Synthesis, Characterization and Biological Evaluation of Novel Substituted 1H-Benzimidazole Derivatives

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Abstract

In order to expand the group of 1H-benzimidazole derivatives, we synthesized several new 1H-benzimidazole derivatives ring containing N-mannich bases. It has been observed that the presence of two (or) more heterocyclic moieties fused or linked enhance the biological profile of drug molecules by many folds. The appropriate carboxylic acids were reacted with O-phenylene diamine to give the corresponding 2-substituted benzimidazole in good to excellent yields by Phillip's reaction. Then, a series of six novel mannich bases of 2-alkyl substituted benzimidazole derivatives were synthesized using mannich reaction by reaction with amines (primary and secondary) and formaldehyde. We reported our results from a study of replacing the N-1 hydrogen of novel benzimidazole derivatives with different types of substitutions like sulphanilamide and piperazine to form N-methyl substituted benzimidazole derivatives by mannich reaction. The structure of the synthesized compounds were elucidated by physical and spectral (UV, IR, 1H NMR and Mass) analysis. The NH band (3164-3385 cm-1) and NH proton signal (δ 4.80 – 5.0 ppm) of 2-substituted 1H-benzimidazole derivatives in IR and 1H NMR spectrum respectively in the synthesized compounds (SR1–SR3), confirmed the formation of 1H-benzimidazole derivatives nucleus.

Keywords: *1H-benzimidazole, N-mannich bases, Phillip's reaction, antimicrobial activity, in-vitro anti-butyrylcholinesterase activity, In-silico studies.*

1. Introduction

As per WHO, Malaria mostly spreads to people through the bites of some infected female Anopheles mosquitoes. Blood transfusion and contaminated needles may also transmit malaria. The first symptoms may be mild, similar to many febrile illnesses, and difficulty to recognize as malaria. Left untreated, P. falciparum malaria can progress to severe illness and death within 24 hours[1]. Imidazole is frequently present in physiologically active natural products, pharmaceuticals, and other heterocyclic compounds since they are abundant in nature.

Because of this, there is now more interest for using Imidazole derivatives to treat a wide range of anti-malarial properties[2]. This is information might be valuable for developing molecules that would be work more effectively on the malarial disease. In the present century due to the advancement and changes in the culture and life style, new diseases are being existed among the human population that the search for better dugs still necessary[3]. Synthesis, new more than ever, is a vital and interesting part of organic chemistry. Organic synthesis has, rightly commanded a good deal of attention from researchers who have to choose from different arrays of synthetic methods. Discovery of new drugs that is therapeutically useful and goes into clinics is a life time dream for medicinal chemist[4]. Carbocyclic (or) heterocyclic ring systems comprise the core of chemical structures of the vast majority of therapeutic agents[5]. The exploitation of a simple molecule with different functionalities for the synthesis of heterocyclic compounds is a worthwhile contribution in the chemistry of heterocycles. Heterocyles dominate medicinal chemistry – the majorities of drugs are heterocyclic or have heterocyclic structural compounds[6].1H-benzimidazoles are remarkably effective compounds both with respect to their degree of virus inhibitory activity and favorable selectivity ratio. Several 1H-benzimidazole derivatives with N-1 substitution showed anti-viral activity against human cytomegalo virus and herpes simplex virus type-1. 2-substituted 1H-benzimidazole derivatives, one of the most important derivatives of 1H-benzimidazole are also known possess varies biological activities[7]. The biological activities of these compounds depend upon the substitution on the 1H-benzimidazole at the N-1 (or) C-2 position. Since 1H-benzimidazole heterocyclic ring[8]. System mimics the purine bases like adenine and guanine of nucleic acids, the N-1 substituted 1H-benzimidazoles may be incorporated into the viral nucleic acids by enzymatic process and subsequently can alter the structure and function of nucleic acids resulting in the inhibition of viral growth[9]. Over the past few decades, Mannich bases of heterocyclic molecules have been grabbing the attention of the synthetic chemists for their wide gamut of biological activities ranging from antibacterial, anti cancer, anti parkinson to anti convulsant, anti inflammatory, analgesic and anti-HIV[10]. The Mannich reaction is one of such. It involves the introduction of single carbon atom by the reaction of an active methylene compound with formaldehyde and a primary amine or secondary amine. Mannich reaction is a simple addition step without final elimination, this presumably reflects the fact that -+NH-R2 is a poorer leaving group than -+OH2[11]. The products of many mannich reactions are referred to as Mannich bases and are themselves useful synthetic intermediates.

In the present research, we decided to synthesize 2-substituted 1H-benzimidazole derivatives by conventional method. Further the study will extended to introduce sulphanilamide and piperazine group substitution on N-1 position of 2-substituted 1H-benzimidazole by Mannich reaction and to screen the newly synthesized compounds for their antibacterial and antifungal activity[12].

2. Materials and Methods

2.1 Materials:

The reagents and solvents were commercially available (Rankem, SD fine, Loba and Fluka). All the solvents used were dried and purified as and when required. Analytical samples were dried in vacuum and they were free of significant impurities on TLC. For antimicrobial activity by using two gram positive bacteria (*Staphylococcus aureus* MTCC 740 and *Bacillus subtilis* MTCC 121) and two gram negative bacteria *(Escherichia coli* MTCC 1302 and *Pseudomonas aeruginosa* MTCC 741) and two fungalorganisms (*Candida albicans* ATCC 24433 and *Trichophyton rubrum* ATCC 2327) were collected from Microbial Resources Division, Kings Institute's of Preventive Medicine, Guindy, Chennai. The nutrient agar medium and sabouraud's dextrose agar medium werepurchased from HI Laboratories Ltd., Mumbai, India.

2.2 Chemical characterization

The synthesized compounds were subjected to characterization biological screening, central anti-nociception activity, peripheral anti-nociception activity, anti-inflammatory activity, antimicrobial activity, antioxidant activity, in-vitro lipid peroxidation inhibitory studies, In-vitro Anti-acetylcholinesterase activity, in-vitro anti-butyrylcholinesterase activity and In-silico studies.

2.2.1 Evaluation of peripheral anti-nociception activity:

Acetic acid-induced writhing, a commonly used method, was utilized to determine the peripheral analgesic activity of the syn- thesized compounds[13].

2.2.2 Evaluation of anti-inflammatory activity:

The anti-inflammatory activity was determine by calculating per cent (%) paw oedema inhibition of standard Diclofenac sodium[14].

2.2.3 Evaluation of antimicrobial activity:

The possible antimicrobial activity of the synthesized benzimi- dazole derivatives was evaluated by the disc diffusion method[15].

2.2.4 Evaluation of antioxidant activity:

Antioxidant activity was evaluated by DPPH scavenging assay method and the results are depicted in Among the synthe- sized compounds[16].

2.2.5 Evaluation of In-silico anti-acetylcholinesterase

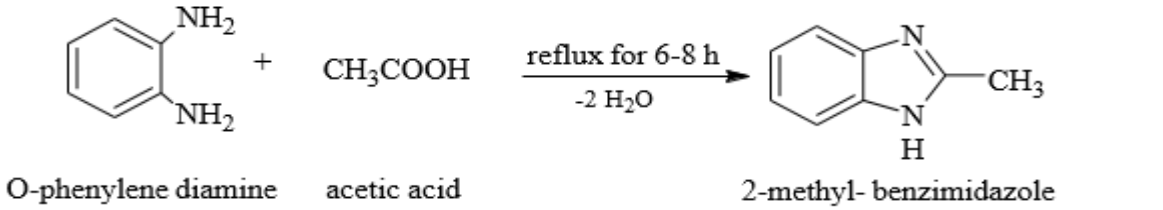
The acetylcholinesterase activity was determined by a colorimetric assay based on Ellman's methodology. The Folin–Ciocalteu colorimetric method was used for total phenolic content determination and the aluminium chloride method for the determination of total flavonoid content. Antioxidant activity assays were performed using the DPPH and FRAP assays[17].

2.2.6 Evaluation of in-vitro lipid peroxidation inhibitory studies:

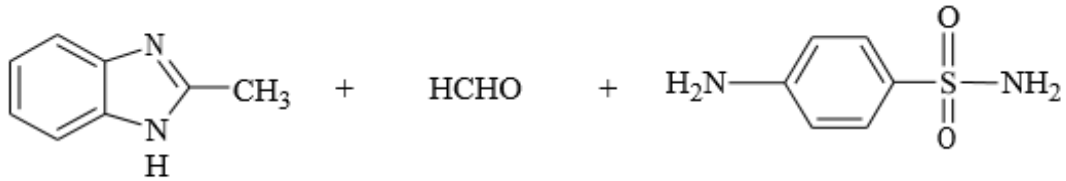
All the synthesized benzimidazole derivatives were subjected to observe the reactivity against non-enzymatic lipid peroxidation using rat brain homogenate. Lipid peroxidation level increased with the addition of Fe^{2+}-ascorbate in the prepared brain homogenate[18].

2.3 Methodology

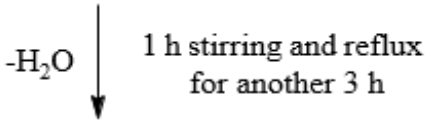
2.3.1 Synthesis of 1-((sulphanilamido) methyl)-2-methyl-benzimidazolesss

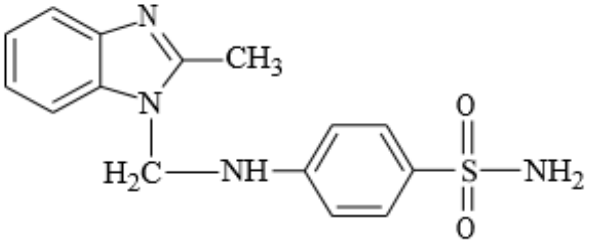


O-phenylene diamine acetic acid 2-methyl- benzimidazole



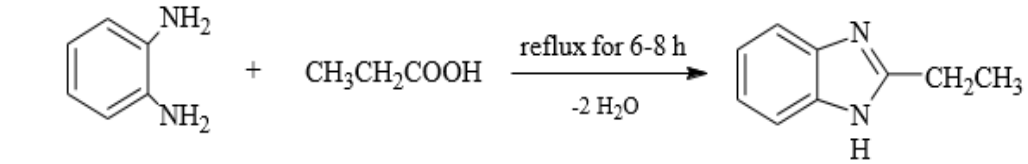
2-methyl benzimidazole Formaldehyde Sulphanilamide



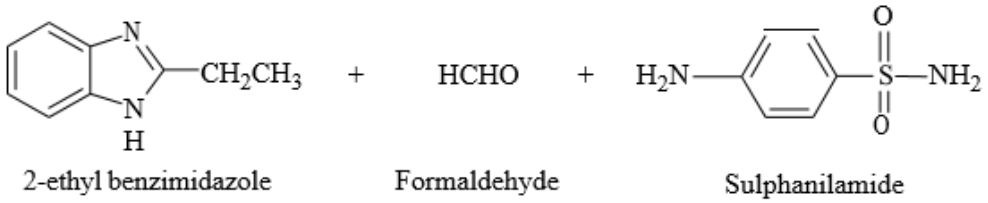


1H-Benzimidazole base of 2-methyl benzimidazole

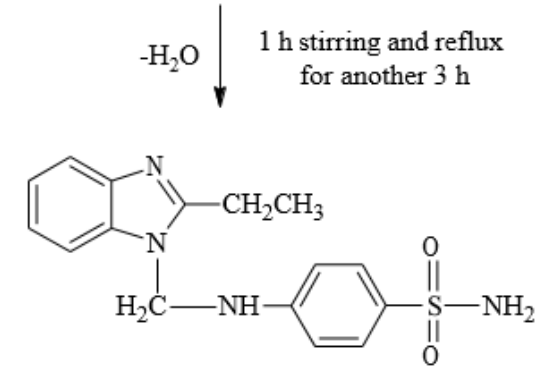
2.3.2 Synthesis of 1-((sulphanilamido) methyl)-2-ethyl-benzimidazole



O-phenylene diamine propionic acid 2-ethyl- benzimidazole

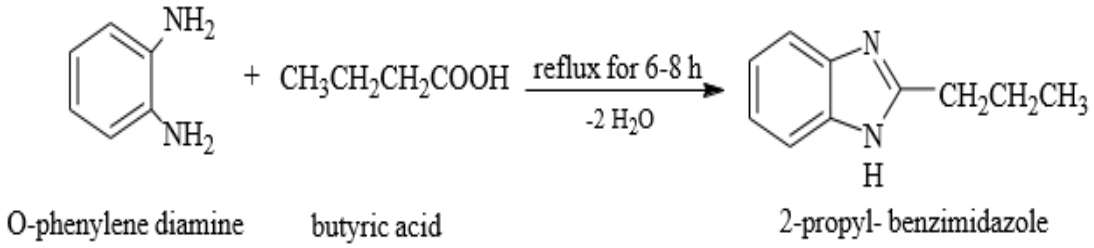


2-ethyl benzimidazole Formaldehyde Sulphanilamide

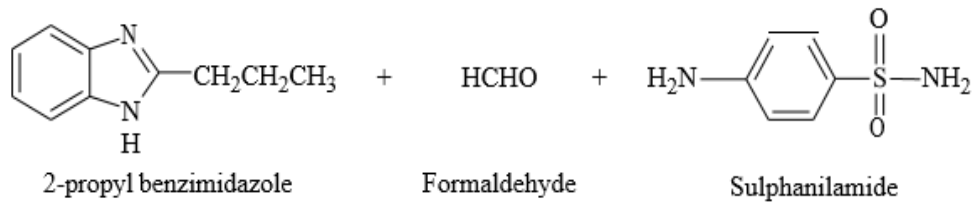


1H-Benzimidazole base of 2-ethyl benzimidazole

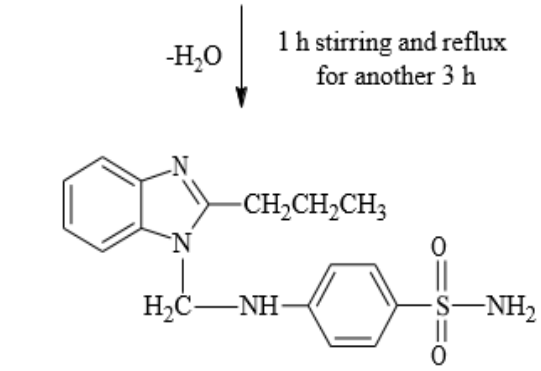
2.3.3 Synthesis of 1-((sulphanilamido) methyl)-2-propyl-benzimidazole



O-phenylene diamine butyric acid 2-propyl- benzimidazole



2-propyl benzimidazole Formaldehyde Sulphanilamide



1H-Benzimidazole base of 2-propyl benzimidazole

3.1Chemical Characterization

A number of compounds were proposed and as we were interested with disubstituted derivatives, we did not synthesize the other monosubstituted derivatives in this series. All synthesized compounds were purified by column chro-matography and the purity of all fractions were ascertained by using TLC. Finally, the purity of the molecule was again confirmed by 1H NMR spectroscopy. These purified samples were used for biological investigation. We coded the sample as 3a, 3b, 3c, 4 and 6 which having good practical yield and appropriate melting point.

3.2 Evaluation of peripheral anti-nociception activity

Table 1. Results of peripheral anti-nociception activity

Sample code	Dose (mg/kg)	Number of Writhing(Mean±SEM)	% Inhibition of Writhing
Control	0	26.8±2.131	–
Standard	0.1ml/10g	2.2±0.583	91.79
3a	25	13.6±0.430	49.25
	50	19.0±1.649	29.10
3b	25	19.6±0.797	26.87
	50	23.2±0.464	13.43
3c	25	12.3±0.831	54.10
	50	15.4±0.697	42.54
4	25	26.0±1.001	2.99
	50	23.1±0.843	13.81
6	25	16.7±0.889	37.69
	50	11.9±0.458	55.60

Acetic acid-induced writhing, a commonly used method, was utilized to determine the peripheral analgesic activity of the synthesized compounds. Among the compounds, compound 6, 3c and 3a showed significant analgesic activity ($P < 0.001$) at 25 mg/kg and 50 mg/kg dose level with writhing inhibition values single dose (25 mg/ kg), double dose (50 mg/kg). respectively (Table 1) compared to 91.79% writing inhibition for the standard Diclofenac. Compound 3b showed moderate analgesic activity at 25 mg/kg dose level with a value of 26.87% of inhibition ($P < 0.001$). Compound 4 did not show any peripheral analgesic activity.

3.3 Evaluation of anti-inflammatory activity

The per cent (%) paw oedema inhibition of standard Diclofenac sodium was 23.73%, 31.15%, 38.93%, 45.39% in 1st, 2nd, 3rd, and 4th hour respectively. From the statistical evaluation, it is evident that the 3b and 3a at 100 mg/kg dose showed significant anti-inflammatory effects from the first hour and onward.

The % paw edema inhibition of 3b was 38.98%, 40.16%, 41.98%, 44.68% and that of 3a was in 1st, 2nd, 3rd and 4th hour respectively. On the other hand, 3c at 100 mg/ kg dose showed moderate anti-inflammatory effect from the first hour and onward. Compound 4 and 6 exhibited mild anti-inflammatory actions.

3.4 Evaluation of antimicrobial activity

The possible antimicrobial activity of the synthesized benzimidazole derivatives was evaluated by the disc diffusion method. Among them, mild antimicrobial activity was found with the zone of inhibition ranging from 7 to 10 mm in the case of compounds 4 and 6, while the zone of inhibition of standard Ciprofloxacin was 40–50 mm. Both compounds showed mild activity against the gram negative bacteria and gram positive bacterial strains. Compounds 3a, 3b and 3c did not show any antimicrobial activity against the microbial strains.

3.5 Evaluation of antioxidant activity

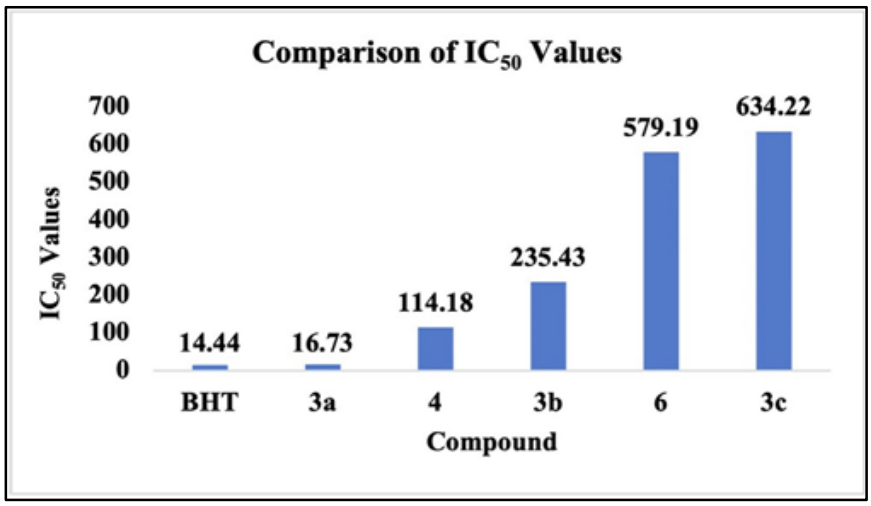


Figure 1. Comparison of antioxidant activities (IC_{50} Values) of Standard and Synthesized Compounds

Antioxidant activity was evaluated by DPPH scavenging assay method and the results are depicted in Among the synthesized compounds, 3a showed very strong antioxidant property..(IC_{50} = 16.73 mg/ml) similar to that of standard BHT (14.44 mg/ml).Compounds, 4 and 3b displayed moderate antioxidant activity having IC_{50} values of 114.18 and 253.43 mg/ml, respectively. On the other hand, Compounds 6 and 3c exhibited very low antioxidant profiles (IC_{50}values of 579.19 and 634.22mg/ml respectively).

3.6 Evaluation of In-vitroAnti-acetylcholinesterase activity

The results of the anti-acetylcholinesterase activity is summarized in fig.The inhibitory activity of synthesized compounds increased with increasing the concentration, and the highest activity was found at 800 mg/ml concentration. Compound 6 showed moderate inhibitory activity against the enzyme acetyl-cholinesterase (IC_{50} = 29.64 mg/ml), whereas 3b and 3a exhibited mild inhibitory activity (IC_{50}=66.03mg/ml and73.15mg/ml respectively).The IC_{50}value of standard drug donepezil was 9.54mg/ml

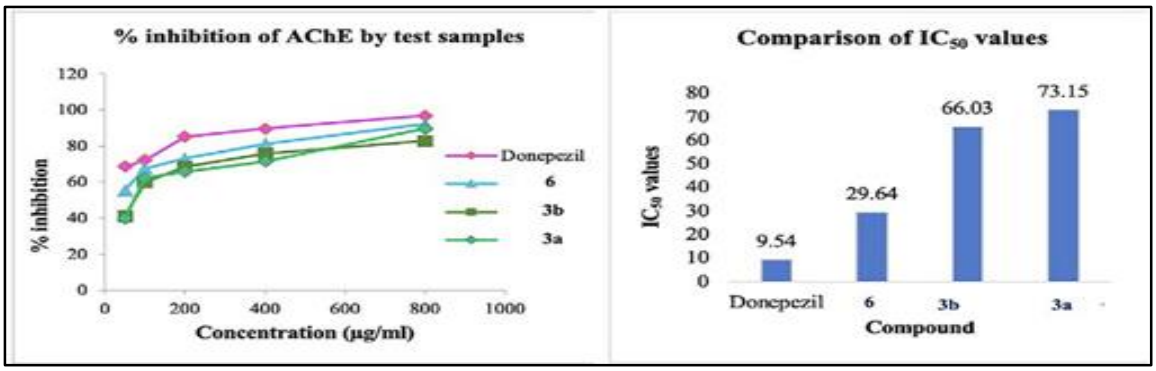


Figure 2. Comparison of the anti-acetylcholinesterase activity of Donepezil (standard) and test samples.The IC50 values of 3b and 3a were 23.42 mg/ml and 15.53 mg/ml, respectively

6.5 Evaluation of in-vitro lipid peroxidation inhibitory studies

All the synthesized benzimidazole derivatives were subjected to observe the reactivity against non-enzymatic lipid peroxidation using rat brain homogenate. Lipid peroxidation level increased with the addition of Fe^{2+}-ascorbate in the prepared brain homoge- nate. The inhibitory activity of the synthesized compounds was found to increase with increasing concentration (Fig. 3). The high- est activity was obtained at the dose of 800 mg/ml. The IC_{50} values of compounds 3a and 4 were found to be 75.53 mg/ml and 98.55 mg/ml, respectively, whereas the value for standard (Catechin) was 59.36 mg/ml (Fig. 3). Compound 3a showed moderate inhibitory and compound 4 showed mild inhibitory activity in lipid peroxidation assay.

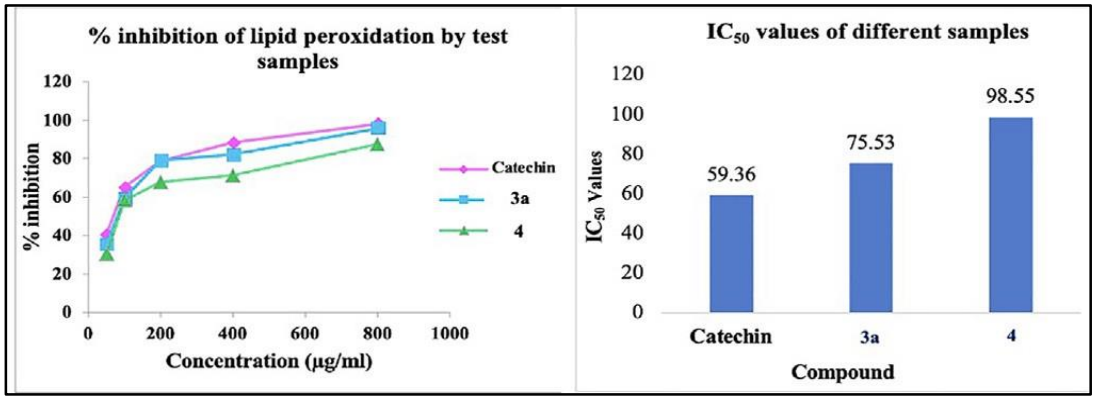


Figure 3.Lipid peroxidation of Catechin (standard) and synthesized compounds at different concentrations.

5. Conclusion

The current interest in the development of new antimicrobial activity has been the mainstay of medicinal intervention against infectious diseases caused by various pathogens. A matter of concern in the treatment of microbial infections is the limited number of efficacious antimicrobial drugs. There is a real perceived need for the discovery of new compounds that are endowed with antimicrobial activities, possibly acting through mechanism of action, to which many clinically relevant pathogens are now resistant.

The compounds were screened for their antibacterial and antifungal activities. The activities reported by means of zone of inhibition in millimeter. All the compounds showed very good antibacterial and antifungal activities at the tested dose level. Also the compounds compared with standard for in-vitro lipid peroxidation inhibitory studies and In-vitro Anti-acetylcholinesterase activity.

6. References

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