Bioorganic & Medicinal Chemistry Letters 20 (2010) 5241–5244

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Hit-to-lead optimization of a series of carboxamides of ethyl 2-amino-4-phenylthiazole-5-carboxylates as novel adenosine A_{2A} receptor antagonists

Anette Graven Sams [a,*], Gitte Kobberøe Mikkelsen [a], Mogens Larsen [a], Lars Torup [b], Lise Tøttrup Brennum [b], Tenna Juul Schrøder [c], Benny Bang-Andersen [a]

[a] Medicinal Chemistry Research, H. Lundbeck A/S, Ottiliavej 9, 2500 Valby, Denmark
[b] In vivo Neuropharmacology, H. Lundbeck A/S, Ottiliavej 9, 2500 Valby, Denmark
[c] Molecular Pharmacology, H. Lundbeck A/S, Ottiliavej 9, 2500 Valby, Denmark

ARTICLE INFO

Article history:
Received 11 June 2010
Revised 25 June 2010
Accepted 29 June 2010
Available online 24 July 2010

Keywords:
A_{2A}
Adenosine
Antagonist
Parkinson's Disease

ABSTRACT

Herein we describe the discovery of a series of novel adenosine A_{2A} receptor antagonists. A successful hit-to-lead optimization of an HTS hit led to replacement of a metabolically labile ester moiety with a heteroaromatic group. A compound from the series, (cyclopropanecarboxylic acid [5-(5-methyl-[1,2,4]oxadiazol-3-yl)-4-phenyl-thiazol-2-yl]-amide, compound **13**), was shown to be effective in reversing haloperidol-induced hypolocomotion, a model of motor dysfunction in Parkinson's Disease.

Adenosine (A) is an important neuromodulator, and its action is mediated via specific receptors, which belong to the family of G-protein coupled receptors. Four adenosine receptors have been cloned and characterized, A_1, A_{2A}, A_{2B} and A_3.[1] The main intracellular signaling pathways involve the formation of cAMP, and signaling through adenosine A_{2A} and A_{2B} receptors leads to activation of adenylate cyclase, whereas signaling through adenosine A_1 and adenosine A_3 receptors leads to inhibition of adenylate cyclase.[2] The adenosine A_{2A} receptor is highly expressed in the striatum, nucleus accumbens, and the olfactory tubercle in humans, while low levels are found in other brain areas. The adenosine A_{2A} receptor is co-expressed with dopamine D_2 receptors in striatum and is involved in the regulation of functional activity of dopamine D_2 receptors, and heterodimerization of A_{2A} and D_2 receptor subtypes inhibit dopamine D_2 receptor function.[3,4] Adenosine A_{2A} receptors have also been shown to modulate the release of GABA in the striatum. By reducing the GABA output, adenosine A_{2A} receptor antagonism helps counteract striatal dopamine depletion and restore normal function in the basal ganglia. Therefore, A_{2A} receptor antagonists may have clinical utility in the treatment of Parkinson's Disease (PD),[3] and extensive work has been done over the past decades to discover potent and selective adenosine A_{2A} receptor antagonists.[5–7] Thus, istradefylline (KW-6002, **1**)[8,9] and preladenant (SCH-420814, **2**)[10] are currently undergoing clinical trials for the treatment of PD.

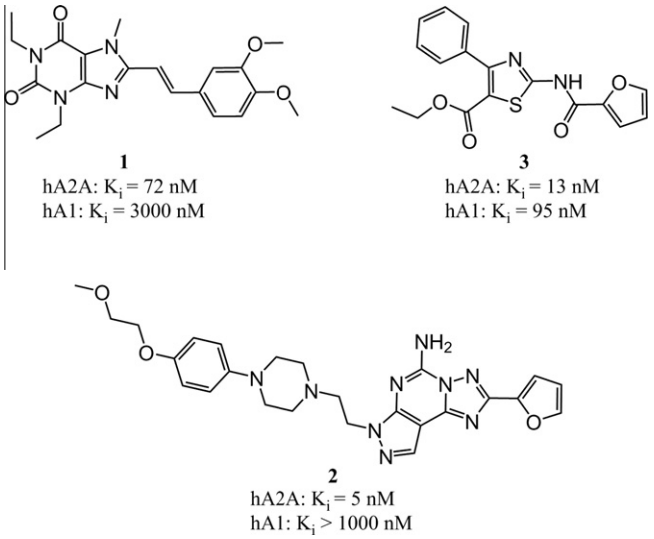
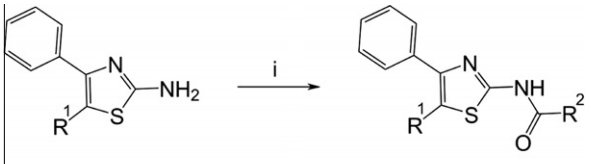


Chart 1. Structures and hA_{2A} and hA_1 binding affinities of istradefylline (KW-6002, **1**), preladenant (SCH-420814, **2**) and HTS hit **3**.

* Corresponding author. Current address: LEO Pharma A/S, Industriparken 55, 2750 Ballerup, Denmark.
E-mail address: anette.sams@leo-pharma.com (A.G. Sams).

0960-894X/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2010.06.138



4a: $R^1 = C(O)^nPr$
b: $R^1 = CH_2OCH_2CH_3$
c: $R^1 = CONHEt$
d: $R^1 = SO_2Et$
e: $R^1 = CN$
f: R^1 = 5-methyl-[1,2,4]oxadiazole-3-yl
g: R^1 = [1,2,4]oxadiazole-3-yl
h: R^1 = 3-methyl-[1,2,4]oxadiazole-3-yl
i: R^1 = 2-ethyl-2*H*-tetrazole-5-yl

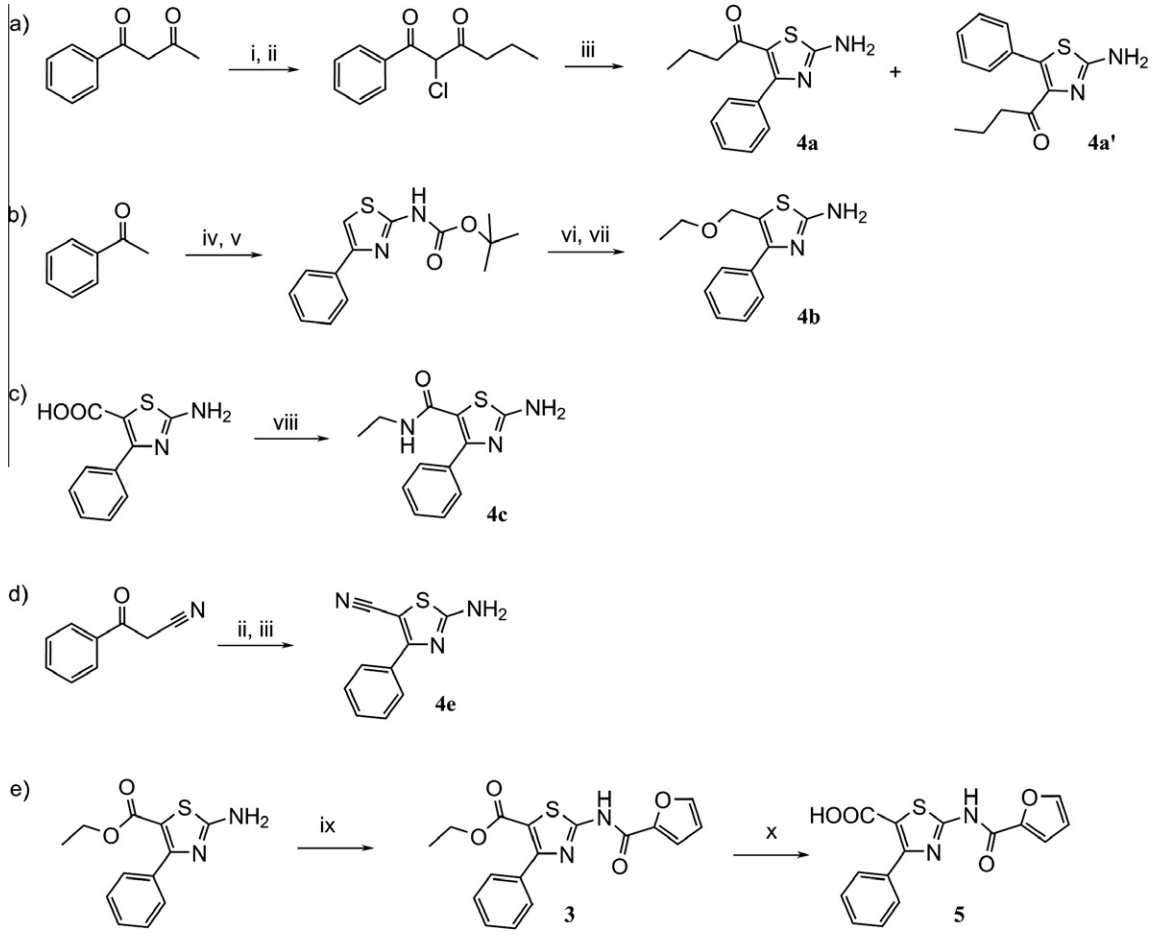
Scheme 1. Reagents: (i) R^2COCl, pyridine, 1,2-dichloroethane.

In our efforts to identify novel A_{2A} receptor antagonists, a screening campaign of a collection of approximately 200,000 compounds was conducted, using a binding assay with [^{3}H] ZM241385 as radioligand and membranes from a commercially available cell line stably expressing rat A_{2A} receptors (MCL-511, PerkinElmer). Among the hits was a series of potent carboxamides of ethyl 2-amino-4-phenylthiazole-5-carboxylate, exemplified by ethyl 2-[(furan-2-carbonyl)-amino]-4-phenylthiazole-5-carboxylate (**3**) (Chart 1). Compound **3** was the most potent among its analogues, binding to the A_{2A} receptor with a K_i value of 13 nM. It had a moderate selectivity toward the hA_1 receptor, as measured by displacement of the radioligand [^{3}H]DPCPX, in membranes from Chinese hamster ovary (CHO) cells stably expressing hA_1 receptors. For comparison, clinically advanced istradefylline (**1**), currently in phase 3 clinical trials, showed a weaker hA_{2A} binding (K_i of 72 nM) and a K_i of 3000 nM for hA_1 receptors. Also, preladenant (**2**), currently in phase 2 clinical trials, displayed a K_i of 5 nM for hA_{2A} receptors and an inhibition of [^{3}H]DPCPX binding of 30% at 2000 nM, indicating a K_i >1000 nM for hA_1 receptors.

The efficacy of **3** as an antagonist at the A_{2A} receptor was verified in a functional cAMP-based assay using AlphaScreen® technology (PerkinElmer) with CHO cells expressing the human A_{2A} receptors, wherein **3** antagonised the effect of the A_{2A} receptor agonist *N*-ethylcarboxamidoadenosine (NECA).

The series of hits represented by **3** constitute a novel structural class of A_{2A} receptor ligands. Previously described A_{2A} receptor antagonists are often classified in two major classes; (1) xanthine like compounds, such as istradefylline (**1**), and (2) the so-called non-xanthine type compounds. Members of the latter classification comprise a structurally diverse group of chemotypes.[5–7] Interestingly, a report on the discovery of a series of 2-amino-5-benzoyl-4-(2-furyl)thiazoles as A_{2A} receptor antagonists, which are also based on the 2-aminothiazole core, recently appeared.[11]

The carboxylic acid ester functionality of **3** made it impossible to test the compound in vivo, as the plasma half-life is extremely

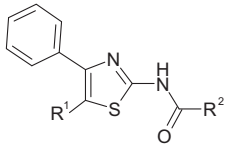


Scheme 2. Reagents and conditions: (i) LDA, EtI, THF, −78 °C, 83%; (ii) SO_2Cl_2, CH_3Cl, 5 °C-reflux; (iii) thiourea, EtOH, reflux; (iv) I_2, thiourea, neat, 100 °C, 60%; (v) Boc_2O, DMF, DMAP, 0–20 °C, 58%; (vi) *n*-BuLi, $ClCH_2OCH_2CH_3$, THF, −78 °C, 33%; (vii) 2 M HCl/diethyl ether, 59%; (viii) EDC, DIPEA, $EtNH_2$, HCl, DMF, 1,2-DCE, 42%; (ix) furan-2-carbonyl chloride, NEt_3, 1,2-DCE, 73%; (x) 2 M NaOH, THF, 60%.

short. Consequently, a hit-to-lead optimization effort was undertaken with the aim of identifying a suitable replacement for the carboxylic acid ester functionality. A parallel synthesis protocol was used to rapidly explore the structure–activity relationship (SAR) of **3**, as outlined in Scheme 1. Hence, 4-phenyl-thiazol-2-ylamines, carrying various substituents at the thiazole 5-position (**4a**–**i**), were acylated with carboxylic acid chlorides in 1,2-dichloroethane (1,2-DCE) in the presence of pyridine. The compounds were purified by preparative HPLC with MS detection. Thus, compounds **6**–**32** were prepared in this manner, whereas compound **5** was prepared from **3** by simple saponification, as shown in Scheme 2. Compound **3** was obtained by acylation of commercially available ethyl 2-amino-4-phenylthiazole-5-carboxylate with furan-2-carboxylic acid chloride in the presence of pyridine. The intermediate **4d** was commercially available. The intermediates **4f**–**i** were synthesised as described in Ref. 12. The preparation of intermediates **4a**–**c**,**e** is outlined in Scheme 2. Thus, treatment of benzoylacetone with lithium diisopropylamide (LDA) followed by ethyl iodide in THF at low temperature, yielded 1-phenyl-hexane-1,3-dione in 83% yield; which was chlorinated with SO_2Cl_2 and then treated with thiourea in refluxing ethanol to afford cyclization and dehydration to yield intermediate **4a**, together with the other isomer **4a**′ in a 1:1 ratio. Separation of the isomers **4a** and **4a**′ proved extremely difficult, and 2% of **4a** was obtained by preparative HPLC purification. For the preparation of intermediate **4b**, 2-amino-4-phenylthiazole was initially obtained in 60% yield from acetophenone, by treatment with thiourea and iodine. Following Boc-protection in 58% yield, the thiazole 5-position was deprotonated with *n*-BuLi at low temperature, and treatment with chloromethyl-ethyl ether (33%) followed by Boc-deprotection in 59% yield, afforded the desired product **4b**. The intermediate **4c** was prepared in 42% yield by a simple amide coupling reaction between commercially available 2-amino-4-phenyl-5-carboxylic acid and ethylamine hydrochloride, in the presence of *N*-ethyl-*N*′-(dimethylaminopropyl)carbodiimide hydrochloride (EDC) and diisopropyl ethyl amine (DIPEA). The intermediate **4e** was prepared in 62% yield from benzoylacetonitrile, by chlorination with SO_2Cl_2 followed by treatment with thiourea in refluxing ethanol.

The affinities of compounds **3**–**32** for hA_{2A} and hA_1 receptors are summarized in Table 1, and expressed as K_i values (nM) or as % inhibition of radioligand binding at 10 μM test concentration. First, the corresponding thiazole-5-carboxylic acid analogue to **3** was shown to be inactive (**5**). We then investigated the relative contribution of either of the oxygen atoms of the R^1 ester group of **3**. Thus, the ethyl ester was first replaced with butan-1-one as R^1 substituent, which led to a slight loss of hA_{2A} receptor affinity and a drop in selectivity versus the hA_1 receptor (**6**), whereas replacing the ethyl ester by a ethyl-methylene ether as the R^1 substituent gave rise to a compound **7** which was selective for the hA_1 receptor, and with 10-fold less affinity for the hA_{2A} receptor when compared to **3**. Hence, the carbonyl oxygen appeared to make the most important contribution to the affinity of **3** toward the hA_{2A} receptor. Replacing the ethyl ester of **3** with ethyl amide or ethyl sulfone at the R^1 position led to a complete loss of affinity at hA_{2A} receptors (**8**, **9**), and replacement by a cyano group led to a substantial loss in hA_{2A} receptor affinity (**10**). We also examined the effect of replac-

Table 1
Chemical structures and binding affinities[a] at hA_{2A} and hA_1 receptors of carboxamides of 2-amino-4-phenyl-thiazoles



Compound	R^1	R^2	hA_{2A}	hA_1
3	CO_2Et	2-Furanyl	13	95
5	COOH	2-Furanyl	0%	n.d.[b]
6	$C(O)^nPr$	2-Furanyl	62	90
7	$CH_2OCH_2CH_3$	2-Furanyl	190	92
8	CONHEt	2-Furanyl	37%	n.d.
9	SO_2Et	2-Furanyl	25%	n.d.
10	CN	2-Furanyl	290	n.d.
11	5-Methyl-[1,2,4]oxadiazole-3-yl	2-Furanyl	700	780
12		Methyl	190	76%
13		Cyclopropyl	28	150
14		Isobutyl	33	82
15		Phenyl	83	45%
16		Benzyl	25	260
17	[1,2,4]Oxadiazole-3-yl	2-Furanyl	410	230
18		Cyclopropyl	120	310
19		Isobutyl	340	77
20		Phenyl	330	300
21		Benzyl	710	1700
22	3-Methyl-[1,2,4]oxadiazole-5-yl	2-Furanyl	200	270
23		Methyl	110	370
24		Cyclopropyl	12	41
25		Isobutyl	48	27
26		Phenyl	190	180
27		Benzyl	57	340
28	2-Ethyl-2*H*-tetrazole-5-yl	2-Furanyl	94	650
29		Cyclopropyl	77	210
30		Phenyl	220	65%
31		Benzyl	460	1200
32		3,4-Dimethoxy-benzyl	45	1600

[a] Expressed as K_i values (nM) or displacement percentage of radioligand at 10 μM test concentration were indicated.
[b] Not determined.

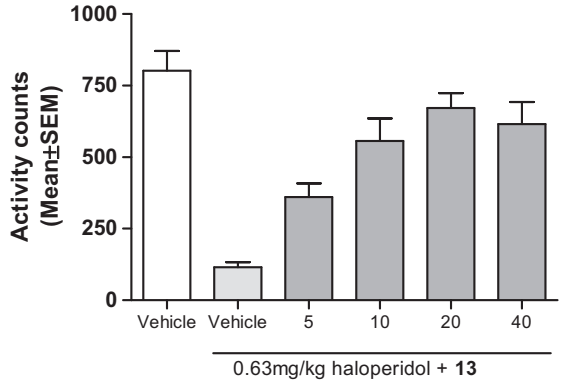


Figure 1. Dose-response of compound **13** in a mouse haloperidol-induced hypolocomotion model of Parkinson's Disease. ED_{50} = 7 mg/kg

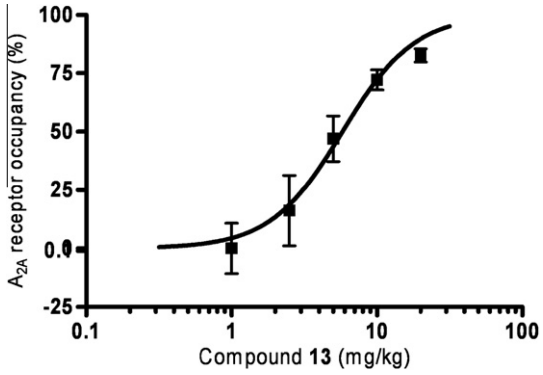


Figure 2. Dose response of displacement of [^{3}H]SCH-442416 by compound **13** in vivo in mice. ED_{50} = 5.8 mg/kg

ing the ethyl ester with a range of five-membered heteroaromatic groups as R^1 substituents. Thus, potent compounds were obtained with R^1 being 5-methyl-[1,2,4]oxadiazole-3-yl or the isomeric 3-methyl-[1,2,4]oxadiazole-5-yl, and for these R^1 substituents, R^2 = cyclopropyl (**13**, **24**), isobutyl (**14**), or benzyl (**16**) yielded compounds with hA_{2A} receptor affinities in the range of **3**. Also, in the case of **13** and **16**, the selectivity toward the hA_1 receptor was comparable to that of **3**. In contrast, the removal of the methyl substituent from the [1,2,4]oxadiazole-3-yl core of these compounds, led to a consistent drop in the hA_{2A} receptor affinities (**17**–**21**). Using 2-ethyl-2*H*-tetrazole-5-yl as the R^1 substituent generally led to less potent compounds, however, a 3,4-dimethoxybenzyl group as the R^2 substituent led to a compound with just fivefold weaker hA_{2A} receptor affinity compared to **3**, yet with an improved selectivity versus the hA_1 receptor (**32**). Hence, **32** show an in vitro receptor profile similar to that of istradefylline (**1**).

Interestingly, the SAR for the R^2 substituent was not parallel for the compounds with a heteroaromatic group as the R^1 substituent compared to those with ethyl ester as the R^1 substituent. Thus, in the heteroaromatic series, R^2 = furan-2-yl did not yield the most potent compounds (**3** vs **11**, **17**, **22**), instead, R^2 = cyclopropyl generally gave the most potent compounds in series defined by heteroaromatic R^1 substituents.

We early on selected compound **13** as a suitable prototype compound to evaluate the in vivo efficacy of this structurally novel series of A_{2A} receptor antagonists, and tested the compound in a mouse haloperidol-induced hypolocomotion model of Parkinson's Disease. In this model, a hypolocomotive state is induced in mice by pre-treatment with the D_2 antagonist haloperidol. Compound **13** dose dependently and fully reversed the hypolocomotive state after po administration, with an ED_{50} value of 7 mg/kg (Fig. 1). For comparison, the two reference compounds istradefylline (**1**) and preladenant (**2**) reversed the hypolocomotive state in the same model with ED_{50} values of 0.13 mg/kg and 0.4 mg/kg, respectively. To verify that the observed behavior was correlated to blockade of A_{2A} receptors, an in vivo binding experiment was performed. The A_{2A} selective ligand [^{3}H]SCH-442416 was dosed to mice iv and the displacement of the radioligand after po administration of compound **13** was measured. Administration of compound **13** dose-dependently displaced [^{3}H]SCH-442416 with an ED_{50} of 5.8 mg/kg (Fig. 2). This value corresponds well to the ED_{50} observed in haloperidol-induced hypolocomotion, suggesting blockade of A_{2A} receptors to be the mechanism of action of the reversal of the haloperidol-induced hypolocomotive state.

In conclusion, a hit-to-lead optimization of a screening hit **3** resulted in the identification of a novel series of A_{2A} receptor antagonists with in vivo efficacy. A compound **13** from the series was selected and was shown to have effect in a model of PD, and to displace a selective A_{2A} receptor radioligand in an in vivo binding experiment with a similar ED_{50} value. The optimization of the hit **3** into a lead series involved the identification of heteroaromatic replacements for the ester functionality of **3**. Thus, incorporation of different heteroaromatics as the R^1 substituent gave compounds with affinities at the hA_{2A} receptor in the same range as the original hit. Interestingly, the observed SAR with respect to the R^2 substituent in the series incorporating heteroaromatic R^1 substituents was not parallel to that in the original series, wherein R^1 = ethyl ester. As a general tendency, the compounds display low selectivity toward the hA_1 receptor, however, it was possible to identify compounds with selectivity over the hA_1 receptor in the same range as istradefylline (**1**).

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.06.138.

References and notes

1. Fredholm, B.-B.; Abbracchio, M. P.; Burnstock, G.; Daly, J. W.; Harden, T. K.; Jacobson, K. A.; Leff, P.; Williams, M. *Pharmacol. Rev.* **1994**, *46*, 143.
2. Olah, M. E.; Stiles, G. L. *Pharmacol. Ther.* **2000**, *85*, 55.
3. Xu, K.; Bastia, E.; Schwarzschild, M. *Pharmacol. Ther.* **2005**, *105*, 267.
4. Simola, N.; Morelli, M.; Pinna, A. *Curr. Pharm. Des.* **2008**, *14*, 1475.
5. Baraldi, P. G.; Tabrizi, M. A.; Gessi, S.; Borea, A. P. *Chem. Rev.* **2008**, *108*, 238.
6. Yuzlenko, O.; Kiec-Kononowicz, K. *Curr. Med. Chem.* **2006**, *13*, 3609.
7. Moro, S.; Gao, Z.-G.; Jacobson, K. A.; Spalluto, G. *Med. Res. Rev.* **2006**, *26*, 131.
8. Jenner, P. *Expert Opin. Invest. Drugs* **2005**, *14*, 729.
9. Hockemeyer, J.; Burbiel, J. C.; Müller, C. E. *J. Org. Chem.* **2004**, *69*, 3308.
10. Neustadt, B. R.; Hao, J.; Lindo, N.; Greenlee, W. J.; Stamford, A. W.; Tulshian, D.; Ongini, E.; Hunter, J.; Monopoli, A.; Bertorelli, R.; Foster, C.; Arik, L.; Lachowicz, J.; Ng, K.; Feng, K.-I. *Bioorg. Med. Chem. Lett.* **2006**, *17*, 1376.
11. Cole, A. G.; Stauffer, T. M.; Rokosz, L. L.; Metzger, A.; Dillard, L. W.; Zeng, W.; Henderson, I. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 378.
12. Larsen, M, Sams, A. G., Mikkelsen, G. K., Bang-Andersen, B. WO2006/032273, 2006.