ejpmr, 2016,3(11), 598-609

SJIF Impact Factor 3.628

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

N-(4-(BENZIMIDAZOLE-2-YL) PHENYL) SULFONAMIDES BASED ON ACID / *N*'-(1*H*-BENZIMIDAZOL-2-YL) PHENYL HYDRAZIDO SULFONAMIDE BENZIMIDAZOLE DERIVATIVES: DESIGN, SYNTHESIS AND BIOLOGICAL EVALUATION

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Article Received on 15/09/2016 **Article Revised on 06/10/2016** **Article Accepted on 27/10/2016**

ABSTRACT

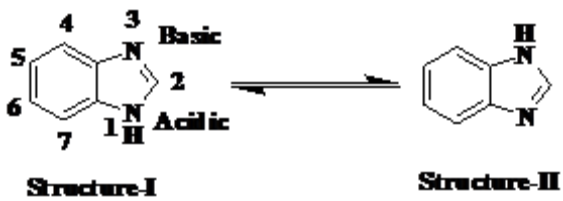
A series of 2-(4-aminophenyl)benzimidazole (2a) based acid / 2-hydrazinobenzimidazole (2b) with the help of hydrazine hydrate which were then reacted with substituted sulfonyl chloride (4a-4f) and mixture of acetic anhydride, pyridine *N*-(4-(benzimidazole-2-yl)phenyl) sulfonamides, *N*'-(1*H*-benzimidazol-2-yl)phenyl hydrazido sulfonamides (5a-5l), respectively. The newly synthesized compounds were screened for their *in-vitro* biological activities including antimicrobial, and antioxidant activity. The Acetyl-CoA carboxylase (ACCase) inhibitor behavior of synthesized compounds was pre-assed via automated docking studies. Finally the whole effort leads to total 10 most promising analogues (**5a, 5b, 5c, 5d, 5e, 5f, 5i, 5j, 5k,** and **5l**) which can be explored further as a template to design more potential agents against ACCase inhibitors.

KEYWORDS: Benzimidazole, Acetyl-CoA carboxylase inhibitors, docking studies, antimicrobial, antioxidant activity.

1. INTRODUCTION

Heterocyclic ring system is ubiquitously present in majority of bioactive molecules ranging from natural to synthetic compounds.[1] The useful pharmacological actions of bioactive compounds are attributed to the presence of heterocyclic ring(s) in their structure (V. Trond HYPERLINK "http://pubs.acs.org/action/doSearch?ContribStored=Hansen%2C+T+V"Hansen et al., 2005). Heterocyclic compounds are well recognized pharmacophore of a large number of medicinal products (J.B. Sperry and D.L. Wright, 2005). HYPERLINK "http://europepmc.org/search;jsessionid=r84qi4ohFllbV4LnnZkP.10?page=1&query=AUTH:%22Wright+DL%22" HYPERLINK "http://europepmc.org/search;jsessionid=r84qi4ohFllbV4LnnZkP.10?page=1&query=AUTH:%22Wright+DL%22 "Heterocyclic compounds are those cyclic compounds whose ring contain besides, carbon, one or more atoms of other elements. The non-carbon atoms such rings are referred to as hetero atoms. HYPERLINK "http://europepmc.org/search;jsessionid=r84qi4ohFllbV4LnnZkP.10?page=1&query=AUTH:%22Wright+DL%22 " Benzimidazole is the heterocyclic compound formed from benzene and imidazole ring containing nitrogen, oxygen, sulfur atoms and its derivatives are of wide interest because of their diverse biological activity and clinical application.[2]

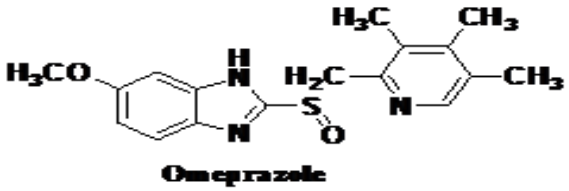
Benzimidazoles were also known as benziminazoles and benzoglyoxalines. These were also named as derivatives of *o*-phenylenediamine, for example, benzimidazole was called methenyl-*o*-phenylenediamine and 2-methyl benzimidazole was called as ethenyl-*o*-phenylenediamine. The systematic numbering of the benzimidazole ring system is shown in structure-1 although benzimidazole is depicated in I as possessing the proton at N1 there actually exists a rapid exchange between the NH- and =N- nitrogen atoms, and two tautomers, I and II, may be drawn for the benzimidazole molecule. Tautomerism occurs through either an intermolecular process involving two or more benzimidazole molecules or through interactions with a protic solvent such as water.[3]



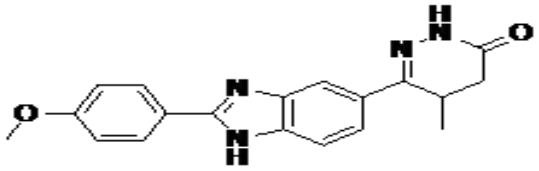
Structure-I Structure-II

They are widely recognized as versatile scaffolds with diverse set of biological activities, such as anti-inflammatory (El-Nezhawy A *et.al.,* 2013), antihypertensive (Shah *et.al.,* 2008), antimicrobial (Ganguly, Yadav *et.al.,* 2014), anticancer (Yoon *et.al.,* 2014), antiprotozoal (Aguayo-Ortiz *et.al.,* (2014) and antiviral (Xue *et.al.,* 2011).Recently, benzimidazole scaffold has emerged as a pharmacophore of choice for designing analgesic and anti-inflammatory agents active on different clinically approved target.

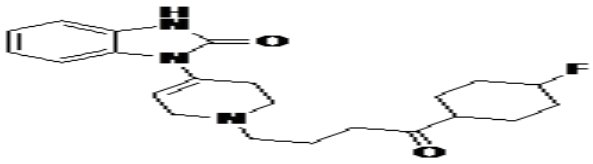
Benzimidazole and its derivatives have evolved as important privileged structures in medicinal chemistry encompassing a diverse range of biological activities including antiparasitic (specifically anthelmintics, e.g., albendazole, mebendazole), antiulcer (proton pump inhibitors (PPIs), e.g., omeprazole), calcium sensitizer (selective inhibitor of phosphodiesterase III (PDE3 e.g., Pimobendan,), antihistaminic (H1-receptor antagonists, e.g., bilastine), anti cancer (nitrogen mustard alkylating agents, e.g., bendamustine), antiemetic/antipsychotics (e.g., droperidol).[4-6]



Omeprazole



Pimobendan



Droperidol

Many researchers have concentrated on the development of small molecules of ACCase inhibitor as antimicrobial activity, because several bacteria such as gram positive, gram negative bacteria are responsible for the microbial diseases.[7] ACCase catalyzes the first step of fatty acid biosynthesis. They are also carried out several biological processes including such as the synthesis and maintenance of cellular membranes. ACCase is a. multicomponent biotin enzyme present in all animals, plants and bacteria. The rate-determining and committed reaction in fatty acid biosynthesis in bacteria is catalyzed by acetyl-CoA carboxylase.[8] The ACCase-catalyzed fatty acid reaction can be divided into two half-reactions shown in Figure 1.[9]

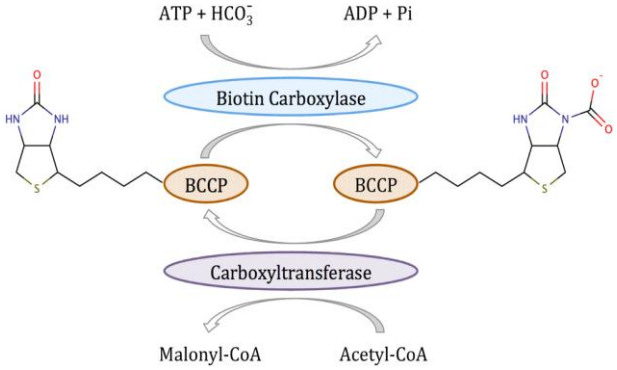


Figure 1: Reaction mechanism of bacterial acetyl-CoA carboxylase

Acetyl coenzyme carboxylase (ACC) is catalyzes the carboxylation of acetyl-CoA to produce malonyl-CoA. In case the first half-reaction, biotin carboxylase (BC) catalyzes the ATP-dependent carboxylation of the vitamin biotin, which *in vivo* is covalently attached to the biotin carboxyl carrier protein (BCCP). In the second half-reaction, carboxyltransferase catalyzes the transfer of the carboxyl group from biotin to acetyl-CoA to form malonyl-CoA, which is the substrate for fatty acid synthase.[10]

2. Experimental

2.1 Measurements

The reagents and solvents were supplied by CDH (New Delhi), Merck (Mumbai, India), S.D. Fine (Mumbai, India) and Qualigens (India). MR-VIS Visual melting point apparatus (LAB India) was used to record the melting points of the synthesized compounds in open end capillary tubes and are uncorrected. The IR spectra were recorded on Hitachi 150-200 spectrophotometer using KBr. ^{1}H-NMR spectra were recorded on Bruker spectropsin DPX-400 MHz in $CDCl_3$ or DMSO-d_6 using [$(CH_3)_4Si$] (TMS) as an internal standard and chemical shift (δ) values are reported in parts per million (ppm). Mass spectral data of the prepared compounds were recorded on a JEOL JMS-D 300 instrument. Perkin-Elmer 240 analyzer was used to perform elemental analyses (C, H, N). Progress of the chemical reaction and the purity of the synthesized compound was checked on silica gel G coated thin-layer chromatography plates in either of the following solvent systems; Toluene: Ethyl acetate: Formic acid (5:4:1, v/v/v) or Petroleum ether: Toluene: Ethyl acetate (5:4:1, v/v/v) or Ethyl acetate: Hexane (3:7, v/v). The spots on TLC were visualized under UV lamp.

2.2 Synthesis of the compounds

2.2.1 Synthesis of 2-(4-aminophenyl) benzimidazole (2a)[11]

Equimolar quantities (0.01mol) of *o*-phenylenediamine, *p*-amino benzoic acid (0.01mol) in 4N HCl (20mL) was refluxed for 30 min. The reaction mixture was poured into at room temp and the solid formed filtered off and recrystallized using absolute alcohol and was obtained pale yellow solid. The cake was the 4-(1*H*-

benzo[*d*]imidazol-2-yl) benzenamine. Melting point of compound was noted using Lab India MR-VIS visual melting point apparatus.

2.2.2 Synthesis of substituted benzene sulphonyl chlorides (4a-f) [12]

Placed benzene or its substituted derivative (0.148mol) in a two-necked flask fitted with a reflux condenser protected with guard tube. Chlorosulphonic acid (0.77mol) in a dropping funnels was added in small portions to the flask with shaking for thorough mixing. After completion of addition, the reaction mixture was refluxed on water-bath for 1 hour. Reaction mixture was cooled to room temperature and oily mixture was poured in a thin stream on to crushed ice with stirring in beaker. Washed with cold water and solid cake was dried. Melting point of compound was determined noted using Lab India MR-VIS visual melting point apparatus.

2.2.3 Synthesis of N-(4-(benzimidazole-2-yl)phenyl) / N'-(1H-benzimidazol-2-yl) phenyl hydrazido sulfonamides (5a-f) [13]

2-(4-Aminophenyl) benzimidazole / *2-Hydrazinobenzimidazole* (0.01mol) was taken in a mixture of pyridine (4ml) and acetic anhydride (20ml) in round bottom flask. To this mixture, we added sulfonyl chloride 10ml. Warm the mixture on water bath for 2 hrs. The completion of reaction was monitored with the help of thin-layer chromatography (TLC) using toluene: ethyl acetate: formic acid (T:E:F-5:4:1) as mobile phase. Melting point of compound was noted using Lab India MR-VIS visual melting point apparatus.

2.2.4 Synthesis of 2-Hydrazinobenzimidazole (2b) [14]

A mixture of 2-mercaptobenzimidazole (50g) and hydrazinehydrate (20 ml) was refluxed for 8-10 hr on wire gauze and cooled to room temperature. The separated crystals were filtered, washed with a little amount of methanol, dried and re-crystallized from methanol and determine the melting point.

4-(1H-Benzo[d]imidazol-2-yl) benzenamine (2a)[15]

TLC–(T:E:F-5:4:1) melting point- 209-211 ^{0}C Colour reddish brown IR (KBr) V_{max} (cm^{-1}):- 3394, 3232 (N-H_2 stretch), 1612 (N-H bend), 2893, 2978 (C-H stretch), 1504, 1396 (C=C stretch). ^{1}H NMR (400MHz, DMSO, in ppm) δ:-7.80 (d, 2H, benzimidazole), 7.78 (d, 2H, benzimidazole), 3.0 (s, 1H, NH benzimidazole), 7.4 (d, 2H, benzene), 7.20 (s, 1H, benzene), 7.10 (s, 1H, benzene), 3.3 (s, 1H, benzene), 2.5 (Ar C-NH_2).

1-(1H-benzo[d]imidazol-2-yl) hydrazine (2b)

TLC–(T:E:F-5:4:1) melting point- 202-204 ^{0}C Colour Gray IR (KBr) V_{max} (cm^{-1}):- 3418, 3333 (N-H stretch) 2839, 2978 (C-H stretch), 1504, 1396 (C=C stretch). ^{1}H NMR (200MHz, $CDCl_3$, in ppm) δ:-7.70 (d, 2H, benzimidazole), 7.26 (d, 2H, benzimidazole), 4.8 (s, 1H, of NH hydrazine), 4.1 (s, 1H, of NH_2 hydrazine), 3.35 (s, 1H, of NH benzimidazole).

N-(4-(1H-benzo[d]imidazol-2-yl)phenyl benezenesulfonamide (5a)

TLC–(T:E:F-5:4:1) melting point- 217-220 ^{0}C Colour white IR (KBr) V_{max} (cm^{-1}):- 3418, 3333 (N-H stretch) 2839, 2978 (C-H stretch), 1504, 1396 (C=C stretch). ^{1}H NMR (400MHz, DMSO, in ppm) δ:- 7.90 (d,2H benzimidazole), 7.26 (d, 2H, benzimidazole), 10.28 (s, 1H, SO_2NH benzimidazole), 7.26 (d, 2H, benzene), 6.52 (d, 2H, benzene), 4.34 (s, 1H, Ar C-NH) 7.93 (d, 2H, benzene).

N-(4-1H-benzo[d]imidazol-2-yl)phenyl-4-bromobenzenesulfonamide (5b)

TLC-(T:E:F-5:4:1) melting point- 230-232 ^{0}C Colour white IR (KBr) V_{max} (cm^{-1}):-3050 (N-H stretch) 2854, 2924 (C-H stretch), 1597, 1458 (C=C stretch), 671 (C-Br stretch). ^{1}H NMR (400MHz, DMSO, in ppm) δ:- 7.90 (d, 2H, benzimidazole), 7.26 (d, 2H, benzimidazole), 10.28 (s, 1H, SO_2NH benzimidazole) , 7.26 (d, 2H, benzene), 6.52 (d, 2H, benzene), 4.34 (s, 1H, Ar C-NH) 7.93 (d, 2H, benzene), 7.80 (d, 2H, benzene).

N-(4-1H-benzo[d]imidazol-2-yl) phenyl-4-methylbromobenzenesulfonamide (5c)

TLC-(T:E:F-5:4:1)melting point- 226-228 ^{0}C Colour white IR (KBr) V_{max} (cm^{-1}):- 3333 3418, (N-H stretch) 2839, (C-H stretch), 1543, 1474 (C=C stretch). ^{1}H NMR (400MHz, DMSO, in ppm) δ:- 7.04 (d, 2H, benzimidazole), 7.22(d, 2H, benzimidazole), 4.34 (s, 1H, Ar C-NH), 10.28 (s, 1H, SO_2NH benzimidazole), 7.6 (d, 2H, benzene), 7.8 (d, 2H, benzene), 8.54 (d, 2H, benzene), 8.18 (s, 1H, benzene) 2.32 (s, 3H, CH_3).

N-(4-1H-benzo[d]imidazol-2-yl) phenyl-3-bromo-4-methylbromobenzenesulfonamide (5d)

TLC-(T:E:F-5:4:1) melting point- 256-258 ^{0}C Colour white IR (KBr) V_{max} (cm^{-1}):- 3050 (N-H stretch) 2854, 2924 (C-H stretch), 1597, 1458 (C=C stretch), 671 (C-Br stretch), 671 (C-Br stretch). ^{1}H NMR (400MHz, DMSO, in ppm) δ:- 7.09 (d, 2H benzimidazole), 7.22(d, 2H, benzimidazole), 4.34 (s, 1H, Ar C-NH), 10.28 (s, 1H, SO_2NH benzimidazole), 7.6 (d, 2H, benzene), 7.8 (d, 2H, benzene), 8.54 (d, 2H, benzene), 8.18 (s, 1H, benzene) 2.32 (s, 3H, CH_3).

N-(4-1H-benzo[d]imidazol-2-yl) phenyl-4-nitrobenzenesulfonamide (5e)

TLC-(T:E:F-5:4:1) melting point- 238-239 ^{0}C Colour white IR (KBr) V_{max} (cm^{-1}):- 3279 (N-H stretch) 2870 (C-H stretch), 1605, 1389 (C=C stretch), 1319 1504 (Ar-N-O stretch).^{1}H NMR (400MHz, DMSO, in ppm) δ:- 7.04 (d, 2H, benzimidazole), 7.22(d, 2H, benzimidazole), 4.34 (s, 1H, Ar C-NH), 10.28 (s, 1H, SO_2NH benzimidazole), 7.6 (d, 2H, benzene), 7.8 (d, 2H, benzene), 8.54 (d, 2H, benzene), 8.18 (s, 1H, benzene).

N-(4-1H-benzo[d]imidazol-2-yl) phenyl-4-fluorobenzenesulfonamide (5f)

TLC-(T:E:F-5:4:1) melting point- 278-280 ^{0}C Colour white IR (KBr) V_{max} (cm^{-1}):- 3350 3267 (N-H stretch),

2870 2950 (C-H stretch), 1605, 1389 (C=C stretch), 1157 (C-F stretch). ^{1}H NMR (400MHz, DMSO, in ppm) δ:- 7.20 (d, 2H, benzimidazole), 7.46 (d, 2H, benzimidazole), 4.51 (s, 1H, Ar C-NH), 10.53 (s, 1H, SO_2NH benzimidazole), 7.78 (d,2H, benzene), 7.80 (s, 1H, benzene) 7.81 (d, 2H, benzene), 7.84 (d, 2H, benzene), 10.53 (s, 1H, benzene).

N-(1H-benzo[d]imidazol-2-yl) benzenesulfonohydrazide (5g)

TLC-(T:E:F-5:4:1) melting point- 212-215 ^{0}C Colour light brown IR (KBr) V_{max} (cm^{-1}):- 3310 3232 (N-H stretch), 2893 2978 (C-H stretch), 1605, 1389 (C=C stretch). ^{1}H NMR (400MHz, DMSO, in ppm) δ:- 7.78 (d, 2H, benzimidazole), 7.36 (d, 2H, benzimidazole), 3.34 (s, 1H, Ar C-NH), 13.2 (s, 1H, SO_2NH benzimidazole), 12.3 (s, 1H, NH of attached imdazole ring) 7.86 (d, 2H, benzene), 7.41 (d, 2H, benzene), 7.23 (s, 1H, benzene).

N-(1H-benzo[d]imidazol-2-yl)-4-bromobenzenesulfonohydrazide (5h)

TLC-(T:E:F-5:4:1) melting point- 222-225 ^{0}C Colour light brown IR (KBr) V_{max} (cm^{-1}):- 3310 3232 (N-H stretch), 2893 2978 (C-H stretch), 1605, 1389 (C=C stretch), 671 (C-F stretch). ^{1}H NMR (400MHz, DMSO, in ppm) δ:- 7.78 (d, 2H, benzimidazole), 7.36 (d, 2H, benzimidazole), 3.34 (s, 1H, Ar C-NH), 13.2 (s, 1H, SO_2NH benzimidazole), 12.3 (s, 1H, NH of attached imdazole ring), 7.86 (d, 2H, benzene), 7.41 (d, 2H, benzene).

N-(1H-benzo[d]imidazol-2-yl)-4-methylbenzenesulfonohydrazide (5i)

TLC-(T:E:F-5:4:1) melting point- 217-219 ^{0}C Colour light brown IR (KBr) V_{max} (cm^{-1}):- 3155 (N-H stretch), 2854 2924 (C-H stretch), 1512, 1458 (C=C stretch). ^{1}H NMR (400MHz, DMSO, in ppm) δ:- 7.78 (d, 2H, benzimidazole), 7.36 (d, 2H, benzimidazole), 3.34 (s, 1H, Ar C-NH), 13.2 (s, 1H, SO_2NH benzimidazole), 12.3 (s, 1H, NH of attached imdazole ring), 7.86 (d, 2H, benzene), 7.41 (d, 2H, benzene), 2.53 (s, 3H, CH_3).

N-(1H-benzo[d]imidazol-2-yl)-3-bromo-4-methylbenzenesulfonohydrazide (5j)

TLC-(T:E:F-5:4:1) melting point- 239-241 ^{0}C Colour light brown IR (KBr) V_{max} (cm^{-1}):- 3148 3109 (N-H stretch), 2932 (C-H stretch), 1512, 1458 (C=C stretch), 650 (C-Br stretch).

^{1}H NMR (400MHz, DMSO, in ppm) δ:- 7.78 (d, 2H, benzimidazole), 7.36 (d, 2H, benzimidazole), 3.34 (s, 1H, Ar C-NH), 13.2 (s, 1H, SO_2NH benzimidazole), 12.3 (s, 1H, NH of attached imdazole ring), 7.34 (s, 1H, benzene), 2.53 (s, 3H, CH_3).

N-(1H-benzo[d]imidazol-2-yl)-4-nitrobenzenesulfonohydrazide (5k)

TLC-(T:E:F-5:4:1) melting point- 231-233 ^{0}C Colour reddish brown IR (KBr) V_{max} (cm^{-1}):- 3255 3109 (N-H stretch), 2924 2854 (C-H stretch), 1512, 1458 (C=C stretch), 1366 (Ar-N-O stretch). ^{1}H NMR (400MHz, DMSO, in ppm) δ:- 7.78 (d, 2H, benzimidazole), 7.36 (d, 2H benzimidazole), 3.34 (s, 1H, Ar C-NH), 13.2 (s, 1H, SO_2NH benzimidazole), 12.3 (s, 1H, NH of attached imdazole ring), 7.86 (d, 2H, benzene), 7.42 (d, 2H, benzene).

N-(1H-benzo[d]imidazol-2-yl)-4-fluorobenzenesulfonohydrazide (5l)

TLC-(T:E:F-5:4:1) melting point- 254-257 ^{0}C Colour light brown IR (KBr) V_{max} (cm^{-1}):- 3255 3109 (N-H stretch), 2924 2854 (C-H stretch), 1512, 1458 (C=C stretch), 1157 (C-F stretch). ^{1}H NMR (400MHz, DMSO, in ppm) δ:- 7.78 (d, 2H, benzimidazole), 7.36 (d, 2H, benzimidazole), 3.34 (s, 1H, Ar C-NH), 13.2 (s, 1H, SO_2NH benzimidazole), 12.3 (s, 1H, NH of attached imdazole ring), 7.86 (d, 2H, benzene), 7.42 (d, 2H, benzene).

3.4 Pharmacological Studies

3.4.1 In vitro antibacterial and antifungal assay

The anti-bacterial activity of the synthesized compounds was evaluated by the agar well diffusion method. All the cultures were adjusted to 0.5McFarland standard, which was visually comparable to a microbial suspension of approximately 1.5 x 108cfu/mL 20 ml of Mullen Hinton agar medium was poured into each Petri plate and the agar plates were swabbed with 100µL inoculate of each test bacterium and kept for 15 minutes for absorption. Using sterile cork borer of 8mm diameter, wells were bored into the seeded agar plates and these were loaded with a 100µl volume with concentration of 2mg/ml of each compound reconstituted in the dimethyl sulphoxide. All the plates were incubated for 24 hrs at 37^{o}C. Anti-bacterial activity of each compound was evaluated by measuring the zone of growth inhibition against the test organisms with zone reader (Hi Antibiotic zone scale). DMSO was used as a negative control as a negative control. This procedure was performed in three replicate plates for each organism. The results of antibacterial and antifungal activity are summarized in Table 2, 3, & 4 with Figure (2-4).

3.4.1.1 Disk diffusion method

This method is also known as *Kirby-Bauer test.* It is very widely used efficient method to determine the extent of growth of microorganism inoculated in a solid agar medium. Test solutions of each sample were prepared at a concentration of 50, 100 µg/ml. Amphotercin-B & Ciprofloxacin was taken as standard for antibacterial as well as antifungal activity at a concentration of 50 µg/ml. Specified media were prepared and sterilized by an autoclave. For antibacterial activity, the media was inoculated with the test organisms (one day old subculture) by uniform mixing and then poured into sterile Petri dishes to a uniform depth and then allowed to cool and solidify at room temperature in an aseptic room. This provided the uniform surface for the growth of bacterium and was used for antimicrobial activity studies. The filter paper disc precontaining drug was

placed on the solidified medium for diffusion of drug in to the agar medium. Plates were kept at room temperature for half an hour for diffusion of the sample into agar media. The organism-inoculated Petri dishes were incubated at specified temperature for specified time. After the incubation period was over, the zones of inhibition produced by the sample in different plates were measured and recorded immediately. The same procedure was done in triplicate.[16]

3.4.2 Antioxidant activity

Synthesized heterocyclic compounds exhibiting good antioxidants activity. The results of antioxidant activity are summarized in Table 4.

3.4.2.1 Evaluation method for reducing power assay

This method is based on the principle of increase in the absorbance of the reaction mixtures. Increase in the absorbance indicates an increase in the antioxidant activity. In this method, the different concentration of test and standard solutions (100, 250, 500 and 1000 µg/ml) were prepared. The antioxidant compounds forms a colored with potassium ferricyanide, trichloro acetic acid and ferric chloride, which is measured at 700nm. Increase in absorbance of the reaction mixture indicates the reducing power of the samples. In the method described by Oyaizu (1986) 2.5ml of 0.2 M phosphate buffer (pH 6.6) and 2.5ml of $K_3Fe(CN)_6$ (1%w/v) are added to 1.0ml of sample dissolved in distilled water. The resulting mixture is incubated at 50℃ for 20 min, followed by the addition of 2.5ml of trichloro acetic acid (10% w/v). the mixture is centrifuged at 3000 rpm for 10 min to collect the upper layer of the solution (2.5ml), mixed with distilled water (2.5) and 0.5ml of $FeCl_3$ (0.1% w/v). The absorbance is then measured at 700nm against blank sample.[17]

3.4.6. Molecular docking analysis

3.4.6.1. Target preparation

The 3D structure of inhibitor Acetyl Co-enzyme carboxylase (ACCs) complex with internal ligand JZL-466 was retrieved (PDB ID-3JZI) from the Protein Data Bank (PDB) (www.rcsb.org). Finally the 3D structure of protein 3JZI imported into the workspace of MVD was used to prepare the protein structure for docking analysis. To prepare the structure all water molecules having more than 5Å specific distance. The standard Molegro algorithm was employed for rendering the missing charges, protonation states, and assigning of polar hydrogen to the receptor. The cavity which has been found associated with the binding of standard drug ciprofloxacin was selected as binding pocket.

3.4.6.2. Preparation of ligand molecules

The ligands (synthesized compounds) were built in Marvin Sketch 5.11.4 in 2D. All explicit hydrogens were added in the structure and molecules were cleaned into 3D, and the conformational energy of molecules was minimized using MMFF94 force field and saved in Marvin Sketch as MDL Molfile (*.mol). After that ligands were imported in MVD, which help in assigning the missing bond orders, charges, bonds, and hybridization states of the imported ligands.

3.4.6.3. Docking studies on MVD

MVD was used for docking studies, which has been considered more accurate than other docking tools comparatively.[18] MVD was used to calculate the interaction energies between ligands and macromolecular systems from the 3D structures of the protein and ligands. The candidates with the best conformation and energy scores were selected. The algorithm used to be the MolDock Score, which is an adaptation of Differential Evolution (DE) algorithm. Finally docking of ligands and protein was performed in docking wizard with the score function Mol dock score. The parameters for docking were set as default, which includes no. of runs 10, maxi-mum iterations 1500, maximum population size 50, but maximum no. of poses was increased to 20.

3.4.6.4. Docking energy calculations

The MolDock score energy, E_{score} is calculated with the help of Eq. 1, where E_{inter} represents ligand-protein interaction energy and E_{intra} is the internal energy of the ligand. Calculation of E_{inter} is done by the help of Eq. 2 whereas E_{intra} is calculated with the help of Eq. 3.[18-19]]

$$E_{inter} = \sum_{i=ligand} \sum_{j=protein} [E_{PLP}(r_{ij}) + 332.0\, q_i q_j / 4r^2_{ij}] \quad (2)$$

$$E_{intra} = \sum_{i=ligand} \sum_{j=protein} [E_{PLP}(r_{ij})] + \sum_{flexible\ bonds} A[1-\cos(m\theta-\theta_0)] + E_{clash} \quad (3)$$

E_{PLP} represents the term "piecewise linear potential" which utilizes two distinct parameters, one for the potential energies of hydrogen words and second for the Vander wall interactions between atoms. The second variable in the Eq. 2 is used for the calculation of electrostatic interactions between charged atoms. The second term in the Eq. 3 is used to calculate torsional energy and θ in the term indicates the torsional angle. The last term E_{clash} in the Eq. 3 is assigned for a penalty term of 1,000 kcal/mol, if the atomic distance between two heavy atoms is smaller than 2.0 Å.

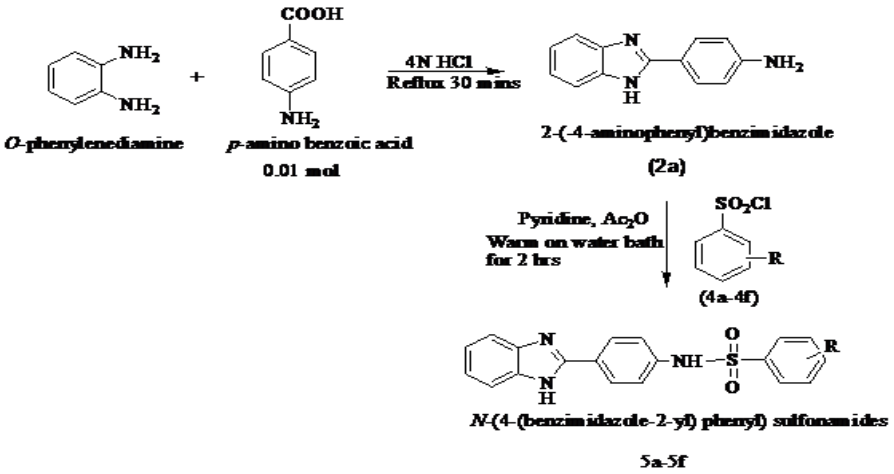
3. RESULT AND DISCUSSION

3.*1 Chemistry*

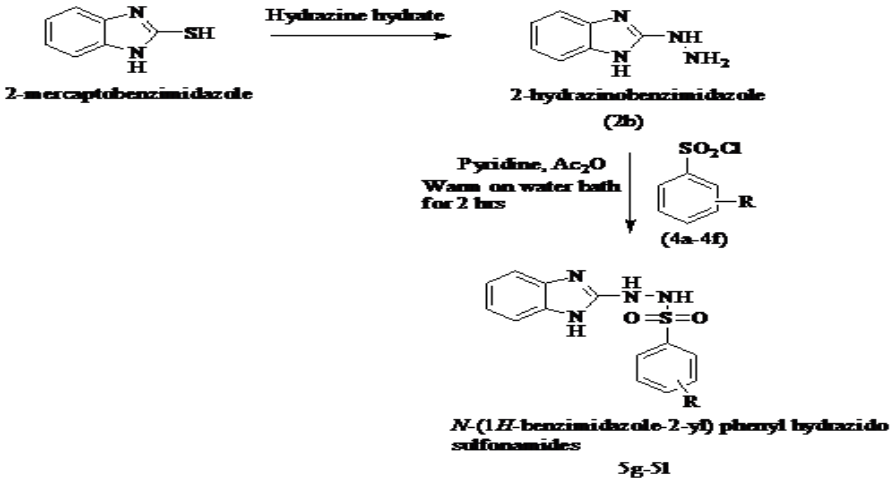
A library of twelve novel compounds possessing oxygen, sulfur or nitrogen as a part of the five membered heterocyclic rings such as benzimidazole and imdazole (Scheme 1) were synthesized in hope of developing potent antimicrobial agents. The title compounds are hybrid molecules of ACCase inhibitor.

In general, the infrared spectral data (IR; cm^{-1}) of 2-(-4-aminophenyl) benzimidazole (2a) showed bands at 3400-3100 stretch, 1620 bending, (amine -NH_2); 1450-1600 (Ar-C=C) 2850-2900 stretch (Ar-C-H). *N*-(4-(Benzimidazole-2-yl) phenyl) sulfonamides (5a-f) showed IR bands at 3410-3130 (benzimidazole N-H),

and 1450-1600 (Ar-C=C). 2-Hydrazinobenzimidazole (2b) showed IR bands at 3400-3150 stretch, 1620 bending, (amine–NH_2), 1450-1600 (Ar-C=C) 2850-2900 stretch (Ar-C-H). N-(1*H*-Benzimidazole)phenyl hydrazido sulfonamides (5g-5l), 3280-3130 stretch (benzimidazole N-H), 1450-1600 (Ar-C=C), 2850-2900 stretch (Ar-C-H). In ^{1}H NMR spectra, signal around 12.3 indicates the formation of benzimidazole ring. The aromatic region values between 6.9-8.25. The physical and spectral data of all the synthesized compounds (5a-f and 5g-l) are presented in Table 1.



Scheme 1: Protocol for the synthesis of title compounds



Scheme 2: Protocol for the synthesis of title compounds

Table 1: Physical data of synthesized *N*-(4-(benzimidazole-2-yl) phenyl) sulfonamides (5a-f) and *N*'-(1*H*-benzimidazol-2-yl) phenyl hydrazido sulfonamides (5g-5l).

Sr. No.	R	Melting point rang	Melting point	Rf Values	Yields (%)
(5a)	H	217-220 ^{0}C	218 ^{0}C	0.64	71.2 %
(5b)	4-Br	230-233 ^{0}C	231 ^{0}C	0.76	67%
(5c)	2-Br-Toulene	226-228 ^{0}C	227 ^{0}C	0.79	65%
(5d)	Toluene	256-258 ^{0}C	257 ^{0}C	0.73	68.5%
(5e)	4-No_2	238-239 ^{0}C	238 ^{0}C	0.75	68.2%
(5f)	4-F	278-280 ^{0}C	279 ^{0}C	0.81	70.12%
(5g)	H	212-215 ^{0}C	213 ^{0}C	0.84	64.23%
(5h)	4-Br	222-225 ^{0}C	223 ^{0}C	0.69	63%
(5i)	2-Br-Toulene	217-219 ^{0}C	218 ^{0}C	0.72	71%
(5j)	Toluene	238-241 ^{0}C	239 ^{0}C	0.74	64.4%
(5k)	4-No_2	231-233 ^{0}C	232 ^{0}C	0.69	66%
(5l)	4-F	254-257 ^{0}C	253 ^{0}C	0.78	65.5%

2.2. Biological evaluation

The synthesized oxygen, nitrogen and sulfur containing heterocyclic compounds were screened for their *in vitro* antibacterial, antifungal, & antioxidant activity the results are compared with the standard drug, Ciprofloxacin, Amphotercin-B & Ascorbic acid.

Antibacterial and antifungal activity

In the present study, 12 chemical compounds were screened for their antibacterial activity and antifungal activity. Tested chemical compounds possessed variable antibacterial activity against both Gram-positive bacteria (*Bacillus subtilis*) and Gram-negative bacteria (*E.coli*).and antifungal activity against (*Candida albicans*). Two compounds namely, V and VIII were unable to exhibit activity against Gram-positive and Gram-negative bacteria (*B. subtilis* and *E.coli*) respectively. On the basis of maximum inhibitory activity shown against Gram positive bacteria, compounds no I, II, X, V, VIII & XII were found to be most effective against *B. subtilis* with zone of inhibition ranging between 7mm & 23mm respectively. Whereas compound I & X with 19mm and 23mm respectively (Table 2). Whereas compounds V and XI with zone of inhibition of 21 mm and 23 mm against Gram- negative bacteria *E.coli* and compounds such as I, II, III, VIII, XI, X, and XII showing maximum zone of inhibition of 19mm, 17mm, 14mm, 13mm, 15mm, 16mm and 12mm respectively (Table 3). The standard drug ciprofloxacin showed zone of inhibition against gram-positive bacteria *B. subtilis* 25mm and 28mm against Gram-negative bacteria *E.coli*. However in case of fungi, ten compounds, I, II, III, IV, V, VI, VII, IX, X and XII were found to be best in inhibiting the growth of yeast, C. Albicans with the zone of inhibition ranging between 16mm, 18mm, 14mm, 21mm, 10mm, 17mm, 14mm, and 23mm while two compounds namely VIII & XII were unable to exhibit activity against antifungal (Table 4). The standard drug Amphotercin-B showed zone of inhibition against fungi 24mm respectively.

Table 2: Antibacterial activity of synthesized compounds against Bacillus subtilis through disk diffusion method

Sample	Zone of inhibition	
	50 µg/ml	**100 µg/ml**
I	**13 mm**	**19 mm**
II	8 mm	13mm
III	6 mm	11 mm
IV	9 mm	15mm
V	9 mm	17 mm
VI	6 mm	13 mm
VII	6mm	10 mm
VIII	0	8 mm
IX	8 mm	14 mm
X	**12 mm**	**23 mm**
XI	6 mm	13 mm
XII	5 mm	11 mm
Ciprofloxacin	**25 mm**	0

Graph-1

FIGURE 2: Antibacterial activity against *Bacillus subtilis*

Table 3: Antibacterial activity of synthesized compounds against *E. coli* through disk diffusion method

Sample	Zone of inhibition	
	50 µg/ml	**100 µg/ml**
I	**16 mm**	**19 mm**
II	5 mm	11 mm
III	0	13mm
IV	7 mm	14 mm
V	0	0
VI	6 mm	13 mm
VII	12 mm	17 mm
VIII	0	8 mm
IX	5 mm	12 mm
X	**10 mm**	**18 mm**
XI	7 mm	13 mm
XII	5 mm	11 mm
Ciprofloxacin	**28 mm**	-

Graph-2
FIGURE 3: Antibacterial activity against *E. coli*

Table 4: Antifungal activity of synthesized compounds against *Candida Albicans* through disk diffusion method

Sample	Zone of inhibition	
	50 µg/ml	**100 µg/ml**
I	**13 mm**	**19 mm**
II	7mm	16mm
III	8 mm	17 mm
IV	9 mm	15 mm
V	**10 mm**	**21 mm**
VI	6 mm	13 mm
VII	6 mm	10 mm
VIII	7 mm	-
IX	8 mm	14 mm
X	**6 mm**	**13mm**
XI	**12 mm**	**23mm**
XII	8 mm	-
Amphotercicin-B	**17 mm**	**24mm**

Graph-3
FIGURE 4: Antibacterial activity against *Candida albicans*

6.13.2 Reducing power assay (RP)
This method is based on the principle of increase in the absorbance of the reaction mixtures. Increase in the absorbance indicates an increase in the absorbance indicates an increase in the antioxidant activity the presence of reducers the antioxidants causes the conversion of the Fe^{3+}/ Ferricyanide complex to the ferrous form. Reducing power of compounds shown in Table 5 show that almost all the compounds shown significant antioxidant effect. Compounds (IV), (V), (VI), (X), (XI) and (XII) have shown excellent absorbance when compared to ascorbic acid. All the remaining compounds also showed significant absorbance. A SAR reveled that heterocyclic moiety is responsible for the antioxidant activity. All these target compounds were also evaluated by graph. The figure 5 shown reducing powers of standard and target compounds, here compounds showed more reducing power than standard.

Table 5: The absorbance and % anti-radical activity of standard (ascorbic acid) and synthesized lead compounds by reducing power assay.

Compounds	Reducing power assay		
	Absorbance 700nm		
	1000µg/ml	**500µg/ml**	**250µg/ml**
Ascorbic acid	0.992	0.899	0.791
(I)	0.680	0.621	0.581
(II)	0.739	0.685	0.623
(III)	0.801	0.732	0.671
(IV)	0.856	0.762	0.690
(V)	0.994	0.923	0.873
(VI)	0.879	0.829	0.774
(VII)	0.641	0.582	0.547
(VIII)	0.703	0.642	0.591
(IX)	0.784	0.723	0.671
(X)	0.851	0.819	0.109
(XI)	0.947	0.856	0.844
(XII)	0.881	0.823	0.782

Graph-5
FIGURE 5: Absorbance level of reducing power of ascorbic acid and target compounds

- **Docking Studies**

Molegro Virtual Docker (MVD) was used to carry out the automated docking studies in order to predict the antimicrobial activity behavior of the synthesized compounds as per the drug receptor interaction basis. The energy score functions and hydrogen bond

interactions formed between the drug and the active amino acid residues were used to predict their binding modes in the cavities (binding site) Acetyl Co-carboxylase enzymes. Ciprofloxacin, the well known broad spectrum antimicrobial drug was used as standard to study the binding conformations of synthesized compounds within the cavity of ACCs protein receptor (PDB ID- 3JZI).

2.2.4.1. Docking studies of synthesized derivatives with ACCs

MVD program allows the flexible docking of ligand with the target. It uses all the flexible bonds of the ligand to produce a number of poses from which the best mode could be selected From docking studies, it was observed that the standard drug ciprofloxacin and internal ligand JZL-466 have mol dock score -123.475 and -157.969 respectively. All the synthesized compounds exhibited better mol dock score value than standard, ranging from -117.567 to -147.049. The compounds no. 5a, 5b, 5c, 5d, 5e, 5f, 5h, 5i, 5j, 5k and 5l have shown highest docking score between -123.852 to -147.049. While the compound 5e, 5h, 5i, 5j, 5k, and 5l exhibited maximum number of H-bond interaction i.e. figure number (6-13) with shown as in Table 6 respectively.

Table 6: MolDock score and bonding interaction of benzimidazole derivatives with amino acids residues using PDB ID: 3JZI for determination of antimicrobial activity.

Compounds	Mol Dock Score	No. of H-bond interaction	H-bond distance (A^0)	Amino acid involved	Structural feature
Ciprofloxacin (Standard drug)-1a	**-123.475**	**4**	3.14 3.46 3.32 3.51	Val365 Gly364 Arg331 Tyr391	O- COOH N-NH N-of piperazine ring O- CO
Internal ligand (JZL-466)-1b	**-157.969**	**7**	3.09 3.15 3.08 2.82 2.66 2.60 3.07	Glu288 Asn290 Gln235 Gln235 His236 Glu276 Glu288	O- OH O- OH O- OH O- OH N-NH N-NH N-NH
5a	-144.632	3	3.03 2.79 3.23	Arg331 Arg331 Tyr391	O – S O – S O – S
5b	-138.778	4	2.77 3.49 3.05 3.22	Met302 Arg331 Arg331 Arg331	N of NH with SO_2 O – S O – S O – S
5c	**-147.649**	**4**	3.09 3.08 3.10 3.48	Tyr391 Arg331 Arg331 Gly364	O – S O – S O – S N of benzimidazole
5d	-145.836	4	3.51 2.84 3.40 3.00	Glu301 Arg331 Arg331 Arg331	N of NH with S O – S O – S O – S
5e	**-147.049**	**7**	3.39 3.07 3.10 3.51 3.42 2.72 2.98	Glu301 Arg331 Arg331 Arg331 Arg331 Arg331 Arg331	N of NH with S O of NO_2 with C O of NO_2 with C N of NO_2 with C O – S O – S O – S
5f	-142.942	5	3.12 3.37 3.46 2.72 2.87	Gly364 Tyr391 Arg331 Arg331 Arg331	N of benzimidazole O – S O – S O – S O – S
5g	-117.567	5	3.33 2.94 3.01 3.54	Arg 331 Arg 331 Arg 331 Met 302	O – S O – S O – S N of NH with S

			2.27	Met 302	N of imdazole ring
5h	-125.445	7	3.42 3.12 2.59 2.46 2.64 2.28 2.12	Gly 362 Try 367 Gly 362 Val 365 Gly.364 Val 365 Val 365	N of imdazole ring N of imdazole ring NH of benzimidazole NH of imdazole ring O - S N of imdazole ring N of imdazole ring
5i	-132.356	7	2.86 3.43 2.80 3.46 2.52 3.40 2.25	Arg 331 Arg 331 Arg 331 Arg 331 Glu 301 Met 302 Met 302	O - S O - S O - S O - S N of NH with S NH of imdazole ring N of NH with S
5j	-123.852	7	3.43 3.09 3.31 3.43 3.33 3.24 3.48	Glu 301 Arg 331 Arg 331 Glu 301 Arg 331 Tyr 391 Arg 331	O - S O - S O - S N of NH with benzimidazole O – S O – S O - S
5k	**-128.345**	**9**	3.07 3.01 3.41 2.73 3.50 2.07 3.38 3.30 3.50	Arg 331 Arg 331 Arg 331 Arg 331 Arg 331 Met 302 Met 302 Glu 301 Glu 301	O of NO_2 with C O of NO_2 with C N of NH with S O - S O – S N of NH with S NH group of attached to benzimidazole ring N of NH with S N of NH with S
5l	-125.72	7	3.23 2.69 2.83 3.41 2.28 3.47 3.34	Arg 331 Arg 331 Arg 331 Arg 331 Met 302 Glu 301 Met 302	O – S O - S O - S O - S N of NH with S N of NH with S N of NH with benzimidazole

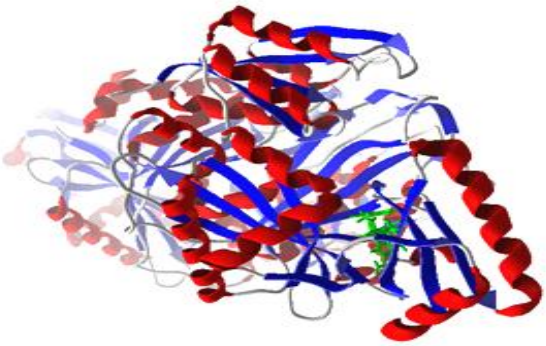


Figure 6: Secondary structure of protein Acetyl Co-enzyme carboxylase inhibitor (PDB ID-3JZI) with co-crystallized JZL 466 (1b) (internal ligand shown in green color).

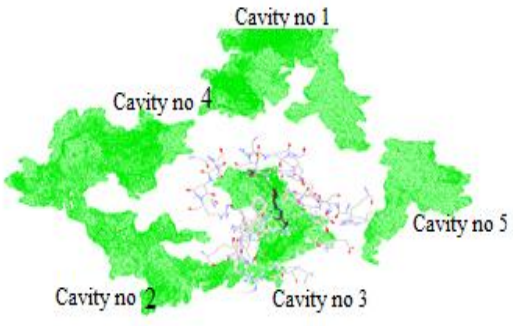
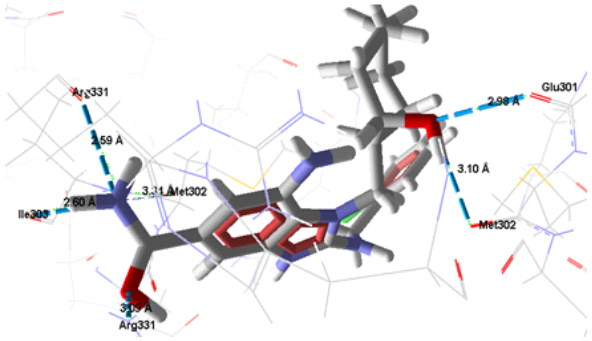


Figure 7: Prediction of cavities in ACCase Total five cavities were predicted (shown in green color). Cavity 3 is shown with active site residues.



Binding of standard JZL 466 (internal ligand)

Figure 8: Binding mode of internal ligand JZL 466 (1b) into Acetyl ACCase inhibitor (3JZI) pocket

It has mol dock score **-157.969** (Table 6) and forms **7** H-bonds (shown as light sky blue dotted lines) with the protein receptor. It forms 2 H-bonds with Met302, 2 H-bond with Arg331, 1 H-bond with Ile305, 2 H-bond with Glu 301.

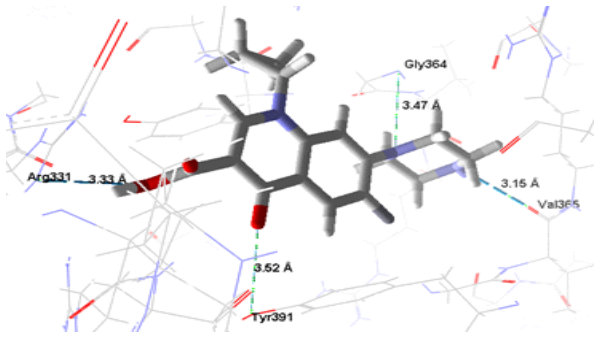


Figure 9: Binding of Ciprofloxacin (Broad spectrum antimicrobial drug used as standard) with 3JZI

Ciprofloxacin (standard drug) **1a** have mol dock score **-123.475** (Table 6) and exhibited **4** H- bonds (shown as light sky blue dotted line) with the receptor protein. It forms **1** H-bond with residue Val365, 1H-bond with residue Arg331, 1H-bond with residue Tyr391, 1H-bond with residue Gly364.

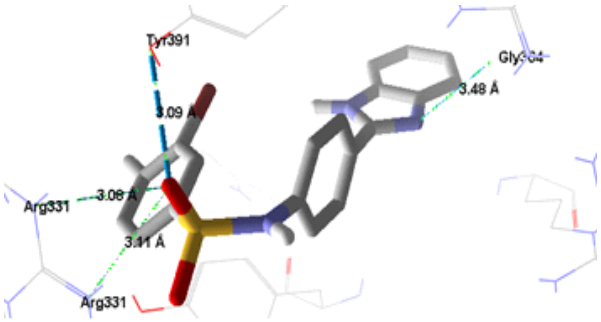


Figure 10: Binding mode of compound 5c into ACCase inhibitor (3JZI) pocket

It has mol dock score **-147.649** (Table 6) and forms **4** hydrogen bonds with the receptor protein (shown as sky blue dotted lines). It forms 2 H-bonds with the residue Arg331, 1 H-bond with Tyr391 and 1H-bonds with Gly364 residue.

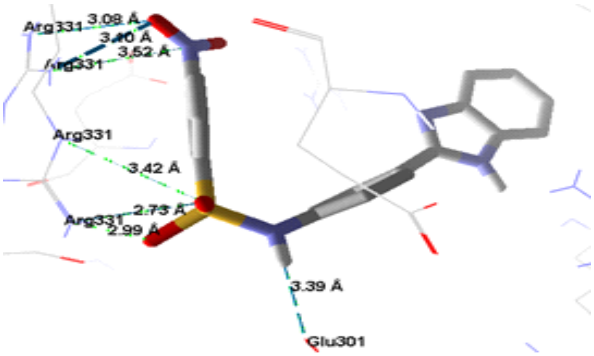


Figure 11: Binding mode of compound 5e into ACCase inhibitor (3JZI) pocket

It has mol dock score **-147.049** (Table 6) and forms **7** H-bonds (shown as light sky blue dotted lines) with the protein receptor. It forms 6 H-bonds with Arg331, 1H-bond with Glu301.

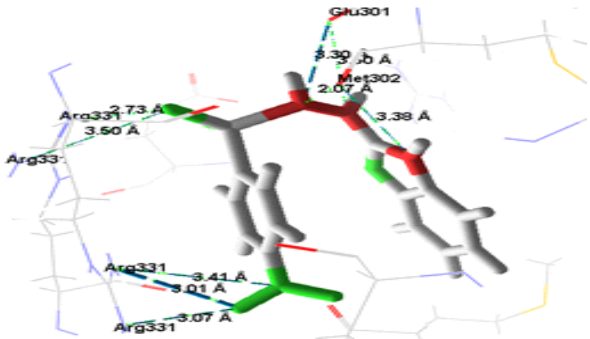


Figure 12: Binding mode of compound 5k into ACCase inhibitor (3JZI) pocket

It has mol dock score **-128.345** (Table 6) and forms **9** hydrogen bonds with the receptor protein (shown as sky blue dotted lines). It forms 5 H-bonds with the residue Arg331, 3 H-bond with Met302 and 1H-bonds with Glu301 residue.

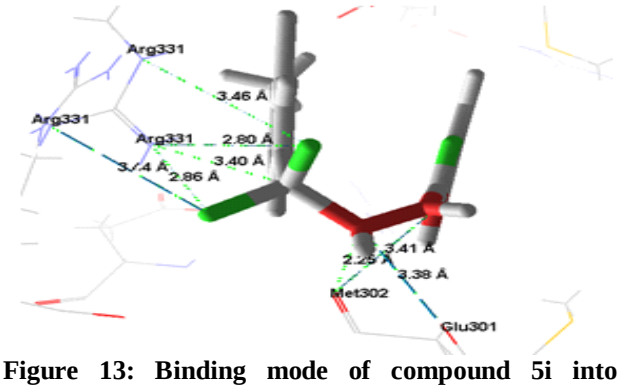


Figure 13: Binding mode of compound 5i into ACCase inhibitor (3JZI) pocket

It has mol dock score **-132.356** (Table 6) and forms **7** hydrogen bonds with the receptor protein (shown as sky blue dotted lines). It forms 4 H-bonds with the residue Arg331, 2 H-bond with Met302 and 1H-bonds with Glu301 residue.

4. CONCLUSION

The present work describes the synthesis of a series of new benzimidazole –based 2-(4-aminophenyl) benzimidazole (2a) based acid / 2-hydrazinobenzimidazole (2b) with the help of hydrazine hydrate which were then reacted with substituted sulfonyl chloride (4a-4f) and mixture of acetic anhydride, pyridine *N*-(4-(benzimidazole-2-yl) phenyl) sulfonamides, *N*'-(1*H*-benzimidazol-2-yl) phenyl hydrazido sulfonamides (5a-5l), respectively Result of *in-vitro* studies demonstrated that almost all compounds showed better antimicrobial and antioxidant activity. ACCase-inhibition was confirmed via automated docking analysis. Almost all *N*-(4-(benzimidazole-2-yl) phenyl) sulfonamides, *N*'-(1*H*-benzimidazol-2-yl) phenyl hydrazido sulfonamides (5a-5l) showed better antimicrobial activity. It was observed that compounds containing nitro and methyl group(s) exhibit higher mol dock score and interaction contain. These compounds also showed better mol dock score and interactions as compared to that of ciprofloxacin. The compounds found to be most potent in both *in-silico* and *in-vivo* anti-inflammatory results are **5a, 5b, 5c, 5d, 5e, 5f, 5i, 5j, 5k,** and **5l**. Therefore, the synthesized compounds may be explored as lead for new therapeutic agents.

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