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A new microwave-assisted, three-component reaction of 5-aminopyrazole-4-carboxylates: Selective synthesis of substituted 5-aza-9-deaza-adenines[1]

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Graphical Abstract

Abstract

A series of 5-aza-9-deaza- analogues of purine was effectively prepared using highly selective annulation of 1,3,5-triazine ring onto 5-aminopyrazole-4-carboxylates via a one-pot, multicomponent, microwave-assisted approach. The products were obtained in good yields and high purity. This catalyst-free method was demonstrated to be scalable and highly reproducible in different microwave reactors. Some structural features of the prepared compounds were studied in details using dynamic NMR spectroscopy and X-ray crystallography.

1. Introduction

Isosterism and bioisosterism have been proven to be efficient strategies in the drug discovery process. The isosteric replacement of biologically important heterocyclic scaffolds with structurally similar heterocycles possessing different arrangements of heteroatoms has been extensively exploited in the design of new therapeutic agents. This strategy, applied to the purine scaffold, has led to the development of several blockbuster drugs and current therapeutic standards (Figure 1) such as sildenafil and vardenafil for the treatment of erectile dysfunction,[2] allopurinol recommended as the first choice for the chronic gout therapy,[3] ibrutinib for the leukaemia therapy,[4] and temozolomide for the treatment of brain cancers.[5] In the area of purine isosteres, we have a sustained interest towards 1,3,5-triazine-based purine-like scaffolds.[6] Among them, 5-azapurine (1,2,4-triazolo[1,5-*a*][1,3,5]triazine) and 5-aza-9-deazapurine (pyrazolo[1,5-*a*][1,3,5]triazine) have been recognised as the most promising skeletons for the construction of new bioactive compounds.[7-9] Recently, pyrazolo[1,5-*a*][1,3,5]triazine derivatives were reported to be useful as cyclin-dependent kinase inhibitors[10], tyrosine threonine kinase inhibitors[11], phosphodiesterase (PDE10 and PDE4) inhibitors[12], cannabinoid (CB_1) receptor antagonists[13], and corticotrophin-releasing factor (CRF_1) receptor antagonists[14]. Their anti-proliferative[15] and antiviral[16] properties have also been well documented.
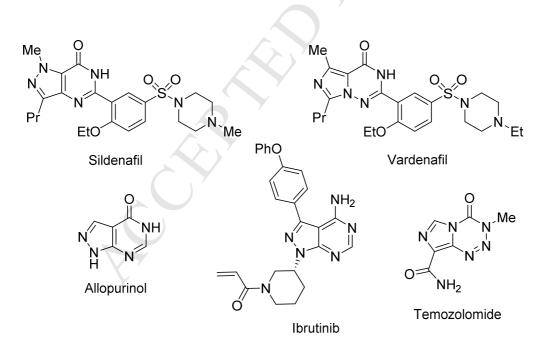

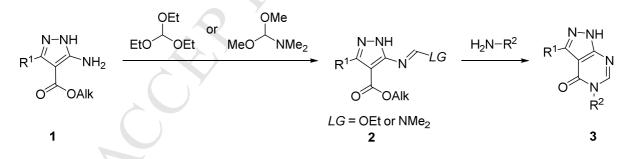**Figure 1.** Therapeutically renowned purine isosteres.

Herein, we report results of our experiments on the synthesis pyrazolo[1,5-*a*][1,3,5]triazines using a multicomponent reaction of 5-aminopyrazole-4-carboxylates (**1**) with trimethyl orthoformate and cyanamide. Possessing several reactive centres in their structure, 5-aminopyrazole-4-carboxylates (**1**) have found many successful applications as building blocks for the construction of fused heterocyclic systems.[17-19] However, no reports on multicomponent reactions of these compounds are available. A combination of multiple reactive centres in 5-aminopyrazole-4-carboxylates (**1**) increases the complexity of potential multicomponent processes making it difficult to predict their outcome and achieve selectivity of the reactions.

The controlled microwave irradiation has been proved to be an excellent tool accelerating reactions in organic synthesis. Many methods for the synthesis of purines and purine-like compounds under microwave irradiation have been reported.[20] Therefore, we also considered application of this fast and efficient way of heating for synthetic transformations described in this paper.

2. Results and Discussion

2.1 Stepwise synthesis of 4-aminopyrazolo[1,5-a][1,3,5]triazine-8-carboxylates (4)

In stepwise approaches, 5-aminopyrazole-4-carboxylates (**1**) were reported to react with triethyl orthoformate or its synthetic equivalents (for example, DMF-DMA) followed by the reaction of the intermediates **2** with *N*-nucleophiles to afford pyrazolo[3,4-*d*]pyrimidin-4-ones (**3**) (Scheme 1).[18,19]

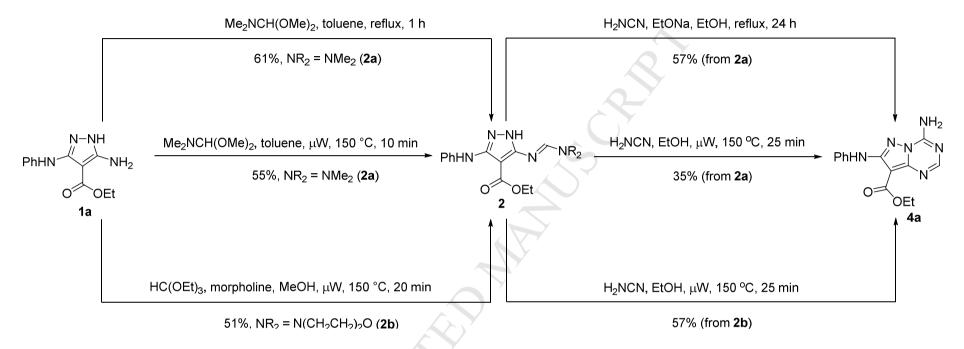**Scheme 1**. Reactions of 5-aminopyrazole-4-carboxylates (**1**) with triethyl orthoformate / DMF-DMA and some *N*-nucleophiles.

Designing our multicomponent reaction, we decided to explore initially a stepwise synthesis of the targeted 4-aminopyrazolo[1,5-*a*][1,3,5]triazine-8-carboxylates (**4**). It was reported recently that aminoazoles could be converted to the corresponding *N,N*-dimethylformamidines, which upon
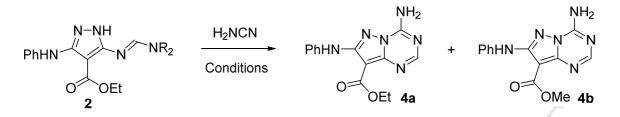
subsequent treatment with cyanamide in the presence of sodium methoxide produced azolo-fused amino-1,3,5-triazines. [1,21] Initially, we applied similar conditions to our model substrate **1a** preparing amidine **2a**, which successfully reacted with cyanamide to afford the desirable product **4a** in 35% overall yield (Scheme 2). Both steps were also performed at higher temperature using microwave irradiation. Amidine **2a** was successfully prepared via the condensation of ethyl 5-amino-3-(phenylamino)pyrazole-4-carboxylate (**1a**) with DMF-DMA under microwave irradiation. To our satisfaction, the subsequent microwave-assisted reaction of amidine **2a** and cyanamide in ethanol lead to the formation of **4a** even in the absence of base. Interestingly, in our attempt to use methanol as a solvent for the reaction of amidine **2a** and cyanamide, a mixture of two different products was isolated: desired compound **4a** and methyl 4-amino-7-(phenylamino)pyrazolo[1,5-*a*][1,3,5]triazine-8-carboxylate (**4b**) (Scheme 3). It appeared that under the reaction conditions, a partial transesterification with the solvent took place replacing the ethyl ester group to the methyl one.

It should be noted that when we performed a one-pot, three-component synthesis of another formamidine (**2b**) from **1a**, triethyl orthoformate, and morpholine under microwave irradiation in methanol following the earlier developed protocol,[22] no transesterification products were isolated at this step (Scheme 2). The microwave-assisted reaction of **2b** with cyanamide in ethanol led to the formation of **4a**. However, the same reaction in methanol afforded a mixture of **4a** and its methyl ester analogue **4b** (Scheme 3).

We proposed that the ester group reactivity increased after the triazine ring annulations and the transesterification took place in the presence of the base (dimethylamine or morpholine), which was released during the reaction. This assumption was further confirmed by the experiment when a similar reaction of **2a** was performed in methanol using a stronger base (sodium methoxide) (Scheme 3). The methyl ester **4b** was isolated from the reaction without even traces of **4a**. Moreover, under these conditions, the transesterification occurred even before the trazine ring closure as indicated by the isolation of a side-product *viz*. methyl 5-amino-3-(phenylamino)pyrazole-4-carboxylate (**1b**) together with **4b**.
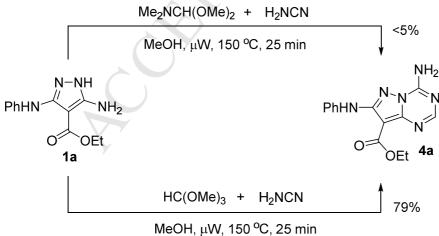
Scheme 2. Reactions of ethyl 5-amino-3-(phenylamino)pyrazole-4-carboxylate (**1a**) with DMF-DMA / trimethyl orthoformate and cyanamide.

Conditions: MeOH, μW, 150 °C, 25 min - **4a**, 15% + **4b**, 15% (from **2a**, NR$_2$ = NMe$_2$);
MeOH, μW, 150 °C, 25 min - **4a**, 49% + **4b**, 5% (from **2b**, NR$_2$ = N(CH$_2$CH$_2$)$_2$O);
MeONa, MeOH, reflux, 24 h - **4b**, 39% (from **2a**, NR$_2$ = NMe$_2$)

Scheme 3. Reactions of *N*-pyrazolylformamidines **2** with cyanamide.

2.2 One-pot, three-component synthesis of 4-aminopyrazolo[1,5-a][1,3,5]triazine-8-carboxylates (4)

Following the successful 1,3,5-triazine ring annulation onto 5-aminopyrazole-4-carboxylates using step-wise approaches, we decided to conduct a one-pot, three-component reaction of 5-aminopyrazole-4-carboxylates (**1a**) with DMF-DMA and cyanamide under microwave irradiation (Scheme 4). To our satisfaction, we found that this reaction in methanol proceeded selectively to form ethyl 4-amino-7-(phenylamino)pyrazolo[1,5-*a*][1,3,5]triazine-8-carboxylate (**4a**) albeit in low isolated yield (<5%). No traces of pyrazolo[3,4-*d*]pyrimidines or methyl ester analogue (**4b**) were detected. The analysis of the crude reaction mixture identified that most of **1a** remained unreacted. The absence of **4b** as a side product in the reaction mixture could be explained by the limited conversion of **1a** to **4a**, which is probably required for the successful transesterification under these conditions.

Scheme 4. One-pot, multicomponent reactions of ethyl 5-amino3-phenylaminopyrazole-4-carboxylate (**1a**).

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In attempts to improve yields and avoid potential base-catalysed transesterification, we performed a similar multicomponent reaction using trimethyl orthoformate instead of DMF-DMA. Under identical conditions, the one-pot, three-component, microwave-assisted reaction of 5-amino-3-phenylaminopyrazole-4-carboxylate (**1a**), trimethyl orthoformate and cyanamide afforded the desired **4a** in good yield (79%). The product **4a** was isolated via simple filtration in high purity without traces of other side products.

Exclusive formation of **4a** confirmed the regio- and chemoselectivity of the multicomponent reaction under these conditions. Variations in the ratio of reagents or changing the solvent did not lead to further improvements in the reaction outcome (Table 1). Notably, the yield of **4a** in the reaction performed using the one-pot, multicomponent format was higher compared to the overall yield in the stepwise approach.
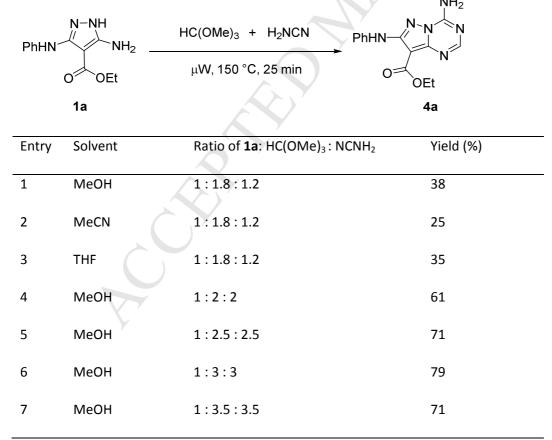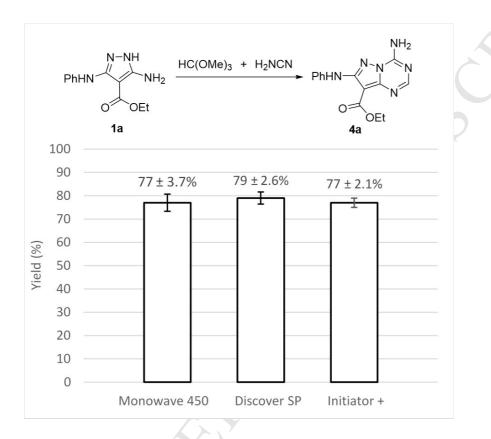
Table 1. Optimization of conditions for the model multicomponent reaction of **1a**.

Entry	Solvent	Ratio of **1a** : HC(OMe)$_3$: NCNH$_2$	Yield (%)
1	MeOH	1 : 1.8 : 1.2	38
2	MeCN	1 : 1.8 : 1.2	25
3	THF	1 : 1.8 : 1.2	35
4	MeOH	1 : 2 : 2	61
5	MeOH	1 : 2.5 : 2.5	71
6	MeOH	1 : 3 : 3	79
7	MeOH	1 : 3.5 : 3.5	71

* The reaction was performed using a Discover SP CEM microwave synthesizer using 1 mmol of **1a** in 2 mL of the specified solvent.

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The three-component reaction of ethyl 5-amino-3-(phenylamino)pyrazole-4-carboxylate (**1a**), trimethyl orthoformate and cyanamide under microwave irradiation was also performed using identical conditions in three different models of microwave synthesizers: Discover SP (CEM), Monowave 450 (Anton Paar), and Initiator+ (Biotage). The reactions were conducted in triplicates in each reactor, demonstrating good reproducibility of the developed method (Figure 2).

* The reaction was performed using 1 mmol of **1a**, 3 mmol of HC(OMe)$_3$ and 3 mmol of H$_2$NCN in 2 mL of MeOH under microwave irradiation at 150 °C for 25 min

Figure 2. One-pot, multicomponent reaction of **1a**, trimethyl orthoformate and cyanamide using three different models of microwave synthesizers.

Scaling up the model multicomponent reaction from 1 mmol to 10 mmol of **1a** under identical microwave conditions afforded the same product **4a** in high purity and similar yield (76%).

Next, the scope of our one-pot, multicomponent reaction was explored using as substrates various substituted methyl and ethyl esters of 5-aminopyrazole-4-carboxylates (**1a-x**), which were prepared

according to the method we developed recently.[23] The one-pot reaction of 5-aminopyrazole-4-carboxylates (**1a-x**) with trimethyl orthoformate and cyanamide under the microwave irradiation was found to proceed efficiently with the formation of desirable products **4a-x** in yields up to 95% (Table 2). A variety of arylamino, alkylamino, and arylalkylamino substituents on the pyrazole ring were well tolerated. The reaction was selective and secondary amino groups of **4a-x** remained intact. No transesterification products were detected when the multicomponent reactions of ethyl 5-aminopyrazole-4-carboxylates were carried out in methanol under these conditions. Overall, the reaction was chemo- and regioselective and afforded interesting purine analogues in a single, relatively short and operationally simple step.

The multicomponent reaction of **1a**, trimethyl orthoformate and cyanamide was also performed under conventional heating. Applying fast heating in the sealed vessel using the Monowave 50 (Anton Paar) reactor to simulate microwave irradiation conditions, we obtained **4a** in 76% yield (1 mmol scale). Therefore, we could not identify any non-thermal microwave effect promoting this reaction.
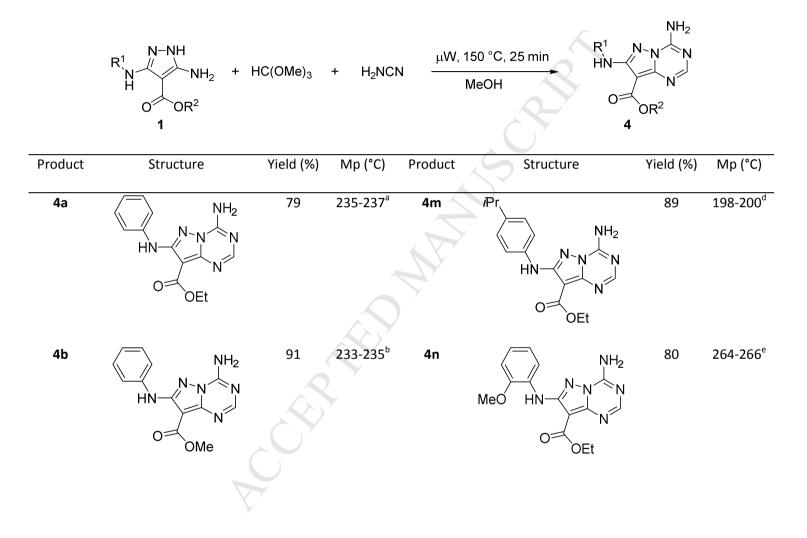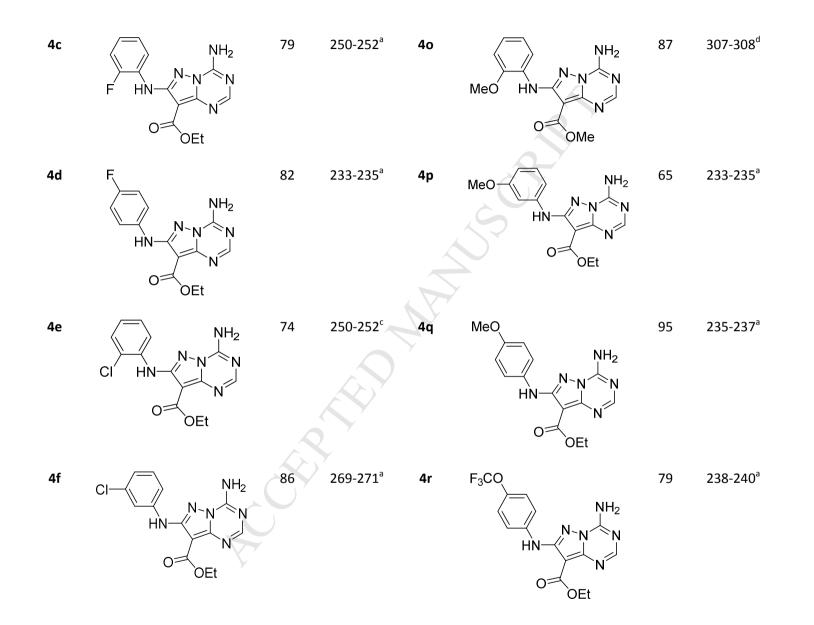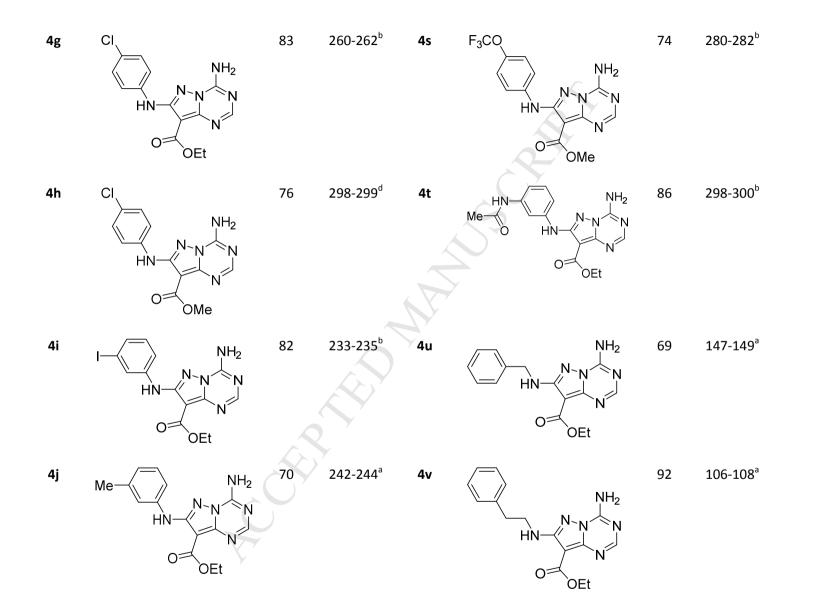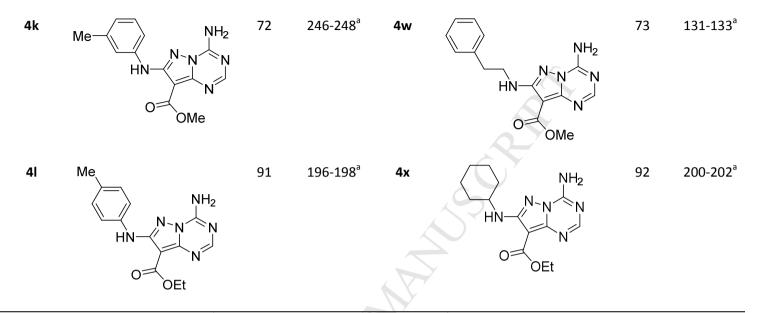
Table 2. One-pot, three-component synthesis of 7-amino-substituted 4-aminopyrazolo[1,5-*a*][1,3,5]triazine-8-carboxylates (**4**).

Product	Structure	Yield (%)	Mp (°C)	Product	Structure	Yield (%)	Mp (°C)
4a		79	235-237[a]	**4m**		89	198-200[d]
4b		91	233-235[b]	**4n**		80	264-266[e]

4c

79   250-252[a]

4o

87   307-308[d]

4d

82   233-235[a]

4p

65   233-235[a]

4e

74   250-252[c]

4q

95   235-237[a]

4f

86   269-271[a]

4r

79   238-240[a]

12

4g 83 260-262[b]

4s 74 280-282[b]

4h 76 298-299[d]

4t 86 298-300[b]

4i 82 233-235[b]

4u 69 147-149[a]

4j 70 242-244[a]

4v 92 106-108[a]

13

4k 72 246-248[a]

4w 73 131-133[a]

4l 91 196-198[a]

4x 92 200-202[a]

Recrystallization solvents: [a] MeOH, [b] MeCN, [c] DMF & MeOH, [e] DMF & H$_2$O, [d] DMF
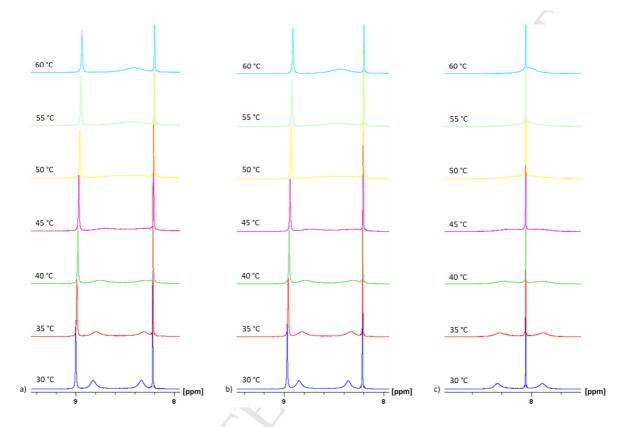
The structure assignments for the prepared 5-aza-9-deaza-adenines **4** were made on the basis of spectral data. The ester group remained intact in the reaction giving characteristic signals for ethoxycarbonyl and methoxycarbonyl groups in NMR spectra, particularly a triplet at 1.33-1.34 ppm and a quartet at 4.34-4.35 ppm or a singlet at 3.75-3.85 ppm for the corresponding alkyl fragment in ^{1}H NMR spectra and a signal of the ester carbonyl carbon at 163.8-164.9 ppm in ^{13}C NMR spectra of compounds **4**. The band at 1630-1692 cm^{-1} in the IR spectra also indicated the ester group presence. The methine proton of the newly formed triazine ring appeared as a singlet at 8.10-8.25 ppm in ^{1}H NMR spectra. The primary amino group on the electron-deficient triazine ring of the prepared 4-aminopyrazolo[1,5-*a*][1,3,5]triazine-8-carboxylates **4** was found to display a hindered rotation of the C-N bond. This restricted rotation made the amino group hydrogen atoms magnetically non-equivalent resulting in appearance of the two broad signals at 7.83-8.50 ppm and 8.10-8.90 ppm in the ^{1}H NMR spectra. Considering polar properties of the solvent (DMSO-d_6), this phenomenon is more likely to be attributed to the high level of delocalization of the electron pair on the amino group nitrogen atom, rather than a potential intramolecular hydrogen bonding between one of the amino group hydrogens with the pyrazole ring nitrogen atom. Moreover, this intramolecular hydrogen bonding was not observed in the solid state. The X-ray crystallography data (*vide infra*) also supported the partial double bond character of the primary amino group, particularly by the significantly shortened distance of its C-N bond (1.319 Å, the shortest C-N bond in the **4a** molecule).

2.3 Dynamic NMR spectroscopy experiments

Recently, Hage et al.[10b] demonstrated an importance of the restricted rotation around the C-N bond for amino groups attached to purine-like scaffolds in the design of CDK5 inhibitors. Particularly, the rotational barrier was estimated for one molecule with the purine scaffold and another one being its 5-aza-9-deaza-isostere (pyrazolo[1,5-*a*][1,3,5]triazine). A nearly ten-fold higher binding affinity of the 5-aza-9-deazapurine towards CDK5 compared to the parent purine molecule was explained on the basis of some stiffening of the rotational barrier around the exocyclic C-N bond for the pyrazolo[1,5-*a*][1,3,5]triazine derivative. Therefore, we performed the variable temperature (dynamic) NMR spectroscopy experiment,[24] enabling the estimation of the free energy barrier for the rotation around the C-N bond of the amino group on the triazine ring, for three representative compounds from the library we prepared.
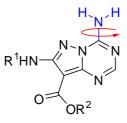
The data were collected for compounds **4a**, **4b** and **4x** at the temperature range 30-60 °C. On heating, the two signals of the amino group in ^{1}H NMR spectra changed their shape and coalesced (Figure 3). The rate constant (*k*) values for the amino group rotation were estimated using the line-

shape analysis of the ^{1}H NMR spectra obtained. Applying the Eyring equation[25], corresponding activation enthalpy (ΔH‡) and entropy (ΔS‡) values were calculated (Table 3). These activation parameters were similar to those determined for 5-azapurine[26] and 5-aza-9-deazapurine[27] derivatives bearing an adenine type of the amino group.

Figure 3. Temperature-dependent shapes of the primary amino group signals in the ^{1}H NMR spectra of compounds **4a** (a), **4b** (b) and **4x** (c).

Table 3. Activation parameters for the rotation of the primary amino group of some 4-aminopyrazolo[1,5-*a*][1,3,5]triazine-8-carboxylates **4**.
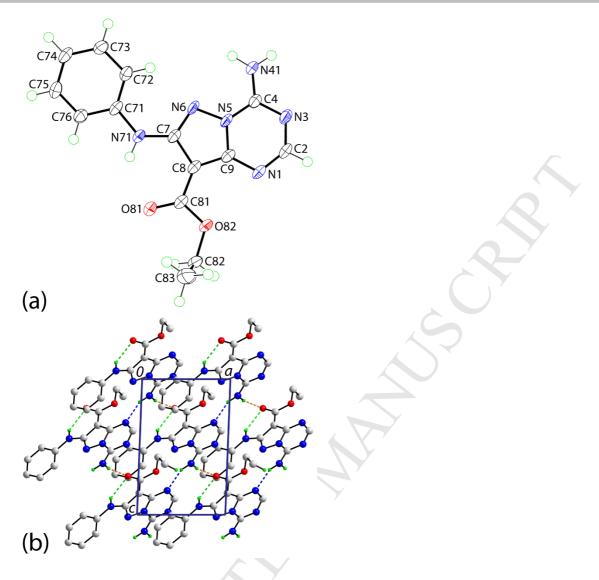

Compound	R^1	R^2	ΔG$^‡_{300}$, kJ mol^{-1}	ΔH‡, kJ mol^{-1}	ΔS‡, J K^{-1} mol^{-1}
4a	Ph	Et	-47.70	3.42	170.4

| **4b** | Ph | Me | -50.18 | 2.11 | 174.3 | |
| **4x** | *cyclo*-Hex | Et | -49.23 | 3.69 | 176.4 | |

*2.4 X-Ray crystallographic study of **4a***

Crystals of a representative product **4a** were obtained and investigated by X-ray crystallography. The molecular structure is shown in Figure 4(a) from which it can be seen that the structure is as determined spectroscopically. The nine-atom fused ring system along with the appended amine group forms a planar residue [r.m.s. deviation of 0.0239 Å] and forms dihedral angles of 8.00(3) and 8.75(8)° with the CO_2 atoms of the ester and the pendent phenyl ring, respectively, indicating to a first approximation, the entire molecule is planar with the exception of the ethyl group which lies prime to the rest of the molecule [the C81–O82–C82–C83 torsion angle = -89.72(17)°]. An intramolecular amine-N7–H…O81(carbonyl) hydrogen bond that closes an S(6) loop is noted[28]. In the crystal, supramolecular layers parallel to [0 1 0] are formed, Figure 4(b), by amine-N41-H…N(triazine), O81(carbonyl) hydrogen bonds[28], indicating the carbonyl-O81 atom accepts two hydrogen bonds.

(a)

(b)

Figure 4. (a) Molecular structure of **4a** showing atom labelling and 70% probability displacement ellipsoids and (b) View of the supramolecular layer parallel to [0 1 0] in the crystal of 4a. The intramolecular amine-N7–H…O81(carbonyl), and intermolecular amine-N41-H…N(triazine) and amine-N41-H…O81(carbonyl) hydrogen bonds are shown as green, blue and orange dashed lines, respectively.

3. Conclusion

We have successfully developed a catalyst-free, highly selective, microwave-assisted method for the multicomponent synthesis of 7-amino substituted 4-aminopyrazolo[1,5-*a*][1,3,5]triazine-8-carboxylates (**4**) employing easily accessible reagents. The method was proven to be practical due to operational economy and simplicity, short reaction time, good yields and high purity of adenine-like products. These features are attractive for organic and medicinal chemists aiming to the preparation

of libraries of purine-related compounds for the drug discovery process. The structural features important for the drug design and development were also estimated for the prepared compounds.

4. Experimental section

4.1 General

Melting points (uncorrected) were determined on a Stuart™ SMP40 automatic melting point apparatus. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Fourier spectrometer (300 MHz) using DMSO-d_6 as a solvent and TMS as an internal reference. IR spectra were recorded using ATR sample base plate diamond Spectrum Two (PerkinElmer) FT-IR spectrometer and in KBr pellets using Varian 640-IR spectrophotometer. Microwave-assisted reactions were carried out in the closed vessel focused single mode using a Discover SP microwave synthesizer (CEM, USA) monitoring reaction temperature by equipped IR sensor. For the method validation, the model reaction was also carried out using Monowave 450 (Anton Paar, Austria) and Initiator+ (Biotage, Sweden) reactors. The sealed vessel reactions under conventional heating were performed in a Monowave 50 (Anton Paar, Austria) reactor.

4.2 Dynamic NMR

Dynamic NMR experiments and lineshape analysis for **4a**, **4b** and **4x** were conducted on a Bruker Fourier spectrometer (300 MHz) using DMSO-d_6 as a solvent and TMS as an internal reference at a temperature range of 27-60 °C. A temperature before coalescence point (27 °C) was chosen to fix parameters for the experiments (Fix Line Broadening parameter at 0.5 Hz). Reaction rate parameters of exchange process at 30-60 °C were obtained using the DNMR Lineshape Fitting module[29] and plotting a graph of ln(k/T) vs 1/T. The activation parameters for the rotation around the C-NH$_2$ bond were calculated using the Eyring equation.

4.3 Synthesis of ethyl 3(5)-phenylamino-5(3)-[((N,N-dimethylamino)methylene)amino]-1H-pyrazole-4-carboxylate (2a) under conventional heating

A mixture of ethyl 5-aminopyrazole-4-carboxylate **1a** (246 mg, 1 mmol) and N,N-dimethylformamide dimethyl acetal (0.2 mL, 1.5 mmol) in toluene (5 mL) was heated under reflux for 1 h. After cooling, the precipitate was filtered and recrystallised from MeOH to give pure formamidine **2a**. White solid, yield 61%, m.p. 150-152 °C (MeOH).

^{1}H NMR (300 MHz, DMSO-d_6): δ 1.24 (3H, t, ^3J = 7.1 Hz, CH$_3$), 2.96 (3H, s, NMe), 3.05 (3H, s, NMe), 4.16 (2H, q, ^3J = 7.1 Hz, CH$_2$), 6.81 (2H, t, ^3J = 7.3 Hz, H-4'), 7.23 (2H, t, ^3J = 7.9 Hz, H-3' and H-5'), 7.54 (2H, d, ^3J = 7.7 Hz, H-2' and H-6'), 7.95 (1H, s, CH), 8.40 (1H, s, NH), 11.75 (1H, s, NH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 13.9 (CH$_3$), 33.5 (CH$_3$), 39.6 (CH$_3$), 58.8 (CH$_2$), 85.9 (CH), 116.0 (C-2' and C-6'), 119.2 (C-4'), 128.7 (C-3' and C-5'), 141.6 (C-1'), 152.1 (C-3), 153.4 (C-5), 158.3 (C-4), 164.9 (C=O). Anal. Calcd. for C$_{15}$H$_{19}$N$_5$O$_2$: C, 59.79; H, 6.36; N, 23.24. Found: C, 59.68; H, 6.41; N, 23.16.

4.4 Synthesis of ethyl 3(5)-phenylamino-5(3)-[((N,N-dimethylamino)methylene)amino]-1H-pyrazole-4-carboxylate (2a) under microwave irradiation

A mixture of ethyl 5-aminopyrazole-4-carboxylate **1a** (246 mg, 1 mmol) with *N,N*-dimethylformamide dimethyl acetal (0.2 mL, 1.5 mmol) in toluene (2 mL) were irradiated in a 10 mL seamless pressure vial using microwave system operating at maximal microwave power up to 150 W at 150 °C for 10 min. After cooling, the precipitate was filtered and recrystallised from MeOH to give compound identical to formamidine **2a** obtained using conventional heating. Yield 55%.

4.5 Synthesis of ethyl 4-amino-7-(phenylamino)pyrazolo[1,5-a][1,3,5]triazine-8-carboxylate (4a) from 2a under conventional heating

To the sodium ethoxide solution prepared by dissolving sodium (60 mg, 2.5 mmol) in abs. EtOH (5 mL), formamidine **2a** (301 mg, 1 mmol) and cyanamide (105 mg, 2.5 mmol) were added. The reaction mixture was heated under reflux for 24 h, cooled and the precipitate was filtered to obtain a compound, which was identical to **4a** prepared using the multicomponent reaction. Yield 57%.

4.6 Synthesis of ethyl 4-amino-7-(phenylamino)pyrazolo[1,5-a][1,3,5]triazine-8-carboxylate (4a) from 2a under microwave irradiation

A mixture of **2a** (301 mg, 1 mmol) with cyanamide (126 mg, 3 mmol) in abs. EtOH (2 mL) were irradiated in a 10 mL seamless pressure vial using microwave system operating at maximal microwave power up to 150 W at 150 °C for 25 min. After cooling, the precipitate was filtered to obtain a compound, which was identical to **4a** prepared using the multicomponent reaction. Yield 35%.

4.7 Synthesis of ethyl 3(5)-phenylamino-5(3)-[(morpholinomethylene)amino]-1H-pyrazole-4-carboxylate (2b) under microwave irradiation

A mixture of ethyl 5-aminopyrazole-4-carboxylate **1a** (246 mg, 1 mmol) with triethyl orthoformate (0.42 mL, 2.5 mmol) and morpholine (0.22 mL, 2.5 mmol) in MeOH (2 mL) were irradiated in a 10 mL seamless pressure vial using microwave system operating at maximal microwave power up to 150 W at 150 °C for 20 min. After cooling, the precipitate was filtered and recrystallised from EtOH to give pure formamidine **2b**. Light yellow solid, yield 51%, m.p. 191-192 °C (EtOH).

^{1}H NMR (300 MHz, DMSO-d_6): δ 1.26 (3H, t, ^3J = 7.1 Hz, CH$_3$), 3.45-3.47 (2H, m, CH$_2$), 3.63-3.66 (4H, m, (CH$_2$)$_2$), 4.18 (2H, q, ^3J = 7.1 Hz, CH$_2$), 6.81 (2H, t, ^3J = 7.3 Hz, H-4'), 7.23 (2H, t, ^3J = 7.9 Hz, H-3' and H-5'), 7.55 (2H, d, ^3J = 7.7 Hz, H-2' and H-6'), 8.00 (1H, s, CH), 8.41 (1H, s, NH), 11.83 (1H, s, NH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 14.1 (CH$_3$), 42.1 (CH$_2$), 48.4 (CH$_2$), 58.9 (CH$_2$), 65.4 (CH$_2$), 66.5 (CH$_2$), 86.0 (CH), 116.1 (C-2' and C-6'), 119.2 (C-4'), 128.7 (C-3' and C-5'), 141.5 (C-1'), 152.2 (C-3), 153.0 (C-5), 157.1 (C-4), 164.9 (C=O). Anal. Calcd. for C$_{17}$H$_{21}$N$_5$O$_3$: C, 59.46; H, 6.16; N, 20.40. Found: C, 59.32; H, 6.22; N, 20.25.

4.8 Synthesis of ethyl 4-amino-7-(phenylamino)pyrazolo[1,5-a][1,3,5]triazine-8-carboxylate (4a) from 2b under microwave irradiation

A mixture of **2b** (343 mg, 1 mmol) with cyanamide (126 mg, 3 mmol) in abs. EtOH (2 mL) were irradiated in a 10 mL seamless pressure vial using microwave system operating at maximal microwave power up to 150 W at 150 °C for 25 min. After cooling, the precipitate was filtered to obtain a compound, which was identical to **4a** prepared using the multicomponent reaction. Yield 57%.

4.9 General method for the microwave-assisted synthesis of 7-amino substituted 4-aminopyrazolo[1,5-a][1,3,5]triazine-8-carboxylates 4

A mixture of 5-aminopyrazole-8-carboxylate **1** (1 mmol), cyanamide (126 mg, 3 mmol) and trimethyl orthoformate (0.35 mL, 3 mmol) in MeOH (2 mL) were irradiated in a 10 mL seamless pressure vial using microwave system operating at maximal microwave power up to 150 W at 150 °C for 25 min. After cooling, the precipitated product **4** was filtered and washed with cold MeOH and recrystallised from a suitable solvent.

4.9.1 Ethyl 4-amino-7-(phenylamino)pyrazolo[1,5-a][1,3,5]triazine-8-carboxylate (**4a**)

White solid, yield 79%; mp 235-237 °C (MeOH); ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.33 (3H, t, ^3J = 7.1 Hz, CH$_3$), 4.34 (2H, q, ^3J = 7.1 Hz, CH$_2$), 6.99 (1H, t, ^3J = 7.3 Hz, H-4'), 7.33 (2H, t, ^3J = 7.9 Hz, H-3' and H-5'), 7.85 (2H, d, ^3J = 7.7 Hz, H-2' and H-6'), 8.22 (1H, s, H-2), 8.35 (1H, br s, NH), 8.84 (1H, br s, NH), 9.00 (1H, s, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 14.4 (CH$_3$), 59.7 (CH$_2$) 86.3 (C-8), 117.6 (C-2' and C-6'), 121.1 (C-4'), 128.9 (C-3' and C-5'), 140.0 (C-1'), 149.0 (C-8a), 149.9 (C-4), 155.5 (C-7), 157.1 (C-2), 164.4 (C=O). IR (ATR): ν 3608 (N-H), 3304 (N-H), 3063 (C-H), 1668 (C=O), 1557, 1511, 1463, 1367, 1259, 1144 cm^{-1}. Anal. Calcd. for C$_{14}$H$_{14}$N$_6$O$_2$: C, 56.37; H, 4.73; N, 28.17. Found: C, 56.21; H, 4.80; N, 28.08.

4.9.2 Methyl 4-amino-7-(phenylamino)pyrazolo[1,5-a][1,3,5]triazine-8-carboxylate (**4b**)

White solid, yield 91%; mp 233-235 °C (MeCN); ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.85 (3H, s, CH$_3$), 6.99 (1H, t, ^3J = 7.4 Hz, H-4'), 7.34 (2H, t, ^3J = 8.0 Hz, H-3' and H-5'), 7.86 (2H, d, ^3J = 7.7 Hz, H-2' and H-6'), 8.21 (1H, s, H-2), 8.36 (1H, br s, NH), 8.86 (1H, br s, NH), 8.97 (1H, s, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 51.0 (CH$_3$), 86.2 (C-8), 117.7 (C-2' and C-6'), 121.2 (C-4'), 128.9 (C-3' and C-5'), 139.8 (C-1'), 149.0 (C-8a), 149.9 (C-4), 155.5 (C-7), 157.1 (C-2), 164.8 (C=O). IR (KBr): ν 3605 (N-H), 3453 (N-H), 3048 (C-H), 1674 (C=O), 1593, 1560, 1447, 1360, 1255, 1145 cm^{-1}. Anal. Calcd. for C$_{13}$H$_{12}$N$_6$O$_2$: C, 54.93; H, 4.25; N, 29.56. Found: C, 54.87; H, 4.31; N, 29.48.

4.9.3 Ethyl 4-amino-7-[(2-fluorophenyl)amino]pyrazolo[1,5-a][1,3,5]triazine-8-carboxylate (**4c**)

White solid, yield 79%; mp 250-252 °C (MeOH); ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.34 (3H, t, ^3J = 7.1 Hz, CH$_3$), 4.35 (2H, q, ^3J = 7.1 Hz, CH$_2$), 6.96-7.04 (1H, m, H-4'), 7.21 (1H, t, ^3J = 7.7 Hz, H-5'), 7.30 (1H, ddd, ^4J = 1.3 Hz, ^3J = 8.1 Hz, $^3J_{HF}$ = 12.0 Hz, H-3'), 8.24 (1H, s, H-2), 8.47 (1H, br s, NH), 8.79 (1H, ddd, ^4J = 1.4 Hz, ^3J = 8.8 Hz, $^4J_{HF}$ = 8.5 Hz, H-6'), 8.90 (1H, br s, NH), 9.30 (1H, d, $^3J_{HF}$ = 3.6 Hz, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 14.3 (CH$_3$), 59.8 (CH$_2$) 86.6 (C-8), 114.3 (d, $^2J_{CF}$ = 18.5 Hz, C-3'), 119.3 (d, $^4J_{CF}$ = 0.9 Hz, C-5'), 121.3 (d, $^3J_{CF}$ = 7.4 Hz, C-4'), 124.9 (d, $^3J_{CF}$ = 3.2 Hz, C-6'), 128.1 (d, $^2J_{CF}$ = 10.2 Hz, C-1'), 148.9 (C-8a), 150.0 (C-4), 151.0 (d, $^1J_{CF}$ = 240.7 Hz, C-2'), 155.1 (C-7), 157.3 (C-2), 164.5 (C=O). IR (ATR): ν 3355 (N-H), 3102 (N-H), 3075 (C-H), 1647 (C=O), 1599, 1551, 1451, 1361, 1259, 1150 cm^{-1}. Anal. Calcd. for C$_{14}$H$_{13}$FN$_6$O$_2$: C, 53.16; H, 4.14; N, 26.57. Found: C, 53.05; H, 4.23; N, 26.44.

4.9.4 Ethyl 4-amino-7-[(4-fluorophenyl)amino]pyrazolo[1,5-a][1,3,5]triazine-8-carboxylate (**4d**)

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Yellow solid, yield 82%; mp 233-235 °C (MeOH); ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.33 (3H, t, ^3J = 7.1 Hz, CH$_3$), 4.34 (2H, q, ^3J = 7.1 Hz, CH$_2$), 7.12 (2H, dd, ^3J = 8.9 Hz, $^3J_{HF}$ = 8.9 Hz, H-3' and H-5'), 7.91 (2H, dd, $^4J_{HF}$ = 4.8 Hz, ^3J = 9.2 Hz, H-2' and H-6'), 8.22 (1H, s, H-2), 8.36 (1H, br s, NH), 8.84 (1H, br s, NH), 8.98 (1H, s, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 14.4 (CH$_3$), 59.6 (CH$_2$) 86.2 (C-8), 115.2 (d, $^2J_{CF}$ = 22.0 Hz, C-3' and C-5'), 119.3 (d, $^3J_{CF}$ = 7.5 Hz, C-2' and C-6'), 136.3 (d, $^4J_{CF}$ = 1.5 Hz, C-1'), 149.0 (C-8a), 149.9 (C-4), 155.5 (C-7), 156.9 (d, $^1J_{CF}$ = 239.1 Hz, C-4'), 157.2 (C-2), 164.3 (C=O). IR (ATR): ν 3459 (N-H), 3327 (N-H), 3043 (C-H), 1658 (C=O), 1563, 1504, 1451, 1364, 1252, 1143 cm^{-1}. Anal. Calcd. for C$_{14}$H$_{13}$FN$_6$O$_2$: C, 53.16; H, 4.14; N, 26.57. Found: C, 53.10; H, 4.18; N, 26.52.

4.9.5 Ethyl 4-amino-7-[(2-chlorophenyl)amino]pyrazolo[1,5-a][1,3,5]triazine-8-carboxylate (*4e*)

Light yellow solid, yield 74%; mp 250-252 °C (DMF & MeOH); ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.34 (3H, t, ^3J = 7.1 Hz, CH$_3$), 4.36 (2H, q, ^3J = 7.1 Hz, CH$_2$), 7.02 (1H, ddd, ^4J = 1.2 Hz, ^3J = 7.7 Hz, ^3J = 7.7 Hz, H-4'), 7.36 (1H, ddd, ^4J = 1.1 Hz, ^3J = 7.4 Hz, ^3J = 8.3 Hz, H-5'), 7.52 (1H, dd, ^4J = 1.4 Hz, ^3J = 8.0 Hz, H-3'), 8.25 (1H, s, H-2), 8.50 (1H, br s, NH), 8.91 (1H, dd, ^4J = 1.3 Hz, ^3J = 8.3 Hz, H-6'), 8.92 (1H, br s, NH), 9.67 (1H, s, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 14.4 (CH$_3$), 59.8 (CH$_2$) 86.6 (C-8), 119.0 (C-6'), 119.8 (C-4'), 121.8 (C-2'), 128.2 (C-5'), 128.8 (C-3'), 136.2 (C-1'), 148.9 (C-8a), 150.0 (C-4), 154.9 (C-7), 157.4 (C-2), 164.5 (C=O). IR (ATR): ν 3473 (N-H), 3370 (N-H), 2919 (C-H), 1659 (C=O), 1587, 1551, 1445, 1313, 1259, 1141 cm^{-1}. Anal. Calcd. for C$_{14}$H$_{13}$ClN$_6$O$_2$: C, 50.53; H, 3.94; N, 25.26. Found: C, 50.46; H, 4.02; N, 25.15.

4.9.6 Ethyl 4-amino-7-[(3-chlorophenyl)amino]pyrazolo[1,5-a][1,3,5]triazine-8-carboxylate (*4f*)

White solid, yield 86%; mp 269-271 °C (MeOH); ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.33 (3H, t, ^3J = 7.1 Hz, CH$_3$), 4.35 (2H, q, ^3J = 7.1 Hz, CH$_2$), 7.01 (1H, ddd, ^4J = 0.8 Hz, ^4J = 2.0 Hz, ^3J = 7.9 Hz, H-4'), 7.33 (1H, t, ^3J = 8.1 Hz, H-5'), 7.86 (1H, t, ^4J = 2.0 Hz, H-2'), 7.92 (1H, ddd, ^4J = 0.8 Hz, ^4J = 2.2 Hz, ^3J = 8.3 Hz, H-6'), 8.23 (1H, s, H-2), 8.47 (1H, br s, NH), 8.86 (1H, br s, NH), 9.10 (1H, s, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 14.4 (CH$_3$), 59.8 (CH$_2$) 86.7 (C-8), 116.4 (C-6'), 117.0 (C-2'), 121.0 (C-4'), 130.5 (C-5'), 133.4 (C-3'), 141.3 (C-1'), 149.1 (C-8a), 150.0 (C-4), 155.2 (C-7), 157.3 (C-2), 164.3 (C=O). IR (ATR): ν 3470 (N-H), 3314 (N-H), 3024 (C-H), 1674 (C=O), 1589, 1549, 1457, 1321, 1244, 1143 cm^{-1}. Anal. Calcd. for C$_{14}$H$_{13}$ClN$_6$O$_2$: C, 50.53; H, 3.94; N, 25.26. Found: C, 50.42; H, 4.06; N, 25.12.

4.9.7 Ethyl 4-amino-7-[(4-chlorophenyl)amino]pyrazolo[1,5-a][1,3,5]triazine-8-carboxylate (*4g*)

White solid, yield 83%; mp 260-262 °C (MeCN); ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.33 (3H, t, ^3J = 7.1 Hz, CH$_3$), 4.34 (2H, q, ^3J = 7.1 Hz, CH$_2$), 7.32 (2H, d, ^3J = 8.9 Hz, H-3' and H-5'), 7.93 (2H, d, ^3J = 9.0 Hz, H-2' and H-6'), 8.22 (1H, s, H-2), 8.39 (1H, br s, NH), 8.86 (1H, br s, NH), 9.05 (1H, s, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 14.3 (CH$_3$), 59.7 (CH$_2$) 86.4 (C-8), 119.4 (C-2' and C-6'), 124.6 (C-4'), 128.5 (C-3' and C-5'), 138.8 (C-1'), 149.0 (C-8a), 149.9 (C-4), 155.3 (C-7), 157.2 (C-2), 164.3 (C=O). IR (ATR): v 3453 (N-H), 3316 (N-H), 3056 (C-H), 1648 (C=O), 1585, 1550, 1492, 1360, 1253, 1146 cm^{-1}. Anal. Calcd. for C$_{14}$H$_{13}$ClN$_6$O$_2$: C, 50.53; H, 3.94; N, 25.26. Found: C, 50.37; H, 4.10; N, 25.11.

4.9.8 Methyl 4-amino-7-[(4-chlorophenyl)amino]pyrazolo[1,5-a][1,3,5]triazine-8-carboxylate (4h)

Light yellow solid, yield 76%; mp 298-299 °C (DMF & H$_2$O); ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.85 (3H, s, CH$_3$), 7.32 (2H, d, ^3J = 8.9 Hz, H-3' and H-5'), 7.93 (2H, d, ^3J = 9.0 Hz, H-2' and H-6'), 8.21 (1H, s, H-2), 8.40 (1H, br s, NH), 8.88 (1H, br s, NH), 9.01 (1H, s, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 51.0 (CH$_3$), 86.3 (C-8), 119.5 (C-2' and C-6'), 124.7 (C-4'), 128.5 (C-3' and C-5'), 138.8 (C-1'), 149.0 (C-8a), 149.9 (C-4), 155.2 (C-7), 157.2 (C-2), 164.6 (C=O). IR (KBr): v 3468 (N-H), 3427 (N-H), 3057 (C-H), 1685 (C=O), 1657, 1587, 1457, 1359, 1257, 1145 cm^{-1}. Anal. Calcd. for C$_{13}$H$_{11}$ClN$_6$O$_2$: C, 48.99; H, 3.48; N, 26.37. Found: C, 48.86; H, 3.60; N, 26.24.

4.9.9 Ethyl 4-amino-7-[(3-iodophenyl)amino]pyrazolo[1,5-a][1,3,5]triazine-8-carboxylate (4i)

Light yellow solid, yield 82%; mp 233-235 °C (MeCN); ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.33 (3H, t, ^3J = 7.1 Hz, CH$_3$), 4.34 (2H, q, ^3J = 7.1 Hz, CH$_2$), 7.11 (1H, t, ^3J = 8.0 Hz, H-5'), 7.33 (1H, ddd, ^4J = 0.9 Hz, ^4J = 1.5 Hz, ^3J = 7.8 Hz, H-4'), 7.91 (1H, dd, ^4J = 1.9 Hz, ^4J = 1.9 Hz, H-2'), 8.19 (1H, ddd, ^4J = 0.8 Hz, ^4J = 2.2 Hz, ^3J = 8.3 Hz, H-6'), 8.23 (1H, s, H-2), 8.39 (1H, br s, NH), 8.85 (1H, br s, NH), 9.01 (1H, s, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 14.4 (CH$_3$), 59.7 (CH$_2$) 86.6 (C-8), 94.6 (C-3'), 117.1 (C-6'), 125.5 (C-2'), 129.9 (C-4'), 130.9 (C-5'), 141.2 (C-1'), 149.0 (C-8a), 149.9 (C-4), 155.1 (C-7), 157.2 (C-2), 164.2 (C=O). IR (KBr): v 3570 (N-H), 3314 (N-H), 3056 (C-H), 1670 (C=O), 1581, 1556, 1455, 1334, 1257, 1144 cm^{-1}. Anal. Calcd. for C$_{14}$H$_{13}$IN$_6$O$_2$: C, 39.64; H, 3.09; N, 19.81. Found: C, 39.48; H, 3.20; N, 19.69.

4.9.10 Ethyl 4-amino-7-[(3-methylphenyl)amino]pyrazolo[1,5-a][1,3,5]triazine-8-carboxylate (4j)

White solid, yield 70%; mp 242-244 °C (MeOH); ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.33 (3H, t, ^3J = 7.1 Hz, CH$_3$), 2.35 (3H, s, CH$_3$), 4.34 (2H, q, ^3J = 7.1 Hz, CH$_2$), 6.80 (1H, d, ^3J = 7.4 Hz, H-4'), 7.21 (1H, t, ^3J = 7.8 Hz, H-5'), 7.53 (1H, s, H-2'), 7.71 (1H, dd, ^4J = 2.0 Hz, ^3J = 8.0 Hz, H-6'), 8.21 (1H, s, H-2), 8.33 (1H,

br s, NH), 8.84 (1H, br s, NH), 8.97 (1H, s, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 14.4 (CH$_3$), 21.0 (CH$_3$), 59.7 (CH$_2$) 86.3 (C-8), 114.8 (C-6'), 117.9 (C-2'), 122.0 (C-4'), 128.8 (C-5'), 138.3 (C-3'), 139.7 (C-1'), 149.0 (C-8a), 149.9 (C-4), 155.6 (C-7), 157.1 (C-2), 164.5 (C=O). IR (ATR): ν 3467 (N-H), 3331 (N-H), 3036 (C-H), 1674 (C=O), 1578, 1550, 1451, 1319, 1262, 1138 cm^{-1}. Anal. Calcd. for C$_{15}$H$_{16}$N$_6$O$_2$: C, 57.68; H, 5.16; N, 26.91. Found: C, 57.54; H, 5.31; N, 26.77.

*4.9.11 Methyl 4-amino-7-[(3-methylphenyl)amino]pyrazolo[1,5-a][1,3,5]triazine-8-carboxylate (**4k**)*

White solid, yield 72%; mp 246-248 °C (MeOH); ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.35 (3H, s, CH$_3$), 3.85 (3H, s, CH$_3$), 6.80 (1H, dd, ^4J = 0.9 Hz, ^3J = 7.5 Hz, H-4'), 7.21 (1H, t, ^3J = 7.8 Hz, H-5'), 7.53 (1H, s, H-2'), 7.72 (1H, dd, ^4J = 2.0 Hz, ^3J = 8.1 Hz, H-6'), 8.21 (1H, s, H-2), 8.34 (1H, br s, NH), 8.86 (1H, br s, NH), 8.93 (1H, s, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 21.0 (CH$_3$), 51.0 (CH$_3$), 86.1 (C-8), 114.8 (C-6'), 118.0 (C-2'), 122.0 (C-4'), 128.8 (C-5'), 138.3 (C-3'), 139.7 (C-1'), 149.1 (C-8a), 149.9 (C-4), 155.5 (C-7), 157.1 (C-2), 164.9 (C=O). IR (KBr): ν 3461 (N-H), 3324 (N-H), 3012 (C-H), 1688 (C=O), 1598, 1568, 1456, 1320, 1263, 1144 cm^{-1}. Anal. Calcd. for C$_{14}$H$_{14}$N$_6$O$_2$: C, 56.37; H, 4.73; N, 28.17. Found: C, 56.26; H, 4.58; N, 28.05.

*4.9.12 Ethyl 4-amino-7-[(4-methylphenyl)amino]pyrazolo[1,5-a][1,3,5]triazine-8-carboxylate (**4l**)*

White solid, yield 91%; mp 196-198 °C (MeOH); ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.33 (3H, t, ^3J = 7.1 Hz, CH$_3$), 2.28 (3H, s, CH$_3$), 4.33 (2H, q, ^3J = 7.1 Hz, CH$_2$), 7.13 (2H, d, ^3J = 8.3 Hz, H-3' and H-5'), 7.73 (2H, d, ^3J = 8.5 Hz, H-2' and H-6'), 8.20 (1H, s, H-2), 8.31 (1H, br s, NH), 8.81 (1H, br s, NH), 8.91 (1H, s, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 14.4 (CH$_3$), 20.4 (CH$_3$), 59.7 (CH$_2$) 86.2 (C-8), 117.7 (C-2' and C-6'), 129.4 (C-3' and C-5'), 130.0 (C-4'), 137.4 (C-1'), 149.1 (C-8a), 149.9 (C-4), 155.7 (C-7), 157.2 (C-2), 164.5 (C=O). IR (ATR): ν 3428 (N-H), 3343 (N-H), 3060 (C-H), 1668 (C=O), 1590, 1554, 1448, 1319, 1259, 1144 cm^{-1}. Anal. Calcd. for C$_{15}$H$_{16}$N$_6$O$_2$: C, 57.68; H, 5.16; N, 26.91. Found: C, 57.52; H, 5.28; N, 26.79.

*4.9.13 Ethyl 4-amino-7-[(4-isopropylphenyl)amino]pyrazolo[1,5-a][1,3,5]triazine-8-carboxylate (**4m**)*

White solid, yield 89%; mp 198-200 °C (DMF & H$_2$O); ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.21 (6H, d, ^3J = 6.9 Hz, (CH$_3$)$_2$), 1.33 (3H, t, ^3J = 7.1 Hz, CH$_3$), 2.87 (1H, m, ^3J = 6.9 Hz, CH) 4.34 (2H, q, ^3J = 7.1 Hz, CH$_2$), 7.19 (2H, d, ^3J = 8.5 Hz, H-3' and H-5'), 7.75 (2H, d, ^3J = 8.6 Hz, H-2' and H-6'), 8.20 (1H, s, H-2), 8.28 (1H, br s, NH), 8.81 (1H, br s, NH), 8.91 (1H, s, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 14.4 (CH$_3$), 24.0

((CH$_3$)$_2$), 32.6 (CH), 59.6 (CH$_2$) 86.2 (C-8), 117.7 (C-2' and C-6'), 126.6 (C-3' and C-5'), 137.6 (C-1'), 141.1 (C-4'), 149.0 (C-8a), 149.8 (C-4), 155.6 (C-7), 157.1 (C-2), 164.5 (C=O). IR (KBr): ν 3346 (N-H), 3144 (N-H), 2959 (C-H), 1680 (C=O), 1591, 1563, 1451, 1358, 1251, 1138 cm^{-1}. Anal. Calcd. for C$_{17}$H$_{20}$N$_6$O$_2$: C, 59.99; H, 5.92; N, 24.69. Found: C, 59.90; H, 6.03; N, 24.58.

*4.9.14 Ethyl 4-amino-7-[(2-methoxyphenyl)amino]pyrazolo[1,5-a][1,3,5]triazine-8-carboxylate (**4n**)*

Yellow solid, yield 80%; mp 264-266 °C (DMF); ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.34 (3H, t, ^3J = 7.1 Hz, CH$_3$), 3.93 (3H, s, OCH$_3$) 4.34 (2H, q, ^3J = 7.1 Hz, CH$_2$), 6.92-7.06 (3H, m, H-3', H-4' and H-5'), 8.22 (1H, s, H-2), 8.38 (1H, br s, NH), 8.67-8.70 (1H, m, H-6'), 8.83 (1H, br s, NH), 9.46 (1H, s, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 14.4 (CH$_3$), 55.8 (OCH$_3$), 59.6 (CH$_2$) 86.6 (C-8), 110.0 (C-3'), 117.4 (C-6'), 120.9 (C-4' and C-5'), 129.2 (C-1'), 146.8 (C-2'), 149.1 (C-8a), 149.9 (C-4), 155.2 (C-7), 157.1 (C-2), 164.0 (C=O). IR (ATR): ν 3396 (N-H), 3330 (N-H), 3127 (C-H), 1637 (C=O), 1594, 1552, 1448, 1355, 1250, 1144 cm^{-1}. Anal. Calcd. for C$_{15}$H$_{16}$N$_6$O$_3$: C, 54.87; H, 4.91; N, 25.60. Found: C, 54.72; H, 5.11; N, 25.46.

*4.9.15 Methyl 4-amino-7-[(2-methoxyphenyl)amino]pyrazolo[1,5-a][1,3,5]triazine-8-carboxylate (**4o**)*

Light yellow solid, yield 87%; mp 307-308 °C (DMF & H$_2$O); ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.85 (3H, s, CH$_3$), 3.93 (3H, s, OCH$_3$), 6.92-7.07 (3H, m, H-3', H-4' and H-5'), 8.20 (1H, s, H-2), 8.41 (1H, br s, NH), 8.65-8.68 (1H, m, H-6'), 8.86 (1H, br s, NH), 9.50 (1H, s, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 51.1 (CH$_3$), 55.9 (OCH$_3$), 86.4 (C-8), 110.0 (C-3'), 117.4 (C-6'), 120.9 (C-4' and C-5'), 129.1 (C-1'), 146.8 (C-2'), 149.0 (C-8a), 149.9 (C-4), 155.3 (C-7), 157.1 (C-2), 164.7 (C=O). IR (KBr): ν 3386 (N-H), 3329 (N-H), 3067 (C-H), 1645 (C=O), 1600, 1560, 1457, 1359, 1258, 1150 cm^{-1}. Anal. Calcd. for C$_{14}$H$_{14}$N$_6$O$_3$: C, 53.50; H, 4.49; N, 26.74. Found: C, 53.33; H, 4.64; N, 26.58.

*4.9.16 Ethyl 4-amino-7-[(3-methoxyphenyl)amino]pyrazolo[1,5-a][1,3,5]triazine-8-carboxylate (**4p**)*

Yellow solid, yield 65%; mp 233-235 °C (MeOH); ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.33 (3H, t, ^3J = 7.1 Hz, CH$_3$), 3.79 (3H, s, OCH$_3$), 4.34 (2H, q, ^3J = 7.1 Hz, CH$_2$), 6.57 (1H, ddd, ^4J = 0.7 Hz, ^4J = 2.3 Hz, ^3J = 8.2 Hz, H-4'), 7.20-7.26 (2H, m, H-2' and H-5'), 7.57 (1H, ddd, ^4J = 0.7 Hz, ^4J = 2.1 Hz, ^3J = 8.1 Hz, H-6'), 8.21 (1H, s, H-2), 8.27 (1H, br s, NH), 8.82 (1H, br s, NH), 9.01 (1H, s, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 14.3 (CH$_3$), 55.0 (OCH$_3$), 59.7 (CH$_2$) 86.4 (C-8), 103.2 (C-2'), 107.1 (C-4'), 110.3 (C-6'), 129.8 (C-5'), 140.9 (C-1'), 149.0 (C-8a), 149.9 (C-4), 155.5 (C-7), 157.1 (C-2), 159.8 (C-3'), 164.5 (C=O). IR (ATR):

ν 3459 (N-H), 3340 (N-H), 2987 (C-H), 1676 (C=O), 1596, 1552, 1448, 1371, 1199, 1081 cm^{-1}. Anal. Calcd. for $C_{15}H_{16}N_6O_3$: C, 54.87; H, 4.91; N, 25.60. Found: C, 54.74; H, 5.02; N, 25.49.

*4.9.17 Ethyl 4-amino-7-[(4-methoxyphenyl)amino]pyrazolo[1,5-a][1,3,5]triazine-8-carboxylate (**4q**)*

Yellow solid, yield 95%; mp 235-237 °C (MeOH); ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.33 (3H, t, ^3J = 7.1 Hz, CH$_3$), 3.75 (3H, s, OCH$_3$), 4.33 (2H, q, ^3J = 7.1 Hz, CH$_2$), 6.89 (2H, d, ^3J = 9.1 Hz, H-3' and H-5'), 7.79 (2H, d, ^3J = 9.1 Hz, H-2' and H-6'), 8.20 (1H, s, H-2), 8.26 (1H, br s, NH), 8.80 (1H, br s, NH), 8.83 (1H, s, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 14.4 (CH$_3$), 55.1 (OCH$_3$), 59.6 (CH$_2$) 86.0 (C-8), 114.0 (C-3' and C-5'), 119.0 (C-2' and C-6'), 133.3 (C-1'), 149.0 (C-8a), 149.8 (C-4), 153.8 (C-4'), 155.7 (C-7), 157.0 (C-2), 164.4 (C=O). IR (ATR): ν 3391 (N-H), 3328 (N-H), 3172 (C-H), 1630 (C=O), 1591, 1560, 1453, 1363, 1247, 1155 cm^{-1}. Anal. Calcd. for $C_{15}H_{16}N_6O_3$: C, 54.87; H, 4.91; N, 25.60. Found: C, 54.80; H, 5.00; N, 25.54.

*4.9.18 Ethyl 4-amino-7-[(4-trifluoromethoxyphenyl)amino]pyrazolo[1,5-a][1,3,5]triazine-8-carboxylate (**4r**)*

White solid, yield 79%; mp 235-237 °C (MeOH); ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.33 (3H, t, ^3J = 7.1 Hz, CH$_3$), 4.34 (2H, q, ^3J = 7.1 Hz, CH$_2$), 7.27 (2H, d, ^3J = 8.3 Hz, H-3' and H-5'), 8.01 (2H, d, ^3J = 9.1 Hz, H-2' and H-6'), 8.23 (1H, s, H-2), 8.39 (1H, br s, NH), 8.87 (1H, br s, NH), 9.10 (1H, s, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 14.3 (CH$_3$), 59.7 (CH$_2$) 86.5 (C-8), 119.0 (C-2' and C-6'), 120.2 (q, $^1J_{CF}$ = 255.1 Hz, CF$_3$), 121.6 (C-3' and C-5'), 139.1 (C-1'), 142.1 (q, $^3J_{CF}$ = 1.6 Hz, C-4'), 149.0 (C-8a), 149.9 (C-4), 155.3 (C-7), 157.2 (C-2), 164.3 (C=O). IR (ATR): ν 3461 (N-H), 3322 (N-H), 3045 (C-H), 1668 (C=O), 1596, 1563, 1454, 1361, 1247, 1147 cm^{-1}. Anal. Calcd. for $C_{15}H_{13}F_3N_6O_3$: C, 47.13; H, 3.43; N, 21.98. Found: C, 47.04; H, 3.32; N, 21.90.

*4.9.19 Methyl 4-amino-7-[(4-trifluoromethoxyphenyl)amino]pyrazolo[1,5-a][1,3,5]triazine-8-carboxylate (**4s**)*

Light yellow solid, yield 74%; mp 280-282 °C (MeCN); ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.85 (3H, s, CH$_3$), 7.27 (2H, d, ^3J = 8.5 Hz, H-3' and H-5'), 8.02 (2H, d, ^3J = 9.1 Hz, H-2' and H-6'), 8.22 (1H, s, H-2), 8.40 (1H, br s, NH), 8.89 (1H, br s, NH), 9.06 (1H, s, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 51.1 (CH$_3$), 86.3 (C-8), 119.1 (C-2' and C-6'), 120.2 (q, $^1J_{CF}$ = 254.8 Hz, CF$_3$), 121.6 (C-3' and C-5'), 139.1 (C-1'), 142.1 (q, $^3J_{CF}$ = 1.8 Hz, C-4'), 149.0 (C-8a), 149.9 (C-4), 155.2 (C-7), 157.2 (C-2), 164.6 (C=O). IR (KBr): ν

3463 (N-H), 3325 (N-H), 3078 (C-H), 1692 (C=O), 1600, 1575, 1459, 1359, 1256, 1145 cm^{-1}. Anal. Calcd. for $C_{14}H_{11}F_3N_6O_3$: C, 45.66; H, 3.01; N, 22.82. Found: C, 45.58; H, 3.10; N, 22.76.

4.9.20. Ethyl 4-amino-7-[(4-acetamidophenyl)amino]pyrazolo[1,5-a][1,3,5]triazine-8-carboxylate (4t)

Grey solid, yield 86%; mp 298-300 °C (MeCN); ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.33 (3H, t, ^3J = 7.1 Hz, CH$_3$), 2.03 (3H, s, CH$_3$), 4.33 (2H, q, ^3J = 7.1 Hz, CH$_2$), 7.56 (2H, d, ^3J = 9.0 Hz, H-3' and H-5'), 7.79 (2H, d, ^3J = 9.0 Hz, H-2' and H-6'), 8.21 (1H, s, H-2), 8.33 (1H, br s, NH), 8.82 (1H, br s, NH), 8.90 (1H, s, NH) 9.82 (1H, s, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 14.4 (CH$_3$), 23.8 (CH$_3$), 59.6 (CH$_2$) 86.1 (C-8), 117.8 (C-2' and C-6'), 119.6 (C-3' and C-5'), 133.2 (C-4'), 135.2 (C-1'), 149.0 (C-8a), 149.8 (C-4), 155.5 (C-7), 157.1 (C-2), 164.4 (C=O), 167.7 (C=O). IR (KBr): ν 3607 (N-H), 3460 (N-H), 3296 (N-H), 3097 (C-H), 1666 (C=O), 1572 (C=O), 1515, 1453, 1367, 1262, 1147 cm^{-1}. Anal. Calcd. for $C_{16}H_{17}N_7O_3$: C, 54.08; H, 4.82; N, 27.59. Found: C, 53.95; H, 4.93; N, 27.43.

4.9.21 Ethyl 4-amino-7-(benzylamino)pyrazolo[1,5-a][1,3,5]triazine-8-carboxylate (4u)

White solid, yield 69%; mp 147-149 °C (MeOH); ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.27 (3H, t, ^3J = 7.1 Hz, CH$_3$), 4.25 (2H, q, ^3J = 7.1 Hz, CH$_2$), 4.60 (2H, d, ^3J = 6.3 Hz, CH$_2$), 6.93 (1H, t, ^3J = 6.3 Hz, NH), 7.24 (1H, t, ^3J = 7.2 Hz, H-4'), 7.33 (2H, t, ^3J = 7.3 Hz, H-3' and H-5'), 7.43 (2H, d, ^3J = 7.0 Hz, H-2' and H-6'), 7.85 (1H, br s, NH), 8.11 (1H, s, H-2), 8.61 (1H, br s, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 14.5 (CH$_3$), 45.2 (CH$_2$), 59.2 (CH$_2$) 85.7 (C-8), 126.8 (C-4'), 127.6 (C-2' and C-6'), 128.3 (C-3' and C-5'), 139.8 (C-1'), 149.6 (C-8a), 149.9 (C-4), 156.5 (C-7), 159.6 (C-2), 164.0 (C=O). IR (ATR): ν 3452 (N-H), 3389 (N-H), 3030 (C-H), 1672 (C=O), 1591, 1562, 1497, 1361, 1252, 1140 cm^{-1}. Anal. Calcd. for $C_{15}H_{16}N_6O_2$: C, 57.68; H, 5.16; N, 26.91. Found: C, 57.61; H, 5.24; N, 26.83.

4.9.22 Ethyl 4-amino-7-(phenethylamino)pyrazolo[1,5-a][1,3,5]triazine-8-carboxylate (4v)

Yellow solid, yield 92%; mp 106-108 °C (MeOH); ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.25 (3H, t, ^3J = 7.1 Hz, CH$_3$), 2.95 (2H, t, ^3J = 7.4 Hz, CH$_2$), 3.61 (2H, q, ^3J = 6.9 Hz, CH$_2$), 4.23 (2H, q, ^3J = 7.1 Hz, CH$_2$), 6.48 (1H, t, ^3J = 5.9 Hz, NH), 7.20-7.32 (5H, m, H-1', H-2', H-3', H-4', H-5'), 7.88 (1H, br s, NH), 8.11 (1H, s, H-2), 8.61 (1H, br s, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 14.4 (CH$_3$), 34.9 (CH$_2$), 43.3 (CH$_2$), 59.0 (CH$_2$) 85.6 (C-8), 126.0 (C-4'), 128.2 (C-2' and C-6'), 128.7 (C-3' and C-5'), 139.4 (C-1'), 149.5 (C-8a), 149.8 (C-4), 156.4 (C-7), 159.4 (C-2), 163.8 (C=O). IR (ATR): ν 3437 (N-H), 3253 (N-H), 3060 (C-H), 1680

(C=O), 1590, 1560, 1497, 1370, 1274, 1138 cm^{-1}. Anal. Calcd. for $C_{16}H_{18}N_6O_2$: C, 58.88; H, 5.56; N, 25.75. Found: C, 58.79; H, 5.64; N, 25.62.

4.9.23 Methyl 4-amino-7-(phenylethylamino)pyrazolo[1,5-a][1,3,5]triazine-8-carboxylate (*4w*)

White solid, yield 73%; mp 131-133 °C (MeOH); ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.95 (2H, t, ^3J = 7.4 Hz, CH$_2$), 3.61 (2H, q, ^3J = 6.9 Hz, CH$_2$), 3.75 (3H, s, CH$_3$), 6.51 (1H, t, ^3J = 5.9 Hz, NH), 7.20-7.32 (5H, m, H-1', H-2', H-3', H-4', H-5'), 7.90 (1H, br s, NH), 8.10 (1H, s, H-2), 8.64 (1H, br s, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 34.9 (CH$_2$), 43.4 (CH$_2$), 50.5 (CH$_3$) 85.4 (C-8), 126.0 (C-4'), 128.2 (C-2' and C-6'), 128.7 (C-3' and C-5'), 139.4 (C-1'), 149.5 (C-8a), 149.9 (C-4), 156.3 (C-7), 159.4 (C-2), 164.2 (C=O). IR (KBr): ν 3465 (N-H), 3391 (N-H), 3024 (C-H), 1688 (C=O), 1657, 1598, 1498, 1361, 1265, 1143 cm^{-1}. Anal. Calcd. for $C_{15}H_{16}N_6O_2$: C, 57.68; H, 5.16; N, 26.91. Found: C, 57.55; H, 5.29; N, 26.78.

4.9.24 Ethyl 4-amino-7-(cyclohexylamino)pyrazolo[1,5-a][1,3,5]triazine-8-carboxylate (*4x*)

White solid, yield 92%; mp 200-202 °C (MeOH); ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.28 (3H, t, ^3J = 7.1 Hz, CH$_3$), 1.28-1.71 (8H, m, ((CH$_2$)$_4$), 1.98-2.01 (2H, m, CH$_2$), 3.69-3.78 (1H, m, *CH*NH), 4.25 (2H, q, ^3J = 7.1 Hz, CH$_2$), 6.31 (1H, t, ^3J = 8.2 Hz, NH), 7.83 (1H, br s, NH), 8.10 (1H, s, H-2), 8.59 (1H, br s, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 14.4 (CH$_3$), 24.3 ((CH$_2$)$_2$), 25.2 (CH$_2$), 32.4 ((CH$_2$)$_2$), 49.9 (*CH*NH), 59.1 (CH$_2$) 85.4 (C-8), 149.5 (C-8a), 149.7 (C-4), 156.3 (C-7), 158.8 (C-2), 164.2 (C=O). IR (ATR): ν 3456 (N-H), 3380 (N-H), 3043 (C-H), 1660 (C=O), 1597, 1561, 1491, 1351, 1258, 1139 cm^{-1}. Anal. Calcd. for $C_{14}H_{20}N_6O_2$: C, 57.68; H, 5.16; N, 26.91. Found: C, 57.57; H, 5.28; N, 26.80.

4.10 X-Ray crystallography of *4a*

Intensity data for **4a** were measured at T = 100(2) K on a SuperNova Dual AtlasS2 diffractometer fitted with Cu Kα radiation so that θ$_{max}$ was 75.1°. Data reduction, including absorption correction, was accomplished with CrysAlis Pro.[30] Of the 13386 measured reflections, 2772 were unique (R$_{int}$ = 0.036) and of these, 2453 data satisfied the $I \geq 2\sigma(I)$ criterion. The structure was solved by direct-methods[31] and refined (anisotropic displacement parameters, C-bound H atoms in the riding model approximation, N-bound H atoms with N–H = 0.88-0.91±0.01 Å and a weighting scheme w = $1/[\sigma^2(F_o^2) + 0.051P^2 + 0.857P]$ where $P = (F_o^2 + 2F_c^2)/3$) on F^2.[32] Based on the refinement of 209 parameters, the final values of R and wR (all data) were 0.043 and 0.114, respectively. The

molecular structure diagram was generated with ORTEP for Windows[33] and the packing diagram with DIAMOND.[34]

Crystal data for $C_{14}H_{14}N_6O_2$ (**4a**): M = 298.31, monoclinic, $P2_1/n$, a = 6.65750(10), b = 20.0193(4), c = 10.2489(2) Å, β = 92.161(2)°, V = 1364.99(4) Å^3, Z = 4, D_x = 1.452 g cm^{-3}, $F(000)$ = 624, μ = 0.854 mm^{-1}. CCDC deposition number: 1817291.

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