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Synthesis, *in vitro* Antimicrobial and Anticancer Evaluation of Some New Pyridazines and Polyfunctionally Substituted Heterocyclic Compounds

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This study aimed for the synthesis of new heterocyclic compounds incorporating sulfamoyl moiety suitable for use as antimicrobial agents *via* versatile and readily accessible *N*-[4-(aminosulfonyl)phenyl]-3-oxobutanamide (**1**). Butanamide coupled with arenediazonium salts to afford hydrazones. The latter reacts with dimethylformamide dimethyl acetal (DMF-DMA) to afford the substituted 1,4-dihydropyridazine. Several new thiophene, pyridine, nicotinamide and pyrazole derivatives have been synthesized by the reactions of butanamide with malononitrile and elemental sulfur, 1,3-diphenylpropenone, arylidenecyanothioacetamide, nitrogen nucleophiles, respectively. Refluxing of butanamide with a mixture of *p*-methoxybenzaldehyde and thiourea afforded 4-(4-methoxyphenyl)-6-methyl-*N*-(4-sulfamoylphenyl)-2-thioxo-1,2-dihydropyrimidine-5-carboxamide which heated with chloroacetyl chloride give *N*-[4-(aminosulfonyl)phenyl]-7-methyl-5-(4-methoxyphenyl)-3-oxo-2,3-dihydro-5*H*-[1,3]thiazolo[3,2-a]pyrimidine-6-carboxamide. Treatment of butanamide with phenyl isothiocyanate afforded the intermediate salt which reacted *in situ* with 2-bromo-1-phenylethanone to afford *N*-[4-(aminosulfonyl)phenyl]-2-(3,4-diphenyl-3*H*-thiazol-2-ylidene)-3-oxobutanamide. Some of the selected products were evaluated for both their *in vitro* antibacterial and antifungal activities and showed promising results. In addition, the anticancer activity of some selected products against human liver (HEPG2) cell line was determined and the results revealed high activities of compounds **5a**, **6** and **14**.

Keywords: Antimicrobial Activity, Anticancer Activity, Butanamide, Pyrazole, Pyridine, Thiophene, Thiazolopyrimidine.

INTRODUCTION

Heterocycles containing sulfonamido moieties have attracted obvious attention due to their significant biological properties and their role as pharmacophores[1-6]. Studies have shown that sulfonamide compounds were used as antibacterial agents[7-13], anticancer[14-16], anti-inflammatory, analgesic agents[17-19], antifungal agents[9,20], and antiviral agents[21], insulin releasing[22], carbonic anhydrase inhibitory[23]. Sulphonamides with varying chemical, pharmacological and antibacterial properties are produced by attaching substituent to the amido group (SO_2NHR) or the amino group ($-NH_2$) of the sulphanilamide nucleus. They inhibit of *p*-aminobenzoic acid utilization as a substrate in the biosynthesis of folic acid[24,25]. Some sulphonamides are also known for their immuno modifying effects[26,27]. In view of these observations and in continuation of our previous work directed to the synthesis of novel heterocyclic compounds of potential biological and pharmacological activities[28-32], we reported here a facile routes for the synthesis of some new hydrazone derivatives, pyrimidine, pyran, pyridine, pyridazine, thiophene, pyrazole, thiazolopyrimidine and thiazole incorporating benzenesulfonamide moiety starting from. *N*-[4-(aminosulfonyl)phenyl]-3-oxobutanamide (**1**) as an excellent building block for the synthesis of the target compounds.

EXPERIMENTAL

All melting points were determined on an Electrothermal Gallenkamp apparatus and are uncorrected. Elemental analyses were carried out at the Microanalytical Centre, Cairo University, Giza, Egypt and results agreed favourably with calculated values. The IR spectra of the synthesized compounds were recorded on a Shimadzu FTIR 8101 PC spectrometer in KBr. The ^{1}H NMR spectra were recorded on a Varian Mercury VXR-300 (300 MHz) instrument in DMSO-d_6 or $CDCl_3$, using TMS as internal standard. The mass spectra were obtained using a GCMS QP1000 ex Shimadzu instrument (EI, 70 eV). The UV spectra were recorded on a Perkin-Elmer Lambda 40 spectrophotometer. The antibacterial, antifungal and anticancer activity assays were carried out in the Medical Mycology Laboratory

of the Regional Center for Mycology and Biotechnology of Al-Azhar University, Cairo, Egypt.

N-[4-(Aminosulfonyl)phenyl]-3-oxobutanamide (**1**) was obtained in accordance with the previously described procedure[33].

Synthesis of hydrazones (3a-c): To the stirred mixture (ice bath) of compound **1** (0.51 g, 2 mmol) and sodium acetate trihydrate (3 g) in ethanol (40 mL) was added a solution of the appropriate aryl diazonium chloride dropwise over a period of 20 min. The latter was prepared as usual by diazotizing the respective aniline (2 mmol) in hydrochloric acid (6 M, 1.2 mL) with sodium nitrite (0.138 g, 2 mmol). After complete addition, the reaction mixture was stirred for further 4 h. The resulting solid was filtered off and recrystallized from ethanol to afford the corresponding coupling products **3a-c**.

***N*-[4-(Aminosulfonyl)phenyl]-2-[(4-methylphenyl)-hydrazono]-3-oxobutanamide (3a):** Yield (80 %), m.p.: 275 °C (ethanol); IR (KBr, ν_{max}, cm^{-1}): 3496 (NH), 3342, 3248 (NH, NH_2), 1693 (C=O), 1662 (C=O); ^{1}H NMR (DMSO-d_6): δ 2.48 (s, 3H, CH_3), 2.49 (s, 3H, CH_3), 7.16–7.41(d, 2H, H Ar), 7.44-7.58 (d, 2H, H Ar), 7.61-7.79 (d, 2H, H Ar) and 7.82-7.84 (d, 2H, H Ar), 8.20 (s, 2H, NH_2), 11.39 (s, 1H, NH), 13.68 (s, 1H, NH)); MS *m/z* (%): 375 (M^++1, 0.3), 374 (M^+, 5.4), 317 (1.5), 309 (4.0), 294 (3.3), 252 (1.1), 226 (8.5), 211 (4.8), 108 (100), 91 (45.3), 73 (33.1), 64 (24.2); UV spectrum (dioxane), λ_{max} nm (log ε): 385 (4.25), 285 (4.35). Anal. calcd. for $C_{17}H_{18}N_4O_4S$ (374.41): C, 54.53; H, 4.85; N, 14.96; S, 8.56. Found: C, 54.50; H, 4.83; N, 14.94; S, 8.52 %.

***N*-[4-(Aminosulfonyl)phenyl]-3-oxo-2-(phenylhydrazono)butanamide (3b):** Yield (90 %), m.p.: 250 °C (ethanol); IR (KBr, ν_{max}, cm^{-1}): 3546 (NH), 3334, 3228 (NH, NH_2), 1685 (C=O), 1630 (C=O); ^{1}H NMR (DMSO-d_6): δ 2.48 (s, 3H, CH_3), 7.31-7.48 (m, 5H, H Ar), 7.56-7.58 (d, 2H, H Ar) and 7.60-7.85 (d, 2H, H Ar), 7.92 (s, 2H, NH_2), 11.32 (s, 1H, NH), 12.21 (s, 1H, NH); MS *m/z* (%): 362 (M^++2, 0.5), 360 (M^+, 5.2), 312 (12.5), 238 (22.1), 192 (36.5), 138 (5.2), 111 (14.3), 87 (14.5), 77 (100); UV spectrum (dioxane), λ_{max} nm (log ε): 380 (4.15), 312 (4.44). Anal. calcd. for $C_{16}H_{16}N_4O_4S$ (360.38): C, 53.32; H, 4.47; N, 15.55; S, 8.90. Found: C, 53.31; H, 4.49; N, 15.50; S, 8.87 %.

***N*-[4-(Aminosulfonyl)phenyl]-2-[(4-chlorophenyl)-hydrazono]-3-oxobutanamide (3c):** Yield (75 %), m.p.: 268 °C (ethanol); IR (KBr, ν_{max}, cm^{-1}): 3478 (NH), 3370, 3264 (NH, NH_2), 1720 (C=O), 1630 (C=O); ^{1}H NMR (DMSO-d_6): δ 2.49 (s, 3H, CH_3), 7.16–7.18 (d, 2H, H Ar), 7.50-762 (d, 2H, H Ar), 7.78-7.80 (d, 2H, H Ar) and 7.86-7.89 (d, 2H, H Ar), 8.22 (s, 2H, NH_2), 11.35 (s, 1H, NH), 13.54 (s, 1H, NH); MS *m/z* (%): 396 (M^++2, 0.71), 394 (M^+, 0.6), 313 (38.6), 239 (5.8), 201 (15.6), 172 (53.2), 139 (100), 112 (14.3), 84 (8.6), 77 (4.5). UV spectrum (dioxane), λ_{max} nm (log ε): 395 (4.23), 320 (4.45). Anal. calcd. for $C_{16}H_{15}ClN_4O_4S$ (394.83): C, 48.67; H, 3.83; Cl, 8.98; N, 14.19; S, 8.12. Found: C, 48.61; H, 3.80; Cl, 8.92; N, 14.18; S, 8.05 %.

Synthesis of 1,4-dihydropyridazine derivatives (5a-c): A mixture of hydrazones **3** (10 mmol) and DMF-DMA (1.32 mL, 10 mmol) in dry dioxane (20 mL) was refluxed for 6 h, then it was left to cool. The resulting solid was filtered off and recrystallized from ethanol to give 1,4-dihydropyridazine derivatives **5a-c**.

***N*-[4-(Aminosulfonyl)phenyl]-1-(4-methylphenyl)-4-oxo-1,4-dihydropyridazine-3-carboxamide (5a):** Yield (80 %), mp 196 °C (ethanol); IR (KBr, ν_{max}, cm^{-1}): 3490 (NH), 3344, 3222 (NH_2), 1695 (C=O), 1630 (C=O); ^{1}H NMR (DMSO-d_6): δ 2.49 (s, 3H, CH_3); 7.23–7.25, 7.45-7.56 and 7.61-7.83 (m, 10H, H Ar), 8.19 (s, 2H, NH_2), 11.40 (s, 1H, NH); MS *m/z* (%): 386 (M^++2, 1.4), 385 (M^++1, 0.4), 384 (M^+, 0.5), 296 (5.1), 227 (82.1), 162 (19.3), 119 (33.1), 106 (65.1), 92 (48.9), 71 (100), 64 (49.5). Anal. calcd. for $C_{18}H_{16}N_4O_4S$ (384.41): C, 56.24; H, 4.20; N, 14.57; S, 8.34. Found: C, 56.18; H, 4.15; N, 14.48; S, 8.30 %.

***N*-[4-(Aminosulfonyl)phenyl]-4-oxo-1-phenyl-1,4-dihydropyridazine-3-carboxamide (5b):** Yield (70 %), m.p.: 190 °C (ethanol); IR (KBr, ν_{max}, cm^{-1}): 3546 (NH), 3334, 3228 (NH_2), 1695 (C=O), 1630 (C=O); ^{1}H NMR (DMSO-d_6): δ 7.16–7.21, 7.41-7.58 and 7.61-7.84 (m, 11H, H Ar), 8.20 (s, 2H, NH_2), 11.39 (s, 1H, NH); MS *m/z* (%): 370 (M^+, 0.6), 368 (M^+-2, 0.5), 313 (0.6), 293 (1.6), 256 (4.7), 213 (2.4), 171 (2.1), 149 (13.1), 88 (100), 58 (27.8). Anal. calcd. for $C_{17}H_{14}N_4O_4S$ (370.38): C, 55.13; H, 3.81; N, 15.13; S, 8.66. Found: C, 55.32; H, 3.90; N, 15.22; S, 8.51 %.

***N*-[4-(Aminosulfonyl)phenyl]-1-(4-chlorophenyl)-4-oxo-1,4-dihydropyridazine-3-carboxamide (5c):** Yield (60 %), m.p.: 190 °C (ethanol); IR (KBr, ν_{max}, cm^{-1}): 3490 (NH), 3345, 3215 (NH_2), 1698 (C=O), 1630 (C=O); ^{1}H NMR (DMSO-d_6): δ 7.45-7.57 and 7.60-7.82 (m, 10H, H Ar), 8.20 (s, 2H, NH_2), 11.19 (s, 1H, NH); MS *m/z* (%): 406 (M^++2, 0.6), 404 (M^+, 0.24), 367 (2.1), 296 (32.4), 252 (10.9), 227 (100), 162 (8.9), 119 (7.1), 108 (4.3), 92 (1.6), 80 (2.4). Anal. calcd. for $C_{17}H_{13}ClN_4O_4S$ (404.82): C, 50.44; H, 3.24; Cl, 8.76; N, 13.84; S, 7.92. Found: C, 50.38; H, 3.20; Cl, 8.71; N, 13.80; S, 7.88 %.

***N*-[4-(Aminosulfonyl)phenyl]-5-amino-4-cyano-3-methylthiophene-2-carboxamide (6):** Elemental sulfur (2.56 g, 10 mmol) and triethylamine (0.5 mL) were added to a solution of acetoacetanilide **1** (2.56 g, 10 mmol) and malononitrile (0.66 g, 10 mmol) in ethanol (30 mL) and the mixture was refluxed for 4 h and then left to cool. The resulting solid was filtered off, washed with water and recrystallized from ethanol. To afford the corresponding *N*-[4-(aminosulfonyl)phenyl]-5-amino-4-cyano-3-methylthiophene-2-carboxamide (**6**). Yield (40 %), m.p.: > 300 °C (ethanol); IR (KBr, ν_{max}, cm^{-1}): 3444 (NH), 3320, 3214 ($2NH_2$), 2210 (CN), 1632 (C=O)[1]; ^{1}H NMR (DMSO-d_6): δ 2.37 (s, 3H, CH_3), 7.22–7.50 (m, 4H, H Ar), 7.79 (s, 4H, $2NH_2$), 9.84 (s, 1H, NH); MS *m/z* (%): 337 (M^++1, 3.5), 336 (M^+, 16), 276 (8), 172 (9), 165 (100), 136 (3), 110 (6), 91 (2). Anal. calcd. for $C_{13}H_{12}N_4O_3S_2$ (336.39): C, 46.42; H, 3.60; N, 16.66; S, 19.06. Found: C, 46.68, H, 3.55; N, 16.54; S, 18.96 %.

Reaction of compound 1 with 1,3-diphenylpropenone, 2-cyano-3-(4-methoxyphenyl)thioacrylamide and *p*-methoxybenzaldehyde: To a solution of 1,3-diphenylpropenone (**7**) (2.08 g, 10 mmol) or 2-cyano-3-(4-methoxyphenyl)thioacrylamide (**9**) (2.18 g, 10 mmol) or *p*-methoxybenzaldehyde (**13**) (1.36 g, 10 mmol) in absolute ethanol (30 mL) was added acetoacetanilide (**1**) (2.56 g, 10 mmol) and few drops of piperidine and the reaction mixture was refluxed for 6 h. The resulting solid was filtered off and recrystallized from ethanol afforded compounds **8**, **12** and **14** respectively.

4-(3-Acetyl-4,6-diphenyl-2-oxopyridin-1(2*H*)-yl)-benzenesulfonamide (8): Yield (30 %); m.p.: 156 ºC (ethanol); IR (KBr, ν_{max}, cm^{-1}): 3370, 3264 (NH_2), 1685 (C=O), 1630 (C=O); ^{1}H NMR (DMSO-d_6): δ 2.49 (s, 3H, CH_3), 5.76 (s, 1H, H pyridine), 6.57–6.61, 6.86 and 7.43-7.46 (m, 14H, H Ar), 7.47 (s, 2H, NH_2); MS *m/z* (%): 444 (M^+, 1.4), 443 (M^+-1, 0.2), 172 (100), 156 (74), 108 (27), 92 (18). Anal. calcd. for $C_{25}H_{20}N_2O_4S$ (444.50): C, 67.55; H, 4.54; N, 6.30; S, 7.21. Found: C, 67.52; H, 4.52; N, 6.24; S, 7.19 %.

***N*-[4-(Aminosulfonyl)phenyl]-5-cyano-2-methyl-4-(4-methoxyphenyl)-6-thionicotinamide (12):** Yield (50 %), m.p.: 210 ºC (ethanol); IR (KBr, ν_{max}, cm^{-1}): 3440 (NH), 3320, 3194 (NH, NH_2), 2222 (CN), 1666 (C=O); ^{1}H NMR (DMSO-d_6): δ 2.52 (s, 3H, CH_3), 3.81 (s, 3H, OCH_3), 7.05-7.08 (d, 2H, H Ar), 7.29 (s, 3H, NH, NH_2), 7.45-7.48 (d, 2H, H Ar), 7.57-7.59 (d, 2H, H Ar), 7.77-8.0 (d, 2H, H Ar), 10.66 (s, 1H, NH); MS *m/z* (%): 454 (M^+, 7), 453 (M^+-1, 14), 289 (29), 262 (19), 204 (100), 195 (17), 182 (13), 121 (25), 112 (53), 102 (16), 86 (12). Anal. calcd. for $C_{21}H_{18}N_4O_4S_2$ (454.52): C, 55.49; H, 3.99; N, 12.33; S, 14.11. Found: C, 55.41; H, 3.86; N, 12.29; S, 14.02 %.

***N*-[4-(Aminosulfonyl)phenyl]-2-acetyl-3-(4-methoxyphenyl)acrylamide (14):** Yield (80 %), m.p.: 175 ºC (ethanol); IR (KBr, ν_{max}, cm^{-1}): 3476 (NH), 3326, 32764 (NH_2), 1698 (C=O), 1645 (C=O); ^{1}H NMR (DMSO-d_6): δ 2.49 (s, 3H, CH_3), 3.76 (s, 3H, OCH_3), 5.77 (s, 1H, CH), 6.86-7.38 (m, 4H, H Ar), 7.40-7.82 (m, 6H, 4H Ar+NH_2), 8.62 (s, 1H, NH); MS *m/z* (%): 374 (M^+, 2), 290 (29), 289 (100), 226 (28), 210 (18), 172 (69), 167 (26), 155 (69), 139 (13), 108 (37), 92 (44). Anal. calcd. for $C_{18}H_{18}N_2O_5S$ (374.41): C, 57.74; H, 4.85; N, 7.48; S, 8.56. Found: C, 57.68; H, 4.78; N, 7.41; S, 8.46 %.

Reaction of compound 14 with hydrazine hydrate and phenyl hydrazine: A mixture of compound **14** (1.87 g, 5 mmol) and hydrazine hydrate (80 %, 3.25 mL) or phenyl hydrazine (0.54 g, 5 mmol) in absolute ethanol (30 mL), was refluxed for 8 h. The solid was filtered off and washed with ethanol and recrystallized from ethanol to afford compounds **15a** & **15b**, respectively.

***N*-[4-(Aminosulfonyl)phenyl]-3-methyl-5-(4-methoxyphenyl)-1*H*-pyrazole-4-carboxamide (15a):** Yield (40 %), m.p.: 126 ºC (ethanol); IR (KBr, ν_{max}, cm^{-1}): 3462 (NH), 3342, 3246 (NH, NH_2), 1640 (C=O); ^{1}H NMR (DMSO-d_6): δ 2.45 (s, 3H, CH_3), 3.84 (s, 3H, OCH_3), 7.07-7.10 (d, 2H, H Ar), 7.31 (s, 2H, NH_2), 7.33-7.36 (d, 2H, H Ar), 7.82-7.85 (d, 2H, H Ar), 7.89-7.92 (m, 3H, H Ar+NH), 8.54 (s, 1H, NH); MS *m/z* (%): 386 (M^+, 4), 270 (47), 267 (27), 241 (14), 161 (41), 149 (23), 134 (18), 121 (100). Anal. calcd. for $C_{18}H_{18}N_4O_4S$ (386.42): C, 55.95; H, 4.70; N, 14.50; S, 8.30. Found: C, 55.88; H, 4.67; N, 14.49; S, 8.20 %.

***N*-[4-(Aminosulfonyl)phenyl]-3-methyl-5-(4-methoxyphenyl)-1-phenylpyrazole-4-carboxamide (15b):** Yield (30 %), 110 ºC (ethanol); IR (KBr, ν_{max}, cm^{-1}): 3452 (NH), 3314, 3256 (NH_2), 1645 (CO); ^{1}H NMR (DMSO-d_6): δ 2.55 (s, 3H, CH_3), 3.88 (s, 3H, OCH_3), 7.00-7.31 and 7.52-7.89 (m, 13H, H Ar), 7.95 (s, 2H, NH_2), 10.21 (s, 1H, NH); MS *m/z* (%): 463 (M^++1, 0.3), 462 (M^+, 0.5), 228 (99), 209 (77), 195 (99), 182 (99), 172 (77), 166 (58), 154 (84), 141 (15), 133 (99), 118 (100), 108 (38), 92 (99), 77 (90), 65 (57). Anal. calcd. for $C_{24}H_{22}N_4O_4S$ (462.52): C, 62.32; H, 4.79; N, 12.11; S, 6.93. Found C, 62.22; H, 4.66; N, 11.91; S, 6.78 %.

***N*-[4-(Aminosulfonyl)phenyl]-6-methyl-4-(4-methoxyphenyl)-2-thio-1,2,3,4-tetrahydropyrimidine-5-carboxamide (16):** To a solution of compound **1** (2.56 g, 10 mmol) and *p*-methoxybenzaldehyde (1.36 g, 10 mmol) in absolute ethanol (40 mL), HCl (0.5 mL) and thiourea (0.76 g, 10 mmol) were added. The reaction mixture was refluxed for 4 h, then left to cool. The resulting solid was filtered off and recrystallized from ethanol. Yield (60 %), m.p.: 255 ºC (ethanol); IR (KBr, ν_{max}, cm^{-1}): 3238-3156 (3NH, NH_2), 1664 (C=O); ^{1}H NMR (DMSO-d_6): δ 2.49 (s, 3H, CH_3), 3.98 (s, 3H, OCH_3), 6.75 (s, 1H, H pyrimidine), 7.11-7.14, 7.52-7.55 and 7.85-7.88 (m, 10H, H Ar+NH_2), 9.86 (s, 1H, NH), 10.01 (s, 1H, NH), 10.20 (s, 1H, NH); MS *m/z* (%): 432 (M^+, 0.5), 408 (44.6), 273 (23.8), 226 (100), 210 (74.1), 195 (23.1), 172 (23.9), 167 (61.8), 151 (8.8), 139 (17.9), 133 (12.9), 92 (29.6), 77 (39.3), 64 (12.1). Anal. calcd. for $C_{19}H_{20}N_4O_4S_2$ (432.52): C, 52.76; H, 4.66; N, 12.95; S, 14.83. Found: C, 52.51; H, 4.63; N, 12.91; S, 14.55 %.

***N*-[4-(Aminosulfonyl)phenyl]-7-methyl-5-(4-methoxyphenyl)-3-oxo-2,3-dihydro-5*H*-[1,3]thiazolo[3,2-a]pyrimidine-6-carboxamide (17):** To a solution of pyrimidine derivative **16** (2.15 g, 5 mmol) in dioxane (20 mL) was added chloroacetyl chloride (0.56 g, 5 mmol). The reaction mixture was refluxed for 2 h, where the reactants dissolved with formation of orange precipitate. The solid product was filtered off and recrystallized from ethanol. Yield (30 %), m.p.: 240 ºC (ethanol); IR (KBr, ν_{max}, cm^{-1}): 3236-3156 (NH, NH_2), 1664 (2C=O); ^{1}H NMR (DMSO-d_6): δ 2.55 (s, 3H, CH_3), 3.83 (s, 3H, OCH_3), 3.88 (s, 2H, CH_2), 5.88 (s, 1H, H pyrimidine), 6.65-6.81 (d, 2H, H Ar), 7.00-7.09 (d, 2H, H Ar), 7.13-7.31 (d, 2H, H Ar), 7.52-7.89 (d, 2H, H Ar), 7.95 (s, 2H, NH_2), 10.21 (s, 1H, NH); MS *m/z* (%): 472 (M^+, 0.6), 471 (M^+-1, 0.1), 290 (100), 226 (22.6), 210 (13.1), 195 (17.1), 172 (24.8), 167 (12.3), 156 (20.5), 108 (5.4), 92 (2.6). Anal. calcd. for $C_{21}H_{20}N_4O_5S_2$ (472.53): C, 53.38; H, 4.27; N, 11.86; S, 13.57. Found: C, 53.35; H, 4.29; N, 11.85; S, 13.52 %.

***N*-[4-(Aminosulfonyl)phenyl]-2-(3,4-diphenyl-3*H*-thiazol-2-ylidene)-3-oxobutanamide (21):** Acetoacetanilide (**1**) (0.522 g, 2 mmol) was added to a stirred solution of KOH (0.11 g, 2 mmol) in DMF (20 mL). The mixture was stirred for 0.5 h and then phenylisothiocyanate (0.27 g, 0.24 mL, 2 mmol) was added. Stirring was continued for 6 h and then 2-bromo-1-phenylethanone (0.398 g, 2 mmol) was added portion wise over a period of 0.5 h. After the addition was complete, the reaction mixture was stirred for additional 12 h, during which the reactant dissolved and a yellow product precipitated. The solid precipitate was filtered off and recrystallized from ethanol. Yield (50 %), m.p.: 250 ºC (ethanol); IR (KBr, ν_{max}, cm^{-1}): 3400, 3254 (NH, NH_2), 1685 (C=O), 1660 (C=O). ^{1}H NMR (DMSO-d_6): δ 2.38 (s, 3H, CH_3), 6.97-7.33 (m, 10H, H Ar), 7.36-7.60 (d, 2H, H Ar), 7.63-7.78 (d, 2H, H Ar), 7.92 (s, 2H, NH_2), 9.61 (s, 1H, H thiazole), 10.49 (s, H, NH); MS *m/z* (%): 492 (M^++1, 2), 491 (M^+, 7), 433 (10.3), 318 (22.7), 293 (100), 251 (13.7), 216 (28.1), 197 (15.1), 172 (21.1), 105 (12.9), 77 (9.1). Anal. calcd. for $C_{25}H_{21}N_3O_4S_2$ (491.58): C, 61.08; H, 4.31; N, 8.55; S, 13.04. Found: C, 61.00; H, 4.28; N, 8.45; S, 12.98 %.

Antimicrobial evaluation: The antibacterial and antifungal activity assays were carried out in the Medical Mycology Laboratory of the Regional Center for Mycology and Biotechnology of Al-Azhar University, Cairo, Egypt using the diffusion plate method[34-36] as follows: a bottomless cylinder containing a measured quantity (1 mL, 5 mg/mL) of the sample is placed on a plate (9 cm diameter) containing a solid bacterial medium (nutrient agar broth) or fungal medium, which has been heavily seeded with a spore suspension of the test organism. After incubation (24 h for bacteria and 5 days for fungi), the diameter of the clear zone of inhibition surrounding the sample is taken as measure of the inhibitory power of the sample against the particular test organism. The solvent used was DMSO and the concentration of the sample used is 100 µg/mL.

Cytotoxic activity: The anticancer activity of all the selected tested compounds were determined against a human liver cancer cell line (HEPG2) using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay and vinblastine was used as a reference drug. Data generated were used to plot a dose response curve of which the concentration of test compounds required to kill 50 % of cell population (IC_{50}) was determined. Cytotoxic activity was expressed as the mean IC_{50} of three independent experiments. The method applied accordance with the previously described procedure[37,38], using Crystal violet stain (1 %). Cells were seeded in 96-well plate at a cell concentration 1×10^4 cells per well in 100 µL of growth medium. Fresh medium containing different concentrations of the test sample was added after 24 h of seeding. Serial two-fold dilutions of the tested chemical compound were added to confluent cell monolayers, dispensed into 96-wel, flat-bottomed microtiter plates using a multichannel pipette. The microtiter plates were incubated at 37 °C in a humidified incubator with 5 % CO_2 for 48 h. Three wells were used for each concentration of the test sample. Control cells were incubated without test sample with DMSO. After incubation of the cells for 24 h at 37 °C, various concentrations of sample (50, 25, 12.5, 6.25, 3.125 and 1.56 µg) were added and the incubation was continued for 48 h and the viable cells yield was determined by a colorimetric method. After the end of incubation period, media were aspirated and the crystal violet solution was added to each well for at least 0.5 h. The stain was removed and the plates were rinsed using tap water until all excess stain is removed. Glacial acetic acid (30 %) was then added to all wells and mixed thoroughly and then the absorbance of the plates was measured after gently shaken on Microplate reader, using a test wavelength of 490 nm. All results were corrected for background absorbance detected in wells without added stain. Treated samples were compared with the cell control in the absence of the tested compounds. All experiments were carried out in triplicate. The cell cytotoxic effect of each tested compound was calculated.

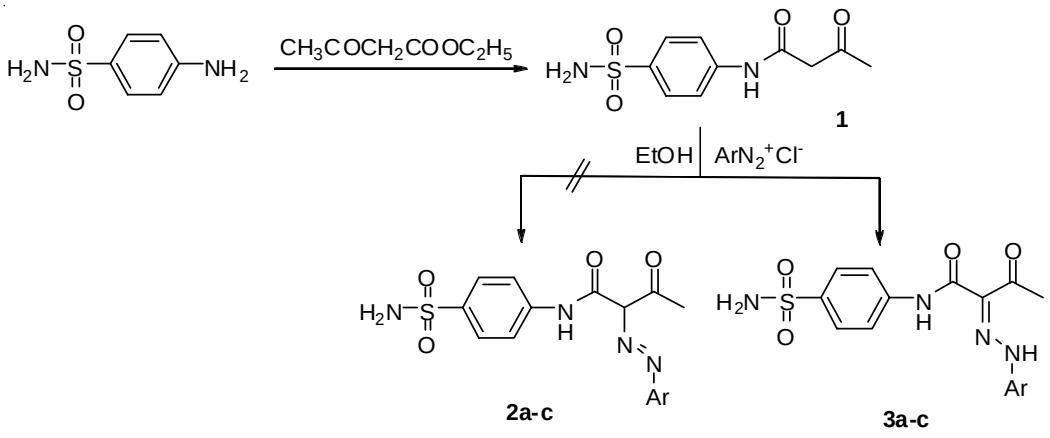
RESULTS AND DISCUSSION

The required starting material *N*-[4-(aminosulfonyl)phenyl]-3-oxobutanamide (**1**) was prepared as previously described[33]. Treatment of compound **1** with diazotized anilines in ethanol buffered with sodium acetate afforded respective arylazo derivatives **3** (**Scheme-I**).

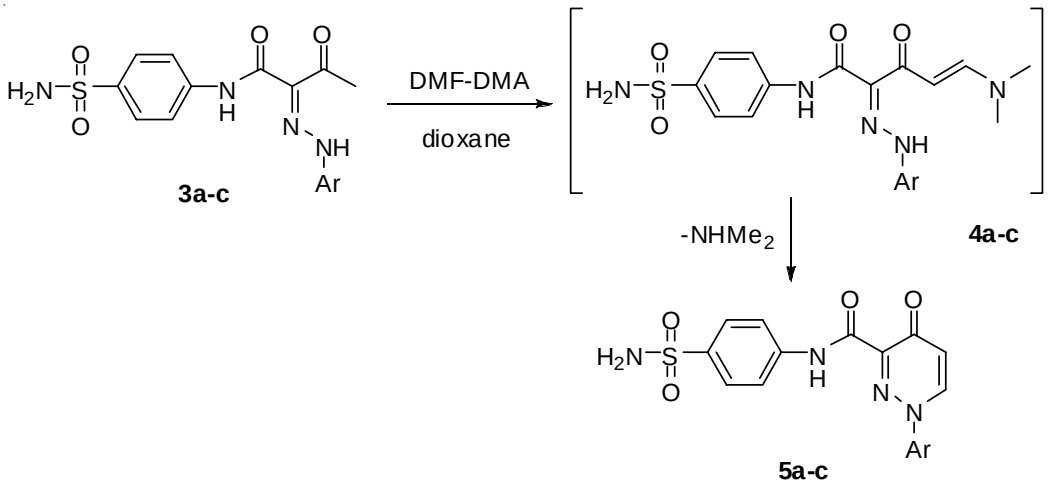
Although latter products can have one of the two isomeric structures **2** or **3**, they were assigned the former structure **3** on the basis of their spectral data. Their ^{1}H NMR spectra reveal, in each case, two characteristic singlet signals in the regions δ 11.32-11.39 and 12.21-13.68 ppm assignable to a two NH protons present in hydrazone isomeric structure **3a-c**[39]. The electronic absorption spectra of **3a-c** in dioxane showed two absorption maxima in the regions 380-395 and 285-320 nm. This absorption pattern is similar to that of hydrazone chromophore[40-42].

In addition, treatment of *N*-[4-(aminosulfonyl)phenyl]-2-arylhydrazono-3-oxobutanamide (**3a-c**) with dimethylformamide dimethylacetal (DMF-DMA) in refluxing dry dioxane afforded the *N*-[4-(aminosulfonyl)phenyl]-1-aryl-4-oxo-1,4-dihydropyridazine-3-carboxamide (**5a-c**) in good yield. This process is assumed to follow a route in which compounds **3a-c** were initially converted to the enamine derivative **4** which underwent sequential electrocyclization and dimethylamine elimination to form compound **5** (**Scheme-II**).

The reactivity of the compound **1** towards malononitrile and elemental sulfur, 1,3-diphenylpropenone, 2-cyano-3-(4-methoxyphenyl)thioacrylamide and *p*-methoxy-benzaldehyde was investigated. Thus the behaviour of the condensation of the compound **1** towards malononitrile and elemental sulfur as potential precursors for 2-amino-3-cyanothiophenes[43] were investigated. Thus, treatment of compound **1** with elemental sulfur and malononitrile, in refluxing ethanol in the presence of catalytic



Scheme-I: Synthesis of hydrazones derivatives



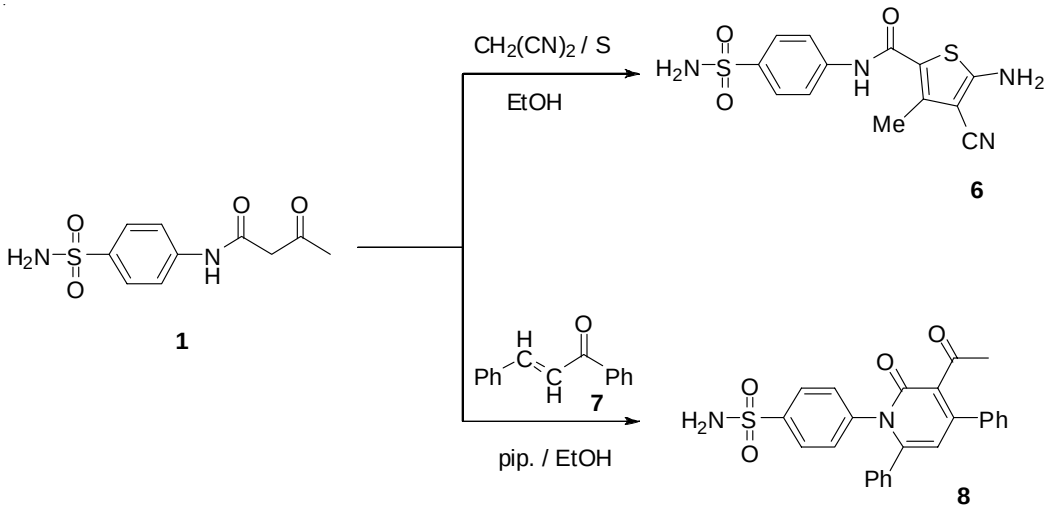
Scheme-II: Synthesis of 1,4-dihydropyridazine derivatives

amount of triethylamine, furnished a single product identified as *N*-[4-(aminosulfonyl)phenyl]-5-amino-4-cyano-3-methylthiophene-2-carboxamide (**6**). The assigned structure can be formed *via* application of Gewald synthesis to alkyl heterocyclic carbonitriles and afforded the thiophene derivative[43] **6** (**Scheme-III**).

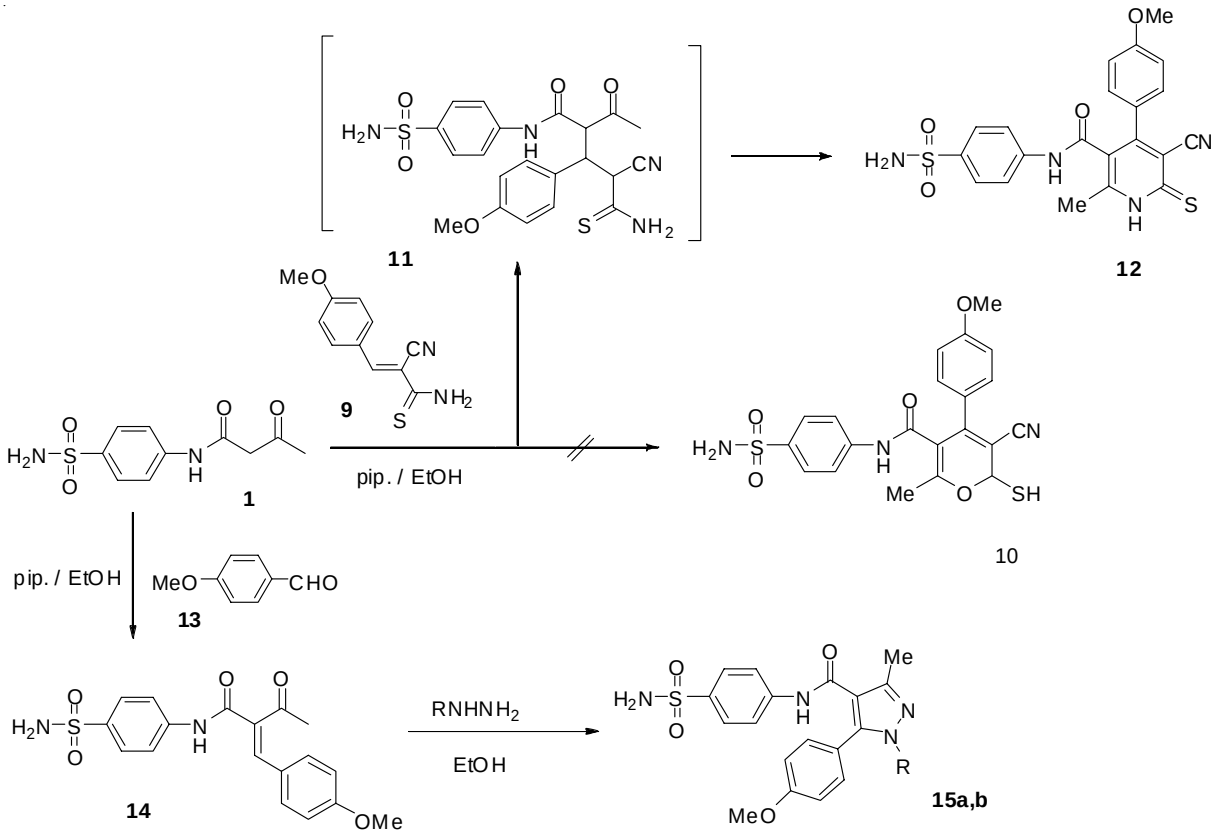
Also, treatment of compound **1** with 1,3-diphenylpropenone (**7**) afforded a yellow crystalline product identified as 4-(3-acetyl-4,6-diphenyl-2-oxopyridin-1(2*H*)-yl)benzenesulfonamide (**8**) (**Scheme-III**). In a similarly manner, the compound **1** reacted with arylidenecyanothioacetamide **9** in refluxing ethanol in the presence of piperidine to give a single product (as examined by TLC) that was identified as *N*-[4-(aminosulfonyl)phenyl]-5-cyano-2-methyl-4-(4-methoxyphenyl)-6-thionicotinamide (**12**) (**Scheme-IV**).

The formation of compound **12** can be explained on the basis of an initial Michael addition of the active methylene moiety in compound **1** to the activated double bond in the compound **9** to afford the acyclic Michael adduct intermediate **11** which undergo intramolecular cyclization *via* loss of H_2O and aromatization takes place during work up *via* air oxidation afforded compound **12** (**Scheme-IV**)[44]. The structure of compound **10** was ruled out on the basis of elemental analysis and spectral data. Also treatment of compound **1** with aromatic aldehyde in ethanol in the presence of catalytic amount of piperidine afforded the corresponding *N*-[4-(aminosulfonyl)phenyl]-2-acetyl-3-(4-methoxyphenyl)acrylamide (**14**). The treatment of compound **14** with hydrazine hydrate or phenyl hydrazine, in refluxing ethanol, afforded the corresponding pyrazole derivatives **15a,b** (**Scheme-IV**). Compound **1** reacts with a mixture of *p*-methoxybenzaldehyde and thiourea in refluxing ethanol in the presence of hydrochloric acid, afforded a yellow crystalline product **16** (**Scheme-V**). Heating of compound **16** with chloroacetyl chloride in refluxing dioxane afforded *N*-[4-(aminosulfonyl)phenyl]-7-methyl-5-(4-methoxyphenyl)-3-oxo-2,3-dihydro-5*H*-[1,3]thiazolo[3,2-a]pyrimidine-6-carboxamide (**17**) (**Scheme-V**).

The nucleophilic addition of compound **1** to equimolar amount of phenyl isothiocyanate **18** in DMF, in the presence of KOH, afforded the corresponding potassium sulfide salt **19** which was not isolated. Heterocyclization of the intermediate **19** with 2-bromo-1-phenylethanone under the same reaction condition, afforded *N*-[4-(aminosulfonyl)phenyl]-2-(3,4-diphenyl-3H-thiazol-2-ylidene)-3-oxobutanamide (**21**) (**Scheme-V**). This result indicates that the reaction of the



Scheme-III: Synthesis of thiophene and oxopyridine derivatives



Scheme-IV

intermediate **19** with 2-bromo-1-phenylethanone proceeds *via* loss of H_2O molecule from the non-isolable intermediate **20** (**Scheme-V**).

Screening for antimicrobial activity: The newly synthesized compounds **3a**, **5a**, **6**, **8**, **12**, **14a,b**, **15a**, **17** and **21** were evaluated for their *in vitro* antibacterial activity against *Streptococcus pneumoniae* (RCMB-010010) and *Bacillis subtilis* (RCMB-010067) as examples of Gram-positive bacteria and *Pseudomonas aeruginosa* (RCMB-010043) and *Escherichia coli* (RCMB-010052) as examples of Gram-negative bacteria. They were also evaluated for their *in vitro* antifungal activity against *Aspergillus fumigatus* (RCMB-02568), *Syncephalastrum racemosum* (RCMB-05922), *Geotricum candidum* (RCMB-05097) and *Candida albicans* (RCMB-05036) fungal strains. Inhibition zone diameter (IZD) in mm was used as criterion for the antimicrobial activity using the diffusion technique[34-36].

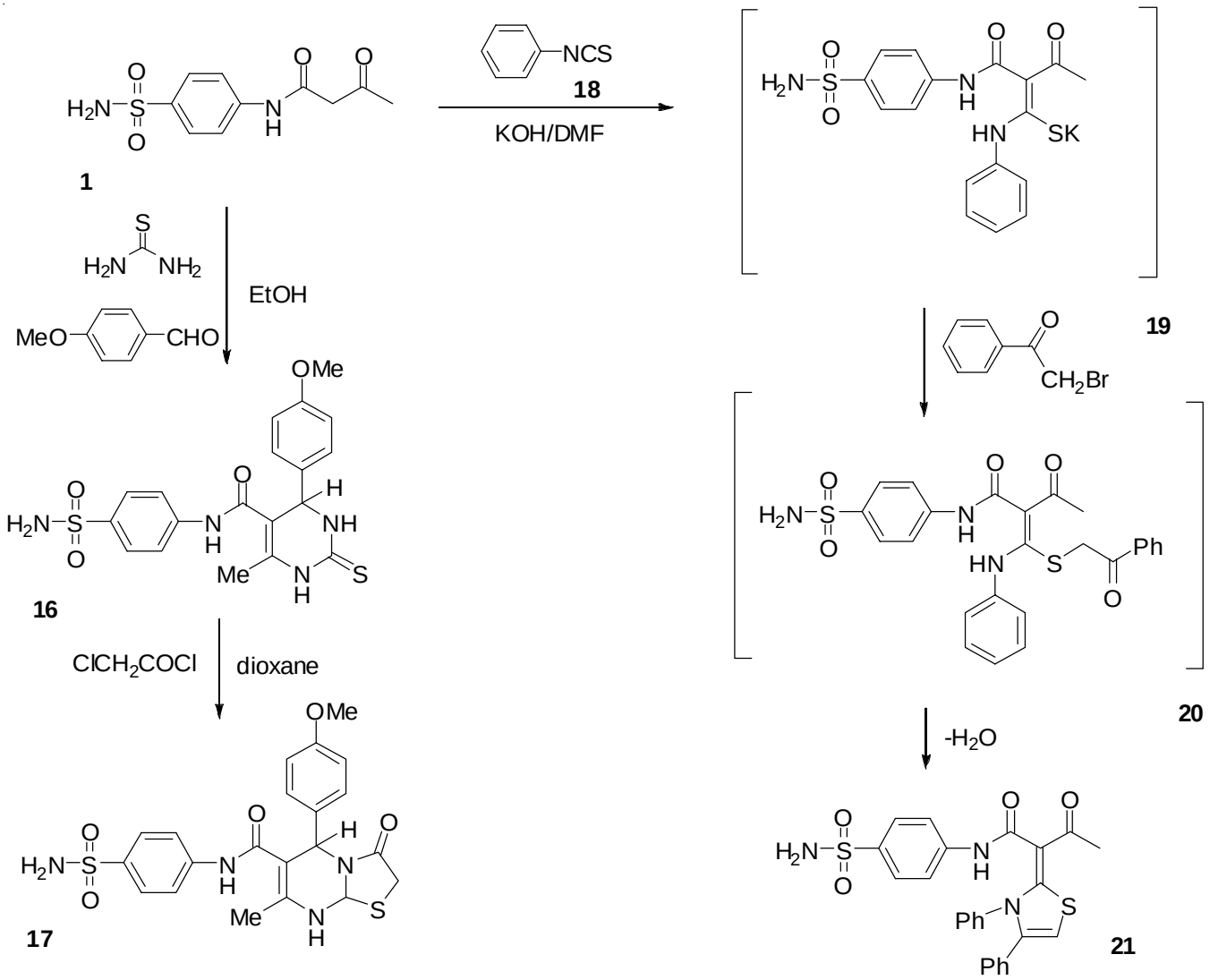
The fungicide amphotericin B and the bactericides ampicillin and gentamycin were used as references to evaluate the potency of the tested compounds under the same conditions. The results are depicted in Table-1.

As seen from Table-1, *Streptococcus pneumoniae* and *Bacillis subtilis* are sensitive to all tested compounds except

TABLE-1
ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIES OF THE SYNTHESIZED COMPOUNDS (**3a, 5a, 6, 8, 12, 14a,b, 15a, 17** AND **21**)

Compound	Inhibition zone diameter (cm)							
	Gram-positive		Gram-negative		Fungi			
	Streptococcus pneumoniae	*Bacillis subtilis*	*Pseudomonas aeruginosa*	*Escherichia coli*	*Aspergillus fumigatus*	*Syncephalastrum racemosum*	*Geotricum candidum*	*Candida albicans*
Standard values*	23.8 ± 0.2	32.4 ± 0.3	17.3 ± 0.1	19.9 ± 0.3	23.7 ± 0.2	19.7 ± 0.2	28.7 ± 0.2	25.4 ± 0.1
3a	12.3 ± 0.58	12.7 ± 0.37	NA	8.5 ± 0.37	17.6 ± 0.58	15.4 ± 0.25	12.6 ± 0.38	NA
5a	16.9 ± 0.58	18.2 ± 0.44	NA	11.9 ± 0.63	15.7 ± 0.33	13.8 ± 0.25	18.3 ± 0.34	NA
6	NA	NA	NA	NA	NA	NA	NA	NA
8	16.7 ± 0.36	19.2 ± 0.27	NA	13.6 ± 0.36	16.8 ± 0.39	13.4 ± 0.58	19.6 ± 0.19	15.9 ± 0.44
12	18.3 ± 0.25	22.6 ± 0.44	13.1 ± 0.32	20.3 ± 0.09	20.6 ± 0.58	16.7 ± 0.33	22.4 ± 0.36	17.6 ± 0.58
14a	18.9 ± 0.44	21.7 ± 0.25	11.6 ± 0.19	15.4 ± 0.39	20.2 ± 0.55	16.3 ± 0.25	22.4 ± 0.58	19.6 ± 0.33
14b	16.3 ± 0.55	18.3 ± 0.25	NA	NA	17.3 ± 0.44	12.6 ± 0.25	19.0 ± 0.58	16.9 ± 0.25
15a	17.5 ± 0.44	19.8 ± 0.63	NA	18.9 ± 0.25	15.3 ± 0.55	13.4 ± 0.35	11.5 ± 0.58	NA
17	12.3 ± 0.58	12.7 ± 0.37	NA	10.8 ± 0.44	17.6 ± 0.58	15.4 ± 0.25	12.6 ± 0.38	NA
21	16.9 ± 0.58	18.2 ± 0.44	NA	11.9 ± 0.63	16.2 ± 0.36	15.0 ± 0.44	17.6 ± 0.58	NA

Data are expressed in the form of mean ± SD. Mean zone of inhibition in mm ± Standard deviation beyond well diameter; (6 mm) produced on a range of environmental and clinically pathogenic microorganism using (5 mg/mL) concentration of tested sample (100 µL was tested).
*The fungicide amphotericin B and the bactericides ampicillin and gentamycin.



Scheme-V

compound **6**; furthermore, *Pseudomonas aeruginosa* is sensitive to compounds **14a** and **12**, while *Escherichia coli* is sensitive to **5a**, **8**, **12**, **14a**, **15a**, **17** and **21** except compound **6**. All tested compounds except compound **6** exhibit antifungal activity against the three tested fungi species *Aspergillus fumigatus*, *Syncephalastrum racemosum* and *Geotricum candidum*. Also the *Candida albicans* strain is sensitive to compounds **8**, **12** and **14a**. The high activity of **8**, **12** and **14a**, **12** and **8** is attributed to the presence of pharmacological active pyridine ring in **8** and **12** and arylidene moiety in compound **14a**. The inactivity of compound **6** against the tested bacteria and fungi is due to the presence of a thiophene ring.

Anticancer activity: The anticancer activity of the synthesized compounds was determined against a human liver (HEPG2) cell line using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay and vinblastine was used as a reference drug. Cytotoxic activity was expressed as the mean IC_{50} of three independent experiments (Table-2). Data generated were used to plot a dose-response curve of which the concentration of test compounds required to kill 50 % of cell population (IC_{50}) was determined. According to Shier *et al.*[45] the compounds exhibiting IC_{50} activity within the range of 10-25 µg/mL are considered weak anticancer drugs, while those of IC_{50} activity between 5 and 10 µg/mL are moderate and compounds of activity below 5.00 µg/mL are considered strong agents. So, compounds **6** is strong, **5a** and **14** are moderate, while compounds **5b**, **8**, **12**, **15a** and **15b** are weak against the studied cell lines (Fig. 1).

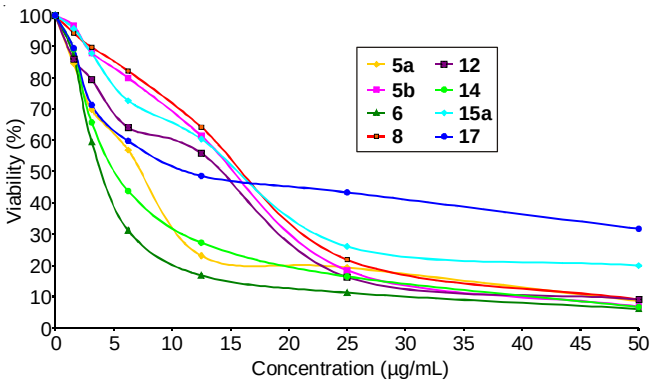


Fig. 1. Effect of the compounds **5a**, **5b**, **6**, **8**, **12**, **14**, **15a** and **17** on cellular viability (HEPG-2 cells)

TABLE-2
VIABILITY VALUES AND IC_{50} OF THE SYNTHESIZED COMPOUNDS AGAINST HEPG-2 CELL LINE

Compounds	Sample concentration (µg/mL) viability (%)							
	50	25	12.5	6.25	3.125	1.56	0	IC_{50} (µg)
Vinblastine	14.38	16.13	24.25	45.13	55.00	72.13	100	4.6
5a	8.58	19.32	23.08	56.84	69.75	84.58	100	7.5
5b	6.80	18.42	61.43	79.96	88.04	96.02	100	15.8
6	6.08	11.23	16.79	31.17	69.38	86.94	100	4.2
8	9.03	21.84	64.21	82.19	89.72	94.38	100	16.7
12	9.12	16.31	55.87	64.10	79.39	86.36	100	14.3
14	6.67	16.43	27.28	43.87	65.69	89.14	100	5.6
15a	13.92	25.06	60.38	72.63	87.84	95.76	100	16.1
15b	31.74	43.38	48.59	59.76	71.24	89.47	100	11.6

Conclusion

Several new hydrazones, acrylamides, pyrazoles, sulfonamide, thionicotinamide, thiophenes, pyrazoles, thiopyrimidine, thiazolo[3,2-a]pyrimidine and thiazoles contain aminosulfonyl moiety were prepared using simple methods *via* a versatile, readily accessible *N*-[4-(aminosulfonyl)phenyl]-3-oxobutanamide (**1**) are demonstrated. The structures of the newly synthesized compounds were proven by spectral methods and they were tested for their antimicrobial activities and anticancer activity was determined against a human liver (HEPG2) cell line. Most of these compounds showed promising activities against both Gram-positive, Gram-negative bacteria and fungi also some compounds are strong anticancer drugs.

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