

Synthesis, (in vitro) Antitumor and Antimicrobial Activity of some Pyrazoline, Pyridine, and Pyrimidine Derivatives Linked to Indole Moiety

Ekhlass Nassar

Department of Chemistry, Faculty of Women for Arts, Science and Education, Ain Shams University, Asma Fahmy Street, Heliopolis, Cairo, Egypt.
ekhlass_nassar@hotmail.com

Abstract: Aldol condensation reaction between 3-indolaldehyde 1 and 4-methoxyacetophenone 2 afforded chalcone compounds 3. This compound was reacted with some different reagents such as hydrazine hydrate, phenyl hydrazine, thiosemicarbazide, hydroxylamine, ethyl cyanoacetate, urea and thiourea to give pyrazolines 4a, 4b, 5a, 5b, 6, oxazoline 7, Michael adduct 8, pyranone 9, and oxo 14a and thiopyrimidine derivatives 14b, respectively. The structures of all the compounds were confirmed by microanalyses and various spectral data. Some of the synthesized new compounds were screened against antitumor and antimicrobial activity. [Journal of American Science. 2010;6(8):338-347]. (ISSN: 1545-1003).

Keywords: Heterocycles, cyclizations, pyrazolines, pyridines, antitumor activity.

1. Introduction

Indole nucleus is incorporated in various natural products such as alkaloids and represented a promising structural class of marine alkaloids based upon their high degree of biological activity 1-5. Indole nucleus was reported to possess a wide variety of biological properties such as anti-tumor 6-10, anti-inflammatory 11-13, anti-convulsant 14, cardiovascular 15, and anti-bacterial 16. In addition, the substitution at 3-position of the indole ring connecting an extra heterocyclic ring such as imidazole (topsentins 17 and nortopsentins 18); dihydroimidazole (discodermindole 3); oxazole (martefrgin 4 amazol 19); oxadiazine (alboinon 20); (didemidines 21) furnished potent agents. Also pyrazole 22, 23, pyridine 24 and pyrimidine 25 derivatives were found to possess a variety of biological activities. Considering the above observations and in connection to previous publications involving the synthesis of new biologically active hetero cycles 26-29. I hope to report here in the synthesis of new 3-substituted indoles incorporating an extra heterocyclic rings such as pyrazole, pyridine and pyrimidine to evaluate their antitumor (anti-proliferative) activities against both of human breast cell line MCF- 7 and liver carcinoma cell line HEPG2 and their antimicrobial activities.

2. Experimental Chemistry

Melting points were determined in open glass capillaries and are uncorrected. Elemental analyses were carried out in the Micro Analytical

Laboratory, Cairo University, Cairo, Egypt, IR spectra of the compounds were recorded on a Perkin-Elmer; spectrophotometer model 1430 using potassium bromide pellets and frequencies were reported in cm^{-1} . The mass spectra were recorded using mass spectrometer HP model MS5988 EI 70eV. ^1H NMR spectra were measured on Varian Mercury 300 MHz spectrometer and chemical shifts were expressed in ppm using TMS as internal standard. Reactions were routinely followed by (TLC).

3-(1H-Indol-3-yl)-1-(4-methoxyphenyl)prop-2-en-1-one 3.

To a stirred mixture of 14.5 g indolaldehyde 1 (100 mmol) and 15.0 g 4-methoxyacetophenone 2 (100 mmol) in 200 mL ethanol at room temperature, 40% NaOH aqueous solution was added portion-wise after which stirring was continued for further 2 hr. The pale yellow precipitate formed was filtered and washed with 3% aqueous HCl, and crystallized from ethanol to give chalcone 3 in 75% yield, mp 124-126°C. IR (KBr): 3162 (NH), 1633 (C=O) cm^{-1} ; ^1H NMR (300 MHz, DMSO-d_6): 3.83 (s, 3H, -OCH₃), 7.01-7.05 (m, 2H, Ar-H), 7.24-7.27 (m, 4H, Ar-H), 7.49-7.52 (m, 2H, Ar-H), 7.53 (d, 1H, $J = 12.9$ Hz, (C=O)(CH=C), 7.92 (d, 1H, $J = 12.9$ Hz (C=O)(C=CH), 8.11-8.30 (m, 1H, Ar-H), 12.3 (s, D₂O-exchangeable, 1H, indole NH) ppm; MS (70 eV): $m/z = 278$ ($\text{M}^+ + 1$, 4.7), 277 (M^+ , 15.0), 144 (100), 116 (39.5), 89 (37.3), 62 (38.6). Anal. Calcd. for C₁₈H₁₅NO₂: C, 77.96; H, 5.45; N, 5.05 Found: C, 78.24; H, 5.59; N, 4.93.

3-(4,5-Dihydro-3-(4-methoxyphenyl)-1H-pyrazol-5-yl)-1H-indole 4a.

To a solution of 2.77 g chalcone **3** (10 mmol) in 50 mL of ethanol, 1.0 mL of hydrazine hydrate (80%) was added and the reaction mixture was refluxed for 4 hr, cooled to -10 °C and left over night. The solid mass separated out was filtered, washed with cold ethanol and crystallized from ethanol to afford pyrazoline derivative **4a** as pale yellow crystals in 78% yield, mp 258-260°C; IR (KBr): $\bar{\nu}$ = 3215, 3170 (2NH), 1618 (C=N) cm^{-1} ; $^1\text{H NMR}$ (300 MHz, DMSO- d_6): = 1.97 (m, 1H), 2.64 (m, 1H), 3.39 (m, 1H), 3.75 (s, 3H, -OCH₃), 6.86-8.36 (m, 9H, Ar-H), 8.90 (s, D₂O-exchangeable, 1H, pyrazoline NH), 11.65 (s, D₂O-exchangeable, 1H, indole NH) ppm; MS (70 eV): m/z = 291 (M^+ , 7.3), 286 (94.9), 257 (100), 142 (36.3), 116 (45.4), 89 (47.2). Anal. Calcd. for C₁₈H₁₇N₃O: C, 74.20; H, 5.88; N, 14.42. Found: C, 74.42; H, 5.67; N, 14.38.

3-(4,5-Dihydro-3-(4-methoxyphenyl)-1-phenyl-1H-pyrazol-5-yl)-1H-indole 4b.

To a solution of 2.77 g of chalcone **3** (10 mmol) in 50 mL of ethanol, 5 mL of acetic acid and 1.1 g of phenyl hydrazine (10 mmol) was added. The reaction mixture was refluxed for 8 hr and left over night. The solid mass separated out was filtered off, washed with ethanol and crystallized from ethanol to give compound **4b** as pale brown crystals in 67% yield, mp 258-260°C; IR (KBr): $\bar{\nu}$ = 3169 (NH), 1633 (C=N) cm^{-1} ; $^1\text{H NMR}$ (300 MHz, DMSO- d_6): = 2.00 (m, 1H), 2.51 (m, 1H), 3.11 (m, 1H), 3.35 (s, 3H, -OCH₃), 6.85-7.00 (m, 2H, Ar-H), 7.18-7.21 (m, 2H, Ar-H), 7.24-7.26 (m, 1H, Ar-H), 7.27-7.30 (m, 4H, Ar-H), 7.47-7.49 (m, 2H, Ar-H), 8.32 (s, 1H, Ar-H), 12.15 (s, D₂O-exchangeable, 1H, indole NH) ppm; MS (70 eV): m/z = 367 (M^+ +1, 3.5), 368 (M^+ , 2.8), 145 (100), 221 (10.7). Anal. Calcd for C₂₄H₂₁N₃O: C, 78.45; H, 5.76; N, 11.44. Found: C, 78.43; H, 5.59; N, 11.48.

Synthesis of pyrazolines 5a, b

This reaction was carried out by the same procedure described in the synthesis of compound **4a** by using acetic acid instead of ethanol in case of **5a** and propanoic acid in case of **5b**.

1-(4,5-Dihydro-5-(1H-indol-3-yl)-3-(4-methoxyphenyl)pyrazol-1-yl)ethanone 5a.

Pale yellow crystals, mp 288-290°C, yield 77%; IR (KBr): $\bar{\nu}$ = 3169 (NH), 1686 (C=O), 1627 (C=N) cm^{-1} ; $^1\text{H NMR}$ (300 MHz, DMSO- d_6): = 1.93 (m, 1H), 2.24 (s, 3H, -CH₃), 2.49 (m, 1H), 3.36 (s, 3H, -OCH₃), 3.83 (m, 1H), 7.14-7.26 (m, 4H, Ar-H), 7.27-7.49 (m, 2H, Ar-H), 7.90-7.92 (m, 1H, Ar-

H), 8.16-8.36 (m, 2H, Ar-H), 11.68 (s, D₂O-exchangeable, 1H, indole NH) ppm; MS (70 eV): m/z = 334 (M^+ +1, 2.4), 333 (M^+ , 2.9), 286 (100), 257 (95.4), 116 (28.0). Anal. Calcd for C₂₀H₁₉N₃O₂: C, 72.05; H, 5.74; N, 12.60. Found: C, 71.87; H, 5.66; N, 12.49.

1-(4,5-Dihydro-5-(1H-indol-3-yl)-3-(4-methoxyphenyl)pyrazol-1-yl)propan-1-one 5b.

Yellow powder, mp 280-281°C, yield 73%; IR (KBr): $\bar{\nu}$ = 3165 (NH), 1675 (C=O), 1630 (C=N) cm^{-1} ; $^1\text{H NMR}$ (300 MHz, DMSO- d_6): = 1.8-2.2 (t, 3H, -CH₃), 2.01 (m, 1H), 2.70 (m, 1H), 2.62-2.74 (q, 2H, -CH₂), 3.43 (m, 1H), 3.78 (s, 3H, -OCH₃), 6.86-6.88 (m, 1H, Ar-H), 6.90-6.93 (m, 2H, Ar-H), 7.16-7.26 (m, 4H, Ar-H), 7.41-7.50 (m, 2H, Ar-H), 11.52 (s, D₂O-exchangeable, 1H, indole NH) ppm; MS (70 eV): m/z = 348 (M^+ +1, 1.8), 347 (M^+ , 5.5), 142 (100), 131 (31.2). Anal. Calcd for C₂₁H₂₁N₃O₂: C, 72.60; H, 6.09; N, 12.10. Found: C, 72.44; H, 6.24; N, 12.26.

4,5-Dihydro-5-(1H-indol-3-yl)-3-(4-methoxyphenyl)pyrazole-1-carbothioamide 6.

To a solution of 2.77 g of chalcone **3** (10 mmol) in 50 mL of ethanol, 1.0g of sodium hydroxide (25 mmol) and 1.2 g of thiosemicarbazide (12 mmol) was added. The mixture was refluxed for 6 hr then left to cool overnight, the formed solid product was filtered off, dried, and then crystallized from ethanol to give compound **6** as yellow powder in 69% yield, mp 228-230 °C; IR (KBr): $\bar{\nu}$ = 3316, 3227 (NH₂, NH), 1606 (C=N), 1251 (C=S) cm^{-1} ; $^1\text{H NMR}$ (300 MHz, DMSO- d_6): = 2.22 (m, 1H), 2.51 (m, 1H), 3.37 (m, 1H), 3.80 (s, 3H, -OCH₃), 7.09-7.20 (m, 2H, Ar-H), 7.23-7.44 (m, 4H, Ar-H), 7.81-8.00 (m, 2H, Ar-H), 8.24-8.31 (m, 1H, Ar-H), 11.11 (s, D₂O-exchangeable, 2H, NH₂), 11.60 (s, D₂O-exchangeable, 1H, indole NH), ppm; MS (70 eV): m/z = 351 (M^+ +1, 1.6), 350 (M^+ , 4.0), 142 (100), 217 (61.3), 200 (54.5), 116 (56.0). Anal. Calcd for C₁₉H₁₈N₄OS: C, 65.12; H, 5.18; N, 15.99; S, 9.15. Found: C, 65.04; H, 5.16; N, 16.12; S, 9.07.

3-(4,5-Dihydro-3-(4-methoxyphenyl)isoxazol-5-yl)-1H-indole 7.

A mixture of 2.77 g of chalcone **3** (10 mmol), 0.7 g of hydroxylamine hydrochloride (10 mmol) and 1.4 g of anhydrous potassium carbonate (10 mmol) in (50 mL) of ethanol was refluxed for 8 hr, then left to cool. The reaction mixture was poured into cold water and the solid product was filtered off, washed with water, dried and finally crystallized from ethanol to afford isoxazole derivative **7** in 68 % yield as pale yellow crystals, mp 138-140 °C; IR (KBr): $\bar{\nu}$ = 3386 (NH), 1640 (C=N) cm^{-1} ; $^1\text{H NMR}$

(300 MHz, DMSO- d_6): δ = 2.11 (m, 1H), 2.52 (m, 1H), 3.47 (m, 1H) 3.72 (s, 3H, -OCH₃), 7.12-7.16 (m, 2H, Ar-H), 7.19-7.23 (m, 4H, Ar-H), 7.43-7.50 (m, 2H, Ar-H), 8.28-8.33 (m, 1H, Ar-H), 11.61 (s, D₂O-exchangeable, 1H, indole NH) ppm; MS (70 eV): m/z = 293 (M^+ +1, 1.3), 292 (M^+ , 4.8), 160 (98.1), 117 (100), 89 (36.6). Anal. Calcd for C₁₈H₁₆N₂O₂: C, 73.95; H, 5.52; N, 9.58. Found: C, 74.11; H, 5.41; N, 9.70.

Ethyl-2-cyano-3-(1H-indol-3-yl)-5-(4-methoxyphenyl)-5-oxopentanoate 8.

To a stirred solution of 2.77 g of chalcone 3 (10 mmol) and 1.3 g of ethyl cyanoacetate (10 mmol) in 50 mL absolute ethanol, a sodium ethoxide solution prepared from 0.23 g sodium metal (10 mmol) and 10 mL absolute ethanol was added. The stirring was continued, at room temperature, for 12 hr. The solid product was collected by filtration, washed with water, dried and finally crystallised from ethanol to afford compound 8 as pale yellow crystals in 80 % yield, mp 240-241 °C; IR (KBr): $\bar{\nu}$ = 3265 (NH), 2222 (C N), 1715 (C=O) cm⁻¹; ¹H NMR (300 MHz, DMSO- d_6): δ = 1.11-1.20 (t, 3H, CH₃), 2.50-2.56 (m, 2H (CH₂-C=O), 3.35 (d, 1H), 3.58 (m, 1H), 3.81 (s, 3H, -OCH₃), 4.37 (q, 2H, CH₂), 6.86-6.90 (m, 1H Ar-H), 6.95-7.00 (m, 2H, Ar-H), 7.18-7.23 (m, 4H, Ar-H), 7.91-7.92 (m, 2H, Ar-H), 11.69 (s, D₂O-exchangeable, 1H, indole NH) ppm; MS (70 eV): m/z = 391 (M^+ +1, 2.3), 390 (M^+ , 8.0), 135 (68.5), 78 (79.8), 62 (100). Anal. Calcd for C₂₃H₂₂N₂O₄: C, 70.75; H, 5.68; N, 7.17. Found: C, 70.61; H, 5.60; N, 7.33.

4-(1H-Indol-3-yl)-6-(4-methoxyphenyl)-2-oxo-2H-pyran-3-carbonitrile 9.

Method A. This compound was synthesized by the same procedure described in the synthesis of compound 8 by refluxing the reaction mixture for 8 hr. The solid that formed after cooling was collected by filtration, washed with water, dried and finally crystallised from ethanol to afford compound 9 as pale yellow crystals in 68% yield, mp 208-209 °C; IR (KBr): $\bar{\nu}$ = 3274 (NH), 2222 (C N), 1688 (C=O) cm⁻¹; ¹H NMR (300 MHz, DMSO- d_6): δ = 3.31 (s, 3H, -OCH₃), 7.12-7.22 (m, 2H, Ar-H), 7.32 (s, 1H, C5), 7.55-7.58 (m, 4H, Ar-H), 7.93-7.96 (m, 2H, Ar-H), 8.54-8.55 (m, 1H, Ar-H), 12.48 (s, D₂O-exchangeable, 1H, indole NH) ppm; MS (70 eV): m/z = 343 (M^+ +1, 2.9), 342 (M^+ , 11.2), 212 (100), 168 (80.4), 140 (64.4), 113 (26.4). Anal. Calcd for C₂₁H₁₄N₂O₃: C, 73.68; H, 4.12; N, 8.18. Found: C, 73.74; H, 4.26; N, 8.03.

Method B. Refluxing 0.78 g of compound 8 (2 mmol) in sodium ethoxide solution prepared from 0.046 g sodium metal (2 mmol) and 50 mL absolute

ethanol for 6 h. the treatment of reaction mixture as mentioned in method A resulted in a compound identical in (mp. and mix. mp. for compound that produced by method A.

General procedure for synthesis of pyridines 11 and 13a-13d

To a solution of 0.69 g of the pyranone 9 (2 mmol) in 30 mL of ethanol, hydrazine hydrate or the appropriate sulfonamides 12a-d (2 mmol) was added. The mixture was refluxed for 6 h. Left to cool, the formed solid product was filtered off, dried, and then crystallized from ethanol/DMF to give compounds 11 and 13a-d, respectively.

1,2-Dihydro-4-(1H-indol-3-yl)-6-(4-methoxyphenyl)-2-oxo-1-aminopyridine-3-carbonitrile 11.

Yellow powder, yield 62%, mp 295-297 °C; IR (KBr): $\bar{\nu}$ = 3320, 3190 (NH₂, NH), 2219 (C N), 1665 (C=O), cm⁻¹; ¹H NMR (300 MHz, DMSO- d_6): δ = 2.51 (s, D₂O-exchangeable, 2H, NH₂), 3.34 (s, 3H, -OCH₃), 7.22-7.29 (m, 2H, Ar-H), 7.33-7.55 (m, 4H, Ar-H), 7.59-7.93 (m, 2H, Ar-H), 9.11 (m, 1H, Ar-H), 11.69 (s, D₂O-exchangeable, 1H, indole NH) ppm; MS (70 eV): m/z = 357 (M^+ +1, 2.5), 356 (M^+ , 10.3), 281 (40.2), 78 (70.3), 62 (100). Anal. Calcd for C₂₁H₁₆N₄O₂: C, 70.77; H, 4.53; N, 15.72. Found: C, 70.59; H, 4.50; N, 15.86.

1,2-Dihydro-4-(1H-indol-3-yl)-6-(4-methoxyphenyl)-2-oxo-1-(4-aminosulfonylphenyl)pyridine-3-carbonitrile 13a.

Pale yellow crystals, yield 65%, mp 248-249 °C; IR (KBr): $\bar{\nu}$ = 3364, 3150 (NH₂, NH), 2221 (C N), 1686 (C=O), 1375 (S=O, asy.) and 1140 (S=O, sym.) cm⁻¹; ¹H NMR (300 MHz, DMSO- d_6): δ = 2.40 (s, D₂O-exchangeable, 2H, sulfonamide NH₂), 3.85 (s, 3H, -OCH₃), 7.04 (s, 1H, C5), 7.24-7.27 (m, 2H, Ar-H), 7.31-7.55 (m, 4H, Ar-H), 7.56-7.74 (m, 2H, Ar-H), 7.91-7.92 (m, 4H, Ar-H), 9.05 (m, 1H, Ar-H), 12.54 (s, D₂O-exchangeable, 1H, indole NH) ppm; MS (70 eV): m/z = 497 (M^+ +1, 3.8), 496 (M^+ , 11.0), 212 (41.8), 168 (100). Anal. Calcd for C₂₇H₂₀N₄O₄S: C, 65.31; H, 4.06; N, 11.28; S, 6.46. Found: C, 65.18; H, 3.92; N, 11.33; S, 6.62.

1,2-Dihydro-4-(1H-indol-3-yl)-6-(4-methoxyphenyl)-2-oxo-1-(4-propylaminosulfonylphenyl)pyridine-3-carbonitrile 13b.

Pale yellow crystals, yield 65%, mp 257-258 °C; IR (KBr): $\bar{\nu}$ = 3358, 3281 (2NH), 2224 (C N), 1686 (C=O), 1370 (S=O, asy.) and 1148 (S=O, sym.) cm⁻¹; ¹H NMR (300 MHz, DMSO- d_6): δ = 1.03 (t, 3H, -CH₃), 1.52 (m, 2H, -CH₂-), 2.10 (s, D₂O-

exchangeable, 1H, sulfonamide NH) 3.35 (t, 2H, -CH₂-), 3.85 (s, 3H, -OCH₃), 6.10 (s, 1H, C5), 6.61-6.66 (m, 2H, Ar-H), 7.27-7.30 (m, 4H, Ar-H), 7.33-7.48 (m, 2H, Ar-H), 7.94-7.97 (m, 4H, Ar-H), 8.54-8.56 (m, 1H, Ar-H), 12.51 (s, D₂O-exchangeable, 1H, indole NH), ppm; MS (70 eV): m/z = 539 (M⁺+1, 3.3), 538 (M⁺, 8.9), 212 (100), 140 (87.5), 63 (65.0). Anal. Calcd for C₃₀H₂₆N₄O₄S: C, 66.90; H, 4.87; N, 10.40; S, 5.95. Found: C, 66.82; H, 4.87; N, 10.29; S, 6.11

1,2-Dihydro-4-(1*H*-indol-3-yl)-6-(4-methoxyphenyl)-2-oxo-1-(4-phenylaminosulfonylphenyl)pyridine-3-carbonitrile 13c.

Pale yellow crystals, yield 71%, mp 220-221 °C; IR (KBr): $\bar{\nu}$ = 3347, 3273 (2NH), 2222 (C N), 1686 (C=O), 1380 (S=O, asy.) and 1148 (S=O, sym.) cm⁻¹; ¹H NMR (300 MHz, DMSO-d₆): = 3.83 (s, 3H, -OCH₃), 6.00 (s, 1H, C5), 6.49-6.54 (m, 2H, Ar-H), 6.95-7.03 (m, 1H Ar-H), 7.07-7.26 (m, 2H, Ar-H), 7.27-7.39 (m, 4H, Ar-H), 7.54-7.58 (m, 2H, Ar-H), 7.92-7.97 (m, 4H, Ar-H), 8.54-8.56 (m, 1H, Ar-H), 9.33 (s, D₂O-exchangeable, 1H, sulfonamide NH), 12.51 (s, D₂O-exchangeable, 1H, indole NH), ppm; MS (70 eV): m/z = 573 (M⁺+1, 3.6), 572 (M⁺, 10.0), 212 (100), 168 (74.5), 140 (70.3). Anal. Calcd for C₃₃H₂₄N₄O₄S: C, 69.22; H, 4.22; N, 9.78; S, 5.60. Found: C, 69.38; H, 4.06; N, 9.70; S, 5.48.

1,2-Dihydro-4-(1*H*-indol-3-yl)-6-(4-methoxyphenyl)-2-oxo-1-[4-(4-methoxyphenyl)aminosulfonylphenyl]pyridine-3-carbonitrile 13d.

Pale yellow crystals, yield 71%, mp 235-236 °C; IR (KBr): $\bar{\nu}$ = 3379, 3285 (2NH) 2220 (C N), 1684 (C=O), 1376 (S=O, asy.) and 1140 (S=O, sym.) cm⁻¹; ¹H NMR (300 MHz, DMSO-d₆): = 3.37 (s, 3H, -OCH₃), 3.65 (s, 3H, -OCH₃), 5.93 (s, 1H, C5), 6.49-6.52 (m, 4H, Ar-H), 6.76-6.78 (m, 2H, Ar-H), 6.93-6.96 (m, 4H, Ar-H), 7.22-7.25 (m, 2H, Ar-H), 7.94-7.96 (m, 4H, Ar-H), 8.53-8.55 (m, 1H, Ar-H), 9.74 (s, D₂O-exchangeable, 1H, sulfonamide NH), 12.52 (s, D₂O-exchangeable, 1H, NH indole) ppm; MS (70 eV): m/z = 603 (M⁺+1, 2.9), 602 (M⁺, 13.3), 241 (100), 169 (73.7), 124 (45.2). Anal. Calcd for C₃₄H₂₆N₄O₅S: C, 67.76; H, 4.35; N, 9.30; S, 5.32. Found: C, 67.71; H, 4.28; N, 9.18; S, 5.40.

General procedure for synthesis of pyrimidines 14a, b

A 2.77 g of chalcone **3** (10 mmol) was added to sodium ethoxide solution [prepared from sodium metal (0.23 g, 10 mmol) and 50 mL of absolute ethanol] then urea or thiourea (10 mmol) was added. The reaction mixture was refluxed for 16 h, then left to cool and poured into crushed ice and neutralized with diluted hydrochloric acid. The

precipitated product was collected by filtration, washed with ethanol and dried. Crystallization from EtOH/DMF afforded the pyrimidine derivatives 14a, b, respectively.

6-(1*H*-Indol-3-yl)-4-(4-methoxyphenyl)pyrimidin-2(1*H*)-one 14a.

Pale yellow crystals, yield 69%, mp 198-200 °C; IR (KBr): $\bar{\nu}$ = 3438, 3170 (2NH), 1669 (C=O), 1633 (C=N) cm⁻¹; ¹H NMR (300 MHz, DMSO-d₆): = 3.83 (s, 3H, -OCH₃), 5.46 (s, 1H, C5), 6.99-7.04 (m, 2H, Ar-H), 7.18-7.22 (m, 4H, Ar-H), 7.49-7.54 (m, 2H, Ar-H), 8.28-8.30 (m, 1H, Ar-H), 9.94 (s, D₂O-exchangeable, 1H, pyrimidin NH), 12.07 (s, D₂O-exchangeable, 1H, indole NH) ppm; MS (70 eV): m/z = 318 (M⁺+1, 2.4), 317 (M⁺, 9.0), 213 (100), 169 (38.4), 140 (42.5), 62 (36.8). Anal. Calcd for C₁₉H₁₅N₃O₂: C, 71.91; H, 4.76; N, 13.24. Found: C, 72.04; H, 4.78; N, 13.38.

6-(1*H*-Indol-3-yl)-4-(4-methoxyphenyl)pyrimidine-2(1*H*)-thione 14b.

Yellow powder, yield 73%, mp 180-182 °C; IR (KBr): $\bar{\nu}$ = 3375, 3165 (2NH), 1630 (C=N) cm⁻¹; ¹H NMR (300 MHz, DMSO-d₆): = 3.35 (s, 3H, -OCH₃), 5.6 (s, 1H, C5), 7.21-7.25 (m, 2H, Ar-H), 7.26-7.49 (m, 4H, Ar-H), 7.54-7.90 (m, 2H, Ar-H), 8.28-8.30 (m, 1H, Ar-H), 8.82 (s, D₂O-exchangeable, 1H, pyrimidin NH), 12.07 (s, D₂O-exchangeable, 1H, indole NH) ppm; MS (70 eV): m/z = 334 (M⁺+1, 2.6), 333 (M⁺, 6.8), 144 (100), 116 (28.7), 89 (24.5). Anal. Calcd for C₁₉H₁₅N₃OS: C, 68.45; H, 4.53; N, 12.60; S, 9.62. Found: C, 68.48; H, 4.66; N, 12.49; S, 9.56.

3-(2-Chloro-6-(4-methoxyphenyl)pyrimidin-4-yl)-1*H*-indole 15.

A solution of 0.63 g of 14a (2 mmol) in 30 mL phosphorus oxychloride was refluxed for 5 hr, then left to cool and poured carefully into crushed ice. The precipitated product was collected by filtration, washed with water and dried. Crystallization from EtOH/DMF afforded compound 15 as violet powder in 68% yield, mp 160-162 °C; IR (KBr): $\bar{\nu}$ = 3217 (NH), 1632 (C=N) cm⁻¹; ¹H NMR (300 MHz, DMSO-d₆): = 3.38 (s, 3H, -OCH₃), 7.11-7.53 (m, 6H, Ar-H), 7.87-8.31 (m, 4H, Ar-H), 12.20 (s, D₂O-exchangeable, 1H, indole NH) ppm; MS (70 eV): m/z = 336 (M⁺+1, 1.9), 335 (M⁺, 5.6), 156 (92.3), 108 (57.5), 92 (100), 64 (78.7). Anal. Calcd for C₁₉H₁₄ClN₃O: C, 67.96; H, 4.20; N, 12.51. Found: C, 67.79; H, 4.34; N, 12.50.

N-(4-Phenylaminosulfonylphenyl)-4-(1*H*-indol-3-yl)-6-(4-methoxyphenyl)pyrimidin-2-amine 16.

This reaction was carried out by the same procedure described in the synthesis of compounds

13a-d. Crystallization of the reaction product from EtOH/DMF afforded compound 16 as faint brown powder in 66% yield, mp 100-101 °C; IR (KBr): $\bar{\nu}$ = 3419, 3349, 3245 (3NH) cm^{-1} ; $^1\text{H NMR}$ (300 MHz, DMSO- d_6): = 3.83 (s, 3H, -OCH₃), 6.5-8.32 (m, 19H, Ar-H), 9.85 (s, D₂O-exchangeable, 1H, NH), 9.93 (s, D₂O-exchangeable, 1H, NH), 12.3 (s, D₂O-exchangeable, 1H, indole NH) ppm; MS (70 eV): m/z = 548 (M^+ +1, 3.7), 547 (M^+ , 12.2), 248 (41.1), 156 (88.8), 108 (56.3), 92 (100), 65 (79.0). Anal. Calcd for C₃₁H₂₅N₅O₃S: C, 67.99; H, 4.60; N, 12.79; S, 5.86. Found: C, 68.13; H, 4.69; N, 12.76; S, 5.66.

Pharmaceutical Applications

Anti-proliferative screening

Cytotoxic activity against human breast carcinoma cell line (MCF-7) and liver carcinoma cell line HEPG-2 in vitro. The method applied is similar to that reported by Skehan et al. 36 using Sulfo-Rhodamine-B stain (SRB). Cells were plated in 96-multiwell plate (104 cells/well) for 24 h before treatment with the test compound to allow attachment of cell to the wall of the plate, different concentration of the compound under test (0, 2.5, 5, and 10 $\mu\text{g/ml}$) were added to the cell monolayer in triplicate wells individual dose, monolayer cells were incubated with the compounds for 48 h at 37°C and in atmosphere of 5 % CO₂, after 48 h, cells were fixed, washed and stained with SRB stain, excess stain was washed with acetic acid and attached stain was recovered with Tris-EDTA buffer, color intensity was measured in an ELISA reader, the relation between surviving fraction and drug concentration is plotted to get the survival curve of each tumor cell line after the specified compound and the IC₅₀ was calculated.

Antimicrobial screening

Applying the agar plate diffusion technique 37, the compounds were screened in vitro for their bactericidal activity against Gram positive bacteria (*Staphylococcus aureus*) and Gram negative bacteria (*Escherichia Coli* and *Pseudomonas aeruginosa*), and for their fungicidal activity against *Fusarium*, *Aspergillus niger* and *Candida albicans*. In this method, a standard 5 mm diameter sterilized filter paper disc impregnated with the compound (0.3 mg/0.1 ml of DMF) was placed on an agar plate seeded with the test organism. The plates were incubated for 24 hours at 37 °C for bacteria and 28 °C for fungi. The zone of inhibition of bacterial and fungi growth around the disc was observed.

3. Results and Discussion:

Chemistry

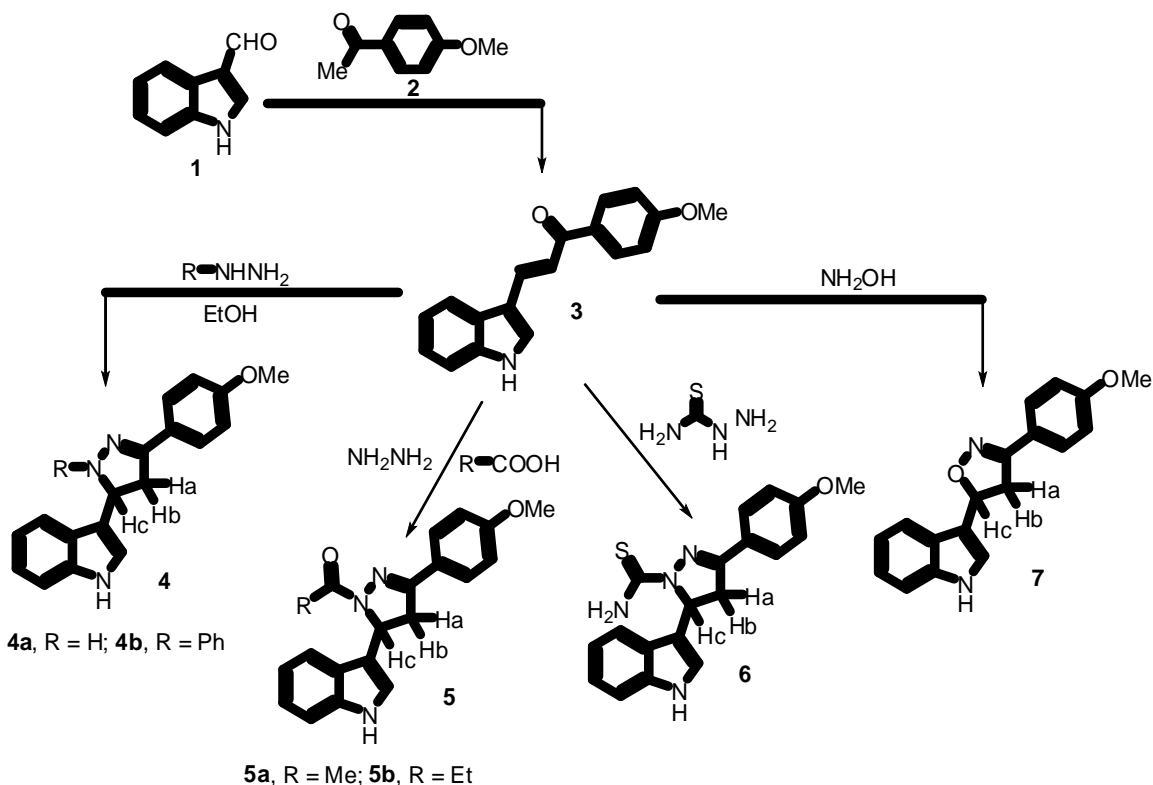
Aldol condensation reaction of 3-indolaldehyde 1 with 4-methoxyacetophenone 2 in ethanolic NaOH solution afforded chalcone 3. Its structure was confirmed by its IR spectrum where it showed a characteristic peak for a conjugated carbonyl group at 1633 cm^{-1} , and also by its $^1\text{H NMR}$ which gave signals at 7.53 (d, 1H, $J = 12.9$ Hz, (C=O)(CH=C), and 7.92 (d, 1H, $J = 12.9$ Hz (C=O) (C=CH). Reaction of chalcone 3 with either hydrazine hydrate or phenyl hydrazine gave the corresponding pyrazoline derivatives 4a, b respectively. Structures of 4a, and 4b were assigned by their IR and $^1\text{HNMR}$ Spectra. Both exhibited C=N stretching vibrations in the region 1618-1630 cm^{-1} , and compound 4a showed an additional absorption band at 3215 cm^{-1} characteristic for pyrazole NH.

$^1\text{H NMR}$ spectra of compounds 4a and 4b showed three multiplets at 2.00, 2.69, and 3.40 ppm resulted from the AMX pattern displayed by two diastereotopic protons at C-4 (H_a and H_b) and one proton (H_c) at C-5 (Scheme 1).

The acyl derivatives 5a, b were prepared either by treating 4a with the corresponding acid chloride, or by reacting chalcone 3 with hydrazine hydrate by changing the reacting medium from ethanol to acetic acid or propanoic acid, respectively. Structures of 5a, b were established by their IR spectra where no characteristic band for NH was detected at 3215 cm^{-1} . The $^1\text{HNMR}$ of compound 5a exhibited a singlet at 2.24ppm (acetyl CH₃ protons), whereas 5b showed a triplet at 2.0 (CH₃CH₂) and quartet at 2.68(CH₃CH₂) respectively.

The formation of pyrazoline 6 was achieved by refluxing chalcone 3 with thiosemicarbazide and sodium hydroxide in ethanol 30. Its structure was confirmed by its IR where absorption peaks appeared at 3316 cm^{-1} and 3227 cm^{-1} , characteristic for NH₂ and NH, respectively, and its $^1\text{H NMR}$ displayed two singlets at = 11.11ppm (D₂O-exchangeable, 2H, NH₂), and 11.60 (D₂O-exchangeable, 1H, indole NH).

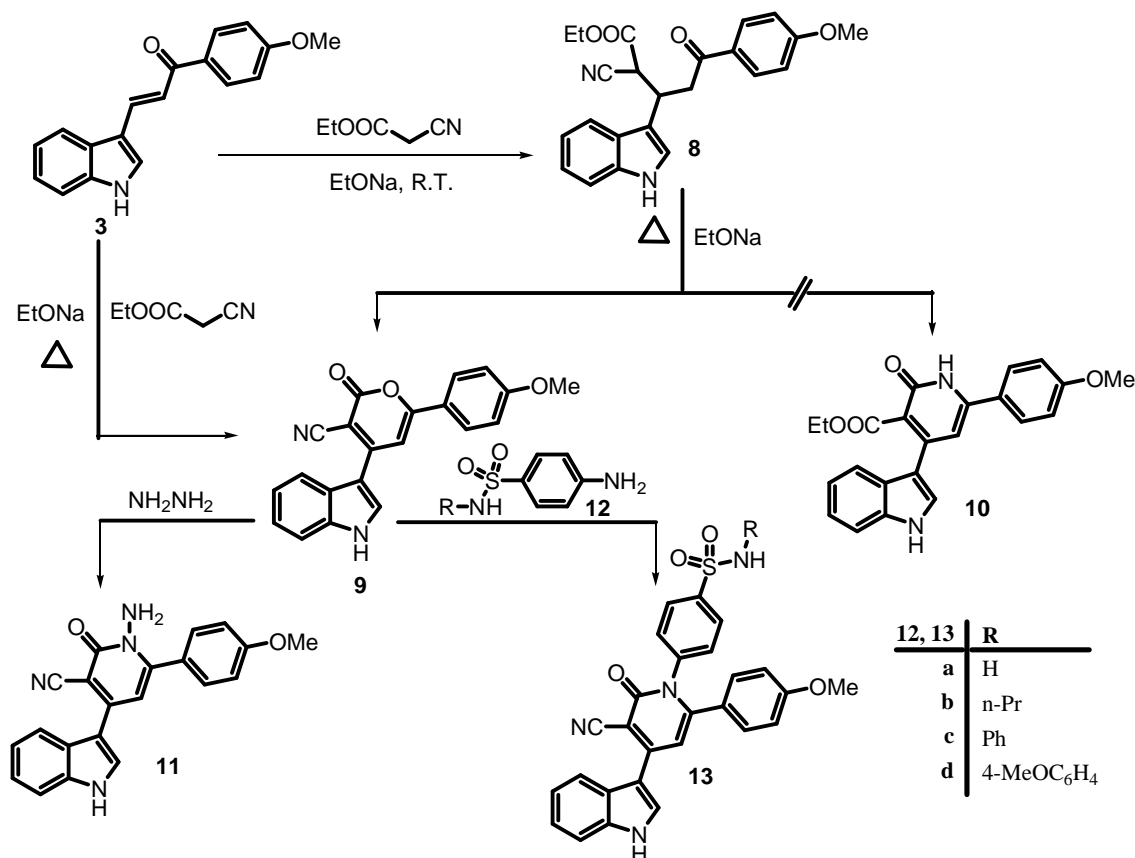
The oxazoline 7 was obtained from refluxing chalcone 3 with hydroxylamine in ethanol, its structure was ascertained by spectral data, where its IR spectrum showed 2 peaks at 3386 and 1640, characteristic for NH and C=N, respectively. Its $^1\text{H NMR}$ displayed a singlet at =11.61 (D₂O-exchangeable, 1H, indole NH). The mass spectrum revealed its molecular ion peak at m/z 292 (M^+) (Scheme 1).



(Scheme 1)

Reaction of chalcone 3 with ethyl cyanoacetate in the presence of sodium ethoxide at room temperature afforded product 8 through *Michael* addition, whereas the similar reaction gave pyranone derivative 9 under the reflux conditions 31 (Scheme 2). The elemental analysis and spectroscopic data confirmed the assigned structure for 9, where its IR displayed a peak at 2222cm^{-1} for C N and disappearance of the band representing the carbonyl of the COOR group. This result led to exclude the formation of an alternative compound 10. Compound 9 could also be obtained by refluxing 8 in ethanol in presence of sodium ethoxide.

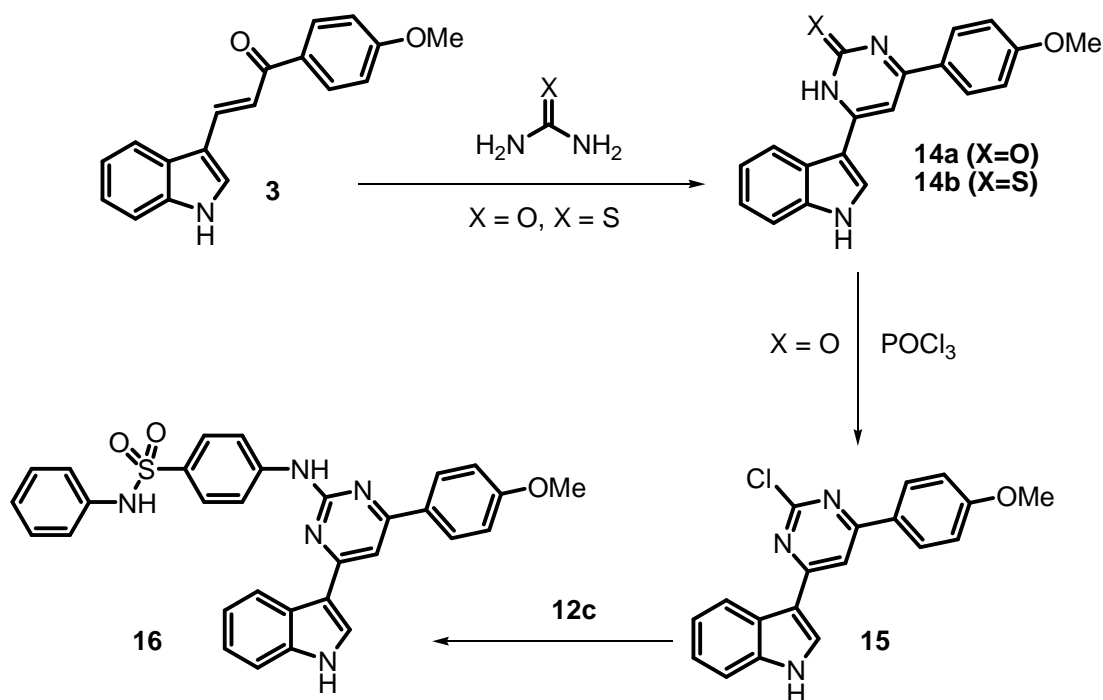
Condensation of 9 with either hydrazinehydrate or sulfonamide derivatives 32-12a-d in refluxing ethanol gave the corresponding pyridinones 11, or 13a-d, respectively (Scheme 2). The structures of these products were ascertained by their spectral data, where their IR spectra showed absorption bands in the region $2219\text{-}2224\text{cm}^{-1}$ and $1665\text{-}1780\text{cm}^{-1}$ characteristic for C N and carbonyl group, respectively. In addition, compounds 13a-d displayed bands at 1375 (S=O, asy.) and 1140 (S=O, sym.) cm^{-1} . Their ^1H NMR spectra were agreed with the structures 11 and 13a-d.



(Scheme 2)

Reflux of chalcone **3** with either urea or thiourea in presence of sodium ethoxide in ethanol afforded the pyrimidine derivatives **14a**, **b**, respectively (Scheme 3). The structure of **14a** was substantiated by its IR, where it revealed bands at 3438, 3170 cm^{-1} for the 2NH groups, 1669 and 1633 cm^{-1} characteristic for C=O, and C=N, respectively. Its ^1H NMR, displayed two singlets at 9.94 for pyrimidin NH and 12.07 for indole NH by ppm. Reflux of compound **14a** in excess phosphorus oxychloride as a reacting medium gave compound **15**

which on its reflux with sulfonamide **12c** in ethanol furnished compound **16** in a moderate yield (Scheme 3). The IR spectrum of compound **16** showed three separate absorption bands at 3419, 3349, and 3245 cm^{-1} characteristic for (3NH). Its ^1H NMR displayed three singlets at 9.85 (D_2O -exchangeable, 1H, NH), 9.93 (D_2O -exchangeable, 1H, NH), and 12.3 (D_2O -exchangeable, 1H, indole NH) by ppm.



Pharmaceutical Applications
Anti-proliferative screening

Compounds 5a, 6, 7, 13a, 13b, 13d and 14b were tested against a human breast carcinoma cell

line (MCF7) and a human liver carcinoma cell line (HEPG2), using 5-Fluorouracil (5-Fluoro-1*H*-pyrimidine-2,4-dione) as a reference drug (Table1). The measurements were carried out in the National Institute of Cancer, Cairo University, Cairo Egypt.

Table1. In vitro cytotoxic activity (IC₅₀) of compounds 5a, 6, 7, 13a, 13b, 13d and 14b and 5-Fluorouracil against a human breast carcinoma cell line (MCF7) and a liver carcinoma cell line (HEPG2)^a

Entry	compound	MCF7 ($\mu\text{g.mL}^{-1}$)	HEPG2 ($\mu\text{g.mL}^{-1}$)
1	5a	1.01	-
2	6	2.62	2.48
3	7	2.55	5.17
4	13a	1.34	4.09
5	13b	0.84	5.30
6	13d	0.60	3.96
7	14b	0.85	2.68
8	5-Fluorouracil	0.67	5.0 ^b

^aIC₅₀ is defined as the concentration which results in a 50% decrease in cell number as compared with that of the control structures in the absence of an inhibitor.

^bThis value of IC₅₀ for the reference drug 5-Fluorouracil against HEPG2 was maintained by the National Institute of Cancer, Cairo University, Cairo, Egypt. (The liver carcinoma cells were found to be more resistant to 5-Fluorouracil as a reference drug).

As shown in Tables 1, it was found that compounds 5a, 6, 7, 13a, 13b, 13d and 14b have significant anti-proliferative activities against human breast cell line MCF-7 compared to the reference standard drug 5-Fluorouracil. Compounds 13b and 13d showed high

activities which can be attributed to the presence of the sulfonamide group, where it was confirmed 33, 34 that compounds containing sulfonamide function group have potent anti-proliferative activities. Compound 13d was found to have a promising activity due to the presence of a methoxy group in the para- position to the sulfonamide group 35. The screened results against liver carcinoma cell line (HEPG2, Table 1) revealed that the tested compounds 6, 7, 13a, 13b, 13d and 14b showed significant activities compared to the reference drug 5-Fluorouracil.

Antimicrobial screening

The synthesized new compounds 4a, 5a, 6, 7, 11, 13a, 13b, 13d and 14b were screened in vitro for

their bactericidal activity against Gram positive bacteria (*Staphylococcus aureus*) and Gram negative bacteria (*Escherichia Coli* and *Pseudomonas aeruginosa*), and for their fungicidal activity against *Fusarium*, *Aspergillus niger* and *Candida albicans* (Table 2). All the screened compounds showed high or moderate bactericidal activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa* compared to that of ciprofloxacin. Compounds 4a, 5a, 6, 13a, 13b and 13d showed a good fungicidal activity, near to that of nystin, against *Fusarium*, all screened compounds except 11 were found to have moderate fungicidal activity against *Candida albicans*.

Table 2. Bactericidal and fungicidal activity of some of the new compounds and ciprofloxacin and nystin.^a

No.	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>	<i>Fusarium</i>	<i>Aspergillus niger</i>	<i>Candida albicans</i>
4a	+++ (32)	-	++ (17)	+++ (31)	-	++ (16)
5a	++ (21)	-	++ (20)	++ (16)	-	++ (18)
6	+++ (30)	-	++ (19)	+++ (27)	-	++ (20)
7	++ (18)	-	++ (17)	-	-	++ (22)
11	++ (15)	-	+++ (27)	-	-	-
13a	+++ (34)	-	+++ (29)	+++ (32)	-	++ (22)
13b	+++ (28)	-	++ (17)	+++ (31)	-	++ (20)
13d	+++ (34)	-	+++ (31)	+++ (30)	-	++ (24)
14b	+++ (29)	-	++ (15)	-	-	++ (20)
Ciprofloxacin	++++	++++	++++	-	-	-
Nystin	-	-	-	++++	++++	++++

^a The activities are based on the diameters of zones of inhibition in mm. One mL of stock solution (5 ug/mL in DMF) was applied in each hole of each paper disk.+: < 15 mm; ++: 15-24 mm; +++: 25-34 mm; ++++: 35-44 mm, etc.

4. Conclusion

Aldol condensation reaction between 3-indolaldehyde and 4-methoxyacetophenone gave a chalcone compound from which some pyrazoline, pyridine, and pyrimidine derivatives linked to indole moiety were obtained and found to have promising antitumor and antimicrobial activities.

Corresponding author

Ekhlass Nassar*
Department of Chemistry, Faculty of Women for Arts, Science and Education, Ain Shams University, Asma Fahmy Street, Heliopolis, Cairo, Egypt.
ekhlass_nassar@hotmail.com

5. References

1. Sakemi, S.; Sun, H. H. *J. Org. Chem.* 1991, 56, 4304.
2. Kawasaki, Y.; Katsuma, H.; Nakayama, Y.; Yamashita, M.; Otha, S. *Heterocycles* 1998, 48, 1887.
3. Sun, H. H.; Sakemi, J. *J. Org. Chem.* 1991, 56, 4307.
4. Vervoort, H. C.; Richards-Gross, S. E.; Fenical, W.; Lee, A. Y.; Clardy, J. *J. Org. Chem.* 1997, 62, 1486.
5. Takahashi, S.; Matsunaga, T.; Hasegawa, C.; Saito, H.; Fujita, D.; Kiuchi, F.; Tsuda, Y. *Chem. Pharm. Bull.* 1998, 46, 1527.
6. Broadbent, T. A.; Broadbent, H. S. *Curr. Med. Chem.* 1998, 5, 337.
7. Broadbent, T. A.; Broadbent, H. S. *Curr. Med. Chem.* 1998, 5, 469.
8. Gamet-Payraastre, L.; Lumeau, S.; Gasc, N.; Cassar, G.; Rollin, P. Tulliez, *J. Anticancer Drugs* 1998, 9, 14.
9. Queiroz, M. R. P.; Abreu, A. S.; Carvalho, M. S. D.; Ferreira, P.M.T.; Nazareth, N.; Nascimento, M. S. *Bioorg & Med. Chem.* 2008, 16, 5584.
10. Cover, C. M.; Hsieh, S. J.; Cram, E. J.; Hong, C.; Riby, J. E.; Bjeldanes, L. F.; Firestone, G. L. *Cancer Res.* 1999, 59, 1244.
11. Misra, U.; Hitkari, A.; Saxena, A. K.; Gurtu, S.; Shanker, K. *Eur. J. Med. Chem.* 1996, 31, 629.
12. Andreani, A.; Rambaldi, M.; Locatelli, A.; Pifferi, G. *Eur. J. Med. Chem.* 1994, 29, 903.
13. Ebeid, M. Y.; Lashine, S. M.; El-Adl, S. M.; Abou Kull, M. E. *Zagazig. J. Pharm. Sci.* 1994, 3, 40.
14. El-Gendy, A. A.; Abdou Naida, A.; El-Taher, S. Z.; El-Banna, H. A. *Alexandria J. Pharm. Sci.* 1993, 7, 99.
15. Kumar, A.; Saxena, K. K.; Gurtu, S.; Sinha, J. N.; Shanker, K. *Indian Drugs* 1986, 24, 1.
16. Dandia, A.; Sehgal, V.; Singh, P. *Indian J. Chem.* 1993, 32B, 1288.
17. Kawasaki, Y.; Yamashita, M.; Otha, S. *Chem. Pharm. Bull.* 1996, 44, 1831.
18. Tsuji, S.; Rinehart, K. L.; Gunasekera, S. P.; Kashman, Y.; Cross, S. S.; Lui, M. S.; Pomponi, S. A.; Diaz, M. C. *J. Org. Chem.* 1988, 53, 5446.
19. Takahashi, S.; Matsunaga, T.; Hasegawa, C.; Saito, H.; Fujita, D.; Kiuchi, F.; Tsuda, Y. *Chem. Pharm. Bull.* 1998, 46, 1527.
20. Guella, G.; Mancini, Y.; N'Diaye, Y.; Pietra, F. *Helv. Chim. Acta.* 1999, 77, 1994.
21. Bergmann, T.; Schories, D.; Steffan, B. *Tetrahedron* 1997, 53, 2055.
22. Farag, A. M.; Mayhoub, A. S.; Barakat, S. E.; Bayomi, A. H. *Bioorg & Med. Chem.* 2008, 16, 881.
23. Diana, P.; Carbone, A.; Barraja, P.; Martorana, A.; Gia, O.; DallaVia, L.; Cirrincione, G. *Bioorg. & Med. Chem. Lett.* 2007, 17, 6134.
24. Son, J.; Zhao, L.; Basnet, A.; Thapa, P.; Karki, R.; Na, Y.; Jahng, Y.; Jeong, T. C.; Jeong, B.; Lee, C.; Lee, E. *Eur. J. Med. Chem.* 2008, 43, 675.
25. Radwana, M. A. A.; El-Sherbiny, M. *Bioorg. Med. Chem.* 2007, 15, 1206.
26. El-Mariah, F. and Nassar, E. *Phosphorus sulfur silicon and relat. Elem.* 2008, 183, 1.
27. El-Mariah, F.; Nassar, E.; Hosny, M. and Deeb, A. *Phosphorus Sulfur Silicon and the Related Elements* 2009, 184, 92-102.
28. Snégaroff, K.; Lassagne, F.; Bentabed-Ababsa, G.; Nassar, E.; Cheikh Sid Ely, S.; Hesse, S.; Perspicace, E.; Derdour, A. and Mongin, F. *Org. Biomol. Chem.* 2009, 7, 4782-4788.
29. Bentabed-Ababsa, G.; Cheikh Sid Ely, S.; Hesse, S.; Nassar, E.; Chevallier, F.; Nguyen, T. T.; Derdour, A. and Mongin, F. *J. Org. Chem.* 2010, 75, 839-847.
30. Abdel-Wahab, B. F.; Abdel-Aziz, H. A.; Ahmed, E. M. *Eur. J. Med. Chem.* 2009, 44, 2632-2635.
31. Shalaby, A. F. A.; Abdulla, M. M.; Amr, A. E.; Hussain, A. A. *Monatshefte für Chemie.* 2007, 138, 1019.
32. Vogel's Textbook of Practical Organic Chemistry (Longman Group Limited, London), 1980, 4th ed., pp. 651.
33. Laixing, H. b.; Lib, Z-R.; Wangb, Y-M.; Wub, Y.; Jiangb, J-D. and Boykina, D. W. *Bioorg. Med. Chem. Lett.*, 1 March 2007, Volume 17, Issue 5, Pages 1193-1196.
34. Hu, L.; Li, Z-R.; Li, Y.; Qu, J.; Ling, Y-H.; Jiang, J-D. and Boykin, D. W. *J. Med. Chem.* 2006, 49, p. 6273.
35. Mólá, W.; Matyjaa, M.; Filipb, B.; Wietrzykb, J. and Boryczka, S. *Bioorg. & Med. Chem.*, 2008, Volume 16, Issue 17, Pages 8136-8141.
36. Skehan, P.; Storeng, R., *J. Nat. Cancer Inst.*, 1990, 82, 1107.
37. Bauer, A. W.; Mkriby, W. W.; Sherris, J. C.; Turck, M., *Am. J. Clin. Pathol.* 1966, 45, 493.

7/1/2010