Molecular Modeling, Synthesis and Antimicrobial Evaluation of New Molecular Hybrids of Tetrazole Derivatives.

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Abstract: A variety of heterocyclic tetrazole derivatives were obtained *via* cyclization of α,β-unsaturated ketones with malononitrile, hydrazine hydrate and (un)substituted phenylhydrazine at different reaction conditions. The intermediate α,β-unsaturated ketones **2a-f** were synthesized *via* base-catalyzed condensation of a tetrazole containing formyl group **1** with different acetophenones. Preliminary antimicrobial screening showed that compounds **3a, 4a, 4b, 6f, 8e and 8f** have promising activity and their MICs values ranged from 23.40 to 46.87 µg/L. Molecular modeling study was done and the *in silico* results were in accordance with the *in vitro* antimicrobial screening.

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Key Words: tetrazoles, antimicrobial activity, molecular modeling.

1. Introduction

The rising prevalence of multi-drug resistant Gram-positive and Gram-negative bacteria continues to provide impetus for the search and discovery of novel antimicrobial agents active against these pathogens.

Tetrazole derivatives are present in many of the bioactive heterocyclic compounds with diverse biological, pharmaceutical and clinical applications such as antibacterial [1-5], antifungal [1,5,6], anti-HIV [7] and analgesic agents [8]. In particular, 5-substituted-1-phenyl-1*H*-tetrazoles were found to be effective antibacterial and antifungal agents [1]. Moreover, certain other nitrogen-bearing heterocycles are known to possess antimicrobial activity, such as pyridines [5,9-11], pyrans [12,13] and pyrazoles [14-17]. Thus, it was envisioned to synthesize new hybrids of tetrazole with pyridine, pyran, or pyrazole with the aim to obtain synergized antimicrobial activity.

The newly synthesized compounds were identified by spectroscopic and elemental analyses and screened for their antimicrobial activity. Moreover, a docking simulation was performed to explore the binding modes of them to different targets in bacteria.

2. Results and Discussion

Condensation of α,β-unsaturated ketones **2a-f** with either hydrazine hydrate or (un)substituted phenylhydrazine to afford 1*H*-pyrazoles and N-(substituted)phenyl-1-*H*-pyrazoles, respectively, are reported in several references [16, 17, 23-26]. Thus, 1*H*-pyrazoles **5a-f**, N-phenyl-1*H*-pyrazoles **6a-f** and N-(4-bromophenyl)-1*H*-pyrazoles **7a-f**, were prepared by reacting α,β-unsaturated ketones **2a-f** with hydrazine hydrate, phenylhydrazine and 4-bromophenylhydrazine, respectively, in refluxing ethanol. Aitmambetov *et al*, (2004) [27] has reported that condensation of hydrazine hydrate with α,β-unsaturated ketones in glacial acetic acid afforded the N-acetyl-1*H*-pyrazoles. Accordingly, the same procedure was adopted for the condensation of α,β-unsaturated ketones **2a-f** with hydrazine hydrate in glacial acetic acid to obtain N-acetyl-1*H*-pyrazoles **8a-f** (scheme 3). All the target compounds were characterized by using thin layer chromatography and melting point techniques. The structures of the newly synthesized compounds were confirmed by spectral data (IR, MS, ^{1}H-NMR and elemental analysis). The chemical profile of the newly synthesized compounds is illustrated in table 1.

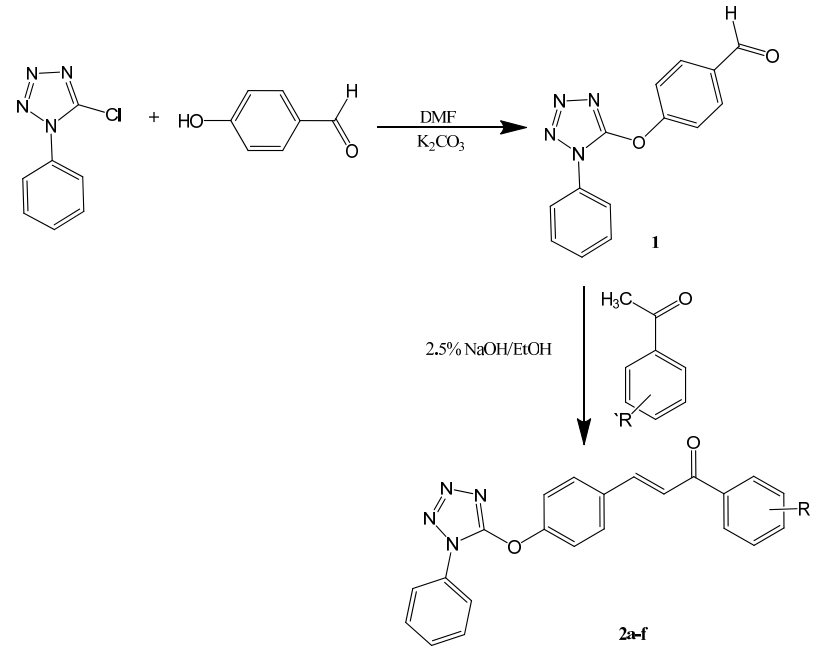
2.1. Chemistry

The starting material **1** was prepared by the method described by Moustafa *et al* (1985), *via* reacting 4-hydroxybenzaldehyde with 5-chloro-1-phenyl-1*H*-tetrazole in DMF, using K_2CO_3 as catalyst [1].

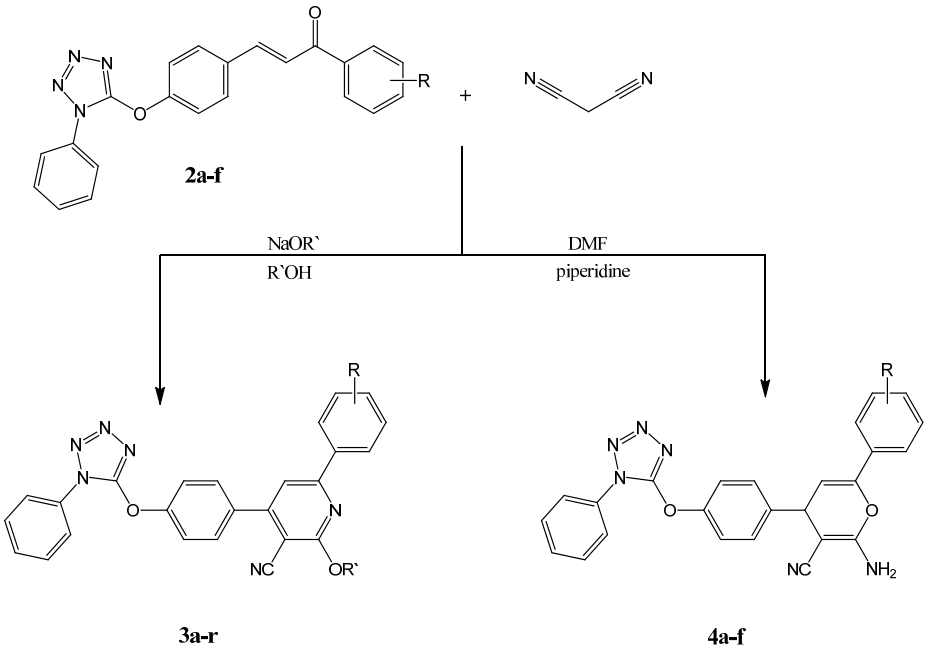
The key intermediates α,β-unsaturated ketones **2a-f** were synthesized through Claisen–Schmidt condensation of equimolar amounts of the appropriate acetophenone and the starting compound **1** under base-catalyzed reaction [1], (scheme 1).

In the present work, two types of heterocyclic rings; pyridine and pyran; were synthesized (scheme

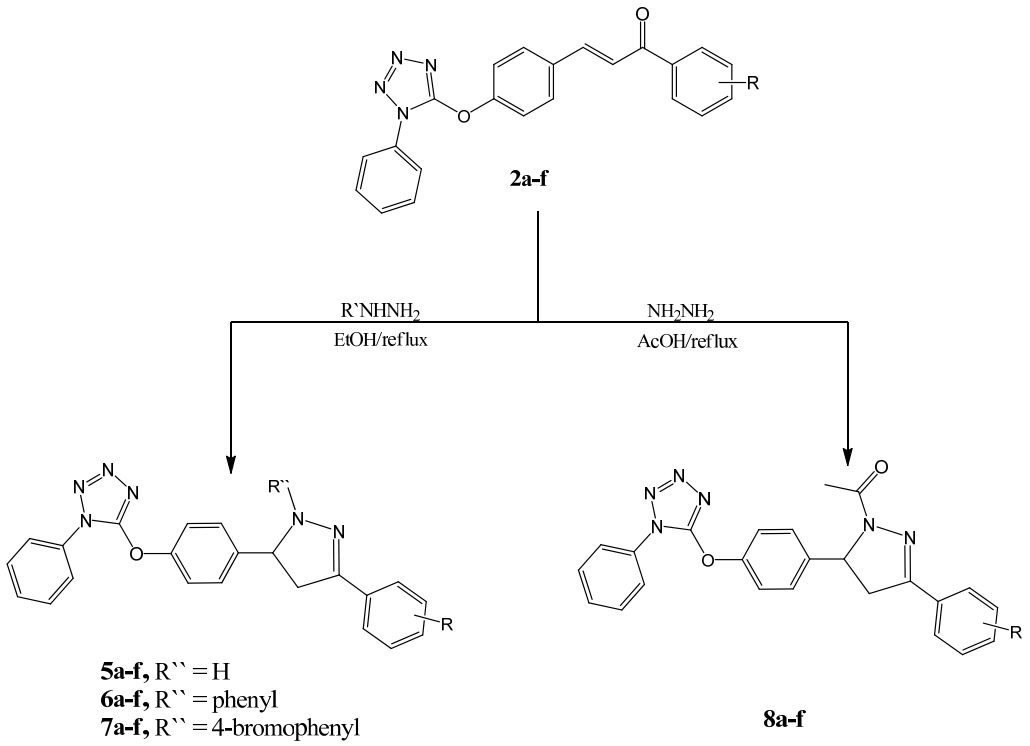
2) by cyclocondensation of the intermediates **2a-f** with malononitrile at different reaction conditions. The one-pot reported procedures [9, 18-21] for preparation of pyridines from α,β-unsaturated ketones **2a-f** and malononitrile in presence of sodium alkoxide-alcohol system was adopted to obtain the corresponding 2-alkoxypyridines **3a-r**. On the other hand, 2-aminopyran derivatives **4a-f** were obtained by adopting the reported procedure [22] in which the α,β-unsaturated ketones **2a-f** were reacted with malononitrile in DMF and in presence of piperidine as catalyst (scheme 2).



Scheme 1



Scheme 2



Scheme 3

Table 1.The Chemical Profile of the Newly Synthesized Compounds.

Compd. No.	R	R`	Mol.fromula	Reaction time	Yield%	mp (°C)
2a	H	----------	$C_{22}H_{16}N_4O_2$	2 h	80	155
2b	4-CH_3	----------	$C_{23}H_{18}N_4O_2$	2 h	73	223
2c	4-Cl	----------	$C_{22}H_{15}ClN_4O_2$	2 h	75	186
2d	4-OCH_3	----------	$C_{23}H_{18}N_4O_3$	2 h	75	150
2e	3-OH	----------	$C_{22}H_{16}N_4O_3$	2 h	60	126
2f	4-NO_2	----------	$C_{22}H_{15}N_5O_4$	2 h	73	133
3a	4-OCH_3	CH_3	$C_{27}H_{20}N_6O_3$	10 min.	70	127
3b	4-OCH_3	C_2H_5	$C_{28}H_{22}N_6O_3$	1 h	67	135
3c	4-OCH_3	C_3H_7	$C_{29}H_{24}N_6O_3$	1.5 h	64	210
3d	4-CH_3	CH_3	$C_{27}H_{20}N_6O_2$	15 min.	68	140
3e	4-CH_3	C_2H_5	$C_{28}H_{22}N_6O_2$	1.5 h	66	190
3f	4-CH_3	C_3H_7	$C_{29}H_{24}N_6O_2$	3 h	62	240
3g	3-OH	CH_3	$C_{26}H_{18}N_6O_3$	30 min.	72	180
3h	3-OH	C_2H_5	$C_{27}H_{20}N_6O_3$	2 h	63	220
3i	3-OH	C_3H_7	$C_{28}H_{22}N_6O_3$	3 h	63	250
3j	H	CH_3	$C_{26}H_{18}N_6O_2$	10 min.	62	165
3k	H	C_2H_5	$C_{27}H_{20}N_6O_2$	30 min.	60	198
3l	H	C_3H_7	$C_{28}H_{22}N_6O_2$	1 h	59	215
3m	4-Cl	CH_3	$C_{26}H_{17}ClN_6O_2$	2 h	58	270
3n	4-Cl	C_2H_5	$C_{27}H_{19}ClN_6O_2$	5 h	55	285
3o	4-Cl	C_3H_7	$C_{28}H_{21}N_6O_2$	6 h	50	300
3p	4-NO_2	CH_3	$C_{26}H_{17}N_7O_4$	4 h	55	230
3q	4-NO_2	C_2H_5	$C_{27}H_{19}N_7O_4$	6 h	52	260
3r	4-NO_2	C_3H_7	$C_{28}H_{21}N_7O_4$	8 h	51	170
4a	4-OCH_3	----------	$C_{26}H_{20}N_6O_3$	24 h	66	217
4b	4-CH_3	----------	$C_{26}H_{20}N_6O_2$	24 h	69	125
4c	3-OH	----------	$C_{25}H_{18}N_6O_3$	24 h	55	225

4d	H	----------	$C_{25}H_{18}N_6O_2$	24 h	59	205
4e	4-Cl	----------	$C_{25}H_{17}ClN_6O_2$	24 h	49	147
4f	4-NO_2	----------	$C_{25}H_{17}N_7O_4$	24 h	45	177
5a	4-OCH_3	----------	$C_{23}H_{20}N_6O_2$	24 h	55	245
5b	4-CH_3	----------	$C_{23}H_{20}N_6O$	24 h	32	188
5c	3-OH	----------	$C_{22}H_{18}N_6O_2$	20 h	41	132
5d	H	----------	$C_{22}H_{18}N_6O$	20 h	22	174
5e	4-Cl	----------	$C_{22}H_{17}ClN_6O$	30 h	33	155
5f	4-NO_2	----------	$C_{22}H_{17}N_7O_3$	48 h	55	310
6a	4-OCH_3	----------	$C_{29}H_{24}N_6O_2$	24 h	18	305
6b	4-CH_3	----------	$C_{29}H_{24}N_6O$	22 h	15	120
6c	3-OH	----------	$C_{28}H_{22}N_6O_2$	24 h	49	350
6d	H	----------	$C_{28}H_{22}N_6O$	24 h	17	344
6e	4-Cl	----------	$C_{28}H_{21}ClN_6O$	20 h	44	191
6f	4-NO_2	----------	$C_{28}H_{21}N_7O_3$	24 h	50	253
7a	4-OCH_3	----------	$C_{29}H_{23}BrN_6O_2$	20 h	52	205
7b	4-CH_3	----------	$C_{29}H_{23}BrN_6O$	24 h	49	277
7c	3-OH	----------	$C_{28}H_{21}BrN_6O_2$	24 h	45	235
7d	H	----------	$C_{28}H_{21}BrN_6O$	20 h	32	295
7e	4-Cl	----------	$C_{28}H_{20}BrClN_6O$	24 h	36	265
7f	4-NO_2	----------	$C_{28}H_{20}BrN_7O_3$	24 h	40	288
8a	4-OCH_3	----------	$C_{25}H_{22}N_6O_3$	24 h	45	340
8b	4-CH_3	----------	$C_{25}H_{22}N_6O_2$	48 h	55	273
8c	3-OH	----------	$C_{24}H_{20}N_6O_3$	48 h	20	315
8d	H	----------	$C_{24}H_{20}N_6O_2$	24 h	39	360
8e	4-Cl	----------	$C_{24}H_{19}ClN_6O_2$	24 h	55	227
8f	4-NO_2	----------	$C_{24}H_{19}N_7O_4$	48 h	50	255

2.2. Molecular Modeling:

A primary molecular modeling study of the newly synthesized derivatives was performed by submitting their structures to the free online site TarFisDock (Target Fishing Dock) [28] to get different binding energy scores with different bacterial targets. Chemical structures were firstly converted into mol2 file format, then submitted to the site. Results from the site were received and carefully analyzed to obtain a conclusive idea about the most appropriate bacterial targets of the examined compounds.

Pyridine derivatives **3a-r** were found to have promising antibacterial activity as revealed by their high negative energy scores of interaction with dihydrofolatereductase whose PDB_ID is (1DRF) which is an important target for antimicrobial drugs belonging to the class of antimetabolites as the enzyme plays important role in the *de novo* purin synthesis [29]. The scores that indicate the interaction energies of these compounds to 1DRF are illustrated in table 2.

Docking calculations were then carried out using the online site DockingServer [30, 31], which perform docking using AutoDock 4.0. Gasteiger partial charges were added to the ligand atoms. Non-polar hydrogen atoms were merged, and rotatable bonds were defined. Essential hydrogen atoms, Kollman united atom type charges and solvation parameters were added with the aid of AutoDock tools [32].

Affinity (grid) maps of 20 Å grid points and 0.375 Å spacing were generated using the Autogrid program [31]. AutoDock parameter set- and distance-dependent dielectric functions were used in the calculation of the van der Waals and the electrostatic terms, respectively.

Table 2.The Interaction Energy Scores of Some Pyridine Derivatives to 1DRF.

Compd. No.	Energy score
3a	-37.87
3b	-37.54
3d	-37.29
3e	-37.11
3g	-37.21
3j	-36.84
3m	-36.58
3p	-35.05

Docking simulations were performed using the Lamarckian genetic algorithm (LGA) and the

Solis and Wets local search method [31]. Initial position, orientation, and torsions of the ligand molecules were set randomly. All rotatable torsions were released during docking. Each docking

experiment was derived from 10 different runs that were set to terminate after a maximum of 250000 energy evaluations. During the search, a translational step of 0.2 Å, and quaternion and torsion steps of 5 were applied.

Table 3 illustrates the results obtained from the docking calculations for pyridine derivatives and the antibacterial agent, trimethoprim which acts on the same target.

Since there is isosteric relationship between pyridine and pyran derivatives, synthesized pyrans were also docked to the same target and their results are shown in table 4. Moreover, pyrazole derivatives were submitted to the free online site (TarFisDock) and their results are shown in table 5. The results showed that the highest negative energy scores were for the target, nicotinic acid mononucleotide adenylyltransferase whose PDB_ID is (1K4M) which is an indispensable enzyme in both *de novo* biosynthesis and salvage of NAD+ and NADP+ and this is absolutely required for cell survival in prokaryotes [33]. Thus, this enzyme represents an attractive target for the development of new broad-spectrum antibacterial agents. Using the previously mentioned DockingServer the pyrazole derivatives were submitted, prepared and docked on the prepared protein (1K4M) and the results obtained were compared to the docked reference substrate NAD+ in addition to an investigational compound (1_02_1) which acts on the same target protein [34]. These results are shown in table 6.

Table 5.The Interaction Energy Scores of Some Pyrazole Derivatives to 1K4M.

Compd. No.	Energy score
5a	-37.01
5b	-37.58
5c	-38.23
5d	-38.25
5e	-39.33
5f	-42.45
8a	-45.26
8b	-39.50

Table 3. Results of Docking of Compounds 3a-r and Trimethoprim to 1DRF.

Compd. No.	Est. Free Energyof Binding Kcal/mol	Est. Inhibition Constant,Ki	vdW+Hbond+desolv. Energy Kcal/mol	Electrostat. Energy Kcal/mol	Total Intermol. Energy Kcal/mol
3a	-9.31	149.38 nM	-11.52	-0.14	-11.38
3b	-9.01	248.23 nM	-11.86	-0.05	-11.81
3c	-8.54	548.92 nM	-10.57	-0.23	-10.80
3d	-8.41	679.35 nM	-11.42	-0.02	-11.43
3e	-7.46	3.42 uM	-10.30	-0.11	-10.41
3f	-6.95	8.09 uM	-9.54	-0.10	-9.64
3g	-7.73	2.14 uM	-10.22	-0.08	-10.30
3h	-7.40	3.79 uM	-10.44	-0.02	-10.46
3i	-7.08	6.47 uM	-9.36	-0.01	-9.35
3j	-7.21	5.17 uM	-9.96	-0.05	-9.91
3k	-7.19	5.37 uM	-10.24	-0.11	-10.35
3l	-7.09	6.36 uM	-9.71	-0.19	-9.91
3m	-6.93	8.31 uM	-9.54	-0.02	-9.52
3n	-6.47	18.06 uM	-9.39	-0.02	-9.37
3o	-6.12	32.89 uM	-8.45	-0.21	-8.66
3p	-6.77	10.87 uM	-9.59	-0.08	-9.52
3q	-6.44	19.17 uM	-8.68	-0.03	-8.65
3r	-5.76	59.47 uM	-7.87	-0.13	-8.00
Trimethoprim	-4.11	977.26 uM	-3.99l	-1.97	-5.96

Table 4. Results of Docking of Pyran Derivatives 4a-f to 1DRF.

Compd. No.	Est. Free Energy of Binding Kcal/mol	Est. Inhibition Constant, Ki	vdW+Hbond+desolv. Energy Kcal/mol	Electrostatic Energy Kcal/mol	Total Intermolec. Energy Kcal/mol
4a	-11.54	3.45 nM	-13.40	-0.19 l	-13.59
4b	-11.12	7.02 nM	-12.61	-0.23	-12.84
4c	-9.45	117.97 nM	-11.80	-0.02	-11.78
4d	-8.86	319.64 nM	-11.68	-0.04	-11.64
4e	-8.66	445.56 nM	-10.59	+0.00	-10.59
4f	-8.08	1.19 uM	-10.33	-0.06	-10.39

Table 6. Results of Docking of Pyrazole Derivatives, NAD+ and (1_2_1) to 1K4M.

Compd. No.	Est. Free Energy of Binding Kcal/mol	Est. Inhibition Constant, Ki Kcal/mol	vdW + Hbond + desolv. Energy Kcal/mol	Electrostat. Energy Kcal/mol	Total Intermolec. Energy Kcal/mol
5a	-8.29	839.47 nM	-9.85	-0.06	-9.91
5b	-8.39	702.26 nM	-9.93	+0.09	-9.85
5c	-8.52	570.68 nM	-9.87	-0.04	-9.91
5d	-8.88	307.80 nM	-10.45	-0.08	-10.53
5e	-9.17	188.88 nM	-11.03	+0.01	-11.01
5f	-9.54	102.16 nM	-11.62	+0.03	-11.60
6a	-8.05	1.25 uM	-10.40	-0.10	-10.49
6b	-8.28	858.13 nM	-10.81	-0.01	-10.82
6c	-7.91	1.60 uM	-9.85	-0.09	-9.94 k
6d	-8.78	364.95 nM	-10.98	-0.07	-11.05
6e	-9.00	254.43 nM	-11.04	+0.06	-10.98
6f	-10.8	12.04 nM	-13.43	+0.17	-13.26
7a	-8.85	327.94 nM	-10.82	-0.01	-10.84
7b	-8.73	398.84 nM	-10.67	-0.08	-10.76
7c	-8.78	363.55 nM	-11.26	-0.04	-11.30
7d	-8.27	862.88 nM	-10.68	+0.08	-10.60
7e	-9.31	150.80 nM	-11.41	-0.12	-11.53
7f	-9.66	82.82 nM	-11.64	+0.12	-11.52
8a	-8.96	270.87 nM	-10.05	-0.04	-10.10
8b	-9.24	170.05 nM	-10.52	-0.07	-10.58
8c	-9.81	64.45 nM	-11.19	-0.11	-11.08
8d	-9.93	52.47 nM	-10.83	-0.06	-10.89
8e	-10.46	21.47 nM	-12.06	-0.10	-12.17
8f	-10.82	11.74 nM	-11.66	-0.13	-11.79
NAD+	-7.70	2.26 uM	-10.11	-0.13	-10.24
(1_02_1)	-8.58	515.08 nM	-10.96	-0.04	-11.01

2.3. Biological Evaluation

The antimicrobial screening of all the newly synthesized compounds was done using cup diffusion technique [35] using Müller-Hinton agar medium for bacteria and Yeast Extract Peptone Dextrose (YPD) medium for fungi. This screening was performed against the Gram-negative *Pseudomonas aeruginosa* and the Gram-positive *Staphylococcus aureus*, in addition to the pathogenic fungi *Candida albicans*. The results of the preliminary antimicrobial testing of the synthesized compounds were compared to the antibacterial agents Benzylpenicillin, Streptomycin and the antifungal drug Fluconazole. Analysis of the inhibition zone diameter data (table 7), proved that the order of sensitivity was the Gram-negative bacteria *Pseudomonas aeruginosa*, the pathogenic fungi *Candida albicans* and to a lesser extent the Gram-positive *Staphylococcus aureus*. The minimal inhibitory concentration (MIC) for the most active compounds, **3a, 4a, 4b, 6f, 8e and 8f** against the same microorganisms used in the primary screening was carried out using the agar dilution method [36] as shown in Table 8.

Comparison between the results of the molecular modeling study and the *in vitro* antimicrobial screening revealed strong correlation between virtual screening and antimicrobial testing.

Pyran derivatives **4a-f** were found to be more active than pyridine derivatives **3a-r.** The order of activity in both series for the R substituent is: 4-OMe > 4-Me > 3-OH > H > 4-Cl > 4-NO_2. On the other hand, R` substitution in compounds **3a-r** showed increasing activity in the order: Me > Et > n-propyl.

In case of pyrazole derivatives **5a-f, 6a-f, 7a-f** and **8a-f,** the antimicrobial activity increased with changing the R substituent in the following order: 4-NO_2> 4-Cl >H > 3-OH > 4-Me > 4-OMe. Substitution on the N^1 atom of the pyrazole ring affected the activity in descending order: acetyl > phenyl > 4-bromophenyl > H.

Figures 1-6 show the structure of compounds **3a, 4a, 4b, 6f, 8e and 8f** docked to their antibacterial targets, these compounds showed the highest negative energy scores and additionally, highest antimicrobial activity.

Table 7. Inibition zone diameter data of the tested compounds.

Compd. No.	Diameter of Inhibition Zone (mm)		
	P. aeruginosa	*S. aureus*	*C. albicans*
5	10	-	12
6	-	8	-
7	12	-	8
3a	20	18	18
3b	16	12	12
3c	-	-	-
3d	16	16	-
3e	10	-	14
3f	-	8	-
3g	14	10	-
3h	12	-	10
3i	8	12	-
3j	14	-	10
3k	10	-	12
3l	-	-	14
3m	10	8	14
3n	10	-	-
3o	-	-	-
3p	8	-	12
3q	-	14	16
3r	10	-	-
4a	22	18	16
4b	18	16	20
4c	16	-	12
4d	12	10	-
4e	-	8	10
4f	-	-	-
5a	8	-	12
5b	-	-	16
5c	-	-	14
5d	-	-	-
5e	14	-	-
5f	14	8	12
6a	-	10	20
6b	-	8	12
6c	10	-	10
6d	10	-	-
6e	16	-	-
6f	22	18	16
7a	-	-	10
7b	10	14	20
7c	-	14	12
7d	12	-	14
7e	12	-	16
7f	16	16	-
8a	-	10	8
8b	-	-	-
8c	14	8	12
8d	16	12	12
8e	18	16	14
8f	24	18	14
Streptomycin	22	NT	NT
Benzylpenicillin	NT	20	NT
Fluconazole	NT	NT	22

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Table 8. MICs of The Most Active Compounds and Reference Drugs.

Compd. No.	Minimal inhibitory concentration (µg/ml)		
	P. aeruginosa	*S. aureus*	*C. albicans*
3a	23.40	46.87	23.40
4a	46.87	23.40	23.40
4b	23.40	46.87	23.40
6f	23.40	23.40	46.87
8e	23.40	46.87	46.87
8f	23.40	23.40	23.40
Streptomycin	11.70	NT	NT
Benzylpenicillin	NT	11.70	NT
Fluconazole	NT	NT	11.70

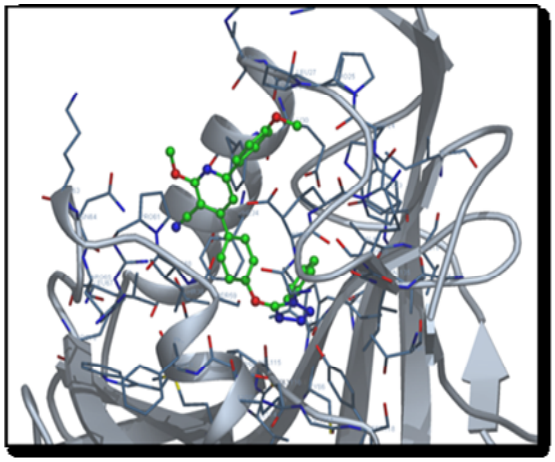

Figure 1. Compound 3a Docked to 1DRF.

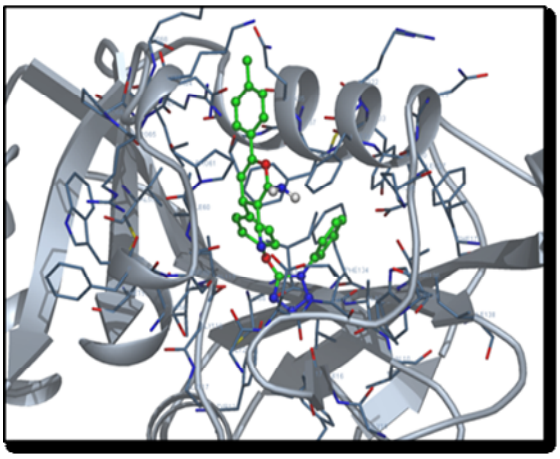

Figure 3. Compound 4b Docked to 1DRF.

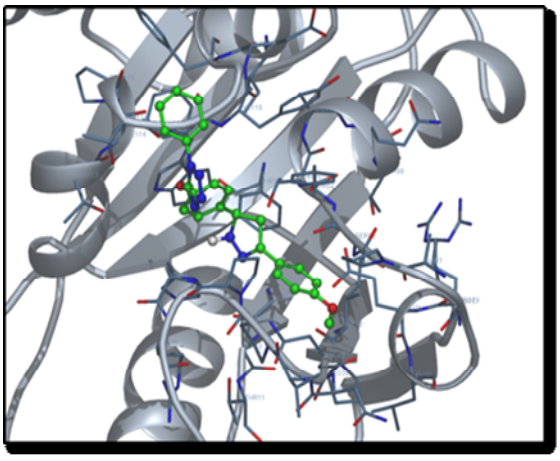

Figure 2. Compound 4a Docked to 1DRF.

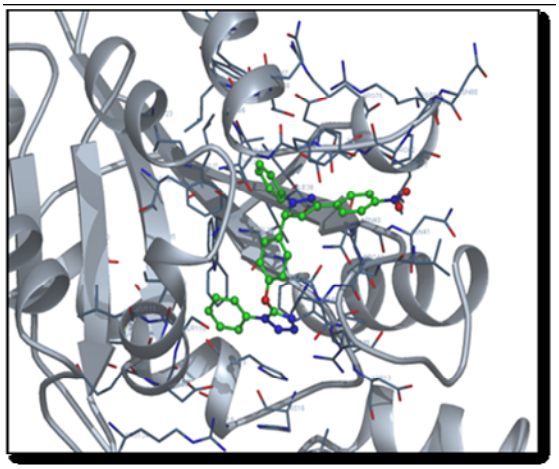

Figure 4. Compound 6f Docked to 1K4M.

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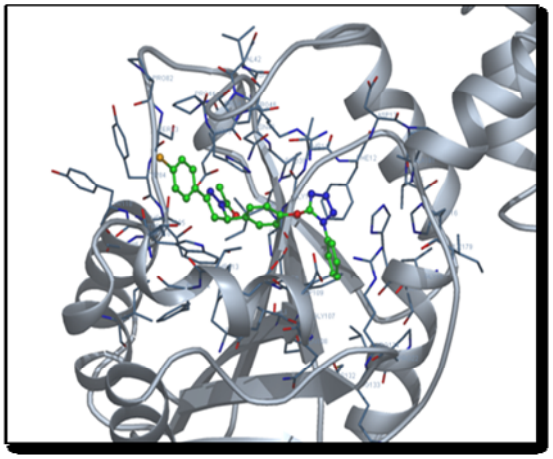

Figure 5. Compound 8e Docked to 1K4M.

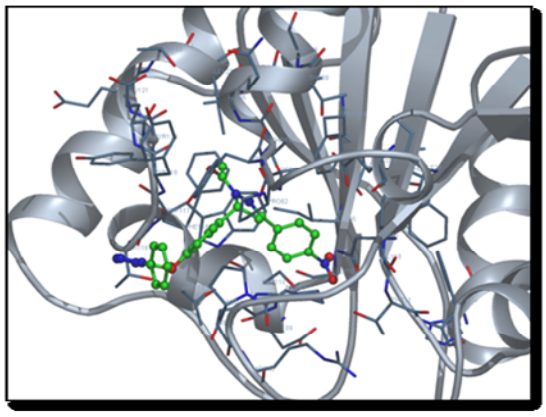

Figure 6. Compound 8f Docked to 1K4M.

3. Conclusion

Three series of tetrazole hybridized with either pyridine, pyran or pyrazole derivatives were synthesized. The antimicrobial activity of the synthesized hybrids revealed pronounced activity against Gram negative microorganisms and fungi. On the other hand, the results of the *in silico* modeling of the synthesized compounds come in agreement with the *in vitro* antimicrobial testing. However, the exact mode of action of these compounds is in need for further investigations.

4. Experimental

4.1. General

Melting points were recorded in Fisher-Jones melting point apparatus and are uncorrected. IR spectra (KBr disks) were recorded using Mattson 5000 FT-IR spectrometer.^{1}H-NMR spectra were obtained on FT-NMR spectrometer (300 MHz) Gemini Varian using TMS as internal reference and DMSO as solvent. MS analyses were performed on JEOL JMS-600H spectrometer. Microanalytical data were performed on a PERKIN-ELMER 2400 C, H, N Elemental Analyzer at the Microanalytical Unit, Cairo University, Egypt. TLC was performed on silica gel coated plates for monitoring the reactions. The following standard organisms used in the antimicrobial screening: *Pseudomonas aeruginosa* (PAO1), *Staphylococcus* aureus (ATCC25923), *Candida albicans* (ATCC66027).

4.2. General method for synthesis of 1-(substituted)phenyl-3-(4-((1-phenyl-1H-tetrazol-5-yl)oxy)phenyl)prop-2-en-1-ones (2a-f)

A mixture of 4-((1-phenyl-1H-tetrazol-5-yl)oxy)benzaldehyde **1** and (un)substituted acetophenones was added to sodium hydroxide solution (10 ml 2.5% in ethanol). The reaction mixture was stirred at room temperature for two hours. Hydrochloric acid 10%was added to the mixture until neutral to litmus, the separated product was filtered and crystallized from ethanol. Compounds **2a-c** were previously synthesized according to the reported procedure [1]. The physical and spectral data of compounds **2d-f** are given below.
IR for compounds **2d-f**: 1140-1155 (C-O),1550-1600 (C=N), 1700-1725 (C=O).

4.2.1. 1-(4-methoxyphenyl)-3-(4-((1-phenyl-1H-tetrazol-5-yl)oxy)phenyl)prop-2-en-1-one (2d)
m/z = 399.2 (M+1). Anal.$C_{23}H_{18}N_4O_3$, Calcd: 69.34, 4.55, 14.06, found: 69.22, 4.52, 14.11.

4.2.2. 1-(3-hydroxyphenyl)-3-(4-((1-phenyl-1H-tetrazol-5-yl)oxy)phenyl)prop-2-en-1-one (2e)
Anal.$C_{22}H_{16}N_4O_3$, calcd: 68.74, 4.20,14.58, found: 68.69, 4.15, 14.42.

4.2.3. 1-(4-nitrophenyl)-3-(4-((1-phenyl-1H-tetrazol-5-yl)oxy)phenyl)prop-2-en-1-one (2f)
Anal.$C_{22}H_{15}N_5O_4$, calcd: 63.92, 3.66, 16.94, found: 63.87, 3.54, 16.85.

4.3. General method for synthesis of 2-alkoxy-6-(substituted)phenyl-4-(4-((1-phenyl-1H-tetrazol-5-yl)oxy)phenyl)nicotinonitriles (3a-r)

A mixture of (un)substituted 1-phenyl-3-(4-((1-phenyl-1H-tetrazol-5-yl)oxy)phenyl)prop-2-en-1-one **2-7**(1mmol) and malononitrile (0.07 gm, 1mmol) was added to 15 ml freshly prepared sodium alkoxide (0.014 mole of sodium in 100 ml of each of absolute methanol, ethanol or n-propanol according to the R` group). The reaction mixture was stirred at room temperature for the appropriate time as illustrated in Table 1. The precipitated product was collected by filteration, washed with ethanol and recrystallized from ethanol/DMF to yield compounds **3a-r**. The physical and spectral data of these compounds are given below.
IR for compounds **3a-r**: 1140-1155 (C-O),1640-1670 (C=N),2230-2250 (C≡N).

4.3.1. 2-methoxy-6-(4-methoxyphenyl)-4-(4-((1-phenyl-1H-tetrazol-5-yl)oxy)phenyl)-nicotinonitrile (3a)

^{1}H-NMR: δ 3.8 (s, 3H, Ar-OCH_3) 4.06 (s, 3H, pyridine-OCH_3) 7.88 (s, 1H, pyridine-H) 7.01-8.06 (m, 13H, ArH). *m/z*= 477 (M+1). Anal.$C_{27}H_{20}N_6O_3$ .Calcd 68.06, 4.23, 17.64, found: 67.95, 4.12, 17.60.

4.3.2. 2-ethoxy-6-(4-methoxyphenyl)-4-(4-((1-phenyl-1H-tetrazol-5-yl)oxy)phenyl)-nicotinonitrile (3b)

m/z= 491.5 (M+1). Anal. $C_{28}H_{22}N_6O_3$Calcd 68.56, 4.52, 17.13, found: 68.40, 4.45, 17.23.

4.3.3. 6-(4-methoxyphenyl)-4-(4-((1-phenyl-1H-tetrazol-5-yl)oxy)phenyl)-2-propoxy-nicotinonitrile (3c)

^{1}H-NMR: δ 0.9 (t, 3H, O-$CH_2CH_2\underline{CH_3}$) 1.74 (m, 2H, O-$CH_2\underline{CH_2}CH_3$) 3.83 (s,3H, O-CH3) 4.46(t, 2H, O-$\underline{CH_2}CH_2CH_3$) 7.88 (s, 1H, pyridine-H) 7.01-8.06 (m, 13H, ArH). Anal. $C_{29}H_{24}N_6O_3$. Calcd: 69.04, 4.79, 16.66, found: 68.95, 4.70, 16.52.

4.3.4. 2-methoxy-4-(4-((1-phenyl-1H-tetrazol-5-yl)oxy)phenyl)-6-(p-tolyl)nicotinonitrile (3d)

m/z = 461.2 (M+1). Anal. $C_{27}H_{20}N_6O_2$. Calcd: 70.42, 4.38, 18.25, found: 70.35, 4.28, 18.23.

4.3.5. 2-ethoxy-4-(4-((1-phenyl-1H-tetrazol-5-yl)oxy)phenyl)-6-(p-tolyl)nicotinonitrile (3e)

^{1}H-NMR: δ 1.32 (t, 3H, O-CH_2-$\underline{CH_3}$) 2.34 (s, 3H, Ar-Me) 4.39 (q, 2H, O-$\underline{CH_2}$-CH_3) 7.88 (s, 1H, pyridine-H) 7.01-8.18(m, 13H, ArH). Anal.$C_{28}H_{22}N_6O_2$ .Calcd: 70.87, 4.67, 17.71, found: 70.72, 4.48, 17.60.

4.3.6. 4-(4-((1-phenyl-1H-tetrazol-5-yl)oxy)-phenyl)-2-propoxy-6-(p-tolyl)nicotinonitrile (3f)

Anal. $C_{28}H_{22}N_6O_2$. Calcd: 70.87, 4.67, 17.71, found: 70.69, 4.67, 17.62.

4.3.7. 6-(3-hydroxyphenyl)-2-methoxy-4-(4-((1-phenyl-1H-tetrazol-5-yl)oxy)phenyl)-nicotinonitrile (3g)

^{1}H-NMR: δ 4.06 (s, 3H, O-$\underline{CH_3}$) 5.35 (s, 1H, OH) 7.88 (s, 1H, pyridine-H) 6.89-8.00 (m, 13H, ArH). Anal. $C_{26}H_{18}N_6O_3$. Calcd: 67.53, 3.92, 18.17, found: 67.33, 3.82, 18.15.

4.3.8. 2-ethoxy-6-(3-hydroxyphenyl)-4-(4-((1-phenyl-1H-tetrazol-5-yl)oxy)phenyl)nicotino-nitrile (3h)

m/z = 477.1 (M+1). Anal. $C_{27}H_{20}N_6O_3$. Calcd: 68.06, 4.23, 17.64, found: 68.20, 4.13, 17.55.

4.3.9. 6-(3-hydroxyphenyl)-4-(4-((1-phenyl-1H-tetrazol-5-yl)oxy)phenyl)-2-propoxy-nicotinonitrile (3i)

Anal. $C_{28}H_{22}N_6O_3$. Calcd: 68.56, 4.52, 17.13, found: 68.44, 4.35, 17.20.

4.3.10. 2-methoxy-6-phenyl-4-(4-((1-phenyl-1H-tetrazol-5-yl)oxy)phenyl)nicotinonitrile (3j)

m/z= 447.2 (M+1). Anal.$C_{26}H_{18}N_6O_2$. Calcd: 69.95, 4.06, 18.82, found: 69.84, 4.13, 17.66.

4.3.11. 2-ethoxy-6-phenyl-4-(4-((1-phenyl-1H-tetrazol-5-yl)oxy)phenyl)nicotinonitrile (3k)

Anal.$C_{27}H_{20}N_6O_2$. Calcd: 70.42, 4.38, 18.25, found: 70.39, 4.32, 18.30.

4.3.12. 6-phenyl-4-(4-((1-phenyl-1H-tetrazol-5-yl)oxy)phenyl)-2-propoxynicotinonitrile (3l)

^{1}H-NMR: δ 0.9 (t, 3H, O-$CH_2CH_2\underline{CH_3}$) 1.74 (m, 2H, O-$CH_2\underline{CH_2}CH_3$) 4.46(t, 2H, O-$\underline{CH_2}CH_2CH_3$) 7.88 (s, 1H, pyridine-H) 7.40-8.10 (m, 14H, ArH). Anal. $C_{28}H_{22}N_6O_2$. Calcd: 70.87, 4.67, 17.71, found: 70.69, 4.55, 17.75.

4.3.13. 6-(4-chlorophenyl)-2-methoxy-4-(4-((1-phenyl-1H-tetrazol-5-yl)oxy)phenyl)nicotino-nitrile (3m)

m/z= 481.5 (M+1), 482.4 (M+2). Anal. $C_{26}H_{17}ClN_6O_2$. Calcd: 64.94, 3.56, 17.48, found: 64.85, 3.44, 17.40.

4.3.14. 6-(4-chlorophenyl)-2-ethoxy-4-(4-((1-phenyl-1H-tetrazol-5-yl)oxy)phenyl)nicotino-nitrile (3n)

m/z = 494.2 (M+1), 495.1(M+2). Ar 11 $C_{27}H_{19}ClN_6O_2$. Calcd: 65.52, 3.87, 16.98, found: 65.40, 3.69, 16.88.

4.3.15. 6-(4-chlorophenyl)-4-(4-((1-phenyl-1H-tetrazol-5-yl)oxy)phenyl)-2-propoxynicotino-nitrile (3o)

Anal.$C_{28}H_{21}ClN_6O_2$. Calcd: 66.08, 4.16, 16.51, found: 66.20, 4.18, 16.54.

4.3.16. 2-methoxy-6-(4-nitrophenyl)-4-(4-((1-phenyl-1H-tetrazol-5-yl)oxy)phenyl)nicotino-nitrile (3p)

^{1}H-NMR: δ 4.06 (s, 3H, O-CH_3) 7.88 (s, 1H, pyridine-H) 7.01-8.20 (m, 13H, ArH). Anal. $C_{26}H_{17}N_7O_4$. Calcd: 63.54, 3.49, 19.95, found: 63.44, 3.45, 19.78.

4.3.17. 2-ethoxy-6-(4-nitrophenyl)-4-(4-((1-phenyl-1H-tetrazol-5-yl)oxy)phenyl)nicotino-nitrile (3q)

m/z = 505.2 (M+1). Anal.$C_{27}H_{19}N_7O_4$. Calcd: 64.15, 3.79, 19.40, found: 64.22, 3.66, 19.35.

4.3.18. 6-(4-nitrophenyl)-4-(4-((1-phenyl-1H-tetrazol-5-yl)oxy)phenyl)-2-propoxynicotino-nitrile (3r)

Anal. $C_{28}H_{21}N_7O_4$. Calcd: 64.73, 4.07, 18.87, found: 64.59, 4.15, 18.89.

4.4. General method for synthesis of 2-amino-6-(substituted)phenyl-4-(4-((1-phenyl-1H-tetrazol-5-yl)oxy)phenyl)-4H-pyran-3-carbo- nitriles (4a-f)

A mixture of (un)substituted 1-phenyl-3-(4-((1-phenyl-1H-tetrazol-5-yl)oxy)phenyl)prop-2-en-1-one **2a-f** (1mmol) and malononitrile (0.07 gm, 1mmol) was added to 15 ml DMF and few drops of piperidine were added. The reaction mixture was stirred at room temperature for 24 hours. The precipitated product was collected by filteration, washed with DMF and recrystallized from ethanol to yield compounds **4a-f**. The physical and spectral data of these compounds are given below.

IR for compounds **4a-f** : 1140-1155 (C-O), 2230-2250 (C≡N),3300-3400 (NH_2).

4.4.1. 2-amino-6-(4-methoxyphenyl)-4-(4-((1-phenyl-1H-tetrazol-5-yl)oxy)phenyl)-4H-pyran-3-carbonitrile (4a)

^{1}H-NMR: δ3.83(s, 3H, O-CH_3) 6.31 (s, 2H, NH_2) 6.55 (d, 1H, *J* = 3.8 Hz, pyran-chiral-H), 6.83 (d, 1H, *J* = 3.7 Hz, pyran-H) 7.50-8.10 (m, 13H, ArH). Anal. $C_{26}H_{20}N_6O_3$. Calcd: 67.23, 4.34, 18.09, found: 67.15, 4.32, 18.04).

4.4.2. 2-amino-4-(4-((1-phenyl-1H-tetrazol-5-yl)oxy)phenyl)-6-(p-tolyl)-4H-pyran-3-carbonitrile (4b)

^{1}H-NMR: δ 2.34 (s, 3H, CH_3) 6.11 (s, 2H, NH_2) 6.34 (d, 1H, *J* = 3.7 Hz, pyran-chiral-H), 6.75 (d, 1H, *J* = 3.6 Hz, pyran-H) 7.50-8.20(m, 13H, ArH). Anal. $C_{26}H_{20}N_6O_2$. Calcd: 69.63, 4.49, 18.74, found: 69.52, 4.44, 18.69.

4.4.3. 2-amino-6-(3-hydroxyphenyl)-4-(4-((1-phenyl-1H-tetrazol-5-yl)oxy)phenyl)-4H-pyran-3-carbonitrile (4c)

^{1}H-NMR: δ 5.33 (s, 1H, OH) 6.21 (s, 2H, NH_2) 6.35 (d, 1H, *J* = 3.6 Hz, pyran-chiral-H), 6.65 (d, 1H, *J* = 3.6 Hz, pyran-H) 7.40-8.20 (m, 13H, ArH). Anal. $C_{25}H_{18}N_6O_3$. Calcd: 66.66, 4.03, 18.66, found: 66.45, 4.09, 18.52.

4.4.4. 2-amino-6-phenyl-4-(4-((1-phenyl-1H-tetrazol-5-yl)oxy)phenyl)-4H-pyran-3-carbonitrile (4d)

Anal. $C_{25}H_{18}N_6O_2$. Calcd: 69.11, 4.18, 19.34, found: 69.20, 4.22, 19.44.

4.4.5. 2-amino-6-(4-chlorophenyl)-4-(4-((1-phenyl 12 1H-tetrazol-5-yl)oxy)phenyl)-4H-pyran-3-carbonitrile (4e)

m/z = 469.2(M+1), 470.4 (M+2). Anal. $C_{25}H_{18}ClN_6O_2$. Calcd: 64.04, 3.65, 17.92, found: 64.16, 3.48, 17.95.

4.4.6. 2-amino-6-(4-nitrophenyl)-4-(4-((1-phenyl-1H-tetrazol-5-yl)oxy)phenyl)-4H-pyran-3-carbonitrile (4f)

m/z = 480.2 (M+1). Anal. $C_{25}H_{17}N_7O_4$. Calcd: 62.63, 3.57, 20.45, found: 62.66, 3.48, 20.33.

4.5. General method for synthesis of 1-phenyl-5-(4-(3-(substituted)phenyl-4,5-dihydro-1H-pyrazol-5-yl)phenoxy)-1H-tetrazoles (5a-f)

A mixture of (un)substituted 1-phenyl-3-(4-((1-phenyl-1H-tetrazol-5-yl)oxy)phenyl)prop-2-en-1-one **2a-f** (1mmol) and hydrazine hydrate (0.1 ml, 2mmol) in ethanol was heated under reflux for the appropriate time as illustrated in table 1. The reaction mixture was cooled and poured onto crushed ice. The solid product was filtered and crystallized from ethanol to yield compounds **5a-f**. The physical and spectral data of these compounds are given below.

IR for compounds **5a-f**: 1600-1650 (C=N), 3350-3400 (N-H).

4.5.1. 5-(4-(3-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-5-yl)phenoxy)-1-phenyl-1H-tetrazole (5a)

^{1}H-NMR: δ 3.40 (m, 2H, C_4 pyrazole), 3.83 (s, 3H, -OCH_3),6.75 (m, 1H, C_5pyrazole),7.40-8.10 (m, 13H, ArH), 10.10 (s, 1H, N-H). Anal.$C_{23}H_{20}N_6O_2$. Calcd: 66.98, 4.89, 20.38, found: 66.88, 4.82, 20.36.

4.5.2. 5-(4-(3-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-5-yl)phenoxy)-1-phenyl-1H-tetrazole (5b)

^{1}H-NMR: δ 2.30 (s, 3H, CH_3), 3.40 (m, 2H, C 12 pyrazole),6.90 (m, 1H, C_5 pyrazole), 7.40-8.10 (m, 13H, ArH), 10.10 (s, 1H, N-H).*m/z* = 396 (M^+).Anal. $C_{23}H_{20}N_6O$. Calcd: 69.98, 5.08, 21.20, found: 69.88, 5.20, 21.11.

4.5.3. 3-(5-(4-((1-phenyl-1H-tetrazol-5-yl)-oxy) phenyl)-4,5-dihydro-1H-pyrazol-3-yl)phenol (5c)

Anal. $C_{22}H_{18}N_6O_2$. Calcd: 66.32, 4.55, 21.09, found: 66.22, 4.40, 21.18.

4.5.4. 1-phenyl-5-(4-(3-phenyl-4,5-dihydro-1H-pyrazol-5-yl)phenoxy)-1H-tetrazole (5d) Anal. $C_{22}H_{18}N_6O$. Calcd: 69.10, 4.74, 21.98, found: 68.98, 4.56, 22.01.

4.5.5. 5-(4-(3-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-5-yl)phenoxy)-1-phenyl-1H-tetrazole (5e)

m/z = 416 (M^+), 418.3(M^++2). Anal. $C_{22}H_{17}ClN_6O$. Calcd: 63.39, 4.11, 20.16, found: 63.25, 4.01, 20.14.

4.5.6. 5-(4-(3-(4-nitrophenyl)-4,5-dihydro-1H-pyrazol-5-yl)phenoxy)-1-phenyl-1H-tetrazole (5f)

m/z = 428.5 (M+1). Anal. $C_{22}H_{17}N_7O_3$. Calcd: 61.82, 4.01, 22.94, found: 61.74, 4.14, 22.85.

4.6. General method for synthesis of 5-(4-(3-((substituted)phenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-5-yl)phenoxy)-1-phenyl-1H-tetrazoles (6a-f)

A mixture of (un)substituted 1-phenyl-3-(4-((1-phenyl-1H-tetrazol-5-yl)oxy)phenyl)prop-2-en-1-one **2a-f** (1mmol) and phenylhydrazine (0.22 ml, 2 mmol) in ethanol was heated under reflux for the appropriate time as illustrated in table 1. The reaction mixture was cooled and poured onto crushed ice. The solid product was filtered and crystallized from ethanol to yield compounds **6a-f**. The physical and spectral data of these compounds are given below.

IR for compounds **6a-f**: 1300-1350 (C-N), 1600-1650 (C=N).

4.6.1. 5-(4-(3-(4-methoxyphenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-5-yl)phenoxy)-1-phenyl-1H-tetrazole (6a)

^{1}H-NMR: δ 3.40 (m, 2H, C_4 pyrazole), 3.30 (s, 3H, -OCH_3),7.01 (m, 1H, C_5 pyrazole), 7.30-8.10 (m, 18H, ArH). Anal. $C_{29}H_{24}N_6O_2$. Calcd: 71.30, 4.95, 17.20, found: 71.22, 4.85, 17.11.

4.6.2. 1-phenyl-5-(4-(1-phenyl-3-(p-tolyl)-4,5-dihydro-1H-pyrazol-5-yl)phenoxy)-1H-tetrazole (6b)

^{1}H-NMR: δ2.50 (s, 3H, CH_3), 3.40 (m, 2H, C_4 pyrazole), 6.95 (m, 1H, C_5 pyrazole), 7.01-8.10 (m, 18H, ArH). Anal. $C_{29}H_{24}N_6O$. Calcd: 73.71, 5.12, 17.78, found: 73.66, 5.22, 17.65.

4.6.3 3-(1-phenyl-5-(4-((1-phenyl-1H-tetrazol-5-yl)oxy)phenyl)-4,5-dihydro-1H-pyrazol-3-yl)phenol (6c)

m/z = 472.20 (M^+). Anal. $C_{28}H_{22}N_6O_2$. Calcd: 70.87, 4.67, 17.71, found: 70.77, 4.62, 17.59.

4.6.4. 5-(4-(1,3-diphenyl-4,5-dihydro-1H-pyrazol-5-yl)phenoxy)-1-phenyl-1H-tetrazole (6d)

Anal. $C_{28}H_{22}N_6O$. Calcd: 73.35, 4.84, 18.33, found: 73.22, 4.75, 18.42.

4.6.5. 5-(4-(3-(4-chlorophenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-5-yl)phenoxy)-1-phenyl-1H-tetrazole (6e)

Anal. $C_{28}H_{21}ClN_6O$. Calcd: 68.22, 4.29, 17.05, found: 68.04, 4.22, 17.11.

4.6.6. 5-(4-(3-(4-nitrophenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-5-yl)phenoxy)-1-phenyl-1H-tetrazole (6f)

m/z =503 (M^+). Anal. $C_{28}H_{21}N_7O_3$. Calcd: 66.79, 4.20, 19.47, found: 66.67, 4.06, 19.39.

4.7. General method for synthesis of 5-(4-(1-(4-bromophenyl)-3-(substituted)phenyl-4,5-dihydro-1H-pyrazol-5-yl)phenoxy)-1-phenyl-1H-tetrazoles (7a-f)

A mixture of (un)substituted 1-phenyl-3-(4-((1-phenyl-1H-tetrazol-5-yl)oxy)phenyl)prop-2-en-1-one **2a-f** (1mmol and 4-bromophenylhydrazine (0.19 gm, 1 mmol) in ethanol was heated under reflux for the appropriate time as illustrated in table 1. The reaction mixture was cooled and poured onto crushed ice. The solid product was filtered and crystallized from ethanol to yield compounds **7a-f**. The physical and spectral data of these compounds are given below.

IR for compounds **7a-f**: 1300-1350 (C-N), 1600-1650 (C=N).

4.7.1. 5-(4-(1-(4-bromophenyl)-3-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-5-yl)- phenoxy)-1-phenyl-1H-tetrazole (7a)

^{1}H-NMR: δ 3.40(m, 2H, C_4 pyrazole), 3.35 (s, 3H, -OCH_3),7.02 (m, 1H, C_5 pyrazole), 7.20-8.10 (m, 17H, ArH). Anal. $C_{29}H_{23}BrN_6O_2$. Calcd: 61.38, 4.09, 14.81, found: 61.22, 4.18, 14.69.

4.7.2. 5-(4-(1-(4-bromophenyl)-3-(p-tolyl)-4,5-dihydro-1H-pyrazol-5-yl)phenoxy)-1-phenyl-1H-tetrazole (7b)

^{1}H-NMR: δ2.50 (s, 3H, CH_3), 3.40(m, 2H, C_4 pyrazole),7.01 (m, 1H, C_5 pyrazole), 7.20-8.10 (m, 17H, ArH).*m/z*= 551.15 (M^+).Anal. $C_{29}H_{23}BrN_6O$. Calcd: 63.16, 4.20, 15.24, found: 63.22, 4.15, 15.19.

4.7.3. 3-(1-(4-bromophenyl)-5-(4-((1-phenyl-1H-tetrazol-5-yl)oxy)phenyl)-4,5-dihydro-1H-pyrazol-3-yl)phenol (7c)

Anal. $C_{28}H_{21}BrN_6O_2$. Calcd: 60.77, 3.82, 15.19, found: 60.65, 3.82, 15.12.

4.7.4. 5-(4-(1-(4-bromophenyl)-3-phenyl-4,5-dihydro-1H-pyrazol-5-yl)phenoxy)-1-phenyl-1H-tetrazole (7d)

Anal. $C_{28}H_{21}BrN_6O_1$. Calcd: 62.58, 3.94, 15.64, found: 62.59, 3.88, 15.54.

4.7.5. 5-(4-(1-(4-bromophenyl)-3-(4-chloro-phenyl)-4,5-dihydro-1H-pyrazol-5-yl)phenoxy)-1-phenyl-1H-tetrazole (7e)

m/z = 571.15(M^+), 573.10(M^++2), 575.10(M^++4).Anal. $C_{28}H_{20}ClBrN_6O$. Calcd: 58.81, 3.53, 14.70, found: 58.77, 3.33, 14.66.

4.7.6. 5-(4-(1-(4-bromophenyl)-3-(4-nitro-phenyl)-4,5-dihydro-1H-pyrazol-5-yl)- phenoxy)-1-phenyl-1H-tetrazole (7f)

Anal. $C_{28}H_{20}BrN_7O_3$. Calcd: 57.74, 3.46, 16.83, found: 57.65, 3.49, 16.75.

4.8. General method for synthesis of 1-(3-(substituted)phenyl-5-(4-((1-phenyl-1H-tetrazol-5-yl)oxy)phenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone (8a-f)

A mixture of (un)substituted 1-phenyl-3-(4-((1-phenyl-1H-tetrazol-5-yl)oxy)phenyl)prop-2-en-1-one **2a-f** (1mmol) and hydrazine hydrate (0.1 ml, 2mmol) in glacial acetic acid was heated under reflux for the appropriate time as illustrated in table 1. The reaction mixture was cooled and poured onto crushed ice. The product was then extracted from aqueous medium by ethyl acetate. For compound **8c**, the product was added to sodium methoxide solution to hydrolyze the formed acetate ester and keep only the acetamide functionality. The solid obtained after concentration was filtered and crystallized from ethanol to yield compounds **8a-f**. The physical and spectral data of these compounds are given below.

IR for compounds **8a-f**: 1600-1650 (C=N), 1700-1725 (C=O).

4.8.1. 1-(3-(4-methoxyphenyl)-5-(4-((1-phenyl-1H-tetrazol-5-yl)oxy)phenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone (8a)

^{1}H-NMR: δ 2.03 (s, 3H, O=C-CH_3), 3.40 (m, 2H, C_4 pyrazole), 3.85 (s, 3H, -OCH_3),7.01 (m, 1H, C_5 pyrazole), 7.20-8.10 (m, 13H, ArH). Anal. $C_{25}H_{22}N_6O_3$. Calcd: 66.07, 4.88, 18.49, found: 66.12, 4.92, 18.37.

4.8.2. 1-(5-(4-((1-phenyl-1H-tetrazol-5-yl)-oxy) phenyl)-3-(p-tolyl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone (8b)

^{1}H-NMR: δ 2.03 (s, 3H, O=C-CH_3), 2.34 (s, 3H, Ar-CH_3), 3.40 (m, 2H, C_4 pyrazole),6.95(m, 1H, C_5 pyrazole), 7.01-8.10 (m, 13H, ArH).Anal. $C_{25}H_{22}N_6O_2$. Calcd: 68.48, 5.06, 19.17, found: 68.49, 5.01, 19.12.

4.8.3. 1-(3-(3-hydroxyphenyl)-5-(4-((1-phenyl-1H-tetrazol-5-yl)oxy)phenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone (8c)

m/z = 440 (M^+). Anal. $C_{24}H_{20}N_6O_3$. Calcd: 65.45, 4.58, 19.08, found: 65.39, 4.42, 19.15.

4.8.4. 1-(3-phenyl-5-(4-((1-phenyl-1H-tetrazol-5-yl)oxy)phenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone (8d)

Anal. $C_{24}H_{20}N_6O_2$. Calcd: 67.91, 4.75, 19.80, found: 67.77, 4.71, 19.77.

4.8.5. 1-(3-(4-chlorophenyl)-5-(4-((1-phenyl-1H-tetrazol-5-yl)oxy)phenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone (8e)

Anal. $C_{24}H_{19}ClN_6O_2$. Calcd: 62.81, 4.17, 18.31, found: 62.75, 4.12, 18.40.

4.8.6. 1-(3-(4-chlorophenyl)-5-(4-((1-phenyl-1H-tetrazol-5-yl)oxy)phenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone (8f)

m/z = 416 (M^+). Anal. $C_{24}H_{19}N_7O_4$. Calcd: 61.40, 4.08, 20.89, found: 61.25, 4.19, 20.80.

4.9. Antimicrobial Testing

Inhibition zone measurements: the tested compounds were dissolved in dimethylsulfoxide at a concentration of 1 mg/ml. The suitable medium (nutrient agar for bacteria and yeast extract peptone dextrose for fungi) was inoculated with the test organisms. A volume of the solution ofeach the test compounds equivalent to 100 mg wasplaced separately in cups, cut in the agar. The plates were incubated at 37 °C for 24 h for bacteria and 48h for fungi, and the resulting inhibition zones were measured (table 7). Dimethylsulfoxide, exhibited no antimicrobial activity against the test organisms and used as a negative control. Minimal inhibitory concentration (MIC) (table 8) was determined using the agar dilution technique [36]. Streptomycin, Benzylpenicillin and Fluconazole were used during the test procedures as reference antibiotics for gram negative, gram positive bacteria and fungi, respectively.

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