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Synthesis and biological evaluation of some novel quinoxalinyl triazole derivatives

Joydeep Mazumder[a], Raja Chakraborty[*a], Saikat Sen[a], Sanjay Vadra[b], Biplab De[c], T. K. Ravi[a]

[a] *Department of Pharmaceutical Chemistry, College of Pharmacy, SRIPMS, Coimbatore, Tamilnadu, India*
[b] *R & D Department, Indswift ltd, Solan, Himachal Pradesh, India*
[c] *Regional Institute of Pharmaceutical Science and Technology, Agartala, Tripura, India*

Abstract

The druglikeness score and bioactivity of the assumed triazole derivatives of the quinaxoline was calculated by determining various parameters as defines by Lipinski's rule. All the derivatives are also screened for bioactivity by calculating the score for GPCR ligand, ion channel modulator, kinase inhibitor, nuclear receptor ligand. The sufficient modification in the structure was done to achieve ideal scores for showing biological activity, which was compared with standard drug. In the present study, 2,3-diphenylquinoxaline-6-carboxilic acid has been prepared from benzyl. Triazole derivatives were prepared by reacting with different aldehyde in presence of ammonium acetate and ammonium hydroxide. The synthesized compounds was characterised by ^{1}H NMR and mass spectral data. All the compounds have been screened for their antibacterial and antifungal activity. Compounds have shown good antifungal activity. Among them fluoro derivative have shown highest activity as it has almost close druglikeness score in compare to the standard fluconazole. No compound has shown antibacterial activity.

Key words: Quinoxaline, Triazole, Lipinski's Rule, Antimicrobial activity.

Introduction

Quinoxaline possesses antimicrobial [1], antimycobacterial [2], antihyperglycemic [3] and antitumor [4] activity. Triazoles are associated with various biological activities like antimicrobial [5], antiallergic [6], antiinflamatory [6], antiviral [7], antioxidant [8] and analgesic [9] activity. In the light of these interesting biological activities, it was our interest to synthesize

some new quinoxaline derivatives bearing triazole moiety. Druglikeness is a qualitative concept used in drug design, which is estimated from the molecular structure before the substance is even synthesized and tested. The calculation of drug-like property can give us better assumption of biological activity of certain molecule. The theoretical calculation and maintain of certain properties of a molecule can fulfill the parameters which are essential to show certain biological activity. Lipinski's rule of five is a rule of thumb to evaluate druglikeness or determine a chemical compound with a certain pharmacological or biological activity that would make it a likely orally active drug in humans. The parameters defined by Lipinski's rule were calculated for the assumed quinoxalinyl-triazole derivatives by Chemsketch11 software and online through www.molinspiration.com, the highest score was calculated and compared with standard drug. The above derivatives were also screened for bioactivity by calculating the score for GPCR ligand, ion channel modulator, kinase inhibitor, nuclear receptor ligand by Molinspiration drug-likeness score online. Sufficient modification of the molecular structure was done to get proper druglikeness score and bioactivity score. The synthesis was done as per **scheme I**.

In the synthesis, benzil reacts with 3,4-diamino benzoic acid to give 2,3-diphenyl-6-carboxylic acid quinoxaline **(1)** which on reaction with phosphorus pentachloride give 2,3-diphenyl quinoxaline-6-carbonyl chloride **(2)**. In the next step 2,3-diphenyl quinoxaline-6-acid hydrazide **(3)** prepared from the previous compound by reacting with hydrazine hydrate. The final targeted compounds, triazole derivatives of quinoxaline **(4a-j)** were synthesized by reacting compound (**3)** with acetic acid and different aromatic aldehydes in presence of ammonium acetate. The structure of the final compounds was confirmed by Mass, ^{1}H NMR spectral and elemental analysis data. The newly synthesized compounds were tested for their antibacterial activity by taking *Staphylococcus aureus, Bacillus sublitis, Escherichia coli* and *Pseudomonas aeruginosa* and antifungal activity against *Candida albicans* and *Aspergillus niger*. Most of the newly synthesized compounds showed excellent activity against *Candida albicans.* The minimum inhibitory concentration of the compounds was determined for antifungal activity.

Results and Discussion

The druglikeness score was calculated by considering partition coefficient (log *P*), molar refractivity, molecular weight, number of heavy atoms, number of hydrogen donor, number of hydrogen acceptor and number of violation. The bioactivity was also calculated for GPCR ligand, ion channel modulator, kinase inhibitor, nuclear receptor ligand. For average organic molecules the probability is if the bioactivity score is more than 0.00 then it is active, if - 0.50 to 0.00 then moderately active, if less than - 0.50 then inactive. The scores were also compared with standard drug score. To achieve an active moiety suitable modification in the structure was done to obtain the proper bioactivity and druglikeness score as defined by Lipinski's rule. The druglikeness score and the calculated value of various parameters of the newly synthesized compounds summarized in the **Table 1** and the bioactivity score for GPCR ligand, ion channel modulator, kinase inhibitor, nuclear receptor ligand for the synthesized final derivatives tabulated in **Table 2**.

Table 1: Calculation of Lipinski's five parameters and M.R value of the compound and compare with their activity

Comp. Code	Active	Highest Score	Log P	Mol. Weight	No. of "H" Donor	No. of "H" Acceptor	No. of Violation	Molar Refractivity (cm^3)
4a	----	-----	6.74	425.49	1	5	1	129.2 ± 0.3
4b	----	-----	7.17	469.98	0	8	1	135.8 ± 0.3
4c	----	-----	7.25	454.51	0	6	1	135.9 ± 0.3
4d	G.P.C.R	0.09	6.98	470.51	1	7	1	137.8 ± 0.3
4e	G.P.C.R	0.16	4.68	443.48	1	5	0	129.2 ± 0.3
4f	G.P.C.R	0.12	7.41	459.9	1	5	1	134.1 ± 0.3
4g	G.P.C.R	0.07	7.37	459.9	1	5	1	134.1 ± 0.3
4h	G.P.C.R	0.02	7.27	454.51	1	6	1	135.9 ± 0.3
4i	G.P.C.R	0.06	8.02	494.38	1	5	1	139.0 ± 0.3
4j	G.P.C.R	0.11	6.85	514.56	0	2	2	149.3 ± 0.3
Std	G.P.C.R	0.25	0.27	306.27	1	7	0	104.3± 0.5

Standard: Fluconazole, G.P.C.R: GPCR ligand

Table 2: The bioactivity score for the synthesized final derivatives

Compound	GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand
4a	0.12	-0.36	-0.11	-0.63
4b	0.05	-0.22	-0.24	-025
4c	0.10	-0.32	-0.12	-0.48
4d	0.09	-0.42	-0.06	-0.12
4e	0.16	-0.36	-0.07	-0.58
4f	0.12	-0.39	-0.11	-0.66
4g	0.07	-0.49	-0.10	-0.71
4h	0.02	-0.31	-0.28	-0.39
4i	0.06	-0.53	-0.10	-0.81
4j	0.11	-0.54	-0.16	-0.21
Standard (Fluconazole)	0.25	-0.02	-0.48	-0.94

In the first step 2,3-diphenyl-6-carboxylic acid quinoxaline **(1)** was synthesized by reacting benzil with 3,4-diamino benzoic acid, yield 82%, melting point 288ºC. The resulting compound on reaction with phosphorus pentachloride give 2,3-diphenyl quinoxaline-6-carbonyl chloride **(2)** on second step, yield 68%, melting point 172ºC. In the next step 2,3-diphenyl quinoxaline-6-acid hydrazide **(3)** prepared from the previous compound by reacting with hydrazine hydrate, yield 62% and melting point 212ºC. In the final step different triazole derivatives of quinoxaline **(4a-j)** was prepared by reacting compound **3** with acetic acid and different aromatic aldehydes in presence of ammonium acetate. The purity of all the above synthesized compounds was

confirmed by single spot in TLC. The solvent system for TLC for the final derivatives was benzene : ethyl acetate : formic acid (4:4:2). The yield, melting point and R_f value of all the final derivatives summarized in the **Table 3.**

Table 3: Physical data of compounds synthesized

Comp. code	Ar	Mol. formula	Melting point (°C)	% yield	R_f value
4a	C_6H_5	$C_{28}H_{19}N_5$	170	64	0.71
4b	$(3\text{-}NO_2)C_6H_5$	$C_{28}H_{18}N_6O_2$	176	68	0.63
4c	$(3\text{-}OCH_3)C_6H_5$	$C_{29}H_{21}N_5O$	172	72	0.83
4d	$(2\text{-}OH,3\text{-}OCH_3)C_6H_5$	$C_{29}H_{21}N_5O_2$	180	76	0.51
4e	$(4\text{-}F)C_6H_5$	$C_{28}H_{18}FN_5$	198	68	0.85
4f	$(4\text{-}Cl)C_6H_5$	$C_{28}H_{18}ClN_5$	192	64	0.72
4g	$(2\text{-}Cl)C_6H_5$	$C_{28}H_{18}ClN_5$	202	60	0.69
4h	$(4\text{-}OCH_3)C_6H_5$	$C_{29}H_{21}N_5O$	186	62	0.53
4i	$[2,4\text{-}(Cl)_2]C_6H_5$	$C_{28}H_{17}Cl_2N_5$	162	72	0.65
4j	$[3,4,5\text{-}(OCH_3)_3]\text{-}C_6H_5$	$C_{31}H_{25}N_5O_3$	154	70	0.67

Solvent system for TLC - Benzene : Ethyl acetate : Formic acid (4 : 4 : 2)

Table 4(a): NMR and Mass spectral data of the compounds (4a-j)

Comp. code	Mass Data *(m/z)*	NMR data (∂, ppm)
4a	426	7.2-7.9(m, 18H, Ar-H), 8.9(s, 1H, N_1-H)
4b	471	7.2-7.8 (m, 17H, Ar-H), 8.5 (s, 1H, N_1-H)
4c	455	7.0-7.9 (m, 17H, Ar-H), 8.6 (s, 1H, N_1-H), 3.7(s, 3H, OCH_3)
4d	472	7.1-7.9 (m, 16H, Ar-H), 8.4 (s, 1H, N_1-H), 3.8(s, 3H, OCH_3), 7.7(s, 1H, OH)
4e	443	7.2-7.9 (m, 17H, Ar-H), 9.3 (s, 1H, N_1-H)
4f	460	7.2-8.0 (m, 17H, Ar-H), 9.1 (s, 1H, N_1-H)
4g	460	7.1-7.9 (m, 17H, Ar-H), 9.2 (s, 1H, N_1-H)
4h	456	7.0-7.7 (m, 17H, Ar-H), 8.6 (s, 1H, N_1-H), 3.8(s, 3H, OCH_3)
4i	495	7.2-7.9 (m, 16H, Ar-H), 9.3 (s, 1H, N_1-H)
4j	517	7.1-7.7 (m, 16H, Ar-H), 8.6 (s, 1H, N_1-H), 3.8(s, 6H, OCH_3) 3.7(s, 3H, OCH_3)

The structure of all newly synthesized compounds was well established by Mass, ^{1}H NMR spectral and elemental analysis data. The data was tabulated in **Table 4a** and **4b**.

Table 4(b): Elemental analysis data of the compounds (4a-j)

Comp. code	Elemental analysis (%) calcd./found		
	C	**H**	**N**
4a	79.04/79.16	4.50/4.54	16.46/16.48
4b	71.48/71.36	3.86/3.79	17.86/17.88
4c	76.47/76.54	4.65/4.77	15.37/15.48
4d	73.35/ 73.51	4.40/4.34	15.27/15.37
4e	75.83/75.67	4.09/4.12	15.79/15.76
4f	73.12/73.23	3.94/3.86	15.23/15.23
4g	73.12/72.90	3.94/3.78	15.23/15.46
4h	76.47/76.33	4.65/4.54	15.37/15.37
4i	68.03/68.31	3.47/3.44	14.17/14.13
4j	72.22/72.34	4.89/4.78	13.58/13.56

Table 5: Quantitative antifungal screening

Comp. code	Zone of inhibition in mm(diameter)			
	Candida albicans		***Aspegilus niger***	
	500 µg /disc	**250 µg /disc**	**500 µg /disc**	**250 µg /disc**
4a	-	-	-	-
4b	-	-	-	-
4c	-	-	-	-
4d	27	25	-	-
4e	28	26	-	-
4f	26	22	-	-
4g	28	26	-	-
4h	28	24	-	-
4i	20	18	-	-
4j	22	18	-	-
DMSO(Blank)	-	-	-	-
Standard	40		40	

Standard (Fluconazole) – 25 µg/disc
(-) indicate no zone of inhibition

In the series, compounds 4d, 4e, 4f, 4g, 4h, 4i, 4j showed good activities against *C. albicans*. The zone of inhibition of compound 4d showed 28 mm in 500 µg/disc and 22 mm in 250 µg/disc, 4e showed 28 mm in 500 µg/disc and 22 mm in 250 µg/disc, 4f showed 28 mm in 500 µg/disc and 22 mm in 250 µg/disc, 4g showed 28 mm in 500 µg/disc and 22 mm in 250 µg/disc, 4h showed 28 mm in 500 µg/disc and 22 mm in 250 µg/disc, 4ishowed 28 mm in 500 µg/disc and 22 mm in 250 µg/disc and 4j showed 28 mm in 500 µg/disc and 22 mm in 250 µg/disc.

The drug likeness score of those compounds was found 0.09, 0.16, 0.12, 0.07, 0.02, 0.06 and 0.11. So in the series, 4e showed highest *in vitro* antifungal activity (28 mm in 500 µg/disc and 22 mm in 250 µg/disc) and showed the highest score (0.16) which was active in GPCR ligand. According to the standard fluconazole which showed the zone of inhibition 30 mm in 25 µg/disc and druglikeness score 0.25 which is also active in GPCR ligand. The activity results were outlined in the **Table 5**.

In vitro determination of minimum inhibitory concentration (MIC) against *Candida albicans*, the results shows that compound 4e and 4g shows highest activity among the series, which inhibits the organism even at the concentration 8 µg/ml. The MIC values were summarized in the **Table 6**.

Table 6: *In vitro* determination of minimum inhibitory concentration (MIC) against *Candida albicans*

Comp. code	Concentration in µg/ml								MIC value (µg/ml)
	572	256	128	64	32	16	8	4	
4d	-	-	-	-	-	-	+	+	16
4e	-	-	-	-	-	-	-	+	8
4f	-	-	-	-	-	+	+	+	32
4g	-	-	-	-	-	-	-	+	8
4h	-	-	-	-	-	-	+	+	16
4i	-	-	-	-	+	+	+	+	64
4j	-	-	-	-	+	+	+	+	64
Control	+	+	+	+	+	+	+	+	
Blank	-	-	-	-	-	-	-	-	

(+) Turbidity, (-) No turbidity

So 4e have the almost close drug likeness score compare to the standard fluconazole. So 4e can consider the lead as antifungal agent among the series.

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druglikeness score to standard fluconazole. So 4e can consider the lead as antifungal agent among the series.

Materials and Methods

Lipinski's rule

The rule was formulated by Christopher A Lipinski in 1997. The rule describe molecular properties important for a drug's pharmacokinetics in the human body, including their absorption, distribution, metabolism and excretion (ADME). The rule is important for drug development where a pharmacologically active lead structure is optimized step-wise for increased activity and selectivity, as well as drug-like properties. The modification of the molecular structure often leads to drugs with higher molecular weight, more rings, more rotatable bond and a higher lipophilicity.

Druglikeness may be defined as a complex balance of various molecular properties and structure features which determine whether particular molecule is similar to the known drugs. These properties, mainly hydrophobicity, electronic distribution, hydrogen bonding characteristics, molecule size and flexibility and presence of various pharmacophoric features influence the behavior of molecule in a living organism, including bioavailability, transport properties, affinity to proteins, reactivity, toxicity, metabolic stability and many others.

Lipinski's rule of five states that, in general, an orally active drug has not more than 5 hydrogen bond donors (OH and NH groups), not more than 10 hydrogen bond acceptors (notably N and O), molecular weight under 500 g/mol, partition coefficient log *P* less than 5, number of violation less than 4.

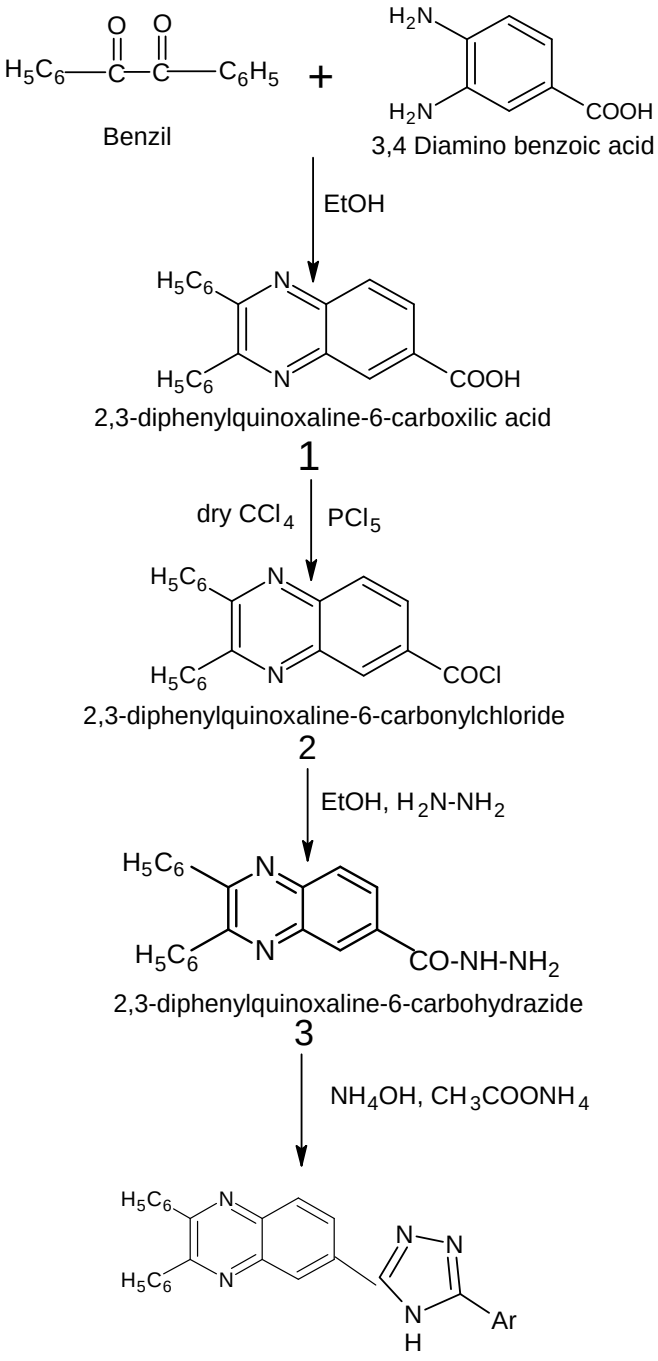
To evaluate druglikeness, Lipinski's rule have spawned many extensions, for example one from a 1999 paper by Arup *et al*. [10]

- Partition coefficient log *P* in - 0.4 to + 5.6 range
- Molar refractivity from 40 to 130
- Molecular weight from 160 to 480
- Number of heavy atoms from 20 to 70

The drugs are also checked for the bioactivity by calculating the activity score for GPCR ligand, ion channel modulator, kinase inhibitor, nuclear receptor ligand. All the parameters were checked with the help of software ChemSketch 11 and Molinspiration drug-likeness score online (www.molinspiration.com). Calculated druglikeness score of each newly synthesized compounds and compared with the specific activity of each compound, and the results were compared with standard drug.

Melting points were determined by open capillary tube method and are uncorrected. All the chemicals and solvents used were of laboratory grade and solvents were purified by suitable method. ^{1}H NMR spectra was recorded using DMSO-d_6 as internal standard at Sastra University, Tamilnadu, India. Mass spectra were recorded at Quest research and training institute,

Bangalore, India. The purity of the products was checked using TLC (Silica Gel-G, Merck). The synthetic approach of the title compounds is outlined in **Scheme I**.



Ar = a:C_6H_5, b:3-(NO_2)-C_6H_4, c:3-(OCH_3)-C_6H_4, d:3-(OCH_3)-4-(OH)-C_6H_3, e:4-F-C_6H_4, f:4-Cl-C_6H_4, g:2-Cl-C_6H_4, h:4-(OCH_3)-C_6H_4, i:2,4-$(Cl)_2$-C_6H_3, j:3,4,5-$(OCH_3)_3$-C_6H_2

Scheme I

Synthesis of 2,3 diphenyl -6-carboxylic acid quinoxaline (1) [11]
Benzil (0.03 mol, 6.24 g) and 3,4 diamino benzoic acid (0.03 mol, 4.56 g) dissolve in 24 ml ethanol separately in 100 ml beaker. Then mix the both solution and warm until cloudiness persist. Then allow to cool, filtered off and recrystallized with ethanol. mp: 288ºC.

Synthesis of 2,3 diphenyl quinoxaline-6-carbonyl chloride (2) [12]
In this reaction, 2,3-diphenyl-6-carboxylic acid quinoxaline (1 mmol, 0.32 g) and phosphorous pentachloride (1 mmol, 0.20 g) were suspended in 10 ml of dry carbontetrachloride and the mixture were stirred continuously on a heating mantle at 50°C till the evolution of hydrochloric acid ceases. The solvent was evaporated in vacuum and the residue was recrystallised from carbontetrachloride to obtain the acid chloride. mp: 172ºC.

Synthesis of 2,3 diphenyl quinoxaline-6-acid hydrazide (3) [13]
A mixture of 2,3 diphenyl quinoxaline-6-carbonyl chloride (0.01 mol, 3.59 g) and excess hydrazine-hydrate (0.04 mol, 2 ml) in ethanol (40 ml) was reflask for 5 h. The solution was then poured into crushed ice water. The solid mass thus obtained was filtered and recrystallized from ethanol. mp: 212ºC**.**

Synthesis of 2,3-phenylquinoxaline - 3-phenyl-4H-1,2,4-triazole (4a-j)
To a solution of **3** (0.1 mol) in acetic acid (20 ml), a pinch of ammonium acetate was added followed by the addition of benzaldehyde (0.1 mol). The mixture was stirred for 24 h at room temperature. The mother liquor on neutralization with ammonia solution gave a solid, which was filtered and recrystallized from ethanol. mp:170ºC. A series of different compound were synthesized by repeating the same procedure with different aldehydes. Structures were confirmed by different spectral data and elemental analysis. The purity of the compounds was confirmed by single spot in TLC.

Antimicrobial activity *[14,15,16]*
The activity was determined using disc diffusion method by measuring the inhibition zone in mm. All the compounds **4(a-j)** were screened *in vitro* antibacterial activity against *Staphylococcus aureus* NCIM 2079, *Bacillus sublitis* NCIM 2063, *Escherichia coli* NCIM 2118 and *Pseudomonas aeruginosa* NCIM 2036 at concentration 500 µg/ml and antifungal activity against *Candida albicans* NCIM 3102 and *Aspergillus niger* NCIM 596 at concentration 500 µg/ml. Standard antibacterial drug ciprofloxin (10 µg/disc) and antifungal drug fluconazole (10 µg/disc) were also tested under similar conditions.

For testing antibacterial activity Mueller-Hinton agar plates were prepared aseptically to get a thickness of 5-6 mm. The plates were dried at 37°C before inoculation. The organism as inoculated in the plates prepared earlier by dipping a sterile swab in the previously standardized inoculum, removing the excess of inoculum by pressing and rotating the swab firmly against the sides of the culture tube above the level of liquid and finally streaking the swab all over the surface of the medium three times, rotating the plate through an angle 60°C after each application. Finally the swab was pressed round the edge of the agar surface. It was allowed to dry at room temperature with the lid closed. The sterile disc containing test drugs, standard and blank were placed on the previously inoculated surface of the Muller-Hinton agar plate. Plates were prepared in triplicate and they were then incubated for 18-24 h at 37°C. Observation were

made for zone of inhibition around the discs and compared with that of the standard. All the compounds synthesized were tested for antibacterial activity against gram positive and gram negative bacteria.

For testing antifungal activity Sabouraud dextrose agar plates were prepared aseptically to get a thickness of 5-6 mm. The plates were dried at 25°C just before inoculation. The organisms were inoculated as same manner as above. Plates were prepared in triplicate and they were incubated at 25°C for 24-48 h, after placing them in the refrigerator for one hour to facilitate uniform diffusion. Observations were made for the zone of inhibition around the discs containing the drug and compared with that of standard drug fluconazole. All the compounds synthesized were tested for antifungal activity.

In vitro MIC determination of synthesized drugs

In vitro MIC determination for the compounds were carried out by two fold serial dilution in liquid RPMI 1640 medium with *Candida albicans*. The concentration range of the drugs screened for MIC determination was 4 to 572 µg/ml. The test organism used was diluted to 1:100 after overnight incubation and a drop (0.01 ml) was used for the MIC determination. Test tubes were sterilized and then labeled with numbers 1 to 8 and 0.5 ml of liquid RPMI 1640 medium which was already prepared using sterilized water was added to each tube. To the first tube 0.5 ml of diluted stock solution (572 µg) was added and serially transferred 0.5 ml through tube no 8 to obtain the quantities indicated. Each pipette was discarded after transfer and a fresh pipette was used for mixing and transferring the mixture to the next tube 0.5 ml was discarded from the 8^{th} tube. The 9^{th} tube was used as the control. With a standardized micropipette a drop of the diluted broth culture of the test organism (approximately 0.01 ml) was added to all the tubes, including the control. The contents of the test tube were mixed gently and incubated at 25°C for 24 h for *S. aureous, B. subtilis, C. albicans* and 48 h for *A. niger* and the results were observed. The above procedure was carried out in duplicate. The MIC was interpreted as the highest dilution of the test compound which shows no colour change.

Conclusion

Quinoxalinyl triazole derivatives were synthesized from benzil and 3,4-diamino benzoic acid using suitable synthetic route. The structures of the compounds were confirmed by spectral studies like MS, ^{1}H NMR, elemental analysis. The purity of the compounds was confirmed by single spot in TLC.

According to druglikeness study compound 2,3-phenylquinoxaline-3-(4-fluoro phenyl)-4H-1,2,4-triazole (**4e)** was selected as a lead moiety among the entire series of the compound as an antifungal agent because of it have similar parameter like standard fluconazole. From the above study it was found that among all the synthesized derivatives compound 4e and 4g shows highest activity among the series against *Candida albicans*, which inhibits the organism even at the concentration 8 µg/ml. In the series, compounds 4d, 4f, 4h, 4i, 4j also showed good activities against *C. albicans.* No compound was found succeptable against *Aspergillus niger.* No compound from the above series has shown significant antibacterial activity tested against *Bacillus subtilis, Escherichia coli, Pseudomonas aeroginosa* and *Staphylococcus aureus.*

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References

[1] M.B. Mohga, A.M. Ashraf, M.R. Hanan, A.E. Afaf, *J. Chin. Chem. Soc.*, **2007**, 54, 469.
[2] E.S. Lainne, J.S. William, C.R. Robert, *J. Med. Chem.*, **2002**, 45, 5604.
[3] P.Y. Pawar, S.B. Bhise, *Indian J. Physiol. Pharmacol.*, **2006**, 50, 431.
[4] W.D. Leslie, J.K. Anthony, J.F. Graeme, C.B. Bruce, A.D. William, *J. Med. Chem*., **1997**, 40, 2040.
[5] J. John, A.S. Bobade, B.G. Khadse, *Indian J. Heterocycl. Chem.*, **2001,** 10, 295.
[6] G. Thomas, D.V. Mehta, R. Tahilramani, J. David, P.K. Talwalker, *J. Med. Chem*., **1971,** 14, 335.
[7] T.W. Joseph, K.R. Roland, P.K. Gyaneshwar, W.S. Robert, *J. Med. Chem*., **1973**, 16 935.
[8] H. Yuksek, S. Kolayh, M. Kucuk, M.O. Yuksek, U. Ocak, E. Sahinbas E. Sivrikaya, M. Ocak, *Indian J. Chem.* **2006,** 45B, 715.
[9] K.C. Asati, S.K. Srivastava, S.D. Srivastava, *Indian J. Chem.*, **2006**, 45B, 526.
[10] K.G. Arup, N.V. Vellarkad, J.W. John, *J. Comb. Chem.*, **1999**, 1, 55.
[11] S. Rostamizadeh, S. Jaafari, *Indian J. Heterocycl. Chem.*, **2001**, 10, 303.
[12] B.S. Furniss, A.J. Hannaford, P.W.G. Smith, A.R. Tatchell editors, Vogel's Text Book of Practical Organic Chemistry, 5th edition, New York, Longman Scientific & Technical, **1989**.
[13] V.K. Pandey, V.D. Gupta, U. Mrinalini, U. Mridula, V.K. Singh, T. Meenal, *Indian J. Chem.*, **2005,** 44B, 158.
[14] J.G. Cappuccino, N. Sherman, Microbiology-A Laboratory Manual, 3rd edition, London, Benjamin Cummings Publishing Company, **1992**.
[15] L. Martinez-Martinez, P. Joyanes, A.I. Suarez, E.J. Perea, *Antimicrob. Agents Chemother.*, **2001,** 45, 2390.
[16] L. Victor, Antibiotics in Laboratory Medicine, 3rd edition, Singapore:Lippincott Williams and Wilkins, **1991**.