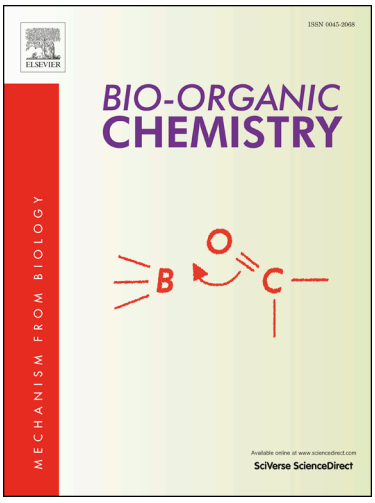

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1,3,4-Oxadiazole/chalcone hybrids: Design, synthesis, and inhibition of leukemia cell growth and EGFR, Src, IL-6 and STAT3 activities

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Abstract

A new series of 1,3,4-oxadiazole/chalcone hybrids was designed, synthesized, identified with different spectroscopic techniques and biologically evaluated as inhibitors of EGFR, Src, and IL-6. The synthesized compounds showed promising anticancer activity, particularly against leukemia, with **8v** being the most potent. The synthesized compounds exhibited strong to moderate cytotoxic activities against K-562, KG-1a, and Jurkat leukemia cell lines in MTT assays. Compound **8v** showed the strongest cytotoxic activity with IC_{50} of 1.95 μM, 2.36 μM and 3.45 μM against K-562, Jurkat and KG-1a leukemia cell lines, respectively. Moreover; the synthesized compounds inhibited EGFR, Src, and IL-6. Compound **8v** was most effective at inhibiting EGFR (IC_{50}= 0.24 μM), Src (IC_{50}= 0.96 μM), and IL-6 (% of control= 20%). Additionally, most of the compounds decreased STAT3 activation.

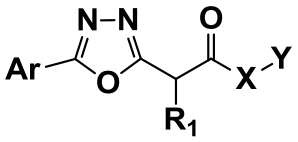
Key words: Leukemia; oxadiazole; chalcone, tyrosine kinases; cytokines; STAT3

1. Introduction

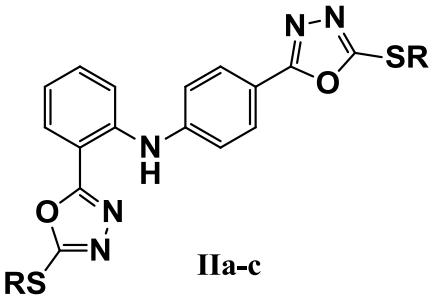
Leukemia is a type of cancer that starts in the blood-forming cells of the bone marrow [1]. Chemotherapeutic agents are found to be effective in killing leukemic cells, however, the 5-year overall survival is very poor [2]. Most patients relapse by tumor regrowth are initiated by chemoresistant leukemia cells. Potential bases for therapeutic resistance in leukemia treatment have been reported [3-5]. The signal transducers and activators of transcription 3 (STAT3) is a critical signaling intermediate in leukemia cells. STAT3 can be activated *via* phosphorylation by tyrosine kinases such as EGFR and Src and cytokines such as IL-6 [6]. The activation of STAT3 in leukemia is associated with poor prognosis [7]. Furthermore, development of chemo-resistant leukemic cells has been attributed to activation of the STAT3 pathway [8-11]. Consequently, significant effort has been committed towards the development of drugs that target STAT3 activators, and includes gefitinib (EGFR inhibitor) [12], dasatinib (Src inhibitor) [13] and tocilizumab (IL-6 inhibitor) [14, 15]. Unfortunately, the targeting of STAT3 activators such as EGFR, Src or IL-6 individually resulted only in moderate clinical efficacy [16-18]. Moreover, resistance develops due to the cross-talk between the pathways that activates STAT3, thus bypassing the inhibition of any one of the activators [8, 19-21]. Recently, efforts have shifted toward the generation of drug candidates that can target two STAT3 activators. For example, Lin *et al* [22] synthesized oxazolo[4,5-g]quinazolin-2(1*H*)-

ones derivatives as dual EGFR and Src inhibitors. Additionally, a series of azaacridine derivatives were synthesized as dual EGFR and Src inhibitors [23]. These dual EGFR and Src inhibitors have the potential to overcome the resistance of leukemia cells to STAT3 inhibition. However, STAT3 activation could occur *via* pathways other than EGFR and Src, such as IL-6 [24-26]. Dual EGFR and Src inhibition may not be sufficient for sustained inhibition of STAT3 activation. Therefore, the development of drugs that target EGFR, Src, and IL-6 may offer a therapeutic advantage.

Oxadiazole derivatives are typical heterocyclic compounds that have exhibited potential as anticancer therapeutics [27-32]. The anti-cancer activities of 1,3,4-oxadiazole involve different mechanisms, and included inhibition of matrix metalloproteinase-9 [33], tubulin polymerization [34], growth factors [30], NF-κB signaling [35], Bcl-2 [36], cell cycle progression at G2/M phase [37], thymidine phosphorylase [38, 39], and STAT3 [40-44]. The potential antitumor effects of oxadiazole derivatives occur mainly *via* the inhibition of STAT3 pathways directly [40] or indirectly [41-44]. A library of 1,3,4-oxadiazole-2-carboxamide compounds was registered in a U.S. patent with the general formula **I** (**Fig. 1**). These compounds inhibited STAT3 at a concentration of 100 μM, as well as inhibited growth of several cancer cell lines, including MDA-MB-468 and human lymphoma cells (SCC-3) [45]. The 2, 4'-bis mercapto-1,3,4-oxadiazole diphenylamine derivative **IIa-c** (**Fig. 1**) showed more potent anticancer activity with IC_{50} less than or equal to 2μM and inhibited EGFR tyrosine kinase activity [41]. The 1,3,4-oxadiazole linked benzimidazole derivative **III a-b** inhibited the EGFR receptor at 0.081 and 0.098 μM, and were more cytotoxic than 5-fluorouracil [42]. The 1,3,4-oxadiazole-2-thione derivative **IV** was the most potent Src kinase inhibitor among 384 tested compounds, with IC_{50} equal to 1.9 μM [44]. The 1,3,4-oxadiazole derivative **V** was also effective in inhibiting the release of IL-1, IL-6 and IL-10 in ConA-stimulated mouse lymph node cells [43].

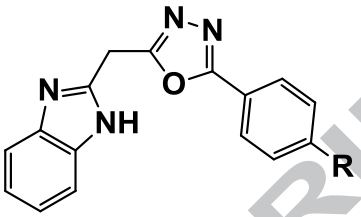


I



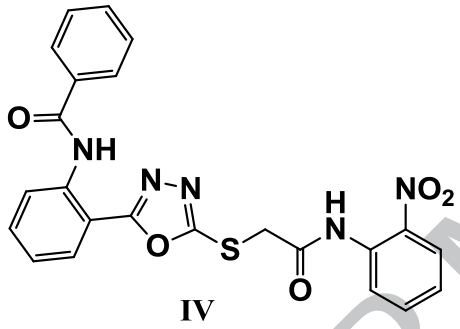
IIa-c

a; R = H, b; R = benzyl, c; R = allyl.

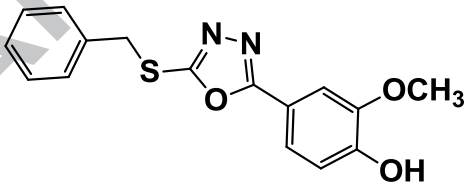


IIIa-b

a; R = 4-Cl, b; R = 4-OCH_3.



IV

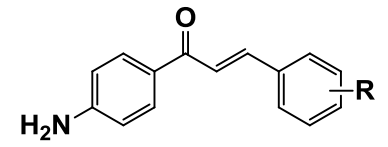


V

Fig. 1. Structures of Compounds **I-V.**

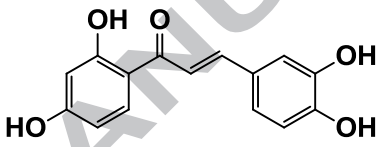
On the other hand, chalcones are precursors of flavonoids and isoflavonoids that exhibited anti-cancer activities, which may be attributed to the inhibition of different molecular targets [46] including EGFR [47, 48], Src family protein [49], IL-6 [50], and others [51]. For example, caspase-3 levels were significantly increased while STAT3 levels were decreased in the leukemia HL60 cell line upon treatment with chalcones **VIa-b (Fig. 2) [**52]. Additionally, Butein **VII** (**Fig. 2**) exerted anticancer effects in human hepatocellular carcinoma through suppression of constitutive and IL-6-induced STAT3 activation, as well as the inhibition of c-Src, JAK1, and JAK2 activation [53]. Moreover, Cardomin **VIII** (**Fig. 2**) exhibited significant anticancer effects in prostate cancer by suppression of STAT3 phosphorylation, nuclear translocation, DNA binding ability, and inhibition of STAT3 dimerization. Computational modeling indicated that Cardomin **VIII** can bind to the Src Homology 2 domain [54]. Several chalcones were derivatized with different bioactive heterocyclic moieties with the goal of generating anticancer agents with increased selectivity, ability to overcome drug resistance, and decreased side effects. 1,2,4-

Triazole/chalcone hybrid **IX** (**Fig. 2**) induced caspase-3 dependent apoptosis in A549 cells through both extrinsic and intrinsic pathways [51]. Additionally, pyrazolo[1,5-a]pyrimidine/chalcone hybrid **X** showed (**Fig. 2**) promising anti-proliferative activity and downregulated EGFR, p-EGFR, STAT3, and p-STAT3 in MDA-MB-231 cells [55]. Moreover; benzo[c]furan-chalcone derivative **XI** (**Fig. 2**) exhibited significant cytotoxicity in MCF-7 cell lines *via* inhibition of tubulin polymerization (IC_{50} = 5.51 × 10^{-5} μM) and EGFR-TK phosphorylation (IC_{50} = 0.09 μM) [56]. The triazoloquinoxaline-chalcone hybrids **XIIa-b** also inhibited EGFR-TK in the submicromolar range (0.039 to 0.083 μM) [57]. Benzimidazole/ chalcone hybrids **XIIIa-b** (**Fig. 2**) are EGFR antagonist that exhibited promising cytotoxicity against the HCT116 and H460 cell lines with IC_{50} ranging from 6.83 -12.52 μM [58].

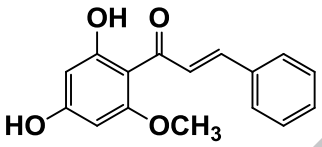


R = a; H or b; R = 2,3-di-OCH_3

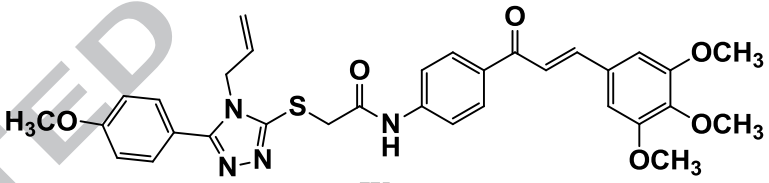
VIa-b



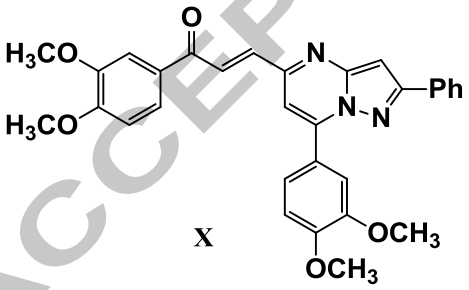
VII



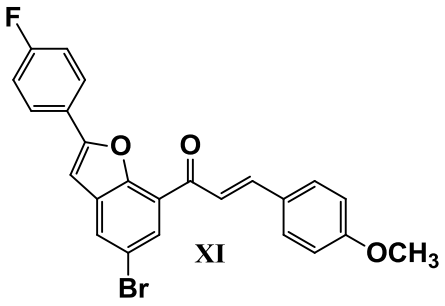
VIII



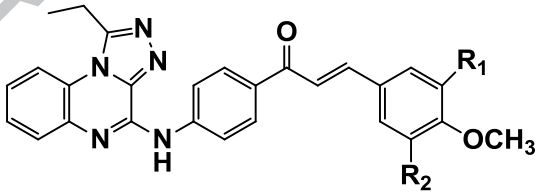
IX



X

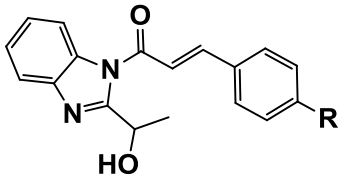


XI



R_1, R_2 = a; H or b; R_1, R_2 = OCH_3

XIIa-b



R = a; H or b; R = OCH_3

XIIIa-b

Fig. 2. Structures of compounds **VI-XIII**.

Based on the studies discussed above, the present study involved the design and synthesis of a series of 1,3,4-oxadiazole/chalcone hybrids (**Fig. 3**) with the combined

abilities to inhibit EGFR, Src, and IL-6. The hybrids were designed such that the oxadiazole or chalcone moieties possess a methoxy group (s) or chlorine as electron donating or as electron withdrawing substituents; respectively. The synthesized compounds were evaluated for their cytotoxic activities against different cancer cell lines, including three different leukemia cell lines (K-562, KG-1a, and Jurkat). Moreover, their inhibitory activities against EGFR, Src, IL-6, and STAT3 were evaluated.

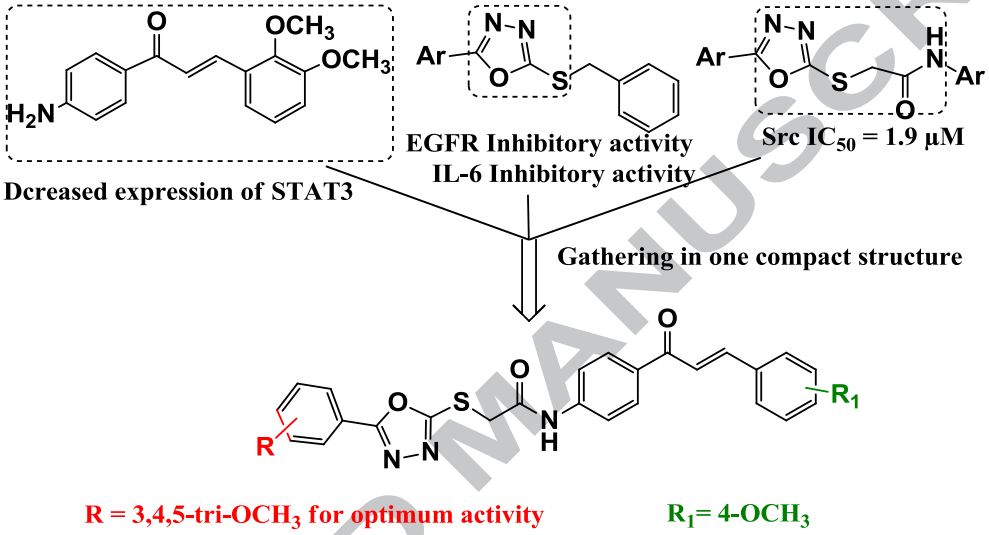
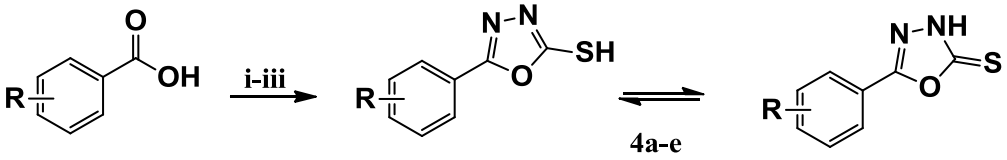


Fig. 3. Pharmacophore of the target compounds.

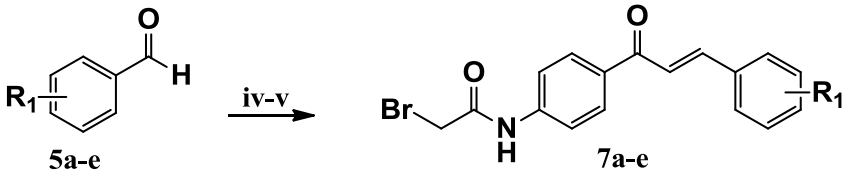
2. Results and discussion

2.1. Chemistry

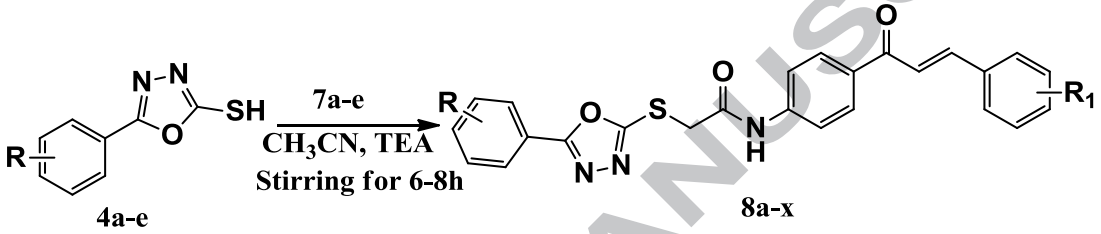
The target compounds and intermediates were prepared as outlined in **Scheme 1**. Alkylation of oxadiazole derivatives **4a-e** with acetylated chalcone derivatives **7a–e** was achieved in acetonitrile in the presence of TEA to generate the target compounds **8a-x** in a good yield ranging from 51 % to 89% [59]. ^{1}H NMR and ^{13}C NMR confirmed the formation of target compounds. ^{1}H NMR spectra of compounds **8a-x** showed a singlet signal at δ: 4.09-4.80 ppm related to (S-C$\underline{H_2}$-CO). Furthermore, the two chalcone protons appear at the aromatic region as doublet signals at δ: 7.81-7.98 ppm and 7.39-7.78 ppm with coupling constant *J* = 15.00-16.00 Hz. The amide proton NH appears as singlet signals at δ 9.74-11.25 ppm. The ^{13}C NMR spectra of compounds **8a-x** revealed the presence of two carbonyl groups appearing at δ: 187.89-189.05 ppm and δ: 166.02-169.22 ppm related to C=O of chalcone and N-C=O, and a characteristic signal of SCH_2 appears at 36.31-37.43 ppm.



R : a) H, b) 4-Cl, c) 4-OCH_3, d) 3,4-Di-OCH_3, e) 3,4,5-Tri-OCH_3.



R_1 : a) H, b) 4-Cl, c) 4-OCH_3, d) 3,4-Di-OCH_3, e) 3,4,5-Tri-OCH_3.



Compd.	R	R_1	Compd.	R	R_1
8a	H	H	**8m**	4-OCH_3	3,4-di-OCH_3
8b	H	4-Cl	**8n**	4-OCH_3	3,4,5-tri-OCH_3
8c	H	3,4-di-OCH_3	**8o**	3,4-di-OCH_3	H
8d	H	3,4,5-tri-OCH_3	**8p**	3,4-di-OCH_3	4-Cl
8e	4-Cl	H	**8q**	3,4-di-OCH_3	4-OCH_3
8f	4-Cl	4-Cl	**8r**	3,4-di-OCH_3	3,4-di-OCH_3
8g	4-Cl	4-OCH_3	**8s**	3,4-di-OCH_3	3,4,5-tri-OCH_3
8h	4-Cl	3,4-di-OCH_3	**8t**	3,4,5-tri-OCH_3	H
8i	4-Cl	3,4,5-tri-OCH_3	**8u**	3,4,5-tri-OCH_3	4-Cl
8j	4-OCH_3	H	**8v**	3,4,5-tri-OCH_3	4-OCH_3
8k	4-OCH_3	4-Cl	**8w**	3,4,5-tri-OCH_3	3,4-di-OCH_3
8l	4-OCH_3	4-OCH_3	**8x**	3,4,5-tri-OCH_3	3,4,5-tri-OCH_3

i) EtOH, Conc H_2SO_4, reflux for 12-18 h. (75.7-87.2 %). ii) $NH_2NH_2H_2O$ (97%), EtOH, reflux 5-8 h, (72.7-80.2 %). iii) 1- CS_2, KOH, EtOH, reflux 12 h.; 2- Conc HCl, (60.0-87.0%). iv) *p*-aminoacetophenone, KOH (60%), EtOH, Stirring 4 h, (67.3-82.9%). v) $BrCH_2COBr$, CH_2Cl_2, K_2CO_3, H_2O, Stirring overnight, (69.7-78%).

Scheme1: Synthesis of the target compounds **8a-x**.

2.2. Biological evaluation

2.2.1. Cytotoxic assays

2.2.1.1. *In vitro* one dose anticancer assay

All the twenty-four synthesized compounds (**8a-x)** were selected by the National Cancer Institute (NCI), USA for *in vitro* anticancer screening. Compounds were screened at 10 μM dose using the Sulforhodamine B colorimetric assay against the full NCI panel of 60 cell lines consisting of nine tumor subpanels, which include leukemia, lung, colon, melanoma, renal, prostate, CNS, ovarian, and breast cancer cell lines.

The compounds tested exhibited promising anticancer activities (**Table 1 and 2 supporting information**) against human cancer cells, particularly leukemia cell lines. Among the synthesized compounds, **8a**, **8n**, **8p**, and **8v** were the most potent (Mean growth inhibition % = 37.77, 27.29, 28.20, and 132.29; respectively).

Compound **8v** exhibited potent cytotoxicities against most of the tested cell lines, with complete cell death observed for 45 used cell lines and % growth inhibition ranging from 101.51- 191.80% (**Table 2 supporting information**).

Six compounds, **8a, 8d, 8m, 8n, 8s,** and **8v** exhibited significant anticancer activity against K-562 cell line with % growth inhibition ranging from 35.80 to 152.18%. Additionally; compounds **8a**, **8d**, **8e**, **8j**, **8k-n**, **8p**, **8s**, **8v**, and **8x** showed anticancer activity against RPMI-8226 cell line with % growth inhibition ranging from 31.29-143.74%. Compounds **8a**, **8d**, **8e**, **8i-k, 8m**, **8n**, **8p**, **8s**, **8v** and **8x** exhibited good anticancer activity against SR cell line with % growth inhibition ranging from 32.85 – 160.49%.

On the other hand, compounds **8a**, **8d, 8l-n,** and **8p** exhibited only moderate anticancer activity against MOLT-4 cell line with % growth inhibition ranging from 31.88– 108.34%.

2.2.1.2 *In vitro* five-dose anticancer assay

Compound **8v** was selected for advanced five-dose testing against the full panel of 58 human tumor cell lines. All of the 58 cell lines, representing nine tumor subpanels, were incubated at five different concentrations of (0.01, 0.1, 1, 10 and 100 μM). The data indicate that compound **8v** exhibited broad-spectrum antitumor activity with GI_{50} ranging from 0.32 to 11 μM, with selectivity ratio ranging between 0.65-1.20 at GI_{50} level (**Table 1**). Moreover, compound **8v** showed the ability to inhibit growth of all

the cell lines tested, with TGI concentration ranging from 1.28 to 23.60 μM. Compound **8v** exhibited LC_{50} with concentrations ranging from 5.28 to > 100 μM.

Table 1. GI_{50} and TGI of compound **8v** against the panel of 58 cell lines representing 9 different cancers tested using NCI's *in vitro* five dose anticancer assay.

Panel/Cell Line	GI_{50} (μM)	TGI (μM)	Panel/Cell Line	GI_{50} (μM)	TGI (μM)
Leukemia			**Melanoma**		
CCRF-CEM	1.84	4.56	**LOX IMVI**	0.32	1.28
HL-60(TB)	2.00	4.10	**MALME-3M**	1.68	3.45
MOLT-4	2.05	5.27	**M14**	1.60	3.30
RPMI-8226	1.73	4.31	**MDA-MB-435**	1.70	3.40
SR	1.86	4.01	**SK-MEL-2**	1.79	4.82
Non-Small Cell Lung Cancer			**SK-MEL-28**	1.74	3.40
A549/ATCC	1.84	3.53	**SK-MEL-5**	2.14	5.24
EKVX	1.79	3.98	**UACC-257**	2.16	4.69
HOP-62	2.13	4.53	**UACC-62**	1.73	3.43
HOP-92	1.93	4.23	**Ovarian Cancer**		
NCI-H226	2.67	5.69	**IGROV1**	1.54	3.75
NCI-H23	1.88	3.97	**OVCAR-3**	1.78	3.38
NCI-H322M	1.59	2.98	**OVCAR-4**	2.00	4.62
NCI-H460	2.00	3.85	**OVCAR-5**	1.72	3.30
NCI-H522	1.55	3.21	**OVCAR-8**	2.32	5.73
Colon Cancer			**NCI/ADR-RES**	2.42	7.03
COLO 205	1.94	3.84	**SK-OV-3**	2.42	5.68
HCC-2998	1.88	3.65	**Renal Cancer**		
HCT-116	0.50	1.94	**786-0**	1.70	3.36
HCT-15	1.63	3.22	**A498**	11.00	23.60
HT29	1.83	3.62	**ACHN**	1.72	3.22
KM12	1.77	3.40	**RXF 393**	1.74	3.54
SW-620	1.86	3.60	**SN12C**	1.83	3.57
CNS cancer			**TK-10**	1.52	3.03
SF-268	1.79	3.96	**UO-31**	1.46	2.97
SF-295	1.77	3.35	**Breast cancer**		
SF-539	1.64	3.03	**MCF7**	1.27	3.68
SNB-19	1.78	3.56	**MDA-MB231/ATCC**	1.82	3.50
SNB-75	1.47	3.15	**HS 578T**	2.65	7.76
U251	1.68	3.18	**BT-549**	1.40	2.81
Prostate Cancer			**T-47D**	2.05	5.08
PC-3	1.75	3.30	**MDA-MB-468**	2.10	5.02
DU-145	1.75	3.21			

2.2.1.3 *In vitro* anti-proliferative assay using K-562, KG-1a, and Jurkat cells

The cytotoxic activities of the synthesized compounds were determined using three different leukemia cell lines (K-562, KG-1a, and Jurkat) and evaluated using MTT assays. Results presented in **Table 2** indicate that most of the synthesized 1,3,4-oxadiazole/chalcone derivatives exhibited strong to moderate cytotoxic activities against the three leukemia cell lines. The relative sensitivities of leukemia cells toward the synthesized compounds were K-562 > Jurkat > KG-1a. Consistent with the NCI results, **8v** showed the strongest cytotoxic activity among the derivatives synthesized, with the relative sensitivities of K-562 (IC_{50} = 1.95 µM) > Jurkat (IC_{50} = 2.36 µM) > KG-1a (IC_{50} = 3.45 µM).

Our structure-activity relationship (SAR) analyses indicate that the type of substitution in both phenyl rings is essential for anticancer activity. For anticancer activity, the substitution of phenyl ring carrying the oxadiazole moiety should be either H, *p*-methoxy or 3,4,5-trimethoxy group (s), with an effective substitution order of 3,4,5-trimethoxy groups > H > *p*-methoxy group. Also, the more effective substitutions of the chalcone phenyl ring are either H, *p*-methoxy or 3,4,5-trimethoxy group (s). For optimal activity, R must be 3,4,5-trimethoxy groups and R_1 must be methoxy group in *p*-position. Thus for good anti-cancer activity, the two phenyl rings should be either unsubstituted or one of the rings should carry *p*-methoxy group, while the second ring should contain 3,4,5-trimethoxy groups.

Table 2. Cytotoxic activity of oxadiazole/chalcone hybrids, **8a-x**, against three leukemia cell lines.

Compound	(IC_{50} μM) ± SD		
	K-562	**KG-1a**	**Jurkat**
8a	7.21±0.35	8.85±0.22	8.47±1.03
8b	75.35±4.61	82.24±4.56	78.71±2.13
8c	69.54±5.15	95.22±3.88	75.22±1.25
8d	22.84±1.32	31.34±1.05	28.28±0.62
8e	93.31±5.21	>100	>100
8f	82.27±3.08	91.54±4.15	88.61±1.35
8g	13.05±1.65	55.64±1.34	41.21±0.91
8h	72.14±3.32	84.64±5.21	78.06±2.10
8i	55.34±4.89	78.95±3.02	62.56±1.37
8j	22.61±2.34	25.22±0.48	31.13±0.46
8k	58.42±3.75	71.07±1.22	62.67±2.71
8l	35.22±2.05	38.32±2.45	33.28±1.22
8m	23.11±0.28	31.54±0.42	28.36±0.85
8n	10.25±0.94	15.97±0.96	13.15±0.08
8o	58.37±5.45	65.64±2.31	94.09±5.31
8p	16.09±1.08	22.52±1.07	17.24±0.79
8q	40.22±2.82	45.29±2.31	31.94±1.30
8r	>100	93.08±1.14	>100
8s	15.14±0.25	32.12±0.64	25.14±0.45
8t	73.25±3.72	84.57±1.28	76.36±1.31
8u	84.22±4.36	95.69±2.48	>100
8v	1.95±0.18	3.45±0.25	2.36±0.47
8w	90.34±2.91	>100	>100
8x	62.15±3.07	71.48±1.18	69.17±0.15
Cisplatin	3.21±0.45	12.15±3.41	15.12±1.35

2.2.2. 1,3,4-oxadiazole/chalcone hybrids inhibited EGFR and Src kinase activities and protein expression.

The oxadiazole/chalcone hybrids **8a-x** inhibited EGFR kinase activity with relatively lower concentrations than that required for the inhibition of Src Kinase activity. The effects of synthesized compounds on EGFR and Src tyrosine kinases are presented in **Table 3**. Compounds **8a**, **8n** and **8v** displayed the highest inhibitory activities against EGFR (IC_{50} = 0.24-2.35 μM) as well as Src (IC_{50} = 0.96-6.24 μM). While compounds **8b**, **8g-8m**, **8p**, **8q**, **8s**, **8t**, and **8x** exhibited moderate inhibitory activities against EGFR (IC_{50} = 10.24-35.15 μM) and Src (IC_{50} = 12.5-44.1 μM). On the other hand, compounds **8c**, **8e**, **8f**, **8o**, **8r**, **8u**, and **8w** showed the lowest activity against EGFR (IC_{50} = 48.34-71.15 μM) and Src (IC_{50} = 50.32-76.2 μM).

Table 3. EGFR and Src kinases inhibitory activity of 1,3,4-oxadiazole/chalcone hybrids**.**

Compound	EGFR (IC_{50} μM)	Src (IC_{50} μM)	Compound	EGFR (IC_{50} μM)	Src (IC_{50} μM)
8a	1.23±0.25	4.5±1.2	**8n**	2.35±0.95	6.24±0.86
8b	35.15±1.36	44.1±3.5	**8o**	71.15±3.25	51.24±5.6
8c	64.35±3.56	57.3±2.4	**8p**	13.26±0.65	13.67±0.89
8d	11.22±1.25	13.41±1.6	**8q**	11.38±1.54	29.48±1.23
8e	59.35±5.63	74.4±3.6	**8r**	63.25±2.36	76.2±3.65
8f	64.68±3.65	50.32±3.5	**8s**	12.35±0.8	15.34±1.25
8g	12.67±1.25	22.14±2.15	**8t**	22.34±2.35	40.21±1.34
8h	30.25±2.35	41.36±4.5	**8u**	55.45±4.35	53.09±5.6
8i	29.34±3.65	43.67±3.5	**8v**	0.24±0.035	0.96±0.2
8j	11.47±1.24	25.34±1.56	**8w**	48.34±2.36	60.34±2.5
8k	18.34±2.35	33.64±2.35	**8x**	20.5±1.35	35.12±3.26
8l	12.34±1.35	24.98±3.46	**Dasatinib**	0.8±0.23	< 0.001
8m	10.24±0.36	12.5±0.3	**Gefitinib**	0.023±0.004	1.25±0.15

To confirm the ability of the synthesized compounds to inhibit EGFR and Src, we evaluated the effects of the most potent compound **8v**, on the expression levels of EGFR, p-EGFR, Src and p-Src in K562 cells using Western blot analysis. As shown in **Fig.4**, compound **8v** downregulated the protein expression levels of p-EGFR,

EGFR, p-Src and Src in a dose-dependent manner. Therefore, it was reasonable to conclude that the anti-proliferative effect of compound **8v** may be attributed to the inhibition of EGFR and Src.

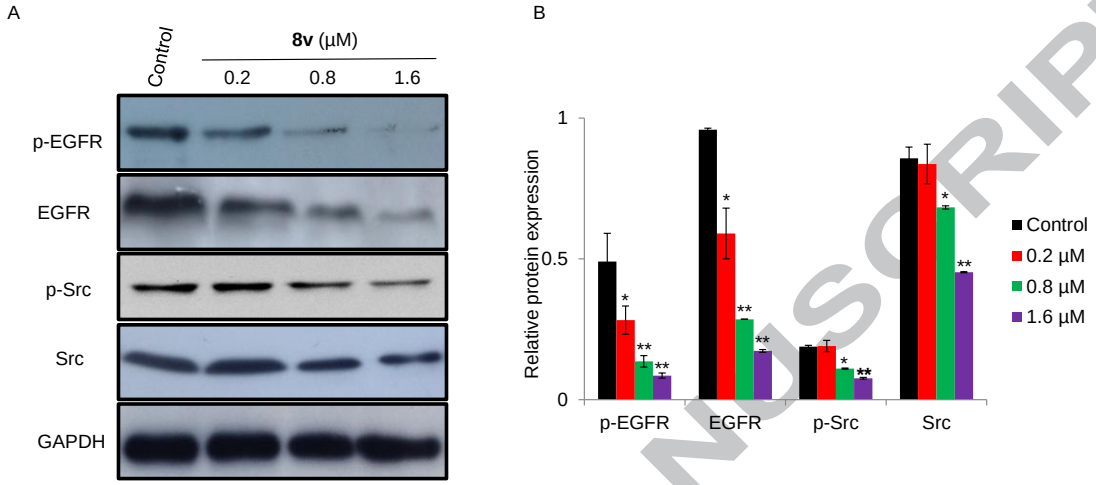


Fig.4. (A) Western blotting analysis of the expression of EGFR, p-EGFR, Src and p-Src in K562 cells treated with 0, 0.2, 0.8, or 1.6 μM oxadiazole chalcone hybrid **8v**. GAPDH served as a loading control. **(B)** The protein expression of EGFR, p-EGFR, Src and p-Src after treatment with different concentrations of compound **8v**. The values are expressed as the mean ± SD based on three different experiments. *, $P < 0.05$ and **, $P < 0.005$ indicate a significant difference compared with vehicle treated cells.

2.2.3. 1,3,4-Oxadiazole/chalcone hybrids inhibited IL-6 production

K562 cells were treated with 10 μM of 1,3,4-oxadiazole/chalcone hybrids **8a-x** for 24 h, after which media were collected to determine IL-6 protein levels. As illustrated in **Fig. 5A**, 1,3,4-oxadiazole/chalcone hybrids decreased IL-6 protein levels. Consistent with relative effectiveness of the compounds in downregulating EGFR and Src, compounds **8a**, **8n**, and **8v** were found to be most effective at downregulating IL-6 levels (% of control = 20-23%). Compounds, **8b**, **8g-8m**, **8p**, **8q**, **8s**, **8t**, and **8x** induced moderate inhibition of IL-6 levels (% of control = 30-75%). On the other hand, compounds **8c**, **8e**, **8f**, **8o**, **8r**, **8u**, and **8w** did not significantly downregulate IL-6 levels (% of control = 82-98%). The initial downregulation of the levels of IL-6

protein were observed 8 hours after treatment with compound **8v,** and IL-6 levels were still downregulated after 48 hours **Fig. 5B**.

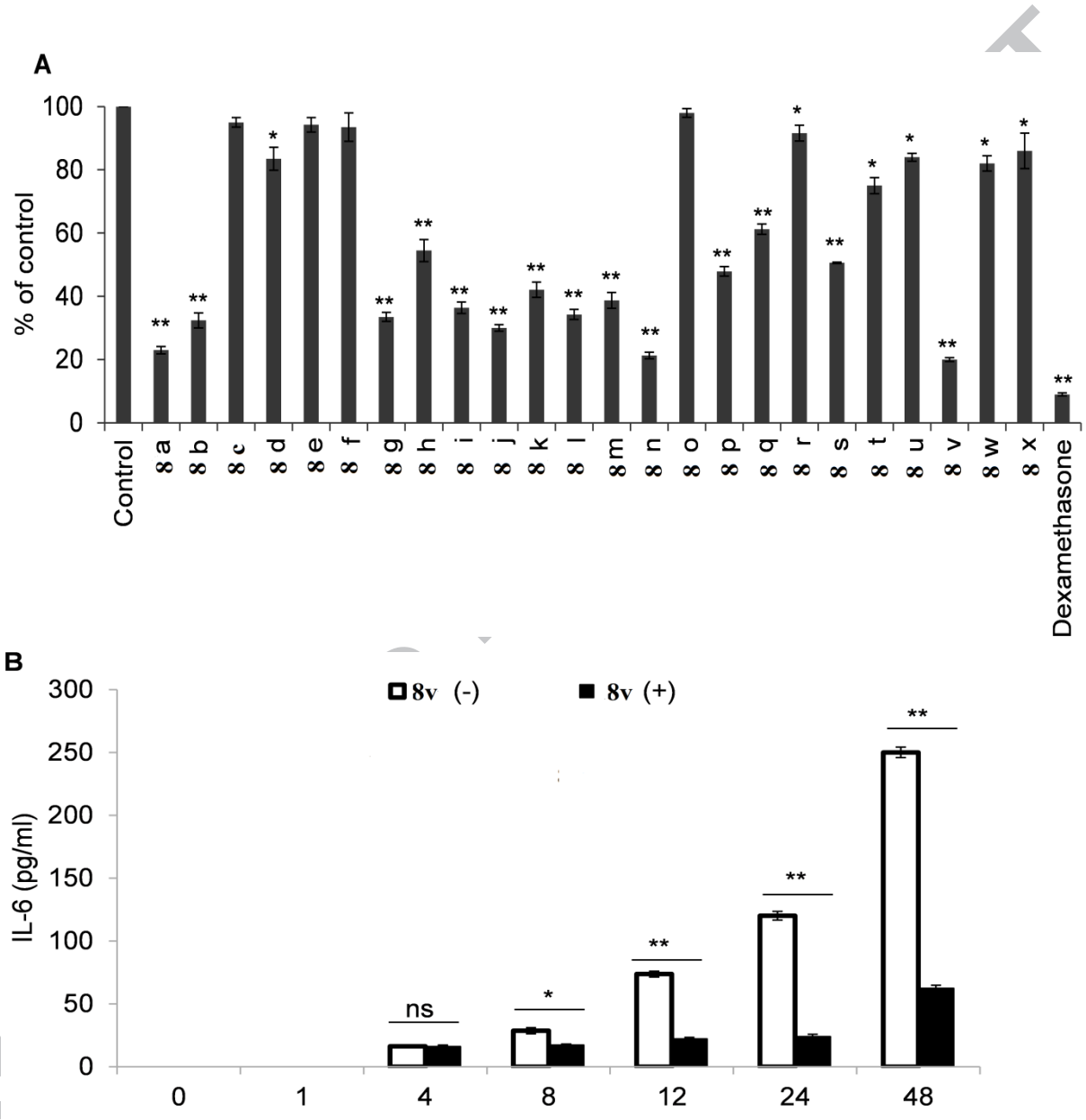


Fig. 5. (A) IL-6 protein expression in K-562 cells 24 h after treatment with 10 µM oxadiazole/chalcone derivatives (**8a-x**) or dexamethasone relative to vehicle treated cells **(B)** Time course effect of **8v** on IL-6 protein expression in K-562 cells treated with 10 µM oxadiazole/chalcone derivative **8v**. The values are expressed as the mean ± SD based on three different experiments. *, $P < 0.05$ and **, $P < 0.005$ indicate a significant difference compared to vehicle treated cells.

2.2.4. 1,3,4-Oxadiazole/chalcone hybrids inhibited STAT3 activity

Because 1,3,4-oxadiazole/chalcone derivatives inhibited EGFR, Src, and IL-6, which a upstream activators of STAT3, the effect of the synthesized compounds on STAT3 activation was evaluated. The results presented in **Fig. 6** indicate that 1,3,4-oxadiazole/chalcone derivatives **8a-x** inhibited STAT3 activation. Compounds **8a**, **8n**, and **8v** were the most effective at inhibiting STAT3 activity. Compounds **8b**, **8g-8m**, **8p**, **8q**, **8s**, **8t**, and **8x** exhibited moderate activities, and the remaining compounds showed low or no activities. Our results also confirmed the type of substitution on phenyl rings influenced activity. For effective STAT3 inhibition, the two phenyl ring should be either unsubstituted or one ring should carry *p*-methoxy group while the second ring should contain 3,4,5-trimethoxy groups.

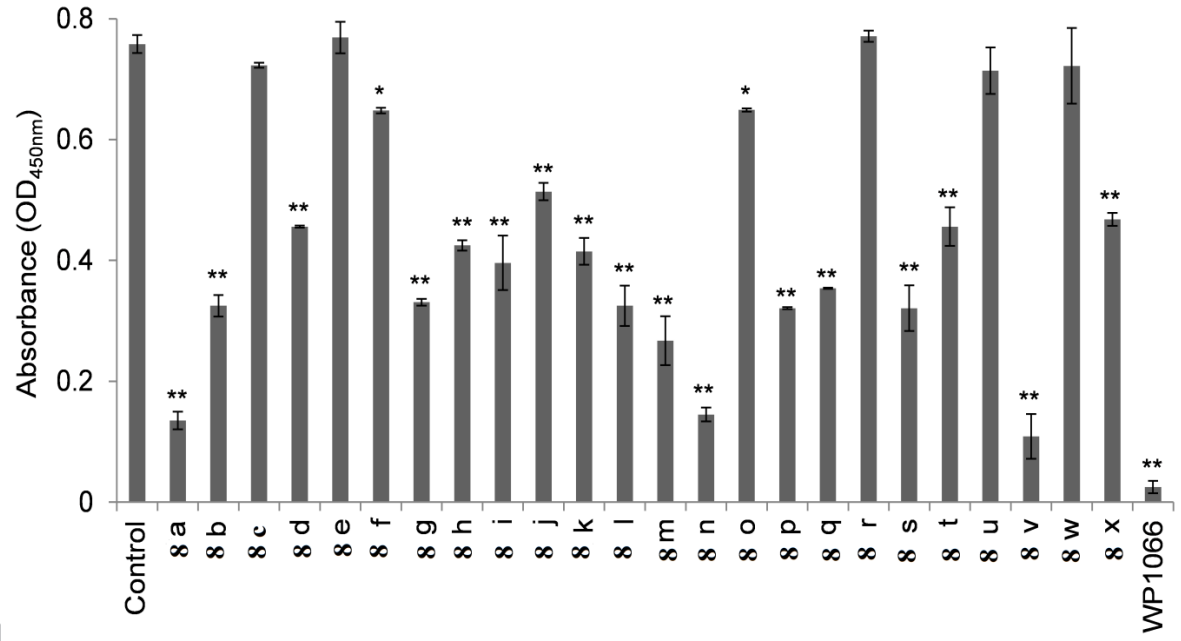


Fig. 6. STAT3 activation assay in K-562 cells treated with 10 µM oxadiazole/chalcone derivatives (**8a-x**), WP1066, or vehicle for 24 h. The values are expressed as the mean ± SD based on three different experiments. *, $P < 0.05$ and **, $P < 0.005$ indicate a significant difference relative to vehicle treated cells.

3. Conclusion

A series of novel 1,3,4-oxadiazole/chalcone hybrids was prepared and identified using different spectroscopic techniques. The compounds we generated showed significant anticancer activity, particularly against leukemia. Compounds **8a, 8n,** and **8v** showed the highest cytotoxicity activity against leukemia cell lines K-562 among the compounds tested. The IC_{50} for the compounds were in the range of 1.95 - 10.25 μM, compared to cisplatin that has an IC_{50} of 3.21 μM. *In vitro* (one dose) anticancer and MTT assays indicated that compound **8v** exhibited the highest activities against human cancer cells. Compounds, **8a**, **8n** and **8v** displayed the most potent or effective inhibitory activity against EGFR (IC_{50} = 0.24-2.35μM), Src (IC_{50} = 0.96-6.24μM), and IL-6 (% of control = 20-23%). Compounds **8a**, **8n**, and **8v** are also the most effective at inhibiting STAT3. It is possible that inhibition of EGFR, Src, and IL-6 by 1,3,4-oxadiazole/chalcone hybrids play roles in the inhibition of STAT3. The inhibition of STAT3 support the potential of 1,3,4-oxadiazole/chalcone hybrids in the treatment of leukemia and prevention of the development of resistance in leukemia cells. However, further studies using *in vivo* models are needed to verify the potential of 1,3,4-oxadiazole/chalcone hybrids as anticancer agents.

4. EXPERIMENTAL SECTION

4.1.Chemistry section:

- Analytical grade chemicals and solvents were used. The progress of the reactions was monitored by thin layer chromatography pre-coated Merck silica gel 60 F254 aluminum sheets.
- Melting points were determined on Stuart electro-thermal melting point apparatus in degree Celsius and were uncorrected.
- ^{1}H spectra were recorded on Burker AG, Switzerland, 500 MHz, Faculty of Pharmaceutical Sciences, Umm Al-Qura University, Mecca, Saudi Arabia; chemical shift (δ) were expressed in ppm relative to TMS (δ=0 PPM) as internal standard and $CDCl_3$ or DMSO-d_6 as a solvent. Chemical shifts (δ) were expressed in parts per million (ppm) and coupling constants (*J*) were expressed in Hertz. The signals were designated as follows: s, singlet; d, doublet; t, triplet; m, multiplet.

- ^{13}C spectra were recorded on Burker AG, Switzerland and 125 MHz, faculty of Pharmaceutical Sciences, Umm Al-Qura University, Mecca, Saudi Arabia using TMS as the reference standard and $CDCl_3$ or DMSO-d_6 as a solvent. Chemical shifts (δ) were expressed in parts per million (ppm).
- LC/MS/MS were carried out using Agilent UPLC/MS/MS 1260 infinity II with 6420 Triple quad LC/MS detector at Faculty of Pharmacy, Minia University, Minia, Egypt
- The purity of the compounds was checked by HPLC.
- Elemental analyses were recorded on Shimadzu GC/ MS-QP5050A, The Regional center for Mycology and Biotechnology, Al-Azhar University, Egypt.

4.1.1. General procedure for the synthesis of substituted ethylbenzoate 2a-e [60].

A mixture of the appropriate substituted benzoic acid **1a-e** (10 mmol), absolute ethanol (20 mL), and concentrated sulfuric acid (2 mL) was heated under reflux for 12-18 h. Excess solvent was removed under reduced pressure. The residue was extracted with ether (2 X 50 mL) and washed with saturated $NaHCO_3$ (2 X 20 mL). The ether layer was dried over anhydrous magnesium sulfate, and ether was evaporated under vacuum to produce the ethyl ester derivatives **2a-e**.

4.1.2. General procedure for the synthesis of substituted benzohydrazides 3a-e [61, 62].

Hydrazine hydrate (97 %, 30 mmol, 1.5 mL) was added to a solution of the isolated esters **2a–e** (10 mmol) in ethanol (20 mL), and the mixture was heated at reflux for 5-8 h. After cooling, the resulting precipitate was filtered off, washed with water, dried, and crystallized from ethanol.

Benzohydrazide (3a) [62].

White solid (77.2 % yield); mp 110-112^{o}C (Reported mp = 112-114 ^{o}C).

4-Chloro-benzohydrazide (3b) [63].

White solid (79.3% yield); mp 195-197 ^{o}C (Reported mp = 189-191^{o}C).

4-Methoxybenohydrazide (3c) [62].

White solid (80.2 % yield); mp 134-137 ^{o}C (Reported mp =136-140^{o}C)

3,4-Dimethoxybenzohydrazide (3d) [62].

White solid (72.7 % yield); mp 145-146 ºC as reported.

3,4,5-Trimethoxybenzohydrazide (3e) [62].

White solid (72.9% yield); mp 158-160^{o}C as reported.

4.1.3. General procedure for the synthesis of 5-aryl--1,3,4-oxadiazole-2(3*H*)-thione derivatives (4a-e) [43, 64].

A mixture of the benzohydrazides **3a-e** (0.05 mol), potassium hydroxide (0.05 mol, 2.81g) and carbon disulfide (0.17 mol, 12.94 g) in 50 mL of absolute ethanol was heated at reflux with stirring for 12 h. The solvent was evaporated under vacuum. The resulting solid was dissolved in water and acidified with 10% HCl. The resulting precipitate was filtered off, washed with water, dried, and recrystallized from ethanol to generate the corresponding oxadiazole derivatives **4a-e** [64].

5-Phenyl-1,3,4-oxadiazole-2(3*H*)-thione (4a) [65].

White solid (76 % yield); mp 215-216^{o}C (Reported mp = 218-220^{o}C).

5-(4-Chlorophenyl) -1,3,4-oxadiazole-2(3*H*)-thione (4b) [65].

Yellowish white solid (87 % yield); mp 172-173^{o}C (Reported mp = 176-178^{o}C).

5-(4-Methoxyphenyl)-1,3,4-oxadiazole-2(3*H*)-thione (4c) [65].

White solid (78 % yield); mp 192-194^{o}C (Reported mp = 198-200^{o}C).

5-(3,4-Dimethoxyphenyl)-1,3,4-oxadiazole-2(3*H*)-thione (4d) [66].

Yellowish white solid (60 % yield); mp 238-239^{o}C (Reported mp = 237-238^{o}C).

5-(3,4,5-Trimethoxyphenyl)-1,3,4-oxadiazole-2(3*H*)-thione (4e) [67].

Yellowish white solid (79 % yield); mp 192-193^{o}C (Reported mp = 186-188^{o}C).

4.1.4. General procedure for the synthesis of 1-(4-aminophenyl)-3-arylprop-2-en-1-one (6a-d) [68].

An equimolar amount of *p*-aminoacetophenone (0.1 mol, 13.51 g) and the appropriate aldehyde **5a-d** (0.1 mol), were dissolved in a minimum amount of ethanol, and aqueous NaOH (0.25 mol, 60%) was then added dropwise. The reaction mixture was stirred in ice bath for 30 min then at rt, until the precipitate was formed within 3 h. The precipitate was filtered off and washed thoroughly with cold distilled water and

cold methanol (2 x 20 mL). The product was recrystallized from absolute ethanol. The structure of the product was confirmed by mp.

1-(4-Aminophenyl)-3-phenyl prop-2-en-1-one (6a) [59]

Buff solid (77.8 % yield); mp 156-157^{o}C (Reported mp = 157-158^{o}C).

1-(4-Aminophenyl)-3-(4-chlorophenyl)prop-2-en-1-one (6b) [68].

Yellow solid (80.3 % yield); mp 159-160^{o}C (Reported mp = 158-159^{o}C).

1-(4-Aminophenyl)-3-(4-methoxyphenyl)prop-2-en-1-one (6c) [68].

Yellow solid (67.3 % yield); mp 115-116^{o}C (Reported mp = 114-115^{o}C).

1-(4-Aminophenyl)-3-(3,4-dimethoxyphenyl)prop-2-en-1-one (6d) [68].

Yellow solid (82.9 % yield); mp 159-160^{o}C (Reported mp = 158-160^{o}C).

1-(4-Aminophenyl)-3-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (6e) [68].

Yellow solid (70.4 % yield); mp 166-168^{o}C as reported.

4.1.5. General procedure for the synthesis of 2-bromo-*N*-(4-(3-arylacryloyl)phenyl)acetamides (7a-e) [59].

To a mixture of the appropriate chalcone **6a-e** (6.30 mmol) in dichloromethane (20 mL) and potassium carbonate (1.302 mmol, 0.18g) in 100 mL water in an ice bath, bromoacetyl bromide (9.20 mmol, 1.856 g) in 30 mL dichloromethane was added in a dropwise manner with stirring over 30 min. Stirring was continued for 2 h at 0^{o}C and at rt overnight. The reaction mixture was extracted with dichloromethane (2 x 60 mL) and the organic layer was washed with distilled water (2 x 40 mL), dried over anhydrous sodium sulfate, filtered, evaporated under vacuum and the residue was recrystallized from ethanol.

2-Bromo-*N*-(4-((*E*)-3-phenylacryloyl)phenyl)acetamide (7a) [59].

Pale yellow powder (70.0 % yield); mp 157-159^{o}C (Reported mp = 157-158^{o}C).

2-Bromo-*N*-(4-(*E*)-3-(4-chlorophenyl)acryloyl)phenyl)acetamide (7b) [68].

Pale yellow powder (71.5 % yield); mp 191-192^{o}C (Reported mp = 190-192^{o}C).

2-Bromo-*N*-(4-((*E*)-3-(4-methoxyphenyl)acryloyl)phenyl)acetamide (7c) [59].

Pale orange crystal (69.70 % yield); mp 155-156^{o}C (Reported mp = 160-162^{o}C).

2-Bromo-*N*-(4-((*E*)-3-(3,4-dimethoxyphenyl)acryloyl)phenyl)acetamide (7d) [59].

Yellow powder (71.0 % yield); mp 150-151^{o}C (Reported mp= 149-150^{o}C).

2-Bromo-*N*-(4-((*E*)-3-(3,4,5-trimethoxyphenyl)acryloyl)phenyl)acetamide (7e) [59].

Yellow powder (78.0 % yield); mp 166-167^{o}C (Reported mp= 160-162^{o}C).

4.1.6. General Procedure for the synthesis of 2-(5-phenyl-1,3,4-oxadiazole-2-ylthio)-*N*-(4-((*E*)-3-phenylacryloyl)phenyl)acetamide derivatives (8a-x).

TEA (0.18 mmol, 0.018 g) was added as a base to an equimolar mixture of **4a-e** (0.09 mmol) and compound **7a-e** (0.09 mmol) in acetonitrile. The reaction mixture was stirred at room temperature until the precipitate formed. The resulting precipitate was filtrated off, and the precipitate was then crystallized with acetonitrile to afford the target compounds [59].

4.1.6.1. *N*-(4-(3-Phenyl)acryloyl)phenyl-2-((5-phenyl-1,3,4-oxadiazol-2-yl)thio)-acetamide 8a

White solid (0.39g, 89 % yield); mp 199-201°C; ^{1}H NMR (500 MHz, DMSO-d_6) - δ (ppm): 10.89 (s, 1H, NH), 8.18 (d, 2H, *J* = 8.0 Hz, Ar-H), 7.96-7.93 (m, 3H, 2 Ar-H and C<u>H</u>=CH), 7.88-7.87 (m, 2H, Ar-H), 7.80 (d, 2H, *J* = 8.0 Hz, Ar-H), 7.73 (d, 1H, *J* = 16.0 Hz, CH=C<u>H</u>), 7.61 (d, 2H, *J* = 6.0 Hz Ar-H), 7.58 (d, 2H, *J* = 6.0 Hz, Ar-H), 7.49-7.46 (m, 2H, Ar-H), 4.42 (s, 2H, CH_2); ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 188.05, 166.14, 165.66, 163.80, 143.99, 143.50, 135.21, 133.16, 132.54, 131.02, 130.50, 129.90, 129.39, 129.31, 126.84, 123.41, 122.40, 119.07, 37.37; LC−MS m/z 440.00 $[M - H]^-$; Anal. Calcd for $C_{25}H_{19}N_3O_3S$: C, 68.01; H, 4.34; N, 9.52; S, 7.26. Found: C, 68.17; H, 4.52; N, 9.80; S, 7.35.

4.1.6.2. *N*-(4-(3-(4-Chlorophenyl)acryloyl)phenyl)-2-((5-phenyl-1,3,4-oxadiazol-2-yl)thio)acetamide 8b

Yellow solid (0.29g, 63 % yield) ; mp 250-252°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 10.85 (s, 1H, NH), 8.20 (d, 2H, *J* = 6.6 Hz, Ar-H), 8.00-7.97 (m, 2H, Ar-H), 7.96-7.93 (m, 3H, 2 Ar-H and CH=C<u>H</u>), 7.79 (d, 2H, *J* = 6.8 Hz, Ar-H), 7.72 (d, 1H, *J* = 15.5 Hz, C<u>H</u>=CH), 7.62-7.58 (m, 3H, Ar-H), 7.54 (d, 2H, *J* = 6.6 Hz, Ar-H), 4.42 (s, 2H, CH_2); ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 187.92, 166.13, 165.67,

163.81, 143.56, 142.51, 135.47, 134.23, 133.07, 132.55, 131.02, 130.56, 129.91, 129.43, 126.85, 123.42, 123.16, 119.07, 37.39; LC−MS m/z 475.10 $[M]^+$; Anal. Calcd for $C_{25}H_{18}ClN_3O_3S$: C, 63.09; H, 3.81; N, 8.83; S, 6.74. Found: C, 63.23; H, 3.97; N, 9.06; S, 6.88.

4.1.6.3. *N*-(4-(3-(3,4-Dimethoxyphenyl)acryloyl)phenyl)-2-((5-phenyl-1,3,4-oxadiazol-2-yl)thio)acetamide 8c

Yellow solid (0.25g, 51% yield) mp 230-231°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 10.84 (s, 1H, NH), 8.19 (d, 2H, *J* = 6.4 Hz, Ar-H), 7.97-7.96 (m, 2H, Ar-H), 7.84 (d, 1H, *J* = 15.2 Hz, CH=C<u>H</u>), 7.80 (d, 2H, *J* = 6.4 Hz, Ar-H), 7.70 (d, 1H, *J* = 15.2 Hz, C<u>H</u>=CH), 7.63-7.57 (m, 3H, Ar-H), 7.55 (s, 1H, Ar-H), 7.39 (d, 1H, *J* = 6.0 Hz, Ar-H), 7.03 (d, 1H *J* = 6.0 Hz Ar-H), 4.42 (s, 2H, CH_2), 3.87 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3); ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 187.89, 166.08, 165.67, 163.82, 151.69, 149.49, 144.53, 143.29, 133.47, 132.55, 130.38, 129.91, 128.05, 126.85, 124.41, 123.42, 119.90, 119.02, 112.01, 111.12, 56.22, 56.07, 37.40; LC−MS m/z 501.90 $[M + H]^+$; Anal. Calcd for $C_{27}H_{23}N_3O_5S$: C, 64.66; H, 4.62; N, 8.38; S, 6.39. Found: C, 64.39; H, 4.80; N, 8.54; S, 6.52.

4.1.6.4. *N*-(4-(3-(3.4.5-Trimethoxyphenyl)acryloyl)phenyl)-2-((5-phenyl-1,3,4-oxadiazol-2-yl)thio)acetamide 8d

Yellow crystals (0.30g, 58 % yield) mp 261-262°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 10.90 (s, 1H, NH), 8.20 (d, 2H, *J* = 7.5 Hz, Ar-H), 7.97 (d, 2H, *J* = 5.2 Hz, Ar-H), 7.91 (d, 1H, *J* = 15.5 Hz, C<u>H</u>=CH), 7.81 (d, 2H, *J* = 7.5 Hz, Ar-H), 7.69 (d, 1H, *J* = 15.5 Hz, CH=C<u>H</u>), 7.65-7.58 (m, 3H Ar-H), 7.23 (s, 2H, Ar-H), 4.43 (s, 2H, CH_2), 3.87 (s, 6H, $2OCH_3$), 3.72 (s, 3H, OCH_3); ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 187.98, 166.13, 165.68, 163.82, 153.57, 144.53, 143.44, 140.11, 133.26, 132.57, 130.77, 130.49, 129.92, 126.84, 123.39, 121.52, 119.04, 106.93, 60.62, 56.60, 37.36; LC−MS m/z 529.90 $[M - H]^-$; Anal. Calcd for $C_{28}H_{25}N_3O_6S$: C, 63.26; H, 4.74; N, 7.90; S, 6.03. Found: C, 63.50; H, 4.87; N, 8.21; S, 6.11.

4.1.6.5. 2-((5-(4-Chlorophenyl)-1,3,4-oxadiazol-2-yl)thio)-*N*-(4-cinnamoyl phenyl)acetamide 8e

Yellow solid (0.30g, 63.3% yield) mp 230-231°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 10.90 (s, 1H, NH), 8.19 (d, 2H, *J* = 7.0 Hz, Ar-H), 7.99-7.97 (m, 2H, Ar-H), 7.93-7.90 (m, 3H, 2 Ar-H and C<u>H</u>=CH), 7.80 (d, 2H, *J* = 7.0 Hz, Ar-H), 7.74 (d, 1H, *J* = 15.5 Hz, CH=C<u>H</u>), 7.66 (d, 2H, *J* = 6.5 Hz, Ar-H), 7.49-7.44 (m, 3H, Ar-H), 4.43 (s, 2H, CH_2); ^{13}CNMR (125 MHz, DMSO-d_6) δ (ppm): 188.05, 166.08, 164.92, 164.11, 143.99, 143.49, 137.24, 135.23, 133.17, 131.02, 130.50, 130.09, 129.39, 129.31, 128.66, 122.41, 122.32, 119.08, 37.38; LC−MS m/z 474.10 $[M - H]^-$; Anal. Calcd for $C_{25}H_{18}ClN_3O_3S$: C, 63.09; H, 3.81; N, 8.83; S, 6.74. Found: C, 63.28; H, 3.75; N, 8.97; S, 6.82.

4.1.6.6. 2-((5-(4-Chlorophenyl)-1,3,4-oxadiazol-2-yl)thio)-*N*-(4-(3-(4-chloro-phenyl)acryloyl)phenyl)acetamide 8f

Yellow solid (0.39g, 77.0% yield) mp 181-182°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 10.86 (s, 1H, NH), 8.18 (d, 2H, *J* = 8.0 Hz, Ar-H), 7.98-7.97 (m, 3H, 2 Ar-H and CH=C<u>H</u>), 7.93 (d, 2H, *J* = 8.0 Hz, Ar-H), 7.78 (d, 2H, *J* = 8.0 Hz, Ar-H), 7.71 (d, 1H, *J* = 15.0 Hz, CH=C<u>H</u>), 7.66 (d, 2H, *J* =7.5Hz, Ar-H), 7.53 (d, 2H *J* = 8.0Hz, Ar-H), 4.42 (s, 2H, CH_2); ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 187.93, 166.09, 164.92, 164.11, 137.25, 135.47, 134.21, 133.06, 131.02, 130.56, 130.09, 129.47, 129.43, 129.01, 128.66, 123.14, 122.31, 119.06, 37.38; LC−MS m/z 509.90 $[M + H]^+$; Anal. Calcd for $C_{25}H_{17}Cl_2N_3O_3S$: C, 58.83; H, 3.36; N, 8.23; S, 6.28. Found: C, 59.17; H, 3.42; N, 8.04; S, 6.12.

4.1.6.7. 2-(5-(4-Chlorophenyl)-1,3,4-oxadiazol-2-ylthio)-*N*-(4-(3-(4-methoxy-phenyl)acryloyl)phenyl)acetamide 8g

Yellow crystals (0.31g, 62 % yield) mp 227-228°C; ^{1}H NMR (500 MHz DMSO-d_6) δ (ppm): 10.83 (s, 1H, NH), 8.17 (d, 2H, *J* = 6.0 Hz, Ar-H), 7.98 (d, 2H, *J* = 5.7 Hz, Ar-H), 7.86 (d, 2H, *J* = 5.7 Hz, Ar-H),, 7.79-7.77 (m, 3H, 2 Ar-H + CH=C<u>H</u>), 7.71 (d, 1H, *J* = 15.00 Hz, C<u>H</u>=CH), 7.67 (d, 2H, *J* = 6.0 Hz, Ar-H), 7.03 (d, 2H, *J* = 5.9 Hz, Ar-H), 4.43 (s, 2H, CH_2), 3.83 (s, 3H, OCH_3); ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 188.83, 166.02, 164.92, 164.12, 161.98, 161.77, 144.95, 143.98, 143.26, 139.02, 131.41, 131.19, 130.33, 130.08, 128.64, 122.31, 119.03, 114.87, 55.84,

37.37; LC−MS m/z 504.00 $[M - H]^-$; Anal. Calcd for $C_{26}H_{20}ClN_3O_4S$: C, 61.72; H, 3.98; N, 8.30; S, 6.34. Found: C, 61.95; H, 4.12; N, 8.59; S, 6.51.

4.1.6.8. 2-(5-(4-Chlorophenyl)-1,3,4-oxadiazol-2-ylthio)-*N*-(4-(3-(3,4-dimethoxyphenyl)acryloyl)phenyl)acetamide 8h

Yellow solid (0.38g, 71.4% yield) mp 215-216°C; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 10.88 (s, 1H, NH), 8.19 (d, 2H, *J* = 6.6 Hz, Ar-H), 7.98 (d, 2H, *J* = 6.1 Hz, Ar-H), 7.84 (d, 1H, *J* = 15.3 Hz, C<u>H</u>=CH), 7.79 (d, 2H, *J* = 6.6 Hz, Ar-H), 7.71-7.66 (m, 3H, 2 Ar-H + CH=C<u>H</u>), 7.55 (s, 1H, Ar-H), 7.39 (d, 1H, *J* = 6.1 Hz, Ar-H), 7.03 (d, 1H, *J* = 6.1 Hz, Ar-H), 4.43 (s, 2H, CH_2), 3.87 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3); ^{13}CNMR (125 MHz, $CDCl_3$) δ (ppm): 187.90, 166.02, 164.93, 164.13, 151.69, 149.49, 144.53, 143.27, 137.25, 133.47, 130.38, 130.09, 128.66, 128.05, 124.41, 122.32, 119.90, 119.02, 112.02, 111.12, 56.22, 56.07, 37.40; LC−MS m/z 534.00 $[M - H]^-$; Anal. Calcd for $C_{27}H_{22}ClN_3O_5S$: C, 60.50; H, 4.14; N, 7.84; S, 5.98. Found: C, 60.38; H, 4.27; N, 8.11; S, 6.15.

4.1.6.9. 2-(5-(4-Chlorophenyl)-1,3,4-oxadiazol-2-ylthio)-*N*-(4-(3-(3,4,5-trimethoxyphenyl)acryloyl)phenyl)acetamide 8i

Yellow crystals (0.40g, 71% yield) mp 261-262°C; ^{1}H NMR (500 MHz,,DMSO-d_6 δ (ppm): 10.85 (s, 1H, NH), 8.20 (d, 2H, *J* = 7.1 Hz, Ar-H), 7.98 (d, 2H, *J* = 6.9 Hz, Ar-H), 7.91 (d, 1H, *J* = 15.4, C<u>H</u>=CH), 7.80 (d, 2H, *J* = 7.1 Hz, Ar-H), 7.71-7.67 (m, 3H, 2 Ar-H + CH=C<u>H</u>), 7.24 (s, 2H, Ar-H), 4.43 (s, 2H, CH_2), 3.87 (s, 6H, $2OCH_3$), 3.72 (s, 3H ,OCH_3); ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 187.96, 166.05, 164.93, 164.13, 153.58, 144.52, 143.41, 140.05, 137.25, 133.29, 130.78, 130.49, 130.10, 128.66, 122.32, 121.53, 119.03, 106.96, 60.62, 57.30, 56.61, 37.41; Anal. Calcd for $C_{28}H_{24}ClN_3O_6S$: C, 59.41; H, 4.27; N, 7.42; S, 5.67. Found: C, 59.58; H, 4.35; N, 7.60; S, 5.74.

4.1.6.10. 2-(5-(4-Methoxyphenyl)-1,3,4-oxadiazol-2-ylthio)-*N*-(4-(3-phenyl acryloyl)phenyl)acetamide 8j

Yellow solid (0.39g, 84% yield) mp 195-197°C; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 9.90 (s, 1H, NH), 8.03 (d, 2H, *J* = 7.0 Hz, Ar-H), 7.96 (d, 2H, *J* = 7.0

Hz, Ar-H), 7.81 (d, 1H, *J* = 15.0 Hz, CH=CH), 7.77-7.75 (m, 1H, Ar-H), 7.66 (d, 2H, *J* = 6.0 Hz, Ar-H), 7.53 (d, 1H, *J* = 15.0, CH=CH), 7.45- 7.42 (m, 4H, Ar-H), 7.02 (d, 2H, *J* = 6.0 Hz, Ar-H), 4.11 (s, 2H, CH_2) , 3.90 (s, 3H, OCH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 189.01, 166.56, 165.84, 164.44, 162.84, 144.59, 141.96, 130.49, 129.92, 128.97, 128.75, 128.47, 121.83, 119.36, 115.17, 114.83, 114.73, 114.12, 55.56, 36.50; Anal. Calcd for $C_{26}H_{21}N_3O_4S$: C, 66.23; H, 4.49; N, 8.91; S, 6.80. Found: C, 66.06; H, 4.62; N, 9.18; S, 6.59.

4.1.6.11. 2-(5-(4-Methoxyphenyl)-1,3,4-oxadiazol-2-ylthio)-*N*-(4-(3-(4-chlorophenyl)acryloyl)phenyl)acetamide 8k

Yellow solid (0.37g, 75% yield) mp 250-251°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 10.83 (s, 1H, NH), 8.19 (d, 2H, *J* = 6.3 Hz, Ar-H), 7.98 (d, 1H, *J* = 16.0 Hz, CH=CH), 7.94 (d, 2H, *J* = 5.7 Hz, Ar-H), 7.89 (d, 2H, *J* = 6.2 Hz, Ar-H), 7.79 (d, 2H, *J* = 6.2 Hz, Ar-H), 7.72 (d, 1H, *J* = 16.0 Hz, CH=CH), 7.54 (d, 2H, *J* = 5.7 Hz, Ar-H), 7.12 (d, 2H, *J* = 6.3 Hz, Ar-H), 4.39 (s, 2H, CH_2), 3.84 (s, 3H, OCH_3); ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 188.04, 166.11, 165.61, 163.62, 153.92, 144.01, 143.48, 141.02, 135.21, 133.17, 131.03, 130.50, 129.40, 129.31, 122.38, 119.06, 118.59, 104.23, 56.59, 37.41; LC−MS m/z 504.00 $[M - H]^-$; Anal. Calcd for $C_{26}H_{20}ClN_3O_4S$: C, 61.72; H, 3.98; N, 8.30; S, 6.34. Found: C, 61.97; H, 4.11; N, 8.54; S, 6.47.

4.1.6.12. 2-(5-(4-Methoxyphenyl)-1,3,4-oxadiazol-2-ylthio)-*N*-(4-((*E*)-3-(4-methoxyphenyl)acryloyl)phenyl)acetamide 8l

Yellow solid (0.37g, 75% yield) mp 183-185°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 10.86 (s, 1H, NH), 8.12 (d, 2H, *J* = 8.0 Hz, Ar-H), 7.86 (d, 2H, *J* = 7.5 Hz, Ar-H), 7.81 (d, 2H, *J* = 8.0 Hz, Ar-H), 7.75-7.71 (m, 3H, Ar-H and CH=CH), 7.68 (d, 1H, *J* = 15.0 *Hz* , CH=CH) 7.08 (d, 2H, *J* = 8.0 *Hz*, Ar-H), 7.00 (d, 2H, *J* = 7.5 Hz, Ar-H), 4.32 (s, 2H, CH_2), 3.82 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3); ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 187.91, 166.15, 165.63, 162.94, 162.53, 161.77, 143.90, 143.32, 133.43, 131.20, 130.33, 128.72, 127.81, 119.85, 119.03, 115.74, 115.33, 114.87, 56.12, 55.99, 37.24; LC−MS m/z 500.10 $[M - H]^-$; Anal. Calcd for

$C_{27}H_{23}N_3O_5S$: C, 64.66; H, 4.62; N, 8.38; S, 6.39. Found: C, 64.49; H, 4.80; N, 8.57; S, 6.21.

4.1.6.13. 2-(5-(4-Methoxyphenyl)-1,3,4-oxadiazol-2-ylthio)-*N*-(4-3-(3,4-dimethoxyphenyl)acryloyl)phenyl)acetamide 8m

Yellow crystals (0.40g, 76.9% yield) mp 193-195°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 10.83 (s, 1H, NH), 8.19 (d, 2H, *J* = 7.3 Hz, Ar-H), 7.90 (d, 2H, *J* = 7.6 Hz, Ar-H), 7.84 (d, 1H, *J* = 15.2 Hz, CH=CH), 7.79 (d, 2H, *J* = 7.3 Hz, Ar-H), 7.70 (d, 1H, *J* = 15.2 Hz, CH=CH), 7.55 (s, 1H, Ar-H), 7.39 (d, 1H, *J* = 7.1 Hz, Ar-H), 7.12 (d, 2H, *J* = 7.6 Hz, Ar-H), 7.03 (d, 1H, *J* = 7.1 Hz, Ar-H), 4.39 (s, 2H, CH_2) , 3.87 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3); ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 187.90, 166.13, 165.64, 162.95, 162.55, 151.70, 149.49, 144.53, 143.30, 133.46, 130.38, 128.73, 128.05, 124.41, 119.90, 119.02, 115.75, 115.36, 112.02, 111.13, 56.22, 56.08, 56.01, 37.38; Anal. Calcd for $C_{28}H_{25}N_3O_6S$: C, 63.26; H, 4.74; N, 7.90; S, 6.03. Found: C, 63.58; H, 4.89; N, 7.81; S, 6.22.

4.1.6.14. 2-(5-(4-Methoxyphenyl)-1,3,4-oxadiazol-2-ylthio)-*N*-(4-(3-(3,4,5-trimethoxyphenyl)acryloyl)phenyl)acetamide 8n

Yellow solid (0.43g, 83.3% yield) mp 220-221°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 10.84 (s, 1H, NH), 8.20 (d, 2H, *J* = 5.7 Hz, Ar-H), 7.91-7.89 (m, 3H, 2 Ar-H and CH=CH), 7.80 (d, 2H, *J* = 5.5 Hz, Ar-H), 7.69 (d, 1H, *J* = 15.3 Hz, CH=CH), 7.24 (s, 2H, Ar-H), 7.12 (d, 2H, *J* = 5.7 Hz, Ar-H), 4.40 (s, 2H, CH_2), 3.87 (s, 6H, 2 OCH_3), 3.85 (s, 3H, OCH_3), 3.72 (s, 3H, OCH_3); ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 187.95, 166.17, 165.63, 162.96, 162.54, 153.58, 144.53, 143.46, 140.10, 133.26, 130.78, 130.50, 128.72, 121.51, 119.01, 115.74, 115.35, 106.93, 56.59, 55.99, 53.91, 37.37; LC−MS m/z 560.10 $[M - H]^-$; Anal. Calcd for $C_{29}H_{27}N_3O_7S$: C, 62.02; H, 4.85; N, 7.48; S, 5.71. Found: C, 61.89; H, 4.97; N, 7.67; S, 5.84.

4.1.6.15. *N*-(4-Cinnamoylphenyl)-2-((5-(3,4-dimethoxyphenyl)-1,3,4-oxadiazol-2-yl)thio)acetamide 8o

Yellow powder (0.41 g, 83.3% yield) mp 200-202°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 11.25 (s, 1H, NH), 8.18 (d, 2H, *J* = 7.3 Hz, Ar-H), 7.96 (d, 1H, *J* = 15.3 Hz, CH=CH), 7.91-7.88 (m, 3H, Ar-H), 7.84 (d, 2H, *J* = 7.3 Hz, Ar-H), 7.73 (d, 1H,

J = 15.3 Hz, CH=CH), 7.55 (d, 1H, *J* = 7.7 Hz, Ar-H), 7.48-7.45 (m, 3H, Ar-H), 7.13 (d, 1H, *J* = 7.7 Hz, Ar-H), 4.45 (s, 2H, CH_2), 3.84 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3); ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 188.05, 166.19, 165.72, 163.00, 152.34, 149.54, 143.99, 143.54, 135.21, 131.02, 130.48, 129.39, 129.31, 122.39, 120.45, 119.60, 119.06, 115.65, 112.46, 109.45, 56.49, 56.18, 37.36; LC−MS m/z 500.10 $[M - H]^-$; Anal. Calcd for $C_{27}H_{23}N_3O_5S$: C, 64.66; H, 4.62; N, 8.38; S, 6.39. Found: C, 64.89; H, 4.76; N, 8.62; S, 6.50.

4.1.6.16. *N*-(4-(3-(4-chlorophenyl)acryloyl)phenyl)-2-((5-(3,4-dimethoxyphenyl)-1,3,4-oxadiazol-2-yl)thio)acetamide 8p

Pale yellow powder (0.32g, 60.7% yield) mp 239-241°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 10.88 (s, 1H, NH), 8.18 (d, 2H, *J* = 8.1 Hz, Ar-H), 7.97 (d, 1H, *J* = 15.6 Hz, CH=CH), 7.93 (d, 2H, *J* = 7.8 Hz, Ar-H), 7.78 (d, 2H, *J* = 8.1 Hz, Ar-H), 7.71 (d, 1H, *J* =15.6 Hz, CH=CH), 7.53 (d, 3H, *J* = 8.4 Hz, Ar-H), 7.42 (s, 1H, Ar-H), 7.12 (d, 1H, *J* = 8.4 Hz, Ar-H), 4.39 (s, 2H, CH_2), 3.83 (s ,3H ,OCH_3), 3.80 (s, 3H, OCH_3); ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 187.94, 166.19, 165.73, 163.01, 152.33, 149.52, 143.57, 142.53, 135.48, 134.19, 131.01, 130.55, 129.60, 129.43, 123.10, 120.45, 119.06, 115.62, 112.44, 109.40, 56.17, 56.08, 37.36; Anal. Calcd for $C_{27}H_{22}ClN_3O_5S$: C, 60.50; H, 4.14; N, 7.84; S, 5.98. Found: C, 60.76; H, 4.23; N, 8.11; S, 6.09.

4.1.6.17. 2-((5-(3,4-Dimethoxyphenyl)-1,3,4-oxadiazol-2-yl)thio)-*N*-(4-(3-(4-methoxyphenyl)acryloyl)phenyl)acetamide 8q

Yellow solid (0.34g, 65.3% yield) mp 189-190°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 10.82 (s, 1H, NH), 8.17 (d, 2H, *J* = 6.4 Hz, Ar-H), 7.86 (d, 2H, *J* = 6.0 Hz, Ar-H), 7.80- 7.77 (m, 3H, 2 Ar-H + CH=CH), 7.71 (d, 1H, *J* = 15.3 Hz, CH=CH), 7.54 (d, 1H, *J* = 6.3 Hz, Ar-H), 7.43 (s, 1H, Ar-H), 7.13 (d, 1H, *J* = 6.3, Ar-H), 7.03 (d, 2H, *J* = 6.4 Hz, Ar-H), 4.40 (s, 2H, CH_2), 3.84 (s, 6H, 2 OCH_3), 3.82 (s, 3H, OCH_3); ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 187.69, 166.23, 165.72, 162.99, 161.76, 152.32, 149.52, 143.97, 143.42, 133.37, 131.21, 130.28, 127.84, 120.47, 119.86, 119.04, 115.64, 114.88, 112.46, 109.46, 56.19, 56.13, 55.86, 37.26; LC−MS m/z 530.20 $[M - H]^-$; Anal. Calcd for $C_{28}H_{25}N_3O_6S$: C, 63.26; H, 4.74; N, 7.90; S, 6.03. Found: C, 63.14; H, 4.89; N, 8.16; S, 6.15.

4.1.6.18. 2-((5-(3,4-Dimethoxyphenyl)-1,3,4-oxadiazol-2-yl)thio)-*N*-(4-(3-(3,4-dimethoxyphenyl)acryloyl)phenyl)acetamide 8r

Yellow solid (0.37 g, 66.0% yield) mp 213-214°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 10.83 (s, 1H, NH), 8.19 (d, 2H, *J* = 6.1 Hz, Ar-H), 7.84 (d, 1H, *J* = 15.2 Hz, CH=CH), 7.79 (d, 2H, *J* = 6.1 Hz, Ar-H), 7.70 (d, 1H, *J* = 15.2 Hz, CH=CH), 7.55-7.51 (m, 2H, Ar-H), 7.43 (s, 1H, Ar-H), 7.39 (d, 1H, *J* = 5.8 Hz, Ar-H), 7.13 (d, 1H, *J* = 6.6 Hz, Ar-H), 7.03 (d, 1H, *J* = 5.8, Ar-H), 4.40 (s, 2H, CH_2), 3.87 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 3.82 (s, 6H, 2 OCH_3); ^{13}C NMR (DMSO-d_6) δ (ppm): 187.90, 166.11, 165.73, 163.02, 152.36, 151.69, 149.56, 149.49, 144.53, 143.30, 133.47, 130.37, 128.05, 124.40, 120.45, 119.89, 119.01, 115.66, 112.47, 112.01, 111.12, 109.47, 56.22, 56.19, 56.11, 56.07, 37.42; LC−MS m/z 562.00 $[M + H]^+$; Anal. Calcd for $C_{29}H_{27}N_3O_7S$: C, 62.02; H, 4.85; N, 7.48; S, 5.71. Found: C, 62.27; H, 4.97; N, 7.62; S, 5.89.

4.1.6.19. 2-((5-(3,4-Dimethoxyphenyl)-1,3,4-oxadiazol-2-yl)thio)-*N*-(4-(3-(3,4,5-trimethoxyphenyl)acryloyl)phenyl)acetamide 8s

Yellow solid (0.42g, 72.2% yield) mp 210-212°C; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 9.86 (s, 1H, NH), 8.02 (d, 2H, *J* = 8.0 Hz, Ar-H), 7.75-7.70 (m, 3H, 2 Ar-H, CH=CH), 7.60 (d, 1H, *J* = 7.0 Hz, Ar-H), 7.53 (s, 1H, Ar-H), 7.40 (d, 1H, *J* = 15.0 Hz, CH=CH), 6.99 (d, 1H, *J* = 8.0, Ar-H), 6.87 (s, 2H, Ar-H), 4.09 (s, 2H ,CH_2), 3.98 (s, 6H, 2OCH_3), 3.94 (s, 6H, 2 OCH_3), 3.92 (s, 3H, OCH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 189.05, 166.62, 165.87, 164.53, 153.48, 152.51, 149.47, 144.83, 141.86, 140.35, 134.15, 130.42, 129.93, 121.19, 120.61, 119.33, 115.26, 111.21, 109.14, 105.60, 61.04, 56.25, 56.21, 56.13, 36.35; LC−MS m/z 590.20 $[M - H]^-$; Anal. Calcd for $C_{30}H_{29}N_3O_8S$: C, 60.90; H, 4.94; N, 7.10; S, 5.42. Found: C, 61.23; H, 4.89; N, 7.24; S, 5.58.

4.1.6.20. *N*-(4-Cinnamoylphenyl)-2-((5-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazol-2-yl)thio)acetamide 8t

Pale yellow crystals (0.38g,71.8% yield) mp 219-221°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 10.84 (s, 1H, NH), 8.19 (d, 2H, *J* = 6.7 Hz, Ar-H), 7.95 (d, 1H, *J* = 15.5 Hz, CH=CH), 7.90-7.88 (m, 2H, Ar), 7.79 (d, 2H, *J* = 6.7 Hz, Ar-H), 7.74

(d, 1H, *J* = 15.5 Hz, C<u>H</u>=CH), 7.49-7.45 (m, 3H, Ar-H), 7.22 (s, 2H, Ar-H), 4.42 (s, 2H, CH_2), 3.84 (s, 6H, $2OCH_3$), 3.74 (s, 3H, OCH_3), ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 188.98, 166.54, 165.74, 164.83, 153.80, 144.64, 141.89, 141.61, 130.52, 129.92, 129.50, 128.97, 128.46, 121.79, 120.06, 119.31, 117.85, 104.11, 61.07, 56.43, 36.39; LC−MS m/z 531.10 $[M]^-$; Anal. Calcd for $C_{28}H_{25}N_3O_6S$: C, 63.26; H, 4.74; N, 7.90; S, 6.03. Found: C, 63.54; H, 4.65; N, 8.12; S, 6.15.

4.1.6.21. *N*-(4-(3-(4-Chlorophenyl)acryloyl)phenyl)-2-((5-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazol-2-yl)thio)acetamide 8u

Yellow solid (0.49g, 87.5% yield) mp 259-261°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 10.85 (s, 1H, NH), 8.19 (d, 2H, *J* = 6.6 Hz, Ar-H), 7.98 (d, 1H, *J* = 16.0 Hz, CH=C<u>H</u>), 7.94 (d, 2H, *J* = 6.4 Hz, Ar-H), 7.79 (d, 2H, *J* = 6.4 Hz, Ar-H), 7.72 (d, 1H, *J* = 16.0 Hz, C<u>H</u>=CH), 7.54 (d, 2H, *J* = 6.6 Hz, Ar-H), 7.22 (s, 2H, Ar-H), 4.80 (s, 2H, CH_2), 3.97 (s, 6H, 2 OCH_3), 3.95 (s, 3H, OCH_3), ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 187.91, 166.12, 165.62, 163.62, 153.93, 143.56, 142.52, 141.03, 135.47, 134.22, 133.08, 131.02, 130.55, 129.43, 123.14, 119.05, 118.59, 104.25, 60.71, 56.60, 37.43; LC−MS m/z 564.10 $[M - H]^-$; Anal. Calcd for $C_{28}H_{24}ClN_3O_6S$: C, 59.41; H, 4.27; N, 7.42; S, 5.67. Found: C, 59.32; H, 4.35; N, 7.68; S, 5.73.

4.1.6.22. *N*-(4-(3-(4-Methoxyphenyl)acryloyl)phenyl)-2-((5-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazol-2-yl)thio)acetamide 8v

Yellow crystals (0.47g,84% yield) mp 211-212°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 10.83 (s, 1H, NH), 8.17 (d, 2H, *J* = 6.9 Hz, Ar-H), 7.86-7.82 (m, 3H, 2 Ar-H + CH=C<u>H</u>), 7.78 (d, 2H, *J* = 7.6 Hz, Ar-H), 7.71 (d, 1H, *J* = 15.2 Hz, C<u>H</u>=CH), 7.22 (s, 2H, Ar-H), 7.03 (d, 2H, *J* = 6.9, Ar-H), 4.42 (s, 2H, CH_2), 3.84 (s, 9H, $3OCH_3$), 3.74 (s, 3H, OCH_3); ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 187.96, 169.22, 165.75, 165.02, 161.78, 153.90, 144.00, 143.22, 140.82, 133.50, 131.21, 130.26, 127.84, 119.84, 119.21, 118.83, 114.88, 104.13, 60.69, 56.62, 55.86, 37.37; LC−MS m/z 561.90 $[M + H]^+$; Anal. Calcd for $C_{29}H_{27}N_3O_7S$: C, 62.02; H, 4.85; N, 7.48; S, 5.71. Found: C, 62.19; H, 4.98; N, 7.67; S, 5.92.

4.1.6.23. *N*-(4-(3-(3,4-Dimethoxyphenyl)acryloyl)phenyl)-2-((5-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazol-2-yl)thio)acetamide 8w

Yellow solid (0.32g, 55.0% yield) mp 168-170°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 10.98 (s, 1H, NH), 8.17 (d, 2H, *J* = 8.0 Hz, Ar-H), 7.83-7.79 (m, 3H, 2 Ar-H + CH=CH), 7.80 (d, 1H, *J* =15.0 Hz, CH=CH), 7.53 (s, 1H, Ar-H), 7.38 (d, 1H, *J* = 8.0 Hz Ar-H), 7.21 (s, 2H, Ar-H), 7.01 (d, 1H *J* = 8.0 Hz, Ar- H), 4.43 (s, 2H, CH_2), 3.86 (s, 6H, 2 OCH_3), 3.83 (s, 6H, 2 OCH_3), 3.73 (s, 3H, OCH_3); ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 188.00, 166.12, 165.61, 163.62, 153.92, 151.68, 149.47, 144.53, 143.35, 141.00, 133.43, 130.35, 128.03, 124.41, 119.88, 119.01, 118.59, 112.00, 111.10, 104.23, 60.70, 56.60, 56.21, 56.06, 37.36; Anal. Calcd for $C_{30}H_{29}N_3O_8S$: C, 60.90; H, 4.94; N, 7.10; S, 5.42. Found: C, 61.23; H, 5.12; N, 7.37; S, 5.39.

4.1.6.24. 2-((5-(3,4,5-Trimethoxyphenyl)-1,3,4-oxadiazol-2-yl)thio)-*N*-(4-(3-(3,4,5-trimethoxyphenyl)acryloyl)phenyl)acetamide 8x

Yellow solid (0.49g, 80% yield) mp 196-198°C; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 9.74 (s, 1H, NH), 8.02 (d, 2H, *J* = 8.1 Hz, Ar-H), 7.74-7.70 (m, 3H, 2 Ar-H + CH=CH), 7.39 (d, 1H, *J* = 15.6 Hz, CH=CH), 7.24 (s, 2H, Ar-H), 6.87 (s, 2H, Ar-H), 4.09 (s, 2H, CH_2), 3.95 (s, 6H, 2 OCH_3), 3.94 (s, 9H, 3 OCH_3), 3.92 (s, 3H, OCH_3), ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 189.03, 166.57, 165.77, 164.79, 153.79, 153.49, 144.84, 141.80, 141.58, 140.44, 134.22, 130.41, 129.91, 121.18, 119.31, 117.87, 105.69, 104.08, 61.07, 61.02, 56.41, 56.25, 36.31; LC−MS m/z 620.10 $[M - H]^-$; Anal. Calcd for $C_{31}H_{31}N_3O_9S$: C, 59.89; H, 5.03; N, 6.76; S, 5.16. Found: C, 59.71; H, 5.21; N, 6.94; S, 5.24.

4.2.Biology section

Cell culture and reagents

Human leukemia cell lines (K-562, KG1a, and Jurkat) were obtained from the American Type Culture Collection (ATCC; Manassas, VA, USA) and were cultured in Iscove's Modified Dulbecco's Medium (IMDM, Sigma-Aldrich) or Roswell Park Memorial Institute Medium (RPMI 1640, Sigma-Aldrich), containing 10% fetal bovine serum (FBS; Sigma-Aldrich) in a humidified atmosphere with 5% CO_2 at 37°C. Cisplatin, dasatinib, dexamethasone, gefitinib, and WP1066 were purchased from Sigma-Aldrich. All chemicals used in this study were analytical or cell-culture grade.

4.2.1. Proliferation assay

The methodology for NCI anticancer screening has been described in detail elsewhere (http://www.dtp.nci.nih.gov). Briefly, the primary anticancer assay was performed at approximately 60 human tumor cell lines panel derived from nine neoplastic diseases, in accordance with the protocol of the Drug Evaluation Branch, National Cancer Institute, Bethesda. Tested compounds were added to the cells at a single concentration (10^{-5} M) and were incubated for 48 h. Endpoint determinations were made with a protein binding dye, SRB. Absorbance was evaluated spectrophotometrically and results for each tested compound were reported as the percent of the growth of the treated cells when compared to untreaed cells.

Compound **8v,** which showed significant cell growth inhibition in the One-Dose Screen, was evaluated against the 58 cell panel at five different concentrations by a dissolving the drug in dimethyl sulfoxide. After drug addition, cells were incubated at 37° C, 5 % CO_2, 95 % air and 100 % relative humidity for 48 h, stained by SRB, and the absorbance was evaluated spectrophotometrically by using an automated plate. The growth percentage was calculated at different drug concentrations levels and at different time points.

4.2.2. Anti-proliferative assay [69, 70].

Cytotoxicity was measured using the MTT assay. Leukemia cells (K-562, KG1a, and Jurkat) were plated at a density of 1×10^4 cells per well in 96-well plates overnight and then treated with vehicle, different concentrations (1, 10, 25, 50, and 100 μM) of 1,3,4-oxadiazole/chalcone derivatives, **8a-x,** or cisplatin (positive control). After 24 h treatment, 20 μL of MTT solution (2mg/mL in phosphate-buffered saline [PBS]) was added to each well and the cells were cultured for another 4 h at 37°C. The medium was aspirated and 150 μL DMSO was added to solubilize MTT formazan crystals. The plates were then shaken, and the optical density was determined at 570 nm using an ELISA plate reader (Model 550, Bio-Rad, USA). At least three independent experiments were performed. The IC_{50} values were calculated using GraphPad Prism 5 (Version 5.01, GraphPad Software, San Diego, CA, USA)

4.2.3. EGFR and Src kinase assay [71].

The EGFR and c-Src kinase assays were carried out in 96-well plates coated with PGT (poly L-glutamic acid L-tyrosine, 4:1, Sigma Aldrich, MO, USA) and incubated at 37°C for 48 h. PGT acts as the substrate for phosphorylation by EGFR and c-Src (Enzo Life Sciences Inc, NY, USA) in the presence of ATP (50 μM). Different concentrations (0.01, 0.1, 1, 10, and 100 μM) of 1,3,4-oxadiazole/chalcone derivatives (**8a-x)**, gefitinib, or dasatinib were added to compete with ATP for binding to ATP-binding site in the kinase domain of EGFR or c-Src. Fifteen ng of EGFR (20 μg/mL) or 6 ng of c-Src (0.1 μg/μL) were added to each well. HRP-conjugated anti-phosphotyrosine antibody (Cell Signaling Technology, Japan) was then used to detect the phosphorylated substrate. The signal was developed by the addition of 3, 3', 5, 5'-tetramethylbenzidine peroxidase substrate (Abcam, Japan) and the colorimetric reaction was monitored at 450 nm using a microplate reader (Bio-Rad, USA). IC_{50} values were calculated using GraphPad Prism 5. Each experiment was carried out at least three times.

4.2.4. Western blot analysis [72].

K562 cells were incubated with different concentrations of compound **8v** (0.2 μM, 0.8 μM and 1.6 μM), or DMSO 24 h. The protein was then collected and measured. For western blot analysis, 50μg of protein was loaded onto a 8 or 10 % SDS-PAGE gel. Proteins separated on the SDS-PAGE gel were transferred to a polyvinylidene fluoride (PVDF) membrane. The PVDF membrane was incubated in a blocking buffer containing 3 % non-fat milk powder, 1 % BSA (Sigma-Aldrich), and 0.5 % Tween-20 in PBS for 1 h. Subsequently, the PVDF membrane was incubated with the suitable and validated primary antibody (Cell Signaling Technology, Danvers, MA, USA) overnight, followed by horseradish peroxidase (HRP)-conjugated IgG (Cell Signaling Technology, Beverly, MA, USA) for 1 h with gentle agitation at room temperature. Detection was carried out using enhanced chemiluminescence (ECL) prime (GE Healthcare, Little Chalfont, UK) and autoradiography with an X-ray film (Konica Minolta Medical Imaging, Wayne, NJ, USA)

4.2.5. IL-6 ELISA [73].

IL-6 cytokine levels in cell-free culture supernatants were determined using Hu-IL6 Cytoset ELISA kit (Biosource International Inc. CA, USA) according to the manufacturer's instructions. Briefly, K-562 cells were treated with DMSO, 10 μM

1,3,4-oxadiazole/chalcone derivatives (**8a-x),** or dexamethasone (positive control) for 24 h. Cell-free medium supernatants were collected for quantification of levels of secreted IL-6 protein via ELISA. The results were expressed as percentages relative to the vehicle control and as mean ± SD based on three independent experiments, each done in triplicate. The time course effect of compound **8v** on IL-6 protein expression was carried out by culturing K-562 cells in 12-well plates. The media were changed when **8v** (10 μM) was added to the cells. Media were collected at 0, 1, 4, 8, 12, 24, and 48 h and used for IL-6 determination by ELISA and expressed as mean ± SD based on three independent experiments, each done in triplicate.

4.2.6. STAT3 activation assay [74].

K562 cells were seeded in 10 cm plates overnight and then treated with vehicle, 10 μM 1,3,4-oxadiazole/chalcone derivatives (**8a-x),** or WP1066 (positive control) for 24 hours. Cells were collected and nuclear fractions were extracted using a Nuclear Extract Kit (Active Motif, Tokyo, Japan) according to the manufacturer's protocol. Nuclear extracts (20 ug) were used to analyze STAT3 activation using the TransAM STAT3 Activation Assay (Active Motif) according to the manufacturer's protocol. The results were expressed as the mean ± SD based on three independent experiments, each done in triplicate.

4.2.7. Statistical analysis

Data were presented as the means ± standard deviations (SD). Student's *t*-test was performed to determine the statistical significance compared to the vehicle treated control. Statistical significance was defined as * $p < 0.05$ or ** $p < 0.005$. Data are representative of three independent experiments.

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Conflicts of Interest

The authors declare no conflicts of interest.

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Highlights

- New 1,3,4-Oxadiazole/Chalcone hybrids **8a-x** were synthesized.
- Compounds **8a**, **8g**, **8n**, **8p**, **8s** and **8v** had shown the highest cytotoxicity against **K-562** cells with IC_{50} ranging from 1.95 to 16.09 µM.
- Compounds, **8a**, **8n** and **8v** displayed the highest inhibition activity against EGFR, Src as well as IL-6.
- The tested hybrids decreased STAT3 activation.

Graphical Abstract