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Short communication

Solvent-free, microwave assisted Knoevenagel condensation of novel 2,5-disubstituted indole analogues and their biological evaluation

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ABSTRACT

A rapid, efficient and environmental benign methodology for the preparation of 2,5-disubstituted indole analogues is developed. 2,5-Disubstituted indole-3-carboxaldehydes (**1a**–**c**) undergo Knoevenagel condensation with barbiturates (**2** & **4**), thiazolidine-2,4-dione (**6**) and 3-methyl-1*H*-pyrazol-5(4*H*)-one (**8**) in solvent-free, NH_4OAc catalyzed, microwave assisted reaction. Structures of the products thus obtained were confirmed by their m.p, Elemental analysis, IR, ^1H NMR, ^{13}C NMR and Mass spectral data. The *in vitro* antioxidant and cytotoxic activities against three tumor cell lines were evaluated and discussed in terms of their structural differences. Among the screened compounds **9b**, **9c**, **7b** and **5b** exhibited excellent antioxidant activity. Compounds **9b**, **9c** and **7b** have shown strong cytotoxicity among the compounds tested.

1. Introduction

Cancer and atherosclerosis, two major causes of death, are due to the salient "free radical" impact. It is possible that endogenous free radical reactions, like those initiated by ionizing radiation, may result in tumors formation. One of the most imperative correlations between reactive oxygen species (ROS) and cancer is the increased death rates from leukemia and malignant neoplasia of the breast, ovaries and rectum is due to the greater impact of lipid peroxidation [1]. There is increasing evidence of the implication of free radicals and reactive oxygen species (ROS) in a variety of diseases and pathophysiological events including inflammation, cancer, myocardial infraction, arthritis and neurodegenerative disorders [2–7]. ROS may cause initiation of lipid peroxidation, direct inhibition of mitochondrial respiratory enzymes, inactivation of glyceraldehyde-3-phosphate dehydrogenase, inhibition of membrane sodium/potassium ATPase activity, inactivation of membrane sodium channel and other oxidative modifications of proteins. All these toxic effects are likely to play a vital role in the pathophysiology of shock, inflammation and ischemia-reperfusion injury [8].

In view of the fact that, indole analogues constitute an important class of therapeutic agents in medicinal chemistry including anticancer [9], antioxidant [10], antirheumatoidal and anti-HIV [11,12]. Some of the indoles inhibit the growth of bladder cancer, cell carcinoma, lung cancer, colon cancer, mammary tumor, prostate cancer and breast tumor cells [13]. Alongside, many indole derivatives are considered as the most potent scavengers of free radicals [14]. They directly scavenge toxic free radicals, such as hydroxyl radical, peroxynitrite anion and hypochlorous acid, reducing macromolecular damage in all organs [15].

In addition, Knoevenagel reactions have prime importance for their pharmacological and biological applications [16]. Organic reactions under solvent-free [17,18] environment have increasingly attracted chemists interest, particularly from the viewpoint of green chemistry [19]. On the other hand, solvent-free reactions are more suitable and successful under microwave activation and several advantages are manifested in this approach [20,21]. The advantages such as short reaction time [22–24], eco-friendly nature and preparative scale synthesis [21,24,25] are making microwave assisted reactions a most acceptable.

In continuation of our ongoing interest in green chemistry of bioactive indoles [26–30]. From the sufficient survey of literature and to the best of our knowledge, first time we herein report, a hitherto 2,5-disubstituted indole analogues in solvent-free, NH_4OAc catalyzed microwave assisted Knoevenagel condensation (Scheme 1). This is rapid, expedient, environmentally benign and free from aforesaid drawbacks.

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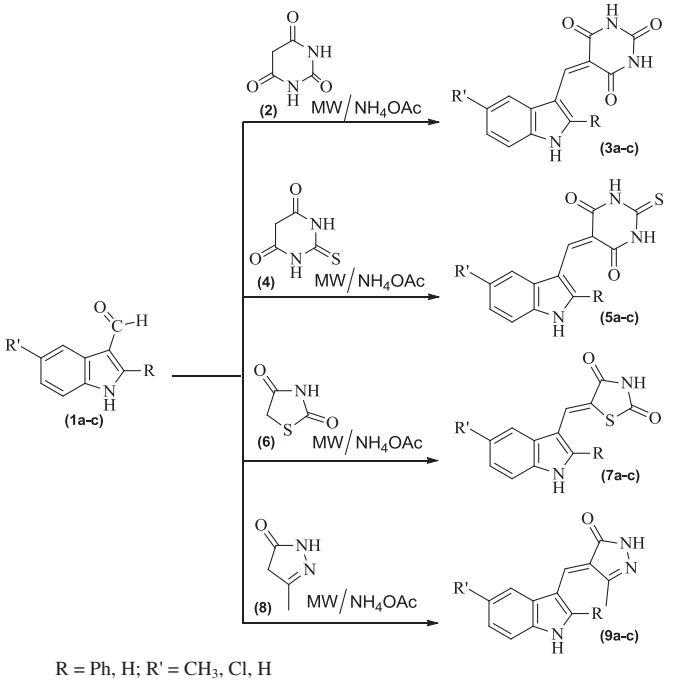

Scheme. 1. Schematic representation for the synthesis of novel indole analogues by Knoevenagel condensation.

2. Chemistry

Avoiding organic solvents during the reactions in organic synthesis leads to a clean, efficient and economical technology (Green Chemistry). There is an increasing interest in the use of environmentally benign reagents and methods. In the present investigation, the Knoevenagel condensation (Scheme 1) was carried out under conventional and microwave irradiation. In microwave assisted synthesis, 2,5-disubstituted indole-3-carboxaldehydes and active methylene compounds (barbiturates/thiazolidine-2,4-dione/3-methyl-1*H*-pyrazol-5(4*H*)-one) in presence of NH_4OAc were irradiated for 5–10 min to produce the Knoevenagel product. The products obtained in good yields with high purity without using any solvent. When the reaction was carried out using conventional heating the products were obtained in moderate yields in 2–4 h (Table 1).

The reaction has also been performed under neat conditions (without solvent support or catalyst). However, this methodology suffers from very low yields and longer in reaction time, sometimes no reaction occurred. This could be made to go successfully using catalyst.

Conventional synthesis suffers from many disadvantages such as use of solvent, long reaction periods, lengthy work up process and low yields with moderate purity (Table 1). Hence, the results accomplished to come up with a new methodology which is novel, rapid, economical and environmentally benign. All the newly synthesized compounds were characterized by elemental analysis, IR, ^{1}H NMR, ^{13}C NMR and Mass spectral data.

The IR spectrum of 5-((5-methyl-2-phenyl-1*H*-indol-3-yl) methylene) pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione (**3a**) has shown a strong absorption at 3444 cm^{-1} corresponding to indole NH. Absorption at 3140 and 3052 cm^{-1} corresponds to pyrimidine NH/NH and an absorption at 1689 and 1575 cm^{-1} corresponds to carbonyl stretching. ^{1}H NMR spectrum of **3a** has exhibited a singlet at δ 11.05 due to indole NH which is also D_2O exchangeable and peaks at δ 10.08 and 10.06 were assigned to pyrimidine NH/NH. A singlet at δ 7.10, doublet at δ 7.25, doublet at δ 7.35 and multiplet between δ 7.50 and 7.65 ppm corresponds to eight aromatic protons present in the molecule. Peaks at δ 2.57 and 7.80 are assigned for the methyl and vicinal protons respectively. ^{13}C NMR spectrum of **3a** has displayed a downfield signals at δ 164 for two symmetric carbonyls and less deshielded peak at δ 154 integrated for the carbonyl flanked by NH of pyrimidine ring and δ 32 integrated for methyl carbon. The mass spectrum of compound **3a** has shown molecular ion peak at *m/z* 346 $[M+1]^+$. The above spectral data supports the formation of compound **3a**. Spectral data of compounds **3a** & **3c** also supported their structures.

Table 1
Comparative study of the synthesis of novel indole analogues.

Entry	Method	Conventional (reflux) microwave power in %	Reaction time (min)	Isolated yield (%)	
				MW	Δ
3a	Δ (AcOH)	Reflux	240	–	67
	MW (Neat)	50	10	21	–
	MW (NH_4OAc)	50	10	90	–
3b	Δ (AcOH)	Reflux	240	–	61
	MW (Neat)	50	10	Nil	–
	MW (NH_4OAc)	50	10	88	–
3c	Δ (AcOH)	Reflux	180	–	62
	MW (Neat)	50	10	Nil	–
	MW (NH_4OAc)	50	05	92	–
5a	Δ (AcOH)	Reflux	240	–	65
	MW (Neat)	50	10	15	–
	MW (NH_4OAc)	50	10	90	–
5b	Δ (AcOH)	Reflux	240	–	65
	MW (Neat)	50	10	Nil	–
	MW (NH_4OAc)	50	10	87	–
5c	Δ (AcOH)	Reflux	180	–	65
	MW (Neat)	50	10	Nil	–
	MW (NH_4OAc)	50	05	90	–
7a	Δ (AcOH)	Reflux	210	–	70
	MW (Neat)	50	10	25	–
	MW (NH_4OAc)	50	10	92	–
7b	Δ (AcOH)	Reflux	210	–	68
	MW (Neat)	50	10	20	–
	MW (NH_4OAc)	50	10	92	–
7c	Δ (AcOH)	Reflux	180	–	70
	MW (Neat)	50	10	15	–
	MW (NH_4OAc)	50	05	95	–
9a	Δ (AcOH)	Reflux	180	–	75
	MW (Neat)	50	05	25	–
	MW (NH_4OAc)	50	05	92	–
9b	Δ (AcOH)	Reflux	180	–	66
	MW (Neat)	50	05	25	–
	MW (NH_4OAc)	50	05	90	–
9c	Δ (AcOH)	Reflux	120	–	70
	MW (Neat)	50	05	25	–
	MW (NH_4OAc)	50	05	94	–

In the IR spectrum of 5-((5-methyl-2-phenyl-1*H*-indol-3-yl) methylene)-dihydro-2-thioxopyrimidine-4,6(1*H*,5*H*)-dione (**5a**), a strong absorption at 3433 cm^{-1} corresponds to indole NH. Absorption at 3034 and 2917 cm^{-1} corresponds to pyrimidine NH/NH and an absorption at 1684 and 1205 cm^{-1} corresponds to C=O and C=S stretching respectively. The ^{1}H NMR spectrum of **5a** has exhibited a singlet at δ 9.91 due to indole NH which is also D_2O exchangeable and peaks at δ 9.10 and 9.20 were assigned to pyrimidine NH/NH. A singlet at δ 7.20, doublet at δ 7.32, doublet at δ 7.50 and multiplet between δ 7.65 and 7.82 ppm corresponds to eight aromatic protons present in the molecule. Peaks at δ 2.55 and 7.96 are assigned for the methyl and vicinal protons respectively. ^{13}C NMR spectrum of **5a** has displayed downfield signals at δ 168 integrated for the C=S flanked by NH of pyrimidine ring, less deshielded peak at δ 157 for two symmetric carbonyls and δ 29 integrated for methyl carbon. The mass spectrum of compound **5a** has shown molecular ion peak at *m/z* 362 $[M+1]^+$. The above spectral data supports the formation of compound **5a**. Spectral data of compounds **5b** & **5c** also supported their structures.

In the IR spectrum of (*Z*)-5-((5-methyl-2-phenyl-1*H*-indol-3-yl) methylene) thiazolidine-2,4-dione (**7a**), an absorption at 3450 cm^{-1} corresponds to indole NH. Absorption at 3134 cm^{-1} corresponds to thiazolidinedione NH and an absorption at 1627 and 1574 cm^{-1} corresponds to carbonyl stretching. The ^1H NMR spectrum of **7a** has exhibited a singlet at δ 11.40 due to indole NH which is also D_2O exchangeable and peak at δ 09.84 is assigned to thiazolidinedione NH. A singlet at δ 7.00, doublet at δ 7.15, doublet at δ 7.27 and multiplet between δ 7.40 and 7.55 ppm corresponds to eight aromatic protons present in the molecule. Peaks at δ 2.60 and 7.70 are assigned for the methyl and vicinal protons respectively. ^{13}C NMR Spectrum of **7a** has displayed a downfield signals at δ 165 and less deshielded peak at δ 159 integrated for C═O of thiazolidine-dione ring and δ 26 integrated for methyl carbon. The mass spectrum of compound **7a** has shown molecular ion peak at *m/z* 335 $[M+1]^+$. The above spectral data supports the formation of compound **7a**. Spectral data of compounds **7b** & **7c** also supported their structures.

The IR spectrum of (4*E*)-3-methyl-4-((5-methyl-2-phenyl-1*H*-indol-3-yl) methylene)-1*H*-pyrazol-5(4*H*)-one (**9a**) has shown a strong absorption at 3195 cm^{-1} corresponding to indole NH. Absorption at 3050 cm^{-1} corresponds to pyrazolone NH and absorption at 1675 cm^{-1} corresponds to carbonyl stretching. ^1H NMR spectrum of **9a** has exhibited singlets at δ 11.50 and 09.90 due to indole NH and pyrazolone NH respectively, which are D_2O exchangeable. A singlet at δ 7.00, doublet at δ 7.25, doublet at δ 7.45 and multiplet between δ 7.64 and 8.00 ppm corresponds to eight aromatic protons present in the molecule. Peaks at δ 2.40, 2.00 and 8.20 are assigned for the methyl, methyl and vicinal protons respectively. ^{13}C NMR Spectrum of **9a** has displayed a downfield signal at δ 163 for carbonyl carbon and δ 23 and 20 integrated for methyl carbons. The mass spectrum of compound **9a** has shown molecular ion peak at *m/z* 316 $[M+1]^+$. The above spectral data supports the formation of compound **9a**. Spectral data of compounds **9b** & **9c** also supported their structures.

3. Results and discussion

3.1. Biological activities

There are some evidences to show that melatonin and other indole derivatives may reduce incidence and the growth of tumors. The known neuroprotective action of melatonin may be due to its antioxidant activity [31]. To understand the antioxidant activity and the role played by the 2,5-disubstituted indole analogues, we have designed and synthesized a series of new indole analogues and evaluated for their activity.

3.1.1. Antioxidant activities

3.1.1.1. Free radical scavenging activity. Free radical scavenging is one of the best known mechanisms by which antioxidants inhibit lipid oxidation. The newly synthesized compounds were screened for free radical scavenging activity by DPPH method [32]. Samples were prepared at concentrations of 10, 50, and 100 μg/100 μl and Butylated hydroxy anisole (BHA) is taken as standard. The incorporation of different heterocycles in to 2,5-disubstituted indole system demonstrated broad spectrum of results. Among the tested compounds, indolylthiazolidinedione **7b**, **7c** and indolylpyrazolone derivatives **9b**, **9c** have shown very good scavenging activity. Whereas, indolylpyrimidine **3b**, **3c** and indolylthioxopyrimidine derivatives **5b**, **5c** have shown moderate activity. The increased activity (**7b**, **7c**, **9b** and **9c**) may be due to the existence of 'Cl' and 'H' substitutions at the fifth position of the indole ring. In contrast, methyl substituted indolyl systems **3a**, **5a**, **7a** and **9a** have shown least activity compared with the standard. Further, the synthesized compounds scavenged the DPPH radical in a concentration dependent manner. Results of percentage of free radical scavenging activity are shown in Fig. 1.

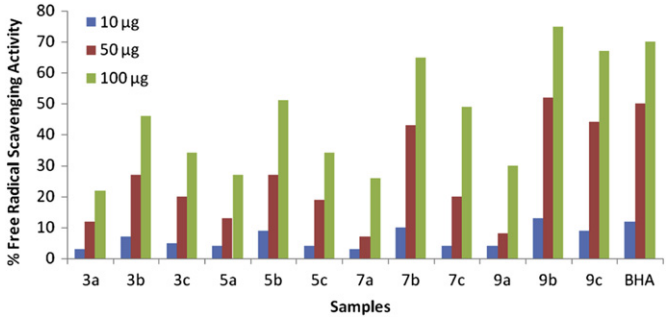

Fig. 1. Free radical scavenging activity of novel indole analogues.

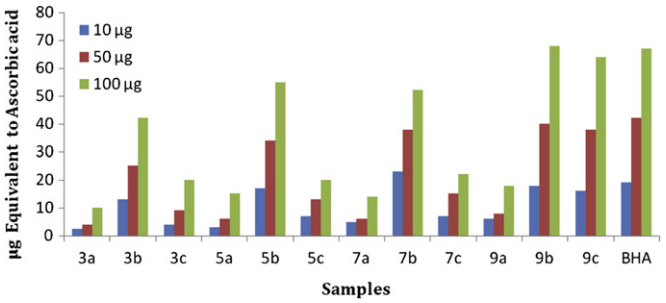

Fig. 2. Total antioxidant capacity of novel indole analogues.

3.1.1.2. Total antioxidant capacity. In total antioxidant activity [33], antioxidant capacities are expressed as equivalents of ascorbic acid. Among the tested indolyl systems, some of the ring systems have shown positive tendency towards the total antioxidant capacity. Pyrazolone analogues have shown strong antioxidant capacity. Whereas, thiazolidinedione, pyrimidine and thioxopyrimidine derivatives have shown moderate to least activity. It was also observed that, the 'Cl' substitution at the 5th position of the indole ring in **3b**, **5b**, **7b** and **9b** is responsible for the improved antioxidant capacity. In contrast, 'CH_3' and 'H' substitutions have shown fewer tendencies. Hence, 5-chloro substituted indolylpyrazolone ring system has emerged as most potent analogue (Fig. 2).

3.1.1.3. Ferric reducing antioxidant power activity. The compounds are also screened for ferric reducing antioxidant activity [34]. Butylated hydroxy anisole (BHA) was used as reference standard. The synthesized compounds reduced Fe^{+3} cations in a concentration dependent manner. Screening results indicated that all the new indole analogues were active ferric reducing antioxidant agents, with varying degrees of potency. As in the previous cases, indolylpyrazolone derivatives **9b** and **9c** have shown excellent

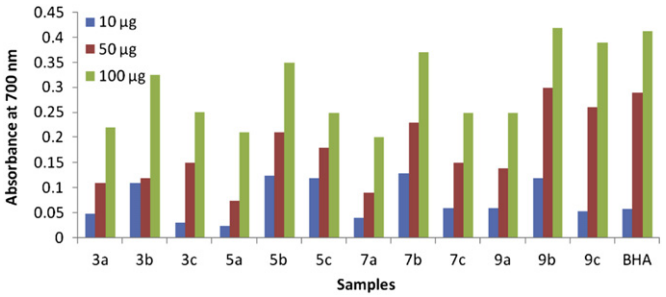

Fig. 3. Ferric reducing antioxidant activity of novel indole analogues.

Table 2
In vitro cytotoxicity of novel indole analogues against various cancer cell lines.

Compound	IC_{50} (μM)		
	A-549 (Lung carcinoma)	HEp-2 (Laryngeal carcinoma)	HeLa (Cervical carcinoma)
3a	NA	NA	NA
3b	27.0	NA	30.0
3c	77.4	NA	74.0
5a	NA	NA	NA
5b	27.5	45.7	23.5
5c	52.3	NA	63.0
7a	NA	NA	NA
7b	4.50	12.1	7.00
7c	87.7	NA	60.7
9a	07.7	26.3	NA
9b	1.00	7.90	2.10
9c	3.40	1.20	33.1
Doxorubicine	0.70	8.70	0.71

NA—Not active and having $IC_{50} > 100$ μM.

ferric reducing activity. Thiazolidinedione, pyrimidine and thioxopyrimidine derivatives have shown moderate to high activity. Alongside, 'Cl' and 'H' substitutions on indole ring have a high impact in improving the activity. Therefore, results clearly signify, the incorporation of methyl substituted pyrazolone ring may play an important role to act as a better ferric reducing antioxidant agent. The results are presented in Fig. 3.

3.1.2. In vitro cytotoxic studies

Evaluation of antitumor cytotoxicity for the synthesized compounds was performed [35] against three different tumor cell lines, A-549 (Lung carcinoma), HEp-2 (Laryngeal carcinoma) and HeLa (Cervical carcinoma) by MTT assay with Doxorubicine as positive reference, the results are presented in Table 2. As can be seen from Table 2, of all the synthesized compounds, **9b** has shown effective cytotoxicity against all the three cell lines HEp-2 (IC_{50} 7.90 μM), A-549 (IC_{50} 1.00 μM) and HeLa (IC_{50} 2.10 μM). Compound **9c** has shown profound cytotoxic activity against HEp-2 (IC_{50} 1.20 μM) and A-549 (IC_{50} 3.40 μM) but failed to show strong tendency against HeLa. Interestingly, 5-Cl and 5-H substituted analogues **9b** and **9c** against HEp-2 have their IC_{50} values falling in the range 1.20–7.90 with better activity than that of the drug Doxorubicine (IC_{50} 8.70). Compound **7b** against all the three cell lines HEp-2 (IC_{50} 12.1 μM), A-549 (IC_{50} 4.50 μM), HeLa (IC_{50} 7.00 μM) and compound **9a** against A-549 (IC_{50} 7.70 μM), HEp-2 (IC_{50} 26.3 μM) have shown moderate cytotoxicity. In contrast, compounds **3b**, **3c**, **5b**, **5c** and **7c** have shown least activity and compounds **3a**, **5a** and **7a** have failed to show any tendency against all the three cell lines. The results clearly signify, indolylpyrazolone system shown markedly strong cytotoxicity against all the three cell lines when compared with other indolyl ring systems. Whereas, the compounds with '5-Cl' and '5-H' substitution has shown some tendency to improve the activity when compared with other substituted indolyl analogues.

4. Conclusions

In conclusion, we have developed a rapid, expedient and environmentally benign microwave assisted Knoevenagel condensation to produce novel indole analogues and evaluated for pharmacological activities. Compounds **9b** and **9c** with indolylpyrazolone system are emerged as most potent analogues by showing strong cytotoxic and antioxidant activities. With these results, it is observed that the pyrazolone ring in the indolylpyrazolone system has enhanced the activity. Consequently, our findings will endow with a great impact on chemists and biochemists for assisting investigations in the indole field in search of novel and greener methodologies.

5. Experimental protocols

5.1. Chemistry

All the chemicals and reagents were purchased from MERCK, Himedia and SD fine chemical companies and are used without further purification. Melting points of the synthesized compounds are determined in open capillaries and are uncorrected. Reactions are monitored by thin-layer chromatography (TLC) on silica gel 60 F_{254} aluminium sheets (MERCK). The mobile phase was chloroform and benzene (3:1) and detection was made using UV light and iodine. IR spectra are recorded in KBr on Perkin–Elmer and FTIR Spectrophotometer (ν_{max} in cm^{-1}). ^1H NMR and ^{13}C NMR spectra recorded on BRUKER AVENE II NMR Spectrometer (Chemical shift in δ ppm downfield from TMS as an internal reference). The Mass spectra are recorded on LC-MSD-Trap-SL instrument. The elemental analysis was determined on FLASH EA 1112 SERIES instrument. All the compounds gave C, H and N analysis within ±0.5% of the theoretical values. Microwave reactions carried out in ONIDA 20STP21 800W multimode microwave oven.

*5.1.1. Typical experimental procedure for the synthesis of 2,5-disubstituted indole-3-carboxaldehydes (**1a**–**c**)*

The precursors 2,5-disubstituted indole-3-carboxaldehydes (**1a**–**c**) were obtained from the Vilsmeier-Haack formylation reaction of 2,5-disubstituted indoles [36].

*5.1.2. Preparation of thiazolidine-2,4-dione (**6**)*

Thiourea (0.048 mol) was added to a stirred solution of chloroacetic acid (0.05 mol) in HCl (8.00 mL, 36%) and the reaction mixture was heated under reflux for 14 h. On cooling the product was precipitated, filtered, dried and crystallized to get thiazolidine-2,4-dione (6); melting point (mp) 122–124 °C [37].

*5.1.3. Preparation of 3-methyl-1H-pyrazol-5(4H)-one (**8**)*

Ethylacetoacetate (0.01 mol) in ethanol (10–15 mL) was cyclised with hydrazine hydrate (0.01 mol) by stirring at room temperature for about 2–3 h. Solid separated out was filtered washed with ethanol and recrystallized from ethanol; melting point (mp) 209–211 °C.

*5.1.4. General procedure for the Knoevenagel condensation products (**3a**–**c**), (**5a**–**c**), (**7a**–**c**) and (**9a**–**c**)*

5.1.4.1. Conventional method. A mixture of 2,5-disubstituted indole-3-carboxaldehyde (**1a**–**c**) (1 mmol), active methylene compound (**2**/**4**/**6**/**8**) (1 mmol) and anhydrous sodium acetate (1 mmol) in acetic acid (6–7 mL) were refluxed for a given time (Table 1). After completion (TLC) the reaction mixture was allowed to cool at room temperature and poured into crushed ice with constant stirring. Crude product was isolated and recrystallized from ethanol to yield target compounds with 61–75% yield (Table 1).

5.1.4.2. Microwave assisted synthesis

5.1.4.2.1. A) Neat reaction. Mixture of 2,5-disubstituted indole-3-carboxaldehyde (**1a**–**c**) (1 mmol) and active methylene compound (**2**/**4**/**6**/**8**) (1 mmol) were powdered, mixed and introduced in an open borosil glass vessel (to decrease internal pressure). This was subjected to microwave irradiation for 10 min with 50% microwave power (Table 1). After completion (TLC) the reaction mixture was brought to room temperature, washed with

aqueous ethanol and dried. The product obtained was very low yields. In some cases reaction did not occur.

5.1.4.2.2. B) Neat with NH_4OAc. Mixture of 2,5-disubstituted indole-3-carboxaldehyde (**1a**–**c**) (1 mmol), active methylene compound (**2**/**4**/**6**/**8**) (1 mmol) and NH_4OAc (1 mmol) were powdered, mixed and introduced in an open borosil glass vessel. This was subjected to microwave irradiation for 5–10 min with 50% microwave power (Table 1). After completion (TLC) the reaction mixture was brought to room temperature, washed with aqueous ethanol and dried. The crude product was recrystallised to get the title compound which was found to be in good purity (TLC) and yield (Table 1).

*5.1.4.3. 5-((5-Methyl-2-phenyl-1H-indol-3-yl)methylene)pyrimidine-2,4,6(1H,3H,5H)-trione (**3a**).* Yield 67% (Ethanol); mp 216–218 °C; IR (KBr) (ν_{max} in cm^{-1}) : 3444, 3140, 3052, 1689, 1575; ^1H NMR (DMSO-d6 + $CDCl_3$) δ (ppm): 11.05 (s, 1H, indole NH), 10.08 (s, 1H, pyrimidine NH), 10.06 (s, 1H, pyrimidine NH), 7.10 (s, 1H, indole Ar–H), 7.25 (d, 1H, $J = 7.8$, indole Ar–H), 7.35 (d, 1H, $J = 7.8$, indole Ar–H), 7.50–7.65 (m, 5H, Ph Ar–H), 7.80 (s, 1H, –CH=), 2.57 (s, 3H, CH_3); ^{13}C NMR(DMSO-d6 + $CDCl_3$) δ (ppm): 164.1(C=O), 154.5(C=O) and 148.9, 136.9, 134.8, 132.1, 129.7, 128.8, 126.6, 123.2, 121.7, 116.5, 114.1, 105 and 32; MS: $m/z = 346$ $[M+1]^+$. Anal. Calcd. for $C_{20}H_{15}N_3O_3$ (345): C, 69.56; H, 4.38; N, 12.17%. Found: C, 69.58; H, 4.26; N, 12.20%.

*5.1.4.4. 5–((5-Chloro-2-phenyl-1H-indol-3-yl)methylene)pyrimidine-2,4,6(1H,3H,5H)-trione (**3b**).* Yield 61% (Ethanol): mp 230–232 °C; IR (KBr) (ν_{max} in cm^{-1}): 3431, 3156, 2916, 1685, 1584; ^1H NMR (DMSO-d6 + $CDCl_3$) δ (ppm): 11.38 (s, 1H, indole NH), 10.35 (s, IH, pyrimidine NH), 10.28 (s, IH, pyrimidine NH), 6.90 (s, 1H, indole Ar–H), 7.12 (d, 1H, $J = 7.8$, indole Ar–H), 7.37 (d, 1H, $J = 7.8$, indole Ar–H), 7.55–7.76 (m, 5H, Ph Ar–H), 7.90 (s, 1H, –CH=); MS: $m/z = 366$ $[M+1]^+$, 368 $[M+3]^+$ (3:1); Anal. Calcd. for $C_{19}H_{12}ClN_3O_3$ (365): C, 62.39; H, 3.31; N, 09.69%. Found: C, 62.42; H, 3.37; N, 09.55%.

*5.1.4.5. 5-((1H-Indol-3-yl)methylene)pyrimidine-2,4,6(1H,3H,5H)-trione (**3c**).* Yield 62% (Ethanol): mp 192–194 °C; IR (KBr) (ν_{max} in cm^{-1}): 3429, 2918, 2849, 1677, 1626; ^1H NMR (DMSO-d6 + $CDCl_3$) (δ ppm): 11.30 (s, 1H, indole NH), 09.80 (s, 1H. pyrimidine NH), 09.70 (s, 1H, pyrimidine NH), 7.12 (d, 1H, $J = 7.7$, indole Ar–H), 7.33 (d, 1H, $J = 7.7$, indole Ar–H), 7.50–7.70 (m, 2H, indole Ar–H), 7.82 (s, 1H, indole Ar–H), 8.00 (s, 1H, –CH=); MS: $m/z = 256$ $[M+1]^+$. Anal. Calcd. for $C_{13}H_9N_3O_3$ (255): C, 61.18; H, 3.55; N, 16.46%. Found: C, 61.25; H, 3.52; N, 16.67%.

*5.1.4.6. 5-((5-Methyl-2-phenyl-1H-indol-3-yl)methylene)-dihydro-2-thioxopyrimidine-4,6(1H,5H)-dione (**5a**).* Yield 65% (Ethanol): mp 232–234 °C; IR (KBr) ν_{max} in cm^{-1} : 3433, 3034, 2917, 1684, 1205; ^1H NMR (DMSO-d6 + $CDCl_3$) in δ ppm: 9.91 (s, 1H, indole NH), 9.10 (s, 1H, pyrimidine NH), 9.20 (s, 1H, pyrimidine NH), 7.20 (s, 1H, indole Ar–H), 7.32 (d, 1H, $J = 7.8$, indole Ar–H), 7.50 (d, 1H, $J = 7.8$, indole Ar–H), 7.65–7.82 (m, 5H, Ph Ar–H), 7.96 (s, 1H, –CH=), 2.55 (s, 3H, CH_3); ^{13}C NMR(DMSO-d6 + $CDCl_3$) in δ ppm: 168.7 (C=S), 157.0 (C=O) and 152.0, 140.7, 137.3, 136.4, 134.0, 133.1, 132.8, 130.7, 127.8, 121.1, 119.0, 116.4, 106.6 and 29.0; MS: $m/z = 362$ $[M+1]^+$. Anal. Calcd. for $C_{20}H_{15}N_3O_2S$ (361): C, 66.46; H, 4.18; N, 11.63%. Found C, 66.52; H, 4.22; N, 11.71%.

*5.1.4.7. 5-((5-Chloro-2-phenyl-1H-indol-3-yl)methylene)-dihydro-2-thioxopyrimidine-4,6(1H,5H)-dione (**5b**).* Yield 65% (Ethanol): mp 215–216 °C; IR (KBr) (ν_{max} in cm^{-1}): 3132, 3085, 2978, 1700, 1214; ^1H NMR (DMSO-d6 + $CDCl_3$) in δ ppm: 10.80 (s, 1H, indole NH), 9.50 (s, 1H, pyrimidine NH), 9.55 (s, 1H, pyrimidine NH),7.20 (s, 1H, indole Ar–H), 7.26 (d, 1H, $J = 7.8$, indole Ar–H), 7.40 (d, 1H, $J = 7.8$, indole Ar–H), 7.57–7.65 (m, 5H, Ph Ar–H), 7.70 (s, 1H, –CH=); MS: $m/z = 382$ $[M+1]^+$ 384 $[M+3]^+$ (3:1). Anal. Calcd. for $C_{19}H_{12}ClN_3O_2S$ (381): C, 59.76; H, 3.17; N, 11.00%. Found: C, 59.77; H, 3.25; N, 11.06%.

5.1.4.8. 5-((1H-Indol-3-yl)methylene)-dihydro-2-thioxopyrimidine-4,6(1H,5H)-dione (5c). Yield 65% (Ethanol): mp 176–178 °C; IR (KBr) (ν_{max} in cm^{-1}): 3411, 2926, 2852, 1719, 1207; ^1H NMR (DMSO-d6 + $CDCl_3$) in δ ppm: 11.17 (s, 1H, indole NH), 09.99(s, 1H, pyrimidine NH), 09.90(s, 1H, pyrimidine NH), 7.20 (d, 1H, $J = 7.7$, indole Ar–H), 7.35 (d, 1H, $J = 7.7$, indole Ar–H), 7.52–7.70 (m, 2H, indole Ar–H), 7.80 (s, 1H, indole Ar–H), 7.90 (s, 1H, –CH=); MS: $m/z = 272$ $[M+1]^+$. Anal. Calcd. for $C_{13}H_9N_3O_2S$ (271): C, 57.55; H, 3.34; N, 15.49%. Found: C, 57.48; H, 3.37; N, 15.65%.

*5.1.4.9. (Z)-5-((5-Methyl-2-phenyl-1H-indol-3-yl)methylene)thiazolidine-2,4-dione (**7a**).* Yield 70% (Ethanol): mp 184–186 °C; IR (KBr) (ν_{max} in cm^{-1}): 3450, 3134, 3065, 1627, 1574; ^1H NMR (DMSO-d6 + $CDCl_3$) in δ ppm: 11.40 (s, 1H, indole NH), 09.84 (s, 1H, thiazolidinedione NH), 7.00 (s, 1H, indole Ar–H), 7.15 (d, 1H, $J = 7.6$, indole Ar–H), 7.27 (d, 1H, $J = 7.6$, indole Ar–H), 7.40–7.55 (m, 5H, Ph Ar–H), 7.70 (s, 1H, –CH=), 2.60 (s, 3H, CH_3); ^{13}C NMR(DMSO-d6 + $CDCl_3$) in δ ppm: 165.6 (C=O), 159.7 (C=O) and 139.9, 136.6, 135.5, 133.3, 132.8, 131.5, 129.6, 126.7, 121.3, 118.7, 116.7, 105.0 and 26; MS: $m/z = 335$ $[M+1]^+$. Anal. Calcd. for $C_{19}H_{14}N_2O_2S$ (334): C, 68.24; H, 4.22; N, 08.38%. Found: C, 68.30; H, 4.30; N, 8.40%.

*5.1.4.10. (Z)-5-((5-Chloro-2-phenyl-1H-indol-3-yl)methylene)thiazolidine-2,4-dione (**7b**).* Yield 68% (Ethanol); mp 195–197 °C; IR (KBr)(ν_{max} in cm^{-1}): 3334, 3127, 2917, 1632, 1583; ^1H NMR (DMSO-d6 + $CDCl_3$) in δ ppm: 11.51 (s, 1H, indole NH), 09.91 (s, 1H, thiazolidinedione NH), 7.20 (s, 1H, indole Ar–H), 7.27 (d, 1H, $J = 7.6$, indole Ar–H), 7.41 (d, 1H, $J = 7.6$, indole Ar–H), 7.55–7.67 (m, 5H, Ph Ar–H), 7.86 (s, 1H, –CH=); MS: $m/z = 355$ $[M+1]^+$, 357 $[M+3]^+$ (3:1), Anal. Calcd. for $C_{18}H_{11}ClN_2O_2S$ (354): C, 60.93; H, 3.12; N, 7.90%. Found: C, 60.98; H, 3.24; N, 7.87%.

*5.1.4.11. (Z)-5-((1H-Indol-3-yl)methylene)thiazolidine-2,4-dione (**7c**).* Yield 70% (Ethanol); mp 218–220 °C; IR (KBr) (ν_{max} in cm^{-1}): 3338, 3027, 2916, 1637, 1567; ^1H NMR (DMSO-d6 + $CDCl_3$) in δ ppm: 10.71 (s, IH, indole NH), 09.50 (s, 1H, thiazolidinedione NH), 6.50 (d, 1H, $J = 7.5$, indole Ar–H), 6.64 (d, 1H, $J = 7.5$, indole Ar–H), 6.77–6.95 (m, 2H, indole Ar–H), 7.12 (s, 1H, indole Ar–H), 7.40 (s, 1H, –CH=); MS: $m/z = 245$ $[M+1]^+$. Anal. Calcd. for $C_{12}H_8N_2O_2S$ (245): C, 59.00; H, 3.30; N, 11.47%. Found: 59.12; H, 3.28; N, 11.56%.

*5.1.4.12. (4E)-3-Methyl-4-((5-methyl-2-phenyl-1H-indol-3-yl)methylene)-1H-pyrazol-5(4H)-one (**9a**).* Yield 75% (Ethanol): mp 235–237 °C; IR (KBr) (ν_{max} in cm^{-1}): 3195, 3050, 2950, 1675, 1600; ^1H NMR (DMSO-d6 + $CDCl_3$) in δ ppm: 11.50 (s, 1H, indole NH), 09.90 (s, 1H, pyrazolone NH), 7.00 (s, 1H, indole Ar–H), 7.25 (d, 1H, $J = 7.6$, indole Ar–H), 7.45 (d, 1H, $J = 7.6$, indole Ar–H), 7.64–8.00 (m, 5H, Ph Ar–H), 8.20 (s, 1H, –CH=), 2.00 (s, 3H, CH_3), 2.40 (s, 3H, CH_3); ^{13}C NMR(DMSO-d6 + $CDCl_3$) in δ ppm: 163.7 (C=O) and 145.3, 141.5, 133.9, 131.2, 130.3, 129.6, 128.3, 127.6, 126.7, 125.7, 124.5, 123.2, 115.7, 114.2, 104.7, 23.4 and 20.0; MS: $m/z = 316$ $[M+1]^+$. Anal. Calcd. for $C_{20}H_{17}N_3O$ (315): C, 76.17; H, 5.43; N, 13.32%. Found: C, 76.10; H, 5.40; N, 13.40%.

*5.1.4.13. (4E)-4-((5-Chloro-2-phenyl-1H-indol-3-yl)methylene)-3-methyl-1H-pyrazol-5(4H)-one (**9b**).* Yield 66% (Ethanol); mp 270–272 °C; IR (KBr)(ν_{max} in cm^{-1}): 3200, 3150, 2900, 1657, 1587; ^1H NMR (DMSO-d6 + $CDCl_3$) in δ ppm: 11.70 (s, 1H, indole NH), 09.70 (s, 1H, pyrazolone NH), 7.00 (s, 1H, indole Ar–H), 7.22 (d, 1H, $J = 7.6$,

indole Ar–H), 7.41 (d, 1H, $J = 7.6$, indole Ar–H), 7.63–7.87 (m, 5H, Ph Ar–H), 8.10 (s, 1H, –CH=), 2.60 (s, 3H, CH_3); MS: $m/z = 337$ $[M+2]^+$, 339 $[M+4]^+$ (3:1), Anal. Calcd. for $C_{19}H_{14}ClN_3O$ (335): C, 67.96; H, 4.20; N, 12.51%. Found: C, 67.92; H, 4.24; N, 12.57%.

*5.1.4.14. (4E)-4-((1H-Indol-3-yl)methylene)-3-methyl-1H-pyrazol-5(4H)-one (**9c**).* Yield 70% (Ethanol); mp 202–204 °C; IR (KBr) (ν_{max} in cm^{-1}): 3190, 2995, 2850, 1660, 1571; ^1H NMR (DMSO-d6 + $CDCl_3$) in δ ppm: 11.10 (s, IH, indole NH), 09.80 (s, 1H, pyrazolone NH), 6.70 (d, 1H, $J = 7.5$, indole Ar–H), 6.84 (d, 1H, $J = 7.5$, indole Ar–H), 7.00–7.37 (m, 2H, indole Ar–H), 7.45 (s, 1H, indole Ar–H), 7.55 (s, 1H, –CH=), 2.6 (s, 3H, CH_3); MS: $m/z = 226$ $[M+1]^+$. Anal. Calcd. for $C_{13}H_{11}N_3O$ (225): C, 69.32; H, 4.92; N, 18.66%. Found: 69.28; H, 4.89; N, 18.60%.

5.2. Biological activities

5.2.1. Antioxidant activities

5.2.1.1. Free radical scavenging activity. Free radical scavenging activity was done by DPPH method [32]. Different concentrations (10 μg, 50 μg and 100 μg) of samples and butylated hydroxy anisole (BHA) were taken in different test tubes. The volume was adjusted to 100 μl by adding MeOH. Five milliliters of 0.1 mM methanolic solution of DPPH was added to these tubes and shaken vigorously. The tubes were allowed to stand at 27 °C for 20 min. The control was prepared as above without any extract. The absorbance of samples were measured at 517 nm. Radical scavenging activity was calculated using the following formula:

$$\%\text{Radical scavenging activity} = [(\text{Control OD} - \text{Sample OD})/(\text{Control OD})] \times 100.$$

5.2.1.2. Total antioxidant capacity. Various concentrations of compounds (10 μg, 50 μg and 500 μg) were taken in a series of test tubes. To this, 1.9 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) was added. The tubes were incubated at 95 °C for 90 min and allowed to cool. The absorbance of each aqueous solution was measured at 695 nm against a blank. Antioxidant capacities are expressed as equivalents of ascorbic acid. Ascorbic acid equivalents are calculated using standard graph of ascorbic acid. The values are expressed as ascorbic acid equivalents in μg per mg of extract.

5.2.1.3. Ferric reducing antioxidant power. Various concentrations of compounds (10 μg, 50 μg and 500 μg) were mixed with 2.5 mL of 200 mmol/L sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide. The mixture was incubated at 50 °C for 20 min. Next, 2.5 mL of 10% trichloroacetic acid (*w/v*) were added. From this solution, 5 mL was mixed with 5 mL of distilled water and 1 mL of 0.1% ferric chloride and absorbance was measured spectrophotometrically at 700 nm. BHA was used as standard.

5.2.2. In vitro cytotoxic studies

5.2.2.1. Cell lines and culture condition. A-549 (Lung carcinoma), HEp-2 (Laryngeal carcinoma) and HeLa (Cervical carcinoma) cells were cultured in RPMI 1640 (Himedia, India) medium supplemented with 10% FCS and containing penicillin 100 U/mL and streptomycin 100 μg/mL at 37 °C in a CO_2 incubator with 5% CO_2.

5.2.2.2. MTT assay. Briefly, the test compounds were diluted in DMSO (0–100 μg/mL) and cytotoxic activity of the compounds onA-549, HEp-2 and HeLa cells (1×10^5 cells/well) were tested by using the cell quantity MTT cell viability assay kit. The wells with only culture medium served as control and the graph is plotted with cell viability against the time period in hours at increasing concentrations of secondary metabolite. The IC_{50} values were calculated by non-linear regression analysis from three independent experiments.

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