ORIGINAL SCIENTIFIC PAPER

CROATICA CHEMICA ACTA

Croat. Chem. Acta **2018**, *91*(*1*), 43–51
Published online: April 2, 2018
DOI: 10.5562/cca3279

HKD 1926

Synthesis of New Azoles and Azolopyrimidines Incorporating Morpholine Moiety as Potent Anti-Tumor Agents

Sobhi M. Gomha,[1,*] Zeinab A. Muhammad,[2] Hassan M. Abdel-aziz,[3] Elham Ezz El-Arab[2]

[1] Department of Chemistry, Faculty of Science, Cairo University, Giza 12613, Egypt
[2] Department of Organic Chemistry, National Organization for Drug Control and Research (NODCAR), Giza 12311, Egypt
[3] Department of Chemistry, Faculty of Science, Bani Suef University, Bani Suef, Egypt
* Corresponding author's e-mail address: s.m.gomha@gmail.com

RECEIVED: December 24, 2017 ✶ REVISED: February 27, 2018 ✶ ACCEPTED: March 7, 2018

Abstract: A new series of morpholinyl-chalcones **2a–d** was prepared by reaction of 2-oxo-N,4-diarylbut-3-enehydrazonoyl chlorides **1a–d** with morpholine. These chalcones were used as a building block for constructing pyrazoles **3a–d** and 3,4-dihydropyrimidine-2(1*H*)-thione **6** *via* their reaction with phenylhydrazine and thiourea, respectively. Moreover, a new series of azolopyrimidine derivatives **11a,b, 15, 17, 19,** and **21** incorporating morpholine moiety were synthesized by reaction of 1-morpholino-4-phenyl-1-(2-phenylhydrazono)but-3-en-2-one (**2a**) with a number of heterocyclic amines in the presence of a catalytic amount of acetic acid. The assigned structures for all the newly synthesized compounds were confirmed on the basis of elemental analyses and spectral data and the mechanisms of their formation were also discussed. All the synthesized compounds were tested for *in vitro* activities against two antitumor cell lines, human lung cancer (A-549) and human hepatocellular carcinoma (HepG-2) compared with the employed standard antitumor drug (cisplatin) and the results revealed that compounds **6, 8c** and **17** have promising activities compared with cisplatin.

Keywords: hydrazonoyl halides, chalcones, pyrazoles, pyrimidines, anticancer activity.

INTRODUCTION

HYDRAZONOYL halides are compounds which have the characteristic functionality –C(X):NNH–, where X is a halogen (Br or Cl). An increasing flow of work has appeared on the chemistry of such a class of compounds. They have recently reawaken interest in their chemistry as they proved to be useful building blocks for one-pot synthesis of a wide variety of heterocycles such as 1,3-thiazoles,[1–4] 1,3,4-thiadiazoles,[5,6] pyrazoles, pyrazolo[3,4-*d*]pyridazines,[7,8] triazolo[4,3-*b*]triazinones,[9,10] triazolo[3,4-*b*]thiadiazines, triazolo[4,3-*b*]tetrazines,[11–13] 1,2,4-triazolo-pyrimidinones,[14–16] pyrimido-tetrazinones, pyrimido-thiadiazinones[17] and benzopyranotriazepines.[18] Literature reveals that morpholine derivatives have found great significance in modern years due to their variety of pharmacological activities including anticancer, anti-inflammatory, analgesic, antidepressant, antifungal, anti-parasitic, antiplatelet, anti-tuberculosis, HIV-protease inhibitors, selective inhibitor of protein kinase C, neuroprotective and anti-malarial.[19–23] In addition, many pyrimidines and triazolopyrimidines are pharmacological scaffolds displaying a wide range of biological activities such as anticancer, antimicrobial, hypoglycemic, CNS depressant, antiallergy, anti-inflammatory, and diuretic activities.[24–33] On the other hand, pyrazoles have been reported to possess a variety of significant and diverse pharmacological activities such as antibacterial, antifungal, anticonvulsant, antiviral, antitubercular, antidepressant, anti-inflammatory, antiamoebic, analgesic and anticancer activity.[34–39] In view of all these reports and in continuation of our previous work in synthesis of bioactive heterocyclic compounds,[40–47] herein, we are interested in synthesizing a new series of pyrazoles and azolopyrimidines using morpholinylchalcones as common precursor and evaluate these compounds for their anticancer activities.

EXPERIMENTAL

Melting points were measured on an Electrothermal IA 9000 series digital melting point apparatus. IR spectra were recorded in potassium bromide discs on Pye Unicam SP 3300 and Shimadzu FTIR 8101 PC infrared spectrophotometers. The ^{1}H and ^{13}C NMR spectra were recorded on a Varian 6 Mercury VX-300 NMR spectrometer (Karlsruhe, Germany). ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) were run in DMSO-d_6 and chemical shifts are expressed in ppm units using TMS as an internal reference. Mass spectra were recorded on a Shimadzu GCMS-QP1000 EX mass spectrometer at 70 eV. Elemental analyses were measured by using a German made Elementar vario LIII CHNS analyzer. Antitumor activity was evaluated at the Regional Center for Mycology and Biotechnology at Al-Azhar University, Cairo, Egypt.

Synthesis of Pyrazoles 3a–d

A mixture of chalcones **2a**–**d** (1 mmol) and phenylhydrazine (0.108 g, 1 mmol) in 15 mL of ethanol containing 1 mL of glacial acetic acid was refluxed for 4–10 h (monitored through TLC). The desired product precipitated from reaction mixture was filtered, washed with ethanol and recrystallized from proper solvent to give the respective pyrazoles **3a**–**d**, respectively.

N′,1,3-TRIPHENYL-1*H*-PYRAZOLE-5-CARBOHYDRAZIDE (3a)

Orange solid, (70 % yield), mp 188–190 °C (EtOH); IR (KBr) ν_{max} 1595 (C=N), 1663 (C=O), 3036, 2927 (C–H), 3263, 3429 (2NH) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 6.67–7.13 (m, 10H, Ar–H), 7.20 (s, 1H, pyrazole-H4), 7.29–7.50 (m, 5H, Ar–H), 9.78 (brs, 1H, NH), 10.87 (brs, 1H, NH); MS *m* / *z* (%) 354 (M$^+$, 2), 293 (4), 178 (17), 148 (5), 118 (12), 92 (46), 77 (54), 69 (42), 65 (30), 43 (100). Anal. Calcd. for $C_{22}H_{18}N_4O$ (354.41): C, 74.56; H, 5.12; N, 15.81. Found: C, 74.39; H, 5.18; N, 15.65 %.

N′,1-DIPHENYL-3-(*P*-TOLYL)-1*H*-PYRAZOLE-5-CARBOHYDRAZIDE (3b)

Orange solid, (60 % yield), mp 203–205 °C (EtOH); IR (KBr) ν_{max} 1597 (C=N), 1671 (C=O), 3050, 2928 (C-H), 3263, 3420 (2NH) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.35 (s, 3H, CH_3), 6.68–7.16 (m, 10H, Ar–H), 7.24 (s, 1H, pyrazole-H4), 7.31-7.76 (m, 4H, Ar–H), 9.58 (brs, 1H, NH), 9.81 (brs, 1H, NH); ^{13}C-NMR (DMSO-d_6): δ 21.1 (CH_3), 112.3, 120.3, 121.0, 121.9, 123.5, 126.1, 126.9, 127.4, 127.9, 128.2, 130.5, 132.7, 133.9, 139.4, 149.7 (Ar–C and C=N), 168.7 (C=O); MS *m* / *z* (%) 368 (M$^+$, 7), 321 (9), 291 (3), 275 (4), 233 (4), 208 (5), 178 (3), 119 (40), 106 (45), 91 (100), 77 (37), 43 (75). Anal. Calcd. for $C_{23}H_{20}N_4O$ (368.44): C, 74.98; H, 5.47; N, 15.21. Found: C, 74.75; H, 5.62; N, 14.99 %.

3-(4-CHLOROPHENYL)-*N*′,1-DIPHENYL-1*H*-PYRAZOLE-5-CARBOHYDRAZIDE (3c)

Orange solid, (60 % yield), mp 206–208 °C (Dioxane); IR (KBr) ν_{max} 1596 (C=N), 1672 (C=O), 3032, 3102 (C–H), 3251, 3280 (2NH) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.35 (s, 3H, CH_3), 6.68–7.24 (m, 10H, Ar–H), 7.89 (s, 1H, pyrazole-H4), 7.38–7.89 (m, 4H, Ar–H), 9.61 (brs, 1H, NH), 9.83 (brs, 1H, NH); MS *m* / *z* (%) 390 (M$^+$+2, 2), 388 (M$^+$, 7), 313 (3), 262 (3), 210 (4), 165 (4), 139 (24), 106 (27), 91 (36), 77 (42), 57 (62), 43 (100). Anal. Calcd. for $C_{22}H_{17}ClN_4O$ (388.86): C, 67.95; H, 4.41; N, 14.41. Found: C, 67.92; H, 4.58; N, 14.33 %.

3-(4-CHLOROPHENYL)-1-PHENYL-*N*′-(*P*-TOLYL)-1*H*-PYRAZOLE-5-CARBOHYDRAZIDE (3d)

Orange solid, (60 % yield), mp 212–214 °C (Dioxane); IR (KBr) ν_{max} 1598 (C=N), 1648 (C=O), 3025 (C–H), 3283, 3236 (2NH) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.89 (s, 3H, CH_3), 6.66–7.19 (m, 9H, Ar–H), 7.39 (s, 1H, pyrazole-H4), 7.58–7.93 (m, 4H, Ar–H), 9.58 (brs, 1H, NH), 9.88 (brs, 1H, NH); ^{13}C-NMR (DMSO-d_6): δ 20.4 (CH_3), 111.6, 112.0, 118.2, 120.5, 121.3, 124.6, 126.2, 126.8, 128.4, 128.8, 130.4, 133.5, 135.9, 149.2, 168.8 (Ar–C and C=N), 168.0 (C=O); MS *m* / *z* (%) 404 (M$^+$+2, 2), 402 (M$^+$, 8), 427 (4), 404 (4), 368 (9), 336 (6), 300 (5), 270 (9), 238 (7), 199 (9), 168 (11), 143 (37), 114 (30), 68 (42), 55 (37), 43 (100). Anal. Calcd. for $C_{23}H_{19}N_4OCl$ (402.88): C, 68.57; H, 4.75; N, 13.91. Found: C, 68.50; H, 4.90; N, 13.65 %.

SYNTHESIS OF 6-(MORPHOLINO-(2-PHENYLHYDRAZONO)METHYL)-4-PHENYL-3,4-DIHYDROPYRIMIDINE-2(1*H*)-THIONE (6)

A mixture of 1-morpholino-4-phenyl-1-(2-phenylhydrazono)but-3-en-2-one (**2a**) (3.35 g, 10 mmol) and thiourea (0.76 g, 10 mmol) in 40 mL of ethanol containing catalytic amount of potassium hydroxide was refluxed for 6 h (monitored through TLC). The reaction mixture was then poured into 2 N HCl, and the solid product was collected by filtration followed by washing with water and EtOH. The crude product was recrystallized from DMF to give pure product of compound **6** as yellowish-white solid in 75 % yield, mp 190–193°C; IR (KBr) ν_{max} 1598 (C=N), 2927, 3054 (C–H), 3165, 3249, 3425 (3NH) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.37–2.40 (m, 4H, 2CH$_2$), 3.41–3.45 (m, 4H, 2CH$_2$), 4.63 (*d*, *J* = 4.1 Hz, 1H, pyrimidine-H6), 6.60 (*d*, *J* = 4.1 Hz, 1H, pyrimidine-H5), 7.14–7.52 (m, 10H, Ar–H), 9.63 (brs, 1H, NH), 10.17 (brs, 1H, NH), 11.13 (brs, 1H, 1NH); MS *m* / *z* (%) 393 (M$^+$, 100), 368 (37), 353 (40), 320 (38), 295 (38), 247 (4), 194 (4), 118 (10), 76 (49), 65 (23), 42 (31). Anal. Calcd. for $C_{21}H_{23}N_5OS$ (393.51): C, 64.10; H, 5.89; N, 17.80. Found: C, 64.40; H, 5.65; N, 17.49 %.

Synthesis of 1,5-Dihydropyrido[2,3-*d*][1,2,4]triazolo[4,3-*a*]pyrimidin-8-yl)-1,5-diphenyl-1*H*-pyrazole-4-carboxylate Derivatives 8a–d

GENERAL PROCEDURE

Triethylamine (0.14 mL, 1 mmol) was added to a mixture of equimolar amounts of thione 6 (0.393 g, 1 mmol) and the appropriate hydrazonoyl halides **7a**–**d** (1 mmol) in dioxane (20 mL) at room temperature. The reaction mixture was refluxed till all of the starting materials have been disappeared and hydrogen sulfide gas ceased to evolve (6–10 h, monitored by TLC). The solvent was evaporated and the residue was triturated with methanol. The solid that formed was filtered and recrystallized from the proper solvent to give the products **8a**–**d**, respectively.

1-(7-(MORPHOLINO(2-PHENYLHYDRAZONO)METHYL)-1,5-DIPHENYL-1,5-DIHYDRO-[1,2,4]TRIAZOLO [4,3-*a*]PYRIMIDIN-3-YL)ETHAN-1-ONE (8a)

Green solid, (70 % yield), mp 219–221 °C (Dioxane); IR (KBr) ν_{max} 1599 (C=N), 1650 (C=O), 3024, 2957 (C–H), 3253 (NH) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.33–2.37 (m, 4H, $2CH_2$), 2.45 (s, 3H, CH_3), 3.44–3.46 (m, 4H, $2CH_2$), 4.67 (*d*, *J* = 4.1 Hz, 1H, pyrimidine-H5), 6.69 (*d*, *J* = 4.1 Hz, 1H, pyrimidine-H6), 7.12–7.61 (m, 15H, Ar–H), 11.20 (brs, 1H, NH); ^{13}C-NMR (DMSO-d_6): δ 24.1 (CH_3), 50.1 (CH), 54.0, 65.6 (CH_2), 113.7, 117.3, 121.2, 122.0, 122.6, 123.3, 124.8, 126.3, 128.2, 128.9, 129.1, 130.2, 132.7, 133.0, 136.2, 139.7, 151.4 (Ar–C and C=N), 193.8 (C=O); MS *m* / *z* (%) 519 (M^+, 7), 491 (11), 458 (10), 372 (18), 355 (14), 321 (54), 293 (100), 249 (16), 54 (38). Anal. Calcd. for $C_{30}H_{29}N_7O_2$ (519.61): C, 69.35; H, 5.63; N, 18.87. Found: C, 69.18; H, 5.71; N, 18.66 %.

1-(7-(MORPHOLINO(2-PHENYLHYDRAZONO)METHYL)-5-PHENYL-1-(*P*-TOLYL)-1,5-DIHYDRO-[1,2,4]TRIAZOLO [4,3-*a*]PYRIMIDIN-3-YL)ETHAN-1-ONE (8b)

Green solid, (75 % yield), mp 213-215 °C (EtOH); IR (KBr) ν_{max} 1597 (C=N), 1676 (C=O), 2976, 3032 (C–H), 3250 (NH) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.30–2.33 (m, 4H, $2CH_2$), 2.28 (s, 3H, CH_3), 2.44 (s, 3H, CH_3), 3.53–3.56 (m, 4H, $2CH_2$), 4.63 (*d*, *J* = 4.1 Hz, 1H, pyrimidine-H5), 6.67 (*d*, *J* = 4.1 Hz, 1H, pyrimidine-H6), 7.07–7.63 (m, 14H, Ar–H), 11.12 (brs, 1H, NH); MS *m* / *z* (%) 533 (M^+, 4), 439 (19), 416 (12), 393 (12), 360 (12), 353 (18), 77 (22), 65 (44), 51 (81), 43 (100). Anal. Calcd. for $C_{31}H_{31}N_7O_2$ (533.64): C, 69.77; H, 5.86; N, 18.37. Found: C, 69.52; H, 5.93; N, 18.20 %.

ETHYL-7-(MORPHOLINO(2-PHENYLHYDRAZONO)METHYL)-1,5-DIPHENYL-1,5-DIHYDRO-[1,2,4]TRIAZOLO [4,3-*a*]PYRIMIDINE-3-CARBOXYLATE (8c)

Green solid, (70 % yield), mp 235–237 °C (DMF); IR (KBr) ν_{max} 1599 (C=N), 1735 (C=O), 2916, 2962 (C-H), 3250 (NH) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.15 (t, *J* = 7.2 Hz, 3H, CH_3), 2.41–2.44 (m, 4H, $2CH_2$), 3.30–3.32 (m, 4H, $2CH_2$), 4.18 (q, *J* = 7.2 Hz, 2H, CH_2), 4.47 (*d*, *J* = 4.1 Hz, 1H, pyrimidine-H5), 6.72 (*d*, *J* = 4.1 Hz, 1H, pyrimidine-H6), 7.00–7.81 (m, 15H, Ar–H), 10.80 (brs, 1H, NH); ^{13}C-NMR (DMSO-d_6): δ 14.8 (CH_3), 50.2 (CH), 54.0, 60.8, 66.2 (CH_2), 114.8, 116.3, 120.3, 120.4, 120.3, 122.3, 123.3, 124.5, 124.9, 126.8, 128.7, 128.8, 129.2, 129.3, 129.4, 140.9, 152.5 (Ar-C and C=N), 166.7 (C=O); MS *m* / *z* (%) 549 (M^+, 8), 488 (27), 455 (24), 421 (9), 405 (12), 384 (40), 368 (96), 323 (25), 135 (39), 75 (100). Anal. Calcd. for $C_{31}H_{31}N_7O_3$ (549.63): C, 67.74; H, 5.69; N, 17.84. Found: C, 67.99; H, 5.55; N, 17.60 %.

ETHYL-7-(MORPHOLINO(2-PHENYLHYDRAZONO)METHYL)-5-PHENYL-1-(*P*-TOLYL)-1,5-DIHYDRO-[1,2,4]TRIAZOLO[4,3-*a*]PYRIMIDINE-3-CARBOXYLATE (8d)

Green solid, (74 % yield), mp 201–203 °C (EtOH); IR (KBr) ν_{max} 1593 (C=N), 1740 (C=O), 2918 (C-H), 3432 (NH) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.16–1.21 (t, *J* = 7.2 Hz, 3H, CH_2CH_3), 2.34–2.37 (m, 4H, $2CH_2$), 2.30 (s, 3H, CH_3), 3.42–3.46 (m, 4H, $2CH_2$), 4.20 (q, *J* = 7.2 Hz, 2H, CH_2CH_3), 4.80 (*d*, *J* = 4.1 Hz, 1H, pyrimidine-H5), 6.89 (*d*, *J* = 4.1 Hz, 1H, pyrimidine-H6), 7.50–8.06 (m, 14H, Ar–H), 11.20 (brs, 1H, NH); MS *m* / *z* (%) 563 (M^+, 1), 499 (16), 447 (11), 422 (9), 398 (30), 370 (17), 355 (67), 326 (17), 294 (34), 90 (11), 77 (100), 50 (21). Anal. Calcd. for $C_{32}H_{33}N_7O_3$ (563.66): C, 68.19; H, 5.90; N, 17.39. Found: C, 68.50; H, 5.60; N, 17.09 %.

General Method for Synthesis of Compounds 11a,b, 15, 17, 19 and 21

A mixture of each of 1-morpholino-4-phenyl-1-(2-phenylhydrazono)but-3-en-2-one (**2a**) (0.335 g, 1 mmol) and the appropriate heterocyclic amine (**9a,b, 14, 16, 18** or **20**) (1 mmol) in ethanol (20 mL) in the presence of catalytic drops of acetic acid was refluxed for 10–15 h (monitored through TLC). The reaction mixture was poured into water and the solid product was collected by filtration followed by washing with ethanol. The crude product was then recrystallized from DMF to give pure products of **11a,b, 15, 17, 19** and **21,** respectively. Compounds **11a,b, 15, 17, 19** and **21** with their physical constants and spectral data are depicted as shown below:

4-((5-PHENYL-[1,2,4]TRIAZOLO[1,5-*a*]PYRIMIDIN-7-YL) (2-PHENYLHYDRAZONO)METHYL)MORPHOLINE (11a)

Yellow solid, (70 % yield), mp 219–220 °C; IR (KBr) ν_{max} 1600 (C=N), 2950, 3055 (C-H), 3247 (NH) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.32–2.34 (m, 4H, $2CH_2$), 3.80–3.83 (m, 4H, $2CH_2$), 7.00–7.46 (m, 10H, Ar-H), 8.01 (s, 1H, triazole-H3), 8.19 (s, 1H, pyrimidine-H5), 10.65 (brs, 1H, NH); ^{13}C-NMR (DMSO-d_6): δ 51.7, 61.4 (CH_2), 115.2, 117.0, 119.2, 121.5, 122.0, 125.3, 127.9, 129.1, 130.3, 132.4, 132.9, 137.5, 144.6, 158.5

(Ar–C and C=N); MS *m* / *z* (%) 399 (M^+, 15), 386 (16), 365 (27), 357 (22), 322 (20), 293 (38), 278 (16), 104 (27), 77 (42), 56 (48), 43 (100), 41 (58). Anal. Calcd. for $C_{22}H_{21}N_7O$ (399.18): C, 66.15; H, 5.30; N, 24.55. Found: C, 65.93; H, 5.71; N, 24.29 %.

4-((2-PHENYLHYDRAZONO)(5-PHENYLTETRAZOLO [1,5-*a*]PYRIMIDIN-7-YL)METHYL)MORPHOLINE (11b)

Yellow solid, (72 % yield), mp 233-235 °C; IR (KBr) ν_{max} 1600 (C=N), 2950, 3018 (C–H), 3245 (NH) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.32–2.34 (m, 4H, $2CH_2$), 3.82-3.85 (m, 4H, $2CH_2$), 7.02–7.49 (m, 10H, Ar–H), 8.09 (s, 1H, pyrimidine-H5), 10.65 (brs, 1H, NH); MS *m* / *z* (%) 400 (M^+, 31), 395 (42), 366 (65), 335 (58), 322 (56), 96 (31), 88 (46), 60 (100). Anal. Calcd. for $C_{21}H_{20}N_8O$ (400.18): C, 62.99; H, 5.03; N, 27.98. Found: C, 62.47; H, 5.38; N, 27.77 %.

4-((2-PHENYLBENZO[4,5]IMIDAZO[1,2-*a*]PYRIMIDIN-4-YL)(2-PHENYLHYDRAZONO)METHYL)MORPHOLINE (15)

Yellow solid, (75 % yield), mp 255–257 °C; IR (KBr) ν_{max} 1600 (C=N), 2948, 3019 (C–H), 3246 (NH) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.30–2.32 (m, 4H, $2CH_2$), 3.71–3.73 (m, 4H, $2CH_2$), 7.14–7.49 (m, 14H, Ar-H), 8.06 (s, 1H, pyrimidine-H5), 10.82 (brs, 1H, NH); ^{13}C-NMR (DMSO-d_6): δ 53.2, 61.1 (CH_2), 115.7, 118.3, 120.1, 120.5, 122.3, 124.1, 124.8, 126.9, 126.9, 129.4, 130.3, 130.8, 133.6, 134.5, 134.8, 137.1, 139.2, 141.6, 159.6 (Ar–C and C=N); MS *m* / *z* (%) 448 (M^+, 21), 372 (32), 357 (100), 349 (56), 333 (56), 305 (64), 294 (53), 264 (83), 217 (36), 200 (23), 159 (27), 110 (48), 74 (82), 44 (82). Anal. Calcd. for $C_{27}H_{24}N_6O$ (448.20): C, 72.30; H, 5.39; N, 18.74. Found: C, 71.77; H, 5.63; N, 18.49 %.

4-((8,10-DIMETHYL-2-PHENYLPYRIDO[2',3':3,4]PYRAZOLO[1,5-*a*]PYRIMIDIN-4-YL)(2-PHENYL-HYDRAZONO)METHYL)MORPHOLINE (17)

Yellow solid, (69 % yield), mp 225–227 °C; IR (KBr) ν_{max} 1612 (C=N), 2910, 2955 (C–H), 3417 (NH) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.30–2.32 (m, 4H, $2CH_2$), 3.69–3.72 (m, 4H, $2CH_2$), 2.34 (s, 3H, CH_3), 2.64 (s, 3H, CH_3), 6.83 (s, 1H, pyridine-H), 6.97–7.57 (m, 10H, Ar–H), 8.15 (s, 1H, pyrimidine-H5),10.67 (brs, 1H, NH); MS *m* / *z* (%) 477 (M^+, 6), 365 (20), 326 (23), 307 (11), 270 (17), 208 (13), 166 (25), 147 (20), 91 (21), 69 (100). Anal. Calcd. for $C_{28}H_{27}N_7O$ (477.23): C, 70.42; H, 5.70; N, 20.53. Found: C, 70.01; H, 6.14; N, 20.27 %.

4-((7-PHENYL-7*H*-THIAZOLO[3,2-*a*]PYRIMIDIN-5-YL)(2-PHENYLHYDRAZONO)METHYL)MORPHOLINE (19)

Yellow solid, (73 % yield), mp 219–221 °C; IR (KBr) ν_{max} 1599 (C=N), 2948, 3018 (C–H), 3246, (NH) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.01–2.22 (m, 4H, $2CH_2$), 3.72–3.75 (m, 4H, $2CH_2$), 4.45 (1H, *d*, *J* = 4.6 *Hz*, pyrimidine-H), 6.59 (1H, *d*, *J* = 4.6 *Hz*, pyrimidine-H), 6.72–7.46 (m, 12H, Ar-H), 10.65 (brs, 1H, NH); MS *m* / *z* (%) 417 (M^+, 7), 322 (100), 293 (52), 278 (16), 252 (50), 246 (12), 144 (8), 76 (9), 42 (38). Anal. Calcd. for $C_{23}H_{23}N_5OS$ (417.53): C, 66.16; H, 5.55; N, 16.77. Found: C, 66.28; H, 5.46; N, 16.59 %.

4-((2-PHENYL-2*H*-BENZO[4,5]THIAZOLO [3,2-*a*]PYRIMIDIN-4-YL)(2-PHENYLHYDRAZONO)METHYL) MORPHOLINE (21)

Yellow solid, (80 % yield), mp 244–246 °C; IR (KBr) ν_{max} 1599 (C=N), 2948, 3017 (C–H), 3245 (NH) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.30–2.31(m, 4H, $2CH_2$), 3.70–3.71 (m, 4H, $2CH_2$), 4.48 (1H, *d*, *J* = 4.6 *Hz*, pyrimidine-H), 6.52 (1H, *d*, *J* = 4.6 *Hz*, pyrimidine-H), 7.02–7.61 (m, 14H, Ar-H), 10.55 (brs, 1H, NH); MS *m* / *z* (%) 483 (M^+, 10), 382 (25), 368 (19), 294 (100), 267 (24), 155 (17), 92 (30), 76 (51), 43 (90). Anal. Calcd. for $C_{27}H_{25}N_5OS$ (467.59): C, 69.35; H, 5.39; N, 14.98. Found: C, 69.30; H, 5.27; N, 14.73 %.

Evaluation of Cytotoxic Effects of Compounds

MAMMALIAN CELL LINES

A-549 cells (human Lung cancer cell line, HepG-2 cells (human Hepatocellular carcinoma) were obtained from VACSERA Tissue Culture Unit.

CHEMICALS USED

Dimethyl sulfoxide (DMSO), crystal violet and trypan blue dye were purchased from Sigma (St. Louis, Mo., USA).

Fetal Bovine serum, DMEM, RPMI-1640, HEPES buffer solution, L-glutamine, gentamycin and 0.25 % Trypsin-EDTA were purchased from Lonza.

CRYSTAL VIOLET STAIN (1 %)

It composed of 0.5 % (*w* / *v*) crystal violet and 50 % methanol then made up to volume with ddH_2O and filtered through a Whatmann No.1 filter paper.

CELL LINE PROPAGATION

The cells were propagated in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10 % heat-inactivated fetal bovine serum, 1 % L-glutamine, HEPES buffer and 50 µg mL^{-1} gentamycin. All cells were maintained at 37 °C in a humidified atmosphere with 5 % CO_2 and were subcultured two times a week.

CYTOTOXICITY EVALUATION USING VIABILITY ASSAY

For cytotoxicity assay, the cells were seeded in 96-well plate at a cell concentration of 1×10^4 cells per well in 100µl of growth medium. Fresh medium containing different concentrations of the test sample was added after 24 h of seeding. Serial two-fold dilutions of the tested chemical compound were added to confluent cell monolayers dispensed into 96-well, flat-bottomed microtiter plates

(Falcon, NJ, USA) using a multichannel pipette. The microtiter plates were incubated at 37 °C in a humidified incubator with 5 % CO_2 for a period of 48 h. Three wells were used for each concentration of the test sample. Control cells were incubated without test sample and with or without DMSO. The little percentage of DMSO present in the wells (maximal 0.1 %) was found not to affect the experiment. After incubation of the cells for at 37 °C, various concentrations of sample were added, and the incubation was continued for 24 h and viable cells yield was determined by a colorimetric method.

In brief, after the end of the incubation period, media were aspirated and the crystal violet solution (1 %) was added to each well for at least 30 minutes. The stain was removed and the plates were rinsed using tap water until all excess stain is removed. Glacial acetic acid (30 %) was then added to all wells and mixed thoroughly, and then the absorbance of the plates were measured after gently shaken on Microplate reader (TECAN, Inc.), using a test wavelength of 490 nm. All results were corrected for background absorbance detected in wells without added stain. Treated samples were compared with the cell control in the absence of the tested compounds. All experiments were carried out in triplicate. The cell cytotoxic effect of each tested compound was calculated**.** The optical density was measured with the microplate reader (SunRise, TECAN, Inc, USA) to determine the number of viable cells and the percentage of viability was calculated as [1-(ODt/ODc)] × 100 % where ODt is the mean optical density of wells treated with the tested sample and ODc is the mean optical density of untreated cells. The relation between surviving cells and drug concentration is plotted to get the survival curve of each tumor cell line after treatment with the specified compound. The 50 % inhibitory concentration (IC_{50}), the concentration required to cause toxic effects in 50 % of intact cells, was estimated from graphic plots of the dose response curve for each conc. using Graphpad Prism software (San Diego, CA. USA).[48]

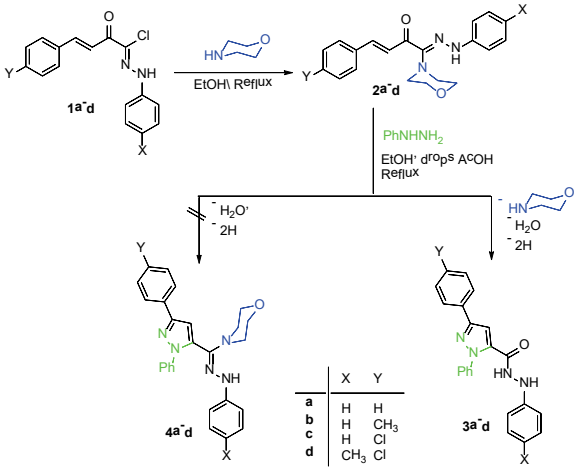
RESULTS AND DISCUSSION

As previously described,[49] compounds **1a–d** reacted with morpholine in ethanol under reflux for 3–6 hrs to give the morpholinohydrazonobutenone derivatives **2a–d** (Scheme 1), which were used as a precursors for synthesis of pyrazoles, pyrimidines, and different fused pyrimidines, such as triazolopyrimidine, tetrazolopyrimidine, thiazolo–pyrimidine, benzothiazolo-pyrimidine benzoimidazo–pyrimidine, and pyridopyrazolopyrimidine derivatives.

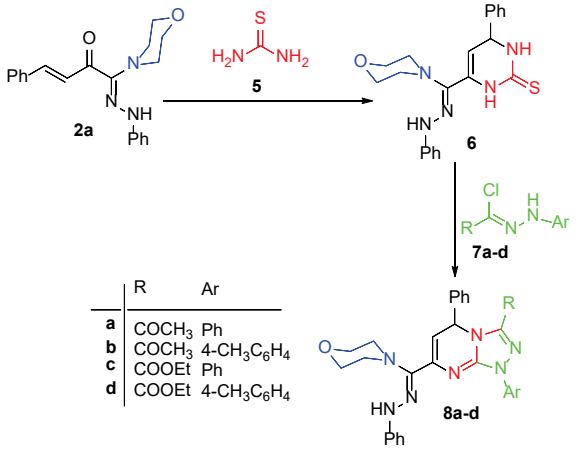
Compounds **2a–d** were refluxed in ethanol containing catalytic amount of acetic acid with phenylhydrazine for 4–10 hrs affording the corresponding pyrazole derivatives **3a–d**, rather than the compounds **4a–d** (Scheme 1), what was confirmed based on the mass spectrum and IR spectrum which showed a peak at 1715 cm^{-1} that revealed the presence of amidic carbonyl group. All the spectral data and elemental analyses of these compounds were in agreement with the proposed structures (see Experiment).

On the other hand, morpholinohydrazinophenyl–butenone derivative **2a** was heated under reflux with thiourea **(5)** and gave the 6-(morpholino(2-phenyl–hydrazono)methyl)-4-phenyl-3,4-dihydropyrimidine-2(1*H*)-thione **(6)** (Scheme 2).

Furthermore, compound **6** with hydrazonoyl chlorides **7a–d** afforded the corresponding sulphur free compounds pyridotriazolopyrimidine **8a–d** (Scheme 2). IR spectrum for 1,5-dihydro-[1,2,4]triazolo[4,3-*a*]pyrimidinone derivative **8a**, as an example, gave peaks at 1650 and 3253 cm^{-1} that correspond to C=O and NH groups. Its ^{1}H-NMR spectrum revealed the presence of only one NH exchangeable hydrogen atom with D_2O. Structures of



Scheme 1. Synthesis of pyrazoles **3a–d**.



Scheme 2. Synthesis of triazolopyrimidines **8a–d**.

compounds **8a–d** were confirmed by spectral data and elemental analyses (see Experimental).

Compound **2a** was then used for preparation of azolopyrimidine compounds *via* its reactions with a variety of heterocyclic amines. For example, reaction of each of compound **2a** with 5-amino-1,2,4-triazole **9a** or 5-amino-1,2,3,4-tetrazole **9b** in the presence of a catalytic amount of acetic acid under reflux afforded triazolo[1,5-*a*]pyrimidine derivative **11**a and tetrazolo[1,5-*a*]pyrimidine derivative **11b**, respectively (Scheme 3). The structure assigned to these products was confirmed based on elemental analysis and spectroscopic methods (IR, ^{1}H NMR and mass spectra) (see Experimental). For example, the IR spectra of products **11a** revealed an absorption band at υ = 3247 due to hydrazine-NH group. Its ^{1}H NMR (DMSO-d_6) showed signals at δ = 2.32–2.34 (m, 4H, 2CH$_2$), 3.80–3.83 (m, 4H, 2CH$_2$), 7.00–7.46 (m, 10H, Ar-H), 8.01 (s, 1H, triazole-H3), 8.19 (s, 1H, pyrimidine-H5), 10.65 (brs, 1H, NH) ppm. The mass spectra of products **11a,b** revealed a molecular ion peak for each one which is consistent with the respective molecular weight.

The suggested mechanism for the formation of compounds **11a,b** was outlined in Scheme 3. It was postulated that the reaction of chalcone **2** with the appropriate heterocyclic amine starts with Michael addition (route a) of the amino group to the double bond of the enone residue of **2a** to give intermediate **10** which undergoes in situ dehydrative cyclization followed by auto-oxidation to give the final azolopyrimidine **11a,b**. The spectral data and elemental analyses data are consistent with the regioisomeric structures **11** and **13**. The isomeric compounds **13** produced by route b were discarded due to difficultness in condensation reaction than Michael addition (route a).[50,51]

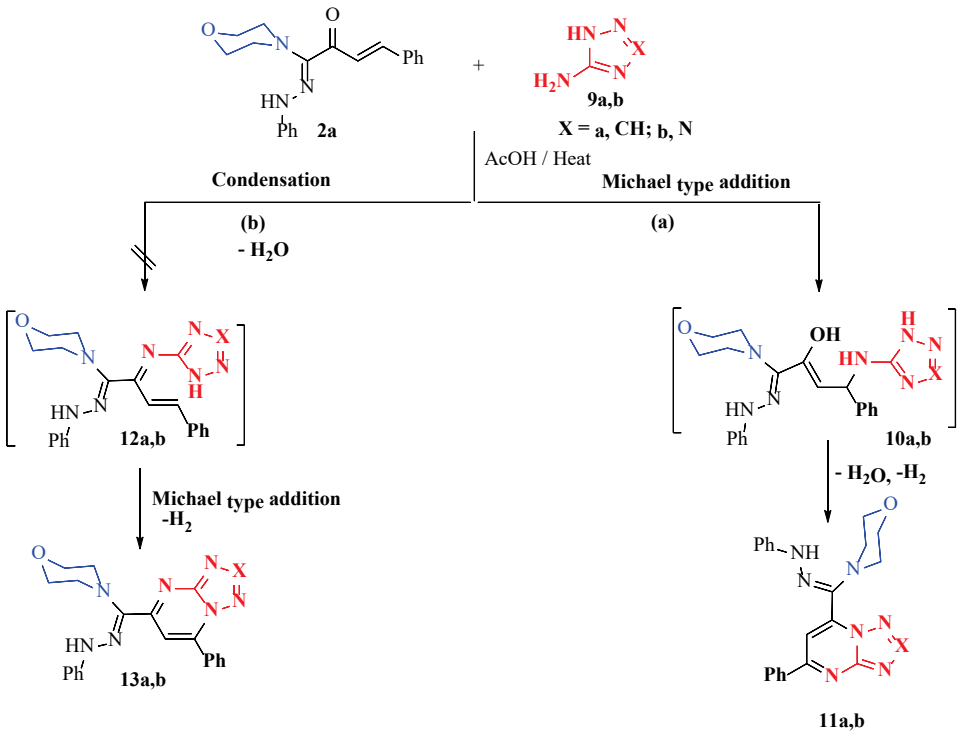
However, an equimolar amount of compound **2a** and 2-aminobenzoimidazole **14** were heated under reflux in ethanol containing a catalytic amount of acetic acid to give benzo[4,5]imidazo[1,2-*a*]pyrimidine derivative **15** (Scheme 4).

Similarly, compound **2a** reacted with pyrazolopyridine amine derivative **16** and yielded 2-phenylpyrido[2',3':3,4]pyrazolo[1,5-*a*]pyrimidine derivative **17** (Scheme 4).

Finally, compound **2a** was heated under reflux with 2-aminothiazole **16** and 2-aminobenzothiazole **20** to afford thiazolo[3,2-*a*]pyrimidine **19** and benzo[4,5]thiazolo[3,2-*a*]pyrimidine derivatives **21**, respectively (Scheme 4). All spectral data and elemental analyses were in agreement with the proposed structures of compounds **15**, **17**, **19** and **21** (see Experimental).

Cytotoxic Activity

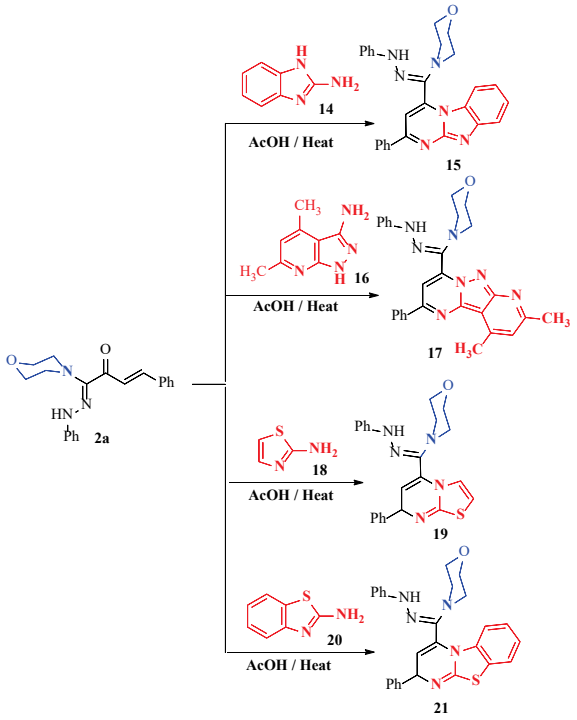
The *in vitro* growth inhibitory activity of the newly synthesized compounds **3a, 6, 8a–d, 11a,b, 15, 17, 19** and



Scheme 3. Synthesis of triazolo- and tetrazolopyrimidines**11a,b**.

21 was investigated against two carcinoma cell lines, human lung cancer cell line (A-549) and human hepatocellular carcinoma cell line (HepG-2), in comparison with the well-known anticancer standard drug (cisplatin) under the same conditions using colorimetric MTT assay. Data generated were used to plot a dose response curve of which the concentration of test compounds required to kill 50 % of cell population (IC_{50}) was determined. The results are depicted in Table 1 and revealed that the descending order of activity of the newly synthesized compounds towards the lung carcinoma cell line (A549) were as follow: **6** > **8c** > **17** > **11a** > **21** > **15** > **11b** > **8d** > **3a** > **19** > **8b** > **8a**.

The descending order of activity of the newly synthesized compounds towards the human Hepatocellular carcinoma cell line (HepG-2) were as follow: **6** > **8c** > **17** > **11a** > **21** > **15** > **8d** > **11b** > **19** > **3a** > **8b** > **8a**.



Scheme 4. Synthesis of fused pyrimidines **15, 17, 19** and **21**.

Structure Activity Relationship (SAR)

Examination of the SAR leads to the following conclusions.

The results revealed that all the tested compounds showed inhibitory activity to the tumor cell lines in a concentration dependent manner.

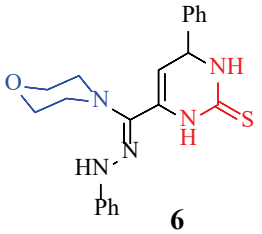
The activities of the synthesized compounds depend on the structural skeleton and electronic environment of the molecules.

Compounds **6** and **8c** were the most active (IC_{50} value of 2.81 ± 0.9, 3.45 ± 5.6 μg mL^{-1}, respectively) against the lung carcinoma cell line (A549), compared with cisplatin reference drug with IC_{50} value of 0.95±0.9 μg mL^{-1}. On the other hand, compounds **8a, 8b, 8d**, **3a** and **11b** have poor inhibitory activity (IC_{50} > 30 μg mL^{-1}) (Figure 1).

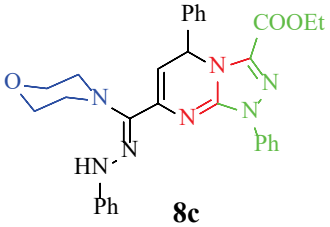
Compounds **6** and **17** were the most active (IC_{50} value of 2.99 ± 1.8, 5.69 ± 9.8 μg mL^{-1}, respectively) against the human hepatocellular carcinoma cell line (HepG-2), compared with cisplatin reference drug with IC_{50} value of 1.40 ± 1.1 μg mL^{-1}. On the other hand, compounds **8a, 8b** and **3a** have poor inhibitory activity (IC_{50} > 30 μg mL^{-1}) (Figure 1).

Among the fused pyrimidine derivatives, triazolopyrimidine **11a** is the most active one against the A549 (IC_{50} = 7.45 ± 0.7 μg mL^{-1}) while the pyridopyrazolo–pyrimidine **17** is the most active one against the HepG-2 cell line (A549) (IC_{50} = 7.45 ± 0.7 μg mL^{-1}).

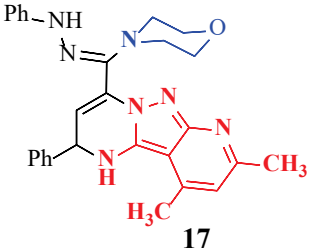
For triazolopyrimidine derivatives **8a–d**: compound **8c** (substituted with COOEt group at position 3) has *in vitro* inhibitory activity more than **8a** (substituted with $COCH_3$ group at position 3).



A-549: 2.81±0.9
HepG2: 2.99±1.8



A-549: 3.45±5.6
HepG2: 6.54±8.2



A-549: 7.33±7.5
HepG2: 5.69±9.8

For Cisplatin:
A-549: 0.95±0.9
HepG2: 1.4±1.1

Figure 1. The most active compounds compared to cisplatin.

Table 1. The *in vitro* inhibitory activity of tested compounds against tumor cell lines expressed as IC_{50} values (μg mL^{-1}) ± standard deviation from three replicates.

Tested compounds	Tumor cell lines		Tested compounds	Tumor cell lines	
	A-549	HepG2		A-549	HepG2
Cisplatin	0.95 ± 0.9	1.4 ± 1.1	**11a**	7.45 ± 0.7	9.78 ± 0.5
3a	32.6 ± 2.8	45.9 ± 3.1	**11b**	35.1 ± 10.8	20.1 ± 9.1
6	2.81 ± 0.9	2.99 ± 1.8	**15**	13.8 ± 1.4	19.0 ± 8.1
8a	326 ± 6.7	376 ± 10.2	**17**	7.33 ± 7.5	5.69 ± 9.8
8b	120 ± 12.1	159 ± 9.8	**19**	24.6 ± 11.8	25.5 ± 7.9
8c	3.45 ± 5.6	6.54 ± 8.2	**21**	9.0 ± 10.8	12.4 ± 8.9
8d	59.5 ± 0.6	24.7 ± 2.9			

CONCLUSION

In this context, a new series of morpholinyl-chalcones was prepared and used as a building block for constructing pyrazoles, 3,4-dihydropyrimidine-2(1*H*)-thione and azolo–pyrimidines *via* their reaction with phenylhydrazine thiourea and a number of heterocyclic amines, respectively. The assigned structures for all the newly synthesized compounds were confirmed on the basis of elemental analyses and spectral data and the mechanisms of their formation were also discussed. Some of the synthesized compounds were tested for *in vitro* activities against the human lung cancer cell lines (A-549) and human hepatocellular carcinoma cell lines (HepG-2) and the results revealed that compounds **6, 8c** and **17** have promising activities compared with cisplatin. Also, the SAR was studied.

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