Synthesis, Antioxidant, Antituomer Activities of Some New Thiazolopyrimidines, Pyrrolothiazolopyrimidines and Triazolopyrrolothiazolopyrimidines Derivatives

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Reaction of 6-amino-2-thiouracil **1** with ethyl bromoacetate yielded ethyl 2-(7-amino-2,5-dioxo-3,5-dihydro-2*H*-thiazolo[3,2-*a*]pyrimidin-6-yl)acetate **2**. Reaction of **2** with sodium ethoxide afforded the pyrrolothiazolopyrimidine derivative **3**. Compound **2** reacted with hydrazine hydrate to give 7-amino-thiazolopyrimidine-carbohydrazide **4**. The latter compound **4** reacted with carbon disulphide to form 7-amino-6-(oxadiazolylmethyl) thiazolopyrimidine **5**. Compound **5** was heated in methanol to yield 9-thioxotriazolopyrrolothiazolopyrimidine **6**. Also, the reaction of **3** with aromatic aldehydes afforded the diarylmethylenepyrrolothiazolopyrimidine derivatives **7a-c**. The latter compounds **7a-c** underwent cyclocondensation with hydroxylamine to give diaryldioxazolopyrrolothiazolopyrimidine derivatives **8a-c**. The new prepared compounds were subjected for antioxidant and antituomer studies, some of these compounds exhibited promising activity.

Keywords: Pyrrolothiazolopyrimidine; Thiazolopyrimidine; Triazolopyrrolothiazolopyrimidine; Antioxidant and antituomer activities.

INTRODUCTION

Thiazolopyrimidines and thiazolidinedionepyrimidines have hypoglycemic and hypolipidemic and antidiabetic activities.[1] Triazolopyrimidines have antifungal activity[2] and pyrimidine derivatives have antileshimanial activity.[3] Thus, these compounds have attracted our attention due to the wide range of biological activities associated with this scaffold. Various related compounds of these pyrimidine derivatives have biological activities ranging from kinase inhibitors, treatment of disease states associated with angiogenesis [plated derived growth factor, PDGFr, fibroplast growth factor, FGFr, and epiderma growth factor, (EGFr),[4] the analogous mitogen-activated protein (CSBP/P38) kinase inhibitor,[5] telomerase inhibitor[6]], for treatment of arthritis, adult respiratory distress syndrome, chronic obstructive pulmonary disease, or Alzheimer's disease.[7] Arylazopyrimidine derivatives showed antitumor activity[8-11] and anticancer activity.[12] Our research group has the interest in the development of synthetic strategy to polyfunctionalized heterocycles. In continuation, this paper describes our approach to the synthesis of thiazolopyrimidines, pyrrolothiazolopyrimidines and triazolopyrrolothiazolopyrimidines.

RESULTS AND DISCUSSION

6-Amino-2-thiouracil (**1**), (mp 320 ^{o}C),[13,14] underwent cyclocondensation by heating under reflux with ethyl bromoacetate,[15] in boiling ethanolic potassium hydroxide,[16] for long times to afford ethyl(7-amino-3,5-dioxo-2,3-dihydro-5*H*-[1,3]thiazolo[3,2-*a*]pyrimidin-6-yl)acetate (**2**). The latter compound underwent cyclocondensation in sodium ethoxide to afford 6,8-dihydropyrrolo[2,3-*d*]thiazolo[3,2-*a*]pyrimidine-3,5,7(2*H*)-trione (**3**) (Scheme I). The IR spectrum compound **2** showed absorption bands at ν 1749 cm^{-1} corresponding to ester CO, as well as the other expected bands for such structure. The IR spectrum of compound **3** revealed the absence of the ester carbonyl band; besides the presence three amidic carbonyl absorptions as well as other expected absorption bands (Experimental section). The ^{1}H NMR spectra of **2** and **3** are given

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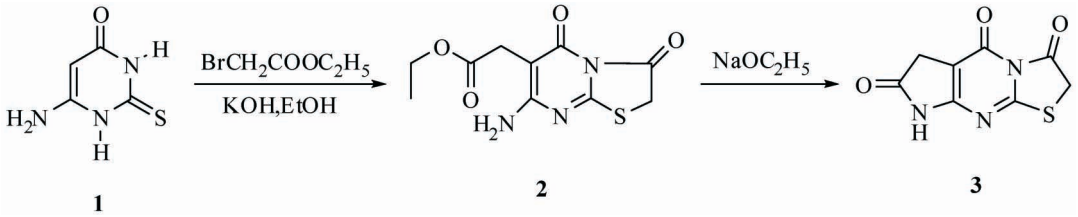
Scheme I

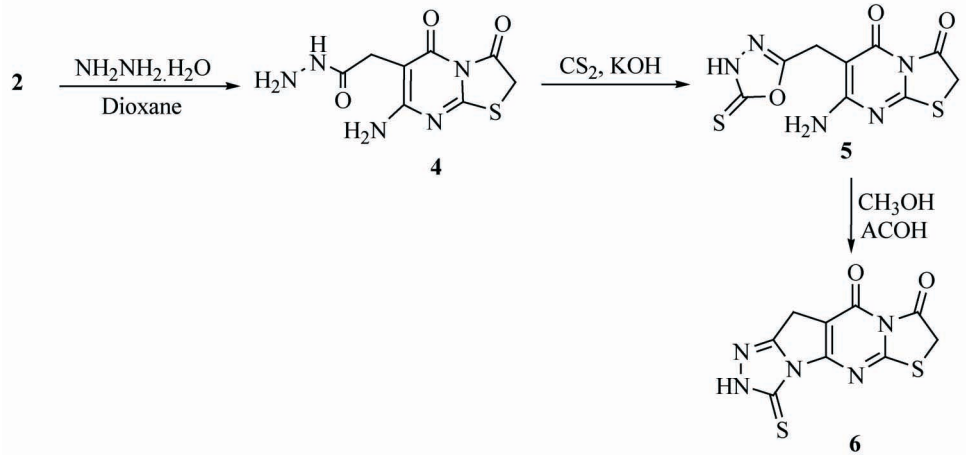


in the experimental section. The Mass spectra of **2** and **3** showed the molecular ion peaks at *m/z* 269 (30.2%) and 223 (55.5%), respectively.

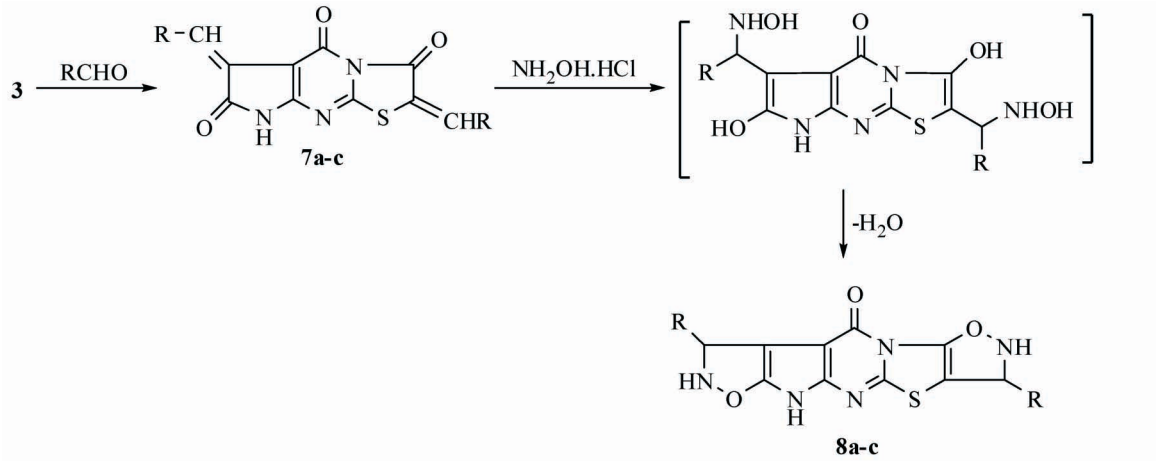
The reaction of the pyrimidinyl acetate derivative **2** with hydrazine hydrate in boiling dioxane produces 2-(7-amino-3,5-dioxo-2,3-dihydro-5*H*-thiazolo[3,2-*a*]pyrimidin-6-yl)acetohydrazide (**4**) in good yield.[17] The latter compound reacted with carbon disulphide to give produce 7-amino-6-[(5-thioxo-4,5-dihydro-1,3,4-oxadiazol-2-yl)-methyl]-5*H*-thiazolo[3,2-*a*]pyrimidine-3,5(2*H*)-dione (**5**). The latter compound, in turn, underwent reductive cyclization upon heating under reflux in methanol where 3-thioxo-2,11-dihydro-3*H*,10*H*-thiazolo[3,2-*a*][1,2,4]triazolo[4′,3′:1,5]pyrrolo[2,3-*d*]pyrimidine-8,10(7*H*)-dione (**6**) was formed,[18,19] (Scheme II). Formation of **6** from **5** may proceed through solvolytic opening of the oxadiazole ring followed by elimination of water to form the new triazole ring. The IR spectrum compound **4** displayed three carbonyl absorption bands, besides the absence of absorption representing ester carbonyl that indicates the formation of the hydrazide moiety. The IR spectrum of compound **5** displayed, among other expected bands, only two carbonyl absorptions. The IR spectrum of compound **6** displayed only one single absorption band in the amino region. The absence of NH_2 absorption indicates that the involvement of NH_2 group in **5** in the formation of the triazole ring in **6**. The ^1H, ^{13}C NMR and mass spectra supported the structures (Experimental section).

Furthermore, pyrrolothiazolopyrimidinetrione **3**, having two active methylene groups, condensed with aromatic aldehydes to afford the 2,6-diarylidene-6,8-dihydropyrrolo[2,3-*d*]thiazolo[3,2-*a*]pyrimidine-3,5,7(2*H*)-triones **7a-c** which underwent cyclocondensation with hydroxylamine in boiling acetic acid, in the presence of anhydrous sodium acetate to furnish 3,9-diaryl-2,3,8,9-tetrahydroisoxazolo [4′,5′:4,5]pyrrolo[2,3-*d*]isoxazolo[5′,4′:4,5]thiazolo[3,2-*a*]pyrimidin-10(6*H*)-ones **8a-c**; the reaction path way involves a double nucleophilec addition of the hydroxyl amine hydrochloride to the ethylenic double bond followed by ring closure *via* elimination two molecules of water,[17] (Scheme III). The IR spectra of compounds **7a-c** displayed absorption bands that correspond to three carbonyl groups, as well as the other expected peaks. IR spectra of **8a-c** showed only one carbonyl absorption band. The benzylic protons of **8a-c** are described as doublets in ^1H-NMR within δ = 4.55-4.57 and 5.21-5.23 due to coupling

Scheme II



Scheme III



a, R= phenyl; **b**, R= *p*-chlorophenyl; **c**, R= *p*-methoxyphenyl

with the adjacent NH, these signals could also represent pairs of singlets from the diastereoisomeric structures. Coupling of the benzylic protons with the adjacent NH groups could be proved by carrying out ^{1}H-NMR in the presence of D_2O. Deuterium exchange with the NH protons caused the benzylic protons signals to appear as singlets. Correct values in elemental analysis of all new compounds are in agreements with the assigned structures.

PHARMACOLOGY

Antioxidant activity

All compounds were tested for antioxidant activity as reflected in the ability to inhibit lipid peroxidation in rat brain and kidney homogenates and rate erythrocyte hemolysis. The pro-oxidant activities of the formed compounds were assayed via their effects on bleomycin-induced DNA damage. Compounds **4** and **5** manifested potent antioxidative activity in the lipid peroxidation assay but no inhibitory activity in the hemolysis assay. On the other hand, compounds **4, 5, 7b, 7c, 8a** and **8b** exhibited significant antioxidant activity and protected the DNA from damage (Table 1).

Anti-tumor Activity using in Vitro Ehrlich used Ascites Assay

The newly synthesized compounds were screened for their antitumor activity. Viability of the cells used in control experiments exceeded 95%. Compound **6** (80.10%) followed by compounds **8b**, **8c**, **8a** (65.14-50.10%) and **2** (48.87%). The other tested compounds showed very weak activity (46.91-25.04%) (Table 2).

Table 1. Anti-oxidant assay for the prepared new compounds

Methods	ABTS	Erythrocyte Hemolysis	Bleomycin-Dependent DNA damage
Compounds [a]	Inhibition (%)	Hemolysis (%)	Absorbance
L-ascorbic acid	88.61	0.85	0.881
Control	0	0	0
2	3.65	4.10	0.858
3	10.97	3.40	0.843
4	83.73	0.80	0.886
5	73.70	1.01	0.886
6	18.60	1.90	0.851
7a	11.90	0.85	0.780
7b	15.20	1.99	0.880
7c	65.20	1.10	0.862
8a	19.50	1.70	0.883
8b	55.20	1.20	0.850
8c	29.90	1.50	0.847

[a] For testing 50 mL of 2 mmol L^{-1} solution in 1 mL methanol/phosphate buffer (1:1, V/V) was used.

Structural Activity Relationship (SAR)

• Novel thiazolopyrimidine derivatives incorporated with substituted carbohydrazide, amino group, oxadiazol, moieties possesses potential for antioxidant activities. Therefore more active compounds (**4**, **5**) because it was give free radical ions these used antioxidant with abnormal cell compared with another compounds.

• Thioxo, triazolo, pyrrolothiazolopyrimidine for example (**6**) showed the highest cytotoxic activity than pyrimidine and pyrimidine derivatives where thioxo-triazole

Table 2. Results of Ehrlich in vitro assay all compounds

Compounds No. [a]	Dead Cells (%)
Control (no drugs)	0
5-Floururacil [b]	99.50
2	48.87
3	45.12
4	40.99
5	46.91
6	80.10
7a	27.92
7b	25.04
7c	32.19
8a	50.10
8b	65.14
8c	55.17

[a] 1 mg mL^{-1} in DMSO/RPMI-1640 (1:10)
[b] 25 mg mL^{-1} in DMSO/RPMI-1640 (1:10)

group increasing the blood inter tissue cell. Hence this compounds for used antitumor agents (cytotoxic activity).

• Diaryl-dioxazolo-pyrrolo-thiazolopyrimidine (ex. **8a-c**) was found to be of moderate to antitumor activities when compared with the pyrimidine derivatives.

• Diarylidene-dihydropyrrolothiazolopyrimidine-triones (ex. **7a-c**) showed the moderate antioxidant activity than pyrimidine derivatives.

• Our prediction is that these compounds with new ring systems show even weaken antioxidant and antitumor activities (Tables 1, 2).

CONCLUSION

New ring systems are prepared. In continuation, this paper describes our interest in the development of synthetic strategy to polyfunctionalized heterocycles such as the synthesis of thiazolopyrimidines, pyrrolothiazolopyrimidines and triazolopyrrolo-thiazolo pyrimidines. The series of compounds (**4, 5, 7b, 7c, 8a, 8b)** exhibited a high antioxidant activity and protect the DNA. The series of compounds (**6**, **8b, 8c, 8a**) proved to have the best cytotoxic activity.

EXPERIMENTAL SECTION

General

All melting points are in degree centigrade and were determined on Gallenkamp electric melting point apparatus. The IR spectra were recorded (KBr) on a Perkin-Elmer 1430 spectrometer (National Research Center and Chemistry Department, Cairo University). The ^{1}H NMR and ^{13}C NMR spectra (δ, ppm) were recorded on JEOL-ECA500 (National Research Center, Egypt) and JEOL JNM-LA-400 FT NMR Spectrometer (Chemistry Department, Cairo University) and chemical shifts were expressed as δ values against TMS as internal standard. Mass spectra were recorded on GCMS-QP 1000 EX Shimadzu Japan (Gas Chromatography-Mass spectrometer). Microanalytical data were obtained at the Microanalytical Center at Cairo University and National Research Center, Egypt.

Ethyl 2-(7-amino-2,5-dioxo-3,5-dihydro-2H-thiazolo-[3,2-a]pyrimidin-6-yl)acetate (2)

A mixture of 6-aminothiouracil (**1**) (1.43 g, 10 mmol) and (ethyl bromoacetate, 20 mmol) was refluxed in ethanol (50 mL) in presence of potassium hydroxide (10 mmol) or (sodium acetate, acetic acid, acetic anhydride) for 20 h (under TLC control). The reaction mixture was cooled with water; the deposited precipitate was filtered off, washed with ethanol, dried, and crystallized from methanol to afford **2** as white crystals (85%), mp 205-207 °C (dec.). IR (KBr) $\nu_{max.}$ 3352, 3177 (NH_2), 1750 (CO, ester group), 1652 (br., 2CO, amidic), 1626 cm^{-1} (C=N); ^{1}H NMR (DMSO-d_6) δ 1.22 (t, 3H, J = 7.10 Hz, CH_3), 3.32 (s, 2H, CH_2), 4.14 (q, 2H, J = 7.15 Hz, CH_2), 4.78 (s, 2H, CH_2, thiazole ring), 6.79 (br., 2H, NH_2, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ 15.20 (1C, CH_3), 32.25 (CH_2), 32.69 (C_2), 52.89 (CH_2O), 81.92 (C_6), 162.57 (C_7), 164.19 (C_{8a}), 165.56 (C_5), 169.83 (C_3), 170.54 (CO, ester group); MS (70 ev, %) *m/z* 271 (M^+ +2, 5.1%), 270 (M^+ +1, 45.5%), 269 (M^+, 30.2%), 241 (100.0%, M^+-CO), 224 (15.0%, M^+-$CH_3CH_2O^·$), 182 (15.1%, M^+-$CH_3CH_2OCOCH_2^·$). Anal. Calc. for $C_{10}H_{11}N_3O_4S$ (269.28): C, 44.60; H, 4.12; N, 15.60; S, 11.91. Found: C, 44.50; H, 4.25; N, 15.53; S, 11.85.

6,8-Dihydropyrrolo[2,3-d]thiazolo[3,2-a]pyrimidine-3,5,7(2H)-trione (3)

A mixture of compound **2** (2.69 g, 10 mmol) was refluxed in ethanol (50 mL) in presence sodium metal (0.23 g, 10 mmol) for 10 h. The reaction mixture was cooled; the deposited precipitate was filtered off, washed with ethanol, the formed salt was dissolve in water and acidified with 10% HCl, the formed precipitate was filtered, dried and crystallized from methanol to afford **3** as yellow crystals (77%); mp >350 °C (dec.). IR (KBr) $\nu_{max.}$ 3419 (NH), 1712 (CO, pyrrole ring), 1663 (br., 2CO, amidic), 1620 cm^{-1} (C=N); ^{1}H NMR (DMSO-d_6) δ 2.93 (s, 2H, CH_2, pyrrole ring), 4.79 (s, 2H, CH_2, thiazole ring), 8.02 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ 33.01 (C_2),

33.20 (C_6), 105.11 (5a), 158.41 (C_{8a}), 164.20 (C_5), 165.31 (C_{11}), 168.80 (C_7), 171.90 (C_3); MS (70 ev, %) *m/z* 225 (M^+ +2, 4.5%), 224 (M^+ +1, 40.5%), 223 (M^+, 55.5%), 195 (6.1%, M^+-CO), 181 (25.2%, M^+-CH_2=C=O), 142 (100.0%, M^+-C_4H_4NO). Anal. Calc. for $C_8H_5N_3O_3S$ (223.21): C, 43.05; H, 2.26; N, 18.83; S, 14.37; Found: C, 43.14; H, 2.30; N, 18.75; S, 14.45.

2-(7-Amino-3,5-dioxo-2,3-dihydro-5H-thiazolo[3,2-a]pyrimidin-6-yl)aceto-hydrazide (4)

A mixture of compound **2** (2.69 g, 10 mmol) and hydrazine hydrate (0.5 mL, 10 mmol) was refluxed in dioxane (50 mL) for 15 h. The reaction mixture was cooled; the deposited precipitate was filtered off, washed with ethanol, dried, and crystallized from ethanol to afford **4** as white crystals (83%); mp 180-182 °C (dec.). IR (KBr) $\nu_{max.}$ 3442-3431 (brs., NH, NH_2), 1712 (CONH) group, 1662 (CO, amide), 1632 (CO, thiazole ring), 1612 cm^{-1} (C=N); ^{1}H NMR (DMSO-d_6) δ 1.90 (s, 2H, CH_2, thiazolo ring), 3.95 (s, 2H, CH_2), 4.48 (br., 2H, NH_2), 5.73 (br., 2H, NH_2), 9.08 (br., 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ 31.30 (C, CH_2), 32.65 (C_2), 81.93 (C_6), 162.10 (C_7), 164.10 (C_{8a}), 165.75 (C_5), 170.40 (C, CO, Carbohydrazide), 171.40 (C_3); MS (70 ev, %) *m/z* 257 (M^+ +2, 4.7%), 256 (M^+ +1, 9.4%), 255 (M^+, 65.5%), 227 (3.5%, M^+-CO), 213 (2.7%, M^+-CH_2=C=O), 142 (100.0%, M^+-$C_4H_7N_3O$). Anal. Calc. for $C_8H_9N_5O_3S$ (255.25): C, 37.64; H, 3.55; N, 27.44; S, 12.56. Found: C, 37.52; H, 3.60; N, 27.50; S, 12.46.

7-Amino-6-[(5-thioxo-4,5-dihydro-1,3,4-oxadiazol-2-yl)-methyl]-5H-thiazolo[3,2-a]pyrimidine-3,5(2H)-dione (5)

To a solution of **4** (2.55 g, 10 mmol) in ethanol (30 mL), KOH (0.5 g) in water (5 mL) and CS_2 (10 mmol) were added. The reaction mixture was heated under reflux till the evolution of hydrogen sulphide ceased. Thereafter, it was cooled, diluted with cold water (30 mL) and acidified with acetic acid. The solid that formed was filtered off, dried and recrystallized from ethanol to afford **5** as yellow crystals (82%); mp 125-127 °C (dec.). IR (KBr) $\nu_{max.}$ 3433-3444 (brs., NH, NH_2), 1676 (CO, amide), 1646 (CO, thiazole ring), 1621 (C=N, oxadiazole ring), 1351-1346 cm^{-1} (C=S); ^{1}H NMR (DMSO-d_6) δ 2.23 (s, 2H, CH_2), 4.15 (s, 2H, CH_2, thiazol ring), 6.12 (br., 2H, NH_2, D_2O exchangeable), 7.33 (br., 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ 31.90 (CH_2), 32.60 (C_2), 81.91 (C_6), 157.80 (C_5, oxadiazole ring), 160.90 (C_7), 163.90 (C_{8a}), 165.55 (C_5), 170.90 (C_3), 173.10 (1C, C=S); MS (70 ev, %) *m/z* 299 (M^+ +2, 9.10%), 298 (M^+ +1, 14.20%), 297 (M^+, 64.30%), 255 (25.15%, M^+-CH_2=C=O), 183 (15.5%, M^+-$C_3H_2N_2OS$), 142 (100.0%, M^+-$C_5H_5N_3OS$). Anal. Calc. for $C_9H_7N_5O_3S_2$ (297.31) C, 36.36; H, 2.37; N, 23.56; S, 21.57. Found: C, 36.41; H, 2.45; N, 23.61; S, 21.49.

3-Thioxo-2,11-dihydro-3H,10H-thiazolo[3,2-a][1,2,4]-triazolo[4′,3′:1,5]pyrrolo-[2,3-d]pyrimidine-8,10(7H)-dione (6)

A suspension of **5** (2.97 g, 10 mmol) in methanol (30 mL) and glacial acetic acid (2 mL) was refluxed for 14 h. The reaction mixture was cooled, the solid so obtained was filtered off dried and recrystallized from methanol to furnish **6** as yellowish color (80%); mp 230-232 °C (dec.). IR (KBr) $\nu_{max.}$ 3446 (br., NH), 1691 (CO, pyrimidine), 1652 (CO, thiazolidinone), 1346 cm^{-1} (C=S); ^{1}H NMR (DMSO-d_6) δ 3.15 (s, 2H, CH_2), 4.47 (s, 2H, CH_2, thiazol ring), 8.66 (brs., 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ 32.90 (C_7), 33.20 (C_{11}), 100.40 (C_{10a}), 158.90 (C_{11a}), 161.90 (C_{4a}), 164.10 (C_{5a}), 165.85 (C_{10}), 171.45 (C_8), 175.55 (C_3); MS (70 ev, %) *m/z* 281 (M^+ +2, 8.40%), 280 (M^{+1} +1, 10.10%), 297 (M^+, 68.50%), 239 (4.70%, M^+-·CH_2CN), 220 (7.10%, M^+-HN=C=O), 205 (8.6%, M^+-C_2H_2OS), 70 (100.0%, OC-N=CO). Anal. Calc. for $C_9H_5N_5O_2S_2$ (279. 31) C, 38.70; H, 1.80; N, 25.07; S, 22.96. Found: C, 38.60; H, 1.71; N, 25.18; S, 22.80.

2,6-Diarylidene-6,8-dihydropyrrolo[2,3-d]thiazolo[3,2-a]pyrimidine-3,5,7(2H)-trione derivatives (7a-c): General procedure

A mixture of compound **3** (2.23 g, 10 mmol), aldehyde derivatives namely; benzaldehyde (2.12 g, 20 mmol), 4-chlorobenzaldehyde (2.80 g, 20 mmol), or 4-methoxybenzaldehyde (2.72 g, 20 mmol), and (0.02 mol) of anhydrous sodium acetate was stirred under reflux in a mixture of glacial acetic acid (30 mL) and acetic anhydride (15 mL) for 5 h. The reaction mixture was allowed to cool to room temperature, poured into cold water (100 mL). The deposited precipitate was filtered off, dried, and crystallized from appropriate solvent to give **7a-c**, respectively.

2,6-Dibenzylidene-6,8-dihydropyrrolo[2,3-d]thiazolo-[3,2-a]pyrimidine-3,5,7(2H)-trione (7a)

Crystallization from dioxane; yellow powder (80%), mp 280-282 °C (dec.). IR (KBr) $\nu_{max.}$ 3432 (brs., NH), 1742 (CO, pyrrole ring), 1690 (CO, amide), 1630 (CO, thiazole ring), 1615 cm^{-1} (C=N); ^{1}H NMR (DMSO-d_6) δ 6.96 (s, 1H, methine), 7.40-7.80 (m, 10H, phenyl), 8.36 (s, 1H, methine), 8.66 (br., 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ 111.91 (C_{5a}), 118.8 (C_2), 126.63 (2C, C_2, C_6, Ph), 126.73 (2C, C_2, C_6, Ph), 127.95 (C_4, Ph), 128.12 (C_4,

Ph), 128.82 (2C, C_3, C_5, Ph), 129.13 (2C, C_3, C_5, Ph), 133.96 (C_6), 134.62 (C, methine), 134.70 (C_1, Ph), 134.90 (C_1, Ph), 142.53 (C, methine), 146.20 (C_{8a}), 158.68 (C_{9a}), 165.55 (C_5), 167.90 (C_7), 172.89 (C_3); MS (70 ev, %) *m/z* 401 (M^+ +2, 6.5%), 400 (M^+ +1, 27.5%), 399 (M^+, 55.5%), 322 (14.5%, M^+-$C_6H_5^{\cdot}$), 237 (45.7%, M^+-C_9H_6OS), 224 (9.10), 209 (35.10%, 237-CO), 145 (100%, C_9H_7NO). Anal. Calc. for $C_{22}H_{13}N_3O_3S$ (399.42): C, 66.15; H, 3.28; N, 10.52; S, 8.03. Found: C, 66.20; H, 3.30; N, 10.60; S, 8.10.

2,6-Di-(4-chlorobenzylidene)-6,8-dihydropyrrolo[2,3-d]-thiazolo[3,2-a]pyrimidine-3,5,7(2H)-trione (7b)

Crystallization from dimethylformamide; brown powder (85%); mp >350 °C (dec.). ^{1}H NMR (DMSO-d_6) δ 6.95 (s, 1H, methine), 7.40 (d, 2H, J = 8.43 Hz, 4-Cl-phenyl), 7.45 (d, 2H, J = 8.40 Hz, 4-Cl-phenyl), 7.48 (d, 2H, J = 8.35 Hz), 7.50 (d, 2H, J = 8.38 Hz), 8.35 (s, 1H, methine), 8.65 (br., NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ 111.96 (C_{5a}), 118.81 (C_2), 126.55 (2C, C_2, C_6, Ph), 126.80 (2C, C_2, C_6, Ph), 128.90 (2C, C_3, C_5, Ph), 129.10 (2C, C_3, C_5, Ph), 131.45 (C_4, Ph), 131.55 (C_4, Ph), 133.96 (C_6), 134.52 (C, methine), 136.56 (C_1, Ph), 138.20 (C_1, Ph), 142.65 (C, methine), 146.50 (C_{8a}), 158.75 (C_{9a}), 165.70 (C_5), 167.80 (C_7), 172.55 (C_3); MS (70 ev, %) *m/z* 471 (M^+ +4, 25.6%), 469 (M^{+1} +2, 50.5%), 467 (M^+, 69.5%), 356 (14.5%, M^+-$^{\cdot}C_6H_4Cl$), 302 (9.7%, M^+-$C_9H_5ClO^{\cdot}$), 111 (100%, C_6H_4Cl). Anal. Calc. for $C_{22}H_{11}Cl_2N_3O_3S$ (468.31): C, 56.42; H, 2.37; N, 8.97; S, 6.85. Found: C, 56.50; H, 2.45; N, 8.85; S, 6.90.

2,6-Di-(4-methoxybenzylidene)-6,8-dihydropyrrolo[2,3-d]thiazolo[3,2-a]pyrimidine-3,5,7(2H)-trione (7c)

Crystallization from dimethylformamide-ethanol mixture; yellow powder (78%); mp >350 °C (dec.). ^{1}H NMR (DMSO-d_6) δ 3.84 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 6.94 (s, 1H, methine), 7.12 (d, 2H, J = 8.42 Hz, 4-methoxy phenyl), 7.15 (d, 2H, J = 8.37 Hz, 4-methoxyphenyl), 7.22 (d, 2H, J= 8.34 Hz), 7.30 (d, 2H, J = 8.41 Hz), 8.33 (s, 1H, methine), 8.64 (br., NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ 55.95 (C, OCH_3), 57.01 (C, OCH_3), 111.95 (C_{5a}), 118.7 (C_2), 125.95 (2C, C_2, C_6, Ph), 126.10 (2C, C_2, C_6, Ph), 127.95 (2C, C_3, C_5, Ph), 128.90 (2C, C_3, C_5, Ph), 130.92 (C_4, Ph), 131.40 (C_4, Ph), 132.98 (C_6), 133.95 (C, methine), 136.90 (C_1, Ph), 137.95 (C_1, Ph), 141.97 (C, methine), 145.96 (C_{8a}), 158.30 (C_{9a}), 165.10 (C_5), 167.30 (C_7), 171.90 (C_3); MS (70 ev, %) *m/z* 461 (M^+ +2, 20.6%), 460 (M^++1, 24.5%), 459 (M^+, 74.0%), 352 (7.5%, M^+-$C_7H_7O^{\cdot}$), 339 (100%, M^+-$C_8H_8O^{\cdot}$). Anal. Calc. for $C_{24}H_{17}N_3O_5S$ (459.47): C, 62.74; H, 3.73; N, 9.15. Found: C, 62.80; H, 3.61; N, 9.23.

3,9-Diaryl-2,3,8,9-tetrahydro-isoxazolo[4′,5′:4,5]pyrrolo[2,3-d]isoxazolo-[5′,4′:4,5]thiazolo[3,2-a]pyrimidin-10-(6H)-one (8a-c): General procedure

A mixture of **7a** (3.99 g, 10 mmol), **7b** (4.68 g, 10 mmol), or **7c** (4.59 g, 10 mmol), hydroxylamine hydrochloride (1.39 g, 20 mmol) and anhydrous sodium acetate (0.82 g, 10 mmol) was stirred under reflux in glacial acetic acid (30 mL) for 5 h. The reaction mixture was allowed to cool to room temperature and poured into cold water (100 mL). The deposited precipitate was filtered off, dried and crystallized from appropriate solvent to give **8a-c**, respectively.

3,9-Diphenyl-2,3,8,9-tetrahydro-isoxazolo[4′,5′:4,5]pyrrolo[2,3-d]isoxazolo[5′,4′:4,5]thiazolo[3,2-a]pyrimidin-10-(6H)-one (8a)

Crystallization from dimethylformamide, brown powder (85%); mp 345-347 °C (dec.). ^{1}H NMR (DMSO-d_6) δ 4.22 (d, 1H, isoxazolo proton), 5.35 (d, 1H, isoxazolo proton), 7.42-7.82 (m, 10H, phenyl), 8.09 (brs., NH, pyrrole proton, D_2O exchangeable), 11.15 (brs., NH, isoxazolo proton, D_2O exchangeable), 11.58 (brs., NH, isoxazolo proton, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ 62.60 (C_9), 65.32 (C_3), 112.38 (C_{3a}), 114.01 (C_{9b}), 114.67 (C_{6a}), 116.98 (9a), 120.30 (C_{5a}), 122.50 (C_4, Ar), 122.90 (C_4, Ar), 128.20 (2C, C_3, C_5, Ar), 128.80 (2C, C_3, C_5, Ar), 129.10 (2C, C_2, C_6, Ar), 129.35 (2C, C_2, C_6, Ar), 135.80 (C_1, Ar), 136.32 (C_1, Ar), 149.90 (C_{11a}), 158.78 (C_{4a}), 166.60 (C_{10}); MS (70 ev, %) *m/z* 431 (M^+ +2, 18.9%), 430 (M^+ +1, 22.6%), 429 (M^+, 50.3%), 352 (7.3%, M^+-$C_6H_5^{\cdot}$), 324 (12.5%, M^+-$C_7H_7N^{\cdot}$), 308 (10.2%, M^+-$C_7H_7NO^{\cdot}$), 121 (100%, C_7H_7NO). Anal. Calc. for $C_{22}H_{15}N_5O_3S$ (429.45): C, 61.53; H, 3.52; N, 16.31; S, 7.47. Found: C, 61.60; H, 3.45; N, 16.25; S, 7.50.

3,9-Di(4-chlorophenyl)-2,3,8,9-tetrahydro-isoxazolo-[4′,5′:4,5]pyrrolo[2,3-d]isoxazolo[5′,4′:4,5]thiazolo[3,2-a]pyrimidin-10-(6H)-one (8b)

Crystallization from dioxane, yellow crystals (83%); mp >350 °C (dec.). ^{1}H NMR (DMSO-d_6) δ 4.20 (d, 1H, J = 6.51 Hz, isoxazolo proton), 5.30 (d, 1H, J = 6.55 Hz, isoxazolo proton), 7.42 (d, 2H, J = 8.40 Hz, 4-Cl-phenyl), 7.45 (d, 2H, J = 8.42 Hz, 4-Cl-phenyl), 7.48 (d, 2H, J = 8.41 Hz, 4-Cl-phenyl), 7.52 (d, 2H, J = 8.39 Hz, 4-Cl-phenyl), 8.10 (brs., NH pyrrole proton, D_2O exchangeable), 11.10 (brs., NH, isoxazolo proton, D_2O exchangeable), 11.47 (brs., NH, isoxazolo proton, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ 60.10 (C_9), 66.05 (C_3), 111.97 (C_{3a}), 114.25

(C_{9b}), 116.53 (C_{6a}), 118.82 (C_{9a}), 121.06 (C_{5a}), 126.30 (2C, C_2, C_6, Ar), 126.38 (2C, C_2, C_6, Ar), 129.40 (2C, C_3, C_5, Ar), 129.49 (2C, C_3, C_5, Ar), 130.80 (C_4, Ar), 131.20 (C_4, Ar), 137.85 (C_1, Ar), 138.10 (C_1, Ar), 149.20 (C_{11a}), 159.05 (C_{4a}), 164.93 (C_{10}); MS (70 ev, %) *m/z* 502 (M^+ +4, 16.9%), 500 (M^+ +2, 30.5%), 498 (M^+, 53.1%), 386 (25.6%, M^+-$C_6H_4Cl^-$), 341 (8.5%, M^+-$C_7H_6ClNO^-$), 111(100%, C_6H_4Cl). Anal. Calc. for $C_{22}H_{13}Cl_2N_5O_3S$ (498.34): C, 53.02; H, 2.63; N, 14.05; S, 6.43. Found: C, 53.15; H, 2.70; N, 14.15; S, 6.50.

3,9-Di(4-methoxyphenyl)-2,3,8,9-tetrahydro-isoxazolo-[4′,5′:4,5]pyrrolo[2,3-d]isoxazolo[5′,4′:4,5]thiazolo[3,2-a]pyrimidin-10-(6H)-one (8c)

Crystallization from dioxane, yellow crystals (81%); mp >350 °C (dec.). ^{1}H NMR (DMSO-d_6) δ 3.83 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 4.21 (d, 1H, J = 6.50 Hz, isoxazolo proton), 5.33 (d, 1H, J = 6.53 Hz, isoxazolo proton), 7.12 (d, 2H, J = 8.41 Hz, 4-methoxyphenyl), 7.17 (d, 2H, J = 8.39 Hz, 4-methoxyphenyl), 7.24 (d, 2H, J = 8.35 Hz, 4-methoxyphenyl), 7.29 (d, 2H, J = 8.40 Hz, 4-methoxyphenyl), 8.12 (brs., NH pyrrole proton, D_2O exchangeable), 11.14 (brs., NH, isoxazolo proton, D_2O exchangeable), 11.52 (brs., NH, isoxazolo proton, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ 56.71 (C, OCH_3), 57.31 (C, OCH_3), 60.31 (C_9), 63.52 (C_3), 111.72 (C_{3a}), 113.99 (2C, C_3, C_5, Ar), 114.21 (2C, C_3, C_5, Ar), 115.32 (C_{9b}), 116.27 (C_{6a}), 118.55 (C_{9a}), 119.36 (C_{5a}), 124.61 (2C, C_2, C_6, Ar), 126.30 (2C, C_2, C_6, Ar), 128.51 (C_1, Ar), 129.45 (C_1, Ar), 149.61 (C_{11a}), 159.31 (C_4, Ar), 160.03 (C_4, Ar), 163.52 (C_{4a}), 165.69 (C_{10}); MS (70 ev, %) *m/z* 491 (M^+ +2, 23.5%), 490 (M^+ +1, 24.7%), 489 (M^+, 68.5%), 461 (54.6%, M^+), 458 (100%, M^+-$OCH_3^·$), 382 (8.5%, M^+-$C_7H_7O^·$). Anal. Calc. for $C_{24}H_{19}N_5O_5S$ (489.50): C, 58.89; H, 3.91; N, 14.31. Found: C, 58.94; H, 3.85; N, 14.25.

PHARMACOLOGICAL SCREENING

Ehrlich cells

Ehrlich cells (Ehrlich ascites carcinoma, EAC) were derived from ascetic fluid from diseased mice (the cells were purchased from the National Cancer institute, Cairo, Egypt).

Antioxidant activity screening assay for erythrocyte hemolysis

The blood was obtained from rats by cardiac puncture and collected in heparinized tubes. Erythrocytes were separated from plasma and the buffy coat was washed three times with 10 volumes of 0.15 M NaCl. During the last wash, the erythrocytes were centrifuged at 2500 rev./min for 10 min to obtain a constantly packed cell preparation. Erythrocyte hemolysis was mediated by peroxyl radicals in this assay system.[20,25] A 10% suspension of erythrocytes in phosphate buffered saline pH 7.4 (PBS) was added to the same volume of 200 mM AAPH solution in PBS containing samples to be tested at different concentrations. The reaction mixture was shaken gently while being incubated at 37 ºC for 2 h. The reaction mixture was then removed, diluted with eight volumes of PBS and centrifuged at 805 × g for 10 min. The absorbance of the supernatant was read at 540 nm. Similarly, the reaction mixture was treated with 8 volumes of distilled water to achieve complete hemolysis, and the absorbance of the supernatant obtained after centrifugation was measured at 540 nm. The data percentage hemolysis was expressed as mean ± standard deviation. L-ascorbic acid was used as a positive control.

Antioxidant activity screening assay (ABTS) method

For each of the investigated compounds (2 mL) of ABTS solution (60 μM) was added to 3 M MnO_2 solution (25 mg/mL) all prepared in phosphate buffer (pH 7, 0.1 M). The mixture was shaken, centrifuged, filtered and the absorbance of the resulting green-blue solution (ABTS radical solution) at λ 734 nm was adjusted to approx. ca. 0.5. Then, 50 μL of (2 mM) solution of the test compound in spectroscopic grade MeOH/phosphate buffer (1:1) was added. The absorbance was measured and the reduction in color intensity was expressed as inhibition percentage. L-ascorbic acid was used as standard antioxidant (positive control). Blank sample was run without ABTS and using MeOH/phosphate buffer (1:1) instead of sample. Negative control was run with MeOH/phosphate buffer (1:1) instead of test compound.[17,21,22,25]

Bleomycin-dependent DNA damage

The assay was done according to Aeschlach [23,25] with minor modifications. The reaction mixture (0.5 mL) contained calf thymus DNA (0.5 mg/mL), bleomycin sulfate (0.05 mg/mL), $MgCl_2$ (5 mM), $FeCl_3$ (50 μM) and samples to be tested at different concentrations. L-ascorbic acid was used as a positive control. The mixture was incubated at 37 ºC for 1 h. The reaction was terminated by addition of 0.05 mL EDTA (0.1 M). The color was developed by adding 0.5 mL TBA (1%, w/v) and 0.5 mL HCl (25%, v/v) followed by

heating at 37 ºC for 15 min. After centrifugation, the extent of DNA damage was measured by increase in absorbance at 532 nm.

Antitumor activity using Ehrlich ascites in vitro assay

Different concentrations of the tested compounds were prepared (100, 50 and 25 g/mL DMSO). Ascites fluid from the peritoneal cavity of the donor animal (contains Ehrlich cells) was aseptically aspirated. The cells were grown partly floating and partly attached in a suspension culture in RPMI 1640 medium, supplemented with 10% fetal bovine serum. They were maintained at 37 ^{o}C in a humidified atmosphere with 5% CO_2 for 2 hrs. The viability of the cells determined by the microscopical examination using a hemocytometer and using trypan blue stain (stains only the dead cells).[24,25]

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