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1-(Phenyl)isoquinoline carboxamides: a novel class of subtype selective inhibitors of thyrotropin-releasing hormone (TRH) receptors

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Abstract—We report the synthesis of and binding to the two subtypes of mouse thyrotropin-releasing hormone (TRH) receptors, TRH-R1 and TRH-R2, of several 1-(phenyl)isoquinoline carboxamide analogues. These analogues showed a degree of selectivity for binding at TRH-R2. These are the first ligands reported that show selective binding to these receptors.
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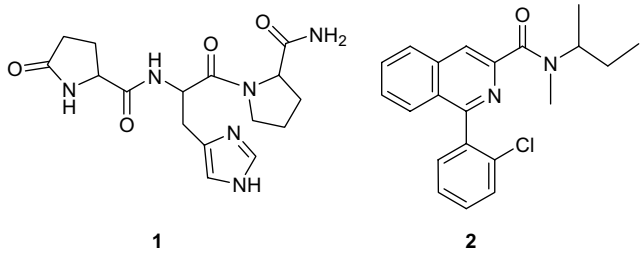
1. Introduction

Thyrotropin-releasing hormone (TRH) (**1**) (Fig. 1), a tripeptide, plays important roles in the regulation of thyroid-stimulating hormone (TSH) and prolactin synthesis and secretion in the pituitary gland, and within the central and peripheral nervous systems.[1] The actions of TRH in rodents are mediated by G protein-coupled receptors (GPCRs) designated TRH-R1 and TRH-R2;[2] a homologue of TRH-R2 has not been observed in humans. The two receptors likely mediate different actions of TRH because they exhibit marked differences in tissue distribution in the rat[3] and TRH-R2 displays higher basal signaling activity than TRH-R1.[4] However, although the two subtypes of TRH receptors are only approximately 50% homologous, they exhibit similar binding affinities for TRH and numerous TRH analogues.[3] Ligands that could distinguish between TRH-R1 and TRH-R2 would be useful in delineating the physiological roles of these receptors.



1 2

Figure 1. Structures of TRH (**1**) and PK 11195 (**2**).

The identification of small molecule ligands that modulate the activity of GPCRs continues to be of high interest based upon established and expectant therapeutic value. TRH has been tested in the clinic for the treatment of several diseases including neurodegeneration, however, the selectivity for the two receptor subtypes has not been addressed and the rapid rate of TRH dispersal in circulation have limited these studies.[5] To date the use of nonpeptidic, small molecule ligands to modulate the activities of TRH receptors has been largely ignored.

Benzodiazepines are among the few small molecules reported to affect TRH receptors. Benzodiazepines act as inverse agonists (or negative antagonists) at TRH-R1

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and TRH-R2[2] but their selectivities have not been tested and their affinities for the receptors are low. Moreover, utilization of a benzodiazepine as a TRH receptor modulator in an intact animal or human would be accompanied by central nervous system depression thereby limiting their usefulness as reagents to study TRH receptor biology. Consequently, novel small molecule modulators of TRH-R1 and TRH-R2 without these liabilities are of interest. Herein, we report a series of novel inhibitors of TRH-R1 and TRH-R2 based upon a 1-(phenyl)-isoquinoline amide scaffold.

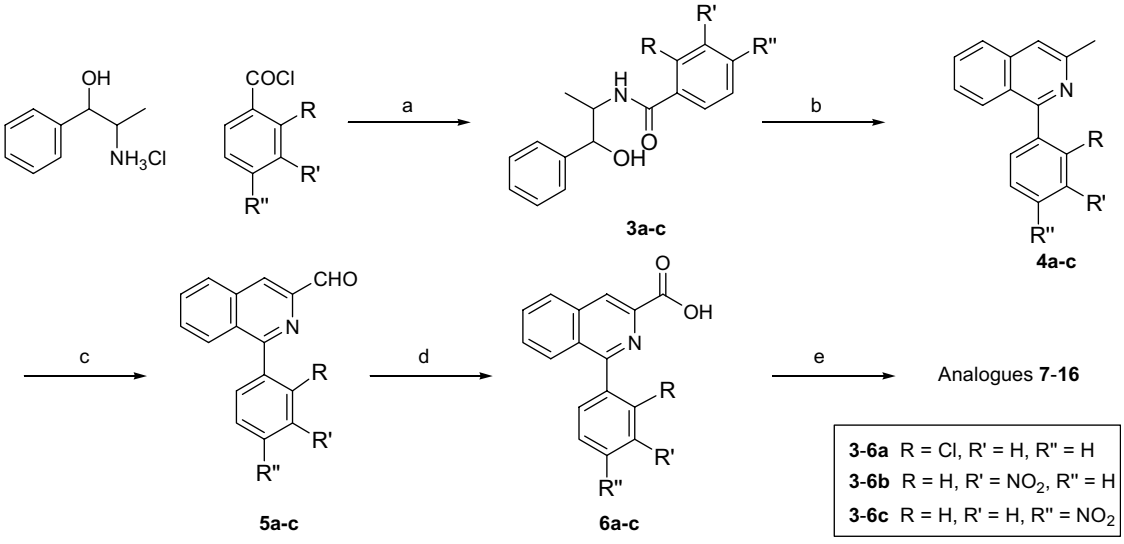
Through random screening we found that analogues of the peripheral benzodiazepine receptor ligand PK 11195 (**2**) (Fig. 1) had TRH inhibitory activity at both TRH-R1 and TRH-R2. Jarvinen et al.[6] had previously reported that PK11195 could compete with binding to TRH receptors in rat anterior pituitary gland, hypothalamus, cortex, and brainstem. Furthermore, we noted that the affinities of these compounds were greater at TRH-R2 than at TRH-R1, which is a transposition from the affinities of centrally acting benzodiazepines (unpublished observations). Aiming to both amplify the affinity for TRH receptors and further establish a degree of selectivity between the two receptors, we synthesized several analogues of this compound examining structure–activity relationships (SAR) at the amide site most specifically.

2. Chemistry

The synthesis employed was a combination of the reported routes of Janin et al. and Manning et al. (Scheme 1).[7] Intermediates **3a**,**b**, and **3c** were achieved via the base catalyzed coupling of norephedrine with 2-chlorobenzoyl chloride, 3-nitrobenzoyl chloride, or 4-nitrobenzoyl chloride. Condensation to the isoquinoline ring was accomplished through treatment with P_2O_5 to provide compounds **4a**,**b**, and **4c**. Oxidation of the benzylic carbon was accomplished through a two-step procedure using selenium oxide to form aldehydes **5a**,**b**, and **5c** followed by silver nitrate oxidation to the carboxylic acids **6a**,**b**, and **6c**. Attempts to oxidize directly to the carboxylic acid via $KMnO_4$ were unsuccessful. The resulting carboxylic acids were derivatized via acid chloride formation followed by coupling with the appropriate amine to yield compounds **7**–**16** (Table 1).[8] Additionally, derivative **17** was achieved via the coupling of the analogous acid and *sec*-butyl amine through the acid chloride.[9]

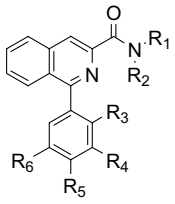
3. Assay results

Having identified PK 11195 as an inhibitor of [^{3}H]MeTRH binding to TRH-R1 and TRH-R2 during our initial screen, we studied the binding of a series of 1-(2-chlorophenyl) isoquinoline carboxamide analogues at a single concentration (100 μM) (Table 1).[10] We found that there were differences among the analogues in their abilities to compete with [^{3}H]MeTRH binding to TRH-R1 versus TRH-R2. In general, analogues with relatively bulky alkyl groups on the amide nitrogen were more potent inhibitors and there appeared to be selectivity in the binding for TRH-R2 versus TRH-R1. Interestingly, this selectivity seemed to be reversed by the inclusion of a nitro group at the 5 position of the 2-chlorophenyl ring. To demonstrate this, we further analyzed the binding of four analogues that contained at least a 4-carbon substituent on the amide nitrogen and with various substitutions on the phenyl ring. Figure 2 illustrates the dose-dependent competition binding of these analogues. Analogue **11**, **13**, and **14** showed greater affinity for TRH-R2 than TRH-R1 whereas analogue **17** did indeed display reversed affinities for TRH-R2 and TRH-R1.[11] The influence of nitro group inclusion seemed to depress the potency of these compounds (analogues **14**, **15**, and **16**) and only in the case of the 2-chloro-5-nitro substituted phenyl ring was the selectivity reversed



Scheme 1. Reagents and conditions: (a) 5% NaOH, CH_2Cl_2; (b) P_2O_5, *o*-dichlorobenzene; (c) SeO_2, *o*-dichlorobenzene; (d) $AgNO_3$, NaOH, EtOH, H_2O; (e) $SOCl_2$, CH_2Cl_2, DMF (one drop) stirred for 5 min followed by the addition of the appropriate amine.

Table 1. Competition of binding of [^{3}H]MeTRH to TRH-R1 and TRH-R2 by **2**, **7**–**17** at 100 μM

	Analogue #	R_1	R_2	R_3	R_4	R_5	R_6	% Competition	
								TRH R1	TRH R2
	**2** (PK 11195)	*sec*-Butyl	Methyl	Cl	H	H	H	44 ± 3.5	56 ± 7.0
	7	Methyl	H	Cl	H	H	H	33 ± 5.5	29 ± 3.2
	8	Ethyl	H	Cl	H	H	H	55 ± 16.8	82 ± 6.9
	9	Propyl	H	Cl	H	H	H	78 ± 4.0	91 ± 1.9
	10	Isopropyl	H	Cl	H	H	H	73 ± 7.8	90 ± 1.5
	11	*t*-Butyl	H	Cl	H	H	H	26 ± 2.7	68 ± 2.9
	12	Butyl	Methyl	Cl	H	H	H	84 ± 5.2	92 ± 2.9
	13	*sec*-Butyl	H	Cl	H	H	H	49 ± 3.2	85 ± 2.9
	14	Isobutyl	H	H	NO_2	H	H	26 ± 1.2	61 ± 2.2
	15	*sec*-Butyl	H	H	NO_2	H	H	21 ± 2.9	31 ± 3.0
	16	*sec*-Butyl	H	H	H	NO_2	H	16 ± 0.3	40 ± 2.6
	17	*sec*-Butyl	H	Cl	H	H	NO_2	65 ± 5.6	33 ± 2.4

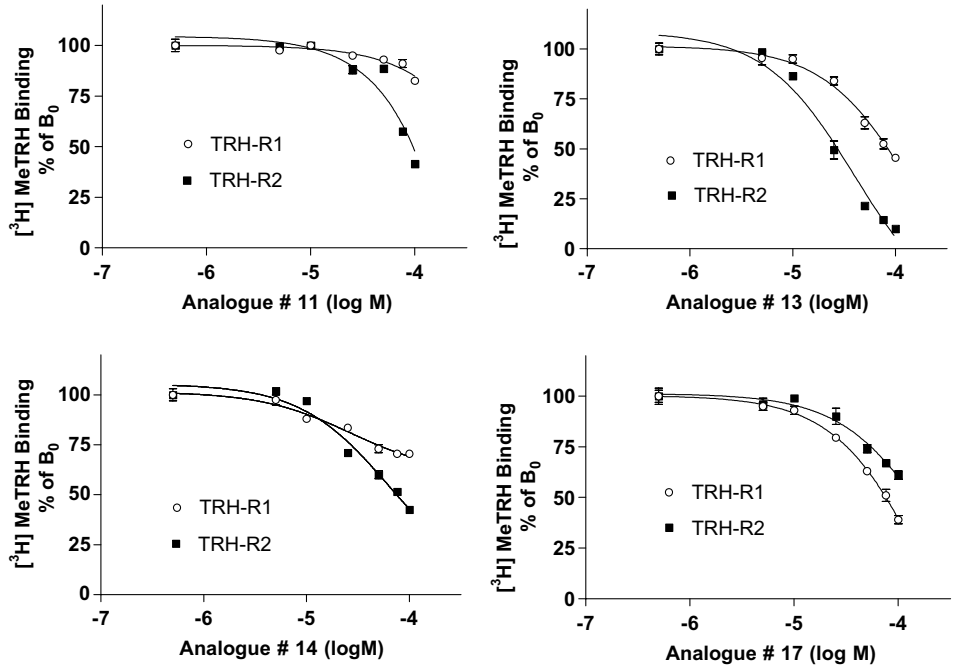


Figure 2. Competition binding curves for **11**, **13**, **14**, **17**.

(Table 1). Studies to determine the generality of this effect are currently underway. An additional 20 analogues of PK 11195 were synthesized and tested to explore additional amide substitutions including larger alkyl groups (i.e., adamantyl), positively charged groups (i.e., spermine), negatively charged groups (i.e., propyl sulfonic acid) and numerous heterocycles (i.e., propyl imidazole); all of which eradicated TRH receptor binding activity (data not shown).

4. Conclusion

Small, nonpeptide molecules that selectively and potently bind and modulate the TRH receptors would be valuable research tools and may have therapeutic potential. In this study we synthesized a series of 1-(phenyl)-isoquinoline carboxamide analogues and found that several showed selectivity for mouse TRH-R2 over TRH-R1. These are the first compounds reported to distinguish between these two subtypes of TRH receptors. In contrast, although a number of peptidic analogues of TRH have been tested,[3,11] none are noted to selectively bind to either TRH receptor subtype. The low molecular weight analogues described here and surveyed in subsequent studies will likely be better probes of TRH receptor function in vivo as they would be predicted to cross the blood–brain barrier and may allow for study of TRH receptors within the central nervous system.

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8. Within the synthesis of **13**, **15**, **16**, and **17** racemic *sec*-butyl amine was utilized. Spectroscopic information: **7** ^{1}H NMR ($CDCl_3$): δ 3.06 (d, J_{HH} = 4.8 Hz, 3H), 7.45–7.51 (m, 3H), 7.57–7.63 (m, 2H), 7.67–7.69 (m, 1H), 7.73–7.79 (m, 1H), 8.05–8.08 (m, 1H), 8.21 (br s, 1H), 8.67 (s, 1H); TOFMS m/e 297.0795 ($M+H^+$) (calculated for $C_{17}H_{14}ClN_2O^+$ 297.0789). Compound **8** ^{1}H NMR ($CDCl_3$): δ 1.27 (t, J_{HH} = 7.5 Hz, 3H), 3.55 (q, J_{HH} = 6.0 Hz, 2H), 7.44–7.53 (m, 3H), 7.56–7.60 (m, 2H), 7.66–7.69 (m, 1H), 7.73–7.78 (m, 1H), 8.04–8.07 (m, 1H), 8.21 (br s, 1H), 8.68 (s, 1H); TOFMS m/e 311.0940 ($M+H^+$) (calculated for $C_{18}H_{16}ClN_2O^+$ 311.0945). Compound **9** ^{1}H NMR ($CDCl_3$): δ 0.98 (t, J_{HH} = 7.5 Hz, 3H), 1.67 (m, 2H), 3.47 (q, J_{HH} = 6.3 Hz, 2H), 7.45–7.53 (m, 3H), 7.57–7.62 (m, 2H), 7.67–7.70 (m, 1H), 7.73–7.78 (m, 1H), 8.04–8.07 (m, 1H), 8.26 (br s, 1H), 8.67 (s, 1H); TOFMS m/e 325.1097 ($M+H^+$) (calculated for $C_{19}H_{18}ClN_2O^+$ 325.1102). Compound **10** ^{1}H NMR ($CDCl_3$): δ 1.29 (d, J_{HH} = 6.6 Hz, 6H), 4.34 (m, 1H), 7.46–7.53 (m, 3H), 7.56–7.61 (m, 2H), 7.66–7.69 (m, 1H), 7.72–7.77 (m, 1H), 8.04–8.07 (m, 2H), 8.67 (s, 1H); TOFMS m/e 325.1111 ($M+H^+$) (calculated for $C_{19}H_{18}ClN_2O^+$ 325.1102). Compound **11** ^{1}H NMR ($CDCl_3$): δ 1.51 (s, 9H), 7.46–7.52 (m, 3H), 7.55–7.60 (m, 2H), 7.67–7.71 (m, 1H), 7.74–7.78 (m, 1H), 8.02–8.05 (m, 1H), 8.15 (br s, 1H), 8.64 (s, 1H); TOFMS m/e 339.1253 ($M+H^+$) (calculated for $C_{20}H_{20}ClN_2O^+$ 339.1258). Compound **12** (partial double bond effect observed) ^{1}H NMR ($CDCl_3$): δ 0.72 (t, J_{HH} = 7.2 Hz, 3H), 0.98 (t, J_{HH} = 7.2 Hz, 3H), 1.08–1.15 (m, 2H), 1.39–1.47 (m, 2H), 1.48–1.61 (m, 2H), 1.63–1.71 (m, 2H), 3.10–3.14 (m, 6H), 3.34–2.48 (m, 2H), 3.51–3.62 (m, 2H), 7.40–7.48 (m, 6H), 7.53–7.59 (m, 4H), 7.63–7.65 (m, 2H), 7.70–7.76 (m, 2H), 7.95–7.97 (m, 2H), 8.10–8.11 (m, 2H), 8.64 (s, 1H); TOFMS m/e 353.1407 ($M+H^+$) (calculated for $C_{21}H_{22}ClN_2O^+$ 353.1415). Compound **13** ^{1}H NMR ($CDCl_3$): δ 0.96 (t, J_{HH} = 7.8 Hz, 3H), 1.26 (d, J_{HH} = 6.6 Hz, 3H), 1.62 (m, 2H), 4.18 (m, 1H), 7.47–7.54 (m, 3H), 7.57–7.62 (m, 2H), 7.66–7.77 (m, 2H), 8.03–8.08 (m, 2H), 8.67 (s, 1H); TOFMS m/e 339.1276 ($M+H^+$) (calculated for $C_{20}H_{20}ClN_2O^+$ 339.1258). Compound **14** ^{1}H NMR ($CDCl_3$): δ 0.99 (d, J_{HH} = 6.6 Hz, 6H), 1.89–1.99 (m, 1H), 3.11–3.19 (m, 2H), 7.52–7.78 (m, 3H), 7.91–8.07 (m, 3H), 8.21 (br s, 1H), 8.33–8.35 (m, 1H), 8.54–8.61 (m, 1H); TOFMS m/e 350.1499 ($M+H^+$) (calculated for $C_{20}H_{20}N_3O^+$ 350.1499). Compound **15** ^{1}H NMR ($CDCl_3$): δ 0.99 (t, J_{HH} = 7.5 Hz, 3H), 1.29 (d, J_{HH} = 6.6 Hz, 3H), 1.60–1.68 (m, 2H), 4.09–4.16 (m, 1H), 7.66–7.71 (m, 1H), 7.75–7.84 (m, 2H), 7.98–8.12 (m, 4H), 8.41–8.45 (m, 1H), 8.60 (s, 1H), 8.70 (s, 1H); TOFMS m/e 350.1489 ($M+H^+$) (calculated for $C_{20}H_{20}N_3O^+$ 350.1499). Compound **16** ^{1}H NMR ($CDCl_3$): δ 0.98 (t, J_{HH} = 7.5 Hz, 3H), 1.28 (d, J_{HH} = 6.9 Hz, 3H), 1.59–1.68 (m, 2H), 4.06–4.14 (m, 1H), 7.65–7.70 (m, 1H), 7.78–7.83 (m, 1H), 7.88–7.92 (m, 2H), 7.96–8.03 (m, 2H), 8.08–8.12 (m, 1H), 8.44–8.47 (m, 2H), 8.69 (s, 1H); TOFMS m/e 350.1503 ($M+H^+$) (calculated for $C_{20}H_{20}N_3O^+$ 350.1499). Compound **17** ^{1}H NMR (DMSO-d_6): δ 1.04 (t, J_{HH} = 7.5 Hz, 3H), 1.35 (d, J_{HH} = 6.6 Hz, 3H), 1.61–1.68 (m, 2H), 4.17–4.28 (m, 1H), 8.05–8.16 (m, 3H), 8.30–8.33 (m, 1H), 8.38–8.46 (m, 1H), 8.62–8.70 (m, 2H), 8.83–8.56 (m, 1H), 9.40 (s, 1H); TOFMS m/e 384.1100 ($M+H^+$) (calculated for $C_{20}H_{19}ClN_3O_3^+$ 384.1109).
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10. Assay description: HEK293EM cells stably expressing either TRH-R1 or TRH-R2 were grown in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum. Cells were selected to express TRH-R1 and TRH R2 at similar concentrations. Competition binding experiments were performed initially using 1 nM [^{3}H][methyl-His]TRH ([^{3}H]MeTRH, DuPont), an analogue of TRH with 5- to10-fold higher affinity for TRH-R1, and a single dose of competing ligand using intact cells at 37 °C for 1 h. Apparent binding inhibitory constants (K_is) were measured at equilibrium using 1 nM [^{3}H]MeTRH and various concentrations of competing ligands. Equilibrium binding constants were derived from competition binding experiments using the formula $K_i = (IC_{50}/(1+([L]/K_d))$, where IC_{50} is the concentration of unlabeled ligand that half-competes with specifically bound [^{3}H]MeTRH. Curves were fitted by nonlinear regression analysis and drawn with the PRISM program 3 (GraphPad Inc.).
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