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Green approach for the synthesis of 3-methyl-1-phenyl-4-((2-phenyl-1H-indol 3-yl)methylene)-1H-pyrazole-5(4H)-ones and their DNA Cleavage, antioxidant, and antimicrobial activities

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Abstract

3-Methyl-1-phenyl-4-((2-phenyl-1*H*-indol-3-yl)methylene)-1*H*-pyrazol-5(4H)-ones (5a-i) was prepared by the condensation reaction of different 3-formyl-2-phenylindole derivatives (2a-i) and 3-methyl-1-phenyl-2-pyrazoline-5-one in quantitative yield by applying various green synthetic methods as grinding, microwave irradiation using different catalysts under solvent-free mild reaction conditions with high product yields. The structures of the synthesized compounds were characterized on the basis of elemental analysis, infrared, ^{1}HNMR, ^{13}C NMR, and mass spectral data. The synthesized compounds were screened for free radical scavenging, antimicrobial, and DNA cleavage activities. Most of the tested compounds belonging to the 3-methyl-1-phenyl-4-((2-phenyl-1*H*-indol-3-yl)methylene)-1*H*-pyrazol-5(4H)-ones series exhibited promising activities.

1 | INTRODUCTION

Heterocycles are useful due to their combination of compact and robust molecular structure with a high degree of molecular diversity that results in properties, which can be finely adjusted to needs of sophisticated application.[1,2]

The heterocycles possessing indole, pyrazole, and isoxaline moiety deserve a special mention due to their versatile use in pharmacological and biochemical studies.[3,4] The important role of Indole nucleus in the field of pharmaceutical chemistry and biochemistry is well-established form last many decades.[5] Chemical studies reveal that the naturally occurring as well as synthetic hallucinogenic drugs such as LSD, harmine, psilocin, psilocybin, and DMT, most of them are derivatives of tryptamine, β-[(3-indolyl)ethyl]amin.[6] The importance of serotonin (5-hydroxytryptamine) has been recognized as a modulator of CNS neurohormonal activity.[7,8] Various biological activities like anticancer,[9,10] antirheumatoidal,[11] anti-HIV,[12] anti-inflammatory,[13] antidiabetic,[14] antihistaminic,[15] anticonvulsant,[16] antihelminthic,[17] antihypertensive,[18] antioxidant,[19] and antimicrobial[20] have been attributed to indole derivatives. Phenyl group at 2-position of indole moiety enhances biological activities like antimicrobial, monoamine oxidase inhibition.[21] The derivatives of 2-phenylindoles exhibits have shown potent CNS depressant activity[22] and cytotoxic activity.[23]

Pyrazole is an important pharmacophore, which exhibits a wide spectrum of biological activities, such as antitumor,[24] antidepressant,[25] antidiabetic,[26] antiamoebic,[27] and COX-2 inhibitor.[28] Incorporation of pyrazoline in indole framework enhances pharmacological importance, according to the review of literature indole derivatives bearing pyrazoline moiety possess diverse biological activities like antitumor,[29] antioxidant,[30] anticancer,[31] antimicrobial,[32] and anti-inflammatory.[33]

DNA cleavage studies have been of great interest for biologists and chemists. The cleaved DNA under

physiological conditions has been developed as artificial nucleases; these synthetic nucleases have provided important tools in the hand of chemists to manipulate DNA and cooperate with molecular biologists.[34] The nature of substituents and interaction of the compound with DNA molecule would help in the design of newer drugs and develop new selective, efficient DNA recognition and cleaving agent.

Free radicals have been implicated in several human diseases such as diabetic's Mellitus, stroke, diabetes, Alzheimer's diseases, atherosclerosis, arthritis, and neurodegenerative Parkinson's disease.[35] Melatonin and serotonin are good free radical scavengers and antioxidant that shows an important role in the immune system. N-substituted indole-2/3-carboxamide and ester derivatives are also showed excellent antioxidant activity against superoxide free radicals.[36]

Reactions in Grindstone Chemistry have initiated itself by grinding crystals of substrate and reagent with the generation of the small amount of energy in the form of heat through friction.[37] Such reactions are easy to handle, comparatively cheaper to operate, and ecologically more favorable. Silica-supported catalysts have great interest and special attention of chemists due to high reactivity, more efficient, eco-friendly nature, recyclable, and easy to handling.[38]

Another solvent-free popular technique is Microwave Chemistry since microwave irradiation is a well-known, eco-friendly, and convenient synthetic method.[39] Montmorillonite KSF showed catalytic activity for this condensation reaction and has many advantages over catalysts like NaOH, KOH such as easy to handle, low cost, and elimination of metal waste. Keeping in view these observations and continuation of our studies towards the synthesis of a variety of bioactive heterocycles,[40] herein, we have designed various strategies to synthesize some noble indolyl pyrazoline derivatives. To make the efficiency and comparative study of these synthetic routes, we have used conventional and solvent-free methods (Grindstone and microwave irradiation). An inexpensive catalyst likes Mg $(HSO_4)_2/SiO_2$ and KSF are used to obtain the target molecule. All the synthesized compound screened for DNA cleavage, free radical scavenging, and antimicrobial activity.

2 | MATERIALS AND METHODS

Melting points of all the synthesized compounds are determined in open capillary tubes and are uncorrected. The infrared (IR) spectra (υ_{max} in cm^{-1}) were recorded on a Perkin Elmer-557 model. Reactions are monitored by thin-layer chromatography (TLC) using silica gel-G as adsorbent, and the spots were visualized using ultraviolet light and iodine. ^1H NMR and ^{13}C NMR spectra were recorded on BRUKER AVENE II 400-MHz NMR spectrometer using tetramethylsilane as an internal standard and DMSO-d_6/$CDCl_3$ as a solvent. The mass spectral data were obtained on a JEOLD-300 spectrometer. Microwave-assisted reactions were carried out in a domestic MW oven (LG MS-194A) operating at 2450 MHz.

2.1 | Biological activities

2.1.1 | Free radical scavenging assays

Free radical scavenging activity was done by α, α-diphenyl-β-picry-hydrazyl radical scavenging (DDPH) assay. The DPPH is a stable free radical and is widely used to assess the free radical scavenging activity of antioxidant compounds. This method is based on the reduction of DPPH in methanol solution in the presence of hydrogen-donating antioxidant due to the formation of the nonradical form DPPH-H (Blois, 1958). This transformation results in a color change from purple to yellow, which was measured by spectrophotometrically. The disappearance of the purple color monitored at 517 nm. The free radical scavenging activity can be measured by using 2, 2-diphenyl-1-picryl-hydrazyl or 2, 2-diphenyl-2-picryl-hydrazyl by the method of McCune and Johns (2002). The reaction mixture (3.0 mL) consists of 1.0 mL of DPPH in methanol (0.3 mM), 1.0 mL of various concentrations of test compounds (50, 75, and 100 μg mL^{-1}), and 1.0 mL of methanol. It incubated for 10 minutes in dark, and then the absorbance was measured at 517 nm. In this assay, the positive controls can be ascorbic acid used as standard; the percentage of inhibition can be calculated using the formula.

$$\text{Inhibition } (\%) = (A_0 - A_1/A_0) \times 100,$$

where A_0 is the absorbance of the control and A_1 is the absorbance of the sample. The test was carried out in triplicate.

2.2 | DNA cleavage analysis

2.2.1 | Preparation of culture media

DNA cleavage experiments were done according to the literature method.[41] Nutrient broth [Peptone, 10; yeast extract, 5; NaCl, 10; in (g/L) was used for culturing of *Escherichia coli*. About 50-mL media was prepared, autoclaved for 15 minutes at 121°C under 15-lb pressures. The autoclaved media were inoculated for 24 h at 37°C.

2.2.2 | Isolation of DNA

The fresh bacterial culture (1.5 mL) was centrifuged to obtain the pellet, which was then dissolved in 0.5 mL of lysis buffer (100 mM tris pH 8.0, 50 mM EDTA, and 10 % SDS). To this, 0.5 mL of saturated phenol was added and incubated at 55°C for 10 minutes, then centrifuged at 10 000 rpm for 10 minutes and to the supernatant, an equal volume of chloroform: isoamyl alcohol (24:1) and 1/20th volume of 3 M sodium acetate (pH 4.8) was added. This was further centrifuged at 10 000 rpm for 10 minutes, and to the supernatant, three volumes of chilled absolute alcohol was 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.

2.2.3 | Agarose gel electrophoresis

The cleavage products were analyzed by agarose gel electrophoresis method [41]. Test samples (1 mg/mL) were prepared in dimethylformamide (DMF). The samples (25 mg) were added to the isolated DNA of *E coli*. The samples were incubated for 2 hours 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 minutes. Removing the gel and stained with 10.0-mg/mL ethidium bromide for 10e 15 minutes, 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. DNA ladder was used 100 to 1000bp (100 bp step up ladder, Merck).

2.3 | Antibacterial assay

Antibacterial bacterial activity is done by Kirby-Bauer well diffusion method. Mueller-Hinton Agar plates were prepared for the antibacterial activity. ATCC Cultures of *E coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 29213) were inoculated in Peptone water and were kept for incubation for 24 hours at 37°C. Inoculum size of bacteria was adjusted using McFarland Turbidity Standard as reference. The bacterial suspensions were compared with 0.5 McFarland Turbidity Standard. Bacterial cultures were swabbed onto the Mueller Hinton Agar surface. About 50 μL of dilution of sample (5a-i), the positive control (Streptomycin (5mg/mL (w/v)) and the negative control (DMSO) was loaded into the respective wells. The antibacterial plates were kept for incubation at 37°C for 24 hours. The zone of inhibition was measured and compared with the controls.

2.4 | Antifungal assay

Antifungal activity was also done by the disk diffusion method. Sabouraud Dextrose Agar plates were prepared for antifungal activity. Cultures of the fungal strain of *Candida albicans* and *Aspergillus niger* were inoculated in Peptone water and were kept for incubation for 24 hours at 37°C. Fungal cultures were swabbed onto the Sabouraud Dextrose Agar surface. About 50 μl of dilution of sample (5a-i), the positive control *Itraconazole* (5mg/well) and the negative control (DMSO), was loaded into the respective wells. The antifungal plates were kept for incubation at 37°C for 24 hours. The antifungal activity of each compound was compared with *Itraconazole* as standard drug. Inhibition zones were measured and compared with the controls.

2.5 | Preparation of silica-supported magnesium hydrogen sulfate

Anhydrous magnesium chloride (5.0 g, 50 mmol) was charged in the 50-mL suction flask equipped with dropping funnel; concentrated sulfuric acid (98 %, 9.43 g, 50 mmol) was added drop wise over the period of 45 minutes with stirring at room temperature. HCl evolved immediately through a funnel and adsorbed by the solution of water and alkali, while the residual HCl eliminated by suction. The $Mg(HSO_4)_2$ (8.36 g) was obtained as white gel and mixed by silica gel (7.65 g) to get the desired catalyst.

2.6 | Synthesis of 2-phenyl-1*H*-indole-3-carbaldehyde (2a-i)

Phosphorus oxychloride (6.0 mmol) was added slowly, with stirring to dimethylformamide (DMF; 5 mL) at 10 to 15°C. The solution was further stirred for 15 minutes at the same temperature until an orange-colored syrupy liquid was formed; to this, 2-phenylindoles (5 mmol) was added in portions with stirring at 40–50°C. The solution was further stirred for 45 minutes and then poured into ice water (100 mL). Sodium hydroxide solution (2 N, 10 mL) was added to it and the mixture was heated on a water bath for 1 hour. It was cooled, filtered, and recrystallized from acetone (80%) to obtain pure compounds (2a-i).

2.7 | Synthesis of 3-methyl-1-phenyl-4-((2-phenyl-1H-indol-3-yl)methylene)-1H-pyrazole-5(4H)-ones. (5a-i)

2.7.1 | General method (i): Solvent-free synthesis of (5a-i)

A mixture of 2-phenyl-1*H*-indole-3-carbaldehyde (2a-i) (3 mmol) and Mg $(HSO_4)_2/SiO_2$(0.5 g) was grinded at room temperature for 2 to 5 minutes in mortar and pestle to generate yellow colored enolate. Then 3-methyl-1-phenyl-2-pyrazoline-5-one (3 mmol) was added to it and grinding continues further to give orange-red colored tacky solid within 10 to 20 minutes. The reaction proceeds exothermically indicated by the rise in temperature (5-10°C). After completion of the reaction (checked by TLC), the mixture was dissolved in CH_2Cl_2 and catalyst was filtered; the residue washed many times with CH_2Cl_2. After evaporation of the solvent under reduced pressure, almost pure compound was obtained. Further purification was achieved by recrystallization from ethanol. The catalyst was washed with diethyl ether, dried at 70°C for 50 minutes, and reused in another reaction. The compounds of the series (5a-i) also synthesized by the above method.

2.7.2 | General method (ii): Microwave-assisted solvent-free synthesis of (5a-i)

The reaction was carried out in domestic microwave (800 W, 2450 MHz). A mixture of 2-phenyl-1*H*-indole-3-carbaldehyde (2a-i) (3 mmol), 3-methyl-1-phenyl-2-pyrazoline-5-one (3 mmol), and montmorillonite KSF (0.4 g) was mixed thoroughly in a pestle mortar. This mixture was then transferred into a conical flask (100 cm^3) and irradiated with microwaves for 5 to 11 minutes. After completion of the reaction (checked by TLC), ethanol was added and the mixture was filtered; the filtrate was concentrated and then the crude product so obtained was recrystallized from ethanol to obtain pure compound (5a-i). The catalyst left as the residue was washed 2 to 3 times with ethanol and dried in vacuum for reuse. The compounds of the series (5a-i) also synthesized by the above method.

2.7.3 | Method (iii) conventional procedure of synthesis (5a-i)

2-Phenyl-1*H*-indole-3-carbaldehyde (2a-i) (3 mmol) and 3-methyl-1-phenyl-2-pyrazoline-5-one (3 mmol) were dissolved in a minimum amount of ethanol (25 mL) and then stirred at room temperature for 5 minutes. Sufficient 2N NaOH solution (40 mL) was added to it, and the whole reaction mixture was further stirred for half an hour, neutralized with 2N HCl (3 mL) diluted with water, and left overnight to get solid compound, which was filtered and recrystallized from ethanol to obtain pure compound. Result and conditions of the synthesis of 3-methyl-1-phenyl-4-((2-phenyl-1*H*-indol-3-yl)methylene)-1*H*-pyrazol-5(4H)-ones (5a-i) are tabulated in Table 1. The compounds of the series (5a-i) also synthesized by the above method.

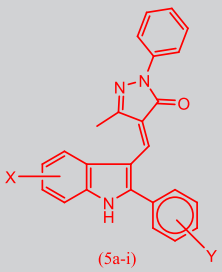
3 | RESULT AND DISCUSSIONS

3.1 | Synthesis

p-Fluorophenylhydrazine was prepared by the method of Chattaway et al.[42] 2-Phenylindole derivatives, which have been prepared by the Fischer indole synthesis and by the method of Joshi et al, were subjected to formylation with phosphorous oxychloride ($POCl_3$) and DMF under Vilsmeier-Haack Formylation reaction. We carried out condensation reaction between various 3-formyl-2-phenylindole derivatives (2a-i) and 3-methyl-1-phenyl-2-pyrazoline-5-one and get desired product 3-methyl-1-phenyl-4-((2-phenyl-1H-indol-3-yl)methylene)-1H-pyrazol-5(4H)-ones (5a-i) (Scheme 1). The reaction was conducted by employing various reaction conditions. In the traditional conventional method, the reactants (compounds 2a-i and appropriate 3-methyl-1-phenyl-4-((2-phenyl-1*H*-indol-3-yl)methylene)-1*H*-pyrazol-5(4H)-ones) were dissolved in ethanol and then aqueous NaOH was added dropwise with continuous stirring. In contrast under solvent-free conditions, the condensation reaction of the same reactants can be carried out by adding and 0.5g amount of Mg $(HSO_4)_2/SiO_2$ under grinded conditions using mortar and pestle to afford target compound (5a-i) in higher yield and lesser time. Grinding together the solid reagents without the addition of catalyst reveals that in some cases, liquid melt is observed, while in others, the discrete crystalline phase of solid reagents remains. More important, upon addition of the catalyst, a rise in temperature (5-10°C) occurs only those systems that exhibit a phase change to a melt. Thus, the existence of a liquid phase is a prerequisite for reaction in these systems. The microwave-assisted reaction using a catalytic amount of KSF clay and solvent-free environment proceeds efficiently and completed within 5 to 11 minutes with excellent yield (78-90%). The result obtained is presented in [Table 1].

The IR spectra of 2-Phenyl-1*H*-indole (1a-i), N—H stretching absorption appears as a strong absorption band in the range of 3460 to 3420 cm^{-1}. The C—N frequencies have been assigned to the 1275 to 970 cm^{-1} region. In ^1H

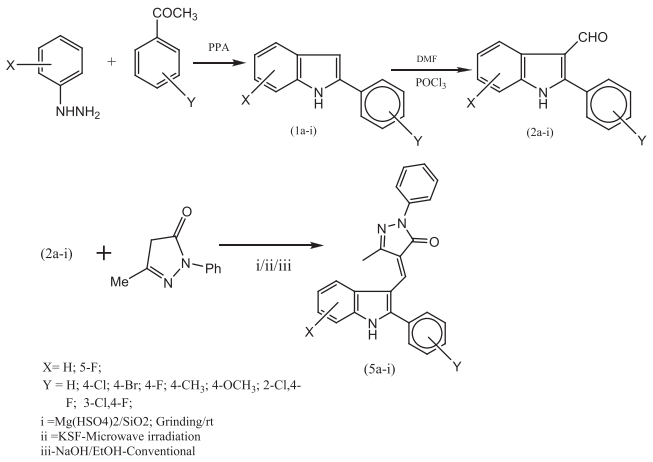
TABLE 1 Result and conditions of the synthesis of 3-methyl-1-phenyl-4-((2-phenyl-1*H*-indol-3-yl)methylene)-1*H*-pyrazol-5(4H)-ones (5a-i)



(5a-i)

			% Yield			Time required			Temp, °C
Compound	**X**	**Y**	**i**	**ii**	**iii**	**I, min**	**II, min**	**III, min**	**II**
5a	H	H	87.8	82.1	68.2	10	5	30	70
5b	H	4-F	92.0	89.3	78.5	10	7	30	60
5c	H	4-Cl	89.2	82.3	75.6	14	8	40	50
5d	H	4-Br	93.4	90.1	67.5	10	11	45	65
5e	H	4-CH_3	88.7	84.5	77.6	11	9	30	60
5f	H	4-OCH_3	87.6	84.4	65.0	10	6	50	75
5g	H	3-Cl, 4-F	84.7	78.5	70.5	13	5	30	55
5h	H	2-Cl, 4-F	91.0	83.0	71.3	12	8	40	60
5i	F	4-F	92.0	87.0	72.4	9	6	30	60

Note. (i) By grinding with Mg $(HSO_4)_2/SiO_2$. (ii) By microwave irradiation with $Mont_mo_rillo_nite$ KSF; reaction was carried out in an LG MS-194A household microwave even with maximum 800W power. (iii) By conventional method.



SCHEME 1 Schematic representation for the synthesis the synthesis of 3-methyl-1-phenyl-4-((2-phenyl-1*H*-indol-3-yl) methylene)-1*H*-pyrazol-5(4H)-ones

NMR spectra of 2-phenyl-1*H*-indole, methine proton at C-3 of indole moiety shows a resonance signal at δ 6.6 ppm, N—H resonance signal appears at δ 8.2 to 8.4 ppm as a broad singlet. Aromatic protons are observed at multiplet from δ 7.8 to 8.0 ppm.

In the IR spectra of 2-phenyl-1*H*-indole-3-carbaldehyde derivatives (2a-i), the absorption band of the N—H stretching is shifted towards lower wave number 3300 to 3150 cm^{-1} due to the presence of –CHO group at position 3- of the indole ring, The strong absorption band in the range 1230-1060 cm^{-1} has been attributed to Ar-F stretching mode. The characteristic absorption due to–CHO group appears 1675 to 1625 cm^{-1}. Analysis of IR spectra of 2-phenyl-1*H*-indole-3-carbaldehyde shows one special feature that the appearance of two broad strong peaks in –NH stretching region. This may be due to the intermolecular hydrogen bonding between the N—H group of one molecule and the –CHO group of the second molecule. In ^{1}H NMR spectra of 2-phenyl-1*H*-indole-3-carbaldehyde derivatives, the N—H resonance signal is observed in the region of δ 10.2 to 11.6 ppm as a broad singlet. Aromatic protons are observed as multiplet from δ 7.2 to 7.9 ppm. Singlet due to –CHO proton appears in the region δ 9 to 10.0 ppm.

In the IR spectra of 3-methyl-1-phenyl-4-((2-phenyl-1*H*-indol-3-yl)methylene)-1*H*-pyrazol-5(4H)-ones (5a-i), the N—H absorption appears as a broad band 3170 to 3010 cm^{-1}. Characteristic absorption due to carbonyl group appears in the range of 1625 to 1605 cm^{-1}. The downfield shift is due to conjugation of the carbonyl group with the olefinic double bond, which results in delocalization of electrons of carbonyl group giving ionic resonance structures. The olefinic double bond (C=C) appears between the range of 1600 to 1525 cm^{-1}. The ^{1}H NMR spectra of 3-methyl-1-phenyl-4-((2-phenyl-1*H*-indol-3-yl)

TABLE 2 Free radical scavenging activity of synthesized compounds (5a-i)

Compound	Concentration, μg mL^{-1}		
	50 Mean ± SD	75 Mean ± SD	100 Mean ± SD
5a	63.46 ± 0.14	64.73 ± 0.19	66.96 ± 0.16
5b	37.45 ± 0.35	39.67 ± 0.39	41.48 ± 0.87
5c	40.43 ± 0.26	42.68 ± 0.22	43.96 ± 0.27
5d	38.36 ± 0.15	40.53 ± 0.34	42.73 ± 0.16
5e	74.26 ± 0.10	76.43 ± 0.18	78.54 ± 0.25
5f	78.72 ± 0.43	80.45 ± 0.12	82.27 ± 0.21
5g	65.25 ± 0.32	67.67 ± 0.23	68.98 ± 0.13
5h	67.34 ± 0.65	69.26 ± 0.14	70.67± 0.34
5i	63.50 ± 0.17	65.72 ± 0.14	67.56 ± 0.37
Ascorbic acid	76.24 ± 0.10	78.48 ± 0.12	80.26± 0.17

methylene)-1*H*-pyrazol-5(4H)-ones (5a-i) exhibit a complex splitting pattern. N—H resonance signal appears as a broad singlet from δ 11.24 to 12.30 ppm. A singlet at *δ* 9.9—8.7 ppm assigned for the vicinal proton. Aromatic proton appears as a complex multiplet in the region δ 7.3 to 8.2 ppm. ^{13}C NMR Spectrum of 3-methyl-1-phenyl-4-((2-phenyl-1*H*-indol-3-yl)methylene)-1*H*-pyrazol-5(4H)-ones has displayed a downfield signal at *δ*182-169for carbonyl carbon and *δ* 23 and 20 integrated for methyl carbon. The mass spectrum of compound 5a has shown ion peak at *m/z* 378 $[M+1]^+$

3.2 | Spectral data of compound

3-methyl-1-phenyl-4-((2-phenyl-1H-indol-3-yl)methylene)-1H-pyrazol-5(4H)-one (5a). Yield 82% (ethanol): mp 235—237°C; IR (υ max, cm^{-1}, KBr): 3150, 3053, 2895, 1630, 1550; ^{1}H NMR (400MHz, DMSO-d_6,δ ppm): 12.32 (s, 1 H indole NH), 9.94 (s, 1H,—CH=), 8.2(d, 1H, indole Ar-H),7.6—7.2 (m, 13H, Ar-H), 2.3(s, 3H, CH_3)^{13}C NMR (DMSO-d_6) in δ (ppm): 182.2 (C=O), 149.8, 140.1, 136.4, 130.1, 127.4, 126.0, 124.8, 122.2, 120.5, 113.9, 112.3 and 21.4; ESI-MS: m/z = 378 $[M + 1]^+$. Anal. Calcd. for $C_{25}H_{19}N_3O$ (377): C, 79.55; H, 5.07; N, 11.13%. Found: C, 79.53; H, 5.09; N, 11.11%

4-((2-(4-fluorophenyl)-1H-indol-3-yl)methylene)-3-methyl-1-phenyl-1H-pyrazol-5(4H)-one (5b). Yield 75% (ethanol): mp 283—285°C; IR (υ max, cm^{-1}, KBr): 3232, 3164, 2870, 1620, 1540; ^{1}H NMR (400MHz, DMSO-d_6, δ ppm): 12.02 (s, 1 H indole NH), 9.24 (s, 1H, —CH=), 8.22(d, 1H, indole Ar-H),7.9—7. 3(m, 12H, Ar-H), 2.27(s, 3H, CH_3)^{13}C NMR (DMSO-d_6) in δ (ppm): 182.5 (C=O), 144.4, 142.1, 133.4, 129.1, 126.3, 124.7, 123.1, 120.6, 118.3, 115.3, 112.5 and 20.3; ESI-MS: m/z = 396$[M + 1]^+$, 397 $[M + 2]^+$ Anal. Calcd. for $C_{25}H_{18}FN_3O$ (395): C, 75.93; H, 4.59; N, 10.63%. Found: C, 75.95; H, 4.43; N, 10.67%

4-((2-(4-chlorophenyl)-1H-indol-3-yl)methylene)-3-methyl-1-phenyl-1H-pyrazol-5(4H)-one (5c). Yield 79% (ethanol): mp 245—248°C; IR (υ max, cm^{-1}, KBr): 3152, 3054, 2896, 1634, 1552; ^{1}H NMR (400MHz, DMSO-d_6, δ ppm): 11.89 (s, 1 H indole NH), 9.84 (s, 1H, —CH=), 8.3(d, 1H, indole Ar-H),7.8—7.2 (m, 12H, Ar-H), 2.33(s, 3H, CH_3)^{13}C NMR (DMSO-d_6) in δ (ppm): 172.8 (C=O), 145.4, 140.1, 137.4, 129.1, 127.3, 125.4, 123.4, 121.6, 120.3, 113.3, 111.5 and 21.3; ESI-MS: m/z = 413 $[M + 2]^+$, 415 $[M + 4]^+$ Anal. Calcd. for $C_{25}H_{18}ClN_3O$ (411): C, 72.90; H, 4.40; N, 10.20%. Found: C, 72.94; H, 4.43; N, 10.24%

4-((2-(4-bromophenyl)-1H-indol-3-yl)methylene)-3-methyl-1-phenyl-1H-pyrazol-5(4H)-one (5d). Yield 89% (ethanol): mp 305—307°C; IR (υ max, cm^{-1}, KBr): 3187, 3068, 2817, 16, 1557; ^{1}H NMR (400MHz, DMSO-d_6, δ ppm): 11.87 (s, 1 H indole NH), 8.98 (s, 1H, —CH=), 8.22(d, 1H, indole Ar-H),7.8—7. 2(m, 12H, Ar-H), 2.24(s, 3H, CH_3)^{13}C NMR (DMSO-d_6) in δ (ppm): 172.5 (C=O), 143.4, 142.1, 137.7, 128.1, 126.2, 124.7,

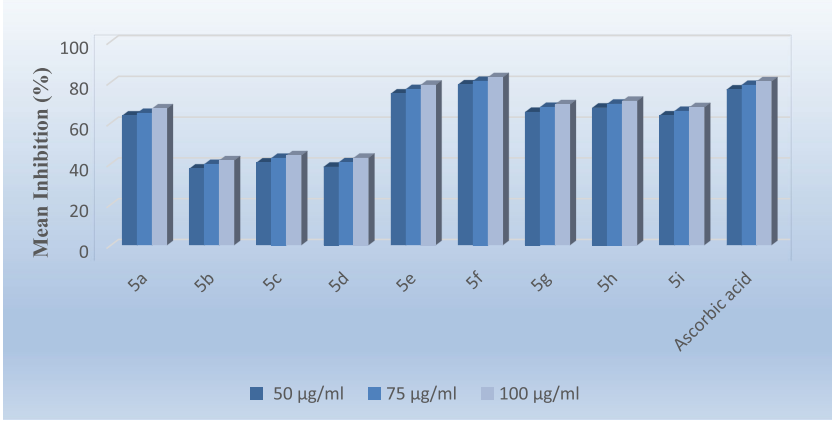


FIGURE 1 Free radical scavenging activity of synthesized compounds (5a-i) [Color figure can be viewed at wileyonlinelibrary.com]

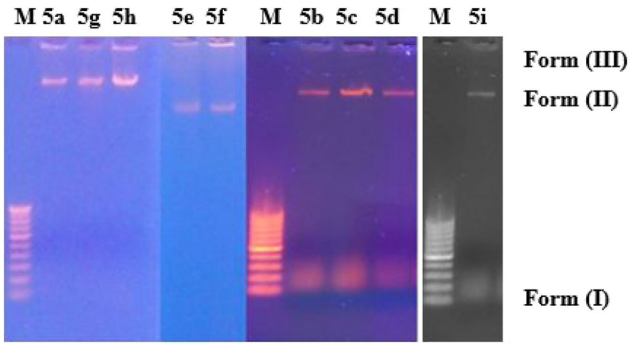


FIGURE 2 DNA cleavage activity of synthesized compounds (5a-i) [Color figure can be viewed at wileyonlinelibrary.com]

123.3, 121.4, 118.2, 115.0, 112.8 and 22.2; ESI-MS: m/z = 456 $[M + 1]^+$, 458 $[M + 2]^+$ Anal. Calcd. for $C_{25}H_{18}FN_3O$ (455): C, 65.80; H, 3.98; N, 9.21%. Found: C, 65.76; H, 3.95; N, 9.23%

3-methyl-1-phenyl-4-((2-p-tolyl-1H-indole-3-yl) methylene)-1H-pyrazol-5(4H)-one (5e). Yield 87% (ethanol): mp 270−272°C; IR (υ max, cm^{-1}, KBr): 3145, 3050, 2890, 1632, 1545; ^{1}H NMR (400MHz, DMSO-d_6, δ ppm): 12.26 (s, 1 H indole NH), 9.92 (s, 1H,−CH=), 8.1(d, 1H, indole Ar-H),7.4−7.1 (m, 12H, Ar-H), 2.3(s, 3H, CH_3), 2.2(s, 3H, CH_3)^{13}C NMR (DMSO-d_6) in δ (ppm): 173.3 (C=O), 148.5, 139.4, 135.2, 130.0, 127.3, 125.7, 123.6, 122.1, 121.4, 113.8, 112.2, 22.1, ; ESI-MS: m/z = 392 $[M + 1]^+$. Anal. Calcd. For $C_{26}H_{21}N_3O$ (391): C, 79.77; H, 5.41; N, 10.73%. Found: C, 79.75; H, 5.39; N, 10.71%

4-((2-(4-methoxyphenyl)-1H-indol-3-yl)methylene)-3-methyl-1-phenyl-1H-pyrazol-5(4H)-one (5f). Yield 89% (ethanol): mp 278−280°C; IR (υ max, cm^{-1}, KBr): 3167, 3064, 2824, 1630, 1560; ^{1}H NMR (400MHz, DMSO-d_6, δ ppm): 12.56 (s, 1 H indole NH), 8.79 (s, 1H, −CH=), 8.34(d, 1H, indole Ar-H),7.7−7. 15(m, 12H, Ar-H), 3.4(s, 3H, CH_3), 2.24(s, 3H, CH_3)^{13}C NMR (DMSO-d_6) in δ (ppm): 177.5 (C=O), 146.4, 143.8, 136.9, 130.1, 128.8, 128.2, 124.4, 123.1, 120.4, 118.2, 115.0, 55.4, and 22.2; ESI-MS: m/z = 408 $[M + 1]^+$, Anal. Calcd. for $C_{26}H_{21}N_3O_2$ (407): C, 76.64; H, 5.19; N, 10.31%. Found: C, 76.62; H, 5.16; N, 10.29%

4-((2-(3-chloro-4-fluorophenyl)-1H-indol-3-yl) methylene)-3-methyl-1-phenyl-1H-pyrazol-5(4H)-one (5g). Yield 82% (ethanol): mp 292−294°C; IR (υ max, cm^{-1}, KBr): 3170, 3065, 2870, 1620, 1547; ^{1}H NMR (400MHz, DMSO-d_6, δ ppm): 12.27 (s, 1 H indole NH), 9.14 (s, 1H,−CH=), 8.72(d, 1H, indole Ar-H),7.9−7. 1(m, 11H, Ar-H), 2.23(s, 3H, CH_3)^{13}C NMR (DMSO-d_6) in δ (ppm): 178.5 (C=O), 147.4, 145.1, 136.7, 126.6, 124.6, 123.7, 122.4, 120.4, 117.2, 113.8, 111.9, and 21.7; ESI-MS: m/z = 431$[M + 2]^+$, 433$[M + 4]^+$ Anal. Calcd. for $C_{25}H_{17}ClFN_3O$ (429): C, 69.85; H, 3.99; N, 8.25%. Found: C, 69.81; H, 3.94; N, 8.28%

TABLE 3 Zone of inhibition (mm) of compounds (5a-i) against tested bacterial strains

		Bacterial strains	
Entry	Compound	*S. aureus* (ATCC 29213)	*Escherichia coli* (ATCC 25922)
1	5a	17.2± 0.6	16.4 ± 0.3
2	5b	18.2 ± 0.4	16.8± 0.4
3	5c	19.4 ± 0.5	18.3 ± 0.2
4	5d	18.9 ± 0.2	17.7 ± 0.3
5	5e	21.3 ± 0.2	22.0 ± 0.6
6	5f	22.1 ± 0.3	22.1 ± 0.4
7	5g	20.0 ± 0.2	19.4± 0.3
8	5h	20.7 ± 0.4	20.4± 0.2
9	5i	19.5 ± 0.4	18.6 ± 0.3
10	Standard	27.5 ± 0.6	24.4 ± 0.3

Note. Streptomycin was used as a standard. About 50 μl/mL of the compound in each well.

4-((2-(2-chloro-4-fluorophenyl)-1H-indol-3-yl) methylene)-3-methyl-1-phenyl-1H-pyrazol-5(4H)-one (5h). Yield 93% (ethanol): mp 300−302°C; IR (υ max, cm^{-1}, KBr): 3182, 3074, 2860, 1650, 1560; ^{1}H NMR (400MHz, DMSO-d_6, δ ppm): 12.30 (s, 1 H indole NH), 9.25 (s, 1H,−CH=), 8.80(d, 1H, indole Ar-H),8.4−7. 4(m, 10H, Ar-H), 2.23(s, 3H, CH_3)^{13}C NMR (DMSO-d_6) in δ (ppm): 178.5 (C=O), 145.4, 143.1, 132.7, 129.6, 126.6, 125.7, 123.4, 120.4, 118.2, 114.3, 112.9, and 21.3; ESI-MS: m/z = 431$[M + 2]^+$, 433$[M + 4]^+$ Anal. Calcd. for $C_{25}H_{17}ClFN_3O$ (429): C, 69.85; H, 3.99; N, 8.25%. Found: C, 69.88; H, 3.98; N, 8.23

4-((5-fluoro-2-(4-fluorophenyl)-1H-indol-3-yl) methylene)-5-methyl-2-phenyl-2,4-dihydro-3H-pyrazol-3-one (5i). Yield 92% (ethanol): mp 149-150°C; IR (υ max, cm^{-1}, KBr): 3240, 3165, 2860, 1620, 1540; ^{1}H NMR (400MHz, DMSO-d_6, δ ppm): 11.97 (s, 1 H indole NH), 9.20 (s, 1H,−CH=), 8.20(d, 1H, indole Ar-H),7.9−7. 3(m, 11H, Ar-H), 2.26(s, 3H, CH_3)^{13}C NMR (DMSO-d_6) in δ (ppm): 182.4 (C=O), 144.0, 142.0, 133.2, 128.4, 126.4, 125.7, 124.1, 120.4, 118.4, 115.5, 112.3 and 20.4; ESI-MS: m/z = 413 $[M]^+$, 414 $[M + 1]^+$ Anal. Calcd. for $C_{25}H_{17}F_2N_3O$ (413): C, 72.63; H, 4.14; N, 10.16 %. Found: C, 72.61; H, 4.16; N, 10.14 %

3.3 | Biological activities

3.3.1 | Free radical scavenging assay

The free radical scavenging activity of synthesized compounds was done by α, α-diphenyl-β-picry-hydrazyl radical

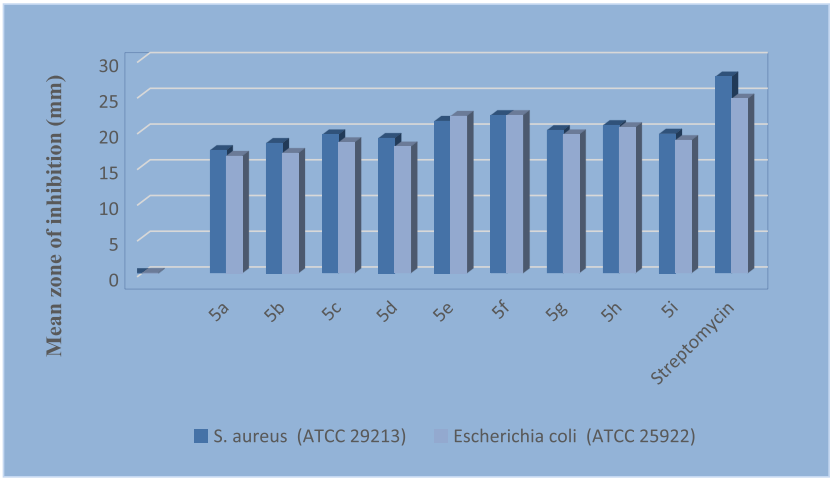


FIGURE 3 Zone of inhibition (mm) of compounds 5a-i against tested bacterial strains [Color figure can be viewed at wileyonlinelibrary.com]

scavenging (DDPH) method. Samples were prepared at concentrations of 50, 75, and 100 μg/mL, and ascorbic acid is taken as standard. The experimental data on the antioxidant activity of the compounds (5a-i) and control drug are presented in Table 2. Among synthesized -CH_3- and -OCH_3- substituted indole derivatives (5e) and (5f) have very good scavenging activity, simple indole derivative (5a) and Di-halogen-substituted indole derivatives (5g), (5h), and (5i) have shown moderate activities and mono-halogen-substituted derivatives (5b), (5c), and (5d) have shown least activity compare with the standard. Therefore, such compounds containing substitutions -CH_3 and -OCH_3 on 2-phenyl indole moiety enhance the free radical scavenging activity. Further, the synthesized compounds scavenged the DDPH radicals in a concentration-dependent manner. The bar graph representation of the percentage of mean free radical scavenging activity is shown in Figure 1.

TABLE 4 Zone of inhibition (mm) of compounds 5a-i against tested fungal strains

		Fungal strains	
Entry	**Compound**	***Candida albicans***	***Aspergillus niger***
1	5a	18.2± 0.6	15.6 ± 0.5
2	5b	19.2 ± 0.2	16.5 ± 0.2
3	5c	21.6 ± 0.3	20.2 ± 0.7
4	5d	20.2 ± 0.5	18.8 ± 0.6
5	5e	22.6 ± 0.2	21.7 ± 0.2
6	5f	23.3 ± 0.4	22.2 ± 0.2
7	5g	20.2 ± 0.5	19.3 ± 0.8
8	5h	21.4 ± 0.3	20.4 ± 0.9
9	5i	21.4 ± 0.5	20.3 ± 0.3
10	Standard	27.4 ± 0.6	23.4 ± 0.2

Note. Itraconazole was used as the standard. About 50 μl/mL of the compound in each well.

3.4 | DNA cleavage analysis

The DNA cleavage activity of 3-methyl-1-phenyl-4-((2-phenyl-1*H*-indol-3-yl)methylene)-1*H*-pyrazol-5(4H)-ones (5a-i) was studied by agarose gel electrophoresis method. The pictures of gels are presented in Figure 2. DNA cleavage study is considered by observing the conversion of super coiled DNA (form I) to nicked DNA (form II) and linear DNA (form III) under anaerobic condition. DNA cleavage was not observed in (lane 1) in which the compound was absent. All the compounds (5a-i) can induce the observable cleavage of the DNA plasmid at the100-μM concentration. At this concentration, all synthesized compounds can promote 70 to 90% conversion of super coiled DNA to nicked and linear DNA. It was observed that DNA cleavage not effective at lower concentrations. The studies revealed that -CH_3- and -OCH_3-substituted indole derivatives (5e) and (5f) exhibit much higher cleaving efficiency shown in the (Figure 2); the super coiled DNA was completely converted to form II and form III, and well defined. The significant activity was shown by simple indole derivative (5a) and Di-halogen-substituted indole derivatives (5g), (5h), and (5i); least activity was shown by mono-halogen-substituted indole derivative (5b), (5c), and (5d). Therefore, compounds having -CH_3 and -OCH_3 substitutions on 2-phenylindole moiety were more capable of abstract hydrogen from deoxyribose sugar to cleave DNA compare than other substituted indole derivative

3.5 | Antibacterial studies

All the synthesized compounds (5a-i) were screened for their antibacterial activity against *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 29213) by

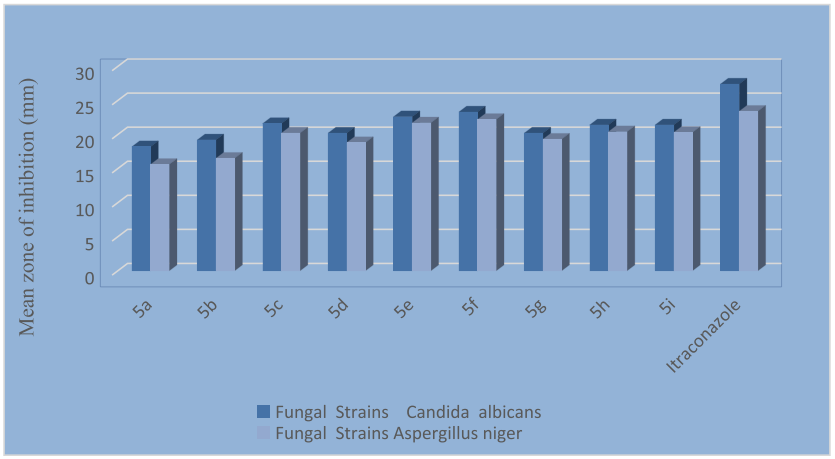


FIGURE 4 Zone of inhibition (mm) of compounds 5a-i against tested fungal strains [Color figure can be viewed at wileyonlinelibrary.com]

Kirby-Bauer well diffusion method with reference to Streptomycin. The observation of antibacterial screening data revealed that all the tested compounds (5a-i) showed promising antibacterial activities. Compound 5e and 5f showed more potent antibacterial activity with the zone of inhibition more than 22 mm; this is due to that the electron-donating methyl and methoxy substituents on indole nucleus have enhanced binding interactions with biological targets. The bacterial zones of inhibition values are summarized in (Table 3). The bar graph representation zone of inhibition (mm) of compounds (5a-i) against tested bacterial strains is shown in Figure 3.

3.6 | Antifungal studies

The antifungal activity of tested compounds (5a-i) was compared with *Itraconazole* (standard), and results are tabulated in Table 4. The antifungal screening of the compounds revealed good to moderate activity. Compound (5e) and (5f) were showed good inhibitory activity against *Candida albicans* and *Aspergillus niger*. The bar graph representation zone of inhibition (mm) of compounds (5a-i) against tested fungal strains is shown in Figure 4.

4 | CONCLUSION

In conclusion, we have designed, synthesized, and characterized a new series of 3-methyl-1-phenyl-4-((2-phenyl-1*H*-indol-3-yl)methylene)-1*H*-pyrazol-5(4H)-ones (5a-h) by various simple, efficient, solvent-free greener methods. The synthesized compounds screened for free radical scavenging activity, DNA cleavage, and antibacterial analysis. Some of the synthesized compounds, viz., -(5e) and (5f) having $-CH_3$ and $-OCH_3$ substituted at indole moiety exhibit as more prominent for all screening analysis. This is because of that the electron-donating substituents on phenyl ring have increased selectivity for their biological targets and enhanced interactions with binding sites. Consequently, there is a good scope to develop a synthetic less harmful drug to reduce oxidative stress and good antioxidant and also capable to inhibit the growth of the pathogens by cleaving their genome.

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