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Quantitative structure-activity relationship study of human A_3 adenosine receptor antagonists: Derivatives of 2-aryl-1,2,4-triazolo [4,3-α]quinoxaline

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Abstract

A quantitative structure-activity relationship (QSAR) study was conducted on the antagonistic activities of derivatives of 2-aryl-1,2,4-triazolo[4,3-α]quinoxaline at the human A_3 adenosine receptor. As per the structural framework, the title analogues were subdivided into two congeneric series, namely the 1,4-dione and the 4-amino-1-one series. A majority of substituents occurred at the *R*- and a limited number at the *X*-positions in both of these series. In the case of the 1,4-dione series, the derived significant QSAR equation revealed that those substituents exhibiting a larger field effect at *R* renders the molecule to more efficiently bind at the receptor site. The study also extrapolated the requirement of electron-donor substituents at the *X*-position which, at present, is regarded as insensitive to any interaction due to limited substitution. However, the *X*-position may be explored in a further synthetic study. From the derived correlation equation for the 4-amino-1-one series, it appeared that a strong electron-withdrawing substituent at *R* will enhance the pK_i value of a compound while a strong electron-donor at this position will have a detrimental effect on it. Based on correlation equations, derived using different electronic parameters, it may be interpreted that the two series of compounds attain different orientation inside the recognition site of the receptor.

Keywords: *2-Aryl-1,2,4-triazlo[4,3-α]quinoxaline derivatives, human A3 receptor antagonist, QSAR analysis, Physico-chemical properties*

Introduction

The biological functions of neuromodulator adenosines are exerted by activation of G-protein-coupled receptors (GPCRs), which are currently classified into A_1, A_{2A}, A_{2B} and A_3 subtypes [1,2]. All the four adenosine receptor (AR) subtypes have been cloned and characterized on a pharmacological level. A_1 or A_{2A} receptors from different species show high amino acid sequence homology (85–95%), while the A_3 subtype exhibits only 74% homology sequence between rat and human or sheep [3,4]. It has been shown that adenylate cyclase inhibition [5] and phospholipase C and D stimulation [6,7] is mediated by activation of the A_3 AR subtype. Moreover, in a rat model it has been demonstrated that the activation of A_3 AR causes the release of inflammatory and allergic mediators from the mast cells [8]. Therefore, A_3 AR antagonists might be useful as anti-inflammatory and anti-asthmatic agent [9] and seems to be involved in cell survival regulation [10], making them promising therapeutics in counteracting ischemia- and aging-associated neurodegeneration [10,11]. Recently, Colotta et al. [12] synthesized new potent and selective human A_3 (hA_3) adenosine receptor antagonists consisting of 2-aryl-1,2,4-triazolo[4,3-α]quinoxaline-1,4-diones and 2-aryl-1,2,4-triazolo[4,3-α]quinoxalin-4-amino-1-ones. The initial structure-activity relationship (SAR) study on these compounds was, however, directed only to alteration of the substituents at the attached 2-phenyl ring or the benzofused moiety, but no rationale has been provided to reduce the trial-and-error factors. Hence, a quantitative SAR (QSAR)

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on these compounds was conducted since QSAR not only provides the rationale for drug design but also enlightens their plausible mechanism of action at molecular level.

Materials and methods

The reported series comprising of 2-aryl-1,2,4-triazolo[4,3-α]quinoxaline-1,4-diones (Figure 1) and 2-aryl-1,2,4-triazolo[4,3-α]quinoxalin-4-amino-1-ones (Figure 2) along with their biological effects are included respectively in Table I and Table II. The biological activity, measured in terms of binding constant, K_i, represents the ability of a compound to displace [^{125}I]-N^6-(4-amino-3-iodobenzyl)-5′-(N-methylcarbamoyl) adenosine ([^{125}I]AB-MECA) from cloned hA_3 receptor stably expressed in Chinese hamster ovary (CHO) cells. For the present work, the K_i values were converted to a negative logarithmic scale (pK_i) on a molar basis. Amongst various attempted substituent parameters [13] governing three major interactions, namely, hydrophobic, electronic, and steric, only the relevant ones are tabulated as the QSAR parameters. The multiple regression analysis (MRA), employing the method of least squares, was used to derive significant correlations between pK_i and the quantifying parameters. Finally, the QSAR equations were subjected to the validation test [14] by the leave-one-out (LOO) method. This method calculates the cross-validated, q^2 index which helps to assess the reliability of the model. For a reasonable QSAR model, q^2 should be greater than 0.6, while a value greater than 0.9 indicates an excellent model.

Results and discussion

Table I lists the compounds where the substituents have been altered at the *R*-position of the 2-phenyl ring, linked to the 1,2,4-triazolo moiety and at the *X*-position of the benzofused framework. However, the later variation is limited only to a few substituents, restricting our exploration of any quantitative parameter for *X*-position. Amongst

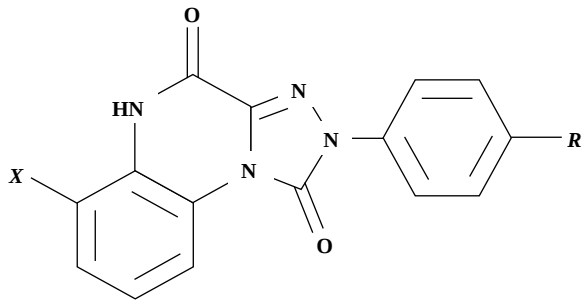

Figure 1. Structure of 2-aryl-1,2,4-triazolo[4,3-a]quinoxaline-1,4-diones.

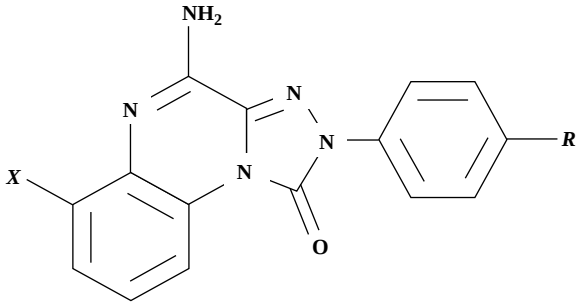

Figure 2. Structure of 2-aryl-1,2,4-triazolo[4,3-a]quinoxalin-4-amino-1-ones.

various attempted physicochemical and structural parameters for the substituents of the *R*-position, the most appealing parameter was the field effect, F_R. The derived correlation between this parameter and the binding affinity, for all the data points (n) in Table I, is shown in Equation (1)

$$pK_i = 5.454(\pm 2.35)F_R + 5.855$$
$$n = 9,\ r = 0.901, s = 0.521,\ F(1,7) = 30.041,$$
$$q^2 = 0.662 \qquad (1)$$

The statistical parameters, r, s and F in this and subsequent equations represent respectively the correlation coefficient, the standard deviation and the *F*-ratio of the variances of calculated to observed activities. The ± data within parenthesis are the 95% confidence interval. Although, the statistical parameters of Equation (1) give significant results and the q^2 index accounts for a reasonable QSAR model, the calculated pK_i value of compound **8** deviates considerably from the observed one. This lone congener having a strong electron withdrawing $-NO_2$ group at *X* will deactivate the molecular framework and the compound may not elicit the desired binding efficacy at to the receptor. Ignoring this compound, the MRA resulted in a much superior correlation Equation (2),

$$pK_i = 5.526(\pm 1.53)F_R + 5.703$$
$$n = 8,\ r = 0.964,\ s = 0.328,$$
$$F(1,\ 6) = 77.823, q^2 = 0.854 \qquad (2)$$

The *r*-value, accounting for 93% ($r^2 = 0.929$) of variance and the *F*-statistics, standing significant at 99% level [$F_{1,6}(0.01) = 13.745$] have both very much increased over that in Equation (1). Additionally, the improved q^2 index now expressed a nearly excellent QSAR model. This equation was further used to calculate pK_i values for the compounds in Table I, which were found in close agreement with the observed ones. Likewise, the predicted pK_i values, obtained from the LOO approach, are included in Table I for comparison. From Equation (2), it appears

Table I. QSAR parameters and binding activities of 2-aryl-1,2,4-triazolo[4,3-α]quinoxaline-1,4-diones (see Figure 1 for structure) at the human A_3 adenosine receptor.

				pK_i(M)[a]		
No.	*R*	X	F_R	Obsd.	Calc. Equation (2)	Prctd. LOO
1	OCH_3	H	0.26	7.80	7.14	7.04
2	NO_2	H	0.67	9.22	9.41	9.80
3	NH_2	H	0.02	5.44	5.81	6.03
4	$N(CH_3)_2$	H	0.10	6.37	6.26	6.22
5	OC_2H_5	H	0.22	6.76	6.92	6.94
6	OH	H	0.29	7.33	7.31	7.30
7	$OCOCH_3$	H	0.41	7.95	7.97	7.97
8	OCH_3	NO_2	0.26	8.33	–[b]	–[b]
9	OCH_3	NH_2	0.26	7.08	7.14	7.15

[a] binding constant, K_i, represents the ability of a compound to displace [^{125}I]-N^6-(4-amino-3-iodobenzyl)-5′-(N-methylcarbamoyl)adenosine ([^{125}I]AB-MECA) from cloned hA_3 receptor stably expressed in Chinese hamster ovary (CHO) cells; taken from Reference [12]; [b] "outlier" compound in the present study.

that the substituents exhibiting a larger field effect at *R* will help the molecule to bind more efficiently at the receptor site. This in turn extrapolates the requirement of electron-donor substituents at the *X*-position. This position, at present, is reported to have a few substituents and is therefore regarded as insensitive to any interaction. However, the position may be explored in further synthetic studies. Based on the conclusions drawn above, a few possible derivatives having higher pK_i values that are not in the original data set are given below:

			pK_i (*M*)
R	*X*	F_R	Calc., Equation (2)
NO	H	0.50	8.47
SO_2CF_3	H	0.73	9.74
SO_2F	H	0.75	9.85
$N(CH_3)_3$	H	0.89	10.62

The listed compounds in Table II, having variations at *R* and *X*, differ slightly in functionality at the 4-position of the 1,2,4-triazolo[4,3-α]quinoxaline moiety compared to that of the compounds in Table I. Thus a quantifying parameter, accounting for the electronic environment, is expected for the substituents at the *R*-position. For this reason, the field parameter, *F*, the resonance parameter, *R*, and electronic parameter, σ, were considered successively in the MRA. However, only the σ parameter accounting for the electron-withdrawing nature of the substituents at *R* could yield a highly significant correlation Equation (3)

$$pK_i = 2.889(\pm 1.22)\sigma_R + 8.201$$
$$n = 7,\ r = 0.938,\ s = 0.228,$$
$$F(1, 5) = 36.915,\ q^2 = 0.698 \qquad (3)$$

The statistical parameters of this equation are in accord with the high level of significance. The *r*-value accounts for 88% of variance, and the *F*-value is significant at 99% level [$F_{1,5}(0.01) = 16.258$]. Additionally, the higher value obtained for q^2 expressed a reasonable QSAR model. The calculated pK_i values using Equation (3) and predicted pK_i values employing the LOO approach were found in close agreement with the observed ones and are included in Table II. From Equation (3), it appears that a strong electron-withdrawing substituent at *R* (i.e., para-substituents of the phenyl ring) will enhance the pK_i

Table II. QSAR parameters and binding activities of 2-aryl-1,2,4-triazolo[4,3-α]quinoxalin-4-amino-1-ones (see Figure 2 for structure) at the human A_3 adenosine receptor.

				pK_i(M)[a]		
No.	*R*	X	F_R	Obsd	Calc. Equation (3)	Prctd. LOO
1	H	NO_2	0.00	8.32	8.20	8.03
2	NH_2	H	−0.66	6.47	6.29	5.95
3	OH	H	−0.37	7.14	7.13	7.13
4	OCH_3	H	−0.27	7.35	7.42	7.43
5	OCH_3	NO_2	−0.27	7.33	7.42	7.44
6	OCH_3	NH_2	−0.27	7.66	7.42	7.38
7	OH	NH_2	−0.37	6.75	7.13	7.20

[a,b] See footnotes under Table I.

value of a compound while a strong electron-donor at this position will cause a detrimental effect. Such inferences guide us to suggest a few unexplored congeners which should be more active than the compounds in the original data set. These are listed below:

			$pK_i(M)$
R	*X*	σ_R	Calc., Equation(3)
OC_2H_5	H	−0.24	7.51
$OCOCH_3$	H	0.31	9.10
SO_2CH_3	H	0.72	10.28
SO_2CF_3	H	0.93	10.89
$N(CH_3)_3$	H	0.82	10.57

The plot showing the variation of observed *vs* calculated and predicted pK_i values for both the tabulated congeners is shown in Figure 3 which may help to understand the goodness of fit and to identify the systematic variation of pK_is. It is important to note that the field parameter, *F*, plays a significant role in ascertaining the binding affinities in analogues belonging to the 1,4-dione series (Table I), while the electronic parameter, σ, emerged as the predominant predictor of the 4-amino-1-one series (Table II). In fact, both these parameters account for the electronic environment generated by the *R* substituents in the compounds of both series. The field parameter, *F*, being intrinsic in nature accounts for the localized effect of the substituents while the electronic parameter, σ, being the linear combination of the field, *F*, and the resonance, *R*, effects accounts for both the localized and delocalized fields. The differing parametric requirements for the compounds of the two series may be attributed to the structural difference with respect to the 1,2,4-triazolo[4,3-α]quinoxaline moiety. The 1,4-dione series consists of a ring –NH–CO– fragment while the 4-amino-1-one series comprise of a $-N = C(NH_2)-$ fragment. In the case of the 1,4-dione series the conjugation is not extended within the entire molecular framework, which may restrict the *R*-substituent to exert only a field effect. On the other hand, in the latter structural moiety, the lone pair on the $-NH_2$ group is involved in the conjugated system and therefore enhances the electron density within molecular framework, leading to the localized and delocalized effects elicited by the *R*-substituents. Such an explanation conforms with the findings [12] that the two series of compounds adopt different orientation inside the recognition site of the receptor.

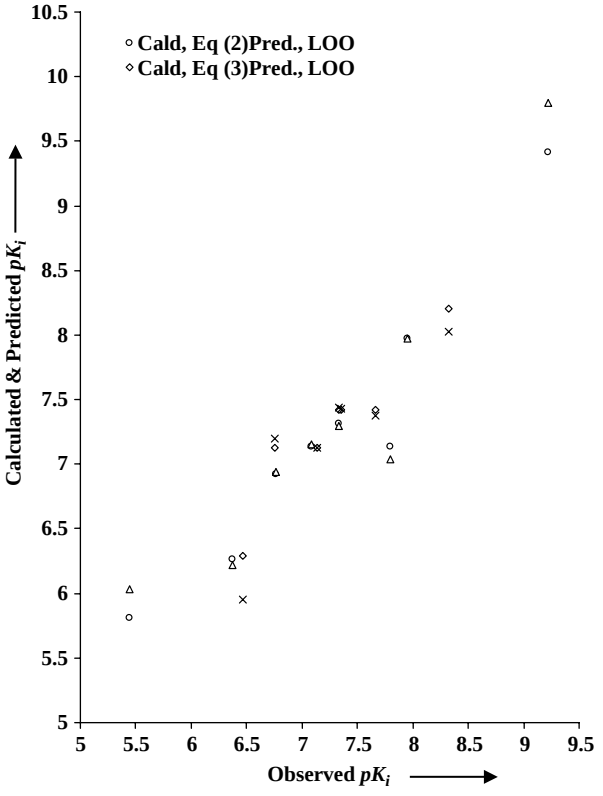

Figure 3. Plot of observed versus calculated and predicted pK_i values.

The aforesaid guidelines may, therefore, provide a basis for rationalizing substituent selection in the future designing of effective inhibitors of human A_3 adenosine. The study may also help in proposing the possible mode of action of the derivatives of 2-aryl-1,2,4-triazlo[4,3-α]quinoxaline at the molecular level.

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