Research Article

L-Proline-Catalyzed Synthesis of Phthalimide Derivatives and Evaluation of Their Antioxidant, Anti-Inflammatory, and Lipoxygenase Inhibition Activities

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A study was planned to synthesize the phthalimide derivatives as phthalimides have versatile biological activities. To synthesize the phthalimide derivatives, initially the reaction was optimized with various catalysts, and L-proline was found to be the best catalyst as it provided excellent yield. A series of phthalimide derivatives was synthesized by facile one-pot reaction of phthalic acid with aryl amines under mild reaction conditions in the presence of L-proline as catalyst. Products were obtained in excellent yields and structurally characterized by 1H, 13C NMR, and mass spectral data. Products 1–7 were evaluated for antioxidant, anti-inflammatory, and lipoxygenase enzyme inhibition activities. Compounds 1 and 4 showed potent antioxidant activity under DPPH with IC50 values 27.3 and 25.0 μM when compared with the standard BHA (IC50 = 44.2 μM), respectively. Compounds 1 and 4 further showed strong lipoxygenase inhibition activity with IC50 values 21.34 and 20.45 μM when compared with standard baicalein (IC50 = 22.60 μM), respectively. Compound 2 was found to be promising and about equal to the used standard aspirin in the inhibition of bovine serum albumin denaturation, while other compounds showed weak-to-moderate % inhibition.

1. Introduction

Heterocyclic compounds have been found to possess important physiological and pharmaceutical properties [1]. Phthalimides and their N-substituted derivatives belongs to the imide ring containing heterocyclic class of compounds which possess extensive biological activities [2]. They have engrossed attention in medicinal chemistry due to their wide range of medicinal applications such as anticonvulsant [3], anti-inflammatory [4], analgesic, hypoglycemic [5], antimicrobial [6, 7], androgen receptor antagonists [8], antitumor [9], anxiolytic [10], anti-HIV-1 [11], anthelmintic [12], and immunomodulatory activities. Phthalimides have also been used as inhibitors of tumor necrosis factor alpha (TNF-α) that plays an important role in different physiological immune systems [13]. Phthalimides have served as starting materials and intermediates for the synthesis of many types of therapeutic synthons, alkaloids, and pharmacophores and also been used as herbicides to control bacterial contamination [14, 15].

Due to wide range of applications in medicinal chemistry, interest is increasing in the synthesis and biological activities of phthalimides and its derivatives. So, we planned to synthesize the N-aryl phthalimides from phthalic acid and aryl amines and evaluate them biologically to find out the novel lead candidates. Herein, we report the synthesis of N-aryl phthalimides 1–7 and their biological screening for antioxidant and anti-inflammatory potentials. All the synthesized compounds have significant antioxidant active, whereas compounds 1 and 4 showed potent antioxidant potential, much better potential as compare to the standard BHA.

2. Materials and Methods

2.1. Experimental. All the reagents and chemicals were of analytical grade purchased from Sigma-Aldrich and used as such. Melting points were measured on a Gallen Kamp
2.2. General Procedure for the Synthesis of Phthalimides through l-Proline-Catalyzed Reaction. The phthalic acid (1.0 mmol), aryl amine (1.0 mmol), and l-proline (0.5 mmol) were sequentially added in ethanol (5.0 mL) into an arbor (round bottom) flask to form a solution. The resultant mixture was stirred at 30°C room temperature for six hours. After completion of the reaction, the reaction mixture was then diluted with EtOAc ethyl acetate and washed with water. The organic phase was dried, filtered, and concentrated to give the pure phthalimide product. Purity of the products was checked by TLC.

2.2.1. N-(4-Methoxyphenyl)isoindoline-1,3-dione 1. Yield: 94%; m.p. 107°C; 1H NMR (500 MHz, CDCl3) δH 7.04 (d, 1H, J = 8.5 Hz), 7.35 (d, 2H, J = 8.5 Hz), 7.80 (m, 2H), 7.96 (m, 2H), 3.88 (s, 3H, OCH3); 13C NMR (125 MHz, CDCl3) δC 167.5×2, 159.2, 134.3×2, 132.0×2, 129.1×2, 129.1×2, 128.1, 126.6×2, 123.7×2, 114.5×2, 55.5. ESIMS m/z C14H9NO2 224.

2.2.2. N-(Phenyl)isoindoline-1,3-dione 2. Yield: 93%; m.p. 101°C; 1H NMR (500 MHz, CDCl3) δH 7.43 (m, 3H), 7.52 (d, 2H, J = 8.0 Hz), 7.83 (dd, 2H, J = 3.0 & 8.5 Hz), 7.99 (dd, 2H, J = 3.0 & 8.5 Hz); 13C NMR (125 MHz, CDCl3) δC 167.3×2, 134.4×2, 131.7×2, 129.1×2, 128.1, 126.6×2, 123.7×3. ESIMS m/z C15H11NO2 234.

2.2.3. N-(4-Chlorophenyl)isoindoline-1,3-dione 3. Yield: 92%; m.p. 105°C; 1H NMR (500 MHz, CDCl3) δH 7.43 (d, 2H, J = 8.5 Hz), 7.51 (d, 2H, J = 8.5 Hz H), 7.82 (dd, 2H, J = 3.0 & 8.0 Hz), 7.98 (dd, 2H, J = 3.0 & 8.0 Hz); 13C NMR (125 MHz, CDCl3) δC 167.0×2, 134.5×2, 133.8, 131.6×2, 130.2, 129.3×2, 127.6×2, 123.8×2. ESIMS m/z C16H10ClNO3 288.

2.2.4. N-(4-nitro)isoindoline-1,3-dione 4. Yield: 95%; m.p. 108.0°C; 1H NMR (500 MHz, CD2OD + CDCl3) δH 7.42 (m, 2H), 7.56 (d, 2H, m), 7.80 (dd, 2H, m), 7.91 (dd, 2H, m); 13C NMR (125 MHz, CD2OD + CDCl3) δC 167.3×2, 134.4×2, 131.7×2, 129.1×2, 128.1×2, 126.6×2, 123.7×2. ESIMS m/z C14H9NO2O4 268.

2.2.5. (p-Tolyl)isoindoline-1,3-dione 5. Yield: 93%; m.p. 102°C; 1H NMR (500 MHz, CDCl3) δH 7.34 (s, 4H), 7.80 (dd, 2H, J = 3.0 & 8.5 Hz), 7.96 (dd, 2H, J = 3.0 & 8.5 Hz), 2.43 (s, 3H); 13C NMR (125 MHz, CDCl3) δC 167.4×2, 138.2, 134.3×2, 131.8×2, 129.8×2, 129.0, 126.4×2, 123.7×2, 21.2. +ESIMS m/z C13H11NO2 238.

2.2.6. (o-Tolyl)isoindoline-1,3-dione 6. Yield: 95%; m.p. 103°C; 1H NMR (500 MHz, CDCl3) δH 7.23 (d, 1H, J = 7.5 Hz), 7.35 (m, 1H), 7.40 (s, 2H), 7.81 (dd, 2H, J = 3.0 & 8.5 Hz), 7.99 (dd, 2H, J = 3.0 & 8.5 Hz), 2.24 (s, 3H); 13C NMR (125 MHz, CDCl3) δC 167.3×2, 136.5, 134.3×2, 132.0, 131.1, 130.6×2, 129.4, 128.7, 126.9 123.7×2, 18.0. +ESIMS m/z C15H11NO2 238.235.

2.2.7. N-(2-Chloro-4-methoxyphenyl)isoindoline-1,3-dione 7. Yield: 94%; m.p. 110°C; 1H NMR (500 MHz, CD2OD + CDCl3) δH 7.44 (brs, 1H), 7.42 (brs, 1H), 7.06 (brs, 1H), 7.80 (brs, 2H), 7.91 (brs, 2H), 3.81 (s, 3H, OCH3); 13C NMR (125 MHz, CD2OD + CDCl3) δC 171.5×2, 158.8, 138.7, 132.0×2, 130.2, 128.3, 126.5, 115.9×2, 135.4×2, 127.7×2, 60.0. +ESIMS m/z C15H14ClNO3 288.

2.3. Biological Assays

2.3.1. Inhibition of Albumin Denaturation Assay. Anti-inflammatory potential as membrane stabilization of the products 1–7 was evaluated by modified method [16]. The reaction mixture consists of test solution (1 mg/ml) and 1% aq. solution of bovine albumin fraction. The pH of the reaction mixture was adjusted by using small amount of HCl. The reaction mixture was incubated for 20 min at 37°C and then heated for 20 min at 51°C. After cooling of the tubes, the turbidity was measured at 660 nm. Aspirin was used as a standard. The experiment was repeated thrice, and the percent inhibition of protein denaturation was calculated as follows:

\[
\% \text{ inhibition} = \left( \frac{\text{Abs control} - \text{Abs}_{\text{test}}}{\text{Abs control}} \right) \times 100, \quad (1)
\]

where Abs control is absorbance without any sample and Abs_{test} is the absorbance of test.

2.3.2. Membrane Stabilization Assay (Heat-Induced Hemolysis). Anti-inflammatory potential as membrane stabilization of the products 1–7 was evaluated by reported method [17]. Red blood cells suspension was prepared as follows: freshly collected human whole blood (10 ml) was transferred to the anticoagulant-containing centrifuge tubes. The tubes were centrifuged for 10 min at 3000 rpm and then washed thrice with normal saline. The blood volume was measured and reconstituted as 10% v/v suspension of normal saline. The reaction mixture (2 ml) was prepared with 1 ml of test sample (1 mg/ml) solution and 1 ml of 10% red blood cells suspension. Aspirin was used as a standard drug. In the control test tube, saline was added instead of test sample. The reaction mixtures were incubated for 30 min at 56°C on
water bath, and then tubes were kept under running tap water for cooling. The reaction mixture was centrifuged for 5 min at 2500 rpm, and the absorbance of released hemoglobin in the supernatant was measured at wavelength 560 nm. The experiment was repeated thrice. The estimation of percentage membrane stability was performed by using this expression:

\[
\text{Percent heat-induced hemolysis} = \frac{\text{OD of control} - \text{OD of test}}{\text{OD of control}} \times 100.
\]

The membrane stabilization percentage or protection was calculated by using this formula:

\[
\text{Percentage protection} = 100 - \frac{\text{OD of control} - \text{OD of test}}{\text{OD of control}} \times 100.
\]

2.4. In Vitro Antioxidant Activities

2.4.1. Hydrogen Peroxide Scavenging Assay. Antioxidant potential of the products 1–7 was measured through the reported spectrophotometric method [18]. 2 mL of hydrogen peroxide H₂O₂ (20 mM) in phosphate buffer saline was added to 1 mL of test compound at concentration ranging from 62.5 to 500 μM. The absorbance was measured at 230 nm after 10 min on ELISA plate reader (SpectraMax Plus 384 Molecular Device, USA) against a blank solution of phosphate buffer without H₂O₂ and compared with the reference compound ascorbic acid. The IC₅₀ values were calculated by using EZ-Fit Enzyme Kinetics Program (Perrella Scientific Inc., Amherst, USA).

\[
\text{% Scavenging of H₂O₂} = \left( \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \right) \times 100.
\]

\[
\text{DPPH scavenging effect(%) or Percent inhibition} = \frac{A_o - A_1}{A_o} \times 100,
\]

where \(A_o\) was the absorbance of control reaction and \(A_1\) was the absorbance of test or standard sample.

2.4.2. DPPH Radical Scavenging Assay. Free radical scavenging activity of the products was measured by 1,1-diphenyl-2-picrylhydrazyl (DPPH) through reported method [19]. In brief, 0.1 mM solution of DPPH in EtOH was prepared. 90 μL of DPPH solution was added to 10 μL of test compound solution that was prepared in ethanol at concentration ranging from 62.5 to 500 μM. The mixture was shaken and allowed to settle down for 30 min at room temperature. The absorbance was measured at wavelength 517 nm by using ELISA plate reader (SpectraMax Plus 384 Molecular Device, USA). Butylated hydroxyanisole was used as standard and experiment was done in triplicate. The IC₅₀ values were then calculated using the EZ-Fit Enzyme Kinetics program (Perrella Scientific Inc., Amherst, USA). The percent DPPH scavenging effect was calculated by using following equation:

3. Results and Discussion

3.1. Chemistry. A series of N-aryl phthalimides was prepared through the reaction of phthalic acid with aryl amines (anilines) in ethanol under mild condition with 1-proline as catalyst at room temperature. Literature shows that mostly N-substituted phthalimides were prepared by the reaction of phthalic anhydride with anilines under somewhat harsh conditions, that is, at high temperature or strong catalyst. However, in this study, N-aryl phthalimides were prepared under mild-conditioned simple reaction in excellent yields with 1-proline catalyst, and this synthetic route is reported for the first time (Scheme 1). Before using this reaction to synthesize the products, the reaction was adjusted and optimized to obtain excellent yield of N-aryl phthalimides by the absence and presence of various catalysts, namely, AlCl₃, CH₃COOH, BBr₃, L-serine, L-arginine, L-lysine, and L-proline. Overall, percentages of products were somewhat better in amino acids while excellent in L-proline without side products (Table 1), and then 1-proline was used as a catalyst to synthesize the products 1–7. The ¹H NMR spectra of products showed signals in the range of δ_H 7.0–7.9 ppm, confirming the synthesis of phthalimide derivatives. The characteristic quaternary carbon signals of phthalimide product appeared at δ_C 167 ppm in the ¹³C NMR spectra, while other signals were observed in the range of δ_C 123–139 ppm. The methoxy group showed NMR signals at δ_H 3.88, δ_C 55.5 and δ_H 3.91, δ_C 60.0 for compounds 1 and 7, respectively. The methyl group of compounds 5 and 6 appeared at δ_H 2.43, δ_C 21.2 and δ_H 2.24, δ_C 18.0 in the NMR spectrum, respectively. All the NMR and ESI data confirmed the synthesis of phthalimide derivatives 1–7.
3.2. Biological Activities. Phthalimides 1–7 were biologically evaluated for their anti-inflammatory, antioxidant, and lipoxygenase enzyme inhibition activities. The anti-inflammatory potential was evaluated by inhibition of albumin denaturation assay, membrane stabilization assay, and lipoxygenase enzyme inhibition assay, while antioxidant potential was evaluated through hydrogen peroxide scavenging assay and DPPH radical scavenging assay.

3.3. Antioxidant Activity. The antioxidant activity of compounds 1–7 was conducted with the DPPH well diffusion and hydrogen peroxide scavenging activities. Phthalimide 2, 3, and 5-7 showed weak DPPH radical scavenging activity, while phthalimide 1 and 4 showed strong activity with IC50 values 21.34 and 20.45 µM, respectively, which has much better potential as compared to the standard BHA (IC50 = 44.2 µM). However, all compounds 1–7 showed weak hydrogen peroxide scavenging activity ranging from 61.0 to 88.5 µM as compared to the standard ascorbic acid (IC50 = 20.77 µM). So, it can be concluded that the phthalimides with the presence of methoxy and nitro groups at the aromatic ring are the reason of significant DPPH radical scavenging activity (Table 2).

3.4. Anti-Inflammatory Activities. In this study, the protein denaturation bioassay was performed for in vitro assessment of anti-inflammatory activity of phthalimide derivatives. Tissue protein denaturation is one of the causes of arthritic and inflammatory diseases. Autoantigen production in certain arthritic diseases may be due to in vivo denaturation of proteins. Agents which can prevent denaturation of protein therefore, would be worthwhile for anti-inflammatory drug development. In our research on the anti-inflammatory activity, ability of compounds to inhibit protein denaturation was calculated. It was effective in inhibiting heat-induced albumin denaturation at concentration 1 mg/ml. Maximum inhibition of 77.73 ± 0.35% was observed for compound 2 at concentration 1 mg/ml. Aspirin as standard anti-inflammatory drug showed the maximum inhibition of 95.89 ± 0.06% at the concentration of 0.20 mg/ml. Compound 2 further showed maximum membrane stabilization inhibition of 83.91 ± 0.06% at 1 mg/ml, while aspirin showed the maximum inhibition of 85.92 ± 0.02 % at 0.20 mg/ml.

3.5. Lipoxygenase (LOX) Inhibition Activity. Among all the synthesized compounds, 1 and 4 showed excellent lipoxygenase enzyme inhibition activity with IC50 values of 21.34 and 20.45 µM, respectively, compared to the standard baicalein with IC50 value 22.6 µM, while rest of the compounds did not show any significant LOX inhibition activity. Phthalimide compounds containing methoxy and nitro groups in the aromatic ring showed strong LOX activity as compared to the presence of chloride and methyl groups in the ring. Thus, it can be concluded that the methoxy and nitro groups in the phthalimide compounds play a key role for the inhibition of enzyme LOX (Table 3).

4. Conclusions

Through the optimization of reaction, phthalimide derivatives were obtained in excellent yields through facile one-pot mild condition reaction of phthalic acid with aryl amines that was catalyzed by l-proline. All the products showed weak-to-moderate antioxidant potential, whereas compounds 1 and 4 showed outstanding and potent antioxidant and lipoxygenase enzyme inhibition potential as compared to the standard drug used. None of the phthalimide derivatives showed cytotoxic activity against all tested cancer cell lines. Among all synthesized compounds, compound 2 was found to be promising and about equal to the used standard aspirin in the inhibition of bovine serum albumin denaturation.
Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Supplementary Materials

\(^{1}\)H-NMR, \(^{13}\)C-NMR, and ESI-MS of compounds 1–7. (Supplementary Materials)

References


