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Novel heterocyclic thyromimetics

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Abstract—Novel heterocycle-fused thyromimetics are presented carrying indoles or indazoles instead of the phenolic group in T3. Potent agonists were identified in both series. SAR trends are examined and found to be mostly consistent with previously published thyromimetics. Moderate THRβ selectivity (approx. 10-fold) was observed in the indole series using isoform-selective transient THR transfection assays

Thyroid hormones are important endocrine signalling hormones that are involved in a number of physiological processes such as lipid metabolism, control of energy expenditure and pre- and neonatal brain development. Molecules mimicking only the beneficial effects of the thyroid hormones and lacking their cardiac side effects (tachycardia and arrhythmia) potentially could be used to treat a number of conditions such as obesity and dyslipidaemia. The tachycardic potential of natural thyroid hormones can at least in part be attributed to the effect on mRNA expression of the pacemaker channel HCN2.[1]

Thyroid hormones are ligands of the nuclear thyroid hormone receptors (THR), which fall into several isoforms and splice variants. The natural hormone T3 (**2**) does not show significant selectivity in binding to any of the THR isoforms. However,[2] tissue distribution and knock-out animal studies as well as results with selective ligands suggest that cardiac side effects can be attributed to the THRα isoform. This provides a rationale for the search for THRβ selective agonists as cardiovascular treatment option.

All attempts to identify therapeutically useful thyromimetics have been structurally derived from the natural hormone probably because the ligand–receptor interaction is very tight and the ligand is completely buried in the binding pocket.[3] Figure 1 depicts thyromimetics, for which selectivity for metabolic activity versus cardiac effects has been described.

GC1(**3**),[4] CGS 26214 (**4**)[5] and L-94901 (**5**)[6] have been described as potent ligands for THR and are void of cardiovascular side effects.

For GC1, modest selectivity for THRβ has been described (6.6-fold), which originated from the oxyacetic acid headgroup.[7] For CGS 26214 data on binding to THR subtypes have not been reported. For L-94901[8] however, it was suggested that liver-selective nucleic transport rather than selective binding to THRβ might be responsible for its selective activity.

As early as 1986[6,9] it had been noted, that modifications of the THR ligands in the outer ring adjacent to the phenol can lead to improved selectivity for metabolic versus cardiovascular effects. Recently, several new chemotypes have been described with selectivity for THRβ.[10–12] The amino acid sequence difference between the inner ligand binding domains of THRα and THRβ is a single amino acid exchange: Ser277 in THRα for Asn331 in THRβ, interacting with the polar head groups of THR ligands. The binding pocket of THRβ seems to be able to accommodate ligands with larger groups on the outer ring by movement of amino acid residues (e.g., Met 442[11]). This flexibility may in part be responsible for increased selectivity.

While indole replacement of the phenol in T3 has appeared in one patent application, neither examples with

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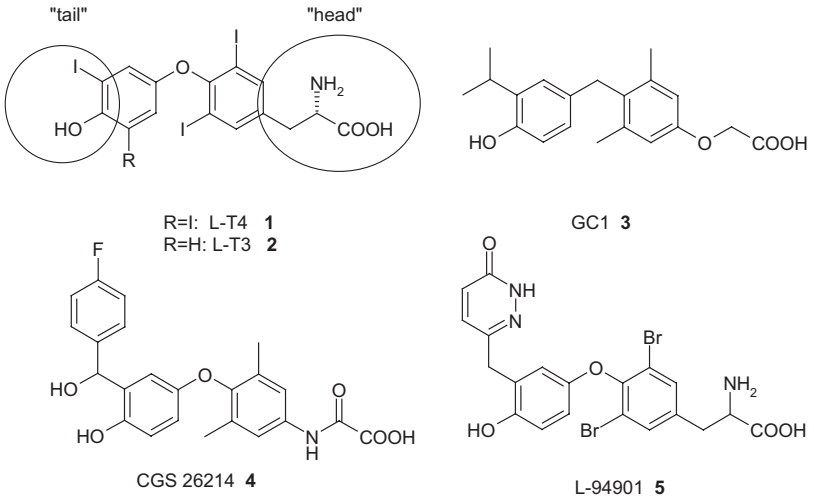


Figure 1. Thyroid hormones L-T4 (**1**) and L-T3 (**2**), GC1 (**3**), CGS 26214 (**4**) and L-94901 (**5**).

substituents in the indole 3-position nor biological activity were reported.[13]

Herein we show that incorporation of indoles and indazoles into the tail group of the biphenylether framework of T3 yields potent, single-digit nanomolar THR agonists. For the indole-type thyromimetics moderate selectivity for THRβ is shown.

Since the hydrogen bond between the phenol and His435 (in THRβ, His381 in THRα) is essential for potent THR interaction, our fused heterocyclic design motif offered the ability to attach substituents in a position similar to the iodine atom in T3, whilst maintaining the hydrogen bond donor capability of the heterocycle. A range of literature-known headgroups was used.

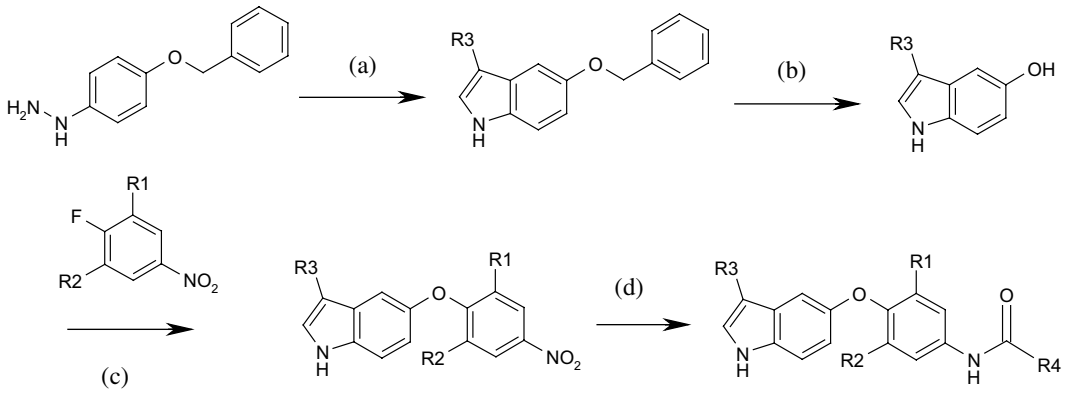
Indoles, indazoles, benzimidazoles and carbazoles were prepared with a typical THR ligand substitution pattern. Benzimidazoles and carbazoles did not show THR agonistic activity. On the other hand, indoles and indazoles demonstrated comparable activity in the single digit nanomolar range.

The syntheses are depicted in Schemes 1–6. The Fischer indole synthesis starting from *para*-benzyloxy phenylhydrazine and the corresponding aldehydes, followed by hydrogenolytic deprotection yields the hydroxyindoles as coupling partners. Nucleophilic substitution is followed by elaboration of the nitro group into the final head group.

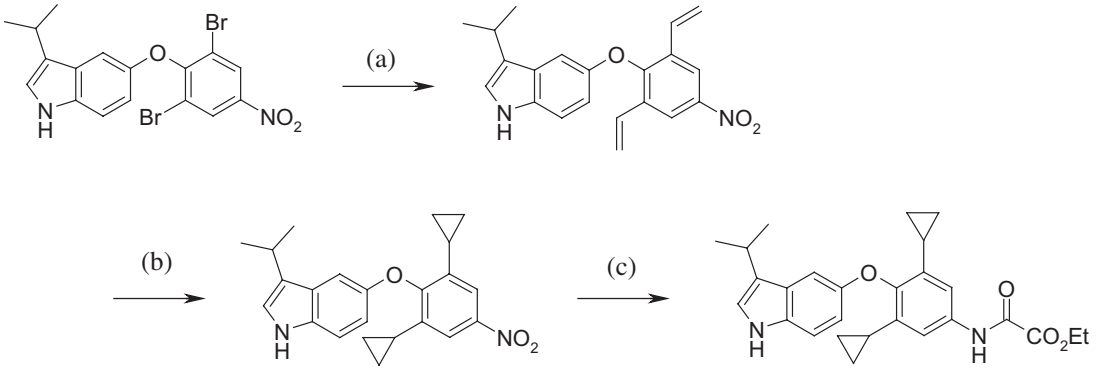
Cyclopropyl is introduced as a substituent on the inner ring via cyclopropanation of the divinyl derivative using diazomethane and subsequent nitrogen extrusion. (Scheme 2) The necessary divinyl precursor is generated from the dibromoderivative using tributylvinylstannan.

For the synthesis of the methylene bridged analogues, an approach was followed that had in part been established during the course of the synthesis of GC1: (Scheme 3).

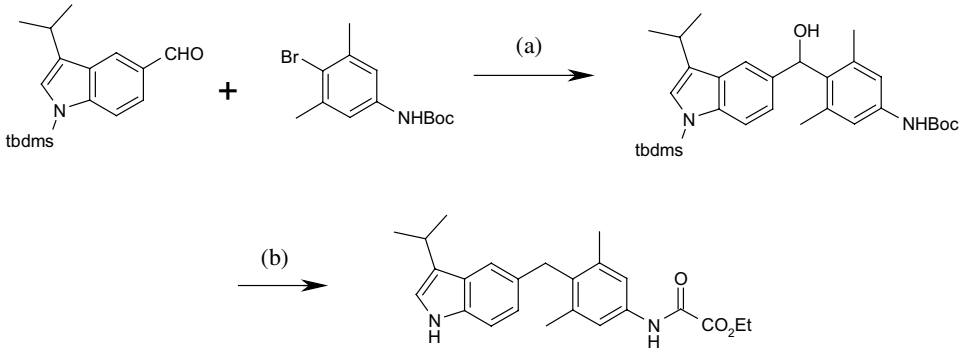
The methylene spacer is constructed from a tertiary alcohol, which was generated from addition of a dilithium-organometallic reagent to an indole benzaldehyde. The dilithiated nucleophile is generated from



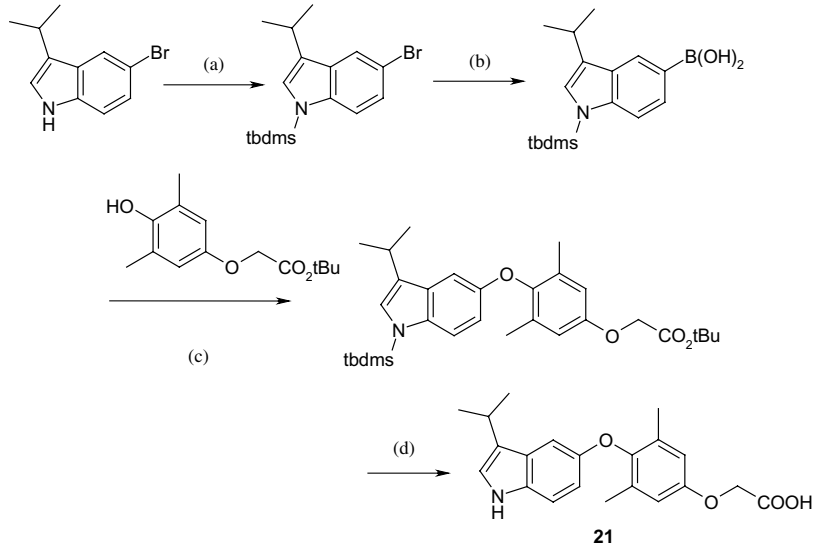
Scheme 1. Synthesis of indole type thyromimetics. Reagents and conditions: (a) cat. H_2SO_4, MeOH, RCH_2CHO, 3 h, RF; (b) Pd/C, MeOH, rt, 1 atm; (c) K_2CO_3, DMSO, rt–125 °C; (d) R_4COCl, Py, NEt_3.



Scheme 2. Synthesis of a dicyclopropyl-thyromimetic. Reagents and conditions: (a) $Pd_2(PPh_3)_4$, vinyl$SnBu_3$, Tol, RF, 20 h, 81%; (b) i. CH_2N_2, 0 °C–rt, 48 h, ii. xylene, RF, 2.5 h 64%; (c) i. $SnCl_2$, 69%, ii. $ClCOCO_2Et$, NEt_3, 96%.



Scheme 3. Synthesis of methylene bridged indole thyromimetics. Reagents and conditions: (a) i. MeLi, THF, −78 °C, ii. *n*-BuLi, THF, −78 °C 13%; (b) i. Et_3SiH, TMSOTf, ii. TFA, iii. $ClCOCO_2Et$, DCM, NEt_3, 27%.

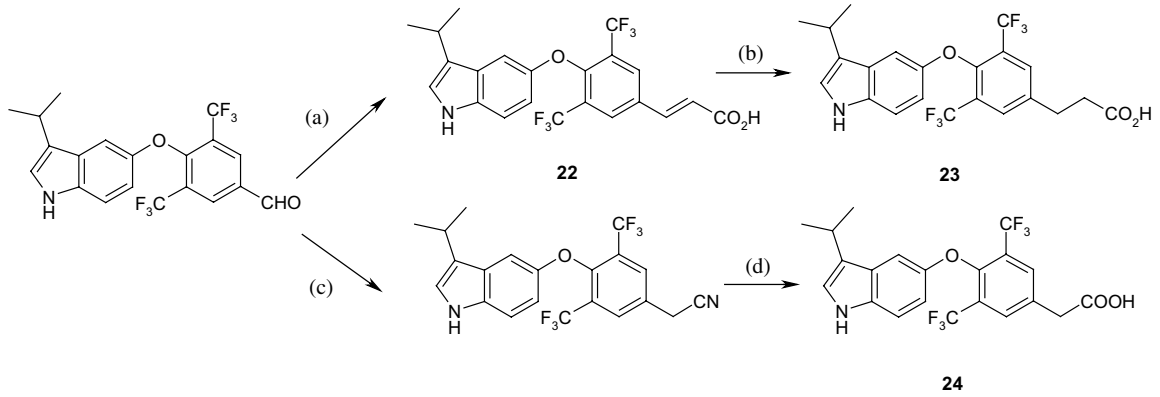


Scheme 4. Synthesis of indole oxyacetic acid thyromimetics. Reagents and conditions: (a) NaH, THF, rt, TBDMSCl, 89%; (b) *n*BuLi, THF, −78 °C, $BOiPr_3$, 58%; (c) $CuOAc_2$, mol. sieves, DCM, Py, NEt_3, 62%; (d) NaOH, 87%.

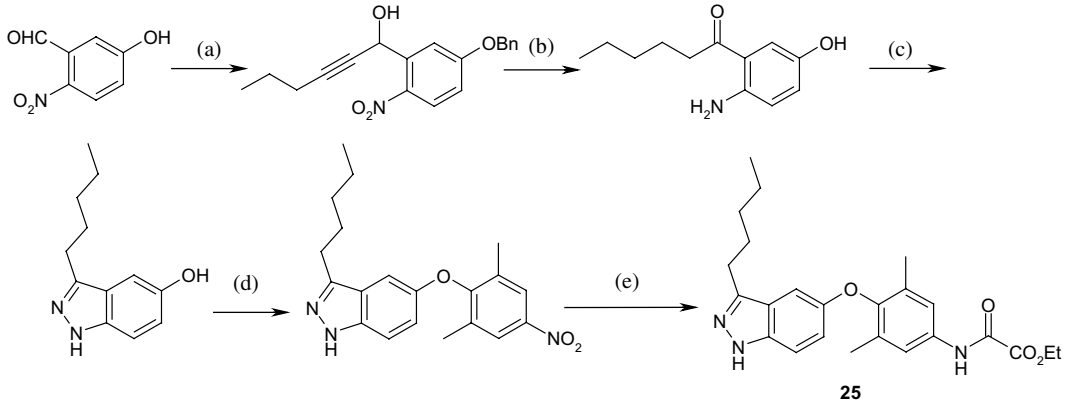
4-bromo-3,5-dimethyl-bocaniline first by deprotonation with methyllithium and subsequent halogen–metal exchange with *n*-BuLi.

The SAR trends for the indole series are shown in Table 1. Potency was determined in a HEP-G2 whole cell assay containing both receptor isoforms.[14]

Consistent with earlier observations in thyromimetic SAR trends,[16] branched alkyl substituents at R3 yield ligands with higher potency, the order of activity is H < Me < iPr (**6**, **7**, **8**). Benzyl (**9**) substituents are also tolerated in this position, however phenyl (**10**) and benzoyl (**11**) groups seem to lack the necessary structural flexibility and result in ligands with considerably

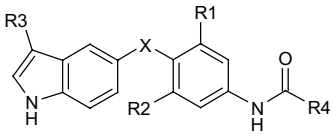


Scheme 5. Synthesis of indole thyromimetics with all-carbon headgroups. Reagents and conditions: (a) i. PPh_3CHCO_2Et, 88%, ii. dioxan, 1 N NaOH, 79%; (b) Pd/C, H_2, 1 atm, 57%; (c) i. $NaBH_4$, MeOH, rt, 97%, ii. PPh_3Br_2, AcCN, Py, 55%, iii. NaCN, DMF, 50 °C, 74%; (d) HOAc, H_2SO_4, 105 °C, 4 h, 15%.



Scheme 6. Indazole thyromimetics. Reagents and conditions: (a) i. BnBr, K_2CO_3, 99%, ii. pentyne, EtMgBr, 45%; (b) MnO_2, 83%, ii. H_2, 91%; (c) i. $NaNO_2$, HCl, ii. $SnCl_2$ 27%; (d) K_2CO_3, DMSO, rt–100 °C, 5 h, 47%; (e) i. H_2, EtOH, Pd/C, 56%, ii. $ClCOCO_2Et$, NEt_3, 77%, iii. cat. NaOEt, EtOH, 70%.

Table 1. EC_{50} data for compounds **6**–**20**



Compd	R1	R2	R3	R4	X	EC_{50}[14] (nM)	Sel[15] (α/β)
6	Me	Me	H	CO_2Et	O	72	—
7	Me	Me	Me	CO_2Et	O	15	—
8	Me	Me	*i*Pr	CO_2Et	O	4	10
9	CF_3	CF_3	4F–Bn	CO_2Et	O	11	—
10	CF_3	CF_3	4F–Ph	CO_2Et	O	115	—
11	CF_3	CF_3	4F–Bz	CO_2Et	O	495	—
12	Me	Me	*i*Pr	CO_2Et	CH_2	18	—
13	Cl	Cl	H	CO_2Et	O	15	—
14	CF_3	CF_3	H	CO_2Et	O	23	—
15	CF_3	CF_3	*i*Pr	CO_2Et	O	3	10
16	Me	Cl	*i*Pr	CO_2Et	O	4	—
17	Cl	Cl	*i*Pr	CO_2Me	O	4	—
18	Br	Br	*i*Pr	CO_2Et	O	1.2	—
19	Vinyl	Vinyl	*i*Pr	CO_2Et	O	7.8	—
20	Me	Cl	*i*Pr	CH_2CO_2Et	O	8	10
2						0.2	0.5

reduced activity. The position of the alkyl substituent on the indole ring seems to be flexible since the 2-methyl and the 3-methyl derivative demonstrate the same activity.

Substituents in the inner ring also exert an influence on the potency, more lipophilic groups result in higher potency (vinyl < Me, CF3, Cl < Br). This had also been demonstrated for KB141 and its dibromo analogue.[10b,17] However, the relatively large difference (approx. 20-fold) reported between dimethyl- and dichloro analogues from an azauracil series[12] was not reflected in the indole series.

Replacement of the bridging oxygen atom with a methylene group (**12**) reduced the activity by a factor of 4. This result is in agreement with findings for very early T3 analogues.[18] However, for GC1 and its ether analogue and for T3 and its methylene analogue the opposite effect was reported, that is, the methylene analogue was more potent.[6,9,19]

Compounds **8**, **15** and **20** demonstrate a 10-fold THRβ selectivity in a functional assay using isoform-selective transient THR transfections.[15] Under these assay conditions, L-T3 (**2**) demonstrates a 2-fold selectivity for the activation of THRα while GC1 displays a 10-fold selectivity for THRβ.

In addition to amide head groups the oxyacetic acid headgroup found in GC1 also was combined with the indole motif. For this purpose, a copper mediated coupling[20] between a phenol and a boronic acid was used as depicted in Scheme 4.

Compound **21** demonstrated somewhat reduced THR agonistic activity (24 nM) compared to the oxamic acid amide analogue (**8**) and 4-fold selectivity in activation of THRβ.

Scheme 5 shows our approach to indole thyromimetics with all-carbon headgroups. Wittig elongation followed by hydrogenation leads to propionic acid derivatives with excellent potency (2.6 nM, **23**). The cinnamic acid precursor demonstrates comparable activity (3.2 nM, **22**). Reduction, bromination and conversion to the cyano headgroup, followed by hydrolysis gave the corresponding acetic acid derivative and the most potent indole thyromimetic in our series (EC_{50} = 0.5 nM, **24**). However, none of these carbon head group derivatives showed THRβ selectivity.

The syntheses of indazole containing thyromimetics and the corresponding hydroxyindazoles are depicted in Scheme 6. Protection of the phenol is followed by a Grignard addition. Oxidation, simultaneous deprotection and reduction of the triple bond delivers the desired amino acetophenon, which is diazotized. The resulting diazoniumion is reduced to the hydrazine, which closes in situ to the indazole ring system.[21] In the case of the indazoles acylation using oxalylethylchloride yields double acylation products, that is, the indazole is also acylated. The indazole nitrogen is liberated using catalytic NaOEt in EtOH.

Indoles and indazoles show a slightly different SAR: for indoles the classical isopropyl substituent showed the highest potency, for the indazoles *n*-pentyl proved to be the optimal substituent (**25**, EC_{50} = 8 nM). This finding has some precedence since *n*-hexyl (equivalent to *n*-pentyl substituents in the indazole series) as a substituent adjacent to the phenol had demonstrated the best activity among linear alkyl chains in a series of 3′substituted 3,5-diiodthyronines.[16]

Our findings, when taken together with other recent reports, show that structural modifications in the head group, the tail group and on the inner ring lead to THR β selective ligands even though the site of amino acid difference interacts only with the head groups of the agonists.

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14. HepG2 (containing both THRα and THRβ) cells were stably transfected with a firefly luciferase vector containing a promoter with a THRE consisting of two inverted palindromic THREs (AGGTCATGACCT) separated by eight nucleotides. Cells were seeded into 96-well plates in Eagle's minimal essential medium supplemented with Glu, non-essential amino acids, insulin, Se and transferrin and were cultivated for 48 h. Test compounds and *at*-retinoic acid were added and the cultures were incubated for 48 h. After lysis, luciferin was added and luciferase activity was measured by a camera system.
15. HUH-7 were transfected in 96-well microtitre plates by a FuGENE-6 mix according to the manufacturer's protocol (Roche Diagnostics) containing an expression vector for either human THRα 1 or human THRβ 1, a human RXR-α expression vector and a firefly luciferase promoter reporter with two THRE as above and a β-galactosidase expression vector for standardization of transfection efficacy. Twenty hour later, test compounds were added and after 48 h incubation period the cells were lysed and luciferase was quantified as above. As controls THR expression vectors were omitted. EC_{50}s were determined by at least three independent transfection assays for either THR. Each transfection assay comprised 96 single transfections used for mean values of induction factor calculation for nine concentrations of the agonist and the solvent control. Standard deviation did not exceed 30% of the respective mean value. Selectivities are reported as ratios EC_{50} (THRα)/EC_{50} (THRβ).
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