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Research paper

Synthesis and biological evaluation of novel pyrazolic chalcone derivatives as novel hepatocellular carcinoma therapeutics

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ABSTRACT

Despite having the second highest mortality associated with cancer, currently Sorafenib is the only FDA-approved chemotherapeutic agent available for liver cancer patients which can only improve survival for few months. In this study, various pyrazolic chalcone analogous compounds were synthesized and evaluated as potential chemotherapeutic agents for the treatment of hepatocellular carcinoma (HCC). Modifying the central pyrazole ring at the C(3)-position with different heteroaryl rings and substituting the C(4)-position of pyrazole with differently substituted chalcone moiety produced fouthy two variant compounds. For all these compounds, cytotoxicity was evaluated using sulforhodamine B assay and real time cell growth tracking, respectively. Based on 50% inhibitory concentration (IC_{50}) values, compounds **39**, **42**, **49**, and **52** were shown to exhibit potent cytotoxic activity against all the cancer cell lines tested, and had better cytotoxic activities than the well-known chemotherapeutic drug 5-FU. Therefore, these compounds were chosen to be further evaluated in a panel of HCC cell lines. Flow cytometric analysis of HCC cells treated with compounds **39**, **42**, **49**, and **52** demonstrated that these compounds caused cell cycle arrest at G2/M phase followed by the apoptotic cell death and impaired cell growth as shown by real-time cell growth surveillance. Consistent with these results, western blotting of HCC cells treated with the compounds resulted in molecular changes for cell cycle proteins, where p21 levels were increased independent of p53 and the levels of the key initiators of mitosis Cyclin B1 and CDK1 were shown to decrease upon treatment. In conclusion, chalcone derivatives **42** and **52** show potent bioactivities by modulating the expression of cell-cycle related proteins and resulting in cell-cycle arrest in the HCC cell lines tested here, indicating that the compounds can be considered as preclinical candidates.

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1. Introduction

Hepatocellular carcinoma (HCC) is the sixth most common cancer and the second most frequent cause of cancer-related mortality [1]. Each year, around five hundred thousand new cases are diagnosed world-wide and nearly 85% of all cases are from developing countries. The 5 year survival rate of HCC patients are low and the incidence of HCC continues to rise (3 fold increase in 20 years). The etiological factors associated with HCC are chronic infection by Hepatitis B virus (HBV) and Hepatitis C virus (HCV), aflatoxin exposure, chronic alcohol intake, and obesity [2,3]. Currently, Sorafenib, a multikinase inhibitor, is the only drug approved by FDA for the treatment of HCC which can only prolong patient survival for a short period of time (2–3 months) [4]. Since liver cancer cells display inherent resistance to conventional chemotherapy and radiotherapy, it is critical to develop novel therapeutic drugs against HCC.

Dysregulation of cell cycle control and cell division is highly correlated with cancer development, growth and progression. Liver cancer develops during chronic liver injury which involves immune response along with cytokines and chemokine activation. Hence during chronic liver disease, cycles of hepatocyte cell death and regeneration are induced which engage the activation of cell cycle proteins [5]. Cyclin-dependent kinases (CDKs) are one of the key

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cellular kinases involved in cell cycle regulation in liver [6]. They act together with their cyclin partners to phosphorylate the Rb protein which helps the release of transcription factors to ensure cell cycle transition. Alterations in the activities of CDKs lead to the transformation of normal cells toward cancerous state [7–12]. Therefore, design and synthesis of therapeutic agents targeting CDKs have been the subject of recent drug discovery research studies and a number of CDK inhibitors have been considered in several clinical trials [13–16]. Hence Palbociclib, which is a selective inhibitor of CDK4 and CDK6, was approved by the FDA to be used in combination with letrozole for the treatment of postmenopausal women with HER2-negative advanced breast cancer [17–20].

Chalcones, 1,3-diaryl-2-propen-1-ones, are naturally occurring intermediates of flavonoid compounds [21]. Chalcones have shown broad range of biological activities such as antioxidant, antibacterial, antifungal, *anti*-HIV, antileishmanial, antifilarial, antimalarial, anti-inflammatory, and anticancer properties [22,23]. It has been determined that many chalcone derivatives exhibit cytotoxic activity in various cancer cell lines through different molecular mechanisms [24,25].

Pyrazoles with their distinctive scaffold possess wide range of anticancer bioactivities [26–31]. Though more widely known for their use in analgesic, antipyretic and anti-inflammatory actions, pyrazoles can also have antileukemic [32,33], antitumor [34,35] and anti-proliferative [36]. Although the pyrazole skeleton is an important building block in mediating its biological effects, the type of peripheral substituents are crucial for their selectivity toward their targets. Studies concerning structure–activity relationships have shown that the cytotoxic potency of the compounds has been highly dependent on the substitution types and patterns on the aryl ring. Thus, 3,4-disubstituted pyrazole analogues, and 3-(imidazol-2-yl)-4-[2-(pyridin-3-yl)-vinyl]-pyrazoles (1), have been reported as cyclin-dependent kinase (CDK) inhibitors and have been shown to inhibit *in vitro* cellular proliferation in various human cancer cells [37]. In another study, Huang et al. showed that *N*-((1,3-diphenyl-1*H*-pyrazol-4-yl)methyl)aniline derivative **2** [38] had inhibitory activity on MCF-7 and B16-F10 cell lines with IC_{50} values of 1.88 and 2.12 μM, respectively. Compound **2** inhibits the CDK2/cyclin E holoenzyme activities with IC_{50} of 0.98 μM (Fig. 1). Recently, (*N*-((1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl)-1,3-diphenyl-1*H*-pyrazole-4-carboxamide derivatives were reported as CDK1/Cdc2 inhibitors [39].

In our previous studies, we reported 1,3-diarylpyrazole derivatives containing thiophene and pyridine heterocycles as significant antiproliferative agents against MCF7, MDA-MB-231, HeLa, Raji and HL60 human cancer cell lines (**3** in Fig. 1) [40,41]. Therefore considering the limited availability of targeted therapies in liver cancer, in this study, the cytotoxicities of pyrazolic chalcone derivatives are investigated in order to identify the potential bioactivities as drug candidates in treatment of HCC. Here we report the synthesis and the biological activities of a new series of 1,3,4-trisubstitutedpyrazoles **12**–**53** in which; *i*) a heteroaryl moiety (3-thienyl, 3-(benzo[d][1,3]dioxol-5-yl, 3-pyridyl, 4-pyridyl) is attached to the central pyrazole ring at position 3; *ii*) phenyl is attached to the pyrazole ring at position 1 and *iii*) chalcone moiety is attached to the central pyrazole ring at position 4.

2. Results and discussion

2.1. Chemistry

The 1,3-diarylpyrazole chalcone derivatives (**12**–**53**) were

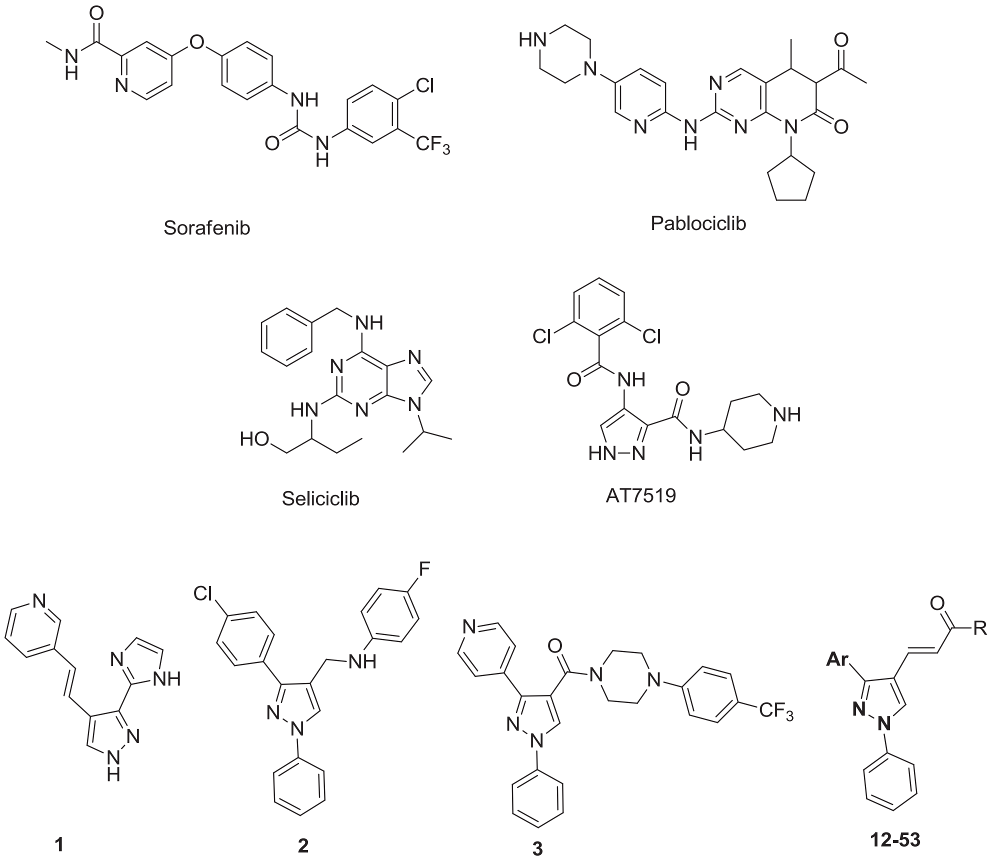


Fig. 1. Structure of hepatotoxic agents and some CDK inhibitors which are already approved or have reached clinical trials for the treatment of cancer; lead CDK inhibitors and title compounds.

synthesized as outlined in Scheme 1. The hydrazone derivatives **4**–**7** were generated by condensing appropriate methyl ketone and phenylhydrazine in the presence of acetic acid in refluxing ethanol. The IR spectra of hydrazone **4**–**7** showed disappearance of the carbonyl peak that belonged to acetyl group of methyl ketone derivatives. This hydrazone derivative was then reacted with $POCl_3$ and DMF resulting in 1,3-diaryl pyrazoles **8**–**11** with aldehyde group at 4 position. 1,3-Diarylpyrazole chalcone analogs **12**–**53** were prepared by condensation of 1-phenyl-3-heteroaryl-1*H*-pyrazole-4-carboxaldehyde derivatives **8**–**11** and corresponding methyl ketones in ethanol solution, applying 40% aqueous NaOH as a catalyst.

All compounds were purified either by automated flash chromatography or recrystallization; or automated flash chromatography then recrystallization and checked for purity by elemental analysis or UPLC (purity was >97%) before being tested in biological assays. The structures of these compounds were confirmed by high-resolution mass spectrometry (HRMS), IR, ^{13}C NMR and ^{1}H NMR spectral data. Elemental analysis results were also provided for eighteen of the title compounds.

2.2. Biological evaluations

2.2.1. Cytotoxicities of the pyrazolic chalcone derivatives in liver cancer cells

Pyrazolic chalcone derivatives (**12**–**53**) were initially screened against Hepatocellular (Huh7), breast (MCF7) and colon carcinoma (HCT116) cells. Bioactivities of each compound were evaluated by sulforhodamine B (SRB) assay. The IC_{50} values after 72 h of treatment with each molecule were calculated in comparison with the well known chemotherapeutic agents such as, nucleobase analogue 5-fluorouracil (5-FU) and nucleoside analogue cladribine. IC_{50} values were in between 0.3 and 29 μM as shown in Table 1.

In general, compounds having thenyl moiety at position 3 on the pyrazole ring (**12**–**19**) displayed great antiproliferative potency on all cancer cell lines with the exception of the benzo[d][1,3]dioxol-5-yl chalcone derivative **19**. Compounds having methoxy substituent at positions 3 and 4 or at 2 and 5 on the phenyl chalconic moiety of the molecules (**16**, **17**) showed IC_{50} values of 0.4–3.4 μM in Huh7, MCF7 and HCT116 cell lines.

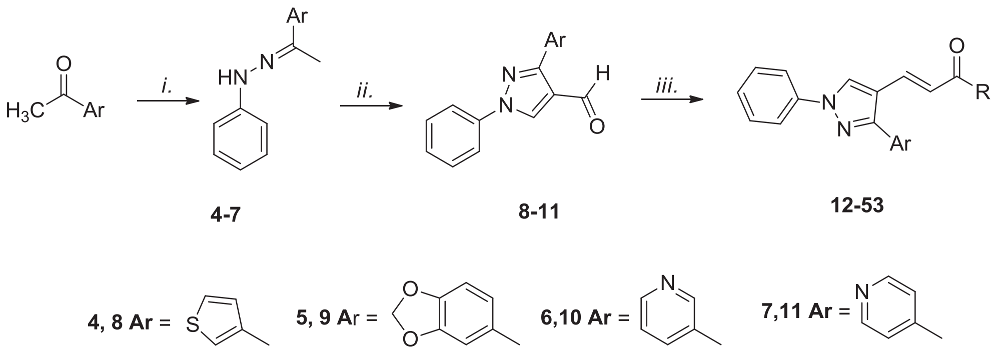
Replacing the thenyl moiety (**12**–**19**) with benzo[d][1,3]dioxol-5-yl ring (**20**–**31**) resulted in a significant decrease in the cytotoxic activity of the compounds which bears methoxy groups on the phenyl chalconic moiety especially against Huh7 (6.1–19.0 μM) and MCF7 (5.8–14.5 μM). Compounds having benzo[d][1,3]dioxol-5-yl moiety at position 3 on the pyrazole ring and heterocyclic rings such as 3-thenyl, 3-/2-pyridyl at the chalconic part (**27**–**29**) showed notable antiproliferative activity against Huh7 (3.2–3.4 μM), MCF7 (2.2–4.9 μM) and HCT116 (3.8–8.9 μM) cell lines. Derivatives **30** (having benzo[d] [1,3]dioxol-5-yl moiety at both on the pyrazole and chalcone part) and **31** (bearing benzo[d][1,3]dioxol-5-yl moiety at position 3 on the pyrazole and 3-pyrolyl moiety at chalcone part) had weak activity for all cell lines used in this study (9.2–27.2 μM).

The 3-/4-pyridyl derivatives at 3 position of pyrazole ring (**32**–**53**) displayed very significant antiproliferative activity on all cancer cell lines (IC_{50} values of below 7 μM) with the exception of compound **46** which bears 3-pyrolyl moiety at chalcone part. Introduction of heterocylic rings at chalcone part as in compounds **33**, **34**, **35**, **36**, **46** did not result in any advantage toward the activity of the compounds. In 3-pyridyl series, regarding the effect of substitution on the phenyl ring (R) at chalcone side, the 2,4,6-trimethoxy derivative **39** has remarkable cytotoxic activity against all tested human cancer cells, especially to Huh7 (Table 1). Compound **42**, the 2,5-dimethoxy derivative were also showed significant activity against all test cell lines (IC_{50} values of 1,3 μM Huh7; 1.5 μM MCF7; 1 μM HCT116). Their analogs in 4-pyridyl series, compounds **49** and **52** were also very active, with IC_{50} values in the range of 0.6–2.6 μM against all tested cell lines following 72 h of treatment. When we compared the IC_{50} values of these compounds to well-known chemotherapeutic drugs, we observed that they were lower than 5-FU and comparable to that of cladribine. Therefore, the compounds which displayed significant cytotoxic effects (**39**, **42**, **49**, **52**) were chosen to be further evaluated in a panel of HCC cell lines composed of Huh7, HepG2, Mahlavu and SNU-475 cells, MCF12A, normal human breast epithelial cells and MRC5, human fetal lung fibroblast cells. Our results showed that pyrazolic chalcone derivatives were cytotoxic to HCC cells, however they were less cytotoxic to MCF12A and MRC-5 cells. (Table 2). Additionally, compound **49** had no cytotoxicity on MRC5 cells.

Recent studies have shown that some chalcone derivatives inhibit cell growth of several human cancer cell lines as tubulin binding and depolymerizing agents [34]. The chalcone analogs of some natural products such as combretastatin have been synthesized and developed by Ducki et al. [42]. These chalcone analogs show potent inhibition of tubulin assembly and possess promising anticancer activity, and are currently under preclinical evaluation [43,44]. Based on this information, we analyzed the effect of compounds **12**–**53** on tubulin polymerization, however no inhibitory effect on tubulin polymerization was observed (data not shown).

2.2.2. Real-time cellular response of hepatocellular carcinoma cells with *39*, *42*, *49* and *52* treatment

Time-dependent cytotoxic activities of pyrazole chalcone derivatives were performed with real time cell electronic sensing (RT-CES) on Huh7, HepG2, Mahlavu and SNU-475 cells. It was shown that all compounds reduced the growth rate of cells compared to



Scheme 1. Synthesis of 1,3-diarylpyrazole chalcone derivatives. Reagents and conditions: *i.*) Phenyl hydrazine, acetic acid, ethanol, 2 h reflux. *ii.*) Dimethyl formamide, $POCl_3$, 50 °C, 4 h *iii.*) methyl ketone derivatives, NaOH, EtOH.

Table 1

Cytotoxicity of compounds **12**–**53** and nucleoside analogs 5-FU and cladribine assessed in different human cancer cells.

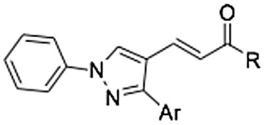
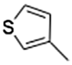
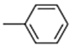
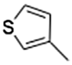
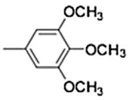
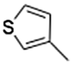
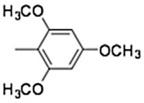
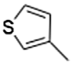
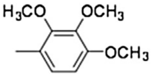
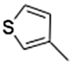
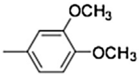
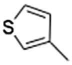
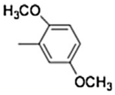
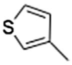
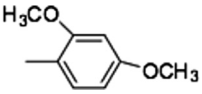
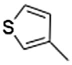
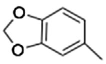
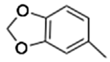
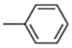
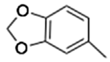
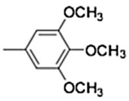
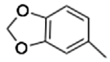
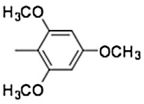
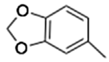
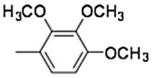
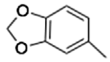
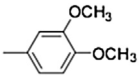
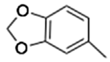
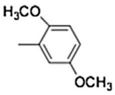
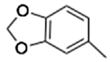
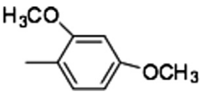
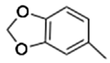
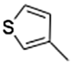
			IC$_{50}$ (μM)		
Compound	Ar	R	Huh7	MCF7	HCT116
12			4.7 ± 0.74	1.9 ± 0.30	5.1 ± 0.55
13		 OCH3 OCH3 OCH3	3.6 ± 0.58	4.9 ± 0.01	3.2 ± 0.65
14		 H3CO OCH3 H3CO	6.1 ± 0.03	2.7 ± 0.54	5.2 ± 1.47
15		 H3CO OCH3 OCH3	4.2 ± 0.47	7.4 ± 0.59	5.8 ± 1.57
16			2.9 ± 0.54	2.8 ± 0.51	1.6 ± 0.32
17			2.1 ± 1.37	0.4 ± 0.25	3.4 ± 0.48
18			3.0 ± 0.54	9.0 ± 1.05	2.2 ± 0.43
19			22.6 ± 0.87	5.3 ± 0.22	12.2 ± 0.78
20			10.5 ± 0.33	11.1 ± 0.59	1.16 ± 0.01
21		 OCH3 OCH3 OCH3	11.7 ± 0.53	10.9 ± 1.25	8.3 ± 1.07
22		 H3CO OCH3 H3CO	6.7 ± 0.21	11.5 ± 0.32	10.8 ± 0.98
23		 H3CO OCH3 OCH3	10.8 ± 0.8	7.4 ± 0.17	3.2 ± 0.54
24			15.4 ± 0.9	11.7 ± 0.37	11.4 ± 0.01
25			6.1 ± 0.11	5.8 ± 0.82	1.3 ± 0.36
26			19.0 ± 1.01	14.5 ± 0.6	8.6 ± 0.9
27			3.2 ± 0.44	4.9 ± 0.04	8.9 ± 0.71

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Table 1 (*continued*)

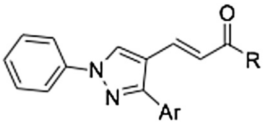
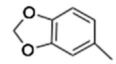
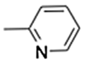
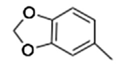
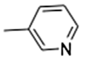
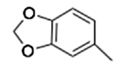
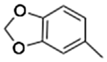
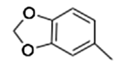
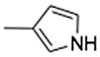
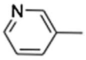
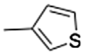
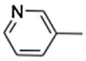
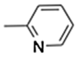
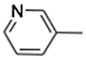
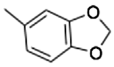
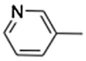
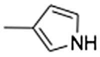
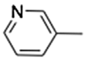
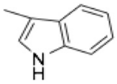
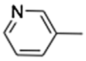
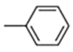
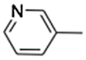
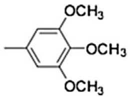
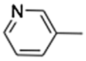
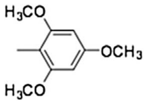
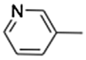
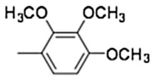
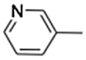
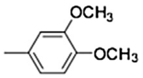
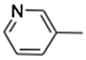
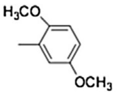
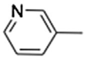
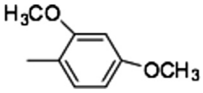
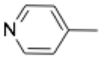
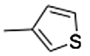
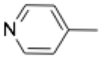
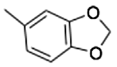
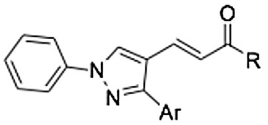
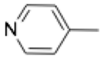
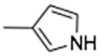
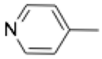
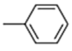
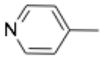
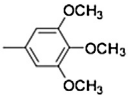
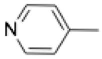
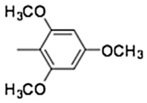
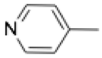
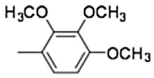
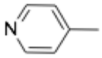
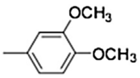
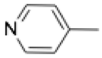
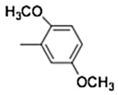
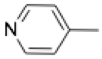
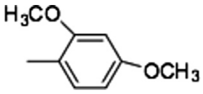
			IC$_{50}$ (μM)		
Compound	Ar	R	Huh7	MCF7	HCT116
28			3.4 ± 0.71	4.1 ± 0.04	6.7 ± 0.47
29			3.4 ± 0.9	2.2 ± 0.55	3.8 ± 0.20
30			9.9 ± 0.77	17.5 ± 0.7	27.2 ± 1.0
31			9.2 ± 0.95	9.9 ± 0.27	20.3 ± 0.56
32			1.7 ± 0.51	0.5 ± 0.04	1.3 ± 0.9
33			6.4 ± 0.8	4.2 ± 0.57	2.1 ± 0.35
34			6.7 ± 0.31	5.6 ± 0.77	1.8 ± 0.67
35			3.3 ± 0.78	6.9 ± 0.05	7.4 ± 1.02
36			3.2 ± 1.07	2.6 ± 0.00	6.4 ± 0.24
37			2.3 ± 0.49	4.1 ± 0.05	2.0 ± 0.71
38			1.9 ± 0.53	14.5 ± 0.06	8.6 ± 0.35
39			0.5 ± 0.17	1.0 ± 0.50	0.3 ± 0.15
40			2.3 ± 1.43	1.4 ± 0.14	1.1 ± 0.46
41			3.1 ± 0.32	1.7 ± 0.84	4.3 ± 1.20
42			1.3 ± 0.16	1.5 ± 0.52	1.0 ± 0.50
43			2.4 ± 0.56	2.3 ± 0.02	1.3 ± 0.75
44			2.2 ± 0.5	1.5 ± 0.58	1.1 ± 0.60
45			0.4 ± 0.14	0.8 ± 0.02	5.3 ± 0.90

Table 1 (*continued*)



Compound	Ar	R	IC$_{50}$ (μM) Huh7	MCF7	HCT116
46			28.8 ± 1.85	13.6 ± 1.47	15.7 ± 0.66
47			1.8 ± 0.51	3.4 ± 0.44	3.1 ± 0.88
48			3.5 ± 0.07	3.5 ± 0.04	0.3 ± 0.29
49			2.6 ± 0.58	1.6 ± 0.66	0.6 ± 0.18
50			3.7 ± 0.03	3.3 ± 0.04	1.3 ± 0.86
51			2.0 ± 0.25	4.3 ± 0.07	1.2 ± 0.32
52			1.7 ± 0.21	1.4 ± 0.38	0.9 ± 0.23
53			4.4 ± 0.52	8.8 ± 0.18	4.4 ± 0.52
5-FU			21.0 ± 0.75	1.4 ± 0.26	18.4 ± 1.1
Cladribine			1.8 ± 0.43	2.0 ± 0.11	0.3 ± 0.15

NI: No inhibition.

Table 2
IC$_{50}$ values of compounds **39, 42, 49 and 52** for HCC cell lines (Huh7, HepG2, Mahlavu and SNU-475) and in epithelial cells (MCF12A and MRC-5).

Compound	IC$_{50}$ Values (μM) Huh7	HepG2	Mahlavu	SNU-475	MCF12A	MRC-5
39	**0.5** ± 0.2	**1.4** ± 0.2	**1.1** ± 0.2	**0.8** ± 0.1	**1.2** ± 0.5	**1.3** ± 0.6
42	**1.3** ± 0.2	**3.4** ± 0.8	**2.0** ± 0.3	**1.9** ± 0.1	**2.4** ± 0.8	**2.7** ± 0.9
49	**2.6** ± 0.6	**1.7** ± 0.1	**1.1** ± 0.1	**1.4** ± 0.3	**8.2** ± 0.6	**NI**
52	**1.7** ± 0.9	**4.8** ± 0.3	**1.6** ± 0.2	**2.0** ± 0.3	**3.0** ± 0.6	**3.1** ± 0.9

NI: No inhibition in cell growth.

the DMSO control (Fig. 2). This real-time growth pattern suggested that the growth inhibition was due to cell cycle arrest, where the cells were neither proliferating nor dying, whereas the cells treated with DMSO continued to proliferate until they reach confluence [45].

2.2.3. Characterization of cell death mechanism induced by pyrazolic chalcone derivatives

HepG2 and SNU475 cells were treated with the compounds for 48 h. In order to determine the death mechanism induced by pyrazolic chalcone derivatives, fluorescent microscopy using Annexin V staining was employed. Compared to DMSO controls, cells treated with all four of the chosen compounds were detached from the surface (Suppl. Fig. 5) and cells with condensed nuclei were positive for Annexin V staining, which indicates apoptotic cell death (Fig. 3a). After 24 h of treatment, PARP cleavage was observed in poorly-differentiated SNU475 cells by western blot analysis compared to well-differentiated HepG2 cells (Fig. 3b) [46].

2.2.4. Induction of G2/M cell cycle arrest by the pyrazolic chalcone derivatives

Since the pattern of cell growth observed from the real-time cell analysis suggested the induction of cell cycle arrest flow cytometry analysis using propidium iodide (PI) staining of DNA was then performed. Following 48hrs of treatment entry to G2/M phase was observed to be increased, which subsequently caused entry into sub-G1 phase at 72 h, especially with compounds **39, 42** and **52** (Fig. 4). Altogether, our results indicate that pyrazolic chalcone derivatives induce cell cycle arrest at the G2/M phase which was followed by apoptotic cell death in hepatocellular carcinoma cell lines.

2.2.5. Analysis of targeted cellular pathways

Cell cycle-related proteins more specifically those that are involved in the G2/M phase were analyzed by western blotting (Fig. 5). CyclinB1 and CDK1 complex was previously shown to be critical for the progression of cells in and out of M phase of the cell

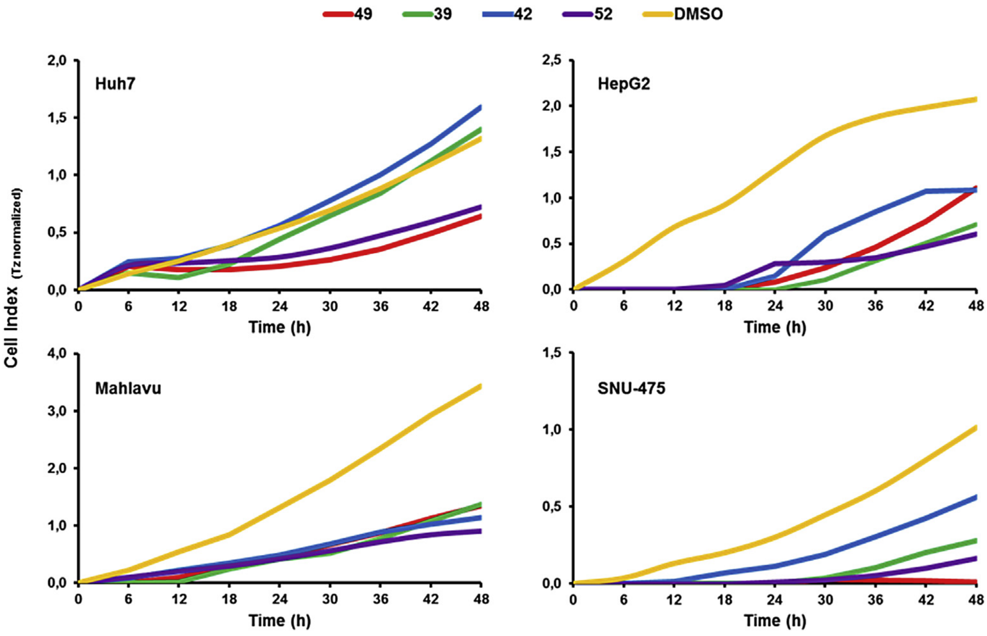


Fig. 2. RT-CES analysis of HCC cell lines treated with compounds **39**, **42**, **49** and **52** and with DMSO control (0.1%) at IC_{100} concentrations for 48 h.

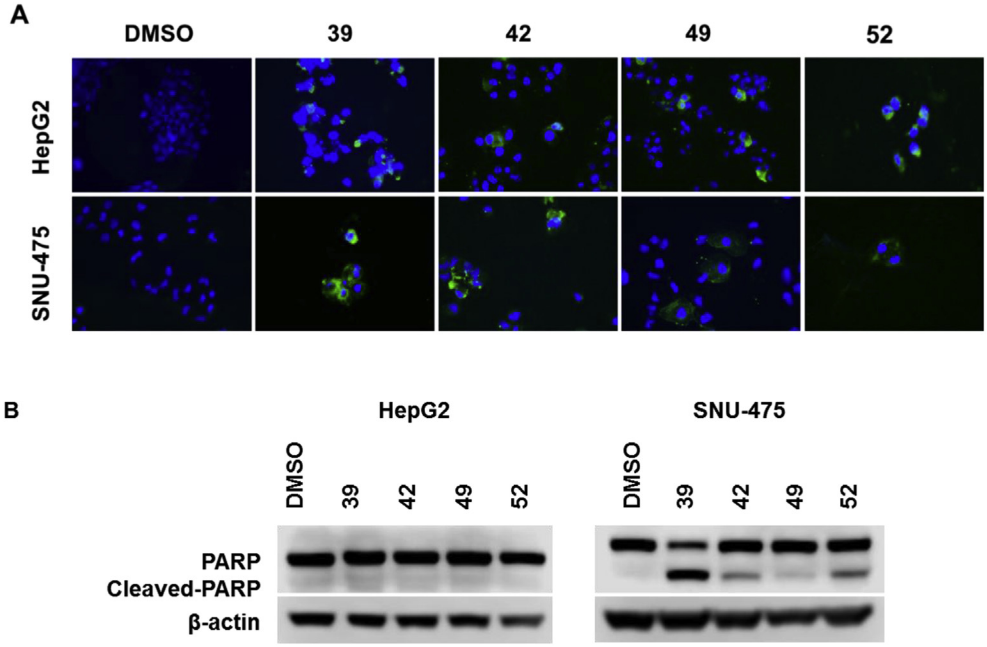


Fig. 3. Detection of apoptosis using fluorescent microscopy and western blotting. **A.** Annexin V staining of HepG2 and SNU-475 cells treated with compounds **39**, **42**, **49** and **52** for 48 h with IC_{100} concentrations. Condensed nuclei appear blue DAPI staining co-localize with Annexin V stained cells appear green. **B.** PARP cleavage after 24 h of treatment as shown by western blot analysis. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

cycle. Upon treatment with pyrazolic chalcone derivatives **42** and **52**, levels of CDK1 were shown to decrease by half in HepG2 cells, whereas a minor change was observed for SNU475 cells. Phospho-Cyclin B1 levels were shown to decrease by half in HepG2 cells, although there was a slight reduction in Cyclin B1 protein levels. It is well known that phosphorylation of Ser147 is necessary for the nuclear translocation of Cyclin B1 during prophase. Therefore, our results are indicate that pyrazole chalcone derivatives could be involved in a mechanism inducing inhibition of Cyclin B1 phosphorylation, which results in a decreased amount of active Cyclin B1, translocating into the nuclei. In addition to this, levels of p21, known to inhibit the activation of CyclinB1/CDK1 complex, were shown to increase by 5–6 fold in HepG2 cells and nearly 2 fold in SNU475 cells upon treatment with compounds **42** and **52**. It was further explored that this change in p21 levels were independent of p53 levels. The phospho-Rb levels were also found to be decreased, further impairing the cell cycle machinery, which could be the implication of secondary effects of pyrazolic chalcone derivatives on cell cycle.

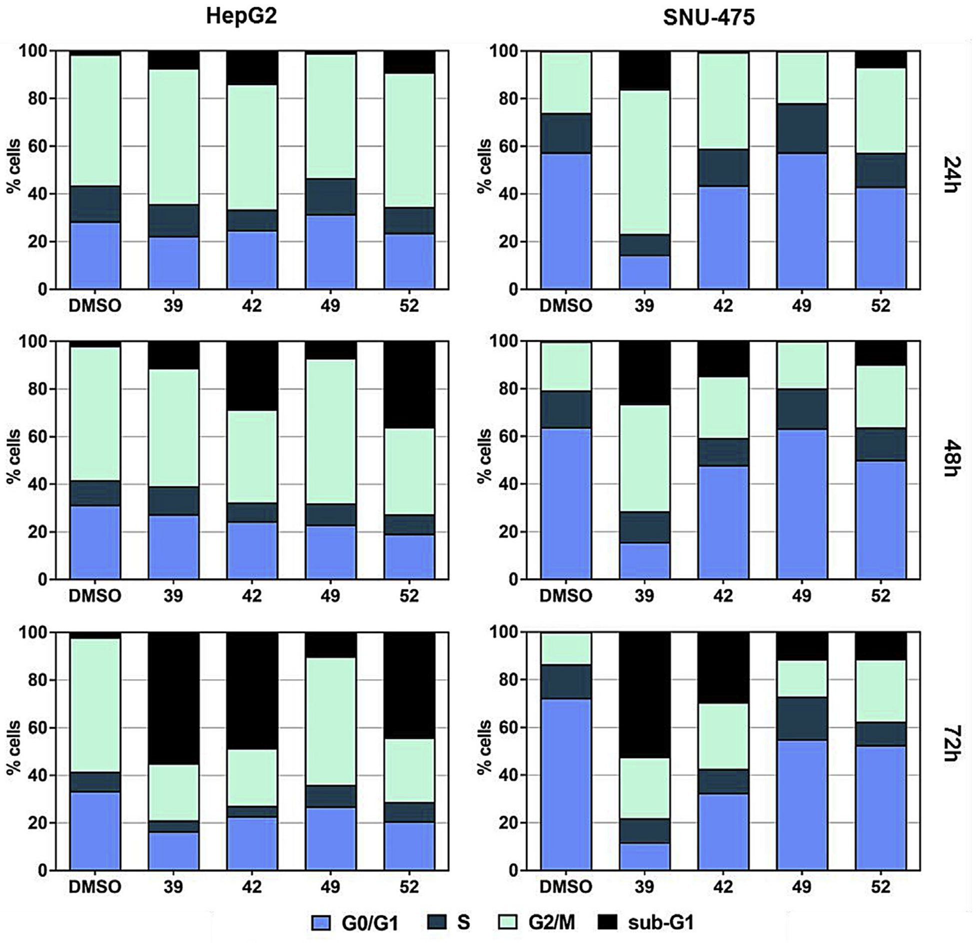


Fig. 4. Cell cycle analysis of HepG2 and SNU475 cells after treatment with compounds **39**, **42**, **49** and **52** and DMSO controls following 24 h, 48 h, and 72 h of treatment.

3. Conclusion

In this study, we synthesized a series of pyrazolic chalcone derivatives and evaluated their antiproliferative activities against human cancer cell lines in comparison with clinically used chemotherapeutics such as 5-FU and Cladribine. The preliminary structure bioactivity relations in these compounds have been established and discussed in results section in detail. The central pyrazole ring was modified at the C(3)-position with different heteroaryl rings and substituted at the C(4)-position with differently substituted chalcone moiety. Based on preliminary screenings the benzo[d][1,3]dioxol-5-yl ring (**20**–**31**) was not tolerated and resulted in mostly inactive or weak inhibitory compounds. Modifications of C(3)-position of central pyrazole ring with 3-/4-pyridyl moiety improved the bioactivity bearing more potent cytotoxic compounds. Therefore compounds **39**, **42**, **49**, and **52** were the most effective derivatives which displayed antiproliferative activity with IC_{50} values smaller than 5 μM against HCC cells used here. Moreover, these compounds were less active on transformed and normal cells (MCF12A and MRC-5). By further investigating their molecular effectors, we showed that compounds **42** and **52** caused cell cycle arrest at the G2/M phase and induced apoptotic cell death (as summarized in Fig. 6). Moreover, decrease in levels of phospho-cyclin B1 at the Ser147 residue upon treatment is worth exploring, since it could provide further information about the mechanism of action of the compounds on cancer cells. Future studies can evaluate the detailed cellular networks that are affected by the use of high throughput genomic screening methods such as transcriptome analysis with next-generation sequencing in the presence of selected compounds. This may allow to identify molecular targets involved in cell cycle for eventual drug design and development in cancer.

4. Experimental

4.1. Chemistry

Melting points were determined with an SMP-II Digital Melting Point Apparatus and are uncorrected (Schorpp Geaetetechnik, Germany). IR spectra were obtained using a Perkin Elmer Spectrum 400 FTIR/FTNIR spectrometer equipped with a Universal ATR Sampling Accessory. ^1H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ or DMSO-d_6 on a Varian Mercury 400 MHz High Performance Digital FT-NMR spectrometer at the NMR facility of the Faculty of Pharmacy, Ankara University or on a Varian Mercury 300 MHz FT-NMR spectrometer at the NMR facility of FARGEM (Pharmaceutical Research and Development Center) Inc. using tetramethylsilane as the internal standard. All chemical shifts were recorded as δ (ppm). High resolution mass spectra data (HRMS) were collected using a Waters LCT Premier XE Mass Spectrometer (high sensitivity orthogonal acceleration time-of-flight instrument) using ESI (+) method. The instrument was coupled to an AQUITY Ultra Performance Liquid Chromatography system (Waters Corporation, Milford, MA, USA). Elemental analyses were performed with a LECO-

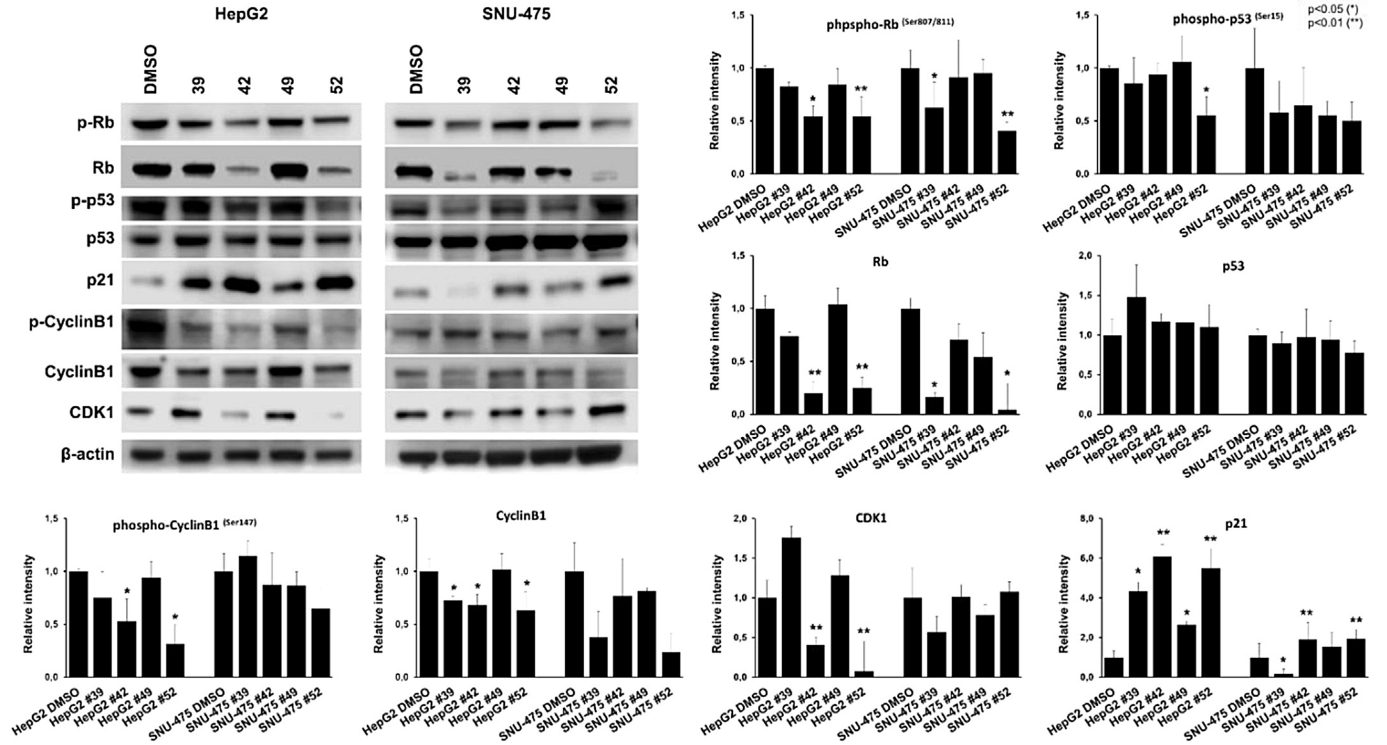


Fig. 5. Representative images from three independent western blot analysis of G2/M arrest proteins in whole lysates of HepG2 and SNU-475 cells treated with compounds **39**, **42**, **49** and **52** in IC$_{100}$ concentrations for 24 h. Graphs demonstrate comparative analysis of protein levels in the presence of compounds normalized to DMSO controls.

932 (C, H, N, S-Elemental Analyzer) at the Faculty of Pharmacy, Ankara University. Flash chromatography was performed with a Combiflash®Rf automated flash chromatography system with RediSep columns (Teledyne-Isco, Lincoln, NE, USA) using DCM-MeOH, hexane-EtOAc or MeOH-EtOAc solvent gradients.

4.1.1. *General procedure for 1,3-diarylpyrazole chalcone synthesis*

To a stirred solution of 1,3-diaryl-1*H*-pyrazole-4-carboxaldehyde (1 mmol) and corresponding methyl ketone (1.05 mmol) in ethanol (5 ml), an aqueous solution of 40% NaOH (2 ml) was added. The resulting solution was heated to 60 °C for 4 h and then allowed to stand overnight at room temperature with continuous stirring. The reaction mixture was poured into water and precipitate was collected by filtration, washed with 50% ethanol, dried, and purified by flash chromatography or recrystallization.

4.1.2. *(E)-1-phenyl-3-(1-phenyl-3-(thiophen-3-yl)-1H-pyrazol-4-yl)prop-2-en-1-one (CAS No: 303773-77-5) (**12**)*

Purified by column flash chromatography (0% → 50% ethyl acetate in hexane). Yield 76%, mp 138–139°C; IR (FTIR/FTNIR-ATR): 1657 cm^{-1} (C=O). ^{1}H NMR (CDCl$_3$, 300 MHz) δ: 8.27 (1H, s), 7.94–7.93 (2H, m), 7.90 (1H, d, *J* = 15.6 Hz), 7.71 (2H, d, *J* = 9.9 Hz), 7.59–7.58 (1H, m), 7.54–7.37 (7H, m), 7.33 (1H, d, *J* = 15.9 Hz), 7.27 (1H, d, *J* = 7.5 Hz). ^{13}C NMR (DMSO-d_6, 400 MHz) δ: 188.68, 148.72, 138.89, 137.67, 134.08, 133.01, 132.64, 129.66, 128.77, 128.57, 128.19, 127.51, 127.23, 127.10, 124.69, 121.39, 118.60, 117.89. HRMS (*m*/*z*): [M+H]$^{+}$ *calcd.* for $C_{22}H_{17}N_2OS$ 357.1062, *found* 357.1064.

4.1.3. *(E)-3-(1-phenyl-3-(thiophen-3-yl)-1H-pyrazol-4-yl)-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (**13**)*

Purified by column flash chromatography (0% → 50% ethyl acetate in hexane). Yield 21%, mp 167–168°C; IR (FTIR/FTNIR-ATR): 1651 cm^{-1} (C=O). ^{1}H NMR (CDCl$_3$, 300 MHz) δ: 8.25 (1H, s), 7.89 (1H, d, *J* = 15.6 Hz), 7.71 (2H, d, *J* = 8.7 Hz), 7.60–7.58 (1H, m), 7.46–7.38 (4H, m), 7.31 (1H, s), 7.25 (1H, d, *J* = 15.6 Hz), 7.19 (2H, s), 3.86 (9H, s).^{13}C NMR (DMSO-d_6, 400 MHz) δ:187.46, 152.91, 148.68, 141.97, 138.89, 133.89, 133.06, 132.72, 129.70, 128.72, 127.57, 127.27, 127.17, 124.77, 121.26, 118.77, 117.89, 106.01, 60.21, 56.23. HRMS (*m*/*z*): [M+H]$^{+}$ *calcd.* for $C_{25}H_{23}N_2O_4S$ 447.1379, *found* 447.1384.

4.1.4. *(E)-3-(1-phenyl-3-(thiophen-3-yl)-1H-pyrazol-4-yl)-1-(2,4,6-trimethoxyphenyl)prop-2-en-1-one (**14**)*

Purified by column flash chromatography (0% → 50% ethyl acetate in hexane). Yield 81%, mp 162.5–163.5 °C; IR (FTIR/FTNIR-ATR): 1626 cm^{-1} (C=O). ^{1}H NMR (CDCl$_3$, 300 MHz) δ: 8.14 (1H, s), 7.67 (2H, d, *J* = 7.8 Hz), 7.48–7.26 (6H, m), 7.19 (1H, d, *J* = 5.1 Hz), 6.73 (1H, d, *J* = 16.2 Hz), 6.09 (2H, s), 3.78 (3H, s), 3.72 (6H, s). ^{13}C NMR (DMSO-d_6, 400 MHz) δ: 193.13, 161.89, 157.85, 148.11, 138.89, 134.47, 132.70, 129.61, 128.72, 128.31, 127.28, 127.16, 127.02, 123.99, 118.62, 117.21, 110.80, 90.91, 55.77, 55.47. HRMS (*m*/*z*): [M+H]$^{+}$ *calcd.* for $C_{25}H_{23}N_2O_4S$ 447.1379, *found* 447.1360.

4.1.5. *(E)-3-(1-phenyl-3-(thiophen-3-yl)-1H-pyrazol-4-yl)-1-(2,3,4-trimethoxyphenyl)prop-2-en-1-one (**15**)*

Purified by recrystallization of ethyl acetate and hexane. Yield 23%, mp 90.5–91 °C; IR (FTIR/FTNIR-ATR): 1649 cm^{-1} (C=O). ^{1}H NMR (CDCl$_3$, 300 MHz) δ: 8.22 (1H, s), 7.78–7.69 (3H, m), 7.59–7.58 (1H, m), 7.46–7.35 (5H, m), 7.30–7.24 (2H, m), 6.71–6.68 (1H, d, *J* = 8.7 Hz), 3.86 (6H, s), 3.82 (3H, s). ^{13}C NMR (DMSO-d_6, 400 MHz) δ: 190.05, 156.39, 152.60, 148.50, 141.64, 138.90, 133.22, 132.71, 129.60, 128.30, 127.47, 127.20, 127.04, 126.32, 125.93, 124.94, 124.57, 118.70, 117.66, 107.88, 61.80, 60.53, 56.07. HRMS (*m*/*z*): [M+H]$^{+}$ *calcd.* for $C_{25}H_{23}N_2O_4S$ 447.1379, *found* 447.1362.

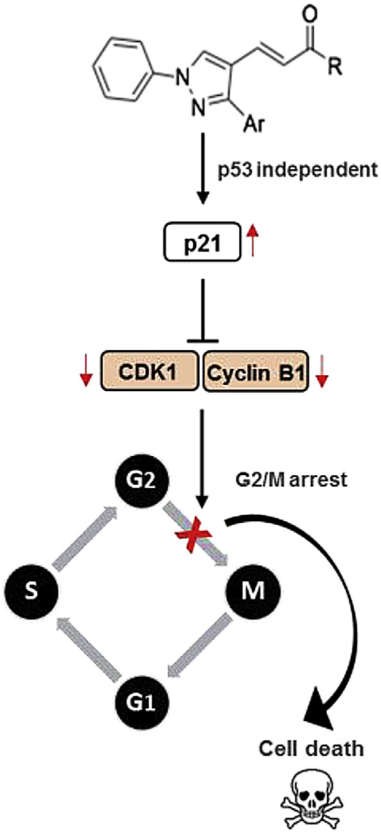


Fig. 6. Representation of molecular mechanisms initiated by pyrazolic chalcone derivatives (**42** and **52**). Upon treatment activation of p21 is initiated independent of p53, which then inhibits CDK1 and Cyclin B1 to form a complex resulting in cell cycle arrest at G2/M phase followed by cell death.

*4.1.6. (E)-1-(3,4-dimethoxyphenyl)-3-(1-phenyl-3-(thiophen-3-yl)-1H-pyrazol-4-yl)prop-2-en-1-one (**16**)*

Purified by recrystallization of ethyl acetate and hexane. Yield 62%, mp 151–152 °C; IR (FTIR/FTNIR-ATR): 1650 cm^{-1} (C=O). ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 8.26 (1H, s), 7.88 (1H, d, $J = 15.6$ Hz), 7.72 (2H, d, $J = 8.1$ Hz), 7.60–7.53 (3H, m), 7.46–7.36 (3H, m), 7.31–7.26 (2H, m), 7.19 (1H, S), 6.85 (1H, d, $J = 8.1$ Hz), 3.90 (6H, s).^{13}C NMR (DMSO-d_6, 400 MHz) δ: 186.89, 153.17, 148.82, 148.59, 138.94, 133.03, 132.77, 130.58, 129.68, 128.46, 127.54, 127.23, 127.07, 124.64, 122.91, 121.35, 118.65, 118.01, 110.87, 110.66, 55.78, 55.59. HRMS (m/z): $[M+H]^+$ calcd. for $C_{24}H_{21}N_2O_3S$ 417.1273, found 417.1273.

*4.1.7. (E)-1-(2,5-dimethoxyphenyl)-3-(1-phenyl-3-(thiophen-3-yl)-1H-pyrazol-4-yl)prop-2-en-1-one (**17**)*

Purified by recrystallization of ethyl acetate and hexane. Yield 73%, mp 105.5–106 °C; IR (FTIR/FTNIR-ATR): 1649 cm^{-1} (C=O). ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 8.18 (1H, s), 7.74 (1H, s), 7.71–7.68 (2H, m), 7.55–7.53 (2H, m), 7.45–7.34 (1H, m), 7.27 (2H, t, $J = 7.5$ Hz), 7.19 (1H, d, $J = 5.1$ Hz), 7.15 (1H, s), 7.09 (1H, d, $J = 3$ Hz), 6.96 (1H, d, $J = 3.3$ Hz), 6.93 (1H, d, $J = 3$ Hz), 3.77 (3H, s), 3.73 (3H, s).^{13}C NMR (DMSO-d_6, 400 MHz) δ: 191.78, 153.00, 151.49, 148.49, 138.88, 133.63, 132.69, 129.61, 129.44, 128.50, 127.44, 127.19, 127.05, 126.29, 124.52, 118.69, 118.02, 117.58, 113.97, 113.64, 56.30, 55.54. HRMS (m/z): $[M+H]^+$ *calcd.* for $C_{24}H_{21}N_2O_3S$ 417.1273, *found* 417.1256.

*4.1.8. (E)-1-(2,4-dimethoxyphenyl)-3-(1-phenyl-3-(thiophen-3-yl)-1H-pyrazol-4-yl)prop-2-en-1-one (**18**)*

Purified by column flash chromatography (0% → 50% ethyl acetate in hexane). Yield 60%, mp 180.5–181 °C; IR (FTIR/FTNIR-ATR): 1642 cm^{-1} (C=O). ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 8.17 (1H, s), 7.78–7.66 (4H, m), 7.58–7.56 (1H, m), 7.46–7.24 (6H, m), 6.50 (1H,d, $J = 10.5$ Hz), 6.42 (1H, s), 3.80 (6H, s). ^{13}C NMR (DMSO-d_6, 400 MHz) δ: 189.28, 163.74, 159.95, 148.40, 138.93, 132.81, 131.87, 131.75, 129.60, 128.28, 127.52, 127.14, 126.98, 126.55, 124.56, 121.45, 118.70, 117.85, 105.85, 98.53, 55.92, 55.57. HRMS (m/z): $[M+H]^+$ *calcd.* for $C_{24}H_{21}N_2O_3S$ 417.1273, *found* 417.1267.

*4.1.9. (E)-1-(benzo[d][1,3]dioxol-5-yl)-3-(1-phenyl-3-(thiophen-3-yl)-1H-pyrazol-4-yl)prop-2-en-1-one (**19**)*

Purified by column flash chromatography (0% → 50% ethyl acetate in hexane). Yield 81% as a colorless oil; IR (FTIR/FTNIR-ATR): 1646 cm^{-1} (C=O). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 8.31 (1H, s), 7.93 (1H, d, $J = 15.6$ Hz), 7.78 (2H, d, $J = 8$ Hz), 7.65–7.64 (1H, m), 7.59 (1H, dd, $J = 2$ Hz, $J = 8$ Hz), 7.52–7.44 (5H, m), 7.36–7.32 (2H, m), 6.88 (1H, d, $J = 8.4$ Hz), 6.06 (2H, s). ^{13}C NMR (DMSO-d_6, 400 MHz) δ: 186.43, 151.47, 148.62, 148.01, 138.92, 133.35, 132.70, 132.32, 129.67, 128.42, 127.49, 127.22, 127.08, 124.61, 124.57, 121.27, 118.59, 117.99, 108.15, 107.65, 102.09. HRMS (m/z): $[M+H]^+$ *calcd.* for $C_{23}H_{17}N_2O_3S$ 401.0960, *found* 401.0954.

*4.1.10. (E)-3-(3-(benzo[d][1,3]dioxol-5-yl)-1-phenyl-1H-pyrazol-4-yl)-1-phenylprop-2-en-1-one (**20**)*

Purified by column flash chromatography (0% → 30% ethyl acetate in hexane). Yield 95%, mp 163.5–164 °C; IR (FTIR/FTNIR-ATR): 1662 cm^{-1} (C=O). ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 8.27 (1H, s), 7.90 (2H, d, $J = 8.7$ Hz), 7.79 (1H, d, $J = 15.6$ Hz), 7.72 (2H, d, $J = 9.6$ Hz), 7.51–7.40 (5H, m), 7.34–7.28 (2H, m), 7.14–7.07 (2H, m), 6.86 (1H, d, $J = 8.1$ Hz), 5.96 (2H, s). ^{13}C NMR (DMSO-d_6, 400 MHz) δ: 189.22, 153.16, 148.28, 148.21, 139.40, 138.15, 134.80, 133.50, 130.14, 129.24, 129.18, 128.66, 127.57, 126.17, 122.91, 121.74, 119.10, 118.10, 109.14, 108.97, 101.86. HRMS (m/z): $[M+H]^+$ *calcd.* for $C_{25}H_{19}N_2O_3$ 395.1396, *found* 395.1399.

*4.1.11. (E)-3-(3-(benzo[d][1,3]dioxol-5-yl)-1-phenyl-1H-pyrazol-4-yl)-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (**21**)*

Purified by column flash chromatography (0% → 30% ethyl acetate in hexane). Yield 53%, mp 163.5–164 °C; IR (FTIR/FTNIR-ATR):1656 cm^{-1} (C=O). ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 8.32 (1H, s), 7.89–7.77 (3H, m), 7.52–7.14 (8H, m), 6.93 (1H, d, $J = 8.1$ Hz), 6.02 (2H, s), 3.93 (9H, s). ^{13}C NMR (DMSO-d_6, 400 MHz) δ: 187.52, 152.90, 152.65, 147.80, 147.72, 141.96, 138.92, 134.11, 133.06, 129.68, 128.80, 127.13, 125.79, 122.47, 121.12, 118.77, 117.60, 108.66, 108.54, 106.00, 101.40, 60.20, 56.21. HRMS (m/z): $[M+H]^+$ *calcd.* for $C_{28}H_{25}N_2O_6$ 485.1713, *found* 485.1716.

*4.1.12. (E)-3-(3-(benzo[d][1,3]dioxol-5-yl)-1-phenyl-1H-pyrazol-4-yl)-1-(2,4,6-trimethoxyphenyl)prop-2-en-1-one (**22**)*

Purified by column flash chromatography (0% → 50% ethyl acetate in hexane). Yield 40%, mp 205–205.5°C; IR (FTIR/FTNIR-ATR): 1650 cm^{-1} (C=O). ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 8.15 (1H, s), 7.67 (2H, d, $J = 7.8$ Hz), 7.44–7.35 (3H, m), 7.29–7.24 (1H, m), 7.05–7.02 (2H, m), 6.81–6.67 (2H, m), 6.07 (2H, s), 5.94 (2H, s), 3.77 (3H, s), 3.73 (6H, s).^{13}C NMR (DMSO-d_6, 400 MHz) δ: 193.40, 161.81, 157.81, 151.95, 147.72, 147.71, 138.92, 135.15, 129.61, 128.60, 128.46, 127.01, 125.59, 122.02, 118.64, 116.84, 110.53, 108.56, 108.16, 101.40, 90.84, 55.73, 55.44. HRMS (m/z): $[M+H]^+$ *calcd.* for $C_{28}H_{25}N_2O_6$ 485.1713, *found* 485.1712.

*4.1.13. (E)-3-(3-(benzo[d][1,3]dioxol-5-yl)-1-phenyl-1H-pyrazol-4-yl)-1-(2,3,4-trimethoxyphenyl)prop-2-en-1-one (**23**)*

Purified by recrystallization of ethyl acetate and hexane. Yield 27%, mp 144.5–145 °C; IR (FTIR/FTNIR-ATR): 1657 cm^{-1} (C=O). ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 8.22 (1H, s), 7.72–7.65 (3H, m), 7.45–7.38 (3H, m), 7.29–7.22 (2H, m), 7.13–7.07 (2H, m), 6.85 (1H, d,

J = 7.8 Hz), 6.69 (1H, d, J = 8.7 Hz), 5.95 (2H, s), 3.85 (6H, s), 3.82 (3H, s). ^{13}C NMR (DMSO-d_6, 400 MHz) δ: 190.03, 156.38, 152.63, 152.45, 147.75, 147.69, 141.64, 138.93, 133.46, 129.60, 128.49, 127.01, 126.30, 125.77, 124.94, 122.40, 118.71, 117.37, 108.61, 108.50, 107.83, 101.37, 61.80, 60.53, 56.06. HRMS (m/z): $[M+H]^+$ *calcd.* for $C_{28}H_{25}N_2O_6$ 485.1713, *found* 485.1711.

*4.1.14. (E)-3-(3-(benzo[d][1,3]dioxol-5-yl)-1-phenyl-1H-pyrazol-4-yl)-1-(3,4-dimethoxyphenyl)prop-2-en-1-one (**24**)*

Purified by column flash chromatography (0% → 30% ethyl acetate in hexane). Yield 45%, mp 158.5–159 °C; IR (FTIR/FTNIR-ATR): 1649 cm^{-1} (C=O). ^{1}H NMR (CDCl$_3$, 300 MHz) δ: 8.26 (1H, s), 7.79 (1H, d, J = 15.3 Hz), 7.71 (2H, d, J = 7.8 Hz), 7.45–7.40 (2H, m), 7.56–7.52 (2H, m), 7.33 (1H, d, J = 15.6 Hz), 7.27 (1H, d, J = 7.5 Hz), 7.14 (1H, s), 7.10 (1H, d, J = 9.6 Hz), 6.88 (1H, s), 6.84 (1H, d, J = 8.4 Hz), 5.96 (2H, s), 3.89 (6H, s). ^{13}C NMR (DMSO-d_6, 400 MHz) δ: 186.93, 153.15, 152.53, 148.81, 147.76, 147.72, 138.96, 133.25, 130.56, 129.66, 128.56, 127.04, 125.81, 122.89, 122.41, 121.19, 118.64, 117.71, 110.86, 110.66, 108.65, 108.50, 101.38, 55.77, 55.57. HRMS (m/z): $[M+H]^+$ *calcd.* for $C_{27}H_{23}N_2O_5$ 455.1607, *found* 455.1606.

*4.1.15. (E)-3-(3-(benzo[d][1,3]dioxol-5-yl)-1-phenyl-1H-pyrazol-4-yl)-1-(2,5-dimethoxyphenyl)prop-2-en-1-one (**25**)*

Purified by column flash chromatography (0% → 50% ethyl acetate in hexane). Yield 28%, mp 125–126°C; IR (FTIR/FTNIR-ATR): 1652 cm^{-1} (C=O). ^{1}H NMR (CDCl$_3$, 300 MHz) δ: 8.18 (1H, s), 7.71–7.68 (2H, d, J = 9.9 Hz), 7.60 (1H, d, J = 16.2 Hz), 7.44–7.39 (2H, m), 7.29–7.24 (1H, m), 7.16 (1H, s), 7.11 (1H, m), 7.08–7.05 (2H, m), 6.94 (1H, d, J = 3.3 Hz), 6.86–6.82 (2H, m), 5.96 (2H, s), 3.77 (3H, s), 3.73 (3H, s). ^{13}C NMR (DMSO-d_6, 400 MHz) δ: 192.04, 153.00, 152.42, 151.37, 147.76, 147.69, 138.91, 134.15, 129.60, 129.41, 128.64, 127.04, 126.16, 125.71, 122.33, 118.71, 117.92, 117.24, 113.92, 113.55, 108.56, 108.44, 101.39, 56.26, 55.54. HRMS (m/z): $[M+H]^+$ *calcd.* for $C_{27}H_{23}N_2O_5$ 455.1607, *found* 455.1608.

*4.1.16. (E)-3-(3-(benzo[d][1,3]dioxol-5-yl)-1-phenyl-1H-pyrazol-4-yl)-1-(2,4-dimethoxyphenyl)prop-2-en-1-one (**26**)*

Purified by recrystallization of ethyl acetate and hexane. Yield 28%, mp 154,8–155.3 °C; IR (FTIR/FTNIR-ATR): 1645 cm^{-1} (C=O). ^{1}H NMR (CDCl$_3$, 300 MHz) δ: 8.17 (1H, s), 7.71–7.62 (4H, m), 7.44–7.39 (2H, m), 7.29–7.22 (2H, m), 7.13–7.07 (2H, m), 6.84 (1H, d, J = 8.1 Hz), 6.49 (1H, d, J = 10.8 Hz), 6.14 (1H, s), 5.95 (2H, s), 3.08 (6H, s). ^{13}C NMR (DMSO-d_6, 400 MHz) δ: 189.51, 163.68, 159.87, 152.33, 147.70, 147.65, 138.96, 132.19, 131.84, 129.57, 128.39, 126.94, 126.41, 125.89, 122.37, 121.43, 118.70, 117.53, 108.60, 108.50, 105.81, 101.36, 98.51, 55.87, 55.54. HRMS (m/z): $[M+H]^+$ *calcd.* for $C_{27}H_{23}N_2O_5$ 455.1607, *found* 455.1607.

*4.1.17. (E)-3-(3-(benzo[d][1,3]dioxol-5-yl)-1-phenyl-1H-pyrazol-4-yl)-1-(thiophen-3-yl)prop-2-en-1-one (**27**)*

Purified by recrystallization of ethyl acetate and hexane. Yield 56%, mp 138.5–139.5 °C; IR (FTIR/FTNIR-ATR): 1659 cm^{-1} (C=O). ^{1}H NMR (CDCl$_3$, 300 MHz) δ: 8.25 (1H, s), 8.01–8.02 (1H, m), 7.78 (1H, d, J = 15.6 Hz), 7.70 (2H, d, J = 8.4 Hz), 7.56 (1H, d, J = 6 Hz), 7.45–7.39 (2H, m), 7.30–7.06 (5H, m), 6.86 (1H, d, J = 7.8 Hz), 5.96 (2H, s). ^{13}C NMR (DMSO-d_6, 400 MHz) δ: 182.77, 152.57, 147.78, 147.72, 142.90, 138.93, 133.31, 133.18, 129.68, 128.46, 127.75, 127.10, 126.93, 125.72, 122.65, 122.39, 118.65, 117.58, 108.66, 108.46, 101.38. HRMS (m/z): $[M+H]^+$ *calcd.* for $C_{23}H_{17}N_2O_3S$ 401.0960, *found* 401.0964.

*4.1.18. (E)-3-(3-(benzo[d][1,3]dioxol-5-yl)-1-phenyl-1H-pyrazol-4-yl)-1-(pyridin-2-yl)prop-2-en-1-one (**28**)*

Purified by recrystallization of ethyl acetate and hexane. Yield 83%, mp 188.5–189.2 °C; IR (FTIR/FTNIR-ATR): 1650 cm^{-1} (C=O). ^{1}H NMR (CDCl$_3$, 300 MHz) δ: 8.88 (1H, d, J = 4.4 Hz), 8.42 (1H, s), 8.14–7.72 (6H, m), 7.46–7.28 (4H, m), 7.16 (1H, s), 7.12 (1H, d, J = 9.9 Hz), 6.88 (1H, d, J = 7.8 Hz), 5.97 (2H, s). ^{13}C NMR (DMSO-d_6, 400 MHz) δ: 188.58, 153.57, 152.91, 149.01, 147.84, 147.75, 138.95, 137.71, 134.72, 129.59, 128.71, 127.50, 127.04, 125.69, 122.56, 122.48, 120.20, 118.75, 117.63, 108.68, 108.61, 101.41. HRMS (m/z): $[M+H]^+$ *calcd.* for $C_{24}H_{18}N_3O_3$ 396.1348, *found* 396.1344.

*4.1.19. (E)-3-(3-(benzo[d][1,3]dioxol-5-yl)-1-phenyl-1H-pyrazol-4-yl)-1-(pyridin-3-yl)prop-2-en-1-one (**29**)*

Purified by column flash chromatography (0% → 50% ethyl acetate in hexane). Yield 10%, mp 174–176.5 °C; IR (FTIR/FTNIR-ATR): 1660 cm^{-1} (C=O). ^{1}H NMR (CDCl$_3$, 400 MHz) δ: 9.17 (1H, s), 8.78 (1H, dd, J = 1.6 Hz, J = 4.8 Hz), 8.36 (1H, s), 8.25 (1H, dt, J = 2 Hz, J = 8 Hz), 7.93–7.89 (1H, d, J = 15.6 Hz), 7.79 (2H, d, J = 7.6 Hz) 7.52–7.48 (2H, m), 7.46–7.42 (1H, m), 7.38–7.32 (2H, m), 7.19 (1H, s), 7.14 (1H, dd, J = 1.6 Hz, J = 7.6 Hz), 6.94 (1H, d, J = 8 Hz), 5.97 (2H, s). ^{13}C NMR (DMSO-d_6, 400 MHz) δ: 187.82, 153.28, 152.86, 149.40, 147.86, 147.77, 138.88, 135.62, 135.01, 132.90, 129.72, 128.92, 127.20, 125.58, 123.99, 122.46, 120.95, 118.65, 117.53, 108.69, 108.49, 101.41. HRMS (m/z): $[M+H]^+$ *calcd.* for $C_{24}H_{18}N_3O_3$ 396.1348, *found* 396.1330.

*4.1.20. (E)-1-(benzo[d][1,3]dioxol-5-yl)-3-(3-(benzo[d] [1,3]dioxol-5-yl)-1-phenyl-1H-pyrazol-4-yl)prop-2-en-1-one (**30**)*

Purified by recrystallization of ethyl acetate and hexane. Yield 51%, mp 169.5–170 °C; IR (FTIR/FTNIR-ATR): 1644 cm^{-1} (C=O). ^{1}H NMR (CDCl$_3$, 300 MHz) δ: 8.25 (1H, s), 7.78 (1H, d, J = 16.5 Hz), 7.71 (2H, d, J = 7.8 Hz), 7.53 (1H, d, J = 9.9 Hz), 7.45–7.40 (3H, m), 7.30–7.07 (4H, m), 6.84 (1H, qd, J = 1.6 Hz, J = 9.3 Hz), 6.00 (2H, s), 5.96 (2H, s). ^{13}C NMR (DMSO-d_6, 400 MHz) δ: 186.44, 152.56, 151.44, 147.99, 147.76, 147.71, 138.94, 133.55, 132.32, 129.65, 128.53, 127.04, 125.75, 124.54, 122.38, 121.09, 118.58, 117.71, 108.63, 108.46, 108.27, 107.65, 102.08, 101.37. HRMS (m/z): $[M+H]^+$ *calcd.* for $C_{26}H_{19}N_2O_5$ 439.1294, *found* 439.1304.

*4.1.21. (E)-3-(3-(benzo[d][1,3]dioxol-5-yl)-1-phenyl-1H-pyrazol-4-yl)-1-(1H-pyrrol-3-yl)prop-2-en-1-one (**31**)*

Purified by column flash chromatography (0% → 50% ethyl acetate in hexane). Yield 11%, mp 200–203°C; IR (FTIR/FTNIR-ATR): 1638 cm^{-1} (C=O). ^{1}H NMR (CDCl$_3$, 400 MHz) δ: 9.50 (1H, s), 8.30 (1H, s), 7.84 (1H, d, J = 15.6 Hz), 7.78 (2H, d, J = 7.6 Hz), 7.50–7.47 (2H, m), 7.36–7.32 (1H, m), 7.21–7.16 (3H, m), 7.08 (1H, s), 6.95–6.92 (3H, m), 6.34–6.32 (1H, m), 6.03 (2H, s). ^{13}C NMR (DMSO-d_6, 400 MHz) δ: 177.61, 152.27, 147.69, 139.01, 132.99, 130.71, 130.53, 129.66, 128.16, 126.97, 126.24, 125.92, 122.72, 122.38, 118.58, 117.75, 116.63, 110.09, 108.66, 108.50, 101.36. HRMS (m/z): $[M+H]^+$ *calcd.* for $C_{23}H_{18}N_3O_3$ 384.1348, found 384.1347.

*4.1.22. (E)-3-(1-phenyl-3-(pyridin-3-yl)-1H-pyrazol-4-yl)-1-(thiophen-3-yl)prop-2-en-1-one (**32**)*

Purified by column flash chromatography (0% → 50% ethyl acetate in hexane). Yield 82%, mp 172–173°C; IR (FTIR/FTNIR-ATR): 1655 cm^{-1} (C=O). ^{1}H NMR (CDCl$_3$, 400 MHz) δ: 8.99 (1H, d, J = 1.6 Hz), 8.69 (1H, dd, J = 1.6 Hz, J = 5.2 Hz), 8.37 (1H, s), 8.07 (1H, dd, J = 1.2 Hz, J = 2.8 Hz), 8.01 (1H, dt, J = 2 Hz, J = 8 Hz), 7.84–7.79 (3H, m), 7.61 (1H, dd, J = 1.2 Hz, J = 4.8 Hz), 7.54–7.50 (2H, m), 7.45–7.35 (3H, m), 7.23 (1H, d, J = 16.4 Hz). ^{13}C NMR (DMSO-d_6, 400 MHz) δ: 182.69, 149.93, 149.68, 148.73, 142.81, 138.84, 135.77, 133.47, 132.40, 129.74, 128.88, 127.91, 127.82, 127.39, 126.92, 123.94, 123.30, 118.81, 118.14. HRMS (m/z): $[M+H]^+$ *calcd.* for $C_{21}H_{16}N_3OS$ 358.1014, *found* 358.1004.

*4.1.23. (E)-3-(1-phenyl-3-(pyridin-3-yl)-1H-pyrazol-4-yl)-1-(pyridin-2-yl)prop-2-en-1-one (**33**)*

Purified by column flash chromatography (0% → 40% ethyl acetate in hexane) then by recrystallization of ethyl acetate and hexane. Yield 11%, mp 187.5–188 °C; IR (FTIR/FTNIR-ATR): 1664 cm^{-1} (C=O). ^1H NMR ($CDCl_3$, 400 MHz) δ: 9.00 (1H, d, *J* = 1.2 Hz), 8.72 (1H, d, *J* = 4 Hz), 8.69 (1H, dd, *J* = 1.6 Hz, *J* = 5.2 Hz), 8.52 (1H, s), 8.18 (1H, d, *J* = 8 Hz), 8.14 (1H, d, *J* = 16 Hz), 8.03 (1H, d, *J* = 7.6 Hz), 7.98–7.94 (1H, m), 7.90–7.86 (1H, m), 7.83–7.77 (1H, m), 7.54–7.34 (6H, m). ^{13}C NMR (DMSO-d_6, 400 MHz) δ: 188.50, 153.43, 150.24, 149.71, 149.01, 148.84, 138.83, 137.73, 135.92, 133.89, 129.63, 129.15, 127.90, 127.55, 127.31, 123.93, 122.49, 120.80, 118.87, 118.14. HRMS (*m*/*z*): $[M+H]^+$ *calcd.* for $C_{22}H_{17}N_4O$ 353.1402, *found* 353.1398.

*4.1.24. (E)-1-(benzo[d][1,3]dioxol-5-yl)-3-(1-phenyl-3-(pyridin-3-yl)-1H-pyrazol-4-yl)prop-2-en-1-one (**34**)*

Purified by column flash chromatography (0% → 50% ethyl acetate in hexane). Yield 82%, mp 204–204.6°C; IR (FTIR/FTNIR-ATR): 1663 cm^{-1} (C=O). ^1H NMR ($CDCl_3$, 400 MHz) δ: 8.99 (1H, d, *J* = 1.6 Hz), 8.69 (1H, dd, *J* = 1.6 Hz, *J* = 5 Hz), 8.37 (1H, s), 8.01 (1H, dt, *J* = 2 Hz, *J* = 5 Hz), 7.83–7.79 (3H, m), 7.56 (1H, dd, *J* = 2 Hz, *J* = 8.4 Hz), 7.54–7.48 (3H, m), 7.44–7.35 (2H, m), 7.31 (1H, s), 6.87 (1H, d, *J* = 8.4 Hz), 6.06 (2H, s). ^{13}C NMR (DMSO-d_6, 400 MHz) δ: 186.40, 151.53, 149.93, 149.65, 148.74, 148.02, 138.85, 135.78, 132.76, 132.22, 129.73, 128.93, 127.94, 127.36, 124.64, 123.93, 121.78, 118.76, 118.27, 108.16, 107.67, 102.12. HRMS (*m*/*z*): $[M+H]^+$ *calcd.* for $C_{24}H_{18}N_3O_3$ 396.1348, found 396.1344.

*4.1.25. (E)-3-(1-phenyl-3-(pyridin-3-yl)-1H-pyrazol-4-yl)-1-(1H-pyrrol-3-yl)prop-2-en-1-one (**35**)*

Purified by column flash chromatography (0% → 2% methanol in ethyl acetate). Yield 15%, mp 211.8–212.5 °C; IR (FTIR/FTNIR-ATR): 1644 cm^{-1} (C=O), ^1H NMR ($CDCl_3$, 400 MHz) δ: 9.55 (1H, s), 9.00 (1H, s), 8.69 (1H, d, *J* = 4.8 Hz), 8.36 (1H, s), 8.01 (1H, dd, *J* = 2 Hz, *J* = 8 Hz), 7.83–7.79 (3H, m), 7.53–7.49 (2H, m), 7.44–7.35 (2H, m), 7.18 (1H, d, *J* = 15.6 Hz), 7.09–7.08(1H, m), 6.95–6.93 (1H, m), 6.34–6.32 (1H, m). ^{13}C NMR (DMSO-d_6, 400 MHz) δ: 177.48, 149.61, 148.76, 138.92, 135.81, 132.95, 129.79, 129.73, 128.60, 128.13, 127.28, 126.40, 123.96, 123.37, 118.75, 118.31, 116.78, 110.07. HRMS (*m*/*z*): $[M+H]^+$ *calcd.* for $C_{21}H_{17}N_4O$ 341.1402, *found* 341.1389.

*4.1.26. (E)-1-(1H-indol-3-yl)-3-(1-phenyl-3-(pyridin-3-yl)-1H-pyrazol-4-yl)prop-2-en-1-one (**36**)*

Purified by column flash chromatography (0% → 30% ethyl acetate in hexane). Yield 17%, mp 267.5–268 °C; IR (FTIR/FTNIR-ATR): 1646 cm^{-1} (C=O). ^1H NMR (DMSO-d_6, 400 MHz) δ: 12.14 (1H, s), 9.33 (1H, s), 8.90 (1H, d, *J* = 1.6 Hz), 8.71 (1H, dd, *J* = 1.6, *J* = 4.8 Hz), 8.51 (1H, s), 8.30 (1H, d, *J* = 6.8 Hz), 8.11 (1H, dt, *J* = 2, *J* = 8 Hz), 7.96 (2H, d, *J* = 7.6 Hz), 7.78 (1H, d, *J* = 15.2 Hz), 7.63–7.41 (6H, m), 7.27–7.20 (2H, m).^{13}C NMR (DMSO-d_6, 400 MHz) δ: 183.26, 149.55, 149.52, 148.70, 138.98, 136.87, 135.70, 134.02, 129.75, 128.62, 128.19, 128.15, 127.26, 125.76, 125.08, 123.94, 123.13, 121.85, 121.66, 118.75, 118.56, 117.64, 112.24. HRMS (*m*/*z*): $[M+H]^+$ *calcd.* for $C_{25}H_{19}N_4O$ 391.1559, *found* 391.1566.

*4.1.27. (E)-1-phenyl-3-(1-phenyl-3-(pyridin-3-yl)-1H-pyrazol-4-yl)prop-2-en-1-on [47] (**37**)*

Purified by flash column chromatography (0% → 25% EtOAc in Hexane). Yield 30%, mp 161–161.8°C; IR (FTIR/FTNIR-ATR): 1664 cm^{-1} (C=O), ^1H NMR ($CDCl_3$, 400 MHz) δ: 8.99 (1H, d, *J* = 1.2 Hz), 8.69 (1H, dd, *J* = 1.6 Hz, *J* = 5.2 Hz), 8.39 (1H, s), 8.02 (1H, dt, *J* = 2 Hz, *J* = 8 Hz), 7.97–7.95 (2H, m), 7.83 (1H, d, *J* = 15.6 Hz), 7.79 (2H, m), 7.59–7.38 (7H, m), 7.37 (1H, d, *J* = 15.6 Hz). ^{13}C NMR (DMSO-d_6, 400 MHz) δ: 188.68, 150.05, 149.70, 148.76, 138.84, 137.57, 135.82, 133.52, 133.14, 129.74, 129.11, 128.80, 128.22, 127.90, 127.39, 123.95, 121.90, 118.79, 118.17. Anal. *calcd* for $C_{23}H_{17}N_3O$ C, 78.61; H, 4.88; N, 11.96. *found:* C, 78.77; H, 4.85; N, 11.58. HRMS (*m*/*z*): $[M+H]^+$ *calcd.* for $C_{23}H_{18}N_3O$ 352.1450; *found* 352.1443.

*4.1.28. (E)-3-(1-phenyl-3-(pyridin-3-yl)-1H-pyrazol-4-yl)-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-on (CAS No: 1287408-93-8) (**38**)*

Purified by flash column chromatography (0% → 25% EtOAc in Hexane). Yield 21%, mp 115–116°C; IR (FTIR/FTNIR-ATR): 1646 cm^{-1} (C=O), ^1H NMR ($CDCl_3$) δ: 8.99 (1H, d, *J* = 1.2 Hz), 8.69 (1H, dd, *J* = 1.6 Hz, *J* = 5.2 Hz), 8.36 (1H, s), 8.02 (1H, dt, *J* = 2 Hz, *J* = 8 Hz), 7.84 (1H, d, *J* = 15.6 Hz), 7.54–7.34 (6H, m), 7.29 (1H, d, *J* = 15.6 Hz), 7.19 (2H, s), 3.92 (6H, s), 3.86 (3H, s). ^{13}C NMR (DMSO-d_6, 400 MHz) δ: 187.51, 152.91, 149.99, 149.72, 148.81, 142.02, 138.84, 135.88, 133.36, 132.95, 129.75, 129.24, 128.01, 127.44, 123.96, 121.82, 118.94, 118.22, 118.14, 106.03, 60.21, 56.23. Anal. *calcd* for $C_{26}H_{23}N_3O_4$ C, 70.73; H, 5.25; N, 9.52; *found:* C, 70.95; H, 5.53; N, 9.12. HRMS (*m*/*z*): $[M+H]^+$ *calcd.* for $C_{26}H_{24}N_3O_4$ 442.1767; *found* 442.1777.

*4.1.29. (E)-3-(1-phenyl-3-(pyridin-3-yl)-1H-pyrazol-4-yl)-1-(2,4,6-trimethoxyphenyl)prop-2-en-1-on (**39**)*

Purified by flash column chromatography (0% → 20% EtOAc in Hexane). Yield 22%, mp 206–207°C; IR (FTIR/FTNIR-ATR): 1625 cm^{-1} (C=O). ^1H NMR (DMSO-d_6, 400 MHz) δ: 9.26 (1H, s), 8.73 (1H, d, *J* = 2 Hz), 8.67 (1H, dd, *J* = 1,6 Hz, *J* = 4.8 Hz), 7.96–7.93 (3H, m), 7.59–7.54 (3H, m), 7.42–7.38 (1H, m), 7.16 (1H, d, *J* = 16 Hz), 6.86 (1H, d, *J* = 16 Hz), 6.29 (2H, s), 3.81 (3H, s), 3.75 (6H, s). ^{13}C NMR (DMSO-d_6, 400 MHz) δ: 193.08, 161.90, 157.89, 149.63, 149.36, 148.53, 138.83, 135.46, 133.98, 129.66, 129.18, 128.92, 127.84, 127.29, 123.85, 118.78, 117.46, 110.57, 90.86, 55.76, 55.44. Anal. *calcd* for $C_{26}H_{23}N_3O_4$ C, 70.73; H, 5.25; N, 9.52; *found*: C, 70.92; H, 5.38; N, 9.48. HRMS (*m*/*z*): $[M+H]^+$ *calcd.* for $C_{26}H_{24}N_3O_4$ 442.1767; *found* 442.1762.

*4.1.30. (E)-3-(1-phenyl-3-(pyridin-3-yl)-1H-pyrazol-4-yl)-1-(2,3,4-trimethoxyphenyl)prop-2-en-1-on (**40**)*

Purified by flash column chromatography (0% → 25% EtOAc in Hexane). Yield 28%, mp 91–92°C; IR (FTIR/FTNIR-ATR): 1648 cm^{-1} (C=O). ^1H NMR ($CDCl_3$, 400 MHz) δ: 8.98 (1H, d, *J* = 1.6 Hz), 8.67 (1H, dd, *J* = 1.6 Hz, *J* = 5.2 Hz), 8.33 (1H, s), 8.02 (1H, dt, *J* = 1.6 Hz, *J* = 8 Hz), 7.79 (2H, m), 7.70 (1H, d, *J* = 15.6 Hz), 7.53–7.37 (5H, m), 7.33 (1H, d, *J* = 15.6 Hz), 6.75 (1H, d, *J* = 8.8 Hz), 3.92 (3H, s), 3.91 (3H, s), 3.87 (3H, s). Anal. *calcd.* for $C_{26}H_{23}N_3O_4$ C, 70.73; H, 5.25; N, 9.52; *found:* C, 70.92; H, 5.17; N, 9.52. HRMS (*m*/*z*): $[M+H]^+$ *calcd.* for $C_{26}H_{24}N_3O_4$ 442.1767; *found* 442.1765.

*4.1.31. (E)-1-(3,4-Dimethoxyphenyl)-3-(1-phenyl-3-(pyridin-3-yl)-1H-pyrazol-4-yl)prop-2-en-1-on (CAS No: 1002609-04-2) (**41**)*

Purified by flash column chromatography (0% → 25% EtOAc in Hexane). Yield 36%, mp 162–163°C; IR (FTIR/FTNIR-ATR): 1655 cm^{-1} (C=O). ^1H NMR ($CDCl_3$, 300 MHz) δ: 8.97 (1H, s), 8.65 (1H, d, *J* = 3.6 Hz), 8.34 (1H, s), 8.03 (1H, d, *J* = 7.8 Hz), 7.79–7.77 (2H, m), 7.76 (1H, d, *J* = 15.9 Hz), 7.55–7.33 (7H, m), 6.85 (1H, d, *J* = 7.8 Hz), 3.91 (6H, s). ^{13}C NMR (DMSO-d_6, 400 MHz) δ: 186.85, 153.22, 149.88, 149.66, 148.82, 148.77, 138.87, 135.81, 132.46, 130.46, 129.73, 128.98, 128.02, 127.34, 123.93, 122.94, 121.85, 118.81, 118.26, 110.87, 110.64, 55.78, 55.57. Anal. *calcd.* for $C_{25}H_{21}N_3O_3$ C, 72.98; H, 5.14; N, 10.21; *found*: C, 72.49; H, 5.05; N, 10.03. HRMS (*m*/*z*): $[M+H]^+$ *calcd.* for $C_{25}H_{22}N_3O_3$ 412.1661; *found* 412.1650.

*4.1.32. (E)-1-(2,5-Dimethoxyphenyl)-3-(1-phenyl-3-(pyridin-3-yl)-1H-pyrazol-4-yl)prop-2-en-1-on (**42**)*

Purified by recrystallization from hexane-ethyl acetate mixture.

Yield 57%, mp 129–130°C; IR (FTIR/FTNIR-ATR): 1656 cm^{-1} (C=O). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 9.29 (1H, s), 8.82 (1H, d, *J* = 1.6 Hz), 8.69 (1H, d, *J* = 4.8 Hz), 8.04 (1H, d, *J* = 8.4 Hz), 7.96 (2H, d, *J* = 8 Hz), 7.59–7.39 (5H, m), 7.28 (1H, d, *J* = 16 Hz), 7.13–7.07 (2H, m), 7.02 (1H, s), 3.80 (3H, s), 3.74 (3H, s). ^{13}C NMR (DMSO-d_6, 400 MHz) δ: 191.72, 152.99, 151.50, 149.74, 149.68, 148.72, 138.81, 135.77, 133.13, 129.66, 129.21, 129.18, 127.98, 127.32, 126.64, 123.86, 118.85, 118.19, 117.80, 113.94, 113.59, 56.25, 55.53. Anal. *calcd* for $C_{25}H_{21}N_3O_3$ C, 72.98; H, 5.14; N, 10.21; *found*: C, 73.31; H, 5.57; N, 10.40. HRMS (*m*/*z*): $[M+H]^+$ *calcd.* for $C_{25}H_{22}N_3O_3$ 412.1661; *found* 412.1649.

*4.1.33. (E)-1-(2,4-Dimethoxyphenyl)-3-(1-phenyl-3-(pyridin-3-yl)-1H-pyrazol-4-yl)prop-2-en-1-on (CAS No: 1002461-21-3) (**43**)*

Purified by recrystallization from acetone-water mixture. Yield 34%, mp 130–131°C; IR (FTIR/FTNIR-ATR): 1646 cm^{-1} (C=O). ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 9.01 (1H, s), 8.63 (1H, d, *J* = 3.4 Hz), 8.24 (1H, s), 8.20 (1H, d, *J* = 7.2 Hz), 7.73–7.68 (2H, m), 7.62 (1H, d, *J* = 15.6 Hz), 7.55–7.53 (2H, m), 7.46 (1H, t, *J* = 7.8 Hz), 7.34–7.32 (1H, m), 7.31 (1H, d, *J* = 15.6 Hz), 6.52 (1H, d, *J* = 8.7 Hz), 6.43 (1H, d, *J* = 1.8 Hz), 3.81 (6H, s). ^{13}C NMR (DMSO-d_6, 400 MHz) δ: 189.16, 163.83, 159.99, 149.61, 148.78, 138.87, 135.84, 131.96, 131.27, 129.64, 129.00, 128.16, 127.24, 126.88, 123.88, 121.28, 118.87, 118.10, 105.89, 98.50, 55.88, 55.57. Anal. *calcd.* for $C_{25}H_{21}N_3O_3$ C, 72.98; H, 5.14; N, 10.21; *found*: C, 73.33; H, 5.38; N, 10.21. HRMS (*m*/*z*): $[M+H]^+$ *calcd.* for $C_{25}H_{22}N_3O_3$ 412.1661; *found* 412.1643.

*4.1.34. (E)-3-(1-phenyl-3-(pyridin-4-yl)-1H-pyrazol-4-yl)-1-(thiophen-3-yl)prop-2-en-1-one (**44**)*

Purified by column flash chromatography (0% → 50% ethyl acetate in hexane) then by recrystallization of ethyl acetate and hexane. Yield 54%, mp 219–220°C; IR (FTIR/FTNIR-ATR): 1655 cm^{-1} (C=O). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 8.74 (2H, d, *J* = 6 Hz), 8.36 (1H, s), 8.08 (1H, d, *J* = 2.8 Hz), 7.90 (1H, d, *J* = 15.6 Hz), 7.79 (2H, d, *J* = 7.6 Hz), 7.67–7.62 (3H, m), 7.52 (2H, t, *J* = 8 Hz), 7.41–7.36 (2H, m), 7.26 (1H, d, *J* = 15.6 Hz). ^{13}C NMR (DMSO-d_6, 400 MHz) δ: 182.68, 150.26, 149.94, 142.76, 139.28, 138.76, 133.55, 132.13, 129.75, 129.15, 127.84, 127.53, 126.91, 123.74, 122.61, 118.88, 118.30. HRMS (*m*/*z*): $[M+H]^+$ *calcd.* for $C_{21}H_{16}N_3OS$ 358.1014, *found* 358.1008.

*4.1.35. (E)-1-(benzo[d][1,3]dioxol-5-yl)-3-(1-phenyl-3-(pyridin-4-yl)-1H-pyrazol-4-yl)prop-2-en-1-one (**45**)*

Purified by column flash chromatography (0% → 50% ethyl acetate in hexane). Yield 60%, mp 148.2–148.8°C; IR (FTIR/FTNIR-ATR): 1656 cm^{-1} (C=O). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 8.73 (2H, d, *J* = 5.6 Hz), 8.36 (1H, s), 7.87 (1H, d, *J* = 15.2 Hz), 7.80 (2H, d, *J* = 7.6 Hz), 7.66 (2H, d, *J* = 6 Hz), 7.59–7.50 (4H, m), 7.38–7.34 (2H, m), 6.88 (1H, d, *J* = 8.4 Hz), 6.06 (2H, s). ^{13}C NMR (DMSO-d_6, 400 MHz) δ: 186.37, 151.47, 150.17, 149.85, 147.95, 139.27, 138.73, 132.41, 132.14, 129.63, 129.13, 127.40, 124.57, 122.52, 122.25, 118.79, 118.36, 108.07, 107.60, 102.03. HRMS (*m*/*z*): $[M+H]^+$ *calcd.* for $C_{24}H_{18}N_3O_3$ 396.1348, *found* 396.1338.

*4.1.36. (E)-3-(1-phenyl-3-(pyridin-4-yl)-1H-pyrazol-4-yl)-1-(1H-pyrrol-3-yl)prop-2-en-1-one (**46**)*

Purified by column flash chromatography (0% → 6% methanol in dichloromethane). Yield 28%, mp 258–258.6°C; IR (FTIR/FTNIR-ATR): 1645 cm^{-1} (C=O). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 9.62 (1H, bs), 8.74 (2H, d, *J* = 4.2 Hz), 8.35 (1H, s), 7.87 (1H, d, *J* = 15.6 Hz), 7.80 (2H, d, *J* = 7.6 Hz), 7.67 (2H, d, *J* = 4.4 Hz), 7.53 (2H, t, *J* = 8 Hz), 7.40–7.36 (1H, m), 7.20 (1H, d, *J* = 15.6 Hz), 7.10 (1H, s), 6.96 (1H, m), 6.35–6.33 (1H, m). ^{13}C NMR (DMSO-d_6, 400 MHz) δ: 177.44, 150.25, 149.65, 139.51, 138.83, 132.93, 129.72, 129.54, 128.85, 127.41, 126.45, 123.83, 122.64, 118.83, 118.49, 116.89, 110.07. HRMS (*m*/*z*): $[M+H]^+$ *calcd.* for $C_{21}H_{17}N_4O$ 341.1402, *found* 341.1400.

*4.1.37. (E)-1-phenyl-3-(1-phenyl-3-(pyridin-4-yl)-1H-pyrazol-4-yl)prop-2-en-1-one (CAS No:1390831-45-4) (**47**)*

Purified by flash column chromatography (0% → 25% EtOAc in Hexane). Yield 36%, mp 215–216 °C; IR (FTIR/FTNIR-ATR): 1656 cm^{-1} (C=O). ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 8.70 (2H, d, *J* = 4.5 Hz), 8.37 (1H, s), 7.96 (2H, d, *J* = 7.5 Hz), 7.86 (2H, d, *J* = 15.6 Hz), 7.78–7.69 (2H, m), 7.58–7.36 (3H, m), 7.58–7.36 (4H, m), 7.22–7.20 (1H, m). ^{13}C NMR (DMSO-d_6, 400 MHz) δ: 188.66, 150.27, 150.07, 139.27, 138.76, 137.52, 133.24, 133.14, 129.74,129.35,128.20, 128.24, 127.52, 122.65, 122.32, 118.85, 118.32. Anal. *calcd.* for $C_{23}H_{17}N_3O$ C, 78.61; H, 4.88; N, 11.96. *found* C, 78.25; H, 4.85; N, 11.82. HRMS (*m*/*z*): $[M+H]^+$ *calcd.* for $C_{23}H_{18}N_3O$ 352.1450; *found* 352.1447.

*4.1.38. (E)-3-(1-phenyl-3-(pyridin-4-yl)-1H-pyrazol-4-yl)-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-on (CAS No: 1390911-60-0) (**48**)*

Purified by flash column chromatography (0% → 25% EtOAc in Hexane). Yield 18%, mp 161–162°C; IR (FTIR/FTNIR-ATR): 1656 cm^{-1} (C=O). ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 8.67 (2H, bs), 8.34 (1H, s), 7.84 (1H, d, *J* = 15.6 Hz), 7.75–7.70 (4H, m), 7.49–7.36 (3H, m), 7.33 (1H, d, *J* = 15.6 Hz), 7.16 (2H, s), 3.87 (9H, s). ^{13}C NMR (DMSO-d_6, 400 MHz) δ: 187.40, 152.91, 150.27, 150.05, 142.08, 139.40, 138.76, 133.03, 132.90, 129.76, 129.44, 127.56, 122.72, 122.22, 119.02, 118.30, 106.08, 60.21, 59.72, 56.23. Anal. *calcd.* for $C_{26}H_{23}N_3O_4$ C, 70.73; H, 5.25; N, 9.52. found C, 70.34; H, 5.63; N, 9.65. HRMS (*m*/*z*): $[M+H]^+$ *calcd.* for $C_{26}H_{24}N_3O_4$ 442.1767; found 442.1757.

*4.1.39. (E)-3-(1-phenyl-3-(pyridin-4-yl)-1H-pyrazol-4-yl)-1-(2,4,6-trimethoxyphenyl)prop-2-en-1-on (**49**)*

Purified by recrystallization from ethanol-water mixture. Yield 68%, mp 192–193°C; IR (FTIR/FTNIR-ATR): 1636 cm^{-1} (C=O). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 9.26 (1H, s), 8.71 (2H, d, *J* = 6 Hz), 7.94 (2H, d, *J* = 8 Hz), 7.57 (2H, t, *J* = 8 Hz), 7.52 (2H, d, *J* = 5.6 Hz), 7.43–7.40 (1H, m), 7.21 (1H, d, *J* = 16 Hz), 6.89 (1H, d, *J* = 16 Hz), 6.33 (2H, s), 3.83 (3H, s), 3.75 (6H, s). ^{13}C NMR (DMSO-d_6, 400 MHz) δ: 193.13, 161.97, 157.89, 150.16, 149.48, 139.21, 138.76, 133.82, 129.68, 129.51, 129.11, 127.43, 122.39, 118.87, 117.66, 110.54, 90.89, 55.79, 55.74. Anal. *calcd.* for $C_{26}H_{23}N_3O_4$ C, 70.73; H, 5.25; N, 9.52. *found*: C, 70.42; H, 5.36; N, 9.41. HRMS (*m*/*z*): $[M+H]^+$ *calcd.* for $C_{26}H_{24}N_3O_4$ 442.1767; *found* 442.1758.

*4.1.40. (E)-3-(1-phenyl-3-(pyridin-4-yl)-1H-pyrazol-4-yl)-1-(2,3,4-trimethoxphenyl)prop-2-en-1-on (**50**)*

Purified by flash column chromatography (0% → 25% EtOAc in Hexane). Yield 30%, mp 160–161°C; IR (FTIR/FTNIR-ATR): 1654 cm^{-1} (C=O). ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 8.66 (2H, d, *J* = 6 Hz), 8.27 (1H, s), 7.74–7.68 (5H, m), 7.48–7.35 (4H, m), 7.33 (1H, d, *J* = 15.6 Hz), 6.70 (1H, d, *J* = 8.7 Hz), 3.87 (3H, s), 3.86 (3H, s), 3.83 (3H, s). ^{13}C NMR (DMSO-d_6, 400 MHz) δ: 189.76, 156.56, 152.75, 150.22, 149.87, 141.65, 139.40, 138.77, 132.28, 129.67, 129.15, 127.45, 126.71, 126.11, 125.07, 122.69, 118.95, 118.10, 107.88, 61.83, 60.53, 56.08. Anal. *calcd.* for $C_{26}H_{23}N_3O_4$ C, 70.73; H, 5.25; N, 9.52. *found*: C, 70.47; H, 5.12; N, 9.37. HRMS (*m*/*z*): $[M+H]^+$ *calcd.* for $C_{26}H_{24}N_3O_4$ 442.1767; *found* 442.1781.

*4.1.41. (E)-1-(3,4-Dimethoxyphenyl)-3-(1-phenyl-3-(pyridin-4-yl)-1H-pyrazol-4-yl)prop-2-en-1-on (CAS No: 1147371-58-1) (**51**)*

Purified by recrystallization from acetone-water mixture. Yield 44%, mp 190–191°C; IR (FTIR/FTNIR-ATR): 1653 cm^{-1} (C=O). ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 8.68 (2H, bs), 8.34 (1H, s), 7.83 (1H, d, *J* = 15.6 Hz), 7.76–7.69 (4H, m), 7.55–7.20 (5H, m), 6.87 (1H, d, *J* = 9 Hz), 3.92 (3H, s), 3.91 (3H, s). ^{13}C NMR (DMSO-d_6, 400 MHz) δ: 186.83, 153.26, 150.26, 149.93, 148.84, 139.40, 138.80, 132.18, 130.43, 129.74, 129.24, 127.49, 123.02, 122.67, 122.30, 118.90, 118.43, 110.87, 110.65, 55.80, 55.58. Anal. calcd for $C_{25}H_{21}N_3O_3$ C, 72.98; H, 5.14; N, 10.21. *found:* C, 72.58; H, 5.12; N, 10.05. HRMS (*m/z*): $[M+H]^+$ *calcd.* for $C_{25}H_{22}N_3O_3$ 412.1661; *found* 412.1651.

4.1.42. (E)-1-(2,5-Dimethoxyphenyl)-3-(1-phenyl-3-(pyridin-4-yl)-1H-pyrazol-4-yl)prop-2-en-1-on (**52**)

Purified by recrystallization from acetone-water mixture. Yield 32%, mp 136–137°C; IR (FTIR/FTNIR-ATR): 1656 cm^{-1} (C=O). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 9.30 (1H, s), 8.73 (2H, d, J = 5.2 Hz), 7.96 (2H, d, J = 8 Hz), 7.63 (2H, d, J = 6 Hz), 7.60–7.56 (2H, m), 7.50 (1H, d, J = 16 Hz), 7.44–7.40 (1H, m), 7.32 (1H, d, J = 16 Hz), 7.15–7.09 (2H, m), 7.04 (1H, d, J = 2.8 Hz), 3.81 (3H, s), 3.75 (3H, s). ^{13}C NMR (DMSO-d_6, 400 MHz) δ: 191.69, 153.01, 151.56, 150.14, 149.86, 139.43, 138.75, 132.83, 129.68, 129.33, 129.21, 127.47, 127.07, 122.68, 118.95, 118.19, 117.98, 114.01, 113.65, 56.30, 55.55. Anal. *calcd.* for $C_{25}H_{21}N_3O_3$ C, 72.98; H, 5.14; N, 10.21. *found:* C, 72.87; H, 5.12; N, 9.88. HRMS (*m/z*): $[M+H]^+$ *calcd.* for $C_{25}H_{22}N_3O_3$ 412.1661; *found* 412.1641.

4.1.43. (E)-1-(2,4-Dimethoxyphenyl)-3-(1-phenyl-3-(pyridin-4-yl)-1H-pyrazol-4-yl)prop-2-en-1-on (CAS No: 1147368-37-3) (**53**)

Purified by recrystallization from acetone-water mixture. Yield 36%, mp 182–183°C; IR (FTIR/FTNIR-ATR): 1644 cm^{-1} (C=O). ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 8.66 (2H, d, J = 4.5 Hz), 8.22 (1H, s), 7.73–7.67 (6H, m), 7.48–7.43 (2H, m), 7.37–7.32 (2H, m), 6.50 (1H, dd, J = 2.1 Hz, J = 8.7 Hz), 6.42 (1H, d, J = 2.4 Hz), 3.80 (3H, s), 3.79 (3H, s). Anal. *calcd* for $C_{25}H_{21}N_3O_3$ C, 72.98; H, 5.14; N, 10.21. *found:* C, 72.67; H, 5.12; N, 9.98. HRMS (*m/z*): $[M+H]^+$ *calcd.* for $C_{25}H_{22}N_3O_3$ 412.1661; *found* 412.1642.

4.2. Biological evaluation

4.2.1. Cells culture

Hepatocellular carcinoma cell lines (Huh7, HepG2 and Mahlavu), human breast carcinoma cells (MCF7) and human colon carcinma cells (HCT116) were grown in Dulbecco's Modified Eagles Medium (DMEM) supplemented with 10% fetal bovine serum (Invitrogen GIBCO), 1% non-essential amino acids (GIBCO, Invitrogen) and SNU-475 were grown in RPMI-1640 media (Invitrogen GIBCO) supplemented with 10% FBS, 2 mM L-glutamine. MCF12A, human breast epithelial cells, were grown in DMEM/HAMS F12 media (Invitrogen GIBCO) supplemented with 10% FBS, 1% NEAA, 100 ng/ml EGF, 500 ng/ml hydrocortisone, and 10 μg/ml insulin. MRC-5, human fetal lung fibroblast cells, were maintained in Minimum Essential Medium (MEM) (Invitrogen GIBCO), with 10% fetal bovine serum (FBS), 2 mM L-glutamine. All media contained 100 units/ml penicillin and streptomycin and cells were maintained at 37 °C in a humidified incubator under 5% CO_2.

4.2.2. NCI-60 sulforhodamine B assay

Cells were plated in 96-well plates (2000–3000 cell/well in 150 μl/well) and grown for 24 h at 37 °C. Treatment of cells with the compounds (dissolved in DMSO) was done in a concentration range of 40 μM-0.3 μM (in triplicates). After 72 h of incubation, cells were fixed with 10% (v/v) trichloroacetic acid (MERCK) for an hour and stained with sulforhodamine B (SRB) solution (50 μl of a 0.4% (m/v) of SRB in 1% acetic acid solution). For the removal of unbound SRB, cells were washed with 1% acetic acid and left for air-drying. Protein bound SRB was solubilized in 10 mM Tris-base prior to absorbance measurement (515 nm) using 96-well plate reader. Cells treated with DMSO alone were used as controls for percent inhibition and IC_{50} calculations.

4.2.3. Real-time cell growth surveillance by electronic sensing (RT-CES)

Real-time cell growth was monitored using the xCELLigence System (Roche Applied Sciences). Human cancer cell lines (1000–3000 cells/well) were seeded into 96-well E-plates. The proliferation of cells were monitored every 30 min for 24 h after seeding, (when cells were in log growth phase) treatments with the compounds were done. The cell index (CI) values were recorded every 10 min for the first 24 h and then every 30 min for the remaining 48 h. The cell growth ratios were calculated by CI_{drug}/CI_{DMSO}.

4.2.4. Detection of apoptosis

Cells were seeded onto coverslips in 6-well plates. After 24 h in culture, cells were treated with the compounds with their IC_{50} concentrations. After 48 h, apoptotic cells were identified by Annexin-V-Fluos (Roche) and DAPI (Santa Cruz) staining according to the manufacturer's recommendations (Roche). Cells were then analyzed using the fluorescence microscope (Nikon Eclipse 50i).

4.2.5. Flow cytometry for cell cycle analysis

Cells were seeded onto 100 mm culture dishes. After 24 h, cells were treated with the compounds with their IC_{100} concentrations. Cells were fixed with ice-cold 70% ethanol for 3 h at −20 °C after 24 h, 48 h and 72 h of incubation. Cell cycle analysis was carried out by PI (Propidium Iodide) staining using MUSE Cell Analyzer according to the manufacturer's recommendations (Millipore).

4.2.6. Western blot analysis

Human cancer cell lines were treated with the compounds (IC_{100} concentrations) or with DMSO control for 24 h. Novex® NuPAGE® Bis-Tris Electrophoresis system was used according to the manufacturer's protocol for all the western blotting analysis. For gel electrophoresis (NuPAGE), 20–50 μg of protein was used per well. Then the proteins were transferred to nitrocellulose membrane via XCell IITM Blot Module. Cyclin-B1 (#554177, BD), Cdc2/Cdk1 (#PC25, Calbiochem), phospho-Cyclin-B1 (Ser147) (bs-3122R, Bioss), p21/WAF1/Cip1 (#05–345, Millipore), p53 (#05–224, Millipore), phospho-p53 (Ser15) (#9286S, Cell Signaling), Rb (#sc102, Santa Cruz), and phospho-Rb (Ser807/811) (#9308S Cell Signaling) antibodies were used in 1:200 to 1:500 5% BSA-TBS-T. β-actin (#A5441, Sigma) antibody was used for equal loading.

Author contributions

S.N.B designed the compounds and their synthesis. M.M.H and F.E performed the chemical synthesis and characterized the compounds. S.N.B and M.M.H contributed to the writing of the manuscript. R.C.A and D.C.K designed the biological experiments and contributed to the writing of the manuscript. D.C.K performed the experiments for biological evaluation of the compounds on HCC cell lines. All authors have given approval to the final version of the manuscript.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.ejmech.2017.02.

002. These data include MOL files and InChiKeys of the most important compounds described in this article.

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