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Synthesis and Antitumor Screening of New 1,7-Diphenyl-3-(1,3-disubstituted-1H-pyrazole-4-carbonyl)-[1,2,4]triazolo[4,3-*a*]pyrimidin-5(1H)-ones

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A new series of 3-(1,3-disubstituted-1H-pyrazole-4-carbonyl)-1,7-diphenyl-[1,2,4]triazolo[4,3-*a*]pyrimidin-5(1H)-ones **4** was prepared by reaction of the enaminone **2** with hydrazonoyl halides **3**. The preliminary screening for antitumor activity of the synthesized compounds was carried out against *Ehrlich Ascites Carcinoma tumor cells*. The results revealed that the studied compounds **4** have low or no antitumor activity towards *EAC tumor cells*.

Key words : Enaminone, Hydrazonoyl halides, Pyrazole, [1,2,4]Triazolo[4,3-*a*]pyrimidine

INTRODUCTION

Various derivatives of 1,2,4-triazolo[4,3-*a*]pyrimidine-5(1H)-one were reported to be useful as calcium-channel-blocking vasodilators, some other have antihypertensive, cardiovascular and anxiolytic activities and others are used as components in photographic materials (Shawali et al., 2007; Barthelmy et al., 1985; Bru-Magniez et al., 1995; Albright et al., 1985; Nakamura et al., 1991). Also, many *N*-arylpyrazole derivatives were reported to have remarkable pharmacological activities as antimicrobial, hypoglycemic, tumor necrosis inhibitors, antithromboembolic disorder, antiangiogeric agents, A3 Adenosine receptor antagonist, neuropeptide Y Y5 receptor antagonists, kinase inhibitor for treatment of type 2 diabetes, hyperlipidemia and obesity, insecticides, thrombopiotinmimetics and anti-inflammatory (Abdel-Gawad et al., 2003; Maekawa et al., 2004; Mercep et al., 2004; Qiao et al., 2004; Baraldi et al., 2003; Stamford et al., 2004; Brown et al., 2004; Stevensons et al., 2004; Heerding, 2004; Cardia et al., 1998; Mullican et al., 1993). These findings attracted our attention towards the synthesis of the title compounds which incorporate both the pyrazole and 1,2,4-triazolo[4,3-*a*]pyrimidine rings and screen their antitumor activity against *Ehrlich Ascites Carcinoma* tumor cells. The latter type of cells were used in this study because they are the only tumor cells that grow in mice available in Egypt. The target compounds have not been reported hitherto. In continuation of our program aimed at exploring the synthetic potentialities of hydrazonoyl halides **3** (Shawali, 1983, 1993; Shawali et al., 1980, 1995, 1980, 2005, 2006, 2007), we investigated reactions of the latter with the enaminone **2** in an attempt to synthesize the target compounds. Although there are many reports dealing with the reactions of hydrazonoyl halides with various enaminones (Shawali et al., 2006), the reactions described herein have not been reported hitherto.

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MATERIALS AND METHODS

Melting points were determined on an electrothermal Gallenkamp apparatus. The IR spectra were recorded in KBr discs using Pye Unicam SP-1000 Spectrophotometer. ^{1}H NMR spectra were recorded in $CDCl_3$ and DMSO-d_6 using a Varian Em-200 MH*z* Spectrometer, and TMS as internal reference. Mass spectra were recorded on AEI MS 30 mass spectrometer operating at 70 eV. Elemental analyses were carried out by the Microanalytical Center of Cairo

University, Giza, Egypt. 3-Acetyl-1,7-diphenyl-[1,2,4] triazolo[4,3-*a*]pyrimidine-5(1H)-one **1** (Shawali et al., 2002) and hydrazonoyl halides **3** were prepared following literature procedures (Shawali et al., 2002; Shawali et al., 1971; Shawali et al., 1973; Wolkoff, 1975; Eweiss et al., 1980).

3-[3-(Dimethylamino)acryloyl]-1,7-diphenyl[1,2,4] triazolo-[4,3-*a*]pyrimidin-5(1H)-one (2)

A mixture of compound **1** (6.6 g, 0.02 mole) and DMF-DMA (2.68 g, 0.023 mole) in dry toluene (20 mL) was refluxed for 6 h then left to cool to room temperature. The precipitate was filtered off, washed with ether, dried and crystallized from ethanol to give the enaminone **2** as orange crystals (6.16 g, 80% yield), mp. 212°C. IR (KBr): ν_{max}/cm^{-1}1685, 1650; ^{1}H NMR ($CDCl_3$) 2.91 (s, 3H, N-CH_3), 3.06 (s, 3H, N-CH_3), 5.36 (d, *J* = 13 H*z*, 1H, =CH), 6.63 (s, 1H, pyrimidine H), 7.42-8.17 (m, 10H, ArH), 8.18 (d, *J* = 13 H*z*, 1H, =CH). MS: *m/z* (%) 385 (M^+, 10), 367 (22), 366 (12), 105 (3), 98 (100), 91 (3), 89 (7), 77 (26), 70 (16). Anal. Calcd for $C_{22}H_{19}N_5O_2$ (385.43) C, 68.56; H, 4.97; N, 18.17. Found: C, 68.38; H, 4.69; N, 18.00%.

1,7-Diphenyl-3-(1,3-disubstituted-1H-pyrazole-4-carbonyl)-[1,2,4]triazolo[4,3-*a*]pyrimidin-5(1H)-ones (4A-G)

General Procedure - To a stirred solution of the appropriate hydrazonoyl halide **3** (0.005 mol) and the enaminone **2** (1.93 g, 0.005 mole) in dioxane (20 mL) was added triethylamine (0.7 mL) dropwise with stirring at room temperature for 6 h. The precipitated triethylamine hydrochloride was filtered off and the filtrate was evaporated under reduced pressure. The residue was triturated with ethanol where the product precipitated. The latter was filtrered, and crystallized form EtOH/dioxane to give the respective product **4**. The compounds **4A-G** prepared are listed below together with their physical constants.

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

Yellow crystals (1.98g, 79%), m.p 249-250°C. IR (KBr): ν_{max}/cm^{-1}, 1705, 1689, 1665. ^{1}H NMR ($CDCl_3$) δ 2.57 (s, 3H, CH_3), 6.58 (s, 1H, pyrimidine H) 7.27–8.34 (m, 15H, ArH), 8.79 (s, 1H, pyrazole H). MS m/z (%) 502 (M^++ 2, 6) 501 (M^++1, 29), 500 (M^+, 81), 457 (27), 367 (16), 213 (100), 178 (24), 129 (22), 116 (12), 104 (25), 98 (34), 77 (80). Anal. Calcd. for $C_{29}H_{20}N_6O_3$ (500.52) C, 69.59; H, 4.03; N, 16.79. Found C, 69.81; H, 3.90; N, 16.77%.

3-[3-Acetyl-1-(4-methylphenyl)pyrazole-4-carbonyl]-1,7diphenyl-[1,2,4]triazolo[4,3-*a*]pyrimidin-5(1H)-one (4Ab)

Pale yellow crystals (1.95 g, 76%), m. p 200°C. IR (KBr): ν_{max}/cm^{-1} 1692, 1675, 1660. ^{1}H NMR ($CDCl_3$) δ 2.46 (s, 3H, CH_3), 2.56 (s, 3H, CH_3), 6.56 (s, 1H, pyrimidine H), 7.27-7.60 (m, 10H, ArH), 7.68 (d, *J* = 8 Hz, 2H, ArH), 8.31 (d, *J* = 8Hz, 2H, ArH), 8.75 (s, 1H, pyrazole-H). MS m/z (%) 515 (M^++ 1, 11) 514 (M^+, 29), 367 (13), 227 (51), 129 (28), 102 (25), 98 (71), 91 (38), 77 (100). Anal. Calcd. for $C_{30}H_{22}N_6O_3$ (514.55) C, 70.03; H, 4.31; N, 16.33. Found C, 70.21; H, 4.03; N, 16.02%.

3-[3-Acetyl-1-(4-chlorophenyl)pyrazole-4-carbony]-1,7-diphenyl-[1,2,4]triazolo[4,3-*a*]pyrimidin-5(1H)-one (4Ac)

Pale yellow crystals (2.27g, 85%) m.p 250°C. IR (KBr): ν_{max}/cm^{-1}, 1691, 1685, 1671. ^{1}H NMR($CDCl_3$) δ 2.50 (s, 3H, CH_3), 6.72 (s, 1H, pyrimidine H), 7.55-8.25(m, 14H, ArH), 9.54 (s, 1H, pyrazole H). MS m/z (%) 536 (M^++ 2, 0.2) 535 (M^++1, 0.7) 534 (M^+, 0.7), 249 (15), 246 (66), 204 (16), 128 (22), 111 (18), 103 (19), 77 (96), 76 (100). Anal. Calcd. for $C_{29}H_{19}ClN_6O_3$ (534.97) C, 65.11, H, 3.58, N, 15.71. Found C, 65.45, H, 3.80, N, 15.59%.

1,7-Diphenyl-3-[3-ethoxycarbonyl-1-phenylpyrazole-4-carbonyl]-[1,2,4]traizolo[4,3-*a*]pyrimidin-5 (1H)-one (4Ba)

Yellow crystals (2.1g, 79%), m.p 178-180°C. IR (KBr): ν_{max}/cm^{-1}, 1695, 1671, 1652. ^{1}H NMR ($CDCl_3$) δ 1.16 (t, *J* = 7 Hz, 3H, CH_3), 4.15 (q, *J* = 7 Hz, 2H, CH_2), 6.63 (s, 1H, pyrimdine H), 7.27-8.34 (m, 15H, ArH), 8.75 (s,1H, pyrazole H). MS m/z (%): 531 (M^++ 1, 9) 530 (M^+, 26), 367 (27), 215 (29), 178 (100), 129 (19), 111 (11), 104 (28), 95 (15), 83 (22), 77 (33). Anal. Calcd. for $C_{30}H_{22}N_6O_4$ (530.55) C, 67.92, H, 4.18, N, 15.84. Found C, 67.70, H, 3.98, N, 16.10%.

1,7-Diphenyl-3-[3-ethoxycarbonyl-1-(4-methylphenyl) pyrazole-4-carbonyl]-[1,2,4]triazolo[4,3-*a*]pyrimidin-5(1H)-one (4Bb)

Pale yellow crystals (2.23 g, 82%) m.p 206-208°C. IR (KBr): ν_{max}/cm^{-1}, 1705, 1698, 1669; ^{1}H NMR ($CDCl_3$) δ 1.16 (t, *J* = 7 Hz, 3H, CH_3), 2.44 (s, 3H, CH_3), 4.16 (q, *J* = 7 Hz, 2H, CH_2) 6.63 (s, 1H, pyrimidine H), 7.27-7.58 and 8.09-8.12 (m, 10H, ArH), 7.66 (d, *J* = 9 Hz, 2H, ArH), 8.29 (d, *J* = 9 H*z*, 2H, ArH), 8.70 (s, 1H, pyrazole H). MS m/z (%): 546 (M^++2, 8) 545 (M^++1, 22), 544 (M^+, 22), 228 (44), 128 (24), 118 (88), 103 (23), 91 (89), 77 (100). Anal. Calcd. for $C_{31}H_{24}N_6O_4$ (544.57) C, 68.37; H, 4.44; N, 15.43. Found C, 68.13; H, 4.70; N, 15.71%.

1,7-Diphenyl-3-[3-ethoxycarbonyl-1-(4-chlorophenyl)pyrazole-4-carbonyl]-[1,2,4]triazolo[4,3-*a*]pyrimidin-5(1H)-one (4Bc)

Pale yellow crystals (2.23g, 79%) m.p 185°C IR (KBr): ν_{max}/cm^{-1}, 1710, 1692, 1640; ^{1}H NMR (DMSO-d$_6$) δ 1.18 (t, *J* = 7 Hz, 3H, CH_3), 4.24 (q, *J* = 7 Hz, 2H, CH_2), 6.63 (s, 1H, pyrimidine H), 7.49-7.71 (m, 10H, ArH), 7.94 (d, *J* = 9 Hz, 2H, ArH), 8.19 (d, *J* = 9 Hz, 2H, ArH), 9.49 (s, 1H, pyrazole H). MS m/z (%) 565 (M^++1, 0.42), 564 (M^+, 0.35) 451 (100), 450 (66), 422 (26), 233 (11), 163 (7), 103 (28), 91 (50), 77 (24). Anal. Calcd. for $C_{30}H_{21}ClN_6O_4$ (564.99) C, 63.78; H, 3.75; N, 14.87. Found C, 63.56; H, 3.63; N, 15.08%.

1,7-Diphenyl-3-[3-ethoxycarbonyl-1-(4-nitrophenyl) pyrazole-4-carbonyl][1,2,4]triazolo[4,3-*a*]pyrimidin-5(1H)-one (4Bd)

Yellow crystals (2.36 g, 82%), m.p 325°C. IR (KBr): ν_{max}/cm^{-1} ,1719, 1681, 1666; ^{1}H NMR ($CDCl_3$) δ 1.18 (t, *J* = 7 Hz, 3H, CH_3), 4.16 (q, *J* = 7 Hz, 2H, CH_2), 6.62 (s, 1H, pyrimidine H), 7.27-8.11 (m, 10H, ArH), 8.28 (d, *J* = 7 Hz, 2H, ArH), 8.41 (d, *J* = 7 Hz, 2H, ArH), 8.90 (s, 1H, pyrazole H). MS m/z (%) 576 (M^++1, 11), 575 (M^+, 21) 475 (12), 261 (11), 259 (39), 213 (28), 148 (20), 128 (27), 117 (11), 103 (52), 91 (20), 77 (83), 76 (100). Anal. Clacd. for $C_{30}H_{21}N_7O_6$ (575.55) C, 62.61, H, 3.68, N, 17.04. Found C, 62.45, H, 3.89, N, 17.06%.

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

Orange crystals (2.28 g, 81%), m.p 190°C. IR (KBr)): ν_{max}/cm^{-1}, 1705, 1690, 1670; ^{1}H NMR ($CDCl_3$) δ 6.48 (s, 1H, pyrimidine H), 6.61-8.13 (m, 20H, ArH), 8.85 (s, 1H, pyrazole H). MS m/z (%) 563 (M^++1, 2), 562 (M^+, 3) 534 (40), 491 (13), 274 (23), 104 (43), 77 (87), 76 (100). Anal. Calcd. for $C_{34}H_{22}N_6O_3$ (562.59) C, 72.59; H, 3.94; N, 14.94. Found C, 72.44; H, 3.76; N, 15.18%.

3-[3-Benzoyl-1-(4-chlorophenyl)pyrazole-4-carbonyl]-1,7-diphenyl-[1,2,4]triazolo[4,3-*a*]pyrimidin-5(1H)-one (4Cc)

Yellow crystals (2.48 g, 83%), m. p 220°C. IR (KBr): ν_{max}/cm^{-1}, 1716, 1676, 1660; ^{1}H NMR (DMSO-d$_6$) δ 6.69 (s, 1H, pyrimidine H), 7.44-7.70 (m, 15H, ArH), 7.93 (d, *J* = 8 Hz, 2H, ArH), 8.13 (d, *J* = 8 Hz, 2H, ArH), 9.60 (s, 1H, pyrazole H). MS m/z (%) 598 (M^++2, 21), 597 (M^++1, 18) 596 (M^+, 67), 491 (34), 309 (72), 287 (25), 143 (18), 138 (25), 128 (15), 118 (15), 111 (22), 103 (24), 77 (100). Anal. Calcd. for $C_{34}H_{21}ClN_6O_3$ (597.04) C, 68.40; H, 3.55; N, 14.08. Found C, 68.32 H, 3.37, N, 13.89%.

1,7-Diphenyl-3-[3-(*N*-phenylaminocarbonyl)-1-(4-methylphenyl-)pyrazole-4-carbonyl][1,2,4]triazolo [4,3-*a*]pyrimidin-5(1H)-one (4Db)

Dark yellow crystals (2.22g, 75%), m.p 296-298°C. IR (KBr): ν_{max}/cm^{-1} 3105, 1698, 1682, 1651; ^{1}H NMR (DMSO-d$_6$) δ 2.38 (s, 3H, CH_3), 6.69 (s, 1H, pyrimidine H), 7.39 (d, *J* = 9 Hz, 2H, ArH), 7.53-7.62 and 8.13-8.16 (m, 15H, ArH), 7.89 (d, *J* = 9 Hz, 2H, ArH), 9.47 (s, 1H, pyrazole H), 10.72 (s, 1H, NH). MS m/z (%) 592 (M^++1, 16), 591 (M^+, 38), 397 (33), 303 (88), 288 (44), 287 (59), 259 (29), 171 (19), 145 (22), 129 (38) 118 (66), 105 (26), 103 (29), 91 (100), 77 (64). Anal. Calcd. For $C_{35}H_{25}N_7O_3$ (591.63) C, 71.06; H, 4.26; N, 16.57. Found C, 71.17 H, 3.89, N, 16.81%.

1,7-Diphenyl-3-[3-(*N*-phenylaminocarbonyl)-1-(4-chlorophenyl)-pyrazole-4-carbonyl][1,2,4]triazolo [4,3-*a*]pyrimidine-5(1H)-one (4Dc)

Yellow crystals (2.51g, 82%), m.p 340°C. IR (KBr): ν_{max}/cm^{-1}, 3105, 1684, 1653; ^{1}H NMR (DMSO-d$_6$) δ 6.69 (s, 1H, pyrimidine H), 7.21 – 7.64 (m, 15H, ArH), 7.67 (d, *J* = 9 Hz, 2H, ArH), 8.06 (d, *J* = 9 Hz, 2H, ArH), 9.54 (s, 1H, pyrazole H), 10.74 (s, 1H, NH). MS m/z (%) 613 (M^++2, 3), (612 (M^++1, 3), 611 (M^+, 6), 367 (16), 325 (11), 324 (11), 323 (22), 262 (12), 204 (11), 138 (31), 129 (38), 116 (15), 105 (35), 98 (85), 91 (35), 77 (100). Anal. Calcd. For $C_{34}H_{22}ClN_7O_3$ (612.05) C, 66.72, H, 3.62, N, 16.02. Found C, 66.61, H, 3.99, N, 15.96%.

1,7-Diphenyl-3-[3-(2-thiophenyl)-1-(4-nitrophenyl) pyrazole-4-carbonyl][1,2,4]triazolo[4,3-*a*]pyrimidin-5(1H)-one (4Ed)

Yellow crystals (2.49g, 85%), m.p > 340°C. IR (KBr): ν_{max}/cm^{-1}, 1697, 1687, 1667; ^{1}H NMR (DMSO-d$_6$) δ 6.72 (s, 1H, pyrimidine H), 7.26-780 and 8.20-8.32 (m, 13H, ArH), 8.10 (d, *J* = 9 Hz, 2H, ArH), 8.40 (d, *J* = 9 Hz, 2H, ArH), 9.54 (s, 1H, pyrazole-H). MS m/z (%) 587 (M^++2, 21), 586 (M^++1, 44), 585 (M^+, 84), 556 (33), 480 (50), 323 (84), 298 (100), 253 (18), 252 (92), 150 (14), 129 (26), 122 (22), 103 (48), 91 (20), 77 (62). Anal. Clacd. for $C_{31}H_{19}N_7O_4S$ (585.61) C, 63.58; H, 3.27; N, 16.74. Found C, 63.40; H, 3.35; N, 16.59%.

1,7-Diphenyl-3-[3-(2-naphthoyl)-1-phenylpyrazole-4-carbonyl]-[1,2,4]triazolo[4,3-*a*]pyrimidin-5(1H)-one (4Fa)

Orange crystals (2.42 g, 79%), m.p 230°C IR (KBr): ν_{max}/cm^{-1}, 1697, 1668, 1622; ^{1}H NMR (DMSO-d$_6$) δ 6.62 (s, 1H, pyrimidine H), 7.43-8.14 (m, 22H, ArH), 8.63 (s, 1H, pyrazole H). MS m/z (%) 613 (M^++1, 21), 612 (M^+, 45), 457 (35), 325 (68), 288 (12), 155 (46), 129 (18), 127 (100), 103 (14), 89 (11), 77 (74). Anal. Calcd. for $C_{38}H_{24}N_6O_3$ (612.65), C, 74.50; H, 3.95; N, 13.72.

Found C, 74.35; H, 3.89; N, 13.98%.

1,7-Diphenyl-3-[3-(2-naphthoyl)-1-(4-methylphenyl) pyrazole-4-carbonyl]-[1,2,4]triazolo[4,3-*a*]pyrimidin-5(1H)-one (4Fb)

Red crystals (2.50 g, 80%), m.p 240-242°C IR (KBr): ν_{max}/cm^{-1} 1697, 1680; ^1H NMR (DMSO-d_6) δ 2.35 (s, 3H, CH_3), 6.66 (s, 1H, pyrimidine H), 7.35 (d, J = 9 Hz, 2H, ArH), 7.55-7.65 and 7.98-8.16 (m, 17H, ArH), 7.77 (d, J = 9 Hz, 2H, ArH), 9.52 (s, 1H, pyrazole H). MS m/z (%) 627 (M^++1, 47), 626 (M^+, 100) 471 (74), 339 (56), 311 (53), 145 (15), 129 (25), 127 (69), 106 (20), 104 (24), 91 (81), 77 (12). Anal. Clacd. for $C_{39}H_{26}N_6O_3$ (626.68) C, 74.75; H, 4.18; N, 13.41. Found C, 74.69; H, 4.11; N, 13.11%.

1,7-Diphenyl-3-[1,3-diphenylpyrazole-4-carbonyl]-[1,2,4]triazolo [4,3-*a*]-pyrimidin-5(1H)-one (4Ga)

Dark white crystals (2.1 g, 78%), m. p 200°C. IR (KBr): ν_{max}/cm^{-1}, 1685, 1670; ^1H NMR (DMSO-d_6) δ 6.63 (s, 1H, pyrimidine H), 7.42-8.27 (m, 20H, ArH), 9.37 (s, 1H, pyrazole H). MS m/z (%) 535 (M^++1, 5), 534 (M^+, 22), 533 (17), 506 (10), 505 (20), 429 (22), 267 (19), 247 (100), 129 (18), 104 (20), 89 (16), 77 (68). Anal. Clacd. for $C_{33}H_{22}N_6O_2$ (534.58) C, 74.15; H, 4.15; N, 15.72. Found C, 74.37; H, 4.35; N, 15.49%.

Pharmacology

Cytotoxic activity against *Ehrlich Ascites Cacinoma cell in vitro*

Ehrlich Ascites Cacinoma cell (EAC), used in this study, was kindly supplied by National Institute of Cancer in Netherland since 1952 and mentained in Swiss albino mice by serial IP transplantation, RPMI 1640 medium (Sigma), *Ehrlich Ascites Cacinoma cell* (EAC) suspension (2.5×10^5/mL), and 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 then used for staining the dead EAC cells (El-Merzabani et al., 1979; El-Merzabani et al., 1979).

A set of sterile test tubes was used, where 2.5×10^5 tumor cells/mL were suspended in phosphate - buffered saline. Then 25, 50, 100 μg/mL from tested compound were added to the suspension, kept at 37°C for 2 h. Trypan blue dye exclusion test was then carried out to calculate the percentage of nonviable cells. Doxorubicin (Adriablastina) is taken as a positive control. The percentage of the non-viable cells were calculated by the following equation :

$$\% \text{ of non-viable cells} = 100\ (N/N_t)$$

Table I. *In vitro* cytotoxic activity of newly synthesized compounds and doxorubicin against (EAC)

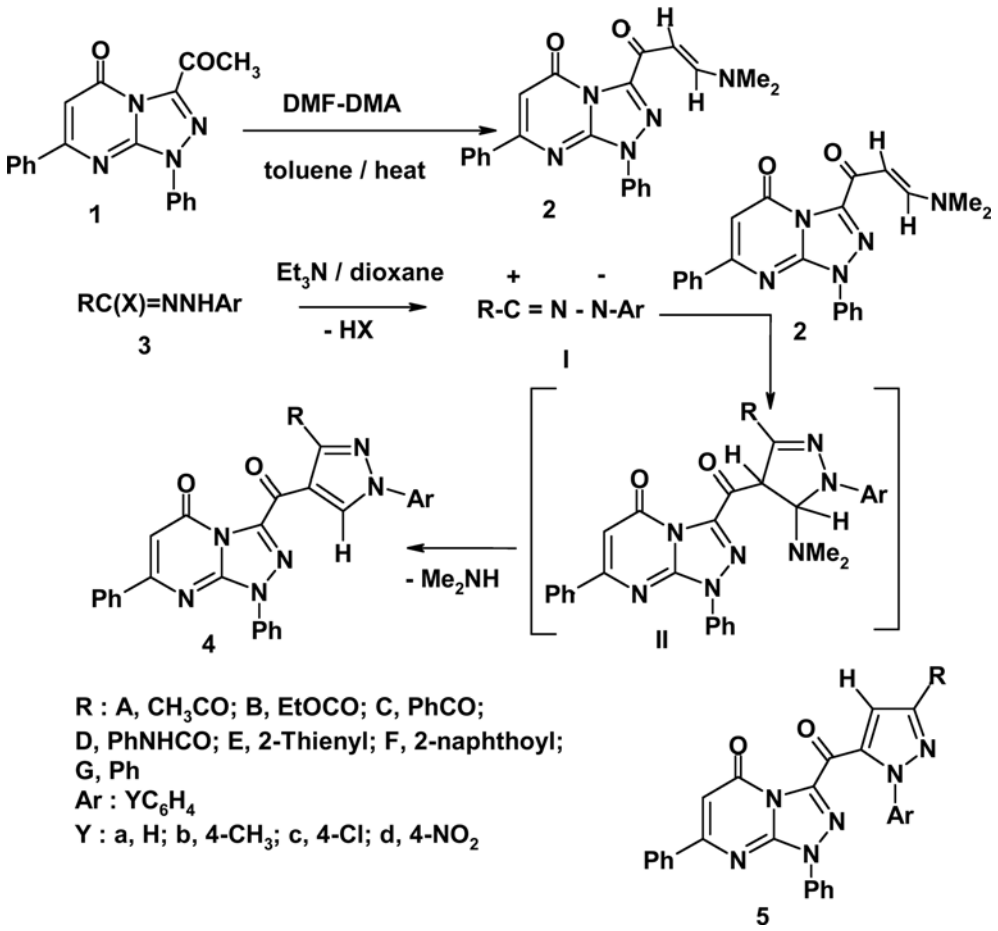
Compound No.	Non-viable cells (%)		
	Concentration (μg/mL)		
	100	50	25
4Aa	10	6	2
4Ab	8	2	1
4Ac	10	2	1
4Ba	8	3	1
4Bc	6	2	1
4Ca	6	3	0
4Cc	8	5	1
4Db	7	4	1
4Dc	6	5	3
4Ed	10	5	2
4Fa	10	3	1
4Ga	8	3	2
Cell control	0	0	0
Doxorubicin	100	55	20

where N is the number of non-viable cells counted, N_t is the total number of cells. The test was repeated four times for each compound. The results are summarized in Table I.

RESULTS AND DISCUSSION

The required new enaminone **2** was prepared in this study by refluxing **1** with DMF-DMA in toluene (Scheme 1). Its structure was confirmed by its spectroscopic data (MS, ^1H NMR and IR) and elemental analysis (see Experimental section). Its ^1H NMR spectrum showed two doublet signals at δ 5.36 and 8.18 with J = 13 Hz assignable to the two olefinic protons. This finding indicates that this enaminone **2** exists in the depicted *trans* configuration (Scheme 1).

Reaction of **2** with each of the hydrazonoyl halides **3A-G** in dioxane in the presence of triethylamine gave, in each case, one isolable product as evidenced by tlc analysis. Both mass spectra and elemental analysis data of the isolated products were consistent with either one of the two isomeric structures **4** or **5** (Scheme 1). On the basis of the ^1H NMR spectra, however, the isolated products were assigned structure **4**. The ^1H NMR spectra of the products revealed, in each case, a singlet signal for the pyrazole ring proton in the region δ 8.63-9.60 assignable to pyrazole-5H. Literature reports indicate that the ^1H NMR spectra of 5- and 4-unsubstituted pyrazoles exhibit the characteristic singlet signals of 5-CH and 4-CH protons at δ 8.35 and 7.30, respectively. Furthermore, spectral simulation for 5- and 4-unsubstituted pyrazoles showed the H-5 and 4-H signals at δ 8.60 and 6.50,



Scheme 1.

respectively (Shawali et al., 1988). In addition, literature reports (Shawali et al., 2006) indicate that reaction of hydrazonoyl halides with various enaminones are regioselective and lead to the formation of 5-unsubstituted pyrazole derivatives.

To account for the formation of the products **4**, it is suggested that the studied reactions of **2** with **3**, proceed via initial dehydrohalogenation of the hydrazonoyl halides **3** to give the respective nitrilimines **I** (Shawali et al., 2006), which in turn cycloadd regioselectively to the enaminone **2**. The resulting cycloadducts **II** undergo *in situ* elimination of dimethylamine under the employed reaction conditions to give compounds **4** as end products (Scheme 1).

Antitumor screening tests

The *in vitro* cytotoxic effects of the newly synthesized compounds **4** and positive control drug doxorubicin (*Adriablastina*) were evaluated at three different concentrations of each against *Ehrlich Ascites Carcinoma* tumor cells (EAC) in the National Institute of Cancer. The results are summarized in Table I. As shown, all compounds have low or no *in vitro* antitumor activity toward *EAC* tumor cells as compared with the reference drug *Doxorubicin*.

CONCLUSION

In conclusion, a simple method for the synthesis of the title heterocyclic derivatives starting from the new enaminone **2** and hydrazonoyl halides **3** is demonstrated. The structures of the newly synthesized compounds **2** and **4** were confirmed by spectral data and elemental analyses. The results of antitumor activity screening of compounds in series **4** revealed that all such compounds exhibited little or no activity against *Ehrlich Ascites Carcinoma* cells in relation to the reference drug *Doxorubicin*.

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