Title:

Pyrazolo[4,3-*e*][1,2,4]triazolo[1,5-*c*]pyrimidines and Structurally Simplified Analogs. Chemistry and SAR Profile as Adenosine Receptor Antagonists.

Running title:

PTPs and their simplified analogs as AR antagonists.

Pyrazolo[4,3-*e*][1,2,4]triazolo[1,5-*c*]pyrimidines and Structurally Simplified Analogs. Chemistry and SAR Profile as Adenosine Receptor Antagonists.

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Abstract: Adenosine was defined as a neuromodulator which exerts its action by interaction with specific G-protein coupled receptor termed adenosine receptors. Adenosine receptors are expressed in several tissues and cells of our body and exist as four different subtypes of these receptors: A_1, A_{2A}, A_{2B} and A_3. In the last years significant efforts were made to obtain highly potent and selective ligands for the four adenosine receptors subtypes. Both agonists and antagonists were used as pharmacological tools to study therapeutic implications of enhancing or blocking the adenosine receptors activity, and some of these compounds have reached clinical phases. The pyrazolo[4,3-*e*][1,2,4]triazolo[1,5-*c*]pyrimidines (PTPs) represent one of the several templates designed as adenosine receptor antagonists. A lot of synthetic work was made on this scaffold in order to obtain potent A_{2A} and A_3 antagonists. Here were reviewed the synthetic approaches followed by both academia and industry to introduce different substituents at different positions of the PTP nucleus, in particular at the 2, 5, 7, 8 and 9 positions. Nevertheless PTP derivatives are tricyclic compounds with a high molecular weight which exhibit limitations such as poor aqueous solubility and difficult synthetic preparation. With the aim to obtain derivatives with the same potency and selectivity of PTP but with better drug-like properties, researchers made structural simplification of this scaffold. Replacement of the pyrazole or triazole rings of PTP led to the [1,2,4]triazolo[1,5-*c*]pyrimidine and pyrazolo[3,4-*d*]pyrimidine derivatives, respectively. Synthetic strategies for these compounds were reported, combined with the SAR profile on the adenosine receptors.

Keywords: pyrazolo[4,3-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine, [1,2,4]triazolo[1,5-*c*]pyrimidine; pyrazolo[3,4-*d*]pyrimidine; adenosine receptors; antagonists; Preladenant.

1. INTRODUCTION

Adenosine receptors (ARs) belong to class A of the G protein-coupled receptors family. A_1, A_{2A}, A_{2B} and A_3 ARs are the four AR subtypes currently identified. [1] The endogenous agonist of these receptors is adenosine, whereas the natural psychotropic compound caffeine is a well-known exogenous AR antagonist. [1,2] The different distribution of the four AR subtypes in cells, tissues and organs of the human body, confers to them different physio-pathological roles. [2] Potent and selective ligands (i.e. agonists and antagonists) at the various ARs are precious pharmacological tools, essential to characterize the ARs under physiological and pathological conditions. In the last decades, several AR antagonists have been reported and some of them were also tested in preclinical and clinical studies. [3] One of the most important class of AR antagonists are the pyrazolo[4,3-*e*][1,2,4]triazolo[1,5-*c*]pyrimidines (PTP, **1a-b**). PTP derivatives have been designed principally as A_{2A} and A_3 AR antagonists (Figure 1). [4] Preladenant (SCH 420814, **2**), an A_{2A} AR antagonist which possesses a PTP scaffold, has reached the phase III clinical trials for the treatment of Parkinson's disease (PD), but failed for lack of efficacy. [5-8] In order to improve the pharmacokinetic properties of PTPs, a molecular simplification approach was applied on this complex tricyclic scaffold. The pyrazole ring of PTP was eliminated leading to the simplified [1,2,4]triazolo[1,5-*c*]pyrimidine (TP, **3**) scaffold. [9-14] Another nucleus which could be considered as a simplified analog, by removal of [1,2,4]triazole ring from PTP scaffold, is the pyrazolo[3,4-*d*]pyrimidine (PP, **4**) nucleus. [15-21] Both of these classes of compounds led to derivatives that behave like adenosine receptor antagonists (Figure 2).

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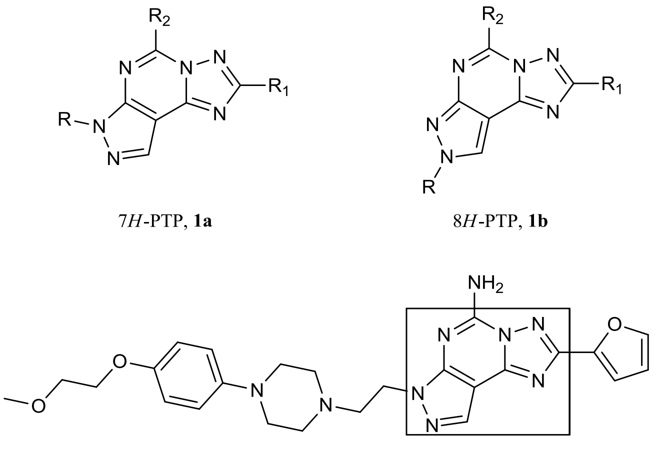
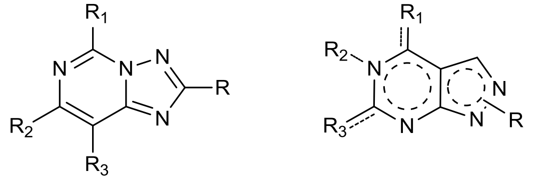


7*H*-PTP, **1a** 8*H*-PTP, **1b**

Preladenant, **2**

Figure 1. Pyrazolo[4,3-*e*][1,2,4]triazolo[1,5-*c*]pyrimidines.



TP, **3** PP, **4**

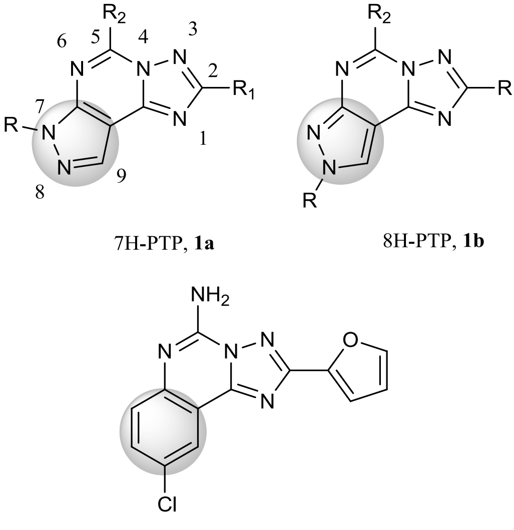
Figure 2. Simplified analogs of the PTP scaffold.

1.1. Adenosine receptors as drug targets

The adenosine receptors are involved in several physiological and pathological processes. [2] As GPCR (G protein-coupled receptor), A_1 and A_3 ARs are coupled with inhibitory G proteins (G_i), which inhibit adenylyl cyclase (AC) reducing the cyclic AMP (cAMP) levels. On the contrary, A_{2A} and A_{2B} ARs are coupled with stimulatory G proteins (G_s), those that activate AC enhancing the cAMP levels. Nevertheless, ARs were found to couple also to other G proteins, such as G_q proteins for the A_{2B} and A_3 ARs, and G_{olf} for the A_{2A} AR in the striatum. [1] In particular, activation of the A_1 AR could be useful for the treatment of cardiac arrhythmias and pain, whereas a block of the receptor activity is beneficial in hypertension, heart and kidney failure. Coronary artery vasodilation is mediated by A_{2A} AR, so agonists are useful as stress agents for myocardial perfusion imaging. Regadenoson is an A_{2A} AR agonist approved by Food and Drug Administration (FDA) for this use. [2] A_{2A} AR is also present in several immune cells and its activation leads to multiple anti-inflammatory effects. On the other hand, antagonists for the hA_{2A} AR were widely studied for the treatment of PD and other neurodegenerative disorders. [22] A_{2B} AR is activated by high levels of adenosine, which are usually reached under pathological conditions such as hypoxia and inflammation. [23] In fact, agonists for the A_{2B} AR are studied for the involvement in promoting ischemic preconditioning, whereas A_{2B} AR antagonists seems to be useful in the treatment of asthma and chronic obstructive pulmonary disease (COPD). [24] Finally, regarding the last characterized among the ARs, the A_3 subtype, there are evidences of a cardioprotective effect mediated by A_3 AR agonists. Instead, for other physio-pathological conditions involving the A_3 AR (e.g. inflammation, cancer and glaucoma) conflicting results on a possible beneficial effect of agonists or antagonists have been obtained. [25]

2. PYRAZOLO[4,3-*e*][1,2,4]TRIAZOLO[1,5-*c*] PYRIMIDINES

In 1993, Gatta *et al.* have reported the first pyrazolo[4,3-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine derivatives (**1a-b**) as A_2 adenosine receptor antagonists. [26] At that time, only few examples of non-xanthine heterocyclic compounds have been found to display antagonistic activity at the A_1 or A_{2A} ARs. Among these compounds CGS 15943 (**5**), a [1,2,4]triazolo[1,5-*c*]quinazoline derivative, is a reference antagonist for A_{2A} AR but it shows low selectivity for the A_{2A} AR versus A_1 AR (Figure 3). Gatta *et al.* explored new chemical entities in order to enhance selectivity at the A_{2A} AR, such as: imidazo[1,2-*c*]pyrazolo[4,3-*e*]pyrimidines, pyrazolo[4,3-*e*][1,2,4]triazolo[1,5-*c*]pyrimidines and [1,2,4]triazolo[5,1-*i*]purines. The pyrazolo[4,3-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine derivatives of this work displayed nanomolar affinity at the A_{2A} AR and represented a starting point for the development of a new class of potent and selective AR antagonists. [26]



7H-PTP, **1a** 8H-PTP, **1b**

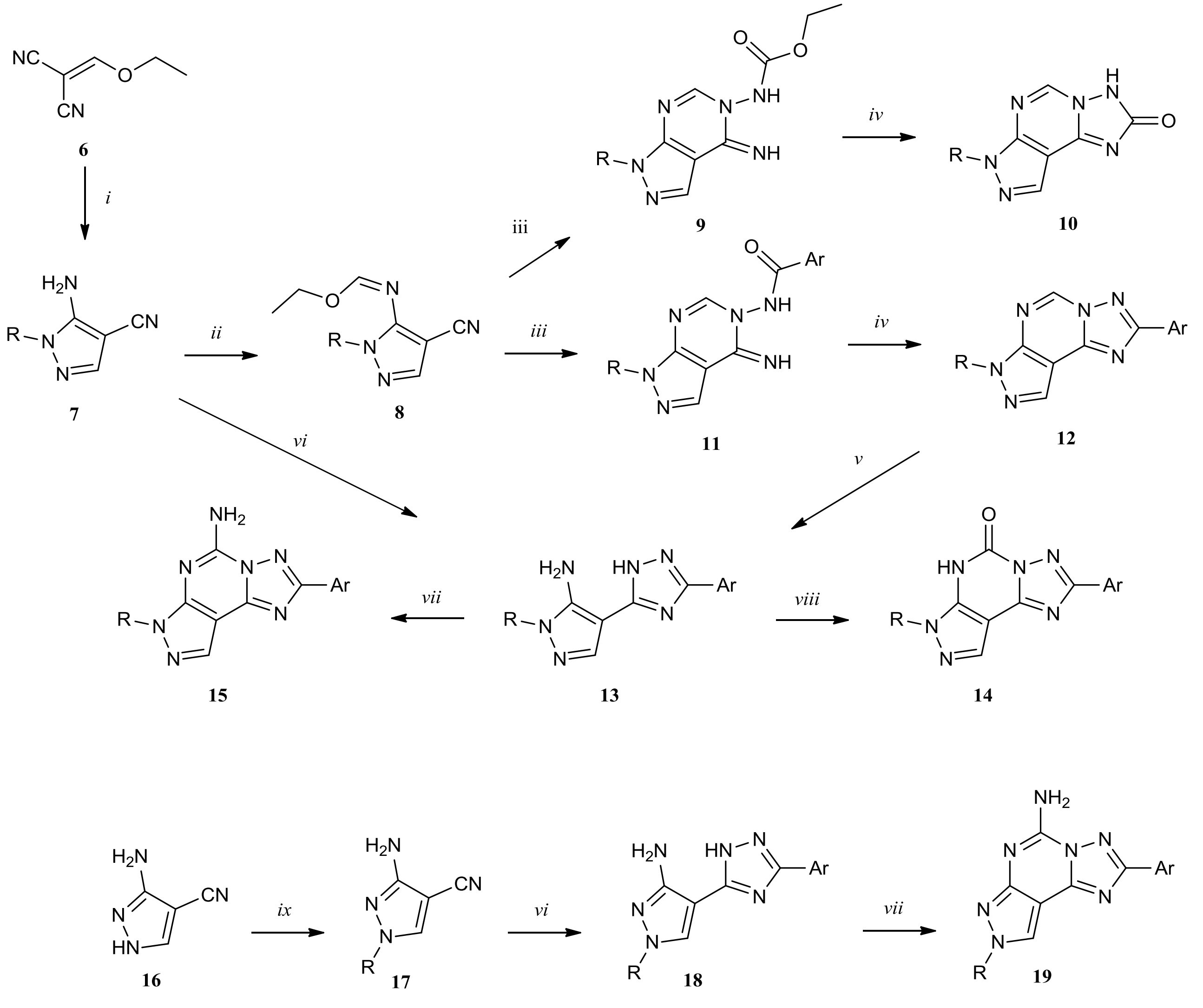
CGS15943, **5**

Figure 3. Comparison between PTP and [1,2,4]triazolo[1,5-*c*]quinazoline derivatives.

2.1. Chemistry

The original work by Gatta *et al.* [26] applied a synthetic approach, reported in Scheme 1, that started from 5-amino-*N*1-fluorobenzyl-1*H*-pyrazole-4-carbonitriles (**7**), which were obtained by reaction of 2-(ethoxymethylene)malononitrile (**6**) with 2- or 4-fluorobenzylhydrazine. [27] Compounds of general formula **7** were refluxed in triethylorthoformate to give the imidate derivatives (**8**) which, by reacting with benzhydrazide or 2-furoic acid hydrazide or ethylcarbazate, in ethylene glycol monomethylether at reflux, provided the corresponding 4-imino-4,5-dihydro-pyrazolo[3,4-*d*]pyrimidine derivatives (**9**, **11**). The PTP nucleus was finally obtained by refluxing compounds of general formula **9** and **11** in diphenylether. The cyclization of derivatives **9** afforded the 7-substituted-3,7-dihydro-2*H*-pyrazolo[4,3-*e*][1,2,4]triazolo[1,5-*c*]pyrimidin-2-one nucleus (**10**), whereas derivatives **11**, which contain an additional aryl group, provided the pyrazolo[4,3-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine (**12**) nucleus substituted at both the 7 and the 2 positions. The following ring opening of the pyrimidine ring in compounds **12**, by 10% hydrochloric acid, gave the (1*H*-1,2,4-triazol-5-yl)-1*H*-pyrazol-5-amines (**13**). An alternative and more convenient route to obtain compounds with general formula **13** was the direct condensation of the appropriated arylhydrazides with the aminopyrazoles **7**, in diphenylether at reflux. The triazolylpyrazoles **13** could be converted in 5-amino-7*H*-pyrazolo[4,3-*e*][1,2,4]triazolo[1,5-*c*]pyrimidines (**15**) by reaction with cyanamide, or they could be alternatively converted to the 6,7-dihydro-5*H*-pyrazolo[4,3-*e*][1,2,4]triazolo[1,5-*c*]pyrimidin-5-one (**14**) derivatives by reaction with ethyl carbamate. Finally, in order to obtain also the 8-substitued analogs, the appropriated starting compound *N*1-fluorobenzyl-3-amino-4-cianopyrazole (**17**) was obtained by alkylation of the 3-amino-4-cyanopyrazole (**16**) with the 2 or 4-fluorobenzylchloride in *N*,*N*-dimethylformamide (DMF) and using potassium carbonate as base. This approach led to a mixture 2:1 of alkylated pyrazoles **7** and **17**. The desired compounds **17**, separated by crystallization, were reacted with the corresponding aryl hydrazide (benzhydrazide or 2-furoic acid hydrazide) in diphenylether under reflux, affording the triazolyl derivatives **18**. As for the previously reported compounds **13**, also derivatives **18** were converted to the final tricyclic compounds (**19**) by reaction with cyanamide, in *N*-methylpyrrolidone at 140-160°C and in the presence of *p*-toluensulfonic acid monohydrate. [26]

With the aim to extensively explore the 7 position of the PTP scaffold, Baraldi *et al.* searched for a common key intermediate that, after alkylation with an appropriate alkyl halide, could afford the desired 7-substituted-PTP. [28] This intermediate has been identified in 2-furan-2-yl-7*H*-pyrazolo[4,3-*e*][1,2,4]triazolo[1,5-*c*]-pyrimidin-5-ylamine (**21**), which has been obtained by treatment of the corresponding *N*7-*tert*-butyl derivative (**20**, prepared following Gatta's approach) with 99% formic acid at 130 °C for 48 h. [29] Unfortunately, this reaction gave a 1:1 mixture

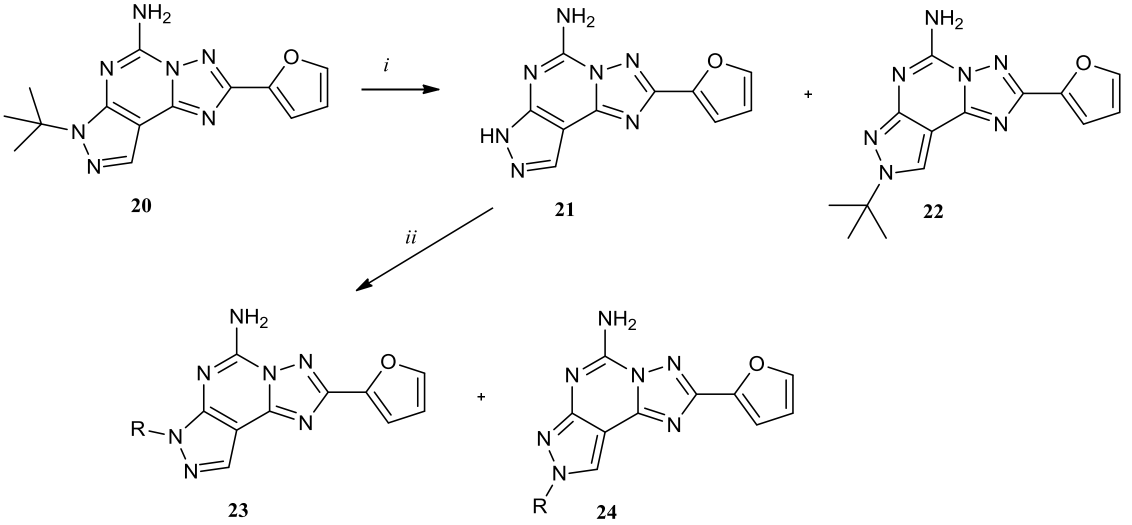


Scheme 1. Reagents: *i*: $RNHNH_2$, EtOH; *ii*: $CH(OC_2H_5)_3$, rfx, 8h; *iii*: $ArCONH_2NH_2$ or $C_2H_5OCONHNH_2$, EGME, rfx, 3h; iv: Ph_2O, rfx; v: 10% HCl (aq), rfx, 3h; *vi*: $ArCONH_2NH_2$, Ph_2O, rfx; *vii*: $CNNH_2$, p-toluensulfonic acid, NMP, 140-160°C; *viii*: $NH_2COOC_2H_5$, 210°C, 3h; *ix*: RCl, DMF, K_2CO_3, 70°C, 3h. R = 4-fluorobenzyl or 2-fluorobenzyl ; Ar = 2-furoyl or phenyl.

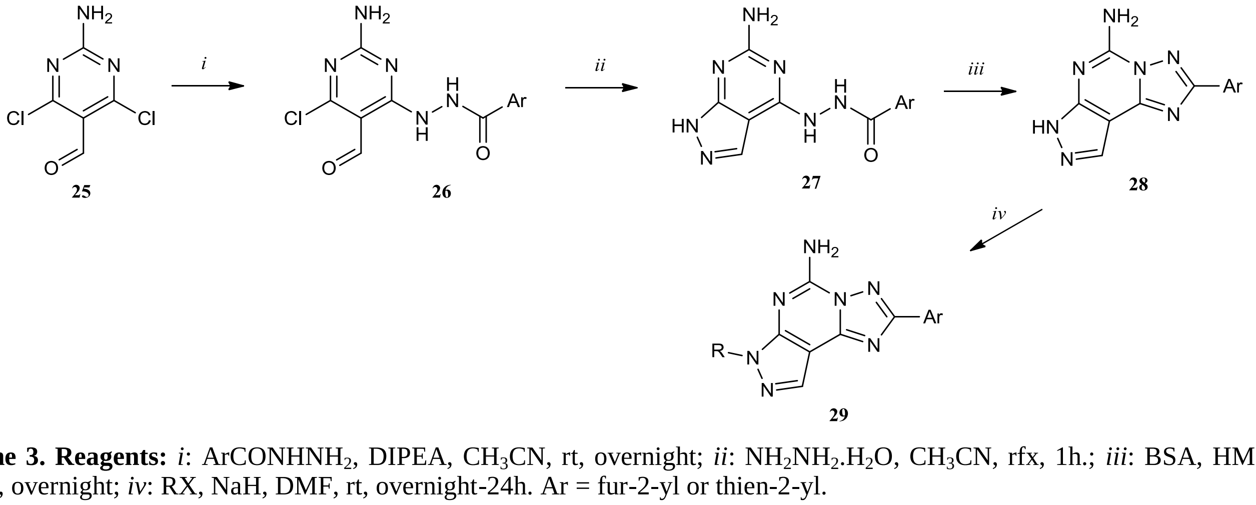
of unsubstituted (**21**) and *N*8-*tert*-butyl (**22**) derivatives (Scheme 2). [30,5] As depicted in Scheme 2, the key intermediate **21** could be easily alkylated on the pyrazole nitrogen by using anhydrous potassium carbonate as base and dry DMF as solvent. The reaction proceeded without competition by the amino group at the 5 position of PTP, but it afforded two regioisomers, alkylated at the 7- (**23**) and 8-positions (**24**) of the PTP nucleus in a 3:1 ratio, respectively. [28] The 7- and 8-alkylated PTP **23-24** were successively separated by column chromatography. This synthetic approach allows the introduction of different alkyl and arylalkyl moieties at the 7 and at the 8 positions, therefore both positions could be investigated at the same time. [28]

Schering has developed a more convenient synthesis (Scheme 3) for the 2-aryl-7*H*-pyrazolo[4,3-*e*][1,2,4]triazolo[1,5-*c*]-pyrimidin-5-ylamines (**28**) compared to the de-*tert*-butylation procedure (Scheme 2), which allows to obtain large amounts of key intermediate in three steps and requires soft purification procedures. [31] The synthesis started from the commercially available pyrimidine derivative **25**, which may be obtained by Vilsmeier's reaction of 2-amino-4,6-dihydroxypyrimidine with $POCl_3$ and DMF. Compound **25** was reacted with aryl hydrazides affording compounds **26**. A first cyclization was performed by treatment with hydrazine in acetonitrile, obtaining the pyrazolo[3,4-*d*]pyrimidines **27**, then, a dehydrative cyclization of **27** with *N*,O-bis(trimethylsilyl)acetamide (BSA) at reflux proceeded with concomitant Dimroth rearrangement to afford the desired products **28**. [31,5] Additionally, pyazole alkylation, by using sodium hydride instead of potassium carbonate, afforded preferentially the *N*7-alkylated derivatives (**29**) with small amounts of *N*8-alkylated regioisomers.

In the same patent also other slightely different synthetic approaches were claimed. [31] As depicted in Scheme 4 the direct cyclization of compound **25** with hydrazine afforded the 4-chloro-1*H*-pyrazolo[3,4-*d*]pyrimidin-6-amine (**30**), which could be in turn alkylated with the appropriated alkylhalide in the presence of NaH and DMF to obtain **31**. As for the alkylation approach used in Scheme 3, also in this case, the use of NaH as the base led principally to *N*1-alkylated compounds, which correspond to the final *N*7-susbtituted PTPs. Subsequent reaction of compounds **31** with hydrazine and the appropriated acylchloride, or the direct reaction with the appropriated arylhydrazide, gave compounds of general formula **33**. Finally, the dehydrative cyclization of compounds **33** afforded the desired *N*7-substituted-PTP derivatives (**34**). When compound **30** was



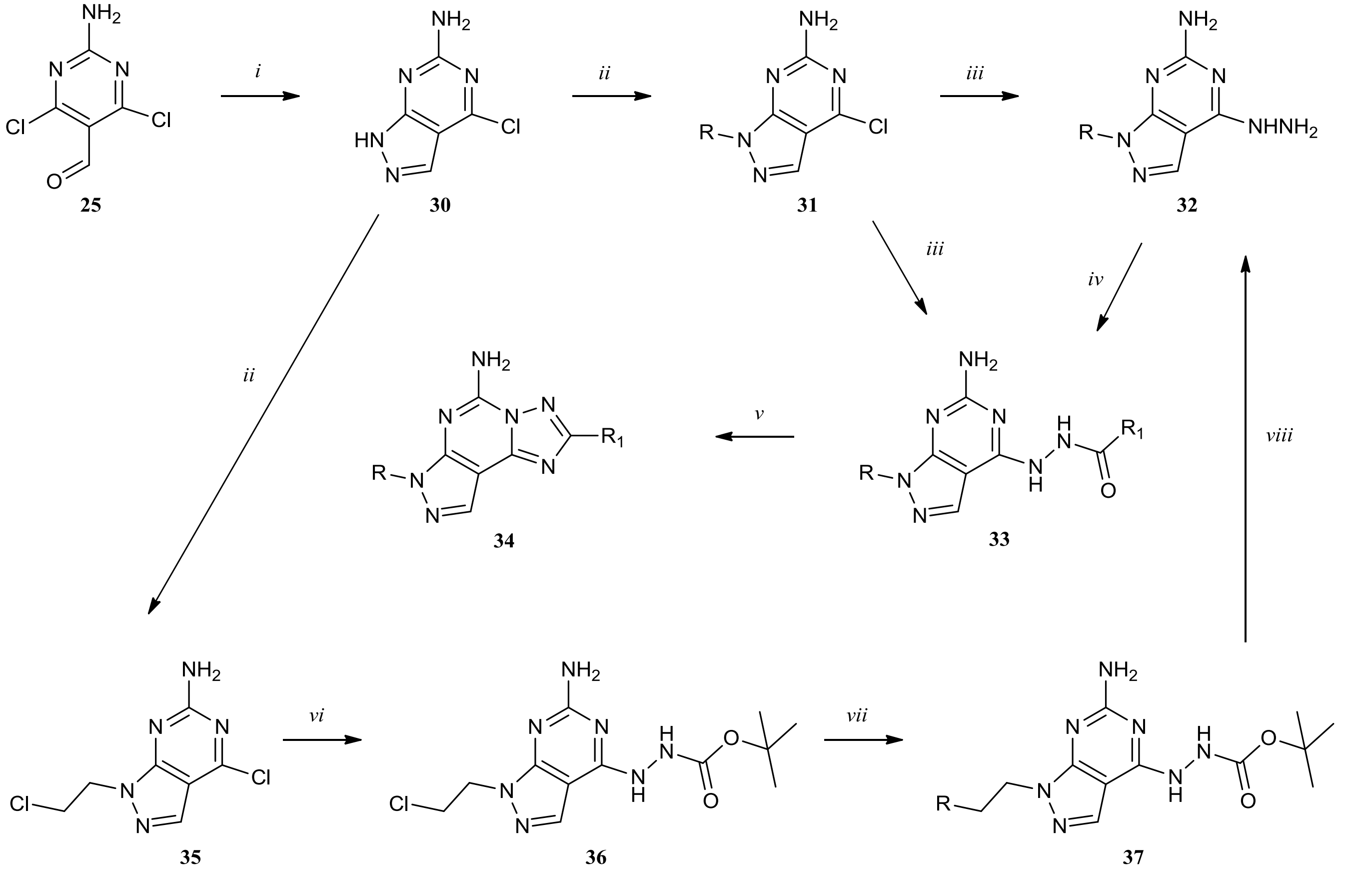
Scheme 2. Reagents: *i*: HCOOH, 130°C, 48h; *ii*: RCl, DMF, K_2CO_3, 100°C, 12h. R = alkyl, arylalkyl.



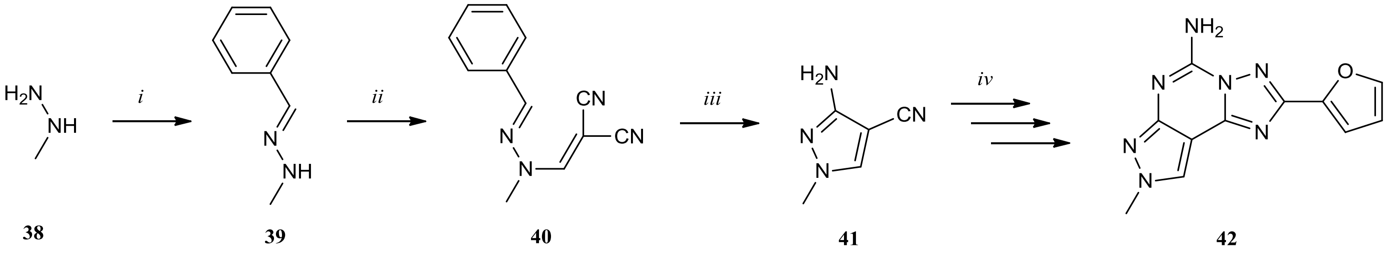
Scheme 3. Reagents: *i*: $ArCONHNH_2$, DIPEA, CH_3CN, rt, overnight; *ii*: $NH_2NH_2.H_2O$, CH_3CN, rfx, 1h.; *iii*: BSA, HMDS, 120°C, overnight; *iv*: RX, NaH, DMF, rt, overnight-24h. Ar = fur-2-yl or thien-2-yl.

alkylated with 1-bromo-2-chloroethane, the obtained derivative **35** was reacted with *tert*-butylcarbazate, which afforded the 4-hydrazino derivative protected on the primary amino group by a Boc (*tert*-butyloxycarbonyl) moiety (**36**). In this way, compound **36** could be further functionalized on the lateral chain at the *N*7 position (e.g. with 1-aryl-piperarazines) (compound **37**) and the subsequent deprotection of the amino moiety with HCl afforded compound **32** which then followed the above mentioned procedure.

All the synthetic pathways hitherto reported for the obtainment of 8-substituted-PTP required the separation of the two isomers of alkylated pyrazole derivatives. In fact, separation occurred at the early stage, such as for the original Gatta's procedure, or at a later stage, such as depicted in Schemes 2 and 3. In addition, a methyl moiety at the 8 position of the PTP scaffold was found to confer selectivity for the A_3 AR subtype. For these reasons, Pastorin *et al.* [32] decided to apply a well-known procedure for the regioselective preparation of the *N*1-methyl-3-amino-4-cyano-pyrazole (**41**). [33,34] Compound **41** was then used as starting material for the synthesis of the desired 8-methyl-PTP (**42**) which allows to easily explore the SAR of these derivatives. [33] The synthesis involved the formation of a hydrazone (**39**) between methylhydrazine (**38**) and benzaldehyde. This initial synthetic step allowed the protection of the non-alkylated nitrogen of methylhydrazine, thus directing the subsequent reaction with ethoxymethylene malondinitrile on the methylated nitrogen (compound **40**).

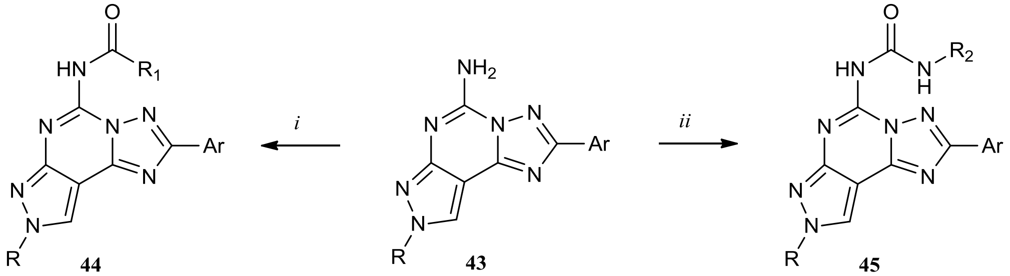


Scheme 4. Reagents: *i*: $NH_2NH_2.H_2O$, DIPEA, DMF, rt, 4h; *ii*: RX, NaH, DMF, 0°C-rt, overnight (for compound **35** $RX=ClCH_2CH_2Br$); *iii*: $NH_2NH_2.H_2O$ or $ArCONHNH_2$, DIPEA, CH_3CN, rt, overnight; *iv*: R_1COCl, DIPEA, DMF, rt, 2h or R_1COOH, HOBt, EDCI, DMF, rt, 1h; *v*: BSA, HMDS, 120°C, overnight; *vi*: $BocNHNH_2$, DMF, 80°C, 18h; *vii*: 1-aryl-piperazine, KI, DMF, 90°C, 68h; *viii*: 4M HCl in dioxane, MeOH-DCM (1:1), rt, 16h. R = arylalkyl, arylthioalkyl, aryloxyalkyl, (4-susbstituted-piperazin-1-yl)alkyl R_1 = Ar, phenyl, pyrrol-2-yl and their substituted derivatives, or nicotinoyl or cyclopent-1-en-1-yl, Ar = fur-2-yl, thien-2-yl.



Scheme 5. Reagents: *i*: PhCHO, EtOH, rfx, 3h; *ii*: $(CN)_2CCHOC_2H_5$, C_6H_6, rfx, 1h; *iii*: HCl conc., EtOH, rfx, 0.5h; *iv*: see scheme 1.

The desired *N*1-methylpyrazole (**41**) was obtained by cyclization of compound **40** in a concentrated solution of hydrochloric acid and then converted to the 2-(furan-2-yl)-8-methyl-8*H*-pyrazolo[4,3-*e*][1,2,4]triazolo[1,5-*c*]pyrimidin-5-amine (**42**) following Gatta *et al.*procedure. [26,32] (Scheme 5)

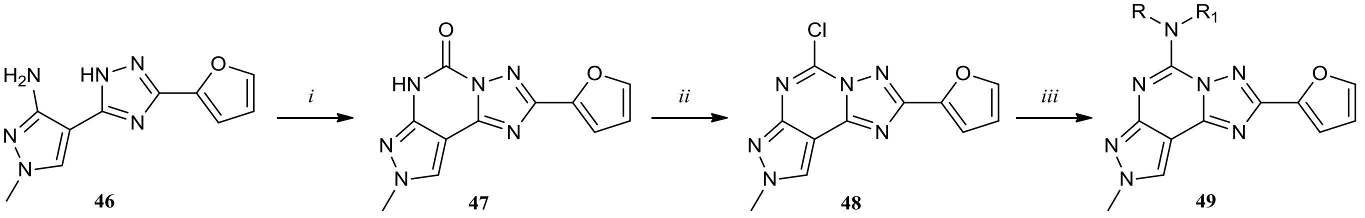


Scheme 6. Reagents: *i*: RCOCl, TEA, THF, rfx, 18h; *ii*: RNCO, rfx, 12-18h. Ar = 2-furyl, Ph or substituted-Ph. R = alkyl or arylalkyl, R_1 =alkyl, aryl, arylalkyl, R_2 = aryl.

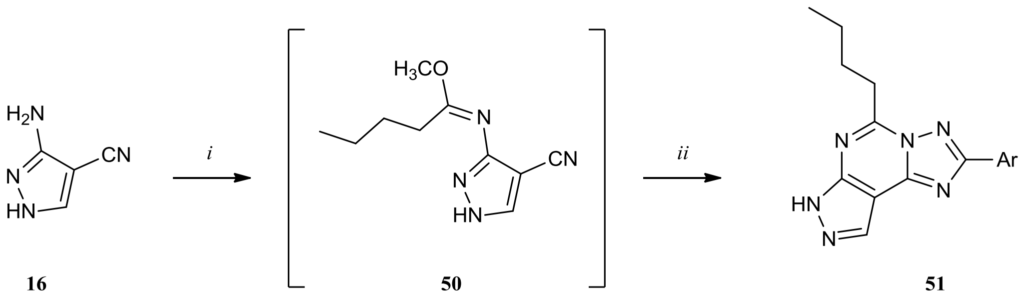
A detailed SAR analysis of the PTP nucleus at the ARs was obtained through investigations on the 5 position of the 8-substituted-PTP scaffold. [30,32,35-41] In particular, the treatment of the 5-amino derivatives (**43**) with various acylchlorides or isocyanates afforded the corresponding 5-amido (**44**) or 5-ureido (**45**) PTP compounds (Scheme 6). In the same conditions, the coupling reaction with isocyanates failed in the case of the 7-substituted-PTP pattern of substitution, probably due to the less nucleophilic character of the amino group at the 5 position. In fact, the nitrogen lone pair of the 5-amino group in 7-substituted derivatives is more extensively delocalized on the heterocyclic ring, than in the 8-substituted derivatives. [35]

An alternative functionalization of the 5 position of PTP was obtained by conversion of the 6,7-dihydro-5*H*-pyrazolo[4,3-*e*][1,2,4]triazolo[1,5-*c*]pyrimidin-5-one (**47**) to the 5-chloro-pyrazolo[4,3-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine derivative (**48**), which could be easily reacted with different moieties containing an amino group. Federico *et al.* [42] reported a method to obtain compound **47** alternate to that of Gatta *et al.* and previously described by Francis *et al.* [44] for the triazoloquinazoline nucleus (Scheme 7). Following this method, the tricyclic urea **47** was prepared by reaction of the corresponding triazolylpyrazole (**46**) with diphosgene in dioxane at reflux. Then, the 5-chloro derivative was obtained by treatment of compound **47** with phosphorous oxychloride, phosphorous pentachloride and pyridine. The simple reaction of the 5-chloro derivative with three equivalents of the desired amino compound, in hot ethanol, gave a large series of 5-aminosubstituted-PTP derivatives (**49**). [42-43]

Finally, Okamura *et al.* reported a one-pot synthesis of fused 1,2,4-triazolo[1,5-*c*]pyrimidines, including pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine derivatives (**51**), which were obtained with very good yields, ranging from 62% to 82% (Scheme 8). PTP derivatives (**51**) were synthesized by condensation of desired iminoesters **50** and arylhydrazide. The iminoester **50** was previously obtained, in the same vessel, by reacting the cyanoaminopyrazole (**16**) with a substituted orthoester (e.g. trimethyl orthovalerate) in the presence of 1% of CSA (*dl*-camphorsulfonic acid) in DMF. [45,46]



Scheme 7. Reagents: *i*: $ClCOOCCl_3$, dry dioxane, 70°C, 1.5h; *ii*: $POCl_3$, PCl_5, Pyr, rfx, 24h; *iii*: R_1RNH_2, EtOH, 110°C, sealed tube, 2h. R = alkyl, cycloalkyl, aralkyl, aminoalkyl, hydroxylalkyl; R_1 = H, alkyl, cycloalkyl, aralkyl, hydroxylalkyl.



Scheme 8. Reagents: *i*: n-BuC$(OCH_3)_3$, 1% CSA, DMF, rt, 1-6h; *ii*: $ArCONHNH_2$ was added in the same vessel, rfx, 1-6h. Ar = Ph or substituted Ph.

2.2. Structure activity relationship

A_{2A} AR antagonists

Gatta and coworkers developed the first series of PTP as AR antagonists. [26] As depicted in Scheme 1, in this preliminary work five different PTP scaffolds have been synthesized: 7-substituted-3,7-dihydro-2*H*-pyrazolo[4,3-*e*][1,2,4]triazolo[1,5-*c*]pyrimidin-2-one (**10**), 2,7-disubstituted-7*H*-pyrazolo[4,3-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine (**12**), 5-amino-2,7-disubstituted-7*H*-pyrazolo[4,3-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine (**15**) and 5-amino-2,8-disubstituted-8*H*-pyrazolo[4,3-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine (**19**). The most promising compounds at the A_{2A} AR were found among the 5-amino-pyrazolo[4,3-*e*][1,2,4]triazolo[1,5-*c*]pyrimidines (**15,19**). In particular, 8-FB-PTP (**52**) showed the highest affinity at the A_{2A} AR (K_i = 1.2 nM), whereas 7-FB-PTP (**53**) displayed a decreased affinity at the A_{2A} AR (K_i = 12 nM) but a better selectivity against the A_1 AR than compound **52** (**53**, A_1/A_{2A} = 15.8 vs **52**, A_1/A_{2A} = 2.8). [26,29] These compounds (**52**,**53**) bear a free amino group at the 5 position, a 4-fluorobenzyl moiety at the 7 or 8 positions and a 2-furyl ring at the 2 position (Figure 4). Further investigations were performed on the pyrazole substitution, maintaining the optimal NH_2 and 2-furyl groups at the 5 and 2 positions, respectively. PTP was substituted on the pyrazole ring, at the 7 and 8 positions, with alkyl or aralkyl moieties, confirming that the 7 substitution improved selectivity at the A_{2A} AR, as clearly demonstrated by comparing compound **54** (Figure 4) with its corresponding 8-β-phenylethyl derivative, which were 52.6 and 3.4 fold selective for the rA_{2A} vs rA_1 ARs compound, respectively. In addition, among 7 substitutions, arylalkyl moieties (e.g. 7-(3-phenyl)propyl, compound **55**, SCH63390) were found to confer higher selectivity at the A_{2A} AR than alkyl moieties (e.g. 7-*n*-butyl, compound **56**). [29,47] Compound **54**, named SCH58261, was synthesized also in its triatiated form, [^{3}H]SCH58261, which demonstrated to label A_{2A} receptors with high affinity (K_D = 0.7 nM) and high specific binding (> 90%). [48] Furthermore, SCH58261 (**54**) maintained antagonism at the A_{2A} AR in *in vivo* cardiovascular models and behave as a central nervous system stimulant, like caffeine. [29]

Nevertheless, compounds **52-56** are highly lipophilic compounds presenting poor solubility in water. In order to enhance the hydrophilic character of PTP derivatives, while retaining high affinity and selectivity at the hA_{2A} AR, several polar moieties have been introduced on the aryl group of the substituent at the 7 position. [49,50] All the synthesized compounds showed a good affinity and selectivity for hA_{2A} AR with different degrees of selectivity over the hA_1 AR. On the other hand, all compounds were inactive at the hA_{2B} and hA_3 ARs. As previously demonstrated, the best length for the side chain is three methylene groups between 7 and the aryl moiety. In fact, promising results were obtained introducing a hydroxyl group at the *para* position of the phenyl ring of SCH63390 (**55**), which increased both affinity and selectivity at the rA_{2A} AR (compound **57**). Also the *para*-methoxy derivative, SCH442316 **58**, demonstrated to be a potent and selective A_{2A} AR antagonist, both at the human and rat receptors (rA_{2A}AR K_i = 5.3 nM, rA_1/rA_{2A} = 487; hA_{2A}AR K_i = 2.7 nM, hA_1/hA_{2A} = 611). [49] Starting from compound **57**, SCH442316 (**58**) can be obtained in its ^{11}C form ([^{11}C]SCH442316), and used as radioligand in positron emission tomography (PET). [51] Amino (compound **59**) and aminomethylene substitutions improved selectivity versus the A_1 AR the polar groups were not sufficient to confer water solubility to the derivatives. On the contrary, the carboxylic acid moiety and, in particular the sulfonic acid group improved water solubility of compounds but affinity at the A_{2A} AR was decreased (e.g. compound **60**). [50]

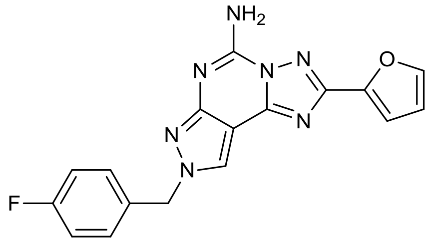
Interestingly, SCH63390 (**55**) was conjugated with a fluorescent moiety, Alexa-Fluor 488, by a *N*-(2-aminoethyl)amido linker, affording MRS5346 (**61**) which was used in A_{2A} adenosine receptor fluorescent polarization assays in place of traditional radioligand based binding studies. MRS5346 (**61**) possessed a K_i affinity value of 111 nM at the hA_{2A} AR, but it was inactive at the other AR subtypes at 10 μM concentration. [52,53]

Because the goal to achieve water solubility by introducing polar group on the phenyl ring of 7-arylalkyl-PTP failed, an additional piperazine ring was introduced as a spacer between the aryl and the alkyl moieties at the 7 position. The aryl ring consists in a phenyl ring variously substituted, in particular at the *para* position. All the developed compounds displayed affinities in the low nanomolar range and retained selectivity over A_1 AR (Figure 5). [5] Considering that compounds were developed as promising therapeutic agents in PD, they were orally employed in the rat haloperidol-induced catalepsy model. The first compound to be tested was the unsubstituted phenyl derivative (**62**), which exhibited a good oral activity at 1h post-dose, but became inactive after 4 h. Inactivation is due to metabolic conversion to the *para*-hydroxyl derivative (**63**) which, despite its high affinity at the A_{2A} AR (hA_{2A}AR K_i = 1.3 nM), shows inactivity at an oral dose of 1 mg/Kg. For these reasons, several derivatives bearing substituted phenyl moieties, able to extend the duration of action, were developed. Among them, 2,4-difluorophenyl derivative (SCH412348, **64**) displayed potent oral activity, but its solubility remained poor. The strategy to improve water solubility of those compounds was to introduce ether-linked substituents. Also in this case, all the derivatives showed nanomolar or subnanomolar affinities at the hA_{2A} AR. The most interesting compound was the 4-methoxyethoxy derivative (**2**), named SCH420814, which exhibited a robust oral anti-cataleptic activity and a solubility in water, as the crystalline free base, of 0.2 μM at native pH (5.1), and 2 mM in 0.01 N HCl. [5] SCH420814 (**2**) was subjected to extensive profiling, reaching also clinical trials under the name of Preladenant (see Section 2.3). [6-8]

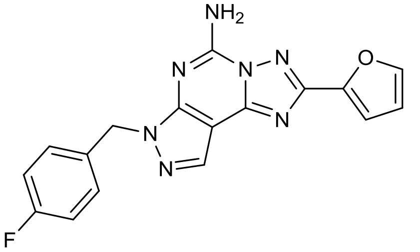
A_3 AR antagonists

The rationale for the development of PTP derivatives as A_3 AR antagonists consists in hybridization between the PTP core, known to behave as antagonist at the A_{2A} ARs, and the arylcarbamoyl moiety of potent rA_3 AR agonists (such as compound **65** in Figure 6), which were known to confer affinity for the rA_3 AR subtype. [54,35] Since the coupling reaction with isocyanates was successful using 8 alkylated compounds, but it failed in the case of the 7 pattern of substitution (see Section 2.1 Chemistry), only 8-substituted derivatives were developed.

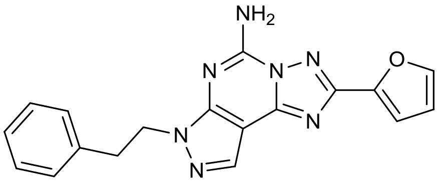
All the synthesized compounds showed high affinity at the hA$_3$ AR (e.g. nanomolar or subnanomolar affinities), with different degrees of selectivity depending on both arylureido and (ar)alkyl substitutions present at the 5 and 8 positions, respectively. Also compounds lacking the phenylcarbamoyl moiety at the N5 position were assayed at the various AR



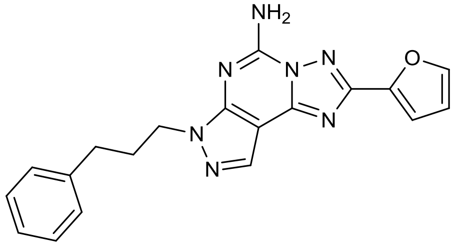
8-FB-PTP, **52**
rA$_1$AR K$_i$=3.3 nM
rA$_{2A}$AR K$_i$=1.2 nM
A$_1$/A$_{2A}$=2.8



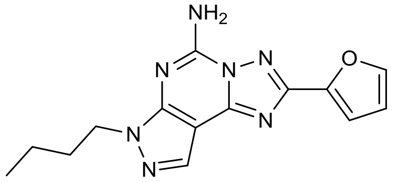
7-FB-PTP, **53**
rA$_1$AR K$_i$=189 nM
rA$_{2A}$AR K$_i$=12.0 nM
A$_1$/A$_{2A}$=15.8



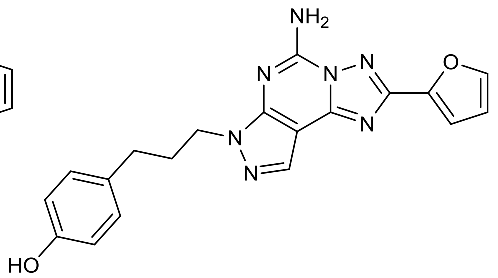
SCH58261, **54**
rA$_1$AR K$_i$=121 nM; hA$_1$AR K$_i$=549 nM
rA$_{2A}$AR K$_i$=2.3 nM; hA$_{2A}$AR K$_i$=1.1 nM
A$_1$/A$_{2A}$= r52.6, h499
hA$_3$AR> 10,000



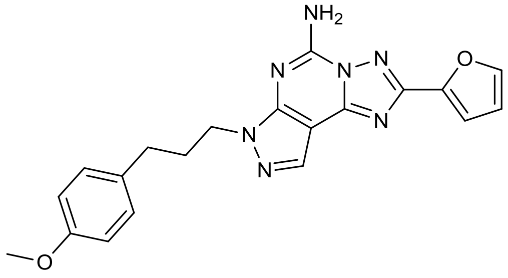
SCH63390, **55**
rA$_1$AR K$_i$=504 nM
rA$_{2A}$AR K$_i$=2.4 nM
A$_1$/A$_{2A}$=210
hA$_3$AR K$_i$> 10,000



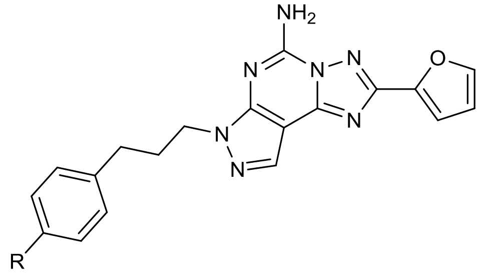
56
rA$_1$AR K$_i$=236 nM
rA$_{2A}$AR K$_i$=8.9 nM
A$_1$/A$_{2A}$=26.5



57
rA$_1$AR K$_i$=741 nM; hA$_1$AR K$_i$=253 nM
rA$_{2A}$AR K$_i$=0.94 nM; hA$_{2A}$AR K$_i$=1.5 nM
hA$_3$AR K$_i$> 10,000

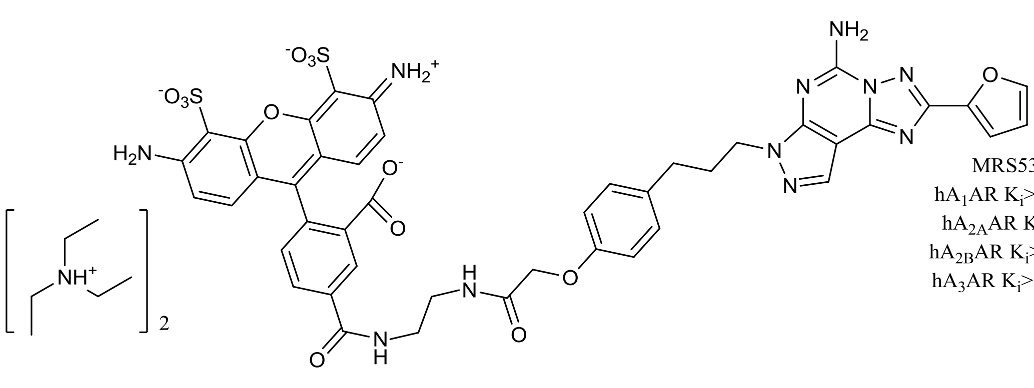


SCH442316, **58**
rA$_1$AR K$_i$=2,825 nM; hA$_1$AR K$_i$=1,650 nM
rA$_{2A}$AR K$_i$=5.3 nM; hA$_{2A}$AR K$_i$=2.7nM
hA$_3$AR K$_i$> 10,000



R=NH$_2$, **59**
hA$_1$AR K$_i$=2,160 nM
hA$_{2A}$AR K$_i$=0.22 nM
hA$_{2B}$AR K$_i$>10,000 nM
hA$_3$AR K$_i$> 10,000 nM

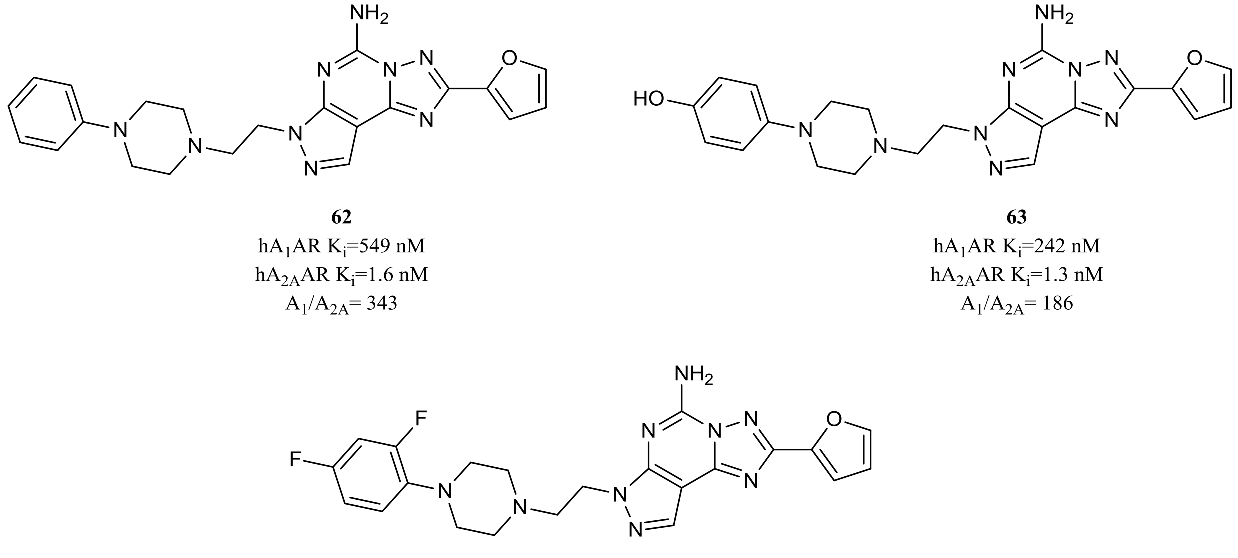
R=SO$_3$H, **60**
hA$_1$AR K$_i$=139 nM
hA$_{2A}$AR K$_i$=140nM
hA$_{2B}$AR K$_i$>10,000 nM
hA$_3$AR K$_i$> 10,000 nM



MRS5346, **61**
hA$_1$AR K$_i$>10,000 nM
hA$_{2A}$AR K$_i$=111 nM
hA$_{2B}$AR K$_i$>10,000 nM
hA$_3$AR K$_i$> 10,000 nM

Figure 4. PTP derivatives as A$_{2A}$ AR antagonists: compounds **52-61.**

subtypes, showing high affinity for the A_{2A} AR, with low selectivity versus the A_1 AR (as observed for 8-FB-PTP (**52**) compound) and low affinity at hA_3 AR subtypes. [30,35] A clear example of this behavior is obtained by comparing compound **66** with compound **67** in Figure 6. Compound **66**, bearing a free amino group at the 5 position, was a poorly selective A_{2A} AR antagonist ($hA_{2A}AR\ K_i = 0.34$ nM; $A_1/A_{2A} = 2.9$), whereas, the introduction of the *para*-methoxyarylcarbamoyl moiety on the amino group at the same position led to a potent and quite selective A_3 AR antagonist (**67**, $hA_3AR\ K_i = 0.98$ nM; $A_1/A_3 = 205$; $A_{2A}/A_3 = 122$). [30,35] An in-depth investigation on the 8 position, by introducing various alkyl and arylalkyl moieties, revealed that small alkyl groups, such as methyl, were the optimal substituents, conferring high affinity at the A_3 AR subtype and good selectivity over the other AR subtypes (i.e. compound **68**, $hA_3AR\ K_i = 0.20$ nM; $A_1/A_3 = 5{,}485$; $A_{2A}/A_3 = 6{,}950$). [30] Starting from the 8-allyl compound **69**, [30] it was possible to obtain the corresponding tritiated derivative, which bears a 8-propyl group, named [^{3}H]-MRE-3008-F20 (**70**), the first selective radioligand for the hA_3 AR behaving as antagonist. [55,56]


62
$hA_1AR\ K_i$=549 nM
$hA_{2A}AR\ K_i$=1.6 nM
A_1/A_{2A}= 343

63
$hA_1AR\ K_i$=242 nM
$hA_{2A}AR\ K_i$=1.3 nM
A_1/A_{2A}= 186

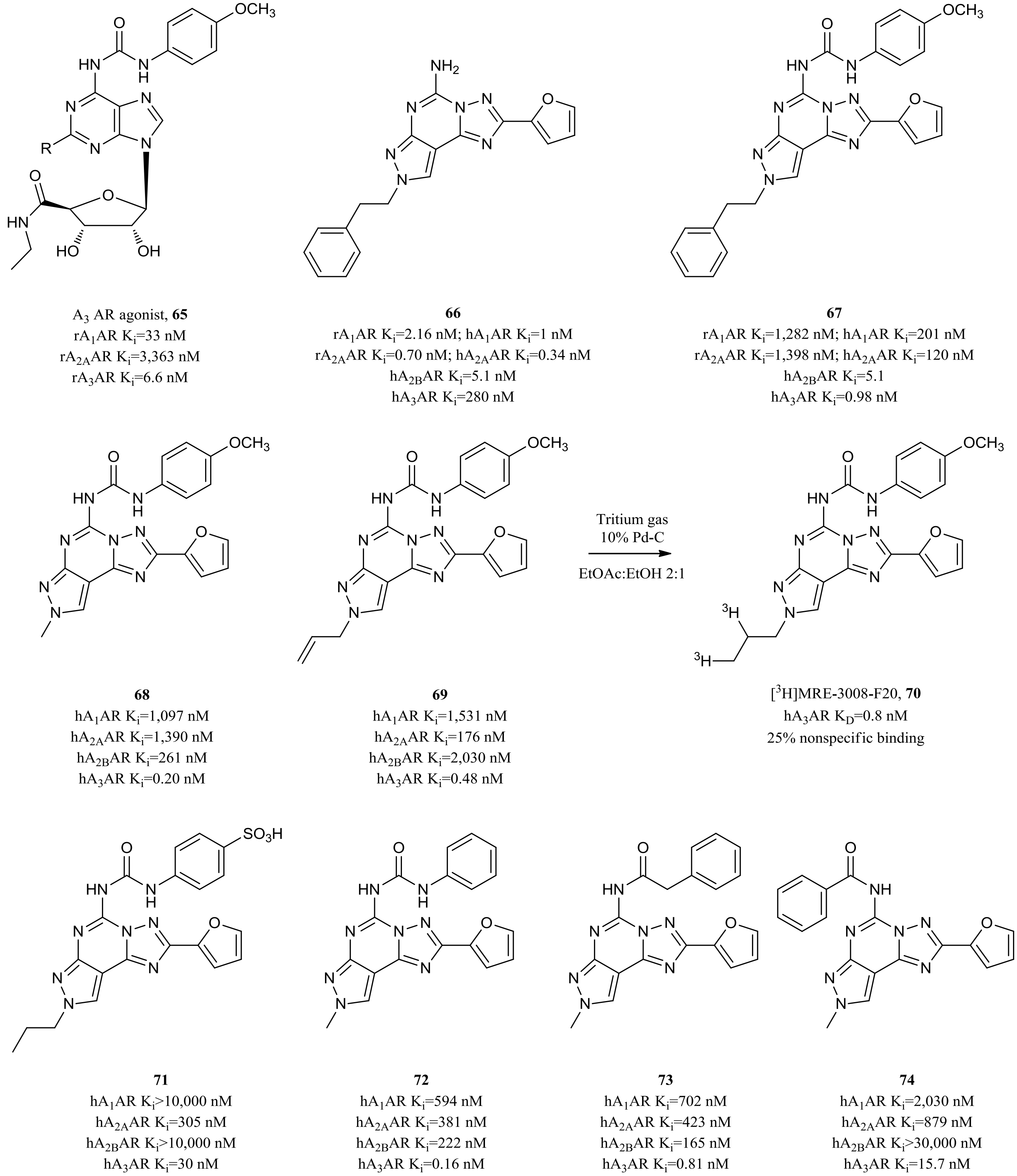
SCH412348, **64**
$hA_1AR\ K_i$>1,000 nM
$hA_{2A}AR\ K_i$=0.6 nM
A_1/A_{2A}> 1,600

Figure 5. PTP derivatives as A_{2A} AR antagonists: compounds **62**-**64**.

Later, also the N5 position was deeply investigated. First of all, different substituents (e.g. chloro, fluoro, bromo, methyl, methoxy, nitro, sulfonic acid, trifluoromethyl) have been introduced at the *ortho*, *meta* and *para* positions of the phenyl ring of the 5-phenylureido group, while maintaining a small alkyl group at the 8 position. Curiously, all the compounds affinity values at A_3 AR were in the range 0.16-3.7 nM, except for derivatives bearing a *para*-sulfonic acid group which dispayed a K_i value above 25 nM (i.e. compound **71**), indicating that *para*-substituent is surrounded by a lipophilic environment inside the A_3 AR binding cleft. Molecular modeling studies on the hA_3 AR suggested that a steric control is present at the *para* and, especially, at the *meta* positions of the phenyl ring. [36] One of the most potent and selective compounds of this series was the unsubstituted compound **72**, which showed a K_i of 0.16 nM at A_3 AR and > 2000-fold selectivity for A_3 over A_1 and A_{2A} ARs. The replacement of phenylureido moiety with a phenylacetyl moiety, maintained affinity at the hA_3 AR, while a slight decreased selectivity was observed (compound **72** vs compound **73** in Figure 6). [36] Instead, the introduction of a shorter amide, such as the benzamide (compound **74**) had a detrimental effect on affinity at the A_3 AR (i.e. compound **74**, $hA_3AR\ K_i = 15.7$ nM). [57] In order to achieve water solubility of these lipophilic compounds, and taking in account the steric control which is present around *para* position, the phenyl ring at the 5 position was replaced with a pyridine ring. In addition, the position and the basicity of pyridine nitrogen were evaluated. [32,37] The most potent compound of the series, which was also the most potent A_3 AR antagonist ever reported (compound **75**), bears the 4-pyridinium moiety as hydrochloride salt. (Figure 7) Moreover, as all the PTP derivatives, also compound **75** proved to be inactive in rats. 1 μM concentration of compound **75** displaced only 35% of the binding of a specific radioligand at rA_3 receptors. [32,37] The 4-pyridylcarbamoyl moiety conferred good water solubility at low pH (<4.0) but poor solubility at physiologic pH. Thus, the replacement of pyridin-4-yl moiety with a 1-(substituted)piperidin-4-yl ring was performed to exploit the higher basicity of this nucleus and the possibility to generate stable, water-soluble salts. The hydrochloride salt of the 1-(cyclohexylmethyl)piperidin-4-yl derivative (**76**, $hA_3AR\ K_i = 9.7$ nM; $A_1/A_3 = 351$; $A_{2A}/A_3 > 515$) showed a solubility of 8 mg/mL at physiological pH and gave a stable aqueous system suitable for intravenous infusion. [41]

More recently, an extensive exploration of a large variety of polyfunctionalized chains (i.e. alkylamino, arylalkylamino, heterocyclic moieties) at the 5-position has been reported. Almost all the synthesized compounds showed affinity for the hA_3 AR in the nanomolar or subnanomolar range, with different levels of selectivity over the other AR subtypes.

The most potent and selective antagonist of the series bears an (S) α-phenylethylamino moiety at the 5 position (**77**, hA_3AR K_i = 0.3 nM, A_1/A_3 = 1,127; A_{2A}/A_3 = 184). Good results in terms of affinity were obtained also with small branched alkylamines and with several benzylamines, however their selectivity versus the other ARs was poor (e.g.



A_3 AR agonist, **65**
rA_1AR K_i=33 nM
$rA_{2A}AR$ K_i=3,363 nM
rA_3AR K_i=6.6 nM

66
rA_1AR K_i=2.16 nM; hA_1AR K_i=1 nM
$rA_{2A}AR$ K_i=0.70 nM; $hA_{2A}AR$ K_i=0.34 nM
$hA_{2B}AR$ K_i=5.1 nM
hA_3AR K_i=280 nM

67
rA_1AR K_i=1,282 nM; hA_1AR K_i=201 nM
$rA_{2A}AR$ K_i=1,398 nM; $hA_{2A}AR$ K_i=120 nM
$hA_{2B}AR$ K_i=5.1
hA_3AR K_i=0.98 nM

68
hA_1AR K_i=1,097 nM
$hA_{2A}AR$ K_i=1,390 nM
$hA_{2B}AR$ K_i=261 nM
hA_3AR K_i=0.20 nM

69
hA_1AR K_i=1,531 nM
$hA_{2A}AR$ K_i=176 nM
$hA_{2B}AR$ K_i=2,030 nM
hA_3AR K_i=0.48 nM

$[^3H]$MRE-3008-F20, **70**
hA_3AR K_D=0.8 nM
25% nonspecific binding

71
hA_1AR K_i>10,000 nM
$hA_{2A}AR$ K_i=305 nM
$hA_{2B}AR$ K_i>10,000 nM
hA_3AR K_i=30 nM

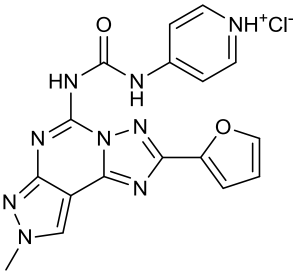
72
hA_1AR K_i=594 nM
$hA_{2A}AR$ K_i=381 nM
$hA_{2B}AR$ K_i=222 nM
hA_3AR K_i=0.16 nM

73
hA_1AR K_i=702 nM
$hA_{2A}AR$ K_i=423 nM
$hA_{2B}AR$ K_i=165 nM
hA_3AR K_i=0.81 nM

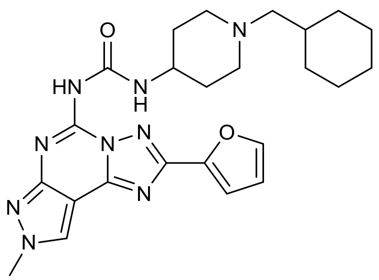
74
hA_1AR K_i=2,030 nM
$hA_{2A}AR$ K_i=879 nM
$hA_{2B}AR$ K_i>30,000 nM
hA_3AR K_i=15.7 nM

Figure 6. PTP derivatives as A_3 AR antagonists: compounds **65-74**.

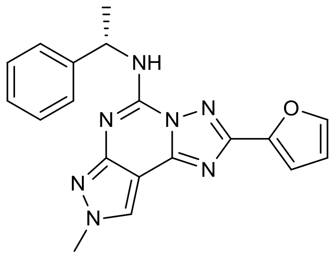
compound **78**, $A_1/A_3 = 25$; $A_{2A}/A_3 = 47.4$). [42]



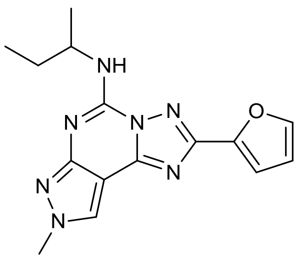
75
$hA_1AR\ K_i=355\ nM$
$hA_{2A}AR\ K_i=110\ nM$
$hA_{2B}AR\ IC_{50}>1,000\ nM$
$hA_3AR\ K_i=0.014\ nM$



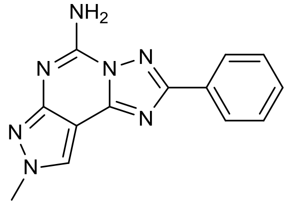
76
$hA_1AR\ K_i=3,408\ nM$
$hA_{2A}AR\ K_i>5,000\ nM$
$hA_{2B}AR\ K_i>5,000\ nM$
$hA_3AR\ K_i=9.7\ nM$



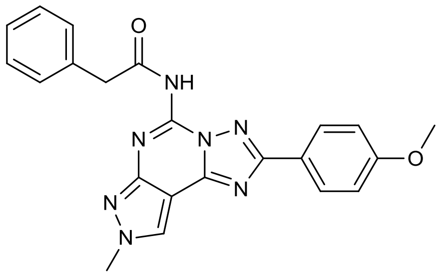
77
$hA_1AR\ K_i=338\ nM$
$hA_{2A}AR\ K_i=55.2\ nM$
$hA_{2B}AR\ K_i>5,290\ nM$
$hA_3AR\ K_i=0.30\ nM$



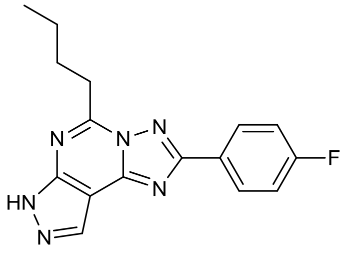
78
$hA_1AR\ K_i=131\ nM$
$hA_{2A}AR\ K_i=248\ nM$
$hA_{2B}AR\ K_i=3,860\ nM$
$hA_3AR\ K_i=5.23\ nM$



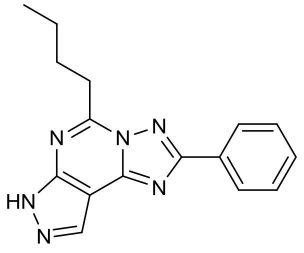
79
$hA_1AR\ K_i=339\ nM$
$hA_{2A}AR\ K_i=121\ nM$
$hA_{2B}AR\ K_i>10,000\ nM$
$hA_3AR\ K_i=75\ nM$



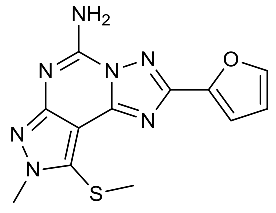
80
$hA_1AR\ K_i>30,000\ nM$
$hA_{2A}AR\ K_i>100,000\ nM$
$hA_{2B}AR\ K_i>10,000\ nM$
$hA_3AR\ K_i=0.241\ nM$



81
$hA_1AR\ IC_{50}=610\ nM$
$hA_{2A}AR\ IC_{50}>10,000\ nM$
$hA_{2B}AR\ IC_{50}>9,400\ nM$
$hA_3AR\ IC_{50}=1.9\ nM$



82
$hA_1AR\ IC_{50}=27\ nM$
$hA_{2A}AR\ IC_{50}=190\ nM$
$hA_{2B}AR\ IC_{50}=2,600\ nM$
$hA_3AR\ IC_{50}=2.1\ nM$



83
$hA_1AR\ K_i=8.4\ nM$
$hA_{2A}AR\ K_i=1.2\ nM$
$hA_{2B}AR\ K_i=10.3\ nM$
$hA_3AR\ K_i=35\ nM$

Figure 7. PTP derivatives as A_3 AR antagonists: compounds **75**-**83**.

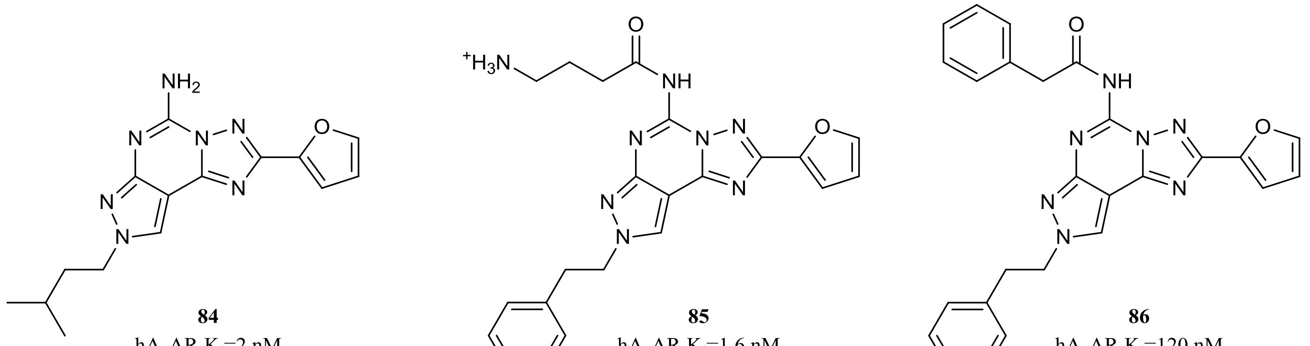
Substitution at position 2 of the PTP tricyclic system has not been deeply explored, being essentially limited to the introduction of a furyl group. Baraldi *et al.* have found that the substitution of the furan ring with phenyl or substituted-phenyl and furyl rings led to a loss of affinity at all the AR subtypes. Remarkably, substitution at the pyrazole ring of PTP nucleus occurred at the 7 rather than at the 8 position. [58] In addition, several reported tricyclic hA_3 AR antagonists bear a substituted phenyl moiety at the position equivalent to that of furan ring in PTP derivatives. [59] For these reasons, Cheong and coll. [39] designed and synthesized a series of PTP bearing a (*para*-substituted)-phenyl ring at the 2 position, while maintaining either methyl or phenyl-ethyl groups at 8 position and a free amine, phenylacetamide or (bis-)benzamide at the 5 position. Interestingly, 5-amino derivatives were unselective AR antagonists (i.e. compound **79**), whereas the 5-phenylacetamido derivatives retained high affinity at the hA_3 AR and showed a remarkably improved selectivity profile toward the other AR subtypes in comparison to the 2-furyl PTP derivatives (e.g. compound **80**, $hA_3AR\ K_i = 0.241$ nM; $A_1/A_3 > 124,000$; $A_{2A}/A_3 > 415,000$ vs compound **73**, $hA_3AR\ K_i = 0.81$ nM; $A_1/A_3 = 877$; $A_{2A}/A_3 = 522$). [39]

Okamura *et al.* developed different fused 1,2,4-triazolo[1,5-*c*]pyrimidine derivatives as A_3 AR antagonists, including 5-*n*-butyl-pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidines. (Figure 7) Concerning the 2 position of the PTP scaffold, different substituted phenyl moieties have been introduced, confirming that the selectivity at the hA_3 AR over the hA_{2A} AR was greater with 4-substituted-phenyl moieties than with 2- or 3- substituted-phenyl groups, or with no substitution (e.g. compound **81** (4-F-Ph), hA$_3$AR K$_i$ = 1.9 nM; A$_1$/A$_3$ = 321; A$_{2A}$/A$_3$ > 5,263 vs compound **82** (Ph), hA$_3$AR K$_i$ = 2.1 nM; A$_1$/A$_3$ = 12.9; A$_{2A}$/A$_3$ = 90.5). [46]

Finally, also the 9 position was examined by Baraldi *et al.* by introducing substituents with different steric and hydrophilic/lipophilic features, such as: alkylamino, arylamino, alkylthio or N-methylpiperazino moieties. These compounds maintained the classical methyl group at the 8 position and the free amino, phenylureido or arylacetamido moieties at the 5 position of the PTP nucleus. [57] Notably, the introduction of a substituent at the 9 position led to a complete loss of selectivity, even if affinity at the ARs in the nanomolar range was maintained. Generally, the methylthio group at the 9 position was the best tolerated, such as in compound **83**, which exhibited K$_i$ values lower than 35 nM at the four AR subtypes. [58]

Antagonists for other AR subtypes

Although a clear SAR profile for the PTP derivatives have been outlined at the A$_{2A}$ and A$_3$ ARs, a similar correlation at the A$_1$ and A$_{2B}$ ARs is still lacking (Figure 8). Concerning both A$_1$ and A$_{2B}$ AR subtypes, potency was achieved but selectivity was very poor. Nevertheless, some common features could be observed in the potent, even if not selective, PTP A$_{2B}$ antagonists. In fact, substitution at the 8 position was preferred to that at the 7 position for binding at the A$_{2B}$ AR; moreover, arylalkyl or branched alkyl moieties were preferred to small alkyl moieties. Compounds **66** (Figure 6) and **84** (Figure 9), bearing a phenylethyl and isopentyl groups at the 8 position, respectively, showed affinity in the nanomolar range at the A$_{2B}$ AR (e.g. **66**, hA$_{2B}$AR K$_i$ = 5.1 nM; **84**, hA$_{2B}$AR K$_i$ = 9.1 nM). [30] A slight improvement of selectivity towards A$_{2A}$ AR was achieved by introducing a γ-aminobutyrylamido or a phenylacetamido group at the 5 position of the PTP nucleus (compounds **85** and **86**), but unfortunately a simultaneous loss of selectivity over the A$_1$ and A$_3$ ARs was observed. [30,60]



84
hA$_1$AR K$_i$=2 nM
hA$_{2A}$AR K$_i$=0.78 nM
hA$_{2B}$AR K$_i$=9.1 nM
hA$_3$AR K$_i$=700 nM

85
hA$_1$AR K$_i$=1.6 nM
hA$_{2A}$AR K$_i$=54 nM
hA$_{2B}$AR K$_i$=27 nM
hA$_3$AR K$_i$=65 nM

86
hA$_1$AR K$_i$=120 nM
hA$_{2A}$AR K$_i$=60 nM
hA$_{2B}$AR K$_i$=35 nM
hA$_3$AR K$_i$=45 nM

Figure 9. PTP derivatives as unselective AR antagonists.

Even regarding the A$_1$ AR subtype, 8-substituted-PTPs showed improved affinity if compared to the 7-substituted derivatives: see compounds **53** and **54** (Figure 4) versus compounds **52** and **66** (Figures 4,6), which bear an arylalkyl group at the 7 and 8 position, respectively. [30] In addition, substitution on the amino group at the 5 position of 8-substitued PTP derivatives seemed to have a detrimental effect in terms of affinity at the A$_1$ AR. In fact, compound **86** (Figure 9), which is the 5-phenylacetyl derivative of compound **66**, displayed a 120-fold detrimental effect on affinity value at the hA$_1$ AR (**86**, hA$_1$AR K$_i$ = 120 nM; **66**, hA$_1$AR K$_i$ = 1 nM). [30,38]

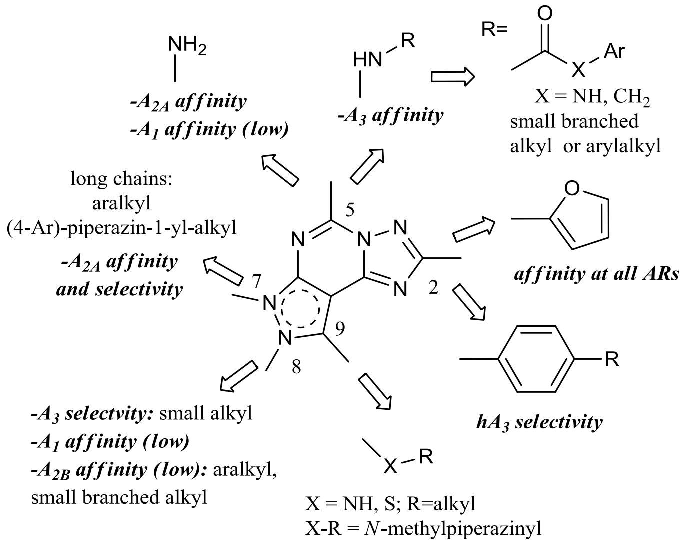
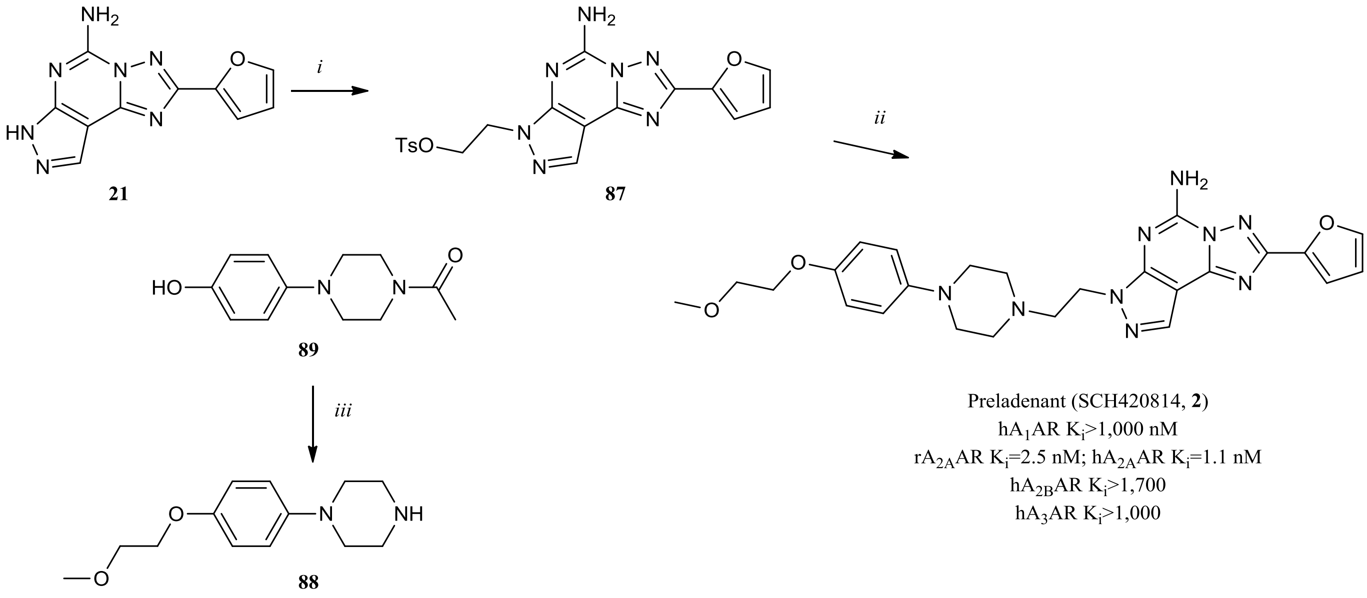


Figure 8. Structure activity relationship of PTP derivatives at ARs.

2.3 Preladenant: a case study

Preladenant (SCH420814, **2**), 2-(furan-2-yl)-7-(2-(4-(4-(2-methoxyethoxy)phenyl)piperazin-1-yl)ethyl)-7*H*-pyrazolo[4,3-*e*][1,2,4]triazolo[1,5-*c*]pyrimidin-5-amine, is a pyrazolo[4,3-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine derivative developed by Schering in a program focused on searching A$_{2A}$ AR antagonists for the treatment of PD. PD is a neurological disorder characterized by bradykinesia, rigidity and tremor. These symptoms are mainly due to degeneration of dopaminergic nigrostriatal neurons and to the resultant depletion of striatal dopamine. [61] In the central nervous system (CNS), the A$_{2A}$ ARs are almost exclusively expressed in dendritic spines of the striatopallidal neurons and colocalize with the D$_2$ receptors. Further studies demonstrated that A$_{2A}$ ARs functionally oppose the actions of dopamine D_2 receptors on GABAergic striatopallidal neurons. In fact, A_{2A} ARs and D_2 receptors have also been found to form heteromers. These findings raised the possibility to use A_{2A} AR antagonists to reduce the postsynaptic effects of dopamine depletion, and in turn diminish the motor deficits of PD. [61,62]

Schering-Plough Research Institute, starting from the observation that SCH58261 (**54**) is a promising A_{2A} AR antagonist with a PTP scaffold, has developed a new series of derivatives with the aim to simultaneously enhance selectivity among the other ARs and improve pharmacokinetic properties of the PTP compounds. A variety of 7-alkylated PTPs were prepared, in particular, 7-(4-arylpiperazin-1-yl)ethyl derivatives were of particular interest. Almost all the synthesized derivatives showed high A_{2A} AR binding affinity, with high selectivity over A_1 AR. Further efforts were directed toward improving solubility by introducing ether-linked substituents at the *para* position of the aryl moiety; these compounds maintained high affinity and selectivity at the hA_{2A} AR. The most promising compound resulted to be SCH420814 (**2**), then named Preladenant (**2**). [5] Preladenant (**2**), whose synthesis is reported in Scheme 9, it is prepared by alkylation of compound **21** (obtained as shown in Scheme 3) with ethylene glycol ditosylate, which afforded the desired tosylate derivative **87** as the major product, along with minor amounts of the 8-alkylated compound. Amination of compound **87** with the *N*-arylpiperazine **88** in DMF at 80 °C afforded Preladenant (**2**) in good yields. The necessary N^1-(4-(2-methoxyethoxy)phenyl)piperazine (**88**) is obtained by reacting the 1-acetyl-4-(4-hydroxyphenyl)piperazine (**90**) with sodium hydride in DMF and then adding the 2-bromo-ethylmethylether. [31]



21 **87** **89** **88**

Preladenant (SCH420814, **2**)
$hA_1AR\ K_i>1{,}000$ nM
$rA_{2A}AR\ K_i=2.5$ nM; $hA_{2A}AR\ K_i=1.1$ nM
$hA_{2B}AR\ K_i>1{,}700$
$hA_3AR\ K_i>1{,}000$

Scheme 9. Reagents: *i*: ethylene glycol ditosylate, NaH, DMF, N_2, rt, 24h; *ii*: compound **88** DMF, 80°C, 20h; *iii*: NaH, DMF, $CH_3OCH_2CH_2Br$, rt, 18h, followed by HCl 6N, rfx, 1h.

In a preliminary profiling, Preladenant (**2**) exhibited high affinity for both human and rat A_{2A} ARs, with K_i values of 1.1 and 2.5 nM, respectively. In addition, the compound was more than 1000-fold selective for hA_{2A} ARs over hA_1, hA_{2B} and hA_3 ARs. In cell-based assays, Preladenant (**2**) blocked adenylate cyclase activity stimulated by the A_{2A} AR agonist CGS21680 with K_b values of 0.7 nM (rat) and 1.3 nM (human), confirming that it is an antagonist. The compound did not show significant binding at a concentration of 10 μM against a panel of 60 other receptors, ion channels and transporters. *In vivo*, SCH420814 dose-dependently reversed haloperidol-induced catalepsy (haloperidol is a D_2 receptor antagonist) in the rat and in the squirrel monkeys. The solubility of Preladenant (**2**) as crystalline free base in water is 0.2 μM at native pH (5.1) and 2 mM in 0.01 N HCl. [5] One of the drawbacks of current dopamine replacement therapies for PD is the development of dyskinesias after chronic activation of D_2 receptors, which correlates with an increased number of A_{2A} ARs. Therefore, treatment with an A_{2A} AR antagonist, by blocking the upregulated A_{2A} AR and avoiding the direct activation of D_2 receptors, may ameliorate PD symptoms while reducing the risk of developing dyskinesias. In rodent models of PD, Preladenant (**2**) demonstrated to be efficacious without leading dyskinesias both as monotherapy or coupled with L-Dopa. In addition, PD patients often suffer depression and Preladenant (**2**) gave positive results in the FST (forced swim test) and TST (tail suspension test) models of behavioral despair, suggesting the potential use of A_{2A} AR antagonists in the treatment of also non-motor PD symptoms. Supported by all these results, Preladenant (**2**) has become an excellent candidate for clinical studies on the treatment of both the motor and non-motor symptoms of PD. [6]

In a phase I study, the safety, tolerability and pharmacokinetics of Preladenant (**2**) at single and multiple oral doses were evaluated. Preladenant (**2**) was generally tolerated up to 200 mg/day after oral administration of both single dose and multiple doses for 10 consecutive days. Preladenant (**2**) reached peak plasma concentrations in about 1 h and then declined rapidly; the effective half-life was about 8 h. [63] A phase II study displayed that 5 and 10 mg Preladenant (**2**) twice daily might be clinically useful to reduce off time in Parkinsonian patients with motor fluctuations who are receiving levodopa and other antiparkinsonian drugs (1 and 2 mg of Preladenant (**2**) are ineffective). Mild transient increases in blood pressure were observed and these are consistent with the expected biological effects of A_{2A} AR inhibition. [64] These results were confirmed by a second phase II study, which evaluated the long-term safety and efficacy of Preladenant (**2**). [65] Preladenant (**2**) was prepared also in its radiolabeled form ^{14}C-preladenant (Preladenant (**2**) was labeled at the carbon at position 2) and orally administered in human healthy subjects in order to study its metabolism and excretion. The identified metabolites of Preladenant have been depicted in Figure 10 and principally consist of O-demethylation and subsequent oxidation, N-dealkylation and furan ring opening. After a single administration of Preladenant (**2**), the dose was principally eliminated in feces (>65%) while a minor part of the dose was eliminated in urine (<10%). In plasma were identified both Preladenant (**2**) and its metabolites **92** and **93**. In the feces, the 14% of the dose is the unaltered drug, while the major metabolites are compounds **92** (29%), **91** (22%) and **90** (6%). In urine Preladenant (**2**) was not detected and the most prevalent metabolite is compound **92** (3.53%). Among the identified metabolites, compounds **92-94** retain high affinity at the hA_{2A} AR. [66]

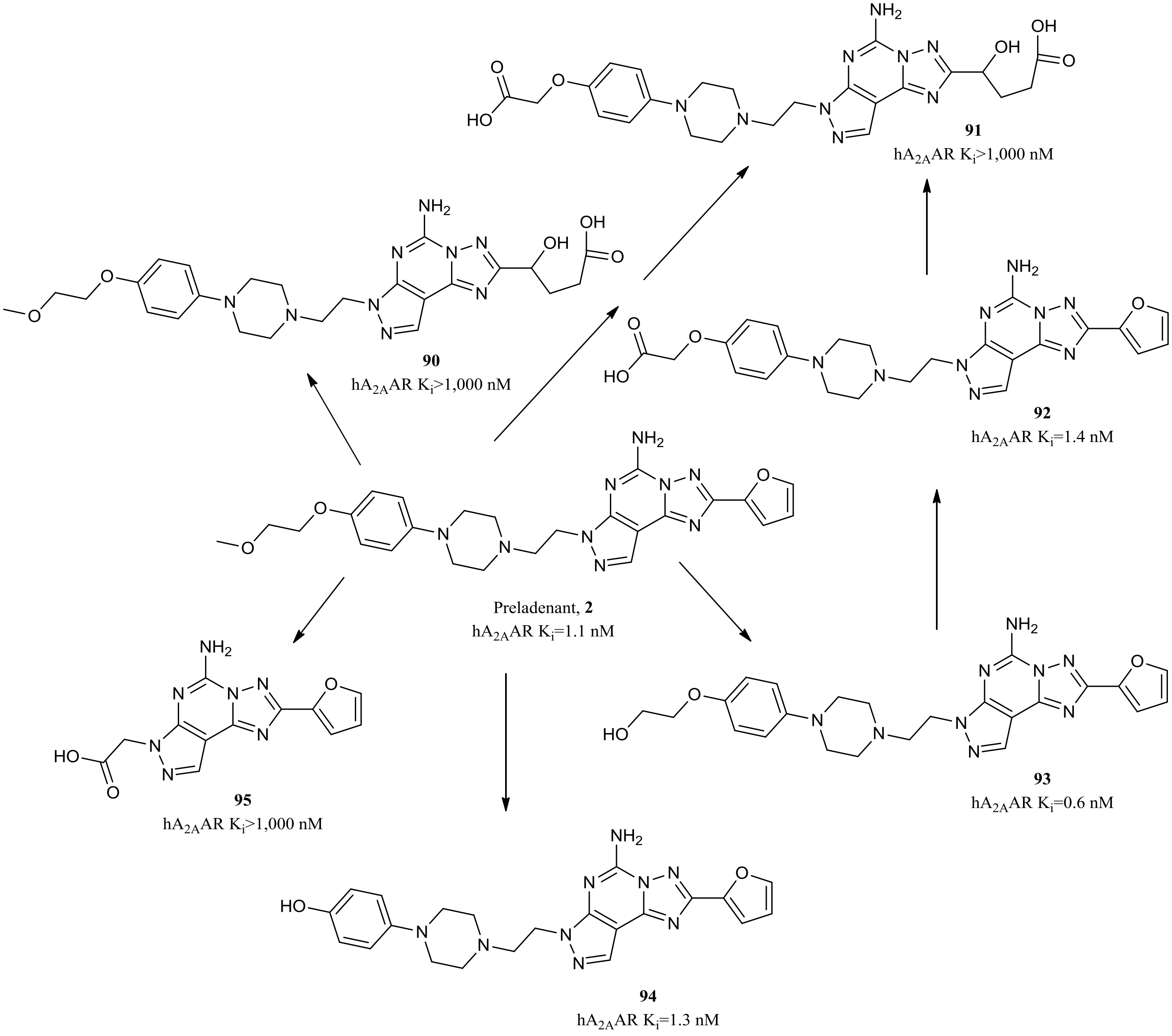
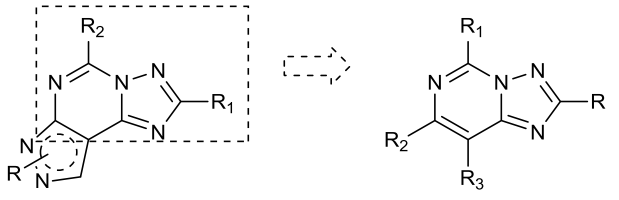


Figure 10. Preladenant metabolites.

Unfortunately, phase III clinical trials of both adjunctive and monotherapy with Preladenant (**2**) in patients with PD, failed to prove drug efficacy. Nevertheless, trials were also unable to identify benefit for rasagiline, the active control; therefore it is difficult to interpret these results and what they mean for Preladenant (**2**) in early Parkinson's disease. [7,8]

3. [1,2,4]TRIAZOLO[1,5-*c*]PYRIMIDINES



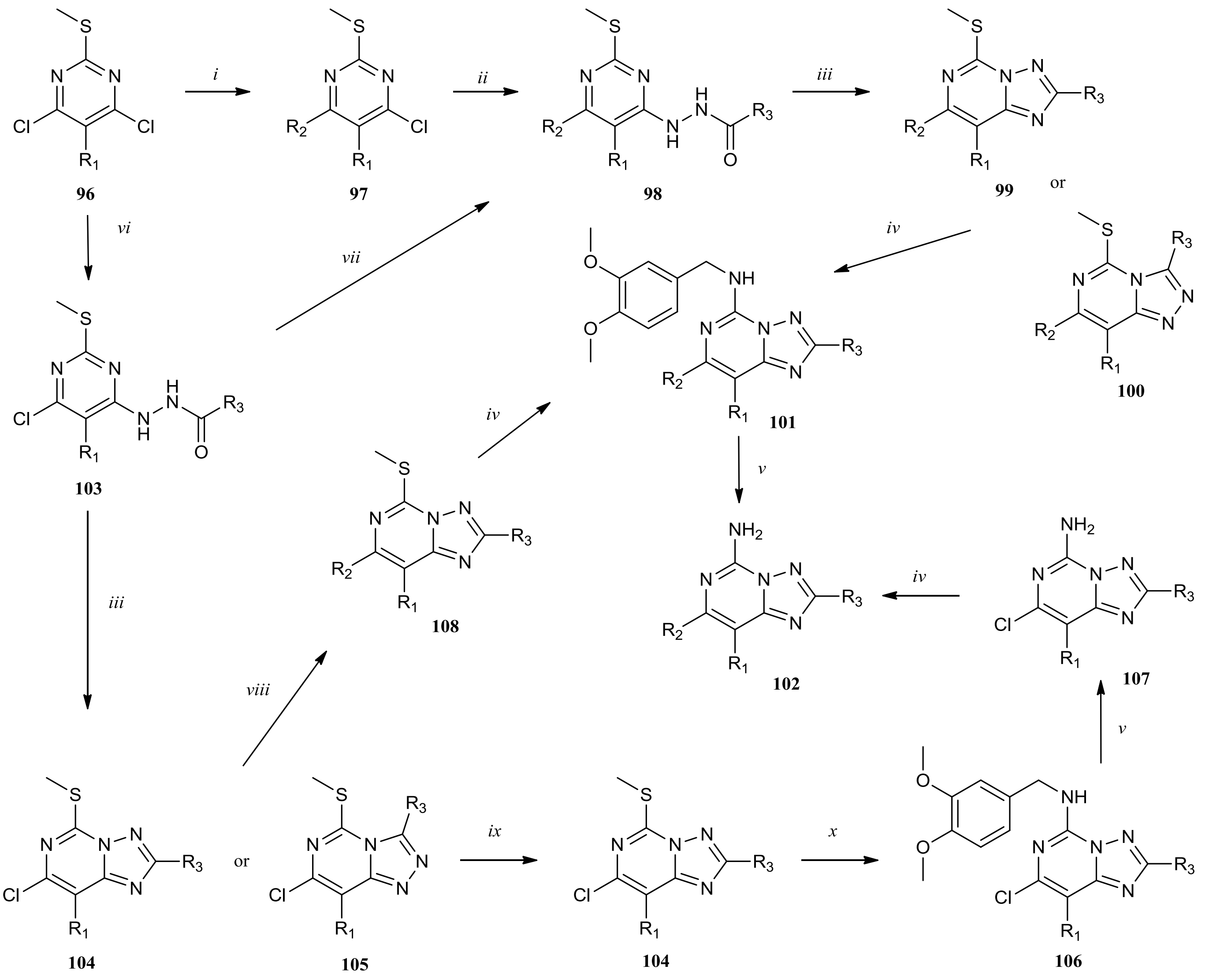
PTP, **1** TP, **3**

Figure 11. TPs as simplified analogs of the PTPs.

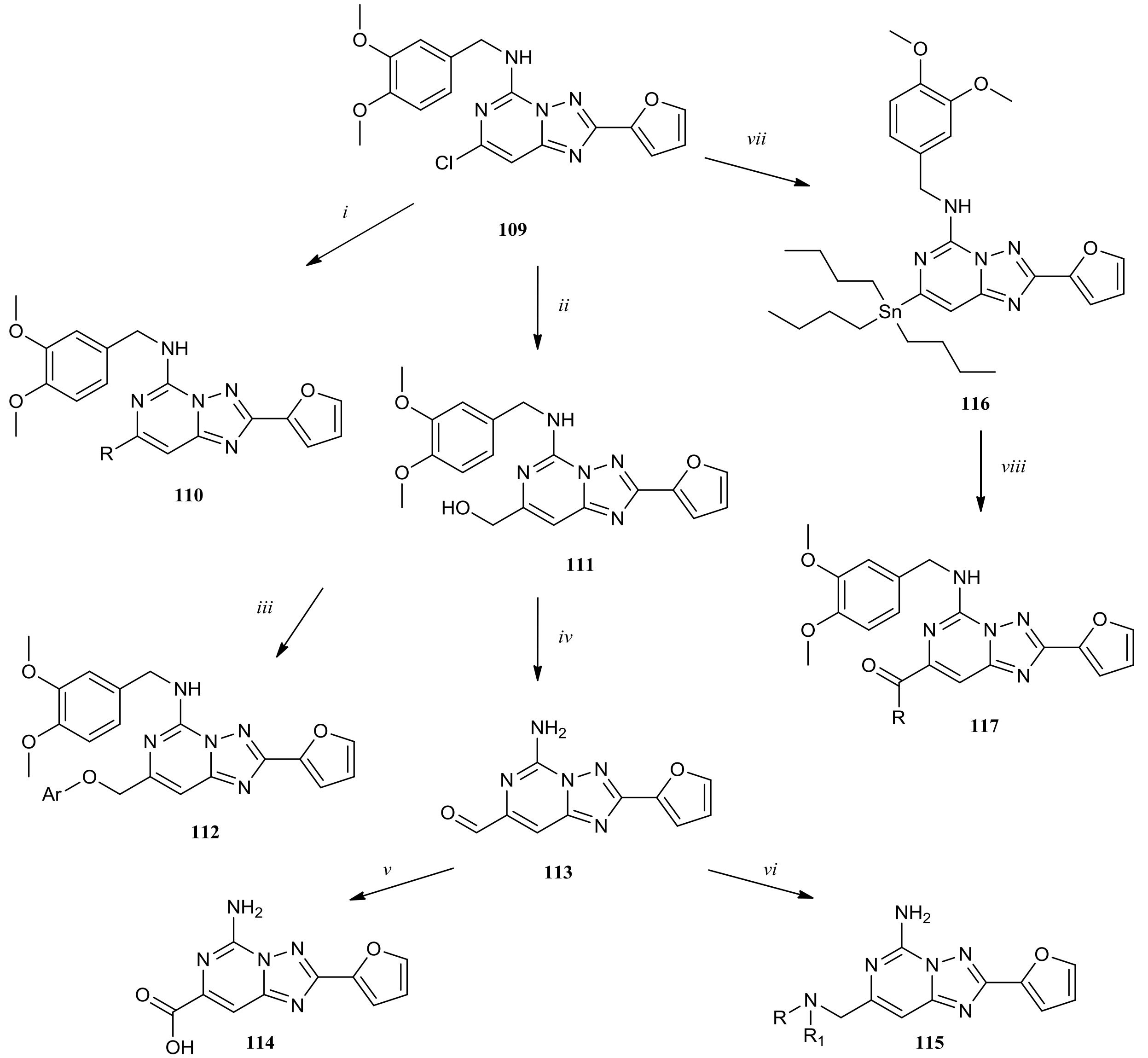
The [1,2,4]triazolo[1,5-*c*]pyrimidine (TP) is a versatile scaffold, which has been reported to bind different protein targets depending on the pattern of substitutions present on its structure, [67,70] including adenosine receptors [9-14]. In fact, TP nucleus (**3**) is a clearly structural simplification of the PTP scaffold (**1**), in which pyrazole ring has been removed. (Figure 11) Here are reviewed chemistry and SAR profile relative to TP derivatives developed as AR antagonists.

3.1. Chemistry

Kyowa Hakko Kogyo Co. was the first company to develop TP derivatives for their use as A_{2A} AR antagonists. [9] The Japanese company deeply explored the chemistry of TP compounds as evidently shown in Schemes 10-12. Several alternative approaches could be used to prepare the TP compounds; in particular, modifications in the series of the reaction steps allow to introduce the desired substituents on the TP nucleus. The starting material could be a compound of general structure **96** (Scheme 10). 4,6-dichloro-2-(methylthio)pyrimidine but also some 4,6-dichloro-5-substituted-2-(methylthio)pyrimidines are commercially available or easily achievable by synthesis.



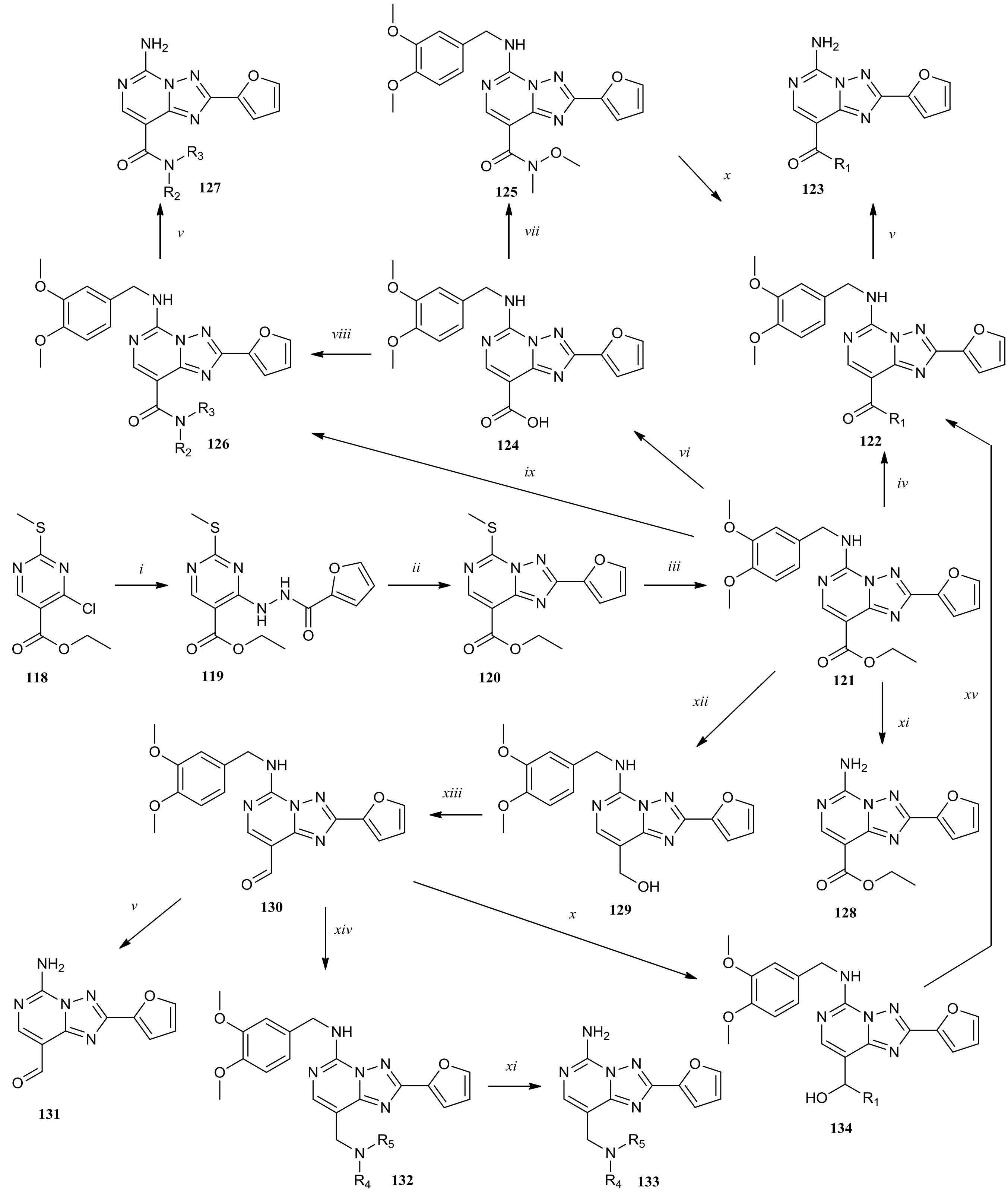
Scheme 10. Reagents: *i*: R_2H, NaH, THF, 0°C-rt, 4h; *ii*: $R_3CONHNH_2$, DBU, DMF (or dioxane-water 3:1), 100°C, 5h; *iii*: P_2O_5, HMDSO, xylene, 90°C, 2h, rfx, 5h; *iv*: veratrylamine or R_2H, DMSO, 140°C, 2-4h; *v*: TFA, anisole, CF_3SO_3H, 0°C-rt, 4h; *vi*: $R_3CONHNH_2$, DBU, THF, rt, 5h; *vii*: R_2H, DMAP, rfx, overnight or R_2H, DMSO, 130°C, overnight; *viii*: R_2H, DBU, THF, rfx, 3h; *ix*: purification by column chromatography; *x*: THF, DBU, 0°c-rt, 1h, veratrylamine, 50°C, 3h; R_1 = H, CH_3 or Ph; R_2 = aryloxy, arylthio, benzylthio, piperidino, morpholino, piperazino, *N1*-substituted-piperazino and various other saturated nitrogen containing heterocycles; R_3 = furyl, thienyl, Ph, 3-pyr, 3-OCH_3Ph.



Scheme 11. Reagents: *i*: $RB(OH)_2$, $(PPh_3)_2PdCl_2$, Na_2CO_3, THF, rfx, 9h, or $(n\text{-}But)_3SnR$, $(PPh_3)_2PdCl_2$, toluene, rfx 9h, with R = Ar, arylalkyl, alkylethynyl, arylethynyl, vinyl; *ii*: a) tert-butyl-dimethyl-(tributylstannylmethoxy)silane, $(PPh_3)_2PdCl_2$, toluene, rfx, 8h, b) TBAF, THF, rt, 1h; *iii*: ArOH, PPh_3 and DEAD, rt, 1-12h or ArOH, (Tributylphosphoranylidene)acetonitrile, THF, 120°C, 19-25h; *iv*: a) TFA, anisole, CF_3SO_3H, rt, 7h, b) MnO_2, DMF, rt, 20h; *v*: a) AcOH, NH_2SO_3H, b) NaOCl, rt, 4h; *vi*: RR_1NH, $Na(CH_3COO)_3BH$, rt, 16h; *vii*: bis(tributyltin), $(PPh_3)_2PdCl_2$, DMF, 100°C, 9h; *viii*: RCOCl, $(PPh_3)_2PdCl_2$, THF, rfx, 10h, with R = Ar.

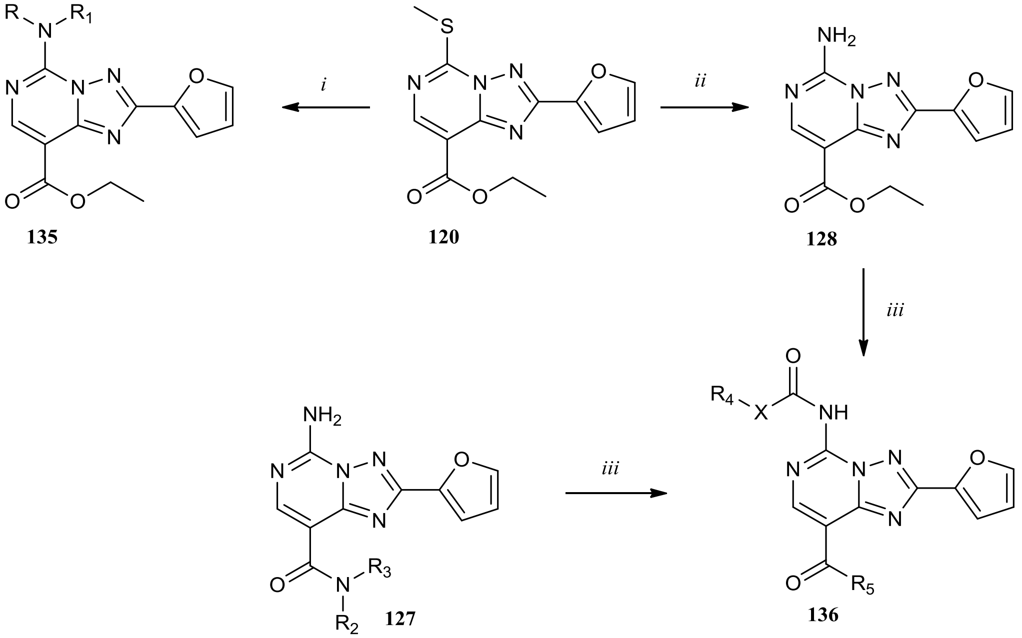
One synthetic approach considered the consecutive substitution of the two chlorine atoms on the pyrimidine ring (**96**): the first with nucleophilic compounds such as amines, alcohols and thiols, obtaining compound **97**, and then the second with the appropriated aryl hydrazide, which afforded compound **98**. These two steps could be inverted (**103**, **98**) and, in both cases, the first substitution was made at room temperature in the presence of a base, whereas the second required heating. The hydrazido derivative **98** underwent dehydrative cyclization with phosphorous pentoxide and hexamethyldisiloxane (HMDSO), which formed the dehydrating agent polyphosphoric acid trimethylsilyl ester (PPSE), in xylene. TP derivative **99** was obtained by Dimroth rearrangement of [1,2,4]triazolo[4,3-*c*]pyrimidine derivative **100**, which generally occurred during the reaction. In some cases, only compound **99** were isolated, indicating that rearrangement was favored. Compound **99** were reacted with veratrylamine in DMSO (dimethylsulfoxide) at 140°C to afford compound **101**, which is a 5-amino-TP derivative protected on the amino group with veratryl moiety. Also this reaction facilitates Dimroth rearrangement, so reaction could also be made on the unpurified mix of compounds **99** and **100**. Deprotection of amino group at the 5 position of compound **101** was obtained by treatment with trifluoroacetic acid (TFA), triflic acid and anisole. This last step led to the desired 5-amino-7,8-disubstituted-TPs (**102**). Alternatively, a chlorine atom of the starting pyrimidine derivative (**96**) could be maintained during all the synthesis, following the same reactions depicted above with slight modifications.

This approach allows access to larger quantities of precursor (**107**) which could be then substituted with the desired substituents at the 7 position. Furthermore, the chlorine atom may be substituted after cyclization step, leading to



Scheme 12. Reagents: *i*: fur-2-yl-CONHNH$_2$, DBU, THF, rt, 5h; *ii*: P$_2$O$_5$, HMDSO, xylene, 90°C, 2h, rfx, 5h; *iii*: veratrylamine, DMSO, 140°C, 2-4h; *iv*: CH$_3$ONHCH$_3$, R$_1$MgBr, THF, -10 -60°C, 13h; *v*: TFA, CF$_3$SO$_3$H, rt, 19h, 50°C, 1.5h; *vi*: LiOH.H$_2$O, EtOH, H$_2$O, rfx, 3h; *vii*: CH$_3$ONHCH$_3$, EDCI, HOBt, DMF, rt, 70h, 50°C, 1.5h; *viii*: R$_2$R$_1$NH, SO$_2$Cl$_2$, pyr, rt, 1h; *ix*: R$_2$R$_3$NH, i-PrMgCl, THF, -5-4°C, 8h; *x*: R$_1$MgBr, THF, rt, 1.5-4h; *xi*: TFA, anisole, CF$_3$SO$_3$H, 0°C-rt, 4h; *xii*: DIBAL, DCM, -78°C, 1h, 0°C, 2h; *xiii*: MnO$_2$, DCM, rt, 2h; *xiv*: R$_4$R$_5$NH, ClCH$_2$CH$_2$Cl, AcOH, Na(CH$_3$COO)$_3$BH, 0°C-rt, overnight; *xv*: PCC, silica gel, DCM, rt, 4h.

compound **108**, which by reaction with veratrylamine gave compound **101**. In conclusion, such synthesis is a very versatile approach, allowing to perform the substitution at the 7 position in any step of the reaction. [9,71]

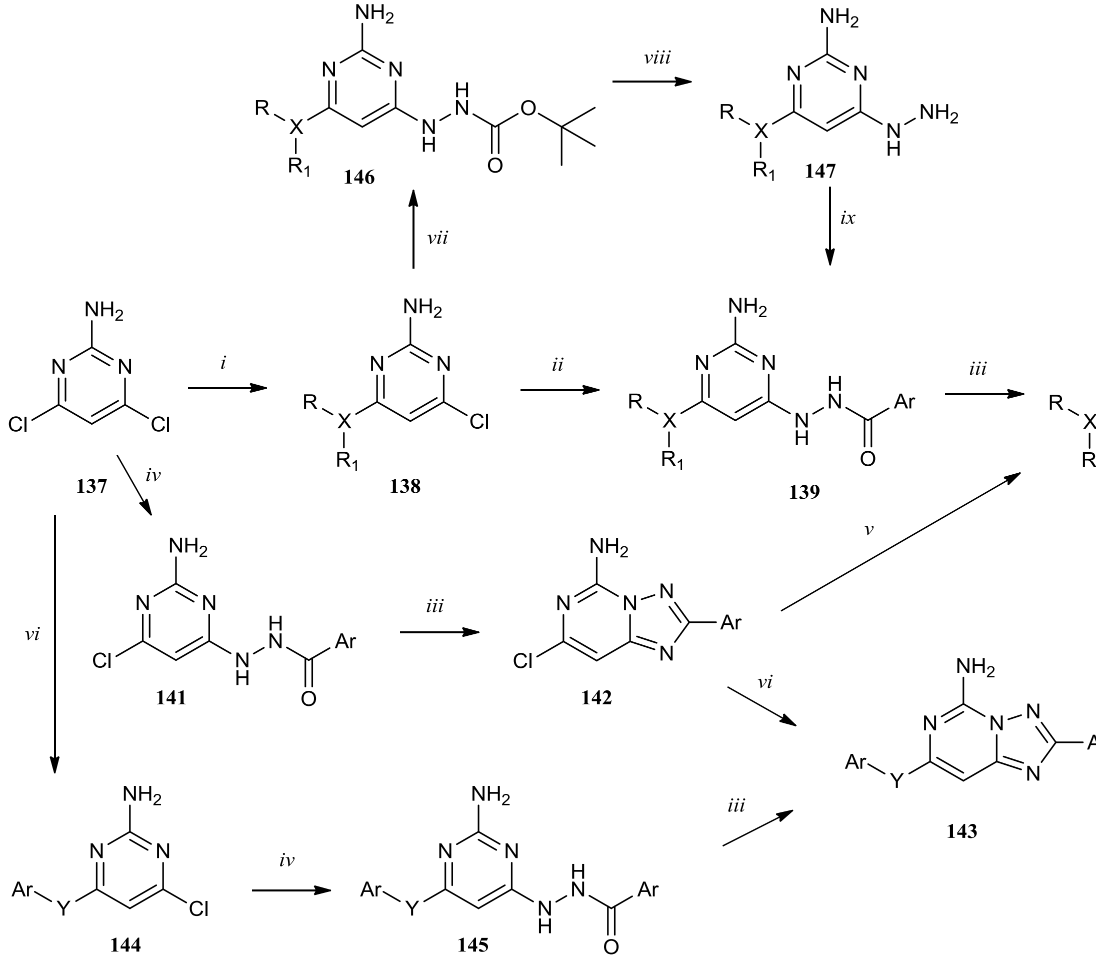


Scheme 13. Reagents: *i*: RR_1NH, EtOH, sealed tube, 120°C, 2h; *ii*: NH_3 7N/MeOH, EtOH, sealed tube, 120°C, 2h; *iii*: R_4NCO, dry THF, rfx, overnight, or R_4CH_2COCl, dry THF, TEA, rfx, overnight. R,R_1,R_2,R_3 = alkyl, arylakyl or RR_1NH are saturated heterocycles with at least one nitrogen as heteroatom. R_4X = $PhCH_2$, PhNH, c-C_6H_{11}-NH.

Scheme 11 shows the most important substitutions, involving the 7 position, obtained using the 7-chloro-N-(3,4-dimethoxybenzyl)-2-(furan-2-yl)-[1,2,4]triazolo[1,5-*c*]pyrimidin-5-amine (**109**) as starting material. Derivatives of general formula **110** have been synthesized by carbon-carbon coupling reactions. This allows the insertion of aryl, arylalkyl, arylethynyl or vinyl moieties at the 7 position, which in turn may be functionalized in order to obtain more complex derivatives.

The Stille reaction between compound **109** and *tert*-butyl-dimethyl-(tributylstannylmethoxy)silane, followed by deprotection of hydroxy group, afforded the 7-hydroxymethyl-TP (**111**). The 7-aldehyde derivative (**113**) was obtained by deprotection of the amino group at the 5 position and oxidation of the hydroxy moiety at the 7 position of compound **111**. Further oxidation of compound **113** afforded the corresponding carboxylic acid (**114**), which may be treated with coupling reagents for the synthesis of amides. On the other hand, reductive amination of compound **113** with the desired amine and sodium triacetoxyborohydride led to 7-(substitutedamino)methyl-TP derivatives (**115**). Finally, the hydroxyl group of compound **111** may be converted by Mitsonobu recation in the corresponding arylether derivatives (**112**). The last example of different substitutions that can be applied at the 7 position of the 7-chloro-TP derivative (**109**) involved its reaction with bis(tributyltin) in order to obtain the corresponding organotin derivative (**116**). Compound **116** is a substrate for Stille reaction and, in fact, the treatment with acylchlorides gave ketone derivatives of general formula **117**. [72]

Instead, TP derivatives bearing different substituents at the 8 position have been obtained using the ethyl 4-chloro-2-(methylthio)pyrimidine-5-carboxylate (**118**) as starting material. Compound **118** is commercially available but it is easily synthesized by condensation between isothiourea and diethyl ethoxymethylene malonate in basic condition, which led to a pyrimidine sodium salt that after treatment with phosphorous oxychloride afforded compound **118**. [73] As displayed in Scheme 12, pyrimidine derivative **118** was converted in the ethyl 5-((3,4-dimethoxybenzyl)amino)-2-(furan-2-yl)-[1,2,4]triazolo[1,5-*c*]pyrimidine-8-carboxylate (**121**) by following the same procedures shown in Scheme 10 for the introduction of hydrazide, and the subsequent dehydrative cyclization and amination at the 5 position. Compound **121** may be deprotected on the amino group to afford compound **128** or modified at the 8 position and subsequently deprotected to afford the final desired compounds. Hydrolysis in basic conditions of the ethyl ester group of compound **121** led to the carboxylic derivative **124**, which was converted to the amido derivatives (**126**) by reaction with mono- or di-substituted amines in the presence of thionyl chloride in pyridine. Instead, the coupling reaction of compound **124** with *N*,O-dimethylhydroxylamine, carried out by the use of EDCI and HOBt as coupling reagents, afforded the Weinreb-Nahm amide **125**. The treatment of compound **125** with a Grignard reagent led to the corresponding ketone derivative (**122**). When R_1 in compound **122** in Scheme 12 is an ethyl, the Weinreb-Nahm ketone synthesis was performed by one-pot reaction directly from the ethyl ester derivative (**121**). Alternatively, compound **121** was reduced to 8-hydroxymethyl (**129**) derivative by diisobutylaluminum hydride, which was in turn oxidized with manganese dioxide to obtain the 8-formyl derivative (**130**), analogously to that reported in Scheme 11 for compound **111** (to obtain compound **113**). By reductive amination of compound **130**, aminomethyl derivatives **132** have been obtained, whereas the Grignard reaction led to the secondary alcohols of general formula **134**. Compounds **134** were oxidized by pyridinium chlorochromate to afford again ketones with general formula **122**. [9,71]

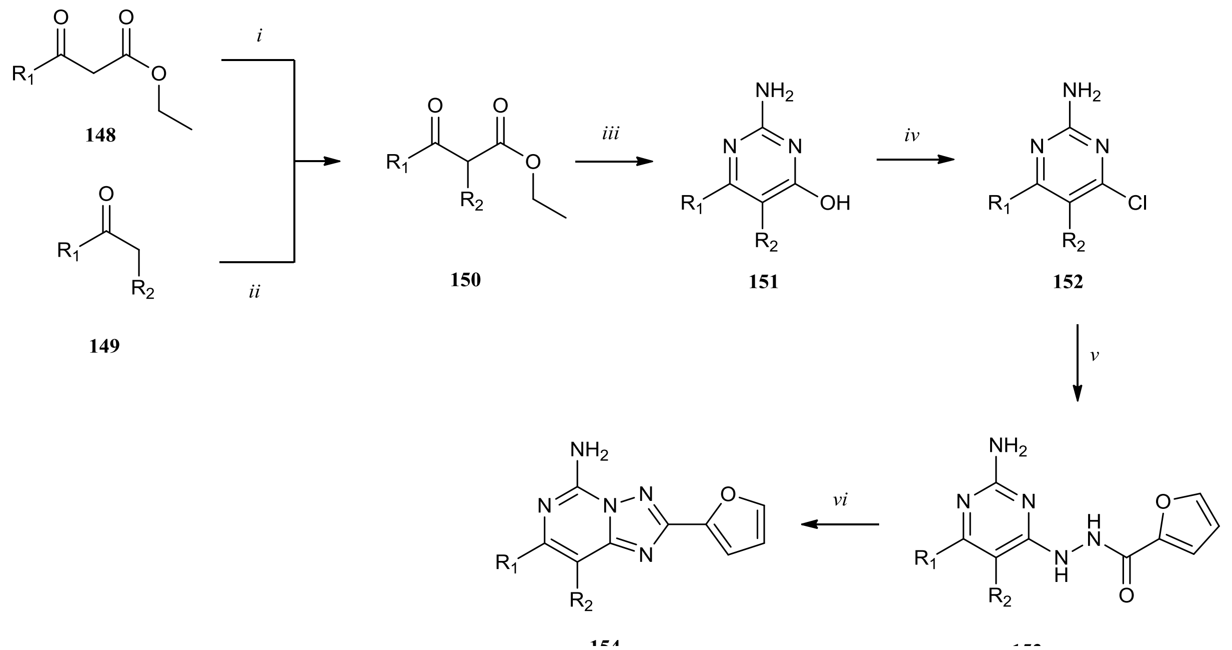


Scheme 14. Reagents: *i*: a) for X = N, RR_1NH, K_2CO_3, EtOH, sealed tube, 90°C, 1h, b) for X = O, ROH, NaH, THF, rfx, 24h, c) for X = S, Na_2S, DMF, RCl, 80°C, 18h; *ii*: aryl hydrazide, 1N HCl, EtOH, sealed tube, 90°C, 16h; *iii*: BSA, 120°C, 18h; *iv*: aryl hydrazide, K_2CO_3, EtOH, sealed tube, 100°C, 18h or aryl hydrazide, BuOH, 90°C, 20h; *v*: for X = N, RR_1NH, DBU, DMF, 140°C, 2h or RR1NH, CsF, DMSO, 110°C; *vi*: $ArYB(OH)_2$, $Pd(PPh_3)_4$, Na_2CO_3, 3/1/1 toluene/ethanol/water, 100°C, 4h, with Y= none or CHCH; *vii*: t-butylcarbazate, 4M HCl/dioxane, EtOH, sealed tube, 100°C, 18h; *viii*: DCM/MeOH, 4M HCl/dioxane, rt, 18h; *ix*: ArCOOH, EDCI, HOBt, NMM, DMF, rt, 3h. Ar = fur-2-yl or 3-CNPh.

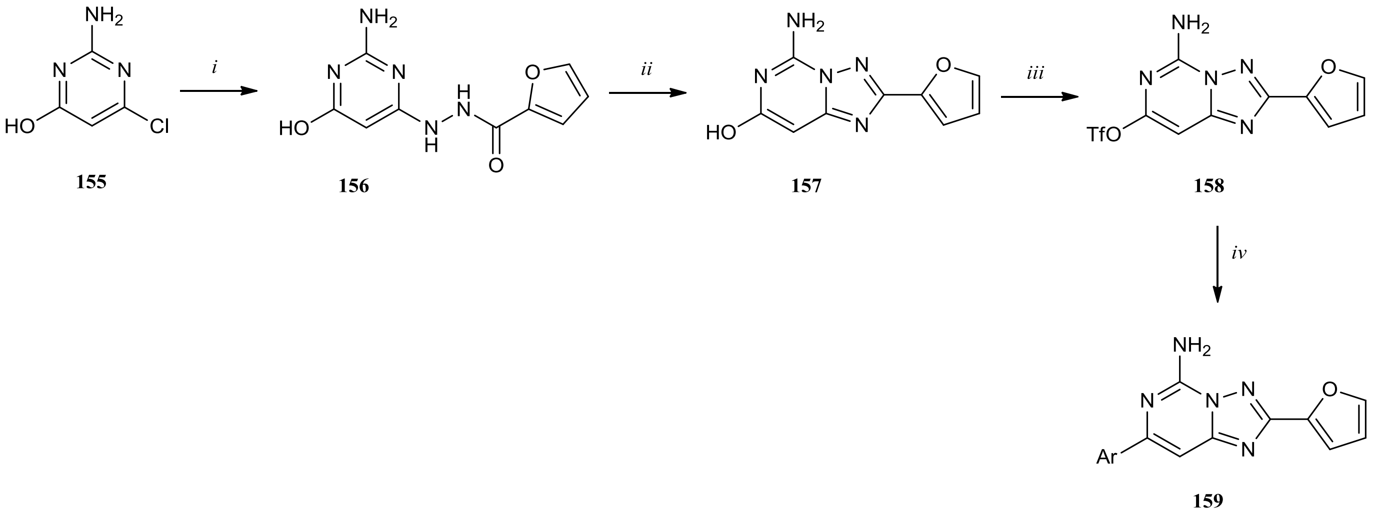
Recently, our group developed 5,8-disubstituted-TPs based on the Kyowa Hakko Kogyo Co. procedure displayed in Scheme 12 (Scheme 13). Procedure was slightly modified in order to introduce different substituents at the 5 position. [14] In fact, compound **120**, which was obtained as in Scheme 12, was reacted with different amines in hot ethanol to afford different 5-amino derivatives (**135**) in good yields. By using ammonia, compound **128** has been directly obtained. Compound **128** or compounds of general formula **127** (obtained as depicted in Scheme 12) were treated with appropriated isocyanates or acylchlorides in order to achieve the corresponding 5-ureido and 5-amido derivatives (**136**). [14]

Schering developed a large series of TP compounds, mainly applying a chemistry similar to those applied by Kyowa Hakko Kogyo Co. As shown in Scheme 14, Schering approach uses directly the 2-amino-4,6-dichloro-pyrimidine (**137**) as starting material. As reported by Kyowa Hakko Kogyo Co., a first chlorine atom was substituted with amines, alcohols, or thiols to give compounds of general formula **138**. In this step, different reagents were employed on the basis of the nucleophile used (i.e. potassium carbonate for amines, sodium hydride for alcohols and sodium sulfide with aryl or alkylchlorides for thiols).

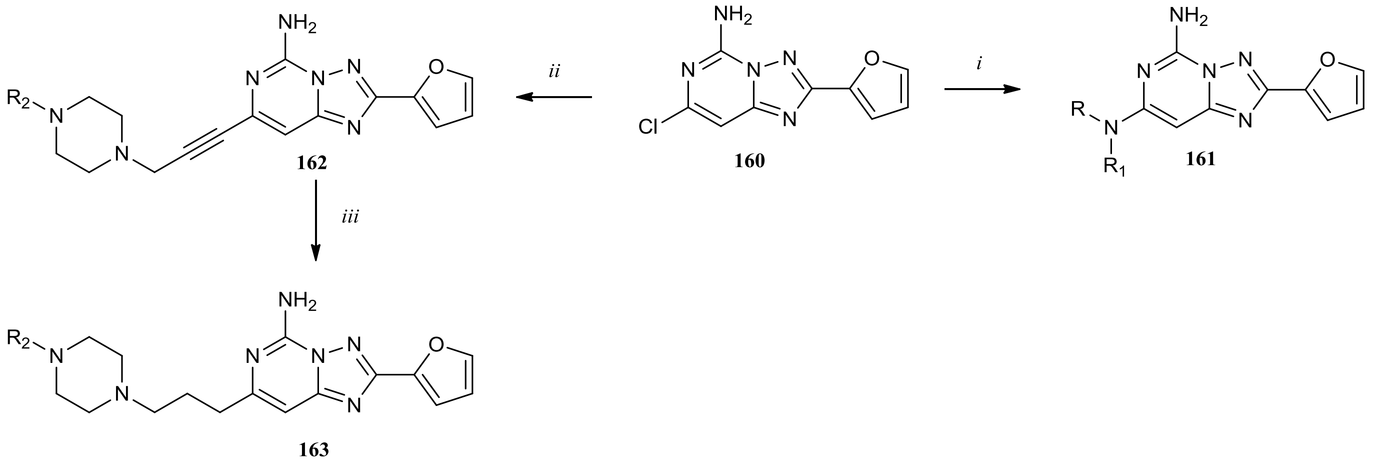
Alternatively, compound **137** may be coupled with alkyl or arylalkyl moieties by Suzuki coupling affording compounds of general formula **144**. Compounds **138**, **144** or the unsubstituted derivative **137** were reacted with the desired arylhydrazide in acidic (**138**) or basic conditions (**137**, **144**) and derivatives **139**, **145** and **141** have been obtained. Compounds **139**, **141** and **145** were then cyclized in dehydrating conditions to the 5-amino-TPs (**140**, **142**, **143**). Schering applied similar cyclization conditions to that used to prepare PTP derivatives, which are BSA at 120°C for 18h. An alternative synthetic route was used to introduce different aryl moieties at the 2 position of the TP nucleus, which involved the reaction between compound **138** and *tert*-butylcarbazate, in solution containing HCl, to form the *N*-Boc-hydrazine derivative **146**. After removal of the Boc protecting group, the resulting 6-hydrazinyl-pyrimidines (**147**) were coupled with the desired carboxylic acid by using EDCI, HOBt and NMM, thus leading again to compounds of



Scheme 15. Reagents: *i*: R_2X, base; *ii*: ClCOOEt, base; *iii*: guanidine carbonate, EtOH, rfx, 20h; *iv*: $POCl_3$, rfx, 2h; *v*: 2-furoic hydrazide, 4M HCl/dioxane, EtOH, sealed tube, 100°C, 18h; *vi*: BSA, 130°C, 6h.



Scheme 16. Reagents: *i*: 2-furoic hydrazide, BuOH, 100°C, 20h; *ii*: BSA, 120°C, 18h; *iii*: triflic anhydride, TEA, DCM, 0°C, N_2, 1h; *iv*: $ArSn(Bu)_3$, $Pd(dppf)Cl_2$, DMF, 80°C, 64h, N_2.



Scheme 17. Reagents: *i*: RR_1NH, CsF, DMSO, 110°C, 18h; *ii*: 1-R_2-4-prop-2-ynyl-piperazine, $Pd(PPh_3)_4$, CuI, PPh_3, TEA, DMF, N_2, 110°C, 18h; *iii*: Pd/C 10%, 1 atm H_2, 1/1 THF/MeOH, rt, 0.5h.

Schering has reported an alternative method to synthetize 7 and 8 aryl or arylalkyl substituted TP derivatives, as shown in Scheme 15. The desired 2-amino-4-chloro-5,6-disubstituted-pyrimidines (**152**) were obtained from condensation between guanidine carbonate and the corresponding β-ketoesters **150**, followed by treatment with phosphorous oxychloride which introduced a chlorine atom in place of hydroxy group of compounds **151** at the 4 position. The desired β-ketoesters **150** can be obtained both by alkylation of a β-ketoesters of general formula **148** and by reaction of ketone derivatives **149** with ethylchloroformate. The pyrimidines **152** were converted to the final desired compounds **154** following the same procedures reported in Scheme 13 with slight modifications. [13,74-75]

Finally, 5-amino-2-(furan-2-yl)-[1,2,4]triazolo[1,5-*c*]pyrimidin-7-ol (**157**) was obtained from 2-amino-6-chloropyrimidin-4-ol (**155**), as shown in Scheme 16. The hydroxyl moiety of compound **157** was treated with triflic anhydride to give compound **158**, which bears the triflate group, a pseudohalide moiety useful for the subsequent Stille cross-coupling reaction. Thus, this reaction represents another way to obtain 5-amino-7-aryl-2-furyl-TP derivatives (**159**). [75]

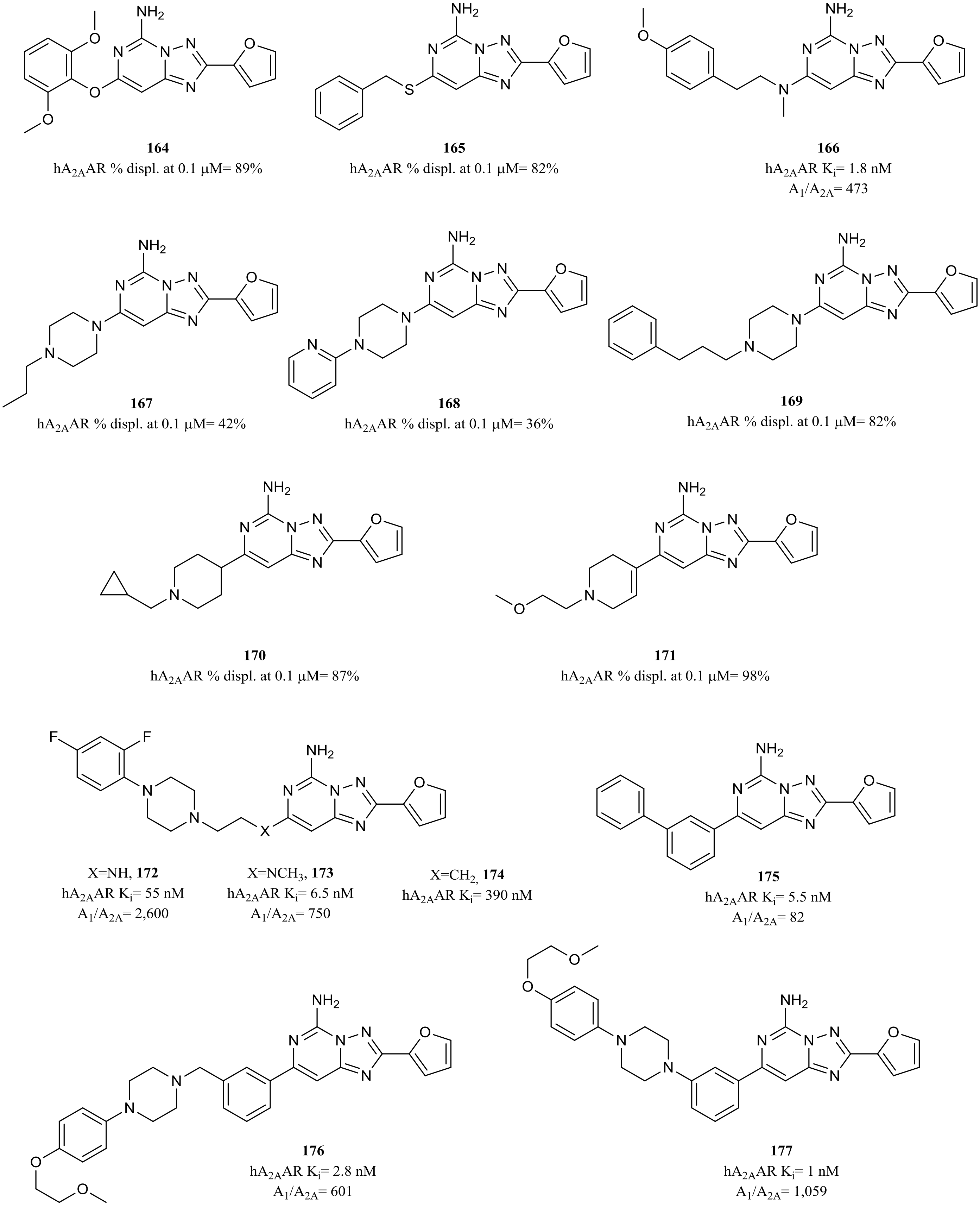
Biogen Idec also investigated the TP scaffold for searching A_{2A} AR antagonists. The company applied a strategy very similar to those reported above. In fact, as shown in Scheme 17, they used the 7-chloro-2-(furan-2-yl)-[1,2,4]triazolo[1,5-*c*]pyrimidin-5-amine (**160**) to introduce different moieties at the 7 position. The 7-susbtituted-amino derivatives (**161**) were obtained as reported above but using cesium fluoride as base. Instead, compound **160**, by Sonogashira cross-coupling reaction with 1-substitited-4-(prop-2-yn-1-yl)piperazine, followed by a catalytic hydrogenation, afforded 7-(4-substitited-piperazin-1-yl)propyl-TP derivatives (**163**). [76-78]

3.2. Structure activity relationship

TP derivatives were almost exclusively reported in patents. In patents, binding data are often incomplete and this makes it difficult to assess the SAR profile of these compounds. All the patents on TP derivatives target A_{2A} AR. Kyowa Hakko Kogyo Co. developed a series of TP mainly maintaining the furyl group at the 2 position and the free amino group at the 5 position of TP scaffold, because these substitutions were known to confer high affinity at the A_{2A} AR subtype in the PTP series (see Section 2.2). The introduction of both phenoxy (**164**) and benzylthio (**165**) moieties led to potent A_{2A} AR antagonists (**164**, hA_{2A} AR displ. at 0.1 µM = 89%; **165**, hA_{2A} AR displ. at 0.1 µM = 82%). Unfortunately, selectivity profile among ARs have not been reported for these compounds. [71] (Figure 12) Neustadt *et al.* developed a series of TPs bearing aryloxy, arylthio and arylamino moieties at the 7 position. They confirmed that all these substitutions led to potent A_{2A} AR antagonists, but only derivatives bearing an additional small alkyl group on the nitrogen at the 7 position displayed good selectivity over the hA_1 AR (i.e. compound **166**). [13] Nevertheless, the most investigated substitution on the TP scaffold has been the piperazino group. Kyowa Hakko Kogyo Co. found that introduction of *N*-alkyl-, *N*-hydroxyalkyl- or *N*-aryl- piperazines at the 7 position of the TP nucleus led to derivatives that displayed poor affinity at the A_{2A} AR (e.g. compound **167,** hA_{2A} AR displ. at 0.1 µM = 42%; compund **168,** hA_{2A} AR displ. at 0.1 µM = 36%). [71] Instead, *N*-arylalkyl-piperazines, with alkyl linkers ranging from 1 to 3 methylene groups (i.e. compound **169**), increased affinity at the A_{2A} AR. Compound **169** was demonstrated to be effective in a MPTP model of PD. [71] Furthermore, piperazine could also be replaced with a piperidine or a 1,2,3,6-tetrahydropyridine, such as in compound **171**, which showed high affinity at the A_{2A} AR. [72] As an extension of the series of piperazine derivatives of [1,2,4]triazolo[1,5-*c*]pyrimidines, Biogen Idec. explored the possibility to introduce a flexible alkyl spacer between the TP nucleus and the piperazine moiety. Compounds **172**, **173** and **174** clearly showed that an aminoethyl spacer gave quite potent and selective A_{2A} AR antagonists (**172**), however methylation at the amino group improved both affinity and selectivity at the A_{2A} AR (compound **173**). Finally, substitution of the amino group with a methylene group had a detrimental effect on affinity at the A_{2A} AR (compound **174**). [76] Schering-Plough Research Institute developed a large series of piperazine and piperidine derivatives using a phenyl ring as a linker between the TP nucleus and the heterocyclic group. Initially, a series of different 7-aryl compounds have been synthesized, demonstrating that an aryl group is well tolerated at the A_{2A} AR, with affinity values in the nanomolar range. Interestingly, compound **175** showed a high affinity at the A_{2A} AR with a good selectivity over the A_1 AR subtype (hA_{2A} AR K_i= 5.5 nM; A_1/A_{2A}= 82). This result suggested to explore substitutions at the *meta* position of the phenyl ring at the 7 position of the TP, thus leading to the synthesis of several derivatives bearing a 4-(phenyl)-piperidine or a 4-(phenyl)-piperazine at this position. The incorporation of the methoxethoxy substituent, as in Preladenant (**2**), seemed particularly beneficial in terms of selectivity over A_1. In fact, compound **176**, which displayed K_i values of 2.8 and 1,680 nM at the A_{2A} an A_1 ARs, respectively, showed good oral activity at 3 mg/kg in a rat catalepsy model. [11] However, these analogs were found to be associated with the blockade of the hERG (human Ether-a-go-go Related Gene) channel, which is related to an augmented risk of cardiac arrhythmia. The basic nitrogen in the side chain at the 7 position was identified as responsible for the inhibition of the hERG channel, thus several derivatives bearing the piperazino moiety directly attached to the phenyl ring were prepared. All the synthesized compounds showed affinity in nanomolar or sub-nanomolar range with a low hERG inhibition. The most potent compound of the series has been the homologue of compound **176** (compound **177**). [12]

Neustadt *et al.* investigated also the 2 position of the TP nucleus, by replacing the furyl group, which is a common structural feature of almost all A_{2A} AR antagonists. Interestingly, 3-cyano-phenyl group maintains affinity and receptor selectivity (**178**, hA_{2A} AR K_i= 0.7 nM, A_1/A_{2A}= 1,071), but failed to show pronounced *in vivo* activity. [13]

Kiowa Hakko Kogyo Co. designed several 7-carbamoyl derivatives of TPs and these compounds were found to be less potent than their corresponding amino derivatives, as observed comparing compound **179** (Figure 13, hA_{2A} AR displ. at 0.1 μM = 65%) with compound **166** (hA_{2A} AR K_i = 1.8 nM). [72] Instead, a small alkyl moiety such an ethyl or



164
$hA_{2A}AR$ % displ. at 0.1 μM= 89%

165
$hA_{2A}AR$ % displ. at 0.1 μM= 82%

166
$hA_{2A}AR$ K_i= 1.8 nM
A_1/A_{2A}= 473

167
$hA_{2A}AR$ % displ. at 0.1 μM= 42%

168
$hA_{2A}AR$ % displ. at 0.1 μM= 36%

169
$hA_{2A}AR$ % displ. at 0.1 μM= 82%

170
$hA_{2A}AR$ % displ. at 0.1 μM= 87%

171
$hA_{2A}AR$ % displ. at 0.1 μM= 98%

X=NH, **172**
$hA_{2A}AR$ K_i= 55 nM
A_1/A_{2A}= 2,600

X=NCH_3, **173**
$hA_{2A}AR$ K_i= 6.5 nM
A_1/A_{2A}= 750

X=CH_2, **174**
$hA_{2A}AR$ K_i= 390 nM

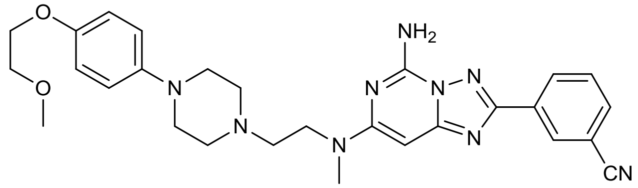
175
$hA_{2A}AR$ K_i= 5.5 nM
A_1/A_{2A}= 82

176
$hA_{2A}AR$ K_i= 2.8 nM
A_1/A_{2A}= 601

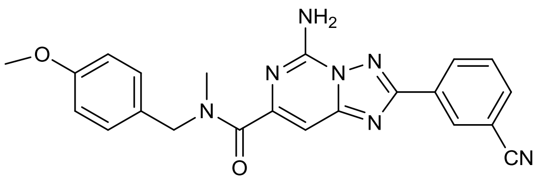
177
$hA_{2A}AR$ K_i= 1 nM
A_1/A_{2A}= 1,059

Figure 12. TP derivatives as A_{2A} AR antagonists: compounds **164-177**.

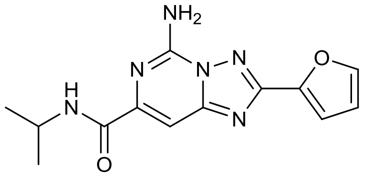
an isopropyl (compound **180**) gave potent A_{2A} AR antagonists. [71] In addition, some 8-substituted derivatives were developed in this series, revealing that small a alkylamido group confers high potency at the A_{2A} AR even when present at the 8 position, such as in the 8-(*N*-ethyl)amido derivative **181**, which displaced 96% of a specific radioligand for the A_{2A} AR at a concentration of 0.1 μM. [71]



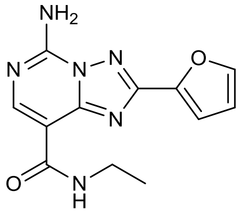
178
$hA_{2A}AR\ K_i$= 0.7 nM
A_1/A_{2A}= 1,071



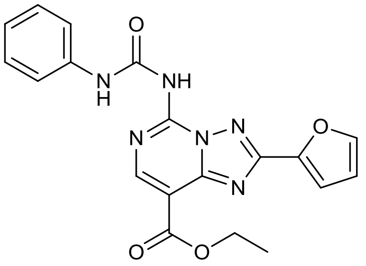
179
$hA_{2A}AR$ % displ. at 0.1 μM= 65%



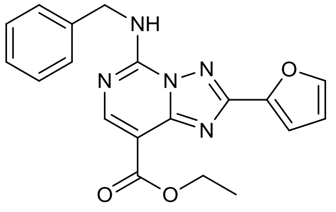
180
$hA_{2A}AR$ % displ. at 0.1 μM= 98%



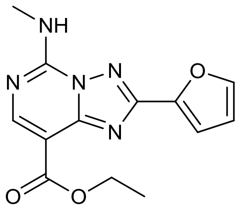
181
$hA_{2A}AR$ % displ. at 0.1 μM= 96%



182
$hA_1AR\ K_i$= 322 nM
$hA_{2A}AR\ K_i$= 4.82 nM
$hA_{2B}AR\ K_i$= 1,900 nM
$hA_3AR\ K_i$= 357 nM



183
$hA_1AR\ K_i$= 940 nM
$hA_{2A}AR\ K_i$= 32.0 nM
$hA_{2B}AR\ K_i$= 2,510 nM
$hA_3AR\ K_i$= 10.0 nM



184
$hA_1AR\ K_i$= 978 nM
$hA_{2A}AR\ K_i$= 104 nM
$hA_{2B}AR\ K_i$= 2,450 nM
$hA_3AR\ K_i$= 4.14 nM

Figure 13. TP derivatives as A_{2A} and A_3 AR antagonists: compounds **178**-**182** and **183**-**184**, respectively.

Recently, a novel series of TP substituted at both the 5 and 8 position was developed and the derivatives were characterized at all the AR subtypes. [14] The introduction of an arylureido moiety at the 5 position (**182**) gave different results in terms of affinity and selectivity depending on the substitutions at the 8 position. The simultaneous presence at the 8 position of an ethoxycarbonyl (**182**) or a methyl carboxamido function led to compounds with quite good affinity and selectivity for the hA_{2A} AR (e.g., compound **182**: hA_{2A} AR K_i = 4.82 nM; hA_{2A}/hA_3 = 0.01; hA_1/hA_{2A} = 66.8), whereas a bigger β-phenethylcarboxamido group or the replacement of arylureido group with a phenylacetamido group produced a decrease of affinity at the hA_{2A} AR. A very different behavior was observed when alkylamino moieties were introduced at the 5 position of 8-ethoxycarbonyl-TP.

In fact, a clear enhancement of affinity at the hA_3 AR was observed for these compounds. Benzylamino moieties led to derivatives displaying good affinity at the hA_3 AR but selectivity is still poor (e.g. compound **183**, hA_3 AR K_i = 10 nM; hA_1/hA_3 = 94; hA_{2A}/hA_3 = 3.2). Better results in terms of both affinity and selectivity at the hA_3 AR were obtained with small alkyl groups at the 5 position, such as for methylamino derivative (**184**) which showed a K_i value of 4.14 nM at the hA_3 AR and was 236- and 25-fold selective against A_1 and A_{2A} ARs, respectively. [14]

The structure activity relationship of TP derivatives is summarized in Figure 14.

4. PYRAZOLO[3,4-*d*]PYRIMIDINES

Over the years, several research groups have been interested in the development of pyrazolo[3,4-*d*]pyrimidines (PPs) as adenosine receptor antagonists. [15-21,79-82] This can easily be attributed to the fact that PPs (**4**) are structurally very similar to the purine nucleus (**185**), whereas the imidazole ring is replaced by a pyrazole ring. In addition, the potent tricyclic adenosine receptor antagonists PTPs (**1**) are PP-fused derivatives, so PPs (**4**) can be considered as a structural simplification of this scaffold. (Figure 15)

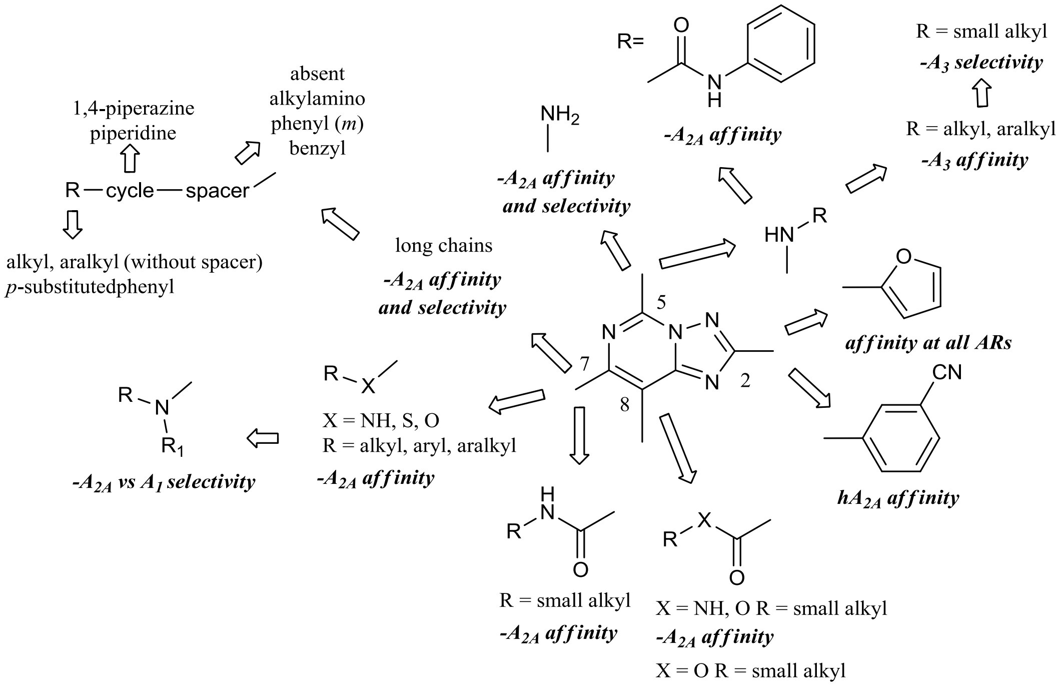


Figure 14. Structure activity relationship of TP derivatives at ARs.

4.1. Chemistry

The most used starting materials for the synthesis of PPs are the 1-substituted-5-amino-1*H*-pyrazole-4-carbonitriles (**7**) or their corresponding regioisomers 3-amino-1-substituted-1*H*-pyrazole-4-carbonitrile (**17**).

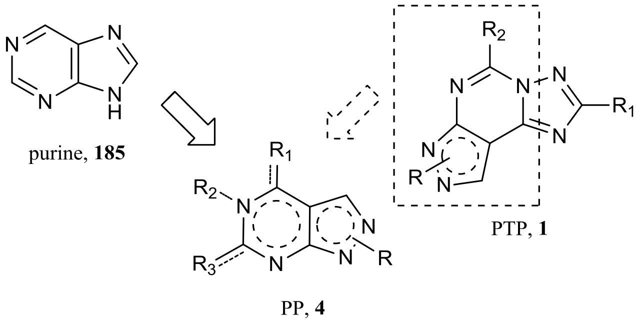
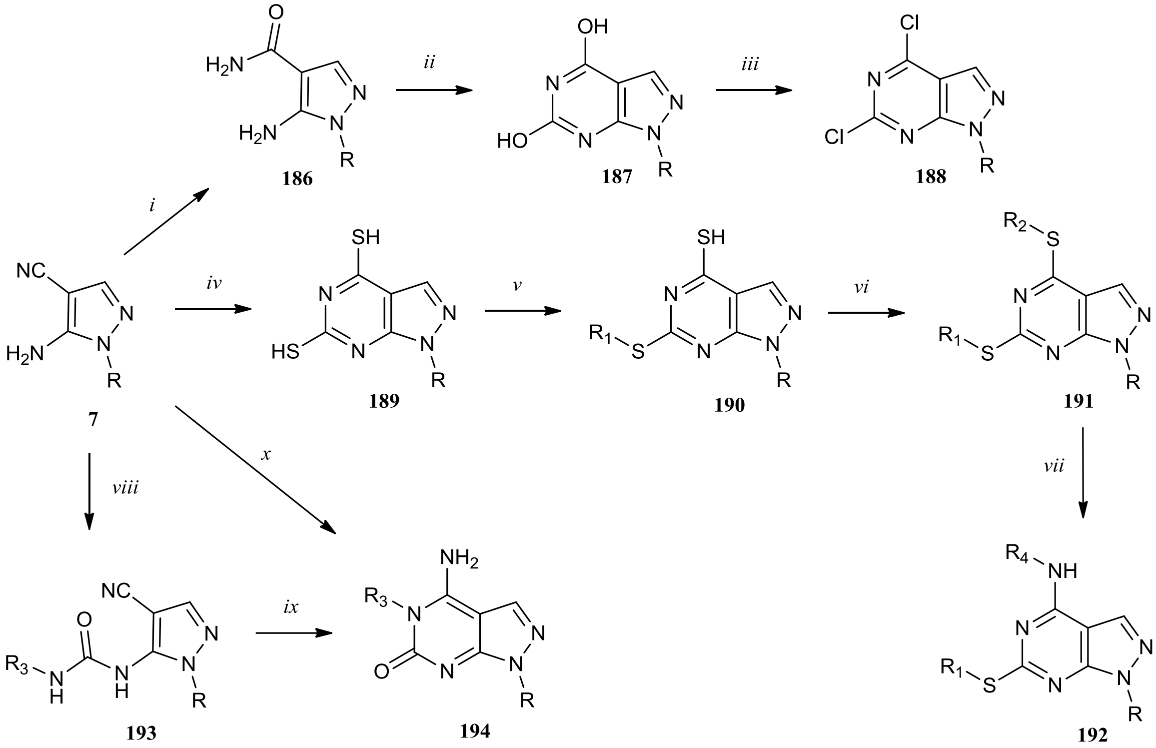


Figure 15. PPs as AR antagonists.

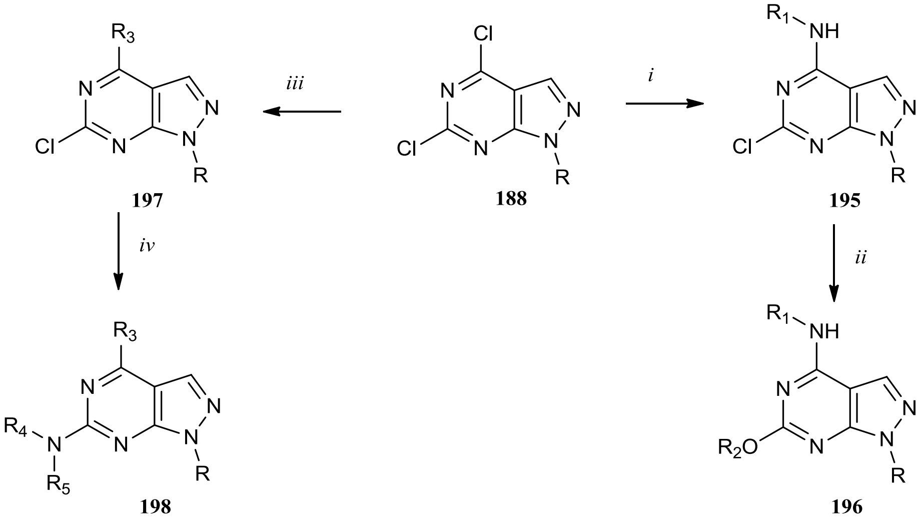
These compounds were obtained as discussed in Section 2.1 and their principal applications for the synthesis of PPs were shown in Scheme 18. The 4,6-dichloro-1-substituted-1*H*-pyrazolo[3,4-*d*]pyrimidine (**188**) could be easily obtained by treatment of the corresponding di-hydroxy derivative (**187**) with phenylphosphoryl dichloride. Compound **187** was obtained by heating the 5-amino-1-substituted-1*H*-pyrazole-4-carboxamide (**186**) with urea. The carboxamido group of compound **186** originated from the conversion of the cyano group of compound **7** in acidic conditions. [18,79] Alternatively, the reaction between compound **7** and potassium ethyl xanthogenate afforded the 4,6-dithiol-PP (**189**), which thiol groups could be successively alkylated (compounds **190** and **191**). If the alkylation at the 4 position was performed using methyliodide, the resulting methylthio derivative could be used to introduce different amino moieties at the 4 position of the PP scaffold (compound **192**). [17,80] Finally, compound **7**, by treatment with an alkyl- or aryl-isocyanate in the presence of a base, such as sodium methoxide or *n*-butyllithium, led to 5-substituted-pyrazolo[3,4-*d*]pyrimidin-6(5*H*)-ones (**194**). This reaction occurred via cyclization of the corresponding 3-ureido derivative, which was confirmed by the isolation of derivative **193**, obtained by addition of the isocyanate without the base. Compound **193** was then effectively converted in compound **194** using ammonium hydroxide. [15]

The 4,6-dichloro-PP (**188**) allows to introduce substitutions of different nature at the 4 and 6 positions of the PP scaffold. For example, the simple reaction between compound **188** and (ar)alkylamino derivatives led to the substitution of the chlorine atom at the 4 position which afforded compounds of general formula **195**. Compounds **195** were in turn converted to compounds **196** by treatment with sodium alkoxide at 90°C. [16] The presence of a halogen atom in compound **188** allows the application of carbon-carbon cross-coupling reactions, for example Gillepsie *et al.* have applied the Stille reaction to compound **188** in order to obtain 4-aryl derivatives (**197**). Again, the second chlorine atom can be substituted by reacting compound **197** with various amines affording compounds of general formula **198** (Scheme 19). [18,79]

PP derivatives were developed also starting from 6-amino-4-chloro-PP (**30**) the synthesis of which is described in Section 2.1 (Scheme 4). Because compound **30** is unsubstituted on the pyrazole ring, the Stille reaction was made on the *N*-Boc protected derivative and the resulting compound was deprotected by hot dimethylamine. These reactions afforded compounds of general formula **199**, which are functionalized on the pyrazole ring by alkylation or by reaction with the appropriated isocyanate, leading to compounds **200** and **201**. Alternatively, alkylation on the pyrazole ring could be performed on compound **30** and, then, the aryl moiety introduced by Suzuki or Negishi coupling as shown in Scheme 20. The alkylation step afforded a mixture of *N*1 and *N*2 alkylated PP derivatives (**202-203**), which was used in the next step without purification, affording a mixture of compounds **204** and **205**. [18,79]

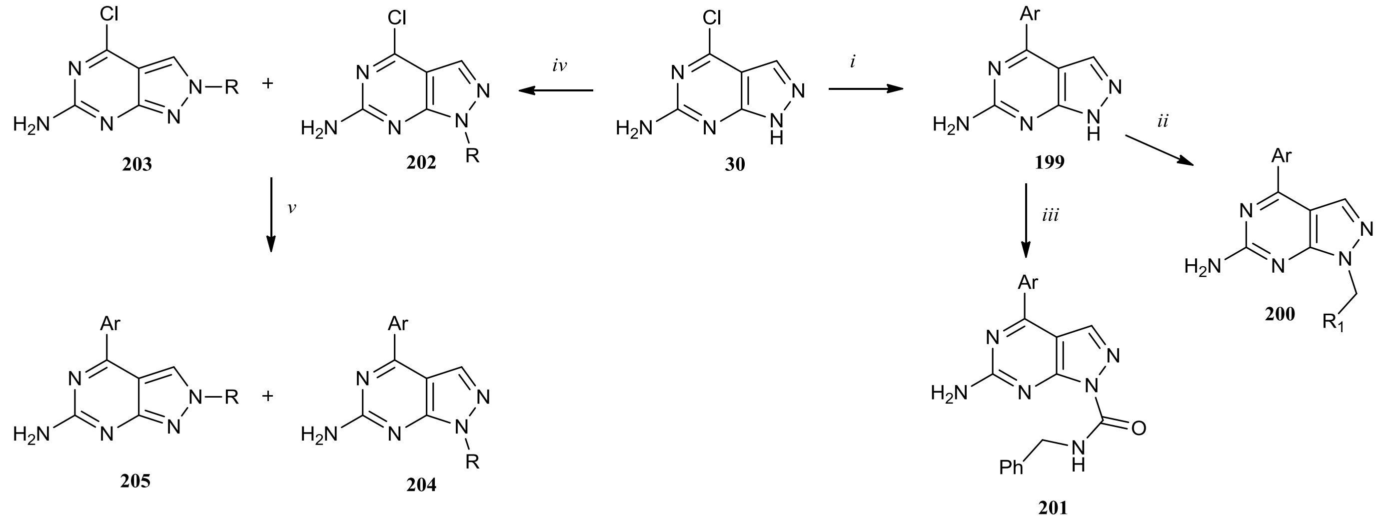


Scheme 18. Reagents: *i*: H_2SO_4 $_{(aq)}$, 60°C, 1h, R= CH_2CH_2Ph; *ii*: urea, neat, 180°C, 1h, R= CH_2CH_2Ph; *iii*: $PhPOCl_2$, 165°C, 16h, R= CH_2CH_2Ph; *iv*: Potassium ethyl xanthogenate, R = Ph; *v*: R_1Br, pyr, rt, R = Ph, R_1 = CH(alkyl)$CONH_2$; *vi*: R_2I, NaOH(aq), rt, R = Ph, R_1 = CH(alkyl)$CONH_2$, R_2 = CH_3; *vii*: NH_2R_4, EtOH, 110°C, sealed tube, 72h. R = Ph, R_1 = CH(alkyl)$CONH_2$, R_4 = H, CH_3; *viii*: R_3NCO, DMF, 80°C, 24h, R = alkyl or aryl, R_3 = CH_3; *ix*: 30% NH_4OH, DMF, rt, 24h; *x*: (method A) R_3NCO, $NaOCH_3$, DMF, 60°C, 12h or (method B) n-BuLi, THF, -70°C, R_3NCO, 1h, -70°C→ rt, 1h, R = alkyl or aryl, R_3 =alkyl or Ph.

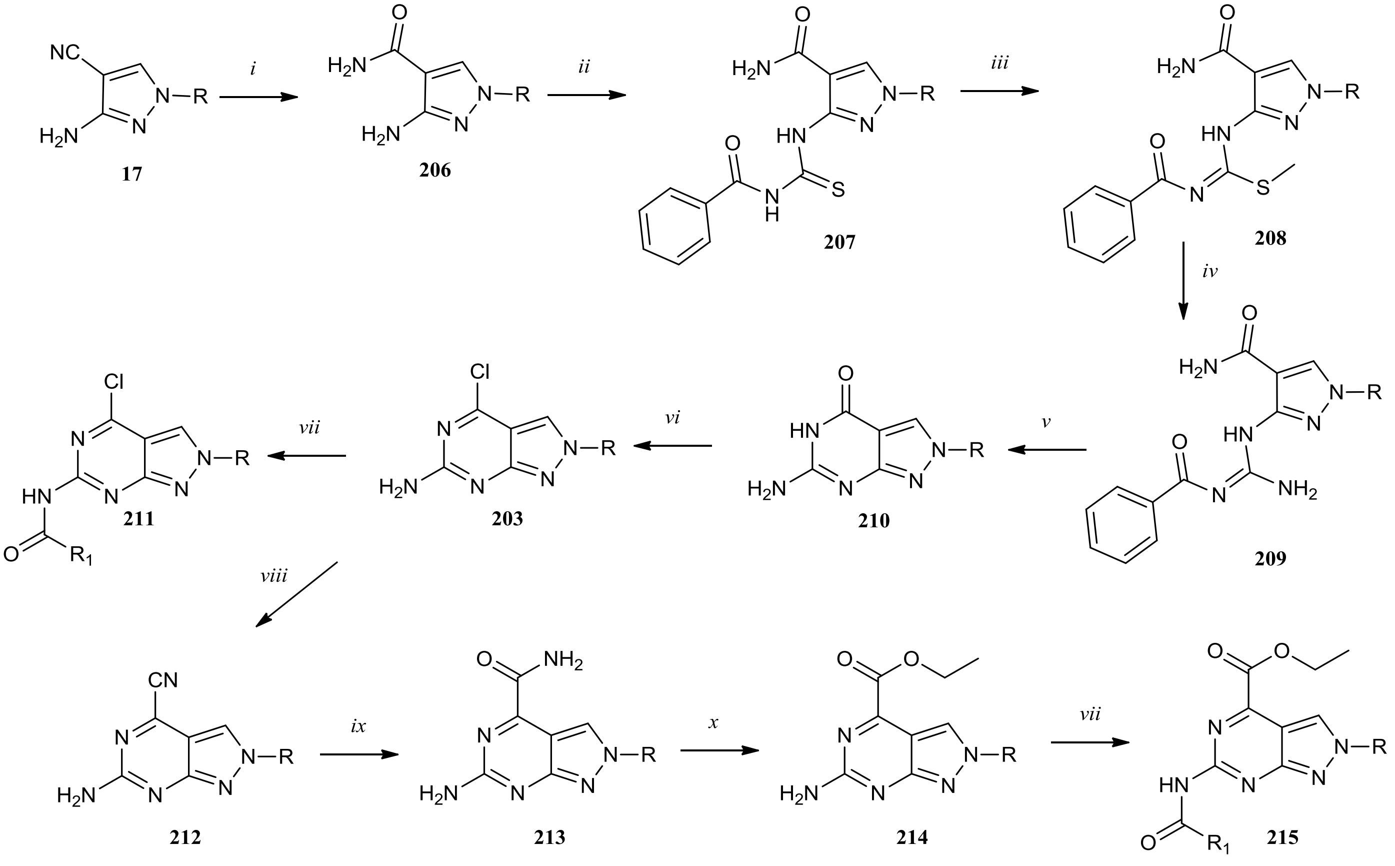


Scheme 19. Reagents: *i*: R_1NH_2, EtOH, rfx; *ii*: $NaOR_2$, 90°C; *iii*: (tributylstannyl)R_3, $PdCl_2(PPh_3)_2$, DMF, rt, R_3 = heteroaryl; *iv*: R_4R_5NH, NMP or iPrOH, Δ, R_4,R_5 = alkyl, hydroxyalkyl.

Venkatesan *et al.* reported a synthetic approach to obtain directly compounds of general formula **203**, starting from the appropriated 3-amino-1-substituted-1*H*-pyrazole-4-carbonitrile (**17**). [20,21] As displayed in Scheme 21, the carboxamide derivatives (**206**) were obtained by treatment of compound **17** with sulfuric acid (as reported in Scheme 18 for the other pyrazole regioisomer). Further, the carboxamide derivatives (**206**) were refluxed with benzoylisothiocyanate in acetone, to obtain the benzoylthioureido derivatives (**207**). Treatment of **207** with methyl iodide in aqueous sodium hydroxide solution gave the corresponding carbamidothioates **208**. The methylthio derivatives (**208**), by reaction with ammonia at 120°C, afforded the benzoylguanidino derivatives (**209**). Hydrolysis of the benzoyl guanidine derivatives under basic conditions allows the cyclization, affording the corresponding 6-amino-2-

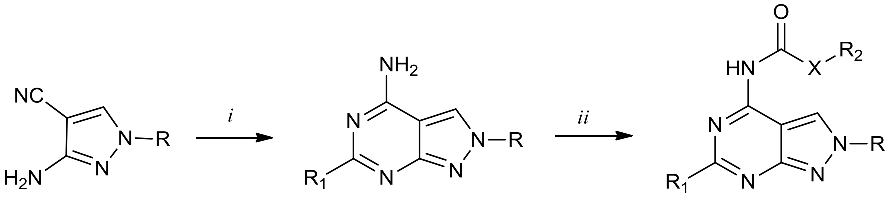


Scheme 20. Reagents: *i*: (A) Boc_2O, TEA, DMAP, DMF, rt, (B) tributylstannyl-aryl, $PdCl_2(PPh_3)_2$, DMF, rt, (C) $(CH_3)_2NH$ (aq), Δ; *ii*: NaH, DMF, 0°C, R_1CH_2Br, rt; *iii*: $PhCH_2NCO$, DMAP, DMF, rt; *iv*: NaH, DMF, BrR, 0°C to rt, R = 2-F-Bn; *v*: $ArB(OH)_2$, $Pd(PPh_3)_4$, $NaHCO_3$, THF, Δ, or ArH, n-BuLi, THF, 1M $ZnCl_2$, $Pd(PPh_3)_4$, -78°C to Δ.



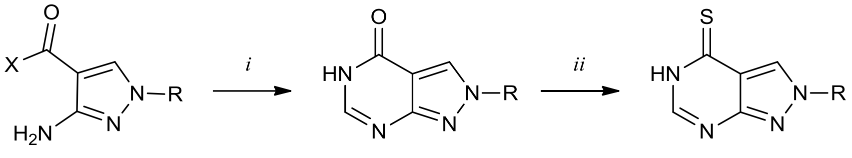
Scheme 21. Reagents: *i*: conc. H_2SO_4, 0°C to rt, 5 h; *ii*: PhCONCS, acetone, rfx, 12 h; *iii*: CH_3I, 0.1 N NaOH, rt, 3 h; *iv*: 2% aq NH_3, DMF, 120°C, sealed tube, 3 h; *v*: 1N NaOH, rfx, 12 h; *vi*: $POCl_3$, dimethylaniline, rfx, 24 h; *vii*: R_1Cl, DIPEA, toluene, rfx, 24 h, R_1= Ar or $ArCH_2$; *viii*: NaCN, p-toluene sulfinate sodium, DMF, 80°C, 2 h; *ix*: K_2CO_3, 30% H_2O_2 (aq), rt, 1 h; *x*: EtOH, conc. H_2SO_4, rfx, 12 h.

alkyl-2*H*-pyrazolo[3,4-*d*]pyrimidin-4(5*H*)-ones (**210**). Compounds **210** were further reacted with phosphoryl oxychloride in the presence of dimethylaniline under reflux condition to form the corresponding chloro analogs (**203**). Compound **212** was prepared by treating compound **203** with sodium cyanide in the presence of sodium *p*-toluene sulfinate as catalyst. The resulting 4-cyano-PP (**212**) was first hydrolyzed to 4-carboxamido-PP (**213**), using aqueous hydrogen peroxide and aqueous potassium carbonate, and then it was esterified to obtain the corresponding 4-carboxylate-PP (**214**). Both compounds **203** and **214** were successively acylated at the free amino group at position 6 of the PP nucleus, by using the desired acylchlorides in the presence of a base (**211**, **215**). [20,21]



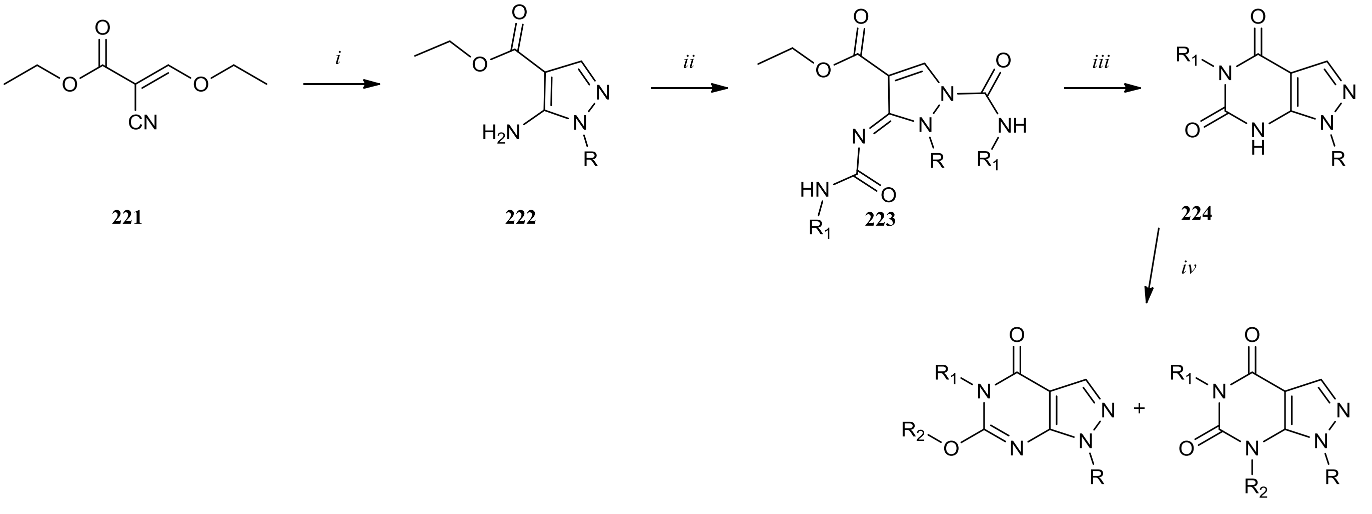
17 **216** **217**

Scheme 22. Reagents: *i*: (for R_1 = Ph) PhCN, t-BuOK, MW, or (for R_1 = H) boiling NH_2CHO, 12 h; *ii*: R_2XCOCl, TEA, MW or R_2NCO, MW, R_2 = aryl.



218 **219** **220**

Scheme 23. Reagents: *i*: NH_2CHO, 100-200°C, 8-16h, X = NH_2, O-alkyl; *ii*: P_2S_5, pyr, 80-115°C, 4-8h or 2,4-bis(4-methoxyphenyl)-1,3-dithia-2,4-diphosphetane-2,4-disulfide, xylene, rfx, 8-24h.

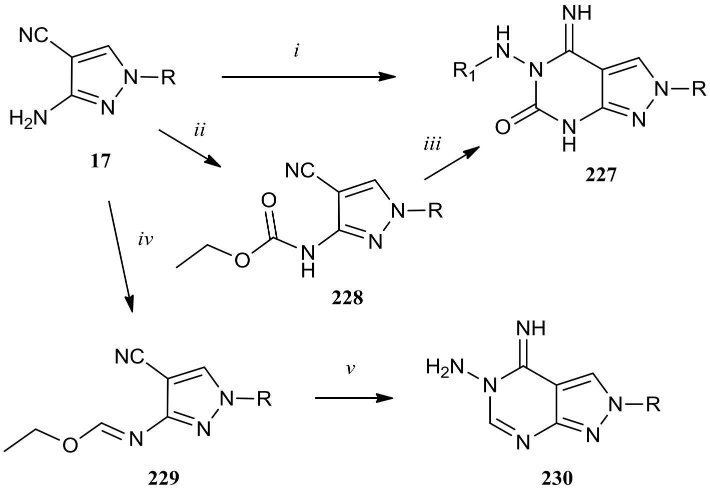


221 **222** **223** **224** **225** **226**

Scheme 24. Reagents: *i*: $RNHNH_2$, EtOH, rfx, 15h; *ii*: R_1NCO, TEA, $CHCl_3$, sealed tube, 120°C, 62h; *iii*: 2N NaOH, rfx, 4 min; *iv*: NaH, DMF, R_2I, 3h, rt.

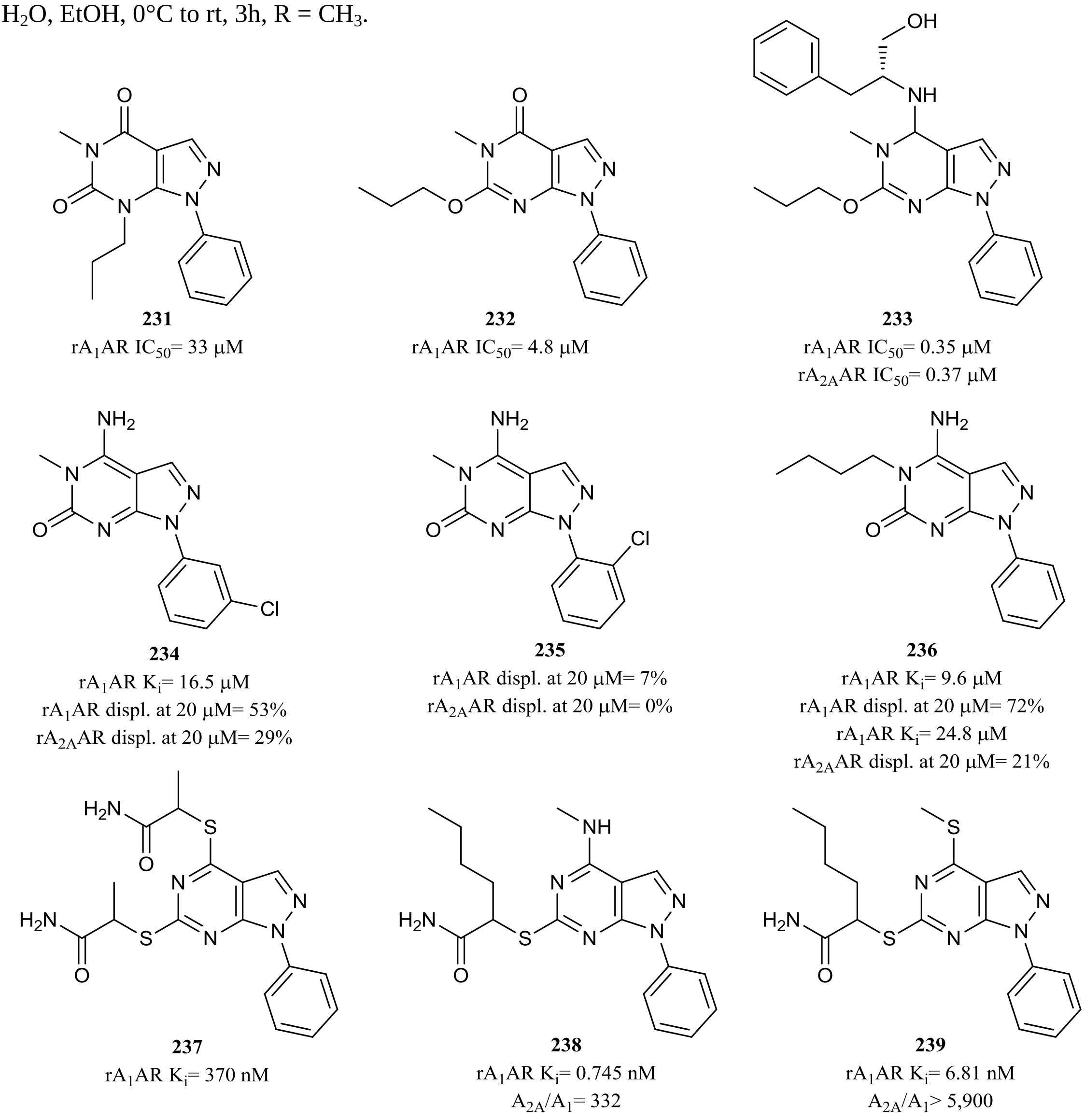
Taliani *et al.* reported a series of 2-substituted-PP as A_3 AR antagonists. [19] As shown in Scheme 22, they used a very simple synthetic approach. Compound **17** was reacted with boiling formamide to afford the 6-unsubstituted-2-substituted-2*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine (**216**, R_1=H). The 6-phenyl-PP derivative (**216**, R_1=Ph) was obtained by reaction with benzonitrile in the presence of potassium *tert*-butoxide and under microwave irradiation. Compounds **216** were converted to the corresponding 4-arylamido and 4-arylureido derivatives (**217**) by treatment with the appropriate acylchloride in the presence of triethylamine or with the appropriate isocyanate, respectively. [19]rnold *et al.* patented a few pyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one (**219**) and pyrazolo[3,4-*d*]pyrimidin-4(5*H*)-thione derivatives (**220**) as anticonvulsive, antiallergic and antiasthmatic agents targeting ARs. [81] Scheme 23 shows that 4-thione derivatives (**220**) were obtained by reaction of compounds of general formula **219** with phosphorous pentasulfide in hot pyridine or with 2,4-bis(4-methoxyphenyl)-1,3-dithia-2,4-diphosphetane-2,4-disulfide in xylene at reflux. Analogously to that reported by Taliani *et al.*, compounds **219** were obtained refluxing compounds of general formula **218** in formamide. The presence of an amido or ester moieties, instead of a cyano group (as in compound **17**) at the 4 position of the pyrazole **218**, led to the introduction of the oxo group (compound **219**), instead of the amino group (**216**) at the 4 position of the PP scaffold. [81]

Peet *et al.* synthesized PP derivatives very similar to xanthines (Scheme 24). They obtained the desired pyrazoles (**222**) by reacting ethyl(ethoxymethylene)cyanoacetate (**221**) with the appropriated substituted-hydrazine. Aminopyrazoles (**222**), when treated with excess methylisocyanate, gave the bisureas of general formula **223**. Cyclization of compounds **223** with aqueous sodium hydroxide gave the 4,6-dione-PPs (**224**). Further alkylation of compounds **224** with alkyliodide and sodium hydride in dimethylformamide gave a mixture of compounds **225** and **226**, easily separated by flash chromatography. [16]



Scheme 25. Reagents: *i*: R_1NHNH_2, EtOH, 80°C, 6h, R= CH_3, EtPh, PrPh, R_1 = H, Ph; *ii*: ClCOOEt, pyr, DMAP, 0°C to rt, 12h, R = CH_3, EtPh, PrPh; *iii*: R_1NHNH_2, EtOH, 80°C, 6h, R = CH_3, R_1 = PhCO; *iv*: $CH(OEt)_3$, rfx, 6h, R = CH_3; *v*: $NH_2NH_2.H_2O$, EtOH, 0°C to rt, 3h, R = CH_3.

Finally, Paira *et al.* developed a series of chelating 4-imino-(2-substituted)-2*H*-pyrazolo[3,4-*d*]-pyrimidines to form an organoruthenium A_3 AR antagonist which was structurally related to PTP. 3-Amino-4-pyrazolecarbonitriles (**17**) were transformed into their corresponding 5-amino-4-imino-2-substitutedamino-4,5-dihydro-2*H*-pyrazolo[3,4-*d*]pyrimidin-6(7*H*)-one (**227**) through a reaction with hydrazine derivatives in EtOH at 80°C. Furthermore, treatment of compound **17** with ethyl chloroformate afforded carbamates (**228**), which in turn were treated with benzhydrazide derivatives in EtOH at 80°C to afford the *N*-(4-imino-2-methyl-6-oxo-6,7-dihydro-2*H*-pyrazolo[3,4-*d*]pyrimidin-5(4*H*)-yl)benzamide (**227**, R_1=PhCO). Pyrazole **17** was also treated directly with triethyl orthoformate under



231
rA_1AR IC_{50}= 33 μM

232
rA_1AR IC_{50}= 4.8 μM

233
rA_1AR IC_{50}= 0.35 μM
$rA_{2A}AR$ IC_{50}= 0.37 μM

234
rA_1AR K_i= 16.5 μM
rA_1AR displ. at 20 μM= 53%
$rA_{2A}AR$ displ. at 20 μM= 29%

235
rA_1AR displ. at 20 μM= 7%
$rA_{2A}AR$ displ. at 20 μM= 0%

236
rA_1AR K_i= 9.6 μM
rA_1AR displ. at 20 μM= 72%
rA_1AR K_i= 24.8 μM
$rA_{2A}AR$ displ. at 20 μM= 21%

237
rA_1AR K_i= 370 nM

238
rA_1AR K_i= 0.745 nM
A_{2A}/A_1= 332

239
rA_1AR K_i= 6.81 nM
A_{2A}/A_1> 5,900

Figure 16. PP derivatives as A_1 AR antagonists.

reflux to give the imidate derivatives (**229**), which were further cyclized with hydrazine hydrate in ethanol to the corresponding PP compounds with general formula **230**. (Scheme 25) [82]

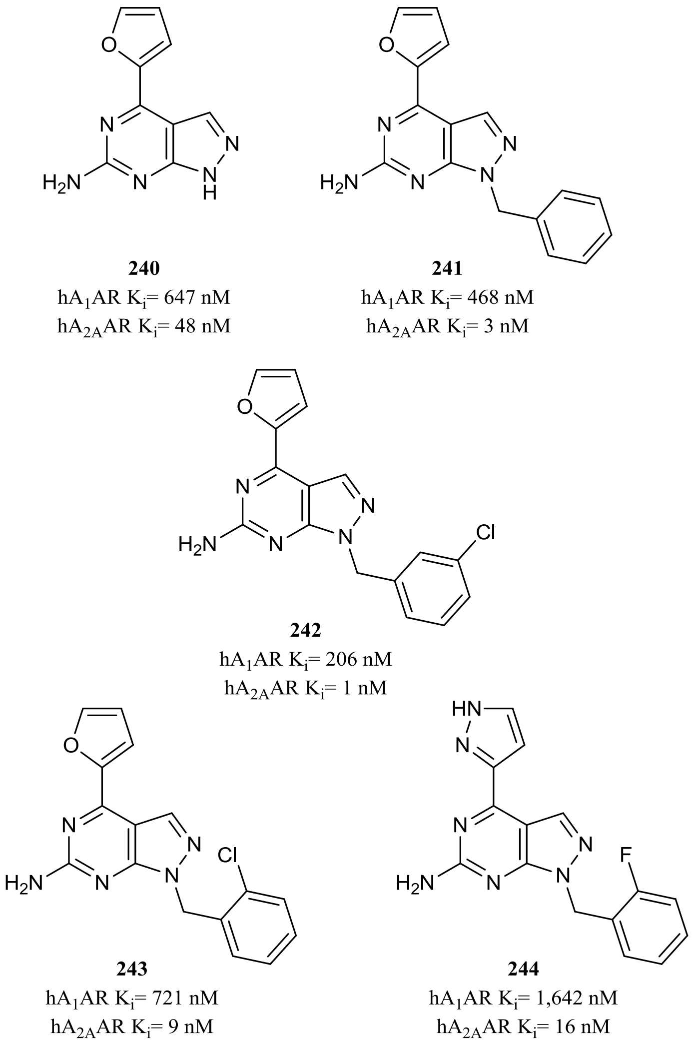


Figure 17. PP derivatives as A_{2A} AR antagonists.

4.2. Structure activity relationship

A_1 AR antagonists.

In a work aimed to develop conformationally restrained (phenylisopropyl)amino-substituted-PPs, Peet and coworkers explored the effect of substituents at the 4 and 6 position of the PP scaffold. [16] In particular, they observed that compound **231**, which is a PP-4,6-dione, showed an IC_{50} of 33 μM at the rA_1 AR, whereas the alkylation at the 6 position (concomitantly to the removal of substituent at *N*7-position) improved the affinity, such as for compound **232** that displayed an about 7-fold increased affinity at the rA_1 AR (IC_{50}= 4.8 μM). Interestingly, compound **233**, which bears a second substitution at the 4 position with an amino moiety, showed a greater affinity at the A_1 AR subtype than compounds **231** and **232** (Figure 16). Instead, Harden and coworkers performed a preliminary investigation on *N*1- and *N*5-substituted PPs. The series involved the variation of the substituent at the *N1*-position with small alkyl groups, phenyl or substituted phenyl moieties (e.g. halogen, NO_2 and NH_2 at *m*, *o* and *p* positions of the phenyl ring), and the variation of the substituent at the *N*5-position with small alkyl moieties or phenyl group.

Binding data at the A_1 AR demonstrated that compounds possessing substituted aromatic groups showed greater affinity than those with alkyl groups at the *N*1-position. Additionally, the investigation on the substituted phenyl moieties showed that chlorine produced a greater response than bromine and fluorine substitutions, whereas nitro or amine substitutions were inactive at a concentration of 20 μM. Secondly, substitution of the aromatic group in the *meta* position gave better affinity values at the rA_1 AR (e.g. compound **234**, displ. at 20 μM = 53%, IC_{50} = 16.5 μM) than *orto* or *para* substitutions (e.g. compound **235**, displ. at 20 μM = 7%). In regard to substitutions at the 5 position, an alkyl moiety showed greater AR affinity than the aromatic group. The most active compound of the series was compound **236** which displayed an IC_{50} of 9.6 μM. Even though an improvement of affinity was achieved, both affinity and selectivity of these compounds were poor.

In 1984, Davies *et al.* developed a series of PPs as purinergic receptor antagonists, in particular, as A_1 AR antagonists. [82] The lead compound, 2,2'-((1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidine-4,6-diyl)bis(sulfanediyl))dipropanamide (**237**), showed an affinity of 370 nM at the A_1 AR. Compound **237** represented the starting point to perform a structural optimization with the aim to improve binding affinity at the A_1 AR. [17] In fact, compound 237 was modified at the 4 position with mercapto, methylthio, and amino groups in order to investigate the hydrogen-bonding and steric tolerance at this position. At the 6 position of the PP scaffold, the 2-mercaptoalkylamide chain was maintained but varying the length of the alkyl chain. [17] From the binding data at rA_1 AR, it was found that the K_i value depended principally on the substituent present at the 4 position and decreased in the order $SH>>SCH_3>NH_2\geq NHCH_3$, with compound **238** being the most potent of the series at the rA_1 AR (rA_1AR Ki = 0.745 nM). Moreover, alkyl chain at α position of the sulfur atom at the 6 position of PP was found to influence affinity at the A_1 AR. In this case, K_i value decreased in the order *i*-Pr>>Et>Pr>Bu, thus indicating that a branched alkyl moiety was unfavorable in terms of affinity, probably due to its steric hindrance. Compounds were tested also at the rA_{2A} AR and binding data suggests that 4-methylthyio compounds were the most promising compounds because they showed nanomolar affinity at the rA_1 AR, with high levels of selectivity over rA_{2A} AR (e.g. compound **239**, rA_1AR K_i = 6.81 nM, $rA_{2A}/rA_1 > 5{,}900$). [17]

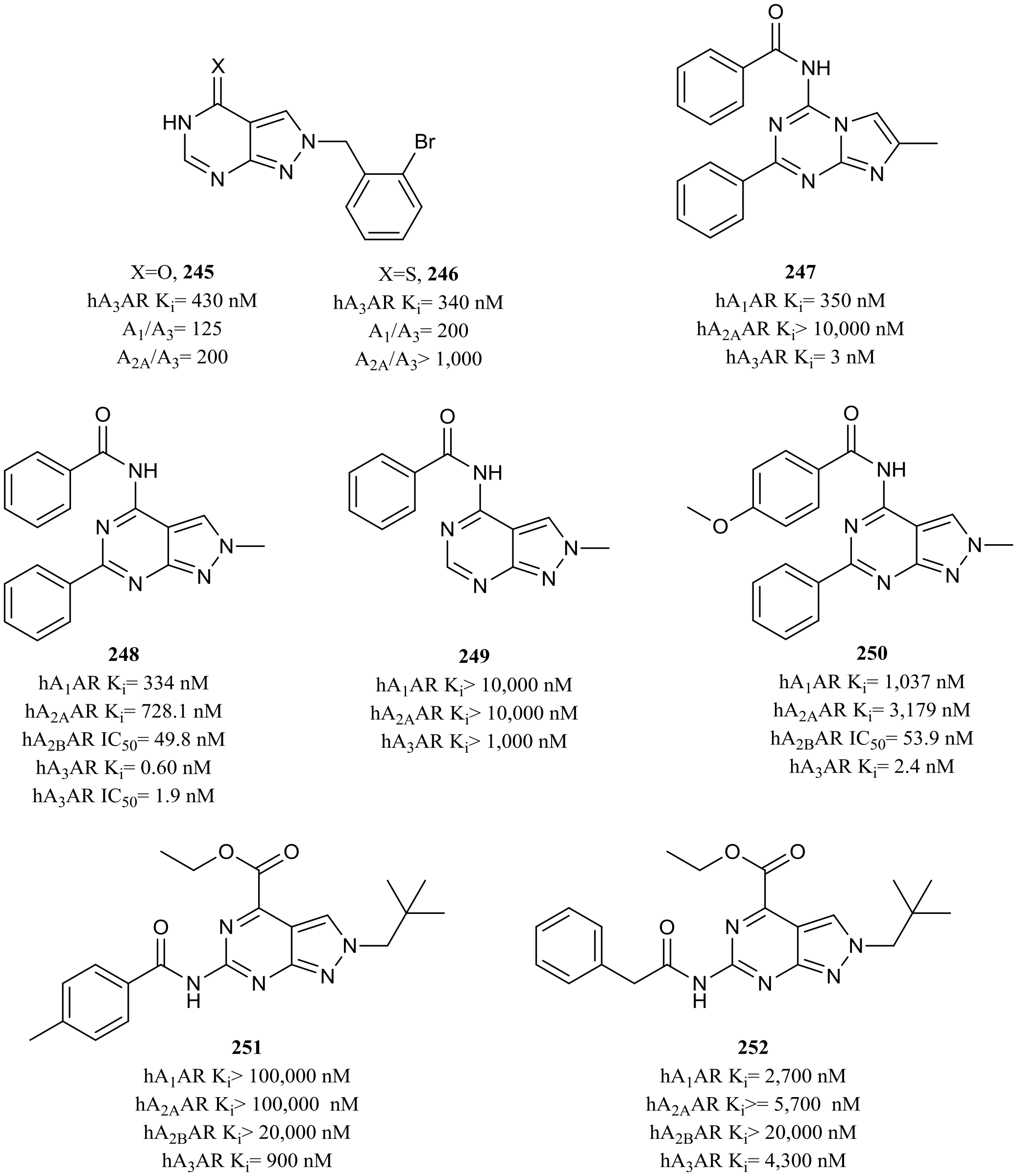
A_{2A} AR antagonists

PPs were explored by Gillespie and collaborators as A_{2A} AR antagonists [18,79] for their evaluation in the treatment of PD. The 4-(furan-2-yl)PP (**240**) was found to possess a K_i of 48 nM at the hA_{2A} AR and was 13-fold selective over A_1 AR subtype. (Figure 17) The introduction of a benzyl moiety at the 1 position of the PP ring (**241**) increased affinity at the A_{2A} AR (compound **241**, hA_{2A} AR K_i = 3 nM). Instead, both substitution of the phenyl ring with various heterocycles and the extension of the linker between phenyl and PP (from 1 to 2 or 3 methylene groups), did not improve affinity. Successively, the effects of substitution around the phenyl ring were explored. Binding data demonstrated that substitution on the *meta*-position (see compound **242**, hA_{2A}

AR K_i = 1 nM) of phenyl ring was preferred to *ortho*- and *para*- substitutions (e.g. compound **243**, hA_{2A} AR K_i = 9 nM), which generally decreased affinity compared to parent compound **241**. Finally, furyl moiety at the 4 position of PP was replaced by other aromatic rings, but all the compounds displayed a reduced affinity at the A_{2A} AR, such as 4-pyrazol-3-yl compound **244** (hA_{2A} AR K_i = 16 nM). [18,79]

A_3 AR antagonists

The first report on development of A_3 AR antagonists with a PP nucleus came from Arnold and its coworkers, which focused on a series of pyrazolo[3,4-*d*]pyrimidin-4(5*H*)-ones and pyrazolo[3,4-*d*]pyrimidine-4(5*H*)-thiones. These compounds bear a substituted benzyl moiety at the 2 position of PP, thus on the 2 position of the pyrazole ring. Substitutions on the phenyl ring were almost limited to the *meta* position. Best results were obtained with halogen substituents, in particular with 2-bromo substituent, both in 4-one (compound **245**, hA_3 AR K_i = 430 nM) and 4-thione series (compound **246**, hA_3 AR K_i = 340 nM). Taliani *et al.* developed a new series of PP based on their previous results on imidazole[1,2-*a*][1,3,5]triazines, which are isosters of PP. [19] The imidazole-[1,2-*a*][1,3,5]triazine **247** showed high affinity at the hA_3 AR and quite good selectivity over A_1 and A_{2A} ARs, however its corresponding PP derivative (**248**) showed higher affinity at the hA_3 AR subtype. For this reason new PP derivatives were synthesized in order to explore the SAR of these compounds at the hA_3 AR. Binding affinity results suggested that phenyl ring at the 6 position was a key feature for recognition at the ARs, since its removal (e.g. compound **249**, hA_3 AR K_i > 1,000 nM) led to poor affinity to all the ARs. At the 2 position, both methyl and benzyl groups are tolerated, while a phenylethyl moiety decreased affinity at the hA_3 AR. Interestingly, compounds bearing a free amino moiety at the 4 position are poorly active at the hA_3 AR and also totally unselective over the other AR subtypes, while the substitution at the 4 position with arylamides or arylureas enhanced both affinity and selectivity at the hA_3 AR. The best compound of the series is compound **250**, which showed a hA_3 $_{Ki}$ value of 0.18 nM.



X=O, **245**
hA_3AR K_i= 430 nM
A_1/A_3= 125
A_{2A}/A_3= 200

X=S, **246**
hA_3AR K_i= 340 nM
A_1/A_3= 200
A_{2A}/A_3> 1,000

247
hA_1AR K_i= 350 nM
$hA_{2A}AR$ K_i> 10,000 nM
hA_3AR K_i= 3 nM

248
hA_1AR K_i= 334 nM
$hA_{2A}AR$ K_i= 728.1 nM
$hA_{2B}AR$ IC_{50}= 49.8 nM
hA_3AR K_i= 0.60 nM
hA_3AR IC_{50}= 1.9 nM

249
hA_1AR K_i> 10,000 nM
$hA_{2A}AR$ K_i> 10,000 nM
hA_3AR K_i> 1,000 nM

250
hA_1AR K_i= 1,037 nM
$hA_{2A}AR$ K_i= 3,179 nM
$hA_{2B}AR$ IC_{50}= 53.9 nM
hA_3AR K_i= 2.4 nM

251
hA_1AR K_i> 100,000 nM
$hA_{2A}AR$ K_i> 100,000 nM
$hA_{2B}AR$ K_i> 20,000 nM
hA_3AR K_i= 900 nM

252
hA_1AR K_i= 2,700 nM
$hA_{2A}AR$ K_i>= 5,700 nM
$hA_{2B}AR$ K_i> 20,000 nM
hA_3AR K_i= 4,300 nM

Figure 18. PP derivatives as A_3 AR antagonists.

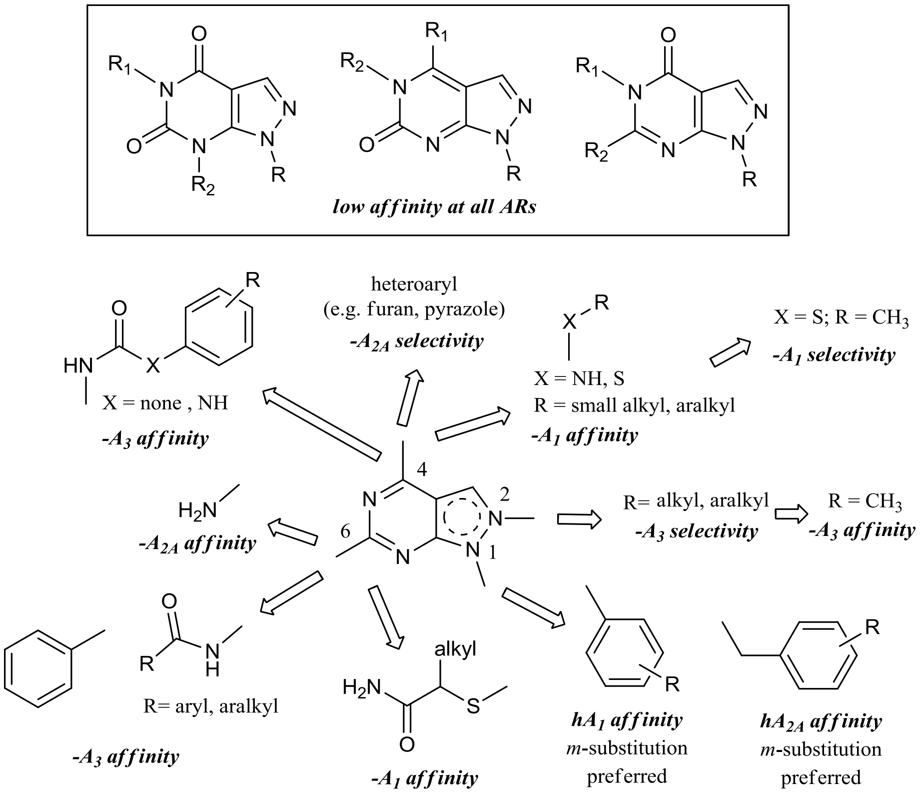


Figure 19. Structure activity relationship of PP derivatives at ARs.

Compounds **248** and **250** were subsequently tested on human glioma U87MG cells, and they were able to counteract the proliferation of glioma cells mediated by A_3 AR agonists. [19]

Recently, Venkatesan *et al.* developed a series of 4-chloro-PP as a simplification of the PTP scaffold, which was well-known to afford potent hA_3 AR antagonists (see Section 2.2). [20] The series investigated both 1 and 6 positions of the PP nucleus. Compounds were active in the micromolar range at the hA_3 AR and almost all are completely unselective. On the contrary, when the chloro moiety at the 4 position was substituted with ethyl carboxylate group, an improvement of affinity and especially of selectivity was observed, as clearly shown in Figure 18 for compound **251** **(**hA_3 AR K_i = 900 nM, A_1/A_3 = 111, A_{2A}/A_3 = 111). [20] A further investigation on these new compounds revealed that neopentyl moiety at the 2 position is preferred over other alkyl of arylalkyl goups. In addition, replacement of the benzoyl group with a phenylacetyl moiety led to a complete loss of selectivity (**252**). [21]

Figure 19 depicts the SAR analysis of PP compounds at ARs.

5. MOLECULAR MODELING STUDIES AT THE ADENOSINE RECEPTORS

5.1 Homology Modelling

Very recently, several X-ray structures of the hA_{2A} AR in complex with agonists and antagonists have become available. [83-90] These structures represent valuable starting points for structure-based drug design approaches. [91] Moreover, they can serve as templates to gather structural information on the other ARs subtypes by means of homology modelling. The success of the homology modelling approach depends upon at least two key factors: the sequence identity and the sequence alignment between the template and the target structure. In the homology modeling of ARs, the sequence alignment is guided by the

Figure 20. Overview of the conserved residues (sticks representation) at the hA_{2A} AR that guide the sequence alignment for the construction of ARs models.

most conserved residues in each transmembrane ™ domain, *i.e.* the TM.50 residue used as reference in the Ballesteros and Weinstein nomenclature system for class A GPCRs [92,93], such as: Asn1.50, Asp2.50, Arg3.50, Trp4.50, Pro5.50, Pro6.50 and Pro7.50 in TM1-7, respectively (Figure 20). As far as the sequence identity is concerned, the hA_{2A} AR shares 55.9%, 67.4%, and 47.7% sequence identity with the hA_1 AR, the hA_{2B} AR, and the hA_3 AR, respectively.

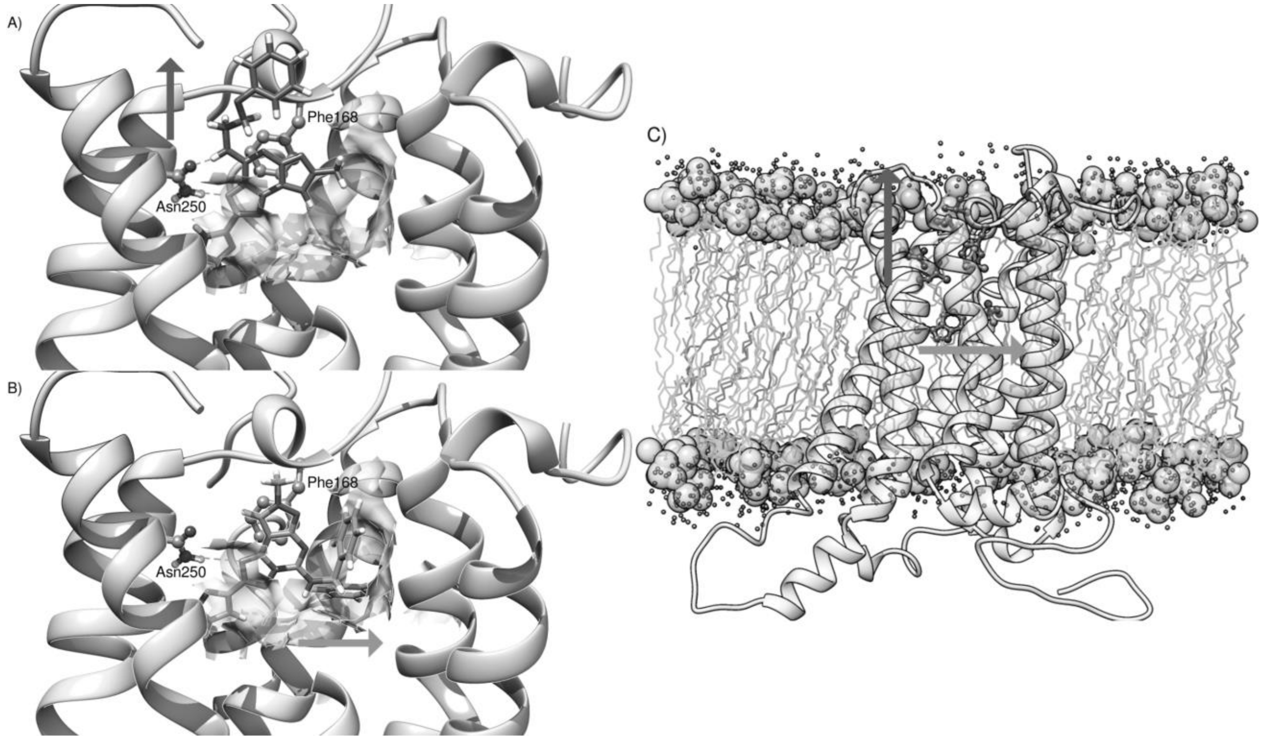


Figure 21. Hypothetical binding modes of example PTP compounds exhibiting BM1 (A) and BM2 (B) inside the hA_3 AR binding site. Poses are viewed from the membrane side facing TM6, TM7, and TM1 with the view of TM7 partially omitted to aid visualization. The side chains of Asn250 (6.55) and Phe168 (EL2) are represented in ball and stick. Hydrogen bond interactions are highlighted as dashed lines, whereas the side chains of residues interacting with the ligand through hydrophobic contacts are displayed as surfaces. An arrow indicating the directionality of the substituent at the PTP N5 position is shown. (C) Schematic representation of the hA_3 AR embedded in a solvated lipid bilayer simulating the cell membrane. The side chains of residues interacting through hydrophobic contacts are displayed as ball and sticks, for BM1 and BM2. Side chains of Asn250 (6.55), Phe168 (EL2), and Trp243 (6.48) are represented in gray ball and stick. Hydrogen atoms are omitted. Readopted from ref [42].

The growing interest in the modeling of GPCRs has prompted the scientific community to release several *ad hoc* web services [94-98] among which "*Adenosiland*", a platform entirely dedicated to ARs. [99] In *Adenosiland*, homology models for all the cloned ARs are freely available. These models were derived from a homology modeling protocol followed by 20 ns of molecular dynamics (MD) simulations in near-native environment (solvated lipid bilayer embedded receptor). The so refined structures represent a structural alternative to homology models. A tool to guide the user in the selection of an appropriate template to build ARs homology model has also been implemented. [100] The models deposited in *Adenosiland* as well as the template searching facility have been exploited to gather macromolecular starting points for the rationalization of SARs of different series of ARs antagonists, such as PTP and TP. [14,42]

5.2 Docking

Provided that a three-dimensional structure - either from experimental data or computationally derived - is available, molecular docking is one of the most widely used structure based drug design techniques. The analysis of docking poses represents a helpful tool to rationalize SARs. With respect to ARs binders, docking studies have been extensively used to elucidate the molecular signatures favoring or affecting the binding of antagonists at the hA_{2A} and the hA_3 ARs. In some cases, docking studies helped in explaining the selectivity profiles exhibited by the most promising synthetized derivatives. Moreover, the analysis of the docking results allows to speculate on the most favorable orientations of the substituents on the scaffolds, thus providing rationale suggestions for further derivatizations.

With regard to this, we have recently reported on the analysis of the 5 position of the PTP scaffold at the hA_3 AR. [42] In particular, we have evidenced the possibility of the scaffold to adopt two different orientations in the binding site according to the nature of the substituent linked to the N5 atom that gives rise to the interaction with different TM domains (Figure 21). Compounds bearing small alkyl and cycloalkyl groups and polar chains prefer a binding mode (BM1, vertical arrow) that exposes the substituent to the extracellular side and envisages the interaction with TM6, TM7, and EL2 (extracellular loop 2). Ligands bearing aromatic rings, bulky substituents as well as those with the double substitution at the N5 atom, preferentially adopt a binding mode (BM2, horizontal arrow) that points the substituent inside the binding cavity interacting with TM2, TM3, and EL2.

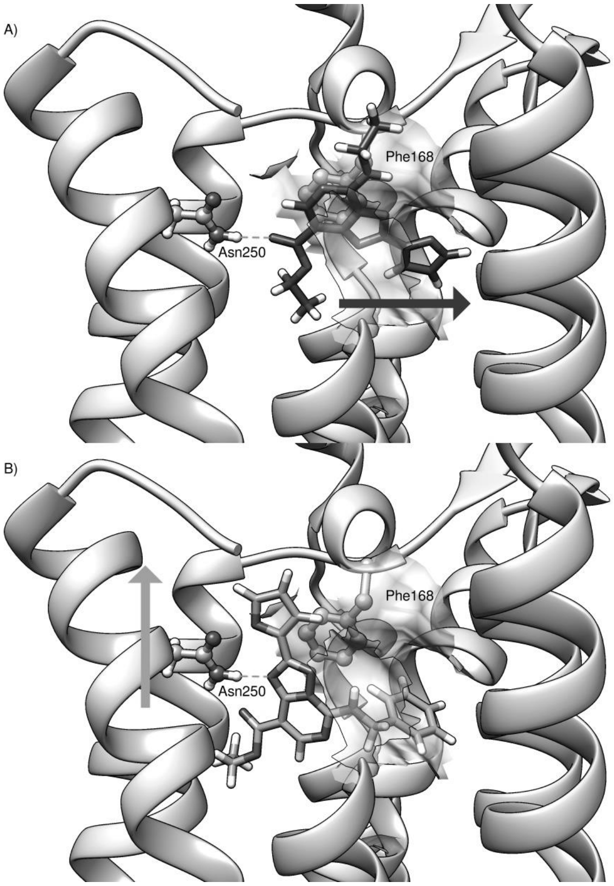


Figure 22. Hypothetical binding modes of example TP compounds exhibiting BM1 (A) and BM2 (B) inside the hA_3 AR binding site. Poses are viewed from the membrane side facing TM6, TM7, and TM1, hydrogen bonds are highlighted as dashed lines, and side chains of Asn250 (6.55) and Phe168 (EL2) are represented in balls and sticks. An arrow indicating the directionality of the furan ring is shown. Readopted from ref. [14].

The possibility for a scaffold to adopt different binding modes depending on the nature and the steric hindrance of the substituents has also emerged through the analysis of the docking poses at the hA_3 AR of a series of 5,8 disubstituted-TP. (Figure 22) [14] Derivatives bearing small alkyl and cycloalkyl groups interact with Asn250 (6.55) through the ethoxycarbonyl group at the 8 position, by pointing the furan ring toward TM2 and exposing the alkyl group to the solvent. Compounds bearing branched chains and benzyl groups interact with Ans250 (6.55) with the N4 of the triazole ring, with the furan ring solvent exposed and the alkyl/benzyl group projected toward TM2.

5.3 Molecular Dynamics

Besides molecular docking, MD is a powerful tool nowadays largely used in medicinal chemistry and drug discovery. [101] With respect to the static analysis proposed by the docking solutions, MD simulations enable to take into account also the flexibility of protein side chains and the interactions mediated by water molecules. The description of these two fundamental aspects in ligand binding is still challenging to be depicted by conventional docking approaches. [102] A strategy to follow the dynamics of ligand-receptor interaction is to compute the so-called the “dynamic scoring function” (DSF), [103] *i.e.* the cumulative sum of electrostatic and hydrophobic contributions to the interaction energy computed between the ligand and selected residues surrounding the binding site over time. This feature has been introduced and exploited to support an apparently less plausible binding mode of a series of 5-alkylaminopyrazolo[4,3-*e*]1,2,4-triazolo[1,5-*c*]pyrimidines at the hA_3 AR. [42] In particular, the two different binding modes described above (Figure 20) have been validated through 60 ns of membrane MD simulations.

CONCLUSIONS

Adenosine receptor antagonists could be useful for the treatment of hypertension, heart and kidney failure, inflammation, neurodegenerative disorders (e.g. PD), asthma, COPD, cancer and glaucoma, depending on the targeted AR subtype. Therefore, the development of antagonists potent and selective for a specific AR subtype is of remarkable importance. PTP represent one of the most important scaffolds among adenosine receptor antagonists. Synthesis of the PTP scaffold was optimized over the years, allowing the development of extended series of derivatives which led to acquisition of a good SAR profile, especially towards the A_{2A} and A_3 AR subtypes. Preladenant (**2**) is a promising PTP derivative that reached phase III clinical trials for the treatment of PD. Unfortunately it failed at this stage of clinical research but the reasons are still unclear. Nevertheless, PTP derivatives are tricyclic compounds with a high molecular weight which exhibit limitations such as poor aqueous solubility and difficult synthetic preparation. TP and PP are bicyclic scaffolds which could be considered as simplified analogs of PTP, possessing a lower molecular weight and thus probably showing better drug-like properties than PTP. TPs were principally developed by industries to obtain A_{2A} AR antagonists. SAR of these compounds was not fully explored, thus, further investigations on this scaffold are needed. Instead, PPs were studied almost exclusively by academia, which apply very different approaches to find potent AR antagonists. The high structural variability of these compounds and the different biological assays used in the various studies, make difficult to obtain a good SAR profile of these derivatives. Molecular modeling studies helped to rationalize the obtained experimental binding data and also to design new optimized derivatives. Both docking and molecular dynamics are powerful tools largely used in medicinal chemistry and drug discovery. Indeed, optimization of the PTP derivatives, and recently also optimization of the TP and PP derivatives, as AR antagonists were strongly supported by molecular modeling studies.

LIST OF ABBREVIATIONS

AC, adenylyl cyclase;

AR, adenosine receptor;

BM, binding mode;

Boc, *tert*-butyloxycarbonyl;

BSA, *N*,O-bis(trimethylsilyl) acetamide;

cAMP, cyclic adenosine monophosphate;

CNS, central nervous system;

COPD, chronic obstructive pulmonary disease;

CSA, camphorsulfonic acid;

DBU, 1,5-diazabiciclo(5.4.0)undec-5-ene;

DCM, dichloromethane;

DEAD, diethyl azodicarboxylate;

DIBAL, diisobutylaluminium hydride;

DIPEA, *N,N*-diisopropylethylamine;

DMAP, 4-dimethylaminopyridine;

DMF, *N*,*N*-dimethylformamide;

DMSO, dimethylsulfoxide;

DSF, dynamic scoring function;

EDCI, N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride;

EGME, ethylene glycol monomethyl ether (2-methoxyethanol);

EL, extracellular loop;

FDA, food and drug administration;

FST, forced swim test;

GPCR, G protein-coupled receptor;

hERG, human Ether-à-go-go-Related Gene potassium channel;

HMDS, hexamethyldisilazane;

HMDSO, hexamethyldisiloxane;

HOBt, hydroxybenzotriazole;

IC_{50}, half maximal inhibitory concentration;

K_i, inhibition constant;

MD, molecular dynamics;

MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine;

NMM, N-methylmorpholine;

NMP, 1-methyl-2-pyrrolidinone;

PCC, pyridinium chlorochromate;

PD, Parkinson's disease;

PP, pyrazolo[3,4-*d*]pyrimidine;

PPSE, polyphosphoric acid trimethyl silyl ester;

PTP, pyrazolo[4,3-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine;

SAR, structure activity relationship;

TEA, triethylamine;

TFA, trifluoroacetic acid;

THF, tetrahydrofuran;

TM, transmembrane;

TP, [1,2,4]triazolo[1,5-*c*]pyrimidine;

TST, tail suspension test.

CONFLICT OF INTEREST

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