



Research Article

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Synthesis of a Potential Anti-Inflammatory Pyrazole Derivative from Hippuric Acid as the Starting Material

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Received: 03 September 2017 Revised: 23 September 2017 Accepted: 04 October 2017

ABSTRACT

The research demonstrated a new method for the synthesis of pyrazole molecules from hippuric acid by a rearrangement reaction and its anti-inflammatory potential. The heterocyclic molecule was synthesized from the substrate; hippuric acid (**1**). The starting material was made to react with o-hydroxy acetophenone (**2**) in pyridine solution using POCl₃ as the condensing agent to form an ester (**3**). The formed ester was subsequently converted into a 1,3-diketone form (**4**) by stirring the ester in pyridine solution in the presence of powdered KOH. The conversion of o-acylated phenolic ester (**3**) into 1,3-diketone (**4**) was mediated by Baker-Venkataraman rearrangement reaction. Lastly, the desired compound; i.e. pyrazole (**5**) was synthesized using an alkaline solution of hydrazine hydrate in good yield. The *in vivo* anti-inflammatory activity of the synthesized derivative was performed by the carrageenan-induced paw edema standard method. The spectroscopic data revealed the possible structures of the synthesized compounds and characterization data were found to be in full agreement with that of the structures. The derivative (**6**) displayed 21.96% at first hr, 34.44% at second hr, and 42.87% at 3rd hr. The activity was found to be quite comparable with that of standard drug indomethacin. The results showed that the produced compound has the perspective to be utilized in inflammatory conditions and arthritis after suitable trails. This study will certainly promote researchers in the rational synthesis of heterocyclic molecules with pronounced biological activity.

Keyword: Pyrazole; Hippuric acid; anti-inflammatory; inflammation; synthesis; rearrangement

INTRODUCTION

The treatment of inflammation involves counteracting the cyclooxygenase enzyme and inflammatory mediators by non-steroidal anti-inflammatory drugs (NSAIDs) [1]. Principally, a long-term therapy leads to several complications; particularly gastrointestinal which involve ulceration and bleeding [2]. These drugs in fact inhibit the cyclooxygenase -1 (COX-1), which catalyzes the formation of arachidonic

acid (AA) to prostaglandins H₂ (PGH₂) comprehensively along with COX-2 in a few cases, as a result of lack of selectivity. Suppression of thromboxane A₂ (TXA₂) production and inhibition of platelet aggregation are the other attributes of NSAIDs [3]. Therefore, it is essential to discover a novel class of anti-inflammatory drugs with equal potential and less adverse effects. The reduced adverse effects and superior safety profile may provide

added advantage for long-term prophylactic use in chronic inflammatory conditions.

Heterocyclic compounds are the foremost choice among the researchers in treating arthritis based inflammatory ailments [4]. At present, nearly 85% of USFDA approved drugs in circulation for treating inflammation are heterocycles. Prominently, experimental molecules with pyridine, pyrimidine, pyrazole, pyrazoline, pyrazolone, etc. ring systems are best known for anti-inflammatory applications [5]. Pyrazole derivatives have gained citadel fame in the last 5 years owing to their diverse applications in fields of pharmaceutical, agrochemistry, and industries [6]. These pharmacoeactive agents play significant position in medicinal chemistry. As far as pharmaceutical applications are concerned, they are very well known for multifarious pharmacological activities like anti-bacterial, anti-fungal, anti-mycobacterial, anti-viral, anti-leishmanial, anti-cancer, anti-inflammatory, analgesic, anti-hypertensive, anti-convulsant, anti-oxidant, anti-diabetic, ulcerogenic, etc. [7, 8].

The present research involved the synthesis of a pyrazole molecule utilizing hippuric acid as the substrate, from which an initially ester intermediate was formed which further rearrange to 1,3-diketone via a name reaction. The formed product was screened for *in vivo* anti-inflammatory activity using carrageenan-induced paw edema standard method. The work involved an elegant and proficient technique to make anti-inflammatory heterocyclic lead.

MATERIALS AND METHODS

Materials

Hippuric acid and o-hydroxy acetophenone were purchased from Sigma-Aldrich Ltd., Germany. Hydrazine hydrate and pyridine were procured from Hi-Media India Ltd., Mumbai. All other chemical derivatives and analytical grade reagents/solvents were purchased from Merck India Ltd., Mumbai.

Instruments

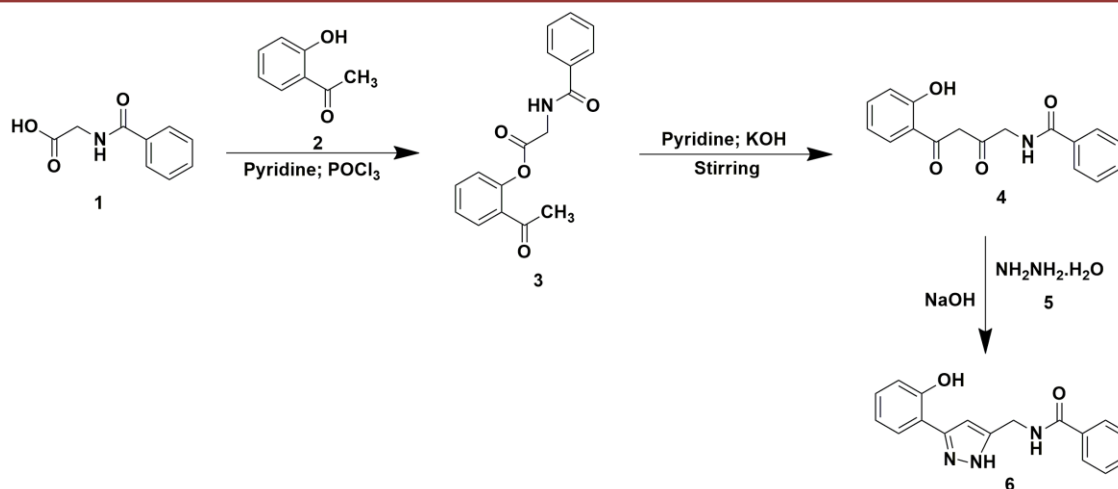
The structure of the proposed pyrazole derivatives was elucidated using the following sophisticated analytical tools: FT-IR spectra, which was obtained on the IRAffinity-1 instrument using KBr disc; $^1\text{H-NMR}$ was performed on Bruker spectropin NMR DPX-300 at 400 MHz; and mass spectra was obtained on JEOL-JMS-DX 303 instrument. The Perfit melting point apparatus was employed to measure the melting point of the derivatives. The progress of the reaction was monitored using Merck silica gel G-coated TLC plates. The information on the approximate elemental composition was procured on Perkin-Elmer 240C analyzer.

Animals

For the screening, permission from the Department Ethical Committee was taken. The male albino rats of weight 180-250 g were employed for the screening. The albino rats were kept in hygienic polypropylene cages in animal house having controlled environment (temperature of 25–26°C, humidity 50–55%, 12/12 hrs light/dark cycles).

Synthesis of target compounds

The heterocyclic molecule was synthesized from the substrate; hippuric acid (**1**). The starting material was made to react with o-hydroxy acetophenone (**2**) in pyridine solution using POCl_3 as the condensing agent to form an ester (**3**). The formed ester was subsequently converted into a 1,3-diketone form (**4**) by stirring the ester in pyridine solution in the presence of powdered KOH. The conversion of o-acylated phenolic ester (**3**) into 1,3-diketone (**4**) was mediated by Baker-Venkataraman rearrangement reaction. Lastly, the desired compound; i.e. pyrazole (**5**) was synthesized using an alkaline solution of hydrazine hydrate in good yield. The **Scheme 1** illustrates the synthetic steps.



Scheme 1: Synthetic protocol for hippuric acid based pyrazole derivative.

Synthetic protocol for 2-acetylphenyl 2-benzamidoacetate (3)

Eqimolar mass (0.01 M) of hippuric acid (**1**) and o-hydroxy acetophenone (**2**) were refluxed in pyridine solution in presence of POCl_3 for 8 hr. The obtained precipitate was collected, washed thoroughly, dried suitably, and recrystallized.

78% yield; FTIR (KBr) ν (cm^{-1}): 3337 (-NH, stretch), 3129 (C-H, aromatic), 1726 (C=O, stretch), 1648 (C=C, aromatic), 1571 (-NH, bending), 1217 (C-N, stretch); $^1\text{H-NMR}$ (δ , ppm, CDCl_3): 8.27 (aliphatic amide, 1H), 7.3-8.1 (aromatic, 9H), 4.58 (aliphatic CH, 2H), 2.67 (methyl group, 3H). MS: M^+ 297. Anal. Calcd. for $\text{C}_{17}\text{H}_{15}\text{NO}_4$: C, 68.68; H, 5.09; N, 4.71. Found: C, 68.29; H, 4.99; N, 4.52.

Synthetic protocol for N-(4-(2-hydroxyphenyl)-2,4-dioxobutyl)benzamide (4)

2-acetylphenyl 2-benzamidoacetate (**3**) was dissolved in pyridine solution and stirred continuously for 6 hr in presence of powder KOH. The obtained precipitate was collected, washed thoroughly, dried suitably, and recrystallized [9].

59% yield; FTIR (KBr) ν (cm^{-1}): 3459 (-OH, stretch), 3322 (-NH, stretch), 3119 (C-H, aromatic), 1734 (C=O, stretch), 1655 (C=C, aromatic), 1603 (-NH, bending), 1288 (C-N, stretch); $^1\text{H NMR}$ (δ , ppm, CDCl_3): 8.19 (aliphatic amide, 1H), 6.7-8.1 (aromatic, 8H), 5.61 (hydroxyl group, 1H), 4.44 (aliphatic CH, 2H). MS: M^+ 297. Anal. Calcd. for $\text{C}_{17}\text{H}_{15}\text{NO}_4$: C, 68.68; H, 5.09; N, 4.71. Found: C, 68.47; H, 5.01; N, 4.66.

Synthetic protocol for N-((3-(2-hydroxyphenyl)-1H-pyrazol-5-yl)methyl)benzamide (6)

Hydrazine hydrate (**5**) (0.01 M) was dissolved in sodium hydroxide solution and the content was cooled to 10°C . N-(4-(2-hydroxyphenyl)-2,4-dioxobutyl)benzamide (**4**) (0.01 M) was added in small portions multiple times continuous stirring for an hour maintaining the temperature. In order to dissolve the inorganic salts, water was added further to the reaction mixture. The content was transferred to the separating funnel and shaken after addition of ether. The layers got differentiated and the aqueous layer was extracted with ether further three times. The ethereal extracts were washed with saturated brine solution, dried suitably, carbonate and ether was removed using a rotary evaporator. The obtained product was dried, additional purified by column chromatographic technique using silica gel and Hexane-ethyl acetate as mobile phase.

37% yield; FTIR (KBr) ν (cm^{-1}): 3439 (-OH), 3305 (-NH, stretch), 3124 (C-H, aromatic), 1740 (C=O, stretch), 1643 (C=C, aromatic), 1599 (-NH, bending), 1225 (C-N, stretch). $^1\text{H NMR}$ (δ , ppm, CDCl_3): 11.21 (heterocyclic amide, 1H), 8.32 (aliphatic amide, 1H), 7.0-8.3 (aromatic, 9H), 5.47 (hydroxyl group, 1H), 4.23 (aliphatic CH, 2H). MS: M^+ 293. Anal. Calcd. for $\text{C}_{17}\text{H}_{15}\text{N}_3\text{O}_2$: C, 69.61; H, 5.15; N, 14.33. Found: C, 69.37; H, 5.02; N, 14.19.

Acute toxicity studies

The purpose of the acute toxicity studies involved establishing a dose which will offer maximum therapeutic efficacy with high *in vivo* safety. The LD₅₀ was determined for the compound **6** in adult male albino rats. The determination involved injection of various gradually increasing doses of the experimental molecule in albino rats and the calculation of the dose based on 50% animal death. For the study, the dose was firstly chose from 25 mg/kg and increased steadily up to 500 mg/kg. [10].

Anti-inflammatory screening

The *in vivo* anti-inflammatory activity of the synthesized derivative was performed by the carrageenan-induced paw edema standard method. The rats were divided into 3 groups of 6 animals each; control, experimental molecule, and standard drug (indomethacin). In an order to lessen the inconsistency of edema, rats were fasted overnight. Before the experiment, 5 mL of water was administered orally per rat. The compound (100 mg/kg b.w.) was suspended in saline solution along with a few drops of Tween 80. The experimental drug was administered orally 1 h before the induction of inflammation. The control group received saline solution having a few drops of Tween 80. The inflammation was produced by injecting 1% carrageenan solution (prepared in saline) into the subplanter region of the right hind paw of rats via the subcutaneous route. The thickness of each rat paw was determined using mercury digital micrometer up to 3 hrs at an interval of 1 hr. The edema was measured from the disparity between the width of injected and non-injected paws. The obtained data were collected and expressed as mean \pm standard error [11].

RESULT AND DISCUSSION

Chemistry

The analytical analyses divulged the fundamental features of the synthesized pyrazole compound. The formation of intermediate **3** was confirmed by the disappearance of hydroxyl peaks in the IR range of 3300-3500 cm⁻¹. Further, the appearance of a prominent peak in the range of 1700-1750 cm⁻¹ implied the presence of two carbonyl moieties in the intermediate compound. The observation was supplementary confirmed by the ¹H-NMR data where no peaks were observed at ~5.5 ppm.

The appearance of the hydroxyl group stated the incorporation of acetophenone moiety with hippuric acid. Correspondingly, the intermediate **4** was confirmed by the re-appearance of hydroxyl group in the analyzed sample in the same range of 3300-3500 cm⁻¹ along with the existence of sharp carbonyl groups. Likewise, the ¹H-NMR indicated the emergence of hydroxyl peak at 5.61 ppm. Secondly, the disappearance of 3 methyl protons at 2.67 ppm also supported the Baker-Venkataraman rearrangement and transformation of intermediate **3** to compound **4**. Finally, the desired pyrazole compound demonstrated by the disappearance of sharp carbonyl groups in the range of 1650-1750 cm⁻¹ and prominent amide functions were seen after 3200 cm⁻¹. The ¹H-NMR highlighted the success of chemical reaction owing to the appearance of heterocyclic amide at 11.21 ppm. The aromatic protons were chiefly located in the range of 6.7-8.3 ppm of the spectra. At last, the mass spectra verified the formation of the intermediates and final pyrazole compound as specified by the base peaks of which exhibited close proximity with the actual molecular weight. Additionally, the appearance of fragment peaks of m/z 100-125 implied the possible fragmented products. The % practical estimation of elemental analyses of the intermediates and analogs was found to be in a very close conformity compared to theoretical values. Thus, the characterization data concluded the subsistence of the desired compound(s).

Determination of LD₅₀ value

The acute toxicity study revealed that the compound **6** was quite safe to be used over a large dose range of 25-500 mg/kg body weight as no sign(s) of toxicity or mortality were observed during the investigation range. The anti-inflammatory potential of the compound of interest was screened at a dose level of 100 mg/kg body weight based on the above experimental data.

Anti-inflammatory activity

The synthesized compound demonstrated an increase in anti-inflammatory activity from 1st hr to 3rd hr. The derivative **6** displayed 21.96% edema inhibition at first hr, 34.44% inhibition of edema at second hr, and 42.87% reduction in 3rd hr (Table 1). The activity was found to be quite comparable with that of standard drug

indomethacin, which presented anti-inflammatory activity of 38.63%, 46.37%, and 61.92%, respectively. The results showed that

the produced compound has the perspective to be utilized in inflammatory conditions and arthritis after suitable trails.

Table 1: Anti-inflammatory potential of the synthesized pyrazole derivative

Group	Dose (mg/Kg)	Percentage (%) inhibition of edema		
		1 hr	2 hr	3 hr
derivative 6	100	21.96 ± 3.57	34.44 ± 4.93	42.87 ± 4.16
Indomethacin	100	38.63 ± 2.86	46.37 ± 2.49	61.92 ± 2.62

n = 6; *ED*₅₀ of 100 mg/kg b.w. in male adult albino mice; *P* < 0.005

CONCLUSION

The research demonstrated a new method for the synthesis of pyrazole molecules from hippuric acid by a rearrangement reaction. The spectroscopic data revealed the possible structures of the synthesized compounds and characterization data were found to be in full agreement with that of the structures. The derivative **6** displayed 21.96% at first hr, 34.44% at second hr, and 42.87% at 3rd hr. The activity was found to be quite comparable with that of standard drug indomethacin. This study will certainly promote researchers in the rational synthesis of heterocyclic molecules with pronounced biological activity.

CONFLICT OF INTEREST

The authors confirmed that there is no conflict of interest for this research paper.

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Cite this article as:

Mrunali Digambar Amdare, Kamna Rajesh Jogdand, Latika Liladhar Kathane, Nutan Ganesh Kuhite, Chitralkha Deorao Padole, Debarshi Kar Mahapatra. Synthesis of a Potential Anti-Inflammatory Pyrazole Derivative from Hippuric Acid as the Starting Material. *J Pharm Chem Biol Sci* 2017; 5(3):216-220