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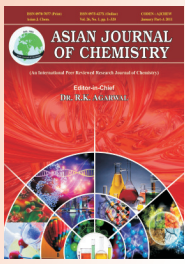

Synthesis, Antibacterial and Cytotoxic Activities of Cyanoenonebenzenesulfonamide, Acetamide and Pyridine-3-carbonitrile Derivatives

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A series of sulfonamides having biologically active acrylamides moieties (**2**, **3**, **5**), penta-2,4-dienamide (**4**), chromene-2-carboxamide (**6**), acetamide derivatives (**7**, **8**) and pyridone derivative (**9**) were prepared. The structure of the synthesized compounds was verified by elemental analyses, IR, ^{1}H NMR and ^{13}C NMR spectra. In addition, the structure compound **9** was confirmed through X-ray crystallography. The antibacterial activities of all the synthesized compounds were evaluated against a panel of multi-drug resistant (MDR) Gram-negative strains of *Escherichia coli*, *Enterobactera erogenes*, *Klebsie-llapneumoniae* and *Pseudomonas aeruginosa* whilst their cytotoxic effects were tested against the leukemia CCRF-CEM and its adriamycin resistant subline CEM/ADR5000 cell lines. Compounds **1**-**5**, **7** and **8** displayed or weak activities on at least one of the ten tested bacterial strains. The best MIC value of 64 µg/mL was obtained with **3** against *E. aerogenes* ATCC 13048. None of the compounds displayed significant cytotoxic effect against the two studied leukemia cell line. Nevertheless, the activity of compounds **4**, **5**, **8** and **9** were better than that of doxorubicin towards the resistant CEM/ADR5000 cell line.

Keywords: Novel sulfonamides, Acetamides, Pyridone, Antibacterial, Anticancer activities.

INTRODUCTION

Sulfonamides are a group of compounds with known pharmacological activities where various derivatives are used as antibacterial[1-3], antiinflammatory[4], anticancer[5,6] and antiviral[7] agents. Some sulfonamides have been reported to show anticancer activity *in vitro* and *in vivo*, including several chromene derivatives[8-11]; In addition, a number of mechanisms have been proposed to explain the anticancer activity of sulfonamides, the most prominent of which is inhibition of carbonic anhydrase[12-14] as suggested by Chegwidden and Spencer[15]. Carbonic anhydrase is upregulated in solid tumors in response to the hypoxic tumor microenvironment and its inhibition leads to reduction in provision of bicarbonate which is needed to combat the deleterious effects of a high rate of glycolytic metabolism. Consequently, tumor growth is reduced when carbonic anhydrase is inhibited. In addition, disruption of microtubule assembly by sulfonamides has also been observed and derivatives in which the NH of the sulfonamide group is substituted by an aryl group have shown potent anticancer activity[16,17]. Also, it was found that the acetamide derivatives constitute an important class of drug, with several types of pharmacological agents possessing anticancer activity[18-20]. A large number of structurally acetamides have ultimately been reported to show substantial anticancer activity *in vitro* and *in vivo*. Considering the ever-growing bacterial resistance there is an immense need to develop new methods to treat bacterial infections. Traditional methods of antibiotic discovery have failed to keep pace with the evolution of this resistance. The pilus is an organelle that is vital for the bacteria in order to adhere to and infect host cells, as well as in establishing biofilms. Disabling these organelles renders the bacteria avirulent. This will at least in theory lead to more tolerated antibiotics and hence slower development of resistance. It has been known for several years that the ring-fused 2-pyridones exhibit biological activity against the pili assembly machinery in 5 uropathogenic *E. coli*, termed the chaperone usher pathway. The chaperone usher pathway is a complex cascade reaction which occurs in the periplasm of Gram-negative bacterium. There are many different types of pili, of which the Type 1 and P-pili involved in the infection of bladder cells and kidneys are the most studied. Pili are built up from repeating protein subunits; these subunits are called Pap in P-pili and Fim in Type 1 pili. One of the key proteins in the

assembly of P pili is the chaperone PapD, which is responsible for the delivery of the pili building blocks from the surface of the inner membrane to the assembly area located at the outer membrane. The 2-pyridone core is an interesting heterocycle which is common to many natural products (Fig. 1) as well as synthetic compounds, which possesses wide spread biological characteristics. The 2-pyridone core is also present in registered pharmaceuticals for instance Corotrop® and Primacor® both are used in the treatment of heart failure. The active ingredient in these drugs is the 2-pyridone based compound milrinone (Fig. 1), which is a phosphodiesterase inhibitor. Sebiprox®, used in treatment of dandruff, also contains a 2-pyridone based active ingredient called ciclopirox (Fig. 1). Compounds containing heteroaromatic rings frequently play an important role as scaffolds of bioactive substances. It is well known that pyridone and its derivatives are among the most popular *N*-heteroaromatic compounds integrated into the structures of many pharmaceutical compounds and their structural units occur in various molecules exhibiting diverse biological activities[21-25]. Based on the above information and as a continuation of our previous work on anticancer[26-28], we report the synthesis of sulfonamides, acetamides and 2-pyridone derivative which is expected to exhibit antibacterial and anticancer activities.

EXPERIMENTAL

Melting points (°C, uncorrected) were determined in open capillaries on a Gallenkemp melting point apparatus (Sanyo Gallenkemp, Southborough, UK). Pre-coated silica gel plates (silica gel 0.25 mm, 60 G F 254; Merck, Germany) were used for thin layer chromatography, dichloromethane/methanol (9.5:0.5 mL) mixture was used as a developing solvent system. IR spectra were recorded in KBr discs using IR-470 Shimadzu spectrometer (Shimadzu, Tokyo, Japan). NMR spectra in (DMSO-d_6) were recorded on Bruker Ac-500 ultra-shield NMR spectrometer (Bruker, Flawil, Switzerland, δ ppm) at 500 MHz, using TMS as internal standard. Elemental analyses were performed on Carlo Erba 1108 Elemental Analyzer (Heraeus, Hanau, Germany). All compounds were within ± 0.4 % of the theoretical values.

Synthesis of benzenesulfonamide derivatives (1-6): 2-cyano-*N*-(4-sulfamoylphenyl) acetamide (**1**). Compound **1** was prepared according to reported method [29].

2-Cyano-3-(4-substituted)-*N*-(4-sulfamoylphenyl) acrylamides (2-5)

General procedure: A mixture of **1** (2.11 g, 0.01 mol) and aromatic aldehydes (0.012 mol) in absolute ethanol (20 mL) containing piperidine (0.5 mL) was refluxed for 4 h. The obtained solid was filtered while hot and crystallized from dioxane to give **2-5**, respectively.

2-Cyano-3-(4-fluorophenyl)-*N*-(4-sulfamoylphenyl) acrylamide (2): Yield % 83; m.p. 265.9 °C; IR (KBr, ν_{max}, cm^{-1}): 3337, 3310, 3290 (NH, NH_2), 3065 (CH arom.), 2966, 2876 (CH aliph.), 2205 (C≡N), 1685 (C=O), 1396, 1184 (SO_2): ^{1}H NMR in (DMSO-d_6) δ: 7.0-8.1 [m, 9H, Ar-H + SO_2NH_2], 8.3 [s, 1H, CH]. 10.9 [s, 1H, NH, D_2O exchangeable]. ^{13}C NMR in (DMSO-d_6): 105.6, 114.3 (2), 114.9, 122.6 (2) 126.7 (2), 127.8 (2), 129.9, 130.8, 133.7, 152.6, 163.1, 164.9. Anal. Calcd; for $C_{16}H_{12}N_3O_3S$ (345.35): C, 55.65; H, 5.50; N, 12.17; found: C, 55.38; H, 5.22; N, 12.51.

2-Cyano-3-(2,4-dinitrophenyl)-*N*-(4-sulfamoylphenyl)-acrylamide (3): Yield % 88; m.p. 215.6 °C; IR (KBr, ν_{max}, cm^{-1}): 3416, 3372, 3286 (NH, NH_2), 3096 (CH arom.), 2976, 2836 (CH aliph.), 2218 (C≡N), 1696 (C=O), 1378, 1161 (SO_2). ^{1}H NMR in (DMSO-d_6); δ: 7.1-8.4 [m, 9H, Ar-H + SO_2NH_2], 8.7 [s, 1H, CH], 11.3 [s, 1H, NH; D_2O-exchangeable]. ^{13}C NMR in (DMSO-d_6): 109.0, 116.7, 118.8, 120.6 (2), 128.1 (2), 128.7, 130.8, 135.9, 138.4, 141.2, 146.7, 150.1, 155.2, 165.3. Anal. Calcd; for $C_{16}H_{11}N_5O_7S$ (417.35): C, 46.05; H, 2.66; N, 16.78; found: C, 46.36; H, 2.43; N, 16.47.

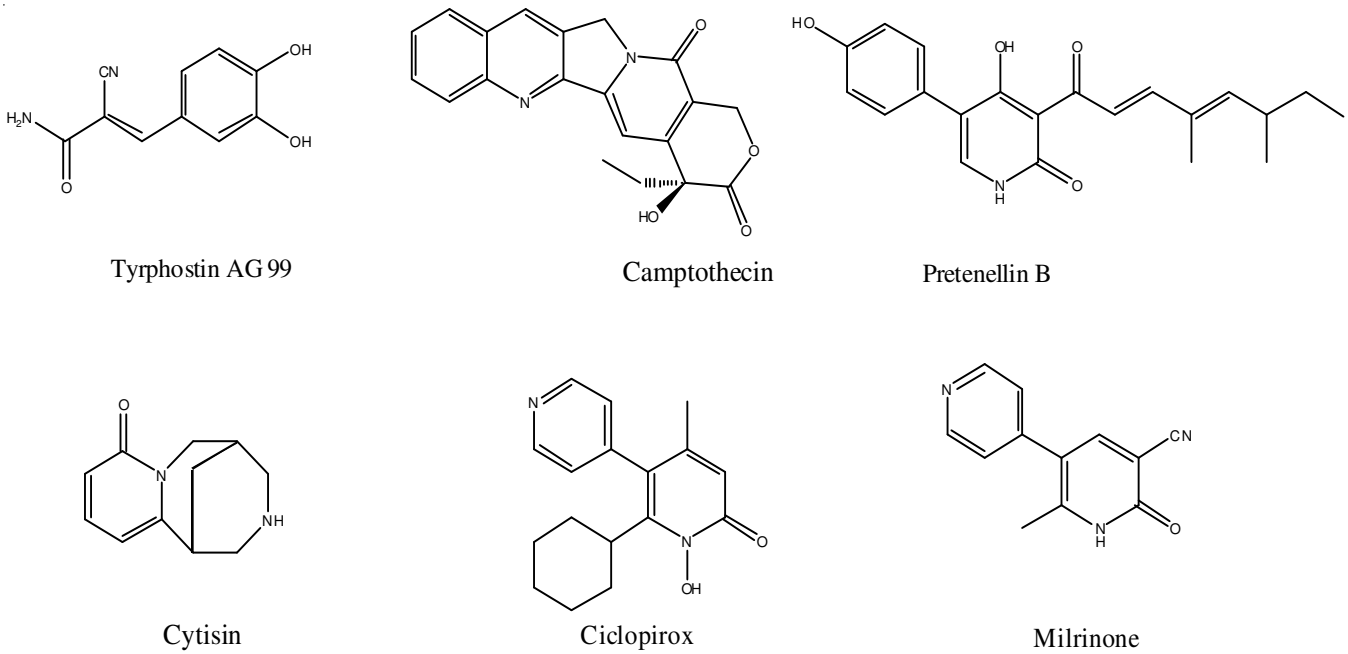


Fig. 1. Examples of naturally occurring 2-pyridones, together with two examples of 2-pyridone based compounds incorporated in registered pharmaceuticals

2-Cyano-5-[4-(dimethylamino)-*N*-(4-sulfamoylphenyl]-penta-2,4-dienamide (4): Yield % 89; m.p. 240.2 °C; IR (KBr, ν_{max}, cm^{-1}): 3360, 3315, 3272 (NH, NH_2), 3091 (CH arom.), 2946, 2860 (CH aliph.), 2212 (C≡N) 1676 (C=O), 1377, 1156 (SO_2). ^{1}H NMR in (DMSO-d_6); δ: 3.0 [s, 6H, $N(CH_3)_2$], 6.6, 6.9 [2d, 2H, CH=CH; J = 7.0, 7.1 Hz], 7.5 [s, 1H, CH], 7.7-8.0 [m, 10H, Ar-H + SO_2NH_2], 10.3 [s, 1H, NH; D_2O-exchangeable]. ^{13}C NMR in (DMSO-d_6): 40.1 (2), 96.7, 112.4 (2), 115.8,122.1 (2),122.8, 126.4, 126.8 (2), 129.3 (2), 130.6, 138.9, 141.5, 149.7, 164.2. Anal. Calcd; for $C_{20}H_{20}N_4O_3S$ (396.46): C, 60.59; H, 5.08; N, 14.13; found: C, 60.31; H, 5.37, N, 13.84.

3-(4-Bromophenyl)-2-cyano-*N*-(4-sulfamoylphenyl)-acrylamide (5): Yield % 79; m.p. 233.5 °C; IR (KBr, ν_{max}, cm^{-1}): 3390, 3309, 3287 (NH, NH_2), 3100 (CH arom.), 2992, 2920 (CH aliph.), 2219 (C≡N) 1689 (C=O), 1336, 1156 (SO_2). ^{1}H NMR in (DMSO-d_6); δ: 7.0-7.9 [m, 10H, Ar-H + SO_2NH_2], 8.4 [s, 1H, CH], 11.2 [s, 1H, NH, D_2O-exchangeable]. ^{13}C NMR in (DMSO-d_6): 108.2, 115.1, 119.8 (2), 121.7, 128.4 (2), 129.6 (2), 130.7 (2), 133.8, 136.7, 140.8, 155.1, 164.6. Anal. Calcd; for $C_{16}H_{12}BrN_3O_3S$ (406.25): C, 47.30; H, 2.98; N, 10.34; found: C, 47.67; H, 2.65; N, 10.08.

3-Oxo-*N*-(4-sulfamoylphenyl)-3*H*-benzo[*f*]chromene-2-carboxamide (6): To a solution of **1** (2.11 g, 0.01 mol) in acetic anhydride (15 mL), 2-hydroxy-1-naphthaldehyde (1.72 g, 0.01 mol) and fused sodium acetate (0.8 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 2 h and the obtained solid was crystallized from acetic acid to give compound **6**.

Yield 79 %; m.p. 287.1 °C; IR (KBr, ν_{max}, cm^{-1}): 3343, 3309, 3275 (NH, NH_2), 3076 (CH arom.), 1700, 1673 (2C=O), 1374, 1184 (SO_2). ^{1}H NMR in (DMSO-d_6); δ: 7.4- 8.1 [m, 12H, Ar-H + SO_2NH_2], 8.5 [s, 1H, CH], 10.3 [s, 1H, NH, D_2O-exchangeable]. ^{13}C NMR in (DMSO-d_6): 116.3, 116.9, 118.2, 120.7 (2), 121.4, 122.6, 125.2, 127.3 (2), 128.2 (2), 131.7 (2), 135.7 (2), 142.6, 154.8, 168.9, 172.0. Anal. Calcd; for $C_{20}H_{14}N_2O_5S$ (394.40): C, 60.91; H, 3.58; N, 7.10; found: C, 61.13; H, 3.81; N, 7.42.

2-Cyano-*N*-(2-ethylphenyl) acetamide (7): A mixture of 2-ethylaniline (1.21 g, 0.01 mol) and ethyl cyanoacetate (1.13 g, 0.01 mol) was fused at 220 °C for 3 h. The reaction mixture was concentrated and cooled. The obtained product was crystallized from ethanol to give compound **7**[33].

2- Cyano-*N*-(3-ethylphenyl) acetamide (8): A mixture of 3-ethylaniline (1.21 g, 0.01 mol) and ethyl cyanoacetate (1.13 g, 0.01 mol) was fused at 220 °C for 3 h. The reaction mixture was concentrated and cooled. The obtained product was crystallized from ethanol to give compound **8**[33].

Yield, 88 %, m.p. 86-88 °C; IR (KBr, ν_{max}, cm^{-1}): 3317 (NH), 3100 (CH. arom.), 2960, 2870 (CH aliph.), 2260 (C≡N), 1670 (C=O). ^{1}H NMR spectrum of **3** in (DMSO-d_6) δ : 1.2 [t, 3H, CH_3], 2.6 [q, 2H, CH_2], 3.6 [s, 2H, CH_2], 4.2 [s, 1H, NH, exchangeable with D_2O], 7.03-7.7 [m, 4H, Ar-H]. Anal. Calcd; for $C_{11}H_{12}N_2O$: C, 70.21; H, 6.38, 14.89; found: C, 70.50; H, 6.10; N, 14.60.

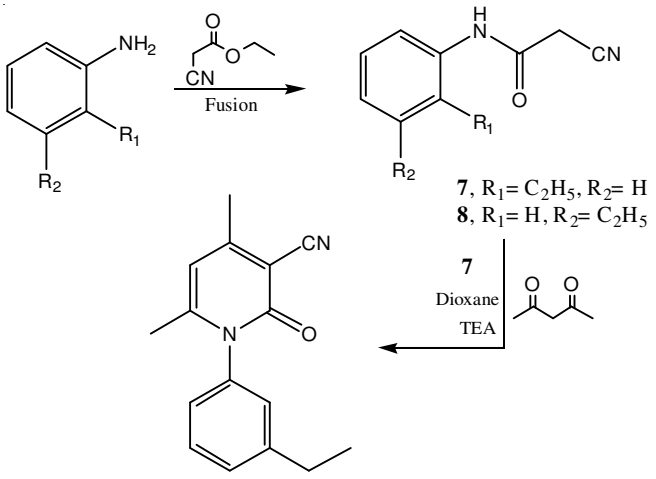
1-(3-Ethylphenyl)-4,6-dimethyl-2-oxo-1,2-dihydropyridine-3-carbonitrile (9): A mixture of 2-cyano-*N*-(3-ethylphenyl)acetamide **3** (1.88 g, 0.01 mol) and acetylacetone (1 g, 0.01 mol) in absolute ethanol (50 mL) containing piperidine (0.5 mL) were refluxed for 5 h. The reaction mixture was triturated with ethanol and the solid obtained was recrystallized from ethanol to give compound **9**[30].

X-Ray analysis: A single-crystal suitable for an X-ray structural analysis was obtained by slow evaporation from an ethanolic solution at room temperature. Compound **9** (Fig. 2), the 1,2-dihydropyridine ring (N1/C1-C5) is essentially planar with a maximum deviation of 0.021 (1) A at atom N1 and almost perpendicular with the benzene ring (C6-C11) with a dihedral angle of 85.33 (8)°. Bond lengths and angles are within the normal ranges and are comparable to those in the related structures[34,35]. The crystal structure is shown in (Fig. 3). The molecules are linked into a one-dimensional chain along the b-axis *via* C4-H4A·O1 interactions[30].

Antibacterial assay: The studied microorganisms included reference (ATCC) and multidrug resistant strains of *Pseudomonas aeruginosa* (PA01, PA124), *Klebsiella pneumoniae* (ATCC11296, KP55), *Escherichia coli* (ATCC8739, AG100ATet, AG102) and *Enterobacter aerogenes* (ATCC-13048, CM64, EA289) obtained from the American Type Culture Collection. Their bacterial feature was previously reported[36]. They were maintained on agar slant at 4 °C and sub-cultured on a fresh Mueller-Hinton Agar plates 24 h prior to any antimicrobial test[37]. The Mueller Hinton Broth (MHB) was used for the minimal inhibitory concentration (MIC) determinations. Chloramphenicol (Sigma-Aldrich, St. Quentin Fallavier, France) was used as reference antibiotics (RA) respectively against bacteria. *p*-Iodonitrotetrazolium chloride (INT, Sigma-Aldrich) was used as microbial growth indicator. The MIC determinations on bacteria was conducted using rapid INT colourimetric assay according to described methods[38] with some modifications. Briefly, the test sample was first of all dissolved in 10 % (v/v) DMSO/MHB to give a final concentration of 512 µg/mL and serially diluted twofold to obtain concentration ranges. 100 µL of each concentration was added in a well (96-well microplate) containing 95 µL of MHB and 5 µL of inoculum (standardized at 1.5×10^6 CFU/mL by adjusting the optical density to 0.1 at 600 nm SHIMADZU UV-120-01 spectrophotometer)[39]. The final concentration of DMSO in the well was less than 3 % (preliminary analyses with 3 % (v/v) DMSO do not alter the growth of the test organisms). The negative control well consisted of 195 µL of MHB and 5 µL of the standard inoculum[40]. The plates were covered with a sterile plate sealer, then agitated to mix the contents of the wells using a plate shaker and incubated at 37 °C for 18 h. The assay was repeated three times in triplicate. The MIC of samples was detected following addition (40 µL) of 0.2 mg/mL *p*-iodonitrotetrazolium chloride and incubation at 37 °C for 0.5 h[37]. Viable microorganisms reduced the yellow dye to a pink colour. MIC was defined as the lowest sample concentration that prevented this change and exhibited complete inhibition of bacterial growth[41].

Cytotoxicity: The resazurin reduction assay[42] was performed to assess the cytotoxicity of the synthesized compounds and doxorubicin towards various sensitive leukemia CCRF-CEM cells and its adriamycin resistant subline CEM/ADR5000. The assay is based on the reduction of the indicator dye, resazurin, to the highly fluorescent resorufin by viable cells. Non-viable cells rapidly lose their metabolic capacity to reduce

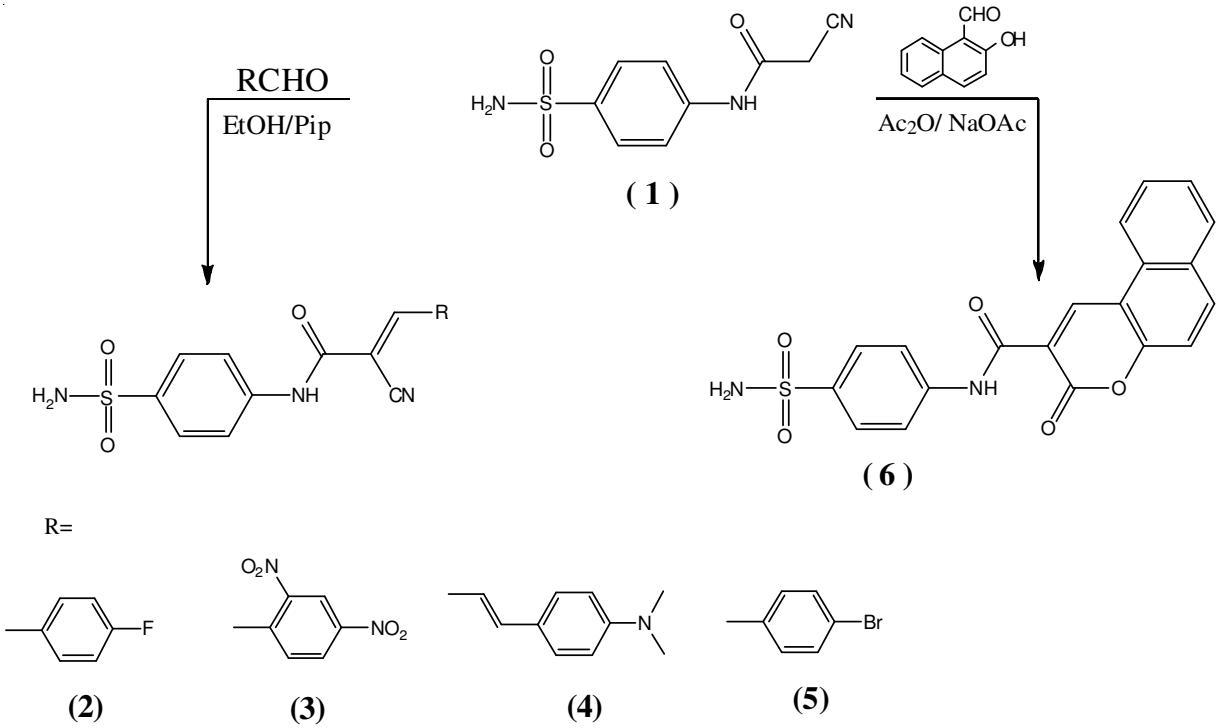
resazurin and, thus, do not produce fluorescent signals anymore. aliquots of 2×10^4 cells per well were seeded in 96-well-plates in a total volume of 100 µL. The studied compound was immediately added in varying concentrations in an additional 100 µL of culture medium to obtain a total volume of 200 µL/well. After 72 h, resazurin (Sigma-Aldrich, Schnelldorf, Germany) (20 µL, 0.01 % w/v) in double distilled H_2O was added to each well and the plates were incubated at 37 °C for 4 h. Fluorescence was measured on an Infinite M2000 ProTM plate reader (Tecan, Crailsheim, Germany) using an excitation wavelength of 544 nm and an emission wavelength of 590 nm. Each assay was done at least twice with six replicates each. The viability was evaluated based on a comparison with untreated cells. IC_{50} values represent the compound concentrations required to inhibit 50 % of cell proliferation and were calculated from a calibration curve by linear regression using Microsoft Excel.



Scheme-II: Formation of acetamide and pyridone derivatives (**7-9**)

RESULTS AND DISCUSSION

The aim of this work was to design and synthesis of a series of sulfonamides having the biologically active cyano enone Michael acceptor moiety (**2-5**), benzochromene (**6**), acetamides (**7**, **8**) and pyridine (**9**) (**Scheme I** and **II**) and evaluation of their antibacterial and anticancer activities. 2-Cyano-*N*-(4-sulfamoylphenyl) acetamide (**1**)[29], is a highly reactive intermediate extensively used for synthesis of heterocyclic compounds. Thus, condensation of compound **1** with aromatic aldehydes in refluxing absolute ethanol in the presence of a catalytic amount of piperidine afforded the corresponding sulfonamide derivatives (**2-5**), respectively, (**Scheme-1**). The structures of the later products were assigned on the basis of their analytical and spectral data. The IR spectra of the reaction products showed in each case four absorption bands corresponding to NH, NH_2 functions in the region 3416-3272 cm^{-1}, in addition to a carbonyl absorption band in the region 1696-1676 cm^{-1}, absorption bands due to C=N in the region 2219- 2205 cm^{-1} and SO_2 functions in the region 1396-1156 cm^{-1}. ^1H NMR spectra of compounds **2-5** in (DMSO-d_6) revealed a singlet in the region 10.3-11.3 ppm corresponding to a NH group. Also, interaction of compound **1** with 2-hydroxy-1-naphthaldehyde in acetic anhydride containing anhydrous sodium acetate furnished the corresponding 3-oxo-*N*-(4-sulfamoylphenyl)-3-*H*-benzo[*f*]chromene-2-carboxamide (**6**). The structure of compound **6** was confirmed on the basis of elemental analysis and spectral data. IR spectrum showed bands at 3343, 3309, 3275 cm^{-1} (NH, NH_2), 1700, 1673 cm^{-1} (2C=O), 1374, 1184 (SO_2). ^1H NMR spectrum in (DMSO-d_6) revealed signals at 8.5 ppm due to the CH group of chromene and 10.3 ppm corresponding to a NH group. Interaction of 2-ethyl or 3-ethylaniline with ethyl cyanoacetate furnished the



Scheme-I: Synthesis of benzenesulfonamide derivatives (**1-6**)

corresponding acetamide derivatives **7** and **8,** respectively. The structure of compounds **7** and **8** was proved in the basis of elemental analysis and spectral data. The corresponding pyridone derivative **9** was obtained *via* reaction of compound **8** with acetylacetone in the presence of acatalytic amount of piperedine. The structure of compound **9** was established by elemental analysis, spectral data. In addition the structure compound **9** was confirmed through X-ray crystallography[30] (Figs. 2, 3).

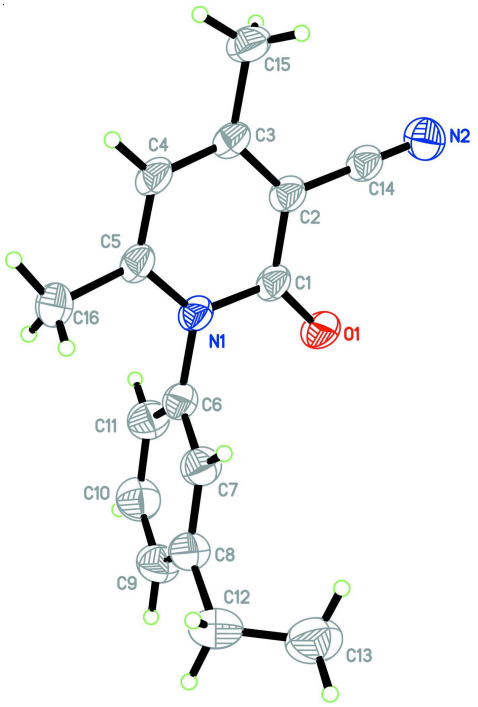


Fig. 2. Molecular structure of compound **8**, showing 30 % probability displacement ellipsoids and the atom-numbering scheme

Biological activity: The results of the antibacterial assays (Table-1) indicated that compounds **1-5**, **7** and **8** displayed moderate (10 < MIC < 100) or weak (100 < MIC < 625) activities[31] on at least one of the ten tested bacterial strains (Table-1). No activity was observed with compounds **6** and **9**. The best MIC value of 64 µg/mL was obtained with 3 against *E. aerogenes* ATCC 13048. However, none of the studied compounds was as active as choramphenicol. In cytotoxicity as says, none of

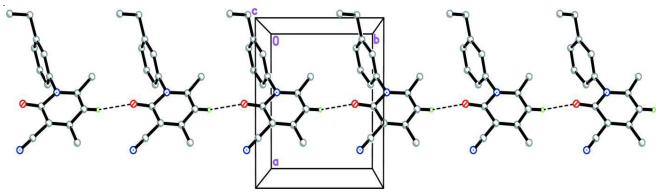


Fig. 3. A crystal packing diagram of compound **8** viewed along the c axis. For the sake of clarity, H atoms not involved in the intermolecular interactions (dashed lines) have been omitted

the compounds displayed significant activities (IC_{50} values below 10 µM)[32] against the two studied leukemia cell line (Table-2). Nevertheless, the activity of compounds **4**, **5**, **8** and **9** (IC_{50} < 40 µg/mL) were better than that of doxorubicin towards the resistant CEM/ADR5000 cell line.

TABLE-2
CYTOTOXICITY OF THE SYNTHESIZED COMPOUNDS AND DOXORUBICIN LEUKEMIA CCRF-CEM AND CEM/ADR5000 CELL LINES AS DETERMINED BY THE RESAZURIN REDUCTION ASSAY

Compound	Leukemia cell line and IC_{50} values (µg/mL)	
	CCRF-CEM	CEM/ADR5000
1	> 40	> 40
2	> 40	> 40
3	> 40	> 40
4	> 40	37.83 ± 0.88
5	> 40	38.60 ± 1.22
6	> 40	> 40
7	> 40	> 40
8	37.67 ± 2.94	38.91 ± 1.28
9	> 40	33.13 ± 7.23
Doxorubicin	0.13 ± 0.01	106.05 ± 7.77

Conclusion

The objective of the present study was to synthesize and evaluate the antibacterial and anticancer activities of some sulfonamide, benzochromene, acetamide and pyridone derivatives. Though none of the studied compound displayed significant antibacterial or cytotoxic activities, some of them such as **3** (against bacteria) as well as **4**, **5**, **8** and **9** (against cancer cell line) could serve as lead molecules to synthesize new derivative with promising effects.

TABLE-1
MINIMAL INHIBITORY CONCENTRATION (MIC) OF THE SYNTHESIZED COMPOUNDS AND CHLORAMPHENICOL ON THE STUDIED MICROORGANISMS

Compound	Microorganisms and MIC (µg/mL)									
	E. coli			*E. aerogenes*			*K. pneumoniae*		*P. aeruginosa*	
	ATCC8739	$AG100A_{Tet}$	AG102	ATCC13048	EA-CM64	EA289	ATCC11296	KP55	PA01	PA124
1	> 512	> 512	512	> 512	> 512	> 512	> 512	> 512	> 512	512
2	> 512	> 512	128	> 512	> 512	> 512	> 512	512	512	> 512
3	128	128	128	64	> 128	> 128	128	128	128	> 128
4	> 512	> 512	> 512	128	> 512	> 512	256	256	> 512	> 512
5	> 512	> 512	> 512	> 512	> 512	> 512	512	> 512	> 512	> 512
6	> 512	> 512	> 512	> 512	> 512	> 512	> 512	> 512	> 512	> 512
7	> 512	> 512	512	> 512	> 512	> 512	> 512	> 512	> 512	512
8	> 512	512	512	> 512	> 512	> 512	> 512	> 512	> 512	> 512
9	> 512	> 512	> 512	> 512	> 512	> 512	> 512	> 512	> 512	> 512
CHL	2	32	16	8	> 256	256	4	64	32	64

CHL: Chloramphenicol

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