European Journal of Medicinal Chemistry 64 (2013) 464–476

Contents lists available at SciVerse ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Synthesis, antibacterial activity and cytotoxicity of new fused pyrazolo[1,5-*a*]pyrimidine and pyrazolo[5,1-*c*][1,2,4]triazine derivatives from new 5-aminopyrazoles

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ARTICLE INFO

Article history:
Received 22 January 2013
Received in revised form
9 April 2013
Accepted 15 April 2013
Available online 23 April 2013

Keywords:
5-Aminopyrazoles
Pyrazolo[1,5-*a*]pyrimidines
Pyrazolo[5,1-*c*][1,2,4]triazines
α,α-Dicyanoketene-*S*,*S*- and *N*,*S*-acetals
Antibacterial activity
Cytotoxicity

ABSTRACT

New 5-aminopyrazoles **2a**–**c** were prepared in high yields from the reaction of known α,α-dicyanoketene-*N*,*S*-acetals **1a**–**c** with hydrazine hydrate under reflux in ethanol. These compounds were utilized as intermediates to synthesize pyrazolo[1,5-*a*]-pyrimidines **3a**–**c**, **4a**–**d**, **5a**–**c**, and **6a**–**c**, as well as pyrazolo[5,1-*c*][1,2,4]triazines **7a**–**c** and **8a**–**c**, by the reaction of 2-[bis(methylthio)methylene]malononitrile, α,α-dicyanoketene-*N*,*S*-acetals **1a**–**b**, acetylacetone, acetoacetanilide as well as acetylacetone, and malononitrile, respectively. Furthermore, cyclization of **2a**–**c** with pentan-2,5-dione yielded the corresponding 5-pyrrolylpyrazoles **9a**–**c**. Moreover, fusion of **2a**–**c** with acetic anhydride resulted in the corresponding 1-acetyl-1*H*-pyrazoles **10a**–**c**. The antibacterial activity and cytotoxicity against Vero cells of several selected compounds are also reported.

1. Introduction

Pyrazolopyrimidine and pyrazolotriazine derivatives constitute an interesting class of heterocycles because of their synthetic versatility, effective biological activities, and pharmacological importance as purine analogs [1–5]. Several derivatives such as 4-hydroxypyrazolopyrimidine (allopurin), which are used in the treatment of hyperuricemia and gout, inhibit *de novo* purine biosynthesis and xanthine oxidase [6]. Various related pyrazolopyrimidine compounds were reported to possess anti-tumor and anti-leukemic activities [7,8]. Substituted pyrazolotriazines are often used in medicine because of their remarkable bactericidal, fungicidal, and antiviral effects [9,10]. Ketene-*S*,*S*- and -*N*,*S*-acetals and related compounds are versatile reagents in the synthesis of polyfunctionalized heterocycles [11–13]. Ketene-*S*,*S* and *N*,*S*-acetals have been paid much attention because they are extensively used for the synthesis of pyrazole derivatives by displacing the methylthio group with bifunctionalized amines, such as hydrazine [14–16]. Accordingly, we report in this paper a novel synthesis of functionalized pyrazolo[1,5-*a*]pyrimidines **3a**–**c**, **4a**–**d**, **5a**–**c**, and **6a**–**c**, as well as pyrazolo[5,1-*c*][1,2,4]triazines **7a**–**c** and **8a**–**c**, by the reaction of respective 2-[bis(methylthio)methylene]malononitrile, α,α-dicyanoketene-*N*,*S*-acetals **1a** and **b**, acetylacetone, and acetoacetanilide as well as acetylacetone and malononitrile with 5-aminopyrazoles **2a**–**c**. The antibacterial activity and toxicity in Vero cells of several selected compounds are also reported.

2. Chemistry

The 5-aminopyrazole intermediates **2a**–**c** were prepared from the cyclocondensation of respective α,α-dicyanoketene-*N*,*S*-acetals 2-[methylthio(morpholino)methylene]malononitrile **1a**, 2-[methylthio(piperidin-1-yl)methylene]malononitrile **1b**, and 2-[methylthio(pyrrolidin-1-yl)methylene]malononitrile **1c** with hydrazine hydrate under reflux in ethanol (Scheme 1). The starting materials **1a**–**c** were prepared *via* the reaction of 2-[bis(methylthio)methylene]malononitrile with an appropriate cyclic secondary amines of morpholine, piperidine, and pyrrolidine in refluxing ethanol

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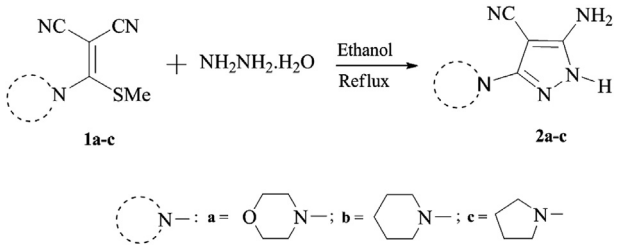
http://dx.doi.org/10.1016/j.ejmech.2013.04.029



Scheme 1. Synthesis of 5-aminopyrazoles **2a**–**c**.

according to a previously reported procedure [17]. The chemical structures of **2a**–**c** were established based on their elemental analysis and spectral data. The IR spectra of **2a**–**c** showed bands between 3222 cm^{-1} and 3453 cm^{-1} for the NH and NH_2 groups, respectively. Their ^1H NMR spectra showed singlet signals with δ values between 4.93 ppm and 6.11 ppm corresponding to the NH_2 protons, whereas the singlet singles with δ values from 10.74 ppm to 11.00 ppm, which can be assigned to the NH protons. The ^{13}C NMR spectra were characterized by signals at δ values of 154.5–154.6 and 158.0–158.6 ppm assigned to respective carbons of C=N and C–NH_2. The structures of **2a**–**c** were supported by their direct infusion mass spectrometry (DIMS) results, which showed molecular ions corresponding to the molecular formulas. For example, the DIMS of 5-amino-3-morpholino-1*H*-pyrazole-4-carbonitrile **2a** showed a molecular ion at $m/z = 193.25$, which corresponds to the molecular formula $C_8H_{11}N_5O$ (193.21).

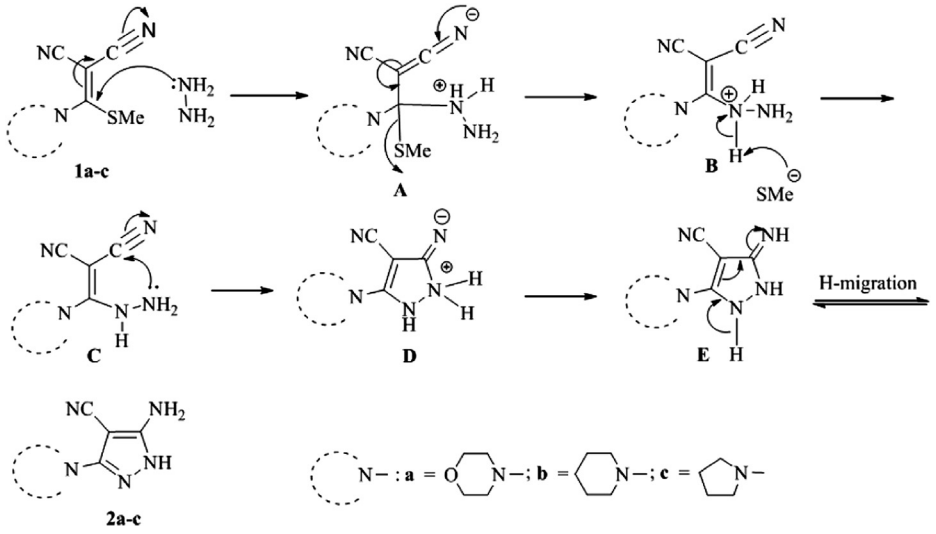
The possible formation of 5-aminopyrazoles is shown in Scheme 2. First, Michael addition to α,α-dicyanoketene-*N*,*S*-acetals **1a**–**c** occurs with lone pair of the NH_2 group in hydrazine to form an intermediate adduct A. Then, the methylthio ion is removed, which results in the formation of intermediate B. This methylthio ion abstracts the proton of the ammonium ion to produce an intermediate C. Subsequently, intermolecular cyclization occurs by the lone pair on the NH_2 group attacking the cyano group to produce intermediate D. The ammonium proton abstraction in D occurs to form E, followed by aromatic-driven 1,5-hydrogen migration to yield products **2a**–**c**.

The structure of 5-amino-3-morpholino-1*H*-pyrazole-4-carbonitrile **2a** was further identified by X-ray diffraction (XRD). The *ORTEP* plot of **2a** and the numbering scheme are illustrated in Fig. 1, which confirmed that the N–H in the pyrazole ring is adjacent to the carbon attached to the amino group. Suitable crystals of **2a** were grown by slow evaporation of MeOH solution of the compound for 48 h. The crystal data and structure refinement results for **2a** are given in Table 1. Compound **2a** crystallized in a triclinic system with space group of $P\bar{1}$ (No. 2). The symmetric unit of **2a** contains two molecules, A and B. Selected bond distances and bond angles are listed in Table 2. The bond lengths and angles in molecules A and B of **2a** are within the normal ranges [18]. The morpholine rings adopt chair conformation in molecules A and B. The mean planes for **2a** in the molecule A were observed in the pyrazole ring N2/N3/C1/C4/C5 and morpholine ring O11/N8/C9/C10/C12/C13, with a maximum deviation of −0.251(1)° at the O11 atom. The mean planes in the molecular B were observed in the pyrazole ring N16/N17/C15/C18/C19 and morpholine ring O25/N22/C23/C24/C26/C27, with a maximum deviation of 0.253(1)° at the O25 atom. The dihedral angles between the mean planes of the morpholine and pyrazole rings in molecules A and B were 10.55(9)° and 18.82(10)°, respectively (see Electronic Supplementary information [19]).

The above mentioned 5-aminopyrazoles **2a**–**c** were used as intermediates for the synthesis of new pyrazolo[1,5-*a*]pyrimidines and pyrazolo[5,1-*c*][1,2,4]triazines. Thus, condensation of **2a**–**c** with α,α-dicyanoketene-*S*,*S*-acetal 2-[bis(methylthio)methylene] malononitrile in refluxing ethanol containing a catalytic amount of triethylamine yielded the corresponding pyrazolo[1,5-*a*]pyrimidines **3a**–**c**. The reaction of 5-aminopyrazoles **2a** and **2c** with α,α-dicyanoketene-*N*,*S*-acetals **1a** and **1b** under similar conditions yielded pyrazolo[1,5-*a*]pyrimidines **4a**–**d** as demonstrated in Scheme 3. The proposed structures of **3a**–**c** and **4a**–**d** were established based on their elemental analysis and spectral data (see Experimental).

The proposed mechanism for the formation of pyrazolo[1,5-*a*]pyrimidines **3a**–**c** and **4a**–**d** is similar to that for the formation of 5-aminopyrazoles **2a**–**c**, except that the final step in the former involves 1,3-hydrogen migration contrary to the 1,5-hydrogen migration in the latter (Scheme 4).

We also attempted to synthesize directly pyrazolo[1,5-*a*]pyrimidines **5a**–**c** and **6a**–**c** by treating 5-aminopyrazoles **2a**–**c** with corresponding 1,3-dicarbonyl compounds, namely, pentane-2,4-dione and acetoacetanilide, in refluxing dimethylformamide containing a catalytic amount of glacial acetic acid as shown in Scheme 5.



Scheme 2. Mechanism for the formation of 5-aminopyrazoles **2a**–**c**.

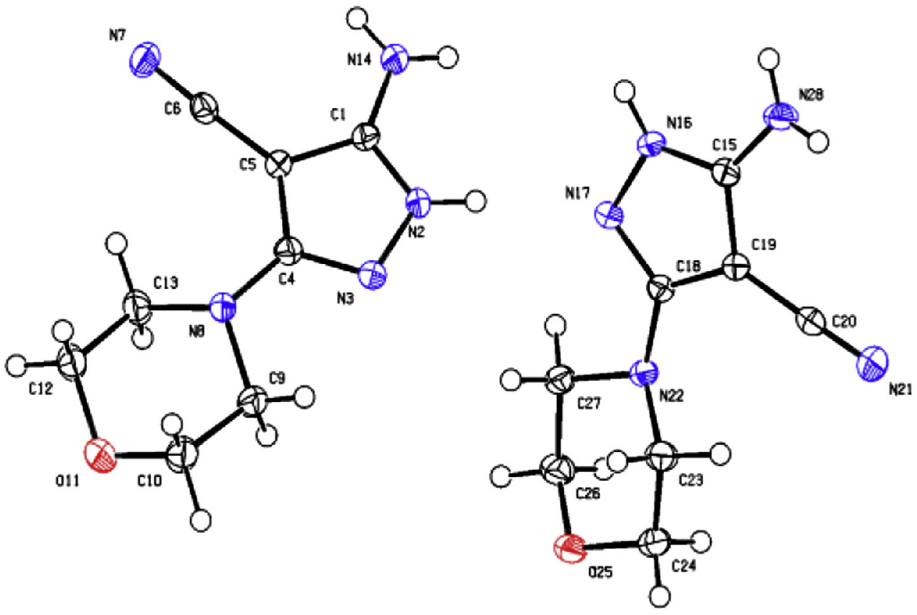


Fig. 1. Single-crystal X-ray analysis. *ORTEP* view of **2a** showing the atom numbering scheme.

The mechanism for the formation of **5a**–**c** and **6a**–**c** is shown in Scheme 6. The conversion involves three major steps, as follows: (i) nucleophilic attack of the NH_2 group from 5-aminopyrazoles on the carbonyl group of acetylacetone and acetoacetanilide, by releasing water molecule, to form intermediate imine A; (ii) intramolecular cyclization occurs by a nucleophilic attack of the NH of pyrazole on the other carbonyl group to form an intermediate adduct B; and (iii) dehydration in B when X = methyl produces **5a**–**c** and elimination of aniline when X = NHPh yields **6a**–**c**.

The structures of the pyrazolo[1,5-*a*]pyrimidines **5a**–**c** and **6a**–**c** were elucidated based on their elemental analyses and spectral data. The characteristic band in the IR spectra of **6a**–**c** is at ν = (3308–3146) cm^{-1} for OH stretching vibrations. The ^1H NMR spectra of **5a**–**c** displayed singlet signals at δ = (2.42–2.63) ppm, which correspond to six protons of two methyl groups. The ^1H NMR spectra of **6a**–**c** displayed singlet signals between δ = (12.89–13.07) ppm because of the OH group. Results from DIMS of **5a**–**c** and **6a**–**c** showed molecular ions corresponding to the molecular formula. For example, the DIMS of 5,7-dimethyl-2-morpholinopyrazolo[1,5-*a*]pyrimidine-3-carbonitrile **5a** showed a molecular ion at m/z = 257.30, which corresponds to the molecular formula $C_{13}H_{15}N_5O$ (257.29). The DIMS of 7-hydroxy-5-methyl-2-(piperidin-1-yl)pyrazolo[1,5-*a*]pyrimidine-3-carbonitrile **6b** showed a molecular ion at m/z = 257.20, which corresponds to the molecular formula $C_{13}H_{15}N_5O$ (257.29).

The diazotized 5-aminopyrazole is an excellent building block for the synthesis of the pyrazolo[5,1-*c*][1,2,4]triazine derivatives [20,21]. Thus, diazotization of 5-aminopyrazoles **2a**–**c** with sodium nitrite and concentrated HCl gave the corresponding diazonium chloride [A], which was coupled with different active methylene compounds, namely, acetylacetone and malononitrile in pyridine to afford the corresponding hydrazono derivatives B and C. When intermediates B and C were refluxed in glacial acetic acid, the target pyrazolo[5,1-*c*][1,2,4]triazine derivatives **7a**–**c** and **8a**–**c** were obtained (Scheme 7).

The mechanism for the formation of pyrazolo[5,1-*c*][1,2,4]triazines **7a**–**c** and **8a**–**c** is presented in Scheme 8. The first step involves the formation of diazotized 5-aminopyrazoles **2′a**–**c**. The second step involves the coupling off the **2′a**–**c** with active methylenes in acetylacetone and malononitrile, in pyridine from 0 °C to 5 °C to yield the corresponding hydrazono intermediates **A** and **B**. During the third step, intramolecular cyclization by nucleophilic attack of nitrogen occurs in the pyrazole ring on the acetyl and cyano groups to form intermediates **A′** and **B′**, followed by proton abstraction to generate intermediate adducts **A″** and **B″**. Finally, aromatic-driven 1,4-dehydration in **A″** forms **7a**–**c**; and 1,5-hydrogen migration in **B″** yields **8a**–**c**.

The structures of **7a**–**c** and **8a**–**c** were elucidated based on their elemental analyses and spectral data. The characteristic band in the

Table 1
Crystal data and structure refinement for **2a**.

Chemical formula	$C_8H_{11}N_5O$	Absorption coefficient	0.83 mm^{-1}
Formula weight	193.22 g mol^{-1}	$F(000)$	408
Color	Colorless	Theta range for data collection	4–71 (°)
Crystal shape	Block	Reflections collected/unique	3337, 3002, R_{int} = 0.023
Size	0.08 × 0.18 × 0.20 mm	Completeness to theta = 25.00	71.023
Temperature	293 K	Max. and min. transmission	T_{min} = 0.862, T_{max} = 0.936
Wavelength	1.54180 Å	Refinement method	Full-matrix least-squares on F^2
Crystal system	Triclinic	Data/restraints/parameters	3322/0/253
Space group	*P*–1 (No. 2)	Goodness-of-fit on F^2	1.11 Full-matrix least-squares on F^2
a, *b*, *c* (Å); α, β, γ (°)	8.5155(4), 10.1546(8), 11.8235(9); 81.712(6), 78.698(5), 66.513(6)	Final *R* indices [$I > 2\sigma(I)$]	R = 0.042, wR = 0.119
Cell volume	917.02(12)	Largest diff. peak and hole	0.24 & −0.28 e $Å^{-3}$
Z	4	Calculated density	1.400 g cm^{-3}

Table 2
Selected bond lengths (Å) and bond angles (°) for **2a**.

Bond	Bond length (Å)	Bond	Bond angle (°)
N2–N3	1.3913(18)	N21–C20–C19	178.64(17)
N3–C4	1.3253(18)	N16–N17–C18	104.70(12)
C1–C5	1.4137(19)	N16–C15–N28	124.17(14)
C5–C6	1.416(2)	N17–C18–C19	111.34(13)
N3–C4	1.3253(18)	C15–C19–C18	104.81(13)
N2–C1	1.333(2)	C18–C19–C20	130.12(15)
N7–C6	1.154(2)	N17–N16–C15	112.49(12)
C4–C5	1.424(2)	N2–N3–C4	104.48 (12)
N8–C9	1.459(2)	N3–C4–C5	111.59 (13)
C9–C10	1.514(2)	N3–C4–N8	119.98(14)
N16–N17	1.3914(17)	C5–C4–N8	128.42 (13)
N17–C18	1.322(2)	C4–C5–C1	104.64 (13)
N22–C18	1.3840(19)	C4–C5–C6	132.06(13)
C19–C20	1.414(2)	C1–C5–C6	123.11(14)
C15–C19	1.397(2)	C5–C6–N7	177.83 (16)
N16–C15	1.3411(19)	C4–N8–C9	113.79 (12)
N8–C13	1.466(2)	N17–N16–C15	112.49(12)
N8–C9	1.459(2)	N16–C15–C19	106.64(13)
O11–C12	1.423(2)	N17–C18–N22	122.64(13)
C12–C13	1.514(2)	N22–C18–C19	125.99(14)

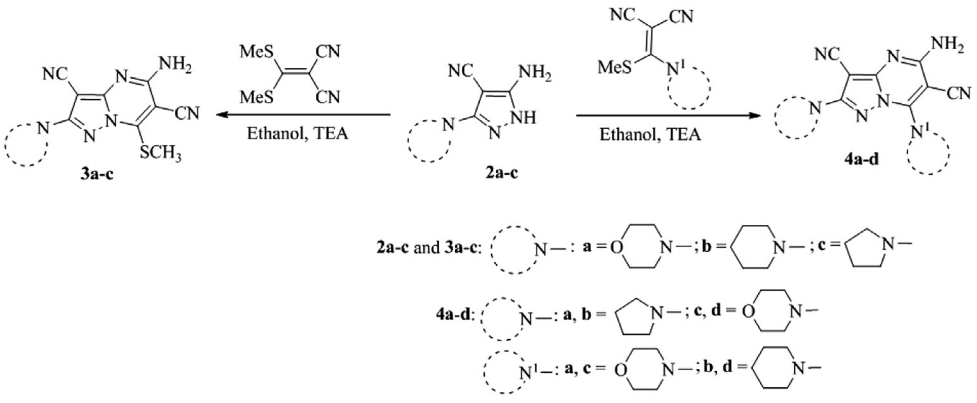
IR spectra of **7a**–**c** and **8a**–**c** is at ν = (1586–1591) and (1585–1598) cm^{-1} for N=N stretching vibrations. Furthermore, displayed bands at ν = (1695–1698) cm^{-1} in **7a**–**c** revealed the carbonyl group, whereas the bands at ν = (3378–3148) cm^{-1} in **8a**–**c** revealed the NH_2 group. The ^1H NMR spectra of **7a**–**c** displayed broad signals at δ = (2.77–3.09) ppm, which corresponds to six protons of two methyl groups. The ^1H NMR spectra of the **8a**–**c** displayed broad signals at δ = (9.16–10.12) ppm caused by the NH_2 group. Results from DIMS of **7a**–**c** showed molecular ions corresponding to the molecular formulas. For examples, the DIMS of 3-acetyl-4-methyl-7-morpholinopyrazolo[5,1-*c*][1,2,4]triazine-8-carbonitrile **7a** showed a molecular ion at m/z = 286.15, which corresponds to the molecular formula $C_{13}H_{14}N_6O_2$ (286.29). The intensity peak at m/z = 200 is due to 3-acetyl-4-methylpyrazolo[5,1-*c*][1,2,4]triazine-8-carbonitrile moiety. The DIMS of 4-amino-7-morpholinopyrazolo[5,1-*c*][1,2,4] triazine-3,8-dicarbonitrile **8a** showed a molecular ion at m/z = 270.15, which corresponds to the molecular formula $C_{11}H_{10}N_8O$ (270.25). The intensity peak at m/z = 212 is due to 4-amino-7-(methyleneamino)pyrazolo[5,1-*c*][1,2,4]triazine-3,8-dicarbonitrile moiety.

The structure of 4-amino-7-morpholinopyrazolo[5,1-*c*][1,2,4] triazine-3,8-dicarbonitrile **8a** was further identified by XRD. The *ORTEP* plot of **8a** and the numbering scheme are presented in Fig. 2. A suitable crystal of **8a** was grown by slow evaporation of DMSO solution of the compound for 72 h. The crystal and structure refinement data for **8a** are listed in Table 3. Compound **8a** crystallized in a monoclinic system with space group *P*21/*c* (No. 14). The asymmetric unit of **8a** contains two molecules, A and B, of the compound and two DMSO solvent molecules. The sulfur atom of one of the DMSO molecules is disordered over two positions, S401 and S402, with equal occupancies. Selected bond distances and bond angles are provided in Table 4. The N112–N113 bond length is 1.3245(19) Å in molecule A and the analog N212–N213 bond length is −1.3219(19) Å in the molecule B, which are longer than the normal N=N bond (1.255 Å). The C110–N109 and C111–N112 bond lengths in molecule A are 1.3515(19) and 1.350(2) Å, respectively. The analog C210–N209 and C211–N212 bond lengths in molecule B are 1.3522(19) and 1.344(2) Å, respectively. These bonds are shorter than the normal C–N bond (1.45 Å). In addition, the C111–C110 bond length in the molecule A is 1.423(2) Å, and the analog C211–C210 bond length in the molecule B is 1.421(2), which are longer than the normal C–C bond (1.34 Å) and close to the C–C single bond (1.45 Å). In addition, the C110–N118 bond length in molecule A is 1.308(2) Å and the analog C210–N218 bond length in molecule B is 1.313(2) Å, which are slightly shorter than the normal C–N bond (1.394 Å) [18]. This condition is due to the electron-donating effect of nitrogen N118 and N218 attached to C110 and C210, respectively, of the pyrimidine rings. The mean planes for **8a** were observed in the morpholines and pyrazolopyrimidine rings in molecules A and B, with a maximum deviation of −0.259(1)° and 0.258(1)° at the O104 and O204 atoms, respectively. The morpholine rings adopted chair conformation. They differed significantly in conformation, as shown by the dihedral angle in the mean planes of the morpholines and pyrazolopyrimidines. The dihedral angles between the mean planes of the morpholines and pyrazolopyrimidines in molecules A and B were 32.32(6)° and 27.54(6)°, respectively (see Electronic Supplementary information [22]).

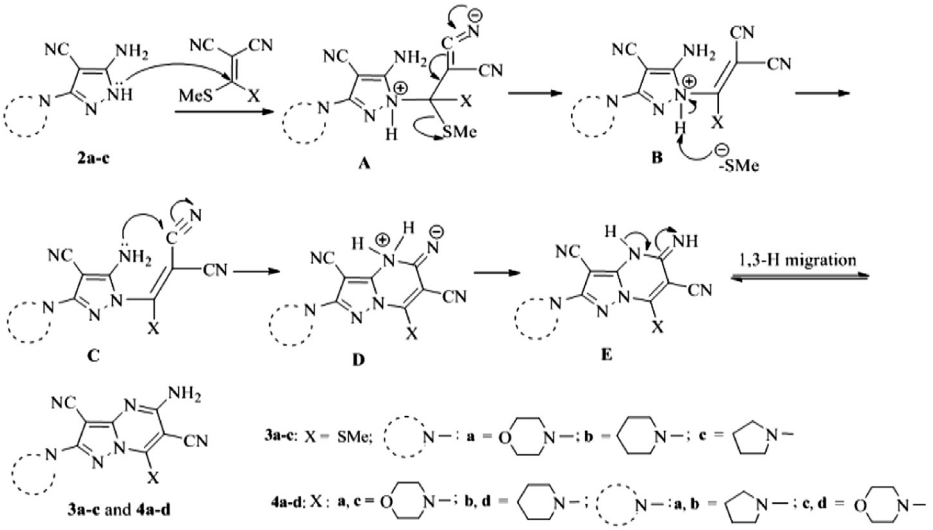
Cyclocondensation reaction of **2a**–**c** with 2,5-hexadione in boiling glacial acetic acid for 5 h produce the corresponding 5-pyrrolylpyrazoles **9a**–**c** as shown in (Scheme 9).

Presumably, the reaction mechanism for **9a**–**c** formation is illustrated in Scheme 10. It involves a nucleophilic attack of the NH_2 group in **2a**–**c** on the carbonyl carbon in 2,5-hexandione, eliminating water, to generate intermediate enamine **A**. Then, intramolecular cyclization by a nucleophilic attack of the incipient NH onto the other carbonyl group in **A** forms an intermediate adduct **B**, followed by dehydration to afford **9a**–**c**.

The structures of **9a**–**c** were established based on their elemental analyses and spectral data. The IR spectra showed the absence of amino group (NH_2) bands, whereas the ^1H NMR spectra showed characteristic signals between δ values of 2.11 ppm–2.12 ppm caused by the two methyl groups [$(CH_3–C=)_2$]. Results



Scheme 3. Synthesis of pyrazolo[1,5-*a*]pyrimidines **3a**–**c** and **4a**–**d**.



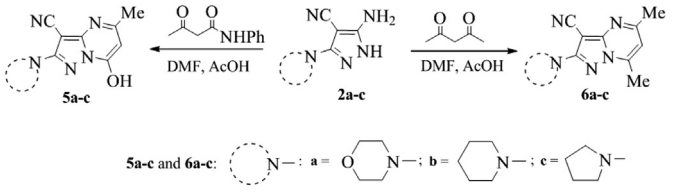
Scheme 4. Mechanism for the formation of pyrazolo[1,5-*a*]pyrimidines **3a**–**c** and **4a**–**d**.

from DIMS of 5-pyrrolylpyrazoles **9a**–**c** showed molecular ions corresponding to the molecular formulas. For example, the DIMS spectrum of 5-(2,5-dimethyl-1*H*-pyrrol-1-yl)-3-morpholino-1*H*-pyrazole-4-carbonitrile **9a** showed a molecular ion at $m/z = 271.20$, which corresponds to a molecular formula $C_{14}H_{17}N_5O$ (271.32).

Fusion of **2a**–**c** with acetic anhydride under reflux for 15 min furnished the corresponding 1-acetyl-5-amino-3-morpholino-1*H*-pyrazole-4-carbonitrile **10a**, 1-acetyl-5-amino-3-(piperidin-1-yl)-1*H*-pyrazole-4-carbonitrile **10b**, and 1-acetyl-5-amino-3-(pyrrolidin-1-yl)-1*H*-pyrazole-4-carbonitrile **10c**, as shown in (Scheme 11).

The structures of **10a**–**c** were established based on the elemental analyses and spectral data. The IR spectra showed the absence of NH bands and displayed bands at ν values between 1702 cm^{-1} and 1707 cm^{-1} caused by the carbonyl group. The ^1H NMR spectra showed characteristic signals at δ values between 1.94 ppm and 2.41 ppm caused by the methyl group attached to carbonyl group (CH_3—CO). Results from the DIMS of **10a–c** showed molecular ions corresponding to the molecular formulas. For example, the DIMS spectrum of 1-acetyl-5-amino-3-morpholino-1*H*-pyrazole-4-carbonitrile **10a** showed a molecular ion at $m/z = 235.25$, which corresponds to a molecular formula $C_{10}H_{13}N_5O_2$ (235.24).

In general, the electronic spectra of all the new synthesized compounds were recorded. The wavelength maxima of the principle absorption peaks in the electronic spectra for compounds with non-fused aromatic parent structure (**2a**–**c**, **9a**–**c**, and **10a**–**c**) occur between 205 and 281 nm whereas their fused aromatic parent structure counter parents (**3a–c**, **4a–d**, **5a–c**, **6a–c**, **7a–c** and **8a–c**) occur between 209 and 306 nm.

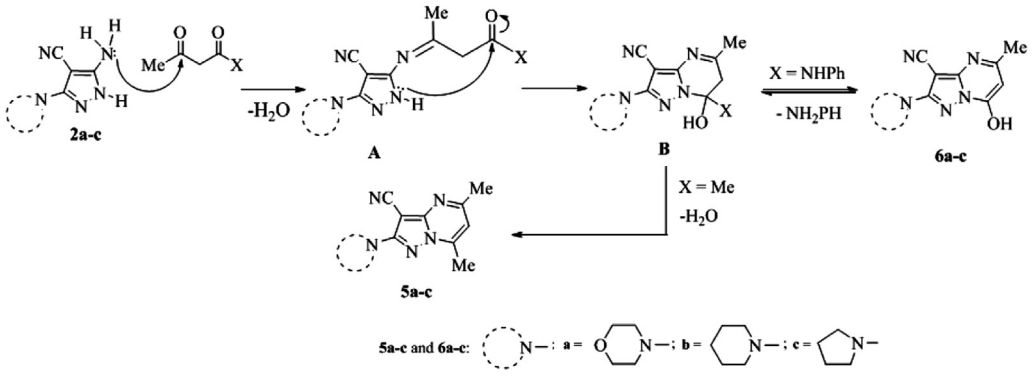


Scheme 5. Synthesis of pyrazolo[1,5-*a*]pyrimidines **5a**–**c** and **6a**–**c**.

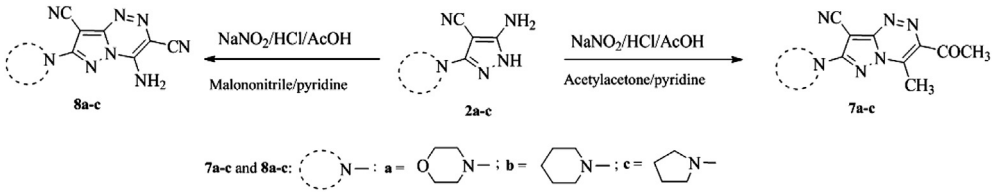
3. Antibacterial evaluation and cytotoxicity assay

3.1. Antibacterial evaluation

The antibacterial activity of the 27 new synthesized compounds was evaluated using the agar diffusion technique [23]. The compounds were prepared and tested at 1 mg/ml concentration in dimethylsulfoxide (DMSO). The tested organisms were Gram-positive bacteria [*Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 11774, Methicillin-resistant *S. aureus* (MRSA) ATCC 43300, *Staphylococcus epidermidis* ATCC 12228, and *Enterococcus faecalis* ATCC 14506] and



Scheme 6. Mechanism for the formation of pyrazolo[1,5-*a*]pyrimidines **5a**–**c** and **6a**–**c**.



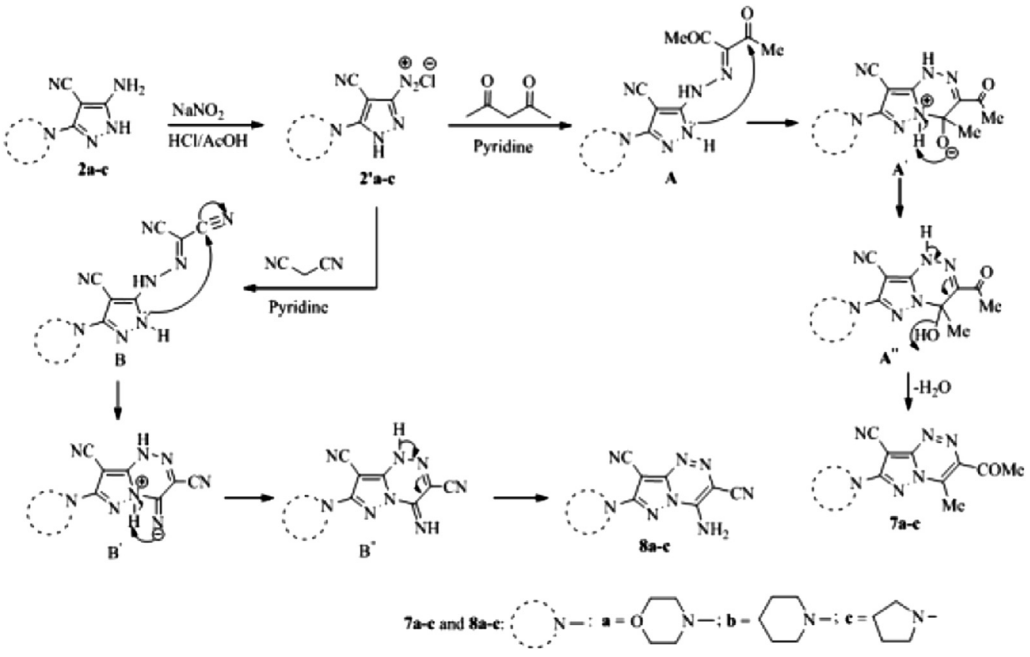
Scheme 7. Synthesis of pyrazolo[5,1-*c*][1,2,4]triazines **7a**–**c** and **8a**–**c**.

Gram-negative bacteria [*Escherichia coli* ATCC 10536, *Pseudomonas aeruginosa* ATCC 10145, *Serratia marcescens* ATCC 13880, and *Salmonella typhimurium* ATCC 51812]. The bacteria were maintained in nutrient agar and Mueller–Hinton agar media, respectively. After 24 h incubation at 37 °C, the diameter (mm) of the inhibition zones was measured. DMSO showed no inhibition zones. Streptomycin (ST) in a concentration of 10 μg was used as reference for antibacterial activities. Minimum inhibitory concentration (MIC) and minimum bacteriocidal concentration (MBC) were determined according to NCCLS (2000) [24] for compounds that showed inhibitory zones in the agar diffusion assay. MIC values were determined as the minimum concentrations that inhibited visible growth of the bacteria, and MBC values were recorded as the lowest concentrations that showed no growth on plate. Only gentamicin was tested for the MIC.

The antibacterial activities are listed in Table 5. The results for 5-aminopyrazoles **2a**–**c** showed 3-amino-5-(piperidin-1-yl)-1*H*-pyrazole-4-carbonitrile **2b** exhibited higher antibacterial activity against *S. aureus* and *E. coli* compared with the other 5-aminopyrazoles **2a** and **2c**. For antibacterial activity in pyrazolo[1,5-*a*]pyrimidines (**3a**–**c**, **4a**–**d**, **5a**–**c**, and **6a**–**c**) and pyrazolo[5,1-*c*][1,2,4]triazines (**7a**–**c** and **8a**–**c**), only **3b**, **5b**, **6a**, **7b**, and **8b** exhibited moderate activities against some of the bacteria tested. Compounds **9a**–**c** and **10a**–**c** also exhibited good antibacterial activity. Compounds 3-(2,5-dimethyl-1*H*-pyrrol-1-yl)-5-morpholino-1*H*-pyrazole-4-carbonitrile **9a** and 3-(2,5-dimethyl-1*H*-pyrrol-1-yl)-5-(piperidin-1-yl)-1*H*-pyrazole-4-carbonitrile **9b** showed pronounced antibacterial activity against *B. subtilis* than 5-(2,5-dimethyl-1*H*-pyrrol-1-yl)-3-(pyrrolidin-1-yl)-1*H*-pyrazole-4-carbonitrile **9c**, but lower than the reference drug streptomycin. For 1-acetyl-5-amino-3-(piperidin-1-yl)-1*H*-pyrazole-4-carbonitrile **10b**, greater inhibitory activity toward *B. subtilis*, *S. aureus*, and *E. coli* was shown compared with 1-acetyl-5-amino-3-(pyrrolidin-1-yl)-1*H*-pyrazole-4-carbonitrile **10c**. The antibacterial activity may be attributed to the aromatic parent structures of the tested compounds. Compounds **2a**–**c**, **9a**–**c**, and **10a**–**c** with non-fused aromatic parent structure displayed evidently higher antibacterial activity than those with fused aromatic parent structure (**3a**–**b**, **4a**–**d**, **5a**–**b**, **6a**–**b**, **7a**–**c**, and **8a**–**c**). The presence of piperidine ring in a structure has been reported to increase antibacterial capability of the compounds [25] and have been demonstrated in this study in **2b**, **3b**, **9b**, **5b**, and **10b**.

The compounds were inactive against several tested bacteria including MRSA, *S. epidermidis*, *E. faecalis*, and *S. typhimurium* by agar diffusion method. The activity of the compounds varies with the type of bacteria tested and nature of the compounds. Some of the compounds showed limited inhibition as a result of their inability to diffuse through the agar. Thus, they must be tested further by broth dilution method to determine the MIC. The antibacterial activity in this study is also probably influenced by the polarity of the compounds. Derivatizations to less polar compounds usually increase the antimicrobial and cytotoxic effects and reduce mutagenicity, in contrast to the effect of introducing hydroxyl group [26].

However, in this study, the MIC values for all compounds were greater than 1 mg/ml. Thus, the compounds cannot be considered as good lead compounds for antibacterial drug compared with the MIC value for positive control (gentamicin, 1 μg/mL) and MBC (2 μg/mL) against *P. aeruginosa*.



Scheme 8. Mechanism for the formation of pyrazolo[5,1-*c*][1,2,4]triazines **7a**–**c** and **8a**–**c**.

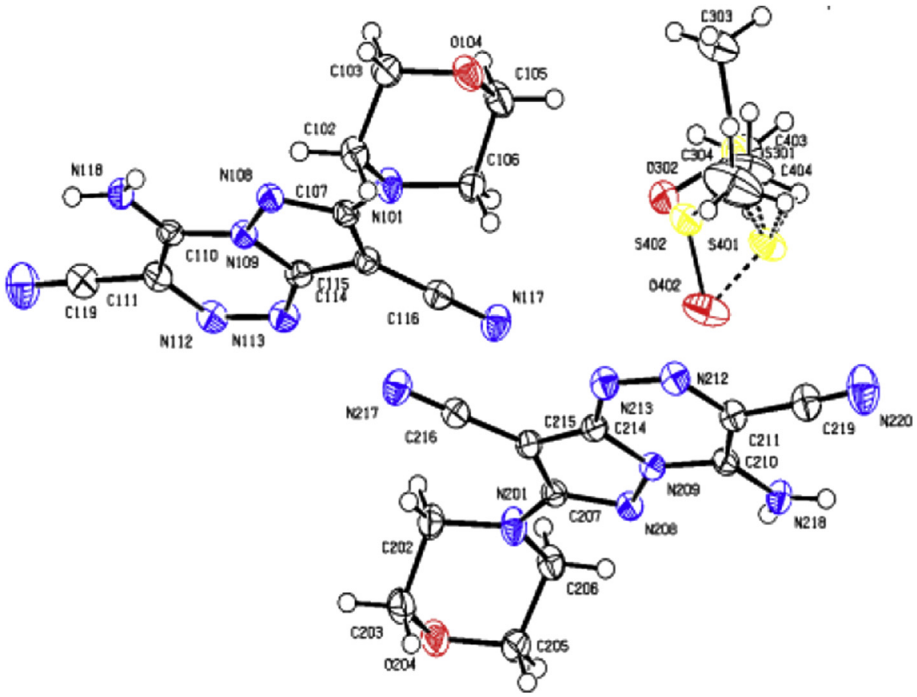


Fig. 2. Single-crystal X-ray analysis. *ORTEP* view of **8a** showing the atom numbering scheme.

3.2. Cytotoxicity assay

The isolated compounds were tested for *in vitro* cytotoxicity using Vero cells by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay [27]. The concentration of stock compound solution was 1.0 mg/ml prepared by dissolving 1 mg compound in 50 μL DMSO and 950 μL 5% FBS-DMEM. A sonicator was used to emulsify the compounds for 40 min before the two-fold dilutions made in 5% FBS-DMEM to produce a compound solution with concentrations of 0.5, 0.25, 0125 and 0.0625 mg/ml. The absorbance for each well was measured at 540 nm in a micro-titer plate reader and percentage of cell viability (CV) was calculated manually using the formula: $CV = [(Abs_{sample} - Abs_{negative\ control})/(Abs_{cell} - Abs_{negative\ control})] \times 100$. A dose–response curve was plotted to enable the calculation of the concentration that kills 50% of the Vero cells (CC_{50}). Selectivity indices were calculated as $SI = IC_{50}/MIC$.

The cytotoxicity evaluation results of the tested compounds as CC_{50} values are shown in Table 6. The compounds were considered safe when their CC_{50}s are higher than 20 μg/ml [28]. However, in terms of selectivity, all the tested compounds have SI less than 10. SI values greater than 10 indicate the therapeutic safety and effectiveness of these drugs as good candidates as antibacterial agents.

4. Experimental section

4.1. Instrumentation

All melting points were determined using a hot stage Gallenkamp melting point apparatus. Infrared spectra were recorded from KBr discs on FT-IR 8300 Shimadzu spectrometer. ^{1}H NMR and ^{13}C NMR spectra were recorded on FT-NMR 400 MHz Joel, ECP, JNM-ECX 500 MHz, Joel and FT-NMR 600 MHz Bruker, AVANCE III spectrometer operating at 400 MHz, 500 MHz and 600 MHz for ^{1}H NMR and at 100 MHz, 125 MHz, 150 MHz for ^{13}C NMR in DMSO-d_6, CD_3CL and AcOH as solvents and using TMS as internal standard. DIMS spectra were recorded on QP5050A Shimadzu apparatus. Elemental analyses of all new compounds were performed on Thermo Finnigan EA1112. Electronic spectra were recorded on a UV-2450 version UV–VIS spectrophotometer and inflections are identified by the abbreviation 'inf'. Single-crystal X-ray diffraction data (XRD) were collected at room temperature with Bruker APEXII CCD a spectrometer. General purpose silica gel of Merck No. 5545 with UV indicator were used in TLC experiments to monitor completion of reactions, in which DCM was used as eluent.

Table 3
Crystal data and structure refinement for **8a**.

Chemical formula	$C_{26}H_{32}N_{16}O_4S_1$	Absorption coefficient	2.00 mm^{-1}
Formula weight	695.83 g mol^{-1}	F(000)	1454.110
Color	Colorless	Theta range for data collection	71.5–3.5 (°)
Crystal shape	Plate-like	Reflections collected/unique	16,257, $R_{int} = 0.000$
Size	0.09 × 0.14 × 0.18 (mm)	Completeness to theta = 25.00	0.99
Temperature	150 K	Max. and min. transmission	$T_{min} = 0.76$, $T_{max} = 0.84$
Wavelength	1.54180 Å	Refinement method	Full-matrix least-squares on F^2
Crystal system	Monoclinic	Data/restraints/parameters	6227/0/444
Space group	$P21/c$ (No. 14)	Goodness-of-fit on F^2	0.98
a, b, c (Å); α, β, γ (°)	8.92233(15), 14.9672(2), 24.4778(4); 90, 98.9113(15), 90	Final R indices [$I > 2\sigma$ (I)]	$R = 0.038$, $wR = 0.101$
Cell volume	3229.37(9) Å^3	Largest diff. peak and hole	0.44–0.31 e Å^{-3}
Z	4	Calculated density	1.431 g cm^{-3}

Table 4
Selected bond lengths (Å) and bond angles (°) for **8a**.

Bond	Bond length (Å)	Bond	Bond length (Å)	Bond	Bond angle (°)
N101–C102	1.464(2)	N201–C202	1.455(2)	N101–C107–N108	119.15(13)
N101–C106	1.456(2)	N201–C206	1.466(2)	N101–C107–C115	129.46(14)
N101–C107	1.344(2)	N201–C207	1.344(2)	N108–C107–C115	111.39(13)
C102–C103	1.509(2)	C202–C203	1.512(2	C107–N108–N109	104.11(12)
C103–O104	1.428(2)	C203–O204	1.420(2)	N108–N109–C110	123.32(12)
O104–C105	1.425(2)	O204–C205	1.432(2)	N108–N109–C114	114.02(12)
C105–C106	1.518(2)	C205–C206	1.512(2)	C110–N109–C114	122.66(13)
C107–N108	1.3461(19)	C207–N208	1.3460(19)	N109–C110–C111	111.96(13)
C107–C115	1.437(2)	C207–C215	1.437(2)	N109–C110–N118	119.15(14)
N108–N109	1.3626(17)	N208–N209	1.3636(17)	N101–C107–N108	119.15(13)
C110–N109	1.3515(19)	C210–N209	1.3522(19)	N101–C107–C115	129.46(14)
N109–C114	1.3747(19)	N209–C214	1.3729(19)	N201–C207–N208	119.21(14)
C111–C110	1.423(2)	C211–C210	1.421(2)	N201–C207–C215	129.33(14)
C110–N118	1.308(2)	C210–N218	1.313(2)	N208–C207–C215	111.45(13)
C111–N112	1.350(2)	C211–N212	1.344(2)	C207–N208–N209	104.09(12)
C111–C119	1.436(2)	C211–C219	1.436(2)	N208–N209–C210	123.90(12)
N112–N113	1.3245(19)	N212–N213	−1.3219(19)	N208–N209–C214	113.78(12)
N113–C114	1.354(2)	N213–C214	1.351(2)	C210–N209–C214	122.22(13)
C114–C115	1.396(2)	C214–C215	1.393(2)	N209–C210–C211	111.99(13)
C115–C116	1.416(2)	C215–C216	1.419(2)	N209–C210–N218	119.56(14)
C116–N117	1.147(2)	C216–N217	1.149(2)	C211–C210–N218	128.45(14)
C119–N120	1.145(2)	C219–N220	1.142(2)	C210–C211–N212	124.76(14)

4.2. General procedure for the synthesis of 5-aminopyrazoles **2a–c**

A mixture of α,α-dicyanoketene-*N*,*S*-acetals **1a–c** (4 mmol) and hydrazine hydrate (1 g, 20 mmol) was refluxed on water-bath for 2 h. Then, 20 mL of ethanol was added, and the reaction mixture was refluxed for further 2 h. The solvent was evaporated and the product was collected, washed with ethanol, dried and recrystallized from methanol to give pure products **2a–c**.

4.2.1. 5-Amino-3-morpholino-1H-pyrazole-4-carbonitrile (**2a**)

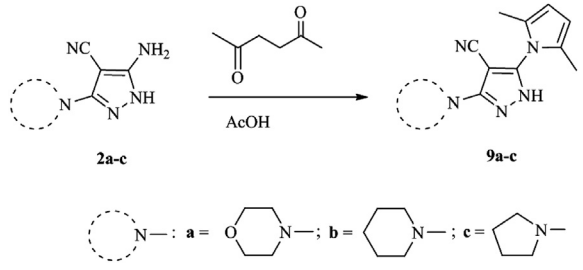
Yield, 97%; colorless crystals; mp 191–192 °C; FT-IR (KBr, cm^{-1}) *ν*: 3442, 3394, 3247 (NH_2/NH), 2196 (CN), 1641, 1600, 1548 (C=C/C=N); ^{1}H NMR (500 MHz, DMSO-d_6) *δ*: 3.05 (br, 4H, N(C<u>H</u>$_2$)$_2$(CH$_2$)$_2$O), 3.59 (t, 4H, N(CH$_2$)$_2$(C<u>H</u>$_2$)$_2$O, *J* = 4.6 Hz), 6.11 (s, 2H, NH_2), 11.00 (s, 1H, pyrazole, NH); ^{13}C NMR (125 MHz, DMSO-d_6) *δ*: 48.6 N(<u>C</u>H$_2$)$_2$(CH$_2$)$_2$O, 62.6 (<u>C</u>–CN), 66.1 N(CH$_2$)$_2$(<u>C</u>H$_2$)$_2$O, 116.9 (CN), 154.6 (C=N), 158.0 (C–NH_2); λ_{max} (MeOH)/nm 211 (log *ε* 4.33), 244 inf (4.05), 264 inf (3.61); DIMS found *m*/*z*: 193.25 (calc. for $C_8H_{11}N_5O$ M^+ requires 193.21); Anal. Calcd. for $C_8H_{11}N_5O$: C 49.73, H 5.74, N 36.25%; found: C 50.06, H 5.46, N 36.34%.

4.2.2. 5-Amino-3-(piperidin-1-yl)-1H-pyrazole-4-carbonitrile (**2b**)

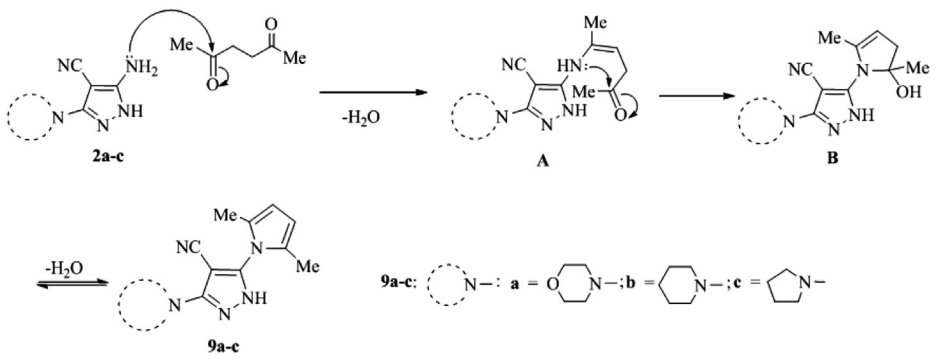
Yield, 88%; colorless crystals; mp 140–142 °C; FT-IR (KBr, cm^{-1}) *ν*: 3435, 3344, 3225 (NH_2/NH), 2193 (CN), 1637 (C=C), 1600 (C=N); ^{1}H NMR (500 MHz, DMSO-d_6) *δ*: 1.50 (br. s, 6H, N(CH$_2$)$_2$(C<u>H</u>$_2$)$_3$), 3.12 (br. s, 4H, N(C<u>H</u>$_2$)$_2$(CH$_2$)$_3$), 6.11 (s, 2H, NH_2), 10.97 (s, 1H, pyrazole, NH); ^{13}C NMR (125 MHz, DMSO-d_6) *δ*: 24.4 (N(CH$_2$)$_2$<u>C</u>H$_2$(CH$_2$)$_2$), 25.3 (N(CH$_2$)$_2$CH$_2$(<u>C</u>H$_2$)$_2$), 49.1 (N(<u>C</u>H$_2$)$_2$CH$_2$(CH$_2$)$_2$), 62.7 (<u>C</u>–CN), 117.2 (CN), 154.5 (C=N), 158.5 (C–NH_2); λ_{max} (MeOH)/nm 212 (log *ε* 4.36), 242 inf (4.10), 263 inf (3.62); DIMS found *m*/*z*: 191.20 (calc. for $C_9H_{13}N_5$ M^+ requires 191.23); Anal. Calcd. for $C_9H_{13}N_5$: C 56.53, H 6.85, N 36.62%; found: C 56.53, H 6.52, N 36.96%.

4.2.3. 5-Amino-3-(pyrrolidin-1-yl)-1H-pyrazole-4-carbonitrile (**2c**)

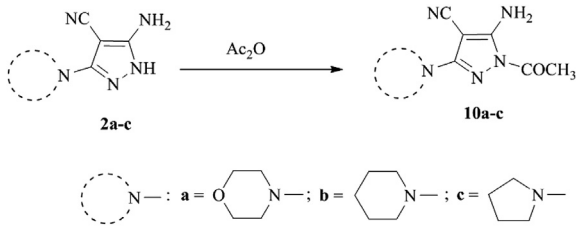
Yield, 87%; colorless crystals; mp 211–212 °C; FT-IR (KBr, cm^{-1}) *ν*: 3440, 3356, 3222 (NH_2/NH), 2189 (CN), 1665, 1613, 1565 (C=C/C=N); ^{1}H NMR (500 MHz, DMSO-d_6) *δ*: 1.76 (s br, 4H,



Scheme 9. Synthesis of 5-pyrrolylpyrazoles **9a–c**.



Scheme 10. Mechanism for the formation of 5-pyrrolylpyrazoles **9a–c**.



Scheme 11. Synthesis of 1-acetyl-5-amino-1*H*-pyrazoles **10a**–**c**.

N(C<u>H</u>$_2$)$_2$(CH$_2$)$_2$), 3.20 (s br, 4H, N(CH$_2$)$_2$(C<u>H</u>$_2$)$_2$), 5.93 (br, 2H, NH$_2$), 10.74 (s, 1H, pyrazole, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 24.8 (N(CH$_2$)$_2$(<u>C</u>H$_2$)$_2$), 47.9 (N(<u>C</u>H$_2$)$_2$(CH$_2$)$_2$), 61.1 (<u>C</u>–CN), 117.1 (CN), 154.5 (C=N), 158.6 (C–NH$_2$); λ_{max} (MeOH)/nm 205 (log ε 4.15), 245 (4.01), 281 (4.20); DIMS found *m*/*z*: 177.20 (calc. for $C_8H_{11}N_5$ M$^+$ requires 177.21); Anal. Calcd. for $C_8H_{11}N_5$: C 54.22, H 6.26, N 39.52%; found: C 54.35, H 5.89, N 39.67%.

4.3. General procedure for the preparation of pyrazolo[1,5-a] pyrimidines **3a**–**c** from the reaction of 5-aminopyrazoles **2a**–**c** with 2-[bis(methylthio)methylene]malononitrile

Pyrazolo[1,5-*a*]pyrimidines **3a**–**c** were prepared according to the literature procedure of Zaharan et al. 2001 [29] as follows: to a solution of 5-aminopyrazoles **2a**–**c** (4 mmol) in absolute ethanol (20 mL), 2-[bis(methylthio)methylene]malononitrile (0.86 g; 4 mmol) and three drops of triethylamine were added. The reaction mixture was refluxed for 5 h. The resulting precipitate was filtered off, dried and crystallized from EtOH:DMF to give pure products **3a**–**c**.

4.3.1. 5-Amino-7-(methylthio)-2-morpholinopyrazolo[1,5-a] pyrimidine-3,6-dicarbonitrile (**3a**)

Yield, 43%; yellow solid; mp >315 °C; IR (KBr, cm^{-1}) ν: 3322 (NH$_2$), 2216 (CN), 1506 (C=C/C=N); ^{1}H NMR (600 MHz, DMSO-d_6) δ: 2.89 (s, 3H, SCH$_3$), 3.48 (t, 4H, N(C<u>H</u>$_2$)$_2$(CH$_2$)$_2$O, J = 4.5 Hz), 3.73 (t, 4H, N(CH$_2$)$_2$(C<u>H</u>$_2$)$_2$O, J = 4.5 Hz), 7.80 (br, 2H, NH$_2$); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 16.6 (SCH$_3$), 47.2 (N(<u>C</u>H$_2$)$_2$(CH$_2$)$_2$O), 62.4 (<u>C</u>–CN), 66.0 (N(CH$_2$)$_2$(<u>C</u>H$_2$)$_2$O), 93.1 (<u>C</u>–CN), 117.0, 117.6 (2CN), 151.0 (=C–N), 156.9 (N–C=N), 160.4 (<u>C</u>–NH$_2$), 164.8 (C–S); λ_{max} (MeOH)/nm 266 (log ε 3.96), 271 (4.60), 346 (3.82); DIMS found *m*/*z*: 315.20 (calc. for $C_{13}H_{13}N_7OS$ M$^+$ requires 315.35); Anal. Calcd. for $C_{13}H_{13}N_7OS$: C 49.51, H 4.16, N, 31.09%; found: C 49.34, H 4.47, N 31.05%.

4.3.2. 5-Amino-7-(methylthio)-2-(piperidin-1-yl)pyrazolo[1,5-a] pyrimidine-3,6-dicarbonitrile (**3b**)

Yield, 34%; green solid; mp 290–291 °C; IR (KBr, cm^{-1}) ν: 3457, 3325, 3150 (NH$_2$), 2208 (CN), 1597 (C=C/C=N); ^{1}H NMR (600 MHz, DMSO-d_6) δ: 1.60 (s, 6H, N(CH$_2$)$_2$(C<u>H</u>$_2$)$_3$), 2.89 (s, 3H, SCH$_3$), 3.51 (s, 4H, N(C<u>H</u>$_2$)$_2$(CH$_2$)$_3$), 7.76 (br, 2H, NH$_2$); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 16.5 (SCH$_3$), 23.8, 24.8 (N(CH$_2$)$_2$(<u>C</u>H$_2$)$_2$<u>C</u>H$_2$), 47.3 (N(<u>C</u>H$_2$)$_2$(CH$_2$)$_3$), 65.2 (<u>C</u>–CN), 83.4 (<u>C</u>–CN), 114.4, 115.3 (2CN), 152.7 (N–C–N), 153.7 (C=N), 157.1 (C–NH$_2$), 160.0 (<u>C</u>–SCH$_3$); λ_{max} (MeOH)/nm 230 (log ε 3.97), 274 (4.54); DIMS found *m*/*z*: 313.15 (calc. for $C_{14}H_{15}N_7S$ M$^+$ requires 313.38); Anal. Calcd. for $C_{14}H_{15}N_7S$: C 53.66, H 4.82, N 31.29%; found: C 53.42, H 4.74, N 30.98%.

4.3.3. 5-Amino-7-(methylthio)-2-(pyrrolidin-1-yl)pyrazolo[1,5-a] pyrimidine-3,6-dicarbonitrile (**3c**)

Yield, 32%; green solid; mp >320 °C; IR (KBr, cm^{-1}) ν: 3455, 3416, 3317, 3127 (NH$_2$), 2206 (CN), 1587 (C=C/C=N); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.92 (s, 4H, N(CH$_2$)$_2$(C<u>H</u>$_2$)$_2$), 2.88 (s, 3H, SCH$_3$), 3.43 (s, 4H, N(C<u>H</u>$_2$)$_2$(CH$_2$)$_2$, DMSO exchangeable), 7.66 (s, 2H, NH$_2$); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 16.3 (SCH$_3$), 25.0 (3CH$_2$), 47.7 (CH$_2$–N), 64.4 (<u>C</u>–CN), 82.7 (<u>C</u>–CN), 114.5, 115.4 (2CN), 152.1 (N–C–N), 153.4 (C=N), 157.1 (C–NH$_2$), 158.0 (<u>C</u>–SCH$_3$); λ_{max} (MeOH)/nm 213 (log ε 4.14), 230 (4.61), 269 (4.17); DIMS found *m*/*z*: 299.10 (calc. for $C_{13}H_{13}N_7S$ M$^+$ requires: 299.10); Anal. Calcd. for $C_{13}H_{13}N_7S$: C 52.16, H 4.38, N 32.75%; found: C 52.19, H 4.29, N 32.71%.

4.4. General procedure for the preparation of pyrazolo[1,5-a] pyrimidines **4a**–**d** from the reaction of 5-aminopyrazoles **2a**,**c** with α,α-dicyanoketene-N,S-acetals **1a**,**b**

Pyrazolo[1,5-*a*]pyrimidines **4a**–**d** were prepared according to the literature procedure of Zaharan et al. 2001 [29] as follows: to a

Table 5
Inhibition zone (mean diameter of inhibition in mm), minimum inhibition concentration (MIC), and minimum bactericidal concentration (MBC) (in mg/ml) of some newly synthesized compounds.

Compound	Gram-positive bacteria						Gram-negative bacteria								
	Bacillus subtilis			*Staphylococcus aureus*			*Escherichia coli*			*Pseudomonas aeruginosa*			*Serratia marcescens*		
	Inhibition zone (mm)	MIC	MBC	Inhibition zone (mm)	MIC	MBC	Inhibition zone (mm)	MIC	MBC	Inhibition zone (mm)	MIC	MBC	Inhibition zone (mm)	MIC	MBC
2a	6 ± 0.56[a]	16	16	11 ± 0.57	16	>16	11 ± 0.57	8	16	7 ± 0.57	8	16	6 ± 0.57	8	8
2b	9 ± 0.55	4	8	15 ± 0.58	16	16	15 ± 0.56	4	8	7 ± 0.57	8	8	6 ± 0.57	2	4
2c	9 ± 0.57	8	8	10 ± 0.57	16	16	9 ± 0.55	8	16	7 ± 0.57	8	16	7 ± 0.00	8	8
3b	10 ± 0.57	16	16	11 ± 0.57	16	16	10 ± 0.55	16	16	7 ± 1.15	8	16	7 ± 1.00	16	16
5b	10 ± 0.58	16	16	12 ± 0.58	16	16	11 ± 0.58	16	16	6 ± 0.00	NT	NT	7 ± 1.15	16	16
6a	9 ± 0.58	8	16	9 ± 0.55	16	16	10 ± 0.57	16	>16	7 ± 0.57	8	>16	6 ± 0.00	NT	NT
7b	10 ± 0.57	16	16	9 ± 0.62	16	16	8 ± 0.57	16	16	6 ± 0.00	NT	NT	6 ± 0.00	NT	NT
8b	11 ± 0.58	16	16	11 ± 0.58	16	16	10 ± 0.58	16	16	7 ± 1.15	16	16	6 ± 0.00	NT	NT
9a	14 ± 0.58	2	4	10 ± 0.58	2	4	10 ± 0.58	4	8	6 ± 0.00	NT	NT	6 ± 0.00	NT	NT
9b	16 ± 0.58	4	8	12 ± 0.58	8	8	7 ± 0.58	8	8	7 ± 0.57	4	8	6 ± 0.00	NT	NT
9c	8 ± 0.58	4	8	7 ± 0.58	8	8	13 ± 0.58	8	16	6 ± 0.00	NT	NT	6 ± 0.00	NT	NT
10b	6 ± 0.57	16	16	10 ± 0.58	16	>16	10 ± 0.58	16	16	6 ± 0.00	NT	NT	6 ± 0.00	NT	NT
10c	14 ± 0.58	16	16	14 ± 0.58	16	>16	16 ± 0.58	8	16	6 ± 0.00	NT	NT	6 ± 0.00	NT	NT
Reference drug	25 ± 0.28[b]			25 ± 0.28[b]			13 ± 0.58[c]			14 ± 0.57[c]			20 ± 0.57[a]		

6 mm is the diameter of the disc.
NT Not tested.
MIC value for positive control (Gentamicin) was 1 μg/mL and MBC was 2 μg/mL against *Pseudomonas aeruginosa*.
[a] Values are mean inhibition zone (mm) ± S.D of results done in triplicate.
[b] Chloramphenicol is the antibiotic used as reference drug against Gram-positive bacteria.
[c] Streptomycin is the antibiotic used as reference drug against Gram-negative bacteria.

Table 6
CC_{50} values and selective index (SI) of tested compounds. ND = not determined as the MIC is not tested.

Compound	CC_{50} (mg/ml)	Selective index				
		Bacillus subtilis	*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Serratia marcescens*
2a	2.34	0.15	0.29	0.29	0.29	0.29
2b	0.55	0.14	0.14	0.14	0.07	0.28
2c	2.5	0.31	0.31	0.31	0.31	0.31
3b	1.75	0.11	0.11	0.11	0.22	0.11
5b	1.25	0.08	0.08	0.08	ND	0.08
6a	0.063	0.01	0.00	0.00	0.01	ND
7b	0.3125	0.02	0.02	0.02	ND	ND
8b	1.4	0.09	0.09	0.09	0.09	ND
9a	0.254	0.13	0.06	0.06	ND	ND
9b	1.4	0.35	0.18	0.18	0.35	ND
9c	0.55	0.14	0.07	0.07	ND	ND
10b	1.015	0.06	0.13	0.13	ND	ND
10c	2.812	0.18	0.18	0.18	ND	ND

solution of 5-aminopyrazoles **2a** and **2c** (4 mmol) in absolute ethanol (20 mL), 2-[methylthio(morpholino)methylene]malononitrile **1a** (0.84 g; 4 mmol) or 2-[methylthio(piperidin-1-yl)methylene]malononitrile **1b** (0.83 g; 4 mmol) and three drops of triethylamine were added. The reaction mixture was refluxed for 48 h. The resulting precipitate was filtered off, dried and crystallized from EtOH:DMF to give pure products **4a**–**d**.

4.4.1. 5-Amino-7-morpholino-2-(pyrrolidin-1-yl)pyrazolo[1,5-a] pyrimidine-3,6-dicarbonitrile (**4a**)

Yield, 47%; green solid; mp >272–270 °C; IR (KBr, cm^{-1}) ν: 3432 (br, NH_2), 2176 (CN), 1621, 1567 (C=C/C=N); ^{1}H NMR (600 MHz, DMSO-d_6) δ: 1.91 (t, 4H, $N(CH_2)_2(\underline{CH_2})_2$, J = 5.6 Hz), 3.39 (t, 4H, $N(\underline{CH_2})_2(CH_2)_2$, J = 5.6 Hz), 3.45 (t, 4H, $N(\underline{CH_2})_2(CH_2)_2O$, J = 5.3 Hz), 3.66 (t, 4H, $N(CH_2)_2(\underline{CH_2})_2O$, J = 5.3 Hz), 7.38 (s, 2H, NH_2); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 25.4 ($N(CH_2)_2(\underline{CH_2})_2$), 47.9 ($N(\underline{CH_2})_2(CH_2)_2$), 50.4, 50.7 ($N(\underline{CH_2})_2(CH_2)_2O$), 66.2 ($N(CH_2)_2(\underline{CH_2})_2O$), 66.8 (C–CN), 68.8 (C–CN), 116.6, 119.2 (2CN), 151.3 (N–C–N), 155.7 (C=N), 157.4 (C–NH_2), 160.2 (=C–N); λ_{max} (MeOH)/nm 226 (log ε 2.99), 276 (2.04); Anal. Calcd. for $C_{16}H_{18}N_8O$: C 56.79, H 5.36, N 33.12%; found: C 57.2, H 5.29, N 33.50%.

4.4.2. 5-Amino-7-(piperidin-1-yl)-2-(pyrrolidin-1-yl)pyrazolo[1,5-a]pyrimidine-3,6-dicarbonitrile (**4b**)

Yield, 46%; green solid; mp >300 °C; IR (KBr, cm^{-1}) ν: 3467, 3305, 3148 (NH_2), 2945, 2863, 2207 (CN), 1642, 1600, 1582 (C=C/C=N); ^{1}H NMR (600 MHz, DMSO-d_6) δ: 1.66 (br. d, 4H, $N(CH_2)_2(\underline{CH_2})_2CH_2$, J = 4.5 Hz), 1.69 (br. d, 2H, $N(CH_2)_2(CH_2)_2\underline{CH_2}$, J = 4.5 Hz), 1.91 (t, 4H, $N(CH_2)_2(\underline{CH_2})_2$, J = 6.5 Hz), 3.46 (t, 4H, $N(\underline{CH_2})_2(CH_2)_2$, J = 6.5 Hz), 3.68 (br, 4H, $N(\underline{CH_2})_2(CH_2)_3$, J = 4.5 Hz), 7.31 (s, 2H, NH_2); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 23.7 ($N(CH_2)_2(CH_2)_2\underline{CH_2}$), 25.3 ($N(CH_2)_2(\underline{CH_2})_2CH_2$), 26.3 ($N(CH_2)_2(\underline{CH_2})_2$), 47.9 ($N(\underline{CH_2})_2(CH_2)_3$), 51.5 ($N(\underline{CH_2})_2(CH_2)_2$), 64.7 (C–CN), 68.7 (C–CN), 116.3, 116.7 (2CN), 151.7 (N–C–N), 155.8 (C=N), 157.4 (C–NH_2), 160.3 (=C–N); λ_{max} (MeOH)/nm 226 (log ε 2.99), 284 (1.97); Anal. Calcd. for $C_{17}H_{20}N_8$: C 60.70, H 5.99, N 33.31%; found: C 61.04, H 5.79, N 33.51%.

4.4.3. 5-Amino-2,7-dimorpholinopyrazolo[1,5-a]pyrimidine-3,6-dicarbonitrile (**4c**)

Yield, 34%; white solid; mp 332 °C; IR (KBr, cm^{-1}) ν: 3381, 3287, 3203 (NH_2), 2211 (CN), 1659 (C=C/C=N); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 4.41 (br, 4H, $N(\underline{CH_2})_2(CH_2)_2O$), 3.72 (br, 4H, $N(\underline{CH_2})_2(CH_2)_2O$), 3.77 (br, 8H, 2[$N(CH_2)_2(\underline{CH_2})_2O$]), 7.45 (s, 2H, NH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 47.1 ($N(\underline{CH_2})_2(CH_2)_2O$), 51.0 ($N(\underline{CH_2})_2(CH_2)_2O$), 65.4 (C–CN), 66.0 ($N(CH_2)_2(\underline{CH_2})_2O$), 66.9 ($N(CH_2)_2(\underline{CH_2})_2O$), 69.5 (C–CN), 116.0, 116.5 (2CN), 151.8 (N–C–N), 156.0 (N–C=N), 159.7 (C–NH_2), 160.4 (=C–N); λ_{max} (MeOH)/nm 225 (log ε 4.08), 270 (4.34), 305 (3.68); Anal. Calcd. for $C_{16}H_{18}N_8O_2$: C 54.23, H 5.12, N 31.62%; found: C 53.99, H 5.16, N 31.51%.

4.4.4. 5-Amino-2-morpholino-7-(piperidin-1-yl)pyrazolo[1,5-a] pyrimidine-3,6-dicarbonitrile (**4d**)

Yield, 30%; white crystals; mp >300 °C; IR (KBr, cm^{-1}) ν: 3317, 3236, 3165 (NH_2), 2203 (CN), 1558 (C=C/C=N); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.67 (br, 6H, $N(CH_2)_2(\underline{CH_2})_3$), 3.41 (br, 4H, $N(\underline{CH_2})_2(CH_2)_3$), 3.68, 3.71 (br, 8H, $N(\underline{CH_2})_2(CH_2)_2O$), 3.71 (br, 8H, $N(CH_2)_2(\underline{CH_2})_2O$), 7.38 (s, 2H, NH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 23.6 ($N(CH_2)_2(CH_2)\underline{CH_2}$), 26.4 ($N(CH_2)_2(\underline{CH_2})CH_2$), 47.1 ($N(\underline{CH_2})_2(CH_2)_2O$), 51.8 ($N(\underline{CH_2})_2(CH_2)_3$), 65.4 (C–CN), 66.0 ($N(CH_2)_2(\underline{CH_2})_2O$), 69.3 (C–CN), 116.1, 116.6 (2CN), 152.2 (N–C–N), 156.1 (N–C=N), 159.7 (C–NH_2), 160.5 (=C–N); λ_{max} (MeOH)/nm 226 (log ε 3.37), 270 (3.57), 3.08 (2.74); Anal. Calcd. for $C_{16}H_{18}N_8O_2$: C 57.94, H 5.72, N 31.80%; found: C 57.89, H 5.78, N 31.82%.

4.5. General procedure for the preparation of pyrazolo[1,5-a] pyrimidines **5a**–**c** and **6a**–**c** from the reaction of 5-aminopyrazoles **2a**–**c** with acetylacetone and acetoacetanilide

Pyrazolo[1,5-*a*]pyrimidines **5a**–**c** and **6a**–**c** were prepared according to the literature procedure Gouda et al. 2010 [20,21] as follows: to a mixture of 5-aminopyrazoles **2a**–**c** (4 mmol) and acetylacetone (0.45 g, 4 mmol) or acetoacetanilide (0.708 g; 4 mmol) in DMF (20 mL) and three drops of glacial acetic acid were added. The resulting mixture was refluxed for 3 h, and allowed to cool at room temperature and then the reaction mixture was poured into crushed-ice, and the separated solid was filtered off, dried well and crystallized from EtOH:DMF to give compounds **5a**–**c** and **6a**–**c**.

4.5.1. 5,7-Dimethyl-2-morpholinopyrazolo[1,5-a]pyrimidine-3-carbonitrile (**5a**)

Yield, 71%; white crystals; mp 212–214 °C; IR (KBr, cm^{-1}) ν: 2210 (CN), 1621, 1571 (C=C/C=N); ^{1}H NMR (600 MHz, DMSO-d_6) δ: 2.44 (s, 3H, C=C–$\underline{CH_3}$), 2.63 (s, 3H, N=C–$\underline{CH_3}$), 3.47 (s, 4H, $N(\underline{CH_2})_2(CH_2)_2O$), 3.69 (s, 4H, $N(CH_2)_2(\underline{CH_2})_2O$), 6.94 (s, 1H, CH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 16.8 (C=C–$\underline{CH_3}$), 24.3 (N=C–$\underline{CH_3}$), 48.0 ($N(\underline{CH_2})_2(CH_2)_2O$), 65.8 ($N(CH_2)_2(\underline{CH_2})_2O$), 66.1 (C–CN), 110.8 (C=$\underline{CH}$), 115.7 (CN), 146.6 (C=$\underline{C}$–CH_3), 152.1 (C=$\underline{C}$–N), 161.0 (N–C=N), 162.2 (N=$\underline{C}$–CH_3); DIMS found *m/z*: 257.30 (calc. for $C_{13}H_{15}N_5O$ M^+ requires: 257.29); λ_{max} (MeOH)/nm 209 (log ε 4.30), 251 (4.51), 316 (3.74); Anal. Calcd. for $C_{13}H_{15}N_5O$: C 60.69, H 5.88, N 27.22%; found: C 60.60, H 5.82, N 27.16%.

*4.5.2. 5,7-Dimethyl-2-(piperidin-1-yl)pyrazolo[1,5-a]pyrimidine-3-carbonitrile (**5b**)*

Yield, 47%; white crystals; mp 209–211 °C; IR (KBr, cm^{-1}) ν: 2199 (CN), 1594, 1580, 1553 (C=C/C=N); ^{1}H NMR (600 MHz, DMSO-d_6) δ: 1.55 (br. s, 2H, N(CH$_2$)$_2$(CH$_2$)$_2$CH$_2$), 1.89 (br. s, 4H, N(CH$_2$)$_2$(CH$_2$)$_2$CH$_2$), 2.42 (s, 3H, C=C–CH$_3$), 2.51 (s, 3H, N=C–CH$_3$), 3.47 (br, 4H, N(CH$_2$)$_2$(CH$_2$)$_2$O), 6.87 (s, 1H, C=CH); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 16.6 (C=C–CH$_3$), 24.0 (N=C–CH$_3$), 25.1 (N(CH$_2$)$_2$(CH$_2$)$_3$), 47.8 (N(CH$_2$)$_2$(CH$_2$)$_2$O), 64.9 (C–CN), 109.8 (C=CH), 115.8 (CN), 145.6 (C=C–CH$_3$), 151.8 (C=C–N), 158.4 (N–C=N), 160.7 (N=C–CH$_3$); DIMS found *m/z*: 255.15 (calc. for $C_{14}H_{17}N_5$ M^+ requires: 255.15); λ_{max} (MeOH)/nm 215 (log ε 4.33), 255 (4.48), 339 (3.78); Anal. Calcd. for $C_{14}H_{17}N_5$ C 65.86, H 6.71, N 27.43%; found: C 65.57, H 6.89, N 27.39%.

*4.5.3. 5,7-Dimethyl-2-(pyrrolidin-1-yl)pyrazolo[1,5-a]pyrimidine-3-carbonitrile (**5c**)*

Yield, 66%; white crystals; mp 209–210 °C; IR (KBr) ν: 2198 (CN), 1578 (C=C/C=N, br), ^{1}H NMR (600 MHz, DMSO-d_6) δ: 1.94 (br. s, 4H, N(CH$_2$)$_2$(CH$_2$)$_2$), 2.46 (s, 3H, C=C–CH$_3$), 2.56 (s, 3H, N=C–CH$_3$), 3.52 (br. s, 4H, N(CH$_2$)$_2$(CH$_2$)$_2$), 6.92 (s, 1H, C=CH); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 16.6 (C=C–CH$_3$), 24.0 (N=C–CH$_3$), 25.0 (N(CH$_2$)$_2$(CH$_2$)$_2$), 47.8 (N(CH$_2$)$_2$(CH$_2$)$_2$), 64.9 (C–CN), 109.8 (C=CH), 115.8 (CN), 145.7 (C=C–CH$_3$), 151.4 (C=C–N), 158.5 (N–C=N), 160.7 (N=C–CH$_3$); DIMS found *m/z*: 241.20 (calc. for $C_{13}H_{15}N_5$ M^+ requires: 241.29); λ_{max} (MeOH)/nm 215 (log ε 4.27), 255 (4.41), 339 (3.71); Anal. Calcd. for $C_{13}H_{15}N_5$: C 64.71, H 6.27, N 29.02%; found: C 64.47, H 6.15, N 29.02%.

*4.5.4. 7-Hydroxy-5-methyl-2-morpholinopyrazolo[1,5-a]pyrimidine-3-carbonitrile (**6a**)*

Yield, 40%; yellow crystals; mp >315 °C; IR (KBr, cm^{-1}) ν: 3638, 3308 (OH), 2216 (CN), 1681, 1644, 1522 (C=C/C=N); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.26 (s, 3H, N=C–CH$_3$), 3.38 (t, 4H, N(CH$_2$)$_2$(CH$_2$)$_2$O, J = 4.8 Hz), 3.72 (t, 4H, N(CH$_2$)$_2$(CH$_2$)$_2$O, J = 4.8 Hz), 5.75 (s, 1H, C=CH), 13.07 (s, 1H, OH); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 18.7 (N=C–CH$_3$), 47.5 (N(CH$_2$)$_2$(CH$_2$)$_2$O), 63.7 (C–CN), 65.8 (N(CH$_2$)$_2$(CH$_2$)$_2$O), 99.4 (=CH), 114.1 (CN), 147.0 (C=C–N), 150.9 (N–C=N), 155.0 (C–CH$_3$), 158.9 (C–OH); λ_{max} (MeOH)/nm 210 inf (log ε 3.98), 247 (4.11), 284 (3.42); Anal. Calcd. for $C_{12}H_{13}N_5O_2$: C 55.59, H 5.05, N 27.01%; found: C 55.51, H 4.95, N 26.90%.

*4.5.5. 7-Hydroxy-5-methyl-2-(piperidin-1-yl)pyrazolo[1,5-a]pyrimidine-3-carbonitrile (**6b**)*

Yield, 56%; white crystals; mp >301 °C; IR (KBr, cm^{-1}) ν: 3290, 3139 (OH), 2199 (CN), 1659, 1628, 1600, 1520 (C=C/C=N); ^{1}H NMR (600 MHz, DMSO-d_6) δ: 1.58 (s, 6H, N(CH$_2$)$_2$(CH$_2$)$_3$), 2.27 (s, 3H, N=C–CH$_3$), 3.42 (s, 4H, N(CH$_2$)$_2$(CH$_2$)$_3$), 5.71 (s, 1H, =CH), 12.92 (s, 1H, OH); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 18.5 (CH$_3$), 24.9 (N(CH$_2$)$_2$(CH$_2$)$_2$CH$_2$), 25.3 (N(CH$_2$)$_2$(CH$_2$)$_2$CH$_2$), 47.2 (N(CH$_2$)$_2$(CH$_2$)$_2$), 63.7 (C–CN), 100.1 (=CH), 114.5 (CN), 147.2 (C=C–N), 150.3 (N–C=N), 154.8 (C–CH$_3$), 158.9 (C–OH); λ_{max} (MeOH)/nm 210 (log ε 4.24), 247 (4.44), 284 (3.80); DIMS found *m/z*: 257.20 (calc. for $C_{13}H_{15}N_5O$ M^+ requires: 257.29); Anal. Calcd. for $C_{13}H_{15}N_5O$: C 60.69, H 5.88, N 27.22%; found: C 60.89, H 5.69, N 27.26%.

*4.5.6. 7-Hydroxy-5-methyl-2-(pyrrolidin-1-yl)pyrazolo[1,5-a]pyrimidine-3-carbonitrile (**6c**)*

Yield, 47%; white crystals; mp >315 °C; IR (KBr, cm^{-1}) ν: 3469, 3146 (OH), 2203, 2209 (CN), 1663, 1601 (C=C/C=N); ^{1}H NMR (600 MHz, DMSO-d_6) δ: 1.93 (t, 4H, N(CH$_2$)$_2$(CH$_2$)$_2$, J = 6.3 Hz), 2.27 (s, 3H, N=C–CH$_3$), 3.47 (t, 4H, N(CH$_2$)$_2$(CH$_2$)$_2$, J = 6.3 Hz), 5.71 (s, 1H, CH), 12.89 (s, 1H, OH); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 19.4 (N=C–CH$_3$), 25.4 (N(CH$_2$)$_2$(CH$_2$)$_2$), 48.5 (N(CH$_2$)$_2$(CH$_2$)$_2$), 62.7 (C–CN), 99.7 (=CH), 114.7 (CN), 147.0 (C=C–N), 149.9 (N–C=N), 154.8 (N=C–CH$_3$), 156.5 (C–OH); λ_{max} (MeOH)/nm 216 (log ε 4.16), 251 (4.37), 284 inf (3.85); DIMS found *m/z*: 243.10 (calc. for $C_{12}H_{13}N_5O$ M^+ requires: 243.26); Anal. Calcd. for $C_{12}H_{13}N_5O$: C 59.25, H 5.39, N 28.79%; found: C 59.30, H 5.68, N 28.60%.

*4.6. General procedure for the preparation of pyrazolo[5,1-c][1,2,4]triazines **7a–c** and **8a–c** from the reaction of 5-aminopyrazoles **2a–c** with acetylacetone and malononitrile*

Pyrazolo[5,1-*c*][1,2,4]triazines **7a–c** and **8a–c** were prepared according to the literature procedure Gouda et al. 2010 [21] as follows: Preparation of the diazonium salt: a solution of sodium nitrite (0.32 g, 4 mmol; in 2 mL water) was gradually added to a well cooled solution of 5-aminopyrazoles **7a–c** (0.84 g, 4 mmol) in a mixture of acetic acid and concentrated HCl [(8:2) 10 mL (1/4) Vol.]. The diazonium salt solution was added dropwise with continuous stirring to cold solution of acetylacetone (0.40 g, 4 mmol) or malononitrile (0.26 g, 4 mmol) in pyridine (10 mL) the reaction mixtures were stirred at 0–5 °C for 2 h and left to stand at room temperature. The separated solid products that obtained were filtered off, dried and crystallized from EtOH:DMF to give products **7a–c** and **8a–c**.

*4.6.1. 3-Acetyl-4-methyl-7-morpholinopyrazolo[5,1-c][1,2,4]triazine-8-carbonitrile (**7a**)*

Yield, 65%; yellow crystals; mp 193–194 °C; IR (KBr, cm^{-1}) ν: 2216 (CN), 1698 (C=O), 1586, 1559, 1504 (C=C/N=N/C=N); ^{1}H NMR (600 MHz, AcOD) δ: 2.91 (s, 3H, CO–CH$_3$), 3.09 (s, 3H, C=C–CH$_3$), 3.90 (t, 4H, N(CH$_2$)$_2$(CH$_2$)$_2$O, J = 4.7 Hz); 3.95 (t, 4H, N(CH$_2$)$_2$(CH$_2$)$_2$O, J = 4. 7 Hz); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 13.8 (C=C–CH$_3$), 28.8 (CO–CH$_3$), 46.6 (N(CH$_2$)$_2$(CH$_2$)$_2$O), 65.9 (C–CN), 66.9 (N(CH$_2$)$_2$(CH$_2$)$_2$O), 114.2 (CN), 138.8 (C–N=N), 142.4 (C–CO), 152.7 (N–C=N), 161.6 (C–CH$_3$), 198.4 (C=O); λ_{max} (MeOH)/nm 210 (log ε 4.10), 230 (4.11), 289 (4.54); DIMS found *m/z*: 286.15 (calc. for $C_{13}H_{14}N_6O_2$ M^+ requires: 286.29); Anal. Calcd. for $C_{13}H_{14}N_6O_2$: C 54.54, H 4.93, N 29.35%; found: C 54.49, H 4.89, N 29.15%.

*4.6.2. 3-Acetyl-4-methyl-7-(piperidin-1-yl)pyrazolo[5,1-c][1,2,4]triazine-8-carbonitrile (**7b**)*

Yield, 75%; colorless; mp 179–180 °C; IR (KBr, cm^{-1}) ν: 2217 (CN), 1691 (C=O), 1589, 1504 (C=C/N=N/C=N); ^{1}H NMR (600 MHz, DMSO-d_6) δ: 1.80–2.02 (br t, 6H, N(CH$_2$)$_2$(CH$_2$)$_3$, J = 7.0 Hz), 2.77 (s, 3H, CH$_3$–CO), 2.90 (s, 3H, C=C–CH$_3$), 3.63 (br t, 4H, N(CH$_2$)$_2$(CH$_2$)$_3$, J = 7.0 Hz); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 13.9 (C=C–CH$_3$), 25.5 (N(CH$_2$)$_2$(CH$_2$)$_3$), 28.9 (CO–CH$_3$), 48.6 (N(CH$_2$)$_2$(CH$_2$)$_3$), 66.3 (C–CN), 114.4 (CN), 138.1 (C–N=N), 142.1 (C–CO), 152.5 (N–C=N), 159.7 (C–CH$_3$), 198.5 (C=O); Anal. Calcd for $C_{14}H_{16}N_6O$: C 59.14; H 5.67; N 29.56%; found: C 59.22; H 5.77; N 29.60%.

*4.6.3. 3-Acetyl-4-methyl-7-(pyrrolidin-1-yl)pyrazolo[5,1-c][1,2,4]triazine-8-carbonitrile (**7c**)*

Yield, 78%; red pale; mp 169–170 °C; IR (KBr, cm^{-1}) ν: 2217 (CN), 1695 (C=O), 1591, 1502, (C=C/N=N/C=N); ^{1}H NMR (600 MHz, DMSO-d_6) δ: 2.01 (t, 4H, N(CH$_2$)$_2$(CH$_2$)$_2$, J = 6.6 Hz), 2.78 (s, 3H, CH$_3$–CO), 2.92 (s, 3H, C=C–CH$_3$), 3.65 (t, 4H, N(CH$_2$)$_2$(CH$_2$)$_2$, J = 6.6 Hz); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 13.8 (C=C–CH$_3$), 25.4 (N(CH$_2$)$_2$(CH$_2$)$_2$), 28.8 (CH$_3$–CO), 48.5 (N(CH$_2$)$_2$(CH$_2$)$_2$), 66.3 (C–CN), 114.3 (CN), 138.1 (C–N=N), 142.1 (C–CO), 152.4 (N–C=N), 159.7 (C–CH$_3$), 198.4 (C=O); λ_{max} (MeOH)/nm 210 (log ε 4.08), 231 (4.06), 294 (4.51); DIMS *m/z* found: 270.20 (calc. for $C_{13}H_{14}N_6O$ M^+ requires: 270.29); Anal. Calcd for $C_{13}H_{14}N_6O$: C 57.77, H 5.22, N 31.09%; found: C 57.76, H 5.32, N 31.14%.

4.6.4. 4-Amino-7-morpholinopyrazolo[5,1-c][1,2,4]triazine-3,8-dicarbonitrile (**8a**)

Yield, 88%, colorless; mp >313 °C (decomposed); IR (KBr, cm^{-1}) ν: 3331, 3184 (NH_2), 2230 (CN), 1656, 1585, 1538 (C=C/C=N/C=C); ^1H NMR (600 MHz, DMSO-d_6) δ: 3.64 (t, 4H, N(CH_2)$_2$(CH_2)$_2$O, J = 4.5 Hz), 3.76 (t, 4H, N(CH_2)$_2$(CH_2)$_2$O, J = 4.5 Hz), 9.29 (br, 2H, NH_2); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 46.8 (N(CH_2)$_2$(CH_2)$_2$O), 65.9 (N(CH_2)$_2$(CH_2)$_2$O), 67.8 (C–CN), 111.1 (C–CN), 114.3, 115.2 (2CN), 141.4 (N–C–N), 152.2 (C–NH_2), 160.6 (N–C=N); λ_{max} (MeOH)/nm 230 (log ε 3.53), 278 (4.06), 360 (3.50); DIMS found m/z: 270.15 (calc. for $C_{11}H_{10}N_8O$ M^+ requires 270.25); Anal. Calcd. for $C_{11}H_{10}N_8O$: C 48.89, H 3.73, N 41.46%; found C 48.82, H 3.76, N 41.39%.

4.6.5. 4-Amino-7-(piperidin-1-yl)pyrazolo[5,1-c][1,2,4]triazine-3,8-dicarbonitrile (**8b**)

Yield, 75%; yellow crystals; mp >286 °C (decomposed); IR (KBr, cm^{-1}) ν: 3305, 3148 (NH_2), 2222 (CN), 1649, 1586 (C=C/C=N/C=C); ^1H NMR (400 MHz, DMSO-d_6) δ: 2.40 (s, 6H, N(CH_2)$_2$(CH_2)$_3$), 4.44 (s, 4H, N(CH_2)$_2$(CH_2)$_3$), 10.02 (br, 2H, NH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 33.3 (N(CH_2)$_2$(CH_2)$_2$$CH_2$), 34.5 (N($CH_2$)$_2$($CH_2$)$_2$$CH_2$), 56.8 (N($CH_2$)$_2$($CH_2$)$_3$), 76.7 (C–CN), 120.2 (C–CN), 124.0, 124.6 (2CN), 150.4 (N–C–N), 161.7 (C–NH_2), 169.6 (N–C=N); λ_{max} (MeOH)/nm 232 (log ε 4.07), 283 (4.51), 360 (4.04); DIMS found m/z: 268.15 (calc. for $C_{12}H_{12}N_8$ M^+ requires 268.28); Anal. Calcd. for $C_{12}H_{12}N_8$: C 53.72, H 4.51, N 41.77%; found: C 53.52, H 4.38, N 41.66%.

4.6.6. 4-Amino-7-(pyrrolidin-1-yl)pyrazolo[5,1-c][1,2,4]triazine-3,8-dicarbonitrile (**8c**)

Yield, 70%; yellow crystals; mp >310 (decomposed); IR (KBr, cm^{-1}) ν: 3378, 3107 (NH_2), 2224 (CN), 2211 (CN), 1648, 1598 (C=C/C=N); ^1H NMR (400 MHz, DMSO-d_6) δ: 1.98 (s br, 4H, N(CH_2)$_2$(CH_2)$_2$), 3.59 (br. s, 4H, N(CH_2)$_2$(CH_2)$_2$), 9.16 (br, 2H, NH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 25.0 (N(CH_2)$_2$(CH_2)$_2$), 47.9 (N(CH_2)$_2$(CH_2)$_2$), 66.8 (C–CN), 110.3 (C–CN), 114.2, 114.9 (2CN), 140.6 (N–C–N), 151.6 (C–NH_2), 158.2 (N–C=N); λ_{max} (MeOH)/nm 232 (log ε 4.19), 280 (4.55), 358 (4.04); DIMS found: m/z 254.20 (calc. for $C_{11}H_{10}N_8$ M^+ requires 254.25); Anal. Calcd for $C_{11}H_{10}N_8$: C 51.96, H 3.96, N 44.07%; found: C 51.95, H 3.84, N 43.86%.

4.7. General procedure for the reaction of 5-aminopyrazoles **2a–c** with 2,5-hexan-di-one

The 5-pyrrolylpyrazoles were prepared according to the literature procedure Gouda et al. 2010 [20,21] as follows: a mixture of 5-aminopyrazoles **2a–c** (4 mmol) and 2,5-hexanedione (0.457 g; 4 mmol) in glacial acetic acid was refluxed for 5 h, then the reaction mixtures was poured into crushed ice, and the separated solid was filtered off and dried well to give **9a–c**.

4.7.1. 5-(2,5-Dimethyl-1H-pyrrol-1-yl)-3-morpholino-1H-pyrazole-4-carbonitrile (**9a**)

Yield, 27%; pale brown; mp 117–119 °C; IR (KBr, cm^{-1}) ν: 3228 (NH/br), 2219 (CN), 1591, 1501 (C=C/C=N); ^1H NMR (600 MHz, CD_3CL) δ: 2.12 (s, 6H, $2CH_3$), 3.37 (t, 4H, N(CH_2)$_2$(CH_2)$_2$O, J = 4.4 Hz), 3.78 (t, 4H, N(CH_2)$_2$(CH_2)$_2$O, J = 4.4 Hz), 5.87 (s, 2H, CH=CH), 10.79 (br, 1H, NH); ^{13}C NMR (150 MHz, AcOD) δ: 11.2 ($2CH_3$), 47.0 (N(CH_2)$_2$(CH_2)$_2$O), 65.5 (N(CH_2)$_2$(CH_2)$_2$O), 74.5 (C–CN), 107.2, 107.4 (CH=CH), 113.5 (CN), 129.0 (C–CH_3), 148.0 (N–C–NH), 154.8 (N–C=N); λ_{max} (CH_3CN)/nm 228 (log ε 3.80), 244 (3.73); DIMS found m/z: 271.20 (calc. for $C_{14}H_{17}N_5O$ M^+ requires 271.32); Anal. Calcd. for $C_{14}H_{17}N_5O$: C 61.98, H 6.32, N 25.81%; found C 61.71, H 6.49, N 25.93%.

4.7.2. 5-(2,5-Dimethyl-1H-pyrrol-1-yl)-3-(piperidin-1-yl)-1H-pyrazole-4-carbonitrile (**9b**)

Yield, 28%; solid brown; mp 120–122 °C; IR (KBr, cm^{-1}) ν: 3232 (NH/br), 2217 (CN), 1607, 1599, 1515 (C=C/C=N); ^1H NMR (600 MHz, CD_3CL) δ: 1.61 (t, 6H, N(CH_2)$_2$(CH_2)$_3$, J = 4.8 Hz), 2.11 (s, 6H, $2CH_3$), 3.31 (t, 4H, N(CH_2)$_2$(CH_2)$_3$, J = 4.8 Hz), 5.85 (s, 2H, 2CH=), 11.05 (br, 1H, NH); ^{13}C NMR (150 MHz, CD_3CL) δ: 12.4 ($2CH_3$), 23.5 (N(CH_2)$_2$$CH_2$($CH_2$)$_2$), 24.9 (N($CH_2$)$_2$$CH_2$($CH_2$)$_3$), 48.2 (N($CH_2$)$_2$$CH_2$($CH_2$)$_3$), 73.4 (C–CN), 107.4 (CH=CH), 114.5 (CN), 129.2 (2C–CH_3), 149.2 (N–C–NH), 154.6 (C=N); λ_{max} (CH_3CN)/nm 230 (log ε 4.18), 246 (4.12); DIMS found: m/z 269.20 (calc. for $C_{15}H_{19}N_5$ M^+ requires 269.34); Anal. Calcd. for $C_{15}H_{19}N_5$: C 66.89, H 7.11, N 26.00%; found: C 66.92, H 6.98, N 26.22%.

4.7.3. 5-(2,5-Dimethyl-1H-pyrrol-1-yl)-3-(pyrrolidin-1-yl)-1H-pyrazole-4-carbonitrile (**9c**)

Brown solid; yield, 28%; mp 112–113 °C; IR (KBr, cm^{-1}) ν: 3172 (NH, br), 2215 (CN), 1611, 1529 (C=C/C=N); ^1H NMR (400 MHz, CD_3CL) δ: 1.92 (t, 4H, N(CH_2)$_2$(CH_2)$_2$, J = 6.4 Hz), 2.11 (s, 6H, $2CH_3$), 3.30 (t, 4H, N(CH_2)$_2$(CH_2)$_2$, J = 6.4 Hz), 5.83 (s, 2H, CH=CH), 11.55 (br, 1H, NH); ^{13}C NMR (100 MHz, CD_3CL) δ: 12.6 ($2CH_3$), 25.7 (N(CH_2)$_2$(CH_2)$_2$), 48.2 (N(CH_2)$_2$(CH_2)$_2$), 71.0 (C–CN), 107.4 (2CH=), 115.2 (CN), 129.2 (C–CH_3), 149.1 (N–C–NH), 152.0 (C=N); λ_{max} (CH_3CN)/nm 230 (log ε 4.18), 245 (4.15); DIMS found m/z: 255.15 (calc. for $C_{14}H_{17}N_5$ M^+ requires 255.32); Anal. Calcd for $C_{14}H_{17}N_5$: C 65.86, H 6.71, N, 27.43%; found: C 65.91, H 6.79, N 27.61%.

4.8. General procedure for the reaction of 5-aminopyrazoles **2a–c** with acetic anhydride

The 1-acetyl-5-amino-1*H*-pyrazoles were prepared according to the literature procedure of Zaharan et al. [29] as follows: a solution of 5-aminopyrazoles **2a–c** (2 mmol) in acetic anhydride (10 mL) was heated under reflux for 15 min. The solid products so formed was collected by filtration and recrystallized from DMF:EtOH to give **10a–c**.

4.8.1. 1-Acetyl-5-amino-3-morpholino-1H-pyrazole-4-carbonitrile (**10a**)

Yield, 95%; white solid; mp 253 °C; IR (KBr, cm^{-1}) ν: 3422, 3172 (NH_2), 2209 (CN), 1703 (CO), 1633 (C=C/C=N); ^1H NMR (600 MHz, AcOD) δ: 1.96 (s, 3H, CH_3–CO), 2.82 (t, 4H, N(CH_2)$_2$(CH_2)$_2$O, J = 4.8 Hz), 3.22 (t, 4H, N(CH_2)$_2$(CH_2)$_2$O, J = 4.8 Hz), 7.47 (s, 2H, NH_2); ^{13}C NMR (150 MHz, AcOD) δ: 23.1 (CH_3–CO), 46.9 (N(CH_2)$_2$(CH_2)$_2$O), 63.8 (C–CN), 65.4 (2 N(CH_2)$_2$(CH_2)$_2$O), 114.6 (CN), 156.2 (C–NH_2), 156.3 (N–C–N=), 172.5 (C=O); λ_{max} (MeOH)/nm 228 (log ε 3.81), 265 (3.67); DIMS found m/z: 235.25 (calc. for $C_{10}H_{13}N_5O_2$ M^+ requires 235.24); Anal. Calcd. for $C_{10}H_{13}N_5O_2$: C 51.06, H 5.57, N 29.77%; found: C 51.32, H 5.69, N 29.65%.

4.8.2. 1-Acetyl-5-amino-3-(piperidin-1-yl)-1H-pyrazole-4-carbonitrile (**10b**)

Yield, 99.43%; colorless crystals; mp 216–218 °C; IR (KBr, cm^{-1}) ν: 3418, 3299 (NH_2), 2213 (CN), 1702 (CO), 1633, 1536 (C=C/C=N); ^1H NMR (400 MHz, DMSO-d_6) δ: 1.55 (s, 6H, N(CH_2)$_2$(CH_2)$_3$), 2.41 (s, 3H, $COCH_3$), 3.30 (s, 4H, N(CH_2)$_2$(CH_2)$_3$), 7.86 (s, 2H, NH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 23.2 ($COCH_3$), 23.8 (N(CH_2)$_2$$CH_2$($CH_2$)$_2$), 24.7 (N($CH_2$)$_2$$CH_2$($CH_2$)$_2$), 47.5 (N($CH_2$)$_2$$CH_2$($CH_2$)$_2$), 63.8 (C–CN), 114.9 (CN), 156.3 (C–NH_2), 156.4 (N–C–N), 172.5 (C=O); λ_{max} (MeOH)/nm 229 (log ε 4.35), 270 (4.17), 306 (3.67); DIMS found m/z: 233.15 (calc. for $C_{11}H_{15}N_5O$ M^+ requires 233.27); Anal. Calcd. for $C_{11}H_{15}N_5O$: C 56.64, H 6.48, N 30.02%; found: C 56.66, H 6.47, N 29.82%.

*4.8.3. 1-Acetyl-5-amino-3-(pyrrolidin-1-yl)-1H-pyrazole-4-carbonitrile (**10c**)*

Yield, 99%; colorless crystals; mp >216–218 °C; IR (KBr, cm^{-1}) ν: 3421, 3311 (NH_2), 2210 (CN), 1707 (CO), 1638, 1579 (C=C/C=N); ^1H NMR (600 MHz, AcOD) δ: 1.40 (t, 4H, $N(CH_2)_2(C\underline{H}_2)_2$, $J = 6.6$ Hz), 1.94 (s, 3H, $COCH_3$), 2.91 (t, 4H, $N(C\underline{H}_2)_2(CH_2)_2$, $J = 6.6$ Hz), 7.40 (s, 2H, NH_2); ^{13}C NMR (150 MHz AcOD) δ: 23.1 ($CO\underline{C}H_3$), 24.8 ($N(CH_2)_2(\underline{C}H_2)_2$), 47.4 ($N(\underline{C}H_2)_2(CH_2)_2$), 63.3 ($\underline{C}$–CN), 114.9 (CN), 154.1 (C–NH_2), 156.1 (N–C–N), 172.3 (C=O); λ_{max} (MeOH)/nm 230 (log ε 3.35), 270 (3.47); DIMS found m/z: 219.25 (calc. for $C_{10}H_{13}N_5O$ M^+ requires 219.24); Anal. Calcd. for $C_{10}H_{13}N_5O$: C 54.78, H 5.98, N 31.94%; found: C 54.85, H 5.94, N 31.82%.

5. Conclusions

In this paper, the synthesis of new pyrazolo[1,5-*a*]-pyrimidines **3a**–**c**, **4a**–**d**, **5a**–**c**, **6a**–**c** and pyrazolo[5,1-*c*][1,2,4]triazines **7a**–**c**, **8a**–**c** by the reaction of 5-aminopyrazoles **2a**–**c** with respective 2-[bis(methylthio)methylene]malononitrile, α,α-dicyanoketene-*N*,*S*-acetals **1a**–**b**, acetylacetone, acetoacetanilide as well as acetylacetone, and malononitrile are reported. Also, the reaction of **2a**–**c** with 2,5-hexanedione afforded the corresponding 5-pyrrolylpyrazoles **9a**–**c**. Also, **2a**–**c** reacted with acetic anhydride to give the corresponding 1-acetyl-1*H*-pyrazoles **10a**–**c**. All procedures for the synthesis of these compounds are very convenient due to the simple procedures, mild conditions, and moderate to high yields. Another advantage is that 5-aminopyrazoles **2a**–**c** was of the same starting material used for the preparation of all those compounds. Some of the prepared compounds showed low to moderate antibacterial activity and not cytotoxic to Vero cells.

Acknowledgments

The authors thank the Ministry of Higher Education Malaysia and Universiti Kebangsaan Malaysia for research grant UKM-GGPM-KPB-098-2010. A scholarship from the Libyan government to WMA is greatly appreciated.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2013.04.029.

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