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Synthesis, Characterization, in-silico ADME, PASS prediction, Molinspiration, Osiris and Toxicological profiling studies of some fluconazole analogues

. [a]Suryawanshi Manjusha B.[*], [a]More Nachiket D. , [b]Chikhale Hemant U. and [a]Patil Sachin V.

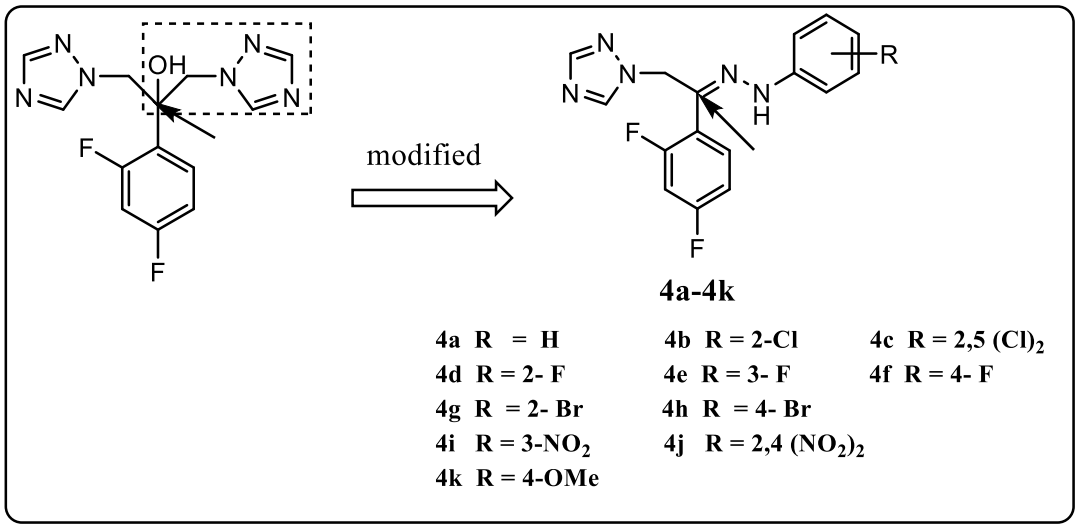
[a]Department of Chemistry, H.P.T Arts and R.Y.K. Science College, Nashik- 422005, MS, India.
[b]Department of Pharmaceutical Chemistry, GES Sir Dr. M.S. Gosavi College of Pharmaceutical Education and Research, Nashik- 422005, MS, India.

[*] Corresponding author. Tel.: +91-8087986846 ; fax : +91-(0253)-2573097

Email:- manjoosha28@gmail.com

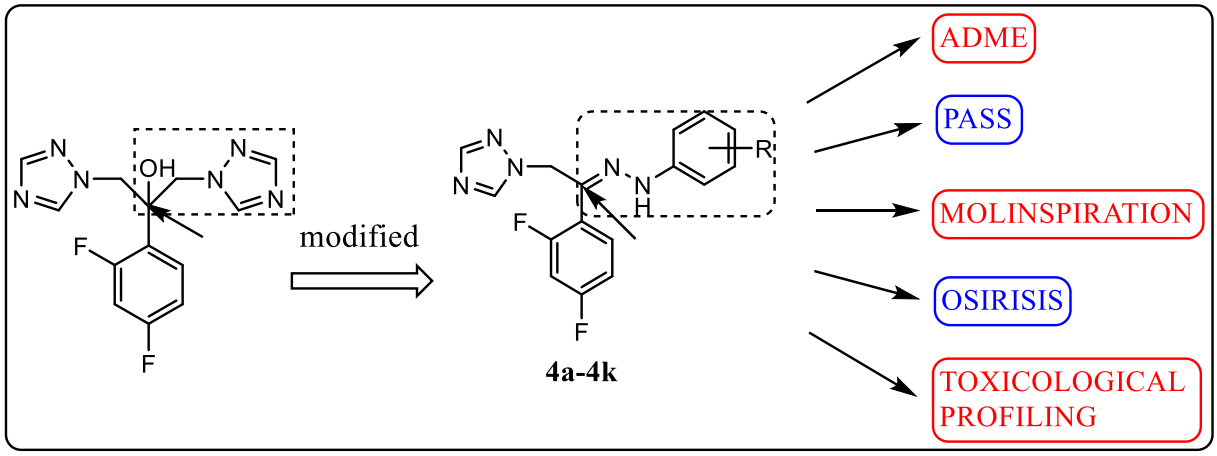
Abstract :

In the present investigation, we focused our interest on the estimation of in silico screening of the fluconazole analogues. We focused our attention on replacement of one of the1,2,4-Triazole rings of fluconazole with substituted hydrazones. Hydrazones are stable to metabolic degradation and are capable of hydrogen bonding, which can be favourable in binding of biomolecular targets and for solubility. The *in silico* studies revealed that **4a (R=H) , 4d (R= 2-Fluoro) and 4e (R= 3-Fluoro)** are a promising lead molecules upon predication of bioactivity scores. PASS online predicted various inhibitions suggestive of anti-fungal activity. All the analogues showed to be mutagen under Ames test. The structures of the synthesized compounds were established on the basis of ^{1}HNMR , ^{13}CNMR and HRMS data. The descriptors obtained from ADME showed good TPSA, absorption, oral bioavailability. This work could be used as an initial approach in identifying potential novel molecules with promising activity and low toxicity.



Key words: *in silico*, hydrazones, PASS analysis, TPSA, antifungal activity.

Graphical Abstract



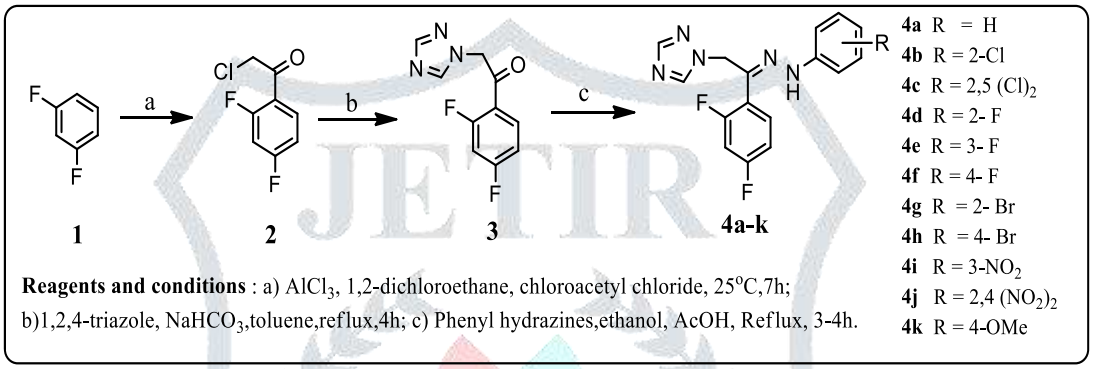
1. Introduction

Antifungal azoles, fluconazole and itraconazole, which are strong inhibitors of lanosterol 14α-demethylase (cytochrome P-45014DM) and orally activehave been widely used in antifungal chemotherapy. In recentyears the developments of resistance to currently available antifungal azoles in *Candida* spp., as well as clinical failures in the treatment of fungal infections have been reported.[1-4] The marketed drugs, such as fluconazole, itraconazole and voriconazoleused in the treatment of invasive fungal infections contain triazole ring.[5-9] Several new azoles, containing 1,2,4-Triazole and 1,3-Difluorobenzene moieties, such as voriconazole, posaconazole, ravuconazoleetc are marketed for clinical trials. These compounds reduce thesynthesis of ergosterol (the main component of fungal membranes)by inhibiting lanosterol14α-demethylase, the cytochrome P-450 dependent enzyme.[10-12] Depletion of ergosterol and the accumulationof 14α-methylated sterols alters membrane fluidity, thereby increasing its permeability. Computational docking experiments indicated that the inhibition of CYP51 involves a coordination bond with iron of the hemegroup, the hydrophilic H-bonding region, the hydrophobic region, and the narrow hydrophobic cleft.[13,14] Several reports on the synthesis and biological activity of structurally modified new analogues of fluconazole are known in the literature.[15] In continuation of our earlier work [16-20] we herein report the design and synthesis of fluconazole analogues with the aim of investigating their antifungal activity. We focused our attention on replacement of one of the1,2,4-Triazole rings of fluconazole with substituted hydrazones. Hydrazones are stable to metabolic degradation and are capable of hydrogen bonding, which can be favourable in binding of biomolecular targets and for solubility. Based on the results of computational docking to the active site of the cytochrome P450 14α-demethylase (CYP51), a series of hydrazones derivatives as analogues of fluconazole were designed, synthesized, and evaluated as antifungal agents. Structures were characterized by ^{1}HNMR ,^{13}CNMR and HRMS analysis.

2. Results and Discussion

2.1 Chemistry

The synthetic route of compounds is outlined in **Scheme 1**. Friedel–Craftacylation of 1,3-difluorobenzene **1** using chloroacetyl chloridein presence of $AlCl_3$ as Lewis acid in 1,2-dichloroethane(DCE) provided the 2-chloro-1-(2,4-difluorophenyl)-ethanone**2** in quantitative yield. In turn, compound **2**was reacted with 1,2,4-Triazole in presence of $NaHCO_3$ togive the key intermediate 1-(2,4-difluorophenyl)-2-[1,2,4]-Triazol-1-yl-ethanone **3**. Further reaction of compound**3** with different substituted phenyl hydrazines gave final compounds **4a-k** . The structures of the title compounds were confirmed by ^{1}H NMR, ^{13}C NMR spectroscopy and HRMS.



Scheme 1: Synthesis of compounds 4a-k

2.2 In silico studies

2.2.1 *In silico* ADME screening

The Topological Polar Surface Area (TPSA) obtained can be related with H-Bonding of the title compounds and showed a very good indication of the bioavailability of the drug candidates. The molecular weight of all the title compounds were under 500 and thus can be regarded as to be easily transported, diffused and transported as compared high molecular analogues. The TPSA of the fluconazole analogues was observed in the range of 55.10 Å to 146.74 Å, which are pretty much in the range below the limit of 160 Å. The percentage of absorption for analogues were obtained by the method proposed by Zhao and et. al. and were in the range of 58 % to 90 %. The lowest absorption was reported for the derivative **4j** (2,4 $(NO_2)_2$) (58.37 %). Thus the % Absorption of rest analogues are indicative of good oral bioavailability. (**Table 1**) The Lipophilicity was indicated by Log P or partition coefficient, the Log P values of all the title compounds were in the range of 2 to 4 thus all were under 5 and was of suggestive of good permeability across the plasma membrane and can be considered as one of the justification of good oral use. The derivative **4i** and **4j** containing NO_2 as a substituent/s violates this behaviour**.** The Swiss ADME also predicted drug-likeness in which various violations were predicted like Lipinski, Ghose, Veber, Egan, Muegge. (**Table 3**) Most of the synthesized analogues showed good bioavailability (0.55), no PAINS alerts and good synthetic accessibility (2.98 - 3.04). In

comparison to Fluconazole and Voriconazole the synthesized derivatives showed good percentage of absorptivity, and excellent LogP values, suggestive of absorbtion to the greater extent. The $LogK_P$ values for the synthesized derivatives have more skin permeability when compared to Fluconazole (-7.92) and Voriconazole (-7.36) . (**Table 2**) All the synthesized molecules showed very good synthetically accessibility (3 to 3.2) except for **4j** (2,4 $(NO_2)_2$). But when compared to Voriconazole all showed very good synthetic accessibility**. (Table 4)**

Compound	MW	R - Bond	H-A	H-D	MR	TPSA	% A	Log (P_o/P_w)
4a	313.30	5	5	1	83.01	55.10	89.99	3.23
4b	347.75	5	5	1	88.02	55.10	89.99	3.81
4c	382.19	5	5	1	93.03	55.10	89.99	4.32
4d	331.30	5	6	1	82.96	55.10	89.99	3.56
4e	331.30	5	6	1	82.96	55.10	89.99	3.51
4f	331.30	5	6	1	82.96	55.10	89.99	3.59
4g	392.20	5	5	1	90.71	55.10	89.99	3.87
4h	392.20	5	5	1	90.71	55.10	89.99	3.86
4i	358.30	6	7	1	91.83	100.92	74.18	2.63
4j	403.30	7	9	1	100.65	146.74	58.37	2.05
4k	342.33	6	6	1	89.50	64.33	86.80	3.25
Fluconazole	306.27	5	7	1	70.71	81.65	80.83	0.88
Voriconazole	349.31	5	8	1	81.19	76.72	82.53	2.40

Table 1: Predicted Pharmacokinetic Descriptors

Compound	GI Absorption	BBB Permeation	PGP Substrate	CYP1A2	CYP2C19	CYP2C9	CYP2D6	CYP3A4	Log Kp (Cm/s)
4a	High	Yes	No	Yes	Yes	Yes	No	No	-5.46
4b	High	Yes	No	Yes	Yes	Yes	No	No	-5.23
4c	High	Yes	No	Yes	Yes	Yes	No	No	-5.99
4d	High	Yes	No	Yes	Yes	Yes	No	No	-5.50
4e	High	Yes	No	Yes	Yes	Yes	No	No	-5.50
4f	High	Yes	No	Yes	Yes	Yes	No	No	-5.50
4g	High	Yes	No	Yes	Yes	Yes	No	No	-5.45
4h	High	Yes	No	Yes	Yes	Yes	No	No	-5.45
4i	High	No	No	Yes	Yes	Yes	No	No	-5.86
4j	Low	No	No	Yes	Yes	Yes	No	Yes	-5.86
4k	High	Yes	No	Yes	Yes	Yes	No	No	-5.67
Fluconazole	**High**	**No**	**No**	**No**	**Yes**	**No**	**No**	**No**	**-7.92**
Voriconazole	**High**	**Yes**	**Yes**	**No**	**No**	**No**	**Yes**	**No**	**-7.36**

Table 2: Predicted absorption parameters of the derivatives

Compound	Lipinski Violations	Ghose Violations	Veber Violations	Egan Violations	Muegge Violations
4a	0	0	0	0	0
4b	0	0	0	0	0
4c	1	0	0	0	1
4d	0	0	0	0	0
4e	0	0	0	0	0
4f	0	0	0	0	0
4g	0	0	0	0	0
4h	0	0	0	0	0
4i	1	0	1	1	0
4j	0	0	0	0	0
4k	0	0	0	0	0
Fluconazole	0	0	0	0	0
Voriconazole	0	0	0	0	0

Table 3: Predicted Drug-likeness of the derivatives

Compounds	Bioavailability	PAINS Alert	Brenk Alert	Lead likeness	Synthetic Accessibility
4a	0.55	0	1	1	2.98
4b	0.55	0	1	1	2.98
4c	0.55	0	1	2	3.03
4d	0.55	0	1	1	3.04
4e	0.55	0	1	1	3.01
4f	0.55	0	1	1	3.01
4g	0.55	0	1	2	3.02
4h	0.55	0	1	2	3.02
4i	0.55	0	3	2	3.21
4j	0.55	0	3	2	**3.47**
4k	0.55	0	1	1	3.04
Fluconazole	0.55	0	0	Yes	2.91
Voriconazole	0.55	0	0	Yes	3.61

Table 4: Predicted Bioavailability and synthetic accessibility of the derivatives

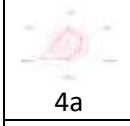
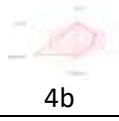
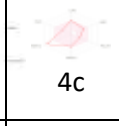
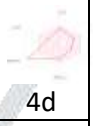
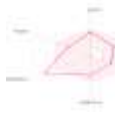
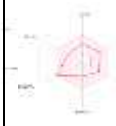
 4a	 4b	 4c	 4d
 4e	 4f	 4g	 4h
 4i	 4j	 4k	
 Fluconazole	 Voriconazole		

Table 5: Bioavailability Radar Graphs

(Pink Area reflects the allowed values of drug likeness properties of analogues)

2.2.2 Prediction of Activity Spectra for Substances (PASS)

The derivatives were screened for their potent biological activities using PASS as an *in silico* tool. The prominent inhibitions inhibited by the analogues were Lanosterol 14α demethylase, CYP51 cytochrome and prominent activities were antifungal, antiepileptic, anticonvulsant. The less prominent were autoimmune disorder and antineoplastic. The Lanosterol 14α demethylase, CYP51 cytochrome were suggestive of antifungal activities. All the inhibitions which showed probability greater than 50% were only considered. The 4b (2-Cl) and 4c (2,5 $(Cl)_2$) analogue exhibited highest probability (0.60 to 0.63) among the other analogues. While 4a, 4d to 4k of them showed probability in the range of (0.50 to 0.60). The anticonvulsant activity was shown by the 4a, 4c, 4e-4h in the range of (0.59 to 0.61) and other showed less probability 4b, 4d, 4j, 4k. The antineoplastic was shown by only 4d (2F) and the autoimmune disorder was shown by only 4a (2H), 4b (2,5 $(Cl)_2$), 4f (4F). The standard and reference molecules chosen showed inhibitions which are only suggestive anti-fungal activities. While when the

predicted inhibitions were compared with the reference molecules, the designed analogues showed other inhibitions and thus suggestive of other biological activities like antiepileptic, antineoplastic and anticonvulsant. **(Table 6)**

Compounds	Antifungal		L14αD		CYP51		Antiepileptic		Anticonvulsant		ADI		Antineoplastic	
	Pa	Pi	Pa	Pi	Pa	Pa	Pi	Pi	Pa	Pi	Pa	Pi	Pa	Pi
4a	0.578	0.021	0.650	0.001	0.547	0.002	0.609	0.007	0.581	0.022	0.548	0.019	NA	NA
4b	0.607	0.018	0.752	0.001	0.524	0.002	0.585	0.008	0.472	0.042	0.445	0.042	NA	NA
4c	0.628	0.016	0.821	0.001	0.548	0.002	0.626	0.006	0.501	0.035	-	-	NA	NA
4d	0.513	0.028	0.674	0.001	0.532	0.002	0.561	0.009	NA	NA	-	-	0.561	0.054
4e	0.538	0.025	0.613	0.001	0.532	0.002	0.621	0.007	0.573	0.023	-	-	0.478	0.078
4f	0.581	0.020	0.658	0.001	0.558	0.002	0.604	0.007	0.569	0.023	0.563	0.017	NA	NA
4g	0.543	0.024	0.626	0.001	0.485	0.002	0.687	0.005	0.539	0.028	-	-	-	-
4h	0.594	0.019	0.636	0.001	-	-	0.653	0.005	0.610	0.018	-	-	-	-
4i	0.592	0.019	0.645	0.001	-	-	0.586	0.008	0.575	0.023	-	-	-	-
4j	0.515	0.028	0.615	0.001	-	-	-	-	-	-	-	-	-	-
4k	0.574	0.021	0.545	0.002	-	-	-	-	-	-	-	-	-	-
Fluconazole	0.726	0.008	0.846	0.001	0.720	0.001	0.411	0.018	0.429	0.053	-	-	-	-
Voriconazole	0.722	0.009	0.713	0.001	0.619	0.001	0.250	0.057	-	-	-	-	-	-

L14αD Lanosterol 14 alpha demethylase inhibitor **ADI** Autoimmune Disorder Treatment **C** **CYP51** Cytochrome P51

Table 6: Prediction of Activity Spectra for Substances (PASS)

2.2.3 Molinspiration Calculation of Bioactivity Score

The predictions of bioactivity of the title compounds was obtained using Molinspiration v. 2018.03 The results are suggestive of that the physiological actions of fluconazole analogues might involve multiple mechanisms and could be due to interactions with GPCR ligands, nuclear receptors, other inhibitions and enzymes. It is clearly evident from the results the interaction of nuclear receptor ligands can be considered to zero or can be neglected as all the values are less than - 0.50. In case of **4f (4F), 4g (2 Br), 4h (3 Br), 4i (3 NO2)** the protease inhibitions can also be neglected as values are less than - 0.50. Thus the **promising derivatives** on the basis of **bioactivity score** (moderate effect) which are been predicted to be acted upon by three mechanisms are derivatives **4a (H), 4d (2F), 4e (3F). (Table 5).**

Compounds	GPCR Ligand	Ion Channel Inhibitor	Kinase Inhibitor	Nuclear Receptor Ligand	Protease Inhibitor	Enzyme Inhibitor
4a	- 0.32	- 0.48	- 0.38	- 0.99	- 0.43	- 0.20
4b	- 0.27	- 0.54	- 0.35	- 0.94	- 0.48	- 0.31
4c	- 0.25	- 0.51	- 0.35	- 0.92	- 0.47	- 0.31
4d	- 0.32	- 0.43	- 0.43	- 0.87	- 0.41	- 0.15
4e	- 0.31	- 0.46	- 0.34	- 0.95	- 0.41	- 0.20
4f	- 0.30	- 0.46	- 0.36	- 0.93	- 0.40	- 0.19
4g	- 0.38	- 0.58.	- 0.46	- 1.00	- 0.55	- 0.30
4h	- 0.40	- 0.54	- 0.41	- 1.05	- 0.52	- 0.27
4i	- 0.43	- 0.50	- 0.47	- 1.01	- 0.51	- 0.29
4j	- 0.37	- 0.54	- 0.51	- 0.99	- 0.52	- 0.29
4k	- 0.34	- 0.53	- 0.39	- 0.92	- 0.44	- 0.24
Fluconazole	0.04	0.01	- 0.09	- 0.23	- 0.09	0.03
Voriconazole	0.23	0.17	0.14	- 0.22	0.02	0.19

Table 7: Predicted Bioactivity Scores from Molinspiration

2.2.4 Osiris Property Explorer

The basis on which **Osiris Property Explorer** predicts is on functional group similarity of the investigated compounds with the in vivo and in vitro case studies of molecules reported earlier to the database. The colour coding is red, yellow and green for the toxicity predictions. The red color is suggestive of high probability to be toxic followed by moderate and low for yellow and green respectively. The all analogues showed drug conform behaviour except for **4a (2H)** (showed high risk) and **4k (4-OMe)** (showed moderate risk). The solubility, drug likeness and drug scores were also obtained but the **standard molecules showed better results as compared to synthesised molecules** and are tabulated in the **Table 8**..

Compound	Solubility	Drug likeness	Drug Score	Mutagenicity	Tumorigenic	reproductive effect	irritant effect
4a	- 3.92	1.70	0.46	Green	Red	Green	Green
4b	- 4.65	3.54	0.75	Green	Green	Green	Green
4c	-5.39	3.85	0.66	Green	Green	Green	Green
4d	- 4.23	3.36	0.81	Green	Green	Green	Green
4e	- 4.23	1.80	0.76	Green	Green	Green	Green
4f	- 4.23	3.03	0.80	Green	Green	Green	Green
4g	- 4.75	- 0.13	0.52	Green	Green	Green	Green
4h	- 4.75	0.85	0.61	Green	Green	Green	Green
4i	- 4.38	-2.28	0.48	Green	Green	Green	Green
4j	- 4.84	- 7.88	0.43	Green	Green	Green	Green
4k	- 3.94	1.38	0.59	Green	Yellow	Green	Green
Fluconazole	-3.73	2.43	0.92	Green	Green	Green	Green
Voriconazole	- 3.23	3.06	0.90	Green	Green	Green	Green

Table 8: Predictions on basis of Osiris Property Explorer

2.2.5. Toxicologically Profiling

All analogues showed mutagen against Ames test. Analogues **4a to 4h showed Carcinogenicity_mouse positive** and **4i to 4k negative**. Analogues **4g and 4h showed positive results for Carcinogenicity_rat and rest analogues were negative**. Only **4g (2-Br) showed low risk** for hERG inhibition and rest all showed hERG showed medium risk. **(Table 9)**

	Algae_at	Ames_test	Cacino_Mouse	Carcino_Rat	dphnia_at	hERG_inhibition	medaka_at	minnow_at	TA100_10RLI	TA100_NA	TA1535_10RLI	TA1535_NA
4a	0.0638	Mutagen	+ve	-ve	0.0686	Medium_risk	0.0083	0.0070	+ve	-ve	+ve	-ve
4b	0.0358	Mutagen	+ve	-ve	0.0273	Medium_risk	0.0015	0.0019	+ve	-ve	+ve	-ve
4c	0.0201	Mutagen	+ve	-ve	0.0108	Medium_risk	0.0002	0.0005	+ve	-ve	+ve	-ve
4d	0.0505	Mutagen	+ve	-ve	0.0538	Medium_risk	0.0052	0.0032	+ve	-ve	+ve	-ve
4e	0.0489	Mutagen	+ve	-ve	0.0556	Medium_risk	0.0055	0.0032	+ve	-ve	+ve	-ve
4f	0.0482	Mutagen	+ve	-ve	0.0575	Medium_risk	0.0058	0.0032	+ve	-ve	+ve	-ve
4g	0.0327	Mutagen	+ve	+ve	0.0215	Medium_risk	0.0010	0.0015	+ve	-ve	+ve	-ve
4h	0.0182	Mutagen	+ve	+ve	0.0240	Medium_risk	0.0012	0.0015	+ve	-ve	+ve	-ve
4i	0.0510	Mutagen	-ve	-ve	0.0706	Medium_risk	0.0091	0.0044	+ve	+ve	+ve	-ve
4j	0.04616	Mutagen	-ve	-ve	0.2203	Low_risk	0.0804	0.0030	+ve	+ve	+ve	-ve
4k	0.0428	Mutagen	-ve	-ve	0.0635	Medium_risk	0.0073	0.0066	+ve	-ve	+ve	-ve
F	0.2218	Mutagen	-ve	+ve	0.6906	Medium_risk	0.6752	0.3601	+ve	+ve	+ve	+ve
V	0.0987	Mutagen	-ve	+ve	0.2087	Medium_risk	0.0695	0.0266	+ve	-ve	+ve	-ve

Table 9 : Toxicological profiling

3.0 EXPERIMENTAL SECTION

3.1 In silico screening

3.1.1 *in silico* ADME screening

Swiss ADME, an online web tool was used for calculation of ADME descriptors. The online tool allows the researchers to either construct the structures in the online tool itself or by feeding the SMILES notation.[21] Construction of structures was preferred, followed by verifying the constructed structures with the SMILES notation obtained from ChemDraw Professional 16.0. SwissADME, allowed to study physiochemical properties, lipophilicity, water solubility, pharmokinetics, drug-likeness, medicinal chemistry. The absorption % was calculated using the method reported by **Zhao et.al. (2002**) by the formula:- % Ab = 109 - [0.345 X TPSA]

3.1.2 Prediction of Activity Spectra for Substances (PASS)

PASS, is also an online web tool, specifically designed for *in silico* evaluation of organic drug like molecule. [22] The SMILES notation obtained from Chem Draw were fed into online web tool for obtaining the various biological activities. The activities obtained are tabulated in Table 6 along with the probability of the specific activity to be active (Pa) and inactive (Pi).

3.1.3. Molinspiration Calculation of Bioactivity Score

Molinspiration Cheminformatics Software v2018.03(*https://www.molinspiration.com/)*
was used for calculating bioactivity scores of all the derivatives.[23] The structures were constructed with the help of ChemDraw Professional 16.0 and the SMILES notations were obtained from there. The SMILES were then fed into the software and the bioactivity score was obtained. The scores predicted from the Molinspiration Cheminformatics Software v2018.03 gave the predictions, which has been tabulated in Table 7. The Molinspiration predicted for various inhibitions like G protein coupled receptor, Ion Channel Modulator, Kinase Inhibitor, Nuclear receptors, Protease inhibitor and enzyme inhibitor. A molecule having bioactivity score more than 0.00 is most likely to exhibit considerable biological activities, while scores between - 0.50 to 0.00 are expected to be moderately active and if score is less than - 0.50 it is presumed to be inactive.

3.1.4 Osiris Property Explorer

Osiris property explorer, online web tool was mainly used to obtain toxicity predictions, solubility and overall drug-likeness of fluconazole analogues.[24] The SMILES of the analogues were fed into the dialog box provided and virtual screening of molecules was performed. The various possible adverse effects of the analogues were shown like tumorgenicity, mutagenicity, effect on reproductive system, irrant system. The properties are shown in 3 colors. The red color indicated high risk of undesired effect, yellow suggestive of moderate toxicity and green colour showed drug conform behavior. The toxicity profiling, solubility, drug likeness and drug scores were also obtained and are tabulated in the Table 8.

3.1.5. Toxicologically Profiling

Toxicological profiling was carried using PreADMET software. [25] The various factors were obtained like Alage_at, Ames_test, carcinogenicity for mouse and rat, daphnia_at, hERG inhibition and other relevant toxic profiles were evaluated.

3.3 General Remarks (Chemistry)

Melting points were determined in open capillaries on a Mel-Temp apparatus and are uncorected. All the reactions were monitored by thin layer chromatography (TLC) on precoated silica gel 60 F254(mesh); spots were visualized with UV light. Merck silica gel(60-120 mesh) was used for column chromatography. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra were recorded on a Brucker Avance II 500 MHzNMR spectrometer in $CDCl_3$/DMSO-d_6 solution using tetramethylsilane as an internal standard. All chemical shifts were recorded in δ (ppm) and the following abbreviations are used: s, singlet; d, doublet; dd, doublet of doublet; t, triplet; m, multiplet. The mass spectra were recorded on Waters, Q-TOF Micromass /ESI-MS at 70eV.

3.3.1. General Procedure for the preparation of 2-chloro-1-(2,4-difluorophenyl)-ethanone (2)

To a solution of 1,3-difluorobenzene (5.7 g, 50 mmol) in1,2-dichloroethane (DCE, 30 ml), anhydrous aluminium chloride (7.98 g, 60 mmol) was added at 25–30 °C and stirred for 30 min. The reaction mixture was then cooled to 0 °C and chloroacetyl chloride (6.21 g, 54 mmol) in DCE (15 ml) was added into it over a period of 30 min at 0–10 °C. The reaction mixture was stirred at 25–30 °C for 7 h and diluted with the DCE (30 ml) and poured into 5% hydrochloric acid(50 ml) at 0–5 °C. The product was extracted with DCE (2 ×50 ml) and the combined organic layer was washed with 5%aqueous $NaHCO_3$ solution (20 ml), water (2 × 20 ml), brine(20 ml) and dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure to yield theproduct**2**, as yellow solid; yield 7.60 g (80%); m.p. 46–48 °C(lit [21]m.p. 46.5 °C).

3.3.2 General Procedure for the preparation of 1-(2,4-difluorophenyl)-2-[1,2,4]- triazol-1-yl-ethanone (3)

A mixture of **2**(9.05 g, 47.5 mmol), 1,2,4-triazole (3.93 g,57.01 mmol), sodium bicarbonate (4.80 g, 57.00 mmol) intoluene (50 ml) was refluxed for 4 h. After the reaction was completed, the reaction mixture was poured into crushed ice and extracted with toluene (2 × 50 ml). The combined organic layer was washed with H_2O (2 × 20 ml), brine (20 ml), dried over anhydrous $Na_2SO_4$and filtered. The filtrate was concentrated under reduced pressure to yield compound **3** as a brown solid; yield 7.30 g (69%), m.p. 104–106 °C (lit[21] m.p.103–105 °C).

3.3.3 General Procedure for the preparation of hydrazones (4a-k)

A mixture of compound **3** (0.01 mol), different substituted phenyl hydrazines (0.01 mol), absolute ethanol (15 ml) and acetic acid (1 ml) was refluxed for 60 min, then allowed to cool at room

temperature. The solid productcollected was re-crystallized from ethanol/DMF were purified by silica gel column chromatography (ethyl acetate: hexane, 1:1).

3.3.4.1. 1-(2,4-difluorophenyl)-2-(1H-1,2,4-triazol-1-yl)ethanone (3)

Yield 69%, White, M.P.103-105^{o}C. ^{1}H NMR (500 MHz, $CDCl_3$): δ8.43 (s, 1H, triazole), 8.11(s, 1H, triazole) , 7.90 (m, 1H, phenyl) , 7.12 (ddd, *J* = 16.4 , 7.2 , 2 Hz,1H, phenyl), 6.81 (ddd , *J*= 13.6 , 13.2 and 2 Hz, 1H, phenyl), 4.59 (s , 2H, methylene) .

^{13}C NMR (125 MHz, $CDCl_3$):δ165.80, 160.29, 151.40, 143.90, 132.60, 121.02, 112.12, 111.06, 100.36, 57.43.

3.3.4.2. 1- (2 - (2,4-difluorophenyl)-2-(2-phenylhydrazono) ethyl) - 1H - 1,2,4-triazole (4a)

Yield 87%, White, M.P. 170-176^{0}C. ^{1}H NMR (500 MHz, $CDCl_3$): δ8.13 (s, 1H, triazole), 7.84(s, 1H, triazole) , 7.32 (bs , NH) , 7.13 - 7.24 (m, 2H, phenyl) , 7.01 - 7.06 (m, 1H, phenyl), 6.96 - 6.99 (m , 4H, phenyl), 6.90 - 6.92 (m, 1H , phenyl) , 5.28 (s , 2H, methylene) .

^{13}C NMR (125 MHz, $CDCl_3$):δ163.40, 161.22, 155.46, 151.22, 143.98, 143.00, 132.41, 129.32, 122.69, 113.92, 113.12, 112.66, 111.36, 51.43.

3.3.4.3. 1-(2-(2-(2-chlorophenyl)hydrazono)-2-(2,4-difluorophenyl)ethyl)-1H-1,2,4-triazole (4b)

Yield 68%, Yellow, M.P. 160 - 165 ^{0}C. ^{1}H NMR (500 MHz, $CDCl_3$): δ10.43 (bs , NH) 8.23 (d,*J* = 2.5 Hz , 1H, triazole), 8.02 (s, 1H, triazole) , 7.79 - 7.84 (ddd,*J* = 11 , 6.5 , 6.5 Hz , 1H, phenyl) , 7.56 - 7.58 (dd,*J* = 8 , 1.5 Hz , 1H, phenyl), 7.32 - 7.34 (ddd , *J* = 9.5 , 8 , 1.5 Hz , 1H , phenyl), 7.23 - 7.26 (ddd , *J* = 8 , 8 , 1.5 Hz , 1H , phenyl) , 6.94 (ddd , *J* = 16 , 16 , 2.5 Hz , 1H , phenyl) , 6.89 - 6.91 (m, 2H, phenyl) , 5.34 (s , 2H, methylene) .

^{13}C NMR (125 MHz, $CDCl_3$):δ164.24, 162.23, 161.43 , 159.46, 151.74, 143.57, 141.15, 135.20, 131.30, 129.49, 127.75, 122.01 , 118.96 , 114.97 , 112.36 , 47.00.

3.3.4.4. 1-(2-(2-(2,4-dichlorophenyl)hydrazono)-2-(2,4-difluorophenyl)ethyl)-1H-1,2,4-triazole (4c)

Yield 67%, Brown ,M.P. 200-205 ^{0}C. ^{1}H NMR (500 MHz, $CDCl_3$): δ10.50 (bs , NH) 8.43 (s, 1H, triazole), 8.12(s, 1H, triazole) , 7.84 (m , 1H, phenyl) , 7.58 (m , 1H, phenyl) , 7.53 (d ,*J* = 1.5 Hz ,1H , phenyl), 7.52 (d, *J* = 7.5 Hz , 1H, phenyl) , 7.39 (dd, *J* = 7.5 , 1.5 Hz ,1H , phenyl) , 6.95 - 6.98 (m, 1H, phenyl) , 5.32 (s , 2H, methylene) .

^{13}C NMR (125 MHz, $CDCl_3$):δ165.45, 162.87, 158.02, 156.54, 147.03, 143.82, 138.19, 131.71, 128.82, 125.28 , 124.93 , 119.36, 114.48, 112.00, 111.50, 52.00.

3.3.4.5 1-(2-(2,4-difluorophenyl)-2-(2-(2-fluorophenyl)hydrazono)ethyl)-1H-1,2,4-triazole (4d)

Yield 85%, Brown ,M.P. 190 ^{0}C. ^{1}H NMR (500 MHz, $CDCl_3$): δ10.50 (bs , NH) 8.23 (s, 1H, triazole), 8.03(s, 1H, triazole) , 7.79 - 7.84 (ddd,*J* = 16 , 15.5, 9 Hz , 1H, phenyl) , 7.53 - 7.56 (dd, *J* = 16 , 8 Hz ,

1H, phenyl), 7.06 - 7.11 (dd ,*J* = 15.5 , 7.5 Hz ,2H , phenyl), 6.93 - 6.96 (dd, *J* = 8 , 7.5 Hz , 1H, phenyl) , 6.87 - 6.88 (m, 2H, phenyl) , 5.32 (s , 2H, methylene) .

^{13}C NMR (125 MHz, $CDCl_3$):δ164.68, 163.03, 157.54, 152.47, 148.58, 144.02, 133.56, 130.36, 127.54, 125.92, 124.21 , 118.65, 113.47, 112.21, 111.47, 51.00.

3.3.4.6. 1-(2-(2,4-difluorophenyl)-2-(2-(3-fluorophenyl)hydrazono)ethyl)-1H-1,2,4-triazole (4e)

Yield 59%, Yellow, M.P. 184-190 ^{0}C.^{1}H NMR (500 MHz, $CDCl_3$): δ10.42 (bs , NH) 8.49 (s, 1H, triazole), 8.29(s, 1H, triazole) , 7.75 - 7.79 (m , 1H , phenyl) , 7.19 (m , 1H , phenyl) , 7.15 (m , 1H , phenyl) , 7.12 (m , 1H , phenyl) , 6.93 - 6.96 (m , 1H , phenyl), 6.60 (m, 1H, phenyl) , 6.58 (m, 1H, phenyl) , 4.98 (s , 2H, methylene) .

^{13}C NMR (125 MHz, $CDCl_3$):δ168.60, 165.03, 155.28, 152.59, 148.03, 144.51, 133.00, 130.50, 129.00, 125.00, 124.49 , 118.66, 113.82, 112.52, 111.44, 50.26.

3.3.4.7. 1-(2-(2,4-difluorophenyl)-2-(2-(4-fluorophenyl)hydrazono)ethyl)-1H-1,2,4-triazole (4f)

Yield 84%, Yellowish green, M.P. 187-190 ^{0}C. ^{1}H NMR (500 MHz, $CDCl_3$): δ10.42 (bs , NH) 8.40 (s, 1H, triazole), 8.22(s, 1H, triazole) , 7.75 - 7.79 (m , 1H , phenyl) , 7.20 - 7.25 (m , 1H , phenyl) , 6.99 (ddd , *J* = 8 , 7.5 , 1.5 Hz , 1H , phenyl) , 6.92 - 6.93 (m, 1H , phenyl) 6.61 (ddd , *J* = 7.5 , 5 , 1.5 Hz , 1H , phenyl) ,4.70 (s , 2H, methylene) .

^{13}C NMR (125 MHz, $CDCl_3$):δ163.42, 161.11, 157.46, 155.59, 151.53 , 143.83, 138.61 , 132.40 , 124.49 , 118.66, 113.82, 112.52, 111.44, 50.26.

3.3.4.8. 1-(2-(2-(2-bromophenyl)hydrazono)-2-(2,4-difluorophenyl)ethyl)-1H-1,2,4-triazole (4g)

Yield 75%, Yellow, M.P. 156 - 160 ^{0}C. ^{1}H NMR (500 MHz, $CDCl_3$): δ8.49 (s, 1H, triazole), 8.34(s, 1H, triazole) , 7.85 (m, 1H, phenyl) , 6.50-7.55 (m, 4H, phenyl), 7.13 (m , 1H , phenyl) , 7.2 (bs, 1H) , 6.95 (m, 1H, phenyl) , 4.96 (s , 2H, methylene) .

^{13}C NMR (125 MHz, $CDCl_3$):δ168.45, 166.27, 157.66, 153.81, 149.98, 144.54, 134.44, 133.32, 128.69, 125.92, 125.03 , 118.25, 113.27, 112.46, 111.42, 51.47.

3.3.4.9. 1-(2-(2-(4-bromophenyl)hydrazono)-2-(2,4-difluorophenyl)ethyl)-1H-1,2,4-triazole (4h)

Yield 54%, White, M.P. 190 ^{0}C. ^{1}H NMR (500 MHz, $CDCl_3$): δ10.39 (bs , NH) 8.65 (s, 1H, triazole), 7.94(s, 1H, triazole) , 7.58 - 7.63 (m , 1H , phenyl) , 7.18 - 7.22 (ddd,*J* = 16.4 , 7.2 , 2Hz, 1H , phenyl) , 7.39-7.42(d , *J* = 8 Hz , 2H , phenyl) , 7.15 - 7.17(d , *J* = 8 Hz , 2H , phenyl) , 7.04 - 7.08 (ddd , *J* = 13.6 , 13.2 , 2 Hz , 1H , phenyl) , 5.57 (s , 2H, methylene) .

^{13}C NMR (125 MHz, $CDCl_3$):δ162.42, 160.88, 156.52, 155.92, 151.20 , 142.00, 137.50 , 132.43 , 123.73 , 118.32, 113.52, 112.78 , 111.75 , 52.26. m/z (70 eV) : 392.0317 [M+1].

3.3.4.10. 1-(2-(2,4-difluorophenyl)-2-(2-(3-nitrophenyl)hydrazono)ethyl)-1H-1,2,4-triazole (4i)

Yield 72%, Dark Yellow, M.P. 200-202 ^{0}C. ^{1}H NMR (500 MHz, $CDCl_3$): δ10.46 (bs , NH) 8.69 (s, 1H, triazole), 8.09(s, 1H, triazole) , 7.95 (m , 1H , phenyl) , 7.74 (ddd , *J* = 7.9 , 2 , 1.5 Hz , 1H, phenyl) , 7.62 (ddd , *J* = 7.9 , 2 , 1.5 Hz , 1H , phenyl) , 7.56 (dd , *J* = 2 , 1.5 Hz , 1H , phenyl) , 7.46 (dd , *J* = 8 , 7.9 Hz, 1H , phenyl) , 7.49 (m , 1H , phenyl) , 7.12 (m , 1H , phenyl) , 5.28 (s , 2H, methylene) .
^{13}C NMR (125 MHz, $CDCl_3$):δ169.20, 165.93, 155.80, 152.21, 148.14, 143.51, 135.70, 133.47, 129.53, 125.14 , 124.25 , 118.66 , 113.82, 112.52, 111.44, 52.26.

3.3.4.11. 1-(2-(2,4-difluorophenyl)-2-(2-(2,4-dinitrophenyl)hydrazono)ethyl)-1H-1,2,4-triazole (4j)

Yield 87%, Yellow,M.P. 210 ^{0}C. ^{1}H NMR (500 MHz, $CDCl_3$): δ10.57 (bs , NH) , 8.91 (d , *J* = 1.5 Hz , 1H , phenyl) , 8.69 (s, 1H, triazole), 8.45 - 8.48 (dd , *J* = 9.5 , 2 Hz , 1H, phenyl) 8.01(s, 1H, triazole) , 7.99 - 8.02 (d ,*J* = 9.5 Hz , 1H, phenyl) , 7.78 - 7.83 (ddd, *J* = 8.5 , 8.5 , 7 Hz , 1H, phenyl), 7.35 - 7.39 (ddd ,*J* = 21 , 11 , 10 Hz ,1H , phenyl), 7.18 - 7.21 (dd, *J* = 8 , 7.5 Hz , 1H, phenyl) , 5.66 (s , 2H, methylene) .
^{13}C NMR (125 MHz, $CDCl_3$): δ164.68, 163.03, 157.54, 152.42, 146.59, 145.63 , 144.46 , 133.64 , 131.92 , 130.88 , 130.76 , 123.26 , 116.46 , 113.67, 105.84 , 54.00.

3.3.4.12. 1-(2-(2,4-difluorophenyl)-2-(2-(4-methoxyphenyl)hydrazono)ethyl)-1H-1,2,4-triazole (4k)

Yield 84%, Yellow. M.P. 205-215^{0}C. ^{1}H NMR (500 MHz, $CDCl_3$): δ10.39 (bs , NH) 8.51 (s, 1H, triazole), 8.02(s, 1H, triazole) , 7.79 - 7.83 (m , 1H , phenyl) , 7.53 (ddd,*J* = 16.4 , 7.2 , 2Hz, 1H , phenyl) , 7.40-7.42(d , *J* = 8 Hz , 2H , phenyl) , 7.25 - 7.27(d , *J* = 8 Hz , 2H , phenyl) , 7.04 - 7.08 (ddd , *J* = 13.6 , 13.2 , 2 Hz , 1H , phenyl) , 5.57 (s , 2H, methylene) , 3.83 (s , 3H , methoxy) .
^{13}C NMR (125 MHz, $CDCl_3$):δ162.42, 160.88, 156.52, 155.92, 151.20 , 142.00, 137.50 , 132.43 , 123.73 , 118.32, 113.52, 112.78 , 111.75 , 55.86 , 51.00.

Conclusion :

We have successfully designed and synthesized eleven Fluconazole derivatives 4a-k. In our previous work we have carried out docking studies for 3PDB ids (5V5Z, 5FSA and 5TZ1) for CYP51. All the synthesized derivatives showed good to moderate antifungal activity. In continuation with this work , we now report herewith in silico ADME, Toxicological profiling and predicted the biological activities using PASS Online webserver and computed the bioactivity scores of the fluconazole analogues .When the predicted inhibitions were compared with the reference molecules, the designed analogues showed other inhibitions and thus suggestive of other biological activities like antiepileptic, antineoplastic and anticonvulsant. The antineoplastic was shown by only 4d (2F) and the autoimmune disorder was shown by only 4a(2H), 4b(2,5($Cl)_2$), 4f (4F). The pharmokinetics study revealed good GI absorption expect 4j (2,4 ($NO_2)_2$, No PGP substrate inhibition and Blood Brain Barrier (BBB) expect for 4i (3 NO_2) and 4j (2,4

$(NO_2)_2$. The designed analogues showed excellent LogP values, suggestive of that each of analogue designed would be absorbed to a greater extent and would be easily distributed by water as compared to the standard antifungal drugs taken as reference. $LogK_P$ values for designed analogues when compared to Fluconazole (-7.92) and Voriconazole (-7.36) suggestive of that the designed analogues have more skin permeability. The bioactivity scores revealed 4a (H), 4d (2F), 4e (3F) as promising candidates. There is also scope of carrying out virtually screening of the analogues with suitable protein for the biological activities like antiepileptic, antineoplastic and anticonvulsant.

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