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A new synthesis of 2,8-disubstituted pyrazolo[4,3-*e*][1,2,4]triazolo [1,5-*c*]pyrimidines

Anton V. Dolzhenko *, Giorgia Pastorin, Anna V. Dolzhenko, Wai Keung Chui

Department of Pharmacy, Faculty of Science, National University of Singapore, 18 Science Drive 4, Singapore 117543, Singapore

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ABSTRACT

A practical synthesis of pyrazolo[4,3-*e*][1,2,4]triazolo[1,5-*c*]pyrimidines, which are key intermediates in the preparation of adenosine receptor antagonists, is developed. The method allows introduction of a variety of aryl substituents at position 2 of the pyrazolo[4,3-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine system via cyclocondensation of 5-amino-4-iminopyrazolo[3,4-*d*]pyrimidine with benzaldehydes accompanied with oxidation by iodobenzene diacetate. Some unexpected reactions are observed and the structures of the products are confirmed using NMR spectroscopy and X-ray crystallography.

Adenosine is a major modulator of cellular response in different organs. The effects of adenosine are mediated through four adenosine receptor subtypes, namely A_1, A_{2A}, A_{2B} and A_3.[1] These subtypes are different in terms of tissue distribution and affinity to adenosine, and have unique pharmacological profiles. Adenosine receptor antagonists have been widely explored as potential therapeutic agents for treatment of nervous system disorders, cardiovascular diseases, and immune and inflammatory disorders.[2]

The pyrazolo[4,3-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine nucleus has been recognized as a promising template for the development of new adenosine receptor antagonists.[3] Through installation of appropriate substituents at R^1 and R^3 in adenosine receptor antagonists **4** (Scheme 1), it was found that it was possible to tune the activity and selectivity of the new agents towards specific adenosine receptor subtypes. However, systematic studies on the effect of the structure of the R^2 substituent on the interaction of **4** with adenosine receptors are not available.

For the synthesis of adenosine receptor antagonists of general structure **4**, the preparation of intermediate **3** is a critical step that limits variation of R^2 (Scheme 1). The cyclocondensation of hydrazides with imidate **2**, prepared from aminopyrazoles **1**, required harsh conditions (260 °C) and chromatographic purification of the products.[4] The poor solubility of **3**, particularly when R^1 = Me and R^3 = Ar, made chromatographic purification a time- and solvent-consuming process.

Therefore, a new method for the preparation of pyrazolo[4,3-*e*][1,2,4]triazolo[1,5-*c*]pyrimidines **3** via a more practical, chromatography-free process under mild reaction conditions would be beneficial for further exploration of the chemistry and pharmacology of adenosine receptor antagonists.

We hypothesized that the reaction of 5-amino-4-imino-pyrazolo[3,4-*d*]pyrimidine (**5**) with aromatic aldehydes followed by oxidation might be a potential approach for the synthesis of pyrazolo[4,3-*e*][1,2,4]triazolo[1,5-*c*]pyrimidines **3** (Scheme 2). A variety of commercially available benzaldehydes allow the introduction of chemical diversity at position 2 of the heterocyclic nucleus.

Imidate **2a**[5] was prepared from aminopyrazole **1a** and triethyl orthoformate using a known procedure (Scheme 3).[4,6] The reaction of **2a** with hydrazine in ethanol on heating was reported[7] to produce 5-amino-4-imino-2-methylpyrazolo[3,4-*d*]pyrimidine (**5**). Surprisingly, our attempt to repeat this synthesis using the conditions described by Baraldi et al.[7] resulted in the formation of a different product.

The downfield shift of the amino group signal at 6.65 ppm as well as the two doublets at 7.47 and 11.83 ppm in the ^{1}H NMR spectrum could indicate the formation of pyrazolotriazepine **6**. However, the large value of the coupling constant for these doublets (J = 11.3 Hz) suggested an arrangement of the NH–CH fragment protons in a *trans*-configuration; this was also confirmed by a 2D NOESY experiment. Finally, using X-ray crystallography (Fig. 1),[8] the structure of the product was revealed as a new fused macrocyclic system, namely 4,12-diamino-2,10-dimethyl-2,8, 10,16-tetrahydrodipyrazolo[3,4-*e*:3′,4′-*l*][1,2,4,8,9,11]hexaazacyclotetradecine (**7**).[9]

We were able to isolate the desired intermediate **5** when the reaction of **2a** with hydrazine was carried out in ethanol at 0–20 °C for 5 h (Scheme 3).[10] Additionally, pure **7** (6–8%) precipitated from the filtrate on standing overnight at ambient temperature. Broadening of the NH and C-4 signals in the NMR spectra of **5** was observed which could be attributed to the dynamic inversion of the imino group configuration.

In the trial reactions of **5** with benzaldehyde (Table 1), we found that varying amounts of **3** formed after initial condensation with

* Corresponding author. Tel./fax: +65 6779 1554.
E-mail address: phada@nus.edu.sg (A.V. Dolzhenko).

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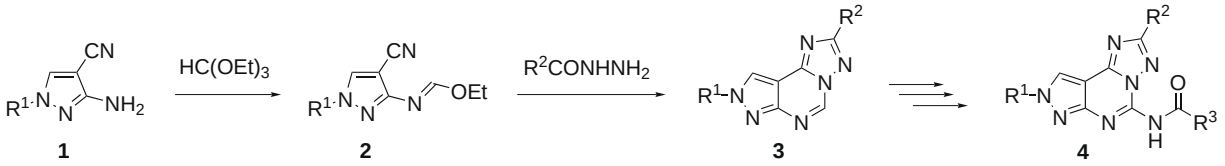
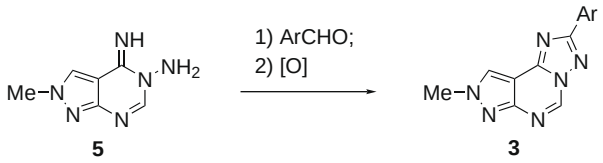


Scheme 1. General synthetic approach to pyrazolo[4,3-*e*][1,2,4]triazolo[1,5-*c*]pyrimidines—antagonists of adenosine receptors.

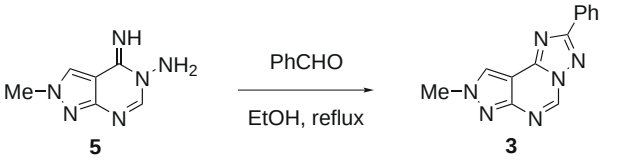


Scheme 2.

the aldehyde even without using an oxidant. Improved results were achieved in the presence of 0.3 equiv of triethylamine. Increasing the quantity of the aldehyde or triethylamine did not improve the reaction yield and did not significantly change the composition of the obtained mixtures.

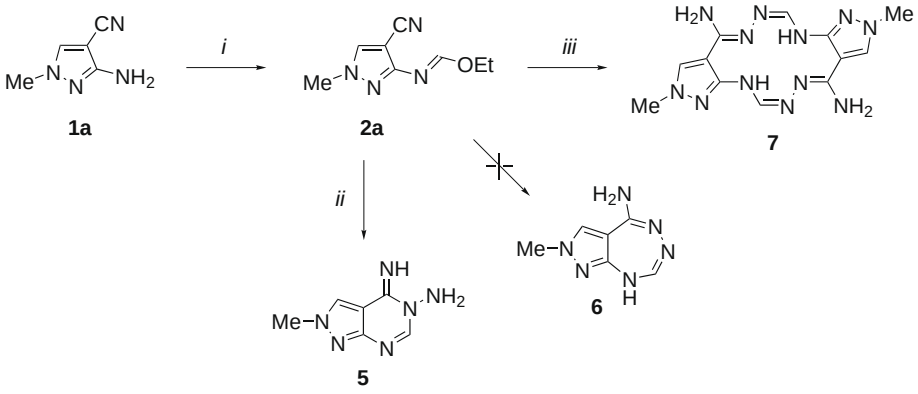
When this method was applied for the reactions of **5** with substituted benzaldehydes, the extent of oxidation with aerial oxygen was strongly dependent on the structure of the aldehydes. In some cases (e.g., Ar = 4-PhC$_6$H$_4$), pure **3** was isolated directly after

Table 1
A study of the reaction of 5-amino-4-iminopyrazolo[3,4-*d*]pyrimidine (**5**) with benzaldehyde



Equiv of PhCHO	Catalyst (equiv)	Combined yield[a] (%)	Quantity of **3** in the mixture[b] (%)
1	—	~40	~50
1	Et_3N (0.1)	~40	~60
1	Et_3N (0.3)	~65	~70
1	Et_3N (1.0)	~60	~70
2	Et_3N (0.3)	~65	~70
1	Piperidine (0.3)	~35	~10

[a] Approximate values without characterization of non-oxidized intermediates.
[b] Estimated by ^{1}H NMR spectroscopy.



Scheme 3. Reagents and conditions: (i) HC(OEt)$_3$ (5–10 equiv), reflux, 8 h (85%); (ii) N_2H_4 (4 equiv), EtOH, 0–20 °C, 5 h (88%); (iii) N_2H_4 (4 equiv), EtOH, reflux, 2 h (62%).

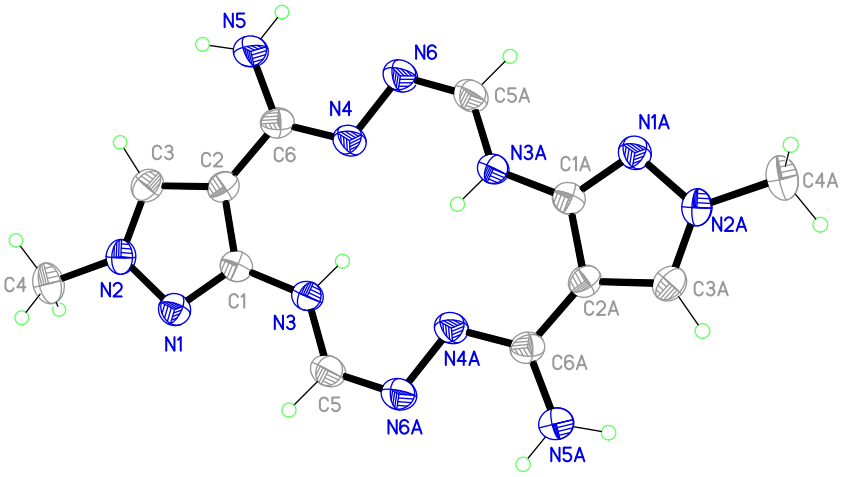
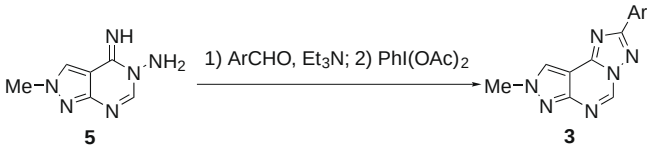
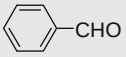
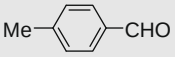
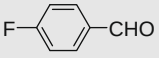
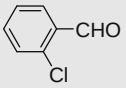
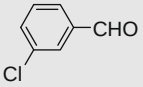
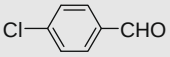
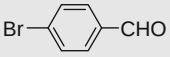
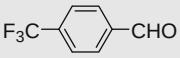
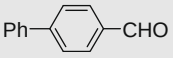
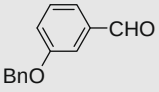
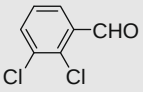
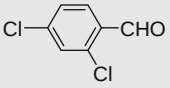
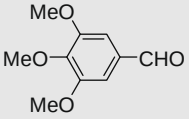


Figure 1. X-ray crystal structure of **7**.

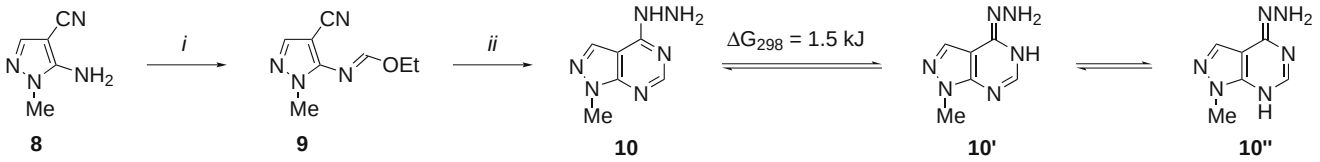
Table 2
Synthesis of 2-aryl-8-methylpyrazolo[4,3-*e*][1,2,4]triazolo[1,5-*c*]pyrimidines (**3**)



5 **3**

3	ArCHO	Mp (°C)	Yield (%)
a		248	60
b		>300	56
c		278–279	53
d		278–279	62
e		235	67
f		284–285	64
g		>300	70
h		>300	92
i		>300	68
j		>300	89[a]
k		243–244	54
l		289–290	90
m		288–289	90
n		252–253	68

[a] Yield after condensation of **5** with the aldehyde, oxidation with $PhI(OAc)_2$ was not required.



Scheme 4. Reagents and conditions: (i) $HC(OEt)_3$ (5–10 equiv), reflux, 6 h (84%); (ii) N_2H_4 (4 equiv), EtOH, rt, 8 h (70%) or reflux, 2 h (86%).

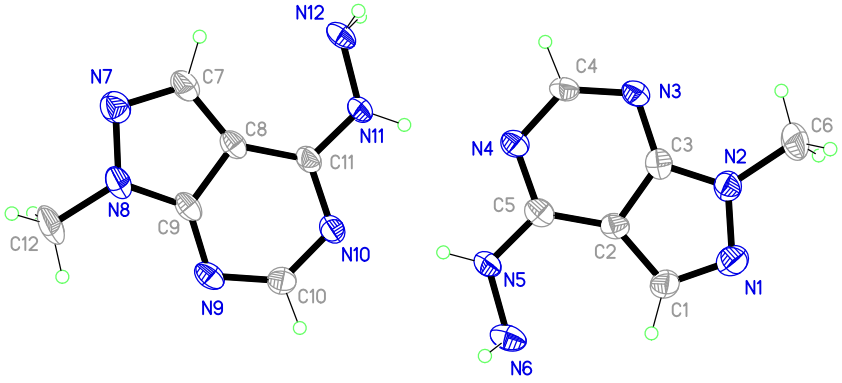


Figure 2. X-ray crystal structure of **10**.

the reaction of **5** with the aldehyde, making further oxidation unnecessary.

Organic hypervalent iodine derivatives have been used extensively as convenient oxidants.[11] The good oxidizing potential, convenience in handling, mild reaction conditions minimizing the possibility of side reactions, benign environmental character and commercial availability are attractive features of hypervalent iodine compounds. Therefore, iodobenzene diacetate[12] was chosen for the oxidation step. The oxidation reaction was performed in acetic acid on the crude mixture of **3** and non-oxidized intermediates. Oxidation was found to proceed under mild conditions, almost quantitatively, affording 2-aryl-8-methylpyrazolo[4,3-*e*][1,2,4]triazolo[1,5-*c*]pyrimidines (**3**) with excellent purity.

In general, the method was found to be practical. Reasonable yields of products **3** were obtained and a variety of readily available aldehydes could be employed (Table 2).[13] The procedure was clean and the products could be used for the synthesis of new potential adenosine receptor antagonists without any additional purification.

Interestingly, the regioisomer of **2**, imidate **9**,[14] prepared from aminopyrazole **8**, reacted differently with hydrazine in ethanol, affording 4-hydrazinopyrazolo[3,4-*d*]pyrimidine (**10**)[15] as a rearrangement product (Scheme 4). The reaction proceeded analogously in all temperature parameters (0–70 °C) applied.

Hydrazino–hydrazono tautomerism of **10** was observed in DMSO-d_6 solution. The tautomeric equilibrium was shifted towards hydrazino form **10** with ΔG_{298} = 1.5 kJ. Due to broadening of the signals in the NMR spectra, it was not possible to differentiate between the two hydrazono forms **10′** and **10″**. They might exist together in a dynamic equilibrium. In the solid state, the product was found to exist exclusively in hydrazino form **10**: two almost identical molecules crystallized together as a dimer (Fig. 2).[16]

In summary, a new synthesis of pyrazolo[4,3-*e*][1,2,4]triazolo[1,5-*c*]pyrimidines, which are important intermediates in the synthesis of adenosine receptor antagonists, was successfully developed. The advantages of the method are simple synthetic procedures and readily available starting materials.

Acknowledgements

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5. *4-Cyano-3-[(ethoxymethylene)amino]-1-methylpyrazole* (**2a**). A mixture of **1a** (2.44 g, 20 mmol) and triethyl orthoformate (30 ml) was heated under reflux for 8 h. Excess triethyl orthoformate was removed under reduced pressure and the residue was recrystallized from AcOEt/hexane. Yield 85%, mp 51–52 °C; LC–MS (APCI) *m*/*z* 179 (MH^+); Anal. Calcd for $C_8H_{10}N_4O$: C, 53.92; H, 5.66; N, 31.44. Found: C, 53.78; H, 5.75; N, 31.27. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.30 (3H, t, *J* 7.2 Hz, CH_2*Me*), 3.79 (3H, s, NMe), 4.28 (2H, q, *J* 7.2 Hz, CH_2), 8.30 (1H, s, H-5), 8.37 (1H, s, N = C*H*OEt); ^{13}C NMR (75 MHz, DMSO-d_6): δ 13.8 (CH_2*Me*), 39.2 (NMe), 62.8 (CH_2), 84.0, 113.7, 137.3 (CH), 156.6, 159.2 (CH).

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9. *4,12-Diamino-2,10-dimethyl-2,8,10,16-tetrahydrodipyrazolo-[3,4-e:3′,4′-l][1,2,4,8,9,11]hexaazacyclotetradecine* (**7**). Hydrazine (2 ml, 40 mmol) was added to a solution of **2a** (1.78 g, 10 mmol) in EtOH (20 ml). The mixture was heated under reflux for 2 h and cooled to rt. The resulting precipitate was filtered and washed with EtOH. Yield 62%, mp 269–270 °C (MeOH); LC–MS (APCI) *m*/*z* 329 (MH^+); Anal. Calcd for $C_{12}H_{16}N_{12}$: C, 43.90; H, 4.91; N, 51.19. Found: C, 43.82; H, 5.02; N, 51.02. ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.73 (6H, s, 2Me), 6.65 (4H, s, $2NH_2$), 7.47 (2H, d, *J* 11.3 Hz, H-7 and -15), 8.02 (2H, s, H-3 and -11), 11.83 (2H, d, *J* 11.3 Hz, H-8 and -16); ^{13}C NMR (75 MHz, DMSO-d_6): δ 38.8 (2Me), 100.4 (2C), 130.6 (2CH), 138.1 (2CH), 147.6 (2C), 152.2 (2C).
10. *5-Amino-4-imino-2-methylpyrazolo[3,4-d]pyrimidine* (**5**). Hydrazine (1.2 ml, 24 mmol) was added to a cold (0–5 °C) solution of **2a** (1.08 g, 6 mmol) in EtOH (20 ml). The mixture was stirred at rt for 5 h. The resulting precipitate was filtered and washed with EtOH to provide **5**. Yield 88%, mp 180 °C (EtOH). LC–MS (APCI) *m*/*z* 165 (MH^+); Anal. Calcd for $C_6H_8N_6$: C, 43.90; H, 4.91; N, 51.19. Found: C, 43.73; H, 5.08; N, 51.05. ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.92 (3H, s, Me), 5.41 (2H, s, NH_2), 7.58 (1H, br s, NH), 7.87 (1H, s, CH), 8.26 (1H, s, CH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 39.5 (Me), 105.4, 128.3 (CH), 149.9 (CH), 151.7 (br s), 155.0. Additionally, **7** (6–8%) precipitated from the filtrate on standing overnight.
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13. *General method for the preparation of 8-methyl-2-aryl-pyrazolo[4,3-e][1,2,4]triazolo[1,5-e]pyrimidines* (**3**). A mixture of **5** (1.64 g, 10 mmol), the appropriate benzaldehyde (10 mmol) and Et_3N (0.42 ml, 3 mmol) in EtOH (30 ml) was heated under reflux overnight. After cooling, the resulting precipitate was filtered, washed with cold EtOH and mixed with AcOH (10 ml). To the resulting mixture was added an equimolar quantity of iodobenzene diacetate. After stirring at rt for 1 h, the precipitate was filtered and washed with EtOH. Analytical samples were recrystallized from DMSO. Data for representative compound **3k**: LC–MS (APCI) *m*/*z* 357 (MH^+); Anal. Calcd for $C_{20}H_{16}N_6O$: C, 67.40; H, 4.53; N, 23.58. Found: C, 67.33; H, 4.60; N, 23.43. ^{1}H NMR (300 MHz, DMSO-d_6): δ 4.20 (3H, s, Me), 5.23 (2H, s, CH_2), 7.20 (1H, dd, *J* 7.9, 1.9 Hz H-4′), 7.30–7.56 (6H, m, H-3′ and Ph), 7.78–7.86 (2H, m, H-2′ and -6′), 8.91 (1H, s, H-9), 9.45 (1H, s, H-5); ^{13}C NMR (75 MHz, DMSO-d_6): δ 40.4 (Me), 69.2 (CH_2), 101.5, 112.6 (CH), 117.3 (CH), 119.4 (CH), 126.2 (CH), 127.5 (2CH), 127.8 (CH), 128.4 (2CH), 130.2 (CH), 131.1, 136.8, 139.8 (CH), 148.4, 153.7, 158.7, 162.9.
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15. *4-Hydrazino-1-methyl-1H-pyrazolo[3,4-d]pyrimidine* (**10**). Mp 243 °C (EtOH); LC–MS (APCI) *m*/*z* 165 (MH^+); Anal. Calcd for $C_6H_8N_6$: C, 43.90; H, 4.91; N, 51.19. Found: C, 43.82; H, 5.02; N, 51.12. ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.89 (3H, s, Me), 4.67* and 4.87 (2H, two br s, NH_2), 8.03* and 8.11 (1H, two br s, H-3), 8.30 (1H, br s, H-6), 9.13 and 9.45* (1H, two br s, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 33.2 and 33.3* (Me), 98.5, 130.8* and 134.6 (two br s, CH), 152.2* and 153.6 (two br s), 155.0 and 155.5* (two br s, CH), 156.8* and 160.5 (two br s). *-signals of minor hydrazone tautomer.
16. Dolzhenko, A. V.; Pastorin, G.; Dolzhenko, A. V.; Tan, G. K.; Koh, L. L. *Acta Cryst.* **2009**, *65E*, o1720–o1721.