

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
3 March 2005 (03.03.2005)

PCT

(10) International Publication Number
WO 2005/018532 A2

- (51) International Patent Classification⁷: **A61K**
- (21) International Application Number:
PCT/SE2004/001224
- (22) International Filing Date: 25 August 2004 (25.08.2004)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:
60/497,312 25 August 2003 (25.08.2003) US

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

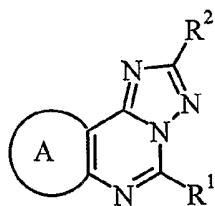
- (71) Applicant (for all designated States except US): **ACTAR AB** [SE/SE]; Franzéngatan 6, S-112 51 Stockholm (SE).
- (72) Inventors; and
- (75) Inventors/Applicants (for US only): **WESTMAN, Jacob** [SE/SE]; Ålands-Västerby, S-740 20 VÄNGE (SE). **NORSTEDT, Gunnar** [SE/SE]; Författarvägen 46, S-16142 BROMMA (SE).
- (74) Agent: **DR LUDWIG BRANN PATENTBYRÅ AB**; P.O.Box 17192, S-104 62 Stockholm (SE).

Published:

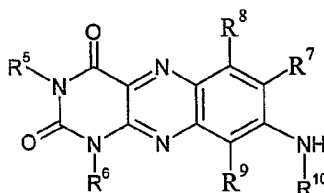
— without international search report and to be republished upon receipt of that report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

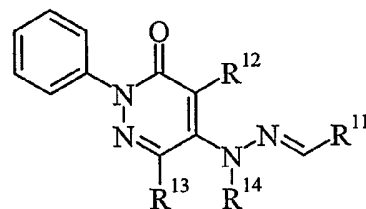
(54) Title: PURINE RECEPTOR BINDING COMPOUNDS



(I)



(II)



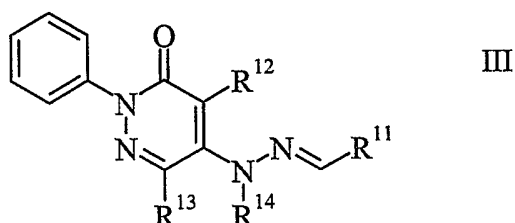
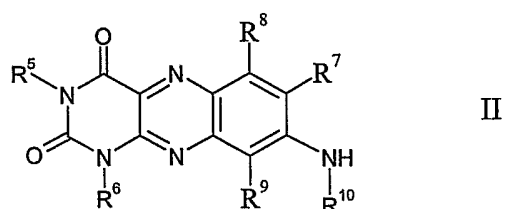
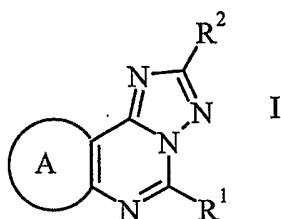
(III)

(57) Abstract: Compounds of formula (I) or a pharmaceutically acceptable salt or prodrug thereof are provided as well as methods of preparation thereof. The use of said compounds as well as of compounds of formulae (II) or (III) in the treatment of disorders mediated by purine receptors.

Purine receptor binding compounds

Field of the invention

The present invention relates to compounds of formula (I), (II) or (III)



or a pharmaceutically acceptable salt or prodrug thereof, wherein A, R¹-R¹⁴, are as defined in the specification.

Compounds of formulae I-III have good affinity and selectivity towards purine receptors, in particular towards the A_{2A}-G_{o1f} complex or the A_{2A}-G_s complex and they act either as agonist or antagonist and can therefore be used for the treatment or prevention of diseases mediated by these receptors.

Background of the invention

Adenosine modulates a wide range of physiological functions by interacting with specific cell surface receptors. The potential of adenosine receptors as drug targets have been known for more than 20 years. There are presently four known receptors for adenosine, which are classified as A₁, A_{2A}, A_{2B} and A₃. All these different receptors show different biological response upon activation or inhibition.

The adenosine A_{2A} receptor belongs to the 7-TM G protein coupled receptor (GPCR) family. When ligands bind and activate these receptors, this induces a change in the conformation of the associated G-protein that in turn influences different second messengers such as cAMP. In this sequence of events the G-protein is essential and it is known that different forms of G-proteins activate numerous biochemical response pathways.

It is well known that the A_{2A} receptor is coupled to G_s and more recently it has been shown that the A_{2A} receptor is also coupled to G_{olf}. The latter is of interest because G_{olf} is expressed at markedly different levels in different tissues. A significant finding in our previous investigations is that G_{olf} is the partner of A_{2A} and present with high density in basal ganglia, and notably in areas where dopamine (DA) transmission is of pathophysiological significance.¹ Evidence suggests that agonists or antagonists of the A_{2A} receptor modulate DA transmission in such a way that antagonists of A_{2A} increase the tone of DA. This is of relevance because DA deficiency is associated to several disorders, notably Parkinson's disease but also other types of chronic CNS disorders such as schizophrenia. In the peripheral system, A_{2A} receptors are present on numerous tissues including platelets, lymphocytes, neutrophils, monocytes, macrophages and mast cells but then coupled to G_s protein.

Given that A_{2A} receptors are present in many different locations and induce several different responses it may be concluded that mere selectivity towards A_{2A} compared to other adenosine receptors may be insufficient to ensure adequate functional selectivity. A truly efficient drug molecule, in addition to having affinity towards A_{2A}, must also be specific towards the different G proteins of interest associated to A_{2A}.

Summary of the invention

According to one aspect, the object of the present invention is to provide new compounds represented by scaffolds with the formula (I) or pharmaceutically acceptable salts or prodrugs thereof.

According to another aspect, the present invention provides a method of preparing said compounds.

According to a further aspect the present invention provides the use of compounds of the formulae I-III as pharmaceutical compositions.

According to a yet further aspect, the invention provides new pharmaceutical compositions comprising said compounds of formulae I-III, or salts or prodrugs thereof.

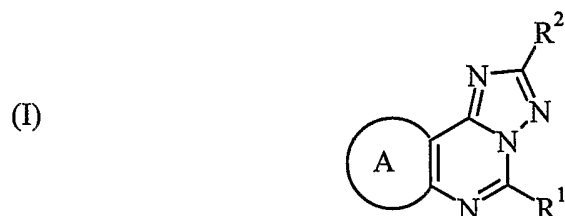
According to still a further aspect the invention provides a method of medical treatment by use of said compounds of formulae I-III.

According to one aspect, the invention provides the use of the inventive compounds, or salts or prodrugs thereof in the manufacture of a medicament for the treatment or prevention of a disorder by blocking of purine receptors, particularly adenosine receptors and more particularly A_{2A} receptors.

Further aspects are as defined in the claims.

Detailed description of the invention

According to a first aspect the present invention provides A compound of formula (I)



or a pharmaceutically acceptable salt or prodrug thereof, wherein

A is a 5- or 6-membered aryl or heteroaryl, optionally substituted with one or more groups selected from unbranched or branched, C1 to C10, more preferably C1 to C6, most preferably C1 to C5 acyclic saturated or unsaturated hydrocarbyl, or C3 to C12, more preferably C5 to C10, most preferably C5 to C7 cyclic saturated or unsaturated hydrocarbyl, optionally substituted with one or more groups Y; halogen; protected or unprotected hydroxy; thio, amino, or carboxyl groups; C6-C14 aryl, optionally substituted with one or more groups Y; 5- to 14-membered heteroaryl with one or more heteroatoms, optionally substituted with one or more

groups Y; 5- to 14-membered non-aromatic heterocycles having one or more heteroatoms, optionally substituted with one or more groups Y;

R^1 represents H; halogen; C1 to C10, more preferably C1 to C6, most preferably C1 to C5 acyclic, or C3 to C12, more preferably C5 to C10, most preferably C5 to C7 cyclic, saturated or unsaturated hydrocarbyl; C6-C14 aryl, optionally substituted with one or more groups Y; 5- to 14-membered heteroaryl having one or more heteroatoms, unsubstituted or substituted with one or more groups Y, C6-C14 aryl; ; 5- to 14-membered non-aromatic heterocycles having one or more heteroatoms, optionally substituted with one or more groups Y;

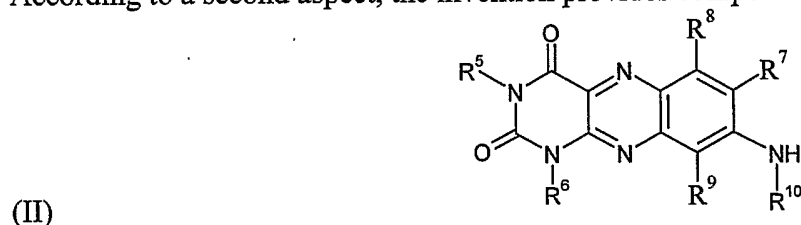
R^2 represents NR^3R^4 , NR^3COR^4 , $NR^3CONR^3R^4$ or NR^3COOR^4 ;

R^3 is H or R^4 ;

R^4 represents C1 to C10, more preferably C1 to C6, most preferably C1 to C5 acyclic, or C3 to C12, more preferably C5 to C10, most preferably C5 to C7 cyclic, saturated or unsaturated hydrocarbyl, optionally substituted with one or more groups Y; C6-C14 aryl, optionally substituted with one or more groups Y; 5- to 14-membered heteroaryl having one or more heteroatoms, optionally substituted with one or more groups Y; 5- to 14-membered non-aromatic heterocycles having one or more heteroatoms, optionally substituted with one or more groups Y; and

Y represents hydroxyl, amine, sulfide, silyl, carboxyl, halogen and C6-C14 aryl.

According to a second aspect, the invention provides compound of formula (II)



or a pharmaceutically acceptable salt or prodrug thereof, wherein

R^5 , R^6 , R^7 , R^8 and R^9 represent the same or different groups selected from H, unbranched or branched C1 to C10, more preferably C1 to C6, most preferably C1 to C5 acyclic saturated or

unsaturated hydrocarbyl, or C3 to C12, more preferably C5 to C10, most preferably C5 to C7 cyclic saturated or unsaturated hydrocarbyl, halogen, C6 to C14 aryl, optionally substituted with one or more groups Y; 5- to 14-membered heteroaryl having one or more heteroatoms, optionally substituted with one or more groups Y; 5- to 14-membered non-aromatic heterocycles;

R¹⁰ represents H, unbranched or branched C1 to C10, more preferably C1 to C6, alkyl; C3 to C12 cycloalkyl, benzyl, halogen, C6 to C14 aryl optionally substituted with one or more groups Y; 5- to 14-membered heteroaryl having one or more heteroatoms, optionally substituted with one or more groups Y; 5- to 14-membered non-aromatic heterocycles; COR⁴; CONR⁴; COOR⁴;

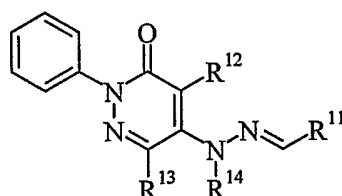
R⁴ represents C1 to C10, more preferably C1 to C6, most preferably C1 to C5 acyclic, or C3 to C12, more preferably C5 to C10, most preferably C5 to C7 cyclic, saturated or unsaturated hydrocarbyl, optionally substituted with one or more groups Y; C6-C14 aryl, optionally substituted with one or more groups Y; 5- to 14-membered heteroaryl with one or more heteroatoms, optionally substituted with one or more groups Y; 5- to 14-membered non-aromatic heterocycles having one or more heteroatoms, optionally substituted with one or more groups Y; and

Y represents hydroxyl, amine, sulfide, silyl, carboxyl, halogen and C6-C14 aryl;

for use as a medicament.

According to a further aspect, the present invention provides a compound of formula (III)

(III)



or a pharmaceutically acceptable salt or prodrug thereof, wherein

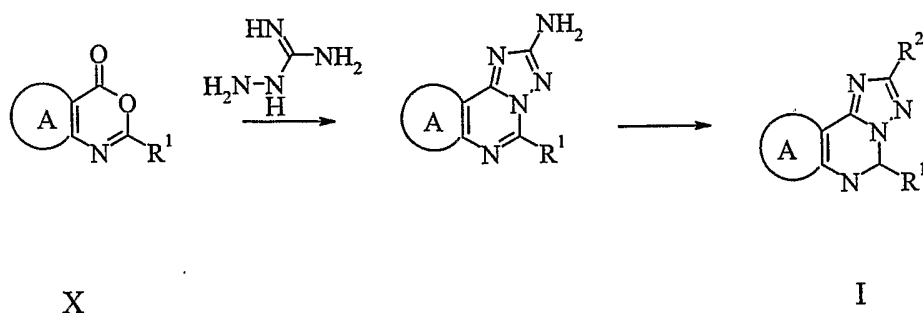
R^{11} , R^{12} , R^{13} and R^{14} represent the same or different groups selected from H, unbranched or branched, C1 to C10, more preferably C1 to C6, most preferably C1 to C5 acyclic saturated or unsaturated hydrocarbyl, or C3 to C12, more preferably C5 to C10, most preferably C5 to C7 cyclic saturated or unsaturated hydrocarbyl, benzyl; halogen, C6 to C14 aryl, optionally substituted with one or more groups Y; 5- to 14-membered heteroaryl having one or more heteroatoms, optionally substituted with one or more groups Y; 5- to 14-membered non-aromatic heterocycles; and

Y represents hydroxyl, amine, sulfide, silyl, carboxyl, halogen and C6-C14 aryl;

for use as a medicament.

The compounds of formulae I-III that have a selectivity towards the A_{2a} - G_{olf} complex will be useful for treating or preventing various diseases such as Parkinson's, Alzheimer's, Huntington's diseases, senile dementia and depression, schizophrenia or other related CNS conditions while compounds of formulae I-III that have a selectivity towards the A_{2a} - G_s complex will be useful as immunomodulators of inflammation and therefore of use in the treatment of conditions involving pathological inflammation.

According to one aspect the invention provides a method of preparing a compound of formula I comprising the steps of :



reacting a compound of formula X, wherein A and R¹ are as defined herein above, with aminoguanidine; and reacting the primary amine function to transform it into NR³R⁴, wherein R³ and R⁴ are as defined herein above.

As used herein the term "alkyl" unless otherwise stated, means an unbranched or branched, acyclic or cyclic, saturated or unsaturated (alkenyl or alkynyl) hydrocarbyl radical. Where cyclic, the alkyl group is preferably C3 to C12, more preferably C5 to C10, most preferably C5-C7. Where acyclic, the alkyl group is preferably C1 to C10, more preferably C1 to C6, more preferably methyl, ethyl, propyl (n-propyl, isopropyl), butyl (branched or unbranched) or pentyl, most preferably methyl.

As used herein, the term "aryl" means an aromatic group, such as phenyl or naphthyl.

As used herein, the term "heteroaryl" means an aromatic group containing one or more heteroatom(s) preferably selected from N, O and S, such as pyridyl, pyrrolyl, quinolinyl, furanyl, thienyl, oxadiazolyl, thiadiazolyl, thiazolyl, oxazolyl, pyrazolyl, triazolyl, imidazolyl, pyrimidinyl, indolyl, pyrazinyl or indazolyl.

As used herein, the term "functional groups" means in the case of unprotected: an hydroxy-thiolo- or amino-function, or a carboxyl group, and in the case of protected: lower alkoxy, N-, O- or S-acetyl, or a carboxyl ester group.

As used herein, the term "non-aromatic heterocycle" means a non-aromatic cyclic group containing one or more heteroatom(s) preferably selected from N, O and S, such as a cyclic amino group such as pyrrolidinyl, piperidyl, piperazinyl, morpholinyl or a cyclic ether group such as tetrahydrofuranyl or monosaccharide.

As used herein the term "halogen" means fluorine, chlorine, bromine or iodine.

As used herein the term "substituted" means that the concerned groups are substituted with one or more functional group(s) such as hydroxyl, amine, sulfide, silyl, carboxylic acid, halogen, aryl, etc.

The compounds of the present invention may be used or administered in combination with one or more additional drugs useful, for example, in the treatment of movement disorder, such as L-DOPA, the components being in the same formulation or in separate formulations for administration simultaneously or sequentially.

Examples of pharmaceutically acceptable addition salts for use in the present inventive pharmaceutical compositions include those derived from mineral acids, such as hydrochloric, hydrobromic, phosphoric, metaphosphoric, nitric and sulphuric acids, and organic acids, such as tartaric, acetic, citric, malic, lactic, fumaric, benzoic, glycolic, gluconic, succinic, and arylsulphonic acids.

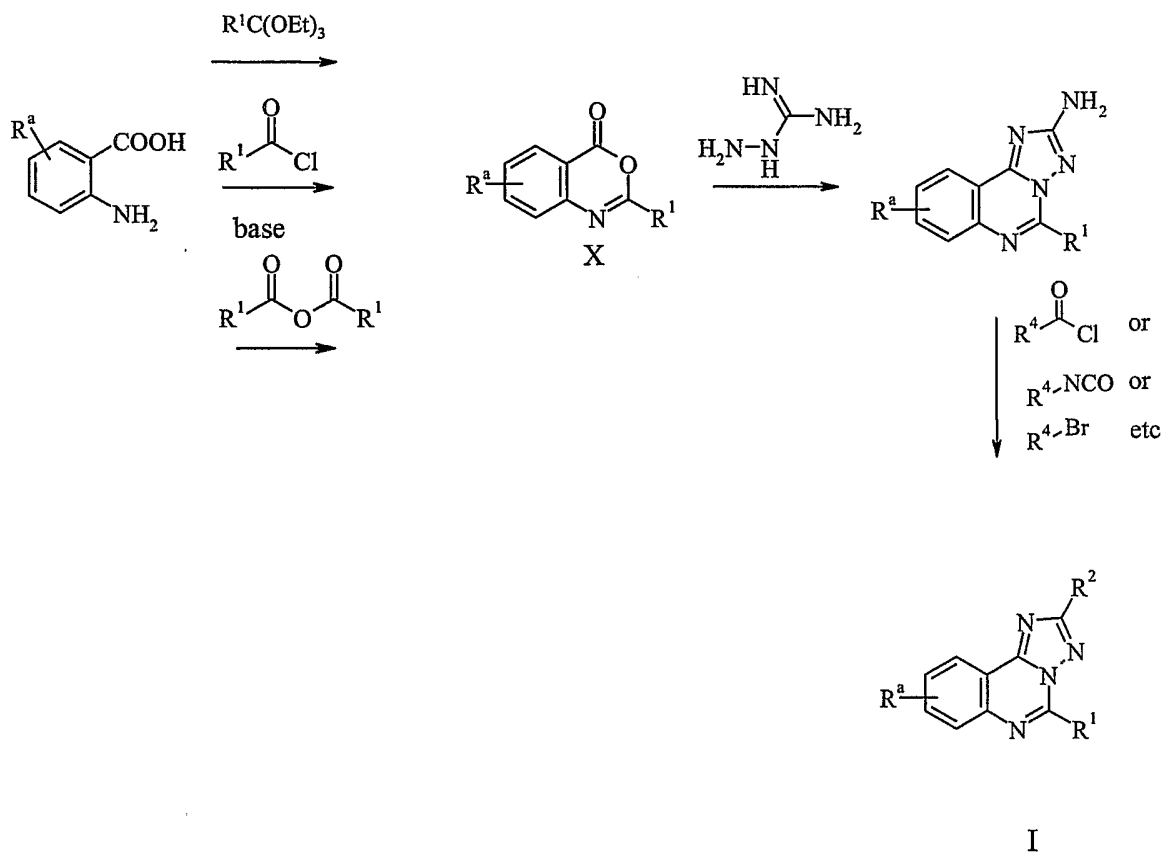
The pharmaceutically acceptable excipients mentioned herein, for example, vehicles, adjuvants, carriers or diluents, are well-known to those who are skilled in the art and are readily available to the public.

The pharmaceutically acceptable carrier may be anyone which is chemically inert to the active compounds and which have no detrimental side effects or toxicity under the conditions of use. Pharmaceutical formulations are found e.g. Remington: "The Science and Practice of Pharmacy," 20th Edn., A.R. Gennaro, Editor, Mack Publishing Co., Easton, PA (2003) (ISBN 0-7817-5025-3). A brief review of methods of drug delivery is also found in e.g. Langer, Science 249:1527-1533 (1990).

The dose administered to an animal, particularly a human, in the context of the present invention should be sufficient to effect a therapeutic response in the animal over a reasonable time frame. One skilled in the art will recognize that dosage will depend upon a variety of factors including the potency of the specific compound, the age, condition and body weight of the patient, as well as the stage/severity of the disease. The dose will also be determined by the route (administration form) timing and frequency of administration. In the case of oral administration the dosage can vary from about 0.01 mg to about 1000 mg per day of a compound of formula (I), (II), (III) or the corresponding amount of a pharmaceutically acceptable salt thereof.

Synthesis of compounds of formula I

According to one aspect of the invention, methods of preparing the inventive compounds of formula I are provided. As an example, the preparation of a compound according to formula I wherein A is phenyl is described:



The first reaction step is the formation of 4*H*-3,1-benzoxazin-4-one (X) which is a central compound. A number of synthetic methods for the preparation of 2-substituted 4*H*-3,1-benzoxazin-4-ones have been described: (i) cyclodehydration of *N*-acylanthranilic acids by acetic anhydrides^{2a-c}, (ii) reaction of anthranilic acid with acid chlorides in pyridine^{3a-b}; (iii) condensation of anthranilic acid with orthoesters⁴.

Depending on the availability of starting materials and reagent the compound X was synthesized in a number of different routes as described below. Several methods were developed and it was found that some methods are more preferable than others depending on the 2-substituent of interest. It was found, for example, that the use of orthoesters are most suitable

for the synthesis of 2-H- and 2-Me- 4*H*-3,1-benzoxazin-4-ones. Trifluoroacetic acid anhydride was used for the synthesis of 2-trifluoromethyl-4*H*-3,1-benzoxazin-4-one and for the synthesis of 2-aryl/heteroaryl-4*H*-3,1-benzoxazin-4-one acid chlorides and pyridine was used.

The products are formed in 30 min to a number of hours under reflux condition depending on the starting material and the substituents. It was found however that the reaction time for all these reaction could be limited to 5 min using microwave assisted heating.

For the synthesis of 2-H- and 2-Me-4*H*-3,1-benzoxazin-4-ones, the anthranilic acid derivatives was treated with an excess of the orthoester. The reaction mixture was heated at 170° C for 5 min. In all cases, upon cooling the products precipitate in a pure form based on GC-MS analysis and used directly in the next step without any further purification step. This reaction proceeds without organic solvent and in the absence of basic or acidic catalyst.

For the synthesis of 2-trifluoromethyl-4*H*-3,1-benzoxazin-4-one, anthranilic acid was dissolved in dichloromethane and treated with a small excess of trifluoroacetic anhydride. The reaction was heated at 120 °C for 5 min. The reaction mixture was poured out in DCM and ice. The organic phase is extracted with water and then dried over MgSO₄. The product is formed in high yield and purity as confirmed by GC-MS analysis. The product is however very moisture sensitive due to the CF₃ -group strong electron withdrawing properties.

For the synthesis of 2-aryl/heteroaryl-4*H*-3,1-benzoxazin-4-ones, anthranilic acid derivatives were heated at 120 °C for 5 min with 1.5 mmol of an aryl acid chloride in a mixture of pyridine and DCM (1:3). After cooling of the reaction mixture, water was added and the reaction mixture was extracted. After drying with MgSO₄ the organic phase was evaporated. The remaining pyridine was co-distilled with toluene. The products were formed in high yield and purity as confirmed by GC-MS. The products were re-crystallized from methanol.

These methods could also be adopted for the formation of compounds of formula X wherein A is other than phenyl, such as by the use of 2- and 3-amino nicotinic acid or 3-amino-pyrazine-2-carboxylic acid for the formation of 4*H*-pyrido-3,1-oxazin-4-ones and 4*H*-pyrazino-3,1-oxazin-4-ones respectively^{3a-b} or the formation of thieno[3,4-d][1,3]oxazin-4-

ones⁵ from the corresponding amino thiophene carboxylic acid. Naphtho[2,3-d][1,3]oxazin-4-ones could be formed from the corresponding 3-amino-naphthalene-2-carboxylic acid⁶. The following steps in the reaction sequence are practicable independently of the identity of A, possibly with adaptive measures within the knowledge of the skilled person, such as protection/deprotection of functional groups.

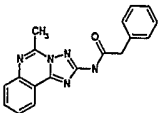
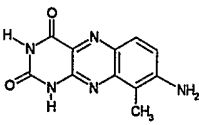
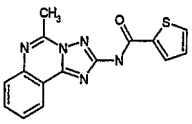
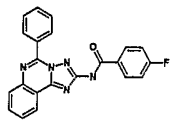
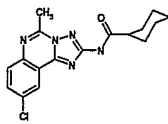
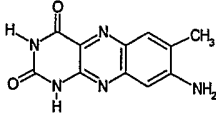
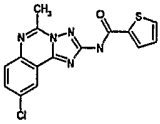
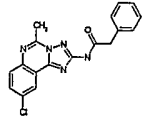
The second step is the formation of 2-amino-5-aryl/alkyl triazolo[1,5-c]pyrimidines. To the best of our knowledge only one publication have described this reaction using benzoxazin-4-ones and amino guanidine hydrochloride and pyridine as solvent for the formation of triazolo[1,5-c]pyrimidines⁷. Under conventional methods the reaction mixture is refluxed for about 10 hours. It was found that when applying microwave heating the reaction time could be reduced dramatically. The benzoxazines were treated with 1.2-1.5 eq. aminoguanidine hydrogencarbonate in pyridine for 15 min at 180 °C. The product is slowly precipitated in pure form (based on GC-MS) from the reaction mixture. The product is filtered and washed with cold hexane or DCM. For some derivatives we had to concentrate the reaction mixture and add MeOH in order to make the product precipitate. No further purification was needed.

The amino function on the 2-amino-5-aryl/alkyl-triazolo[1,5-c]pyrimidine derivatives could finally be coupled with a number of derivatives to form secondary amines, amides, carbamates, ureas etc. Methods for the formation of secondary amines, tertiary amines, amides, carbamates, and ureas from primary amines are well established and well known for the person skilled in the art. Below the *N*-acylation is described.

The 2-amino-5-aryl/alkyl-triazolo[1,5-c]pyrimidine derivatives were dissolved in MeCN/Pyridine 3:1 and 1.5 equiv. of the acid chloride was added. The reaction mixture was heated at 170 °C for 5 min. Half of the produced products precipitated from the reaction mixture in a pure form. The rest of the compound was purified with preparative HPLC. 90% of the produced compound was found in a purity>95%. The rest of the compounds was found in >90% purity.

Screening data - examples

Compounds, as disclosed herein, were analyzed for A2A receptor binding properties using methods described below.

ID #	Article #	Structure	Purification method and Final purity	Ratio	Ki nM Striatum	Ki nM Leukocytes
175	ACT-002		Crystallisation Purity: 97.7	0.4	146.5	359.4
407				0.42	30	71
182	ACT-009		HPLC purity: 98.4%	0.52	539.5	1033
189	ACT-015		Crystallisation purity: 97.5%	0.68	318.9	467.5
234	ACT-059		HPLC Purity: 95.6%	0.77	543.2	704.1
406				0.87	294	336
237	ACT-062		HPLC Purity: 95.6%	1.07	727.8	679.1
233	ACT-058		HPLC Purity: 95.6%	1.09	201.5	184.1

196 ACT-022		HPLC Purity: 95.6%	1.1	134	120.9
197 ACT-023		Crystallisation Purity: 98.4%	1.15	795.9	692.3
177 ACT-004		Crystallisation Purity: 97.4%	1.22	594	486.6
236 ACT-061		HPLC Purity: 96%	2.1	739.1	348.5
214 ACT-039		Crystallisation Purity: 96.3%	2.5	2475	985.5
183 ACT-010		HPLC Purity: 100% 1H-NMR	3.25	716.5	220.3
307			5.03	1118	222

The examples describe the structure of different compounds and how they bind A_{2a} receptors coupled to G_s (Ki leukocytes) and A_{2a} receptors coupled to G_{o1f} (Ki striatum). The ratio indicates selectivity of binding where a low ratio indicates selectivity towards A_{2a}-G_{o1f} coupled receptors and a high ratio indicates selectivity towards A_{2a}-G_s coupled receptors.

Experimental Part

Biology

Animals

The pigs (20-25 kg) were from Swedish Meat, Uppsala, Sweden.

Tissue preparation

Striatum

The brain striatum was dissected out and the tissue was homogenized using an Ultra-Turrax, 3x15s at full speed in 50 mM Tris-HCl buffer, pH 7.4. The homogenate was centrifuged at 30,000 g for 50 min at 4°C and resuspended in the 50 mM Tris-HCl buffer. Finally the striatal membrane preparation was frozen in aliquots at - 20°C until used.

Leukocytes

Ficoll-Paque PLUS: Heparin-treated blood (0.2 ml/10 ml whole blood) was mixed with an equal volume of PBS and 4 ml of the diluted blood was carefully layered over 3 ml of Ficoll-Paque PLUS in a 15 ml centrifuge tube. After a centrifugation at 400g for 30 min at room temperature in a swing-out rotor, the white leukocyte rich layer in the middle of the tube was transferred to other tubes with 3 volumes of PBS and pelleted by centrifugation at 400g for 15 min at room temperature. The supernatant was removed and the pellet was resuspended in 6 ml of PBS and centrifuged at 400g for 15 min at room temperature. Finally, the pellet was resuspended in 0.4 ml of 50 mM Tris-HCl buffer containing 5 mM MgCl₂, pH 7.4, and frozen at -20°C.

The protocol for the Ficoll-Paque PLUS method was scaled up, since a large amount of leukocytes was needed in later assays. 1200 ml of heparin-treated blood was diluted with an equal volume of PBS. 10 ml of Ficoll-Paque PLUS was added to 50 ml centrifuge tubes and 15 ml of the diluted blood was carefully layered over. A white leukocyte-rich layer in the middle of the tubes was created after a centrifugation at 400g for 30 min at room temperature

in a swing-out rotor. Then the same isolation procedure as for the small volume of blood, as described above, was followed.

The leukocytes were thawed on ice, sonicated (3 times 5 sec), diluted in 50 mM Tris-HCl buffer containing 5 mM MgCl₂, pH 7.4, and then centrifuged at 50,000 g for 30 min at 4°C. The supernatant was decanted, the pellet was resuspended in 5 ml of the 50 mM Tris-HCl buffer and centrifuged at 50,000 g for 20 min at 4°C. The resulting pellet was resuspended in 5 ml of the 50 mM Tris-HCl buffer. Aliquots of the leukocyte membrane preparation were then rapidly frozen and stored at -20°C.

Protein measurement

The protein concentrations were determined according to the method of Lowry *et al.*⁸ with bovine serum albumin as reference standard.

Radioligand binding assay

Adenosine deaminase (2 units/ml) was added to the membrane preparation for 1 h at 37°C to remove the endogenous adenosine before the binding assays.

Saturation binding studies

The assays were carried out in triplicate and in a final volume of 300 µl. The membranes (25 µg/well for striatum) (100 µg/well for leukocytes) were incubated with 10 different concentrations of [³H]-ZM241385 ranging from 0.10 nM to 7.5 nM. To measure the total ligand binding, the membrane suspension was incubated with radioligand in Tris-HCl buffer, pH 7.4, containing 5 mM MgCl₂ for 1 h at 4°C. The non-specific binding was determined in the presence of 100 µl 50 µM 5'-N-ethylcarboxamidoadenosine (NECA). After 1 h of incubation, the samples were harvested by filtration through Whatman GF/B filters with a semi-automatic cell harvester (Skatron A/S, Norway). Each filter was rinsed with 6 ml of cold 50 mM Tris-HCl buffer, pH 7.4. Then the filters were punched out into scintillations vials and the radioactivity was determined in an LKB 1209 RackBeta Liquid Scintillation Counter with 3 ml Ready Safe scintillation liquid. The binding data were analysed with the GraphPad Prism pro-

gramme (GraphPAD, San Diego, CA, USA) for the determination of dissociation constants (K_d) and the number of receptors expressed as a B_{max} value.

Competition binding studies

In the competition assays, at least seven different concentrations of the substances were used. The assays were carried out in triplicate in a final volume of 300 μ l. The concentration of [3 H]-ZM241385 was 1,5 nM – 2,5 nM and the concentration of the striatal and leukocytes membranes were 25 μ g/assay and 100 μ g/assay respectively. After incubation for 1 h at 4°C, the samples were filtered through Whatman GF/B filters with the same semi-automatic cell harvester system that were used in the saturation binding assays. The inhibitory binding constant, K_i was calculated from the IC_{50} values according to the Cheng and Prusoff equation⁹ $K_i = IC_{50} / ((1 + [C^*]) / K_d^*)$ where $[C^*]$ is the concentration of the radioligand and K_d^* its dissociations constant.

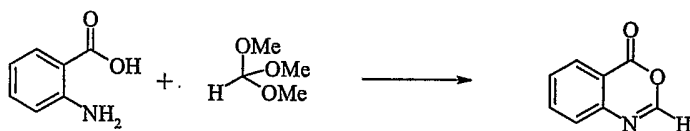
Chemistry

The microwave-assisted reactions were performed in a SmithSynthesizerTM, a single mode microwave cavity, from Personal Chemistry. NMR spectra were recorded in $CDCl_3$ or $DMSO-d_6$ at 25 °C, using a JEOL at 270 MHz (1H). All NMR spectra recorded were in agreement with the postulated structures. HPLC-MS chromatograms and spectra were obtained with an Agilent 1100 system with a diode array detector. Purity is reported based on the % area of the UV-peak (See table above) . The analytical GCMS were obtained with a Varian. All starting reagents were of the best grade available (Aldrich or Lancaster) and were used without purification. It should also be noted that the products could be formed using conventional heating.

Examples

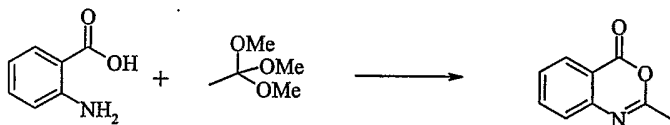
Example 1. Synthesis of benzoxazin-4-one using orthoesters

1.1. Synthesis of 4*H*-3,1-benzoxazin-4-one



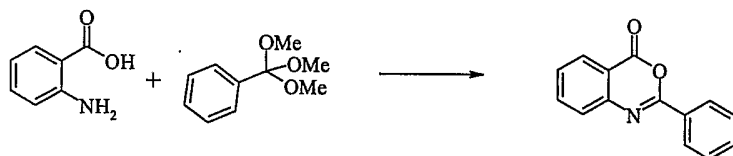
1 g of anthranilic acid (MW:137, 7.3 mmol) and 2.0 mL of trimethylorthoformate (1.94 g, MW: 106, d: 0.970, 18.5 mmol, 2.5 eq.) are heated under microwave conditions for 5 min at 160 °C. After irradiation the excess of reagent is evaporated to dryness. Since the product is very hygroscopic no further purification is performed. The product is formed in high yield and purity and is confirmed by GC-MS.

1.2 Synthesis of 2-methyl-4*H*-3,1-benzoxazin-4-one



The product is formed by the use of trimethylorthoacetate in the same way as described above. The product was precipitated from the reaction mixture and filtered and dried in a desiccator. The product is formed in quantitatively yield and purity and is confirmed by GC-MS.

1.3. Synthesis of 2-benzyl-4*H*-3,1-benzoxazin-4-one

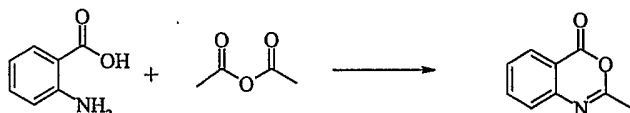


0.5 g Anthranilic acid (MW:137, 3.65 mmol) and 2.0 mL trimethylorthobenzoate (2.122 g, MW:182, d: 1.061, 11.65 mmol, 3.2 eq.) is heated under microwave conditions for 5 min at

160 °C. The reaction mixture is after irradiation evaporated in order to withdraw the formed MeOH. Hexane is then added to the reaction mixture and the product is precipitated over night. The product is formed but a noncyclic bi-product is also formed (+MeOH).

Example 2. Synthesis of benzoxazin-4-one using acetic anhydride

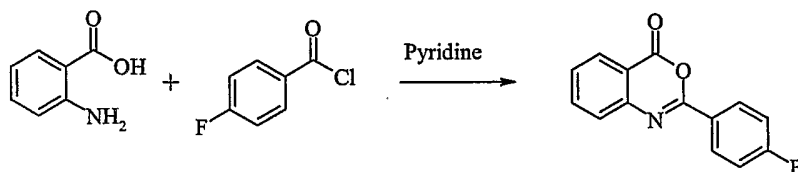
2.1. Synthesis of 2-methyl-4*H*-3,1-benzoxazin-4-one



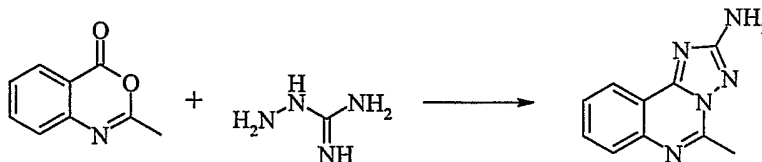
0.5 g anthranilic acid (MW:137, 3.65 mmol) and 2.0 mL acetic acid anhydride (excess) is heated under microwave conditions for 5 min at 160 °C. The excess of reagent is after irradiation evaporated to dryness. Based on GC analysis the use of orthoester gives a purer reaction.

Example 3. Synthesis of benzoxazin-4-one using acid chlorides

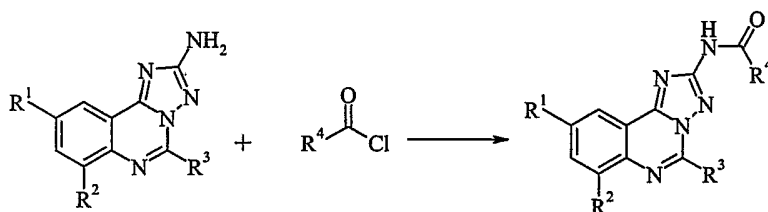
3.1 Synthesis of 2-(*p*-F-benzyl)-4*H*-3,1-benzoxazin-4-one



137 mg Anthranilic acid (MW:137, 1.0 mmol) is heated at 120 °C for 5 min with 177 μ L 4-fluoro benzoyl chloride (1.5 mmol, MW:158.56, d:1.34), 0.5 mL pyridine and 1.5 mL DCM. Water is then added to the reaction mixture and the reaction mixture is extracted. The organic phase is dried with MgSO_4 . Filtered and the solvent is evaporated. The pyridine is co-distilled with toluene. The product is formed in high yield and purity and confirmed by GC-MS. The product is re-crystallized from methanol.

Example 4. Synthesis of 2-amino-5-aryl/alkyl triazolo[1,5-c] quinazoline**4.1 Synthesis of 2-amino-5-methyl-triazolo[1,5-c] quinazoline**

The benzoxazine (1 mmol) is treated with 1.2 mmol aminoguanidine hydrogencarbonate in 2.5 ml pyridine for 15 min at 180 °C under microwave conditions. The reaction mixture is cooled to room temperature. The product is slowly precipitated in pure form (based on GC-MS) from the reaction mixture. The product is filtered and washed with cold hexane or DCM. For some derivatives we had to concentrate the reaction mixture and add MeOH in order to make the product to precipitate.

4.2 Synthesis of 2-amido-5-alkyl/aryl-triazolo[1,5-c] quinazoline

General procedure: The amino-triazolo-pyrimidines were dissolved in a mixture of MeCN/pyridine 3:1, 1.5 eq. of the acid chloride was added and the reaction mixture was heated at 170 °C for 5 min. The products were either precipitated from the reaction mixture in pure form and filtered or was purified with preparative LC-MS.

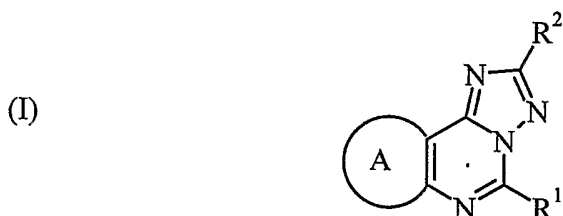
The overall yields vary from 30 to 60% depending on the isolation procedure. No further optimisation of the yield was performed. The purity however was always higher than 90% and mostly over 95%.

References

1. Kull, B.; Svenningsson, P.; Fredholm, B. B. *Mol. Pharmacol.* **2000**, 58 (4), 771-777.
- 2a Parkanyi, C.; Liang Yuan, H.; Strömberg, B. H. E.; Evenzahav, A. *J. Heterocycl.Chem.* **1992**, 749-753.
- 2b. Parkanyi, C.; Schmidt, D. S. *J. Heterocycl.Chem.* **2000**, 725-729.
- 2c. Forsch, R. A.; Wright, J. E.; Rosowsky, A. *Bioorg. Med. Chem.* **2002**, 2067-2076.
- 3a. Hurd, C.; Bethune, V. G. *J. Org. Chem.* **1970**, 1471 –1475.;
- 3b. Errede, L. A.; Oien, H. T.; Yarian, D. R. *J. Org. Chem.* **1977**, 12-18.
4. Khajavi, M. S.; Montazari, N.; Hosseini, S. S. S. *J. Chem. Res.* **1997**, 286-287.
5. Shinkwin, A. E.; Whish, W. J. D.; Threadgill, M. D. *Bioorg. Med. Chem.* **1999**, 7, 297-308.
6. Zhang, W., Liu, R.; Huang, Q.; Zhang, P.; Koehler, K. F.; Harris, B.; Skolnick, P-: Cook, J.M. *Eur. J. Med. Chem.* **1995**, 30, 483-496.
7. Ried, W.; Valentin, J. *Chem Ber.* **1968**, 2106-2116.
8. Lowry, O. H.; Randall, R. J. *J. Biol. Chem.* **1951**, 193, 265-275.
9. Cheng, Y-C.; Prusoff, W. H. *Biochem. Pharmacol.* **1973**, 22, 3099-3108.

Claims

1. A compound of formula (I)



or a pharmaceutically acceptable salt or prodrug thereof, wherein

A is a 5- or 6-membered aryl or heteroaryl, optionally substituted with one or more groups selected from unbranched or branched, C1 to C10, more preferably C1 to C6, most preferably C1 to C5 acyclic saturated or unsaturated hydrocarbyl, or C3 to C12, more preferably C5 to C10, most preferably C5 to C7 cyclic saturated or unsaturated hydrocarbyl, optionally substituted with one or more groups Y; halogen; protected or unprotected hydroxy; thio, amino, or carboxyl groups; C6-C14 aryl, optionally substituted with one or more groups Y; 5- to 14-membered heteroaryl with one or more heteroatoms, optionally substituted with one or more groups Y; 5- to 14-membered non-aromatic heterocycles having one or more heteroatoms, optionally substituted with one or more groups Y;

R¹ represents H; halogen; C1 to C10, more preferably C1 to C6, most preferably C1 to C5 acyclic, or C3 to C12, more preferably C5 to C10, most preferably C5 to C7 cyclic, saturated or unsaturated hydrocarbyl; C6-C14 aryl, optionally substituted with one or more groups Y; 5- to 14-membered heteroaryl having one or more heteroatoms, optionally substituted with one or more groups Y, C6-C14 aryl; 5- to 14-membered non-aromatic heterocycles having one or more heteroatoms, optionally substituted with one or more groups Y;

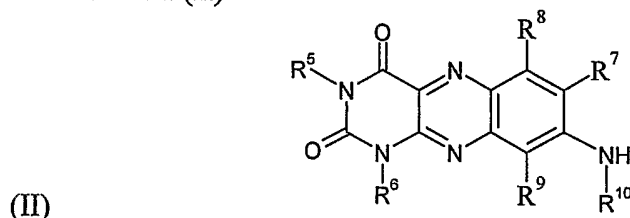
R² represents NR³R⁴, NR³COR⁴, NR³CONR³R⁴ or NR³COOR⁴;

R³ is H or R⁴;

R⁴ represents C1 to C10, more preferably C1 to C6, most preferably C1 to C5 acyclic, or C3 to C12, more preferably C5 to C10, most preferably C5 to C7 cyclic, saturated or unsaturated hydrocarbyl, optionally substituted with one or more groups Y; C6-C14 aryl, optionally substituted with one or more groups Y; 5- to 14-membered heteroaryl having one or more heteroatoms, optionally substituted with one or more groups Y; 5- to 14-membered non-aromatic heterocycles having one or more heteroatoms, optionally substituted with one or more groups Y; and

Y represents hydroxyl, amine, sulfide, silyl, carboxyl, halogen and C6-C14 aryl.

2. A compound of formula (II)



or a pharmaceutically acceptable salt or prodrug thereof, wherein

R⁵, R⁶, R⁷, R⁸ and R⁹ represent the same or different groups selected from H, unbranched or branched C1 to C10, more preferably C1 to C6, most preferably C1 to C5 acyclic saturated or unsaturated hydrocarbyl, or C3 to C12, more preferably C5 to C10, most preferably C5 to C7 cyclic saturated or unsaturated hydrocarbyl, halogen, C6 to C14 aryl, optionally substituted with one or more groups Y; 5- to 14-membered heteroaryl having one or more heteroatoms, optionally substituted with one or more groups Y; 5- to 14-membered non-aromatic heterocycles;

R¹⁰ represents H, unbranched or branched C1 to C10, more preferably C1 to C6, alkyl; C3 to C12 cycloalkyl, benzyl, halogen, C6 to C14 aryl optionally substituted with one or more groups Y; 5- to 14-membered heteroaryl having one or more heteroatoms, optionally substituted with one or more groups Y; 5- to 14-membered non-aromatic heterocycles; COR⁴; CONR⁴; COOR⁴;

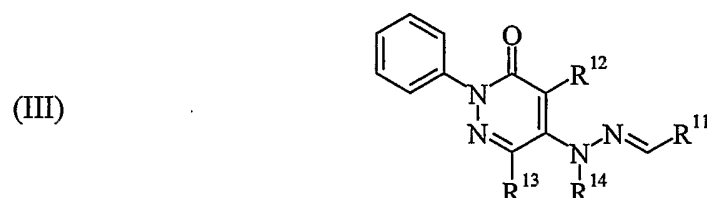
R⁴ represents C1 to C10, more preferably C1 to C6, most preferably C1 to C5 acyclic, or C3 to C12, more preferably C5 to C10, most preferably C5 to C7 cyclic, saturated or unsaturated

hydrocarbyl, optionally substituted with one or more groups Y; C6-C14 aryl, optionally substituted with one or more groups Y; 5- to 14-membered heteroaryl with one or more heteroatoms, optionally substituted with one or more groups Y; 5- to 14-membered non-aromatic heterocycles having one or more heteroatoms, optionally substituted with one or more groups Y; and

Y represents hydroxyl, amine, sulfide, silyl, carboxyl, halogen and C6-C14 aryl;

for use as a medicament.

3. A compound of formula (III)



or a pharmaceutically acceptable salt or prodrug thereof, wherein

R^{11} , R^{12} , R^{13} and R^{14} represent the same or different groups selected from H, unbranched or branched, C1 to C10, more preferably C1 to C6, most preferably C1 to C5 acyclic saturated or unsaturated hydrocarbyl, or C3 to C12, more preferably C5 to C10, most preferably C5 to C7 cyclic saturated or unsaturated hydrocarbyl, benzyl; halogen, C6 to C14 aryl, optionally substituted with one or more groups Y; 5- to 14-membered heteroaryl having one or more heteroatoms, optionally substituted with one or more groups Y; 5- to 14-membered non-aromatic heterocycles; and

Y represents hydroxyl, amine, sulfide, silyl, carboxyl, halogen and C6-C14 aryl;

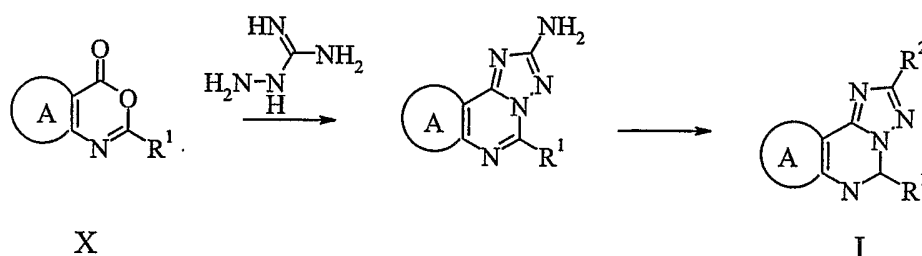
for use as a medicament.

4. A compound according to any of the claims 1-3 wherein any heteroatom in any heteroaryl or non-aromatic heterocycle is selected from N, O and S.

5. A compound according to any of the claims 1-4, wherein any heteroaryl is selected from pyridyl, pyrrolyl, quinolinyl, furanyl, thienyl, oxadiazolyl, thiadiazolyl, thiazolyl, oxazolyl, pyrazolyl, triazolyl, imidazolyl, pyrimidinyl, indolyl, pyrazinyl or indazolyl.

6. A compound according to claims 1-5 wherein any non-aromatic heterocycle is selected from pyrrolidinyl, piperidyl, piperazinyl, morpholinyl or a cyclic ether such as tetrahydrofuranlyl or a monosaccharide.

7. Process for the preparation of compounds of formula (I) according to claim 1, comprising the steps of :



reacting a compound of formula (X), wherein A and R¹ are as defined in claim 1, with aminoguanidine; and reacting the primary amine function to transform it into NR³R⁴, wherein R³ and R⁴ are as defined in claim 1.

8. Process according to claim 7 comprising the use of microwave dielectric heating.

9. A pharmaceutical composition containing a therapeutically effective amount of a compound according to any of the claims 1-6 or a pharmaceutically acceptable salt or prodrug thereof, and at least one pharmaceutically acceptable excipient.

10. A method of treatment or prevention of a disease wherein compounds that bind to purine receptors influence the course of the disease, comprising administration of a therapeutically effective amount of a compound according to any of claims 1-6 to a patient in the need of such treatment.

11. A method according to claim 10, wherein the purine receptors are adenosine receptors.

12. A method according to claim 11, wherein the adenosine receptors are A_{2A} receptors.
13. A method according to claim 11, wherein the disease is related to the adenosine A_{2A}-G_{olf} receptor complex.
14. A method according to claim 12, wherein the disease is related to the adenosine A_{2A}-G_s receptor complex.
15. A method according to claim 13, wherein the disease is selected from Parkinson's disease, Huntington's disease, Alzheimer's disease, schizophrenia, depression or other related CNS disorders.
16. A method according to claim 14, wherein the disease is inflammation.
17. A method according to any of the claims 10-16 wherein one or more additional drugs useful in the treatment of movement disorders or inflammation is/are furthermore administered.