# Synthesis, Characterization and Antimicrobial Activity of Piperidine Derivatives

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**Summary:** Synthesis of various piperidine derivatives having important biological and pharmacological potentials has been discussed in the past. In present study we reported the synthesis of benzoyl and sulphonyl derivatives by taking Piperidine-4-carboxamide as principal molecule. These compounds were characterized by various spectroscopic techniques such as NMR, FTIR and Mass spectrometry. Elemental composition was explored using CHN analyzer. Antimicrobial activity study of the synthesized compounds was performed using disc diffusion method. Dissociation constant (pKa) of the synthesized compounds were determined by potentiometric titration method. In addition. The findings of the study predicted good absorption of these newly synthesized compounds. Besides, compound III showed good antifungal activity which can be helpful in pharmacokinetics and pharmacodynamics approaches of antibiotics.

**Keywords:** Piperidine, Pharmacological, Potentiometric titration, Absorption, Pharmacokinetics.

## Introduction

Heterocyclic compounds embody an important class of organic compounds having vital relevance for pharmaceuticals, biomedical and biological sciences owing to their interesting biological properties [1, 2]. Over the years, they gain enormous research interest in the fields of drug discovery and design for curing of a variety of different diseases [1-3].

Among heterocyclic compounds, Piperidine has played significant role in drug discovery process [3]. Several researchers reported the potential of piperidine containing compounds with antibacterial activity [3, 4]. Many piperidine derivatives are explored as precursors for the design and synthesis of various drug formulations owing to their excellent biological properties such as antimicrobial, antifungul, antiviral, cytotoxic, anti-inflammatory, analgesic, anaesthetic etc. [5-7].

The success of piperidine as drug precursor motivated us to explore some novel piperidine derivatives having potentially superior properties. In the present work, we explored the synthesis of benzoyl and sulphonyl derivatives of piperidine by taking piperidine-4-carboxamide as principal molecule [8]. The synthesized compounds were characterized using spectrometric techniques and their antimicrobial and antifungal efficacy was evaluated against various standard Gram positive, Gram negative bacterial and fungal strains. The dissociation constant (pKa) is an important parameter used to find the ionized and unionized forms of compounds at respective pH to predict their absorption and excretion [9, 10]. We also explored the dissociation constant (pKa) values of the synthesized piperidine derivatives using a standard potentiometric titration method [11, 12] and the obtained values were compared with the predicted values obtained using ACD software.

## Experimental

All reagents and solvents were purchased from Sigma Aldrich and used without further purification. Reactions were observed by TLC and were visualized under ultraviolet lamp at 254 nm. Gallenkamp melting point apparatus was used to determine the melting points of the products whereas solid silica gel was used for drying. Cecil CE-7200 and Shimadzu IR 8900 spectrophotometers were used to record the Ultraviolet (UV) and Infra-Red (IR) spectra respectively. Varian Massen MAT 312A spectrometer was used to determine Mass spectra (MS). Nuclear Magnetic Resonance ($^1$HNMR) spectra were recorded on a Bruker AM-500 spectrometer, pH-Meter of Jenway, Germany 3510 was used to perform potentiometric titration.

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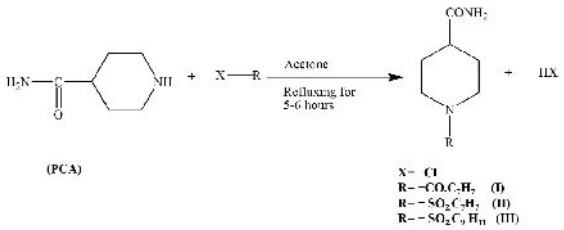
*Synthesis procedure*

Equimolar solutions of Piperidine-4-carboxamide (PCA) and substituted sulphonyl and benzoyl halides in acetone were mixed and refluxed (Scheme-1). The precipitates (I-III) thus obtained separated by filtration, washed thoroughly with hot acetone and recrystallized in methanol to obtain the pure crystals. The compounds were then kept in vacuum desiccators for drying. All compounds showed solubility in ethanol and acetic acid. The chemical structures of the synthesized compounds (I-III) confirmed by NMR FTIR, mass spectrometery and elemental analysis data, all are depicted in Scheme-2. The spectroscopic and elemental analysis data is mentioned below.



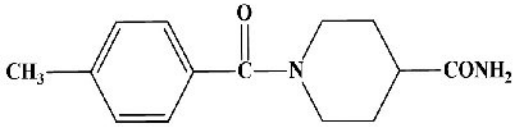

Scheme-1: Synthesis of derivatives.

*Analytical and Spectral Data:*

*Compound **I** (1-(3'-methylbenzoyl) piperidine-4- carboxylic acid amide):*IR(KBr)cm$^{-1}$:3384, 3203, 2817, 2364, 1668, 1367, 900. MS m/z: 282.5 ($M^{+1}$[$C_{14}H_{18}N_2O_2^+$].HCl). $^1$H-NMR (MeOD), 500MHz) δ ppm: 2.4 (s, 1H, H-4), δ 1.84,(M,4 H, H-3, H-5), δ 3.4(m, 4H, H-2, H-6), δ 7.4 (s, 2H, H-8, H-12) δ 7.9(s, 2H, *H*-9, H-11) δ 2.14 (s, Ar-$CH_3$).CHN Analysis: Calculated (%) C-59.46, H-6.72, N-9.91, Found C-59.20, H-6.78, N-9.92

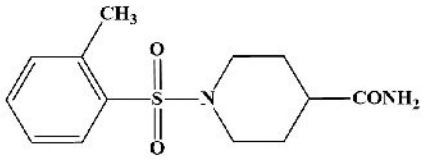
*Compound **II** (1-(Toluene-2-sulfonyl)-piperidine-4-carboxylic acid amide):*IR(KBr)cm$^{-1}$: 3389, 3103, 2935,1669, 1620, 1428, 1320,1251, 1157, 926, 725.MS m/z: 282 ($M^{+1}$, $C_{13}H_{18}N_2O_3S$).$^1$H-NMR($d_6$-DMSO, 400MHz)δ ppm: 2.24-2.28 (M, 1H,H-4), 1.63-1.70 (4H, H-3, H-5), 2.81-2.88 (t,4h, h-2, h-6) J=12.352, 12.376), 7.1 (s, 1H, H-9), 7.3 (s, 1H, H-10), 2.39 (s, 3H, $CH_3$), 7.42-7.44(d, 2H, H-11, *J*=8.108) 7.59-7.61 (d, 2H, H-12, *J*= 8.208).CHN Analysis (%): Calculated C-55.31, H-6.38, N-9.93, Found C-55.31, H-6.75, N-9.97.

*Compound **III** (1-(2', 4',6'-Trimethyl-benzenesulfonyl)-piperidine-4-carboxylic acid amide):* IR(KBr)cm$^{-1}$: 3386, 3104,2933, 1666, 1615, 1426, 1367, 1252, 1152,844, 793.MS m/z: 310($M^{+1}$, $C_{15}H_{22}N_2O_3S$), H-NMR($d_6$-DMSO, 400MHz) δ ppm: 2.24-2.28 (m, 1H,H-4), 1.63-1.70 (4H, H-3, H-5), 2.81-2.88 (t,4H, H-2, H-6) *J*=12.352, 12.376), 2.26 (s, 9H, 3$CH_3$), 7.06(S, 2H, H-9, H-11).CHN Analysis (%): Calculated C-58.06, H-7.09, N-9.03, Found C-57.73, H-7.08, N-9.10.



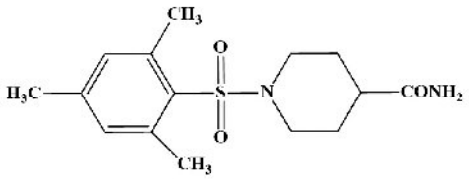
**(I)**

***1-(3'-methyl benzoyl) piperidine-4- carboxylic acid amide***



**(II)**

***1-(Toluene-2-sulfonyl)-piperidine-4-carboxylic acid amide***



**(III)**

***1-(2', 4',6'-Trimethyl-benzenesulfonyl)-piperidine-4-carboxylic acid amide***

Scheme-2: Chemical Structures of the Synthesized Compounds.

*In-vitro Antimicrobial and Antifungal Activity*

Antimicrobial potential of the synthesized analogs (I-III) was evaluated using Agar Disc Diffusion Method [13, 14]. Five (05) Gram positive (*Bacillus subtilis, Bacilluscereus, Staphylococcus aureus, Staphylococcus epidermidis, Cornye bacteriumdiptheriae*) and three (03) Gram negative (*Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi*) bacterial strains were selected and seeded over a previously sterilized nutrient agar. A 10µl aliquot of the sample was placed on the dried discs of Whattman and incubated at 37°C for 24 hours. Later, the zones of inhibition were measured. Discs having DMSO were used as a control sample.

For in-vitro antifungal activity, eight (08) fungal strains (*Trichphytonlongiformis, Trichomonasruburum, Candida albicans, Candida glabrata, Aspergillusflavus, Aspergillusnigar, Microsporumcanis, Fusariumsolani*) were employed.

*Determination of pKa*

Potentiometric Titration Method was adopted for the determination of pKa values of the synthesized compounds. Briefly, 0.01M solutions of compounds (I-III) were prepared in the carbon dioxide ($CO_2$) free distilled water. The temperature was maintained at 25±0.5°C and the titrations were performed on a pre-calibrated potentiometer using 0.1M NaOH solution under constant stirring. The ionic strength of solutions was maintained by 0.1M KCl solution. The pKa was calculated for each compound with the help of titration curves obtained.

Using ACD Labs software, the predicted pKa values of compounds (I-III) were also determined and are listed in Table-1.

**Result and Discussion**

*Chemistry:* Physical properties of the synthesized derivatives (I-III) are listed in Table-1. Whereas characterization of the synthesized derivatives were performed by using FTIR, NMR, mass spectrometry and elemental analyzer. Proposed structures are listed in Scheme-2.

*pKa Values:* The *pKa* values of the synthesized compounds I-III calculated using potentiometric titration method and predicted using ACD Labs software are all listed in Table-1 and the respective titration curves are showed in Fig. 1. It can be seen that the compound I, which is a benzoyl derivative exhibit a higher pKa value compared to the sulfonyl derivatives (compound II and III). The observed higher value for compound I is likely caused by the presence of methyl group on the benzoyl ring (Scheme-2). In the same way, sulphonyl derivative (III) showed slightly higher pKa compared to the compound II which is likely caused by the presence of three methyl groups at different positions on sulfonyl ring (Scheme-2). Besides, the pKa values of the derivatives were also influenced by the nature of benzoyl and sulfonyl ring. Since benzoyl moiety is less acidic than sulphonyl, therefore the pKa values of benzoyl derivative was higher than that of the sulphonyl derivatives. Remarkably, the predicted p*K*a values obtained using ACD software program were found to be in close agreement with experimental values obtained using potentiometric titration method.

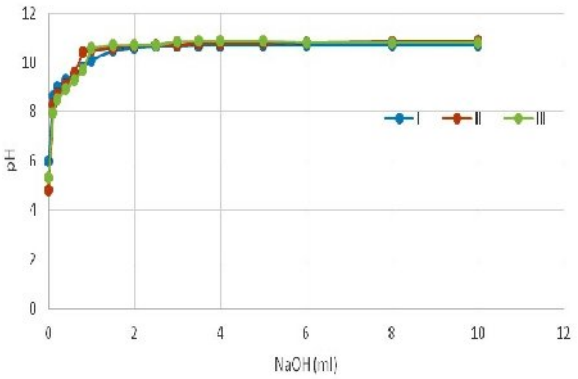



Fig. 1: Potentiometric Titration Curves of synthesized derivatives (**I - III**).

*In-vitro Antimicrobial Activity:* Antimicrobial activity of synthesized compounds performed by disc diffusion method and the obtained results are mentioned in Table-2 and 3. The activity tables reflected that the parent molecule was totally devoid of any antibacterial action against all the tested Gram positive, Gram negative bacterial and fungal strains. The synthesized compounds also did not show any activity against the same. On the other hand, compound III having three methyl groups at sulphonyl ring displayed significant antifungal effects against the fungal strain *Aspergillusflavus* compared to the compound II, with only one methyl group. The observed antifungal activity is even higher than the control compounds *Isonipecotamide* and *Miconazole* (Table-3). The observed antimicrobial activity can be explained on the basis of the bulkiness of the molecule which affected its lipophilicity due to which compound III was not completely ionized. The antifungal effects are likely due to large unionized part of the compound [15-17].

Table-1: Physical Characteristic of Compounds **I** to **III**.

| Compound | | Appearance | Mol. Formula | Mol. Wt (g/mol) | Yield %5 | M.P (°C) | Solubility | pKa Values | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | Pot. Titration | ACD Labs |
| Benzoyl derivate | I | Off White powder | $C_{14}H_{19}N_2O_2Cl$ | 282.5 | 22 | 195 | Ethanol, DMSO | 7.96 | 6.85±0.62 |
| Sulfonyl derivatives | II | Off white powder | $C_{13}H_{18}N_2O_3S$ | 282.0 | 58 | 210 | Ethanol, DMSO | 7.06 | 6.80±0.50 |
| | III | White powder | $C_{15}H_{22}N_2O_3S$ | 310.0 | 54 | 213 | Ethanol, DMSO | 7.20 | 6.80±0.50 |

Table-2: *In-vitro* antibacterial activity of Compounds **I-III**.

| | Zone of inhibitions (mm) | | | | |
|---|---|---|---|---|---|
| Bacteria | Standard | Isonipecotamide | Compound I | Compound II | Compound III |
| Gram positive | | | | | |
| *Bacillus subtilis* | Ampicillin 0 | 0 | 0 | 0 | 0 |
| *Bacilluscereus* | Ampicillin 0 | 0 | 0 | 0 | 0 |
| *Staphylococcus aureus* | Tetracycline 20 | 0 | 0 | 0 | 0 |
| *Staphylococcus epidermidis* | Tetracycline 20 | 0 | 0 | 0 | 0 |
| *Cornyebacteriumdiptheriae* | Tetracycline 25 | 0 | 0 | 0 | 0 |
| Gram negative | | | | | |
| *Escherichia coli* | Tetracycline 22 | 0 | 0 | 0 | 0 |
| *Pseudomonas aeruginosa* | Ampicillin 12 | 0 | 0 | 0 | 0 |
| *Salmonella typhi* | Ampicillin 22 | 0 | 0 | 0 | 0 |

**0** = absence of measurable inhibitory action, × = activity not performed, 14 = better activity
12 = good activity, 10 = moderate activity

Table-3: In-vitro antifungal activity of Compounds **I-III.**

| | Zones of Inhibition (mm) | | | | |
|---|---|---|---|---|---|
| Fungi | Isonipecotamide | Miconazole | Compound I | Compound II | Compound III |
| *Trichphytonlongiformis* | 0 | 95 | 0 | 0 | 0 |
| *Trichomonasruburum* | 8 | | 0 | 0 | 0 |
| *Candida albicans* | 0 | 90 | 8 | 0 | 0 |
| *Candida glabrata* | 0 | 110.8 | 0 | 0 | 0 |
| *Aspergillusflavus* | 0 | 20.22 | 0 | 0 | 30 |
| *Aspergillusnigar* | 0 | 90 | 0 | × | × |
| *Microsporumcanis* | 0 | 98.4 | 0 | 20 | 10 |
| *Fusariumsolani* | 0 | 73.25 | 0 | 0 | 5 |

**0**= absence of measurable inhibitory action, ×=activity not done, 14=better activity
12=good activity, 10=moderate activity

## Conclusion

In this work we demonstrated the synthesis of benzoyl and sulphonyl derivatives using Piperidine-4-carboxamide as principal molecule. The synthesized compounds were all thoroughly characterized by spectroscopic techniques. Besides, the in vitro antimicrobial activity, antifungal activity and the corresponding dissociation constants (pKa) were also determined.

In conclusion, among the three compounds synthesized, the compound III exhibited significant antifungal activity against the fungal strain *Aspergillusflavus*. The synthesized compounds were found to be either weak acids or nearly neutral compounds where more pKa reflects lower ionization at physiological pH (7.4). It was further found that the lipophilicity of the compounds has significant effects on the ionization of the molecules. Compounds with higher lipophilicity exhibited less ionization. This finding is in agreement with the earlier reported studies where it was shown that the antibacterial activity is largely due to unionized form of the drug.

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