

Synthesis, characterization and biological evaluation of piperidine-4-carboxamide derivatives in mice.

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Abstract—Present work comprises of synthesis of sulfonamide and amide derivatives of piperidine-4-carboxamide via amino-dechlorination and amino-de-alkoxylation reaction. Structure of the analogues was confirmed by different techniques i.e. IR and ^1H NMR. Piperidine derivatives had found to be potent dopamine reuptake inhibitor. Synthesized derivatives were also shown to relieve pain and to achieve analgesia in mice. The antibacterial activity of the derivatives was also assessed with the parent against a series of Gram-positive and Gram-negative bacteria. The synthesized compounds showed diverse antimicrobial profile among which most compounds possessed a comparable or better activity in comparison to the parent.

Index Terms— Piperidine-4-carboxamide, dopamine reuptake inhibitor, analgesia, antibacterial activity.

1 INTRODUCTION

PIPERIDINE alkaloids constitute a large family of compounds many of which exhibit a wide range of physiological activities [1],[2]. These physiological activities of many substituted piperidines prompted the scientists to design simple methods for the synthesis of piperidine carboxamide derivatives.

Analgesic and anti-inflammatory drugs are the most common products used in many of disease for relief of pain and inflammation. It has been found very difficult to treat chronic pain using the treatments currently available. Therefore, the development of new analgesics has always been one of the main aim [3]. Some 3-phenyl piperidine derivatives have shown significant analgesic activities. Some 1-amidino-3-amino-2-hydroxy piperidine derivatives were reported which possessed antiviral activities [4]. Advances in piperidine field are likely to provide better compounds capable of dealing with the resistant strains. These research efforts have been rewarded by very significant improvements in antibacterial potency as well as *in vivo* efficacy. In the light of above mentioned briefly discussed research findings we prompted to prepare sulfonamide and amide derivative from piperidine molecule, by treating piperidine-4-carboxamide with 4-nitrobenzene sulfonyl chloride and methyl propionate respectively in order to assess the biological efficacy and behavioral activity. The present study enlightens the synthesis, spectroscopic analysis (including IR and ^1H NMR), and evaluation of behavioral and biological activities of piperidine-4-carboxamide derivatives in mice.

2 EXPERIMENTAL

2.1 Materials And Methods

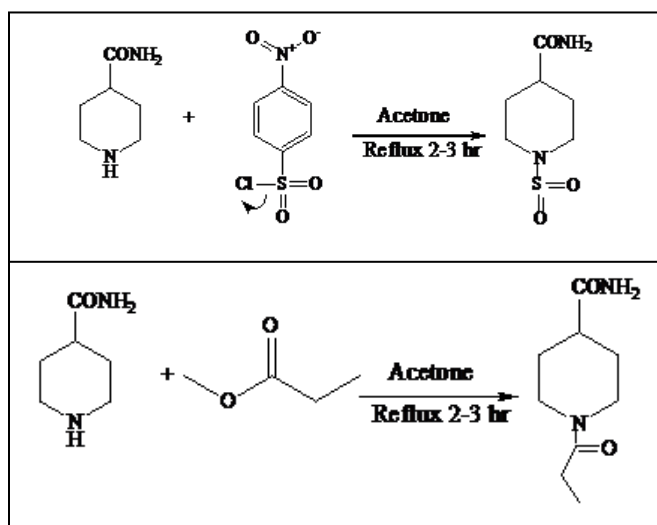
Reactions were monitored by TLC using pre-coated silica gel, GF-254 and were visualized under ultraviolet light at 254nm and 360nm on HP UVIS Desaga (Heidelberg). Silica gel G 60 (0.040-0.063 mm) was also used for preparing analytical thin layer plates.

All melting points were recorded on Gallenkamp melting point apparatus and were are uncorrected. Solid calcium sulphate (anhydrous) from E. Merck was used for drying methanol, ethanol and DMSO. U.V spectra were taken on spectrophotometer. Infra Red (IR) spectra were measured on a IR 460 spectrophotometer using KBr disc. Electron Impact Mass spectra (EIMS) were determined on Varain massen spectrometer. Proton Nuclear magnetic resonance (^1H NMR) spectra were recorded in d_6 -DMSO on Bruker AM-300 spectrometer operating at 300 MHz. Chemical shifts (δ) were reported in parts per million (ppm) and coupling constant J in Hertz (Hz).

2.2 General Procedure For Preparation Of Derivatives

Piperidine-4-carboxamide, 4-nitrobenzene sulfonyl chloride and methyl propionate were dissolved in acetone in equimolar quantities separately in conical flasks and then mixed together in a round bottom flask. The reaction mixtures were stirred by magnetic stirrer and refluxed for about 4 to 5 hours. Completion of the reactions were monitored by TLC with CHCl_3 -MeOH (in varying proportions) system. The resulting precipitates of products were filtered and washed with warm acetone to remove the unreacted starting materials. The products thus obtained were recrystallized with pure ethanol. The pure

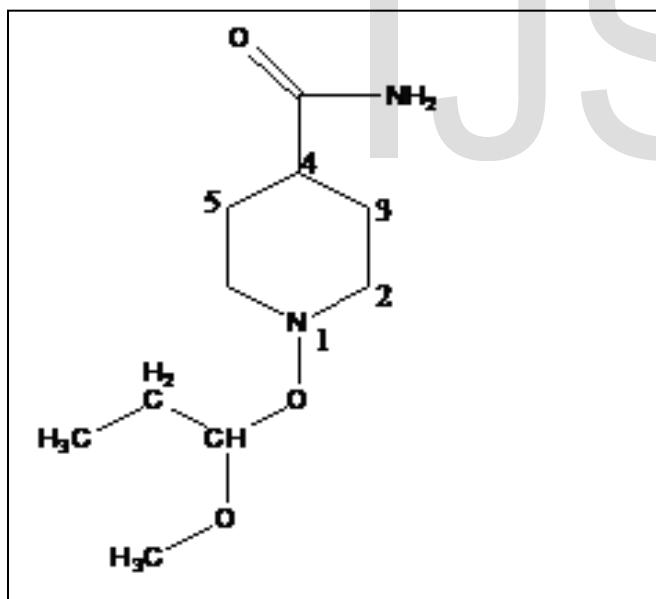
compounds were dried in vacuum desiccator over anhydrous calcium sulphate.



2.3 Characterization of synthesized compounds and Physical Data

The structures of synthesized compounds were elucidated by UV, IR, EIMS and Proton NMR spectral techniques. The spectral and physical data are given as under.

1-(1-Methoxy-propoxy)-piperidine-4-Carboxylic acid amide (SS2)



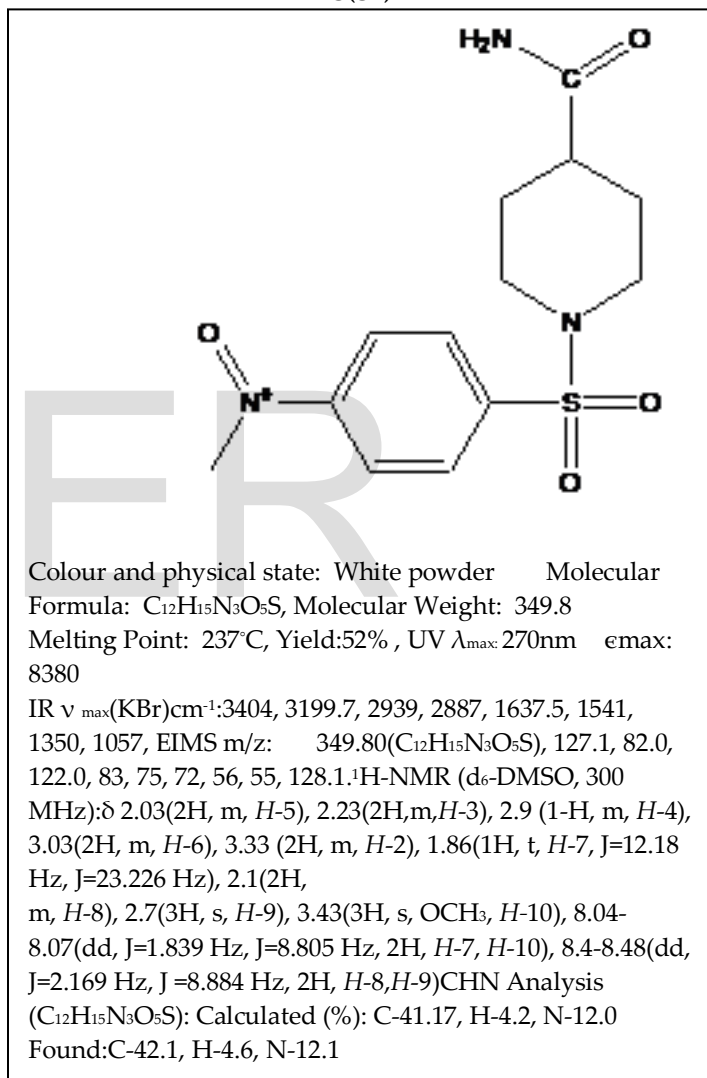
Color and physical state: Off white powder Molecular Formula: $C_{10}H_{22}N_2O_3$, Molecular Weight: 216

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Melting Point: $170^{\circ}C$, Yield: 49%, $UV\lambda_{max}$: 220nm

ϵ_{max} : 539, $IR\nu_{max}(KBr)cm^{-1}$: 3384, 2947, 2792, 2914, 2617, 2520, 1651, 1450, 1365, 1299, 1342, EIMS:m/z: 216 ($C_{10}H_{20}N_2O_3$), 141, 100, 94, 73, 72, 70, 71, 57, 56, ^{55}H NMR (d_6 -DMSO, 300 MHz): δ : 2.03(2H, m, H-5), 2.33(2H, m, H-3), 2.9(1H, m, H-4) 3.03 (2H, m, H-6), 3.33 (2H, m, H-2), 1.86(1H, t, H-7, J=12.18 Hz, J=23.26 Hz), 2.1(2H, m, H-8), 2.7 (3H, s, H-9), 3.43(3H, s, OCH₃, H-10)CHN Anal ($C_{10}H_{20}N_2O_3$): Calculated (%): _____ 216.28, C-55.418, H-9.221, N-12.95 Found:C-55.6, H-9.26, N-12.165

1-(4-Nitro-benzene sulfonyl piperidine-4-Carboxylic acid amide S(S4)



2.4 Methodology of Pharmacological Evaluations Analgesic activity

This activity was carried out on white Albino mice of either sex (locally bred) weighing between 20-30 gm. The compounds were tested for their analgesic activity as antinociceptive effect against thermal stimuli (tail flick method)[5]. Groups of five animals were maintained under standard 12 hours light/12 hours dark at temperature $25\pm 1^{\circ}C$

(room temperature), fed with frequent diet and water. Derivatives were dissolved in water and injected to the test animals intraperitoneally at the doses of 50mg/kg body weight. Pethidine (50 mg/kg) was used as a standard drug. The initial readings were taken immediately before administration of test compounds and standard drugs (0 minutes) and then 30, 60, 90, 120, 150 and 180 minutes after the administration of compounds. The criteria of analgesia was the difference in post drug and pre drug latency which was greater than two times the pre drug average latency[6]. Mean increase in latency after drug administration or Analgesia TFLD was calculated as follows:

Analgesia TFLD = Post drug tail flick latency - pre drug tail flick latency)

Analgesic activity was expressed as TFLD ± SEM in terms of seconds. Statistical analysis was performed using student t-test and values were considered significant or highly significant, P<0.05 or P<0.01 respectively. All statistical procedures were performed according to the method[7].

Behavioral Studies

Compounds were dissolved in water for injection and injected to the test animals intraperitoneally (i.p.) at the dose of 50mg/Kg body weight. Water for injection was injected by the same route to the control animals. The open field apparatus consisted of a square area 24 x 24 cm with 14 cm height. Floor of the apparatus was divided by lines into 25 equal squares. The mice were exposed to the open field after 30 minutes after receiving injection. The activity was scored as number of squares crossings with all four paws for 5 minutes.

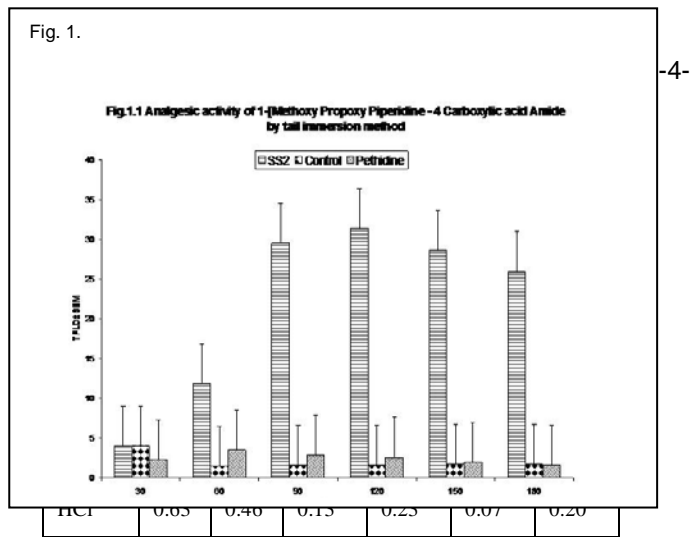
Antimicrobial Screening

The test compounds were evaluated for the antimicrobial activity by disc diffusion method[8]. Different Gram negative and Gram positive organisms were seeded over previously sterilized nutrient agar. The zones of inhibition were measured around the dried discs of Whattmann containing 10µl of the test sample. Blank discs containing DMSO were used as control. The plates were incubated at 37°C for 24 hours.

3. Result and Discussion

The antinociceptive test used in this work is chosen to test nociceptive stimuli namely cutaneous thermic (thermal) which is the characteristic of central narcotic analgesics. An increase in the central inhibitory neurotransmitter, γ-aminobutyric acid (GABA) explains the antinociceptive effects [9] Tail-flick response is essentially a spinal reflex[10]. It has already been shown that tail-flick method is specifically used for screening the strong narcotic effects [11]. Involvement of μ and κ-opioid receptor can also be suggested[12]. The effect on tail-flick response provides a confirmation of this central effect since, the assay is specific for opioid induced antinociceptive effect (9).Results of antinociceptive effects of substituted derivatives of piperidine-4-carboxamide were shown in the tables 1 and 2 showing the analgesia of varying degrees and its duration by tail immersion method [13]. Result shows the effects produced by the compound SS2 demonstrated highly significant effects. The onset of action was early reaches to

maximum in 120 minutes and effect lasted upto 180 minutes showing long duration of action. It is also interesting to note that it seems to be more potent as compared to pethidine



Significant difference by student't test when *p<0.05 and highly significant when **p<0.01 as compared to control n /group = 5

Table – 2:Analgesic Effect of 1-(4-Nitrobenzene-sulfonyl) piperidine-4-carboxylic acid amide (SS4)

Treatment (IP)	Mean Increase in Latency after drug administration ± SEM					
	30 min	60 min	90 min	120 min	150 min	180 min
Control	1.52 ± 0.6	1.56 ± 1.62	1.63 ± 1.65	1.65 ± 1.66	1.68 ± 1.69	1.7 ± 1.69
SS4	4.82 ± 1.04 ± 7	14.6 ± 4** ± 2.30	31.53 ± 7.04	28.91 ± 5.29	28.62 ± 6.40	25.18 ± 5.39
Pethidine	2.26 ± *	3.52 ± **	2.82 ± *	2.57 ± *	1.92 ± *	1.57 ± *
HCl	0.63	0.46	0.13	0.23	0.07	0.20

Significant difference by student't test when *p<0.05 and highly significant when **p<0.01 as compared to control n / group = 5

Table: 3: Effect of Piperidine-4-Carboxamide Derivatives (SS2&SS4) on behavior in Open Field Test in mice

Treatment (IP)Dose (50 mg/Kg)	Number of squares cross	t-test	Latency to move (sec)	t-test
Control	38 ± 1.91		1 ± 0	
SS2	75.42** ± 0.54	3.909 P<0.01	21.8** ± 21.04	5.509 P<0.01
SS4	2.54** ± 1.307	1.428 P<0.01	0.31 ± 1.29	0.781 P<0.01

Values are mean ± SD. (n=5), 30 minutes after injection. Significant differences by student t test when *p<0.05, and highly significant when **p>0.01 as compared to control.

Results of compound SS4 also demonstrated highly signifi-

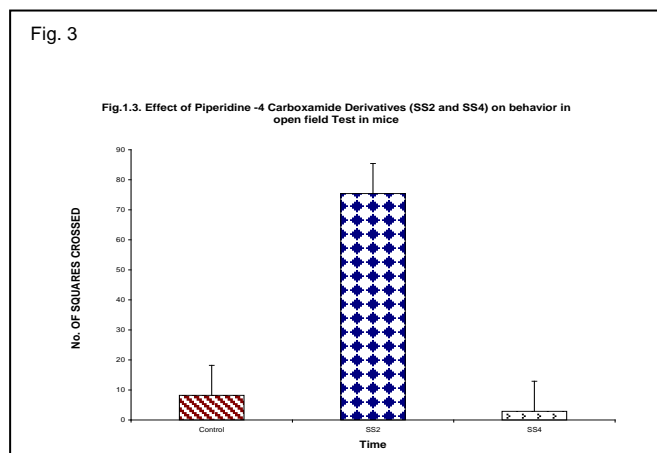
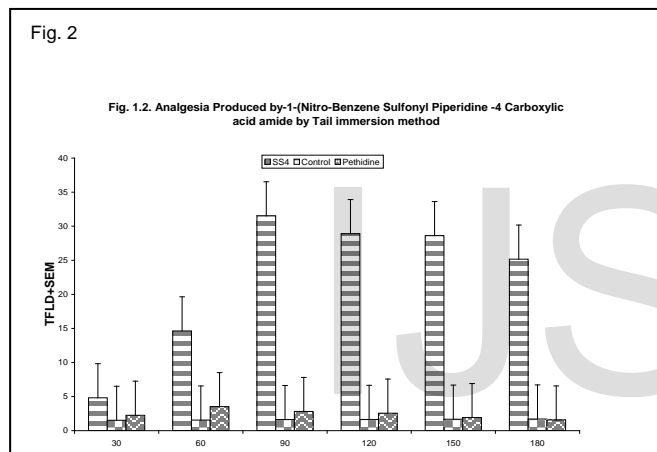
Table – 4: Antibacterial Activity of synthetic compounds against gram-positive organisms and their zones of inhibition in mm.

Synthetic compound	Name of bacterial species (gram-positive)				
	Bac-sub-tilis	Bac-cere-us	Staph-aure-us	Staph-epider-midis	Corynebacte-rium diiphtherae
SS2	12	12	10	-	-
SS42	14	12	10	10	10

Activity key=No of zone of inhibition=14=Better activity=12=good activity=10=moderate activity

Table – 5 Antibacterial Activity of synthetic compounds against gram-negative organisms and their zones of inhibition in mm.

	Name of bacterial species (gram-negative)
	Salmonella-typhi
SS2	12
SS4	10



cant analgesic effects. Again the onset of action is fast and the duration is longer lasting. It is shown that analgesia started within 15 minutes, reaches to maximum in 90 minutes and decreasing slowly but still highly significant effects can be seen upto 180 minutes.

It is well established that locomotor activity can show positive effects in the antidepressant tests [14]. Therefore, a precise method was established to determine the effect on the mobility and the open field activity produced by the synthesized compounds. Open field activity of the newly synthesized derivatives SS2 and SS4 are presented in table 3.

It has already been studied in our synthetic lab that the parent compound Piperidine-4-carboxamide was devoid of hyper or hypomotility [15] while the synthesized compounds possessed significant activity. The compound SS2, produced hypermotility in animals and showed highly significant activity ($P < 0.01$) at the dose of 50mg/kg body weight depicted by the number of squares crossed. Whereas, the compound SS4 produced hypomotility in the animals.

The primary mechanism underlying behavioral effects including locomotion stimulation was thought to be due to its ability to bind to dopamine receptors [16]. In agreement with the dopamine hypothesis, piperidine derivatives had been found to inhibit dopamine reuptake and motor effects. It can also be suggested that besides the inhibition of dopamine reuptake,

other mechanisms might also play a modulatory role in increasing the locomotion in animals.

Antibacterial activity of compound against Gram positive organisms SS4 appeared more effective as compared to SS2, as observed against *Bacillus* sp. with 14 mm zone of inhibition and other bacteria including *Bacillus cereus*, *S. aureus*, *S. epidermidis* & *C. diphtherae* were also inhibited by SS4, It has shown 12 mm & 10 mm zones of inhibition respectively. Overall activity of SS4 against gram positives is good as compared to SS2. SS2 has shown 12 mm to 10 mm zone of inhibition against *Bacillus subtilis*, *B. cereus* and *S. aureus* respectively. Antibacterial activities of test samples against Gram negatives indicate that *S. typhi* is the only susceptible organism among all tested strains. Both synthetic compounds SS2 and SS4 have indicated same antibacterial potential against gram negatives

4 CONCLUSION

It can be concluded that if the study proceeded further, the duration of action could be determined. This long duration shows that drug was not metabolized upto 180 minutes or it may be possible that the metabolized components might also be responsible to produce analgesia. Hypothesis, piperidine derivatives had been found to inhibit dopamine reuptake and motor effects. It can also be suggested that besides the inhibition of dopamine reuptake, other mechanisms might also play a modulatory role in increasing the locomotion in animals. A complete overview of the results of Pharmacological and Biological activities of both of these novel derivatives clearly demonstrates that both of them could be proved as most active molecules, although further exploration of these compounds is required to make them safe and potent therapeutic agents.

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