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SYNTHESIS OF NEW FUNCTIONALIZED 3-SUBSITITUTED [1,2,4]TRIAZOLO [4,3-*a*]PYRIMIDINE DREIVATIVES: POTENTIAL ANTIHYPERTENSIVE AGENTS

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Abstract: A convenient synthesis of a series of thiosemicarbazide, 1,3,4-oxadiazole, 1,3,4-thiadiazole, thiazole, 1,2,4-triazole, pyrazole and dioxoisoindoline derivatives incorporating 1,2,4-triazolo[4,3-*a*]pyrimidine *via* the reaction of the readily accessible 1,5-dihydro-5-oxo-1,7-diphenyl-1,2,4-triazolo[4,3-*a*]pyrimidine-3-carbohydrazide (**2**) with the appropriate reagents is described. The newly synthesized compounds were found to possess antihypertensive and diuretic activities compared to captopril and furosemide as reference controls, respectively.

Keywords: triazolo[4,3-*a*]pyrimidine, thiosemicarbazide, 1,3,4-oxadiazole, 1,3,4-thiadiazole, thiazole, dioxoisoindoline, antihypertensive, diuretic activities

Hypertension described as a 'silent killer' increases the incidence of cardiovascular diseases. Blockers have been widely used since more than four decades for the treatment of hypertension. However, these drugs are contraindicated in asthmatics and diabetics (1, 2), so we need continuously to test new compounds as antihypertensive drugs to improve the therapeutic variety. Various derivatives of 1,2,4-triazolo[4,3-*a*]pyrimidine were reported to be useful as calcium-channel-blocking vasodilators, some have antihypertensive (3), cardiovascular (4, 5) and anxiolytic (6) activities. Furthermore, several other publications have also pointed out the biological importance of oxadiazole, thiazole and thiadiazole derivatives (7–9). In view of these reports and in continuation of our interest in the synthesis of a variety of heterocyclic systems for biological evaluation (10–15), we describe herein a facile synthesis of some new 1,2,4-triazolo[4,3-a]pyrimidine derivatives substituted at position-3 with thiosemicarbazide, 1,3,4-oxadiazole, 1,3,4-thiadiazole, thiazole, 1,2,4-triazole, pyrazole and dioxoisoindoline derivatives (that contain cyclic and/or opened amide moieties) using ethyl 1,5-dihydro-5-oxo-1,7-diphenyl-1,2,4-triazolo[4,3-*a*]pyrimidine-3-carboxylate (**1**) as a starting material (14). The synthesized compounds were evaluated for their antihypertensive and diuretic activities compared to captopril and furosemide as reference controls, respectively.

EXPERIMENTAL

All melting points were measured on a Gallenkamp melting point apparatus. The infrared spectra were recorded on a Pye Unicam SP 3-300 and Shimadzu FT IR 8101 PC infrared spectrophotometers. The ^{1}H NMR spectra were recorded in dimethyl sulfoxide (DMSO-d_6) at 300 MHz on a Varian Mercury VXR-300 NMR spectrometer. Chemical shifts are related to that of the solvent. Mass spectra were recorded on a Shimadzu GCMS-QP 1000 EX mass spectrometer at 70 eV. Elemental analyses were carried out at the Micro-analytical Center of Cairo University, Giza, Egypt. The starting ethyl 1,5-dihydro-5-oxo-1,7-diphenyl-1,2,4-triazolo[4,3-*a*]pyrimidine-3-carboxylate (**1**) was prepared as previously reported (14).

1,5-Dihydro-5-oxo-1,7-diphenyl-1,2,4-triazolo[4,3-*a*]pyrimidine-3-carbohydrazide (**2**)

A mixture of hydrazine hydrate 80% (6 mL) and ethyl 1,2,4-triazolo[4,3-*a*]pyrimidine-3-car-

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boxylate **1** (5 g, 13.8 mmol) in absolute ethanol (20 mL) was stirred at room temperature for 10 h. The white solid precipitate was collected by filtration, washed with ethanol, dried and crystallized from ethanol to give white crystals of the corresponding acid hydrazide **2**. Yield 96 %; m.p. 235-237°C. IR (KBr, cm^{-1}): 3310, 3237, 3112 (NH, NH_2), 1699, 1674 (2 C=O). ^{1}H NMR (DMSO-d_6, δ, ppm): 4.49 (s, 2H, NH_2 D_2O- exchangeable), 6.83 (s, 1H, pyrimidine H), 7.54–8.28 (m, 10H, ArH), 10.62 (s, 1H, NH D_2O-exchangeable). MS: *m*/*z* (%): 347 (M^+ +1, 23), 346 (M^+, 68), 315 (17), 77 (100). Analysis: for $C_{18}H_{14}N_6O_2$ (346.35) calcd.: C, 62.42; H, 4.07; N, 24.27%; found: C, 62.30; H, 4.10; N, 24.20%.

1-(1,5-Dihydro-5-oxo-1,7-diphenyl-1,2,4-triazolo[4,3-*a*]pyrimidine-3-carbonyl)-thiosemicarbazide (**3**)

A mixture of **2** (2.1 g, 6 mmol), potassium thiocyanate (0.87 g, 9 mmol) and hydrochloric acid (3 mL) in methanol (20 mL), was refluxed for 3 h with stirring. The reaction mixture was evaporated under reduced pressure and the residue was heated in methanol (50 mL) for 1 h. The obtained solid was filtered off, washed with water, dried and crystallized from ethanol to afford colorless crystals of **3**. Yield 78%; m.p.: 240–242°C. IR (KBr, cm^{-1}): 3334, 3278, 3206, 3120 (2 NH, NH_2), 1680, 1670 (2 C=O). ^{1}H NMR (DMSO-d_6, δ, ppm): 4.1 (s, br, 2H, NH_2, D_2O-exchangeable), 6.74 (s, 1H, pyrimidine H) 7.55–8.21 (m, 10H, ArH), 9.85, 11.33 (s, br, 2H, 2NH, D_2O-exchangeable). MS: *m*/*z* (%): 405 (M^+, 14), 287 (20), 218 (100), 77 (74). Analysis: for $C_{19}H_{15}N_7O_2S$ (405.44) calcd.: C, 56.29; H, 3.73; N, 24.18; S,7.91%; found: C, 56.35; H, 3.80; N, 24.10; S, 7.95%.

3-(5-Mercapto-4*H*-1,2,4-triazol-3-yl)-1,7-diphenyl-1,2,4-triazolo[4,3-*a*]pyimidin-5(1*H*)-one (**4**)

A suspension of **3** (0.4 g, 1 mmol) in potassium hydroxide solution (10 mL, 7%) was refluxed for 3 h. The reaction mixture was cooled then acidified with 10% hydrochloric acid to pH 6. The solid that formed was filtered off, washed with water, dried and finally crystallized from ethanol to afford white solid of **4**. Yield 68%; m.p. 295–296°C. IR (KBr, cm^{-1}): 3124, 3100 (2NH), 1656 (C=O). ^{1}H NMR (DMSO-d_6, δ, ppm): 6.47 (s, 1H, pyrimidine H), 7.13–8.08 (m, 10H, ArH), 9.60, 9.71 (s, 2H, 2NH D_2O-exchangeable). MS: *m*/*z* (%): 387 (M^+, 11), 354 (25), 259 (14), 186 (18), 144 (15), 77 (100). Analysis: for $C_{19}H_{13}N_7$ OS (387.43) calcd.: C, 58.90; H, 3.38; N, 25.31; S, 8.28%; found: C, 58.95; H, 3.48; N, 25.38; S, 8.35%.

3-(5-Amino-1,3,4-oxadiazol-2-yl)-1,7-diphenyl-1,2,4-triazolo[4,3-*a*]pyrimidin-5-(*1H*)-one (**5**)

A solution of **3** (0.4 g, 1 mmol) in pyridine (10 mL) was refluxed with stirring till evolution of hydrogen sulfide ceased. The reaction mixture was left overnight at room temperature and poured into cold water. The formed precipitate was filtered off, washed with water, dried and finally crystallized from ethanol to afford white solid of **5**. Yield 78%; m.p. 263–365°C. IR (KBr, cm^{-1}): 3201, 3100 (NH_2), 1680 (C=O). ^{1}H NMR (DMSO-d_6, δ, ppm): 4.23 (s, br, 2H, NH_2 D_2O-exchangeable), 6.73 (s, 1H, pyrimidine H), 7.60–8.28 (m, 10H, ArH). MS: *m*/*z* (%): 371 (M^+, 80), 347 (20), 256 (32), 180 (46), 77 (100). Analysis: for $C_{19}H_{13}N_7O_2$ (371.36) calcd.: C, 61.45; H, 3.53; N, 26.40%; found: C, 61.50; H, 3.48; N, 26.45%.

1,7-Diphenyl-3-(5-amino-1,3,4-thiadiazol-2-yl)-1,2,4-triazolo[4,3-*a*]pyrimidin-5-(1*H*)-one **(6)**

A solution of **3** (0.4 g, 1 mmol) in conc. sulfuric acid (5 mL) was stirred at room temperature for 4 h. The reaction mixture was poured into cold water. The obtained solid was collected by filtration, washed with water, dried and crystallized from DMF to give green solid of **6**. Yield 75 %; m.p. 150–152°C. IR (KBr, cm^{-1}): 3277, 3198 (NH_2), 1678 (C=O). MS: *m*/*z* (%): 387 (M^+, 17), 312 (15), 287 (23), 129 (38), 77 (100). Analysis: for $C_{19}H_{13}N_7OS$ (387.43) calcd.: C, 58.90; H, 3.38; N, 25.31; S, 8.28%; found: C, 59.00; H, 3.42; N, 25.25; S, 8.14%.

1-(1,5-Dihydro-5-oxo-1,7-diphenyl-1,2,4-triazolo[4,3-*a*]pyrimidin-3-carbonyl)-phenylthiosemicarbazide (**7**)

A mixture of **2** (3.46, 10 mmol) and phenyl isothiocyanate (1.35 g, 10 mmol) in absolute ethanol (40 mL) was refluxed for 3 h. After cooling, the formed solid was filtered off and crystallized from ethanol/DMF to give fine white crystals of phenyl thiosemicarbazide **7.** Yield 85%; m.p. 259–261°C. IR (KBr, cm^{-1}): 3275, 3228, 3116 (3NH), 1683, 1677 (2C=O). ^{1}H NMR (DMSO-d_6, δ, ppm): 6.74 (s, 1H, pyrimidine H) 7.55–8.21 (m, 15H, ArH), 7.93, 8.74, 11.34 (s, 3H, 3NH D_2O-exchangeable). MS: *m*/*z* (%): 481 (M^+, 15), 463 (20), 312 (15), 77 (100). Analysis: for $C_{25}H_{19}N_7O_2S$ (481.54) calcd.: C, 62.36; H, 3.98; N, 20.36; S, 6.66%; found: C, 62.40; H, 3.91; N, 20.26; S, 6.60%.

1,7-Diphenyl-3-(5-phenylamino-1,3,4-thiadiazol-2-yl)-1,2,4-triazolo[4,3-*a*]pyrimidin-5-(1*H*)-one (**8**)

A solution of **7** (0.48 g, 1 mmol) in conc. sulfuric acid (5 mL) was stirred for 4 h at room tem-

perature. The reaction mixture was poured into cold water; the formed precipitate was filtered off, washed with water, dried and crystallized from ethanol to give green solid of **8**. Yield 75%; m.p. 285–287°C. IR (KBr, cm^{-1}): 3190 (NH), 1696 (C=O)**.** MS: *m*/*z* (%): 463 (M^+, 100), 312 (15), 233 (23), 128 (38), 77 (73). Analysis: for $C_{25}H_{17}N_7OS$ (463.52) calcd.: C, 64.78; H, 3.70; N 21.15; S, 6.92%; found: C, 64.83; H, 3.82; N, 21.10; S, 7.00%.

1,7-Diphenyl-3-(5-phenylamino-1,3,4-oxadiazol-2-yl)-1,2,4-triazolo[4,3-*a*]pyrimidin-5-(1*H*)-one (**9**)

A solution of **7** (0.48 g, 1 mmol) in dry pyridine (5 mL) was refluxed for 12 h. The reaction mixture was left overnight at room temperature and poured into cold water. The formed precipitate was collected by filtration, washed with water, dried and finally crystallized from ethanol to give white crystals of **9**. Yield 78%; m.p. 230–232°C. IR (KBr, cm^{-1}): 3220 (NH), 1684 (C=O). ^{1}H NMR (DMSO-d_6, δ, ppm): 6.68 (s, 1H, pyrimidine H), 7.60–8.28 (m, 15H, ArH), 8.11 (s, br, 1H, NH- D_2O-exchangeable). MS: *m*/*z* (%): 447 (M^+, 25), 345 (95), 302 (50), 260 (40), 91 (100), 77 (55). Analysis: for $C_{25}H_{17}N_7O_2$ (447.45) calcd.: C, 67.11; H, 3.83; N, 21.91%; found: C, 67.20; H, 3.89; N, 21.81%.

3-(5-Mercapto-4-phenyl-4*H*-1,2,4-triazol-3-yl)-1,7-diphenyl-1,2,4-triazolo[4,3-*a*]pyrimidin-5-(1*H*)-one (**10**)

A solution of **7** (4.81 g, 10 mmol) in potassium hydroxide solution (5%, 25 mL) was refluxed for 3 h. The resulting solution was treated with charcoal, filtered and cooled. The filtrate was acidified with hydrochloric acid to pH 5–6 and the formed solid was filtered off, dried, and finally crystallized from ethanol/dioxane to afford yellow crystals of **10**. Yield 85%; m.p. 280–281°C. IR (KBr, cm^{-1}): 3256 (NH), 1696 (C=O). ^{1}H NMR (DMSO-d_6, δ, ppm): 6.70 (s, 1H, pyrimidine-H) 7.40–8.21(m, 15 H, ArH), 13.10 (s, br, 1H, NH D_2O-exchangeable). MS: *m*/*z* (%): 463 (M^+, 47), 312 (5), 233 (36), 128 (33), 77 (100). Analysis: for $C_{25}H_{17}N_7OS$ (463.52) calcd.: C, 64.78; H, 3.70; N, 21.15; S, 6.92%; found: C, 64.70; H, 3.78; N, 21.05; S, 6.88%.

3-(5-Methylthio-4-phenyl-4*H*-1,2,4-triazol-3-yl)-1,7-diphenyl-1,2,4-triazolo[4,3-*a*]pyrimidin-5-(1*H*)-one (**11**)

To a solution of compound **10** (2.3 g, 5 mmol) in ethanolic solution of sodium ethoxide [prepared from sodium metal (0.11 g, 5 mg-atom) in ethanol (20 mL)], methyl iodide (0.71 g, 5 mmol) was added with stirring at room temperature. The reaction mixture was refluxed for 2 h. The solution was concentrated, cooled, diluted with water and left to stand overnight. The obtained precipitate was filtered off, washed with water, dried and crystallized from ethanol to afford white crystals of **11**. Yield 70%; m.p. 236–237°C. IR (KBr, cm^{-1}): 1695 (C=O)**.** ^{1}H NMR (DMSO-d_6, δ, ppm): 2.45 (s, 3H, SCH_3), 6.78 (s, pyrimidine H), 7.31–7.65 (m, 15H, ArH). MS: *m*/*z* (%): 477 (M^+, 16), 401 (14), 77 (100). Analysis: for $C_{26}H_{19}N_7OS$ (477.55) **c**alcd.: C, 65.39; H, 4.01; N, 20.53; S, 6.71%; found: C, 65.41; H, 4.09; N, 20.43; S, 6.78%.

3-(5-Hydrazino-4-phenyl-4*H*-1,2,4-triazol-3-yl)-1,7-diphenyl-1,2,4-triazolo[4,3-*a*]pyrimidin-5-(1*H*)-one (**12**)

A mixture of **10** or **11** (3 mmol of each) and hydrazine hydrate (80%, 5 mL) was refluxed for 5 h. The reaction mixture was concentrated under reduced pressure, the solid obtained was filtered off, dried and crystallized from ethanol to afford white solid of **12**. Yield 65%; m.p 275–276°C. IR (KBr, cm^{-1}): 3280 3200, 3120 (NH_2, NH), 1679 (C=O)**.** ^{1}H NMR (DMSO-d_6, δ, ppm): 4.49 (s, br, 2H, NH_2, D_2O-exchangeable), 6.67 (s, 1H, pyrimidine H), 7.21–7.43 (m, 15H, ArH), 9.65 (s.1H, NH, D_2O-exchangeable). MS: *m*/*z* (%): 461 (M^+, 50), 286 (36), 77 (100). Analysis: for $C_{25}H_{19}N_9O$ (461.49) calcd.: C, 65.07; H, 4.15; N, 27.32%; found: C, 65.11; H, 4.09; N, 27.43%.

1,5-Dihydro-5-oxo-1,7-diphenyl-*N*-(3,4-diphenyl-3*H*-thiazol-*(2E)*-ylidene-1,2,4-triazolo[4,3-*a*]pyrimidin-3-carbohydrazide (**13**)

A mixture of **7** (0.48 g**,** 1 mmol) and phenacyl bromide (0.2 g, 1 mmol) in absolute ethanol (10 mL), and few drops of triethylamine as a catalyst, was heated under reflux for 3 h. After cooling, the obtained product was filtered off, washed with ethanol, dried and crystallized from ethanol to afford orange crystals of **13**. Yield 78%; m.p. 220–223°C. IR (KBr, cm^{-1}): 3128 (NH), 1689, 1665 (2C=O)**.** ^{1}H NMR (DMSO-d_6, δ, ppm): 6.54 (s, 1H, thiazole H), 6.81 (s, 1H, pyrimidine H), 7.21–8.23 (m, 20H, ArH), 11.85 (s, br, 1H, NH). MS: *m*/*z* (%): 581 (M^+, 14), 446 (31), 328 (17), 252 (42), 102 (100), 77 (52). Analysis: for $C_{33}H_{23}N_7O_2S$ (581.6) calcd.: C, 68.14; H, 3.99; N, 16.86; S, 5.51%; found: C, 68.20; H, 3.89; N, 16.80; S, 5.54%.

Potassium salt of thiosemicarbazide derivative (**14**)

To a cold solution of **2** (3.46 g, 10 mmol) in ethanol (25 mL) and potassium hydroxide (0.84 g,

15 mmol), carbon disulfide (1.14 g, 15 mmol) was added. The reaction mixture was stirred at room temperature for 8 h., and the precipitate formed was collected by filtration and washed with dry diethyl ether to give yellow solid of potassium salt **14** in 75% yield. The obtained potassium salt was used for the next reactions without further purification. IR (KBr, cm^{-1}): 3280, 3150 (2NH), 1695, 1675 (2C=O).

3-(5-Mercapto-1,3,4-oxadiazol-2-yl)-1,7-diphenyl-1,2,4-triazolo[4,3-*a*]pyrimidin-5(1*H*)-one (**15**)

Method A: A mixture of potassium hydroxide (0.84 g, 15 mmol), acid hydrazide **2** (3.46 g, 10 mmol) in ethanol (50 mL) and carbon disulfide (1.14 g, 15 mmol) was refluxed for 5 h. After cooling, the reaction mixture was diluted with water and acidified with hydrochloric acid to pH ~5. The precipitated solid was filtered off, washed with water, dried and crystallized with ethanol to give yellow crystals of **15** with 75% yield.

Method B: A solution of potassium hydroxide (0.84 g, 15 mmol) and potassium salt of the thiosemicarbazide **14** (4.60 g, 10 mmol) in absolute ethanol (50 mL) was refluxed for 4 h, diluted with water and acidified with HCl to pH ~5. The precipitated solid was filtered off, washed with water, dried and crystallized from ethanol to give **15**. Yield 90%; m.p. 230–231°C. IR (KBr, cm^{-1}): 3102 (NH), 1676 (C=O). MS: *m*/*z* (%): 388 (M^+, 40), 256 (17), 180 (41), 77 (100). Analysis: for $C_{19}H_{12}N_6O_2S$ (388.41) calcd.: C, 58.76; H, 3.11; N, 21.64; S, 8.26%; found: C, 58.80; H, 3.19; N, 21.52; S, 8.20%.

3-(5-Mercapto-4-amino-4*H*-[1,2,4]-triazol-3-yl)-1,7-diphenyl[1,2,4]triazolo[4,3-*a*]pyrimidin-5(1*H*)-one (**16**)

A solution of **14** or **15** (10 mmol of each) in hydrazine hydrate (5 mL) was refluxed for 3 h. The obtained solid was filtered off, washed with water, dried and crystallized from ethanol/DMF to give green solid of **16**. Yield 70%; m.p. 255–256°C. IR (KBr, cm^{-1}): 3301, 3217, 3177(NH_2, NH), 1679 (C=O). ^{1}H NMR (DMSO-d_6, δ, ppm): 4.50 (s, 2H, NH_2 D_2O-exchangeable), 6.58 (s, 1H, pyrimidine H), 7.37–7.56 (m, 10H, ArH), 12.30 (s, br, 1H, NH D_2O-exchangeable). MS: *m*/*z* (%) 402 (M^+, 14), 323 (32), 218 (100), 117 (23), 77 (74) Analysis: for $C_{19}H_{14}N_8OS$ (402.44) calcd.: C, 56.71; H, 3.51; N, 27.84; S, 7.97%; found: C, 56.75; H, 3.48; N, 27.78; S, 7.90%.

Reactions of acid hydrazide **2** with dicarbonyl compounds

To a mixture of acid hydrazide **2** (3.46 g, 10 mmol) and acetylacetone or ethyl acetoacetate (10 mmol) in ethanol (20 mL), one drop of piperidine was added. The reaction mixture was refluxed for 6 h., The formed precipitate was filtered off, dried and crystallized from dilute ethanol to afford compounds **17** or **18**.

3-(3,5-Dimethylpyrazole-1-carbonyl) 1,7-diphenyl-1,2,4-triazolo[4,3-*a*]pyrimidin-5-(*1H*)-one (**17**)

White solid, yield 67%; m.p. 223°C. IR (KBr, cm^{-1}): 1693, 1663 (2C=O). ^{1}H NMR (DMSO-d_6, δ, ppm): 1.95, 2.06 (s, 6H, $2CH_3$), 4.8 (s, 2H, pyrazole H), 6.78 (s, 1H, pyrimidine H), 7.37–7.56 (m, 10H ArH), 9.38 (s, 1H, NH D_2O-exchangeable). MS: *m*/*z* (%): 410 (M^+, 10), 371 (22), 315 (36), 287 (11), 77 (100). Analysis: for $C_{23}H_{18}N_6O_2$ (410.44) calcd.: C, 67.31; H, 4.42; N, 20.48%; found: C, 67.40; H, 4.45; N, 20.31%.

3-(3-Methylpyrazole-5-oxo-1-carbonyl)-1,7-diphenyl-1,2,4-triazolo[4,3-*a*]pyrimidin-5(*1H*)-one (**18**)

White solid, yield 78%; m.p. 180–181°C. IR (KBr, cm^{-1}): 1710, 1697 (3C=O). MS m/*z* (%): 412 (M^+, 10), 77 (100). Analysis: for $C_{22}H_{16}N_6O_3$ (412.41) calcd.: C, 64.07; H, 3.91; N, 20.38%; found: C, 64.12; H, 3.95; N, 20.42%.

Reactions of acid hydrazide **2** with carboxylic acid anhydride

General procedure

A mixture of **2** (0.34 g 1 mmol) and the appropriate carboxylic acid anhydride, namely phthalic anhydride or 2,3,4,5-tetrachlorophthalic anhydride (1 mmol) in glacial acetic acid (10 mL) was heated under reflux for 2 h. The reaction mixture was cooled, then poured into ice cold sodium bicarbonate solution. The separated product was filtered off, washed with water, dried and crystallized from ethanol/dioxane to afford the corresponding imide derivatives **19** and **20**, respectively.

1,5-Dihydro-5-oxo-*N*-(1,3-dioxoisoindolin-2-yl)-1,7-diphenyl-1,2,4-triazolo[4,3-*a*]pyrimidin-3-carboxamide (**19**)

White crystals, yield 85%; m.p. 213–214°C. IR (KBr, cm^{-1}): 3310 (NH), 1730, 1715, 1678 (3C=O). ^{1}H NMR (DMSO-d_6, δ, ppm): 6.95 (s, 1H, pyrimidine H) 7.63–8.68 (m, 14H, ArH), 12.95 (s, br, 1H, NH D_2O-exchangeable). MS: *m*/*z* (%): 476 (M^+, 10), 77 (100). Analysis: for $C_{26}H_{16}N_6O_4$ (476.46) calcd.: C, 65.54; H, 3.38; N, 17.64%; found: C, 65.60; H, 3.45; N, 17.50%.

N-(4,5,6,7-Tetrachloro-1,3-dioxoisoindolin-2-yl)-1,5-dihydro-5-oxo-(1,7-diphenyl-1,2,4-triazolo[4,3-*a*]pyrimidin-3-carboxamide (**20**)

White crystals, yield 85%; mp 283–285°C. IR (KBr, cm^{-1}): 3109 (NH), 1749, 1713, 1670 (3C=O).

Table 1. The relative antihypertensive potency of the tested compounds to captopril.

Compd. no.	Relative potency*	LD_{50} [mg/kg]**	ED_{50} [mg/kg]
2	6.17	345.76 ± 0.23	4.38 ± 0.013
3	5.87	254.76 ± 0.24	4.60 ± 0.012
4	5.55	231.11 ± 0.20	4.86 ± 0.011
7	6.58	234.78 ± 0.16	4.10 ± 0.009
8	1.36	456.76 ± 0.25	19.85 ± 0.008
10	4.24	234.76 ± 0.11	6.37 ± 0.006
11	4.47	433.77 ± 0.15	6.04 ± 0.005
14	5.05	345,87 ± 0.20	5.35 ± 0.002
15	5.28	321.11 ± 0.14	5.11 ± 0.003
16	1.00	543.54 ± 0.11	27.00 ± 0.002
18	4.58	112.56 ± 0.12	5.90 ± 0.001

* captopril = 1, ** captopril = 623.50 mg/kg

Table 2. The relative diuretic potency of the tested compound to furosemide.

Compd. no.	Relative potency*	ED_{50} [mg/kg]
2	20.14 ± 0.08	0.10 ± 0.00001
3	18.45 ± 0.10	0.11 ± 0.00001
4	16.61 ± 0.09	0.12 ± 0.00001
7	21.87 ± 0.08	0.09 ± 0.000001
8	2.12 ± 0.10	0.94 ± 0.0002
10	8.88 ± 0.11	0.23 ± 0.00003
11	9.09 ± 0.10	0.22 ± 0.00002
14	13.21 ± 0.12	0.15 ± 0.00001
15	15.00 ± 0.09	0.13 ± 0.00001
16	1.06 ± 0.11	1.89 ± 0.0002
18	11.23 ± 0.08	0.18 ± 0.0001

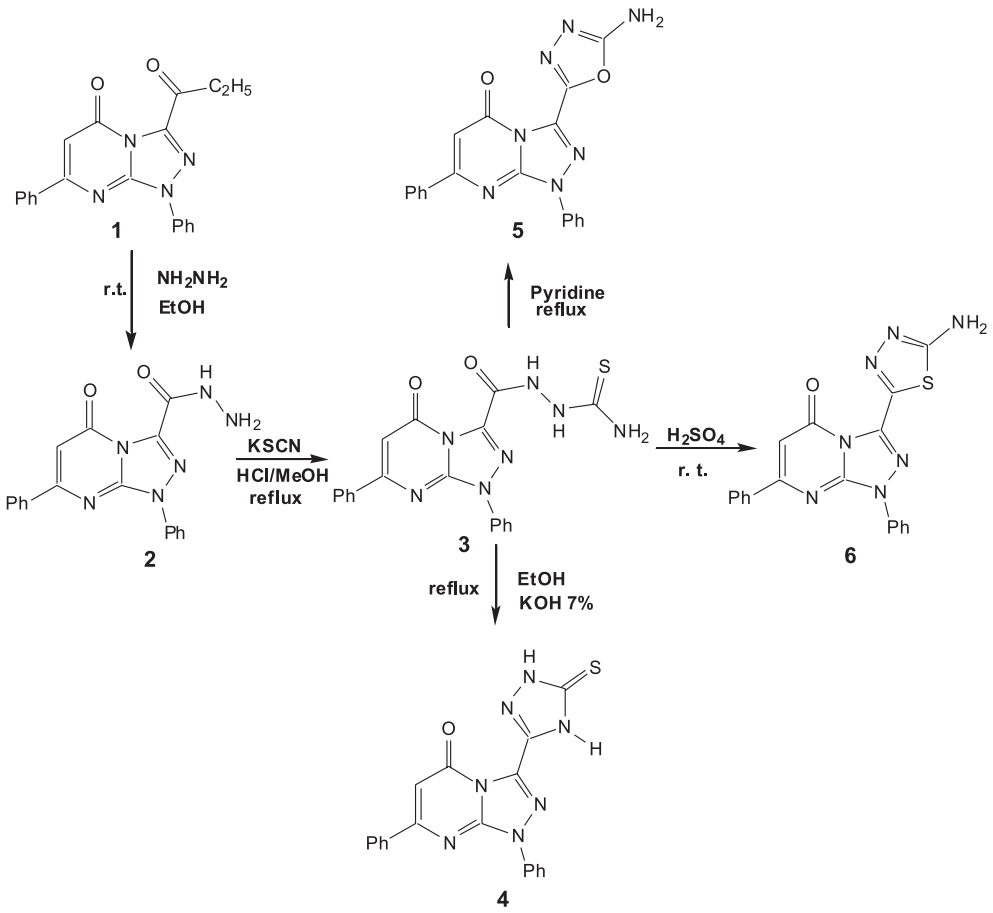
*furosemide = 1 (furosemide LD_{50} = 253 mg/kg)

^{1}H NMR (DMSO-d_6, δ, ppm): 6.94 (s, 1H, pyrimidine H), 7.62–8.26 (m, 10H, ArH), 12.93 (s, br, 1H, NH D_2O-exchangeable). MS: *m*/*z* (%): 614 (M^+, 10), 315 (100), 287 (36), 129 (21) 77 (22). Analysis: for $C_{26}H_{12}Cl_4N_6O_4$ (614.24) calcd.: C, 50.84; H, 1.97; N, 13.68%; found: C, 50.72; H, 1.85; N, 13.60%.

Biological testing

Antihypertensive activity

In this study, we used captopril (Sigma, St. Louis, MO), a known ACE inhibitor, as the positive control. Also, we used in this study 17- to 20-week-old male spontaneously hypertensive rats (SHR) weighing 300 to 350 g. These animals were obtained from Charles River Laboratories España S.A. (Barcelona, Spain). The rats remained at a temperature of 23°C with 12 h light/dark cycles, consumed tap water and a standard rat diet (A04; anlab, Barcelona, Spain) *ad libitum* during the experiments. Four different doses of the β-casein peptide (LHLPLP) (0.5, 1, 2, and 3 mg/kg) and 2 different doses of LVYPFPGPIPNSLPQNIPP (3 and 6 mg/kg) were administered to the SHR. High doses (10 mg/kg) of the remaining sequences were also administered to these animals. Each of the tested compounds (1 mmol/L of the corresponding water solution) was orally administered by gastric intubation between 09:00 and 10:00 h. To carry out similar experiments, captopril (50 mg/kg) served as the positive control and 1 mL of distilled water served as the negative control. The systolic blood pressure (SBP) and the diastolic blood pressure (DBP) of the



Scheme 1.

rats were measured by the tail cuff method before administration of the different products and also 2, 4, 6, 8, and 24 h after administration. The original method for measuring arterial blood pressure using the tail cuff (16) provides only SBP values, but the equipment used in this study is (5001; Leticia, Hospitalet, Barcelona, Spain) that have a high-sensitivity pulse transducer coupled with an accurate microprocessor program, which allowed us to distinguish between SBP and DBP. The indirect measurement of blood pressure with this equipment is basically sphygmomanometer and the process is the same as that used in blood pressure measurements in humans. Before the measurement, the rats were held at 30°C for 10 min to make the pulsations of the tail artery detectable, and the values of SBP and DBP were obtained by the average reading from 3 measurements.

All the experiments mentioned were performed as authorized for scientific research (European Directive 86/609/CEE and Royal Decree 223/1988) by the Spanish Ministry of Agriculture, Fisheries and Food. The antihypertensive activities of tested compounds are listed in Table 1.

Diuretic activity

The tested compounds were screened for diuretic activity by Kau method (17). 120 Swiss albino mice weighing between 20–25 g were divided into twelve groups, each group containing ten mice. The animals were placed in metabolic cages. Food and water were withdrawn 12 h prior to the experiment. Some of the synthesized compounds were administered orally to the animals of group IV–XII at a dose of 3 mg/kg body weigh), respectively. Negative control animals of group I were supplied with saline water containing 0.1% Tween-80. The positive control animals of group II and group III were given urea (500 mg/kg body weight) and standard diuretic drug furosemide (3 mg/kg body weight), respectively. The urinary output of each group was recorded at different time intervals

from the graduated urine chamber of the metabolic cage. After 4 h, the animals were taken out of the cage to measure the cumulated volume of urine excreted by each group. The diuretic activity of tested compounds is listed in Table 2.

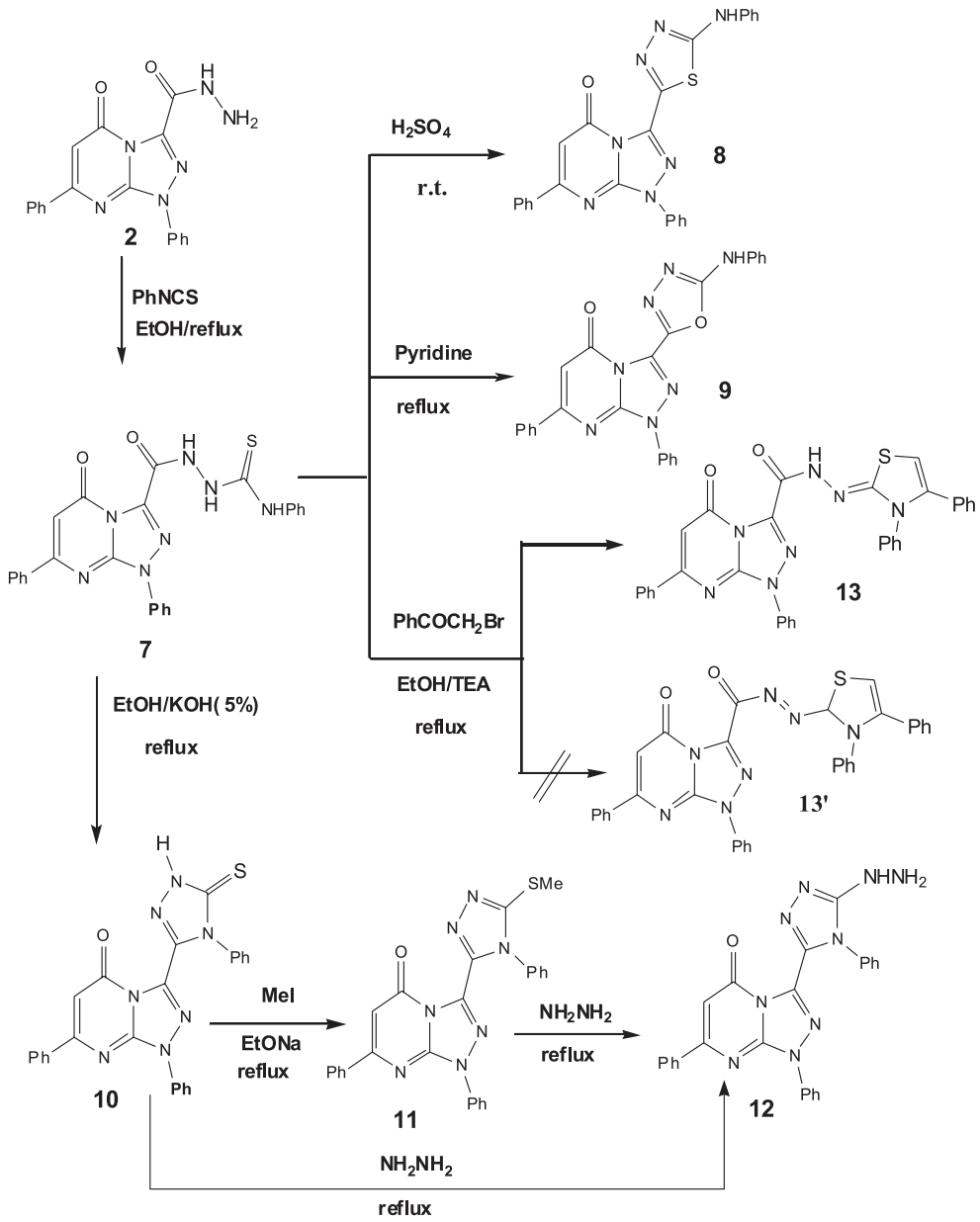
Determination of acute toxicity

LD_{50} values were determined by using male albino rats and injecting them with different increasing doses of agents. Doses that killed 50% of the tested animals were calculated according to Austen et al. (18) (Table 1).

RESULTS AND DISCUSSION

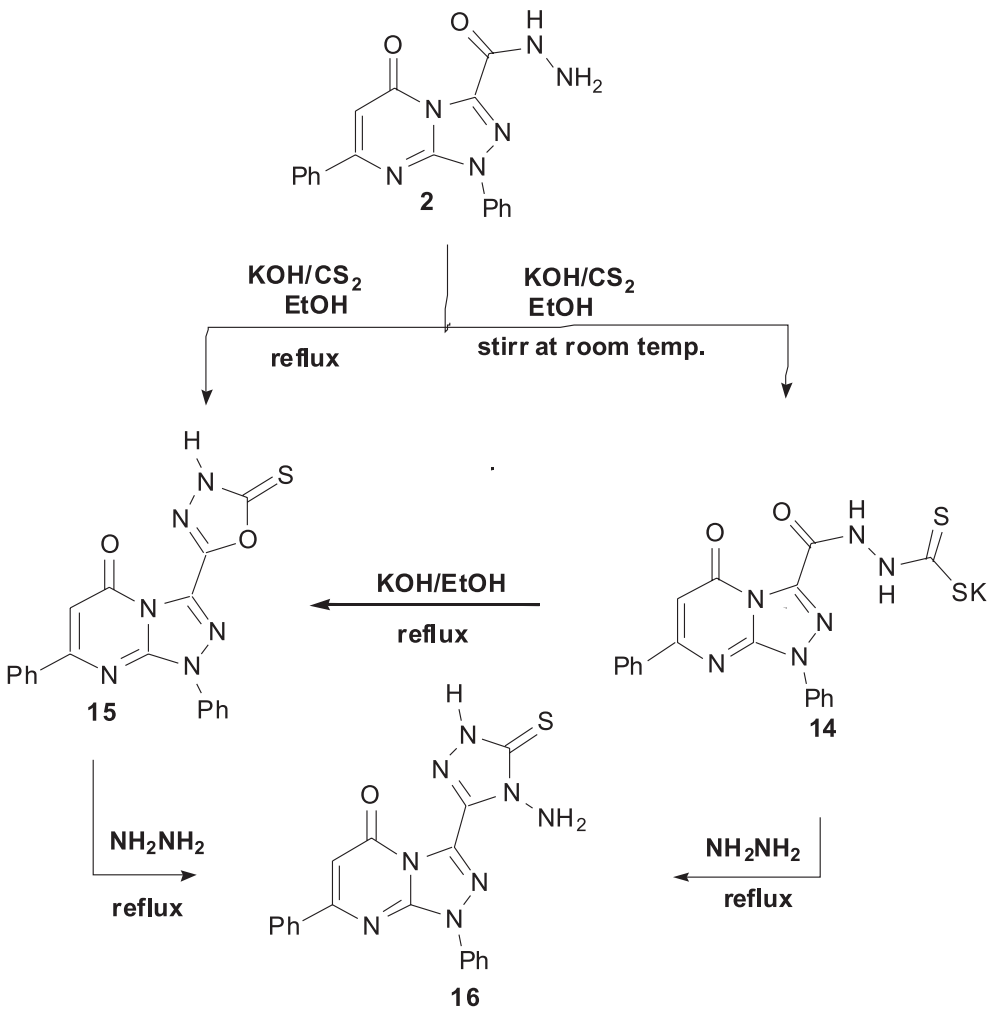
Chemistry

Acid hydrazide can be considered as useful intermediate for the formation of several heterocyclic compounds such 1,3,4-oxadiazoles, 1,2,4-triazoles and 1,3,4-thiadiazoles (19–22). Therefore,

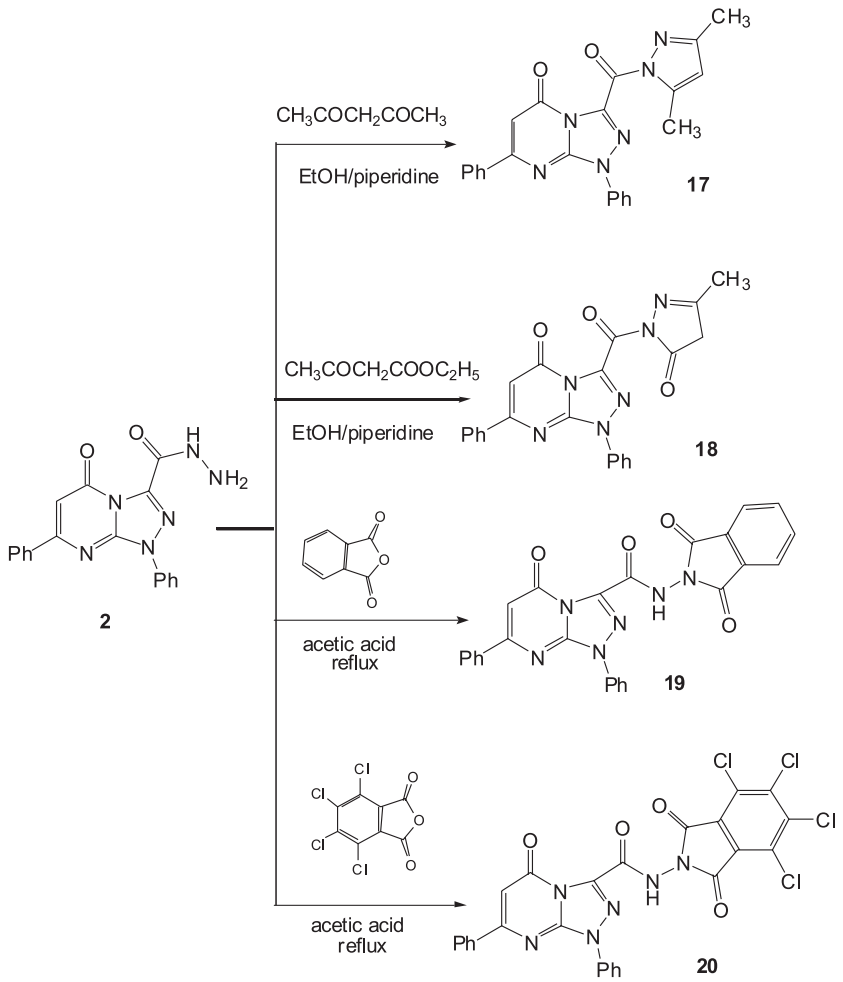


Scheme 2.

treatment of ethyl 1,5-dihydro-5-oxo-1,7-diphenyl-1,2,4-triazolo[4,3-*a*]pyrimidine-3-carboxylate (**1**) (14) with hydrazine hydrate, in refluxing ethanol, afforded the corresponding acid hydrazide **2** in close to quantitative yield. The ^{1}H NMR of acid hydrazide **2** revealed D_2O-exchangeable signals at δ 4.49 and 10.62 ppm due to NH_2 and NH protons, respectively. Treatment of the acid hydrazide **2** with potassium thiocyanate in refluxing methanol, in the presence of hydrochloric acid, afforded the 1-(1,5-dihydro-5-oxo-1,7-diphenyl-1,2,4-triazolo[4,3-*a*]pyrimidine-3-carbonyl)thiosemicarbazide (**3**). The ^{1}H NMR spectrum of **3** revealed D_2O-exchangeable signals at δ 4.1, 9.85 and 11.33 ppm due to NH_2 and 2 NH protons, respectively. Its mass spectrum revealed a molecular ion peak at *m*/*z* 405. Compound **3** was cyclized *via* oxidative cyclization in basic medium (7% KOH), under reflux condition with subsequent acidification to give 3-(5-mercapto-4*H*-1,2,4-triazol-3-yl)-1,7-diphenyl-1,2,4-triazolo[4,3-*a*]pyrimidin-5-(1*H*)-one (**4**). Further reflux of compound **3** with dry pyridine afforded a product identified as 3-(5-amino-1,3,4-oxadiazol-2-yl)-1,7-diphenyl-1,2,4-triazolo[4,3-*a*]pyrimidin-5-(1*H*)-one (**5**) (Scheme 1). Dehydrative cyclization of compound **3**, in the presence of conc. sulfuric acid, led to the corresponding 1,7-diphenyl-3-(5-amino-1,3,4-thiadiazol-2-yl)-1,2,4-triazolo[4,3-*a*]pyrimidin-5-(1*H*)-one (**6**), which was separated as green solid soluble with difficulty in most organic solvents (Scheme 1). The structure of the synthesized products **4**, **5** and **6** were established on the basis of elemental analysis and spectral data. For example, the absence of absorption band of carbonyl group attached to the triazole



Scheme 3.



Scheme 4.

moiety, in the IR spectra of compounds **4**–**6** (Scheme 1) confirmed the intramolecular cyclization of compound **3**. ^{1}H NMR spectrum of compound **4** revealed D_2O-exchangeable signals at δ 9.6 and 9.71 ppm due to 2 NH protons and its mass spectrum revealed a molecular ion peak at *m*/*z* 387. The ^{1}H NMR of compound **5** revealed D_2O-exchangeable signals at δ 4.23 ppm due to NH_2 protons. Its mass spectrum revealed a molecular ion peak at *m*/*z* 371.

Treatment of acid hydrazide **2** with phenyl isothiocyanate in refluxing ethanol, afforded a product identified as 1-(1,5-dihydro-5-oxo-1,7-diphenyl-1,2,4-triazolo[4,3-*a*]pyrimidine-3-carbonyl)phenyl-thiosemicarbazide (**7**). The latter product undergoes intramolecular cyclization when treated with sulfuric acid, dry pyridine and KOH (5%) to afford compounds **8**, **9** and **10**, respectively (Scheme 2). Treatment of (**10**) with methyl iodide in the presence of sodium ethoxide solution, afforded a product identified as 3-(5-methyl thio-4-phenyl-4*H*-1,2,4-triazol-3-yl)-1,7-diphenyl-1,2,4-triazolo[4,3-*a*]pyrimidin-5-(1*H*)-one (**11**). Treatment of compound **10** or **11** with hydrazine hydrate under reflux condition afforded the same product, identical in all respects (m.p. and spectral data) and identified as 3-(5-hydrazino-4-phenyl-4*H*-1,2,4-triazol-3-yl)-1,7-diphenyl-1,2,4-triazolo[4,3-*a*]pyrimidin-5(1*H*)-one (**12**) (Scheme 2). The ^{1}H NMR spectrum of compound **12** revealed D_2O-exchangeable signals at δ 4.49 and 9.69 ppm due to NH_2 and NH protons. Treatment of compound **7** with phenacyl bromide in refluxing ethanol, in the presence of triethylamine, afforded 1,5-dihydro-5-oxo-1,7-dipheny-l*N*-(3,4-

diphenyl-3*H*-thiazol-(2*E*)-ylidene-1,2,4-triazolo[4,3-*a*]pyrimidine-3-carbohydrazide (**13**) as shown in Scheme 2. The structure of compound **13** was confirmed and the alternative structure **13**' was excluded on the basis of elemental analysis and spectral data. For example, the ^{1}H NMR spectrum of compound **13** revealed singlet signals at δ 6.54, 11.85 ppm due to CH protons α to the sulfur atom of the thiazolidine ring and NH proton, respectively.

Treatment of acid hydrazide **2** with carbon disulfide in ethanol, in the presence of potassium hydroxide at room temperature, resulted in the formation of potassium salt **14**, which on treatment with ethanolic potassium hydroxide afforded a product identified as 3-(5-mercapto-1,3,4-oxadiazole-2-yl)-1,7-diphenyl-1,2,4-triazolo[4,3-*a*]pyrimidin-5(1*H*)-one (**15**) (Scheme 3). Formation of oxadiazole **15** can be discussed according to the related literature (16–18). Furthermore, treatment of potassium salt **14** with hydrazine hydrate, in refluxing ethanol, afforded the corresponding 3-(5-mercapto-4-amino-4*H*-1,2,4-triazol-3-yl)-1,7-diphenyl-1,2,4-triazolo[4,3-*a*]pyrimidin-5(1*H*)-one **16** (Scheme 3). It's ^{1}H NMR spectrum revealed two singlet signals (D_2O-exchangeable) at δ 4.50 and 12.30 ppm due to NH_2 and NH protons, respectively. In addition, 1,3,4-oxadiazole can be easily converted into the corresponding 4-amino-1,2,4-triazole under the action of hydrazine hydrate (20, 21).

Condensation reaction of acid hydrazide **2** with dicarbonyl compounds namely, acetylacetone and ethyl acetoacetate, in the presence of catalytic amount of piperidine afforded the corresponding substituted pyrazole derivatives 3-(3,5-dimethylpyrazole-1-carbonyl)-1,7-diphenyl-1,2,4-triazolo[4,3-*a*]pyrimidin-5(1*H*)-one (**17**) and 3-(3-methylpyrazole-5-oxo-1-carbonyl)-1,7-diphenyl-1,2,4-triazolo[4,3-*a*]pyrimidin-5(1*H*)-one (**18**), respectively (Scheme 4). The structure of compounds **17** and **18** were confirmed based on the related literature (22, 23) and spectral data of the synthesized products.

Finally, condensation of acid hydrazide **2** with acid anhydrides, namely phthalic anhydride and 2,3,4,5-tetrachlorophthalic anhydride, in refluxing glacial acetic acid, yielded the corresponding imides **19** and **20**, respectively (Scheme 4). The structures of the synthesized products **19** and **20** were established on the basis of elemental analysis and spectral data.

Biological activity

Antihypertensive activity

The acute toxicity of the tested compounds was assayed by the determination of their LD_{50} (Table 1). From the observed data (Table 1), it has been noticed that many of the newly tested compounds showed considerable antihypertensive activity

We used the angiotensin converting enzyme inhibitor ACEI model to investigate the antihypertensive effect of the newly syntesized compounds and using captopril as reference control standard against the activity of the tested compounds compared by determining the ED_{50} of both the tested compounds and captopril then determine the relative potency, which is the ratio of ED_{50} of captopril to ED_{50} of the tested compound. From the observed data (Table 1), it can be noticed that many of the newly tested compounds showed considerable antihypertensive activity This activity is arranged in a descending manner of activity relative to the reference drug captopril as **7**, **2**, **3**, **4**, **15**, **14**, **18**, **11**, **10**, **8** and **16**, respectively.

Diuretic activity

To assess the diuretic activity, the tested compounds were screened by KAU method (21). Determination of the urinary output as measuring tool for diuretic activity was done using furosemide as refrence controled standard against the activity of the tested compounds compared by determining the ED_{50} of both the tested compounds and furosemide then determine the relative potency, which is the ratio of ED_{50} of furosemide to ED_{50} of the tested compounds. All the tested compounds showed potent diuretic activity and they are arranged in a descending manner of activity to the reference drug furosemide as follows: **7**, **2**, **3**, **4**, **15**, **14**, **18**, **11**, **10**, **8** and **16,** respectively (Table 2).

CONCLUSION

The thiosemicarbazide and triazole thione moiety attached to triazolopyrimidine system is essential for both antihypertensive and diuretic effects. Compounds **2**, **3, 4** and **7** showed four of the highest observed activities. Thus, these compounds can be considered as new class and lead compounds in this field. Further studies are in progress on the same compounds to increase their activities and understand their QSAR. The overall results of the present study can be considered very promising in the perspective of new drugs discovery, with respect to the medical importance.

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