IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
Impact Factor: 7.53
Volume 4, Issue 1, September 2024

Design, Synthesis, and Antimicrobial Evaluation of Novel Schiff Bases Derived from Thymol

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Abstract: *Schiff bases, a significant class of organic compounds, are synthesized through the reaction of a primary amine with an aldehyde or ketone under specific conditions. In this study, we report the synthesis of novel Schiff bases derived from thymol (2-Isopropyl-5-methylphenol). The synthesis began with the esterification of thymol to produce (2-Isopropyl-5-methylphenoxy)-acetic acid ethyl ester (1), followed by hydrazination to yield (2-Isopropyl-5-methylphenoxy)-acetic acid hydrazide (2). The hydrazide (2) was then treated with various aromatic aldehydes to synthesize a series of Schiff bases (3A-J). The chemical structures of these compounds were elucidated using infrared (IR) spectroscopy, proton nuclear magnetic resonance (1H-NMR), and mass spectrometry. The synthesized Schiff bases were evaluated for their antibacterial and antifungal activities using standard screening methods. The results indicated that all synthesized compounds exhibited varying degrees of antimicrobial activity. Among the series, compound 3C showed the highest antibacterial and antifungal potency, suggesting it could be a promising candidate for further development. This study highlights the potential of Schiff bases as bioactive compounds with broad-spectrum antimicrobial properties. The structure-activity relationship (SAR) suggests that the presence of specific substituents on the aromatic aldehyde moiety could enhance biological activity. Further investigation into the mechanisms of action and optimization of these Schiff bases could contribute to the development of novel antimicrobial agents.*

Keywords: Schiff bases, thymol, synthesis, antibacterial activity, antifungal activity, aromatic aldehydes, IR spectroscopy, 1H-NMR

I. INTRODUCTION

Infection is a major category of human disease and skilled management of antimicrobial drugs is of the first importance. Infectious diseases caused by bacteria and fungi affect millions of people worldwide. The accomplishment of antimicrobial agents, ranging from direct killing of invading pathogens to immune response modulation and other complex biological responses, has stimulated research and clinical interest for more than two decades. However the area is still flourishing due to emerging discoveries in the functions, roles and regulation of antimicrobial agents. Antimicrobial agents kill microorganisms or inhibit their growth and they can be grouped according to the microorganisms against which they act on; antibacterials are used against bacteria and antifungals are used against fungi [1]. Microbial infection is a leading cause of global disease burden with high morbidity and mortality especially in the developing world. A matter of concern in the treatment of microbial infection is the limited number of efficacious antimicrobial agents, which clearly highlights the urgent need of novel antimicrobial agents [2]. Schiff bases are some of the most widely used organic compounds [3]. Schiff bases are condensation products of primary amines with carbonyl compounds and they were first reported by Schiff in 1864 [4]. Since then a variety of methods have been described for the synthesis of imines. Structurally, a Schiff base (also known as imine or azomethine) is a nitrogen analogue of an aldehyde or ketone in which the carbonyl group (>C=O) has been replaced by an imine or azomethine group. The common structural feature of these compounds is the azomethine group with a general formula RHC=N-R1, where R and R1 are alkyl, aryl, cycloalkyl or heterocyclic groups which may be variously substituted. These compounds are also known as anils, imines or azomethines [3]. Schiff bases are some of the most widely used organic compounds. Schiff bases have also been shown to exhibit a broad range of biological activities, including antifungal, antibacterial, antimalarial, antiproliferative, anti-inflammatory, antiviral, and antipyretic properties [5-7]. Imine or

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azomethine groups are present in various natural, naturally derived, and non-natural compounds. The imine group present in such compounds has been shown to be critical to their biological activities [8-11].

A.K. Chakraborti *et al*demonstrated that the efficiency of synthesizing Schiff bases is dependent on the use of highly electrophilic carbonyl compounds and strongly nucleophilic amines. They proposed as an alternative the use of substances that function as Bronsted- Lowry or Lewis acids to activate the carbonyl group of aldehydes, catalyze the nucleophilic attack by amines, and dehydrate the system, eliminating water as the final step [12]. N.K.Fuloria *et al* synthesized imines from haloaryloxy moiety. The novel series of compounds were elucidated on the basis of spectral studies and screened for antibacterial and antifungal activities [13,14]. A. Deep *et al* designed and characterized hydrazone derivatives of biphenyl-4-carboxylic acid. All compounds exhibited promising antimicrobial activity [15]. H.M.A. Sharif *et al*. synthesized Schiff bases of salicylaldehyde with different amines, and screened for anti-microbial activities [16]. A. Iqbal *et al*. combined benzaldehyde with amoxicillin to produce active antibacterial Schiff bases [17]. Thus from the literature review & market demand, it is observed that Schiff bases (imines) possesses wide variety of biological activities like antimicrobial, antitubercular, antimalarial and antiviral. Among various biological activities of several Schiff bases molecules synthesized in literature, the antimicrobial activity was found to be very prominent. Hence it was thought worthwhile to study and synthesize compounds possessing imino group and investigate their structural modification. The aim of present study was to synthesize and screen Schiff bases of thymol for antimicrobial activity.

II. MATERIAL AND METHODS

2.1. General

Melting points of newly synthesized compounds were determined in open capillary tubes. Compounds were routinely checked for their purity on silica gel G (Merck) thin layer chromatography (TLC) plates. Iodine chamber and UV lamp were used for visualization of TLC spots. IR spectra were recorded (in KBr) on Schimadzu FTIR Spectrophotometer. ^{1}H NMR spectra were recorded on Bruker DPX-300 NMR spectrometer in DMSO-d_6 using tetramethylsilane (TMS) as an internal standard and mass spectra on LCMS 2010 EV SHIMADZU Mass spectrometer.

2.2. Test microorganisms and medium

The synthesized compounds were screened for antibacterial activity using disk diffusion method. The antibacterial activity of the synthesized compounds was evaluated against Gram-positive bacteria *Staphylococcus aureus* (MTCC 737), and Gram-negative bacteria*Escherichia coli* (MTCC 452) and antifungal activity using*Candida albicans* (MTCC 227). The Nutrient agar and Sabouraud's agar medium were used to test the sensitivity of bacterial and fungal strains respectively against synthesized compounds.

2.3. Chemistry

2.3.1. Synthesis of (2-Isopropyl-5-methyl-phenoxy)-acetic acid ethyl ester (1)

A mixture of thymol (0.1mol), ethylchloro acetate (0.1 mol) and anhydrous potassium carbonate (0.15 mol) in dried acetone was refluxed for 24 h. Resultant mixture was distilled off and poured on to ice-cold water and stirred. Residue was extracted with ether and the extract was dried over anhydrous sodium sulphate and was purified under reduced pressure to yield compound **1**; yield (55.63%), b.p. (240^0c), IR (KBr) cm^{-1}: 1745 C=O of ester, 1181 for C-O of ester.

2.3.2. Synthesis of (2-Isopropyl-5-methyl-phenoxy)-acetic acid hydrazide (2)

A mixture of compound **1** (0.05mol) and hydrazine hydrate (0.075 mol) in ethanol was refluxed for 8 h and after distilling off the solvent the residue was recrystallized from methanol to yield compound **2**; yield (72.00%), m.p. (98^0c), IR (KBr) cm^{-1}: 3318**,** 3210 for N-H and NH_2, 1678 for C=O of amide, 1504 for N-H bending of amide, 1254 and 1065 for C-O of phenyl ether.

2.3.3. General procedure for the synthesis of Schiff bases (3A-3J)

A mixture of compound **2** (0.001 mol) and different aromatic aldehydes (0.001 mol) was refluxed for 8 h using ethanol and glacial acetic acid. Crystals formed were washed with ice-cold water, dried and recrystallized from methanol to yield compounds **3A-3J**.

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2.3.4. Spectral data of synthesized compounds (3A-3J)

2.3.4.1.*(2-Isopropyl-5-methyl-phenoxy)-acetic acid (4-pyrrolidin-1-yl-benzylidene)-hydrazide* (3A).IR (KBr, cm^{-1}) 3194 for N-H, 1697 for C=O of amide, 1609 for C=N of imines, 1528 for N-H bending of amide, 1258 and 1072 for C-O of phenyl ether. **^{1}H NMR** (DMSO-d_6, 300MHz) δ ppm: 1.16-1.17 (6H, d, for CH_3 of isopropyl), 1.96-1.97 (4H, dd, for pyrrolidine-H), 2.22-2.25 (3H, s, for Ar-CH_3), 3.27-3.28 (4H, s, for pyrrolidine-H), 4.59-4.60 (1H, sept, for Me_2CH), 5.06 (2H, s, for O-CH_2-CO), 6.55-7.50 (7H, m, for Ar-H), 7.87 (1H, s, for N=CH), 11.24 (1H, s, for NH). **ESI-MS**: MS: m/z 379 (M^+).

2.3.4.2.*(2-Isopropyl-5-methyl-phenoxy)-acetic acid benzylidene-hydrazide* (3B). IR (KBr, cm^{-1}) 3198 for N-H, 1670 for C=O of amide, 1605 for C=N of imines, 1539 for N-H bending of amide, 1254 and 1045 for C-O of phenyl ether. **^{1}H NMR** (DMSO-d_6, 300MHz) δ ppm: 1.16-1.17 (6H, d, for CH_3 of isopropyl), 2.23-2.25 (3H, s, for Ar-CH_3), 3.30-3.33 (1H, sept, for Me_2CH), 5.13 (2H, s, for O-CH_2-CO), 6.66-6.76 (3H, m, for Ar-H), 7.06-7.71 (5H, m, for Ar-H), 8.02 (1H, s, for N=CH), 11.57 (1H, s, for NH). **ESI-MS**: MS: m/z 310 (M^+).

2.3.4.3. *(2-Isopropyl-5-methyl-phenoxy)-acetic acid (5-nitro-thiophen-2-ylmethylene)-hydrazide* (3C). IR (KBr, cm^{-1}) 3109 for N-H, 1682 for C=O of amide, 1612 for C=N of imines, 1528 for N-H bending of amide, 1250 and 1038 for C-O of phenyl ether. **^{1}H NMR** (DMSO-d_6, 300MHz) δ ppm: 1.22-1.24 (6H, d, for CH_3 of isopropyl), 2.26-2.28 (3H, s, for Ar-CH_3), 3.10 (1H, sept, for Me_2CH), 5.10 (2H, s, for O-CH_2-CO), 6.64-7.12 (3H, m, for Ar-H), 7.50-7.54 (2H, d, for thiophene-H), 8.12 (1H, s, for N=CH), 11.24 (1H, s, for NH). **ESI-MS**: MS: m/z 361 (M^+).

2.3.4.4. *(2-Isopropyl-5-methyl-phenoxy)-acetic acid (1-methyl-1H-indol-3-ylmethylene)-hydrazide* (3D). IR (KBr, cm^{-1}) 3179 for N-H, 1686 for C=O of amide, 1620 for C=N of imines, 1543 for N-H bending of amide, 1254 and 1045 for C-O of phenyl ether. **^{1}H NMR** (DMSO-d_6, 300MHz) δ ppm: 1.20-1.23 (6H, d, for CH_3 of isopropyl), 2.28-2.30 (3H, s, for Ar-CH_3), 3.12 (1H, sept, for Me_2CH), 3.69 (3H, s, for N-CH_3), 5.10 (2H, s, for O-CH_2-CO), 6.74-7.34 (7H, m, for Ar-H), 7.57 (1H, t, for pyrrole-H), 8.18 (1H, s, for N=CH), 11.12 (1H, s, for NH). **ESI-MS**: MS: m/z 363 (M^+).

2.3.4.5. *(2-Isopropyl-5-methyl-phenoxy)-acetic acid (4-imidazol-1-yl-benzylidene)-hydrazide* (3E). IR (KBr, cm^{-1}) 3113 for N-H, 1697 for C=O of amide, 1609 for C=N of imines, 1524 for N-H bending of amide, 1261 and 1061 for C-O of phenyl ether. **^{1}H NMR** (DMSO-d_6, 300MHz) δ ppm: 1.16-1.17 (6H, d, for CH_3 of isopropyl), 2.22-2.25 (3H, s, for Ar-CH_3), 3.12 (1H, sept, for Me_2CH), 5.06 (2H, s, for O-CH_2-CO), 6.66-6.76 (3H, m, for Ar-H), 7.47 (1H, dd, for imidazole-H), 7.82-7.84 (2H, ddd, for Ar-H), 7.88-7.90 (1H, dd, for imidazole-H), 8.04-8.07 (2H, ddd, for Ar-H), 8.41 (1H, s, for N=CH), 8.93-8.95 (1H, dd, for imidazole-H), 11.24 (1H, s, for NH). ESI-MS: MS: m/z 376 (M^+).

2.3.4.6. *(2-Isopropyl-5-methyl-phenoxy)-acetic acid (1-methyl-1H-imidazol-2-ylmethylene)-hydrazide* (3F). IR (KBr, cm^{-1}) 3132 for N-H, 1701 for C=O of amide, 1609 for C=N of imines, 1524 for N-H bending of amide, 1285 and 1069 for C-O of phenyl ether. **^{1}H NMR** (DMSO-d_6, 300MHz) δ ppm: 1.16-1.17 (6H, d, for CH_3 of isopropyl), 2.25 (3H, s, for Ar-CH_3), 3.10 (1H, sept, for Me_2CH), 3.69 (3H, s, for N-CH_3), 4.93 (2H, s, for O-CH_2-CO), 6.49-6.76 (3H, m, for Ar-H), 7.58-7.70 (2H, dd, for imidazole-H), 8.21 (1H, s, for N=CH), 11.14 (1H, s, for NH). **ESI-MS**: MS: m/z 314 (M^+).

2.3.4.7. *(2-Isopropyl-5-methyl-phenoxy)-acetic acid (6-methyl-pyridin-2-ylmethylene)-hydrazide* (3G). IR (KBr, cm^{-1}) 3059 for N-H, 1717 for C=O of amide, 1624 for C=N of imines, 1539 for N-H bending of amide, 1250 and 1080 for C-O of phenyl ether. **^{1}H NMR** (DMSO-d_6, 300MHz) δ ppm: 1.20-1.23 (6H, d, for CH_3 of isopropyl), 2.28-2.30 (3H, s, for Ar-CH_3), 2.52-2.54 (3H, s, for pyridine-CH_3), 3.10 (1H, sept, for Me_2CH), 5.10 (2H, s, for O-CH_2-CO), 6.74-7.34 (6H, m, for Ar-H), 8.18 (1H, s, for N=CH), 11.12 (1H, s, for NH). **ESI-MS**: MS: m/z 325 (M^+).

2.3.4.8. *(2-Isopropyl-5-methyl-phenoxy)-acetic acid (4-trifluoromethyl-benzylidene)-hydrazide* (3H). IR (KBr, cm^{-1}) 3202 for N-H, 1701 for C=O of amide, 1605 for C=N of imines, 1516 for N-H bending of amide, 1242 and 1069 for C-O of phenyl ether. **^{1}H NMR** (DMSO-d_6, 300MHz) δ ppm: 1.22-1.24 (6H, d, for CH_3 of isopropyl), 2.26-2.28 (3H, s, for Ar-CH_3), 3.12 (1H, sept, for Me_2CH), 4.90 (2H, s, for O-CH_2-CO), 6.50-6.76 (3H, m, for Ar-H), 7.41-7.66 (4H, m, for Ar-H), 8.02 (1H, s, for N=CH), 10.58 (1H, s, for NH). **ESI-MS**: MS: m/z 378 (M^+).

2.3.4.9. *(2-Isopropyl-5-methyl-phenoxy)-acetic acid (4-trifluoromethoxy-benzylidene)-hydrazide* (3I). IR (KBr, cm^{-1}) 3198 for N-H, 1693 for C=O of amide, 1605 for C=N of imines, 1508 for N-H bending of amide, 1261 and 1072 for C-O of phenyl ether. **^{1}H NMR** (DMSO-d_6, 300MHz) δ ppm: 1.24-1.26 (6H, d, for CH_3 of isopropyl), 2.26-2.28 (3H, s, for Ar-CH_3), 3.14 (1H, sept, for Me_2CH), 5.10 (2H, s, for O-CH_2-CO), 6.56-6.76 (3H, m, for Ar-H), 7.12-7.20 (2H, m, for Ar-H), 7.42-7.50 (2H, m, for Ar-H), 8.10 (1H, s, for N=CH), 11.67 (1H, s, for NH). **ESI-MS**: MS: m/z 394 (M^+).

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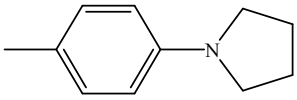
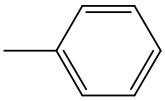
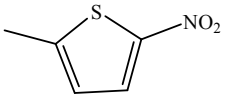
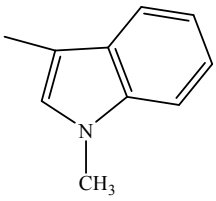
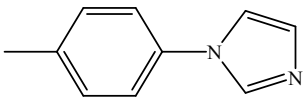
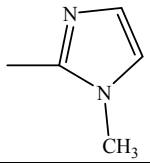
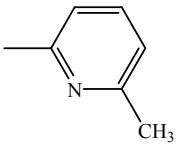
2.3.4.10. ***(2-Isopropyl-5-methyl-phenoxy)-acetic acid (2-hydroxy-benzylidene)-hydrazide*** **(3J). IR** (KBr, cm^{-1}) 3290 for O-H, 3190 for N-H, 1686 for C=O of amide, 1597 for C=N of imines, 1555 for N-H bending of amide, 1292 and 1030 for C-O of phenyl ether. **^{1}H NMR** (DMSO-d_6, 300MHz) δ ppm: 1.16-1.17 (6H, d, for CH_3 of isopropyl), 2.23-2.25 (3H, s, for Ar-CH_3), 3.30 (1H, sept, for Me_2CH), 4.90 (2H, s, for O-CH_2-CO), 5.20 (1H, s, for aromatic C-OH), 6.50-6.76 (3H, m, for Ar-H), 7.06-7.71 (4H, m, for Ar-H), 8.02 (1H, s, for N=CH), 11.57 (1H, s, for NH). **ESI-MS**: MS: m/z 326 (M^+).

Table – 1: Physical characteristics of synthesized compounds 3A-J

Compound	Ar' (aryl group)	Molecular Weight (Mol. formula)	Yield (%)	m.p. (^{0}c)
3A		379.49 ($C_{23}H_{29}N_3O_2$)	88.46	220
3B		310.39 ($C_{19}H_{22}N_2O_2$)	78.42	147
3C		361.41 ($C_{17}H_{19}N_3O_4S$)	72.64	152
3D		363.46 ($C_{22}H_{25}N_3O_2$)	68.00	137
3E		376.45 ($C_{22}H_{24}N_4O_2$)	65.00	124
3F		314.38 ($C_{17}H_{22}N_4O_2$)	75.78	193
3G		325.40 ($C_{19}H_{23}N_3O_2$)	62.28	149

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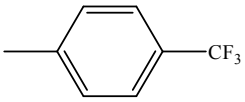
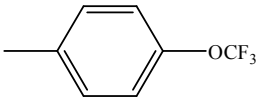
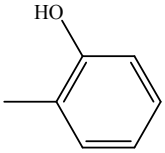
3H		378.39 ($C_{20}H_{21}N_2O_2F_3$)	59.78	176
3I		394.39 ($C_{20}H_{21}N_2O_3F_3$)	72.12	185
3J		326.39 ($C_{19}H_{22}N_2O_3$)	80.22	142

Screening for biological activity: The synthesized compounds **3A-3J** were screened for antibacterial activity using *Staphylococcus aureus* (MTCC 737), *Escherichia coli* (MTCC 452) and antifungal activity using*Candida albicans* (MTCC 227) by disk diffusion method at a concentration of 2 mg/mL using DMF as solvent. Ampicillin 1 mg/mL and fluconazole 2.5 mg/mL were used as standards. The results were recorded using ampicillin and fluconazole as standards are given inTable-2.

Table 2: Antimicrobial activity of compounds 3A-3J

Compound number	Zone of inhibition in mm		
	Antibacterial activity		Antifungal activity
	S. aureus	*E. coli*	*C. albicans*
3A	20	20	12
3B	18	18	11
3C	24	24	16
3D	22	22	13
3E	17	18	11
3F	24	23	15
3G	19	18	12
3H	17	16	10
3I	19	18	12
3J	21	20	13
Ampicillin	25	24	---
Fluconazole	---	---	17

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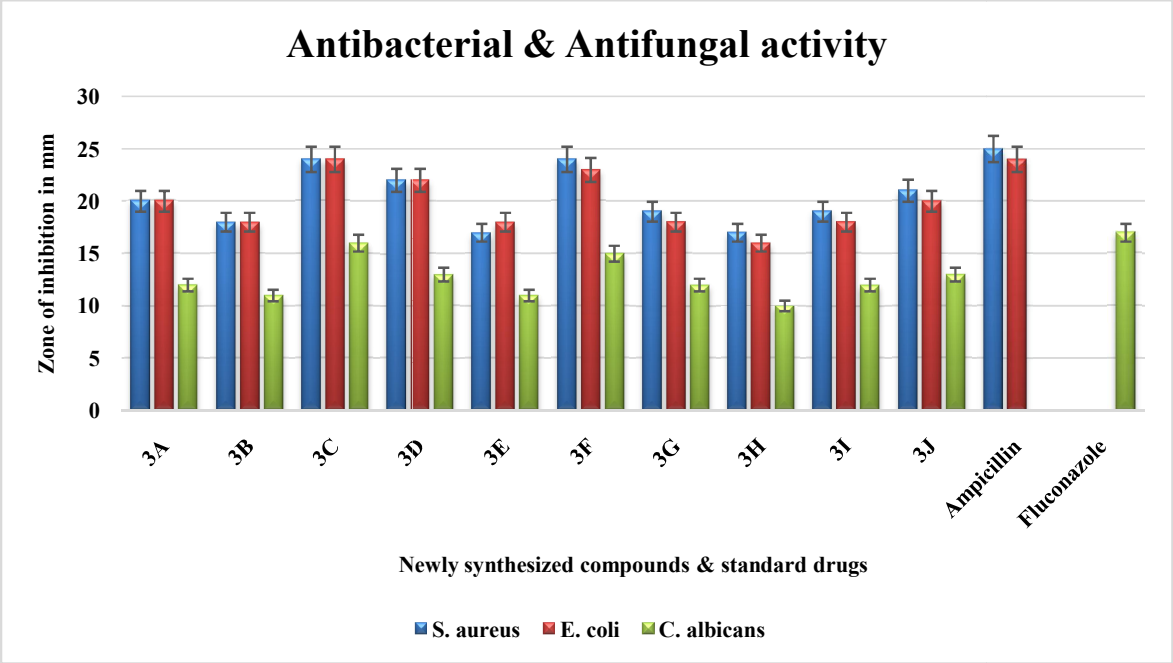
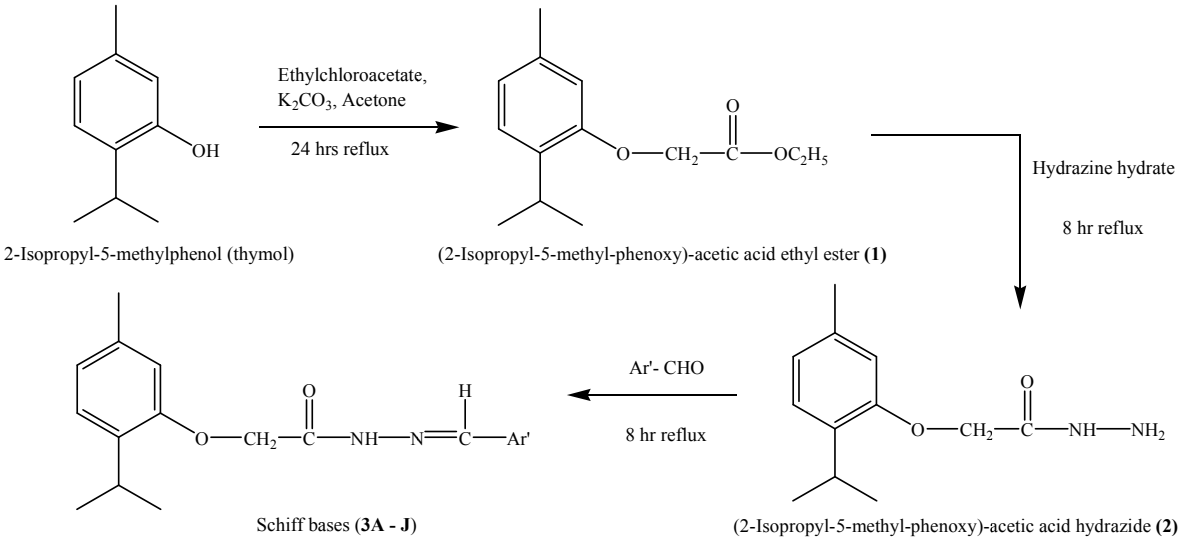


III. RESULTS AND DISCUSSION

The synthetic route is outlined in Scheme-I. (2-Isopropyl-5-methyl-phenoxy)-acetic acid ethyl ester (**1**) was synthesized by refluxing 2-Isopropyl-5-methylphenol (thymol) with ethylchloroacetate in dry acetone. Compound **1** on reacting with hydrazine hydrate gave (2-Isopropyl-5-methyl-phenoxy)-acetic acid hydrazide (**2**). Condensation of **2** with various aromatic aldehydes afforded the potent antibacterial and antifungal Schiff bases (**3A-3J**). Physical data of **3A-3J** are given in Table-1. The structures of all compounds were characterized by spectral analysis. All the synthesized compounds **3A-3J** had shown good antibacterial and antifungal activity (Table-2).



where Ar'-CHO = aromatic & heterocyclic aldehydes

Scheme – I: Conversion of thymol into Schiff bases

First of all nucleophilic substitution reaction take place between 2-Isopropyl-5-methylphenol (thymol) and ethylchloroacetate in the basic medium to produce the corresponding ethyl ester (**1**).

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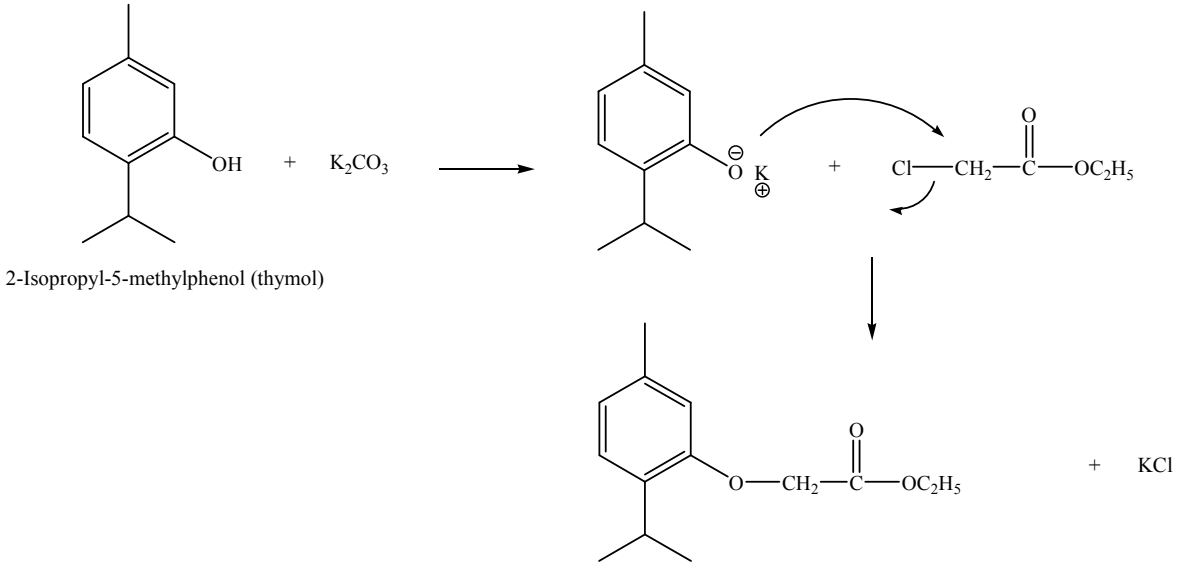
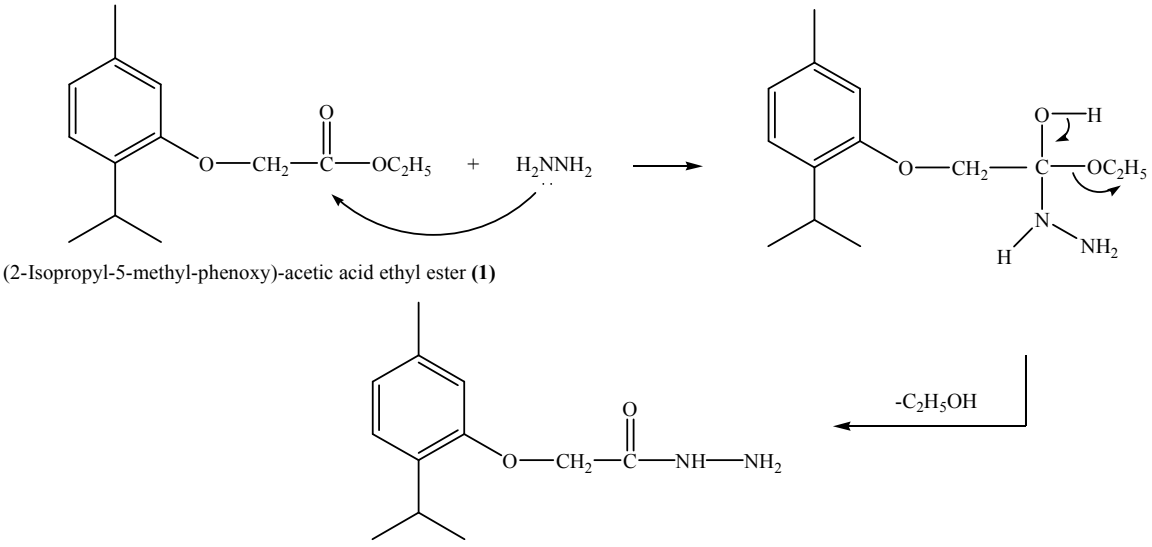


2-Isopropyl-5-methylphenol (thymol)

(2-Isopropyl-5-methyl-phenoxy)-acetic acid ethyl ester **(1)**

The second step involves the synthesis of acid hydrazide through the reaction between hydrazine and the ethyl ester **(1)**. The reaction proceeds by nucleophilic substitution of hydrazine to the ethyl ester carbonyl group to give the corresponding hydrazide **(2)**.

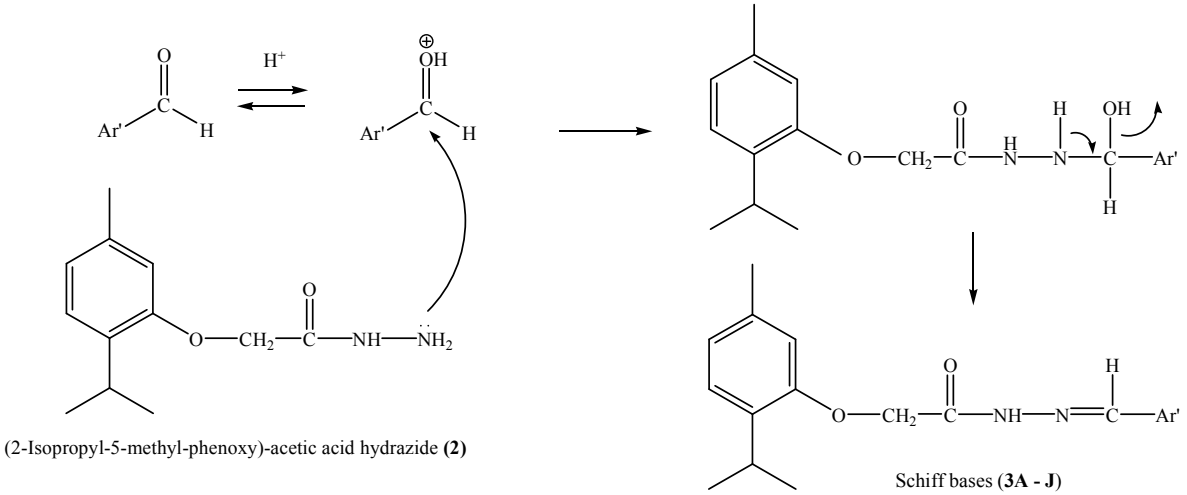


(2-Isopropyl-5-methyl-phenoxy)-acetic acid ethyl ester **(1)**

(2-Isopropyl-5-methyl-phenoxy)-acetic acid hydrazide **(2)**

The final step involves nucleophilic attack by the basic nitrogen compound on carbonyl carbon. Protonation of carbonyl oxygen in presence of acid makes carbonyl carbon more susceptible to nucleophilic attack. The addition product undergoes dehydration to produce Schiff bases **(3A-J)**.

IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
Impact Factor: 7.53
Volume 4, Issue 1, September 2024



(2-Isopropyl-5-methyl-phenoxy)-acetic acid hydrazide **(2)**

Schiff bases (**3A - J**)

IV. CONCLUSION

The successful synthesis of Schiff bases was confirmed through FTIR spectroscopy, 1H-NMR, and mass spectrometry, revealing the presence of characteristic functional groups and validating the structures. Antimicrobial evaluation of the synthesized compounds (3A to 3J) demonstrated significant antibacterial and antifungal activities. Among the derivatives, compound 3C exhibited the highest potency, comparable to standard antibiotics such as ampicillin and fluconazole, particularly against Staphylococcus aureus, Escherichia coli, and Candida albicans. Compounds 3D and 3F also showed notable antimicrobial effects. The highest activity was observed in Schiff bases containing a nitro thiophene moiety, underscoring the potential of these derivatives as promising candidates for further development as antimicrobial agents. These findings suggest that the synthesized Schiff bases could serve as a valuable scaffold for new antimicrobial therapeutics.

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