UTILITY OF SOME SULPHA DRUGS IN SYNTHESIS OF NEW BIOLOGICALLY ACTIVE HETEROCYCLIC COMPOUNDS : SYNTHESIS AND ANTICANCER ACTIVITY OF SOME NOVEL THIOSEMICARBAZIDE DERIVATIVES.

E. M. AHMED, N. M. TAHA AND N. M. S. NADY

Department of Chemistry, Faculty of Science (Girls), Al-Azhar University, Cairo, Egypt.

Abstract

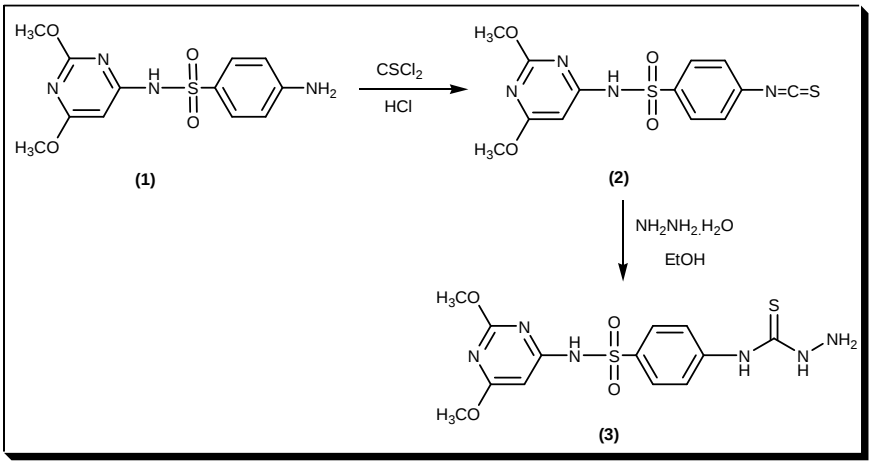
N-(4-(N-(2,6-dimethoxypyrimidin-4-yl)sulfamoyl)phenyl) hydrazine carbothioamide novel thiosemicarbazide (3) was used as starting material for synthesis of some novel Diacetyl hydrazine-carbothioamide (4), 1,3,4-Thiadiazol (7), 1,2-bis(carbothioamide) (9), 3,5-di-oxopyrazolidine (10), oxo-4,5-dihydropyrazole (11), 3,4-dihy-drophthalazine (15) and 3,6-dioxopiperazine (16).Where we found that, 1,2-bis(carbothioamide) (5), 1,2,4-triazole (6), carbamothioyl formohydrazonate (8) have anticancer activity of drug (s) using (E.A.C).

Introduction

The small-ring compounds such as three membered sulfur heterocycles and polyheterocycles may find use in cancer chemotherapy and should be evaluated for their anticancer activity[1-3]. The polarization of the isothiocyanate group in the manner indicated clearly shows the electrophilic character of the central carbon atom. Most of the chemistry of isothiocyanates is based on reaction of N=C bond with substrates A-B, A=B, A=B-C=D as well 1,3 and 1,4-dipolar compound resulting in the formation of widely differing acyclic and cyclic reaction products[4-16]. Pyrimidine derivatives are important class of hetero aromatic ring system that finds extensive use in the pharmaceutical industry. pyrimidines are reported to show anti bacterial[17-20], antifungal[21-25] and anticancer effeus[26]. Furthermore, it was found that pyrimidines act as intermediates for agricultural microbicides and herbicides. In view of the above mentioned findings and in continuation of our interest in biologically active compounds[27-30], we report herein the synthesis of some novel thiosemicarbazide,1,2,4-triazol (6), carbamothioylformohydrazonate (8), 1,2-bis(carbothioamide) (9), 3,5-dioxo-pyr-azolidine (10), oxo-4,5-dihydropyrazole (11), azomethine (13), 3,4-dihydrophthalazine (15) and 3,6-dioxopiperazine (16) derivatives.

Results and Discussion

Isothiocyanate derivatives are useful and widely used building blocks in the synthesis of nitrogen, sulfur and oxygen heterocyclic compounds, organometallic compounds of academic, pharmaceutical and industrial interest.[16,31] Isothiocyanatosulfonamides (2) were synthesized by treatment of 4-amino-N-(2,6-dimethoxy-pyri-midin-4-yl)benzenesulfonamide (1) with thiophosgene in the presence of dilute hydrochloric acid at room temperature, Scheme 1.



Scheme 1

The reaction of isothiocyanate derivative **(2)** with some nitrogen and oxygen nucleophiles was investigated. Thus, treat-ment of isothiocyanate derivative **(2)** with hydrazine hydrate in ethanol at room temperature gave the novel thiosemicarbazide derivative[32-34] **(3)**, Scheme **1**. The structure of thiosemicarbazide **(3)** was established by elemental analysis and spectral data.

Refluxing the thiosemicarbazide derivative **(3)** in acetic anhy-dride furnished Diacetyl hydrazinecarbothioamide derivative **(4).** the monoacetylthiosemicarbazide **(5)** and 1,3,4-thiadiazole derivatives **(6)** were ruled out based on elemental analyses and spectral data.

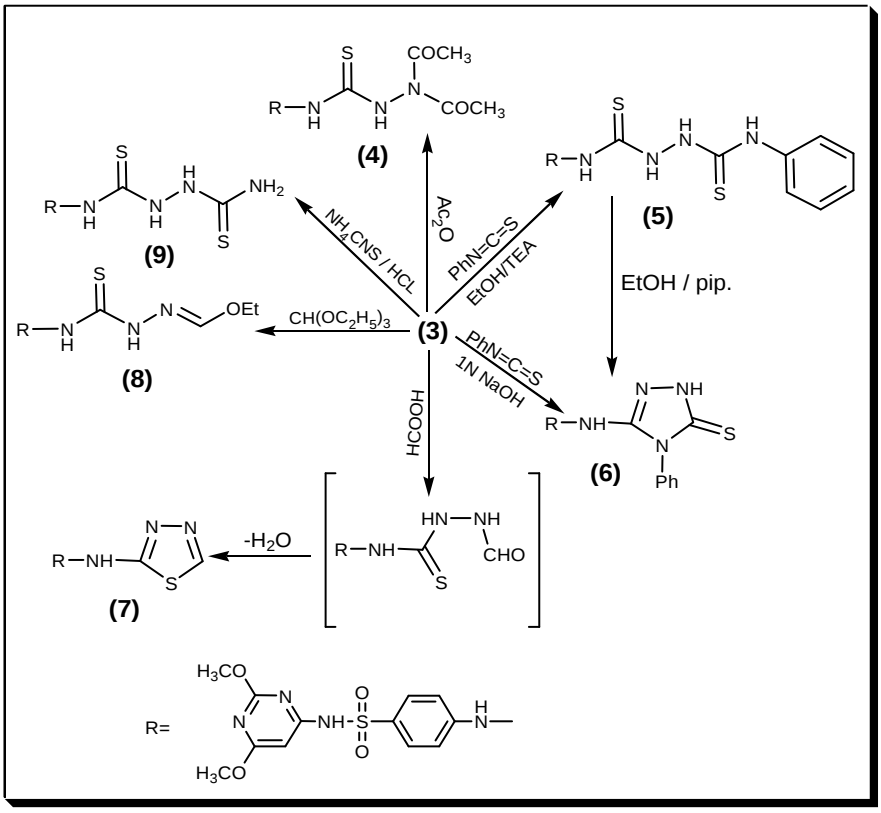
1,2-Bis(carbothioamide) derivative **(5)** was obtained by treat-ment of thiosemicarbazide derivative **(3)** with phenyl isothiocyanate in refluxing ethanol containing triethylamine, Scheme 2.

1,2,4-Triazole derivative **(6)** was obtained via reaction of the thiosemihydrazide **(3)** with phenyl isothiocyanate in presence of NaOH (1N). Another route for

obtaining 1,2,4-triazole derivative was via cyclocondensation of **(5)** in ethanol containing piperidine, Scheme 2.

Refluxing of thiosemicarbazide derivative **(3)** with formic acid afforded 1,3,4-thiadiazole derivative **(7)**.

The formation of thiadiazole derivative **(7)** was assumed to proceed via the initial formation of formyl intermediate followed by intramolecular cyclization through loss of water molecule[35]. The reaction of thiosemicarbazide derivative **(3)** with triethylorthofor-mate under reflux temperature afforded carbamothioyl formo-hydrazonate derivative **(8)**. 1,2-Bis(carbothioamide) derivative **(9)** was obtained by reaction of **(3)** with ammonium thiocyanate, Scheme 2.



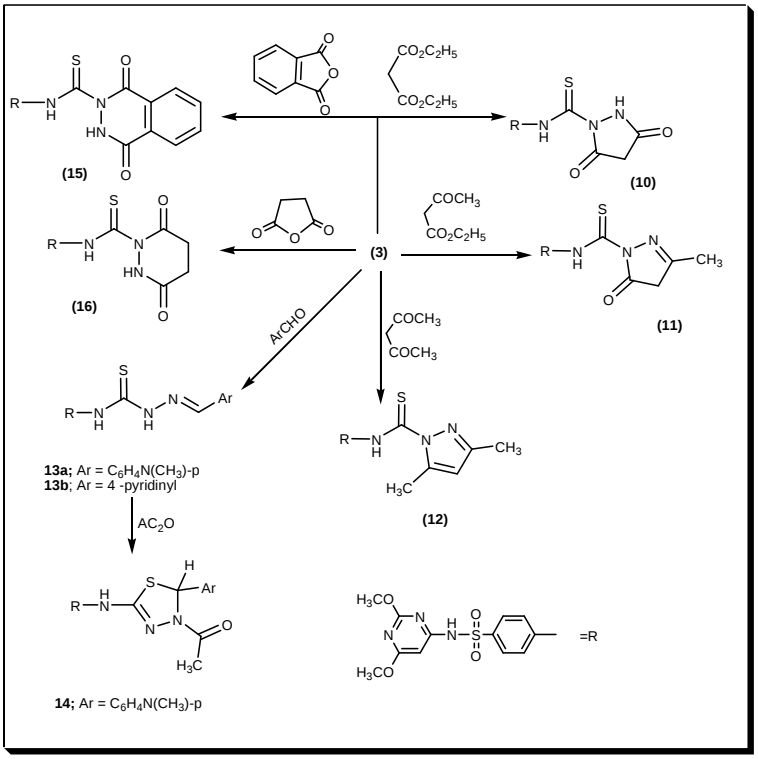
Scheme 2

3,5-di-oxopyrazolidine **(10)** was prepared through interaction of the thiosemicarbazide **(3)** with diethylmalonate in presence of sodium ethoxide, Scheme 3.

oxo-4,5-dihydropyrazole derivatives **(11)** and **(12)** were obtained by treatment of thiosemicarbazide derivative **(3)** with ethylacetoacetate and 2,4-pentandione in refluxing ethanol containing triethylamine, Scheme 3.

Condensation of thiosemicarbazide derivative **(3)** with aromatic aldehydes afforded the hydrazinecarbothioamides **(13a,b)**. The structures of **(13a,b)** were proved by analytical data and IR measurements which revealed the absence of NH_2. Refluxing of **(13a)** with acetic anhydride yielded the corresponding 1,3,4-thiadiazole derivatives **(14)**, Scheme 5**.** The structures of **(14)** were proved by analytical data and IR measurements which revealed the absence of NH and presence of absorption band for C=O at 1693 and 1681 cm^{-1} for compounds **14**.

Finally, interaction of **(3)** with phthalic anhydride yielded the corresponding the phthalazine derivative **(15)**. The structure of **(15)** was proved by analytical data and IR measurements which revealed the absence of NH_2 and presence of absorption band for C=O group. Similarly, interaction of **(3)** with succinic anhydride yielded the corresponding dioxopiperazine derivative **(16)**, Scheme 3.



Scheme 3

Experimental

Melting points are uncorrected and were determined on a Stuart melting point apparatus. Elemental analyses were determined on a Perkin Elmer 240 (microanalyses) in Microanalytical Laboratory, Cairo University, Giza, Egypt. IR spectra were recorded on a Shimadzu 440 Infrared Spectrophotometer (Shimadzu) Japan using KBr technique. UV spectra were recorded using ATl Unicam–UV-VIS Aurora scan.1HNMR Spectra were recorded on a BRUKER Proton NMR-Avance 300 (300MHz), in DMSO-d_6 as a solvent, using tetramethyl silane (TMS) as internal standard. Mass spectra were run on HP MODEL MS – 5988.

4-Isothiocyanato-N-(2,6-dimethoxypyrimidin-4-yl)benzenesulfonamide (2).

4-Amino-N-(2,6-dimethoxypyrimidin-4-yl)benzenesulfonamide **1** (0.01 mole) was dissolved in (200 mL) H_2O containing (50 mL) of concentrated HCl. To this (0.012 mole) of $CSCl_2$ was added in one portion. Stirring was begin immediately and continued until all of the red color of $CSCl_2$ had disappeared (1hr) and the product was precipitate as a white crystals. The resulting solid was filtered off, dried and recrystallized from acetone to give **2**.

Yield: 97%; MP. 170–172ºC; IR (KBr) *ν* (cm^{-1}): 3445 (NH), 3000 (CH-arom.), 2030 (NCS), 1586 (C=N), 1345, 1163 (SO_2) and 1090 (C=S); MS: m/z: 353(M^+1; 17.49%), 292 (7.92%), 256 (6.45%), 198 (9.52%), 158 (100%; base peak), 97 (21.66%), and 77 (14.87%). Anal. calcd. For $C_{13}H_{12}N_4O_4S_2$ (352): C, 44.31; H, 3.43; N, 15.90. Found: C, 43.91; H, 3.52; N, 15.62.

N-(4-(N-(2,6-dimethoxypyrimidin-4-yl)sulfamoyl)phenyl)hydrazinecarbothioamide (3).

Hydrazine hydrate (0.01 mole) was added to a solution of **2** (0.01 mole) in ethanol (50 mL). The reaction mixture was stirred for 3h until gave white precipitate. The product was recrystallized from ethanol to give **3**.

Yield: 90%; MP. 218–220ºC; IR (KBr) *ν* (cm^{-1}): 3346, 3291, 3196 (NH/NH_2), 3106 (CH-arom.), 1597 (C=N), 1348, 1157 (SO_2) and 1078 (C=S). ^{1}HNMR: δ 3.7 (hum,6H,2OCH_3),6.76 (s,1H, pyrimidine-H), and 7.68-7.94 (m, 6H, Ar-H). Anal. calcd. for $C_{13}H_{16}N_6O_4S_2$ (384): C, 40.62; H, 4.20; N, 21.86. Found: C, 40.83; H, 4.39; N, 21.58.

2,2-diacetyl-N-(N-(2,6-dimethoxypyrimidin-4-yl)sulfamoyl)-phenyl)hydrazinecarbothioamide (4).

A solution of **3** (0.01 mole) in acetic anhydride (15 mL) was heated under reflux for 24h. After cooling the solid product thus formed was collected and recrystallized from ethanol to give **4**.

Yield: 66%; m.p. 240-242ºC; IR (KBr) *v* (cm^{-1}): 3346, 3291, 3196 (NH/NH_2),3106(CH-arom.),22927(CH-alipha.)1694(C=O)1592(C=N), 1348,1157(SO_2)and1087(C=S).MS :m/z(%)(468.75M,6.04),437(7.15)

391(7.96)283(37.91)213(100)198(32.42),66(78.02).Anal.calcd.for $C_{17}H_{20}N_6O_6S_2$ (468): C, 43.58; H, 4.30; N, 17.94. Found: C, 43.82; H, 4.49; N, 17.66.

N^1-(4-(N-(2,6-dimethoxypyrimidin-4-yl)sulfamoyl)phenyl)-N^2-phenyl hydrazine-1,2-bis(carbothioamide) (5).

A mixture of **3** (0.01 mole) and phenyl isothiocyanate (0.01 mole) in ethanol (20 mL), containing 3 drops of triethylamine was heated under reflux for 10h and then cooled, poured into crushed ice water, the obtained solid was recrystallized from dioxane to give **5**.

Yield: 80%; m.p. 160-162ºC; IR (KBr) *v* (cm^{-1}): 3327, 3280, 3101 (3NH), 3085 (CH-arom), 2920 (CH-aliph), 1595 (C=N), 1306, 1162 (SO_2) and 1083 cm^{-1} (C=S). MS: m/z: 519 (M^+; 9.41%), 502 (11.76%), 437 (17.65%), 347 (22.35%), 314 (14.12%), 238 (31.76%), 213 (38.82%), 107 (51.76%), 65 (100%). Anal. calcd. for $C_{20}H_{21}N_7O_4S_3$ (519): C, 46.23; H, 4.07; N, 18.87. Found: C, 46.47; H, 4.21; N, 18.59.

N-(2,6-dimethoxypyrimidin-4-yl)-4-(4-phenyl-5-thioxo-4,5-dihydro-1H-1,2,4-triazol-3-ylamino)benzenesulfonamide (6).

Method A

A mixture of **3** (0.01 mole) and phenyl isothiocyanate (0.01 mole) in (1N) sodium hydroxide (20 mL) was refluxed for 6h and then cooled, poured into crushed ice water/HCl, the obtained solid was recrystallized from ethanol.

Method B

A mixture of **5** (0.01 mole) and pyridine (1 mL) in ethanol (10ml) was refluxed for 6h and then cooled, acidified with HCl, the obtained solid was recrystallized from ethanol to give **6**.

Yield: 82%;MP. 360-362ºC; IR (KBr) *v* (cm^{-1}): 3275 (NH), 3085 (CH-arom), 2955 (CH-aliph.), 1602 (C=N) and 1094 cm^{-1} (C=S). MS: m/z: 485 (M^+; 2.23%), 424 (2.23%), 368 (8.61%), 313 (10.37%), 236 (9.25%), 213 (5.10%) and 119 (24.08%), 57 (100%). Anal. calcd. for $C_{20}H_{19}N_7O_4S_2$ (485): C, 49.47; H, 3.94; N, 20.19. Found: C, 49.68; H, 4.03; N, 19.91.

4-(1,3,4-Thiadiazol-2-ylamino)-N-(2,6-dimethoxypyrimidin-4-yl)benzene sulfonamide (7).

A solution of **3** (0.01 mole) in formic acid (20 mL) was refluxed for 24 h. The reaction mixture was cooled and the precipitate was filtered and recrystallized from ethanol to give **7**.

Yield: 90%; m.p. 190-192ºC; IR (KBr) *ν* (cm^{-1}): 3251, 3387 (2NH), 3089 (CH-arom), 2995 (CH-aliph.), 1595 (C=N) and 1310, 1155 cm^{-1} (SO_2). ^{1}HNMR: δ 3.44 (hump, 6H, $2OCH_3$), 6.76 (s, 1H, pyimidine-H), 7.56, 8.15 (m, 5H, Ar-H), 9.0 (s, 1H, thiadiazole). Anal. calcd. for $C_{14}H_{14}N_6O_4S_2$ (394): C, 42.63; H, 3.58; N, 21.31. Found: C, 42.87; H, 3.77; N, 21.12.

Ethyl N'-4-(N-(2,6-dimethoxypyrimidin-4-yl)sulfamoyl)phenyl carbamothioylformohydrazonate (8).

A solution of **3** (0.01 mole) in triethylorthoformate (20 ml) was heated under reflux for 2h. The reaction mixture was filtered while hot and recrystallized from ethanol to give **8**.

Yield: 85%; m.p. 230-232ºC; IR (KBr) *ν* (cm^{-1}): 3425, 3254 (2NH), 3087 (CH-arom), 2994 (CH-aliph.),1595 (C=N) and 1311,1156 cm^{-1} (SO_2). MS: m/z: 442 (M^+2; 0.4%), 368 (9.71%), 340 (16.91%), 264 (13.31%), 239 (15.83%), 183 (87.77%) and 119 (81.29%), 76 (100%). Anal. calcd. for $C_{16}H_{20}N_6O_5S_2$ (440): C, 43.63; H, 4.58; N, 19.08. Found: C, 43.87; H, 4.72; N, 18.80.

N^1-(4-(N-(2,6-dimethoxypyrimidin-4-yl)sulfamoyl)phenyl) hydrazine-1,2-bis(carbothioamide) (9).

A mixture of **3** (0.01 mole) and ammonium thiocyanate (0.03 mole), conc. HCl (4 ml) was refluxed in ethanol (100ml) for 18hr. and then cooled, the obtained solid was dried and recrystallized from ethanol to give **9**.

Yield: 60%; m.p. 200-202ºC; IR (KBr) *ν* (cm^{-1}): 3271, 3209, 3116 (NH, NH_2), 3062 (CH-arom), 2858 (CH-aliph.), 1593 (C=N) and 1199 cm^{-1} (SO_2). MS: m/z: 444(M^{+1}; 4.12%), 368 (9.71%), 313 (15.46%), 255 (13.40%), 213 (43.99%), 183 (40.55%) and 95(46.74%), 55(100%). Anal. calcd. for $C_{14}H_{17}N_7O_4S_3$ (443): C, 37.91; H, 3.86; N, 22.11. Found: C, 38.15; H, 4.05; N, 21.83.

N-(4-(N-(2,6-dimethoxypyrimidin-4-yl)sulfamoyl)phenyl)-3,5-dioxopyrazolidine-1-carbothioamide (10).

To a solution of 0.5g sodium in 25ml of absolute ethanol, diethylmalonate (0.01mole) was added first and then thiosemicarbazide derivative **(3)** (0.01 mole). The reaction mixture was refluxed for 8h. It was cooled and dissolved in water (50ml), then filtered to remove unreacted material, acidified with 10% hydrochloric

acid. The precipitate was filtered off and washed with cooled water, which then recrystallized from ethanol to give **10**.

Yield: 75%; m.p. 270-272ºC; IR (KBr) *v* (cm^{-1}): 3239, 3112 (2NH), 2924 (CH-aliph.), 1715, 1626 (2C=O) 1597 (C=N) and 1383, 1153 cm^{-1} (SO_2). MS: m/z: 452 (M^+; 3.73%), 301 (2.17%), 332 (2.17%), 225 (13.00%), 180 (100%) 153 (0.2%). Anal. calcd. for $C_{16}H_{16}N_6O_6S_2$ (452): C, 42.47; H, 3.56; N, 18.57. Found: C, 42.23; H, 3.75; N, 18.38.

N-(4-(N-(2,6-dimethoxypyrimidin-4-yl)sulfamoyl)phenyl)-3-methyl-5-oxo-4,5-dihydropyrazole-1-carbothioamide (11).

A mixture of **3** (0.01 mole) and ethylacetoacetate (0.01mole), in absolute ethanol (50 ml) and triethylamine (0.5ml) was refluxed for 7h. then cooled, the precipitate was filtered off and the obtained solid was recrystallized from ethanol to give **11**.

Yield: 77%; m.p. 175-177ºC; IR (KBr) *v* (cm^{-1}): 3443, 3103 (2NH), 2926 (CH-aliph.), 1701 (C=O), 1598 (C=N) and 1316, 1156 cm^{-1} (SO_2). MS: m/z: 451(M^{+1}; 0.4%), 368 (8.42%), 313 (5.53%), 255 (17.90%), 213 (100%), 183 (11.55%), 123 (24.67%), 92 (35.56%). ^{1}HNMR: δ 1.25 (s, 3H, CH_3), 3.74 (hump, 6H, $2OCH_3$), 5.96 (s, 2H, CH_2), 6.77 (s, 1H, pyrimidine-H), 7.69, 7.97 (d-d, 5H, Ar-H). Anal. calcd. for $C_{17}H_{18}N_6O_5S_2$ (450): C, 45.32; H, 4.03; N, 18.66. Found: C, 45.11; H, 4.22; N, 18.38.

N-(4-(N-(2,6-dimethoxypyrimidin-4-yl)sulfamoyl)phenyl)-3,5-dimethyl-1H-pyrazole-1-carbothioamide (12).

A mixture of **3** (0.01 mole) and acetylacetone (0.01mole), in absolute ethanol (50 ml) was refluxed for 7h then allowed to cool, the precipitate was filtered off and the obtained solid was recrystallized from ethanol to give **12**.

Yield: 82%; m.p. 210-212ºC; IR (KBr) *v* (cm^{-1}): 3297 (NH), 3086 (CH-arom), 2921 (CH-aliph.), 1600 (C=N), 1327, 1153 (SO_2). MS: m/z: 449 (M^{1+}; 5.16%), 368 (11.46%), 320 (12.32%), 255 (26.07%), 213 (100%), 193 (14.04%), 123 (53.58%), 55 (47.85%). Anal. calcd. for $C_{18}H_{20}N_6O_4S_2$ (448): C, 48.20; H, 4.49; N, 18.74. Found: C, 48.43; H, 4.68; N, 18.55.

N-(4-(N-(2,6-diethoxypyrimidin-4-yl)sulfamoyl)phenyl)-2-(4-(di-methylamino) benzylidene) hydrazinecarbothioamide (13a),
N-(4-(N-(2,6-dimethoxypyrimidin-4-yl)sulfamoyl)phenyl)-2-(pyridin-4-ylmethylene)hydrazinecarbothioamide (13b).

A mixture of **3** (0.01 mole) and aromatic aldehyde (0.01mole), in ethanol (20 ml), containing 3 drops of triethylamine was heated under reflux for 8h and then

cooled, poured into crushed ice water, the obtained solid was recrystallized from dioxane to give **13a,b**.

13a: Yield: 87%; m.p. 130-132ºC; IR (KBr) *ν* (cm^{-1}): 3370 (NH), 3068 (CH-arom), 2920 (CH-aliph.), 1597 (C=N), 1360, 1153 (SO_2) and 1082 (C=S). MS: m/z: 513 (M-2; 0.4%), 433 (15.73%), 256 (21.35%), 179 (22.47%), 136 (21.35%) 69(100). Anal. calcd. for $C_{22}H_{25}N_7O_4S_2$ (515): C, 51.25; H, 4.89; N, 19.02. Found: C, 51.49; H, 5.03; N, 18.83.

13b: Yield: 86%; m.p. 160-162ºC; IR (KBr) *ν* (cm^{-1}): 3430 (NH), 3070 (CH-arom), 2921 (CH-aliph.), 1598 (C=N), 1153, 1355 (SO_2) and 1088 cm^{-1} (C=S). MS: m/z: 473 (M^+; 0.4%), 474 (M+1; 26.15%), 430 (100%), 462 (78.89%), 415 (53.46%), 403 (67.88%), 383 (26.16%), 105 (16%). Anal. calcd. for $C_{19}H_{19}N_7O_4S_2$ (473): C, 48.19; H, 4.04; N, 20.71. Found: C, 48.43; H, 4.23; N, 20.43.

Formation of 1,3,4-thiadiazole derivatives (14):

A mixture of **13a** (0.01 mole) and acetic anhydride (20 ml) was reflux for 3h. Excess acetic anhydride and acetic acid were removed under reduced pressure and the residue so formed was recrystallized from dioxane to give **14**

14: Yield: 43%; m.p. 90-92ºC; IR (KBr) *ν* (cm^{-1}): 3399, 3257 (3NH), 3103 (CH-arom), 2936 (CH-aliph.), 1693 (C=O), 1591 (C=N) and 1325, 1162 cm^{-1} (SO_2). MS: m/z: 558 (M+1; 0.4%), 387 (9.71%), 248 (16.91%), 223 (13.31%), 155 (15.83%), 73 (100%). Anal. calcd. for $C_{24}H_{27}N_7O_5S_2$ (557): C, 51.69; H, 4.88; N, 17.58. Found: C, 51.45; H, 4.69; N, 17.39.

Formation of compounds (15) and (16): General procedure:

A mixture of **3** (0.01 mole) and phthalic anhydride or succinic anhydride (0.01 mole) in acetic anhydride (50 ml) was refluxed for 7h, then cooled, the precipitate was filtered off the obtained solid were recrystallized from dioxane to give **15** and **16**.

N-(4-(N-(2,6-dimethoxypyrimidin-4-yl)sulfamoyl)phenyl)-1,4-dioxo-3,4-dihydrophthalazine-2(1H)-carbothioamide (15).

Yield: 70%; m.p. 140-142ºC; IR (KBr) *ν* (cm^{-1}): 3444 (NH), 2924 (CH-aliph.), 1738, 1627 (2C=O), 1596 (C=N), and 1080 cm^{-1} (C=S). ^{1}HNMR: δ 3.38, 4.1 (2s, 6H, $2OCH_3$), 6.75 (s, 1H, pyrimidine-H), 7.55-8.12 (d-d, 4H, Ar-H). Anal. calcd. for $C_{21}H_{18}N_{6-}O_6S_2$ (514): C, 49.02; H, 3.53; N, 16.33. Found: C, 49.26; H, 3.72; N, 16.14.

N-(4-(N-(2,6-dimethoxypyrimidin-4-yl)sulfamoyl)phenyl)-3,6-dioxo piperazine-1-carbothioamide (16).

Yield: 60%; m.p. 120-122ºC; IR (KBr) *v* (cm^{-1}): 3325 (NH), 3038 (CH arom.), 2931 (CH-aliph.), 1693 (2C=O) and 1085 cm^{-1} (C=S). ^{1}HNMR: δ 3.8 (hump, 6H, $2OCH_3$), 4.29, 4.53 (2d, 4H, $2CH_2$), 6.72 (s, 1H, pyrimidine-H) and 7.68-8.05 (m, 4H, Ar-H). Anal. calcd. for $C_{17}H_{18}N_6O_6S_2$ (466): C, 43.77; H, 3.89; N, 18.02. Found: C, 43.53; H, 3.75; N, 17.83.

Antitumor activity of the (E.A.C) :

.The method used is that of trypan blue exclusion

Reagents :

1- RPMI 1640 medium (sigma).

2- Ehrlich Ascites Carcinoma cells (EAC) suspension ($2.5x10^6$/ml).

3- Trypan blue dye; A stock solution was prepared by dissolving one gram of the dye in distilled water (100 ml). The working solution was then prepared by diluting (1 ml) of the stock solution with (9 ml) of distilled water. The stain was used then for staining the dead EAC cells.

4- The data of tested compounds are summarized in (Table 1).

Procedure :

1 ml of tumor cells which is drawn from mice bearing (E.A.C).

1- EAC cells were obtained by needle aspiration of the ascetic fluid from preinoculated mice under aseptic conditions.[36]

2- The cells were tested for viability and contamination by staining certain cell volume of this fluid by an equal volume of the working solution of trypan blue dye.[38,39]

3- The ascetic fluid was diluted with saline (1:10) to contain $2.5x10^6$ cells on a hemocytometer.

4- In a set of sterile test tubes 0.1 ml of tumor cells suspension, 0.8 ml RPMI 1640 media and 0.1 ml of each tested compound (corresponding to 100, 50 and 25 µg/ml) were mixed. The test tubes were incubated at 37ºC for 2hr. Trypan blue exclusion test[37,38] was carried out to calculate the percentage of non-viable cells. Compounds producing more than 70% non viable cells are considered active.

5- Doxorubicin (Adriablastina)^R is taken as a reference.

$$\%\ \text{of non-viable cells} = \frac{\text{No. of non viable}}{\text{Total No. of cells}} \times 100$$

Screening test

Antitumor Activity of the Drug(s) using (E.A.C)

Compd. No.	Non-viable cells (%) Concentration (µg/ml)		
	100	50	25
4	0	0	0
5	40	20	10
6	50	30	10
7	0	0	0
8	**30**	10	0
11	10	0	0
13c	20	10	0
16	20	10	0
Doxrubicin	100	55	20

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