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Design, synthesis, *in vitro* antimicrobial and cytotoxic evaluation of novel 1,2,3-selena/thiadiazolyltetrazole derivatives†

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A new series of 2,5-disubstituted tetrazoles and 1,2,3-selena/thiadiazolyl-2*H*-tetrazole derivatives were synthesized and evaluated for their *in vitro* antimicrobial and cytotoxic activities against pathogenic strains. The preliminary screening results indicated that some of the compounds demonstrated moderate to good antibacterial and antifungal activities, comparable to the first-line drugs. Compounds **6a** and **6b** exhibited pronounced cytotoxicity against Hep G2 and MCF-7 cell lines. The chemical structures of all the newly synthesized compounds were characterized by means of spectral and elemental analyses.

1. Introduction

Selenium containing heterocyclic systems have attracted continuing interest over the years because of their varied potential pharmaceutical properties.[1–4] Among selenium containing heterocyclic compounds, 1,2,3-selenadiazoles and their derivatives exhibit antibacterial,[5] antifungal,[1,2,6] antitumor,[7] antiaflatoxigenic,[8] antihemostatic,[9] and insecticidal activities.[10] Some of them are also used as immunomodifiers, cytokine inducers, enzyme inhibitors, virucides,[11] and chemotherapeutic agents.[12] It has been found that the introduction of a 1,2,3-selenadiazole ring to molecules of known biologically active compounds changes their activities and in some cases leads to an increase in their biological action.[13]

Compounds with a 1,2,3-thiadiazole moiety have been found to exhibit various pharmacological properties like antibacterial,[14] antiHIV,[15] antiviral, fungicidal,[16] insecticidal,[17] and herbicidal activity.[18] Some of the 1,2,3-thiadiazoles possesses platelet aggregation inhibitory activity in humans[19] and some of them are used in agricultural chemistry as inducers of systemic acquired resistance (SAR) in plants.[20]

Tetrazole is an increasingly popular functionality and represents an important class of heterocycles which exhibit a wide range of applications in medicinal as well as synthetic chemistry.[21] Tetrazoles can be used as isosteric replacements for carboxylic acids in drug design,[22] in coordination chemistry as ligands, in material applications including rocket propellants,[23] explosives,[24] and in agriculture and pharmaceuticals.[25] Tetrazole derivates are well known as compounds with a high level of biological activity.[26–32] An advantage of tetrazolic acids over carboxylic acids is that they are resistant to many biological metabolic degradation pathways. It was also noticed that toxic properties of a drug can decrease through the introduction of a tetrazole ring into the molecule.[22] Furthermore, tetrazole moieties are important synthons in synthetic organic chemistry.[33]

Prompted by these observations, it was speculated that designing and synthesis of a new series of tetrazole embedded selenadiazole and thiadiazole derivatives would be worthwhile. Furthermore, after extensive literature search, it was observed that, till date no effort has been made to combine these vital moieties as a single molecular scaffold and to investigate their antimicrobial and cytotoxic studies. So, keeping this observation in view and in continuation of our research on the synthesis of biologically significant heterocycles,[34] we herein, report the synthesis and bioactivity of some new 1,2,3-selena/thiadiazolyl-2*H*-tetrazoles which entailed the union of two biologically active nuclei, *viz.*, tetrazole and selena/thiadiazole. The newly synthesized compounds were screened for their *in vitro* antimicrobial and cytotoxic activities.

2. Chemistry

The reaction sequences employed for synthesis of intermediates and target compounds are shown in Scheme 1. The starting materials used in the present study namely 5-aryl tetrazoles **2a–d** were prepared following a previously reported literature procedure.[35] Alkylation of these tetrazoles with 4-chloro/methoxy phenacyl bromide in refluxing acetone containing anhydrous potassium carbonate afforded the ethanone derivatives **3a–h** in good yields. A literature survey

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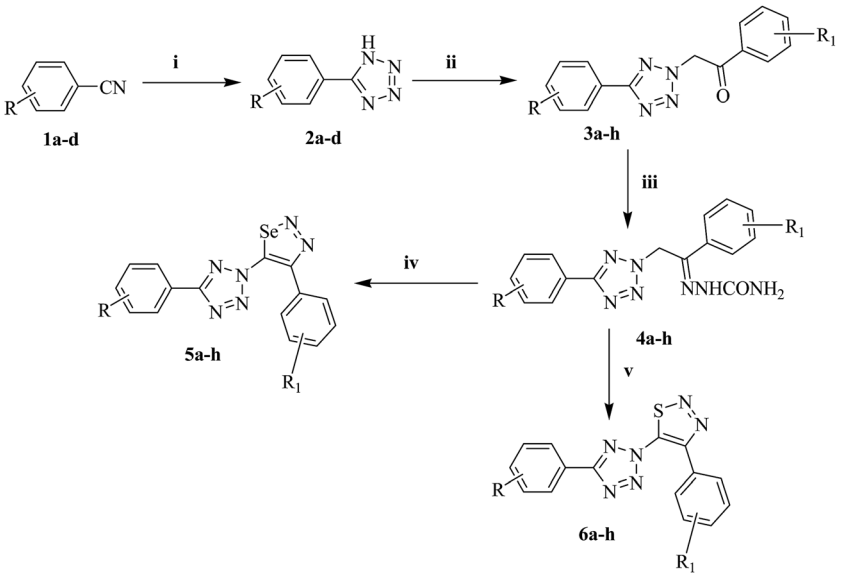

Scheme 1 Schematic representation for the synthesis of 1,2,3-selena/thaidiazolyltetrazoles. Reagent and conditions: (i) NaN_3, DMF, NH_4Cl, 120 °C, 7 h; (ii) K_2CO_3, 4-substituted phenacyl bromide, acetone, reflux, 8 h; (iii) $NH_2NHCONH_2 \cdot HCl$, AcONa, MeOH, reflux, 5 h; (iv) SeO_2, AcOH, reflux, 6 h; (v) $SOCl_2$, 0–5 °C, 30 min, RT, 5 h. (a) R = H, R_1 = 4-Cl; (b) R = 4-Cl, R_1 = 4-Cl; (c) R = 2-Cl, R_1 = 4-Cl; (d) R = 4-CH_3, R_1 = 4-Cl; (e) R = H, R_1 = 4-OCH_3; (f) R = 4-Cl, R_1 = 4-OCH_3; (g) R = 2-Cl, R_1 = 4-OCH_3; (h) R = 4-CH_3, R_1 = 4-OCH_3.

revealed that alkylation of 5-substituted tetrazoles affords generally mixtures of 1,5- and 2,5-regioisomers,[30,36,37] which could be separated by silica gel chromatography.[38,39] However in the present study, compounds **3a–h** were purified by fractional crystallization from diethyl ether followed by crystallization from methanol. Furthermore, ^{1}H NMR studies of disubstituted tetrazoles have shown that protons of CH_2 group attached to N_1 of 1,5-disubstituted tetrazoles are more shielded than the corresponding protons of 2,5-disubstituted tetrazoles.[40–42] Moreover, their ^{13}C NMR spectra have shown that the tetrazole-C5 carbon atom of 1,5-disubstituted tetrazoles is more shielded than the corresponding carbon of the 2,5-disubstituted tetrazoles.[40–42] Based on these reported observations, compounds **3a–h** were designated as 2,5-disubstituted tetrazoles as evidenced from its ^{1}H NMR that exhibited a signal for N–CH_2 protons at δ 6.74–6.66 ppm, whereas its ^{13}C NMR spectrum showed a signal for tetrazole-C5 atom at δ 164.34–164.15 ppm. These *N*-substituted tetrazoles **3a–h** on treatment with semicarbazide hydrochloride to obtain corresponding semicarbazone derivatives **4a–h** which were then subjected to reaction with selenium dioxide and thionyl chloride to furnish the corresponding selena and thiadiazolyltetrazoles (**5a–h** and **6a–h**) in good yields. Structures of all the newly synthesized compounds were established on the basis of elemental analyses, IR, mass, ^{1}H NMR and ^{13}C NMR data. The synthesized compounds were also screened for their antimicrobial and cytotoxic activities.

3. Biological activity

3.1. Antimicrobial activity

The *in vitro* antimicrobial activity of the newly synthesized compounds was done using agar disc-diffusion method.[43] The antibacterial activity of the synthesized compounds **3a–h**, **4a–h** and **5a–h** was tested against the Gram-positive bacteria such as *Bacillus subtilis*, *Staphylococcus aureus*, and the Gram-negative bacteria *i.e. Pseudomonas aeuroginosa*, *Escherichia coli* using nutrient agar medium. The antifungal activity of the compounds was screened against *Candia albicans* and *Aspergillus niger* using potato dextrose agar medium. The minimum inhibitory concentration (MIC) was performed using microdilution susceptibility method.[44] Ciprofloxacin was used as a standard antibacterial drug and fluconazole was used as standard antifungal drug. The observed data on the antimicrobial activity of the tested compounds and control drugs are given in Tables 1 and 2.

3.2. Cytotoxicity

The cancer cell lines Hep G2 and MCF-7, which were used in the present study, were purchased from National Centre for Cell Sciences, Pune, India. The cell lines were grown aseptically using Dulbecco's modified eagles medium (DMEM) enriched with 10% fetal bovine serum and 100 U mL^{-1} penicillin, 75 U mL^{-1} streptomycin at 37 °C, pH 7.2 and 5% CO_2 atmosphere. After attaining 80% confluence, the cells were trypsinized with 0.25% trypsin–EDTA and diluted with media to a fixed number of cells (10^4 cells per well in 100 μL culture medium).

4. Results and discussion

4.1. Chemistry

The IR spectrum of compound **3a** exhibited characteristic absorption band around 1698 cm^{-1} accounting for carbonyl group. The absorption bands observed at 1589 and 1451 cm^{-1} attributed to tetrazole ring. The M + 1 peak at *m*/*z* 299 in mass spectrum of compound **3a** found to be in conformity with its molecular formula of the assigned structure. The ^{1}H NMR

Table 1 Antimicrobial activity of synthesized compounds **3a–h**, **4a–h** and **5a–h**

		Zone of inhibition (mm)					
		Gram +ve bacteria		Gram −ve bacteria		Fungi	
Compound	Conc. (μg per disc)	*B. subtilis*	*S. aureus*	*P. aeuroginosa*	*E. coli*	*C. albicans*	*A. niger*
3a	100	11	10	12	11	14	15
	200	13	12	14	13	15	15
3b	100	11	26	25	12	14	23
	200	14	28	27	12	16	26
3c	100	26	15	10	14	17	17
	200	28	17	14	16	18	19
3d	100	15	16	15	18	16	14
	200	19	19	18	18	18	16
3e	100	11	12	15	11	12	14
	200	14	16	18	13	15	15
3f	100	16	10	16	26	11	10
	200	18	12	18	29	13	13
3g	100	13	11	16	24	17	15
	200	15	14	18	27	19	17
3h	100	12	16	13	14	18	16
	200	15	17	15	16	18	18
4a	100	10	11	11	11	14	13
	200	12	13	12	14	16	17
4b	100	16	30	31	12	32	10
	200	18	34	34	15	34	13
4c	100	25	32	30	10	18	31
	200	28	35	33	14	18	34
4d	100	12	11	10	11	12	30
	200	16	14	15	14	17	33
4e	100	11	12	14	15	11	13
	200	13	15	14	16	15	16
4f	100	18	17	16	27	12	14
	200	20	19	18	29	16	17
4g	100	16	15	18	25	14	15
	200	19	18	18	28	19	18
4h	100	15	18	16	15	16	15
	200	17	18	18	17	16	18
5a	100	12	30	31	14	15	13
	200	15	33	35	18	17	16
5b	100	34	32	31	42	13	35
	200	40	34	35	44	17	39
5c	100	13	32	31	41	36	16
	200	19	34	33	43	40	17
5d	100	15	24	23	14	23	12
	200	18	28	26	19	27	15
5e	100	11	15	16	15	13	14
	200	14	18	16	18	15	16
5f	100	26	18	17	19	23	11
	200	29	20	22	22	27	13
5g	100	24	12	13	15	13	22
	200	28	15	16	17	15	26
5h	100	13	14	15	17	17	16
	200	15	17	18	19	19	18
Ciprofloxacin	100	35	38	37	42		
	200	41	44	42	45		
Fluconazole	100					38	36
	200					42	39

spectrum of the compound **3a** showed singlet at δ 6.74 ppm corresponding to characteristic CH_2 proton. ^{13}C NMR spectrum of **3a** exhibited a distinctive signal at δ 58.99 ppm for carbon of CH_2, δ 164.34 for tetrazole carbon and δ 190.45 ppm for carbonyl (C=O) carbon. In IR spectrum of compound **4a**, the appearance of bands at 3423, 3307 and 3248 cm^{-1} ($-NH_2$ & –NH) accounted for the formation of semicarbazone. The M + 1 base peak at m/z 356 confirmed the structure of compound **4a**. ^1H

Table 2 Minimum inhibitory concentration (MIC, μg mL^{-1}) of synthesized compounds **3a–h**, **4a–h** and **5a–h**

	Gram +ve bacteria		Gram −ve bacteria		Fungi	
Compound	*B. subtilis*	*S. aureus*	*P. aeuroginosa*	*E. coli*	*C. albicans*	*A. niger*
3a	400	400	400	400	400	400
3b	400	100	200	400	400	100
3c	200	400	400	400	400	400
3d	400	400	400	400	400	400
3e	400	400	400	400	400	400
3f	400	400	400	200	400	400
3g	400	400	400	100	400	400
3h	400	400	400	400	400	400
4a	400	400	400	400	400	400
4b	400	50	25	400	25	400
4c	100	25	25	400	400	50
4d	400	400	400	400	400	50
4e	400	400	400	400	400	400
4f	400	400	400	100	400	400
4g	400	400	400	100	400	400
4h	400	400	400	400	400	400
5a	400	50	50	400	400	400
5b	**6.25**	50	25	**6.25**	400	**6.25**
5c	400	25	50	**6.25**	**6.25**	400
5d	400	200	100	400	200	400
5e	400	400	400	400	400	400
5f	100	400	400	400	100	400
5g	200	400	400	400	400	100
5h	400	400	400	400	400	400
Ciprofloxacin	6.25	6.25	6.25	6.25		
Fluconazole					6.25	6.25

NMR spectrum of **4a** displayed one singlet at δ 6.18 ppm corresponding to the proton of CH_2, a broad singlet at 6.82 ppm related to the NH_2 group and another one singlet at 10.38 ppm assignable for NH proton. The ^{13}C NMR of **4a** exhibited peaks at δ 46.53, 156.70, and 164.14 ppm corresponding to carbons of CH_2, tetrazole and amide ($-CONH_2$) respectively. The disappearance of CO, NH and NH_2 absorptions in IR spectrum of **5a** accounted for selenadiazole ring formation. The structure of compound **5a** was further confirmed by ^1H NMR and ^{13}C NMR. In ^1H NMR spectrum, the absence of signals at δ 6.18, 6.82 and 10.38 ppm due to the protons of CH_2, NH_2 and NH respectively confirmed the formation of **5a**. In ^{13}C NMR, the appearance of signals at δ 174.45 and 176.79 ppm due to C_4 and C_5 of selenadiazole ring confirmed the cyclization of semicacarbazone **4a** into 1,2,3-selenadiazole **5a**. The compound **5a** was well confirmed by its mass spectrum which showed M + 1 peak at *m*/*z* 388, 100% which is in agreement with the molecular formula $C_{15}H_9ClN_6Se$. The formation of cyclized product **6a** was established using IR, ^1H NMR and ^{13}C NMR spectra. In IR spectrum, the absence of bands due to NH_2, NH and C=O groups accounted for the formation of thiadiazole ring. In addition, ^1H NMR spectra revealed the disappearance of peaks due to protons of CH_2, NH_2 and NH. The formation of thiadiazole ring was further supported by ^{13}C NMR spectra. In ^{13}C NMR spectra, the absence of signals due to carbons of CH_2 and C=O as well as appearance of signals around δ 173.37 and 175.63 ppm assignable to C_4 and C_5 of thiadiazole ring, confirmed the cyclization of semicacarbazone **4a** into 1,2,3-thiadiazole **6a**. The formation of **6a** was further confirmed by its mass spectrum, which showed M + 1 peak at *m*/*z* 341, 100% which is in agreement with their molecular weight. The elemental analysis values are in good agreement with theoretical data. Similarly, all the compounds were characterized on the basis of spectral data and elemental analysis. A full characterization details were provided in Experimental section.

4.2. Biological results

The results of *in vitro* antimicrobial studies of the compounds **3a–h**, **4a–h**, **5a–h** and the standard drugs are represented in Tables 1 and 2 The screening results indicated that the most of the synthesized compounds showed significant antimicrobial activity from moderate to good activity.

The results of antibacterial activity in Table 2 showed that intermediates **3a–h** and **4a–h** exhibited weak or no antibacterial efficacy against all the bacterial strains. In addition, some of the chloro substituted compounds among **4a–h** exerted relatively better activities in inhibiting the growth of tested strains in comparison with the compounds **3a–h**. Moreover, the bioactivity of target compounds **5a–h** were improved by the introduction of selenadiazole ring into the substituted tetrazolylsemicarbazones, which indicated that the selenadiazole ring was helpful for enhancing antibacterial activity. The types of substituents on the phenyl ring of tetrazole and selenadiazole ring exhibited no significant effects on biological

activity. Moreover, substituents with chlorine on the 4-position of phenyl ring of selenadiazole moiety (**5b** and **5c**) gave excellent activity against the tested bacterial strains than others and comparable activity with standard drug ciprofloxacin (MIC: 6.25 μg mL^{-1}). Hence, among all the synthesized compounds, only **5b** exhibited excellent activity against *E. coli* (ZI: 42, 44 mm; MIC: 6.25 μg mL^{-1}) and *B. subtilis* (ZI: 34, 40 mm; MIC: 6.25 μg mL^{-1}) and compound **5c** also showed excellent activity against *E. coli* (ZI: 41, 43 mm; MIC: 6.25 μg mL^{-1}). Compounds **4b**, **4c**, **5a**, **5b**, and **5c** were displayed good activity against *S. aureus* and *P. aeuroginosa*. Compounds **3c**, **4c**, **5f**, and **5g** were showed moderate activity towards *B. subtilis*. Compounds **3f**, **3g**, **4f**, and **4g** exhibited moderate activity against *E. coli* and compounds **3b** and **5d** showed moderate activity towards *S. aureus* and *P. aeuroginosa*.

The zone of inhibition (ZI) and MIC values for the *in vitro* antifungal studies of the compounds **3a–h**, **4a–h**, **5a–h** and the standard are also given in Tables 1 and 2. The antifungal evaluation *in vitro* revealed that the activities of the compounds were relatively weak in comparison to their antibacterial activities. The chlorobenzyl substituted target compounds **5a–h** exhibited moderate to good antifungal activities against the strains. Especially, among the tested compounds the target 4-chlorobenzyl substituted compounds **5b** and **5c** displayed comparable antifungal efficiency against tested fungi *A. niger* (ZI: 35, 39 mm; MIC: 6.25 μg mL^{-1}) and *C. albicans* (ZI: 36, 40 mm; MIC: 6.25 μg mL^{-1}) respectively in comparison with the reference drug fluconazole (MIC: 6.25 μg mL^{-1}). Compound **4b** showed good activity against *C. albicans* whereas compounds **4c** and **4d** exhibited good activity against *A. niger*. Compounds **5d** and **5f** displayed moderate activity against *C. albicans* whereas compounds **3b** and **5g** showed moderate activity against *A. niger*. On the other hand, compounds **3a**, **3d**, **3e**, **3h**, **4a**, **4e**, **4h**, **5e**, and **5h** were displayed least activity against both bacterial and fungal strains.

Compounds **4a–h** and **6a–h** were assayed for their cytotoxic activity against Hep G2 and MCF-7 cell lines by MTT assay.[45] The results were summarized in Table 3. The structure activity relationship of the compounds showed that compounds with chloro substituent on thiadiazole ring exhibited good activity compared to other tested compounds.

Among the tested compounds, compound **6a** was found to be active against Hep G2 cell line (IC_{50}: 48.83 μL) whereas compound **6b** exhibited good activity against both Hep G2 (IC_{50}: 43.19 μL) and MCF-7 (IC_{50}: 47.15 μL) cell lines. Compounds **4c**, **4d**, **4h** and **6c** showed potency against both the cell lines. Remaining compounds displayed no activity against the cell lines tested.

We believe that presence of selena/thiadiazolyl ring was responsible for increase in the activities and particularly, presence of –Cl group to phenyl ring of tetrazole and selena/thiadiazolyl moiety was more responsible for the increase of antimicrobial and cytotoxic activities compared to other groups. From these results it is clear that substituent group play a definite role in the biological activity of these molecules.

Table 3 *In vitro* cytotoxic activity of compounds **4a–h** and **6a–h** against human cancer lines Hep G2 and MCF-7

		IC_{50} (μL)	
Entry	Compound	Hep G2	MCF-7
1	**4a**	—	—
2	**4b**	—	—
3	**4c**	64.88	115.53
4	**4d**	78.95	165.53
5	**4e**	—	—
6	**4f**	—	—
7	**4g**	—	—
8	**4h**	91.14	141.41
9	**6a**	**48.83**	**65.78**
10	**6b**	**43.19**	**47.15**
11	**6c**	54.54	74.78
12	**6d**	—	—
13	**6e**	—	—
14	**6f**	—	—
15	**6g**	—	—
16	**6h**	—	—
17	Cisplatin	33.69	21.69

5. Conclusion

In this study, a new class of heterocycles such as *N*-substituted tetrazoles (**3a–h** and **4a–h**) and its cyclized derivatives **5a–h** and **6a–h** (1,2,3-selena/thiadiazolyltetrazoles) were synthesized and characterized by spectral and elemental analyses. All the synthesized compounds were evaluated for their preliminary antimicrobial and cytotoxic activities. The maximum antimicrobial activity was observed with compounds **5b** and **5c** whereas compound **6a** and **6b** exhibited appreciable cytotoxicity.

6. Experimental

6.1. Chemistry

Melting points were recorded on a Stuart SMP30 melting point apparatus and were uncorrected. Column chromatography was performed using silica-gel (60–120 mesh size) purchased from Thomas Baker and Thin Layer Chromatography (TLC) was carried out using aluminium sheets pre-coated with silica gel $60F_{254}$ purchased from Merck. IR spectra (KBr) were obtained using a PerkinElmer Spectrum 100 FT-IR Spectrometer. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on a Bruker WM-400 spectrometer in DMSO-d_6 with TMS as an internal standard. Mass spectra were carried out on a JEOL SX-102 spectrometer. CHN analysis was done by Carlo Erba EA 1108 automatic elemental analyzer. The chemicals and solvents used were of commercial grade and were used without further purification unless otherwise stated.

6.1.1. General procedure for the synthesis of 1-4-aryl-2-(5-aryl-2*H*-tetrazol-1-yl)ethanone (3a–h). A mixture of compound **2a–d** (0.005 mol), phenacyl bromide (0.005 mol) and anhydrous K_2CO_3 (0.010 mol) in acetone (25 mL) was stirred at reflux temperature for 8 h. After completion of the reaction

(monitored by TLC), the reaction mixture was poured into water (50 mL). The solid separated was filtered, washed with water, and triturated with diethyl ether. The separated solid was filtered, dried and crystallized from methanol to furnish **3a–h**.

*6.1.1.1. 1-(4-Chlorophenyl)-2-(5-phenyl-2H-tetrazol-1-yl)ethanone (**3a**).* White crystals, yield 1.28 g (81%), m.p. 151–154 °C; IR (KBr, cm^{-1}): 2973, 1698, 1589, 1451; ^1H NMR (400 MHz, DMSO-d_6): 6.74 (s, 2H, N–CH_2), 7.57–7.61 (m, 3H, ArH), 7.72 (d, 2H, ArH), 8.08–8.11 (m, 4H, ArH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): 58.99, 126.34, 126.74, 129.17, 129.33, 130.30, 130.67, 132.45, 139.50, 164.34, 190.45 ppm; ESI-MS (*m*/*z*): 299 $(M + 1)^+$; anal. calcd for $C_{15}H_{11}ClN_4O$: C, 60.31; H, 3.71; N, 18.76. Found: C, 60.24; H, 3.76; N, 18.72.

*6.1.1.2. 1-(4-Chlorophenyl)-2-(5-(4-chlorophenyl)-2H-tetrazol-1-yl)ethanone (**3b**).* White solid, yield 1.34 g (81%), m.p. 125–128 °C; IR (KBr, cm^{-1}): 2989, 1700, 1588, 1453; ^1H NMR (400 MHz, DMSO-d_6): 6.66 (s, 2H, N–CH_2), 7.24 (d, 2H, ArH), 7.62 (d, 2H, ArH), 8.00–8.08 (m, 4H, ArH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): 57.88, 127.92, 128.71, 129.84, 130.67, 133.56, 134.82, 135.43, 139.84, 164.32, 189.43 ppm; ESI-MS (*m*/*z*): 334 $(M + 1)^+$; anal. calcd for $C_{15}H_{10}Cl_2N_4O$: C, 54.07; H, 3.03; N, 16.82. Found: C, 54.16; H, 3.13; N, 16.73.

*6.1.1.3. 1-(4-Chlorophenyl)-2-(5-(2-chlorophenyl)-2H-tetrazol-1-yl)ethanone (**3c**).* White crystals, yield 1.29 g (78%), m.p. 161–162 °C; IR (KBr, cm^{-1}): 2994, 1708, 1583, 1456; ^1H NMR (400 MHz, DMSO-d_6): 6.68 (s, 2H, N–CH_2), 7.23–7.30 (m, 4H, ArH), 7.76–7.80 (m, 4H, ArH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): 57.82, 127.64, 128.38, 128.92, 129.57, 130.28, 131.34, 132.53, 133.31, 135.94, 140.28, 164.28, 189.53 ppm; ESI-MS (*m*/*z*): 334 $(M + 1)^+$; anal. calcd for $C_{15}H_{10}Cl_2N_4O$: C, 54.07; H, 3.03; N, 16.82. Found: C, 54.13; H, 3.09; N, 16.78.

*6.1.1.4. 1-(4-Chlorophenyl)-2-(5-p-tolyl-2H-tetrazol-1-yl) ethanone (**3d**).* White crystals, yield 1.32 g (85%), m.p. 165–168 °C; IR (KBr, cm^{-1}): 2998, 1703, 1586, 1457; ^1H NMR (400 MHz, DMSO-d_6): 2.38 (s, 3H, CH_3), 6.68 (s, 2H, N–CH_2), 7.12 (d, 2H, ArH), 7.64 (d, 2H, ArH), 7.98–8.02 (m, 4H, ArH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): 20.98, 57.74, 123.96, 126.47, 127.93, 128.64, 129.98, 133.62, 134.74, 135.53, 141.12, 164.32, 189.15 ppm; ESI-MS (*m*/*z*): 313 $(M + 1)^+$; anal. calcd for $C_{16}H_{13}ClN_4O$: C, 61.44; H, 4.19; N, 17.91. Found: C, 61.49; H, 4.26; N, 17.83.

*6.1.1.5. 1-(4-Methoxyphenyl)-2-(5-phenyl-2H-tetrazol-1-yl)-ethanone (**3e**).* White crystals, yield 1.24 g (85%), m.p. 131–133 °C; IR (KBr, cm^{-1}): 2984, 1703, 1589, 1454; ^1H NMR (400 MHz, DMSO-d_6): 3.92 (s, 3H, OCH_3), 6.72 (s, 2H, N–CH_2), 7.02 (d, 2H, ArH), 7.26 (d, 2H, ArH), 7.92–8.08 (m, 5H, ArH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): 55.69, 58.34, 114.23, 125.93, 126.82, 129.55, 130.67, 131.24, 133.54, 163.42, 164.32, 190.48 ppm; ESI-MS (*m*/*z*): 295 $(M + 1)^+$; anal. calcd for $C_{16}H_{14}N_4O_2$: C, 65.30; H, 4.79; N, 19.04. Found: C, 65.22; H, 4.72; N, 19.09.

*6.1.1.6. 1-(4-Methoxyphenyl)-2-(5-(4-chlorophenyl)-2H-tetrazol-1-yl)ethanone (**3f**).* White solid, yield 1.36 g (83%), m.p. 181–184 °C; IR (KBr, cm^{-1}): 2994, 1700, 1600, 1573, 1456; ^1H NMR (400 MHz, DMSO-d_6): 3.89 (s, 3H, OCH_3), 6.66 (s, 2H, N–CH_2), 7.15 (d, 2H, ArH), 7.65–7.67 (d, 2H, ArH), 8.05–8.11 (m, 4H, ArH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): 55.69, 58.70, 114.26, 125.64, 126.55, 128.07, 129.43, 130.81, 135.26, 163.37, 164.16, 189.28 ppm; ESI-MS (*m*/*z*): 329 $(M + 1)^+$; anal. calcd for $C_{16}H_{13}ClN_4O_2$: C, 58.45; H, 3.99; N, 17.04. Found: C, 58.39; H, 3.92; N, 17.10.

*6.1.1.7. 1-(4-Methoxyphenyl)-2-(5-(2-chlorophenyl)-2H-tetrazol-1-yl)ethanone (**3g**).* White solid, yield 1.18 g (72%), m.p. 117–120 °C; IR (KBr, cm^{-1}): 2998, 1707, 1592, 1458; ^1H NMR (400 MHz, DMSO-d_6): 3.89 (s, 3H, OCH_3), 6.68 (s, 2H, N–CH_2), 7.15 (d, 2H, ArH), 7.53–7.62 (m, 2H, ArH), 7.68–7.71 (dd, 1H, ArH), 7.93–7.96 (dd, 1H, ArH), 8.07 (d, 2H, ArH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): 55.70, 58.70, 114.27, 125.92, 126.56, 127.67, 130.75, 130.83, 131.32, 131.83, 131.90, 162.15, 164.15, 189.24 ppm; ESI-MS (*m*/*z*): 329 $(M + 1)^+$; anal. calcd for $C_{16}H_{13}ClN_4O_2$: C, 58.45; H, 3.99; N, 17.04. Found: C, 58.36; H, 3.88; N, 17.13.

*6.1.1.8. 1-(4-Methoxyphenyl)-2-(5-p-tolyl-2H-tetrazol-1-yl)-ethanone (**3h**).* White crystals, yield 1.23 g (80%), m.p. 139–140 °C; IR (KBr, cm^{-1}): 2991, 1699, 1588, 1457; ^1H NMR (400 MHz, DMSO-d_6): 2.39 (s, 3H, CH_3), 3.89 (s, 3H, OCH_3), 6.62 (s, 2H, N–CH_2), 7.10–7.16 (m, 4H, ArH), 7.33–7.40 (m, 2H, ArH), 7.60 (d, 2H, ArH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): 20.94, 55.68, 58.54, 114.25, 120.73, 124.08, 126.60, 128.04, 129.78, 130.86, 140.30, 155.02, 164.22, 189.38 ppm; ESI-MS (*m*/*z*): 309 $(M + 1)^+$; anal. calcd for $C_{17}H_{16}N_4O_2$: C, 66.22; H, 5.23; N, 18.17. Found: C, 66.15; H, 5.29; N, 18.21.

6.1.2. General procedure for the synthesis of 1-1-(4-aryl-2-(5-aryl-2*H*-tetrazol-1-yl)ethylidene)semicarbazide (4a–h). A mixture of compound **3a–h** (0.003 mol), semicarbazide hydrochloride (0.0036 mol) and sodium acetate (0.006 mol) in methanol (15 mL) was heated to reflux for 5 h. After the reaction was completed, the reaction mixture was poured into crushed ice, the solid thus separated was filtered, washed with water and dried to afford **4a–h**.

*6.1.2.1. 1-(1-(4-Chlorophenyl)-2-(5-phenyl-2H-tetrazol-1-yl)-ethylidene)semicarbazide (**4a**).* White solid, yield 0.87 g (82%), m.p. 212–215 °C; IR (KBr, cm^{-1}): 3423, 3307, 3248, 2964, 1695, 1591, 1451; ^1H NMR (400 MHz, DMSO-d_6): 6.18 (s, 2H, N–CH_2), 6.82 (brs, 2H, NH_2), 7.40 (d, 2H, ArH), 7.53–7.55 (m, 3H, ArH), 7.90 (d, 2H, ArH), 7.98–8.01 (m, 2H, ArH), 10.38 (s, 1H, NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): 46.53, 126.32, 126.50, 127.88, 128.32, 129.29, 130.69, 133.45, 134.54, 135.35, 156.70, 164.14 ppm; ESI-MS (*m*/*z*): 356 $(M + 1)^+$; anal. calcd for $C_{16}H_{14}ClN_7O$: C, 54.01; H, 3.97; N, 27.56. Found: C, 54.08; H, 3.92; N, 27.51.

*6.1.2.2. 1-(1-(4-Chlorophenyl)-2-(5-(4-chlorophenyl)-2H-tetrazol-1-yl)ethylidene)semicarbazide (**4b**).* White solid, yield 0.91 g (78%), m.p. 80–83 °C; IR (KBr, cm^{-1}): 3433, 3317, 3246, 3090, 1692, 1588, 1453; ^1H NMR (400 MHz, DMSO-d_6): 6.14 (s, 2H, N–CH_2), 6.64 (brs, 2H, NH_2), 7.12 (d, 2H, ArH), 7.62 (d, 2H, ArH), 7.82 (d, 2H, ArH), 8.02 (d, 2H, ArH), 10.28 (s, 1H, NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): 46.58, 127.63, 128.42, 129.73, 130.54, 133.52, 134.74, 135.26, 139.68, 156.64, 163.14 ppm; ESI-MS (*m*/*z*): 391 $(M + 1)^+$; anal. calcd for $C_{16}H_{13}Cl_2N_7O$: C, 49.25; H, 3.36; N, 25.13. Found: C, 49.20; H, 3.32; N, 25.19.

*6.1.2.3. 1-(1-(4-Chlorophenyl)-2-(5-(2-chlorophenyl)-2H-tetrazol-1-yl)ethylidene)semicarbazide (**4c**).* White solid, yield 0.87 g (75%), m.p. 216–218 °C; IR (KBr, cm^{-1}): 3427, 3343, 3257, 3072, 1689, 1584, 1450; ^1H NMR (400 MHz, DMSO-d_6): 6.14 (s, 2H, N–CH_2), 6.66 (brs, 2H, NH_2), 7.04–7.12 (m, 4H, ArH), 7.61–7.70 (m, 4H, ArH), 10.21 (s, 1H, NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6):

46.51, 127.52, 128.44, 129.43, 130.14, 131.22, 132.41, 133.28, 134.61, 135.86, 139.93, 155.82, 163.22 ppm; ESI-MS (*m*/*z*): 391 (M + 1)$^+$; anal. calcd for $C_{16}H_{13}Cl_2N_7O$: C, 49.25; H, 3.36; N, 25.13. Found: C, 49.17; H, 3.28; N, 25.21.

*6.1.2.4. 1-(1-(4-Chlorophenyl)-2-(5-p-tolyl-2H-tetrazol-1-yl)-ethylidene)semicarbazide (**4d**).* White solid, yield 0.89 g (81%), m.p. 202–205 °C; IR (KBr, cm^{-1}): 3413, 3341, 3244, 3078, 1687, 1590, 1452; ^{1}H NMR (400 MHz, DMSO-d_6): 2.36 (s, 3H, CH_3), 6.15 (s, 2H, N–CH_2), 6.78 (brs, 2H, NH_2), 7.33–7.40 (m, 4H, ArH), 7.87–7.91 (m, 4H, ArH), 10.33 (s, 1H, NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): 20.96, 46.47, 123.76, 126.25, 127.86, 128.30, 129.80, 133.45, 134.56, 135.44, 140.45, 156.67, 164.21 ppm; ESI-MS (*m*/*z*): 370 (M + 1)$^+$; anal. calcd for $C_{17}H_{16}ClN_7O$: C, 55.21; H, 4.36; N, 26.51. Found: C, 55.12; H, 4.29; N, 26.58.

*6.1.2.5. 1-(1-(4-Methoxyphenyl)-2-(5-phenyl-2H-tetrazol-1-yl)-ethylidene)semicarbazide (**4e**).* White solid, yield 0.84 g (80%), m.p. 137–140 °C; IR (KBr, cm^{-1}): 3433, 3321, 3249, 3088, 1693, 1590, 1454; ^{1}H NMR (400 MHz, DMSO-d_6): 3.86 (s, 3H, OCH_3), 6.08 (s, 2H, N–CH_2), 6.72 (brs, 2H, NH_2), 6.98 (d, 2H, ArH), 7.14 (d, 2H, ArH), 7.66–7.92 (m, 5H, ArH), 10.32 (s, 1H, NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): 46.59, 55.58, 113.21, 124.86, 125.98, 129.47, 130.76, 132.87, 133.54, 156.99, 160.02, 163.56 ppm; ESI-MS (*m*/*z*): 352 (M + 1)$^+$; anal. calcd for $C_{17}H_{17}N_7O_2$: C, 58.11; H, 4.88; N, 27.90. Found: C, 58.02; H, 4.81; N, 27.98.

*6.1.2.6. 1-(1-(4-Methoxyphenyl)-2-(5-(4-chlorophenyl)-2H-tetrazol-1-yl)ethylidene)semicarbazide (**4f**).* White solid, yield 0.88 g (77%), m.p. 188–191 °C; IR (KBr, cm^{-1}): 3441, 3347, 3249, 3012, 1698, 1566, 1418; ^{1}H NMR (400 MHz, DMSO-d_6): 3.74 (s, 3H, OCH_3), 6.15 (s, 2H, N–CH_2), 6.69 (brs, 2H, NH_2), 6.89 (d, 2H, ArH), 7.61 (d, 2H, ArH), 7.81 (d, 2H, ArH), 8.00 (d, 2H, ArH), 10.13 (s, 1H, NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): 46.66, 55.13, 113.73, 125.38, 127.59, 128.08, 129.43, 135.31, 136.41, 156.90, 159.82, 163.21 ppm; ESI-MS (*m*/*z*): 386 (M + 1)$^+$; anal. calcd for $C_{17}H_{16}ClN_7O_2$: C, 52.92; H, 4.18; N, 25.41. Found: C, 52.97; H, 4.23; N, 25.49.

*6.1.2.7. 1-1-(4-Methoxyphenyl)-2-(5-(2-chlorophenyl)-2H-tetrazol-1-yl)ethylidene semicarbazide (**4g**).* White solid, yield 0.80 g (70%), m.p. 143–146 °C; IR (KBr, cm^{-1}): 3443, 3340, 3254, 3087, 1693, 1592, 1453; ^{1}H NMR (400 MHz, DMSO-d_6): 3.74 (s, 3H, OCH_3), 6.12 (s, 2H, N–CH_2), 6.54 (brs, 2H, NH_2), 7.02 (d, 2H, ArH), 7.52–7.80 (m, 4H, ArH), 8.00 (d, 2H, ArH), 10.11 (s, 1H, NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): 46.43, 55.10, 113.43, 124.86, 126.43, 127.58, 128.31, 129.72, 130.57, 131.61, 131.99, 156.71, 159.81, 163.20 ppm; ESI-MS (*m*/*z*): 386 (M + 1)$^+$; anal. calcd for $C_{17}H_{16}ClN_7O_2$: C, 52.92; H, 4.18; N, 25.41. Found: C, 52.98; H, 4.25; N, 25.46.

*6.1.2.8. 1-(1-(4-Methoxyphenyl)-2-(5-p-tolyl-2H-tetrazol-1-yl)-ethylidene)semicarbazide (**4h**).* White solid, yield 0.81 g (74%), m.p. 141–144 °C; IR (KBr, cm^{-1}): 3433, 3347, 3249, 3084, 1696, 1590, 1454; ^{1}H NMR (400 MHz, DMSO-d_6): 2.36 (s, 3H, CH_3), 3.73 (s, 3H, OCH_3), 5.83 (s, 2H, N–CH_2), 6.41 (brs, 2H, NH_2), 6.98 (d, 2H, ArH), 7.29–7.35 (m, 4H, ArH), 7.88 (d, 2H, ArH), 10.12 (s, 1H, NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): 20.94, 46.53, 55.12, 114.55, 122.28, 123.94, 127.59, 128.08, 129.75, 136.58, 140.35, 156.90, 159.81, 164.14 ppm; ESI-MS (*m*/*z*): 366 (M + 1)$^+$; anal. calcd for $C_{18}H_{19}N_7O_2$: C, 59.17; H, 5.24; N, 26.83. Found: C, 59.11; H, 5.20; N, 26.88.

6.1.3. General procedure for the synthesis of 5-aryl-1-(4-aryl-1,2,3-selenadiazol-5-yl)-1*H*-tetrazole (5a–h). Compound **4a–h** (0.001 mol) was dissolved in glacial acetic acid (10 mL) and to this selenium dioxide (0.0012 mol) was added portionwise. Then the reaction mixture was refluxed for 6 h, after completion of the reaction (monitored by TLC), the reaction mixture was cooled to room temperature. After cooling, the selenium metal deposited was filtered and the filtrate was poured into ice-cold water and the solid obtained was filtered, washed thoroughly with water then with aqueous sodium carbonate solution and again with water. The residue was dried and purified by column chromatography (silica gel 60–120 mesh, hexane : ethylacetate, 7 : 3) to obtain **5a–h**.

*6.1.3.1. 1-(4-(4-Chlorophenyl)-1,2,3-selenadiazol-5-yl)-5-phenyl-2H-tetrazole (**5a**).* Red solid, yield 0.30 g (78%), m.p. 91–94 °C; IR (KBr, cm^{-1}): 1632, 1586, 1566, 1490, 1442, 1390, 1300, 1287; ^{1}H NMR (400 MHz, DMSO-d_6): 7.54 (d, 2H, ArH), 7.71 (t, 3H, ArH), 8.05 (d, 2H, ArH), 8.11–8.14 (m, 2H, ArH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): 126.53, 127.81, 129.24, 129.86, 130.92, 133.06, 133.75, 136.54, 139.93, 169.14, 174.45, 176.79 ppm; ESI-MS (*m*/*z*): 388 (M + 1)$^+$; anal. calcd for $C_{15}H_9ClN_6Se$: C, 46.47; H, 2.34; N, 21.68. Found: C, 46.40; H, 2.26; N, 21.74.

*6.1.3.2. 5-(4-Chlorophenyl)-1-(4-(4-chlorophenyl)-1,2,3-selenadiazol-5-yl)-2H-tetrazole (**5b**).* Brown solid, yield 0.31 g (74%), m.p. 88–91 °C; IR (KBr, cm^{-1}): 1627, 1582, 1556, 1481, 1432, 1387, 1308, 1291; ^{1}H NMR (400 MHz, DMSO-d_6): 7.14 (d, 2H, ArH), 7.68 (d, 2H, ArH), 8.02–8.10 (m, 4H, ArH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): 127.84, 128.82, 129.93, 130.54, 133.67, 134.71, 135.67, 139.94, 167.82, 174.43, 176.62 ppm; ESI-MS (*m*/*z*): 423 (M + 1)$^+$; anal. calcd for $C_{15}H_8Cl_2N_6Se$: C, 42.68; H, 1.91; N, 19.91. Found: C, 42.61; H, 1.83; N, 19.98.

*6.1.3.3. 5-(2-Chlorophenyl)-1-(4-(4-chlorophenyl)-1,2,3-selenadiazol-5-yl)-2H-tetrazole (**5c**).* Red solid, yield 0.29 g (69%), m.p. 72–75 °C; IR (KBr, cm^{-1}): 1622, 1585, 1559, 1483, 1438, 1391, 1304, 1286; ^{1}H NMR (400 MHz, DMSO-d_6): 7.16–7.25 (m, 4H, ArH), 7.76–7.84 (m, 4H, ArH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): 127.54, 128.32, 128.73, 129.54, 130.24, 131.43, 132.60, 133.42, 135.92, 140.03, 167.82, 174.73, 176.21 ppm; ESI-MS (*m*/*z*): 423 (M + 1)$^+$; anal. calcd for $C_{15}H_8Cl_2N_6Se$: C, 42.68; H, 1.91; N, 19.91. Found: C, 42.59; H, 1.85; N, 19.96.

*6.1.3.4. 1-(4-(4-Chlorophenyl)-1,2,3-selenadiazol-5-yl)-5-p-tolyl-2H-tetrazole (**5d**).* White solid, yield 0.28 g (71%), m.p. 64–67 °C; IR (KBr, cm^{-1}): 1630, 1580, 1563, 1491, 1441, 1383, 1306, 1291; ^{1}H NMR (400 MHz, DMSO-d_6): 2.22 (s, 3H, CH_3), 7.51 (d, 2H, ArH), 7.58–7.63 (m, 4H, ArH), 7.97 (d, 2H, ArH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): 20.62, 125.58, 128.17, 128.92, 129.14, 129.82, 130.26, 133.57, 136.39, 139.53, 169.08, 174.96, 176.52 ppm; ESI-MS (*m*/*z*): 402 (M + 1)$^+$; anal. calcd for $C_{16}H_{11}ClN_6Se$: C, 47.84; H, 2.76; N, 20.92. Found: C, 47.92; H, 2.70; N, 20.98.

*6.1.3.5. 1-(4-(4-Methoxyphenyl)-1,2,3-selenadiazol-5-yl)-5-phenyl-2H-tetrazole (**5e**).* White solid, yield 0.28 g (75%), m.p. 54–56 °C; IR (KBr, cm^{-1}): 1625, 1582, 1566, 1488, 1440, 1384, 1301, 1288; ^{1}H NMR (400 MHz, DMSO-d_6): 3.81 (s, 3H, OCH_3), 7.02–7.14 (m, 4H, ArH), 7.78–7.92 (m, 5H, ArH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): 55.62, 114.18, 126.12, 129.68, 130.78, 132.68, 135.42, 137.14, 159.96, 169.56,

174.92, 176.64 ppm; ESI-MS (*m*/*z*): 384 $(M + 1)^+$; anal. calcd for $C_{16}H_{12}N_6OSe$: C, 50.14; H, 3.16; N, 21.93. Found: C, 50.08; H, 3.10; N, 21.98.

*6.1.3.6. 5-(4-Chlorophenyl)-1-(4-(4-methoxyphenyl)-1,2,3-selenadiazol-5-yl)-2H-tetrazole (**5f**).* White solid, yield 0.29 g (70%), m.p. 84–87 °C; IR (KBr, cm^{-1}): 1632, 1602, 1574, 1513, 1444, 1398, 1308, 1252; ^1H NMR (400 MHz, DMSO-d_6): 3.76 (s, 3H, OCH_3), 6.98 (d, 2H, ArH), 7.46 (d, 2H, ArH), 7.59 (d, 2H, ArH), 7.96 (d, 2H, ArH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): 55.59, 113.74, 120.62, 123.17, 125.81, 129.96, 131.08, 135.43, 159.91, 169.32, 174.83, 176.67 ppm; ESI-MS (*m*/*z*): 418 $(M + 1)^+$; anal. calcd for $C_{16}H_{11}ClN_6OSe$: C, 46.01; H, 2.65; N, 20.12. Found: C, 46.07; H, 2.61; N, 20.17.

*6.1.3.7. 5-(2-Chlorophenyl)-1-(4-(4-methoxyphenyl)-1,2,3-selenadiazol-5-yl)-2H-tetrazole (**5g**).* Brown solid, yield 0.28 g (69%), m.p. 131–134 °C; IR (KBr, cm^{-1}): 1630, 1596, 1572, 1493, 1431, 1390, 1306, 1277; ^1H NMR (400 MHz, DMSO-d_6): 3.84 (s, 3H, OCH_3), 7.18–7.24 (m, 4H, ArH), 7.94–8.02 (m, 4H, ArH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): 55.62, 113.14, 124.84, 126.63, 127.42, 130.15, 130.76, 131.06, 131.92, 132.34, 159.87, 169.36, 174.76, 176.62 ppm; ESI-MS (*m*/*z*): 418 $(M + 1)^+$; anal. calcd for $C_{16}H_{11}ClN_6OSe$: C, 46.01; H, 2.65; N, 20.12. Found: C, 46.10; H, 2.60; N, 20.16.

*6.1.3.8. 1-(4-(4-Methoxyphenyl)-1,2,3-selenadiazol-5-yl)-5-p-tolyl-2H-tetrazole (**5h**).* White solid, yield 0.26 g (66%), m.p. 151–154 °C; IR (KBr, cm^{-1}): 1624, 1588, 1559, 1486, 1444, 1391, 1307, 1288; ^1H NMR (400 MHz, DMSO-d_6): 2.32 (s, 3H, CH_3), 3.82 (s, 3H, OCH_3), 7.22–7.30 (m, 4H, ArH), 7.58–7.70 (m, 4H, ArH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): 20.92, 55.61, 113.82, 120.64, 123.87, 126.59, 128.12, 129.53, 131.32, 140.15, 159.88, 169.43, 174.86, 176.59 ppm; ESI-MS (*m*/*z*): 398 $(M + 1)^+$; anal. calcd for $C_{17}H_{14}N_6OSe$: C, 51.39; H, 3.55; N, 21.15. Found: C, 51.32; H, 3.49; N, 21.20.

6.1.4. General procedure for the synthesis of 5-aryl-1-(4-aryl-1,2,3-thiadiazol-5-yl)-2*H*-tetrazole (6a–h). Compound **4a–h** (0.001 mol) was added portion wise to the thionyl chloride (3 mL) at 0–5 °C for 30 min. The reaction mixture was allowed to stir at RT for 5 h and then decomposed with saturated ice cold sodium carbonate solution. A gummy product was obtained, which was solidified on treatment with petroleum ether and purified by column chromatography (silica gel 60–120 mesh, hexane : ethyl acetate, 7 : 3) to obtain **6a–h**.

*6.1.4.1. 1-(4-(4-Chlorophenyl)-1,2,3-thiadiazol-5-yl)-5-phenyl-2H-tetrazole (**6a**).* Red solid, yield 0.25 g (76%), m.p. 82–85 °C; IR (KBr, cm^{-1}): 1630, 1549, 1522, 1478, 1427, 1375, 1307, 1278; ^1H NMR (400 MHz, DMSO-d_6): δ 7.51 (d, 2H, ArH), 7.69 (t, 3H, ArH), 8.04 (d, 2H, ArH), 8.08–8.12 (m, 2H, ArH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 126.51, 127.78, 129.20, 129.78, 130.88, 133.00, 133.59, 136.42, 139.91, 168.92, 173.37, 175.63 ppm; ESI-MS (*m*/*z*): 341 $(M + 1)^+$; anal. calcd for $C_{15}H_9ClN_6S$: C, 52.87; H, 2.66; N, 24.66. Found: C, 52.95; H, 2.77; N, 24.58.

*6.1.4.2. 5-(4-Chlorophenyl)-1-(4-(4-chlorophenyl)-1,2,3-thiadiazol-5-yl)-2H-tetrazole (**6b**).* Brown solid, yield 0.27 g (73%), m.p. 74–76 °C; IR (KBr, cm^{-1}): 1623, 1567, 1521, 1450, 1426, 1366, 1311, 1283; ^1H NMR (400 MHz, DMSO-d_6): δ 7.12 (d, 2H, ArH), 7.67 (d, 2H, ArH), 8.00–8.08 (m, 4H, ArH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 127.84, 128.82, 129.93, 130.54, 133.67, 134.71, 135.67, 139.94, 167.88, 174.31, 176.50 ppm; ESI-MS (*m*/*z*): 376 $(M + 1)^+$; anal. calcd for $C_{15}H_8Cl_2N_6S$: C, 48.01; H, 2.15; N, 22.40. Found: C, 48.11; H, 2.22; N, 22.33.

*6.1.4.3. 5-(2-Chlorophenyl)-1-(4-(4-chlorophenyl)-1,2,3-thiadiazol-5-yl)-2H-tetrazole (**6c**).* Brown solid, yield 0.25 g (70%), m.p. 100–103 °C; IR (KBr, cm^{-1}): 1624, 1578, 1517, 1451, 1411, 1376, 1304, 1279; ^1H NMR (400 MHz, DMSO-d_6): δ 7.14–7.24 (m, 4H, ArH), 7.74–7.82 (m, 4H, ArH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 127.38, 128.41, 128.67, 129.51, 130.18, 131.34, 132.52, 133.26, 135.78, 140.11, 167.76, 174.62, 176.21 ppm; ESI-MS (*m*/*z*): 376 $(M + 1)^+$; anal. calcd for $C_{15}H_8Cl_2N_6S$: C, 48.01; H, 2.15; N, 22.40. Found: C, 48.09; H, 2.24; N, 22.31.

*6.1.4.4. 1-(4-(4-Chlorophenyl)-1,2,3-thiadiazol-5-yl)-5-p-tolyl-2H-tetrazole (**6d**).* Red solid, yield 0.23 g (67%), m.p. 80–83 °C; IR (KBr, cm^{-1}): 1639, 1586, 1488, 1447, 1401, 1351, 1302, 1250; ^1H NMR (400 MHz, DMSO-d_6): δ 2.20 (s, 3H, CH_3), 7.50 (d, 2H, ArH), 7.57–7.60 (m, 4H, ArH), 7.96 (d, 2H, ArH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 20.60, 125.55, 128.07, 128.90, 129.10, 129.79, 130.24, 133.51, 136.32, 139.47, 169.06, 174.95, 176.55 ppm; ESI-MS (*m*/*z*): 355 $(M + 1)^+$; anal. calcd for $C_{16}H_{11}ClN_6S$: C, 54.16; H, 3.12; N, 23.69. Found: C, 54.09; H, 3.21; N, 23.61.

*6.1.4.5. 1-(4-(4-Methoxyphenyl)-1,2,3-thiadiazol-5-yl)-5-phenyl-2H-tetrazole (**6e**).* Red solid, yield 0.22 g (69%), m.p. 61–63 °C; IR (KBr, cm^{-1}): 1625, 1552, 1511, 1478, 1420, 1387, 1317, 1289; ^1H NMR (400 MHz, DMSO-d_6): δ 3.84 (s, 3H, OCH_3), 7.01–7.12 (m, 4H, ArH), 7.76–7.90 (m, 5H, ArH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 55.60, 114.19, 126.14, 129.49, 130.58, 132.62, 135.37, 137.11, 159.83, 169.44, 173.69, 175.51 ppm; ESI-MS (*m*/*z*): 337 $(M + 1)^+$; anal. calcd for $C_{16}H_{12}N_6OS$: C, 57.13; H, 3.60; N, 24.98. Found: C, 57.24; H, 3.68; N, 24.89.

*6.1.4.6. 5-(4-Chlorophenyl)-1-(4-(4-methoxyphenyl)-1,2,3-thiadiazol-5-yl)-2H-tetrazole (**6f**).* White solid, yield 0.27 g (74%), m.p. 89–91 °C; IR (KBr, cm^{-1}): 1632, 1602, 1574, 1513, 1444, 1416, 1373, 1252; ^1H NMR (400 MHz, DMSO-d_6): δ 3.81 (s, 3H, OCH_3), 6.98 (d, 2H, ArH), 7.44 (d, 2H, ArH), 7.58 (d, 2H, ArH), 7.95 (d, 2H, ArH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 55.56, 113.71, 120.53, 123.13, 125.82, 129.87, 131.04, 135.35, 159.84, 169.26, 173.61, 175.84 ppm; ESI-MS (*m*/*z*): 371 $(M + 1)^+$; anal. calcd for $C_{16}H_{11}ClN_6OS$: C, 51.82; H, 2.99; N, 22.66. Found: C, 51.74; H, 2.87; N, 22.78.

*6.1.4.7. 5-(2-Chlorophenyl)-1-(4-(4-methoxyphenyl)-1,2,3-thiadiazol-5-yl)-2H-tetrazole (**6g**).* Red solid, yield 0.24 g (66%), m.p. 111–113 °C; IR (KBr, cm^{-1}): 1631, 1587, 1564, 1481, 1424, 1372, 1311, 1264; ^1H NMR (400 MHz, DMSO-d_6): δ 3.83 (s, 3H, OCH_3), 7.16–7.22 (m, 4H, ArH), 7.92–8.00 (m, 4H, ArH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 55.60, 113.19, 124.78, 126.66, 127.41, 130.18, 130.71, 131.04, 131.88, 132.31, 159.76, 169.33, 174.24, 176.54 ppm; ESI-MS (*m*/*z*): 371 $(M + 1)^+$; anal. calcd for $C_{16}H_{11}ClN_6OS$: C, 51.82; H, 2.99; N, 22.66. Found: C, 51.91; H, 2.87; N, 22.60.

*6.1.4.7.1. 1-(4-(4-Methoxyphenyl)-1,2,3-thiadiazol-5-yl)-5-p-tolyl-2H-tetrazole (**6h**).* Brown solid, yield 0.21 g (62%), m.p. 62–65 °C; IR (KBr, cm^{-1}): 1628, 1573, 1547, 1472, 1431, 1383, 1312, 1261; ^1H NMR (400 MHz, DMSO-d_6): δ 2.30 (s, 3H, CH_3), 3.84 (s, 3H, OCH_3), 7.22–7.28 (m, 4H, ArH), 7.56–7.70 (m, 4H, ArH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 20.90, 55.64, 113.77, 120.62, 123.82, 126.48, 128.18, 129.47, 131.30, 140.11, 159.78,

169.36, 174.63, 176.47 ppm; ESI-MS (m/z): 351 $(M + 1)^+$; anal. calcd for $C_{17}H_{14}N_6OS$: C, 58.27; H, 4.03; N, 23.98. Found: C, 58.38; H, 4.13; N, 23.87.

6.2. Biological protocol

6.2.1. Antimicrobial activity. Preliminary *in vitro* antimicrobial activity of compounds **3a–h**, **4a–h** and **5a–h** was performed using agar disc-diffusion method. Sterile filter paper discs (6 mm diameter) impregnate with a solution of test compound solution in DMSO of specific concentration 100 μg and 200 μg per disc were carefully placed on the agar culture plates that had been previously inoculated separately with the microorganisms. The plates were incubated at 37 °C and the diameter of the growth inhibition zones were measured after 24 h in case of bacteria and after 48 h in case of fungi. The MICs of the compound assays were carried out using microdilution susceptibility method. Ciprofloxacin was used as reference antibacterial agent. Fluconazole was used as reference antifungal agent. The test compounds, ciprofloxacin and fluconazole were dissolved in DMSO at concentration of 800 μg mL^{-1} and further two-fold serial dilutions to obtain final concentrations of 400, 200, 100, 50, 25, 12.50, and 6.25 μg mL^{-1}. The microorganism suspensions were inoculated to the corresponding wells. The plates were incubated at 36 °C for 24 h and 48 h for bacteria and fungi, respectively. The minimum inhibitory concentrations (MIC, μg mL^{-1}) of the compounds were determined as the lowest concentration of each chemical compounds in the tubes with no turbidity *i.e.* no growth of inoculated bacteria or fungi.

6.2.2. MTT assay. *In vitro* cytotoxicity of the newly synthesized compounds was determined by MTT assay. 100 μL of medium containing 10 000 cell per well were seeded in each well of 96 well plates and incubated overnight in a CO_2 incubator at 37 °C, 5% CO_2. Following incubation the cells were treated with different concentrations of each compound and incubated for 24 h. After incubation the medium was removed and 10 μL of MTT reagent (5 mg mL^{-1}) was added to each well. The plates were again incubated for 3 h and at the end of the incubation period, the medium was removed and DMSO (5 mL) was added to each well. Rock the plates at room temperature for a minimum period of 1 h to solubilize the formazan dye. The percentage of viable cells in each well was calculated from absorbance of purple colored formazan crystals read at 560 nm using micro plate reader. All experiments were carried out in triplicates maintaining a control (with solvent only) and a standard (with commercial drug cisplatin).

The percentage of inhibition of each compound was calculated using the following formula:

$$\% \text{ inhibition} = (\text{mean absorbance of treated cells/mean absorbance of control}) \times 100$$

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