Pharmacological Activities and One Pot Two Step Synthesis of Novel (E)-N-(1-methyl-4-oxoimidazolidin-2-ylidene)benzamide

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Abstract: The aim of the present work was to synthesize a compound from biological waste product i-e Creatinine and to develop an easy way of synthesis unlike the conventional methods which is time consuming and requires large amount of solvent along with great deal of efforts and to explore its pharmacological importance on scientific ground. The title compound showed highly significant analgesic activity in animal model at both parameters, In acetic acid induced writhing test the result were highly significant i-e 92.03% (P<0.001) while in hotplate method the compound showed 86.11% (P<0.001) activity at 30 mg/kg body weight against Diclofenac Sodium and Pentazocine used as standard respectively. For the toxicity study, the compound was orally administered to the Swiss albino mice according to the OECD guideline 423. There was no lethality or toxic symptoms observed for all the tested doses throughout the 14-day period.

Key words: Analgesic - Benzamide - Creatinine

INTRODUCTION

Most of the synthetic and natural compounds contains amide. They have their activities due to amide bond [1]. About 25% of available drugs contain amid linkages [2] such as, ritonavir [3], anti-bacterial [4] and anti-tubercular [5]. In 1832, benzamide were first discover by Liebig and Woehler [6]. Numerous benzamide derivatives were screened for different pharmacological activities like antiemetic, anti-ulcer, prokinetics [7], anti-nepathic pain [8], analgesic, antipytetic, anti-inflammatory [9], anti-tuberculosis, antibacterial, antifungal [10] 5-HT4 agonist [11], congestive obstructive pulmonary disease [12] HDAC-inhibitory activity [13] and gastro esophageal reflux disease (GERD) [14]. In some of the derivatives EPS (extrapyramidal syndrome) side effect was observed, Lien et al. have proposed a model which can separate coplanar hydrophobic region and positively charged tertiary nitrogen (N+) from a more or less by a three carbon distance to associate EPS with the structures of various bytoufenone, benzamide and phenothiazine derivatives. Since then, many new benzamide derivatives have been designed tested and marketed by several pharmaceutical companies [15].

Benzamides are reported for analgesic, antimicrobial, antitumor and antioxidant potentials herein we report the synthesis, analgesic and acute toxicity, screening of Novel (E)-N-(1-methyl-4-oxoimidazolidin-2-ylidene)benzamide derivative. The benzamide was synthetized by stirring equimolar quantities of potassium thiocyanates, benzoil chloride to produce isothiocyanates. Followed by addition of creatinine in equimolar quantity, using acetone as solvent at 40 ºC (Scheme 1).
MATERIALS AND METHODS

Chemistry: All chemicals and solvents were brands of Merck and Sigma Aldrich. On Gallan Kamp (Electrothermal/Barnstead) melting point (mp) apparatus mp of the compound was determined in open capillaries and are uncorrected. Thin-layer chromatography (TLC) on Merck silica gel 60 F254 aluminum sheets (Merck; Darmstadt, Germany) was used to monitor the reaction progress, using ethyle acetate and n-hexane (1:1) as solvent system and observed under UV light (254/365 nm). Bruker AV spectrometer was used for recording H1 NMR and C13NMR spectra at 300 MHz and 75 MHz, in CDCl3 as a solvent and internal standard tetramethylsilane (TMS) was used. Chemical shift values (δ) were mentioned in ppm. FTS 3000 MX, Bio-Rad Merlin Fourier Transform Infra Red spectrophotometer was used to record IR spectra. Using KBr pellets for recording spectra.

General Procedure for the Synthesis of (E)-N-(1-methyl-4-oxoimidazolidin-2-ylidene) Benzamide: The compound was synthesized by reacting 5 mmol benzoyl chloride solution in 30 ml acetone add dropwise to 5 mmol of potassium thiocyanate solution in 15 ml acetone. The potassium chloride was filtered off from benzoyl isothiocyanate which was then treated with 5 mmol solid creatinine to afford the desired benzamide derivative. The reaction progress was monitored on thin layer chromatography (TLC), after completion of reaction the mixture was left to cool and filtered. The filtrate was evaporated under reduce pressure and was crystallized in absolute ethanol.

The chemical formula of the compound is C11H11N2O2

(E)-N-(1-methyl-4-oxoimidazolidin-2-ylidene) Benzamide Orange crystalline solid, Yield 90%, mp 289 ºC, Rf 0.5.1H NMR (300 MHz, CDCl3), δ: 11.03 (s, 1H, =N-H), 8.29 (m, 2H, ArH) and 7.50 (m, 3H, ArH), 3.98 (s, 2H, =C-H2) and 3.29 (s, 3H, =C-H3);

13C NMR (75 MHz, DMSO), δ: 30.94 (N-CH3), 51.75 (N-CH3), 128.56 (ArC), 129.61 (ArC), 132.26 (ArC), 137.46 (ArC attached to Carbonyl group), 158.82 (C attached to three Nitrogen), 172.27(C=O), 174.72(Ar-C=O);

IR (KBR) 1567.3 (C=N), 1604.0 (C=O, amide), 1754.2 (C=O, carbonyl) and 3310.7 (N-H, secondary amine)

Study Design and Pharmacological Screening

Analgesic Activities: The analgesic potentials of the benzamide derivative were determined by two different methods:

- Peripheral analgesic screening
- Central analgesic screening

Peripheral Analgesic Screening: The mice were divided into four separate groups (AI-AIV) of six mice each. AI was kept as reference/control group. All received the diclofenac sodium 50 mg/kg body weight and was kept as standard. Benzamide derivative was administered to AIII group at 15mg/kg of body weight and AIV was administered at 30 mg/kg body weight. The last two groups were kept as sample groups. Acetic acid 0.6 % was injected intra peritoneal after 15 minutes of administration of drugs to the mice and were placed on plate surface. The mice were observed after 3 minutes of acetic acid injection for 30 minutes continuously. Total number of writhes were counted of each mice. The percent inhibition of analgesia was calculated by the following formula [16,17].

The percent analgesic activity = {(S1 – S2) ÷ S1} × 100
Whereas S1 is control group, S2 is test group

Central Analgesic Screening: The mice were divided into four separate groups (TI-TIV) of six mice each. TI was kept as reference/control group and was given 0.5 % W/V Tween80 2 ml/kg body weight in normal saline. TII received the pentazocine 10 mg/kg body weight and was kept as standard. TIII was administered with benzamide derivative at 15mg/kg of body weight and TIV was administered at 30 mg/kg body weight. The last two
groups were kept as sample groups and the mice were observed for tail withdrawal effects from hot water at subsequent reaction times i-e 15, 30, 45, 60, 75 and 90 minutes respectively. The mean reaction time of standard and sample group was compared with reference group and percent nociceptive activity was calculated using the following formula:

\[
\text{The percent analgesic activity} = \left(\frac{S1 - S2}{S1}\right) \times 100
\]

Where S2 pretreatment reactions time and the S1 post treatment reactions time

**Acute Toxicity:** The acute toxic effects of benzamide derivative was determined by using Lorke (1983) procedure with slight modification were mice of either sex were divided into three groups i-e ATI-ATIII containing six animals each and benzamide derivative was administered at a dose of 50, 75 and 150 mg/kg body weight in the 1st stage. At 2nd stage group ATIV and ATV were administered a dose of 200 and 300 mg/kg body weight. In stage 3rd group ATVI and ATVII were given a dose of 500 and 750 mg/kg body weight. In each and every stage of study the animal were observed for one day to calculate LD₅₀ of compound, (the concentration at which half animals are killed in a group) [16].

**RESULTS AND DISCUSSION**

**Analgesic Activities**

**Peripheral Analgesic Screening:** The peripheral analgesic screening was carried out for the compound using acetic acid inducing writhing assay in mice and results were presented as “mean increase in latency after drug administration ± SEM” comparative to control and percent reduction in writhing reflexes.

Writhing test is a chemical technique for pain induction of peripheral origin in mice by injecting irritant substance to animals such as acetic acid. The peritoneal receptors are considered to be partially involved in the contraction response of abdomen [20-23] and is proposed to be related with prostanoids overall, e.g. lipooxygenase [22] along with prostaglandin E₂ and prostaglandin E₂ are observed in high concentration in peritoneal fluid [23]. The writhing assay are not assessed in human and assumed to be a reflexive test with no clinical complements. Writhing assay is of moral concern because it produces severe pain in animals [24]. Acetic acid have irritant property which cause concentrated pain and result in episodic retraction of abdomen and hind limb stretching. It also triggers the CNS to release prostaglandin which inturn maximize the nociceptors sensitivity [25,33]. Substance that increase threshold to noxious or external painful stimuli by reducing pain sensation generated by thermal, physical, pressure and chemical method are known as analgesic [26]. NSAIDs play key role in treatment of inflammation condition like soft tissue lesions, rheumatoid arthritis, respiratory infection, fever and oral cavity lesion which are assumed to be the indirect action of acetic acid [27,28]. Diclofenac sodium is a commonly used NSAID usually in treatment/management of ankylosing spondylitis, rheumatoid arthritis and osteo-arthritis [29,30]. Because of its anti-inflammatory and analgesic properties [29] by inhibiting prostaglandins production, diclofenac reduces arthritic pain, inflammation and swelling [30,31].

Due to above mentioned importance we had screen the benzamide derivative for its analgesic activity and the recorded data is tabulated in Table 1. The benzamide derivative showed 87.58%(P<0.001) and 92.03%(P<0.001) at 15 mg/kg and 30 mg/kg body weight which is highly significant activity at both doses, but slightly lower than Diclofenac sodium which was 93.98% (P<0.001) at 50 mg/kg body weight. It suggests that the activity is due to the amide linkage and phenyl ring, [32] like phenacetin and paracetamole.

Further, benzamide derivative should be subjected for further analysis to its pharmacological significance. The result from the observed data support that the benzamide derivative may be inhibitor of cyclooxygenase/lipoxygenase and by decreasing the production of prostaglandin E₂ may be because of its interaction with transduction mechanism in primary afferent nociceptors.

**Tail immersion Test (Central):** Pain and inflammation are associated with many clinical condition of pathophysiology, for example vascular diseases, cancer and arthritis [34]. The tail immersion are highly accepted method for opiate analgesic discoveries along with spinal origin analgesic drugs [18,22]. In animals models the compound presented significant to highly significant results at different doses towards the thermal stimulus, at 30mg/kg body weight the compound almost showed similar activity to standard drug after 60min of administration at which point the response time increased from 0.88 second to 1.34 seconds at 15mg/kg body weight the compound did showed significant result which are tabulated in table 2.
Table 1: Peripheral analgesic assay

<table>
<thead>
<tr>
<th>Test compounds</th>
<th>Dose mg/kg B.W.</th>
<th>Mean writhing ±SEM</th>
<th>% Analgesic Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tween80</td>
<td></td>
<td>52.33±0.422</td>
<td>0.00</td>
</tr>
<tr>
<td>Diclofenac Sod. (STD)</td>
<td>50</td>
<td>3.67±0.494***</td>
<td>92.98</td>
</tr>
<tr>
<td>CT</td>
<td>Dose 1</td>
<td>15</td>
<td>6.50±0.428***</td>
</tr>
<tr>
<td></td>
<td>Dose 2</td>
<td>30</td>
<td>4.17±0.307***</td>
</tr>
</tbody>
</table>

Table 2: Tail immersion data of the compound

<table>
<thead>
<tr>
<th>Reaction time in seconds ±SEM</th>
<th>% Analgesic Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>CT</td>
<td>CT</td>
</tr>
<tr>
<td>Time interval</td>
<td>15mg/kg</td>
</tr>
<tr>
<td>15min</td>
<td>0.65 ± 0.084</td>
</tr>
<tr>
<td>30min</td>
<td>0.70 ± 0.113</td>
</tr>
<tr>
<td>45min</td>
<td>0.70 ± 0.101</td>
</tr>
<tr>
<td>60min</td>
<td>0.72 ± 0.109</td>
</tr>
<tr>
<td>75min</td>
<td>0.69 ± 0.108</td>
</tr>
<tr>
<td>90min</td>
<td>0.69 ± 0.126</td>
</tr>
</tbody>
</table>

All values are expressed as Mean±SEM (n=6), * non-significant P >0.05
* Significant P <0.05, ** more significant P <0.01, *** highly significant P<0.001

Table 3: Acute toxicity data of synthesized compounds

<table>
<thead>
<tr>
<th>Sample</th>
<th>Stages</th>
<th>Dose mg/Kg</th>
<th>Animals died</th>
<th>LD₅₀</th>
</tr>
</thead>
<tbody>
<tr>
<td>CT</td>
<td>Stage-I</td>
<td>50</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>75</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>100</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Stage-II</td>
<td>15</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>200</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Stage-III</td>
<td>500</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>750</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

The compound was tested upto 750mg/kg body weight and was found safe.

Fig. 1: Tail immersion response
All the observed data was statically analyzed using One-way ANOVA followed by Dunnett’s test. Which revealed that at 15 mg/kg body weight, the compound presented 76.39% activity $P<0.01$ more significant results and at 30 mg/kg, the compound presented 86.11% activity $P<0.001$ highly significant results compared to pentazocine, standard drug, 90.28% activity $P<0.001$ highly significant at 60 minutes respectively.

The results presented that the benzamide derivative had highest activity and comparable or slightly less than that of pentazocine. Nevertheless, it’s worth to mention that the benzamide derivative had excellent central analgesic effects. Which are graphically represented in Fig 1.

**Acute Toxicity:** The compound was tested for possible acute toxic effects and the results are tabulated in the Table 3.

**CONCLUSIONS**

(E)-N-(1-methyl-4-oxoimidazolidin-2-ylidene) benzamide was characterized by different spectroscopic methods i-e $^1$HNMR, $^{13}$CNMR and IR spectroscopy which confirmed the hydrogen and carbon skeleton of the compound respectively whereas the IR spectroscopy confirmed the bond stretch/ functional group of the title compound.

The compound has demonstrated encouraging analgesic activity at both parameters in animal model of nociception. Our present work also revealed that the compound is safe for consumption with high LD$_{50}$ value. This study requires further attention to investigate the mechanism of action of (E)-N-(1-methyl-4-oxoimidazolidin-2-ylidene) benzamide and its role in the pain inhibitory mechanisms of peripheral and central nervous system.

**ACKNOWLEDGEMENTS**

The authors are great to University Of Malakand Chakdara, Lower Dir, KPK, Pakistan for making the resources available to carry out this research work.

**REFERENCES**


