Bioorganic & Medicinal Chemistry Letters xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis and biological evaluation of thienopyrimidine derivatives as GPR119 agonists

Moon-Kook Jeon [a,†], Kyu Myung Lee [a,†], Il Hyang Kim [a], Yoon Kyung Jang [a,b], Seung Kyu Kang [a], Jun Mi Lee [a], Kwan-Young Jung [a], Jaladi Ashok Kumar [a], Sang Dal Rhee [a], Won Hoon Jung [a], Jin Sook Song [a], Myung Ae Bae [a], Kwang Rok Kim [a], Jin Hee Ahn [a,b,*]

[a] Drug Discovery Division, Korea Research Institute of Chemical Technology, Republic of Korea
[b] Department of Medicinal and Pharmaceutical Chemistry, University of Science and Technology, 305-333, Republic of Korea

ARTICLE INFO

Article history:
Received 3 June 2014
Revised 6 July 2014
Accepted 8 July 2014
Available online xxxx

Keywords:
Diabetes
Thienopyrimidine
GPR119
Treatment

ABSTRACT

A series of thienopyrimidine derivatives was synthesized and evaluated for their GPR119 agonistic ability. Several thienopyrimidine derivatives containing R^1 and R^2 substituents displayed potent GPR119 agonistic activity. Among them, compound **5d**, which is a prototype, showed good in vitro activity with an EC_{50} value of 3 nM and human and rat liver microsomal stability. Compound **5d** exhibited no CYP inhibition and induction, Herg binding, or mutagenic potential. Compound **5d** showed increase insulin secretion in beta TC-6 cell and lowered the glucose excursion in mice in an oral glucose-tolerance test.

Diabetes is a serious metabolic disorder that occurs when the pancreas does not produce enough insulin, or the body cannot effectively use existing insulin.[1] Hyperglycemia (high blood glucose) can lead to various health consequences such as kidney damage, heart disease, stroke, nerve damage and blindness. Type 2 diabetes mellitus (T2DM, or noninsulin dependent), is the most common form of diabetes caused by insulin resistance, and loss of pancreatic β-cell function and approximately 95% diabetic patients are suffering from type 2 diabetes. This health burden is growing at an alarming rate, and it is estimated that there are approximately 350 million diabetic people globally. The prevalence of the disease is expected to escalate to 439 million by 2030.[2–4] Although, a variety of treatments are available for T2DM, many patients are unable to achieve their target HbA1c level.[5] Considerable attention has been focused on overcoming this public health challenge worldwide. Hence, there is a strong need for novel approaches to achieve better glycemic control and normoglycemia. Strategies that promote significant glycemic control by limiting hypoglycemia and cardiovascular side effects by enhancing insulin secretion in a glucose dependent manner could offer robust treatment for T2DM.

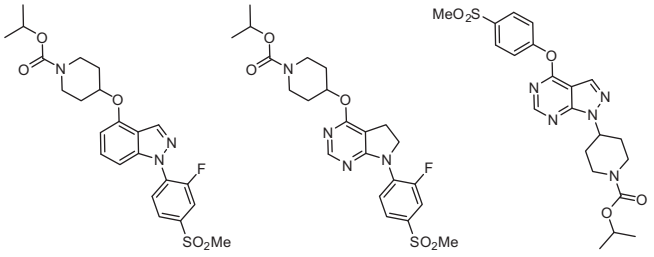


Figure 1. Representative bicyclic GPR119 agonists.

GPR119 is a member of the class A G protein-coupled receptor (GPCR) family, and it is highly expressed in pancreatic β-cells and intestinal endocrine cells.[6–8] Upon activation by its endogenous ligand, intracellular cAMP accumulates and adenylate cyclase activation enhances the effect of glucose-stimulated insulin secretion (GSIS) and GLP-1 release. Thus GPR119 represents a promising target for the treatment of type 2 diabetes and obesity owing to its ability to improve glucose homoeostasis while concurrently slowing gastric emptying, reducing food intake and promoting weight loss.[9,10]

Endogenous ligands for GPR-119 have been identified including lysophosphatidylcholine (LPC) and oleoylethanolamide (OEA).[9–11] Moreover, numerous small molecule GPR119 agonists have been

* Corresponding author.
E-mail address: jhahn@krict.re.kr (J.H. Ahn).
† These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.bmcl.2014.07.020
0960-894X/

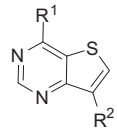
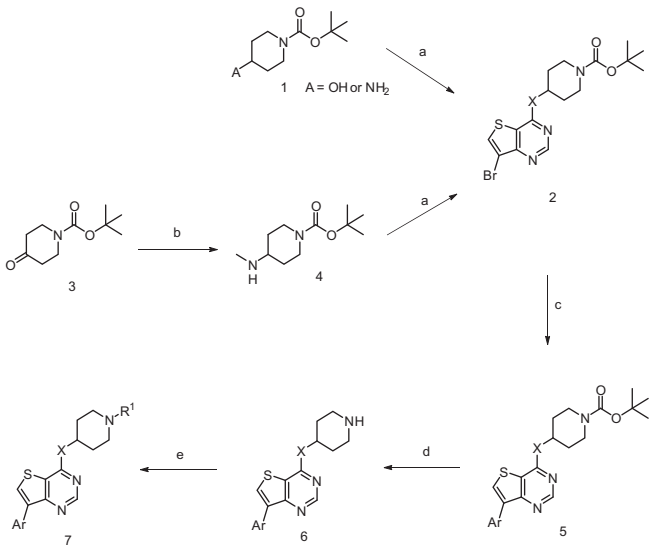


Figure 2. Structure of thienopyrimidine scaffold.



Scheme 1. Reagents and conditions: (a) 6-Bromo-4-chlorothieno[3,2-*d*]pyrimidine, A = OH, NaH, THF room temperature, 12 h; A = NH_2, DMSO, room temperature, 12 h; (b) 40% methylamine in methanol, $NaBH(OAc)_3$, 1,2-dichloroethane, room temperature, 12 h; (c) $Pd(PPh_3)_4$, Na_2CO_3, arylboronic acid, 1,4-dioxane, 110 °C, 24 h; (d) 4 M HCl in dioxane, room temperature, 2 h; (e) carbamate, alkylchloroformate, triethylamine, CH_2Cl_2, room temperature, 1 h; amide: acylhalide, triethylamine, CH_2Cl_2, DMF, room temperature, 12 h; pyrimidine; 2-chloro-5-ethylpyrimidine, triethylamine, DMF, 130 °C, 3 h.

identified in recent years. Among them, structurally rigid bicyclic compounds were identified as promising scaffolds. These bicyclic analogues exhibited potent GPR119 agonistic activity, efficacy and PK profiles (Fig. 1).[12–16]

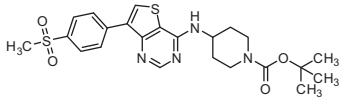
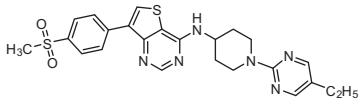
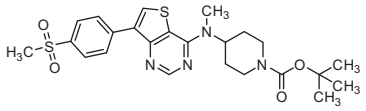
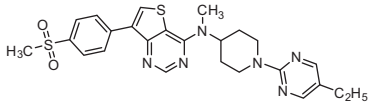
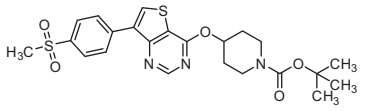
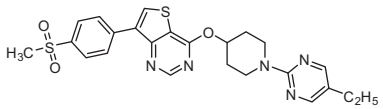
This prompted us to look for an alternate bicyclic scaffold, as a result, we identified the thienopyrimidine scaffold.[17] In this present work we wish to report the synthesis and biological evaluation of thienopyrimidine derivatives as GPR119 agonists (Fig. 2).

The general method for compound synthesis is outlined in Scheme 1. As shown in Scheme 1, commercially available Boc protected piperidine derivative **1** was coupled with 6-bromo-4-chlorothieno[3,2-*d*]pyrimidine to yield coupled product **2**, which was treated with diverse aryl boronic acids by Suzuki coupling in the presence of palladium catalyst afforded compound **5** with good yield. Meanwhile, Boc-protected piperidinone **3** was converted to 4-methylaminopiperidine derivative **4** via reductive amination, and it was then coupled with 6-bromo-4-chlorothieno[3,2-*d*]pyrimidine, followed by Suzuki reaction to yield compound **5**. Deprotection of compound **5** with 4 M HCl afforded compound **6**, which was derivatized by diverse electrophiles to give the final thienopyrimide derivative **7**.

Thus synthesized thienopyrimidine derivatives were evaluated in vitro for GPR119 agonistic activity, and the results are summarized in Tables 1–3. First, we fixed the 4-methylsulfonylphenyl substituent at the R^2 position on the thienopyrimidine ring, and derivatized at the R^1 position. As shown in Table 1, **5a** and **7a** showed weak agonistic activities; however, the introduction of a Boc-protected *N*-methylpiperidine to thienopyrimidine **5b** exhibited good in vitro activity with an EC_{50} value of 39 nM. Also, pyrimidine substituted *N*-methylpiperidine derivative **7b** displayed moderate potency (EC_{50} = 1200 nM). 4-Oxypiperidine derivatives **5c** and **7c** also activated GPR119 with EC_{50} values of 100 nM and 240 nM, respectively.

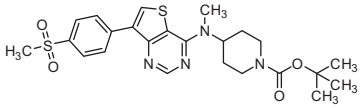
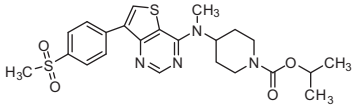
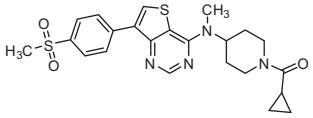
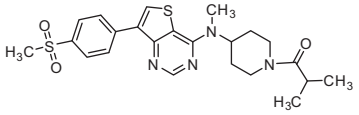
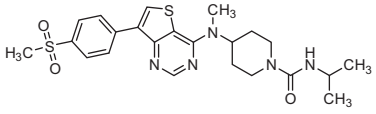
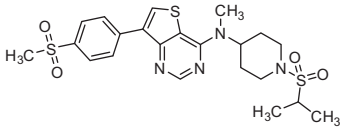
Based on the data shown in Table 1, we further derivatized the R^1 position with an *N*-methylaminopiperidine group. As shown in

Table 1
In vitro GPR119 agonist activity of thienopyrimidine derivatives

Compound	Structure	% Activation at 1 μM[a]	Human EC_{50} (nM)
5a		49	ND[b]
7a		49	ND
5b		76	39
7b		67	1200
5c		64	100
7c		75	240

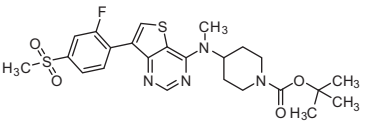
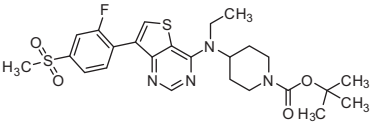
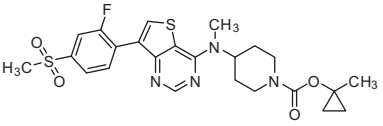
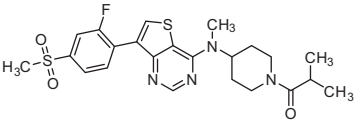
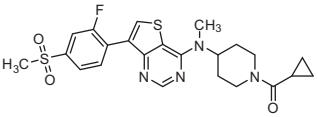
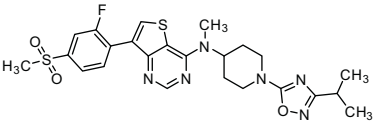
[a] Activation relative to GSK1292263.
[b] Not determined.

Table 2
In vitro GPR119 agonist activity of 4-methylaminopiperdinothienopyrimidine derivatives

Compound	Structure	% Activation at 1 μM[a]	Human EC_{50} (nM)
5b		76	39
7d		67	82
7e		72	173
7f		60	781
7g		49	ND[b]
7h		70	1200

[a] Activation relative to GSK1292263.
[b] Not determined.

Table 3
In vitro GPR119 agonist activity of 4-methylaminopiperdinothienopyrimidine derivatives

Compound	Structure	% Activation at 1 μM[a]	Human EC_{50} (nM)
5d		94	3
7i		45	ND
7j		105	26
7k		115	140
7l		65	ND[b]
7m		>50	ND

[a] Activation relative to GSK1292263.
[b] Not determined.

Please cite this article in press as: Jeon, M.-K.; et al. *Bioorg. Med. Chem. Lett.* (2014), http://dx.doi.org/10.1016/j.bmcl.2014.07.020

Table 4
Stability, CYP, hERG, AMES and cytotoxicity of compound **5d**

Assay	Results
Liver microsomal stability (Human)	>95% parent was remained after 30 min incubation
Liver microsomal stability (rat)	>95% parent was remained after 30 min incubation
CYP inhibition	1A2: no inhibition 2A6: no inhibition 2C19: no inhibition 2C9: IC_{50} = 76.85 μM 3A4: no inhibition 2D6: no inhibition 2B6: no inhibition
CYP induction	1A2: no induction 2B6: no induction 2C9: no induction 2E1: no induction 3A4: no induction
Herg	13% at 10 μM (binding assay)
Mini AMES	TA100: no mutation TA98: no mutation
Cytotoxicity	NIH 3T3: IC_{50} = 49.77 μM L929: IC_{50} = 65.79 μM HFL-1: IC_{50} >100 μM CHO-K1: IC_{50} = 53.37 μM

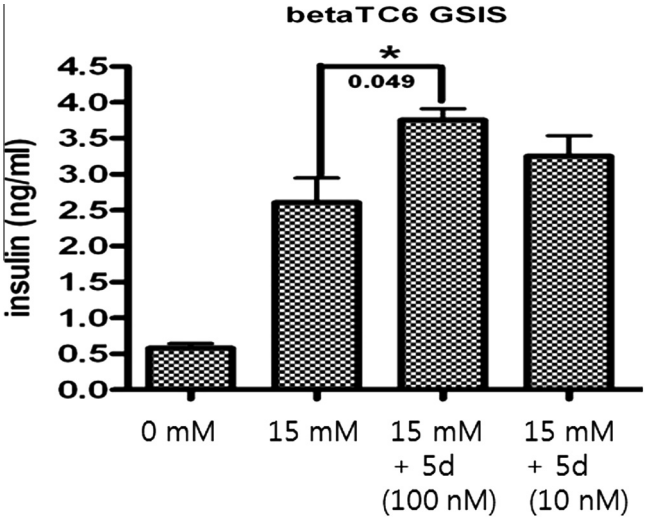


Figure 3. Glucose stimulated insulin secretion in TC-6 cells. *P <0.05.

Table 2, isopropyl carbamate **7d** was also active with an EC_{50} value of 82 nM. Amide derivatives (**7e** and **7f**) were weaker than carbamate derivatives (**5b** and **7d**). Introduction of an isopropyl urea moiety resulted in loss of activity (**7g**). Also, isopropyl sulfonamide (**7h**) showed moderated GPR119 agonistic activity.

Diverse substituted aryl and heteroaryl groups were introduced at the R^2 position of the thienopyrimidine ring. As expected, methylsulfonyl substituted aryl or heteroaryl derivatives showed good in vitro activity (data not shown). Therefore, we focused on R^2 modification with 2-fluoro-4-methylsulfonylphenyl group, and the results were summarized in Table 3. *tert*-Butyl and isopropyl carbamate (**5d**) showed good in vitro potency with EC_{50} value of 3 nM. However, the introduction of an ethyl group resulted in loss of activity (compound **7i**). Methylcyclopropyl carbamate (**7j**) exhibited good activity (EC_{50} = 26 nM), whereas other amides (**7k** and **7l**) and oxadiazole (**7m**) resulted in a loss of activity.

From the results of our in vitro data, **5d** was selected as a prototype compound. Next, compound **5d** was investigated for its stability, ability to induce/inhibit CYP, Herg binding and cytotoxicity. As shown in Table 4, compound **5d** is metabolically stable in human and rat liver microsomes, with over 95% of the parent compound remaining after 30 min incubation. In CYP inhibition/induction assays with several CYP subtypes, compound **5d** did not significantly inhibit or induce CYP. Compound **5d** showed no Herg binding (13% inhibition at 10 μM) and mutagenic potential in the AMES assay.

To evaluate that **5d** has direct effects on beta cells, insulin secretion was measured in the pancreatic beta cell line, TC-6. As can be seen in Figure 3, exposure to **5d** at concentrations ranging from 10 nM to 100 nM increased glucose-stimulated insulin secretion (GSIS) in a dose-dependent manner in TC-6 cells.

To evaluate in vivo efficacy, oral glucose tolerance tests (OGTT) were performed using compound **5d**. Plasma glucose levels were determined based on the AUC of the glucose concentration, and they were significantly reduced at 15 mg/kg dose (Fig. 4).

In conclusion, we identified a series of thienopyrimidine derivatives as GPR119 agonists. Several thienopyrimidine derivatives with R^1 and R^2 substituents were found to be potent GPR119 agonists. Among them, compound **5d** was the most active with an EC_{50} value of 3 nM and showed good human and rat liver microsomal stability. Compound **5d** exhibited no CYP inhibition and induction, hERG binding, or mutagenic potential. Compound **5d** induced increased insulin secretion from beta cell and reduced the AUC of glucose in vivo OGTT. We are currently producing further modification of this prototype that will be examined in the near future.

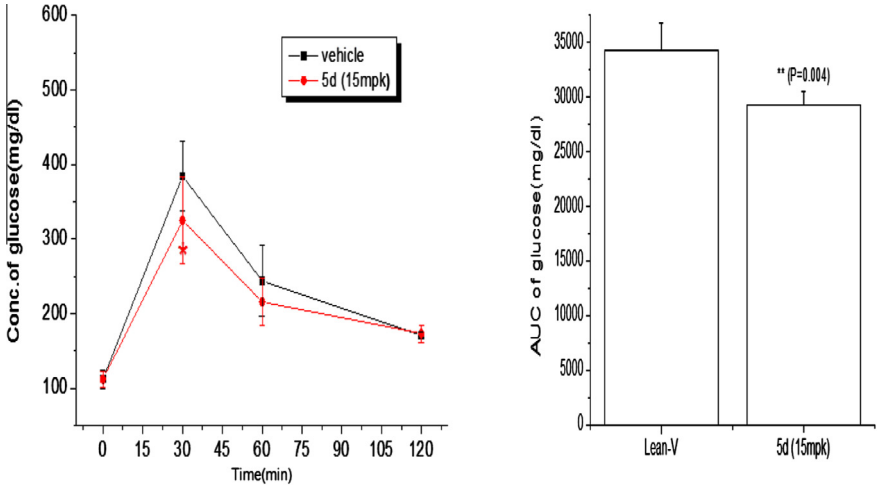


Figure 4. In vivo oral glucose tolerance test of compound **5d** in normal mice. Results are expressed as means ± SEM for n = 7 mice/group. *P <0.05, **P <0.01.

Acknowledgments

This research was supported by the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT & Future Planning (2012-0019773) and a grant of the Korea Health technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare (A111345).

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.07.020.

References and notes

1. DeFronzo, R. A.; Bonadonna, R. C.; Ferrannini, E. *Diabetes Care* **1992**, *15*, 318.
2. Scully, T. *Nature* **2012**, *485*, S2.
3. Danaei, G.; Finucane, M. M.; Lu, Y.; Singh, G. M.; Cowan, M. J.; Paciorek, C. J.; Lin, J. K.; Farzadfar, F.; Khang, Y.-H.; Stevens, G. A.; Rao, M.; Ali, M. K.; Riley, L. M.; Robinson, C. A.; Ezzati, M. *Lancet* **2011**, *378*, 31.
4. Shaw, J. E.; Sicree, R. A.; Zimmet, P. Z. *Diabetes Res. Practice* **2009**, *87*, 4.
5. Koro, C. E.; Bowlin, S. J.; Bourgeois, N.; Fedder, D. O. *Diabetes Care* **2004**, *27*, 17.
6. Chu, Z. L.; Jones, R. M.; He, H.; Carroll, C.; Gutierrez, V.; Lucman, A.; Moloney, M.; Gao, H.; Mondala, H.; Bagnol, D.; Unett, D.; Liang, Y.; Demarest, K.; Semple, G.; Behan, D. P.; Leonard, J. *Endocrinology* **2007**, *148*, 2601.
7. Fredrikson, R.; Hoglund, P.; Gloriam, D. E. I.; Lagerstrom, M. C.; Schioth, H. B. *FEBS Lett.* **2003**, *554*, 381.
8. Sakamoto, Y.; Inoue, H.; Kawakami, S.; Miyawaki, K.; Miyamoto, T.; Mizuta, K.; Itakura, M. *Biochem. Biophys. Res. Commun.* **2006**, *351*, 474.
9. (a) Jones, R. M.; Leonard, J. N.; Buzard, D. J.; Lehmann, J. *Expert Opin. Ther. Pat.* **2009**, *19*, 1339; (b) Fyfe, M. C. T.; McCormack, J. G.; Overton, H. A.; Procter, M. J.; Reynet, C. *Expert Opin. Drug Disc.* **2008**, *3*, 403.
10. Overton, H. A.; Babbs, A. J.; Doel, S. M.; Fyfe, M. C. T.; Gardner, L. S.; Griffin, G.; Jackson, H. C.; Procter, M. J.; Rasamison, C. M.; Tang-Christensen, M.; Widdowson, P. S.; Williams, G. M.; Reynet, C. *Cell Metab.* **2006**, *3*, 167.
11. Soga, T.; Ohishi, T.; Matsui, T.; Saito, T.; Matsumoto, M.; Takasaki, J.; Matsumoto, S.; Kamohara, M.; Hiyama, H.; Yoshida, S.; Momose, K.; Ueda, Y.; Matsushime, H.; Kobori, M.; Furuichi, K. *Biochem. Biophys. Res. Commun.* **2005**, *326*, 744.
12. Semple, G.; Ren, A.; Fioravanti, B.; Pereira, G.; Calderon, I.; Choi, K.; Xiong, Y.; Shin, Y.-J.; Gharbaoui, T.; Sage, C. R.; Morgan, M.; Xing, C.; Chu, Z-L.; Leonard, J. N.; Grottick, A. J.; Al-Shamma, H.; Liang, Y.; Demarest, K. T.; Jones, R. M. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 3134.
13. Katamreddy, S. R.; Carpenter, A. J.; Ammala, C. E.; Boros, E. E.; Brashear, R. L.; Briscoe, C. P.; Bullard, S. R.; Caldwell, R. D.; Conlee, C. R.; Croom, D. K.; Hart, S. M.; Heyer, D. O.; Johnson, P. R.; Kashatus, J. A.; Minick, D. J.; Peckham, G. E.; Ross, S. A.; Roller, S. G.; Samano, V. A.; Sauls, H. R.; Tadepalli, S. M.; Thompson, J. B.; Xu, Y.; Way, J. M. *J. Med. Chem.* **2012**, *55*, 10972.
14. Gillespie, P.; Goodnow, R. A., Jr.; Saha, G.; Bose, G.; Moulik, K.; Zwingelstein, C.; Myers, M.; Conde-Knape, K.; Pietranico-Cole, S.; So, S.-S. *Bioorg. Med. Chem. Lett.* **2014**, *24*, 949.
15. Alper, P.; Azimioara, M.; Cow, C.; Mutnick, D.; Nikulin, V.; Michellys, P.-Y.; Wang, Z.; Reding, E.; Paliotti, M.; Li, J.; Bao, D.; Zoll, J.; Kim, Y.; Zimmerman, M.; Groessel, T.; Tuntland, T.; Jeseph, S. B.; McNamara, P.; Seidel, H. M.; Epple, R. *Bioorg. Med. Chem. Lett.* **2014**, *24*, 2383.
16. Wang, Y.; Yu, M.; Zhu, J.; Zhang, J.; Kayser, F.; Medina, J. C.; Siegler, K.; Conn, M.; Shan, B.; Grillo, M. P.; Liu, J.; Coward, P. *Bioorg. Med. Chem. Lett.* **2014**, *24*, 1133.
17. Thienopyrimidine based patents were published including our patent, however, detailed biological data and SAR results were not disclosed. (WO2012154009, WO2012093809, WO2011159657).