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Research Article

Anti-Gram-Positive and Anti-Gram-Negative Pathogen Killing Potentials of 3-[((4-(1,3-dioxoisoindolin-2-yl)phenyl)hydroxyl) methylene amino]-4-Methyl Benzoic Acid

Disha M Dhabarde[1], Prachi Shambharkar[2], Manish A Kamble[3], Jagdish R Baheti[4], Debarshi Kar Mahapatra[5]

[1]Assistant Professor, [2]Student, Department of Pharmaceutical Chemistry, Kamla Nehru College of Pharmacy, Nagpur, Maharashtra, India.
[3]Assistant Professor, [4]Principal & Professor, Department of Pharmacognosy, Kamla Nehru College of Pharmacy, Nagpur, Maharashtra, India.
[5]Assistant Professor, Department of Pharmaceutical Chemistry, Dadasaheb Balpande College of Pharmacy, Nagpur, Maharashtra, India.

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Corresponding Author:
Debarshi Kar Mahapatra, Department of Pharmaceutical Chemistry, Dadasaheb Balpande College of Pharmacy, Nagpur, Maharashtra, India.
E-mail Id:
dkmbsp@gmail.com
Orcid Id:
https://orcid.org/0000-0002-3986-0337
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A B S T R A C T

Inspired from the available biological data of several imperative therapeutic scaffolds in several medicinal chemistry databases, phthalimide-based compounds were identified and their Schiff's base-based hybridized molecules were designed. In one of the recent rational exploration, 3-[((4-(1,3-dioxoisoindolin-2-yl)phenyl) hydroxyl)methylene-amino]-4-methyl benzoic acid was synthesized in a two-step reaction, characterized comprehensively by using a few sophisticated spectroscopic instruments such as FT-IR spectroscopy, Mass spectroscopy, and ^{1}H-NMR spectroscopy, as well as CHN Analyzer, and ultimately screened for its anti-bacterial potentials against gram-negative microbial species (*Escherichia coli*) and gram-positive microbial species (*Staphylococcus aureus*) using the conventional procedure. The chemical structure of the proposed molecule was completely recognized by sophisticated spectroscopic instruments. The compound exhibited an incredible anti-bacterial activity against anti-*E. coli* and expressed a fair bacterial activity against anti-*S. aureus* in *in-vitro* anti-microbial screening model, but with a very low potency. The compound was found to be more effective towards Gram-negative species. The investigation pointed towards several prospective for the development of broad-spectrum anti-microbial drugs against resistant pathogenic strains by looking insights into the unexplored class of phthalimide-based hybrid Schiff's base compound.

Keywords: Phthalimide, Schiff's base, *Escherichia coli*, *Staphylococcus aureus*, Anti-microbial, Anti-bacterial

Introduction

In this era, the whole world population is panic with the anti-microbial drug resistance.[1] The human civilization has witnessed several challenges; there was a time when the drug was not available and the majority of the cure was due to precautions and preventions,[2] which was followed by an era where some budding prospects lie with therapeutics,[3] that was trailed by a period where the market was flooded with drug products and the mainstream population was happy to be equipped with 'therapeutic weapons' as research was on thrust,[4] new computational tools were available,[5] new therapeutic drug-targets were found,[6] several unexplored classes were investigated by robotic and artificial intelligence approaches, etc.[7] But in the due course of time, the situation abruptly changed and these pathogens such as bacteria, fungi, viruses, worms, amoeba, etc. became smart owing to native xenobiotic neutralizing mechanistic pathways and situation-oriented self-defense learning approaches.[8] Now, the modern situation is an interesting era where the self-claimed modern-day humans are equipped with massive 'therapeutic weapons' in context to quantities but the majority of them are now completely obsolete in tackling the super-smart pathogens.[9]

Inspired from the available biological data of several imperative therapeutic scaffolds in several medicinal chemistry databases, phthalimide-based compounds were identified and their Schiff's base-based hybridized molecules were designed. In one of the recent rational exploration, 3-[((4-(1,3-dioxoisoindolin-2-yl)phenyl) hydroxyl)methylene-amino]-4-methyl benzoic acid was synthesized in a two-step reaction, characterized comprehensively by using a few sophisticated spectroscopic instruments such as FT-IR spectroscopy, Mass spectroscopy, and ^{1}H-NMR spectroscopy, as well as CHN Analyzer, and ultimately screened for its anti-bacterial potentials against gram-negative microbial species (*Escherichia coli*) and gram-positive microbial species (*Staphylococcus aureus*) using the conventional procedure.

Materials and Methods

Chemicals and Instrumentation

The analytical grade 99.9% pure chemicals, reactants, starting material, and solvents were procured solely from Sigma Aldrich, Germany via a local seller at Nagpur, Maharashtra. Double distilled water (Borosil®, India) was used in this experiment. The progress of the chemical reaction was checked by utilizing the Merck® pre-coated Silica gel-G TLC plates. Elemental analysis (PerkinElmer 2400 model), Mass Spectroscopy (MICROMASS® Q-TOF model), Fourier-transformed infrared spectroscopy (Shimadzu® IR-Affinity-1 model), and Proton (^{1}H)-Nuclear Magnetic Resonance (NMR) Spectroscopy (Bruker® Avance-II model) were employed for the characterization purpose.

Synthesis of Target Compounds

The chemical reaction involved the reacting phthalimide (1) and 4-chlorobenzoic acid (2) where HCl (hydrochloride) moiety gets eliminated during the process, as hydrogen (H) gets removed from (1) and chlorine (Cl) from (2). In the succeeding reaction process, a Schiff's base component was created by prompt reaction of the carbonyl (C=O) group (electrophilic nature) situated in the 4-benzamidobenzoic acid (3) with the active amino (-NH_2) group (nucleophilic nature) present in the 3-amino-4-methyl benzoic acid (4) to form azomethine (C=N) component, 3-[((4-(1,3-dioxoisoindolin-2-yl)phenyl)hydroxyl)methylene amino]-4-methyl benzoic acid (5) (Figure 1). The mechanism of chemical reaction entailed an attack of the carbonyl carbon (3) with the amine (4) of the reactant to form (5).[10]

Synthesis of 4-(1,3-dioxoisoindolin-2-yl)-benzoic acid (3)

The solution of equimolar concentration (0.01 M) of phthalimide (1) and 4-chloro benzoic acid (2) were made to react in presence of anhydrous sodium carbonate (0.01 M) was stirred at room temperature for 6 hr duration. The solvent was evaporated under rotary evaporator, the collected precipitate was washed thoroughly with cold water, and recrystallized from ethanol to get the desired intermediate product.

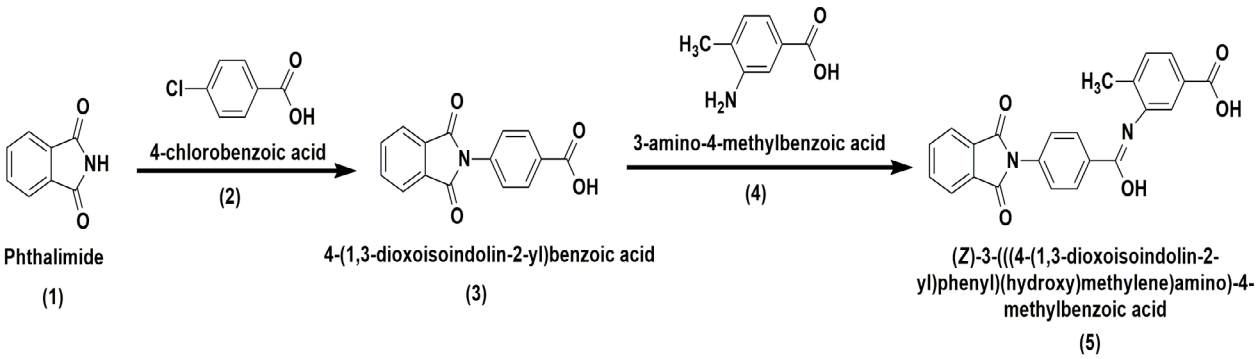

Figure 1.Synthesis of Schiff's base containing phthalimide hybrid compound

Off-white powder; 89% yield; m.p.: 212-214°C; FTIR (KBr) υ (cm^{-1}): 3399 (-OH, stretching), 3137 (C-H, aromatic), 1701 (C=O, stretching), 1606 (C=C, aromatic), 1252 (C-N, stretching), 1225 (C-O); ^{1}H-NMR (δ, ppm, $CDCl_3$): 11.16 (Hydroxyl, 16, 1H), 7.3-8.1 (Aromatic, 8H). MS: M^+ 267. Anal. Calcd. for $C_{15}H_9NO_4$: C, 67.42; H, 3.39; N, 5.24. Found: C, 66.81; H, 3.01; N, 4.92.

Synthesis of 3-[((4-(1,3-dioxoisoindolin-2-yl)phenyl)hydroxyl) methylene-amino]-4-methyl benzoic acid (5)

An equimolar quantity (0.01 M) of 4-(1,3-dioxoisoindolin-2-yl)-benzoic acid (3) and 3-amino-4-methyl benzoic acid (4) were made to refluxed for 6 hr period in the presence of glacial acetic acid (8-10 drops) under ethanolic environment. The reaction content was further cooled down in the presence of crushed ice to precipitate the desired product. The acquired product was washed thoroughly with water, air-dried, and properly recrystallized from absolute ethanol.

Light yellow powder; 76% yield; m.p.: 180-182°C; FTIR (KBr) υ (cm^{-1}): 3361 (-OH, stretching), 3146 (C-H, aromatic), 1732 (C=O, stretching), 1689 (C=N, stretching), 1623 (C=C, aromatic), 1474 (-CH_3), 1355 (C-N, stretching), 1236 (C-O); ^{1}H-NMR (δ, ppm, $CDCl_3$): 11.14 (Hydroxyl, 25, 1H), 7.6-8.9 (Aromatic, 11H), 2.47 (Methyl, 19, 3H), 2.11 (Hydroxyl, 17, 1H). MS: M^+ 400. Anal. Calcd. for $C_{23}H_{16}N_2O_5$: C, 69.00; H, 4.03; N, 7.00. Found: C, 68.08; H, 3.82; N, 6.57.

Anti-Microbial Screening

The anti-microbial potential of 3-[((4-(1,3-dioxoisoindolin-2-yl)phenyl)hydroxyl)methylene-amino]-4-methyl benzoic acid against *Escherichia coli* (*E. coli*, MTCC 2961) and *Staphylococcus aureus* (*S. aureus,* MTCC 3160) was evaluated using the disc diffusion method.

In Dimethyl Sulfoxide (DMSO), the novel compound was dissolved, soaked further on a Whatman filter paper (no. 1), placed carefully over the microbial culture agar plates, and at last incubated at temperature 37±1ºC for 24 hrs duration.[11] The minimum inhibitory concentration (MIC) was estimated by utilizing the agar streak dilution method. The microbial suspension of concentration (10^6 CFU/mL) was formed by serial dilution with DMSO and relocated into the Petri dish up to 5 mm depth at 40-50ºC temperature. The protocol was performed in a triplicate manner and the average value of MIC was computed. Ciprofloxacin served as the positive control and DMSO act as negative control.[12]

Results and Discussion

Chemistry

The chemical structure of the novel phthalimide-based Schiff's base compound was ascertained properly by making the utilization of sophisticated instruments such as ^{1}H-NMR spectroscopy, FT-IR spectroscopy, and Mass spectroscopy. The FT-IR spectrum of the compound revealed some key features that partially supported the formation of the proposed molecule. The most imperative point of the compound, azomethine (-C=N) that presented the linkage of 4-(1,3-dioxoisoindolin-2-yl)-benzoic acid with 3-amino-4-methyl benzoic acid was observed dominantly at 1689 cm^{-1}. In addition to it, the appearance of the peaks representing the methyl (-CH_3) group at 1474 cm^{-1} and hydroxyl (-OH) group at 3361 cm^{-1} also supported the step-2 of the chemical reaction. The phthalimide portion was ascertained from the carbonyl group at 1732 cm^{-1}. The absorption peaks located at 3146 cm^{-1} and 1623 cm^{-1} corresponded to the C-H and C=C components of the three aromatic rings. Finally, the central aromatic component and its substitution or

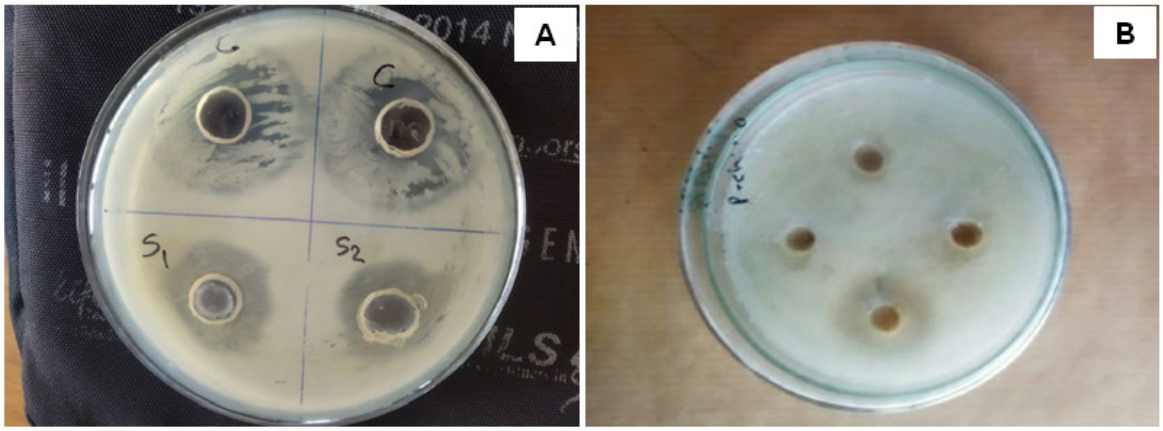

Figure 2.Anti-bacterial activity of the 3-[((4-(1,3-dioxoisoindolin-2-yl)phenyl)hydroxyl)methylene-amino]-4-methyl benzoic acidagainst (A) E. coli (B) S. aureus

The bacterial species were cultured under the laminar air-flow conditions in the nutrient broth media by using the Muller Hinton Agar medium. The cultured bacterial species were selectively relocated into the microbial culture agar plates.

groups, specifically C-O and C-N were established from the absorption peaks at 1236 cm^{-1} and 1355 cm^{-1}, respectively.

On analysis of the compound using the ^{1}H-NMR technique, the proposed chemical structure was completely

determined. The signals obtained from the aromatic three-rings protons (11 hydrogens) were seen characteristically in the range of 7.6-8.9 ppm. The key features of the compounds; *viz*. hydroxyl group and methyl group were confirmed from the recorded proton signals that prevailed at 2.11 ppm and 2.47 ppm, respectively. Furthermore, the mass spectroscopy technique displayed the sharp emergence of compound base peak (M^+) at m/z 400 which matched corresponding with the compound molecular mass that finally concluded the formation of the desired product. Multiple fragmented peaks of varied molecular mass (m/z range of 150-250) appeared in the mass spectra that represented the fragmented components such as phthalimide, benzoic acid, amino(phenyl)methanol, etc. The elemental analysis data that was procured through analyzer experimentally, nearly matched with the computed theoretically values (~5% difference) that indicated the formation of the desired compound.

Anti-microbial Screening

The novel phthalimide-based Schiff's base compound, 3-[((4-(1,3-dioxoisoindolin-2-yl)phenyl)hydroxyl)methylene-amino]-4-methyl benzoic acid (5) demonstrated an incredible anti-bacterial activity against anti-*E. coli* (ZOI = 19.8 mm, MIC = 500 µg/mL), the Gram-negative strain (Figure 2A) and expressed a fair bacterial activity against anti-*S. aureus* (ZOI = 13.9 mm, MIC = 500 µg/mL), the Gram-positive strain (Figure 2B) as compared to ciprofloxacin (ZOI = 31.5 mm, MIC = 6.25 µg/mL), the standard drug. The novel compound expressed a little promising activity against both the Gram-positive strain and Gram-negative strain as compared to the marketed product, ciprofloxacin.

Conclusion

With an intention to discover novel compounds that may possibly be potential in overcoming the associated resistance, the current exploration exclusively focuses toward investigating the pharmacotherapeutically privileged anti-microbial activity of the molecule 3-[((4-(1,3-dioxoisoindolin-2-yl)phenyl)hydroxyl)methylene-amino]-4-methyl benzoic acid against both Gram-positive (*S. aureus*) and Gram-negative (*E. coli*) strains. The chemical structure of the proposed phthalimide-based molecule was completely recognized by making use of few sophisticated spectroscopic instruments such as FT-IR spectroscopy, Mass spectroscopy, and ^{1}H-NMR spectroscopy, as well as CHN Analyzer. The compound exhibited an incredible anti-bacterial activity against anti-*E. coli* and expressed a fair bacterial activity against anti-*S. aureus* in *in-vitro* anti-microbial screening model, but with a very low potency. The compound was found to be more effective towards Gram-negative species. The investigation pointed towards several prospective for the development of broad-spectrum anti-microbial drugs against resistant pathogenic strains by looking insights into the unexplored class of phthalimide-based hybrid Schiff's base compound. Although, pre-clinical studies and clinical investigations are fundamentally required to determine the beneficial effects, adverse effects, as well as establishing the structure-activity-relationship (SAR) of the whole series that will open avenues for better pharmacotherapy.

Conflict of Interest: None

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