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Synthesis and biological evaluation of pyrimidine derivatives with diverse azabicyclic ether/amine as novel GPR119 agonist

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ABSTRACT

A class of novel pyrimidine derivatives bearing diverse conformationally restricted azabicyclic ether/amine were designed, synthesized and evaluated for their GPR119 agonist activities against type 2 diabetes. Most compounds exhibited superior hEC_{50} values to endogenous lipid oleoylethanolamide (OEA). Analogs with 2-fluoro substitution in the aryl ring showed more potent GPR119 activation than those without fluorine. Especially compound **27m** synthesized from *endo*-azabicyclic alcohol was observed to have the best EC_{50} value (1.2 nM) and quite good agonistic activity (112.2% max) as a full agonist.

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disease characterized by hyperglycemia due to impaired insulin secretion and insulin resistance.[1] The number of people with T2DM worldwide is more than 300 million, and the prevalence is rapidly increasing.[2,3] Long-term complications such as heart disease, organ failure, and lower limb amputations are the major risk factor for T2DM patients.[4] Although a variety of treatments are available for T2DM, many patients are unable to achieve their target glycemic control.[5] Therefore, new drugs with novel mode of action that exhibit improved efficacy and safety relative to current available medications are clearly needed.

G Protein coupled receptor 119 (GPR119) is a class A type receptor, which is expressed primarily in pancreatic β-cells and the K and L cells of the gastrointestinal tract.[6,7] Some endogenous natural agonists of GPR119, such as oleoylethanolamide (OEA) and *N*-oleoyldopamine (OLDA), have been identified and investigated for their biological effects.[8,9] However, because of their instability and weak activity, it's not practical to develop it directly as a clinical drug. GPR119 agonists could stimulate secretion of the incretins, glucagon-like peptide-1(GLP-1) and glucose-dependent insulinotropic peptide (GIP) from L-cells *in vivo*, and increase the release of insulin from pancreatic β-cells.[10–14] These results significantly indicate GPR119 agonists have a dual mechanism for lowing plasma glucose and potential diabetes control.

Arena researchers disclosed the first potent and oral small molecule agonist of GPR119, in the form of AR231453 (Fig. 1).[15] Compound AR231453 displayed the strong agonistic activity (EC_{50} = 0.68 nM) and improved oral glucose tolerance in wild-type mice but not in GPR119 deficient mice.[16] Following with this enthusiasm, many pharmaceutical companies and institutes were pursuing GPR119 agonists for the treatment of type 2 diabetes.[17–23] To date, some GPR119 agonists have been progressed to the clinical phases (APD668, APD597, PSN821, GSK1292263,

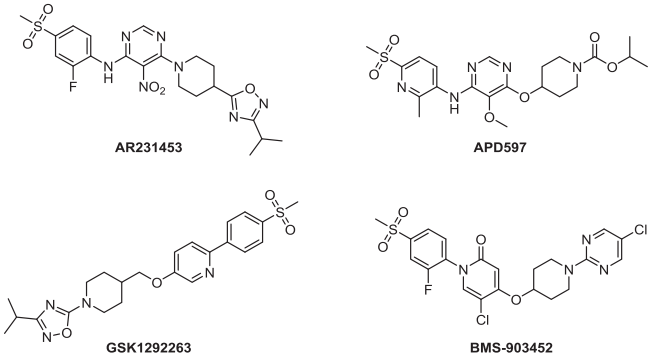

Fig. 1. Some representative structures of GPR119 agonists.

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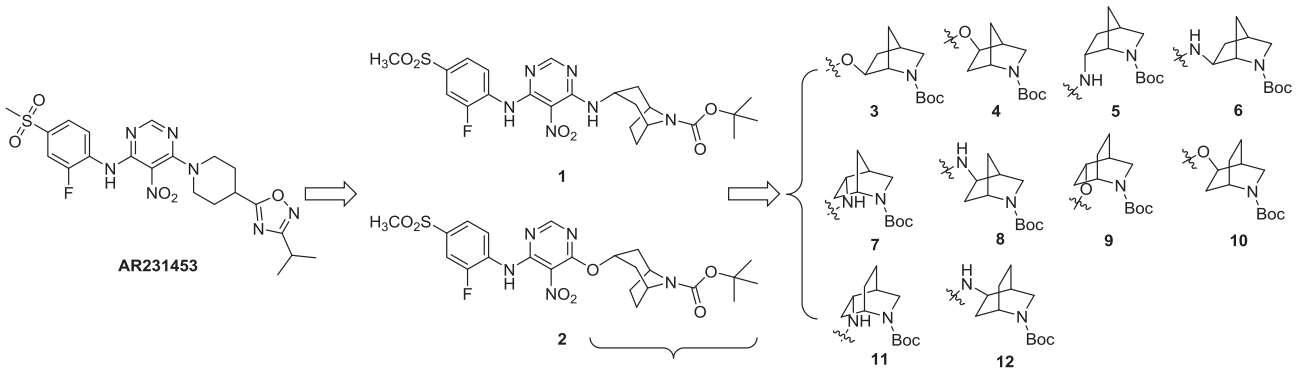

Fig. 2. The target compounds.

MBX-2982, DS-8500a, BMS-903452, LEZ763, ZYG-19) as shown in Fig. 1.[24–30]

In our efforts to discover small molecule full agonist of GPR119, pyrimidine compound AR231453 was selected as lead structure. As disclosed in our previously papers, derivatives **1** and **2** bearing *endo*-nortropanol/amine exhibited strong and full GPR119 agonistic activities (EC_{50} in the nanomolar range, Fig. 2).[31,32] Based on the exciting results, optimization of lead compound was conducted via retaining 5-nitropyrimidine and replacing piperidine with conformation restricted diverse azabicyclic ethers or amines. We estimated that introduction of rigid fragments like *endo*/*exo* azabicyclic rings to the ligands that reduced the conformational flexibility to make an ideal conformation and best recognition by the receptor. Herein, we report synthesis of a series of 5-nitropyrimidine derivatives with *endo*/*exo* azabicyclic fragments as potential GPR119 agonists for the treatment of type 2 diabetes.

The azabicyclic intermediates **3**–**12** were synthesized following the procedures and conditions as shown in Scheme 1. The Diels-Alder reaction of cyclopentadiene, ammonium chloride and formaldehyde in water gave alkene compound, which was then protected by a Boc group. Transformation of alkene **13** into a mixture of alcohol **3** and **4** was achieved using a hydroboration-oxidation reaction. Amines **5**–**8** were prepared from alcohols **3** and **4** via a 3 steps sequence of PCC oxidation followed by reductive amination and debenzylation.[33,34] The azabicyclic rings **9**–**12** were generated from ketone **20** via similar methods with intermediates **3**–**8**. The ketone **20** was obtained by the aza Diels-Alder reaction,[35] which was converted to compound **21** via replacement of *p*-methoxyphenyl group (PMP) with Boc group.[36] Reduction of **21** with $NaBH_4$ gave a mixture alcohol **9** and **10** with a ratio of 1.2/1. The synthetic pathway of amines **11**–**12** was same with **5**–**8** by reductive amination and debenzylation. The stereochemistry of all intermediates **3**–**12** was determined based on ^1H NMR data and published procedures.[33–37] The methyne contiguous with nitrogen or oxygen in bicyclic configuration isomers showed different splitting signal in ^1H NMR spectra.

The synthesis of 5-nitropyrimidine analogs **27a**–**t** was outlined in Scheme 2. 4,6-Dichloro-5-nitropyrimidine, 4-methylsulfonylaniline and 2-fluoro-4-methylsulfonylaniline were prepared according to previously reported procedures.[38–40] Reaction of 4,6-dichloro-5-nitropyrimidine and substituted aniline in DMF yielded compounds **25** and **26**, following by treatment with diverse azabicyclic alcohol or amine to afford target compounds **27a**–**t**.

Analogs **27a**–**t** were evaluated for their abilities to activate the human GPR119 in a cell-based cAMP assay, which were expressed in EC_{50} and %max values. The EC_{50} values represent the concentration of the tested compounds for 50% cAMP stimulation of oleoylethanolamide (OEA), while the %max values present the relative response (%) of the tested compounds compared to the maximal effect of OEA.[41]

Table 1 illustrated the biological results of compounds **27a**–**t**. Among these analogs, compounds bearing 2-fluoro-4-methylsulfonyl aniline group showed more potent GPR119 activation activities than 4-methylsulfonylaniline group. And most compounds synthesized from *endo*-azabicyclic moiety exhibited superior EC_{50} values and stronger agonistic activities comparing with those containing *exo*-azabicyclic moiety. Especially, compounds **27c**, **27i**, **27m**, **27n**, **27q**, **27r**, **27s**, **27t** displayed strong EC_{50}s (single digit nM). However, derivatives **27c**, **27n**, **27r**, **27s**, **27t** were observed middle level %max values and proved as partial agonists. Moreover, several derivatives only with *endo*-azabicyclic scaffold were proved as full agonists basted on %max values. Furthermore, compound **27q** bearing *endo*-azabicyclic amine **11** revealed the potent EC_{50} value (1.8 nM) with good efficacy (104.3% max). And compound **27m** containing *endo*-azabicyclic alcohol showed the quite good efficacy (112.2% max) with best EC_{50} value (1.2 nM).

In summary, we discovered a new series of 5-nitropyrimidine analogs with diverse aza-bicyclic ether or amine as GPR119 gonists for treatment of type 2 diabetes. As a result, most derivatives exhibited the significant GPR119 activation activities. All compounds containing 2-fluoro-4-methylsulfonyl aniline fragment showed more potent GPR119 agonistic activities than those with 4-methylsulfonylaniline group, which indicated fluorine atom as a hydrogen bond receptor was benefit for the activation activity. And analogs bearing *endo*-azabicyclic scaffold exhibited better %max values and were proved as full agonists comparing with *exo*-azabicyclic moiety, which implied that *endo*-azabicyclic moiety might be "agonist conformation". It is exciting that compounds **27c**, **27i**, **27m**, **27n**, **27q**, **27r**, **27s**, **27t** displayed single digit nM of EC_{50} Values. Notably, the analog **27q** showed potent agonistic activity (104.3% max) with strong EC_{50} value (1.8 nM) while the analog **27m** revealed maximum agonistic activity (112.2% max) with quite good EC_{50} value (1.2 nM). These results encourage us to search other heterocyclic structures as parents ring with *endo*-azabicyclic moiety to investigate the structure activity relationship of ligands with GPR119. The follow-up studies and results will be reported in due course.

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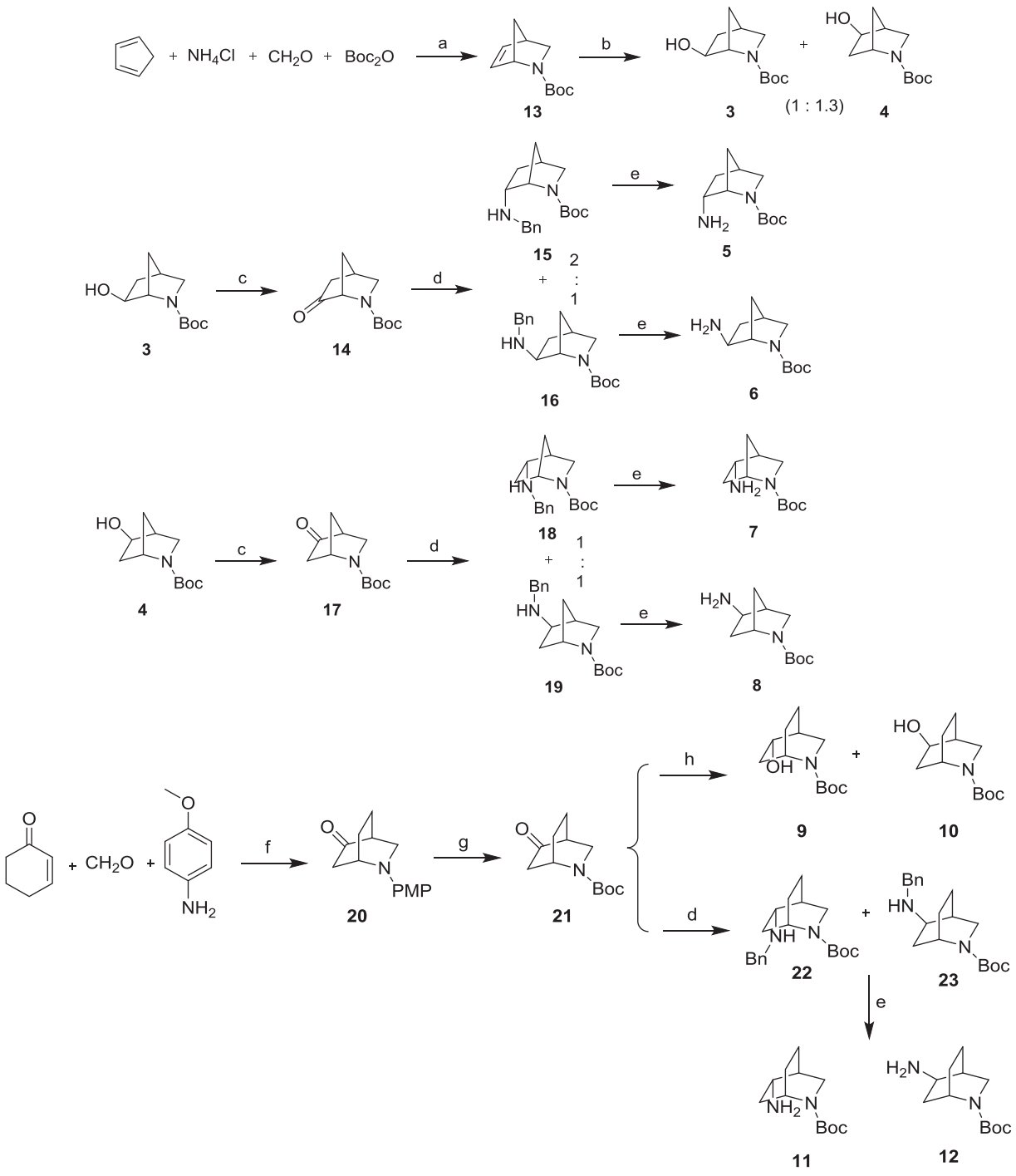
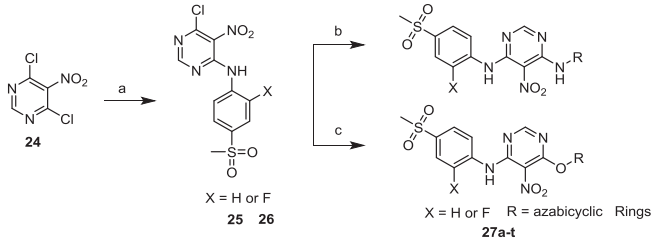


Scheme 1. Reagents and conditions: (a) NaOH, H_2O, rt, 18 h; (b) *i*: $NaBH_4$, $(CH_3)_2SO_4$, THF, rt, 3 h, under N_2; *ii*: KOH, H_2O_2, rt, 0.5 h; (c) PCC, CH_2Cl_2, rt, 6 h; (d) $BnNH_2$, $NaBH_3CN$, CH_2Cl_2, rt, overnight; (e) $Pd(OH)_2/C$, H_2, MeOH, rt, overnight; (f) L-proline, DMSO, 50 °C, 24 h. (g) trichloro isocyanuric acid, sulfuric acid, CH_3CN, rt, overnight; then Boc_2O, rt, 4 h. (h) $NaBH_4$, MeOH, 0 °C–rt, 3 h.



Scheme 2. Reagents and conditions: (a) DIPEA, DMF, 0 °C–rt, 2 h; (b) R-NH_2, DIPEA, THF, rt, 5 h; (c) R-OH, LiHMDS, THF, 0 °C–rt, 5 h.

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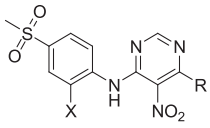
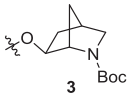
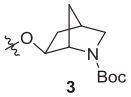
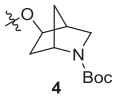
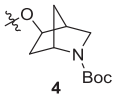
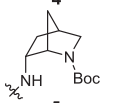
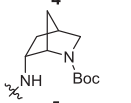
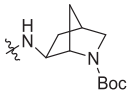
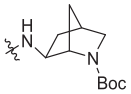
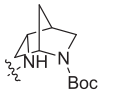
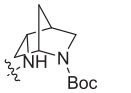
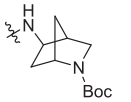
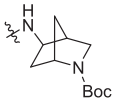
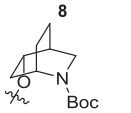
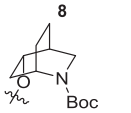
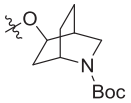
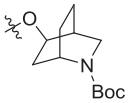
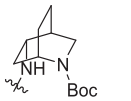
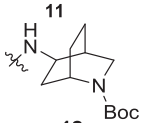
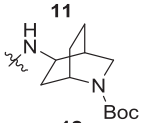
Table 1
GPR119 agonist activities of compounds **27a-t**.



X = H or F
R = Azabicyclic alcohols or amines

	X	R	hGPR119 activity			X	R	hGPR119 activity	
			EC_{50}[a] (μM)	%max[b]				EC_{50}[a] (μM)	%max[b]
27a	F	 3	0.0777	52.6	**27b**	H	 3	0.315	58.4
27c	F	 4	0.0056	86.7	**27d**	H	 4	0.110	100.6
27e	F	 5	0.053	76.7	**27f**	H	 5	>1	38.9
27g	F	 6	0.236	63.4	**27h**	H	 6	>10	21.6
27i	F	 7	0.0049	97	**27j**	H	 7	0.0482	94.3
27k	F	 8	>10	34.8	**27l**	H	 8	>10	25.1
27m	F	 9	0.0012	112.2	**27n**	H	 9	0.0031	71.9
27o	F	 10	>10	32.9	**27p**	H	 10	>10	44.7
27q	F	 11	0.0018	104.3	**27r**	H	 11	0.0056	78.9
27s	F	 12	0.0053	63.2	**27t**	H	 12	0.0064	58.1
OEA			**2.2**	**100**					

[a] EC_{50}: concentration for 50% cAMP stimulation of OEA.
[b] %max: cAMP stimulation% compared to maximal effect of OEA.

Acknowledgements

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41. HEK293 cells (4×10^3 cells/well) were seeded on 96 half-well plates and incubated for 24 h. The cells were transected with GPR119 expression plasmid (OriGene Technologies, Inc., USA) using Lipofectamine and Plus reagent (Life Technologies Corporation., USA). After 24 h, transfected cells were incubated with compounds dissolved in assay buffer (KRBH buffer containing 0.1% BSA and 500 μM 3-isobutyl-1-methylxanthine) for 60 min at 37 °C. Subsequently, cells were harvested with lysis buffer (50 mM phosphate buffer containing 1 M KF and 1.25% Triton X-100, pH 7.0) for 10 min at room temperature and the assay was performed using the cAMP homogeneous time-resolved fluorescence kit (CIS bio international, France).