Design, Synthesis, Biological Activity and Molecular Modeling of New Heterocyclic Tetrazole Derivatives

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Abstract: A series of new tetrazole heterocyclic derivatives were designed and synthesized through reaction of 5-chloro-1-phenyl-1*H*-tetrazole with 4-hydroxybenzoic acid hydrazide to give the key intermediate 4-((1-phenyl-1*H*-tetrazole-5-yl)oxy)benzohydrazide, which was condensed with different aromatic and heterocyclic aldehydes to give a series of tetrazole *N*-aroylhydrazone derivatives **6-19** followed by reflux with thioglycolic acid or acetic anhydride to give the targeted tetrazole 4-thiazolidinones **20-29** and 2,5-oxadiazoline derivatives **30-40**. Selected compounds were tested for their *in vitro* antimicrobial and anticancer activity. Compounds with the most pronounced antimicrobial activity **3, 14, 26**, and **35**, in addition to **36** were tested for their *in vitro* cytotoxic activity against (HuH-7) and (CaCo-2) cell lines. Compound **14** and **35** were the most active against (CaCo-2) cell line with IC_{50} of 4.2µM and 9.8µM, respectively, while compound **35** was the most active against (HuH-7) cell line with IC_{50} of 24µM. On the other hand, incorporation of 1-phenyl-tetrazole moiety into propyl paraben **2** led to three times increase in the antifungal activity. A molecular modeling study was performed on propyl paraben **2** and its tetrazole derivative **3**.

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1. Introduction

The discovery process of new antimicrobial agents has flourished from 1930-1970 but, since then, it has been significantly slow[1]. Over the past two decades, there have been a continuous struggle between the development of new antimicrobial agents and the emergence of antimicrobial resistance[2]. The death rate for patients with resistant infections is about twice that in patients with non-resistant infections[1]. Moreover, there has been a rapid pervasion of fungal infections due to the increasing number of immunocompromised patients. Aids, cancer and organ transplant patients are immunosuppressed and are very susceptible to high risk of oral and systemic fungal infections like candidiasis caused by *Candida albicans*[3, 4]. On the other hand, the WHO has considered cancer as a leading cause of death, as it represents around 13% of all deaths worldwide in 2008 and expected to continue rising, with an estimated 13.1 million deaths in 2030[5]. Therefore, there is an urgent need for the development and discovery of new potent, less toxic and broad spectrum antimicrobial as well as anticancer agents, with new mechanisms of action to avoid resistance with currently used chemotherapeutic agents.

Cancer patients under chemotherapy are mostly immunosuppressed and highly susceptible to microbial infections. Co-administration of more than one drug is very common in the treatment of cancer patients with accompanied microbial infections. Treatment protocol is more complicated in case of patients with impaired kidney or liver function. In this case the concept of monotherapy by a single drug which possesses dual activity of both anticancer and antimicrobial is favorable from pharmacotherapeutic and pharmacoeconomic points[6].

Along the last two decades, tetrazole moiety has attracted a considerable attention as non-classical bioisostere of many functional groups, such as carboxylic acid group[7], ester group[8] and cis-peptide linkage[9]. Incorporation of tetrazoles into a drug molecule usually retain the biological activity profile with a substantial increase in the metabolic stability[10-12], bioavailability and potency due to increasing lipophilicity[7-9].

Pharmacologically, tetrazole containing compounds are reported to possess diverse chemotherapeutic activities as antibacterial[13-15], antimycobacterial[16], antifungal [17, 18], antiviral[19], anti-parasitic[20] and anticancer potentials.[21-26]

Parabens are esters of *p*-hydroxybenzoic acid (PHBA) that are used in more than 13,200 cosmetic formulations as preservatives[27]. Generally they are rapidly absorbed, metabolized and excreted[28]. Parabens are more active against fungi than bacteria and their activity as antimicrobial agents have been correlated to their lipophilicity[29]. Furthermore, hydrazone derivatives are known for their antimicrobial[30, 31] and anticancer activities[32]. A

dual antifungal and anticancer activity was reported [24, 25] for a scaffold of (5-mercapto-1-methyl-1*H*-tetrazole) or (5-mercapto-1-phenyl-1*H*-tetrazole) linked to hydrazone moiety through an aliphatic chain.

On the other hand, much attention was given to the antimicrobial [33, 34] and anticancer [35] properties of thiazolidinone derivatives. A scaffold of 1,5-disubstituted tetrazole linked to 4-thiazolidinone moiety was found to exhibit promising antimicrobial activity[36]. It is also known that oxadiazole derivatives are involved in many antimicrobial [37] and anticancer [38] scaffolds. Promising antimicrobial activity was reported for a scaffold of biphenyltetrazole with 1,3,4-oxadiazole derivatives[13]. Moreover, the anticancer activity of 2,5-diaryl-1,3,4-oxadiazoline derivatives as Comberstatin-A4 analogs has been under focus as the oxadiazoline ring provides the optimal non-planar conformational geometry exactly like Comberstatin-A4 for the interaction with colchicine binding site[39].

Inspired by the above mentioned findings, incorporation of 1-pheny-tetrazole moiety into propyl paraben was envisioned, hoping that this hybridization would increase lipophilicity and consequently antimicrobial activity of propyl paraben. A molecular modeling study of propyl paraben and its tetrazole derivative, as well as, a comparison of their physicochemical properties and biological activity was investigated. One of the most important concepts of drug design is the covalent conjugation of biologically active moieties, acting by different mechanisms that would lead, in a favorable case, to synergism that provides compounds with improved activity. Accordingly, it was thought worthwhile to combine the structural features of 1,5-disubstituted-tetrazole moiety with hydrazones and some derived thiazolidinones and oxadiazoles by covalent conjugation through an aromatic ring linker. The targeted compounds were evaluated for their *in vitro* antimicrobial and anticancer potential.

2. Results and Discussion

2.1 Chemistry

The starting key intermediate 4-((1-phenyl-1*H*-tetrazol-5-yl)oxy)benzohydrazide **5** was synthesized in a good yield through two-stepped procedure involving hydrazinolysis of propyl 4-hydroxybenzoate ester **2** with hydrazine hydrate to give the corresponding acid hydrazide 4-hydroxybenzoic acid hydrazide **4**, then reaction with 5-chloro-1-phenyl-1*H*- tetrazole **1** in presence of potassium carbonate anhydrous (scheme 1). An earlier trial to synthesize the starting intermediate **5** through reaction of propyl 4-hydroxybenzoate **2** with 5-chloro-1-phenyl-1*H*-tetrazole **1** followed by hydrazinolysis of the formed propyl ester product **3** failed to produce the target key intermediate **5.** This might be attributed to the relative unstability of the formed propyl ester (M.p. 35**°** C). Condensation of the key intermediate **5** with different aromatic and heterocyclic aldehydes successfully furnished the targeted compounds of tetrazole *N*-aroylhydrazone derivatives **6-19** (scheme 1).

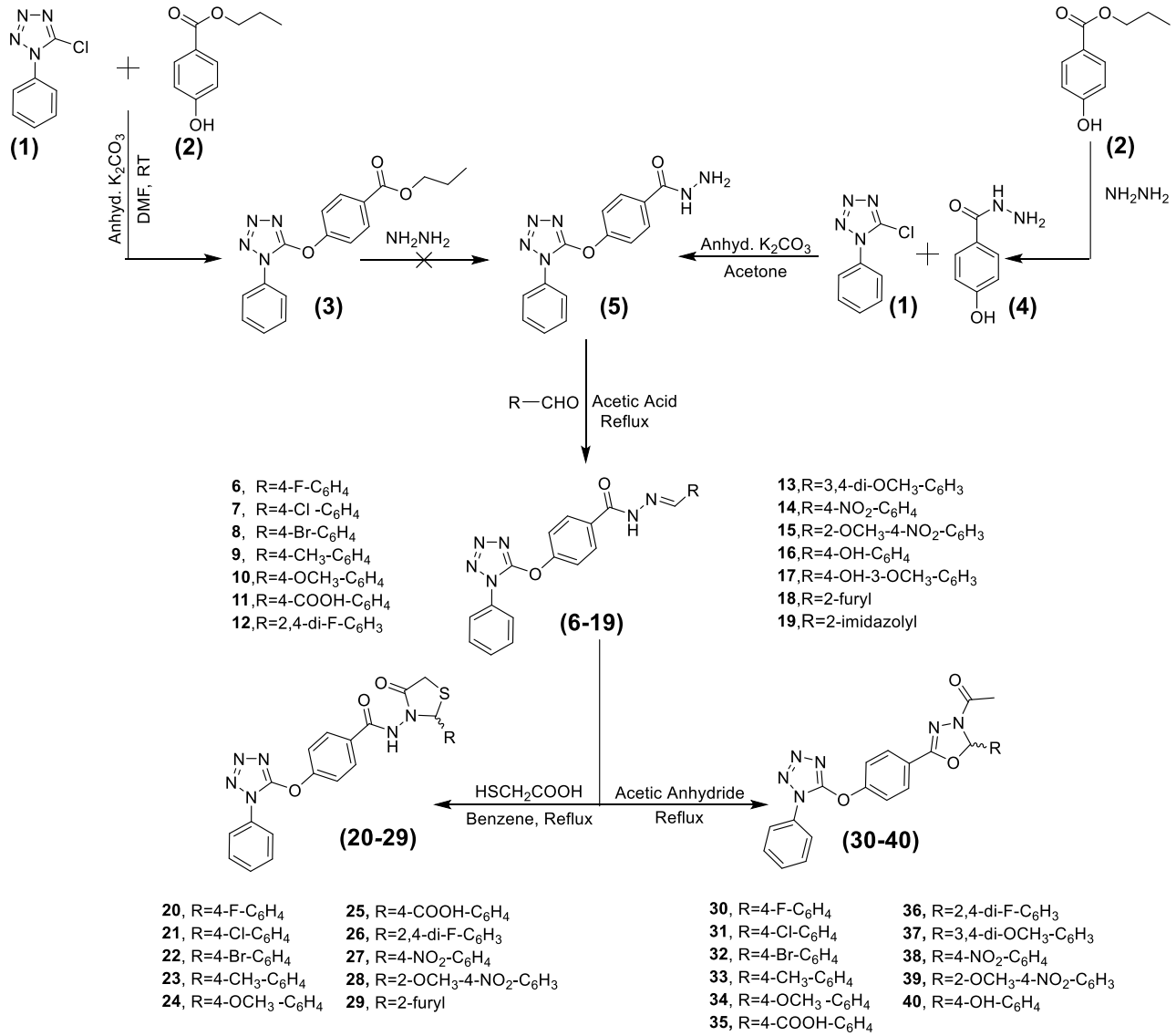
Characteristic IR absorption peaks of the synthesized aroylhydrazones **6-19** were observed at 1667 cm^{-1} for hydrazide carbonyl (C=O), and at 3195 cm^{-1} for hydrazide amide (N–H). ^{1}H NMR showed a singlet for azomethine proton (N=CH) at δ 8.01–8.69 ppm and a singlet for the amide proton (NH) at (δ 11.20–12.35 ppm), which are characteristics for aroylhydrazones.

The targeted tetrazole 4-thiazolidinone derivatives (±) N-(2-(substituted-phenyl)-4-oxothiazolidin-3-yl)-4-((1-phenyl-1*H*-tetrazol-5-yl)oxy)benzamide **20-26** and the analog **29** were synthesized through reaction of tetrazole *N*-aroylhydrazone derivatives **6-12** and **18** with thioglycolic acid in benzene under reflux (scheme 1). Compounds **27&28** were obtained through carrying out the reaction in thioglycolic acid only in absence of solvent due to the high polarity of nitro group in the corresponding tetrazole *N*-aroylhydrazone derivatives **14&15**, that hindered their solubility in benzene and subsequently their reaction with thioglycolic acid. Characteristic IR bands for thiazolidinone derivatives were observed at 1724cm^{-1} and 3150cm^{-1} for (C=O) carbonyl group and the amide (NH) of the ring, respectively. Absorption peak at 1652 cm^{-1} for (C=O) carbonyl group of aroylhydrazone was also observed. ^{1}H NMR showed disappearance of the azomethine proton singlet (N=CH-) of aroylhydrazone and appearance of two doublet of doublets and a singlet at (δ 5.89 - 6.16 ppm)for the two methylene protons and the methine proton of the ring, respectively.

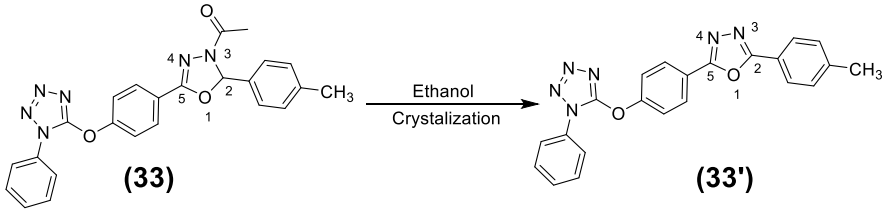
A series of tetrazole 2,5-disubstituted-oxadiazoline derivatives **30-40** (±) 1-(2-(substituted-phenyl)-5-(4-((1-phenyl-1*H*-tetrazol-5-yl)oxy) phenyl)-1,3,4-oxadiazol-3(2H)-yl) ethan-1-one were synthesized as depicted in (scheme 1) through refluxing the tetrazole N-aroyl-hydrazone derivatives **6-16** with acetic anhydride. Formation of 2,5-disubstituted-oxadiazoline ring was proved in ^{1}H NMR by the absence of the singlets of the azomethine (N=CH-) and amidic nitrogen (CO-NH) protons of the starting compounds, and the appearance of singlet at (δ 2.12 -2.44 ppm) for the acetyl group and a singlet for the proton at the C2 of the oxadiazoline ring at (δ 7.05 - 7.36 ppm).

It is worth-mentioning that although the molecular ion peak M^{+1} of crude compound **33** was 441.3 (as expected), yet the molecular ion peak after crystallization in ethanol was found to be M^{+1} 397.1. This was attributed to the loss of acetyl group at N3

and the proton at C2 during crystallization, followed by formation of a double bond between C2 and N3. This assumption has been confirmed by the behavior of ^{1}H NMR of compound **33** before and after crystallization, where after crystallization, the two singlets for the acetyl protons and the proton at C2 have vanished**.** The deacetylated derivative was given number **33'** (scheme 2).



Scheme 1



Scheme 2

2.2 Biological activity

2.2.1 Antimicrobial activity

Selected compounds were evaluated for their *in vitro* antimicrobial activity. Test microorganisms in this evaluation includes *Staphylococcus epidermidis* ATCC 12228 and *Bacillus subtilis* ATCC 35021 as examples of Gram-positive bacteria, *Escherichia coli* ATCC 25922 and *Proteus mirabilis* ATCC 25933 as

examples of Gram-negative bacteria, and *Candida albicans* ATCC 76615 as an example of fungi. Agar well diffusion method[40] was used for determination of the preliminary antibacterial and antifungal activity. Ciprofloxacin and ketoconazole were used as reference antibacterial and antifungal agents, respectively, while dimethyl sulfoxide (DMSO) 80% was used as a negative control. Inhibition zones diameter (mm) of tested compounds were measured. Minimum inhibitory concentration (MIC) was determined for the most active compounds using two-fold broth microdilution method[41, 42]. Antimicrobial activity of propyl paraben **2** compared to its tetrazole derivative **3** was investigated to explore the effect of incorporation of tetrazole moiety into propyl paraben on its antimicrobial activity. Their MIC_{90} values were determined as a measure of their fungicidal activity against *Candida albicans* ATCC 26555 at the Micro-analytical Center, Faculty of Science, Cairo University, Egypt.

Inhibition zones of tested compounds are listed in table 1**.** Results revealed that most of the tested compounds demonstrated good antimicrobial activity against all the test microorganisms. In general, the tested compounds were found to be more active against fungi than bacteria.

In Gram-positive bacteria, compound **35** was the most active against *S. epidermidis and B. subtilis*, with inhibition zones larger than that of ciprofloxacin, whereas the inhibition zones of compounds **6, 9, 12, 14, 17, 26** against *B. subtilis* were found to be equal to that of ciprofloxacin. In Gram-negative bacteria compounds **20** and **26** were the most active against *E. coli*, while compounds **3, 21** and **22** were the most active against *P. mirabilis*. Compound **33'** demonstrated the highest antifungal activity with inhibition zone larger than that of ketoconazole, while Compound **14** demonstrated inhibition zone equal to that of ketoconazole. Compounds **3, 26, 36, 38** and **40** showed very good antifungal activity with inhibition zones comparable to that of ketoconazole.

The most active compounds **3**, **14**, **26**, **33'** and **35** with the highest total inhibition zones against all test microorganisms were selected for determination of their (MICs) figure 1. As shown in table 2, Compound **14** was the most active against *S. epidermidis* and *P. mirabilis* with MIC of 10µg/mL ($2.32x10^{-2}$ µmol/mL) for both of them, while compound **35** was the most active against *B. subtilis* and *E.coli* with MIC values of 10µg/mL ($2.12x10^{-2}$ µmol/mL) and 5µg/mL ($1.06x10^{-2}$ µmol/mL), respectively. Regarding activity against *C. albicans*, compounds **26** and **33'** seems to be the most potent with equal activity when their MIC values are expressed in µg/mL as both have MIC of 5 µg/mL, but when their MIC values are expressed in µmol/mL, compound **26** would be found to be more potent with MIC of ($1.01x10^{-2}$ µmol/mL), while that of compound **33'** equals ($1.26x10^{-2}$ µmol/mL). Compounds that seems to have equal MIC values when expressed in (µg/mL) are actually completely different when expressed in (µmol/mL).

From the obtained antimicrobial data, it was difficult to deduce a clear structure activity correlation due to close range of inhibitory activity of the tested compounds. However, it was obvious in aroylhydrazones that 4-nitro substituent was the most favorable along all the synthesized substituents, being the most active against all tested organisms. On the other hand, 4-methyl and 2-imidazolyl derivatives showed also a comparable activity to that of 4-NO_2. The activity order of aroylhydrazones regarding their R substituent is 4-NO_2 > 4-CH_3 > 2-imidazolyl. In 4-thiazolidinone derivatives, nitro derivative did not show the same characteristic antimicrobial activity as in aroylhydrazones. Substituents of 2,4-difluoro, 4-Br and 4-Cl were the most active in a descending order of 2,4-diF > 4-Br > 4-Cl, whereas substituents of 4-COOH, 4-CH_3 and 2,4-diF showed the highest activity in oxadiazoles.

On the other hand, inhibition zones (mm) of propyl paraben **2** compared to its tetrazole derivative **3**, depicted in table 1, showed a comparable inhibition zones against both Gram-positive and Gram-negative bacteria. Both compounds showed almost equal large inhibition zones against *Candida albicans*. Determination of their (MIC_{90}) values, known as minimal fungicidal concentrations (MFC) revealed that compound **3** as antifungal is more potent three times than propyl paraben with MIC_{90} value of 0.14 µmol/mL, while that of propyl paraben is 0.43 µmol/mL. Incorporation of 1-phenyl tetrazole moiety in propyl paraben significantly increased the antifungal activity. Investigation of physicochemical, steric and electrostatic properties of propyl paraben and its tetrazole derivative was performed and discussed in molecular modeling section.

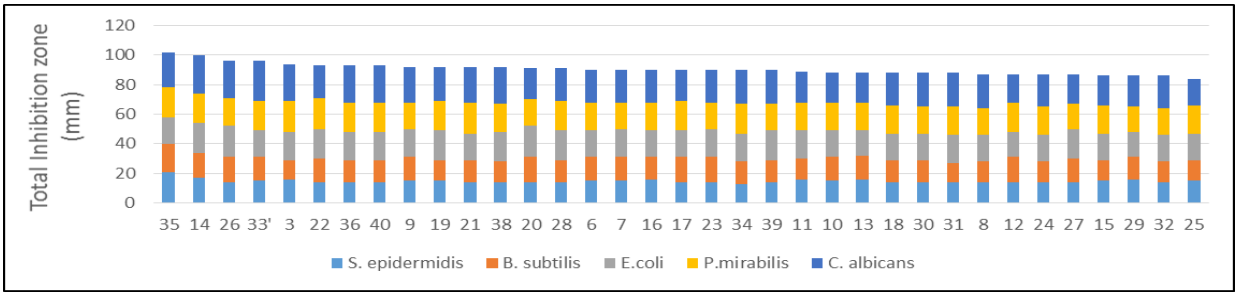


Figure 1: Tested compounds against their total inhibition zones of all test organisms in descending order.

Table 1. Antimicrobial activity data of tested compounds.

	Inhibition zone diameter in (mm)						
Comp. No.	Gram-positive bacteria			Gram-negative bacteria			Fungi
	Staph. epidermidis (ATCC 12228)	*Bacillus subtilis* (ATCC 35021)		*Escherichia coli* (ATCC 25922)	*Proteus mirabilis* (ATCC 25933)		*Candida albicans* (ATCC 76615)
2	16	13		19	21		**25**
3	15	17		18	23		**24**
6	15	16		18	19		22
7	15	16		19	18		22
8	14	14		18	18		23
9	15	16		19	18		24
10	15	16		18	19		20
11	16	14		19	19		21
12	14	**17**		17	20		19
13	16	16		17	19		20
14	17	**17**		20	20		**26**
15	15	14		18	19		20
16	16	15		18	19		22
17	14	**17**		18	20		21
18	14	15		18	19		22
19	15	14		20	20		23
20	14	**17**		**21**	18		21
21	14	15		18	**21**		**24**
22	14	16		20	**21**		22
23	14	**17**		19	18		22
24	14	14		18	19		22
25	15	14		18	19		18
26	14	**17**		**21**	19		**25**
27	14	16		20	17		20
28	14	15		20	20		22
29	16	15		17	17		21
30	14	15		18	18		23
31	14	13		19	19		23
32	14	14		18	18		22
33'	15	16		18	20		**27**
34	13	15		19	20		23
35	**21**	**19**		18	20		24
36	14	15		19	20		**25**
38	14	14		20	19		**25**
39	14	15		20	18		23
40	14	15		19	20		**25**
Cipro[1]	**19**	**17**		**24**	**30**		---
Keto[2]	---	---		---	---		**22**

Table 2: The MIC's of the most active compounds.

Comp. No.	Minimal Inhibitory Concentration µg/mL (µmol/mL)					
	Gram-positive bacteria		Gram-negative bacteria			Fungi
	S. epidermidis (ATCC12228)	*B. subtilis* (ATCC35021)	*E. coli* (ATCC25922)	*P. mirabilis* (ATCC25933)		*C. albicans* (ATCC76615)
3	20 ($6.16x10^{-2}$)	20 ($6.16x10^{-2}$)	10 ($3.08x10^{-2}$)	20 ($6.16x10^{-2}$)		10 ($3.08x10^{-2}$)
14	**10 ($2.32x10^{-2}$)**	20 ($4.65x10^{-2}$)	10 ($2.32x10^{-2}$)	**10 ($2.32x10^{-2}$)**		10 ($2.32x10^{-2}$)
26	20 ($4.04x10^{-2}$)	20 ($4.04x10^{-2}$)	20 ($4.04x10^{-2}$)	20 ($4.04x10^{-2}$)		**5 ($1.01x10^{-2}$)**
33'	20 ($3.08x10^{-2}$)	20 ($3.08x10^{-2}$)	20 ($3.08x10^{-2}$)	20 ($3.08x10^{-2}$)		5 ($1.26x10^{-2}$)
35	20 ($4.25x10^{-2}$)	**10 ($2.12x10^{-2}$)**	**5 ($1.06x10^{-2}$)**	20 ($4.25x10^{-2}$)		20 ($4.25x10^{-2}$)
Cipro[1]	0.03 ($9.05x10^{-5)}$	0.03 ($9.05x10^{-5}$)	0.156 ($4.7x10^{-4}$)	0.156 ($4.7x10^{-4}$)		---
Keto[2]	---	---	---	---		0.19 ($3.5x10^{-4}$)

[1]Ciprofloxacin, [2]Ketoconazole

2.2.2 Anticancer activity

It has been pointed out in the introduction part that this work aims to design and synthesize new compounds with dual antimicrobial and anticancer activity. Prompted by this target, the most active compounds as antimicrobials, **3, 14, 26 & 35,** in addition to, compound **36** were tested for their *in vitro* anticancer activity against two cell lines, hepatocellular carcinoma cells (HuH-7) and colorectal carcinoma cells (CaCo-2) using sulphorhodamine B assay method. The most active compounds as anticancer would have consequently a good dual activity as antimicrobial and anticancer. IC50 values of tested compounds against (CaCo-2) and (HuH-7) cell lines are listed in tables 3,4, respectively.

Table 3: IC_{50} of tested compounds against CaCo-2 cell line.

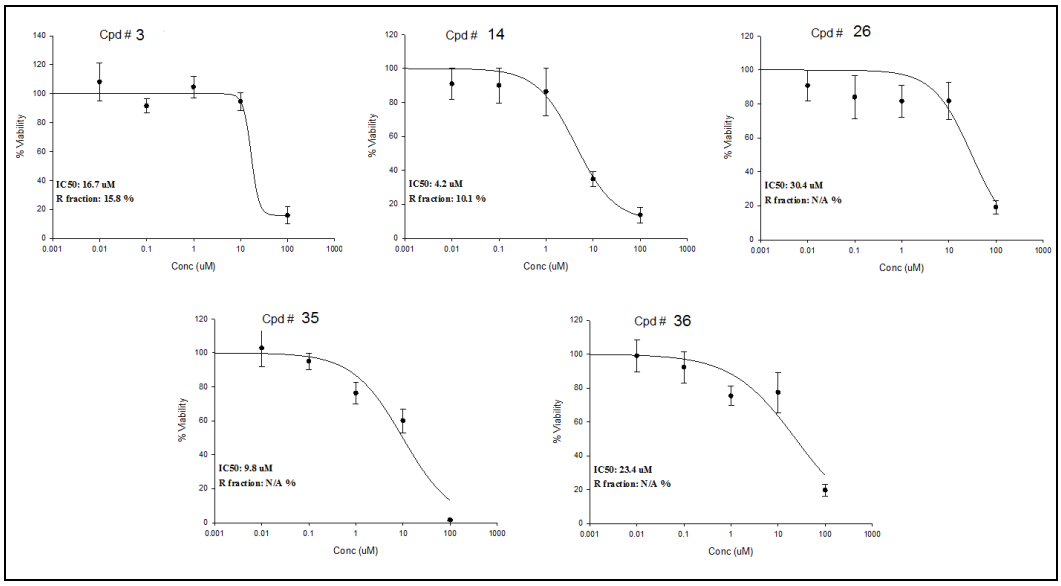
Comp.	CaCo-2	
	IC_{50}	*R fraction%
3	16.7	15.8
14	**4.2**	10.1
26	30.4	N/A
35	**9.8**	N/A
36	23.4	N/A

Results revealed that compound **14** and **35** have very significant activity against (CaCo-2) cell line with IC_{50} of 4.2µM and 9.8µM, respectively. Although both compounds **14** and **26** showed IC_{50} of 10.1µM against (HuH-7) cell line, results weren't considered significant due to large resistant fractions of 35.4% and 45.1%, respectively. Compound **35** was the most active against (HuH-7) cell line with IC_{50} of 24µM and resistant fraction of 0.0%. Therefore, compounds **14** and **35** are the most active. Dose-response curve of tested compounds against (Caco-2) and (HuH-7) cell lines are shown in figures 2 and 3**,** respectively.

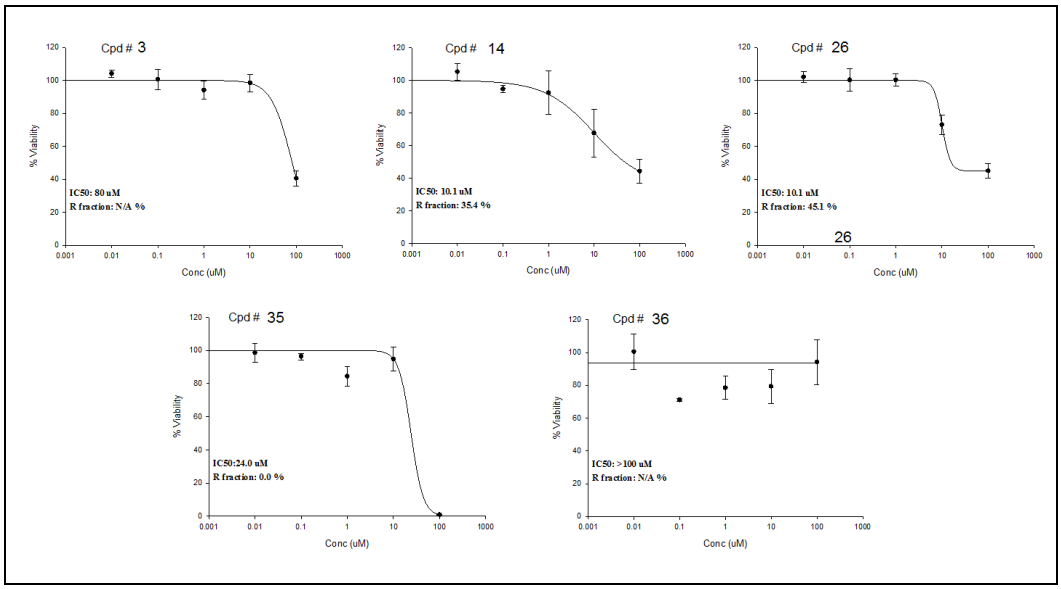
Table 4: IC_{50} of tested compounds against HuH-7cell line.

Comp.	HuH-7	
	IC_{50}	*R fraction%
3	80.0	N/A
14	10.1	35.4
26	10.1	45.1
35	**24.0**	0.0
36	>100	N/A

Based on the above findings, it is concluded that compounds **14** and **35** are the most active as antimicrobial and anticancer. These two compounds represent a promising leads for further optimization to synthesize new potent chemotherapeutic agents with dual activity for achieving the concept of monotherapy.



Figures 2: Dose-response curve of tested compounds against Caco-2 cell line.



Figures 3: Dose-response curve of tested compounds against HuH-7 cell line.

2.3 Molecular modeling

As shown in biological activity section, incorporation of 1-phenyl tetrazole moiety into propyl paraben led to three times increase in the antifungal activity. A molecular modeling approach was carried out to investigate how this incorporation affected lipophilic, electronic, hydrogen bonding and steric hindrance properties. Propyl paraben and its tetrazole derivative were prepared, overlayed and aligned using SYBYL-X 2.1[43] molecular modeling package. As shown in figure 4, 1-phenyl-tetrazole moiety represents the only structural difference between propyl paraben **2** and its tetrazole derivative **3**. Their lipophilicity LP, electrostatic potential EP and hydrogen bonding HB maps were calculated onto their molecular surfaces.

Tetrazole propyl paraben derivative **3** has a ClogP value of 3.91**,** while that of propyl paraben **2** is 3.04. LP maps are in accordance to the calculated lipophilicity, as they have shown large brown hydrophobic region corresponding to 1-phenyl-tetrazole moiety in tetrazole derivative **3**, while in propyl paraben no significant hydrophobic region were observed figure 5(a).

The EP maps showed red to yellow colors for high electron density regions of ester carbonyl and phenolic **OH** in propyl paraben, while in its tetrazole derivative **3** 1-phenyl-tetrazole moiety and ester

carbonyl showed the highest electron density regions. Regions of high electron density in tetrazole derivative are larger than that in propyl paraben figure 5(b).

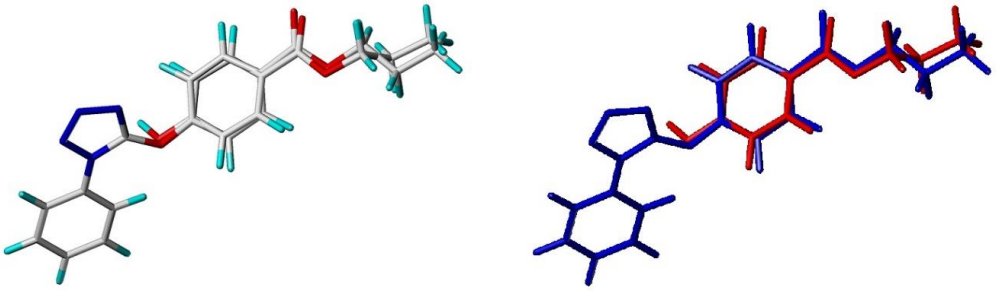


Figure 4: Propyl paraben 2 and its tetrazole derivative 3 aligned and overlayed.

Hydrogen bonding greatly contributes to the binding affinity of any drug to its target receptor. Incorporation of 1-phenyl tetrazole moiety increased number of HB acceptors from 3 to 7 with the loss of the only one HB donor.

Bulkiness of the incorporated 1-phenyl-tetrazole moiety in tetrazole derivative **3** compared to that of phenolic **OH** in propyl paraben was measured and expressed as the total atomic steric hindrance. 1-phenyl-tetrazole moiety and phenolic **OH** group have steric hindrance of 13.98 and 0.71, respectively, showing large difference in their volumes.

It is concluded that difference in structural features contributes to the difference in physicochemical properties and subsequently the activity. As potency of tetrazole derivative **3** is three times that of propyl paraben **2**, a correlation between structure and activity assumes that, incorporation of bulky lipophilic group with high electron density and HB acceptors in place of phenolic **OH** is more favorable for good binding with high affinity. Compound **3** is a promising lead for optimization to synthesize new antifungal agents with potent activity.

3. Conclusion

Three new series of tetrazole hybrids with either aroylhydrazone, 4-thiazolidinone or 2,5-oxadiazoline derivatives were synthesized. The anticancer activity of the synthesized compounds was more significant than their antimicrobial activity, however, their activity against fungi and Gram-negative bacteria was very characteristic. In silico studies of the conjugation product **3** obtained by reaction of propyl paraben **2** with 1-phenyl-tetrazole moiety showed an increase in its lipophilicity (ClogP) with accompanied 3 times increase in its antifungal activity than the parent paraben **2**. This finding is in concordance with the reported increase of antifungal activity of parabens in relation to their lipophilicity.

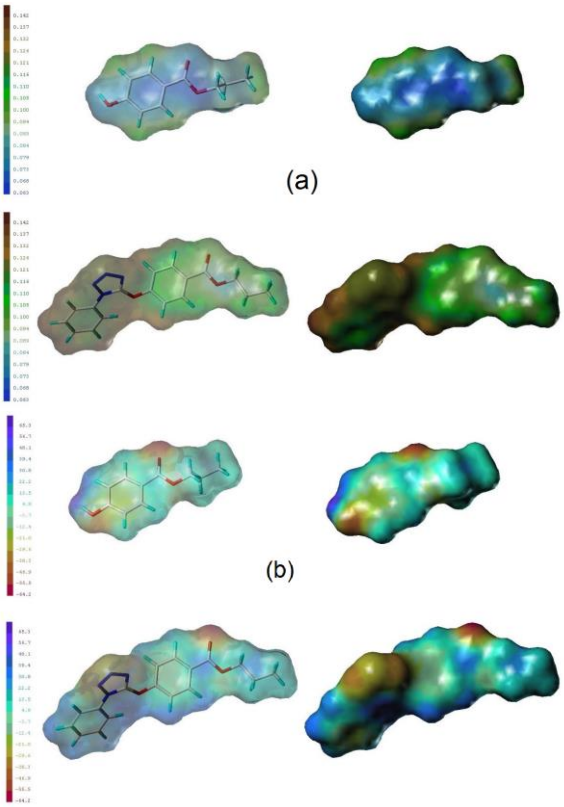


Figure 5: transparent and opaque LP (a) and EP (b) colored maps of propyl paraben and its tetrazole derivative 3 (light gray: carbon, red: oxygen, blue: nitrogen cyan: hydrogen).

4. Experimental

4.1 Chemistry

All chemicals, reagents and solvents were purchased from commercial suppliers and used as received. Melting points were determined using Barnstead electrothermal melting point apparatus and were uncorrected. Reaction monitoring and checking purity of compounds was done by using Merck TLC precoated silica gel 60 GF_{254} plates, using mobile

phase mixture of chloroform/ methanol in a ratio of (9.5/0.5). Spots were detected by exposure to UV lamp at λ_{254} nm. Infrared (IR) spectra were recorded on Perkin Elmer FT-IR system (Spectrum GX) using KBr plate technique and the results were expressed in wave number (cm^{-1}).^{1}H NMR was obtained on 600 MHz Bruker Advance DPX600 spectrometer at Faculty of science, King Abdulaziz University, using tetramethylsilan as internal reference and (DMSO-d6) or ($CDCl_3$) as solvents (Chemical shifts in δ, ppm). Splitting patterns were expressed as follows: s: singlet; d: doublet; t: triplet; sex: sextet; m: multiplet; br: broad; dd: doublet of doublets; tt: triplet of triplets. The mass spectra were obtained with electrospray ionization (ESI) technique using Agilent 6320 Ion Trap HPLC-ESI-MS system. Microanalyses (C, H, N, S) was performed at the Microanalytical Unit, Cairo University, Egypt.

4.1.1 Synthesis of propyl 4-((1-phenyl-1H-tetrazol-5-yl)oxy)benzoate (3)

A solution of 5-chloro-1-phenyl tetrazole **1** (0.90 g, 5 mmol) in DMF (5mL) was added dropwise over a period of 15 min to a well stirred solution of 4-hydroxypropyl benzoate **2** (0.90 g, 5 mmol) in DMF (10 mL) containing anhydrous potassium carbonate (0.69 g, 5 mmol). Stirring at room temperature was maintained for further 8 h and the reaction mixture was then poured onto ice water. The separated solid was filtered, washed with water, dried and crystallized from appropriate solvent. White crystals (ethanol). Yield: 72.6%; mp: 35-36 ° C; IR (KBr, cm^{-1}): 2965, 2932 (CH aromatic), 2865, 2832 (CH aliphatic), 1735 (C=O), 1599, 1532 (C=C aromatic), 1290 (C-O-C); ^{1}H NMR (δ, ppm, $CDCl_3$): 1.03 (t, 3H, *J* = 7.8Hz, CH_3), 1.80 (six, 2H, *J* = 7.2Hz, OCH_2-**CH_2**-CH_3), 4.30 (t, 2H, *J* = 6.6Hz, O**CH_2**-CH_2-CH_3), 7.52 (d, 2H, *J* = 9Hz, Ar-H), 7.54 (tt, 1H, *J* = 7.8, 2.4Hz, Ar-H), 7.58-7.63 (m, 2H, Ar-H), 7.78 (d, 2H, *J* = 8.4Hz, Ar-H), 8.15 (d, 2H, *J* = 9Hz, Ar-H); ESI-MS *m/z* (Rel. Int.): 325.2 (M^{+1}), 255.1 (100), 297.2 (30).

4.1.2 Synthesis of 4-hydroxybenzoic acid hydrazide (4)

To a stirred warm solution of hydrazine hydrate (20 mL), 4-hydroxypropyl benzoate **2** (14.41 g, 80 mmol) was added portion wise. The reaction was heated under reflux for 1h, then allowed to cool. The separated solid was filtered, washed with successive portions of cold water, dried and crystalized from appropriate solvent to give white crystals of reported compound **4**[156,157]. White crystals (ethanol/water). Yield: 98.5%; mp: 265 ° C[156,157]; ^{1}H NMR (δ, ppm, DMSO-d_6): 4.37 (brs, 2H, NH_2), 6.75 (d, 2H, *J* = 8.4Hz, Ar-H), 7.66 (d, 2H, *J* = 9Hz, Ar-H), 9.47 (s, 1H, NH), 9.91 (brs, 1H, OH); ESI-MS *m/z* (Rel. Int.): 151 (M^-).

4.1.3 Synthesis of 4-((1-phenyl-1H-tetrazol-5-yl)oxy)benzohydrazide (5)

An equimolar mixture of 4-hydroxybenzoic acid hydrazide **4** (7.60 g, 50 mmol) and 5-chloro-1-phenyl tetrazole **1** (9.03 g, 50 mmol) in acetone (100 mL) containing anhydrous potassium carbonate (6.91 g, 50 mmol) was heated under reflux for 12 h. The reaction mixture was allowed to cool and poured onto ice cold water. The separated solid was filtered, dried and crystallized from appropriate solvent. White crystals (ethanol). Yield: 80%; mp: 165 ° C; IR (KBr, cm^{-1}): 3391,3254 (NH), 1648 (C=O), 1548, 1496 (C=C aromatic), 1280 (C-O-C); ^{1}H NMR (δ, ppm, DMSO-d_6): 4.5 (brs, 2H, NH_2), 7.60 (m, 3H, ArH), 7.63-7.67 (m, 2H, ArH), 7.92 (d, 2H, ArH), 7.97 (d.2H, ArH), 9.87 (s, 1H, NH); ESI-MS *m/z* (Rel. Int.): 297.1 (M^+), 280.2 (19), 269.1 (14.28), 252.1 (71.4), 237.1 (9.52), 227.1 (100), 211 (23.8), 182.1 (4.76), 168.1 (19), 133.1 (28.56).

4.1.4 General procedure for synthesis of (E)- N'-(substituted-benzylidene/heterocyclic)-4-((1-phenyl -1H-tetrazol-5- yl)oxy) benzohydrazide (6-19).

An equimolar mixture of appropriate aromatic aldehyde (10 mmol) and hydrazide derivative **5** (2.96 g, 10 mmol) in glacial acetic acid (10 mL) was heated under reflux until extensive precipitation was seen. The reaction mixture was then allowed to cool and poured onto ice cold water. The separated solid was filtered, washed with water, dried and crystallized from appropriate solvent.

In case of compound **19**, no precipitate was observed. Reaction mixture was maintained under reflux for 30 min, then allowed to cool, poured onto ice cold water and neutralized by 10% sodium bicarbonate solution to separate the product.

4.1.4.1 (E)-N'-(4-fluorobenzylidene)-4-((1-phenyl-1H-tetrazol-5-yl)oxy) benzohydrazide (6). White crystals (acetic acid). Yield: 79%; mp: 194-196 ° C; IR (KBr, cm^{-1}): 3233 (NH), 1700 (C=O), 1633 (N=C), 1533,1500 (C-C)Ar, 1283 (C-O)ether; ^{1}H NMR (δ, ppm, $CDCl_3$: DMSO-d_6): 7.02 (t, 2H, *J* = 8.4Hz, Ar-H), 7.47-7.49 (m, 3H, Ar-H), 7.54 (t, 2H, *J* = 7.8Hz, Ar-H), 7.69 (d, 2H, *J* = 8.4Hz, Ar-H), 7.72 (d, 2H, *J* = 8.4Hz, Ar-H), 7.99 (d, 2H, *J* = 8.4Hz, Ar-H), 8.35 (s, 1H, N=CH), 11.68 (s, 1H, CO-NH); ESI-MS *m/z* (Rel. Int.): 403.1 (M^+), 375.1 (100), 333.1 (73), 236.1 (69.2), 211.1 (32.1). Anal. Calcd for $C_{21}H_{15}FN_6O_2$ (402.39): C, 62.68; H, 3.76; N, 20.89.Found: C, 62.45; H, 4.01; N, 20.57.

4.1.4.2 (E)-N'-(4-chlorobenzylidene)-4-((1-phenyl-1H-tetrazol-5-yl)oxy) benzohydrazide (7). White crystals (acetic acid). Yield: 97%; mp: 219-221 ° C; IR (KBr, cm^{-1}): 3300 (NH), 1650 (C=O), 1584 (N=C), 1533, 1500 (C-C)Ar, 1266 (C-O)ether; ^{1}H NMR (δ, ppm, $CDCl_3$: DMSO-d_6): 7.27 (d, 2H, *J* = 8.4Hz, Ar-H), 7.45-7.48 (m, 3H, *J* = 8.4Hz, Ar-H), 7.53 (t, 2H, *J*

= 7.8Hz, Ar-H), 7.63 (d, 2H, *J* = 8.4Hz, Ar-H), 7.70 (d, 2H, *J* = 8.4Hz, Ar-H), 7.98 (d, 2H, *J* = 8.4Hz, Ar-H), 8.33 (s, 1H, N=CH), 11.63 (s, 1H, CONH); ESI-MS *m/z* (Rel. Int.): 420.2 (M^{+2}), 391.3 (100), 349.3 (94.6), 274.2 (13.15), 254.3 (47.43), 236.5 (84.16), 211.6 (26.3)Anal. Calcd for $C_{21}H_{15}ClN_6O_2$ (418.84): C, 60.22; H, 3.61; N, 20.07. Found: C, 59.88; H, 3.48; N, 19.97.

4.1.4.3 (E)-N'-(4-bromobenzylidene)-4-((1-phenyl-1H-tetrazol-5-yl)oxy) benzohydrazide (8). White crystals (acetic acid). Yield: 84.9%; mp: 214-216 ° C; IR (KBr, cm^{-1}): 3300 (NH), 1658 (C=O), 1584 (N=C), 1533, 1500 (C-C)Ar, 1266 (C-O)ether; ^{1}H NMR (δ, ppm, $CDCl_3$: DMSO-d_6): 7.43 (d, 2H, *J* = 8.4Hz, Ar-H), 7.47 (t, 3H, *J* = 8.4, Ar-H), 7.53 (t, 2H, *J* = 8.4Hz, Ar-H), 7.57 (d, 2H, *J* = 8.4Hz, Ar-H), 7.70 (d, 2H, *J* = 7.8Hz, Ar-H), 7.99 (d, 2H, *J* = 8.4Hz, Ar-H), 8.32 (s, 1H, N=CH), 11.67 (s, 1H, CONH); ESI-MS *m/z* (Rel. Int.): 463.3 (M), 435.2 (100), 393.2 (60), 236.2 (60), 211.3 (23), 182.1 (10). Anal. Calcd for $C_{21}H_{15}BrN_6O_2$ (463.30): C, 54.44; H, 3.26; N, 18.14. Found: C, 54.29; H, 3.10; N, 18.36.

4.1.4.4 (E)-N'-(4-methylbenzylidene)-4-((1-phenyl-1H-tetrazol-5-yl)oxy) benzohydrazide (9). White crystals (acetic acid). Yield: 75.8%; mp: 184-186 ° C; IR (KBr, cm^{-1}): 3266 (NH), 1683 (C=O), 1616 (N=C), 1532, 1491 (C-C)Ar, 1275 (C-O)ether; ^{1}H NMR (δ, ppm, DMSO-d_6): 2.34 (s, 3H, CH_3), 7.27 (d, 2H, *J* = 8.4Hz, Ar-H), 7.61 (tt, 1H, *J* = 7.8, 2.4Hz, Ar-H), 7.62 (d, 2H, *J* = 8.4Hz, Ar-H), 7.66-7.68 (m, 2H, Ar-H), 7.73-7.75 (m, 2H, Ar-H), 7.85 (d, 2H, *J* = 7.8Hz, Ar-H), 8.03 (d, 2H, *J* = 9Hz, Ar-H), 8.41 (s, 1H, N=CH), 11.86 (s, 1H, CO-NH); ESI-MS *m/z* (Rel. Int.): 399.1 (M^+), 371.1 (100), 329.1 (48.2), 236.1 (87.3), 211.1 (33.2), 118.1 (22). Anal. Calcd for $C_{22}H_{18}N_6O_2$ (398.43): N, 21.09. Found: N, 21.06.

4.1.4.5 (E)-N'-(4-methoxybenzylidene)-4-((1-phenyl-1H-tetrazol-5-yl)oxy) benzohydrazide (10)

White crystals (acetic acid). Yield: 83%; mp: 194-196 ° C; IR (KBr, cm^{-1}): 3216 (NH), 1632 (C=O), 1600 (N=C), 1533,1483 (C-C)Ar, 1300 (C-O)ether; ^{1}H NMR (δ, ppm, DMSO-d_6): ($CDCl_3$: DMSO-d_6): 3.75 (s, 3H, OCH_3), 6.83 (d, 2H, *J* = 8.4Hz, Ar-H), 7.45- 7.48 (m, 3H, Ar-H), 7.53 (t, 2H, *J* = 7.8Hz, Ar-H),7.62 (d, 2H, *J* = 8.4Hz, Ar-H), 7.71 (d, 2H, *J* = 7.8Hz, Ar-H), 7.98 (d, 2H, *J* = 8.4Hz, Ar-H), 8.29 (s, 1H, N=CH), 11.49 (s, 1H, CO-NH); ESI-MS *m/z* (Rel. Int.): 415.1 (M^+), 345 (10), 287 (50), 254 (30), 237 (100), 211.1 (11), 134 (23). Anal. Calcd for $C_{22}H_{18}N_6O_3$ (414.43): C, 63.76; H, 4.38; N, 20.28. Found: C, 63.84; H, 4.34; N, 20.23.

4.1.4.6(E)-4-((2-(4-((1-phenyl-1H-tetrazol-5-yl) oxy)benzoyl)hydrazono) methyl)benzoic acid (11).

White crystals (ethanol). Yield: 78.6%; mp: 259-261 ° C; IR (KBr, cm^{-1}): 3233 (NH), 1683 (C=O) carboxyl, 1650 (C=O) amide, 1566 (N=C), 1533, 1483 (C-C)Ar, 1266 (C-O)ether; ^{1}H NMR (δ, ppm, DMSO-d_6): 7.61 (tt, 1H, *J* = 7.2Hz, Ar-H), 7.66 (d, 2H, *J* = 7.8Hz, Ar-H), 7.68-7.70 (m, 3H, Ar-H), 7.84-7.86 (m, 4H, Ar-H), 8.01 (d, 2H, *J* = 8.4Hz, Ar-H), 8.05 (d, 2H, *J* = 9Hz, Ar-H), 8.5 (s, 1H, N=CH), 12.1 (s, 1H, CONH), 13.12 (s, 1H, COOH); ESI-MS *m/z* (Rel. Int.): 429.2 (M^+), 429.2 (56.42), 401.1 (100), 359.1 (52.08), 237 (47.74), 130 (26.04); Anal. Calcd for $C_{22}H_{16}N_6O_4$ (428.41): C, 61.68; H, 3.76; N, 19.62. Found: C, 62.05; H, 3.76; N, 20.00.

4.1.4.7 (E)-N'-(2,4-difluorobenzylidene)-4-((1-phenyl-1H-tetrazol-5-yl)oxy)benzohydrazide (12)

White crystals (acetic acid). Yield: 85%; mp: 206-208 ° C; IR (KBr, cm^{-1}): 3283 (NH),1683 (C=O), 1616 (N=C), 1533, 1483 (C-C)Ar, 1266 (C-O)ether; ^{1}H NMR (δ, ppm, $CDCl_3$: DMSO-d_6): 6.78 (td, 1H, *J* = 9.6, 1.8Hz, Ar-H), 6.85 (td, 1H, *J* = 7.8, 1.8Hz, Ar-H), 7.46-7.48 (m, 3H, Ar-H), 7.53 (t, 2H, *J* = 7.8, Ar-H), 7.71 (d, 2H, *J* = 7.8Hz, Ar-H), 8.02 (d, 2H, *J* = 9Hz, Ar-H), 8.05 (dd, 1H, *J* = 7.2Hz, Ar-H), 8.59 (s, 1H, N=CH), 11.77 (s, 1H, CONH); ESI-MS *m/z* (Rel. Int.): 421.2 (M^+), 393.2 (87.46), 351.1 (94.12), 237 (100), 140 (24.99)Anal. Calcd for $C_{21}H_{14}F_2N_6O_2$ (420.38): C, 60.00; H, 3.36; N, 19.99. Found: C, 59.81; H, 3.40; N, 19.84.

4.1.4.8 (E)-N'-(3,4-dimethoxybenzylidene)-4-((1-phenyl-1H-tetrazol-5-yl)oxy)benzohydrazide (13)

White crystals (acetic acid). Yield: 87.6%; mp: 215-217 ° C; IR (KBr, cm^{-1}): 3272 (NH),1663 (C=O),1600 (N=C), 1545 ,1490 (C-C) Aromatic, 1272 (C-O) ether;^{1}H NMR (δ, ppm, $CDCl_3$): 3.90 (s, 3H, OCH_3), 3.92 (s, 3H, OCH_3), 6.84 (d, 1H, *J* = 8.4Hz, Ar-H), 7.11 (dd, 1H, *J* = 8.4, 1.8Hz, Ar-H), 7.4 (d, 2H, *J* = 9Hz, Ar-H), 7.44 (s, 1H, Ar-H), 7.54(tt, 1H, *J* = 7.2, 1.2Hz, Ar-H), 7.60 (t, 2H, *J* = 7.8Hz, Ar-H), 7.78 (d, 2H, *J* = 7.8Hz, Ar-H), 7.9 (d, 2H, *J* = 8.4Hz, Ar-H), 8.27 (s, 1H, N=CH), 10.28 (s, 1H, CONH); ESI-MS *m/z* (Rel. Int.): 445.2 (M^+), 417.2 (49.98), 237 (100), 164 (23.32). Anal. Calcd for $C_{23}H_{20}N_6O_4$ (444.45): C, 62.16; H, 4.5; N, 18.91. Found: C, 62.35; H, 4.18; N, 18.78.

4.1.4.9 (E)-N'-(4-nitrobenzylidene)-4-((1-phenyl-1H-tetrazol-5-yl)oxy)benzohydrazide (14). Yellow crystals (acetic acid). Yield: 86.7%; mp: 252-254 ° C; IR (KBr, cm^{-1}): 3236 (NH), 1672 (C=O), 1582 (N=C), 1536, 1490 (C-C)Ar, 1327 (N-O), 1254 (C-O)ether; ^{1}H NMR (δ, ppm, DMSO-d_6): 7.61-7.62 (tt, 1H, *J* = 7.8Hz, Ar-H), 7.66-7.68 (m, 4H, Ar-H), 7.85 (d, 2H, *J* = 7.8Hz, Ar-H), 8.00 (d, 2H, *J* = 8.4Hz, Ar-H), 8.06 (d, 2H, *J* = 8.4Hz, Ar-H), 8.3 (d, 2H, *J* = 9Hz, Ar-H), 8.54 (s, 1H, N=CH), 12.25 (s, 1H, CO-NH); ESI-MS *m/z* (Rel. Int.): 430.2 (M^+). Anal. Calcd for $C_{21}H_{15}N_7O_4$ (429.40): C, 58.74; H, 3.52; N, 22.83. Found: C, 58.93; H, 3.28; N, 22.49.

4.1.4.10 (E)-N'-(2-methoxy-4-nitrobenzylidene)-4-((1-phenyl-1H-tetrazol-5-yl)oxy)benzohydrazide (15). Yellow crystals (acetic acid). Yield: 87.7%; mp: 240-242 ° C; IR (KBr, cm^{-1}): 3237 (NH), 1690 (C=O), 1582 (N=C), 1545, 1490 (C-C)Ar, 1345 (N-O), 1272 (C-O)ether; ^{1}H NMR (δ, ppm, DMSO-d_6): 4.00 (s, 3H, OCH_3), 7.61 (tt, 1H, *J* = 7.2, 1.2Hz Ar-H), 7.66 (d, 2H, *J* = 7.8Hz, Ar-H), 7.69 (d, 2H, *J* = 9Hz, Ar-H), 7.85-7.87 (m, 3H, Ar-H), 7.91 (dd, 1H, *J* = 8.4, 1.8Hz, Ar-H), 8.07 (d, 2H, *J* = 9Hz, Ar-H), 8.10 (d, 1H, *J* = 8.4Hz, Ar-H), 8.81 (s, 1H, N=CH), 12.19 (s, 1H, CONH); ESI-MS *m/z* (Rel. Int.): 460.1(M^+), 432.1 (100), 390.1 (33.32), 251.1 (66.64). Anal. Calcd for $C_{22}H_{17}N_7O_5$ (459.42): C, 57.52; H, 3.73; N, 21.34. Found: C, 57.19; H, 3.73; N, 21.60.

4.1.4.11 (E)-N'-(4-hydroxybenzylidene)-4-((1-phenyl-1H-tetrazol-5-yl)oxy)benzohydrazide (16)

White crystals (acetic acid). Yield: 75.3%; mp: 232-234 ° C; IR (KBr, cm^{-1}): 3272 (NH), 3200 (OH), 1654 (C=O), 1600 (N=C), 1527, 1490 (C-C)Ar, 1263 (C-O)ether; ^{1}H NMR (δ, ppm, DMSO-d_6): 6.85 (d, 2H, *J* = 8.4Hz, Ar-H), 7.58 (d, 2H, *J* = 8.4, Ar-H), 7.63 (tt, 1H, *J* = 7.8Hz, Ar-H), 7.68-7.71 (m, 4H, Ar-H), 7.87 (d, 2H, *J* = 7.2Hz, Ar-H), 8.04 (d, 2H, *J* = 9Hz, Ar-H), 8.36 (s,1H, N=CH), 9.99 (s, 1H, OH), 11.77 (s, 1H, CO-NH); ESI-MS *m/z* (Rel. Int.): 401.1 (M^+), 373.1 (60), 331.1 (32), 254 (35), 236.1 (100). 211.1 (23). Anal. Calcd for $C_{21}H_{16}N_6O_3$ (400.40): C, 63.00; H, 4.03; N, 20.99. Found: C, 62.78; H, 3.88; N, 20.72.

4.1.4.12 (E)-N'-(4-hydroxy-3-methoxybenzylidene) -4-((1-phenyl-1H-tetrazol-5-yl)oxy) benzohydrazide(17). White crystals (ethanol). Yield: 69%; mp: 215-217 ° C; IR (KBr, cm^{-1}): 3302 (OH), 3236 (NH), 1655 (C=O), 1590 (N=C), 1543, 1497 (C-C) Aromatic, 1282(C-O)ether ;^{1}H NMR (δ, ppm, DMSO-d_6): 3.83 (s, 3H, OCH_3), 6.83 (d, 1H, *J* = 7.8Hz, Ar-H), 7.08 (dd, 1H, *J* = 7.8, 1.8Hz, Ar-H), 7.31 (d, 1H, *J* = 1.8Hz, Ar-H), 7.61 (tt, 1H, *J* = 7.8, 1.2Hz, Ar-H), 7.65-7.68 (m, 4H, Ar-H), 7.85 (d, 2H, *J* = 7.8Hz, Ar-H), 8.02 (d, 2H, *J* = 8.4Hz, Ar-H), 8.33 (s, 1H, N=CH), 9.57 (s, 1H, OH), 11.74 (s, 1H, CONH); ESI-MS *m/z* (Rel. Int.): 431.1 (M+), 403.1 (85.87), 361.1 (5.5), 286 (8.44), 237 (100), 150 (22.72).Anal. Calcd for $C_{22}H_{18}N_6O_4$ (430.42): C, 61.39; H, 4.22; N, 19.53. Found: C, 61.17; H, 3.99; N, 19.41.

4.1.4.13 (E)-N'-(furan-2-ylmethylene)-4-((1-phenyl-1H-tetrazol-5-yl)oxy)benzohydrazide (18)

White crystals (ethanol). Yield: 62.7%; mp: 175-177 ° C; IR (KBr, cm^{-1}): 3236 (NH), 1636 (C=O), 1544,1490 (C-C)Ar, 1272 (C-O) ether; ^{1}H NMR (δ, ppm, $CDCl_3$): 6.46 (dd, 1H, *J* = 1.8Hz, furan-CH), 6.62 (s, 1H, furan-CH),7.41 (d, 2H, *J* = 7.8Hz, Ar-H), 7.49 (d, 1H, *J* = 0.6Hz, furan CH), 7.58-7.62 (m, 3H, Ar-H), 7.77 (d, 2H, *J* = 7.8Hz, Ar-H), 7.79 (d, 2H, *J* = 7.8Hz, Ar-H), 8.42 (s, 1H, N=CH), 10.50 (s, 1H, CONH), 11.28 (s, 1H, CONH); ESI-MS *m/z* (Rel. Int.): 375.1 (M^+), 347.1 (10.2), 305.1 (40.3), 237 (100), 211.1 (40).Anal. Calcd for $C_{19}H_{14}N6O_3$ (374.36): C, 60.96; H, 3.77; N, 22.45. Found: C, 60.66; H, 3.57; N, 22.65.

4.1.4.14 (E)-N'-((1H-imidazol-2-yl)methylene)-4-((1-phenyl-1H-tetrazol-5-yl) oxy) benzohydrazide (19). White crystals (ethanol). Yield: 71.9%; mp: 258-260 ° C; IR (KBr, cm^{-1}): 3236 (NH), 1654 (C=O), 1600 (N=C), 1544,1490, (C-C)Ar,1272 (C-O) ether;^{1}H NMR (δ, ppm, DMSO-d_6): 7.15 (s, 2H, imidazole-H), 7.61 (tt, 1H, *J* = 7.2, 1.8Hz, Ar-H), 7.65-7.7 (m, 4H, Ar-H), 7.85 (d, 2H, *J* = 8.4Hz, Ar-H), 8.04 (d, 2H, *J* = 9Hz, Ar-H), 8.34 (s, 1H, N=CH), 11.91 (s, 1H, CONH), 12.86 (s, 1H, imidazole-NH); ESI-MS *m/z* (Rel. Int.): 375.1 (M^+), 237 (100), 209 (10.2).Anal. Calcd for $C_{18}H_{14}N_8O_2$ (374.36): C, 57.75; H, 3.77; N, 29.93. Found: C, 58.00; H, 3.65; N, 30.05.

4.1.5 General synthesis of (±) N-(2-(substituted-phenyl)-4-oxothiazolidin-3-yl)-4-((1-phenyl-1H-tetrazol-5-yl)oxy)benzamide (20-29)

To a well stirred solution of appropriate aroylhydrazone derivatives **6-12&18** (5mmol) in dry benzene (10 mL), mercaptoacetic acid (1 mL) in dry benzene (5 mL) was added. Reaction mixture was heated under reflux for 6-10 h, then allowed to cool and the solvent was evaporated under reduced pressure. The separated sticky residue was boiled with hot water for 15 min, then allowed to cool. The separated solid was filtered, washed with sodium bicarbonate 10% several times, then washed with water, dried and crystallized from appropriate solvent.

In case of compounds **27&28**, a mixture of appropriate aroylhydrazone **14&15** (5mmol) in mercaptoacetic acid (5 mL) was heated at 80 ° C for 24 h. Reaction mixture was allowed to cool and neutralized by sodium carbonate solution (10%). The separated solid was filtered, washed with boiling water (30 mL), dried and crystallized from appropriate solvent.

4.1.5.1N-(2-(4-fluorophenyl)-4-oxothiazolidin-3-yl)-4-((1-phenyl-1H-tetrazol-5-yl)oxy)benzamide (20). White crystals (ethanol). Yield: 70.3%; mp: 166-168 ° C; IR (KBr, cm^{-1}): 3259 (NH), 1716 (C=O), 1673 (C=O), 1542,1489 (C-C)Ar, 1281 (C-O)ether;^{1}H NMR (δ, ppm, $CDCl_3$): 3.73 (dd, 1H, *J* = 16.2, 1.2Hz, thiazolidinone CH_2), 3.89 (dd, 1H, *J* = 16.2, 1.2Hz, thiazolidinone CH_2), 6.04 (s, 1H, thiazolidinone CH), 7.06 (t, 2H, *J* = 8.4Hz, Ar-H), 7.33 (d, 2H, *J* = 9Hz, Ar-H), 7.41-7.44 (m, 2H, Ar-H), 7.53 (tt, 1H, *J* = 7.2, 1.2Hz, Ar-H), 7.59 (tt, 2H, *J* = 7.8Hz, Ar-H), 7.67 (d, 2H, *J* = 9Hz, Ar-H), 7.75 (d, 2H, *J* = 6.4Hz Ar-H), 9.09 (s, 1H, CONH); ESI-MS *m/z* (Rel. Int.): 477.1 (M+1), 403.1 (26.66), 375.1 (6.66), 237 (100), 195.1 (13.33). Anal. Calcd for $C_{23}H_{17}FN_6O_3S$ (476.49): C,

57.98; H, 3.60; N, 17.64; S, 6.73. Found: C, 57.92; H, 3.30; N, 17.67; S, 6.90.

4.1.5.2 N-(2-(4-chlorophenyl)-4-oxothiazolidin-3-yl)-4-((1-phenyl-1H-tetrazol-5-yl)oxy)benzamide (21). White crystals (ethanol/chloroform). Yield: 85.47%; mp: 176-178 ° C; IR (KBr, cm^{-1}): 3252 (NH), 1719 (C=O), 1675 (C=O), 1543, 1489 (C-C)Ar, 1290 (C-O)ether ;^{1}H NMR (δ, ppm, DMSO-d_6): 3.79 (dd, 1H, *J* = 16.2, 1.2Hz, thiazolidinone CH_2), 3.91 (dd, 1H, *J* = 16.2, 1.8Hz, thiazolidinone CH_2), 5.89 (s, 1H, thiazolidinone CH), 7.36 (d, 2H, *J* = 8.4Hz, Ar-H), 7.39 (d, 2H, *J* = 8.4Hz, Ar-H),7.42 (d, 2H, *J* = 9Hz, Ar-H),7.53 (t, 1H, *J* = 7.8Hz, Ar-H), 7.59 (t, 2H, *J* = 8.4Hz, Ar-H), 7.73(d, 2H, *J* = 9Hz, Ar-H), 7.75 (d, 2H, *J* = 8.4Hz, Ar-H), 8.41 (s, 1H, CONH); ESI-MS *m/z* (Rel. Int.): 493.3 (M+1), 237.1 (100), 195.2 (100). Anal. Calcd for $C_{23}H_{17}ClN_6O_3S$ (492.94): C, 56.04; H, 3.48; N, 17.05; S, 6.50. Found: C, 56.05; H, 3.22; N, 17.15; S, 6.27.

4.1.5.3 N-(2-(4-bromophenyl)-4-oxothiazolidin-3-yl)-4-((1-phenyl-1H-tetrazol-5-yl)oxy)benzamide (22). White crystals (ethanol/chloroform). Yield: 86.9%; mp: 190-192 ° C; IR (KBr, cm^{-1}): 3245 (NH), 1718 (C=O) 1674 (C=O), 1543, 1488 (C-C)Ar, 1290 (C-O)ether ;^{1}H NMR (δ, ppm, DMSO-d_6): (DMSO-d_6): 3.82 (d, 1H, *J* = 15.6Hz, thiazolidinone CH_2), 3.97 (dd, 1H, *J* = 15.6, 1.8Hz, thiazolidinone CH_2), 5.92 (s, 1H, thiazolidinone CH), 7.46 (d, 2H, *J* = 8.4Hz, Ar-H), 7.57- 7.66 (m, 7H, Ar-H), 7.82 (d 2H, *J* = 7.2Hz, Ar-H), 7.86 (d, 2H, *J* = 9Hz, Ar-H), 10.83 (s, 1H, CONH); ESI-MS *m/z* (Rel. Int.): 537.3 (M), 463.2 (14.28), 435.2 (9.52), 237.1 (100), 195.2 (19.04). Anal. Calcd for $C_{23}H_{17}BrN_6O_3S$ (537.39): C, 51.41; H, 3.19; N, 15.64; S, 5.97. Found: C, 51.45; H, 3.19; N, 15.82; S, 5.93.

4.1.5.4 N-(4-oxo-2-(p-tolyl)thiazolidin-3-yl)-4-((1-phenyl-1H-tetrazol-5-yl)oxy)benzamide (23)

White crystals (ethanol). Yield: 64.13%; mp: 166-168 ° C; IR (KBr, cm^{-1}): 3237 (NH), 1715 (C=O), 1673 (C=O), 1542, 1491 (C-C)Ar, 1289 (C-O)ether ;^{1}H NMR (δ, ppm, DMSO-d_6): 2.35 (s, 3H, CH_3), 3.74 (dd, 1H, *J* = 16.2, 1.8Hz, thiazolidinone CH_2), 3.89 (d, 1H, *J* = 16.2Hz, thiazolidinone CH_2), 6.02 (s, 1H, thiazolidinone CH), 7.19 (d, 2H, *J* = 7.8Hz, Ar-H), 7.31 (d, 2H, *J* = 7.8Hz, Ar-H), 7.36 (d, 2H, *J* = 8.4Hz, Ar-H), 7.53 (tt, 1H, *J* = 7.8, 2.4Hz, Ar-H), 7.57-7.60 (m, 2H, Ar-H), 7.70 (d, 2H, *J* = 9Hz, Ar-H), 7.75 (d, 2H, *J* = 7.8Hz, Ar-H), 8.65 (s, 1H, CONH).; ESI-MS *m/z* (Rel. Int.): 473.3 (M^+), 399.2 (20), 371.3 (10), 237.1 (100). Anal. Calcd for $C_{24}H_{20}N_6O_3S$ (472.52): N, 17.7; S, 6.78. Found: N, 18.00; S, 6.46.

4.1.5.5 N-(2-(4-methoxyphenyl)-4-oxothiazolidin-3-yl)-4-((1-phenyl-1H-tetrazol-5-yl)oxy)benzamide (24). White crystals (ethanol). Yield: 48.7%; mp: 180-182 ° C; IR (KBr, cm^{-1}): 3242 (NH), 1715 (C=O), 1672 (C=O), 1541,1493 (C-C)Ar, 1219 (C-O)ether;^{1}H NMR (δ, ppm, DMSO-d_6): ($CDCl_3$): 3.72 (dd, 1H, *J* = 16.2,1.2Hz, thiazolidinone CH_2), 3.79 (s, 3H, OCH_3), 3.88 (dd, 1H, 16.2, 1.2Hz, thiazolidinone CH_2), 6.01 (s, 1H, thiazolidinone CH), 6.88 (d, 2H, *J* = 9Hz, Ar-H), 7.35 (t, 4H, *J* = 7.2Hz, Ar-H), 7.52 (tt, 1H, *J* = 7.8, 1.2Hz, Ar-H), 7.58 (t, 2H, *J* = 8.4Hz, Ar-H), 7.69 (d, 2H, *J* = 8.4Hz, Ar-H), 7.75 (d, 2H, *J* = 8.4Hz, Ar-H), 8.83 (s, 1H, CONH); ESI-MS *m/z* (Rel. Int.): 489.1 (M^+), 415.1 (60.71), 381.1 (96.42), 237 (100). Anal. Calcd for $C_{24}H_{20}N_6O_4S$ (488.52): C, 59.01; H, 4.13; N, 17.20; S, 6.56. Found: C, 58.88; H, 3.91; N, 16.98; S, 6.33.

4.1.5.6 4-(4-oxo-3-(4-((1-phenyl-1H-tetrazol-5-yl)oxy)benzamido)thiazolidin-2-yl)benzoic acid (25). White crystals (ethanol). Yield: 75.4%; mp: 102-104 ° C; IR (KBr, cm^{-1}): 3231 (NH), 1714 (C=O), 1663 (C=O), 1533, 1483 (C-C)Ar, 1266 (C-O)ether ;^{1}H NMR (δ, ppm, DMSO-d_6): 3.83 (d, 1H, *J* = 15.6Hz, thiazolidinone CH_2), 3.99 (dd, 1H, *J* = 15.6, 1.8Hz, thiazolidinone CH_2), 6.00 (s, 1H, thiazolidinone CH), 7.58 (tt, 1H, *J* = 7.8, 1.2Hz, Ar-H), 7.60-7.66 (m, 6H, Ar-H), 7.81 (d, 2H, *J* = 7.8Hz, Ar-H), 7.86 (d, 2H, *J* = 8.4Hz, Ar-H), 7.93-7.94 (d, 2H, *J* = 8.4Hz, Ar-H), 10.86 (s, 1H, CONH), 12.98 (s, 1H, COOH); ESI-MS *m/z* (Rel. Int.): 503.1 (M^+), 503 (13.04), 485.1 (100), 429.1 (17.39), 237 (43.47), 195 (8.69). Anal. Calcd for $C_{24}H_{18}N_6O_5S$ (502.51): C, 57.37; H, 3.61; N, 16.72; S, 6.38. Found: C, 57.49; H, 3.34; N, 16.49; S, 6.50.

4.1.5.7 N-(2-(2,4-difluorophenyl)-4-oxothiazolidin-3-yl)-4-((1-phenyl-1H-tetrazol-5-yl)oxy)benzamide (26). White crystals (ethanol). Yield: 84.9%; mp: 131-133 ° C; IR (KBr, cm^{-1}): 3232 (NH),1668 (C=O), 1595 (C=O), 1537, 1494 (C-C)Ar, 1266 (C-O)ether ;^{1}H NMR (δ, ppm, DMSO-d_6): (DMSO-d_6): 3.83 (d, 1H, *J* = 16.2Hz, thiazolidinone CH_2), 3.96 (dd, 1H, *J* = 16.2, 1.8Hz, thiazolidinone CH_2), 6.09 (s, 1H, thiazolidinone CH), 7.15 (td, 1H, *J* = 7.8, 2.4Hz, Ar-H), 7.28 (td, 1H, *J* = 15, 2.4Hz, Ar-H), 7.59 (tt, 1H, *J* = 7.2, 1.2Hz, Ar-H), 7.62 (d, 2H, *J* = 9Hz, Ar-H), 7.64 (d, 2H, *J* = 7.2Hz, Ar-H), 7.69 (dd, 1H, *J* = 9, 7.8Hz, Ar-H), 7.82 (d, 2H, *J* = 7.8Hz, Ar-H), 7.87 (d, 2H, *J* = 9Hz, Ar-H), 10.88 (s, 1H, CONH); ESI-MS *m/z* (Rel. Int.): 495.1 (M^+), 421.1 (41.66), 237 (100), 195 (16.66). Anal. Calcd for $C_{23}H_{16}F_2N_6O_3S$ (494.48): C, 55.87; H, 3.26; N, 17.00; S, 6.48. Found: C, 56.19; H, 3.50; N, 17.61; S, 6.55.

4.1.5.8 N-(2-(4-nitrophenyl)-4-oxothiazolidin-3-yl)-4-((1-phenyl-1H-tetrazol-5-yl)oxy)benzamide (27)

Yellow crystals (ethanol). Yield: 68.8%; mp: 127-129 ° C; IR (KBr, cm^{-1}): 3261 (NH), 1718 (C=O), 1676 (C=O), 1523,1491 (C-C)Ar, 1283 (C-O) ether. ;^{1}H NMR (δ, ppm, DMSO-d_6): 3.85 (d, 1H, *J* = 16.2 Hz, thiazolidinone CH_2), 4.03 (dd, 1H, *J* = 16.2, 1.8Hz, thiazolidinone CH_2), 6.08 (s, 1H,

thiazolidinone CH), 7.59 (tt, 1H, *J* = 7.2, 1.2Hz, Ar-H), 7.61 (d, 2H, *J* = 9Hz, Ar-H), 7.65 (t, 2H, *J* = 7.8Hz, Ar-H), 7.79 (d, 2H, *J* = 9Hz, Ar-H), 7.81 (d, 2H, *J* = 7.8Hz, Ar-H), 7.86 (d, 2H, *J* = 9Hz, Ar-H), 8.23 (d, 2H, *J* = 8.4Hz, Ar-H), 10.90 (s, 1H, CONH); ESI-MS *m/z* (Rel. Int.): 504.1 (M^+), 318.3 (25), 274.2 (45), 237 (100), 195 (85), 178 (35), 150 (45). Anal. Calcd for $C_{23}H_{17}N_7O_5S$ (503.49): C, 54.87; H, 3.40; N, 19.47; S, 6.37. Found: C, 54.70; H, 3.19; N, 19.19; S, 6.16.

4.1.5.9 N-(2-(2-methoxy-4-nitrophenyl)-4-oxothiazolidin-3-yl)-4-((1-phenyl-1H-tetrazol-5-yl)oxy)benzamide (28). Yellow crystals (ethanol). Yield: 40.37%; mp: 205-207 ° C; IR (KBr, cm^{-1}): 3216 (NH), 1716 (C=O), 1653 (C=O), 1531,1500 (C-C)Ar, 1292 (C-O) ether ;^{1}H NMR (δ, ppm, DMSO-d_6): 3.70 (d, 1H, *J* = 16.2Hz, thiazolidinone CH_2), 3.9 (dd, 1H, *J* = 16.2, 1.8Hz, thiazolidinone CH_2), 3.93 (s, 3H, OCH_3), 6.16 (s, 1H, thiazolidinone CH), 7.59 (tt, 1H, *J* = 7.2Hz, Ar-H), 7.62-7.66 (m, 4H, Ar-H), 7.75 (d, 1H, *J* = 8.4Hz, Ar-H), 7.79 (d, 1H, *J* = 2.4Hz, Ar-H), 7.81 (d, 2H, *J* = 7.8Hz, Ar-H), 7.88 (dd, 1H, *J* = 8.4, 2.4Hz, Ar-H), 7.89 (d, 2H, *J* = 8.4, Ar-H), 10.96 (s, 1H, CONH); ESI-MS *m/z* (Rel. Int.): 534.1 (M^+), 475.3 (19.17), 453.3 (100), 265.1 (20.55), 227.1 (28.77), 150.1 (39.73). Anal. Calcd for $C_{24}H_{19}N_7O_6S$ (533.52): C, 54.03; H, 3.59; N, 18.38; S, 6.01. Found: C, 53.80; H, 3.27; N, 18.09; S, 5.86.

4.1.5.10 N-(2-(furan-2-yl)-4-oxothiazolidin-3-yl)-4-((1-phenyl-1H-tetrazol-5-yl)oxy)benzamide (29)

White crystals (ethanol). Yield: 61%; mp: 187-189 ° C; IR (KBr, cm^{-1}): 3253 (NH), 1705 (C=O), 1666 (C=O), 1539, 1489 (C-C)Ar, 1280 (C-O)ether ;^{1}H NMR (δ, ppm, DMSO-d_6): 3.77 (d, 1H, *J* = 15.6Hz, thiazolidinone CH_2), 3.89 (dd, 1H, *J* = 15.6, 1.2Hz thiazolidinone CH_2), 5.98 (s, 1H, thiazolidinone CH), (dd, 1H, *J* = 9, 1.8Hz, furan CH), 6.60 (d, 1H, *J* = 3.6Hz, furan CH), 7.59 (tt, 1H, *J* = 7.2.1.8Hz, Ar-H), 7.63-7.67 (m, 4H, Ar-H), 7.73 (d, 1H, *J* = 0.6Hz furan CH), 7.83 (d, 2H, *J* = 8.4Hz, Ar-H), 7.91 (d, 2H, *J* = 9Hz, Ar-H), 10.93 (s, 1H, CONH); ESI-MS *m/z* (Rel. Int.): 449.1 (M^+), 375.1 (100), 237 (100). Anal. Calcd for $C_{21}H_{16}N_6O_4S$ (448.46): C, 56.24; H, 3.60; N, 18.74; S, 7.15. Found: C, 56.04; H, 3.39; N, 18.48; S, 6.88.

4.1.6 General synthesis of (±) 1-(2-(substituted-phenyl)-5-(4-((1-phenyl-1H-tetrazol-5-yl)oxy)phenyl)-1,3,4-oxadiazol-3(2H)-yl)ethan-1-one (30-40)

A mixture of appropriate aroylhydrazone **6-16** (5mmol) in acetic anhydride (10 mL) was heated under reflux for 2 h. The reaction mixture was allowed to cool, poured onto ice cold water and kept in refrigerator overnight. The separated solid was filtered, washed with water, dried and crystallized from appropriate solvent.

4.1.6.1 1-(2-(4-fluorophenyl)-5-(4-((1-phenyl-1H-tetrazol-5-yl)oxy)phenyl)-1,3,4-oxadiazol-3(2H)-yl)ethan-1-one (30). White crystals (ethanol). Yield: 36.36%; mp: 96-98 ° C; IR (KBr, cm^{-1}): 1665 (C=O), 1540,1501(C-C)Ar; ^{1}H NMR (δ, ppm, DMSO-d_6): 2.26 (s, 3H, CH_3 acetyl), 7.22 (s, 1H, oxadiazole CH), 7.27 (t, 2H, *J* = 8.4Hz, Ar-H), 7.55 (dd, 2H, *J* = 9, 5.4Hz, Ar-H), 7.60 (tt, 1H, *J* = 7.8, 1.8Hz, Ar-H), 7.66 (t, 2H, *J* = 7.8Hz, Ar-H), 7.69 (d, 2H, *J* = 8.4Hz, Ar-H), 7.84 (d, 2H, *J* = 7.8Hz, Ar-H), 7.95 (d, 2H, *J* = 9Hz, Ar-H); ESI-MS *m/z* (Rel. Int.): 445.2 (M^+), 403.3 (10), 385.1 (30), 321.2 (100), 293.3 (30), 235.2 (32). Anal. Calcd for $C_{23}H_{17}FN_6O_3$ (444.43): C, 62.16; H, 3.86; N, 18.91. Found: C, 61.90; H, 3.58; N, 19.22.

4.1.6.2 1-(2-(4-chlorophenyl)-5-(4-((1-phenyl-1H-tetrazol-5-yl)oxy)phenyl)-1,3,4-oxadiazol-3(2H)-yl)ethan-1-one (31). White crystals (ethanol). Yield: 34.5%; mp: 112-114 ° C; IR (KBr, cm^{-1}): 1663 (C=O), 1547,1504 (C-C)Ar,;^{1}H NMR (δ, ppm, $CDCl_3$): 2.36 (s, 3H, CH_3 acetyl), 7.07 (s, 1H, oxadiazole CH), 7.38 (d, 2H, *J* = 8.4Hz, Ar-H), 7.43 (d, 2H, *J* = 8.4Hz, Ar-H), 7.53-7.57 (m, 3H, Ar-H), 7.61 (t, 2H, *J* = 8.4Hz, Ar-H), 7.78 (d, 2H, *J* = 8.4Hz, Ar-H), 7.98 (d, 2H, *J* = 9Hz, Ar-H). ; ESI-MS *m/z* (Rel. Int.): 461.2 (M^+), 401.2 (40), 321.2 (100), 393.2 (40), 235.2 (40). Anal. Calcd for $C_{23}H_{17}ClN_6O_3$ (460.88): C, 59.94; H, 3.72; N, 18.24. Found: C, 60.19; H, 3.57; N, 18.18.

4.1.6.3 1-(2-(4-Bromophenyl)-5-(4-((1-phenyl-1H-tetrazol-5-yl)oxy)phenyl)-1,3,4-oxadiazol-3(2H)-yl)ethan-1-one (32). White crystals (ethanol). Yield: 40.36%; mp: 102-104 ° C; IR (KBr, cm^{-1}): 1661 (C=O), 1545,1503 (C-C)Ar,;^{1}H NMR (δ, ppm, DMSO-d_6): ($CDCl_3$): 2.35 (s, 3H, CH_3 acetyl), 7.05 (s, 1H, oxadiazole CH), 7.36 (d, 2H, *J* = 9Hz, Ar-H), 7.53-7.57 (m, 5H, Ar-H), 7.60 (t, 2H, *J* = 8.4Hz, Ar-H), 7.78 (d, 2H, *J* = 8.4Hz, Ar-H), 7.98 (d, 2H, *J* = 9Hz, Ar-H). ; ESI-MS *m/z* (Rel. Int.): 505.3 (M), 445.1 (30), 321.2 (100), 293.2 (32), 235.2 (55). Anal. Calcd for $C_{23}H_{17}BrN_6O_3$ (505.33): C, 54.67; H, 3.39; N, 16.63. Found: C, 54.38; H, 3.40; N, 16.90.

4.1.6.4 1-(5-(4-((1-phenyl-1H-tetrazol-5-yl)oxy) phenyl)-2-(p-tolyl)-1,3,4-oxadiazol-3(2H)-yl)ethan-1-one (33). White crystals (ethyl acetate). Yield: 43.2%; mp: 130-132 ° C; ^{1}H NMR (δ, ppm, DMSO-d_6): ($CDCl_3$): 2.12 (s, 3H, CH_3 acetyl), 2.44 (s, 3H, CH_3 tolualdehyde), 7.32 (d, 2H, *J* = 8.4Hz, Ar-H),7.48 (d, 2H, *J* = 8.4Hz, Ar-H), 7.51-7.55 (m, 2H, Ar-H & oxadiazole CH), 7.58 (t, 2H, *J* = 7.8Hz, Ar-H), 7.76-7.78 (m, 4H, Ar-H), 7.93 (d, 2H, *J* = 8.4Hz, Ar-H); ESI-MS *m/z* (Rel. Int.): 441.3 (M^+), 381.1 (33.33), 321.2 (100), 293.2 (33.33), 235.2 (32).

4.1.6.5 2-(4-((1-phenyl-1H-tetrazol-5-yl)oxy) phenyl)-5-(p-tolyl)-1,3,4-oxadiazole (33')

White crystals (ethanol). Yield: 10%; mp: 130-132 ° C; ^{1}H NMR (δ, ppm, DMSO-d_6): ($CDCl_3$): 2.45 (s,

3H, CH3 tolualdehyde), 7.35 (d, 2H, *J* = 7.8Hz, Ar-H), 7.55 (tt, 1H, *J* = 7.2, 1.2Hz, Ar-H), 7.62 (t, 2H, *J* = 8.4Hz, Ar-H), 7.65 (d, 2H, *J* = 9Hz, Ar-H), 7.80 (d, 2H, *J* = 8.4Hz, Ar-H), 8.03 (d, 2H, *J* = 8.4Hz, Ar-H), 8.24 (d, 2H, *J* = 9Hz, Ar-H); ESI-MS *m/z* (Rel. Int.): 397.1 (M^+), 369.1 (100), 326.1 (70), 252 (10). Anal. Calcd for $C_{22}H_{16}N_6O_2$ (396.41): N, 21.20. Found: C, N, 21.05.

4.1.6.6 1-(2-(4-methoxyphenyl)-5-(4-((1-phenyl-1H-tetrazol-5-yl)oxy)phenyl)-1,3,4-oxadiazol-3(2H)-yl)ethan-1-one (34)

White crystals (ethanol). Yield: 27%; mp: 119-121 º C; IR (KBr, cm^{-1}): 1660 (C=O), 1540, 1544, 1504 (C-C) Ar ;^{1}H NMR (δ, ppm, DMSO-d_6): ($CDCl_3$): 2.12 (s, 3H, CH_3 acetyl), 3.89 (s, 3H, OCH_3), 7.00 (d, 2H, *J* = 8.4Hz, Ar-H), 7.05 (s, 1H oxadiazole CH), 7.49 (d, 2H, *J* = 9Hz, Ar-H), 7.52 (tt, 1H, *J* = 7.8, 1.2Hz, Ar-H), 7.59 (t, 2H, *J* = 8.4Hz, Ar-H), 7.78 (d, 2H, *J* = 7.8Hz, Ar-H), 7.84 (d, 2H, *J* = 8.4Hz, Ar-H), 7.92 (d, 2H, *J* = 8.4Hz, Ar-H); ESI-MS *m/z* (Rel. Int.): 457.2 (M+), 397.1 (20), 307.2 (100).Anal. Calcd for $C_{24}H_{20}N_6O_4$ (456.46): C, 63.15; H, 4.42; N, 18.41. Found: C, 63.07; H, 4.44; N, 18.65.

4.1.6.7 4-(3-acetyl-5-(4-((1-phenyl-1H-tetrazol-5-yl)oxy)phenyl)-2,3-dihydro-1,3,4-oxadiazol-2-yl)benzoic acid (35)

White crystals (ethanol). Yield: 49.9%; mp: 136-138 º C; IR (KBr, cm^{-1}): 1718 (C=O), 1633 (C=O) 1537 , 1502(C-C)Ar;^{1}H NMR (δ, ppm, DMSO-d_6): (DMSO-d_6): 2.27 (s, 3H, CH_3 acetyl), 7.27 (s, 1H, oxadiazole CH), 7.59 (tt, 1H, *J* = 7.8, 1.2Hz, Ar-H), 7.61 (d, 2H, *J* = 8.4Hz,Ar-H), 7.65 (t, 2H, *J* = 7.8Hz, Ar-H), 7.69 (d, 2H, *J* = 9Hz, Ar-H), 7.84 (d, 2H, *J* = 7.8Hz, Ar-H), 7.96 (d, 2H, *J* = 9Hz, Ar-H), 8.00 (d, 2H, *J* = 8.4Hz, Ar-H), 13.12 (s, 1H, COOH); ESI-MS *m/z* (Rel. Int.): 471.1 (M^+), 410.8 (25), 320.9 (100), 235.1 (25).Anal. Calcd for $C_{24}H_{18}N_6O_5$ (470.45): C, 61.27; H, 3.86; N, 17.86. Found: C, 61.65; H, 4.15; N, 16.93.

4.1.6.8 1-(2-(2,4-difluorophenyl)-5-(4-((1-phenyl-1H-tetrazol-5-yl)oxy)phenyl)-1,3,4-oxadiazol-3(2H)-yl)ethan-1-one (36)

White crystals (ethanol). Yield: 58.6%; mp: 127-129 º C; IR (KBr, cm^{-1}): 1667 (C=O), 1538,1498(C-C)Ar;^{1}H NMR (δ, ppm, DMSO-d_6): ($CDCl_3$): 2.37 (s, 3H, CH_3 acetyl), 6.87 (td, 1H, *J* = 9,2.4Hz, Ar-H), 6.91 (td, 1H, *J* = 8.4, 2.4Hz, Ar-H), 7.21 (s, 1H, oxadiazole CH), 7.39 (dd, 1H, *J* = 7.8, 1.8Hz, Ar-H), 7.53-7.55 (m, 3H, Ar-H), 7.60 (t, 2H, *J* = 8.4Hz), 7.78 (d, 2H, *J* = 7.8Hz, Ar-H), 7.96 (d, 2H, *J* = 9Hz, Ar-H); ESI-MS *m/z* (Rel. Int.): 463.1 (M^+), 421.1 (28.57), 403.1 (64), 321.1 (100), 293.1 (50), 235 (64.2). Anal. Calcd for $C_{23}H_{16}F_2N_6O_3$ (462.42): N, 18.17. Found: N, 18.25.

4.1.6.9 1-(2-(3,4-dimethoxyphenyl)-5-(4-((1-phenyl-1H-tetrazol-5-yl)oxy)phenyl)-1,3,4-oxadiazol-3(2H)-yl)ethan-1-one (37)

White crystals (ethanol). Yield: 52%; mp: 134-136 º C; IR (KBr, cm^{-1}): 1592 (C=O), 1532,1491 (C-C)Ar.;^{1}H NMR (δ, ppm, DMSO-d_6): ($CDCl_3$): 2.13 (s, 3H, CH_3 acetyl), 3.87 (s, 3H, OCH_3), 3.94 (s, 3H, OCH_3), 6.98 (d, 2H, *J* = 8.4Hz, Ar-H), 6.99 (d, 1H, *J* = 8.4Hz, Ar-H), 7.04 (s, 1H, oxadiazole CH), 7.05 (d,1H, *J* = 1.8Hz, Ar-H), 7.46 (dd, 1H, *J* = 1.8Hz,Ar-H), 7.51-7.66 (m, 5H, Ar-H), 8.23 (d, 2H, *J* = 9Hz, Ar-H). ; ESI-MS *m/z* (Rel. Int.): 487.1 (M^+), 445.1 (100), 307.1 (100).Anal. Calcd for $C_{25}H_{22}N_6O_5$ (486.49): C, 61.72; H, 4.56; N, 17.28. Found: C, 61.64; H, 4.38; N, 17.38.

4.1.6.10 1-(2-(4-nitrophenyl)-5-(4-((1-phenyl-1H-tetrazol-5-yl)oxy)phenyl)-1,3,4-oxadiazol-3(2H)-yl)ethan-1-one (38)

Yellow crystals (ethanol). Yield: 68%; mp: 177-179 º C; IR (KBr, cm^{-1}): 1671 (C=O), 1526,1495 (C-C)Ar.;^{1}H NMR (δ, ppm, DMSO-d_6): 2.27 (s, 3H, CH_3 acetyl), 7.36 (s, 1H, oxadiazole CH), 7.60 (tt, 1H, *J* = 7.2, 1.2Hz, Ar-H), 7.66 (tt, 2H, *J* = 8.4Hz, Ar-H), 7.7 (d, 2H, *J* = 9Hz, Ar-H), 7.79 (d, 2H, *J* = 9Hz, Ar-H), 7.84 (d, 2H, *J* = 7.8Hz, Ar-H), 7.97 (d, 2H, *J* = 9Hz, Ar-H), 8.28 (d, 2H, *J* = 7.2, Ar-H). ; ESI-MS *m/z* (Rel. Int.): 494.1(M+Na), 512 (44.44), 494.2 (100), 452 (77.77), 424.1 (100) Anal. Calcd for $C_{23}H_{17}N_7O_5$ (471.43): C, 58.60; H, 3.63; N, 20.80. Found: C, 58.85; H, 3.38; N, 20.73.

4.1.6.11 1-(2-(2-methoxy-4-nitrophenyl)-5-(4-((1-phenyl-1H-tetrazol-5-yl)oxy)phenyl)-1,3,4-oxadiazol-3(2H)-yl)ethan-1-one (39)

Yellow crystals (ethanol). Yield: 56%; mp: 145-147 º C; IR (KBr, cm^{-1}): 1662 (C=O), 1527(C-C)Ar.;^{1}H NMR (δ, ppm, DMSO-d_6): (DMSO-d_6): 2.68 (s, 3H, CH_3 acetyl), 3.94 (s, 3H, OCH_3), 7.35 (s, 1H, oxadiazole CH), 7.59 (tt, 1H, *J* = 7.2, 1.2Hz, Ar-H), 7.62-7.69 (m, 5H, Ar-H), 7.83-7.86 (m, 4H, Ar-H), 7.91 (d, 2H, *J* = 9Hz, Ar-H); ESI-MS *m/z* (Rel. Int.): 502.1 (M^+), 460.1 (100), 432.1 (25.92), 315 (11.11), 293.1 (11.11), 150.1 (14.81).Anal. Calcd for $C_{24}H_{19}N_7O_6$ (501.46): C, 57.49; H, 3.82; N, 19.55. Found: C, 57.48; H, 3.89; N, 19.29.

4.1.6.12 1-(2-(4-hydroxyphenyl)-5-(4-((1-phenyl-1H-tetrazol-5-yl)oxy)phenyl)-1,3,4-oxadiazol-3(2H)-yl)ethan-1-one (40)

White crystals (ethyl acetate/methanol). Yield: 9%; mp: 155-157 º C; IR (KBr, cm^{-1}): 3200 (OH), 1667 (C=O), 1539,1501 (C-C)Ar; ^{1}H NMR (δ, ppm, DMSO-d_6): (DMSO-d_6): 2.27 (s, 3H, CH_3 acetyl), 7.19 (d, 2H, *J* = 8.4Hz, Ar-H), 7.22 (s, 1H, oxadiazole CH), 7.52 (d, 2H, *J* = 9Hz, Ar-H), 7.58-7.61 (m, 1H, Ar-H), 7.64-7.67 (t, 2H, *J* = 7.8Hz, Ar-H) 7.69 (d, 2H, *J* = 9Hz, Ar-H), 7.84 (d, 2H, *J* = 9Hz, Ar-H), 7.96 (d, 2H, *J* = 9Hz, Ar-H), 11.94 (s, 1H, OH); ESI-MS *m/z* (Rel. Int.): 443.3 (M^+), 415.3 (10), 373.3 (100), 237.2 (10).Anal. Calcd for $C_{23}H_{18}N_6O_4$ (442.44): C, 62.44; H, 4.10; N, 19.00. Found: C, 62.57; H, 4.00; N, 18.96.

4.2 Biological activity

4.2.1 Antimicrobial activity

4.2.1.1 Inhibition zone measurement

Stock solutions of tested compounds were prepared in molar concentrations [44] of (10 µmol/mL in 80% DMSO). The organisms used for study includes both Gram-positive (*S. epidermidis*, *B. subtilis*), Gram-negative (*E. coli*, *P. mirabilis*) and yeast like fungi (*C. albicans*). Cultures for experiments were prepared and used for inoculation of media. Mueller-Hinton agar and Sabouraud Dextrose media were used for bacteria and fungi, respectively.

The inoculated media was transferred to petri dishes and allowed to cool and solidify at room temperature. Wells (7mm in diameter) were then made in the solidified agar using a sterile cork borer and loaded with 180µL (10 µmol/mL) of each of the tested compound. Plates were then incubated at 37**°** C for 24h for bacteria and for 48h for fungi. DMSO (80%) was used as a negative control. Ciprofloxacin (1 µg/mL) and ketoconazole (50 µg/mL) were used as standards for antibacterial and antifungal activity, respectively. After incubation time, antimicrobial activity was evaluated as a measure of the average diameter of zone of inhibition against the test organisms and compared to that of the reference standards.

4.2.1.2 Minimal inhibitory concentration (MIC) measurement

Minimal inhibitory concentrations (MIC) of the most active compounds were measured using two-fold broth microdilution method[41, 42]. Test was carried out using Muller-Hinton Broth and Sabouraud Liquid Medium for determination of antibacterial and antifungal activity, respectively. Stock solutions of the tested compounds were prepared in DMSO at concentration of 1600 µg/mL to obtain final concentrations in the range of (80, 40, 20,…0.156µg/mL). Ciprofloxacin and ketoconazole were also prepared by two-fold serial dilution with starting concentrations at 5 µg/mL and 50 µg/mL, respectively. The microorganisms' suspensions at 10^6 CFU/mL concentration were inoculated to the corresponding wells. The last two wells of each column were used as positive and negative controls, respectively. The 96-microwell plates were incubated at 37ºC for 24h. The MICs of the compound were recorded as the lowest concentration where no viability was observed in the wells of 96-microwell plates after incubation for 24 h.

4.2.1.3 Determination of MIC_{90} of propyl paraben 2 and its tetrazole derivative 3 as antifungal agents

MIC_{90} of propyl paraben **(2)** and its tetrazole derivative **(3)** was determined by a whole-cell assay in a 96-well microtitre format. Candida cells with an initial cell optical density at 600 nm (OD_{600}) of 0.001 in Sabouraud dextrose broth (SDB, Difco) medium were inoculated with serial dilutions (0-320 µg/mL) of the two compounds in the SDB medium. Growth inhibition was measured by determining the OD_{600} after 48 h. The lowest concentration at which a compound led to an OD_{600} of ≤ 0.010 was determined as the (MFC) minimum fungicidal concentration of the compound; the concentration that causes 90% growth inhibition (IC_{90}) of the compound was determined[45].

4.2.2 Anticancer activity

4.2.2.1 Methodology

The cytotoxicity of test compounds were tested against hepatocellular carcinoma cells (HuH-7) and colorectal carcinoma cells (CaCo-2) by SRB assay as previously described [46]. Exponentially growing cells were collected using 0.25% Trypsin-EDTA and plated in 96-well plates at 1000-2000 cells/well. Cells were exposed to serial concentrations of test compounds for 72 h and subsequently fixed with TCA (10%) for 1 h at 4 ºC. After several washings, cells were exposed to 0.4% SRB solution for 10 min in dark place and subsequently washed with 1% glacial acetic acid. After drying overnight, Tris-HCl was used to dissolve the SRB-stained cells and color intensity was measured at 540 nm.

4.2.2.2 Data analysis

The dose response curve of compounds was analyzed using E_{max} model.

$$\%\,\text{Cell viability} = (100 - R) \times \left(1 - \frac{[D]^m}{K_d{}^m + [D]^m}\right) + R$$

Where R is the residual unaffected fraction (the resistance fraction), [D] is the drug concentration used, K_d is the drug concentration that produces a 50% reduction of the maximum inhibition rate and m is a Hill-type coefficient. IC_{50} was defined as the drug concentration required to reduce absorbance to 50% of that of the control (i.e., $K_d = IC_{50}$ when R=0 and E_{max} =100-R) [47].

4.3 Molecular modeling

Propyl paraben **2** and its tetrazole derivative **3** were drawn on ChemSketch [48], Advanced Chemistry Development, Inc. (ACD/Labs) Freeware and saved as sdf file. The file was optimized with Prepare Ligand application in SYBYL- X 2.1[43] program as a Quick 3D Job. The generated 3D structures were further optimized using Minimize Energy computational tool with the following adjusted parameters (Method: Powell, Initial Optimization: None; Termination: Gradient at 0.0005 kcal/mol; Max Iterations: 10000; Force Filed: MMFF94s; charges: MMFF94; dielectric Function: Constant; Rest of Parameters: SYBYL- X Default). The energy minimized compounds were then aligned using alignment tool in SYBYL- X through fitting atom

approach. Lipophilicity was calculated and Connolly surface was created for the two compounds and colored according to LP, EP and HB properties. For measuring the bulkiness of phenolic **OH** and 1-phenyl tetrazole moiety in propyl paraben **2** and its tetrazole derivative **3**, respectively, the two compounds were drawn in MarvinSketch [49] freeware, then their total atomic steric hindrance was calculated as a measure of their bulkiness.

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