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Research Article

SYNTHESIS AND PHARMACOLOGICAL EVALUATION OF NOVEL BENZOYL DERIVATIVES OF PIPERIDINE-4-CARBOXAMIDE

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ABSTRACT

In this research program, the synthesis and pharmacological evaluation of two novel derivatives of Piperidine-4-carboxamide, 1-(3,5-dinitrobenzoyl)piperidine-4-carboxamide (**I**) and 1-(3,4,5-trimethoxybenzoyl)piperidine-4-carboxamide (**II**) were reported. They were synthesized by condensing the parent molecule with substituted benzoyl chlorides. The structural elucidation of these newly synthesized derivatives was performed using UV Visible, IR, ^{1}HNMR, EIMS. The products were then assessed on different pharmacological parameters such as analgesic, antimicrobial, antioxidant, and anxiolytic activities. It was observed that compound **II** displayed good analgesic profile. Both compounds possessed least to moderate antibacterial effects against tested strains of gram positive and gram negative bacteria. Compound **I** expressed moderate antifungal activity against some filamentous fungi and yeast while compound **II** possessed least activity for fungi only. Both compounds were inactive as antioxidants and also failed to produce remarkable change in behavior at the dose 50mg/Kg body weight. SAR was also established.

Keywords: Piperidine-4-carboxamide, Antimicrobial activity, Analgesic activity, Antioxidant activity.

INTRODUCTION

Piperidine is naturallyobtained from *Piper nigrum* L.[1], different other methods have also been reported for its synthesis[2]. The structure is the building block of many important bioactive molecules such as morphine and scopolamine[3].Researchers have notified that the substituted piperidine derivatives possessed a broad spectrum of pharmacological applications. The synthetic analogues were found active against different bacterial and fungal strains[4-5]. Different studies have also reported the derivatives with interesting antinociceptive and antidepressant activities[6-8].

In the current decade, numerous of the piperidine derivatives have been synthesized by our research fellows with diverse biological activities[9-11]. In continuation of our goal, the research work is aspired to synthesize two more hybrids of Piperidine-4-carboxamideand evaluated their pharmacological potential.

MATERIALS AND METHODS

Reagents and Chemicals: Piperidine-4-carboxamide, 3,5-dinitrobenzoylchloride, 3,4,5-trimethoxybenzoylchloride were purchased from Sigma Aldrich, London. Analytical grade acetone, ethanol and hexane were obtained from E. Merck and distilled prior to use to ensure extra purity.TLC plates of E. Merck's precoated silica gel GF-254 were also used.

Instruments for Structural elucidation

Hitachi U-3200 spectrophotometer was used to record the Ultraviolet (UV) spectra of the synthesized compounds of in methanol. IR spectra were measured on Shimadzu IR 460

spectrophotometer using KBr disc. Electron Impact Mass spectra (EIMS) were determined on Massen spectrophotometer MAT 311A.Proton Nuclear magnetic resonance (^{1}HNMR) spectra were recorded in MeOD on AVANCE AV 500 spectrometer, operating at 500MHz. Chemical shifts (ppm), multiplicity (s=singlet, d=doublet, t=triplet, m=multiplet), coupling constant *J* (Hz), number of protons (1H=one proton, 2H= two protons, 3H= three protons) were used to report the data.

Experimental Animals

20-30g male albino mice were purchased from Aga Khan University and Hospital, Karachi and housed for about three days in the same environmental condition with free access to water and standard rodent diet.

Treatment of Mice

Test compounds **I**, **II** and Pethidine HCl (dissolved in Water for Injection) were administered intraperitoneally to the test and standard mice respectively at the dose of 50mg/Kg body weight. Control group receiving only vehicle always run parallel to the test groups.

The study was performed according to the guidelines provided by Committee on Animal Research and Ethics (CARE) (https://www.apa.org/science/leadership/care/care-animal-guidelines.pdf)

General procedure for Synthesis of Compounds I and II

Equimolar quantities of Piperdine-4-caroboxamide **1** and substituted benzoyl chlorides were dissolved in acetone and then mixed in a separate flask with constant heating and stirring for 4-5 hrs **(Scheme 1)**. The reaction response was observed via TLC plates using a solvent system of $CHCl_3$:MeOH. Gravity filtration was used to obtain the precipitates of the products which were then washed with hot acetone, recrystallized with methanol and kept in vacuum desicator for drying.

Pharmacological Evaluation of the synthesized compounds

Determination of Analgesic Activity

The compounds were tested for their antinociceptive effect against thermal stimuli (tail flick method) according to the method of Di Stasi et al.[12].Briefly, mice were held in a suitable restrainer with whole tail extending out. A 2-3cm marked area of the tail was immersed in a water bath (51°C). The animals were noted for pre-drug and post drug latency time for a test period of 180 min. Tail Flick latency difference (TFLD) was used to measure the analgesia produced by test and standard drugs and calculated as:

Analgesia TFLD =
(post drug TFL – pre drug TFL)

Statistical Analysis

Analgesic activity was expressed as TFLD±SEM in term of seconds.

Determination of Behavioral activity (Open field test)

In the open field apparatus, a square area of 24×24cm with walls 14cm high, lines dividing the floor into 25 equal squares, mice were observed for 5mins to determine the number of square crossing with all four paws after 30min of receiving injection[13].

Determination of Antimicrobial activity (Ivitro)

The evaluation of anti-microbial activity was based on the disc diffusion method[14]**.** Dried discs of Whattman containing 10µL of the test sample were used for measuring the zones of inhibition in mm. Blank discs containing DMSO served as control. Plates were then incubated at 37°C for 24hrs.

Determination of Antioxidant activity

Method reported by Lee et al. was used to determine the antioxidant activity of the synthesized derivatives[15]**.** Reaction mixture containing 10µL of the test sample prepared in DMSO and 90µL of 1,1-diphenyl-2-picrylhydrazyl (DPPH) prepared in ethanol were added in 96-wellµL plates (final concentrations of samples were 200µg/mL and that of DPPH was 300µM), and incubated at 37°C for 30mins. Absorbance was measured at 515nm using spectrophotometer Percent inhibition of samples was determined by comparison with control group as:

$$\% \text{ inhibition} = \frac{\text{absorbance of the control} - \text{absorbance of the sample} \times 100}{\text{Absorbance of the control}}$$

Ascorbic acid was used as standard control. The calculated EC_{50} value was used to represent the concentration of sample required to scavenge 50% of DPPH.

RESULT

Physical and structural information of the two newly synthesized derivatives were provided in Table **1**. Figure **1** was showing shown the observed analgesia in seconds (±SEM).The results of antibacterial, antifungal and antioxidant screening were presented in Table **2,3** and **4** respectively. Table **5** was reporting the behavioural study of the two compounds.

DISCUSSION

Chemistry

The UV spectra of compounds **I** and **II** exhibited λ_{max} value at 255nm and 256nm respectively. The IR spectra of both the derivatives displayed absorption bands at 3382-3384cm^{-1}indicating the presence of NH amide ($CONH_2$) while the carbonyl linkage(C=O) was appeared at around 1667-1670cm^{-1}. Peaks around 1625-1628cm^{-1},1416-1430cm^{-1} and 2939-2950cm^{-1}confirmed the stretching bands of C=C, $CH_2$and C-H of aromatic rings. CN bands were found at 1130-1186cm^{-1}.

EIMS of the synthesized products **I** and **II** showed M^+ at 322.0 suggesting a molecular formula of $[C_{13}H_{14}N_4O_6]$ and $[C_{16}H_{22}N_2O_5]$ respectively.

The ^{1}H-NMR spectra of both the derivatives showed protons of amine group at δ6.0ppm. In both the spectra, protons of piperidine ring appeared at different positions; proton of $C_1$was found at δ 2.5-2.59ppm, protons of $C_2$and C_3 were present at δ1.80-1.90ppm and δ2.009-2.039ppm respectively. C_5 and C_6 protons showed their presence at δ3.02-3.08 and 3.40-3.44ppmrespectively. ^{1}H-NMR spectrum of compound **I** exhibited signals at δ8.652-8.656 and 9.048-9.075ppm corresponds to C_7, C_8 and C_9 of phenyl ring. In compound **II**, C_7, C_8protons of phenyl ring were observed at δ6.60ppm. Spectrum of the same compound possessed protons of methoxy groups around δ3.78-3.852ppm.

Pharmacological Screening

Analgesic Activity

Pain is a question not yet answered by remedy. According to WHO, “90% of diseases are associated with pain”[16]. Today, the study of new chemical moieties that are effective in the management of pain is one of the vital objectives. The traditional curative strategy for pain comprises of non steroidal anti-inflammatory drugs (NSADs) and opioids, both are associated with serious side effects[17].

Figure**1** showed the results of tail flick latency after drug administration. It is evident that compound **I** at dose of 50mg/Kg body weight did not produce any significant analgesia throughout the experiment. Compound **II** showed significant nociceptive effects with early onset of action having TFLD value of 1.89±0.374, reaching to a maximum at 60min (TFLD value of 2.864±0.380) and highly significant effect persisted up to 150min. Similar effect was observed for treating the mice with Pethidine HCl at 50mg/kg which served as a positive control of the experiment.

The SAR reveals that the two compounds differ in substitutents attached to benzene ring at different positions. Compound **I** has two nitro groups at meta position and it was found devoid of analgesia. Compound **II** with pronounced analgesic activity has three methoxy groups at meta and para positions. Therefore, it might be suggested that the methoxy group was responsible for analgesia and not the nitro group.

Invitro Antimicrobial Evaluation

The problem of antibiotic resistance has become a worldwide concern and highly demands the emergence of new novel antimicrobial agents[18].

According to table**2**, it can be observed that both the tested compounds **I** and **II** were evaluated as moderately active against gram positive bacteria *B. subtillus* and *B. cereus* while they were inactive against *S. aureua* and *S. epidermidis*. The compounds also exhibited least to moderate antibacterial effects for gram negative species *E.coli, S. typhi* and *P. aeroginosa*. Therefore, it is concluded that the antibacterial profile of the two newly synthesized molecules reflected almost a similar pattern, indicating that the attachment of nitro group in derivative **I** and methoxy group in derivative **II** might be accountable for the equivalent activity.

According totable **3**, it can be seen that among filamentous fungi compound **I** showed moderate activity while compound **II** displayed least activity against *A. niger* and *rhizopus specie*whereas no activity was observed against *A. flavus* and *penicillium specie*. Only compound **I** possessed activity against yeast *C. albicans* and *S. cervaciae*. These findings suggested that in compound **I** dinitro groups were responsible to produce least to moderate activity against some species of filamentous fungi and yeast while trimethoxy groups in compound **II** were not showing promising activity against filamentous fungi and yeast.

Antioxidant Activity

Free radicals with odd unpaired electron are associated with number of serious disorders. The substance acting as free radical scavengers are called antioxidants. These antioxidants combat oxidative substances and protect the body from damaging by other free radicals [19].

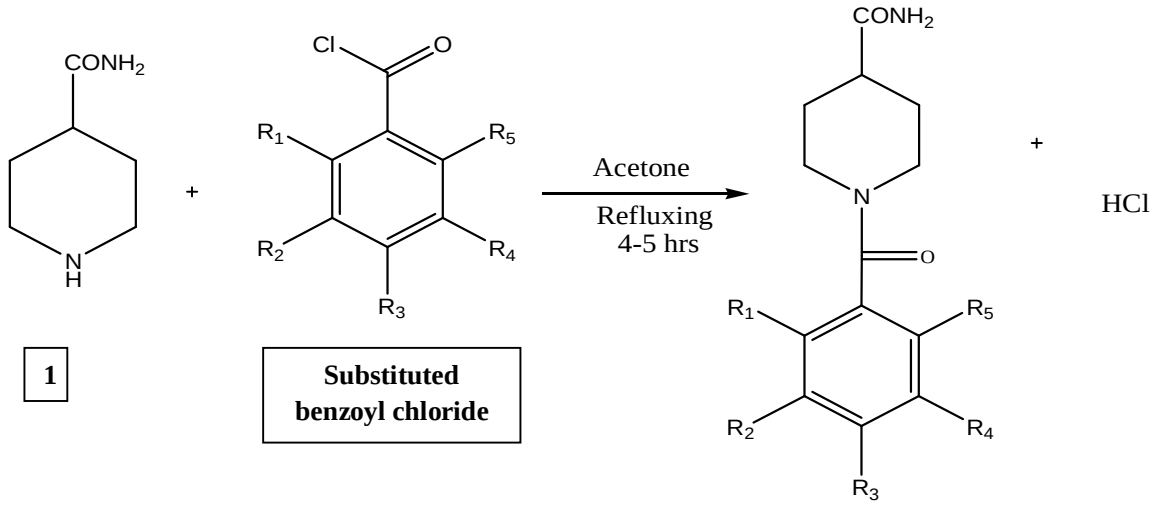
The results shown in Table **3** indicated that the synthesized derivatives **I** and **II** did not show noteworthy %inhibition and therefore it can be predicted that both dinitro and trimethoxy substitutions are devoid of antioxidant activity by this method.

Behavioural Assessment

Table **5** provides the number of squares crossed during open field test. When the synthesized compounds were tested at doses of 50mg/kg, they did not exhibit pronounce motor activity or did not have any remarkable change in exploratory activity throughout the experiment. From the observed results it might be predicted that dinitro group in compound **I** and trimethoxy group in compound **II** did not produce significant behaviour change.

CONCLUSION

Drug design is a part of medicinal chemistry. On the basis of some positive results, it is proposed that further work should be carried out on both of these compounds (**I** and **II**) to explore the new molecules with enhanced therapeutic effects in future. Negative results do not mean that these compounds are totally ineffective or useless rather they need further exploitation by different methods at other doses.

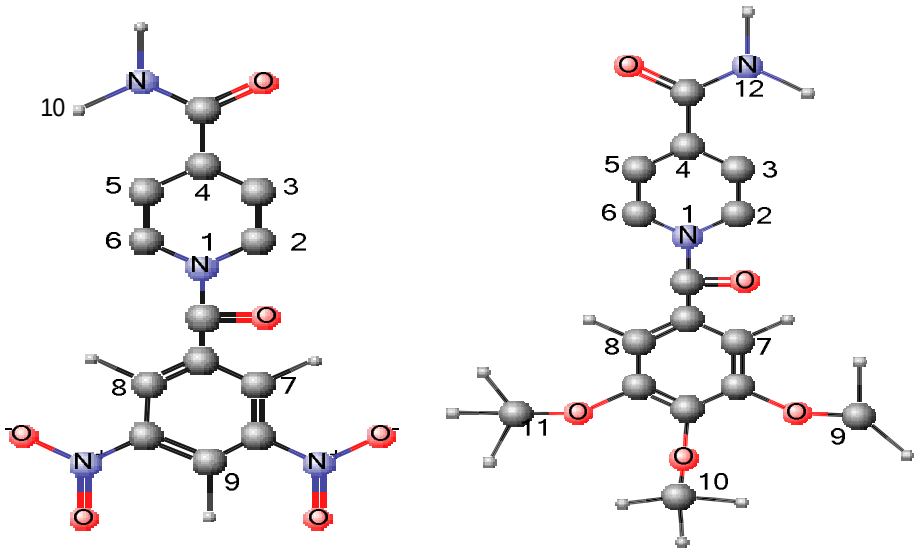


1

Substituted benzoyl chloride

I; R_1, R_3, R_5 = H, R_2, R_4 = NO_2
II; R_1,R_5 = H, R_2, R_3, R_4 = OCH_3

I-II



I II

Scheme. 1: Synthesis of Piperidine-4-carboxamide derivatives

Table 1: Physical and Structural Data of the synthesized derivatives I-II

Compounds	State	M.P. (°C)	UV λ_{max} (nm)	IR υ_{max} (cm^{-1})	EI-MS M^+	H^1NMR δ (ppm)
1-(3,5-dinitrobenzoyl)piperidine-4-carboxamide	White crystalline powder	154±2	255	3384.9($CONH_2$), 2948.9 (C-H), 1667.0(C=O), 1625.8(C=C), 1430.9(CH_2), 1186.3(C-N),	322 [$C_{13}H_{14}N_4O_6$]	1.80-1.90(m, 2H, H-2), 2.009-2.039 (dd, 2H, H-3, *J*=15), 2.5-2.59 (m, 1H, H-4), 3.02-3.08 (m, 2H, H-5), 3.40-3.44 (m, 2H, H-6), 8.652-8.656(d, *J*=2, 2H, H-7, H-8), 9.048-9.057 (t, 1H, H-9, *J*=5), 6.0 (s, 2H, H-10)
1-(3,4,5-trimethoxybenzoyl)piperidine-4-carboxamide	White to offwhite crystals	140±1	256	3382.7($CONH_2$), 2939.7(C-H), 1670.07(C=O), 1627.9(C=C), 1416.0(CH_2), 1130.6(C-N),	322 [$C_{16}H_{22}N_2O_5$]	1.825-1.90(m, 2H, H-2), 2.009-2.039 (dd, 2H, H-3, *J*=15), 2.5-2.59 (m, 1H, H-4), 3.02-3.05 (m, 2H, H-5), 3.40-3.41 (m, 2H, H-6), 6.69 (s, 2H, H-7, H-8), 3.852(s, 6H, H-9, H-11, $ArOCH_3$), 3.78 (s, 3H, H-10, $ArOCH_3$ 6.0 (s, 2H, H-12)

Table 2: In vitro Antibacterial Screening of compounds I & II

Bacteria	Zone of inhibition (mm)	
Gram +ve strains	**Compound I**	**Compound II**
Bacillus subtillus	10	10
Bacilus cereus	10	10
Staphylococcus aureus	--	-
Staphylococcus epidermidis	-	-
Gram -ve strains		
E.coli	9	10
Salmonella typhi	10	9
Pseudomonas aeroginosa	9	9

9 = least activity
10 = moderate activity
- = No activity

Table 3: In vitro Antifungal Screening of compounds I & II

	Zone of inhibition (mm)	
	Compound I	**Compound II**
Filamentous fungi		
Aspergillus niger	10	9
Aspergillus flavor	-	-
Rhizopus specie	10	9
Penicillium specie	-	-
Yeast		
Candida albicans	10	-
Sacharomyces cervaciae	10	-

9 = least activity
10 = moderate activity
- = No activity

Table 4: Anti-oxidant activity of compounds I & II in terms of %inhibition

Compound	% inhibition
Piperidine-4-carboxamide	19%
I	17%
II	-

Table 5: Open field Activity of compounds I-II

Compound	Mean number of squares ± SEM
I	88.2 ± 3.045
II	84.4 ± 2.987

Significant difference by Student'test: *p<0.05 and **p<0.01

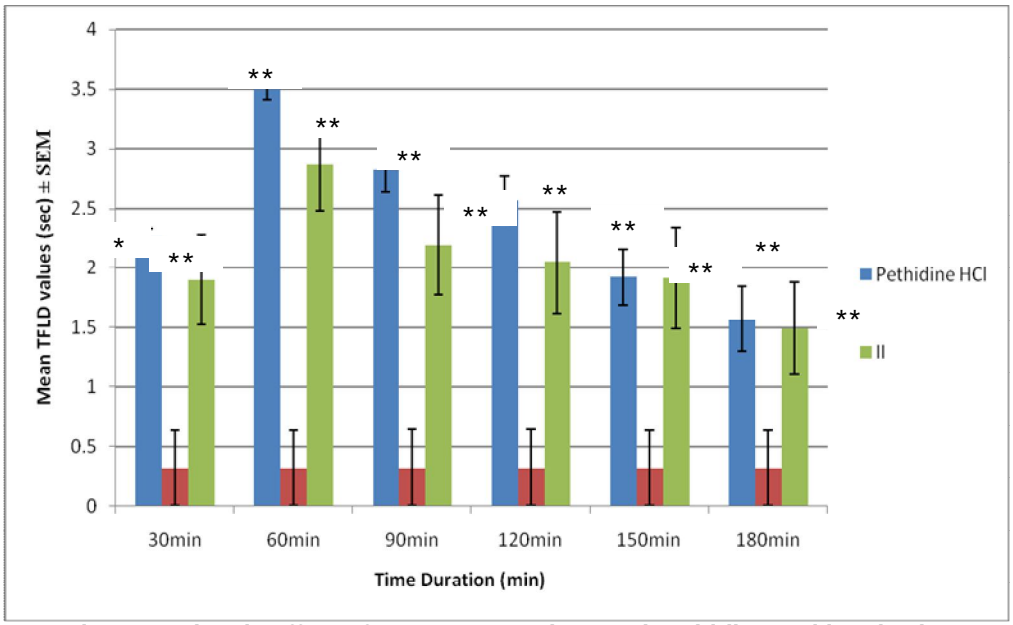


Fig. 1: Analgesic effect of test compound I, II and Pethidine HCl in mice by tail immersion method

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