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The Synthesis and Evaluation of Dihydroquinazolin-4-ones and Quinazolin-4-ones as Thyroid Stimulating Hormone Receptor Agonists

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Abstract

We herein describe the rapid synthesis of a diverse set of dihydroquinazolin-4-ones and quinazolin-4-ones, their biological evaluation as thyroid stimulating hormone receptor (TSHR) agonists, and SAR analysis. Among the compounds screened, **8b** was 60-fold more potent than the hit compound **1a**, which was identified from a high throughput screen of over 73,000 compounds.

Thyroid-stimulating hormone (TSH) is an α/β heterodimeric glycoprotein hormone that is produced in the anterior pituitary gland and controls function and proliferation of thyroid follicular cells upon interaction with the TSH receptor (TSHR).^{1–3} TSHR is a G-protein coupled receptor that belongs to the glycoprotein receptor family.⁴ Two closely related members of this family include LHCGR (Luteinizing Hormone/Chorionic Gonadotropin Receptor)⁵ and FSHR (Follicle Stimulating Hormone Receptor).^{6,7} TSHR was first identified in the thyroid cell plasma membrane in 1966,⁸ and since that time has been found in bone,⁹ brain,¹⁰ kidney,¹¹ testis,¹² endometrium¹³ and the immune system.¹⁴ The primary function of TSHR in thyroid follicular cells is the regulation of thyroid hormone synthesis, as well as thyrocyte size and number,^{7,15} but the roles of TSHR in the other organs and tissues are not fully understood. The identification of a potent and selective small molecule TSHR agonist could provide a valuable tool for researchers interested in elucidating the roles and importance of this receptor in a variety of tissues. A small molecule TSHR agonist could also be clinically valuable. Recombinant TSH is currently used for patients with thyroid cancer receiving thyroid hormone suppression therapy, but it is difficult to produce and must be administered intramuscularly.¹⁶ A small molecule agonist could serve as an orally bioavailable alternative to recombinant TSH with advantages for therapeutic applications.

There are several reports of TSHR monoclonal antibody agonists,^{17–19} but very few of small molecule TSHR agonists. Thienopyrimidine **Org 41841** (Fig. 1) was first described in 2002 as a partial LHCGR agonist and was later found to be a partial TSHR agonist with an EC₅₀ of 7700 nM compared to 220 nM for LHCGR.²⁰ This finding was followed with a series of synthesized analogues for SAR studies. Some analogues were LHCGR selective with low μ M potency, but none were TSHR selective.^{21,22} Upon screening a library of 73,180 compounds (PubChem Assay ID: 1401), dihydroquinazolin-4-one **1a** (Figure 1) was

identified as a TSHR agonist.^{23,24} The dihydroquinazolin-4-one chemotype is present in some known drugs and bioactive compounds, such as the diuretics fenquizone²⁵ and metolazone,²⁶ but had not been previously identified as a TSHR modulator. More importantly, **1a** was a selective TSHR agonist with no detectable LHR or FSHR efficacy and was therefore selected for further studies. We previously reported that dihydroquinazolin-4-one **1b** was a full TSHR agonist with *in vivo* efficacy in mice.²⁴ In this paper, we describe the synthesis and structure-activity relationship studies that were undertaken to evaluate a library of dihydroquinazolin-4-one **1a** analogues.

A convergent route was developed for the rapid synthesis of **1a** analogues (Scheme 1). The western fragment **3** was synthesized either *via* coupling of R³NH₂ with an acid **2** or ring opening of isatoic anhydride **4**. The eastern fragment **7** was synthesized *via* the benzylation of **6** with **5**. This product was combined with fragment **3** in the presence of Yb(OTf)₃ to afford dihydroquinazolin-4-ones **1** or **8**.²⁷ Quinazolin-4-ones **9** were obtained by oxidation of dihydroquinazolin-4-ones **1** using either DDQ²⁸ or MnO₂.²⁹

Our SAR studies first investigated substitution on the amide and several clear trends were observed (R³, Table 1). First, there was little tolerance for hydrophilic groups, such as the amine (**1k**), alcohol (**1l**) or pyridine (**1i**), at this site. Second, while lipophilic groups are generally well tolerated, there appeared to be steric constraints. The benzyl group (**1g**) performed the best out of all analogues, but either lengthening (phenethyl **1m**) or shortening (phenyl **1f**) the tether between the aryl ring and amide resulted in a 2 and 10-fold reduction in potency, respectively. Finally, aliphatic groups were tolerated (*n*-Bu **1h**), albeit not especially potent, but a lone hydrogen (H **1j**) resulted in a very low potency and decreased efficacy. Among the tested R³ analogues, the benzyl group (**1g**) was the most potent with a 4-fold higher potency than the hit compound **1a**. Based upon these findings, the optimization of other sites was pursued with R³ fixed as benzyl.

Aryl ring substitution on the western fragment of the molecule was next explored with a focus on the site ortho to the carbonyl (R², Table 1). Analogues with H (**1g**) or F (**1e**) were equipotent, but the OH (**1b**) analogue resulted in a 3-fold improvement in potency. The free phenol was found to be critical for activity because when it was replaced with a methyl ether (**1c**) there was a sharp drop in potency. The orientation of the phenol was also critical for activity because when it was moved *meta* to the carbonyl (**1d**) the potency returned to the same level as the unsubstituted analogue (**1g**). This can be rationalized with a model. In our previously published modeling study, we proposed that **1b** bound to the transmembrane helical bundle of TSHR. Both the R² OH and the carbonyl group of the dihydroquinazolin-4-one were predicted to participate in hydrogen bonding with Asparagine N5.47. This prediction was supported by a point mutation study where **1b** lost significant activity with the N5.74A mutation.²⁴ In our analogues, the OH at R¹ was not appropriately situated to engage in hydrogen bonding with Asparagine N5.47 and the R² OMe analogue (**1c**) had no hydrogen available for hydrogen bonding. The phenol analogue (**1b**) was the most potent analogue identified from this series. Removal of the R⁴ methyl ether (**1n**) resulted in significant losses of potency and efficacy and hence the methyl ether was maintained in all subsequent analogues.

Substitution on the eastern fragment was next explored (Table 2). There was little tolerance for change on the eastern most ring. The lead compound substituted the R⁶ site with NHAc. When this site was instead substituted with a OMe (**1o**), CN (**1r**) or H (**1p**) there were losses in potency and efficacy. When NHAc was moved to the R⁵ position (**1q**) there were also sharp drops in potency and efficacy. Among the tested analogues, the R⁶ as NHAc motif of the original hit could not be altered without detrimental effects on the activity. Y was also explored in this series. All of the previous analogues maintained Y as oxygen, but when the

oxygen was replaced with a sulfur group there was up to a 5-fold increase in potency (**8b** vs **1b**, **8a** vs **1g**). In fact, **8b** was the most potent compound evaluated from our library and was also found to maintain selectivity for TSHR over LHR and FSHR.

In solution, dihydroquinazolin-4-one analogues were slowly oxidized by air to the corresponding quinazoline-4-ones. The dihydroquinazolin-4-ones suffer from poor stability in acidic aqueous conditions due to hydrolysis. For example, compound **1b** degraded at pH = 2 with a half-life of 3 h.²⁴ Quinazoline-4-ones **9a–c** (Table 3) were prepared to preemptively address these issues that could limit the application of these analogues. Although there was a modest decrease in potency of all the oxidized analogues, these compounds still maintained potency in the sub- μ M range and had the advantage of improved stability over dihydroquinazolin-4-ones (Table 3). The selectivity of **9c** was also tested and it was found to be selective for TSHR over LHR and FSHR.

In conclusion, we have successfully synthesized a library of analogues for SAR study. Among them, **1b**, **8a**, and **8b** were at least 10-fold more potent than the hit **1a**. In addition, we identified several quinazolin-4-one TSHR agonists (**9a–c**) with improved stability and sub- μ M potency.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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27. General procedure for the synthesis of 1,2-dihydroquinazolin-4-ones **1** and **8**. A solution of **7** (400 μ mol) in EtOH (6 mL) was treated with benzamide **3** (440 μ mol) and ytterbium trifluoromethanesulfonate (200 μ mol) and heated to 80 $^{\circ}$ C for 2–6 h. The reaction mixture was concentrated under reduced pressure and purified *via* column chromatography on Silica-gel. The isolated product was triturated with Et₂O to afford the desired product. Example: **1e** ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 1.98 (s, 3 H), 3.79 (s, 3 H), 3.80 (d, *J* = 15.6 Hz, 1H), 4.93 (s, 2 H), 5.20 (d, *J* = 15.3 Hz, 1 H), 5.67 (br.s., 1 H), 6.31 – 6.51 (m, 2 H), 6.85 (d, *J* = 8.6 Hz, 2 H), 6.99 (d, *J* = 8.4 Hz, 1 H), 7.08 – 7.35 (m, 7 H), 7.38 (s, 1 H), 7.45 (d, *J* = 8.6 Hz, 2 H), 7.65 (br. s., 1 H), 9.75 (s, 1 H); HPLC: *t*_R = 5.58 min, UV₂₅₄ = 96%; HRMS (ESI): *m/z* calcd for C₃₁H₂₈FN₃O₄ [M+Na]⁺ 548.1956, found 548.1967.
28. Procedure for the synthesis of quinazolin-4-ones **9a** and **9b**. To a solution of 1,2-dihydroquinazolin-4-ones **1** (200 μ mol) in EtOH (5 mL) was added DDQ (240 μ mol in 4 mL of acetonitrile). After stirring at r.t. for 4 h, the mixture was concentrated under reduced pressure and purified *via* column chromatography on silica gel to give the desired products. **9a**: ¹H NMR (400 MHz, CHLOROFORM-*d*) δ 2.15 (s, 3 H), 3.95 (s, 3 H), 5.12 (s, 2 H), 5.19 (s, 2 H), 6.12 (d, *J* = 2.7 Hz, 1 H), 6.22 – 6.28 (m, 1 H), 6.93 (d, *J* = 9.0 Hz, 2 H), 7.00 (d, *J* = 8.6 Hz, 1 H), 7.12 (br. s., 1 H), 7.24 (s, 1 H), 7.39 (d, *J* = 9.0 Hz, 2 H), 7.46 – 7.56 (m, 2 H), 7.64 – 7.82 (m, 2 H), 8.33 (d, *J* = 8.2 Hz, 1 H); HPLC: *t*_R = 5.49 min, UV₂₅₄ = 95%; HRMS (ESI): *m/z* calcd for C₂₉H₂₅N₃O₅ [M+1]⁺ 496.1872, found 496.1873. **9b**: ¹H NMR (400 MHz, CHLOROFORM-*d*) δ 2.13 (s, 3 H), 3.91 (s, 3 H), 5.04 (s, 2 H), 5.26 (s, 2 H), 6.81 – 7.04 (m, 5 H), 7.11 – 7.26 (m, 5 H), 7.38 (d, *J* = 9.0 Hz, 2 H), 7.46 – 7.58 (m, 2 H), 7.71 – 7.83 (m, 2 H), 8.36 (d, *J* = 7.8 Hz, 1 H); HPLC: *t*_R = 5.73 min, UV₂₅₄ = 98%; HRMS (ESI): *m/z* calcd for C₃₁H₂₇N₃O₄ [M+1]⁺ 506.2086, found 506.2082.
29. Procedure for the synthesis of quinazolin-4-one **9c**. To a solution of **1b** (15.0 mg, 29 μ mol) in 2 mL of DMSO solution was added MnO₂ (87 mg, 0.29 mmol). The mixture was heated at 80 $^{\circ}$ C for 12 h. The solid was filtered and the solvent was removed under reduced pressure. The residue was purified by chromatography on silica gel (7%–60% ethyl acetate in hexanes) to yield **9c** (8.1 mg, 54%). ¹H NMR (400 MHz, CHLOROFORM-*d*) δ ppm 2.14 (s, 3 H), 3.90 (s, 3 H), 5.04 (s, 2 H), 5.20 (s, 2 H), 6.79 – 7.01 (m, 6 H), 7.08 (br.s., 1H), 7.20 – 7.25 (m, 4 H), 7.27 – 7.42 (m, 3 H),

7.52 (d, J=2.0 Hz, 1 H), 7.64 (t, J=8.1 Hz, 1 H), 11.69 (s, 1 H); HPLC: t_R = 6.23 min, UV_{254} = 98%; HRMS (ESI): m/z calcd for $C_{31}H_{27}N_3O_5$ $[M+1]^+$ 522.2027, found 522.2028.

30. ELISA cAMP assay: Transiently transfected HEK-EM293 cells or cells stably expressing TSHR, were seeded into 96-well plates at a density of 50,000 cells/well in DMEM containing 10% FBS. Cells were cultured for 24 h before incubation for 1 h in serum-free DMEM containing 1 mM 3-isobutyl-1-methylxanthine (Sigma) and TSH, or small-molecule ligands in a humidified 5% CO_2 incubator at 37 $^{\circ}C$. Following aspiration of the media, cells were lysed using lysis buffer of the cAMP-Screen Direct system (Applied Biosystems). The cAMP contents of the cell lysates were determined using the method described in the manufacturer's protocol.

Org 41841 **1a** **1b**

Figure 1.
TSHR agonists

Scheme 1.

Reagents and conditions: (a) DIPEA, $R^3\text{NH}_2$, 2-chloro-1,3-dimethylimidazolinium chloride, CH_2Cl_2 ; (b) $R^3\text{NH}_2$, CH_3CN (or DMA), 12 h; (c) K_2CO_3 , CH_3CN , 150 °C, 30 min; (d) $\text{Yb}(\text{OTf})_3$, **3**, EtOH, 80 °C, 2–6 h; (e) DDQ, r.t., 1 h or MnO_2 , DMSO, 80 °C, 12 h.

Table 1

SAR of R1–R4

Compound	R1	R2	R3	R4	EC ₅₀ (μM)	E _{max} (%)
1a	H	H		OMe	1.16	101
1b	H	OH	Bn	OMe	0.09	100
1c	H	OMe	Bn	OMe	4.48	49.1
1d	OH	H	Bn	OMe	0.33	93.3
1e	H	F	Bn	OMe	0.28	94.7
1f	H	H	Ph	OMe	5.09	75.1
1g	H	H	Bn	OMe	0.29	99.8
1h	H	H	<i>n</i> -Bu	OMe	0.83	112
1i	H	H		OMe	3.98	70.3
1j	H	H	H	OMe	n.d.	39.5
1k	H	H		OMe	n.d.	10.1
1l	H	H		OMe	n.d.	19.7
1m	H	H		OMe	0.43	102.5
1n	H	H	Bn	H	1.86	36.8

Activities (EC₅₀) in μM were obtained from the Elisa assay. E_{max} is expressed as % of the maximal response of **1b**, set at 100%. ³⁰ n.d. = not determined

Table 2

SAR of Y, R5 and R6

Compound	R2	R5	R6	Y	EC ₅₀ (μM)	E _{max} (%)
1b	OH	H	NHAc	O	0.09	100
1g	H	H	NHAc	O	0.29	99.8
1o	H	H	OMe	O	2.16	46.3
1p	H	H	H	O	2.57	25.6
1q	H	NHAc	H	O	4.34	24.8
1r	H	H	CN	O	n.d.	11.7
1s	H	F	H	O	n.d.	7.4
8a	H	H	NHAc	S	0.08	95.7
8b	OH	H	NHAc	S	0.018	69.6

Activities (EC₅₀) in μM were obtained from the Elisa assay. E_{max} is expressed as % of the maximal response of **1b**, set at 100%. ³⁰ n.d.= not determined

Table 3

Comparison of oxidized and unoxidized analogues

Compound	R ²	R ³	X	EC ₅₀ (μM)	E _{max} (%)
1a	H		NH	1.16	101
9a	H		N	0.93	59
1g	H	Bn	NH	0.29	99.8
9b	H	Bn	N	0.96	97.4
1b	OH	Bn	NH	0.09	100
9c	OH	Bn	N	0.55	98.6

Activities (EC₅₀) in μM were obtained from the Elisa assay. E_{max} is expressed as % of the maximal response of **1b**, set at 100%.³⁰