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NOVEL HETEROCYCLIC DERTVATIVES OF 2-QUINOLINONE ASSOCIATED WITH ANTIBACTERIAL AND ANTITUMOR POTENCIES

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Abstract – 3-(2,2-Dichloroacetyl)-4-hydroxy-1-methylquinolin-2(1*H*)-one (**2**) was utilized to obtain several new quinolinones bearing imidazole, quinoxaline, 1,2,4-triazine, pyridotriazine and triazolotriazine heterocycles as substituents at position 3. Condensation of compound **2** with aminotriazine **11** afforded the iminone **12** which was subjected to annellation reactions leading to some new quinolinones fused at face [c]; *viz.* pyrazole, pyrimidine and benzodiazepine. Structure of the new compounds was confirmed by spectral and elemental analyses. The synthesized compounds were screened *in vitro* for their antimicrobial and antitumor activities.

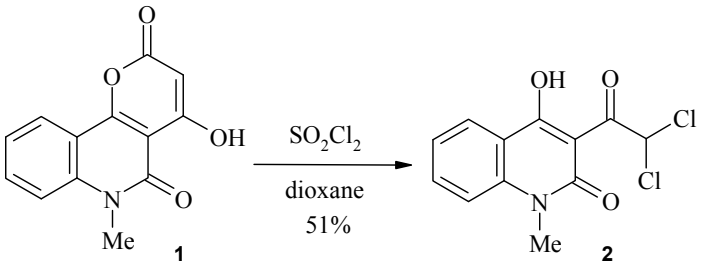
INTRODUCTION

The chemistry of quinolinone derivatives has been of increasing interest since many of these compounds have found useful chemotherapeutic agents. For example, quinolinones revealed potential hepatitis C polymerase inhibitors.[1,2] Also, many quinolinone derivatives were found active as microsomal prostaglandin E2 synthase-1[3] and selective iNOS.[4] Many quinolinone derivatives have been found active as antibacterial,[5,6] anti-hepatitis B virus (HBV) activities,[7,8] anti-HIV-1 agents.[9] Combination of pyrazole nucleus with quinoline moiety, in one molecular framework, was reported in literature to possess biological activity.[10-12] Also, pyrimido[5,4-*c*]quinolin-5(1*H*)-ones showed antioxidant and toxicological activities.[13] Many pyrimidoquinolines have antiviral activity at nanomolar concentrations against HIV-1,[14] and exhibited good antibacterial and antifungal activity.[15] In addition, imidazole derivatives display efficacy in human tumor xenograft models.[16] On the other hand, 1,2,4-triazine derivatives have been shown to have significant antitumor activities with simultaneous non-toxicity to human normal cell line and HSF cells.[17] Furthermore, 1,2,4-triazine derivatives exhibited comparable antibacterial potencies

in vitro as that of ampicillin,[18] and significant broad cytotoxic activity against cancer cell in low micromolar range.[19] Based on these findings, it was of interest to introduce a new series of quinoline and its fused systems incorporated with the above biologically active moieties, especially 1,2,4-triazine, for the evaluation of their antimicrobial and antitumor properties.

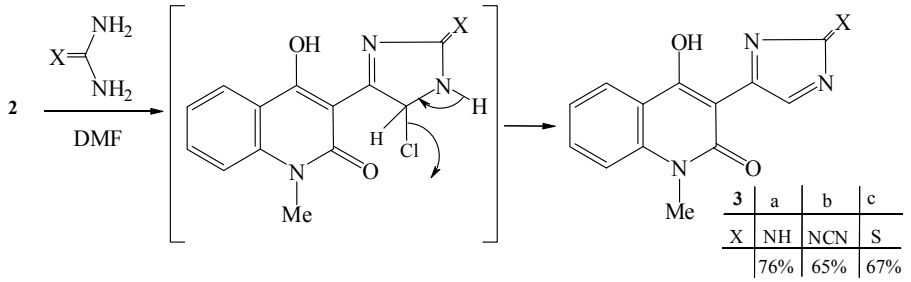
RESULTS AND DISCUSSION

The dichloroacetyl derivative **2** was prepared by heating pyranoquinolinedione **1** with sulfuryl chloride in dioxane (Scheme 1).[20,21]



Scheme 1

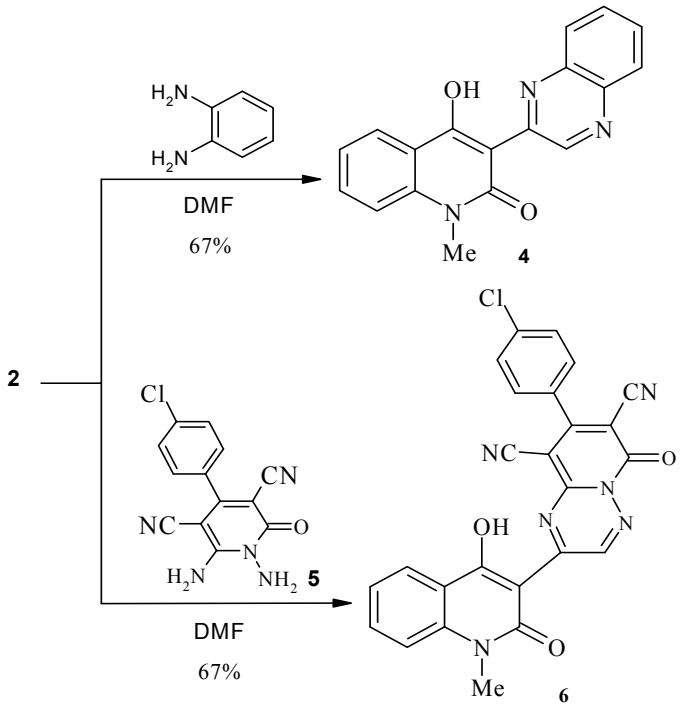
Reaction of compound **2** with some 1,3-binucleophilic reagents, *namely;* guanidine hydrochloride, cyanoguanidine, and thiourea, was carried out under reflux in DMF, to afford the corresponding 3-imidazolylquinolinones **3a–c**, respectively (Scheme 2). The ^{1}H-NMR spectrum of compound **3a** revealed the presence of broad singlet at 9.74 ppm (NH). Moreover, its mass spectrum recorded the molecular ion peak at *m/z* 254 which agree well with the molecular formula and supports the identity of the structure. Also, the IR spectrum of compound **3b** showed a characteristic absorption band due to the nitrile function at 2213 cm^{-1}. While the IR spectrum of compound **3c** showed a characteristic absorption band due to thioxo group at 1219 cm^{-1}.



Scheme 2

Condensation of compound **2** with 1,4-*N*,*N*-binucleophiles such as *o*-phenylenediamine and 1,6-diaminopyridine derivative **5**,[22] under reflux in DMF, afforded the quinoxalinylquinolinone **4** and the

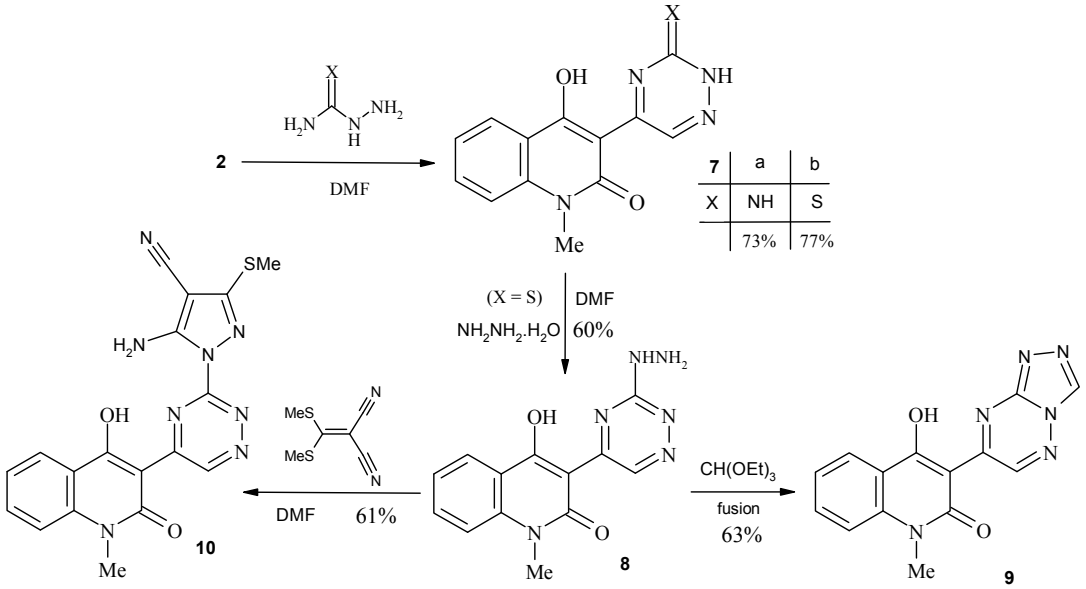
pyridotriazine derivative **6**, respectively (Scheme 3). The ^{1}H-NMR spectrum of compound **4** showed characteristic singlet signal at 8.30 ppm due the $CH_{quinoxalin}$, in addition to the presence of aromatic protons. While in the ^{1}H-NMR spectrum of compound **6** the integral count of protons appeared in the aromatic region revealed the presence of nine aromatic protons due to benzo protons of quinoline, 1,4-disubstituted benzene protons, and C3-H of pyridotriazine. IR spectrum of the compound **6** exhibits absorption bands at 2219, 2291 cm^{-1} due to two nitrile groups and 1686, 1660 cm^{-1} due to $C{=}O_{pyridone}$ and $C{=}O_{quinolinone}$, respectively.



Scheme 3

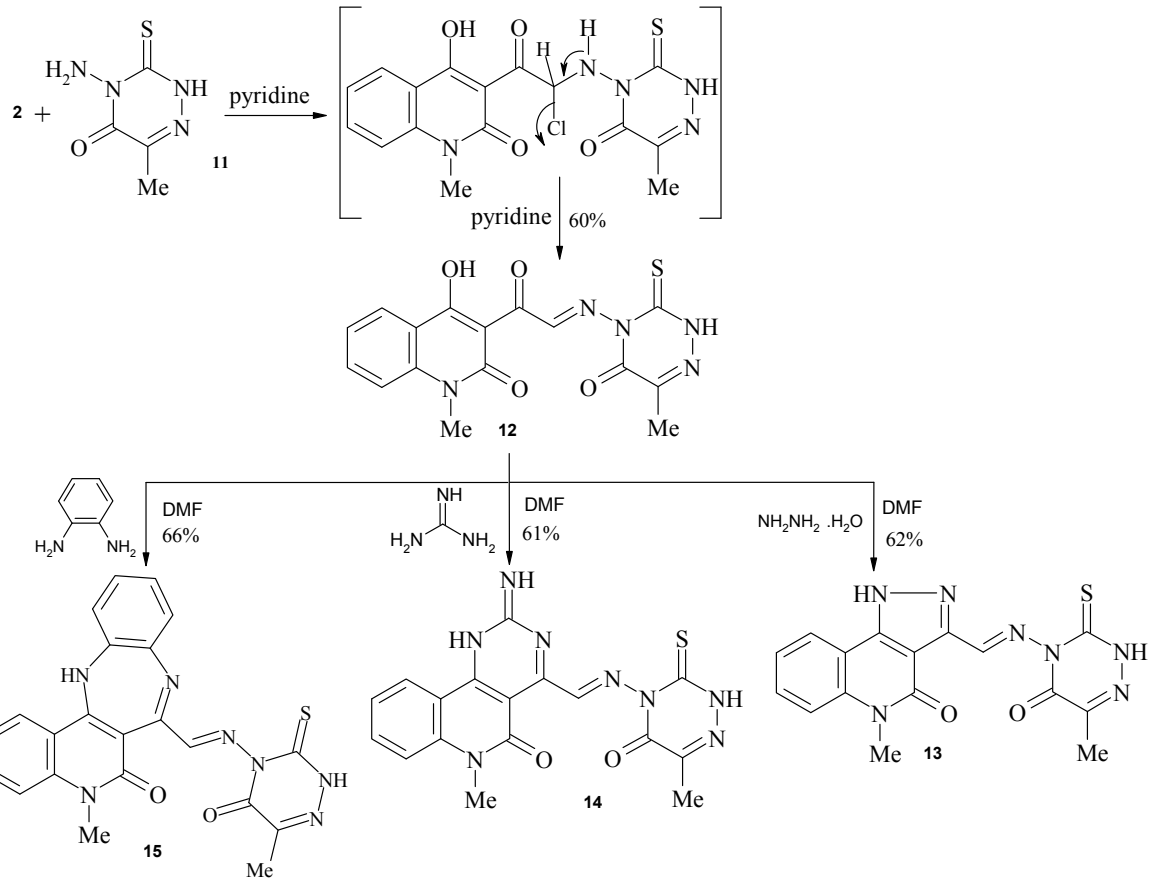
Reaction of compound **2** with aminoguanidine and thiosemicarbazide as 1,4-binucleophiles, under reflux in DMF, yielded the corresponding 1,2,4-triazine derivatives **7a,b**, respectively (Scheme 4). IR spectra of compounds **7a** and **7b** showed characteristic absorption bands due to the NH groups at 3191 and 3126 cm^{-1}, respectively. The ^{1}H-NMR spectra of compounds **7a** and **7b** showed characteristic singlets at 8.16 ppm and 8.24 ppm, respectively, which were assigned to the proton of 1,2,4-triazine. In addition to the deuterium exchangeable proton signal due to the N2-H, which appeared at 10.51 ppm and 12.67 ppm of **7a** and **7b**, respectively. Also, the mass spectrum of compound **7a** revealed the molecular ion peak at *m/z* 269 and the base peak at *m/z* 242 which was in agreement with its molecular mass after loss of one molecule of HCN. In addition, the mass spectrum of **7b** revealed a molecular ion peak at *m/z* 286 [M^+] as the base peak, which is in good accordance with the molecular formula. Hydrazinolysis of the 1,2,4-triazine derivative **7b** in DMF produced the hydrazinotriazine **8** (Scheme 4). The elemental analysis

of the later compound showed absence of sulfur in the product revealing the replacement of the thiol group. IR spectrum exhibited stretching vibrational bands at 3411, 3335 cm^{-1} due to NH_2 and at 3191, 3100 cm^{-1} due to OH and NH groups. Also, ^{1}H-NMR spectrum displayed three characteristic signals due to exchangeable protons 8.43 ppm (NH_2), 9.99 ppm (NH) and 13.36 ppm (OH). Moreover, the mass spectrum revealed the molecular ion peak at *m/z* 284 [M^+] as the base peak. Thermal cyclocondensation of the hydrazinopyrimidine **8** with triethyl orthoformate, under fusion condition, was carried out to get the triazolotriazine derivative **9** (Scheme 4). The ^{1}H-NMR spectrum of the product **9** revealed the disappearance of the NH_2 signal, in addition to the appearance of singlet at 8.24 ppm assigned to the characteristic $CH_{triazole}$. Reaction of the hydrazinotriazine **8** with [bis(methylthio)methylene]malononitrile, in DMF under reflux, gave the compound **10**. The IR spectrum of the reaction product **10** showed characteristic absorption bands at 3323, 3183 (NH_2), 2214 (C≡N), 1671(C=O). ^{1}H-NMR spectrum of the product **10** showed a new singlet signal at 2.88 ppm due to three protons of SCH_3 group, in addition to a broad signal at 5.22 ppm due to two protons of NH_2 group.



Scheme 4

Reaction of the dichloroacetyl derivative **2** with 4-aminotriazine **11**[23] was carried out in boiling pyridine. Interestingly, the only product that obtained revealed absence of chlorine in its molecular formula according to findings of elemental and mass spectral analysis. In addition, IR spectrum showed three different strong absorption vibrations at 1709, 1667, and 1632 cm^{-1} indicating the presence of acetyl C=O. Moreover, ^{1}H-NMR spectrum revealed an azomethine C-H singlet signal at 5.93 ppm. These results indicate that a nucleophilic replacement took place with leaving of a chlorine atom which is followed by elimination of hydrogen chloride leading to the α-iminone **12** (Scheme 5).



Scheme 5

α-Iminone **12**, as interesting starting material, was allowed to react with some binucleophilic reagents to prepare some new quinolinones fused at face [c] with pyrazole, pyrimidine and benzodiazepine heterocycles. Therefore, treatment of *α*-iminone **12** with hydrazine hydrate, guanidine and *o*-phenylenediamine, in DMF under reflux, afforded the pyrazoloquinolinone **13**, pyrimidoquinolinone **14** and benzodiazepinoquinolinone **15**, respectively (Scheme 5). The IR spectra of the three later products did not reveal the absorption bands due to C4-OH and acetyl C=O vibrational frequencies that supports the cyclization process. Moreover, the ^{1}H-NMR spectrum of compound **13** showed signals due to two exchangeable protons characteristic for $NH_{pyrazole}$ and $NH_{triazine}$ at 10.53 and 13.83 ppm. Mass spectrum of compound **13** exhibited a molecular ion peak at *m/z* 367, corresponding to its calculated molecular formula. The ^{1}H- NMR spectrum of compound **14** revealed the appearance of three exchangeable protons due to three different N-H signals at 8.78, 11.37 and 12.23 ppm. Mass spectrum of compound **14** revealed a peak at 394 assigned to molecular ion peak. While, the ^{1}H-NMR spectrum of compound **15** showed eight aromatic protons, in addition to two characteristic exchangeable singlet signals at 9.95 and 12.79 ppm due the 2 NH protons.

Biological activity

Antimicrobial Activity

The results depicted in Table 1 showed various activities against all species of microorganisms which suggest that the variations in the structures affect on the growth of the microorganisms. Thus, we can conclude from these results:

1) Most of the prepared compounds showed a low to high antimicrobial activity towards Gram positive bacteria, Gram negative bacteria and the fungal strain (Table 1).
2) Compounds **3c**, **7b**, **9** and **12** showed higher significant activity than the reference antibiotic Doxymycin against *Staphylococcus aureus* as Gram positive bacteria.
3) Compounds **3c** and **7b** showed equal activity to the standard antibiotic Doxymycin against *Proteus vulgaris* and *Klebsiella* as Gram negative bacteria, while compound **12** showed higher activity.
4) Compounds **3c**, **9** and **10** showed higher activity than the standard Fluconazole as fungus strain.
5) The microbial activity of compounds **3c** and **12** is due to the presence of two bioactive heterocycles as 1,2,4-triazine and quinolinone. Compounds **9** and **10** are good antimicrobial due to presence of triazolotriazine and pyrazolyltriazine, respectively.

Table 1. The antimicrobial activity of the newly synthesized compounds

Compound No.	Diameter of inhibition zone [a] (mm), Conc. (100 μg mL^{-1})			
	S.aureus (Gram +ve)	*P.vulgaris (Gram –ve)*	*Klebsiella (Gram –ve)*	*C.albicans (Fungal strain)*
3a	3	8	5	2
3b	7	3	6	4
3c	17	10	15	18
4	8	8	6	3
6	3	7	10	4
7a	6	9	6	8
7b	16	10	15	2
8	8	2	11	4
9	19	9	12	19
10	14	8	4	16
12	35	20	16	4
13	12	7	9	5
14	11	5	11	9
15	10	3	12	11
Doxymycin[b]	15	10	15	-
Fluconazole[b]	-	-	-	16

[a] 12 mm or less: resistant or no inhibition, 13–17 mm: moderate inhibition, 18 mm or more: strong inhibition.
[b] The concentration of used standard drugs was 100 μg/mL.

The minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC, μg/mL) of the most active compounds **3c**, **7b**, **9**, **10**, and **12** against the selected bacterial and fungal strains were determined (Table 2). The minimum inhibitory concentration (MIC) for these compounds show that these compounds may be considered promising for the development of new antimicrobial agents.

Table 2. The minimum inhibitory concentration (MIC, μg/mL) of the synthesized compounds **3c**, **7b**, **9**, **10**, and **12**

The selected organisms	The minimum inhibitory concentration (MIC)					
	3c	**7b**	**9**	**10**	**12**	**standard**[a]
Staphylococcus aureus	>50	>50	25	>50	>50	7
Proteus vulgaris	>50	>50	>50	>50	25	12.5
Klebsiella	8	25	>50	6	7	12.5
Candida albicans	>50	>50	2.5	7	5	5

[a] Doxymycin and Fluconazole were used as standard drugs against bacterial and fungal strains, respectively..

Antitumor activity

The antitumor activity of some of the newly synthesized compounds was investigated against Ehrlich ascites carcinoma cells (EAC). The Antitumor efficacy of the compounds against EAC cell lines was demonstrated compared to doxorubicin. The obtained results revealed that compounds exhibited IC_{50} values less than the used standard drug (doxorubicin). The triazinylquinolinones **7b**, **12**, **13**, and **14** displayed higher activity (less IC_{50} values). The reason for the higher reactivity of the triazinylquinolinones can be explained by the presence of two bioactive heterocycles, quinolinone and 1,2,4-triazinone, in one molecular frame, especially 6-methyl-5-oxo-3-thioxo-1,2,4-triazinone moiety in compounds **12-14** which is known with its activity towards tumor cells.[24] It has been reported that quinolones are known as potent antitumor agents because they target topoisomerase II enzyme and are considered as therapeutic promise.[25] In addition, 1,2,4-triazines show promising cytotoxic effect against different several tumor cell lines.[26] Also, El-Naggar *et al*.,[27] found that 1,2,4-triazines significantly reduce tumor growth in EAC bearing mice. The most potent compound in this series is compound **12** due to presence of pyrazole ring fused with quinolone. Xia *et al*.[28] deduced that pyrazoloquinolones have the highest cytotoxic activity against tumor cells in comparison with other qiunolone derivatives due to highest affinity towards topoisomerase II enzyme.

Table 3. Tumor cell growth inhibition expressed as inhibitory concentration IC_{50} (μM) in the presence of the most active compounds

Compound	[a]IC_{50} (in μg/mL)
7b	29.6
12	27.1
13	33.6
14	29.8
Doxorubicin	39.5

[a] IC_{50} -the molar concentration that inhibits tumor cell growth to 50%;

EXPERIMENTAL

Melting points were determined on a digital Stuart SMP3 apparatus. Infrared spectra were measured on Perkin-Elmer 293 spectrophotometer (cm^{-1}), using KBr disks. ^{1}H-NMR spectra were measured on Gemini-300BB spectrometer 300 MHz (at 75 MHz for ^{13}C), or Jeol Eca-500 MHz (at 125 MHz for ^{13}C) using DMSO-d_6 or $CDCl_3$ as a solvent and TMS (δ) as the internal standard. Mass spectra were obtained using GC-2010 Shimadzu GC-Mass spectrometer (70 eV). Elemental microanalyses were performed on a Perkin–Elmer CHN-2400 analyzer. Compounds **1, 5** and **12** are obtained according to literature methods[20,22,23]

3-(2,2-Dichloroacetyl)-4-hydroxy-1-methylquinolin-2(1*H*)-one (2)

To a suspension of compound **1** (2.43 gm, 10 mmol) in dioxane (20 mL) sulfuryl chloride (3.3 mL, 40 mmole) was added portionwise, while the temperature was not allowed to rise above 50 °C, where it was then kept for additional 10 min. The mixture was quickly heated to the boil and poured into ice-water (100 mL). The solid obtained was filtered and crystallized from toluene to give **2** as yellow crystals, mp 188-190 °C (3.22 g, 51%).[21]

General procedure for the preparation of compounds 3a-c

The 1,3-binucleophile (10 mmol) was added in one portion to a solution of compound **2** (2.85 g, 10 mmol) in DMF (25 mL) and the mixture was heated under reflux for 4 h. The solid obtained, after cooling was filtered off and then dried and recrystallized.

1-Methyl-4-hydroxy-3-(2-imino-2*H*-imidazol-4-yl)quinolin-2(1*H*)-one (3a)

Obtained from guanidine hydrochloride (0.94 g), crystallized from acetic acid as yellow crystals, mp 273-275 °C (1.93 g, 76%). IR (KBr, cm^{-1}): 3366 (OH), 3206 (NH), 3030 ($CH_{arom.}$), 2917 ($CH_{aliphatic}$), 1668 ($C{=}O_{quinolinone}$), 1622 (C=N), 1586 (C=C). ^{1}H-NMR (500 MHz, DMSO-d_6), δ: 3.49 (s, 3H, NCH_3), 7.21 (t, 1H, J = 7.2 Hz, H-6), 7.59–7.67 (m, 2H, H-8 and H-7), 7.91(s, 1H, $CH_{imidazole}$), 8.06 (d, 1H, J = 8.0 Hz, H-5), 9.74 (s, 1H, NH exchangeable with D_2O), 12.59 (s, 1H, OH exchangeable with D_2O). M/z (relative

intensity): 255 [M^+ +1, 6], 254 [M^+, 33], 228 (20), 221 (11), 200 (18), 188 (25), 175 (100), 172 (21), 147 (24), 146 (20), 134 (23), 115 (14), 106 (14), 104 (24), 77 (34). Anal. Calcd for $C_{13}H_{10}N_4O_2$ (254.24): C, 61.41; H, 3.96; N, 22.04%. Found: C, 61.44; H, 3.83; N, 22.01%.

1-Methyl-4-hydroxy-3-(2-cyanoiminoimidazol-4-yl)quinolin-2(1*H*)-one (3b)

Obtained from cyanoguanidine (0.84 g), crystallized from DMF as yellow crystals, mp 262-264 °C (1.81 g, 65%). IR (KBr, cm^{-1}): 3400 (OH), 3080 ($CH_{arom.}$), 2924, 2855 ($CH_{aliphatic}$), 2213 (C≡N), 1629 ($C=O_{quinolinone}$), 1611 (C=N). ^{1}H-NMR (500 MHz, DMSO-d_6), δ: 3.48 (s, 3H, CH_3), 7.32 (t, 1H, J = 7.4 Hz, H-6), 7.64 (d, 1H, J = 8.0 Hz, H-8), 7.89 (t, 1H, J = 7.2 Hz, H-7), 8.01 (d, 1H, J = 8.2 Hz, H-5), 8.15 (s, 1H, $CH_{imidazole}$), 14.25 (s, 1H, OH exchangeable with D_2O). ^{13}C-NMR (125 MHz, DMSO-d_6), δ: 30.2, 86.3, 111.1, 117.7, 118.5, 121.9, 123.2, 125.1, 133.9, 135.7, 140.5, 149.1, 152.5, 152.6. M/z (relative intensity): 279 [M^+, 42], 266 (95), 253 (42), 225 (59), 199 (17), 188 (50), 185 (76), 146 (11), 140 (44), 132 (14), 115 (25), 105 (28), 94 (100). Anal. Calcd for $C_{14}H_9N_5O_2$ (279.26): C, 60.21; H, 3.25; N, 25.08%. Found: C, 60.14; H, 3.22; N, 25.04%.

1-Methyl-4-hydroxy-3-(2-thioxo-1,2-dihydroimidazol-4-yl)quinolin-2(1*H*)-one (3c)

Obtained from thiourea (0.76 g), crystallized from acetic acid as yellow crystals, mp 255-257 °C (1.81 g, 67%). IR (KBr, cm^{-1}): 3393 (OH), 3074 ($CH_{arom.}$), 2977, 2833 ($CH_{aliphatic}$), 1666 ($C=O_{quinolinone}$), 1624 (C=N), 1219 (C=S). ^{1}H-NMR (300 MHz, DMSO-d_6), δ: 3.63 (s, 3H, NCH_3), 7.23 (t, 1H, J = 7.0 Hz, H-6), 7.67 (d, 1H, J = 8.2 Hz, H-8), 7.89 (t, 1H, J = 7.2 Hz, H-7), 8.05 (d, 1H, J = 8.2 Hz, H-5), 8.2 (s, 1H, $CH_{imidazole}$), 13.85 (s, 1H, OH exchangeable with D_2O). Anal. Calcd for $C_{13}H_9N_3O_2S$ (271.26): C, 57.55; H, 3.34; N, 15.49; S, 11.80%. Found: C, 57.21; H, 3.12; N, 15.04%.

1-Methyl-4-hydroxy-3-(quinoxalin-2-yl)quinolin-2(1*H*)-one (4)

A mixture of compound **2** (2.85 g, 10 mmol) and *o*-phenylenediamine (1.1 g, 10 mmol), in DMF (25 mL), was heated under reflux for 3 h. The solid obtained during heating was filtered and crystallized from acetic acid to give **4** as brown crystals, mp 276–279 °C (2.03 g, 67%). IR (KBr, cm^{-1}): 3411 (OH), 3070 ($CH_{arom.}$), 2979, 2937 ($CH_{aliphatic}$), 1654 ($C=O_{quinoline}$), 1600, 1567 (C=N and C=C). ^{1}H-NMR (500 MHz, DMSO-d_6), δ: 3.63 (s, 3H, NCH_3), 7.24 (t, 1H, J = 7.2 Hz, H-6), 7.28 -7.83 (m, 6H, Ar-H), 8.06 (d, 1H, J = 8.0 Hz, H-5), 8.30 (s, 1H, $CH_{quinoxaline}$), 13.85 (s, 1H, OH exchangeable with D_2O). ^{13}C-NMR (125 MHz, DMSO-d_6), δ: 28.2, 98.7, 111.8, 114.25, 115.8, 119.3, 121.6, 126.0, 128.4, 129.3, 130.0, 132.8, 134.7, 137.8, 144.0, 144.5, 146.2, 153.2. M/z (relative intensity): 304 [M^++1, 5], 303 [M^+, 81], 293 (13), 288 (14), 285 (37), 281 (36), 280 (20), 253 (38), 252 (30), 250 (100), 249 (17), 220 (34), 218 (36), 214 (31), 202 (20), 185 (25), 136 (38), 134 (50), 128 (29), 118 (25), 97 (48), 77 (65). Anal. Calcd for $C_{18}H_{13}N_3O_2$ (303.32): C, 71.28; H, 4.32; N, 13.85%. Found: C, 71.24; H, 4.23; N, 13.74%.

1-Methyl-4-hydroxy3-(8-(4-chlorophenyl)-6-oxo-6*H*-7,9-dicarbonitrilepyrido[1,2-*b*][1,2,4]triazin-2-yl)quinolin-2(1*H*)-one (6)

A mixture of compound **2** (2.85 g, 10 mmol) and 1,6-diamino-4-(4-chlorophenyl)-2-oxo-1,2-dihydropyridine-3,5-dicarbonitrile (**5**) (2.85 g, 10 mmol) in DMF (25 mL), was heated under reflux for 3 h. The solid obtained during heating was filtered and crystallized from DMF to give **6** as white crystals, mp 291-293 °C (3.22 g, 67%). IR (KBr, cm^{-1}): 3357 (OH), 2973, 2930 ($CH_{aliphatic}$), 2291, 2219 (2C≡N), 1686 ($C=O_{pyridone}$), 1660 ($C=O_{quinolinone}$), 1612 (C=N), 1583 (C=C). ^{1}H-NMR (300 MHz, DMSO-d_6), δ: 3.61 (s, 3H, N*CH*$_3$), 7.34 (t, 1H, *J* = 6.0 Hz, H-6), 7.44 (d, 2H, Ar-H), 7.56 (d, 1H, *J* = 6.4 Hz, Ar-H), 7.82 (m, 3H, Ar-H), 8.13 (d, 1H, *J* = 8.4 Hz, H-5), 8.18 (s, 1H, $CH_{pyridotriazine}$), 12.81 (bs, 1H, OH exchangeable with D_2O). M/z (relative intensity): 482 [M^++2, 7], 481 [M^++1, 35], 480 [M^+, 100], 479 [M^+-1, 10], 453 (27), 452 (27), 424 (15), 396 (3), 382 (2), 265 (37), 266 (12), 240 (5), 225 (2), 200 (22), 146 (10), 132 (14), 120 (8), 105 (5), 104 (6), 77(15). Anal. Calcd for $C_{25}H_{13}ClN_6O_3$ (480.87): C, 62.44; H, 2.72; Cl 7.37; N, 17.48%. Found: C, 62.44; H, 2.73; N, 17.44%.

General procedure for the preparation of compounds 7a and **7b**

The 1,4-binucleophile (10 mmol) was added in one portion to a solution of compound **2** (2.85 g, 10 mmol) in DMF (25 mL) and the reaction mixture was heated under reflux for 4 h. The solid obtained, after cooling was filtered off and then dried and recrystallized.

1-Methyl-4-hydroxy-3-(3-imino-2,3-dihydro-1,2,4-triazin-5-yl)quinolin-2(1*H*)-one (7a)

Obtained from aminoguanidine nitrate (1.37 g), crystallized from DMF as pale yellow crystals, mp 292-294 °C (1.96 g, 73%). IR (KBr, cm^{-1}): 3411 (OH), 3191 (NH), 3070 ($CH_{arom.}$), 2979, 2937 ($CH_{aliphatic}$), 1654 ($C=O_{quinoline}$), 1600, 1567 (C=N). ^{1}H-NMR (300 MHz, DMSO-d_6), δ: 3.61 (s, 3H, NCH_3), 7.30 (t, 1H, *J* = 6.8 Hz, H-6), 7.51 (d, 1H, *J* = 8.0 Hz, H-8), 7.86 (t, 1H, *J* = 7.0 Hz, H-7), 8.07 (d, 1H, *J* = 8.0 Hz, H-5), 8.16 (s, 1H, $H_{triazine}$), 9.02 (s, 1H, =NH exchangeable with D_2O), 10.51 (s, 1H, NH exchangeable with D_2O), 13.93 (s, 1H, OH exchangeable with D_2O). M/z (relative intensity): 269 [M^+, 8], 242 (M^+-HCN, 100), 241 (91), 228 (15), 214 (14), 200 (25), 175 (85), 172 (22), 159 (24), 147 (31), 146 (27), 134 (50), 132 (35), 115 (26), 106 (30), 105 (37), 104 (63), 77(16). Anal. Calcd for $C_{13}H_{11}N_5O_2$ (269.26): C, 57.99; H, 4.12; N, 26.01%. Found: C, 57.44; H, 4.09; N, 26.04%.

1-Methyl-4-hydroxy-3-(3-thioxo-2,3-dihydro-1,2,4-triazin-5-yl)quinolin-2(1*H*)-one (7b)

Obtained from thiosemicarbazide (0.91 g), crystallized from DMF as pale brown crystals, mp 282-284 °C (2.20 g, 77%). IR (KBr, cm^{-1}): 3400 (OH), 3126 (NH), 3030 ($CH_{arom.}$), 2917 ($CH_{aliphatic}$), 1668 ($C=O_{quinolinone}$), 1623 (C=N). ^{1}H-NMR (500 MHz, DMSO-d_6), δ: 3.62 (s, 3H, NCH_3), 7.24 (t, 1H, *J* = 7.2 Hz, H-6), 7.45 (d, 1H, *J* = 8.0 Hz, H-8), 7.67 (t, 1H, *J* = 7.2 Hz, H-7), 8.01 (d, 1H, *J* = 8.0 Hz, H-5), 8.24 (s, 1H, $CH_{triazine}$), 12.67 (s, 1H, NH exchangeable with D_2O), 14.36 (s, 1H, OH exchangeable with D_2O). ^{13}C-NMR (125 MHz, DMSO-d_6), δ: 28.6, 102.3, 113.5, 115.3, 122.6, 124.9, 131.3, 136.0, 141.8, 154.1, 159.6, 165.0, 171.5. M/z (relative intensity): 287 [M^++1, 19], 286 [M^+, 100], 273 (31), 258 (21), 257 (62), 256 (21), 240 (13), 225 (12), 200 (22), 175 (15), 146 (9), 134 (19), 132 (16), 115 (12), 105 (28), 104 (32),

77(50). Anal. Calcd for $C_{13}H_{10}N_4O_2S$ (286.31): C, 54.54; H, 3.52; N, 19.57, S, 11.20%. Found: C, 54.44; H, 3.53; N, 19.16%.

1-Methyl-4-hydroxy-3-(3-hydrazino-2,3-dihydro-1,2,4-triazin-5-yl)quinolin-2(1*H*)-one (8)

A mixture of compound **7b** (2.86 g, 10 mmol) and hydrazine hydrate (0.58 mL, 12 mmol), in DMF (30 mL), was heated under reflux for 4 h. The solid deposited after cooling was filtered and crystallized from DMF to give compound **8** as white crystals, mp above 300 °C (1.70 g, 60%). IR (KBr, cm^{-1}): 3411, 3335, (NH_2), 3191, 3100 (OH, NH), 3070 ($CH_{arom.}$), 2979, 2937 ($CH_{aliphatic}$), 1654 ($C=O_{quinoline}$), 1600, 1567 (C=N and C=C). ^{1}H-NMR (300 MHz, DMSO-d_6), δ: 3.44 (s, 3H, NCH_3), 7.26 (t, 1H, J = 7.2 Hz, H-6), 7.53 (d, 1H, J = 8.0 Hz, H-8), 7.71 (t, 1H, J = 7.2 Hz, H-7), 7.93 (d, 1H, J = 8.0 Hz, H-5), 8.25 (s, 1H, $CH_{triazine}$), 8.43 (s, 2H, NH_2 exchangeable with D_2O), 9.99 (s, 1H, NH exchangeable with D_2O), 13.36 (s, 1H, OH exchangeable with D_2O). M/z (relative intensity): 285 [M^++1, 17], 284 [M^+, 100], 256 (25), 255 (6), 254 (11), 216 (96), 215 (19), 214 (69), 172 (51), 146 (66), 132 (57), 115 (29), 105 (14), 104 (22), 77(67). Anal. Calcd for $C_{13}H_{12}N_6O_2$ (284.28): C, 54.93; H, 4.25; N, 29.56%. Found: C, 54.84; H, 4.23; N, 29.54%.

1-Methyl-4-hydroxy-3-[1,2,4-triazolo[4,3-*b*][1,2,4]triazin-7-yl]quinolin-2(1*H*)-one (9)

A solution of compound **8** (2.84 g, 10 mmol) in triethyl orthoformate (8.16 mL, 48 mmol) was heated under fusion for 2 h. The precipitate so formed on hot was filtered and crystallized from acetic acid to give compound **9** as pale yellow crystals, mp above 300 °C (1.85 g, 63%). IR (KBr, cm^{-1}): 3446 (OH), 3075 ($CH_{arom.}$), 2977, 2934 ($CH_{aliphatic}$), 1673 ($C=O_{quinolinone}$), 1634 (C=N), 1598 (C=C). ^{1}H-NMR (300 MHz, DMSO-d_6), δ: 3.47 (s, 3H, NCH_3), 7.24 (t, 1H, J = 6.0 Hz, H-6), 7.47 (d, 1H, J = 8.0 Hz, H-8), 7.67 (t, 1H, J = 6.4 Hz, H-7), 7.99 (d, 1H, J = 8.4 Hz, H-5), 8.01, 8.24 (2s, 2H, $H_{triazine}$ and $H_{triazole}$), 14.36 (bs, 1H, OH exchangeable with D_2O). ^{13}C-NMR (75 MHz, DMSO-d_6), δ: 28.2, 99.3, 113.3, 117.0, 118.9, 124.7, 125.4, 139.0, 141.5, 154.1, 156.4, 159.4, 163.0, 166.4. Anal. Calcd for $C_{14}H_{10}N_6O_2$ (294.27): C, 57.14; H, 3.43; N, 28.56%. Found: C, 57.12; H, 3.33; N, 28.54%.

5-Amino-1-[5-(4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-yl)-[1,2,4]triazine-3-yl]-3-methylsulfanyl-1*H*-pyrazole-4-carbonitrile (10)

A mixture of compound **8** (2.84 g, 10 mmol) and [bis(methylthio)methylene]malononitrile (1.70 gm, 10 mmol), in DMF (25 mL) was heated under reflux for 4 h. The reaction solution was left to cool at room temperature and the solid so formed was filtered and crystallized from dioxane to give compound **10** as pale yellow crystals, mp above 300 °C (2.48 g, 61%). IR (KBr, cm^{-1}): 3400 (OH), 3323, 3183 (NH_2), 3080 ($CH_{arom.}$), 2924, 2855 ($CH_{aliphatic}$), 2214 (C≡N), 1671($C=O_{quinolinone}$), 1629 (C=N), 1611 (C=C). ^{1}H-NMR (300 MHz, DMSO-d_6), δ: 2.88 (s, 3H, SCH_3), 3.61 (s, 3H, NCH_3), 5.22 (bs, 2H, NH_2 exchangeable with D_2O), 7.22 (t, 1H, J = 6.2 Hz, H-6), 7.49 (d, 1H, J = 8.2 Hz, H-8), 7.77 (t, 1H, J = 6.4

Hz, H-7), 8.13 (d, 1H, *J* = 8.0 Hz, H-5), 8.23 (s, 1H, $CH_{triazine}$), 12.28 (bs, 1H, OH exchangeable with D_2O). Anal. Calcd for $C_{18}H_{14}N_8O_2S$ (406.43): C, 53.20; H, 3.47; N, 27.57; S, 7.89%. Found: C, 53.12; H, 3.43; N, 27.44; S, 7.85%.

1-Methyl-4-hydroxy-3-[2-(6-methyl-5-oxo-3-thioxo-2,3dihydro-1,2,4-triazin-4(5*H*)-ylimino)acetyl]-quinolin-2(1*H*)-one (12)

A mixture of compound **2** (2.85 g, 10 mmol) and aminotriazine **11** (1.58 g, 10 mmol), in pyridine (30 mL), was heated under reflux for 4 h. Then, the solution was filtered and the clear solution was acidified by dilute HCl. The precipitate so formed was filtered, washed several times with water, air dried and crystallized from ethanol to give **12** as yellow crystals, mp 236-237 °C (2.22 g, 60%). IR (KBr, cm^{-1}): 3419 (OH), 2981, 2934 ($CH_{aliphatic}$), 1709 (C=O), 1667, 1632 ($C=O_{triazinone}$ and $C=O_{quinolinone}$), 1599, 1575 (C=N and C=C). ^{1}H-NMR (300 MHz, DMSO-d_6), δ: 3.23 (s, 3H, $CH_{3\ triazine}$), 3.68 (s, 3H, N*CH*$_3$), 5.93 (s, 1H, *CH*=N), 7.20 (t, 1H, *J* = 6.2 Hz, H-6), 7.44 (d, 1H, *J* = 8.0 Hz, H-8), 7.61 (t, 1H, *J* = 6.4 Hz, H-7), 7.89 (d, 1H, *J* = 8.4 Hz, H-5), 11.39 (bs, 1H, NH exchangeable with D_2O), 13.23 (bs, 1H, OH exchangeable with D_2O). ^{13}C-NMR (75 MHz, DMSO-d_6), δ: 13.6, 28.6, 61.8, 102.6, 113.8, 115.5, 117.0, 122.8, 125.2, 136.2, 142.0, 153.3, 160.1, 163.0, 171.8, 190.4. M/z (relative intensity): 371 [M^+, 16], 343 (17), 326 (23), 292 (16), 278 (16), 275 (27), 269 (60), 241 (14), 223 (32), 197 (30), 175 (20), 174 (22), 158 (7), 146 (43), 136 (16), 132 (79), 114 (13), 105 (27), 104 (33), 77(79), 63 (100). Anal. Calcd for $C_{16}H_{13}N_5O_4S$ (371.38): C, 51.75; H, 3.53; N, 18.86, S, 8.63%. Found: C, 51.74; H, 3.53; N, 18.74; S, 8.53%.

General procedure for the preparation of compounds 13, 14 and 15

Hydrazine hydrate (12 mmol), guanidine hydrochloride (10 mmol) and/or *o*-phenylenediamine (10 mmol) was added to a solution of compound **12** (3.71 g, 10 mmol) in DMF (25 mL) and the reaction mixture was refluxed for 4 h. The solid obtained after cooling was filtered off and then dried and recrystallized to give compounds **13**, **14** and **15** respectively.

5-Methyl-3-[(6-methyl-5-oxo-3-thioxo-2,5-dihydro-3*H*-1,2,4-triazin-4-ylimino)methyl]-1,5-dihydro-pyrazolo[4,3-*c*]quinolin-4-one (13)

Obtained from hydrazine hydrate (0.58 mL, 12 mmol), crystallized from DMF as yellow crystals, mp above 300 °C (2.27 g, 62%). IR (KBr, cm^{-1}): 3292 (NH), 2978, 2870 ($CH_{aliphatic}$), 1662, 1630 ($C=O_{triazinone}$ and $C=O_{quinolinone}$), 1603, 1572 (C=N and C=C). ^{1}H-NMR (300 MHz, DMSO-d_6), δ: 2.88 (s, 3H, $CH_{3\ triazine}$), 3.63 (s, 3H, N*CH*$_3$), 5.57 (s, 1H, *CH*=N), 7.28 (t, 1H, *J* = 6.2 Hz, H-8), 7.32 (d, 1H, *J* = 8.0 Hz, H-6), 7.77 (t, 1H, *J* = 6.4 Hz, H-7), 8.13 (d, 1H, *J* = 8.4 Hz, H-9), 10.53 (bs, 1H, NH exchangeable with D_2O), 13.83 (bs, 1H, NH exchangeable with D_2O). ^{13}C-NMR (75 MHz, DMSO-d_6), δ: 16.2, 28.9, 62.9, 98.2, 114.5, 118.2, 119.9, 120.7, 122.2, 124.2, 128.5, 135.5, 145.4, 153.7, 155.6, 176.7. M/z (relative intensity): 367 [M^+, 25], 366 [M^+-1, 20], 326 (27), 303 (25), 278 (26), 258 (20), 255(26), 249 (21), 201

(21), 175 (21), 148 (31), 134 (22), 115 (26), 105 (22), 104 (27), 86(100), 77 (36). Anal. Calcd for $C_{16}H_{13}N_7O_2S$ (367.39): C, 52.31; H, 3.57; N, 26.69, S, 8.73%. Found: C, 52.21; H, 3.53; N, 26.64; S, 8.63%.

2-Imino-6-methyl-4-[(6-methyl-5-oxo-3-thioxo-2,5-dihydro-3*H*-1,2,4-triazin-4-ylimino)methyl]-2,6-dihydro-1*H*-pyrimido[5,4-*c*]quinolin-5-one (14)

Obtained from guanidine hydrochloride (0.94 g, 10 mmol), crystallized from acetic acid as pale yellow crystals, mp above 300 °C (2.40 g, 61%). IR (KBr, cm^{-1}): 3253 (NH), 3072 ($CH_{arom.}$), 2977, 2932 ($CH_{aliphatic}$), 1674, 1634 ($C{=}O_{triazinone}$ and $C{=}O_{quinolinone}$), 1599, 1569 (C=N and C=C). ^{1}H-NMR (300 MHz, DMSO-d_6), δ: 2.88 (s, 3H, $CH_{3\ triazine}$), 3.61 (s, 3H, NCH_3), 5.86 (s, 1H, *CH*=N), 7.22 (t, 1H, J = 6.4 Hz, H-9), 7.43 (d, 1H, J = 8.4 Hz, H-7), 7.67 (t, 1H, J = 6.2 Hz, H-8), 8.10 (d, 1H, J = 8.0 Hz, H-10), 8.78 (bs, 1H, NH exchangeable with D_2O), 11.37 (bs, 1H, NH exchangeable with D_2O), 12.23 (bs, 1H, NH exchangeable with D_2O). M/z (relative intensity): 394 [M^+, 11], 393 [M^+-1, 13], 379 (11), 365 (12), 331 (14), 322 (100), 321 (60), 294 (65), 268 (46), 266 (22), 257 (11), 240 (13), 189 (11), 175 (10), 146 (23), 132 (33), 132 (16), 115 (14), 108 (25), 105 (12), 93 (22), 77 (42). Anal. Calcd for $C_{17}H_{14}N_8O_2S$ (394.41): C, 51.77; H, 3.58; N, 28.41; S, 8.13%. Found: C, 51.74; H, 3.53; N, 28.14; S, 8.09%.

8-Methyl-6-[(6-methyl-5-oxo-3-thioxo-2,5-dihydro-3*H*-1,2,4-triazin-4-ylimino)methyl]-13*H*-benzo-[1,5]diazepino[6,5-*c*]quinolin-7(8*H*)-one (15)

Obtained from *o*-phenylenediamine (1.1 g, 10 mmol), crystallized from acetic acid as pale yellow crystals, mp above 300 °C (2.91 g, 66%). IR (KBr, cm^{-1}): 3294, 3252 (NH), 2980, 2934 ($CH_{aliphatic}$), 1670, 1632 ($C{=}O_{triazinone}$ and $C{=}O_{quinolinone}$), 1603, 1583 (C=N and C=C). ^{1}H-NMR (300 MHz, DMSO-d_6), δ: 3.15 (s, 3H, $CH_{3\ triazine}$), 3.63 (s, 3H, NCH_3), 5.87 (s, 1H, *CH*=N), 7.23 (t, 1H, J = 6.4 Hz, Ar-H), 7.36 (d, 1H, J = 8.2 Hz, Ar-H), 7.42 (t, 1H, J = 6.4 Hz, Ar-H), 7.86-8.13 (m, 5H, Ar-H), 9.95 (s, 1H, NH exchangeable with D_2O). 12.79 (bs, 1H, NH exchangeable with D_2O). ^{13}C-NMR (75 MHz, DMSO-d_6), δ: 16.2, 29.1, 62.1, 92.2, 113.6, 114.3, 115.7, 117.0, 119.6, 127.2, 128.1, 129.2, 129.7, 130.4, 134.5, 138.5, 146.2, 152.2, 152.6, 157.3, 165.2, 174.9. Anal. Calcd for $C_{22}H_{17}N_7O_2S$ (443.49): C, 59.58; H, 3.86; N, 22.11, S, 7.23%. Found: C, 59.54; H, 3.83; N, 22.02.

Antimicrobial Activity

The standardized disc agar diffusion method[29] was followed to determine the activity of the synthesized compounds against the sensitive organisms *Staphylococcus aureus* as a Gram positive bacterium, *Proteus vulgaris* and *Klebsiella* as Gram negative bacteria and *Candida albicans* as a fungus strain. The antibiotic Doxymycin and Fluconazole were used in concentration 100 μg mL^{-1}, as references for antibacterial and antifungal agents. The compounds were dissolved in DMSO which has no inhibition activity to get concentration of 100 μg mL^{-1}. The test was performed on medium potato dextrose agars (PDA) which

contain infusion of 200 g potatoes, 6 g dextrose and 15 g agar.[30] Uniform size filter paper disks (3 disks per compound) were impregnated by equal volume (10 μL) from the specific concentration of dissolved tested compounds and carefully placed on inoculated agar surface. After incubation for 36 h at 27 °C in the case of bacteria and for 48 h at 24 °C in the case of fungi, inhibition of the organisms was measured and used to calculate mean of inhibition zones.

Antitumor activity

EAC cells were maintained by weekly intraperitoneal transplantation of 2.5 X 10^5 cells in mice. The tumor is characterized by a moderately rapid growth, which leads to the death of the mice in about 20 days due to the distal metastasis. Ascites was withdrawn under aseptic conditions from the peritoneal cavity of tumor bearing mice by needle aspiration after 7 days of EAC cells inoculation. To adjust the number of EAC cells/mL, tumor cells obtained were diluted several times with normal saline. EAC viable cells were counted by trypan blue exclusion method where 10 μl trypan blue (0.05%) was mixed with 10 μl of the cell suspension. Within 5 min, the mixture was spread onto haemocytometer, covered with a cover slip and then the cells were examined under microscope. Dead cells are blue stained viable cells are not.[31] Cell suspension was adjusted to contain 2.5 X 10^5 viable cells/ml. EAC cells, RPMI medium drugs, and DMSO were added in sterile test tubes according to trypan blue exclusion method.[31] The cells were incubated for two hours at 37 °C under a constant over lay of 5% CO_2. EAC viable cells were counted by trypan blue exclusion using haemocytometer as mentioned above. The cell surviving fraction was calculated from the relation T/C; where T and C represent the number of viable cells in a unit volume and the number of total (viable + dead) cells in the same unit volume, respectively.

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