Supplementary Information

Sustainable route to antiviral furano-chalcones via microwave-assisted solvent-free synthesis with recyclable MgO

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Supplementary: Experimental

S.1.1 Furano-Chalcones purification

Since purification was a prerequisite for conducting biological activity assays, the four synthesized compounds (**3a, 3b, 3c, and 3d**) were isolated, and the corresponding GC-MS and ^{1}H-NMR/^{13}NMR spectra of purified products are herein reported.

In particular, Furano-chalcones (**3a, 3b, 3c and 3d**) purification was carried out by Flash-chromatography using a PuriFlash® 5.050 system (Interchim, Montluçon, France). The purification was performed on C18 packed columns (12 g; Daily purity, Sepachrom srl, Rho, Milan) at a flow rate of 15 mL·min^{-1}. Before purification, each crude sample (**3a, 3b, 3c** and **3d**) (200 mg) was mixed with 0.5 g of C18 silica and packed into a precolumn. A gradient elution was employed using distilled water (A) and methanol (B) (**Table S1)**.

Table S1. PuriFlash® elution gradient for chalcone purification**.**

CV	% A (H_2O)	% B (MeOH)
0	95	5
8	60	40
12	30	70
16 - 20	25	75
22 - 24	0	100

The purified chalcones (**3a, 3b, 3c and 3d**) were analysed by GC-MS, NMR (^{1}H-NMR and ^{13}C-NMR), and UV-Vis.

S.1.2 Virus titration for biological assays. HSV-2 virus stock was titrated by plaque assay, and virus titer was quantified counting viral plaques via crystal violet staining (0.1% crystal violet in 20% ethanol). ZIKV, HRV and IFV stocks were titrated via focus assays and infected foci or cells were quantified by means of the indirect immunochemistry procedure, using a primary mouse monoclonal antibody directed to flavivirus protein E (D1-4G2-4-15 (4G2), Novus Biological, Centennial, CO) or a primary antibody specific to HRV-A1 (18758, QED Bioscience Inc., San Diego, CA) or specific to IFV-A nucleoprotein (ab20343, Abcam, Cambridge, UK) and a secondary antibody peroxidase-conjugated AffiniPure F(ab')2 Fragment Goat Anti-Mouse IgG (H+L) (Jackson ImmunoResearch Laboratories Inc., West Grove, PA). Viral titers were expressed as focus-/plaque-forming units per mL (FFU/mL and PFU/mL). All cell-based experiments were performed with DMEM supplemented with 2% (v/v) FBS in a humidified 5% CO_2 atmosphere at 37 °C (for HSV-2, ZIKV and IFV-A) or at 34 °C (for HRV).

S.1.3 Statistical analyses for biological assays. The results are reported as mean values of three independent experiments performed in duplicate ± standard error of the mean (SEM). EC_{50} (half-maximal effective concentration) and CC_{50} (half-maximal cytotoxic concentration) values and their respective 95% confidence intervals (CIs) were calculated with regression analysis by fitting a variable slope–sigmoidal dose–response curve, using GraphPad Prism 10.0 (GraphPad Software, Boston, MA, USA). Selectivity indexes (SIs) of compounds were calculated as the ratio CC_{50}/EC_{50}.

Supplementary: Results

S.2.1 Reaction optimization

The MgO-catalyzed Claisen-Schmidt condensation was optimized under solvent-free, microwave-assisted conditions using acetophenone (1 mmol) and different aldehydes (1 mmol) with 14 wt% MgO. At 90 °C no conversion was observed, while at 120 °C only HMF showed significant reactivity (65% yield of **3c**). Temperatures ≥150 °C and 2 h reaction time provided high conversion and selectivity for all substrates (**Table S2**).

Table S2. Optimization of MW-assisted, solvent-free, MgO-catalyzed Claisen-Schmidt condensations.[a]

Entry	Ketone	Aldehyde	Product	Temp. (°C)	Time (h)	Conv. (%)[b]	Sel. (%)	Yield (%)[c]
1	**1**	FA (**2a**)	**3a**	90	2	0	-	0
2				120	2	0	-	0
3				150	1.5	9	100	9
4				150	2	51	100	51
5				180	1.5	33	100	33
6				180	2	65	99	64
7		MF (**2b**)	**3b**	90	2	0	-	0
8				120	2	2	100	2
9				150	1.5	13	100	13
10				150	2	63	99	63
11				180	1.5	55	100	55
12				180	2	83	99	83
13		HMF (**2c**)	**3c**	90	2	0	-	0
14				120	2	69	94	65
15				150	1.5	60	100	60
16				150	2	90	93	84
17				180	1.5	87	99	86
18				180	2	99	86	85
19		Benzaldehyde (**2d**)	**3d**	90	2	0	-	0
20				120	2	13	100	13
21				150	2	59	100	59
22				180	2	79 (99)[d]	98 (100)[d]	77 (99)[d]
23		Benzaldehyde (**2d**)[e]		180	2	70	99	69

[a] Reaction conditions: acetophenone (1 mmol), aldehyde (1 mmol), MgO 14 wt% stirred for 2 h and heated to the respective temperature. [b] Conversion is referred to acetophenone. [c] Determined by GC-MS. [d] Reaction performed in EtOH. [e] HTc 14 wt% used as catalyst instead of MgO.

S.2.2. Scale-up

The optimized conditions were applied to a threefold scale-up (3 mmol), maintaining good conversion and selectivity, with HMF remaining the most reactive substrate (78% yield of **3c**) and less reactive aldehydes showing slightly lower yields (**Table S3**).

Table S3. Scale-up performance of MgO-catalyzed Claisen-Schmidt condensations under microwave-assisted, solvent-free conditions.[a]

Entry	Ketone	Aldehyde	Product	Conv. (%)[b]	Sel. (%)	Yield (%)[c]
1	**1**	FA (**2a**)	**3a**	59	100	59
2		MF (**2b**)	**3b**	58	97	56
3		HMF (**2c**)	**3c**	99	79	78
4		Benzaldehyde (**2d**)	**3d**	48	100	48

[a] Reaction conditions: acetophenone (3 mmol), aldehyde (3 mmol), MgO 14 wt% stirred for 2 h at 150°C. [b] Conversion is referred to acetophenone. [c] Determined by GC-MS.

S.3 Furano-Chalcones characterization

The purified chalcones (**3a, 3b, 3c and 3d**) were analysed by GC-MS, NMR (^{1}H-NMR and ^{13}C-NMR), and UV-Vis.

S.3.1. GC-MS chromatograms of product 3a, 3b, 3c, and 3d.

GC-MS analyses were performed on an Agilent Technologies 6850 Network GC System (Agilent Technologies, Santa Clara, CA) equipped with a 5973 Network Mass Selective Detector and a 7683B Automatic Sampler, using a capillary column (HP-5MS; length 30 m; i.d. 0.25 mm; film thickness 0.25 µm). In detail, for the GC-MS analysis, the oven temperature program was set as follows: initial temperature of 50 °C for 5 min, ramped to 100 °C at 10 °C/min for 1 min, ramped to 230 °C at 20 °C/min for 1 min, ramped to 300 °C at 20 °C/min for 5 min, and held for 15 min. The injector temperature was set at 250 °C, with a split ratio of 20:1. Helium was used as the carrier gas at a constant flow rate of 24 mL/min. Chromatograms and the corresponding MS spectra of products **3a, 3b, 3c and 3d** are reported herein (Figures S1–S4).

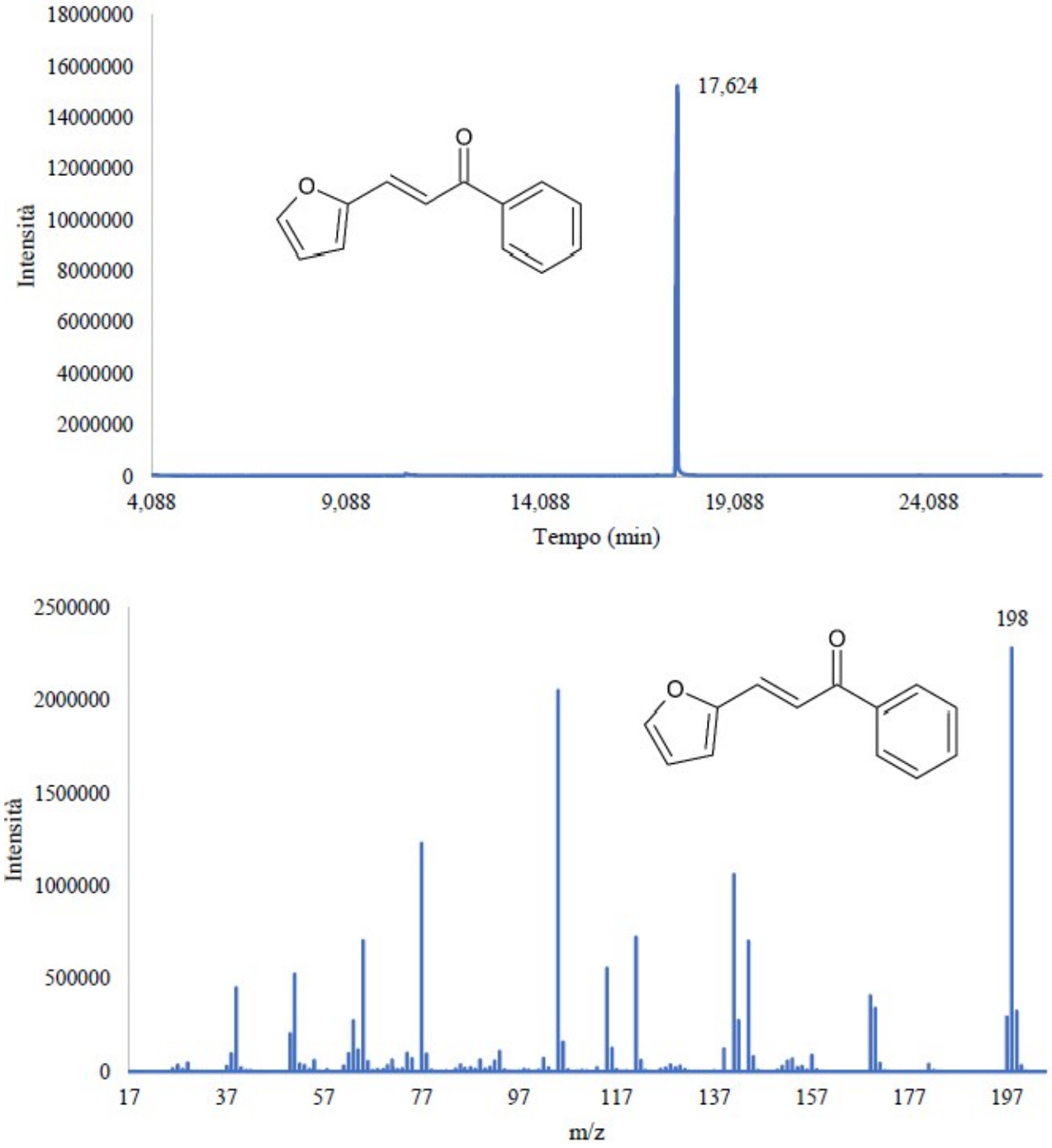

Figure S1 GC-MS of purified (2E)-3-(furan-2-yl)-1-phenylprop-2-en-1-one **(3a)**.

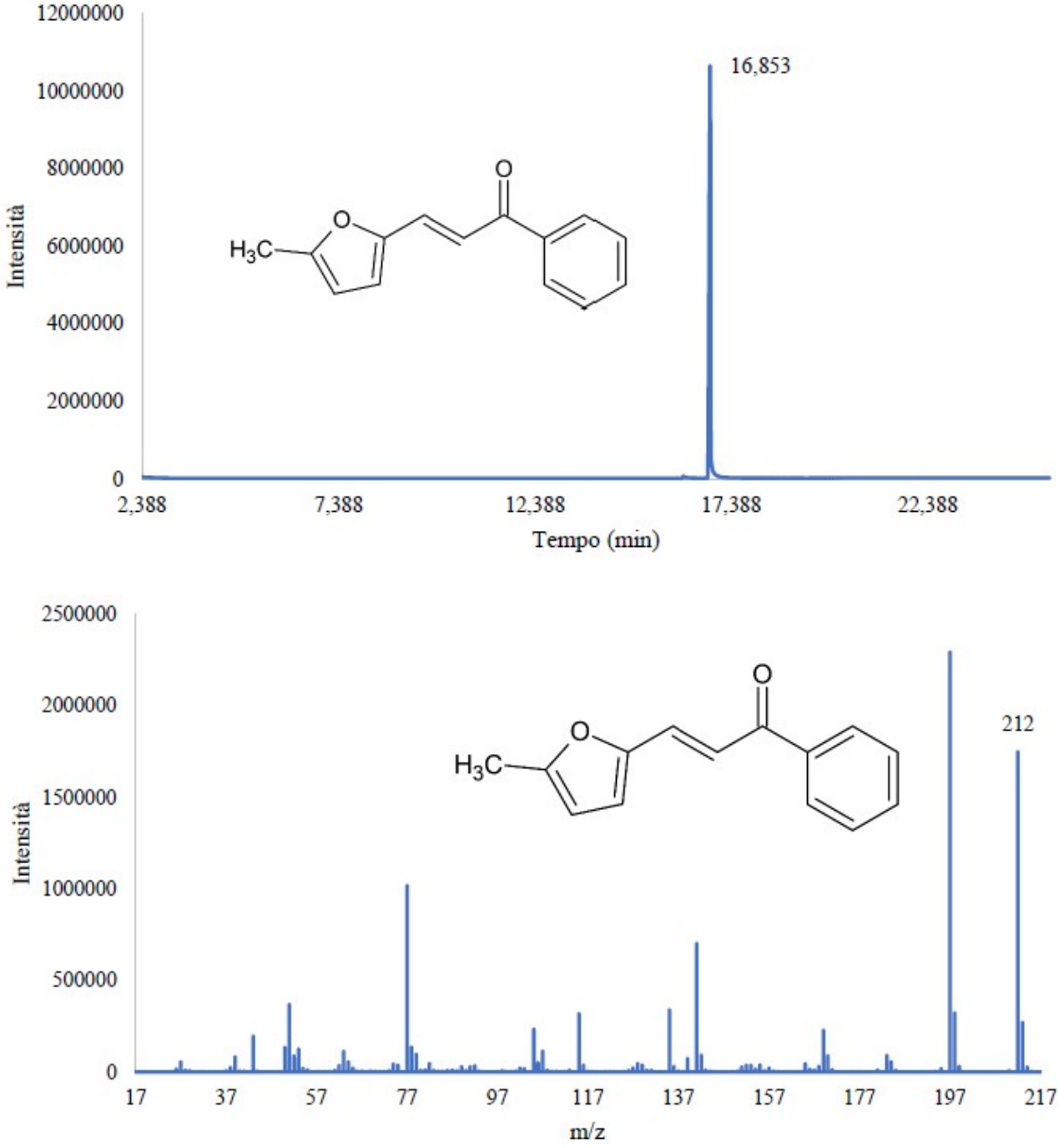

Figure S2 GC-MS of purified (2E)-3-(5-methylfuran-2-yl)-1-phenylprop-2-en-1-one **(3b)**.

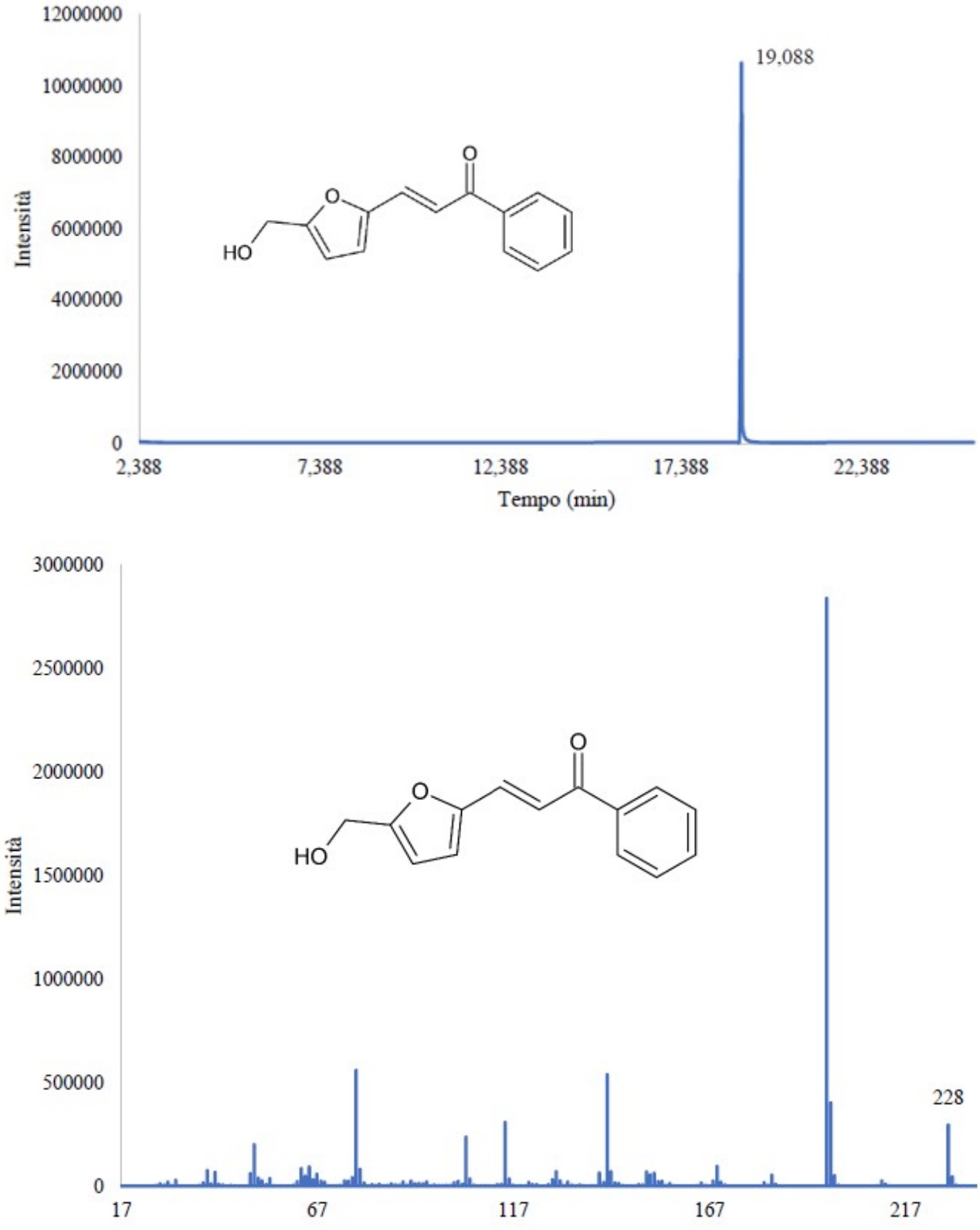

Figure S3 GC-MS of purified (2E)-3-[5-(hydroxymethyl)furan-2-yl]-1-phenylprop-2-en-1-one **(3c)**.

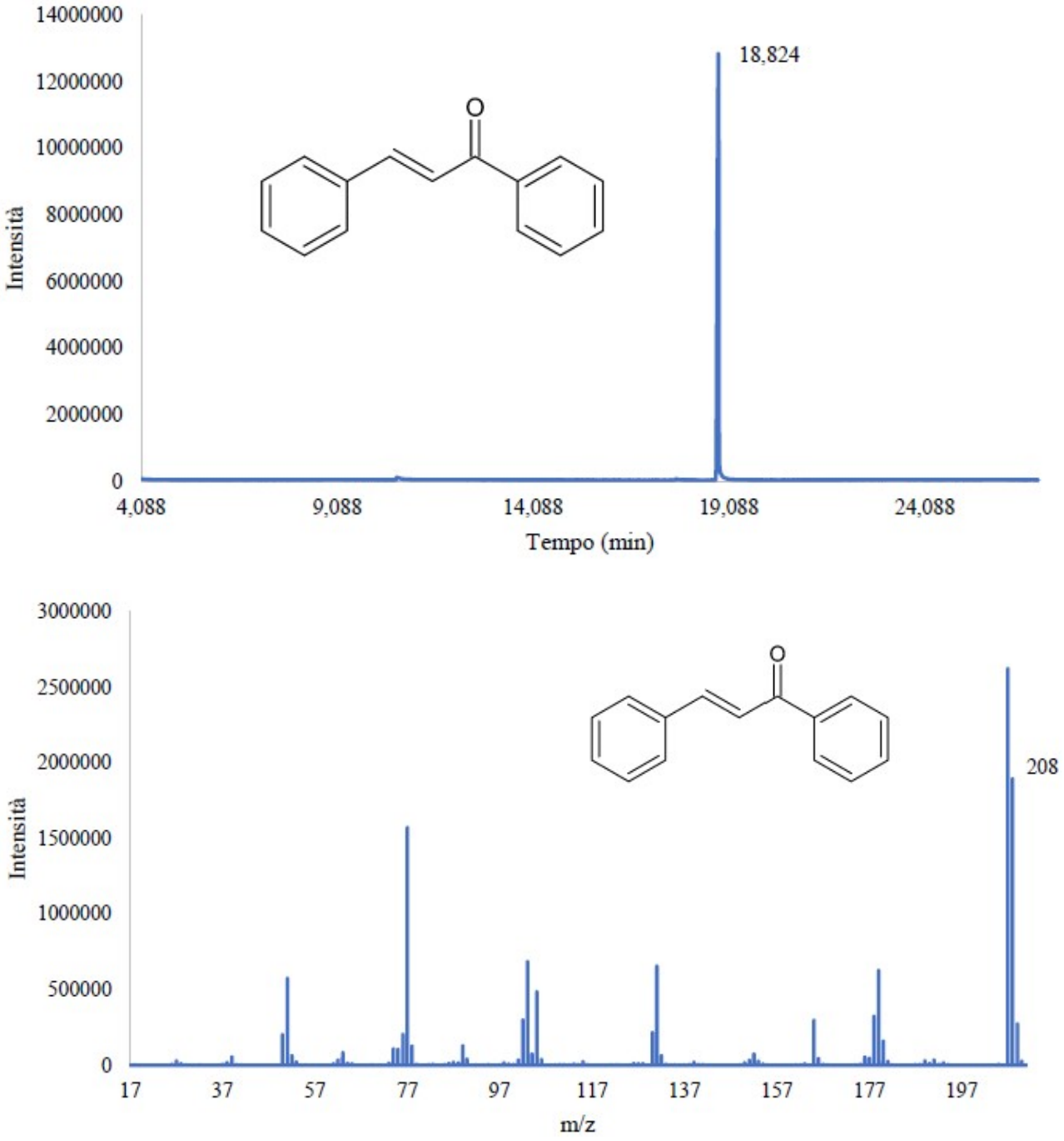

Figure S4 GC-MS of purified (2E)-1,3-diphenylprop-2-en-1-one **(3d)**.

S.3.2. NMR spectra of product 3a, 3b, 3c, and 3d.

^{1}H and ^{13}C NMR spectra of the purified products were recorded in $CDCl_3$ using a JEOL JNM-ECZR 600 MHz nuclear magnetic resonance spectrometer (JEOL USA, Inc.). NMR spectra of corresponding **3a, 3b, 3c** and **3d** chalcones are reported below (Figures S5–S12).

(2E)-3-(furan-2-yl)-1-phenylprop-2-en-1-one (3a)

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.04–8.02 (m, 2H, Ar-H), 7.61–7.57 (m, 3H, Ar-H), 7.53 (d, J = 15.6 Hz, 1H, CH=CH), 7.50 (dd, J = 3.5, 0.8 Hz, 1H, furan-H), 7.45 (d, J = 15.6 Hz, 1H, CH=CH), 6.72 (dd, J = 1.8, 0.8 Hz, 1H, furan-H), 6.52 (dd, J = 3.5, 1.8 Hz, 1H, furan-H).

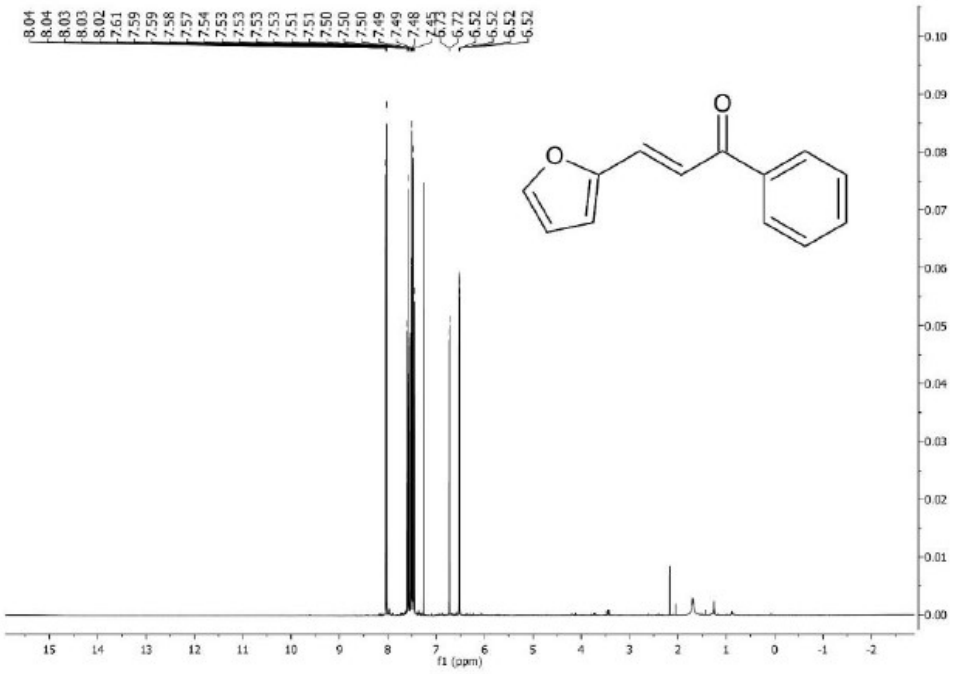

Figure S5 – 1H NMR of (2E)-3-(furan-2-yl)-1-phenylprop-2-en-1-one **(3a).**

^{13}C NMR (151 MHz, $CDCl_3$) δ 190.00, 151.83, 145.07, 138.30, 132.91, 130.83, 128.76, 128.58, 119.46, 116.38, 112.82.

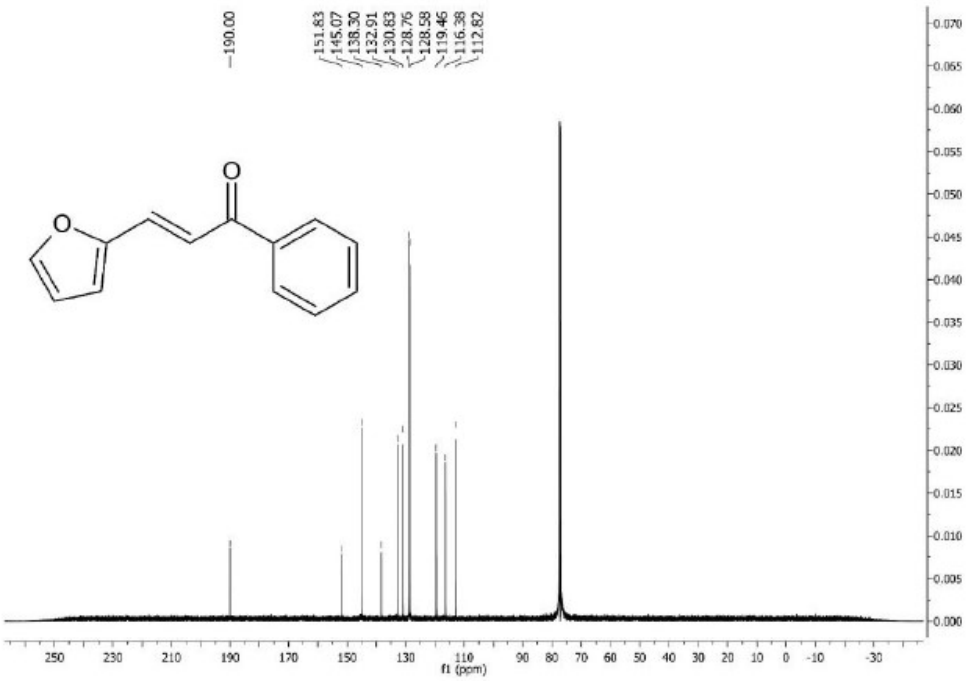

Figure S6 – ^{13}C NMR of (2E)-3-(furan-2-yl)-1-phenylprop-2-en-1-one **(3a).**

(2E)-3-(5-methylfuran-2-yl)-1-phenylprop-2-en-1-one (3b)

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.04–8.03 (m, 2H, Ar-H), 7.58 (d, J = 15.6 Hz, 1H, CH=CH), 7.56–7.48 (m, 3H, Ar-H), 7.39 (d, J = 15.6 Hz, 1H, CH=CH), 6.63 (dd, J = 3.5, 1.8 Hz, 1H, furan-H), 6.14–6.13 (m, 1H, furan-H), 2.40 (s, 3H, CH_3).

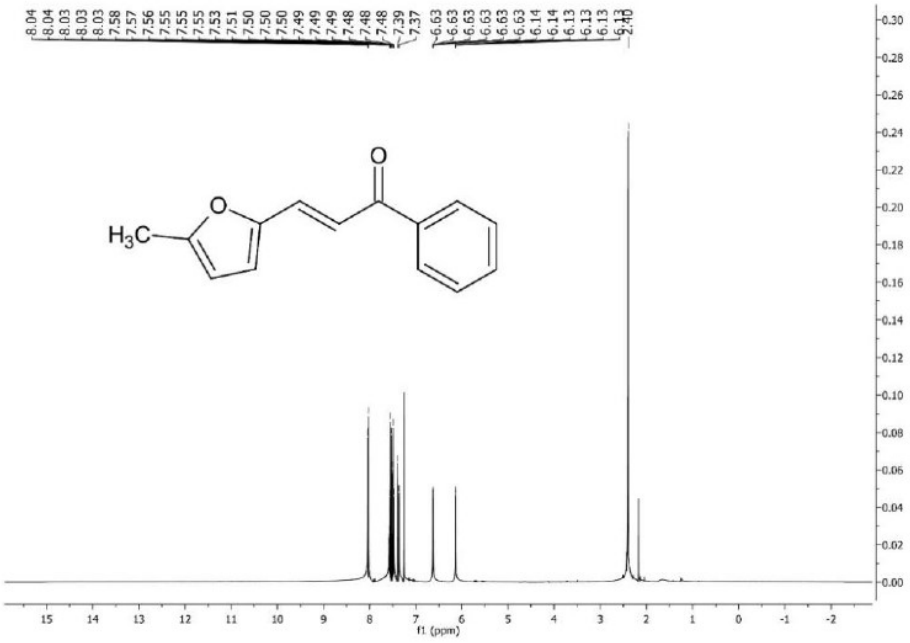

Figure S7 – ^{1}H NMR of (2E)-3-(5-methylfuran-2-yl)-1-phenylprop-2-en-1-one **(3b).**

^{13}C NMR (151 MHz, $CDCl_3$) δ 190.06, 156.07, 150.51, 138.54, 132.71, 130.97, 128.69, 128.53, 118.42, 117.67, 109.51, 14.18.

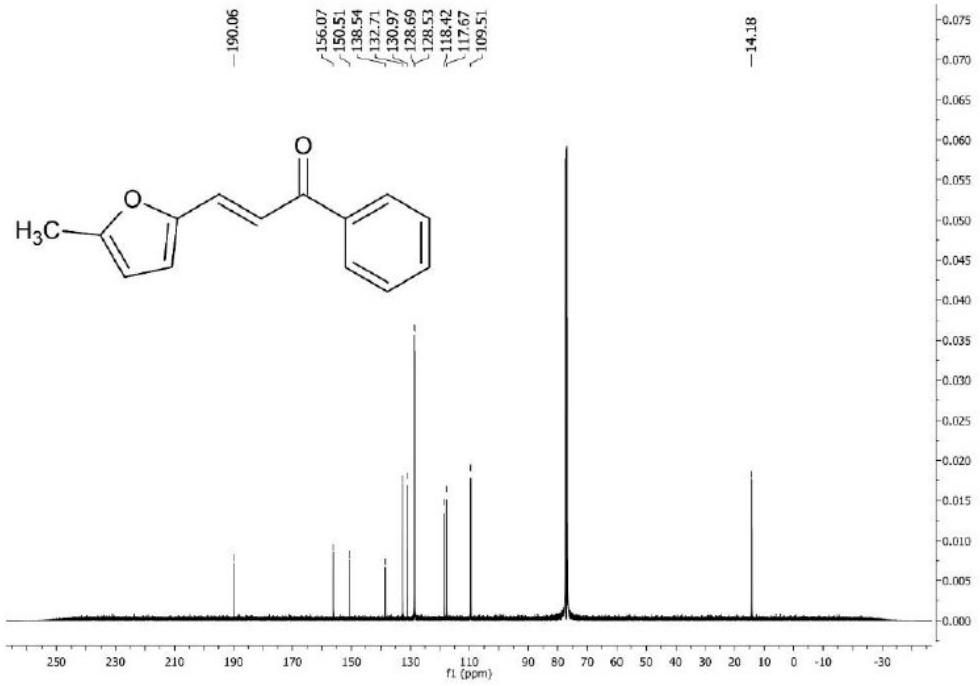

Figure S8 – ^{13}C NMR of (2E)-3-(5-methylfuran-2-yl)-1-phenylprop-2-en-1-one **(3b).**

(2E)-3-[5-(hydroxymethyl)furan-2-yl]-1-phenylprop-2-en-1-one (3c)

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.04–8.02 (m, 2H, Ar-H), 7.59–7.49 (m, 3H, Ar-H), 7.47 (d, J = 15.6 Hz, 1H, CH=CH), 7.44 (d, J = 15.6 Hz, 1H, CH=CH), 6.67–6.66 (m, 1H, furan-H), 6.42–6.41 (dd, J ≈ 3.5, 1.8 Hz, 1H, furan-H), 4.69 (s, 2H, CH_2OH), 1.92 (br s, 1H, OH).

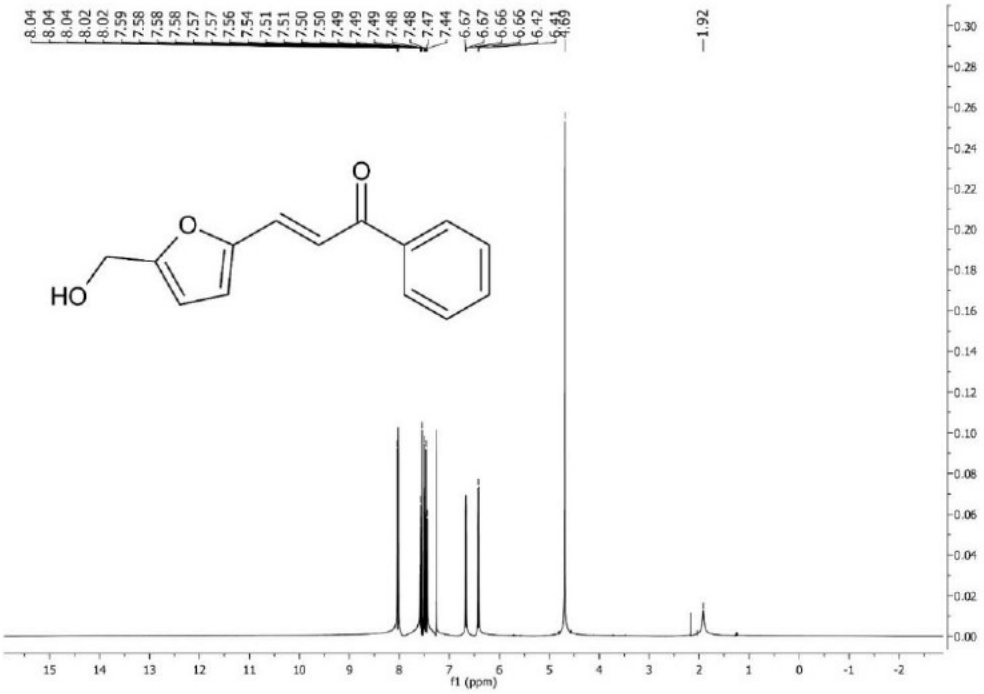

Figure S9 – ^{1}H NMR of (2E)-3-[5-(hydroxymethyl)furan-2-yl]-1-phenylprop-2-en-1-one **(3c).**

^{13}C NMR (151 MHz, CDCl3) δ 189.95, 156.88, 151.74, 138.23, 132.97, 130.69, 128.76, 128.60, 119.34, 117.39, 110.80, 57.84.

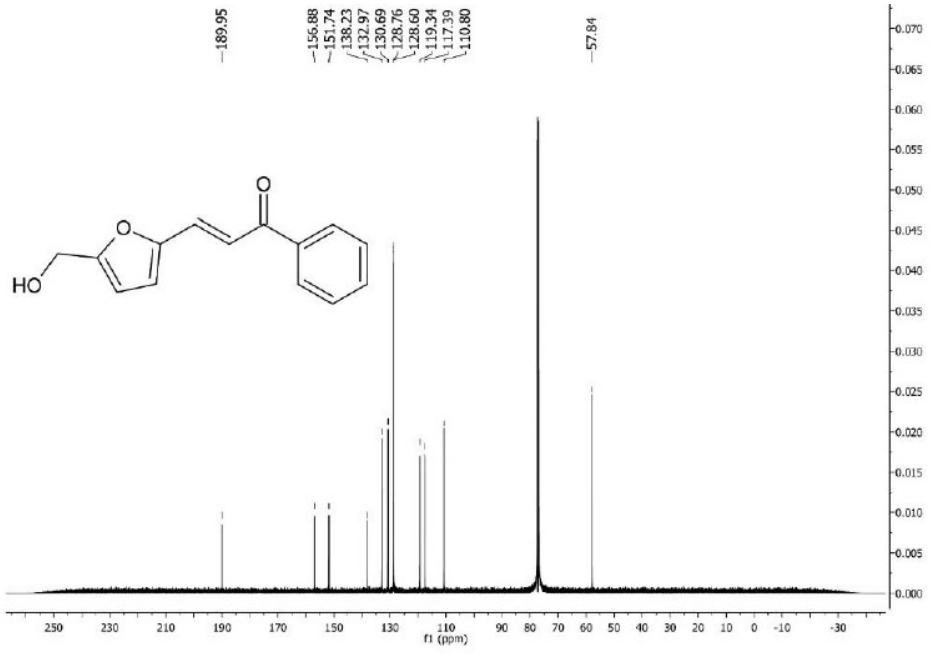

Figure S10 – ^{13}C NMR of (2E)-3-[5-(hydroxymethyl)furan-2-yl]-1-phenylprop-2-en-1-one **(3c).**

(2E)-1,3-diphenylprop-2-en-1-one (3d)

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.03–8.02 (m, 2H, Ar-H), 7.83 (d, J = 15.6 Hz, 1H, CH=CH), 7.66–7.61 (m, 1H, Ar-H), 7.55–7.50 (m, 2H, Ar-H), 7.51–7.41 (m, 5H, Ar-H), 7.44 (d, J = 15.6 Hz, 1H, CH=CH).

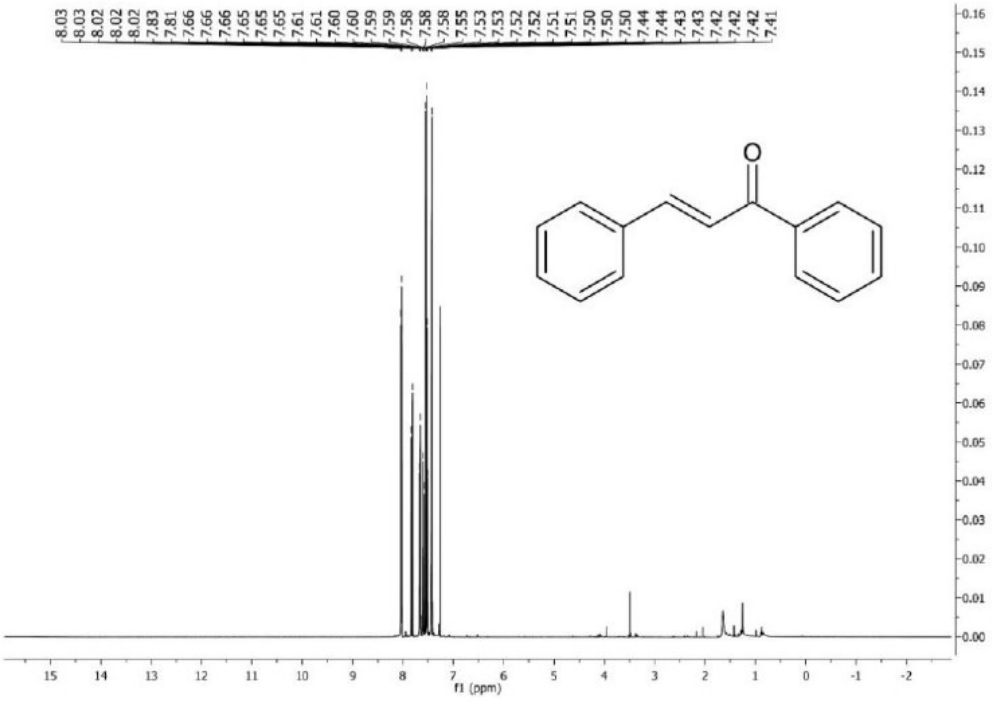

Figure S11 – ^{1}H NMR of (2E)-1,3-diphenylprop-2-en-1-one **(3d).**

^{13}C NMR (151 MHz, $CDCl_3$) δ 190.74, 145.01, 138.37, 135.04, 132.94, 130.70, 129.11, 128.78, 128.66, 128.60, 122.26.

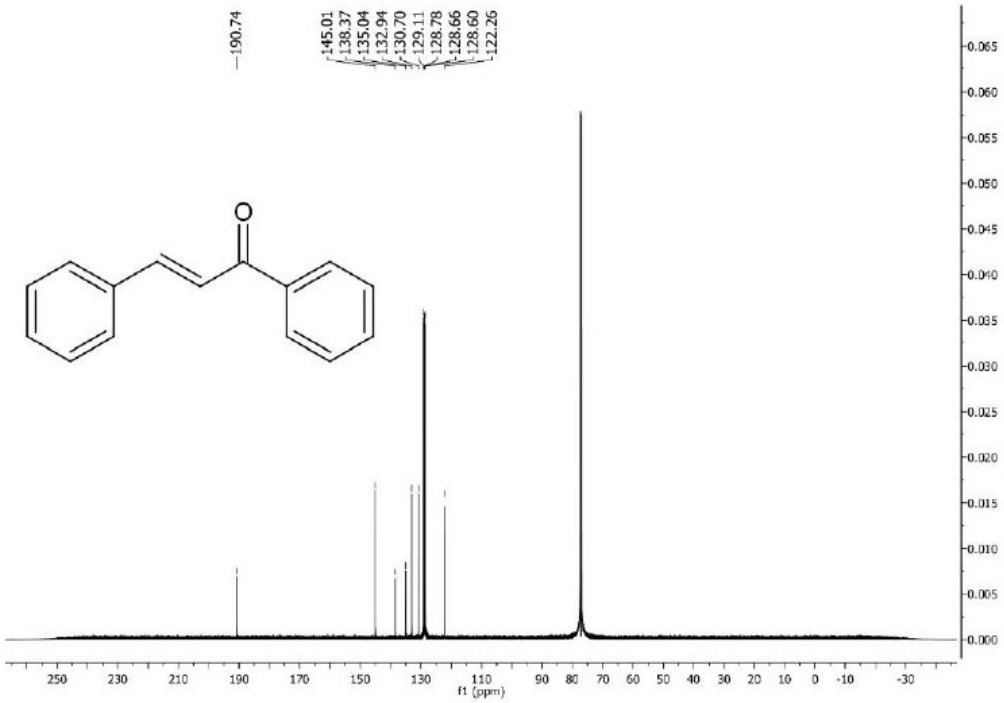

Figure S12 – ^{13}C NMR of (2E)-1,3-diphenylprop-2-en-1-one (3d).

S.4 Characterization of the MgO catalyst for solvent-free MW-assisted Claisen-Schmidt condensation of acetophenone with 3a, 3b, 3c and 3d

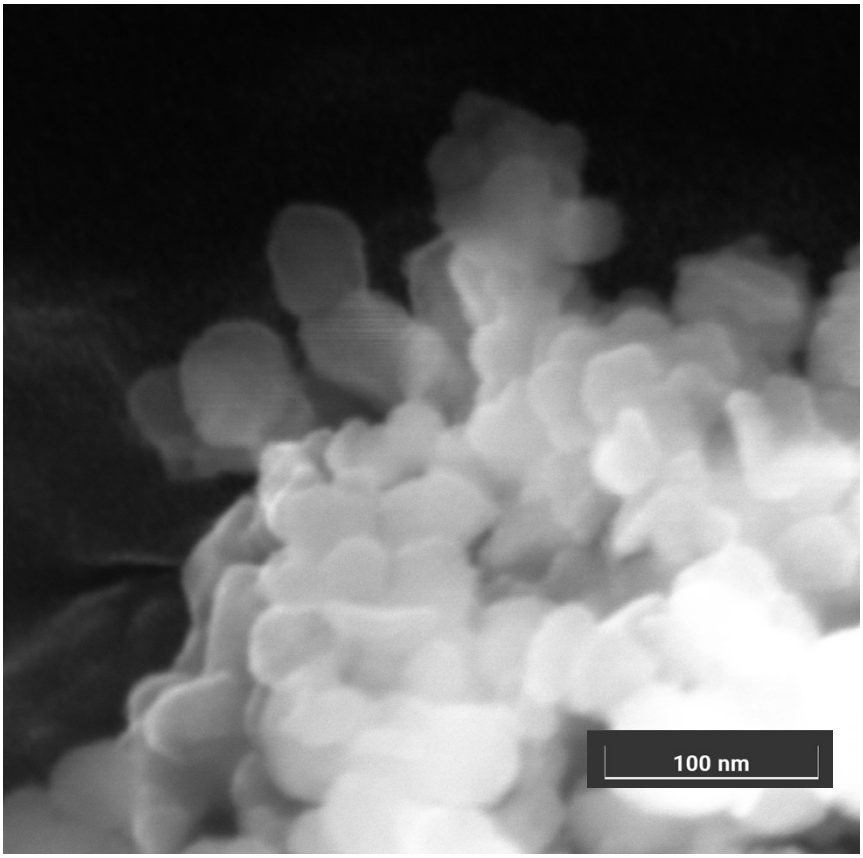

Figure S13. FESEM representative SE image of MgO before reaction. Instrumental magnification: 1057000×.

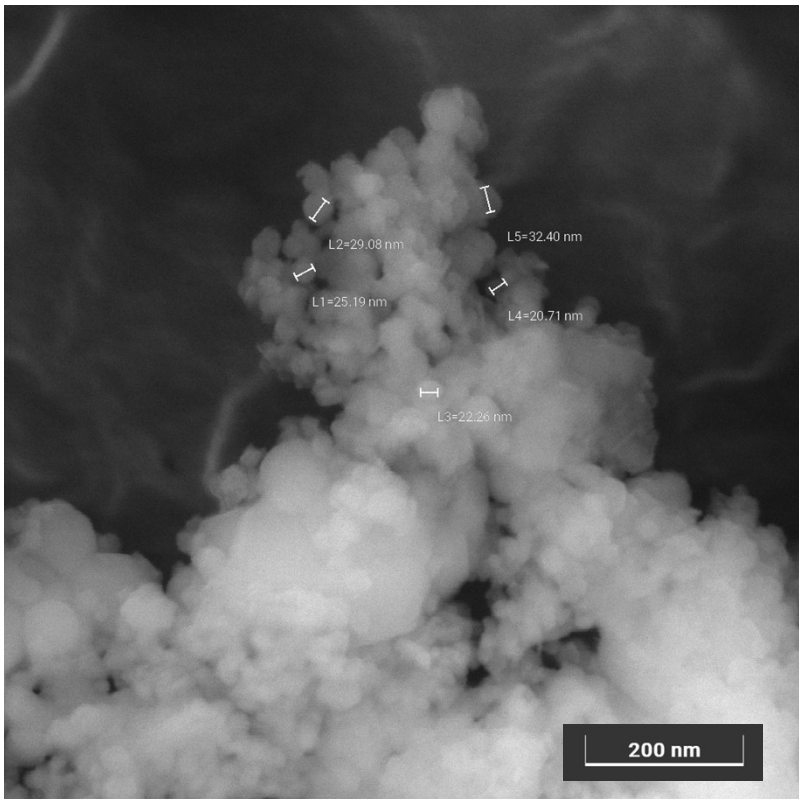

Figure S14. FESEM representative SE image of MgO before reaction and after calcination at 450 °C. Instrumental magnification: 280000×.

S.5 Green Metrics

Table S4. Green Star Score for the MW-assisted and solvent-free Claisen Smith protocol

#	Principle	Score	Technical Motivation
P1	Prevention	3	E-factor close to zero (excluding water). Absence of reaction and extraction solvents (assuming a simple work-up).
P2	Atom Economy	3	Atom Economy (AE) is very high (>90%).
P3	Less Hazardous Chemical Synthesis	2	MgO (an inert solid) is used instead of corrosive liquid bases like NaOH or KOH.
P4	Designing Safer Chemicals	2	Furan-chalcones are bioactive molecules of pharmaceutical interest, not industrial toxins.
P5	Safer Solvents and Auxiliaries	3	Maximum Score. The solvent-free approach is considered the "gold standard" of the fifth principle.
P6	Design for Energy Efficiency	1	To be honest: 150-180°C results in a score of 1 according to Ribeiro et al. However, the text specifies that Microwaves (MW) optimize energy consumption.
P7	Use of Renewable Feedstocks	3	Furfural and HMF are derived from agricultural waste (hemicellulose and cellulose). It is a "bio-based" process.
P8	Reduce Derivatives	3	Direct synthesis in a single step (one-pot), without the use of protecting groups.
P9	Catalysis	3	Use of a heterogeneous catalyst (MgO) which is recyclable and non-stoichiometric.
P10	Design for Degradation	2	The products are organic and potentially biodegradable, unlike persistent polymers.

P11	Real-time Analysis for Pollution Prevention	2	Modern MW reactors allow for precise control of T and P during the reaction, preventing deviations.
P12	Inherently Safer Chemistry for Accident Prevention	3	The absence of flammable solvents at 150°C eliminates the risk of overpressure and fires typical of organic solvents.

Table S5. Green Star Score for the conventional synthesis (Solvent+NaOH)

#	Principle	Score	Technical Motivation (Traditional Method)
P1	Prevention	1	Production of salts (NaCl) and large volumes of waste solvents.
P2	Atom Economy	3	The reaction is the same, but the process is less efficient.
P3	Less Hazardous Chemical Synthesis	1	Use of corrosive bases (NaOH) and strong acids (HCl).
P4	Designing Safer Chemicals	2	The products are identical.
P5	Safer Solvents and Auxiliaries	1	Use of ethanol and extraction solvents (e.g., EtOAc or DCM).
P6	Design for Energy Efficiency	3	Low temperature.
P7	Use of Renewable Feedstocks	2	Furfural and HMF are derived from agricultural waste (hemicellulose and cellulose). It is a "bio-based" process.
P8	Reduce Derivatives	3	No protecting groups are used.
P9	Catalysis	1	NaOH is not recyclable; it is neutralized and destroyed.
P10	Design for Degradation	2	Identical to the alternative method.
P11	Real-time Analysis for Pollution Prevention	1	Manual monitoring (TLC/Sampling).
P12	Inherently Safer Chemistry for Accident Prevention	1	Use of flammable solvents and corrosive reagents.

S.6 Biological activity

Table S6. Effect on cellular viability of compounds, expressed as CC_{50} (µg/mL) and 95% confidence intervals.

Compound	HeLa cells	Vero cells	MDCK cells	MRC-5 cells	HFF-1 cells
3a	46.8 (40.5 to 51.7)	44.1 (39.2 to 49.5)	50.2 (45.2 to 53.6)	n.t.	n.t.
3b	54.0 (49.2 to 62.1)	37.7 (32.1 to 41.0)	56.9 (52.8 to 60.7)	38.8 (34.2 to 41.8)	18.7 (16.4 to 21.6)
3c	73.4 (68.2 to 78.0)	93.0 (82.3 to 100.2)	81.0 (75.1 to 86.0)	86.2 (81.1 to 90.5)	19.8 (18.2 to 22.0)
3d	<12.5	<12.5	<12.5	<12.5	<12.5

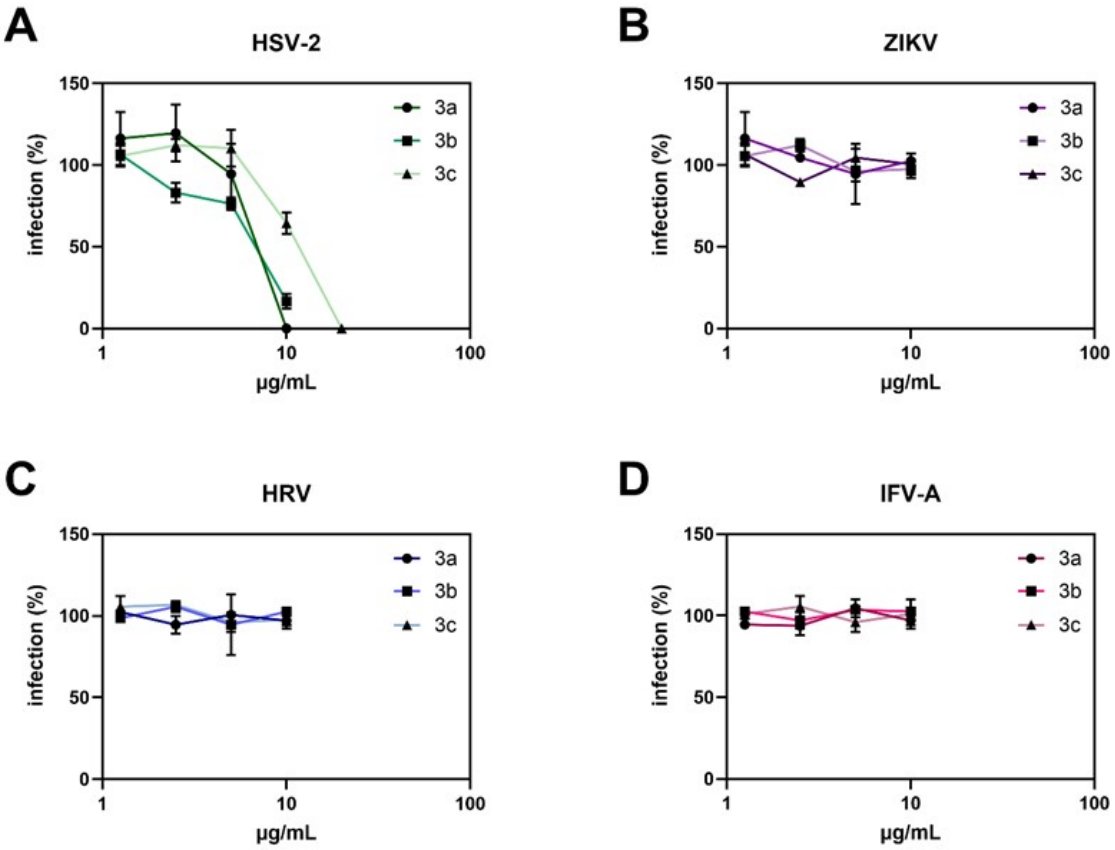

Figure S15. Antiviral activity of compounds **3a**, **3b**, **3c**. Antiviral activity was evaluated in cells infected with herpes simplex virus type 2 (HSV-2, panel A), zika virus (ZIKV, panel B), human rhinovirus (HRV, panel C) and influenza virus type A (IFV-A, panel D), in presence of increasing concentrations of each compound. Virus infectivity was assessed at 24 h post-infection, and percentages of infection in treated samples were calculated by comparing to control untreated samples. Values are presented as mean ± standard error of the mean (SEM); n = 3.