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Short Communication

SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF FUNCTIONAL ANALOGUES OF FLUCONAZOLE

MRUNMAYEE P.TORASKAR[1]*, VILASRAO J. KADAM[1], VITHAL M. KULKARNI[2]

[1]DEPARTMENT OF PHARMACEUTICAL CHEMISTRY, SEC-8,C.B.D. BELAPUR, NAVI MUMBAI 400614, [2]POONA COLLEGE OF PHARMACY, ERANDWANE, PUNE-38. E mail: rupalitoraskar@yahoo.com, vmkulkarni60@gmail.com

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ABSTRACT

Reaction of 2'[(1H-1,2,4-triazol-1-yl)methyl]-2-(2,4-diflurophenyl)oxirane **2** with 5-aryl 4-amino-3-mercapto-1,2,4-triazoles **1** yielded related functional analogues of fluconazole **3a-3f.**Structures of various compounds were established on the basis of spectral data. These compounds were screened for in vitro antimicrobial activity .The results were discussed further.

Keywords: Fluconazole, 1, 2, 4-Triazole, CYP450

INTRODUCTION

Invasive fungal infections have increased in frequency and severity over the last two decades as a result of an increasing no. of immunocompromised hosts.[1]Widespread use of antifungal therapies for curative purposes has been developed to overcome the threat of candida colonization but also has led to development of resistance to current therapy.[2]

Triazoles are known to displace lanosterol from lanosterol 14-α-demethylase, a cytochrome P-450dependent enzyme and block the biosynthesis of ergosterol, an essential component of fungal cell membranes.[3] In particular focus has been paid to the triazole derivatives because of their broad antifungal spectrum and low toxicity. However, the current antifungal therapy suffers from drug related toxicity, severe drug resistance, non optimal pharmacokinetics and serious drug drug interaction[4, 5]. Therefore there is need for safe, novel and effective antifungal compound.

3,5-disubstituted-1,2,4-triazole and its derivatives have been reported to possess wide spectrum of activities ranging from antibacterial [6]antiinflammatory[7],anticonvulsant[8],antineoplastic[9],antimalarial[10]an danticancer[11].Our present study reports the synthesis and antimicrobial activities of new fluconazole analogues.

MATERIAL AND METHODS

Preparation of 5-Mercapto-4-amino-3-(aryl)-1, 2, 4-triazole [12, 13] **1** was done by reported procedure which was schematically shown in step 1. Also preparation of 2-[(1H-1, 2, 4-triazole-1-yl) methyl]-2-(2, 4-difluorophenyl)oxirane was done by reported procedure[14] **2**(step2). 2-(2,4-difluorophenyl)-1-{[3-aryl-5-mercapto-4H-1,2,4-triazol-4-yl]amino}-3-(1H-1,2,4-triazol-1-yl)propan-2-ol **(3a- 3f)**were prepared by reacting mixture of oxirane (0. 01 mole), methanol (20 ml), NaOH (0.01mole) and triazole **1** (0.012 mole) under reflux with stirring for 8 hrs. The solvent was evaporated under reduced pressure. The residue was triturated with water (50 ml) and extracted with ethyl acetate (100 ml x 3). The combined organic layers were washed with water (100ml x 3) and brine (100 ml x 3), dried over anhydrous magnesium sulphate and filtered. The solvent was evaporated under reduced pressure. The residue was purified by silica gel column chromatography (Ethyl acetate: hexane, 1:1).

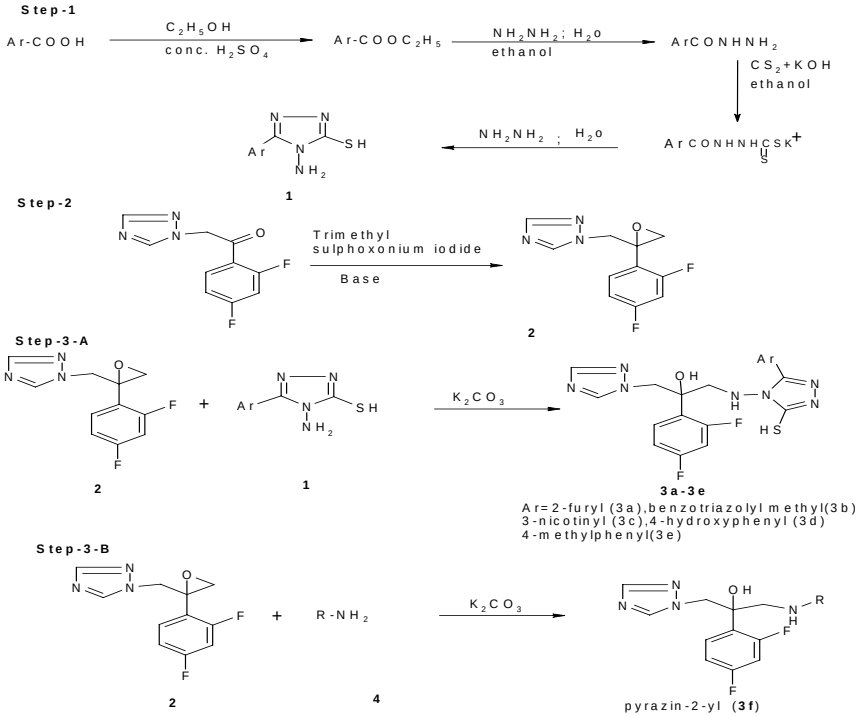

Fig. 1: Scheme of synthesis

2-(2,4-difluorophenyl)-1-{[3-(2-furyl)-5-mercapto-4*H*-1,2,4-triazol-4-yl]amino}-3-(1*H*-1,2,4-triazol-1-yl)propan-2-ol(3a) Empirical formula $C_{17}H_{15}F_2N_7O_2S$, Yield 30%. IR(cm^{-1}):ν_{max} 3500 (OH *str*),2927(NH str),756(disubstitutedphenyl),1421(SH),1207(-C-N-C);^{1}H NMR (δ ppm) :6.7(1H),4.1,3.85(4H),8.58(1H),13(1H)

2-(2,4-difluorophenyl)-1-{[3-(benztriazolyl methyl)-5-mercapto-4*H*-1,2,4-triazol-4-yl]amino}-3-(1*H*-1,2,4-triazol-1-yl)propan-2-ol(3b) Empirical formula $C_{20}N_{10}$ $H_{18}F_2OS$, Yield 65%. IR(cm^{-1}): ν_{max} 3600 (OH *str*),2925(NH),1616(C-H str),1421(SH),1137(C-N-C);^{1}H NMR (δ ppm): 6.5(1H),8.5(1H),7.-7.8,4.9,4.1(4H)

2-(2,4-difluorophenyl)-1-[(3-pyridin-3-yl-5-sulfanyl-4*H*-1,2,4-triazol-4-yl)amino]-3-(1*H*-1,2,4-triazol-1-yl)propan-2-ol(3c) Empirical formula $C_{18}H_{16}F_2N_8OS$, Yield 40%IR(cm^{-1}): ν_{max} 3500 (OH *str*),2927(NH),1612(C-H *str*), 1137(C-N-C);^{1}H NMR (δ ppm):14(1H),7.1-7.7,8.7(1H),5.7(1H)

4-(4-{[2-(2,4-difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl]amino}-5-sulfanyl-4*H*-1,2,4-triazol-3-yl)phenol(3d) Empirical formula $C_{19}H_{17}F_2N_7O_2S$, Yield 35%. IR(cm^{-1}): ν_{max} 3500 (OH *str*),2927(NH),1612(C-H *str*),^{1}H NMR (δ ppm):13.74(1H),12.33(1H),5.3-5.7(4H)4.57(1H)7.2-7.7,8.4(1H)

2-(2,4-difluorophenyl)-1-{[3-mercapto-5-(4-methylphenyl)-4*H*-1,2,4-triazol-4-yl]amino}-3-(1*H*-1,2,4-triazol-1-yl)propan-2-ol(3e) E.F $C_{20}H_{19}F_2N_7OS$,Yield 40%,IR(cm^{-1}): 3600 (OH *str*),2925(NH),1616(C-H str),1421(SH),1137(C-N-C);^{1}H NMR(δ ppm) : 6.5(1H),8(1H),7-7.8 , 4.9,4.1(4H),13(1H)

2-(2,4-difluorophenyl)-1-(pyrazin-2-ylamino)-3-(1*H*-1,2,4-triazol-1-yl)propan-2-ol(3f) E.F $C_{15}H_{14}F_2N_6O$, Yield 50% IR(cm^{-1}): 3500 (OH *str*),2925(NH),1616(C-H *str*),1137(C-N-C);^{1}H NMR(δ ppm) : 6.8(1H),8.2(1H),7-7.8, 4.4(4H).

RESULTS AND DISCUSSION

The I.R. spectrum of oxirane **2** showed an absorption band at 1506 cm^{-1} for -CH_2 bending in the ring. The absorption band observed at 1137 cm^{-1} could be attributed to –C-N-C group. Further I.R. spectrum of analogues **3a** and **3b** exhibited absorption band at 3500 cm^{-1} and 3600 cm^{-1} for OH respectively indicating opening of oxirane ring to an alcohol. In the ^{1}H NMR spectrum of compound **3b** OH proton was resonated at δ 4.9 as a singlet and –CH_2 protons were resonated at δ 4.1.

All the compounds were screened for in vitro antibacterial and antifungal activity using cup-plate agar diffusion method [15] by measuring the zone of inhibition in mm. Streptomycin and Fluconazole were used as standards respectively .The compounds were screened against *E.coli, S.aureus* , *P. aeruginosa, Candida albicans* and *Aspergillus niger* at concentrations 150, 100, 75 and 50 µg/ml. The plates were incubated at 37 ^{0}C for 24 hrs.The zone of inhibition in mm was observed and measured. The results of antimicrobial activity were presented in Table 1.

Compound 3a, 3b, 3e and 3f showed moderate antibacterial and antifungal activity. Molecular modeling studies were performed using Glide software available within Schrodinger ver.9.1.[16] The enzyme structure of CYP-450 was downloaded from PDB (PDB1EA1).Docking of 3a and 3f was performed on CYP450. Fluconazole binds to active site of CYP450 through formation of co-ordinate bond of iron from heme with third nitrogen of one of the triazoles.The docking scores for 3a (-7.93)and 3f(-10.04) were observed to be closer to that of fluconazole (-8.90).

Table1: Antimicrobial activity data of compounds 3a-3f

Compounds	150 µg/ml					100 µg/ml					75 µg/ml					50 µg/ml				
	EC	PA	SA	CA	AN	EC	PA	SA	CA	AN	EC	PA	SA	CA	AN	EC	PA	SA	CA	AN
3a	30	35	30	24	29	25	29	25	30	24	25	30	20	22	20	20	20	20	20	20
3b	32	25	27	18	15	20	22	20	12	16	22	22	25	20	12	20	20	25	15	20
3c	12	12	10	10	9	10	10	8	9	9	9	9	7	8	7	6	6	6	6	6
3d	8	10	8	12	10	6	8	6	9	6	6	6	7	8	8	7	7	7	6	6
3e	18	30	30	30	22	15	24	30	25	24	20	18	20	22	20	19	18	20	18	18
3f	35	23	30	24	30	30	20	28	20	30	25	20	25	22	28	20	22	20	16	20
Streptomycin	35	35	35	-	-	35	35	35	-	-	35	35	35	-	-	35	35	35	-	-
Fluconazole	-	-	-	30	30	-	-	-	30	30	-	-	-	30	30	-	-	-	30	30

Zone of Inhibition in mm

E.C= *E.coli,* P.A= *P.aeruginosa,* S.A = *S.aureus,* C.A= *Candida albicans,* A.N= *Aspergillus niger*

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