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Novel Benzimidazole based Hydrazide-hydrazone Compounds: Synthesis, Characterization and Antimicrobial Assessment

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This work presents a comprehensive study on the design, synthesis, spectral characterization and antimicrobial assessment of new hydrazide-hydrazone incorporated benzimidazole compounds (**6a-p**). The synthesis of these compounds (**6a-p**) involved the condensation of benzimidazole derivative, 4-(1-methyl-5-nitro-1*H*-benzo[*d*]imidazol-2-yl)butane hydrazide with substituted aromatic aldehydes, utilizing an efficient and environmentally benign synthetic route. The IR, NMR and mass spectrometry were among the spectroscopic methods used to characterize the novel synthesized substances to confirm their chemical structures. The antimicrobial properties of the synthesized benzimidazole-based hydrazide-hydrazone compounds (**6a-p**) were systematically assessed against a panel of pathogenic microorganisms, including two Gram-positive and two Gram-negative bacteria and three fungi. The potency of antimicrobial drugs was assessed by determining their minimum inhibitory concentrations (MIC). The findings demonstrate that compounds **6k** and **6p** have moderated antibacterial action against positive-Gram bacteria *S. pyogenus* (MTCC 442) and *S. aureus* (MTCC 96), but considerable antimicrobial activity against Gram-negative bacteria *E. coli* (MTCC 443) and *P. aeruginosa* (MTCC 1688). Although every drug exhibits mild to moderate antifungal efficacy against *A. niger* (MTCC 282), *A. clavatus* (MTCC 1323) and *C. albicans* (MTCC 227). Furthermore, *in silico* prediction of compounds pharmacokinetic properties was also conducted.

Keywords: Benzimidazole, Hydrazide-hydrazone, Antibacterial activity, Antifungal activity.

INTRODUCTION

Over the past few years, antimicrobial resistance (AMR) has emerged as a major issue in public health around the world. The efficiency of currently available antimicrobial drugs is seriously threatened by the emergence of resistant strains of bacteria, viruses, fungi and parasites. There has never been a greater need for an ongoing, careful search for novel antimicrobial drugs [1,2]. When bacteria develop defenses against drugs meant to eradicate or manage them, it is known as antimicrobial resistance. Resistance strains have developed more quickly as a result of the overuse and misuse of antibiotics in agriculture, human healthcare and animal healthcare [2,3].

Heterocyclic compounds, characterized by their diverse structures and versatile chemical reactivity, have emerged as promising candidates in the development of antimicrobial agents [4]. The medicinal value of benzimidazole compounds has received a lot of attention lately since their diverse pharmacological properties. Benzimidazole, a bicyclic heterocyclic ring system, serves as a crucial scaffold in the development of pharmaceutical agents with promising therapeutic potential. This class of compounds has exhibited several biological properties, such as antimicrobial [5-9], anti-inflammatory [10,11], antiviral [12,13], anticancer [14-18] and anthelminthic properties [19]. The benzimidazole moiety is present in the core structures of several commercially available medications, including as omeprazole (proton pump inhibitor), flubendazole (anthelmintic), thiabendazole (antifungal), telmisartan (antihypertensive), Hoechst33342 (antiviral and antitumor), albendazole (antibacterial), bendamustine (anticancer), benzitramide (analgesic), mizolastine (H3 antagonist) and nocodazole (anticancer) [20,21] (as displayed in Fig. 1).

Derivatives of hydrazide-hydrazone exhibit a broad range of biological actions, including antimicrobial [22-25], anticancer [26-29], anti-inflammatory [30], antiviral [31], antiprotozoal [32] and anticonvulsant [33] effects. They are found

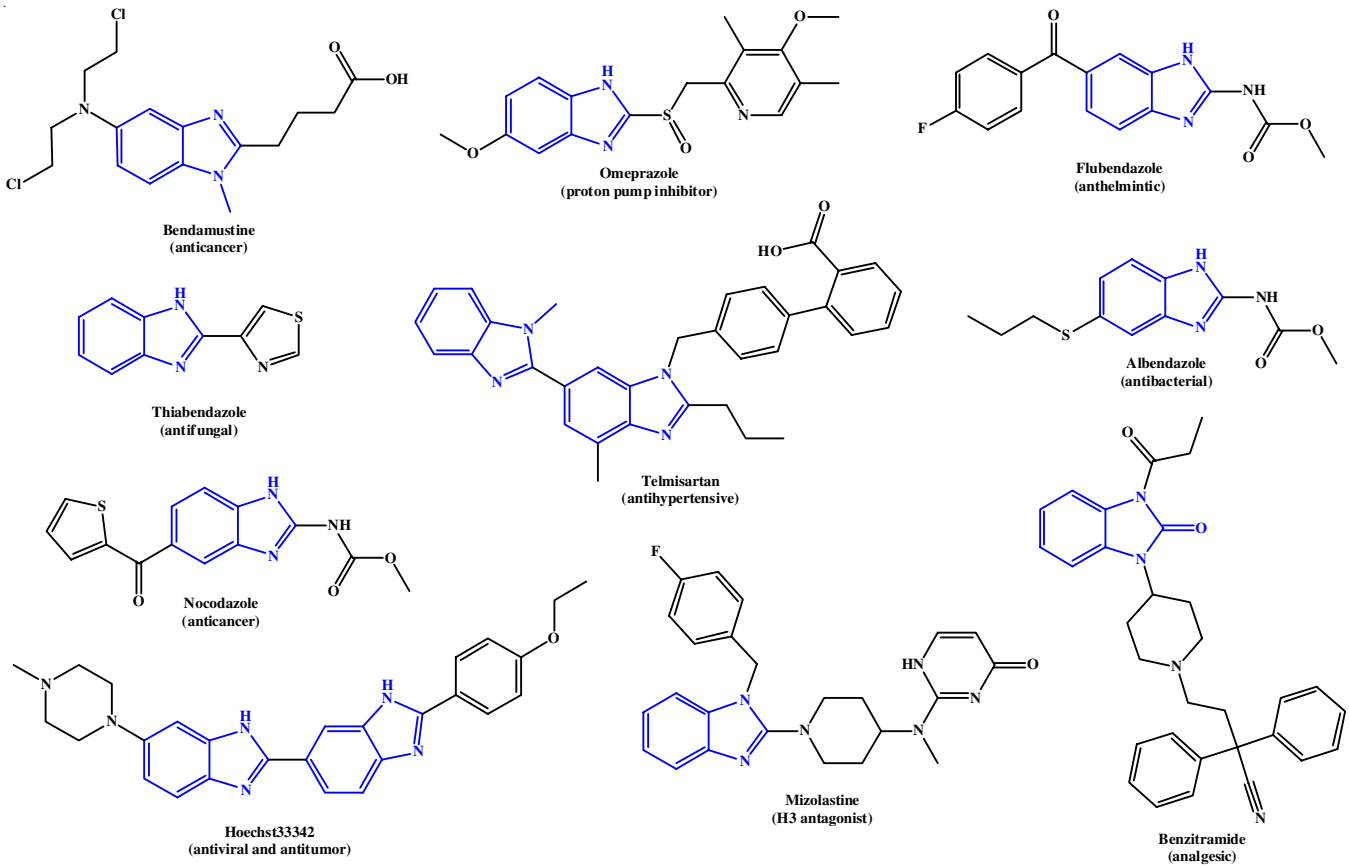


Fig. 1. Structures of some benzimidazole based drugs

in numerous bioactive compounds. Consequently, many medicinal chemists create different hydrazide-hydrazones and assess their biological activity. The biological characteristic of this family of chemicals that is most mentioned in scientific literature is antibacterial activity [34]. Moreover, other frequently employed chemotherapeutic drugs bearing hydrazide-hydrazone moiety include nitrofurazone [35], furazolidone [36,37] and nitrofurantoin [38,39] (as displayed in Fig. 2).

Considering these informations and the need to discover new powerful antimicrobial drugs, we are synthesizing a range of novel heterocyclic compounds (**6a-p**) using benzimidazole and hydrazide-hydrazone as its structural constituents in the current effort. Along with their *in silico* pharmacokinetic parameters (ADME) predicted, their *in vitro* antimicrobial efficacy against four distinct bacterial strains and three different fugal stains was also examined.

EXPERIMENTAL

The chemicals employed in the synthesis were all of laboratory quality and the solvents used were of commercial grade and used as such. The uncorrected melting points of synthetic compounds were ascertained using the utilization of the open capillary method. Using UV light (254 nm) to highlight the spots on Merck 60 F-254 silica gel aluminum plates, thin-layer chromatography (TLC) was utilized to track the progress of the reaction. A Shimadzu-8400 FT-IR spectrometer was used to gather the spectra and an Applied Biosystems-API 2000 LC/MS/MS was used to get the mass spectra. ^{1}H & ^{13}C NMR spectra of the derivatives were recorded using the Bruker Advance Neo 400 MHz spectrometer; the chemical shifts were reported in ppm (δ units).

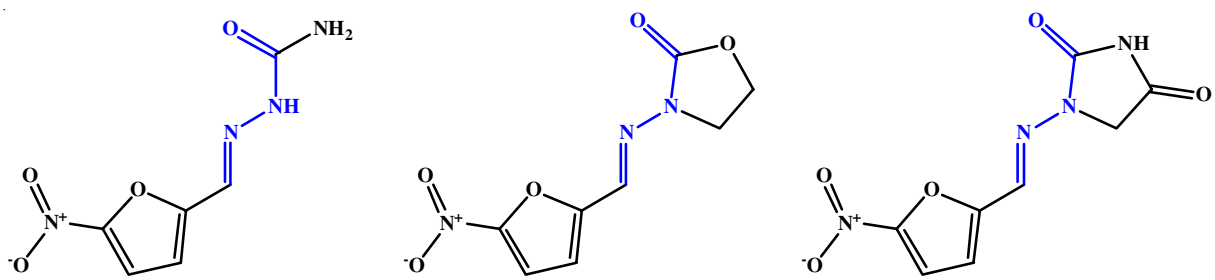


Fig. 2. Structure of some drugs containing hydrazide-hydrazone moiety

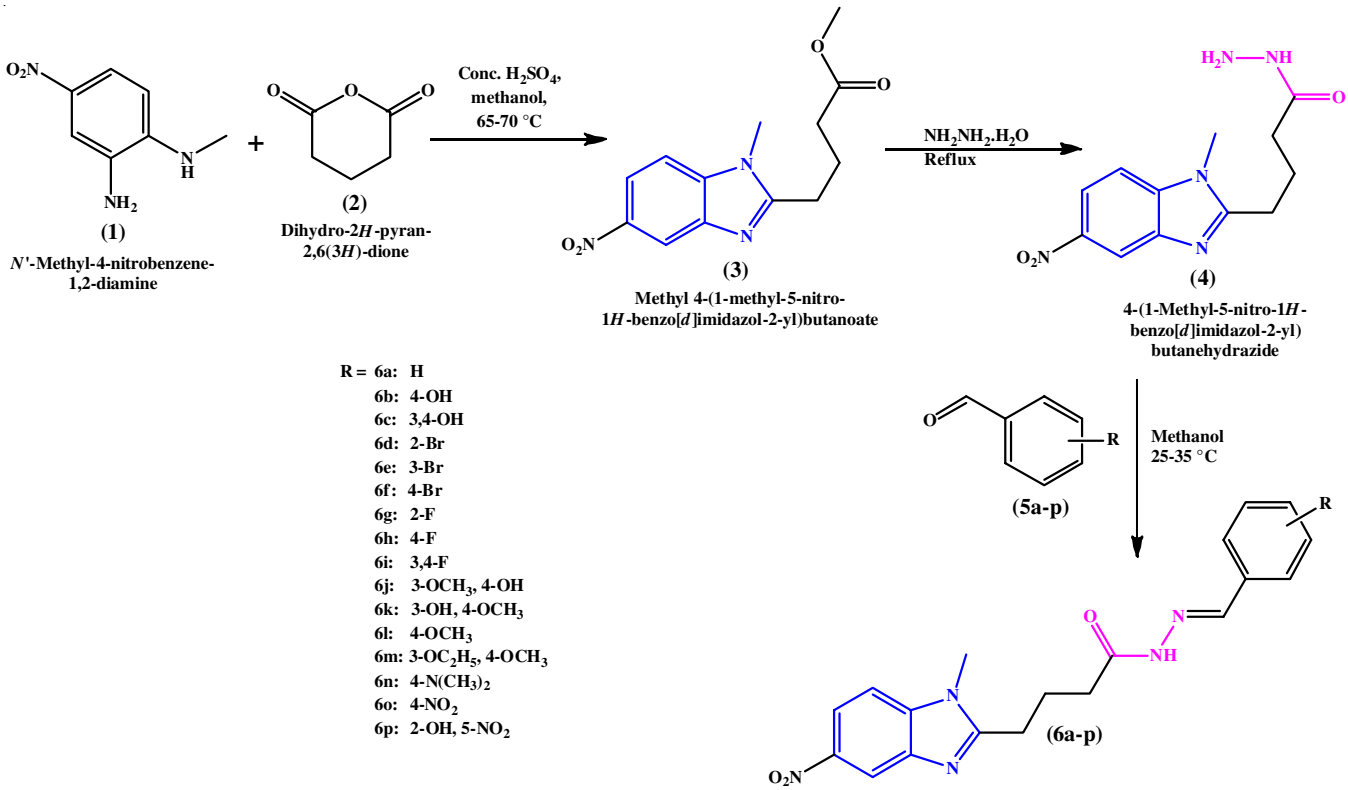
Synthesis of methyl 4-(1-methyl-5-nitro-1*H*-benzo[*d*]-imidazol-2-yl) butanoate (3): *N*-Methyl-4-nitrobenzene-1,2-diamine (**1**) (0.01 mol) and (20 mL) methanol was taken (0.012 mol) dihydro-2*H*-pyran-2,6(3*H*)-dione (glutaric anhydride) (**2**) was charged in reaction mixture. Reaction mass continuously stirred at 65-75 ºC for 2-3 h (0.012 mol) conc. H_2SO_4 was slowly added in reaction mixture. Reaction mass and continuously stirred at 65-75 ºC. TLC using MDC:methanol (9.5:0.5) as a mobile phase was used for reaction monitoring. Filtered the suspension and wet solid was washed by (4 mL) methanol. Obtained solid **3** was dried under vacuum in oven at 45-50 ºC.

Synthesis of 4-(1-methyl-5-nitro-1*H*-benzo[*d*]imidazol-2-yl)butane hydrazide (4): Compound **3** (0.01 mol) and aqueous hydrazine hydrate (30 mL, 80% w/w) was mixed and reflux the reaction mass for 12 h. TLC in methanol:MDC (0.2: 9.8) as a mobile phase was used to monitor a progress of the reaction. Gradually cool the reaction mixture. Filtered the solid, washed with 5 mL of water and then dried in oven at 45-50 ºC. Yield: 60%, m.f.: $C_{12}H_{15}N_5O_3$ (*m.w.*: 277.28). IR (KBr, ν_{max}, cm^{-1}): 3327.32 & 3300.31 (N–H *str.*), 3061.13 (arom. C–H *str.*), 2945.40 (alkyl C–H *str.*), 2906.82 (alkyl C–H *str.*), 1618.33 (C=N), 1518.03 (N–H bend.), 1475.59 (arom. C–C *str.*), 1435.09 (arom. C–C), 1329.00 (C–N *str.*). ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm: 8.90 (s, 1H), 8.36 (d, *J* = 2.1 Hz, 1H), 8.06 (dd, *J* = 8.9, 2.2 Hz, 1H), 7.66 (d, *J* = 8.9 Hz, 1H), 4.09 (s, 2H), 3.73 (s, 3H), 2.83 (t, *J* = 7.4 Hz, 2H), 2.10 (t, *J* = 7.2 Hz, 2H), 2.00-1.89 (m, 2H). ^{13}C NMR ($CDCl_3$, 400 MHz) δ_C ppm: 171.25, 159.42, 142.32, 141.31, 140.33, 117.27, 114.37, 110.21, 32.63, 30.10, 26.17, 22.51. Mass: *m/z* 278.44 $(M+1)^+$.

General procedure for the synthesis of 4-(1-methyl-5-nitro-1*H*-benzo[*d*]imidazol-2-yl)butane hydrazide (6a-p): A solution of compound **4** (0.01 mol) and various substituted benzaldehyde (**5a-o**, 0.012 mol) in methanol (30 mL) at 25-35 ºC stirred for 18 h. The completion of the reaction was look altered by TLC using methanol:MDC (0.2:9.8) as a mobile phase. The separated solid was filtered and recrystallized by methanol to obtain a crude product (**6a-p**) (**Scheme-I**). The stated procedure was foll-owed to synthesize the entire library of the titled compounds (**6a-p**) and characterized with various spectral analyses.

***N′*-Benzylidene-4-(1-methyl-5-nitro-1*H*-benzo[*d*]-imidazol-2-yl)butane hydrazide (6a):** Yield: 65%, m.p.: 176-178 ºC, m.f.: $C_{19}H_{19}N_5O_3$ (*m.w.*: 365.39). IR (KBr, ν_{max}, cm^{-1}): 3188.44 (N–H *str.*), 3113.21 (arom. C–H *str.*), 2956.97 (alkyl C–H *str.*), 2835.45 (alkyl C–H *str.*), 1680.05 (C=O *str.* amide), 1599.04 (C=N), 1518.03 (N–H bend.), 1479.45 (arom. C–C *str.*), 1423.51 (arom. C–C *str.*), 1332.86 (C–N *str.*). ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm: 11.36 (d, *J* = 36.9 Hz, 1H), 8.46 (d, *J* = 1.8 Hz, 1H), 8.18-7.94 (m, 2H), 7.73 (t, *J* = 8.9 Hz, 1H), 7.68-7.55 (m, 2H), 7.47-7.33 (m, 3H), 3.83 (d, *J* = 5.0 Hz, 3H), 3.06-2.95 (m, 2H), 2.83 (t, *J* = 7.2 Hz, 1H), 2.40 (t, *J* = 7.1 Hz, 1H), 2.13 (dd, *J* = 14.4, 7.2 Hz, 2H). ^{13}C NMR ($CDCl_3$, 400 MHz) δ_C ppm: 174.47, 168.66, 160.04, 146.28, 143.01, 141.87, 140.87, 134.76, 130.10, 129.20, 127.41, 127.06, 117.75, 114.87, 110.72, 31.76, 30.56, 26.71, 21.98. Mass: *m/z* 366.67 $(M+1)^+$.

***N′*-(4-Hydroxybenzylidene)-4-(1-methyl-5-nitro-1*H*-benzo[*d*]imidazol-2-yl)butane hydrazide (6b):** Yield: 59%, m.p.: 222-224 ºC, m.f.: $C_{19}H_{19}N_5O_4$ (*m.w.*: 381.39). IR (KBr, ν_{max}, cm^{-1}): 3612.79 (O-H *str.*), 3184.34 (N–H *str.*), 3083.49 (arom. C–H *str.*), 2967.16 (alkyl C–H *str.*), 2864.41 (alkyl C–H *str.*), 1673.37 (C=OH amide), 1593.49 (C=N), 1517.95 (N–H bend.), 1464.21 (arom. C–C *str.*), 1409.46 (arom. C–C*str.*),



Scheme-I: Synthetic route of novel benzimidazole based hydrazide-hydrazone compounds (6a-p)

1324.67 (C–N *str.*). ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm: 9.48 (s, 1H), 8.50 (s, 1H), 8.39 (d, *J* = 1.1 Hz, 2H), 7.96 (d, *J* = 26.4 Hz, 2H), 7.49-7.31 (m, 2H), 6.87-6.69 (m, 2H), 3.85-3.80 (m, 3H), 2.81-2.76 (m, 2H), 2.34-2.29 (m, 2H), 2.04-1.90 (m, 2H). ^{13}C NMR ($CDCl_3$, 400 MHz) δ_C ppm: 170.94, 159.84, 158.32, 148.64, 139.31, 138.18, 137.74, 129.72, 129.72, 126.92, 116.78, 115.60, 115.60, 114.03, 111.07, 33.76, 31.72, 29.50, 19.00. Mass: *m/z* 382.65 $(M+1)^+$.

***N′*-(3,4-Dihydroxybenzylidene)-4-(1-methyl-5-nitro-1*H*-benzo[*d*]imidazol-2-yl)butane hydrazide (6c):** Yield: 62%, m.p.: 130-132 ºC, m.f.: $C_{19}H_{19}N_5O_5$ (*m.w.*: 397.38). IR (KBr, ν_{max}, cm^{-1}): 3618.19 (O-H *str.*), 3186.14 (N–H *str.*), 3086.15 (arom. C–H *str.*), 2968.15 (alkyl C–H *str.*), 2867.19 (alkyl C–H *str.*), 1678.64 (C=O *str.* amide), 1597.16 (C=N), 1513.97 (N–H bend.), 1464.56 (arom. C–C *str.*), 1404.64 (arom. C–C *str.*), 1326.14 (C–N *str.*). ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm: 8.46 (s, 1H), 8.36 (d, *J* = 1.5 Hz, 2H), 7.99 (s, 1H), 7.92 (d, *J* = 11.4 Hz, 2H), 7.01 (s, 1H), 6.79 (s, 1H), 6.62 (s, 1H), 3.86-3.81 (m, 3H), 2.81-2.76 (m, 2H), 2.35-2.30 (m, 2H), 2.03-1.90 (m, 2H). ^{13}C NMR ($CDCl_3$, 400 MHz) δ_C ppm: 170.94, 159.84, 148.19, 147.92, 145.53, 139.31, 138.18, 137.74, 127.98, 121.02, 116.78, 116.63, 115.92, 114.03, 111.07, 33.76, 31.72, 29.50, 19.00. Mass: *m/z* 398.45 $(M+1)^+$.

***N′*-(2-Bromobenzylidene)-4-(1-methyl-5-nitro-1*H*-benzo[*d*]imidazol-2-yl)butane hydrazide (6d):** Yield: 64%, m.p.: 190-192 ºC, m.f.: $C_{19}H_{18}N_5O_3Br$ (*m.w.*: 444.28). IR (KBr, ν_{max}, cm^{-1}): 3186.48 (N–H *str.*), 3082.64 (arom. C–H *str.*), 2964.37 (alkyl C–H *str.*), 2849.25 (alkyl C–H *str.*), 1676.15 (C=O *str.* amide), 1597.16 (C=N), 1517.56 (N–H bend.), 1474.46 (arom. C–C *str.*), 1405.75 (arom. C–C *str.*), 1325.64 (C–N *str.*). ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm: 8.48 (s, 1H), 8.42 (s, 1H), 7.96 (d, *J* = 29.7 Hz, 2H), 7.47 (d, *J* = 3.0 Hz, 2H), 7.37 (s, 1H), 7.20 (s, 1H), 3.90-3.85 (m, 3H), 2.76-2.71 (m, 2H), 2.41-2.36 (m, 2H), 2.08-2.03 (m, 2H). ^{13}C NMR ($CDCl_3$, 400 MHz) δ_C ppm: 170.94, 159.84, 145.72, 139.31, 138.18, 137.74, 133.42, 132.59, 128.04, 127.79, 127.06, 124.60, 116.78, 114.03, 111.07, 33.76, 31.72, 29.50, 19.00. Mass: *m/z* 445.67 $(M+1)^+$.

***N′*-(3-Bromobenzylidene)-4-(1-methyl-5-nitro-1*H*-benzo[*d*]imidazol-2-yl)butane hydrazide (6e):** Yield: 58%, m.p.: 180-182 ºC, m.f.: $C_{19}H_{18}N_5O_3Br$ (*m.w.*: 444.28). IR (KBr, ν_{max}, cm^{-1}): 3184.58 (N–H *str.*), 3086.21 (arom. C–H *str.*), 2968.55 (alkyl C–H *str.*), 2856.67 (alkyl C–H *str.*), 1670.41 (C=O *str.* amide), 1595.18 (C=N), 1514.17 (N–H bend.), 1473.66 (arom. C–C *str.*), 1406.15 (arom. C–C *str.*), 1327.07 (C–N *str.*). ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm: 11.48 (d, *J* = 43.7 Hz, 1H), 8.45 (d, *J* = 2.0 Hz, 1H), 8.13 (dd, *J* = 10.2, 3.6 Hz, 1H), 8.01 (d, *J* = 63.0 Hz, 1H), 7.82 (d, *J* = 27.0 Hz, 1H), 7.73 (dd, *J* = 13.1, 8.9 Hz, 1H), 7.61 (dt, *J* = 14.1, 7.3 Hz, 2H), 7.37 (dt, *J* = 22.8, 7.8 Hz, 1H), 3.84 (d, *J* = 3.6 Hz, 3H), 3.06-2.95 (m, 2H), 2.83 (t, *J* = 7.2 Hz, 1H), 2.41 (t, *J* = 7.1 Hz, 1H), 2.13 (p, *J* = 7.1 Hz, 2H). ^{13}C NMR ($CDCl_3$, 400 MHz) δ ppm: 170.94, 159.84, 145.72, 139.31, 138.18, 137.74, 133.42, 132.59, 128.04, 127.79, 127.06, 124.60, 116.78, 114.03, 111.07, 33.76, 31.72, 29.50, 19.00. Mass: *m/z* 446.58 $(M+1)^+$.

***N′*-(4-Bromobenzylidene)-4-(1-methyl-5-nitro-1*H*-benzo[*d*]imidazol-2-yl)butane hydrazide (6f):** Yield: 65%, m.p.: 196-198 ºC, m.f.: $C_{19}H_{18}N_5O_3Br$ (*m.w.*: 444.28). IR (KBr, ν_{max}, cm^{-1}): 3187.34 (N–H *str.*), 3085.65 (arom. C–H *str.*), 2966.24 (alkyl C–H *str.*), 2843.16 (alkyl C–H *str.*), 1666.27 (C=O *str.* amide), 1598.21 (C=N), 1516.24 (N–H *bend.*), 1479.14 (arom. C–C *str.*), 1406.15 (arom. C–C *str.*), 1323.24 (C–N *str.*). ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm: 8.30 (d, *J* = 19.0 Hz, 2H), 7.91 (d, *J* = 16.5 Hz, 2H), 7.51-7.32 (m, 4H), 3.85-3.80 (m, 3H), 2.68-2.63 (m, 2H), 2.40-2.35 (m, 2H), 2.05-2.00 (m, 2H). ^{13}C NMR ($CDCl_3$, 400 MHz) δ_C ppm: 170.94, 159.84, 148.64, 139.31, 138.18, 137.74, 132.49, 132.49, 132.38, 129.31, 129.31, 123.99, 116.78, 114.03, 111.07, 33.76, 31.72, 29.50, 19.00. Mass: *m/z* 445.67 $(M+1)^+$.

***N′*-(2-Fluorobenzylidene)-4-(1-methyl-5-nitro-1*H*-benzo[*d*]imidazol-2-yl)butane hydrazide (6g):** Yield: 67%, m.p.: 188-190 ºC, m.f.: $C_{19}H_{18}N_5O_3F$ (*m.w.*: 383.38). IR (KBr, ν_{max}, cm^{-1}): 3227.64 (N–H *str.*), 3084.44 (arom. C–H *str.*), 3059.64 (arom. C–H *str.*), 2966.24 (alkyl C–H *str.*), 1662.67 (C=O *str.* amide), 1592.34 (C=N), 1521.64 (N–H *bend.*), 1448.19 (arom. C–C *str.*), 1423.64 (arom. C–C *str.*), 1336.72 (C–N *str.*). ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm: 8.49 (s, 1H), 8.35 (s, 1H), 7.97 (d, *J* = 28.8 Hz, 2H), 7.52 (s, 1H), 7.29 (s, 1H), 7.19 (s, 1H), 7.03 (s, 1H), 3.91-3.86 (m, 3H), 2.76-2.71 (m, 2H), 2.40-2.35 (m, 2H), 2.08-2.03 (m, 2H). ^{13}C NMR ($CDCl_3$, 400 MHz) δ_C ppm: 170.94, 160.39, 159.84, 149.22, 139.31, 138.18, 137.74, 130.79, 129.40, 125.34, 124.55, 117.39, 116.78, 114.03, 111.07, 33.76, 31.72, 29.50, 19.00. Mass: *m/z* 384.26 $(M+1)^+$.

***N′*-(4-Fluorobenzylidene)-4-(1-methyl-5-nitro-1*H*-benzo[*d*]imidazol-2-yl)butane hydrazide (6h):** Yield: 66%, m.p.: 184-188 ºC, m.f.: $C_{19}H_{18}N_5O_3F$ (*m.w.*: 383.38). IR (KBr, ν_{max}, cm^{-1}): 3227.56 (N–H *str.*), 3084.67 (arom. C–H *str.*), 3058.34 (arom. C–H *str.*), 2963.36 (alkyl C–H *str.*), 1659.15 (C=O *str.* amide), 1583.46 (C=N), 1519.94 (N–H bend.), 1439.57 (arom. C–C *str.*), 1436.16 (arom. C–C *str.*), 1334.67 (C–N *str.*). ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm: 9.46 (s, 1H), 8.53 (s, 1H), 8.39 (s, 1H), 7.96 (d, *J* = 27.2 Hz, 2H), 7.58-7.53 (m, 2H), 7.07-7.00 (m, 2H), 3.85-3.80 (m, 3H), 2.82-2.77 (m, 2H), 2.33-2.28 (m, 2H), 2.00-1.90 (m, 2H). ^{13}C NMR ($CDCl_3$, 400 MHz) δ_C ppm: 170.94, 161.27, 159.84, 148.64, 139.31, 138.18, 137.74, 130.72, 130.37, 130.37, 116.78, 115.52, 115.52, 114.03, 111.07, 33.76, 31.72, 29.50, 19.00. Mass: *m/z* 384.28 $(M+1)^+$.

***N′*-(3,4-Difluorobenzylidene)-4-(1-methyl-5-nitro-1*H*-benzo[*d*]imidazol-2-yl)butane hydrazide (6i):** Yield: 65%, m.p.: 176-178 ºC, m.f.: $C_{19}H_{17}N_5O_3F_2$ (*m.w.*: 401.37). IR (KBr, ν_{max}, cm^{-1}): 3229.14 (N–H *str.*), 3086.14 (arom. C–H *str.*), 3057.38 (arom. C–H *str.*), 2969.35 (alkyl C–H *str.*), 1677.16 (C=O *str.* amide), 1571.56 (C=N), 1505.64 (N–H bend.), 1429.46 (arom. C–C *str.*), 1404.26 (arom. C–C *str.*), 1331.87 (C–N *str.*). ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm: 8.35 (s, 1H), 8.27 (s, 1H), 7.92 (d, *J* = 17.9 Hz, 2H), 7.34 (s, 1H), 7.20 (s, 1H), 7.01 (s, 1H), 3.85-3.80 (m, 3H), 2.73-2.68 (m, 2H), 2.42-2.37 (m, 2H), 2.06-2.01 (m, 2H). ^{13}C NMR ($CDCl_3$, 400 MHz) δ_C ppm: 170.94, 159.84, 150.45, 147.92, 147.51, 139.31, 138.18, 137.74, 132.39, 124.36, 118.26, 116.93, 116.78, 114.03, 111.07, 33.76, 31.72, 29.50, 19.00. Mass: *m/z* 402.65 $(M+1)^+$.

***N′*-(4-Hydroxy-3-methoxybenzylidene)-4-(1-methyl-5-nitro-1*H*-benzo[*d*]imidazol-2-yl)butane hydrazide (6j):** Yield: 58%, m.p.: 200-204 ºC, m.f.: $C_{20}H_{21}N_5O_5$ (*m.w.*: 411.41). IR (KBr, ν_{max}, cm^{-1}): 3612.79 (O-H *str.*), 3180.72 (N–H *str.*),

3091.99 (arom. C–H *str.*), 2980.12 (alkyl C–H *str.*), 2852.81 (alkyl C–H *str.*), 1668.48 (C=O *str.* amide), 1612.54 (C=N), 1514.17 (N–H bend.), 1475.59 (arom. C–C *str.*), 1458.23 (arom. C–C *str.*), 1348.29 (C–N *str.*), 1288.49 (C-O-C *str.*), 1033.88 (C-O-C *str.*). ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm: 9.79 (s, 3H), 8.47 (s, 3H), 8.34 (s, 3H), 7.96 (d, *J* = 29.5 Hz, 6H), 7.05 (s, 2H), 6.96 (s, 4H), 6.78 (s, 3H), 3.87-3.79 (m, 18H), 2.69-2.64 (m, 6H), 2.39-2.34 (m, 6H), 1.98-1.93 (m, 5H). ^{13}C NMR ($CDCl_3$, 400 MHz) δ_C ppm: 170.94, 159.84, 148.36, 148.12, 147.92, 139.31, 138.18, 137.74, 127.81, 122.57, 116.78, 115.00, 114.03, 111.26, 111.07, 56.79, 33.76, 31.72, 29.50, 19.00. Mass: *m/z* 412.77 $(M+1)^+$.

***N′*-(3-Hydroxy-4-methoxybenzylidene)-4-(1-methyl-5-nitro-1*H*-benzo[*d*]imidazol-2-yl)butane hydrazide (6k):** Yield: 60%, m.p.: 132-136 ºC, m.f.: $C_{20}H_{21}N_5O_5$ (*m.w.*: 411.41). IR (KBr, ν_{max}, cm^{-1}): 3618.28 (O-H *str.*), 3185.62 (N–H *str.*), 3098.49 (arom. C–H *str.*), 2985.34 (alkyl C–H *str.*), 2846.78 (alkyl C–H *str.*), 1669.67 (C=O *str.* amide), 1624.64 (C=N), 1518.64 (N–H bend.), 1472.34 (arom. C–C *str.*), 1456.39 (arom. C–C *str.*), 1346.54 (C–N *str.*), 1275.46 (C-O-C *str.*), 1036.49 (C-O-C *str.*). ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm: 11.18 (d, *J* = 29.9 Hz, 1H), 9.51 (d, *J* = 21.7 Hz, 1H), 8.48-8.42 (m, 1H), 8.14 (dd, *J* = 8.2, 2.9 Hz, 1H), 7.93 (d, *J* = 21.9 Hz, 1H), 7.73 (dd, *J* = 12.4, 9.0 Hz, 1H), 7.19 (d, *J* = 35.8 Hz, 1H), 7.05-6.96 (m, 1H), 6.79 (dd, *J* = 18.2, 8.1 Hz, 1H), 4.13 (q, *J* = 5.2 Hz, 1H), 3.83 (d, *J* = 4.7 Hz, 3H), 3.78 (d, *J* = 23.8 Hz, 3H), 3.17 (d, *J* = 5.2 Hz, 1H), 3.06-2.95 (m, 2H), 2.80 (t, *J* = 7.1 Hz, 1H), 2.37 (t, *J* = 7.1 Hz, 1H), 2.19-2.08 (m, 2H). ^{13}C NMR ($CDCl_3$, 400 MHz) δ_c ppm: 173.99, 168.32, 159.78, 148.79, 146.76, 142.55, 140.55, 126.01, 122.25, 121.06, 117.50, 115.81, 114.59, 110.33, 109.69, 55.72, 33.54, 30.28, 26.57, 21.77. Mass: *m/z* 412.77 $(M+1)^+$.

***N′*-(4-Methoxybenzylidene)-4-(1-methyl-5-nitro-1*H*-benzo[*d*]imidazol-2-yl)butane hydrazide (6l):** Yield: 70%, m.p.: 182-186 ºC, m.f.: $C_{20}H_{21}N_5O_4$ (*m.w.*: 395.41). IR (KBr, ν_{max}, cm^{-1}): 3264.62 (N–H *str.*), 3064.76 (arom. C–H *str.*), 2986.48 (alkyl C–H *str.*), 2844.15 (alkyl C–H *str.*), 1687.15 (C=O *str.* amide), 1593.54 (C=N), 1517.76 (N–H bend.), 1476.48 (arom. C–C *str.*), 1456.18 (arom. C–C *str.*), 1347.64 (C–N *str.*), 1243.45 (C-O-C *str.*), 1032.49 (C-O-C *str.*). ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm: 9.57 (s, 1H), 8.58 (s, 1H), 8.49 (s, 1H), 7.98 (s, 1H), 7.92 (s, 1H), 7.58-7.40 (m, 2H), 7.03-6.86 (m, 2H), 3.86-3.78 (m, 6H), 2.64-2.59 (m, 2H), 2.37-2.32 (m, 2H), 1.93-1.88 (m, 2H). ^{13}C NMR ($CDCl_3$, 400 MHz) δ_c ppm: 170.94, 160.10, 159.84, 148.64, 139.31, 138.18, 137.74, 129.12, 129.12, 127.06, 116.78, 114.32, 114.32, 114.03, 111.07, 56.04, 33.76, 31.72, 29.50, 19.00. Mass: *m/z* 396.67 $(M+1)^+$.

***N′*-(3-Ethoxy-4-methoxybenzylidene)-4-(1-methyl-5-nitro-1*H*-benzo[*d*]imidazol-2-yl)butane hydrazide (6m):** Yield: 60%, m.p.: 184-188 ºC, m.f.: $C_{22}H_{25}N_5O_5$ (*m.w.*: 439.46). IR (KBr, ν_{max}, cm^{-1}): 3268.16 (N–H *str.*), 3066.64 (arom. C–H *str.*), 2978.15 (alkyl C–H *str.*), 2835.45 (alkyl C–H *str.*), 1684.37 (C=O *str.* amide), 1594.17 (C=N), 1516.57 (N–H bend.), 147.49 (arom. C–C *str.*), 1456.46 (arom. C–H *str.*), 1346.19 (C–N *str.*), 1230.46 (C-O-C *str.*), 1041.24 (C-O-C *str.*). ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm: 9.47 (s, 1H), 8.55 (s, 1H), 8.38 (s, 1H), 7.99 (s, 1H), 7.92 (s, 1H), 7.24 (d, *J* = 1.3 Hz, 2H), 6.98 (s, 1H), 4.10-3.95 (m, 2H), 3.85-3.80 (m, 3H), 3.80-3.75 (m, 3H), 2.83-2.78 (m, 2H), 2.34-2.29 (m, 2H), 2.00-1.90 (m, 2H), 1.41-1.36 (m, 3H). ^{13}C NMR ($CDCl_3$, 400 MHz) δ_C ppm: 170.94, 159.84, 151.77, 149.24, 147.92, 139.31, 138.18, 137.74, 128.91, 121.80, 116.78, 114.52, 114.03, 111.95, 111.07, 64.52, 56.79, 33.76, 31.72, 29.50, 19.00, 13.83. Mass: *m/z* 440.26 $(M+1)^+$.

***N′*-(4-(Dimethyl amino)benzylidene)-4-(1-methyl-5-nitro-1*H*-benzo[*d*]imidazol-2-yl)butane hydrazide (6n):** Yield: 68%, m.p.: 180-184 ºC, m.f.: $C_{21}H_{24}N_6O_3$ (*m.w.*: 40.45). IR (KBr, ν_{max}, cm^{-1}): 3229.18 (N–H *str.*), 3086.41 (arom. C–H *str.*), 3055.16 (arom. C–H *str.*), 29.76.15 (alkyl C–H *str.*), 1681.15 (C=O *str.* amide), 1584.16 (C=N), 1567.15 (N–H bend.), 1437.19 (arom. C–C *str.*), 1436.31 (arom. C–C *str.*), 1336.15 (C–N *str.*). ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm: 8.32 (s, 1H), 8.15 (s, 1H), 7.90 (d, *J* = 17.3 Hz, 2H), 7.45-7.27 (m, 2H), 6.74-6.56 (m, 2H), 3.84-3.79 (m, 3H), 2.92-2.87 (m, 6H), 2.69-2.64 (m, 2H), 2.42-2.37 (m, 2H), 2.06-2.01 (m, 2H). ^{13}C NMR ($CDCl_3$, 400 MHz) δ_C ppm: 170.94, 159.84, 153.88, 148.64, 139.31, 138.18, 137.74, 128.77, 128.77, 120.97, 116.78, 114.03, 111.07, 110.96, 110.96, 41.91, 41.91, 33.76, 31.72, 29.50, 19.00. Mass: *m/z* 409.46 $(M+1)^+$.

4-(1-Methyl-5-nitro-1*H*-benzo[*d*]imidazol-2-yl)-*N′*-(4-nitrobenzylidene)butane hydrazide (6o): Yield: 59%, m.p.: 194-198 ºC, m.f.: $C_{19}H_{18}N_6O_5$ (*m.w.*: 410.38). IR (KBr, ν_{max}, cm^{-1}): 3229.46 (N–H *str.*), 3086.67 (arom. C–H *str.*), 3054.67 (arom. C–H *str.*), 2967.14 (alkyl C–H *str.*), 1679.15 (C=O *str.* amide), 1586.17 (C=N), 1513.24 (N–H bend.), 1449.15 (arom. C–C *str.*), 1439.45 (arom. C–C *str.*), 1336.41 (C–N *str.*). ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm: 8.36 (s, 1H), 8.28-8.12 (m, 3H), 7.92 (d, *J* = 16.0 Hz, 2H), 7.82-7.68 (m, 2H), 3.85-3.80 (m, 3H), 2.69-2.64 (m, 2H), 2.40-2.35 (m, 2H), 2.05-2.00 (m, 2H). ^{13}C NMR ($CDCl_3$, 400 MHz) δ_C ppm: 170.94, 159.84, 148.64, 148.02, 139.94, 139.31, 138.18, 137.74, 127.95, 127.95, 124.48, 124.48, 116.78, 114.03, 111.07, 33.76, 31.72, 29.50, 19.00. Mass: *m/z* 411.55 $(M+1)^+$.

***N′*-(2-Hydroxy-5-nitrobenzylidene)-4-(1-methyl-5-nitro-1*H*-benzo[*d*]imidazol-2-yl)butane hydrazide (6p):** Yield: 61%, m.p.: 220-224 ºC, m.f.: $C_{19}H_{18}N_6O_6$ (*m.w.*: 426.3). IR (KBr, ν_{max}, cm^{-1}): 3627.28 (O-H *str.*), 3212.62 (N–H *str.*), 3049.57 (arom. C–H *str.*), 2976.14 (alkyl C–H *str.*), 2844.81 (alkyl C–H *str.*), 1679.67 (C=O *str.* amide), 1626.44 (C=N), 1513.44 (N–H bend.), 1474.64 (arom. C–C *str.*), 1452.69 (arom. C–C *str.*), 1342.92 (C–N *str.*). ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm: 9.97 (s, 1H), 8.47 (s, 1H), 8.33 (d, *J* = 19.7 Hz, 2H), 8.04 (d, *J* = 30.2 Hz, 2H), 8.00 (s, 1H), 7.96 (d, *J* = 30.0 Hz, 2H), 7.20 (s, 1H), 3.89-3.84 (m, 3H), 2.73-2.68 (m, 2H), 2.42-2.37 (m, 2H), 2.04-1.99 (m, 2H). ^{13}C NMR ($CDCl_3$, 400 MHz) δ_C ppm: 170.94, 161.72, 159.84, 148.29, 139.67, 139.31, 138.18, 137.74, 125.59, 124.04, 123.11, 119.00, 116.78, 114.03, 111.07, 33.76, 31.72, 29.50, 19.00. Mass: *m/z* 427.49 $(M+1)^+$.

Antimicrobial properties: Using a manual *in vitro* bacterial susceptibility test called the broth dilution method, the minimal inhibitory concentration (MIC) of each synthetic hydrazide-hydrazone derivative (**6a-p**) was determined. The amount of antimicrobial drugs required to stop the growth of germs was determined numerically using this method [40]. *Pseudomonas aeruginosa* (MTCC 1688), *Escherichia coli* (MTCC 443),

Streptococcus pyogenes (MTCC 442) and *Staphylococcus aureus* (MTCC 96) were the common reference strains selected to determine antibacterial effectiveness. The standard stains *Aspergillus niger* (MTCC 282), *Candida albicans* (MTCC 227) and *Aspergillus clavatus* (MTCC 1323) were used to assess the efficacy of antifungals. For all the standard stains, Institute of Microbial Technology, Chandigarh, India was the source. For every bacterial strain, a standard inoculum was created in order to use the microdilution method to obtain an inoculum size of roughly 10^8 CFU mL^{-1} in each well. Synthesized compounds were diluted with DMSO to produce a stock solution of 2000 µg/mL. During primary screening, the synthesized compounds were examined at 250 µg/mL, 500 µg/mL and 1000 µg/mL concentrations. Mueller-Hinton broth (MH broth) was diluted two-fold (2×) and then poured into microtiter plates to ensure that each test well had the required concentration. After that, the microtiter plates were incubated for a full day at 37 ºC. To every test and growth control well, 50 (µL) of a bacterial suspension of a standard inoculum size was introduced. All the microorganisms tested were exposed to the synthetic compounds that had worked well in the first screening in a second series of dilutions. The initial screening's active ingredients were further diluted to yield concentrations of 6.250, 12.5, 25, 50, 100 and 200 µg/mL. In the meantime, an antibiotic-free control tube was made and the inoculum was evenly spread over one-fourth of a plate that contained a test organism-appropriate media. By determining this control organism's Minimum Inhibitory Concentration (MIC), the precision of the drug concentrations was confirmed. After 24 h of incubation at 37 ºC, the turbidity of the solution was measured to monitor the growth of bacteria. The highest concentration at which at least 99% of the target organism was inhibited was the MIC, according to research findings [41,42].

In silico study

ADME analysis: A synthetic molecule must be low in toxicity, reach its intended location in the body in adequate quantities and keep its active state for as long as necessary to facilitate the intended biological event in order to be considered a potential medicine [43]. Only a small percentage of the various synthesized molecules meet those requirements. A significant portion of new compounds fail the tests for their physico-chemical qualities. Prediction of the ADME qualities is crucial in predicting the hit chemicals. The specifications pertaining to absorption, distribution, metabolism and excretion–abbreviated ADME–can be found using different techniques. It has been discovered that predicting ADME parameters early in the drug discovery process greatly lowers the percentage of pharmacokinetic-related clinical phase failures. A reliable substitute for the conventional experimental method of determining ADME parameters is offered by computer models. Swiss ADME (http://www.swissadme.ch) is a unique web tool made available to the public by the Molecular Modelling Group of the Swiss Institute of Bioinformatics in Switzerland which, depends on a compound's structural attributes, compute and forecast its pharmacochemical qualities, pharmacokinetics, drug-likeness and medicinal chemistry appropriateness [34,35,44].

Utilizing the SwissADME web tool, an *in silico* analysis of the synthesized compounds (**4** and **6a-p**) was conducted in order to predict drug-likeness by six physico-chemical properties *viz*. lipophilicity, size, polarity, solubility, flexibility and saturation–are the basis of the bioavailability radar [36,37]. Additionally anticipated by the BOILED-egg model for human gastrointestinal absorption (HIA) and blood brain barrier (BBB) penetration [38]. The synthesized compounds' structures were sketched in ChemDraw Ultra 12.0 and translated into SMILES format for prediction.

RESULTS AND DISCUSSION

The novel hydrazones of 4-(1-methyl-5-nitro-1*H*-benzo[*d*]-imidazol-2-yl)butane hydrazide (**6a-p**) were synthesized and characterized successfully (**Scheme-I**). In first step, *N*-methyl-4-nitrobenzene-1,2-diamine (**1**) and dihydro-2*H*-pyran-2,6-(3*H*)-dione (**2**) were coupled in presence of conc. H_2SO_4, resulting in the cyclization of benzimidazole derivative, methyl 4-(1-methyl-5-nitro-1*H*-benzo[*d*]imidazol-2-yl)butanoate (**3**). Compound **3** was refluxed in hydrazine hydrate to achieve 4-(1-methyl-5-nitro-1*H*-benzo[*d*]imidazol-2-yl)butane hydrazide (**4**). In final step, compound **4** was condensed with different aromatic aldehydes (**5a-p**), resulting in the desired hydrazide-hydrazone derivatives as potential antimicrobial agents (**6a-p**).

Antibacterial assay: The investigations conducted on the antibacterial and antifungal actitives of recently generated scaffolds (**6a-p)** against a range of pathogens are compiled in (Table-1). In depth analysis, in Gram-negative, *E. coli* (MTCC 443), compound **6k** displayed potency having an MIC of 50 µg/mL; which is like the standard drug (chloramphenicol). On the other hand, in Gram-negative, *P. aeruginosa* (MTCC 1688), similar potency has been exhibited by two compounds, *i.e.*, compounds **4** and **6o** by having MIC of 50 µg/mL. While against Gram-positive strain *S. pyogenes* (MTCC 442) and *S. aureus* (MTCC 96) no compounds show activity similar or higher than standard drugs.

Antifungal assay: The antifungal activity values of synthesized compounds (**6a-p**) are displayed in Table-1. In *C. albicans* (MTCC 227), compounds **6b-g** and **6j-p** displayed good activity, having MICs of 250 µg/mL or 500 µg/mL. In comparison to griseofulvin having MIC-500 µg/mL, as a standard drug those are similar or slightly higher than it. Surprisingly, none of the compounds were found to be having activity similar or higher than a standard drug against fungal stains *A. niger* and *A. clavatus*.

***In silico* analysis:** Predicted for the blood brain barrier (BBB) permeation and human gastrointestinal absorption (HIA) by BOILED-egg model by SwissADME web base tool and the results are presented in Table-2, which is the function of WLOGP *vs.* TPSA. The graphic representation (Fig. 3) indicates that there is a high likelihood of brain penetration in the yellow zone and passive absorption by the gastrointestinal tract in the white region. Red dots are not P-gp (PGP–) substrate, while blue dots are anticipated to be an active efflux made by P-gp (PGP+).

Drug likeness of all the synthesized derivatives (**6a-p**) were checked by SwissADME web base tool by predicting

TABLE-1
ANTIMICROBIAL ACTIVITIES OF SYNTHESIZED DERIVATIVES (**6a-p**)

Compd.	Antibacterial activity				Antifungal activity		
	Gram-negative		Gram-positive				
	E. coli	*P. aeruginosa*	*S. aureus*	*S. pyogenus*	*C. albicans*	*A. niger*	*A. clavatus*
4	62.5	50	100	250	500	500	500
6a	125	62.5	250	125	1000	1000	1000
6b	250	100	125	100	250	1000	1000
6c	100	125	100	250	250	500	500
6d	125	250	100	250	500	1000	1000
6e	62.5	100	125	125	250	500	1000
6f	100	125	62.5	100	500	1000	1000
6g	125	100	125	250	500	1000	1000
6h	125	125	250	250	1000	>1000	>1000
6i	100	125	250	250	1000	1000	1000
6j	125	250	125	250	500	1000	1000
6k	50	100	100	100	250	500	500
6l	100	100	250	125	500	>1000	>1000
6m	100	125	500	500	500	1000	1000
6n	125	250	125	250	250	500	1000
6o	62.5	50	125	100	250	500	500
6p	100	125	250	250	500	1000	1000
Chloramphenicol	50	50	50	50	–	–	–
Ciprofloxacin	25	25	50	50	–	–	–
Norfloxacin	10	10	10	10	–	–	–
Nystatin	–	–	–	–	100	100	100
Greseofulvin	–	–	–	–	500	100	100

TABLE-2
PHYSICO-CHEMICAL PROPERTY FOR BOILED-EGG METHOD

Compd.	m.w.	WLOGP	TPSA (Å)	PGP subtract	GI absorption	BBB permeant
4	277.28	0.79	118.76	No	High	No
6a	365.39	2.95	105.10	No	High	No
6b	381.39	2.66	125.33	No	High	No
6c	397.38	2.37	145.56	Yes	Low	No
6d	444.28	3.72	105.10	No	High	No
6e	444.28	3.72	105.10	No	High	No
6f	444.28	3.72	105.10	No	High	No
6g	383.38	3.51	105.10	No	High	No
6h	383.38	3.51	105.10	No	High	No
6i	401.37	4.07	105.10	No	High	No
6j	411.41	2.67	134.56	Yes	High	No
6k	411.41	2.67	134.56	Yes	High	No
6l	395.41	2.96	114.33	No	High	No
6m	439.46	3.36	123.56	No	High	No
6n	408.45	3.02	108.34	No	High	No
6o	410.38	2.86	150.92	No	Low	No
6p	426.38	2.57	171.15	Yes	Low	No

the physico-chemical properties like lipophilicity, size, polarity, solubility, flexibility and saturation. In the bioavailability radar pink area (Fig. 4) represent the optimum range for each property which are as below. The range of lipophilicity as an XLOGP3 is -0.7 to 5.0. M.W. Size ranges from 150 to 500 g/mol, while the polarity in the TPSA range of 20-130 Å. Solubility as log S, with a maximum of 6 (Table-3) and at least 0.25% of the carbons in the sp^3 hybridization are saturated. Flexibility: there should be no more than 9 rotatable bonds.

Conclusion

This study reports that a hybrid series of 4-(1-methyl-5-nitro-1*H*-benzo[*d*]imidazol-2-yl)butane hydrazide-hydrazone derivatives (**6a-p**) was successfully synthesized with a good to average yield and characterized by spectral analysis. The antibacterial activity against Gram-positive and Gram-negative bacterial stains, antifungal activity against some fugal stains and *in silico* predicted the physico-chemical properties. *In vitro* antibacterial study revealed that all the synthesized compounds

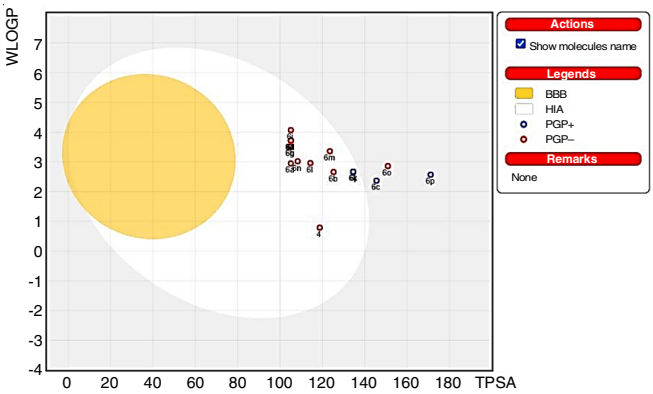


Fig. 4. Brain or Intestinal EstimateD permeation method (BOILED-egg) predictive graphical representation of synthesized derivatives (**6a-p**)

showed moderate antibacterial activity, which support the importance of the compound as an antibacterial drug candidate. While *in vitro* antifungal study revealed that all compounds show moderate antifungal activity against one fungal stain out of three tested stains while poor activity against remaining two fungal stains. Only compounds **6k** and **6o** inhibit Gram-bacteria probable due to presence of -OH (hydroxy) and -NO_2 (nitro) group. From their physico-chemical studies all the compounds show the good gastrointestinal absorption and no blood brain barrier permeability and show drug-likeness characteristics.

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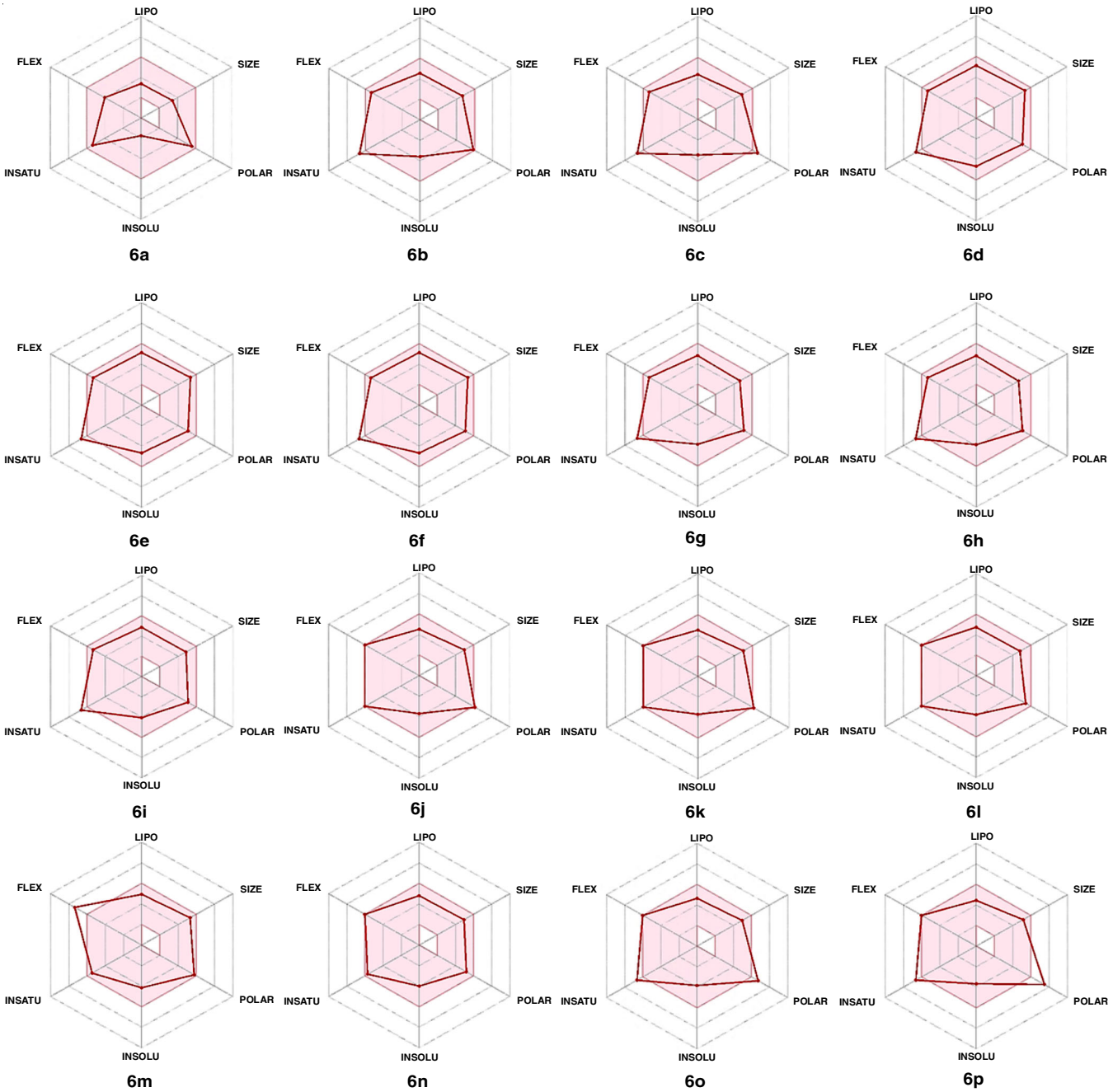

Fig. 5. Bioavailability radar, A glance at a drug-likeness of synthesized derivatives (**6a-p**)

TABLE-3
PHYSICO-CHEMICAL PROPERTY FOR BIOAVAILABILITY RADAR

Compd.	XLOGP3	m.w.	TPSA (Å)	ESOL Log S	Fraction Csp3	#Rotatable bonds
4	0.42	277.28	118.76	-1.76	0.33	6
6a	2.80	365.39	105.10	-3.75	0.21	8
6b	2.45	381.39	125.33	-3.62	0.21	8
6c	2.09	397.38	145.56	-3.48	0.21	8
6d	3.49	444.28	105.10	-4.66	0.21	8
6e	3.49	444.28	105.10	-4.66	0.21	8
6f	3.49	444.28	105.10	-4.66	0.21	8
6g	2.90	383.38	105.10	-3.91	0.21	8
6h	2.90	383.38	105.10	-3.91	0.21	8
6i	3.00	401.37	105.10	-4.07	0.21	8
6j	2.42	411.41	134.56	-3.69	0.25	9
6k	2.42	411.41	134.56	-3.69	0.25	9
6l	2.77	395.41	114.33	-3.83	0.25	9
6m	3.11	439.46	123.56	-4.14	0.32	11
6n	2.92	408.45	108.34	-3.99	0.29	9
6o	2.63	410.38	150.92	-3.82	0.21	9
6p	2.28	426.38	171.15	-3.68	0.21	9

providing the laboratory facilities. Thanks are also due to O_2h discovery, Ahmedabad and Indrashil University, Kadi, Gujarat, for spectral analysis support and Microcare Laboratory, Surat for antimicrobial analysis.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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