Research Article

A Novel Multi-Component Reaction of Indoles and Formyl Furochromone was Described for the Synthesis of Indoles Derivatives with Expected Antitumor Activity

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ABSTRACT

A new strategy for synthesis of 3-diaryl indoles (4_{a-c}) was developed through $FeCl_3$ as Lewis acid catalyzed three-component aza-Friedel-Crafts reactions of aldehyde (1) tertiary aromatic amines (2) and indole derivatives (3_{a-c}) in one-pot. A simple and efficient synthesis of compounds (5a-d) were achieved via Schiff's base reaction of 3-diaryl indole (4_b) with various aldehydes namely benzaldehyde, hydroxylbenzaldehyde, isonicotinaldehyde and furoaldehyde respectively. Compounds (6a-c) were obtained by the reaction of compound (4c) with different halide compounds namely 3-bromoprop-1-ene, iodoethane and benzyl chloride respectively. The reaction of cyclohexyl isocyanide (7) with aldehyde (1) and indole (3_c) has given compound (8) in one step. While, the reaction of cyclohexyl isocyanide (7) with aldehyde (1) has given compound (9) which reacted with indole (3_c) yielded compounds (10). Five selected indole derivatives 4a, 4b, 5b, 5d and 6a were subjected to a screening system for investigation of their antitumor potency against breast (MCF7) and liver (HEPG2) cell lines. Moreover, the biochemical effects of the selected indole derivatives on some enzymes such as aspartate and alanine aminotransferase (AST and ALT) and alkaline phosphatase (ALP), in addition to albumin, globulins, creatinine, total lipids, cholesterol, triglycerides and bilirubin in serum of mice were studied in comparison to 5-Flurouracil and Doxorubicin. The antitumor activity results indicated that most of the selected indole derivatives showed moderate growth inhibition activity against liver (HEPG2) cell line. Moreover, compound (6a), showed anti-proliferative activity against both breast and liver cell lines.

Keywords: Cyclohexyl isocyanide, Aza-Friedel-Crafts reaction and Schiff's base. Breast and Liver Cancer cell lines

INTRODUCTION

Multicomponent reactions (MCRs) are of increasing importance in organic and medicinal chemistry, as high degrees of molecular diversity can be introduced by these reactions in a very fast, efficient, and time saving manner without the isolation of any intermediates.[1]

As a result, considerable attention paid to the development of new and improved one-pot multicomponent reactions in recent years.[2] Multicomponent reactions offer a tangible way to access both chemically and structurally diverse class of molecules.[1]

Moreover, multicomponent organic transformations accomplish by readily available, inexpensive catalysts/reagents are invariably attractive.[3] Indoles are important structural units in many natural products and their derivatives are known to possess various biological properties,[4] such as, antibactenrial, antioxidative, and insecticidal activities, also some indole derivatives uses as antibiotics in pharmaceuticals.[5] Indole frame work is present in plethora of bioactive molecules such as etodolac,[6] indometacin,[7] and reserpine,[8] are used for treating inflammation, hypertension, and psychiatric disorders.

The presence of indole skeleton plays a vital role in determining anticancer activity of tryprostatin A, an antimitotic agent used in the treatment of multidrug resistant tumours.[9] 3-Alkyl substituted indoles are useful in the total synthesis of paraherquamide A[10] anthelmintic agent; clavicipitic acid,[11] a derailment product of ergot alkaloid biosynthesis; indolmycin,[12] a novel antibioticveiutamine,[13] a marine alkaloid etc.

A great deal of work devotes to the study of indole derivatives since this moiety is found in a wide variety of natural and synthetic.

MATERIALS AND METHODS

Experimental

Chemistry

All melting points are uncorrected and were taken on electro-thermal capillary melting point apparatus. The melting points were measured in degrees centigrade and determined using BucHI 510 apparatus.

Elemental analyses were carried out in the micro analytical unit of the National Research Centre.

IR spectra were recorded on a Mattson-5000 FTIR spectrometer using KBr Wafer technique.[1] H-NMR spectra were determined on a Varian-Gemini-300 MHz and Jeol-Ex-300 MHz NMR spectrometer using TMS as an internal standard with (chemical shift δ= 0 ppm).

Mass Spectra were determined on Finnigan MatSSQ 7000 mode: EI, 70Ev (Thermo Inst. Sys. Inc., USA). The purity of

the synthesized compounds was tested by thin layer chromatography (TLC), Merck plates.

TLC Silica gel 60 F_{254} 25 Aluminum sheets 20 x 20 cm.

General Procedure for the Preparation of Compounds (4_{a-c})

A mixture of indole derivatives, namely 2-thiophen-2yl)-1H-indole,4-(1H-indole-2-yl)benzylamine and 1H-indole (3a-c)(1.0mmol), aldehyde(1) (1.0mmol),N,N-dimethyleaniline (1.0mmol), $FeCl_3$ (0,1mmol) and 1,2-dichloroethane (2mmol) was stirred and refluxed in an oil bath at 100°C for 24h under nitrogen, left to cool to room temperature, diluted with aqu.$NaHCO_3$, the resulting solid obtained was recrystallyzed from ethanol.

6-((4-(dimethylamino)phenyl)((S)-2-(thiophen-2-yl)-1H-indol-3-yl)methyl)-4,9-dimethoxy-5H-furo[3,2-g]chromen-5-one(4a)

Crystallized from ethanol pale gray solid m.p.160 °C, yield 75%. Analysis: for $C_{34}H_{28}N_2O_5S$ M.Wt., 576.66 calcd C, 70.82; H, 4.89; N, 4.86; S, 5.56 Found: C, 80.13; H, 4.90; N, 4.88; S, 5.55 IR (KBr, cm^{-1}): 3149 (NH) and 1638 (C=O); ^{1}HNMR (DMSO-d_6, δ, ppm): 2.47 (s, 6H, 2 CH_3), 3.77, 3.32 (ss, 6H, 2OCH_3), 6.46 (s, 1H, methyl), 7.14 (t, 1H, thiophene ring), 7.40-6.46 (m, 8H, arom.), 7.62, 7.28 (dd, 2H, J= 1.8 thiophene ring), 7.80, 7.28 (dd, 2H, J=2.01 furan ring), 8.11 (s, 1H, H_7) 11.70 (s, 1H, NH exchangeable with D_2O).

6-((2-(4-aminophenyl)-1H-indol-3-yl)(4-(dimethylamino)phenyl)methyl)-4,9-dimethoxy-5H-furo[3,2-g]chromen-5-one (4b)

Crystallized from ethanol, dark blue solid, m.p170 °C yield 80% Analysis: for $C_{36}H_{31}N_3O_5$ M.Wt. 585.65, calcd: C, 73.83; H, 5.34; N, 7.17 Found: C, 73.07; H, 5.30; N, 7.10; IR (KBr, cm^{-1}) br. band at 3455-3150 (NH, NH_2) and 1611 (C=O). ^{1}H-NMR (DMSO-d_6, δ, ppm): 2.56 (s, 6H, 2CH_3), 3.86, 3.75 (s, 6H, 2OCH_3), 4.02 (s, 2H, NH_2 , exchangeable with D_2O), 6.35 (s, 1H, methyl), 7.21-6.93 (m, 12H, arom.), 7.48, 6.61 (dd, 2H, J=2.0 furan ring), 8.07 (s, 1H, H_7) and 10.84 (s, 1H, NH exchangeable with D_2O).

6-((4-(dimethylamino) phenyl) (indolin-3-yl) methyl)-4, 9-dimethoxy-5H-furo [3,2-g] chromen-5-one (4c)

Crystallized from ethanol, dark violet solid, m.p.165 °C, yield 80%. Analysis: for $C_{30}H_{26}N_2O_5$ Mol. Wt.: 494.45 calcd: C, 72.86; H, 5.30; N, 5.66 Found: C, 72.26; H, 5.32; N, 5.62; IR (KBr, cm^{-1}), 337, 3299 (NH) and 1611 (C=O). ^{1}H-NMR (DMSO-d_6, δ, ppm): 2.47 (s, 6H, 2 CH_3), 3.36, 3.89 (s, 6H, 2OCH_3), 7.33, 7.01 (m, 8H, arom.), 7.77, 6.96 (dd, 2H, J=2.01, furan ring), 8.08 (s, 1H, H_7) and 10.85 (s, 1H, NH exchangeable with D_2O), MS: (m/z)494.18 (7%), 117(100%), 90.15 (90%).

General Procedure for the Preparation of Compounds (5a-d)

In one-pot reaction, a mixture of compound (4b) (1.0 mmol) in 30ml ethanol was refluxed then added aldehyde derivatives namely benzaldehyde, p-hydroxybenzaldehyde, isonicotin aldehyde and furaldehyde(1:1 mol) and few drops of acetic acid for 2-4h after the reaction completed it poured on ice to get the products (5a-d) which then crystallized from ethanol.

6-((2-(4-(benzylideneamino)phenyl)1H-indol-3-yl)(4-(dimethylamino)phenyl)methyl)-4,9-dimethoxy-5H-furo[3,2-g]chromen-5-one (5a)

Crystallized from ethanol, violet solid, m.p. >290 °C, yield 90%. Analysis: for $C_{43}H_{35}N_3O_5$, Mol. Wt.: 673.27 calcd: C, 76.63; H, 5.24; N, 6.24 Found: C, 76.65; H, 5.26; N, 6.22; IR (KBr, cm^{-1}) band at 3150 (NH), 1607 (C=N) and1600-1680 (C=O). ^{1}H-NMR (DMSO-d6, δ, ppm): 2.46 (s, 6H, 2 CH_3), 3.81 (s, 6H, 2OCH_3), 4.78 (s, 1H, CH), 7.31, 6.87 (dd, 2H, J=2.01, furan ring), 7.33-6.93 (m, 17H, arom.), 8.07 (s, 1H, H_7) 8.39 (s, 1H, CH=N) and10.81 (s, 1H, NH exchangeable with D_2O).

6-((2-(4-(benzylideneamino)phenol)indol-3-yl)(4-(dimethylamino)phenyl)methyl)-4,9-dimethoxy-5H-furo[3,2-g]chromen-5-one (5b)

Crystallized from ethanol, violet solid, m.p.>290 °C, yield 90%. Analysis: for $C_{43}H_{35}N_3O_6$ Mol. Wt.: 689.25 calcd: C, 74.88; H, 5.11; N, 6.09 Found: C, 74.87; H, 5.13; N, 6.07; IR (KBr, cm^{-1}) br. band at 3600-3300 (NH, OH), 1608 (C=N) and 1600-1680 (C=O). ^{1}H-NMR (DMSO-d6, δ, ppm): 2.12 (s, 6H, 2 CH_3), 3.73 (s, 6H, 2OCH_3), 4.71 (s, 1H, CH), 5.00 (s, 1H, NH exchangeable with D_2O), 7.60, 7.00 (dd, 2H, J=1.8, furan ring), 7.60-7.91 (m, 17H, arom.), 8.10 (s, 1H, H_7), 8.39 (s, 1H, CH=N) and 10.02 (s, 1H, OH exchangeable with D_2O).

6-((2-(4-pyridine-4-yl)methyleneamino)phenyl)-1H-indol-3-yl)(4-(dimethylamino)phenyl)methyl)-4,9-dimethoxy-5H-furo[3,2-g]chromen-5-one (5c)

Crystallized from ethanol, violet solid, m.p.>290 °C, yield 90%. Analysis: for $C_{42}H_{34}N_4O_5$ Mol. Wt.: 674.74, calcd: C, 73.08; H, 6.30; N, 7.10 Found: C, 73.06; H, 6.31; N, 7.11 IR (KBr, cm^{-1}) 3150 (NH), 1608 (C=N) and 1600-1680 (C=O). ^{1}H-NMR (DMSO-d6, δ, ppm): 2.12 (ss, 6H, 2 CH_3), 3.73 (s, 6H, 2OCH_3), 4.71 (s, 1H, CH), 7.60, 7.00 (dd, 2H, J=1.8, furan ring), 8.10 (s, 1H, H_7), 7.60-7.91 (m, 16H, arom.), 8.39 (s, 1H, CH=N) and 10.02 (s, 1H, NH exchangeable with D_2O).

6-((2-(4-((furan-2-yl)methyleneamino)phenyl)-1H-indol-3yl)(4-(dimethylamino)phenyl)methyl)-4,9-dimethoxy-5H-furo[3,2-g]chromen-5-one (5d)

Crystallized from ethanol, violet solid, m.p.>290 °C, yield 90%. Analysis: for $C_{41}H_{33}N_3O_6$ Mol. Wt.: 663.72 calcd: C, 74.19; H, 5.01; N, 6.33 Found: C, 74.17; H, 5.03; N, 6.31 IR (KBr, cm^{-1}) 3150 (NH), 1608 (C=N) and 1600-1680 (C=O). ^{1}H-NMR (DMSO-d6, δ, ppm): 2.12 (ss, 6H, 2 CH_3), 3.73 (s, 6H, 2OCH_3), 4.71 (s, 1H, CH), 7.60, 7.00 (dd, 4H, J= 1.8, furan ring), 8.10 (s, 1H, H_7), 7.60-7.91 (m, 12H, arom.), 8.39 (s, 1H, CH=N) and 10.02 (s, 1H, NH exchangeable with D_2O).

General Procedure for the Preparation of Compounds (6a-c)

A mixture of compound (4c) (1.0mmol) in DMF (5ml) unhydrous pot. carbonate, halide derivatives namely 3-bromopropene, iodoethan and benzyl chloride.

The reaction mixture was refluxed for 3-4 h, after compellation the reaction it poured on to crashed ice to get the products (6a-c) which then crystallized from ethanol.

6-((4-(dimethylamino)phenyl)(indolin-3-yl)methyl)-4,9-dimethoxy-5H-furo[3,2-g]chromen-5-one (6a)

Crystallized from ethanol, violet solid, m.p.>290 °C, yield 90%. Analysis: for $C_{33}H_{30}N_2O_5$ Mol. Wt.: 534.46 calcd: C, 74.14; H, 5.66; N, 5.24 Found: C, 74.16; H, 5.65; N, 5.23 IR (KBr, cm^{-1}) 1614 (C=O); ^{1}H NMR (DMSO-d6, δ, ppm): 2.46, 2.47 (ss, 6H, 2 CH_3), 3.76, 4.06 (s, 6H, 2OCH_3), 4.53 (s, 1H, methyl), 4.63, 4.30 (d, 2H, CH_2), 5.70 (d, 2H, =CH_2), 5.81 (m, 1H, CH=), 6.77 (s, 1H, CH indole), 7.31, 6.87 (dd, 2H, J=2.01, furan ring), 7.33-6.93 (m, 8H, arom.) and 8.07 (s, 1H, H7).

6-((4-(dimethylamino)phenyl)(1-ethylindolin-3-yl)methyl)-4,9-dimethoxy-5H-furo[3,2-g] chromen-5-one (6b)

Crystallized from ethanol, violet solid, m.p.290 °C, yield 90%. Analysis: for $C_{32}H_{30}N_2O_5$ Mol. Wt.: 522.59, calcd: C, 73.55; H, 5.79; N, 5.36 Found: 73.57; H, 5.78; N, 5.35 IR (KBr, cm^{-1}) 1608 (C=O); ^{1}H NMR (DMSO-d6, δ, ppm): 1.26 (t, 3H, CH_3), 2.06, 2.07 (ss, 6H, 2 CH_3), 3.74, 3.76 (s, 6H, 2OCH_3), 3.98 (q, 2H, CH_2), 4.54 (1H, CH methyl), 6.57 (s, 1H, CH indole), 7.31, 6.87 (dd, 2H, J=2.01, furan ring), 7.33-6.93 (m, 8H, arom.), 7.87 (s, 1H, H_7).

6-((1-benzyl-1H-indol-3-yl)(4-(dimethylamino)phenyl)methyl)-4,9-dimethoxy-5H-furo[3,2-g]chromen-5-one (6c)

Crystallized from ethanol, violet solid, m.p.>290 °C, yield 90%. Analysis: for $C_{37}H_{32}N_2O_5$ Mol. Wt.: 584.66, calcd: C, 76.01; H, 5.52; N, 4.79 Found: C, 76.02; H, 5.50; N, 4.80 IR (KBr, cm^{-1}) 1634 (C=O); ^{1}H NMR (DMSO-d6, δ, ppm) 2.96, 2.89 (ss, 6H, 2 CH_3), 3.76, 3.71 (s, 6H, 2OCH_3) 4.93 (s, 1H, CH methyl), 5.11 (s,2H,CH_2), 6.01 (s, 1H, CH indole), 7.01, 6.99 (dd, 2H, J=2.0, furan ring), 8.03 (s, 1H, H_7) and 7.46-7.00 (13H, arom.).

General Procedure for the Preparation of Compound (8)

A mixture of indole (3c) (1.0 mmol), aldehyde (1) (1.0mmol) and isocyanide (7) (0.5 mmol) in ethanol in the presence of excess of ammonium acetate (2ml) which was added drop by drop the reaction mixture was refluxed for 6-8h at room temperature.

The solid was filtered off and recrystallized from ethanol to afford the pure product.

6-(2-cyclohexyimino)-1-(1H-indol-3-yl) ethyl)-4,9-dimethoxy-5H-furo[3,2-g]chromen-5-one (8)

Crystallized from methanol, brown solid m.p. 125 °C yield (80%). Analysis: for $C_{29}H_{28}N_2O_5$, Mol. Wt.: 484.54, calcd: C, 77.88; H, 5.28; N, 5.78 Found: C, 77.86; H, 5.29; N, 5.79 IR (KBr, cm^{-1}): 3283 (NH), 1609 (C=O) and (C=N); ^{1}H-NMR (DMSO-d_6, δ, ppm): 1.87- 1.19 (m, 11H, 1NCH, 5CH_2 cyclohexane), 3.50 (s, 1H, methyl), 3.78 (s, 6H, 2OCH_3), 6.80 (s, 1H, = CH indole), 7.50 (s, 1H, CH=N) 7.31, 6.93 (dd, 2H, J=2.0, furan ring), 8.07 (s, 1H, H_7), 7.76-6.93 (m, 4H, arom.**)** and 10.81 (s, 1H, NH exchangeable with D_2O)**.**

General Procedure for the Preparation of Compound (9)

To a magnetically stirred solution of formyl furochromone (1) (2.0 mmol) in dry CH_2Cl_2 (10 ml) in screw-capped vial, was added cyclohexyl isocyanide (7) (1.0 mmol) via a syringe at room temperature (25 °C). The reaction mixture was then stirred for 4 days and the completion of reaction was confirmed by TLC (EtOAc / hexane 1:1). Then, the resulting solid were filtered off and washed with acetone (5 ml) to yield (9). The dried product thus obtained showed single spot on TLC and was pure enough for all analytical purposes.

6-((14Z)-((18E)-3-(cyclohexylimino)-5,8-dimethoxy-9-oxo-3H-furo[3,4-b]chromen-1(9H)-furo[7,6]-4,9-dimethoxy-5H-furo[3,2-g]chromen-5-one(9)

Crystallized from acetone yellow solid m.p.90 °C yield (80%). Analysis: for $C_{35}H_{29}NO_{11}$, Mol. Wt.: 639.60, calcd: C, 65.72; H, 4.57; N, 2.19 Found: C, 65.73; H, 4.56; N, 2.20 IR (KBr, cm^{-1}): 1664 (C=O), 1613 (C=C) and 1546 (C=N). ^{1}H-NMR (DMSO-d_6, δ, ppm): 1.19-1.87 (m, 11H, 5CH_2, NCH, cyclohexane), 3.86, 3.96 (4s, 12H, 4OCH_3), 6.00 (s, 1H, methyl), 6.93, 7.31 (dd, 2H, J=2.0, furan ring) and 8.07 (s, 1H, H_7)**.**

General Procedure for the Preparation of Compound (10)

A mixture of compound (9) (1.0, mmol) and 1-*H*-indole (3c) (1.0 mmol) in CH_2Cl_2, the reaction mixture was refluxed for 4-6h and tested by TLC till completed evaporate the solvent and the precipitate was collected then crystallized from ethanol.

3-(Cyclohexylimino)-6-[(4,9-dimethoxy-6 (furan-5H-furo [3,2-g] chromen-5-one- 1-methylene 6-(1-1H-indole-3-yl)vinyl)-4,9-dimethoxy-5H-furo[3,2-g]chromen-5-one (10)

Crystallized from ethanol yellow solid m.p. 95 °C yield (80%). Analysis: for $C_{43}H_{34}N_2O_{11}$, Mol. Wt.: 754.22, calcd: C, 68.43; H, 4.54; N, 3.71 Found: C, 68.44; H, 4.55; N, 3.70 IR (KBr, cm^{-1}): 3123 (NH), 1664 (C=O), 1613 (C=C), and 1546 (C=N). ^{1}H-NMR (DMSO-d_6, δ, ppm): 1.19-1.87 (m, 11H, 5CH_2, NCH, cyclohexane), 3.86, 3.96 (4s, 12H, 4OCH_3), 6.67 (s, 1H, CH indole), 7.31, 6.93 (dd, 2H, J=2.0, furan ring), 7.36-6.33 (m, 4H, arom.**),** 7.67 (s, 1H, H_7) and 10.11 (s, 1H, NH exchangeable with D_2O).

Anticancer Testing

A series of novel of indole derivatives that possessing a broader spectrum of antitumor activity and fewer toxic

side effects than traditional anticancer drugs have been investigated.

Five selected indole derivatives (compounds 4a, 4b, 5b, 5d and 6a) were subjected to a screening system for investigation of their antitumor potency against breast (MCF7) and liver (HEPG2) cell lines. Moreover, the biochemical effects of the selected indole derivatives on some enzymes such as aspartate and alanine aminotransferases (AST and ALT) and alkaline phosphatase (ALP), in addition to albumin, globulins, creatinine, total lipids, cholesterol, triglycerides and bilirubin in serum of mice were studied in comparison to 5-Flurouracil and Doxorubicin.

Measurement of Potential Cytotoxicity by SRB Assay

The selected indole derivatives (compounds 4a, 4b, 5b, 5d, 6a) were subjected to a screening system for evaluation of their antitumor activity against Breast MCF7 and Liver HEPG2 cancer cell lines in comparison to the known anticancer drugs: 5-FU and DOX.

Potential cytotoxicity of the selected indole derivatives was tested using the method of Skehan[14] as follows:

Cells were plated in 96-multiwell plate (10^4 cells/well) for 24 h before treatment with the compound(s) to allow attachment of cells to the wall of the plate. Different concentrations of the compound under test (0, 1, 2.5, 5, 10 and 50 µg/ml) were added to the cell monolayer. Triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the compound(s) for 48 h at 37 ^{o}C and in an atmosphere of 5% CO_2. Cultures were then fixed with trichloroacetic acid and stained for 30 minutes with 0.4% (wt/vol) sulforhodamine B (SRB) dissolved in 1% acetic acid. Unbound dye was removed by four washes with 1% acetic acid, and protein-bound dye was extracted with 10 mM unbuffered Tris base [tris hydroxymethyl aminomethane] for determination of optical density in a computer-interfaced, 96-well micro titer plate reader.

The SRB assay results were linear with the number of cells and with values for cellular protein measured by both the Lowry and Bradford assays at densities ranging from sparse sub confluence to multilayered supra confluence. The signal-to-noise ratio at 564 nm was approximately 1.5 with 1,000 cells per well.

The relation between surviving fraction and drug concentration is plotted to get the survival curve of both cancer cell lines after the specified compound.

Biochemical Analysis

Animals

Male albino mice weighing 18-20 g were used in the present study. Mice were divided into three main groups as follows:

- Group (1): Untreated or control group (5 mice).
- Group (2): divided into two subgroups (5 mice for each subgroup) and treated with 5-FU or DOX as reference anticancer drugs.
- Group (3): divided into eight subgroups (5 mice for each subgroup) and treated with the selected indole derivatives.

Treatment

- Group (1): each mouse was given a single intraperitoneal injection of 0.1ml DMSO.
- Group (2): each mouse was given a single intraperitoneal injection of 0.1ml containing 12 mg/kg body weight 5-FU or DOX dissolved in sterile water.
- Group (3): each mouse was given a single intraperitoneal injection of 0.1ml containing 12 mg/kg body weight of the s selected indole derivatives (compounds 4a, 4b, 5b, 6a, 5d) respectively) dissolved in DMSO.

Blood was collected after 7 days from all mice groups.

The biochemical effects of the selected indole derivatives (compounds 4a, 4b, 5b, 5d, 6a), on some liver enzymes such as aspartate and alanine aminotransferases (AST and ALT)[15] and alkaline phosphatase (ALP)[16], were done using blood auto analyzer (Olympus AV 400, Japan).

Moreover, albumin[17], globulins[18] and creatinine[19], total lipids[20], cholesterol[21], triglycerides[22] and bilirubin[23] in serum of mice were evaluated in comparison to 5-FU and DOX.

Statistical analysis of the results was performed using Chi-square values (SPSS computer program).

Results of the biochemical investigations indicated that 5-fluorouracil and Doxorubcin caused significant changes in the level of all parameters tested while treatment with selected compounds showed slight, moderate or no significant changes.

RESULTS AND DISCUSSION

Chemistry

The indole nucleus was an important substructure found in numerous natural alkaloids.[24,25] Multicomponent reactions (MCRs) involving at least three starting materials in a one-pot. Reactions were remain the most efficient method of synthesis of heterocyclic[26] Isocyanides-based multicomponent reactions (IMCRs), such as the Passerini and the Ugi reactions were very useful for the diversity-oriented synthesis of collections of compounds. They were allowed a dramatic increase of structural complexity in just one step, introducing at the same time three or more diversity inputs with a high degree of atom economy.[27] As part of our ongoing efforts devoted to iron-catalyzed organic reactions,[3] herein we wished to report the first genuinely and highly efficient $FeCl_3$ as Lewis acid catalyzed one-pot three-component

aza-Friedel-Crafts reaction of indoles, aldehydes and tertiary aromatic amines. The reactions generated were corresponding 3-diarylmethyl indole derivatives (4a-c), in good yield under mild reaction conditions (scheme 1)[28].

Also, compound (4b) has been reacted with different aldehydes namely benzaldehyde, hydroxylbenzaldehyde, isonicotinaldehyde and furaldehyde respectively via Schiff's base reaction to yield compounds (5a-d) (scheme 2)[29]. The alkylation and arylation reaction of compound (4c) with different halide compounds namely 3-bromoprop-1-ene, iodoethane and benzyl chloride respectively) has been yielded compounds (6a-c) (scheme 3)[30].

The outstanding position of IMCRs can be traced back to the exceptional reactivity of the functional group of the isocyanide.[31] In connection with our previous research on indole, guided by observation that the presence of two or more different heterocyclic moieties in a single molecule often enhances the biocidal remarkably, we investigated a three-component reaction of cyclohexyl isocyanides (7), aldehyde (1) and indole (3c) which were afforded compound (8) in one step,[32] scheme (4). Also, the reaction of formyl furochromone (1), with cyclohexyl isocyanide (7) in dry dichloromethane at room temperature has been reported to give compound (9),[33] (scheme 4). At the reaction of compound (9) with indole lead to compound (10).

Anticancer Activity

Preliminary screening of the selected indole derivatives showed that all selected compounds exhibited a slight to moderate growth inhibition activities on the tested cell lines between 1-50 µg/ml concentrations in comparison to the known anticancer drugs: 5-Flurouracil and Doxorubicin. Table 1 indicated the cytotoxic activity of the newly synthesized indole derivatives (compounds 1-8) against Breast MCF7 and liver HEPG2 cancer cell lines in comparison to the traditional anticancer drugs: 5-FU and DOX. It can be deduced from the results that compound 6a was the most active and induced a reasonable growth inhibition, in a dose-dependent manner against Breast MCF7 and liver HEPG2 cancer cell lines when compared to 5-FU and DOX (IC_{50} equals 19.4 and 43 µg/ml).

Table 1: Effect of some selected newly synthesized compounds on liver (HEPG2) and breast (MCF7) carcinoma cell lines.

COMPOUND	CELL LINS	
	HEPG2(IC50)	MCF7(IC50)
5-flurouracil	5	0.67
Doxorubicin	3.56	6.71
4a	44	-ve
4b	-ve	-ve
5b	-ve	-ve
6a	43	19.4
5d	40.9	-ve

Effect of Antitumour Compounds on the Biochemical Parameters

Data obtained in Table 2 presents the liver enzymatic activities (ALT, AST and ALP) in serum of control and treated groups of mice.

Table 2: Biochemical effects of treatment with 5-FU, DOX and indole derivatives on serum ALT, AST and ALP in mice.

Biochemical Parameters Mice Groups	Alanine amino transferase	Aspartate amino transferase	Alkaline phosphatase
	Mean ± SD ALT (IU/ml)	Mean ± SD AST (IU/ml)	Mean ± SD ALP (k.k./dl)
Control	43.5 ± 2.03	108.32 ± 4.19	18.70 ± 1.10
5-FU	51.4 ± 9.02	130.43 ± 8.9	25.485 + 6.03
P<	0.001	0.001	0.001
Doxorubcin	59.2 ± 12.03	147.2 ± 16.3	30.317 ± 5.1
P<	0.001	0.001	0.001
4a	42.4 ± 4.05	109.8 ± 12.09	17.4 ± 1.07
P<	n.s.	n.s.	n.s.
4b	40.6 ± 3.	119.1 ± 10.1	19.5 ± 1.03
P<	n.s.	0.01	n.s.
5b	38.6 ± 3.6	110.5 ± 7.5	19.4 ± 1.7
P<	n.s.	n.s.	n.s.
6a	51.9 ± 4.3	124 ± 8.5	24.7 ± 2.01
P<	0.01	0.01	0.01
5d	37.4 ± 3.2	123.1 ± 8.2	18.03 ± 1.2
P<	n.s.	0.01	n.s.

*Data are expressed as Mean + S.D.P> 0.05 insignificant, P< 0.01: significant, P< 0.001: highly significant, n.s. : non significant

The results showed that the values recorded for AST and ALT were significantly higher ($P < 0.001$) with 5-FU and DOX treated groups of mice than the control. On the other hand, treatment with the new indole derivatives (compounds 4a, 4b, 5b, 6a and 5d), caused inverse effects, where most values recorded for AST and ALT were no significant (n.s.) or slightly higher ($P < 0.01$) in comparison to control. Moreover, the recorded data showed that ALP activities were significantly increased ($P < 0.001$) with the treatment of 5-Fu and DOX, while there were no significant changes in ALP activities upon treatment with the new compounds.

Data listed in table 3 demonstrates the comparison between the levels of total lipids, cholesterol, triglycerides and bilirubin in serum of treated mice and the control group. It can be deduced from the present data that 5-FU and DOX caused a significant increase in the level of these parameters while treatment with the

selected compounds showed slight or no significant changes.

Table 3: Biochemical effects of treatment with 5-FU, DOX and indole derivatives on serum total lipids, cholesterol, triglycerides and bilirubin in mice.

Biochemical Parameters Mice Groups	Total Lipids (mg/dl)	Cholestrol (mg/dl)	Triglycerides (mg/dl)	Bilirubin (mg/dl)
Control	323.41 ± 27.1	94.32 ± 13.5	108.7 ± 16.8	0.63 ± 0.04
5-FU	378.2 ± 31.4	105.9 ± 11.7	126.5 ± 19.4	0.75 ± 0.10
P<	0.001	0.001	0.001	0.001
Doxorubcin	366.7 ± 6.10	109.3 ± 14.2	137.8 ± 17.10	0.81± 0.19
P<	0.001	0.001	0.001	0.001
4a	319.7 ± 26.3	93.9 ± 9.4	106.7 ± 12.1	0.50 ± 0.03
P<	n.s.	n.s.	n.s.	0.01
4b	325.4 ± 22.1	96.4 ± 8.3	110.3 ± 11.2	0.49 ± 0.02
P<	n.s.	n.s.	n.s.	0.01
5b	325.1 ± 22.8	97.1 ± 6.8	111.4 ± 8.6	0.51 ± 0.06
P<	n.s.	n.s.	n.s.	0.01
6a	340.1 ± 28.1	106.3 ± 9.3	120.2 ± 9.8	0.74 ± 0.1
P<	0.01	0.01	0.01	0.01
5d	330.3 ± 23.5	96.7 ± 8.5	119.6 ± 9.4	0.67 ± 0.09
P<	n.s.	n.s.	0.01	n.s.

Data are expressed as Mean + S.D.P> 0.05 insignificant, P< 0.01: significant, P< 0.001: highly significant, n.s. : non significant

Table 4 represents a comparison between the levels of albumin, globulins and creatinine in serum of control and treated groups of mice.

It is clear from the results in the table that there was a slight increase in the level of albumin and creatinine and globulins in the 5-FU and DOX treated groups of mice while there were slight or non significant changes in the other treated groups.

Cytotoxic drugs remain the main stay of cancer chemotherapy and are being administered with novel ways of therapy such as inhibitors of signals[34].

It is therefore important to discover novel cytotoxic agents with spectra of activity and toxicity that differ from those current agents. It is well known that chemotherapy aims to destroy the cancer cells with various types of chemicals.

The substances used are supposed to target mainly the cancer cells and doses are calculated to minimize the collateral damage to surrounding tissues, which nevertheless occurs[35].

This kind of treatment increases the entropy of the organism, suppresses the immune system, and forms a toxic cell environment which may destroy surrounding healthy cells[36]. So it is important to minimize curing doses to the least amount possible as well as trying to minimize the side effects of these drugs. For this, novel derivatives of indole possessing a broader spectrum of antitumor activity and fewer toxic side effects than 5-FU and DOX have been sought.

The antitumor activities of such compounds were assessed against HEPG2 cancer cell line in comparison to the traditional anticancer drugs: 5-FU and DOX. Regarding the antitumor activity study, some of the selected compounds showed reasonable antitumor activity in comparison to 5-FU and DOX.

Moreover, study of the induced biochemical parameters of the tested compounds in mice showed insignificant differences relative to the control group which indicates a moderate margin of safety for the selected compounds.

Comparable to 5-FU and DOX, a dose augmentation of compound 6a, searching for possible higher potency, seems, consequently, realizable without undesirable implications.

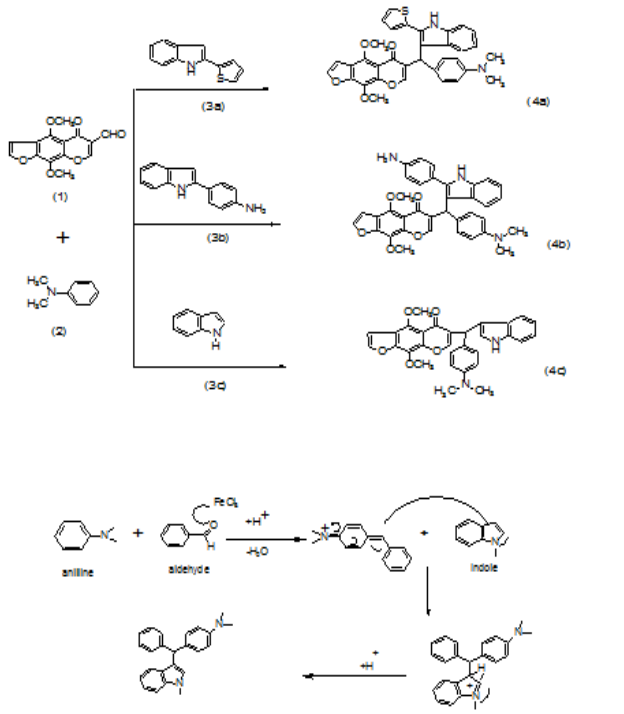
Furthermore, the selected compounds have important potential advantages over 5-FU and DOX because of their lower toxicity and their ability to induce lower biochemical parameters changes.

These results are in agreement with Espinosa[37]and Kamalakannan and Venkappayya[38], who reported that novel derivatives of 5-FU possessing a broader spectrum of antitumor activity and fewer toxic side effects than 5-FU.

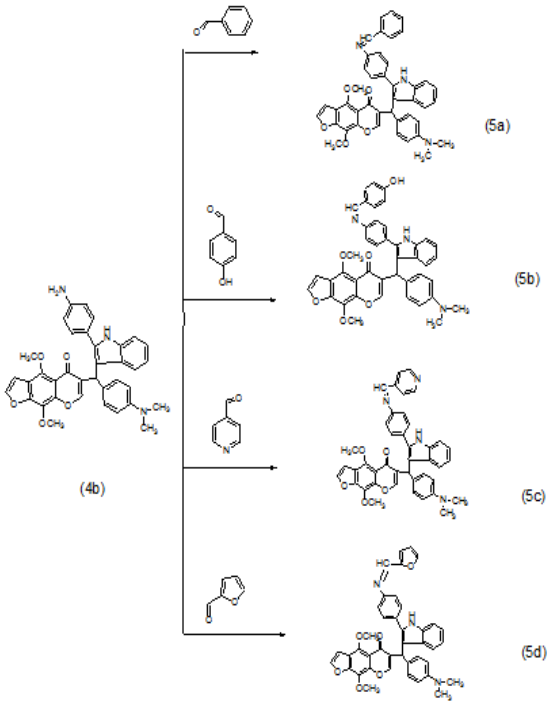
Table 4: Biochemical effects of treatment with 5-FU, DOX and indole derivatives on serum albumin, globulin, creatinine in mice.

Biochemical Parameters Mice Groups	Albumin (mg/dl)	Globulin (mg/dl)	Creatinine (mg/dl)
Control	5.6 ± 0.5	4.3 ± 0.3	0.69 ± 0.03
5-FU	6.4 ± 0.4	5.7 ± 0.4	0.81 ± 0.04
P<	0.01	0.01	0.01
Doxorubcin	6.37 ± 0.5	5.9 ± 0.5	0.78 ± 0.03
P<	0.01	0.01	0.01
4a	5.8 ± 0.5	5.2 ± 0.3	0.66 ± 0.04
P<	n.s.	n.s.	n.s.
4b	5.3 ± 0.3	4.8 ± 0.2	0.62 ± 0.03
P<	n.s.	n.s.	n.s.
5b	5.5 ± 0.1	4.7 ± 0.3	0.65 ± 0.05
P<	n.s.	n.s.	n.s.
6a	5.8 ± 0.3	6.5 ± 0.4	0.74 ± 0.05
P<	n.s.	0.01	0.01
5d	5.8 ± 0.3	4.2 ± 0.3	0.68 ± 0.04
P<	n.s.	n.s.	n.s.

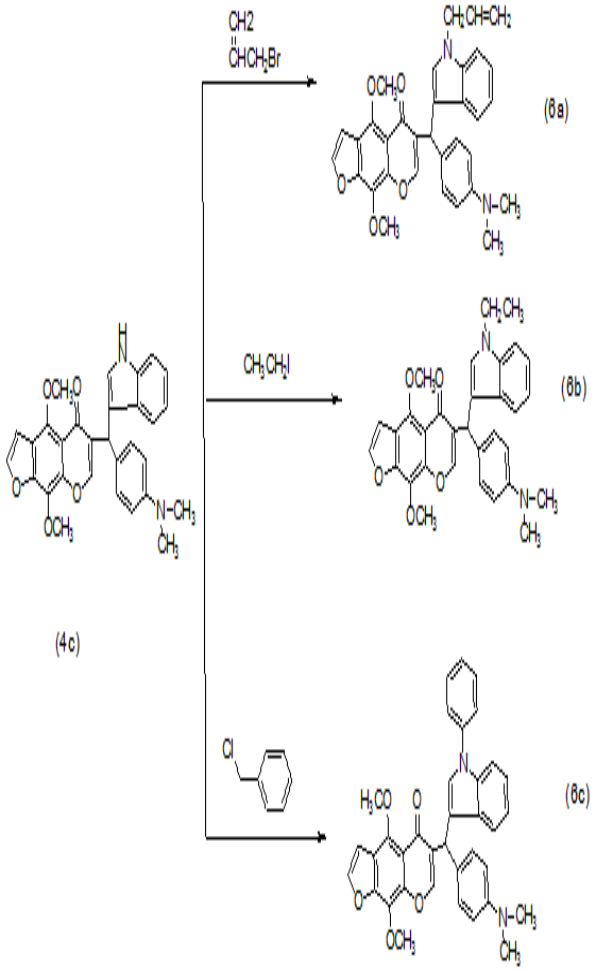
Data are expressed as Mean + S.D.P> 0.05 insignificant, P< 0.01: significant, P< 0.001: highly significant, n.s. : non significant



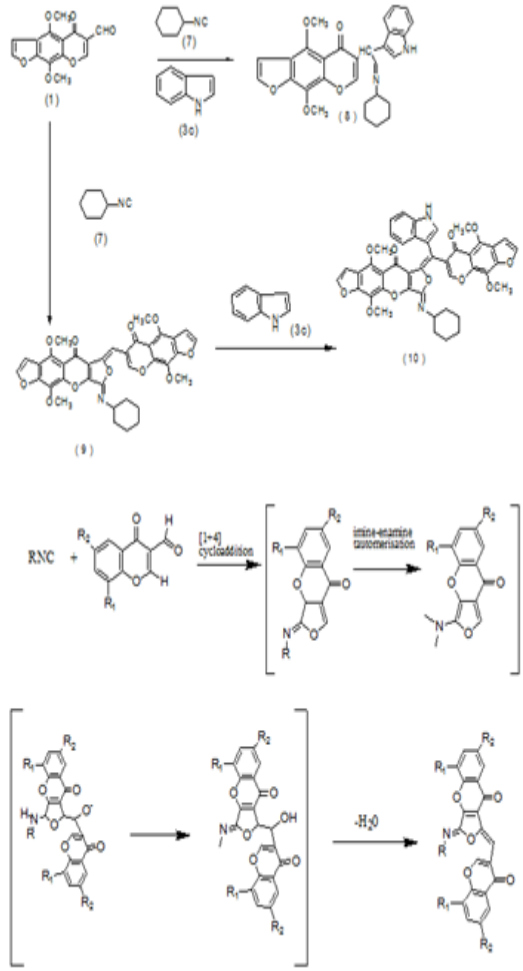
Scheme 1: Possible mechanism for the formation of product 4c



Scheme 2



Scheme 3



Scheme 4: Possible mechanism for the formation of product 9

CONCLUSION

As a part of our research and related heterocyclic ring system and our attempts to identify new target compounds for future development as antitumor, we previously reported the synthesis and biological of a series of substituted indole derivatives that have various heterocyclic ring system.

These compounds were selected for further structure modification in an effort to obtain more potent compounds.

In the present investigation, we report the design and synthesis of new series of indoles which include in their structures various heterocyclic moieties directly attached to the furochromon nucleus.

The goal of this work in making these structure changes was to explore the significance of the spacer on the respective biological activity as anticancer. In addition, our study was extended to the synthesis of additional derivatives containing a substituted indole moiety directly attached to furochromone nucleus.

The anticancer activity data indicate that compound 7 was the most active and induced a reasonable growth inhibition, in a dose-dependent manner against HEPG2 when compared to 5-FU and DOX.

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