http://dx.doi.org/10.5012/bkcs.2012.33.9.2985

Synthesis of New Heterocycles Derived from 3-(3-Methyl-1*H*-indol-2-yl)-3-oxopropanenitrile as Potent Antifungal Agents

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Received January 31, 2012, Accepted June 16, 2012

New thiazoline derivatives **7a-c**, and thiophenes **9a-c** linked to indole moiety were easily prepared *via* the reaction of the acrylamide derivative **3** with phenacyl bromides **4a-c**, depending on the reaction conditions. In addition, the reaction of compound **3** with hydrazonoyl chlorides **11a-f** afforded a series of 1,3,4-thiadiazole derivatives **13a-f**. Moreover, coupling of 3-(3-methyl-1*H*-indol-2-yl)-3-oxopropanenitrile (**2**) with the diazonium salts of 3-phenyl-5-aminopyrazole **16** or 3-amino-1,2,4-triazole **17** gave the corresponding hydrazones **18** and **19**, respectively. Cyclization of the latter hydrazones yielded the corresponding pyrazolo[5,1-*c*]-1,2,4-triazine and 1,2,4-triazolo[5,1-*c*]-1,2,4-triazine derivatives **20** and **21**, respectively. The structures of the synthesized compounds were assigned on the basis of elemental analysis, IR, ^{1}H NMR and mass spectral data. All the synthesized compounds were tested for *in vitro* activities against certain strains of fungi such as *Aspergillus niger*, *Aspergillus nodulans*, *Alternaria alternate*. Compounds showed marked inhibition of fungal growth nearly equal to the standards.

Key Words : 2-Cyanoacetyl-3-methyl-indole, Hydrazonoyl chlorides, Thiazoles, 1,3,4-Thiadiazoles, Antifungal activity

Introduction

The indole nucleus is probably one of the most widely distributed heterocyclic ring systems found in nature, since many of indole containing natural and synthetic products such as reserpine, vincristine, indolmicine, mitomycines, pindolol, dolasetrone mesylate, indomethacine and sumatriptan are being used for the treatment of various illnesses.[1]

Indole derivatives have been reported to exhibit antifungal,[2-7] antibacterial,[2-4,8,9] antiphage,[2] antiproliferative,[10] optimal inhibitory,[11] anticholinergic,[12] antiviral,[13] antitumor,[14] antiinflammatory,[15] and antihypertensive[16] activities and also as plant growth regulators.[17] In view of the above mentioned findings and as continuation of our efforts in the synthesis of new biologically active heterocycles,[18-22] we report herein the synthesis of some new heterocycles with the indole moieties.

Experimental Section

All melting points were measured on Electro thermal IA 9000 series digital melting point apparatus. The IR spectra were recorded in potassium bromide discs on a Pye Unicam SP 3300 and Shimadzu FT IR 8101 PC infrared spectrophotometer. The NMR Spectra were recorded at 270 MHz on a Varian Mercury VX-300 NMR spectrometer. ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) were run in deuterated dimethylsulphoxide (DMSO-d_6). Chemical shifts were related to that of the solvent. Mass Spectra were recorded on a Shimadzu GCMS-QP1000 EX mass spectrometer at 70 eV. Elemental analyses and the biological evaluation of the products were carried out at the Microanalytical Centre of Cairo University, Giza, Egypt. All reactions were followed by TLC (Silica gel, Aluminum Sheets 60 F254, Merck). Ethyl 3-methyl-1*H*-indole-2-caboxylate **1**[23] Phenacyl bromides **4a-c**[24] and hydrazonoyl chlorides **11a-f**[25,26] were prepared as reported in the literature.

3-(3-Methyl-1*H*-indol-2-yl)-3-oxopropanenitrile (2). To ethyl 3-methyl-1*H*-indole-2-carboxylate (**1**) (10 (19.9 g, 0.1 mol) and acetonitrile (4.1 mL, 0.1 mol) in dry benzene (250 mL) and dimethylformamide (10 mL), was added sodium hydride (4.8 g, 60%). The reaction mixture was refluxed for 4h, and then allowed to cool to room temperature. The solid formed was collected by filtration, washed with ether and dried. This material was dissolved in water and then neutralized with concentrated hydrochloric acid to pH 7. The precipitated product was collected by filtration, washed with water and dried. Recrystallisation from ethanol gave compound **2** in 63% yield as yellow solid, mp 148 °C; IR (KBr) ν (cm^{-1}) 3402 (NH), 2221 (CN), 1670 (CO); ^{1}H NMR ($CDCl_3$) δ 2.51, (s, 3H, indole CH_3), 3.66, (s, 2H, CH_2), 7.25-8.21 (m, 4H, ArH), 11.54 (s, 1H, indole NH); MS *m/z* (%): 199 (M^++1, 14), 198 (M^+, 100), 128 (32), 104 (35), 77 (62), 66 (45). Anal. calcd. for $C_{12}H_{10}N_2O$ (198.08): C, 72.71; H, 5.08; N, 14.13; Found. C, 72.53; H, 5.00; N, 13.92%.

3-Mercapto-2-(2-methyl-1*H*-indole-3-carbonyl)-3-phenylamino)acrylonitrile (3). To an ice-cooled suspension of finely powdered potassium hydroxide (1.1 g, 0.02 mol) in dry DMF (5 mL), 2-cyanoacetylindole **2** (1.98 g, 0.01 mol) and then the phenyl isothiocyanate (1.35, 0.01 mol) were added in portions with stirring. After complete addition, stirring was continued at room temperature for an over-

night. The reaction mixture was then poured onto ice/cold H_2O and acidified with 0.1 N HCl to pH 3-4. The obtained precipitate was filtered, washed with H_2O, dried, and crystallized from ethanol to give the acrylamide **2** in 85% yield as yellow solid, mp 156 ºC; IR (KBr) ν (cm^{-1}) 3402, 3220 (2NH), 2218 (CN), 1702 (CO); ^{1}H NMR ($CDCl_3$) δ 2.52, (s, 3H, indole CH_3), 7.31-8.24 (m, 9H, ArH), 9.36 (s, 1H , NH), 11.81 (s, 1H , indole NH), 14.14 (s, 1H, SH); MS *m/z* (%): 334 (M^++1, 7), 333 (M^+, 29), 248 (45), 165 (42), 104 (35), 77 (100), 66 (33). Anal. calcd. for $C_{19}H_{15}N_3OS$ (333.09): C, 68.45; H, 4.53; N, 12.60; Found. C, 68.35; H, 4.50; N, 12.48%.

Reaction of Acrylamide Derivative 3 with Phenacyl Bromides 4a-c in Absence of TEA.

General Procedure: A mixture of **3** (0.333 g, 1 mmol) and the appropriate phenacyl bromides **4a-c** (1 mmol) in ethanol (20 mL) was stirred at room temperature for 5 h. The reaction mixture was poured into 50 mL of cold water. The resultant solid products were collected by filtration and recrystallized from the proper solvent to give corresponding thiazole derivatives **7a-c**.

2-(3,4-Diphenylthiazol-2(3*H*)-ylidene)-3-(3-methyl-1*H*-indol-2-yl)-3-oxopropanenitrile (7a): Yield 78%; yellow solid; mp 288 °C. IR (KBr): ν 3402 (NH), 2216 (CN), 1674 (CO) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.53 (s, 3H, indole CH_3), 6.45 (s, 1H, thiazole C5-H), 6.98-7.58 (m, 14H, ArH), 11.83 (s, 1H, indole NH); MS *m/z* (%): 434 (M^++1, 11), 433 (M^+, 100), 319 (32), 130 (19), 77 (56), 51 (26). Anal. Calcd for $C_{27}H_{19}N_3OS$ (433.12): C, 74.80; H, 4.42; N, 9.69; Found C, 74.72; H, 4.32; N, 9.39%.

3-(3-Methyl-1*H*-indol-2-yl)-3-oxo-2-(3-phenyl-4-*p*-tolylthiazol-2(3*H*)-ylidene)propanenitrile (7b): Yield 76%; yellow solid; mp 292 °C. IR (KBr): ν 3402 (NH), 2219 (CN), 1670 (CO) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.23 (s, 3H, CH_3), 2.52 (s, 3H, indole CH_3), 6.44 (s, 1H, thiazole C5-H), 6.98-7.58 (m, 13H, ArH), 11.81 (s, 1H, indole-NH); MS *m/z* (%): 448 (M^++1, 5), 447 (M^+, 100), 319 (42), 130 (11), 77 (68), 51 (46). Anal. Calcd for $C_{28}H_{21}N_3OS$ (447.14): C, 75.14; H, 4.73; N, 9.39; Found C, 75.04; H, 4.79; N, 9.13%.

2-(4-(4-Chlorophenyl)-3-phenylthiazol-2(3*H*)-ylidene)-3-(3-methyl-1*H*-indol-2-yl)-3-oxopropanenitrile (7c): Yield 78%; yellow solid; mp 302 °C. IR (KBr): ν 3408 (NH), 2220 (CN), 1670 (CO) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.52 (s, 3H, indole CH_3), 6.45 (s, 1H, thiazole C5-H), 6.98-7.58 (m, 13H, ArH), 11.88 (s, 1H , indole NH); MS *m/z* (%): 469 (M^++2, 4), 468 (M^++1, 10), 467 (M^+, 100), 319 (32), 130 (27), 77 (54), 51 (56). Anal. Calcd for $C_{27}H_{18}ClN_3OS$ (467.09): C, 69.30; H, 3.88; N, 8.98; Found C, 69.13; H, 3.78; N, 8.78%.

Reaction of Acrylamide Derivative 3 with Phenacyl Bromides 4a-c in Presence of TEA.

General Procedure: To a mixture of **3** (0.333 g, 1 mmol) and the appropriate phenacyl bromides **4a-c** (1 mmol) in ethanol (20 mL), was added triethylamine (0.5 mL, 10 mmol) at room temperature. The reaction mixture was heated under reflux until all the starting material was consumed (2-4 h, monitored by TLC). The solid that formed, after cooling, was filtered and recrystallized from DMF to give corresponding thiophene derivatives **9a-c**.

5-Benzoyl-4-(3-methyl-1*H*-indol-2-yl)-2-(phenylamino)-thiophene-3-carbonitrile (9a): Yield 84%; yellow solid; mp 232 °C. IR (KBr): ν 3402, 3217 (2NH), 2218 (CN), 1691 (CO) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.53 (s, 3H, indole-CH_3), 6.98-7.58 (m, 14H, ArH), 9.32 (s, 1H, NH), 11.83 (s, 1H, indole NH); MS *m/z* (%): 434 (M^++1, 19), 433 (M^+, 58), 356 (21), 319 (14), 130 (9), 77 (100), 51 (26). Anal. Calcd for $C_{27}H_{19}N_3OS$ (433.12): C, 74.80; H, 4.42; N, 9.69; Found C, 74.72; H, 4.32; N, 9.39%.

4-(3-Methyl-1*H*-indol-2-yl)-5-(4-methylbenzoyl)-2-(phenylamino)thiophene-3-carbonitrile (9b): Yield 82%; yellow solid; mp = 246 °C. IR (KBr): ν 3402, 3234 (2NH), 2218 (CN), 1694 (CO) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.16 (s, 3H, CH_3), 2.53 (s, 3H, indole CH_3), 6.92-7.57 (m, 13H, ArH), 9.35 (s, 1H, NH), 11.83 (s, 1H, indole NH); MS *m/z* (%): 448 (M^++1, 11), 447 (M^+, 48), 356 (41), 319 (12), 77 (100), 51 (32). Anal. Calcd for $C_{28}H_{21}N_3OS$ (447.14): C, 75.14; H, 4.73; N, 9.39; Found C, 75.00; H, 4.57; N, 9.18%.

5-(4-Chlorobenzoyl)-4-(3-methyl-1*H*-indol-2-yl)-2-(phenylamino)thiophene-3-carbonitrile (9c): Yield 80%; yellow solid; mp = 258 °C. IR (KBr): ν 3400, 3232 (2NH), 2218 (CN), 1694 (CO) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.53 (s, 3H, indole CH_3), 6.92-7.78 (m, 13H, ArH), 9.36 (s, 1H, NH), 11.87 (s, 1H, indole NH); MS *m/z* (%): 469 (M^++2, 3), 468 (M^++1, 11), 467 (M^+, 26), 319 (42), 77 (100), 51 (22). Anal. Calcd for $C_{27}H_{18}ClN_3OS$ (467.09): C, 69.30; H, 3.88; N, 8.98; Found C, 69.13; H, 3.78; N, 8.78%.

Reaction of the Thioacetanilide Derivative 3 with Hydrazonoyl Chlorides 11a-f. To a solution of the thioacetanilide derivative **3** (0.333 g, 1 mmol) in absolute ethanol (20 mL), the appropriate hydrazonoyl chlorides **11a-f** (1 mmol) were added, in the presence of triethylamine (0.3 mL). The reaction mixture was refluxed for 4 h and then allowed to cool. The formed solid product was filtered off, washed with ethanol and recrystallized from EtOH/DMF to afford the corresponding thiadiazole derivatives **13a-f**.

2-(5-Acetyl-3-phenyl-1,3,4-thiadiazol-2(3*H*)-ylidene)-3-(3-methyl-1*H*-indol-2-yl)-3-oxopropanenitrile (13a): Yield 86%; pale yellow solid; mp 230 °C. IR (KBr): ν 3348 (NH), 2206 (CN), 1678, 1646 (2CO) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.31 (s, 3H, CH_3), 2.54 (s, 3H, indole CH_3), 6.98-7.97 (m, 9H, ArH), 11.88 (s, 1H, indole NH); MS *m/z* (%): 401 (M^++1, 8), 400 (M^+, 100), 356 (21), 319 (22), 128 (16), 77 (66), 51 (12). Anal. Calcd for $C_{22}H_{16}N_4O_2S$ (400.10): C, 65.98; H, 4.03; N, 13.99. Found C, 65.76; H, 4.00; N, 13.69%.

2-(5-Acetyl-3-*p*-tolyl-1,3,4-thiadiazol-2(3*H*)-ylidene)-3-(3-methyl-1*H*-indol-2-yl)-3-oxopropanenitrile (13b): Yield 82%; pale yellow solid; mp = 238 °C. IR (KBr): ν 3352 (NH), 2206 (CN), 1678, 1652 (2CO) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.11 (s, 3H, CH_3), 2.31 (s, 3H, CH_3), 2.54 (s, 3H, indole CH_3), 6.98-7.92 (m, 8H, ArH), 11.83 (s, 1H, indole NH); MS *m/z* (%): 415 (M^++1, 12), 414 (M^+, 100), 356 (25), 319 (29), 105 (42), 77 (46), 51 (12). Anal. Calcd for $C_{23}H_{18}N_4O_2S$ (414.12): C, 66.65; H, 4.38; N, 13.52. Found C, 66.58; H, 4.31; N, 13.45%.

2-(5-Acetyl-3-(4-chlorophenyl)-1,3,4-thiadiazol-2(3*H*)-ylidene)-3-(3-methyl-1*H*-indol-2-yl)-3-oxopropanenitrile (13c): Yield 88%; pale yellow solid; mp 243 °C. IR (KBr): ν 3350 (NH), 2206 (CN), 1677, 1651 (2CO) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.30 (s, 3H, CH_3), 2.52 (s, 3H, indole CH_3), 6.96-7.98 (m, 8H, ArH), 11.87 (s, 1H, indole NH); MS *m/z* (%): 435 (M^++2, 14), 434 (M^++1, 43), 433 (M^+, 100), 356 (33), 319 (19), 281 (13), 105 (42), 77 (53), 51 (9). Anal. Calcd for $C_{22}H_{15}ClN_4O_2S$ (434.06): C, 60.76; H, 3.48; N, 12.88. Found C, 60.66; H, 3.42; N, 12.59%.

Ethyl 5-(1-Cyano-2-(3-methyl-1*H*-indol-2-yl)-2-oxoethylidene)-4-phenyl-4,5-dihydro-1,3,4-thiadiazole-2-carboxylate (13d): Yield 72%; yellow solid; mp 162 °C. IR (KBr): ν 3402 (NH), 2210 (CN), 1708, 1652 (2CO) cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.38 (t, 3H, CH_3, J = 7.4 Hz), 2.51 (s, 3H, indole CH_3), 4.26 (q, 2H, CH_2, J = 7.4 Hz), 7.10-7.96 (m, 9H, ArH), 11.76 (s, 1H, indole NH); MS *m/z* (%): 431 (M^++1, 21), 430 (M^+, 35), 337 (100), 164 (23), 106 (41), 77 (26). Anal. Calcd for $C_{23}H_{18}N_4O_3S$ (430.11): C, 64.17; H, 4.21; N, 13.01. Found C, 63.13; H, 4.09; N, 12.93%.

Ethyl 5-(1-Cyano-2-(3-methyl-1*H*-indol-2-yl)-2-oxoethylidene)-4-*p*-tolyl-4,5-dihydro-1,3,4-thiadiazole-2-carboxylate (13e): Yield 74%; yellow solid; mp 168 °C. IR (KBr): ν 3402 (NH), 2212 (CN), 1708, 1651 (2CO) cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.38 (t, 3H, CH_3, J = 7.4 Hz), 2.33 (s, 3H, CH_3), 2.51 (s, 3H, indole CH_3), 4.28 (q, 2H, CH_2, J = 7.4 Hz), 7.10-7.96 (m, 8H, ArH), 11.77 (s, 1H, indole NH); MS *m/z* (%): 445 (M^++1, 26), 444 (M^+, 100), 371 (17), 313 (21), 158 (29), 105 (43), 77 (29). Anal. Calcd for $C_{24}H_{20}N_4O_3S$ (444.13): C, 64.85; H, 4.54; N, 12.60. Found C, 64.68; H, 4.44; N, 12.51%.

Ethyl 4-(4-Chlorophenyl)-5-(1-cyano-2-(3-methyl-1*H*-indol-2-yl)-2-oxoethylidene)-4,5-dihydro-1,3,4-thiadiazole-2-carboxylate (13f): Yield 78%; yellow solid; mp 178 °C. IR (KBr): ν 3402 (NH), 2218 (CN), 1712, 1654 (2CO) cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.39 (t, 3H, CH_3, J = 7.4 Hz), 2.52 (s, 3H, indole CH_3), 4.31 (q, 2H, CH_2, J = 7.4 Hz), 7.13-7.96 (m, 8H, ArH), 11.82 (s, 1H, indole NH); MS *m/z* (%): 446 (M^++2, 12), 445 (M^++1, 26), 444 (M^+, 100), 371 (17), 313 (21), 158 (29), 105 (43), 77 (29). Anal. Calcd for $C_{23}H_{17}ClN_4O_3S$ (464.07): C, 59.42; H, 3.69; 7.63; N, 12.05. Found C, 59.32; H, 3.62; 7.63; N, 11.95%.

Synthesis of Hydrazones 18 and 19.

General Procedure: To a stirred cold solution of compound **2** (0.37 g, 2 mmol) in pyridine (25 mL) was added the diazonium salts of 3-phenyl-5-aminopyrazole **16** or 3-amino-1,2,4-triazole **17** (2 mmol) portion-wise over a period of 30 min at 0-5 °C. The reaction mixture was kept in an ice box overnight and then diluted with water. The solid that precipitated was filtered off, washed with water and dried. Recrystallization from ethanol/DMF gave the corresponding hydrazones **18** and **19**, respectively.

2-(3-Methyl-1*H*-indol-2-yl)-2-oxo-*N*′-(4-phenyl-1*H*-pyrazol-5-yl)acetohydrazonoyl cyanide (18): Yield 80%; yellow solid; mp 270 °C. IR (KBr): ν 3420, 3226, 3198 (3NH), 2218 (CN), 1624 (CO) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.50 (s, 3H, indole CH_3), 6.92-7.88 (m, 10H, ArH and pyrazole H), 7.96 (s, 1H, NH), 11.89 (s, 1H, indole NH), 12.03(s, 1H, hydrazone NH); MS *m/z* (%): 369 (M^++1, 12), 368 (M^+, 19), 210 (100), 158 (53), 130 (42), 91 (100), 77 (32). Anal. Calcd for $C_{21}H_{16}N_6O$ (368.14): C, 68.47; H, 4.38; N, 22.81. Found C, 68.38; H, 4.29; N, 22.74%.

2-(3-Methyl-1*H*-indol-2-yl)-2-oxo-*N*′-(1*H*-1,2,4-triazol-5-yl)acetohydrazonoyl Cyanide (19): Yield 84%; yellow solid; mp 292 °C. IR (KBr): ν 3421, 3220, 3190 (3NH), 2218 (CN), 1624 (CO) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.52 (s, 3H, indole CH_3), 7.12-7.91 (m, 5H, ArH and triazole H), 7.80 (s, 1H, NH), 11.88 (s, 1H, indole NH), 12.06 (s, 1H, hydrazone NH); MS *m/z* (%): 294 (M^++1, 11), 293 (M^+, 100), 164 (23), 130 (24), 77 (34). Anal. Calcd for $C_{14}H_{11}N_7O$ (293.10): C, 57.33; H, 3.78; N, 33.43. Found C, 57.14; H, 3.69; N, 33.23%.

Cyclization of Hydrazones 18 and 19. A solution of compound **18** or **19** (0.01 mol) in pyridine (20 mL, 99%) was refluxed for 3 h, cooled, poured onto ice-water to give a precipitate, which was filtered off, dried and recrystallized from DMF to afford compounds **20** and **21**, respectively.

(4-Amino-8-phenylpyrazolo [5,1-*c*][1,2,4]triazin-3-yl) (3-methyl-1*H*-indol-2-yl)methanone (20): Yield 78%; yellow solid; mp 312 °C. IR (KBr): ν 3402-3200 (NH and NH_2), 1626 (CO) cm^{-1}. ^1H NMR (DMSO-d_6) δ 2.51 (s, 3H, indole-CH_3), 6.92 (s, 2H, NH_2), 7.01-7.91 (m, 10H, ArH and pyrazole H), 11.82 (s, 1H, indole NH). MS *m/z* (%): 369 (M^++1, 17), 368 (M^+, 100), 197 (26), 158 (62), 77 (48). Anal. Calcd for $C_{21}H_{16}N_6O$ (368.14): C, 68.47; H, 4.38; N, 22.81. Found C, 68.37; H, 4.27; N, 22.62%.

(4-Amino-[1,2,4]triazolo[5,1-*c*][1,2,4]triazin-3-yl)(3-methyl-1*H*-indol-2-yl)methanone (21): Yield 74%; yellow solid; mp 342 °C. IR (KBr): ν 3402-3200 (NH and NH_2), 1626 (CO) cm^{-1}. ^1H NMR (DMSO-d_6) δ 2.52 (s, 3H, indole-CH_3), 6.88 (s, 2H, NH_2), 7.12-7.91 (m, 5H, ArH and triazole H), 11.89 (s, 1H, indole NH). MS *m/z* (%): 294 (M^++1, 15), 293 (M^+, 72), 210 (22), 130 (100), 77 (24). Anal. Calcd for $C_{14}H_{11}N_7O$ (293.10): C, 57.33; H, 3.78; N, 33.43. Found C, 57.14; H, 3.69; N, 33.23%.

Antifungal Activity Assay. Compounds **7a-c** and **13a-f** were assayed for their antifungal activity against *Candida albicans* (ATCC 10231), *Aspergillus fumigatus* (HIC 6094), *Trichophytonrubrum* (IFO 9185) and *Trichophyton mentagrophytes* (IFO 40996) in DMSO by disc diffusion, broth dilution methods.[27]

For the antifungal assay, Sabourands agar media was prepared by dissolving peptone (1 g), D-glucose (4 g) and agar (2 g) in distilled water (100 mL) and adjusting the pH to 5.7. Normal saline was used to make a suspension of spore of fungal strain for lining. A loopful of particular fungal strain was transferred to 3 mL saline to get a suspension of corresponding species. Twenty milliliters of agar media was poured into each petri-dish, excess of suspension was decanted and the plates were dried by placing in an incubator at 37 °C for 1 h. Using an agar punch wells were made and each well was labeled. A control was also prepared in triplicate and maintained at 37 °C for 3-4 d. The *C. albicans* was grown for 48 h at 28 °C in YPD broth (1% yeast extract, 2%

Table 1. Antifungal Activity of Compounds **7a-c** and **13a-f**

Compound	Minimal inhibitory concentration in μg/mL (zone of inhibition in mm)[a]			
	C. albicans	*A. fumigatus*	*T. rubrum*	*T. mentagrophytes*
7a	26 (10)	30 (9)	35 (12)	28 (16)
7b	25 (15)	20 (16)	25 (17)	22 (16)
7c	18 (17)	22 (16)	24 (16)	28 (9)
13a	22 (12))	28 (9)	32 (11)	26 (8)
13b	25 (10)	25 (8)	36 (10)	25 (15)
13c	30 (10)	32 (10)	26 (10)	30 (12)
13d	14 (23)	18 (22)	20 (18)	18 (21)
13e	30 (12)	30 (10)	40 (14)	30 (14)
13f	15 (21)	19 (20)	22 (19)	18 (23)
Fluconazole	16 (22)	18 (20)	20 (22)	16 (20)

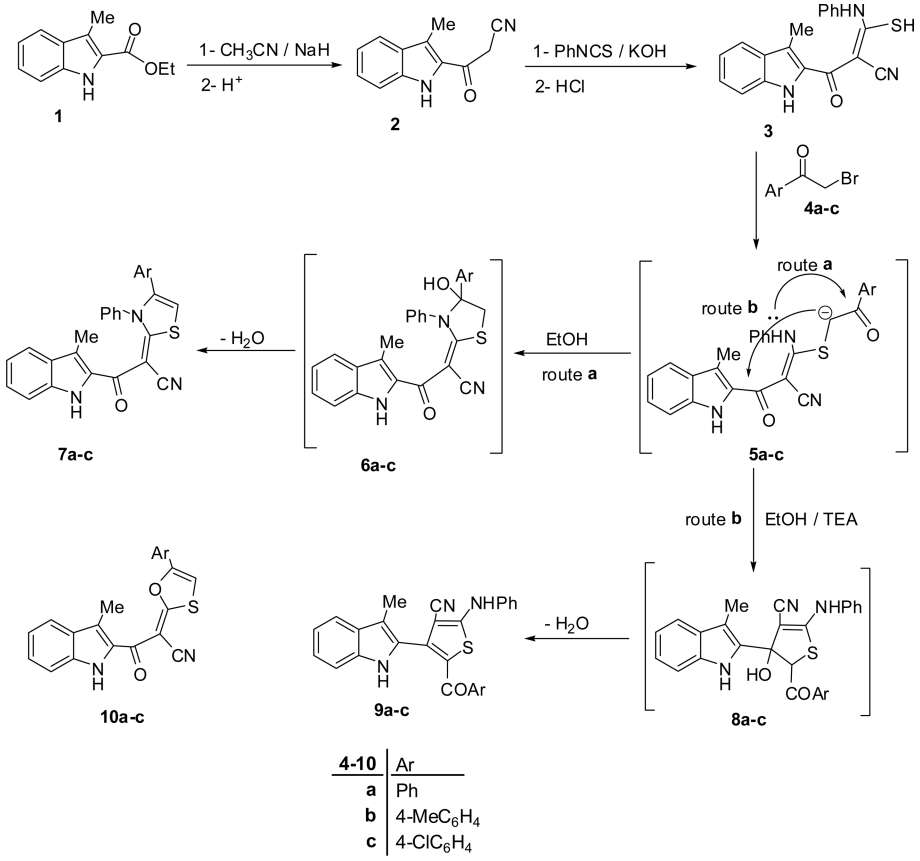
[a]The values in parentheses indicate the zone of inhibition.

peptone and 2% dextrose), harvested by centrifugation and then washed twice with sterile distilled water. *A. fumigatus*, *T. rubrum* and *T. mentagrophytes* were plated in potato dextrose agar (PDA) (Difco) and incubated at 28 °C for two weeks. Spores were washed three times with sterile distilled water and resuspended in distilled water to obtain an initial inoculums size of 105 spores/mL. Each test compound was dissolved in DMSO and diluted with potato dextrose broth (Difco) to prepare serial two-fold dilutions in the range 100 to 0.8 μg/mL. Ten microliters of the broth containing about 10^3 (for yeast) and 10^4 (for filamentous fungi) cells/mL of test fungi was added to each well of a 96-well microtiter plate. Culture plates were incubated for about 48-72 h at 28 °C. The inhibition zone and minimal inhibitory concentration (MIC) were determined and compared with the standard drug fluconazole (Table 1).

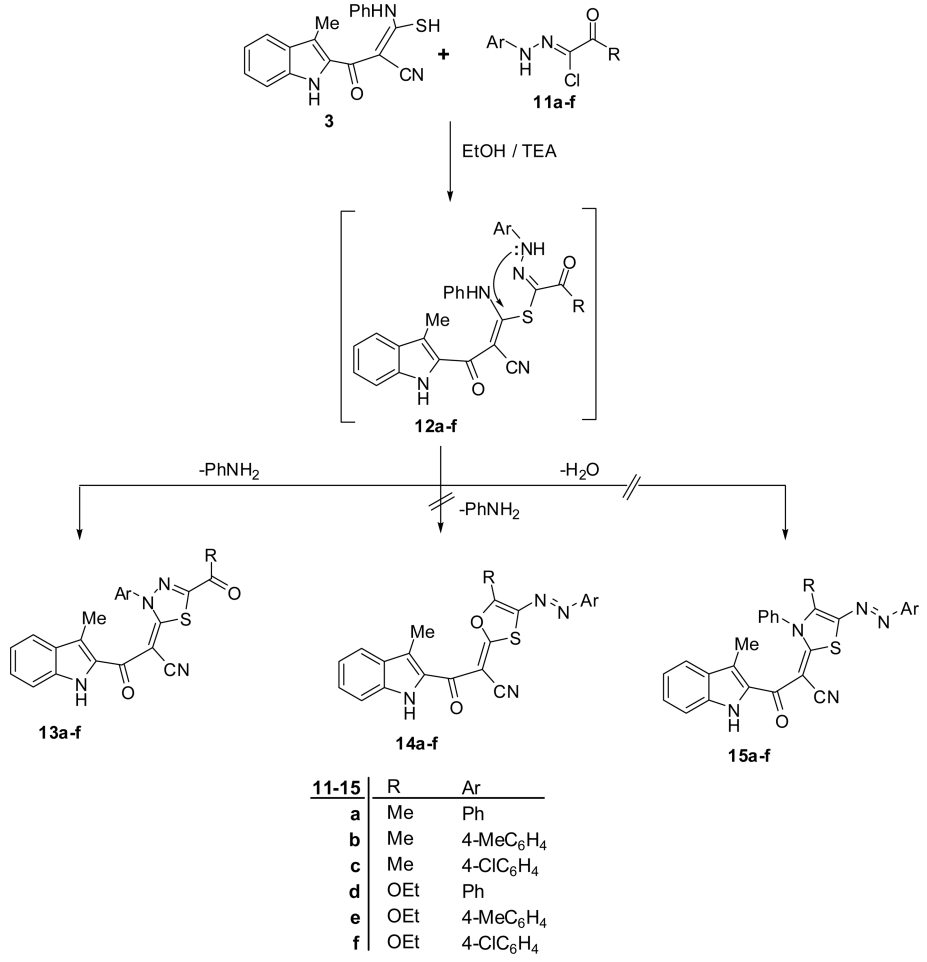
Results and Discussion

Refluxing the mixture of ethyl 2-methyl-1*H*-indole-3-carboxylate (**1**) and acetonitrile in the presence of sodium hydride in dry benzene afforded the cyanoacetyl derifatives **2** (Scheme 1). The base-catalyzed addition of cyanoacetyl derivative **2** to phenyl isothiocyanate in dry DMF at room temperature followed by acidification with dilute HCl afforded the corresponding acrylamide derivative **3**. The structure of compound **3** was elucidated on the basis of spectroscopic data and microanalysis. For example, the mass spectrum of **3** showed the molecular ion peak at *m/z* (%) = 333 (M^+, 29). The ^{1}H NMR spectrum showed the disappearance of singlet signal assignable for methylene group in the precursor **2**, and the appearance of D_2O exchangeable NH and SH singlet signals at δ 9.36 and δ 14.14 ppm, respectively.

Reaction of **3** with the appropriate phenacyl bromide **4a-c** in ethanol without basic catalyst produced the corresponding thiazoline derivatives **7a-c** rather than **9a-c** or **10a-c** (Scheme 1). On the other hand, it has been found that when



Scheme 1. Reaction of acrylamide derivative **3** with phenacyl bromides **4a-c**.



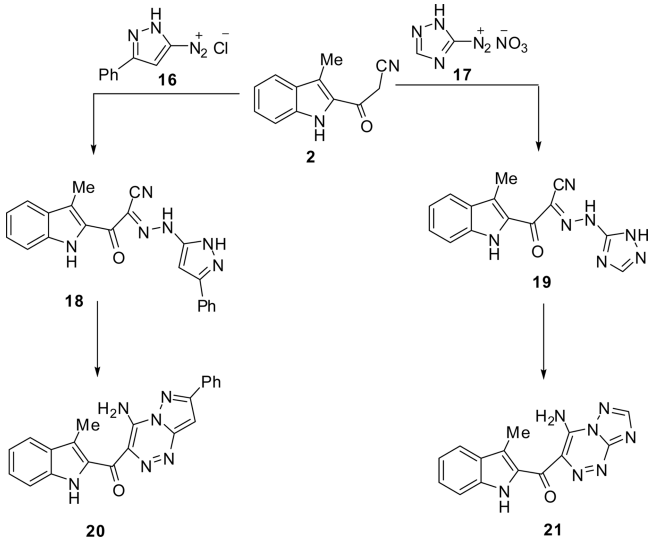
11-15	R	Ar
a	Me	Ph
b	Me	4-MeC_6H_4
c	Me	4-ClC_6H_4
d	OEt	Ph
e	OEt	4-MeC_6H_4
f	OEt	4-ClC_6H_4

Scheme 2. Reaction of acrylamide derivative **3** with hydrazonoyl halides **11a-f**.

phenacyl bromides **4a-c** reacted with **3** in boiling ethanol containing a catalytic amount of triethylamine afforded the thiophene derivatives **9a-c**. The formation of **7a-c** is assumed to proceed *via* first elimination of HBr to give the open acyclic intermediate **5a-c**. Dehydration of these intermediates under the employed reaction conditions gave the isolated products **7a-c**. The structural assignments of **7a-c** and **9a-c** were based on analytical and spectral data. The ^{1}H-NMR spectra of compound **7a-c** revealed singlet signal at δ 6.45 due to the thiazole-H_5 proton. The mass spectra of these products **7a-c** and **9a-c** showed the molecular ion peaks at the expected *m/z* values (see Experimental section). These results were consistent with previous report on the reaction of various acrylamide derivatives with phenacyl bromides.[28-30]

The reaction of **3** with hydrazonoyl chlorides **11a-f** in refluxing ethanol and in the presence of a catalytic amount of triethylamine afforded the corresponding 1,3,4-thiadiazole derivatives **13a-f** rather than the compounds **14a-f** and/or **15a-f** (Scheme 2). These results indicate that the reaction of **3** with hydrazonoyl chlorides **11a-f** proceeds *via* the loss of HCl followed by elimination of aniline molecule from the non-isolable intermediate **12a-f**, respectively. The formation of the latter yielded the final products **13a-f**. The formation of the latter 1,3,4-thiadiazoles is in the line with previous reports.[31]

The structures of **13a-f** were confirmed on the basis of spectroscopic data and elemental analyses. For example, the ^{1}H NMR spectra showed the disappearance of D_2O exchange-



Scheme 3. Synthesis of pyrazolotriazine and triazolotriazine derivatives **20** and **21** from **2**.

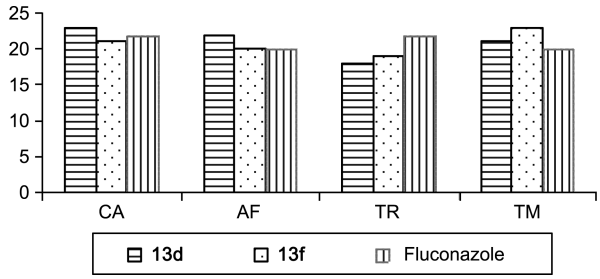


Figure 1. Comparison of antifungal activity (MIC Values) of selected compounds with Fluconazole d *C. albicans* (*CA*), *A. fumigatus* (*AF*), *T. rubrum* (*TR*), *T. mentagrophytes* (*TM*).

able NH and SH singlet signals at δ 9.36 and δ 14.14 ppm, and the appearance of $COCH_3$ or COOEt signals at δ 2.11-2.30 and δ 1.38, 4.26-4.31 ppm, respectively. Their IR spectra showed the disappearance of the NH group, and revealed in each case a carbonyl band in the region 1677 or 1708 cm^{-1}.

Moreover, coupling of the title compound **2** with the diazonium chloride of 3-phenyl-5-aminopyrazole **16** or diazonium nitrate of 3-amino-1,2,4-triazole **17** at 0-5 °C,[32,33] gave the corresponding hydrazones **18** or **19**, respectively (Scheme 3). Cyclization of the hydrazones **18** or **19** was accomplished under boiling pyridine to yield the corresponding fused triazines **20** or **21**, respectively.

The structure of the hydrazones **18** and **19** were elucidated on the basis of their spectral data (IR, MS and ^{1}H NMR) and also by independent cyclization into **20** and **21**, respectively (Scheme 3). Compounds **18** and **19** underwent an intramolecular cyclization upon boiling in pyridine *via* the addition of their endocyclic NH to the triple bond of a nitrile function to afford the corresponding pyrazolo[5,1-*c*]-1,2,4-triazine and 1,2,4-triazolo[5,1-*c*]-1,2,4-triazine derivatives **20** and **21**. Their IR spectra revealed the disappearance of band corresponding to nitrile absorption and the presence of amino and carbonyl functions, whereas their ^{1}H NMR showed the appearance of a D_2O exchangeable signal due to the amino functions.[32,33]

Antifungal Screening. The antifungal screening data reveals that many of the newly synthesized compounds were active with moderate to good antifungal activity (Table 1). The compounds **13d** and **13f** showed the highest activity towards all tested strains. The compound **7c** showed good antifungal activity towards *C. albicans*, *A. fumigatus* and *T. rubrum*, and the compound **7b** also showed potent activity towards *A. fumigatus*, *T. rubrum* and *T. mentagrophytes*. The comparison of MIC values of the selected compounds **13a-f** and standard drug against different fungi is presented in Figure 1.

Conclusions

In conclusion, a new series of heterocyclic compounds, thiazoline **7a-c**, thiophenes **9a-c** and 1,3,4-thiadiazole derivatives **13a-f**, were synthesized and assayed for their antifungal activity. Among them, the compounds **13d**, **13f**, **7c** and **7b** exhibited potent inhibitory activity towards all tested fungi.

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