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Synthesis and Biological Evaluation of Some Novel Hydrazones Carrying Benzimidazole and Pyrene/Vanillin Moiety

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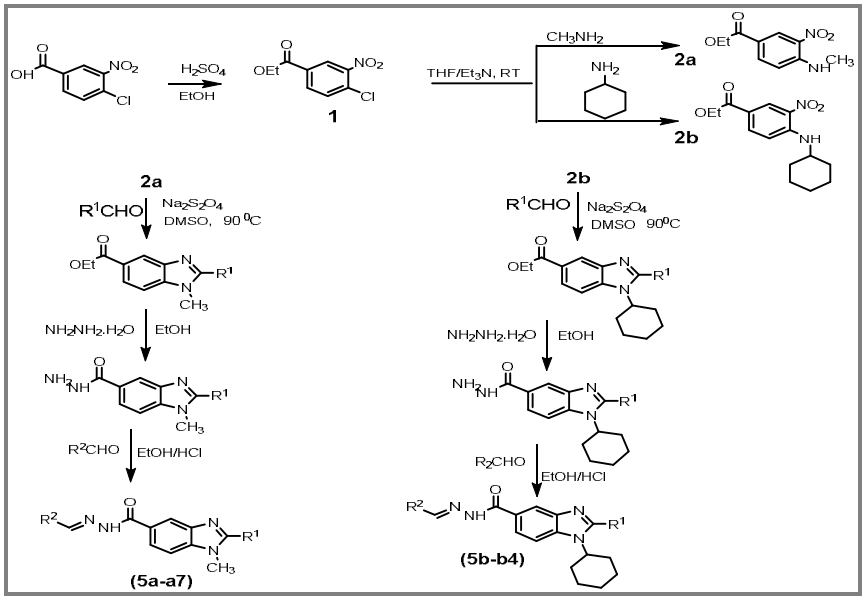
ABSTRACT

Novel series of hydrazones carrying benzimidazole moiety are prepared by the condensation benzimidazole carbohydrazide carrying pyrene/vanillin with appropriate aldehydes. The novel hydrazones so prepared were characterized by Elemental analyses, UV, IR, ^{1}H NMR, and Mass spectral studies. These novel compounds were screened for the antibacterial activity against S. aureus,S. mutans, P. aeruginosa and E. coli, and antifungal activity against A. niger and C. albicans sps. and antioxidant activity by DPPH method. The results revealed that most of the compounds exhibited good antibacterial and antifungal properties at 75 µL mL^{-1}. Besides, they showed good antioxidant activity as compared with standard Butylated hydroxy anisole.

Graphical Abstract



Synthetic route of final compound 5a-a6, 5b-54

Keywords: Benzimidazole, Pyrene, Hydrazones, Antimicrobial agent, Antioxidant property.

INTRODUCTION

Contagious diseases are causing problems throughout the world mainly due to the resistance developed by the microorganisms against the known drugs and hence become a major health problem [**1**]. One way to fight this challenge is, the appropriate usage of the commercially available antibiotics and the other is the improvement or discovery of new anti-microbial agents [**2**]. Compounds containing imine bonds like benzimidazole carbohydrazide have been intensively synthesized for various reasons, one of which is for their biological activities [**3–5**]. Benzimidazole containing compounds, exhibit various pharmacological activities such as anti-cancer [**6**], anti-bacterial [**7, 8**], anti-fungal [**9, 10**], analgesic [**11**] and anti-viral [**12**], antioxidant [**13–15**] etc. Some of the hydrazones have found useful as antibacterial [**16**] cardiovascular applications [**17**], while some other derivatives have been synthesized and tested for inhibition of HIV-1 infection [**18**]. Also, the benzimidazole ring system is a key structure in drug discovery and has become the key component in many medicinally important compounds. The most commonly applied method for preparing of benzimidazole derivative uses 1, 2-diamino benzene as a synthetic precursor [**19**]. For example, benzimidazole containing drugs available in the market currently include: Albendazole, Mebendazole, Thiabendazole, Ridinalazole, Cyclobendazole, etc.

Therefore, in the present communication, we are reporting the synthesis of novel hydrazones bearing benzimidazole and their antimicrobial and antioxidant activities.

MATERIALS AND METHODS

The progress of the reaction was once monitored with the aid of TLC (Merck Silica Gel 60 F–254 thin layer plates). The chemicals had been bought from Sigma-Aldrich or Hi-Media and have been used after distillation/recrystallization. All the solvents were dried by using standard drying techniques mentioned in the literature. Melting points have been measured on an open capillary tube in the Innovative DTC-967A digital melting point tools and are uncorrected. IR Spectra were recorded as KBr pellets on a Shimadzu FT-IR 157 spectrophotometer. All the NMR spectra have been recorded on a Bruker Avance II spectrometer. Chemical shifts (δ) are referred to in phrases of ppm and J-coupling constants are given in Hz. Mass spectra are recorded in a Shimadzu LCMS-8030. C H N analysis was performed with Vario-EI Elementar-III model analyzer.

General procedure for the preparation of compound (1): A mixture of 4-chloro -3-nitrobenzoic acid (0.07 moles), ethyl alcohol (30 mL), and concentrated sulfuric acid (3 mL), was contained in a round-bottom flask of 250 mL. The reaction mixture was refluxed at 70°C for 8 h and tested by TLC (EtOAc: Hexane 30 percent). After the reaction was finished, the mixture was refrigerated and poured into ice-cold water. The precipitate was then formed, filtered, and washed with water and air-dried four times. Finally, to afford a pale-yellow substance it was recrystallized from ethanol. [54-56°C (Lit. 53-55°C); yield 79 %]

General procedure for the preparation of compounds (2a and 2b): To a stirred solution of ethyl 4-chloro-3-nitrobenzoate (0.04 mmol) in triethylamine (0.05 mol) and tetrahydrofuran (15 mL), appropriate primary amine (0.1 mmol) was added drop wise over 30 min. The reaction mixture was once stirred at room temperature for 24 h. The solid formed was filtered, collected, and dried, and recrystallized from ethyl alcohol. [**2a:** m.p 90-92^{o}C; yield 92% and **2b**: m.p 72-74^{o}C; yield 94%]

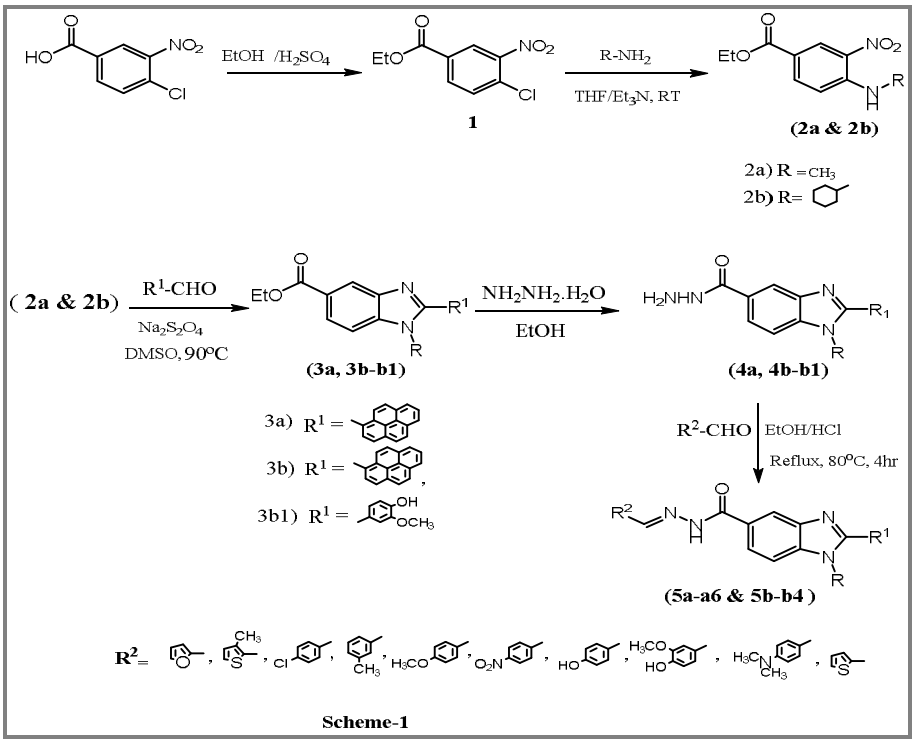
General procedure for the preparation of compounds (3a, 3b and 3b1): A mixture containing ethyl 4-amino substituted -3-nitrobenzoate (0.01 mmol), appropriate aldehydes (0.01 mol), sodium dithionite (0.024 mmol) was taken in DMSO and refluxed with stirring for 4 hr. at 90°C. The reaction completion was monitored by TLC. After completion of the reaction mixture mass was cooled to room temperature, and then poured on to crushed ice. The precipitate that obtained used to be filtered, washed with water, and purified by means of recrystallization from ethanol. Compounds so prepared are [**3a**: m.p 132-34°C, yield 75%; **4b**: m.p 78-80°C, yield 72%; **5b**: m.p 142-45°C, yield 70%]

General procedure for the preparation of compounds (4a, 4b and **4b1):** A mixture of ethyl 4-aminobenzoate (0.001 mol) and hydrazine hydrate (0.002 mol) was refluxed in 15 mL absolute ethanol for 7 h. The excess alcohol was released, concentrated, cooled, and poured onto crushed ice. The solid mass thus separated was dried, and recrystallized from ethanol. [6a: m.p 195-97°C, yield 77%; 7b: m.p 231-33°C, yield 82%; 8b: m.p 185-87°C, yield 75%]

General procedure for the preparation of compounds (5a-a6 and **5b-b4):** An appropriate mixture of equimolar quantities of benzimidazole-5-carbohydrazide **(4a or 4b or 4b1)** (0.01mol), substituted benzaldehydes (0.01 mol) and 2-3 drops of conc. HCl acid was mixed together taken to 20 mL of absolute ethanol and refluxed for 4 h. The reaction completion was monitored by TLC. The resultant mixture was cooled to room temperature. Then the precipitate obtained, was filtered at the filter pump and washed several times with cold water. The crude product was further precipitated by recrystallized from ethanol.

RESULTS AND DISCUSSION

The synthetic routes for the synthesis of targeted compounds are given in scheme 2. The characterization data of the newly synthesized compounds are given in table 1. Esterification of 4-chloro-3-nitrobenzoic acid with ethanol gave the corresponding **(1).** The ester when treated with appropriate aniline with tetrahydrofuran in presence of triethylamine as catalyst at room temperature gave the amino derivative **(2a)** and **(2b)** in 70-95% yields; followed with a reaction of substituted aldehydes by the one-pot nitro reductive cyclization using sodium dithionite in DMSO yielded compounds (**3a, 3b and 3b1**), and then the reaction of compounds (**3a, 3b and 3b1**) with a hydrazine hydrate in ethanol afforded a carbohydrazide bearing benzimidazole derivatives (**4a, 4b and 4b1**). Finally, novel carbohydrazide bearing benzimidazole were refluxed with differently substituted aldehydes in ethanol and a few drops of hydrochloric acid provided the Schiff's base products of hydrazide containing benzimidazole compounds (**5a-a6 and 5b-b4**). The structural elucidation of these newly synthesized compounds was performed by elemental analysis, IR, UV/Vis, ^{1}H NMR, and Mass spectral studies.



Scheme 2. Synthetic path of novel synthesized compounds (**5a-a6** and **5b-b4).**

Table 1. Characterization data of novel synthesized compounds **(5a-a6 & 5b-b4)**

Compound	R_2	Yield (%)	M.P. (^{0}C)	Mol. Formula (Mol.Wt.)	CHN analysis Found (Calc.)		
					C	H	N
5a	Furyl	82	182-84	$C_{30}H_{20}N_4O_2$ (468.52)	76.89 (76.91)	4.25 (4.30)	11.90 (11.96)
5a1	3-methylthiophenyl	88	222-25	$C_{31}H_{22}N_4OS$ (498.60)	74.70 (74.68)	4.43 (4.45)	11.32 (11.24)
5a2	4-chlorophenyl	86	284-86	$C_{32}H_{21}ClN_4O$ (513.60)	74.89 (74.92)	4.09 (4.13)	10.81 (10.92)
5a3	3-methoxyphenyl	83	276-78	$C_{33}H_{24}N_4O_2$ (508.58)	77.96 (77.94)	4.74 (4.76)	11.11 (11.02)
5a4	4-methoxyphenyl	76	293-96	$C_{33}H_{24}N_4O_2$ (508.58)	78.31 (77.94)	5.24 (4.76)	11.21 (11.02)
5a5	4-nitrophenyl	66	287-89	$C_{32}H_{21}N_5O_3$ (523.55)	73.60 (73.41)	4.20 (4.04)	13.33 (13.38)
5a6	4-Hydroxy-3-methoxy phenyl	70	270-72	$C_{33}H_{24}N_4O_3$ (524.58)	75.60 (75.56)	4.50 (4.61)	10.79 (10.68)
5b	4-(dimethylamine)phenyl	78	184-86	$C_{39}H_{35}N_5O$ (589.74)	79.50 (79.43)	5.82 (5.98)	11.81 (11.88)
5b1	4-Hydroxy-3-methoxy phenyl	84	178-80	$C_{38}H_{32}N_4O_3$ (592.72)	77.01 (76.75)	5.69 (5.76)	9.38 (9.42)
5b2	4-Hydroxy-3-methoxy phenyl	87	143-46	$C_{29}H_{30}N_4O_5$ (514.58)	67.62 (67.69)	5.75 (5.88)	10.78 (10.89)
5b3	Hydroxyphenyl	75	145-47	$C_{33}H_{24}N_4O_3$ (524.58)	75.52 (75.56)	4.57 (4.61)	10.73 (10.68)
5b4	Thiophenyl	46	190-92	$C_{26}H_{26}N_4O_3S$ (474.58)	65.51 (65.80)	5.42 (5.52)	11.54 (11.81)

The IR spectrum of compound (5a-a6 and **5b-b4):** IR spectra of all the final synthesized compounds NH bands corresponding to the carbohydrazide were observed while the band characteristic of hydrazide NH_2 disappeared. In typical example, the IR spectrum of benzimidazole incorporates synthesized compound**5a2**, a broad absorption band characteristic of the N-H group was observed at δ, 3385 cm^{-1}. The amide carbonyl C=O) absorption band was observed at 1660 cm^{-1}. The other prominent absorption bands observed are at 1628 cm^{-1} and 1568 cm^{-1} are due to the stretching of >C=N- and>C=C< bonds respectively. Similarly the IR spectra of other carbohydrazide bearing benzimidazole carrying pyrene **5a3** and **5a4** showed their prominent absorption bands at 1662 cm^{-1}, 1633cm^{-1}, 1564 cm^{-1}, and 1659 cm^{-1}, 1628cm^{-1}, 1573cm^{-1} due to the stretching of(C=O), (C=N) and (C=C) bonds respectively.

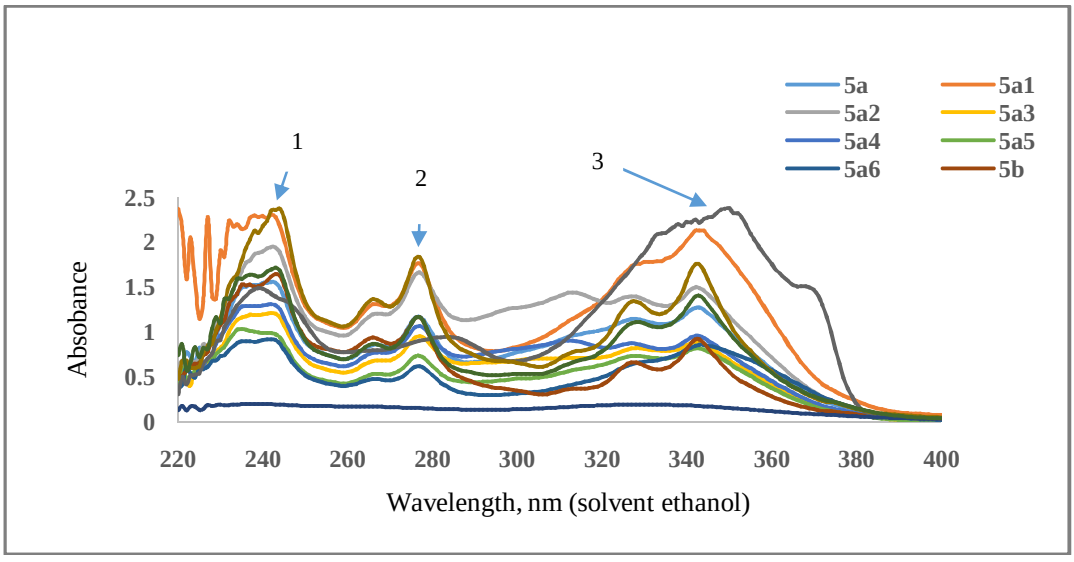


Figure 1. UV spectra of compounds (**5a-a6** and **5b-b4).**

When infrared light interact with molecules, causes to undergo vibrational transitions, the shorter wavelength, higher energy radiation in the UV (200-400 nm) and visible (400-700 nm) range of the electromagnetic spectrum causes many organic molecules to undergo electronic transitions. Because of this phenomenon, UV/Vis is a method used in organic chemistry in order to identify the presence of free electrons or double (π) bonds within a molecule. The UV spectra of all synthesized compounds are shown in figure 1 above.

In the UV spectrum of compound **9c** showed three absorption bands of λ_{max} (solvent ethanol) 242 nm and 277 nm (π→π*) and 342 nm (n→π*) electronic transition respectively. The existence of three peaks indicates the existence of several absorbing species. As conjugated pi systems become larger, the energy gap for a π - π* transition becomes increasingly narrow, and the wavelength of light absorbed correspondingly becomes longer.

The ^{1}HNMR (400MHz, DMSO-d_6) spectrum further confirmed the structure of proposed for the synthesized compounds **5a-a6** and **5b-b4**. As an example, the ^{1}HNMR spectra of **5a2** the methyl protons of benzimidazole (N-CH_3) come into resonance as a singlet at δ, 3.76 ppm whereas aromatic protons appeared at δ, 7.52-8.54 ppm accounting for 17 protons. The N-H proton signal was observed at δ, 12.06 ppm. The protons of the p-chlorophenyl ring appeared as two doublets at δ, 7.54, and 7.56 ppm respectively. Similarly, the ^{1}HNMR spectra of carbohydrazide containing benzimidazole linked pyrene/vanillin **5a-a6** and **5b-b4** were also in conformity with the proposed structure.

Furthermore, the evidence for the formation of newly synthesized compounds was also obtained by recording their mass spectra. For instance, the mass spectrum of carbohydrazide bearing benzimidazole carrying pyrene **5a2** was showed the molecular ion peak (M^++1) at m/z 513.20/515.96 with the relative intensity of 3:1, which is in agreement with the molecular formula $C_{32}H_{21}ClN_4O$, thereby confirming the formation of carbohydrazide bearing benzimidazole carrying pyrene.

Spectral Data of synthesized compounds

(E)-N'-(furan-2-ylmethylene)-1-methyl-2-(pyren-2-yl)-1H-benzo[d]imidazole-5-carbohydrazide (5a): M.F: $C_{30}H_{20}N_4O_2$; M.Wt: 468.52; yield 82%, MP 182-84°C; IR (KBr, m, cm^{-1}): 3396 (-NH), 3215 (-Ar CH), 3035 (-Ali CH), 1653 (-C=O), 1626 (-C=N), 1550 (-C=C), and 1060 (-C-N); ^{1}HNMR: (DMSO-d_6, 400 MHz), δ 3.76 (s, 3H, N-CH_3 Protons), δ 6.66 (d, J=7.96Hz, 1H, pyrene-H-2), δ 6.95 (d, J=8.28Hz, 1H, pyrene-H-10), δ 7.86-8.51 (m, 13H, Pyrene and Aromatic Protons), δ 11.92 (s, 1H, -CH=N), and δ 11.92 (s, 1H, -NH).

(E)-1-methyl-N'-((3-methylthiophen-2-yl)methylene)-2-(pyren-2-yl)-1H-benzo[d]imidazole-5-carbohydrazide (5a1): M.F: $C_{31}H_{22}N_4OS$; M.Wt: 498.60; yield 88%, MP 222-225°C; IR (KBr, m, cm^{-1}): 3414 (-NH), 3037 (-Ali CH), 3226 (-Ar CH), 1656 (-C=O), 1633 (-C=N), 1565 (-C=C), and 1062 (-C-N); ^{1}HNMR: (DMSO-d_6, 400 MHz), δ 2.35 (s, 3H, C-CH_3 protons), 3.79 (s, 3H, N-CH_3 protons), 6.99 (d, J=4.96Hz, 1H, pyrene-H-2), δ 7.58 (d, J=4.88Hz, 1H, pyrene-H-10), δ 7.93-8.53 (m, 13H, Pyrene and Aromatic Protons), δ 8.66 (s, 1H, -CH=N,), and δ 11.94 (s, 1H, -NH) .

(E)-N'-(4-chlorobenzylidene)-1-methyl-2-(pyren-2-yl)-1H-benzo[d]imidazole-5-carbohydrazide (5a2): M.F: $C_{32}H_{21}ClN_4O$; M.Wt: 513.00 yield 86%, MP 284-286°C; IR (KBr, m, cm^{-1}): 3385 (-NH), 3047 (-Ali CH), 3218 (-Ar CH), 1659 (-C=O), 1627 (-C=N), 1568 (-C=C), 1027 (-C-N) and 745 (-C-Cl); ^{1}HNMR: (DMSO-d_6, 400 MHz), δ 3.76 (s, 3H, N-CH_3 protons), 7.54 (d, J=7.32Hz, 2H, pyrene-H-2), δ 7.79 (d, J=6.96Hz, 2H, pyrene-H-10), δ 7.86-8.51 (m, 13H, Pyrene and Aromatic Protons), δ 11.92 (s, 1H, -CH=N), and δ 11.92 (s, 1H, -NH).

(E)-N'-(3-methoxybenzylidene)-1-methyl-2-(pyren-2-yl)-1H-benzo[d]imidazole-5-carbohydrazide (5a3): M.F: $C_{33}H_{24}N_4O_2$; M.Wt: 508.58 yield 83%, MP 276-278°C; IR (KBr, m, cm^{-1}): 3400 (-NH), 3042 (-Ali CH), 3218 (-Ar CH), 1662 (-C=O),1633 (-C=N), 15642 (-C=C), and 1033 (-C-N); ^{1}HNMR: (DMSO-d_6, 400 MHz), δ 3.83 (s, 3H, C-OCH_3 protons), 3.88 (s, 3H, N-CH_3 protons), 6.99 (d, J=7.40Hz, 1H, pyrene-H-2), δ 7.58 (d, J=7.94Hz, 1H, pyrene-H-10), δ 7.03-8.59 (m, 16H, Pyrene and Aromatic Protons), δ 8.62 (s, 1H, -CH=N), and δ 12.31 (s, 1H, -NH).

(E)-N'-(4-methoxybenzylidene)-1-methyl-2-(pyren-2-yl)-1H-benzo[d]imidazole-5-carbohydrazide (5a4): M.F: $C_{33}H_{24}N_4O_2$; M.Wt: 508.58 yield 76%, MP 293-296°C; IR (KBr, m, cm^{-1}): 3388 (-NH), 3030 (-Ali CH), 3222 (-Ar CH), 1659 (-C=O), 1628 (-C=N), 1573 (-C=C), and 1027 (-C-N); ^{1}HNMR: (DMSO-d_6, 400 MHz), δ 2.36 (s, 3H, C-OCH_3 protons), 3.78 (s, 3H, N-CH_3 protons), 7.29 (d, J=7.48Hz, 2H, pyrene-H-2), δ 7.58 (d, J=7.66Hz, 2H, pyrene-H-10), δ 7.65-8.50 (m, 13H, Pyrene and Aromatic Protons), δ 8.52 (s, 1H, -CH=N), and δ 11.97 (s, 1H, -NH).

(E)-N'-(4-nitrobenzylidene)-1-methyl-2-(pyren-2-yl)-1H-benzo[d]imidazole-5-carbohydrazide (5a5): M.F: $C_{32}H_{21}N_5O_3$; M.Wt: 523.55 yield 66%, MP 287-289°C; IR (KBr, m, cm^{-1}): 3237 (-NH), 2988 (-Ali CH), 3222 (-Ar CH), 1663 (-C=O), 1585 (-C=N), 1564 (-C=C), and 1027 (-C-N); ^{1}HNMR: (DMSO-d_6, 400 MHz), δ 3.86 (s, 3H, N-CH_3 protons), 6.99 (d, J=8.02Hz, Hz, 1H, pyrene-H-2), δ 7.58 (d, J=8.68Hz, 1H, pyrene-H-10), δ 7.93-8.53 (m, 13H, Pyrene and Aromatic Protons), and δ 8.69 (s, 1H, -CH=N), and δ 12.49 (s, 1H, -NH).

(E)-N'-(4-hydroxy-3-methoxybenzylidene)-1-methyl-2-(pyren-2-yl)-1H-benzo[d]imidazole-5-carbohydrazide (5a6): M.F: $C_{33}H_{24}N_4O_3$; M.Wt: 524.58 yield 70%, MP 270-272°C; IR (KBr, m, cm^{-1}): 3465 (-OH), 3375 (-NH),3158 (-Ali CH), 3222 (-Ar CH), 1651 (-C=O),1606 (-C=N), 1582 (-C=C), and 1026 (-C-N); ^{1}HNMR: (DMSO-d_6, 400 MHz), δ 3.65 (s, 3H, OCH_3 protons), 3.82 (s, 3H, N-CH_3 protons), 6.99 (d, 1H, pyrene-H-2), δ 7.58 (d, 1H, pyrene-H-10), δ 7.93-8.53 (m, 13H, Pyrene and Aromatic Protons), and δ 8.66 (s, 1H, -CH=N), and δ 11.96 (s, 1H, -NH).

(E)-1-cyclohexyl-N'-(4-(dimethylamino)benzylidene)-2-(pyren-2-yl)-1H-benzo[d]imidazole-5-carbohydrazide (5b): M.F: $C_{39}H_{35}N_5O$; M.Wt: 589.74 yield 78%, mp 184-186°C; IR (KBr, m, cm^{-1}): 3215 (-NH), 3034 (-Ali CH), 2934 & 2855 (-Ar CH), 1705 (-C=O), 1595 (-C=N), 1512 (-C=C), and 1016 (-C-N); ^{1}HNMR: (DMSO-d_6, 400 MHz), δ 1.50-2.35 9 (m, 6H, N-cyclohexyl protons)2.35 (s, 3H, N-CH_3 protons), 3.79 (s, 3H, N-cyclohexyl protons), 6.99 (d, 1H, pyrene-H-2), δ 7.58 (d, 1H, pyrene-H-10), δ 7.93-8.53 (m, 13H, Pyrene and Aromatic Protons), and δ 8.66 (s, 1H, -CH=N).

(E)-1-cyclohexyl-N'-(4-hydroxy-3-methoxybenzylidene)-2-(pyren-2-yl)-1H-benzo[d]imidazole-5-carbohydrazide (5b1): M.F: $C_{38}H_{32}N_4O_3$; M.Wt: 592.69 yield 84%, mp 178-180°C; IR (KBr, m, cm^{-1}): 3389 (-OH), 3213 (-NH), 3044 (-Ali CH), 2934 & 2865 (-Ar CH), 1703 (-C=O), 1595 (-C=N), 1512 (-C=C), and 1015 (-C-N); ^{1}HNMR: (DMSO-d_6, 400 MHz), δ 1.24 (m, 3H, cyclohexyl), 1.36-1.43 (m, 1H, cyclohexyl), 1.68 (m, 4H, cyclohexyl), 2.51–2.55 (m, 2H, cyclohexyl), and 3.83–3.95 (m, 1H, N–CH of cyclohexyl), 4.37 (s, 3H, OCH_3),), 6.87 (d, J=7.68Hz,1H, pyrene-H-2), δ 7.12 (d, J=7.60,1H, pyrene-H-10), δ 7.26-8.51 (m, 13H, Pyrene and Aromatic Protons), δ 8.57 (s, 1H, -CH=N) and δ 11.84 (s, 1H, -NH).

(E)-1-cyclohexyl-N'-(4-hydroxy-3-methoxybenzylidene)-2-(4-hydroxy-3-methoxyphenyl)-1H-benzo[d]imidazole-5-carbohydrazide (5b2): M.F: $C_{29}H_{30}N_4O_5$; M.Wt: 514..58 yield 87%, mp 143-145°C; IR (KBr, m, cm^{-1}): 3240 (-OH),3157 (-NH), 3049 (-Ali CH), 2941 & 2853 (-Ar CH), 1747 (-C=O), 1626 (-C=N), 1585 (-C=C), and 1067 (-C-N); ^{1}HNMR: (DMSO-d_6, 400 MHz), δ 1.25 (m, 3H, cyclohexyl), 1.34-1.41 (m, 1H, cyclohexyl), 1.72 (m, 4H, cyclohexyl), 2.50–2.45 (m, 2H, cyclohexyl), and 3.80–3.92 (m, 1H, N–CH of cyclohexyl), 3.86 (s, 6H, OCH_3),), δ 7.26-8.51 (m, 11H, Aromatic Protons), and δ 8.57 (s, 1H, -CH=N) and δ 11.86 (s, 1H, -NH).

(E)-N'-(4-hydroxybenzylidene)-1-cyclohexyl-2-(4-hydroxy-3-methoxyphenyl)-1H-benzo[d] imidazole-5-carbohydrazide (5b3): M.F: $C_{28}H_{28}N_4O_4$; M.Wt: 484.55 yield 75%, mp 145-147°C; IR (KBr, m, cm^{-1}): 3354 (-OH),3154 (-NH), 3042 (-Ali CH), 2933 & 2855 (-Ar CH), 1705 (-C=O), 1591 (-C=N), 1479 (-C=C), and 1072 (-C-N), ^{1}HNMR: (DMSO-d_6, 400 MHz), δ 1.36 (m, 3H, cyclohexyl), 1.45 (m, 1H, cyclohexyl), 1.68-2.00 (m, 4H, cyclohexyl), 2.18–2.33 (m, 2H, cyclohexyl), and 3.92 (m, 1H, N–CH of cyclohexyl), 4.36-4.41 (t, 3H, OCH_3),), 6.85 (dd, J=8.12Hz,1H, hydroxyl phenyl-H-3 &5), δ 7.60-8.59 (m, 13H, Aromatic Protons), δ 8.68 (s, 1H, -CH=N), and δ 11.76 (s, 1H, -NH).

(E)-1-cyclohexyl-2-(4-hydroxy-3-methoxyphenyl)-N'-((thiophen-2-yl) methylene)-1H-benzo [d] imidazole-5-carbohydrazide (5b4): M.F: $C_{26}H_{26}N_4O_3S$; M.Wt: 474.57 yield 46%, mp 190-192°C; IR (KBr, m, cm^{-1}): 3350 (-OH),3154 (-NH), 3046 (-Ali CH), 2936 & 2858 (-Ar CH), 1709 (-C=O), 1620 (-C=N), 1589 (-C=C), 1065 (-C-N), and 629 (-C-S), ^{1}HNMR: (DMSO-d_6, 400 MHz), δ 1.32 (m, 3H, cyclohexyl), 1.43 (m, 1H, cyclohexyl), 2.10 (m, 4H, cyclohexyl), 2.15–2.32 (m, 2H, cyclohexyl), and 3.83 (m, 1H, N–CH of cyclohexyl), 4.25 (t, 3H, OCH_3),), δ 7.58-8.54 (m, 10H, Aromatic Protons), δ 8.58 (s, 1H, -CH=N), and δ 11.68 (s, 1H, -NH).

Antimicrobial Activity: The synthesized compounds (**5a-a6** and **5b-b4**) have been evaluated for their in vitro antibacterial activity against (*S. mutans* and *S. aureus*) Gram positive bacteria, (*P. aeruginosa* and *E. coli*) Gram negative bacteria and antifungal activity against *A. niger* and *C. albicans* by the Disc diffusion method [**20**], and the results of antimicrobial activity are given in Table 2 and 3. Compound 5a, 5a1, 5a3, 5a6, 5b1, 5b2, 5b3, and 5b4 showed excellent antifungal activities for all the tested pathogen with minimal inhibitory concentration (MIC) that ranged between 26 and 30 µL mL^{-1} whereas the remaining compounds showed moderate antifungal activities with MIC ranging between 12 and 25 µL mL^{-1} against *A. niger* and *C. albicans* having a MIC value of 24-32 and 35-40 µL mL^{-1} respectively. Compound 5a6, 5b2 and 5b3 showed good antibacterial activities against all the tested pathogen including Gram-positive and Gram-negative bacteria with minimal inhibitory concentration (MIC) that ranged between 18-20, 13-18 and 13-18 µL mL^{-1} respectively, whereas the others showed moderate antibacterial activities with MIC ranging between 10 and 15 µL mL^{-1} against (*S. aureus, S. mutans, E. coli*, and *P aeruginosa*) (30-34, 30-32, 30-38 and 40-43) µL mL^{-1} respectively. Among all the compounds tested, compound 5a6 showed an excellent (MIC) 30 µg mL^{-1} against *C. albicans*. All the synthesized compounds showed better antifungal activity than antibacterial activity. However, compound 5a1 and 5a2 did not show inhibition against bacteria *E. coli*, as observed by the absence of an inhibition zone (data not shown).

Table 2. The antibacterial activity evaluation data of newly synthesized compounds **(5a-a6 & 5b-b4)**

Tested Pathogenic Bacteria	Addition of Compound into plate (µL mL^{-1})	Diameter of zone of inhibition (in mm) for tested compounds											
		5a	**5a1**	**5a2**	**5a3**	**5a4**	**5a5**	**5a6**	**5b**	**5b1**	**5b2**	**5b3**	**5b4**
S. aureus	5	-	-	-	-	-	-	-	-	-	-	-	-
	10	-	-	-	-	-	-	-	-	-	-	-	-
	25	-	-	-	-	-	-	13	-	-	-	-	-
	50	-	-	-	-	-	-	13	-	10	13	-	18
	75	12	10	14	12	10	12	15	10	12	15	10	20
Ciprofloxacin		**30**	**32**	**32**	**34**	**32**	**32**	**34**	**32**	**30**	**32**	**32**	**32**
S.mutans	5	-	-	-	-	-	-	-	-	-	-	-	-
	10	-	-	-	-	-	-	-	-	-	-	-	-
	25	-	-	-	-	-	-	13	-	-	-	-	-
	50	-	-	-	-	-	-	15	-	10	13	-	13
	75	12	13	14	13	13	12	18	10	15	18	10	15
Ciprofloxacin		**30**	**30**	**32**	**30**	**30**	**32**	**32**	**32**	**30**	**32**	**32**	**32**
E. coli	5	-	-	-	-	-	-	-	-	-	-	-	-
	10	-	-	-	-	-	-	-	-	-	-	-	-
	25	-	-	-	-	-	-	-	-	-	-	-	-
	50	-	-	-	-	-	-	-	-	10	-	10	12
	75	15	-	-	16	10	15	16	10	15	10	13	15
Ciprofloxacin		**30**	**30**	**34**	**34**	**30**	**34**	**34**	**35**	**34**	**35**	**38**	**38**
P aeruginosa	5	-	-	-	-	-	-	-	-	-	-	-	-
	10	-	-	-	-	-	-	-	-	-	-	-	-
	25	-	-	-	-	-	-	-	-	-	-	-	-
	50	-	12	12	13	15	15	13	10	10	12	-	10
	75	15	13	15	18	20	18	20	13	12	13	15	12
Ciprofloxacin		**43**	**42**	**43**	**42**	**43**	**42**	**43**	**40**	**42**	**43**	**42**	**43**

Where: (-) = Resistant (no activity)

Table 3. The antifungal activity evaluation data of novel synthesized compounds **(5a-a6** and **5b-b4)**

Tested Pathogenic Fungi	Addition of Compound into plate (µL mL^{-1})	Diameter of zone of inhibition (in mm) for tested compounds											
		5a	5a1	5a2	5a3	5a4	5a5	5a6	5b	5b1	5b2	5b3	5b4
	5	-	-	-	-	-	-	-	-	-	-	-	-
	10	-	-	-	-	-	-	-	-	-	-	-	-
C. albicans	25	15	12	13	12	12	12	12	18	13	14	-	13
	50	22	20	23	20	18	17	16	20	24	20	15	20
	75	23	28	25	28	20	20	30	25	28	23	20	26
Fluconazole		**38**	**40**	**40**	**38**	**38**	**38**	**35**	**38**	**35**	**34**	**35**	**38**
	5	-	-	-	-	-	-	-	-	-	-	-	-
	10	-	-	-	-	-	-	-	-	-	-	-	-
A. niger	25	18	13	-	18	13	-	-	-	-	18	16	20
	50	20	18	13	20	18	15	13	13	18	20	20	25
	75	25	20	15	23	20	20	20	18	20	25	26	28
Ciprofloxacin		**30**	**32**	**30**	**32**	**30**	**28**	**25**	**28**	**24**	**25**	**26**	**25**

Where: (-) = Resistant (no activity)

Antioxidant Activity: Antioxidants are substances that may guard cells against the harm brought on by unstable molecules called regarded as free radicals. DPPH test, which is primarily based on the capacity of DPPH, a stable free radical, to decolorize within the presence of antioxidants, is an immediate and reliable technique for finding out. Radical scavenging action. Free radical scavenging activity of the test compounds was carried out based on the scavenging activity of stable DPPH and the results were compared with the standard antioxidant Butylated Hydroxy Anisole, abbreviated as BHA. The DPPH (1, 1-diphenyl-2-picrylhydrazyl) assay is used as a preliminary test to investigate the antioxidant activity of the synthesized compounds. All the test samples analyte has shown good antioxidant activity in comparison to standard BHA at 100 µg mL^{-1}. The results of DPPH free radical scavenging activity are shown in table 4.

$$\textbf{Free radical scavenging activity (\%)} = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \times 100$$

Table 4. The antioxidant activity evaluation data of novel synthesized compounds (**5a-a6 & 5b-b4**)

Compound (100 µg mL^{-1})	% free radical scavenging	Compound (100 µg mL^{-1})	% free radical scavenging
5a	50.88	5b	51.75
5a1	40.79	5b1	39.12
5a2	53.51	5b2	46.86
5a3	47.81	5b3	37.44
5a4	47.81	5b4	41.36
5a5	51.32	BHA	93.42
5a6	48.68	Control	0.00

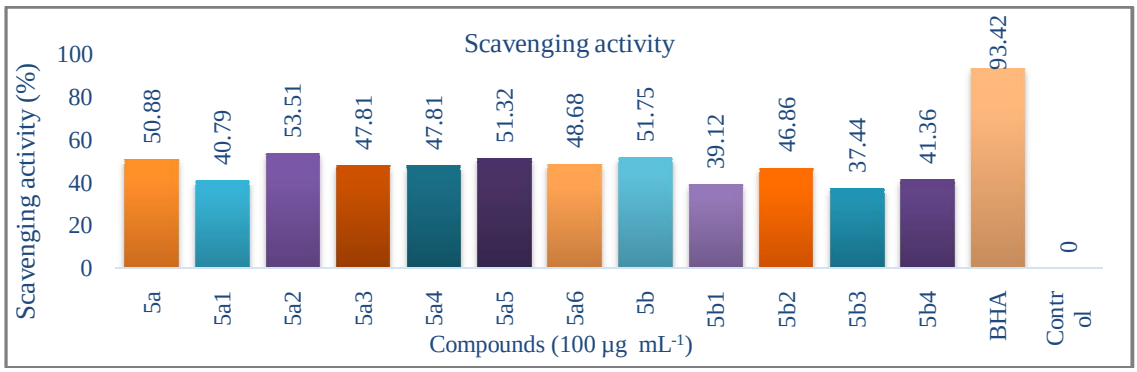


Figure 2. Pictorial representation for DPPH scavenging assay of the compounds (5a-a6 and 5b-b4).

APPLICATION

The synthesized novel compounds were evaluated for their potential antibacterial, antifungal and antioxidant activities. The results of antibacterial and antifungal screening data revealed that the synthesized compounds showed considerable and varied activity against the tested microorganisms. They also demonstrated a moderate antioxidant activity in comparison to standard BHA. The results opened that these derivatives seemed as promising active pharmacophore. More studies should undertake to determine the degree of a variety of biological activities.

CONCLUSION

In this study, 12 unknown novel compounds **(5a-a6** and **5b-b4)** were successfully synthesized in excellent yields. The new series of carbohydrazide bearing benzimidazole and pyrene/vanillin so synthesized were evaluated for antibacterial, antifungal, and antioxidant activities. The structures of the compounds were determined by IR, UV/VIS, ^{1}H-NMR, and MS spectral data. All the synthesized heterocyclic compounds have exhibited excellent antifungal, good antibacterial, and significant antioxidant activity.

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