

Solvent-free synthesis and *in vitro* cytotoxicity of fluorinated chalcones against HepG2 cells

Van Thi Bich Pham,^a Hien Van Nguyen,^a Ha Quoc An Than,^b Trung Thanh Pham,^c and Hao Minh Hoang^{*c}

^aDepartment of Chemistry, Nong Lam University, Ho Chi Minh City, Vietnam

^bInstitute of Applied Materials Science, Vietnam Academy of Science and Technology, Ho Chi Minh City, Vietnam

^cDepartment of Chemical Technology, Ho Chi Minh City University of Technology and Education, Ho Chi Minh City, Vietnam

Email: haohm@hcmute.edu.vn

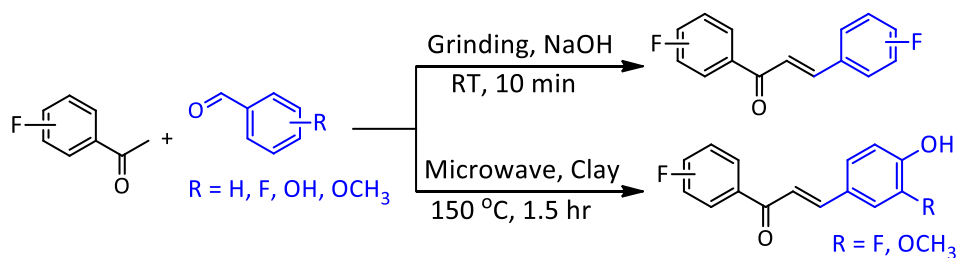
Received 08-19-2024

Accepted 11-06-2024

Published on line 11-14-2024

Abstract

A series of chalcones (**1a-1g**, **2a-2g**, **3a-3g**) containing fluoro substituents at various positions of both rings were synthesized via Claisen-Schmidt condensation using solvent-free grinding and microwave-assisted methods. Among synthesized chalcones, eleven structures (**1f**, **2b**, **2d-2g**, **3b-3d**, **3f-3g**) are novel compounds. Based on the fact that fluorinated organic compounds displayed promising anticancer activities, fluorinated chalcones were assayed for cytotoxicity against HepG2 cancer cells by determining their IC₅₀ values. We found three structures (**1c**, IC₅₀ = 63.38±6.76 μM; **1e**, IC₅₀ = 59.33±1.10 μM; **1g**, IC₅₀ = 43.18±6.54 μM) showing highest the antiproliferative activities. The *in vitro* results indicated the effects of the position of fluoro substituents on the cytotoxic activity. In particular, the scaffold of 4'-F chalcone might be regarded as playing a crucial role in the promising anticancer inhibitory effects towards HepG2 cells.



Keywords: Grinding; microwave-assisted method; fluorinated chalcones; anticancer; HepG2 cells.

Cite as *Arkivoc* 2024 (7) 202412272

DOI: <https://doi.org/10.24820/ark.5550190.p012.272>

Page 1 of 13

©AUTHOR(S)

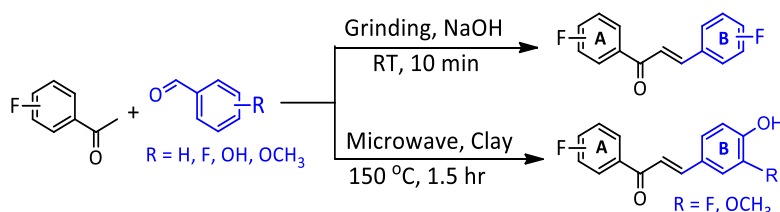
Introduction

Chalcone analogues derived from chalcone skeleton consisting of two aromatic rings joined by a three-carbon α,β -unsaturated ketone. Chalcones are commonly synthesized by the base-catalyzed Claisen-Schmidt condensation of acetophenones and aryl aldehydes. The green chemistry approach to this reaction by grinding together of the reactants with solid NaOH in the absence of solvent at room temperature has proceeded smoothly.¹⁻³ However, our group has experienced the disadvantages in the isolation of hydroxy chalcones by grinding method, which was consistent with the results reported.⁴⁻⁶ It was detrimental to the purity of the desired product due to low yields and side reactions under basic medium. In order to overcome limitations of base-catalyzed protocols for the synthesis of the required chalcones, clays as solid catalysts have been explored as effective candidates for C-C-coupling reactions.⁷⁻⁹ An aspect of green chemistry, microwave-assisted green method has been highly efficient in the field of organic synthesis due to the reduction of side-products.¹⁰ Therefore, in a series of reactions focusing on the synthesis of *trans*-chalcones, the eco-friendly protocol involved solvent-free, clay-based catalyst under microwave irradiation has been developed and applicable to various substrates.⁸

Chalcones exhibited promising *in vitro* and *in vivo* anticancer activities.¹¹⁻¹⁵ Hence, chalcone skeleton is a valuable template for the development of novel chalcone-based anticancer agents. Majority of chalcones have been identified from plants containing hydroxy (-OH) and methoxy (-OCH₃) substituents on aromatic rings while instances having fluorine (F) have been rarely isolated. Halogenated chalcones exhibited an increase in the antiproliferative activity and among halogens, fluorine displays promising effects as medicinal chemistry targets.^{14,16,17} As established in the literature, replacement of fluorine in aromatic C-H in biologically active molecules has been shown to demonstrate improved biological activity in multiple cases.¹⁸⁻²³ The significant activity results can be attributed to the presence of C-F bonds, which resulted in higher affinities for binding to target molecules, bonding interactions, metabolic stabilities and physical properties.^{24,25}

The anticancer activity of fluorinated chalcones against human hepatocarcinoma cell line (HepG2) have been reported. Chalcone with trimethoxy groups at positions 2,4,6 in ring A and 2',5'-difluoro groups in ring B exhibited a significant 2.2-fold increase of HepG2 inhibitory activity compared to non-fluorinated chalcone.²⁴ Being motivated by these findings, Polat et al. designed trimethoxy chalcones with fluoro groups at different positions on ring B to evaluate the anticancer activities. Investigating the cytotoxic effects of these compounds, the synthesized fluorinated chalcones were found to be sensitive to HepG2 cancer cells and *meta*-F scaffold mainly contributed to the antiproliferative activity.²⁰

In continuation of our studies on fluorinated organic compounds as anticancer agents, our group is focusing on fluorinated chalcones. We selected fluoro acetophenones and benzaldehydes as starting materials to synthesize chalcones containing fluorine in both ring A and B via Claisen-Schmidt condensation under solvent-free, solid-catalyzed and microwave-assisted conditions (Scheme 1). Fluorinated chalcones were subsequently assayed for the cytotoxic potency against HepG2 cancer cells.



Scheme 1. General synthesis for fluorinated chalcones.

Results and Discussion

Fluorinated chalcones herein have been synthesized using grinding or microwave-assisted methods under solvent-free condition. Synthesis of fluoro-substituted chalcones (**1a-1e**, **2a-2e**, **3a-3e**) (Figure 1) was achieved by grinding together equivalent molar ratios of fluorine-containing acetophenones and benzaldehyde analogues. Two reagents were ground for 10 min in a solvent-free environment prior to adding solid NaOH. The reaction mixture was further ground for 10 min for C-C coupling reaction. The crude products were purified using flash column chromatography (CC) to afford the expected chalcones.

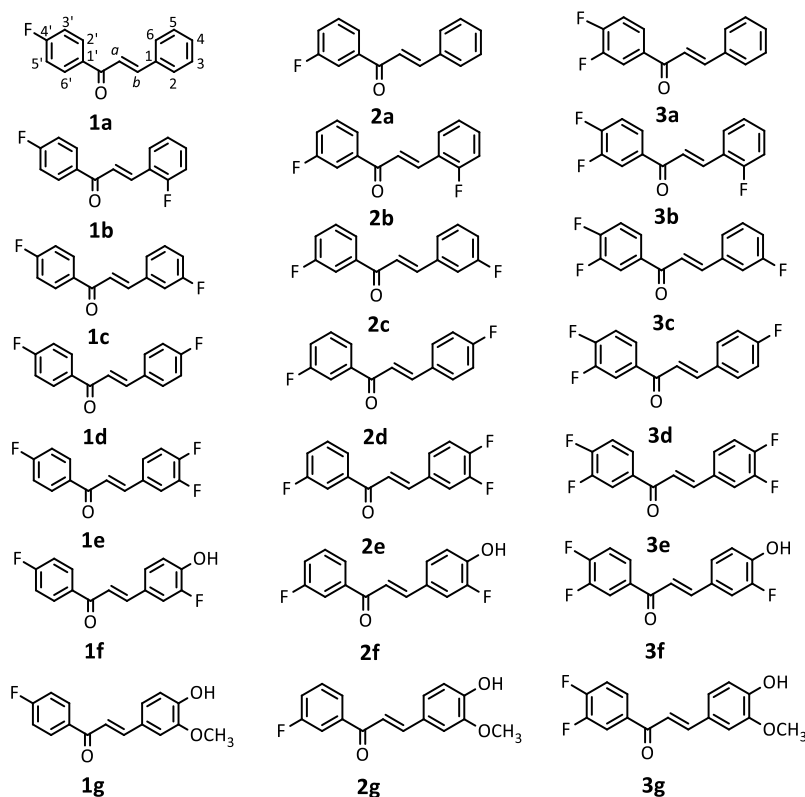


Figure 1. Chemical structures of fluorinated chalcones (**1a-1g**; **2a-2g**, **3a-3g**).

In a previous procedure for (*E*)-chalcone synthesis, we tried a classical procedure as a reference by stirring a mixture composing of acetophenone, benzaldehyde and solid NaOH in ethanol at reflux condition.⁶ The reaction was monitored by thin layer chromatography (TLC). After stirring for 24 h, the conversion was determined by TLC (see the Supplementary Information). However, besides starting materials due to incomplete conversion, TLC displayed spots, which can be attributed to side-products. To obtain the pure (*E*)-chalcone, the crude mixture was purified by silica gel CC. Unfortunately, after elucidating the structures of isolated products by analyzing ¹H NMR data, the desired (*E*)-chalcone was obtained in yield of 5%, while the Michael addition product was isolated in 41% yield (¹H NMR data of Michael product was found in the Supplementary Information). In addition, in solution chemistry, the interactions between reactants or/and reactant-product play crucial roles in the final outcome. Therefore, the Cannizzaro products formed by self-oxidation-reduction reaction of benzaldehyde in basic condition can be found in our case.

By eliminating the solvent, we created a solvent-free environment for the reaction by grinding reactants and solid NaOH together. During work-up and having a Michael product in hand, we easily

determined the presence of the Michael product in this procedure. Therefore, the effect of the grinding process on the reaction product must be considered. We found that the procedure involving grinding fluoro acetophenones and benzaldehydes for 10 min prior to adding solid NaOH gave the expected chalcones as main products in 32-81% yields (Table 1).

In our previous results, the base-catalyzed synthesis of chalcones with hydroxyl groups in ring B was unfavorable due to the enolate formation from hydroxy benzaldehydes, which hinders the nucleophilic attack to form the expected chalcones.⁴⁻⁶ Keeping these observations in mind, we aimed at synthesizing hydroxy chalcones through the use of montmorillonite K10 (MK10) clay as a solid catalyst, solvent-free and microwave-assisted condition. The clay-catalyzed Claisen-Schmidt condensation afforded chalcones **1f**, **1g**, **2f**, **2g**, **3f**, **3g** in yields of 33-46% (Table 1). MK10 clay acts as an acidic catalyst in the aldol condensation due to the presence of inorganic metal ions, which function as Lewis acids to promote the reaction. It was believed that these ions located near the carbonyl oxygen facilitate the enolisation of the aryl ketones by coordination.^{8,26,27}

In general, the use of grinding method for the preparation of chalcones (**1a-1e**, **2a-2e**, **3a-3a**) resulted in higher isolated yields (32-81%) when compared to clay-catalyzed, microwave irradiation procedure, in which the crude products were subjected to purification by CC to afford expected hydroxy chalcones (**1f**, **1g**, **2f**, **2g**, **3f**, **3g**) in 33-46% yields. Nevertheless, we successfully synthesized desired hydroxy chalcones, which have not been described in literature.^{1,8} Furthermore, our protocol avoided the protection step of phenolic groups by protecting reagents such as methoxyethoxymethyl (MEM) or methoxymethyl (MOM) ether.^{28,29}

The structure of fluorinated chalcones was elucidated by using NMR and HRESIMS/ESIMS spectra. The ¹H and ¹³C-NMR signals were assigned. The appearance of two doublet signals in the ¹H-NMR spectra in a range from δ 7.3 to δ 7.8 ppm with coupling constants (³J(H-H) of ~15 Hz) demonstrated a *trans* configuration in synthesized chalcones containing fluorines.

Fluorinated chalcones were evaluated *in vitro* for their cytotoxicity towards hepatocellular carcinoma (HepG2) using MTT assay. Doxorubicin was tested as a positive control to assess test validity. The cytotoxic effect was expressed as the growth inhibitory concentration (IC₅₀, μ M) values (Table 1). In our assay, compounds showing < 50% activities in a range from 2 to 128 μ g/mL were considered "not detectable". The results are presented as IC₅₀ values in Table 1. It could be observed that the majority of fluorinated chalcones demonstrated cytotoxic activity against HepG2 cells with IC₅₀ values in a range from 43.18 to 321.47 μ M, except for compound **3a**. Chalcones are known for inhibitory activity against multiple targets as they possess the electrophilic α,β -unsaturated ketone as a Michael acceptor, which can cross link with the -SH group of cysteine residues in protein/enzyme targets besides other cellular nucleophiles.^{30,31} In other words, the cellular toxicity of chalcones can be proposed regarding the Michael acceptor mechanism in which chalcones inhibited Phase 2 detoxification enzymes (e.g. glutathione S-transferases (GST)) by forming a covalent bond with -SH nucleophile.³²⁻³⁶

Table 1. Isolated yields and cytotoxicities of fluorinated chalcones against HepG2 cancer cell line

Fluorinated chalcones	Yield (%)		Mp (°C)	IC ₅₀ ±SD (μM)
	Grinding	Microwave		
1a	50		80-81	82.31±3.89
1b	72		77-79	77.92±1.31
1c	43		80-81	63.38±6.76
1d	67		102-104	94.11±7.78
1e	49		117-119	59.33±1.10
1f		46	205-207	87.90±5.57
1g		41	102-104	43.18±6.54
2a	58		62-64	80.94±1.10
2b	81		65-67	91.20±6.84
2c	58		83-84	72.68±2.58
2d	71		131-133	77.60±6.02
2e	46		94-96	71.93±3.47
2f		33	186-188	142.69±7.53
2g		34	83-85	84.57±5.14
3a	51		81-82	Not detectable
3b	67		79-81	77.73±3.70
3c	58		101-102	67.84±3.70
3d	62		110-112	89.59±5.49
3e	32		132-133	85.45±3.17
3f		39	206-208	321.47±9.46
3g		36	135-137	85.01±5.72
Doxorubicin (a positive control)				28.70±2.21

It is not trivial to define the exact role of fluorine in enhancing the bioactivity of fluorine-containing molecules.^{37,38} The structure-activity relationship (SAR) study has been considered as a straightforward solution to interpret the effects of fluoro group. In an attempt to improve the understanding of the influence of fluorine on the anticancer effects, Padhye et al. reported that the antiproliferative activities of fluoro

chalcones against human pancreatic BxPC-3 cancer cells were improved by 2- to 7-fold over the non-fluorinated structures. It has been reported that the fluorination of ring B of chalcone leading to the metabolic stability of the C–F bond is responsible for the enhanced antiproliferative property.²² Nakamura et al. also reported an increase in antiproliferative activity of chalcones with the addition of fluorine substituents on the B ring of chalcones in their study.²³

The X-ray data of the ligand-protein complex revealed that the aromatic C-F units interacted favorably with >N-H and >C=O groups of residues at active site of protein via hydrogen bonds and C-F...C=O contacts, respectively, which provided valuable insights into the nature of effective F environments in target proteins.³⁹⁻⁴¹ In addition, the aromatic fluorination affected the electronic nature of the aromatic rings, which are prone to a pronounced π - π stacking interaction with phenylalanine residue.^{42,43}

In general, compounds **1c** ($IC_{50} = 63.38 \pm 6.76 \mu M$), **1e** ($IC_{50} = 59.33 \pm 1.10 \mu M$), **2c** ($IC_{50} = 72.68 \pm 2.58 \mu M$), **2e** ($IC_{50} = 71.93 \pm 3.47 \mu M$), **3c** ($IC_{50} = 67.84 \pm 3.70 \mu M$) containing *meta*-fluoro group in ring B have lower IC_{50} values compared to the others, except for **1g** ($IC_{50} = 43.18 \pm 6.54 \mu M$), suggesting that the *meta*-F substitution into ring B might boost up the cytotoxicity. Fluorinated chalcones **1e**, **2e** and **3e** showed close or lower activities compared to compounds **1c**, **2c**, **3c**, indicating that the introduction of the *para*-fluoro group in ring B has no apparent effect on activity against HepG2 cells.

At the same time, chalcones (**1f**, $IC_{50} = 87.90 \pm 5.57 \mu M$; **2f**, $IC_{50} = 142.69 \pm 7.53 \mu M$; **3f**, $IC_{50} = 321.47 \pm 9.46 \mu M$) bearing *para*-OH group in ring B exhibited decreased activity against HepG2 growth, suggesting that replacing fluorine with a hydroxyl group had a negative impact on the cytotoxicity of compounds **1f**, **2f**, and **3f**. In order to evaluate the contribution of *meta*-F model on activity, except **1g**, the replacement of -F with -OCH₃ group (**2g**, $IC_{50} = 84.57 \pm 5.14 \mu M$; **3g**, $IC_{50} = 85.01 \pm 5.72 \mu M$) resulted in no improvement on growth inhibition in HepG2 cells. Remarkably, chalcone **1g** was found to be the most effective inhibition activity on HepG2 cells with $IC_{50} = 43.18 \pm 6.54 \mu M$ in our assay. The observation might be attributable to the presence of *para*-F group in ring A. Considering IC_{50} values, most structures **1a-1g** showed close or lower IC_{50} values compared to the others, suggesting the crucial effect of *para*-F motif on the inhibitory activity against HepG2 cells. Finally, more fluorination on both ring A and B, **3e** ($IC_{50} = 85.45 \pm 3.17 \mu M$) for example, resulted in no significant difference in cytotoxicity.

Conclusions

In summary, we synthesized 21 fluorinated chalcones in solvent-free condition and evaluated their *in vitro* cytotoxicities against HepG2 cancer cells. Fluorinated chalcones were synthesized from Claisen-Schmidt condensation reactions between acetophenone analogues and benzaldehydes. Chalcones (**1a-1e**, **2a-2e**, **3a-3e**) were prepared in 32-81% yields using solid NaOH as base by grinding method, while compounds (**1g-1f**, **2g-2f**, **3g-3f**) were synthesized in yields of 33-46% employing MK10 clay as a solid catalyst under microwave irradiation. Novel fluorinated chalcones (**1f**, **2b**, **2d-2g**, **3b-3d**, **3f-3g**) along with known compounds were elucidated by NMR and mass spectroscopic analysis. The *in vitro* observation revealed that fluorinated chalcones bearing 4'-F groups in ring A or 3-F in ring B contributed to the cytotoxicity. However, the more fluorination in both rings led to insignificant inhibition toward HepG2 cells. On the basis of our finding, it can be concluded that the skeleton of 4'-F chalcone might act as a pharmacophore on fluorinated chalcone structures against HepG2 cells.

Experimental Section

Chemicals – General

All chemicals used for synthesis and purification were purchased from suppliers without further purification. Acetophenone (99%, China), 3'-fluoroacetophenone (99%, Acros Organics), 4'-fluoroacetophenone (99%, Acros Organics), 3',4'-difluoroacetophenone (96%, Fisher), benzaldehyde (99%, Fisher), 2-fluorobenzaldehyde (97%, Acros Organics), 3-fluorobenzaldehyde (98%, Acros Organics), 4-fluorobenzaldehyde (98%, Acros Organics), 3,4-difluorobenzaldehyde (96%, Acros Organics), 3-fluoro-4-hydroxybenzaldehyde (98%, Fisher), 4-hydroxy-3-methoxybenzaldehyde (98%, China) were utilized as starting materials for the synthetic procedures of fluorinated chalcones. Sodium hydroxide (NaOH, 99%, China) and montmorillonite K10 (surface area 220 – 270 m²/g, Sigma-Aldrich) are used as catalysts in synthetic procedures by grinding and microwave-assisted methods, respectively. *n*-Hexane (> 99%, J. T. Baker), dichloromethane (DCM, > 99%, J. T. Baker) and ethyl acetate (EtOAc, > 99%, J. T. Baker) as solvents were stored in 4.0 L glass bottles.

Analytical methods

The ¹H and ¹³C NMR spectra were recorded on a Brüker Avance at 600 MHz and 150 MHz, respectively. Chemical shifts δ (ppm) were referenced to residual protonated solvent signals of CDCl₃: δ 7.26 ppm (¹H); 77.16 ppm (¹³C), DMSO-*d*₆: δ 2.50 ppm (¹H); 39.52 ppm (¹³C). The proton and carbon signals were abbreviated as s (singlet), d (doublet), dd (doublet of doublet), t (triplet), td (triplet of doublet), m (multiplet). HRESIMS/ESIMS spectra were recorded on a 1100 Series LC-MSD-Trap-SL mass spectrometer (Agilent Technologies, USA). The products were monitored and purified by using thin layer chromatography-TLC (silica gel 60 F₂₅₄, Merck) and flash column chromatography-CC (silica gel 0.035 – 0.070 mm, Merck). The UV-detection was carried out at λ = 254 and 366 nm to visualize spots on TLC.

Chemical synthesis

Grinding procedure for synthesis of 1a-1e, 2a-2e, 3a-3e

An open mortar (Agate mortar (inner diameter × outer diameter × depth = 100 × 120 × 30 mm) with pestle) was charged with fluorinated methyl ketones (5.0 mmol, 1.0 equiv) and aromatic aldehydes (5.0 mmol, 1.0 equiv). Two reagents were ground for 10 min before adding solid NaOH (5.0 mmol, 1.0 equiv). The mixture was subsequently ground with a pestle at room temperature for 10 min. The consumption of starting materials was evidenced by TLC. The reaction was added with a volume of distilled water (15 mL) and acidified to pH 6 with HCl 6 M and finally transferred to a separatory funnel 125 mL. The aqueous phase was extracted with DCM (3×15 mL). The combined organic layers were dried over Na₂SO₄, filtered and the solvent was removed under reduced pressure. The crude product was purified via flash CC (eluent: *n*-hexane/EtOAc = 98/2 → 90/10).

(*E*)-3-(2-Fluorophenyl)-1-(3-fluorophenyl)prop-2-en-1-one (2b). Yield 81%; yellow powder; mp 65-67 °C; ¹H NMR (600 MHz, CDCl₃): δ_{H} 7.13-7.16 (m, 1H, H-3), 7.20 (td, *J*(H,H) 7.8 Hz, *J*(H,H) 0.6 Hz, 1H, H-5), 7.28-7.30 (m, 1H, H-4'), 7.38-7.42 (m, 1H, H-6), 7.47-7.51 (m, 1H, H-4), 7.58 (d, *J*(H,H) 16.2 Hz, 1H, H- α), 7.63 (td, *J*(H,F) 7.8 Hz, *J*(H,H) 1.8 Hz, 1H, H-3'), 7.70-7.72 (m, 1 H, H-6'), 7.90 (dd, *J*(H,F) 7.8 Hz, *J*(H,H) 0.6 Hz, 1H, H-2'), 7.90 (d, *J*(H,H) 15.6 Hz, 1H, H- β). ¹³C NMR (150 MHz, CDCl₃): δ_{C} 115.30 (d, *J*(C,F) 21.0 Hz, 1C, C-6'), 116.32 (d, *J*(C,F) 21.0 Hz, 1C, C-3), 119.99 (d, *J*(C,F) 21.0 Hz, 1C, C-4'), 122.83 (d, *J*(C,F) 12.0 Hz, 1C, C-1), 124.20 (d, *J*(C,F) 7.5 Hz, 1C, C-2'), 124.27 (1C, C- α), 124.59 (d, *J*(C,F) 3.0 Hz, 1C, C-5), 129.97 (d, *J*(C,F) 3.0 Hz, 1C, C-3'), 130.31 (d, *J*(C,F) = 7.5 Hz, 1C, C-4), 132.07 (d, ³*J*(C,F) 7.5 Hz, 1C, C-6), 138.31 (1C, C- β), 140.21 (d, *J*(C,F) 7.5 Hz, 1C, C-1'), 161.01 (d, *J*(C,F) 166.5 Hz, 1C, C-2), 162.70 (d, ¹*J*(C,F) 160.5 Hz, 1C, C-5'), 189.17 (1C, >C=O). HRESIMS calcd. for [M + H]: 245.0778; found: *m/z* 245.0770 ([M + H]⁺).

(E)-1-(3-Fluorophenyl)-3-(4-fluorophenyl)prop-2-en-1-one (2d). Yield 71%; white solid; mp 131-133 °C; ^1H NMR (600 MHz, CDCl_3): δ_{H} 7.14 (dd, $J(\text{H},\text{F})$ 9.0 Hz, $J(\text{H},\text{H})$ 8.4 Hz, 1H, H-3,5), 7.28-7.30 (m, 1H, H-4'), 7.39 (d, $J(\text{H},\text{H})$ 15.6 Hz, 1H, H- α), 7.47-7.51 (m, 1H, H-3'), 7.63 (d, $J(\text{H},\text{H})$ 9.0 Hz, 1H; H-2), 7.64 (d, $J(\text{H},\text{H})$ 9.0 Hz, 1H, H-6), 7.68-7.71 (m, 1H, H-6'), 7.78 (d, $J(\text{H},\text{H})$ 15.6 Hz, 1H, H- β), 7.79 (m, 1H, H-2'). ^{13}C -NMR (150 MHz, CDCl_3): δ_{C} 115.27 (d, $J(\text{C},\text{F})$ 25.5 Hz, 1C, C-6'), 116.17-116.31 (d, $J(\text{C},\text{F})$ 21.0 Hz, 2C, C-3,5), 119.77 (d, $J(\text{C},\text{F})$ 21.0 Hz, 1C, C-4'), 121.31 (1C, C- α), 124.13 (d, $J(\text{C},\text{F})$ 3.0 Hz, 1C; C-2'), 130.29 (d, $J(\text{C},\text{F})$ 9.0 Hz, 1C, C-3'), 130.51 (d, $J(\text{C},\text{F})$ 9.0 Hz, 2C, C-2,6), 130.99 (d, $J(\text{C},\text{F})$ 3.0 Hz, 1C, C-1), 140.29 (d, $J(\text{C},\text{F})$ 6.0 Hz, 1C, C-1'), 144.25 (1C, C- β), 162.12 (d, $J(\text{C},\text{F})$ 246.0 Hz, 1C, C-4), 163.42 (d, $J(\text{C},\text{F})$ 250.5 Hz, 1C, C-5'), 188.96 (1C, >C=O). HRESIMS calcd. for $[\text{M} + \text{H}]^+$: 245.0778; found: m/z 245.0775 ($[\text{M} + \text{H}]^+$).

(E)-3-(3,4-Difluorophenyl)-1-(3-fluorophenyl)prop-2-en-1-one (2e). Yield 46%; white solid; mp 94-96 °C; ^1H NMR (600 MHz, CDCl_3): δ_{H} 7.20-7.23 (m, 1H, H-5), 7.29-7.30 (m, 1H, H-6), 7.37 (m, 1H, H-6), 7.37 (d, $J(\text{H},\text{H})$ 16.2 Hz, 1H, H- α), 7.46-7.52 (m, 2H, H-3',4'), 7.68-7.69 (m, 1H, H-6'), 7.71 (d, $J(\text{H},\text{H})$ 15.6 Hz, 1H, H- β), 7.78-7.80 (m, 1H, H-2'). ^{13}C NMR (150 MHz, CDCl_3): δ_{C} 115.23 (d, $J(\text{C},\text{F})$ 27.0 Hz, 1C, C-6'), 116.55 (d, $J(\text{C},\text{F})$ 21.0 Hz, 1C, C-4'), 117.93 (d, $J(\text{C},\text{F})$ 25.5 Hz, 1C; C-5), 119.99 (d, $J(\text{C},\text{F})$ 25.5 Hz, 1C, C-6), 122.39 (1C, C- α), 122.39 (d, $J(\text{C},\text{F})$ 3.0 Hz, 1C, C-2'), 125.39 (dd, $J(\text{C},\text{F})$ 7.5 Hz, $J(\text{C},\text{F})$ 4.5 Hz, 2C; C-2), 130.37 (d, $J(\text{C},\text{F})$ 9.0 Hz, 1C, C-3'), 131.94 (1C, C-1), 140.03 (d, $J(\text{C},\text{F})$ 7.5 Hz, 1C, C-1'), 143.05 (1C, C- β), 149.67 (dd, $J(\text{C},\text{F})$ 162.0 Hz, $J(\text{C},\text{F})$ 16.5 Hz, 1C, C-3), 151.66 (dd, $J(\text{C},\text{F})$ 183.0 Hz, $J(\text{C},\text{F})$ 16.5 Hz, 1C, C-4), 161.96 (d, $J(\text{C},\text{F})$ 295.5 Hz, 1C, C-5'), 188.59 (1C, >C=O). HRESIMS calcd. for $[\text{M} + \text{H}]^+$: 263.0684; found: m/z 263.0678 ($[\text{M} + \text{H}]^+$).

(E)-1-(3,4-Difluorophenyl)-3-(2-fluorophenyl)prop-2-en-1-one (3b). Yield 67%; yellow powder; mp 79-81 °C; ^1H NMR (600 MHz, CDCl_3): δ_{H} 7.13-7.17 (m, 1H, H-3), 7.20 (t, $J(\text{H},\text{H})$ 7.2 Hz, 1H, H-4), 7.27-7.32 (m, 1H, H-3'), 7.39-7.43 (m, 1H, H-6), 7.56 (d, $J(\text{H},\text{H})$ 15.6 Hz, 1H, H- α), 7.62-7.65 (m, 1H, H-4), 7.80-7.83 (m, 1H, H-2'), 7.86-7.89 (m, 1H, H-6'), 7.90 (d, $J(\text{H},\text{H})$ 15.6 Hz, 1H, H- β). ^{13}C -NMR (150 MHz, CDCl_3): δ_{C} 116.32 (d, $J(\text{C},\text{F})$ 22.5 Hz, 1C, C-3), 117.50 (d, $J(\text{C},\text{F})$ 18.0 Hz, 1C, C-3'), 117.84 (d, $J(\text{C},\text{F})$ 19.5 Hz, 1C, C-6'), 122.70 (d, $J(\text{C},\text{F})$ 12.0 Hz, 1C, C-1), 123.54 (1C, C- α), 124.59 (d, $J(\text{C},\text{F})$ 3.0 Hz, 1C, C-5), 125.40 (dd, $J(\text{C},\text{F})$ 7.5 Hz, $J(\text{C},\text{F})$ 4.5 Hz, 1C, C-2'), 130.06 (d, $J(\text{C},\text{F})$ 3.0 Hz, 1C, C-4), 132.15 (d, $J(\text{C},\text{F})$ 9.0 Hz, 1C, C-6), 135.11 (1C, C-1'), 138.54 (1C, C- β), 149.65 (d, $J(\text{C},\text{F})$ 249.0 Hz, 1C, C-5'), 152.65 (d, $J(\text{C},\text{F})$ 255.0 Hz, 1C, C-5'), 161.01 (d, $J(\text{C},\text{F})$ 255.0 Hz, 1C, C-2), 187.72 (1C, >C=O). HRESIMS calcd. for $[\text{M} + \text{H}]^+$: 263.0684; found: m/z 263.0662 ($[\text{M} + \text{H}]^+$).

(E)-1-(3,4-Difluorophenyl)-3-(3-fluorophenyl)prop-2-en-1-one (3c). Yield 58%; white solid; mp 101-102 °C; ^1H NMR (600 MHz, CDCl_3): δ_{H} 7.12-7.15 (m, 1H, H-2), 7.28-7.32 (m, 1H, H-3'), 7.34-7.35 (m, 1H, H-4), 7.40-7.43 (m, 2H, H-5,6), 7.43 (d, $J(\text{H},\text{H})$ 15.6 Hz, 1H, H- α), 7.77 (d, $J(\text{H},\text{H})$ 16.2 Hz, 1H, H- β), 7.80-7.83 (m, 1H, H-2'), 7.85-7.89 (m, 1H, H-6'). ^{13}C NMR (150 MHz, CDCl_3): δ_{C} 114.50 (d, $J(\text{C},\text{F})$ 25.5 Hz, 1C, C-4), 117.56 (d, $J(\text{C},\text{F})$ 21.0 Hz, 1C, C-6'), 117.70 (d, $J(\text{C},\text{F})$ 21.0 Hz, 1C, C-2), 117.97 (d, $J(\text{C},\text{F})$ 25.5 Hz, 1C, C-3'), 122.06 (1C, C- α), 124.66 (d, $J(\text{C},\text{F})$ 3.0 Hz, 1C, C-6), 125.36 (dd, $J(\text{C},\text{F})$ 9.0 Hz, $J(\text{C},\text{F})$ 4.5 Hz, 1C, C-2'), 130.60 (d, $J(\text{C},\text{F})$ 9.0 Hz, 1C, C-5), 134.99 (1C, C-1), 136.78 (d, $J(\text{C},\text{F})$ 9.0 Hz, 1C, C-1'), 144.22 (1C, C- β), 149.20-154.50 (2C, C-4',5'), 162.10 (d, $J(\text{C},\text{F})$ 295.5 Hz, 1C, C-3), 187.38 (1C, >C=O). HRESIMS calcd. for $[\text{M} + \text{H}]^+$: 263.0684; found: m/z 263.0688 ($[\text{M} + \text{H}]^+$).

(E)-1-(3,4-Difluorophenyl)-3-(4-fluorophenyl)prop-2-en-1-one (3d). Yield 62%; white solid; mp 110-112 °C; ^1H NMR (600 MHz, CDCl_3): δ_{H} 7.11-7.14 (dd, $J(\text{H},\text{H})$ 8.4 Hz, $J(\text{H},\text{F})$ 9.0 Hz, 2H, H-3,5), 7.27-7.32 (m, 1H, H-3'), 7.37 (d, $J(\text{H},\text{H})$ 15.6 Hz, 1H, H- α), 7.63 (d, $J(\text{H},\text{H})$ 9.0 Hz, 1H, H-2), 7.64 (d, $J(\text{H},\text{H})$ 9.0 Hz, 1H, H-6), 7.79 (d, $J(\text{H},\text{H})$ 15.6 Hz, 1H, H- β), 7.80-7.82 (m, 1H, H-2'), 7.85-7.88 (m, 1H, H-6'). ^{13}C NMR (150 MHz, CDCl_3): δ_{C} 116.21 (d, $J(\text{C},\text{F})$ 21.0 Hz, 2C, C-3,5), 117.64 (d, $J(\text{C},\text{F})$ 18.0 Hz, 1C, C-2'), 117.81 (d, $J(\text{C},\text{F})$ 16.5 Hz, 1C, C-6'), 120.67 (1C, C- α), 125.31 (d, $J(\text{C},\text{F})$ 4.5 Hz, 1C, C-2'), 130.49 (2C, C-2,6), 130.88 (1C, C-1), 135.21 (1C, C-1'), 144.45 (1C, C- β), 163.47 (d, $J(\text{C},\text{F})$ 250.5 Hz, 1C, C-4), 187.49 (1C, >C=O). HRESIMS calcd. for $[\text{M} + \text{H}]^+$: 263.0684; found: m/z 263.0676 ($[\text{M} + \text{H}]^+$).

Microwave-assisted procedure for synthesis of 1f-1g, 2f-2g, 3f-3g

Firstly, an open mortar was charged with fluorinated acetophenones (5.0 mmol, 1.0 equiv), aromatic aldehydes containing hydroxyl group (5.0 mmol, 1.0 equiv) and montmorillonite K10 clay catalyst (1.0 g, 0.2 g/mmol) and subsequently ground with a pestle at room temperature for 20 min. Next, the mixture was transferred to a 10 mL-vial vessel sealed with 10 mL cap and heated at 150 °C under microwave irradiation (Microwave Reactor Discover 2.0, CEM, USA). "Standard" method (Vessel type: Pyrex; Control type: Standard; Temperature: 150 °C; Time: 1.5 hr) was used to carry out microwave-assisted synthesis. The reaction was cooled down, diluted with a total volume of 45 mL DMC or EtOAc. The catalyst was removed by filtration using a Büchner funnel and the organic phase was dried over Na₂SO₄, filtered and the solvent was removed under reduced pressure. The crude product was purified via flash CC (eluent: *n*-hexane/EtOAc = 95/5 → 80/20).

(E)-3-(3-Fluoro-4-hydroxyphenyl)-1-(4-fluorophenyl)prop-2-en-1-one (1f). Yield 46%; yellow powder; mp 205-207 °C; ¹H NMR (600 MHz, CDCl₃): δ_H 7.05 (dd, *J*(H,H) 8.4 Hz, *J*(H,F) 9.0 Hz, 2H, H-5), 7.18 (dd, *J*(H,H) 8.4 Hz, *J*(H,F) 8.4 Hz, 2H, H-3',5'), 7.33 (d, *J*(H,H) 7.8 Hz, 1H, H-6), 7.35 (d, *J*(H,H) 15.6 Hz, 1H, H-α), 7.39 (dd, *J*(H,F) 11.4 Hz, *J*(H,H) 1.8 Hz, 1H, H-2), 7.70 (d, *J*(H,H) 15.6 Hz, 1H, H-β), 8.03 (d, *J*(H,H) 9.0 Hz, 1H, H-2'), 8.04 (d, *J*(H,H) 9.0 Hz, 1H, H-6'). ¹³C NMR (150 MHz, CDCl₃): δ_C 114.85 (d, ²*J*(C,F) 22.5 Hz, 1C, C-2), 115.68-115.85 (d, *J*(C,F) 25.5 Hz, 2C, C-3',5'), 120.53 (1C, C-α), 126.12 (1C, C-5), 130.74 (1C, C-1), 130.99-131.07 (d, *J*(C,F) 12.0 Hz, 2C, C-2',6'), 134.56 (1C, C-1'), 143.70 (1C, C-β), 145.80 (1C, C-4), 152.07 (1C, C-3), 188.54 (1C, >C=O). HRESIMS calcd. for [M + H]: 261.0727; found: *m/z* 261.0710 ([M + H]⁺).

(E)-3-(3-Fluoro-4-hydroxyphenyl)-1-(3-fluorophenyl)prop-2-en-1-one (2f). Yield 33%; yellow powder; mp 186-188 °C; ¹H NMR (600 MHz, CDCl₃): δ_H 7.05 (dd, *J*(H,H) 9.0 Hz, *J*(H,F) 8.4 Hz, 1H, H-5), 7.27-7.29 (m, 1H, H-4'), 7.30 (d, *J*(H,H) 15.6 Hz, 1H, H-α) 7.32 (dd, *J*(H,H) 7.8 Hz, *J*(H,H) 1.2 Hz 1H, H-6), 7.39 (dd, *J*(H,F) 10.8 Hz, *J*(H,H) 1.8 Hz, 1H, H-2), 7.47-7.50 (m, 1H, H-3'), 7.67-7.70 (m, 1H, H-6'), 7.71 (d, *J*(H,H) 15.6 Hz, 1H, H-β), 7.78-7.79 (m, 1H, H-2'). ¹³C-NMR (150 MHz, CDCl₃): δ_C 114.96 (d, *J*(C,F) 16.5 Hz, 1C, C-2), 115.19 (d, *J*(C,F) 22.5 Hz, 1C, C-6'), 117.78 (1C, C-5), 119.71 (d, *J*(C,F) 22.5 Hz, 1C, C-4'), 120.5 (1C, C-α), 124.09 (d, *J*(C,F) 3.0 Hz, 1C, C-2'), 126.24 (d, *J*(C,F) 3.0 Hz, 1C, C-6), 128.11 (d, *J*(C,F) 6.0 Hz, 1C, C-1), 130.26 (d, *J*(C,F) 7.5 Hz, 1C, C-3'), 140.32 (d, *J*(C,F) 7.5 Hz, 1C, C-1'), 144.27 (1C, C-β), 146.08 (d, *J*(C,F) 15.0 Hz, 1C, C-4), 150.34 (1C, C-4), 152.30 (1C, C-3), 162.09 (d, *J*(C,F) 246.0 Hz, 1C, C-5'), 188.92 (1C, >C=O). HRESIMS calcd. for [M + H]: 261.0727; found: *m/z* 261.0710 ([M + H]⁺).

(E)-1-(3-Fluorophenyl)-3-(4-hydroxy-3-methoxyphenyl)prop-2-en-1-one (2g). Yield 34%; yellow powder; mp 83-85 °C; ¹H NMR (600 MHz, CDCl₃): δ_H 3.97 (s, 3H, -OCH₃), 6.96 (d, *J*(H,H) 7.8 Hz, 1H, H-5), 7.13 (d, *J*(H,H) 1.8 Hz, 1H, H-2), 7.22 (dd, *J*(H,H) 7.8 Hz, *J*(H,H) 1.8 Hz, 1H, H-6), 7.27-7.28 (m, 1H, H-4'), 7.30 (d, *J*(H,H) 15.6 Hz, 1H, H-α), 7.46-7.49 (m, 1H, H-3'), 7.68-7.70 (m, 1H, H-6'), 7.52 (d, *J*(H,H) 15.6 Hz, 1H, H-β), 7.78-7.79 (m, 1H, H-2'). ¹³C NMR (150 MHz, CDCl₃): δ_C 56.08 (1C, -OCH₃), 114.96 (1C, C-2), 115.19 (d, *J*(C,F) 21.0 Hz, 1C, C-6'), 119.29 (1C, C-α), 119.46 (d, *J*(C,F) 21.0 Hz, 1C, C-4'), 123.64 (1C, C-6), 124.08 (d, *J*(C,F) 3.0 Hz, 1C, C-2'), 127.31 (1C, C-1), 130.20 (d, *J*(C,F) 7.5 Hz, 1C, C-3'), 140.69 (1C, C-1'), 145.97 (1C, C-β), 146.88 (1C, C-3), 148.59 (1C, C-4), 162.08 (d, *J*(C,F) 246.0 Hz, 1C, C-5'), 189.25 (1C, >C=O). HRESIMS calcd. for [M + H]: 273.0927; found: *m/z* 273.0930 ([M + H]⁺).

(E)-1-(3,4-Difluorophenyl)-3-(3-fluoro-4-hydroxyphenyl)prop-2-en-1-one (3f). Yield 39%; yellow powder; mp 206-208 °C. ¹H NMR (600 MHz, CDCl₃-DMSO-*d*₆): δ_H 5.56 (1H, OH), 7.04 (dd, *J*(H,H) 8.4 Hz, *J*(H,F) 8.4 Hz, 1H, H-5), 7.28-7.41 (m, 3H, H-6,2,3'), 7.30 (d, *J*(H,H) 15.6 Hz, 1H, H-α), 7.72 (d, *J*(H,H) 15.6 Hz, 1H, H-β), 7.79-7.80 (m, 1H, H-2'), 7.84-7.87 (m, 1H, H-6'). ¹³C-NMR (150 MHz, CDCl₃-DMSO-*d*₆): δ_C 115.27 (d, *J*(C,F) 22.5 Hz, 1C, C-3'), 117.30 (d, *J*(C,F) 22.5 Hz, 1C, C-2), 117.45 (d, *J*(C,F) 21.0 Hz, 1C, C-6'), 118.17 (d, *J*(C,F) 3.0 Hz, 1C, C-5), 118.74 (1C, C-α), 125.23 (1C, C-2'), 126.23 (1C, C-6), 126.43 (d, *J*(C,F) 7.5 Hz, 1C, C-1), 135.35 (1C, C-1'), 144.86

(1C, C-β), 148.03 (1C, C-3), 148.13 (1C, C-4), 150.63 (1C, C-5'), 165.80 (1C, C-4'), 187.28 (1C, >C=O). HRESIMS calcd. for [M + H]: 279.0633; found: *m/z* 279.0615 ([M + H]⁺).

(E)-1-(3,4-Difluorophenyl)-3-(4-hydroxy-3-methoxyphenyl)prop-2-en-1-one (3g). Yield 36%; yellow solid; mp 135-137 °C. ¹H NMR (600 MHz, CDCl₃): δ_H 3.96 (s, 3H, -OCH₃), 5.95 (s, 1H, -OH), 6.96 (d, *J*(H,H) 8.4 Hz, 1H, H-5), 7.12 (d, *J*(H,H) 1.8 Hz, 1H, H-2), 7.22 (dd, *J*(H,H) 8.4 Hz, *J*(H,H) 1.8 Hz, 1H, H-6), 7.28 (d, *J*(H,H) 15.6 Hz, 1H, H-α), 7.28-7.29 (m, 1H, H-3'), 7.76 (d, *J*(H,H) 15.6 Hz, 1H, H-β), 7.79-7.81 (m, 1H, H-2'), 7.84-7.87 (m, 1H, H-6'). ¹³C-NMR (150 MHz, CDCl₃): δ_C 56.07 (1C, -OCH₃), 110.14 (1C, C-2), 114.97 (1C, C-5) 117.39 (d, *J*(C,F) 18.0 Hz, 1C, C-3'), 117.71 (d, *J*(C,F) 18.0 Hz, 1C, C-6'), 118.57 (1C, C-α), 123.63 (1C, C-6), 125.23 (1C, C-2'), 127.18 (1C, C-1), 135.56 (1C, C-1'), 146.12 (1C, C-β), 146.88 (1C, C-3), 148.66 (1C, C-4), 187.71(1C, >C=O). HRESIMS calcd. for [M + H]: 291.0833; found: *m/z* 291.0822 ([M + H]⁺).

HepG2 cell cytotoxic assay

All fluorinated chalcones were evaluated *in vitro* for their cytotoxic activities against human hepatocellular carcinoma (HepG2) cells using the modified MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma-Aldrich, USA) method with Doxorubicin as a positive control to assess test validity. HepG2 (ATCC, HB-8065TM, USA) cell line was seeded in DMEM (Dulbeccos Modified Eagle Medium) supplemented with 10% FBS (Fetal Bovine Serum) and the other components. Cells (3×10⁵) were grown in a 37 °C humidified incubator with 5% CO₂. Compounds were completely dissolved in dimethylsulfoxide 10% in medium (DMSO, Aldrich 99.9%) at a concentration of 20 mg/mL. Each sample solution was diluted by culture medium into final concentrations of 128, 32, 8, 2 µg/mL in the wells. Each concentration was tested in four separate wells per 96-well plate and in duplicate (4×2). The concentration of positive control Doxorubicin used was 0.01 mM. The concentration of DMSO in tested wells was 0.5%. Growing cells were inoculated at the appropriate concentration (190 µL volume) into each well of the 96-well plate. Sample solutions were applied (10 µL volume) to triplicate culture wells, and cultures were incubated for 72 hours at 37 °C. MTT was prepared at 5 mg/mL and 10 µL added to microculture wells. After 4 hours incubation at 37 °C, 210 µL of culture medium were removed from each well, and formazan product was dissolved in 100 µL of DMSO. The optical density (OD) at 540 nm was measured with a BioTek microplate reader. The percent inhibition I (%) of HepG2 inhibitory activity was calculated using the equation:

$$I (\%) = [(OD_{\text{Culture medium with cells}} - OD_{\text{Sample}}) / (OD_{\text{Culture medium with cells}} - OD_{\text{Culture medium without cells}})] \times 100$$

The IC₅₀ (the half-maximal inhibitory concentration) was determined from plot of I% vs inhibitor concentration.

Acknowledgements

This work was supported by the Nong Lam University-Ho Chi Minh City, Vietnam under Grant number CS-CB23-KH-01.

Supplementary Material

The ¹H, ¹³C NMR, HSQC and HRESIMS/ESIMS spectra of all fluorinated chalcones are available in the Supplementary Material. The details of spectral data of known compounds **1a-1e**, **1g**, **2a**, **2c**, **3a**, **3e** are added. In addition, TLC result of the base-catalyzed reaction between acetophenone and benzaldehyde after stirring

for 24 h at reflux condition and ^1H NMR spectrum of the Michael addition product of this reaction are also found in the Supplementary Material.

References

1. Rateb, N. M.; Zohdi, H. F. *Synth. Commun.* **2009**, *39*, 2789.
<https://doi.org/10.1080/00397910802664244>.
2. Radhakrishnan, S. K.; Shimmon, R. G.; Conn, C.; Baker, A. T. *Chem. Biodivers.* **2016**, *13*, 531.
<https://doi.org/10.1002/cbdv.201500168>.
3. Rahman, A. F. M. M.; Ali, R.; Jahng, Y.; Kadi, A. A. *Molecules* **2012**, *17*, 571.
<https://doi.org/10.3390/molecules17010571>.
4. Wachter-Jurcsak, N.; Radu, C.; Redin, K. *ChemInform* **1998**, *29*, chin.199834168.
<https://doi.org/10.1002/chin.199834168>.
5. Dhar, D. N. *The Chemistry of Chalcones and Related Compounds*; Wiley: New York, 1981.
6. Van, P. T. B.; Huy, L. T.; Thuy, N. T. B.; Nga, V. T.; Tam, L. M.; Phuong, H.; Hao, H. M. *Vietnam J. Chem.* **2023**, *61*, 325.
<https://doi.org/10.1002/vjch.202200151>.
7. Ballini, R.; Bosica, G.; Ricciutelli, M.; Maggi, R.; Sartori, G.; Sartorio, R.; Righi, P. *Green Chem.* **2001**, *3*, 178.
<https://doi.org/10.1039/b101355f>.
8. Rocchi, D.; González, J.; Menéndez, J. *Molecules* **2014**, *19*, 7317.
<https://doi.org/10.3390/molecules19067317>.
9. Castanheiro, R. A. P.; Pinto, M. M. M.; Cravo, S. M. M.; Pinto, D. C. G. A.; Silva, A. M. S.; Kijjoa, A. *Tetrahedron* **2009**, *65*, 3848.
<https://doi.org/10.1016/j.tet.2009.03.019>.
10. Kappe, C. O. *Angew. Chem. Int. Ed.* **2004**, *43*, 6250.
<https://doi.org/10.1002/anie.200400655>.
11. Mahapatra, D. K.; Bharti, S. K.; Asati, V. *Eur. J. Med. Chem.* **2015**, *98*, 69.
<https://doi.org/10.1016/j.ejmech.2015.05.004>.
12. Sharma, V.; Kumar, V.; Kumar, P. *Anticancer Agents Med. Chem.* **2013**, *13*, 422.
<https://doi.org/10.2174/1871520611313030006>.
13. Karthikeyan, C.; Narayana Moorthy, N. S. H.; Ramasamy, S.; Vanam, U.; Manivannan, E.; Karunagaran, D.; Trivedi, P. *Recent Patents Anticancer Drug Discov.* **2014**, *10*, 97.
<https://doi.org/10.2174/1574892809666140819153902>.
14. Gao, F.; Huang, G.; Xiao, J. *Med. Res. Rev.* **2020**, *40*, 2049.
<https://doi.org/10.1002/med.21698>.
15. Srinivasan, B.; Johnson, T. E.; Xing, C. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 555.
<https://doi.org/10.1016/j.bmcl.2010.10.063>.
16. Dias, T. A.; Duarte, C. L.; Lima, C. F.; Proença, M. F.; Pereira-Wilson, C. *Eur. J. Med. Chem.* **2013**, *65*, 500.
<https://doi.org/10.1016/j.ejmech.2013.04.064>.
17. Mohamed, M. F.; Ibrahim, N. S.; Ibrahim, S. A.; El-Manawaty, M. A.; El-Hallouty, S. M.; Hassaneen, H. M.; Abdelhamid, I. A. *Polycycl. Aromat. Compd.* **2023**, *43*, 268.
<https://doi.org/10.1080/10406638.2021.2014535>.
18. Henmi, K.; Hiwatashi, Y.; Hikita, E.; Toyama, N.; Hirano, T. *Biol. Pharm. Bull.* **2009**, *32*, 1109.

- <https://doi.org/10.1248/bpb.32.1109>.
19. Gomes, M.; Muratov, E.; Pereira, M.; Peixoto, J.; Rosseto, L.; Cravo, P.; Andrade, C.; Neves, B. *Molecules* **2017**, *22*, 1210.
<https://doi.org/10.3390/molecules22081210>.
20. Polat, M. F.; Anil, D. A.; Ozkemahli, G.; Turkmenoglu, B.; Hepokur, C.; Burmaoglu, S.; Algul, O. *ChemistrySelect* **2023**, *8*, e202301189.
<https://doi.org/10.1002/slct.202301189>.
21. Lim, Y. H.; Oo, C. W.; Koh, R. Y.; Voon, G. L.; Yew, M. Y.; Yam, M. F.; Loh, Y. C. *Drug Dev. Res.* **2020**, *81*, 994.
<https://doi.org/10.1002/ddr.21715>.
22. Padhye, S.; Ahmad, A.; Oswal, N.; Dandawate, P.; Rub, R. A.; Deshpande, J.; Swamy, K. V.; Sarkar, F. H. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 5818.
<https://doi.org/10.1016/j.bmcl.2010.07.128>.
23. Nakamura, C.; Kawasaki, N.; Miyataka, H.; Jayachandran, E.; Kim, I. H.; Kirk, K. L.; Taguchi, T.; Takeuchi, Y.; Hori, H.; Satoh, T. *Bioorg. Med. Chem.* **2002**, *10*, 699.
[https://doi.org/10.1016/S0968-0896\(01\)00319-4](https://doi.org/10.1016/S0968-0896(01)00319-4).
24. Burmaoglu, S.; Algul, O.; Anil, D. A.; Gobek, A.; Duran, G. G.; Ersan, R. H.; Duran, N. *Bioorg. Med. Chem. Lett.* **2016**, *26*, 3172.
<https://doi.org/10.1016/j.bmcl.2016.04.096>.
25. Park, B. K.; Kitteringham, N. R.; O'Neill, P. M. *Annu. Rev. Pharmacol. Toxicol.* **2001**, *41*, 443.
<https://doi.org/10.1146/annurev.pharmtox.41.1.443>.
26. Nagendrappa, G. *Appl. Clay Sci.* **2011**, *53*, 106.
<https://doi.org/10.1016/j.clay.2010.09.016>.
27. Dintzner, M. R.; Little, A. J.; Pacilli, M.; Pileggi, D. J.; Osner, Z. R.; Lyons, T. W. *ChemInform* **2007**, *38*, chin.200722117.
<https://doi.org/10.1002/chin.200722117>.
28. Khupse, R. S.; Erhardt, P. W. *J. Nat. Prod.* **2007**, *70*, 1507.
<https://doi.org/10.1021/np070158y>.
29. Kostopoulou, I.; Tzani, A.; Polyzos, N.-I.; Karadendrou, M.-A.; Kritsi, E.; Pontiki, E.; Liargkova, T.; Hadjipavlou-Litina, D.; Zoumpoulakis, P.; Detsi, A. *Molecules* **2021**, *26*, 2777.
<https://doi.org/10.3390/molecules26092777>.
30. De Freitas Silva, M.; Pruccoli, L.; Morroni, F.; Sita, G.; Seghetti, F.; Viegas, C.; Tarozzi, A. *Molecules* **2018**, *23*, 1803.
<https://doi.org/10.3390/molecules23071803>.
31. Zhuang, C.; Zhang, W.; Sheng, C.; Zhang, W.; Xing, C.; Miao, Z. *Chem. Rev.* **2017**, *117*, 7762.
<https://doi.org/10.1021/acs.chemrev.7b00020>.
32. Maydt, D.; De Spirt, S.; Muschelknautz, C.; Stahl, W.; Müller, T. J. J. *Xenobiotica* **2013**, *43*, 711.
<https://doi.org/10.3109/00498254.2012.754112>.
33. Kenari, F.; Molnár, S.; Perjési, P. Reaction of Chalcones with Cellular Thiols. *Molecules* **2021**, *26*, 4332.
<https://doi.org/10.3390/molecules26144332>.
34. Dinkova-Kostova, A. T.; Massiah, M. A.; Bozak, R. E.; Hicks, R. J.; Talalay, P. *Proc. Natl. Acad. Sci.* **2001**, *98*, 3404.
<https://doi.org/10.1073/pnas.051632198>.
35. Gan, F.-F.; Kaminska, K. K.; Yang, H.; Liew, C.-Y.; Leow, P.-C.; So, C.-L.; Tu, L. N. L.; Roy, A.; Yap, C.-W.; Kang, T.-S.; Chui, W.-K.; Chew, E.-H. *Antioxid. Redox Signal.* **2013**, *19*, 1149.

- <https://doi.org/10.1089/ars.2012.4909>.
36. Srinivasan, B.; Johnson, T. E.; Lad, R.; Xing, C. *J. Med. Chem.* **2009**, *52*, 7228.
<https://doi.org/10.1021/jm901278z>.
37. Smart, B. E. *J. Fluor. Chem.* **2001**, *109*, 3.
[https://doi.org/10.1016/S0022-1139\(01\)00375-X](https://doi.org/10.1016/S0022-1139(01)00375-X).
38. Isanbor, C.; O'Hagan, D. *J. Fluor. Chem.* **2006**, *127*, 303.
<https://doi.org/10.1016/j.jfluchem.2006.01.011>.
39. Paulini, R.; Müller, K.; Diederich, F. *Angew. Chem. Int. Ed.* **2005**, *44*, 1788.
<https://doi.org/10.1002/anie.200462213>.
40. Olsen, J. A.; Banner, D. W.; Seiler, P.; Wagner, B.; Tschopp, T.; Obst-Sander, U.; Kansy, M.; Müller, K.; Diederich, F. *ChemBioChem* **2004**, *5*, 666.
<https://doi.org/10.1002/cbic.200300907>.
41. DerHovanesian, A.; Doyon, J. B.; Jain, A.; Rablen, P. R.; Sapse, A. M. *Org. Lett.* **1999**, *1*, 1359.
<https://doi.org/10.1021/ol9908326>.
42. Dubowchik, G. M.; Vrudhula, V. M.; Dasgupta, B.; Ditta, J.; Chen, T.; Sheriff, S.; Sipman, K.; Witmer, M.; Tredup, J.; Vyas, D. M.; Verdoorn, T. A.; Bollini, S.; Vinitzky, A. *Org. Lett.* **2001**, *3*, 3987.
<https://doi.org/10.1021/ol0166909>.
43. Kim, C.-Y.; Chang, J. S.; Doyon, J. B.; Baird, T. T.; Fierke, C. A.; Jain, A.; Christianson, D. W. *J. Am. Chem. Soc.* **2000**, *122*, 12125.
<https://doi.org/10.1021/ja002627n>.

This paper is an open access article distributed under the terms of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>)