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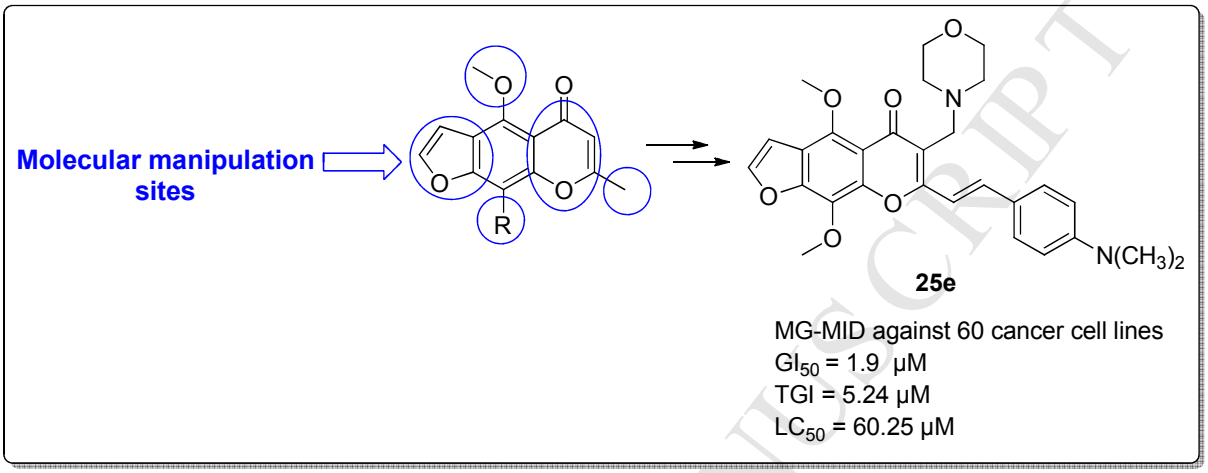
Graphical abstract



Highlights

- Novel semisynthetic flavonoid derivatives were prepared as antiproliferative agents
- **9a**, **9b**, **25c** and **25e** showed the lowest results for GI_{50}, TGI and LC_{50}
- Kinase inhibitory ability against CDK2/cyclin E1, CDK4/cyclin D1 and GSK-3β
- Mice-bearing EAC solid tumor was used to test the *in vivo* antitumor activity of **25e**
- **25e** caused decrease in tumor size, increase in GSK-3β and decrease in cyclin D1

Design, Synthesis and Structure-Activity Relationship of Novel Semi-synthetic Flavonoids as Antiproliferative Agents

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ABSTRACT: Various flavonoid scaffold based derivatives *viz* furochalcones (**3a-e**, **6a-d** and **9a-d**), furoflavones (**10a-d**, **11a-d**, **12a-d**, **18a**&**b**), flavones (**21a-d**), furoaurones (**13a**,**b**, **14a-d** and **15a-d**) and 7-styrylfurochromones (**22a-d** and **25a-e**) were designed and synthesized. The novel compounds were evaluated for their antiproliferative activity against a panel of 60 cancer cell lines comprising 9 types of tumors. Ten compounds belonging to the major subgroups of flavonoids *viz* furochalcones (**3a**, **3d**, **6b**, **9a** and **9b**), furoflavones (**12a** and **12c**), furoaurones (**15d**), styrylfurochromones (**25b** and **25e**) showed very promising activity. These active compounds were also evaluated *in vitro* as kinase inhibitors against CDK2/cyclin E1, CDK4/cyclin D1 and GSK-3β and the best inhibition was displayed against GSK-3β with the allylfurochalcone derivative **9b** exhibiting 80% decrease in GSK-3β catalytic activity. On the other hand, the styrylfurochromone **25e** interestingly showed a 13% enhancement of GSK-3β catalytic power and a 12% reduction in CDK4/cyclin D1 activity. Finally, the *in vivo* anti-tumor activity of **25e** was evaluated against breast cancer induced in mice. The results showed a profound anti-tumor effect of **25e** that accompanies a significant increase and decrease in the levels of GSK-3β and cyclin D1, respectively.

1. Introduction

Flavonoids represent an important class of naturally occurring compounds present in dietary plants and herbal remedies [1]. The role of dietary flavonoids in cancer prevention is well established and widely discussed. Many mechanisms of action have been identified including cell cycle arrest, induction of apoptosis, inhibition of angiogenesis, antioxidant effect and inhibition of some functional enzymes like cyclin-dependent kinases, tyrosine kinases, aromatases, topoisomerases and glycogen phosphorylases [2-10]. Chemically, flavonoids are classified into several subclasses among them are flavones (2-phenyl-*4H*-benzopyran-4-ones), aurones (structural isomers of flavones; 2-benzylidene benzofuran-3(*2H*)-ones), 2-styryl chromones (vinylogues of flavones) and o-hydroxychalcones (the flexible analogs of aurones and flavones). Many natural products belonging to the flavonoid subclasses possess anticancer activity like quercitin (**I**) and apigenin (**II**) (flavones), hamiltrone (**III**) (aurone), hamilcone (**IV**) (chalcone) and hormothamnione (**V**) (styrylchromone) (Figure 1) [11-15]. In addition, flavopiridol (L86–8275) (**VI**) (Figure 1), a semisynthetic flavones currently under clinical development for management of chronic lymphocytic leukemia, exhibited cytotoxic activity against several types of human cell lines associated with potent inhibition of cyclin-dependent kinases (CDKs) CDK2/cyclin E1 and CDK4/cyclin D1 as well as glycogen synthase kinase 3-beta (GSK-3β) [10,16]. While CDKs 2 and 4 are important regulators progression through cell cycle, GSK-3β plays a profound regulatory rule in cell transduction pathways responsible for various cellular functions including differentiation, growth, proliferation, motility and survival. Dysregulation of CDKs, cyclins and GSK-3β expression leads to many pathological transformations leading to neoplasia, tumorigenesis and cancer progression [17,18]. With regards to GSK-3β, available evidences indicate that it may function as a ‘‘tumor suppressor” for certain types of tumors, such as skin and mammary tumors and as a ‘‘tumor promoter” for other types, such as colon and pancreatic cancers [19].

On the other hand, the observed over-expression of cyclin D1 in a variety of tumor types, including mammary carcinogenesis, suggests that tumor promotion is mediated by cyclin D1 dysregulation [20,21]. Furthermore, in mammary cancer, an association has been established between active GSK-3β and cyclin D1 cellular levels. Being a direct target of GSK-3β, at high levels of GSK-3β, cyclin D1 gets rapidly phosphorylated at Thr286 with subsequent promotion of its proteasomal degradation leading to initiation of cellular apoptotic mechanisms and halting tumor progression.

Since the anticancer activity of furoflavonoids has not been extensively studied, this investigation deals with the design, synthesis and evaluation of antiproliferative activity of some semi-synthetic furoflavonoids including furoflavones, furoaurones, furostyrylchromones and furochalcones derived from khellin and visnagin. It also deemed of interest to study the correlation between introducing various substituents on the furoflavonoid scaffold and the cytotoxic activity of these compounds. The design criteria behind the preparation of these new derivatives focused on the introduction of variable functionalities on different sites of the furoflavonoid backbone scaffold and depiction of structure activity relationships through studying the subsequent effect of the adopted molecular manipulations on the cytotoxic activity of this class of compounds. Initially, 8-bromofuroflavonoids were synthesized as certain bromoflavones and bromochalcones were reported to possess cytotoxic activity against various tumor cell lines [22,23]. Also, the 8-allylfuroflavonoid analogs were prepared since the allyl group is considered as a precursor for acetic acid moiety where recent data indicated that flavone acetic acid possesses a broad range of activity against many tumor types [24-26]. On the other hand, alteration of furoflavonoid backbone scaffold between the bioisosteres furoflavones, furoaurones, furostyrylchromones and furochalcones was carried out to study the impact of ring size and/or cyclic versus non-cyclic alterations on the biological behavior of these derivatives. Finally, 6-morpholinomethyl substituted

furoflavonoids were also prepared since previous studies showed that Mannich bases can enhance the cytotoxicity [27,28]. The synthesized compounds were subjected to screening against a panel of 60 cell lines representing 9 cancer types *viz* leukemia, lung, colon, CNS, melanoma, ovarian, renal, prostate and breast. Literature search showed that flavonoid based pharmaceutically active molecules mainly elicit their anticancer activity through inhibition of kinases specially cyclin-dependent kinases and GSK-3β [20,21]. On this basis, the new flavonoids were screened for their potential kinase inhibitory activity against CDK2/cyclin E1, CDK4/cyclin D1 and GSK-3β which gave interesting results. Also, the *in vivo* effect of **25e**, the most active compound in this study, on mammary tumor induced in mice along with evaluation of its role as a modulator of GSK-3β and cyclin D1 was performed. To our knowledge this is the first report of antiproliferative semi-synthetic flavonoids displaying this mechanism of action.

Figure 1

2. Results And Discussion

2.1. Chemistry

Schemes I-VI depict the pathways for the synthesis of the intermediates and target compounds. The key starting furochalcones **3a-e,** which are important intermediates for the synthesis of flavonoids, were obtained from the natural furochromone **1** through hydrolytic alkaline cleavage of the γ-pyrone ring to furnish the acylated hydroxybenzofuran **2**, followed by Claisen-Schmidt condensation with the appropriate aromatic aldehydes [29,30]. Similarly, the bromochalcones **6a-d** were obtained from the semisynthetic bromofurochromone **4** in two consecutive steps involving alkaline hydrolysis with 15% KOH [31] to give the acylated bromohydroxybenzofuran **5** followed by a Claisen-Schmidt condensation with different aromatic aldehydes [32]. On the other hand, the allylfurochalcones **9a-d** were prepared from

2 by *O*-allylation with allyl bromide to give the allyl ether **7** [33] followed by Claisen rearrangement through refluxing with *N*,*N*-diethylaniline to give **8** that upon condensation with aromatic aldehydes produced the target compounds (Scheme 1).

Synthesis of the methoxylated furoflavones **10a-d**, **11a-d** and **12a-d** is illustrated in scheme **II** where the target compounds were obtained from the furochalcones **3a-e**, **6a-d** and **9a-d**, respectively. This was achieved through oxidative cyclization with freshly sublimed SeO_2 in n-butanol. It is noteworthy that in the case of allylchalcones **9a-d**, SeO_2 led to oxidization of the allyl group to an acrylaldehyde moiety along with the oxidative cyclization to furnish derivatives **12a-d** [34]. Spectral analysis of **12a-d** were in accordance with the proposed structures where their I.R. spectra showed a band at around 1700 cm^{-1} corresponding to the aldehydic carbonyl group and their ^{1}HNMR showed a peak around 9.5 ppm attributed to the aldehydic proton. On the other hand, subjecting the chalcones **3d**, **3e**, **6a-d** and **9a-d** to oxidative cyclization using 30% H_2O_2 and 20% NaOH, the furoaurones **13a**, **13b**, **14a-d** and **15a-d** were obtained, respectively, predominantly without any trace of their structural furoflavone isomers. This can be mechanistically attributed, as depicted in Figure 2, to the presence of the 4-methoxy substituent which directs ring closure to formation of the 5-membered ring (aurones) rather than the 6-membered ring (flavones) favoring bond angles that would allow less steric hindrance between the *O*-substituent (OCH_3) and the carbonyl group. However, in absence of the OCH_3 substituent, previous reports showed that hydroxychalcones were oxidized to 3-hydroxyflavones [35]. The structures of the aurones were elucidated through I.R. which showed the presence of bands at the range of 1692-1697cm^{-1} corresponding to the carbonyl group which appears at 1640-1650 cm^{-1} in case of flavones. Aurones also gave a negative $FeCl_3$ test which confirms the failure of formation of 3-hydroxyfuroflavones. Furthermore, ^{1}HNMR of the aurones, unlike the 3-

hydroxyfuroflavone derivatives, showed peaks at around 6.7 ppm attributed to the vinyl proton with absence peaks signifying the presence of the hydroxyl group.

Figure 2

Scheme **III** describes the synthesis of the phenolic furoflavone analogues **18a**&**b**. This was achieved starting by demethoxylation of furoflavones **10a**&**b** using HBr to give the desmethyl counterparts**16a**&**b** [36]. Then, the latter were subjected to *O*-allylation to give the allyl ether analogues **17a**&**b**. Finally, Claisen rearrangement of derivatives **17** gave the allyl hydroxyfuroflavones **18a**&**b** [33].

Additionally, the methoxylated allylflavones **21a-d** were synthesized from **10a-d** in three steps as shown in scheme **IV**. The first step involves oxidative cleavage of the furan ring with $K_2Cr_2O_7$ in H_2SO_4 [36] to give the hydroxy aldehydes **19a-d** which were then *O*-allylated to yield derivatives **20a-d**. Finally, Claisen rearrangement gave the target allyl flavones **21a-d** [33].

Scheme **V** depicts the synthesis of 8-bromo-7-styrylfurochromones **22a-d** from 9-bromovisnagin **4** through condensation with various aromatic aldehydes using sodium ethoxide as a basic catalyst [37].

Finally, the 6-morpholinomethyl-7-styryl furochromones **25a-e** were obtained by subjecting ω-acetyl khellinone **23** [38] to Mannich reaction using morpholine hydrochloride and formalin which affected cyclization giving the 7-methyl-6-morpholinomethylfurochrome **24** [39] that was then subjected to Claisen-Schmidt condensation with different aromatic aldehydes in ethanolic solution of sodium ethoxide to furnish the target compounds (Scheme **VI**).

2.2. Biological Screening

In vitro cytotoxicity studies on tumoral cells. Forty three of the synthesized flavonoids were selected by the national cancer institute (NCI, Bethesda, MD, USA) for the *in vitro* disease-oriented human cell screening panel assay for their cytotoxic activity. A primary single-dose (10 μM) assay was performed by incubating each test compound with the full panel of the 60 tested cell lines. A 48 h continuous drug exposure protocol was used and a sulphorhodamine B (SRB) protein assay was employed to estimate cell viability or growth [40]. The data obtained are a mean-graph of the percentage growth inhibition (GI %) caused by the tested compounds (Table S1).

Furthermore, ten flavonoids, which satisfied pre-determined threshold inhibition criteria, were chosen to progress to a five dose (0.01-100 μM) screening assay and data obtained were used to create a log concentration percent growth inhibition curve. The selected compounds belong to the major subgroups of flavonoids *viz* furochalcones (**3a**, **3d**, **6b**, **9a** and **9b**), furoflavones (**12a** and **12c**), furoaurones (**15d**), styrylfurochromones (**25b** and **25e**). During the course of this screening, the response parameters corresponding to compound concentration leading to 50% decrease in net cell growth (GI_{50}), compound concentration resulting in total growth inhibition (TGI) and compound concentration affecting net 50% loss in initial cells at the end of the 48h incubation period (LC_{50}) were calculated for each cell line. Subpanel and full panel mean-graph mid-point values (MG-MID) were also determined which represent the average GI_{50}, TGI or LC_{50} values of all cell lines in the full panel.

Table 1 illustrates the GI_{50} (μM) of the ten tested compounds. With regards to the sensitivity of the individual cell lines, all the ten compounds except **3d** showed an obvious activity ($GI_{50} < 2$ μM) against one or more of the tested cell lines.

On leukemia cell lines, compound **12c** showed the highest activity against RPMI-8226 with GI_{50} 1.37 µM; **12a**, **12c**, **25b** and **25e** displayed activity against CCRF-CEM with GI_{50} in the range of 1.09-1.85 µM. Compound **25e** proved to be potent toward all the tested leukemia subpanels with GI_{50} range of 1.17-1.85 µM.

On non-small cell lung cancer cell lines, compounds **3a**, **6b**, **9a**, **9b**, **25b**, **25c** and **25e** exhibited distinctive activity against HOP-92 subpanel with GI_{50} range of 0.24-1.69 µM. Also, **9a**, **9b** and **25c** showed remarkable activity against NCI-H226 with GI_{50} range of 1.48-1.52 µM. Furthermore, compound **25e** exhibited good cytotoxic activity against NCI-H322M and NCI-H522 with GI_{50} 1.65 and 1.83 µM, respectively and compound **25b** displayed cytotoxicity against NCI-H522 with GI_{50} 1.77 µM.

Regarding colon cancer cell lines, compound **6b** and **25e** were the most potent against Colo-205 with GI_{50} 1.76 and 1.66 µM, respectively. Compounds **9a** and **9b** showed good activity against HCC-2998 with GI_{50} values of 1.73 and 1.79 µM, respectively. Compound **12a** and **12c** were highly active against HCT-116 with GI_{50} 1.7 and 1.06 µM, respectively. Finally, the rest of the examined cell lines were best inhibited by only one of the tested compounds where **12c** inhibited HCT-15 with GI_{50} 1.73 µM, **9b** inhibited HT29 with a GI_{50} of 1.89 µM and **25e** inhibited KM12 with a GI_{50} of 1.73 µM.

On CNS cancer cell lines, compounds **9a** and **9b** revealed superior activity against SF-539 with a GI_{50} of 1.71 and 1.79 µM, respectively. Compounds **6b**, **9a**, **15d**and **25e** were all active against SNB-75 with a GI_{50} range of 1.39-1.8 µM. Lastly, SF-295 growth was inhibited by **25e** with GI_{50} of 1.82 µM.

With reference to melanoma cancer cell lines, derivatives **3a**, **9a**, **9b**, **12a** and **12c** showed potent activity against LOXIMVI with GI_{50} range of 1.08-1.69 µM. On the other hand, **25b** and **25e** were active against SK-MEL-2 with GI_{50} of 1.65 and 1.39 µM,

respectively. Derivatives **12c**, **25b** and **25e** were effective against both SK-MEL-5 with a GI_{50} range of 1.09-1.69 µM and UACC-62 with a GI_{50} range of 1.77-1.9 µM.

Regarding ovarian cancer, the subpanel IGROV-1 was sensitive towards **25b** and **25e** with GI_{50} values 1.94 and 1.79 µM, respectively. While OVCAR-3 was responsive to **6b** (GI_{50} 1.91 µM), **9b** (GI_{50} 1.97 µM) and **25e** (GI_{50} 1.76 µM), NCI/ADR-RES was sensitive to **9a** with IC_{50} of 1.88 µM. Finally, **6b** affected SK-OV-3 displaying a relatively low GI_{50} of 1.11 µM.

Concerning the renal cancer subpanel cell lines, the flavonoid **25e** was active against 786-O, CAKI-1, SKF393 and UO-31 with IC_{50} values 1.93, 1.89, 1.81 and 1.43 µM, respectively. Also, compounds **9a** affected A498 cell line at a GI_{50} of 1.74 µM. Lastly, RXF393 was responsive to **9a**, **9b** and **25e** (GI_{50} range 1.72-1.85 µM).

As for prostate cancer, compound **25e** was active against the two subpanels PC-3 and DU-145 with GI50 1.69 and 1.67 µM, respectively.

On breast cancer, compound **25e** was active against the three subpanels MCF-7, HF478T and MDA-MB-468 with GI_{50} range of 1.68-1.88 µM. Finally, compound **12c** displayed activity towards MCF-7 (GI_{50} 1.63 µM); while **25b** was effective against HS-578T (GI_{50} 1.9 µM).

2.3. SAR findings

Table 2 displays the median growth inhibitory concentration (Med-GI_{50}, µM) of the ten most active flavonoids in this study. Concerning the major subgroups of the synthesized flavonoids, the styrylfurochromones represented by compounds **25b** and **25e**, showed superior activity against all the tested cell lines with GI_{50} range of 1.53-4.30 µM. The most active member in this series was **25e** with GI_{50} range of 1.53-2.31 µM.

Furthermore, chalcones represent the second subgroup of flavonoids displaying good cytotoxic activity specially derivatives **3a**, **6b**, **9a** and **9b**. The most active members were **9a** and **9b** carrying a 7-allyl substituent with GI_{50} range of 2.25-4.3 µM. Compound **9b**, possessing a 4-chloro substituent on ring B of the chalcone, showed increased activity against colon, melanoma, ovarian and prostate cell lines compared to its dechloro analogue **9a**. Replacement of the 7-allyl group in **9b** with a bromo substituent furnished compound **6b** which in comparison to **9b** displayed superior activity towards leukemia and prostate cancer cell lines and nearly equal activity against colon and ovarian cell lines. On the other hand, comparison of the chalcones **9a** with the 7-deallyl analog **3a** revealed that the presence of the allyl group markedly increased the activity against all tested cell lines (GI_{50} range of 2.25-4.3 µM for **9a** versus 3.55-7.45 µM for **3a**). Finally, the presence of the 2-methoxy group in ring B as represented by **3d** markedly decreased the cytotoxic activity (GI_{50} range of 16.6-40.4 µM) compared to the demethoxy congener **3a**.

Regarding the flavone subgroup, the most active members of which are **12a** and **12c**, a clear cytotoxic activity was displayed against all tested cell lines with GI_{50} ranging between 1.96 and 13.01 µM. It was evident that the presence of the 4-methoxy group in the isolated phenyl ring in **12c** increased the activity against all tested cell lines except non-small cell lung cancer which slightly decreased. Comparing the chalcone **9a** (GI_{50} range of 2.25-4.3 µM) with its cyclized flavone congener **12a** (GI_{50} range of 2.33-13.01µM) revealed that the flexible analog **9a** was much more active against all tested cell lines except for leukemia which showed nearly equal sensitivity.

Lastly in this regards, concerning the aurones subgroup, the most active member in this series was found to be **15d** which elicited a remarkable activity against all tested cell lines (GI_{50} range of 3.42-6.69 µM).

Relating to the broad spectrum antitumor activity, the results revealed that all ten tested compounds exhibited variable effective degree of GI_{50}, TGI and LC_{50} (MG-MID) (Table 3). Compound **25e** showed the highest activity at the two levels GI_{50} and TGI with values 1.9 and 5.24 µM, respectively. Yet, **25e** showed an LC_{50} of 60.25 µM which was lower than that of derivatives **9a** and **9b** the most active at this level, displaying values of 37.15 and 41.68 µM, respectively. It is worth mentioning though that the most active derivative in this study **25e** exhibited high LC_{50} (< 10 µM) against some subpanels, for example, leukemia HL-60(TB) (9.7 µM); small cell lung cancer NCI-H460 (8.48 µM); colon cancer Colo-205 (5.87 µM), HT-29 (6.68 µM); CNS cancer SNB-75 (5.75 µM) and melanoma MALME-3M (8.65 µM), SK-MEL-2 (5.89 µM), SK-MEL-5 (4.99 µM) and UACC-62 (7.61 µM) (data not shown).

Finally, compound **3d** was the least active at the three levels with GI_{50}, TGI and LC_{50} (MG-MID) of 38.9, 89.12 and 100 µM, respectively.

2.4. Kinase inhibition assay

The profiling data for the ten most cytotoxic compounds in this study against the 3 kinases CDK2, CDK4 and GSK-3β as potential molecular targets was performed. Profiling results for CDK2/Cyclin E1 protein kinase showed weak inhibition of the target by compounds **3d**, **6b**, and **12a** compared to control (Table 4).

Regarding the profiling data against CDK4/Cyclin D1 protein kinase target, weak inhibition of the target activity was observed by the flavonoids **3a**, **6b**, **15d**, **25b** and **25e** compared to control with percentage inhibition of enzyme activity between 6 to 15% (Table 4).

Finally, profiling data against GSK-3β protein kinase target showed weak to potent inhibition of the target activity by eight of the ten tested compounds and to our surprise weak

stimulation was observed by the two derivatives belonging to the styrylfurochromone class (**25b** and **25e**) (Table 4). Compound **9b** showed the highest inhibition of GSK-3β activity at 20 μM concentration of the compound where the GSK-3β activity was inhibited by 80% compared to control. The compounds **12a** and **15d** also showed good inhibition of GSK-3β activity by 58% and 56%, respectively, compared to control. Derivatives **3a**, **3d**, **6b**, **9a** and **12c** showed variable degrees of enzyme inhibition with percentages ranging from 11 to 38%.

To our surprise and in contrast to the other tested flavonoid derivatives, the styrylfurochromones **25b** and **25c** both caused stimulation of enzyme activity by 5 and 13%, respectively. Special interest was given to **25e**, the most potent compound in this study (MG-MID GI_{50} and TGI of 1.9 and 5.24 μM, respectively). We decided to go further with *in vivo* studies with **25e** in an attempt to correlate the observed high *in vitro* antiproliferative activity with the unexpected stimulation of GSK3-β activity.

*2.5. Effect of **25e** on the growth of Ehrlich solid tumor*

Treatment of mice with **25e** (3 mg/kg; i.p.) and (6 mg/kg; i.p.) daily for 5 days significantly inhibited the growth of Ehrlich solid tumor, the tumor size was 62.1% and 57.1% of that of control mice, respectively (Figure 3). While the final tumor size in mice receiving **25e** (3 mg/kg; i.p.) for 5 days was non-significantly changed when compared to the initial volume, the reduction in mice receiving **25e** (6 mg/kg; i.p.) for the same period was significant when compared to the initial volume. Our results showed that treatment of mice-bearing Ehrlich solid tumor with **25e** resulted in significant decrease in the tumor size in a dose-dependent manner when compared to the control group.

Figure 3

*2.6. Effect of **25e** on Cyclin D1 and GSK-3β in tumor tissue*

Treatment of mice with **25e** at a low dose (3 mg/kg; i.p.) and high dose (6 mg/kg; i.p.) daily for 5 days significantly declined the tumor level of cyclin D1 by 40.6% and 59.1%, (Figure 4) while the level of GSK-3β increased by 101.5% and 197.2%, respectively compared to untreated group (Figure 5). It can be concluded that the tumor suppressor effect of **25e** on Ehrlich solid tumor is mediated, in part, through increase in GSK-3β and decrease in cyclin D1 levels. These results are in consistence with the studies of Dong *et al.* [41] and Altiok *et al.* [42] that attributed the antitumor of new antitumor compounds against human breast cancer to their ability to promote a decline in cyclin D1 level accompanied by increased level of GSK-3β which phosphorylates cyclin D1 leading eventually to its degradation.

Figures 4 and 5

3. Conclusion

In this study a library of 50 semisynthetic flavonoids *viz* furochalcones, furoflavones, furoaurones and styrylfurochromones was synthesized to investigate their antiproliferative activity. Results of the *in vitro* screening performed against a panel of 60 cell lines belonging to nine types of cancer revealed that ten compounds possessed promising broad spectrum *in vitro* antiproliferative activity. Four compounds, **9a**, **9b**, **25c** and **25e**, showed the lowest results for GI_{50}, TGI and LC_{50} (MG-MID). The ten most active compounds were also tested for their potential kinase inhibitory activity against the 3 protein kinases CDK2/cyclin E1, CDK4/cyclin D1 and GSK-3β and showed variable degrees of enzyme inhibition with the exception of **25c** and **25e** which unexpectedly caused stimulation of GSK-3β activity. Furthermore, the *in vivo* antitumor activity of **25e** was assessed against mice-bearing Ehrlich solid tumor and resulted in significant decrease in the tumor size in a dose-dependent manner accompanied by significant increase in GSK-3β and decrease in cyclin D1 levels. These results suggest that the effect of **25e** on tumor tissue is likely related to its ability to reduce

cyclin D1 level through activation of GSK-3β activity that phosphorylates cyclin D1 thus promoting its proteolysis. Further investigation into the exact mechanism of action of these flavonoid derivatives and specially the styrylfurochromone ones is underway.

4. Experimental

4.1. Chemistry

Melting points are uncorrected and determined in one end open capillary tubes using Gallen Kamp melting point apparatus MFB-595-010M (GallenKamp, London, England). Microanalysis was carried out at the microanalytical unit, Faculty of Science, Cairo University. All compounds were within ± 0.4 % of the theoretical values. IR spectra were determined using KBr discs (cm^{-1}) on Shimadzu Infrared Spectrometer IR-435 (Shimadzu, Kyoto, Japan), Perkin-Elmer FT-IR 1650 (Perkin-Elmer, Waltham, Massachusetts 02451, USA) and Mattson Genesis II FTIR^{TM} Spectrometer (Mattson, Madison, WI, USA). ^{1}H-NMR (DMSO-d_6, D_2O) δ *ppm* spectra were determined using Joel NMR Varian Gemini 200 A Spectrometer (Joel, Tokyo, Japan) and Varian Mercury VX-300 MHz NMR Spectrometer (Varian, Oxford, England). Mass spectra were recorded using Hewlett Packard Varian (Varian, Polo, USA) and Shimadzu Gas Chromatograph Mass spectrometer-QP 1000 EX (Shimadzu, Kyoto, Japan). TLC were carried out using Art.DC-Plastikfolien, Kieselgel 60 F254 sheets (Merck, Darm-stadt, Germany), the developing solvents were CCl_4/ $CH_3COOC_2H_5$ (9:1) or (4:1) and the spots were visualized by Vilber Lourmat 77202 (Vilber, Marne La Vallee, France).

4.1.1. General procedure for the synthesis of 1-(6-hydroxy-4-methoxy-1-benzofuran-5-yl)-3-(substituted)phenylprop-2-en-1-ones ***(3a-e)***

To a mixture of **(2)** (1 g, 4.6 mmol) and 30% sodium hydroxide solution (4 ml); a solution of the appropriate aromatic aldehydes (5.5 mmol) in alcohol (5 ml) was added, the resulting red solution was allowed to stand for 24h at room temperature, diluted with water to 100 ml, then acidified with glacial acetic acid. The formed precipitate was collected by filtration, dried and recrystallized from ethanol [43].

Compounds **3a-c** were obtained as previously reported in the literature [43].

*4.1.1.1. 1-(6-Hydroxy-4-methoxy-1-benzofuran-5-yl)-3-(2-methoxyphenyl)prop-2-en-1-one **(3d)***

$C_{19}H_{16}O_5$ (324.33), Yield % 93, m.p.134-145°C; IR (KBr, cm^{-1}): 3421 (OH), 1624 (C=O). MS (m/z) rel.intensity: 324 [M^+, 65.76], 77 (100). ^{1}H-NMR (DMSO-d_6) δ ppm: 3.86 (s, 3H, OCH_3), 4.06 (s, 3H, OCH_3) 6.82 (s, 1H, C^7-H), 7.20 (d, *J* = 15.9 Hz, 1H, C<u>H</u>=CHCO), 7.21 (d, *J* = 2.1 Hz, 1H, furan), 7.64-7.72 (m, 3H, 2H benz + 1H CH=C<u>H</u>CO), 7.74 (d, *J* = 8.3 Hz, 2H, benz), 7.88 (d, *J* = 2.1 Hz, 1H, furan), 10.55 (s,1H, OH, exch. with D_2O). Anal.Calcd. for $C_{19}H_{16}O_5$: C, 70.36; H, 4.97. Found: C, 70.75; H, 4.76.

*4.1.1.2. 1-(6-Hydroxy-4-methoxy-1-benzofuran-5-yl)-3-(4-methylphenyl)prop-2-en-1-one **(3e)***

$C_{19}H_{16}O_4$ (308.33), Yield %93, mp.115-117°C; IR (KBr, cm^{-1}): 3430 (OH), 1628 (C=O), MS (m/z) rel.intensity: 308 [M^+, 100]. Anal.Calcd. for $C_{19}H_{16}O_4$: C, 74.01; H, 5.23. Found: C, 74.55; H, 4.94.

*4.1.2. General procedure for the synthesis of 1-(7-bromo-6-hydroxy-4-methoxy-1-benzofuran-5-yl)-3-substituted-phenyl prop-2-en-1-ones **(6a-d)***

These derivatives were prepared employing the procedure adopted for derivatives **3** starting with the bromoacetyl derivative **5** [32].

4.1.2.1. 1-(7-Bromo-6-hydroxy-4-methoxy-1-benzofuran-5-yl)-3-phenylprop-2-en-1-one ***(6a)***

$C_{18}H_{13}BrO_4$ (373.2), Yield % 85, m.p. 138-140 °C; IR (KBr, cm^{-1}): 3414 (OH), 1630 (C=O). MS (m/z) rel.intensity: 374 [M^+ + 2, 11.8], 372 [M^+, 12.3], 103 (100). ^{1}H-NMR (DMSO-d_6) δ ppm: 4.03 (s, 3H, OCH_3), 7.44-7.56 (m, 7H, 5H benz + 1H CH=CHCO + 1H furan), 7.73 (d, *J* = 2.1 Hz, 1H, furan), 8.01 (d, *J* = 15.87 Hz, 1H, CH=CHCO), 11.30 (s, 1H, OH, exch. with D_2O). Anal.Calcd. for $C_{18}H_{13}BrO_4$: C, 57.93; H, 3.51. Found: C, 58.11; H, 3.52.

4.1.2.2. 1-(7-Bromo-6-hydroxy-4-methoxy-1-benzofuran-5-yl)-3-(4-chlorophenyl)prop-2-en-1-one ***(6b)***

$C_{18}H_{12}BrClO_4$ (407.64), Yield % 83, m.p. 173-175°C; IR (KBr, cm^{-1}): 3398 (OH), 1626 (C=O). MS (m/z) rel.intensity: 408 [M^+ + 4, 16.2], 406 [M^+ + 2, 10.8], 404 [M^+, 5.3], 53 (100). ^{1}H-NMR (DMSO-d_6) δ ppm: 4.08 (s, 3H, OCH_3), 7.34 (d, *J* = 2.1 Hz, 1H, furan), 7.39 (d, *J* = 15.8 Hz, 1H, CH=CHCO), 7.46-7.57 (m, 3H, 2H benz +1H CH=CHCO), 7.88 (d, *J*= 8.4Hz, 2H, benz), 7.97 (d, *J* = 2.1Hz, 1H, furan) 11.25 (s,1H, OH, exch. with D_2O). Anal.Calcd. for $C_{18}H_{12}BrClO_4$: C, 53.03; H, 2.97. Found: C, 53.13; H, 2.51.

4.1.2.3. 1-(7-Bromo-6-hydroxy-4-methoxy-1-benzofuran-5-yl)-3-(4-methoxy-phenyl) prop-2-en-1-one ***(6c)***

$C_{19}H_{15}BrO_5$ (403.22), Yield % 87, m.p. 168-170°C; IR (KBr, cm^{-1}): 3444 (OH), 1630 (C=O). MS (m/z) rel.intensity: 404 [M^+ + 2, 16.03], 402 [M^+, 19.02], 269(100). ^{1}H-NMR (DMSO-d_6) δ ppm: 3.81 (s, 3H, OCH_3), 4.09 (s, 3H, OCH_3), 7.30 (d, *J* = 15.8 Hz, 1H, CH=CHCO), 7.36 (d, *J* = 2.1 Hz, 1H, furan), 7.42-7.48 (m, 3H, 2H benz +1H CH=CHCO), 7.49 (d, *J* = 8.4 Hz, 2H, benz), 8.0 (d, *J* = 2.1 Hz, 1H, furan), 11.27 (s, 1H, OH, exch. with D_2O). Anal.Calcd. for $C_{19}H_{15}BrO_5$: C, 56.59; H, 3.75. Found: C, 56.62; H, 4.15.

4.1.2.4. 1-(7-Bromo-6-hydroxy-4-methoxy-1-benzofuran-5-yl)-3-(2-methoxy-phenyl)prop-2-en-1-one ***(6d)***

$C_{19}H_{15}BrO_5$ (403.22), Yield % 83, m.p. 160-162°C; IR (KBr, cm^{-1}): 3430 (OH), 1624(C=O). Ms (m/z) rel.intensity: 404 [M^+ + 2, 4.1], 402 [M^+, 3.8], 111 (100). ^{1}H-NMR (DMSO-d_6) δ ppm: 3.83 (s, 3H, OCH_3), 4.08 (s, 3H, OCH_3) 7.33 (d, *J* = 15.84 Hz, 1H, C<u>H</u>=CHCO), 7.40 (d, *J* = 2.1 Hz, 1H, furan), 7.61-7.69 (m, 3H, 2H, benz +1H, CH=C<u>H</u>CO), 7.75 (d, *J* = 8.3 Hz, 2H, benz), 8.17 (d, *J* = 2.1 Hz, 1H, furan), 11.20 (s,1H, OH, exch. with D_2O). Anal.Calcd. for $C_{19}H_{15}BrO_5$: C, 56.59; H, 3.75. Found: C, 56.66; H, 4.0.

4.1.3. General procedure for the synthesis of 1-(7-allyl-6-hydroxy-4-methoxy-1-benzofuran-5-yl)-3-(substituted)phenyl-prop-2-en-1-ones ***(9a-d)***

These derivatives were prepared employing the procedure adopted for derivatives **3** starting with the allylfuroacetophenone derivative **8** [36]**.** Derivative **9a** was obtained as previously reported [36].

4.1.3.1. 1-(7-Allyl-6-hydroxy-4-methoxy-1-benzofuran-5-yl)-3-(4-chlorophenyl)prop-2-en-1-one ***(9b)***

$C_{21}H_{17}ClO_4$ (368.08), Yield % 94, m.p. 115-117°C; IR (KBr, cm^{-1}): 3420 (OH), 1624 (C=O). MS (m/z) rel.intensity: 368 [M^+, 20.96], 102 (100). ^{1}H-NMR (DMSO-d_6) δ ppm: 3.56 (d, *J*= 5.4Hz, 2H, -C<u>H$_2$</u>CH=CH_2), 4.12 (s, 3H, OCH_3), 5.02 (dd, 1H, -CH_2CH=C<u>H$_2$</u>, J_{gem} = 1.80 Hz, J_{cis} = 10.17 Hz), 5.10 (dd, 1H, -CH_2CH=C<u>H$_2$</u>, J_{gem} = 1.80 Hz, J_{trans} = 17.13 Hz), 6.01-6.31 (m, 1H, -CH_2C<u>H</u>=CH_2), 7.28-7.95 (m, 8H, 4H benz + 2H furan + 2H α,β-H protons), 12.00 (s, 1H, OH, exch. with D_2O). Anal.Calcd. for $C_{21}H_{17}ClO_4$: C, 68.36; H, 4.65. Found: C, 68.40; H, 4.50.

4.1.3.2. 1-(7-Allyl-6-hydroxy-4-methoxybenzofuran-5-yl)-3-(2-methoxyphenyl)prop-2-en-1-one ***(9d)***

$C_{22}H_{20}O_5$ (364.13), Yield %82, m.p. 122-124°C; IR (KBr, cm^{-1}): 3320 (OH), 1639(C=O). MS (m/z) rel.intensity: 364 [M^+, 51.41], 91 (100). ^{1}H-NMR (DMSO-d_6) δ ppm: 3.62 (d, J= 5.4Hz, 2H, -C<u>H$_2$</u>CH=CH$_2$) , 3.88 (s, 3H, OCH$_3$), 4.11 (s, 3H, OCH$_3$), 4.96 (dd, 1H, -CH$_2$CH=C<u>H$_2$</u>, J_{gem} = 1.80 Hz, J_{cis} = 10.2 Hz), 4.97 (dd, 1H, -CH$_2$CH=C<u>H$_2$</u>, J_{gem} = 1.80 Hz, J_{trans} = 17.2 Hz), 6.2-6.6 (m, 1H, -CH$_2$C<u>H</u>=CH$_2$), 7.03-7.86 (m, 8H, 4H benz + 2H furan + 2H α,β-protons), 12.11 (s, 1H, OH exch. with D_2O). Anal.Calcd. for $C_{22}H_{20}O_5$: C, 72.51; H, 5.53. Found: C, 72.76; H, 5.60.

4.1.4. General procedure for the synthesis of 4-methoxy-7-(substituted)phenyl-furo[3,2-g]chromen-5-ones ***(10a-d)***

A mixture of freshly sublimed selenium dioxide (9 mmol) and the appropriate chalcone **(3a-d)** (3.4 mmol) in n-butanol (15 ml) was heated under reflux for 24h after-which the obtained turbid solution was filtered while hot to remove the selenium metal. The filtrate was concentrated and left to cool to produce a precipitate which was filtered, dried and recrystallized from ethanol [34].

Derivatives **10a-c** were obtained as previously reported [34].

4.1.4.1. 4-Methoxy-7-(2-methoxyphenyl)-5H-furo[3,2-g]chromen-5-one ***(10d)***

$C_{19}H_{14}O_5$ (322.31), Yield %79, m.p. 165-167°C; IR (KBr, cm^{-1}): 3421 (OH), 1643 (C=O). MS (m/z) rel.intensity: 322 [M^+, 85.54], 293 (100). ^{1}H-NMR ($CDCl_3$) δ ppm: 3.96 (s, 3H, OCH$_3$), 4.21 (s, 3H, OCH$_3$), 7.03-7.13 (m, 4H, 1H C^9-H + 1H furan + 2H benz), 7.37 (s, 1H, C^6-H), 7.45 (t, J = 8.4 Hz, 1H, benz), 7.61 (d, J = 2.1 Hz, 1H, furan), 7.93 (d, J = 8.4 Hz, 1H, benz). Anal.Calcd. for $C_{19}H_{14}O_5$: C, 70.80; H, 4.38. Found: C, 70.64; H, 4.29.

4.1.5. General procedure for the synthesis of 9-bromo-4-methoxy-7-(substituted)phenyl-furo[3,2-g]chromen-5-ones ***(11a-d)***

The target compounds were obtained from chalcones **6a-d** employing the procedure described for synthesis of derivatives **9**.

4.1.5.1. 9-Bromo-4-methoxy-7-phenyl-5H-furo[3,2-g]chromen-5-one ***(11a)***

$C_{18}H_{11}BrO_4$ (371.18), Yield % 62, m.p. 228-230°C; IR (KBr, cm^{-1}): 1640 (C=O). MS (m/z) rel.intensity: 372 [M^+ + 2, 98.4], 370 [M^+, 100]. ^{1}H-NMR (DMSO-d_6) δ ppm: 4.02 (s, 3H, OCH_3), 6.90 (s, 1H, C^6-H), 7.41 (d, *J* = 2.1 Hz, 1H, furan), 7.56-7.59 (m, 3H, benz), 8.11 (d, *J* = 8.1 Hz, 2H, benz) 8.16 (d, *J* = 2.1 Hz, 1H, furan). Anal.Calcd. for $C_{18}H_{11}BrO_4$: C, 58.24; H, 2.99. Found: C, 58.10; H, 3.0.

4.1.5.2. 9-Bromo-7-(4-chlorophenyl)-4-methoxy-5H-furo[3,2-g]chromen-5-one ***(11b)***

$C_{18}H_{10}BrClO_4$ (405.63), Yield % 63, m.p. 278-280°C; IR (KBr, cm^{-1}): 1647 (C=O). MS (m/z) rel.intensity: 408 [M^+ + 4, 1.2], 406 [M^+ + 2, 1.1], 404 [M^+, 0.5], 77 (100).^{1}H-NMR (DMSO-d_6) δ ppm: 4.10 (s, 3H, OCH_3), 6.93 (s, 1H, C^6-H), 7.42 (d, *J* = 2.1 Hz, 1H, furan), 7.50 (d, *J* = 8.2 Hz, 2H, benz), 8.12-8.32 (m, 3H, 2H benz + 1H furan). Anal.Calcd. for $C_{18}H_{10}BrClO_4$: C, 53.03; H, 2.48. Found: C, 52.70; H, 2.57.

4.1.5.3. 9-Bromo-4-methoxy-7-(4-methoxyphenyl)-5H-furo[3,2-g]chromen-5-one ***(11c)***

$C_{19}H_{13}BrO_5$ (401.21), Yield % 60, m.p. 261-263°C; IR (KBr, cm^{-1}): 1645 (C=O). MS (m/z) rel.intensity: 402 [M^+ +2, 69.24], 400 [M^+, 77.38], 132 (100). ^{1}H-NMR (DMSO-d_6) δ ppm: 3.85 (s, 3H, OCH_3), 4.08 (s, 3H, OCH_3), 6.81 (s, 1H, C^6-H), 7.12 (d, *J* = 8.2 Hz, 2H, benz), 7.41 (d, *J* = 2.1 Hz, 1H, furan), 8.10 (d, *J* = 8.2 Hz, 2H, benz), 8.17 (d, *J* = 2.1 Hz, 1H, furan). Anal.Calcd. for $C_{19}H_{13}BrO_5$: C, 56.88; H, 3.27. Found: C, 56.79; H, 3.57.

4.1.5.4. 9-Bromo-4-methoxy-7-(2-methoxyphenyl)-5H-furo[3,2-g]chromen-5-one ***(11d)***

$C_{19}H_{13}BrO_5$ (401.21), Yield % 61, m.p. 235-237°C; IR (KBr, cm^{-1}): 1640 (C=O). MS (m/z) rel.intensity: 402 [M^+ + 2, 44.3], 400 [M^+, 42.6], 106 (100). ^{1}H-NMR (DMSO-d_6) δ ppm: 3.96 (s, 3H, OCH_3), 4.11 (s, 3H, OCH_3), 6.92 (s, 1H, C^6-H), 7.19 (t, *J* = 8.2 Hz, 1H, benz), 7.27 (d, *J* = 8.2 Hz, 1H, benz), 7.43 (d, *J* = 2.1 Hz, 1H, furan), 7.58 (t, *J* = 8.2 Hz, 1H, benz), 8.07 (d, *J* = 8.2 Hz, 1H, benz), 8.17 (d, *J* = 2.1 Hz, 1H, furan). Anal.Calcd. for $C_{19}H_{13}BrO_5$: C, 56.88; H, 3.27. Found: C, 56.70; H, 3.10.

4.1.6. General procedure for the synthesis of 3-(4-methoxy-5-oxo-7-(substituted)phenyl-5H-furo[3,2-g]chromen-9-yl)acrylaldehydes ***(12a-d)***

A mixture of freshly sublimed selenium dioxide (9 mmol) and the appropriate chalcone **(9a-d)** (3.4 mmol) in n-butanol (15 ml) was heated under reflux for 24h. The obtained turbid solution was filtered while hot to remove the selenium metal. The filtrate was concentrated and left to cool to produce a precipitate which was filtered, dried and recrystallized from ethanol.

4.1.6.1. 3-(4-Methoxy-5-oxo-7-phenyl-5H-furo[3,2-g]chromen-9-yl)acrylaldehyde ***(12a)***

$C_{21}H_{14}O_5$ (346.33), Yield % 65, m.p. 225-227°C; IR (KBr, cm^{-1}): 1722 (CHO) 1654 (C=O). ^{1}H-NMR (DMSO-d_6) δ ppm: 4.21 (s, 3H, OCH_3), 6.87 (s, 1H, C^6-H), 7.45 (dd, *J* = 7.8 Hz, 1H, -CH=C<u>H</u>-CHO), 7.59 (d, *J* = 2.1 Hz, 1H, furan), 7.62 (d, *J* = 8.2 Hz, 2H, benz), 8.07-8.21 (m, 3H, benz), 8.24 (d, 1H, *J* = 7.8 Hz, -C<u>H</u>=CH-CHO), 8.11 (d, *J* = 2.1 Hz, 1H, furan), 9.88 (d, *J* = 7.8 Hz, 1H, -CH=CH-C<u>H</u>O). Anal.Calcd. for $C_{21}H_{14}O_5$: C, 72.83; H, 4.07. Found: C, 72.79; H, 4.45.

4.1.6.2. 3-[7-(4-Chlorophenyl)-4-methoxy-5-oxo-5H-furo[3,2-g]chromen-9-yl]acrylaldehyde ***(12b)***

$C_{21}H_{13}ClO_5$ (380.78), Yield % 62, m.p. 202-203°C; IR (KBr, cm^{-1}): 1716 (CHO) 1645 (C=O). ^{1}H-NMR (DMSO-d_6) δ ppm: 4.21 (s, 3H, OCH_3), 6.88 (s, 1H, C^6-H), 7.14 (d, *J*= 8.2 Hz, 2H, benz), 7.23 (dd, *J* = 7.8 Hz, 1H, -CH=CH-CHO), 7.61 (d, *J* = 2.1 Hz, 1H, furan), 8.08 (d, *J* = 8.2 Hz, 2H, benz), 8.13 (d, *J* = 7.8 Hz, 1H, -CH=CH-CHO), 8.23 (d, *J* = 2.1 Hz, 1H, furan), 9.89 (d, *J* = 7.8 Hz, 1H, -CH=CH-CHO). Anal.Calcd. for $C_{21}H_{13}ClO_5$: C, 66.24; H, 3.44. Found: C, 66.63; H, 3.75.

4.1.6.3. 3-[4-Methoxy-7-(4-methoxyphenyl)-5-oxo-5H-furo[3,2-g]chromen-9-yl]acryl-aldehyde ***(12c)***

$C_{22}H_{16}O_6$ (376.36), Yield %60, m.p. 218-220°C; IR (KBr, cm^{-1}): 1720 (CHO), 1639 (C=O). ^{1}H-NMR (DMSO-d_6) δ ppm: 3.86 (s, 3H, OCH_3), 4.19 (s, 3H, OCH_3), 6.73 (s, 1H, C^6-H), 7.08 (d, *J* = 8.2 Hz, 2H, benz), 7.17 (dd, *J* = 7.8 Hz, 1H, -CH=CH-CHO), 7.42 (d, *J* = 2.1 Hz, 1H, furan), 7.99 (d, *J* = 8.2 Hz, 2H, benz), 8.13 (d, *J* = 7.8 Hz, 1H, -CH=CH-CHO), 8.17 (d, *J* = 2.1 Hz, 1H, furan), 9.85 (d, *J* = 7.8 Hz, 1H, -CH=CH-CHO). Anal.Calcd. for $C_{22}H_{16}O_6$: C, 70.21; H, 4.29. Found: C, 70.59; H, 4.61.

4.1.6.4. 3-[4-Methoxy-7-(2-methoxyphenyl)-5-oxo-5H-furo[3,2-g]chromen-9-yl]acryl-aldehyde ***(12d)***

$C_{22}H_{16}O_6$ (376.36), Yield %62, m.p. 175-177°C; IR (KBr, cm^{-1}): 1725 (CHO), 1650 (C=O). ^{1}H-NMR (DMSO-d_6) δ ppm: 3.83 (s, 3H, OCH_3), 4.13 (s, 3H, OCH_3), 6.56 (s, 1H, C^6-H), 7.00 (d, *J* = 8.2 Hz, 2H, benz), 7.04 (dd, *J* = 7.8 Hz, 1H, -CH=CH-CHO), 7.32 (d, *J* = 2.1 Hz, 1H, furan), 7.98 (d, *J* = 8.2 Hz, 2H, benz), 8.04 (d, *J* = 7.8 Hz, 1H, -CH=CH-CHO), 8.06 (d, *J* = 2.1 Hz, 1H, furan), 9.73 (d, *J* = 7.8Hz, 1H, -CH=CH-CHO). Anal.Calcd. for $C_{22}H_{16}O_6$: C, 70.21; H, 4.29. Found: C, 69.93; H, 3.98.

4.1.7. General procedure for the synthesis of 2-(substituted)benzylidene-4-methoxyfuro[3,2-f][1]benzofuran-3(2H)-ones ***(13a & 13b)***

To a mixture of the appropriate chalcone **(3d** or **3e)** (10 mmol), ethanol (30 ml) and 20% aqueous solution of sodium hydroxide (30 ml), 30% hydrogen peroxide solution (6 ml) was added with continuous stirring at room temperature. The reaction mixture was allowed to stand overnight and acidified with acetic acid. The solid separated was filtered, washed with water and crystallized from benzene.

4.1.7.1. 4-Methoxy-2-(2-methoxybenzylidene)furo[3,2-f][1]benzofuran-3(2H)-one ***(13a)***

$C_{19}H_{14}O_5$ (322.31), Yield % 80, m.p. 197-199°C; IR (KBr, cm^{-1}): 1693 (C=O). MS (m/z) rel.intensity: 322 [M^+, 53.3], 290 (100). ^{1}H-NMR ($CDCl_3$) δ ppm: 3.90 (s, 3H, OCH_3), 4.41 (s, 3H, OCH_3), 7.04 (s, 1H, C^8-H), 6.96 (s, 1H, vinyl proton), 7.33-7.51 (m, 5H, 4H benz + 1H furan), 8.26 (d, *J* = 2.1 Hz, 1H, furan). Anal.Calcd. for $C_{19}H_{14}O_5$: C, 70.80; H, 4.38. Found: C, 70.59; H, 4.41.

4.1.7.2. 4-Methoxy-2-(4-methylbenzylidene)furo[3,2-f][1]benzofuran-3(2H)-one ***(13b)***

$C_{19}H_{14}O_4$ (306.31), Yield % 80, m.p. 197-199°C; IR (KBr, cm^{-1}): 1693 (C=O). MS (m/z) rel.intensity: 306 [M^+, 100]. ^{1}H-NMR ($CDCl_3$) δ ppm: 2.36 (s, 3H, CH_3), 4.33 (s, 3H, OCH_3), 6.73 (s, 1H, C^7-H), 7.30 (s, 1H, vinyl proton), 7.31-7.35 (m, 3H, 2H benz + 1H furan), 7.86 (d, *J* = 8.4 Hz, 2H, benz), 7.97 (d, *J* = 2.1 Hz, 1H, furan). Anal.Calcd. for $C_{19}H_{14}O_4$: C, 74.50; H, 4.61. Found: C, 74.29; H, 4.27.

4.1.8. General procedure for the synthesis of 8-bromo-2-(substituted)benzylidene-4-methoxyfuro[3,2-f][1]-benzofuran-3(2H)-ones ***(14a-d)***

These compounds were obtained starting with chalcones **6a-d** adopting the procedure described for preparation of derivatives **13**.

4.1.8.1. 2-Benzylidene-8-bromo-4-methoxyfuro[3,2-f][1]benzofuran-3(2H)-one ***(14a)***

$C_{18}H_{11}BrO_4$ (371.18), Yield % 82, m.p. 213-215°C; IR (KBr, cm^{-1}): 1697 (C=O). MS (m/z) rel.intensity: 372 [M^+ +2, 25.8], 370 [M^+, 24.6], 90 (100). ^{1}H-NMR (DMSO-d_6) δ ppm: 4.34 (s, 3H, OCH_3), 6.84 (s, 1H, vinyl), 7.45 (t, *J* = 8.2 Hz, 1H, benz), 7.45-7.54 (m, 3H, 2H, benz + 1H, furan), 7.98 (d, *J* = 8.2 Hz, 2H, benz), 8.06 (d, *J* = 2.1 Hz, 1H, furan). Anal.Calcd for $C_{18}H_{11}BrO_4$: C, 58.24; H, 2.99. Found: C, 58.41; H, 2.95.

4.1.8.2. 8-Bromo-2-[4-chlorobenzylidene]-4-methoxyfuro[3,2-f][1]benzofuran-3(2H)-one ***(14b)***

$C_{18}H_{10}BrClO_4$ (405.63), Yield % 86, m.p. 223-225 °C; IR (KBr, cm^{-1}): 1692 (C=O). MS (m/z) rel.intensity: 408 [M^+ + 4, 23.1], 406 [M^+ + 2, 76.9], 404 [M^+, 60.3] 99 (100). ^{1}H-NMR (DMSO-d_6) δ ppm: 4.40 (s, 3H, OCH_3), 6.94 (s, 1H, vinyl), 7.54 (d, *J* = 2.1 Hz, 1H, furan), 7.65 (d, *J* = 8.6 Hz, 2H, benz), 8.05 (d, *J* = 8.6 Hz, 2H, benz), 8.18 (d, *J* = 2.1 Hz, 1H, furan). Anal.Calcd for $C_{18}H_{10}BrClO_4$: C, 53.03; H, 2.48. Found: C, 53.15; H, 2.48.

4.1.8.3. 8-Bromo-2-[4-methoxybenzylidene]-4-methoxyfuro[3,2-f][1]benzofuran-3(2H)-one ***(14c)***

$C_{19}H_{13}BrO_5$ (401.21), Yield % 87, mp.233-235 °C; IR (KBr, cm^{-1}): 1690 (C=O). MS (m/z) rel.intensity: 402 [M^+ + 2, 97], 400 [M^+, 100]. ^{1}H-NMR (DMSO-d_6) δ ppm: 3.83 (s, 3H, OCH_3), 4.31 (s, 3H, OCH_3), 6.78 (s, 1H, vinyl), 7.05 (d, *J* = 8.4 Hz, 2H, benz), 7.38 (d, *J* = 2.1 Hz, 1H, furan), 7.91 (d, *J* = 8.4 Hz, 2H, benz), 8.04 (d, *J* = 2.1 Hz, 1H, furan). Anal.Calcd. for $C_{19}H_{13}BrO_5$: C, 56.88; H, 3.27. Found: C, 57.10; H, 3.12.

4.1.8.4. 8-Bromo-2-[2-methoxybenzylidene]-4-methoxyfuro[3,2-f][1]benzofuran-3(2H)-one ***(14d)***

$C_{19}H_{13}BrO_5$ (401.21), Yield % 81, m.p. 280-230 °C; IR (KBr, cm^{-1}): 1697 (C=O). MS (m/z) rel.intensity: 402 [M^+ +2, 23.5], 400 [M^+, 22.5], 89 (100). ^{1}H-NMR (DMSO-d_6) δ ppm: 3.91

(s, 3H, OCH_3), 4.34 (s, 3H, OCH_3), 7.12-7.16 (m, 3H, 1H, vinyl + 2H, benz), 7.44-7.48 (m, 2H, 1H, benz + 1H, furan), 8.09 (d, *J* = 2.1 Hz, 1H, furan), 8.19 (d, *J* = 8.2 Hz, 1H, benz). Anal.Calcd for $C_{19}H_{13}BrO_5$: C, 56.88; H, 3.27. Found: C, 57.20; H, 3.45.

4.1.9. General procedure for the synthesis of 8-allyl-2-(substituted)benzylidene-4-methoxyfuro[3,2-f][1]benzofuran-3(2H)-ones ***(15a-d)***

To a mixture of the appropriate chalcone **(9a-d)** (10 mmol), ethanol (30 ml) and 20% aqueous solution of sodium hydroxide (30 ml) at room temperature, was added 30% hydrogen peroxide solution (6 ml) with continuous stirring. The reaction mixture was allowed to stand overnight and acidified with dilute acetic acid. The solid separated was filtered, washed with water and crystallized from ethanol.

4.1.9.1. 8-Allyl-2-[benzylidene]-4-methoxyfuro[3,2-f][1]benzofuran-3(2H)-one ***(15a)***

$C_{21}H_{16}O_4$ (332.1), Yield % 82, m.p. 163-165°C; IR (KBr, cm^{-1}): 1697 (C=O). MS (m/z) rel.intensity: 332 [M^+, 100]. ^{1}H-NMR (DMSO-d_6) δ ppm: 3.72 (d, *J* = 5.4 Hz, 2H, -C<u>H</u>$_2$ –CH=CH_2), 4.31 (s, 3H, OCH_3), 5.14 (dd, 1H, -CH_2–CH=C<u>H</u>$_2$, J_{gem} = 1.9 Hz, J_{cis} = 10.10 Hz), 5.23 (dd, 1H, -CH_2–CH=C<u>H</u>$_2$, J_{gem} = 1.9 Hz, J_{trans} = 16.8 Hz), 6.23 (m, 1H, -CH_2 –C<u>H</u>=CH_2), 6.76 (s, 1H, vinyl proton), 7.42 (d, *J* = 8.4 Hz, 2H, benz), 7.56 (d, *J* = 2.1 Hz, 1H, furan), 7.94-7.98 (m, 4H, 3H benz + 1H furan). Anal.Calcd. for $C_{21}H_{16}O_4$: C, 75.89; H, 4.85. Found: C, 76.00; H, 5.03.

4.1.9.2. 8-Allyl-2-[4-chlorobenzylidene]-4-methoxyfuro[3,2-f][1]benzofuran-3(2H)-one ***(15b)***

$C_{21}H_{15}ClO_4$ (366.79), Yield % 85, m.p. 170-172°C; IR (KBr, cm^{-1}): 1692 (C=O). MS (m/z) rel.intensity: 367 [M^+ + 1, 0.74], 53 (100). ^{1}H-NMR (DMSO-d_6) δ ppm: 3.68 (d, *J* = 5.4 Hz, 2H, -C<u>H</u>$_2$–CH=CH_2), 4.23 (s, 3H, OCH_3), 5.10 (dd, 1H, -CH_2–CH=C<u>H</u>$_2$, J_{gem} = 2.0 Hz, J_{cis} = 10.2 Hz), 5.13 (dd, 1H, -CH_2–CH=C<u>H</u>$_2$, J_{gem} = 2.0 Hz, J_{trans} = 17.1 Hz), 6.11 (m, 1H, -CH_2–

C<u>H</u>=CH_2), 6.75 (s, 1H, vinyl proton), 7.3 (d, *J*= 8.4Hz, 2H, benz), 7.56 (d, *J*= 2.1Hz, 1H, furan), 7.99-8.00 (m, 3H, 2H benz + 1H furan). Anal.Calcd. for $C_{21}H_{15}ClO_4$: C, 68.76; H, 4.12. Found: C, 69.03; H, 4.14.

4.1.9.3. 8-Allyl-2-[4-methoxybenzylidene]-4-methoxyfuro[3,2-f][1]benzofuran-3(2H)-one ***(15c)***

$C_{22}H_{18}O_5$ (362.38), Yield % 80, m.p. 150-152°C; IR (KBr, cm^{-1}): 1695(C=O). MS (m/z) rel.intensity: 362 [M^+, 100]. ^{1}H-NMR (DMSO-d_6) δ ppm: 3.68 (d, *J* = 5.4 Hz, 2H, -C<u>H</u>$_2$–CH=CH_2), 3.83 (s, 3H, OCH_3), 4.28 (s, 3H, OCH_3), 5.10 (dd, 1H, -CH_2 –CH=C<u>H</u>$_2$, J_{gem} = 2.1 Hz, J_{cis} = 10.2 Hz), 5.15 (dd, 1H,-CH_2–CH=C<u>H</u>$_2$, J_{gem} = 2.1 Hz, J_{trans}= 17.3 Hz), 5.97-6.15 (m, 1H, -CH_2–C<u>H</u>=CH_2), 6.74 (s, 1H, vinyl proton), 7.08 (d, J=8.4Hz, 2H, benz), 7.30 (d, *J* = 2.1 Hz, 1H, furan), 7.92 (d, *J* = 8.4 Hz, 2H, benz), 8.00 (d, *J* = 2.1 Hz, 1H, furan). Anal.Calcd. for $C_{22}H_{18}O_5$: C, 72.92; H, 5.01. Found: C, 72.81; H, 4.91.

4.1.9.4. 8-Allyl-2-[2-methoxybenzylidene]-4-methoxyfuro[3,2-f][1]benzofuran-3(2H)-one ***(15d)***

$C_{22}H_{18}O_5$ (362.38), Yield % 78, m.p. 128-130°C; IR (KBr, cm^{-1}): 1693 (C=O). ^{1}H-NMR (DMSO-d_6) δ ppm: 3.67 (d, *J* = 5.4 Hz, 2H, -C<u>H</u>$_2$–CH=CH_2), 3.90 (s, 3H, OCH_3), 4.30 (s, 3H, OCH_3), 5.15 (dd, 1H,-CH_2CH=C<u>H</u>$_2$, J_{gem} = 2.1 Hz, J_{cis} = 10.2 Hz), 5.20 (dd, 1H,-CH_2CH=C<u>H</u>$_2$, J_{gem} = 2.1 Hz, J_{trans} = 17.2 Hz), 6.05-6.20 (m, 1H, -CH_2C<u>H</u>=CH_2), 7.04 (s, 1H, vinyl proton), 7.12-7.29 (m, *J* = 8.4 Hz, 2H, benz), 7.39 (d, *J* = 2.1 Hz, 1H, furan), 7.42 (t, *J* = 8.4 Hz, 1H, benz), 7.99 (d, *J* = 2.1 Hz, 1H, furan), 8.18 (d, *J* = 8.4 Hz, 1H, benz). Anal.Calcd. for $C_{22}H_{18}O_5$: C, 72.92; H, 5.01. Found: C, 72.85; H, 4.92.

4.1.10. General procedure for the synthesis of 9-allyl-4-hydroxy-7-substituted phenyl-5H-furo[3,2-g]chromen-5-ones ***(18a & 18b)***

A mixture of **(16a** or **16b)** [44] (10 mmol), allyl bromide (1.45 g, 12 mmol) and anhydrous potassium carbonate (3 g) in acetone (70 ml) was refluxed for 24h, then filtered while hot and the residue was washed repeatedly with small portions of hot acetone. Evaporating the combined filtrate gave an oily residue of the allyloxy derivatives **(17a** or **17b)**. The latter, without purification, was refluxed for 2h with *N*,*N*-diethylaniline (20 ml), then allowed to stand at room temperature for 5h. The reaction mixture was poured onto a mixture of crushed ice (100 g) and conc. HCl (20 ml) with good stirring. The resulting precipitate was collected by filtration, washed with water, dried and recrystallized from ethanol.

*4.1.10.1. 9-Allyl-4-hydroxy-7-phenyl-5H-furo[3,2-g]chromen-5-one **(18a)***

$C_{20}H_{14}O_4$ (318.32), Yield %70, m.p. 149-151°C; IR (KBr, cm^{-1}): 3421 (OH), 1643 (C=O). MS (m/z): 318 [M^+, 97.60], 261 (100). ^{1}H-NMR ($CDCl_3$) δ ppm: 3.82 (d, J = 5.6 Hz, 2H, -C$\underline{H_2}$–CH=CH_2), 5.12 (dd, 1H, -CH_2–CH=C$\underline{H_2}$, J_{gem} = 2.2 Hz, J_{cis} = 9.9 Hz), 5.16 (dd, 1H, -CH_2 –CH=C$\underline{H_2}$, J_{gem} = 2.2 Hz, J_{trans} = 16.5 Hz), 6.16 (m, 1H, -CH_2–C$\underline{H}$=CH_2), 6.65 (s, 1H,C^6-H), 7.24 (d, J = 8.4 Hz, 2H, benz), 7.73-7.79 (m, 4H, 3H benz + 1H furan), 7.89 (d, J = 2.1 Hz, 1H, furan), 13.55 (s, 1H, OH, exch. with D_2O). Anal.Calcd. for $C_{20}H_{14}O_4$: C, 75.46; H, 4.43. Found: C, 74.86; H, 4.69.

*4.1.10.2. 9-Allyl-7-(4-chlorophenyl)-4-hydroxy-5H-furo[3,2-g]chromen-5-one **(18b)***

$C_{20}H_{13}ClO_4$ (352.77), Yield % 72, m.p. 160-162°C; IR (KBr, cm^{-1}): 3420 (OH), 1639 (C=O). MS (m/z): 352 [M^+, 100]. ^{1}H-NMR ($CDCl_3$) δ ppm: 3.85 (d, J = 5.6 Hz, 2H, -C$\underline{H_2}$–CH=CH_2), 5.09 (dd, 1H, -CH_2–CH=C$\underline{H_2}$, J_{gem} = 2.2 Hz, J_{cis} = 9.9 Hz), 5.12 (dd, 1H, -CH_2 –CH=C$\underline{H_2}$, J_{gem} = 2.2 Hz, J_{trans} = 16.5 Hz), 6.12 (m, 1H, -CH_2–C$\underline{H}$=CH_2), 6.69 (s, 1H,C^6-H), 7.27 (d, J = 8.4 Hz, 2H, benz), 7.63-7.69 (m, 3H, 2H benz + 1H furan), 7.86 (d, J = 2.1 Hz, 1H, furan), 13.52 (s, 1H, OH, exch. with D_2O). Anal.Calcd. for $C_{20}H_{13}ClO_4$: C, 68.09; H, 3.71. Found: C, 67.86; H, 3.43.

4.1.11. General procedure for the synthesis of 7-hydroxy-5-methoxy-2-(substituted)phenyl-4-oxo-4H-chromene-6-carbaldehydes ***(19a-d)***

To mixture of the appropriate 4-methoxyfurochromone **(10a-d)** (3.4 mmol), glacial acetic acid (10 ml) and 25% sulfuric acid (20 ml) at 50°C was added 30% potassium dichromate solution (6.7 ml). After a few minutes, colorless precipitate began to separate out. The mixture was left to cool at room temperature, diluted to 100 ml with water and filtered. The residue obtained was dried and recrystallized from ethanol [36].

Derivative **19a** was obtained as previously reported in the literature [36].

4.1.11.1. 2-(4-Chlorophenyl)-7-hydroxy-5-methoxy-4-oxo-4H-chromene-6-carbaldehyde ***(19b)***

$C_{17}H_{11}ClO_5$ (330.72), Yield % 85, m.p. 240-243°C; IR (KBr, cm^{-1}): 3417 (OH), 1720 (CHO), 1640 (C=O). ^{1}H-NMR (DMSO-d_6) δ ppm: 3.97 (s, 3H, OCH_3), 6.91 (s, 1H, C^8-H), 7.00 (s, 1H, C^3-H), 7.65 (d, *J* = 8.4 Hz, 2H, benz), 8.11 (d, *J* = 8.4 Hz, 2H, benz), 10.28 (s, 1H, CHO), 12.01 (s,1H, OH, exch. with D_2O). Anal.Calcd. for $C_{17}H_{11}ClO_5$: C, 61.74; H, 3.35. Found: C, 62.1; H, 3.62.

4.1.11.2. 7-Hydroxy-5-methoxy-2-(4-methoxyphenyl)-4-oxo-4H-chromene-6-carbaldehyde ***(19c)***

$C_{18}H_{14}O_6$ (326.3), Yield %78, m.p. 223-225°C; IR (KBr, cm^{-1}): 3421(OH), 1718 (CHO), 1654 (C=O). ^{1}H-NMR (DMSO-d_6) δ ppm: 3.96 (s, 3H, OCH_3), 4.07 (s, 3H, OCH_3), 6.74 (s, 1H, C^8-H), 7.36 (s, 1H, C^3-H), 7.10 (d, *J* = 8.4Hz, 2H, benz), 8.01 (d, *J* = 8.4Hz, 2H, benz), 10.26 (s, 1H, CHO), 11.98 (s,1H, OH, exch. with D_2O). Anal.Calcd. for $C_{18}H_{14}O_6$: C, 66.62; H, 4.32. Found: C, 66.27; H, 4.51.

4.1.11.3. 7-Hydroxy-5-methoxy-2-(2-methoxyphenyl)-4-oxo-4H-chromene-6-carbaldehyde ***(19d)***

$C_{18}H_{14}O_6$ (326.3), Yield % 75, m.p. 192-194°C; IR (KBr, cm^{-1}): 3394 (OH), 1735 (CHO), 1647 (C=O). ^{1}H-NMR (DMSO-d_6) δ ppm: 3.68 (s, 3H, OCH_3), 4.13 (s, 3H, OCH_3), 6.84 (s, 1H, C^8-H), 6.95 (s, 1H, C^3-H), 7.12-7.29 (m, 2H, benz), 7.61 (d, *J* = 8.2 Hz, 1H, benz), 7.95 (d, *J* = 8.2 Hz, 1H, benz), 10.32 (s, 1H, CHO), 12.04 (s, 1H, OH, exch. with D_2O). Anal.Calcd. for $C_{18}H_{14}O_6$: C, 66.62; H, 4.32. Found: C, 66.96; H, 4.67.

4.1.12. General procedure for the synthesis of 8-allyl-7-hydroxy-5-methoxy-2-(substituted) phenyl-4-oxo-4H-chromene-6-carbaldehydes ***(21a-d)***

A mixture of **(19a-d)** (10 mmol), allyl bromide (1.45 g, 12 mmol) and anhydrous potassium carbonate (3 g) in acetone (70 ml) was refluxed for 16h, then filtered while hot and the residue was washed repeatedly with small portions of hot acetone. Evaporating the combined filtrate gave **(20a-d)**. The oily residue **(20a-d)** was refluxed for 2h with *N*,*N*-diethylaniline (20 ml), then allowed to stand at room temperature for 5h. The reaction mixture was poured in a mixture of crushed ice (100 g) and conc. HCl (20 ml) with good stirring. The resulting precipitate was collected by filtration, washed with water, dried and recrystallized from ethanol.

4.1.12.1. 8-Allyl-7-hydroxy-5-methoxy-4-oxo-2-phenyl-4H-chromene-6-carbaldehyde ***(21a)***

$C_{20}H_{16}O_5$ (336.34), Yield % 63, m.p. 107-109°C; IR (KBr, cm^{-1}): 3410 (OH), 1718 (CHO), 1647 (C=O). MS (m/z) rel.intensity: 336 [M^+, 4.64], 102 (100). ^{1}H-NMR (DMSO-d_6) δ ppm: 3.49 (d, *J* = 5.4 Hz, 2H, -C<u>H</u>$_2$–CH=CH_2), 4.04 (s, 3H, OCH_3), 5.05 (dd, 1H, -CH_2–CH=C<u>H</u>$_2$, J_{gem} = 1.9 Hz, J_{cis} =9.96 Hz), 5.09 (dd, 1H, -CH_2–CH=C<u>H</u>$_2$, J_{gem} = 1.9 Hz, J_{trans} = 15.8 Hz), 5.80-5.97 (m, 1H, -CH_2–C<u>H</u>=CH_2), 7.04 (s, 1H, C^3-H), 7.58-7.62 (m, 3H, benz), 8.05 (d, *J* = 8.4 Hz, 2H, benz), 10.27 (s, 1H, CHO), 12.56 (s, 1H, OH, exch. with D_2O). Anal.Calcd for $C_{20}H_{16}O_5$: C, 71.42; H, 4.79. Found: C, 71.81; H, 4.41

4.1.12.2. 8-Allyl-2-(4-chlorophenyl)-7-hydroxy-5-methoxy-4-oxo-4H-chromene-6-carbaldehyde ***(21b)***

$C_{20}H_{15}ClO_5$ (370.78), Yield % 67, m.p. 116-118°C; IR (KBr, cm^{-1}): 3417 (OH), 1720 (CHO), 1647 (C=O), MS (m/z) rel.intensity: 370 [M^+, 36.23], 338 (100). ^{1}H-NMR (DMSO-d_6) δ ppm: 3.49 (d, J = 5.4 Hz, 2H, -C<u>H$_2$</u>–CH=CH$_2$), 4.01 (s, 3H, OCH_3), 5.08 (dd, 1H, -CH$_2$–CH=C<u>H$_2$</u> , J_{gem} = 1.9 Hz, J_{cis} =9.96 Hz), 5.11 (dd, 1H, -CH$_2$–CH=C<u>H$_2$</u> , J_{gem} = 1.9 Hz, J_{trans} = 15.8 Hz), 5.81-5.99 (m, 1H, -CH$_2$–C<u>H</u>=CH$_2$), 7.02 (s, 1H, C^3-H), 7.50 (d, J = 8.4 Hz, 2H, benz), 8.02 (d, J = 8.4 Hz, 2H, benz), 10.25 (s, 1H, CHO), 12.56 (s, 1H, OH, exch. with D_2O). Anal.Calcd. for $C_{20}H_{15}ClO_5$: C, 64.79; H, 4.08. Found: C, 65.12; H, 4.43.

4.1.12.3. 8-Allyl-7-hydroxy-5-methoxy-2-(4-methoxyphenyl)-4-oxo-4H-chromene-6-carbaldehyde ***(21c)***

$C_{21}H_{18}O_6$ (366.36), Yield % 69, m.p.125-127°C; IR (KBr, cm^{-1}): 3415 (OH), 1708 (CHO), 1643 (C=O), MS (m/z) rel.intensity: 366 [M^+, 30.51], 337 (100). ^{1}H-NMR (DMSO-d_6) δ ppm: 3.33 (d, J = 5.4 Hz, 2H, -C<u>H$_2$</u>–CH=CH$_2$), 3.83 (s, 3H, OCH_3), 4.20 (s, 3H, OCH_3), 4.99 (dd, 1H, -CH$_2$–CH=C<u>H$_2$</u>, J_{gem} =2.1 Hz, J_{cis} = 10.2 Hz), 5.05 (dd, 1H, -CH$_2$–CH=C<u>H$_2$</u>, J_{gem} = 2.1 Hz, J_{trans} = 17.2 Hz), 5.88-6.17 (m, 1H, -CH$_2$–C<u>H</u>=CH$_2$), 7.10 (s, 1H, C^3-H), 7.89-7.92 (m, 4H, benz), 10.26 (s, 1H, CHO), 12.61 (s, 1H, OH, exch. with D_2O). Anal.Calcd. for $C_{21}H_{18}O_6$: C, 68.85; H, 4.95. Found: C, 68.46; H, 4.57.

4.1.12.4. 8-Allyl-7-hydroxy-5-methoxy-2-(2-methoxyphenyl)-4-oxo-4H-chromene-6-carbaldehyde ***(21d)***

$C_{21}H_{18}O_6$ (366.36), Yield % 60, m.p. 114-116°C; IR (KBr, cm^{-1}): 3415 (OH), 1717 (CHO), 1643(C=O), MS (m/z) rel.intensity: 366 [M^+, 21.17], 324 (100). ^{1}H-NMR (DMSO-d_6)δ ppm: 3.49 (d, J= 5.4Hz, 2H, -C<u>H$_2$</u>–CH=CH$_2$), 3.81 (s, 3H, OCH_3), 4.0 (s, 3H, OCH_3), 4.97 (dd, 1H, -CH$_2$ –CH=C<u>H$_2$</u>, J_{gem} = 2.0 Hz, J_{cis} = 10.1 Hz), 5.10 (dd, 1H, -CH$_2$ –CH=C<u>H$_2$</u>, J_{gem} =2.0 Hz,

J_{trans} = 16.8 Hz), 5.84-6.18 (m, 1H, -CH_2 –C<u>H</u>=CH_2), 7.02 (s, 1H, C^3-H), 7.31-7.60 (m, 4H, benz), 10.27 (s, 1H, CHO), 12.64 (s, 1H, OH, exch. with D_2O). Anal.Calcd. for $C_{21}H_{18}O_6$: C, 68.85; H, 4.95. Found: C, 68.61; H, 4.50.

4.1.13. General procedure for the synthesis of 9-bromo-7-{-2-[4-(substituted)phenyl]vinyl}-4-methoxy-5H-furo[3,2-g]chromen-5-ones ***(22a-d)***

To a cooled solution of 9-bromovisnagin **(4)** (3.01 g, 1 mmol) and (1.2 mmol) of the appropriate aromatic aldehyde in absolute ethyl alcohol (60 ml) was added a cooled solution of sodium (2.5 mmol) in absolute ethyl alcohol (30 ml). The small amount of the solid that separated was brought into solution by slight warming and the reaction mixture was kept overnight at room temperature. The precipitate formed was filtered off, washed with a little alcohol and crystallized from ethyl alcohol.

4.1.13.1. 9-Bromo-4-methoxy-7-[2-phenylvinyl]-5H-furo[3,2-g]chromen-5-one ***(22a)***

$C_{20}H_{13}BrO_4$ (397.22), Yield % 75, m.p. 220-222°C; IR (KBr, cm^{-1}): 1640 (C=O). MS (m/z) rel.intensity: 398 [M^+ + 2, 11.5], 396 [M^+, 10.8], 77 (100). ^{1}H-NMR (DMSO-d_6) δ ppm: 4.07 (s, 3H, OCH_3), 6.33 (s, 1H, C^3-H), 7.18 (d, *J* = 16.2 Hz, 1H, CH=C<u>H</u>), 7.31-7.48 (m, 4H, 1H furan + 3H benz), 7.54-7.70 (m, 3H, 1H C<u>H</u>=CH + 2H benz), 8.16 (d, *J* = 2.1 Hz, 1H, furan). Anal.Calcd. for $C_{20}H_{13}BrO_4$: C, 60.47; H, 3.30. Found: C, 60.63; H, 3.20.

4.1.13.2. 9-Bromo-7-[2-(4-chlorophenyl)vinyl]-4-methoxy-5H-furo[3,2-g]chromen-5-one ***(22b)***

$C_{20}H_{12}BrClO_4$ (431.66), Yield % 78, m.p. 195-197°C; IR (KBr, cm^{-1}): 1655 (C=O). MS (m/z) rel.intensity: 432 [M^+ + 2, 0.05], 430 [M^+, 0.04], 268 (100). ^{1}H-NMR (DMSO-d_6) δ ppm: 4.04 (s, 3H, OCH_3), 6.28 (s, 1H, C^3-H), 7.15 (d, *J* = 16.2 Hz, 1H, CH=C<u>H</u>), 7.29-7.41 (m,

3H, 1H furan + 2H benz), 7.54-7.70 (m, 3H, 1H C<u>H</u>=CH + 2H benz), 8.19 (d, *J* = 2.1 Hz, 1H, furan). Anal.Calcd. for $C_{20}H_{12}BrClO_4$: C, 55.65; H, 2.80. Found: C, 55.32; H, 2.54.

4.1.13.3. 9-Bromo-4-methoxy-7-[2-(4-methoxyphenyl)vinyl]-5H-furo[3,2-g]chromen-5-one ***(22c)***

$C_{21}H_{15}BrO_5$ (427.24), Yield % 76, m.p. 203-205°C; IR (KBr, cm^{-1}): 1660 (C=O). MS (m/z) rel.intensity: 428 [M^+ + 2, 12.46], 426 [M^+, 13.37], 115(100). ^{1}H-NMR ($CDCl_3$) δ ppm: 3.87 (s, 3H, OCH_3), 4.16 (s, 3H, OCH_3), 6.29 (s, 1H, C^3-H), 6.66 (d, *J* = 8.1 Hz, 2H, benz), 6.93 (d, *J* = 16.6 Hz, 1H, CH=C<u>H</u>), 7.06 (d, *J* = 2.1 Hz, 1H, furan), 7.07-7.27 (m, 3H, 1H C<u>H</u>=CH + 2H benz), 7.70 (d, *J* = 2.1 Hz, 1H, furan). Anal.Calcd. for $C_{21}H_{15}BrO_5$: C, 59.04; H, 3.54. Found: C, 59.33; H, 3.31.

4.1.13.4. 9-Bromo-7-{2-[4-(dimethylamino)phenyl]vinyl}-4-methoxy-5H-furo[3,2-g]chromen-5-one ***(22d)***

$C_{22}H_{18}BrNO_4$ (440.29), Yield % 73, m.p. 198-200°C; IR (KBr, cm^{-1}): 1636 (C=O). MS (m/z) rel.intensity: 441 [M^+ + 2, 47.7], 439 [M^+, 45.5], 211 (100). ^{1}H-NMR (DMSO-d_6) δ ppm: 2.93 (s, 6H, $N(CH_3)_2$), 4.06 (s, 3H, OCH_3), 6.19 (s, 1H, C^3-H), 6.75 (d, *J* = 8.2 Hz, 2H, benz), 6.87 (d, *J* = 16.6 Hz, 1H, CH=C<u>H</u>), 7.30 (d, *J* = 2.1 Hz, 1H, furan), 7.50-7.58 (m, 3H, 1H, C<u>H</u>=CH + 2H, benz), 8.15 (d, *J* = 2.1 Hz, 1H, furan). Anal.Calcd. for $C_{22}H_{18}BrNO_4$: C, 60.01; H, 4.12; N, 3.18. Found: C, 60.07; H, 4.15; N, 3.02.

4.1.14. General procedure for the synthesis of 4,9-dimethoxy-6-(morpholin-4-ylmethyl)-7-(2-substituted-phenylvinyl)-5H-furo[3,2-g]chromen-5-ones ***(25a-e)***

These compounds were obtained starting with **24** [37,38] employing the same procedure described for the synthesis of derivatives **23**.

4.1.14.1. 4,9-Dimethoxy-6-(morpholin-4-ylmethyl)-7-(2-phenylvinyl)-5H-furo[3,2-g]chromen-5-one ***(25a)***

$C_{26}H_{25}NO_6$ (447.48), Yield % 74, m.p. 148-150°C; IR (KBr, cm^{-1}): 1629 (C=O). MS (m/z) rel.intensity: 372 [M^+ - C_6H_5, 7.4], 56 (100). ^{1}H-NMR (DMSO-d_6) δ ppm: 2.49 (t, *J* = 4.2 Hz, 4H, $2CH_2$), 3.52 (t, *J* = 4.2 Hz, 4H, $2CH_2$), 3.62 (s, 2H, CH_2), 3.96 (s, 3H, OCH_3), 4.19 (s, 3H, OCH_3), 7.24 (d, *J* = 16.1 Hz, 1H, CH=C<u>H</u>), 7.51-7.75 (m, 6H, 5H benz + 1H, furan), 7.78 (d, *J* = 2.1 Hz, 1H, furan), 8.10 (d, *J*= 16.1Hz, 1H, C<u>H</u>=CH). Anal.Calcd. for $C_{26}H_{25}NO_6$: C, 69.79; H, 5.63; N, 3.13. Found: C, 69.51; H, 5.90; N, 3.25.

4.1.14.2. 7-[2-(4-Chlorophenyl)vinyl]-4,9-dimethoxy-6-(morpholin-4-ylmethyl)-5H-furo[3,2-g]chromen-5-one ***(25b)***

$C_{26}H_{24}ClNO_6$ (481.92), Yield % 77, m.p. 177-179°C; IR (KBr, cm^{-1}): 1628 (C=O). MS (m/z) rel.intensity: 483 [M^+ + 2, 12.5], 481 [M^+, 4.6], 396 [100]. ^{1}H-NMR (DMSO-d_6) δ ppm: 2.45 (t, *J* = 4.2 Hz, 4H, $2CH_2$), 3.51 (t, *J* = 4.2 Hz, 4H, $2CH_2$), 3.53 (s, 2H, CH_2), 3.96 (s, 3H, OCH_3), 4.18 (s, 3H, OCH_3), 7.22 (d, *J* = 16.2 Hz, 1H, CH=C<u>H</u>), 7.50-7.56 (m, 3H, 2H benz + 1H furan), 7.78 (d, *J* = 8.2 Hz, 2H, benz), 7.79 (d, *J* = 2.1 Hz, 1H, furan), 8.04 (d, *J* = 16.2 Hz, 1H, C<u>H</u>=CH). Anal.Calcd. for $C_{26}H_{24}ClNO_6$: C, 64.80; H, 5.02; N, 2.91. Found: C, 64.82; H, 4.43; N, 2.77.

4.1.14.3. 4,9-Dimethoxy-7-[2-(4-methoxyphenyl)vinyl]-6-(morpholin-4-ylmethyl)-5H-furo[3,2-g]chromen-5-one ***(25c)***

$C_{27}H_{27}NO_7$ (477.51), Yield % 82, mp. 159-161°C; IR (KBr, cm^{-1}): 1640 (C=O). MS (m/z) rel.intensity: 477 [M^+, 10.44], 392 (100). ^{1}H-NMR (DMSO-d_6) δ ppm: 2.48 (t, *J* = 4.2 Hz, 4H, $2CH_2$), 3.53 (t, *J* = 4.2 Hz, 4H, $2CH_2$), 3.61 (s, 2H, CH_2), 3.83 (s, 3H, OCH_3), 3.97 (s, 3H, OCH_3), 4.19 (s, 3H, OCH_3), 7.06 (d, *J* = 16.0 Hz, 1H, CH=C<u>H</u>), 7.21 (d, *J* = 8.4 Hz, 2H, benz), 7.39-7.57 (m, 3H, 2H, benz + 1H, furan), 7.68 (d, *J* = 2.1Hz, 1H, furan), 8.07 (d, *J*=

16.0Hz, 1H, C<u>H</u>=CH). Anal.Calcd. for $C_{27}H_{27}NO_7$: C, 67.91; H, 5.70; N, 2.93. Found: C, 67.64; H, 5.70; N, 2.90.

4.1.14.4. 4,9-Dimethoxy-7-[2-(2-methoxyphenyl)vinyl]-6-(morpholin-4-ylmethyl)-5H-furo[3,2-g]chromen-5-one ***(25d)***

$C_{27}H_{27}NO_7$ (477.51), Yield % 80, m.p. 151-153°C; IR (KBr, cm^{-1}): 1615 (C=O). MS (m/z) rel.intensity: 477 [M^+, 21.1], 199 (100). ^{1}H-NMR (DMSO-d_6) δ ppm: 2.49 (t, *J* = 4.2 Hz, 4H, $2CH_2$), 3.59 (t, *J* = 4.2 Hz, 4H, $2CH_2$), 3.93 (s, 2H, CH_2), 3.93 (s, 3H, OCH_3), 3.97 (s, 3H, OCH_3), 4.19 (s, 3H, OCH_3), 7.03 (t, *J* = 8.4 Hz, 1H, benz), 7.23 (d, *J* = 16.2 Hz, 1H, CH=C<u>H</u>), 7.41-7.51 (m, 3H, 2H, benz + 1H, furan), 7.56 (d, *J* = 8.4 Hz, 1H, benz), 7.95 (d, *J* = 2.1 Hz, 1H, furan), 8.09 (d, *J* = 16.2 Hz, 1H, C<u>H</u>=CH). Anal.Calcd. for $C_{27}H_{27}NO_7$: C, 67.91; H, 5.70; N, 2.93. Found: C, 68.25; H, 5.46; N, 3.03.

4.1.14.5. 4,9-Dimethoxy-7-[2-(4-dimethylaminophenyl)vinyl]-6-(morpholin-4-ylmethyl)-5H-furo- [3,2-g]chromen-5-one ***(25e)***

$C_{28}H_{30}N_2O_6$ (490.55), Yield % 78, m.p. 163-165°C; IR (KBr, cm^{-1}): 1615 (C=O). MS (m/z) rel.intensity: 490 [M^+, 8.04], 254 (100). ^{1}H-NMR ($CDCl_3$) δ ppm: 2.64 (s, 6H, $N(CH_3)_2$), 2.64 (t, *J* = 4.2 Hz, 4H, $2CH_2$), 3.70-3.72 (m, 6H, 4H $2CH_2$ + 2H CH_2), 4.09 (s, 3H, OCH_3), 4.26 (s, 3H, OCH_3), 7.03 (d, *J* = 16.2 Hz, 1H, CH=C<u>H</u>), 7.03 (d, *J* = 8.4 Hz, 2H, benz), 7.27-7.54 (m, 3H, 2H, benz + 1H, furan), 7.62 (d, *J* = 2.1 Hz, 1H, furan), 7.66 (d, *J* = 16.2 Hz, 1H, C<u>H</u>=CH). Anal.Calcd. for $C_{28}H_{30}N_2O_6$: C, 68.65; H, 6.16; N, 5.71. Found: C, 68.61; H, 6.01; N, 5.84.

4.2. In vitro antitumor screening in NCI (Bethesda, MD, USA)

The chosen compounds were subjected to the *in vitro* disease-oriented human cells screening panel assay to be evaluated for their anticancer cytotoxic activity. A primary *in vitro* one-dose anticancer assay was performed using the full NCI 60 cell panel in accordance with the

current protocol of the Drug Evaluation Branch, NCI, Bethesda, MD, USA. In this protocol, all compounds submitted to the NCI 60 cell screen, are tested initially at a single high dose in the full NCI 60 cell panel including leukemia, non-small cell lung, colon, CNS, melanoma, ovarian, renal, prostate and breast cancer cell lines. These cell lines were incubated with one concentration (10 μM) for each tested compound. A 48h continuous drug exposure protocol was used, and a sulphorhodamine B (SRB) protein assay was employed to estimate cell viability or growth [40]. The data obtained are a mean graph of the percent growth of treated cells, and presented as percentage growth inhibition (GI %) caused by the test compounds.

Only compounds which satisfy pre-determined threshold inhibition criteria set forth by the Development Therapeutic Program (DTP) were selected for progress to the five-dose screen. This was performed under sterile conditions, where cell lines were grown in RPMI 1640 media (Gibco, NY, USA) supplemented with 10% fetal bovine serum (Biocell, CA, USA); 5×10^4 cell/ml was used to test the growth inhibition activity of the synthesized compounds. Concentrations of the compounds ranging from 0.01 to 100 μM were prepared in buffer saline. Each compound was initially solubilized in dimethyl sulfoxide (DMSO), however, each final dilution contained less than 1% DMSO. Solutions of different concentrations (0.2 ml) were pipetted into separate wells of a microtiter tray in duplicates. Cell culture (1.8 ml) containing a cell population of 6×10^4 cells/ml was pipetted into each well. Controls, containing only phosphate buffer, saline and DMSO at identical dilutions, were also prepared in the same manner. These cultures were incubated in a humidified incubator at 37 °C. The incubator was supplied with 5% CO_2 atmosphere. After 48 h, cells in each well were diluted 10 times with saline and counted by using a coulter counter. The counts were corrected for the dilution. The GI_{50}, TGI, LC_{50} results are listed in Tables 1-4.

4.3. Kinase inhibition assay

To determine whether the activity of the synthesized flavonoids is related to their enzyme inhibition ability of protein kinase targets, profiling of the most active compounds in this study against CDK2, CDK4 and GSK-3β was performed. The kinase assay was carried out at a single concentration (20 μM) in duplicate and the data enlisted in Table 4 corresponds to the average of both experiments.

CDK2, CDK4 and GSK-3β were cloned, expressed and purified. The selected compounds were used to make stock solutions which were then diluted to form the assay solutions used for profiling against the selected kinases.

A radioisotope assay format was used for profiling evaluation of the kinase targets and all assays were performed in a designated radioactive working area. Protein kinase assays were performed at ambient temperature for 20-30 minutes in a final volume of 25 μl according to the following assay reaction recipe:

Component 1: 5 μl of diluted active protein kinase target (~10-50 nM final protein concentration in the assay)

Component 2: 5 μl of stock solution of substrate (1-5 μg of peptide or protein substrate)

Component 3: 5μl of kinase assay buffer or protein kinase activator in kinase assay buffer

Component 4: 5 μl of compound (100 μM) or 10% DMSO

Component 5: 5 μl of ^{33}P-ATP (250 M stock solution, 0.8 Ci)

The assay was initiated by the addition of ^{33}P-ATP and the reaction mixture incubated at ambient temperature for 30 minutes. After the incubation period, the assay was terminated by spotting 10 μl of the reaction mixture onto Multiscreen phosphocellulose P81 plate. The plate was washed 3 times for approximately 15 minutes each in a 1% phosphoric acid solution. The radioactivity on the P81 plate was counted in the presence of scintillation fluid in a Trilux scintillation counter.

Blank control was set up for each protein kinase target which included all the assay components except the addition of the appropriate substrate (replaced with equal volume of assay dilution buffer). The corrected activity for each kinase target was determined by removing the blank control value.

The results for profiling of the ten selected compounds against CDK2, CDK4 and GSK-3β protein kinase targets are displayed as % inhibition compared to control and presented in Table 4.

*4.4. Evaluation of antitumor effects of **25e** on Ehrlich solid tumor*

Tumor was generated in female BALB/mice purchased from National Cancer Institute, Egypt. Ehrlich ascites carcinoma (EAC) cells were implanted subcutaneously (S.C.) by inoculation of (2×10^5 tumor cells/mouse) into the left hind legs at the volume of 0.2 ml of saline. Tumors were permitted to grow for the following 2 wk. The animals received i.p. injections of saline (for control), **25e** (3 mg/kg) and **25e** (6 mg/kg), daily for 5 days. Tumor sizes were measured by two-dimensional measurements using a vernier caliper. Tumor size was calculated by the formula $V = 0.4 \times a(b)^2$, where "a" and "b" are the long and short axes of tumor, respectively [45]. Initial and final tumor sizes were calculated on the first treatment day (just before dosing) and the last treatment day (24 hours after the last dose), respectively.

4.5. Determination of cyclin D1 and GSK-3β levels in tumor tissue

Tumors were excised, washed with cold saline and dried carefully with filter paper. For each sample, 10% homogenate was prepared in phosphate buffer saline. Quantitative determination of cyclin D1 and GSK-3β was carried out using competitive enzyme-linked immunosorbent assay (ELISA; Cytoimmune Science Inc., MD). Samples were run in duplicate according to the manufacturer's instructions.

4.6. Statistical analysis

Data are expressed as mean ± standard error of mean (SEM). Comparisons among different groups were performed by one-way analysis of variance ANOVA followed by Tukey–Kramer test. Comparison within the same group, before and after treatment was performed using paired student t-test The Graphpad Software Instat (version 9) was used to carry out the statistical analysis.

Notes

The authors declare no conflict of financial interest.

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Abbreviatons Used

δ: chemical shift in parts per million downfield from tetramethylsilane
µM: micro Molar
°C: degrees Celsius
Compd: compound
CDK: cyclin-dependent kinase
DMSO-d_6: dimethyl sulfoxide deuterated
g: gram(s)
h: hour(s)
J: coupling constant (in NMR spectrometry)

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Figure Captions

- **Figure 1:** Structures of some anticancer flavonoid derivatives.
- **Figure 2:** Oxidative cyclization pathway of 1-(6-hydroxy-4-methoxybenzofuran)-3-phenyl-prop-2-en-1-ones.
- **Figure 3:** Effect of **25e** on the growth of Ehrlich solid tumor; Mice were treated with **25e** (3 mg/kg; i.p.) and (6 mg/kg; i.p.) daily for 5 days. Tumor volume was determined before and at the end of treatment period. Data are presented as the mean ± SEM, n = 6. (a) indicates significant difference from control group. $p < 0.05$; ANOVA, Tukey-Kramer test. (b) indicates significant difference from the same group before treatment. $p < 0.05$; paired Student's t-test.
- **Figure 4:** Effect of **25e** on the tumor level of cyclin D1; Mice were treated with **25e** (3 mg/kg; i.p.) and (6 mg/kg; i.p.) daily for 5 days. The level of cyclin D1 was determined. Data are presented as the mean ± SEM, n = 6. (a) indicates significant difference from control group. (b) indicates significant difference from the low dose group. $p < 0.05$; ANOVA, Tukey-Kramer test.
- **Figure 5:** Effect of **25e** on the tumor level of GSK-3β; Mice were treated with **25e** (3 mg/kg; i.p.) and (6 mg/kg; i.p.) daily for 5 days. The level of GSK-3β was determined. Data are presented as the mean ± SEM, n = 6. (a) indicates significant difference from control group. (b) indicates significant difference from the low dose group. $p < 0.05$; ANOVA, Tukey-Kramer test.
- **Scheme I.** Reagents and conditions: (a) KOH, reflux, 1h; (b) ArCHO, ethyl alcohol, reflux, 24h; (c) allyl bromide, anhyd. K_2CO_3, dry acetone, reflux, 24h; (d) N,N-diethylaniline, reflux, 2h.
- **Scheme II.** Reagents and conditions: (a) SeO_2, n-butanol, reflux, 24h; (b) H_2O_2, NaOH, ethyl alcohol, reflux, 24h.
- **Scheme III.** Reagents and conditions: (a) 48% HBr, glacial acetic acid, reflux 1h; (b) allyl bromide, K_2CO_3, acetone, reflux 24h; (c) N,N-diethylaniline, reflux 2h.
- **Scheme IV.** Reagents and conditions: (a) $K_2Cr_2O_7$, H_2SO_4, glacial acetic acid, reflux 1h; (b) allyl bromide, K_2CO_3, acetone, reflux 24h; (c) N,N-diethylaniline, reflux 2h.
- **Scheme V.** Reagents and conditions: (a) ArCHO, Na, ethyl alcohol, 24h.
- **Scheme VI.** Reagents and conditions: (a) morpholine hydrochloride, formalin, ethyl alcohol, 24h; (b) ArCHO, Na, ethyl alcohol, 24h.

Table (1): Growth inhibitory concentration (GI_{50}, µM) of the most active flavonoids.

Cancer cell line	Compound									
	3a	**3d**	**6b**	**9a**	**9b**	**12a**	**12c**	**15d**	**25b**	**25e**
Leukemia										
CCRF-CEM	-	9.72	2.66	2.75	3.33	1.85	1.09	3.44	1.78	1.17
HL-60(TB)	-	-	1.72	-	-	2.33	2.72	-	-	1.55
K-562	4.43	-	2.88	2.87	3.25	3.21	2.8	3.51	3.28	1.85
MOLT-4	3.36	-	2.09	2.17	2.61	2.06	1.84	3.52	2.84	1.58
RPMI-8226	3.15	23.5	2.78	1.88	3.04	2.24	1.37	3.51	1.94	1.49
SR	3.27	-	-	2.15	2.77	-	-	3.16	-	-
Non-small cell Lung cancer										
A549/ATCC	8.62	>100	6.72	4.41	4.7	21.5	18.5	4.12	2.85	2.83
EKVX	12.5	33.71	4.18	6.13	7.23	-	-	4.41	2.85	2.24
HOP-62	11.3	19.3	6.82	5.32	2.31	2.49	2.98	4.98	3.24	2.33
HOP-92	1.64	2.98	1.39	1.33	1.69	11.5	16.8	2.07	1.28	0.24
NCI-H226	6.03	25.1	2.84	1.48	1.52	4.14	2.93	3.22	2.79	2.06
NCI-H23	6.12	22.4	3.25	2.52	2.78	2.44	2.8	2.88	3	2.56
NCI-H322M	13.6	>100	5.22	3.19	4.88	9.69	5.06	5.93	2.54	1.65
NCI-H460	3.02	>100	2.99	2.79	3.2	11.3	4.7	3.46	2.05	2.02
NCI-H522	4.29	>100	2.8	2.15	1.88	17.6	34.6	3.16	1.77	1.83
Colon cancer										
Colo-205	15.7	>100	1.76	5.96	4.44	27.7	27.6	7.64	2.76	1.66
HCC-2998	3.76	>100	2.5	1.73	1.79	16.1	13.7	2.85	2.85	2.62
HCT-116	3.72	40.4	2.83	3.03	2.74	1.7	1.06	3.29	2.92	2.11
HCT-15	5.25	>100	3.43	2.91	3.4	2.52	1.73	3.6	2.78	2.25
HT-29	6.34	>100	4.11	3.01	1.89	25.1	18.1	3.98	2.69	2.2
KM-12	3.71	>100	3.49	4.06	3.72	16.5	12.3	3.49	2.1	1.73
SW-620	3.52	>100	2.71	3.32	2.56	2.16	2.3	4.24	3.32	2.38
CNS cancer										
SF-268	4.05	22.2	3.5	2.8	3.25	4.23	4.38	3.73	2.81	2.22
SF-295	7.83	16.6	6.67	2.44	2.77	13.6	3.4	3.67	2.01	1.82
SF-539	3.22	24.3	-	1.71	1.79	3.59	4.14	5.45	-	-
SNB-19	7.33	30.8	8.24	2.85	2.85	12	3.16	5.83	-	1.98
SNB-75	2.42	7.49	1.8	1.39	2.01	8.3	3.13	1.44	2.41	1.39
U251	4.39	18.9	3.22	2.36	2.3	3.06	2.53	3.53	3.05	2.49
Melanoma										
LOXIMVI	1.69	30.1	2.2	1.56	1.63	1.48	1.08	3.13	2.18	-
MALME-3M	-	18.9	2.61	5.8	2.74	3.47	3.79	19.8	2.24	2.08

M14	8.89	>100	4.82	5.36	5.08	3.32	2.24	8.19	2.76	2.34
MDA-MB-435	3.21	5.69	3.05	2.78	2.06	4.83	2.81	3.12	2.05	2.05
SK-MEL-2	12.4	>100	4.16	3.04	3.97	10.6	9.06	6.06	1.65	1.39
SK-MEL-28	7.03	>100	9.86	2.49	2.32	2.73	2.52	5.21	2.17	2.3
SK-MEL-5	3.87	>100	3.33	2.66	2.07	3.04	1.69	3.91	1.24	1.09
UACC-257	7.01	>100	4.98	2.56	2.39	11.5	3.63	6.41	2.35	2.54
UACC-62	4.15	23.7	3.47	2.74	2.88	5.45	1.77	4.14	1.9	1.86
Ovarian cancer										
IGROV-1	3.51	26	3.04	3.35	3.46	17.5	13.6	5.27	1.94	1.79
OVCAR-3	3.76	47.4	1.91	2.46	1.97	5.19	3.92	3.28	2.34	1.76
OVCAR-4	5.4	>100	3.28	2.89	2.77	3.2	3.8	3.34	2.75	2.47
OVCAR-5	5.67	>100	6.04	3.22	2.8	22.3	11.7	3.57	2.76	2.59
OVCAR-8	3.63	53.8	3.43	3.13	3.04	2.86	2.83	3.87	2.78	5.15
NCI/ADR-RES	2.42	29.6	3.07	1.88	2.23	3.01	2.84	2.82	3.12	2.63
SK-OV-3	14.1	21.3	1.11	8.08	5.96	6.34	4.73	5.45	3.56	2.72
Renal cancer										
786-0	16.2	35.3	2.99	4.12	4.83	26.9	16.8	6.29	3.09	1.93
A498	7.97	46	3.92	1.74	2.77	7.44	17.2	5.81	-	2.34
ACHN	4.51	30	5.09	3.79	2.31	20.8	19.3	4.32	2.47	2.16
CAKI-1	8.07	>100	3.35	2.8	2.61	15.4	5.03	3.15	2.45	1.89
RXF 393	3.56	14.9	2.37	1.85	1.72	4.26	2.15	2.47	2.36	1.81
SN12C	4.48	>100	3.77	3.79	4.96	2.71	2.32	4.85	3.05	2.45
TK-10	11.2	21.9	3.48	4.96	3.62	-	-	6.5	5.32	2.46
UO-31	3.14	>100	2.59	2.89	2.88	3.63	3.17	4.98	-	1.43
Prostate Cancer										
PC-3	7.01	>100	2.94	3.68	3.81	3.23	2.58	5.25	2.5	1.69
DU-145	3.81	23.5	4.07	4.92	3.85	10.7	4.53	3.19	2.63	1.67
Breast Cancer										
MCF-7	3.52	9.56	2.36	2.73	2.51	2.72	1.63	2.92	2.1	1.88
MDA-MB-231/ATCC	4.38	33.1	4.33	3.11	4.3	4.1	4.22	4.59	2.92	2.22
HS-578T	2.76	38.2	-	3.9	4.02	7.2	10.4	5.29	1.9	-
BT-549	7.72	28.2		2.63	3	2.88	2.14	4.44	2.38	-
HF478T	-	-	3.12	-	-	-	-	-	-	1.68
PC-549	-	-	2.54	-	-	-	-	-	-	2.87
T-47D	11.05	55.9	3.38	4.11	3.29	2.14	2.03	4.34	4.31	2.21
MDA-MB-468	6.77	10.3	2.13	3.07	2.27	7.02	3.58	2.46	2.12	1.76

Table (2): Median GI_{50} (µM) of *in vitro* cytotoxic screening of the most active flavonoids.

Cancer cell Line	Compound									
	3a	**3d**	**6b**	**9a**	**9b**	**12a**	**12c**	**15d**	**25b**	**25e**
Leukemia	3.55	16.6	2.42	2.36	3	2.33	1.96	3.42	2.46	1.53
Non-small cell lung	7.45	20.69	4.02	3.25	3.35	10.08	11.04	3.8	2.48	1.97
Colon	6	40.4	2.97	3.43	2.93	13.01	10.01	4.15	2.77	2.13
CNS	4.82	20.04	4.68	2.25	2.49	7.46	3.45	3.94	2.57	1.98
Melanoma	6.03	19.75	4.27	3.22	2.79	5.15	3.17	6.69	2.06	1.98
Ovarian	5.49	35.6	3.12	3.57	3.17	8.62	6.2	3.94	2.75	2.73
Renal	7.39	29.6	3.44	3.24	3.21	11.59	9.42	4.77	3.12	2.05
Prostate	5.41	23.5	3.5	4.3	3.83	6.96	3.55	4.22	2.56	1.68
Breast	6.03	27.26	2.97	3.25	3.23	4.34	4	4	2.62	2.31

Table (3): Median GI_{50}, TGI and LC_{50} (µM) for the most active flavonoids.

MG-MID	Compound									
	3a	**3d**	**6b**	**9a**	**9b**	**12a**	**12c**	**15d**	**25b**	**25e**
GI_{50}	5.24	38.9	3.63	2.95	2.88	5.62	4.16	4.07	2.45	1.9
TGI	18.62	89.12	28.84	11.22	10.96	35.48	26.3	58.88	33.11	5.24
LC_{50}	54.95	100	87.09	41.68	37.15	87.09	72.44	100	87.09	60.25

Table (4): Results of *in vitro* enzyme assay against the kinases CDK2, CDK4 and GSK-3β (percentage activity change).

Tested Kinase	Compound									
	3a	**3d**	**6b**	**9a**	**9b**	**12a**	**12c**	**15d**	**25b**	**25e**
CDK2/cyclin E1	-1	-6	-5	0	-3	-6	0	0	0	0
CDK4/cyclin D1	-13	0	-15	0	0	0	0	-9	-6	-12
GSK-3β	-28	-17	-30	-38	-80	-58	-11	-56	5	13

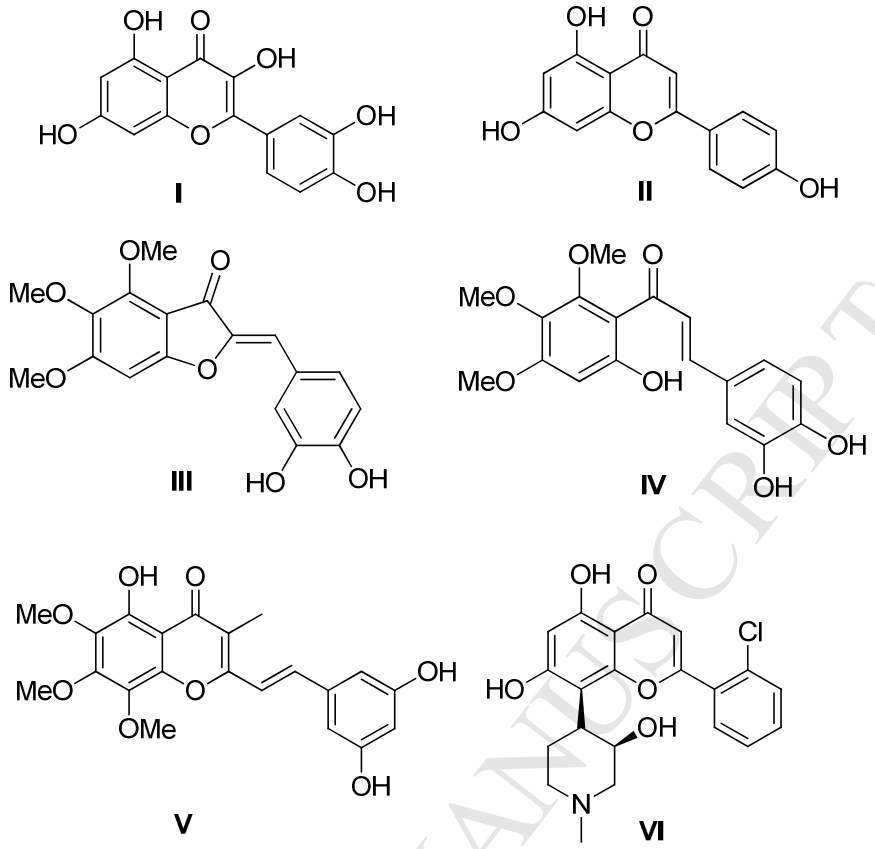


Figure 1: Structures of some anticancer flavonoid derivatives

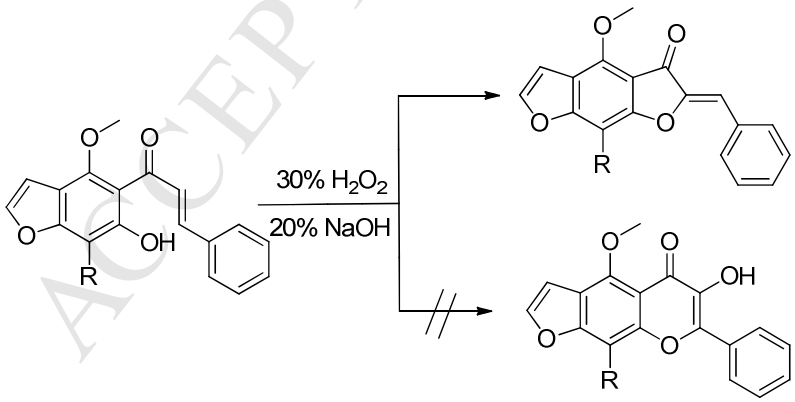


Figure 2: oxidative cyclization pathway of 1-(6-hydroxy-4-methoxybenzofuran)-3-phenylprop-2-en-1-ones

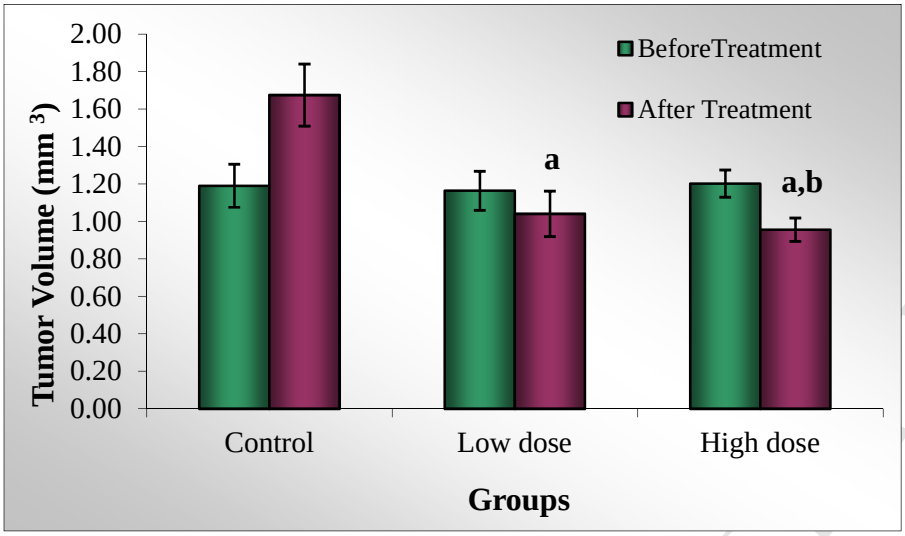


Figure 3: Effect of **25e** on the growth of Ehrlich solid tumor; Mice were treated with **25e** (3 mg/kg; i.p.) and (6 mg/kg; i.p.) daily for 5 days. Tumor volume was determined before and at the end of treatment period. Data are presented as the mean ± SEM, n = 6. (a) indicates significant difference from control group. $p < 0.05$; ANOVA, Tukey-Kramer test. (b) indicates significant difference from the same group before treatment. $p < 0.05$; paired Student's t-test.

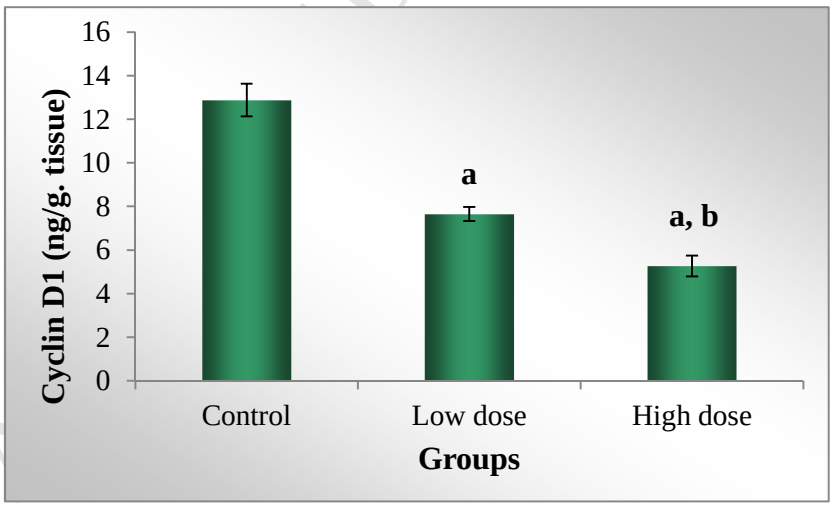


Figure 4: Effect of **25e** on the tumor level of cyclin D1; Mice were treated with **25e** (3 mg/kg; i.p.) and (6 mg/kg; i.p.) daily for 5 days. The level of cyclin D1 was determined. Data are presented as the mean ± SEM, n = 6. (a) indicates significant difference from control group. (b) indicates significant difference from the low dose group. $p < 0.05$; ANOVA, Tukey-Kramer test.

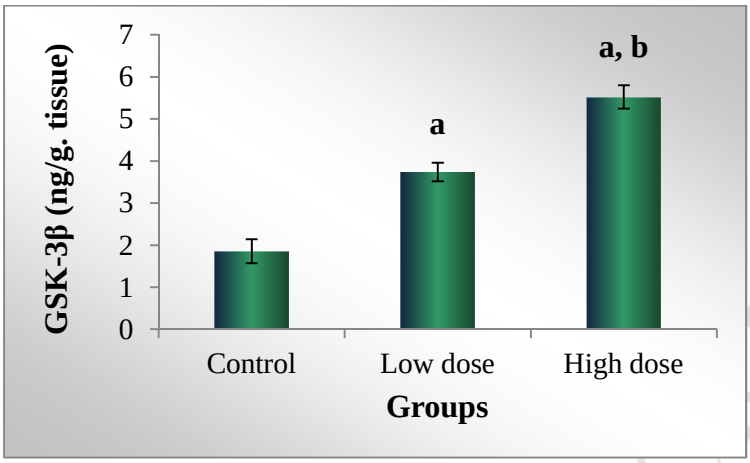
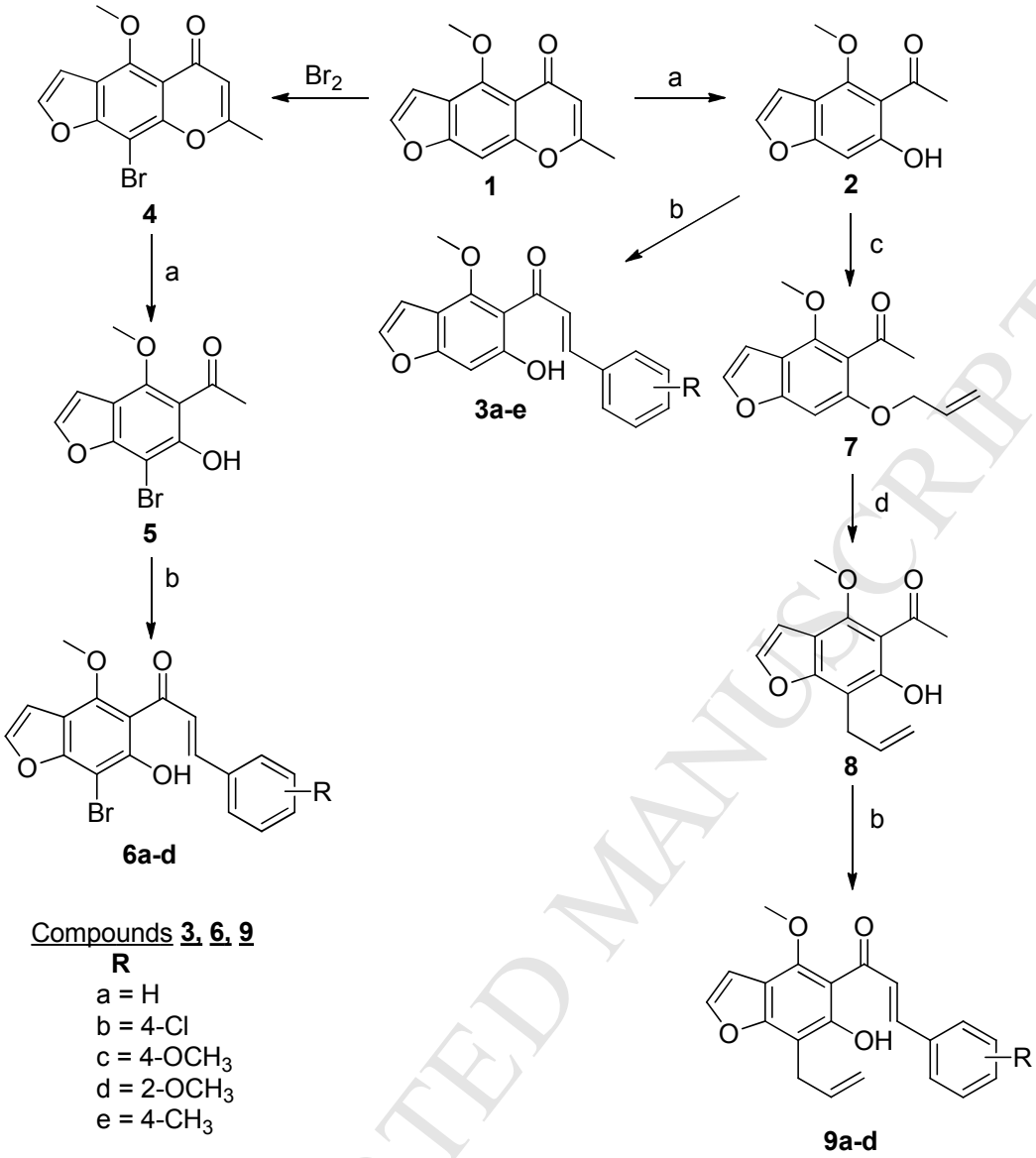


Figure 5: Effect of **25e** on the tumor level of GSK-3β; Mice were treated with **25e** (3 mg/kg; i.p.) and (6 mg/kg; i.p.) daily for 5 days. The level of GSK-3β was determined. Data are presented as the mean ± SEM, n = 6. (a) indicates significant difference from control group. (b) indicates significant difference from the low dose group. $p < 0.05$; ANOVA, Tukey-Kramer test.

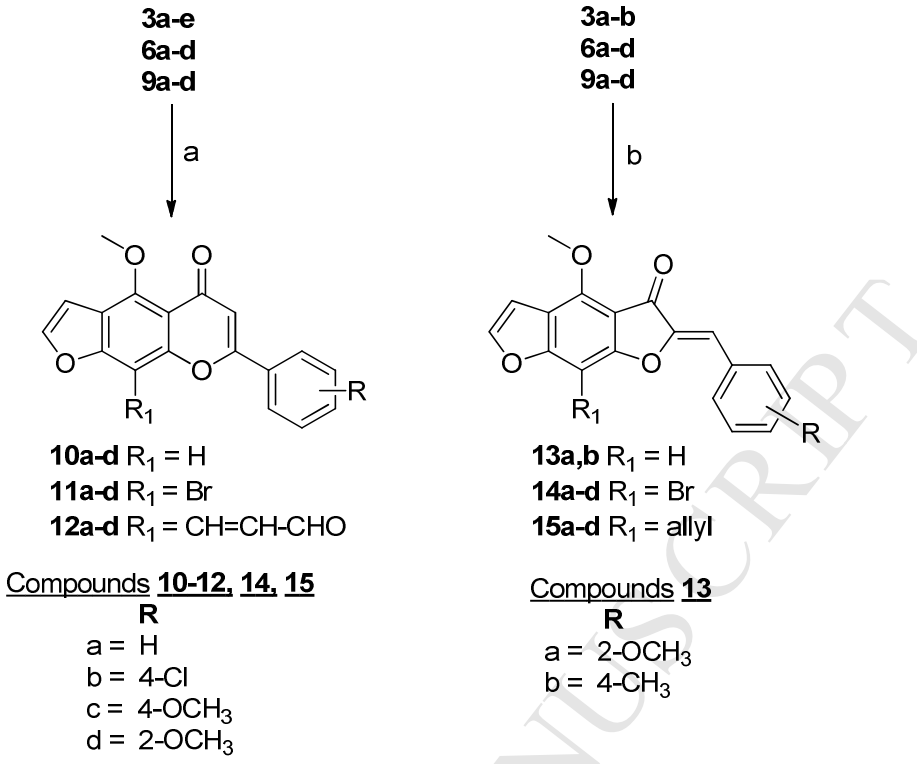


Compounds **3, 6, 9**

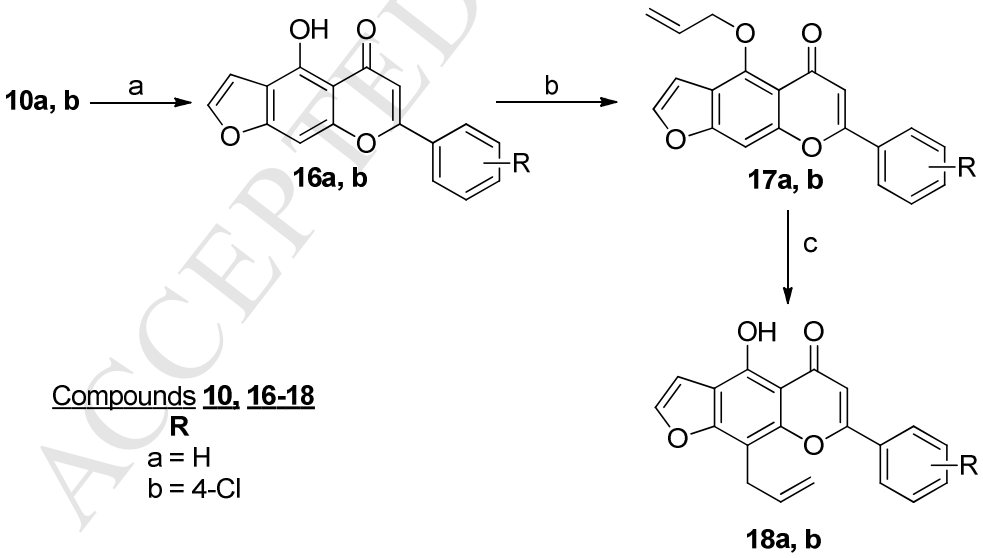
R

a = H
b = 4-Cl
c = 4-OCH_3
d = 2-OCH_3
e = 4-CH_3

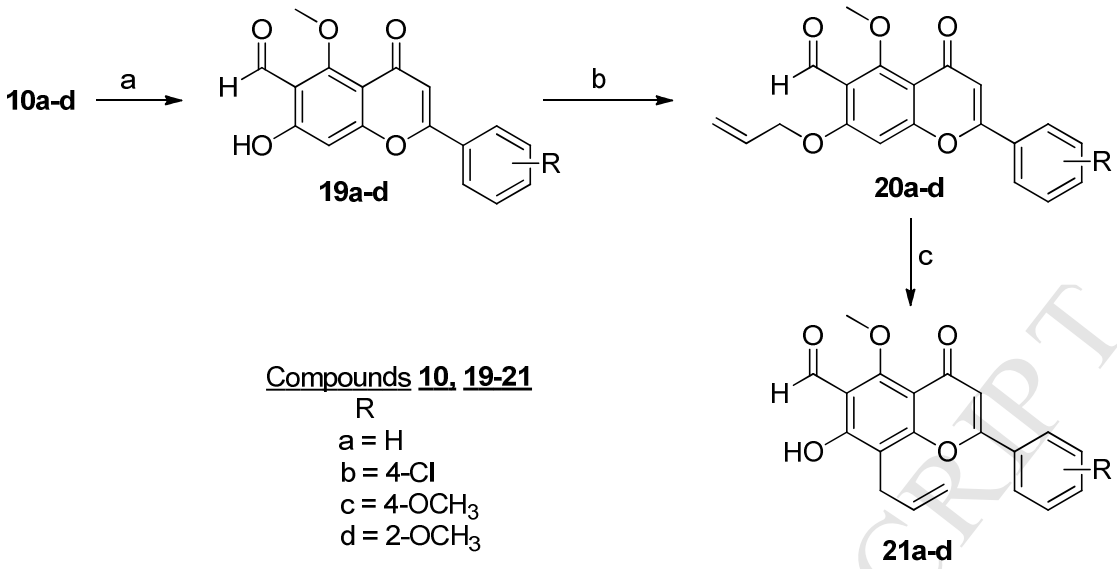
Scheme I. Reagents and conditions: (a) KOH, reflux, 1h; (b) ArCHO, ethyl alcohol, reflux, 24h; (c) allyl bromide, anhyd. K_2CO_3, dry acetone, reflux, 24h; (d) *N,N*-diethylaniline, reflux, 2h.



Scheme II. Reagents and conditions: (a) SeO_2, n-butanol, reflux, 24h; (b) H_2O_2, NaOH, ethyl alcohol, reflux, 24h.



Scheme III. Reagents and conditions: (a) 48% HBr, glacial acetic acid, reflux 1h; (b) allyl bromide, K_2CO_3, acetone, reflux 24h; (c) *N,N*-diethylaniline, reflux 2h.



Compounds **10, 19-21**

R

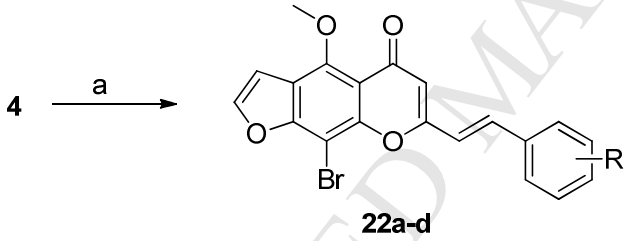
a = H

b = 4-Cl

c = 4-OCH_3

d = 2-OCH_3

Scheme IV. Reagents and conditions: (a) $K_2Cr_2O_7$, H_2SO_4, glacial acetic acid, reflux 1h; (b) allyl bromide, K_2CO_3, acetone, reflux 24h; (c) *N,N*-diethylaniline, reflux 2h.



Compounds **22**

R

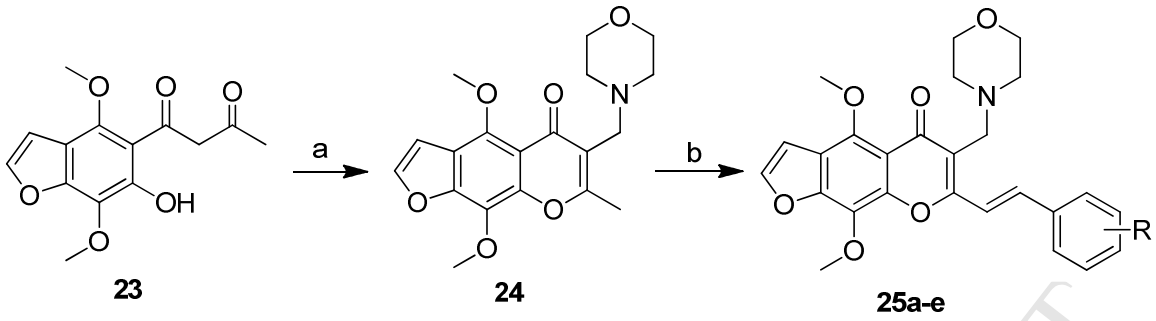
a = H

b = 4-Cl

c = 4-OCH_3

d = 4-$N(CH_3)_2$

Scheme V. Reagents and conditions: (a) ArCHO, Na, ethyl alcohol, 24h.



23 **24** **25a-e**

Compounds **25**
R
a = H
b = 4-Cl
c = 4-OCH_3
d = 2-OCH_3
e = 4-$N(CH_3)_2$

Scheme VI. Reagents and conditions: (a) morpholine hydrochloride, formalin, ethyl alcohol, 24h; (b) ArCHO, Na, ethyl alcohol, 24h.

Design, Synthesis and Insights into the Structure-Activity Relationship of Novel Semi-synthetic Flavonoids as Antiproliferative Agents

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KEYWORDS: furochalcones, furoflavones, furoaurones, furostyrylfurochromones, cytotoxicity, kinase inhibition.

Table (S1): Results of one-dose (10 μM) *in vitro* anticancer screen against a panel of 60 cell lines **(NCI, Bethesda, MD, USA).**

Compound	*Cell Panel*	*Sub-panel (% Growth Inhibition)*
3a	**Leukemia**	CCRF-CE (65), MOLT-4 (89), RPMI-8226 (86), SR (72)
	Melanoma	MDA-MB-435 (68)
	Colon cancer	HCT-116 (68), KM12 (64)
	CNS cancer	SNB-75 (86)
	Ovarian cancer	NCI/ADR-RES (68)
	Renal cancer	UO-31 (60)
	Prostate Cancer	DU-145 (75)
	Breast Cancer	MCF-7 (76)
3b	**Leukemia**	CCRF-CEM (60), RPMI-8226 (66), SR (63)
	Breast Cancer	MCF-7 (64), T-47D (67)
3d	**Leukemia**	CCRF-CEM (63), MOLT-4 (66), RPMI-8226 (86), SR (62)
	Non-small cell lung cancer	NCI-H460 (61)
	Colon cancer	HCT-116 (66), KM12 (63)
	Melanoma	MDA-MB-435 (74)
	Prostate Cancer	DU-145 (78)
	Breast Cancer	MCF-7 (60), MDA-MB-468 (84)
10c	**CNS cancer**	SF-295 (60), U251 (62)
	Renal cancer	UO-31 (62)
11b	**Ovarian cancer**	OVCAR-4 (72)
14a	**Leukemia**	CCRF-CEM (79), HL-60(TB) (64), MOLT-4 (85), RPMI-8226 (94), SR (79)
	Non-small cell lung cancer	NCI-H23 (78), NCI-H460 (83), NCI-H522 (62)
	Colon cancer	HCT-116 (77), HCT-15 (60), HT29 (72), SW-620 (60)
	CNS cancer	SF-295 (65), SF-539 (75), U251 (73)
	Melanoma	LOXIMV (65), MDA-MB-435 (74), SK-MEL-2 (88), SK-MEL-5 (67)
	Ovarian cancer	OVCAR-3 (86), OVCAR-8 (70), NCI/ADR-RES (79)
	Renal cancer	CAKI-1 (87), RXF 393 (93), TK-10 (61)
	Prostate Cancer	PC-3 (66)
	Breast Cancer	MCF-7 (84), BT-549 (66), MDA-MB-468 (93)
14b	**Leukemia**	CCRF-CEM (79), HL-60(TB) (74), MOLT-4 (85), RPMI-8226 (91), SR (82)
	Non-small cell lung cancer	NCI-H522 (64)
	Colon cancer	HCT-116 (83), HT29 (63), SW-620 (63)
	CNS cancer	SF-295 (67), U251 (62
	Melanoma	LOXIMV (65), MALME-3M (65), MDA-MB-435 (82), SK-MEL-2 (84), UACC-257 (61)
	Ovarian cancer	OVCAR-3 (67), NCI/ADR-RES (61)
	Renal cancer	CAKI-1 (80), RXF 393 (78)
	Prostate Cancer	PC-3 (69)
	Breast Cancer	MCF-7 (79), T-47D (60), MDA-MB-468 (80)
14c	**Leukemia**	SR (60)
	CNS cancer	SF-539 (60)
	Breast Cancer	MCF-7 (60)
	Leukemia	MOLT-4 (67), RPMI-8226 (71), SR (68)
	Colon cancer	HCT-116 (62)
	Renal cancer	CAKI-1 (61)
15d	**Leukemia**	CCRF-CEM (63), RPMI-8226 (71)
	Non-small cell lung cancer	NCI-H23 (80), NCI-H460 (69)
	Colon cancer	HCT-116 (79)
	CNS cancer	SNB-75 (64)

	Melanoma	LOXIMV (62)
	Ovarian cancer	OVCAR-8 (61), NCI/ADR-RES (65)
	Renal cancer	CAKI-1 (62), RXF 393 (75)
	Prostate Cancer	DU-145 (65)
	Breast Cancer	MCF-7 (67)
18c	**Non-small cell lung cancer**	HOP 92 (60)
20b	**Leukemia**	HL-60(TB) (75), K-562 (76), RPMI-8226 (96)
	Non-small cell lung cancer	HOP-92 (97), NCI-H23 (61), NCI-H460 (61), NCI-H522 (93)
	Colon cancer	HCT-116 (89), SW-620 (69)
	CNS cancer	U251 (68)
	Melanoma	LOXIMV (99), MDA-MB-435 (73), SK-MEL-2 (75)
	Ovarian cancer	IGROVI (62), OVCAR-3 (81), OVCAR-8 (66), NCI/ADR-RES (78)
	Renal cancer	786-0 (65), TK-10 (73), UO-31 (74)
	Prostate Cancer	PC-3 (76)
	Breast Cancer	MCF-7 (85), T-47D (72), MDA-MB-468 (91)
20c	**Non-small cell lung cancer**	HOP 92 (66)
22c	**Non-small cell lung cancer**	NCI-H522 (62)
25b	**Leukemia**	CCRF-CEM (81), HL-60(TB) (60), MOLT-4 (93), RPMI-8226 (60)
	Non-small cell lung cancer	HOP-92 (95), NCI-H460 (88)
	CNS cancer	SF-268 (67)
	Ovarian cancer	OVCAR-3 (68), OVCAR-8 (60)
	Renal cancer	ACHN (66), CAK-1 (61), UO-31 (61)
	Prostate Cancer	PC-3 (72)
	Breast Cancer	MDA-MB-468 (86)
	Renal cancer	ACHN (66), CAK-1 (61), UO-31 (61)
	Prostate cancer	PC-3 (72)
	Breast Cancer	MDA-MB-468 (86)
25c	**Leukemia**	CCRF-CEM (87), MOLT-4 (66), RPMI-8226 (61)
	Melanoma	SK-MEL-5 (84)
25e	**Leukemia**	CCRF-CEM (95), HL-60(TB) (64), K-562 (84), MOLT-4 (88), RPMI-8226 (80)
	Non-small cell lung cancer	NCI-H23 (80), NCI-H522 (84)
	Colon cancer	HCT-116 (82), HCT-15 (67), NHT29 (82), KM12 (73)
	CNS cancer	SNB-75 (99), U251 (69)
	Melanoma	LOXIMV (65), M14 (63), UACC-257 (76)
	Ovarian cancer	IGROVI (78), OVCAR-3 (88), OVCAR-8 (80), NCI/ADR-RES (87)
	Renal cancer	786-0 (95), ACHN (89), UO-31 (63)
	Prostate Cancer	PC-3 (87), DU-145 (93)
	Breast Cancer	MDA-MB-468 (70)

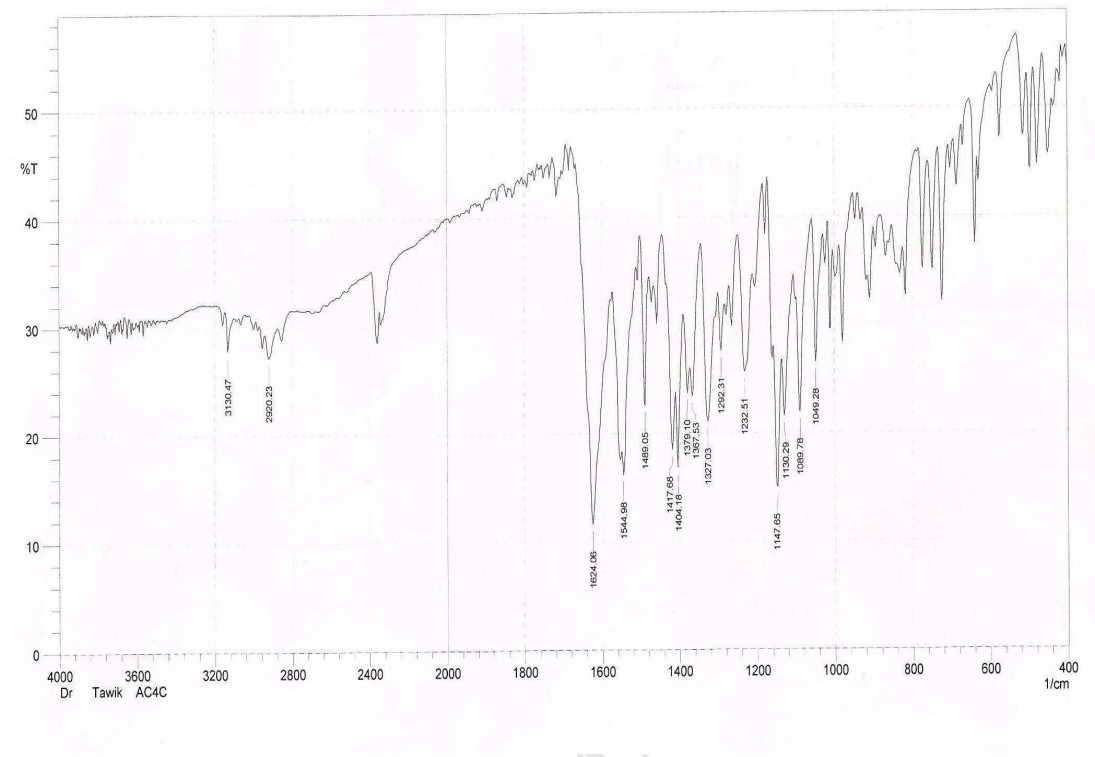


Figure S1: I.R. spectrum of compound **9b**.

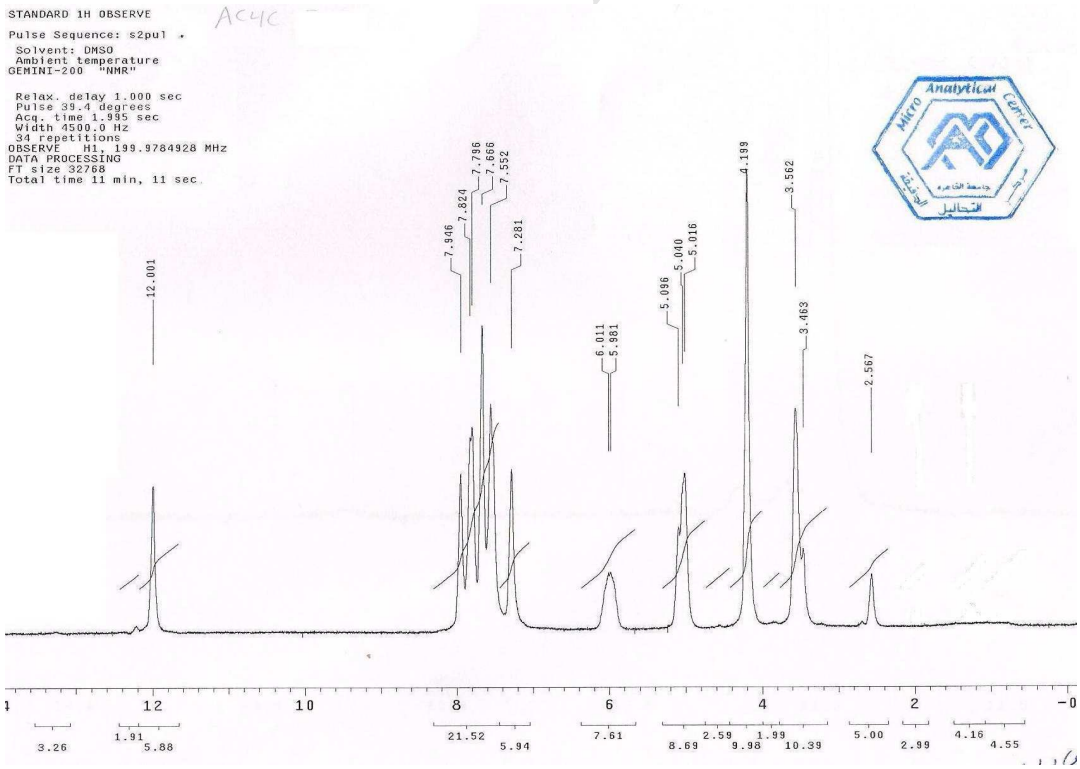


Figure S2: 1HNMR spectrum of compound **9b**.

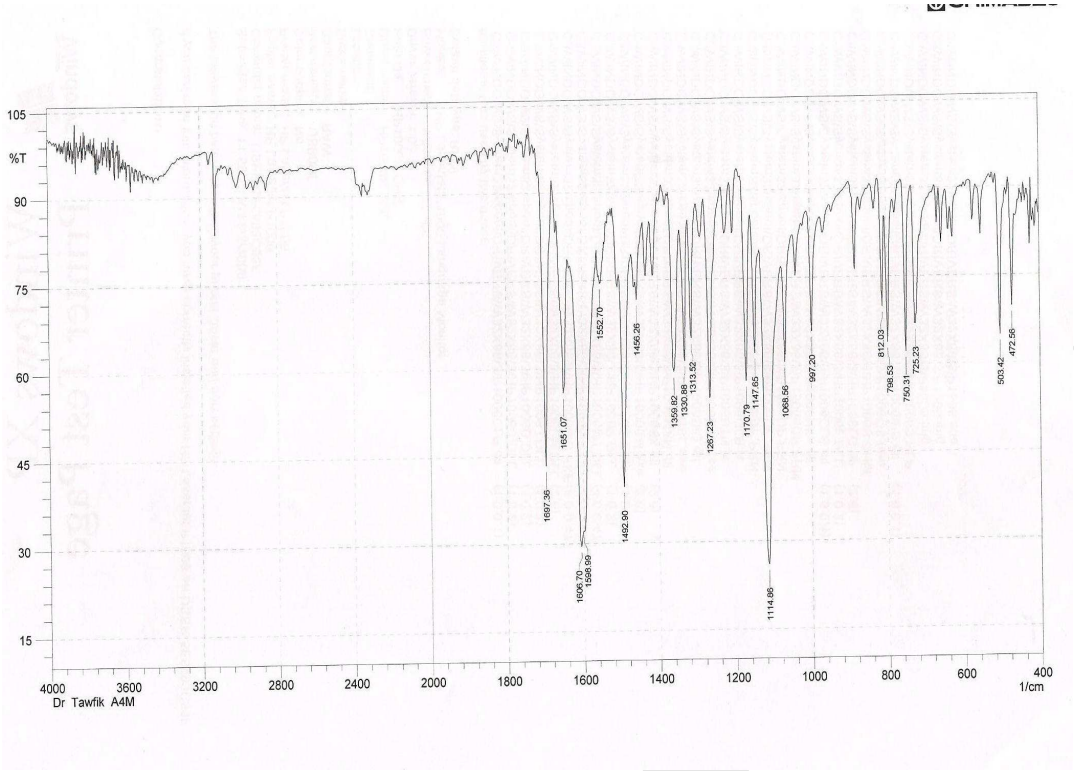


Figure S3: I.R. spectrum of compound **13b**.

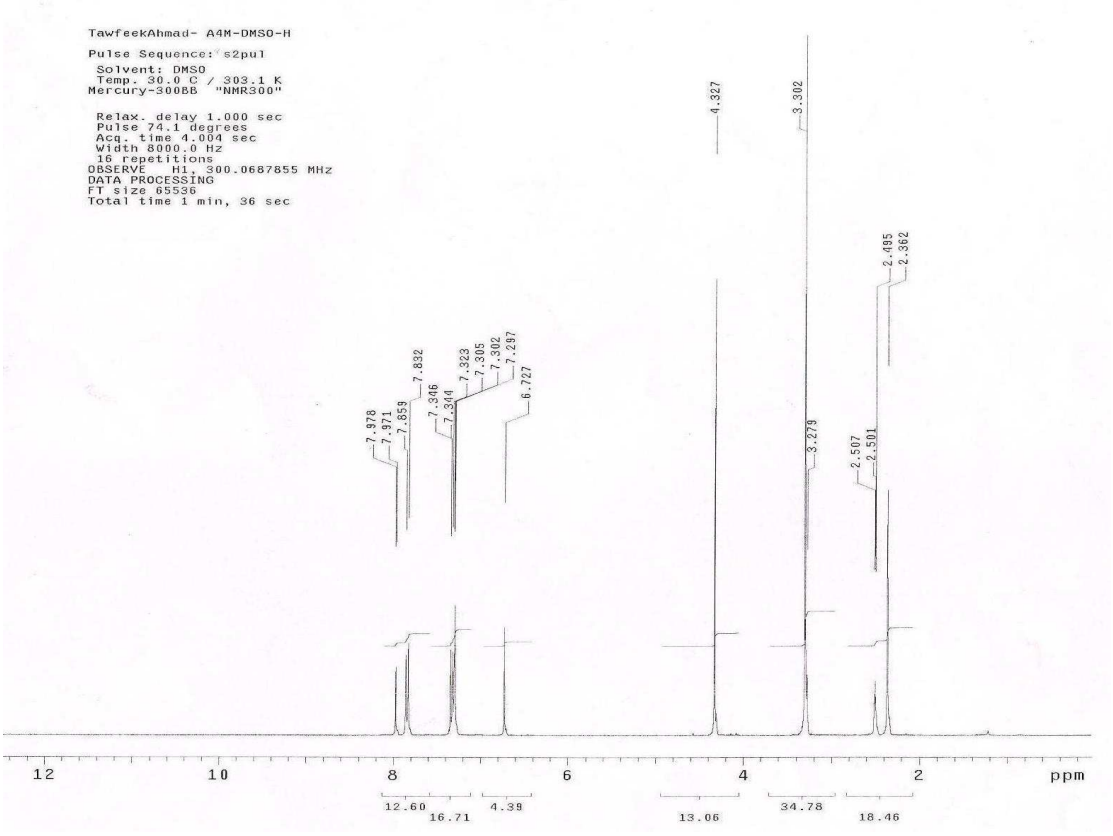


Figure S4: 1HNMR spectrum of compound **13b**.

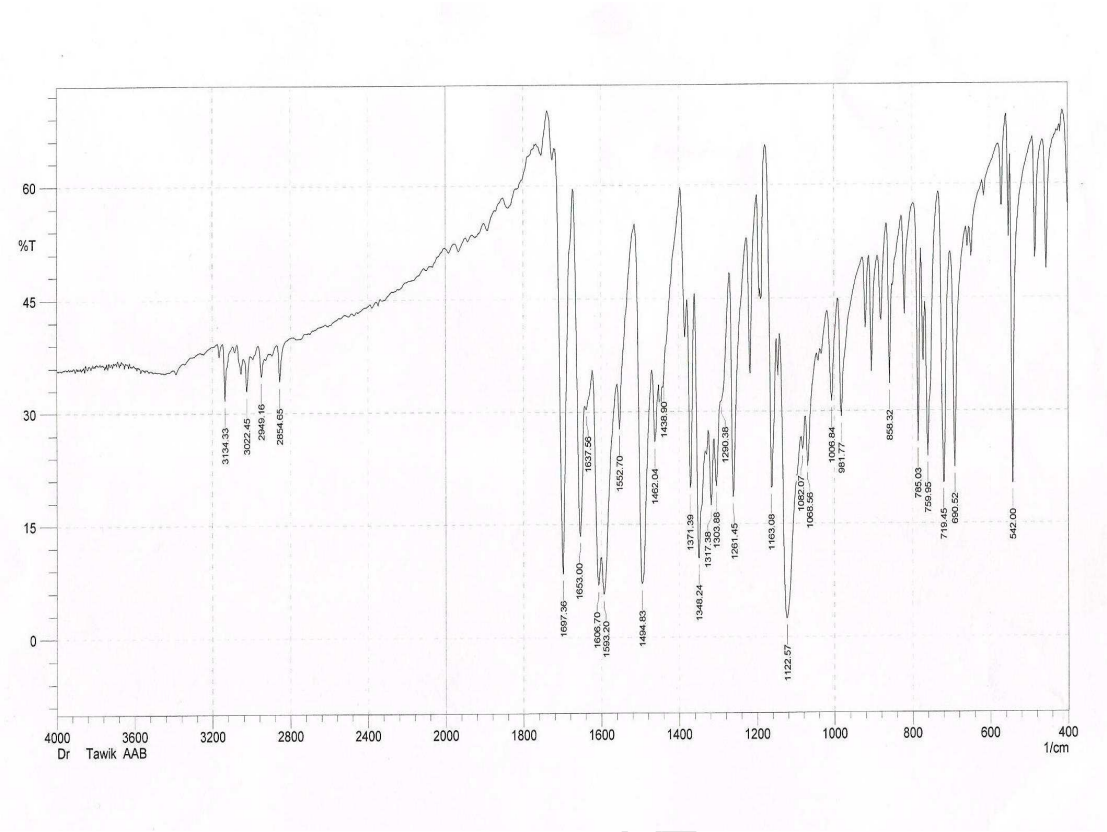


Figure S5: I.R. spectrum of compound **15b**.

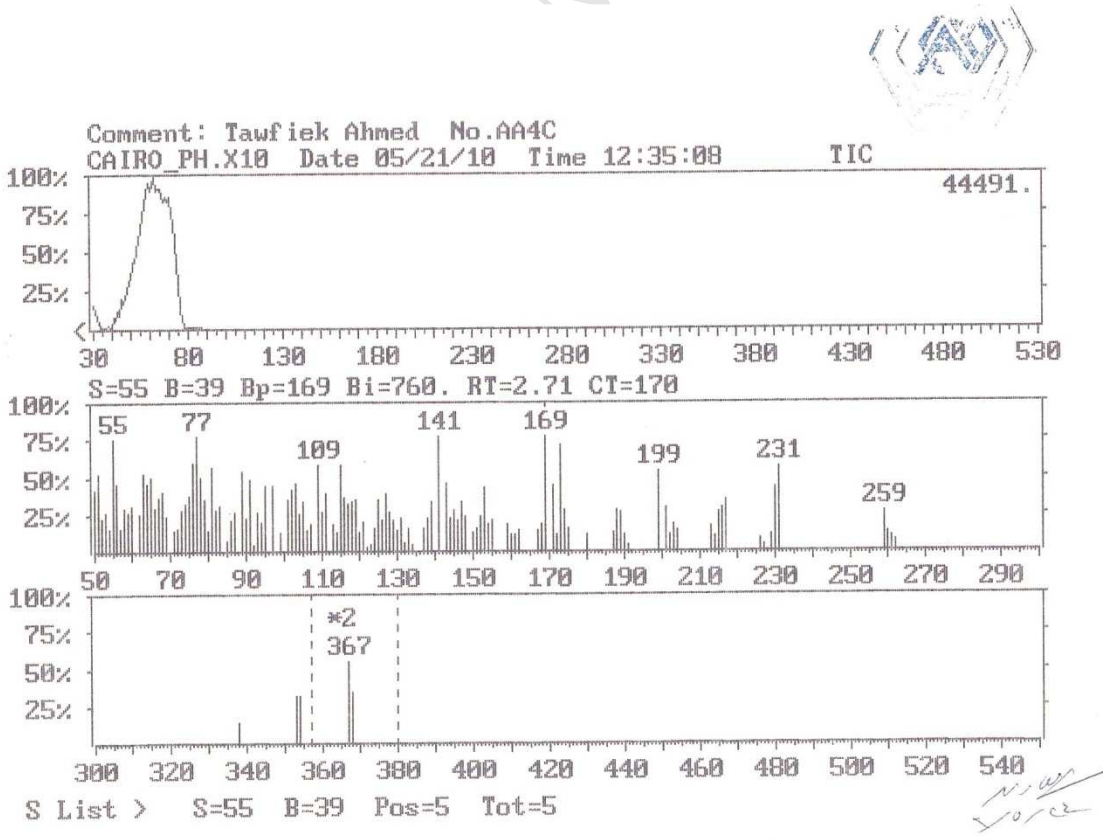


Figure S6: Mass spectrum of compound **15b**.

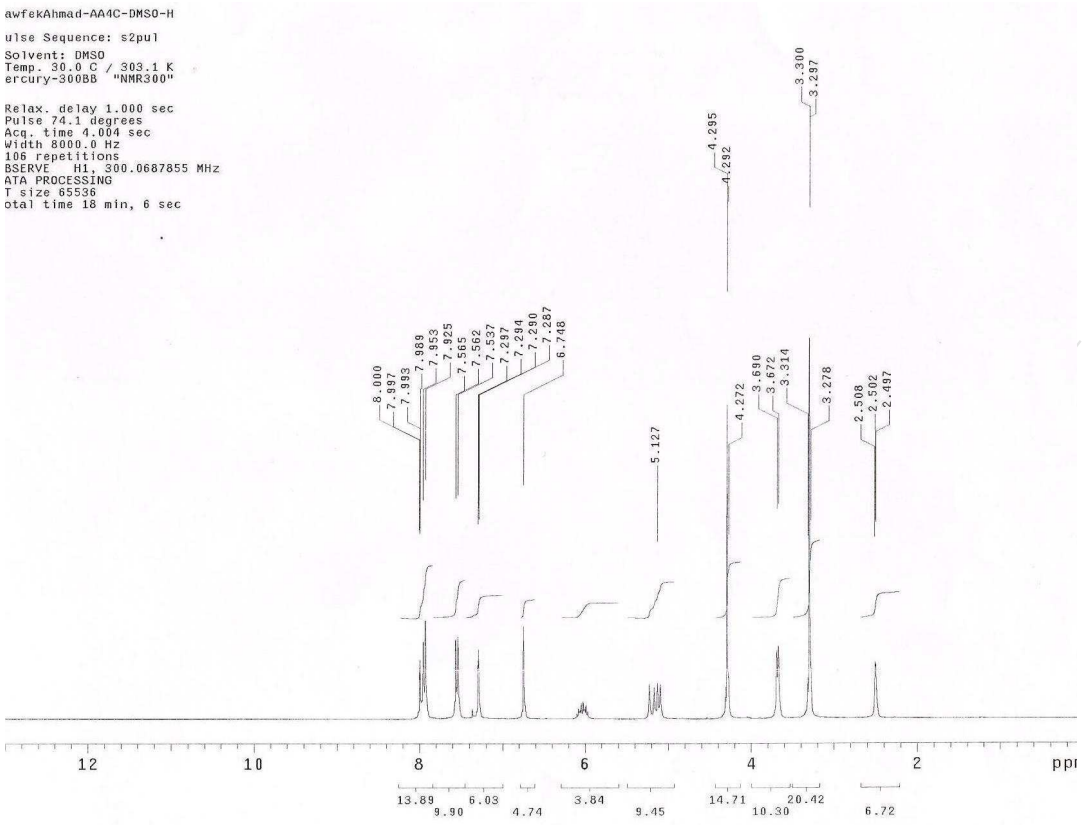


Figure S7: 1HNMR spectrum of compound **15b**.

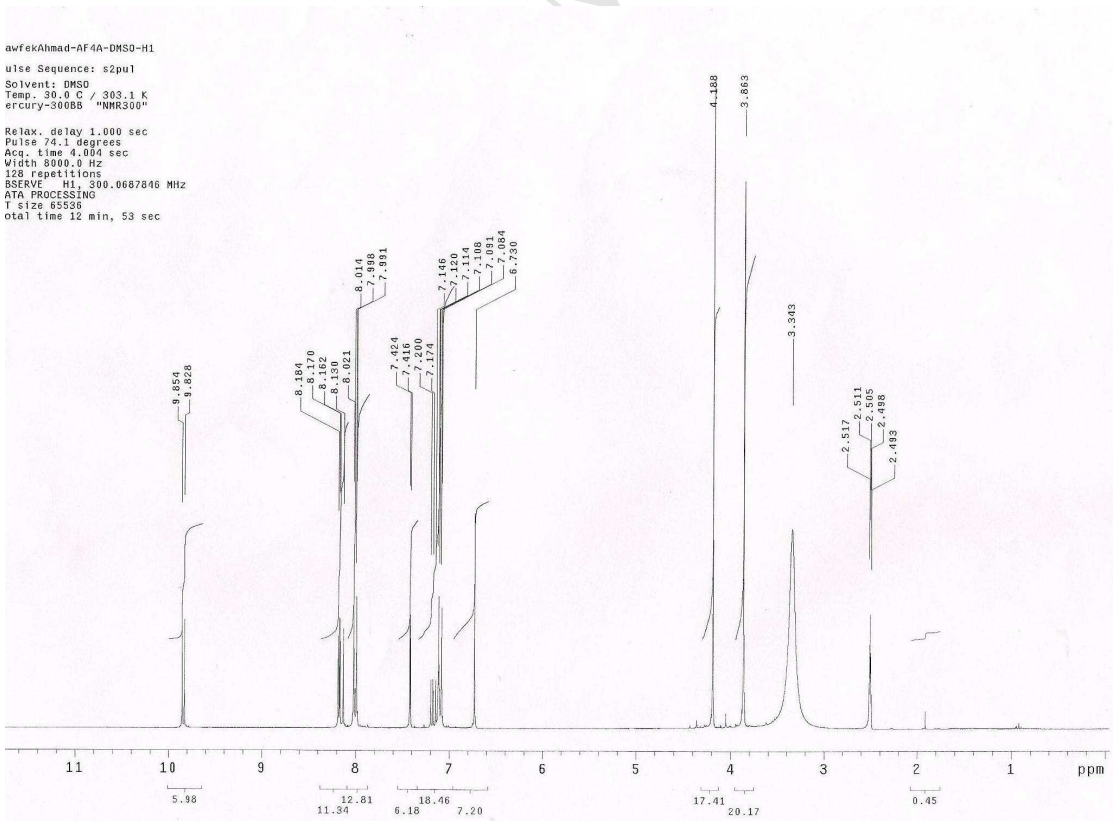


Figure S8: 1HNMR spectrum of compound **12c**.

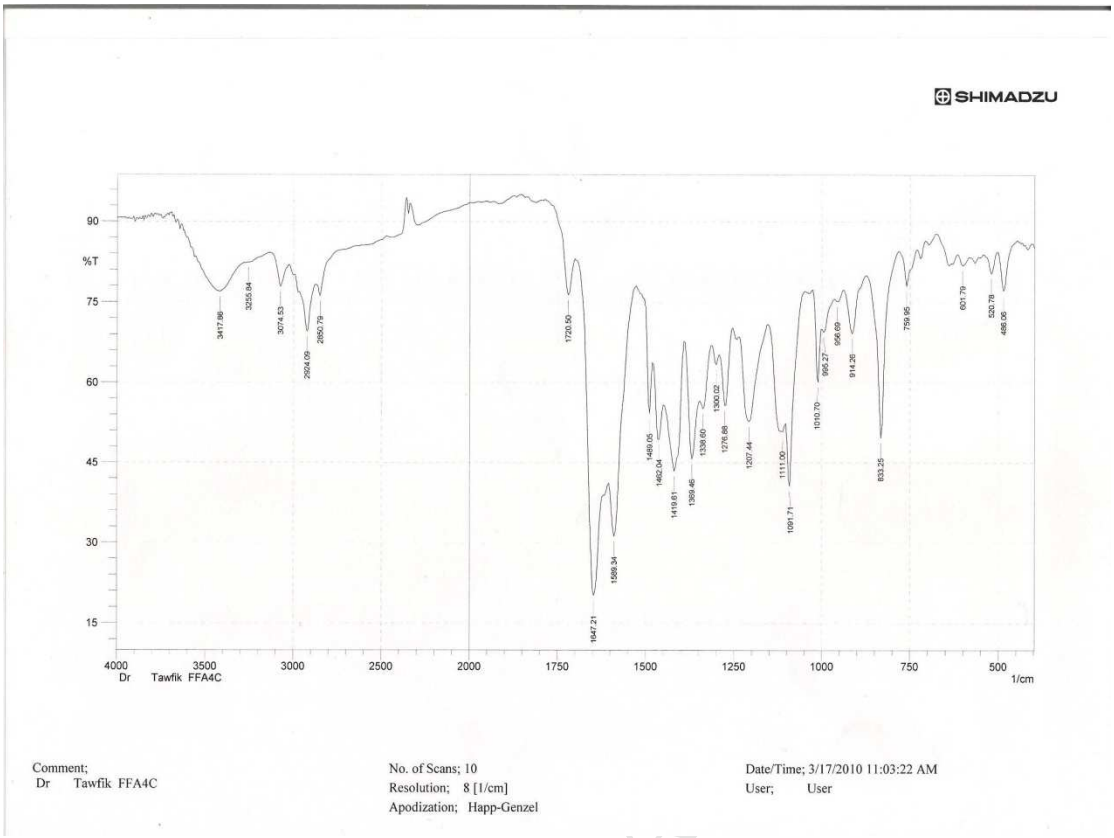


Figure S9: I.R. spectrum of compound **21c**.

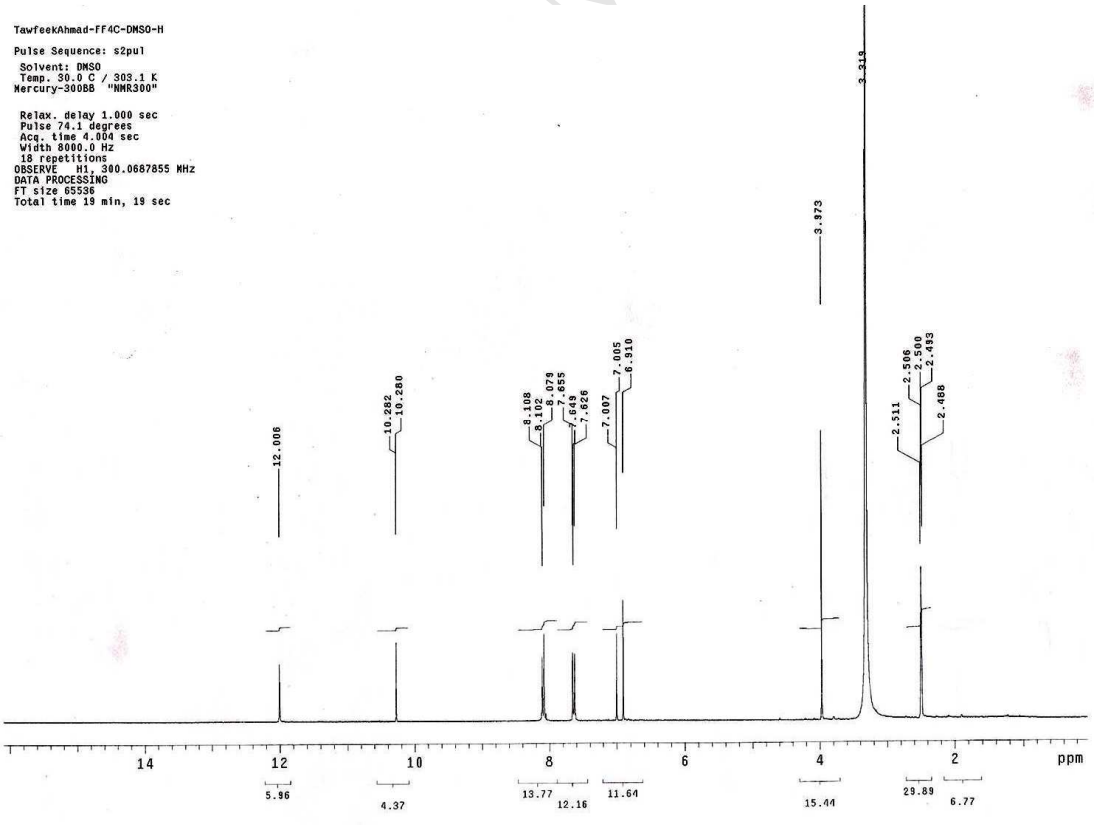


Figure S10: 1HNMR spectrum of compound **21c**.

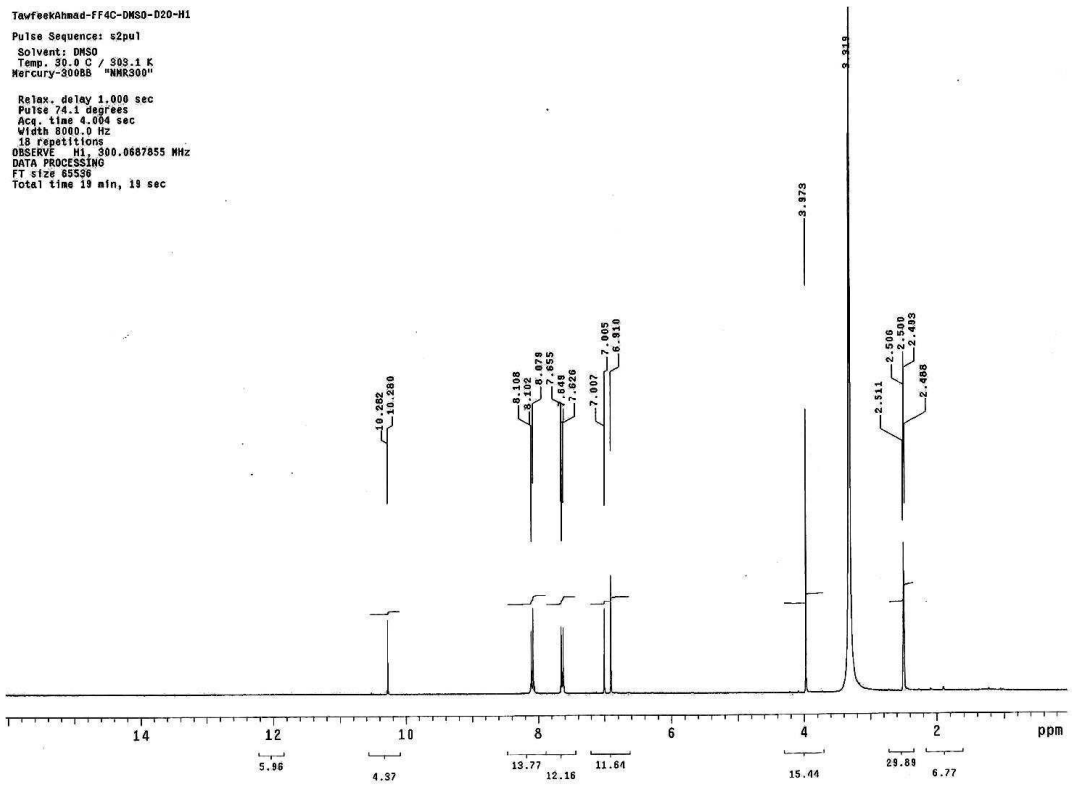
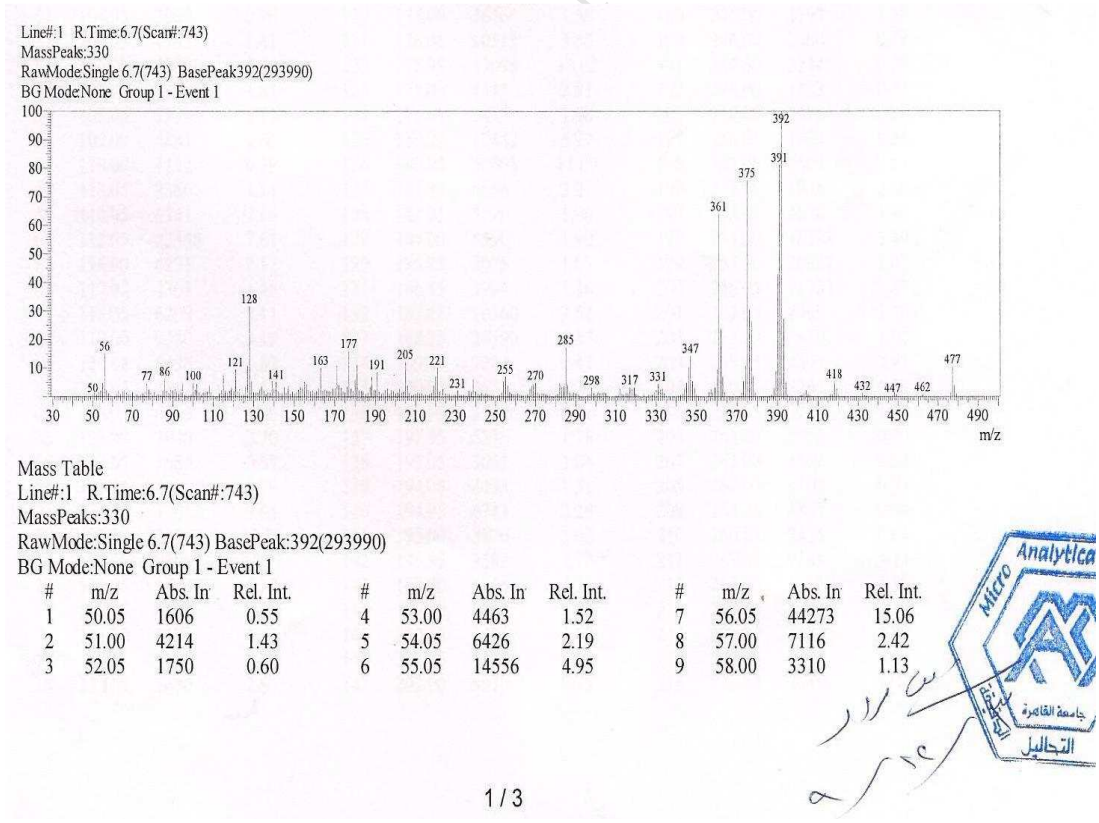


Figure S11: 1HNMR spectrum of compound **21c/D_2O**.



Mass Table

Line#:1 R.Time:6.7(Scan#:743)

MassPeaks:330

RawMode:Single 6.7(743) BasePeak:392(293990)

BG Mode:None Group 1 - Event 1

#	m/z	Abs. In	Rel. Int.	#	m/z	Abs. In	Rel. Int.	#	m/z	Abs. In	Rel. Int.
1	50.05	1606	0.55	4	53.00	4463	1.52	7	56.05	44273	15.06
2	51.00	4214	1.43	5	54.05	6426	2.19	8	57.00	7116	2.42
3	52.05	1750	0.60	6	55.05	14556	4.95	9	58.00	3310	1.13

1 / 3

Figure S12: Mass spectrum of compound **25c**.

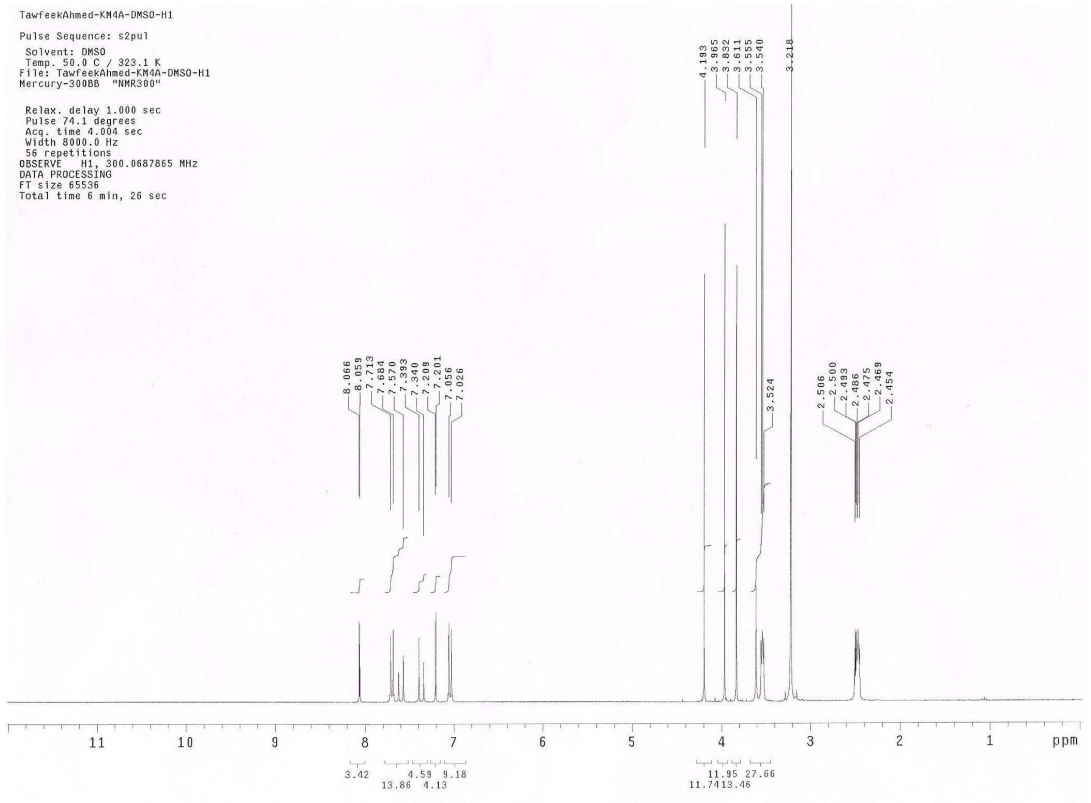


Figure S13: Mass spectrum of compound **25c**.