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Research Article

MULTICOMPONENT ONE-POT SYNTHESIS OF NOVEL INDOLE ANALOGUES AS POTENT ANTIOXIDANT AGENTS

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ABSTRACT

Objective: The purpose of this study was to design and synthesize innovative multicomponent one-pot indole analogues that would be effective antioxidants for the body.

Methods: A novel series of indolyl-pyrimidine derivatives were synthesized and characterized by spectrum analysis, and their antioxidant activity and DPPH, total antioxidant capacity, ferric reducing antioxidant power methods, and DNA cleavage activity were examined.

Results: Compound **6a** displayed promising free radical scavenging and total antioxidant properties. Compound **6b** has demonstrated excellent ferric reducing activity, which is due to the presence of a "CH_3" substitution at five position of indole. When compared to a standard DNA marker, compound **6a** demonstrated the highest DNA cleavage activity at desired concentrations.

Conclusion: We have synthesized novel pyrimidine analogues containing an indole moiety to investigate drug-like molecules. We have devised that a method that is simple, multicomponent, has a short reaction time, and is ecologically benign.

Keywords: Indole, Multicomponent reaction, Antioxidant, DNA cleavage activities.

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INTRODUCTION

The concept of green chemistry has centered on multicomponent reactions (MCRs), in which at least three reactants react to produce a single product that allows the formation of several new bonds [1]. MCRs in organic synthesis have provided numerous interesting and novel modifications [2]. The MCR [3] is a reaction that occurs when three or more reactants combine in a single pot to produce a single product. There are a number of bioactive compounds being synthesized using these reactions [4]. The microwave technique aids in the acceleration of reactions, the efficiency of heating, the absence of side reactions, the high purity of the product, and the environmental friendliness [5]. Synthesis of small molecules using microwave irradiation in a shorter time [6]. Heterocyclic compounds have a high degree of structural diversity and have proven to be broadly and cost-effectively useful as therapeutic agents [7]. Heterocycles have piqued the interest of many synthetic chemists due to their abundance in natural products, and medicinal chemistry is still developing new methods for their synthesis [8]. Indole derivatives have been discovered in a diverse range of natural products, including alkaloids, peptides, and a variety of synthetic compounds [9,10]. Indole alkaloids' recognition of the plant growth hormone, heteroauxin [11], and the essential amino acid tryptophan [12] as indole derivatives has boosted this research. Indole is a very weak base, because it is an electron-rich or -excessive system and the nitrogen atom contributes two electrons to the overall system [13]. Substituted indoles are biologically interesting compounds with antibacterial, antifungal, antiviral, and protein kinase inhibitory properties [14]. Pyrimidines are one of the most important heterocycles with remarkable pharmacological activities, because they are a necessary component of all cells and thus all living matter [15]. Pyrimidine derivatives have been reported to be anti-microbial [16], analgesic, antiviral, anti-inflammatory [17], anti-HIV [18], anti-tubercular [19], anti-tumor [20], anti-neoplastic [21], anti-malarial [22], and hypnotic drugs [23]. The ongoing search for novel agents that target pathological processes in human carcinogenesis has resulted in the development of small molecules that may modulate cell cycle [24].

Free radicals are highly reactive chemical species that contain one or more unpaired electrons. It changes the chemical reactivity of an atom or molecule and is usually more reactive than the corresponding non-radical, and they act as electron acceptors. This loss of electrons is referred to as oxidation, and free radicals are referred to as oxidizing agents, because they cause other molecules to donate their electrons [25].

The hydroxyl radical (OH), superoxide radical (O_{2^-}), and hydroperoxyl radical are examples of free radicals (HO_{2^-}). Antioxidants are compounds that prevent the oxidation of other molecules by delaying the initiation or propagation of oxidizing reactions [26]. Antioxidants act as "free radical scavengers," removing free radicals and inhibiting oxidation [27]. Oxygen free radicals, also known as reactive oxygen species (ROS), are byproducts of normal cellular metabolism [28]. As oxidative stress, ROS can cause damage to cell structure, including lipids and membranes, proteins, and nucleic acids [29]. Oxidative damage/stress, which is associated with ROS, is thought to be involved in a variety of pathological conditions such as diabetes, ageing, atherosclerosis, inflammation, carcinogenesis [30,31], and neurodegenerative [32] disorders such as Parkinson's, Alzheimer's, and amyotrohic lateral sclerosis [33]. These literature reviews encourage us to synthesize novel indole analogues, and the development of simple, efficient, and environmentally friendly approaches for the synthesis of indole derivatives is highly desirable [34-38].

EXPERIMENTAL SECTION

Chemistry

Materials and Methods

All reagents were purchased commercially and purified further using conventional techniques. The melting points were determined using

an uncorrected open capillary technique. Thin-layer chromatography with silica gel-G coated Al plates (Merck) was used to determine the purity of the compounds, and spots were observed by exposing the dried plates to iodine vapors. The IR (KBr pellet) spectra were acquired using an FT-IR spectrometer from Perkin-Elmer (Spectrum ONE). The ^{1}H and ^{13}C NMR (DMSO-d6) spectra were acquired using BRUKER NMR 500 and 125 MHz spectrometers, respectively, and the chemical shift values are presented in ppm (scale) using tetramethylsilane as an internal reference. Electron impact mass spectral measurements were performed at 70 eV on a JEOL GC match spectrometer. Flash EA 1112 series elemental analyzer was used to conduct the analyses.

Experimental

The precursors 3,5-disubstituted indol-2-carboxyhydrazide **(1)** were obtained from 3,5-disubstituted indol-2-carboxylates [34] and 2,5-disubstituted indole-3-carboxaldehydes **(5a-c)** were synthesized by Vilsmeier-Haack formylation reaction of 2,5-disubstituted indoles [35].

General Procedure for the synthesis of 2-(5-bromo-3-phenyl-1H-indole-2-carbonyl) hydrazinecarbothioamide (3)

To a solution of 5-bromo-3-phenyl-indol-2-carboxyhydrazide **1 (**0.01mol) in ethanol, ammonium thiocyanate **2 (**0.01mol) and few drops of hydrochloric acid are added to the reaction mixture. The mixture was refluxed 5–6 h. The completion of the reaction was monitored by TLC. After the completion, the reaction mixture was poured to a beaker containing 100 mL of ice-cold water, filtered, washed with water, and dried. The crude product was crystallized from ethanol.

General Procedure for the synthesis of Ethyl 1-(5-bromo-3-phenyl-1H-indole-2-carboxamido)-4-(5-chloro-2-phenyl-1H-indol-3-yl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (6a-c).

Conventional method

After adding a solution of 2-(5-bromo-3-phenyl-1H-indole-2-carbonyl) hydrazinecarbothioamide **3 (**0.01 mol), ethyl acetoacetate **4 (**0.01 mol), substituted indole aldehyde **5 (**0.05 mol), and a catalytic amount of MK10 Clay, the reaction mixture is refluxed for 6–8 h in ethanol as the solvent. TLC was used to monitor the reaction's conclusion. The reaction mixture was then poured into a beaker containing ice-cold water. The crude products obtained in this manner were filtered and crystallized to generate the named compounds. **6a-c.**

Microwave oven method

A solution of 2-(5-bromo-3-phenyl-1H-indole-2-carbonyl) hydrazinecarbothioamide **3 (**0.01 mol), ethyl acetoacetate **4 (**0.01 mol), substituted indole aldehyde **5 (**0.05 mol), and a catalytic amount of MK10 Clay was added and mixed before being introduced into a Borosil sample crucible. This was microwave irradiated for 8–10 min at 70% microwave intensity. Following completion of the TLC analysis, the reaction mixture was cooled to room temperature, washed with ethanol, and recrystallized to obtain the title compounds. **6a-c.**

Ethyl 1-(5-bromo-3-phenyl-1H-indole-2-carboxamido)-4-(5-chloro-2-phenyl-1H-indol-3-yl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (6a)

Yield 60 and 85%, m.p. 265-67°C; FT-IR (KBr) (cm^{-1}): 3342 (NH), 3295 (NH), 3195 (NH), 3158 (CONH), 1738 (COOEt), 1628 (C=N), 1205 (C=S); ^{1}H NMR (DMSO-d_6) δ: 12.66, 12.32 (s, 1H, 2X indole-NH), 8.46 (s, 1H, Prymidin-NH D_2O exchangeable), 8.46 (s, 1H, CONH D_2O exchangeable), 6.89–7.86 (m, 7H, Ar-H), 4.43 (q, 2H, CH_2), 3.32 (t, 3H, CH_3), 2,35 (s, 3H, CH_3); ^{13}C-NMR (DMSO-d6): δ 184.3, 167.2, 161.9, 152.8, Aromatic carbons 144.6-111.8, 104, 6, 61.8, 47.2, 14.7, 10.2; Mass: m/z 737 $[M]^+$, 739 [M+2], 741 [M+4]; Anal. Calcd. for $C_{37}H_{29}N_5O_3SBrCl$ (737.086), C, 76.13, H, 3.95, N, 9.48. Found: C, 76.17, H, 3.92, N, 9.45%.

Ethyl 1-(5-bromo-3-phenyl-1H-indole-2-carboxamido)-4-(5-methyl-2-phenyl-1H-indol-3-yl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (6b)

Yield 65 and 88%, m.p. 210-12°C; FT-IR (KBr) (cm^{-1}): 3344 (NH), 3292 (NH), 3198 (NH), 3155 (CONH), 1737 (COOEt), 1628 (C=N), 1204 (C=S); ^{1}H NMR (DMSO-d_6) δ: 12.68, 12.30 (s, 1H, 2X indole-NH), 8.47 (s, 1H, Prymidin-NH D_2O exchangeable), 8.47 (s, 1H, CONH D_2O exchangeable), 6.90-7.85 (m, 7H, Ar-H), 4.44 (q, 2H, CH_2), 3.29 (t, 3H, CH_3), 2,38 (s, 3H, CH_3), 2.01 (s, 3H, CH_3); ^{13}C NMR (δ ppm): Mass: m/z 717 $[M]^+$, 719 [M+2]; ^{13}C-NMR (DMSO-d6): δ 184.6, 167.1, 161.7, 152.9, Aromatic carbons 144.4–111.7, 104.3, 61.2, 47.3, 21.6, 14.4, 10.1; Mass: m/z 717 $[M]^{+\cdot}$, 719 [M+2]; Anal. Calcd. for $C_{38}H_{30}N_5O_3SBr$ (717.141), C, 63.51, H, 4.49, N, 9.74. Found: C, 63.55, H, 4.44, N, 9.77%.

Ethyl 1-(5-bromo-3-phenyl-1H-indole-2-carboxamido)-4-(2-phenyl-1H-indol-3-yl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (6c)

Yield 65 and 88%, m.p. 210-12°C; IR cm^{-1}: 3342 (Indole-NH), 3295 (Indole-NH), 3195 (Pyrimidin-NH), 3158 (CONH), 1738 (Ester), 1628 (C=N), 1203 (C=S); ^{1}H NMR (δ ppm): 12.66, 12.32 (s, 1H, 2X indole-NH), 8.46 (s, 1H, Prymidin-NH D_2O exchangeable), 8.46 (s, 1H, CONH D_2O exchangeable), 6.89-7.86 (m, 7H, Ar-H), 4.43 (q, 2H, CH_2), 3.32 (t, 3H, CH_3), 2,35 (s, 3H, CH_3); ^{13}C NMR (δ ppm): Mass: m/z 703 $[M]^+$, 705 [M+2]; ^{13}C-NMR (DMSO-d6): δ 184.2, 167.8, 161.9, 152.7, Aromatic carbons 144.8–111.9, 104.6, 61.3, 47.7, 14.2, 10.2; Mass: m/z 703 $[M]^+$, 705 [M+2]; Anal. Calcd. for $C_{37}H_{30}N_5O_3SBr$ (703.125), C, 63.07 H, 4.29, N, 9.94. Found: C, 63.09 H, 4.31, N, 9.99%.

Antioxidant activity

Free radical scavenging activity

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) technique was used to assess free radical scavenging activity. Different concentrations of samples and butylated hydroxy anisole (BHA) were taken in separate test tubes (10 µg, 50 µg, and 100 µg). MeOH was added to get the volume to 100 µl. Five milliliters of 0.1mM DPPH methanolic solution was added to each of these test tubes and vigorously shaken. For 20 min, the tubes were left to stand at 27°C. The control was prepared in the same manner as described above, but without samples. At 517 nm, the absorbances of the samples were determined. The activity of radical scavenging was determined using the following formula:

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

Total antioxidant capacity

Antioxidant capacity in total determined the total antioxidant capacity. A series of test tubes were filled with samples at various concentrations (10µg, 50 µg, and 100 µg). This was followed by the addition of 1.9 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The tubes were incubated for 90 min at 95°C and then allowed to cool. At 695 nm, the absorbance of each aqueous solution was determined in comparison to a blank. Antioxidant capacity is represented in ascorbic acid equivalents. Equivalents of ascorbic acid are computed using the standard graph of ascorbic acid. The data are presented in terms of ascorbic acid equivalents µg/mg of samples.

Ferric reducing antioxidant power

The antioxidant activity of ferric reducing agents was determined according to samples at various concentrations (10 µg, 50 µg, and 100 µg) were combined with 2.5 mL of 200 mm/L sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide. For 20 min, the mixture was incubated at 50°C. Following that, 2.5 mL of 10% trichloroacetic acid (w/v) was added. 5 mL of this solution was combined with 5 mL distilled water and 1 mL 0.1% ferric chloride, and the absorbance at 700 nm was determined spectrophotometrically. BHA was employed as a control.

DNA cleavage activity

Preparation of culture media

DNA cleavage experiments were done according to the literature [39]. Nutrient broth (peptone, 10; yeast extract, 5; NaCl, 10; in [g/l]) was used

for culturing of *Calf-thymus*. 50 mL 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.

Isolation of DNA

The freshly prepared 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, 50mM 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 3M sodium acetate (pH 4.8) were 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 (10mM tris pH 8.0, 1mM EDTA) and stored in cold condition.

Agarose gel electrophoresis

Cleavage products were analyzed by agarose gel electrophoresis method. Test samples (1 µg/mL) were prepared in DMF. The samples (50 µg and 100 µg) were added to the isolated DNA of Calf-thymus. 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, 0.5M EDTA/1L) and finally loaded on agarose gel and passed the constant 50V 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 VilberLourmat Gel documentation system and then photographed to determine the extent of DNA cleavage. The results are compared with standard DNA marker.

RESULTS AND DISCUSSION

Chemistry

5-bromo-3-phenyl-indol-2-carboxyhydrazide **1** (0.01 mol), ammonium thiocyanate **2** (0.01 mol), and few drops of hydrochloric acid in ethanol to under reflux temperature to afford 2-(5-bromo-3-phenyl-1Hindole-2-carbonyl)hydrazinecarbothioamide **3a (Scheme 1).** The synthesis of ethyl 1-(5-bromo-3-phenyl-1*H*-indole-2-carboxamido)-4-(5-chloro-2-phenyl-1*H*-indol-3-yl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate **6a-c** is carried out by both conventional and microwave method. In conventional method, the reaction of 2-(5-bromo-3-phenyl-1*H*-indole-2-carbonyl)hydrazinecarbothioamide **3 (**0.01 mol), ethyl acetoacetate **4 (**0.01 mol), and substituted indole aldehyde **5 (**0.05 mol) and a catalytic amount of MK10 Clay is added and refluxed for 6–8 h in ethanol to yield the titled compound **6a-c (Scheme 2)**. The same reaction is carried out by microwave method by the reaction of 2-(5-bromo-3-phenyl-1H-indole-2-carbonyl) hydrazine carbothioamide **3 (**0.01 mol), ethyl acetoacetate **4 (**0.01 mol), and substituted indole aldehyde **5 (**0.05 mol) and a catalytic amount of MK10 Clay is mixed and irradiated for 8–10 min with 70% microwave power to yield the titled compounds. The formation of products was monitored by TLC. All the newly synthesized compounds were characterized by IR, ^{1}HNMR, and mass spectroscopic techniques(Table 1).

The IR spectrum of compound **6a** showed a strong absorption peak at 3342 cm^{-1}, 3295 cm^{-1} corresponding to two indole NH, absorption peak at 3195 cm^{-1}, and 3158 cm^{-1} corresponding to pyrimidine NH and CONH, absorption peak at1738 cm^{-1}corresponds to ester carbonyl and absorption at 1628 cm^{-1} and 1203 cm^{-1} which corresponding to C=N and C=S functions, respectively. The ^{1}H NMR spectrum of **6a**, the signal appeared as singlet at δ 12.66 ppm, 12.32 ppm due to two indole NH, singlet at δ 8.46 ppm and δ 8.43 ppm pyrimidine NH and CONH which are D_2O exchangeable. Signal resonated as multiplet between δ 6.89 and 7.86 ppm which corresponds to seventeen aromatic protons. Signal appeared as a Quartet at δ 4.43 ppm is assigned for the CH_2 protons, a signal resonated as triplet at δ 3.32 ppm corresponds to methyl protons and singlet at δ 2.35 ppm assigned for methyl protons. The mass spectrum of compound **6a** has shown molecular ion peak at m/z 739 [M]$^+$, 741[M+2], 743[M+4] which is corresponding to molecular weight and isotopic peak of the compound. These spectral data support the formation of the titled compound **6a.** Similarly, other compounds in the series were synthesized.

Biological evaluation

Antioxidant activity

Free radical scavenging activity

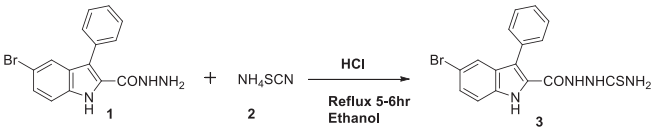
The DPPH technique was used to test the target compounds for free radical scavenging activity. The samples were prepared at concentrations of 10, 50, and 100 µg/100 µL, with BHA serving as the standard. In a methanolic solution, DPPH is a stable free radical. Due to the unpaired electron in DPPH, it has a significant absorption maximum in the visible area at 517 nm (purple color). Among the substances studied, **6a** shown extremely promising free radical scavenging capability. The presence of halogen substitution at the five locations of both indoles accounts for the enhanced activity. Compounds **6b** with a methyl group at another indole ring demonstrated moderate activity, while compounds **6c** shown the least activity when compared to the standard. Fig. 1 shows a bar graph representation of the proportion of free radical scavenging activity.

Total antioxidant capacity

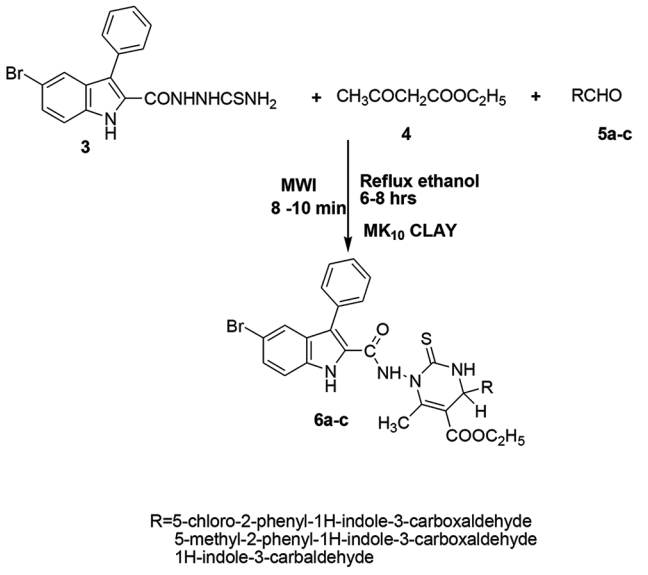
Total antioxidant activity was tested on the newly synthesized substances. Antioxidant capacity is measured in terms of ascorbic acid equivalents. Among the examined compounds, **6a**, which is halogen substituted at the fifth position of both indoles, demonstrated a very high overall antioxidant capacity. When compared to the standard, compounds having methyl substitution at the fifth position of the indole ring and no substitution at the second and fifth positions had the lowest overall antioxidant capacity. Fig. 2 depicts the overall antioxidant activity results.

Ferric reducing antioxidant power activity

The ferric reducing antioxidant activity of the new compounds was tested. The standard was BHA. Compound 6b exhibits high ferric reduction antioxidant activity, whereas other indole counterparts exhibit moderate to low activity. The presence of a methyl group at



Scheme 1: Schematic route for the synthesis of compound 3



Scheme 2: Schematic route for the synthesis of compounds (6a-c)

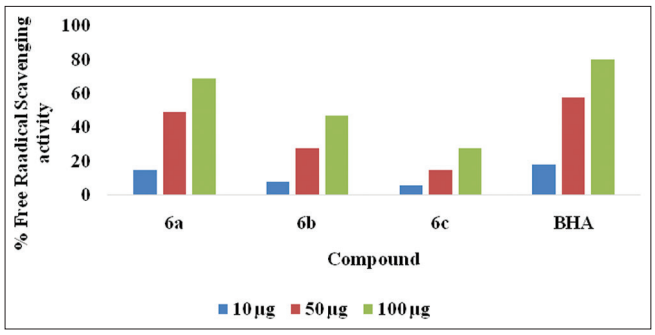


Fig. 1: Free radical scavenging activity of compounds 6a-c

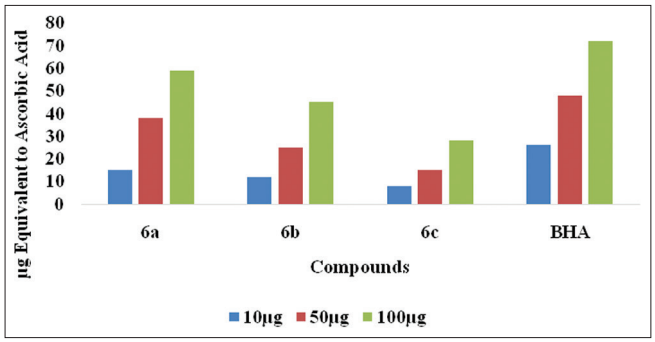


Fig. 2: Total antioxidant capacity of compounds 6a-c

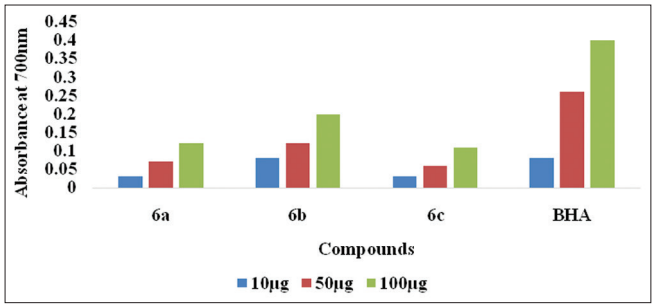


Fig. 3: Ferric reducing antioxidant power activity of compounds 6a-c

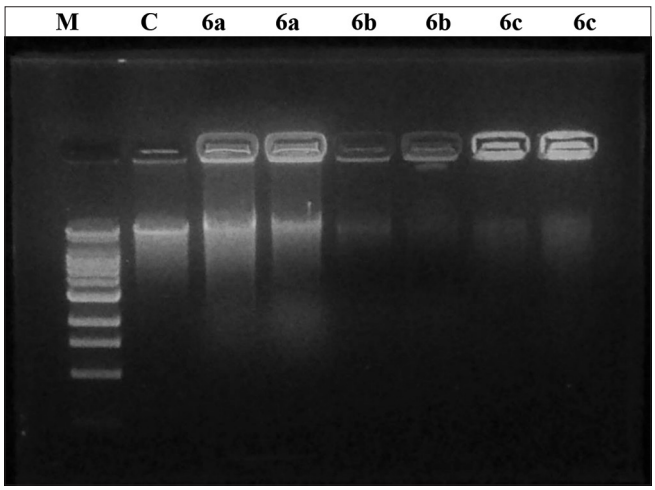


Fig. 4: DNA cleavage activity of compounds 6a-c

the fifth position of the indole ring acts as a superior electron donor, enhancing the compounds' reducing power activity. The results are shown in Fig. 3.

Table 1: Physical data of the target compound 6a-c

Comp. No.	M.P. (°C)	Yield (%)	
		Conventional method	Microwave method
6a	210–12	65	88
6b	265–67	60	85
6c	200–02	50	70

DNA cleavage activity

DNA cleavage tests have been performed on all of the new chemicals. Used gel electrophoresis to measure the DNA cleavage activity. Fig. 4 depicts images of the gels. When compared to a standard DNA marker, compound **6a** demonstrated the maximum activity at 50 µg and 100 µg, whereas the remaining compounds demonstrated moderate activity at 50 µg and 100 µg.

CONCLUSION

In this study, we synthesized new pyrimidine analogues containing an indole moiety to investigate drug-like molecules. We created a simple, MCR with a short reaction time, an environmentally friendly technique, and an excellent yield of all produced chemicals. MK10 Klay has proven to be an effective catalyst. When compared to the standard, compound **6a** showed promising free radical scavenging and total antioxidant activity, with the maximum activity attributed to the presence of halogen at position five of the indole ring. Compound **6b** has demonstrated strong ferric reducing activity, which is attributable to the presence of a "CH_3" substitution at five positions of indole. Compound **6a** demonstrated the strongest DNA cleavage activity at both doses, whereas the remaining compounds demonstrated moderate activity.

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AUTHORS' CONTRIBUTIONS

Parveen R. is mainly involved in the design and execution; the experimental section, results and discussion, and analysis section were performed by Prabhakar W. Chavan, who is the supervisor of the overall work. Dr. Jaishree B. and Dr. Prashant C. Hanamshetty were involved in biological screening and results.

CONFLICTS OF INTEREST

The authors state that they have no conflicts of interest.

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