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SYNTHESIS AND EVALUATION OF 2-(1H-BENZO[D]IMIDAZOL-1-YL) ACETAMIDE DERIVATIVES FOR ANTIMICROBIAL ACTIVITY

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Abstract

The objective of this current work was to synthesized some 2-(1H-benzo[d]imidazol-1-yl)acetamide derivatives and evaluating the antibacterial activity of the synthesized compounds in gram positive and gram negative bacteria. The synthesis was accomplished in two steps that included synthesis of acetate derivative of benzimazole and converting the acetate to acetamide by reaction with appropriate amine. The antibacterial activity of the synthesized compounds was determined using zone of inhibition method against gram positive (*S. aureus, B. subtilis*) and gram negative bacteria (*E. coli*). The results revealed that the antibacterial action of the synthesized compounds was dose dependent. The compounds were mild to moderately antibacterial. The presence of small substituents in the compounds was not favorable for antibacterial activity (2a, & 2b) whereas aromatic as well as heteroaromatic ring in the amine favored antibacterial action (2c, 2d & 2e). The substitution of one hydrogen of the amino group with aromatic ring favored antibacterial action against gram positive bacteria (2c). On the other hand the presence of two aromatic rings on the nitrogen led to poor activity against gram positive bacteria (2d) while the activity was optimum against gram negative bacteria. It was evident from the results that the incorporation of heteroromatic ring as part of the amine group was highly beneficial for activity against both gram positive as well as gram negative bacteria.

Keywords

Benzimidazole, acetamide, antibacterial, disc diffusion, characterization

Introduction

Bacterial resistance to existent antibiotherapy is a perpetual internationally-recognized problem [1]. Year after year, there is a continuous need for novel antibacterial drugs and this research and development efforts recently resulted in few new drugs or combination of drugs proposed for the use into the clinic.

Over the last few years Plazomicin, eravacycline, sarecycline, omadacycline, rifamycin and imipenem, cilastatin and relebactam combination, pretomanid, lefamulin, cefiderocol have been approved by the USFDA for antimicrobial therapy.

Literature exhibited that Benzimidazole derivatives have been widely investigated for antimicrobial actions. Small molecules have lately been the focus of synthesis for targeting various receptors for treatment of diseases [2-11].

Hence it was hypothesized that synthesizing small molecules based on the bnenzimidazole nucleus might be beneficial for antimicrobial action. The objective of the present research work was to synthesize small N-substituted benzimidazole derivatives and evaluated the anti bacterial action of the synthesized compounds against gram positive and gram negative bacteria in culture medium.

Material and Methods

All the reagents and chemicals were used as obtained without any purification or drying except potassium carbonate which was made anhydrous by drying in hot air oven.

The scheme adopted for the synthesis of the benzimidazole acetamide derivatives has been presented in Figure 1.

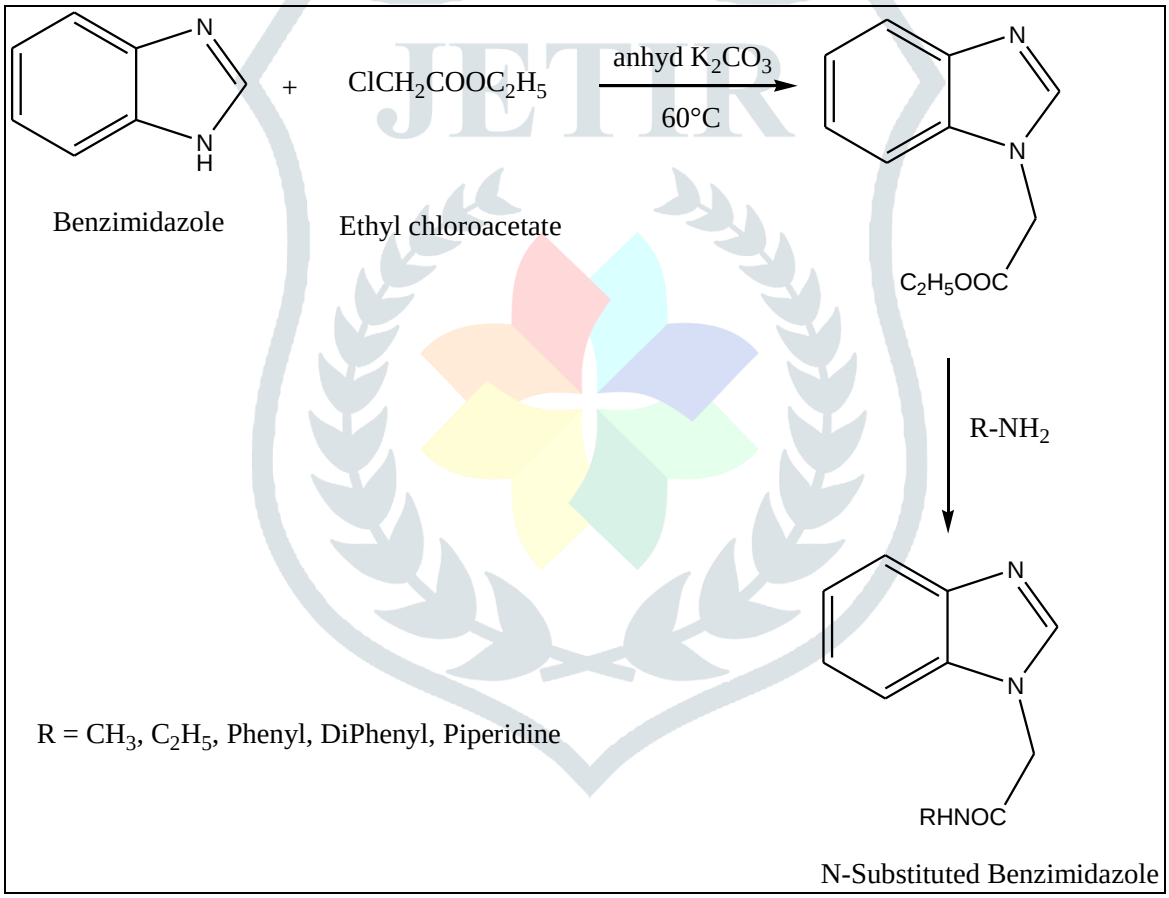

Figure 1 Scheme for synthesis of benzimidazole derivatives

Synthesis of ethyl 2-(1H-benzo[d]imidazol-1-yl)acetate

Anhydrous K_2CO_3 was added to a solution of the benzimidazole (10 mmol) and ethylchloroacetate (10 mmol) dissolved in anhydrous DMF in a round bottom flask and allowed the reaction to refluxed for 1-2 hr. After the completion of reaction checked by TLC, crushed ice was added into reaction mixture compounds got ppt out. Filter the solid and washed with cold water [2].

General method of synthesis of N-substituted benzimidazole

Compound obtained from step 1 (10 mmol) was dissolved in 15 ml methanol and to it was added corresponding amine (20 mmol). The reaction mixture was refluxed for 2-3 h and after completion of reaction the product got precipitated out. The crystals formed were filtered, washed and after drying recrystallized from methanol [12].

Chemical characterization

The melting point determination of each of the synthesized compounds was carried out instrumentally using open capillary method and is uncorrected. In order to determine solubility of the synthesized compounds, 1 mg of the compound was weighed and shaken with 1 mL of solvent (non polar to polar) taken in test tubes. The samples were dried completely and mixed with IR grade potassium bromide previously dried in an oven. The dispersion was the fed on to the sample holder of the IR spectrophotometer and spectrum was obtained

Antibacterial activity

Antibacterial acivity [13]

The compounds synthesized during the present investigation were screened for their antibacterial activity. The antibacterial tests were conducted on four common microorganisms viz. *Bacillus subtilis, Streptococcus aureus and Escherichia coli* which are the representative types of gram positive and gram-negative organisms respectively. The antibacterial activity of the compounds was assessed by disc diffusion.

Test solution

20 mg of the synthesized compounds were dissolved separately in 20 ml methanol. 1 ml of this solution was diluted to 10 ml with methanol. 0.5 ml (50 µg) and 1 ml (100 µg) of this solution was further diluted upto 2 ml by addition of methanol to obtain a solution of 25 and 50 µg/ml strength. These sample solutions were sterilized test tubes. These test compounds (25, 50 and 100 µg/ml) were soaked on small circular disc of 5 mm. Ciprofloxacin was used as the standard drug at concentration of 50 and 100µg/ml prepared in distilled water.

Assay procedure

The sterilized media (nutrient agar) was cooled to 45°C with gentle shaking for uniform cooling and then inoculated with 18-24 h old bacterial subculture under aseptic conditions in a laminar air flow bench and mixed well by gentle shaking. This was poured in to sterile Petri dishes and allowed to set. After solidification all the Petri dishes were transferred to laminar flow unit and the test sample discs were carefully kept on the solidified media by using sterilized forceps. These Petri dishes were kept in the laminar air flow unit undisturbed for one-hour diffusion at room temperature and then for incubation at 37°C for 24 h in an incubator. The extent diameter of inhibition after 24 h was measured as the zone of inhibition in millimeters (mm).

Results and Discussion

Chemistry

Synthesis of the desired compounds was carried out by utilizing the scheme depicted in Figure 1. The scheme was optimized by varying the molar concentrations of the reactants and the reaction time so that maximum yield could be achieved for the compounds. The optimized reaction conditions for the first steps was 1:1 ratio of benzimidazole and ethylchloroacetate while in the second step a ratio 1:2 ratio of the step 1 product and amine was most suitable to obtain the maximum yield of the title compounds.
The R_f value obtained from TLC, melting point and percent yield of the synthesized compounds is depicted in Table 1.

Table 1 TLC, melting point and percent yield

Compound Code	Color	R_f Value	Melting Point	% Yield
2a	Brown	0.72	263-265°C	72
2b	Brown	0.56	251-253°C	74
2c	Yellow	0.71	246-248°C	69
2d	Brown	0.63	271-274°C	64
2e	Pale Yellow	0.57	286-289°C	63

The compounds were soluble in methanol (2c,2d,2e), chloroform (2a-2e) and DMSO (2a, 2c) whereas all of them were insoluble in water.

The spectral studies (NMR, Mass and IR) were conducted to confirm the structure of the synthesized compounds. The spectra were obtained for the samples and the interpretation of each spectrum was carried out to ascertain the formation of desired bonds and incorporation of the functional groups.

The FTIR spectra of all the compounds exhibited stretching and bending vibrations for CH, C=O, C-N whereas N-H stretching peaks were prominent in the corresponding compounds containing free NH (2a, 2b, 2c). The proton NMR spectra yielded shifts for aromatic protons, the proton of iminie as well as the CH_2 proton of the benzyl group. The mass spectra revealed molecular ion peaks in compound 2e.

2-(1H-benzo[d]imidazol-1-yl)-N-methylacetamide, 2a

IR (cm^{-1}): 3138.07 (N-H stretching), 3026.05 (C-C stretching), 2751 (C-H stretching), 1706 (C=O stretching), 1603 (C=C stretching), 1475 (C-H bend), 1316 (C-N stretching)

^{1}HNMR (δ ppm): 8.176 (CH imine), 7.1-7.8 (CH, aromatic), 3.41 (CH_2), 0.81 (CH_3)

Mass (m/z): 189.2 (calculated)

2-(1H-benzo[d]imidazol-1-yl)-N-ethylacetamide, 2b

IR (cm^{-1}): 3356.71 (N-H stretching), (3036.76 C-C stretching), 2756 (C-H stretching), 1699 (C=O stretching), 1603 (C=C stretching), 1480 (C-H bend), 1318 (C-N stretching)

^{1}HNMR (δ ppm): 8.182 (CH imine), 7.1-7.8 (CH, aromatic), 3.45 (CH_2), 1.36 (CH_2), 0.93 (CH_3)

Mass (m/z): 203.2 (calculated)

N-phenyl-1H-benzo[d]imidazole-1-carboxamide, 2c

IR (cm^{-1}): 3413 (N-H stretching), 2755 (C-H stretching), 1713 (C=O stretching), 1586 (C=C stretching), 1481 (C-H bend), 1323 (C-N stretching)

^{1}HNMR (δ ppm): 8.176 (CH imine), 7.1-7.8 (CH, aromatic), 3.47 (CH_2)

Mass (m/z): 237.3 (calculated)

N,N-diphenyl-1H-benzo[d]imidazole-1-carboxamide, 2d

IR (cm^{-1}): 2759 (C-H stretching), 1682 (C=O stretching), 1575 (C=C stretching), 1462 (C-H bend), 1321 (C-N stretching)

^{1}HNMR (δ ppm): 8.16 (CH imine), 7.1-7.8 (CH, aromatic), 3.41 (CH_2)

Mass (m/z): 313.3 (calculated)

(1H-benzo[d]imidazol-1-yl)(piperidin-1-yl)methanone, 2e

IR (cm^{-1}): 3026 (C-H stretching), 1680 (C=O stretching), 1589 (C=C stretching), 1484 (C-H bend), 1313 (C-N stretching)

^{1}HNMR (δ ppm): 8.11 (CH imine), 7.1-7.8 (CH, aromatic), 3.36 (CH_2)

Mass (m/z): 229.3 (M^+ peak)

Antibacterial Action

The antibacterial activity of the synthesized benzimimdazole derivatives was determined measuring the zone of inhibition in the agar plate. Three levels of drug concentration (25, 50 & 100 µg/mL) of the synthesized compounds were tested for antibacterial action using ciprofloxacin as the standard drug for antibacterial action. The zone of inhibition of the test compounds is presented in table 2.

Table 2 Antibacterial activity of synthesized compounds

Compound Code	Zone of Inhibition (mm)*								
	B. subtilis			***S. auerus***			***E.coli***		
	25µg	50µg	100µg	25µg	50µg	100µg	25µg	50µg	100µg
2a	7	8	10	6	8	9	7	9	12
2b	7	9	12	6	9	13	9	13	16
2c	9	12	**19**	8	15	**19**	8	11	15
2d	8	10	15	7	9	13	11	18	**23**
2e	13	16	**20**	11	14	**20**	13	**19**	**25**
Ciprofloxacin	15	**21**	**33**	13	**22**	**27**	15	**23**	**35**

* Below 12 mm – poor activity; 13-18 mm – moderate activity & above 18 mm – optimal activity

The results revealed that the antibacterial action of the synthesized compounds was dose dependent. The compounds were mild to moderately antibacterial. The presence of small substituents in the compounds was not favorable for antibacterial activity (2a, & 2b) whereas aromatic as well as heteroaromatic ring in the amine favored antibacterial action (2c, 2d & 2e). The substitution of one hydrogen of the amino group with aromatic ring favored antibacterial action against gram positive bacteria (2c). On the other hand the presence of two aromatic rings on the nitrogen led to poor activity against gram positive bacteria (2d) while the activity was optimum against gram negative bacteria. It was evident from the results that the incorporation of heteroromatic ring as part of the amine group was highly beneficial for activity against both gram positive as well as gram negative bacteria.

Conclusion

The benzimidazolyl acetamide derivatives were synthesized using two step reaction approach and were obtained in good yields and of sufficient purity. The synthesized compounds exhibited mild to moderate antibacterial action against gram negative and gram positive bacteria. The study of a congeneric series of benzimidazolyl acetamide compounds, determining it IC_{50} values and performing QSAR analysis would be highly helpful in designing newer antimicrobial compounds that would overcome the problems of resistance to antibiotics by the microbes.

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