Synthesis, Characterization and Pharmacological Evaluation of Antimicrobial Activity of Novel 1,2,4-Triazole Derivatives

[1]Ashish G Lonkar*, [2]Balaji N Thakare, [2]Ashish D Tale, [3]Yuvraj L Pandhare

[1]P. Wadhwani College of Pharmacy, Yavatmal-445001, (MS) India

[2]MUPS College of Pharmacy, Degaon, Tq. Risod, Dist.Washim-444506, (MS) India

[3]MUPS Institute of Pharmacy, Degaon, Tq. Risod, Dist.Washim-444506, (MS) India

Abstract : The 1,2,4-Triazole nucleus and there fused heterocyclic derivatives are associated with various pharmacological activities and are potential linker moieties. The derivatives of 2,4-Dihydro-4-{4-[4-(4-(3-Chloropropan-One)Phenyl)-1-Piperazinyl] Phenyl}-2-(1-Methylpropyl)-3h-1,2,4-Triazole-3-One were synthesized and the structure of synthesized compound confirmed by TLC and spectroscopic techniques like 1H NMR, IR and mass spectrometry. All the synthesized compounds were screened for antibacterial and anti-fungal activities using agar well diffusion method against *Escherichia coli, Staphylococcus aureus, Pseudomonas aerugenosa* and *Klepsiella pneumoniae* bacterial strain and *Candida albicans,* and *Aspergillus Niger* pathogenic fungi at various concentration such as 50 µg/ml, 100 µg/ml, 200 µg/ml, 250 µg/ml and 300 µg/ml. The reference standard use for bacterium was Amikacine sulphate (10 µg/ml) and for fungi was ketoconazole (15 µg/ml). It has been observed that the entire tested compound's showed mild to moderate activity against tested bacteria and fungi. Some of them have good activity at observed concentration.

Key words- Triazole, 1H NMR, IR, mass spectrometry, antibacterial and antifungal.

INTRODUCTION

Since resistance to antimicrobial drugs is widespread therefore, it is necessary to search for and synthesize new classes of antimicrobial compounds that are effective against pathogenic microorganisms that have developed resistance to the antibiotics. Moreover, from the pharmacoeconomic cost-efficiency viewpoint, and seeking better patient compliance, antimicrobial agents with high therapeutic effect, high safety, and minimum adverse effects are considerably desirable. Therefore, there is an urgent need to develop novel antimicrobial and antitubercular chemotherapeutic agents [1].

Now a day's research is concentrated towards the introduction of new and safe therapeutic agents of clinical importance. The heterocycles are enjoying their importance as being the center of activity. The nitrogen containing heterocycles are found in abundance in most of the medicinal compounds. The success of imidazole as an important moiety of number of medicinal agents led to introduction of the triazoles. The triazoles are said to be the isosters of imidazoles in which the carbon atom of imidazole is isosterically replaced by nitrogen [2].

This interesting group of compound i.e. 1,2,4-Triazole nucleus is associated with various pharmacological activities and are potential linker moieties has diverse biological activities such as anti-microbial activity, Anti-malarial, Antifungal, anti-inflammatory, fungicidal, herbicidal, pesticidal, analgesic, anticonvulsant, anti-HIV etc. Keeping this observation in view we had decide to synthesize 1,2,4-triazole derivatives.

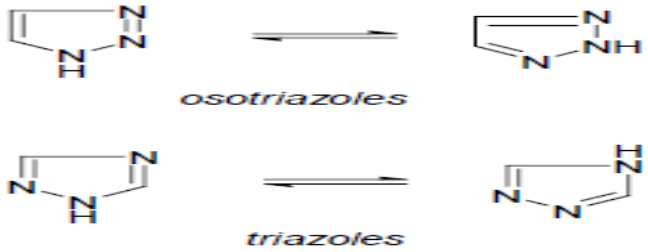
Chemistry of triazole

Triazole is a five membered heterocyclic compound containing two carban atom and three nitrogen atom. According to the position of nitrogen atoms the triazoles are exists in isomeric forms.

Two structural isomeric triazoles are known, the 1,2,3-(1,2,5-) and the 1,2,4-(1,3,4-) the former being known as *osotriazole*, and the latter as *triazole.* Each exists in two dissimilar tautomeric forms. The different isomers are characterized by the position of the nascent hydrogen. Thus 1,2,4-triazoles are exist in two forms i.e. 1*H* and 4*H*.

Replacement of the imino hydrogen atom by an alkyl or aryl group prevents the tautomerism and thereby gives rise to the possibility of two 1-substituted triazoles and two 1-substituted osotriazoles.

Triazole moiety is an important and frequent insecticide, agrochemical structure feature of many biological active compound as cytocrome p450 enzyme inhibitors and peptide analog inhibitor. The azole class of antifungal agent is chemical either an imidazole or a triazole group joined to an asymmetric carbon atom as their functional pharmacophore treatment for these infection.



Azole like antifungal agent are Ketoconazole, Fluconazole, Voriconazole and Ptraconazole 1,2,4-triazole are as analgesic antiasthmatic, antibacterial, anticholinergic activity. They are aromatic ring compounds similar to the azole, pyrazole and imidazole but with an additional nitrogen atom in the ring structure. In the last few decades, the chemistry of 1,2,4-triazoles nucleus and their fused heterocyclic derivatives has received considerable attention owing to their synthetic and effective biological importance. For example, a large number of 1,2,4-triazole-containing ring system have been incorporated into a wide variety of therapeutically interesting drug candidates including anti-inflammatory, CNS stimulants sedatives, antianxiety, antimicrobial agents and antimycotic activity such as fluconazole, intraconazole, voriconazole [3]. Also, there are known drugs containing the 1,2,4-triazole group e.g. **Triazolam, Alprazolam, Etizolam**, and **Furacylin**. Moreover, sulphur containing heterocycles represent an important group of sulphur compounds that are promising for use in practical applications. Among these heterocycles, the mercapto- and thione-substituted 1,2,4-triazole ring systems have been well studied and so far a variety of biological activities have been reported for a large number of their derivatives, such as antibacterial, antifungal, antitubercular, antimycobacterial, anticancer, diuretic and hypoglycemic properties [4-8].

Anti-Microbial Activity

The earliest evidence of successful chemotherapy is from ancient Peru, where the Indians used bark from the Cinchona tree to treat malaria. Modern chemotherapy has been dated to the work of Paul Ehrlich in Germany, who sought systematically to discover effective agents to treat trypanosomiasis and syphilis. Ehrlich postulated that it would be possible to find chemicals that were selectively toxic for parasites but not toxic to humans. Progress in the development of novel antibacterial agents has been great, but the development of effective, nontoxic antifungal and antiviral agents has been slow. Amphotericin B, isolated in the1950s, remains an effective antifungal agent, although newer agents such as fluconazole are now widely used. An antimicrobial is a substance that kills or inhibits the growth of microbes such as bacteria (antibacterial activity), fungi (antifungal activity) and viruses (antiviral activity). Any attempt to discuss the chemotherapeutic properties of heterocyclic compounds must, of necessity, be confined to a limited aspect of the subject. Therefore, the present discussion will be limited to monocyclic compounds with 5-membered ring. By definition, this includes not only compounds with a single 5-membered ring but also substances with two or more rings, one of which must be six membered. a membrane-polyene complex that alters the membrane permeability, resulting in the polyene antibiotics, which apparently act by binding to membrane sterols, contain a rigid hydrophobic center and a flexible hydrophilic section [10-12].

MATERIALS AND METHODS

Chemicals and instruments

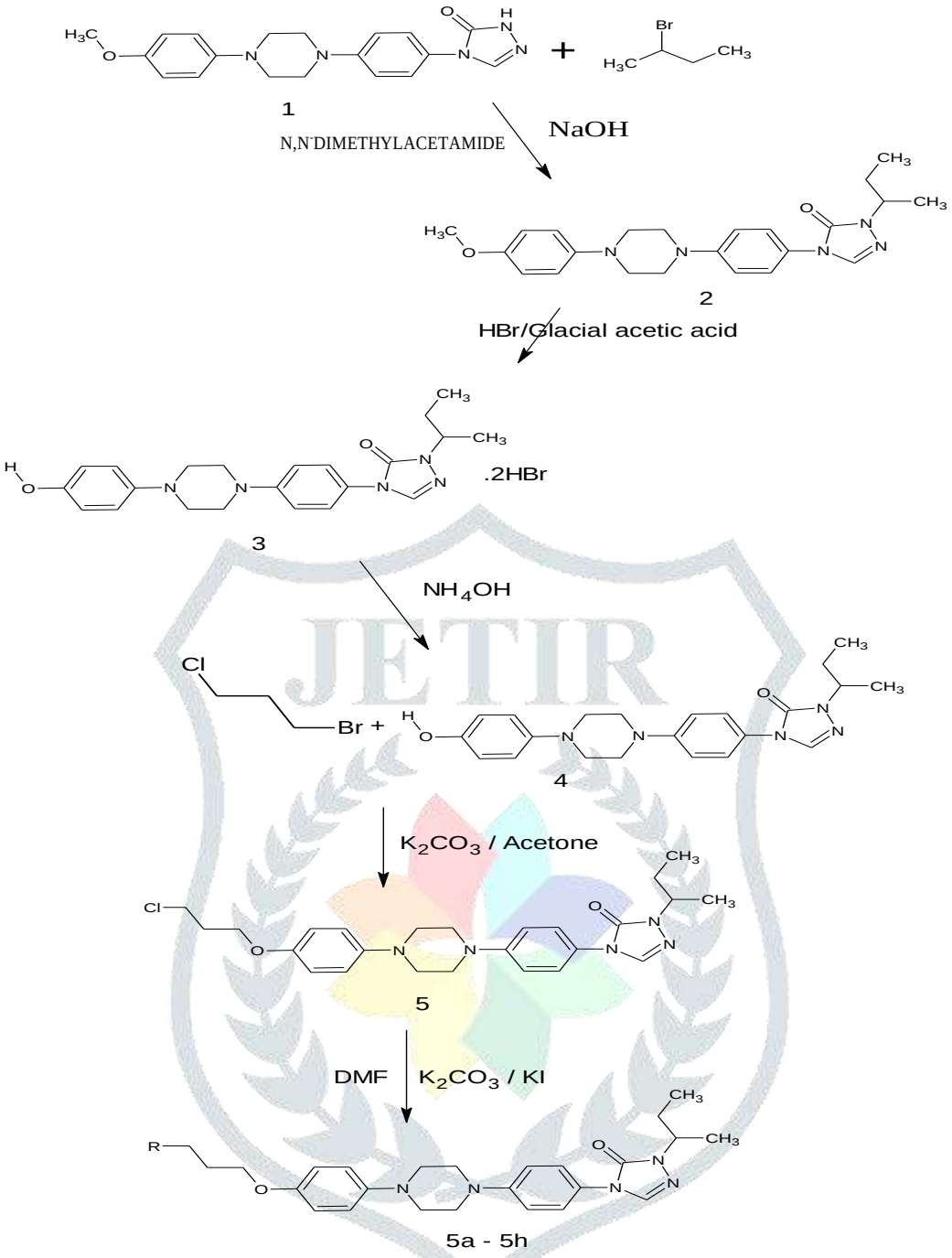
Potassium carbonate (Ramnath & co.ltd), Piperazine (Spectrochem private Ltd, Mumbai), Nmethyl Piperazine (Cata pharma Ltd, Mumbai), Benzyl amine, Potassium iodide (Sam Fine chem. Ltd, Mumbai), Tri-ethylamine (Merck specialities Pvt. Ltd), 1-benzyl piperazine (Spectrochem private Ltd, Mumbai), Di-methyl formamide(RCI Labscane Ltd), 1-bromo-3-chloro propane, 4-methoxy aniline, 4-chloro aniline, Sodium triazole, Mamentine HCl, Ter-butyl amine, Acetone, Potato dextrose agar (LOBA chemicals, Mumbai) and instruments Hot air Oven (Bio-technics india), MASS Spectrophotometer (DART), FTIR Spectrophotometer (Shimadzu), NMR Spectrophotometer (Brucker), Autoclave (Hicon), Incubator (Rolex) and calibrated glass wares were used throughout the work respectively.

Preparation of 1,2,4-Ttriazole derivatives (Summarized in Fig. 1 Scheme of synthesis):

Preparation Of 2, 4-Dihydro-4-{4-[4-(4-Methoxyphenyl)-1-Piperazinyl] Phenyl}-2-(1-Methylpropyl)-3h-1,2,4-Triazole-3-One (2)

The process consist of an alkylation of 2,4-di hydro-4-[4-[4-(4-methoxyphenyl)-1-piperazinyl]phenyl]-3H-1,2,4-triazol-3-one (1 mol) with 2-bromobutane (1 mol) in the presence of a sodium hydroxide solution (2.5 mol) and using N,N-dimethyl acetamide as the solvent. After completion of the reaction, the reaction mixture is treated with activated carbon. After filtration the product is crystallizes by addition of water and seeding. The crystallization is completed by cooling. The crystallized product is isolated, washed with water and dried.

Scheme:



Where R,

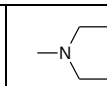
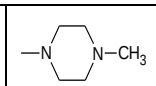
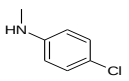
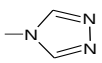
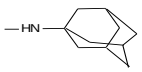
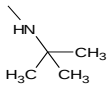
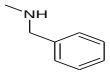
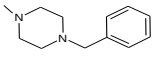
5a		5b		5c		5d	
5e		5f		5g		5h	

Fig. 1: Scheme of Synthesis

Preparation Of 2, 4-Dihydro-4-{4-[4-(4-Hydroxyphenyl)-1-Piperazinyl]Phenyl}-2-(1-Methylpropyl)-3h-1,2,4-Triazole-3-One (3 & 4)

The process consist of demethylation of 2,4-di hydro-4-[4-[4-(4-methoxyphenyl)-1-piperazinyl]phenyl]-3H-1,2,4-triazol-3-one (1 mol) in a mixture of glacial acetic acid, hydrobromic acid (2.5 mol) and water. 2, 4-dihydro-4-{4-[4-(4-hydroxyphenyl)-1-piperazinyl]phenyl}-2-(1-methylpropyl)-3h-1,2,4-triazole-3-oneis first crystallized as dihydrobromic acid salt. The crystallized product is isolated and washed with methanol. The wet dihydrobromic acid salt of 2,4-dihydro-4-{4-[4-(4-hydroxyphenyl)-1-piperazinyl]phenyl}-2-(1-methyl propyl)-3H-1,2,4-triazole-3-one is converted to the free base with ammonium hydroxide, using methanol as a solvent**.** The product is isolated, washed with methanol and dried.

Preparation Of 2,4-Dihydro-4-{4-[4-(4-(3-Chloropropan-One)Phenyl)-1-Piperazinyl] Phenyl}-2-(1-Methylpropyl)-3h-1,2,4-Triazole-3-One (5)

The process consist of O-alkylation of 2,4-dihydro-4-{4-[4-(4-hydroxyphenyl)-1-piperazinyl]phenyl}-2-(1-methylpropyl)-3H-1,2,4-triazole-3-one (1 mol).with 3-chloro-1-bromo propyl (2.5 mol) in the presence of potassium carbonate (2.25 mol) and acetone as a solvent. The reaction is carried out at reflux temperature for 24 h. The product is then precipitated by addition of water and washed with salt solution. The precipitate was then extracted with dichloro methane and the product was obtained by distillation of dichloro methane. The product is then dried under vacuum [14].

Preparation Of Triazole Derivatives (5a-5h)

The synthesis consist of amination of 2, 4-dihydro-4-{4-[4-(4-(3-chloropropyl)phenyl)-1-piperazinyl]phenyl}-2-(1-methylpropyl)-3h-1,2,4-triazole-3-one (1 mol) with different aromatic amines(1.2 mol) (5a-h)mentioned in above table. The amination was done in the presence of potassium iodide (1.2 mol) and potassium carbonate (4 mol) and dimethylformamide as solvent. .The reaction was carried out at 85^0 for 24-30 h [15-18].

Physical and Spectral Data of Synthesised Compounds

The synthesised intermediates and final derivatives were characterized by physical data like nature, coloure, percentage practical yield (% P.Y.) retention factor (Rf), solubility and FTIR, 1H NMR, Mass spectroscopy. Molecular formula and formula weight were calculated by using ChemSktech (version 12). All compounds were solid in nature **(Table 1 and Fig. 2-6)**. *Solubility of compound:* All compounds were found to be insoluble in water, sparingly soluble in toluene and freely soluble in dichloromethane. Thin layer chromatography was carried out by using readymade thin layer chromatographic plates, for all the compounds by using Stationary phase as silica gel and Mobile phase as chloroform: methanol (9.4:0.6).

Table 1: physical data of synthesized compounds

Comp No.	Molecular formula	Mol. Wt	Color	% yield	R_f value
2	$C_{23}H_{29}N_5O_2$	407	white	85	0.65
3	$C_{22}H_{27}N_5O_2$	393	faint red	80	0.39
4	$C_{25}H_{32}N_5O_2Cl$	469.5	pale red	97	0.69
5	$C_{29}H_{41}N_7O_2$	519	pale yellow	70	0.62
5a	$C_{30}H_{43}N_7O_2$	533	pale yellow	65	0.59
5b	$C_{32}H_{40}N_6O_3$	556	pale red	67	0.65
5c	$C_{36}H_{41}N_6O_2$	589	creamy white	30	0.67
5d	$C_{29}H_{42}N_6O_2$	506	pale yellow	62.99	0.56
5e	$C_{31}H_{37}N_6O_2Cl$	560.5	pale red	67.46	0.64
5f	$C_{36}H_{48}N_7O_2$	610	pale brown	58.18	0.51
5g	$C_{27}H_{34}N_8O_2$	502	pale yellow	64.50	0.63
5h	$C_{32}H_{40}N_6O_2$	540	pale red	59.36	0.53

Comp. 2. FTIR(KBr)cm^1: 1690(c=ostr), 3121(ArC-Hstr), 1509(ArC-Cst), 1327(C-Nstr), 1229(AliC-Nstr), 1611(ArC-Cstr), 3054(Ar C-H str). ^{1}H NMR($CDCl_3$): 0.88(t,3H), 1.38(d,3H), 1.69(m,1H), 1.82(m,1H), 3.22(t,4H), 3.35(t,4H), 3.78(s,3H), 4.26(m,1H), 6.85(d,2H), 6.95(d,2H), 7.01(d,2H), 7.41(d,2H), 7.62(s,1H). Mass (m/z): 407

Comp. 3. FTIR(KBr)cm^1: 1693(c=ostr), 3137(ArC-Hstr), 1510(ArC-Cstr), 1326(C-Nstr), 1226(AliC-Nstr), 1607(ArC-Cstr), 3060(Ar C-H str), 3342(O-H str). ^{1}H NMR($CDCl_3$): 0.88(t,3H), 1.39(d,3H), 1.68(m,1H), 1.83(m,1H), 3.19(t,4H), 4.27(t,4H), 4.27(m,1H),6.81(d,2H), 6.69(d,3H), 7.01(d,2H), 7.41(d,2H), 7.62(s,1H), Mass (m/z): 393

Comp. 4. FTIR(KBr)cm^1: 1683(c=ostr), 3130(ArC-Hstr), 1511(ArC-Cstr), 1317(C-Nstr), 1229(AliC-Nstr), 1614(ArC-Cstr), 3068 (Ar C-H str), 2876(AliC-C str). ^{1}H NMR($CDCl_3$): 0.88(t,3H), 1.38(d,3H), 1.72(m,1H), 1.72(m,1H), 3.24(t,4H), 3.36(t,4H), 4.31(m,1H), 6.85(d,2H), 6.93(d,2H), 7.01(d,2H), 7.41(d,2H), 7.62(s,1H), 2.19(t,2H), 3.72(t,2H), 4.06(t, 2H). Mass (m/z): 469

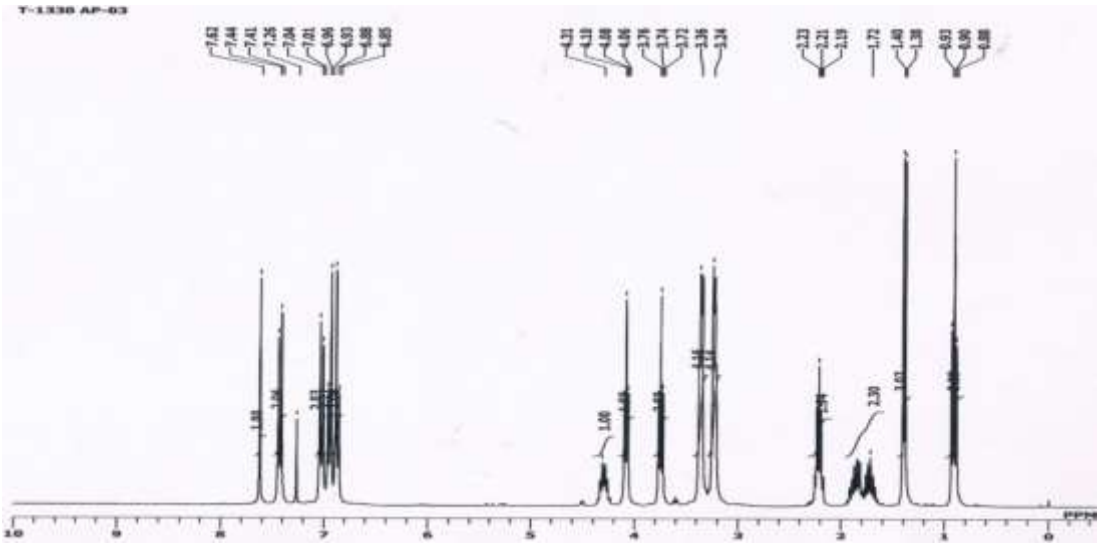


Fig. 2: 1H NMR of Comp. 4

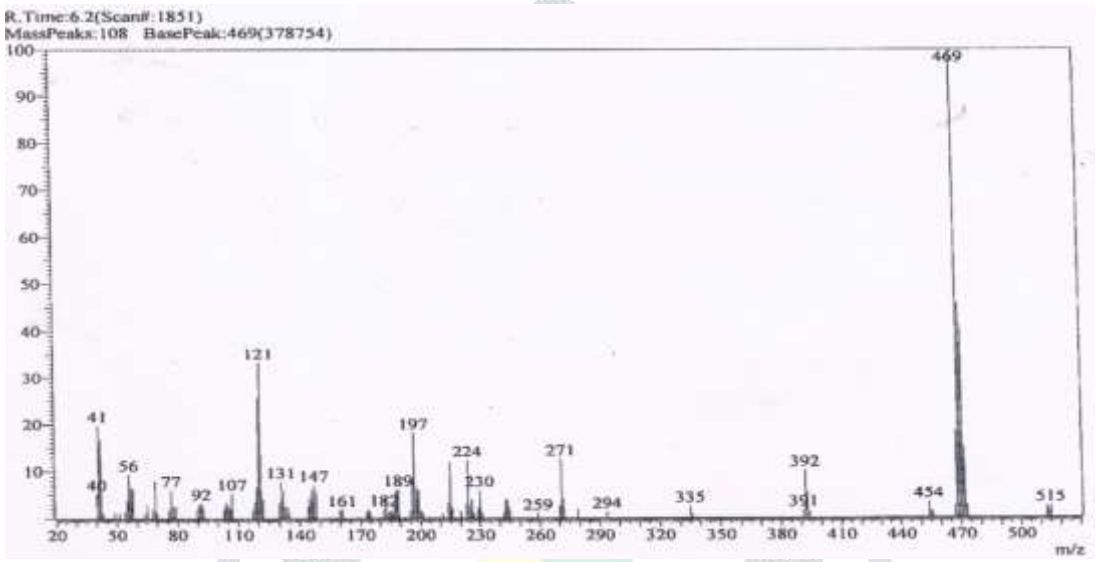


Fig. 3: Mass spectra of Comp. 4

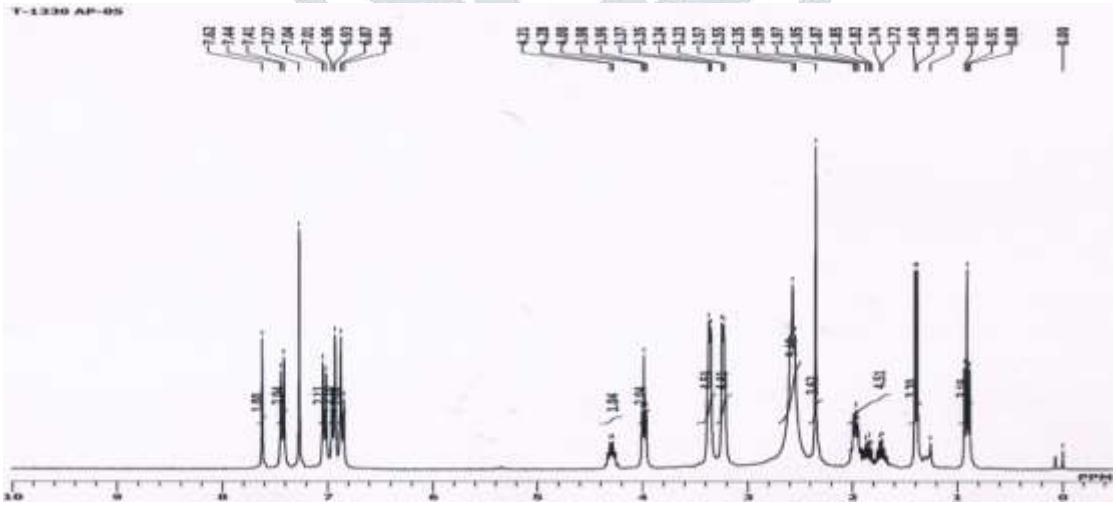


Fig. 4: 1H NMR of Comp. 5a

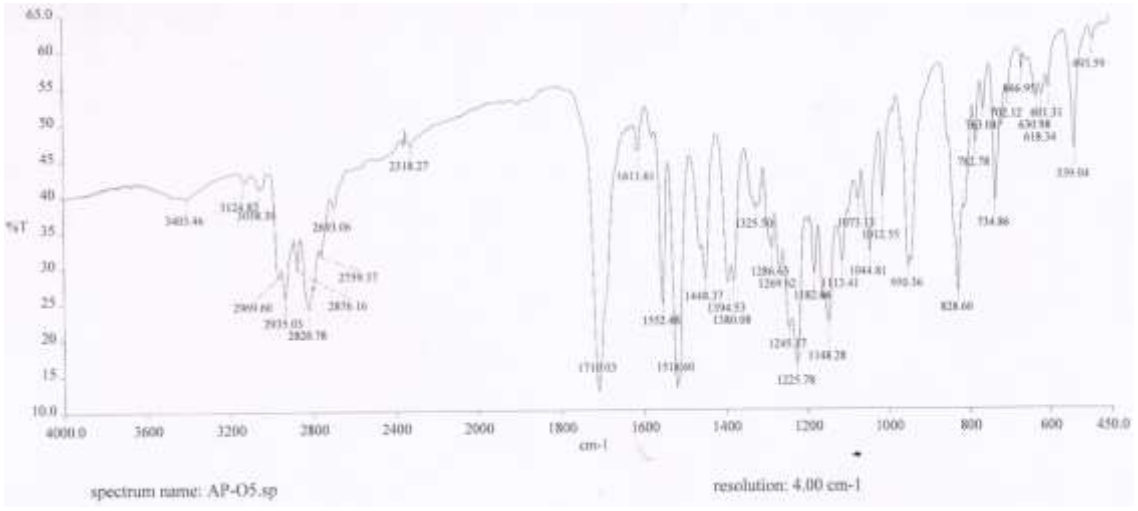


Fig. 5: IR spectra of Comp. 5a

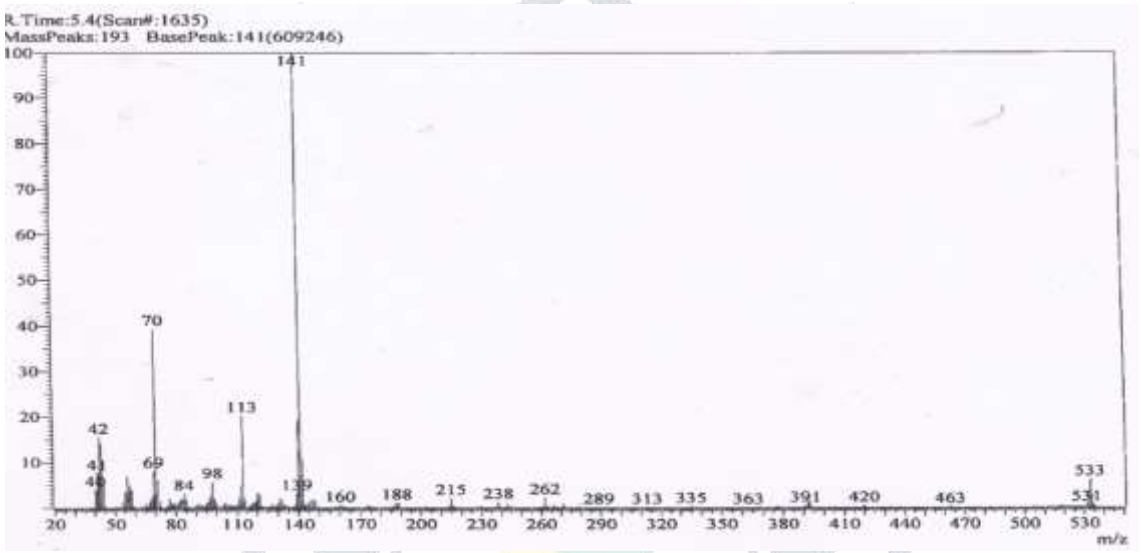


Fig. 6: Mass spectra of Comp. 5a

Comp. 5a. FTIR(KBr)cm[1]: 1698(c=ostr), 3121(ArC-Hstr), 1511(ArC-Cstr) 1324(C-Nstr), 1227(AliC-Nstr), 1608(ArC-Cstr), 3044 (Ar C-H str), 2875(AliC-C str), 1324(ArC-N str). ^{1}H NMR($CDCl_3$): 0.81(t,3H), 0.86(d,3H), 1.65(m,1H), 1.65(m,1H), 3.17(t,4H),3.29(t,4H), 4.31(m,1H), 6.85(d,2H), 6.93(d,2H), 7.01(d,2H), 7.41(d,2H), 7.62(s,1H), 2.19(t,2H), 3.72(t,2H), 4.06(t,2H), 1.19(m,8H), 1.77(s, 1H). Mass (m/z): 523

Comp. 5b. FTIR(KBr)cm[1]: 1710(c=ostr), 3124(ArC-Hstr), 1518(ArC-Cstr), 1325(C-Nstr), 1225(AliC-Nstr), 1611(ArC-Cstr), 3058 (Ar C-H str), 2876(Ali C-C str). ^{1}H NMR($CDCl_3$): 0.88(t,3H), 1.26(d,3H), 1.82(m,1H), 1.95(m,1H), 3.23(t,4H), 3.35(t,4H), 4.28(m,1H), 6.85(d,2H), 6.93(d,2H), 7.01(d,2H), 7.41(d,2H), 7.62(s,1H), 2.19(t,2H), 3.72(t,2H), 4.06(t,2H), 2.35(s,3H), 2.55(m, 8H). Mass (m/z): 533

Comp. 5c. FTIR(KBr)cm[1]: 1691(c=ostr), 2966(ArC-Hstr), 1514(ArC-Cstr), 1320(C-Nstr), 1228(AliC-Nstr), 1600(ArC-Cstr), 2966 (Ar C-H str), 2826(AliC-C str), 0735(C-Cl str). ^{1}H NMR($CDCl_3$): 0.88(t,3H), 1.38(d,3H), 1.69(m,1H), 1.82(m,1H), 3.23(t,4H), 3.34(t,4H), 4.28(m,1H), 6.85(d,2H), 6.93(d,2H), 7.01(d,2H), 7.41(d,2H), 7.62(s,1H), 2.19(t,2H), 3.72(t,2H), 4.06(t,2H), .4.28(s,1H), 6.93(m,4H). Mass (m/z): 560

Comp. 5d. FTIR(KBr)cm[1]: 1698(c=ostr), 3121(ArC-Hstr), 1511(ArC-Cstr), 1324(C-Nstr), 1227(AliC-Nstr), 1608(ArC-Cstr), 3044 (Ar C-H str), 2875(AliC-C str). ^{1}H NMR($CDCl_3$): 0.87(t,3H), 1.37(d,3H), 1.71(m,1H), 1.74(m,1H), 3.24(t,4H), 3.36(t,4H), 4.31(m,1H), 6.85(d,2H), 6.93(d,2H), 7.01(d,2H), 7.41(d,2H), 7.62(s,1H), 2.19(t,2H), 3.72(t,2H), 4.06(t,2H), 7.95(s,2H). Mass (m/z): 502

Comp. 5e. FTIR(KBr)cm[1]: 1708(c=ostr), 3122(ArC-Hstr), 1519(ArC-Cstr), 1324(C-Nstr), 1227(AliC-Nstr), 1610(ArC-Cstr), 3060 (Ar C-H str), 2875(Ali C-C str). ^{1}H NMR($CDCl_3$): 0.88(t,3H), 1.38(d,3H), 1.72(m,1H), 1.72(m,1H), 3.24(t,4H), 3.36(t,4H), 4.31(m,1H), 6.85(d,2H), 6.93(d,2H), 7.01(d,2H), 7.41(d,2H), 7.62(s,1H), 2.19(t,2H), 3.72(t,2H), 4.06(t,2H), 4.01(s,1H). Mass (m/z): 601

Comp. 5f. FTIR(KBr)cm[1]: 1684(c=ostr), 3122(ArC-Hstr), 1513(ArC-Cstr), 1320(C-Nstr), 1225(AliC-Nstr), 1612(ArC-Cstr), 3056 (Ar C-H str), 2854(AliC-C str). ^{1}H NMR($CDCl_3$): 0.88(t,3H), 1.38(d,3H), 1.74(m,1H), 1.74(m,1H), 3.23(t,4H), 3.36(t,4H), 4.28(m,1H), 6.85(d,2H), 6.93(d,2H), 7.01(d,2H), 7.41(d,2H), 7.62(s,1H), 2.19(t,2H), 3.72(t,2H), 4.06(t,2H), 1.19(s,9H), 3.23(s, 1H). Mass (m/z): 506

Comp. 5g. FTIR(KBr)cm^{1}: 1698(c=ostr), 3121(ArC-Hstr), 1511(ArC-Cstr), 1324(C-Nstr), 1227(AliC-Nstr), 1608(ArC-Cstr), 3044 (Ar C-H str), 2875(AliC-C str). ^{1}H NMR($CDCl_3$): 0.88(t,3H), 1.38(d,3H), 1.72(m,1H), 1.72(m,1H), 3.24(t,4H), 3.36(t,4H), 4.31(m,1H), 6.85(d,2H), 6.93(d,2H), 7.01(d,2H), 7.41(d,2H), 7.62(s,1H), 2.19(t,2H), 3.72(t,2H), 4.06(t,2H), 1.98(m,1H), 4.02(m,2H), 6.87(d,2H), 7.26(m,3H). Mass (m/z): 540

Comp. 5h. FTIR(KBr)cm^{1}: 1708(c=ostr), 3122(ArC-Hstr), 1519(ArC-Cstr), 1324(C-Nstr), 1227(AliC-Nstr), 1610(ArC-Cstr), 3060 (Ar C-H str), 2875(AliC-C str). ^{1}H NMR($CDCl_3$): 0.88(t,3H), 1.38(d,3H), 1.72(m,1H), 1.72(m,1H), 3.24(t,4H), 3.36(t,4H), 4.31(m,1H), 6.87(d,3H), 6.93(d,2H), 7.01(d,2H), 7.41(d,2H), 7.62(s,1H), 2.19(t,2H), 3.72(t,2H), 4.06(t,2H), 2.50(s,8H), 3.51(s,2H), 7.31(d,2H). Mass (m/z): 609

RESULT AND DISCUSSION

In the present study twelve novel molecules were synthesized, characterized and evaluated for the antibacterial as well as antifungal activity. The synthesis was carried out according to literature survey, the starting material use for the synthesis of triazole derivatives first checked for their moisture content and the reaction was carried out in basic condition to obtained 2,4-dihydro-4-{4-[4-(4-methoxyphenyl)-1-piperazinyl] phenyl}-2-(1-methylpropyl)-3H-1,2,4-triazole-3-one. Readymade TLC plates were use for in process reaction monitoring. The hydroxyl derivative was obtained by demethylation of 2,4-dihydro-4-{4-[4-(4-methoxyphenyl)-1-piperazinyl]phenyl}-2-(1-ethylpropyl)-3H-1,2,4-triazole-3-one. The color compound was prepared in mild basic condition as too strong base may hamper the piperazine ring structure. As in the chloro compound chlorine atom is highly electrophylic and active. As per literature different aromatic amino substitution made to prepare series of 1,2,4-Triazole derivatives. During the synthesis some impurities were observed along with the derivative which can be removed by using Colum chromatography.

The synthesized derivatives were confirmed by spectral data e.g. IR spectroscopy, NMR spectroscopy and Mass spectroscopy. Every compound shows carbonyl (=C=O) stretching in between 1690-1710 cm^{-1},aromatic C-H stretching in 3121-2966 cm^{-1},aromatic C-C stretching in the range of 1509-1520 cm^{-1}, aromatic C-N stretching in the range of 1300-1330 cm^{-1},aliphatic C-C stretching at 2854 cm^{-1}.The NMR spectral data was matching with the predetermine structures, some compounds shows trace amount of impurity. And further it confirmed by mass spectra as molecular weight is exactly matching with peaks observed.

CONCLUSION

All the synthesized compounds were screened for antibacterial and anti-fungal activities using agar well diffusion method under culture condition given in **Table 2,** against *Escherichia coli, Staphylococcus aureus, Pseudomonas aerugenosa* and *Klepsiella pneumoniae* bacterial strain and *Candida albicans,* and *Aspergillus Niger* pathogenic fungi at various concentration such as 50 µg/ml, 100 µg/ml, 200 µg/ml, 250 µg/ml and 300 µg/ml. The reference standard use for bacterium was Amikacine sulphate (10 µg/ml) and for fungi was ketoconazole (15 µg/ml). It has been observed that the entire tested compound's showed mild to moderate activity against tested bacteria and fungi. Some of them have good activity at observed concentration **(Table 3 and 4)**.

Table 2: Culture conditions for inoculum preparation

Organism	Suitable Medium	Incubation Temp(^{O}C)	Inoculum Incubation Time	Microbial Recovery Incubation Time
Escherichia coli	Soybean–Casein Digest Broth; Soybean–Casein Digest Agar	32.5 ± 2.5	18 to 24 h	3 to 5 d
Pseudomonas aeruginosa	Soybean–Casein Digest Broth; Soybean–Casein Digest Agar	32.5 ± 2.5	18 to 24 h	3 to 5 d
Staphylococcus aureus	Soybean–Casein Digest Broth; Soybean–Casein Digest Agar	32.5 ± 2.5	18 to 24 h	3 to 5 d
Klepsiella pneumoniae	Soybean–Casein Digest Broth; Soybean–Casein Digest Agar	32.5 ± 2.5	18 to 24 h	3 to 5 d
Candida albicans	Sabouraud Dextrose Agar; Sabouraud Dextrose Broth	22.5 ± 2.5	44 to 52 h	3 to 5 d
Aspergillus niger	Sabouraud Dextrose Agar; Sabouraud Dextrose Broth	22.5 ± 2.5	6 to 10 d	3 to 7 d

Table 3: Zone of inhibition of compounds at various concentrations against bacteria

Comp	Conc. (µg/ml)	Zone of inhibition (in mm)				Comp	Zone of inhibition (in mm)			
		EC	*SA*	*PA*	*KP*		*EC*	*SA*	*PA*	*KP*
	50	-	-	-	13		-	-	-	-
	100	09	-	12	11		-	-	08	10
2	200	-	09	-	12	5c	12	11	11	-
	250	10	-	13	13		12	11	-	11
	300	12	-	14	13		13	12	14	12
	50	06	08	08	09		-	-	-	-
	100	-	09	-	-		08	07	-	10
3	200	11	-	-	-	5d	-	10	9	11
	250	12	11	13	12		-	12	10	10
	300	-	12	14	13		10	-	11	11
	50	-	-	10	10		-	09	-	07
	100	10	-	12	11		-	-	-	-
4	200	-	12	10	11	5e	11	-	12	-
	250	-	-	11	-		12	11	-	10
	300	14	13	-	12		13	12	15	13
	50	-	06	-	-		-	07	07	06
	100	-	-	09	10		07	-	08	06
5	200	10	-	12	10	5f	-	09	-	-
	250	12	10	13	-		-	-	-	-
	300	13	12	15	13		09	10	10	09
	50	-	-	-	-		-	-	-	08
	100	-	08	-	09		-	-	09	-
5a	200	10	-	-	-	5g	10	11	-	-
	250	09	-	12	11		12	13	11	12
	300	11	12	13	12		13	-	12	14
	50	07	-	-	-		-	05	-	-
	100	09	09	-	-		07	-	10	08
5b	200	12	13	11	10	5h	-	10	13	09
	250	-	-	-	-		-	-	16	13
	300	13\	15	13	-		13	14	17	-
	50	**12**	**18**	**09**	**11**					
	100	**12**	**19**	**12**	**11**					
STD	200	**14**	**20**	**12**	**11**					
	250	**15**	**21**	**16**	**14**					
	300	**18**	**22**	**18**	**16**					

Where, *EC :-Escherichia coli, SA :-Staphylococcus aureus PA :-Pseudomonas aeruginos,, KP:-Klepsiella pneumonia, STD :-Amikacin sulphate(10 µg /ml), Solvent:-D.M.S.O.*

Table 4: Zone of inhibition of compounds at various concentrations against fungi

Comp.	Cons (µg/ml)	Zone of inhibition (in mm)		Comp.	Zone of inhibition (in mm)	
		CA	*AN*		*CA*	*AN*
	50	-	08		06	09
	100	10	09		-	08
2	200	11	10	5c	10	13
	250	11	-		12	12
	300	12	11		12	20
	50	05	-		06	09
	100	07	10		08	10
3	200	09	10	5d	10	-
	250	10	11		11	12
	300	11	12		13	12

	50	07	09		-	08
	100	10	12		-	09
4	200	-	12	5e	08	09
	250	11	13		10	10
	300	13	15		10	11
	50	-	-		-	08
	100	09	-		10	10
5	200	09	08	5f	-	11
	250	09	09		12	13
	300	11	11		11	16
	50	07	-		07	-
	100	09	-		-	09
5a	200	-	09	5g	09	10
	250	09	10		12	12
	300	11	11		12	14
	50	06	-		08	09
	100	-	09		09	-
5b	200	11	10	5h	09	10
	250	-	10		12	11
	300	12	12		14	14
	50	**10**	**18**			
	100	**15**	**22**			
STD	200	**16**	**24**			
	250	**18**	**28**			
	300	**21**	**30**			

Where, *CA:-Candida albicans, STD:-Ketoconazole(15* µg */ml),AN:-Aspergillus niger, Solvent:-Dimethylsulfoxide.*

Conflicts of interest

The authors declare that there are no conflicts of interest.

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