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Design, synthesis and biological evaluation of 1,3,4-oxadiazoles/thiadiazoles bearing pyrazole scaffold as antimicrobial and antioxidant candidates

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A series of semicarbazones, thiosemicarbazones, 1,3,4-oxadiazoles/thiadiazoles bearing pyrazole scaffold were designed and synthesized. All the synthesized new compounds were characterized by ^{1}H NMR, ^{13}C NMR, MS and elemental analysis. The synthesized compounds were screened to probe their *in vitro* antimicrobial activity against bacteria and fungi species. The structure-activity relationship of the synthesized compounds was studied. The compounds displayed good to excellent potency against tested microorganisms. The *in vitro* antioxidant activities of the 1,3,4-oxadiazoles/thiadiazoles were evaluated by DPPH, hydroxyl and nitric oxide radical scavenging assay. Among the tested compounds, compound with chloro substitution showed good antioxidant potential.

1. Introduction

The pyrazole motif makes up the core structure of numerous biologically active compounds. Compounds bearing pyrazole nucleus exhibit versatile range of biological activities such as antimicrobial[1], anti-inflammatory[2], analgesic[3] and GABA receptor antagonists and insecticides.[4] In addition to the diverse biological activities of pyrazoles, other heterocycles in association with pyrazoles play a prime role in chemical and pharmacological fields. The prevalence of pyrazole cores in biologically active molecules has stimulated the need for elegant and efficient ways to make these heterocyclic lead.

Further, semicarbazone and thiosemicarbazone are excellent prototypes for the design and development of novel amino oxadiazole and thiadiazole respectively. In addition semicarbazone have received significant attention from pharmaceutical industry due to their wide spectrum of biological activity such as anticonvulsant,[5] antioxidant,[6] antimicrobial activities[7] etc. Compounds containing 1,3,4-oxadiazole core were known to possess pharmacological properties such as antimicrobial,[8] COX-2 inhibitors and anti-inflammatory, antitumor and analgesic.[9] The widespread use of 1,3,4-oxadiazoles

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as a scaffold in medicinal chemistry established this moiety as a member of the privileged structures class, among them the synthesis of 2-amino-5-substituted-1,3,4-oxadiazole has received a lot of interest.

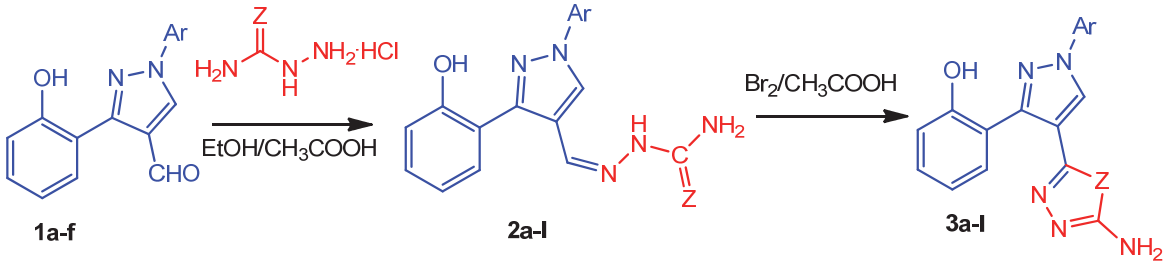
Sulpha drugs are well recognized for their various physiological activities. Thiosemicarbazone derivatives have been the focus of medicinal chemists because of their potential biological activities.[10] Thiosemicarbazones are very useful intermediate for the development of molecules of pharmaceutically important molecules, as well as they themselves possess antimicrobial, antiviral,[11] anti-HIV[12] and anticancer[13] properties. 1,3,4-Thiadiazoles have gained importance as they constitute the structural features of many bioactive compounds. These compounds are of great interest in chemistry owing to their bioactivity of certain plant growth regulating effects as well as antimicrobial activity.[14] 1,3,4-Thiadiazoles possess a wide range of therapeutic activities like antioxidant, anticancer, anti-inflammatory and hyperlipidaemia.[15]

In the pursuit and design of new drugs, the development of hybrid molecules through the combination of different pharmacophores in one frame may lead to compounds with interesting biological profiles. In view of these facts and as a part of our extensive research program, the synthesis of 1,3,4-oxadiazole and 1,3,4-thiadiazole derivatives incorporating with pyrazole nucleus as hybrid molecule possessing antimicrobial and antioxidant activity is aimed.

2. Result and discussion

2.1 Chemistry

The precursor 3-(2-hydroxyphenyl)-1-phenyl-1*H*-pyrazole-4-carbaldehydes, **1a-f** were synthesized from our earlier reported method.[16] The synthetic route used to obtain the target 1,3,4-oxadiazole and 1,3,4-thiadiazole containing pyrazole moiety is outlined in **Scheme 1**. The synthetic strategy involves the preparation of semicarbazones, **2a-f** and thiosemicarbazones, **2g-l** by the condensation of 3-(2-hydroxyphenyl)-1-phenyl-1*H*-pyrazole-4-carbaldehydes, **1a-f** with semicarbazide hydrochloride and thiosemicarbazide hydrochloride respectively. The oxidative cyclization of semicarbazones, **2a-f** and thiosemicarbazones, **2g-l** lead to the formation of 1,3,4-oxadiazolyl pyrazoles, **3a-f** and 1,3,4-thiadiazolyl pyrazoles, **3g-l**.



3.a) Ar=C_6H_5, Z=O; b) Ar=4-ClC_6H_4, Z=O; c) Ar=2-$CH_3C_6H_4$, Z=O; d) Ar=2-$OCH_3C_6H_4$, Z=O; e) Ar=2,4-$(CH_3)_2C_6H_3$, Z=O; f) Ar=2,4-$(NO_2)_2C_6H_3$, Z=O; .g) Ar=C_6H_5, Z=S; h) Ar=4-ClC_6H_4, Z=S; i) Ar=2-$CH_3C_6H_4$, Z=S; j) Ar=2-$OCH_3C_6H_4$, Z=S; k) Ar=2,4-$(CH_3)_2C_6H_3$, Z=S; l) Ar=2,4-$(NO_2)_2C_6H_3$, Z=S.

Scheme 1. Synthesis of 1,3,4-oxadiazolyl/thiadiazolyl pyrazoles

The synthesized new compounds were characterized by spectral analysis before being evaluated for their *in vitro* antimicrobial and antioxidant activities. In ^{1}H NMR spectra, compounds **2a-f** showed signals in the region δ 4.810-4.835 ppm, δ 6.625-6.652 ppm and δ 8.010-8.115 ppm which were assigned to NH_2, $CONH_2$ and CH=N protons respectively. Compounds **2g-l** showed the signals in the region δ 2.212-2.430 ppm, δ 4.515-4.621 ppm and δ 8.142-8.244 ppm were assigned to NH_2, CSNH and CH=N protons respectively. The signals observed as singlet in the region δ 10.20-10.40 ppm for the CHO protons of **1a-l** were absent in **2a-l**. In ^{13}C NMR spectra, the $CONH_2$ and C=N carbons of **2a-f** appeared in the region δ 156.62-158.45 ppm and δ 143.42-143.63 ppm while, $CSNH_2$ and C=N

carbons of **2g-l** appeared at the region δ 178.64-179.22 ppm and δ 142.75-143.18 ppm respectively. These spectral data support the formation of semicarbazones **2a-f** and thiosemicarbazones **2g-l**.

The compounds **3a-l** showed a singlet for two protons in the region δ 3.566-4.112 ppm for NH_2 protons in their ^1H NMR spectra. The signals are observed for CONH/CSNH and CH=N protons of **2a-l** were found absent. In their ^{13}C NMR spectra, the signals due to two N=C-O and N=C-S carbons appeared in the region δ 164.15-176.14 ppm. Further, all compounds showed signals due to aromatic, substituent protons and carbons in the expected region. Synthesized new molecules showed M+1 ion peak as a base peak in their mass spectra. Further, the analytical data obtained for the compounds **3a-l** were in good agreement with theoretically calculated data. All these spectral and analytical results confirmed the formation of the products.

2.2. Antimicrobial activity

Microbial studies of synthesized compounds were assessed by minimum inhibitory concentration (MIC) by serial dilution method[17]. The compounds were screened for their antimicrobial activities against Gram-negative bacteria species *Escherichia coli, Pseudomonas aeruginosa,* Gram-positive bacteria *Staphylococcus aureus,* fungi species *Aspergillus nigar, Aspergillus flavus* and *Candila albicans*. The experiments were carried out in triplicate; the results were taken as a mean of three determinations. Known antibiotics ciprofloxacin and fluconazole were used as standards for antibacterial and antifungal studies respectively. The results of MIC's were summarized in **Table 1** and **Table 2**.

Table 1. MIC's of the test compounds **2a-l** against bacterial and fungal species

Compound No.	Minimum inhibitory concentration (MIC's) in µg/mL					
	S. aureus	*E. coli*	*P. aeruginosa*	*A. niger*	*A. .flavus*	*C. albicans*
2a	25	25	50	25	25	50
2b	25	12.5	12.5	12.5	25	25
2c	50	25	25	25	25	50
2d	25	25	25	25	50	50
2e	25	12.5	25	25	25	25
2f	50	75	100	75	75	---
2g	25	50	12.5	25	50	25
2h	12.5	12.5	12.5	25	25	12.5
2i	25	12.5	12.5	25	50	25
2j	50	25	12.5	25	50	25
2k	50	25	12.5	25	50	50
2l	50	75	100	75	75	100
ciprofloxacin	25	12.5	12.5	----	----	----
fluconazole	----	----	----	12.5	25	25

The synthesized semicarbazones and thiosemicarbazones exerted a wide range of modest to good *in vitro* antibacterial activity against the tested organisms. Compounds **2a, 2g** having no substitutions, and **2d, 2j** with methoxy substitution on the aromatic ring showed moderate activity against tested species. Compounds **2c, 2e** and **2k** having CH_3 substituent showed moderate activity and compound **2i** containing CH_3 substitution exhibited equal activity compared with that of standard. It has been interesting from the results of the study that chloro substitution in the synthesized compounds enhanced the activity to the greater extent. **2b** demonstrated excellent activity against all and **2h** against *S.aureus* organisms. Nitro substitution present in compounds **2f** and **2l** retarded the inhibitory effect against the organism tested.

Compounds **2a** and **2g** showed moderate antifungal activity against the tested species. Compounds **2c, 2e, 2i** and **2k** having methyl and **2d, 2j** having methoxy substitution showed moderate activity. Compounds **2b** and **2h** having chloro substitution exhibited inhibition to a remarkable extent; while **2f** and **2l** with electron withdrawing nitro substitution showed lesser activity against the tested organisms.

Table 2. MIC's of the test compounds **3a-l** against bacterial and fungal species

Compound No.	Minimum inhibitory concentration (MIC's) in µg/mL					
	S. aureus	*E. coli*	*P. aeruginosa*	*A. niger*	*A. flavus*	*C. albicans*
3a	25	25	25	25	25	50
3b	25	12.5	12.5	12.5	25	25
3c	25	12.5	25	25	25	50
3d	25	12.5	25	25	25	50
3e	25	25	25	25	50	25
3f	50	75	100	75	100	50
3g	25	25	50	25	25	50
3h	12.5	12.5	12.5	12.5	25	25
3i	25	50	12.5	25	50	25
3j	50	12.5	25	12.5	25	50
3k	50	25	25	25	25	50
3l	50	100	75	100	50	50
ciprofloxacin	25	12.5	12.5	----	----	----
fluconazole	----	----	----	12.5	25	25

The synthesized new 1,3,4-oxadiazoles and 1,3,4-thiadiazoles demonstrated moderate to excellent antibacterial and antifungal activity by inhibiting the tested organisms. Compounds, **3a, 3g** showed moderate activity, chloro substituted compound **3b** showed excellent antibacterial activity against all the tested organisms. Compound **3h** showed the highest activity against *Staphylococcus aureus* compared with standard ciprofloxacin. Compounds **3c, 3e, 3i** and **3k** having methyl substitution exhibited moderate to good activity, **3d** and **3j** having methoxy substitution showed good activity, Compounds **3f** and **3l** having nitro substitution exhibit lesser activity against the organisms tested.

Compounds **3a, 3g** having no substitution, and compounds **3d, 3j** having methoxy substitution exhibited moderate activity against the fungal species tested. However, compounds **3c, 3e, 3i** and **3k** having methyl substitution showed moderate to good activity. Chloro substitution present in **3b** and **3h** demonstrated excellent activity and **3f** and **3l** having nitro substitution exhibited lesser activity against the fungal organisms tested.

In an attempt to interpret and correlate the molecular parameters of the small molecules with the potency of inhibition against the various microorganisms, detailed quantitative structure-activity relationship (QSAR) analysis was carried out. Physicochemical parameters for the small molecules were computed[18] and both pair-wise and multivariate analysis was carried out as specified in the literature[19,20]. Further, parameters like LogP, aromatic bond content, molecular weight, number of atoms and bonds were negatively correlated with inhibition potency.

Analysis of the results indicates that the small-molecule features that likely contribute to increased potency of inhibition vary across different microorganisms. This is an encouraging observation since specific variation of a particular molecular feature would lead to increased specificity towards a particular kind of microorganism. Further, this analysis also points out to the parameters that can be modulated to increase the potency of these compounds in general across the different microorganisms employed. However, care must be exercised in interpreting these results given the small sample size that was employed across compounds **2a-l** and **3a-l** and the fact that MIC was considered as the dependent variable.

The effect of substitution in the aromatic ring of synthesised compounds has been studied based on their *in vitro* antimicrobial activity results. Monochloro substitution in carbazone, **2b** and thiosemicarbazine, **2h**; 1, 3, 4-oxadiazole, **3b** and 1, 3, 4-thiadiazole, **3h** bearing pyrazole scaffold showed good antimicrobial activity. Among these scaffolds, ortho substitution **3b** and **3h** showed high efficiency then para substitution **2b** and **2h**, in antimicrobial. So this suggest that ortho monochloro

substitution plays a very vital role in hamper the cellular architecture of *E. coli, P. aeruginosa, S. aureus,* fungi species *A. niger, A. flavus* and *C. albicans*. Results suggest that **3h** could actively inhibit the growth of gram positive (*S. aureus*) and gram negative (*E. coli* and *P. aeruginosa*).

Comparative analysis illustrate that, among **2b**/**3b** and **2h**/**3h** shows that, sulfur moiety in 1, 3, 4-thiadiazoles, **2h**/**3h** act as potent inhibitor for both gram positive as well as gram negative bacteria. In case of moderate electronegative elements like sulfur and chlorine containing compounds, **3h** showed better *in vitro* activities, comparatively then at of higher electronegative element oxygen, **3b**. Therefore, the substitution and position of chloro plays a very important role in enhancing the bioactivities of the compound. Thus the *in vitro* data suggest that monochloro substitution at ortho position, **3h** is most favorable for enhancing the antimicrobial activity.

2.3. Antioxidant activities

2.3.1. DPPH radical scavenging activity

Antioxidants are characterized by their ability to scavenge free radicals. Proton radical scavenging action is an important attribute of antioxidants, which are measured by DPPH scavenging assay. This assay was performed by a method reported by Renuka *et al.*[17] 1 mL of DPPH solution (0.1 mM in 95% methanol) was mixed with different aliquots of test samples (25, 50, 75 and 100 µg/ml) in methanol. The mixture was shaking vigorously and allowed to stand for 20 min at room temperature. The absorbance was read against blank at 517 nm in an ELICO SL 159 UV visible spectrophotometer. The free radical scavenging potential was calculated as a percentage (I %) of DPPH decoloration using the equation:

$$I\% \text{ of scavenging} = (A_0\text{-}A_1/A_0) \times 100$$

where A_0 is the absorbance of the control reaction mixture excluding the test compounds, and A_1 is the absorbance of the test compounds. Tests were carried out in triplicate and the results are expressed as I% ± Standard Deviations and were summarized in **Table 3**.

2.3.2. Nitric oxide radical scavenging assay

This assay was performed by a method reported by Padmaja *et al.*[21] Nitric oxide (NO) was generated from sodium nitroprusside (SNP) and it was measured by the Griess reaction. Nitric oxide was generated by the sodium nitroprusside in phosphate buffer at physiological pH and then nitric oxide was reacted with oxygen, produced the nitrite ions, which can be estimated by the Griess Reagent. 1 mL of Sodium nitroprusside (10 mM), 1.5 ml of phosphate buffer (pH 7.4) was mixed with the test solution (25, 50, 75 and 100 µg/ml) and incubated 25 °C for 150 min, to this 1 mL of Griess reagent (1 % sulfanilamide in 2 % phosphoric acid and 0.1% *N*-(1-naphthyl) ethylenediaminedihydrochloride) was added and allowed to stand for 3 min, the absorbance of the chromatophore was read at 546 nm. Ascorbic acid was used as standard. The experiments were carried out at four different concentrations in triplicates and the results are expressed as I% ± Standard Deviations and were summarized in **Table 4**.

2.3.3. Hydroxyl radical scavenging assay

Hydroxy radical scavenging assay was carried out according to the reported procedure.[22] Product formed by the degraded deoxyribose was on heating with thiobarbituric acid (TBA) form a pink colored chromogen. This confirms the formation of $OH^{\cdot}$. The addition of the tested compound with the reaction mixture, they distant the hydroxy radicals from the deoxyribose and prevent their degradation. This experiment was performed by mixing 0.1 mL of phosphate buffer; 0.2 mL of 2-deoxyribose, test solution (25, 50, 75 and 100 µg/ml), 0.1 mL of H_2O_2 (10 mM), 0.1 mL of ascorbic acid (1 mM), 0.1

mL of EDTA and 0.01 mL of $FeCl_3$ (100 mM) was incubated at 37 °C for 60min. Thereafter, the reaction was terminated by adding 1 mL of cold 2.8% trichlroacetic acid and the reaction product was measured by adding 1 mL of 1% thiobarbituric acid (1g in 100mL of 0.05 N NaOH) in boiling water for 15 min. The absorbance was measured at 535 nm. BHA was used as a positive control. Decreased absorbance of the reaction mixture indicates increased hydroxyl radical scavenging activity. The experiment was carried out in triplicate and the results were expressed as I% ± standard deviations and were summarized in **Table 5**.

Table 3. Antioxidant activity of compounds **3a-l** in DPPH method

Test samples	% Radical Scavenging activity			
	25 (µg/mL)	50 (µg/mL)	75 (µg/mL)	100 (µg/mL)
3a	26.53±0.23	36.80±0.34	38.91±0.15	50.19.±0.96
3b	33.89±0.84	43.22±0.98	49.57±0.46	52.67±0.25
3c	23.71±0.52	33.25±0.28	38.93±0.34	46.44±0.24
3d	28.81±0.32	37.54±0.37	42.28±0.44	49.23±0.61
3e	25.45±0.19	35.71±0.54	39.11±0.40	48.43±0.50
3f	18.60±0.29	28.67±0.42	35.27±0.35	46.25±0.53
3g	26.48±0.20	36.60±0.51	39.82±0.26	50.90±0.55
3h	35.69±0.58	46.92±0.32	51.58±0.51	54.83±0.72
3i	25.00±0.34	35.04±0.27	39.84±0.17	47.75±0.24
3j	29.20±0.41	37.85±0.27	43.58±0.36	50.78±0.73
3k	25.83±0.39	35.75±0.34	40.19±0.31	48.85±0.73
3l	22.05±0.29	24.33±0.19	28.47±0.53	37.68±0.71
Ascorbic acid	25.68±0.32	34.94±0.40	39.42±0.37	49.46±0.45

A freshly prepared DPPH solution shows a deep purple color with an absorption maximum at 517 nm. Changes in the purple color to yellow indicate decreased in the absorbance. This is because of the antioxidant molecule reduce the DPPH free radical through donation of a hydrogen atom. Hence, instantaneously or concomitant decrease in absorbance was found, which indicates that the more potent antioxidant activity of the compound. **Table 3** shows all the newly synthesized compounds were exhibited moderate to good activity because of their H-donating capacity. Compounds **3b** and **3h** having chloro substituent and compounds **3d** and **3j** having methoxy substituent showed the stronger DPPH scavenging activity than others. Nitro substituent compound **3f** and **3l** have shown less activity compared with the standard ascorbic acid, while the remaining compounds exhibited moderate activity.

Table 4. Antioxidant activity of compounds **3a-l** in nitric oxide method

Test samples	% Radical Scavenging activity			
	25 (µg/mL)	50 (µg/mL)	75 (µg/mL)	100 (µg/mL)
3a	09.06±0.64	18.96±0.69	32.56±0.47	39.14±0.18
3b	16.57±0.39	23.95±0.25	41.27±0.29	46.31±0.18
3c	11.12±0.51	20.41±0.55	33.52±0.37	39.70±0.59
3d	11.10±0.48	19.13±0.56	37.49±0.24	39.28±0.73
3e	11.09±0.48	20.12±0.54	34.00±0.39	39.69±0.62
3f	08.19±0.88	17.29±0.52	28.15±0.39	32.53±0.53
3g	08.93±0.22	17.79±0.43	33.28±0.49	39.96±0.20
3h	19.63±0.22	25.66±0.25	41.00±0.47	48.45±0.58
3i	11.71±0.25	21.78±0.30	34.89±0.85	39.78±0.47
3j	14.19±0.35	22.22±0.22	36.13±0.68	39.89±0.24
3k	13.02±0.46	21.94±0.35	35.14±0.44	39.73±0.42
3l	10.09±0.46	17.66±0.55	25.78±0.36	34.78±0.71
Ascorbic acid	11.05±0.41	19.49±0.27	35.06±0.33	38.81±0.39

Nitric oxide plays a significant role in inflammatory processes. In the immunological system, it fights against tumor cells and infectious agents. During inflammatory reactions, nitric oxide is produced by the inducible enzyme nitric oxide synthase in cells like macrophages and renal cells after stimulation by lipopolysaccharide. NO react with oxygen or superoxide anion radical to form even stronger oxidant

peroxynitrite.[20] Compounds, **3b, 3h** having chloro substitution in the phenyl ring showed greater ability to scavenge NO radical. Compounds, **3f, 3l** having nitro substituent showed least activity compare to standard and the remaining compounds displayed moderate activity.

Table 5. Antioxidant activity of compounds **3a-l** in Hydroxyl radical method

Test samples	% Radical Scavenging activity			
	25 (µg/mL)	50 (µg/mL)	75 (µg/mL)	100 (µg/mL)
3a	08.75±0.60	14.34±0.81	28.96±0.77	32.68±0.62
3b	20.21±0.40	24.79±0.49	38.00±0.88	40.89±0.78
3c	11.54±0.41	16.46±0.39	29.24±0.84	32.06±0.52
3d	12.88±0.22	18.82±0.68	31.58±0.72	35.89±0.52
3e	11.96±0.21	16.46±0.37	29.26±0.83	32.05±0.52
3f	02.73±0.54	12.33±0.60	15.63±0.38	30.32±0.89
3g	08.63±0.18	13.11±0.35	29.73±0.18	32.01±0.38
3h	24.32±0.40	28.54±0.51	36.04±0.77	42.43±0.32
3i	12.10±0.44	18.55±0.38	31.53±0.34	33.05±0.93
3j	13.45±0.71	19.36±0.32	31.97±0.55	34.91±0.37
3k	11.84±0.21	19.24±0.34	31.61±0.31	33.34±0.52
3l	06.46±0.47	12.61±0.33	24.58±0.84	30.74±0.58
BHA	09.32±0.37	14.34±0.63	27.40±0.63	32.62±0.54

The hydroxy radical is a highly reactive free radical formed in biological systems and it is able to damage the biomolecule found in living cells.[23] The hydroxy radical has the ability to break DNA and cause strand breakage, which contributes to carcinogenesis, mutagenesis and cytotoxicity. In the present investigation, compounds **3a-l** were found to be stronger to weak hydroxyl radical scavenging activity. Among the samples studied, compound **3b** and **3h** exhibited the remarkable capacity for scavenging hydroxyl radical which was significantly higher than that of the standard of BHA. Remaining compounds exhibited moderate activity.

Based on their *in vitro* antioxidant activity results, the effect of substitutions in the phenyl ring has been studied. Chloro substitution in carbazone, **2b** and thiosemicarbazine, **2h**; 1, 3, 4-oxadiazole, **3b** and 1, 3, 4-thiadiazole, **3h** bearing pyrazole scaffold showed good antioxidant activities. Among them, ortho substitution in **3b, 3h** showed greater antioxidant efficiency then para substitution in **2b, 2h**.

The hydroxyl group present parental scaffolds (1, 3, 4-oxadiazoles/thiadiazoles) acts as good free radicals scavenger. Sulfur-antioxidant paradox is well established in many bioactives like glutathione, thioredoxin and glutaredoxin efficiently form a line of defense against reactive oxygen and nitrogen species[24]. Hence sulfur containing compounds are known to be a very important in maintaining redox potentials in the system. Thus the *in vitro* data suggest that monochloro substitution at ortho position, **3h** is most favorable for enhancing the antioxidant activity. Whereas in case of mono methyl substitution or mono/di nitro substitutions no appreciable amount of activity, suggesting that hydrophobicity in case of **3c** and **3i** and increased electronegative atoms in case of **3f** and **3l** doesn't have any role in enhancing antioxidant activity of the presented novel 1, 3, 4-oxadiazoles/thiadiazoles bearing pyrazole scaffolds.

3. Conclusion

The simple easy accessible procedure for the synthesis of 1,3,4-oxadiazole and 1,3,4-thiadiazole incorporating pyrazole nucleus and their *in vitro* antimicrobial and antioxidant activity results revealed the significance of the study. All newly synthesized compounds exhibited moderate to good antimicrobial activity against the tested microorganisms, compounds having chloro substituent demonstrated potent antimicrobial activity. Compounds **3b** and **3h** showed significant antioxidant

activity in all the assays. The compounds, particularly **4b** exhibited greater activity in comparison to the standard drug. The SAR study of the synthesized compounds remains the topic of interest.

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4. Experimental

4.1 Materials and methods

Melting points were determined by an open capillary tube method and are uncorrected. Purity of the compounds was checked on thin layer chromatography (TLC) plates pre-coated with silica gel using the solvent system ethyl acetate: n-hexane (1:4 v/v). The spots were visualized under iodine vapors and UV light. The ^{1}H NMR and ^{13}C NMR spectra was recorded on a Spect 500 MHz and 125.6 MHz spectrophotometer respectively using DMSO as solvent and TMS as internal standard. The chemical shifts are expressed in δ ppm. Mass spectra were obtained on Shimadzu LCMS-2010A spectrophotometer (ESI). Elemental analysis was obtained on a Thermo Finnigan Flash EA 1112 CHN analyzer. Purification of compounds was done by column chromatography on silica gel (70-230 mesh, Merck).

4.2. General procedure

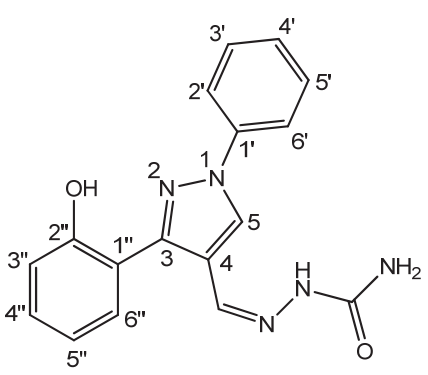
*General procedure for the synthesis of semicarbazones, **2a-f** and thiosemicarbazones, **2g-l**.*

To a solution of semicarbazide hydrochloride (1.115 g, 0.01 mol) and 3-(2-hydroxyphenyl)-1-aryl-1*H*-pyrazole-4-carbaldehyde, **1a-f** (2.64 g, 0.01 mol) in ethyl alcohol, 3-4 drops of acetic acid was added. The mixture was refluxed on a water bath for 2-3 h, the progress of the reaction was checked by TLC. After completion of the reaction, the mixture was poured in to crushed ice and mixed well; the solid separated was filtered, washed with water and recrystallized from ethyl alcohol to obtain the products **2a-f** in 80-88% yield. Under similar conditions **1a-f** with thiosemicarbazide hydrochloride yielded **2g-l** in 80-86%.

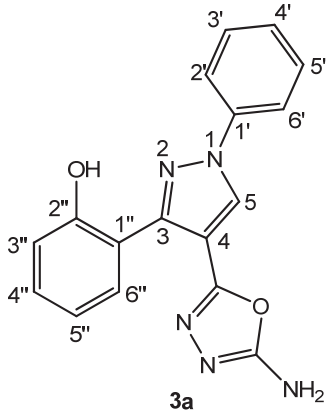
*General procedure for the synthesis of 1,3,4-oxadiazolyl pyrazole and 1,3,4-thiadiazolyl pyrazole **3a-l**.*

2-(4-(5-Amino-1,3,4-oxadiazol/thiadiazol-2-yl)-1-aryl-1*H*-pyrazol-3-yl)phenol **3a-l** were prepared by the oxidative cyclization of substituted semicarbazones/thiocarbazones **2a-l** (0.01 mol) and sodium acetate were dissolved in 25mL glacial acetic acid taken in a two necked round bottomed flask fitted with a dropping funnel which was supplied with (0.01 mol) of bromine dissolved in (8 mL) of glacial acetic acid. Bromine was added drop wise with stirring magnetically. The reaction mixture was stirred at room temperature for 2-3 h. The progress of the reaction was monitored by TLC, after completion of the reaction the solution was poured into crushed ice and swirled well. The resulting solid was filtered, washed with water and dried under vacuum to obtain a crude product, which was purified by column chromatography on silica gel (60-120 mesh) using ethyl acetate and hexane (1:4 v/v) as eluent.

4.3. Physical and Spectral Data



2a



3a

1-((3-(2-Hydroxyphenyl)-1-phenyl-1*H*-pyrazol-4-yl)methylene)semicarbazone, (2a)

Obtained as pale yellow solid in 88% yield (2.82 g), m. p. 190-192 ºC; ^{1}H NMR (500 MHz, DMSO-*d*$_6$) δ (ppm): 4.822 (s, 2H, -NH_2), 6.611 (s, 1H, NH), 6.921 (s, 1H, pyrazole-H), 7.132-7.481 (m, 9H, Ar-H), 8.014 (s, 1H, N=CH), 8.653 (s, 1H, OH); ^{13}C NMR (125.7 MHz, DMSO-d_6) δ (ppm): 112.34 (1C, C-4), 115.38 (1C, C-3''), 119.54 (2C, C-2'& C-6'), 121.46 (1C, C-1''), 122.34 (1C, C-5J''), 125.13 (1C, C-4'), 127.31 (1C, C-6''), 128.92 (2C, C-3'& C-5'), 129.63 (1C, C-4''), 131.89 (1C, C-3), 138.48 (1C, C1'), 143.65 (1C, C=N), 148.23 (1C, C-5), 153.76 (1C, C-2''), 156.62 (1C, C=O). MS (*m/z*): 322 (M+1). Anal. Calcd. for $C_{17}H_{15}N_5O_2$ (%): C, 63.54; H, 4.71; N, 21.79. Found: C, 63.78; H, 4.50; N, 21.98.

1-((1-(4-Chlorophenyl)-3-(2-hydroxyphenyl)-1*H*-pyrazol-4-yl)methylene)semicarbazone, (2b).

Obtained as a pale yellow solid in 87% yield (3.08 g), m. p. 183-184 ºC; ^{1}H NMR (500 MHz, DMSO-*d*$_6$) δ (ppm): 4.810 (s, 2H, NH_2), 6.632 (s, 1H, NH), 6.824 (s, 1H, pyrazole-H), 7.152-7.293 (m, 4H, Ar-H), 7.384 (d, 2H, H-2'& H-6'), 7.526 (d, 2H, H-3'& H-5'), 8.118 (s, 1H, N=CH), 8.615 (s, 1H, OH); MS (*m/z*) : 357 (M+2 ^{37}Cl), 355 (M^{+} ^{35}Cl). Anal. Calcd. for $C_{17}H_{14}ClN_5O_2$ (%): C, 57.39; H, 3.97; N, 19.68. Found: C, 57.64; H, 3.68; N, 19.85.

1-((3-(2-Hydroxyphenyl)-1-*o*-tolyl-1*H*-pyrazol-4-yl)methylene)semicarbazone, (2c).

Obtained as a pale yellow solid in 85% yield (2.85 g), m. p. 173-174 ºC; ^{1}H NMR (500 MHz, DMSO-*d*$_6$) δ (ppm): 2.327 (s, 3H, CH_3), 4.814 (s, 2H, NH_2), 6.621 (s, 1H, NH), 6.827 (s, 1H, pyrazole-H), 7.152-7.254 (m, 4H, Ar-H), 7.324 (d, 1H, H-3'), 7.418 (t, 2H, H-4' & H-5'), 7.481 (d, 1H, H-6'), 8.146 (s, 1H, N=CH), 8.621 (s, 1H, OH). ^{13}C NMR (125.7 MHz, DMSO-d_6) δ (ppm): 15.85 (1C, CH_3), 111.72 (1C, C-4), 115.87 (1C, C-3''), 119.24 (1C, C-6'), 120.52 (1C, C-1''), 122.64 (1C, C-5''), 123.93 (1C, C-2'), 125.85 (1C, C-4'), 126.37 (1C, C-5'), 127.22 (1C, C-6''), 128.47 (1C, C-3'), 129.46 (1C, C-4''), 131.27 (1C, C-3), 138.54 (1C, C1'), 143.42 (1C, C=N), 149.84 (1C, C-5), 154.33 (1C, C-2''), 158.47 (1C, C=O). Anal. Calcd. for $C_{18}H_{17}N_5O_2$ (%): C, 64.47; H, 5.11; N, 20.88. Found: C, 64.23; H, 5.38; N, 20.69.

1-((3-(2-Hydroxyphenyl)-1-(2-methoxyphenyl)-1*H*-pyrazol-4-yl)methylene)semicarbazone, (2d).

Obtained as a pale yellow solid in 82% yield (2.87 g), m. p. 166-167 ºC; ^{1}H NMR (500 MHz, DMSO-*d*$_6$) δ (ppm): 3.525 (s, 3H, OCH_3), 4.821 (s, 2H, NH_2), 6.628 (s, 1H, NH), 6.883 (s, 1H, pyrazole-H), 7.151-7.262 (m, 4H, Ar-H), 7.316 (d, 1H, H-3'), 7.412 (t, 2H, H-4' & H-5'), 7.463 (d, 1H, H-6'), 8.013 (s, 1H, N=CH), 8.612 (s, 1-H, OH). Anal. Calcd. for $C_{18}H_{17}N_5O_3$ (%): C, 61.53; H, 4.88; N,19.93. Found: C, 61.72; H, 4.63; N, 19.54.

1-((3-(2-Hydroxyphenyl)-1-(2,4-dimethylphenyl)-1*H*-pyrazol-4-yl)methylene)semicarbazone, (2e).

Obtained as a pale yellow solid in 84% yield (2.93 g), m. p. 164-166 ºC; ^{1}H NMR (500 MHz, DMSO-*d*$_6$) δ (ppm): 2.357 (s, 6H, 2CH$_3$), 4.834 (s, 2H, NH$_2$), 6.652 (s, 1H, NH), 6.823 (s, 1H, pyrazole-H), 7.113-7.244 (m, 4H, Ar-H), 7.312 (s, 1H, H-3'), 7.383 (d, 1H, H-5'), 7.437 (d, 1H, H-6'), 8.114 (s, 1-H, N=CH), 8.562 (s, 1-H, OH). Anal. Calcd. for $C_{19}H_{19}N_5O_2$ (%): C, 65.32; H, 5.48; N, 20.04. Found: C, 65.68; H, 5.23; N, 20.25.

1-((3-(2-Hydroxyphenyl)-1-(2,4-dinitrophenyl)-1*H*-pyrazol-4-yl)methylene)semicarbazone, (2f).

Obtained as a pale yellow solid in 80% yield (3.28g), m. p. 204-205 ºC; MS (*m/z*): 412 (M+1). Anal. Calcd. for $C_{17}H_{13}N_7O_6$ (%): C, 49.64; H, 3.19; N, 23.84. Found: C, 49.23; H, 3.42; N, 23.33.

1-((3-(2-Hydroxyphenyl)-1-phenyl-1*H*-pyrazol-4-yl)methylene)thiosemicarbazone, (2g).

Obtained as a pale yellow solid in 86% yield (2.89 g), m. p. 170-172 ºC; ^{1}H NMR (500 MHz, DMSO-*d*$_6$) δ (ppm): 2.214 (s, 2H, NH$_2$), 4.5 (s, 1H, NH), 6.2 (s, 1H, pyrazole-CH), 7.05-7.41 (m, 9H, Ar-H), 8.20 (s, 1H,N=CH), 8.53 (s, 1H, OH). Anal. Calcd. for $C_{17}H_{15}N_5OS$ (%): C, 60.52; H, 4.48; N, 20.76. Found: C, 60.81; H, 4.68; N, 20.55.

1-((1-(2-Chlorophenyl)-3-(2-hydroxyphenyl)-1-phenyl-1*H*-pyrazol-4-yl)methylene)thiosemicarbazone, (2h).

Obtained as a pale yellow solid in 80% yield (2.96 g), m. p. 168-169 ºC; ^{1}H NMR (500 MHz, DMSO-*d*$_6$) δ (ppm): 2.431 (s, 2H, NH$_2$), 4.618 (s, 1H, NH), 6.227 (s, 1H, pyrazole-H), 7.141-7.327 (m, 4H, Ar-H), 7.418 (d, 2H, H-2' & H-6'), 7.562 (d, 2H, H-3'& H-5'), 8.142 (s, 1H, N=CH), 8.651 (s, 1H, OH);MS (*m/z*): 373 (M+2 ^{37}Cl), 371(M^{+} ^{35}Cl). Anal. Calcd. for $C_{17}H_{14}ClN_5OS$ (%): C, 54.91; H, 3.79; N, 18.83. Found: C, 54.73; H, 3.94; N, 19.04.

1-((3-(2-Hydroxyphenyl)-1-*o*-tolyl-1*H*-pyrazol-4-yl)methylene)thiosemicarbazone, (2i).

Obtained as a pale yellow solid in 84% yield (2.94 g), m. p. 184-185 ºC; MS (*m/z*) 352 (M+1), Anal. Calcd. for $C_{18}H_{17}N_5OS$ (%): C, 61.52; H, 4.88; N, 19.93. Found: C, 61.82; H, 4.53; N, 20.54.

1-((3-(2-Hydroxyphenyl)-1-(2-methoxyphenyl)-1*H*-pyrazol-4-yl)methylene)thiosemicarbazone, (2j).

Obtained as a pale yellow solid in 86% yield (3.15 g), m. p. 169-170 ºC; ^{13}C NMR (125.7 MHz, DMSO-d$_6$) δ (ppm): 56.81 (1C, OCH$_3$), 111.62 (1C, C-4), 114.28 (1C, C-3'), 115.37 (1C, C-3''), 119.64 (1C, C-6'), 120.71 (1C, C-1''), 121.63 (1C, C-5'), 122.82 (1C, C-5''), 124.84 (1C, C-1'), 126.23 (1C, C-4'), 127.58 (1C, C-6''), 129.15 (1C, C-4''), 130.83 (1C, C-3), 142.74 (1C, C=N), 145.26 (1C, C-2'), 150.17 (1C, C-5), 154.58 (1C, C-2''), 179.26 (1C, C=S). MS (*m/z*): 352 (M+1). Anal. Calcd. for $C_{18}H_{17}N_5O_3$ (%): C, 61.53; H, 4.88; N, 19.93. Found: C, 61.81; H, 4.42; N, 19.52.

1-((3-(2-Hydroxyphenyl)-1-(2,4-dimethylphenyl)-1*H*-pyrazol-4-yl)methylene)thiosemicarbazone, (2k).

Obtained as a pale yellow solid in 81% yield (2.95 g), m. p. 188-190 ºC; ^{13}C NMR (125.7 MHz, DMSO-d$_6$) δ (ppm): 16.87 (1C,CH$_3$), 21.84 (1C, CH$_3$), 112.18 (1C, C-4), 115.83 (1C, C-3''), 119.26 (1C, C-6'), 120.53 (1C, C-1''), 122.31 (1C, C-5''), 124.57 (1C, C-2'), 125.29 (1C, C-5'), 127.16 (1C,

C-6''), 128.94 (1C, C-4''), 130.11 (1C, C-3), 131.82 (1C, C-3'), 135.12 (1C, C-1'), 136.23 (1C, C-4'), 143.14 (1C, C=N), 150.51 (1C, C-5), 155.27 (1C, C-2''), 178.63 (1C, C=S). MS (*m/z*): 366 (M+1). Anal. Calcd. for $C_{19}H_{19}N_5OS$ (%): C, 62.44; H, 5.24; N, 19.16. Found: C, 62.21; H, 5.47; N, 19.41.

1-((3-(2-Hydroxyphenyl)-1-(2,4-dinitrophenyl)-1*H*-pyrazol-4-yl)methylene)thiosemicarbazone, (2l).

Obtained as a pale yellow solid in 86% yield (3.67 g), m. p. 196-197 ºC; Anal. Calcd. for $C_{17}H_{13}N_7O_5S$ (%): C, 47.77; H, 3.07; N, 22.94. Found: C, 47.96; H, 3.42; N, 22.53.

2-(4-(5-Amino-1,3,4-oxadiazol-2-yl)-1-phenyl-1*H*-pyrazol-3-yl)phenol, (3a).

Obtained as a pale yellow solid in 85% yield (2.71 g), m. p. 180-182 ºC; ^{1}H NMR (500 MHz, DMSO-*d*$_6$) δ (ppm): 4.039 (s, 2H, NH_2), 6.528 (s, 1H, pyrazole CH), 7.121-7.457 (m, 9H, Ar-H), 8.273 (s, 1H, OH).^{13}C NMR (125.7 MHz, DMSO-d_6) δ (ppm): 110.23 (1C, C-4), 116.21 (1C, C-3''), 120.19 (2C, C-2' & C-6'), 120.85 (1C, C-1''),121.57 (1C, C-5''), 125.46 (1C, C-4'), 127.31 (1C, C-6''), 128.76 (2C, C-3' &C-5'), 129.62 (1C, C-4''), 131.28 (1C, C-3), 139.54 (1C, C1'), 145.86 (1C, C-5), 154.84 (1C, C-2''), 164.12 (1C, $C=N_{oxadiazole}$), 176.45 (1C, C-NH_2). MS (*m/z*): 320 (M+1). Anal. Calcd. for $C_{17}H_{13}N_5O_2$ (%): C, 63.94; H, 4.10; N, 21.93. Found: C, 63.28; H, 4.53; N, 21.62.

2-(4-(5-Amino-1,3,4-oxadiazol-2-yl)-1-(4-chlorophenyl)-1*H*-pyrazol-3-yl)phenol, (3b).

Obtained as a pale yellow solid in 78% yield (2.75 g), m. p.193-195 ºC; ^{1}H NMR (500 MHz, DMSO-*d*$_6$) δ (ppm): 4.128 (s, 2H, NH_2), 6.484 (s, 1H, pyrazole CH), 7.182-7.524 (m, 4H, Ar-H), 8.216 (s, 1H, OH).^{13}C NMR (125.7 MHz, DMSO-d_6) δ (ppm): 110.85 (1C, C-4), 115.83 (1C, C-3''), 120.42 (1C, C-1''), 121.83 (1C, C-5''), 122.38 (2C, C-2' & C-6'), 128.24 (1C, C-6''), 129.62 (2C, C-3' &C-5'), 130.45 (1C, C-4''), 131.56 (1C, C-3), 133.22 (1C, C-4'), 136.46 (1C, C1'), 144.79 (1C, C-5), 155.85 (1C, C-2''), 164.91 (1C, $C=N_{oxadiazole}$), 175.22 (1C, C-NH_2). MS (*m/z*): 355 (M+2 ^{37}Cl), 353 (M^+ ^{35}Cl). Anal. Calcd. for $C_{17}H_{12}ClN_5O_2$ (%): C, 57.72; H, 3.42; N, 19.80. Found: C, 57.45; H, 3.78; N, 19.34.

2-(4-(5-Amino-1,3,4-oxadiazol-2-yl)-1-*o*-tolyl-1*H*-pyrazol-3-yl)phenol, (3c).

Obtained as a pale yellow solid in 76% yield (2.53 g), m. p. 156-158 ºC; ^{1}H NMR (500 MHz, DMSO-*d*$_6$) δ (ppm): 2.322 (s, 3H, CH_3), 4.086 (s, 2H, NH_2), 6.571 (s, 1H, pyrazole CH), 7.125-7.323 (m, 8H, Ar-H), 8.214 (s, 1H, OH). ^{13}C NMR (125.7 MHz, DMSO-d_6) δ (ppm):15.86 (1C, CH_3), 109.74 (1C, C-4), 116.23 (1C, C-3''), 120.94 (1C, C-1''), 121.66 (1C, C-5''), 123.68 (1C, C-2'), 122.57 (1C, C-6'), 128.76 (1C, C-6''), 129.54 (1C, C-3') 125.45 (1C, C-5'), 129.43 (1C, C-4''), 131.72 (1C, C-3), 125.83 (1C, C-4'), 137.64 (1C, C1'), 145.43 (1C, C-5), 154.91 (1C, C-2''), 165.32 (1C, $C=N_{oxadiazole}$), 176.72 (1C, C-NH_2). MS (*m/z*): 334 (M+1). Anal. Calcd. for $C_{18}H_{15}N_5O_2$ (%): C, 64.86; H, 4.54; N, 21.01. Found: C, 64.47; H, 4.93; N, 21.43.

2-(4-(5-Amino-1,3,4-oxadiazol-2-yl)-1-(2-methoxyphenyl)-1*H*-pyrazol-3-yl)phenol, (3d).

Obtained as a pale yellow solid in 81% yield (2.82 g), m. p. 161-162 ºC; ^{1}H NMR (500 MHz, DMSO-*d*$_6$) δ (ppm): 3.657 (s, 3H, OCH_3), 4.126 (s, 2H, NH_2), 6.537 (s, 1H, pyrazole CH), 7.028-7.354 (m, 8H, Ar-H), 8.216 (s, 1H, OH). ^{13}C NMR (125.7 MHz, DMSO-d_6) δ (ppm): 55.81 (1C, OCH_3), 110.82 (1C, C-4), 114.24 (1C, C-3'), 115.45 (1C, C-3''), 119.22 (1C, C-1''), 121.34 (1C, C-5''), 121.87 (1C, C-6'),122.53 (1C, C-5'), 124.64 (1C, C1'), 126.71 (1C, C-4'), 128.17 (1C, C-6''), 129.63 (1C, C-4''), 131.94 (1C, C-3), 143.61 (1C, C-2'), 144.88 (1C, C-5), 155.44 (1C, C-2''), 165.76 (1C, $C=N_{oxadiazole}$), 175.61 (1C, C-NH_2). MS (*m/z*): 350 (M+1). Anal. Calcd. for $C_{18}H_{15}N_5O_3$ (%): C, 61.89; H, 4.33; N, 20.05. Found: C, 61.52; H, 4.76; N, 20.48.

2-(4-(5-Amino-1,3,4-oxadiazol-2-yl)-1-(2,4-dimethylphenyl)-1*H*-pyrazol-3-yl)phenol, (3e).

Obtained as a pale yellow solid in 77% yield (2.67 g), m. p. 158-160 ºC; ^{1}H NMR (500 MHz, DMSO-*d*$_6$) δ (ppm): 2.362 (s, 6H,2CH$_3$), 4.112 (s, 2H, NH$_2$), 6.563 (s, 1H, pyrazole CH), 6.984-7.363 (m, 7H, Ar-H), 8.271 (s, 1H, OH). ^{13}C NMR (125.7 MHz, DMSO-d$_6$) δ (ppm): 16.24 (1C, CH$_3$), 22.49 (1C, CH$_3$), 110.23 (1C, C-4), 116.88 (1C, C-3''), 120.13 (1C, C-6'), 120.85 (1C, C-1''), 121.86 (1C, C-5''), 124.59 (1C, C-2'), 127.54 (1C, C-5'), 128.16 (1C, C-6''), 129.47 (1C, C-4''), 131.34 (1C, C-3), 132.23 (1C, C-3'), 134.47 (1C, C1'), 136.46 (1C, C-4'), 145.35 (1C, C-5), 155.98 (1C, C-2''), 164.87 (1C, C=N$_{oxadiazole}$), 176.32 (1C, C-NH$_2$). MS (*m/z*) 348 (M+1). Anal. Calcd. for C$_{19}$H$_{17}$N$_5$O$_2$(%): C, 65.69; H, 4.93; N, 20.16. Found: C, 65.24; H, 4.58; N, 20.53.

2-(4-(5-Amino-1,3,4-oxadiazol-2-yl)-1-(2,4-dinitrophenyl)-1*H*-pyrazol-3-yl)phenol, (3f).

Obtained as a pale yellow solid in 78% yield (3.19 g), m. p. 188-189 ºC; ^{1}H NMR (500 MHz, DMSO-*d*$_6$) δ (ppm): 4.129 (s, 2H, NH$_2$), 6.726 (s, 1H, pyrazole CH), 7.128-7.644 (m, 7H, Ar-H), 8.251 (s, 1H, OH).^{13}C NMR (125.7 MHz, DMSO-d$_6$) δ (ppm): 111.49 (1C, C-4), 117.37 (1C, C-3''), 118.35 (1C, C-3'), 121.33 (1C, C-1''), 121.87 (1C, C-5''), 122.54 (1C, C-6'), 126.74 (1C, C-5'), 128.66 (1C, C-6''), 131.47 (1C, C-4''), 131.92 (1C, C-3), 138.72 (1C, C-2'), 142.47 (1C, C1'), 146.25 (1C, C-5), 146.48 (1C, C-4'), 154.81 (1C, C-2''), 165.64 (1C, C=N$_{oxadiazole}$), 176.93 (1C, C-NH$_2$). MS (*m/z*): 410 (M+1). Anal. Calcd. for C$_{17}$H$_{11}$N$_7$O$_6$ (%): C, 49.88; H, 2.71; N, 23.95. Found: C, 50.21; H, 3.22; N, 23.62.

2-(4-(5-Amino-1,3,4-thiadiazol-2-yl)-1-phenyl-1*H*-pyrazol-3-yl)phenol, (3g).

Obtained as a pale yellow solid in 82% yield (2.74 g), m. p. 192-195 ºC; ^{1}H NMR (500 MHz, DMSO-*d*$_6$) δ (ppm): 3.982 (s, 2H, NH$_2$), 6.424 (s, 1H, pyrazole CH), 7.145-7.362 (m, 9H, Ar-H), 8.183 (s, 1H, OH).^{13}C NMR (125.7 MHz, DMSO-d$_6$) δ (ppm): 112.81 (1C, C-4), 117.68 (1C, C-3''), 120.54 (2C, C-2' & C-6'), 121.69 (1C, C-1''), 122.35 (1C, C-5''), 126.28 (1C, C-4'), 128.26 (1C, C-6''), 128.62 (1C, C-4''), 129.83 (2C, C-3' & C-5'), 132.14 (1C, C-3), 138.66 (1C, C1'), 146.35 (1C, C-5), 155.42 (1C, C-2''), 162.54 (1C, C-NH$_2$), 174.22 (1C, C=N$_{oxadiazole}$). MS (*m/z*): 336 (M+1). Anal. Calcd. for C$_{17}$H$_{13}$N$_5$OS (%): C, 60.88; H, 3.91; N, 20.88. Found: C, 60.57; H, 4.26; N, 20.42.

2-(4-(5-Amino-1,3,4-thiadiazol-2-yl)-1-(4-chlorophenyl)-1*H*-pyrazol-3-yl)phenol, (3h).

Obtained as a pale yellow solid in 80% yield (2.95 g), m. p. 174-176 ºC; ^{1}H NMR (500 MHz, DMSO-*d*$_6$) δ (ppm): 3.966 (s, 2H, NH$_2$), 6.465 (s, 1H, pyrazole CH), 7.162-7.433 (m, 8H, Ar-H), 8.117 (s, 1H, OH). ^{13}C NMR (125.7 MHz, DMSO-d$_6$) δ (ppm): 112.32 (1C, C-4), 116.73 (1C, C-3''), 122.76 (2C, C-2' & C-6'), 120.44 (1C, C-1''), 121.81 (1C, C-5''), 132.63 (1C, C-4'), 127.37 (1C, C-6''), 129.35 (1C, C-4''), 128.46 (2C, C-3' & C-5'), 131.78 (1C, C-3), 137.46 (1C, C1'), 145.29 (1C, C-5), 155.92 (1C, C-2''), 161.81 (1C, C-NH$_2$), 175.31 (1C, C=N$_{oxadiazole}$). MS (*m/z*): 371 (M+2 ^{37}Cl), 369 (M^{+} ^{35}Cl). Anal. Calcd. for C$_{17}$H$_{12}$ClN$_5$OS (%): C, 55.21; H, 3.27; N, 18.94. Found: C, 55.53; H, 3.64; N, 19.32.

2-(4-(5-Amino-1,3,4-thiadiazol-2-yl)-1-*o*-tolyl-1*H*-pyrazol-3-yl)phenol, (3i).

Obtained as a pale yellow solid in 76% yield (2.65 g), m. p. 163-165 ºC; ^{1}H NMR (500 MHz, DMSO-*d*$_6$) δ (ppm): 2.376 (s, 3H, CH$_3$), 3.943 (s, 2H, NH$_2$), 6.481 (s, 1H, pyrazole CH), 7.084-7.352 (m, 8H, Ar-H), 8.131 (s, 1H, OH). ^{13}C NMR (125.7 MHz, DMSO-d$_6$) δ (ppm): 16.76 (1C, CH$_3$), 111.42 (1C, C-4), 115.22 (1C, C-3''), 118.21 (1C, C-6'), 121.67 (1C, C-1''), 122.73 (1C, C-5''), 123.88 (1C, C-2'), 125.25 (1C, C-4'), 125.94 (1C, C-5'), 128.64 (1C, C-6''), 130.26 (1C, C-4''), 131.43 (1C, C-3), 132.69 (1C, C-3'), 139.22 (1C, C1'), 146.54 (1C, C-5), 154.66 (1C, C-2''), 162.89 (1C, C-NH$_2$),

174.87 (1C, C=$N_{oxadiazole}$). MS (*m/z*): 350 (M+1). Anal. Calcd. for $C_{18}H_{15}N_5OS$ (%): C, 61.87; H, 4.33; N, 20.04. Found: C, 61.46; H, 4.68; N, 20.34.

2-(4-(5-Amino-1,3,4-thiadiazol-2-yl)-1-(2-methoxyphenyl)-1*H*-pyrazol-3-yl)phenol, (3j).

Obtained as a pale yellow solid in 79% yield (2.88 g), m. p. 171-172 ºC; ^{1}H NMR (500 MHz, DMSO-*d*$_6$) δ (ppm): 3.524 (s, 3H,OCH_3), 3.917 (s, 2H, NH_2), 6.436 (s, 1H, pyrazole CH), 7.141-7.355 (m, 8H, Ar-H), 8.156 (s, 1H, OH). ^{13}C NMR (125.7 MHz, DMSO-d_6) δ (ppm): 55.42 (1C, OCH_3), 110.86 (1C, C-4), 113.82 (1C, C-3'), 116.94 (1C, C-3''), 120.57 (1C, C-1''), 121.18 (1C, C-6'), 122.23 (1C, C-5'), 122.94 (1C, C-5''), 124.36 (1C, C1'), 128.21 (1C, C-4'), 127.52 (1C, C-6''), 130.61 (1C, C-4''), 131.77 (1C, C-3), 143.95 (1C, C-2'), 145.42 (1C, C-5), 153.93 (1C, C-2''), 161.52 (1C, C-NH_2), 175.48 (1C, C=$N_{oxadiazole}$). MS (*m/z*): 366 (M+1). Anal. Calcd. for $C_{18}H_{15}N_5O_2S$ (%): C, 59.16; H, 4.14; N, 19.17%. Found: C, 58.78; H, 4.43; N, 19.46.

2-(4-(5-Amino-1,3,4-thiadiazol-2-yl)-1-(2,4-dimethylphenyl)-1*H*-pyrazol-3-yl)phenol, (3k).

Obtained as a pale yellow solid in 78% yield (2.83 g), m. p. 181-183 ºC; ^{1}H NMR (500 MHz, DMSO-*d*$_6$) δ (ppm): 2.356 (s, 6H, $2CH_3$), 3.954 (s, 2H, NH_2), 6.418 (s, 1H, pyrazole CH), 7.102-7.374 (m, 7H, Ar-H), 8.184 (s, 1H, OH).^{13}C NMR (125.7 MHz, DMSO-d_6) δ (ppm): 16.23 (1C, CH_3), 23.18 (1C, CH_3), 111.65 (1C, C-4), 114.82 (1C, C-3''), 119.42 (1C, C-1''), 120.74 (1C, C-6'), 122.51 (1C, C-5''), 124.36 (1C, C-2'), 127.27 (1C, C-5'), 128.11 (1C, C-6''), 130.35 (1C, C-4''), 132.18 (1C, C-3), 133.45 (1C, C-3'), 134.32 (1C, C-4'), 136.22 (1C, C1'), 146.28 (1C, C-5), 155.43 (1C, C-2''), 162.88 (1C, C-NH_2), 176.1 (1C, C=$N_{oxadiazole}$). MS (*m/z*): 364 (M+1). Anal. Calcd. for $C_{19}H_{17}N_5OS$ (%): C, 62.79; H, 4.17; N, 19.27. Found: C, 62.47; H, 4.45; N, 19.63.

2-(4-(5-Amino-1,3,4-thiadiazol-2-yl)-1-(2,4-dinitrophenyl)-1*H*-pyrazol-3-yl)phenol, (3l).

Obtained as a pale yellow solid in 76% yield (3.23 g), m. p. 191-192 ºC; ^{1}H NMR (500 MHz, DMSO-*d*$_6$) δ (ppm): 3.982 (s, 2H, NH_2), 6.485 (s, 1H, pyrazole- CH), 7.101-7.480 (m, 7H, Ar-H), 8.168 (s, 1H, OH). ^{13}C NMR (125.7 MHz, DMSO-d_6) δ (ppm): 111.22 (1C, C-4), 116.85 (1C, C-3''), 118.68 (1C, C-3'), 120.36 (1C, C-1''), 121.85 (1C, C-5''), 122.48 (1C, C-6'), 126.45 (1C, C-5'), 128.52 (1C, C-6''), 130.21 (1C, C-4''), 132.88 (1C, C-3), 139.86 (1C, C1'), 140.21 (1C, C-2'), 146.35 (1C, C-5), 147.40 (1C, C-4'), 155.81 (1C, C-2''), 162.63 (1C, C-NH_2), 175.32 (1C, C=$N_{oxadiazole}$). MS (*m/z*): 426 (M+1). Anal. Calcd. for $C_{17}H_{11}N_7O_5S$ (%): C, 48.00; H, 2.61; N, 23.05%. Found: C, 48.31; H, 2.21; N, 22.74.

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