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Discovery of novel pyrimidine and malonamide derivatives as TGR5 agonists

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ABSTRACT

Takeda G-protein-coupled receptor 5 (TGR5) is a promising molecular target for metabolic diseases. A series of 4-(2,5-dichlorophenoxy)pyrimidine and cyclopropylmalonamide derivatives were synthesized as potent agonists of TGR5 based on a bioisosteric replacement strategy. Several compounds exhibited improved potency, compared to a reference compound with a pyridine scaffold. The pharmacokinetic profile of the representative compound **18** was considered moderate.

TGR5 (Takeda G-protein-coupled receptor 5) is a G protein-coupled receptor (GPCR) for which bile acids (Bas) are the endogenous ligands.[1] TGR5 is expressed in various tissues, particularly in the liver, gallbladder, intestine, spleen, and brain. Activation of TGR5 can stimulate intestinal enteroendocrine cells to secrete glucagon-like peptide-1 (GLP-1), which itself plays multiple physiological roles in the modulation of glucose homeostasis such as glucose-dependent stimulation of insulin, suppression of glucagon release, slowing of gastric emptying, and appetite suppression.[2] Furthermore, it also increases energy expenditure in brown adipose tissues and muscles by increasing thyroid hormone activity,[3] and reduces expression of pro-inflammatory cytokines such as TNF-α by preventing nuclear translocation of NF-κB.[4] Thus, TGR5 agonists have been proposed as potential drugs for the treatment of metabolic diseases and cholestatic liver disease.[5] Indeed, some TGR5 agonists have been recently reported,[6] and can be classified into two categories. The first class of TGR5 agonists possesses structural similarity with BAs, and include naturally occurring TGR5 agonists such as lithocholic acid (LCA)[7] and 6α-ethyl-23(*S*)-methylcholic acid (INT-777),[8] the latter of which is a selective TGR5 agonist being developed as an anti-diabetic agent. The second class of TGR5 agonists consists of non-bile acids including isoxazole (**1**),[9] pyrazole (**2**)[10] and nicotinamide (**3**)[11] (Fig. 1). At present, none of new chemical entities have entered clinical trials, and further investigation is required to identify more molecules with good pharmacokinetic (PK) and pharmacodynamic (PD) profiles to take this therapeutic field a step forward. Expansion of the chemical pool for TGR5 agonists could increase the possibility for finding drug candidates. Herein, we described the discovery of novel and potent TGR5 agonists through a bioisostere replacement strategy.

We commenced our studies on the discovery of novel TGR5 agonists based on pyridine compound **3**,[11] which has been reported to show a high potency but unsatisfactorily high clearance. Unlike the reported strategy to optimize PK/PD properties,[11] we planned to introduce pyrimidine or cyclopropylmalonamide moieties instead of the pyridine backbone as a bioisosteric strategy (Fig. 2). Replacement of pyridine with pyrimidine was expected to improve log*P*; *c*log *P* values of pyridine **3** and the corresponding pyrimidine **16b** are 4.97 and 4.02, respectively. Specifically, we surmised that incorporation of cyclopropyl group may restrict the number of available conformations and allow two side chains of amide groups to be placed nearby, similar to the two substituents in the pyridine of compound **3**.

N-linked pyrimidine derivatives **9** and **11** were synthesized as outlined in Scheme 1. The key intermediate **7** was obtained from

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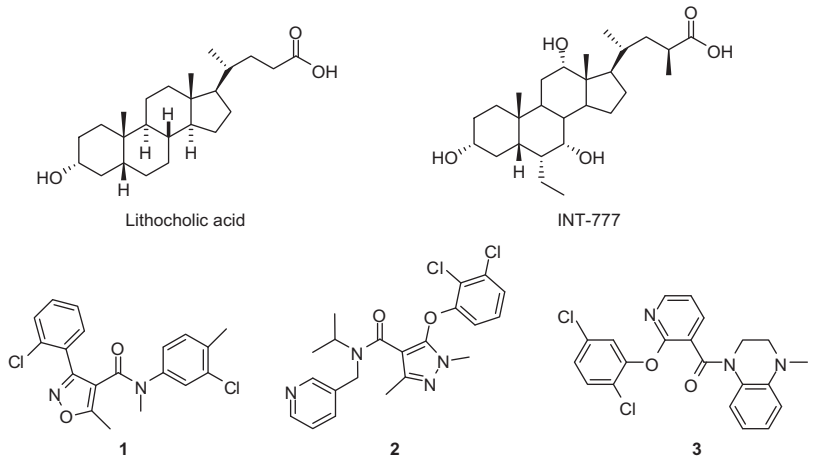


Figure 1. Structures of the reported TGR5 agonists.

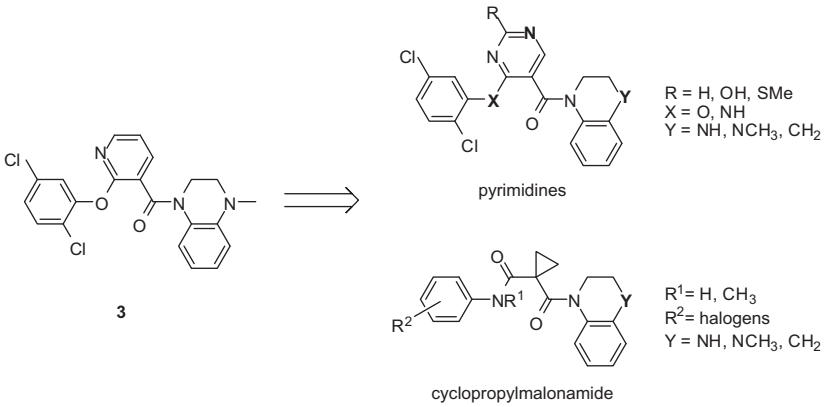


Figure 2. Design of pyrimidine and malonamide derivatives based on the known potent TGR5 agonist pyridine **3**.

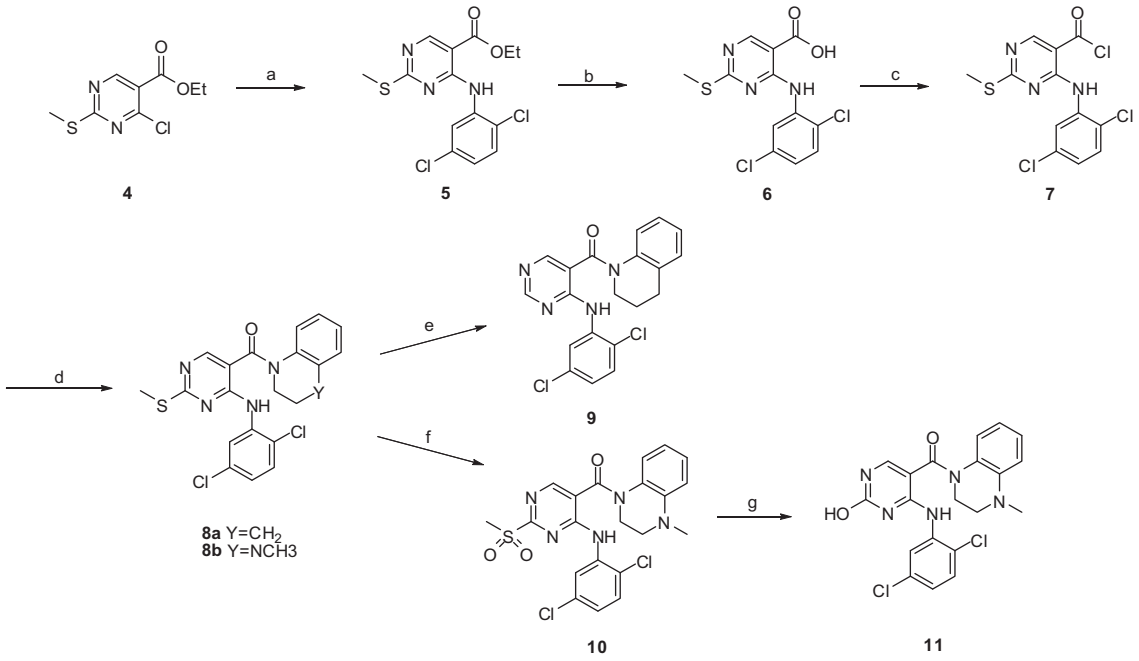
the commercially available compound **4**. Sequential addition of dichloroaniline and hydrolysis, followed by transformation of the acid into acid chloride provided compound **7**. Amide formation of **7** with 1,2,3,4-tetrahydroquinoline or 1,2,3,4-tetrahydroquinoxaline gave compounds **8a** and **8b**, respectively. Removal of the methylthio group from **8a** by Raney-Ni afforded derivative **9**. In addition, 2-hydroxypyrimidine derivative **11** was obtained by sequential treatment with *m*CPBA and AcOH.

Next, O-linked pyrimidine derivatives were synthesized as outlined in Scheme 2. Similar to the synthetic methods described for the N-linked pyrimidine derivatives, nucleophilic substitution of compound **4** with 2,5-dichlorophenol followed by hydrolysis gave acid **13**, which was readily converted to acid chloride **14** in the presence of thionyl chloride. Amide formation of the intermediate **14** with various aryl amines gave **15a–c** and **19**. Derivatives **16a–b** and **20** were prepared by reduction of **15a–b** and **19** with Raney-Ni, respectively. Subsequent oxidation and hydroxylation of **15b** furnished derivative **18**.

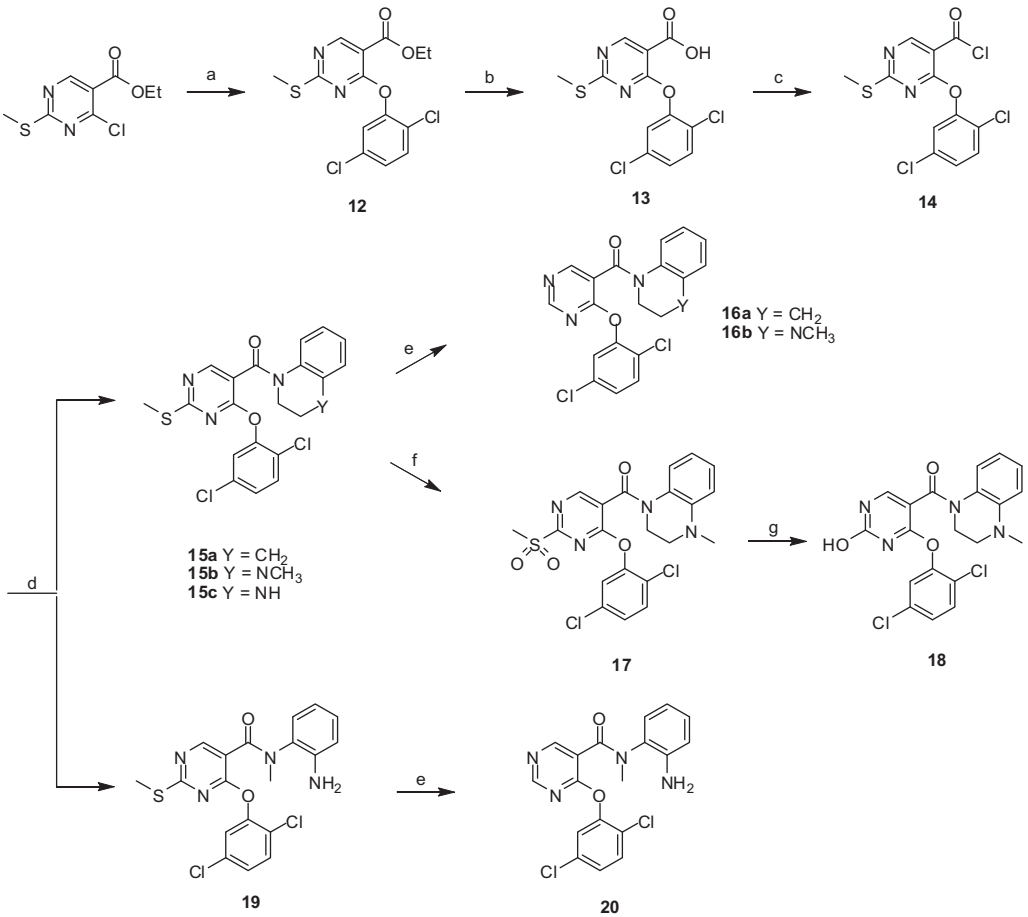
Synthesis of cyclopropylmalonamide derivatives **26a–d** was carried out as described in Scheme 3. The acid **21** was readily converted into the corresponding acid chloride **22** with thionyl chloride, which was coupled with anilines to furnish amides **23a–d**. Finally, malonamides **26a–d** were efficiently obtained using the same procedure as that used for the formation of the first amide.

The newly synthesized agonists of TGR5 were applied to HEK293 cells stably expressing TGR5 and their activity was evaluated by measuring intracellular cAMP levels (Table 1).[12] In the pyrimidine series, the TGR5 agonistic activity of derivatives (**9** and **16a**) containing a tetrahydroquinoline group was greatly reduced. The N-demethylated derivative **15c** exhibited low activity, and compound **20** containing an open ring structure did not demonstrate agonistic activity. Remarkably, pyrimidine derivative **16b** exhibited increased activity compared to that shown by the parent compound **3**, indicating that our bioisosteric approach for replacing pyridine with pyrimidine was effective. The 2-hydroxypyrimidine **18** also showed excellent activity for TGR5 and was as potent as **16b**. O-linked compound **18** exhibited a higher potency than the corresponding N-linked derivative **11**. Compound **15b** bearing a methylthio group instead of a hydroxyl group exhibited slightly lower activity than the hydroxyl derivative **18**. Among cyclopropyl malonamides, it was notable that derivative **26c** displayed as similar potency as the parent compound **3,** despite the negligible agonistic activities of the other malonamide derivatives. A significant drop in activity was also observed for derivatives **26a** and **26b** with, as indicated in Figure 2, methylene and secondary amine group at the Y position, respectively. Such decreased activity was even seen for the malonamide derivative series, suggesting that pyrimidine and malonamide derivatives may be similarly positioned in the TGR5 binding site unless the groups in the Y position are largely involved in nonspecific binding with off-targets.

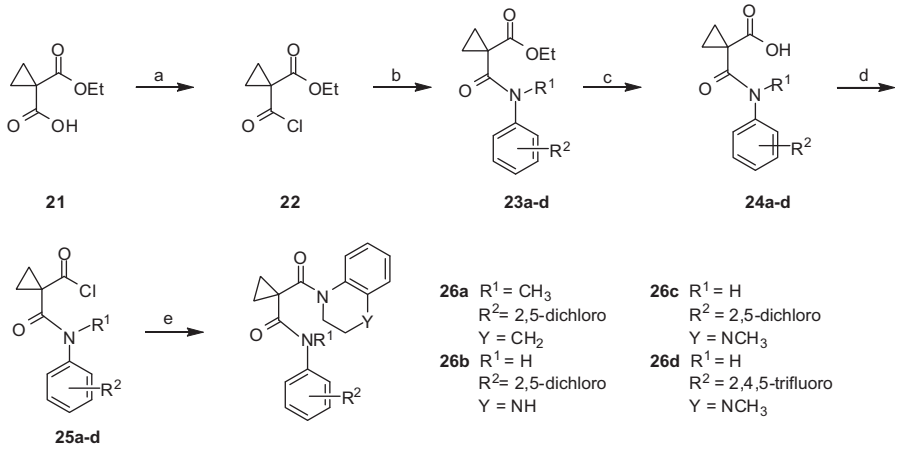
The three potent compounds **16b**, **18** and **26c** were evaluated for CYP inhibition prior to their in vivo PK study. As shown in Table 2, compound **18** displayed an advantage over **16b** and **26c** with regard to CYP2C19 inhibition even though all of these com-



Scheme 1. Reagents and conditions: (a) 2,5-dichloroaniline, *i*Pr_2EtN, *t*-BuOH, reflux,85%; (b) 1 N-NaOH, MeOH, 93%; (c) thionyl chloride, CH_2Cl_2, reflux; (d) 1,2,3,4-tetrahydroquinone for **8a** or 1-methyl-1,2,3,4-tetrahydroquinoxaline for **8b**, NEt_3, rt, 82% for 2 steps; (e) Raney-Ni, THF, rt, 80%; (f) *m*CPBA, CH_2Cl_2, rt, 65%; (g) AcOH, reflux, 68%.



Scheme 2. Reagents and conditions: (a) NaH, DMF, 2,5-dichlorophenol, rt, 85%; (b) 1 N-NaOH, MeOH, 92%; (c) thionyl chloride, CH_2Cl_2, reflux; (d) 1,2,3,4-tetrahydroquinone for **15a** or 1-methyl-1,2,3,4-tetrahydroquinoxaline for **15b** or 1,2,3,4-tetrahydroquinoxaline for **15c**, Et_3N, rt, 64–70% for 2 steps; (e) Raney-Ni, THF, rt; 70–73%; (f) *m*CPBA, CH_2Cl_2, rt, 67%; (g) AcOH, reflux, 40%.



Scheme 3. Reagents and conditions: (a) thionyl chloride, CH_2Cl_2, reflux; (b) 1,2,3,4-tetrahydroquinoline or 1,2,3,4-tetrahydroquinoxaline or 1-methyl 1,2,3,4-tetrahydroquinoxaline, Et_3N, rt, 85% for 2 steps; (c) 1 N-NaOH, MeOH, 92%; (d) thionyl chloride, CH_2Cl_2, reflux; (e) anilines, Et_3N, rt, 70–85% for 2 steps.

Table 1
Activity of compounds in HEK293-TGR5 cells[12]

Compound	EC_{50}* (nM)
INT-777	293
3	6.1
9	>1000
11	20
15b	12
15c	41
16a	48
16b	3.5
18	3.9
20	>1000
26a	>1000
26b	178
26c	5.1
26d	122

* The experiment was performed in triplicate and repeated at least three times independently.

Table 2
CYP inhibition by compounds, **16b**, **18** and **26c**

Compounds	IC_{50}* (μM)				
	CYP3A4	CYP1A2	CYP2C9	CYP2C19	CYP2D6
16b	0.25	20	1.30	1.61	20
18	0.26	20	1.29	16.72	20
26c	0.27	50	1.54	0.78	9.62
Positive control**	<0.062	3.62	0.18	1.90	<0.006

* IC_{50} values were determined using human liver microsomes purchased from BD biosciences.
** CYP3A4; ketoconazole, CYP1A2; furafylline, CYP2C9; sulfaphenazole, CYP2C19; tranylcypromine, CYP2D6; quinidine.

pounds showed similar inhibitory activity. Therefore, **18** was chosen and subjected to an in vivo PK study using ICR mice. Its pharmacokinetic profile is summarized in Table 3. At an oral dose of 10 mg/kg, the bioavailability (F) and $t_{1/2}$ of **18** were 13.9% and 2.83 h, respectively. According to the previous report,[11] the parent compound **3** is expected to exhibit a high in vivo clearance. However, compound **18** exhibited a moderate in vivo clearance. The overall PK profile of the pyrimidine **18** appeared to be acceptable, suggesting that the pyrimidine scaffold could be considered valuable in further investigations.

In summary, using a bioisostere replacement strategy, the pyrimidine analogs **16b** and **18** and the cyclopropyl malonamide derivative **26c** were found to be potent TGR5 agonists. In addition, **18** exhibited a moderate PK profile. Although no dramatic improvements were observed, the approach used in this study appeared to be an effective strategy for optimizing PK/PD profile.

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Table 3
Pharmacokinetic properties of compound **18** in male ICR mice

Routes	Dose (mg/kg)	AUC_{0-7h} (ng h/mL)	C_{max} (ng/mL)	T_{max} (h)	$t_{1/2}$ (h)	V_d (L/kg)	Cl (L/h/kg)	MRT_{last} (h)	F (%)
iv	5	1429.77	–	–	1.45	7.31	3.49	0.2	–
po	10	397.47	236.40	1.0	2.83	90.5	22.15	1.97	13.9

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12. *cAMP assay in HEK293-TGR5 cells*:[2,6b] The human TGR5 stable cell line, HEK293-TGR5 was purchased from Creative Biogene (USA). The cells were dispended into 96 well plates and incubated for 48 h. The day of the experiment, compounds were diluted in KRB (Krebs Ringer Bicarbonate) buffer containing 1 mM 3-isobutyl-1-methylxanthine (IBMX). Cells were washed in KRB buffer containing 1 mM IBMX and treated for 1 h at 37 °C with the diluted compounds. Following compound treatment, cAMP levels of lysed cells were measured using the Catch Point cAMP Fluorescent Assay Kit (Molecular Devices, Sunnyvale, CA) and assay was conducted according to the manufacturer's instructions.