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Synthesis and Antiproliferative Activity of 2,4-Bis(indol-3-*yl*)pyrrole Derivatives: Marine Nortopsentin Analogs

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Abstract

In this study, a series of nortopsentin analogs (2,4-bis(indol-3-*yl*)pyrrole derivatives) were designed, synthesized, and tested for their in vitro cytotoxic activity against three cell lines: human prostate adenocarcinoma (PC-3), human ovary adenocarcinoma (SKOV3), and human Dukes' type B colorectal adenocarcinoma (LS-174T). Compounds **5a**, **5e**, **5h**, **5j**, and **5k** had stronger antiproliferative activity against SKOV3, compound **5h** and **5b** against LS-174T, and compound **5e** against PC-3 than the known doxorubicin drug. As a result, this work provides the framework for further research into 2,4-bis(indol-3-*yl*)pyrrole derivatives as antiproliferative drugs.

Keywords: Indole; bisindole; nortopsentin; pyrrole; antiproliferative; PC-3; SKOV3; LS-174T.

1. Introduction

Indole, and bis-indole derivatives are important skeletons of various biologically active candidates [1-3]. In many synthetic pharmaceutical compounds indole ring moiety is the main component unit [4-6], and the World Drug Index indicated that 74 indole molecules act as drug applicants. Furthermore, indoles and bis-indoles are common units in various natural product compounds and possess wide spectrum biological activities such as antitumor, antiviral, antimicrobial, and anti-inflammatory activities [7-12].

Furthermore, the indole core is found in a wide range of therapeutic compounds with a variety of pharmacological properties, including anticancer [13], antioxidant [14], antirheumatoid [15], anti-HIV, and antibacterial action [16]. The anticancer drugs vinblastine and vincristine were the most prominent indole units in these categories [17]. There are also a variety of indole compounds with different pharmacological properties. Non-steroidal anti-inflammatory drugs having an indole ring, such as Indomethacin and Etodolac, have been revealed to own antioxidant properties [18-21]. One important finding is that various indole compounds have antioxidant properties [22, 23]. Melatonin derivatives, for example, are particularly effective in scavenging ROS and RNS [24, 25]. Moreover, in recent years, pyrrole and its derivatives have been identified as key units in medical research, with a variety of useful bioactivities such as antibacterial, antioxidant, and antibiotic agents [26-30].

The development of pharmaceutical compounds possess an *N*-heterocyclic ring has been motivated by organic products. The pyrrole moiety is the most commonly detected heterocycle in

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pharmacological research databases, and it has a variety of therapeutic effects. [31-33].

Nortopsentins AC (Fig. 1) were identified from the marine sponge *Spongosorites ruetzler* and revealed cytotoxicity against P388 cells as well as antifungal activity against *Candida albicans in vitro* [34]. The use of marine bis-indoles as lead targets for discovering novel medications has gained interest in medicinal chemistry due to their interesting biological activities and distinct structures [35-39].

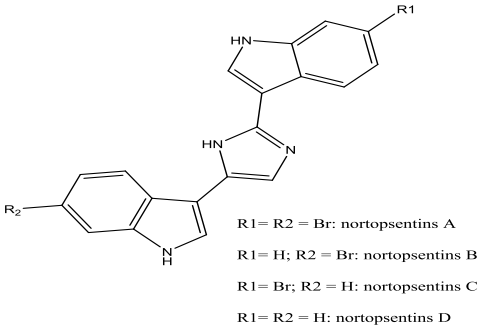


Fig. 1: Structures of Nortopsentins A−C

Because of the importance of bisindole and pyrrole moieties in the detection and development of antitumor drugs, we characterized a hybrid of the two molecules, bisindole-pyrrole derivatives, and tested their anticancer potency against three cell lines: human prostate adenocarcinoma; metastatic cells (PC-3), human ovary adenocarcinoma (SKOV3) and human dukes' type B, colorectal adenocarcinoma (LS174T).

2. Experimental section

2.1. General Considerations

All commercially available reagents and solvents in this research were of analytical grade purity and procured from Merck, Germany, and Sigma-Aldrich. Melting point (ºC) was measured on the XT-5 microscopic apparatus. IR spectra which measured on an IS10 spectrometer (ν in cm^{-1}) using KBr disk were done at Cairo University. MS (EI) m/z analysis was done via a Thermo Scientific DCQII. ^{1}HNMR and ^{13}CNMR spectra were recorded on a Varian (Inova 500 MHz) spectrometer and chemical shifts were expressed in (ppm) using tetramethylsilane (TMS) as the internal standard. The formation of the compounds was monitored by thin-layer chromatography (TLC) Merck silica gel (60 F_{254}), (TLC/n-hexane:EtOAc; 7:3).

General procedure for the synthesis of bis(indol-3-yl)pyrrole derivatives (5a-l): A mixture of α-cyano chalcones **3** (1 mmol)**,** appropriate aldehydes **4** (1 mmol), and ammonium acetate (4 mmol) was refluxed in acetic acid at 120 ºC for 16 h (controlled by (TLC/n-hexane:EtOAc; 7:3). Then, the mixture was cooled and the precipitated was recrystallized from EtOH/DMF. **5-(4-Cyanophenyl)-2,4-di(*1H*-indol-3-*yl*)-*1H*-pyrrole-3-carbonitrile** ***(5a):*** Recrystallized using EtOH/DMF, yellow (81%); mp 252 ºC; IR (KBr) mmax/cm^{-1} 3112-3320 (3 NH), 2210, 2224 (2CN); ^{1} H NMR (500 MHz, DMSO d_6): δ 7.26–7.28 (m, 4H, bis-indole H5, H6), 7.53-7.55 (dd, J = 8.6, 1.2 Hz, 2H, bis-indole H7), 7.87 (dd, 2H, 4-CN-Ph), 8.02 (dd, 2H, 4-CN-Ph), 8.21 (dd, J = 8.4, 1.1 Hz, 2H, bis-indole H4), 8.58 (s, 2H, bis-indole-H2), 11.89 (brs, NH, pyrrole), 12.21, 12.23 (brs, 2NH, bis-indole); ^{13}C NMR (DMSO-d_6) δ/ppm: 136.78 (2C), 136.31, 132.16 (2C), 129.06 (2C), 128.70, 128.65 (2C), 128.04, 126.42 (2C), 124.56, 124.23, 121.64, 121.20, 119.68, 118.30, 118.21 (2C), 117.34, 112.69, 111.34, 111.12 (2C), 98.94, 68.93; MS-EI (m/z %): 423 [M^+]; Calcd. for : $C_{28}H_{17}N_5$: C, 79.42; H, 4.05; N, 16.54. Found: C, 79.46; H, 4.01; N, 16.52.

2,4,5-tri(*1H*-Indol-3-*yl*)-*1H*-pyrrole-3-carbonitrile (5b):
Recrystallized using EtOH/DMF, yellow (74%); mp 280-281ºC; IR (KBr) mmax/cm^{-1} 3120-3392 (2 NH), 2216 (CN); ^{1} H NMR (500 MHz, DMSO d_6): δ 7.25–7.27 (m, 6H, tri-indole H5, H6), 7.50-7.54 (dd, J = 8.6, 1.2 Hz, 3H, tri-indole H7), 8.16-8.18 (dd, J = 8.4, 1.1 Hz, 3H, tri-indole H4), 8.56-8.58 (s, 3H, tri-indole-H2), 11.65 (brs, NH, pyrrole), 12.31-12-33 (brs, 3NH, tri-indole); ^{13}C NMR (DMSO-d_6) δ/ppm: 136.87(3C), 128.60 (3C), 128.54, 124.21 (3C), 121.76 (3C), 121.43 (3C), 119.25 (3C), 117.88, 118.13 (2C), 117.52, 111.14 (3C), 100.11, 98.33, 69.14; MS-EI (m/z %): 437 [M^+]; Calcd. for : $C_{29}H_{19}N_5$: C, 79.61; H, 4.38; N, 16.01. Found: C, 79.68; H, 4.36; N, 16.03.

5-(4-Bromophenyl)-2,4-di(*1H*-indol-3-*yl*)-*1H*-pyrrole-3-carbonitrile (5c):
Recrystallized using EtOH/DMF, yellow (83%); mp 268-269ºC; IR (KBr) mmax/cm^{-1} 3126-3396 (3 NH), 2210 (CN); ^{1} H NMR (500 MHz, DMSO d_6): δ 7.27–7.29 (m, 4H, bis-indole H5, H6), 7.51-7.53 (dd, J = 8.6, 1.2 Hz, 2H, bis-indole H7), 7.55 (dd, 2H, 4-Br-Ph), 7.76 (dd, 2H, 4- Br-Ph), 8.20 (dd, J = 8.4, 1.1 Hz, 2H, bis-indole H4), 8.56 (s, 2H, bis-indole-H2), 11.63 (brs, NH, pyrrole), 12.35 (brs, 2NH, bis-indole); ^{13}C NMR (DMSO-d_6) δ/ppm: 136.65 (2C), 132.14 (2C), 130.88, 128.86 (2C), 128.74, 128.61 (2C), 128.11, 126.42 (2C), 124.56, 124.22, 123.40, 121.64, 121.20, 119.62, 118.31, 118.20, 117.64, 111.17 (2C), 110.06, 95.43, 68.76; MS-EI (m/z %): 478 [M^{+}+ 2, 50%]; 476 [$M^{+\cdot}$ 50%]; Calcd. for : $C_{27}H_{17}BrN_4$: C, 67.93; H, 3.59; N, 11.74. Found: C, 67.97; H, 3.56; N, 11.72.

2,4-di(*1H*-Indol-3-*yl*)-5-(2-nitrophenyl)-*1H*-pyrrole-3-carbonitrile (5d):
Recrystallized using EtOH/DMF, yellow (78%); mp 243-245ºC; IR (KBr) mmax/cm^{-1} 3126-3396 (3 NH),

2218 (CN), 1550, 1350 (NO_2); ^{1}H NMR (500 MHz, DMSO d_6): δ 7.24–7.26 (m, 4H, bis-indole H5, H6), 7.50-7.52 (dd, *J* = 8.6, 1.2 Hz, 2H, bis-indole H7), 7.73 (m, 1H, 2-NO_2-Ph), 7.91 (m, 2H, 2-NO_2-Ph), 8.02 (m, 1H, 2-NO_2-Ph), 8.20 (dd, *J* = 8.4, 1.1 Hz, 2H, bis-indole H4), 8.59 (s, 2H, bis-indole-H2), 11.60 (brs, NH, pyrrole), 12.36 (brs, 2NH, bis-indole); ^{13}C NMR (DMSO-d_6) δ/ppm: 148.53, 136.49 (2C), 135.45, 132.43, 129.58, 128.71, 128.45 (2C), 125.63, 124.65 (2C), 124.41, 121.78 (2C), 119.68 (2C), 118.30, 118.21 (2C), 117.38, 111.37, 111.10 (2C), 102.78, 96.45, 69.12; MS-EI (m/z %): 443 [M^+]; Calcd. for : $C_{27}H_{17}N_5O_2$: C, 73.13; H, 3.86; N, 15.79. Found: C, 73.16; H, 3.83; N, 15.72.

5-(4-(Dimethylamino)phenyl)-2,4-di(*1H*-indol-3-*yl*)-*1H*-pyrrole-3-carbonitrile (5e): Recrystallized using EtOH/DMF, yellow (80%); mp 241-242ºC; IR (KBr) mmax/cm^{-1} 3126-3396 (3 NH), 2216 (CN); ^{1}H NMR (500 MHz, DMSO d_6): δ 3.11 (2s, 6H, N-Me_2), 7.12 (dd, 2H, 4-NMe_2-Ph), 7.24–7.26 (m, 4H, bis-indole H5, H6), 7.50-7.52 (dd, *J* = 8.6, 1.2 Hz, 2H, bis-indole H7), 7.59 (dd, 2H, 4-NMe_2-Ph), 8.20 (dd, *J* = 8.4, 1.1 Hz, 2H, bis-indole H4), 8.59 (s, 2H, bis-indole-H2), 11.60 (brs, NH, pyrrole), 12.36 (brs, 2NH, bis-indole); ^{13}C NMR (DMSO-d_6) δ/ppm: 154.74, 136.67 (2C), 128.86, 128.46 (2C), 128.40 (2C), 124.86 (2C), 121.72 (2C), 121.46 (2C), 121.32, 119.82 (2C), 118.28, 117.51, 112.71 (2C), 111.23 (2C), 111.13, 102.23, 96.78, 69.54, 42.08 (2C); MS-EI (m/z %): 441 [M^+]; Calcd. for : $C_{29}H_{23}N_5$: C, 78.89; H, 5.25; N, 15.86. Found: C, 78.94; H, 5.20; N, 15.82.

2,4-di(*1H*-Indol-3-*yl*)-5-(4-nitrophenyl)-*1H*-pyrrole-3-carbonitrile (5f):

Recrystallized using EtOH/DMF, yellow (84%); mp 271-272ºC; IR (KBr) mmax/cm^{-1} 3126-3396 (3 NH), 2210, 2218 (2CN), 1560, 1350 (NO_2); ^{1}H NMR (500 MHz, DMSO d_6): δ 7.26–7.29 (m, 4H, bis-indole H5, H6), 7.50-7.53 (dd, *J* = 8.6, 1.2 Hz, 2H, bis-indole H7), 7.90 (dd, 2H, 4-NO_2-Ph), 8.16 (dd, 2H, 4-NO_2-Ph), 8.20 (dd, *J* = 8.4, 1.1 Hz, 2H, bis-indole H4), 8.58 (s, 2H, bis-indole-H2), 11.61 (brs, NH, pyrrole), 12.34 (brs, 2NH, bis-indole); ^{13}C NMR (DMSO-d_6) δ/ppm: 148.18, 137.95, 136.83 (2C), 128.70, 128.64, 128.46 (2C), 128.11, 126.32 (2C), 124.86, 124.53 (2C), 121.64 (2C), 121.24 (2C), 119.81 (2C), 118.35, 117.64, 111.16 (2C), 110.02, 95.48, 68.65; MS-EI (m/z %): 443 [M^+]; Calcd. for : $C_{27}H_{17}N_5O_2$: C, 73.13; H, 3.86; N, 15.79. Found: C, 73.18; H, 3.81; N, 15.74.

5-(4-Chlorophenyl)-2,4-di(*1H*-indol-3-*yl*)-*1H*-pyrrole-3-carbonitrile (5g):

Recrystallized using EtOH/DMF, yellow (82%); mp 273-274ºC; IR (KBr) mmax/cm^{-1} 3120-3390 (3 NH), 2212 (CN); ^{1}H NMR (500 MHz, DMSO d_6): δ 7.25–7.28 (m, 4H, bis-indole H5, H6), 7.50-7.52 (dd, *J* = 8.6, 1.2 Hz, 2H, bis-indole H7), 7.55 (dd, 2H, 4-Cl-Ph), 7.91 (dd, 2H, 4-Cl-Ph), 8.20 (dd, *J* = 8.4, 1.1 Hz, 2H, bis-indole H4), 8.58 (s, 2H, bis-indole-H2), 11.69 (brs, NH, pyrrole), 12.41 (brs, 2NH, bis-indole); ^{13}C NMR (DMSO-d_6) δ/ppm: 136.69 (2C), 134.32, 130.01, 129.36 (2C), 128.92 (2C), 128.56, 128.43 (2C), 124.82, 123.40, 121.61 (2C), 121.42 (2C),, 119.62 (2C), 118.21, 117.72, 111.18 (2C), 111.02, 99.08, 96.08, 69.41; MS-EI (m/z %): 432 [M^+, 66%], 434 [M^++2, 33%]; Calcd. for: $C_{27}H_{17}ClN_4$: C, 74.91; H, 3.96; N, 12.94. Found: C, 64.96; H, 3.92; N, 12.90.

2,4-di(*1H*-Indol-3-*yl*)-5-(4-methoxyphenyl)-*1H*-pyrrole-3-carbonitrile (5h):

Recrystallized using EtOH/DMF, yellow (78%); mp 240-241ºC; IR (KBr) mmax/cm^{-1} 3125-3394 (3 NH), 2212 (CN); ^{1}H NMR (500 MHz, DMSO d_6): δ 3.83 (s, 3H, OMe), 7.11 (dd, 2H, 4- OMe-Ph), 7.25–7.27 (m, 4H, bis-indole H5, H6), 7.51-7.53 (dd, *J* = 8.6, 1.2 Hz, 2H, bis-indole H7), 7.56 (dd, 2H, 4- OMe-Ph), 8.20 (dd, *J* = 8.4, 1.1 Hz, 2H, bis-indole H4), 8.60 (s, 2H, bis-indole-H2), 11.68 (brs, NH, pyrrole), 12.39 (brs, 2NH, bis-indole); ^{13}C NMR (DMSO-d_6) δ/ppm: 160.07, 136.62 (2C), 128.77 (2C), 128.56 (2C), 128.41 (2C), 124.83 (2C), 121.70 (2C), 121.49 (2C), 119.45 (2C), 118.27, 117.56, 114.65 (2C), 111.21 (2C), 111.10, 101.14, 95.19, 69.03, 56.06; MS-EI (m/z %): 428 [M^+]; Calcd. for : $C_{28}H_{20}N_4O$: C, 78.49; H, 4.70; N, 13.08; O, 3.73. Found: C, 78.54; H, 4.66; N, 13.07.

2,4-di(*1H*-Indol-3-*yl*)-5-(thiophen-2-*yl*)-*1H*-pyrrole-3-carbonitrile (5i):

Recrystallized using EtOH/DMF, yellow (70%); mp 241-243ºC; IR (KBr) mmax/cm^{-1} 3130-3396 (3 NH), 2221 (CN); ^{1}H NMR (500 MHz, DMSO d_6): δ 7.13 (m, 1H, thiophene H4), 7.25–7.28 (m, 4H, bis-indole H5, H6), 7.46 (m, 1H, thiophene H3), 7.50-7.53 (dd, *J* = 8.6, 1.2 Hz, 2H, bis-indole H7), 7.57 (m, 1H, thiophene H2), 8.14 (dd, *J* = 8.4. 1.1 Hz, 2H, bis-indole H4), 8.55 (s, 2H, bis-indole-H2), 11.71 (brs, NH, pyrrole), 12.43 (brs, 2NH, bis-indole); ^{13}C NMR (DMSO-d_6) δ/ppm: 140.06, 136.62 (2C), 128.72 (2C), 128.61, 128.54, 128.41 (2C), 128.09, 127.61 (2C), 124.63 (2C), 121.72, 121.42 (2C), 119.62 (2C), 118.21, 111.18 (2C), 99.08, 96.08, 69.41; MS-EI (m/z %): 404 [M^+]; Calcd. for : $C_{25}H_{16}N_4S$: C, 74.24; H, 3.99; N, 13.85; S, 7.93. Found: C, 74.26; H, 3.93; N, 13.89.

5-(2,4-Dichlorophenyl)-2,4-di(*1H*-indol-3-*yl*)-*1H*-pyrrole-3-carbonitrile (5j):

Recrystallized using EtOH/DMF, yellow (83%); mp 252-253ºC; IR (KBr) mmax/cm^{-1} 3120-3390 (3 NH), 2212 (CN); ^{1}H NMR (500 MHz, DMSO d_6): δ 7.25–7.28 (m, 4H, bis-indole H5, H6), 7.35 (s, 1H, 2,4-diCl-Ph), 7.48 (d, 1H, 2,4-diCl-Ph), 7.50-7.52 (dd, *J* = 8.6, 1.2 Hz, 2H, bis-indole H7), 8.01 (d, 1H, 2,4-diCl-Ph), 8.20 (dd, *J* = 8.4, 1.1 Hz, 2H, bis-indole H4), 8.59 (s, 2H, bis-indole-H2), 11.72 (brs, NH, pyrrole), 12.42 (brs, 2NH, bis-indole); ^{13}C NMR (DMSO-d_6) δ/ppm: 136.64 (2C), 135.76, 133.65,

131.36, 130.23, 12.74, 128.58, 128.45 (2C), 128.33, 127.67, 124.81, 121.64 (2C), 121.43 (2C),, 119.66 (2C), 118.20, 117.71, 111.14 (2C), 111.04, 100.05, 97.12, 69.48; S-EI (m/z %): 466 [M^+, 96%], 468 [M^++2, 65%], 470 [M^++4, 33%]; Calcd. for: $C_{27}H_{16}Cl_2N_4$: C, 69.39; H, 3.45; N, 11.99. Found: C, 69.42; H, 3.43; N, 12.03.

2,4-di(*1H*-Indol-3-*yl*)-5-phenyl-*1H*-pyrrole-3-carbonitrile (5k):

Recrystallized using EtOH/DMF, yellow (80%); mp 247-249ºC; IR (KBr) mmax/cm^{-1} 3120-3393 (3 NH), 22182 (CN); ^{1} H NMR (500 MHz, DMSO d_6): δ 7.26–7.28 (m, 4H, bis-indole H5, H6), 7.46 (m, 1H, Ph), 7.50-7.52 (dd, *J* = 8.6, 1.2 Hz, 2H, bis-indole H7), 7.68 (d, 2H, Ph), 7.83 (m, 2H, Ph), 8.20 (dd, *J* = 8.4, 1.1 Hz, 2H, bis-indole H4), 8.59 (s, 2H, bis-indole-H2), 11.74 (brs, NH, pyrrole), 12.46 (brs, 2NH, bis-indole); ^{13}C NMR (DMSO-d_6) δ/ppm: 136.64 (2C), 131.87, 130.02 (2C), 129.74 (2C), 128.42 (2C), 128.32, 127.60 (2C), 124.84, 121.61 (2C), 121.45 (2C),, 119.68 (2C), 118.21, 117.56, 111.12 (2C), 111.03, 100.03, 98.45, 69.19; S-EI (m/z %): 398 [M^+]; Calcd. for: $C_{27}H_{18}N_4$: C, 81.39; H, 4.55; N, 14.06. Found: C, 81.46; H, 4.51; N, 14.03.

2.2. Cytotoxicity screening

2.2.1. Cell culture

Three cell lines including human Prostate adenocarcinoma; metastatic cells (PC-3), human ovary adenocarcinoma (SKOV3) and human dukes' type B, colorectal adenocarcinoma (LS174T), were obtained from the American Culture Collection (ATCC). Cells were conserved in RPMI-1640 complemented with (100 µg/mL), penicillin (100parts/mL) and warmth-deactivated fetal bovine serum (10% v/v) in a moistened, 5% (v/v) CO_2 atmosphere at 37 ^{0}C.

2.2.2. Cytotoxicity assay

Three cells lines were preserved with six different concentrations of each complex (0.01, 0.1, 1, 10, 100 and 1000 mg); cells (control) were added. Cells were incubated with each concentration at 72 h and fixed with TCA (10% w/v) for 1 h at 4 ^{0}C. Three cells lines were washed many times and marked by 0.4% (w/v) SRB solution for 10 min in a dark room. The additional of stain was take out and removed with 1% (v/v) acetic acid. The SRB-marked cells were dry overnight, and subsequently dissolved with Tris-HCl and the color strength was studied in a micro plate reader at 540 nm. The relation between viability ratio of each growth cell line and tested molecule concentrations was examined to obtain the IC_{50} by Sigma Plot 12.0 software [40].

3. Results and discussion

1. 3.1. Chemistry

Synthesizing a variety of heterocyclic compounds for biological studies is one of our key program techniques [41-45]. We recently reported [46] the synthesis of a new series of indolylpyrrole derivatives using a multicomponent reaction of the known cyanochalcones **3**, [47-50], selected aldehydes **4**, and ammonium acetate in refluxing acetic acid, which was chemically confirmed by a green, high yields, and efficient method *via* a reaction of compound **1** with available benzoin **6** and excess ammonium acetate in refluxing H_2O/EtOH (Fig. 2) [51, 52].

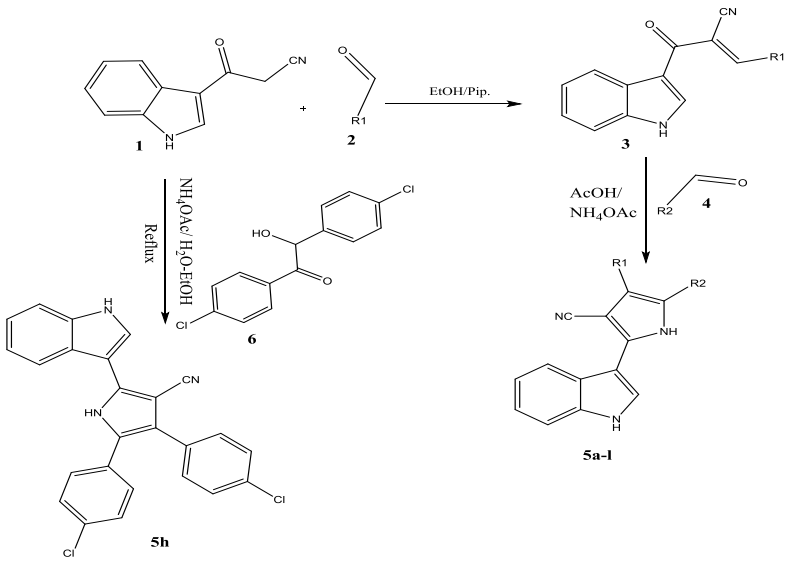
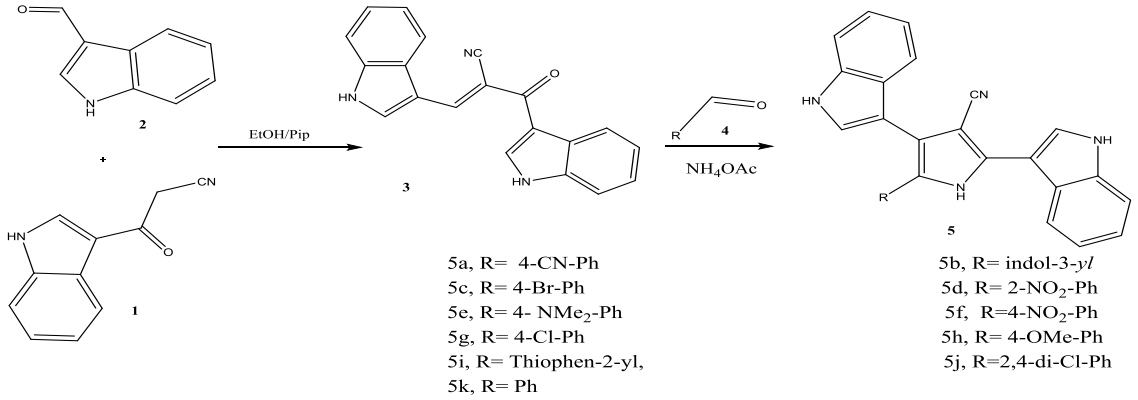


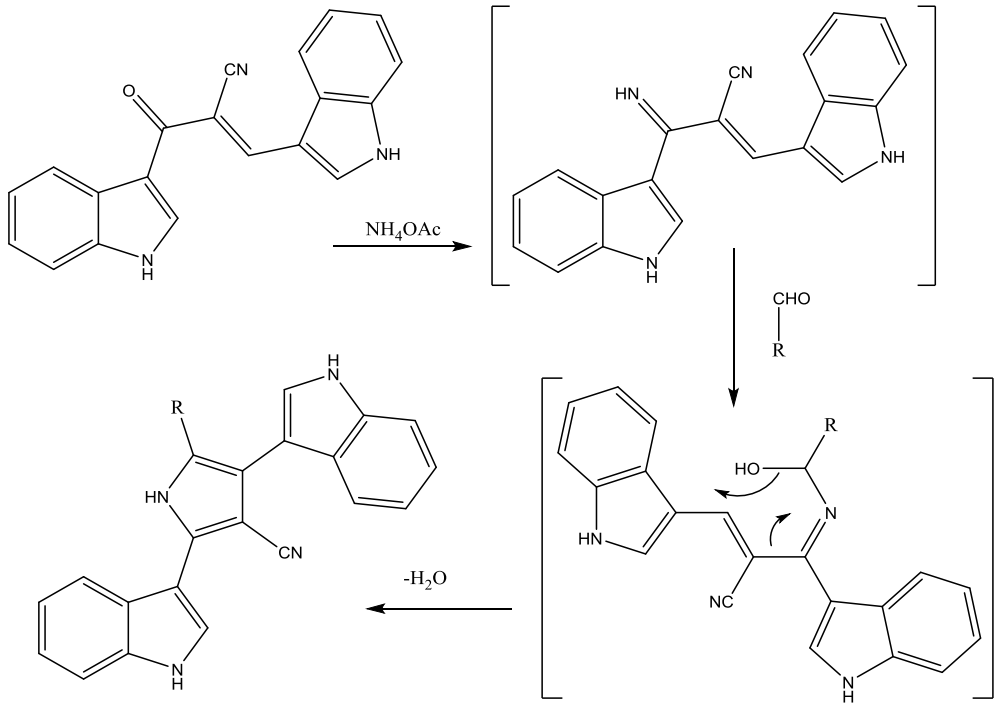
Fig. 2: Our previous work for the synthesis of new indolylpyrrole derivatives.

As illustrated in Scheme 1, a multicomponent reaction of (*E*)-3-(*1H*-indol-3-*yl*)-2-(*1H*-indole-3-carbonyl)acrylonitrile**,** α-cyano chalcones **3**, different available aldehydes **4**, and ammonium acetate in refluxing acetic acid yielded a new series of 2,4-bis(indol-3-*yl*)pyrrole derivatives **5a-j**



Scheme 1. Synthesis of new bis(indol-3-*yl*)pyrrole derivatives

The structures of the target compounds (**5a-l**) was confirmed using the spectroscopic studies and elemental analysis (experimental units), e.g., the IR of compound **5a** revealing bands at 3112-3320, 2210, and 2224 cm^{-1} for the three NH, and two CN groups, respectively, along with the loss of the C=O group. Additionally, ^{1}H NMR revealed three broad peak (exchangeable D_2O) at 11.89 and 12.21, 12.23 ppm of three NH groups, and the loss of distinguishing olefinic proton of chalcone **3a** (see Fig. 3). Furthermore, ^{13}C NMR data and mass spectroscopy reinforced the suggested construction of compound **5a** (m/z 423, M+). The proposed mechanism for the building of bis(indol-3-*yl*)pyrrole derivatives was illustrated in Scheme 2.



Scheme 2 The proposed mechanism of the new 2,4-bis(indol-3-*yl*)pyrrole derivatives

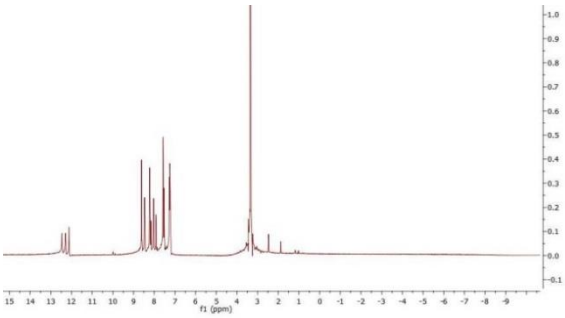


Fig. 3. H^1-NMR of compound **5a**

3.2. Cytotoxicity activities

All the target compounds (**5a-k**) were evaluated for their *in vitro* cytotoxic activity. The cytotoxic activity was realized by SRB (Sulforhodamine B colorimetric) assay towards three cell lines comprising human Prostate adenocarcinoma; metastatic cells (PC-3), human ovary adenocarcinoma (SKOV3) and human dukes' type B, colorectal adenocarcinoma (LS-174T), over a concentration range of 0.01 to 1000µg/ml. Most of the compounds exhibited variable cytotoxic activity. The tested compounds exhibited various cytotoxic traits against solid tumor cells. The compounds **5a, 5b, 5c, 5e, 5g, and 5j** have the most effective profile against PC-3, SKOV3 and LS174T cells with IC_{50} values in the 0.5 ± 0.2 to 4.7 ± 0.2µg/ml range, and also, compounds **5f** against PC-3 cells with IC_{50} 4.6 ± 0.3µg/ml as well as compounds **5d**, **5h**, **5i**, and **5j** have the similar stronger effect against SKOV3 cells with IC_{50} values in the 2.7 ± 0.7, 2.1 ± 0.4, 1.5 ± 0.3, and 0.5 ± 0.2 µg/ml range respectively, also compounds **5d,** and **5h** displayed toxic effect towards LS174T cells with IC_{50} values in the 4.4 ± 0.3, and 2.1 ± 0.14µg/ml range respectively (Table 1 and Fig. 4).

Table 1: The IC_{50} of bis(indol-3-*yl*)pyrroles (5a-k) against three tumor cell lines.

Compounds	IC_{50} (µg/ml)		
	PC-3	**SKOV3**	**LS-174T**
5a	2.9 ± 0.2	1.8 ± 0.1	2.6 ± 0.1
5b	3.4 ± 0.2	2.8 ± 0.1	2.2 ± 0.04
5c	2.3 ± 0.1	3.3 ± 0.2	3.1 ± 0.1
5d	14.3 ± 1.3	2.7 ± 0.7	4.4 ± 0.3
5e	1.6 ± 0.5	1.8 ± 0.2	3.3 ± 0.1
5f	4.6 ± 0.3	7.3 ± 0.3	7.8 ± 3.1
5g	4.7 ± 0.2	2.4 ± 0.1	2.8 ± 0.1
5h	7.9 ± 1.4	2.1 ± 0.4	2.1 ± 0.14
5i	15.9 ± 2	1.5 ± 0.3	9.6 ± 0.3
5j	4.01 ± 0.8	0.5 ± 0.2	3.8 ± 0.2
5k	5.8 ± 0.4	2.1 ± 0.13	5.1 ± 0.1
Doxorubicin	2.1 ± 0.1	2.2 ± 0.02	2.4 ± 1.2

In addition, compound **5k** has significance inhibiting proliferation of PC-3, and LS-174T cells with IC_{50}'s 5.8 ± 0.4 and 5.1 ± 0.1µg/ml respectively, and compound **5f** has similar effect against SKOV3 and LS-174T cells with IC50's 7.3 ± 0.3, and 7.8 ± 3.1µg/ml respectively as well as compound **5h** against PC-3 cells with IC_{50} 7.9 ± 1.4µg/ml. On the other hand, compounds **5d, and 5i** displayed a moderate effect against PC-3 cells with IC_{50}s 14.3 ± 1.3, and 15.9 ± 2µg/ml respectively (Table 1 and Fig. 4).

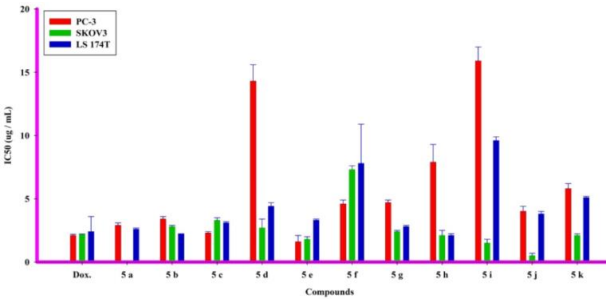


Fig.4. The IC_{50}s of 2,4-bis(indol-3-*yl*)pyrroles (**5a-k**) comparison with chemotherapy (doxorubicin) after 72 hr. incubation with three human adenocarcinoma cells (PC-3, SKOV3, and LS-174T).

4. Conclusions

The three-component process has been used to build a new series of bis(indol-3-*yl*)pyrrole derivatives *via* multicomponent reaction of α-cyano chalcones, appropriate aldehydes, and ammonium acetate in refluxed acetic acid. The chemical structures of the synthesized compounds were established with spectroscopic studies and then evaluated for their *in vitro* cytotoxic activity by SRB assay against three cell lines including human Prostate adenocarcinoma; metastatic cells (PC-3), human ovary adenocarcinoma (SKOV3) and human dukes' type B, colorectal adenocarcinoma (LS-174T). In summary, an antiproliferative activity of compounds **5a, 5e, 5h, 5j,** and **5k** against SKOV3, and compounds **5h** and **5b** against LS-174T as well as compound **5e** against PC-3 is higher than the doxorubicin drug activity. Therefore, this work presents a groundwork for extra research of certain bis(indol-3-*yl*)pyrrole derivatives as antiproliferative agents.

From the structure-activity relationship (SAR) point of view, it is observed that the 4-NMe_2-Phenyl substituted as compound **5e** increase the activity against both human Prostate adenocarcinoma; metastatic cells (PC-3), and human ovary adenocarcinoma (SKOV3) more than doxorubicin drug. Also, the 4-OMe-Phenyl derivatives as compound **5h** enhance the activity

against both human ovary adenocarcinoma (SKOV3) and human dukes' type B, colorectal adenocarcinoma (LS-174T) more than doxorubicin drug.

DECLARATION OF COMPETING INTEREST

The authors declare that they have no conflict of interest.

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5. REFERENCES

1- Higuchi, K, Kawasaki, T, (2007) Simple indole alkaloids and those with a non- rearranged monoterpenoid unit. Nat. Prod. Rep. 24(4), 843-868. doi.org/10.1039/B516351J

2- Lichman, BR, (2021) The scaffold-forming steps of plant alkaloid biosynthesis. Nat. Prod. Rep. 38(1), 103-129. doi.org/10.1039/D0NP00031K

3- Banerjee,T, Kar, D, Krishna, PR, Prabhakar, S, Nomula, R, Mallula, VS, Bandyopadhyay, A, (2015) A novel triazine-aryl-bis-indole derivative inhibits both phosphodiesterase IV and expression of cell adhesion molecules. RSC advances, 5(86), 70271-70281. doi.org/10.1039/C5RA11495K

4- Ölgen, S, Kaebler, A, Nebioğlu, D, Jose, J, (2007) New potent indole derivatives as hyaluronidase inhibitors. Chemical biology & drug design, 70(6), 547-551. doi: 10.1111/j.1747-0285.2007.00590.x

5- Smart, BP, Oslund, RC, Walsh, LA, Gelb, MH, (2006) The first potent inhibitor of mammalian group X secreted phospholipase A2: elucidation of sites for enhanced binding. J. Med. Chem. 49(10), 2858-2860. doi.org/10.1021/jm060136t

6- Hopkins, CR, Czekaj, M, Kaye, SS, Gao, Z, Pribish, J, Pauls, H, Levell, J, (2005) Design, synthesis, and biological activity of potent and selective inhibitors of mast cell tryptase. Bioorg. Med. Chem. Lett. 15(11), 2734-2737. DOI: 10.1016/j.bmcl.2005.04.002

7- Bao, B, Sun, Q, Yao, X, Hong, J, Lee, CO, Cho, HY, Jung, JH, (2007) Bisindole Alkaloids of the Topsentin and Hamacanthin Classes from a Marine Sponge Spongositessp. J. Nat. Prod. 70, 2−8. doi: 10.1021/np060206z

8- Bartik, K, Braekman, J-C, Daloze, D, Stoller, C, Huysecom, J, Vandevyver, G, Ottinger, R, (1987) Topsentins, new toxic bis-indole alkaloids from the marine sponge Topsentia genitrix. Can. J. Chem. 65, 2118−2121. doi:10.1139/v87-352

9- Tsujii, S, Rinehart, KL, Gunasekera, SP, Kashman, Y, Cross, SS, Lui, MS, Pomponi, SA, Diaz, MC, (1988) Topsentin, bromotopsentin, and dihydrodeoxybromotopsentin: antiviral and antitumor bis(indolyl)imidazoles from Caribbean deep-sea sponges of the family Halichondriidae. Structural and synthetic studies. J. Org. Chem. 53, 5446−5453. doi.org/10.1021/jo00258a009

10- Morris, SA, Andersen, RJ, (1989) Nitrogenous metabolites from the deep water sponge Hexadella sp. Can. J. Chem. 67, 677−681. doi.org/10.1139/v89-102

11- Murray, L, Lim, T, Hooper, J, Capon, R, (1995) Isobromotopsentin: a new bis(indole) alkaloid from a deep-water marine sponge Spongosorites sp. Aust. J. Chem. 48, 2053−2058. doi.org/10.1071/CH9952053

12- Shin, J, Seo, Y, Cho, KW, Rho, J-R, Sim, CJ, (1999) New Bis(Indole) Alkaloids of the Topsentin Class from the Sponge Spongosorites genitrix. J. Nat. Prod. 62, 647−649. DOI: 10.1021/np980507b

13- Chen, I, Safe, S, Bjeldanes, L, (1996) Indole-3-carbinol and diindolylmethane as aryl hydrocarbon (Ah) receptor agonists and antagonists in T47D human breast cancer cells. Biochemical pharmacology, 51(8), 1069-1076. doi.org/10.1016/0006-2952(96)00060-3

14- Suzen, S, Buyukbingol, E, (2000) Anti-cancer activity studies of indolalthiohydantoin (PIT) on certain cancer cell lines. Il Farmaco. 55(4), 246-248. doi.org/10.1016/S0014-827X(00)00028-8

15- Giagoudakis, G, Markantonis, SL, (2005) Relationships between the concentrations of prostaglandins and the nonsteroidal antiinflammatory drugs indomethacin, diclofenac, and ibuprofen. Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy. 25(1), 18-25. doi.org/10.1592/phco.25.1.18.55618

16- Suzen, S, Buyukbingol, E, (1998) Evaluation of anti-HIV activity of 5-(2-phenyl-3′-indolal)-2-

thiohydantoin. Il Farmaco. 53(7), 525-527. doi.org/10.1016/S0014-827X(98)00053-6

17- Ferguson, PJ, Phillips, JR, Selner, M, Cass, CE, (1984) Differential activity of vincristine and vinblastine against cultured cells. Cancer research. 44(8), 3307-3312. PMID: **6744266**

18- Shirinzadeh, H, Ghalia, M, Tascioglu, A, Adjali, FI, Gunesacar, G, Gurer-Orhan, H, Suzen, S, (2020) Bioisosteric modification on melatonin: synthesis of new naphthalene derivatives, in vitro antioxidant activity and cytotoxicity studies. Brazilian Journal of Pharmaceutical Sciences, 56. doi.org/10.1590/s2175-97902019000418124

19- Shirinzadeh, H, Ince, E, Westwell, AD, Gurer-Orhan, H, Suzen, S, (2016) Novel indole-based melatonin analogues substituted with triazole, thiadiazole and carbothioamides: studies on their antioxidant, chemopreventive and cytotoxic activities. Journal of enzyme inhibition and medicinal chemistry, *31*(6), 1312-1321. doi.org/10.3109/14756366.2015.1132209

20- Shirinzadeh, H, Neuhaus, E, Erguc, EI, Aliyev, AT, Gurer-Orhan, H, Suzen, S, (2020) New indole-7-aldehyde derivatives as melatonin analogues; synthesis and screening their antioxidant and anticancer potential. Bioorg. Chem., *104*, 104219. https://doi.org/10.1016/j.bioorg.2020.104219

21- Mukhopadhyay, D, Dasgupta, P, Roy DS, Palchoudhuri, I Chatterjee, S Ali, Dastidar,SG, (2016) A Sensitive In vitro Spectrophotometric Hydrogen Peroxide Scavenging Assay using 1,10-Phenanthroline, Free Rad Antiox 6, 124–132. doi.org/10.5530/fra.2016.1.15

22- Suzen S, (2007) Antioxidant Activities of Synthetic Indole Derivatives and Possible Activity Mechanisms. Book: Topics in Heterocyclic Chemistry, Bioactive Heterocycles V. Khan, M.T.H. (Ed.), Spinger-Verlag Berlin Heidelberg., 11, 145-178. doi.org/10.1007/7081_2007_074

23- Estevão, MS, Carvalho, LC, Ribeiro, D, Couto, D, Freitas, M, Gomes, A, Ferreira, LM, Fernandes, E, Marques, MB, (2010) Antioxidant activity of unexplored indole derivatives: synthesis and screening, Europ. J. Med. Chem. 45 4869–4878. doi.org/10.1016/j.ejmech.2010.07.059

24- Galano, A, (2016) Computational-aided design of melatonin analogues with outstanding multifunctional antioxidant capacity, RSC Adv. (6), 22951–22963. DOI: 10.1039/c6ra00549g

25- Suzen, S, (2015) Evaluation of synthetic melatonin analog antioxidant compounds. Book: Melatonin: Therapeutic Value and Neuroprotection. Srinivasan V, Gobbi G, Shillcutt S.D, Suzen S (Eds). Publisher: Boca Raton: Taylor & Francis, Chapter 21, 259- 269. ISBN 9780367378288

26- Biava, M, Fioravanti, R, Porretta, GC, Deidda, D, Maullu, C, Pompei, R, (1999) New pyrrole derivatives as antimycobacterial agents analogs of BM212. Bioorg. Med. Chem.. 9(20), 2983-2988. doi.org/10.1016/S0960-894X(99)00510-7.

27- MacLean, PD, Chapman, EE, Dobrowolski, SL, Thompson, A, Barclay, LC, (2008) Pyrroles as antioxidants: solvent effects and the nature of the attacking radical on antioxidant activities and mechanisms of pyrroles, dipyrrinones, and bile pigments. J. org. Chem. 73(17), 6623-6635. doi.org/10.1021/jo8005073

28- Cantrell, CL, Groweiss, A, Gustafson, KR, Boyd, MR, (1999) A new staurosporine analog from the prosobranch mollusk Coriocella nigra. Nat. Prod. Lett. 14(1), 39-46. https://doi.org/10.1080/10575639908045433

29- Lehuédé, J, Fauconneau, B, Barrier, L, Ourakow, M, Piriou, A, Vierfond, JM, (1999) Synthesis and antioxidant activity of new tetraarylpyrroles. European journal of medicinal chemistry. 34(11), 991-996. doi.org/10.1016/S0223-5234(99)00111-7

30- Hidalgo, FJ, Nogales, F, Zamora, R, (2003) Effect of the pyrrole polymerization mechanism on the antioxidative activity of nonenzymatic browning reactions. J. agr. food Chem. 51(19), 5703-5708. doi.org/10.1021/jf034369u

31- Petri, GL, Spanò, V, Spatola, R, Holl, R, Raimondi, MV, Barraja, P, Montalbano, A, (2020) Bioactive pyrrole-based compounds with target selectivity. Eur. Med. Chem., 112783. doi.org/10.1016/j.ejmech.2020.112783

32- Kilic-Kurt Z, Bakar-Ates F, Aka Y, Kutuk O, (2019) Design, synthesis and *in vitro* apoptotic mechanism of novel pyrrolopyrimidine

derivatives. Bioorg. Chem., 83:511–519. doi: 10.1016/j.bioorg.2018.10.060

33- Bortolozzi R, Carta D, Prà MD, Antoniazzi G, Mattiuzzo E, Sturlese M, Di Paolo V, Calderan L, Moro S, Hamel E, Quintieri L, Ronca R, Viola G, Ferlin MG, (2019) Evaluating the effects of fluorine on biological properties and metabolic stability of some antitubulin 3-substituted 7-phenyl-pyrroloquinolinones. Eur. J. Med. Chem.;178:297–314. doi: 10.1016/j.ejmech.2019.05.092

34- Sakemi, S, Sun, HH, (1991) Nortopsentins A, B, and C. cytotoxic and antifungal imidazolediylbis[indoles] from the Sponge Spongosorites ruetzleri. J. Org. Chem., 56, 4304−4307. doi.org/10.1021/jo00013a044

35- Carbone, D, Parrino, B, Cascioferro, S, Pecoraro, C, Giovannetti, E, Di Sarno, V, Diana, P, (2021) 1, 2, 4-Oxadiazole Topsentin Analogs with Antiproliferative Activity against Pancreatic Cancer Cells, Targeting GSK3β Kinase. *ChemMedChem*, *16*(3), 537-554. doi.org/10.1002/cmdc.202000752

36- Cascioferro, S, Attanzio, A, Di Sarno, V, Musella, S, Tesoriere, L, Cirrincione, G, Parrino, B, (2019) New 1, 2, 4-oxadiazole nortopsentin derivatives with cytotoxic activity. *Marine drugs*, *17*(1), 35. doi: 10.3390/md17010035

37- Carbone, A, Parrino, B, Barraja, P, Spano, V, Cirrincione, G, Diana, P, Maier, A, Kelter, G, Fiebig, HH, (2013) Synthesis and antiproliferative activity of 2,5-bis(3′-indolyl)pyrroles, analogues of the marine alkaloid nortopsentin. Mar. Drugs, 11, 643−654. doi: 10.3390/md11030643

38- Diana, P, Carbone, A, Barraja, P, Kelter, G, Fiebig, HH, Cirrincione, G, (2010) Synthesis and antitumor activity of 2,5-bis(3′-indolyl)- furans and 3,5-bis(3′-indolyl)-isoxazoles, nortopsentin analogues. Bioorg. Med. Chem., 18, 4524−4529. DOI: 10.1016/j.bmc.2010.04.061

39- Kamel, M. M., Abdel-hameid, M. K., El-Nassan, H. B., & El-Khouly, E. A. (2020). Recent advances in the synthesis and biological applications of nortopsentin analogs. *Chemistry of Heterocyclic Compounds*, *56*, 499-502. doi:10.1007/s10593-020-02687-4

40- Jeong-Chae, L., Hak-Ryul, K., Ju, K., Yong-Suk, J., (2002). Antioxidant property of an ethanol extract of the stem of Opuntia ficusindica var. saboten. J. Agr. Food Chem. 50, 6490–6496. https://doi.org/10.1021/jf020388c.

41- Muhammad, ZA, Radwan, MA, Farghaly, TA, Gaber, HM, Elaasser, MM, (2019) Synthesis and antitumor activity of novel [1, 2, 4, 5]-tetrazepino [6, 7-b] indole derivatives: marine natural product Hyrtioreticuline C and D analogues. Mini reviews in medicinal chemistry. 19(1), 79-86. DOI: 10.2174/1389557518666180724094244

42- El-Nezhawy, AO, Eweas, AF, Radwan, MA, El-Naggar, TB, (2016) Synthesis and Molecular Docking Studies of Novel 2-Phenyl-4-Substituted Oxazole Derivatives as Potential Anti-cancer Agents. J. Het. Chem. 53(1), 271-279. https://doi.org/10.1002/jhet.2422

43- Radwan, MA, Abbas, EM, (2009) Synthesis of some pyridine, thiopyrimidine, and isoxazoline derivatives based on the pyrrole moiety. Monatshefte für Chemie-Chemical Monthly. 140(2), 229-233. doi.org/10.1007/s00706-008-0061-y

44- Ghorab, M, Taha, NM, Radwan, MA, Amin, NE, Shehab, M, Faker, IM, (2008) Dapson in Heterocyclic Chemistry, Part I: Novel Synthesis of Sulfone Biscompounds for Antimicrobial and Antitumor Activities. Phosphorus, Sulfur, and Silicon. 183(12), 2891-2905. doi.org/10.1080/10426500802505408

45- Kalmouch, A., Rdwan, M., M Omran, M., Sharaky, M., & O Moustafa, G. (2020). Synthesis of novel 2, 3'-bipyrrole derivatives from chalcone and amino acids as antitumor agents. *Egyptian Journal of Chemistry*, *63*(11), 3-6. DOI: 10.21608/ejchem.2020.27117.2560

46- Radwan, MA, Al Rugaie, O, Al Abdulmonem,W, Alfaifi, MY, Elbehairi, S, (2021) Synthesis and cytotoxic activity of new indolylpyrrole derivatives. Arabian Journal of Chemistry. doi.org/10.1016/j.arabjc.2021.103209

47- Radwan, MA, Alminderej, FM, Awad, HM, (2020) One-pot multicomponent synthesis and cytotoxic evaluation of novel 7-substituted-5-(*1H*-indol-3-*yl*) tetrazolo [1, 5-a] pyrimidine-6-carbonitrile. *Molecules*. 25(2), 255. doi:10.3390/molecules25020255

48- Quiroga, J, Trilleras, J, Sanchez, A.I, Insuasty, B, Abonia, R, Nogueras, M, Cobo, J (2009)

Regioselective three-component synthesis of indolylpyrazolo [3, 4-b] pyridines induced by microwave and under solvent-free conditions. *Letters in Organic Chemistry*, *6*(5), 381-383.

49- Kamalraja, J, Sowndarya, R, Perumal, PT, (2014). A Greener Approach for the Regioselective Synthesis of Multifunctionalized Indolylpyrrole and Indolyltriazolylpyrrole Hybrids via Michael Addition of α-Azido Ketones. *Synlett*, *25*(15), 2208-2212

50- Li, L, Xu, H, Dai, L, Xi, J, Gao, L, Rong, L, (2017) An efficient metal-free cascade process for the synthesis of 4-arylpyrimido [1, 2-b] indazole-3-carbonitrile derivatives. Tetrahedron, *73*(36), 5358-5365

51- Tamaddon, F, Amirpoor, F, (2013) Improved Catalyst-Free Synthesis of Pyrrole Derivatives in Aqueous Media. Synlett, 24(14), 1791-1794.

52- Bhat, SI, Trivedi, DR, (2013) A catalyst-and solvent-free three-component reaction for the regioselective one-pot access to polyfunctionalized pyrroles. Tetrahedron Letters. 54(41), 5577-5582