Heterocyclic Letters
Vol. 12| No.3|653-668|May-July|2022
ISSN : (print) 2231–3087 / (online) 2230-9632
CODEN: HLEEAI
http://heteroletters.org

SYNTHESIS, DOCKING AND BIOLOGICAL EVALUATION OF N-[4-(1H-BENZIMIDAZOL-2-YL)-PHENYL]-3-(SUBSTITUTED)-ACRYLAMIDE DERIVATIVES AS ANTIMICROBIAL, ANTHELMINTIC AND ANTIOXIDANT AGENTS

Narasimha K[1], Durgaprasad Kemisetti[2*], Faruk Alam[2], Ega Jagadish Kumar[1], Biplab Kumar Dey[2]

[1]*Faculty of Pharmaceutical Science, Chaitanya Deemed to be University, Hanamkonda, Warangal, Telangana-500001*
[*2]*Faculty of Pharmaceutical Science, Assam Down Town University, Panikhaiti, Gandhinagar, Guwahati, Assam-781026*
[*]*Corresponding Author: Dr. Durgaprasad Kemisetti*
E.Mail: kdp251999@gmail.com
ORCID ID: 0000-0003-2081-1794

ABSTRACT

A new series of N-[4-(1H-benzimidazol-2-yl)-phenyl]-3-(substituted)-acryl amide (chalcones) derivatives were synthesized by condensation of *p*-amino benzoic acid and *o*-phenylene diamine. Further the acetylated product of benzimidazole derivatives undergoes Claisen-Schimdt condensation with aryl aldehydes to produce corresponding chalcones. The structures of synthesized compounds were characterized by FT-IR, ^{1}H NMR and ESI-MS spectral data. The *in-vitro* biological activities of the test compounds (**5a-5j**) were screened for antimicrobial, antifungal, antioxidant and anthelmintic activities. Among the compounds **5a, 5d** and **5g** showed more significant antimicrobial activity, antifungal, antioxidant and anthelmintic. By docking on to PDB ID: 1A9U and 3FLY, confirmed that **5a, 5d** and **5g** possess higher anthelmintic activity. The *in-vitro* antioxidant activity was evaluated by DPPH. All the synthesized compounds have shown free radical scavenging and anthelmintic activity in dose dependent manner.

KEYWORDS: Benzimidazole, Docking, Antimicrobial, Antioxidant, Anthelmintic activity.

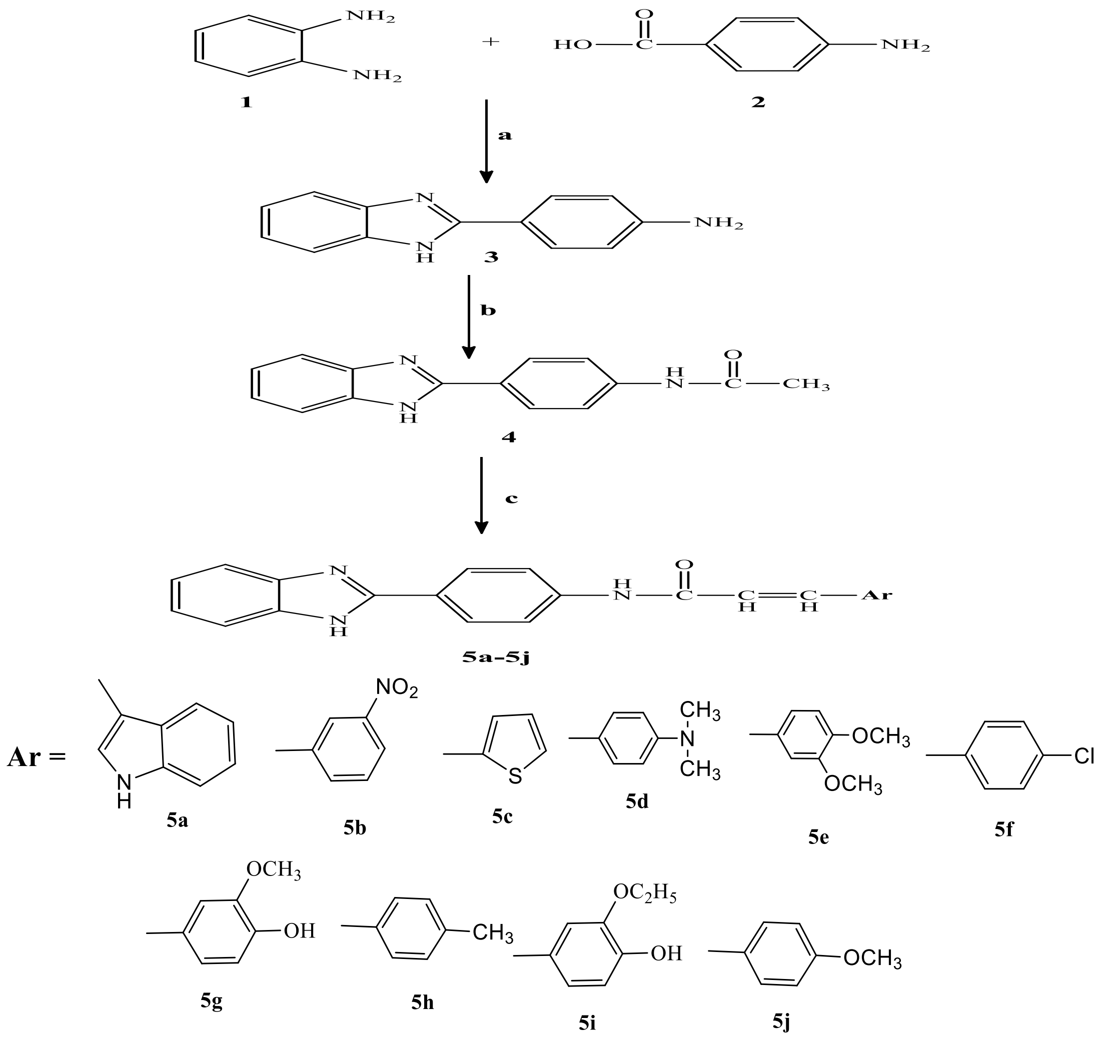
INTRODUCTION:

The benzimidazole nucleus which is a useful structure for research and development of new pharmaceutical molecules has received much attention in the last decade. The benzimidazole still remain one of the most versatile classes of compounds, therefore are useful substructures for further molecular exploration[i]. They exhibit a wide range of biological activities and this molecule is a constituent of vitamin-B_{12}[ii]. Literature review revealed that benzimidazole, possess diverse chemotherapeutic activities such as antimicrobial[iii-iv], antiviral[v-vi], anti-HIV[vii], anti-mycobacterial[viii], anticancer[ix-x], anti-inflammatory[xi], and anticonvulsant[xii]. Benzimidazole is a potent inhibitor of PARP-1 and PARP-2 that is currently under development by KuDOS Pharmaceuticals[xiii]. These are a relatively new class of benzodiazepine receptor ligands that range in efficacy from antagonist to full agonist[xiv]. Additional analogs have displayed inhibitory activity against EGFR, VEGFR-2 and PDGFR kinase inhibitors[xv]. Well known examples of approved benzimidazole-based drugs like omeprazole (Prilosec, a proton-pump inhibitor)[xvi], candesertan (anti-hypertensive, an angiotensin II receptor antagonist)[xvii], mebendazole (treatment of worm infestations)[xvii] and astemizole (non-sedative anti-histamine)[xix].

Owing to the immense important and varied biological activities exhibited by the benzimidazole, it was aimed in our present investigation to design and synthesize some novel benzimidazole (**5a-5j**) derivatives as depicted in **Scheme**. In this context we have prepared a series of N-[4-(1H-Benzimidazol-2-yl)-phenyl]-3-(substituted) acrylamide derivatives by condensation and cyclization of *o*-phenylene diamine with *p*-amino benzoic acid. Further the acetylated product was treated with different aryl aldehydes to give the titled compounds. These synthesized compounds were evaluated for their *in-vitro* biological activities.

EXPERIMENTAL SECTION:

The IR spectra of the compounds were recorded on Perkin-Elmer 1600 spectrophotometer, FT-IR spectrometer using KBr disc. ^{1}H NMR was recorded on Bruker advance-300 MHz instrument using TMS as an internal standard (chemical shifts in δ, ppm). Mass spectra were recorded on LC-MSD-Trap-SL using ESI-MS method. The purity of the compounds was checked on silica gel-coated aluminum sheets by thin-layer chromatography. Melting points (m.p.) were determined in open capillaries, using Toshniwal melting point apparatus, expressed in ^{0}C and are uncorrected. Column chromatography was performed by using Qualigen's silica gel for column chromatography (60–120 mesh). All the solvents were AR grade and were distilled before use. Indole-3-carboxyaldehyde was obtained from Merck (India). All substituted aldehydes were purchased from Sd. Fine Chemicals. Fluconazole and streptomycin were purchase from Himedia (Mumbai, India). DPPH (α,α-diphenyl-β-picryl hydrazyl) was purchased from Sigma Chemical Company (St. Louis, MO, USA) and H_2O_2 from Merck, India. The standard cultures of test microorganisms were obtained from Department of Microbiology, Kakatiya University, Warangal, Telangana, India.



Reagents: (a) 4N HCl, aq $NaHCO_3$; (b) $CHCl_3$, Ac_2O, (c) KOH, Ethanol, substituted aldehydes.

Chemistry:

General procedure for Synthesis of 2-(4-aminophenyl) benzimidazole (3):

2-(4-aminophenyl) benzimidazole (**3**) was synthesized by the condensation of (0.01 mol) *o*-phenylene diamine and (0.01 mol) *p*-amino benzoic acid in 40 ml 4(N) Hydrochloric acid and refluxed for 4 hrs, then cooled at room temperature. The pH was adjusted to 7.2 using sodium hydroxide pellets. The resulting solid was filtered and washed with water, dried in vacuum and recrystallized with methanol[xx]. The completion of the reaction was monitored by thin layer chromatography using solvent system chloroform: methanol. The yield of 2-(4-aminophenyl) benzimidazole was found to be 78.5%.

Synthesis of N-(4-(1H-benzo[d]imidazol-2-yl) phenyl) acetamide (4):

Dissolve 0.01 mol of 2-(4-aminophenyl) benzimidazole (**3**) in chloroform (50 ml) and acetic anhydride (0.01 mol) was added drop wise with constant stirring at 5 to 10ºC. The reaction mixture was stirred for 4 hrs. The excess solvent was distilled off and the solid product was filtered, dried and recrystallized from ethanol to give N-(4-(1H-benzo[d]imidazol-2-yl) phenyl) acetamide.

Synthesis of Benzimidazolyl chalcones Derivatives (5a-5j):

Dissolve 0.01 mol of N-(4-(1H-benzo[d]imidazol-2-yl) phenyl) acetamide (**4**) in ethanol (30 ml) and various aromatic aldehydes (0.01 mol) were taken and then an aqueous solution of KOH (2%, 5 ml) added to it. The reaction mixture was refluxed for 5 hrs and then the excess solvent was removed by vacuum distillation and then it was poured into crushed ice and acidified with dilute HCl. The solid separated was filtered and recrystallized by using ethanol.

N-[4-(1H-benzimidazol-2-yl)-phenyl]-3-(1H-indol-3yl)-acrylamide (5a):

M.F: $C_{24}H_{20}N_4O$, M Wt: 380.44, % yield 76 %, 156-158^{0}C; IR, cm^{-1} (KBr):3395 cm^{-1} (-NH *str*), 3112cm^{-1} (-NH), 3060-2900cm^{-1} (-CH *str*, Ar), 1699 cm^{-1} (C=O *str*), 1647 cm^{-1} (-CH=CH-), 1594 cm^{-1} (-NH),1433cm^{-1} (C=N *str*), 1331,1293cm^{-1} (C-N *str*), 990-860 cm^{-1} (-NH). ^{1}H NMR, δ ppm data (DMSO-d_6)**:**δ10.5 (s, 1H, -NH), δ10.1 (d, 1H, -NH , indole), δ9.30 (s, 1H, - NH-C=O), δ7.55 (d, 1H, -CH=CH), δ7.46-7.70 (d, 4H), δ7.26-7.70 (m, 4H), δ7.00 (m, 4H, indole), δ6.84 (d, 1H, -CH=CH). Mass (ESI-MS): m/z 377 (M^+,100%), 378(M+1, 30%).

N-[4-(1H-benzimidazol-2yl)-phenyl]-3-(3-nitro-phenyl)-acrylamide (5b):

M.F: $C_{22}H_{16}N_4O_3$, M.Wt: 384.38, % yield 77 %, mp. 136-138^{0}C; IR, cm^{-1} (KBr):3395 cm^{-1} (-NH *str*), 3112 (-NH), 3060-2900 (C-H *str*, Ar), 1699 (C=O *str*), 1660-1640 (-CH=CH-), 1620-1590 (-NH), 1620-1535 (–NO_2), 1620-1430(C=N *str*), 1600,1610 (-C=C-,Ar), 1375-1275 (s*ym*,-NO_2), 764 & 694 (Ar-H) . ^{1}H-NMR (DMSO) δ ppm: 10.6-11.3 (s, 1H, -NH), 8.33-9.38 (s, 1H, -NH-C=O), 7.47-8.23 (d, 4H,Ar-H), δ7.46-7.70 (d, 4H, Ar-H), δ7.26-7.70 (m, 4H), δ7.09 &7.66 (d, 1H,-CH=CH). Mass (ESI-MS): m/z 384 (M^+, 100%), 385 (M+1, 24.8%).

N-[4-(1H-benzo[d]imidazol-2-yl) phenyl]-3-(thiophen-2-yl)acrylamide (5c):

M.F: $C_{20}H_{15}N_3OS$, M. Wt: 345.41, % yield 89 %, mp. 126-128^{0}C; IR, cm^{-1} (KBr): 3320 (-NH *str*), 3210 (-NH), 3090 (C-H *str*, Ar), 1700 (C=O *str*), 1660-1650 (-CH=CH-), 1620-1535 (–NO_2), 1640-1430(C=N *str*), 1600,1610 (-C=C-,Ar), 1375-1275 (s*ym*,-NO_2), 740 & 690 (Ar-H). ^{1}H-NMR (DMSO) δ ppm: 11.20 (s, 1H, -NH-C=O), 10.6 (s, 1H, -NH), 7.32-8.05 (d, 4H, Ar-H), δ7.46-7.70 (d, 4H, Ar-H), δ7.26-7.70 (m, 4H), δ7.10 (d, 1H,-CH=CH), δ7.55 (d, 1H,-CH=CH). Mass (ESI-MS): m/z 384 (M^+, 100%), 385 (M+1, 24.8%).

N-[4-(1H-benzimidazol-2-yl)-phenyl]-3-(4-dimethylamino-phenyl)-acrylamide (5d):

M.F: $C_{24}H_{22}N_4O$, M. Wt: 382.45, % yield 84 %, mp. 156-157^{0}C; IR, cm^{-1} (KBr):3395 cm^{-1} (N-H str in –NH-C=O), 3112cm^{-1} (N-H in ring), 3060-2900cm^{-1} (C-H str in Ar), 2853 cm^{-1},2808 cm^{-1} (C-H str in –CH_3), 1699 cm^{-1} (C=O str), 1620-1590 cm^{-1} (N-H bend), 1620-1430 cm^{-1} (C=N str in ring), 1586 cm^{-1} (-CH=CH-), 1585,1555,1527 cm^{-1} (-C=C- in Ar), 1365 cm^{-1} (C-H out of plane), 855 cm^{-1} (Ar-H). ^{1}H NMR, δppm (DMSO- d_6): 10.9 (s, 1H, -NH), 8.54 (s, 1H, -NH-C=O), 7.55 (d, 1H, -CH=CH), 7.46-7.70 (d, 4H,Ar-H), 7.26-7.70 (m, 4H), 6.84 (d, 1H, -CH=CH), 6.54-7.12 (d, 4H, Ar-H), 2.85 (s, 3H, -NCH_3). Mass (ESI-MS): m/z 382 (M^+, 100%), 383 (M+1,28%).

N-[4-(1H-benzimidazol-2-yl)-phenyl]-3-(3,4-dimethoxy-phenyl)-acrylamide (5e):

M.F: $C_{24}H_{21}N_3O_3$, M. Wt: 399.44, % yield 91 %, mp. 172-174^{0}C; IR, cm^{-1} (KBr):3415cm^{-1}(-NH), 3395 (-NH), 3060-2900 (C-H *str*), 2845 (C-H *str*, –OCH_3), 1670 (C=O str), 1620-1590 (-NH), 1620-1430 (C=N, *str*),1586 (-CH=CH-), 1585, 1550, (-C=C-, Ar), 1275,1021 (C-O-C *str*), 840-960(Ar-H), 607(Ar-H).^{1}H NMR, δ ppm (DMSO- d_6): 11.3 (s, 1H, -NH),9.36 (s, 1H, -NH-C=O), 7.55 (d, 1H, -CH=CH),7.46-7.70 (d, 4H, Ar-H), 7.26-7.70 (m, 4H),6.84 (d, 1H, -CH=CH), 6.61-6.75 (m, 3H, Ar-H), 3.73 (s, 3H, -OCH_3). Mass (ESI-MS): m/z 399 (M^+, 100%), 400 (M+1,28%).

N-[4-(1H-benzimidazol-2-yl)-phenyl]-3-(4-chloro-phenyl)-acrylamide (5f):

M.F: $C_{22}H_{16}ClN_3O$, M. Wt: 373.83, % yield 89 %, mp. 129-131^{0}C; IR,cm^{-1} (KBr): 3395 (-NH *str*), 3112 (-NH), 3060-2900 (C-H *str*, Ar), 1670 (C=O *str*), 1620-1590 (-NH), 1620-1430 (C=N), 1586 (-CH=CH-), 1585,1555,1527 (-C=C-, Ar), 750&690(Ar-H), 633 (C-Cl). ^{1}H

NMR, δ ppm (DMSO- d_6):10.6 (s, 1H, -NH),9.56 (s, 1H, -NH-C=O), 7.55 (d, 1H, -CH=CH), 7.46-7.70 (d, 4H,Ar-H), 7.26-7.70 (m, 4H),7.22-7.24 (d, 4H, Ar-H), 6.84 (d, 1H, -CH=CH). Mass (ESI-MS): m/z 373 (M^+, 100%), 375 (M+1, 28%).

N-(4-(1H-benzoimidazol-2-yl)phenyl)-3-(4-hydroxy-3-methoxyphenyl)acrylamide (5g):
M.F: $C_{23}H_{19}N_3O_3$, M. Wt: 385.41, % yield 78 %, mp. 133-135^{0}C; IR,cm^{-1} (KBr): 3400 -3380(-NH *str*), 3130-3070 (C-H *str*, Ar), 3570-3200 (-OH, broad), 2830 (CH_3-O-,*str*), 1680 (C=O *str)*, 1620-1590 (-NH), 1509-1610 (C=N), 1615 (-C=C-, Ar), 1410 (-OH, Phenol), 1200 (C-O, *str*), 710-690(Ar-H). ^{1}H NMR, δ ppm (DMSO- d_6): 11.06 (s, 1H, -NH-C=O), 10.9 (s, 1H, -NH), 9.02 (s,1H,-OH),8.01 (d, 1H, -CH=CH), 7.86-7.20 (m,11H, Ar-H), 6.84 (d, 1H, -CH=CH), 4.25(s,3H,-CH_3). Mass (ESI-MS): m/z 385 (M^+, 100%), 386 (M+1, 20%).

N-(4-(1H-benzoimidazol-2-yl)phenyl)-3-(p-tolyl)acrylamide (5h):
M.F: $C_{23}H_{19}N_3O$, M. Wt: 353.41, % yield 78 %, mp. 158-160^{0}C; IR,cm^{-1} (KBr): 3400 -3300(-NH *str*), 3100 (C-H *str*, Ar), 2970 (-CH_3,*str*), 1605 (C=O *str)*, 1610-1590 (-NH), 1510-1600 (C=N), 1615 (-C=C-, Ar), 1350-1330 (C-H, *bending*), 710-690(Ar-H). ^{1}H NMR, δ ppm (DMSO- d_6): 10.92 (s, 1H, -NH-C=O), 10.9 (s, 1H, -NH), 8.22 (d, 1H, -CH=CH), 7.64-6.93 (m,12H, Ar-H),6.24 (d, 1H, -CH=CH), 2.78 (s,3H,-CH_3). Mass (ESI-MS): m/z 353 (M^+, 100%), 354 (M+1, 68%).

N-(4-(1H-benzo[d]imidazol-2-yl)phenyl)-3-(3-ethoxy-4-hydroxyphenyl)acrylamide (5i):
M.F: $C_{24}H_{21}N_3O_3$, M. Wt: 399.44, % yield 88 %, mp. 166-168^{0}C; IR,cm^{-1} (KBr): 3400 -3380(-NH *str*), 3130-3070 (C-H *str*, Ar), 2830 (CH_3-O-,*str*), 1680 (C=O *str)*, 1620-1590 (-NH), 1509-1610 (C=N), 1615 (-C=C-, Ar), 1200 (C-O, *str*), 710-690(Ar-H). ^{1}H NMR: δ 7.95 – 7.53 (m, 7H), 7.47 – 7.15 (m, 4H), 7.05 – 6.76 (m, 3H), 6.35 (d, J = 15.0 Hz, 1H), 4.10 (d, J = 77.0 Hz, 3H), 1.41 (d, J = 11.9 Hz, 3H). MF: $C_{24}H_{21}N_3O_3$, m/z: 399.16 (100.0%), 400.16 (26.0%).

N-(4-(1H-benzo[d]imidazol-2-yl)phenyl)-3-(4-methoxyphenyl)acrylamide (5j):
M.F: $C_{23}H_{19}N_3O_2$, M. Wt: 369.41, % yield 81 %, mp. 169-171^{0}C; IR,cm^{-1} (KBr): 3400 -3380(-NH *str*), 3130-3070 (C-H *str*, Ar), 2830 (CH_3-O-,*str*), 1680 (C=O *str)*, 1620-1590 (-NH), 1509-1610 (C=N), 1615 (-C=C-, Ar), 1200 (C-O, *str*), 710-690(Ar-H). ^{1}H NMR, δ ppm (DMSO- d_6): 11.02 (s, 1H, -NH-C=O), 10.6 (s, 1H, -NH), 7.82 (d, 1H, -CH=CH), 7.86-7.20 (m,12H, Ar-H), 6.62 (d, 1H, -CH=CH), 4.25(s,3H,-CH_3). Mass (ESI-MS): m/z 369 (M^+, 100%), 370 (M+1, 20%).

Protocol of Molecular Docking:
The software used for finding whether a molecule can be a drug or not is by Lipinski rule of five. It gives information about Molecular weight, Hydrogen bond donor, hydrogen bond acceptor, logP value and Molar refractivity. Molsoft L.L.C Drug Likeness and Molecular Property Prediction gives information about a molecule i.e Molecular formula, Molecular weight, Hydrogen bond acceptor, Hydrogen bond donor, MolLogP, MolLogS, MolPSA, MolVol, pKa, BBB Score, Number of stereo centers and Drug Likeness Score. OSIRIS Property explorer provides information whether a molecule, if synthesized causes any toxicity effect by showing in the window on the screen, Green color indicates non- toxic whereas Red color toxic. Apart from this other information like solubility, TPSA, clogP, Drug Likeness and Drug Score are available. PASS (Prediction of Activity Spectra for Substances) online software predicts whether a molecule is biologically active, as value *Pa* and if inactive as *Pi*. Drug activity ADME, Physicochemical parameters, GI Absorption, Drug likeness, GPCR ligand, Ion channel modulator, Kinase inhibitor, Nuclear receptor ligand, Protease inhibitor and Enzyme inhibitor and Bioavailablity score were found out using Molinspiration and SWISS ADME softwares[xxi-xliii].

For molecular docking, the software used was Autodock 4.0/4.2, as it was user compatible software which was most widely used for Protein-Ligand binding. It can be used in any of the three methods like Rigid Body Docking, Flexible Ligand Docking and Flexible ligand and protein respectively[xxi-xliii].

Molecular properties, Druglikeness, Bioactivity with respect to GPCR ligand, Ionchannel modulator, Kinase Inhibitor, Nuclear receptor ligand, Protease inhibitor and Enzyme inhibitor, were found out by using Molinspiration software[xxvi-xxx].

Physicochemical properties like GI Absorption, Solubility, Inhibitor, Bioavailability and ADME properties were found by SWISS ADME software[xxvi-xxx].

All allowed torsions, for the ligands were set as flexible. Molecular docking study was executed to understand the probable binding interactions of the synthesized compounds (ligands) onto the active site of the receptors 1A9U and 3FLY, respectively.

All the hetero atoms including water molecules and bound ligands in PDB crystal structures were removed from the receptors. After adding polar hydrogen and charges, the receptor was set as rigid with no flexible bonds.

The docking glide score, free binding energy (*using Prime MM-GBSA method*), hydrogen bonding and π–π interactions with the surrounding Amino acids were studied to elucidate the binding affinities and appropriate alignment of all the ligands onto the active site of 1A9U and 3FLY, respectively. The best-suited conformations of ligands, which were successful in reversing the protein in its original conformation and produced maximum dock score, were studied precisely[xxi-xliii].

***Antimicrobial activity*:**

The antimicrobial activity was performed by agar disc-diffusion technique[xliv] against Gram-positive bacteria including *Staphylococcus aureus, Bacillus subtilis* and *L.bacillus* and Gram-negative bacteria including *Escherichia Coli, Pseudomanas aeruginosa* and *Salmonella paratyphi*. Antifungal activity was screened against *Pencillin notatum, Aspergillus flavus, Candida albicans* and *Aspergillus niger*. The inhibition zone was measured in mm using streptomycin against bacteria and Flucanazole for fungi as standards in dimethyl sulphoxide (DMSO). DMSO showed no inhibition zone. Each compound and standard drugs were diluted obtaining 1000 µg/ml concentration, as a stock solution. All the compounds were tested at a concentration of 50 µg/ml and 100 µg/ml. Each experiment was repeated twice and the average of the two determinations was recorded.

***DPPH radical scavenging method*:**

The total antioxidant activity was measured by the DPPH radical scavenging assay method[xlv]. The radical scavenging activity of plant extracts against stable DPPH radical (DPPH*) was determined. L-Ascorbic acid was used as the reference compound. The antioxidant activity is expressed in terms of IC_{50} (concentration of the extract / reference compound required to inhibit DPPH radical formation by 50%).The results were expressed as IC_{50} values (the concentration of test required to scavenge 50% free radicals).

Nitric oxide ($NO^{\cdot}$) radical scavenging method:

Nitric oxide generated from sodium nitroprusside in an aqueous solution at physiological *p*H, interacts with the Griess reagent and the absorbance of the chromophore formed was measured at 546 nm using spectrophotometry[xlvi]. The reaction mixture (5.0 ml) containing SNP (5 mM) in phosphate buffered saline (pH 7.3), with or without the plant extract at different concentrations, was incubated at 25^{0}C for 180 min in front of a visible polychromatic light source (25W tungsten lamp). Generation of $NO^{\cdot}$ free radical thus interacted with oxygen to produce the nitrite ion ($NO^{\cdot}$) which was assayed at 30 min intervals by mixing 1.0 ml of incubation mixture with an equal amount of Griess reagent (1% sulfanilamide in 5% phosphoric acid and 0.1% naphthylethylenediaminedihydrochloride).

***Anthelmintic Activity*:**

The synthesized compounds were screened for anthelmintic activity by using Indian earth worms *Pheretima posthuma* obtained from Department of Botany, Kakatiya University. The earthworms were divided into groups of six earthworms approximately of equal size were selected randomly for the present study[xlvii].

Albendazole is diluted with normal saline solution to obtain 0.1, 0.2, 0.5 and 1 % (*m/V*) served as standard and was poured into Petri dishes. The synthesized compounds were dissolved in minimal quantity of ethanol and diluted to prepare four concentrations of 0.1, 0.2, 0.5 and 1 % (*m/V*) of each compound. Normal saline served as a control. The compounds were evaluated by the time taken for complete paralysis and death of earthworms. The mean lethal time for each test compound was recorded and compared with standard drug. The time taken by worms to become motionless was noted as paralysis time. Death was concluded when the worms lost their motility followed with fading away of their body colour.

RESULTS AND DISCUSSION:

The key intermediate used for the synthesis of both series of the final compounds was 2-(4-aminophenyl) benzimidazole (3), which in turn prepared by the reaction of *o*-phenylene diamine (1) with *p*-amino benzoic acid (2) in the presence of 4N HCl. The reaction of compound (3) with different aryl aldehydes in absolute ethanol gave benzimidazole Schiff bases (6a-6j). And the N-[4-(1H-Benzimidazol-2-yl)-phenyl]-3-(aryl) acryl amides (5a-5j) were prepared by treating acetylated product of benzimidazole with aryl aldehyde using Claisen-schimdt condensation. 2-(4-aminophenyl)benzimadole (**3**) was prepared following the procedure previously described [23-24]. The physical data of N-(4-(1H-benzo[d]imidazol-2-yl) phenyl) acetamide (**4**) were comparable.

IR spectra of these compounds showed the presence of characteristic absorption peaks around 3395 cm^{-1}(-NH-C=O-), 1700 to 1670 cm^{-1} (-C=O-, str), 1660-1640 cm^{-1} (-CH=CH-), 1620-1590 cm^{-1}(N-H bend) 1620-1430 cm^{-1} (C=N). ^{1}H NMR spectra revealed that the compounds shows peaks at δ10.5-11.9 (s, 1H,-NH), δ7.46-7.70 (m, 4H, benzene), δ7.26-7.70 (m, 4H, Hetero aromatic), δ6.84 and 7.55 corresponding to two protons of (-CH=CH-). ^{13}C NMR spectra revealed that the compounds shows peaks at δ 168.2 (C of C=O), δ 163.2 (N=C), δ 153.6 (C-N), δ 122.9-141.5 (C=C in hetero aromatic), δ 113.2-129.9 (C=C in aromatic), δ 43.6 ($-CH_3$, aliphatic).

All the synthesized compounds were subjected to Lipinski rule of five, the compounds along with standard (Albendazole), was following the rule without violation.

The synthesized compounds 5a-5j and standard when subjected to OSIRIS toxicity, all the compounds along with standard (5a, 5b, 5c, 5e, 5f, 5g, 5h, 5i) exhibited non-toxic (mutagenicity, tumorigenicity, reproductive toxicity and irritating effects), whereas 5d has exhibited mutagenic and tumorigenic effect, 5j had shown irritant and reproductive effect and Albendazole had shown reproductive effect. Drug score for compound 5a and Albendazole were found to be 0.41 and 0.26 respectively.

The derivatives along with standard drug, when subjected for PASS Online, for Anthelmintic activity the values obtained *Pa* and *Pi* for compound 5a and standard were 0.493, 0.018 and 0.847, 0.002 respectively.

As per the obtained results from the SWISS ADME software for the synthesized compounds, along with standard, all the compounds were found to possess moderate to lower solubility, higher GI absorption and similar bioavailability score (0.55). However, all the compounds were inhibiting the enzymes, CYP1A2 other than compounds like 5b and 5f i.e no action on CYP1A2.

The bioactivity score > 0.00, the compound has considerable bioactivity, if the activity ranges from 0.00-0.50, then moderate activity and if activity is < -0.50, then compound is inactive[xxvi-xxx].

From the docking result using two proteins (PDB ID: 1A9U and 3FLY), has been found that the Sub Rank and Rank Grep Pattern values for all the test and standards compounds were found to be 1(one) (Table 1a and 1b).

Table 1a: Binding energies of test compounds and standard (Std. Albendazole)

PDB ID: 1A9U	5a	5b	5c	5d	5e	5f	5g	5h	5i	5j	Std.
Run	5	2	10	2	9	6	1	1	9	3	1
Binding Energy (kcal/mol)	**-8.01**	-6.50	-6.90	-7.57	-7.18	-7.69	-7.29	64.36	-6.75	-7.17	-6.33
Cluster RMSD	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Reference RMSD	46.59	68.45	75.87	41.57	41.88	42.21	41.55	44.71	40.74	41.47	46.17

Table 1b: Binding energies of test compounds and standard (Std. Albendazole)

PDB ID: 3FLY	5a	5b	5c	5d	5e	5f	5g	5h	5i	5j	Std.
Run	9	1	3	10	2	2	1	3	1	3	4
Binding Energy (kcal/mol)	**-7.23**	-6.80	-7.14	-6.18	-2.71	-6.93	-6.91	-6.79	-6.82	-6.62	-5.78
Cluster RMSD	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Reference RMSD	36.41	35.29	26.46	27.13	36.51	37.04	35.70	25.63	25.66	35.67	19.35

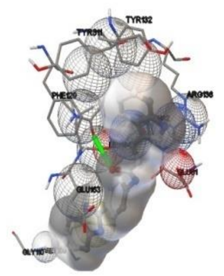
Compound **5a** had lowest binding energy against 1A9U and 3FLY were found to be **-8.01** and **-7.23** kcal/mol, respectively as compared to Albendazole -6.33 and -5.78 respectively (Table 1a and 1b), which indicated a good binding pose or very high binding affinity towards the receptor binding site. Compound **5d** had lower energy, but higher energy than **5a** against 1A9U and 3FLY was found to be -7.57 and -6.18 kcal/mol. Compound **5g** had lower energy, but higher energy than **5a** and **5d** against 1A9U and 3FLY was found to be -7.29 and -6.91kcal/mol. **Albendazole** had lower energy, but higher energy than **5a, 5d** and **5g** against 1A9U and 3FLY was found to be -6.33 and -5.78 kcal/mol.

The results of 1A9U and 3FLY interacting amino acids for the synthesized compounds and standard were tabulated in Table 2 and Figure 1.

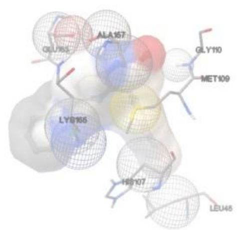
Table 2: Interacting Amino acids for test and standard compounds (Std. Albendazole)

Interacting Amino acids			
Comp	**1A9U**	**Comp**	**3FLY**

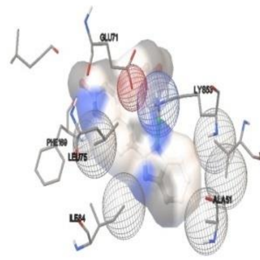
5a	TYR132, ARG136, GLU81, ASN82, LEU164, GLU163, PHE129, TYR311, MET109, GLY110 (1 Hydrogen UNL O1).	5a	LYS53, LEU104, VAL30, ALA51, THR106, LEU75, ILE84, GLY110,ALA111(NoHydrogens)
5d	LyS165, HIS107, LEU48, MET109, GLY110, ALA157, GLU163 (1 Hydrogen LYS165)	5d	PHE348, LYS76, VAL349, LEU86, PRO351, LYS79
5g	LYS53, VAL38, ALA51, ILE84, LEU75, PHE169, GLU71 (1 Hydrogen LYS53)	5g	ASP292, LEU246, PRO266, LYS267, LEU291, LEU289, VAL239(1Hydrogen UNL O1)
Std.	THR132, GLN133, ASN82, LYS165, GLU163 (1 Hydrogen UNL1, N1)	Std.	GLU163, TYR311, ARG136, ASN82, GLU81, LYS165 (2 Hydrogens UNL1,O1, UNL1, O1)



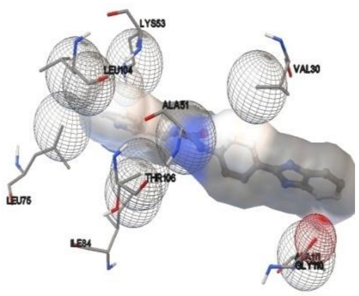
5a (1A9U)



5d (1A9U)

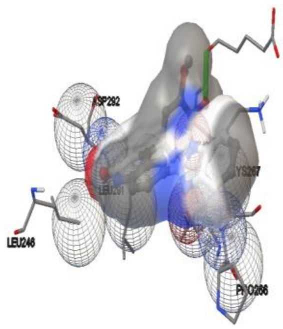


5g (1A9U)

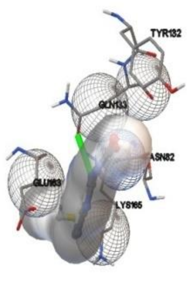


5a (3FLY)

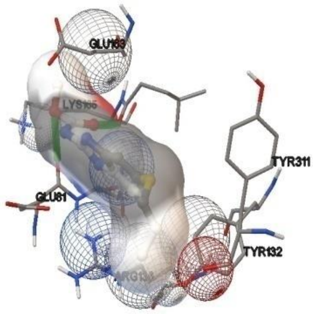
5d (3FLY)



5g (3FLY)



Albendazole (1A9U)



Albendazole (3FLY)

Figure 1: Docking of 5a, 5d, 5g and Albendazole with interactions on PDB ID: 1A9U and 3FLY

***In-vitro* antimicrobial activity:**
The results of antibacterial activity (the zone of inhibition) are presented in Figure 2a and Figure 2b, the results revealed that all newly synthesized compounds were exhibited potent antibacterial activity. In general, compounds **5a, 5d** and **5g** exhibited more pronounced antibacterial activity than the other test compounds against both Gram positive and Gram negative bacteria. Among all the compounds tested **5a** exhibited remarkable antibacterial activity against the Gram positive *Bacillus subtilis, Saphylococcus aureus* and Gram negative *Escherichia coli* as compared with Streptomycin. Compounds **5c** and **5f** showed good activity against *Lactobacillus* and *Escherichia Coli, Pseudomanas aeruginosa* & *Salmonella paratyphi*. Moreover, compounds **5b, 5c, 5e** and **5i** were moderate active and **5f, 5h** and **5j** wild mild active against the tested microorganism.
The compounds **5a-5j** were also tested against *Pencillin notatum, Aspergillus flavus, Candida albicans* and *Aspergillus niger* for their antifungal activity and most of the compounds indicated significant antifungal activity by agar disc diffusion and their zone of inhibition results are presented in Figure 2. From the results, it is evident from the screening data compounds **5a** and **5d** were more effective against tested microorganisms and their potencies were comparable against standard drug fluconazole. However none of the compounds were superior to standard used against any fungi.
Furthermore, when the comparison for the compounds was made between bacteria and fungi it was observed that the different derivatives of benzimidazole found to be more active against bacteria than fungi and among different fungi as listed, it was observed that the compounds are more active against gram positive bacteria than the gram negative ones.

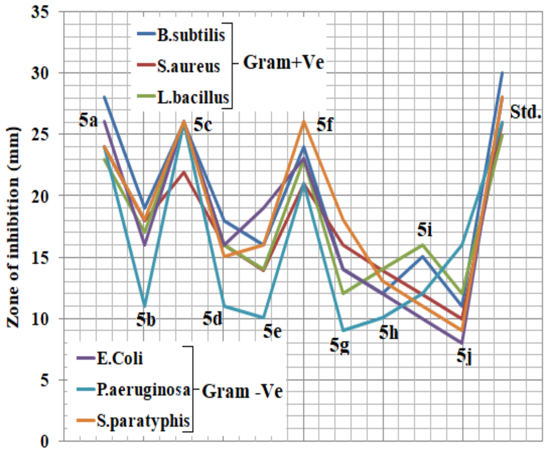


Figure 2a

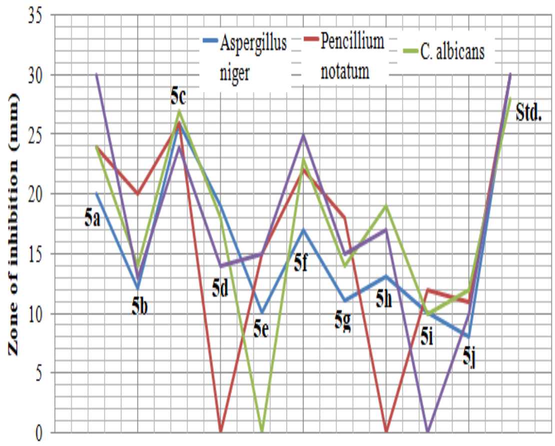


Figure 2b

Figure 2: Antibacterial and antifungal activity of test compound**.**

All values are the mean of three measurements and expressed as mean ±SD, NA= No Activity found; Std.; Streptomycin and Flucanazole

Antioxidant activity:
Free radical scavenging activity of all the synthesized compounds performed using DPPH method and the results were found in Figure 3. All the synthesized compounds produced a concentration dependant scavenging of free radical, DPPH and NO. The IC_{50} values of all the test compounds were found between 113.21µg/ml and 245.23µg/ml. Among all the test compounds, compound **5a, 5d,** and **5g** having more potent antioxidant activity (IC_{50} values) against DPPH and NO free radicals (Figure 3), where as others were showing moderate to poor activity compared to standards one. The IC_{50} values of the test compounds were found

to be significant as compared to that of standard, Ascorbic acid. The effect of antioxidants on DPPH radical scavenging was thought to be due to their hydrogen donating ability. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The reduction capability of DPPH radicals was determined by decrease in its absorbance at 517 nm induced by antioxidants.

The absorbance of the chromophore (purple azo dye) formed during the diazotization of nitrite ions with sulphanilamide at 546 nm and the nitrite generated in the presence or absence of the test compounds were estimated using a standard curve based on sodium nitrite solutions of known concentrations. Each experiment was carried out at least three times and the data presented as an average of three independent determinations.

In our study, the antioxidant activities of all the compounds were positively correlated as correlation coefficients (*R*) and coefficients (R2) = 0.250, 0.420 and 0.063, 0.177 in NO and DPPH assays, respectively. All *R* values were positive at the $p < 0.05$ significance level. This indicates that the two antioxidant assays are suitable and reliable for assessing the total antioxidant potentials of the test compounds. All the synthesized compounds although possess moderate antioxidant activities only few were highlighted due to presence of various electron donating and electron withdrawing groups as well they were violating certain rules of Lipinski etc.

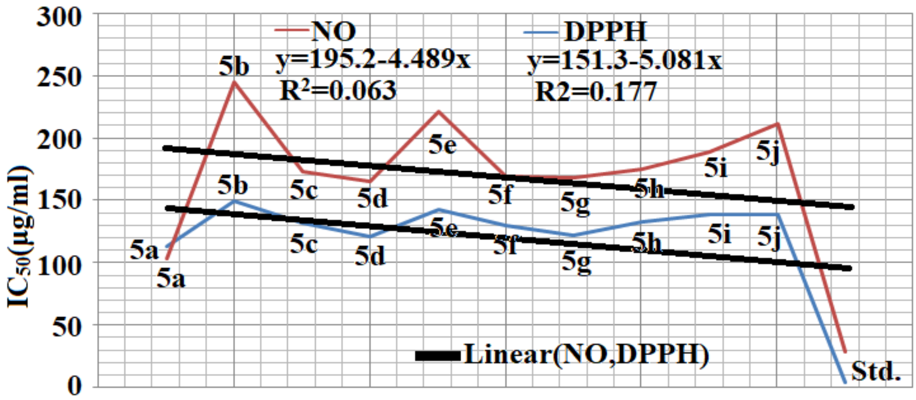


Figure 3: Antioxidant activity of synthesized compounds using DPPH and NO assay method

All values are the mean of three measurements and expressed as mean ± SD; Radical scavenging activity by DPPH (2, 2-diphenyl-2-picrylhydrazyl hydrate) and NO (Nitric oxide) assay is expressed in IC_{50} (Concentration of the compounds/ solution required to inhibit DPPH and NO radical formation by 50%); Significance level at $p<0.05$ significant, using ANOVA test; Std.=Ascorbic acid.

Anthelmintic activity:

The synthesized compounds **5a-5j**, were subjected to anthelmintic activity against *Pheretima posthuma*. The compounds were tested for four concentrations i.e., 0.1, 0.2, 0.5, and 1.0 % (mg/ml), is given in Figure 4a-4c. All the benzimidazole derivatives showed significant anthelmintic activity and the compounds **5a, 5d** and **5g** has more potent anthelmintic activity. All other compounds possess mild to moderate anthelmintic activity. When a comparison is made between the compounds **5a** and **5d** or **5g**, it appears that compounds with heterocyclic group (benzopyrole) are more active than the compounds having, tertiary nitrogen with electro donating group and electronegative substituent group bonded to the phenyl ring through an unsaturated chain. This was further confirmed by comparing the data for compounds **5d** and **5g**. These compounds were found to be active in order as **5a** > **5d** > **5g** where, **a** represented substituted benzopyrole, **d** represented presence of para di-methyl amine and **g** indicates the hydroxy group at *para* position. So, it was observed that compounds possessing heterocyclic ring as in **5a** is more potent than un-substituted /substituted phenyl ring as **5d** or **5g**.

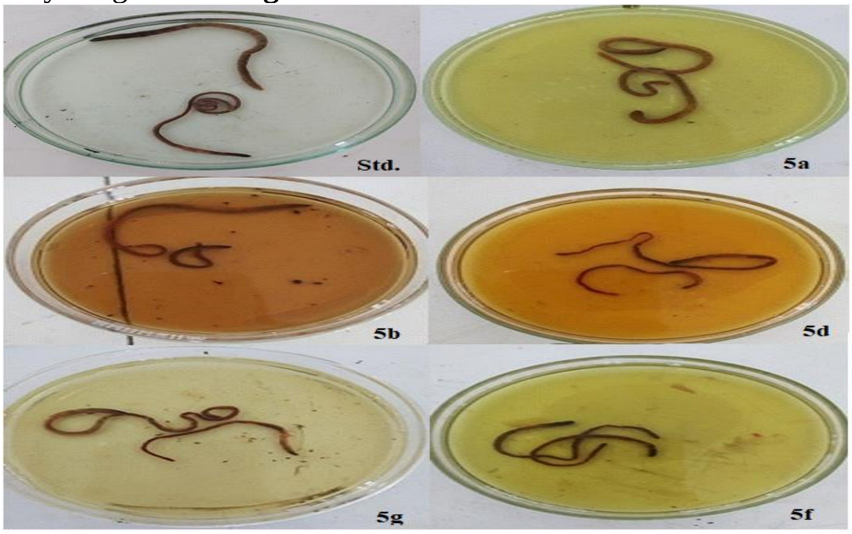


Figure 4a: Anthelmintic activity of 0.5 mg/ml concentration

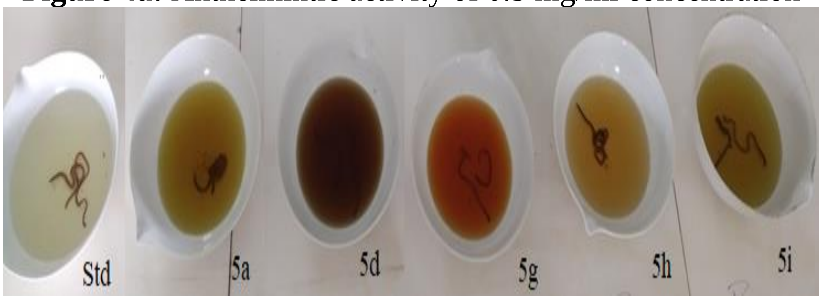


Figure 4b: Anthelmintic activity of 1 mg/ml concentration

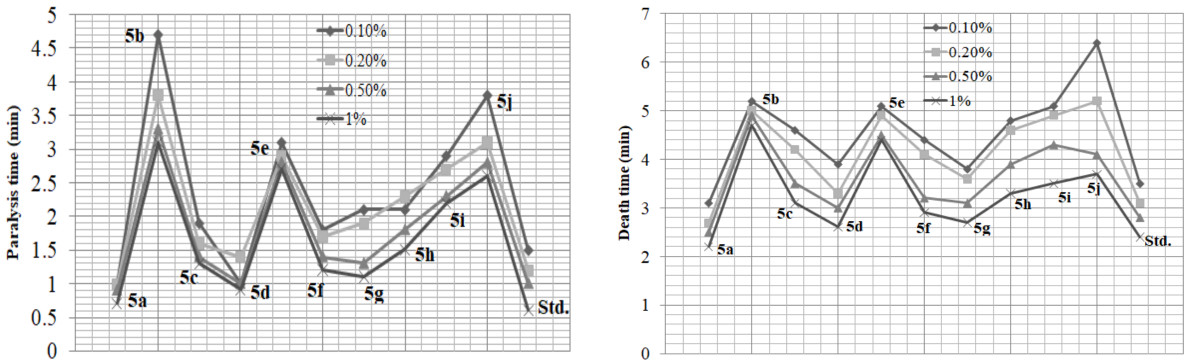


Figure 4c: Comparison of paralyzing and death time of worms (min.)

All values are the mean of three measurements and expressed as mean ±SD, Significance level at $p<0.05$ significant, using ANOVA test; Std.; Albendazole

CONCLUSION:

A series of novel benzimidazole derivatives have been synthesized and characterized by spectral data. All the compounds were evaluated for *in-vitro* biological activities i.e., antimicrobial, antifungal, antioxidant and anthelmintic activities. Benzimidazole chalcones has shown mild to moderate antimicrobial activity. Overall observation from the results of the antimicrobial, antifungal, antioxidant and anthelmintic activities of the synthesized compounds revealed that compounds with indole nucleus, *p*-dimethyl amino benzene and *p*-hydroxyl, *m*-methoxy disubstituted benzene linked to the end of vinyl group of chalcone are more active than the remaining compounds. This confirmed various substituents like electron with drawing group on benzimidazole nucleus leads to novel biological activities as well as increase the hydrophilicity of the synthesized compounds might be presence of secondary/tertiary nitrogen atom and hydroxyl group in their structures. *In silico* docking also confirmed 5a, 5d and 5g compounds have shown higher biological activities due to list binding energy and highest affinity towards receptors. The above 3 compounds (5a, 5d, and 5g) may become lead compounds for their biological properties and to explore them for further activities by the upcoming researchers.

ACKNOWLEDGEMENTS
The authors are thankful to the Chancellor, Vice Chancellor, Dean Faculty of Pharmaceutical Sciences, Assam Down Town University Guwahati and Chaitanya Deemed to be University, Warangal, Kakatiya University, Warangal, for providing the facilities required to carry out this research work.

CONFLICTS OF INTEREST
The authors declare no Conflict of Interest in publishing the research article.

REFERENCES:

i. Ansari K.F.; Lal C.; Khitoliya. R. K.; Synthesis and biological activity of some triazole-bearing benzimidazole derivatives; J-Serb.Chem.SOC.; 2011,**76(3),** 341-352. DOI: 10.2298/JSC100301029A.

ii. Malleshappa Noolvi.; Suresh Agrawal.; Harun Patel.; Aravind Badiger.; Monika Gaba.; Azit Zambre.; Synthesis, antimicrobial and cytotoxic activity of novel azitidine-2-one derivatives of 1H-Benzimidazole; Arabian Journal of chemistry.; 2014,**7,** 219-226 DOI:10.1016/j.arabjc.2011.02.011.

iii. Meral Tuncbilek.; Tulug Kiper.; Nurten Altanlar.; Synthesis and *in-vitro* Antimicrobial activity of some novel substituted Benzimidazole derivatives having potent activity against MRSA; European Journal of Medicinal Chemistry.; 2009, **44(3),** 124-133. DOI: 10.1016/j.ejmech.2008.06.026.

iv. Parmender Singh Rathee.; Ritu Dhanka.; Sunny Bharadwaj.; Monika Gupta.; Rakesh Kumar.; Synthesis and Antimicrobial studies of novel Benzimidazole derivatives; Journal of applied Pharmaceutical Science.; 2011, **01,** 127-130.

v. Ansari K.F.; Lal C.; Synthesis, Physicochemical properties and Antimicrobial activity of some new Benzimidazole derivatives; European journal of medicinal chemistry.; 2009, **44(10),** 4028-4033. DOI: 10.1016/j.ejmech.2009.04.037.

vi. Gulgun Ayhan-Kilcigil.; Nurten Altanlar.; Synthesis and antimicrobial activities of some new benzimidazole derivatives; IL Farmaco.; 2003, **58(12),** 1345-1350. DOI: 10.1016/S0014-827X(03)00190-3.

vii. Sahoo Biswa Mohan.; Behera T.P.; Ravi Kumar B.V.V.; Microwave irradiation versus conventional method: synthesis of benzimidazolyl chalcones derivatives; International Journal of ChemTech Research.; 2010, **2,** 1634-1637.

viii. Gigani yaseen.; Jadhav Sudhakar.; Design, Synthesis and Antimicrobial activity of 2-mercapto Benzimidazole derivatives; International journal of pharma and bio sciences.; 2010, **1(4),** 281-286.

ix. Baviskar B.A.; Shiradkar M.R.; Deokate U.A.; Khadabadi S.S.; Synthesis and Antimicrobial activity of some novel Benzimidazolyl chalcones; E- Journal of chemistry.; 2009, **6(1),** 196-200. DOI:10.1155/2009/746292.

x. Srivastava Y.K.; Janardan Singh yadav.; Microwave assisted rapid and efficient synthesis, charectarization and pharmacological evaluation of some novel benzimidazole assembled 1,5-benzodiazepine and 1,5-benzothiazepine derivatives; Scholars Research Library, Der Pharmacia letter.; 2011, **3,** 248-291.

xi. Yogendra K. Srivastava.; Janardan Singh Yadav.; A facile synthesis and antimicrobial activity of some new 2-substituted benzimidazole derivatives carrying pyridine; Pelagia Research Library, Der Chemica Sinica.; 2011, **2,** 1-7.

xii. Janardian Singh Yadav.; Srivastava Y.K.; An efficient microvave assisted synthesis of some novel 1,4 Dizepene derivatives as possible antimicrobial agents; Rasayan J.Chem.; 2010, **3,** 726-730.

xiii. Hosamani M.; Ramya V. Shingalapur.; Rangappa S. Keri.; Synthesis and evaluation of *in-vitro* antimicrobial and antitubercular activity of 2-styryl benzimidazoles; European Journal of Medicinal chemistry.; 2009, **44,** 4244-4248.

xiv. Sahar M. Badr.; Hassan M Eisa.; Alaa-eldin M Barghash.; Synthesis and Antimicrobial activity of certain Benzimidazole and fused Benzimidazole derivatives; Indian journal of chemistry.; 2010, **49B,** 1515-1525.

xv. Gopalakrishna Aridoss.; Shanmugasundaram Amirthaganesan.; Nanjundan Ashok kumar.; Jong Tae Kim.; Kwon Taek lim.; Senthamaraikannan kabilan.; Yeon Tae Jeong.; A facile synthesis, antibacterial and antitubercular studies of some piperidin-4-one and tetrahydropyridine derivatives; Bioorganic & Medicinal Chemistry Letters.; 2008, **18,** 6524-6548. DOI: 10.1016/j.bmcl.2008.10.045.

xvi. Mishra A.K.; Goutham V.; Guptha A.; Bansal R.; Kumar S.; Synthesis and Antimicrobial activity of some new Benzimidazole derivatives- an overview; Journal of Pharmacy Research.; 2010, **3,** 371-378.

xvii. See S.; Stirling A.L.; Candesartan cilexetil: an angiotensin II-receptor blocker; Am J Health Syst Pharm.; 2000, **57(8),** 739-746. DOI: 10.1093/ajhp/57.8.739.

xviii. Bhavneet Bharti.; Sahul Bharti.; Sumeeta Khurana.; Worm Infestation: Diagnosis, Treatment and Prevention; Indian J Pediatr.; 2017,1-8. DOI: 10.1007/s12098-017-2505-z.

xix. Wihl J.A.; Petersen B.N.; Petersen LN.; Gundersen G. K.; Bresson K.; Mygind N.; Effect of the non-sedative H1-receptor antagonist astemizole in perennial allergic and nonallergic rhinitis; Journal of Allergy and Clinical Immunology.; **75(6),** 1985, 720-727, DOI: 10.1016/0091-6749(85)90100-9.

xx. Gurusamy Mariappan.; Rajib Hazarika.; Faruk Alam.; Rashmi Karki.; Uddhav Patangia.; Shyamalendu Nath.; Synthesis and biological evaluation of 2-substituted benzimidazole derivatives; Arabian Journal of Chemistry.; 2015, **8,** 715–719. DOI: 10.1016/j.arabjc.2011.11.008.

xxi. Sai Krishna G.; Kumara Swamy D.; Sirisha K.; Sai Santhoshi K.; Durga Prasad K.; Synthesis and Pharmacological Evaluation of New Thiazole Derivatives as Anthelmintic Agents; Int. J. Pharm. Sci. Nanotech.; 2020, **13(5),** 5075-5081. DOI:10.37285/ijpsn.2020.13.5.4.

xxii. Bodige S.; Ravula P.; Gulipalli K.Ch; Endoori S.; Koteswara Rao Cherukumalli P.; Narendra Sharat Chandra J. N.; Seelam N.; Design, Synthesis, Antitubercular and Antibacterial activities of 1,3,5-triazinyl carboxamide derivatives and *In Silico* docking studies. Russian. J. Gen. Chem. 2020, **90(7),** 1322-1330. DOI:10.1134/S1070363220070208.

xxiii. Gulipalli K Ch.; Ravula P.; Bodige S.; Endoori S.; Koteswara Rao Cherukumalli P.; Narendra Sharat Chandra J. N.; Seelam N.; Synthesis and Anticancer activity of

Novel urea and thiourea bearing Thiophene-2-carboxalate derivatives; Russian. J. Gen. Chem.; 2020, **90(7),** 1336-1344. DOI:10.1134/S1070363220070221.

xxiv. Reddy A.S.; Pati S.P.; Kumar P.P.; Pradeep H.N.; Sastry G.N.; Virtual screening in drug discovery- A computational perspective; Curr. Protein. Peptide. Sci.; 2007, **8(4),** 329-351. DOI:10.2174/138920307781369427.

xxv. Kaushik Hatti S.; Chandregowda V.; Venkateswara Rao G.; Anil K.; Chandrasekara Reddy G.; *In-silico* interaction studies of Quinazoline derivatives for their inhibitory action on both wild and mutant EGFRs; J. Prot. Bioinfor.; 2009, **2(3),** 126-130. DOI:10.4172/jpb.1000069.

xxvi. Anju LS. T.; Jyoti H.; Novel Mannich bases of 4-thiazolidinone derivatives as Antitubercular Agents; Int. J. Res. Pharm. Chem.; 2014, **4(2),** 351-359.

xxvii. Arul K.; Anton Smith A.; *In-silico* Design, synthesis and *in vitro* Antitubercular activity of Novel 1,2,4-triazole derivatives; Int. J. Pharm. Pharm. Sci.; 2014, **6(11),** 213-217.

xxviii. Shashank Shekhar Mishra.; Chandra Shekhar Sharma.; Hemendra Pratap Singh.; Harshda Pandiya.; Neeraj Kumar.; *In Silico* ADME, Bioactivity and toxicity parameters calculation of some selected Anti-Tubercular drugs; Int. J. Pharm. Phytopharmacol. Res.; 2016, **6(6),** 77-79. DOI:10.24896/eijppr.2016661.

xxix. Katharigatta Venugopala N.; Sandeep Chandrashekharappa.; Pran Kishore Deb.; Christophe Tratrat.; Melendhran Pillay.; Deepak Chopra.; *et. al*.; Anti-Tubercular activity and molecular docking studies of Indolizine derivatives targeting mycobacterial InhA enzyme; J. Enz. Inhib. Med. Chem.; 2021, **36(1),** 1472-1487. DOI:10.1080/14756366.2021.1919889.

xxx. Misbaudeen Abdul-Hammed.; Ibrahim Olaide Adedotun.; Victoria Adeola Falade.; Adewusi John Adepoju.; Sabitu Babatunde Olasupo.; Modinat Wuraola Akinboade.; Target-based drug discovery, ADMET profiling and bioactivity studies of antibiotics as potential inhibitors of SARS-CoV-2 main protease (M^{pro}); Virus. Dis.; 2021, **32(4),** 642-656. DOI:10.1007/s13337-021-00717-z.

xxxi. Jarrahpour A.; Fathi J.; Mimouni M.; Ben Hadda T.; Sheik J.; Chohan ZH. Pervez.; Petra, Osiris and Molinspiration (POM) together as a successful support in drug design: antibacterial activity and biopharmaceutical characterization of some azo Schiff bases; Med. Chem. Res.; 2012, **21(8),** 1984-1990. DOI:10.1007/s00044-011-9723-0.

xxxii. Filimonov D.A.; Lagunin A.A.; Gloriozova T.A.; Rudik A.V.; Druzhilovskii D.S.; Pogodin P.V.; Poroikov V.V.; Prediction of the biological activity spectra of organic compounds using the pass online web resource; Chem. Heterocycl. Compd.; 2014, **50,** 444-457. DOI:10.1007/s10593-014-1496-1.

xxxiii. Geronikaki A.; Babaev E.; Dearden J.; Dearden W.; Filimonov D.; Galaeva I.; Krajneva V.; Lagunin A.; Macaev F.; Molodavkin G.; Poroikov V.; Pogrebnoi S.; Saloutin V.; Stepanchikova A.; Stingaci E.; Tkach N.; Vlad L.; Voronina T.; Design, Synthesis, computational and biological evaluation of new anxiolytics; Bioorg. Med. Chem.; 2004, **12(24),** 6559-6568. DOI:10.16/j.bmc.2004.09.016.

xxxiv. Goel R.K.; Kumar V.; Mahajan M.P.; Quinazolines revised: search for novel anxiolytic and GABAergic agents; Bioorg. Med. Chem. Lett.; 2005, **15,** 2145-2148. DOI:10.1016/j.bmcl.2005.02.023.

xxxv. Holtje H. D.; Sippl W.; Rognan D.; Folkers G., *Molecular Modelling Basic Principles and Applications*. *Wiley VCH publishers*, Germany, 2nd Edition.; 2003, p. 1-240.

xxxvi. Estari Mamidala.; A Practical Manual.; *Molecular Docking Studies in Drug Discovery, A Practical Manual Global Science Publishing Group*, 1st Edition.; 2019, p. 1-107.

xxxvii. Douglas Kitchen B.; Helene D.; John Furr R.; Jurgen B.; Docking and scoring in virtual screening and drug discovery: Methods & Applications; Nat. Rev. Drug. Dis.; 2004, **3,** 935-949. DOI:10.1038/nrd1549.

xxxviii. Lipinski C.A.; Lombardo F.; Dominy B.W.; Feeney P.J.; Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings; Adv. Drug. Del. Rev.; 2001, **46(1-3),** 3-26. DOI:10.1016/s0169-409x(00)00129-0.

xxxix. Parasuraman S.; Prediction of activity spectra for substances; J. Pharmacol. Pharmacotherap.; 2011, **2(1),** 52-53. DOI:10.4103/0976-500X.77119.

xl. Thomas L.; Matthias R.; Computational methods for biomolecular docking; Curr. Opin. Struct. Biol.; 1996, **6(3),** 402-406. DOI:10.1016/S0959-440X(96)80061-3.

xli. Krzysztof Z .T.; Natalia K.; Biernasiuk A.; Malm A.; Salat K.; Furgata A.; Dzitko K.; Bekier A.; Baranowska A.T.; Paneth A., Thiazoles with cyclopropyl fragments as antifungal, anticonvulsant, and anti *Toxoplasma gondii* agents: Synthesis, toxicity, evaluation and molecular docking study; Med. Chem. Res.; 2018, **27(9),** 2125-2140. DOI:10.1007/s00044-018-2221-x.

xlii. Alexandra Quek.; Nur Kartinee Kassim.; Amin Ismail.; Muhammad Alif.; Mohammad Latif.; Khozirah Shaari.; Dai Chuan Tan.; Pei Cee Lim.; Identification of Dipeptidyl peptidase-4 and α-Amylase inhibitors from *Melicopeglabra* (Blume) T.G. Hartley (Rutaceae) using Liquid Chromatography Tandem Mass Spectrometry, *In vitro* and *In Silico* Methods; Molecules.; 2021, **26(1),** 1-16. DOI:10.3390/molecules26010001.

xliii. Kim B.R.; Kim H.; Choi I.; Kim J.B.; Jin C.; Han A.R.; DPP-IV Inhibitory Potentials of Flavonol Glycosides Isolated from the Seeds of *Lens culinaris*: *In Vitro* and *Molecular Docking* Analyses; Molecules.; 2018, **23(8),** 1998. DOI:10.3390/molecules23081998.

xliv. Faruk Alam.; Ruhul Amin.; Synthesis and Pharmacological Activity of Some Pyrazolone Derivatives; Journal of Pharmaceutical Research International.; 2020, **32(10),** 46-55. DOI: 10.9734/JPRI/2020/v32i1030492.

xlv. Pallab Kalita.; Simanta Medhi.; Sudarshana Borah.; Bhargav G. K.; Faruk Alam.; Evaluation of Anti Inflammatory and Anti Oxidant Activities of *Osbeckia crinite*; Journal of Pharmaceutical Research International.; 2022, **34(8B),** 38-46. DOI: 10.9734/jpri/2022/v34i8B35482.

xlvi. Faruk Alam.; A Study on the Antimicrobial and Antioxidant Activities of Some New 1, 3, 4-Thiadiazole Derivatives; International Journal of ChemTech Research.; 2015, **7(5),** 2520-2531.

Faruk Alam.; Synthesis and Biological Evaluation of Some Pyrazole-based Mannich Bases; Research J. Pharm. and Tech.; 2019, **12(9),** 225-4230. DOI: 10.5958/0974-360X.2019.00726.1.

Received on May 17, 2022.