Bioorganic & Medicinal Chemistry Letters 18 (2008) 4298–4302

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Discovery of piperidine-aryl urea-based stearoyl-CoA desaturase 1 inhibitors

Zhili Xin *,†, Hongyu Zhao, Michael D. Serby, Bo Liu, Mei Liu, Bruce G. Szczepankiewicz, Lissa T. J. Nelson, Harriet T. Smith, Tom S. Suhar, Rich S. Janis, Ning Cao, Heidi S. Camp, Christine A. Collins, Hing L. Sham, Teresa K. Surowy, Gang Liu

Abbott Laboratories, Global Pharmaceutical Research and Development, 100 Abbott Road, Abbott Park, IL 60064, USA

ARTICLE INFO

Article history:
Received 8 June 2008
Revised 23 June 2008
Accepted 25 June 2008
Available online 28 June 2008

Keywords:
SCD1 inhibitors
Desaturation index

ABSTRACT

A series of structurally novel stearoyl-CoA desaturase1 (SCD1) inhibitors has been identified via molecular scaffold manipulation. Preliminary structure–activity relationship (SAR) studies led to the discovery of potent, and orally bioavailable piperidine-aryl urea-based SCD1 inhibitors. 4-(2-Chlorophenoxy)-*N*-[3-(methyl carbamoyl)phenyl]piperidine-1-carboxamide **4c** exhibited robust in vivo activity with dose-dependent desaturation index lowering effects.

© 2008 Elsevier Ltd. All rights reserved.

The incidence of obesity in industrial societies has increased dramatically over the past 30 years. Serious consequences of this trend include a concurrent rise in the number of individuals with type 2 diabetes mellitus, a projected decrease in the average life-span of individuals in Western societies, and increasing pressure on national health care systems to manage the complications resulting from obesity.[1] Although obesity is clearly linked to chronic over-nutrition, many people find it difficult to achieve long-term weight loss through diet alone. Pharmaceutical agents that could safely promote weight loss and allow individuals to maintain an ideal body weight would offer an important option to those struggling with obesity.

Stearoyl-CoA desaturase (SCD) is a microsomal enzyme that catalyzes the rate-limiting step in the biosynthesis of monounsaturated fatty acids from saturated fatty acids, in conjunction with cytochrome b_5 reductase, cytochrome b_5, and the cofactors NADH.[2–4] The preferred substrates for SCD are palmitoyl(C16:0)-CoA and stearoyl (C18:0)-CoA, which are converted to palmitoleoyl (C16:1)-CoA and oleoyl (C18:1)-CoA, respectively. The products, palmitoleic and oleic acids are major components of cell membrane phospholids, circulating triglycerides, and cholesterol esters. Four mouse SCD (SCD1, SCD2, SCD3, and SCD4)[5] and two human SCD genes[6,7] have been characterized. Human SCD1 shows 85% homology to the murine SCD1. SCD1 is abundantly expressed in liver and adipose tissue and is tightly regulated by dietary and hormonal factors including glucose, insulin, cholesterol and polyunsaturated fatty acids.[8,9] There is ample evidence indicating that SCD1 plays an important role in lipid metabolism[10] and body weight control.[11] Higher SCD1 activity in humans has been correlated with elevated plasma triglyceride levels.[12] SCD1 deficiency in mice, either naturally deficient Asebia mice[13] or laboratory-created SCD1 knockout mice (SCD1−/−),[14] has been shown to reduce body adiposity, increase insulin sensitivity, and impart resistance to diet-induced obesity.[15] Despite the fact that SCD1−/− mice consume more food than normal mice, they clearly show a leaner phenotype. The SCD1−/− mice also have lower levels of hepatic cholesterol esters and triglycerides.[16] Inhibition of SCD1 activity via antisense oligonucleotide knockdown of gene expression in diet induced obese (DIO) mice resulted in reduced adiposity, improved hepatic steatosis and increased energy expenditure.[17] These observations make SCD1 a potential therapeutic target for the treatment of obesity and diabetes.

Recently, a number of patent applications from Xenon Pharmaceuticals[18,19] and Merck Frosst[20] have been published, covering a few series of small molecules that modulate SCD1 activity and regulate plasma lipid levels. The SAR studies of SCD1 inhibition based on a pyridazine carboxamide template have been described by our co-workers from Abbott Laboratories.[21,22] Here, we present the discovery of a different class of SCD1 inhibitors with good in vitro potency and in vivo activities.

The nicotinamides (Fig. 1) represent one class of SCD1 inhibitors disclosed by investigators at Xenon. We synthesized compound **1a** (Fig. 1), and determined an IC_{50} of 0.4 μM vs murine liver microsomal SCD1. In the course of searching for SCD1 inhibitors with a new scaffold, we decided to replace the central aminopyridine of

* Corresponding author. Tel.: +1 617 914 1110; fax: +1 617 679 3635.
E-mail address: zhili.xin@biogenidec.com (Z. Xin).
† Present address: Biogen Idec, 14 Cambridge Center, Cambridge, MA 02142-1453, USA.

0960-894X/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2008.06.088

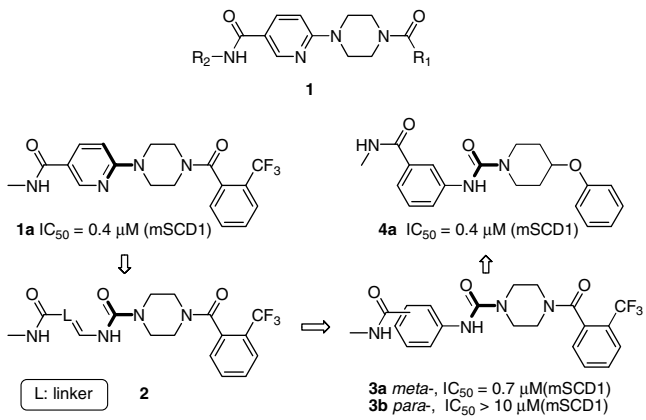


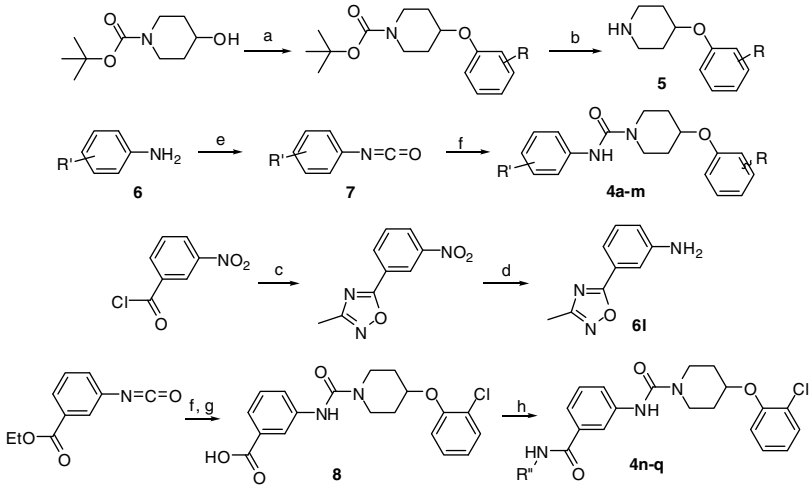
Figure 1. Rational design of novel piperidine-aryl urea-based stearoyl-CoA desaturase 1 (SCD1) inhibitors

1a with a urea group. This modification allowed the maximum conservation of the polarity and geometry of the aminopyridine in **1a** yet led to a new chemotype. Although trans-olefin appears to be a rational linkage to hold the rest of molecule in place, **2** is obviously not a suitable synthetic target due to its instability. We thus introduced a benzene ring next to the urea. Both *meta* and *para* substituted derivatives (**3a**, **3b**) have been constructed to probe the favorite orientation. **3a**, with *meta*-methylamide, provided a very promising IC_{50} against mSCD1 (0.7 μM), while *para*-substituted analog **3b** failed to demonstrate any potency in the same assay. We quickly found that piperazine benzamide can be successfully replaced by piperidine ether without losing any potency (**4a**, IC_{50} = 0.4 μM). Compound **4a** features some advantageous characteristics as our lead compound. It carries several open sites that can be readily modified to build SAR; the piperidine-aryl urea-based scaffold has not been exploited extensively, and its analogs might have improved pharmacokinetic profiles.

The synthesis of newly designed SCD1 inhibitors is straightforward and is outlined in Scheme 1. Mitsunobu reaction was followed by Boc-deprotection with HCl/dioxane to produce 4-phenoxypiperidine **5**. Compound **4a–m** was synthesized through a two-step, one-pot reaction. Thus, readily available aniline **6** was transformed to isocyanate **7** in the presence of triphosgene. Treatment of intermediate **7** in situ with appropriate 4-phenoxypiperidine **5** proceeded smoothly to afford urea **4a–m**. In the case of **4l**, aniline **6l** was prepared from 3-nitrobenzoylchloride via a cyclization[23] and subsequent reduction. Compounds that were further derivatized through the amide modification were prepared from an acid core **8**, which was obtained by addition of 4-(2-chlorophenoxy)piperidine to commercially available ethyl 3-isocyanatobenzoate followed by hydrolysis of the ester. Coupling of acid **8** and requisite amine mediated by TBTU in DMF with Hunig's base provided the desired amide **4n–q**.

For the characterization of SCD1 inhibitors, both mouse SCD1 and human SCD1 enzymatic activities have been measured. As a source of human SCD1 activity, recombinant human SCD1 and accessory protein cytochrome b_5 and cytochrome b_5 reductase were cloned and expressed using baculovirus/insect cell express systems. Structure–activity relationship studies were carried out and the results are summarized in Table 1. In general, the potency of inhibitors using the human recombinant sources of enzymes (hSCD1) was lower than using mouse SCD1 (mSCD1), which may reflect the difference between human SCD1 and mouse SCD1. However, the rank order of IC_{50} was about the same in both assays. We will discuss our SAR primarily based on mSCD1 data, since in vivo activity of our SCD1 inhibitor was evaluated in mouse model.

The optimization began with an examination of substitution on phenoxy ring. With the *ortho*-substituted analogues, the IC_{50}s steadily decreased from H (**4a**, 370 nM) and F (**4b**, 98 nM) to bulkier halogen groups, such as Cl and Br (**4c** and **4d**, <4 nM). Consistent with the SAR trend observed for the pyridazine series,[21] increased lipophilicity on the phenoxy ring, especially on *ortho*-position, correlates with improved inhibitory activity. As can be observed, introduction of 2-methyl group resulted in an inhibitor (**4e**, 17 nM) with >20-fold improvement in potency. The impact was less pronounced with relatively more hydrophilic methoxy analogue (**4f**, 81 nM). *Meta*-substitution was also well tolerated as exemplified with compound **4g** (20 nM), although not as optimal as *ortho*-substituted analogue **4c** (<4 nM). To explore any additional lipophilic interaction, we introduced a fluoride at the 4, 5 or 6-position on the basis of **4c**. As shown in Table 1, 2,4-di-substituted analogue **4j** was able to maintain most of the potency observed with **4c**, whereas 2,6-di-substitution surrendered all the gain generated from 2-mono-substitution (**4h**, 330 nM vs **4a**, 370 nM). The co-presence of F at 5-position was found to be beneficial as 2,5-analogue **4i** exhibited about twofold potency improvement in human SCD1 (**4i**, hSCD1 IC_{50} = 18 nM vs **4c**, hSCD1 IC_{50} = 37 nM).



Scheme 1. Reagents and conditions: (a) phenols, Ph_3P, DEAD, THF, rt; (b) 4 N HCl in dioxane, rt; (c) acetamide oxime, Et_3N, CH_3CN, microwave 150 °C, 15 min; (d) $SnCl_2 \cdot 2H_2O$, ethanol, reflux, 1 h; (e) triphosgene, diisopropyethylamine, THF, rt, 5 min; (f) 4-phenoxypiperidine, THF, rt, 1 h; (g) 2 N NaOH, THF, MeOH, rt, overnight; (h) corresponding amines, TBTU, diisopropylethylamine, DMF, rt.

Table 1
SAR summary of SCD-1 inhibitors **4a–q**

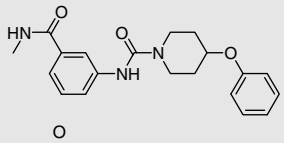
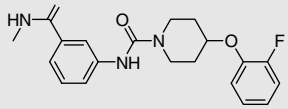
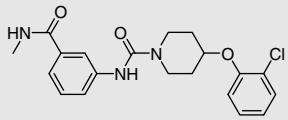
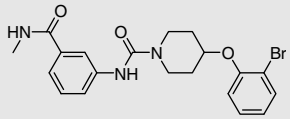
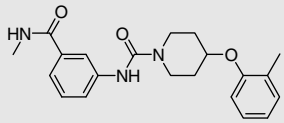
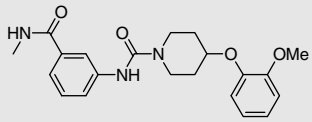
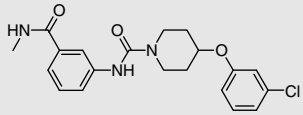
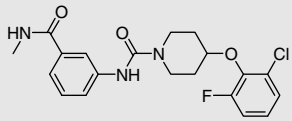
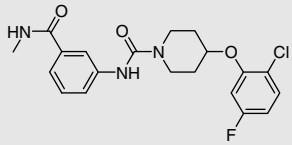
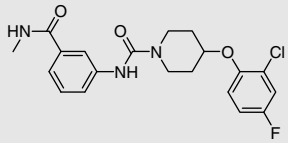
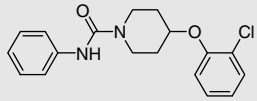
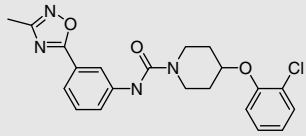
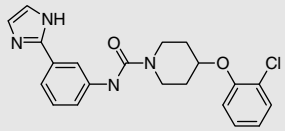
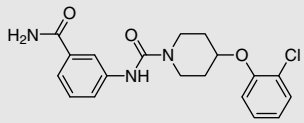
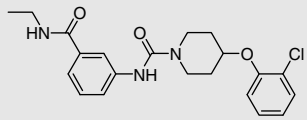
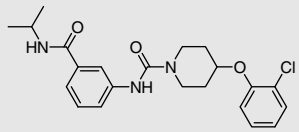
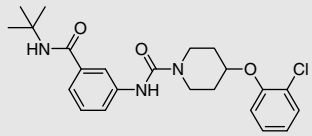
Compound	Structure	mSCD1 IC_{50} (μM)[a]	hSCD1 IC_{50} (μM)[a]
4a		0.37 ± 0.25	4.0 ± 1.1
4b		0.098 ± 0.047	0.68 ± 0.23
4c		<0.004	0.037 ± 0.017
4d		<0.004	0.030 ± 0.013
4e		0.017 ± 0.013	0.12 ± 0.01
4f		0.081 ± 0.039	1.9 ± 0.2
4g		0.020	0.27 ± 0.04
4h		0.33 ± 0.11	9.7 ± 0.3
4i		<0.004	0.018 ± 0.011
4j		0.006 ± 0.000	0.058 ± 0.037
4k		0.59 ± 0.13	7.5 ± 1.1
4l		0.008 ± 0.002	0.11 ± 0.03

Table 1 (*continued*)

Compound	Structure	mSCD1 IC_{50} (μM)[a]	hSCD1 IC_{50} (μM)[a]
4m		0.010 ± 0.006	0.36 ± 0.10
4n		0.010 ± 0.00	0.059 ± 0.012
4o		0.008 ± 0.002	0.15 ± 0.09
4p		0.81 ± 0.18	7.1 ± 2.9
4q		>10	>10

[a] Values are geometric means of at least two experiments, standard error is given in parentheses.

In general, this series of SCD1 inhibitors features favorable pharmacokinetic profiles in rodents, characterized by modest volume of distribution and high oral bioavailability in mice (**4c**, CL_p = 0.4 L/h/kg; V_{ss} = 0.4 L/kg; F = 92%).

Based on the encouraging results associated with compound **4c**, we next investigated the replacement of amide group with a heterocyclic moiety to further improve SCD1 inhibitory activity. This modification yielded a couple of analogues with good retention of IC_{50} (**4l**, 8 nM; **4m**, 10 nM), although they offered no potency advantage over **4c**. The presence of a functional group on the aryl 3-position seems to be important to possess high potency, as unsubstituted analogue showed dramatic loss of activity (**4k**, 0.6 μM). Fine-tuning of amide derivatives indicated that only primary amide or small group of secondary amide such as ethylamide was favorable with comparable IC_{50} to **4c**. Bulky group was detrimental to the activity as **4q** showed no inhibition at 10 μM.

Since SCD1 activity depends on cytochrome b_5 and cytochrome b_5 reductase, we established a selectivity assay to exclude the possibility that compounds identified as SCD1 inhibitors may actually inhibit the co-enzyme. The lack of inhibition above the concentration of 10 μM supports the direct SCD inhibition by our urea-based chemotype, represented by compound **4c**.

We next investigated the selectivity profiles of our potent inhibitors. Excellent selectivity over kinases was routinely achieved. **4c**

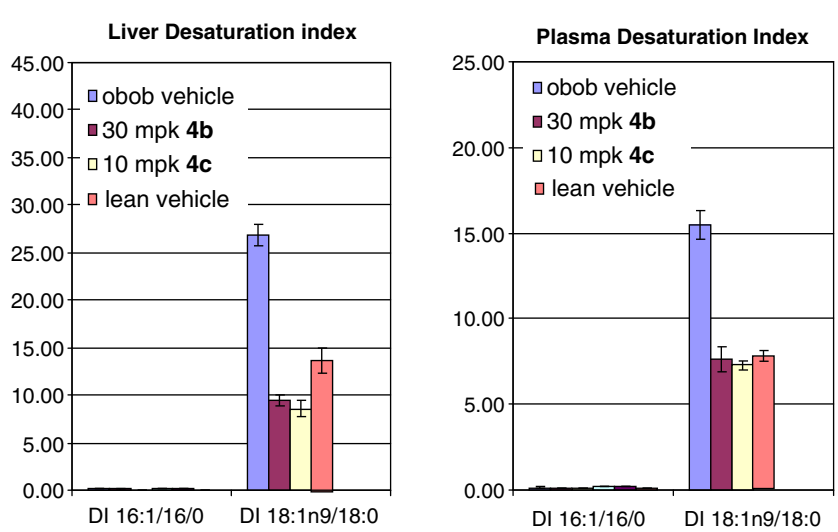


Figure 2. Liver and plasma triacylglycerol desaturation index lowering effect with the treatment of SCD1 inhibitors **4b** and **4c** in ob/ob mice.

was also tested in a ^{3}H-Dofetilide binding assay and showed no significant hERG channel blockade activity (IC_{50} > 100 μM).

SCD1 inhibitors **4b** and **4c** were evaluated in a 5-day efficacy study using ob/ob mice. A desaturation index, calculated as the ratio of 16:0/16:1n7 or 18:0/18:1n9, was used as in vivo biomarker for activity. The lipid classes examined include cholesterol ester, diacylglycerol, free fatty acid, free cholesterol, total phospholipids, and triacylglycerol. **4b** (30 mpk bid) and **4c** (10 mpk bid) consistently reduced desaturation indices of all these lipid categories to lean level, or even lower. Figure 2 showed the desaturation index (18:0/18:1n9) lowering effect with the treatment of SCD1 inhibitors **4b** and **4c**. A dose dependent triglyceride desaturation index reduction by **4c** was also observed in ob/ob mice (data not shown). These observations demonstrated a clear correlation between in vitro activity and in vivo desaturation index.

In conclusion, we have discovered a series of highly potent and selective SCD1 inhibitors with excellent pharmacokinetic profiles based on de novo design. Representative SCD1 inhibitors from this class demonstrated in vivo efficacy with dose-dependent desaturation index lowering effect. The pharmacology data from chronic studies will be reported in due time.

Acknowledgments

We thank the R4EK staff for pharmacokinetic studies, the R46Y protein expression/purification group for providing insect cells co-expressing human SCD1, human cytochrome b_5 and human cytochrome b_5 reductase.

Supplementary data

Experimental procedures for the synthesis and spectral characterization of reported compounds **4a–q** in Table 1, the detailed protocols of mouse SCD1 and human SCD1 assays can be found in the online version. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.06.088.

References and notes

1. Olshansky, S. J.; Passaro, D. J.; Hershaw, R. C.; Layden, J.; Carnes, B. A.; Brody, J.; Hayflick, L.; Butler, R. N.; Allison, D. B.; Ludwig, D. S. *N. Engl. J. Med.* **2005**, *352*, 1138.
2. Miyazaki, M.; Ntambi, J. M. *Prostaglandins Leukot. Essent. Fatty Acids* **2003**, *68*, 113.
3. Ntambi, J. M. *Prog. Lipid Res.* **1995**, *34*, 139.
4. Enoch, H. G.; Catala, A.; Strittmatter, P. *J. Biol. Chem.* **1976**, *251*, 5095.
5. Miyazaki, M.; Jacobson, M. J.; Man, W. C.; Cohen, P.; Asilmaz, E.; Friedman, J. M.; Ntambi, J. M. *J. Biol. Chem.* **2003**, *278*, 33904. and the references cited therein.
6. Zhang, L.; Ge, L.; Parimoo, S.; Stenn, K. S.; Prouty, S. M. *Biochem. J.* **1999**, *340*, 255 (b).
7. (a) Wang, J.; Yu, L.; Schmidt, R. E.; Su, C.; Huang, X.; Gould, K.; Cao, G. *Biochem. Biophys. Res. Commun.* **2005**, *332*, 735; (b) Zhang, S.; Yang, Y.; Shi, Y. *Biochem. J.* **2005**, *388*, 135.
8. Waters, K. M.; Ntambi, J. M. *J. Biol. Chem.* **1994**, *269*, 27773.
9. Ntambi, J. M. *J. Lipid Res.* **1999**, *40*, 1549.
10. Cohen, P.; Ntambi, J. M.; Friedman, J. M. *Curr. Drug Targets Immune Endocr. Metabol. Disord.* **2003**, *3*, 71.
11. Dobrzyn, A.; Ntambi, J. M. *Trends Cardiovasc. Med.* **2004**, *14*, 77.
12. Attie, A. D.; Krauss, R. M.; Gray-Keller, M. P.; Brownlie, A.; Miyazaki, M.; Kastelein, J. J.; Lusis, A. J.; Stalenhoef, A. F.; Stoehr, J. P.; Hayden, M. R.; Ntambi, J. M. *J. Lipid Res.* **2002**, *43*, 1899.
13. Zheng, Y.; Eilersten, K. J.; Ge, L.; Zhang, L.; Sundberg, J. P.; Prouty, S. M.; Stenn, K. S.; Parimoo, S. *Nat.Genet.* **1999**, *23*, 268.
14. Ntambi, J. M.; Miyazaki, M.; Stoehr, J. P.; Lan, H.; Kendziorski, C. M.; Yandell, B. S.; Song, Y.; Cohen, P.; Friedman, J. M.; Attie, A. D. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 11482.
15. Ntambi, J. M.; Miyazaki, M. *Prog. Lipid Res.* **2004**, *43*, 91.
16. Miyazaki, M.; Kim, Y. C.; Gray-Keller, M. P.; Attie, A. D.; Ntambi, J. M. *J. Biol. Chem.* **2000**, *275*, 30132.
17. Jiang, G.; Li, Z.; Liu, F.; Ellsworth, K.; Dallas-Yang, Q.; Wu, M.; Ronan, J.; Esau, C.; Murphy, C.; Szalkowski, D.; Bergeron, R.; T.Doebber; Zhang, B. B. *J. Clin. Invest.* **2005**, *115*, 1030.
18. Abreo, M.; Chafeev, M., Chakka, N., Chowdhury, S.; Fu, J.; Gschwend, H. W.; Holladay, M. W.; Hou, D.; Kamboj, R.; Kodumuru, V.; Li, W.; Liu, S.; Raina, V.; Sun, S.; Sun, S.; Sviridov, S.; Tu, C.; Winther, M. D.; Zhang, Z. WO 2005/011655..
19. Fu, J.; Kodumuru, V.; Sun, S.; Winther, M.; Fine, R. M.; Harvey, D. F.; Klebansky, B.; Gray-Keller, M. P.; Gschwend, H. W.; Li, W. US 2005/0119251.
20. Li, C. S.; Ramtohul, Y. K.; Huang, Z.; Lachance, N. WO 2006/130986.
21. Liu, G.; Lynch, J. K.; Freeman, J.; Liu, B.; Xin, Z.; Zhao, H.; Serby, M. D.; Kym, P. R.; Suhar, T. S.; Smith, H. T.; Cao, N.; Yang, R.; Janis, R. S.; Krauser, J. A.; Cepa, S. P.; Beno, D. W. A.; Sham, H.; Collins, C. A.; Surowy, T. K.; Camp, H. S. *J. Med. Chem.* **2007**, *50*, 3086.
22. Zhao, H.; Serby, M. D.; Smith, H. T.; Cao, N.; Suhar, T. S.; Surowy, T. K.; Camp, H. S.; Collins, C. A.; Sham, H.; Liu, G. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 3388.
23. Chiou, S.; Shine, H. J. *J. Heterocycl. Chem.* **1998**, *26*, 125.