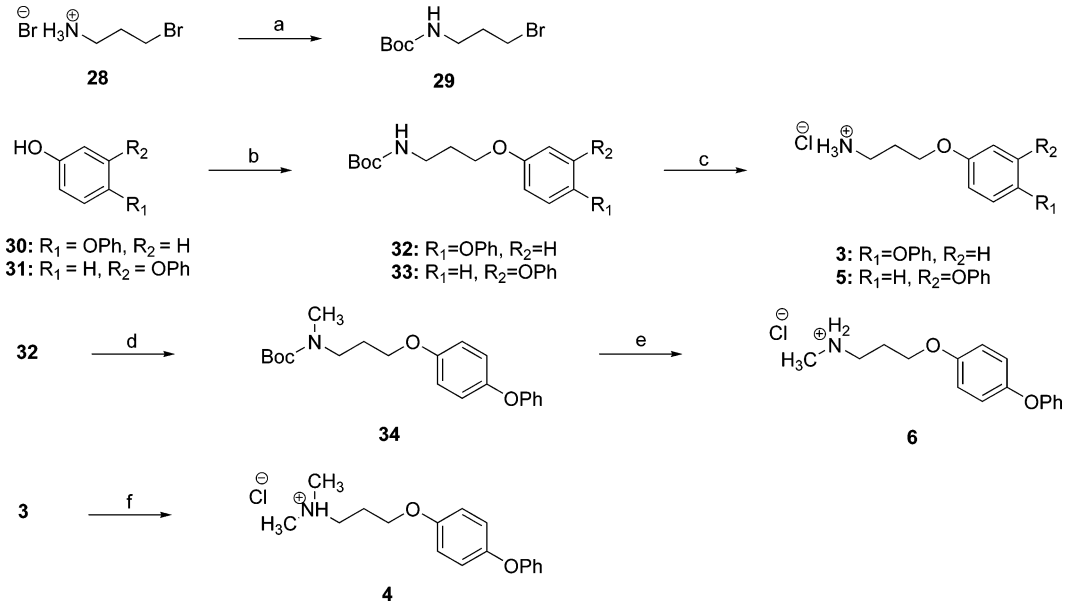


Figure 2. Structures of β-adrenergic receptor (alprenolol, pindolol, and propranolol) and dopamine receptor (metoclopramide, nafadotride, sulpiride, SKF 38393, and SCH 23390) ligands used as models for $TAAR_1$ ligand design.

Scheme 1. Synthesis of Aryloxypropylamines[a]



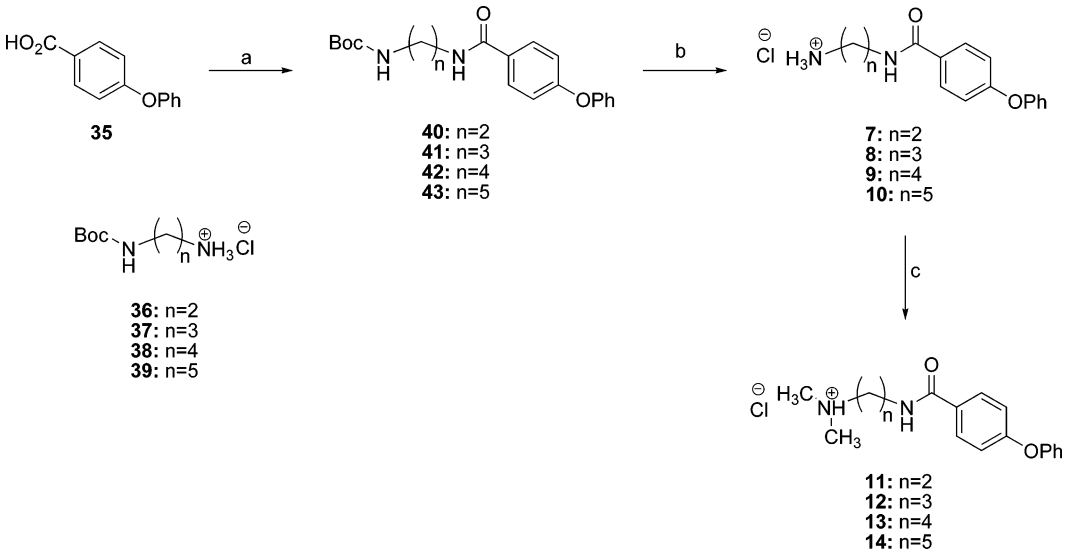
[a] Reagents and conditions: (a) Boc_2O, $NaHCO_3$, THF/H_2O, 78%. (b) (i) NaH, DMF, 0 °C; (ii) **29**, DMF, 83−91%. (c) 3 N HCl (anhydrous in EtOAc), 53−75%. (d) (i) NaH, DMF, 0 °C. (ii) MeI, 62%. (e) 3 N HCl (anhydrous in EtOAc), 90%. (f) (i) K_2CO_3, H_2O; (ii) H_2CO, HCO_2H, 80 °C; (iii) 3 N HCl (anhydrous in EtOAc), 76%.

amide nitrogen was varied from two to five carbons. Reaction of the acyl chloride of 4-phenoxybenzoic acid and previously reported monoprotected alkyldiamines **36**−**39**[7] provided amides **40**−**43**. Boc deprotection yielded the hydrochloride salts of **7**−**10** (ET-2 to ET-5) in poor to excellent yields. Dimethylated **11**−**14** (ET-7 to ET-10) were synthesized through an Eschweiler−Clarke reaction.

The β-phenylphenoxyphenethylamines (Scheme 3) and tetrahydrobenzazepines (Scheme 4) were modeled after the prototypical dopamine receptor ligands SKF 38393 and SCH 23390 (Figure 2). Compounds **15** (ET-13) and **16** (ET-14) have a β-phenyl ring alone, while **17** (ET-17) and **18** (ET-20) possess a seven-membered ring in addition to the β-phenyl ring. The reaction of the organolithium species of 4-bromodiphenyl ether (**44**) with benzaldehyde and phenyl lithium with 3-phenoxybenzaldehyde (**45**) gave the dibenzylic alcohols **46** and **47**, respectively (Scheme 3). Initial attempts at converting this alcohol into a nitrile group by nucleophilic displacement of the corresponding mesylate with sodium cyanide were unsuccessful due to the sterically congested nature of the dibenzylic alcohol. Generating the chloride followed by reaction with $TiCl_4$ and trimethylsilylcyanide (TMSCN) successfully provided nitriles **48** and **49**.[8] Nitrile reduction with lithium aluminum hydride and treatment with acid yielded the hydrochloride salts **15** and **16** in poor to modest yield.

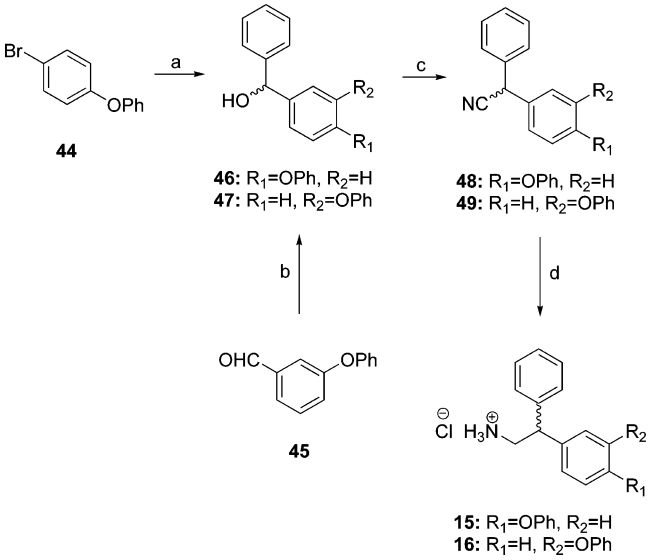
Tetrahydrobenzazepines **53** and **55** were synthesized following the published procedure for known compounds **52** and **54** (Scheme 4).[9] Reacting 3-methoxyphenethylamine (**50**) with neat

Scheme 2. Synthesis of Benzamidoalkylamines[a]



[a] Reagents and conditions: (a) (i) $SOCl_2$, DCM, DMF, reflux; (*ii*) **36**−**39**, pyridine, DCM, 65−90%. (b) 3 N HCl (anhydrous in EtOAc), 96−100%. (c) (i) K_2CO_3, H_2O; (ii) H_2CO, HCO_2H, 80 °C; (iii) 3 N HCl (anhydrous in EtOAc), 11−98%.

Scheme 3. Synthesis of β-Phenylphenoxyphenethylamines[a]



[a] Reagents and conditions: (a) (i) *n*BuLi, THF, −78 °C; (ii) benzaldehyde, 97%. (b) PhLi, THF, −78 °C, 86%. (c) (i) $SOCl_2$, DCM; (ii) TMSCN, $TiCl_4$, DCM, 90−98%. (d) (i) $LiAlH_4$, THF, reflux; (ii) 3 N HCl (anhydrous in EtOAc), 26−47%.

styrene oxide gave benzylic alcohol **51**. Treatment with trifluoroacetic acid in the presence of catalytic amounts of sulfuric acid induced cyclization to form **53**. Compound **53** was obtained as an inseparable mixture with an unknown product. Methyl ether deprotection with boron tribromide followed by acid exposure gave pure hydrochloride salts of **55**. Boc protection of **54** and **55** followed by copper(II)-mediated coupling with phenylboronic acid provided biaryl ethers **58** and **59**.[10] Boc deprotection yielded **17** (ET-17) and **18** (ET-20) as hydrochloride salts.

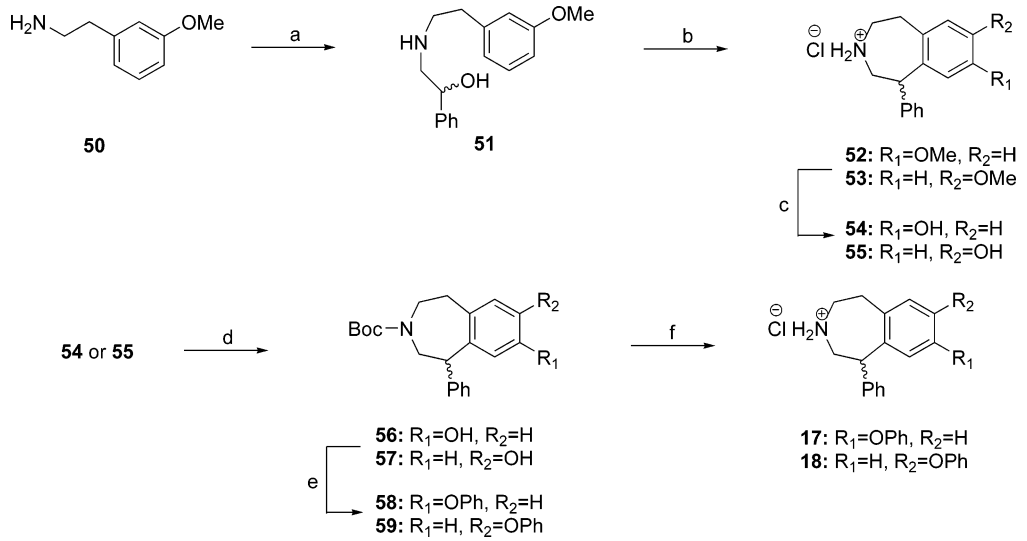
To further explore the steric constraints around the β-carbon, additional phenoxyphenethylamine derivatives possessing smaller and bulkier substituents at the β-carbon were synthesized (Scheme 5). Triisopropyl- (**62**) or *tert*-butyldimethylsilyl- (**61**) protected 4-hydroxybenzylnitrile was treated with lithium diisopropylamine and methyl iodide or benzyl bromide to give **63**, **64**, or **65**. The nitrile was reduced with $LiAlH_4$−$AlCl_3$[11] and subsequently reacted with Boc anhydride to provide Boc-protected amines **66**−**68**. Silyl deprotection with TBAF followed by copper(II)-mediated biaryl ether formation with phenylboronic acid yielded **72**−**74**. Acid deprotection resulted in biaryl ether hydrochloride salts **19** (MM-7), **20** (MM-10), and **21** (MM-13) in excellent yields.

Compounds **22** (MM-14) and **23** (MM-15) were both synthesized from commercially available 4-phenoxybenzaldehyde (**75**). Piperidine-catalyzed condensation reaction with Meldrum's acid provided intermediate **76**. Michael addition of phenylacetylide and trimethylsilyl acetylide gave **77** and **78**, respectively, in good yields.[12] Acetal deprotection and thermal decarboxylation yielded acids **79** and **80**. Base-mediated deprotection of **80** gave terminal alkyne **81**. Curtius rearrangement followed by acid exposure resulted in hydrochloride salts **22** and **23** in low yields.

Previously, we found that having a methyl group rather than an iodine group at the 3-position of **1** was well tolerated in both $rTAAR_1$ and $mTAAR_1$.[2] Compared to **1**, the potency of 3-methylthyronamine was only 2-fold lower in $rTAAR_1$ and essentially unaffected in $mTAAR_1$. The phenoxynaphethylamines (Scheme 6) were developed as another halogen-free derivative to further probe the binding pocket occupied by the iodine group of **1**. Copper(II)-mediated coupling of 4-hydroxynaphthadehyde (**82**) with phenylboronic acid provided biaryl ether aldehyde **83**. Following $NaBH_4$ reduction, the resulting alcohol was converted into a nitrile by treatment with thionyl chloride followed by reaction with $TiCl_4$ and TMSCN. Nitrile **85** was then reduced with $LiAlH_4$ and exposed to acid to generate hydrochloride salt **24** (ET-21) in modest yield.

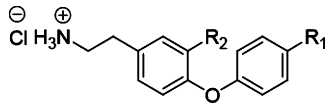
A phenyl group was incorporated into the β-carbon of the naphethylamine scaffold to determine if adding a phenyl ring would enhance the potency of the naphethylamine scaffold as observed with **15** and **16**. Reaction of commercially available 4-methoxynaphthaldehyde (**86**) with phenylmagnesium bromide quantitatively provided dibenzylic alcohol **87**. Treatment with thionyl chloride followed by $TiCl_4$ and TMSCN provided nitrile **88**. Nitrile reduction with $LiAlH_4$ and exposure to acid gave the primary amine hydrochloride salt **26** (ET-32). Methyl ether deprotection with BBr_3 and acid treatment precipitated naphthol **25** (ET-31). The Boc-protected amine **89** was subjected to

Scheme 4. Synthesis of Tetrahydrobenzazepines[a]



[a] Reagents and conditions: (a) styrene oxide, reflux, 36%. (b) TFA, H_2SO_4, reflux. (c) (i) BBr_3, DCM; (ii) 3 N HCl (anhydrous in EtOAc), 40%. (d) Boc_2O, $NaHCO_3$, THF/H_2O, 61−81%. (e) $PhB(OH)_2$, $Cu(OAc)_2$, *i*Pr_2EtN, pyridine, 4 Å molecular sieves, DCM, 94−100%. (f) 3 N HCl (anhydrous in EtOAc), 85−90%.

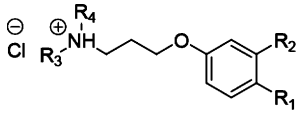
Table 1. Activity of **1** and **2** on $rTAAR_1$ and $mTAAR_1$



			$rTAAR_1$			$mTAAR_1$		
compd	R_1	R_2	EC_{50}[a] ± SEM (nM)	E_{max}[b] ± SEM (%)	N[c]	EC_{50}[a] ± SEM (nM)	E_{max}[b] ± SEM (%)	N[c]
1	OH	I	33 ± 3	100 ± 0	5	314 ± 43	100 ± 0	5
2	H	H	63 ± 7	93 ± 4	3	420 ± 66	85 ± 4	3

[a] Values represent the average of N independent experiments in triplicate and were calculated by use of Prism software as described in the Materials and Methods section. [b] E_{max} is the maximum stimulation achieved at a concentration of 10 μM and was calculated by use of Prism software. E_{max} = 100% is defined as the activity of compound **1** at 10 μM. [c] N is the number of independent experiments in triplicate that were performed and used to calculate the EC_{50} and E_{max} values.

Table 2. Activity of Aryloxypropylamines **3**−**6** on $rTAAR_1$ and $mTAAR_1$



					$rTAAR_1$			$mTAAR_1$		
compd	R_1	R_2	R_3	R_4	EC_{50}[a] ± SEM (nM)	E_{max}[b] ± SEM (%)	N[c]	EC_{50}[a] ± SEM (nM)	E_{max}[b] ± SEM (%)	N[c]
3	OPh	H	H	H	758 ± 104	80 ± 3	2	433 ± 154	92 ± 2	2
4	OPh	H	CH_3	CH_3	>1000	36 ± 6	2	464 ± 173	62 ± 2	2
5	H	OPh	H	H	373 ± 9	90 ± 7	2	885 ± 549	78 ± 13	2
6	OPh	H	CH_3	H	370 ± 70	46 ± 12	2	66 ± 12	113 ± 2	2

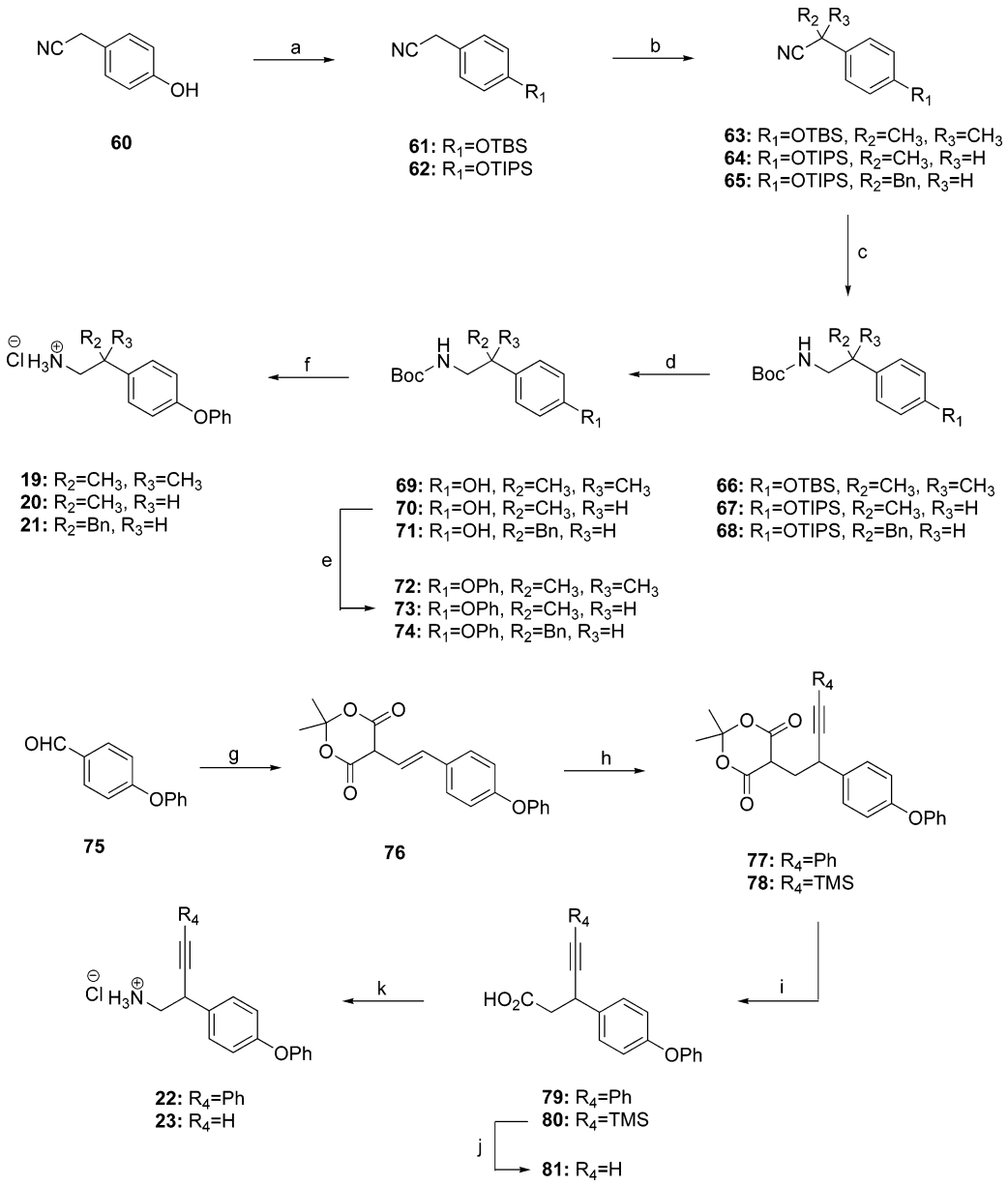
[a−c] See footnotes for Table 1.

copper(II)-mediated coupling to install the biaryl ether group (**90**). Acid deprotection provided hydrochloride salt **27** (ET-33) in good yield.

Receptor Activation. As previously reported, both $rTAAR_1$ and $mTAAR_1$ are coupled to stimulatory G proteins and thus induce cAMP production in HEK293 stable cell lines upon agonist exposure.[3,4] Representative dose-response curves of **1** in $rTAAR_1$ and $mTAAR_1$ are shown in Figure 3. It should be noted that the measured EC_{50} values of **1** (Table 1) are approximately 2- and 3-fold higher in $rTAAR_1$ (EC_{50} = 33 ± 3 nM) and $mTAAR_1$ (EC_{50} = 314 ± 43 nM), respectively, compared to previously reported values due to a change in assay from a radioactive cAMP format to an enzyme fragment complementation (EFC) format.[13,14] However, the rank order potency of compounds was consistent between the two assays. Additionally, the EFC assay appears to have a better signal range, providing the capacity to distinguish compounds that are more efficacious than **1**.

Inserting an oxymethylene bridge between the aromatic and ethylamine portions of **2** had different effects on $rTAAR_1$ and $mTAAR_1$ (Table 2). For $rTAAR_1$, having an oxymethylene bridge was detrimental. Compared to **2**, the potency of **3** decreased ~12-fold (EC_{50} = 758 ± 104 nM, E_{max} = 80% ± 3%). Monomethylating the amine of **3** (**6**) improved the potency ~2-fold but decreased efficacy (EC_{50} = 370 ± 70 nM, E_{max} = 46% ± 12%). Dimethylation of **3** decreased both potency and efficacy (EC_{50} > 1 μM, E_{max} = 36% ± 6%). This trend was consistent with previous SAR for $rTAAR_1$, suggesting that

Scheme 5. Synthesis of β-Substituted Phenoxyphenethylamines[a]



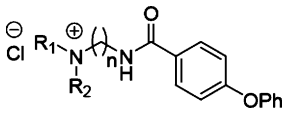
[a] Reagents and conditions: (a) TBSCl or TIPSCl, imidazole, THF, 0 °C, 84−98%. (b) LDA, MeI or BnBr, −78 °C to rt, 75−98%. (c) (i) $LiAlH_4$, $AlCl_3$, THF; (ii) Boc_2O, $NaHCO_3$, THF/H_2O, 46−86%. (d) TBAF, THF, 0 °C, 91−96%. (e) $PhB(OH)_2$, $Cu(OAc)_2$, *i*Pr_2EtN, pyridine, 4 Å molecular sieves, DCM, 37−46%. (f) 3 N HCl (anhydrous in EtOAc), 92−94%. (g) Meldrum's acid, piperidine, benzene, reflux, 70%. (h) PhCCLi or TMSCCH and *n*BuLi, THF, −78 °C, 76−77%. (i) (i) 3 N HCl (anhydrous in EtOAc); (ii) dimethyl acetamide, 135 °C, 73−98%. (j) NaOH, MeOH, 76%. (k) (i) $ClCO_2Et$, NaN_3, Et_3N, acetone; (ii) toluene, reflux; (iii) 3 N HCl (anhydrous in EtOAc), 36−38%.

monomethylation of the amine can enhance potency and is preferred over dimethylation.[2] Interestingly, moving the phenoxy group of **3** from the para to the meta position (**5**) increased potency ~2-fold and efficacy ~10% (EC_{50} = 373 ± 9 nM, E_{max} = 90% ± 7%).

For $mTAAR_1$, the oxymethylene bridge was well tolerated (Table 2). Compound **3** (EC_{50} = 433 ± 154 nM, E_{max} = 92% ± 2%) was just as potent and efficacious as **2**. Monomethylation of the amine (**6**) was very beneficial, enhancing the potency ~6.5-fold and increasing efficacy by ~21% (EC_{50} = 66 ± 12 nM, E_{max} = 113% ± 2%). Compound **6** was also more potent than **1** by ~4-fold. Dimethylated **4** activated $mTAAR_1$ with same potency as **3** but had a lower efficacy (EC_{50} = 464 ± 173 nM, E_{max} = 62% ± 2%). This is also consistent with our previous results showing that $mTAAR_1$ can better accommodate a dimethylamine moiety compared to $rTAAR_1$.[2] The decrease in efficacy without a concomitant reduction in potency observed by dimethylating the amine of **3** (**4**) suggests that it may be possible to convert $mTAAR_1$ agonists into antagonists by simply adjusting the alkylation state of the amine. This may also apply to $rTAAR_1$, as monomethylation of **3** (**6**) decreased the efficacy but improved potency. Contrary to $rTAAR_1$, having the phenoxy group at the meta position appears to be detrimental for $mTAAR_1$ since the potency and efficacy of **5** decreased ~2-fold and ~14% (EC_{50} = 885 ± 549 nM, E_{max} = 78% ± 13%), respectively, compared to **3**.

In general, compounds based on the benzamidoalkylamine scaffold were poor agonists for $rTAAR_1$ (Table 3). All primary and secondary amine derivatives (**7**−**14**) activated $rTAAR_1$ with EC_{50} values greater than 1 μM and efficacies equal to or less than 69%. These benzamidoalkylamine analogues were also poor agonists for $mTAAR_1$ except for **14**. Compound **14** was ~4- and ~3-fold more potent (EC_{50} = 109 ± 6 nM) for $mTAAR_1$ compared to **2** and **1,** respectively. The efficacy of

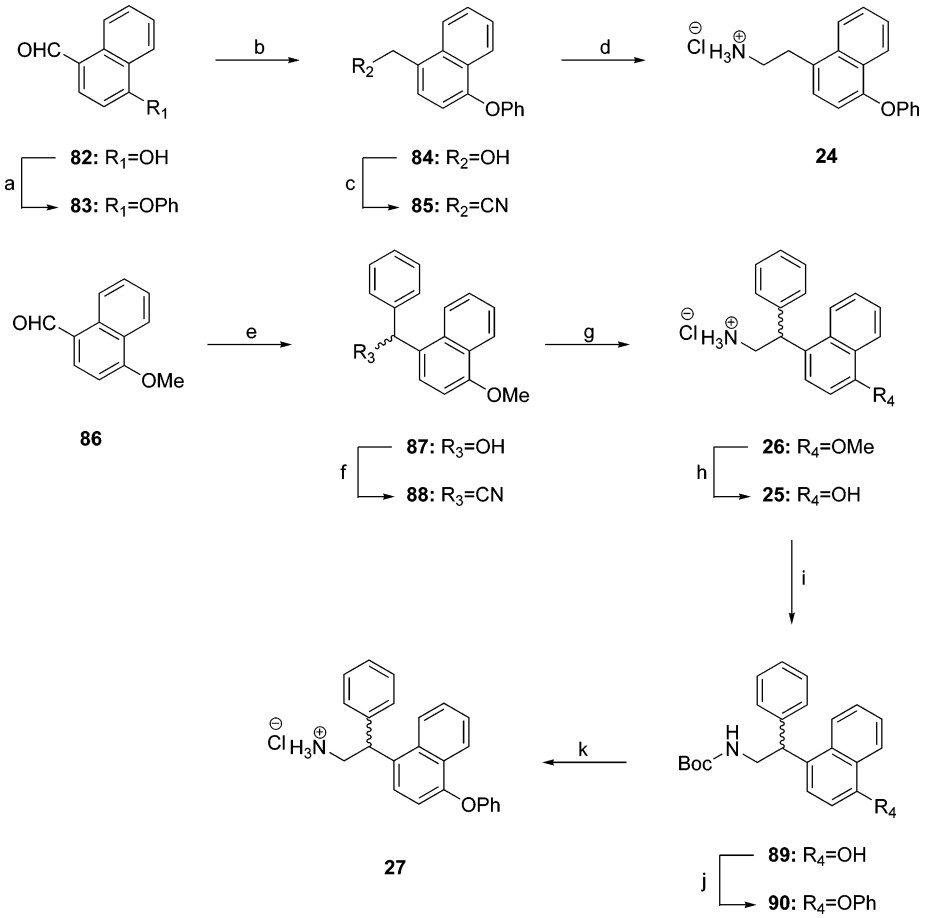
Table 3. Activity of Benzamidoalkylamines **7**−**14** on $rTAAR_1$ and $mTAAR_1$



				$rTAAR_1$			$mTAAR_1$		
compd	*n*	R_1	R_2	EC_{50}[a] ± SEM (nM)	E_{max}[b] ± SEM (%)	*N*[c]	EC_{50}[a] ± SEM (nM)	E_{max}[b] ± SEM (%)	*N*[c]
7	2	H	H	>1000	4 ± 2	2	>1000	17 ± 5	2
8	3	H	H	>1000	2 ± 1	2	>1000	2 ± 1	2
9	4	H	H	>1000	31 ± 2	2	>1000	1 ± 1	2
10	5	H	H	>1000	69 ± 7	2	905 ± 261	96 ± 5	2
11	2	CH_3	CH_3	>1000	5 ± 4	2	>1000	7 ± 2	2
12	3	CH_3	CH_3	>1000	2 ± 4	2	>1000	0 ± 2	2
13	4	CH_3	CH_3	>1000	1 ± 4	2	>1000	3 ± 1	2
14	5	CH_3	CH_3	>1000	44 ± 8	2	109 ± 6	82 ± 4	3

[a−c] See footnotes for Table 1.

Scheme 6. Synthesis of β-Substituted Naphethylamines[a]



[a] Reagents and conditions: (a) $PhB(OH)_2$, $Cu(OAc)_2$, iPr_2EtN, pyridine, 4 Å molecular sieves, DCM, 39%. (b) $NaBH_4$, EtOH, 95%. (c) (i) $SOCl_2$, DCM; (ii) TMSCN, $TiCl_4$, DCM, 54%. (d) (i) $LiAlH_4$, THF, reflux; (ii) 3 N HCl (anhydrous in EtOAc), 54%. (e) PhMgBr, THF, ∼100%. (f) (i) $SOCl_2$, DCM; (ii) TMSCN, $TiCl_4$, DCM, 97%. (g) (i) $LiAlH_4$, THF, reflux; (ii) 3 N HCl (anhydrous in EtOAc), 31%. (h) (i) BBr_3, DCM; (ii) 3 N HCl (anhydrous in EtOAc), 72%. (i) Boc_2O, $NaHCO_3$, THF/H_2O, 61%. (j) $PhB(OH)_2$, $Cu(OAc)_2$, iPr_2EtN, pyridine, 4 Å molecular sieves, DCM, 55%. (k) 3 N HCl (anhydrous in EtOAc), 84%.

14 (E_{max} = 82% ± 4%) was comparable to that of **2**. Compounds with maximum efficacy less than 20% were not antagonists for $rTAAR_1$ or $mTAAR_1$ as determined in competition assays measuring their ability to inhibit compound **1**-induced cAMP production (data not shown).

Appending a phenyl ring from the β-carbon of **2** was preferred by $rTAAR_1$ but not $mTAAR_1$ (Table 4). The potency of **15** (EC_{50} = 28 ± 2 nM, E_{max} = 103% ± 4%) for $rTAAR_1$ was equivalent to that of **1** and ∼2-fold more potent than **2**. Moving the phenoxy group to the meta position (**16**) further increased potency (EC_{50} = 19 ± 2 nM) and efficacy (E_{max} = 131% ± 7%). For $mTAAR_1$, the β-carbon phenyl substituent was unfavorable, giving compounds with potencies greater than 1 μM and efficacies equal to or less than 35% for both compounds. Compounds **15** and **16**, as well as other compounds with stereogenic centers (**17**, **18**, **20**−**23**, and **25**−**27**), were evaluated as racemic mixtures.

Cyclizing the amine to conformationally restrict the β-carbon phenyl group gave tetrahydrobenzazepines (**17** and **18**) that were equally weak agonists for both $rTAAR_1$ and $mTAAR_1$ (Table

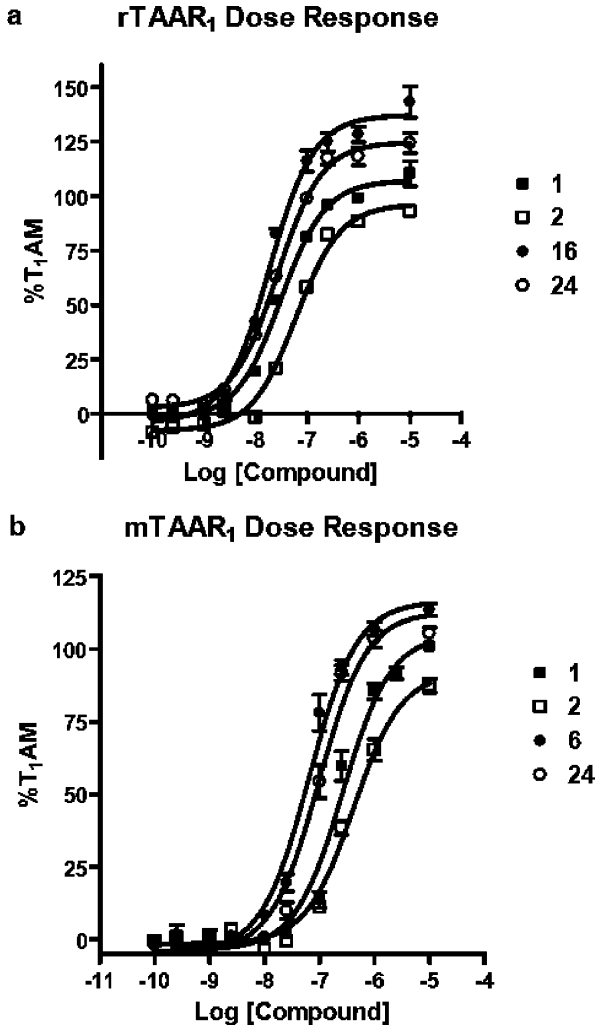


Figure 3. Representative dose-response curves of potent agonists for (a) $rTAAR_1$ and (b) $mTAAR_1$ stably expressed in HEK293 cells. (a) Dose−response curves of **1** (■), **2** (□), **16** (●), and **24** (○) for $rTAAR_1$. (b) Dose−response curves of **1** (■), **2** (□), **6** (●), and **24** (○) for $mTAAR_1$. Data reported were normalized to **1** and expressed as a percentage of compound **1** activity (% T_1AM). Dose−response curves were plotted and EC_{50} values were calculated with Prism software as described in the Materials and Methods section.

5). The potencies of **17** and **18** were greater than 1 μM and the efficacies did not surpass 86% for either receptor.

Increasing the size of the substituent at the β-carbon of **2** to a benzyl (**21**) or phenylethynyl (**23**) was well tolerated by $rTAAR_1$ but not $mTAAR_1$ (Table 6). Compound **21** was ~2-fold less potent (EC_{50} = 140 ± 77 nM) but equally as efficacious (E_{max} = 92% ± 3%) as **2** for $rTAAR_1$. Compound **23** was equipotent (EC_{50} = 62 ± 15 nM) and more efficacious (E_{max} = 119% ± 3%) compared to **2**. In $mTAAR_1$, both **21** and **23** were weak agonists.

Smaller β-carbon substituents were also better tolerated in $rTAAR_1$ compared to $mTAAR_1$ (Table 6). Adding one or two methyl groups to the β-carbon of **2** decreased the potency for $rTAAR_1$ by 4-fold (EC_{50} = 250 ± 132 nM and >1 μM for **20** and **19**, respectively). Monomethyl **20** was comparably efficacious (E_{max} = 94% ± 7%) to **2**, whereas dimethyl **19** was significantly less efficacious (E_{max} = 13% ± 2%). Interestingly, a small unsaturated acetylene group at the β-carbon (**22**) slightly enhanced the potency (EC_{50} = 41 ± 4 nM) without significantly changing the efficacy (E_{max} = 105% ± 14%) of **2** for $rTAAR_1$. For $mTAAR_1$, all of these derivatives activated poorly (EC_{50} ≥ 1 μM) and had lower efficacies (E_{max} ≤ 72%). It should be noted that **22** displayed some level of antagonist activity in competition assays with **1,** decreasing cAMP induction of **1** at $mTAAR_1$ ~30% at a dose of 10 μM (data not shown).

Converting the inner ring of **2** from benzene to naphthalene increased the potency and efficacy for both $rTAAR_1$ and $mTAAR_1$ (Table 7). Compound **24** was ~2-fold more potent (EC_{50} = 26 ± 1 nM) and ~20% more efficacious (E_{max} = 113% ± 5%) than **2**. For $mTAAR_1$, **24** was ~20% more efficacious and ~4-fold more potent (EC_{50} = 100 ± 22 nM) than **2**. A phenyl ring at the β-carbon (**27**) decreased the potency of **24** for $rTAAR_1$ ~2-fold. Compound **27** activated $rTAAR_1$ with a potency of 52 ± 4 nM and an efficacy of 100% ± 5%. Changing the phenoxy group of **27** to a methoxy (**26**) or hydroxyl (**25**) group decreased potency of **25** and **26** ~5−14-fold (EC_{50} = 716 ± 269 nM and 270 ± 66 nM, respectively) and efficacy ~10−30% (E_{max} = 89% ± 3% and 71% ± 4%, respectively). All the β-carbon phenyl derivatives of **24** (**25**−**27**) were at least 10-fold less potent at $mTAAR_1$ (EC_{50} = 1 μM) compared to **2**. When **25**−**27** were screened for antagonist activity in competition assays, only **27** displayed some degree of antagonism, inhibiting compound **1**-induced cAMP production at $mTAAR_1$ ~50% at a dose of 10 μM (data not shown). The observed activity of all compounds (**3**−**27**) tested were found to be $TAAR_1$−dependent, as all compounds showed no cAMP accumulation when screened in an empty vector cell line (data not shown).

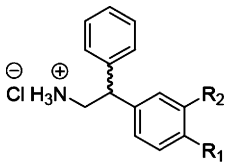
Discussion

We have previously shown that the potency of **1** for both rat and mouse $TAAR_1$ can be improved by changing the methylation state of the amine and/or modifying the outer-ring portion of the phenoxyphenethylamine core scaffold.[2] The pharmacological survey by Bunzow et al.[3] showed that $rTAAR_1$ could be activated by phenethylamine analogues, amphetamines, ergolines, and aminergic GPCR (dopamine, adrenergic, and serotonin receptors) drugs with a structurally diverse range of ethylamine segments. In this study, we synthesized a number of phenyltyramine derivatives with structural modifications in the ethylamine section to explore the SAR and determine if structural variations in this region would lead to agonists for rat and mouse $TAAR_1$ that were more potent than **1**.

The $TAAR_1$ activity data showed that rat and mouse $TAAR_1$ can tolerate prominent structural features commonly found in the alkylamine fragment of existing βAR and dopamine receptor agonists and antagonists; however, distinct $TAAR_1$ species preferences are evident. Extending the distance between the charged amine and the aromatic ring of **2** by inserting an oxymethylene bridge or an amide linker was tolerated by $mTAAR_1$ but not $rTAAR_1$. On the other hand, appending a phenyl ring at the β-carbon of **2** was detrimental for $mTAAR_1$ activation but beneficial for $rTAAR_1$ activation. The preference for hydrocarbon functional groups with some degree of unsaturation at the β-carbon indicates that the binding pocket of $rTAAR_1$ around this position is primarily hydrophobic in nature. Conversely, the tolerance for ether and amide groups in the ethylamine chain of **2** suggests a more polar binding pocket in the same region of $mTAAR_1$.

The two receptors also responded differently to changes in the position of the phenoxy group. In general, the potency and efficacy of compounds increased when the phenoxy group was shifted from the para to the meta position in $rTAAR_1$, as observed with the change in activity from **3** to **5** and **15** to **16**. The same modification gives the opposite effect in $mTAAR_1$,

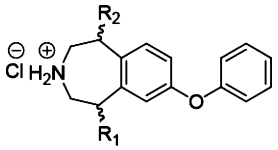
Table 4. Activity of β-Phenylphenoxyphenethylamines **15** and **16** on $rTAAR_1$ and $mTAAR_1$



			$rTAAR_1$			$mTAAR_1$		
compd	R_1	R_2	$EC_{50}{}^a \pm$ SEM (nM)	$E_{max}{}^b \pm$ SEM (%)	N^c	$EC_{50}{}^a \pm$ SEM (nM)	$E_{max}{}^b \pm$ SEM (%)	N^c
15	OPh	H	28 ± 2	103 ± 4	3	>1000	35 ± 8	3
16	H	OPh	19 ± 2	131 ± 7	3	>1000	15 ± 4	3

[a–c] See footnotes for Table 1.

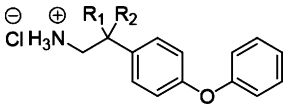
Table 5. Activity of Tetrahydrobenzazepines **17** and **18** on $rTAAR_1$ and $mTAAR_1$



			$rTAAR_1$			$mTAAR_1$		
compd	R_1	R_2	$EC_{50}{}^a \pm$ SEM (nM)	$E_{max}{}^b \pm$ SEM (%)	N^c	$EC_{50}{}^a \pm$ SEM (nM)	$E_{max}{}^b \pm$ SEM (%)	N^c
17	Ph	H	>1000	32 ± 8	2	>1000	22 ± 3	2
18	H	Ph	>1000	86 ± 2	2	>1000	45 ± 5	2

[a–c] See footnotes for Table 1.

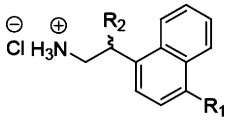
Table 6. Activity of β-Substituted Phenoxyphenethylamines **19**−**23** on $rTAAR_1$ and $mTAAR_1$



			$rTAAR_1$			$mTAAR_1$		
compd	R_1	R_2	$EC_{50}{}^a \pm$ SEM (nM)	$E_{max}{}^b \pm$ SEM (%)	N^c	$EC_{50}{}^a \pm$ SEM (nM)	$E_{max}{}^b \pm$ SEM (%)	N^c
19	CH_3	CH_3	>1000	13 ± 2	2	>1000	18 ± 4	2
20	CH_3	H	250 ± 132	94 ± 7	2	>1000	72 ± 7	2
21	Bn	H	140 ± 77	92 ± 3	2	>1000	31 ± 9	2
22	CCH	H	41 ± 4	105 ± 14	3	>1000	2 ± 5	2
23	CCPh	H	62 ± 15	119 ± 3	3	929 ± 314	79 ± 9	2

[a–c] See footnotes for Table 1.

Table 7. Activity of Naphethylamines **24**−**27** on $rTAAR_1$ and $mTAAR_1$



			$rTAAR_1$			$mTAAR_1$		
compd	R_1	R_2	$EC_{50}{}^a \pm$ SEM (nM)	$E_{max}{}^b \pm$ SEM (%)	N^c	$EC_{50}{}^a \pm$ SEM (nM)	$E_{max}{}^b \pm$ SEM (%)	N^c
24	OPh	H	26 ± 1	113 ± 5	3	101 ± 22	104 ± 3	3
25	OH	Ph	716 ± 269	89 ± 3	2	>1000	14 ± 4	2
26	OMe	Ph	270 ± 66	71 ± 4	2	>1000	14 ± 4	2
27	OPh	Ph	52 ± 4	100 ± 6	3	>1000	0 ± 2	2

[a–c] See footnotes for Table 1.

leading to less potent and efficacious compounds. Confining the ethylamine chain of **15** and **16** in a seven-membered ring (**17** and **18**) to restrict the conformational orientations of the β-carbon phenyl group was extremely detrimental to $rTAAR_1$, decreasing potency greater than 35-fold.

Transforming the phenethylamine ring of **2** into a naphthyl ring was equally beneficial, improving the potency and efficacy for $rTAAR_1$ and $mTAAR_1$ to the level of **1** or better. The additional benzene ring in the naphthyl group of **24** most likely occupies the iodine binding pocket in $TAAR_1$ and is thus a good isosteric replacement for the iodine present in **1**. Contrary to the phenoxyphenethylamine scaffold, appending a phenyl group at the β-carbon of the phenoxynaphethylamine scaffold decreased the potency for $rTAAR_1$. Having the bulkier naphthyl group for an inner ring could possibly limit the available torsional conformations of **27** and affect its ability

to position the β-phenyl ring in the optimal orientation inside the binding pocket of $rTAAR_1$, resulting in decreased potency. The considerable decrease in potency observed with hydroxyl (**25**) or methoxy (**26**) substituents in place of the phenoxy group illustrates the significant contribution of the biaryl ether moiety to the potency of the phenoxynaphethylamine scaffold.

The observed SAR preferences between the rat and mouse $TAAR_1$ were quite interesting, given that the two receptors are 93% similar. Since the rodent receptors are only 83−85% similar to the human receptor, the SAR of the ethylamine portion of **1** for the human $TAAR_1$ ($hTAAR_1$) may be different from that of the rat or mouse $TAAR_1$. Compound **1** has recently been found to be significantly less potent for $hTAAR_1$ by Wainscott et al.[15] when they investigated the pharmacological profile of $hTAAR_1$.

Conclusion

The present study demonstrates that it is possible to enhance the potency of thyronamines for both rat and mouse $TAAR_1$ by incorporating functionalities in the ethylamine portion of the phenoxyphenethylamine scaffold. Rat and mouse $TAAR_1$ have different structural preferences in this region of the scaffold, with $rTAAR_1$ favoring unsaturated hydrocarbon groups and $mTAAR_1$ preferring functional groups that are polar and hydrogen-bond acceptors. Despite this species variability, transforming the inner ring of the phenoxyphenethylamine scaffold into a naphthyl group was equally beneficial to both receptors, mostly likely acting as an excellent isosteric replacement for the iodophenyl inner ring of **1**.

Materials and Methods

General. ^1H and ^{13}C NMR spectra were taken on a Varian 400 (400 and 100 MHz, respectively). Data reported are calibrated to internal tetramethylsilane (TMS; 0.0 ppm) for all solvents unless otherwise noted and are reported as follows: chemical shift, multiplicity (app = apparent, br = broad, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublet of doublet), coupling constant, and integration. High-resolution mass spectrometry (HRMS) with electrospray ionization was performed by the Mass Spectrometry Laboratory, School of Chemical Sciences, University of Illinois at Urbana−Champaign. Inert atmosphere operations were conducted under argon passed through a drierite drying tube in flame-dried or oven-dried glassware unless otherwise noted. Anhydrous tetrahydrofuran (THF), dichloromethane (DCM), diethyl ether, pyridine, and diisopropylethylamine were filtered through two columns of activated basic alumina and transferred under an atmosphere of argon gas in a solvent purification system designed and manufactured by Seca Solvent Systems. Anhydrous *N*,*N*-dimethylformamide (DMF) was obtained by passing through two columns of activated molecular sieves. All other anhydrous solvents and reagents were purchased from Aldrich, Sigma−Aldrich, Fluka, Alfa Aesar, or Acros and were used without any further purification unless otherwise stated. Final compounds were judged to be > 95% pure by ^1H NMR analysis and confirmed HPLC. HPLC was performed on an Agilent 1200 Series LC system [using a Waters XTerra Phenyl 3.5 μm (3.0 × 50 mm) column] with a gradient of 0−90% acetonitrile (0.1% TFA) over 8 min and 0−100% methanol (0.05% TFA) over 8 or 10 min.

In Vitro cAMP Assays. After incubation in fresh medium for at least 2 h, HEK293 cells stably transfected with either $rTAAR_1$ or $mTAAR_1$ were harvested in Krebs−Ringer−HEPES buffer (KRH) and preincubated with 200 μM 3-isobutyl-1-methylxanthine (IBMX) for 20−30 min. Cells were incubated in KRH with 133 μM IBMX and 3 μL of the test compound, forskolin (10 μM), or vehicle (dimethyl sulfoxide, DMSO) for 1 h at 37 °C (300 μL total volume). The cells were boiled for 20 min after addition of 100 μL of 0.5 mM sodium acetate buffer. The cell lysate was centrifuged to remove cellular debris, and an aliquot (30 μL) was transferred to an opaque, flat-bottom 96-well plate (Corning 3917). The cAMP content of the aliquot was measured by use of the Hithunter cAMP XS kit (DiscoveRX, Fremont, CA). The plate was shaken on a titer plate shaker for 2 min after addition of 20 μL of cAMP XS antibody/lysis mix. After incubation in the dark for 1 h, 20 μL of cAMP XS ED reagent was added and the plate was shaken for 2 min. After another hour of incubation in the dark, 40 μL of cAMP XS EA/CL substrate mix was added and the plate was shaken for 2 min. The plate was allowed to incubate in the dark for 18 h before luminescence was measured (three readings/well at 0.33 s/reading) on an Analyst AD assay detection system (LJL Biosystems) or a Packard Fusion microplate reader. Data were reported relative to **1** and expressed as % T_1-AM. Concentration−response curves were plotted and EC_{50} values were calculated with Prism software (GraphPad, San Diego, CA). Standard error of the mean was calculated from the EC_{50} and E_{max} values of each independent triplicate experiment by use of Prism Software.

General Procedure for *t*-Boc Deprotection. The *t*-Boc-protected amine (1.40 mmol) was dissolved in a 3 N anhydrous HCl solution in ethyl acetate (3 mL), and the reaction mixture was stirred at room temperature for 2−16 h. The reaction was exposed to diethyl ether and the resulting amine hydrochloride salts were washed with diethyl ether. If the amine hydrochloride salts did not form a precipitate, the diethyl ether/ethyl acetate solution was concentrated under reduced pressure and rinsed with diethyl ether to give the hydrochloride salts.

General Procedure for N,N-Dimethylation of Amine Hydrochloride Salt. The amine hydrochloride salt (0.22 mmol) was dissolved in water, treated with potassium carbonate (>0.22 mmol), and extracted with dichloromethane. The organic layer was dried over $MgSO_4$, filtered, and concentrated under reduced pressure to give the free amine. A solution of free amine (0.22 mmol), formic acid (>1.10 mmol, 88% in water solution), and formaldehyde (>1.10 mmol, 37% in water solution) was then stirred at 80 °C for ∼20 h. After cooling to room temperature, the reaction was diluted with water, made basic (pH ∼10) with potassium carbonate, and extracted with dichloromethane. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure to give the crude product. The crude mixture was treated with a solution of 3 N anhydrous HCl in ethyl acetate (1 mL) and exposed to diethyl ether, and the resulting amine hydrochloride salts were washed with diethyl ether. If the amine hydrochloride salts did not form a precipitate, the diethyl ether/ethyl acetate solution was concentrated under reduced pressure and rinsed with diethyl ether to give the hydrochloride salt.

General Procedure for the Reduction of Nitrile to Amine Hydrochloride. To a suspension of lithium aluminum hydride (26.7 mmol) in THF (56 mL) at 0 °C was added a solution of nitrile (6.66 mmol) in THF (10 mL). After refluxing under argon for 24 h, the reaction was quenched with water (1.014 mL), 10% aqueous sodium hydroxide (2.028 mL), and water (3.043 mL). The reaction was filtered to remove the precipitated aluminum salts. The filtrate was washed with water and brine and extracted with ethyl acetate. The organic layer was dried over $MgSO_4$, filtered, and concentrated under reduced pressure to give the crude product. The crude mixture was treated with a 3 N anhydrous HCl solution in ethyl acetate (5−10 mL) and exposed to diethyl ether, and the resulting amine hydrochloride salts were washed with diethyl ether. If the amine hydrochloride salts did not form a precipitate, the diethyl ether/ethyl acetate solution was concentrated under reduced pressure and rinsed with diethyl ether to give the hydrochloride salts.

General Procedure for Curtius Rearrangement. To a solution of acid (500 mg, 1.46 mmol), ethylchloroformate (0.16 mL, 1.61 mmol), and triethylamine (0.2 mL, 1.46 mmol) in acetone (10 mL) at 0 °C was added a solution of sodium azide (210 mg, 3.21 mmol) in water (2 mL). The reaction was stirred for 1 h and then diluted

with ether. The reaction was washed with water and brine, dried over $MgSO_4$, and concentrated under reduced pressure. The crude product was dissolved in toluene (20 mL) and refluxed for 1 h. After cooling to ambient temperature, the crude mixture was concentrated in vacuo. The crude product was dissolved in dioxane (5 mL), concentrated HCl (3 mL), and water (3 mL) and then refluxed for 1 h. After cooling to ambient temperature, the crude mixture was diluted with ether, washed with water and brine, dried over $MgSO_4$, and concentrated under reduced pressure.

3-[(4-Phenoxyphenyl)oxy]propylamine Hydrochloride (3). Refer to general procedure for *t*-Boc deprotection described above: 0.12 g, 53% yield). ^{1}H NMR (400 MHz, methanol-d_4) δ 7.29 (t, J = 8.0 Hz, 2H), 7.04 (t, J = 7.4 Hz, 1H), 6.96 (s, 4H), 6.89 (d, J = 7.6 Hz, 2H), 4.11 (t, J = 5.8 Hz, 2H), 3.16 (t, J = 7.2 Hz, 2H), 2.14 (m, 2H). HRMS (EI$^+$) m/z for $C_{15}H_{17}NO_2$ [M + H]$^+$: calcd, 244.1338; found, 244.1343.

***N,N*-Dimethyl-3-[(4-phenoxyphenyl)oxy]propylamine Hydrochloride (4).** Refer to general procedure for N,N-dimethylation to amine hydrochloride salt described above; 0.17 g, 76% yield. ^{1}H NMR (400 MHz, methanol-d_4) δ 7.30 (t, J = 8.0 Hz, 2H), 7.04 (t, J = 7.4 Hz, 1H), 6.96 (s, 4H), 6.89 (d, J = 8.0 Hz, 2H), 4.10 (d, J = 5.8 Hz, 2H), 3.32 (m, 2H), 2.92 (s, 6H), 2.21 (m, 2H). HRMS (EI$^+$) m/z for $C_{17}H_{21}NO_2$ [M + H]$^+$: calcd, 272.1651; found, 272.1641.

3-[(3-Phenoxyphenyl)oxy]propylamine Hydrochloride (5). Refer to general procedure for *t*-Boc deprotection described above: 0.76 g, 75% yield. ^{1}H NMR (400 MHz, methanol-d_4) δ 7.35 (t, J = 8.2 Hz, 2H), 7.24 (t, J = 8.4 Hz, 1H), 7.12 (t, J = 7.4 Hz, 1H), 6.98 (d, J = 7.6 Hz, 2H), 6.70 (app d, J = 7.6 Hz, 1H), 6.57 (app s, 1H), 6.56 (app s, 1H), 4.08 (t, J = 5.8 Hz, 2H), 3.13 (t, J = 7.2 Hz, 2H), 2.12 (m, 2H). HRMS (EI$^+$) m/z for $C_{15}H_{17}NO_2$ [M + H]$^+$: calcd, 244.1338; found, 244.1339.

***N*-Methyl-3-[(4-phenoxyphenyl)oxy]propylamine Hydrochloride (6).** Refer to general procedure for *t*-Boc deprotection described above: 0.94 g, 90% yield. ^{1}H NMR (400 MHz, methanol-d_4) δ 7.29 (t, J = 8.0 Hz, 2H), 7.04 (t, J = 7.4 Hz, 1H), 6.96 (s, 4H), 6.90 (app t, J = 7.4 Hz, 2H), 4.11 (t, J = 5.8 Hz, 2H), 3.23 (t, J = 7.2 Hz, 2H), 2.74 (s, 3H), 2.17 (m, 2H). HRMS (EI$^+$) m/z for $C_{16}H_{18}NO_2$ [M + H]$^+$: calcd, 258.1494; found, 258.1503.

2-(4-Phenoxybenzamido)ethylamine Hydrochloride (7). Refer to general procedure for *t*-Boc deprotection described above: 0.25 g, ~100% yield. ^{1}H NMR (400 MHz, methanol-d_4) δ 7.88 (d, J = 8.8 Hz, 2H), 7.41(t, J = 7.8 Hz, 2H), 7.20 (t, J = 7.4 Hz, 1H), 7.05 (d, J = 8.0 Hz, 2H), 7.01 (d, J = 8.8 Hz, 2H), 3.66 (t, J = 5.8 Hz, 2H), 3.17 (t, J = 6.0 Hz, 2H); ^{13}C NMR (400 MHz, methanol-d_4) δ 170.5, 162.5, 157.2, 131.2, 130.6, 129.1, 125.6, 121.0, 118.4, 41.4, 38.8. HRMS (EI$^+$) m/z for $C_{15}H_{16}N_2O_2$ [M + H]$^+$: calcd, 257.1290; found, 257.1293.

3-(4-Phenoxybenzamido)propylamine Hydrochloride (8). Refer to general procedure for *t*-Boc deprotection described above: 0.23 g, 96% yield. ^{1}H NMR (400 MHz, methanol-d_4) δ 7.84 (d, J = 8.8 Hz, 2H), 7.41 (t, J = 8.1 Hz, 2H), 7.20 (t, J = 7.3 Hz, 1H), 7.06 (d, J = 7.8 Hz, 2H), 7.01 (d, J = 8.8 Hz, 2H), 3.49 (t, J = 6.6 Hz, 2H), 2.98 (t, J = 7.3 Hz, 2H), 1.94 (quintet, J = 7.0 Hz, 2H). HRMS (EI$^+$) m/z for $C_{16}H_{18}N_2O_2$ [M + H]$^+$: calcd, 271.1447; found, 271.1447.

4-(4-Phenoxybenzamido)butylamine Hydrochloride (9). Refer to general procedure for *t*-Boc deprotection described above: 0.21 g, 97% yield. ^{1}H NMR (400 MHz, methanol-d_4) δ 7.82 (d, J = 9.3 Hz, 2H), 7.41 (t, J = 8.1 Hz, 2H), 7.20 (t, J = 7.3 Hz, 1H), 7.05 (d, J = 8.8 Hz, 2H), 7.00 (d, J = 8.8 Hz, 2H), 3.42 (t, J = 6.6 Hz, 2H), 2.98 (t, J = 7.1 Hz, 2H), 1.71 (m, 4H). HRMS (EI$^+$) m/z for $C_{17}H_{20}N_2O_2$ [M + H]$^+$: calcd, 285.1603; found, 285.1603.

5-(4-Phenoxybenzamido)pentylamine Hydrochloride (10). Refer to general procedure for *t*-Boc deprotection described above: 0.13 g, 98% yield. ^{1}H NMR (400 MHz, methanol-d_4) δ 7.81 (d, J = 8.8 Hz, 2H), 7.41 (t, J = 8.1 Hz, 2H), 7.20 (t, J = 7.6 Hz, 1H), 7.05 (d, J = 8.8 Hz, 2H), 7.00 (d, J = 9.3 Hz, 2H), 3.39 (t, J = 6.8 Hz, 2H), 2.93 (t, J = 7.8 Hz, 2H), 1.69 (m, 4H), 1.46 (m, 2H). HRMS (EI$^+$) m/z for $C_{18}H_{22}N_2O_2$ [M + H]$^+$: calcd, 299.1760; found, 299.1767.

***N,N*-Dimethyl-2-(4-phenoxybenzamido)ethylamine Hydrochloride (11).** Refer to general procedure for N,N-dimethylation fo amine hydrochloride salt described above: 0.09 g, 98% yield. ^{1}H NMR (400 MHz, methanol-d_4) δ 7.87 (d, J = 8.8 Hz, 2H), 7.42(t, J = 7.8 Hz, 2H), 7.21 (t, J = 7.6 Hz, 1H), 7.05 (d, J = 8.8 Hz, 2H), 7.02 (d, J = 9.2 Hz, 2H), 3.74 (t, J = 5.8 Hz, 2H), 3.37 (t, J = 5.8 Hz, 2H), 2.98 (s, 6H). HRMS (EI$^+$) m/z for $C_{17}H_{20}N_2O_2$ [M + H]$^+$: calcd, 285.1603; found, 285.1609.

***N,N*-Dimethyl-3-(4-phenoxybenzamido)propylamine Hydrochloride (12).** Refer to general procedure for N,N-dimethylation fo amine hydrochloride salt described above: 0.12 g, 76% yield. ^{1}H NMR (400 MHz, methanol-d_4) δ 7.86 (d, J = 8.8 Hz, 2H), 7.41 (t, J = 8.1 Hz, 2H), 7.20 (t, J = 7.6 Hz, 1H), 7.05 (d, J = 7.8 Hz, 2H), 7.01 (d, J = 8.8 Hz, 2H), 3.48 (t, J = 6.6 Hz, 2H), 3.19 (t, J = 7.6 Hz, 2H), 2.91 (s, 6H), 2.03 (m, 2H). HRMS (EI$^+$) m/z for $C_{18}H_{22}N_2O_2$ [M + H]$^+$: calcd, 299.1760; found, 299.1757.

***N,N*-Dimethyl-4-(4-phenoxybenzamido)butylamine Hydrochloride (13).** Refer to general procedure for N,N-dimethylation to amine hydrochloride salt: 0.16 g, 11% yield. ^{1}H NMR (400 MHz, methanol-d_4) δ 7.82 (d, J = 8.8 Hz, 2H), 7.41 (t, J = 7.8 Hz, 2H), 7.20 (t, J = 7.4 Hz, 1H), 7.06 (d, J = 7.6 Hz, 2H), 7.00 (d, J = 8.8 Hz, 2H), 3.43 (t, J = 6.8 Hz, 2H), 3.19 (t, J = 8.0 Hz, 2H), 2.89 (s, 6H), 1.77 (m, 2H), 1.69 (m, 2H). HRMS (EI$^+$) m/z for $C_{19}H_{24}N_2O_2$ [M + H]$^+$: calcd, 313.1916; found, 313.1925.

***N,N*-Dimethyl-5-(4-phenoxybenzamido)pentylamine Hydrochloride (14).** Refer to general procedure for N,N-dimethylation fo amine hydrochloride salts: 0.09 g, 31% yield. ^{1}H NMR (400 MHz, methanol-d_4) δ 7.82 (d, J = 8.8 Hz, 2H), 7.41 (t, J = 8.0 Hz, 2H), 7.20 (t, J = 7.6 Hz, 1H), 7.04 (d, J = 8.4 Hz, 2H), 7.00 (d, J = 8.8 Hz, 2H), 3.40 (t, J = 6.0 Hz, 2H), 3.13 (t, J = 7.8 Hz, 2H), 2.88 (s, 6H), 1.78 (m, 2H), 1.69 (m, 2H), 1.46 (m, 2H). HRMS (EI$^+$) m/z for $C_{20}H_{26}N_2O_2$ [M + H]$^+$: calcd, 327.2073; found, 327.2087.

2-(4-Phenoxyphenyl)-2-phenylethylamine Hydrochloride (15). Refer to general procedure for the reduction of nitrile to amine hydrochloride described above: 0.24 g, 47% yield. ^{1}H NMR (400 MHz, methanol-d_4) δ 7.22−7.35 (m, 9H), 7.04 (t, J = 7.6 Hz, 1H), 6.89−6.92 (m, 4H), 4.22 (t, J = 8.0 Hz, 1H), 3.56 (d, J = 8.0 Hz, 1H). HRMS (EI$^+$) m/z for $C_{20}H_{19}NO$ [M + H]$^+$: calcd, 290.1545; found, 290.1559.

2-(3-Phenoxyphenyl)-2-phenylethylamine Hydrochloride (16). Refer to general procedure for the reduction of nitrile to amine hydrochloride described above: 2.79 g, 26% yield. ^{1}H NMR (400 MHz, methanol-d_4) δ 7.34 (m, 10H), 7.12 (app t, J = 7.3 Hz, 1H), 6.98 (m, 1H), 6.86 (dd, J = 8.1, 2.7 Hz, 1H), 4.26 (t, J = 8.1 Hz, 1H), 3.62 (d, J = 8.3 Hz, 2H). HRMS (EI$^+$) m/z for $C_{20}H_{19}NO$ [M + H]$^+$: calcd, 290.1545; found, 290.1548.

8-Phenoxy-1-phenyl-2,3,4,5-tetrahydro-3-benzazepine Hydrochloride (17). Refer to general procedure for *t*-Boc deprotection described above: 0.50 g, 90% yield. ^{1}H NMR (400 MHz, methanol-d_4) δ 7.40 (t, J = 7.6 Hz, 2H), 7.32 (app t, J = 7.4 Hz, 2H), 7.27 (m, 2H), 7.19 (d, J = 7.6 Hz, 2H), 7.07 (t, J = 7.4 Hz, 1H), 6.87 (d, J = 7.6 Hz, 2H), 6.81 (dd, J = 8.4, 2.4 Hz, 1H), 6.40 (d, J = 1.6 Hz, 1H), 4.61 (d, J = 8.4 Hz, 1H), 3.72 (m, 2H), 3.48 (dd, J = 11.8, 7.6 Hz, 2H), 3.18 (m, 2H). HRMS (EI$^+$) m/z for $C_{22}H_{21}NO$ [M + H]$^+$: calcd, 316.1701; found, 316.1713.

7-Phenoxy-1-phenyl-2,3,4,5-tetrahydro-3-benzazepine Hydrochloride (18). Refer to general procedure for *t*-Boc deprotection described above: 0.10 g, 85% yield. ^{1}H NMR (400 MHz, methanol-d_4) δ 7.44 (t, J = 7.6 Hz, 3H), 7.35 (app t, J = 8.1 Hz, 3H), 7.23 (d, J = 8.1, 2H), 7.12 (t, J = 7.3 Hz, 1H), 7.00 (dd, J = 8.8, 1.0 Hz, 1H), 6.90 (s, 1H), 6.77 (s, 2H), 4.60 (dd, J = 8.6, 1.7 Hz, 1H), 3.66−3.84 (m, 2H), 3.22 (m, 2H), 3.09 (m, 2H). HRMS (EI$^+$) m/z for $C_{22}H_{21}NO$ [M + H]$^+$: calcd, 316.1701; found, 316.1702.

2-Methyl-2-(4-phenoxyphenyl)propan-1-amine Hydrochloride (19). Refer to general procedure for *t*-Boc deprotection described above: white solid, 46 mg, 92% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.72 (br s, 3H), 7.44−7.37 (m, 4H), 7.14 (t, J = 7.6 Hz, 1H), 7.02−6.99 (m, 4H), 3.32 (s, 2H), 1.35 (s, 6H); ^{13}C NMR (100 MHz, methanol-d_4) δ 158.45, 157.91, 139.98, 130.91, 128.65, 124.61, 119.99, 51.74, 38.03, 26.97. HRMS (EI$^+$) m/z for $C_{16}H_{19}NO$ [M + H]$^+$: calcd, 241.1467; found, 241.1457.

2-(4-Phenoxyphenyl)propan-1-amine Hydrochloride (20). Refer to general procedure for *t*-Boc deprotection described above: white solid, 95 mg, 93% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.82 (s, 2H), 7.40 (app t, J = 8.2 Hz, 2H), 7.31 (d, J = 7.0 Hz, 2H), 7.14 (app t, J = 8.2 Hz, 1H), 7.00 (m, 4H), 3.02 (m, 1H), 1.26 (d, J = 6.0 Hz, 3H); ^{13}C NMR (100 MHz, methanol-d_4) δ 158.59, 158.14, 137.99, 130.88, 129.71, 124.49, 120.33, 119.89, 46.88, 38.94, 19.99. HRMS (EI$^+$) m/z for $C_{15}H_{17}NO$ [M + H]$^+$: calcd, 227.1310; found, 227.1306.

2-(4-Phenoxyphenyl)-3-phenylpropan-1-amine Hydrochloride (21). Refer to general procedure for *t*-Boc deprotection described above: white solid, 70 mg, 94% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.79 (s, 2H), 7.38 (t, J = 8.0 Hz, 2H), 7.25 (d, J = 8.6 Hz, 2H), 7.22 (d, J = 7.5 Hz, 2H), 7.14 (m, 2H), 7.07 (d, J = 7.5 Hz, 2H), 6.95 (t, J = 7.5 Hz, 4H), 3.29 (m, 1H), 3.11 (m, 3H), 2.82 (dd, J = 8.6, 13.6 Hz, 1H); ^{13}C NMR (100 MHz, methanol-d_4) δ 158.58, 158.23, 139.94, 135.54, 130.87, 130.64, 130.17, 129.37, 127.46, 124.49, 120.28, 119.84, 46.95, 45.01, 41.78. HRMS (EI$^+$) m/z for $C_{21}H_{21}NO$ [M + H]$^+$: calcd, 303.1623; found, 303.1621.

2-(4-Phenoxyphenyl)-4-phenylbut-3-yn-1-amine Hydrochloride (22). Refer to general procedure for Curtius rearrangement described above. The crude product was purified via flash SiO_2 chromatography [eluted with methanol/ethyl acetate (1:4)] to give the pure product **22** as a white solid (185 mg, 36% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.19 (s, 4H), 7.53 (t, J = 8.3 Hz, 4H), 7.41 (m, 5H), 7.16 (t, J = 7.4 Hz, 1H), 7.05 (t, J = 8.9 Hz, 4H), 4.36 (t, J = 6.9 Hz, 1H), 3.21 (m, 2H); ^{13}C NMR (100 MHz, methanol-d_4) δ 158.85, 158.29, 132.84, 130.95, 130.36, 129.76, 129.48, 124.74, 123.75, 120.17, 120.12, 86.95, 46.29, 37.23. HRMS (EI$^+$) m/z for $C_{22}H_{19}NO$ [M + H]$^+$: calcd, 313.1467; found, 313.1367.

2-(4-Phenoxyphenyl)but-3-yn-1-amine Hydrochloride (23). Refer to general procedure for Curtius rearrangement described above. The crude product was purified via flash SiO_2 chromatography [eluted with methanol/ethyl acetate (1:4)] to give the pure product **23** as a white solid (71 mg, 38% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.99 (s, 3H), 7.44 (t, J = 8.8 Hz, 2H), 7.40 (d, J = 8.4 Hz, 2H), 7.16 (t, J = 7.4 Hz, 1H), 7.03 (m, 4H), 4.08 (t, J = 6.0 Hz, 1H), 3.50 (d, J = 2.6 Hz, 1H), 3.12 (m, 2H). HRMS (EI$^+$) m/z for $C_{16}H_{15}NO$ [M + H]$^+$: calcd, 237.1154; found, 237.1149.

2-(1-Phenoxynaphthalen-4-yl)ethylamine (24). Refer to general procedure for reduction of nitrile to amine hydrochloride: 0.13 g, 54% yield. ^{1}H NMR (400 MHz, methanol-d_4) δ 8.24 (d, J = 8.8 Hz, 1H), 8.12 (d, J = 8.8 Hz, 1H), 7.66 (app t, J = 7.6 Hz, 1H), 7.55 (app t, J = 7.6 Hz, 1H), 7.36 (m, 3H), 7.13 (app t, J = 7.3 Hz, 1H), 7.02 (d, J = 7.3 Hz, 2H), 6.88 (d, J = 7.8 Hz, 1H), 3.43 (t, J = 7.6 Hz, 1H), 3.27 (t, J = 7.3 Hz, 2H). HRMS (EI$^+$) m/z for $C_{18}H_{17}NO$ [M + H]$^+$: calcd, 264.1388; found, 264.1393.

2-(1-Hydroxynaphthalen-4-yl)-2-phenylethylamine Hydrochloride (25). To a solution of **26** (0.24 g, 0.77 mmol) in dry $CHCl_3$ (7 mL) at 0 °C was added BBr_3 (2.43 mL, 1.0 M solution in DCM). After stirring for 1 h at room temperature, the reaction was cooled to 0 °C and anhydrous MeOH (7.65 mL) was added. The reaction was refluxed in an open mouth flashed for 20 min before evaporation of the solvent. The crude mixture was diluted with EtOAc and made alkaline with potassium carbonate. The organic layer was washed with water and brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. Treatment with 3 N anhydrous HCl in ethyl acetate and exposure to diethyl ether precipitated the hydrochloride salts of **25** as a brownish solid (0.16 g, 72%). ^{1}H NMR (400 MHz, methanol-d_4) δ 8.27 (d, J = 8.4 Hz, 1H), 8.01 (d, J = 9.0 Hz, 1H), 7.44 (m, 3H), 7.35 (m, 4H), 7.23 (m, 1H), 6.89 (d, J = 7.9 Hz, 1H), 5.03 (t, J = 8.0 Hz, 1H), 3.70 (m, 2H). HRMS (EI$^+$) m/z for $C_{18}H_{17}NO$ [M + H]$^+$: calcd, 264.1388; found, 264.1388.

2-(1-Methoxynaphthalen-4-yl)-2-phenylethylamine Hydrochloride (26). Refer to general procedure for the reduction of nitrile to amine hydrochloride described above: 0.26 g, 31% yield. ^{1}H NMR (400 MHz, methanol-d_4) δ 8.28 (m, 1H), 8.04 (d, J = 7.9 Hz, 1H), 7.47 (m, 3H), 7.35 (m, 4H), 7.24 (m, 1H), 6.97 (d, J = 8.1 Hz, 1H), 5.05 (t, J = 8.0 Hz, 1H), 4.03 (s, 3H), 3.72 (m, 2H). HRMS (EI$^+$) m/z for $C_{19}H_{19}NO$ [M + H]$^+$: calcd, 278.1545; found, 278.1550.

2-(1-Phenoxynaphthalen-4-yl)-2-phenylethylamine Hydrochloride (27). Refer to general procedure for *t*-Boc deprotection described above: slightly yellow solid, 0.058 g, 84% yield. ^{1}H NMR (400 MHz, methanol-d_4) δ 8.24 (m, 1H), 8.16 (d, J = 7.9 Hz, 1H), 7.56 (m, 1H), 7.50 (m, 2H), 7.37 (m, 6H), 7.25 (m, 1H), 7.14 (m, 1H), 7.04 (m, 2H), 6.95 (d, J = 8.1 Hz, 1H), 5.13 (t, J = 7.9 Hz, 1H), 3.74 (m, 2H). HRMS (EI$^+$) m/z for $C_{24}H_{21}NO$ [M + H]$^+$: calcd, 340.1701; found, 340.1716.

Acknowledgment. This research was supported by a grant from the National Institutes of Health (Grant DK52798 to T.S.S.).

Supporting Information Available: Experimental procedures for all intermediates and HPLC data for final compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Scanlan, T. S.; Suchland, K. L.; Hart, M. E.; Chiellini, G.; Huang, Y.; Kruzich, P. J.; Frascarelli, S.; Crossley, D. A.; Bunzow, J. R.; Ronca-Testoni, S.; Lin, E. T.; Hatton, D.; Zucchi, R.; Grandy, D. K. 3-Iodothyronamine is an endogenous and rapid-acting derivative of thyroid hormone. *Nat. Med.* **2004**, *10* (6), 638−42.

(2) Hart, M. E.; Suchland, K. L.; Miyakawa, M.; Bunzow, J. R.; Grandy, D. K.; Scanlan, T. S. Trace amine-associated receptor agonists: synthesis and evaluation of thyronamines and related analogues. *J. Med. Chem.* **2006**, *49* (3), 1101−12.

(3) Bunzow, J. R.; Sonders, M. S.; Arttamangkul, S.; Harrison, L. M.; Zhang, G.; Quigley, D. I.; Darland, T.; Suchland, K. L.; Pasumamula, S.; Kennedy, J. L.; Olson, S. B.; Magenis, R. E.; Amara, S. G.; Grandy, D. K. Amphetamine, 3,4-methylenedioxymethamphetamine, lysergic acid diethylamide, and metabolites of the catecholamine neurotransmitters are agonists of a rat trace amine receptor. *Mol. Pharmacol.* **2001**, *60* (6), 1181−8.

(4) Borowsky, B.; Adham, N.; Jones, K. A.; Raddatz, R.; Artymyshyn, R.; Ogozalek, K. L.; Durkin, M. M.; Lakhlani, P. P.; Bonini, J. A.; Pathirana, S.; Boyle, N.; Pu, X.; Kouranova, E.; Lichtblau, H.; Ochoa, F. Y.; Branchek, T. A.; Gerald, C. Trace amines: identification of a family of mammalian G protein-coupled receptors. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98* (16), 8966−71.

(5) Lindemann, L.; Ebeling, M.; Kratochwil, N. A.; Bunzow, J. R.; Grandy, D. K.; Hoener, M. C. Trace amine-associated receptors form structurally and functionally distinct subfamilies of novel G protein-coupled receptors. *Genomics* **2005**, *85* (3), 372−85.

(6) Pine, S. H.; Sanchez, B. L. Formic acid−formaldehyde methylation of amines. *J. Org. Chem.* **1971**, *36* (6), 829−32.

(7) Mattingly, P. G. Mono-Protected Diamines-N$^\alpha$-*tert*-Butoxycarbonyl α,ω-Alkanediamine Hydrochlorides from Amino-alcohols. *Synthesis* **1990**, (4), 366−368.

(8) Kraxner, J.; Hubner, H.; Gmeiner, P. Azepino- and diazepinoindoles: Synthesis and dopamine receptor binding profiles. *Arch. Pharm.* **2000**, *333* (9), 287−292.

(9) Shah, J. H.; Izenwasser, S.; Geter-Douglass, B.; Witkin, J. M.; Newman, A. H. (±)-(*N*-alkylamino)benzazepine analogs: novel dopamine D1 receptor antagonists. *J. Med. Chem.* **1995**, *38* (21), 4284−93.

(10) Evans, D. A.; Katz, J. L.; West, T. R. Synthesis of Diaryl Ethers through the Copper-Promoted Arylation of Phenols with Arylboronic Acids. An Expedient Synthesis of Thyroxine. *Tetrahedron Lett.* **1998**, *39* (19), 2937−2940.

(11) Finholt, A. E.; Bond, A. C.; Schlesinger, H. I. Lithium Aluminum Hydride, Aluminum Hydride and Lithium Gallium Hydride, and Some of their Applications in Organic and Inorganic Chemistry. *J. Am. Chem. Soc.* **1947**, *69* (5), 1199−1203.
(12) Kruse, L. I.; Kaiser, C.; DeWolf, J. W. E.; Chamber, P. A.; Goodhart, P. J.; Ezekiel, M.; Ohlstein, E. H. β-Substituted Phenethylamines as High-Affinity Mechanism-Based Inhibitors of Dopamine β-Hydroxylase. *J. Med. Chem.* **1988**, *31* (4), 704−706.
(13) Eglen, R. M. Enzyme fragment complementation: a flexible high throughput screening assay technology. *Assay Drug Dev. Technol.* **2002**, *1* (1, Pt. 1), 97−104.
(14) Eglen, R. M.; Singh, R. Beta galactosidase enzyme fragment complementation as a novel technology for high throughput screening. *Comb. Chem. High Throughput Screening* **2003**, *6* (4), 381−7.
(15) Wainscott, D. B.; Little, S. P.; Yin, T.; Tu, Y.; Rocco, V. P.; He, J. X.; Nelson, D. L. Pharmacologic characterization of the cloned human trace amine-associated receptor1 (TAAR1) and evidence for species differences with the rat TAAR1. *J. Pharmacol. Exp. Ther.* **2007**, *320* (1), 475−85.

JM0700417