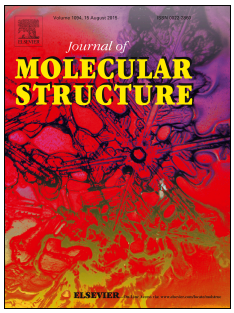
Accepted Manuscript



Design, synthesis, molecular docking and biological screening of *N*-ethyl-*N*-methylbenzenesulfonamide derivatives as effective antimicrobial and antiproliferative agents

Shimaa M. Abd El-Gilil

PII: S0022-2860(19)30443-0

DOI: https://doi.org/10.1016/j.molstruc.2019.04.048

Reference: MOLSTR 26420

To appear in: *Journal of Molecular Structure*

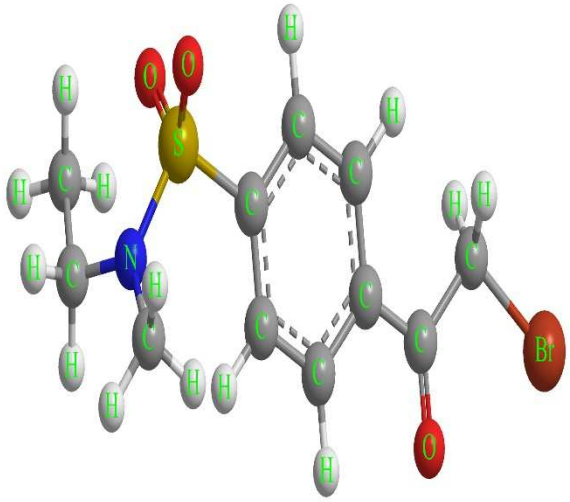
Received Date: 4 March 2019

Revised Date: 9 April 2019

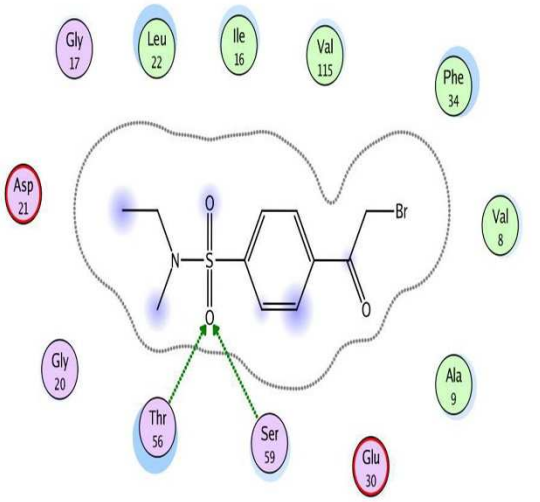
Accepted Date: 10 April 2019

Please cite this article as: S.M. Abd El-Gilil, Design, synthesis, molecular docking and biological screening of *N*-ethyl-*N*-methylbenzenesulfonamide derivatives as effective antimicrobial and antiproliferative agents, *Journal of Molecular Structure* (2019), doi: https://doi.org/10.1016/j.molstruc.2019.04.048.

GRAPHICAL ABSTRACT



3D structure of starting material **2**



2D Ligand-protein interactions in compound **2-DHFR** X-ray complex

Design, synthesis, molecular docking and biological screening of *N*-ethyl-*N*-methylbenzenesulfonamide derivatives as effective antimicrobial and antiproliferative agents

Shimaa M. Abd El-Gilil

Department of Pharmaceutical Organic Chemistry, Faculty of Pharmacy (Girls), Al-Azhar University, Nasr City, 11754, Cairo, Egypt

ABSTRACT

Sulfonamides are the most famous agents, which have been utilized for preparation of effective antiproliferated agents. Therefore, this article describes the synthesis of new series of *N*-ethyl-*N*-methylbenzenesulfonamide derivatives having various biologically active moieties such as, thiazoles **3**, **4**, **11**, **12**, **14**, **15**, **21**, 1,3,4-thiadiazine **6**, imidazo[2,1-*b*]thiazole **8**, 2-oxo-2*H*-chromene **17**, and 3-oxo-3*H*-benzo[*f*]chromene **19**, starting with 4-(2-bromoacetyl)-*N*-ethyl-*N*-methylbenzenesulfonamide (**2**), that was synthesized from the interaction of 4-acetyl-*N*-ethyl-*N*-methylbenzenesulfonamide (**1**) with bromine under stirring in dioxane/diethylether mixture. The newly structures were be proved *via* their elemental analysis and spectral data. However, they were also screened for their cytotoxic activity against two different human cell lines, alveolar adenocarcinoma carcinoma (lung) (A-549) and liver carcinoma (HepG2) and antimicrobial. Compound **8** having imidazo[2,1-*b*]thiazole moiety exhibited the most potent cytotoxic activity against (A-549) cell line (SI; 30.77). While, compound **11** having 2-cyanomethyl thiazole moiety showed significant cytotoxic activity against (HepG2) cell line (SI; 67.11). On the other hand, compound **9** having 4-chlorophenyl moiety exerted significant antimicrobial activity more than the reference drugs. Molecular Operating Environment (MOE) was performed for the synthesized compounds to study their mode of action as inhibitors against DHFR enzyme active sites.

Keywords:

N-ethyl-*N*-methylbenzenesulfonamide, Thiazole, Imidazo[2,1-*b*]thiazole, Anticancer, Antimicrobial, Molecular docking

1. Introduction

Thiazole, 1,3,4-thiadiazine and imidazo[2,1-*b*]thiazole derivatives were reported as antimicrobial [1,2], anti-inflammatory [3] and antiviral [4-6] agents. Moreover, they acted like antihistaminic [7], antiparasitic [8], besides as calming down agents [9]. Furthermore, thiazoles were widely used in the dye and photographic industry [10]. Additionally, sulfonamides displayed antibacterial [11-14], antifungal [15], insulin releasing [16-18], carbonic anhydrase inhibitor [19-22], hypoglycemic [23], anesthetic [24], anti-tumor [25,26], anti-cancer [27] and anti-inflammatory [28,29] activities. Some active sulfonamides as anti-bacterial are also known for their immune modifying effects [30,31]. Therefore, taking in consideration the various reports and in continuation of the previous work [32-37], it was aimed to synthesized substituted benzenesulfonamide conjugated with thiazole, 1,3,4-thiadiazine, imidazo[2,1-*b*]thiazole, 2-oxo-2*H*-chromene, 3-oxo-3*H*-benzo[*f*]chromene moieties that are required to medicinal chemistry utilizing phenacyl bromide derivative **2** as a backbone.

2. Materials and methods

2.1. Chemistry

An apparatus called electrothermal melting temperature has been used to measure melting points, which are uncorrected. IR spectra were determined as KBr discs using IR-470 Shimadzu spectrometer, (ν, cm^{-1}). ^{1}H and ^{13}C NMR spectra were monitored on Bruker 300 *MHz* spectrometer in DMSO-d_6. Mass spectra were run on a Shimadzu GC/MS-QP 5000 instrument at 70 *eV*. Elemental analysis were done on Carlo Erba 1108 CHN Analyzer. All compounds were within ±0.4 % of the theoretical values. Besides, the reported 4-acetyl-*N*-ethyl-*N*-methylbenzenesulfonamide (**1**) was prepared according to

the method specified in the literature [38]. **Table 8,** lists the physical and analytical data of the newly prepared compounds.

*2.1.1. 4-(2-Bromoacetyl)-N-ethyl-N-methylbenzenesulfonamide **(2)***

To a stirred solution of acetophenone derivative **1** (2.41 g, 0.01 mol) in a 30 mL mixture of dioxane/diethylether (1:2), drops of bromine (1.59 g, 0.01 mol) were added, leave on side, then poured on cold water, filter the product and recrystallized from ethanol to give **2**. Yield, 87 %. IR (KBr, cm^{-1}): ν_{max} = 3056 (CH arom.), 2909 (CH aliph.), 1701 (C=O), 1333, 1160 (SO_2), 1179 (C-S). ^{1}H NMR: δ *ppm*: 1.08 (t, 3H, CH_3 ethyl), 2.71 (s, 3H, CH_3), 3.23 (q, 2H, CH_2 ethyl), 4.63 (s, 2H, CH_2), 7.87, 8.63 (dd, 4H, Ar-H, *J* = 8.7 *Hz*). ^{13}C NMR: δ *ppm*: 11.7 (CH_3 ethyl), 31.9 (CH_2), 34.0 (CH_3), 43.8 (CH_2 ethyl), [129.5 (2C), 131.4 (2C), 139.3, 146.5] (6 arom. C's), 189.3 (C=O). MS *m/z* (%): 321 [M^++2] (25); 319 [M^+] (36); 306 (100); 304 (92); 263 (26); 261 (24); 226 (41); 199 (20); 197 (21); 162 (16); 148 (16); 122 (4); 120 (8); 118 (25); 106 (20); 104 (47); 90 (29); 89 (34); 77 (6); 75 (36); 65 (4); 63 (13).

*2.1.2. 4-(2-Aminothiazol-4-yl)-N-ethyl-N-methylbenzenesulfonamide **(3)***

2.1.2.1. Procedure A; in dioxane (40 mL), a triturated mix of I_2 (2.53 g, 0.01 mole) and thiourea (1.52 g, 0.02 mole) was added to acetophenone derivative **1** (2.41 g, 0.01 mol). Reflux with stirring was continued for 8 h. The obtained solid was washed with aqueous sodium thiosulfate to remove excess iodine and then with water. After that, in hot water, dissolve the obtained solid and filter while hot. Also, NH_3. H_2O was added to precipitate 2-aminothiazole derivative **3**, which dried and recrystallized from ethanol, Yield, 41 %.

2.1.2.2. Procedure B; a solution of phenacyl bromide derivative **2** (3.20 g, 0.01 mol) and thiourea (0.76 g, 0.01 mole) in ethanol was refluxed for 2 h. Then add (5 mL) of pyridine with continuation of reflux for 5 h. Collect the gotten solid and recrystallized from dioxane. Yield, 79 %. IR (KBr, cm^{-1}): ν_{max} = 3300, 3284 (NH_2), 3093 (CH arom.), 2935 (CH aliph.), 1550 (C=N), 1364, 1181 (SO_2), 1179 (C-S). ^{1}H NMR: δ *ppm*: 1.04 (t, 3H, CH_3 ethyl), 2.69 (s, 3H, CH_3), 3.27 (q, 2H, CH_2 ethyl), 6.91 (s, 1H, CH-thiazole), 7.53 (s,

2H, NH_2 transferrable with D_2O), 7.82, 8.67 (dd, 4H, Ar-H, J = 9.1 Hz). ^{13}C NMR: δ *ppm*: 11.9 (CH_3 ethyl), 35.6 (CH_3), 39.7 (CH_2 ethyl), 106.9 (C5-thiazole), [127.6 (2C), 129.7 (2C), 137.3, 138.5] (6 arom. C's), 154.4 (C4-thiazole), 169.6 (C2-thiazole). MS *m/z* (%): 299 [M^++2] (3); 298 [M^++1] (4); 297 [M^+] (32); 282 (14); 239 (33); 191 (15); 176 (15); 175 (100); 133 (8); 121 (7); 105 (10); 104 (10); 89 (17); 64 (7); 52 (8); 51 (8).

2.1.3. N-Ethyl-4-(2-hydrazinylthiazol-4-yl)-N-methylbenzenesulfonamide **(4)**

Reflux an ethanolic solution of thiosemicarbazide (0.91 g, 0.01 mol) and **2** (3.20 g, 0.01 mol) for 1 h. Filter off the product and recrystallized from ethanol/benzene to give **4**. Yield, 55 %. IR (KBr, cm^{-1}): ν_{max} = 3390, 3345 (NH_2), 3270 (NH), 3090 (CH arom.), 2981 (CH aliph.), 1590 (C=N), 1343, 1170 (SO_2), 1179 (C-S). ^1H NMR: δ *ppm:* 1.03 (t, 3H, CH_3 ethyl), 2.61 (s, 3H, CH_3), 3.32 (q, 2H, CH_2 ethyl), 5.09 (br, 2H, NH_2 transferrable with D_2O), 7.34 (s, 1H, CH-thiazole), 7.97, 8.01 (dd, 4H, Ar-H, J = 8.7 *Hz*), 11.20 (s, 1H, NH transferrable with D_2O). ^{13}C NMR: δ *ppm*: 12.1 (CH_3 ethyl), 35.2 (CH_3), 37.4 (CH_2 ethyl), 106.0 (C5-thiazole), [124.9 (2C), 128.6 (2C), 134.1, 137.5] (6 arom. C's), 153.2 (C4-thiazole), 176.6 (C2-thiazole). MS *m/z* (%): 313 [M^++1] (1); 312 [M^+] (2); 298 (4); 255 (5); 239 (3); 224 (6); 199 (3); 191 (4); 175 (8); 160 (16); 149 (3); 133 (4); 129 (6); 127 (7); 109 (21); 107 (21); 101 (19); 96 (14); 89 (8); 82 (100); 81 (42); 80 (98); 79 (42); 74 (14); 64 (10); 60 (17); 45 (18).

2.1.4. N-Ethyl-N-methyl-4-(2-(phenylamino)-6H-1,3,4-thiadiazin-5-yl)benzene-sulfonamide **(6)**

An equimolar mixture of **2** (3.20 g, 0.01 mol) and *N*-phenylhydrazinecarbothioamide (1.67 g, 0.01 mol) was heated under reflux for 4 h in ethanol (40 mL). Collect the solid that formed while heating and recrystallized from ethanol to give **6**. Yield, 43 %. IR (KBr, cm^{-1}): ν_{max} = 3215 (NH), 3065 (CH arom.), 2912 (CH aliph.), 1584 (C=N), 1356, 1160 (SO_2), 1179 (C-S). ^1H NMR: δ *ppm:* 1.04 (t, 3H, CH_3 ethyl), 2.72 (s, 3H, CH_3), 3.36 (q, 2H, CH_2 ethyl), 4.41 (s, 2H, CH_2-thiadiazine), 6.51-8.17 (m, 9H, Ar-H), 9.82 (s, 1H, NH transferrable with D_2O). ^{13}C NMR: δ *ppm:* 11.3 (CH_3 ethyl), 27.3 (C6-

thiadiazin), 38.1 (CH_3), 39.2 (CH_2 ethyl), [121.6 (2C), 123.1, 128.8 (2C), 130.1 (2C), 131.2 (2C), 138.4, 140.1, 144.1] (12 arom. C's), 154.3 (C2-thiadiazin), 168.7 (C5-thiadiazin). MS *m/z* (%): 388 [M^+] (4); 341 (6); 307 (10); 306 (95); 304 (89); 286 (9); 284 (19); 278 (9); 258 (10); 212 (10); 149 (6); 148 (7); 100 (100); 99 (9); 73 (29); 55 (16); 53 (22); 46 (11).

2.1.5. N-Ethyl-4-(imidazo[2,1-b]thiazol-5-yl)-N-methylbenzenesulfonamide **(8)**

An equimolar mixture of phenacyl bromide derivative **2** (3.20 g, 0.01 mol) and 2-aminothiazole (1 g, 0.01 mol) was heated under reflux for 3 h, in absolute ethanol (30 ml). Solid product was collected and recrystallized from dioxane to give **8**. Yield, 46 %. IR (KBr, cm^{-1}): ν_{max} = 3099 (CH arom.), 2965 (CH aliph.), 1583 (C=N), 1358, 1151 (SO_2), 1179 (C-S). ^{1}H NMR: δ *ppm*: 1.03 (t, 3H, CH_3 ethyl), 2.71 (s, 3H, CH_3), 3.34 (q, 2H, CH_2 ethyl), 7.56-8.17 (m, 6H, Ar-H + CH=CH of thiazole), 8.01 (s, 1H, CH-imidazole). ^{13}C NMR: δ *ppm:* 11.2 (CH_3 ethyl), 35.6 (CH_3), 37.4 (CH_2 ethyl), 121.5, 122.4, 124.2, [125.5 (2C), 128.4 (2C), 134.5, 137.3] (6 Arom. C's), 131.1, 148.4. MS *m/z* (%): 321 [M^+] (2); 306 (4); 263 (6); 228 (5); 227 (3); 226 (22); 199 (13); 197 (8); 179 (5); 124 (1); 119 (4); 108 (5); 105 (4); 104 (12); 101 (7); 100 (90); 99 (20); 89 (14); 82 (96); 81 (42); 80 (100); 79 (44); 76 (18); 73 (60); 72 (16); 58 (33); 57 (8).

2.1.6. 4-(2-((4-Chlorophenyl)amino)acetyl)-N-ethyl-N-methylbenzenesulfonamide **(9)**

An ethanolic solution of **2** (3.20 g, 0.01 mol) and *p*-chloroaniline (1.52 g, 0.012 mol) was refluxed for 4 h, the product was recrystallize from ethanol to give **9**. Yield, 62 %. IR (KBr, cm^{-1}): ν_{max} = 3201 (NH), 3086 (CH arom.), 2927 (CH aliph.), 1681 (C=O), 1369, 1141 (SO_2), 1179 (C-S), 715 (C-Cl). ^{1}H NMR: δ *ppm*: 1.04 (t, 3H, CH_3 ethyl), 2.69 (s, 3H, CH_3), 3.34 (q, 2H, CH_2 ethyl), 4.50 (s, 2H, CH_2), 6.61, 7.32 (dd, 4H, Ar-H of chlorophenyl, *J* = 8.2 *Hz*), 7.81, 8.24 (dd, 4H, Ar-H of benzenesulfonamide, *J* = 8.5 *Hz*), 10.22 (s, 1H, NH transferrable with D_2O). ^{13}C NMR: δ *ppm:* 11.5 (CH_3 ethyl), 32.5 (CH_3), 36.5 (CH_2 ethyl), 63.1 (CH_2), [115.3 (2C), 128.4, 129.5 (2C), 131.2 (2C), 132.3 (2C), 139.2, 146.2, 148.4] (12 ArC's), 197.7 (C=O). MS *m/z* (%): 366 [M^+] (6); 364 (5);

359 (15); 328 (8); 313 (21); 312 (89); 307 (100); 277 (10); 226 (20); 214 (13); 208 (16); 207 (25); 199 (37); 185 (49); 171 (12); 163 (21); 152 (12); 150 (11); 142 (12); 140 (29); 138 (31); 127 (16); 119 (15); 111 (19); 105 (88); 104 (58); 91 (25); 78 (37); 77 (41).

2.1.7. 4-(2-Cyanoacetyl)-N-ethyl-N-methylbenzenesulfonamide **(10)**

To an ethanolic solution of **2** (3.20 g, 0.01 mol) and potassium cyanide (0.65 g, 0.01 mol) was added and heated under reflux for 5 h, upon reflux, a yellowish white crystals were gotten, filtered and recrystallized from ethanol to give **10**. Yield, 37 %. IR (KBr, cm^{-1}): ν_{max} = 3075 (CH arom.), 2978 (CH aliph.), 2202 (C≡N), 1627 (C=O), 1363, 1155 (SO_2), 1179 (C-S). ^{1}H NMR: *δ ppm:* 1.03 (t, 3H, CH_3 ethyl), 2.69 (s, 3H, CH_3), 3.35 (q, 2H, CH_2 ethyl), 3.71 (s, 2H, CH_2), 7.79, 8.31 (dd, 4H, Ar-H, *J* = 10.4 *Hz*). ^{13}C NMR: *δ ppm:* 12.2 (CH_3 ethyl), 27.6 (CH_2), 36.3 (CH_3), 39.8 (CH_2 ethyl), [124.4 (2C), 127.5 (2C), 139.1, 147.3] (6 arom. C's), 136.2 (C≡N), 189.6 (C=O). MS *m/z* (%): 267 [M^++1] (10); 266 [M^+] (12); 264 (10); 263 (64); 238 (31); 226 (18); 215 (13); 208 (16); 198 (100); 186 (13); 184 (46); 175 (44); 169 (11); 166 (12); 159 (15); 149 (15); 134 (12); 121 (20); 120 (13); 119 (11); 105 (16); 103 (13); 91 (18); 89 (30); 77 (20); 76 (14); 64 (73); 60 (15); 59 (20).

2.1.8. 4-(2-(Cyanomethyl)thiazol-4-yl)-N-ethyl-N-methylbenzenesulfonamide **(11)**

A mixture of **2** (3.20 g, 0.01 mol) and 2-cyanoethanethioamide (1.00 g, 0.01 mol) in ethanol was heated under reflux for 3 h. The obtained solid was recrystallized from ethanol/benzene to give **11**. Yield, 81 %. IR (KBr, cm^{-1}): ν_{max} = 3097 (CH arom.), 2981 (CH aliph.), 2198 (C≡N), 1597 (C=N), 1336, 1160 (SO_2), 1179 (C-S). ^{1}H NMR: *δ ppm*: 1.04 (t, 3H, CH_3 ethyl), 2.71 (s, 3H, CH_3), 3.31 (q, 2H, CH_2 ethyl), 4.62 (s, 2H, CH_2), 7.79 (s, 1H, H5-thiazole), 7.13, 8.01 (dd, 4H, Ar-H, *J* = 8.6 *Hz*). ^{13}C NMR: *δ ppm:* 11.3 (CH_3 ethyl), 22.1 (CH_2), 35.2 (CH_3), 38.3 (CH_2 ethyl), 104.3 (C5-thiazole), 116.1 (C≡N), [122.3 (2C), 124.1 (2C), 132.4, 137.2] (6 arom. C's), 151.1 (C4-thiazole), 169.1 (C2-thiazole). MS *m/z* (%): 321 [M^+] (6); 305 (25); 263 (26); 215 (10); 200 (13); 198 (100);

172 (22); 159 (41); 134 (11); 132 (38); 93 (15); 91 (4); 90 (11); 89 (98); 87 (8); 85 (4); 79 (21); 77 (8); 75 (12); 52 (24); 51 (23).

2.1.9. N'-(4-Chlorophenyl)-4-(4-(N-ethyl-N-methylsulfamoyl)phenyl)thiazole-2-carbo-hydrazonoyl cyanide **(12)**

2.1.9.1. Procedure A; to an ethanolic icy solution of **11** (3.21 g, 0.01 mol) containing sodium acetate (6.55 g, 0.09 mol), *p*-chlorobenzenediazonium chloride was added drop wise (prepared under stirring at 0-5 ^{o}C upon adding *p*-chloroaniline (1.26 g, 0.01 mol) to sodium nitrite (0.68 g, 0.01 mol) in 5 mL concentrated hydrogen chloride). The product recrystallized from dioxane to give **12**. Yield, 86 %.

2.1.9.2. Procedure B; an equimolar mixture of **2** (3.20 g, 0.01 mol) and 2-amino-*N*'-(4-chlorophenyl)-2-thioxoacetohydrazonoyl cyanide (2.37 g, 0.01 mol) was refluxed in 40 mL ethanol for 2 h. The product obtained was filtered to give the compound **12**, Yield, 73 %. IR (KBr, cm^{-1}): ν_{max} = 3170 (NH), 3097 (CH arom.), 2935 (CH aliph.), 2218 (C≡N), 1597 (C=N), 1345, 1151 (SO_2), 1179 (C-S), 741 (C-Cl). ^{1}H NMR : δ *ppm* : 1.04 (t, 3H, CH_3 ethyl), 2.71 (s, 3H, CH_3), 3.32 (q, 2H, CH_2 ethyl), 7.07, 7.24 (dd, 4H, Ar-H, of chlorophenyl, *J* = 8.6 *Hz*), 7.51 (s, 1H, H5-thiazole), 7.81, 8.01 (dd, 4H, Ar-H, of benzenesulfonyl, *J* = 8.6 *Hz*), 11.94 (s, 1H, NH, Discharged with D_2O). ^{13}C NMR: δ *ppm:* 11.9 (CH_3 ethyl), 35.3 (CH_3), 37.6 (CH_2 ethyl), 112.2 (C5-thiazole), [115.2 (2C), 124.3, 125.4 (2C), 126.7 (2C), 128.1 (2C), 133.4, 136.2, 143.4] (12ArC's), 122.3 (C≡N), 156.1 (C4-thiazole), 157.2 (C2-thiazole), 159.1 (C=N). MS *m/z* (%): 460 [M^++1] (1); 459 [M^+] (9); 302 (3); 255 (16); 228 (7); 225 (12); 224 (23); 222 (30); 205 (14); 160 (14); 152 (25); 147 (38); 128 (21); 114 (29); 112 (100); 77 (56); 75 (17); 74 (12); 64 (53); 52 (26); 51 (33).

2.1.10. 4-(2-(1-Cyano-2-(4-fluorophenyl)vinyl)thiazol-4-yl)-N-ethyl-N-methylbenzene-sulfonamide **(14)**

2.1.10.1. Procedure A; to an ethanolic solution of mixture thiazolylacetonitrile derivative **11** (3.21 g, 0.01 mol) and *p*-fluorobenzaldehyde (1.23 g, 0.01 mol), add few drops of

piperidine and refluxed for 4 h. The solid was recrystallized from ethanol/benzene to give **14**. Yield, 80 %.

2.1.10.2. Procedure B; an equimolar mixture of **2** (3.20 g, 0.01 mol) and 2-cyano-3-(4-fluorophenyl)prop-2-enethioamide (2.04 g, 0.01 mol) was refluxed in 50 mL ethanol for 3 h. The solid was filtered and recrystallized. Yield, 67 %. IR (KBr, cm^{-1}): ν_{max} = 3107 (CH arom.), 2974 (CH aliph.), 2218 (C≡N), 1593 (C=N), 1365, 1181 (SO_2), 1179 (C-S), 1170 (C-F). ^{1}H NMR: *δ ppm*: 1.03 (t, 3H, CH_3 ethyl), 2.72 (s, 3H, CH_3), 3.37 (q, 2H, CH_2 ethyl), 7.22, 7.81 (dd, 4H, Ar-H of fluorophenyl, *J* = 8.6 *Hz*), 7.99, 8.49 (dd, 4H, Ar-H of benzenesulfonyl, *J* = 8.5 *Hz*), 7.51 (s, 1H, H5-thiazole), 9.02 (s, 1H, CH=C). ^{13}C NMR: *δ ppm:* 11.6 (CH_3 ethyl), 35.4 (CH_3), 37.6 (CH_2 ethyl), 110.3 (C=CH), 112.4 (C5-thiazole), [116.2 (2C), 123.6 (2C), 125.7 (2C), 132.1 (2C), 133.3, 137.1, 138.2, 164.3] (12ArC's), 120.1 (C≡N), 153.2 (C4-thiazole), 156.3 (C2-thiazole), 159.1 (C=CH). MS *m/z* (%): 428 [M^++1] (11); 427 [M^+] (50); 413 (7); 412 (36); 306 (17); 304 (100); 208 (3); 206 (10); 175 (25); 158 (10); 152 (20); 140 (4); 139 (5); 89 (23).

2.1.11. 4-(2-(1-Cyano-2-(dimethylamino)vinyl)thiazol-4-yl)-N-ethyl-N-methylbenzene-sulfonamide **(15)**

Compound **11** (3.21 g, 0.01 mol) was heated under reflux for 4 h in xylene (40 mL) and DMF-DMA (1.19 g, 0.01 mol). The collected solid washed with ether, and recrystallized from benzene to give **15**. Yield, 53 %. IR (KBr, cm^{-1}): ν_{max} = 3038 (CH arom.), 2924 (CH aliph.), 2211 (C≡N), 1590 (C=N), 1355, 1161 (SO_2), 1179 (C-S). ^{1}H NMR: *δ ppm:* 1.04 (t, 3H, CH_3 ethyl), 2.72 (s, 3H, CH_3), 2.82 (s, 6H, $(CH_3)_2N$), 3.35 (q, 2H, CH_2 ethyl), 7.31 (s, 1H, CH=C), 7.81 (s, 1H, H5-thiazole), 8.18, 8.71 (dd, 4H, Ar-H, *J* = 8.5 *Hz*). ^{13}C NMR: *δ ppm:* 11.2 (CH_3 ethyl), 35.1 (CH_3), 37.2 (CH_2 ethyl), 44.6 (2C, $(CH_3)_2N$), 84.3 (C=CH), 112.2 (C5-thiazole), 115.2 (C≡N), [128.3 (2C), 129.2 (2C), 134.1, 137.4] (6 arom. C's), 153.2 (C4-thiazole), 156.3 (C2-thiazole), 158.6 (C=CH). MS *m/z* (%): 378 [M^++2] (10); 377 [M^++1] (18); 376 [M^+] (100); 254 (33); 253 (46); 239 (9); 237 (9); 220 (12); 211 (18); 198 (13); 180 (11); 127 (15); 89 (15).

2.1.12. N-Ethyl-N-methyl-4-(2-(2-oxo-2H-chromen-3-yl)thiazol-4-yl)benzenesulfonamide **(17)**

2.1.12.1. Procedure A; to an ethanolic solution containing few drops of piperidine, a mixture of **11** (3.21 g, 0.01 mol) and *o*-hydroxybenzaldehyde (1.22 g, 0.01mol) was heated under reflux for 4 h. The collected product was recrystallized from ethanol/benzene to give **17**. Yield, 77 %.

2.1.12.2. Procedure B; an ethanolic solution of equimolar mixture of **2** (3.20 g, 0.01 mol) and 2-oxo-2*H*-chromene-3-carbothioamide (2.04 g, 0.01 mol) was heated under reflux for 2 h. Filter the solid and recrystallized. Yield, 57 %. IR (KBr, cm^{-1}): ν_{max} = 3093 (CH arom.), 2935 (CH aliph.), 1658 (C=O), 1600 (C=N), 1364, 1180 (SO_2), 1276, 1037 (C-O-C), 1179 (C-S). ^{1}H NMR: *δ ppm*: 1.03 (t, 3H, CH_3 ethyl), 2.72 (s, 3H, CH_3), 3.36 (q, 2H, CH_2 ethyl), 7.04 (s, 1H, H5-thiazole), 7.38-7.52 (m, 4H, Ar-H), 7.81, 8.01 (dd, 4H, Ar-H, *J* = 8.5 *Hz*), 9.08 (s, 1H, H4-coumarin). ^{13}C NMR: *δ ppm:* 11.8 (CH_3 ethyl), 32.5 (CH_3), 36.7 (CH_2 ethyl), 113.2 (C5-thiazole), [114.2, 118.5, 123.3, 125.2 (2C), 128.6 (2C), 129.2, 130.8, 131.1, 134.5, 141.3, 144.2, 156.1] (14ArC's), 157.1 (C4-thiazole), 158.2 (C2-thiazole), 163.7 (C=O). MS *m/z* (%): 427 [M^++1] (9); 426 [M^+] (17); 425 (71); 410 (26); 408 (72); 306 (14); 304 (22); 303 (100); 288 (17); 286 (14); 259 (5); 258 (16); 151 (37); 134 (11); 89 (34).

2.1.13. N-Ethyl-N-methyl-4-(2-(3-oxo-3H-benzo[f]chromen-2-yl)thiazol-4-yl)benzene-sulfonamide **(19)**

An ethanolic solution of a mixture of thiazolylacetonitrile derivative **11** (3.21 g, 0.01 mol) and *o*-hydroxy-1-naphthaldehyde (1.73 g, 0.01 mol) containing drops of pipredine was heated under reflux for 6 h. Filter the product obtained and recrystallized from acetic acid to give **19**. Yield, 69 %. IR (KBr, cm^{-1}): ν_{max} = 3062 (CH arom.), 2873 (CH aliph.), 1660 (C=O), 1590 (C=N), 1333, 1179 (SO_2), 1208, 1042 (C-O-C), 1179 (C-S). ^{1}H NMR: *δ ppm:* 1.04 (t, 3H, CH_3 ethyl), 2.72 (s, 3H, CH_3), 3.35 (q, 2H, CH_2 ethyl), 7.36 (s, 1H, H5-thiazole), 7.91-8.22 (m, 10H, Ar-H), 8.86 (s, 1H, H1-benzocoumarin). ^{13}C NMR: *δ ppm:* 11.2 (CH_3 ethyl), 32.6 (CH_3), 37.1 (CH_2 ethyl), 112.0 (C5-thiazole), [113.5, 118.2, 121.2, 122.9, 125.7, 126.9 (2C), 128.0 (2C), 129.1, 130.0, 130.9, 131.7, 132.1, 135.3,

138.9, 144.8, 154.1] (18ArC's), 155.8 (C4-thiazole), 156.8 (C2-thiazole), 164.3 (C=O). MS *m/z* (%): 478 [M^++2] (5); 477 [M^++1] (8); 476 [M^+] (15); 475 (14); 459 (23); 457 (31); 354 (16); 353 (14); 336 (23); 252 (6); 240 (9); 238 (8); 198 (5); 193 (56); 191 (13); 177 (31); 176 (25); 175 (28); 165 (22); 164 (57); 162 (19); 160 (10); 150 (11); 149 (22); 139 (29); 128 (14); 133 (28); 132 (12).

2.1.14. 2-Cyano-2-(4-(4-(N-ethyl-N-methylsulfamoyl)phenyl)thiazol-2-yl)ethanedithioic acid **(21)**

Carbon disulfide (0.75 g, 0.01 mol) was added slowly to an icy solution of compound **11** (3.21 g, 0.01 mol) in 25 mL *N,N*-dimethylformamide containing potassium hydroxide (1.12 g, 0.02 mol). Left mix for 24 h at room temperature followed by trituration with 40 mL icy water, neutralized with 1N hydrogen chloride. Wash the collected solid with excess water, and recrystallized from ethanol to give **21**. Yield, 44 %. IR (KBr, cm^{-1}): ν_{max} = 3094 (CH arom.), 2927 (CH aliph.), 2591 (SH), 2218 (C≡N), 1613 (C=N), 1366, 1172 (SO_2), 1344 (C=S), 1179 (C-S). ^{1}H NMR: *δ ppm:* 1.04 (t, 3H, CH_3 ethyl), 1.69 (s, 1H, SH), 2.73 (s, 3H, CH_3), 3.37 (q, 2H, CH_2 ethyl), 5.34 (s, 1H, CH), 7.71 (s, 1H, H5-thiazole), 7.98, 8.92 (dd, 4H, Ar-H, *J* = 8.5 *Hz*). ^{13}C NMR: *δ ppm:* 11.4 (CH_3 ethyl), 35.3 (CH_3), 37.5 (CH_2 ethyl), 59.3 (CH), 110.3 (C5-thiazole), 113.6 (C≡N), [125.8 (2C), 126.5 (2C), 134.5, 137.4] (6ArC's), 158.4 (C4-thiazole), 167.7 (C2-thiazole), 224.6 (C=S). MS *m/z* (%): 397 [M^+] (1); 368 (1); 348 (1); 339 (2); 334 (1); 326 (3); 324 (13); 320 (25); 310 (19); 306 (48); 282 (16); 297 (45); 263 (28); 224 (45); 200 (10); 199 (60); 159 (100); 132 (40); 89 (64); 64 (26); 63 (16); 58 (21); 52 (12).

3. *In-vitro* anticancer screening

3.1. Cytotoxicity Assessment

All novel compounds were evaluated for their cytotoxicity toward two human cell lines, alveolar adenocarcinoma carcinoma (lung) (A-549) and liver carcinoma (HepG2). Doxorubicin (DOX) was a reference control, (**Tables 1-5**).

3.2. Cytotoxicity evaluation using viability assay

Cell lines were purchased from the American Type Culture collection and their accession number as follows: A-549 (ATCC CCL-185™) lung carcinoma cell line, and HepG2 (ATCC® HB-8065™) liver carcinoma cell line. Exponentially, place cells for 24 h in 96-well plates, and then add fresh medium which containing different concentration of the tested sample. Sequential two-fold dilutions of the tried sample were added using a multichannel pipette. Moreover, all cells were cultivated at 37 ^{o}C, 5 % CO_2 and 95 % moisture. In addition, incubation of control cells occurred at 37 ^{o}C. However, after incubation for 24 h different concentrations of tested sample were added (50, 25, 12.5, 6.25, 3.125 & 1.56 µg/L) and continued the 48 h incubation, then, add 1 % crystal violet solution to each well for 0.5 h to examine viable cells. Rinse the wells using water until no stain. After that, add 30 % glacial acetic acid to all wells with shaking plates on Microplate reader (TECAN, Inc.) to measure the absorbance, using a test wavelength of 490 nm. Besides, compare the treated samples with the control cell. The cytotoxicity was estimated by IC_{50} in µM. Additionally, selectivity index (SI), the concentration ratio which causes death of 50 % in baby hamster kidney (BHK) cell line (CC_{50}) divided to the concentration which causes death of 50 % in human carcinoma (IC_{50}) [39-42] was also determined.

4. *In Vitro* Antimicrobial evaluation

Bacterial and fungal strains were obtained from culture collection of the Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University. The testing of the efficacy of all novel derivatives was carried out by standardized disc-agar diffusion method [43]. Using Mueller Hinton Agar. Activation of various strains of bacteria occurred by using a loop that full of bacterial strain in the broth and incubated for 24 h at 37^{o} C. Furthermore, 0.1 mL of the suspension of strains was poured on the agar, spread well and left to solidify. Moreover, using a sterile cork, about 0.9 cm cut was made and were filled completely with the tested compound solution. The wells were incubated at 37

^{o}C for 24 h. Each assay was done in triplicate. ZOI was determined using the mean value. Tested compound giving high ZOI value reflecting its significant antibacterial activity. The tested strains included three strains of gram-positive bacteria, namely; *Staphylococcus aureus (RCMB 010027)*, *Streptococcus pneumoniae (RCMB 010010)* and *Bacillus subtilis (RCMB 010067)* compared to *Ampicillin* as a reference drug. In addition to four strains of gram-negative bacteria, namely; *Pseudomonas aeruginosa (RCMB 010043)*, *Mycobacterium tuberculosis (RCMB 010120)*, *Klebsiella pneumoniae (RCMB 0010093)* and *Escherichia coli (RCMB 010052) ATCC 25955* compared to *Gentamicin* as a reference drug. Besides two different fungal strains namely; *Aspergillus fumigatus (RCMB 02568)* and *Candida albicans (RCMB 05036)* compared to *Amphotericin B* as a control drug, (**Table 6**).

5. Docking assay

5.1. Materials

The study was done in the Department of Pharmaceutical Organic Chemistry, Faculty of Pharmacy (Girls), Al-Azhar University, on an Intel(R) Core(TM) i7-3632QM 2.20 *GHz* processor, 8.00 GB memory with windows 7 Ultimate operating system using Molecular Operating Environment (MOE 2015.10; Chemical Computing Group, Montreal, Canada) as the computational software. All the minimizations were performed with MOE until a RMSD gradient of 0.05 Kcal mol^{-1} Å^{-1} with MMFF94X force field.

5.2. General Methodology

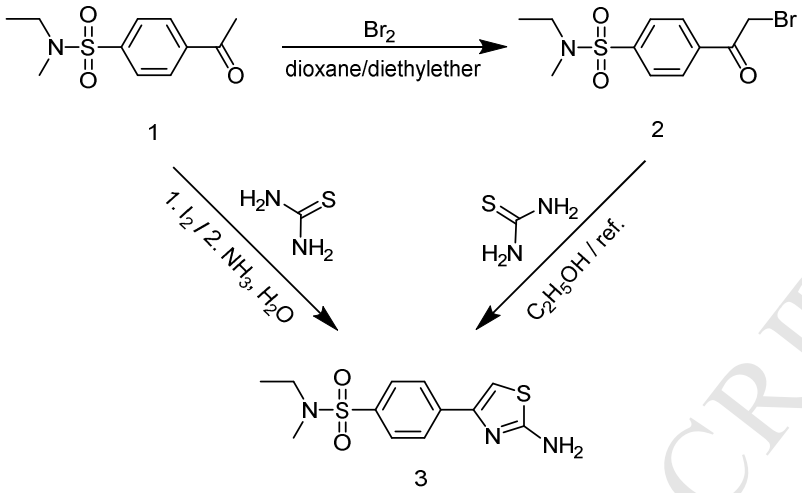
It depends on downloading from Protein Data Bank (PDB ID: 1BID), the bounding of 3D structure of doxorubicin (DOX) to DHFR enzyme (PDB ID: 4DFR). Hydrogen atoms were further added to the structure of the enzyme. In addition, molecules of water and bound ligands were deleted manually and their energy was be minimized. MOE-Alpha site finder has been used to generate the active site. However, the alpha spheres created Dummy atoms. Besides, docking of ligands via the active sites

of DHFR was obtained using MOE-Dock and MMFF94X molecular mechanics force field for 8000 connections. Furthermore, the conformation having the lowest energy was chosen and forced to minimization of energy using MMFF94X force field. Docking results were mentioned as Score. E_conf; The energy of the conformer. E_place; Score from the placement stage E_score1; Score from the 1strescoring stage. E_score2; Score from the 2nd rescoring stage. E_refine; Score from the refinement stage.

6. Results and discussion

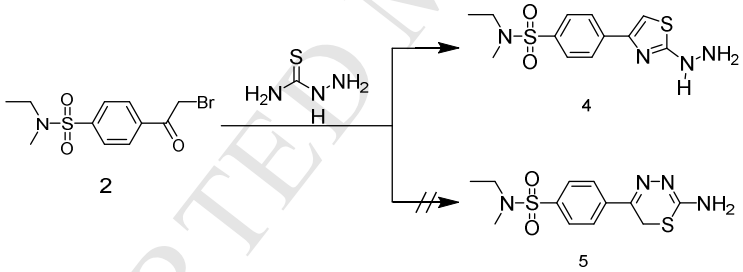
6.1. Chemistry

4-(2-bromoacetyl)-*N*-ethyl-*N*-methylbenzenesulfonamide (**2**) was synthesized by reaction of 4-acetyl-*N*-ethyl-*N*-methylbenzenesulfonamide (**1**) with bromine, which was employed as a building unit for synthesis of novel thiazole **(3)** (**Scheme 1**). The active function that attached to *N*-ethyl-*N*-methylbenzenesulfonamide derivatives determined the degree of its pharmacological activity. ^{1}H NMR spectrum of **2** displayed a singlet at δ = 4.63 *ppm* due to active CH_2 of bromoacetyl group. Besides, ^{13}C NMR spectrum exhibited a vital signal at δ = 31.9 *ppm* corresponding to active CH_2 group. However, MS spectrum showed molecular ion peaks at *m/z* = 319 and 321 corresponding bromine isotopes. On the other hand, 2-Aminothiazole derivative **3**, was obtained by two various pathways. One of them included refluxing of compound **1** with thiourea and iodine. Besides that, the solid product has low yield, iodine has pollution problems. Therefore, the effort extended to other pathway at which in ethanolic solution compound **2** was refluxed with thiourea, this method gave yield about 79 %. IR spectrum of **3** exhibited forked bands at ν = 3300, 3284 cm^{-1} mentioned to NH_2 function. ^{1}H NMR spectrum shown a singlet of one proton at δ = 6.91 *ppm*, attributed to CH-thiazole and deuterium oxide transferrable signal at δ = 7.53 *ppm*, referred to NH_2 protons. However, ^{13}C NMR spectrum of **3** displayed signals at δ = 106.9 and 169.6 *ppm* due to C5-thiazole and C2-thiazole, respectively.



Scheme 1. Synthesis of starting material **2** and 2-aminothiazole derivative **3**.

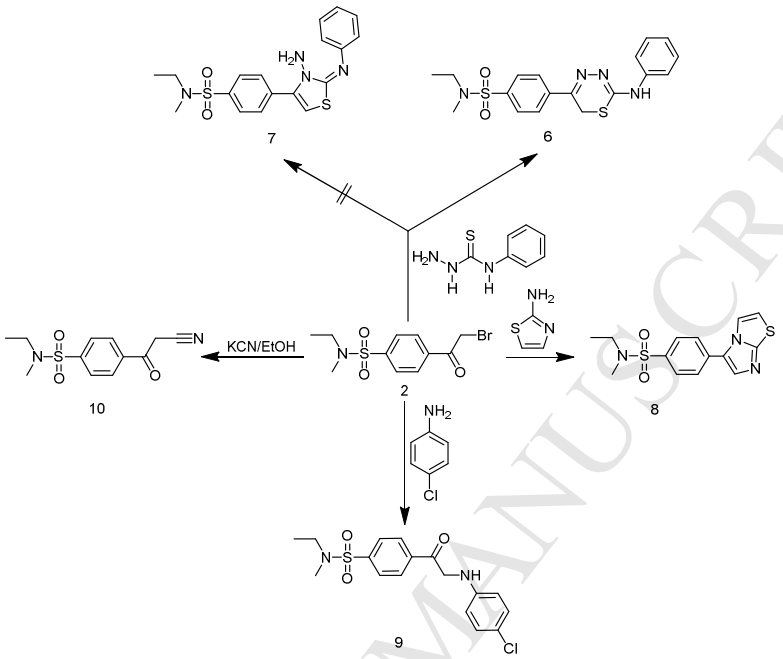
Moreover, alteration of compound **2** into different heterocyclic compound when reacted with thiosemicarbazide were unsuccessful, as the reaction afforded also thiazole derivative **4**, instead of the expected 2-aminothiadiazine derivative **5**, which confirmed by IR spectrum (as it showed band at ν = 3269 cm^{-1} conforming to NH function) and ^{1}H NMR spectrum (as it indicated a singlet at δ = 11.20 *ppm*) (**Scheme 2**).



Scheme 2. Synthesis of 2-hydrazinylthiazole derivative **4**.

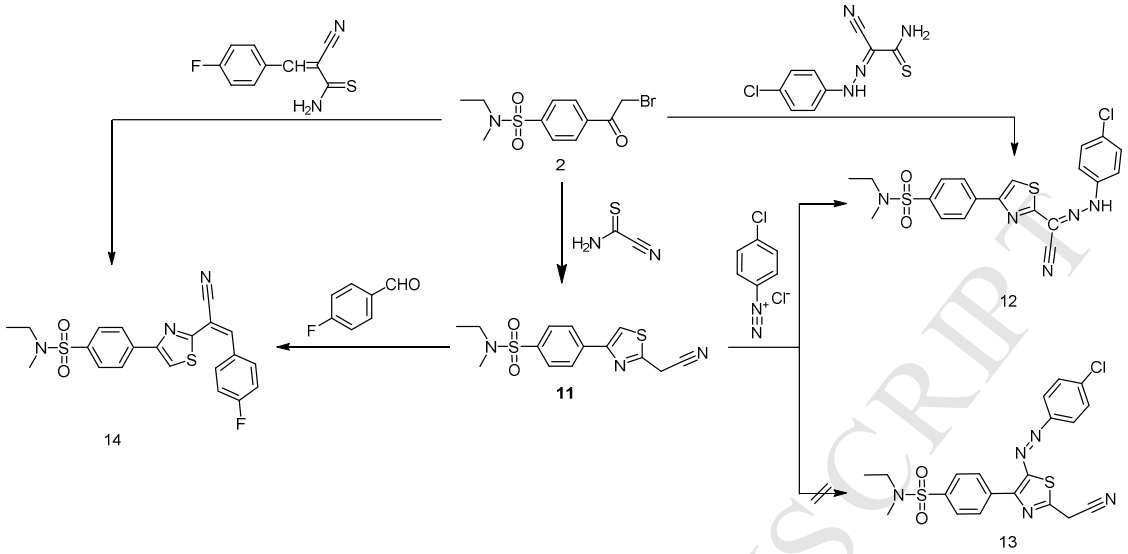
However, thiadiazin derivative **6** was obtained *via* the reaction of compound **2** with *N*-phenylhydrazinecarbothioamide. While, the other expected structure 4-(3-amino-2-(phenylimino)-2,3-dihydrothiazol-4-yl)-*N*-ethyl-*N*-methylbenzene-sulfonamide (**7**) was ruled out depending on spectral data. IR spectrum of **6** showed a specific band at ν = 3215 cm^{-1} attributed to NH moiety. Moreover, ^{13}C NMR spectrum revealed carbons of thiadiazine moiety at δ = 27.3 *ppm*. Besides, the MS spectrum confirmed structure **6** as it showed *m/z* = 388. The goal was raised to focus on different fused heterocyclic rings activity. Therefore, refluxing of compound **2** with 2-aminothiazole in ethanolic solution afforded imidazothiazole derivative **8**. Also, reaction of **2** with 4-chloroaniline and potassium cyanide afforded compounds **9** and **10**, respectively. Thus, IR spectrum of **10**

showed an absorption band at ν = 2220 cm^{-1} corresponding to cyano function. Additionally, ^{1}H NMR spectrum showed a singlet at δ = 3.71 *ppm* corresponding to active methylene group. (**Scheme 3**).



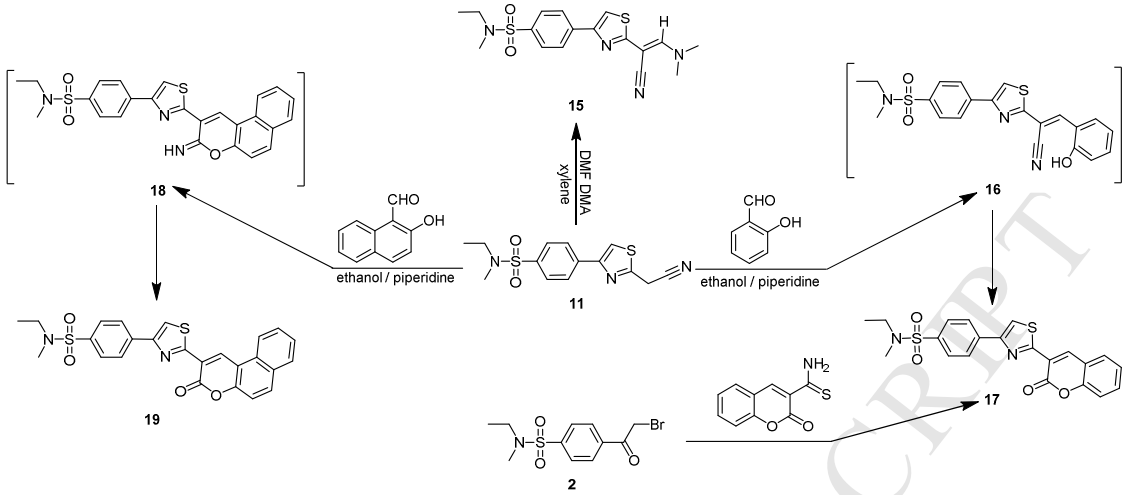
Scheme 3. Synthesis of compounds **6**, **8**-**10** from starting material **2**.

Our aim was extended to synthesis other thiazole derivatives *via* refluxing of **2** with 2-cyanoethanethioamide in ethanolic solution to afford 4-(2-(cyanomethyl)thiazol-4-yl)-*N*-ethyl-*N*-methylbenzenesulfonamide **11**. IR, ^{1}H NMR and ^{13}C NMR spectra confirmed the product. As methylene group is established to be very reactive. Therefore, coupling of equimolar amount of **11** with 4-chlorobenzenediazonium chloride in cold ethanolic solution (0-5 ^{o}C) containing sodium acetate, yellow crystalline solid of one of two structures **12** or **13** be possible. Spectral data provided a definite support for only structure **12** as IR spectrum exhibited band at ν = 3170 cm^{-1} due to NH function. Besides, thiazole proton was appeared as a singlet at δ = 7.51 *ppm* in ^{1}H NMR spectrum. Additionally, other pathway could be used for preparation of compound **12** through refluxing of phenacyl bromide derivative **2** with 2-amino-*N*'-(4-chlorophenyl)-2-thioxoacetohydrazonoyl cyanide. Moreover, condensation of compound **11** with 4-fluorobenzaldehyde afforded thiazolylacrylonitrile derivative **14**. However, refluxing of compound **2** with 2-cyano-3-(4-fluorophenyl)prop-2-enethioamide gave also compound **14**. (**Scheme 4**).



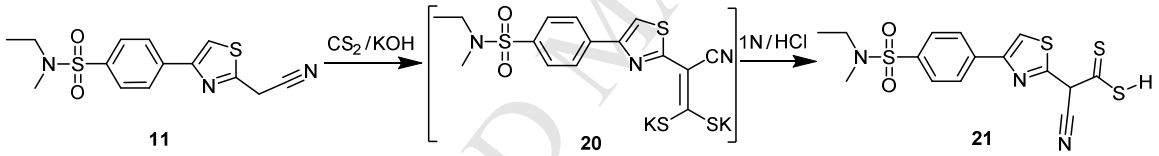
Scheme 4. Strategy synthesis of novel sulfonamide thiazole derivatives **11**, **12** and **14** utilizing **2** as a starting material.

Furthermore, refluxing of **11** with DMF-DMA in xylene afforded compound **15**, which confirmed by ^{1}H NMR and ^{13}C NMR spectra. Our goal was also discovered the condensation of **11** with *o*-phenolic aldehydes [44], such as *o*-hydroxybenzaldehyde and *o*-hydroxy-1-naphthaldehyde. Therefore, refluxing of **11** with *o*-hydroxybenzaldehyde in ethanolic solution containing few drops of piperidine afforded compound **17**. In addition, it can be prepared *via* Knovenagel condensed intermediate **16**, at which intramolecular cyclization occurred *via* Michael-type addition reaction [45] followed by formation of carbonyl group by hydrolysis of the imino group. Similar hydrolysis reactions have been reported [46,47]. Another pathway at which phenacyl bromide derivative **2** was refluxed with 2-oxo-2*H*-chromene-3-carbothioamide in ethanol can also synthesize it. In the same manner, upon reaction of **11** with 2-hydroxy-1-naphthaldehyde, it gave the intermediate benzo[*f*]chromen-3-imine derivative **18** which by hydrolysis afforded benzo[*f*]chromen-3-one derivative **19**. (**Scheme 5**).



Scheme 5. Synthesis of newly compounds **15**, **17** and **19**.

At room temperature, in dry DMF and in presence of KOH, reaction of **11** with carbon disulfide can be proceeded and gave potassium dithiolate salt **20** which upon treatment with 1N hydrogen chloride, it transformed into ethanedithioic acid derivative **21**, which confirmed by spectral data. (**Scheme 6**).



Scheme 6. Strategy synthesis of newly sulfonamide ethanedithioic acid derivative **21** from 2-cyanomethylthiazole derivative **11**.

6.2. In vitro anticancer activity

Activity of the prepared compounds toward (A-549) and (HepG2) cell lines was depends on their IC_{50} values which tabulated in (**Tables 1 and 2**); respectively as well as (**Charts 1 and 2**); respectively. While, CC_{50} values against baby hamster kidney (BHK) cell line represented in (**Table 3**). Furthermore, CC_{50}, IC_{50} and $SI=CC_{50}/IC_{50}$ of the tested compounds toward lung (A-549), (HepG2) cell lines compared to human normal baby hamster kidney (BHK) cell lines are tabulated in (**Tables 4 and 5**); respectively as well as (**Charts 3 and 4**); respectively. From the obtained results in the previous (**Tables 1-5**) and adopting that increasing selectivity index SI values give a selective toxicity against cancer cells, it can be noted that, compound **3** bearing 2-amino thiazole moiety exerted significant increase in cytotoxicity against (HepG2) cell line having SI value 64.52, while it showed comparable activity against lung cancer cell line (A-549), SI value 4.00,

compared to the reference drug doxorubicin having SI values 22.90 and 5.51, respectively. Moreover, Imidazo[2,1-*b*]thiazole derivative **8** (IC_{50} = 1.55 μg/mL) exhibited strong anticancer activity against lung cancer cell line (A-549) and revealed SI value 30.77, however, it showed moderate activity against (HepG2) cell line (IC_{50} = 10.6 μg/mL) having SI value 4.50 compared to doxorubicin (IC_{50} = 20.23 and 4.87 μg/mL; SI value 5.51 and 22.90), respectively. Furthermore, compound **9** bearing 4-chlorophenyl moiety resulted in improvement in activity against lung cancer cell line (A-549), SI value 5.94, while SI value of doxorubicin 5.51. However, it revealed nearly activity to doxorubicin (SI value 22.90) toward (HepG2) cell line (SI value 17.77). Additionally, the presence of 2-cyanomethyl thiazole moiety as in compound **11** showing increase in cytotoxic activity against (HepG2) cell line (SI value 67.11), while it showed comparable activity against lung cancer cell line (A-549), SI value 4, compared to the reference drug doxorubicin having SI values 22.90 and 5.51, respectively. Moreover, 4-fluorophenyl derivative **14** showed marked increase in anticancer activity against both lung cancer (A-549) and (HepG2) cell lines exerting SI values 6.82 and 25.98, respectively. Beside, compound **17** having 2-oxo-2*H*-chromen moiety exhibited slightly better anticancer activity against both lung cancer (A-549) and (HepG2) cell lines exerting SI values 5.93 and 31.17, respectively. On the other hand, the presence of 2-cyano ethanedithioic acid moiety as in compound **21** indicated significant increase in anticancer activity against (HepG2) cell line (SI value 35.78), however, it exists a promising activity as doxorubicin toward lung cancer cell line (A-549), SI value 4. It can be concluded that according to SI values of the synthesized compounds, the cytotoxicity order toward lung cancer (A-549) cell line is: **8** > **14** > **9** > **17** > DOX > **10** > **6** > **2**, **11**, **19**, **15**, **21**, **12**, **3** > **4**. While, the order against (HepG2) cell line is: **11** > **3** > **21** > **17** > **14** > DOX > **10** > **9** > **12** > **2** > **8** > **19**, **15** > **6** > **4**.

6.3. Antimicrobial screening

Novel tested compounds were screened for the preliminary antimicrobial efficacy toward various microorganisms representing Gram +ve bacteria (*Staphylococcus aureus*, *Streptococcus pneumonia* and *Bacillis subtilis*), Gram -ve bacteria (*Pseudomonas*

aeruginosa, *Mycobacterium tuberculosis*, *Klebsiella pneumoniae* and *Escherichia coli*), fungi (*Aspergillus fumigatus* and *Candida albicans*). The obtained results in (**Table 6**) as well as (**Chart 5**) showed that Compound **9** exhibited dual activities as antibacterial and antifungal agents on all tested microbial strains. Regarding antibacterial activities, it can be clearly observed that, compounds **2**, **3**, **4**, **6** and **8** showed increase activities against two strains of Gram-negative bacteria; *Mycobacterium tuberculosis* and *Klebsiella Pneumoniae*. All results were compared to the reference drugs.

6.4. Docking and molecular modeling

A well-founded procedure used to expect the compound interaction with the most suitable binding site. Various types of interactions between ligand and amino acid were possibly determined. The main enzymes that were concerned in studying antimicrobial and anticancer activities are thymidylate synthase and dihydrofolate reductase [48,49]. All dock runs were done by Molecular Operating Environment (MOE) module to observe the cytotoxicity of the tested compounds. This work revealed DHFR interaction with its substrate DOX and with the following representative active anticancer compounds **2-4, 6, 8-12, 14, 15, 17, 19** and **21** using MOE program [50] on DHFR active sites. The energy scores were tabulated in (**Table 7**).

6.4.1. Docking of DOX into DHFR; illustrated that (OH) function of tetracene acted as a H-bond donor to backbone Asp 21 with 2.65 Å having a strength of 1.1 %. However, (OH) function of acetyl pyran acted as a H-bond acceptor with Ser 59 (3.02 Å) having a strength of 16.2 %. Moreover, (NH_2) function acted as a H-bond donor with Glu 30 (3.31 Å) with strength of 5 %. Furthermore, there is arene-arene contact among phenyl ring of tetracene and Phe 31. In addition to, hydrophobic interactions involving: Val 8, Gly 20, Asp 21, Leu 22, Phe 31, Phe 34, Thr 56, Ile 60, Pro 61, Asn 64, Leu 67, Val 115 and Tyr 121, **Fig. 1**.

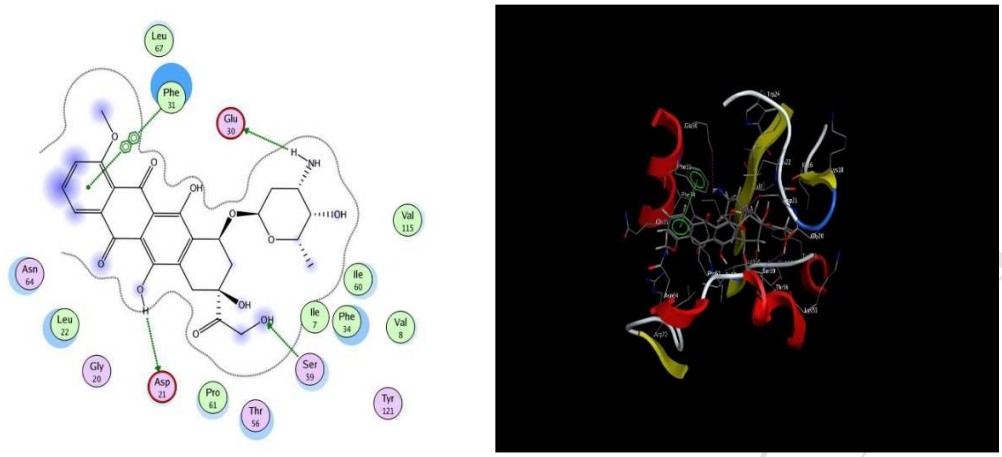


Fig. 1. Docking of DOX into DHFR

*6.4.2. Docking of compounds **2-4**, **6**, **8-12**, **14**, **15**, **17** and **21** into DHFR;* (**Figures 2-13** and **15**), respectively (**supplementary data**).

*6.4.3. Docking of compound **19** into DHFR;* In a similar manner, it proved that H-bond acceptor interactions were established between either (O) atom of (C=O) function and Ser 59 (2.82 Å) having a strength of 10.3 % or one (O) atom of (SO_2) function and Arg 70 (2.52 Å) with a strength of 70.2 %. Besides, hydrophobic interactions among: Val 8, Leu 22, Phe 31, Phe 34,Gln 35, Thr 56, Ser 59, Ile 60, Pro 61, Asn 64, Leu 67, Lys 68, Arg 70, Val 115 and Tyr 121, **Fig. 14**.

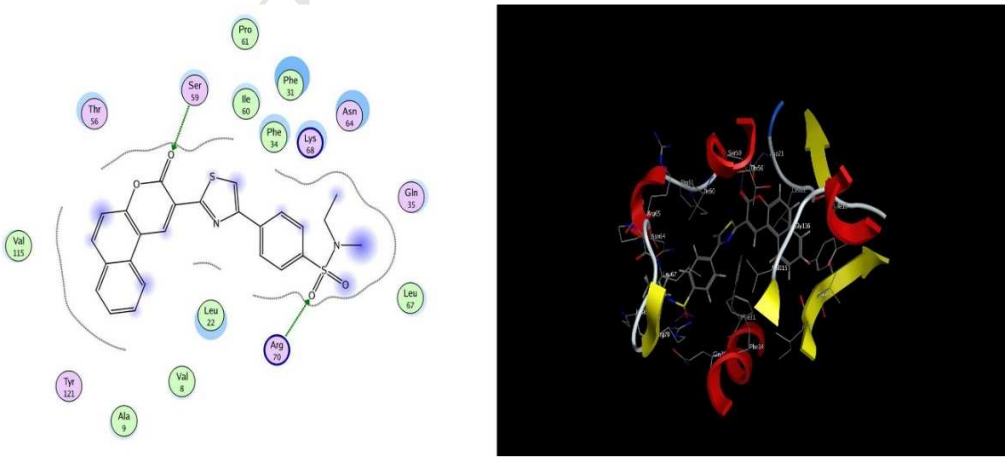


Fig. 14. Docking of Compound **19** into DHFR

6.5. Conclusion of docking assay:

Docking study exerted various interactions between the target and ligand, which are expected to have better anticancer activity. The compounds under investigation are **2-4, 6, 8-12, 14, 15, 17, 19** and **21**. Particularly noteworthy, only compounds **19**, **17**, **12**, **14**, **15** and **6**, were suggested to exhibit their efficacy *via* inhibition of the active sites of DHFR enzyme (**Table 7**). It was clarified that, score energy of the tested compounds follows the order **19 > 17 > 12 > 14 > 15 > 6 > 9 > 21 > 4 > 11 > 3 > 8 > 10 > 2** (**Chart 6**).

6.6. Structure Activity Relationship (SAR)

For the well understanding of the antitumor and antimicrobial activities, SAR of the tested compounds has to be indicated it proved that; thiazole derivatives as in compounds **3, 11, 14** and **21** existed marked increase in anticancer activity against human liver cancer (HepG2) cell line, while introduction of imidazo moiety to thiazole derivatives (imidazo[2,1-*b*]thiazole as in compound **8**) lead to significant increase in the anticancer activity against human lung cancer (A-549) cell line, and marked decrease in the activity against (HepG2) cell line. Additionally, introduction of bulky group as 2-oxo-2*H*-chromene in compound **17** showed anticancer activity against both cell lines more than DOX as a reference drug. Moreover, the presence of 1,3,4-thiadiazine as in compound **6** exerted significant decrease in anticancer activity but showed promising antibacterial activity especially against gram negative bacteria. On the other hand, the presence of 4-chlorophenyl moiety, compound **9** showed marked increase in both anticancer and antimicrobial activities.

7. Conclusion

This article proved that compounds having 4-chlorophenyl moiety **9** exhibited dual activities, antimicrobial and anticancer especially against human lung cancer (A-549) cell line in comparable to the positive reference doxorubicin. In addition, compound

2 exhibited antibacterial action toward Gram-positive bacteria. However, compound **3** showed excellent antibacterial and cytotoxic activities against (HepG2) cell line. Compound **8** showed promising cytotoxic activity toward human lung cancer (A-549) cell line, while, compound **11** exhibited its activity toward human liver cancer (HepG2) cell line. The compounds exerted their cytotoxic action *via* inhibition of the active sites of DHFR.

References

[1] N.P. Prajapati, K.D. Patel, R.H. Vekariya, H.D. Patel, D.P. Rajani, Thiazole fused thiosemicarbazones: Microwave-assisted synthesis, biological evaluation and molecular docking study, J. Mol. Struct. 1179 (2019) 401-410.

[2] K.Z. Łączkowski, K. Motylewska, A.B. Łączkowska, A. Biernasiuk, K. Misiura, A. Malm, B. Fernandez, Synthesis, antimicrobial evaluation and theoretical prediction of NMR chemical shifts of thiazole and selenazole derivatives with high antifungal activity against Candida spp., J. Mol. Struct. 1108 (2016) 427-437

[3] Q. Lu, Q. Yu, Y. Zhu, J. Weng, J. Yuan, D. Hu, J. Chen, X. Liu, C. Tan, Novel stilbene analogues containing thiazole moiety: Synthesis, biological evaluation and docking study, J. Mol. Struct. 1180 (2019) 780-786

[4] H. Osman, S.K. Yusufzai, M.S. Khan, B.M. Abd Razik, O. Sulaiman, S. Mohamad, J.A. Gansau, M.O. Ezzat, T. Parumasivam, M.Z. Hassan, New thiazolyl-coumarin hybrids: Design, synthesis, characterization, X-ray crystal structure, antibacterial and antiviral evaluation, J. Mol. Struct. 1166 (2018) 147-154

[5] M. Haroon, T. Akhtar, M. Yousuf, M.W. Baig, M.N. Tahir, L. Rasheed, Synthesis, spectroscopic characterization and crystallographic behavior of ethyl 2-(4-methyl-(2-benzylidenehydrazinyl))thiazole-4-carboxylate: experimental and theoretical (DFT) studies, J. Mol. Struct. 1167 (2018) 154-160.

[6] D. Romani, M.J. Marquez, M.B. Marquez, S.A. Brandan, Structural, topological and vibrational properties of an isothiazole derivatives series with antiviral activities, J. Mol. Struct. 1100 (2015) 279-289.

[7] A. Barakat, S.M. Soliman, A.M. Al-Majid, M.A.M. ShahidulIslam, Y.A.M.M. Elshaier, H.A. Ghabbour, New spiro-oxindole constructed with pyrrolidine/thioxothiazolidin-4-one derivatives: Regioselective synthesis, X-ray crystal structures, Hirshfeld surface analysis, DFT, docking and antimicrobial studies, J. Mol. Struct. 1152 (2018) 101-114.

[8] K. Anichina, A. Mavrova, D. Yancheva, J. Tsenov, R. Dimitrov, Tautomerism and isomerism in some antitrichinellosis active benzimidazoles: Morphological study in polarized light, quantum chemical computations, J. Mol. Struct. 1150 (2017) 179-187.

[9] S. Carberry, E. Molloy, S. Hammel, G. O'Keeffe, G.W. Jones, K. Kavanagh, S. Doyle, Gliotoxin effects on fungal growth: mechanisms and exploitation, Fungal Genet. Biol. 49 (2012) 302-312.

[10] M.R. Maliyappa, J. Keshavayya, N.M. Mallikarjuna, P.M. Krishna, N. Shivakumara, T. Sandeep, K. Sailaja, M.A. Nazrulla, Synthesis, characterization, pharmacological and computational studies of 4,5,6,7-tetrahydro-1,3-benzothiazole incorporated azo dyes, J. Mol. Struct. 1179 (2019) 630-641.

[11] M. Danish, A. Bibi, K. Gilani, M.A. Raza, M. Ashfaq, M.N. Arshad, A.M. Asiri, K. Ayub, Antiradical, antimicrobial and enzyme inhibition evaluation of sulfonamide derived esters; synthesis, X-Ray analysis and DFT studies, J. Mol. Struct. 1175 (2019) 379-388.

[12] P. Shafieyoon, E. Mehdipour, Y.S. Mary, Synthesis, characterization and biological investigation of glycine-based sulfonamide derivative and its complex: Vibration assignment, HOMO-LUMO analysis, MEP and molecular docking, J. Mol. Struct. 1181 (2019) 244-252.

[13] M. Rocha, O.E. Piro, G.A. Echeverria, A.C. Pastoriza, M.A. Sgariglia, J.R. Soberon, D.M. Gil, Co(II), Ni(II) and Cu(II) ternary complexes with sulfadiazine and dimethylformamide: Synthesis, spectroscopic characterization, crystallographic study and antibacterial activity, J. Mol. Struct. 1176 (2019) 605-613

[14] B. Eren, A. Unal, F. Ozdemir-Kocak, Combined experimental and theoretical studies on the chemical and spectroscopic properties of an antimicrobial N-(Phenyl)dimethyldisulfonimide, J. Mol. Struct. 1175 (2019) 542-550.

[15] A.S. Kumar, J. Kudva, S.M. Kumar, U. Vishwanatha, V. Kumar, D. Naral, Synthesis, characterization, crystal structure, Hirshfeld interaction and bio-evaluation studies of 4-amino quinazoline sulfonamide derivatives, J. Mol. Struct. 1167 (2018) 142-153.

[16] D.T. Nguyen, V.H.G. Phan, D.S. Lee, T. Thambi, D.P. Huynh, Bioresorbable pH- and temperature-responsive injectable hydrogels-incorporating electro sprayed particles for the sustained release of insulin, Polym. Degrad. Stabil. 162 (2019) 36-46.

[17] N. Ghareb, N.M. El-Sayed, R. Abdelhameed, K. Yamada, M.S. Elgawish, Toward a treatment of diabesity: Rational design, synthesis and biological evaluation of benzene-sulfonamide derivatives as a new class of PTP-1B inhibitors, Bioorg. Chem. 86 (2019) 322-338.

[18] Z. Iqbal, G. Morahan, M. Arooj, A.N. Sobolev, S. Hameed, Synthesis of new arylsulfonylspiroimidazolidine-2′,4′-diones and study of their effect on stimulation of insulin release from MIN6 cell line, inhibition of human aldose reductase, sorbitol accumulations in various tissues and oxidative stress, Eur. J. Med. Chem. 168 (2019) 154-175.

[19] L. Chen, G. Luo, Facile synthesis of acyl sulfonamides from carboxyic acids using the Mukaiyama reagent, Tetrahedron Lett. 60 (2019) 268-271.

[20] J. Zhang, J. Li, H. Su, Y. Zhao, X. Zeng, M. Hu, W. Xiao, X. Mao, H-bonding effect of oxyanions enhanced photocatalytic degradation of sulfonamides by g-C3N4 in aqueous solution, J. Hazard. Mater. 366 (2019) 259-267.

[21] D.O. Ozgun, H. Gul, C. Yamali, H. Sakagami, I. Gulcin, M. Sukuroglu, C.T. Supuran, Synthesis and bioactivities of pyrazoline benzensulfonamides as carbonic anhydrase and acetylcholinesterase inhibitors with low cytotoxicity, Bioorg. Chem. 84 (2019) 511-517.

[22] M. Krasavin, A. Shetnev, T. Sharonova, S. Baykov, S. Kalinin, A. Nocentini, V. Sharoyko, G. Poli, T. Tuccinardi, S. Presnukhina, T.B. Tennikova, C.T. Supuran, Continued exploration of 1,2,4-oxadiazole periphery for carbonic anhydrase-targeting primary arene sulfonamides: Discovery of subnanomolar inhibitors of membrane-bound hCA IX isoform that selectively kill cancer cells in hypoxic environment, Eur. J. Med. Chem. 164 (2019) 92-105.

[23] S. Soltani, P. Magri, M. Rogalski, M. Kadri, Charge-transfer complexes of hypoglycemic sulfonamide with π-acceptors: Experimental and DFT-TDDFT studies, J. Mol. Struct. 1175 (2019) 105-116.

[24] H.Z. Ozsoy, Carbonic anhydrase enzymes: Likely targets for inhalational anesthetics, Med. Hypotheses 123 (2019) 118-124.

[25] A.A.M. Abdel-Aziz, A. Angeli, A.S. El-Azab, M.E.A. Hammouda, M.A. El-Sherbeny, C.T. Supuran, Synthesis and anti-inflammatory activity of sulfonamides and carboxylates incorporating trimellitimides: Dual cyclooxygenase/carbonic anhydrase inhibitory actions, Bioorg. Chem. 84 (2019) 260-268.

[26] Y. Li, J. Xiao, Q. Zhang, W. Yu, M. Liu, Y. Guo, J. He, Y. Liu, The association between anti-tumor potency and structure-activity of protein-kinases inhibitors based on quinazoline molecular skeleton, Bioorg. Med. Chem. 27 (2019) 568-577.

[27] M. Salehi, M. Kubicki, M. Galini, M. Jafari, R.E. Malekshah, Synthesis, characterization and crystal structures of two novel sulfa drug Schiff base ligands derived sulfonamide and molecular docking study, J. Mol. Struct. 1180 (2019) 595-602.

[28] R. Sribalan, G. Banuppriya, M. Kirubavathi, V. Padmini, Synthesis, biological evaluation and in silico studies of tetrazole-heterocycle hybrids, J. Mol. Struct. 1175 (2019) 577-586.

[29] R. Venkatesh, S. Kasaboina, N. Jain, S. Janardhan, U.D. Holagunda, L. Nagarapu, Design and synthesis of novel sulphamide tethered quinazolinone hybrids as potential antitumor agents, J. Mol. Struct. 1181 (2019) 403-411.

[30] P. Schulz, S. Robak, J. Dastych, A.K. Siwicki, Influence of bacteriophages cocktail on European eel (Anguilla anguilla) immunity and survival after experimental challenge, Fish Shellfish Immun. 84 (2019) 28-37.

[31] C. Zhao, K.P. Rakesh, L. Ravidar, W. Fang, H. Qin, Pharmaceutical and medicinal significance of sulfur (SVI)-Containing motifs for drug discovery: A critical review, Eur. J. Med. Chem. 162 (2019) 679-734.

[32] M.S. Bashandy, S.M. Abd El-Gilil, Synthesis, molecular docking and anti-human breast cancer activities of novel thiazolylacetonitriles and thiazolylacrylonitriles and their derivatives containing benzenesulfonylpyrrolidine moiety, Heterocycles, 92 (2016) 431-469.

[33] K.F. Debbabi, S.A. Al-harbi, H.M. Al-saidi, E. Aljuhani, S.M. Abd El-gilil, M.S. Bashandy, Study of reactivity of cyanoacetohydrazonoethyl-N-ethyl-N-methyl benzenesulfonamide: preparation of novel anticancer and antimicrobial active heterocyclic benzenesulfonamide derivatives and their molecular docking against dihydrofolate reductase, J. Enzyme Inhib. Med. Chem. 31 (2016) 7-19.

[34] S.A. Al-Harbi, M.S. Bashandy, H.M. Al-Saidi, A.A.A. Emara, S.M. Abd El-Gilil, Spectroscopic properties, anti-colon cancer, antimicrobial and molecular docking studies of silver(I), manganese(II), cobalt(II) and nickel(II) complexes for 2-amino-4-phenylthiazole derivative, Eur. J. Chem. 7 (2016) 421-430.

[35] M.T. Sarg, M.M. Koraa, A.H. Bayoumi, S.M. Abd El-Gilil, Synthesis of Pyrroles and Condensed Pyrroles as Anti-Inflammatory Agents with Multiple Activities and Their Molecular Docking Study, Open J. Med. Chem., 5 (2015) 49-96.

[36] A.H. Halawa, S.M. Abd El-Gilil, A.H. Bedair, M. Shaaban, M. Frese, N. Sewald, E.M. Eliwa, A.M. El-Agrody, Synthesis, biological activity and molecular modeling study of new Schiff bases incorporated with indole moiety, Z. Naturforsch. 72 (2017) 467-475.

[37] A.H. Halawa, S.M. Abd El-Gilil, A.H. Bedair, E.M. Eliwa, M. Frese, N. Sewald, M. Shaaban, A.M. El-Agrody, Synthesis of diverse amide linked bis-indoles and indole

derivatives bearing coumarin-based moiety: cytotoxicity and molecular docking investigations, Med. Chem. Res. 27 (2018) 796-806.

[38] M.M. Abdelall, M.S. Bashandy, A. El-morsy, Heteroaromatization with sulfonamido phenyl ethanone: part (IV). synthesis and antimicrobial activity of new N-ethyl-N-methylbenzenesulfonamide, 1,3,4-thiadiazole, 1,3,4-thiadiazine, 4-oxothiazolidine and pyrazolo[5,1-c][1,2,4]triazine derivatives, Al-Azhar Bull. Sci. 21 (2010) 325-343.

[39] P. Skehan, R. Storegng, D. Scudiero, A. Monks, J. McMahon, D. Vistica, J.T. Warren, H. Bokesch, S. Kenney, M.R. Boyd, New Colorimetric Cytotoxicity Assay for Anticancer-Drug Screening, J. Natl. Cancer Inst. 82 (1990) 1107-1112.

[40] M. Al-Qubaisi, R. Rozita, S. Yeap, A. Omar, A. Ali, N.B. Alitheen, Selective cytotoxicity of Goniothalamin against hepatoblastoma HEPG2 cells. Molecules, 16 (2011) 2944-2959.

[41] J.D. Chellaian, J. Johnson, Spectral characterization, electrochemical and anticancer studies on some metal (Li) complexes containing tridentate quinoxaline Schiff base. Spectrochim. Acta A Mol. Biomol. Spectrosc. 127 (2014) 396-404.

[42] A. Suga, T. Narita, L. Zhou, H. Sakagami, K. Satoh, H. Wakabayashi, Inhibition of NO production in LPS-stimulated mouse macrophage-like cells by benzo[b]cyclohept[e][1,4]oxazine and 2-aminotropone derivatives, In Vivo, 23 (2009) 691-697.

[43] Y. Saintigny, K. Makienko, C. Swanson, M.J. Emond, J.R. Monnat, Homologous recombination resolution defect in Werner syndrome, Mol. Cell Biol. 2 (2004) 6971-6978.

[44] M.A.A. Elneariry, T.M. Abdel-Rahman, A.M. Hammad, 2-(1,3-Benzothiazol-2-yl)ethanethioamides in heterocyclic synthesis: novel synthesis of pyridine, pyrido[2,3-d]pyrimidine, coumarin, thiazolone and triazole derivatives, J. Chem. Res. (S) (1998) 684-685.

[45] S.M. Sayed, Nitriles in heterocyclic synthesis: reactivity of 2-(benzothiazol-2-ylmethyl)-1,3-thiazol-4(5H)-one towards αβ-Unsaturated Nitriles, J. Chinese Chem. Soc. 50 (2003) 1061-1073.

[46] K. Sato, T. Amakasu, Coumarins V. acid-catalyzed reaction of phenols with beta-oxonitriles, J. Org. Chem. 33 (1968) 2446-2450.

[47] O. Meth-Cohn, B. Tarnowski, A useful synthon for sulphur heterocycles; I. the synthesis of thiocoumarins, Synthesis, (1978) 56-58.

[48] Q.R. Du, D.D. Li, Y.Z. Pi, J.R. Li, J. Sun, F. Fang, W.Q. Zhong, H.B. Gong, H.L. Zhu, Novel 1,3,4-oxadiazole thioether derivatives targeting thymidylate synthase as dual anticancer/antimicrobial agents. Bioorg. Med. Chem. 21 (2013) 2286-2297.

[49] K.N. Rao, S.R. Venkatachalam, Dihydrofolate reductase and cell growth activity inhibition by the β-carboline-benzoquinolizidine plant alkaloid deoxytubulosine from alangium lamarckii: its potential as an antimicrobial and anticancer agent. Bioorg. Med. Chem. 7 (1999) 1105-1110.

[50] S. Vilar, G. Cozza, S. Moro, Medicinal chemistry and the molecular operating environment (MOE): Application of QSAR and molecular docking to drug discovery. Curr. Top. Med. Chem. 8 (2008) 1555-1572.

Table 1

Six dose growth inhibition percentages and IC_{50} values of the test compounds against lung (**A-549)** cell line.

Comp. No.	Validity for sample Conc. (µg/mL)						IC_{50}
	50	25	12.5	6.25	3.125	1.56	
2	78.94 ± 1.5	90.88 ± 1.1	97.12 ± 0.7	98.96 ± 0.6	100	100	> 50
3	74.91 ± 1.5	86.48 ± 1.2	93.02 ± 0.9	98.74 ± 0.6	100	100	> 50
4	86.21 ± 0.97	93.78 ± 0.34	98.04 ± 0.08	100	100	100	> 100
6	38.92 ± 1.42	47.81 3.97	63.26 ± 4.58	81.57 2.15	90.84 0.66	96.22 0.57	23.2
8	16.86 ± 0.32	20.97 0.35	28.74 ± 0.62	35.18 ± 0.72	43.67 ± 0.39	49.86 ± 1.62	1.55
9	39.31 ± 2.8	53.26 ± 3.9	70.49 ± 4.6	81.61 ± 1.1	89.54 ± 0.9	96.32 ± 0.7	30.8
10	12.24 ± 0.74	27.68 ± 1.8	64.07 ± 4.3	78.29 ± 1.5	89.62 ± 1.1	94.53 ± 0.8	18.5
11	67.23 ± 4.5	84.31 ± 1.3	91.89 ± 1.1	96.04 ± 0.7	99.14	100	> 50
12	83.48 ± 1.4	91.57 ± 0.8	97.72 ± 0.6	99.08	100	100	> 50
14	34.16 ± 2.7	47.23 ± 3.3	59.88 ± 4.1	74.06 ± 1.5	86.52 ± 1.1	94.39 ± 0.9	22.3
15	79.34 ± 1.5	88.86 ± 1.2	95.69 ± 0.7	99.17	100	100	> 50
17	28.98 ± 1.9	39.75 ± 2.8	54.27 ± 3.4	69.16 ± 4.6	81.34 ± 1.1	90.63 ± 0.9	16.2
19	76.52 ± 1.5	89.63 ± 1.1	94.71 ± 0.8	98.54 ± 0.6	100	100	> 50
21	60.37 ± 4.1	76.49 ± 1.5	85.13 ± 1.1	91.57 ± 0.9	95.68 ± 0.8	98.72 ± 0.6	> 50
DOX	34.67 ± 2.8	45.96 ± 3.9	59.08 ± 5.1	70.44 ± 5.0	84.49 ± 2.7	98.43 ± 0.8	20.23

Table 2

Six dose growth inhibition percentages and IC_{50} values of the test compounds against (**HepG-2**) cell line

Comp. No.	Validity for sample Conc. (µg/mL)						IC_{50}
	50	25	12.5	6.25	3.125	1.56	
2	41.98 ± 3.1	68.17 ± 4.6	84.54 ± 1.1	91.36 ± 0.9	97.82 ± 0.6	100	42.3
3	6.62 ± 0.97	14.58 ± 1.2	27.85 ± 1.9	40.97 ± 3.3	49.81 ± 4.1	64.02 ± 4.6	3.1
4	75.64 1.32	88.17 ± 0.89	94.82 ± 0.46	98.75 ± 0.13	100	100	> 100
6	43.68 1.84	52.94 ± 2.09	71.82 ± 1.64	86.55 ± 0.73	92.89 ± 0.57	98.17 ± 0.21	32.9
8	19.41 0.53	27.65 ± 0.82	46.29 ± 2.93	58.38 ± 1.86	71.56 ± 0.62	80.92 ± 0.71	10.6
9	8.92 ± 1.33	20.84 ± 1.5	39.71 ± 2.8	68.45 ± 4.6	82.96 ± 1.3	93.14 ± 0.9	10.3
10	7.47 ± 0.78	15.39 ± 1.3	28.57 ± 1.7	45.42 ± 3.9	59.13 ± 4.5	74.28 ± 1.5	5.21
11	7.18 ± 0.97	13.32 ± 0.74	24.93 ± 1.7	37.25 ± 2.7	48.94 ± 3.3	60.41 ± 4.1	2.98
12	32.54 ± 2.1	43.87 ± 3.4	57.18 ± 4.2	68.09 ± 4.6	83.51 ± 1.1	97.42 ± 0.6	19.2
14	3.23 ± 0.51	23.12 ± 1.7	34.06 ± 2.5	47.18 ± 3.9	69.41 ± 4.4	78.53 ± 1.2	5.85
15	81.56 ± 1.3	92.43 ± 0.8	98.75 ± 0.6	100	100	100	> 50
17	5.95 ± 0.62	13.72 ± 1.13	21.66 ± 1.7	38.09 ± 2.8	49.54 ± 3.4	68.27 ± 4.2	3.08
19	72.42 ± 1.5	89.57 ± 1.2	94.71 ± 0.9	98.42 ± 0.6	100	100	> 50
21	6.42 ± 0.96	15.71 ± 1.4	28.54 ± 2.3	43.35 ± 3.9	74.96 ± 1.5	88.53 ± 1.2	5.59
DOX	6.78 ± 1.60	16.92 ± 2.01	23.90 ± 2.1	41.34 ± 3.1	52.57 ± 3.9	71.90 ± 5.1	4.87

Table 3

Six dose growth inhibition percentages and CC_{50} values of the test compounds against **(BHK)** cell line

Comp. No.	Validity for sample Conc. (µg/mL)						CC_{50}
	50	25	12.5	6.25	3.125	1.56	
2	91.35 ± 0.9	96.27 ± 0.7	98.94 ± 0.6	100	100	100	> 200
3	93.74 ± 0.9	97.62 ± 0.7	99.08	100	100	100	> 200
4	96.23 ± 0.71	100	100	100	100	100	> 100
6	75.42 ± 1.64	88.74 ± 0.62	96.25 ± 0.37	100	100	100	> 100
8	48.19 ± 3.53	67.54 ± 1.82	78.93 ± 0.71	91.46 ± 0.32	98.78 ± 0.12	100	47.7
9	80.26 ± 1.3	88.41 ± 1.1	93.27 ± 0.9	97.89 ± 0.6	100	100	183
10	61.49 ± 4.1	69.25 ± 4.6	81.43 ± 1.2	91.29 ± 0.9	97.83 ± 0.7	98.17 ± 0.6	93.8
11	86.38 ± 1.2	92.49 ± 0.9	98.13 ± 0.6	100	100	100	> 200
12	97.42 ± 0.6	99.08	100	100	100	100	> 200
14	72.46 ± 1.5	85.92 ± 1.1	93.41 ± 0.8	98.78 ± 0.6	100	100	152
15	96.13 ± 0.7	99.42	100	100	100	100	> 200
17	62.37 ± 4.1	79.12 ± 1.5	87.54 ± 1.1	95.21 ± 0.8	98.89 ± 0.6	100	96
19	93.21 ± 0.9	98.74 ± 0.6	100	100	100	100	> 200
21	93.26 ± 1.1	98.73 ± 0.6	100	100	100	100	> 200
DOX	65.47 ± 3.7	81.44 ± 2.6	90.32 ± 1.9	97.26 ± 0.3	100	100	111.5

Table 4

CC_{50}, IC_{50} (in µg/mL and µM) and selective index (**SI)** of the synthesized compounds against human alveolar adenocarcinoma (**A-549**) and human normal baby hamster kidney **(BHK)** cell lines.

Comp. No.	CC_{50} (µg/mL)	CC_{50} (µM)	IC_{50} (µg/mL)	IC_{50} (µM)	SI
2	> 200	624.61	> 50	156.15	4.00
3	> 200	672.49	> 50	168.12	4.00
4	> 100	320.09	> 100	320.09	1.00
6	> 100	257.39	23.2	59.72	4.31
8	47.7	148.40	1.55	4.82	30.77
9	183	498.83	30.8	83.96	5.94
10	93.8	352.21	18.5	69.47	5.07
11	> 200	622.24	> 50	155.56	4.00
12	> 200	434.81	> 50	108.70	4.00
14	152	355.55	22.3	52.16	6.82
15	> 200	531.21	> 50	132.80	4.00
17	96	225.08	16.2	37.98	5.93
19	> 200	419.67	> 50	104.92	4.00
21	> 200	503.07	> 50	125.77	4.00
DOX	111.5	205.14	20.23	37.22	5.51

Table 5

CC_{50}, IC_{50} (in µg/mL and µM) and selectivity index (**SI**) of the synthesized compounds against **(HepG-2)** and human normal baby hamster kidney **(BHK)** cell lines.

Comp. No.	CC_{50} (µg/mL)	CC_{50} (µM)	IC_{50} (µg/mL)	IC_{50} (µM)	SI
2	> 200	624.61	42.3	132.10	4.73
3	> 200	672.49	3.1	10.42	64.52
4	> 100	320.09	> 100	320.09	1.00
6	> 100	257.39	32.9	84.68	3.04
8	47.7	148.40	10.6	32.98	4.50
9	183	498.83	10.3	28.08	17.77
10	93.8	352.21	5.21	19.56	18.00
11	> 200	622.24	2.98	9.27	67.11
12	> 200	434.81	19.2	41.74	10.42
14	152	355.55	5.85	13.68	25.98
15	> 200	531.21	> 50	132.80	4.00
17	96	225.08	3.08	7.22	31.17
19	> 200	419.67	> 50	104.92	4.00
21	> 200	503.07	5.59	14.06	35.78
DOX	111.5	205.14	4.87	8.96	22.90

Table 6

Antimicrobial activity of newly tested compounds

Comp. No.	Fungi		Bacteria						
			Gram-positive			Gram-negative			
	Aspergillus fumigatus (RCMB 02568)	Candida albicans (RCMB 05036)	Staphylococcus aureus (RCMB 010027)	Streptococcus pneumoniae (RCMB 010010)	Bacillis subtilis (RCMB 010067)	Pseudomonas aeruginosa (RCMB 010043)	Mycobacterium tuberculosis (RCMB 010120)	Klebsiella pneumoniae (RCMB 0010093)	Escherichia coli (RCMB 010052)
2	21.7 ± 0.58	---	21.9 ± 0.41	23.7 ± 0.44	24.2 ± 0.25	16.8 ± 0.58	17.2 ± 0.25	18.9 ± 0.44	20.4 ± 0.58
3	20.3 ± 0.44	---	21.5 ± 0.25	21.9 ± 0.44	23.4 ± 0.32	---	20.1 ± 0.25	20.5 ± 0.21	21.8 ± 0.37
4	17.3 ± 0.58	---	16.2 ± 0.72	20.4 ± 0.63	21.1 ± 0.58	---	17.4 ± 0.44	19.2 ± 1.2	21.3 ± 0.63
6	18.9 ± 0.63	---	19.2 ± 1.2	21.3 ± 0.58	23.2 ± 0.25	---	18.9 ± 0.25	21.1 ± 0.72	20.9 ± 0.63
8	22.3 ± 1.2	---	20.4 ± 0.72	23.1 ± 0.58	25.2 ± 0.63	---	21.3 ± 0.58	21.3 ± 0.63	23.4 ± 0.63
9	24.4 ± 0.37	20.3 ± 0.58	29.3 ± 0.25	25.9 ± 0.58	27.2 ± 0.32	25.8 ± 0.58	19.2 ± 0.44	20.3 ± 0.44	26.5 ± 0.37
10	---	---	---	---	---	---	---	---	---
11	---	---	---	---	---	---	---	---	---
12	13.6 ± 0.58	---	16.4 ± 0.37	18.2 ± 0.44	19.2 ± 0.63	---	16.1 ± 0.37	17.4 ± 0.25	18.2 ± 0.82
14	---	---	---	---	---	---	---	---	---
15	---	---	---	---	---	---	---	---	---
17	---	---	---	---	---	---	---	---	---
19	---	---	---	---	---	---	---	---	---
21	16.2 ± 0.58	---	16.9 ± 0.58	18.3 ± 0.37	20.4 ± 0.44	---	15.2 ± 0.25	16.4 ± 0.44	18.5 ± 0.58
St[a]	23.7 ± 0.10	21.9 ± 0.12	28.9 ± 0.14	25.3 ± 0.58	26.4 ± 0.34	26.3 ± 0.15	16.3 ± 0.58	17.3 ± 0.12	27.3 ± 0.44

Mean zone of inhibition in mm ± standard deviation (S.D.).

[a] Standard controls for the microorganisms are "*Amphotericin B*" for the Fungi, "*Ampicillin*" for the Gram-positive bacteria and "*Gentamicin*" for the Gram-negative bacteria.

---: No activity.

Table 7

Docking score energy of the selective newly synthesized compounds

Comp. No.	Score	E_conf	E_place	E_score1	E_score2	E_refine
2	-15.0213	34.17024	-81.7005	-9.03719	-15.0213	-18.9901
3	-16.8163	34.661	-82.8206	-10.5749	-16.8163	-13.0949
4	-17.3838	45.53849	-74.898	-10.7264	-17.3838	-5.01795
6	-20.247	79.83461	-105.076	-11.1418	-20.247	14.14417
8	-16.7075	51.06242	-64.5982	-9.54607	-16.7075	4.626528
9	-20.0809	67.44415	-84.9201	-10.3409	-20.0809	-4.32465
10	-15.3252	38.44773	-58.584	-9.0367	-15.3252	-3.89987
11	-17.3081	33.7631	-83.2258	-9.84283	-17.3081	-32.0938
12	-22.5328	80.3844	-110.264	-11.2471	-22.5328	1.456523
14	-22.0103	70.73538	-90.1287	-11.2382	-22.0103	-1.35123
15	-20.9875	66.40745	-102.369	-10.3949	-20.9875	3.773701
17	-22.9127	80.69428	-106.101	-10.9483	-22.9127	-1.78558
19	-25.8317	96.38638	-119.775	-11.4462	-25.8317	6.946416
21	-18.8596	54.23026	-84.3471	-11.492	-18.8596	-9.88955

Table 8

Physical and analytical data of all newly synthesized compounds

Comp. No.	M. Formula	M. Wt.	Color	M. P. (ºC)	Calculated (Found) %			
					C	H	N	S
2	$C_{11}H_{14}BrNO_3S$	320.20	White	63-64	41.26 (41.14)	4.41 (4.39)	4.37 (4.41)	10.01 (9.88)
3	$C_{12}H_{15}N_3O_2S_2$	297.40	White	91-92	48.46 (48.39)	5.08 (4.87)	14.13 (14.28)	21.56 (21.73)
4	$C_{12}H_{16}N_4O_2S_2$	312.41	White	95-96	46.13 (46.26)	5.16 (5.30)	17.93 (18.01)	20.53 (20.44)
6	$C_{18}H_{20}N_4O_2S_2$	388.51	Yellowish white	103-105	55.65 (55.71)	5.19 (5.04)	14.42 (14.36)	16.51 (16.60)
8	$C_{14}H_{15}N_3O_2S_2$	321.42	White	130-132	52.32 (52.45)	4.70 (4.91)	13.07 (12.83)	19.95 (20.02)
9	$C_{17}H_{19}ClN_2O_3S$	366.86	White	82-83	55.66 (55.57)	5.22 (5.10)	7.64 (7.79)	8.74 (8.80)
10	$C_{12}H_{14}N_2O_3S$	266.32	Yellowish white	70-72	54.12 (54.28)	5.30 (5.47)	10.52 (10.68)	12.04 (11.82)
11	$C_{14}H_{15}N_3O_2S_2$	321.42	White	201-202	52.32 (52.10)	4.70 (4.81)	13.07 (12.93)	19.95 (20.01)
12	$C_{20}H_{18}ClN_5O_2S_2$	459.97	Yellow	220-221	52.22 (52.16)	3.94 (3.99)	15.23 (15.19)	13.94 (13.83)
14	$C_{21}H_{18}FN_3O_2S_2$	427.51	Yellowish white	215-217	59.00 (59.16)	4.24 (4.19)	9.83 (9.73)	15.00 (14.89)
15	$C_{17}H_{20}N_4O_2S_2$	376.50	Yellowish white	172-173	54.23 (54.15)	5.35 (5.26)	14.88 (14.78)	17.03 (17.19)
17	$C_{21}H_{18}N_2O_4S_2$	426.51	Yellowish white	273-274	59.14 (59.05)	4.25 (4.18)	6.57 (6.39)	15.04 (14.90)
19	$C_{25}H_{20}N_2O_4S_2$	476.57	Brown	299-300	63.01 (62.95)	4.23 (4.11)	5.88 (5.92)	13.46 (13.29)
21	$C_{15}H_{15}N_3O_2S_4$	397.56	White	212-213	45.32 (45.15)	3.80 (3.74)	10.57 (10.30)	32.26 (32.33)

HIGHLIGHTS

- Synthesis of new series of *N*-ethyl-*N*-methylbenzenesulfonamide.
- The newly structures were elucidated through their elemental analysis and spectral data.
- All the newly synthesized compounds screened for two different human cancer cell lines, A-549 and HepG2 and exhibited good results.
- Compound **9** having 4-chlorophenyl moiety exerted significant antimicrobial activity more than the reference drugs
- Molecular docking of the synthesized compounds showed various interactions with the active sites of dihydrofolate reductase enzyme.

Chart 1

Growth inhibition diagram of the tested compounds against (**A-549)** cell line.

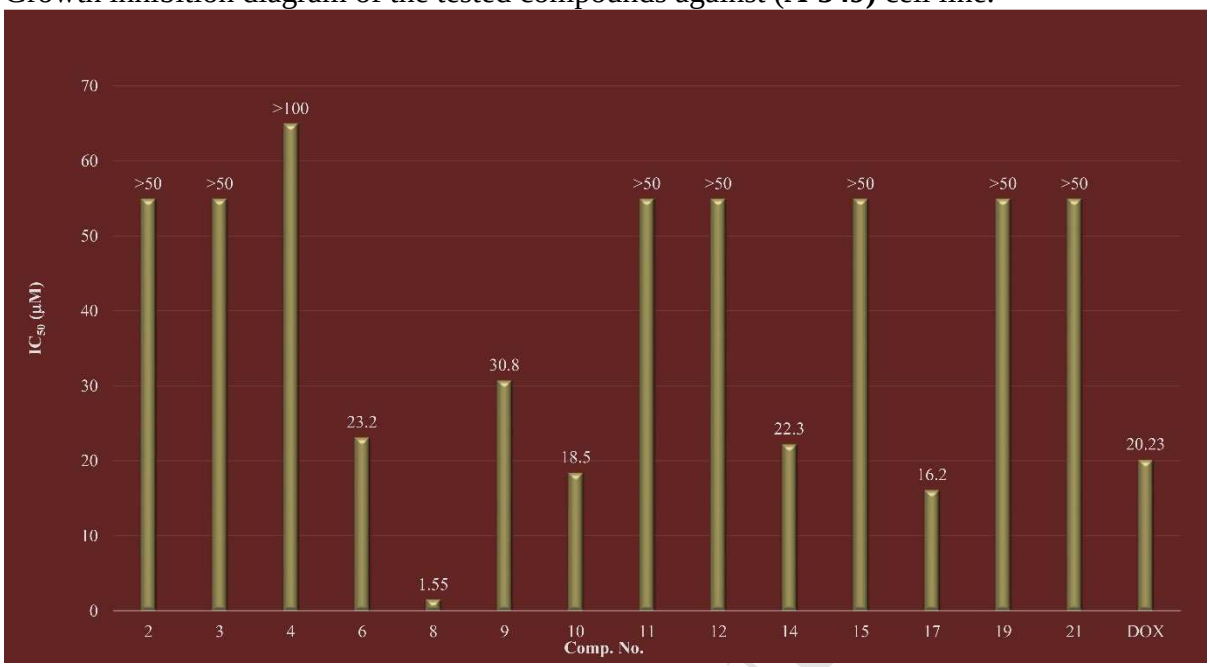


Chart 2

Growth inhibition diagram of the tested compounds against **(HepG-2)** cell line.

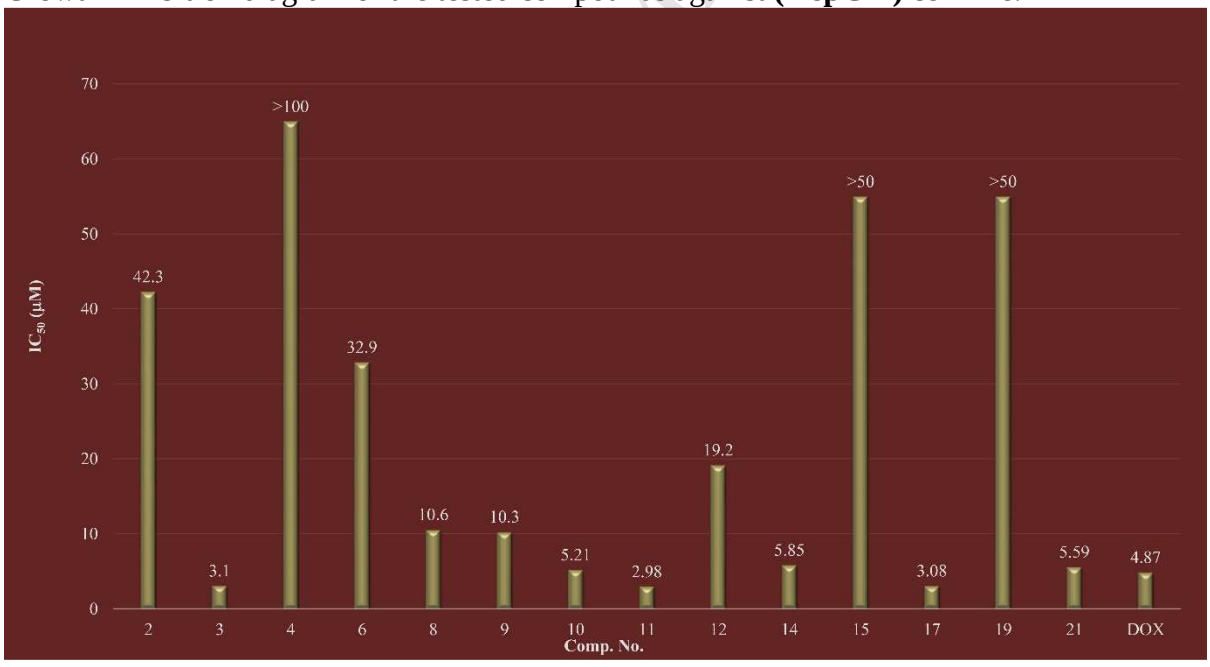


Chart 3

Selective index (**SI**) of the tested compounds against human alveolar adenocarcinoma carcinoma (**A-549**) cell line and human normal baby hamster kidney **(BHK)** cell line

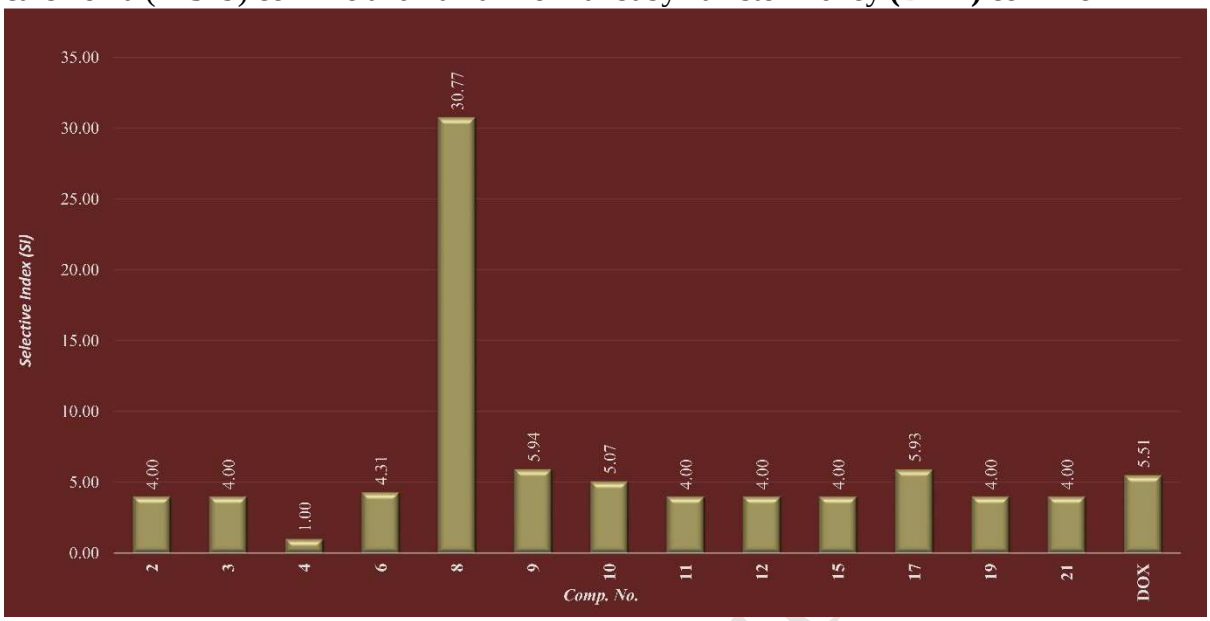


Chart 4

Selective index (**SI)** of the synthesized compounds against human liver carcinoma **(HepG-2)** cell line and human normal baby hamster kidney **(BHK)** cell line

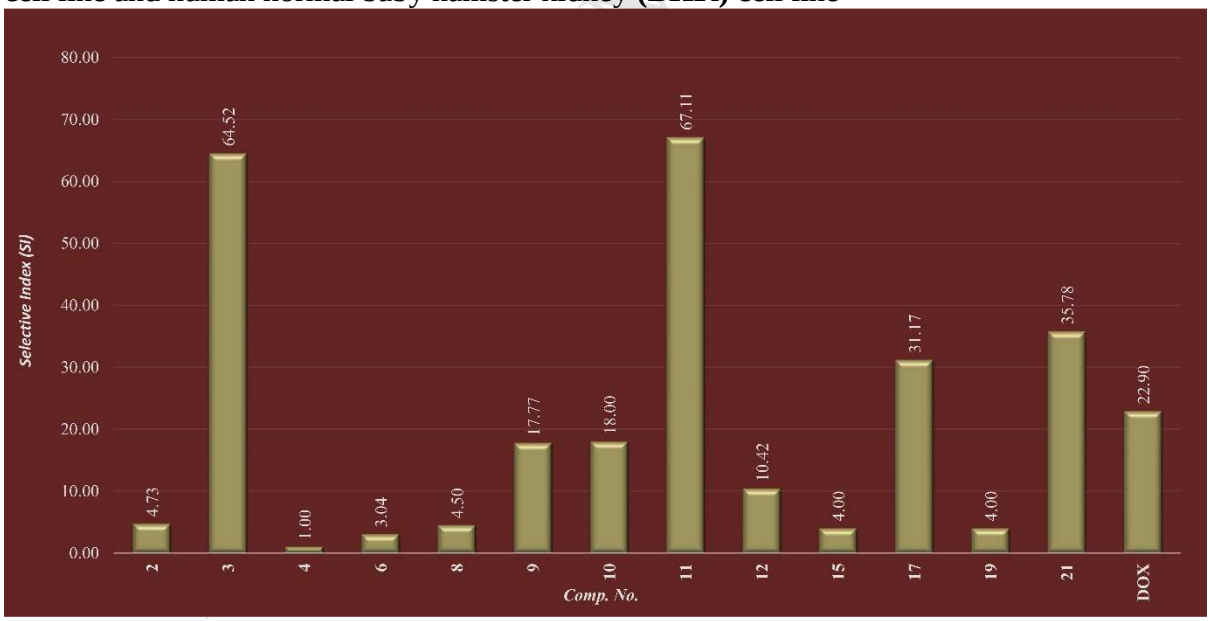


Chart 5

Comparison of the antimicrobial activity of the synthesized compounds

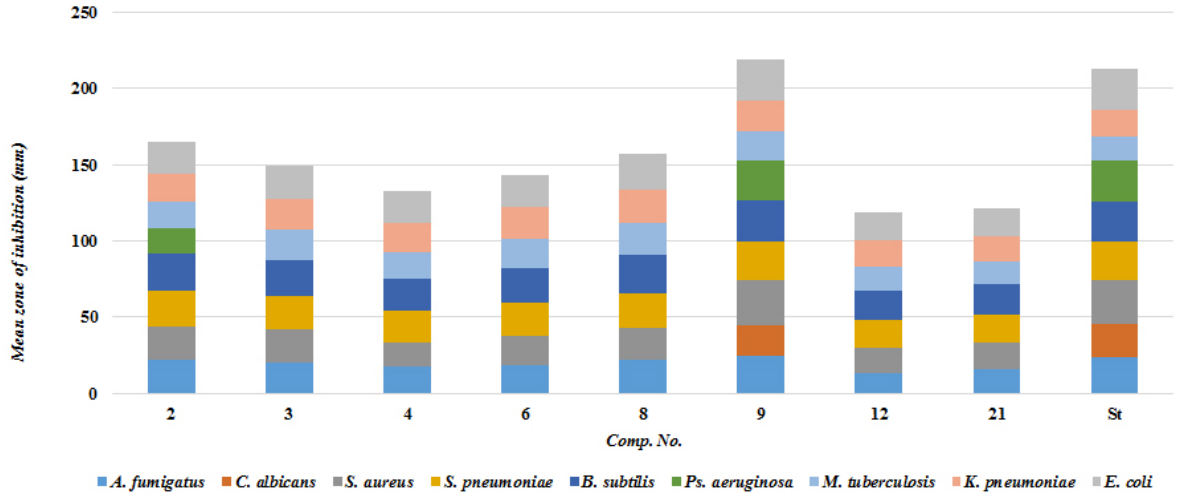


Chart 6

Order of docking score energy of the selective newly synthesized compounds