

Received:

20 June 2018

Revised:

19 August 2018

Accepted:

14 February 2019

Cite as: Rangappa Santosh, Ashwini Prabhu, Mukunthan K. Selvam, Panchangam M. Krishna, Gundibasappa K. Nagaraja, Punchappady D. Rekha. Design, synthesis, and pharmacology of some oxadiazole and hydroxypyrazoline hybrids bearing thiazoyl scaffold: antiproliferative activity, molecular docking and DNA binding studies. Heliyon 5 (2019) e01255. doi: 10.1016/j.heliyon.2019.e01255



Design, synthesis, and pharmacology of some oxadiazole and hydroxypyrazoline hybrids bearing thiazoyl scaffold: antiproliferative activity, molecular docking and DNA binding studies

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Abstract

A series of oxadiazole (**7a-l**) and hydroxypyrazoline derivatives (**8a-l**) incorporating thiazole were synthesized and characterized by spectral analysis (¹H-NMR, ¹³C-NMR, Mass, and FT-IR). The synthesized compounds were screened for their *in vitro* cytotoxicity against MDA-MB231 and HT-29 human cell lines. Conjugates **7d**, **7e**, **7f**, **7i**, **7l**, **8a**, **8b**, **8i** and **8l** exhibited significant antiproliferative activity on both MDA-MB231 and HT-29 cell lines. Flow cytometric analysis reveals that, **7i** arrests both cells lines at G₀/G₁ phase

whereas **8i** induced G0/G1 arrest only in the HT-29 cells. Furthermore, Computational interaction studies of **7i** and **8i** exhibited its capacity of being a plausible CDK2 and BCL-2 inhibitor respectively. In addition, DNA binding of the synthesized compounds and DNA docking of **7i** and **8i** demonstrated the ability to interact with DNA. Compounds **7i** and **8i** causes' remarkable growth inhibition of MDA-MB231 and HT-29 cells but compound **8i** was considerably effective against HT-29 cells. Overall these compounds can be practiced for further drug development.

Keyword: Organic chemistry

1. Introduction

As per WHO, the burden of cancer will increase to 23.6 million new cases each year by 2030 [1]. Breast cancer is the most frequently diagnosed cancer and the leading cause of death in females, whereas colorectal cancer is the third most commonly diagnosed cancer in males and females [2]. The treatments for these cancers still remain a challenging task as chemotherapy is often ineffective, because of the intrinsic drug resistance to these tumours [3]. There is evidence to indicate that colorectal cancer cells are self-sufficient in growth signals, which escapes from apoptosis [4] Therefore, it is imperative to develop more effective drugs. Apoptosis is a morphologically and biochemically driven process, while impaired apoptosis and defects in the regulation of the cell cycle are hallmarks that contribute to cancer growth and aggressiveness [5]. With progressing knowledge of oncogenesis and apoptosis, comes an appreciation of the role played by cell-cycle regulation, in malignant transformation. Modulation of the cell cycle also contributes to chemotherapy resistance. The cyclin-dependent kinases1 (CDK1), cyclin-dependent kinases2 (CDK2) and DNA, the essential engines of the cell cycle, are therefore rational therapeutic targets. Over the last several years, a new class of anticancer therapy has been developed and extensively tested against these targets.

Modern studies have shown a significant fascinate in thiazole derivatives, due to far-reaching biological activities, such as anticonvulsant activity [6], anti-HIV [7], anti-diabetic [8], anti-alzheimer [9], antimalarial [10], antimicrobial [11], anti-inflammatory [12], antiproliferative against MiaPaCa-2 cell line [13], antiproliferative against gastric carcinoma cells [14], antiproliferative against diffuse malignant peritoneal mesothelioma cell lines, a very aggressive form of cancer [15] and CDK1 inhibitory activity of thiazol [16]. Most of the pharmaceutical drugs such as Fanetizole, Meloxicam [17], Tiazofurin [18] and Ritonavir [19] (Fig. 1) contains thiazole rings.

On the other hand, there are a bunch of reports on 1,3,4-oxadiazoles exhibiting various pharmacological activities, such as anti-diabetic [20], antihypertension

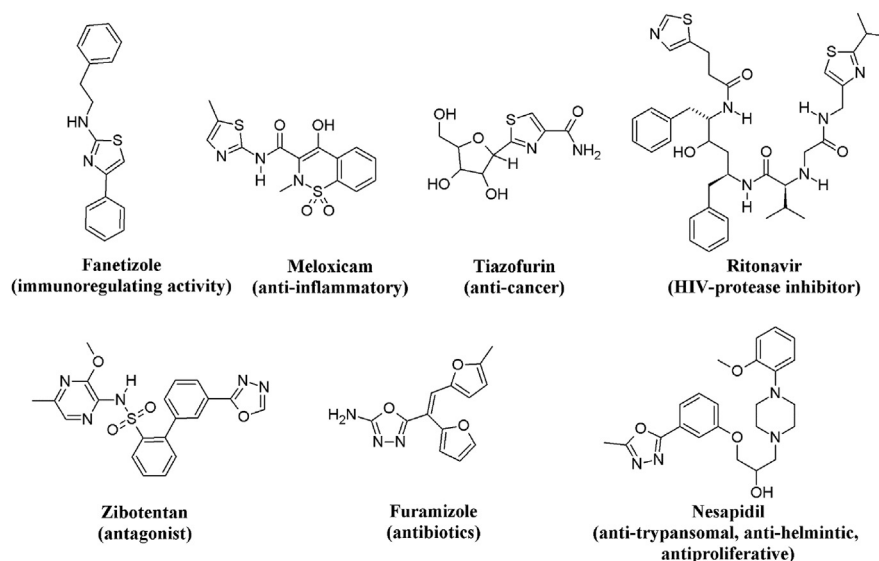


Fig. 1. Commercially available drugs containing thiazole and 1,3,4- oxadiazole.

[21], analgesic [22], antiviral [23], anticonvulsant [24], antifungal [25] antibacterial [26], anticancer [27], anti-glycation [28], anti-inflammatory [29], antimicrobial [30], and ulcerogenic [31]. Compounds containing oxadiazole units such as Nesapidil, Furamizole, and Zibotentan [32] (Fig. 1) are currently used in clinical medicines. Additionally, pyrazolines incorporated with a variety of functional groups or substituents are found in many important biologically active compounds and significant research on this species has been carried out. They exhibit a wide range of biological activities such as antidepressant, anti-inflammatory, antimicrobial, and anticancer effects etc. In accordance with literature, pyrazoline derivatives are not useful in treatment of various cancer types, including lung, breast, colon, rectum, brain, stomach, liver, bladder, pancreas, bone, mouth, esophagus, cervix and prostate cancers, and also some of them act as cancer chemopreventive agents [33, 34, 35, 36, 37, 38]. Numerous analysis shown, pyrazoline derivatives were reported as epidermal growth factor receptor tyrosine kinase (EGFR-TK) inhibitors [39], COX-2/B-Raf inhibitors [40], aurora kinase inhibitors [41], tubulin assembling inhibitors [42], telomerase inhibitors [43]. Provoked by above-mentioned observations and in continuation of our search for potent and less toxic antiproliferative agents, we have aspired to introduce some hybrids by combining thiazole-oxadiazole and thiazole-hydroxypyrazoline pharmacophores in a molecular framework.

Dinesh *et al.* [44] published anticancer activity of hydroxypyrazoline against MCF-7, MDA-MB-cancer cell lines. Samir Bondock *et al.* [45] reported 1,3,4-oxadiazole with antitumor activities against HepG2, WI-38, VERO, MCF-7 cancer cell lines, N.C. Desai *et al.* [11] analyzed 1,3,4-oxadiazole clubbed thiazole with anticancer activity against HeLa cell lines and M.F. Hassan *et al.* [46] disclosed DNA binding studies of 1,3,4-oxadiazole and 1,3,4-thiadiazole derivatives.

Based on literature survey, the interaction of thiazole-hydroxypyrazoline derivatives in a molecular framework has so far not been studied whereas there exists a report on some of the thiazole-oxadiazole derivatives as antitubercular agent [47] but not reported as antiproliferative agents. This flurry encouraged us on synthesis, purification and characterization of thiazole-oxadiazoles and thiazole-hydroxypyrazoline class of compounds, for relatively safe alternatives to ameliorate the clinical consequences of the breast and colorectal cancers.

2. Results and discussion

2.1. Chemistry

The multistep reaction sequence for the synthesis of the objective compounds **7a-l** and **8a-l** (Table 1) are framed in Fig. 2. 3-Cyanopyridine was taken as a starting material which on treatment with H₂S gas in the presence of triethylamine in absolute alcohol, gave pyridine-3-carbothioamide **1**, which on further reaction with ethyl-2-chloroacetoacetate yielded ethyl-4-methyl-2-(pyridin-3-yl)-1,3-thiazole-5-carboxylate **2**. Compound **2** was further refluxed with hydrazine hydrate to give an intermediate 5-methyl-2-(pyridin-3-yl)-1,3-thiazole-4-carbohydrazide **3**. Among the title compounds **7a-l** were obtained by refluxing intermediate **3** with different substituted acids **6a-l**, in presence of POCl₃ which was confirmed by characteristic FT-IR, ¹H-NMR ¹³C-NMR and LC-MS spectroscopic techniques. The absorption bands in the range 653, 1060 and 1568 cm⁻¹ corresponds to (C-S), (C-O-C) and (C=N) respectively. The ¹H-NMR spectrum of **7h** showed eleven different types of protons at δ 2.46 (s, 3H, -CH₃), 7.42 (t, 1H(H₃), *J* = 6 Hz), 7.55 (d, 2H(2H₅), *J* = 5.6 Hz), 8.02 (dd, 1H(H₄), *J* = 6 Hz and *J* = 1.2 Hz), 8.57–8.64 (3H, (8.58 (dd, 1H(H₂), *J* = 6 Hz and *J* = 0.8 Hz), 8.64 (d, 2H(2H₆), *J* = 6 Hz), 8.88 (d, 1H(H₁), *J* = 1.2 Hz).

¹³C-NMR spectra exhibited different types of carbons, thereby confirming the structure of **7h**. The LC-MS spectrum of **7h** revealed the presence of molecular ion peak at 322 (M+1), which was in agreement with the molecular weight of the respective compound. Scaffolds **8a-l** were obtained by condensation of chalcone-dibromides **5a-l** (which were obtained from chalcones **4a-l** by well-known Claisen-Schmidt reaction) with **3** in presence of catalytic amount of triethylamine using ethanol as solvent. Target scaffolds **8a-l** were confirmed by characteristic FT-IR, NMR and LC-MS spectroscopic technique. Peaks at 3359, 1631, 1602 and 698 corresponds to (O-H), (C=O), (C=N) and (C-S) respectively. The ¹H-NMR spectra of **8g** showed that hydroxyl proton resonated as a singlet at δ 5.2. The methylene protons of hydroxypyrazoline ring appeared as two doublets at δ 3.7 and 3.4 with a germinal coupling constant (*J* = 18.4 Hz) indicating the magnetic non-equivalence of the two protons of the CH₂ group adjacent to a chiral centre. A sharp singlet at

Table 1. Derivatives of **7a-l** and **8a-l**.

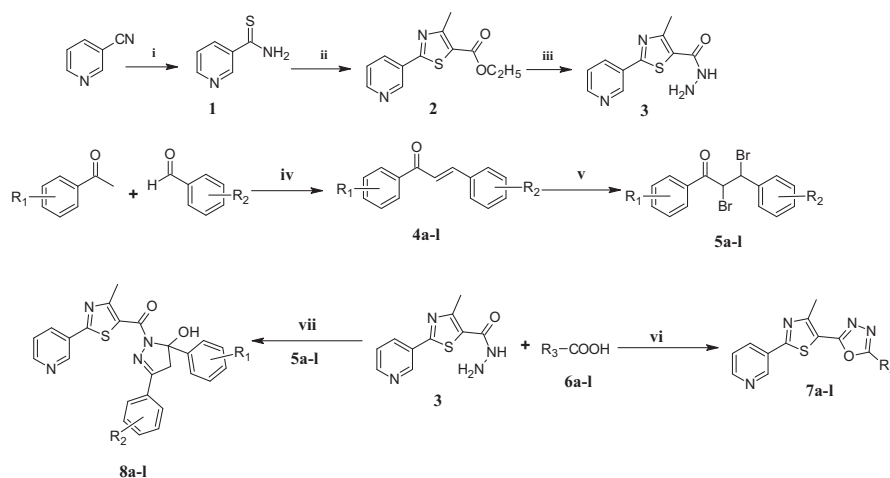
Code	R ₁	R ₂	R ₃
7a	-	-	furfuryl
7b	-	-	6-bromonaphthyl
7c	-	-	4-CH ₃ -Ph
7d	-	-	4-NH ₂ -Ph
7e	-	-	2-Cl,4-NO ₂ -Ph
7f	-	-	2-Chloropyridyl
7g	-	-	4-Cl-Ph
7h	-	-	4-Pyridyl
7i	-	-	3,4-OCH ₃ -Ph
7j	-	-	3,4-Cl-Ph
7k	-	-	-CH ₂ -Ph
7l	-	-	-CH ₂ C(Cl) ₃
8a	4-CH ₃	3-Cl	-
8b	4-CH ₃	3-NO ₂	-
8c	4-CH ₃	4-CH ₃	-
8d	2,4-Cl	3,4-OCH ₃	-
8e	4-CH ₃	4-F	-
8f	4-OCH ₃	4-F	-
8g	4-F	4-F	-
8h	4-F	4-OCH ₃	-
8i	4-F	3-Cl	-
8j	4-Cl	4-F	-
8k	4-OCH ₃	4-Cl	-
8l	4-OCH ₃	4-Br	-

δ 2.80 and 5.28 is assigned for methyl and OH protons. Other aromatic protons of **8g** resonated as complex multiples at δ 7.06 to 7.78. Moreover, ¹³C-NMR spectra of **8g** confirmed the presence of pyrazoline ring in which singlet at δ 29.3 and 94.6 are due to the sp [3] carbon of C-4 and C-5 respectively. Singlet at 19.0 and 165.6 are due to methyl and carbonyl carbon respectively. whereas, other aromatic carbon appeared in the expected region. Molecular ion peak at 477.61 (M+1), which was in agreement with the molecular weight of **8g** confirmed the structure.

3. Biological activity

3.1. *In vitro* cytotoxicity

As per the IC₅₀ data (Table 2), nine derivatives, **7d**, **7e**, **7f**, **7i**, **7l**, **8a**, **8b**, **8i**, and **8l**, have shown significant inhibition on both MDA-MB231 and HT 29 cancer cell



- (i) H_2S , EtOH, TEA, RT, 8 hrs (ii) Ethyl-2-chloroacetate, EtOH, 65 °C, 8 hrs
 (iii) N_2H_4 , EtOH, 90 °C, 10 hrs (iv) NaOH, EtOH, RT, 6 hrs (v) Br_2 , glacial acetic acid, RT, 700 rpm.
 (vi) POCl_3 , 90 °C, 10 hrs (vii) TEA, EtOH, 65 °C

Fig. 2. The synthetic route to compounds **7a-l** and **8a-l**.

lines. Considering the IC_{50} values for **7a-l** and **8a-l**, we tried to link a correlation between the cytotoxicity and molecular structure, by looking at the position and nature of the functional groups on the thiazole-oxadiazole and thiazole-pyrazoline derivatives. The presence of dimethoxy (3,4-dimethoxybenzyl), trichloromethyl, nitro combined with chloro (2-chloro-4-nitrobenzyl), 3-chloropyridyl and amine (4-aminobenzyl) in position 5 on the oxadiazole ring, correspond to compounds **7i**, **7l**, **7e**, **7f**, and **7d**, respectively which exhibited highest antiproliferative activity. On the other hand, the presence of methyl (**7c**), chloro (**7g**), dichloro (**7j**), phenyl (**7k**), pyridine (**7h**), furan (**7a**) and bromo (**7b**), decreased the antiproliferative efficiency. The viability of MDA-MB231 and HT 29 cell lines decreases with an increase in the concentration of the thiazole-oxadiazole derivatives **7a-l**. The presence of 3-chlorobenzyl, 4'-methylbenzyl (**8a**), 3-nitrobenzyl, 4-methylbenzyl (**8b**), 3-chlorobenzyl, 4'-fluorobenzyl (**8i**), and 4-bromobenzyl, 4'-methoxybenzyl (**8l**) on the hydroxypyrazoline ring, exhibited highest antiproliferative activity on both the cell lines. However, the combination of 3,4-dimethoxybenzyl, 2,4'-dichlorobenzyl (**8d**), 4-fluorobenzyl, 4'-methoxybenzyl (**8f**), 4-fluorobenzyl, 4'-fluorobenzyl (**8g**) and 4-chlorobenzyl, 4'-methoxybenzyl (**8k**) were effective towards HT-29. On the other hand combination of 4-methylbenzyl, 4'-methylbenzyl (**8c**), 4-fluorobenzyl, 4'-methylbenzyl (**8e**), 4-methoxybenzyl, 4'-fluorobenzyl (**8h**) and 4-fluorobenzyl, 4'-chlorobenzyl (**8j**) decreased the antiproliferative efficiency. The viability of MDA-MB231 and HT 29 cell lines decreases with an increase in the concentration of the hydroxypyrazoline derivatives **8a-l**. Due to the significant antiproliferative activity of **7i** on both MDA-MB231 (IC_{50} : $10.2 \pm 0.02 \mu\text{M}$) and HT 29 (IC_{50} : $25.91 \pm 1.12 \mu\text{M}$) cell lines and **8i** MDA-MB231 (IC_{50} : $29.50 \pm 1.26 \mu\text{M}$) and HT 29 (IC_{50} : $20.32 \pm 1.23 \mu\text{M}$) was studied further.

Table 2. Cytotoxicity IC₅₀ (μM) of **7a-l** and **8a-l**.

Code	MDA-MB231	HT 29
7a	141.53 ± 1.89	33.26 ± 2.85
7b	48.88 ± 0.13	52.34 ± 2.85
7c	36.35 ± 1.25	42.35 ± 1.12
7d	35.67 ± 0.13	32.04 ± 0.89
7e	19.88 ± 0.06	36.31 ± 1.23
7f	30.05 ± 0.12	29.49 ± 2.16
7g	46.31 ± 0.05	175.19 ± 5.64
7h	306.99 ± 2.56	105.91 ± 4.45
7i	10.2 ± 0.02	25.91 ± 1.12
7j	33.87 ± 0.89	202.5 ± 5.64
7k	133.69 ± 4.56	215.38 ± 6.89
7l	16.89 ± 0.89	56.98 ± 0.86
8a	41.90 ± 2.35	39.07 ± 1.12
8b	52.05 ± 1.23	36.29 ± 1.12
8c	73.33 ± 2.54	307.35 ± 0.12
8d	38.92 ± 1.12	343.36 ± 1.23
8e	123.66 ± 2.35	700.60 ± 1.16
8f	39.10 ± 3.89	856.70 ± 8.53
8g	42.45 ± 1.25	946.20 ± 12.13
8h	385.11 ± 3.58	395.91 ± 2.56
8i	29.50 ± 1.26	20.32 ± 1.23
8j	118.88 ± 3.32	590.40 ± 2.56
8k	28.01 ± 1.23	231.42 ± 4.12
8l	24.78 ± 2.25	26.64 ± 1.16

3.2. Flow cytometry assay

To investigate the effect of compound **7i** and **8i** on the progression of cell cycle, MDA-MB231 cells and HT-29 cells were treated with its IC₅₀ concentrations, 10.2 and 25.91 μM respectively for **7i** similarly 20.32 and 29.50 μM for **8i**. Cell cycle distribution was analyzed after appropriate gating of cell populations in FL-2-Area vs FL-2-Width plot of PI fluorescence. The compound **7i** was able to induce G0/G1 arrest in treated MDA-MB231 cells, 48 hrs after the treatment. The percentage of G0/G1 cells increased significantly from 41.33% in control (untreated) to 96.73% in cells after treatment with the test compound. These results suggest that the compound **7i** brings about changes in the first phase of the cell cycle and mitosis. The compound also induced G0/G1 arrest in the HT-29 cells, thereby indicating its antiproliferative action on colorectal cancer. Analogously, **8i** did not induce cell cycle arrest in treated MDA-MB231 cells, 48 h after the treatment. No difference was

incurred in the DNA content in different phases of cell cycle (G0/G1, S, and G2/M) compared to control, indicating that the compound did not induce cytotoxicity via cell cycle arrest in MDA-MB231 cells. But the compound **8i** induced G0/G1 arrest in the HT-29 cells. The percentage of G0/G1 phase increased from 42.36 to 71.1 % compared to that of control cells. There was a significant reduction in the cellular DNA content of S and G2/M phases compared to that of control cells. These results indicate the antiproliferative action of **8i** on the colorectal cancer cells via cell cycle arrest (Fig. 3).

3.3. Molecular docking studies

As per flowcytometric results CDK-2 (Siev) and BCL-2 proteins were selected for molecular docking of compounds **7i** and **8i** respectively. Compounds **7i** (Chemscore 3.90) and **8i** (Chemscore 3.70) showed good interaction within the binding site of CDK-2 protein and BCL-2. A stable hydrogen bonding was observed with the active site amino acid LYS89 and N4 of **7i**. This interaction is similar to the Roniciclib (Chemscore 5.01) interactions with ASP86 NH of CDK-2 protein. Likewise with the active site amino acid ARG124 and N4 of **8i**. Which is similar to the Navitoclax (Chemscore 2.71) interactions with the BCL-2 protein. The presence of lead **7i** and **8i** in proximity to an active pocket of a target site CDK-2 and BCL-2 proteins respectively shows a better non-H bonding interaction efficacy. Hydrophilic sulphur present in the ligand **7i** interacts with the ILeu10 amino acid. Similarly, hydrophilic GLN141, HIS84 interacts with O17, O28, and N12 group of the ligand. On the other hand, Sulphur, fluorine, and chlorine present in ligand **8i** interact with the ALA146, PHE101 and valine130 amino acids respectively. Thus, GLU138 interacts with N17

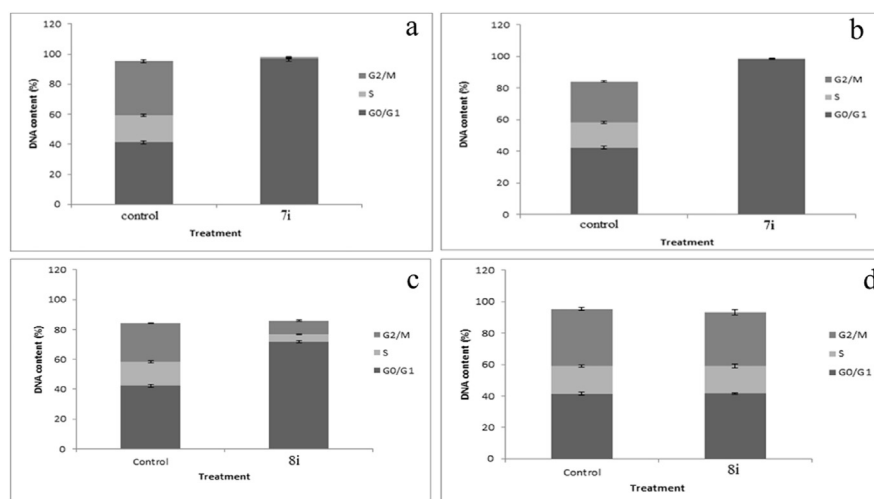


Fig. 3. Flow cytometric analysis in (a) MDA-MB231 after treatment with conjugate, **7i**, (b) HT 29 after treatment with conjugate, **7i**, (c) HT 29 after treatment with conjugate, **8i**, (d) MDA-MB231 after treatment with conjugate, **8i**.

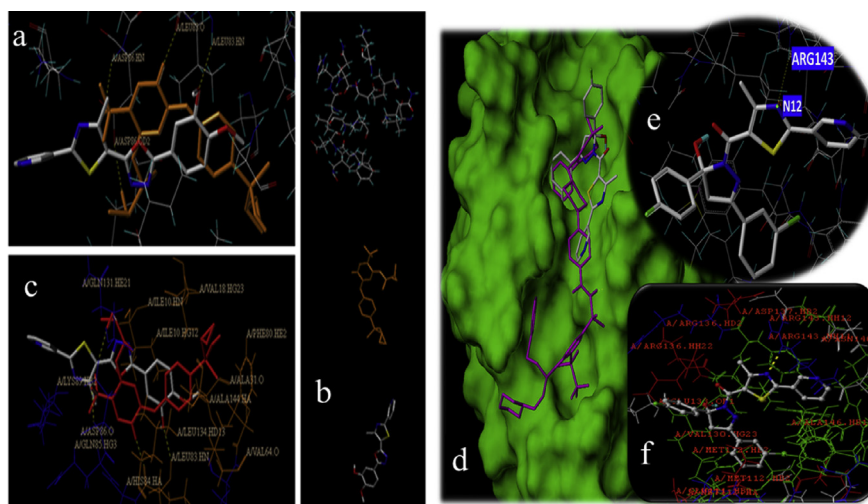


Fig. 4. (a) Binding of Compounds **7i** and Roniciclib (brown) with protein target site, (b) Ligand **7i** and Roniciclib binding orientation, (c) non-H Hydrogen bond interactions between **7i** with binding sites of amino acids, (d) Binding of compound **8i** and navitoclax (Pink) with protein target site, (e) Shows compound **8i** hydrogen bonding with binding site amino acid, (f) Non-Hydrogen bond interactions between **8i** with binding site amino acids.

and O14 groups of the ligand. From this result, it is inferred that **7i** and **8i** molecules are a promising cancer inhibitor of synthetic origin (Fig. 4).

3.4. DNA binding studies

The Interactions of prepared compounds **7a-l** and **8a-l** with CT-DNA was monitored by absorption titrations using UV-visible spectrophotometer in 290–340 nm range. In the presence of increasing amount of DNA, the spectra of **7a-l** and **8a-l** (except **7e**,

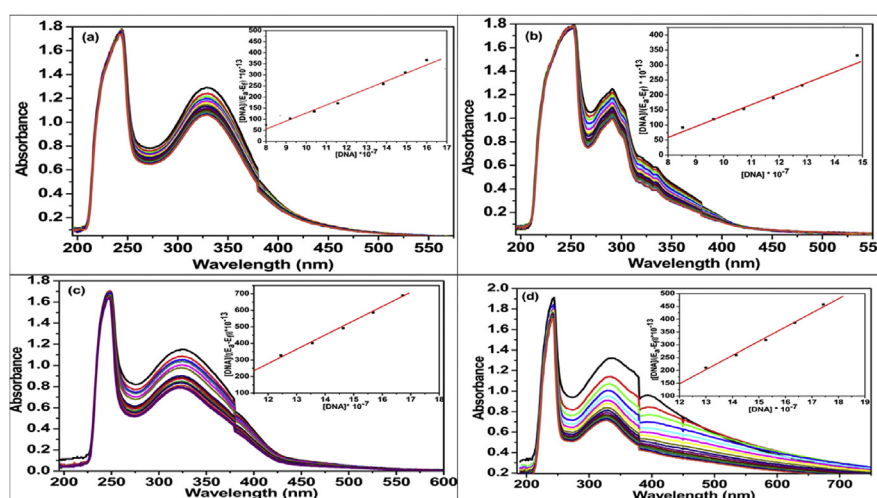


Fig. 5. Absorption spectra of (a) **7a**, (b) **7b**, (c) **8a**, and (d) **8c** in the absence [Top curve in each] and presence [subsequent curve] of increasing concentration of CT-DNA.

7j and **8b**, because of forming precipitation in the buffer) compounds, showed a decrease in the intensity of the band, but the bands were shifted to either lower wavelength or higher wavelength region. The change in absorbance values with increasing amounts of CT-DNA was used to calculate the binding constant of **7a-l** and **8a-l**. Due to the strong stacking interaction between an aromatic chromophore and the base pairs of DNA, the binding constants concluded that **7a-l** and **8a-l** interacted with CT-DNA through intercalation mode, which is well supported by the available literature for similar kind of compounds [48, 49, 50, 51, 52]. It is also indicated that the compound form adducts with DNA through intercalation and was stabilized by hydrophobic and hydrogen bond interactions [53, 54, 55, 56]. Hence, the compounds revealed a stronger binding affinity towards DNA double helix (Fig. 5, Table 3).

Table 3. Wavelength shifts, % hypochromism (H%) and binding constants of **7a-l** and **8a-l** using calf thymus DNA.

Compound	Free	Bound	$\Delta\lambda_{max}$ (nm) ^a	H% ^b	$K_b \times 10^6$ M ⁻¹ ^c
7a	329	328	1	15.33	5.83
7b	291	291	0	14.91	4.34
7c	301	300	1	33.17	7.74
7d	311	313	2	18.93	4.01
7e	-	-	-	-	-
7f	328	326	2	11.13	7.26
7g	318	311	7	8.14	7.53
7h	334	332	2	11.06	3.29
7i	340	332	8	44.03	13.8
7j	-	-	-	-	-
7k	323	325	2	7.18	4.65
7l	323	320	3	11.43	3.34
8a	326	320	6	30.61	3.70
8b	-	-	-	-	-
8c	337	325	12	46.12	3.71
8d	305	312	7	52.52	5.85
8e	320	313	7	38.09	3.28
8f	307	303	4	34.07	2.79
8g	308	311	3	30.07	3.53
8h	300	297	3	49.54	3.93
8i	329	316	13	31.11	4.06
8j	300	314	14	35.60	3.39
8k	313	319	6	17.74	2.52
8l	315	325	10	59.58	4.27

^a $\Delta\lambda_{max}$ = (Bound – Free).

^b $H\% = [(A_f - A_b)/A_f] \times 100$, Where A_f and A_b represent the absorbance of free and bound compounds

^c K_b = Intrinsic binding constant.

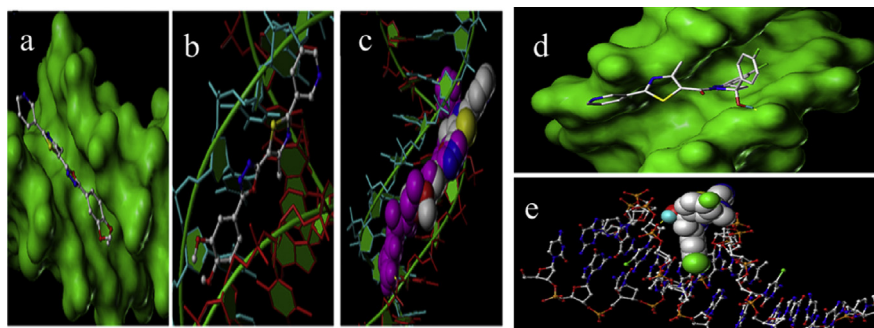


Fig. 6. (a) Represents the docked complex of the **7i** complex with DNA. (b) Binding orientation of compound **7i** in the groove of DNA. (c) The DNA binding with netropsin (CPK structure) is shown docked into DNA (stick structure), (d) Binding orientation of compound **8i** in the minor groove of DNA. (e) The DNA binding compound **8i** (CPK structure) is shown docked into DNA (stick structure). The H bond is shown as (yellow) line.

3.5. DNA docking studies

The compound **7i** and **8i** have been docked with a double-helical B-DNA of sequence: C-G-C-G-A-A-T-T-C-G-C-G (Fig. 6). The results obtained from experiments proposed that the synthesized compound interacted with DNA grooves and intercalation. The compounds **7i** and **8i** fits snugly within the minor groove in the A-A-T-T center. The mode of intercalation of this complex between the DNA base pairs is primarily due to effective stacking forces between the aromatic nucleus and DNA bases [57]. Molecular docking simulation was used to gain an insight of preferential docked location and orientation of the complexes within the DNA groove. It is confirmed that the proposed structure **7i** and **8i** should be capable of binding to DNA base sequences.

4. Conclusion

A series of oxadiazole derivatives **7a-l** and hydroxypyrazoline derivatives **8a-l** incorporating thiazole were synthesized with agreeable yield and their structures were confirmed by spectroscopic techniques. The *in vitro* cytotoxicity was evaluated for **7a-l** and **8a-l** on two human cell lines, MDA-MB231 and HT-29. Among the series, compounds **7i** and **8i** exhibited the most potent antiproliferative activity on both MDA-MB231 and HT-29 cell lines. It was also observed that **7i** showed cell cycle arrest in **G0/G1** phase on both cell lines. Whereas, **8i** revealed the cell cycle arrest in **G0/G1** phase on the colorectal cancer cells. Further, **7i** (Chemscore 3.90) and **8i** (Chemscore 3.70) showed good interaction within the binding site of the CDK-2 protein (5iev) and BCL-2 respectively. In addition, **7a-l** and **8a-l** (except **7e**, **7j** and **8b** due to the formation of precipitation in the buffer) interacted with CT-DNA via intercalation mode. Furthermore, DNA docking indicated that **7i** and **8i** interacted with DNA grooves and intercalation. Overall, these compounds act as multi-

targeting agents, as they can interact with protein target cdk (**7i**) and BCL-2 (**8i**) as well as DNA, which are the essential engines for the cell cycle. Thus, these compounds can be further studied as antiproliferative lead structures, to lay the foundation for developing anticancer drugs.

5. Experimental

5.1. Materials and methods

All the reagents for the present study were purchased from commercial suppliers of Sigma-Aldrich, Spectrochem India and Himedia. Melting points were determined in an open capillary tube and were uncorrected. Thin layer chromatography (Merck silica gel 60 F254 coated aluminium plates) confirmed the purity of the products. Synthesised compounds were characterized by $^1\text{H-NMR}$, $^{13}\text{C-NMR}$, FT-IR, LC-MS and elemental analysis. FT-IR spectrum was recorded on Shimadzu-FTIR Infrared spectrometer (γ_{max} in cm^{-1}). $^1\text{H-NMR}$ (400 MHz) and $^{13}\text{C-NMR}$ spectrum, was recorded on a Bruker Advance II 400 spectrometer, with 5mm PABBO BB-1H Tubes, using DMSO/ CDCl_3 as a solvent, using TMS as internal standard (Chemical shift in δ ppm). Elemental analysis was carried out by using VARIOEL-III (Elemental analyze system GmbH). LC-MS was obtained by using Agilent 1200 series LC and MicromassZQ spectrometer. DNA binding studies were carried out using ELICO SL 159 UV-VIS Spectrophotometer.

5.2. Synthesis of pyridine-3-carbothioamide (1)

Pyridine-3-carbothioamide [58] **1** was synthesized by passing H_2S to a stirring solution (750 rpm) of 3-cyanopyridine in presence of trimethylamine (TEA) at RT for 8 hrs. The progress of the reaction was monitored by TLC. The precipitate formed in the reaction medium was filtered and recrystallized from ethanol.

5.3. Synthesis of ethyl 4-methyl-2-(pyridin-3-yl)-1,3-thiazole-5-carboxylate (2)

Mixture of Pyridine-3-carbothioamide **1** (2 g, 0.0144 mol) and ethyl-2-chloroacetoacetate (2.04 g, 0.0144 mol) was refluxed at 65°C for 8 hrs. The progress and completion of the reaction was confirmed by TLC. The reaction mixture was cooled and poured into ice cold water, the precipitate formed was filtered and recrystallized from ethanol.

5.4. Ethyl 4-methyl-2-(pyridin-3-yl)-1,3-thiazole-5-carboxylate (2)

Yield 89%; m.p. $162-165^\circ\text{C}$; IR (cm^{-1}): 694 (C-S), 1085 (C-O-C), 1579 (C=N), 1720 (C=O), 2932 (C-H), 3044 (Ar C-H); $^1\text{H-NMR}$ (400 MHz,

CDCl₃, δ ppm): 1.3 (t, 3H, -CH₃, J = 4.8 Hz), 2.49 (s, 3H, -CH₃), 4.39 (q, 2H, -CH₂-, J = 9.6 Hz and J = 4.4 Hz), 7.47 (t, 1H(H₃), J = 6.4 Hz), 8.07 (dt, 1H(H₄), J = 6 Hz and J = 0.8 Hz), 8.64 (dd, 1H(H₂), J = 6 Hz and J = 1.2 Hz), 8.98 (d, 1H(H₁), J = 0.8 Hz); ¹³C-NMR (100 MHz, DMSO, δ ppm): 14.5 (-CH₃), 17.6 (-CH₃), 61.8 (-CH₂-), 122.5, 124.7, 128.59, 134.4, 147.6, 152.3, 160.8, 161.6, 166.4. LC-MS, (m/z): 249.08 (M+1), Anal. Calcd. for C₁₂H₁₂N₂O₂S (248.30): C, 58.05; H, 4.87; N, 11.28. Found: C, 58.11; H, 4.82; N, 11.16. Figs. S1–S4.

5.5. Synthesis of 4-methyl-2-(pyridin-3-yl)-1,3-thiazole-5-carbohydrazide (3)

Compound **2** (2 g, 0.01 mol) was treated with hydrazine hydrate (1.6 g, 0.05 mol) and refluxed at 90 °C for 10 hrs, formation of product was confirmed by TLC, which was obtained by cooling the reaction mixture overnight and recrystallized from ethanol.

5.6. 4-Methyl-2-(pyridin-3-yl)-1,3-thiazole-5-carbohydrazide (3)

Yield 93%; m.p. 148–151 °C; IR (cm⁻¹): 671 (C-S), 1589 (NH₂ Scissoring), 1620 (C=O), 2848 (C-H), 2916 (Ar C-H), 3352, 3332 (-NH₂); ¹H-NMR (400 MHz, DMSO, δ ppm): 2.49 (s, 3H, -CH₃), 4.60 (d, 2H, -NH₂, J = 4.8 Hz), 7.58 (dd, 1H(H₃), J = 7.6 Hz and J = 4.8 Hz), 8.31 (d, 1H(H₄), J = 8 Hz), 8.71 (t, 1H, J = 4.8 Hz, NH), 9.12 (d, 1H(H₂), J = 1.6 Hz), 9.65 (s, 1H(H₁)); ¹³C-NMR (100 MHz, DMSO, δ ppm): 17.4, 124.8, 126.1, 128.9, 134.2, 147.4, 151.8, 155.3, 161.3, 163.4. LC-MS, m/z: 235.04 (M+1), Anal. Calcd. For C₁₀H₁₀N₄OS (234.27): C, 51.27; H, 4.30; N, 23.91. Found: C, 51.16; H, 4.32; N, 23.88. Figs. S5–S8.

5.7. Procedure for the synthesis of chalcone (4a-l)

4a-l are prepared by Claisen Schmidt condensation between substituted acetophenones and substituted aldehydes in presence of NaOH as base at RT. The product formed was confirmed by TLC and poured into ice cold water, the precipitate formed was filtered and recrystallized from ethanol.

5.8. Procedure for the synthesis of chalcone dibromides (5a-l)

To a solution of chalcones **4a-l** (0.01 mol) in glacial acetic acid (50 mL), bromine (0.01 mol) in glacial acetic acid (25 mL) was added slowly with vigorous stirring (700 rpm) at RT for 12 hrs. The reaction mixture was poured into ice cold water the precipitate formed was filtered and recrystallized from ethanol.

5.9. Procedure for the synthesis of hydroxypyrazolines derivatives (8a-l)

To a mixture of chalcone dibromides **5a-l** (0.1 mol) in absolute ethanol (7.5 mL) 4-methyl-2-(pyridin-3-yl)-1,3-thiazole-5-carbohydrazide **3** (0.1 mol) and triethylamine (1 mL) were added and the reaction mixture was heated under reflux for 10 hrs on a water bath. The contents were cooled and poured into ice cold water. The resulting hydroxypyrazolines derivatives **8a-l** were collected by filtration and recrystallized from ethanol.

5.10. General procedure for the synthesis of (7a-l)

To a mixture of substituted aromatic and aliphatic acids **6a-l**, (0.01 mol) in POCl₃ (15 mL) 5-methyl-2-(pyridin-3-yl)-1,3-thiazole-4-carbohydrazide **3** (0.01 mol) was added. The reaction mixture was heated under reflux at 90 °C for 10 hrs on an oil bath. The contents were cooled, neutralized by sodium bicarbonate and poured into crushed ice. The resulting substituted thiazole-oxadiazoles **7a-l**, were collected by filtration and recrystallized from ethanol.

5.10.1. 3-{5-[5-(furan-2-yl)-1,3,4-oxadiazol-2-yl]-4-methyl-1,3-thiazol-2-yl}pyridine (7a)

Yield 78%; mp 180–182 °C; IR (cm⁻¹): 649 (C-S), 1041 (C-O-C), 1460 (C=C), 1525 (C=N), 2953 (C-H), 3037 (Ar C-H); ¹H-NMR (400 MHz, CDCl₃, δ ppm): 2.46 (s, 3H, -CH₃), 6.50 (t, 1H, O-CH = CH-CH = C, *J* = 6.4 Hz), 6.95 (dd, 1H, O-CH = CH-CH = C, *J* = 6 Hz), 7.40 (t, 1H(H₃), *J* = 6.8 Hz), 7.78 (dd, 1H, O-CH = CH-CH = C, *J* = 6 Hz and 1.2 Hz), 8.07 (dt, 1H(H₄), *J* = 6.8 Hz and *J* = 1.2 Hz), 8.64 (dd, 1H(H₂), *J* = 6.2 Hz and *J* = 0.8 Hz), 8.98 (d, 1H(H₁), *J* = 2.8 Hz); ¹³C-NMR (100 MHz, CDCl₃, δ ppm): 17.3 (-CH₃), 113.9, 116.7, 120.6, 123.3, 127.8, 133.0, 139.6, 147.0, 148.2, 149.8, 152.3, 157.4, 161.2, 168.3; LC-MS, (m/z): 311 (M+1); Anal. Calcd. For C₁₅H₁₀N₄O₂S (310.41): C, 58.05; H, 3.25; N, 18.05. Found: C, 58.07; H, 3.27; N, 18.10.

5.10.2. 3-{5-[5-(6-bromonaphthalen-1-yl)-1,3,4-oxadiazol-2-yl]-4-methyl-1,3-thiazol-2-yl}pyridine (7b)

Yield 65%; m.p. 205–207 °C; IR (cm⁻¹): 658 (C-S), 1042 (C-O-C), 1463 (C=C), 1566 (C=N), 2931 (C-H), 3041 (Ar C-H); ¹H-NMR (400 MHz, CDCl₃, δ ppm): 2.50 (s, 3H, -CH₃), 7.44–8.99 (m, 10H); ¹³C-NMR (100 MHz, CDCl₃, δ ppm): 17.35 (-CH₃), 120.3, 120.5, 120.6, 123.3, 124.9, 126.7, 127.3, 127.8, 128.4, 129.4, 129.8, 130.1, 132.9, 133.0, 148.9, 149.8, 152.3, 161.2, 165.2, 168.3; LC-

MS, (m/z): 449.99 (M+), 451.99 (M+2); Anal. Calcd. For C₂₁H₁₃BrN₄OS (449.32): C, 56.13; H, 2.92; N, 12.47. Found: C, 56.10; H, 2.94; N, 12.49.

5.10.3. 3-{4-methyl-5-[5-(4-methylphenyl)-1,3,4-oxadiazol-2-yl]-1,3-thiazol-2-yl}pyridine (7c)

Yield 73%; m.p. 210–212 °C; IR (cm⁻¹): 696 (C-S), 1016 (C-O-C), 1492 (C=C), 1577 (C=N), 2960 (C-H), 3039 (Ar C-H); ¹H-NMR (400 MHz, CDCl₃, δ ppm): 2.34 (s, 3H, -CH₃), 2.47 (s, 3H, -CH₃), 7.29 (d, 2H(2H₆), *J* = 6 Hz), 7.42 (t, 1H(H₃), *J* = 6 Hz), 7.52 (d, 2H(2H₅), *J* = 5.6 Hz), 8.02 (t, 1H(H₄), *J* = 6 Hz), 8.59 (dd, 1H(H₂), *J* = 6 Hz and *J* = 5.6 Hz), 8.8 (d, 1H(H₁), *J* = 1.2 Hz); ¹³C-NMR (100 MHz, CDCl₃, δ ppm): 17.57(-CH₃), 21.69(-CH₃), 115.4, 120.6, 123.93, 126.9, 128.8, 129.9, 133.8, 142.6, 147.9, 151.6, 157.1, 159.2, 164.4, 165.4; LC-MS, (m/z): 334 (M+); Anal. Calcd. For C₁₈H₁₄N₄OS (334.39): C, 64.65; H, 4.22; N, 16.75. Found: C, 64.62; H, 4.25; N, 16.72. Figs. S13–S16.

5.10.4. 4-{5-[4-methyl-2-(pyridin-3-yl)-1,3-thiazol-5-yl]-1,3,4-oxadiazol-2-yl}aniline (7d)

Yield 70%; m.p. 236–238 °C; IR (cm⁻¹): 673 (C-S), 1037 (C-O-C), 1471 (C=C), 1573 (C=N), 2941 (C-H), 3045 (Ar C-H), 3286, 3221 (-NH₂); δ ¹H-NMR (400 MHz, CDCl₃, δ ppm): 2.47 (s, 3H, -CH₃), 4.16 (s, 2H, -NH₂), 6.73 (d, 2H(2H₆), *J* = 6 Hz), 7.36–7.44 (7.38 (d, 2H(2H₅), *J* = 6 Hz), 7.44 (t, 1H(H₃), *J* = 6.8 Hz), 8.03–8.05 (dt, 1H(H₄), *J* = 6.1 Hz, *J* = 1.2 Hz), 8.61 (dd, 1H(H₂), *J* = 6.4 Hz and *J* = 1.2 Hz), 8.90 (d, 1H(H₁), *J* = 1.2 Hz); ¹³C-NMR (100 MHz, CDCl₃, δ ppm): 16.6 (-CH₃), 109.5, 114.5, 118.0, 127.6(d), 134.9(d), 137.0, 140.2, 149.2, 151.3, 156.2, 161.3, 165.8; LC-MS, (m/z): 336 (M+1); Anal. Calcd. For C₁₇H₁₃N₅OS (335.38): C, 60.88; H, 3.91; N, 20.88. Found: C, 60.85; H, 3.93; N, 20.85.

5.10.5. 3-{5-[5-(2-chloro-4-nitrophenyl)-1,3,4-oxadiazol-2-yl]-4-methyl-1,3-thiazol-2-yl}pyridine (7e)

Yield 86%; m.p. 203–205 °C; IR (cm⁻¹): 3032 (Ar C-H), 2958 (C-H), 1575 (C=N), 1516 & 1346 (N-O), 696 (C-S); ¹H-NMR (400 MHz, CDCl₃, δ ppm): 2.46 (s, 3H, -CH₃), 7.42 (t, 1H(H₃), *J* = 6 Hz), 7.8 (d, 1H(H₇), *J* = 6 Hz), 8.02 (dt, 1H(H₄), *J* = 6 Hz and *J* = 2.4 Hz), 8.19 (dd, 1H(H₆), *J* = 5.6 Hz and *J* = 1.8 Hz), 8.37 (d, 1H(H₅), *J* = 1.2 Hz), 8.59 (dd, 1H(H₂), *J* = 6 Hz and *J* = 1.2 Hz), 8.88 (d, 1H(H₁), *J* = 1.2 Hz); ¹³C-NMR (100 MHz, CDCl₃, δ ppm): 17.7(-CH₃), 114.6, 122.0, 123.6, 126.6, 128.0, 132.0, 133.9, 134.1, 147.9, 149.6, 151.9, 158.3, 160.8, 161.2, 166.5; LC-MS, m/z: 399.99 (M+), 402.99 (M+2);

Anal. Calcd. For $C_{17}H_{10}ClN_5O_3S$ (399.81): C, 51.07; H, 2.52; N, 17.57. Found: C, 51.91; H, 2.56; N, 17.14. Figs. S17–S20.

5.10.6. 3-chloro-4-{5-[4-methyl-2-(pyridin-3-yl)-1,3-thiazol-5-yl]-1,3,4-oxadiazol-2-yl}pyridine (7f)

Yield 90%; m.p. 199–201 °C; IR (cm^{-1}): 696 (C-S), 1076 (C-O-C), 1346 (N-O, symmetric), 1516 (N-O, asymmetric), 1575 (C=N), 2958 (C-H), 3032 (Ar C-H); 1H -NMR (400 MHz, $CDCl_3$, δ ppm): 2.49 (s, 3H, -CH₃), 7.39 (dd, 1H(H₃), J = 6.4 Hz and J = 1.2 Hz), 7.71 (d, 1H(H₇), J = 6 Hz), 8.09 (dt, 1H(H₄), J = 6.2 Hz, J = 1.2 Hz), 8.54 (dd, 1H(H₂), J = 6.6 Hz and J = 1.2 Hz), 8.63 (s, 1H(H₆)), 8.73 (s, 1H(H₅), 8.84 (d, 1H(H₁), J = 1.2 Hz); ^{13}C -NMR (100 MHz, $CDCl_3$, δ ppm): 17.8(-CH₃), 120.6, 120.7, 123.3, 126.7, 127.8, 128.0, 133.0, 147.7, 148.9, 149.1, 149.8, 152.3, 161.2, 164.8, 168.3. LC-MS, (m/z): 355.81 (M⁺), 357.92 (M+2); Anal. Calcd. For $C_{16}H_{10}ClN_5OS$ (355.80): C, 54.01; H, 2.83; N, 19.68. Found: C, 54.04; H, 2.80; N, 19.65.

5.10.7. 3-{5-[5-(4-chlorophenyl)-1,3,4-oxadiazol-2-yl]-4-methyl-1,3-thiazol-2-yl}pyridine (7g)

Yield 85%; m.p. 121–123 °C; IR (cm^{-1}): 667 (C-S), 1064 (C-O-C), 1488 (C=C), 1543 (C=N), 2945 (C-H), 3031 (Ar C-H); 1H -NMR (400 MHz, $CDCl_3$, δ ppm): 2.41 (s, 3H, -CH₃), 7.39–7.44 (dd, 3H(H₃, 2H₆), J = 6.2 Hz, J = 12.4 Hz), 7.51 (d, 2H(2H₅), J = 6 Hz), 8.16 (dt, 1H(H₄), J = 6.4 Hz, J = 1.2 Hz), 8.63 (dd, 1H(H₂), J = 6.1 Hz), 8.91 (d, 1H(H₁), J = 0.8 Hz); ^{13}C -NMR (100 MHz, $CDCl_3$, δ ppm): 17.5(-CH₃), 120.6, 122.8, 123.3, 127.8, 128.8, 128.8, 129.2, 129.2, 133.0, 135.7, 148.9, 149.8, 152.3, 161.2, 163.7, 168.3; LC-MS, (m/z): 354.8 (M⁺), 356.3 (M+2); Anal. Calcd. For $C_{17}H_{11}ClN_4OS$ (354.81): C, 57.55; H, 3.12; N, 15.79. Found: C, 57.52; H, 3.09; N, 15.76.

5.10.8. 3-{4-methyl-5-[5-(pyridin-4-yl)-1,3,4-oxadiazol-2-yl]-1,3-thiazol-2-yl}pyridine (7h)

Yield 79%; m.p. 116–118 °C; IR (cm^{-1}): 653 (C-S), 1060 (C-O-C), 1469 (C=C), 1568 (C=N), 2949 (C-H), 3035 (Ar C-H); 1H -NMR (400 MHz, $CDCl_3$, δ ppm): 2.46 (s, 3H, -CH₃), 7.42 (t, 1H(H₃), J = 8 Hz), 7.55 (d, 2H(2H₅), J = 5.6 Hz), 8.02 (dd, 1H(H₄), J = 6 Hz and J = 1.2 Hz), 8.57–8.64 (3H, (8.58 (dd, 1H(H₂), J = 6 Hz and J = 0.8 Hz), 8.64 (d, 2H(2H₆), J = 6 Hz), 8.88 (d, 1H(H₁), J = 1.2 Hz); ^{13}C -NMR (100 MHz, $CDCl_3$, δ ppm): 17.7(-CH₃), 114.6, 120.2, 123.9, 130.5, 133.9, 147.9, 151.0, 151.8, 158.2, 160.5, 162.4, 166.2; LC-MS, (m/z): 322 (M+1); Anal. Calcd.

For $C_{16}H_{11}N_5OS$ (321.35): C, 59.80; H, 3.45; N, 21.79. Found: C, 59.76; H, 3.41; N, 21.82. Figs. S9–S12.

5.10.9. 3-{5-[5-(3,4-dimethoxyphenyl)-1,3,4-oxadiazol-2-yl]-4-methyl-1,3-thiazol-2-yl}pyridine (7i)

Yield 83%; m.p. 126–128 °C; IR (cm^{-1}): 634 (C-S), 1139 (C-O-C), 1465 (C=C), 1510 (C=N), 2939 (C-H), 3047 (Ar C-H); 1H -NMR (400 MHz, DMSO, δ ppm): 2.47 (s, 3H, -CH₃), 3.80 (s, 3H, -OCH₃), 3.83 (s, 3H, -OCH₃), 7.00 (d, 1H(H₇), J = 6 Hz), 7.19 (d, 1H(H₅), J = 1.2 Hz), 7.20–7.22 (dd, 1H(H₆), J = 6 Hz, J = 1.2 Hz), 7.42 (t, 1H(H₃), J = 6 Hz), 8.00–8.02 (dt, 1H(H₄), J = 6 Hz, J = 1.2 Hz), 8.57–8.59 (dd, 1H(H₂), J = 1.2 Hz), 8.87 (d, 1H(H₁), J = 0.8 Hz); ^{13}C -NMR (100 MHz, CDCl₃, δ ppm): 17.5(-CH₃), 56.0(-OCH₃), 56.1(-OCH₃), 109.5, 111.2, 115.4, 115.9, 120.5, 133.7, 147.8, 149.5, 151.6, 152.3, 157.0, 159.1, 164.2, 165.3; LC-MS, (m/z): 381.07 (M+1); Anal. Calcd. For $C_{19}H_{16}N_4O_3S$ (380.42): C, 59.99; H, 4.24; N, 14.73. Found: C, 59.96; H, 4.21; N, 14.76. Figs. S21–S24.

5.10.10. 3-{5-[5-(3,4-dichlorophenyl)-1,3,4-oxadiazol-2-yl]-4-methyl-1,3-thiazol-2-yl}pyridine (7j)

Yield 90%; m.p. 218–220 °C; IR (cm^{-1}): 676 (C-S), 1091 (C-O-C), 1490 (C=C), 1549 (C=N), 2947 (C-H), 3034 (Ar C-H); 1H -NMR (400 MHz, CDCl₃, δ ppm): 2.52 (s, 3H, -CH₃), 7.48 (m, 4H(H₃, H₅, H₆, H₇)), 8.08 (dt, 2H(H₄), J = 6 Hz and J = 1.2 Hz), 8.67 (dd, 1H(H₂), J = 6.2 Hz and J = 1.2 Hz), 8.93 (d, 1H(H₁), J = 1.6 Hz); ^{13}C -NMR (100 MHz, CDCl₃, δ ppm): 17.85, 120.6, 123.3, 125.5, 127.8, 128.7, 128.8, 129.9, 133.0, 133.2, 133.6, 148.9, 149.8, 152.3, 161.2, 163.7, 168.3; LC-MS, (m/z): 389.30 (M+), 391.23 (M+2), 393.41 (M+4); Anal. Calcd. For $C_{17}H_{10}Cl_2N_4OS$ (389.25): C, 52.45; H, 2.59; N, 14.39. Found: C, 52.47; H, 2.56; N, 14.41.

5.10.11. 3-[5-(5-benzyl-1,3,4-oxadiazol-2-yl)-4-methyl-1,3-thiazol-2-yl]pyridine (7k)

Yield 71%; m.p. 190–192 °C; IR (cm^{-1}): 690 (C-S), 1009 (C-O-C), 1485 (C=C), 1569 (C=N), 2957 (C-H), 3029 (Ar C-H); 1H -NMR (400 MHz, CDCl₃, δ ppm): 2.43 (s, 3H, -CH₃), 3.93 (s, 2H, -CH₂-), 7.15–7.42 (m, 5H(2H₅, 2H₆, H₇)), 7.39 (t, 1H(H₃), J = 8 Hz), 7.97 (dt, 1H(H₄), J = 6.1 Hz and J = 1.2 Hz), 8.48 (dd, 1H(H₂), J = 6.4 Hz and J = 0.8 Hz), 8.86 (d, 1H(H₁), J = 0.8 Hz); ^{13}C -NMR (100 MHz, CDCl₃, δ ppm): 17.2, 33.9, 120.6, 123.3, 127.8, 128.6, 128.7, 128.7, 128.9, 133.0, 134.0, 148.9, 149.8, 152.3, 161.2, 165.6, 168.3; LC-MS, (m/z): 335.51 (M+1); Anal. Calcd. For $C_{18}H_{14}N_4OS$ (334.39): C, 64.65; H, 4.22; N, 16.75. Found: C, 64.68; H, 4.24; N, 16.79.

5.10.12. 3-{4-methyl-5-[5-(trichloromethyl)-1,3,4-oxadiazol-2-yl]-1,3-thiazol-2-yl}pyridine (7l)

Yield 80%; m.p. 169–171 °C; IR (cm⁻¹): 677 (C-S), 1103 (C-O-C), 1483 (C=C), 1565 (C=N), 2961 (C-H), 3050 (Ar C-H); ¹H-NMR (400 MHz, CDCl₃, δ ppm): 2.56 (s, 3H, -CH₃), 7.46 (t, 1H(H₃), *J* = 7.8 Hz), 8.07 (dt, 1H(H₄), *J* = 6.6 Hz and *J* = 1.2 Hz), 8.64 (dd, 1H(H₂), *J* = 6.2 Hz and *J* = 1.2 Hz), 8.89 (d, 1H(H₁), *J* = 0.8 Hz); ¹³C-NMR (100 MHz, CDCl₃, δ ppm): 17.61, 92.85, 120.60, 123.32, 127.82, 133.01, 148.94, 149.87, 152.31, 161.26, 162.34, 168.30; LC-MS, (m/z): 361.81 (M⁺), 363.27 (M+2), 365.43 (M+4), 368.18 (M+6); Anal. Calcd. For C₁₂H₇Cl₃N₄OS (361.63): C, 39.85; H, 1.95; N, 15.49. Found: C, 39.88; H, 1.97; N, 15.46.

5.10.13. 3-(4-methylphenyl)-5-(3-chlorophenyl)-5-hydroxy-4,5-dihydro-1H-pyrazol-1-yl[4-methyl-2-(pyridin-3-yl)-1,3-thiazol-5-yl]methanone (8a)

Yield 80%; m.p. 106–108 °C; IR (cm⁻¹): 682 (C-S), 1593 (C=N), 1631 (C=O), 2845 (Ar C-H), 2918 (C-H), 3152 (O-H); ¹H-NMR (400 MHz, CDCl₃, δ ppm): 2.30 (s, 3H, -CH₃), 2.74 (s, 3H, -CH₃), 3.46 (d, 1H, -CH₂-, *J* = 18.4 Hz), 3.76 (d, 1H, -CH₂-, *J* = 18.4 Hz), 5.25 (d, 1H, O-H, *J* = 16 Hz), 7.24–9.16 (m, 12H); ¹³C-NMR (100 MHz, CDCl₃, δ ppm): 17.9(-CH₃), 20.0(-CH₃), 49.1, 94.1, 119.9, 122.8, 123.8, 125.9, 127.4, 128.0, 128.3, 128.6, 129.2, 129.7, 131.6(d), 132.9, 134.0, 137.2, 139.0, 141.9, 146.9, 150.4, 150.9, 160.0, 162.4, 166.7; LC-MS, (m/z): 487.9 (M⁺), 490.4 (M+1); Anal. Calcd. For C₁₂H₇Cl₃N₄OS (488.98): C, 63.86; H, 4.33; N, 11.46. Found: C, 63.80; H, 4.29; N, 11.42. Figs. S25–S27.

5.10.14. 3-(4-methyl)-5-(3-nitrophenyl)-5-hydroxy-4,5-dihydro-1H-pyrazol-1-yl[4-methyl-2-(pyridin-3-yl)-1,3-thiazol-5-yl]methanone (8b)

Yield 84%; m.p. 108–110 °C; IR (cm⁻¹): 698 (C-S), 1527 (N=O), 1602 (C=N), 1654 (C=O), 2842 (C-H), 2916 (Ar C-H), 3163 (O-H); ¹H-NMR (400 MHz, CDCl₃, δ ppm): 2.34 (s, 3H, -CH₃), 2.79 (s, 3H, -CH₃), 3.54 (d, 1H, -CH₂-, *J* = 18.4 Hz), 3.86 (d, 1H, -CH₂-, *J* = 18.4 Hz), 5.25 (d, 1H, O-H, *J* = 16 Hz), 7.19–9.29 (m, 12H); ¹³C-NMR (100 MHz, CDCl₃, δ ppm): 16.6(-CH₃), 21.1(-CH₃), 47.4, 92.6, 122.9, 124.8, 125.3, 124.3, 127.2, 128.9, 129.4, 129.4, 132.0, 137.2, 134.5, 134.9, 135.3, 137.0, 139.0, 144.1, 147.1, 148.3, 149.2, 150.4, 151.35; LC-MS, (m/z): 499.35 (M⁺); Anal. Calcd. For C₁₂H₇Cl₃N₄OS (499.54): C, 62.51; H, 4.24; N, 14.02. Found: C, 62.59; H, 4.25; N, 14.07. Figs. S28–S30.

5.10.15. 3-(4-methylphenyl)-5-(4-methylphenyl)-5-hydroxy-4,5-dihydro-1H-pyrazol-1-yl[4-methyl-2-(pyridin-3-yl)-1,3-thiazol-5-yl]methanone (8c)

Yield 82%; m.p. 88–90 °C; IR (cm⁻¹): 676 (C-S), 1592 (C=N), 1641 (C=O), 2847 (C-H), 2912 (Ar C-H), 3157 (O-H); ¹H-NMR (400 MHz, CDCl₃, δ ppm): 2.36 (s, 3H, -CH₃), 2.68 (d, 6H, 2CH₃), 2.80 (s, 3H, -CH₃), 3.48 (dd, 1H, -CH₂-, *J* = 18.4 Hz and *J* = 6.8 Hz), 3.79 (dd, 1H, -CH₂-, *J* = 18.4 Hz and *J* = 6.8 Hz), 5.26 (s, 1H, O-H), 7.26–9.33 (m, 12H); ¹³C-NMR (100 MHz, CDCl₃, δ ppm): 18.98(-CH₃), 19.65(-CH₃), 19.7(-CH₃), 50.1, 92.0, 121.0(d), 127.1(d), 128.9, 129.4, 129.6, 133.9, 138.2, 140.2, 143.1, 147.9, 151.3, 152.2, 160.9(d), 163.0, 163.3(d), 165.5, 167.6, 167.6; LC-MS, (m/z): 468.81 (M⁺); Anal. Calcd. For C₁₂H₇Cl₃N₄OS (468.57): C, 69.21; H, 5.16; N, 11.96. Found: C, 69.15; H, 5.21; N, 11.88.

5.10.16. 3-(2,4-dichlorophenyl)-5-(3,4-dimethoxyphenyl)-5-hydroxy-4,5-dihydro-1H-pyrazol-1-yl[4-methyl-2-(pyridin-3-yl)-1,3-thiazol-5-yl]methanone (8d)

Yield 79%; m.p.122–124 °C; IR (cm⁻¹): 698 (C-S), 1224 (C-O-C), 1605 (C=N), 1627 (C=O), 2851 (C-H), 2928 (Ar C-H), 3206 (O-H); ¹H-NMR (400 MHz, CDCl₃, δ ppm): 2.38 (s, 3H, -CH₃), 3.56 (dd, 1H, -CH₂-, *J* = 18.4 Hz and *J* = 6.8 Hz), 3.82 (s, 3H, -OCH₃), 3.84 (s, 3H, -OCH₃), 3.88 (dd, 1H, -CH₂-, *J* = 18.4 Hz and *J* = 6.8 Hz), 5.14 (s, 1H, O-H), 7.45–9.45 (m, 10H); ¹³C-NMR (100 MHz, CDCl₃, δ ppm): 17.2(-CH₃), 49.8, 51.0, 51.2, 91.1, 116.3, 121.0, 126.2, 127.1, 128.4, 128.1, 128.9, 128.9, 129.4, 129.6, 132.9, 138.5, 139.4, 141.1, 149.9, 152.2, 153.2, 161.9, 163.1, 164.2, 165.3, 166.3, 168.6, 169.6; LC-MS, (m/z): 569.66 (M⁺), 571.62 (M+1), 573.60 (M+4); Anal. Calcd. For C₁₂H₇Cl₃N₄OS (569.46): C, 56.95; H, 3.89; N, 9.84. Found: C, 56.93; H, 3.88; N, 9.80.

5.10.17. 3-(4-methylphenyl)-5-(3-fluorophenyl)-5-hydroxy-4,5-dihydro-1H-pyrazol-1-yl[4-methyl-2-(pyridin-3-yl)-1,3-thiazol-5-yl]methanone (8e)

Yield 94%; m.p.124–126 °C; IR (cm⁻¹): 698 (C-S), 1605 (C=N), 1623 (C=O), 2848 (C-H), 2917 (Ar C-H), 3306 (O-H); ¹H-NMR (400 MHz, CDCl₃, δ ppm): 2.39 (s, 3H, -CH₃), 2.81 (s, 3H, -CH₃), 3.59 (d, 1H, -CH₂-, *J* = 18.4 Hz), 3.91 (d, 1H, -CH₂-, *J* = 18.4 Hz), 5.23 (s, 1H, O-H), 7.26–9.31 (m, 12H); ¹³C-NMR (100 MHz, CDCl₃, δ ppm): 18.6(-CH₃), 20.6, 49.0, 92.3, 116.4(d), 122.0, 127.0, 128.35, 128.7(d), 129.0, 129.4, 134.0, 138.1, 140.4, 143.0, 148.0, 151.5, 152.4, 161.0, 163.0, 163.4, 165.6, 167.6, 167.8; LC-MS, (m/z): 473.61 (M⁺); Anal.

Calcd. For $C_{12}H_7Cl_3N_4OS$ (472.53): C, 66.09; H, 4.48; N, 11.86. Found: C, 66.14; H, 4.49; N, 11.77.

5.10.18. 3-(4-methoxyphenyl)-5-(4-fluorophenyl)-5-hydroxy-4,5-dihydro-1H-pyrazol-1-yl[4-methyl-2-(pyridin-3-yl)-1,3-thiazol-5-yl]methanone (8f)

Yield 81%; m.p. 160–162 °C; IR (cm^{-1}): 698 (C-S), 1226 (C-O-C), 1605 (C=N), 1621 (C=O), 2845 (C-H), 2921 (Ar C-H), 3296 (O-H); 1H -NMR (400 MHz, $CDCl_3$, δ ppm): 2.77 (s, 3H, $-CH_3$), 3.54 (d, 1H, $-CH_2-$, $J = 18.4$ Hz), 3.81 (s, 3H, $-OCH_3$), 3.86 (d, 1H, $-CH_2-$, $J = 18.4$ Hz), 5.14 (s, 1H, O-H), 7.21–9.43 (m, 12H); ^{13}C -NMR (100 MHz, $CDCl_3$, δ ppm): 18.9($-CH_3$), 47.9, 50.0, 50.3, 95.0, 116.3(d), 121.0(d), 127.1(d), 128.4, 128.9(d), 128.9, 129.4, 129.6, 133.9, 138.2, 140.2, 143.15, 147.9, 151.3, 152.2, 160.9(d), 163.0, 163.3(d), 165.5, 167.6, 167.6; LC-MS, (m/z): 488.70 (M+); Anal. Calcd. For $C_{12}H_7Cl_3N_4OS$ (488.53): C, 63.92; H, 4.33; N, 11.47. Found: C, 63.84; H, 4.30; N, 11.45. Figs. S31–S33.

5.10.19. 3-(4-fluorophenyl)-5-(4-fluorophenyl)-5-hydroxy-4,5-dihydro-1H-pyrazol-1-yl[4-methyl-2-(pyridin-3-yl)-1,3-thiazol-5-yl]methanone (8g)

Yield 90%; m.p. 163–165 °C; IR (cm^{-1}): 698 (C-S), 1602 (C=N), 1631 (C=O), 2958 (C-H), 3053 (Ar C-H), 3359 (O-H); 1H -NMR (400 MHz, $CDCl_3$, δ ppm): 2.80 (s, 3H, $-CH_3$), 3.43 (d, 1H, $-CH_2-$, $J = 18.4$ Hz), 3.79 (d, 1H, $-CH_2-$, $J = 18.4$ Hz), 5.28 (s, 1H, O-H), 7.06–9.22 (m, 12H); ^{13}C -NMR (100 MHz, $CDCl_3$, δ ppm): 19.0($-CH_3$), 48.1($-CH_2-$), 94.6, 115.7, 116.4, 120.8, 123.9, 125.9(d), 126.9(d), 129.0, 133.9, 139.0, 148.0, 151.5, 152.2, 161.0(d), 161.4, 163.0(d), 163.5, 165.6, 167.8; LC-MS, (m/z): 477.61 (M+1); Anal. Calcd. For $C_{12}H_7Cl_3N_4OS$ (476.49): C, 63.02; H, 3.81; N, 11.76. Found: C, 63.05; H, 3.80; N, 11.77. Figs. S34–S37.

5.10.20. 3-(4-fluorophenyl)-5-(4-methoxyphenyl)-5-hydroxy-4,5-dihydro-1H-pyrazol-1-yl[4-methyl-2-(pyridin-3-yl)-1,3-thiazol-5-yl]methanone (8h)

Yield 71%; m.p. 74–76 °C; IR (cm^{-1}): 693 (C-S), 1221 (C-O-C), 1602 (C=N), 1625 (C=O), 2844 (C-H), 2913 (Ar C-H), 3302 (O-H); 1H -NMR (400 MHz, $CDCl_3$, δ ppm): 2.68 (s, 3H, $-CH_3$), 3.52 (dd, 1H, $-CH_2-$, $J = 18.4$ Hz), 3.77 (s, 3H, $-OCH_3$), 3.83 (dd, 1H, $-CH_2-$, $J = 18.4$ Hz), 5.11 (s, 1H, O-H), 7.19–9.40 (m, 12H); ^{13}C -NMR (100 MHz, $CDCl_3$, δ ppm): 18.9($-CH_3$), 49.9, 50.0, 94.9, 116.29(d), 127.0, 128.9(d), 128.8, 129.5, 133.8, 138.2, 140.2, 143.0, 147.8, 151.2, 152.2, 160.8(d), 163.0, 163.3(d), 165.6, 167.7, 167.5; LC-MS, (m/z):

488.70 (M⁺); Anal. Calcd. For C₁₂H₇Cl₃N₄OS (488.53): C, 63.92; H, 4.33; N, 11.47. Found: C, 63.84; H, 4.30; N, 11.45.

5.10.21. 3-(4-fluorophenyl)-5-(3-chlorophenyl)-5-hydroxy-4,5-dihydro-1H-pyrazol-1-yl[4-methyl-2-(pyridin-3-yl)-1,3-thiazol-5-yl]methanone (8i)

Yield 64%; m.p. 161–163 °C; IR (cm⁻¹): 694 (C-S), 1599 (C=N), 1631 (C=O), 2938 (C-H), 3044 (Ar C-H), 3359 (O-H); ¹H-NMR (400 MHz, CDCl₃, δ ppm): 2.31 (s, 3H, -CH₃), 3.55 (d, 1H, -CH₂-, *J* = 18.4 Hz), 3.83 (d, 1H, -CH₂-, *J* = 18.4 Hz), 5.16 (s, 1H, O-H), 7.31–9.28 (m, 12H); ¹³C-NMR (100 MHz, CDCl₃, δ ppm): 19.4(-CH₃), 49.3, 92.4, 117.7, 118.4, 121.7, 124.7, 126.9(d), 127.9(d), 130.1, 134.9, 140.2, 149.3, 152.5, 153.8, 162.3(d), 162.9, 164.1(d), 164.4, 165.8, 167.5; LC-MS, (m/z): 493.44 (M⁺), 495.56 (M+1); Anal. Calcd. For C₁₂H₇Cl₃N₄OS (492.95): C, 60.91; H, 3.68; N, 11.37. Found: C, 60.92; H, 3.67; N, 11.22.

5.10.22. 3-(4-chlorophenyl)-5-(4-fluorophenyl)-5-hydroxy-4,5-dihydro-1H-pyrazol-1-yl[4-methyl-2-(pyridin-3-yl)-1,3-thiazol-5-yl]methanone (8j)

Yield 92%; m.p. 154–156 °C; IR (cm⁻¹): 695 (C-S), 1600 (C=N), 1629 (C=O), 2936 (C-H), 3045 (Ar C-H), 3357 (O-H); ¹H-NMR (400 MHz, CDCl₃, δ ppm): 2.75 (s, 3H, -CH₃), 3.51 (dd, 1H, -CH₂-, *J* = 18.4 Hz and *J* = 6.8 Hz), 3.76 (dd, 1H, -CH₂-, *J* = 18.4 Hz, and *J* = 6.8 Hz), 5.23 (s, 1H, O-H), 7.25–9.21 (m, 12H); ¹³C-NMR (100 MHz, CDCl₃, δ ppm): 19.4(-CH₃), 49.4, 92.4, 117.8, 118.4, 121.76, 124.7, 126.8(d), 127.8(d), 130.0, 134.9, 140.5, 149.3, 152.5, 153.8, 162.4(d), 162.8, 164.22(d), 164.3, 165.8, 167.5; LC-MS, (m/z): 493.52 (M⁺), 495.47 (M+1); Anal. Calcd. For C₁₂H₇Cl₃N₄OS (492.95): C, 60.91; H, 3.68; N, 11.37. Found: C, 60.92; H, 3.67; N, 11.22.

5.10.23. 3-(4-methoxyphenyl)-5-(4-chlorophenyl)-5-hydroxy-4,5-dihydro-1H-pyrazol-1-yl[4-methyl-2-(pyridin-3-yl)-1,3-thiazol-5-yl]methanone (8k)

Yield 77%; m.p. 85–87 °C; IR (cm⁻¹): 697 (C-S), 1225 (C-O-C), 1602 (C=N), 1624 (C=O), 2842 (C-H), 2915 (Ar C-H), 3298 (O-H); ¹H-NMR (400 MHz, CDCl₃, δ ppm): 2.80 (s, 3H, -CH₃), 3.55 (dd, 1H, -CH₂-, *J* = 18.4 Hz and *J* = 6.8 Hz), 3.79 (s, 3H, -OCH₃), 3.86 (dd, 1H, -CH₂-, *J* = 18.4 Hz and *J* = 6.8 Hz), 5.14 (s, 1H, O-H), 7.20–9.31 (m, 12H); ¹³C-NMR (100 MHz, CDCl₃, δ ppm): 18.6(-CH₃), 50.0, 50.2, 94.9, 116.2(d), 121.1(d), 127.2(d), 128.4, 128.9(d), 128.9, 129.4, 129.7, 133.9, 138.2, 140.2, 143.1, 147.9, 151.3, 152.3, 160.9(d), 163.2, 163.4(d), 165.4, 167.5, 167.6; LC-MS, (m/z): 505.81 (M⁺),

507.78 (M+2); Anal. Calcd. For C₁₂H₇Cl₃N₄OS (504.98): C, 61.84; H, 4.19; N, 11.09. Found: C, 61.81; H, 4.18; N, 11.07.

5.10.24. 3-(4-methoxyphenyl)-5-(4-bromophenyl)-5-hydroxy-4,5-dihydro-1H-pyrazol-1-yl[4-methyl-2-(pyridin-3-yl)-1,3-thiazol-5-yl]methanone (8l)

Yield 89%; m.p. 143–145 °C; IR (cm⁻¹): 692 (C-S), 1223 (C-O-C), 1601 (C=N), 1620 (C=O), 2842 (C-H), 2914 (Ar C-H), 3301 (O-H); ¹H-NMR (400 MHz, CDCl₃, δ ppm): 2.71 (s, 3H, -CH₃), 3.52 (dd, 1H, -CH₂-, *J* = 18.4 Hz and *J* = 6.8 Hz), 3.77 (s, 3H, -OCH₃), 3.84 (dd, 1H, -CH₂-, *J* = 18.4 Hz and *J* = 6.8 Hz), 5.11 (s, 1H, O-H), 7.18–9.38 (m, 12H); ¹³C-NMR (100 MHz, CDCl₃, δ ppm): 18.6 (-CH₃), 50.0, 50.2, 94.9, 116.2(d), 121.0(d), 127.2(d), 128.4, 128.8(d), 128.9, 129.4, 129.6, 133.8, 138.2, 140.2, 143.1, 147.9, 151.3, 152.2, 160.8(d), 163.0, 163.3(d), 165.4, 167.5, 167.6; LC-MS, (m/z): 549.61 (M+), 551.73 (M+2); Anal. Calcd. For C₁₂H₇Cl₃N₄OS (549.43): C, 56.84; H, 3.85; N, 10.20. Found: C, 56.88; H, 3.83; N, 10.22.

5.11. MTT assay

MDA-MB231 (triple negative breast cancer) and HT-29 (Colorectal cancer) cells, were procured from National Centre for Cell Sciences, Pune. They were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% foetal bovine serum and 1% antibiotic-antimycotic solution. The cells were maintained at 37 °C and 5% CO₂ levels. They were used for the experiments after three consecutive passages. Cytotoxicity of 1,3,4-Oxadiazole derivatives was assessed by MTT assay (Mosmann, 1983). Briefly, MDA-MB231 and HT-29 cells were seeded onto 96 well microtiter plates at a density of 5,000 cells/well and incubated overnight in the humidified atmosphere. The test compounds were added at concentrations of 6.25, 12.5, 25, 50 and 100 μM. 48 hrs after drug addition, 100 μL of MTT solution (1 mg/mL) was added to the wells, 4 hrs following which the formazan crystals formed were, solubilized in DMSO. Absorbance was recorded at 570 nm and percentage cytotoxicity was calculated in comparison with control.

5.12. Flow cytometry

To concentrate the impact of compounds **7i** and **8i** on different phase of MDA-MB231 and HT 29 cell cycle, Flow cytometric studies were carried out. Cells were seeded at a density of 0.5 million cells in 60 mm dishes. 24 hrs after seeding, they were treated with IC₅₀ concentration of the drug **7i** and **8i**, 48 hrs following drug addition, cells were harvested by trypsinization, washed with ice-cold PBS and fixed with 70% chilled ethanol. Fixed cells were stored at -20

°C for 48 hrs. Cells in ethanol were centrifuged at 3000 rpm for 20 minutes, ethanol was removed and washed with PBS. Cells were incubated with 200 μL of 50 $\mu\text{g/mL}$ solution of RNase A at 37 °C for 5 hrs and added with 1 μL Propidium iodide. Cell cycle analysis was done using analytical flow cytometer (Guava EasyCyte, Merck Millipore).

5.13. Molecular docking studies

The crystal structures from PDB 5iev of Cyclin-dependent kinase 2 with Roniclib (for **7i**) and 4LVT from PDB of B-cell lymphoma 2 (BCL-2) with Navitoclax (for **8i**) was used for the study. The protein was prepared by removing all water molecules and adding all hydrogen atoms. The ligands **7i** and **8i** was docked into the active sites using the molecular docking software SYBYL ver 7.3 (Tripos, L.P.) Surflex-Dock (BioPharmics LLC.) with the default parameters. The proprietary software is licensed to Manipal Institute of Technology, Manipal University, India. Surflex-Dock is a program for calculating the docking modes of small molecules into protein-binding sites. In this study, we have used ChemScore, a scoring function that is derived from regression against ligand-receptor binding free energies. In the docking process, the active site was defined. For each ligand, 20 conformations were generated ($40 \times 20 = 800$ conformations) and then docked into M1 mAChR.

5.14. DNA binding studies

Electronic absorption spectroscopy, is one of the most common techniques for the investigation of the binding mode of small molecules to DNA [59]. All the experiments involving the binding of prepared compounds with CT-DNA, were carried out in double distilled water. A solution of CT-DNA in 50 mM NaCl/5 mM Tris–HCl (pH 7.2) buffer, gave a UV absorbance at 260 and 280 nm and was found to be 1.8–1.9, indicating that the DNA was sufficiently free of protein [55]. A concentrated stock solution of DNA, was prepared in 5 mM Tris–HCl/50 mM NaCl in water at pH 7.0 and the concentration of CT-DNA was determined per nucleotide by taking the absorption coefficient ($6,600 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$) at 260 nm. The compounds (except **5e** and **5j**, because of forming precipitation in buffer) were dissolved in DMF solution for all the experiments. Absorption titration experiments, were performed with a fixed concentration of the compound (10–25 μM), and increasing concentration of DNA (0–350 μM). After equilibrium was reached (ca. 5 min), the spectra were recorded against an analogous blank solution containing the same concentration of DNA. To enable quantitative comparison of the DNA binding affinities, the intrinsic binding constant (K_b) of the complexes for binding with CT-DNA were obtained by the following equation [46].

$$\frac{[DNA]}{(\epsilon_a - \epsilon_b)} = \frac{[DNA]}{(\epsilon_a - \epsilon_f)} + \frac{1}{K_b(\epsilon_b - \epsilon_f)}$$

Where [DNA] is the concentration of DNA in base pair ϵ_a, ϵ_b and ϵ_f corresponds to the molar extinction coefficients of apparent, bound and free metal complexes respectively. A plot of $\frac{[DNA]}{(\epsilon_a - \epsilon_b)}$ versus [DNA], gave a slope $\frac{1}{(\epsilon_b - \epsilon_f)}$ and an intercept equal to $\frac{1}{K_b(\epsilon_b - \epsilon_f)}$; K_b is the ratio of slope to the intercept.

5.15. DNA docking

The crystal structure 6BNA [56] from PDB with netropsin was used for the study. The DNA structure was prepared by removing the bound netropsin molecule and removing all water molecules to avoid potential interference with the docking. The binding site was defined using an atom in the center of the DNA molecule and was large enough, that it encompassed netropsin binding site.

Declarations

Author contribution statement

Rangappa Santosh: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Gundibasappa K. Nagaraja, Mukunthan K. Selvam, Ashwini Prabhu: Performed the experiments.

Punchappady D. Rekha: Conceived and designed the experiments.

Panchangam M. Krishna: Conceived and designed the experiments; Analyzed and interpreted the data.

Funding statement

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Competing interest statement

The authors declare no conflict of interest.

Additional information

Supplementary content related to this article has been published online at <https://doi.org/10.1016/j.heliyon.2019.e01255>.

Acknowledgements

The authors are thankful to Department of Chemistry, Mangalore University for providing laboratory facilities for performing the experiments.

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