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Thyroid receptor ligands. Part 8: Thyromimetics derived from N-acylated-α-amino acid derivatives displaying modulated pharmacological selectivity compared with KB-141

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Abstract—Based on the scaffold of the pharmacologically selective thyromimetic **2b**, structurally a close analog to KB-141 (**2a**), a number of novel N-acylated-α-amino acid derivatives were synthesized and tested in a TR radioligand binding assay as well as in a reporter cell assay. On the basis of TRβ$_1$-isoform selectivity and affinity, as well as affinity to the reporter cell assay, **3d** was selected for further studies in the cholesterol-fed rat model. In this model **3d** revealed an improved therapeutic window between cholesterol and TSH lowering but decreased margins versus tachycardia compared with **2a**.

Hyperlipidemia is a well-established risk factor for atherogenesis and cardiovascular disease, and thus one of the leading causes for morbidity and mortality in the developed world.[1] The active endogenous thyroid hormone (L-T$_3$, **1**) (Fig. 1) is a potent lipid lowering agent but cannot be used therapeutically in patients with hyperlipidemia, mainly due to the side-effect of tachycardia. There exist two subtypes of thyroid hormone receptors (TRs), α and β, unequally distributed in the body. TRα is most abundant in the heart. Most effects of **1** on the heart are mediated through TRα, while the majority of actions in the liver are mediated through TRβ. The prospects for the treatment of metabolic diseases such as dyslipidemia with TRβ-selective ligands would be enhanced by avoidance of cardiovascular acceleration mediated through TRα.[2] Moreover, as evident from the older literature, tissue selectivity can also contribute significantly to pharmacological selectivity.[3]

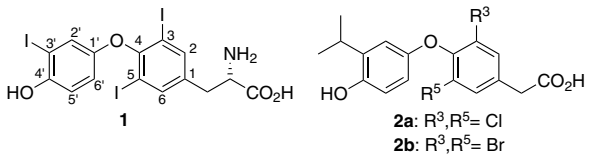

Figure 1. Chemical structure of L-T$_3$ (**1**) including ring-numbering, and KB-141 (**2a**) and **2b**.

We have previously shown that TRβ$_1$-selectivity can be achieved both in vitro and in vivo when the R^1-amino acid side-chain of **1** is truncated to acetic acid and the iodine atoms at the R^3 and R^5-positions are substituted with chlorine or bromine to generate KB-141 (**2a**) and **2b** (Fig. 1). These compounds were 14 and 10 times selective for TRβ over TRα, respectively, ratios that were further propagated in vivo.[4]

Keywords: KB-141; Thyromimetic; α-Amino acid; Binding assay; TRβ-selective; Cholesterol-fed rat model; Cholesterol; Thyroid stimulating hormone; Tachycardia.
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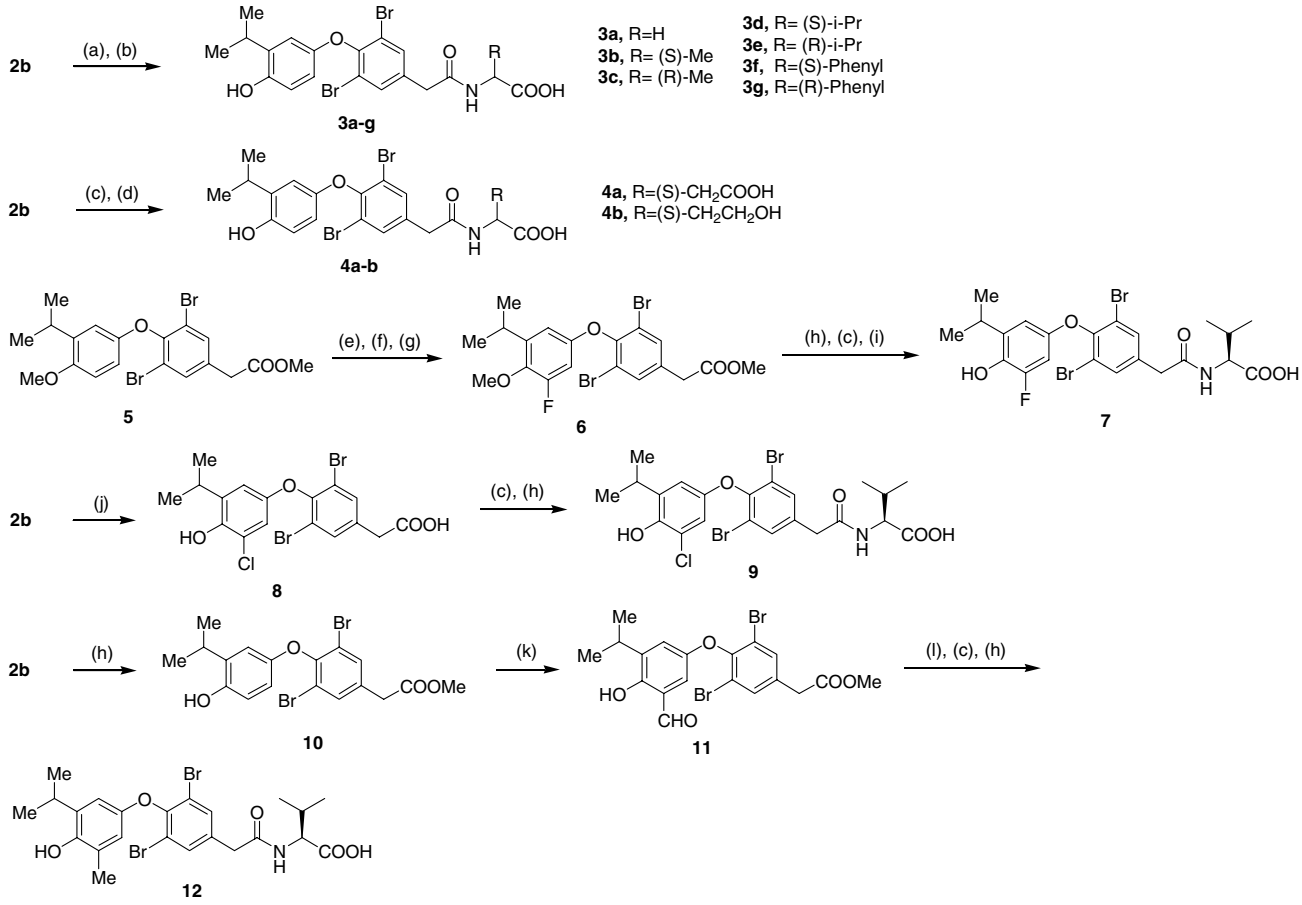

Scheme 1. Reagents and conditions: (a) EDCI, HBT, CH_2Cl_2 + carboxylic acid ester of amino acid, TEA, 40 °C; (b) NaOH, EtOH, 40 °C; (c) EDCI, HBT, DMF + carboxylic acid ester of amino acid, DMF, rt; (d) NaOH, EtOH, rt; (e) HNO_3, C_6H_6, rt; (f) sodiumhydrosulfite, EtOH, Δ; (g) $NOBF_4$, CH_2Cl_2, *o*-xylen, Δ; (h) LiOH, THF, rt; (i) $BF_3{\cdot}SMe_2$, CH_2Cl_2, rt; (j) benzyltrimethylammonium tetrachloroiodate acetic acid, rt; (k) $SnCl_4$, trioctyl amine, toluene, rt + paraformaldehyde; (l) hydrazine hydrate, EtOH, rt + KOH, ethylene glycol, 150 °C.

As **2a** had a similar tissue-distribution as **1**, its selectivity for cholesterol lowering versus tachycardia could be explained by virtue of its TRβ$_1$-selectivity.[5] On the other hand, TRβ$_1$ activation can be expected to reduce thyroid stimulating hormone (TSH) levels and, therefore, reduce the endogenous production of **1**. This may be an issue in tissues not accessible to TRβ$_1$-selective agonists, leading to a paradoxical local tissue hypothyroidism.[6] Indeed, TSH was lowered in vivo with **2a**, at doses even lower than for cholesterol lowering.[5] Consequently, in order to avoid TSH-lowering induced by both **1** and **2a**, a selective agent may require some degree of liver selectivity (or lack of systemic effects) or, alternatively, selective receptor modulation.

In the present study, we wanted to investigate both the in vitro and in vivo consequences when the R^1-acetic acid group was condensed with α-amino acids. As is evident from the literature, since the TR receptor is highly accommodative of a variety of R^1-side-chains, this flexibility can be expected to encompass larger R^1-chains than acetic acid. Since the therapeutic ratio of **2b** was almost within the same range as **2a**, we also decided to maintain R^3 and R^5-position as bromines particularly as the starting material could be prepared with facility and in larger yields.[4] Consequently, a set of thyromimetics was prepared as outlined in Scheme 1 where various α-amino acids were N-acylated with the R^1-acetic acid group of **2b** by expedient coupling with protected amino acids to generate the end products **3a**–**g** and **4a** and **4b** in low to moderate yields (18–52%). Introduction of R$^{5'}$-substituents was achieved by more elaborate routes: R$^{5'}$-F (**7**) from intermediate **6** via a nitration–reduction–diazotization sequence; R$^{5'}$-*Cl* (**9**) from intermediate **8** via regioselective chlorination; and R$^{5'}$-*Me* (**12**) from intermediate **11** via regioselective formylation followed by reduction. Total yields for the preparation of **7**, **9**, and **12** were 8%, 18%, and 18%, respectively.

The results of a radioligand binding assay for the human TRα1 and TRβ1, as well as a reporter cell assay employing CHOK1-cells (Chinese hamster ovary cells) stably transfected with hTRα$_1$ or hTRβ$_1$ and an alkaline phosphatase reporter gene downstream thyroid response element (TRAFα$_1$ and TRAFβ$_1$),[7] are summarized in Table 1. All new ligands (**3a**–**g**, **4a**, **4b**, **7**, **9**, and **12**) exhibited weaker TRβ$_1$-binding (IC$_{50}$s) as well as TRAFβ$_1$ affinities (EC$_{50}$s) compared with **2b**. However, in general TRβ$_1$-binding selectivity was conserved due to a concomitant reduction in affinity for TRα$_1$. The naturally occurring amino acid stereochemistry (*S*-form) was moderately favored in isomeric pairs **3b** and **3c** and **3d**

Table 1. Thyroid hormone receptor binding affinities (IC_{50}), TRAF affinities (EC_{50}), and efficacy of **1**, and synthetic thyromimetics **2a**, **2b**, **4a**–**i**, and **9a**–**c** [a]

Compound	*h*TRα_1 IC_{50} [b]	*h*TRβ_1 IC_{50} [b]	α_1/β_1 [c]	TRAFα EC_{50} [d]	TRAFβ EC_{50} [d]
1	0.24	0.26	0.54	—	—
2a	25 ± 1.7	1.1 ± 0.029	14	1.7	0.78
2b	1.4 ± 0.44	0.095 ± 0.23	8.7	0.38	0.20
3a	26 ± 1.2	2.7 ± 0.41	5.9	103 (75)	42 (85)
3b	10	1.3	4.5	7.2 (81)	2.6 (92)
3c	83	5.9	8.3	15 (83)	7.6 (100)
3d	4.3 ± 0.22	0.27 ± 0.0018	9.4	1.4 (78)	0.77 (92)
3e	9.8 ± 1.0	0.71 ± 0.067	8.2	2.3 (88)	1.2 (100)
3f	7.5	0.78	5.7	Nt	Nt
3g	5.5 ± 0.29	0.60 ± 0.070	6.0	1.5 (65)	0.73 (85)
4a	460 ± 38	290 ± 23	0.9	1500 (72)	1300 (84)
4b	168	26	3.8	66 (74)	38 (88)
7	88 ± 12	12 ± 0.50	4.3	17 (78)	11 (88)
9	670 ± 50	1400 ± 16	0.29	65 (21)	210 (31)
12	1500 ± 73	2600 ± 240	0.34	170 (13)	1100 (27)

[a] All IC_{50} and EC_{50} values are expressed as nM and the data for **1**, **2a**, and **2b** are taken from Ref. 4.
[b] The IC_{50} values for **2a**, **2b**, **3a**, **3d**, **3e**, **3g**, **4a**, **7**, **9**, and **12** are expressed as mean IC_{50}s ± SE, while IC_{50}s for the other compounds are average means of two runs with variability of the measurements with an average of ±25%.
[c] Normalized selectivity: (IC_{50} hTRα_1)/(IC_{50} hTRβ_1 × 1.7). For an explanation, see Ref. 4.
[d] EC_{50} values are expressed as nM and are all calculated means of duplicate runs with an average variability of the measurements of approximately ±25%. Values within parentheses denote % agonism.

and **3e**; but no preference was observed for **3f** and **3g**. Anyway, only the (*S*)-enantiomers of analogs **4a**, **4b**, **7**, **9**, and **12** were investigated. The introduction of additional polar groups in the amino acid chain, either H-bond acceptors (**4a**) or H-bond donators (**4b**), resulted in significant loss of affinity for both TR-binding and TRAF-binding, but unexpectedly retained agonist efficacy in TRAF.

Affinity and agonist efficacy progressively diminished as the size of the $R^{5'}$-substituent increased suggesting that there exists a maximal size of a ligand that can be accommodated by the receptor with retained TR agonism (F (**7**) < Cl (**9**) < Me (**12**)).[8] In keeping with this hypothesis, evaluation of **12** for antagonism in TRAF revealed that 1100 nM concentration of **12** produced 50% inhibition of L-T_3 and 70% efficacy as an antagonist.[9]

Overall, based on optimal affinity for TR-binding, TRAF-affinity as well as TRβ_1-selectivity, **3d** was selected for in vivo studies in the cholesterol-fed rat model.[5]

Dose–response data for **1**, **2a**,[5] and **3d** are shown in Table 2. The data are shown as ED_{15} for heart rate (dose causing 15% increase from vehicle), ED_{50} for cholesterol (dose causing 50% suppression from vehicle), and TSH suppression (dose causing 30% suppression from vehicle). Compared with **2a**, potency for cholesterol lowering was retained with **3d**, slightly increased for tachycardia, and decreased for TSH-lowering. The potency ratios (= 'therapeutic windows') clearly show the selectivity for these compounds by dividing the ED_{15} for heart rate/ED_{50} cholesterol and ED_{30} for TSH/ED_{50} cholesterol. When normalized in this manner, it can be seen that **2a** has a larger margin for cholesterol lowering versus tachycardia, while **3d** has a larger margin for cholesterol versus TSH-lowering. In contrast, **1** exhibits absolutely no separation between cholesterol and TSH-lowering versus tachycardia in this animal model.[5]

Table 2. ED-values and potency ratios for **1**, **2a**, and **3d** in the cholesterol-fed rat model[a]

Compound	ED_{15} HR/ED_{50} Chol	ED_{30} TSH/ED_{50} Chol
1	31/21 = 1.5	6.7/21 = 0.3
2a	2900/72 = 40 (27-fold vs **1**)	32/72 = 0.4 (1.3-fold vs **1**)
3d	1580/82 = 19 (13-fold vs **1**)	242/82 = 3.0 (10-fold vs **1**)

[a] ED-values are expressed as nmol/kg/day. Administration was per oral and the experimental procedures in Ref. 5 were followed. Full dose–response data and procedure for calculation of ED-values can be found in 'Supplementary data'.

Consideration of the potency ratios above suggests that **3d** might be a selective thyroid hormone receptor modulator (STRM) in relation to **2a,** since increased liver-selectivity for **3d** alone would not be sufficient to explain the increased separation between TSH and cholesterol-lowering as the separation versus tachycardia actually decreased.

In conclusion, based on the scaffold of **2b**, a number of α-amino acid derivatives were prepared. Among these compounds **3d** was found to exhibit optimal affinity, TRβ_1-selectivity, and agonist activity. In cholesterol-fed rats, **3d** displayed potential STRM behavior in relation to **2a**, which warrants further examination of this compound in relevant in vitro and in vivo models.

Supplementary data

Supplementary data, including experimental procedures, dose–response data in rats, and analytical data,

associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.05.049.

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8. The inductive effect of the $R^{5'}$-position might also be considered. When the $R^{5'}$-position is substituted with a fluorine atom, then the pK_a of the $R^{4'}$-hydroxyl group is lowered by approximately 1.5 log units (HL/L+H = 8.99, ACD/pK_a DB: 4.04, http://www.acdlabs.com). Considering the importance of the $R^{4'}$-hydroxyl group for functional activity (agonism/antagonism), a change of its pK_a may have consequences. The cell-assay (TRAF) experiment is, however, conducted in a cell culture media at pH 7.4. Consequently, the larger fraction of the $R^{4'}$-hydroxyl group is still unionized.
9. For the use of the TRAF-assay in 'antagonist-mode', see: (a) Carlsson, B.; Singh, B. N.; Temciuc, M.; Nilsson, S.; Li, Y.-L.; Mellin, C.; Malm, J. *J. Med. Chem* **2002**, *45*, 623; (b) Koehler, K.; Gordon, S.; Brandt, P.; Carlsson, B.; Bäcksbro-Saeidi, A.; Apelqvist, T.; Agback, P.; Grover, G. J.; Nelson, W.; Grynfarb, M.; Färnegårdh, M.; Rehnmark, S.; Malm, J. *J. Med. Chem.* **2006**, *49*, 6635; (c) Hedfors, Å.; Appelqvist, T.; Carlsson, B.; Bladh, L.-B.; Litten, C.; Agback, P.; Grynfarb, M.; Koehler, K. F.; Malm, J. *J. Med. Chem.* **2005**, *48*, 3114.