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PII: S0960-894X(15)00083-9
DOI: http://dx.doi.org/10.1016/j.bmcl.2015.01.062
Reference: BMCL 22402

To appear in: *Bioorganic & Medicinal Chemistry Letters*

Received Date: 18 December 2014
Revised Date: 26 January 2015
Accepted Date: 27 January 2015

Please cite this article as: Mikkelsen, G.K., Langgård, M., Schrøder, T.J., Kreilgaard, M., Jørgensen, E.B., Brandt, G., Griffon, Y., Boffey, R., Bang-Andersen, B., Synthesis and SAR studies of analogues of 4-(3,3-dimethyl-butyrylamino)-3,5-difluoro-*N*-thiazol-2-yl-benzamide (Lu AA41063) as adenosine A_{2A} receptor ligands with improved aqueous solubility, *Bioorganic & Medicinal Chemistry Letters* (2015), doi: http://dx.doi.org/10.1016/j.bmcl.2015.01.062

Synthesis and SAR studies of analogues of 4-(3,3-dimethyl-butyrylamino)-3,5-difluoro-*N*-thiazol-2-yl-benzamide (Lu AA41063) as adenosine A_{2A} receptor ligands with improved aqueous solubility

Gitte Kobberøe Mikkelsen[a], Morten Langgård[b] , Tenna Juul Schrøder[c], Mads Kreilgaard[a], Erling B. Jørgensen[d], Guillaume Brandt[e], Yann Griffon[e], Ray Boffey[e] and Benny Bang-Andersen[a]

[a]*Departments of Discovery Chemistry & DMPK,* [b]*Computational Chemistry,* [c]*Molecular Pharmacology, and* [d]*Biologics & Pharmaceutical Science Department, H. Lundbeck A/S, 9 Ottiliavej, DK-2500 Valby, Denmark;* [e]*Argenta Discovery Ltd, 8-9 The Spire Green Centre Harlow CM19 5TR, UK*

* Corresponding author. Gitte Kobberøe Mikkelsen, H. Lundbeck A/S, Ottiliavej 9, 2500 Valby, Denmark. Email: gmi@lundbeck.com.

Abstract:

An adenosine A_{2A} receptor antagonist may be useful for the treatment of Parkinson's disease. Synthesis and structure-activity studies starting from (4-(3,3-dimethylbutanamido)-3,5-difluoro-*N*-(thiazol-2-yl)benzamide (Lu AA41063, **4**) led to a novel series of human (h) A_{2A} receptor antagonists with improved aqueous solubility. Compound **22** was identified as a key representative from the series, displaying submicromolar hA_{2A} receptor affinity and excellent aqueous solubility. Compound **22** also displayed good in vitro pharmacokinetic properties and is considered a good starting point for further lead optimisation toward hA_{2A} receptor antagonists with improved druggability properties.

Keywords: hA_{2A}, solubility

The neurotransmitter adenosine interacts with the four seven transmembrane G protein-coupled receptors A_1, A_{2A}, A_{2B} and A_3.[1] The adenosine receptors are located in several tissues, including the central nervous system (CNS)[2] and adenosine A_{2A} receptors are predominantly expressed in the dopamine rich areas of the CNS such as the striatum. Currently, the interactions between A_{2A} and D_2 receptors are of great interest in the treatment for Parkinson´s disease (PD), which involves a decrease in dopamine levels. The A_{2A} receptors interact tonically and antagonistically with the D_2 receptors, causing a decrease in affinity of the D_2 receptors for dopamine upon stimulation. Thus, an A_{2A} receptor antagonist may be useful as a monotherapy for the treatment of PD. Alternatively, A_{2A} receptor antagonists may be capable of enhancing the effect of clinically used dopamine receptor agonists for example by increase the time-period of dopaminergic drug response.[3,4]
There has been an extensive effort over the past decade to synthesise novel and selective A_{2A} receptor antagonists,[5, 6] and recently istradefylline (KW-6002, **1**)[7] was launched under the name Nouriast® as the first antiparkinsonian agent based on A_{2A} receptor antagonism. Other companies such as Vernalis (i.e. V-81444 (**2**)) and Roche currently have A_{2A} receptor antagonists in Phase II clinical trials.

((Figure 1, single-column width))

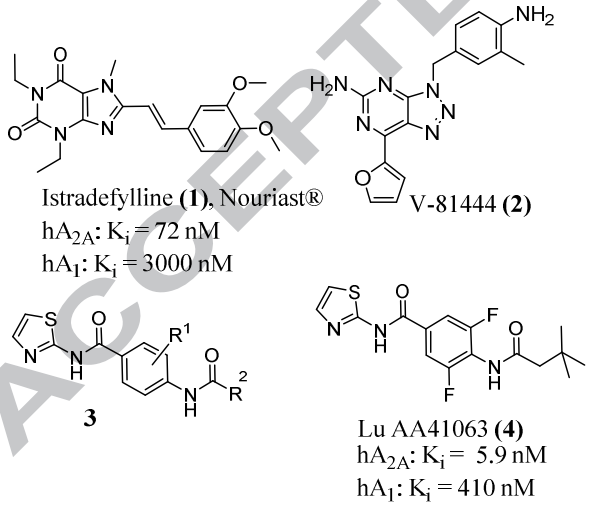


Figure 1. Structures and human (h) A_{2A} and hA_1 receptor binding affinities of istradefylline (KW-6002, **1**), V-81444 (**2**) from Vernalis, and Lu AA41063 (**4**) that is derived from general structure **3**.[9]

Common for many A_{2A} receptor antagonists are their planar aromatic structure, which often results in low aqueous solubility. This has complicated the development of drugs for this target in general. Several groups have reported different attempts to improve the aqueous solubility of A_{2A} receptor antagonists.[13, 14]

We have previously reported a new class of potent and selective human (h) A_{2A} receptor antagonists of the general structure **3** (Figure 1).[9] Among these compounds, Lu AA41063 (**4**) and its water soluble prodrug was identified as a highly selective hA_{2A} receptor antagonist with effect in an *in vivo* model of PD. However, this series of compounds in general suffer from poor aqueous solubility, which complicated the solid dose formulation.[9] For example the aqueous solubility of compound **4** is 1 µg/mL at pH 7.4. To avoid the complications of prodrug formulation we explored the possibilities to significantly improve the aqueous solubility of compounds based on the general structure of **3**.

We set out to investigate the structure-activity and structure-solubility relationships within general structure **3**, in order to identify space that would allow for the introduction of solubilising groups at positions with only limited effect on hA_{2A} receptor affinity.

From previously disclosed SAR within this series it was known that modifications around the aminothiazole were not tolerated.[9] An in-house homology model of the A2A receptor based on the published experimental model 1UPE (PDB code) was used to rationalize the observation. Figure 2 shows compound **4** docked into the later published crystal structure PDB: 3EML structure. This model confirmed our hypothesis that the aminothiazole is orientated inwards not allowing substitution and the alkyl chain pointing in the direction of the extracellular domain.

((Figure 2, single-column width))

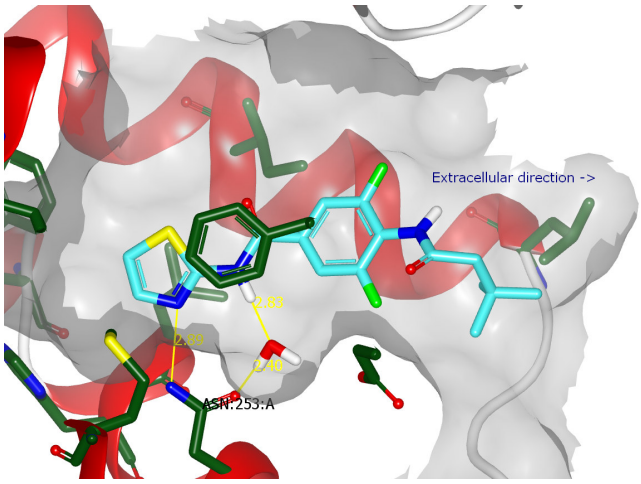


Figure 2. Docking of compound **4** docked into the later published crystal structure PDB: 3EML structure

Firstly, taking compounds **5a** and **5b** (Figure 3) as starting points for our effort , we investigated the effect of breaking the planarity of general structure **3** by reducing the carbonyl groups to methylene linkers. The replacement of the thiazolyl carbonyl moiety in compound **5a** with a methylene spacer (compound **6**) resulted in complete loss of hA_{2A} receptor affinity. Replacement of the other carbonyl group in compound **5b** with a methylene spacer resulted in compound **7** with a substantially weaker hA_{2A} receptor affinity when compared to compound **5b**.

Introducing a nitrogen atom at the 2- or 3-position of the central benzene ring led to compounds **8** and **9**. Compound **9** was inactive at the hA_{2A} receptor, while compound **8** lost a factor of 10 when compared to **5a**. This could be an acceptable starting point if the aqueous solubility would have increased significantly. However, the aqueous solubility of compound **8** was only 10 µg/mL and this was considered to be insufficient for a starting point for further optimization. Introduction of a hydroxy group in the neo-pentyl group (compound **10**) resulted in significant loss of hA_{2A} receptor affinity compared to compound **5a,b**.

((Figure 3 , double-column width))

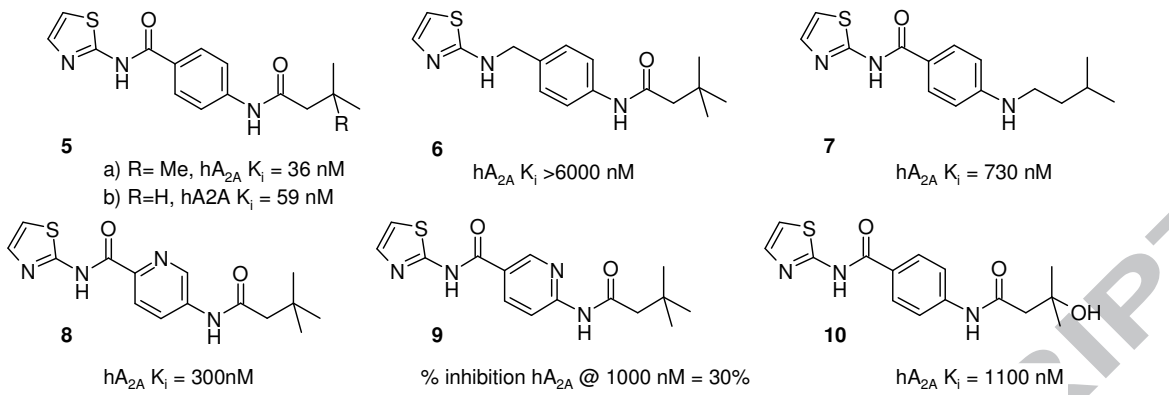


Figure 3. Structures human (h) A_{2A} receptor binding affinities for compounds **5a** and **5b** and structural modifications thereof.

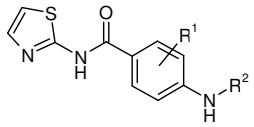
Next we turned to examine the effect of introducing a more basic nitrogen atom in the right hand side amide alkyl chain (Table 1). Previous SAR investigations had taught us that the β-branching of the alkyl chain was necessary to achieve high affinity for the hA_{2A} receptor.[9] Replacing the β-carbon atom in **11** by a nitrogen atom resulted in a complete loss of hA_{2A} receptor affinity (compound **12**). Similarly, replacing one of the carbon atoms in the cyclopentane ring of **13** by a nitrogen atom led to significant reduction of hA_{2A} receptor affinity (compounds **14** and **15**). The docked pose of **4** (Figure 2) suggests that to reach the solvent exposed region of the receptor, a longer linking group would be needed between the core and the polar moieties. Therefore we extended the R^2 alkyl group in order to probe if a basic nitrogen atom could be introduced at a more distant position from the central benzene ring, using non-β-branched model systems to probe this hypothesis. In short, replacing the terminal carbon atom in compound **16** with a nitrogen atom resulted in complete loss of affinity to the hA_{2A} receptor (compound **17**), whereas the homologue **18** with a chain of four carbon atoms between the carbonyl and the basic nitrogen atom displayed a hA_{2A} receptor affinity of 830 nM. Compounds with even longer carbon chains of five and seven carbon atoms between the carbonyl and the nitrogen atom showed increasing affinity for the hA_{2A} receptor (compounds **19** and **20**).

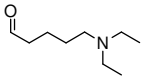
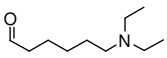
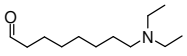
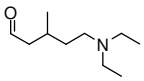
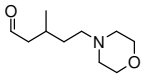
Reintroducing a methyl group at the β-position of compound **18** resulted in compound **21**, which showed a 7-fold improvement in hA_{2A} receptor affinity when compared to **18**. Compound **21** showed submicromolar hA_{2A} receptor affinity (K_i = 110 nM). Restricting the

terminal ethyl groups in a morpholine ring resulted in the equipotent compound **22**, which had a good selectivity toward the hA_1 receptor (IC_{50} > 2000 nM).

((Table 1, single-column width))

Table 1. Compound **5a** and right hand side analogues.



Cpd	R^1	R^2	A_{2A} K_i (nM) or %inhib.[a]
5a	H		36
11	3-Me		67
12	3-Me		1%
13	H		67
14	H		6600
15	H		>2100
16	H		190
17	H		-18%
18	H		830
19	H		240
20	H		130
21	H		110
22	H		110

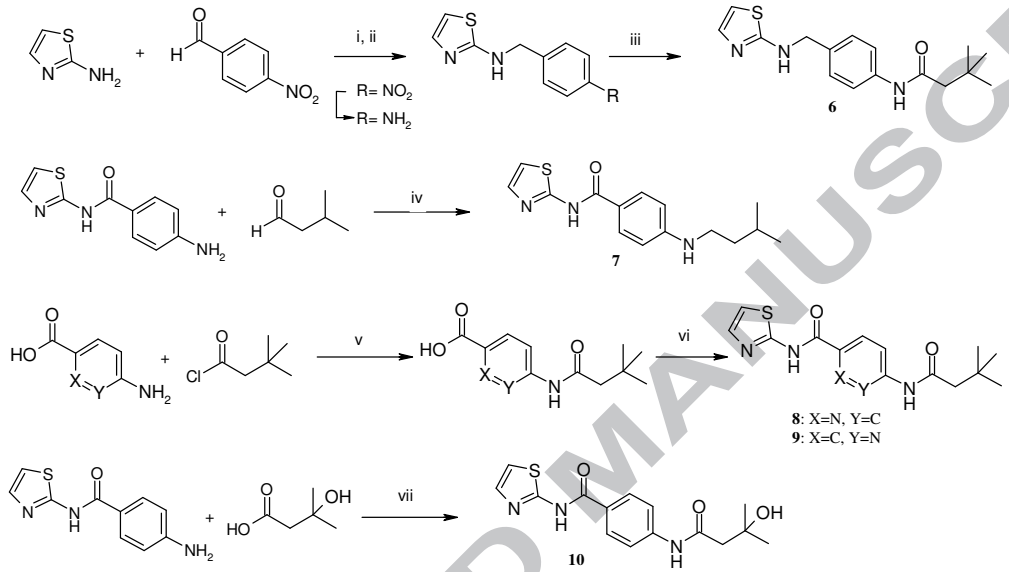
[a]Expressed as K_i values (nM) or percentage displacement of radioligand at 1000 nM test concentration where indicated.

Compound **22** showed no significant inhibition of CYP1A2, CYP2C9, CYP2C19 and CYP3A4 liver enzymes (IC_{50} > 40 µM) as well as CYP2D6 (IC_{50} = 18 µM), and displayed low in vitro clearance in human liver microsomes (hCl_{int} = 0.62 L/kg/h). The aqueous solubility at pH=7.4 of compound **22** was significantly higher (116 µg/mL) than for Lu AA41063 (**4**) (1 µg/mL). Consequently this compound was considered the overall best

compound of the series and a good starting point for further investigations towards druggable hA_{2A} receptor antagonists.

Compound **5a, 5b, 11, 13** and **16** were synthesised as previously described[9, 11].

((Scheme 1, double-column width))



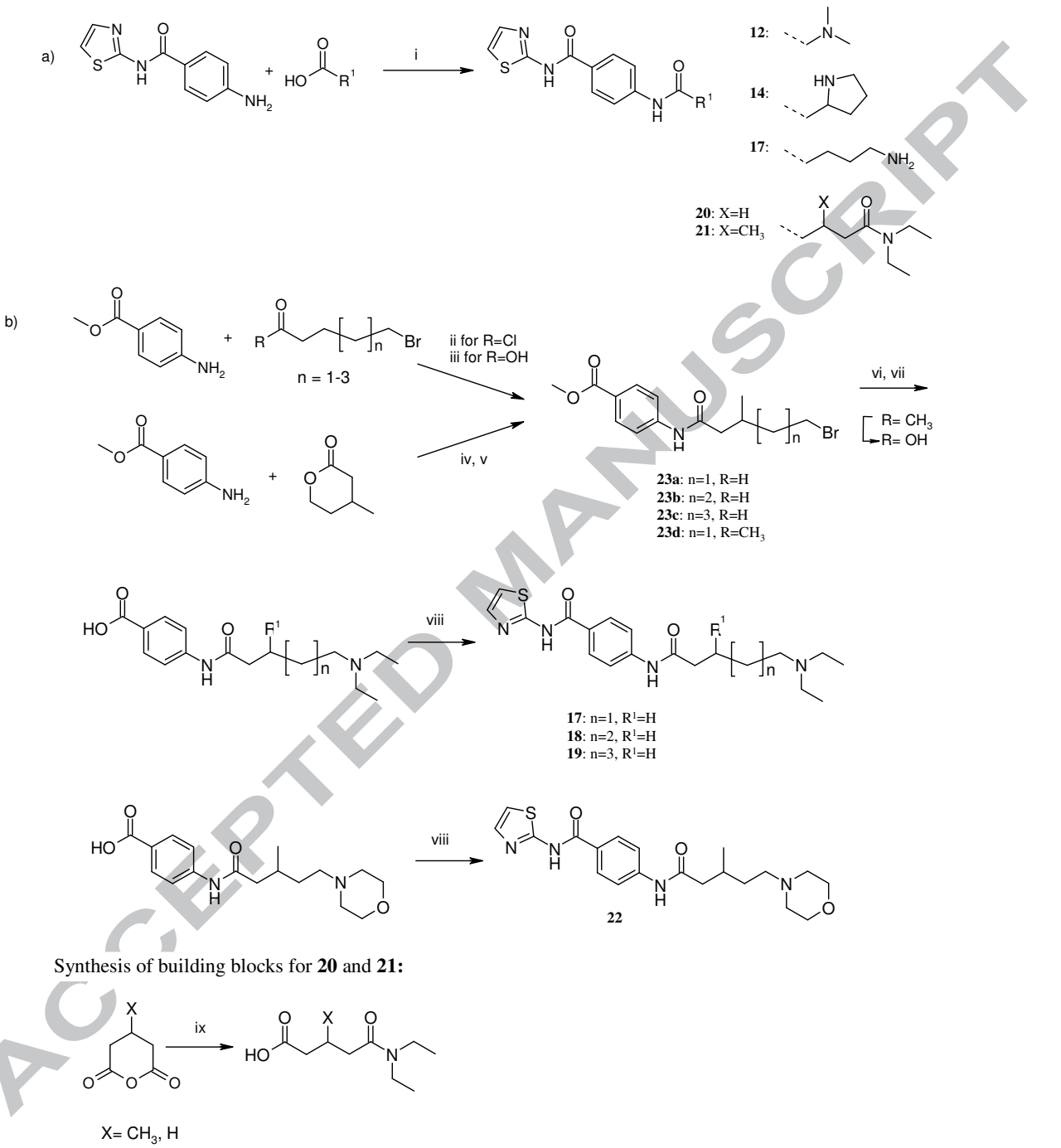
Scheme 1. Reaction conditions: (i) $NaBH(OAc)_3$, AcOH, 1,2-DCE; (ii) Zn, EtOH, HCl, 40°C; (iii) 3,3-dimethyl-butyryl chloride, pyridine, 0° to r.t.; (iv) $NaBH(OAc)_3$, AcOH, 1,2-DCE, DMSO; (v) pyridine, 0° to r.t.; (vi) $(COCl)_2$, 2-aminothiazole, pyridine 1,2-DCE, DMF; (vii) HATU, DIPEA, DMF, r.t.

Compounds **6-10** were synthesised in the following manner (Scheme 1). Reductive amination of 4-nitro-benzaldehyde with 2-aminothiazole, followed by reduction of the nitro group and acylation using 3,3-dimethyl-butyryl chloride gave compound **6**.

Compound **7** was synthesised by reductive amination of 3-methyl-butyraldehyde with 4-amino-*N*-thiazol-2-yl-benzamide.[9] Acylation of 5-amino-pyridine-2-carboxylic acid or 6-amino-nicotinic acid with 3,3-dimethyl-butyryl chloride followed by treatment with oxalylchloride and subsequent reaction with 2-aminothiazole gave the compounds **8** and **9**.

Coupling of 4-amino-*N*-thiazol-2-yl-benzamide[9] with 3-hydroxy-3-methyl-butyric acid gave compound **10**.

((Scheme 2, double-column width))



Synthesis of building blocks for **20** and **21:**

Scheme 2. Reaction conditions: (i) For compound **12**, **14**, **16**: DMF, HATU, *N,N*-diisopropylethylamine, overnight, room temperature followed by treatment with TFA for boc protected compounds. For compound **20**, **21**: DMF, EDC, *N,N*-diisopropylethylamine, 60°C, 3 days (ii) NEt_3, DCM, 0°C to room temperature; (iii) DCM, EDCI, DMAP, room temperature; (iv) $AlCl_3$, DCE (v) CBr_4, polystyrene supported PPh_3, DCM ; (vi) CH_3CN, KI, K_2CO_3, diethylamine or morpholine, reflux, overnight; (vii) THF, H_2O, $LiOH{\cdot}H_2O$, room

temperature overnight; or 1M LiOH in methanol, room temperature, overnight; (viii) DCM, EDCI, DMAP, 2-aminothiazole, room temperature; ix: THF, Et_2N, r.t.

Compounds **12, 14** and **17-22** were synthesised in the following manner (Scheme 2). The basic nitrogen atom in the amide alkyl chain was introduced by different methods. Compound **12, 14**, **17, 20** and **21** were synthesised by coupling of 4-amino-*N*-thiazol-2-yl-benzamide[10] with carboxylic acids. The boc group was removed from amino acids that contained a boc group, after the coupling to 4-amino-*N*-thiazol-2-yl-benzamide. Intermediate **23a, 23b** and **23c** were synthesised by acylation of 4-amino-benzoic acid methyl ester using acid chlorides or by coupling with carboxylic acids. The intermediate **23d** was synthesized by treatment of 4-amino-benzoic acid methyl ester with $AlCl_3$ and 4-methyl-tetrahydro-pyran-2-one. The formed alcohol was converted to the intermediate bromide **23d** by treatment with CBr_4 and polystyrene supported triphenylphosphine in DCM. The compounds **17-19** and **22** were obtained by alkylation of the intermediates **23a**, **23b**, **23c** and **23d** with diethylamine or morpholine followed by hydrolysis of the methyl ester using LiOH in THF/water, the final coupling with 2-aminothiazole gave the compounds **17-19** and **22**. The building blocks for synthesis of **20** and **21** were synthesised by reaction of glutaric anhydride or 3-methylglutaric anhydride with diethylamine in THF at room temperature.

In summary, synthesis and structure-activity and -solubility relationship studies of analogues of Lu AA41063 (**4**) have resulted in the identification of a series of new adenosine A_{2A} receptor ligands, including specifically compound **22** that contain a basic nitrogen atom in the amide alkyl chain of the of 4-carboxamido-*N*-thiazol-2-yl-benzamide scaffold. Compound **22** showed submicromolar hA_{2A} receptor affinity, good selectivity toward the hA_1 receptor, as well as good in vitro microsomal stability. Furthermore, compound **22** was devoid of any CYP enzyme inhibition, and it showed significant better aqueous solubility than **4**. Compound **22** is considered a good starting point for further optimisation toward druggable A_{2A} receptor antagonists.

Acknowlegdement

Thanks to Anette Graven Sams for fruitfull medchem discussions.

Abbreviations

HATU, 2-(1H-7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate
EDC, N-ethyl-N'-dimethylaminopropyl-carbodiimide hydrochloride,
HOBt, 1-hydroxy-benzotriazole
DMAP 4-dimethylaminopyridine
DMF, dimethylformamide
DMSO dimethyl sulfoxide
1,2-DCE 1,2-dichloroethane
AcOH acetic acid

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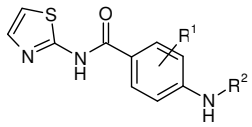
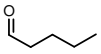
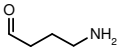
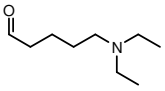
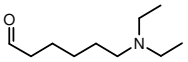
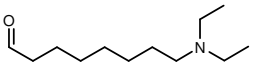
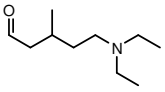
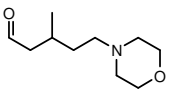
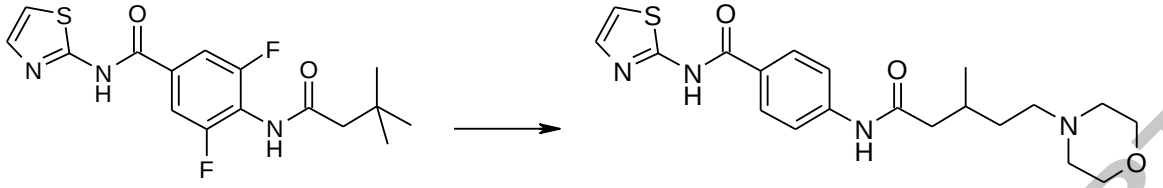
Table 1. Compound **5a** and right hand side analogues.



Cpd	R^1	R^2	A_{2A} K_i (nM) or %inhib.[a]
5a	H		36
11	3-Me		67
12	3-Me		1%
13	H		67
14	H		6600
15	H		>2100
16	H		190
17	H		-18%
18	H		830
19	H		240
20	H		130
21	H		110
22	H		110

[a]Expressed as K_i values (nM) or percentage displacement of radioligand at 1000 nM test concentration where indicated.

Graphical abstract:



Lu AA41063 **(4)**

hA_{2A}: K_i = 5.9 nM
aq. solubility @ pH 7.4 = 1μg/mL

22

hA_{2A}: K_i = 110 nM
aq. solubility @ pH 7.4 =116 μg/mL