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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\textsubscript{1}, A\textsubscript{2A}, A\textsubscript{2B} and A\textsubscript{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\textsubscript{2A} and A\textsubscript{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 derivatives that behave like adenosine receptor antagonists (Figure 1). [4] Preladenant (SCH 420814, 2), an A\textsubscript{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 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\textsubscript{1}, A\textsubscript{2A}, A\textsubscript{2B} and A\textsubscript{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 pharmaceutical 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\textsubscript{2A} and A\textsubscript{3} AR antagonists (Figure 1). [4] Preladenant (SCH 420814, 2), an A\textsubscript{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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The adenosine receptors are involved in several physiological and pathological processes. [2] As GPCR (G protein-coupled receptor), A1 and A3 ARs are coupled with inhibitory G proteins (G(i)), which inhibit adenylyl cyclase (AC) reducing the cyclic AMP (cAMP) levels. On the contrary, A2A and A3B 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(s) proteins for the A2B and A3 ARs, and G(i) for the A2A AR in the striatum. [1] In particular, activation of the A2A 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 vasodilatation is mediated by A2A AR, so agonists are useful as stress agents for myocardial perfusion imaging. Regadenoson is an A2A AR agonist approved by Food and Drug Administration (FDA) for this use. [2] A2A AR is also present in several immune cells and its activation leads to multiple anti-inflammatory effects. On the other hand, antagonists for the hA2A AR were widely studied for the treatment of PD and other neurodegenerative disorders. [22] A2B 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 A2B AR are studied for the involvement in promoting ischemic preconditioning, whereas A2B 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 A3 subtype, there are evidences of a cardioprotective effect mediated by A3 AR agonists. Instead, for other physio-pathological conditions involving the A3 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 A2 adenosine receptor antagonists. [26] At that time, only few examples of non-xanthine heterocyclic compounds have been found to display antagonistic activity at the A1 or A2A ARs. Among these compounds CGS 15943 (5), a [1,2,4]triazolo[1,5-c]quinazoline derivative, is a reference antagonist for A2A AR but it shows low selectivity for the A2A AR versus A1 AR (Figure 3). Gatta et al. explored new chemical entities in order to enhance selectivity at the A2A 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 A2A AR and represented a starting point for the development of a new class of potent and selective AR antagonists. [26]

![Figure 1. Pyrazolo[4,3-e][1,2,4]triazolo[1,5-c]pyrimidines.](image1)

![Figure 2. Simplified analogs of the PTP scaffold.](image2)

![Figure 3. Comparison between PTP and [1,2,4]triazolo[1,5-c]quinazoline derivatives.](image3)
compounds of general formula 9 and 11 in diphenylether. The cyclization of derivatives 9 afforded the 7-substituted-3,7-dihydro-2H-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 (1H-1,2,4-triazol-5-yl)-1H-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-7H-pyrazolo[4,3-e][1,2,4]triazolo[1,5-c]pyrimidin-5-one derivatives by reaction with ethyl carbamate. Finally, in order to obtain also the 8-substituted analogs, the appropriated starting compound N1-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-7H-pyrazolo[4,3-e][1,2,4]triazolo[1,5-c]-pyrimidin-5-ylamine (21), which has been obtained by treatment of the corresponding N7-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: RNHNH₂, EtOH; ii: CH(OCC₂H₅)₃, rfx, 8h; iii: ArCONH₂NH₂ or C₆H₅OCOHNH₂, EGME, rfx, 3h; iv: Ph₂O, rfx; v: 10% HCl (aq), rfx, 3h; vi: ArCONH₂NH₂, Ph₂O, rfx; vii: CNNH₂, p-toluensulfonic acid, NMP, 140-160°C; viii: NH₂COOC₂H₅, 210°C, 3h; ix: RCl, DMF, K₂CO₃, 70°C, 3h. R = 4-fluorobenzyl or 2-fluorobenzyl ; Ar = 2-furol or phenyl.
of unsubstituted (21) and N8-tert-butyl (22) derivatives (Scheme 2). 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. 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.

Schering has developed a more convenient synthesis (Scheme 3) for the 2-aryl-7H-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. 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 POCl3 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,N-bis(trimethylsilyl)acetamide (BSA) at reflux proceeded with concomitant Dimroth rearrangement to afford the desired products 28. Additionally, pyazole alkylation, by using sodium hydride instead of potassium carbonate, afforded preferentially the N7-alkylated derivatives (29) with small amounts of N8-alkylated regioisomers.

In the same patent also other slightly different synthetic approaches were claimed. As depicted in Scheme 4 the direct cyclization of compound 25 with hydrazine afforded the 4-chloro-1H-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 N1-alkylated compounds, which correspond to the final N7-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 N7-substituted-PTP derivatives (34). When compound 30 was


Scheme 3. Reagents: i: ArCONHNH2, DIPEA, CH3CN, rt, overnight; ii: NH2NH2.H2O, CH3CN, 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 \( N7 \) position (e.g. with 1-aryl-piperazinines) (compound 37) and the subsequent deprotection of the amino moiety by HCl afforded compound 32 which then followed the above mentioned procedure.

All the synthetic pathways hitherto reported for the obtaining 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 N1-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 methyldihyrazine (38) and benzaldehyde. This initial synthetic step allowed the protection of the non-alkylated nitrogen of methyldihyrazine, thus directing the subsequent reaction with ethoxymethylene malononitrile on the methylated nitrogen (compound 40).

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\text{Scheme 4. Reagents:} \ i: \ NH_2NH_2.H_2O, \text{ DIPEA, DMF, rt, 4h;} \ ii: \text{ RX, NaH, DMF, 0°C-rt, 1h;} \ iii: \text{ RX=ClCH_2CH_2Br;} \ iv: \text{ R,COCl, DIPEA, DMF, rt, 2h or R,COOH, HOBt, EDCI, DMF, rt, 1h;} \ v: \text{ 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 = arlyalkyl, arlythioalkyl, aryloxalkyl, (4-substituted-piperazin-1-yl)alkyl R = Ar, phenyl, pyrrol-2-yl and their substituted derivatives, or nicotinoyl or cyclopent-1-en-1-yl, Ar = fur-2-yl, thien-2-yl.}
\]

\[
\text{Scheme 5. Reagents:} \ i: \text{ PhCHO, EtOH, rfx, 3h;} \ ii: \text{ (CN)_2CCHOC_2H_5, C_6H_6, rfx, 1h; iii: HCl conc., EtOH, rfx, 0.5h; iv: see scheme 1.}
\]
The desired N1-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-8H-pyrazolo[4,3-e][1,2,4]triazolo[1,5-c]pyrimidin-5-amine (42) following Gatta et al. procedure. [26,32] (Scheme 5)

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-5H-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 with the cyanoaminopyrazole (16) with a substituted orthoester (e.g. trimethyl orthoformate) in the presence of 1% of CSA (dl-camphorsulfonic acid) in DMF. [45,46]

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, R1 = alkyl, ary, arylalkyl, R2 = ary.

Scheme 7. Reagents: i: ClCOOCCI3, dry dioxane, 70°C, 1.5h; ii: POCl3, PCl3, Pyr, rfx, 24h; iii: R1RNH2, EtOH, 110°C, sealed tube, 2h. R = alkyl, cycloalkyl, aralkyl, aminoalkyl, hydroxylalkyl; R1 = H, alkyl, cycloalkyl, aralkyl, hydroxylalkyl.

Scheme 8. Reagents: i: n-BuC(OCH3)3, 1% CSA, DMF, rt, 1-6h; ii: ArCONHNH2 was added in the same vessel, rfx, 1-6h. Ar = Ph or substituted Ph.

2.2. Structure activity relationship
A2A 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-2H-pyrazolo[4,3-e][1,2,4]triazolo[1,5-c]pyrimidin-2-one (10), 2,7-disubstituted-2H-pyrazolo[4,3-e][1,2,4]triazolo[1,5-c]pyrimidine (12), 5-amino-2,7-disubstituted-7H-pyrazolo[4,3-e][1,2,4]triazolo[1,5-c]pyrimidine (15) and 5-amino-2,8-disubstituted-8H-pyrazolo[4,3-e][1,2,4]triazolo[1,5-c]pyrimidine (19). The most promising compounds at the A2A 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 A2A AR (Ki = 1.2 nM), whereas 7-FB-PTP (53) displayed a decreased affinity at the A2A AR (Ki = 12 nM) but a better selectivity against the A1 AR than compound 52 (53, A1/A2A = 15.8 vs 52, A1/A2A = 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 NH2 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 A2A 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 rA2A AR vs rA1 AR compounds, 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 A2A AR than alkyl moieties (e.g. 7-n-butyl, compound 56). [29,47] Compound 54, named SCH58261, was synthesized also in its triitated form, [3H]SCH58261, which demonstrated to label A2A receptors with high affinity (KD = 0.7 nM) and high specific binding (> 90%). [48] Furthermore, SCH58261 (54) maintained antagonism at the A2A 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 hydrophobic character of PTP derivatives, while retaining high affinity and selectivity at the hA2A 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 hA2A AR with different degrees of selectivity over the hA1 AR. On the other hand, all compounds were inactive at the hA3B and hA1 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 rA2A AR (compound 57). Also the para-methoxy derivative, SCH442316 58, demonstrated to be a potent and selective A2A AR antagonist, both at the human and rat receptors (rA2A AR Ki = 5.3 nM, rA1/rA2A = 487; hA2A AR Ki = 2.7 nM, hA1/hA2A = 611). [49] Starting from compound 57, SCH442316 (58) can be obtained in its 11C form ([11C]SCH442316), and used as radioligand in positron emission tomography (PET). [51] Amino (compound 59) and aminomethylene substitutions improved selectivity versus the A1 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 A2A 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 A2A adenosine receptor fluorescent polarization assays in place of traditional radioligand based binding studies. MRS5346 (61) possessed a Ki affinity value of 111 nM at the hA2A 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-aryalkyl-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 A1 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 A2A AR (hA2A AR Ki = 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 hA2A 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]

A1 AR antagonists

The rationale for the development of PTP derivatives as A1 AR antagonists consists in hybridization between the PTP core, known to behave as antagonist at the A2A ARs, and the arylcarbamoyl moiety of potent rA1 AR agonists (such as compound 65 in Figure 6), which were known to confer affinity for the rA1 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 position of substitution (see Section 2.1 Chemistry), only 8-substituted derivatives were developed.
All the synthesized compounds showed high affinity at the hA3 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

Figure 4. PTP derivatives as A2A AR antagonists: compounds 52-61.
subtypes, showing high affinity for the A2A AR, with low selectivity versus the A1 AR (as observed for 8-FB-PTP (52) compound) and low affinity at hA3 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 A2A AR antagonist (hA2A/AR Kᵢ = 0.34 nM; A₁/A₂A = 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 A3 AR antagonist (67, hA3AR Kᵢ = 0.98 nM; A₁/A₃ = 205; A₂A/A₃ = 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 A3 AR subtype and good selectivity over the other AR subtypes (i.e. compound 68, hA3AR Kᵢ = 0.20 nM; A₁/A₃ = 5,485; A₂A/A₃ = 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 [³H]-MRE-3008-F20 (70), the first selective radioligand for the hA3 AR behaving as antagonist. [55,56]

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 A3 AR were in the range 0.16-3.7 nM, except for derivatives bearing a para-sulfonic acid group which displayed a Kᵢ value above 25 nM (i.e. compound 71), indicating that para-substituent is surrounded by a lipophilic environment inside the A₃ AR binding cleft. Molecular modeling studies on the hA3 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 Kᵢ of 0.16 nM at A3 AR and > 2000-fold selectivity for A3 over A1 and A2A ARs. The replacement of phenylureido moiety with a phenylacetyl moiety, maintained affinity at the hA3 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 A₃ AR (i.e. compound 74, hA₃AR Kᵢ = 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 A3 AR antagonist ever reported (compound 75), bears the 4-pyridinum 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 for rA3 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, hA₃AR Kᵢ = 9.7 nM; A₁/A₃ = 351; A₂A/A₃ > 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 hA3 AR in the nanomolar or subnanomolar range, with different levels of selectivity over the other AR subtypes.

**Figure 5.** PTP derivatives as A₂A AR antagonists: compounds 62-64.
The most potent and selective antagonist of the series bears an (S) α-phenylethylamino moiety at the 5 position (77, hA3AR $K_i = 0.3$ 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. Figure 6. PTP derivatives as $A_3$ AR antagonists: compounds 65-74.)
compound 78, \( \text{A}_1/\text{A}_3 = 25; \) \( \text{A}_{2\alpha}/\text{A}_3 = 47.4 \). [42] Interestingly, 5-amino derivatives were unselective AR antagonists (i.e. compound 79), whereas the 5-phenylacetamido derivatives retained high affinity at the \( \text{hA}_3 \) 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, \( \text{hA}_3 \) AR \( K_i = 0.241 \text{ nM} \); \( \text{A}_1/\text{A}_3 > 124,000 \); \( \text{A}_{2\alpha}/\text{A}_3 > 415,000 \) vs compound 73, \( \text{hA}_3 \) AR \( K_i = 0.81 \text{ nM} \); \( \text{A}_1/\text{A}_3 = 877 \); \( \text{A}_{2\alpha}/\text{A}_3 = 522 \)). [39]

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 \( \text{hA}_3 \) 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.

![Figure 7. PTP derivatives as A_3 AR antagonists: compounds 75-83.](image-url)

Figure 7. PTP derivatives as \( \text{A}_3 \) AR antagonists: compounds 75-83.
with no substitution (e.g. compound 81 (4-F-Ph), hA2AR $K_i$ = 1.9 nM; A1hA3 = 321; A2A2hA3 > 5,263 vs compound 82 (Ph), hA2AR $K_i$ = 2.1 nM; A1hA3 = 12.9; A2A2hA3 = 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 methythio 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 A2A and A1 ARs, a similar correlation at the A1 and A2B ARs is still lacking (Figure 8). Concerning both A1 and A2B 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 A2B antagonists. In fact, substitution at the 8 position was preferred to that at the 7 position for binding at the A2B 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 A2B AR (e.g. 66, hA2BhA3 $K_i$ = 5.1 nM; 84, hA2BhA3 $K_i$ = 9.1 nM). [30] A slight improvement of selectivity towards A2A AR was achieved by introducing a $\gamma$-aminobutyrylamido or a substituted PTP derivatives seemed to have a detrimental effect in terms of affinity at the A1, AR. In fact, compound 86 (Figure 9), which is the 5-phenylacetamido derivative of compound 66, displayed a 120-fold detrimental effect on affinity value at the hA1 AR (86, hA1hA3 $K_i$ = 120 nM; 66, hA1hA3 $K_i$ = 1 nM). [30,38]

![Figure 8. Structure activity relationship of PTP derivatives at ARs.](image_url)

**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-(2-methoxyethoxy)phenyl)piperazin-1-yl)ethyl)-7H-pyrazolo[4,3-c][1,2,4]triazolo[1,5-c]pyrimidin-5-amine, is a pyrazolo[4,3-c][1,2,4]triazolo[1,5-c]pyrimidine derivative developed by Schering in a program focused on searching A2A 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 A2A ARs are almost exclusively expressed in dendritic spines of the striatopallidal neurons and colocalize with the D2 receptors. Further studies demonstrated that A2A ARs functionally oppose the actions

![Figure 9. PTP derivatives as unselective AR antagonists.](image_url)

**Figure 9. PTP derivatives as unselective AR antagonists.**
of dopamine D₂ receptors on GABAergic striatopallidal neurons. In fact, A₂A ARs and D₂ receptors have also been found to form heteromers. These findings raised the possibility to use A₂A 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 A₂A 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-aryl)piperazin-1-yl)ethyl derivatives were of particular interest. Almost all the synthesized derivatives showed high A₂A AR binding affinity, with high selectivity over A₁ 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 hA₂A 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 N₁-(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-bromoethylmethylether. [31]

In a preliminary profiling, Preladenant (2) exhibited high affinity for both human and rat A₂A ARs, with Kᵢ values of 1.1 and 2.5 nM, respectively. In addition, the compound was more than 1000-fold selective for hA₂A ARs over hA₁, hA₃B and hA₃ ARs. In cell-based assays, Preladenant (2) blocked adenylate cyclase activity stimulated by the A₂A AR agonist CGS21680 with Kᵢ 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 D₂ 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 D₂ receptors, which correlates with an increased number of A₂A ARs. Therefore, treatment with an A₂A AR antagonist, by blocking the upregulated A₂A AR and avoiding the direct activation of D₂ 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 A₂A 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

![Scheme 9. Reagents:](image-url)

- **i:** ethylene glycol ditosylate, NaH, DMF, N₂, rt, 24h;
- **ii:** compound 88 DMF, 80°C, 20h;
- **iii:** NaH, DMF, CH₂OCH₂CH₂Br, rt, 18h, followed by HCl 6N, rfx, 1h.
ineffective). Mild transient increases in blood pressure were observed and these are consistent with the expected biological effects of A2A 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 [14C]-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 hA2A AR. [66]

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

![Figure 10. Preladenant metabolites.](image-url)
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 is a clearly structural simplification of the PTP scaffold, 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 A2A 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-

![Scheme 10. Reagents:](image-url)

fact, TP nucleus (3) is a clearly structural simplification of the PTP scaffold (1), in which pyrazole ring has been dichloro-5-substituted-2-(methylthio)pyrimidines are commercially available or easily achievable by synthesis.
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-CONHNH₂, DBU, THF, rt, 5h;  
ii: P₂O₅, HMDSO, xylene, 90°C, 2h, rfx, 5h;  
iii: veratrylamine, DMSO, 140°C, 2-4h;  
iv: CH₃ONHCH₃, R₄MgBr, THF, -10 to -60°C, 13h;  
v: TFA, CF₃SO₂H, rt, 19h, 50°C, 1.5h;  
vi: LiOH·H₂O, EtOH, H₂O, rfx, 3h;  
vii: CH₃ONHCH₃, EDCI, HOBt, DMF, rt, 70h, 50°C, 1.5h;  
viii: R₂R₃NH, SO₃Cl₂, pyr, rt, 1h;  
ix: R₂R₃NH, i-PrMgCl, THF, -5 to -4°C, 8h;  
x: R₄MgBr, THF, rt, 1.5-4h;  
x: TFA, anisole, CF₃SO₂H, 0°C-rt, 4h;  
x: DIBAL, DCM, -78°C, 1h, 0°C, 2h;  
x: MnO₂, DCM, rt, 2h;  
x: R₄R₅NH, CICH₂CH₂Cl, AcOH, Na(CH₃COO)₃BH, 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]

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 HOBr 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 R1 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 disobutylaluminum 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

Scheme 13. Reagents:  

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 hydroxy group of compound 111 may be converted by Missonobu reaction 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 acrylchlorides gave ketone derivatives of general formula 117. [72]
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]

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. Compounds 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]

Scheme 14. Reagents: i: a) for X = N, RR,NH, K₂CO₃, EtOH, sealed tube, 90°C, 1h, b) for X = O, ROH, NaH, THF, rfx, 24h, c) for X = S, Na₂S, DMF, RCl, 80°C, 18h; ii: aryl hydrazide, 1N HCl, EtOH, sealed tube, 90°C, 16h; iii: BSA, 120°C, 18h; iv: aryl hydrazide, K₂CO₃, EtOH, sealed tube, 100°C, 18h or aryl hydrazide, BuOH, 90°C, 20h; v: for X = N, RR,NH, DBU, DMF, 140°C, 2h or RR1NH, CsF, DMSO, 110°C; vi: ArYB(OH)₂, Pd(PPh₃)₄, Na₂CO₃, 3/1 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-y1 or 3-CNPh.

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 arylhydrazine 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;  
v: 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$_1$NH, CsF, DMSO, 110°C, 18h;  
ii: 1-R$_2$-4-prop-2-ynyl-piperazine, Pd(PPh$_3$)$_4$, Cul, 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 synthesize 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-furly-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-substituted-amine derivatives (161) were obtained as reported above but using cesium fluoride as base. Instead, compound 160, by Sonogashira cross-coupling reaction with 1-substituted-4-(prop-2-yn-1-yl)piperazine, followed by a catalytic hydrogenation, afforded 7-(4-substituted-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 benzlythio (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 TP's bearing arylxoy, 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%; compound 168, hA$_{2A}$ AR displ. at 0.1 µM = 36%). [71] Instead, N-aryalkyl-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 piperidino 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 aminethoxyl 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$_{hA}$ = 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)piperidino 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. [71] 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, hA2A AR displ. at 0.1 µM = 65%) with compound 166 (hA2A AR Ki = 1.8 nM). [72] Instead, a small alkyl moiety such an ethyl or

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**Figure 12.** TP derivatives as A2A AR antagonists: compounds 164-177.
an isopropyl (compound 180) gave potent A<sub>2A</sub> AR antagonists. In addition, some 8-substituted derivatives were developed in this series, revealing that small an alkylamido group confers high potency at the A<sub>2A</sub> 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<sub>2A</sub> AR at a concentration of 0.1 µM. [71]

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<sub>2A</sub> AR (e.g., compound 182; hA<sub>2A</sub>AR K<sub>i</sub> = 4.82 nM; hA<sub>2A</sub>/hA<sub>1</sub> = 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<sub>2A</sub> 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<sub>3</sub> AR was observed for these compounds. Benzylamino moieties led to derivatives displaying good affinity at the hA<sub>3</sub> AR but selectivity is still poor (e.g. compound 183, hA<sub>3</sub>AR K<sub>i</sub> = 10 nM; hA<sub>1</sub>/hA<sub>3</sub> = 94; hA<sub>2A</sub>/hA<sub>3</sub> = 3.2). Better results in terms of both affinity and selectivity at the hA<sub>3</sub> AR were obtained with small alkyl groups at the 5 position, such as for methylamino derivative (184) which showed a K<sub>i</sub> value of 4.14 nM at the hA<sub>3</sub> AR and was 236- and 25-fold selective against A<sub>1</sub> and A<sub>2A</sub> 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)

4.1. Chemistry

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

![Figure 15. PPs as AR antagonists.](image)

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-1H-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-1H-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-dithiolo-PP (189), which thiol groups could be successively alkylated (compounds 190 and 191). If the alkylation at the 4 position was performed using methylidide, 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(5H)-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 Gillespie 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 aryl derivatives (198).
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 N1 and N2 alkylated PP derivatives (202-203), which was used in the next step without purification, affording a mixture of compounds 204 and 205. [18,79]

Venkatesan et al. reported a synthetic approach to obtain directly compounds of general formula 203, starting from the appropriated 3-amino-1-substituted-1H-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) Boc₂O, TEA, DMAP, DMF, rt, (B) tributylstannyl-aryl, PdCl₂(PPh₃)₂, DMF, rt, (C) (CH₃)₂NH (aq), Δ; ii: NaH, DMF, 0°C, R₃CH₂Br, rt; iii: PhCH₃NCO, DMAP, DMF, rt; iv: NaH, DMF, BrR, 0°C to rt, R = 2-F-Bn; v: ArB(OH)₂, Pd(PPh₃)₄, NaHCO₃, THF, Δ, or ArH, n-BuLi, THF, 1M ZnCl₂, Pd(PPh₃)₄, -78°C to Δ.

Scheme 21. Reagents: i: conc. H₂SO₄, 0°C to rt, 5 h; ii: PhCONCS, acetone, rfx, 12 h; iii: CH₃I, 0.1 N NaOH, rt, 3 h; iv: 2% aq NH₃, DMF, 120°C, sealed tube, 3 h; v: 1N NaOH, rfx, 12 h; vi: POCl₃, dimethylaniline, rfx, 24 h; vii: R₃Cl, DIPEA, toluene, rfx, 24 h, Rᵢ = Ar or ArCH₂; viii: NaCN, p-toluene sulfinylate sodium, DMF, 80°C, 2 h; ix: K₂CO₃, 30% H₂O₂ (aq), rt, 1 h; x: EtOH, conc. H₂SO₄, rfx, 12 h.
alkyl-2H-pyrazolo[3,4-d]pyrimidin-4(5H)-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]

Taliani et al. reported a series of 2-substituted-PP as A3 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-2H-pyrazolo[3,4-d]pyrimidin-4-amine (216, R1=H). The 6-phenyl-PP derivative (216, R1=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] Arnold et al. patented a few pyrazolo[3,4-d]pyrimidin-4(5H)-one (219) and pyrazolo[3,4-d]pyrimidin-

[Diagrams and schemes are not transcribed but are described in the text.]
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 alkylidene and sodium hydride in dimethylformamide gave a mixture of compounds 225 and 226, easily separated by flash chromatography. \[16\]

Finally, Paira et al. developed a series of chelating 4-imino-(2-substituted)-2H-pyrazolo[3,4-d]-pyrimidines to form an organoruthenium A\textsubscript{1} 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-2H-pyrazolo[3,4-d]pyrimidin-6(7H)-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 benzohydrazide derivatives in EtOH at 80°C to afford the N-(4-imino-2-methyl-6-oxo-6,7-dihydro-2H-pyrazolo[3,4-d]pyrimidin-5(4H)-yl)benzamide (227, R\textsubscript{1}PhCO). Pyrazole 17 was also treated directly with triethyl orthoformate under

![Scheme 25. Reagents: i: R\textsubscript{1}NHNH\textsubscript{2}, EtOH, 80°C, 6h, R=CH\textsubscript{3}, EtPh, PrPh, R\textsubscript{1}=H, Ph; ii: CICOOEt, pyr, DMAP, 0°C to rt, 12h, R=CH\textsubscript{3}, EtPh, PrPh; iii: R\textsubscript{1}NHNH\textsubscript{2}, EtOH, 80°C, 6h, R=CH\textsubscript{3}, R\textsubscript{1}=PhCO; iv: CH(OEt)\textsubscript{3}, rfx, 6h, R=CH\textsubscript{3}; v: NH\textsubscript{2}NH\textsubscript{2}.H\textsubscript{2}O, EtOH, 0°C to rt, 3h, R=CH\textsubscript{3}.](image)

![Figure 16. PP derivatives as A\textsubscript{1} AR antagonists.](image)
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]

![Chemical Structures](https://example.com/structures.png)

**Figure 17.** PP derivatives as A2A AR antagonists.

### 4.2. Structure activity relationship

#### A1/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 rA1 AR, whereas the alkylation at the 6 position (concomitantly to the removal of substituent at N7-position) improved the affinity, such as for compound 232 that displayed an about 7-fold increased affinity at the rA1 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 A1 AR subtype than compounds 231 and 232 (Figure 16). Instead, Harden and coworkers performed a preliminary investigation on N1- and N5-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 N5-position with small alkyl moieties or phenyl group.

Binding data at the A1 AR demonstrated that compounds possessing substituted aromatic groups showed greater affinity than those with alkyl groups at the N1-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 rA1 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 A1 AR antagonists. [82] The lead compound, 2,2”-((1-phenyl-1H-pyrazolo[3,4-d]pyrimidine-4,6-diyl)bis(sulfanediyl))dipropanamide (237), showed an affinity of 370 nM at the A1 AR. Compound 237 represented the starting point to perform a structural optimization with the aim to improve binding affinity at the A1 AR. [17] In fact, compound 237 was modified at the 4 position with mercapro, 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 rA1 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\(_2\)NH\(_2\)>>NHCH\(_3\), with compound 238 being the most potent of the series at the rA1 AR (rA1 AR K\(_i\) = 0.745 nM). Moreover, alkyl chain at a position of the sulfur atom at the 6 position of PP was found to influence affinity at the A1 AR. In this case, K\(_i\) value decreased in the order t-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 rA2A AR and binding data suggests that 4-methylthio compounds were the most promising compounds because they showed nanomolar affinity at the rA1 AR, with high levels of selectivity over rA2A AR (e.g. compound 239, rA1 AR K\(_i\) = 6.81 nM, rA2A/rA1 > 5,900). [17]

#### A2A AR antagonists

PPs were explored by Gillespie and collaborators as A2A 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 hA2A AR and was 13-fold selective over A1 AR subtype. (Figure 17) The introduction of a benzyl moiety at the 1 position of the PP ring (241) increased affinity at the A2A AR (compound 241, hA2A 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, hA2A AR...
AR $K_i = 1$ nM) of phenyl ring was preferred to ortho- and para- substitutions (e.g. compound 243, $h_{A2A} AR K_i = 9$ 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 ($h_{A2A} AR K_i = 16$ 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-$4(5H)$-ones and pyrazolo[3,4-$d$]pyrimidine-$4(5H)$-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, $h_{A3} AR K_i = 430$ nM) and 4-thione series (compound 246, $h_{A3} AR K_i = 340$ nM). Taliani et al. developed a new series of PP based on their previous results on imidazole[1,2-$\alpha$][1,3,5]triazines, which are isosters of PP. [19] The imidazole-[1,2-$\alpha$][1,3,5]triazine 247 showed high affinity at the $h_{A3}$ AR and quite good selectivity over $A_1$ and $A_{2A}$ ARs, however its corresponding PP derivative (248) showed higher affinity at the $h_{A3}$ AR subtype. For this reason new PP derivatives were synthesized in order to explore the SAR of these compounds at the $h_{A3}$ 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, $h_{A3} AR K_i > 1,000$ 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 $h_{A3}$ AR. Interestingly, compounds bearing a free amino moiety at the 4 position are poorly active at the $h_{A3}$ AR and also totally unsselective over the other AR subtypes, while the substitution at the 4 position with arylamides or arylureas enhanced both affinity and selectivity at the $h_{A3}$ AR. The best compound of the series is compound 250, which showed a $h_{A3} K_i$ value of 0.18 nM.

Figure 18. PP derivatives as $A_3$ AR antagonists.
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 A3 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 hA3 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 hA3 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 (hA3 AR Ki = 900 nM, A1/A3 = 111, A2A/A3 = 111). [20] A further investigation on these new compounds revealed that neopentyl moiety at the 2 position is preferred over other alkyl of arylalkyl groups. 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 hA2A 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...
most conserved residues in each transmembrane TM 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 hA2A AR shares 55.9%, 67.4%, and 47.7% sequence identity with the hA1 AR, the hA2B AR, and the hA3 AR, respectively.

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 hA2A and the hA3 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 hA3 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

Figure 21. Hypothetical binding modes of example PTP compounds exhibiting BM1 (A) and BM2 (B) inside the hA3 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 hA3 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].
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 hA3 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 hA3 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 Asn250 (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 hA3 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 A2A and A3 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 A2A 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-butyloxy carbonyl;
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;
IC₅₀, half maximal inhibitory concentration;
Kᵢ, 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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