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P_{2T} Purinoceptor Antagonists. A QSAR Study of Some 2-Substituted ATP Analogues

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Abstract

FPL67085MX represents the first in a class of novel, highly potent and selective P_{2T} purinoceptor antagonists which are inhibitors of adenosine diphosphate (ADP)-induced platelet aggregation in-vitro.

In an early series of compounds we studied the effect of variation of the adenine 2-substituent on potency and derived quantitative structure-activity relationships (QSARs) between the properties of the molecules and their biological activity. This work has recently been revisited using comparative molecular-field analysis (CoMFA) and the comparison of the predictions from the two methods is discussed along with their relative merits in terms of compound design.

The model suggests that the receptor for these molecules has a narrow lipophilic cleft, which is occupied by the adenine 2-substituent.

FPL67085MX (Burnstock 1971) represents the first in a class of novel, highly potent and selective P_{2T}-purinoceptor antagonists which are inhibitors of adenosine diphosphate (ADP)-induced platelet aggregation in-vitro. The proposal by Burnstock (1971) that ATP was not only the principal storage form for energy in mammalian systems, but also the endogenous agonist at a previously unrecognised family of important cell surface receptors (now known as the P_2-purinoceptors), was not widely accepted at the time it was made in the early 1970s. Indeed, only one academic group investigated matters further. This research collaboration (led by Cusack & Hourani (1981)) confirmed the hypothesis, and extended the range of ATP analogues that were examined as possible tools for the characterisation of the various receptor subtypes.

Four P_2-purinoceptor subtypes had been recognized at the time the work described here was begun. These were designated P_{2X}, P_{2Y}, P_{2Z} and P_{2T}. The P_{2X} subtype is found principally on the external surface of the smooth muscle of the vasculature and mediates vasoconstriction. P_{2Y} is found on the endothelium of blood vessels and mediates vasodilation. P_{2Z} is found on the surface of mast cells and neutrophils and stimulation causes a controlled increase in permeability of the cell membrane. The P_{2T}-purinoceptor is found only on platelets (and their precursors megakaryocytes), and stimulation causes platelet aggregation (Burnstock & Kennedy 1985). Under normal circumstances this is an essential haemostatic process. Pathophysiologically, it is the first step toward thrombosis, stroke and myocardial infarction, and inhibition of platelet aggregation is a very attractive therapeutic approach to such events. It was known that, unlike the other subtypes, where both ATP and ADP are equipotent agonists, at the P_{2T}-purinoceptor, ADP is an agonist and ATP is an antagonist. These nucleotides are released in high concentration from sites of tissue damage on the vasculature; they are rapidly metabolized by phosphatase enzymes on circulating cell surfaces, ATP being converted to ADP, AMP and finally adenosine. The structure of ATP was taken as the starting point for a synthetic programme seeking an antagonist of ADP at the P_{2T} purinoceptor, with no activity at the other subtypes and which would not be degraded to release an agonist species.

The triphosphate chain was stabilized against ectonucleotidase attack by incorporation of a dihalomethylene unit in place of the oxygen of a phosphoric anhydride link, and substitution on the adenine ring was found to modulate the binding affinity of the molecule to the receptor. Using compounds from this series it was possible to demonstrate the pivotal role of ADP at the P_{2T}purinoceptor in platelet aggregation (Fig. 1), and to confirm the therapeutic potential of an antagonist at this receptor (Humphries et al 1994). FPL67085MX was selected from the compounds made for testing, and is currently in full development as a novel antithrombotic agent.

In an early series of compounds (Fig. 2, $X = CBr_2$) we studied the effect of variation of the adenine 2-substituent on potency and derived quantitative structure-activity relationships (QSARs) between the properties of the molecules and their biological activity. This work has recently been revisited using comparative molecular-field analysis (CoMFA) and the comparison of the predictions from the two methods is discussed along with their relative merits in terms of compound design.

Methods

Molecular modelling

The coordinates (Cambridge Crystallographic Database (CSSR code ADENTPO2) of ATP were modified using

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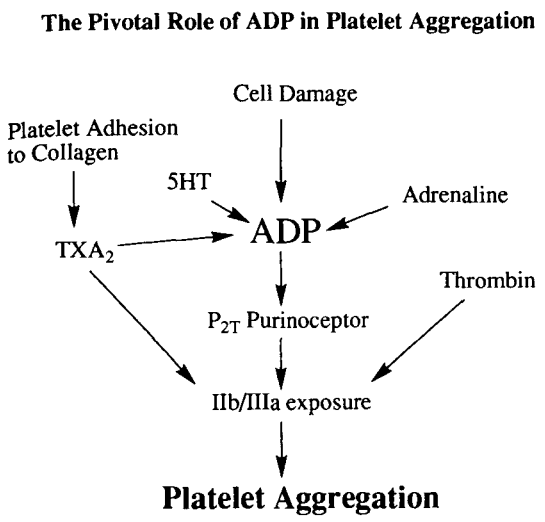

FIG. 1. The pivotal role of ADP in platelet aggregation.

Chem-X (developed and distributed by Chemical Design Ltd, Chipping Norton, UK) to give a series of 2-substituted 9-methyl adenines (Fig. 2 and Table 1) as models of the appropriate triphosphates. Semi-empirical quantum-mechanical calculations were performed with the molecular orbital package MOPAC 5.0 (J. P. P. Stewart and F. J. Steiler, QCPE 455, Indiana University), using the PM3 Hamiltonian. All molecules were subjected to full geometry optimization within MOPAC. The adenine ring was kept constant throughout the series by using the same skeleton for all compounds. The 2-substituent was manually built to give maximum overlay in an arbitrary low-energy conformation.

Multiple linear regression (MLR)

The variables (or descriptors) used in this study encode information about important electronic and geometric features of the molecules. The electronic descriptors were all derived from MOPAC calculations and include atom-centred partial charges, orbital energies and dipole moments. The geometric properties of the 2-substituent were described using a set of steric parameters (calculated using a modified form of the STERIMOL program (Verloop et al 1976)) and, in addition, the volume and surface area of the substituent. The modified STERIMOL parameters, S_{up}, S_{left}, S_{right}, S_{down} were introduced in order

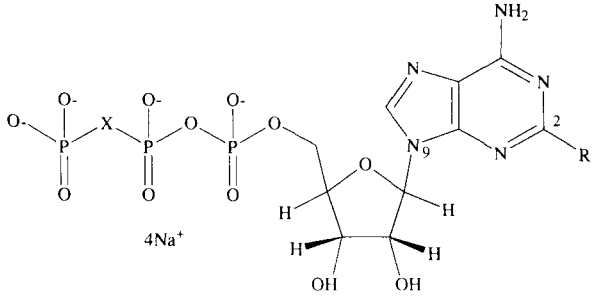

FIG. 2. Chemical structure of ADP analogues.

Table 1. Biological data. The P_{2T}-purinoceptor antagonist potency of the compounds was measured as the concentration of drug required to give a 50% inhibition of the aggregation of human washed platelets when stimulated by ADP (100 mM).

Compound	R	pIC50
1	H	5·0
2	$S(CH_2)_2CH_3$	9·3
3	$S(CH_2)_4CH_3$	10·0
4	2-Thiophenyl	5·0
5	Phenyl	5·0
6	NH_2	4·0
7	$(CH_2)_3CH_3$	5·5
8	$NHCOCH_3$	4·0
9	SCH_2CH_3	7·0
10	$O(CH_2)_2CH_3$	6·1

to represent the dimensions of the substituents in the same orientation as each other. S_{up} represents the orthogonal distance between two parallel lines; the adenine-2-substituent bond and a line tangential to the Van der Waals radius of the 2-substituent (see Fig. 3). The macroscopic property logP was also included for each 2-substituent. A total of 32 descriptors were calculated for each of the 9 molecules in the training set and these were used to construct a table with 9 compounds and 32 independent variables. The statistics package RS/1 (BBN Software Products, UK), was used to perform forwards and backwards stepwise multiple linear regression analysis on this table.

Comparative molecular field analysis (CoMFA)

The structures built for the MLR analysis were imported into Sybyl version 6.04 (Tripos Associates Inc.) and atom-typed. CoMFA (Cramer et al 1988) fields were created using an sp3 carbon probe carrying a unit positive charge. The default grid spacing of 2 Å was used except where stated otherwise, with a box clearance of 4 Å and a distance-dependent dielectric. Partial least-squares (PLS) analysis was performed using the default 2 kcal column filtering, and the maximum number of components included was

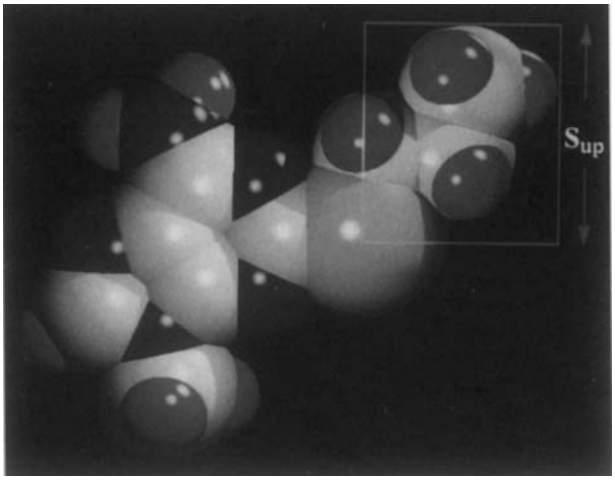

FIG. 3. Space-filling model of 2-thiopropyl adenine showing physical significance of parameter S_{up}.

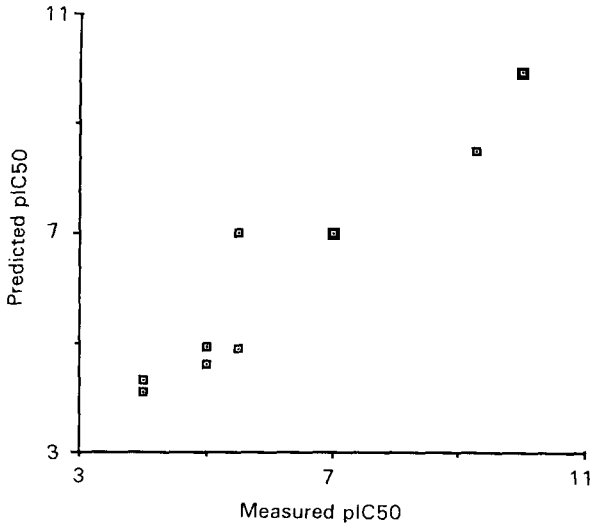

FIG. 4. Graph showing predicted vs measured activity for the MLR analysis.

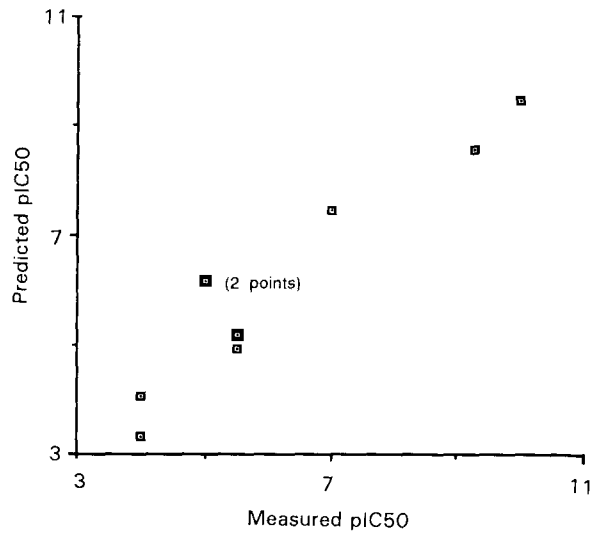

FIG. 5. Graph showing predicted vs measured activity for the COMFA analysis.

the default five. Combined electrostatic/steric columns were used along with CoMFA standard scaling. Cross-validation was performed using 4 groups (3 groups of 2, one group of 3).

Results

Multiple linear regression

The first step in this analysis was to produce a table of correlations of the biological activity (pIC50) with the molecular descriptors in order to reveal which of the properties had the most influence on the potency of the molecules. This showed the steric descriptor S_{up} (Fig. 3) to be the variable most correlated with the P_{2T}-purinoceptor antagonist activity of the compounds (correlation coefficient 0·84) and produced the following regression equation:

$$pIC50 = 1{\cdot}099 * S_{up} + 2{\cdot}06 \quad n = 9 \quad F = 16{\cdot}5 \quad r^2 = 0{\cdot}7 \tag{1}$$

Of the remaining variables not in the model the partial atomic charge on N_9 (QN_9) was found to be most significantly correlated with the biological activity (and not significantly correlated with S_{up}) and adding this descriptor explains a further 18% of the variance to give (2):

$$pIC50 = 0{\cdot}82 * S_{up} + 158{\cdot}9 * QN_9 + 34{\cdot}4 \quad n = 9$$
$$F = 21{\cdot}4 \quad r^2 = 0{\cdot}88 \tag{2}$$

Table 2. CoMFA results.

Field-fit	no	no	yes	yes	yes
Column filtering (kcal)	2·0	2·0	5·0	2·0	0·2
Grid spacing (Å)	2	1	2	2	2
q^2 (2 components)	0·67	0·71	0·36	0·41	0·55
r^2	0·91	0·92	0·78	0·87	0·9
Predicted pIC50 (cmpd **10**)	6·8	6·7	7·2	7·9	7·8

At this point no more variables were found to have any significant correlation with the biological activity and equation 2 was selected as the final equation. The predictions from this equation are plotted against the experimental data in Fig. 4.

Comparative molecular field analysis

A cross-validated $r^2(q^2)$ of 0·67 (SDEP = 1·2) was obtained with 2 components and 2 kcal filtering (60 remaining columns). Cramer et al (1993) have demonstrated that a cross-validated r^2 of 0·45 is obtained about 1% of the time for 10 compounds with 100 variables (columns). This indicates that our model is statistically significant and likely to be reliably predictive. We were interested to see if reducing the grid-spacing, or using field-fitting on the molecules would give a better model. The steric fields around the adenine ring would be identical for all compounds, but the electrostatic fields will be different and field fitting might increase the likelihood of finding a correlation in this region, particularly if charge on N9 is important as expected from the MLR results. The results in Table 2 indicate that grid-spacing does not affect the results but field-fitting to the most active compound, followed by unrestrained minimization, gave a worse result. It was thought that this may be due to additional noise in the field columns caused by mis-alignment of the ring systems. Changing the filtering did not give a better result – apparently the model is irretrievably degraded, and the additional noise cannot be removed without losing useful information. The predicted activities using no cross-validation are plotted against the real activities in Fig. 5. The CoMFA fields are shown in Fig. 6 along with compounds **3** and **5** (2-thiopentyl (pIC50 = 10·0) and phenyl (pIC50 = 5·0)). Field A represents steric grid points that correlate positively with activity (bulk good for activity). Field B shows an area where bulk is bad for activity, and field C shows a region where positive charge is good for activity. The CoMFA confirms the MLR conclusion that

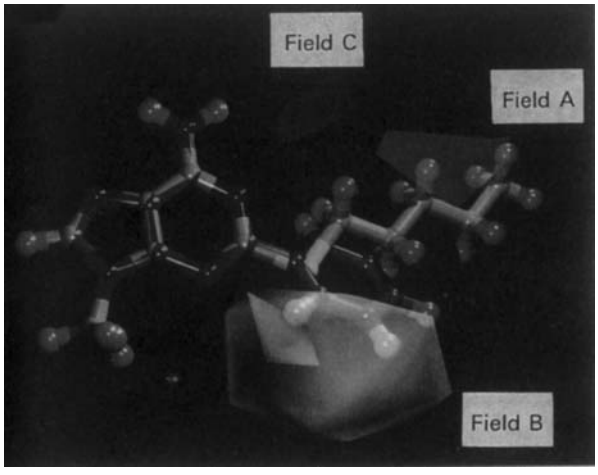

FIG. 6. Superposition of compounds **3** and **5** along with the COMFA fields.

bulk extending linearly away from the base gives active compounds. It provides additional information that lateral bulk as shown by the phenyl ring is poor for activity and that positive charge about the methylene attached to sulphur is good for activity. No field density was seen about N9.

Discussion

Variation of the 2-substituent in FPL67085MX analogues gives a change in P_{2T}-purinoceptor antagonist activity of 6 orders of magnitude. Both traditional Hansch analysis and CoMFA analysis have been used to derive equations that describe this variation and allow us to design novel potent antagonists. For compound **10,** MLR predicts pIC50 6·06; CoMFA predicts pIC50 6·8. The measured value of the pIC50 is 6·1 which is well within the error in the CoMFA analysis as displayed by the standard error in prediction (SDEP = 1·2).

The model indicates that the receptor for these molecules has a narrow lipophilic cleft, which is occupied by the adenine 2-substituent. Molecules which fail to put any volume into this pocket—for example the 2-H and 2-NH2 substituted compounds—have low activity, as do molecules such as the 2-phenyl compound in which there is lateral bulk. The 2-substituent is the major controlling influence on the activity of the compounds. The physical rôle of the charge on N9 in the MLR is more difficult to explain. The charge on this atom may be solely a reflection of the change of the electronic nature of the various 2-substituents. No substituent constants such as Hammett sigma values or partial atomic charges were used to describe the electronic nature of the 2-substituent (only partial atomic charges for atoms on the adenine ring were used in the regression due to the difficulty in mapping charges from one substituent to another). CoMFA is able to directly sample the electrostatics in the region about the 2-substituent and no correlation is seen with ring field points.

In terms of suggesting compounds to make, both methods give similar predictions for increased extended bulk, although the CoMFA display provides a more easily interpreted model and may in this case provide a better rationale for the effect of electrostatics.

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