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Original article

Synthesis, antioxidant and DNA cleavage activities of novel indole derivatives

J.S. Biradar*, B.S. Sasidhar, R. Parveen

Central Research Lab, Department of Chemistry, Gulbarga University, Gulbarga, 585106 Karnataka, India

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ABSTRACT

A new series of novel indole derivatives containing barbitone moiety (**5a**-**i**) are synthesized by simple and efficient condensation of chalcones (**3a**-**i**) with barbituric acid (**4**). The synthesized compounds are screened for their antioxidant (free radical scavenging, total antioxidant capacity and ferric reducing antioxidant power) and DNA cleavage activities were evaluated. Among the synthesized compounds (**5a**), (**5d**) and (**5g**) exhibited excellent antioxidant activity and all the tested compounds in the series have exhibited promising DNA cleavage activities. The structures of the synthesized compounds are assigned on the basis of elemental analysis, IR, ^{1}H NMR, ^{13}C NMR and mass spectral data.

1. Introduction

There is increasing evidence of the implication of free radicals and reactive oxygen species in a variety of diseases and pathophysiological events including inflammation, cancer, myocardial infraction, arthritis and neurodegenerative disorders [1–3]. The serious consequence of free radical action on biological systems is a multiple complex aspects of their intervention in a series of inflammatory [4] and nutritional diseases [5,6]. Many anti-inflammatory agents are known to act through the scavenging of oxygen radicals [7,8]. Deoxyribonucleic acid (DNA) is the primary target molecule for most anticancer and antiviral therapies [9,10]. The design of molecules that can bind with DNA provides a formidable opportunity for chemist with molecules which can offer new prospects for controlled manipulation of genome.

Indole derivatives constitute an important class of therapeutic agents in medicinal chemistry including anticancer [11], antioxidant [12], antirheumatoidal and anti-HIV [13,14] and also play a vital role in the immune system [16,17]. Many indole derivatives are considered as the most potent scavenger of free radicals [15]. Artificial receptors for biologically active molecules have attracted attention from the viewpoint of molecular recognition [18]. Derivatives of barbituric acid are important members of the pyrimidine family, as they are suitable for this purpose. Further many barbituric acid derivatives are known to possess a wide range of activities, such as hypnotics [19–22]

It was envisaged that the two pharmacophores if linked together (Scheme 1) would generate novel molecular templates which are likely to exhibit interesting biological properties in animal models. Owing to the importance and in continuation of our work on synthesis of biologically active compounds [23–25], we focused on the investigation of antioxidant properties of pharmacologically active compounds and several experimental protocols have been developed for this purpose and excellent results have been obtained.

2. Chemistry

A typical synthetic strategy employed to obtain the title compounds (**5a**–**i**) in excellent yields is depicted in Scheme 1. In the present investigation, substituted indole-3-carboxaldehydes (**1a**–**c**) were obtained from 2,5-disubstituted indoles formed by Bischler's method were subjected to formylation under Vilsmeier–Haack reaction conditions with phosphorous oxychloride and dimethylformamide [26]. Various substituted indole-3-carboxaldehydes (**1a**-**c**) were reacted with substituted acetophenones (**2a**–**c**) in basic media to get chalcones (**3a**–**i**) by reported method [27]. The condensation of chalcones (**3a**–**i**) with barbituric acid in acetic acid gave (**5a**–**i**) [28]. All the newly synthesized compounds were characterized by IR, ^{1}H NMR, ^{13}C NMR and mass spectroscopic data. The IR spectrum of 5-(3-(1H-indol-3-yl)-1-phenylallylidene) pyrimidine-2,4,6(1H,3H,5H)-trione (**5a**) showed a strong absorption

* Corresponding author. Tel.: +91 8472 263292; fax: +91 8472 248632.
E-mail address: jsbiradar@yahoo.com (J.S. Biradar).

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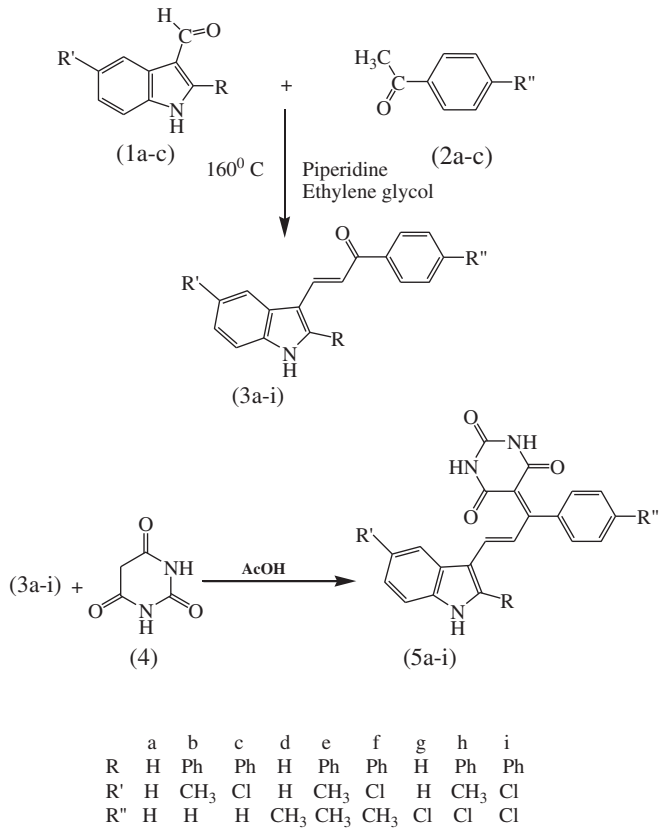


	a	b	c	d	e	f	g	h	i
R	H	Ph	Ph	H	Ph	Ph	H	Ph	Ph
R'	H	CH_3	Cl	H	CH_3	Cl	H	CH_3	Cl
R"	H	H	H	CH_3	CH_3	CH_3	Cl	Cl	Cl

Scheme 1. Schematic representation for the synthesis of novel indole derivatives from barbituric acid.

at 3408 cm^{-1} corresponding to indole NH, absorption at 2918 and 2849 cm^{-1} corresponds to pyrimidine NH/NH, and absorption at 1685 cm^{-1} corresponds to carbonyl stretching. The ^1H NMR spectrum of (**5a**) has exhibited a singlet at δ 11.19 due to indole NH which is also D_2O exchangeable. Peaks at δ 10.72 and 10.69 were assigned to pyrimidine NH/NH and doublet for vicinal protons has merged with aromatic multiplet between δ 6.7 and 8.0 ppm. The mass spectrum of compound **5a** has shown molecular ion peak at m/z 359.3 [m + 2]. The various new indole derivatives synthesized during the present investigation are listed in Table 1.

3. Results and discussion

3.1. Biological activities

All the newly synthesized indole derivatives (**5a**–**i**) are screened for their biological activities such as antioxidant (free radical scavenging, total antioxidant capacity and ferric reducing antioxidant power) and DNA cleavage activities were studied by agarose gel electrophoresis method.

3.1.1. Antioxidant activities

3.1.1.1. Free radical scavenging activity. The newly synthesized compounds were screened for free radical scavenging activity by DPPH method [29]. The samples were prepared at concentrations of 10, 50, and 100 μg/100 μl, and butylated hydroxy anisole (BHA) is taken as standard. Simple indole derivatives, **5a**, **5d** and **5g** have very good scavenging activity. Chloro-substituted indole derivatives **5c**, **5f** and **5i** have shown moderate activities and methyl derivatives **5b**, **5e** and **5h** have shown least activity compared with the standard. The bar graph representation of percentage of free radical scavenging activity is shown in Fig. 1.

3.1.1.2. Total antioxidant capacity. Total antioxidant activity was performed to all the newly synthesized compounds [30]. Antioxidant capacities are expressed as equivalents of ascorbic acid. Among the tested compounds, **5a**, **5d** and **5g** which are simple indole derivatives have shown very good activity and remaining compounds shown less activity. The results of total antioxidant activity were shown in Fig. 2.

3.1.1.3. Ferric reducing antioxidant power activity. All the novel compounds were screened for ferric reducing antioxidant activity [31]. Butylated hydroxy anisole (BHA) was used as standard. Simple indole derivatives **5a**, **5d** and **5g** have shown more promising activity and other derivatives have shown moderate activities. The results are presented in Fig. 3.

3.2. DNA cleavage activity

The DNA cleavage activity was determined using gel electrophoresis by Sambrook et al. [32]. The pictures of the gels are presented in Fig. 4. The gel after electrophoresis clearly revealed that, all the tested compounds did act on the DNA as little tailing in the bands can be observed in treated samples. The difference was observed in bands of all the compounds compared to the control DNA. This shows that the control DNA alone does not show any apparent cleavage as the compounds did. With this, it can be concluded that the compound inhibits the growth of the pathogenic organism by cleaving the genome.

4. Conclusion

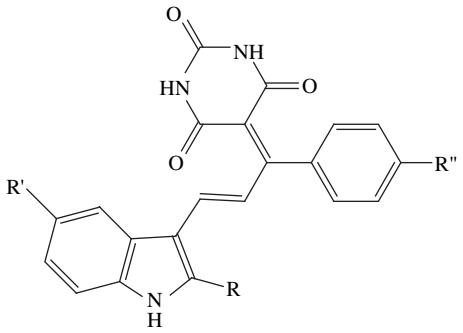
In conclusion, we have synthesized novel indole derivatives (**5a**–**i**) and evaluated these compounds for their antioxidant and DNA cleavage activities. Most of them demonstrated a broad spectrum of antioxidant activities. The simple indole derivatives **5a**, **5d** and **5g** were concluded as most potent derivatives in all the three cases. DNA cleavage studies revealed that the test compounds in the series have exhibited promising cleavage activity. With these excellent results, it can be concluded that the simple indole and chlorine at the fifth position of indole moiety enhanced the activity. Therefore, our findings will provide a great impact on chemists and biochemists for further investigations in the indole field in search of molecules possessing potent antioxidant and anticancer activities. Based on these results, selected novel compounds are being screened for anticancer activity which will be reported in due course.

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 (1:1) 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 on BRUKER AVENE II 400-MHz NMR Spectrometer (Chemical shift in δ ppm down field from TMS as an internal reference). The mass spectra are recorded on LC-MSD-Trap-SL instruments. 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.

Table 1
Physical and analytical data of the indole – baritone derivatives.



Entry	Product[a]	*R*	*R′*	*R″*	Yield[b] (%)	m.p.[c] (°C)	Mol. formula/Mol. Wt.	Elem. Analysis (Cal./Found)		
								C	H	N
1	**5a**	H	H	H	67	190–192	$C_{21}H_{15}N_3O_3$ 357	70.58 70.52	4.23 4.26	11.76 11.80
2	**5b**	Ph	CH_3	H	69	195–197	$C_{28}H_{21}N_3O_3$ 447	75.15 75.10	4.73 4.78	09.39 09.43
3	**5c**	Ph	Cl	H	57	200–203	$C_{27}H_{18}ClN_3O_3$ 467	69.31 69.26	3.88 3.84	08.98 09.07
4	**5d**	H	H	CH_3	61	167–169	$C_{22}H_{17}N_3O_3$ 371	71.15 71.13	4.61 4.64	11.31 11.29
5	**5e**	Ph	CH_3	CH_3	67	210–213	$C_{29}H_{23}N_3O_3$ 461	75.47 75.50	5.02 5.05	09.10 09.06
6	**5f**	Ph	Cl	CH_3	55	250–252	$C_{29}H_{20}ClN_3O_3$ 481	69.78 69.76	4.18 4.20	08.72 08.76
7	**5g**	H	H	Cl	56	272–274	$C_{21}H_{14}ClN_3O_3$ 391	64.37 64.40	3.60 3.54	10.72 10.70
8	**5h**	Ph	CH_3	Cl	68	243–245	$C_{28}H_{20}ClN_3O_3$ 481	69.78 69.74	4.18 4.21	08.72 08.75
9	**5i**	Ph	Cl	Cl	67	267–270	$C_{27}H_{17}Cl_2N_3O_3$ 502	64.55 64.50	3.41 3.45	08.36 08.39

[a] Products were characterized by IR, NMR, MS and elemental analysis.
[b] Isolated yields.
[c] Melting points are uncorrected.

*5.1.1. Typical experimental procedure for the synthesis of 2,5-substituted lindole-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.

*5.1.2. Experimental procedure for the synthesis of 3-(2,5-substituted-1H-indole-3-yl)-1-phenyl prop-2-en-1-one (**3a**–**i**)*

Various substituted indole-3-carboxaldehydes (**1a**–**c**) were reacted with substituted acetophenones (**2a**–**c**) in ethylene glycol with catalytic amount of piperidine to yield chalcones (**3a**–**i**) [27].

*5.1.3. General procedure for the synthesis of (**5a**–**i**)*

To a solution of (**3a**–**i**) (0.01 mol) in 6–7 mL acetic acid, barbituric acid (**4**) (0.01 mol) was added. The reaction mixture was then refluxed for 6–7 h. After completion (TLC) the reaction mixture was poured into crushed ice with constant stirring. Crude product was isolated and recrystallized from suitable solvents to yield target compounds (**5a**–**i**).

*5.1.3.1. 5-((E)-3-(1H-indol-3-yl)-1-phenylallylidene)pyrimidine-2,4,6 (1H,3H,5H)-trione (**5a**).* Yield 67% (Benzene): mp 190–192 °C; IR (KBr) ν_{max} in cm^{-1}: 3408, 2918, 2849 1685, 1589; ^1H NMR

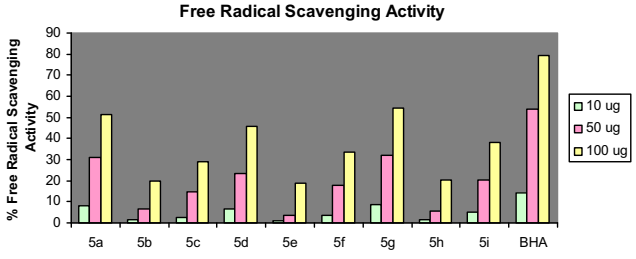


Fig. 1. Free radical scavenging activity of the synthesized compounds (**5a**–**i**).

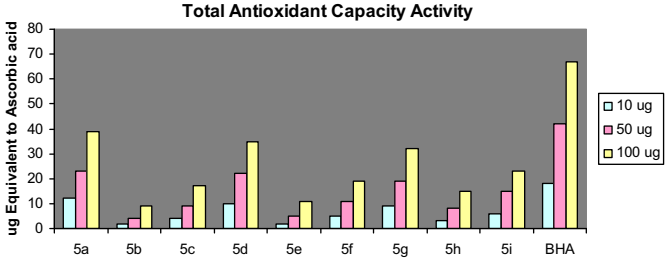


Fig. 2. Total antioxidant capacity of the compounds (**5a**–i).

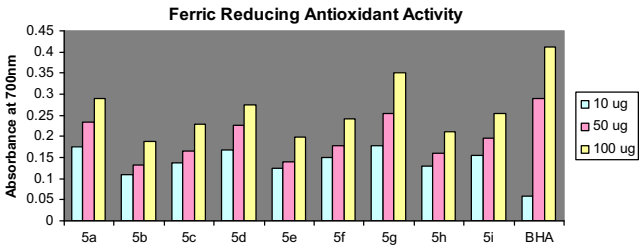


Fig. 3. Ferric reducing antioxidant activity of the compounds (**5a**–**i**).

(DMSO-d_6 + $CDCl_3$) in δ ppm: 11.19 (*s*, 1H, indole NH), 10.72 (*s*, 1H, pyrimidine NH), 10.69 (*s*, 1H, pyrimidine NH), 6.7–8.0 (*m*, 12H, 10Ar-H, 2CH=CH–); ^{13}C NMR(DMSO-d_6 + $CDCl_3$) in δ ppm: 163.1(C=O), 153.5(C=O) and 146.9, 134.9, 133.8, 131.1, 128.7, 127.8, 125.6, 122.2, 121.7, 120.0 and 115.1; MS: $m/z = 359.3$ $[M+2]^+$. Anal. Calcd. for $C_{21}H_{15}N_3O_3$: C, 70.58; H, 4.23; N, 11.76%. Found: C, 70.52; H, 4.26; N, 11.80%.

*5.1.3.2. 5-((E)-3-(5-methyl-2-phenyl-1H-indol-3-yl)-1-phenylallylidene)pyrimidine-2,4,6(1H,3H,5H)-trione (**5b**).* Yield 69% (Benzene): mp 195–197 °C; IR (KBr) ν_{max} in cm^{-1}: 3433, 3051, 2924, 1693, 1599; ^{1}H NMR (DMSO-d_6 + $CDCl_3$) in δ ppm: 11.2 (*s*, 1H, indole NH), 9.99 (*s*, IH, pyrimidine NH), 9.94 (*s*, IH, pyrimidine NH), 6.8–8.0 (*m*, 15H, 13Ar-H, 2CH=CH–), 2.4 (*s*, 3H, CH_3); MS: $m/z = 445.4$ $[M-2]^+$. Anal. Calcd. for $C_{28}H_{21}N_3O_3$: C, 75.15; H, 4.73; N, 09.39%. Found: C, 75.10; H, 4.78; N, 09.43%.

*5.1.3.3. 5-((E)-3-(5-chloro-2-phenyl-1H-indol-3-yl)-1-phenylallylidene)pyrimidine-2,4,6(1H,3H,5H)-trione (**5c**).* Yield 57% (benzene): mp 200–203 °C; IR (KBr) ν_{max} in cm^{-1}: 3296, 3063, 2916, 1693, 1643; ^{1}H NMR (DMSO-d_6 + $CDCl_3$) in δ ppm: 10.73 (*s*, 1H, indole NH), 10.43 (*s*, 1H, pyrimidine NH), 10.41 (*s*, 1H, pyrimidine NH), 6.7–8.6 (*m*, 15H, 13Ar-H, 2CH=CH–); MS: $m/z = 469.3$ $[M+2]^+$. Anal. Calcd. for $C_{27}H_{18}ClN_3O_3$: C, 69.31; H, 3.88; N, 08.98%. Found: C, 69.26; H, 3.84; N, 09.07%.

*5.1.3.4. 5-((E)-3-(1H-indol-3-yl)-1-p-tolylallylidene)pyrimidine-2,4,6(1H,3H,5H)trione (**5d**).* Yield 61% (Ethanol): mp 167–169 °C; IR (KBr) ν_{max} in cm^{-1}: 3408, 3055, 2918, 1685, 1597; ^{1}H NMR (DMSO-d_6 + $CDCl_3$) in δ ppm: 11.59 (*s*, 1H, indole NH), 9.89 (*s*, 1H, pyrimidine NH), 9.88 (*s*, 1H, pyrimidine NH), 7.1–8.2 (*m*, 11H, 9Ar-H, 2CH=CH–); ^{13}C NMR (DMSO-d_6 + $CDCl_3$) in δ ppm: 163.7 (C=O), 152.0 (C=O), 27.2 (CH_3) and 147.0, 134.7, 134, 1131.1, 128.5, 127.8, 127.5, 124.8, 122.1, 117.0 and 114.4; MS: $m/z = 372.3$ $[M+1]^+$. Anal. Calcd. for $C_{22}H_{17}N_3O_3$: C, 71.15; H, 4.61; N, 11.31%. Found C, 71.13; H, 4.64; N, 11.29%.

*5.1.3.5. 5-((E)-3-(5-methyl-2-phenyl-1H-indol-3-yl)-1-p-tolylallylidene)pyrimidine-2,4,6(1H,3H,5H)-trione (**5e**).* Yield 67% (Ethanol): mp 210–213 °C; IR (KBr) ν_{max} in cm^{-1}: 2957, 2918, 2849, 1697, 1591; ^{1}H NMR (DMSO-d_6 + $CDCl_3$) in δ ppm: 11.10 (*s*, 1H, indole NH), 9.75 (*s*, 1H, pyrimidine NH), 9.70 (*s*, 1H, pyrimidine NH), 7.1–8.2 (*m*, 14H, 12Ar-H, 2CH=CH–), 2.3 (s, 3H, CH_3); MS: $m/z = 460.5$ $[M-1]^+$. Anal. Calcd. for $C_{29}H_{23}N_3O_3$: C, 75.47; H, 5.02; N, 09.10%. Found: C, 75.50; H, 5.05; N, 09.06%.

*5.1.3.6. 5-((E)-3-(5-chloro-2-phenyl-1H-indol-3-yl)-1-p-tolylallylidene)pyrimidine-2,4,6(1H,3H,5H)-trione (**5f**).* Yield 55% (Ethanol): mp 250–252 °C; IR (KBr) ν_{max} in cm^{-1}: 2959, 2918, 2849, 1684, 1651; ^{1}H NMR (DMSO-d_6 + $CDCl_3$) in δ ppm: 11.01 (*s*, 1H, indole NH), 10.21(*s*, 1H, pyrimidine NH), 10.12 (*s*, 1H, pyrimidine NH), 7.0–8.1 (*m*, 14H, 12Ar-H, 2CH=CH–); MS: $m/z = 482.4$ $[M+1]^+$. Anal. Calcd. for $C_{29}H_{20}ClN_3O_3$: C, 69.78; H, 4.18; N, 08.72%. Found: C, 69.76; H, 4.20; N, 08.76%.

*5.1.3.7. 5(E)-1-(4-chlorophenyl)-3-1H-indol-3-yl) allylidene)pyrimidine-2,4,6 (1H,3H,5H)-trione (**5g**).* Yield 56% (Ethanol): mp 272–274 °C; IR (KBr) ν_{max} in cm^{-1}: 3027, 2976, 2849, 1684, 1604; ^{1}H NMR (DMSO-d_6 + $CDCl_3$) in δ ppm: 11.20 (*s*, 1H, indole NH), 10.20 (*s*, 1H, pyrimidine NH), 10.19 (*s*, 1H, pyrimidine NH), 7.1–7.5 (*m*, 11H, 9Ar-H, 2CH=CH–), 2.2 (*s*, 3H, CH_3); ^{13}C NMR(DMSO-d_6 + $CDCl_3$) in δ ppm: 164.6 (C=O), 155.7 (C=O) and 147.5, 138.9, 137.8, 131.7, 129.3, 128.7, 128.5 124.6, 121.7, 120.7 and 119.3; MS: $m/z = 393.1$ $[M+2]^+$. Anal. Calcd. for $C_{21}H_{14}ClN_3O_3$: C, 64.37; H, 3.60; N, 10.72%. Found: C, 64.40; H, 3.54; N, 10.70%.

*5.1.3.8. 5((E)-1-(4-chlorophenyl)-3-(5-methyl-2-phenyl-1H-indol-3-yl) allylidene) pyrimidine-2,4,6 (1H,3H,5H)-trione (**5h**).* Yield 68% (Ethanol): mp 243–245 °C; IR (KBr) ν_{max} in cm^{-1}: 2987, 2918, 2849, 1695, 1606; ^{1}H NMR (DMSO-d_6 + $CDCl_3$) in δ ppm: 11.10 (*s*, 1H, indole NH), 10.37 (*s*, 1H, pyrimidine NH), 10.31 (*s*, 1H, pyrimidine NH), 7.2–8.2 (*m*, 14H, 12Ar-H, 2CH=CH–), 2.5 (*s*, 3H, CH_3); MS: $m/z = 482.8$ $[M+1]^+$. Anal. Calcd. for $C_{28}H_{20}ClN_3O_3$: C, 69.78; H, 4.18; N, 08.72%. Found: C, 69.74; H, 4.21; N, 08.75%.

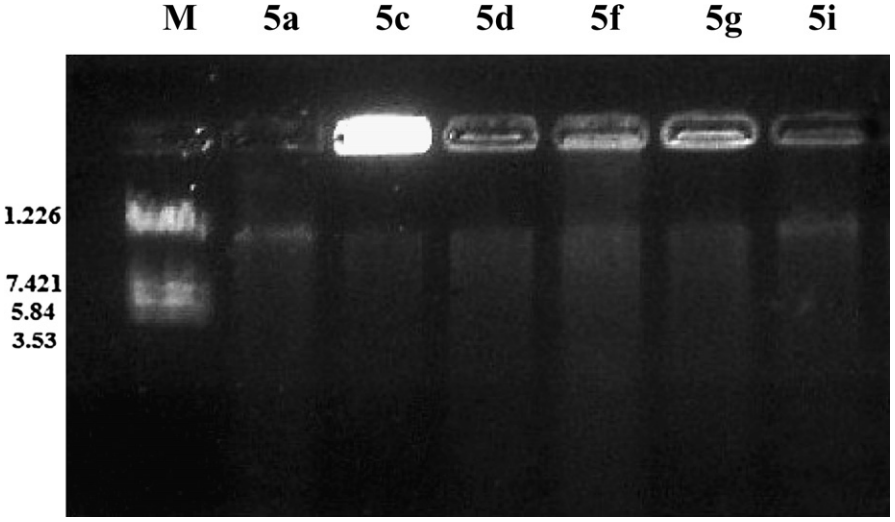


Fig. 4. DNA cleavage activity of novel indole derivatives **5a**, **5c**, **5d**, **5f**, **5g** and **5i**.

*5.1.3.9. 5-((E)-3-(5-chloro-2-phenyl-1H-indol-3-yl)-1-(4-chlorophenyl)allylidene) pyrimidine-2,4,6(1H,3H,5H)-trione (**5i**).* Yield 67% (Ethanol): mp 267–270 °C; IR (KBr) ν_{max} in cm^{-1}: 3227, 2918, 2849, 1716, 1647; ^1H NMR (DMSO-d_6 + $CDCl_3$) in δ ppm: 10.91 (*s*, IH, indole NH), 10.48 (*s*, 1H, pyrimidine NH), 10.40 (*s*, 1H, pyrimidine NH), 7.0–8.2 (*m*, 14H, 12Ar-H, 2CH=CH–), 2.4 (*s*, 3H, CH_3); MS: *m*/*z* = 502.2 $[M-1]^+$. Anal. Calcd. for $C_{27}H_{17}Cl_2N_3O_3$: C, 64.55; H, 3.41; N, 08.36%. Found: C, 64.50; H, 3.45; N, 08.39%.

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 [29]. 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 absorbances of samples were measured at 517 nm. Radical scavenging activity was calculated using the following formula:

% Radical scavenging activity = [(Control OD − Sample OD)/(Control OD)] × 100.

5.2.1.2. Total antioxidant capacity. Various concentrations of extracts (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 extracts (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 oC 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.

5.3. DNA cleavage activity

5.3.1. Preparation of culture media

DNA cleavage experiments were done according to the literature [32]. Nutrient broth [peptone, 10; yeast extract, 5; NaCl, 10; in (g/l)] was used for culturing of *Escherichia coli*. Fifty-milliliter media was prepared, autoclaved for 15 min at 121 °C under 15 lb pressure. The autoclaved media were inoculated for 24 h at 37 °C.

5.3.2. Isolation of DNA

The fresh bacterial culture (1.5 ml) is centrifuged to obtain the pellet which is then dissolved in 0.5 ml of lysis buffer (100 mM tris pH 8.0, 50 mM EDTA, 10% SDS). To this 0.5 mL of saturated phenol was added and incubated at 55 °C for 10 min, then centrifuged at 10,000 rpm for 10 min and to the supernatant, equal volume of chloroform: isoamyl alcohol (24:1) and 1/20th volume of 3 M sodium acetate (pH 4.8) was added. Centrifuging at 10,000 rpm for 10 min and to the supernatant, 3 volumes of chilled absolute alcohol were added. The precipitated DNA was separated by centrifugation and the pellet was dried and dissolved in TAE buffer (10 mM tris pH 8.0, 1 mM EDTA) and stored in cold condition.

5.3.3. Agarose gel electrophoresis

Cleavage products were analyzed by agarose gel electrophoresis method [32]. Test samples (1 mg/ml) were prepared in DMF. The samples (25 mg) were added to the isolated DNA of *E. coli*. The samples were incubated for 2 h at 37 °C and then 20 ml of DNA sample (mixed with bromophenol blue dye at 1:1 ratio) was loaded carefully into the electrophoresis chamber wells along with standard DNA marker containing TAE buffer (4.84 g tris base, pH 8.0, 0.5 M EDTA/1 L) and finally loaded on agarose gel and passed the constant 50 V of electricity for 30 min. Removing the gel and stained with 10.0 mg/ml ethidium bromide for 10–15 min, the bands were observed under Vilber Lourmat Gel documentation system and then photographed to determine the extent of DNA cleavage. The results are compared with standard DNA marker.

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