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DESIGN, SYNTHESIS AND IN-VIVO ANTI-TUMOUR ACTIVITIES OF SOME NOVEL 2-((1H-INDOL-4-YL) OXY)-1-(4,5-DIPHENYL-1H-PYRAZOL-1-YL) ETHANONE DERIVATIVES

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ABSTRACT

The synthesis of new series of 2-((1H-indol-4-yl)oxy)-1-(4,5-diphenyl-1H-pyrazol-1-yl) ethanone was carried out by employing the general scheme developed in our laboratory using appropriate reagents. In the present work substituted Pyrazoles (4a-j) were synthesized by three steps. In the first step synthesis of chalcones by reacting substituted aldehydes with acetophenone yielded chalcones (I) and in second step synthesis of acetohydrazide by successive reactions of esterification and hydrazones with 4-hydroxy indole yielded 2-((1H-indol-4-yl)oxy)acetohydrazide (III). Finally the synthesis of title compound by condensation of (I) and (III) with ethanol in the formation of 2-((1H-indol-4-yl)oxy)-1-(4,5-diphenyl-1H-pyrazol-1-yl) ethanone. The final compounds were purified by recrystallization from appropriate solvents and the structures were characterized by spectral analysis IR, ^{1}H NMR, MASS spectra. Then the synthesized compounds were screened for the in-vivo anti-tumour activity conducted using the standard methodologies.

KEY WORDS: Chalcone, Acetohydrazide, Pyrazole, Anti-tumour activity.

INTRODUCTION

Pyrazole[1] derivatives are well established in the literature as important biologically active heterocyclic compounds. These derivatives are the subject of many research studies due to their widespread potential biological activities such as anti-inflammatory[2], antipyretic[3], antimicrobial[4], antiviral[5], antitumour[6,7], anticonvulsant[8], antihistaminic[9], antidepressant[10], insecticides[10] and fungicides.[11]

Although pyrazoles are widely distributed in nature and it is possible to obtain them by extractive techniques, homogenous and heterogeneous synthesis procedures are frequently employed. Moreover, pyrazoles have been extensively studied for their broad spectrum of biological activities, analgesic[12], anti-inflammatory[13], antipyretic, anti-arrhythmic, tranquilizing, muscle relaxing, psychoanaleptic, and anti-convulsant, monoamineoxidase inhibiting, anti-diabetic and anti-bacterial activities.

The indole framework is widely distributed in compounds with significant biological and pharmacological relevance. The development of synthetic methodologies leading to indole derivatives has attracted much attention among organic chemists. The carbon-carbon bond formation at the C-3 of indole takes advantage of the electron rich nature of this position which can be viewed as possessing enamine like character. Furthermore 3-substituted indoles are components of many drugs and are commonly found in molecules of pharmaceutical interest in a variety of therapeutic areas.

Cancer refers to a group of more than a hundred different diseases that are characterized by deoxyribonucleic acid damage that causes abnormal cell growth and development. Cancer has become an increasingly serious burden around the world. There are approximately 24.6 million people living with cancer in year 2006 almost 7 million people die each year. By 2020, patients with cancer will be estimated as 30 million, where as mortality rate will be over 10 million a year and the estimated number of new cases annually is expected to rise 10 millions in 2002 to 16 million by 2020 if this trend continues. Sixty percent of these will occur in the less developed parts of the world. The number of a 22% increase in cancer incidence and mortality with four most frequent cancers being breast, lung, colorectal and stomach, while four most deadly cancers are lung, liver, colorectal and stomach.[14] Besides breast cancer in women, ovarian cancer is still one of cancer found in high incident and diagnosed in the late of disease. Although some progress has been made in cancer diagnosis and treatment, the high incidence and low survival rate of patient have still been reported. The development of new therapeutic approach remains one of the most challenging in cancer research.

MATERIALS AND METHODS

All the reactions were carried out under prescribed laboratory conditions. The solvents and reagents used in the synthetic work were of laboratory reagent grade and were purified by distillation and crystallization techniques wherever necessary. The final products were

purified by recrystallization. Melting points were determined by open capillary method and were uncorrected. The aromatic aldehydes were obtained commercially.

1. Synthesis of E-chalcone: (1a-j)

An equimolar mixture of acetophenone and substituted aromatic aldehyde (0.01 mol) were dissolved in minimum amount of alcohol. Sodium hydroxide solution (0.02mol) was added slowly and the mixture stirred for 2hr until the entire mixture becomes very cloud. Then the mixture was poured slowly into 400 ml of water with constant stirring and kept in refrigerator for 24 hours. The precipitate obtained was filtered, washed and recrystallized from ethanol.

2. Synthesis of ethyl 2-((1H-indol-4-yl)oxy)acetate: (2)

An equimolar mixture of 4-hydroxy indole, ethyl chloroacetate and anhydrous potassium carbonate (0.02mol) in dry acetone (60 ml) was refluxed on a water bath for 24 hr. The inorganic solid was filtered and the excess solvent was removed on a rota vapour.

3. Synthesis of 2-((1H-indol-4-yl)oxy)acetohydrazide: (3)

To a suspension of (**2**) (0.01 mol) in absolute ethanol (200 ml), hydrazine hydrate (99%, 0.015 mol) was added and the reaction mixture was refluxed for 15hr. The solution was concentrated and allowed to cool overnight. The resulting solid obtained was filtered, washed with cold ethanol, dried and recrystalized from ethanol. The compound was separated as brown crystals.

4. Synthesis of 2-((1H-indol-4-yl)oxy)-1-(4,5-diphenyl-1H-pyrazol-1-yl)ethanone: (4a-j)

2-((1H-indol-4-yl)oxy)acetohydrazide **(3)** (0.1 mol) and a mixture of (E)-chalcone **(1)** was dissolved in alcohol (15 ml) and reflux for 6hrs. After completion of the reaction mixture was allowed to stay at room temperature for 30min and the solid that separated was collected, washed with cold water. The product was dried in vacuum.

Table 1: List of pyrazole derivatives prepared (4a-j):

Compound	IUPAC Name
4a	2-((1H-indol-4-yl)oxy)-1-(4,5-diphenyl-1H-pyrazol-1-yl)ethanone
4b	2-((1H-indol-4-yl)oxy)-1-(4-(4-hydroxyphenyl)-5-phenyl-1H-pyrazol-1-yl)ethanone
4c	2-((1H-indol-4-yl)oxy)-1-(4-(4-dimethylaminophenyl)-5-phenyl-1H-pyrazol-1-yl)ethanone
4d	2-((1H-indol-4-yl)oxy)-1-(4-(4-hydroxy-2-methoxyphenyl)-5-phenyl-1H-pyrazol-1-yl)ethanone
4e	2-((1H-indol-4-yl)oxy)-1-(5-phenyl-4-(2,4,6-trimethoxyphenyl)-1H-pyrazol-1-yl)ethanone

4f	2-((1H-indol-4-yl)oxy)-1-(4-(4-chlorophenyl)-5-phenyl-1H-pyrazol-1-yl)ethanone
4g	2-((1H-indol-4-yl)oxy)-1-(4-(4-nitrophenyl)-5-phenyl-1H-pyrazol-1-yl)ethanone
4h	2-((1H-indol-4-yl)oxy)-1-(4-(2-methoxyphenyl)-5-phenyl-1H-pyrazol-1-yl)ethanone
4i	2-((1H-indol-4-yl)oxy)-1-(4-(3-nitrophenyl)-5-phenyl-1H-pyrazol-1-yl)ethanone
4j	2-((1H-indol-4-yl)oxy)-1-(4-(3-methoxyphenyl)-5-phenyl-1H-pyrazol-1-yl)ethanone

Table 2: Physical constants data of synthesized compounds

Compound	R	Mol. formula	% Yield	R_f	M.P.
4a	H	$C_{25}H_{21}N_3O_2$	75	0. 54	135
4b	2-OH	$C_{25}H_{21}N_3O_3$	66	0. 68	54
4c	4-$N(CH_3)_2$	$C_{27}H_{26}N_4O_2$	78	0.56	57
4d	3-OH, 4- OCH_3	$C_{26}H_{23}N_3O_4$	80	0.65	79
4e	2,4,6- $(OCH_3)_3$	$C_{28}H_{27}N_3O_5$	62	0.74	117
4f	4-Cl	$C_{27}H_{20}ClN_3O_2$	67	0.70	122
4g	4-NO_2	$C_{25}H_{20}N_4O_4$	74	0.62	77
4h	2- OCH_3	$C_{26}H_{23}N_3O_3$	82	0.58	111
4i	3-NO_2	$C_{25}H_{20}N_4O_4$	56	0.64	91
4j	3-OCH_3	$C_{26}H_{23}N_3O_3$	61	0.61	126

SPECTRAL DATA OF SYNTHESIZED COMPOUNDS

2-((1H-indol-4-yl)oxy)-1-(4,5-diphenyl-1H-pyrazol-1-yl)ethanone (4a)

IR (KBr) cm^{-1} 3121, 3116, 2962, 2803, 1576,1467, 1385, 1242, 1161, 1066, 919, 822, 765. ^1H NMR (DMSO D_6, 400 MHz) ppm 8.76-6.18 (m, 11H, Ar), 4.86 (s, 2H, OCH_2).

2-((1H-indol-4-yl)oxy)-1-(4-(4-hydroxyphenyl)-5-phenyl-1H-pyrazol-1-yl)ethanone (4b)

IR (KBr) cm^{-1} 3436, 3133, 3022, 2963, 2849, 1357, 1369, 1556, 1567, 1483, 1355, 1327, 1263, 1147, 1052, 927, 813, 773 ^1H NMR (DMSO D_6, 400 MHz) ppm 8.58- 6.49 (m, 10H, Ar), 5.04 (s, 2H, OCH_2), 3.53 (b, s, 1H, OH).

2- ((1H-indol-4-yl) oxy)-1-(4- (4- N,N - dimethylaminophenyl) - 5-phenyl -1H - pyrazol-1-yl) ethanone (4c)

IR (KBr) cm^{-1} 3117, 3015, 2961, 2871, 1383, 1364, 1562, 1524, 1453, 1373, 1365, 1259, 1158, 1063, 931, 813, 774. ^1H NMR (DMSO D_6, 400 MHz) ppm 8.49 -6.51 (m, 10 H, Ar), 5.02 (s, 2H, OCH_2), 2.23 (s, 6H, -$N(CH_3)_2$).

2-((1H-indol-4-yl)oxy)-1-(4- (4- hydroxy- 2 -methoxyphenyl) - 5 - phenyl-1H - pyrazol-1-yl) ethanone (4d)

IR (KBr) cm^{-1} 3359, 3123, 3019, 2971, 2882, 1375, 1359, 1551, 1517, 1457, 1383, 1369, 1253, 1145, 1055, 924, 816, 781.^1H NMR (DMSO D_6, 400 MHz) ppm 8.65 -6.53 (m, 9H, Ar), 4.97 (s, 2H, OCH_2), 4. 43 (b, s, 1H, -3-OH of phenyl), 3.91 (s, 3H, 2-OCH_3 of phenyl).

2-((1H-indol-4-yl)oxy)-1-(5-phenyl-4-(2,4,6-trimethoxyphenyl)-1H-pyrazol-1-yl)ethanone (4e)

IR (KBr) cm^{-1} 3121, 3022, 2949, 2871, 1382, 1355, 1549, 1523, 1455, 1381, 1359, 1262, 1159, 1053, 930, 815, 781. ^1H NMR (DMSO D_6, 400 MHz) ppm 8.62-5.61 (m, 9 H, Ar), 4.83 (s, 2H, OCH_2), 3.43 (s, 9H, - $(OCH_3)_3$).

2-((1H-indol-4-yl)oxy)-1-(4-(4-chlorophenyl) - 5-phenyl - 1H-pyrazol-1-yl) ethanone (4f)

IR (KBr) cm^{-1} 3093, 3005, 2968, 2817, 1554, 1472, 1388, 1263, 1165, 1073, 933, 826, 781. ^1H NMR (DMSO D_6, 400 MHz) ppm 8.35-6.39 (m, 10H, Ar), 5.18 (s, 2H, OCH_2).

2-((1H-indol-4-yl)oxy) -1-(4 - (4-nitrophenyl) - 5-phenyl-1H-pyrazol-1-yl) ethanone (4g)

IR (KBr) cm^{-1} 3113, 3026, 2955, 2853, 1376, 1347, 1555, 1523, 1483, 1381, 1359, 1256, 1153, 1068, 933, 814, 775. ^1H NMR (DMSO D_6, 400 MHz) ppm 8.68- 6.51 (m, 11H, Ar), 5.07 (s, 2H,OCH_2).

2-((1H-indol-4-yl)oxy)-1-(4-(2-methoxyphenyl)-5-phenyl-1H-pyrazol-1-yl)ethanone (4h)

IR (KBr) cm^{-1} 3117, 3015, 2961, 2871, 1383, 1364, 1562, 1524, 1453, 1373, 1365, 1259, 1158, 1063, 931, 813, 774. ^1H NMR (DMSO D_6, 400 MHz) ppm 8.49 -6.51 (m, 10 H, Ar), 5.02 (s, 2H, OCH_2), 2.23 (s, 6H, $-N(CH_3)_2$).

2-((1H-indol-4-yl)oxy)-1-(4-(3 - nitrophenyl) - 5 - phenyl-1H-pyrazol-1-yl) ethanone (4i

IR (KBr) cm^{-1} 3113, 3026, 2955, 2853, 1376, 1347, 1555, 1523, 1483, 1381, 1359, 1256, 1153, 1068, 933, 814, 775. ^1H NMR (DMSO D_6, 400 MHz) ppm 8.68- 6.51 (m, 11H, Ar), 5.07 (s, 2H,OCH_2).

2-((1H-indol-4-yl)oxy)-1-(4-(3-methoxyphenyl)-5-phenyl-1H-pyrazol -1 -yl)ethanone (4j)

IR (KBr) cm^{-1} 3359, 3123, 3019, 2971, 2882, 1375, 1359, 1551, 1517, 1457, 1383, 1369, 1253, 1145, 1055, 924, 816, 781.^1H NMR (DMSO D_6, 400 MHz) ppm 8.65 -6.53 (m, 9H, Ar), 4.97 (s, 2H, OCH_2), 4. 43 (b, s, 1H, -3-OH of phenyl), 3.91 (s, 3H, 3-OCH_3 of phenyl).

EVALUATION OF IN VIVO ANTI-TUMOUR ACTIVITIES OF SYNTHESIZED COMPOUNDS

Tumor cells and Inoculation

Earlich Ascites Carcinoma (EAC) cells were obtained through the courtesy of Amla Cancer Research Centre, Thrissur, Kerala state, India. These cells were maintained in Swiss albino

mice by weekly intraperitoneal inoculation of $1x10^6$ cells/mouse i.e., the viable EAC cells were counted (Trypan blue indicator) under microscope and were adjusted at $2x10^6$ cell/ml.

Preparation of test samples

A suspension of the synthesized compounds was prepared in 0.5 % tween 80 so as to obtain the dosage forms in the concentration of 2mg/ml. These suspensions were administered orally to the animals with the help of intragastric catheter at the dose 20mg/kg of body weight.

Preparation of Standard drug formulation

The standard drug 5-Fluorouracil (5-FU) 100mg was suspended in 100 ml of 0.5% tween 80 and administered orally to the animals with the help of intragastric catheter at the dose 20 mg/kg of body weight.

4.3.1 Experimental design

The Swiss albino mice divided into twelve groups of three animals each. All animals were inoculated with 0.2 ml of EAC cells per 10 g body weight of mice on the zero day expect Group I animals which are served as normal control received only 0.5ml of 5% tween 80. A day of incubation was allowed for multiplication of the cells. Fourteen days dose of synthesized compounds (20 mg/kg body weight), 5- Fluorouracil (20 mg/kg body weight) were given orally to the animals belongs group III to XII with the help of intragastric catheter respectively from the first day up to the 14th day with 24 h intervals. Group II animals served as tumour control and received 0.5 ml of tween 80. Food and water withheld 18 h before scarifying the animals. On 15th day animals were killed by cervical dislocation. The liver was excised, rinsed in ice-cold normal saline solution followed by ice-cold 10% KCl solution, blotted, dried and weighed. Histological observation of liver tissues was carried out using the standard procedure. The haemotological and hepatoprotective were carried out in serum.

Antitumor activity of the extracts was measured in EAC animals with respect to the following parameters

i. Tumour volume

The mice were dissected and the ascitic fluid was collected from the peritoneal cavity. The volume was measured by taking it in a graduated centrifuge tube and packed cell volume was determined by centrifuging at 1000 rpm for 5 min.

ii. Tumour cell count

The ascetic fluid was taken in a WBC pipette and diluted 100 times. Then a drop of the diluted cell suspension was placed on the Neubauer counting chamber and the number of cells in the 64 small squares was counted.

ii. Viable / non-viable tumour cell count

The cells were then stained with trypan blue (0.4% in normal saline) dye. The cells that did not take up the dye were viable and those that took the stain were nonviable. These viable and nonviable cells were counted.

RESULTS AND DISCUSSION

The parameter selected in this study is to investigate the anti-tumor activity of the synthesized compounds in EAC infected mice. The transplantable tumor cells namely Ehrilich Ascite Carcinoma (EAC) cells were used. These tumors in the ascites form are fast growing and kill the host cells within the period of 3-4 weeks approximately after tumor transplantation with 2×10^6 cells. The tumor uptake by the host was manifested by a very high initial growth rate followed by a gradual decline with progressive accumulation of ascites fluid (Lag phase).

The anti-tumor activity of the synthesized compounds was assessed by determining their anti-cancer activity through the tumor volume, total cell count (viable and non-viable), packed cell volume.[16,17] In addition, the hematological parameters like Hb%, RBC and WBC count, were also assessed in the tumor bearing mice. Regular rapid increase in ascetic fluid tumor volume was measured in EAC tumor bearing mice. The tumor cells directly draw the nutrition from ascetic fluid; it does mean the ascetic fluid continuously supply the nutritional requirement to tumor cells[18]. Treatment with synthesized compounds suppressed the tumor volume and decreased the body weight of tumor bearing mice by reducing the secretion of ascetic fluid which is necessary for the growth of tumor cells.

The major problems in the cancer chemotherapy are myelosuppression and anemia. The reduction of RBC and Hb % in tumor bearing mice lead to the formation of anemic condition and this may be either due to iron deficiency or due to hemolytic or myelopathic conditions[19]. The treatment with synthesized compounds restored the Hb %, RBC and WBC count more or less to normal levels. These findings clearly indicate that synthesized compounds possess effective protective properties on the haemopoietic system.

The tumor cell volume, packed cell volume and viable cell count showed significant increase in EAC bearing mice control compared to normal group. Administration of synthesized compounds showed a significant reversal of these changes towards the normal values. The same effect was also exhibited by the standard 5-fluorouracil treatment. The results are tabulated in table 3

Table 3: Effect of administration of synthesized compounds (20 mg/kg, orally for 14 days) & 5- Fluorouracil (20mg/kg, orally for 14 days) treatment on the Tumor volume, packed cell volume, viable and Non-viable cells.

Sl. No.	Particulars /Groups	Tumour volume (ml)	Packed cell volume (ml)	Viable cells	Non-viable cells
1	Normal control	--	--	--	--
2	EAC control	3.58±0.14	2.45±0.17	8.75±0.25	0.75±0.25
3	EAC + 4a	2.10±0.05**	2.30±0.04**	3.10±0.4**	0.85±0.25**
4	EAC + 4b	2.50±0.04**	2.25±0.06**	3.15±0.25**	0.50±0.29**
5	EAC + 4c	1.50±0.09**	1.01±0.03**	1.75±0.25**	0.75±0.25**
6	EAC + 4d	1.75±0.04**	1.05±0.03**	3.05±0.41**	1.25±0.25**
7	EAC + 4e	1.90±0.04*	1.55±0.03**	2.25±0.48**	0.85±0.25**
8	EAC + 4f	2.30±0.05**	1.30±0.04**	3.00±0.4**	0.95±0.25**
9	EAC + 4g	3.50±0.04**	1.25±0.06**	3.15±0.25**	0.50±0.29**
10	EAC + 4h	2.30±0.09**	1.51±0.03**	2.75±0.25**	1.15±0.25**
11	EAC + 4i	1.35±0.04**	0.95±0.03**	1.60±0.41**	0.75±0.25**
12	EAC + 4j	2.20±0.04**	2.35±0.06**	3.15±0.25**	0.50±0.29**
13	EAC + 5FU	0.90±0.04*	0.55±0.03**	1.25±0.48**	0.75±0.25**

Values are expressed as mean ± SEM, n=three rats in each group,

** $p<0.001$ compared to tumour control.

The anti-tumor property of the synthesized compounds was assessed by studying the different hematological parameters. Hematological parameters of EAC infected mice were found to be remarkably altered compared to normal mice. The WBC count was increased but the hemoglobin percentages which were found to be decreased with modest change of the total number of RBCs. Treatment with ethanolic extract at the dose of 200mg/kg b. w changed these altered parameters to the normal values. The altered hematological parameters for synthesized compounds were given in Table 4.

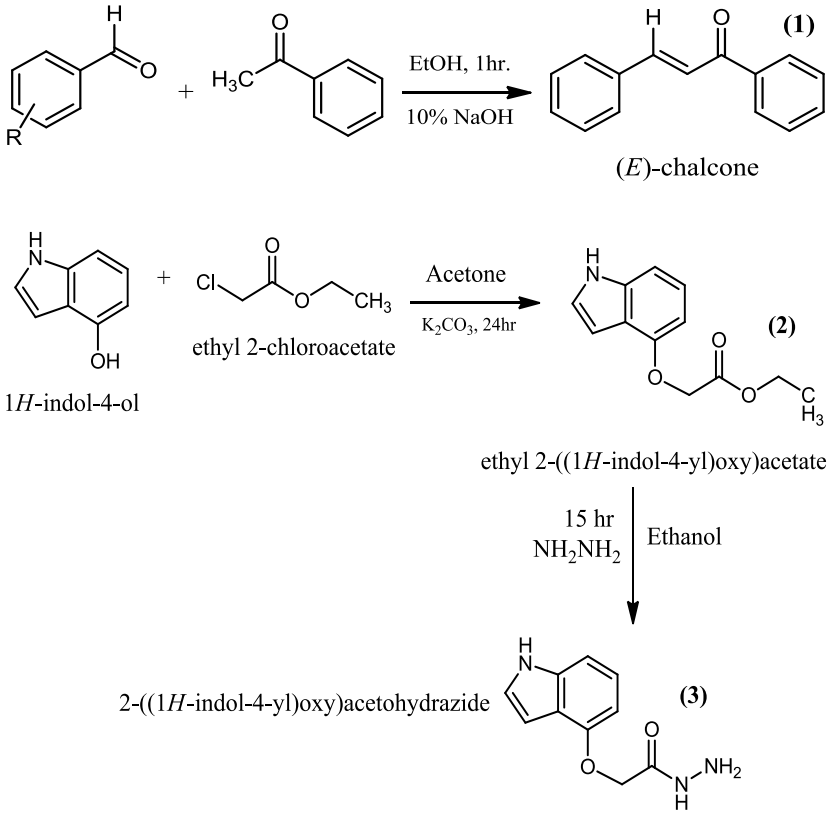
Table 4: Effect of administration of synthesized compounds (20 mg/kg, orally for 14 days) & 5-Fluorouracil (20mg/kg, orally for 14 days) treatment on Haematological parameters of EAC bearing mice

Sl. No.	Particulars /Groups	Hb content (g/dl)	Total RBC count 10^6cells/mm^3	Total WBC count 10^3cells/mm^3	Lymphocytes (%)	Neutrophils (%)	Monocytes (%)
1	Normal control	13.15±0.10	6.00±0.09	5.32±0.09	73.50±1.71	25.00±1.68	1.50±0.29
2	EAC control	09.20±0.11*	3.65±0.14*	9.72±0.13*	24.25±1.31*	74.25±1.55*	1.40±0.29
3	EAC + 4a	09.12±0.12**	4.90±0.15**	6.80±0.11**	49.25±1.55**	69.50±1.18**	1.52±0.29
4	EAC + 4b	10.10±0.19**	4.18±0.11**	6.30±0.15**	53.25±1.15**	72.00±1.18**	1.45±0.25
5	EAC + 4c	12.93±0.19**	5.16±0.16**	5.87±0.16**	71.75±1.21**	28.15±1.08**	1.48±0.29
6	EAC + 4d	11.12±0.05	3.85±0.10	6.30±0.09	45.75±0.85	43.00±0.82	1.35±0.25
7	EAC + 4e	10.12±0.12**	3.80±0.15**	6.85±0.11**	59.25±1.55**	49.50±1.18**	1.52±0.29
8	EAC + 4f	10.13±0.19**	4.38±0.11**	7.80±0.15**	43.25±1.15**	42.00±1.18**	1.45±0.25
9	EAC + 4g	12.12±0.05	4.85±0.10	6.40±0.09	35.75±0.85	33.00±0.82	1.55±0.25
10	EAC + 4h	09.43±0.19**	4.16±0.16	7.17±0.16**	41.75±1.21**	48.15±1.08**	1.48±0.29
11	EAC + 4i	12.96±0.19**	5.38±0.11**	5.80±0.15**	73.25±1.15**	27.05±1.18**	1.45±0.25
12	EAC + 4j	09.34±0.12**	3.90±0.15**	6.90±0.11**	46.25±1.55**	59.50±1.18**	1.52±0.29
13	EAC + 5FU	13.12±0.05	5.85±0.10	7.30±0.09	75.75±0.85	23.00±0.82	1.25±0.25

Values are mean S.E.M. where n=6.

*p< 0.001statistically significant when compared with normal group.

**p < 0.001statistically significant when compared with EAC control group.



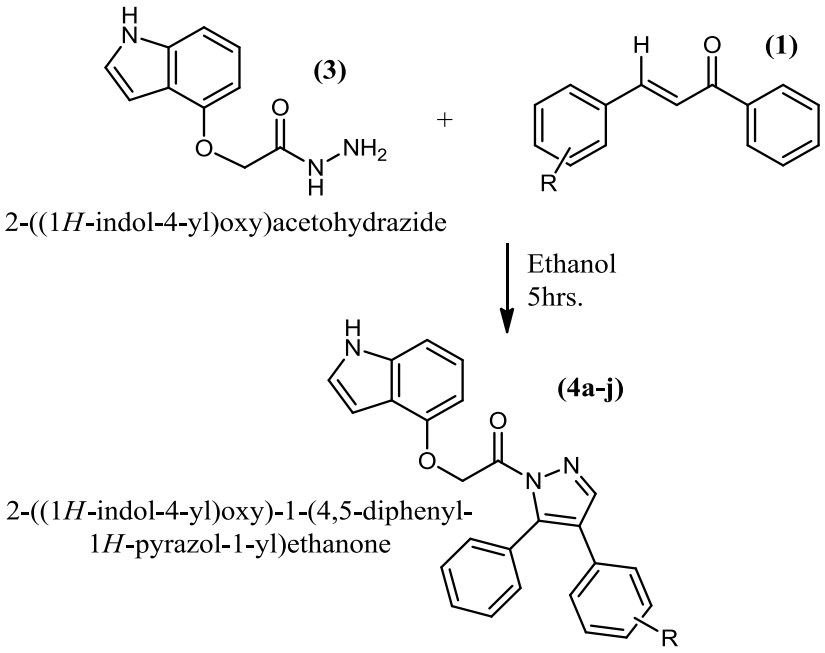


Figure 2: Scheme of Synthesis

CONCLUSION

In the present study, series of pyrazole derivatives from 4-hydroxy indole have been synthesized and confirmed through the spectral data. Further, they have been screened for in-vivo anti-tumour activity. It was concluded that these synthesized compounds have the potential of being useful in the treatment of such disorders for which they have been screened in the present study.

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