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Original article

Synthesis and cytotoxicity evaluation of biaryl-based chalcones and their potential in TNFα-induced nuclear factor-κB activation inhibition

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ABSTRACT

A series of biaryl-based chalcones were designed as a combination of the natural chalcone and biphenyl moieties, and synthesized by two step chemistry involving Knoevenagel reaction and microwave assistant Suzuki coupling. Sulforhodamine B (SRB) assay was performed to evaluate the cell viability inhibitory abilities of these compounds against five cancer cell lines (A549, CNE2, SW480, MCF-7, and HepG2) from different tissues. Their Nuclear Factor-κB (NF-κB) nuclear translocation inhibitory activities were further investigated by High Content Analysis (HCA) based assay. Most of the compounds showed moderate to strong anticancer and NF-κB nuclear translocation inhibition activities and potent compounds were found.

1. Introduction

Natural occurring or synthetic chalcones (1, 3-diary-2-propene-ones) have been found exhibiting various biological activities, especially in anti-inflammatory and anticancer [1–8]. Large numbers of efforts have been made to elucidate the mechanisms of chalcone-based compounds for their anticancer activities. Although interference of microtubule formation [6,7,9,10] was suggested to be one mechanism for the anticancer activity of chalcones, their potential of inhibiting Nuclear Factor-κB (NF-κB) activation pathway, which was thought to play an important role in the process leading from inflammation to carcinogenesis [11–13], attracted much attention [14,15]. Typically, NF-κB is involved in most of inflammation response; recent advances have revealed important roles of NF-κB signaling in oncogenesis. NF-κB is commonly overexpressed and constitutively activated in different types of tumors [16–18]. Constitutive activation of NF-κB promotes tumor proliferation, invasion and metastasis [19,20], allows the malignant cells to escape apoptosis, and therefore contributes to the radiation and chemotherapeutic resistance and leads to failure of therapy [21–24].

The chalcone core (**1**, Fig. 1) was found to inhibit the NF-κB related survival system and contributed to its antiproliferative activity in T24 and HT-1376 cells [8]. Many natural chalcones which were reported exhibiting anticancer activity, such as butein (**2**) [25], cardamonin (**3**) [26], isoliquiritigenin (**4**) [27], Licochalcone A (**5**) [28] and Xanthohumol (**6**) [29], were proven to be potent NF-κB inhibitors though IκBα kinase β (IKKβ) blockage and/or other biological processes in various cell lines. Some synthetic analogue of chalcone, for example MX781 (**7**) which is characterized by a large substituted group on the *m*- position of one aromatic ring of chalcone, was also reported with potent IKKβ inhibitory activity and induce significant apoptosis [29]. The NF-κB inhibitory properties of these compounds are considered at least partially other explanations for their anticancer activities due to the close linkage between inflammation and tumorigenesis [11,12,30]. The development of highly active and clinically promising chalcone-based therapeutic anticancer agents would be promising way for chemotherapy of malignant tumor. Nevertheless, investigations focused on both NF-κB inhibitory and anticancer activities of novel chalcone derivatives remain rarely reported.

We have focused on the NF-κB inhibitors for anticancer agents discovery for years, in our previous work [31], we found 4-arylidene curcumin analogues exhibiting potent anticancer activities, and mechanism studies revealed that the NF-κB inhibition contributed to their anticancer abilities. We herein report the synthesis of a series of new biaryl-based chalcones, which were further investigated on their activity of NF-κB inhibition and cancer cell growth inhibition against a panel of cancer cell lines (A549,

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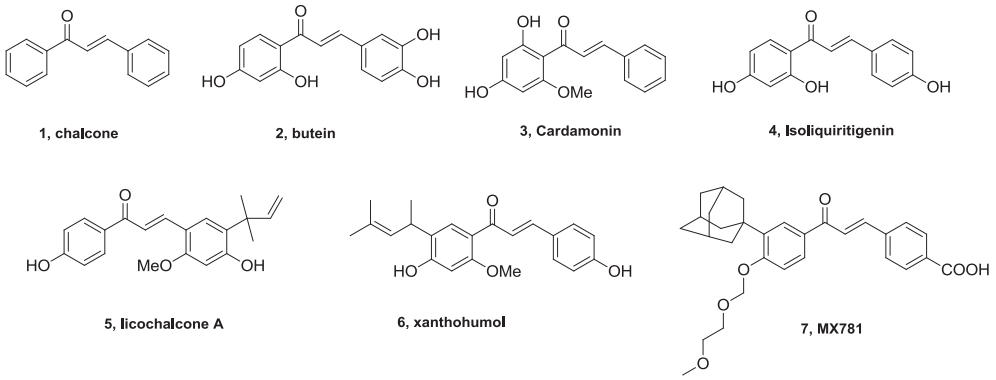


Fig. 1. Structure of representative chalcone-based NF-κB inhibitors.

CNE2, SW480, MCF-7, HepG2), by high content analysis technology based NF-κB translocation assay coupled with anti-proliferation studies.

2. Results and discussion

2.1. Chemistry

The biaryl-based chalcones were designed as a combination of typical chalcone moiety with a biphenyl moiety. The biphenyl moiety occurred in many natural products (eg. **8**–**10**, Fig. 2) and were found exhibiting a wide variety of biological activities, including anticancer [32–35], antiangiogenic [32,34] and antiviral [36] etc. Furthermore, different substitutions were adopted to expand the structure diversity in current work.

Biaryl-based chalcones **12**–**20** were synthesized according to the sequence of reactions as illustrated in Scheme 1. Microwave assistant Suzuki coupling of (3-fluorophenyl) boronic acid with 1-(3-bromophenyl) ethanone led to the biphenyl intermediate **11**, followed by chlorotrimethylsilane (TMSCl) catalyzed knoevenagel condensation to yield the target products. B ring aryl-substituted chalcones **22** and **23** were obtained by similar procedures (Scheme 2).

For the synthesis of biaryl-based chalcones **25**–**35**, the reaction sequences illustrated in Scheme 3 were taken up. Interestingly, by using 6 equiv. KOH as catalyst, all the acetyl groups were successfully removed at knoevenagel condensation step in synthesis of **25** and **28**–**35** except that in **27** synthesis. We found large amount of products precipitated during synthesis of the precursor of **27** (compound **26**), distinguished from others. The precipitate of **26** may prevent the deprotection of acetyl group in the following step. The remove of acetyl group in **26** was achieved by dissolved the product in a mixture of EtOH and H_2O under 70 °C with NaOH as catalyst, yield **27**. For the synthesis of **37**–**41**, similar reaction procedures were used (Scheme 4).

Biaryl-based chalcones **45**–**48** were synthesized by reactions illustrated in Scheme 5. A NaOH (*aq*) catalyzed knoevenagel condensation yield the chalcone intermediates **42**–**44**, followed by microwave assistant Suzuki coupling with aryl-substituted boronic acids led to the target products **45**–**48**.

In total 38 compounds were obtained successfully and 33 compounds **12**–**20**, **22**–**41**, **45**–**48** were identified as new compounds.

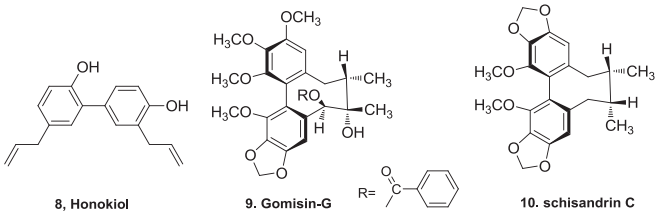


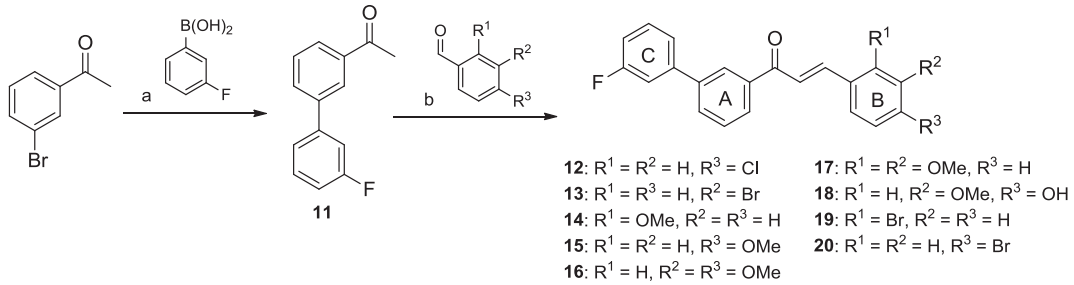
Fig. 2. Structure of representative biphenyl-based natural products.

2.2. Biological evaluation

2.2.1. Antiproliferative activity against five human cancer cell lines

After obtain the proposed compounds, all final compounds **12**–**20**, **22**–**23**, **25**–**35**, **37**–**41**, **45**–**48** along with chalcone intermediates **42**–**44** were first tested for their antiproliferative activities against five tumor cell lines (human lung carcinoma cell line, A549; human nasopharyngeal carcinoma (NPC) cell line, CNE2; human colon cell line, SW480; human breast adenocarcinoma cell line, MCF-7; human hepatoma cell line, HepG2) by measuring the amount of total cell protein with the sulforhodamine B (SRB) assay. For comparison, dose–response experiments were carried out to obtain IC_{50} values for each compound, cell viability were measured and calculated after incubated the compounds with different cells for 48 h. As summarized in Table 1, most of the tested compounds show moderate to potent antiproliferative activities against targeted cell lines. Generally, compounds with electron withdraw group F, Cl or Br on aromatic B ring (ring A, B and C are defined in Scheme 1) of the aryl-substituted chalcones (**12**–**13**, **19**–**20**, **22**, **33**–**34**) exhibit relative lower activities than the electron donation group OH or OCH_3 substituted analogs with several exceptions. By contrast, the 3′- position F substituted on C ring of biaryl moiety show relative higher activities than the 4′- position F substituted analogs except the analogs with halogen substitution on B ring; for example, **45**–**46** have lower activities against all cancer cell lines than that of **15**–**16**, indicating the importance of F substitution position on ring C. Furthermore, 3′,4′-dimethoxy substitution on the C ring is more potent than 4′-F substitution by comparing the data of **28**–**30** with **38**–**40** respectively. On the other hand, although the amino group substitution on the A ring of chalcone increased the activities slightly by comparing the activity of **25**, **27** with that of **45**, **46** respectively, the effects of amino group substitution seems not so significant since the other amino group substituted compounds have no obvious increased cytotoxicity in general, this conclusion can also be supported by the cytotoxicity data of **26** and **27**, the acylation of amino group on **27** does not effect its activity apparently.

Noticeably, compounds with *p*- position OCH_3 substitution on aromatic B ring generally have higher activities than others, and an additional *m*-position OH substitution on B ring significantly increase their activities, compounds **35** and **47** show strongest



Scheme 1. Reagents and conditions: (a) $PdCl_2$(dppf), 2 M (*aq*) K_2CO_3, dioxane, microwave heating, 150 °C, 20 min; (b) TMSCl, DMF, 100 °C, 20 h.

cytotoxicity against A549 cells comparing that of **25**, **27**–**34** and **12**–**20** respectively, the most potent compound **47** ($IC_{50} = 0.50$ μM) show more than 10 times potent than the precursor chalcone **44** ($IC_{50} = 5.88$ μM). Furthermore, compound **38** has good activity against A549, MCF-7 and HepG2, and **15** and **16** showed strong inhibition activities toward all cell lines, especially for CNE2 cell line with IC_{50} values 0.74 μM and 0.77 μM respectively.

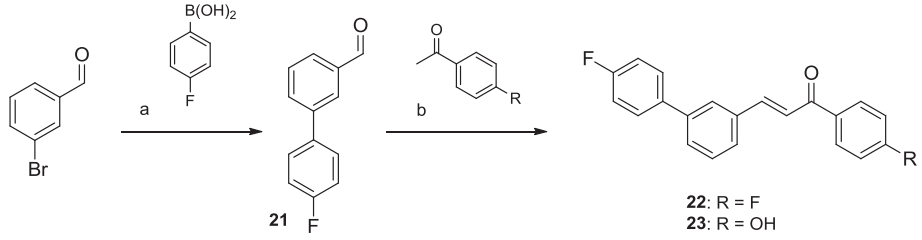
2.2.2. Inhibition of TNFα-induced nuclear translocation of NF-κB

NF-κB is a transcription factor that is activated in response to certain stimulations, such as TNFα, and is associated with the activation of many cellular defense genes [37]. Normally, NF-κB is in the cytoplasm and it should be activated and translocated into the nucleus to induce specific gene expression; to monitor the activation status of NF-κB, a high content analysis (HCA) based assay was used to visualize the dynamic movement of the NF-κB p65 subunit between the cytoplasm and nucleus under various conditions. A549 cells in a 384-well plate format were treated with tested compounds before TNFα was added to stimulate the nucleus translocation of NF-κB p65 subunit, an automated fluorescence imaging system for high content analysis was employed to monitor the cytoplasm/nucleus localization of NF-κB in response to TNFα. IC_{50} values of tested compounds were summarized in Table 2, As shown in Table 2, most of the tested compounds (**12**–**14**, **16**–**19**, **23**, **26**, **28**–**32**, **37**–**41**, **47**) show strong to moderate inhibitory activities ($IC_{50} = 1.70$ μM–35.28 μM) to TNFα induced NF-κB activation while others (**15**, **20**, **22**, **25**, **27**, **33**–**34**, **42**–**46**, **48**) present no obvious activities ($IC_{50} > 50$ μM). The C ring 3′- F substituted analogs show relative higher activities than the 4′- F substituted analogs with several exceptions (**15** and **20**), this phenomena seems generally to be consistent with their cytotoxicity; However, unlike their cytotoxicity, most of the amino substituted compounds **25**–**34** and **37**–**41** exhibiting good activities in NF-κB inhibition except **25** and **33**–**34**. Representative images from the most potent compound **38** ($IC_{50} = 1.70$ μM) were shown in Fig. 3, these images clearly showed that the nucleus translocation of NF-κB was blocked after addition of **38**.

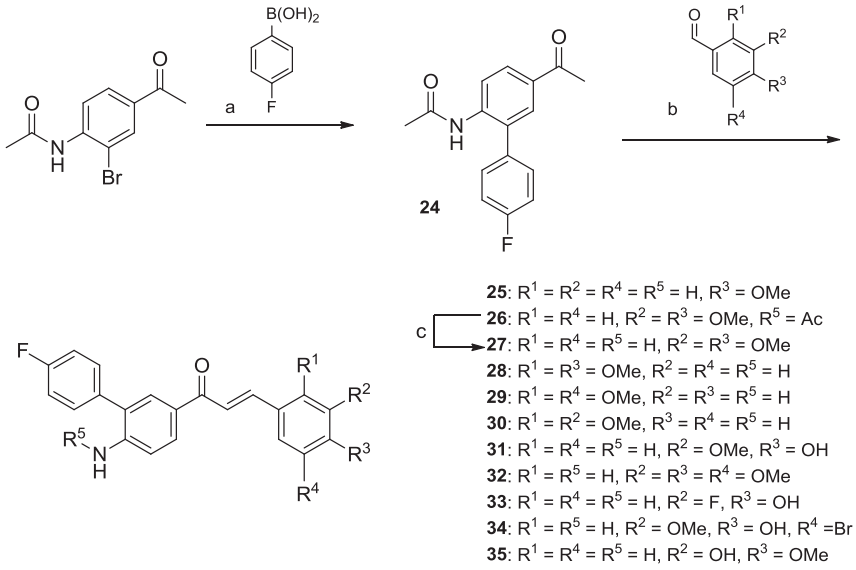
It should be pointed out that the NF-κB activation inhibition assay was carried out on A549 based model, significantly, data in Table 1 and Table 2 suggested that A549 growth suppression of more than 50% tested compounds are good consistent with their NF-κB inhibitory properties, indicating a close correlation of the NF-κB inhibitory activity and cytotoxicity. The apparent inconsistence revealed by the rest compounds is most probably due to a multi-targeting effect of tested compounds on the overall growth suppression phenotype; their inhibition of other targets in addition to NF-κB could be also response for the suppression of cell growth. Furthermore, the cytotoxicity varied along with the cancer cell type. It is reasonable that the efficacy of the test compounds on cell survival is also determined by cell's genetic backgrounds. How these novel chalcone analogs inhibit cancer cell growth remains to be established, which requires extensive research in the future.

3. Conclusions

In summary, a series of new biaryl-based chalcone derivatives have been discovered as potential anticancer agents. Although some biaryl-chalcones and their biological activities [38–41] have been reported, their anticancer activities were rarely studied. Many reports have revealed the close correlations of inflammation with carcinogenesis; our work found that most of the biary-based chalcone derivatives exhibit significant NF-κB inhibitory activity and cytotoxicity to a panel of cancer cell lines. Based on our investigation, the NF-κB inhibition might be one of potential mechanisms for their anticancer activities though the detail mechanism remains to be discovered. Remarkably, Human NPC is referred to as *Cantonese cancer*, extremely occurred in Guangdong province accounting for 18% of all cancers in China [42,43]. It occurs in about 25 cases per 100,000 people in this region, 25 times higher than the rest of the world [43]. Our study found that **15** and **16** exhibit potent inhibitory activities toward CNE2 cell growth with some extend of selectivity to other cell lines, suggesting that **15** and **16** could be served as leads for novel anti-NPC drug discovery; furthermore, several compounds such as **35**, **38**, **47** and **48**, were found to be potent leads against different cancer cell line. Overall, our findings in this work offered a good start point and the novel potent biaryl-based chalcones may represent new opportunities for anticancer drug development, such works are scheduled on our works.



Scheme 2. Reagents and conditions: (a) $PdCl_2$(dppf), 2 M (*aq*) K_2CO_3, dioxane, microwave heating, 150 °C, 20 min; (b) TMSCl, DMF, 100 °C, 20 h.



Scheme 3. Reagents and conditions: (a) $PdCl_2$(dppf), 2 M (*aq*) K_2CO_3, dioxane, microwave heating, 150 °C, 20 min; (b) KOH, EtOH, rt; (c) NaOH, EtOH/H_2O, 70 °C, 8 h.

4. Experiments

4.1. General

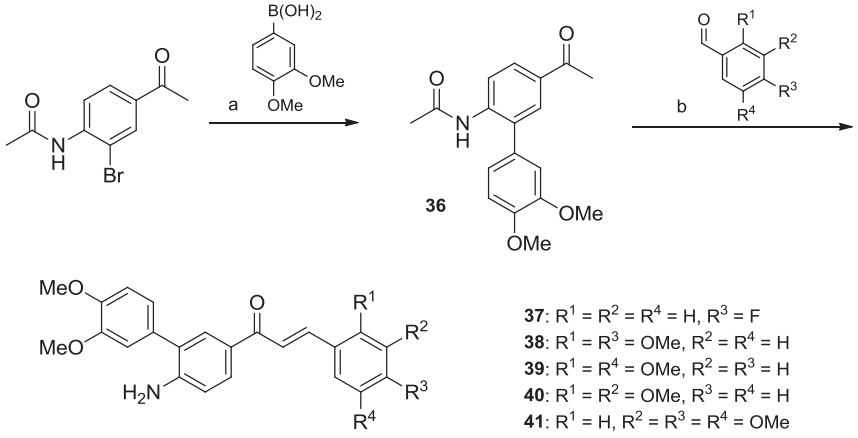
All commercial reagents and solvents were purchased from vendors and were used without further purification or distillation. Microwave reactions were performed in a Biotage Initiator 2.5 microwave reactor. Reactions were monitored by TLC on a glass plate coated with silica gel with fluorescent indicator (GF_{254}). Column chromatography was performed on silica gel (200–300 mesh). ^{1}H NMR and ^{13}C NMR spectra were recorded using TMS as an internal standard with Burker BioSpin Ultrashield 400 NMR system or Varian INOVA 500NB. The Purities of compounds used for biological evaluation (>95%) were determined on a DIONEX Ultimate 3000 HPLC System (Chromeleon SR9 Build 2673); column, Acclaim®120C18, 5 μm, 4.6 × 250 mm; flow rate, 1 ml/min; samples were eluted with a gradient from certain concentration of A to 95% A over 30 min, whereas the solvent A was CH_3CN in double-deionized H_2O with 0.1% trifluoroacetic acid (TFA), using UV monitor at 254 nm for detection. For compounds **16**–**18**, start from 70% A; for compounds **12**–**15**, **19** and **20**, start from 80% A; for compounds **22**, **23** and **42**–**44**, start from 60% A; for compounds **25**–**35**, **37**–**41** and **45**–**48**, start from 40% A. Compounds purities were calculated as the percentage peak area of the analyzed compound, retention times (t_R) were calculated in minutes. A list of all purities is given in the Supporting Information, Table S1. High resolution mass spectra (HRMS) were recorded on Shimadzu LCMS-IT-TOF.

4.2. General procedure for the preparation of *11*, *21*, *24* and *36*

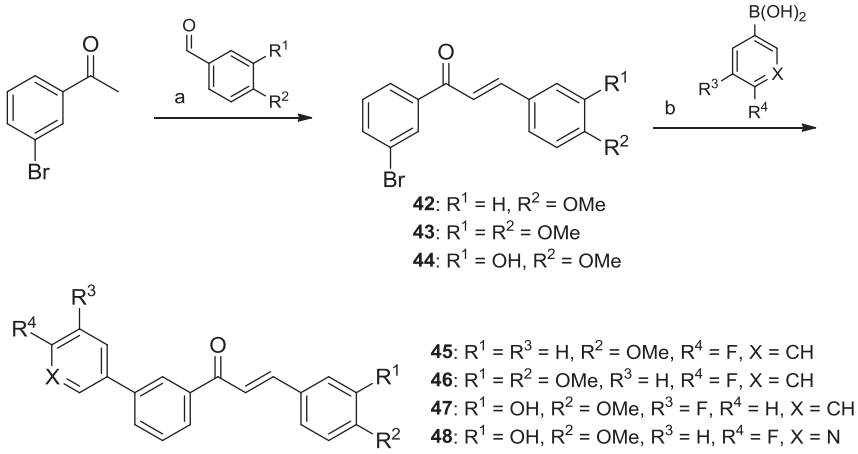
The procedure is exemplified by the preparation of **11**.

4.2.1. 1-(3′-Fluoro-[1,1′-biphenyl]-3-yl)ethanone (*11*)

A previously described Suzuki-Miyaura coupling condition [44] was used with slight modification. A mixture of 1-(3-bromophenyl) ethanone (133 μL, 199 mg, 1.0 mmol), (3-fluorophenyl)boronic acid (182 mg, 1.3 mmol), dichloro(1,1′-bis(diphenylphosphino)ferrocene)palladium(II)-dichloromethane adduct (50 mg, 0.06 mmol), 2M(*aq*) K_2CO_3(1.5 ml), and 1,4-dioxane (1.5 ml) was heated in a sealed vial in a microwave reactor at 150 °C for 20 min. After cooling, 10 ml water was added, and the mixture was extracted with EtOAc (10 ml) by three times. The combined organic layer was washed until neutral and dried over anhydrous sodium sulfate. After removal of the solvent in vacuum, the crude product was purified by column chromatography to yield light yellow liquid 150 mg (70.02%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.15 (t, $J = 1.8$ Hz, 1H),



Scheme 4. Reagents and conditions: (a) $PdCl_2$(dppf), 2 M (*aq*) K_2CO_3, dioxane, microwave heating, 150 °C, 20 min; (b) KOH, EtOH, rt.



Scheme 5. Reagents and conditions: (a) 6 M NaOH (*aq*), EtOH, rt; (b) $PdCl_2$(dppf), 2 M (*aq*) K_2CO_3, dioxane, microwave heating, 150 °C, 20 min.

7.94 (ddd, J = 7.8, 1.6, 1.2 Hz, 1H), 7.75 (ddd, J = 7.7, 2.0, 1.1 Hz, 1H), 7.53 (t, J = 7.7 Hz, 1H), 7.44–7.36 (m, 2H), 7.30 (ddd, J = 10.1, 2.7, 1.5 Hz, 1H), 7.10–7.03 (m, 1H), 2.65 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 197.89, 164.34, 161.89, 142.28, 142.21, 140.23, 140.20, 137.57, 131.51, 130.43, 130.35, 129.12, 127.72, 126.68, 122.74, 122.72, 114.63, 114.42, 114.05, 113.83, 26.58; HRMS calcd for $C_{14}H_{11}OF$ $[M + Na]^+$: 237.0692, found 237.0692.

*4.2.2. 4′-Fluoro-[1,1′-biphenyl]-3-carbaldehyde (**21**)*

Yellow oil, 80.72%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.08 (s, 1H), 8.05 (s, 1H), 7.85 (d, J = 7.6 Hz, 1H), 7.81 (d, J = 7.8 Hz, 1H), 7.64–7.56 (m, 3H), 7.20–7.12 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 192.18, 164.06, 161.60, 141.08, 136.96, 135.80, 135.77, 132.80, 129.54, 128.80, 128.72, 127.77, 116.01, 115.79; HRMS calcd for $C_{13}H_9OF$ $[M + H]^+$: 201.0716, found 201.0718.

Table 1
Antiproliferative activity of the target compounds against five cancer cell lines[a].

Compound	Human cancer cell lines, IC_{50} (μM)				
	A549	CNE2	SW480	MCF-7	HepG2
12	15.44 ± 0.45	9.06 ± 0.51	16.85 ± 0.23	18.15 ± 0.27	10.41 ± 0.23
13	9.12 ± 0.19	6.55 ± 0.59	8.61 ± 0.41	12.65 ± 1.34	6.98 ± 0.94
14	15.51 ± 0.73	10.40 ± 0.87	16.64 ± 0.07	18.92 ± 0.13	15.36 ± 1.43
15	2.44 ± 0.27	0.74 ± 0.10	4.51 ± 0.80	3.08 ± 0.06	1.72 ± 0.48
16	1.94 ± 0.16	0.77 ± 0.14	2.97 ± 1.03	4.62 ± 1.19	1.17 ± 0.08
17	8.90 ± 0.09	10.00 ± 1.27	13.50 ± 0.88	11.67 ± 0.82	9.20 ± 2.77
18	9.90 ± 0.47	5.69 ± 0.08	6.74 ± 0.49	14.20 ± 1.00	6.83 ± 0.84
19	12.44 ± 1.47	8.91 ± 0.16	14.79 ± 1.20	19.98 ± 0.62	12.83 ± 2.72
20	19.83 ± 1.86	10.45 ± 0.66	18.19 ± 1.27	18.32 ± 0.78	9.92 ± 0.59
22	12.23 ± 1.10	7.39 ± 1.22	13.54 ± 2.92	14.12 ± 1.14	12.30 ± 4.39
23	8.36 ± 0.15	5.51 ± 0.14	12.02 ± 0.98	10.83 ± 1.60	5.12 ± 0.11
25	4.31 ± 0.14	1.60 ± 0.04	7.23 ± 0.42	7.58 ± 2.58	3.75 ± 1.02
26	7.38 ± 0.09	2.74 ± 0.03	5.35 ± 0.82	15.01 ± 1.94	5.63 ± 0.74
27	8.63 ± 0.16	3.51 ± 0.40	6.64 ± 1.20	14.96 ± 2.71	3.78 ± 0.36
28	14.37 ± 0.38	6.29 ± 0.65	12.38 ± 0.30	10.82 ± 2.78	7.74 ± 1.12
29	7.04 ± 0.09	6.71 ± 0.38	7.83 ± 0.68	8.92 ± 2.52	4.01 ± 0.70
30	13.34 ± 1.10	11.32 ± 2.79	12.5 ± 0.50	13.29 ± 3.77	7.37 ± 0.71
31	12.23 ± 0.97	5.75 ± 0.57	8.35 ± 1.09	10.53 ± 3.23	4.30 ± 0.32
32	6.07 ± 0.09	4.60 ± 0.83	6.12 ± 0.22	9.03 ± 0.30	4.21 ± 0.15
33	24.52 ± 0.21	18.09 ± 3.44	31.70 ± 0.76	18.27 ± 2.04	11.70 ± 1.52
34	>50	25.50 ± 3.98	22.86 ± 5.15	40.51 ± 9.49	4.73 ± 0.03
35	0.94 ± 0.04	NA	NA	NA	NA
37	18.00 ± 0.48	7.93 ± 1.18	14.7 ± 0.70	19.44 ± 0.37	8.96 ± 0.54
38	2.21 ± 0.02	8.34 ± 1.65	9.30 ± 1.97	6.49 ± 0.43	2.13 ± 0.12
39	6.20 ± 0.76	6.59 ± 0.84	6.49 ± 0.73	6.33 ± 0.34	2.10 ± 0.07
40	6.54 ± 0.05	6.60 ± 0.57	8.33 ± 0.53	10.22 ± 0.52	5.36 ± 0.06
41	4.62 ± 0.09	4.33 ± 0.75	5.63 ± 0.03	6.58 ± 0.46	2.68 ± 1.16
42	34.21 ± 2.46	NA	NA	NA	NA
43	9.43 ± 1.06	NA	NA	NA	NA
44	5.88 ± 0.25	NA	NA	NA	NA
45	11.23 ± 0.46	5.55 ± 0.01	8.35 ± 0.08	16.35 ± 0.34	5.65 ± 0.04
46	11.78 ± 0.65	5.55 ± 0.01	6.49 ± 0.21	11.55 ± 0.47	5.37 ± 0.34
47	0.50 ± 0.08	NA	NA	NA	NA
48	2.06 ± 0.16	NA	NA	NA	NA

NA: Not tested.
[a] Data shown are the average values from at least two independent experiments with standard error (SE).

Table 2
Inhibition activity of target compounds on NF-κB activation/translocation[a].

Compound	IC_{50} (μM)	Compound	IC_{50} (μM)	Compound	IC_{50} (μM)
12	24.39 ± 6.65	**25**	>50	**38**	1.70 ± 0.35
13	11.21 ± 1.53	**26**	2.65 ± 0.41	**39**	3.19 ± 1.00
14	35.28 ± 5.43	**27**	>50	**40**	11.90 ± 3.24
15	>50	**28**	7.99 ± 1.35	**41**	5.44 ± 2.12
16	33.44 ± 7.28	**29**	10.29 ± 5.50	**42**	>50
17	24.05 ± 3.63	**30**	13.81 ± 3.89	**43**	>50
18	12.87 ± 0.10	**31**	6.83 ± 2.35	**44**	>50
19	13.65 ± 0.32	**32**	11.62 ± 2.88	**45**	>50
20	>50	**33**	>50	**46**	>50
22	>50	**34**	>50	**47**	9.59 ± 2.88
23	12.41 ± 2.33	**37**	21.26 ± 4.91	**48**	>50

[a] Data shown are the average values from two independent experiments with standard error (SE).

4.2.3. *N-(5-Acetyl-4′-fluoro-[1,1′-biphenyl]-2-yl)acetamide (**24**)*

White power, 89.65%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.48 (d, J = 8.4 Hz, 1H), 7.96 (dd, J = 8.6, 2.1 Hz, 1H), 7.82 (d, J = 2.1 Hz, 1H), 7.40–7.32 (m, 2H), 7.25–7.13 (m, 3H), 2.59 (s, 3H), 2.06 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 196.94, 168.45, 164.09, 161.62, 139.32, 133.13, 133.09, 132.82, 131.17, 131.09, 130.71, 130.34, 129.35, 120.59, 116.64, 116.42, 26.50, 24.81; HRMS calcd for $C_{16}H_{14}NO_2F$ $[M + Na]^+$: 294.0906, found 294.0899.

4.2.4. *N-(5-Acetyl-3′,4′-dimethoxy-[1,1′-biphenyl]-2-yl)acetamide (**36**)*

White power, 88.20%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.51 (d, J = 8.5 Hz, 1H), 7.94 (dd, J = 8.6, 2.1 Hz, 1H), 7.85 (d, J = 2.1 Hz, 1H), 7.43 (s, 1H), 7.00 (d, J = 8.2 Hz, 1H), 6.92 (dd, J = 8.2, 2.0 Hz, 1H), 6.85 (d, J = 2.0 Hz, 1H), 3.96 (s, 3H), 3.90 (s, 3H), 2.59 (s, 3H), 2.06 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 196.66, 168.28, 149.23, 148.84, 139.22, 132.17, 131.23, 129.95, 129.20, 128.46, 121.20, 120.02, 112.06, 111.46, 55.74, 55.64, 26.14, 24.44; HRMS calcd for $C_{18}H_{19}NO_4$ $[M - H]^-$:312.1236, found 312.1230.

4.3. *General procedure for the preparation of **12-20**, **22** and **23***

TMSCl catalyzed Knoevenagel condensations were used [45]. The procedure is exemplified by the preparation of **12**. Other products were synthesized by the corresponding acetophenones and the corresponding aldehydes.

4.3.1. *(E)-3-(4-Chlorophenyl)-1-(3′-fluoro-[1,1′-biphenyl]-3-yl) prop-2-en-1-one (**12**)*

Intermediate **11** (428.5 mg, 2 mmol), 4-chlorobenzaldehyde (281.1 mg, 2 mmol) were place in a 40 mL pressure tube and dissolved in DMF (3 mL). TMSCl (770 μL, 6 mmol) was added to the solution. The tube was thoroughly sealed and heated at 100 °C for 20 h on a Carousel 12 Plus™ reaction station (Radleys Discovery Technologies). After cooling, the tube was opened and 15 mL water was added. Then the mixture was extracted with EtOAc (15 ml) by three times, the combined organic layer was washed with water (10 mL) by two times and dried over anhydrous sodium sulfate. After removal of the solvent in vacuum, the crude product was purified by column chromatography to yield light yellow power, 471 mg (69.94%); HPLC t_R = 13.01 min; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.20 (t, J = 1.7 Hz, 1H), 8.01 (ddd, J = 7.7, 1.7, 1.1 Hz, 1H), 7.80 (d, J = 15.7 Hz, 1H), 7.79 (ddd, J = 7.7, 1.8, 1.1 Hz, 1H), 7.60 (t, J = 7.7 Hz, 1H), 7.60 (dd, J = 8.8, 2.1 Hz, 2H), 7.53 (d, J = 15.7 Hz, 1H), 7.47–7.39 (m, 2H), 7.41 (dd, J = 8.8, 2.1 Hz, 2H), 7.34 (ddd, J = 10.4, 2.6, 1.4 Hz, 1H), 7.12–7.07 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 190.06, 164.56, 162.11, 143.78, 142.58, 142.50, 140.69, 140.67, 138.80, 136.69, 133.40, 131.58, 130.62, 130.54, 129.79, 129.39, 129.36, 127.95, 127.22, 123.02, 122.99, 122.42, 114.89, 114.68, 114.39, 114.17; HRMS calcd for $C_{21}H_{14}OFCl$ $[M + Na]^+$: 359.0615, found 359.0610.

4.3.2. *(E)-3-(3-Bromophenyl)-1-(3′-fluoro-[1,1′-biphenyl]-3-yl) prop-2-en-1-one (**13**)*

Light yellow power, 82.15%; HPLC t_R = 13.91 min; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.20 (t, J = 1.6 Hz, 1H), 8.01 (ddd, J = 7.7, 1.7, 1.1 Hz, 1H), 7.82–7.80 (m, 1H), 7.80 (ddd, J = 7.7, 1.8, 1.1 Hz, 1H), 7.76 (d, J = 15.7 Hz, 1H), 7.60 (td, J = 7.7, 0.4 Hz, 1H), 7.58–7.55 (m, 2H), 7.54 (d, J = 15.7 Hz, 1H), 7.47–7.41 (m, 2H), 7.35 (ddd, J = 10.4, 2.8, 1.4 Hz, 1H), 7.31 (t, J = 7.9 Hz, 1H), 7.12–7.07 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 189.86, 164.54, 162.10, 143.41, 142.52, 142.45, 140.70, 140.68, 138.64, 137.01, 133.46, 131.66, 131.02, 130.62, 130.59, 130.53, 129.37, 127.98, 127.40, 127.22, 123.22, 123.18, 123.02, 122.99, 114.89, 114.68, 114.38, 114.16; HRMS calcd for $C_{21}H_{14}OFBr$ $[M + Na]^+$: 403.0110, found 403.0104.

4.3.3. *(E)-1-(3′-Fluoro-[1,1′-biphenyl]-3-yl)-3-(2-methoxyphenyl) prop-2-en-1-one (**14**)*

Yellow oil, 33.13%; HPLC t_R = 10.88 min; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.21 (t, J = 1.6 Hz, 1H), 8.16 (d, J = 15.9 Hz, 1H), 8.01 (ddd, J = 7.7, 1.7, 1.2 Hz, 1H), 7.78 (ddd, J = 7.7, 1.9, 1.1 Hz, 1H), 7.66 (dd, J = 7.7, 1.7 Hz, 1H), 7.62 (d, J = 15.9 Hz, 1H), 7.58 (td, J = 7.7, 0.4 Hz, 1H), 7.45–7.42 (m, 2H), 7.40 (ddd, J = 8.4, 7.4, 1.7 Hz, 1H), 7.37–7.34 (m, 1H), 7.11–7.05 (m, 1H), 7.01 (td, J = 7.4, 0.6 Hz, 1H), 6.96 (d, J = 8.3 Hz, 1H), 3.93 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 191.13, 164.52, 162.08, 158.90, 142.71, 142.64, 140.90, 140.41, 140.39, 139.23, 132.01, 131.13, 130.54, 130.46, 129.25, 129.22, 128.00, 127.30, 123.91, 122.97, 122.94, 122.92, 120.87, 114.73, 114.52, 114.31, 114.09, 111.34, 55.62; HRMS calcd for $C_{22}H_{17}O_2F$ $[M + Na]^+$: 355.1110, found 355.1108.

4.3.4. *(E)-1-(3′-Fluoro-[1,1′-biphenyl]-3-yl)-3-(4-methoxyphenyl) prop-2-en-1-one (**15**)*

Yellow power, 65.81%; HPLC t_R = 9.41 min; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.19 (t, J = 1.7 Hz, 1H), 8.00 (ddd, J = 7.7, 1.7, 1.1 Hz, 1H), 7.83 (d, J = 15.6 Hz, 1H), 7.77 (ddd, J = 7.7, 1.9, 1.1 Hz, 1H), 7.63 (dd, J = 9.1, 2.4 Hz, 2H), 7.58 (t, J = 7.7 Hz, 1H), 7.44 (d, J = 15.6 Hz, 1H), 7.47–7.42 (m, 2H), 7.35 (ddd, J = 10.1, 2.7, 1.3 Hz, 1H), 7.11–7.06 (m, 1H), 6.95 (dd, J = 9.1, 2.4 Hz, 2H), 3.86 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 190.38, 164.53, 162.09, 161.90, 145.17, 142.71, 142.63, 140.52, 139.28, 131.19, 130.56, 130.45, 129.24, 127.88, 127.62, 127.14, 123.01, 122.99, 119.71, 114.77, 114.56, 114.36, 114.14, 55.52; HRMS calcd for $C_{22}H_{17}O_2F$ $[M + H]^+$: 333.1291, found 333.1299.

4.3.5. *(E)-3-(3,4-Dimethoxyphenyl)-1-(3′-fluoro-[1,1′-biphenyl]-3-yl)prop-2-en-1-one (**16**)*

Yellow oil, 56.35%; HPLC t_R = 9.48 min; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.19 (t, J = 1.6 Hz, 1H), 8.00 (ddd, J = 7.7, 1.6, 1.1 Hz, 1H), 7.80 (d, J = 15.6 Hz, 1H), 7.77 (ddd, J = 7.7, 1.9, 1.1 Hz, 1H), 7.58 (td, J = 7.7, 0.4 Hz, 1H), 7.47–7.42 (m, 2H), 7.41 (d, J = 15.6 Hz, 1H), 7.37–7.32 (m, 1H), 7.26 (dd, J = 8.4, 2.0 Hz, 1H), 7.17 (d, J = 2.0 Hz, 1H), 7.11–7.05 (m, 1H), 6.91 (d, J = 8.4 Hz, 1H), 3.95 (s, 3H), 3.93 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 190.25, 164.39, 161.94, 151.54, 149.24, 145.38, 142.52, 142.44, 140.35, 140.32, 139.11, 131.06, 130.45, 130.36, 129.10, 127.77, 127.75, 126.97, 123.26, 122.86, 122.84, 119.83, 114.64, 114.43, 114.19, 113.97, 111.13, 110.28, 55.94, 55.94; HRMS calcd for $C_{23}H_{19}O_3F$ $[M + H]^+$: 363.1396, found 363.1396.

4.3.6. *(E)-3-(2,3-Dimethoxyphenyl)-1-(3′-fluoro-[1,1′-biphenyl]-3-yl)prop-2-en-1-one (**17**)*

Yellow power, 26.38%; HPLC t_R = 11.99 min; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.20 (t, J = 1.6 Hz, 1H), 8.13 (d, J = 15.9 Hz, 1H), 8.01 (ddd, J = 7.7, 1.6, 1.1 Hz, 1H), 7.78 (ddd, J = 7.7, 1.8, 1.1 Hz, 1H), 7.62 (d, J = 15.9 Hz, 1H), 7.59 (t, J = 7.8 Hz, 1H), 7.43 (m, 2H), 7.36–7.32 (m,

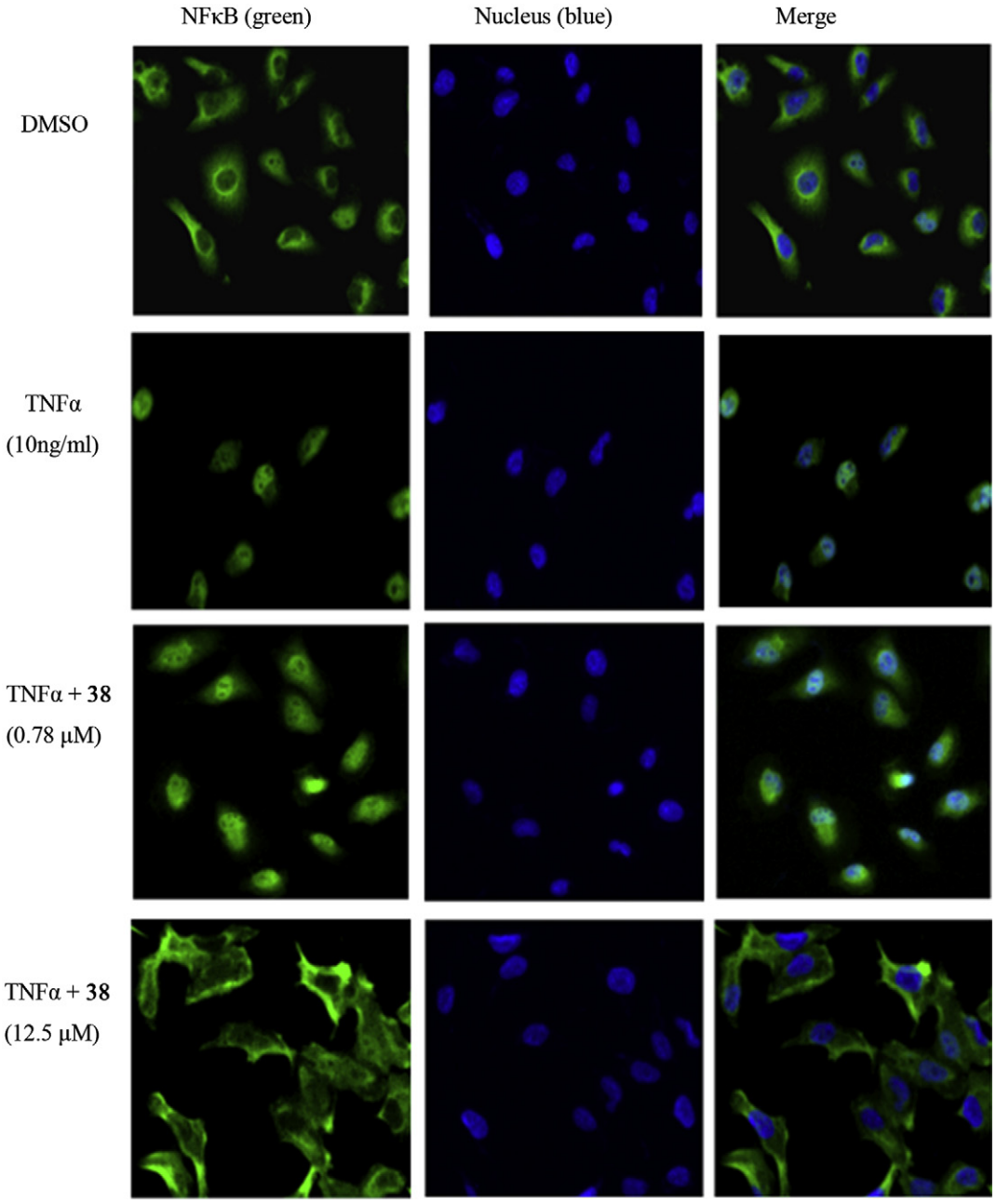


Fig. 3. Inhibition of TNFα induced NF-κB activation by biary-based chalcone **38**. Example images of NF-κB subcellular localization. A549 cells were treated with compounds or vehicle (DMSO) for 2 h, followed by stimulation with TNFα (10 ng/ml) for 30 min. In the vehicle (DMSO) treatment, NF-κB is located at cytoplasm, upon TNFα treatment, NF-κB is activated and translocated to the nucleus. Preincubation of the cells with increasing concentrations of compound **38** inhibited the TNFα-induced NF-κB translocation to the nucleus.

1H), 7.30 (dd, J = 8.0, 1.2 Hz, 1H), 7.11 (t, J = 8.0 Hz, 1H), 7.09–7.05 (m, 1H), 6.99 (dd, J = 8.0, 1.2 Hz, 1H), 3.90 (s, 3H), 3.89 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 190.81, 164.55, 162.10, 153.34, 149.11, 142.69, 142.61, 140.55, 140.53, 140.21, 139.07, 131.34, 130.59, 130.50, 129.29, 129.12, 128.02, 127.31, 124.35, 123.70, 123.01, 122.98, 119.79, 114.79, 114.59, 114.44, 114.35, 114.13, 61.44, 56.01; HRMS calcd for $C_{23}H_{19}O_3F$ $[M + H]^+$: 363.1396, found 363.1400.

*4.3.7. (E)-1-(3′-Fluoro-[1,1′-biphenyl]-3-yl)-3-(4-hydroxy-3-methoxyphenyl)prop-2-en-1-one (**18**)*

Yellow oil, 55.03%; HPLC t_R = 6.82 min; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.19 (t, J = 1.8 Hz, 1H), 8.00 (ddd, J = 7.7, 1.6, 1.1 Hz, 1H), 7.79 (d, J = 15.6 Hz, 1H), 7.77 (ddd, J = 7.7, 1.6, 1.1 Hz, 1H), 7.58 (t, J = 7.7 Hz, 1H), 7.47–7.42 (m, 2H), 7.40 (d, J = 15.6 Hz, 1H), 7.37–7.32 (m, 1H), 7.24 (dd, J = 8.2, 1.9 Hz, 1H), 7.14 (d, J = 1.9 Hz, 1H), 7.11–7.06 (m, 1H), 6.97 (d, J = 8.2 Hz, 1H), 5.97 (s, 1H), 3.97 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 190.63, 164.53, 162.09, 148.62, 147.00, 145.84, 142.68, 142.61, 140.54, 140.52, 139.26, 131.22, 130.57, 130.49, 129.23, 127.90, 127.46, 127.14, 123.59, 123.01, 122.98, 119.72, 115.08, 114.78, 114.57, 114.36, 114.14, 110.35, 56.14; HRMS calcd for $C_{22}H_{17}O_3F$ $[M + H]^+$: 349.1240, found 349.1235.

*4.3.8. (E)-3-(2-Bromophenyl)-1-(3′-fluoro-[1,1′-biphenyl]-3-yl) prop-2-en-1-one (**19**)*

Light yellow power, 42.39%; HPLC t_R = 13.52 min; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.20 (t, J = 1.7 Hz, 1H), 8.17 (d, J = 15.7 Hz, 1H), 8.01 (ddd, J = 7.7, 1.8, 1.1 Hz, 1H), 7.80 (ddd, J = 7.7, 1.8, 1.1 Hz, 1H), 7.76 (dd, J = 7.8, 1.6 Hz, 1H), 7.65 (dd, J = 8.0, 1.2 Hz, 1H), 7.60 (t,

J = 7.7 Hz, 1H), 7.44 (d, J = 15.7 Hz, 1H), 7.46–7.41 (m, 2H), 7.38 (td, J = 7.8, 1.2 Hz, 1H), 7.37–7.33 (m, 1H), 7.27 (td, J = 7.8, 1.6 Hz, 1H), 7.12–7.06 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 190.38, 164.51, 162.07, 143.65, 142.51, 142.43, 140.59, 140.57, 138.58, 135.02, 133.66, 131.56, 130.59, 130.50, 129.34, 128.10, 128.02, 127.85, 127.35, 126.04, 125.06, 122.98, 122.96, 114.84, 114.63, 114.33, 114.11; HRMS calcd for $C_{21}H_{14}OFBr$ $[M + Na]^+$: 403.0110, found 403.0118.

4.3.9. (E)-3-(4-Bromophenyl)-1-(3′-fluoro-[1,1′-biphenyl]-3-yl) prop-2-en-1-one (**20**)

Light yellow power, 69.55%; HPLC t_R = 13.93 min; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.19 (t, J = 1.6 Hz, 1H), 8.00 ((ddd, J = 7.7, 1.7, 1.0 Hz, 1H), 7.79 ((ddd, J = 7.7, 1.7, 1.0 Hz, 1H), 7.78 (d, J = 15.7 Hz, 1H), 7.60 (t, J = 7.7 Hz, 1H), 7.55 (d, J = 15.7 Hz, 1H), 7.58–7.51 (m, 4H), 7.47–7.41 (m, 2H), 7.36–7.32 (m, 1H), 7.12–7.07 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 190.03, 164.55, 162.10, 143.83, 142.56, 142.48, 140.68, 140.66, 138.77, 133.81, 132.35, 131.58, 130.62, 130.53, 129.98, 129.36, 127.94, 127.21, 125.06, 123.01, 122.98, 122.49, 114.88, 114.67, 114.38, 114.16; HRMS calcd for $C_{21}H_{14}OFBr$ $[M + Na]^+$: 403.0110, found 403.0124.

4.3.10. (E)-3-(4′-Fluoro-[1,1′-biphenyl]-3-yl)-1-(4-fluorophenyl) prop-2-en-1-one (**22**)

White power, 35.31%; HPLC t_R = 20.45 min; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.12–8.03 (m, 2H), 7.87 (d, J = 15.7 Hz, 1H), 7.78 (s, 1H), 7.65–7.52 (m, 5H), 7.50 (t, J = 7.7 Hz, 1H), 7.23–7.12 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 188.76, 167.00, 164.47, 164.03, 161.58, 144.86, 141.19, 136.61, 136.57, 135.44, 134.57, 134.54, 131.27, 131.18, 129.57, 129.35, 128.90, 128.82, 127.28, 127.20, 122.06, 116.01, 115.97, 115.79, 115.75; HRMS calcd for $C_{21}H_{14}OF_2$ $[M + Na]^+$: 343.0910, found 343.0917.

4.3.11. (E)-3-(4′-Fluoro-[1,1′-biphenyl]-3-yl)-1-(4-hydroxyphenyl) prop-2-en-1-one (**23**)

White power, 37.74%; HPLC t_R = 11.68 min; ^{1}H NMR (400 MHz, Acetone) δ 8.15–8.08 (m, 3H), 7.99 (d, J = 15.6 Hz, 1H), 7.86–7.76 (m, 4H), 7.73–7.67 (m, 1H), 7.55 (t, J = 7.7 Hz, 1H), 7.31–7.20 (m, 2H), 7.01–6.95 (m, 2H); ^{13}C NMR (100 MHz, Acetone) δ 188.13, 164.71, 162.80, 162.28, 143.57, 141.47, 137.58, 137.55, 136.92, 131.97, 131.12, 130.34, 129.85, 129.77, 129.41, 128.33, 127.66, 123.41, 116.53, 116.31, 116.21; HRMS calcd for $C_{21}H_{15}O_2F$ $[M - H]^-$: 317.0978, found 317.0984.

4.4. General procedure for the synthesis of **25**, **26**, **28-35** and **37-41**

A mixture of the corresponding acetophenone (1 equiv) and aldehyde (1 equiv) in anhydrous EtOH was stirred at room temperature for 10 min. Then solid KOH (6 equiv) was added. The reaction mixture was stirred at room temperature until aldehyde was consumed as monitored by TLC. After that, HCl (10%) was added until pH 4 was obtained. The mixture was concentrated in vacuum to remove EtOH, and further extracted with EtOAc. The EtOAc layer was dried over anhydrous sodium sulfate. After removal of the solvent in vacuum, the residue was purified by column chromatography to give corresponding chalcones.

4.4.1. (E)-1-(6-Amino-4′-fluoro-[1,1′-biphenyl]-3-yl)-3-(4-methoxyphenyl)prop-2-en-1-one (**25**)

Yellow power, 54.15%; HPLC t_R = 19.27 min; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92 (dd, J = 8.4, 2.1 Hz, 1H), 7.84 (d, J = 2.1 Hz, 1H), 7.78 (d, J = 15.6 Hz, 1H), 7.61–7.56 (m, 2H), 7.47–7.41 (m, 3H), 7.20–7.14 (m, 2H), 6.94–6.89 (m, 2H), 6.79 (d, J = 8.4 Hz, 1H), 4.20 (s, 2H), 3.85 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 188.19, 163.64, 161.48, 161.18, 148.40, 143.20, 134.49, 134.45, 131.81, 130.98, 130.90, 130.12, 130.10, 129.00, 128.12, 125.68, 119.70, 116.20, 115.99, 114.72, 114.45, 55.46; HRMS calcd for $C_{22}H_{18}NO_2F$ $[M - H]^-$: 346.1243, found 346.1238.

4.4.2. (E)-N-(5-(3-(3,4-Dimethoxyphenyl)acryloyl)-4′-fluoro-[1,1′-biphenyl]-2-yl)acetamide (**26**)

Yellow power, 79.57%; HPLC t_R = 14.71 min; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.51 (d, J = 8.3 Hz, 1H), 8.04 (dd, J = 8.6, 2.1 Hz, 1H), 7.90 (d, J = 2.1 Hz, 1H), 7.78 (d, J = 15.6 Hz, 1H), 7.42–7.36 (m, 3H), 7.26–7.20 (m, 4H), 7.15 (d, J = 2.0 Hz, 1H), 6.90 (d, J = 8.4 Hz, 1H), 3.95 (s, 3H), 3.93 (s, 3H), 2.07 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 188.97, 168.51, 163.99, 161.52, 151.52, 149.29, 145.02, 138.90, 134.00, 133.19, 133.15, 131.17, 131.09, 130.93, 130.58, 129.22, 127.87, 123.22, 120.68, 119.57, 116.54, 116.32, 111.20, 110.33, 56.03, 56.02, 24.75; HRMS calcd for $C_{25}H_{22}NO_4F$ $[M - H]^-$: 418.1455, found 418.1444.

4.4.3. (E)-1-(6-Amino-4′-fluoro-[1,1′-biphenyl]-3-yl)-3-(2,4-dimethoxyphenyl)prop-2-en-1-one (**28**)

Yellow power, 53.25%; HPLC t_R = 19.69 min; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.05 (d, J = 15.7 Hz, 1H), 7.91 (dd, J = 8.4, 2.1 Hz, 1H), 7.84 (d, J = 2.1 Hz, 1H), 7.57 (d, J = 8.6 Hz, 1H), 7.53 (d, J = 15.7 Hz, 1H), 7.47–7.42 (m, 2H), 7.20–7.14 (m, 2H), 6.78 (d, J = 8.4 Hz, 1H), 6.52 (dd, J = 8.5, 2.4 Hz, 1H), 6.47 (d, J = 2.4 Hz, 1H), 4.18 (s, 2H), 3.88 (s, 3H), 3.85 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 188.64, 163.30, 162.68, 160.85, 160.03, 148.39, 138.63, 134.45, 134.42, 131.60, 130.73, 130.65, 130.18, 129.92, 128.72, 125.26, 119.90, 117.20, 115.92, 115.71, 114.54, 105.39, 98.24, 55.39, 55.31; HRMS calcd for $C_{23}H_{20}NO_3F$ $[M - H]^-$: 376.1349, found 376.1341.

4.4.4. (E)-1-(6-Amino-4′-fluoro-[1,1′-biphenyl]-3-yl)-3-(2,5-dimethoxyphenyl)prop-2-en-1-one (**29**)

Yellow power, 70.22%; HPLC t_R = 19.98 min; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (d, J = 15.8 Hz, 1H), 7.93 (dd, J = 8.4, 2.1 Hz, 1H), 7.86 (d, J = 2.1 Hz, 1H), 7.58 (d, J = 15.8 Hz, 1H), 7.49–7.42 (m, 2H), 7.22–7.15 (m, 3H), 6.93 (dd, J = 9.0, 3.0 Hz, 1H), 6.88 (d, J = 9.0 Hz, 1H), 6.80 (d, J = 8.4 Hz, 1H), 4.22 (s, 2H), 3.87 (s, 3H), 3.82 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 188.75, 163.65, 161.19, 153.63, 153.29, 148.37, 138.64, 134.48, 134.45, 131.99, 130.99, 130.91, 130.26, 129.02, 125.69, 125.12, 123.12, 116.68, 116.23, 116.01, 114.71, 113.83, 112.57, 56.26, 55.98; HRMS calcd for $C_{23}H_{20}NO_3F$ $[M + Na]^+$: 400.1325, found 400.1320.

4.4.5. (E)-1-(6-Amino-4′-fluoro-[1,1′-biphenyl]-3-yl)-3-(2,3-dimethoxyphenyl)prop-2-en-1-one (**30**)

Yellow power, 73.74%; HPLC t_R = 19.31 min; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06 (d, J = 15.8 Hz, 1H), 7.92 (dd, J = 8.4, 2.1 Hz, 1H), 7.84 (d, J = 2.1 Hz, 1H), 7.60 (d, J = 15.8 Hz, 1H), 7.48–7.40 (m, 2H), 7.26 (dd, J = 8.0, 1.4 Hz, 1H), 7.21–7.14 (m, 2H), 7.07 (t, J = 8.0 Hz, 1H), 6.94 (dd, J = 8.1, 1.4 Hz, 1H), 6.79 (d, J = 8.4 Hz, 1H), 4.23 (s, 2H), 3.88 (s, 3H), 3.86 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 188.56, 163.61, 161.15, 153.29, 148.82, 148.53, 138.12, 134.42, 134.38, 131.99, 130.94, 130.86, 130.25, 129.58, 128.77, 125.61, 124.24, 123.72, 119.65, 116.20, 115.99, 114.72, 113.90, 61.36, 55.96; HRMS calcd for $C_{23}H_{20}NO_3F$ $[M + Na]^+$: 400.1326, found 400.1320.

4.4.6. (E)-1-(6-Amino-4′-fluoro-[1,1′-biphenyl]-3-yl)-3-(4-hydroxy-3-methoxyphenyl)prop-2-en-1-one (**31**)

Yellow power, 27.13%; HPLC t_R = 13.31 min; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92 (dd, J = 8.4, 2.1 Hz, 1H), 7.84 (d, J = 2.1 Hz, 1H), 7.75 (d, J = 15.5 Hz, 1H), 7.47–7.42 (m, 2H), 7.39 (d, J = 15.5 Hz, 1H), 7.21 (ddd, J = 8.1, 1.9, 0.4 Hz, 1H), 7.20–7.14 (m, 2H), 7.11 (d, J = 1.9 Hz, 1H), 6.94 (d, J = 8.2 Hz, 1H), 6.79 (d, J = 8.4 Hz, 1H), 5.94 (s, 1H), 4.22 (s, 2H), 3.95 (s, 3H); ^{13}C NMR (100 MHz, Acetone) δ 187.41, 164.14, 161.71, 150.64, 149.84, 148.66, 143.69, 136.13, 136.10, 132.36, 131.93, 131.85, 130.75, 128.78, 128.50, 125.59, 124.03, 121.04, 120.05, 116.60,

116.39, 116.10, 115.24, 111.89, 56.37; HRMS calcd for $C_{22}H_{18}NO_3F$ $[M - H]^-$: 362.1192, found 362.1189.

*4.4.7. (E)-1-(6-Amino-4′-fluoro-[1,1′-biphenyl]-3-yl)-3-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (**32**)*

Yellow power, 31.94%; HPLC t_R = 17.48 min; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.93 (dd, J = 8.4, 2.1 Hz, 1H), 7.84 (d, J = 2.1 Hz, 1H), 7.72 (d, J = 15.5 Hz, 1H), 7.47–7.42 (m, 2H), 7.41 (d, J = 15.5 Hz, 1H), 7.21–7.14 (m, 2H), 6.85 (s, 2H), 6.80 (d, J = 8.4 Hz, 1H), 4.23 (s, 2H), 3.91 (s, 6H), 3.89 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 188.03, 163.64, 161.18, 153.53, 148.58, 143.59, 140.24, 134.36, 134.33, 131.85, 130.98, 130.90, 130.89, 130.24, 128.72, 125.72, 121.25, 116.24, 116.03, 114.64, 105.71, 61.06, 56.35; HRMS calcd for $C_{24}H_{22}NO_4F$ $[M - H]^-$: 406.1455, found 406.1464.

*4.4.8. (E)-1-(6-Amino-4′-fluoro-[1,1′-biphenyl]-3-yl)-3-(3-fluoro-4-hydroxyphenyl)prop-2-en-1-one (**33**)*

Yellow power, 43.05%; HPLC t_R = 13.84 min; ^{1}H NMR (400 MHz, DMSO) δ 7.93 (dd, J = 8.6, 2.1 Hz, 1H), 7.83 (d, J = 2.1 Hz, 1H), 7.81 (dd, J = 14.7, 1.8 Hz, 1H), 7.76 (d, J = 15.4 Hz, 1H), 7.54 (d, J = 15.4 Hz, 1H), 7.52–7.46 (m, 2H), 7.44 (dd, J = 8.4, 1.5 Hz, 1H), 7.35–7.27 (m, 2H), 6.96 (t, J = 8.7 Hz, 1H), 6.82 (d, J = 8.6 Hz, 1H), 5.79 (s, 2H); ^{13}C NMR (100 MHz, DMSO) δ 186.11, 162.69, 160.27, 152.49, 150.40, 150.09, 147.32, 147.20, 141.27, 141.25, 135.01, 134.98, 131.75, 131.06, 130.98, 130.01, 127.05, 126.98, 126.59, 126.57, 126.40, 123.87, 120.29, 117.82, 117.79, 115.82, 115.61, 115.44, 114.31; HRMS calcd for $C_{21}H_{15}NO_2F_2$ $[M - H]^-$: 350.0993, found 350.0991.

*4.4.9. (E)-1-(6-Amino-4′-fluoro-[1,1′-biphenyl]-3-yl)-3-(3-bromo-4-hydroxy-5-methoxyphenyl)prop-2-en-1-one (**34**)*

Yellow power, 53.25%; HPLC t_R = 17.09 min; ^{1}H NMR (400 MHz, DMSO) δ 9.96 (s, 1H), 7.99 (dd, J = 8.6, 1.8 Hz, 1H), 7.82 (d, J = 1.8 Hz, 1H), 7.81 (d, J = 15.4 Hz, 1H), 7.66 (d, J = 1.3 Hz, 1H), 7.55 (d, J = 15.4 Hz, 1H), 7.52–7.43 (m, 3H), 7.31 (t, J = 8.9 Hz, 2H), 6.83 (d, J = 8.6 Hz, 1H), 5.79 (s, 2H), 3.91 (s, 3H); ^{13}C NMR (100 MHz, DMSO) δ 186.06, 162.66, 160.24, 150.40, 148.43, 145.72, 141.23, 134.96, 134.93, 131.62, 131.02, 130.94, 130.15, 127.61, 126.32, 125.51, 123.94, 120.62, 115.80, 115.59, 114.12, 111.22, 109.60, 56.51; HRMS calcd for $C_{22}H_{17}NO_3FBr$ $[M - H]^-$: 440.0298, found 440.0289.

*4.4.10. (E)-1-(6-Amino-4′-fluoro-[1,1′-biphenyl]-3-yl)-3-(3-hydroxy-4-methoxyphenyl)prop-2-en-1-one (**35**)*

Yellow power, 56.88%; HPLC t_R = 13.25 min; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90 (d, J = 8.4 Hz, 1H), 7.82 (s, 1H), 7.71 (d, J = 15.5 Hz, 1H), 7.47–7.37 (m, 3H), 7.26–7.23 (m, 1H), 7.16 (t, J = 8.0 Hz, 2H), 7.10 (d, J = 8.3 Hz, 1H), 6.84 (d, J = 8.2 Hz, 1H), 6.77 (d, J = 8.4 Hz, 1H), 5.71 (s, 1H), 4.21 (s, 2H), 3.91 (s, 3H); ^{13}C NMR (100 MHz, Acetone) δ 164.13, 161.70, 150.65, 150.40, 147.66, 143.29, 136.11, 136.08, 132.45, 131.91, 131.83, 130.78, 129.70, 128.76, 125.60, 122.78, 120.72, 116.58, 116.37, 115.31, 114.62, 112.21, 56.27; HRMS calcd for $C_{22}H_{18}NO_3F$ $[M + H]^+$: 364.1343, found 364.1344.

*4.4.11. (E)-1-(6-Amino-3′,4′-dimethoxy-[1,1′-biphenyl]-3-yl)-3-(4-fluorophenyl)prop-2-en-1-one (**37**)*

Yellow power, 49.38%; HPLC t_R = 17.35 min; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92 (dd, J = 8.4, 2.1 Hz, 1H), 7.88 (d, J = 2.1 Hz, 1H), 7.76 (d, J = 15.6 Hz, 1H), 7.64–7.59 (m, 2H), 7.49 (d, J = 15.6 Hz, 1H), 7.12–7.05 (m, 2H), 7.01 (dd, J = 8.2, 1.9 Hz, 1H), 6.98 (d, J = 7.9 Hz, 1H), 6.97 (d, J = 2.1 Hz, 1H), 6.79 (d, J = 8.4 Hz, 1H), 4.31 (s, 2H), 3.94 (s, 3H), 3.91 (s, 3H); ^{13}C NMR (100 MHz, DMSO) δ 186.11, 164.32, 161.85, 150.61, 148.89, 148.10, 140.47, 131.84, 131.81, 131.66, 131.07, 130.94, 130.85, 129.76, 126.04, 124.91, 122.33, 122.31, 121.02, 115.89, 115.67, 114.03, 112.51, 112.29, 55.58, 55.43; HRMS calcd for $C_{23}H_{20}NO_3F$ $[M - H]^-$: 376.1349, found 376.1348.

*4.4.12. (E)-1-(6-Amino-3′,4′-dimethoxy-[1,1′-biphenyl]-3-yl)-3-(2,4-dimethoxyphenyl)prop-2-en-1-one (**38**)*

Yellow power, 56.27%; HPLC t_R = 16.34 min; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92 (dd, J = 8.4, 2.1 Hz, 1H), 7.88 (d, J = 2.1 Hz, 1H), 7.76 (d, J = 15.6 Hz, 1H), 7.64–7.59 (m, 2H), 7.49 (d, J = 15.6 Hz, 1H), 7.12–7.05 (m, 2H), 7.01 (dd, J = 8.2, 1.9 Hz, 1H), 6.98 (d, J = 7.9 Hz, 1H), 6.97 (d, J = 2.1 Hz, 1H), 6.79 (d, J = 8.4 Hz, 1H), 4.31 (s, 2H), 3.94 (s, 3H), 3.91 (s, 3H); ^{13}C NMR (100 MHz, DMSO) δ 186.11, 164.32, 161.85, 150.61, 148.89, 148.10, 140.47, 131.84, 131.81, 131.66, 131.07, 130.94, 130.85, 129.76, 126.04, 124.91, 122.33, 122.31, 121.02, 115.89, 115.67, 114.03, 112.51, 112.29, 55.58, 55.43; HRMS calcd for $C_{23}H_{20}NO_3F$ $[M - H]^-$: 376.1349, found 376.1348.

*4.4.13. (E)-1-(6-Amino-3′,4′-dimethoxy-[1,1′-biphenyl]-3-yl)-3-(2,5-dimethoxyphenyl)prop-2-en-1-one (**39**)*

Yellow power, 53.88%; HPLC t_R = 16.70 min; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 (d, J = 15.8 Hz, 1H), 7.91 (dd, J = 8.3, 2.1 Hz, 1H), 7.88 (d, J = 2.0 Hz, 1H), 7.59 (d, J = 15.8 Hz, 1H), 7.16 (d, J = 3.0 Hz, 1H), 7.02 (dd, J = 8.2, 1.9 Hz, 1H), 6.97 (d, J = 1.9 Hz, 1H), 6.97 (d, J = 8.2 Hz, 1H), 6.91 (dd, J = 9.0, 3.0 Hz, 1H), 6.86 (d, J = 9.0 Hz, 1H), 6.78 (d, J = 8.3 Hz, 1H), 4.28 (s, 2H), 3.94 (s, 3H), 3.91 (s, 3H), 3.85 (s, 3H), 3.81 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 188.65, 153.52, 153.17, 149.33, 148.64, 148.59, 138.36, 131.76, 131.02, 129.90, 128.66, 126.46, 125.01, 123.04, 121.34, 116.61, 114.45, 113.66, 112.49, 112.31, 111.70, 56.16, 56.01, 56.00, 55.86; HRMS calcd for $C_{25}H_{25}NO_5$ $[M - H]^-$: 418.1654, found 418.1644.

*4.4.14. (E)-1-(6-Amino-3′,4′-dimethoxy-[1,1′-biphenyl]-3-yl)-3-(2,3-dimethoxyphenyl)prop-2-en-1-one (**40**)*

Yellow power, 55.24%; HPLC t_R = 15.97 min; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 (d, J = 15.8 Hz, 1H), 7.92 (dd, J = 8.4, 2.1 Hz, 1H), 7.88 (d, J = 2.1 Hz, 1H), 7.61 (d, J = 15.8 Hz, 1H), 7.26 (dd, J = 7.9, 1.2 Hz, 1H), 7.07 (t, J = 8.0 Hz, 1H), 7.01 (dd, J = 8.2, 1.9 Hz, 1H), 6.97 (d, J = 1.8 Hz, 1H), 6.97 (d, J = 8.2 Hz, 1H), 6.94 (dd, J = 8.2, 1.3 Hz, 1H), 6.78 (d, J = 8.4 Hz, 1H), 4.29 (s, 2H), 3.94 (s, 3H), 3.91 (s, 3H), 3.88 (s, 3H), 3.87 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 188.49, 153.18, 149.32, 148.76, 148.71, 148.58, 137.85, 131.78, 130.96, 129.90, 129.51, 128.46, 126.42, 124.16, 123.66, 121.30, 119.50, 114.46, 113.79, 112.27, 111.69, 61.28, 55.99, 55.97, 55.87; HRMS calcd for $C_{25}H_{25}NO_5$ $[M - H]^-$: 418.1654, found 418.1645.

*4.4.15. (E)-1-(6-Amino-3′,4′-dimethoxy-[1,1′-biphenyl]-3-yl)-3-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (**41**)*

Yellow power, 56.14%; HPLC t_R = 14.21 min; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92 (dd, J = 8.4, 2.1 Hz, 1H), 7.88 (d, J = 2.1 Hz, 1H), 7.72 (d, J = 15.5 Hz, 1H), 7.43 (d, J = 15.5 Hz, 1H), 7.02 (dd, J = 8.2, 1.8 Hz, 1H), 6.98 (d, J = 8.2 Hz, 1H), 6.97 (d, J = 1.8 Hz, 1H), 6.85 (s, 2H), 6.79 (d, J = 8.4 Hz, 1H), 4.30 (s, 2H), 3.94 (s, 3H), 3.91 (s, 9H), 3.89 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 187.90, 153.37, 149.28, 148.83, 148.57, 143.28, 140.01, 131.59, 130.86, 130.80, 129.86, 128.30, 126.40, 121.27, 121.19, 114.37, 112.26, 111.63, 105.54, 60.90, 56.19, 55.94, 55.92; HRMS calcd for $C_{26}H_{27}NO_6$ $[M - H]^-$: 448.1760, found 448.1751.

*4.5. Synthesis of (E)-1-(6-amino-4′-fluoro-[1,1′-biphenyl]-3-yl)-3-(3,4-dimethoxyphenyl)prop-2-en-1-one (**27**)*

To a mixture of 40 ml EtOH and 10 ml H_2O, compound **26** (419 mg, 1.0 mmol) and NaOH (80 mg, 2.0 mmol) were added, and the mixture was refluxed at 70 °C for 8h. Then the mixture was concentrated in vacuum and extracted with EtOAc. The organic layer was dried over anhydrous sodium sulfate. After removal of the solvent in vacuum, the residue was purified by column chromatography to yield yellow power 235.1 mg (62.35%); HPLC t_R = 16.49 min; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.93 (dd, J = 8.4, 2.1 Hz, 1H), 7.85 (d, J = 2.1 Hz, 1H), 7.76 (d, J = 15.5 Hz, 1H), 7.47–7.39 (m,

2H), 7.41 (d, J = 15.7 Hz, 1H), 7.23 (dd, J = 8.4, 1.9 Hz, 1H), 7.21–7.15 (m, 2H), 7.15 (d, J = 1.9 Hz, 1H), 6.89 (d, J = 8.3 Hz, 1H), 6.79 (d, J = 8.4 Hz, 1H), 4.21 (s, 2H), 3.94 (s, 3H), 3.92 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 188.00, 163.44, 160.99, 151.07, 149.14, 148.48, 143.41, 134.33, 134.30, 131.67, 130.83, 130.75, 130.05, 128.62, 128.20, 125.48, 122.77, 119.71, 116.06, 115.85, 114.56, 111.12, 110.26, 55.94, 55.91; HRMS calcd for $C_{23}H_{20}NO_3F$ $[M - H]^-$: 376.1349, found 376.1340.

4.6. Synthesis of chalcones **42–44** by the Claisen–Schmidt condensation between acetophenones derivatives and appropriate aromatic aldehydes

In general, acetophenone (5 mmol) was added to equimolar of aromatic aldehyde and dissolved in EtOH. To the solution, 6 M NaOH (*aq*) (2 ml) was added and the reaction mixture was stirred at room temperature until aldehyde was consumed by monitoring with TLC. Then HCl (10%) was added until pH 4 was obtained. The mixture was concentrated in vacuum to remove EtOH, and further extracted with EtOAc. The EtOAc layer was dried over anhydrous sodium sulfate. After removal of the solvent in vacuum, the crude product was purified by recrystallization with EtOH to give corresponding chalcones.

4.6.1. (E)-1-(3-Bromophenyl)-3-(4-methoxyphenyl)prop-2-en-1-one (**42**)

Light yellow power, 85.69%; HPLC t_R = 14.21 min; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.13 (t, J = 1.7 Hz, 1H), 7.96–7.89 (m, 1H), 7.79 (d, J = 15.6 Hz, 1H), 7.69 (ddd, J = 7.9, 1.9, 1.0 Hz, 1H), 7.61 (d, J = 8.7 Hz, 2H), 7.37 (s, J = 7.9 Hz, 2H), 6.95 (d, J = 8.8 Hz, 2H), 3.86 (s, 3H).

4.6.2. (E)-1-(3-Bromophenyl)-3-(3,4-dimethoxyphenyl)prop-2-en-1-one (**43**)

Light yellow power, 89.25%; HPLC t_R = 16.08 min; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.13 (t, J = 1.8 Hz, 1H), 7.95–7.89 (m, 1H), 7.77 (d, J = 15.6 Hz, 1H), 7.70 (ddd, J = 7.9, 1.9, 1.0 Hz, 1H), 7.38 (t, J = 7.9 Hz, 1H), 7.31 (d, J = 15.6 Hz, 1H), 7.24 (dd, J = 8.3, 2.0 Hz, 1H), 7.16 (d, J = 2.0 Hz, 1H), 6.91 (d, J = 8.3 Hz, 1H), 3.96 (s, 3H), 3.94 (s, 3H).

4.6.3. (E)-1-(3-Bromophenyl)-3-(3-hydroxy-4-methoxyphenyl) prop-2-en-1-one (**44**)

Yellow power, 85.62%; HPLC t_R = 12.88 min; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.13 (s, 1H), 7.92 (d, J = 7.7 Hz, 1H), 7.75 (d, J = 15.6 Hz, 1H), 7.70 (d, J = 8.8 Hz, 1H), 7.38 (t, J = 7.8 Hz, 1H), 7.32 (d, J = 15.6 Hz, 1H), 7.29 (d, J = 1.8 Hz, 1H), 7.15 (dd, J = 8.3, 1.7 Hz, 1H), 6.88 (d, J = 8.3 Hz, 1H), 5.67 (s, 1H), 3.95 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 189.00, 149.28, 146.05, 145.80, 140.26, 135.45, 131.45, 130.20, 128.24, 126.96, 123.02, 122.96, 119.50, 113.32, 110.73, 56.07; HRMS calcd for $C_{16}H_{13}O_3Br$ $[M + H]^+$: 333.0121, found 333.0117.

4.7. General procedure for synthesis of **45-48**

The procedure is exemplified by the synthesis of **45.**

4.7.1. (E)-1-(4′-Fluoro-[1,1′-biphenyl]-3-yl)-3-(4-methoxyphenyl) prop-2-en-1-one (**45**)

A mixture of **42** (317 mg, 1.0 mmol), (4-fluorophenyl)boronic acid (182 mg, 1.3 mmol), dichloro(1,1′-bis(diphenylphosphino) ferrocene)palladium(II)-dichloromethane adduct (50 mg, 0.06 mmol), 2 M (aq) K_2CO_3 (2.0 ml), and 1,4-dioxane (2.0 ml) was heated in a sealed vial in a microwave reactor at 150 °C for 20 min. After cooling, HCl (10%) was added until pH 4 was obtained and the mixture was extracted with EtOAc (10 ml) by three times. The combined EtOAc layer was washed until neutral and dried over anhydrous sodium sulfate. After removal of the solvent in vacuum, the crude product was purified by column chromatography to yield yellow power 233.3 mg (70.23%); HPLC t_R = 26.23 min; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.17 (t, J = 1.7 Hz, 1H), 8.01–7.94 (m, 1H), 7.83 (d, J = 15.6 Hz, 1H), 7.77–7.72 (m, 1H), 7.64–7.54 (m, 5H), 7.44 (d, J = 15.6 Hz, 1H), 7.20–7.14 (m, 2H), 6.98–6.92 (m, 2H), 3.87 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 190.43, 163.96, 161.84, 161.51, 145.06, 140.67, 139.10, 136.40, 131.02, 130.37, 129.07, 128.88, 128.80, 127.55, 127.26, 126.90, 119.66, 115.89, 115.70, 114.48, 55.37; HRMS calcd for $C_{22}H_{17}O_2F$ $[M + H]^+$: 333.1285, found 333.1288.

4.7.2. (E)-3-(3,4-Dimethoxyphenyl)-1-(4′-fluoro-[1,1′-biphenyl]-3-yl)prop-2-en-1-one (**46**)

Yellow power, 60.56%; HPLC t_R = 23.63 min; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.16 (t, J = 1.8 Hz, 1H), 7.97 (d, J = 7.7 Hz, 1H), 7.80 (d, J = 15.6 Hz, 1H), 7.77–7.72 (m, 1H), 7.64–7.54 (m, 3H), 7.41 (d, J = 15.6 Hz, 1H), 7.26 (dd, J = 8.4, 1.8 Hz, 1H), 7.20–7.13 (m, 3H), 6.91 (d, J = 8.3 Hz, 1H), 3.95 (s, 3H), 3.94 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 190.32, 163.90, 161.45, 151.56, 149.29, 145.26, 140.64, 139.07, 136.38, 136.35, 130.96, 129.00, 128.83, 128.75, 127.81, 127.21, 126.84, 123.21, 119.94, 115.86, 115.65, 111.19, 110.38, 55.95, 55.92; HRMS calcd for $C_{23}H_{19}O_3F$ $[M + H]^+$: 363.1391, found 363.1379.

4.7.3. (E)-1-(3′-Fluoro-[1,1′-biphenyl]-3-yl)-3-(3-hydroxy-4-methoxyphenyl)prop-2-en-1-one (**47**)

Yellow power, 76.58%; HPLC t_R = 20.51 min; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.20 (s, 1H), 8.00 (d, J = 7.7 Hz, 1H), 7.78 (d, J = 8.4 Hz, 1H), 7.78 (d, J = 15.5 Hz, 1H), 7.58 (t, J = 7.7 Hz, 1H), 7.47–7.40 (m, 2H), 7.43 (d, J = 15.6 Hz, 1H), 7.35 (d, J = 10.2 Hz, 1H), 7.31 (d, J = 2.0 Hz, 1H), 7.16 (dd, J = 8.3, 2.0 Hz, 1H), 7.09 (ddd, J = 5.9, 4.2, 2.2 Hz, 1H), 6.89 (d, J = 8.3 Hz, 1H), 5.69 (s, 1H), 3.95 (s, 3H); ^{13}C NMR (100 MHz, DMSO) δ 188.89, 163.93, 161.51, 150.41, 146.70, 144.82, 141.94, 141.86, 139.35, 138.72, 131.18, 130.93, 130.84, 129.45, 127.90, 127.67, 126.61, 123.11, 122.38, 119.52, 114.97, 114.65, 114.43, 113.91, 113.69, 111.87, 55.67; HRMS calcd for $C_{22}H_{17}O_3F$ $[M + H]^+$: 349.1234, found 349.1232.

4.7.4. (E)-1-(3-(6-Fluoropyridin-3-yl)phenyl)-3-(3-hydroxy-4-methoxyphenyl)prop-2-en-1-one (**48**)

Yellow power, 78.23%; HPLC t_R = 14.84 min; ^{1}H NMR (400 MHz, Acetone) δ 8.62 (s, 1H), 8.43 (s, 1H), 8.35 (td, J = 8.3, 2.4 Hz, 1H), 8.19 (d, J = 7.7 Hz, 1H), 7.96 (d, J = 7.5 Hz, 1H), 7.82 (d, J = 15.4 Hz, 1H), 7.81 (d, J = 9.8 Hz, 1H), 7.76 (d, J = 15.7 Hz, 1H), 7.70 (t, J = 7.6 Hz, 1H), 7.40 (d, J = 1.6 Hz, 1H), 7.28 (d, J = 8.3 Hz, 1H), 7.22 (dd, J = 8.6, 2.8 Hz, 1H), 7.03 (d, J = 8.2 Hz, 1H), 3.92 (s, 3H); ^{13}C NMR (100 MHz, Acetone) δ 189.57, 151.03, 147.83, 147.00, 146.85, 145.63, 141.34, 141.26, 140.35, 138.08, 131.90, 130.41, 129.28, 128.86, 127.78, 123.48, 120.52, 114.83, 112.27, 110.50, 110.12; HRMS calcd for $C_{21}H_{16}NO_3F$ $[M + H]^+$: 350.1187, found 350.1194.

4.8. Cell culture

Cells (human lung carcinoma cell line, A549; human nasopharyngeal carcinoma cell line, CNE2; human colon cell line, SW480; human breast adenocarcinoma cell line, MCF-7; human hepatoma cell line, HepG2.) were grown in tissue culture flasks in complete growth medium (RPMI-1640 medium with 2 mM glutamine, pH 7.4, supplemented with 10% fetal bovine serum, 100 μg/ml streptomycin and 100 units/ml penicillin) in a carbon dioxide incubator (37 °C, 5% CO_2, 90% Relative Humidity).

4.9. In vitro cytotoxicity assay

Cytotoxicity study was determined using 96-well tissue culture plates. Sulforhodamine B (SRB) assay [46,47] was performed to evaluate cell viability. It measures cellular protein content to

determine cell density. Compounds stock solutions (10 mM) were serially diluted with DMSO, and then various concentrations of compounds in DMSO were further diluted with complete growth medium to obtain working solutions. The final DMSO concentration was 0.5%. Cells were plated at 5000 cells/well. The cells were allowed to grow in carbon dioxide incubator (37 °C, 5% CO_2, 90% RH) for 24 h. Then test compounds in complete growth medium (100 μL) were added to the wells in triplicate. The plates were further incubated for 48 h. The cell growth was stopped by gently layering trichloroacetic acid (50%, 50 μL) on top of the medium in all the wells. The plates were incubated at 4 °C for 1 h to fix the cells attached to the bottom of the wells. The liquid of all the wells was gently pipetted out and discarded. The plates were washed five times with distilled water and air-dried. The plates were stained with SRB dye (0.4% in 1% acetic acid, 100 μL) for 30 min. The plates were washed five times with 1% acetic acid and then air-dried. The adsorbed dye was dissolved in Tris base solution (150 μL, 10 mM, pH 10.4) and plates were gently shaken for 1 h on an orbital shaker. The optical density (OD) was recorded on a TECAN infinite® M200 pro multimode reader at 515 nm. The cell growth was determined by subtracting mean OD value of respective blank from the mean OD value of experimental set. Percent growth in presence of test compounds was calculated considering the growth in absence of any test compounds as 100%. Plot % control of cell viability against the test compound concentration by plot concentrations on a log scale and fit the data by using nonlinear regression in GraphPad Prism 5, the absolute IC_{50} values was obtained from the dose–response curves. All data are average values from triplicate samples, and the experiments were repeated at least two times.

4.10. High content screening based NF-κB translocation assay

Cellomics® NF-κB Activation HCS Reagent Kit (Thermo Scientific), in combination with the ArrayScan HCS Reader (Thermo Scientific) were used to perform the assay, and the enclosed experimental protocol was followed. A549 cells were plated in 384-well plates (PerkinElmer, Packard ViewPlate®, Product No. 6007460) at 1250 cells/25 μL/well and grown for 24 h. Test compounds in DMSO were diluted with complete growth medium to obtain working solutions. Then test compounds in complete growth medium (25 μL) were added to the wells in duplicate. The final DMSO concentration was 0.5%. The plates were incubated at 37 °C for 2 h. TNFα (5 μL) was added (10 ng/mL, final, Sigma–Aldrich, St.Louis, MO) to cells to stimulate NF-κB translocation for 30 min. Cells were then fixed with pre-warmed (37 °C) paraformaldehyde (25 μL, 4%, Thermo Scientific, Product No. 28906) for 10 min. Then cells were permeabilized with permeabilization buffer (Triton X-100) for 10 min, washed twice with PBS. Rabbit anti-p65 NF-κB antibody was added and incubated at room temperature for 1 h. Cells were washed three times and incubated with DyLight™ 488-conjugated goat anti-rabbit IgG (stain NF-κB) along with Hoechst 33342 (stain nucleus) at room temperature for 1 h. After washing, 50 μL PBS was added and the plates were evaluate on ArrayScan HCS Reader (DyLight 488 conjugates: excitation at 494 nm, emission at 532 nm, Hoechst Dye: excitation at 350 nm, emission at 461 nm). The ArrayScan HCS Reader and the Cytoplasm to Nucleus Translocation BioApplication software were used to plate handing, focusing, cell image acquisition, analysis, and quantification of NF-κB activation. The levels of NF-κB translocation were calculated and expressed as the difference between average fluorescence intensity in the nucleus and in the cytoplasm. After stimulation with TNFα, the inhibitory effect of test compounds on TNFα induced NF-κB translocation was expressed as a percentage of fluorescence intensity difference (in nucleus and in cytoplasm) in control wells (TNFα only) after subtracting background (no TNFα). The IC_{50} of test compounds in this NF-κB translocation assay stands for the concentration of a compound required to induce 50% inhibition. All data are average values from duplicate samples, and the experiments were repeated twice.

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Appendix. Supplementary material

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ejmech.2012.02.023.

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