

## Synthesis, Antitumor and Anti-inflammatory Activities of 2-thienyl-3-substitued Indole Derivatives

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**Abstract:** A series of 2-thienyl-3-substitued indole derivatives (1-11) were synthesized. Their structures were established on the basis of elemental and spectral (IR, <sup>1</sup>HNMR and Mass spectral data) studies. All the synthesized compounds have been tested for their antitumor activity against breast cancer cell line (MCF-7) using doxorubicin as a standard drug (IC<sub>50</sub>=2.97 µg/ml). Most of the prepared compounds showed good to moderate antitumor activity. The most potent one was 10b (IC<sub>50</sub>=2.6 µg/ml). Twelve of the prepared compounds were evaluated for their anti-inflammatory activity (against carrageenan induced oedema in albino rats at a dose of 50 mg/kg body weight using indomethacin at a dose of 25mg/kg body weight as a standard drug) and ulcerogenic effect. Compound 9a exhibited 55.31% inhibition with ulcerogenic activity less than that of indomethacin.

**Key words:** 2-Thienylindole • Antitumor • Anti-Inflammatory • Ulcerogenic • MCF-7 • Indole Carboxaldehyde

### INTRODUCTION

Indole moiety is probably the most widely spread nitrogen heterocycle in nature. It is an essential part of the amino acid tryptophan and the neurotransmitter serotonin. The indole scaffold is also found in a manifold of naturally occurring plant based alkaloids. Indole derivatives have displayed versatile pharmacological properties including their antitumor [1-4], antiviral [5-7], anti-inflammatory [8-11], anti-hyperlipidemic [12], hypoglycemic [13], anti-hypertensive [14], anti-asthmatic [15] and anti-tubercular [16] activities.

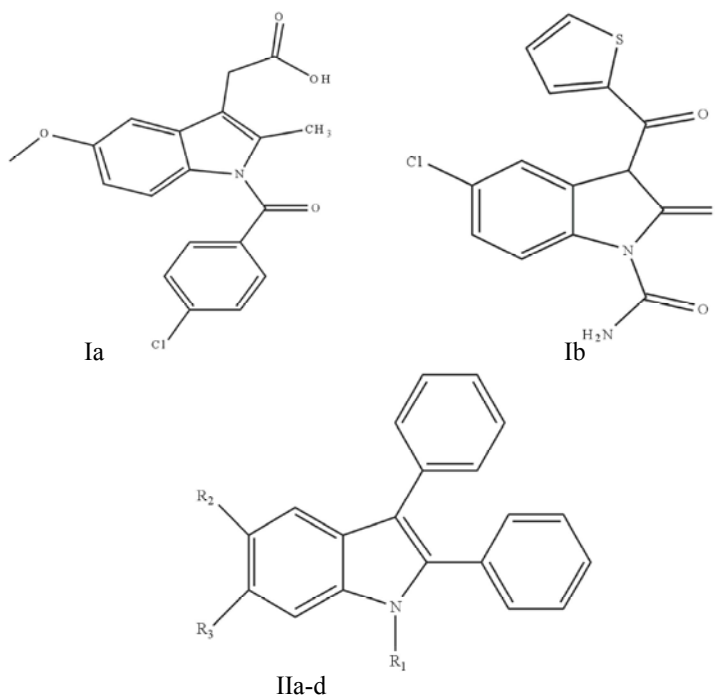
Acute, chronic inflammation and different types of arthritis are the inflammatory disorders and constitute a big blow to humanity. Continual research for newer non-steroidal anti-inflammatory drugs is the only way to fortify against this awful threat.

The discovery of indomethacin Ia and tenidap Ib as successful agents for clinical treatment of inflammatory disorders [17,18] has led us to explore the indole moiety

as a hopeful target for designing more potent and safer anti-inflammatory agents. 2,3- Diphenylindole derivatives IIa-d exert potent anti-inflammatory and analgesic activities [19-23] as well.

Although a large number of anticancer drugs are currently used, cancer still remains a major threat to the public health. The search for new drugs with higher therapeutic index, lower capacity to induce resistance and lower capability to cause side effects such as bone marrow depression, immune suppression and regrowth of tumor cells remains an active field of investigation in medicinal chemistry.

Derivatives of 2-aryl-3-substitued indoles IIIa-d were found to possess significant activities against breast cancer cell line (MCF-7) [24,25]. Encouraged by these findings, it was thought that it would be worthwhile to design and synthesize new 2,3-disubstitued indoles by isosteric replacement of the 2-phenyl moiety in IIIa-d with the 2-thienyl ring and varying the substitutions at position 3 of the indole moiety, schemes 1, 2.

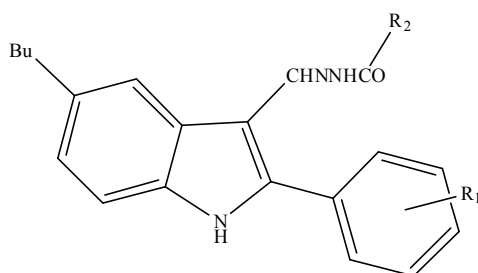


(Iia)  $R_1 = \text{COCH}_2\text{Cl}$ ,  $R_2 = \text{OCH}_3$ ,  $R_3 = \text{H}$

(Iib)  $R_1 = \text{Me}$ ,  $R_2 = 3\text{-aryl-1, 3-thiazolidin-4-one-2-yl}$ ,  $R_3 = \text{OCH}_3$

(Iic)  $R_1 = \text{H}$ ,  $R_2 = \text{OCH}_3$ ,  $R_3 = 3\text{-aryl-1, 3-oxazolin-5-one-4-methylene}$

(Iid)  $R_1 = \text{H}$ ,  $R_2 = \text{OCH}_3$ ,  $R_3 = \text{benzopyran}$



(IIIa)  $R_1 = 4\text{-OMe}$ ,  $R_2 = 4\text{-Me phenyl}$  (IIIc)  $R_1 = 4\text{-OMe}$ ,  $R_2 = 3\text{-N}$

(IIIb)  $R_1 = 4\text{-OMe}$ ,  $R_2 = 4\text{-OMe phenyl}$ , (IIId)  $R_1 = 4\text{-Et}$ ,  $R_2 = 3\text{-F-phenyl}$

All the synthesized compounds were evaluated as antitumor agents against breast cancer cell line (MCF-7) and some of them have been tested for their anti-inflammatory and ulcerogenic activities.

## MATERIALS AND METHODS

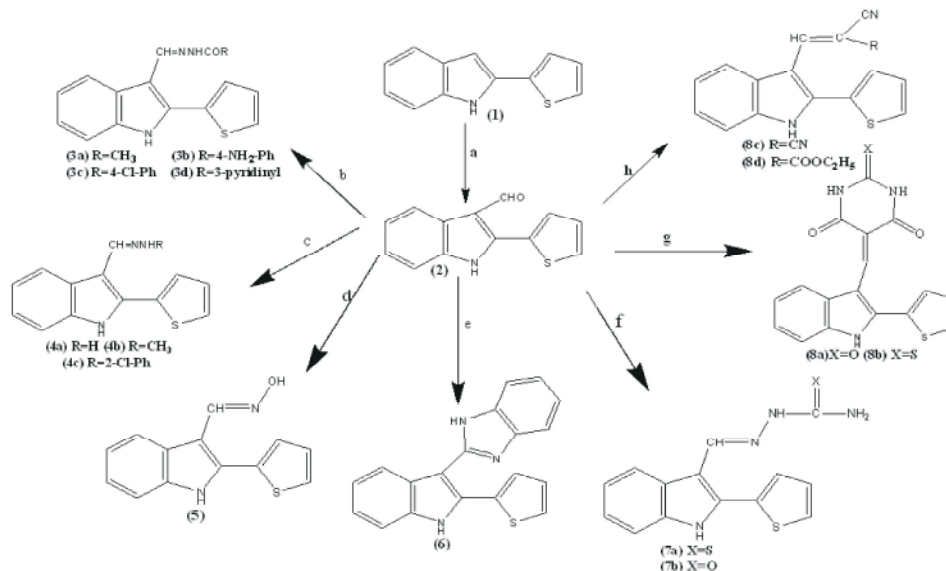
**Experimental Section:** Melting points are uncorrected and were recorded by open capillary tube method using on Electro-thermal Melting Point apparatus. Infra red

spectra (KBr) were recorded on Jasco FT/IR 6100 Fourier Transformer spectrophotometer.  $^1\text{H}$ NMR spectra were recorded on JEOL EX 500 MHZ spectrometer. The chemical shifts were expressed in  $\delta$ , ppm units using trimethylsilane as the internal standard. Mass spectra were recorded on GC/MS FINNIGAN MAT SSQ 7000 digital DEC 3000 & JEOL JMS-AX 500 mass spectrometers. Reaction time was monitored by Thin Layer Chromatography, using Merck Silica Gel Plates with fluorescent indicator system. The spots were visualized using Vilber Lourmet ultraviolet lamp at  $\lambda = 254$  nm. The adopted solvent system was Petroleum ether 60-80°C: ethyl acetate (8:2). Elemental analysis was performed on Vario El Elementar.

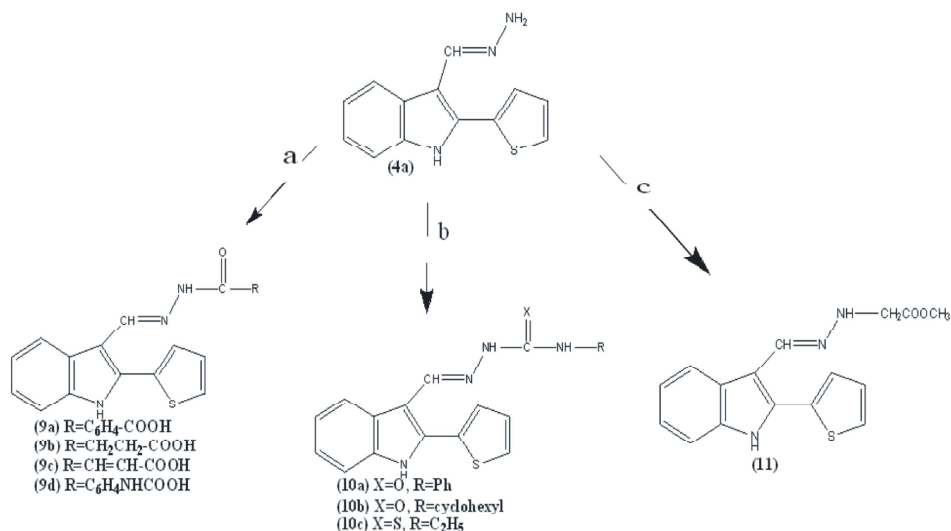
2-(Thiophen-2-yl)-1H-indole (1) and 2-(thiophen-2-yl)-1H-indole-3-carbaldehyde (2) were synthesized according to the reported procedures [26,27].

## Synthesis of the Compounds

**General Procedure for the Synthesis of Compounds (3a-d):** Compound 2 (0.5g, 2mmol) and the appropriate acid hydrazide derivative(2.5mmol) were refluxed in absolute ethanol ( 20 ml) in the presence of 2-3 drops of glacial acetic acid for 2-4 hrs. The solution was



Scheme 1: Reagents and conditions: a:  $\text{POCl}_3$ , DMF; b:  $\text{RCONHNH}_2$ , absolute ethanol/acetic acid; c:  $\text{R-NHNH}_2$ , absolute ethanol/acetic acid; d:  $\text{NH}_2\text{OH} \cdot \text{HCl}$ , absolute ethanol, pyridine; e: o-phenylenediamine, absolute ethanol/ $\text{NH}_3$ ; f: thiosemicarbazide or semicarbazide  $\text{HCl}$ , absolute ethanol/acetic acid; g: barbituric/thiobarbituric acid (s), absolute ethanol/piperidine; h: malononitrile / ethylcyanoacetate, absolute ethanol/TEA.



Scheme 2: Reagents and conditions: a: acid anhydrides, acetic acid; b: isocyanates or isothiocyanate, ethyl acetate/TEA; c: methylbromoacetate, dry acetone/anhydrous  $\text{K}_2\text{CO}_3$ .

concentrated to half its volume, cold water was then added to the hot mixture where a precipitate was formed, filtered and crystallized from the appropriate solvent.

#### Synthetic Schemes of the Compounds

**N'-((2-(Thiophen-2-yl)-1H-indol-3-yl) methylene) acetohydrazide(3a):** Yellow solid, 53 % yield, m.p. 250-

251°C (absolute ethanol). IR (KBr)  $\text{cm}^{-1}$ : 3297(NH indole); 3158(N-H); 2919(CH aliphatic); 1655(C=O); 575(C=N).  $^1\text{H-NMR}$  ( $\text{DMSO-d}_6$ ) ( $\delta$ , ppm): 2.23(s, 3H,  $\text{CH}_3$ ); 7.12-7.24(m, 3H, thiophene and indole protons); 7.37-7.39 (d, 1H, thiophene proton,  $J=10$ ); 7.46(m, 1H, indole proton); 7.77 (m, 1H, thiophene proton); 8.15-8.16 (d, 1H, indole proton,  $J=5\text{Hz}$ ); 8.49 (s, 1H,

CH=N); 10.97 (s, 1H, N-NH, D<sub>2</sub>O exchangeable); 11.12 (s, 1H, NH indole, D<sub>2</sub>O exchangeable). MS: m/z (%): M<sup>+</sup>, 237 (100). Elemental analysis (C<sub>13</sub>H<sub>13</sub>N<sub>3</sub>OS) (283.35) calc./found (%) C(63.53/63.13), H (4.60/4.91), N (14.87/14.43), S (11.31/11.54).

**4-amino-n'-((2-(Thiophen-2-yl)-1H-indol-3-yl)methylene) benzo Hydrazide (3b):** Yellow solid, 77 % yield, m.p. 230-232°C (absolute ethanol). IR (KBr) cm<sup>-1</sup>: 3405(NH indole, NH<sub>2</sub>); 3204(N-NH); 2920(CH aliphatic); 1651(C=O amidic); 1609(C=N); 1452(C=C aromatic). <sup>1</sup>H-NMR (DMSO-d<sub>6</sub>) (δ, ppm): 1.19 (s, 2H, NH<sub>2</sub>); 7.19-7.30(m, 4H, thiophene and indole protons); 7.42-7.43(m, 2H, aromatic protons); 7.6 (m, 2H, thiophene and indole protons); 7.82-7.83 (d, J=5Hz, 2H, aromatic protons); 8.40-8.41 (d, J=5Hz, 1H, indole proton); 9.18 (s, 1H, CH=N); 12.01(s, 1H, NH indole, D<sub>2</sub>O exchangeable). MS(m/z) 361 (M<sup>+</sup>+1). Elemental analysis (C<sub>20</sub>H<sub>16</sub>N<sub>4</sub>OS) (360.43) calc./found (%) C (66.61/66.21), H (4.46/5.00), N (15.51/15.92), S (8.91/8.79).

**4-chloro-n'-((2-(Thiophen-2-yl)-1H-indol-3-yl)methylene) benzo Hydrazide (3c):** Yellow solid, 73 % yield, m.p. 220-223°C (petroleum ether 60-80°C). IR(KBr) cm<sup>-1</sup>: 3398 (NH indole); 3199(N-NH); 1649 (C=O amidic); 1607 (C=N); 1449 (C=C aromatic). <sup>1</sup>H-NMR (DMSO-d<sub>6</sub>) (δ, ppm): 7.16-7.30(m, 4H, thiophene and indole protons); 7.42-7.43(d, 2H, aromatic protons); 7.59-7.61(d, 2H, thiophene and indole protons, J=10); 7.82-7.83(d, 2H, aromatic protons, J=5); 8.40-8.41(d, 1H, indole proton, J=5 Hz); 9.18 (s, 1H, CH=N); 10.97 (s, 1H, N-NH, D<sub>2</sub>O exchangeable); 12.02(s, 1H, NH indole, D<sub>2</sub>O exchangeable). Elemental analysis (C<sub>20</sub>H<sub>14</sub>ClN<sub>3</sub>OS) (379.86) calc./found (%) C (63.24/63.60), H (3.71/3.65), N (11.00/10.5), S (8.44/8.61), Cl (9.33/9.10).

**N'-((2-(Thiophen-2-yl)-1H-indol-3-yl) methylene) nicotinedhydrazide (3d):** Whitish yellow solid, 74 % yield, m.p. 146-149°C (petroleum ether 60-80°C). IR(KBr) cm<sup>-1</sup>: 3417(NH indole); 3207(N-NH); 1635 (C=O amidic); 1598 (C=N). <sup>1</sup>H-NMR (DMSO-d<sub>6</sub>) (δ, ppm): 7.15-7.80(m, 9H, indole, thiophene and pyridine protons); 8.39-8.40(d, 1H, pyridine proton, J=5); 8.74 (s, 1H, CH=N); 8.92(s, 1H, pyridine); 11.83(s, 1H, N-NH, D<sub>2</sub>O exchangeable); 11.90(s, 1H NH indole, D<sub>2</sub>O exchangeable). MS (m/z) (%) M<sup>+</sup>+2, 348, (11.6). Elemental analysis (C<sub>19</sub>H<sub>14</sub>N<sub>4</sub>OS) (346.41) calc./found (%) C(65.80/65.61), H (4.07/4.41), N (16.13/16.51), S (9.21/9.48).

**General Procedure for the Synthesis of Compounds (4a-c):** Compound 2 (0.5 g, 2 mmol) and the appropriate hydrazine derivative (2.5mmol) were refluxed in absolute ethanol (20 ml) in the presence of a catalytic amount of glacial acetic acid for 2-5 hrs. The reaction mixture was concentrated to half its volume, poured onto ice/water and the precipitate was filtered and crystallized from petroleum ether (60-80°C).

**3-(Hydrazonomethyl)-2-(Thiophen-2-yl)-1H-indole(4a):** Canary yellow solid, 77 % yield, m.p. 235-236°C. IR(KBr) cm<sup>-1</sup>: 3402 (NH indole, NH<sub>2</sub>). <sup>1</sup>H-NMR (DMSO-d<sub>6</sub>) (δ, ppm): 7.17-7.30 (m, 3H, indole and thiophene protons); 7.42-7.44(d, 1H, thiophene proton, J=10Hz); 7.58-7.60 (d, 1H, indole proton, J=10 Hz); 7.81-7.83 (d, 1H, thiophene proton, J=10Hz); 8.36-8.38(d, 1H, indole proton, J=10Hz); 9.18 (s, 1H, CH=N); 12.02 (s, 1H, NH indole, D<sub>2</sub>O exchangeable). MS (m/z)(%) M<sup>+</sup>-2, 239 (5); M<sup>+</sup>+1, 242(0.44). Elemental analysis (C<sub>13</sub>H<sub>11</sub>N<sub>3</sub>S) (241.31) calc./found (%) C (64.7/64.9), H (4.59/5.03), N (17.41/17.38), S (13.29/13.27).

**3-(2-methylhydrazono)methyl)-2-(Thiophen-2-yl)-1H-indole (4b):** Orange solid, 34 % yield, m.p. 110-112°C. IR (KBr) cm<sup>-1</sup> : 3391 (NH indole, NH-CH<sub>3</sub>), 2920 (CH<sub>2</sub>aliphatic), 1611 (C=N). <sup>1</sup>H-NMR (DMSO-d<sub>6</sub>) (δ, ppm): 2.86 (s, 3H, CH<sub>3</sub>); 7.11-7.30 (m, 3H, thiophene and indole protons); 7.42 (m, 1H, thiophene proton); 7.60 (m, 1H, thiophene proton); 7.82 (m, 1H, indole proton); 8.41 (m, 1H, indole proton); 9.18 (s, 1H, CH=N); 11.83 (s, 1H, NH-CH<sub>3</sub>, D<sub>2</sub>O exchangeable); 12.02 (s, 1H, NH indole, D<sub>2</sub>O exchangeable). MS (m/z) (%) M<sup>+</sup>, 255 (2.2); M<sup>+</sup>-1, 254 (5.6) ; 62 (100). Elemental analysis (C<sub>14</sub>H<sub>13</sub>N<sub>3</sub>S) (255.34) calc./found (%) C (65.85/65.87), H (5.13/5.11), N (16.46/16.48), S (12.56/12.53).

**3-((2-(2-chlorophenyl)hydrazono)methyl)-2-(Thiophen-2-yl)-1H-indole (4c):** Pale yellow solid, 65 % yield, m.p. 150-153°C IR (KBr) cm<sup>-1</sup>: 3398 (NH indole, N-NH), 1596 (C=N). <sup>1</sup>H-NMR (DMSO-d<sub>6</sub>) (δ, ppm): 6.71 (m, 1H, aromatic proton); 7.16-7.28 (m, 4H, indole and aromatic protons); 7.38-7.396 (d, 1H, aromatic proton, J=8Hz); 7.52 (m, 2H, indole and thiophene protons); 7.75 (m, 1H, thiophene proton); 8.29-8.31 (d, 1H, indole proton, J=10Hz); 8.79 (s, 1H, CH=N); 9.73 (s, 1H, N-NH, D<sub>2</sub>O exchangeable) ; 11.65 (s, 1H, NH indole, D<sub>2</sub>O exchangeable). MS (m/z) (%) M<sup>+</sup>, 361 (1.8); 227 (88); 199 (31); 63 (100). Elemental analysis (C<sub>15</sub>H<sub>14</sub>ClN<sub>3</sub>S) (351.85) calc./found (%) C (64.86/64.66), H (4.01/4.04), N (11.94/11.90), S (9.11/9.14), Cl (10.08/10.12).

**2- (Thiophen-2-yl)-1H-indole-3-carbaldehyde Oxime(5):**

Compound 2 ( 0.5 g, 2m mmol) and hydroxyl amine hydrochloride (2.5 mmol) were refluxed in ethanol (20 ml) in the presence of a catalytic amount of pyridine for 6 hrs. The mixture was then poured onto cold water and neutralized with dilute HCl to give brownish yellow precipitate which was collected, filtered and crystallized from petroleum ether (60-80°C).

Yield 32%, m. p. 167-169 °C. IR (KBr)  $\text{cm}^{-1}$ : 3385 (NH indole, OH), 1629 (C=N).  $^1\text{H-NMR}$  (DMSO- $\text{d}_6$ ) ( $\delta$ , ppm): 7.08-7.22 (m, 3H, indole and thiophene protons); 7.37 (m, 1H, thiophene proton); 7.46 (m, 1H, indole proton); 7.72 (m, 1H, thiophene proton); 8.015-8.029 (d, 1H, indole proton,  $J=7\text{Hz}$ ); 8.48 (s, 1H, CH=N); 10.81 (s, 1H, OH,  $\text{D}_2\text{O}$  exchangeable); 11.73 (s, 1H, NH indole,  $\text{D}_2\text{O}$  exchangeable). Elemental analysis ( $\text{C}_{13}\text{H}_{10}\text{N}_2\text{OS}$ ) (242.30) calc. /found (%) C (64.44/64.21), H (4.16/4.19), N (11.56/11.50), S (13.23/13.20).

**2- (2- (Thiophen-2-yl)-1h-indole-3-yl)-1H-benzo[d] Imidazole (6):**

Compound 2 (0.5g, 2mmol) and o-phenylenediamine (2.5mmol) were refluxed in absolute ethanol (30 ml) in the presence of ammonium hydroxide at 70 °C for 20 hrs. The reaction mixture was then concentrated to half its volume and poured onto water where a pale yellow precipitate was collected and crystallized from petroleum ether 60-80°C.

Yield 58%, m. p. 125-126 °C IR (KBr)  $\text{cm}^{-1}$ : 3384 (NH indole, NH benzimidazole); 3059 (CH aromatic) ; 1616 (C=N); 3385 (NH indole, OH); 1629 (C=N).  $^1\text{H-NMR}$  (DMSO- $\text{d}_6$ ) ( $\delta$ , ppm): 7.09-7.20 (m, 5H, indole, thiophene and benzimidazole protons); 7.45-7.46 (d,  $J=5\text{ Hz}$ , 1H, thiophene proton) ; 7.55-7.57 (m, 3H, benzimidazole and thiophene protons); 7.70-7.75 (m, 2H, indole protons); 8.10 (s, 1H, NH benzimidazole); 11.90 (s, 1H, NH indole,  $\text{D}_2\text{O}$  exchangeable). MS (m/z) (%)  $\text{M}^+$ , 315 (24);  $\text{M}^+ - 1$ , 314 (100); 200 (19%). Elemental analysis ( $\text{C}_{19}\text{H}_{13}\text{N}_3\text{S}$ ) (315.39) calc./found (%) C (72.36/72.78), H (4.15/3.86), N (13.32/13.65), S (10.17/10.30).

**General Procedure for the Synthesis of Compounds**

**(7a,b):** Compound 2 (0.5g, 2mmol) and the appropriate semi/thiosemicarbazide ( 2 mmol) were refluxed in absolute ethanol ( 20 ml ) in the presence of 5 drops of glacial acetic acid for 2 hrs. The desired products were precipitated upon addition of cold water, filtered and crystallized from absolute ethanol.

**2- (2- (Thiophen-2-yl)-1H-indol-3-yl)methylene hydrazinecarbothio Amide (7a):**

Yellow solid, 77 % yield, m.p. 230-232°C. IR (KBr)  $\text{cm}^{-1}$ : 3396-3435 (NH indole,  $\text{NH}_2$ ); 3152-3208 (N-NH); 1590 (C=N); 1535 (C=C aromatic); 1284 (C=S).  $^1\text{H-NMR}$  (DMSO- $\text{d}_6$ ) ( $\delta$ , ppm): 7.11 (m, 1H, indole proton); 7.18 (m, 1H, indole proton); 7.25 (m, 1H, thiophene proton); 7.37 (m, 1H, thiophene proton); 7.48 (m, 1H, indole proton); 7.78-7.79 (d, 1H, thiophene proton,  $J=5\text{ Hz}$ ); 8.06 (s, 2H,  $\text{NH}_2$ ) ; 8.25-8.27 (d, 1H, indole proton,  $J=10\text{Hz}$ ); 8.64 (s, 1H, CH=N); 11.27 (s, 1H, N-NH,  $\text{D}_2\text{O}$  exchangeable); 11.85 (s, 1H, NH indole,  $\text{D}_2\text{O}$  exchangeable). MS (m/z) (%)  $\text{M}^+ - 1$ , 299 (0.32) ; 222 (100%). Elemental analysis ( $\text{C}_{14}\text{H}_{12}\text{N}_4\text{S}_2$ ) (300.40) calc./found (%) C (55.97/56.30), H (4.03/4.09), N (18.65/19.00), S (21.35/21.50).

**2- (2- (Thiophen-2-yl)-1H-indol-3-yl) methylene hydrazine Carboxamide (7b):**

Pale yellow solid, 80 % yield, m.p. 226-227°C IR (KBr)  $\text{cm}^{-1}$ : 3335-3412 ( $\text{NH}_2$ , NH indole); 3203 (N-NH); 1638 (C=N, C=O).  $^1\text{H-NMR}$  (DMSO- $\text{d}_6$ ) ( $\delta$ , ppm): 6.19 (s, 2H,  $\text{NH}_2$ ); 7.10-7.24 (m, 3H, indole and thiophene protons); 7.35-7.44 (m, 2H, indole and thiophene protons); 7.72-7.74 (d, 1H, thiophene proton,  $J=10\text{Hz}$ ); 8.18-8.20 (d, 1H, indole proton,  $J=10\text{Hz}$ ); 8.38 (s, 1H, CH=N); 9.97 (s, 1H, N-NH,  $\text{D}_2\text{O}$  exchangeable); 11.68 (s, 1H, NH indole,  $\text{D}_2\text{O}$  exchangeable). MS (m/z) (%)  $\text{M}^+$ , 284 (1.5)

$\text{M}^+ + 1$ , 285 (12.7). Elemental analysis ( $\text{C}_{14}\text{H}_{12}\text{N}_2\text{OS}$ ) (284.34) calc./found (%) C (59.14/59.01), H (4.25/4.29), N (19.70/19.50), S (11.28/11.53).

**General Procedure for the Synthesis of Compounds**

**(8a-d):** Compound 2 (0.5g, 2mmol) and the active methylene derivative (2mmol) were refluxed in absolute ethanol (20 ml) in the presence of few drops of piperidine or triethylamine for 3-5 hrs. The reaction mixture was concentrated to half its volume and diluted with cold water where a precipitate was formed, filtered and crystallized from the appropriate solvent. Compound 8a required further purification using preparative TLC and the adopted solvent system was petroleum ether: ethyl acetate 6:4.

**5- (2- (Thiophen-2-yl)-1h-indol-3-yl) methylene pyrimidine-2,4,6 (1H,3h,5h)-trione (8a):**

White solid, 16% yield, m.p. 210-212°C (acetone). IR (KBr)  $\text{cm}^{-1}$ : 3417 (NH indole, 2NH); 1649 (O=C-NH); 1584 (C=C). MS (m/z) (%)  $\text{M}^+$ , 337 (1); 199 (66). Elemental analysis ( $\text{C}_{17}\text{H}_{11}\text{N}_3\text{O}_3\text{S}$ ) (337.35) calc./found (%) C (60.52/61.11), H (3.29/3.64), N (12.46/12.31), S (9.50/9.86).

**5- ( (2- (Thiophen-2-yl)-1H-indol-3-yl)methylene)-2-thioxo Dihydropyrimidine-4,6 (1H,5h)-dione (8b):** Brick red solid, 32 % yield, m.p. 215-217°C (petroleum ether 60-80°C). IR (KBr)  $\text{cm}^{-1}$ : 3300-3400 (NH indole, 2NH); 1646 (O=C amidic).  $^1\text{H-NMR}$  (DMSO- $d_6$ ) ( $\delta$ , ppm): 6.95 (m, 1H, indole proton); 7.05 (m, 1H, indole proton); 7.10 (m, 1H, thiophene proton); 7.33 (m, 1H, thiophene proton); 7.48 (m, 2H, thiophene and indole protons); 8.02 (m, 1H, indole proton); 8.42 (s, 1H, CH=C); 12.22 (br, s, 2H, 2NH pyrimidine,  $\text{D}_2\text{O}$  exchangeable); 12.94 (s, 1H, NH indole,  $\text{D}_2\text{O}$  exchangeable). MS (m/z) (%)  $\text{M}^+ + 1$ , 354 (1); 199 (50); 61 (100); 43 (81). Elemental analysis ( $\text{C}_{17}\text{H}_{11}\text{N}_3\text{O}_2\text{S}_2$ ) (353.42) calc./found (%) C (57.77/57.95), H (3.14/3.40), N (11.89/11.78), S (18.15/18.11).

**2- ( (2- (Thiophen-2-yl)-1H-indol-3-yl) methylene) malononitrile (8c):** Brown solid, 81 % yield, m.p. 180-181°C (absolute ethanol). IR (KBr)  $\text{cm}^{-1}$ : 3260 (NH indole); 2218 (CN); 1564 (C=C).  $^1\text{H-NMR}$  (DMSO- $d_6$ ) ( $\delta$ , ppm): 7.26-7.35 (m, 3H, thiophene and indole protons); 7.51-7.53 (d, 1H, indole proton,  $J = 10$  Hz); 7.59-7.60 (d,  $J = 5$  Hz, 1H, thiophene proton); 7.96 (m, 2H, indole and thiophene protons); 8.19 (s, 1H, CH=C); 13.00 (s, 1H, NH indole,  $\text{D}_2\text{O}$  exchangeable). MS (m/z) (%)  $\text{M}^+ - 2$ , 273 (80);  $\text{M}^+ - 1$ , 274 (17); 61 (100). Elemental analysis ( $\text{C}_{16}\text{H}_9\text{N}_3\text{S}$ ) (275.33) calc./found (%) C (69.80/70.30), H (3.29/3.75), N (15.26/14.96), S (11.65/11.99).

**Ethyl 2-cyano-3- (2- (Thiophen-2-yl)-1H-indol-3-yl)acrylate (8d):** Brown solid, 71 % yield, m.p. 181-184°C (absolute ethanol). IR (KBr)  $\text{cm}^{-1}$ : 3277 (NH indole); 2921 (CH aliphatic); 2216 (CN); 1712 (COO).  $^1\text{H-NMR}$  (DMSO- $d_6$ ) ( $\delta$ , ppm): 1.27 (m, 3H,  $\text{CH}_3$ ); 4.27 (m, 2H,  $\text{CH}_2$ ); 7.24-7.31 (m, 3H, thiophene, indole protons); 7.51 (m, 2H, thiophene, indole protons); 7.93-7.95 (d,  $J = 10$  Hz, 1H, thiophene proton); 8.04-8.06 (d,  $J = 10$  Hz, 1H, indole proton); 8.49 (s, 1H, CH=C); 12.85 (s, 1H, NH indole,  $\text{D}_2\text{O}$  exchangeable). MS (m/z) (%)  $\text{M}^+$ , 322 (12);  $\text{M}^+ + 2$ , 324 (1). Elemental analysis ( $\text{C}_{18}\text{H}_{14}\text{N}_2\text{O}_2\text{S}$ ) (322.38) calc./found (%) C (67.06/66.55), H (4.38/4.76), N (8.69/8.99), S (9.95/10.30).

**General Procedure for the Synthesis of Compounds (9a-d):** Compound 4a (0.48gm, 2 mmol) and the appropriate acid anhydride (2.5mmol) were refluxed in glacial acetic acid (10 ml) for 4-6 hrs. The solution was left to concentrate at room temperature, water was added and the formed precipitate was collected, washed with water and crystallized from petroleum ether 60-80°C.

**2- (2- ( (2- (Thiophen-2-yl)-1H-indol-3-yl) methylene) hydrazine Carbonyl) benzoic Acid (9a):** Dark grey solid, 33 % yield, m.p. 158-159°C. IR (KBr)  $\text{cm}^{-1}$ : 3011 (NH indole, -NH hydrazide); 2500-3300 (br, strong, OH of COOH); 1739 (C=O of COOH); 1659 (C=O amidic); 1582 (C=N).  $^1\text{H-NMR}$  (DMSO- $d_6$ ) ( $\delta$ , ppm): 7.25-8.03 (m, 13H, indole, thiophene, aromatic, NH hydrazide and CH=N); 10.21 (s, 1H, COOH,  $\text{D}_2\text{O}$  exchangeable); 12.38 (s, 1H, NH indole,  $\text{D}_2\text{O}$  exchangeable); MS (m/z) (%)  $\text{M}^+$ , 389 (0.37); 226 (100); 199 (28.4). Elemental analysis ( $\text{C}_{21}\text{H}_{15}\text{N}_3\text{O}_3\text{S}$ ) calc./found (%) C (64.77/64.49), H (3.88/3.51), N (10.79/10.84), S (8.23/7.55).

**4-oxo-4- (2- ( (2- (Thiophen-2-yl) - 1H-indol-3-yl) methylene) Hydrazinyl) Butanoic Acid (9b):** Yellowish brown solid, 50 % yield, m.p. 106-108°C (absolute ethanol). IR (KBr)  $\text{cm}^{-1}$ : 3151 (NH indole, -NH hydrazide); 2500-3300 (br, strong, OH of COOH); 1704 (C=O of COOH); 1626 (C=O amidic); 1581 (C=N).  $^1\text{H-NMR}$  (DMSO- $d_6$ ) ( $\delta$ , ppm): 1.79 (m, 2H,  $\text{CH}_2\text{COOH}$ ); 2.71 (m, 2H,  $\text{CH}_2\text{-C=O}$ ); 7.20-7.86 (8H's, indole, thiophene and N-NH protons); 8.15 (s, 1H, CH=N); 10.20 (s, 1H, COOH,  $\text{D}_2\text{O}$  exchangeable); 12.31 (s, 1H, NH indole,  $\text{D}_2\text{O}$  exchangeable). MS (m/z) (%)  $\text{M}^+$ , 341 (0.48); 227 (100); 199 (55). Elemental analysis ( $\text{C}_{17}\text{H}_{15}\text{N}_3\text{O}_3\text{S}$ ) (341.38) calc./found (%) C (59.81/59.59), H (4.43/4.67), N (12.31/12.81), S (9.39/9.11).

**4-oxo-4- (2- ( (2- (Thiophen-2-yl)-1H-indol-3-yl)methylene) Hydrazinyl)but-2-enoic Acid (9c):** Brown solid, 53 % yield, m.p. 94-96°C. IR (KBr)  $\text{cm}^{-1}$ : 3445 (NH indole, N-NH indole); 3100-3500 (OH of COOH); 2920 (CH aromatic); 1725 (C=O of COOH); 1625 (C=N, C=O amidic).  $^1\text{H-NMR}$  (DMSO- $d_6$ ) ( $\delta$ , ppm): 6.17 (s, 1H, N-NH,  $\text{D}_2\text{O}$  exchangeable); 7.20-8.14 (m, 10H, indole thiophene, CH=N,  $\text{CH}=\text{CH}$  protons); 10.21 (s, 1H, COOH,  $\text{D}_2\text{O}$  exchangeable); 12.38 (s, 1H, NH indole,  $\text{D}_2\text{O}$  exchangeable). MS (m/z) (%)  $\text{M}^+$ , 339 (2); 199 (30). Elemental analysis ( $\text{C}_{17}\text{H}_{13}\text{N}_3\text{O}_3\text{S}$ ) (339.37) calc./found (%) C (60.17/60.69), H (3.86/3.65), N (12.38/12.98), S (9.45/9.40).

**2- (2- ( (2- (Thiophen-2-yl)-1H-indol-3-yl) methylene) hydrazine Carbonyl)phenyl)carbamic Acid (9d):** Dark grey solid, 38 % yield, m.p. 100-103°C. IR (KBr)  $\text{cm}^{-1}$ : 3416 (NH indole, -NH hydrazide); 3000-3300 (OH of COOH); 1676 (C=O of COOH); 1608 (C=O amidic, C=N).  $^1\text{H-NMR}$  (DMSO- $d_6$ ) ( $\delta$ , ppm): 1.19 (s, 1H, NH-C=O,  $\text{D}_2\text{O}$  exchangeable); 6.95 (s, 1H, NH-COOH,  $\text{D}_2\text{O}$  exchangeable); 7.07-8.15 (m, 11 H's, indole, thiophene and aromatic protons); 8.62 (s, 1H, CH=N); 11.23 (s, 1H, COOH,  $\text{D}_2\text{O}$  exchangeable).

exchangeable); 11.50 (s, 1H, NH indole, D<sub>2</sub>O exchangeable). MS (m/z) (%) M<sup>+</sup>, 404 (1); 199 (20). Elemental analysis (C<sub>18</sub>H<sub>14</sub>N<sub>2</sub>O<sub>2</sub>S) calc./found (%) C (62.36/62.70), H (3.99/4.30), N (13.85/13.20), S (7.93/7.45).

**General Procedure for the Synthesis of Compounds (10a-c):** Compound 4a (0.48gm, 2mmol) and the appropriate isocyanate /isothiocyanate derivative (2.5 mmol) were refluxed in ethyl acetate (25 ml) in the presence of 3-5 drops of triethylamine for 4 hrs. The reaction mixture was then cooled; the formed precipitate was filtered and crystallized from the appropriate solvent. Compound 10b required further purification using preparative TLC and the adopted solvent system was petroleum ether: ethyl acetate 6:4.

**N-phenyl-2- ((2-(Thiophen-2-yl)-1H-indol-3-yl)methylene) hydrazine Carboxamide (10a):** Brown solid, 25 % yield, m.p. 114-116°C (petroleum ether 60-80°C). IR (KBr) cm<sup>-1</sup>: 3325 (NH indole); 3282, 3194 (-NH hydrazide, NH-phenyl); 1649 (C=O amidic); 1596 (C=N); 1541 (C=C). <sup>1</sup>H-NMR (DMSO-d<sub>6</sub>) (δ, ppm): 6.91-8.48 (12 H's, indole, thiophene and aromatic protons); 8.67 (s, 1H, CH=N); 10.49 (s, 1H, NH-phenyl, D<sub>2</sub>O exchangeable); 11.82 (s, 1H, N-NH, D<sub>2</sub>O exchangeable); 12.04 (s, 1H, NH indole, D<sub>2</sub>O exchangeable). MS (m/z) (%) M<sup>+</sup>+2, 362 (31); 314 (100). Elemental analysis (C<sub>20</sub>H<sub>16</sub>N<sub>4</sub>OS) (360.43) calc./found (%) C (66.65/66.98), H (4.47/4.98), N (15.54/15.80), S (8.90/8.58).

**N-cyclohexyl-2- ((2-(Thiophen-2-yl)-1H-indol-3-yl)methylene) Hydrazinecarboxamide (10b):** White solid, 20 % yield, m.p. 135-138°C (acetone). IR (KBr) cm<sup>-1</sup>: 3400 (NH indole); 3327 (-NH hydrazide, NH-cyclohexyl); 2927 (CH aliphatic); 1653 (C=O amidic, C=N). <sup>1</sup>H-NMR (DMSO-d<sub>6</sub>) (δ, ppm): 1.10-2.08 (m, 11H's cyclohexyl protons); 3.26 (s, 1H, NH-cyclohexyl, D<sub>2</sub>O exchangeable); 7.19 (m, 1H, indole proton); 7.24 (m, 1H, indole proton); 7.25 (m, 1H, thiophene proton); 7.37-7.39 (d, 1H, J=10Hz, thiophene proton); 7.43 (m, 1H, indole proton); 7.75-7.76 (d, 1H, J=5Hz, thiophene proton); 8.05-8.07 (d, 1H, J=10Hz, indole proton); 8.36 (s, 1H, CH=N); 10.10 (s, 1H, N-NH, D<sub>2</sub>O exchangeable); 11.76 (s, 1H, NH indole, D<sub>2</sub>O exchangeable). MS (m/z) (%) M<sup>+</sup>, 366 (30); 199 (13). Elemental analysis (C<sub>20</sub>H<sub>22</sub>N<sub>4</sub>OS) (366.48) calc./found (%) C (65.55/65.86), H (4.43/3.92), N (12.31/12.81), S (9.39/9.11).

**N-ethyl-2- ((2-(Thiophen-2-yl)-1H-indol-3-yl)methylene) hydrazine Carbothioamide (10c):** Yellow solid, 25 % yield, m.p. 126-129°C (petroleum ether 60-80°C). IR (KBr) cm<sup>-1</sup>: 3359 (NH indole); 3164 (N-NH); 2954 (CH aliphatic); 1601

(C=N); 1226 (C=S). <sup>1</sup>H-NMR (DMSO-d<sub>6</sub>) (δ, ppm): 1.16 (m, 3H, CH<sub>3</sub>); 3.63 (q, 2H, CH<sub>2</sub>); 7.19-7.25 (m, 3H, indole and thiophene protons); 7.480-7.486 (d, J=3Hz, 1H, thiophene proton); 7.75 (s, 1H, NH-ethyl); 7.95 (m, 2H, indole and thiophene protons); 8.25-8.26 (d, J=5Hz, 1H, indole proton); 8.63 (s, 1H, CH=N); 11.26 (s, 1H, N-NH, D<sub>2</sub>O exchangeable); 11.84 (s, 1H, NH indole, D<sub>2</sub>O exchangeable). MS (m/z) (%) M<sup>+</sup>, 328 (5); 292 (9). Elemental analysis (C<sub>16</sub>H<sub>16</sub>N<sub>4</sub>S<sub>2</sub>) (328.46) calc./found (%) C (58.51/58.99), H (4.90/4.59), N (17.06/17.72), S (19.52/19.69).

**Methyl 2- ((2-(Thiophen-2-yl)-1H-indol-3-yl)methylene) Hydrazinyl Acetate (11):** Compound 4a (0.48gm, 2mmol) and methyl bromoacetate (0.3ml, 2mmol) were refluxed in dry acetone (25 ml) in the presence of potassium carbonate (0.408 gm, 3mmol) on a water bath for 6 hrs. The reaction mixture was filtered, the filtrate was concentrated to half its volume and cold water was added where a yellow precipitate was obtained and crystallized from petroleum ether 40-60°C.

70 % yield, m.p. 91-93°C. IR (KBr) cm<sup>-1</sup>: 3375 (NH indole, N-NH); 2947 (CH aliphatic); 1746 (COO ester); 1593 (C=N). <sup>1</sup>H-NMR (DMSO-d<sub>6</sub>) (δ, ppm): 2.46 (s, 3H, OOC-CH<sub>3</sub>); 3.62 (s, 2H, O-CH<sub>2</sub>-COO); 5.06 (s, 1H, N-NH, D<sub>2</sub>O exchangeable); 7.28-8.00 (m, 7H, indole and thiophene protons); 8.33 (s, 1H, CH=N); 12.00 (s, 1H, NH indole, D<sub>2</sub>O exchangeable). MS (m/z) (%) M<sup>+</sup>-1 312 (5); 253 (19); 62 (100). Elemental analysis (C<sub>16</sub>H<sub>15</sub>N<sub>3</sub>O<sub>2</sub>S) (313.37) calc./found (%) C (61.32/61.88), H (4.82/4.98), N (13.41/13.80), S (10.23/10.70).

**Antitumor Activity:** Antitumor activity was performed in the National Cancer Institute Cairo University using the method of Skehan *et al.* [28, 29]

Cells were plated in 96-multiwell plate (10<sup>4</sup> cells/ well) for 24 hrs before treatment with the compounds to allow attachment of the cells to the wall of the plate. Different concentrations of the compounds under test (0, 12.5, 25, 50 μg/ml) were added to the cell monolayer. Triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the compounds for 48 hrs at 37°C and in atmosphere of 5% CO<sub>2</sub>. After 48 hrs, cells were fixed, washed and stained with (Sulforhodamine B) 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 was added. IC<sub>50</sub> values of the tested compounds were calculated by Graph Pad Prism 5 software.

**Anti-Inflammatory Activity:** The anti-inflammatory activity testing was performed according to the method of Winter *et al.* [30].

Rats of either sex weighing 150-180 g were divided into 14 groups of 6 animals each. Oedema was induced in the left hind paw of all rats by subcutaneous injection of 0.1 ml of 1% (w/v) carrageenan in distilled water into their footpads. One group was kept as control and was given the respective volume of the solvent (DMSO). Twelve groups were pretreated with the test drugs, dissolved in DMSO, in a dose of 50 mg/ kg body weight, given orally 1 h before the carrageenan injection. The last group was given indomethacin (Indocid®) in a dose of 25 mg/ kg body weight, orally as a standard reference. The paw volume of each rat was measured using digital caliber before carrageenan injection and then hourly for 4 hrs post administration of the compounds.

The oedema rate and inhibition rate of each group were calculated as follows:

$$\text{Oedema rate (E) \%} = 100 (V_t - V_0 / V_0)$$

$$\text{Inhibition rate (I) \%} = 100 (E_c - E_t / E_c)$$

Where:

- $V_0$  is the volume before carrageenan injection (ml).
- $V_t$  is the volume at t hour after carrageenan injection (ml).
- $E_c$  is the oedema rate of control group.
- $E_t$  is the oedema rate of treated group.

**Ulcerogenic Effect:** Male rats (150-180) were fasted overnight and orally given the tested compounds (50mg/kg). 5 hrs later, animals were killed; their stomachs were removed, opened along the greater curvature and the number of ulcers assessed by the method of Corell *et al.* [31]. A separate group which received indomethacin (25mg/kg) as an ulcerogenic drug was used as a positive control. The results were compared with a DMSO treated group.

## RESULTS AND DISCUSSION

**Chemistry:** Compound 1 was reported to be obtained via Fischer indole synthesis using polyphosphoric acid and formylated using Vilsmeier Haack formylation method ( $\text{POCl}_3/\text{DMF}$ ) [26, 27] to give the starting compound 2. The indole hydrazide derivatives 3a-d were obtained by the condensation of the formyl derivative 2 with different acid hydrazides in ethanol using drops of glacial acetic acid. IR spectrum of compound 3d showed the

disappearance of aldehydic absorption band at  $1707\text{ cm}^{-1}$  and the appearance of absorption bands at 1598,  $1635\text{ cm}^{-1}$  corresponding to  $\text{C}=\text{N}$  and amidic  $\text{C}=\text{O}$  respectively.  $^1\text{H-NMR}$  spectrum of 3d revealed the disappearance of aldehydic proton singlet at 10.21 ppm and appearance of a singlet at 8.74 ppm attributed to  $(\text{CH}=\text{N})$  and two exchangeable singlets at 11.83 and 11.90 ppm integrated for the two protons of  $(\text{N-NH})$  and  $(\text{NH indole})$  respectively. Compound 2 reacted with various hydrazines in ethanol using drops of glacial acetic acid to give indole hydrazone derivatives 4a-c. IR spectra of the whole series 4a-c revealed the lack of the carbonyl absorption bands, in addition to the appearance of bands at 1596-1611 corresponding to  $\text{C}=\text{N}$  and bands at 3391-3402 attributed to  $\text{NH}$ . Mass spectrum of 4c illustrated a molecular ion peak ( $\text{M}^+$ ) at  $m/z$  361.  $^1\text{H-NMR}$  spectrum of 4c exhibited a singlet signal at 8.79 ppm attributed to  $(\text{CH}=\text{N})$ , two exchangeable singlets at 9.73 and 11.65 ppm corresponding to  $(\text{N-NH})$  and  $(\text{NH indole})$  respectively. The carboxaldehyde oxime 5 was obtained by reacting the starting formyl derivative 2 with hydroxyl amine hydrochloride in absolute ethyl alcohol in the presence of pyridine.  $^1\text{H NMR}$  showed a new exchangeable signal at 10.81 ppm corresponding to  $\text{OH}$  proton of the oxime group. The 3-substituted indole derivative 6 was prepared via the reaction of the formyl derivative 2 with o-phenylenediamine in ethanol in presence of ammonia solution. IR spectrum of compound 6 showed the absence of the carbonyl absorption band, the presence of absorption bands at  $3384\text{ cm}^{-1}$  corresponding to  $(\text{NH indole}, \text{NH benzimidazole})$ . Its mass spectrum revealed a molecular ion peak ( $\text{M}^+$ ) at  $m/z$  315 and a peak at  $m/z$  314 ( $\text{M}^+-1$ ) as a base peak. The hydrazinecarbothioamide and oxamide derivatives 7a, b were prepared by the reaction of the formyl derivative 2 with thiosemicarbazide and semicarbazide  $\text{HCl}$  respectively by condensation in absolute ethanol in presence of drops of glacial acetic acid. IR spectrum of 7a revealed the absence of the carbonyl absorption band and appearance of absorption bands at  $3396\text{-}3435\text{ cm}^{-1}$  corresponding to  $\text{NH}_2\text{NH}_2$ ,  $1590\text{ cm}^{-1}$  attributed to  $\text{C}=\text{N}$  disclosed a peak  $m/z$  at 299 ( $\text{M}^+-1$ ). In order to obtain different biologically active nuclei associated with the indole nucleus, the formyl derivative 2 was condensed with active methylene compounds in ethyl acetate in the presence of piperidine or triethylamine as a catalyst to give compounds 8a-d. IR spectrum of compound 8d showed absorption bands at 2216 and  $1712\text{ cm}^{-1}$  corresponding to  $\text{CN}$  and carboxylic ester groups respectively. Mass spectrum of compound 8d revealed a molecular ion peak ( $\text{M}^+$ ) at  $m/z$  322.  $^1\text{H-NMR}$  spectrum of



8d demonstrated 2 multiplets at 1.27 and 4.27 ppm attributed to protons of ( $\text{CH}_2\text{-CH}_3$ ) and ( $\text{-CH}_2\text{-CH}_3$ ) respectively, a singlet at 8.49 ppm related to ( $\text{CH=C}$ ) and an exchangeable singlet at  $\delta$  12.85 corresponding to ( $\text{NH}$  indole).

As seen in scheme 2, the unsubstituted hydrazone 4a derivative was allowed to react with different acid anhydrides in glacial acetic acid to give indole hydrazone carbonyl acid derivatives 9a-d. IR spectrum of compound 9b showed a new strong broad absorption band at 2500-3300  $\text{cm}^{-1}$  attributed to OH of carboxylic group and two additional bands at 1704  $\text{cm}^{-1}$  and 1626  $\text{cm}^{-1}$  corresponding to C=O (carboxylic and amidic) respectively. Mass spectrum of compound 9b showed a molecular ion peak ( $\text{M}^+$ ) at m/z 341 corresponding to the molecular weight of the compound.  $^1\text{H-NMR}$  spectrum of compound 9b showed 2 multiplets at 1.79 and 2.71 ppm referring to ( $\text{-CH}_2\text{-COOH}$ ) and ( $\text{-CH}_2\text{-C=O}$ ) respectively, a singlet at 8.15 ppm attributed to ( $\text{CH=N}$ ) and two exchangeable singlets at 10.20 and 12.31 ppm integrated for the two protons of ( $\text{COOH}$  and  $\text{NH}$  indole) respectively. Upon reaction of compound 4a with isocyanate / isothiocyanate derivatives, indole hydrazone carboxamide and thioamide derivatives 10a-c were formed. IR spectrum of compound 10c showed an additional absorption band at 1226  $\text{cm}^{-1}$  corresponding to C=S. Mass spectrum of 10c revealed a molecular ion peak ( $\text{M}^+$ ) at m/z 328 corresponding to the molecular weight of the compound.  $^1\text{H-NMR}$  spectrum of 10c showed a multiplet at 1.16 ppm integrated for ( $\text{-CH}_3$ ), a quartet at 3.63 ppm attributed to ( $\text{-CH}_2\text{-}$ ) as new signals. Methyl bromoacetate as a halo ester derivative was refluxed with 4a in dry acetone in the presence of anhydrous potassium carbonate to give indole methylene hydrazinyl acetate derivative 11. IR spectrum showed appearance of a new absorption band at 1746  $\text{cm}^{-1}$  representing C=O of the ester. Mass spectrum disclosed a peak m/z at 312 ( $\text{M}^+-1$ ).  $^1\text{H-NMR}$  spectrum revealed two new singlets at 2.46 and 3.62 ppm integrated for ( $\text{OOC-CH}_3$ ) and ( $\text{O-CH}_2\text{-COO}$ ) respectively.

**Antitumor Activity:** All the synthesized compounds 1-11 were tested for their cytotoxic activity against MCF-7 cell line and compared with the standard drug doxorubicin. Cytotoxic activities of the tested compounds on MCF-7 (human breast cancer cell line) were expressed as  $\text{IC}_{50}$  ( $\mu\text{g/ml}$ ), Table 1 where  $\text{IC}_{50}$  ( $\mu\text{g/ml}$ ): dose of the compound which reduces survival to 50%. All the tested compounds showed remarkable anticancer activities against breast cell line MCF-7. However, one compound 10b was highly

Table 1:  $\text{IC}_{50}$  ( $\mu\text{g/ml}$ ) values of the tested compounds

Comp.	$\text{IC}_{50}$ ( $\mu\text{g/ml}$ )	Comp.	$\text{IC}_{50}$ ( $\mu\text{g/ml}$ )
1	7.8	8a	4.4
2	10.6	8b	3.6
3a	8.6	8c	18.4
3b	6.4	8d	4.6
3c	7	9a	5.4
3d	5.8	9b	13.6
4a	15	9c	4.4
4b	8.6	9d	13.4
4c	4	10a	9.4
5	4.8	10b	2.6
6	4.6	10c	4.8
7a	3.8	11	38.4
7b	7.8	Doxorubicin	2.97

potent ( $\text{IC}_{50}=2.6 \mu\text{g/ml}$ ), twelve compounds showed good antitumor activity ( $\text{IC}_{50}$  ranges from 3.6-6.4  $\mu\text{g/ml}$ ), eleven compounds showed moderate activity ( $\text{IC}_{50}$  ranges from 7-18.4  $\mu\text{g/ml}$ ) and only one compound showed weak activity ( $\text{IC}_{50}=38.4 \mu\text{g/ml}$ ).

3-formyl derivative 2 ( $\text{IC}_{50}=10.6 \mu\text{g/ml}$ ) was found to be less potent than 2-thienyl indole 1 ( $\text{IC}_{50}=7.8 \mu\text{g/ml}$ ). All the prepared hydrazide based indole derivatives 3a-d revealed better activities than the starting compound 2. Compound 3d having isonicotinyl moiety ( $\text{IC}_{50}=5.8 \mu\text{g/ml}$ ) exhibited the best activity in this series. The 4-aminobenzohydrazide derivative 3b ( $\text{IC}_{50}=6.4 \mu\text{g/ml}$ ) was slightly more active than the 4-chloro analogue 3c ( $\text{IC}_{50}=7 \mu\text{g/ml}$ ). It may be attributed to the electronic nature of the substituent at p-position of the benzene ring. On the other hand, aliphatic substitution in 3a ( $\text{IC}_{50}=8.6 \mu\text{g/ml}$ ) was the least active in this series. Among the hydrazone based indole derivatives, 4c (o-Cl phenyl hydrazone derivative) was the most active ( $\text{IC}_{50}=4 \mu\text{g/ml}$ ) while the unsubstituted hydrazone derivative 4a ( $\text{IC}_{50}=15 \mu\text{g/ml}$ ) was less potent than the starting 3-formyl derivative 2. The methyl hydrazone 4b ( $\text{IC}_{50}=8.6 \mu\text{g/ml}$ ) showed moderate activity. Carboxaldehyde oxime derivative 5 ( $\text{IC}_{50}=4.8 \mu\text{g/ml}$ ) displayed better activity than the formyl 2. Incorporation of a new heterocyclic moiety (benzimidazole) at  $\text{C}_3$  of the indole ring as seen in 6 ( $\text{IC}_{50}=4.6 \mu\text{g/ml}$ ) highly increased the activity. Generally, reaction of 2 with thiosemi/semicarbazides increased the activity and bioisosteric replacement of sulphur atom in compound 7a ( $\text{IC}_{50}$ : 3.8  $\mu\text{g/ml}$ ) by an oxygen atom to give compound 7b ( $\text{IC}_{50}$ : 7.8  $\mu\text{g/ml}$ ) decreased the cytotoxic activity. Reaction of 2 with active methylene derivatives greatly increased

Table 2: Anti- inflammatory effect of the tested compounds

Groups	1 h		2 hrs		3 hrs		4 hrs	
	Edema (%)	Inhibition (%)	Edema (%)	Inhibition (%)	Edema (%)	Inhibition (%)	Edema (%)	Inhibition (%)
Control	35.4±1.19 <sup>b</sup>	----	43.7±3.74 <sup>b</sup>	----	45.2±4.01 <sup>b</sup>	----	47.1±2.36 <sup>b</sup>	----
2 (50 mg/kg)	22.4±1.49 <sup>a</sup>	36.50	25.7±1.28 <sup>a</sup>	41.34	29.7±1.41 <sup>a</sup>	34.32	30.7±2.95 <sup>a</sup>	34.87
3b (50 mg/kg)	21.3±1.48 <sup>a</sup>	42.92	27.0±0.88 <sup>a</sup>	35.66	27.4±1.73 <sup>a</sup>	33.52	28.0±1.74 <sup>a</sup>	33.75
3c (50 mg/kg)	24.3±1.39 <sup>a</sup>	34.81	33.7±1.89 <sup>a</sup>	19.69	33.5±2.54 <sup>a</sup>	18.59	34.2±2.29 <sup>a</sup>	19.16
4a (50 mg/kg)	32.1±2.69 <sup>b</sup>	9.31	34.7±3.47 <sup>a</sup>	20.72	35.0±3.05 <sup>a</sup>	22.63	37.5±2.59 <sup>a</sup>	20.50
6 (50 mg/kg)	25.4±2.15 <sup>a</sup>	28.20	33.4±1.20 <sup>a</sup>	23.73	41.5±2.92 <sup>b</sup>	7.04	44.8±2.83 <sup>b</sup>	4.31
7a (50 mg/kg)	22.8±1.15 <sup>a</sup>	35.54	32.7±1.78 <sup>a</sup>	25.27	32.2±1.57 <sup>a</sup>	28.87	32.8±1.90 <sup>a</sup>	30.39
7b (50 mg/kg)	28.0±2.00 <sup>a</sup>	20.68	39.2±2.26 <sup>b</sup>	10.43	40.9±1.73 <sup>b</sup>	9.61	41.6±1.84 <sup>b</sup>	11.76
8d (50 mg/kg)	25.7±1.38 <sup>a</sup>	31.14	28.2±2.50 <sup>a</sup>	32.63	29.2±2.60 <sup>a</sup>	28.97	29.5±2.53 <sup>a</sup>	30.13
9a (50 mg/kg)	18.1±1.54 <sup>ab</sup>	51.58	19.8±1.36 <sup>ab</sup>	52.85	19.2±1.62 <sup>ab</sup>	53.35	18.9±1.09 <sup>ab</sup>	55.31
9b (50 mg/kg)	21.7±1.43 <sup>a</sup>	41.86	24.6±1.69 <sup>a</sup>	41.39	25.2±1.27 <sup>a</sup>	38.69	26.6±2.11 <sup>a</sup>	37.15
9c (50 mg/kg)	26.1±0.77 <sup>a</sup>	30.10	33.1±2.10 <sup>a</sup>	20.97	34.0±1.12 <sup>a</sup>	17.46	32.3±1.60 <sup>a</sup>	23.59
9d (50 mg/kg)	19.9±1.51 <sup>a</sup>	46.82	22.7±1.37 <sup>a</sup>	45.92	26.0±1.13 <sup>a</sup>	36.80	26.4±1.83 <sup>a</sup>	37.62
Indomethacin (25 mg/kg)	25.0±0.78 <sup>a</sup>	29.29	30.6±1.32 <sup>a</sup>	29.93	31.1±2.03 <sup>a</sup>	31.34	32.1±2.28 <sup>a</sup>	31.89

Values represent the mean ± S.E. of six rats for each group.

<sup>a</sup>  $P < 0.05$ : Statistically significant from control (Dunnett's test).

<sup>b</sup>  $P < 0.05$ : Statistically significant from indomethacin (Dunnett's test).

Table 3 : Ulcerogenic activity

Groups	Ulcer score	
	Number	Severity
Control	0	0
2 (50 mg/kg)	3.2±0.37 <sup>ab</sup>	4.8±0.37 <sup>ab</sup>
3b (50 mg/kg)	0	0
9a (50 mg/kg)	4.4±0.51 <sup>ab</sup>	5.8±0.86 <sup>ab</sup>
9b (50 mg/kg)	0	0
9d (50 mg/kg)	0	0
Indomethacin (25 mg/kg)	7.8±0.86 <sup>a</sup>	14.0±0.55 <sup>a</sup>

Values represent the mean ± S.E. of six rats for each group.

<sup>a</sup>  $P < 0.05$ : Statistically significant from control (Kruskal-Wallis followed by Mann-Whitney test).

<sup>b</sup>  $P < 0.05$ : Statistically significant from indomethacin (Kruskal-Wallis followed by Mann-Whitney test).

the antitumor activity except the reaction with malononitrile. Barbituric/Thiobarbituric acid derivatives 8a,b ( $IC_{50}$ : 4.4 and 3.6  $\mu$ g/ml respectively) showed good antitumor activities. It appears that thio derivative was more potent than its oxo analogue. Compound 8d ( $IC_{50}$ =4.6  $\mu$ g/ml) exhibited good activity.

Reaction of 4a ( $IC_{50}$ =15  $\mu$ g/ml) with different acid anhydrides gave compounds 9a-d with varied antitumor activity. Compounds 9a (phthalyl derivative) and 9c (malyl derivative) showed the most potent activity ( $IC_{50}$ =5.4 and 4.4  $\mu$ g/ml respectively) whereas compounds 9b (having succinyl moiety) and 9d (isatoyl derivative) were nearly equipotent ( $IC_{50}$ =13.6 and 13.4  $\mu$ g/ml respectively). Reaction of 4a with isocyanate or isothiocyanate derivatives increased the activity. Derivative 10b

(cyclohexyl derivative) ( $IC_{50}$ : 2.6  $\mu$ g/ml) showed the greatest antitumor activity. Substitution of the cyclohexyl ring in 10b with a phenyl ring as seen in compound 10a ( $IC_{50}$ : 9.4  $\mu$ g/ml) greatly reduced the activity. Derivative 10c bearing an ethyl group ( $IC_{50}$ : 4.8  $\mu$ g/ml) showed good antitumor activity. Reaction of compound 4a with methyl bromoacetate as a halo ester gave compound 11 ( $IC_{50}$ : 38.4  $\mu$ g/ml) having the weakest activity.

**Anti-inflammatory Activity:** Twelve of the newly synthesized compounds 2, 3b-c, 4a, 6, 7a-b, 8d, 9a-d were tested for their anti-inflammatory activity against carrageenan induced oedema in albino rats using indomethacin as a reference standard and the results are given in Table 2.

After 4 hrs from administration of the tested compounds, five compounds 9a, 9d, 9b, 2 and 3b were found to be more active than indomethacin (31.89%) giving inhibition of oedema of 55.31%, 37.62%, 37.15%, 34.87%, 33.75% respectively. While two compounds 7a, 8d showed similar activity to that of indomethacin giving inhibition of 30.39%, 30.13% respectively. Finally, five compounds 9c, 4a, 3c, 7b and 6 were weaker than indomethacin showing inhibition of 23.59%, 20.5%, 19.16%, 11.76% and 4.31% respectively.

**Ulcerogenic Effect:** The most active compounds 2, 3b, 9a, 9b, 9d were subjected to ulcer index study. Compounds 3b, 9b, 9d were found to have no ulcerogenic effect while compounds 2, 9a showed ulcerogenic activity to some

extent but still obviously less ulcerogenic than reference drug (indomethacin). Compound 9a has proved to be potent as an anti-inflammatory agent with less ulcerogenic effect. So, that compound might be a promising lead drug.

In a summary, all the synthesized indole derivatives showed anti-tumor activity against MCF-7 cell line and compound 10b was a promising lead. Some compounds were tested for anti-inflammatory activity and compounds 9a showed interesting anti-inflammatory activity with less ulcerogenicity than Indomethacin.

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