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A FASILE SYNTHESIS OF 1- (2, 3 – DICHLOROPHENYL)-4-(1-METHYL-3-PHENYL-1H-1,2,4-TRIAZOL 5-YL) PIPERAZINE MOTIFS AND ITS ANTIMICROBIAL ACTIVITY

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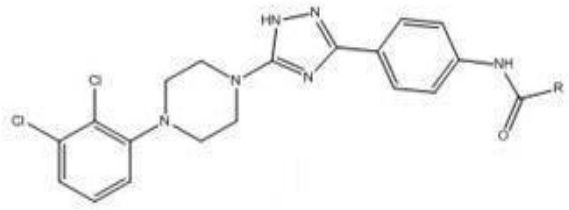


Where R= Alkyl/Aryl group

7a = -CH3	7f = $-C_6H_5$
7b = $-C_2H_5$	7g = $-C_6H_5Cl$
7c = $-C_3H_7$	7h = $-C_6H_5F$
7d = $-CH(CH_3)_2$	7i = $-SC_4H_3$
7e = $-CH(CH_2)_2$	7j = $-NC_5H_5$

ABSTRACT

A Series of 1-(2,3-dichlorophenyl)-4-(1-methyl-3-phenyl-1H-1,2,4-triazol-5-yl) piperazine derivatives have been synthesized, in this study by efficient synthetic protocol. The structures of the compounds were elucidated with the aid of IR, ^1H NMR, Mass spectroscopy and elemental analysis. The synthesized all compounds were evaluated for their in vitro antibacterial activity against gram positive and gram negative bacterial strains and three fungal species.

Key words: 1, 2, 4 – triazol, piperazine, antimicrobial activity, MIC.

Introduction

Piperazine is an interesting heterocyclic moiety as constituent of several biologically active molecules.The polar nitrogen atoms in the piperazine ring confer bioactivity to molecules and enhance favorable interaction with macromolecules [1,2].

Piperazine ring has been found to exhibit wide spectrum of biological activities and it is used in many drugs against different diseases. Some are known to exhibit antihypertensive [3], antiinflammatory [4], antiallergenic [5], antitussive [6], antibacterial [7], antiserotonic [8],antipsychotic [9], anti-influenza [10], anticancer [11], antischizophernia [12], or central nervous system CNS-depressant activity [13].

That azoles and its derivatives are associated with various biological activities as antifungal, antibacterial,[14-17] anti-inflammatory,[18-20] herbicidal,[21] anthelmintic,[22-24] CNS depressant,[25-27] antitumor agent,[28] anticancer,[29-31] antiparasitic,[32] analgesic,[33-34] anticonvalsant,[35] antipyretic and antihypertensive

activity.[36] Itoh Manabu et al [37] proved that 3-arylamino-1,2,4-triazole (1) derivative is remarkably stable in a human serum and is a highly promising pharmaceutical agent.

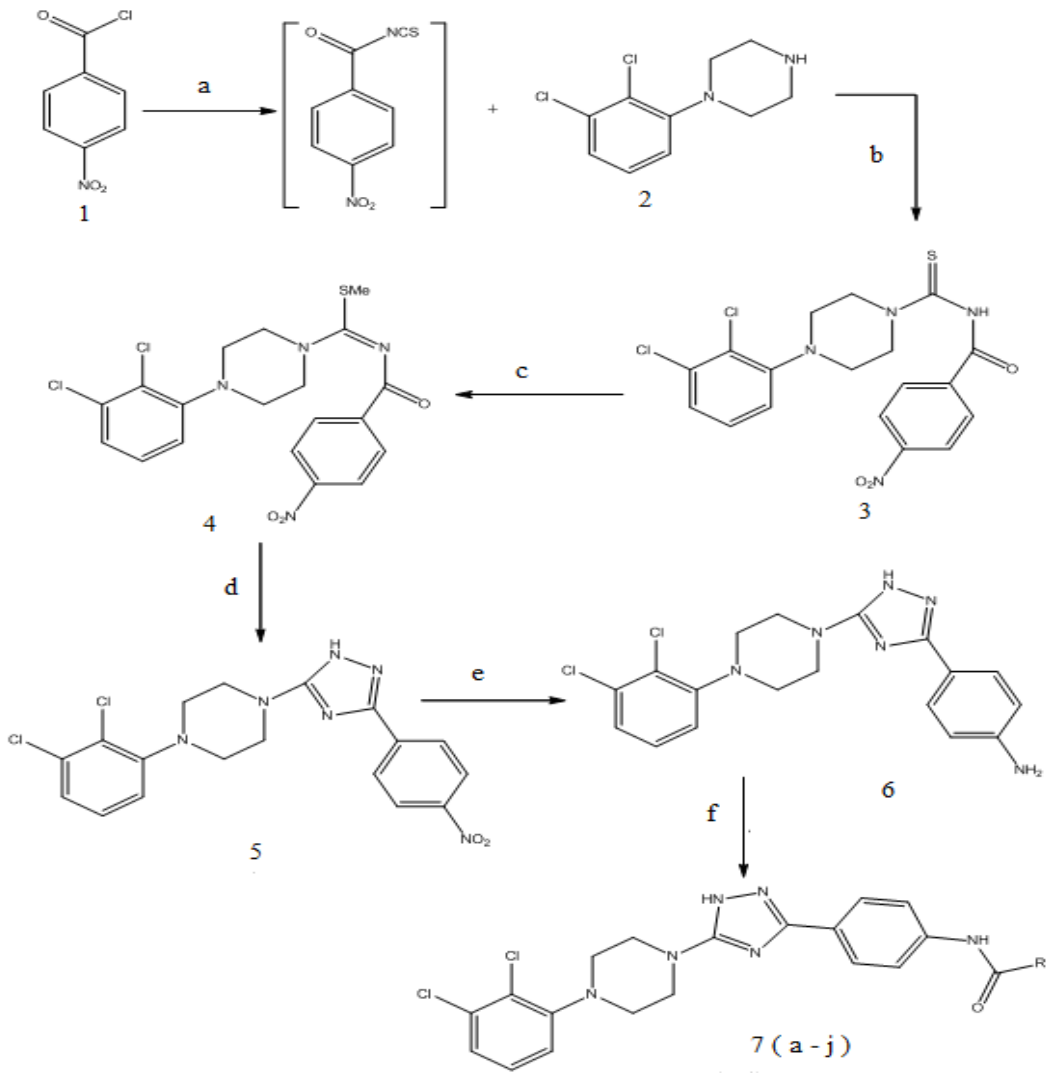
Importance of 1,2,4-triazoles as analytical reagents [38] Substituted 1,2,4-triazoles find many useful applications. Some of them are used as analytical reagents for determination of boron,[39]antimony[40] and cobalt.[41] Other triazoles find many synthetic uses as halogenating agents[42] or as activating polymeric reagents[43]. Now 1,2,4- triazoles derivatives are widely used as biocides [44] and as antifungal agents.[45] Several 1,2,4-triazoles derivatives find applications as photographic reagents.

Looking to the pharmaceutical applications of 1,2,4-triazoles derivatives, in this section we have planned to synthesized some biologically active heterocyclic compounds which contain triazole based on piperazine moiety.

Results and Discussion

Chemistry: The Synthetic route of compounds 7(a – j) are outline in scheme – 1,para – Nitro benzoyl chloride was converted to N-(4-(2,3-dichlorophenyl) piperazine-1-carbonothioyl)-4-nitrobenzamide (3) by treatment with 1-(2,3 dichlorophenyl) piperazine in presence of acetone. Compound N-(4-(2,3-dichlorophenyl) piperazine-1-carbonothioyl)-4-nitrobenzamide reacts with methyl iodide and anhydrous K2CO3 in DMF gives methyl 4-(2,3-dichlorophenyl)-N-(4-nitrobenzoyl) piperazine-1-carbimidothioate (4) in good yield. Methyl 4-(2,3-dichlorophenyl) -N-(4-nitrobenzoyl) piperazine-1-carbimidothioate (4), was reflux with Hydrazine hydrate in Ethanol gives 1-(2,3 dichlorophenyl)-4-(3-(4-nitrophenyl)-1H-1,2,4-triazol-5-yl) piperazine (5) and further reduced by using iron powder in acetic acid to obtained 4-(5-(4-(2,3-dichlorophenyl)piperazin-1-yl)-1H-1,2,4-triazol-3-yl)aniline (6). Compound 4-(5-(4-(2,3-dichlorophenyl)piperazin-1-yl)-1H-1,2,4-triazol-3-yl)aniline (6) on reaction with triethyl amine in dichloromethane followed by acetyl chlorides gives the final compounds N-(4-(5-(4-(2,3-dichlorophenyl)piperazin-1-yl)-1H-1,2,4-triazol-3-yl) phenyl)acetamide [7(a – j)].

Scheme – 1



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7b = $-C_2H_5$ 7g = $-C_6H_5Cl$

7c = -C_3H_7 7h = -C_6H_5F

7d = -$CH(CH_3)_2$ 7i = -SC_4H_3

7j = - NC_2H_5

Scheme – 1 Reagent and condition :(a) NH4NCS,Acetone,15 min,reflux,Yield-80% **(b)** 1-(2,3 dichlorophenyl) piperazine hydrochloride,30 min,reflux,yield-80 % **(c)** MeI,K2CO3,DMF,RT,1Hr,Yield-73% **(d)** Hydrazine hydrate(NH2 NH2 H2O),ethanol,reflux,2 hrs,yield-73% **(e)** Acetic acid,Ferric powder,RT,4-5 Hrs,yield-66% **(f)** trimethyl amine ,MDC,Alkyl/aryl chloride,30 min,10-15°C,2-3 hrs RT, yield-53-73%.

Table 1

Phisical data of compound 7(a-j)

Compound	-R	Mol. Formula	Mol. Weight	mp ^oC	% yield
7a	-CH3	$C_{20}H_{20}Cl_2N_6O$	411	198-200	60
7b	-C_2H_5	$C_{21}H_{22}Cl_2N_6O$	445	205-207	65
7c	-C_3H_7	$C_{22}H_{24}Cl_2N_6O$	459	186-189	71
7d	-$CH(CH_3)_2$	$C_{22}H_{24}Cl_2N_6O$	459	181-183	58
7e	-$CH(CH_2)_2$	$C_{22}H_{22}Cl_2N_6O$	457	191-194	53
7f	-C_6H_5	$C_{25}H_{22}Cl_2N_6O$	493	211-215	73
7g	-C_6H_5Cl	$C_{25}H_{21}Cl_3N_6O$	527.5	229-232	64
7h	-C_6H_5F	$C_{25}H_{21}Cl_2FN_6O$	511	240-243	55
7i	-SC_4H_3	$C_{23}H_{20}Cl_2N_6OS$	499	261-265	61
7j	-NC_5H_5	$C_{24}H_{21}Cl_2N_7O$	494	231-236	58

Biological Activities. The newly synthesized derivatives were evaluated for their in vitro antibacterial activity against E. Coli MTCC 600, Proteus Mirabilis MTCC 9242,Bacillus Pumilus MTCC9584, BacillusCereus MTCC 9762, Saccharomyces Cerevisiae MTCC-2928, Aspergilus Flavus MTCC-2206, Trycoderma Viride MTCC-2150 by micro broth dilution methods. The MIC values are given in Table 2. The standard drug used for antibacterial activity was ciprofloxacin. Mueller Hinton Broth was used as nutrient medium for bacteria and sabouraud dextrose broth for fungal to grow. Inoculums size for test strain was adjusted to 108 CFU/ mL by comparing the turbidity. The serial dilutions were prepared in primary and secondary screening.

The target compounds and standard drugs were dissolved in DMSO-water (1:1 v/v) at a concentration of 2.0 mg/mL. In primary screening, 500 µg/mL, 250 µg/mL and 125 µg/mL concentrations of the synthesized drugs were taken. Data were not taken for the initial solution because of the high DMSO concentration (10%). The active synthesized drugs found in this primary screening were further tested in a second set of dilution against all microorganisms. In secondary screening, the drugs found active in primary screening were similarly diluted to obtain 100 µg/mL, 50 µg/ mL, 25 µg/mL, 12.5 µg/mL and 6.25 µg/mL concentrations. The inoculated wells were incubated overnight at 37°C in a humid atmosphere. The highest dilution showing at least 99% inhibition zone is taken as MIC.

Table 2

Phisical data of compound 7(a-j)

Compound	Minimal Bactercidal Concentration(µg/ml) and Zone of Inhibition in (mm)				Minimal Fungicidal concentration (MFC) (µg/ml)		
	Escherichia Coli MTCC 600	Proteus Mirabilis MTCC 9242	Bacillus Pumilus MTCC 9584	Bacillus Cereus MTCC 9762	Saccharomyces Cerevisiae MTCC-2928	Aspergilus Flavus MTCC-2206	Trycoderma Viride MTCC-2150
7a	1000(22)	250(24)	500(16)	500(28)	500	500	1000
7b	125 (27)	125(31)	1000(32)	1000(33)	500	500	500
7c	--	--	62.5(23)	62.5(21)	1000>	1000>	500
7d	250(22)	500(27)	500(21)	250(20)	500	1000	1000
7e	500(24)	1000(28)	--	1000(25)	1000>	1000>	1000
7f	--	1000(23)	1000(17)	--	1000>	500	500
7g	500(18)	62.5(21)	250(18)	125(18)	500	500	1000
7h	1000(26)	--	--	62.5(14)	500	1000>	1000
7i	--	500(19)	1000(20)	1000(19)	1000>	500	500
7j	62.5(17)	1000(19)	500(26)	--	500	1000>	500
Ciprofloxacin	62.5(35)	62.5(35)	62.5(35)	62.5(35)	--	--	--
Erythromycin	125(34)	125(34)	125(34)	62.5(34)	--	--	--
Aphotericin	--	--	--	--	125	125	250

B							

In 1-(2,3-dichlorophenyl)-4-(1-methyl-3-phenyl-1H-1,2,4-triazol-5-yl)piperazine derivatives (7a-j), compounds 7c have similar activity against *Bacillus pumilis* compared to all standard drugs whereas compounds 7a, 7d and 7g were moderately active than standard drugs. In the case of *Escherichia* coli, except for compounds 7a, 7e, 7g and 7h, all the compounds were moderately active as standard drugs except 7j showed similar activity. In the case of *Proteus mirabilis*, compounds 7b and 7g had better activity than standard drugs whereas others compounds were found to be less active than standard drugs. In the case of *Bacillus cereus*, compounds 7c and 7h was the most potent among the other synthesized compounds.

Conclusions

Present study reports synthesis of novel 4-(5-(4-(2,3-dichlorophenyl)piperazin-1-yl)-1H-1,2,4-triazol-3-yl) aniline and successively amide coupling with different Aryl/alkyl chloride. The antibacterial screening of newly synthesize compounds was carried out against eight different organism, and some of compounds exhibits moderate to excellent activity. Many known drugs posses Piperazine nucleus as active core and it well known to shows the potential pharmacological activities. The antibacterial screening suggests that the newly synthesized compound 7c having Alkyl amide substitution exhibited good to excellent activity against all the tested microorganisms except

Experimental Section

Melting points were determined in open capillary tubes in a Hicon melting point apparatus and are uncorrected .All the Fourier Transform Infra Red (FT-IR) spectra were recorded in KBr pellets on a Shimadzu-8400S spectrometer. The 1 H-NMR spectra were taken on a Bruker- Spectrospin DXC(300MHz)NMR spectrometer. Chemical shifts are expressed in ppm relative to tetramethylsilane (TMS),used as an internal standard. The purity of

the compounds was checked by thin layer chromatography (TLC) on merck Silica Gel 60 F254 precoated sheets. Iodine chamber and UV lamp were used for the visualization of TLC spots.

Preparation of N-(4-(2,3-dichlorophenyl)piperazine-1-carbonothioyl)-4-nitrobenzamide (3)

To a solution of NH4SCN (4.9 g, 0.064 mol) in acetone (50 ml) was added slowly p-nitro benzoylchloride (10 g, 0.054 mol) under dry condition within 10 min. After completion of addition reaction mixture was refluxed for 15 min. A solution of 1-(2,3-dichlorophenyl)piperazine (12.47 g, 0.054 mol) in acetone (20 ml) was added to above stirred suspension at such a rate that reflux gently. After completion of addition reaction mixture was refluxed further for 30 min. Reaction was cooled and poured in water, and resulting yellow solid separated by filtration. Solid was recrystalized in isopropanol giving pale yellow solid as compound (3) (18.8 g, yield 80%).

Preparation of methyl 4-(2,3-dichlorophenyl)-N-(4-nitrobenzoyl) piperazine-1-carbimidothioate (4)

A mixture of N-(4-(2,3-dichlorophenyl)piperazine-1-carbonothioyl)-4-nitrobenzamide (3) (18 g, 0.041 mol), methyl iodide (6.94 g, 0.049 mol) and anhydrous K2CO3 (13.5 g, 0.098 mol) in DMF (90 ml) was stirred at rt for 1 h. Reaction mixture was poured in water (200 ml) with stirring. The resulting light brown solid was filtered, washed with water and dried. This was crystallized from isopropanol giving off-white solid as compound (4) (14.3 g, 73.0 %).

Preparation of 1-(2,3 dichlorophenyl)-4-(3-(4-nitrophenyl)-1H-1,2,4-triazol-5-yl) piperazine (5)

To a solution of methyl 4-(2,3-dichlorophenyl)-N-(4-nitrobenzoyl) piperazine-1-carbimidothioate (4) (14.0 g, 0.031 mol) and Hydrazine hydrate (3.1 g, 0.062 mol) in ethanol (70 ml) was refluxed for 2 h. Reaction was cooled and poured in water, and resulting yellow solid separated by filtration. Solid was recrystalized in IPA giving yellow solid as compound (5) (9.3 g, 73.0 %).

Preparation of 4-(5-(4-(2,3-dichlorophenyl)piperazin-1-yl)-1H-1,2,4-triazol-3-yl)aniline (6)

To a solution of 1-(2,3-dichlorophenyl)-4-(3-(4-nitrophenyl)-1H-1,2,4-triazol-5-yl) piperazine (5) (9 g, 0.021 mol) in acetic acid (50 ml) was added iron powder (2 g) stir at room temperature for 4-5 h. Reaction was poured in water and basify by addition of sat. sodium carbonate to pH 7.5-8. Solid products extracted with ethyl acetate, wash with water and distilled out to get light brown. Solid was recrystalized in methanol giving pure compound (6) (5.5 g, 66.0 %).

Preparation of N-(4-(5-(4-(2,3-dichlorophenyl)piperazin-1-yl)-1H-1,2,4-triazol-3-yl) phenyl) aryl/alkyl amide (7a-j)

To a solution of 4-(5-(4-(2,3-dichlorophenyl)piperazin-1-yl)-1H-1,2,4-triazol-3-yl)aniline (5) (200 mg, 0.514 mmol), triethyl amine (0.143 ml, 1.02 mmol) in dichloromethane (10 ml) was added aryl/alkyl chloride (0.060 ml, 0.771 mol) slowly at 10-150C within 30 min. Reaction mixture allow to stir at room temperature for 2-3 h. Reaction was poured in water and extracted with dichloromethan. Dichloromethane layer washed with sat. sodium bicarbonate, water and dried under sodium sulphate. Solvent was evaporated completely to get pale yellow solid as compound (7a-j) (132 mg, 60.0 %). Ana Obs.: C-55.60%, H-4.72%, N-19.43%; Calc. for C20H20Cl2N6O: C-55.69%, H-4.67%, N-19.48%.

The monitoring of reaction and purity of compounds were checked on TLC aluminium sheet silica gel 60 F245 (E.Merck) using hexane-ethyl acetae (5:5 V/V) and methanol-chloroform (2:8 V/V) as mobile phase and visualize under U.V light 254 nm.

Other compounds of the series (7b-j) were prepared by using similar method and their physical data are recorded in Table-1.

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Reference :

1. Todorovic A & Luevano H C, Peptides, 26**,** 2026,**[2005].**

2. Donnelly L E & Roger D F, Curr Opin Drug Discov Develop, 6,

815,[**2003].**

3. Shiv K., Agarwal, K. S. Anil, Sur R.N., R.C. Srimal and N.D. Bhola, Ind. J. Chem. , 29B,80, **[1990].**

4. David B.R. Wilfred J.F. and Dallas K.B. J. Heterocyclic Chem., 32(3),707, **[1995]**.

5. Orjales A., Bordell M., Rubio V., J. Heterocyclic Chem., 18,157, **[1981].**

6. Schickaneder H., Moersdorf P., Engler H. and Arbens K.,Ger.Offen. DE3, (1987);607,941,Chem. Abstr., 107(25), 236741w, **[1987].**

7. Araki K., Kuroda T., Uemri S., Noriguchi A., Ikeda Y., Hirayama F., Yokoyama Y., Iwao E. and Yakushiji T., J. Med. Chem., 36(10),1356, **[1993].**

8. Irikura T., Masuzawa K., Uchid H. and Naruse K. (Kyorin Pharmaceutical Co. Ltd.), Japan 70,09, 543, **[1970].**

9. Yerich J. P., New J.S., Lobeck W.G., Dextraze P., Bernstein E., Taylor D. P., Yocca F.D., Eison M.S. and Temple D.L.J., J. Med. Chem.., 35(24),4516, **[1992].**

10. C.D. Lunsford and A.D.Gal, Ger offen (1971); 2,120,367, Chem. Abstr., 76,72551x, **[1972].**

11. Landquist J., Ger Offen **[1970];**1, 941,567, Chem. Abst., 23-26, 12159r,

[1970].

12. Katawaka M., Hino K. and Ochi Y., Dainippon Pharmaceutical , Japan A2 12p, **[1995].**

13. Rober B.M., J. Heterocyclic Chem., 17,753, **[1980].**

14. Joshi K.C.,Jain R.,Dandia A.& Sharma K.,Ind.J.Chem.28B,698,**[1989]**.

15. Purohit M.&Srivastava S.K.,J.Ind.Chem.Soc.,68,168, **[1991].**

16. Desai P.S & Desai K.R.;J.Ind.Chem.Soc.,70,175, **[1993].**

17. Purohit M.& Srivastava S.K.; .Ind.J Pharm.Sci.,54**,**25,**[1993].**

18. Sawhney S.N..Dharam Vir & Gupta A.;Ind.J.Chem,.29B, 1107,**[1990].**

19. Goro Tsukamato & Kaichiro Yashino,Yoshihiko Kohno,Hiroshi Ohtaka,Hajime Kagaya & Keizo Ito;J.Med. Chem 23,734, **[1980].**

20. Kumar V.& Reddy M.;Indian Drugs,23**,**98, **[1985].**

21. Ware G.W.&Freeman W.A.;Chem.Abstr.,88, **[1978].**

22. Bhaduri A.P.,Visen K.S.,MIshra A.,Gupta S.& Sen A.B.; J.Med. Chem., **27,** 1083, **[1984].**

23. Sharma S.& Choerles E.S.;Proc.Drug Res.,26, 9,**[1982].**

24. Shamm A.M.,Seth M.& Bhaduri A.P.,Ind.J.Chem..25B**,**395,**[1986].**

25. Nagar S.,Augony T.K.& Parmar S.S.;J.Pharma Sci.,62,178,**[1973].**

26. Singh S.P., Augony T.K.& Parmar S.S.;J.Pharma Sci.,63,960,**[1974].**

27. Dubey Rashmi,Sayed Abuzar & Katiyar J.C.;J.Med.Chem.,28**,**1748,**[1985].**

28. Lalezari I.,Gomea L.A. & Khorshid M.;J.Heterocyclic Chem.,27,687,**[1990].**

29. Bennet L.& Baker H.I.;J.Org.Chem.,22,707,**[1957]**

30. Nishio H.,Yamamota I.,Kaziya K. & Han O.K., Chem. Pharm.Bull., 17**,**539, **[1969].**

31. El-Dawy M.A.& Hozzoa A.B.;J.Pharm Sci.,72,45 [1983**].**

32. Yomanka H.,Sakamoto Y.,Shiozawa A.& Adacjo I.;Chem.Abstr.,91,47525r, **[1979].**

33. Wamhoff H.;Comprehensive Heterocyclic Chem,Pergamon Press ,Oxford, 5,788 ,**[1984].**

34. Bochi R.J.,Chabala J.C. & Fischer M.H.;Chem.Abstr.,104,19592,**[1986].**

35. Parmar S.S.,Gupta A.K.,Singh H.H. & Gupta T.K.;J.Med.Chem.15, 999,**[1972].**

36. Meyer W.E.,Toncufick A.S & Marqui H.;J.Med.Chem..32**,**593,**[1989].**

37. Itoh Manabu .Ohta Masahiko,Miyazaki Yutaka,Sawama Yuka, Matsumoto Shigeki & Yamsaki Fumiaki; Patent no: WO2007088895,**[2007].**

38. http://www.pharmainfo.net/exclusive/reviews/triazoles_:_as_a_ promising _ medicinal _agents/

39. Temle C.J.;Chem.Hetyrocycl.Compd.,37**,**1**[1981].**

40. Polya J.B.& Woodruff M.;Aust.J.Chem.,26**,**1585**[1973].**

41. Calzolari C.& Favretto L.;Analyst,93**,**494**[1968].**

42. Polya J.B.;Comprehensive Heterocyclic Chemistry,5,Pergamon Press,733 **[1984].**

43. Mokotoff M.,Jhao M.,Roth S.M.,Shelley J.A.,Slavoski J.N.& Kouttab N.M.;J.Med.Chem.,33**,**354**[1990].**

44. Mastsumoto O.& Uekawa T.,Jpn Kokai;Tokkyo Koho (2000), jp (2000239262) , (20000905),13pp,Kishimoto T.,Yamada Y.,Iwasa T. & Matsuda M.,Jpn Kokai Tokkyo Kohojp(07330742), (19951219), 10pp,**[1995].**

45. Oita S.&Uchida T.,jpn Kokai Tokkyo Koho(1998),jp(1021-2287),

(19980811), 7Ppp,Nakamura Y.,Komatsu H.& Yoshihara H.,jpn.Kokai

Tokkyo koho jp(08059675),(19960305),5pp, **[1996].**