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Synthesis of New Pyrazolo [5, 1-*c*] [1, 2, 4] triazines from 5-Aminopyrazole and Study Biological Activity and Cytotoxicity

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Abstract: Treatment of 2-[bis(methylthio)methylene]malononitrile 1 with *p*-Chloroaniline afforded 2-[(4-chlorophenylamino) (methylthio) methylene] malononitrile 2a, of which upon reaction with hydrazine hydrate yielded 5-amino-3-(4-chlorophenylamino)-1*H*-pyrazole-4-carbonitrile 3. This compound was utilized as a key intermediate for the synthesis of pyrazolo [5, 1-*c*] [1, 2, 4] triazines by diazotization of 3 with nitrous acid at low temperature afforded 3-(4-chlorophenylamino)-1*H*-pyrazole-4-carbonitrile-5-diazonium chloride 3', in which coupling with active methylene of acetylacetone and malononitrile in the presence of pyridine gave the hydrazone derivatives, which cyclized directly upon addition of acetic acid to produce respective pyrazolo [5, 1-*c*] [1, 2, 4] triazine derivatives 7 and 8. Finally, treatment of 3 with acetic anhydride gave the acetyl amide derivative 9. The antibacterial and antifungal activities, as well cytotoxicity against Breast cancer cells (MCF7) of some selected compounds are also reported.

Keywords: Pyrazolo [5, 1-*c*] [1, 2, 4] triazines, *p*-Chloroaniline, 5-Aminopyrazole, Antibacterial Activity, Antifungal Activity, Cytotoxicity

1. Introduction

5-Aminopyrazoles have gained significant interest for synthetic organic chemists in the development of new biological active molecules of the pharmacological importance [1-5]. They have been used in the preparation of polyfunctionalized heterocyclic compounds such as pyrazolopyrimidines and pyrazolotriazines [6-9]. Various related compounds of pyrazolopyrimidines have antitumor and anti-leukemic activities [10, 11]. On the other hand, substituted pyrazolotriazines are often used in medicine due to their pronounced bactericidal, fungicidal and antiviral effects [12, 13]. Cyclization of 5-aminopyrazoles with ketene-*S, S* and *N, S*-acetals is the most widely used route for the synthesis of pyrazolotriazines [6-9]. Accordingly, we report in this paper synthesis of functionalized pyrazolo [5, 1-*c*] [1, 2, 4] triazines 7 and 8 by the reactions of 5-aminopyrazole 3 with respective acetylacetone and malononitrile. The antibacterial, antifungal, cytotoxicity testing results of two compounds is also included.

2. Materials and Methods

2.1. Chemistry

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 and FT-NMR 600 MHz Bruker, AVANCE III spectrometer operating at 400 MHz, and 600 MHz for ^{1}H NMR and at 100 MHz, 150 MHz for ^{13}C NMR in DMSO-d_6 as solvent and using TMS as

internal standard. DIMS spectra were recorded on QP5050A Shimadzu apparatus. X-ray diffraction (XR-D) data 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.

2.1.1. Synthesis of 2-[(4-Chloropzhenylamino) (Methylthio) Methylene] Malononitrile (2a)

A mixture of 2-[bis (methylthio) methylene] malononitril 1 (1.7 g, 0.01 mol), *p*-chloroaniline (1.27 g, 0.01 mol), and three drops of triethylamine (TEA) was refluxed in absolute ethanol 30 mL on oil-bath in the presence balloon of nitrogen. The reaction mixture was refluxed for one week. The solvent was evaporated and the product was collected, washed with ethanol, dried and recrystallized from ethanol to give pure 2-[(4-chlorophenylamino) (methylthio) methylene] malononitrile 2a.

Yield, 48%; colorless crystals; mp 165-167°C; FT-IR (KBr, cm^{-1}) *v*: 3246 (NH–Ar), 3010 (H–Ar), 2200, 2189 (2CN), 1585, 1526, 1500 (C=C/C=N); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.50 (s, 3H, SCH_3), 7.34 (d, 2H, 2H–Ar, *J*=8.04), 7.49 (d, 2H, 2H–Ar, *J*=8.04), 10.58 (s, 1H, NH–Ar); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 16.4 (SCH_3), 53.9 (C–2CN), 116.9 (2CN), 126.2 (2C–H, Ar), 129.7 (2C–H, Ar), 131.2 (C–Cl), 137.9 (C–NH, Ar), 172.4 (S–C–NH).

2.1.2. General Procedure for the Synthesis of 5-amino-3-(4-Chlorophenylamino)-1H-Pyrazole-4-Carbonitrile (3)

A mixture of 2-[(4-chlorophenylamino) (methylthio) methylene] malononitrile 2a (0.998, 4 mmol) and hydrazine hydrate (1.00 g, 20 mmol) was refluxed on water-bath for 2 hours. Then, 20 mL of ethanol was added, and the reaction mixture was refluxed for further 2 hours. The solvent was evaporated and the product was collected, washed with ethanol, dried and recrystallized from methanol to give pure product 3.

Yield, 97%; whit solid; mp 240-242°C; FT-IR (KBr, cm^{-1}) *v*: 3430, 3254 (NH_2/ NH–Ar), 2915 (H-Ar), 2212 (CN), 1622, 1611, 1584 (C=C/C=N); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 6.30 (s, 2H, NH_2, pyrazole), 7.20 (d, 2H, 2H–Ar, *J*=8.8 Hz), 7.50 (d, 2H, 2H–Ar, *J*=8.8 Hz), 8.54 (s, 1H, NH, pyrazole), 11.19 (s, 1H, NH-Ar); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 63.6 (C–CN), 115.7 (CN), 118.2 (2C=H, Ar), 122.9 (C–Cl), 128.8 (2C=H, Ar), 142.2 (C–NH, Ar), 151.2 (NH–C–N), 153.5 (C–NH_2).

2.1.3. General Procedure for the Preparation of Pyrazolo [5, 1-c] [1, 2, 4] Triazines 4 and 5

To a solution each of acetyl acetone (0.20 g; 2 mmoL) or malononitrile (0.13 g; 2 mmoL) in pyridine (5 mL), an ice-cooled solution of the appropriate diazonium solution [prepared by the addition of a solution of sodium nitrite (0.138 g; 2 mmol) in water (2 mL) to the required 5-aminopyrazole 3 (0.467 g; 2 mmol) in hydrochloric acid and acetic anhydride [(2:8) 10 mL (1/4) vol.] was added drop wise with stirring. Stirring was maintained for overnight. Each the precipitated product was filtered, washed with water, dried and crystallized from EtOH-DMF (2:2) afforded compounds 7 and 8.

*i. 3-acetyl-7-(4-chlorophenylamino)-4-methylpyrazolo [5, 1-*c*] [1, 2, 4] triazine-8-carbonitrile (*4)

Yield, 60%; brown solid; mp 269°C decomposed; IR (KBr, cm^{-1}) *v*: 3302 (NH), 2234 (CN), 1696 (C=O), 1629, 1585, 1566 (C=C/N=N/C=N); ^{1}H NMR (600 MHz, DMSO-d_6) δ: 2.82 (s, 3H, C=C–CH_3), 3.02 (s, 3H, CO–CH_3), 7.43 (d, 2H, 2CH=, Ar, *J*=9.0 Hz), 7.80 (d, 2H, 2CH=, Ar, *J*=9.0 Hz), 10.36 (s, 1H, NH, Ar); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 14.02 (C=C–CH_3), 28.9 (CO–CH_3), 69.1 (C–CN), 112.5 (CN), 121.1 (2CH=, Ar), 126.9 (=C–Cl, Ar), 138.9 (C–NH), 139.8 (C–N=N), 142.2 (C–CO), 151.1 (HN–C=N), 158.1 (C–CH_3), 198.4 (C=O); DIMS found *m/z*: 326.75 (calc. for $C_{15}H_{11}ClN_6O$ M^+ requires: 326.74).

*ii. 4-amino-7-(4-chlorophenylamino) pyrazolo [5, 1-*c*] [1, 2, 4] triazine-3, 8-dicarbonitrile (*5)

Yield, 75%; yellow crystals; mp > 336°C; IR (KBr, cm^{-1}) *v*: 3294 (NH), 3089 (CH–Ar), 2809 (CH, aliphatic), 2228 (CN), 1639, 1583 (C=C/N=N/C=N); ^{1}H NMR (600 MHz, DMSO-d_6) δ: 2.73 (s, 3H, N–CH_3, DMF), 2.89 (s, 3H, N–CH_3, DMF), 7.32 (d, 2H, 2CH=, Ar, *J*=15.0 Hz), 7.94 (s, 1H, CH, DMF), 7.98 (d, 2H, 2CH=, Ar, *J*=15.0 Hz), 9.48 (br, 2H, NH_2), 9.97 (s, 1H, NH, Ar); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 31.2 (N–CH_3, DMF), 36.3 (N–CH_3, DMF), 69.6 (C–CN), 111.1 (C–CN), 112.8 (CN), 115.4 (CN), 120.5 (2CH=, Ar), 125.7 (=C–Cl, Ar), 128.9 (2CH=, Ar), 139.4 (C–NH), 141.9 (C–N=N), 150.7 (C–NH_2), 156.2 (HN–C=N), 162.8 (H–C=O, DMF); DIMS found *m/z*: 310.10 (calc. for $C_{13}H_7ClN_8$ M^+ requires: 310.05).

2.1.4. General Procedure the Preparation of 1-Acetyl-5-Amino-3-(4-Chlorophenylamino)-1H-Pyrazole-4-Carbonitrile (6)

These were prepared according to the literature procedure [14] as follows: A solution of 5-aminopyrazole 3 *(*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 product 9.

Yield, 84%; whit solid; mp 295-296°C; IR (KBr, cm^{-1}) *v*: 3416, 3263 (NH/NH_2), 2211 (CN), 1703 (CO), 1602 (C=C/C=N); ^{1}H NMR (600 MHz, DMSO) δ: 2.56 (s, 3H, CH_3–CO), 7.31 (d, 2H, 2CH=Ar), 7.69 (d, 2H, 2CH=Ar), 8.00 (s, 2H, NH_2), 9.10 (s, 1H, NH); ^{13}C NMR (150 MHz, DMSO) δ: 23.8 (CH_3–CO), 64.7 (C–CN), 113.7 (CN), 119.6 (2H, 2CH=, Ar, *J*=13.2 Hz), 124.7 (H, Cl–C=, Ar), 128.9 (2H, 2CH=, Ar, *J*=13.2 Hz), 140.4 (C–N–H), 151.0 (NH–C=N), 155.2 (C–NH_2), 172.9 (C=O); DIMS found *m/z*: 275.65 (calc. for $C_{12}H_{10}ClN_5O$ M^+ requires 275.69).

2.2. Antibacterial and Antifungal Evaluations

The synthesized compounds were evaluated for their antibacterial and antifungal activities using the agar diffusion technique [15] to determine which antibiotic (sample given) are most successful in treating bacteria or fungal infections. The response (sensitivity/resistance) of microbes against

antimicrobial compounds various to each other. Microbes used in this test are: three bacterial [*Staphylococcus aureus S276*, *Staphylococcus epidermidis S276*, and *Pseudomonas aeruginosa 15442*] and two fungi: [*Aspergillusbrasiliensis ATCC 1640* and *Aspergillusniger UPMC 393*]. The test is carried out by placing 6 mm diameter of paper disc containing antibiotic onto a plate which microbes are growing. The microbe culture is standardized to 0.5 McFarland standards which is approximately 10^8 cells. Not more than 6 discs should be placed on the same agar plate. Streptomycin standard are used for each bacteria and Nystatin standard are used for fungi. The plates are inverted and incubate at 30-37°C for 18-24 hours, 24-48 or until sufficient growth has occurred. After incubation, each plate is examined. The diameters of the zones of complete inhibition (as judged by the unaided eye) are measured, including the diameter of the disc. Zones are measured to the nearest whole millimeter, using sliding calipers or a ruler, which is held on the back of the inverted Petri plate.

2.3. Cytotoxicity Assay

Some of the selected synthesized compounds were also tested against human breast adenocarcinoma (MCF-7) cell lines by using the MTT assay. Human MCF-7 breast adenocarcinoma cell line was procured from ATCC. The cells were cultured in a humid environment at 37°C and 5% CO_2 as a monolayer in DMEM (Dulbecco's Modified Eagles Medium; US Biological) supplemented with 10% FBS (Fetal Bovine Serum; Bioclot) and 1% penicillin/streptomycin (Invitrogen). Cells were grown up to 85-90% confluence and harvested using 0.25% trypsin/EDTA solution before sub-cultured onto 96-well plates. Cells were then treated with different compounds at a final concentration ranging from 0.47-30 µg/mL for 24 hrs. Stock solutions were prepared in dimethylsulfoxide (DMSO) and stored at 20°C until used. The 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) colorimetric assay developed [16] with modification was used to screen the cytotoxic activity of compounds. Briefly, 100µl of the MCF-7 cells (1 x 105 cells/mL) were subculture onto sterile flat-bottomed 96 well plates and exposed to 7 different concentrations (30.00, 15.00, 7.50, 3.75, 1.87, 0.93 and 0.47 µg/mL) of each compound for 24 h. After the completion of Incubation in 37°C, 5% CO_2 incubator, 20 µL of MTT reagent (Invitrogen) in 5.0 mg/mL phosphate buffered saline (PBS) was added to each well and further incubated for 3 h at 37°C, 5% CO_2 incubator. MTT solution was then removed before 100 µL of DMSO (Sigma Aldrich) were added to each well and mix thoroughly to dissolve the blue formazan crystals. Further incubation was carried out for 20 min. Finally, the optical density (OD) of each well was measured on ELISA reader at 570 nm (test wavelength) and 630 nm (reference wavelength). This cytotoxixity test was performed in two independent experiments, each time in triplicate. The percentage of cytotoxicity compared to the untreated cells was determined. The percentage of viability against each compound concentration were plotted to determine the CC_{50} value (the concentration at which 50% cell proliferation is inhibited).

The percentage of cells viability was calculated in relative with the number of viable cells as a percentage of control by defining the absorbance at 570 nm for the control as 100%.

3. Results and Discussions

3.1. Chemistry

The 5-amino-3-[4-chlorophenylamino]-1*H*-pyrazole-4-carbonitrile intermediate 3 was prepared from cyclocondensation of respective α, α-dicyanoketene-*N, S*-acetal 2-[(4-chlorophenylamino) (methylthio) methylene] malononitrile 2a with hydrazine hydrate under reflux in ethanol for four hours (figure 1). The starting material 2a was prepared *via* the reaction of 2-[bis (methylthio) methylene] malononitrile 1 with an aromatic amine of *p*-choroaniline under reflux in ethanol in the presence of balloon of nitrogen gas for one week.

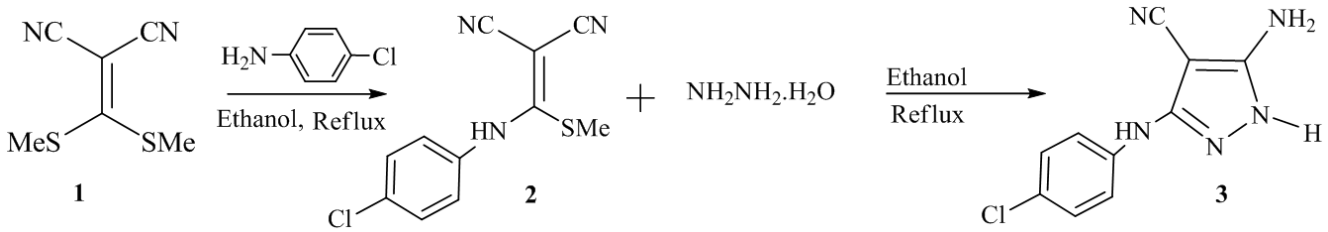

Figure 1. *Synthesis of 5-aminopyrazole 3.*

The possible formation of the 5-aminopyrazole 3 is shown in figure 2. First, Michael addition to 2-[(4-chlorophenylamino) (methylthio) methylene] malononitrile 2a occurs with a lone pair of the NH_2 group in hydrazine to form an intermediate adduct A. Then, the methylthio anion is removed, which results in the formation of intermediate B. This methylthio anion abstracts a proton of the ammonium ion to produce an intermediate C. Subsequently, intermolecular cyclization occurs by a lone pair on the remaining NH_2 group attacking the carbon on cyano group to produce intermediate D. The ammonium proton intramolecular abstraction in D occurs (1, 3-hydrogen migration) to form E, followed by aromatic-driven 1, 5-hydrogen migration to yield product 3.

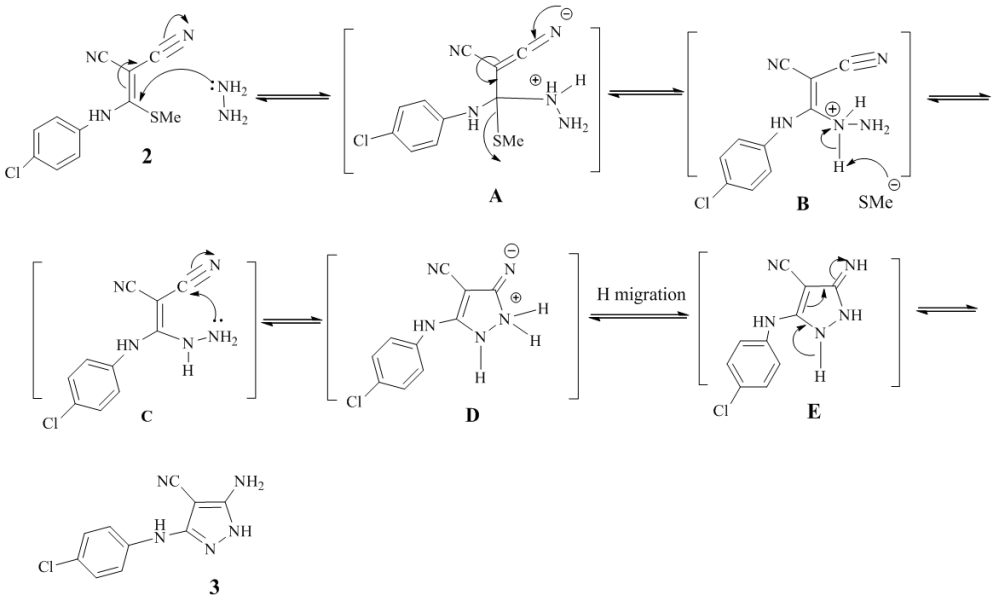

Figure 2. *Mechanism for formation of 5-aminopyrazole 3.*

The chemical structure of the compound 3 was established on the basis of its spectral data. IR spectrum of 3 showed bands at ν 3430, 3254 cm^{-1} for NH and NH_2 groups. The NH_2 protons in ^1H NMR spectrum were at δ 6.30 ppm, whereas the two singlet signals at δ 8.54 and 11.19 ppm assignable to the respective ArNH and cyclo-NH protons. The ^{13}C NMR spectrum was characterized by signals at δ 151.2 and 153.5 ppm assigned to respective aromatic carbons of NH–C=N and C–NH_2. In addition, signals between δ 118.2-142.2 ppm assigned to the other carbons of aromatic rings.

The structure of compound 2a was identified by X-ray diffraction analysis. The molecular structure and the numbering scheme of 2a are presented in Figure 3. Suitable crystals of 2a were grown by slow evaporation from MeOH solution after 48 hours. The crystal data and structure refinement results for 2a are given in Table 1. Compound 2a crystallized in a monoclinic system with space group of *P21/n*. Some selected bond distances and bond angles for 2a are given in Table 2. The bond lengths and angles of the molecule are within in the normal ranges [17]. The phenyl ring, (C1–C6), in compound 2a is essentially planar with a maximum deviation of 0.000 (17) Å, for atom C2. The N2/N3/C8/C9/C10 fragment is essentially planar with a maximum deviation of 0.000 (12) Å, for atom C8, and the dihedral angle between the mean plane and the phenyl ring is 69.8 (9)° (see Electronic Supplementary Information [18]). In crystal packing of compound 2a, the molecules are connected by weak C–H···N and N–H···N intermolecular hydrogen bonds forming one-dimensional chains along the *b* axis (Figure 4).

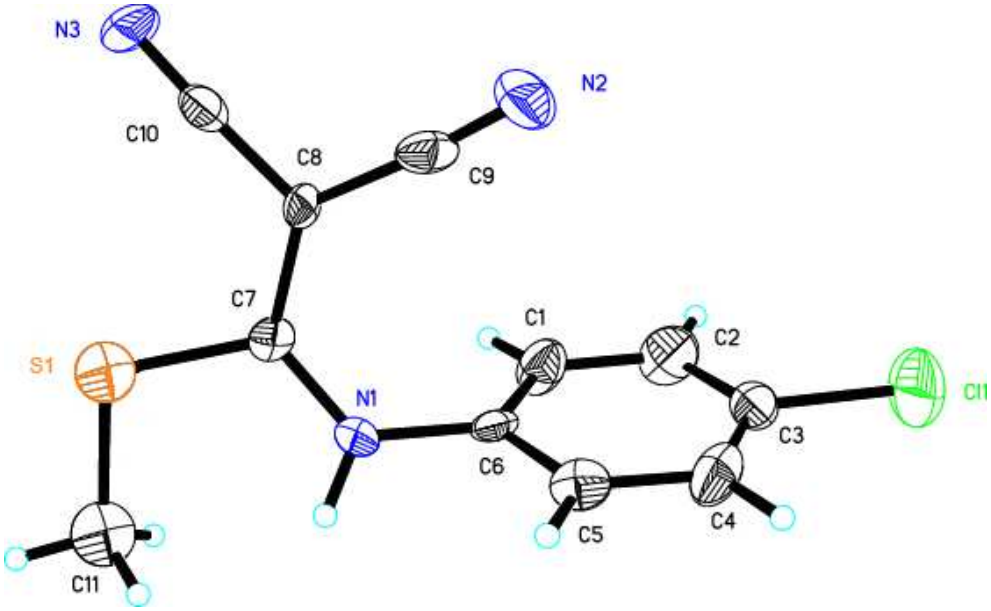

Figure 3. *The molecular structure of 2a with 50% probability displacement ellipsoids.*

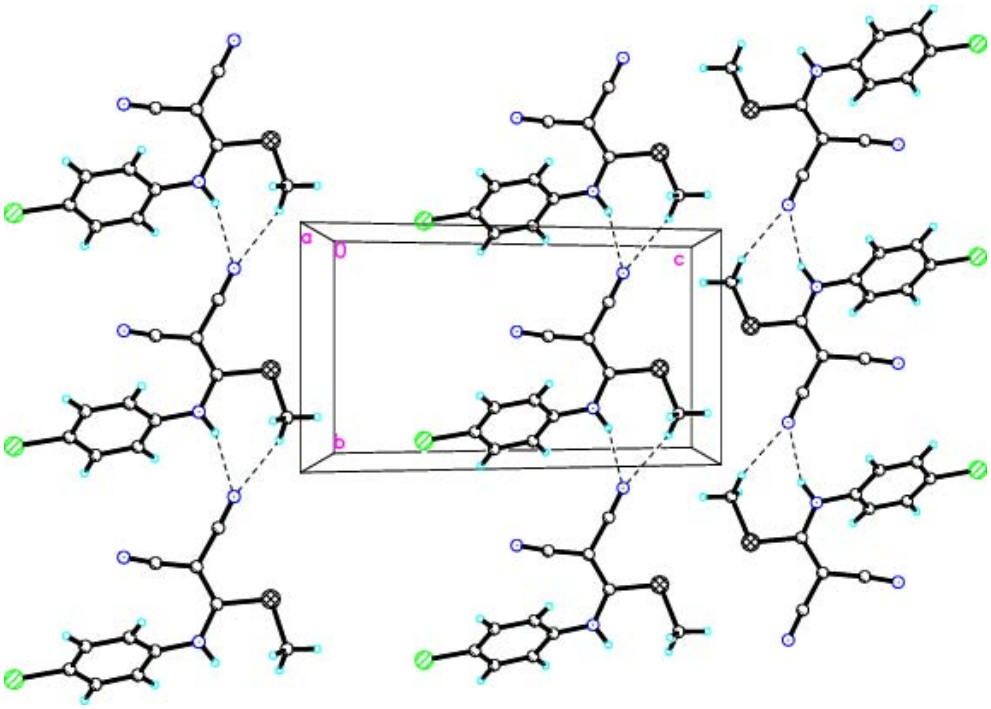

Figure 4. *Packing diagrams of compound 2a, viewed down the b axis. The dashed lines denote C-H···N and N-H···N hydrogen bonds.*

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

	Crystal 2a		**Crystal 2a**
Chemical formula	C_{11} H_8 Cl N_3 S	Absorption coefficient	0.268 mm^{-1}
Formula weight	249.72 g mol^{-1}	F (000)	512
Colour	Yellowish	Theta range for data collection	3.2-28.3 (°)
Crystal shape	Block	Reflections collected / unique	17124, 2684, R_{int}=0.077
Size mm	0.31 x 0.25 x 0.35	Completeness to theta=25.00	71.025
Temperature	293 K	Max. and min. transmission	T_{min}=0.923, T_{max}=0.939
Wavelength	0.71073Å	Refinement method	Full-matrix least-squares on F^2
Crystal system	Monoclinic	Data/ restraints/ parameters	3322/0/253
Space group	*P21/n*	Goodness-of-fit on F^2	1.36 Full-matrix least-squares on F^2
a, b, c (Å);	12.2861 (9) 7.4046 (5) 13.6025 (9)	Final R indices [I>2 σ (I)]	*R*=0.2808, *wR*=0.5367
α, β, γ ( )°	90, 109.282 (3), 90	Largest diff. peak and hole	-1.64, & 2.48 e $Å^{-3}$
Cell volume	1168.05 (14)	Calculated density	1.4393 (2) $g.cm^{-3}$
Z	4		

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

Bond	**Bond length (Å)**	**Bond**	**Bond angle (°)**
Cl1–C1	1.737 (15)	C7–S1–C11	104.5 (7)
S1–C7	1.741 (12)	C4–N1–C7	126.8 (11)
S1–C11	1.748 (15)	Cl1–C1–C2	118.3 (13)
N1–C4	1.435 (17)	N1–C4–C3	120.0 (13)
N1–C7	1.333 (16)	S1–C7–N1	119.8 (9)
N2–C9	1.133 (19)	N1–C7–C8	125.3 (11)
N3–C10	1.13 (2)	S1–C7–C8	114.9 (9)
C7–C8	1.386 (16)	C7–C8–C9	121.3 (11)
C8–C9	1.429 (19)	C7–C8–C10	125.3 (11)
C8–C10	1.443 (19)	C9–C8–C10	112.8 (11)

The above mentioned 5-aminopyrazole 3 was used as intermediates for the synthesis of pyrazolo [5, 1-*c*] [1, 2, 4] triazines, on other hand the diazotized of 5-aminopyrazoles is an excellent building block for the synthesis of the pyrazolo [5, 1-*c*] [1, 2, 4] triazine derivatives [19, 20]. Thus, diazotization of 5-aminopyrazole 3 with sodium nitrite and conc. HCl gave the corresponding 3-(4-chlorophenylamino)-1*H*-pyrazole-4-carbonitrile-5-diazonium chloride 3', which was coupled with different active methylene compounds, namely; acetylacetone and malononitrile in pyridine to afford the corresponding hydrazono derivatives A and B, respectively. When the intermediates A and B were refluxed in glacial acetic acid, the target 3-acetyl-7-(4-chlorophenylamino)-4-methylpyrazolo [5, 1-*c*] [1, 2, 4] triazine-8-carbonitrile 4 and 4-amino-7-(4-chlorophenylamino) pyrazolo [5, 1-*c*] [1, 2, 4] triazine-3, 8-dicarbonitrile 5 were obtained. The formation of 4 and 5 may be interpreted through the nuclophilic attack of ring nitrogen on acetyl or cyano groups (Figure 5).

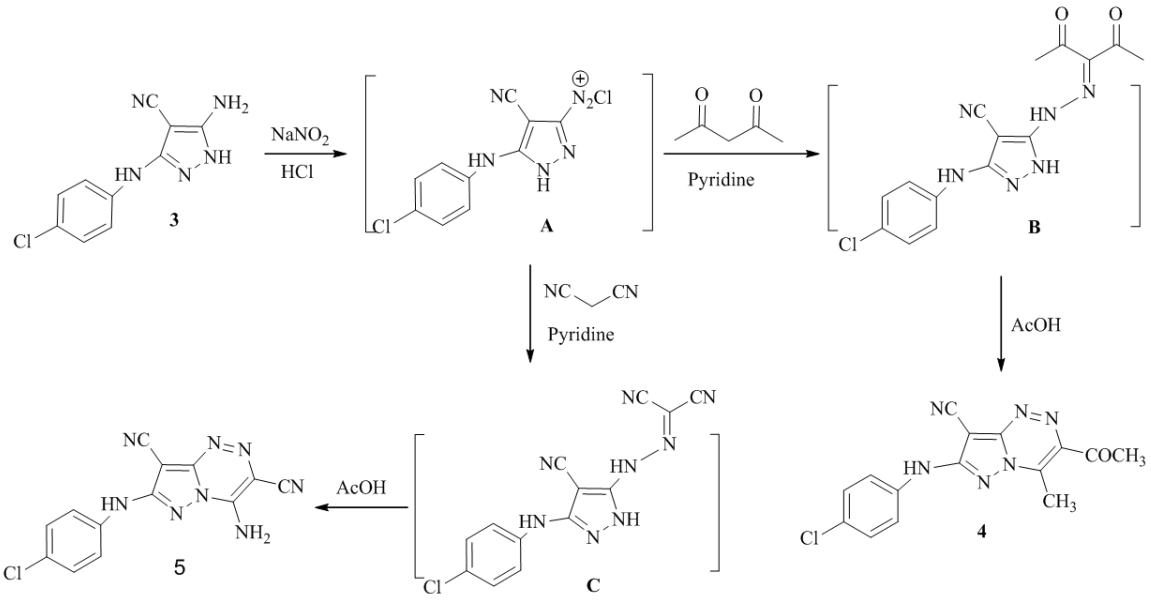

Figure 5. *Synthesis of pyrazolo [5, 1-c] [1, 2, 4] triazines 4 and 5.*

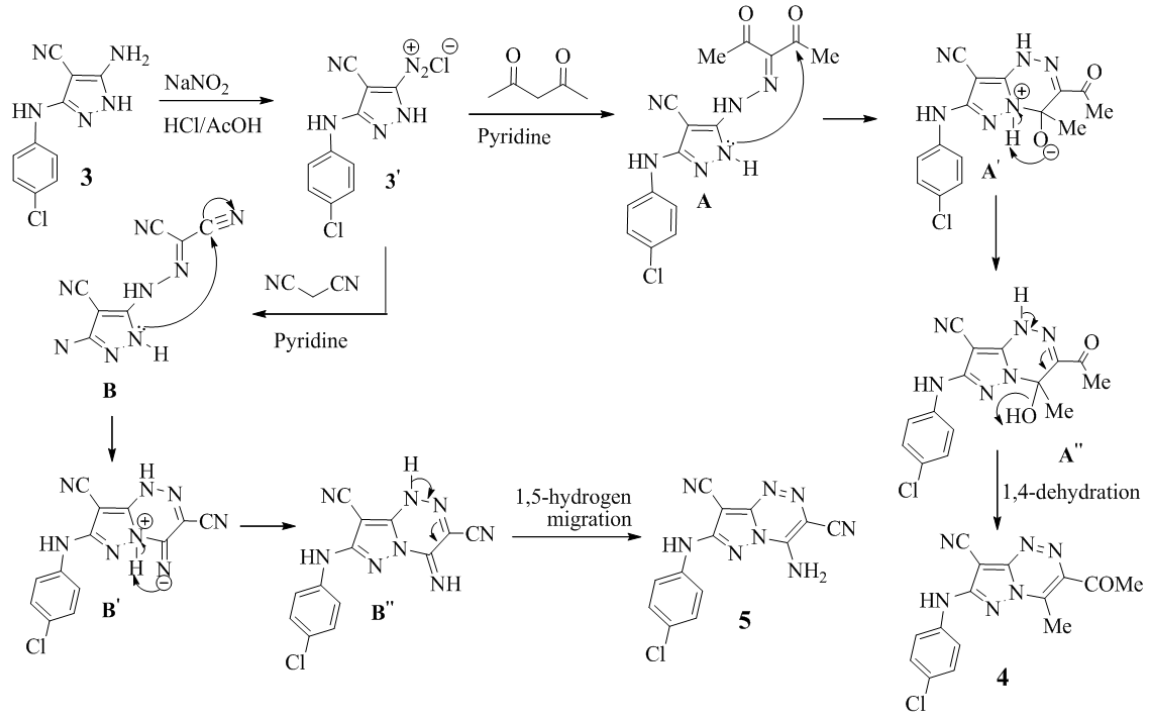

Figure 6. *Mechanism for formation of pyrazolo [5, 1-c] [1, 2, 4] triazines 4 and 5.*

The structures of 4 and 5 were elucidated on the basis of spectral data. The characteristic absorption band in the IR spectra of 4 and 5 is at ν 1583-1585 cm^{-1} for N=N stretching vibrations. Furthermore, displayed band at ν 1696 cm^{-1} in the compound 4 revealed to the carbonyl group and the NH_2 group in the compound 5 disappeared in the IR spectrum due to formation of hydrogen bonding with DMF, which used as solvent to purification of compound 5 and appeared only band at 3293 cm^{-1} for NH group. The ^1H NMR spectra of the compound 4 displayed broad signals at δ 2.82-3.02 ppm, which corresponding to six protons of two methyl groups. The ^1H NMR spectra of the compound 8 displayed broad signal at δ 9.97 ppm due to NH group attached to aromatic ring. While the signal at δ 9.48 ppm due to NH_2 group, which form the hydrogen bonding with oxygen of DMF. The structures of the isolated products 4 and 5 were supported by their direct infusion mass spectrometry (DIMS) results, which showed molecular ions corresponding to the molecular formula. The DIMS of compound 4 showed a molecular ion at *m/z*=326.75 which corresponds to the molecular formula

$C_{15}H_{11}ClN_6O$ (326.74). The DIMS of compound 5 showed a molecular ion at *m/z*=310.10 which correspond to the molecular formula $C_{13}H_7ClN_8$ M^+ requires 310.05. The molecular ion at *m/z*=73.10 which correspond to the molecular formula of DMF C_3H_7NO M^+ requires 73.07.

The mechanism for the formation of pyrazolo [5, 1-*c*] [1, 2, 4] triazines 4 and 5 is presented in Figure 6. The first step involves the formation of 3-(4-chlorophenylamino)-1*H*-pyrazole-4-carbonitrile-5-diazonium chloride 3'. The second step involves the coupling off the 3' with active methylenes as 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 4; and 1, 5-hydrogen migration in B" yields 5.

Fusion of the compound 3 with acetic anhydride under reflux for 15 minutes furnished the corresponding 1-acetyl-5-amino-3-(4-chlorophenylamino)-1*H*-pyrazole-4-carbonitrile 6 (Figure 7). The structures of the compound 6 were established on the basis of spectral data. The IR spectrum showed the absence of NH band and display band at υ 1703 cm^{-1} due to carbonyl group, while the ^{1}H-NMR spectra showed characteristic signal at δ 2.56 ppm due to methyl protons attached to carbonyl group (CH_3–CO). The structure of the isolated product 6 was supported by their direct infusion mass spectrometry (DIMS) result, which showed molecular ion corresponding to the molecular formula. The DIMS spectrum of 9 showed a molecular ion at *m/z*=275.65, which corresponding to a molecular formula $C_{12}H_{10}ClN_5O$ M^+ requires 275.69.

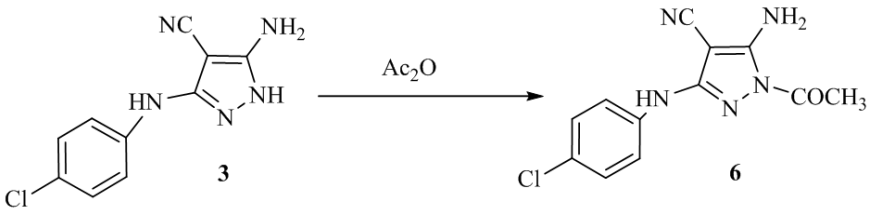

Figure 7. *Synthesis of 1-acetyl-1H-pyrazole 6.*

3.2. Antibacterial, Antifungal Evaluations and Cytotoxicity Assay

The antibacterial and antifungal activities results are listed in Table 3. The results for the pyrazolo [5, 1-c] [1, 2, 4] triazine 5 showed moderate against tested bacteria and fungi. It was also demonstrated in this study that the compound 6 exhibited good antibacterial and antifungal activities.

Table 3. *Inhibition zone (mean diameter of inhibition in mm) as a criterion of antibacterial and antifungal activities of the synthesized compounds.*

	Inhibition zone (mm)[c]				
Compound	**Antibacterial evaluation**			**Antifungal evaluation**	
	Staphylococcus aureus S276	***Staphylococcus eperdermidis S273***	***Pseudomonas aeruginosa 15442***	***Aspergillusniger UPMC 393***	***Aspergillusbrasiliensis ATCC16404***
5	7±0.57	9±0.57	8±0.55	9±0.58	7±0.58
6	10±0.58	10±0.58	11±0.58	10±0.58	10±0.58
[a]Streptomycin	20	23	27	-	-
[b]Nystatin	-	-	-	20	23

[a]Setreptomycin as reference drug for bacteria.
[b]Nystatin as reference drug for fungi.
[c]Values are mean inhibition zone (mm) ± S. D of results done in triplicate.
6 mm is the diameter of the disc

Cytotoxicity results of two tested compounds are summarized in Table 4. The CC_{50} value was graphically obtained by plotting the percentage growth inhibition against the corresponding different concentrations of the test compound used. The CC_{50} values for compounds 8 and 9 have CC_{50} value of more than 30 µg/ ml. According to Chandrashekar et al [21], CC_{50} value of more than 20 µg/ml can be considered as non cytotoxic.

Table 4. *Cytotoxicity (CC_{50}) of two compounds against Human MCF-7 cells.*

Compounds	CC50 value (µg/ml)
5	>30
6	>30

4. Conclusions

We could successfully synthesized new pyrazolo [5, 1-*c*] [1, 2, 4] triazines from 5-aminopyrazole 3. In this paper, we synthesized new pyrazolo [5, 1-*c*] [1, 2, 4] triazines 4, 5 by the reaction of 5-aminopyrazole 3 with respective malononitrile, and acetyl acetone. Also, 5-aminopyrazole 3 reacted with acetic anhydride gave the corresponding 1-acetyl-1*H*-pyrazole 6. 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-aminopyrazole 3 was of the same

starting material used for the preparation of all those compounds. Some of the prepared compounds shown unpromising antibacterial, antifungal activities and cytotoxicty.

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