International Journal of ChemTech Research
CODEN(USA): IJCRGG ISSN : 0974-4290
Vol.4, No.2, pp 532-538, April-June 2012

Synthesis and Biological Evaluation of some Substituted 4-Piperidones

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Abstract: Some substituted 4-piperidones were synthesized by Mannich condensation reaction between substituted aromatic aldehydes, ethyl methyl ketone and ammonium acetate in presence of ethanol. The synthesized compounds were characterized and screened for local anesthetic, antibacterial and antifungal activities. Compound M3 showed significan local anesthetic activity almost near to that of the standard. Compound F4 showed moderate activity compared to that of the standard. Other analogues were found to be less active. A few of the synthesized compounds were found to be effective against Gram (+) ve and Gram (–) ve microorganisms, while none of the compounds showed activity against *C. albicans* and *A.niger*.
Key words: 4-piperidones, Antibacterial, Antifungal, Local anesthetic.

Introduction

4-Piperidones have been known to possess a large number of biological activities like local anesthetic [1], antimicrobial [2], analgesic [3] and CNS depressant [4]. Earlier studies have been carried out by substitution in 2, 3 and 6 positions and evaluate the resulting compounds for their biological activity. It was proposed, therefore, that a new series of substituted 4-piperidones would result in compounds with potent biological activity.

The objective of the present study was, therefore, to synthesise 2,6- aryl/hetero aryl disubstituted and 3-methyl 4- piperidone derivatives, by Mannich condensation of substituted aromatic/hetero aromatic aldehydes, ethyl methyl ketone and ammonium acetate, characterize by spectral studies and screen them for their antibacterial, antifungal and local anesthetic activities.

1. Materials and Methods

All the chemicals used were of synthetic grade and procured from S.D.Fine chemicals Mumbai, India. Silica Gel G, Carbon Tetrachloride, and petroleum ether were of AR grade and procured from LOBA CHEMIE Mumbai, India. Cultures of microorganisms were procured from NCL, Pune, India.

Melting points of the synthesized compounds were determined in open capillary tubes and are uncorrected. IR spectra were recorded on ABB BOMEM FTIR Spectrometer using KBr pellets. ^{1}H NMR spectra were recorded in DMSO δ_6 on VARIAN MERCURY YH-300 model NMR spectrometer. Thin layer chromatography was performed using plates coated with silica gel G of 0.25mm thickness. Carbon tetrachloride: Petroleum ether (40-60°C) in the ratio of 4:1was used as the eluent. Spots were visualized through the U.V.Iight chamber and Iodine chamber.

1.1. General Procedure for Compounds F1-F5.

The procedure reported by Noller and Baliah was followed to prepare these compounds [5]. Compounds F1 to F5 were prepared by heating carefully to simmering a mixture of ethyl methyl ketone (0.1 mole), dry ammonium acetate (0.1 mole), and substituted benzaldehyde (0.1 mole) and furfuraldehyde (0.1 mole) in presence of ethanol (30ml). The flask was kept at room temperature for 12

h. Dry ether (50ml) was then added followed by concentrated hydrochloric acid (30ml) and cooled in ice water. The precipitated hydrochloride was filtered, washed with ethanol and ether mixture (1:5) and transferred to a one litre beaker. The hydrochloride was suspended in acetone and basified with strong ammonia solution. On dilution with excess of water the base seperated out. It was filtered, washed repeatedly with water and dried. The crude piperidin-4-one was recrystallized using absolute ethanol. A similar procedure was adopted to obtain compounds M1-M6 by taking a mixture of ethyl methyl ketone and substituted benzaldehyde.

1.2. Local Anesthetic Studies by Nerve block anesthesia (Sollman method) [7]

39 Frogs were divided in to 13 groups of 3 each. The upper spinal cord of the animals was destroyed by pithing. The abdomen was opened and the organs were removed to expose the spinal cord and to make a pouch. The test samples (2 ml) were applied in to the pouch with the help of a cotton ball. The control animals received 2 ml of saline, the standard animals received 2 ml of Lignocaine (1% w/v) and the test animal received 2 ml of various test samples (1% w/v). The foot withdrawal time was determined at 0, 2, 4, 6, 8 and 10 min after the drug application by immersing the right leg in a beaker containing 0.1N HCl. After noting down the withdrawal time, the leg was washed thoroughly with normal saline before taking the next reading. The data obtained was tabulated and the results were analyzed by comparing with the control values.

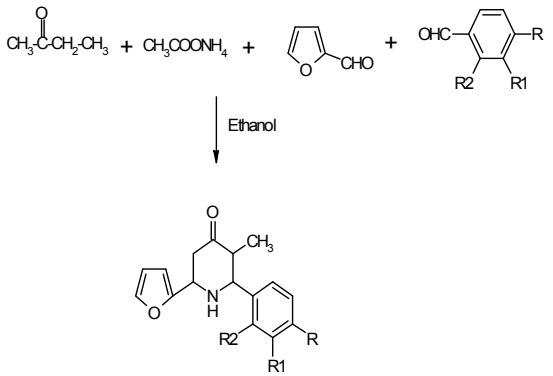
1.3. Antibacterial studies

Antibacterial studies were carried out by cup plate method [8]. The nutrient agar medium was prepared in distilled water (28g in 1000ml). It was then sterilized in an autoclave. In the meanwhile petriplates and pipettes were sterilized in an hot air oven. The sterilized medium was then poured into petriplates and the suspension of microbial cultures was poured in to the petriplates. Wells were then prepared. To these wells 0.1ml of the compound to be tested is added aseptically at the concentrations of 25µg/0.1ml and 50µg/0.1ml and ofloxacinan 50µg/0.1ml in DMSO as the standard. Then the plates were kept for incubation at 37° C for 2 h in an incubator.

1.4. Antifungal Studies

The antifungal activity of the compounds was tested against C.aibicans using sabourad dextrose agar medium [9]. The sterilized (autoclaved at 120°c for 30min) medium (40°-50°c) was inoculated with the suspension of microorganism and poured into petriplates to give a depth of 3-4 mm. The test compounds at two concentrations (25µg/0.1ml and 50µg0.1ml in DMSO) were placed on the medium. The plates were pre incubated for 1h at room temperature and incubated at 37°C for 48h for antifungal activity. Ketoconazole of same concentration as test compounds, was used as the standard. The zone of inhibition was measured in mm.

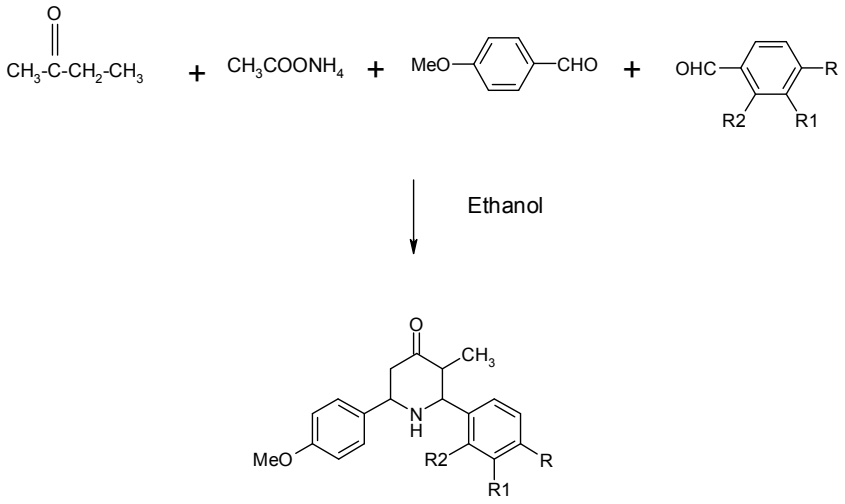
Synthetic Scheme 1.



Compounds F1-F5

COMPOUNDS	R	R1	R2
F1	H	H	H
F2	OH	H	H
F3	OCH_3	H	H
F4	Cl	H	H
F5	H	OH	H

Synthetic Scheme 2.



Compounds M1-M6

COMPOUNDS	R	R1	R2
M1	H	H	H
M2	OH	H	H
M3	Cl	H	H
M4	H	OH	H
M5	$N(CH_3)_2$	H	H
M6	OH	OCH_3	H

Table 1. Physical Data of Synthesised Compounds

Compounds	MP (^oC)	Rf Value	Yield (%)
F1	220	0.24	49.21
F2	194	0.22	59.04
F3	199	0.29	22.77
F4	192	0.28	84.77
F5	202	0.25	33.23
M1	97	0.20	71.18
M2	104	0.15	34.53
M3	103	0.17	81.99
M4	90	0.21	47.99
M5	112	0.14	14.70
M6	102	0.19	07.33

Table 2a. Spectral Data of Synthesized Compounds F1 to F5.

Compound	IR (KBr) cm^{-1}	^1H NMR (DMSO-*d6*) δ ppm
F1	1724(C=O), 2939(N-H), 818(Ar-H)	9.86 (s,1H;N-H),7.07-7.83 (m,8H;Ar-H),3.51-3.58 (m,2H;2,6-H),2.57-2.71 (m,3H;3,5-H),0.88 (s,3H;$3CH_3$)
F2	1721(C=O), 2977(N-H), 834(Ar-H), 3306(OH)	9.95 (s,1H;N-H),6.23-7.82 (m,7H;Ar-H),3.46-3.88 (m,2H;2,6-H),2.03-2.83 (m,3H;3,5-H),0.86 (s,3H;$3CH_3$),11.53 (s,1H;OH)
F3	1723(C=O), 2936(N-H), 828(Ar-H)	9.80 (s,1H;N-H),6.54-7.86 (m,7H;Ar-H),3.09-3.85 (m,2H;2,6-H),2.49-2.65 (m,3H;3,5-H),0.86 (s,3H;$3CH_3$)
F4	1723(C=O), 2910(N-H), 825(Ar-H)	9.78 (s,1H;N-H),6.53-7.78 (m,7H;Ar-H),3.04-3.87 (m,2H;2,6-H),2.16-2.72 (m,3H;3,5-H),0.86 (s,3H;$3CH_3$)
F5	1711(C=O), 2933(N-H), 816(Ar-H), 3042(OH)	9.84 (s,1H;N-H),6.65-7.89 (m,7H;Ar-H),3.03-3.85 (m,2H;2,6-H),2.03-2.98 (m,3H;3,5H),0.67 (s,3H;$3CH_3$),10.23 (s,1H;OH)

Table 2b. Spectral Data of Synthesized Compounds M1 to M6.

Compound	IR (KBr) cm^{-1}	^1H NMR (DMSO-*d6*) δ ppm
M1	1722(C=O), 2936(N-H), 832(Ar-H),	9.87 (s,1H;N-H),6.34-7.11 (m,9H;Ar-H),3.27-3.94 (m,2H;2,6-H),2.35-2.62 (m,3H;3,5H),0.81 (s,3H;$3CH_3$)
M2	1723(C=O), 2936(N-H), 830(Ar-H), 3418(OH)	9.84 (s,1H;N-H),6.65-7.82 (m,8H;Ar-H),3.09-3.97 (m,2H;2,6-H),2.03-2.69 (m,3H;3,5H),0.86 (s,3H;$3CH_3$),11.06 (s,1H;OH)
M3	1722(C=O), 2913(N-H), 828(Ar-H),	9.74 (s,1H;N-H),6.37-7.17 (m,8H;Ar-H),3.33-3.92 (m,2H;2,6-H), 2.41-2.72 (m,3H;3,5H),0.86 (s,3H;$3CH_3$).
M4	1721(C=O), 2936(N-H), 835(Ar-H), 3066(OH)	9.76 (s,1H;N-H),6.24-7.98 (m,8H;Ar-H),3.24-3.99 (m,2H;2,6-H),2.09-2.69 (m,3H;3,5H),0.85 (s,3H;$3CH_3$),11.42 (s,1H;OH)
M5	1722(C=O), 2915(N-H), 828(Ar-H),	9.79 (s,1H;N-H),6.81-7.99 (m,8H;Ar-H),3.24-3.99 (m,2H;2,6-H),2.09-2.69 (m,3H;3,5H),0.85 (s,3H;$3CH_3$)
M6	1722(C=O), 2936(N-H), 829(Ar-H),	9.78 (s,1H;N-H),6.81-6.92 (m,7H;Ar-H),3.79-3.99 (m,2H;2,6-H),2.57-2.97 (m,3H;3,5H),0.88 (s,3H;$3CH_3$).

Table 3. Mass Spectra

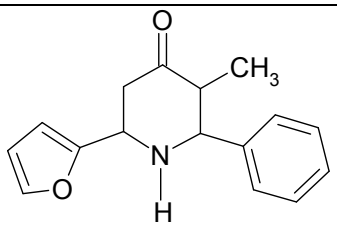
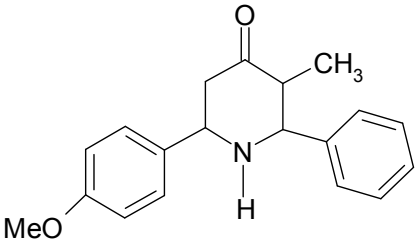
Compound Code	Structure	m/z
F1		255 (M^+), 212,198,184,170, 146,123, 104,96
M1		295 (M^+), 281,224,207,134,91,73, 65,44

Table 4. Local anesthetic activity of the synthesized compounds

Compounds	Mean Foot Withdrawal Time(In Sec)					
	0MIN	2MIN	4MIN	6MIN	8MIN	10MIN
F1	1.16(±0.1)	2.83(±0.1)	3.00(±0.2)	3.66(±0.2)	4.00(±0.2)	4.00(±0.1)
F2	1.08(±0.1)	2.33(±0.2)	3.00(±0.1)	3.08(±0.2)	4.33(±0.3)	5.66(±0.3
F3	1.66(±0.2)	3.00(±0.2)	3.66(±0.2)	4.83(±0.2)	5.83(±0.3)	6.50(±0.3)
F4	1.50(±0.1)	3.16(±0.3)	3.66(±0.2)	4.16(±0.2)	6.55(±0.2)	7.00(±0.3)
F5	1.16(±0.3)	2.00(±0.2)	2.00(±0.1)	2.66(±0.1)	3.00(±0.3)	3.16(±0.2)
M1	1.16(±0.2)	2.00(±0.3)	2.00(±0.1)	2.66(±0.1)	3.00(±0.2)	3.16(±0.2)
M2	1.00(±0.1)	1.66(±0.2)	2.33(±0.2)	3.00(±0.2)	3.16(±0.2)	3.16(±0.2)
M3	1.00(±0.2)	3.83(±0.1)	4.00(±0.3)	5.66(±0.3)	6.50(±0.3)	7.50(±0.3)
M4	1.00(±0.2)	2.16(±0.2)	2.83(±0.2)	3.00(±0.2)	3.00(±0.2)	3.16(±0.1)
M5	1.00(±0.1)	3.83(±0.2)	4.00(±0.3)	4.16(±0.2)	4.16(±0.2)	4.83(±0.2)
M6	1.33(±0.2)	3.00(±0.2)	3.83(±0.2)	5.83(±0.3)	6.00(±0.3)	6.16(±0.2)
Control	1.33(±0.2)	1.66(±0.1)	2.00(±0.2)	1.66(±0.1)	1.33(±0.1)	1.33(±0.1)
Standard	6.16(±0.2)	10(±0.3)	10(±0.3)	10(±0.3)	10(±0.2)	10(±0.3)

Values are Mean ± SD, n=3

Fig.1. Evaluation of Local anesthetic activity.

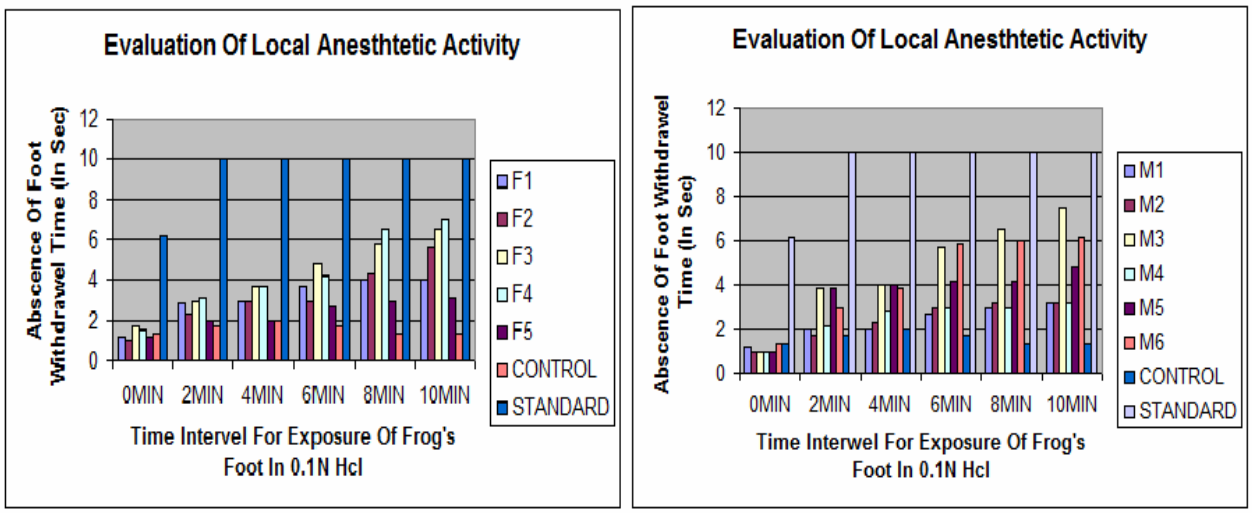
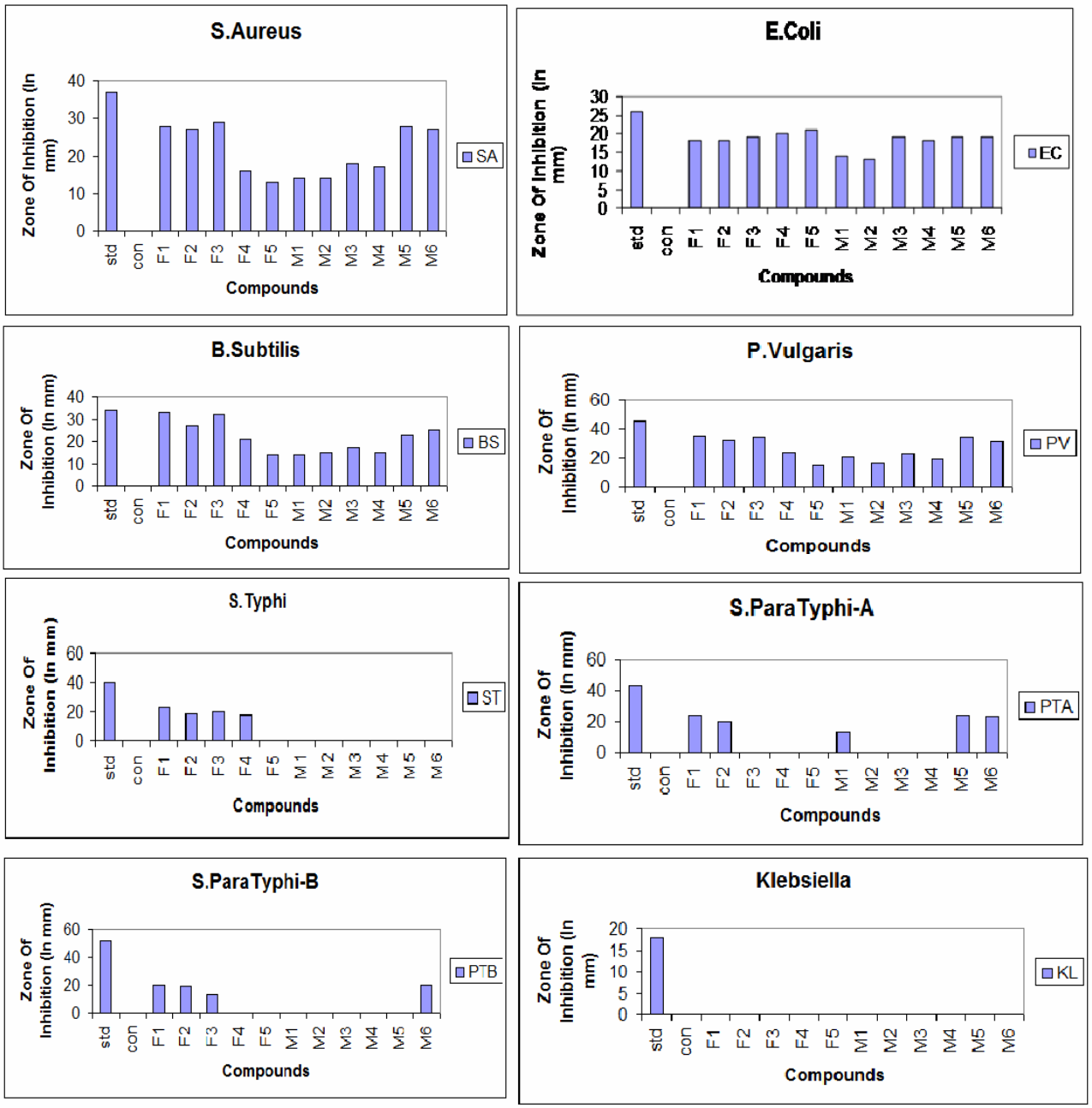


Fig.2. Evaluation of Antimicrobial activity.



2. Results and Discussion

2.1. Chemistry of synthesized compounds

Scheme 1 shows the synthesis compounds F1 to F5 and Scheme 2 shows the synthesis of compounds M1 to M6. The melting points, Rf values and the percentage yield obtained for the compounds are given in Table 1. The compounds were characterized by IR and ^{1}H NMR spectral analysis and the data obtained are given in Table 2a and 2b. Compounds F1 and M1 were also characterized by mass spectra. The data reveal that the synthesised piperidine-4-ones show the expected characteristic absorption bands for N-H, C=O, and O-H bonds. The piperidine ring adopts a chair confirmation. Hence a number of signals for axial protons, equatorial protons and the protons in the substituent are obtained in the ^{1}H NMR spectra of the substituted piperidine-4-ones. The mass spectral peaks of the compounds F1 and M1 was confirmed by corresponding m/z peaks and shown in Table 3.

2.2. Local anesthetic activity

All the synthesised compounds were tested for their local anesthetic activity and the data obtained is given in Table 4 and Figure 1. The data reveal that all the compounds possess moderate local anesthetic activity.

The compound M3 shows activity nearest to that of the standard. Compound F4 shows moderate activity compared to that of the standard. Phenyl and hydroxyl substituted 4-piperidones show less activity and 4-methoxy, 2-hydroxy-3-methoxy substituted compounds show moderate activity. Furyl substituent at position 6 does not influence local anesthetic activity, whereas 4-methoxy phenyl substituent at position 6 leads to moderate activity.

2.3. Antimicrobial Activity

The antimicrobial activity of all the synthesized compounds F1-F5, and M1-M6 are shown in Figure 2. The data reveal that all the compounds possess moderate activity against the some of the Gram (+) ve and Gram (–) ve microorganisms at the concentration studied. But all the compounds are inactive against *Klebsiella.* Compounds F5, M1-M6 were found to be inactive against S*almonella typhi.* When compared with the standard all the compounds show moderate activity against *S.aureus, E.coli, B.subtilis, P.Vulgaris.* All the synthesized 4-piperidones show no zone of inhibition against *Aspergillus Niger*, *Candida Albicans* at the concentration of 50µg/0.1 ml.

3. Conclusion

In the present study, efforts were made to synthesize a new series of piperidine-4-ones and evaluate their biological activities. All the newly synthesized compounds were screened for their local anesthetic and antimicrobial activity. Among them F4 and M3 show good local anesthetic activity. It is also observed that as the time increases local anesthetic activity increases. The substituent at position 2 (p-chloro phenyl) shows better local anesthetic activity than others.

All the compounds synthesised show antibacterial activity against tested organisms. Compounds F3 and M5 are effective against *S.aureus* and F5 is effective against *E.coli.* F1 and more effective against *B.subtilis.* F3 and M5 were found to be effective against *P.vulgaris*. However, these compounds were found to possess negligible activity against *S.typhi, S.Para typhi-A, S.Para typhi-B and Klebsiella.* Analogs containing para methoxy phenyl (F3), ortho hydroxy phenyl (F2), para dimethyl amino phenyl (M5) groups at position 2 have better activity as compared with others. None of the compounds show activity against *C.albicans, A.niger.*

Acknowledgements

The authors wish to acknowledge Mr.Kakade, Krishna Institute of Medical Sciences, Karad, for providing statistical assistance.

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