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Synthesis and SAR of 2-aryl-3-aminomethylquinolines as agonists of the bile acid receptor TGR5

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ABSTRACT

Optimization of a screening hit from uHTS led to the discovery of TGR5 agonist **32**, which was shown to have activity in a rodent model for diabetes.

TGR5 (also known as GPBAR1 or M-BAR)[1] is a G*s*-coupled GPCR mainly expressed in the GI tract, gall bladder, spleen, lung, and placenta.[2] It is responsive to stimulation by bile acids, for example cholic acid (CA, **1**).[3,4] Ligand binding activates adenylyl cyclase which leads to the elevation of intracellular cAMP and subsequent activation of mitogen-activated protein kinase pathways. Activation of TGR5 by bile acids has been shown to regulate a number of metabolic processes, including hormonal control of energy expenditure.[5,6] For example, mice fed a high fat diet containing 0.5% CA gained less weight than control mice on a high fat diet alone. Consistent with this finding, TGR5 knockout mice show significant fat accumulation and weight gain when given a high fat diet.[7,8] In addition, it has also been reported that stimulation of STC-1 cells (murine enteroendocrine cells) with bile acids increased glucagon like peptide-1 (GLP-1) secretion.[9] GLP-1 improves glucose homeostasis by several mechanisms including stimulating pancreatic insulin secretion and inhibiting glucagon secretion. Thus, a small molecule TGR5 agonist may be beneficial for the treatment of type 2 diabetes and obesity. Due to the activity of bile acids on a number of receptors,[10] there is a need for a selective TGR5 agonist to determine the pharmacological consequences of TGR5 activation in vivo. One approach has been to modify bile acids to yield more selective compounds, of which compound **2** (Fig. 1) is a representative example.[11–14] Our approach, described herein, has been to develop a synthetic TGR5 agonist.[15]

Functional ultra-high throughput screening of the Kalypsys compound file employing HEK-293 cells stably expressing human TGR5 (hTGR5) and measuring intracellular cAMP as the primary endpoint resulted in the identification of several series of TGR5 agonists, including a set of quinolines represented by **3** (Fig. 2).[16] Quinoline **3** displayed an EC_{50} of ~10 μM on the human receptor but was significantly less potent (≫10 μM) on the mouse receptor (mTGR5), perhaps as a consequence of the relatively weak se-

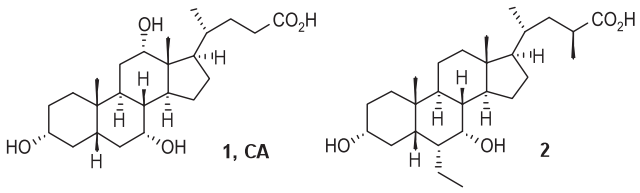

Figure 1. Cholic acid (CA, **1**) and a TGR5 selective bile acid **2**.

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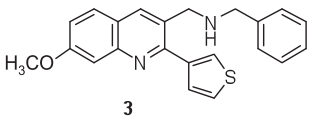


3

Figure 2. Screening hit.

quence homology (83%) between the two species.[17] Given our desire to assess the pharmacology of TGR5 compounds in mouse models the identification of compounds with improved mTGR5 activity became our initial objective.

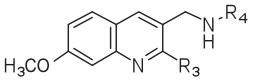
A straightforward synthesis of **3** that allowed rapid access to analogs was developed (Scheme 1).[18] Thus, substituted anilines **4** were acetylated using acetic anhydride to give acetamides **5** which were then reacted with DMF and $POCl_3$ under Vilsmeier–Haack conditions to afford chloroquinolines **6**. Addition of various aryl groups were then incorporated via either Suzuki or Stille type couplings to give quinoline-aldehydes **7**, which were then subjected to standard reductive amination conditions with a variety of amines to afford the desired TGR5 agonists **8**. In general, high yields were obtained for all steps.

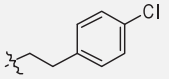
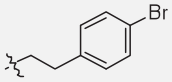
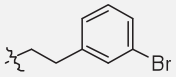
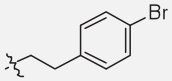
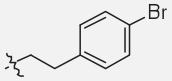
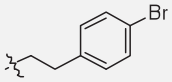
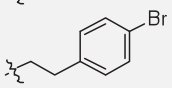
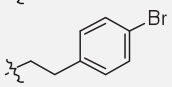
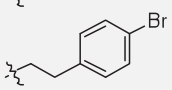
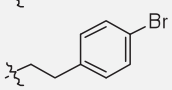
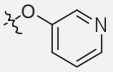
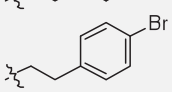
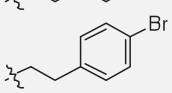
Hydroxyl analogs (**10**) were accessed by demethylation of the corresponding methoxy quinolines **9** using boron tribromide in good yields (Scheme 2).

Our SAR studies initially focused on the pendant amine of **3** (R^4). We prepared a library using a broad range of amines; some of the key compounds described in Table 1. In general, *ortho*- and *meta*-substituted benzyl amines were inactive (data not shown). However, we discovered that *para*-substitution both greatly improved hTGR5 activity but more importantly also improved mTGR5 activity.

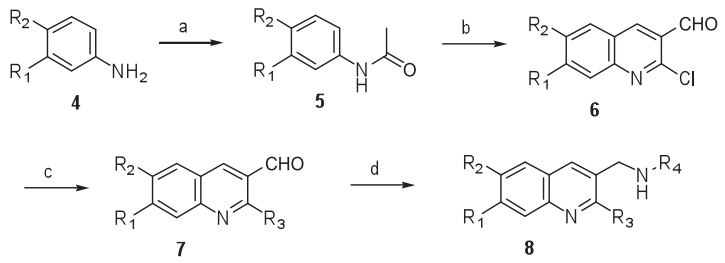
In particular 4-CH_3 (**11**) and 4-Cl (**12**) substitution gave potent compounds (0.88 μM and 0.58 μM on the human and 3.9 μM and 4.3 μM on the mouse receptors, respectively, more than an order

Table 1
SAR around the amine and aryl groups

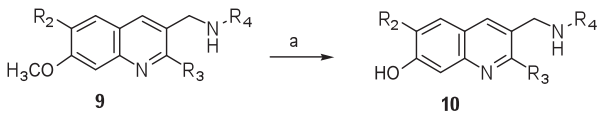


Compd	R^4	R^3	hTGR5 EC_{50}[a] (μM)	mTGR5 EC_{50}[a] (μM)
1	—	—	6	5.3
2	—	—	0.14	0.25
3	$-CH_2Ph$	3-Thiophenyl	11.0	>10
11	$-CH_2C_6H_4(4\text{-}CH_3)$	3-Thiophenyl	0.88	3.9
12	$-CH_2C_6H_4(4\text{-}OCH_3)$	3-Thiophenyl	1.1	8.5
13	$-CH_2C_6H_4(4\text{-}Cl)$	3-Thiophenyl	0.58	4.3
14		3-Thiophenyl	0.097	3.7
15		3-Thiophenyl	0.065	3.2
16		3-Thiophenyl	2.8	13
17		2-Thiophenyl	0.58	4.6
18		3-Furanyl	0.71	6.1
19		2-Furanyl	0.50	4.0
20		Phenyl	0.49	8.1
21		4-Pyridyl	0.044	3.7
22		3-Pyridyl	0.065	5.0
23			>10	>10
24			5.0	>10
25			2.7	>10

[a] Values are means of three experiments, standard deviations were within 20% of reported values.

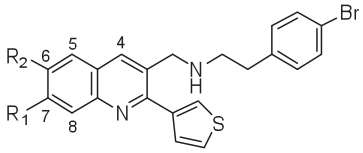


Scheme 1. Reagents and conditions: (a) Ac_2O, DMAP, CH_2Cl_2, 70–90%. (b) $POCl_3$, DMF, 90 °C 30–85%. (c) $R_3B(OH)_2$, $Pd(PPh_3)_2Cl_2$, 2 M Na_2CO_3, DME, 90 °C or $R_3Sn(nBu)_3$, DMF, 90 °C, 40–70%. (d) R_4NH_2, $NaHB(OAc)_3$, AcOH, *i*PrOH, 75–95%.



Scheme 2. Reagents and conditions: (a) BBr_3, CH_2Cl_2, 80–90%.

Table 2
SAR on the quinoline ring



Compd	R^1	R^2	hTGR5 EC_{50}[a] (μM)	mTGR5 EC_{50}[a] (μM)
15	$-OCH_3$	–H	0.065	3.2
26	$-CH_3$	–H	5.0	>10
27	–Cl	–H	6.9	>10
28	–OEt	–H	4.5	9.4
29	$-OCF_3$	–H	>10	>10
30	$-CO_2H$	–H	>10	>10
31	–H	–H	5.5	>10
32	–OH	–H	5.1	0.28
33	–H	$-OCH_3$	>10	>10
34	–H	–OH	0.082	1.6
35	$-OCH_3$	–OH	0.43	0.32
36	$-OCH_3$	$-OCH_3$	>10	>10
37	–OH	$-OCH_3$	>10	>10
38	–OH	–OH	>10	>10

[a] Values are means of three experiments, standard deviations were within 20% of reported values.

of magnitude improvement over the initial lead and comparable to the potency of cholic acid). Extension of the benzyl amines to phenethylamines such as **14** and **15** led to a further enhancement in potency, especially on the human receptor. Compound **15**, which displayed an EC_{50} of 65 nM on the human receptor and 3.2 μM on the mouse receptor, became our point for further optimization. We next focused on the 3-thiophenyl ring (R^3) with the results summarized in Table 1. Other five-membered heterocyclic rings, such as the 2-thiophenyl (**17**), 3-furanyl (**18**), and 2-furanyl (**19**) were somewhat less potent than **15**. Thiazole (**24**) and oxazole (**25**) substitution led to a large loss in potency. A phenyl group (**20**) was tolerated, showing comparable potency to the furan and thiophene substituted compounds (hTGR5 EC_{50} of 0.49 μM, mTGR5 of 8.1 μM). Pyridyl substitution showed activity comparable to **15**, with 4-pyridyl compound **21** (hTGR5 EC_{50} of 44 nM) and 3-pyridyl compound **22** (hTGR5 EC_{50} of 65 nM) both being among the most active human compounds tested. Lastly, a compound with a heteroatom linker, as in hydroxypyridine **23**, showed no activity.

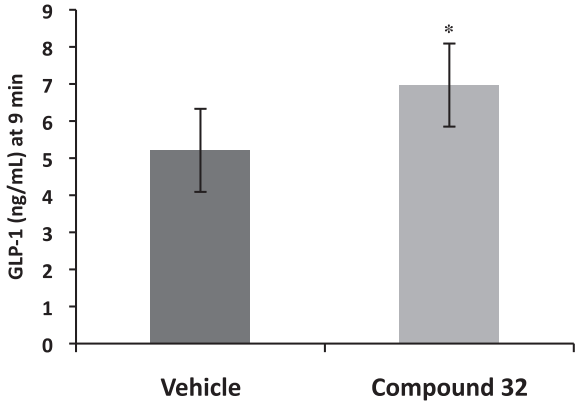


Figure 3. Compound **32** evokes increased GLP-1 release in DIO mice following dextrose bolus. *p <0.05 for vehicle.

We next turned our attention to the SAR around the quinoline ring, using **15** as a starting point (Table 2). In general, substitution at the 4, 5, 6, and 8 positions of the quinoline were not tolerated (data not shown), with the exceptions at the 6 position noted below. Examining a range of substituents at the 7 position, we initially found that only methoxy was well tolerated. Methyl (**26**), chloro (**27**), ethoxy (**28**), trifluoromethoxy (**29**), carboxylic acid (**30**), and hydrogen (**31**) all gave only weakly potent or inactive compounds. Interestingly, demethylation of **15** to give phenol **32** gave a compound that was considerably more active on the mouse receptor (0.28 μM vs 3.2 μM) but less active on the human receptor (5.1 μM vs 0.065 μM). Due to our interest in developing a mouse active compound for in vivo studies, we investigated this effect further. As with other groups, methoxy substitution at the 6 position (**33**) gave an inactive compound. Intriguingly, demethylation to 6-substituted phenol **34** gave a compound that was again

Table 3
Selected mouse PK parameters for compounds **15**, **26**, and **37**[a]

Compd	Cl_p[b] (mL/min/kg)	V_d[b] (L/kg)	C_{max}[c] (μg/mL)	AUC[c] (μg h/mL)	$t_{1/2}$[c] (h)	%*F*
32	38	2.4	0.37	0.40	4.4	9
35	29	1.4	0.50	0.86	7.7	10

[a] Compounds dosed 3 mpk iv and 30 mpk po.
[b] iv.
[c] po.

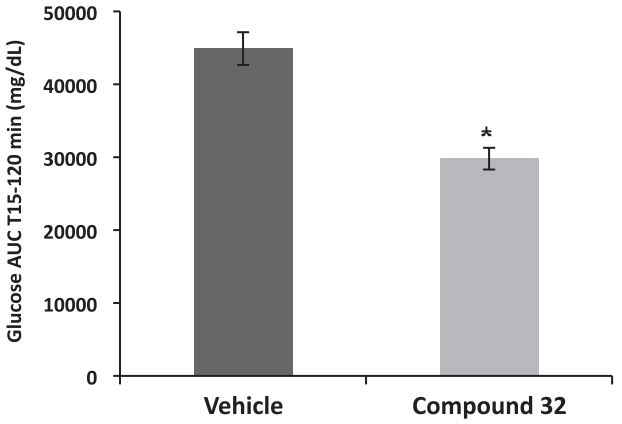


Figure 4. Compound **32** promotes increased glucose clearance in DIO mice during OGTT. $^{*}p$ <0.01 for vehicle.

active on both the human (0.082 μM) and mouse receptor (1.6 μM). Combining these results led us to compound **35**, which displayed potency both on the human (0.43 μM) and mouse (0.32 μM) receptor. Other combinations of methoxy and phenol groups were not active (**36**–**38**).

Mouse pharmacokinetics studies were carried out on **32** and **35** (Table 3). In general, these compounds demonstrated moderate to high clearance, with modest bioavailability. However, the exposure after oral dosing of 30 mpk was sufficient to investigate the effect of the compounds in vivo.

To test the hypothesis that activation of TGR5 stimulates GLP-1 release, diet-induced obese (DIO) mice were dosed with compound **32** prior to an oral glucose tolerance test (OGTT). As shown in Figure 3, a 30 mpk oral dose of **32** increases plasma GLP-1 following an oral glucose challenge.[19,20]

We also examined the effect of **32** during an oral glucose tolerance test (OGTT). As shown in Figure 4, acute dosing of **32** enhanced glucose clearance significantly, consistent with our previous finding of elevated plasma GLP-1 levels.[21]

In conclusion we have developed a series of quinolines that are both potent and selective[22] agonists of human and mouse TGR5. Activation of TGR5 was shown to increase GLP-1 secretion and enhance glucose clearance in vivo, warranting a further investigation of TGR5 agonists as potential therapeutics for the treatment of type 2 diabetes. Future reports from our group will focus on the further optimization of these molecules as well as a more in depth discussion of the pharmacology studies.

Acknowledgments

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16. HEK293 cells stably expressing human or mouse TGR5 were established by stably transfecting HEK-293 cells with an expression vector (pcDNA 3.1, Invitrogen) inserted with human TGR5 cDNA using Fugene6 (Roche, Indianapolis, IN) according to conventional methods. Cells were grown in DMEM (Invitrogen, Carlsbad, CA) supplemented with 10% FBS, 1% penicillin/streptomycin under geneticin selection. To assess the activity of test compounds, cells were harvested using non-enzymatic cell dissociation buffer (Invitrogen, Carlsbad, CA), seeded in DMEM supplemented with 0.1% FBS at 0.8×10^6/mL and incubated overnight at 37 °C in an atmosphere of 10% CO2 and 95% humidity. The next day, test agents were added to cells in the presence of 1 mM IBMX and incubated for 30 minutes at 37 °C. Intracellular cAMP was then measured by TR-FRET using a commercially available LANCE kit (Perkin–Elmer, Boston MA). All compounds were tested in 20-point dose response, $n = 3$. Compounds were full agonists on both the hTGR5 and mTGR5 receptors, comparable to cholic acid.
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18. Complete experimental procedures and spectral data can be found in: Pinkerton, A. B.; Kabakibi, A.; Herbert, M. R.; Siegel, D. L. WO2008/097976.
19. Preliminary data presented at: Guha, M.; Pinkerton, A.; Banerjee, U.; Dhamija, S.; Anderson, J.; Herbert, M.; Siegel, D.; Hoffman, T.; Staszewski, L.; Cayanan, C.; Shiau, A.; Rao, T.; Noble, S.; Kabakibi, A. National Meeting of the American Diabetes Association, San Francisco, CA, June, 2008.
20. Compound **32** as a bis-HCl salt (30 mpk) in CMC (0.5%)/Tween 20 (0.25%) was dosed orally to DIO mice ($n = 6$ mice per time point, fasted for 18 h) 15 min prior to an oral dextrose bolus (2 g/kg in saline). Blood was collected from the tail vein at 3, 6, 9, 12, 15, and 20 min post dose. To prevent the degradation of GLP-1, a sub-optimal dose of vildagliptin (0.1 mpk) was given 25 min prior to dextrose dosing. Plasma GLP-1 was measured by ELISA. Data is represented as plasma GLP-1 level at 9 min post dextrose dose.
21. Compound **32** bis-HCl salt (30 mpk) in CMC (0.5%)/Tween 20 (0.25%) was dosed orally to DIO mice ($n = 6$ mice per time point, fasted for 18 h) 15 min prior to an OGTT (2 g/kg dextrose in saline). Blood was collected from the tail vein at 0, 15, 20, 30, 60, and 120 min post OGTT, and plasma glucose level was measured using a hand-held glucose meter. Data is represented as glucose AUC post OGTT.
22. Compounds **32** and **35** were screened in a CEREP panel of approximately 30 targets that could potentially affect glucose or GLP-1 levels and their activity was found to be <50% of optimal at 10 μM confirming a TGR5 mechanism of action.