Original Article

Synthesis, Characterization, Molecular Docking Studies and Antimicrobial Evaluation of N-Benzimidazol-1-Yl-Methyl-Benzamide Derivatives

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ABSTRACT

N-benzimidazol-1-yl-methyl-benzamide derivatives (3a-3x) were synthesized by Mannich reaction and evaluated for *in vitro* antimicrobial activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Staphylococcus aureus, Candida albicans* and *Aspergillus niger*. The structures of novel target compounds were elucidated by spectral and analytical techniques. Among the synthesized derivatives, **3o** *N-[2-(2-chloro-phenyl)-benzimidazol-1-ylmethyl]-benzamide,* **3q** *N-[2-(4-chloro-phenyl)-benzimidazol-1ylmethyl]-benzamide* and **3r** *N-[2-(2-bromo-phenyl)-benzimidazol-1-ylmethyl]-benzamide* were found to be most effective antimicrobial compounds. Clotrimazole and ciprofloxacin were used as reference antimicrobial agents. Further, *in silico* studies were carried out to define the interaction of the title compounds with microbial protein.

Key words: Mannich bases, Benzimidazole, Antibacterial activity, Antifungal activity, Docking study.

INTRODUCTION

Antimicrobial resistance is an evolving predicament in treating the patients, and causes several deaths every year.[1] The main cause of microbial resistance is the mutations or transfer of resistant genes between organisms.[2] Resistant microbes may require other medications or elevated doses associated with more side effects, some of which may be life alarming. The chronic use of antibiotics is escalating the rates of infections, due to antibiotic resistance. As resistance to antibiotics becomes more common, the development of novel, effective and inimitable antimicrobial agents is the only superlative way to conquer microbial resistance and develop effective therapies.

Despite of several attempts to build up new structural analogues in the seek for more effective antimicrobials, the benzimidazoles still remain the most flexible class of compounds against microbes.[3-5] 2- substituted benzimidazole and its derivatives have attracted great attention for the past few decades due to their chemotherapeutic values and therefore, are useful compounds for further molecular discovery. These derivatives show biological activities such as antimicrobial,[6] anti-allergic,[7] PARP (poly ADP ribose polymerase) inhibitors- as anticancer agents,[8] as cytomegalovirus (HCMV) inhibitors,[9] antiulcer,[10] anti inflammatory,[11] and as antihistaminics.[12]

On the other side mannich bases are the end products of mannich reaction and are known as beta amino ketone carrying compounds.[13] Mannich bases are very reactive because of the introduction of basic functional group which renders the derivative in aqueous solvent and can be changed into several other compounds.[14] Literature

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revealed that mannich bases have been reported to possess analgesic,[15] anti-inflammatory,[16,17] anticancer,[18,19] anti-convulsant,[20] antibacterial,[21,22] antifungal,[22,23] and several other activities.

Keeping the above facts in mind and as part of continuation of our research on Mannich bases,[24] we hereby report the synthesis and antimicrobial evaluation of mannich bases of 2-substituted benzimidazole derivatives. Their structural configuration was elucidated with the elemental and spectral analysis. Synthesized novel compounds derived from mannich reaction were further evaluated for antimicrobial activity and then screened by *in silico* method to study drug receptor interaction between novel compounds and microbial protein.

MATERIALS AND METHODS

Chemicals were procured from Sigma Aldrich, USA. Melting points of the synthesized compounds were determined by open capillary tubes and were uncorrected. The purity of the synthesized compounds was determined by thin layer chromatography on precoated silica gel G plates with visualization by iodine vapours/ UV chamber. Infrared spectra (KBr pellets) were recorded on Perkin Elmer Spectrum FTIR spectrophotometer. Proton NMR spectra were recorded on Bruker Avance II 400 NMR Spectrometer using DMSO-*d6* as a solvent and TMS (tetra methyl silane) as internal standard (chemical shift in δ ppm). Elemental analyses were carried out using Eager Xperience CHN analyzer at Panjab University, Chandigarh. Mass spectra were taken on Q-TOF MICROMASS Spectrometer (WATERS, USA).

Chemistry

Mannich Bases of 2-substituted benzimidazole were synthesized by the reaction of 2-substituted benzimidazole (secondary amine), formalin and benzamide (active hydrogen compound) (3a-x). 2-substituted benzimidazoles (2a-x) were prepared by the reaction of ortho-phenylenediamine with substituted carboxylic acid and with substituted aromatic aldehyde respectively.

General schemes for the synthesis of compounds

General method

The title compounds were prepared by the following steps.

Synthesis of 2-substituted benzimidazoles from ortho-phenylenediamine dihydrochloride[2(a-f),2h,2j,2v]

2-substituted benzimidazoles were synthesized by the reaction of o-phenylenediamine dihydrochloride with substituted carboxylic acid by the method described in literature.[25]

Synthesis of 2-substituted benzimidazoles from ortho-phenylenediamine [2(k-x),2i,2g]

2- substituted benzimidazoles were synthesized by the reaction of o-phenylenediamine and substituted benzaldehyde by the procedure reported in literature.[26-28]

Synthesis of mannich base of 2-substituted benzimidazoles with benzamide [3(a-x)]

2- substituted benzimidazole (0.01 mole) was added to the ethanolic solution of benzamide (0.01mole). Formaldehyde (37%) (0.01 mole) was added and the reaction mixture was then adjusted to the pH of 3.5 with conc. HCl. The mixture was kept at efficient ice cooling for half an hour. Then it was refluxed with stirring at 80°C for 10-12 hrs. Formaldehyde (37%) was added to the reaction mixture in portions for completion of the reaction. End of reaction was monitored by TLC. Solvent system- $CHCl_3$: CH_3OH ; 9.5: 0.5. Reaction mixture was kept in refrigerator overnight. Solvent was evaporated under reduced pressure and product was collected, washed with water and recrystallized from ethanol.

Similar type of procedures and equimolar quantities of reactants were used and 24 novel compounds were synthesized. Synthetic route for synthesis of novel compounds is shown in the general scheme. Physical data of synthesized compounds is given in Table 1.

Spectral data

N-(Benzimidazol-1-yl methyl)-benzamide. (3a)

White crystals, mp 198-199ºC, *IR (KBr, cm-1)* 3307 (N-H), 1486 (C=N), 3053 C-H(CH_2), 2965 (C-H, Ar.) 1526 (C=C), 1634 (C=O), 675-870 (CH bend.Ar). *1H NMR* (400 MHz, DMSO-d_6, δ ppm) 7.39-7.91 (m, 10H, ArH), 4.98 (s, 2H, NCH_2N), 8.70 (s, 1H, NH). *Anal Calcd* for $C_{15}H_{13}N_3O$: C 71.70 , H 5.21, N 16.72 ; found C 70.84, H 5.14, N 15.94.

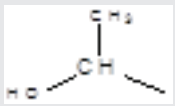
N-(2-methyl-benzimidazol-1-yl methyl)-benzamide (3b)

White crystals, mp 180-182ºC, *IR (KBr, cm-1)* 3308 (N-H), 1487 (C=N), 3055 C-H(CH_2), 2966 (C-H, Ar.), 1526 (C=C), 1634 (C=O), 675-870 (CH bend.Ar). *1H NMR* (400 MHz, DMSO-d_6, δ ppm) 7.3-7.9 (m, 9H, ArH), 4.89 (s, 2H, NCH_2N), 8.8 (s, 1H, NH), 2.80 (s, 3H, CH_3) *Anal Calcd* for $C_{16}H_{15}N_3O$: C 72.43, H 5.7, N 15.84; found C 69.84, H 5.54, N 15.69. MS: m/z = 266.2 (M+1).

N-(2-chloromethyl-benzimidazol-1-yl methyl)-benzamide (3c)

Yellow crystals; mp. 190-191ºC, *IR (KBr, cm-1)* 3308 (N-H), 1485 (C=N), 3055 C-H(CH_2), 2966 (C-H, Ar.) 1526 (C=C), 1634 (C=O), 789 (C-Cl), 675-870 (CH bend.Ar). *1HNMR* (400 MHz, DMSO-d_6, δppm),

Table 1: Physical data of synthesized compounds

Compound	R	Molecular formula	Molecular weight	Melting Point(ºC)	Yield (%)	R_f value
3a	H-	$C_{15}H_{13}N_3O$	251.28	198-199	66.2	0.78
3b	CH_3-	$C_{16}H_{15}N_3O$	265.31	180-182	62.4	0.69
3c	$ClCH_2$-	$C_{16}H_{14}ClN_3O$	299.75	190-191	76.5	0.59
3d	C_2H_5-	$C_{17}H_{17}N_3O$	279.34	210-212	64.2	0.64
3e	C_3H_8-	$C_{18}H_{19}N_3O$	293.36	208-210	62.5	0.77
3f	C_4H_{10}-	$C_{19}H_{21}N_3O$	307.39	212-214	60.6	0.91
3g	NH_2-	$C_{15}H_{14}N_4O$	266.30	205-206	58.4	0.90
3h		$C_{17}H_{17}N_3O_2$	295.34	190-192	56.2	0.65
3i	SH-	$C_{15}H_{13}N_3OS$	283.35	170-172	52.7	0.91
3j	$SHCH_2$-	$C_{16}H_{15}N_3OS$	297.38	212-214	51.8	0.84
3k	C_6H_5-	$C_{21}H_{17}N_3O$	327.38	195-197	80.4	0.95
3l	(2-N-C_5H_4)-	$C_{20}H_{16}N_4O$	328.37	218-220	72.5	0.77
3m	(3-N-C_5H_4)-	$C_{20}H_{16}N_4O$	328.37	205-206	79.7	0.85
3n	(2-OH-C_6H_4)-	$C_{21}H_{17}N_3O_2$	343.38	180-182	75.3	0.84
3o	(2-Cl-C_6H_4)-	$C_{21}H_{16}ClN_3O$	361.82	190-191	80.0	0.87
3p	(3-Cl-C_6H_4)-	$C_{21}H_{16}ClN_3O$	361.82	200-202	71.3	0.86
3q	(4-Cl-C_6H_4)-	$C_{21}H_{16}ClN_3O$	361.82	195-196	73.6	0.75
3r	(2-Br-C_6H_4)-	$C_{21}H_{16}BrN_3O$	406.28	190-192	75.4	0.81
3s	(3-Br-C_6H_4)-	$C_{21}H_{16}BrN_3O$	406.28	192-194	76.7	0.80
3t	(4-Br-C_6H_4)-	$C_{21}H_{16}BrN_3O$	406.28	195-196	78.6	0.77
3u	(4-NO_2-C_6H_4)-	$C_{21}H_{16}N_4O_3$	372.38	180-183	79.2	0.64
3v	(-C_6H_5- CH_2)-	$C_{22}H_{19}N_3O$	341.41	170-172	64.6	0.70
3w	(4-F-C_6H_4)-	$C_{21}H_{16}FN_3O$	345.37	230-231	72.7	0.76
3x	(2-NH_2-C_6H_4)-	$C_{21}H_{18}N_4O$	342.39	206-208	78.5	0.88

Stationary phase: Silica gel G, Mobile phase for TLC : chloroform: methanol (9.5:0.5)

7.4-8.1 (m, 9H, ArH), 4.92 (s, 2H, NCH_2N), 9.0 (s, 1H, NH), 5.19 (s, 2H, CH_2). Anal Calcd for $C_{16}H_{14}ClN_3O$: C 64.11, H 4.71, N 14.02; found C 65.06, H 4.41, N 13.9. MS: m/z = 300.2 (M+1).

N-(2-ethyl-benzimidazol-1-yl methyl)-benzamide (3d)

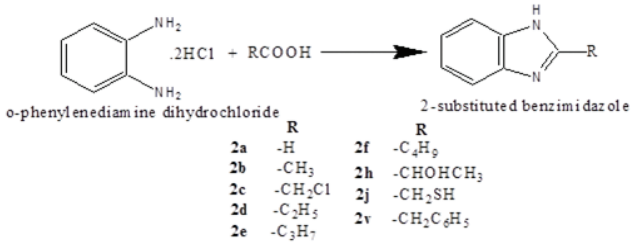
White crystals, mp 210-212ºC, *IR (KBr, cm-1)* 3308 (N-H), 1486 (C=N), 3019 C-H(CH_2), 2967 (C-H, Ar.) 1526 (C=C), 1634 (C=O), 675-870 (CH bend.Ar). *1HNMR* (400 MHz, DMSO-d_6, δppm), 7.3-7.9 (m, 9H, ArH), 4.99 (s, 2H, NCH_2N), 8.8 (s, 1H, NH), 3.27 (q, 2H, CH_2, J=7.6 Hz), 1.56 (t, 3H, CH_3, J=7.6 Hz). Anal Calcd for $C_{17}H_{17}N_3O$: C 73.10, H 6.13 N 15.04; found C 71.06, H 5.98, N 14.9. MS: m/z = 279.2 (M+1).

N-(2-propyl-benzimidazol-1-yl methyl)-benzamide (3e)

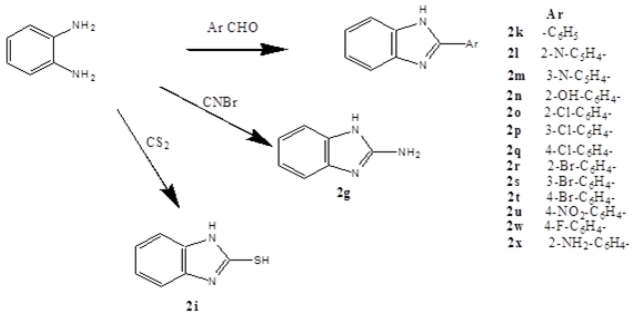
White crystals, mp 208-210ºC, *IR (KBr,cm-1)* 3308 (N-H), 1487 (C=N), 3056 C-H(CH_2), 2967 (C-H,Ar.) 1527 (C=C), 1634 (C=O), 675-870 (CH bend.Ar). *1HNMR* (400MHz, DMSO-d_6, δppm), 7.40-8.12 (m, 9H, ArH), 4.93 (s, 2H, NCH_2N), 8.9 (s, 1H, NH), 2.96 (t, 2H, CH_2, J=7.6 Hz), 1.93 (sextet, 2H, CH_2, J=7.6 Hz), 1.0 (t, 3H, CH_3, J=7.2 Hz). Anal Calcd for $C_{18}H_{19}N_3O$: C 73.69, H 6.53, N 14.32; found C 75.4, H 5.96 N 14.15.

N-(2-butyl-benzimidazol-1-yl methyl)-benzamide (3f)

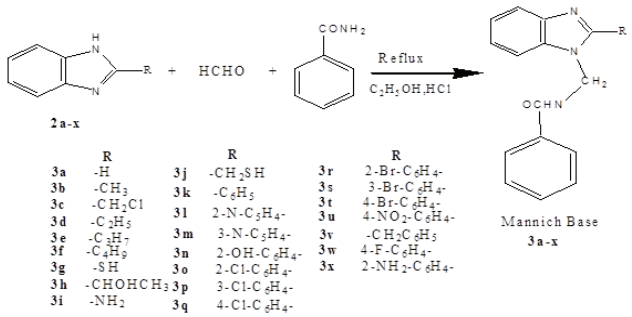
White crystals, mp 212-214ºC, *IR (KBr, cm-1)* 3307 (N-H), 1486 (C=N), 3055 C-H(CH_2), 2966 (C-H, Ar.) 1527 (C=C), 1635 (C=O), 675-870 (CH bend.Ar). *1H NMR* (400MHz, DMSO-d_6, δppm), 7.18-8.06 (m, 9H, ArH), 4.97 (s, 2H, NCH_2N), 8.84 (s, 1H, NH), 0.93 (t, 3H, CH_3, J=7.2 Hz), 1.40 (sextet, 2H, CH_2, J=7.6 Hz), 1.82 (p, 2H, CH_2, J= 2.16 Hz), 2.93 (t, 2H, CH_2, J=7.6 Hz). Anal Calcd for $C_{19}H_{21}N_3O$: C 74.24, H 6.89, N 13.67; found C 69.4, H 6.59, N 12.43.



Scheme 1a: Synthesis of 2-substituted benzimidazoles using o-phenylene diamine dihydrochloride.



Scheme 1b: Synthesis of 2-substituted benzimidazoles using o-phenylene diamine.



Scheme 2: Synthesis of mannich bases from 2-substituted benzimidazoles.

N-(2-amino-benzimidazol-1-yl methyl)-benzamide (3g)

Yellow crystals; mp. 205-206ºC, *IR (KBr, cm-1)* 3305 (N-H), 1484 (C=N), 3165 C-H(CH_2), 3060 (C-H, Ar.) 1529 (C=C), 1638 (C=O), 675-870 (CH bend.Ar). *1H NMR* (400MHz, DMSO-d_6, δppm), 7.21-7.96 (m, 9H, ArH), 7.97 (s,1H, NH_2), 4.90 (s, 2H, NCH_2N), 9.03(s, 1H, NH), 9.6 (s, 1H, NH_2). Anal Calcd for $C_{15}H_{14}N_4O$: C 67.65, H 5.30 , N 21.04 ; found C 65.3, H 4.92, N 20.3.

N-2-[(1-hydroxy-ethyl)-benzimidazol-1-ylmethyl]-benzamide (3h)

White crystals, mp 190-192ºC *IR (KBr, cm-1)* 3308 (N-H), 1487 (C=N), 3056 C-H(CH_2), 2967 (C-H, Ar.) 1527 (C=C), 1634 (C=O), 675-870 (CH bend.Ar). *1H NMR* (400MHz, DMSO-d_6, δppm), 7.39-7.92 (m, 9H, ArH), 4.90 (s, 2H, NCH_2N), 8.97 (s, 1H, NH), 5.70 (br, s, 1H, OH), 1.59 (d, 3H CH_3, J= 6.4 Hz), 5.03 (1H, q, CH, J= 6.8 Hz). Anal Calcd for $C_{17}H_{17}N_3O_2$: C 69.14, H 5.80, N 14.23 ; found C 68.3, H 5.42, N 13.92.

N-(2-mercapto-benzimidazol-1-ylmethyl)-benzamide (3i)

White crystals, mp 270-272ºC, *IR (KBr, cm-1)* 3305 (N-H), 1378 (C=N), 3178 C-H(CH_2), 3056 (C-H, Ar.) 1526 (C=C), 1635 (C=O), 675-870 (CH bend.Ar).*1H NMR* (400MHz, DMSO-d_6, δppm), 7.26-7.95 (m, 9H, ArH), 4.95 (s, 2H, NCH_2N), 8.86 (s, 1H, NH), 3.4 (s, 1H, SH). Anal Calcd for $C_{15}H_{13}N_3OS$: C 63.58, H 4.62, N 14.83; found C 62.3, H 4.42, N 14.62.

N-(2-mercaptomethyl-benzimidazol-1-ylmethyl)-benzamide (3j)

White crystals, mp 212-214ºC, *IR (KBr, cm-1)* 3308 (N-H), 1487 (C=N), 3056 C-H(CH_2), 2966 (C-H, Ar.) 1526 (C=C), 1634 (C=O), 675-870 (CH bend.Ar). *1H NMR* (400MHz, DMSO-d_6, δppm), 7.4-8.12 (m, 9H, ArH), 4.92 (s, 2H, NCH_2N), 8.98 (s, 1H, NH), 3.39 (s, 1H, SH), 4.18 (s, 2H, CH_2). Anal Calcd for $C_{16}H_{15}N_3OS$: C 64.62, H 5.08, N 14.13; found C 63.3, H 4.96, N 13.92. MS: m/z = 298.2 (M+1).

N-(2-phenyl-benzimidazol-1-ylmethyl)-benzamide (3k)

Brown crystals; mp. 195-197 ºC, *IR (KBr, cm-1)* 3308 (N-H), 1488 (C=N), 3056 C-H(CH_2), 2965 (C-H, Ar.) 1526 (C=C), 1634 (C=O), 675-870 (CH bend.Ar). *1H NMR* (400MHz, DMSO-d_6, δppm), 7.4-8.32 (m, 14H, ArH), 4.93 (s, 2H, NCH_2N), 9.0 (s, 1H, NH). Anal Calcd for $C_{21}H_{17}N_3O$: C 77.04, H 5.23, N 12.84 ; found C 76.3, H 5.20, N 12.72. MS: m/z = 329.2 (M+1).

N-(2-pyridin-2-yl-benzimidazol-1-ylmethyl)-benzamide (3l)

Off white crystals; mp. 218-220ºC, *IR (KBr, cm-1)* 3308 (N-H), 1447 (C=N), 3056 C-H(CH_2), 2967 (C-H, Ar.) 1527 (C=C), 1634 (C=O), 675-870 (CH bend.Ar). *1H NMR* (400MHz, DMSO-d_6, δppm), 7.39-7.91 (m, 13H, ArH), 4.9 (s, 2H, NCH_2N), 8.8 (s, 1H, NH). Anal Calcd for $C_{20}H_{16}N_4O$: C 73.15, H 4.91, N 17.06 ; found C 73.3, H 4.20, N 16.72.

N-(2-pyridin-3-yl-benzimidazol-1-ylmethyl)-benzamide (3m)

Yellow crystals; mp. 205-206ºC, *IR (KBr, cm-1)* 3308 (N-H), 1486 (C=N), 3053 C-H(CH_2), 2966 (C-H,Ar.), 1527 (C=C), 1635 (C=O), 675-870 (CH bend.Ar). *1H NMR* (400MHz, DMSO-d_6,δppm), 7.2-8.96 (m, 13H, ArH), 4.9 (s, 2H, NCH_2N), 9.5 (s, 1H, NH). Anal Calcd for $C_{20}H_{16}N_4O$: C 73.15, H 4.9, N 17.6 ; found C 72.06, H 5.6, N 16.92. MS: m/z = 329.3 (M+1).

N-[2-(2-hydroxy-phenyl)-benzimidazol-1-ylmethyl]-benzamide (3n)

White crystals; mp. 180-182ºC, *IR (KBr, cm-1)* 3310 (N-H), 1491 (C=N), 3057 C-H(CH_2), 2964 (C-H, Ar.) 1527 (C=C), 1633 (C=O), 675-870 (CH bend.Ar). *1H NMR* (400MHz, DMSO-d_6,δppm), 7.04-8.18 (m, 13H, ArH), 4.91 (s, 2H, NCH_2N), 9.0 (s, 1H, NH), 13.6 (br,s, 1H, OH). Anal Calcd for $C_{21}H_{17}N_3O_2$: C 73.45 , H 4.99, N 12.24 ; found C 72.06, H 4.63, N 11.92. MS: m/z = 344.8 (M+1).

N-[2-(2-chloro-phenyl)-benzimidazol-1-ylmethyl]-benzamide (3o)

White crystals; mp. 190-191ºC, *IR (KBr, cm-1)* 3307 (N-H), 1485 (C=N), 3048 C-H(CH_2), 2963 (C-H, Ar.) 1526 (C=C), 1634 (C=O), 770 (C-Cl), 675-870 (CH bend.Ar). *1H NMR* (400MHz, DMSO-d_6, δppm), 7.41-8.9 (m, 13H, ArH), 4.91 (s, 2H, NCH_2N), 9.01(s, 1H, NH). Anal Calcd for $C_{21}H_{16}ClN_3O$: C 69.71, H 4.46, N 11.61; found C 68.5, H 4.31, N 10.2. MS: m/z = 362.2 (M+1), 363(M+2).

N-[2-(3-chloro-phenyl)-benzimidazol-1-ylmethyl]-benzamide (3p)

White crystals; mp. 200-202ºC, *IR (KBr, cm-1)* 3309 (N-H), 1485 (C=N), 3034 C-H(CH_2), 2953 (C-H,Ar.) 1527 (C=C), 1633 (C=O), 765 (C-Cl), 675-870 (CH bend.Ar). *1H NMR* (400MHz, DMSO-d_6, δppm), 7.46-8.7 (m, 13H, ArH), 4.98 (s, 2H, NCH_2N), 8.9 (s, 1H, NH). Anal Calcd for $C_{21}H_{16}ClN_3O$: C 69.71, H 4.46, N 11.61; found C 68.5, H 4.81, N 11.2.

N-[2-(4-chloro-phenyl)-benzimidazol-1-ylmethyl]-benzamide (3q)

White crystals; mp. 195-196ºC, *IR (KBr, cm-1)* 3307 (N-H), 1485 (C=N), 3045 C-H(CH_2) 2952 (C-H, Ar.) 1525 (C=C), 1635(C=O), 764 (C-Cl) 675-870 (CH bend.Ar). *1H NMR* (400MHz, DMSO-d_6, δppm), 7.35-8.6 (m, 13H, ArH), 4.96 (s, 2H, NCH_2N), 8.90 (s, 1H, NH). Anal Calcd for $C_{21}H_{16}ClN_3O$: C 69.71, H 4.46, N 11.61 ; found C 68.52, H 4.31, N 10.8. MS: m/z = 363.9 (M+2).

N-[2-(2-bromo-phenyl)-benzimidazol-1-ylmethyl]-benzamide (3r)

White crystals; mp. 190-192ºC, *IR (KBr, cm-1)* 3309 (N-H), 1488 (C=N), 3056 C-H(CH_2), 2967 (C-H, Ar.) 1527 (C=C), 1635 (C=O), 869 (C-Br), 675-870 (CH bend.Ar). *1H NMR* (400MHz, DMSO-d_6, δppm), 7.4-8.1 (m, 13H, ArH), 4.91(s, 2H, NCH_2N), 8.9 (s, 1H, NH). Anal Calcd for $C_{21}H_{16}BrN_3O$: C 62.08, H 3.97, N 10.34; found C 63.3, H 2.98, N 9.43. MS: m/z = 406.2 (M+1), 407.3 (M+2).

N-[2-(3-bromo-phenyl)-benzimidazol-1-ylmethyl]-benzamide (3s)

White crystals; mp. 192-194ºC, *IR (KBr, cm-1)* 3307 (N-H), 1487 (C=N), 3034 C-H(CH_2), 2966 (C-H, Ar.) 1525 (C=C), 1634 (C=O), 865 (C-Br), 675-870 (CH bend.Ar). *1H NMR* (400MHz, DMSO-d_6, δppm), 7.1-8.7 (m, 13H, ArH), 4.98(s, 2H, NCH_2N), 9.0 (s, 1H, NH). Anal Calcd for $C_{21}H_{16}BrN_3O$: C 62.08, H 3.97, N 10.34 ; found C 61.3, H 3.90, N 9.49.

N-[2-(4-bromo-phenyl)-benzimidazol-1-ylmethyl]-benzamide (3t)

Off white crystals; mp. 195-196ºC, *IR (KBr, cm-1)* 3308 (N-H), 1486 (C=N), 3056 C-H(CH_2), 2967 (C-H, Ar.) 1527 (C=C), 1635 (C=O), 865(C-Br), 675-870 (CH bend.Ar). *1H NMR* (400MHz, DMSO-d_6, δppm), 7.2-8.6 (m, 13H, ArH), 4.97 (s, 2H, NCH_2N), 8.89 (s, 1H, NH). Anal Calcd for $C_{21}H_{16}BrN_3O$: C 62.08, H 3.97, N 10.34 ; found C 61.9, H 2.96, N 9.82.

N-[2-(4-nitro-phenyl)-benzimidazol-1-ylmethyl]-benzamide (3u)

Yellow crystals; mp. 180-183ºC, *IR (KBr, cm-1)* 3308 (N-H), 1463 (C=N), 3058 C-H(CH_2), 2966 (C-H, Ar.) 1524 (C=C), 1634 (C=O), 747 (C-NO_2), 675-870 (CH bend.Ar). *1H NMR* (400MHz, DMSO-d_6, δppm), 7.24-8.46 (m, 13H, ArH), 4.94 (s, 2H, NCH_2N), 8.90 (s, 1H, NH). Anal Calcd for $C_{21}H_{16}N_4O_3$: C 67.73, H 4.33, N 15.05; found C 66.3, H 4.28, N 14.96. MS: m/z = 373.7 (M+1).

N-(2-benzyl-benzimidazol-1-ylmethyl)-benzamide (3v)

White crystals; mp. 170-172ºC, *IR (KBr, cm-1)* 3308 (N-H), 1487 (C=N), 3054 C-H(CH_2), 2966 (C-H, Ar.) 1525 (C=C), 1634 (C=O), 675-870 (CH bend.Ar). *1H NMR* (400MHz, DMSO-d_6, δppm), 7.24-7.93 (m, 14H, ArH), 4.93(s, 2H, NCH_2N), 8.62 (s, 1H, NH), 4.53 (s, 2H, CH_2). Anal Calcd for $C_{22}H_{19}N_3O$: C 77.40, H 5.61, N 12.31; found C 76.3, H 5.49, N 11.94.

N-[2-(4-flouro-phenyl)-benzimidazol-1-ylmethyl]-benzamide (3w)

White crystals; mp. 230-231ºC, *IR (KBr, cm-1)* 3310 (N-H), 1458 (C=N), 3055 C-H(CH_2), 2927 (C-H, Ar.) 1525 (C=C), 1634 (C=O), 848 (C-F), 675-870 (CH bend. Ar). *1H NMR* (400MHz, DMSO-d_6, δppm), 7.4-8.48 (m, 13H, ArH), 4.90 (s, 2H, NCH_2N), 9.03 (s, 1H, NH). Anal Calcd for $C_{21}H_{16}FN_3O$: C 73.03, H 4.67, N 12.17; found C 72.7, H 3.98, N 11.82. MS: m/z = 346.3 (M+1).

N-[2-(2-amino-phenyl)-benzimidazol-1-ylmethyl]-benzamide (3x)

Yellow crystals; mp. 206-208ºC, *IR (KBr, cm-1)* 3310 (N-H), 1486 (C=N), 3086 C-H(CH_2), 2959 (C-H, Ar.)

1525 (C=C), 1634 (C=O), 675-870 (CH bend.Ar). *1H NMR* (400MHz, DMSO-d_6, δppm), 6.52-7.95 (m, 13H, ArH), 4.98 (s, 2H, NCH_2N), 8.79 (s, 1H, NH), 4.0 (s, 2H, NH_2). Anal Calcd for $C_{21}H_{18}N_4O$: C 73.67, H 5.30, N 16.36; found C 71.9, H 4.96, N 15.82. MS: m/z = 343.5 (M+1).

Antimicrobial Evaluation

The synthesized compounds were screened for their *in vitro* antimicrobial activity against two gram positive bacterial strains: *Staphylococcus aureus* (MTCC 96), *Bacillus subtilis* (MTCC 121), two gram negative bacterial strains: *Escherichia coli* (MTCC 40), *Pseudomonas aeruginosa* (MTCC 2453), and two fungal strains: *Candida albicans* (MTCC 183) and *Aspergillus niger* (MTCC 404). The serial dilution technique was used for evaluation of antimicrobial activity. Stock solution of synthesized compounds was prepared in dimethyl sulphoxide (100 μg/ml). Nutrient broth (I.P.) and sabouraud dextrose broth media (I.P.)[29] were used for bacteria and fungi respectively. Ciprofloxacin and clotrimazole were used as standard drugs for antibacterial activity and antifungal activity respectively. Sterlized media (1 ml) was transferred into sterile test tubes. Stock solution (100 μg/ml) (1ml) was put in one tube and serially diluted to give concentrations of 50, 25, 12.5, 6.25, 3.125 and 1.56 μg/ml. 0.1ml suspension of bacteria and fungus in saline was added to all test tubes and were then incubated at 37 ± 1ºC for 24 hours (bacteria) and 25ºC for 72 hrs (fungi). The test mixture contained 10^6 organisms/ml (CFU/ml). Macroscopic examination of inoculated culture tubes was done for turbidity. MIC was the lowest concentration at which microbial growth attenuated and no turbidity was seen in the tube. Same procedure was followed for reference drugs and the experiment was done in triplicate.

Molecular Docking studies

To get more insight into the binding interaction of the synthesized compounds, molecular docking studies were performed using Autodock Vina software. Marvin Sketch application of ChemAxon was used for drawing all the ligands employed in this study. The 3D structures of enoyl reductase-NAD^+-triclosan complex (PDB id: 1C14) was procured from Protein Data Bank (www.rcsb.org).[30] Before performing the docking, protein receptor was prepared by merging all the non-polar hydrogens and removing the water of crystallization using the same graphical interface. Autodock tool uses the hybrid global-local search algorithm which is a big improvement in the genetic algorithm for the best confirmations of legends. Then the protein was defined for the generation of active site, i.e. grid with specific dimensions. The parameters of grid box are given in Table 2. The Autodock Vina uses a hybrid scoring function (empirical + knowledge based function) for evaluating binding affinity of ligands with the receptor.[31]

A set of 24 compounds was screened for antimicrobial activity by molecular docking simulations using PDB id : 1C14. The screening results were further compared with the *in-vitro* studies for their drug receptor interaction.

RESULTS AND DISCUSSION

Chemistry: In this study, 24 novel compounds incorporating benzimidazole scaffold were prepared and screened for antimicrobial activity. The reaction order for the synthesis of title compounds is given in general schemes. 2-substituted benzimidazoles were prepared by the reaction of ortho-phenylenediamine dihydrochloride with substituted carboxylic acid (Scheme 1a) and by the reaction of ortho-phenylene diamine with substituted aromatic aldehyde, carbon disulphide and cynogen bromide (Scheme 1b). Then Mannich bases were prepared by the reaction of Benzamide (active hydrogen compound), secondary amine (2-substituted benzimidazole), formaldehyde and conc. hydrochloric acid (Scheme 2). The structures of all the compounds were elucidated by spectral analysis. From IR spectra, the appearance of peaks at 3308-3310 cm^- and 1400-1500 cm^- indicated the presence of NH of carboxamide and C=N stretching of benzimidazole respectively. From NMR spectra, a 2 protons singlet at 4.8-4.99 ppm confirmed the presence of CH_2 attached to NH in all the synthesized compounds. The emergence of sharp singlet at 8.0-8.9 ppm ascertained the presence of NH of carboxamide in all the synthesized compounds. The appearance of multiplet at 6.30-8.0 ppm confirmed the presence of aromatic and hetero-aromatic protons. The calculated molecular weight of the synthesized compounds was comparable with observed *m/e* value. Hence, the calculated values of the projected structures were found to be in good confirmity with the data obtained experimently.

Antimicrobial Evaluation of test compounds: From Table 3, it was found that amongst all synthesized compounds, **3o, 3q, 3r, 3t, 3u** and **3w** were most significantly active against bacterial strains as compared to standard ciprofloxacin and rest of the compounds showed moderate activity.

Structure activity relationship revealed that compound **3o** with its electron withdrawing Cl group at 2 position of phenyl ring which is substituted at 2-position of benzimidazole showed better activity against *P. auregenosa, S. aureus, B. subtilis* and similar effect against *E. coli* than standard ciprofloxacin. Compound **3q** with its elec-

tron withdrawing Cl group at 4 position of benzene ring, showed improved activity against P.auregenosa, S. aureus and similar effect against *E. coli* and *B. subtilis* as compared to reference drug ciprofloxacin. Compound **3r** with its elecron withdrawing bromo group at 2 position of phenyl ring showed better results against *P. aerugenosa* and similar effect against all the rest of the three strains when compared with ciprofloxacin. Compound **3t, 3u**, **3w** with electron withdrawing Br, NO_2 and F groups at 4 position of phenyl ring respectively showed better activity against *P. auregenosa* and *B. subtilis* than standard drug ciprofloxacin.

From Table 4, it was found that amongst all the synthesized compounds, **3o, 3q** and **3r** with electron withdrawing Cl and Br groups at ortho and para positions of benzene ring respectively were most significantly active against fungal strains and rest of the compounds showed moderate activity as compared to standard clotrimazole. Hence, it is concluded that substitutions on heteroaromatic rings with electron withdrawing groups like Cl, Br, NO_2, and F at 2 and 4 positions of phenyl rings showed enhanced antimicrobial activity as compared to reference drugs. No inhibitory effect was found for DMSO used as control.

Molecular Docking Studies: After *in vitro* antimicrobial evaluation, to understand the interaction of synthesized

Table 2: x, y, z coordinates of grid box (PDB ID: 1C14)

center_x	-1.723
center_y	32.185
center_z	145.55
size_x	40
size_y	40
size_z	40

Table 3: Minimum inhibitory concentration (MIC) (μg/ml) of the synthesized compounds against Gram positive and Gram negative bacteria

Compound	*E .coli (MTCC 40)*	*P.aeruginosa (MTCC2453)*	*S.aureus (MTCC 96)*	*B.subtilis (MTCC121)*
3a	12.5	12.5	12.5	12.5
3b	12.5	12.5	12.5	12.5
3c	6.25	12.5	12.5	12.5
3d	12.5	12.5	12.5	12.5
3e	12.5	12.5	12.5	12.5
3f	12.5	6.25	12.5	12.5
3g	12.5	12.5	12.5	12.5
3h	6.25	12.5	12.5	12.5
3i	12.5	6.25	12.5	6.25
3j	6.25	12.5	12.5	12.5
3k	6.25	6.25	12.5	12.5
3l	6.25	6.25	12.5	12.5
3m	12.5	12.5	6.25	12.5
3n	6.25	12.5	6.25	12.5
3o	3.125	6.25	3.125	3.125
3p	6.25	12.5	6.25	12.5
3q	3.125	6.25	3.125	6.25
3r	3.125	6.25	6.25	6.25
3s	12.5	6.25	12.5	12.5
3t	6.25	6.25	6.25	6.25
3u	6.25	6.25	3.125	6.25
3v	12.5	12.5	12.5	12.5
3w	6.25	6.25	12.5	3.125
3x	6.25	12.5	12.5	6.25
Ciprofloxacin	3.125	12.5	6.25	6.25
Control	-	-	-	-

Table 4: Minimum inhibitory concentration (MIC) (μg/ml) of the synthesized compounds against fungal strains

Compound	*C. albicans* (MTCC 183)	*A.niger* (MTCC 404)
3a	25	25
3b	25	25
3c	12.5	12.5
3d	25	25
3e	12.5	25
3f	25	25
3g	25	25
3h	25	25
3i	12.5	12.5
3j	25	25
3k	12.5	6.25
3l	12.5	6.25
3m	12.5	12.5
3n	6.25	12.5
3o	3.125	3.125
3p	6.25	12.5
3q	3.125	6.25
3r	6.25	3.125
3s	12.5	25
3t	6.25	6.25
3u	6.25	6.25
3v	25	25
3w	6.25	6.25
3x	6.25	12.5
Clotrimazole	6.25	6.25
Control	-	-

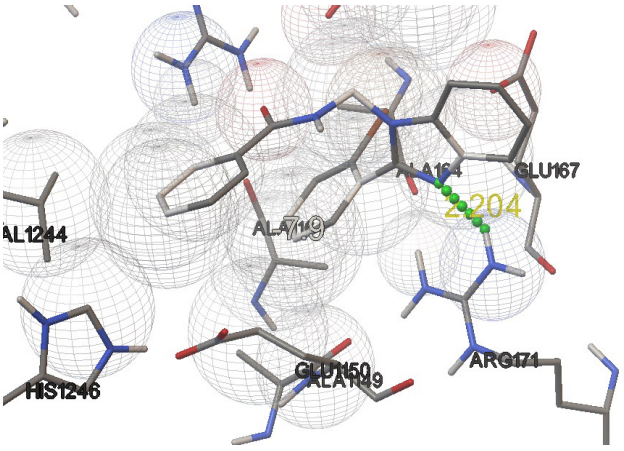


Figure 2: Binding pose for compound 3r (dock score -7.9 kcal/mol) within the domain of microbial receptor showing hydrogen bonding in dashed green line.

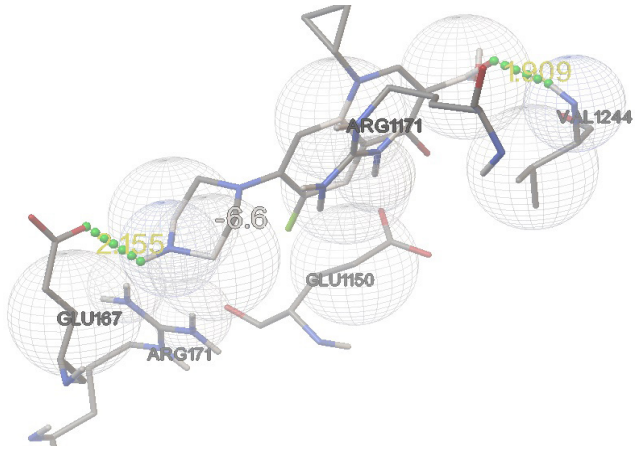


Figure 3: Binding pose for Standard Ciprofloxacin (dock score -6.6 kcal/mol) within the domain of microbial receptor showing two hydrogen bonding in dashed green line.

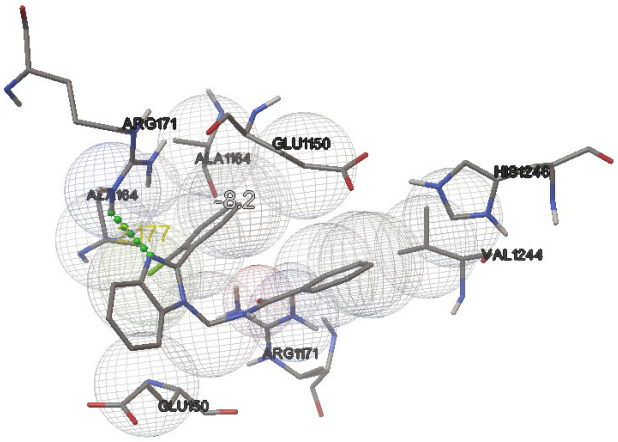


Figure 1: Binding pose for compound 3o (dock score -8.2 kcal/mol) within the domain of microbial receptor showing hydrogen bonding in dashed green line.

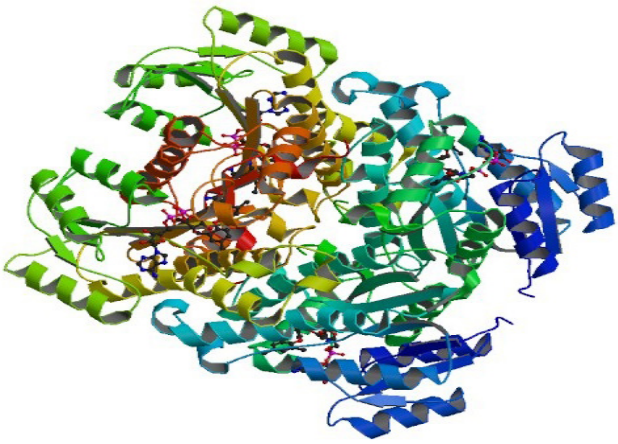
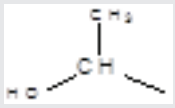


Figure 4: Secondary structure of PDB ID-1C14.

Table 5: Data of dock score of synthesized compounds and interactive amino acids for antimicrobial activity

Comp	R	Dock score	Number of hydrogen bonds	Amino acids involved in bonding
3a	H-	-7.0	-	-
3b	CH_3-	-7.2	-	-
3c	$ClCH_2$-	-7.0	1	Gln1040
3d	C_2H_5-	-6.5	-	-
3e	C_3H_8-	-7.5		
3f	C_4H_{10}-	-7.6	-	-
3g	NH_2-	-7.0	1	Gln1040
3h		-6.8	-	-
3i	SH-	-6.5	-	-
3j	$SHCH_2$-	-6.5	-	-
3k	C_6H_5-	-7.7	1	Arg171
3l	(2-N-C_5H_4)-	-7.1	1	Gln40
3m	(3-N-C_5H_4)-	-7.3	1	Arg1171
3n	(2-OH-C_6H_4)-	-8.1	1	Arg171
3o	(2-Cl-C_6H_4)-	-8.2	1	Arg171
3p	(3-Cl-C_6H_4)-	-7.9	-	-
3q	(4-Cl-C_6H_4)-	-8.1	-	-
3r	(2-Br-C_6H_4)-	-7.9	1	Arg171
3s	(3-Br-C_6H_4)-	-7.8	1	Gln1040
3t	(4-Br-C_6H_4)-	-8.1	-	-
3u	(4-NO_2-C_6H_4)-	-8.0	-	-
3v	(-C_6H_5- CH_2)-	-7.9	-	-
3w	(4-F-C_6H_4)-	-7.8	1	Arg171
3x	(2-NH_2-C_6H_4)-	-8.1	1	Arg171
Internal ligand	ciprofloxacin	-6.6	2	Glu167, Val1244

compounds with microbial protein, *in silico* study was performed using molecular docking. Considering 1C14 as target, the series of compounds were docked to get the best *in silico* confirmations in the domain of 1C14 protein. Binding affinities of the synthesized compounds were evaluated by using docking program autodock vina, which also optimised the antimicrobial activities of synthesized compounds as possible microbial inhibitors. During analysis, H-bonding and dock score were taken as two important parameters for obtaining the hits among the set of compounds. From the *in silico* study, it was revealed that the compounds were having higher dock score than that of standard ciprofloxacin (-6.6 kcal/mol). The main amino acids which played a vital role in interaction are Gln1040, Arg171, Gln40, Arg 1171. Among the series, compound **3o** has displayed best dock score of -8.2 kcal/mol with 1 H-bond, length of 2.177 A°, shown in Figure 1. Compound **3r** also displayed good dock score of -7.9 kcal/mol with 1 H bond, length of 2.204 A°, shown in Figure 2. The data of dock score and interactive amino acids of all the compounds of the series is provided in Table 5. The interactions of standard with the target protein and secondary structure of the receptor are displayed in Figure 3 and Figure 4 respectively. From the docking study, it was confirmed that the compounds substituted with halogen has displayed best interactions with the receptor among the series.

CONCLUSION

Synthesis of N-(Benzimidazol-1-yl methyl)-benzamide derivatives was done using classical mannich reaction. Their structures were elucidated by spectral

analysis. Synthesized compounds were then screened for *in vitro* antibacterial and antifungal activity. Also molecular docking studies were done and their inhibitory activity was tested against PDB ID: 1C14 microbial protein. Both the theory (Docking) and practice (MIC values) demonstrate that N-(Benzimidazol-1-yl methyl)-benzamide derivatives act as potent inhibitor of 1C14 microbial protein. All the synthesized derivatives showed momentous antimicrobial activity against bacterial strains *E.coli* (MIC 12.5-3.125 µg/ml), *P. aeruginosa* (MIC 12.5-3.125 µg/ml), *S.aureus* (MIC 12.5-3.125 µg/ml) and *B.subtilis* (MIC 12.5-3.125 µg/ml) in comparison to reference drug ciprofloxacin and against fungal strains, *C.albicans* (MIC 25-3.125 µg/ml) and *A. niger* (MIC 25-3.125 µg/ml) when compared with clotrimazole as standard antimicrobial agent. Amongst all the synthesized compounds, **3o**, **3q**, **3r**, **3t**, **3u and 3w** with its electron withdrawing substitutions (2-chloro, 4-chloro, 2-bromo, 4-bromo, 4-nitro and 4-flouro groups respectively) on aromatic rings were the most active compounds against the bacterial strains. Compounds **3o and 3q** with chloro substitution at 2 and 4 position of phenyl ring respectively and compound **3r** with its bromo substitution at 2 position of phenyl ring were the most active compounds against fungal strains. Hence it is concluded that N-(Benzimidazol-1-yl methyl)-benzamide derivatives with electron withdrawing substituents could be used for designing more potent antimicrobial agents. Further studies of these derivatives are highly suggested to judge the safety of novel compounds.

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CONFLICT OF INTEREST

The author declares that there is no conflict of interests.

ABBREVIATIONS USED

MS: Mass Spectrometry; **FTIR:** Fourier transform infrared spectroscopy; **NMR:** Nuclear magnetic resonance; **IP:** Indian Pharmacopoeia; **TLC:** Thin Layer Chromatography; **DMSO-d_6 :** Deuterated Dimethyl Sulphoxide; **MIC:** Minimum inhibitory concentration; **Int.lgn:** Internal ligand; **PDB:** Protein Data Bank; **MTCC:** Microbial Type culture collection.

REFERENCES

1. Goker H, Kus C, Boykin DW, Yildiz SN and Altanlar N. Synthesis and Antimicrobial Activity of Some New 2-Phenyl-N-substituted Carboxamido-1H-benzimidazole Derivatives. Arch Pharm Med Chem. 2001;334(5):148-52.
2. Woodford N and Ellington MJ. The emergence of antibiotic resistance by mutation. Clinical Microbiology and Infection : the Official Publication of the Eur Society Clinical Microbiology and Infectious Diseases. 2007;13(1):5-18.
3. Pawar NS, Dalal DS, Shimpi SR, Mahulikan PP. Studies of antimicrobial activity of N-alkyl and N-acyl 2-(4-thiazolyl)-1H-benzimidazoles. Eur J Pharm Sci. 2004;21(2-3):115-8.
4. Goker H, Kus C, Boykin DW, Yildiz S and Altanlar N. Synthesis of some new 2-substituted-phenyl-1H-benzimidazole-5-carbonitriles and their potent activity against Candida species. Bioorg Med Chem. 2002;10(8):2589-96.
5. Mohammad BG, Hussein MA, Abdel-Alim AA and Hashem M. Synthesis and antimicrobial activity of some new 1-alkyl-2-alkylthio-1,2,4-triazolobenzimidazole derivatives. Arch Pharm Res. 2006;29(1):26-33.
6. Deep A, Jain S, Sharma PC, Mittal SK, Phogat P and Malhotra M. Synthesis, characterization and antimicrobial evaluation of 2, 5-disubstituted-4-thiazolidinone derivatives. Arabian J of Chem. 2014;7(3):287-91.
7. Nakano H, Inoue T, Kawasaki N, Miyatka H, Miyatka H, Taguchi T and Satoh T. Synthesis and Biological activites of novel antiallergic agents with 5-lipooxygenase inhibiting action. Bioorganic Med Chem. 2000;8(2):373-80.
8. White AW, Almassy R, Calvert AH and Golding BT. Resistance modifying agents, Synthesis and Biological properties of Benzimidazole inhibitors of DNA repair enzyme Poly(ADP Ribose) polymerase. J Med Chem. 2000;43:4084-97.
9. Zhu Z, Lippa B and Drach JC. Design Synthesis and biological evaluation of tricyclic nucleosides (Dimensional probes) as analogues of certain antiviral polyhalogenated Benzimidazole Ribonucleosides. J Med Chem. 2000;43(12):2430-7.
10. Bariwal JB, Shah AK, Kathiravan MK, Somani RS, Jagtap JR and Jai KS. Synthesis and antiulcer activity of novel pyrimidylthiomethyl and Pyrimidylsulfinylmethyl benzimidazoles as potential reversible proton pump inhibitors. Ind J Pharm Ed Res. 2008;42(3):225-31.
11. Mariappan G, Hazarika R, Alam F, Karki R, Patangia U and Nath S. Synthesis and biological evaluation of 2-substituted benzimidazole derivatives. Arabian J of Chem. 2015;8:715-9.
12. Spasov AA, Yozhitsa IN and Bugaeva LI. Benzimidazole derivatives: Spectrum of Pharmacological activity and toxicological properties (a review). Pharm Chem Journal. 1999;33(5):232-43.
13. March J. Advanced Organic Chemistry: Reaction Mechanisms and Structure, Fourth edition. A. Wiley-Interscience Publication, New York; John Wiley and Sons:900-902.1992.
14. Achar KC, Hosamani KM and Seetharamareddy HR. *In vivo* analgesic and anti-inflammatory activities of newly synthesized benzimidazole derivatives. Eur J Med Chem. 2010;45(5):2048-54.
15. Malinka W, Swiatek P, Filipek B, Sapa J, Jezierska A and Koll A. Synthesis, analgesic activity and computational study of new isothiazolopyridines of mannich base type. Farmaco. 2005;60(11-12):961-8.
16. Kalluraya B, Chimbalkar RM and Hedge JC. Anticonvulsant activity of nicotinyl/isonicotinyl substituted 1,2,4-triazol-5-thione Mannich bases. Indian Journal of Heterocyclic Chemistry. 2005;15(1):15-8.
17. Koksal M, Gokhan N, Kupeli E, Yesilada E and Erdogan H. Analgesic and anti-inflammatory activities of some new mannich bases of 5- nitro-2-benzoxazolinones. Archives of Pharmacal Research. 2007;30(4):419-24.
18. IvanovaY, Momekov G, Petrov O, Karaivanova M and Kalcheva V. Cytotoxic Mannich bases of 6-(3-aryl-2-propenoyl)-2-(3H)-benzoxazolones. Eur J Med Chem. 2007;52(11-12):1382-7.
19. Gul HI, Vepsalainen J, Gul M, Erciyas E and Hanninen O. Cytotoxic activities of mono and bis mannich bases derived from acetophenone against Renca and Jurkat cells. Pharmaceutica Acta Helvetiae. 2000;74(4):393-8.
20. Vashishtha SC, Zello GA, Nienaber KH. Cytotoxic and anticonvulsant aryloxy aryl mannich bases and related compounds. Eur J Med Chem. 2004;39(1):27-35.

21. Ashok M, Holla BS and Poojary BC. Convenient one pot synthesis and antimicrobial evaluation of some new Mannich bases carrying 4-methylthiobenzyl moiety. Eur J Med Chem. 2007;42(8):1095-1.
22. Pandeya SN, Sriram D, Nath G and De Clercq. Synthesis , antibacterial, antifungal and anti-HIV activities of norfloxacin Mannich bases. Eur J Med Chem. 2000;35(2):249-55.
23. Singh BN, Shukla SK and Singh M. Synthesis and biological activity of sulphadiazine schiff's bases of isatin and their N-Mannich bases. Asian J of Chemistry. 2007;19(7):5013-8.
24. Babbar R, Pathak DP, Jain N, Jain S. Synthesis and anti-inflammatory activity of mannich bases of nicotinamide with diclofenac and mefenamic acid. Der Pharma Chemica. 2012;4(5):2024-8.
25. Furniss BS, Hannaford AJ, Smith PWG and Tatchell R. Vogel's Text book of Practical Organic Chemistry. 5th edition. Longman scientific and technical England;1989.
26. Devmurari VP, Pandey S, Goyani MB, Jivani NP. Synthesis and antibacterial evaluation of 2- some substituted benzimidazole-1-carbodithionate derivatives. Inter J Chem Tech Res. 2010;2(1):598-605.
27. Simonov AM, Anisimova VA. Synthesis and transformation of 2- amino benzimidazoles. (Review). Chemistry of Heterocyclic Compounds. 1979;15(7):705-23.
28. Wang ML, Liu BL. Synthesis of 2- mercaptobenzimidzole from the reaction of o-phenylene diamine and carbon disulphide in the presence of potassium hydroxide. Journal of Chinese Institute of Chemical Engineers. 2007;38(2):161-7.
29. Pharmacopoeia. Pharmacopoeia of India. vol. II. Ministry of Health Department, Govt. of India, New Delhi :1996.
30. Shah UA, Wagh NK, Deokar HS, Kadam SS, and Kulkarni VM. Design, synthesis, pharmacological screening and molecular docking of biphenyl analogues as antiinflammatory agents (Part-I). Journal of Pharma and Bio Sciences. 2010;1(4):501.
31. Trott O, Olson AJ. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading. J Comput Chem. 2010;31(2):455-61.

SUMMARY

- A series of N-benzimidazol-1-yl-methyl-benzamide derivatives (3a-3x) were synthesized by Mannich reaction and evaluated for *in vitro* antimicrobial activity against various bacterial and fungal strains. The novel target compounds were further characterized by spectral and analytical techniques. Among the synthesized derivatives, 3o N-[2-(2-chloro-phenyl)-benzimidazol-1-ylmethyl]-benzamide, 3q N-[2-(4-chloro-phenyl)-benzimidazol-1ylmethyl]-benzamide and 3r N-[2-(2-bromo-phenyl)-benzimidazol-1-ylmethyl]-benzamide were found to be most effective antimicrobial compounds. Clotrimazole and ciprofloxacin were used as reference antimicrobial agents. Molecular docking studies were also performed to describe the interaction of the title compounds with microbial protein. Hence it is summarized that N-(Benzimidazol-1-yl methyl)-benzamide derivatives with electron withdrawing substituents might be used for designing more effective antimicrobial agents.