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SYNTHESIS, CHARACTERIZATION AND ANALGESIC ACTIVITY OF VARIOUS INDOLE RYLSEMICARBAZONE & THIOSEMICARBAZONE DERIVATIVES

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ABSTRACT

The formylation of indole **(a)** using Vilsmeier-Haack reaction affords the 1-H-indole-3-carboxaldehyde **(1)** & the treatment of various p-substituted anilines **(b-d)** with sodium cyanate in the presence of glacial acetic acid results in p-substituted phenylureas **(e-g)**. The 4-arylsemicarbazides **(h-j)** were synthesized by refluxing the p-substituted phenylureas**(e-g)** with hydrazine hydrate. The condensation of compound **(1)** with compounds **(h-j)** lead to the formation of 4-arylsemicarbazone derivatives of indole **(2-4)**. The Mannich bases **(5-10)** of above condensed products **(2-4)** were prepared by refluxing them with various secondary amines i.e. dimethylamine, diethylamine & with formaldehyde in the presence of glacial acetic acid. In another reaction the compound **(1)** was condensed with thiosemicarbazide **(k)** to give (2E)-2-(1H-indoyl-3-ylmethylidene) hydrazine carbothioamide **(11)** & it **(11)** was further reacted with several secondary amines, formaldehyde & glacial acetic acid to form Mannich bases **(12-13)**. All the synthesized compounds were characterized by IR, NMR & elemental analysis. These compounds were screened for their analgesic activity using acetic acid induced writhing test & hot plate test. Some of these compounds **(2, 4-5, 10** and **12)** exhibit promising activities.

Keywords: **Indole; Semicarbazone; Thiosemicarbazone; Analgesic activity.**

INTRODUCTION

Indole derivatives are an important class of organic heterocycles because of their potential activity as well as a part of several alkaloids. Indole derivatives are reported to be effective in CNS disorders such as convulsion and depression [1]. Indole and its analogs constitute the active class of the compounds possessing wide spectrum of biological activities such as antimicrobial [2], anti-inflammatory [3], antidiabetic [4], antiviral [5], anticancer [6] etc. Indole derivatives are also very much used in the management of pain [7-8]. Earlier, the pain was often considered as a simple response by the brain to a noxious stimulus in the periphery; this pain information was then transmitted via a well-known 'pain' pathways. The biological processes involved in pain perception are simply a 'stimulus-response' relationship. Nonsteroidal anti-inflammatory drugs (NSAIDs) are most commonly used for the treatment of peripheral analgesia and also have one of their actions as reduction in the peripheral inflammatory response.

Generally NSAIDs provide their anti-inflammatory & analgesic action by blocking the cyclo-oxygenase pathway. Most notably COX-1 and COX-2 have been sequenced, but a COX-3 isoform that is a splice variant of COX-1 has recently been described. It has been found that acetaminophen (Paracetamol) has minimal effects on peripheral COX-1 or COX-2 in vitro, were as exert central analgesic actions through inhibition of COX-3.[9]

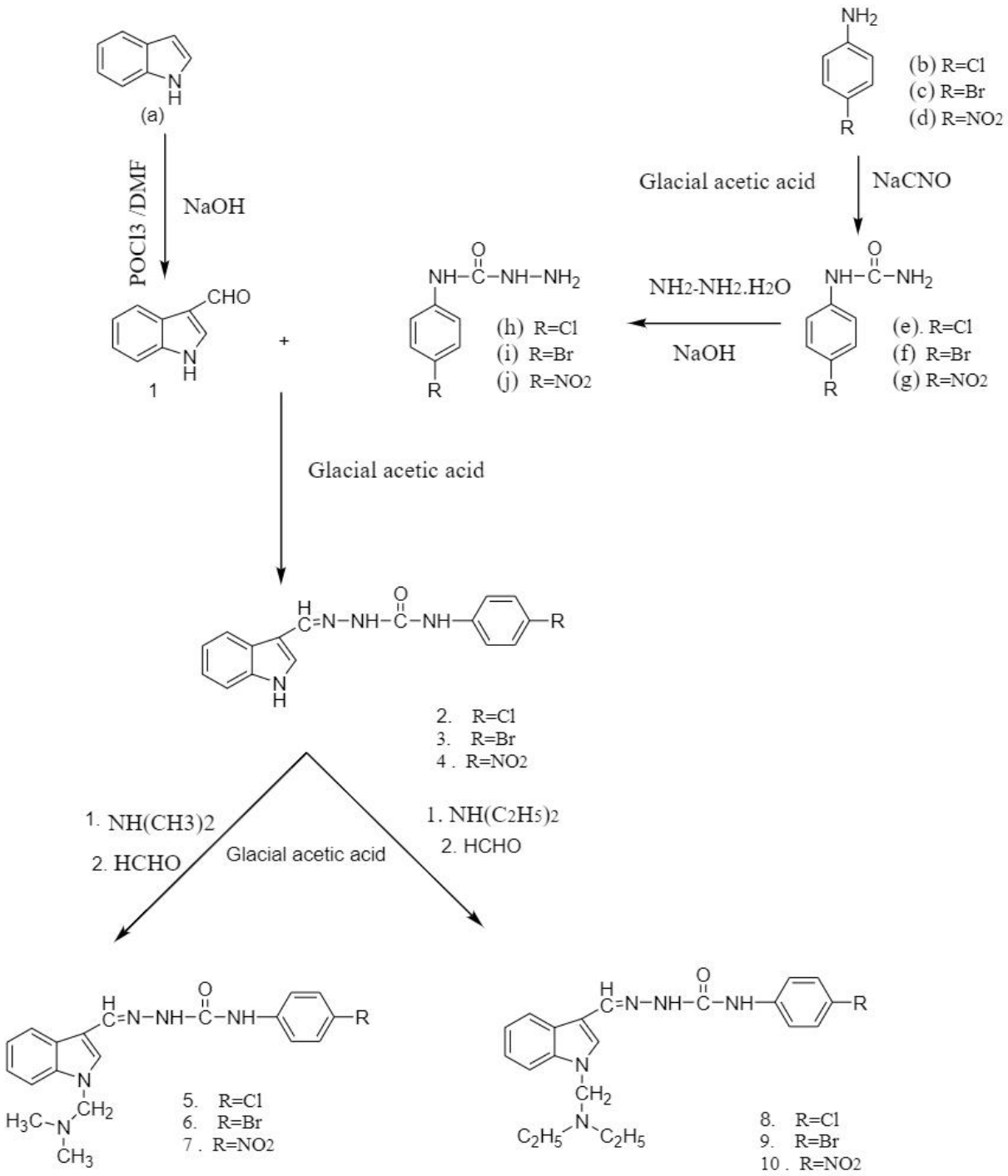
As well as the peripheral action of NSAIDs, there is increasing evidence that they exert their analgesic effects through central mechanisms involved in the development and maintenance of spinal cord sensitization. Opioid receptors modulate nociceptive input at many sites in the CNS, although functionally these can be divided into two main sites i.e. supraspinal and spinal sites of analgesia.[10]

Some evidences suggest that the hydrazone moiety present in some compounds possess a pharmacophoric character for the inhibition of COX. According to these results, analgesic profile of new series of heterocyclic N-acylarylhydrazones were described by lima *et al* [11]. Thus the biological importance of indole & arylsemicarbazones [12, 13] in the management of pain prompted us to synthesize a few hydrazones derivative of indole which carry the -NHN=CH-Ar moiety

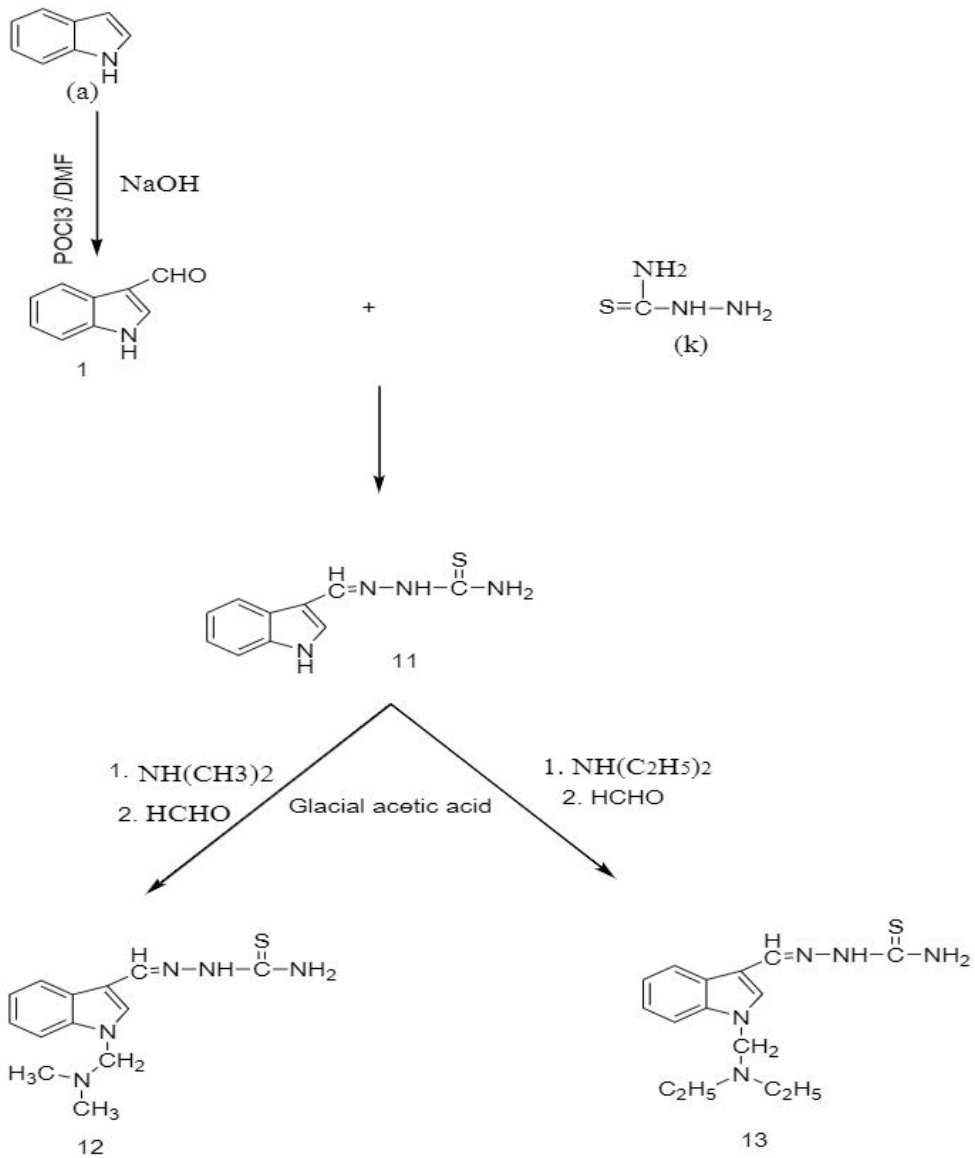
Chemistry

The target compounds were synthesized as outlined in scheme 1 & scheme 2. The starting material indole **(a)** was procured from a commercial vendor and allowed to undergo Vilsmeier-Haack reaction in the presence of POCl3 & DMF to produce 1-H-indole-3-carboxaldehyde **(1).** In the next step various p-substituted anilines **(b-d)** were allowed to react with sodium cyanate in the presence of glacial acetic acid to yield p-substituted phenylureas **(e-g),** which were further treated with an equimolar quantity of hydrazine hydrate in a NaOH solution to form 4-arylsemicarbazides**(h-j).** The final compounds **(2-4)** were obtained by condensing 1-H-indole-3-carboxaldehyde with p-chloroarylsemicarbazide, p-bromoarylsemicarbazide & p-nitroarylsemicarbazides respectively. The Mannich bases **(5-10)** were finally synthesized by refluxing the above compounds **(2-4)** with dimethylamine & diethylamine respectively in the presence formaldehyde & acetic acid.

In scheme 2, the 1-H-indole-3-carboxaldehyde **(1)** was condensed with thiosemicarbazide **(k)** to give 2-(1*H*-indol-3-ylmethylidene)hydrazinecarbothioamide**(11)** **.** The Mannich bases **(12-13)** of the compound (**k)** were also prepared by refluxing it with several secondary amines in formaldehyde using acetic acid as a solvent.


(a)
POCl3 /DMF
NaOH
CHO
1
+
NH2
(b) R=Cl
(c) R=Br
(d) R=NO2
Glacial acetic acid
NaCNO
NH-C-NH2
(e). R=Cl
(f) R=Br
(g) R=NO2
NH2-NH2.H2O
NaOH
NH-C-NH-NH2
(h) R=Cl
(i) R=Br
(j) R=NO2
Glacial acetic acid
2. R=Cl
3. R=Br
4 . R=NO2
1. NH(CH3)2
2. HCHO
Glacial acetic acid
1. NH(C2H5)2
2. HCHO
5. R=Cl
6. R=Br
7 . R=NO2
8. R=Cl
9. R=Br
10 . R=NO2

Scheme -1



Scheme -2

Pharmacology

The analgesic activity was determined by the hot –plate test (central analgesic), and acetic acid induced writhing assay (peripheral analgesic) [13, 14, 15]. The results (table 1 and 2) revealed that all the tested compounds exhibited significant activity. Most of the tested compounds have nearly the same activity as the reference drug (indomethacin) in peripheral analgesic assays and the remaining tested compounds have good activities in both central and peripheral analgesic assays. Few compounds show even more activity than reference one in peripheral analgesic assay.

Table 1 Percent analgesic activity (Peripheral, writhing test).

Groups	No. of writhings response in mice	% Analgesic activity
Control	62±0.24	----------
2	16.66±0.30	73.12*
3	24.33±0.30	60.75*
4	23.50±0.31	62.09*
5	14.67±0.39	76.33*
6	26.50±0.39	57.25*
7	32.66±0.45	47.33*
8	27.5±0.39	55.64*
9	31.33±0.45	49.46*
10	19±0.24	69.35*
11	34.25±0.96	44.75*
12	21.67±0.77	65.04*
Diclofenac	6.67±0.30	89.24*
Indomethacin	21.83±0.28	64.47*

Each value represents the mean ±SEM (n=6) Significant levels *p<0.01 as compared with respective control

Table 2 Central analgesic activity (hot-plate test)

Group	Reaction time (min.)			
	0 min.	30 min.	60 min.	90 min.
Control	3.00±0.24	3.00±0.24	3.33±0.30	3.6±0.19
2	3.33±0.19	7.00±0.24**	7.50±0.20**	7.00±0.24**
3	3.50±0.20	5.67±0.30**	6.00±0.24**	5.3±0.19**
4	3.33±0.19	6.67±0.30**	6.67±0.30**	6.3±0.19**
5	3.00±0.24	8.00±0.24**	8.67±0.30**	8.5±0.20**
6	3.00±0.24	4.33±0.30*	6.33±0.30**	4.3±0.19
7	3.33±0.19	4.50±0.20**	5.00±0.24**	4.8±0.28*
8	3.00±0.24	4.00±0.24	4.50±0.39	4.00±0.24
9	3.33±0.19	5.50±0.39**	4.00±0.62	4.1±0.28
10	3.33±0.19	7.50±0.39**	8.33±0.30**	7.00±0.24**
11	3.00±0.24	4.00±0.33	6.00±0.24**	4.5±0.20
12	3.00±0.30	5.00±0.30**	6.00±0.40**	5.50±0.39**
13	3.00±0.33	4.67±0.30**	4.33±0.39	4.33±0.30
Morphine	3.33±.19	9.00±0.24**	12.50±0.20**	9.8±0.28**

Values represent the mean ± SEM of six animals for each group. *significant at p<0.05 , **significant at p<0.01 (Dunnett's test)

Results & discussion

The 4-arylsemicarbazone derivatives of indole ring were screened for their analgesic activity using both central analgesic and peripheral analgesic assays. The compounds 2, 5 & 12 were found to

be good analgesic when compared with reference drug i.e., Diclofenac & even more active than another reference drug Indomethacin using peripheral analgesic assay. The compound 5 was found to be most active among all the screened compounds using acetic acid induced writhing test. The evaluation of all the synthesized derivatives against hot-plate test revealed that the compounds 2,3,4,5, 6, 7, 10, 11 & 12 were active central analgesics & compound 5 was the most active among all the derivatives tested for the central analgesic activity.

Conclusion

The results showed that the compound 5 is an active compound against both Peripheral and Central analgesic assays. The other active compounds such as 2 & 5 revealed that chloro substituted aryl rings along with indole nucleus possess potent analgesic activity. Thus the present investigation offers the synthesis of newer potent analgesics having indole ring along with 4-aryl substituted semicarbazones & thosemicarbazones.

Materials and methods

Animals

The healthy Swiss albino mice of both sexes weighing 25-30 g were taken for the study. The animals were kept in large spacious hygienic cages during the course of experimental period. The animals had free access to standard commercial diet and water ad libitum and were kept in rooms maintained at 22±1^{0}C with 12h light dark cycle.

Analgesic activity using hot plate test

The experiment was carried out using Glassman's method [16], using hot plate apparatus, maintained at 55±0.5 ^{0}C. The mice were divided into 14 groups of 6 animals each. The reaction time of mice to the thermal stimulus was the time interval between placing the animal above the hot plate and it licked its hind paw or jumped. The reaction time was measured prior to administration of synthesized compounds and drug administration (0 min). Group 1 was kept as normal control. The synthesized compounds were injected subcutaneously to mice of groups 2-14 at a dose of 5mg/kg. Mice of group 14 served as standard and were treated with morphine sulphate 5mg/kg. The reaction time was again measured at 0, 30, 60, 90 min. after the treatment. To avoid tissue damage to the mice paws, cut-off time for the response to the thermal stimulus was set at 60s. The increase in the reaction time against control was calculated.

Analgesic activity (acetic acid induced writhing response model)

The compounds were selected for investigating their analgesic activity in acetic acid induced writhing response in Swiss albino mice, following the method of Koster *et al,* [17]. The 14 groups of mice having 6 each were used for the experiment. The 1st group served as control and received only vehicle & the group's between 2-12 received the tested compounds. The last two groups received reference drugs as diclofenac at a dose of 20 mg/kg & indomethacin at a dose of 10mg/kg [18]. After 30 min, each mouse was administered 0.6% of an aqueous solution of acetic acid (10mL/kg) and the mice were then kept in transparent boxes for observation. The numbers of writhes were counted for 20min after acetic acid injection. The number of writhes in each treated groups was compared to that of control group. The number of writhes was recorded and the percentage protection was calculated using the following ratio: % protection = (control mean- treated mean/control mean) ×100.

Experimental

All melting points are in degree centigrade and were determined on Jindal melting point apparatus and were uncorrected. The reactions were monitored and the purity of the products was checked by Thin Layer Chromatography (TLC) using Chloroform:Methanol(9:1) as solvent system(Table-3). The IR spectra were recorded (KBr) on Jasco FT-IR 6100 spectrophotometer. ^{1}H NMR were recorded on Bruker Avauce II 300MHz NMR spectrometer using DMSO-d6 as a solvent. Tetramethylsilane serves as internal standard in ^{1}H NMR(Table-5). Elemental analysis was done on Vario EL-III analyser(Table-6).

***General synthesis of 1-H-indole-3-carboxaldehyde* (1)**

The solution of indole **(a)** was prepared in DMF in a beaker. In an ice bath dimethyl formamide is taken & to it POCl3 was added drop wise by maintaining temperature below 10 ^{0}C. The mixture was then stirred for 0.5 h. To this reaction mixture, solution of indole **(a)** was added at a temperature below 10^{0}C. The temperature was raised to 35^{0}C and maintained for 1h. The reaction mixture was cooled again to 10^{0}C & made alkaline by the addition of NaOH solution. The suspension formed was heated to 60^{0}C and cooled to room temperature. The final compound was filtered and washed with water [19].

***General synthesis of 4-substituted aromatic ureas* (e-g)**

The synthesis was performed by using the well-known procedure described by Pandeya *et al*.[20] Various 4-substituted anilines (0.1 mol) **(b-d)** were dissolved in 10ml of glacial acetic acid and to it 50 ml of water was added. To this solution an equimolar (0.1 mol) quantity of sodium cyanate in 50 ml of warm water was mixed with stirring. The reaction mixture was kept for 30 minutes and crystals were collected, recrystallized from absolute ethanol.

Synthesis of 4-substituted aryl semicarbazide derivatives **(h-j)**

To a solution of substituted ureas in 100 ml of ethanol, an equimolar quantity (0.01mol) of hydrazine hydrate was added. The reaction mixture was made alkaline by adding NaOH and refluxed for 1.5h and cooled in ice and the product was filtered and recrystallized from ethanol.

General synthesis of indole semicarbazone derivatives (2-4)

To a solution of semicarbazide of substituted amine in ethanol, 1-2 ml of glacial acetic acid was added. To this solution an equimolar quantity of 1-H-indole-3-carboxaldehyde was added. The reaction mixture was refluxed for 1.5h. The resultant product was filtered and recrystallized from ethanol.

General synthesis of Mannich bases of indole semicarbazone derivatives (5-10)

The Mannich bases of indole semicarbazone derivatives were prepared by refluxing them with formaldehyde, and various secondary amines such as dimethylamine & diethylamine respectively in the presence of glacial acetic acid for 3h. The resultant products were cooled at room temperature and filtered recrystallized with ethanol.

General synthesis of indole thiosemicarbazone derivatives & their Mannich bases (11-13)

To the solution of 1-H-indole-3-carboxaldehyde in glacial acetic acid an equimolar quantity of thosemicarbazide **(k)** was added and the reaction mixture was refluxed for 1.5h to form condensation products. The final product was filtered and recrystallized using ethanol. The Mannich bases of above condensed products were also prepared by refluxing the 2-(1*H*-indol-3-ylmethylidene)hydrazinecarbothioamide **(11)** with dimethylamine & diethylamine respectively in the presence of formaldehyde and glacial acetic acid for 3h. The products were filtered and recrystallized with ethanol.

Table 3 Physico-chemical data of synthesized derivatives

Derivatives	Molecular formula	Molecular weight	Melting point (^{0}C)	Rf value	% yield
1	C_9H_7N0	145.157	193-195	0.88	81%
2	$C_{16}H_{13}ClN_4O$	312.75	240-243	0.77	72%
3	$C_{16}H_{13}BrN_4O$	356.03	272-275	0.82	83%
4	$C_{16}H_{13}N_5O_3$	323.10	118-120	0.88	80%
5	$C_{19}H_{20}ClN_5O$	369.85	48-52	0.66	70%
6	$C_{19}H_{20}BrN_5O$	414.30	58-60	0.68	82%
7	$C_{19}H_{20}N_6O_3$	380.16	65-67	0.90	84%
8	$C_{21}H_{24}ClN_5O$	397.90	75-78	0.66	66%
9	$C_{21}H_{24}BrN_5O$	442.35	52-55	0.78	75%
10	$C_{21}H_{24}N_6O_3$	408.45	57-59	0.88	78%
11	$C_{10}H_{10}N_4S$	218.27	95-97	0.72	84%
12	$C_{13}H_{17}N_5S$	275.35	58-60	0.82	88%
13	$C_{15}H_{21}N_5S$	303.42	67-70	0.77	77%

Table 4 IUPAC Names of synthesized derivatives

Derivatives	IUPAC Name
1	1-H-indole-3-carboxaldehyde
2	(2E)-N-(4-chlorophenyl)-2-(1H-indol-3-ylmethylidene)hydrazinecarboxamide
3	(2E)-N-(4-bromophenyl)-2-(1H-indol-3-ylmethylidene)hydrazinecarboxamide
4	(2E)-2-(1H-indol-3-ylmethylidene)-N-(4-nitrophenyl)hydrazinecarboxamide
5	(2E)-N-(4-chlorophenyl)-2-({1-[(dimethylamino)methyl]-1H-indol-3-yl}methylidene)hydrazinecarboxamide
6	(2E)-N-(4-bromophenyl)-2-({1-[(dimethylamino)methyl]-1H-indol-3-yl}methylidene)hydrazinecarboxamide
7	(2E)-2-({1-[(dimethylamino)methyl]-1H-indol-3-yl}methylidene)-N-(4-nitrophenyl)hydrazinecarboxamide
8	(2E)-N-(4-chlorophenyl)-2-({1-[(diethylamino)methyl]-1H-indol-3-yl}methylidene)hydrazinecarboxamide
9	(2E)-N-(4-bromophenyl)-2-({1-[(diethylamino)methyl]-1H-indol-3-yl}methylidene)hydrazinecarboxamide
10	(2E)-2-({1-[(diethylamino)methyl]-1H-indol-3-yl}methylidene)-N-(4-nitrophenyl)hydrazinecarboxamide
11	(2E)-2-(1H-indol-3-ylmethylidene)hydrazinecarbothioamide
12	(2E)-2-({1-[(dimethylamino)methyl]-1H-indol-3-yl}methylidene)hydrazinecarbothioamide
13	(2E)-2-({1-[(diethylamino)methyl]-1H-indol-3-yl}methylidene)hydrazinecarbothioamide

Table 5 Spectral analysis of synthesized derivatives

Derivatives	IR analysis	^{1}H NMR
1	3460(NH),1720(C=O),1540(C=C Aromatic ring), 3050(=C-H ring stretch), 2750cm^{-1}(-C-H stretch)	9.93(1H,s,CHO),12.22(1H,s,NH),8.35(1H,S,H^2) 7.21(2H,br,H^5 and H^6),8.15(1H,s,H^4),7.54(1H,s,H^7)
2	3460(NH),3350(Ar-NH), 1610(C=N),1680(C=O), 820(Ar-H),3280(-CONH)	9.18(s,1H,=NNH),5.8(s,1H,Ar-NH),5.96(s,1H,CONH) 7.84(s,1H,CH=N), 12.22(1H,s,NH)
3	3480(NH),3360(Ar-NH), 1618(C=N),1700(C=O), 825(Ar-H),3260(-CONH)	9.12(s,1H,=NNH),6.1(s,1H,Ar-NH),5.89(s,1H,CONH) 7.78(s,1H,CH=N), 12.19(1H,s,NH)
4	3450(NH),3345(Ar-NH), 1605(C=N),1671(C=O), 834(Ar-H) 3275(-CONH)	9.14(s,1H,=NNH),6.15(s,1H,Ar-NH),5.85(s,1H,CONH) 7.71(s,1H,CH=N), 12.20(1H,s,NH)
5	3330(Ar-NH), 1612(C=N),1660(C=O), 836(Ar-H) 3300(-CONH)	9.11(s,1H,=NNH),6.25(s,1H,Ar-NH),5.75(s,1H,CONH) 7.69(s,1H,CH=N), 4.92(s,2H,-CH2)
6	3348(Ar-NH), 1610(C=N),1678(C=O), 840(Ar-H), 3288(-CONH)	9.18(s,1H,=NNH),6.33(s,1H,Ar-NH),5.88(s,1H,CONH) 7.79(s,1H,CH=N), 4.93(s,2H,-CH2)
7	3350(Ar-NH), 1620(C=N),1698(C=O), 833(Ar-H), 3260(-CONH)	9.01(s,1H,=NNH),6.22(s,1H,Ar-NH),5.77(s,1H,CONH) 7.54(s,1H,CH=N), 4.91(s,2H,-CH2)
8	3355(Ar-NH), 1622(C=N),1688(C=O), 834(Ar-H), 3310(-CONH)	8.98(s,1H,=NNH),5.78(s,1H,Ar-NH),5.93(s,1H,CONH) 7.77(s,1H,CH=N), 4.97(s,2H,-CH2)
9	3360(Ar-NH), 1640(C=N),1695(C=O), 822(Ar-H), 3270(-CONH)	9.17(s,1H,=NNH),5.66(s,1H,Ar-NH),5.97(s,1H,CONH) 7.56(s,1H,CH=N), 4.93(s,2H,-CH2)
10	3346(Ar-NH), 1616(C=N),1714(C=O), 836(Ar-H), 3286(-CONH)	9.11(s,1H,=NNH),5.8(s,1H,Ar-NH),5.93(s,1H,CONH) 7.22(s,1H,CH=N), 4.96(s,2H,-CH2)
11	3360(Ar-NH), 3460(NH),1618(C=N), 7.24(s,1H,CH=N) 1274(C=S), 832(Ar-H),	9.16(s,1H,=NNH),6.13(s,1H,Ar-NH), 12.45(1H,s,NH)
12	3350(Ar-NH), 1624(C=N),1290(C=S), 836(Ar-H)	9.11(s,1H,=NNH),6.23(s,1H,Ar-NH),) 7.45(s,1H,CH=N) 4.92(s,2H,-CH2)
13	3340(Ar-NH), 1615(C=N),1267(C=S), 7.45(s,1H,CH=N) 842(Ar-H)	9.11(s,1H,=NNH),6.23(s,1H,Ar-NH),) 4.91(s,2H,-CH2)

Table 6 Elemental analysis of synthesized derivatives

Derivatives	Calculated	Found
2	C (61.64%)H(4.19%)Cl(11.34%)N(17.91%)O(5.12%)	C (61.66%)H(4.22%)Cl(11.35%)N(17.97%)O(4.80%)
3	C (53.80%) H(3.67%)Br(22.37%)N(15.68%)O(4.48%)	C (53.84%) H(3.69%)Br(22.34%)N(15.59%)O(4.54%)
4	C (59.44%)H(4.05%)N(21.66%)O(14.85%)	C (59.49%)H(4.01%)N(21.68%)O(14.83%)
5	C (61.70%)H(5.45%)Cl(9.59%)N(18.94%)O(4.33%)	C (61.74%)H(5.46%)Cl(9.53%)N(18.89%)O(4.36%)
6	C (55.08%) H(4.87%)Br(19.29%)N(16.90%)O(3.86%)	C (55.03%)H(4.88%)Br(19.31%)N(16.88%)O(3.87%)
7	C (59.99%)H(5.30%)N(22.09%)O(12.62%)	C (60.01%)H(5.28%)N(22.15%)O(12.65%)
8	C (63.39%)H(6.08%)Cl(8.91%)N(17.60%)O(4.02%)	C (63.41%)H(6.11%)Cl(8.87%)N(17.58%)O(4.00%)
9	C (57.02%) H(5.47%)Br(18.06%)N(15.83%)O(3.62%)	C (57.05%) H(5.49%)Br(18.01%)N(15.85%)O(3.58%)
10	C (61.75%)H(5.92%)N(20.58%)O(11.75%)	C (61.79%)H(5.96%)N(20.63%)O(11.70%)
11	C (55.02%)H(4.62%)N(25.67%)S(14.69%)	C (55.06%)H(4.65%)N(25.61%)S(14.73%)
12	C (56.07%)H(6.22%)N(25.43%)S(11.64%)	C (56.11%)H(6.23%)N(25.41%)S(11.69%)
13	C (59.38%)H(6.98%)N(23.08%)S(10.57%)	C (59.41%)H(6.01%)N(23.09%)S(10.63%)

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