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Herbicide potential of new phytotoxins structurally based on plant allelochemicals

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ABSTRACT

Agriculture requires the development of new herbicides to control weeds. The synthesis of molecules totally or partially based on the combination of basic allelochemical structures viz., chalcone, pyrimidine and diphenyl ether skeletons may provide phytotoxic compounds more active and environmentally friendly than present herbicides. In this work, chloride aryl pyrimidine benzyl ether (CAPBE) compounds **4a-4f** and diphenyl pyrimidine propenone (DPPP) compounds **6a-6g** were synthesized and tested on barnyardgrass (*Echinochloa cruss-galli*) and rape (*Brassica napus*). These compounds were designed by combining the pyrimidine-diphenyl ether and pyrimidine-chalcone structures, respectively. The herbicidal activity was tested in Petri dish bioassays at 100 mg/L, 10 mg/L and 1 mg/L. The CAPBE compound **4b** bearing a 2,4-fluoride substitution in the benzyl ring, exerted the strongest herbicidal activity at 100 mg/L. The CAPBE structure by itself and its benzyl substituted derivatives 4-NO_2 (**4c**) and 4-OCF_3 (**4e**) had the strongest herbicidal activity to barnyardgrass, while the remaining CAPBE compounds, were similarly inhibitory to both test species. The DPPP compounds showed selective herbicidal activity on rape, with compound **6a** as the most phytotoxic. However, phytotoxicity decreased with substitutions in the phenyl propenone moiety of the DPPP skeleton. Compounds **4b** and **6a** deserve further investigations as herbicide lead molecules.

Key words: Allelochemicals, Biginelli reaction, *Brassica napus*, diphenyl ether, *Echinokchloa crus-galli*, herbicide activity, petri dish bioassay, phytotoxic compounds, pyrimidine, rape.

INTRODUCTION

Worldwide demand for herbicides is continuously increasing mainly in underdeveloped countries, which suffer from lack of workers for hand weeding and need to increase crop yields (22). Hence, in the near future, there will be great need for new high efficiency and environmentally friendly herbicides (8). Several phytotoxic compounds can be combined to achieve this purpose, including those based on plant allelochemicals such as the pyrimidine derivatives, chalcones and diphenyl ethers (21). The pyrimidine derivatives currently include both natural and synthetic molecules that can be used not only as herbicides but also insecticides, fungicides and plant growth regulators (17). Their activity has been enhanced by introducing acyl, acyl thiourea or thioether groups into the pyrimidine ring, or withdrawing electron groups (CF_3, NO_2, Cl and F) from other structural parts of pyrimidine

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derivatives. In case of diphenyl ethers, some halogenated molecules of this type isolated from plants, are suspected phytotoxins (27). In fact, the diaryl template greatly enhanced the physical and chemical properties of some herbicides, and provides several advantages, [improvement of biological activity, reduction in toxicity to mammals and crops, increase in photostability with longer efficacy and delay in evolution of weed resistance (2)]. Aromatic ether compounds containing a heterocyclic ring had high herbicidal activity, particularly when they contain a nitrogen heterocyclic compound such as the pyrimidine (3,10,26). Regarding the chalcone molecule, it has a 1,3-diaryl propenone structure, which exists in many plants (28). Its structure contains α, β-unsaturated ketone, which has good flexibility and can interact with a variety of cell enzymes. It has wide range of applications in medicinal chemistry due to its biological activities including antibacterial (7), anti-inflammatory (9), anti-malarial (5) and anti-tumor activities (16). Some chalcone derivatives showed anti-pinworm (15), antiallergic (11), chemophophylactic (14,19,24) and allelopathic activities (4). In this work, we synthesized pyrimidine-aryl benzyl ether and pyrimidine-chalcone hybrids to obtain chloride aryl pyrimidine benzyl ether (CAPBE) molecules and diphenyl pyrimidine propenone (DPPP) compounds, respectively. The molecules were synthesized by using active substructure splicing and bioisosterism methods. Different substituents were introduced into the target molecules to obtain compounds with enhanced biological activity.

MATERIALS AND METHODS

The study was conducted at Zhejiang Provincial Key Laboratory of Chemical Utilization of Forestry Biomass, Zhejiang A＆F University, Lin'an, Zhejiang Province, China. [30.23 °N, 119.52 °E, Elevation: 1192 m]. The area has a subtropical monsoon climate, with average daily maximum temperature (33 °C) in hot summer and average daily minimum temperature (4 °C) in cold winter, the mean annual rainfall: 1613.9 mm. This study done in 2020 includes design, synthesis, structural characterization and activity tests of compounds.

I. General Procedure

^{1}H NMR spectra were recorded at Varian Mercury 400 or 600 MHz spectrometers. Chemical shifts (δ) were reported in ppm quoted relative to internal tetramethylsilane (internal standard, 0.0 ppm) with the coupling constants (J) given in Hz. ^{13}C NMR spectra were recorded with the same spectrometer operating at 100/150 MHz with complete proton decoupling (internal standard $CDCl_3$: 77.0 ppm). Splitting patterns were assigned s = singlet, d = doublet, t = triplet, etc. Mass spectra were obtained with a Finnigan Trace MS spectrometer. Melting points (mp.) were obtained on a digital melting point apparatus (Bibby-Electrothermal) without correction model. Unless otherwise noted, commercial materials were used. All reactions were monitored by TLC analysis on silica gel coated plates. Flash column chromatography was performed by using 200-300 mesh silica gel.

II. General procedure for the synthesis of pyrimidine derivatives

Firstly, 3,4-dihydropyrimidine-2-ketone **2a** and **2b** were prepared by using the Biginelli reaction between 4-chloro/4-trifluoromethyl substituted benzaldehyde,

acetylacetone and urea (20). Then, the intermediates **2a** and **2b** were oxidized to give the 2-hydroxy pyrimidine intermediates **3a** and **3b**. On the one hand, 2-hydroxy pyrimidine intermediate **3a** underwent nucleophilic substitution reaction with substituted benzyl bromide under alkaline conditions to produce the target product pyrimidine benzyl ethers **4a**-**4f** with 62~71 % yields. On the other hand, the 2-hydroxy pyrimidine intermediate **3b** underwent nucleophilic substitution and aldol condensation reactions in sequence to give the target product pyrimidine chalcones **6a**-**6g** with 50~83 % yields. The total synthetic routes are shown in Figure 1.

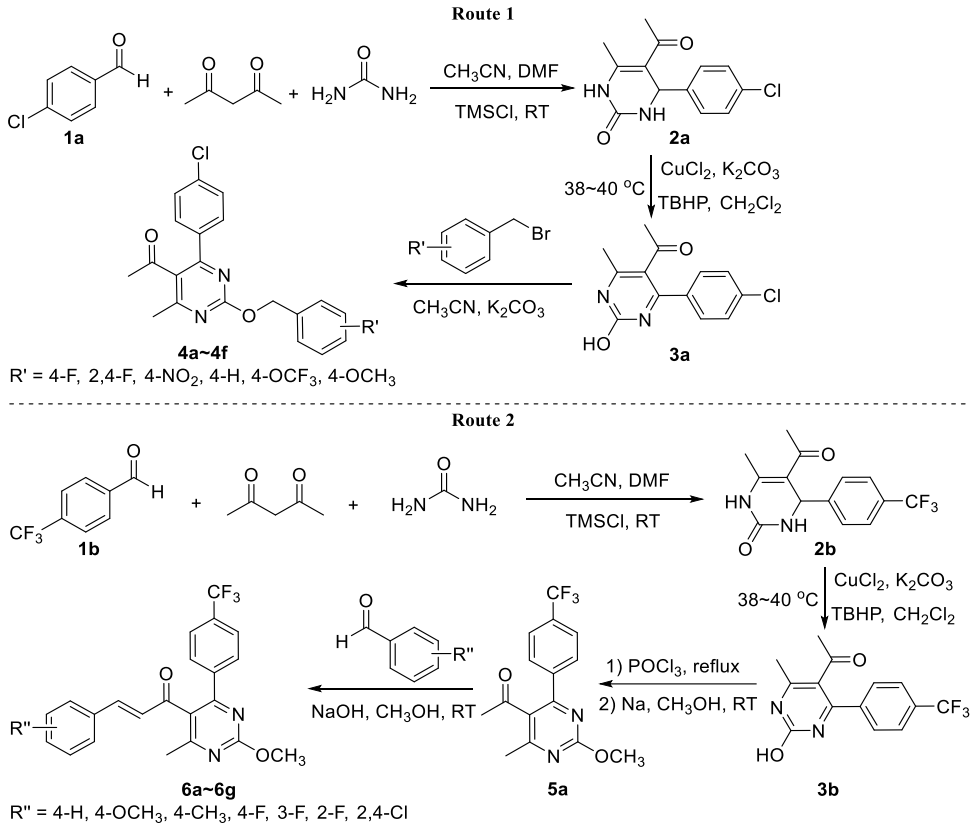

Figure 1. Total synthesis routes of pyrimidine derivatives

III. Synthesis of 3,4-dihydropyrimidine-2-ketones 2a and 2b

Acetylacetone (10 mmol, 1.0 g), *p*-chlorobenzaldehyde **1a** (10 mmol, 1.4 g) or 4-trifluoromethyl benzaldehyde **1b** (10 mmol, 1.74 g), urea (12 mmol, 0.72 g), acetonitrile (8 mL) and DMF (4 mL) were successively added into a 100 mL round-bottom flask, and stirred at room temperature for about 10 min until the solid was dissolved. Chlorotrimethylsilane (TMSCl, 7 mmol, 0.9 mL) was added dropwise into the flask within

30 min. The reaction solution turned from colourless to turbid and then to yellow as the reaction progress, and a white solid was precipitated after 3 h. Intermediates **2a** and **2b** were obtained by vacuum filtering and washing with acetonitrile as white powdery solids gave yields of 75 % and 62 %, respectively.

IV. Oxidation of 3,4-dihydropyrimidin-2-one derivatives

3,4-Dihydropyrimidin-2-ketone **2a** (15 mmol, 3.97 g) or **2b** (15 mmol, 4.47 g) and CH_2Cl_2 (250 mL) were added into a 100 mL two-necked flask, followed by stirring and heating under reflux until the sample was dissolved. Then, $CuCl_2$ (0.75 mmol, 0.10 g) and anhydrous K_2CO_3 (7.5 mmol, 1.04 g) were added into the flask. When the solution reached the refluxing temperature of 70-80 °C, *tert*-butyl hydroperoxide (TBHP, 75 mmol, 7.2 mL) was added dropwise to the flask. The solution turned from white turbid to clear greenish colour with addition of TBHP. After refluxing for 24 h, the mixture was left to cool at room temperature. The solution was washed successively with saturated $NaHSO_3$ solution (2×100 mL) and saturated saline solution (3×100 mL), dried with anhydrous Na_2SO_4 and concentrated in vacuo to give a residue. The residue was purified by column chromatography on silica gel with petroleum ether/ethyl acetate (1:20, v/v) as eluent to yield 78 % and 76 % of the intermediates 2-hydroxy pyrimidine **3a** and **3b** as faint yellow solids, respectively.

V. Synthesis of CAPBE compounds 4a~4f

2-Hydroxypyrimidine **3a** (2 mmol, 0.53 g) and 10 mL of acetonitrile were added into a 50 mL round bottom flask. Then, the mixtures were stirred and heated under reflux until the solid was dissolved. After that, anhydrous K_2CO_3 (3 mmol, 0.42 g) and substituted benzyl bromide (2.4 mmol, 1.2 equiv.) was added in succession. The reaction progress was monitored by thin layer chromatography (TLC). Upon completion of the reaction, the mixture was cooled and poured into a 250 mL separating funnel and 100 mL of water was added. The aqueous layer was extracted with CH_2Cl_2 (3 × 20 mL), and the organic extracts were combined, washed with saturated saline solution (3 × 100 mL), dried with anhydrous sodium sulfate, and evaporated to dryness under reduced pressure. The residue was purified by column chromatography on silica gel with petroleum ether/ethyl acetate (1:20, v/v) as eluent to give the target compounds 62~71 % yields.

(i). 1-[4-(4-Chloro-phenyl)-2-(4-fluoro-benzyloxy)-6-methyl-pyrimidin-5-yl]-ethenone (4a): White solid in 62 % yield. Mp.: 135.6~136.6 ℃. ^{1}H-NMR (400 MHz, $CDCl_3$, ppm) δ 7.58-7.56 (m, 2H, Ar-H), 7.50-7.44 (m, 4H, Ar-H), 7.07-7.03 (t, *J* = 7.8 Hz, 2H, Ar-H), 5.47 (s, 2H, -O-CH_2-), 2.49 (s, 3H, H_3C-Ar), 2.07 (s, 3H, H_3C-CO-Ar); ^{13}C-NMR (100 MHz, $CDCl_3$, ppm) δ 204.02, 167.67, 163.73, 163.50, 163.42, 161.28, 137.08, 135.64, 131.95, 130.18, 127.67, 115.35, 115.13, 68.59, 32.22, 22.57; EI-MS m/z: 370.29 (M^+, 28.11), 109.12 (100.00).

(ii). 1-[4-(4-Chloro-phenyl)- 2-(2,4-difluoro-benzyloxy) -6-methyl-pyrimidin-5-yl]-ethanone (4b): Yellow solid in 64 % yield. Mp.: 122.7~123.4 ℃. ^{1}H-NMR (400 MHz, $CDCl_3$, ppm) δ 7.61-7.59 (m, 2H, Ar-H), 7.55-7.55 (m, 1H, Ar-H), 7.46 (d, *J* = 7.3 Hz, 2H, Ar-H), 6.88-6.81 (m, 2H, Ar-H), 5.52 (s, 2H, -O-CH_2-), 2.50 (s, 3H, H_3C-Ar), 2.08 (s, 3H, H_3C-CO-Ar); ^{13}C-NMR (100 MHz, $CDCl_3$, ppm) δ 203.97, 167.74, 164.01,

163.51, 163.27, 162.39, 162.27, 161.71, 161.59, 159.89, 159.77, 137.11, 135.54, 131.76, 127.77, 119.48, 119.33, 119.28, 111.26, 111.05, 104.03, 103.77, 103.52, 62.60, 32.19, 22.54; EI-MS m/z: 388.35 (M^+, 33.82), 127.11 (100.00).

(iii). 1-[4-(4-Chloro-phenyl)- 6-methyl-2-(4-nitro-benzyloxy)-pyrimidin- 5-yl]- ethanone (4c): Yellow solid in 63 % yield. Mp.: 138.8~140.0 ℃. ^{1}H-NMR (400 MHz, $CDCl_3$, ppm) δ 8.22 (d, *J* = 7.4 Hz, 2H, Ar-H), 7.70 (d, *J* = 7.6 Hz, 2H, Ar-H), 7.56 (d, *J* = 7.7 Hz, 2H, Ar-H), 7.45 (d, *J* = 7.4 Hz, 2H, Ar-H), 5.62 (s, 2H, -OCH_2-), 2.51 (s, 3H, H_3C-Ar), 2.09 (s, 3H, H_3C-CO-Ar); ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ 203.85, 167.88, 163.56, 163.07, 147.48, 143.63, 135.37, 128.01, 123.59, 67.75, 32.17, 22.56; EI-MS m/z: 397.31 (M^+, 53.89), 246.18 (100.00).

(iv). 1-[2-Benzyloxy-4-(4-chloro-phenyl)-6-methyl-pyrimidin-5-yl]-ethanone (4d): Yellow solid in 65 % yield. Mp.: 129.3~130.6℃. ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 7.57 (d, *J* = 7.7 Hz, 2H, Ar-H), 7.51 (d, *J* = 7.9 Hz, 2H, Ar-H), 7.45 (d, *J* = 7.6 Hz, 2H, Ar-H), 7.39-7.32 (m, 3H, Ar-H), 5.51 (s, 2H, -O-CH_2-),2.49 (s, 3H, H_3C-Ar), 2.06 (s, 3H, H_3C-CO-Ar); ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ 204.17, 167.65, 163.56, 163.46, 137.04, 136.17, 135.69, 130.22, 129.11, 128.33, 128.28, 128.05, 127.57, 69.31, 32.27, 22.60; EI-MS m/z: 352.25 (M^+, 70.77), 246.15 (100.00).

(v). 1-[4-(4-Chloro-phenyl)-6-methyl-2-(4-trifluoromethoxy-benzyloxy)-pyrimidin-5-yl]-ethanone (4e): Yellow oil in 71 % yield. ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 7.56 (t, *J* = 15.7 Hz, 4H, Ar-H), 7.46 (d, *J* = 7.8 Hz, 2H, Ar-H), 7.21 (d, *J* = 7.9 Hz, 2H, Ar-H), 5.51 (s, 2H, -O-CH_2-), 2.50 (s, 3H, H_3C-Ar), 2.07 (s, 3H, H_3C-CO-Ar); ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ 203.97, 167.73, 163.50, 163.34, 148.87, 137.10, 135.57, 134.95, 130.15, 129.67, 129.12, 127.76, 124.16, 121.60, 120.83, 119.05, 116.49, 68.30, 32.17, 22.54; EI-MS m/z: 436.33 (M^+, 47.12), 175.14 (100.00).

(vi). 1-[4-(4-Chloro-phenyl)-2- (3-methoxy-benzyloxy)- 6-methyl-pyrimidin- 5-yl]-ethenone (4f): Colorless oil in 67 % yield. ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 7.57 (d, *J* = 8.0 Hz, 2H, Ar-H), 7.43 (d, *J* = 8.0 Hz, 2H, Ar-H), 7.28 (t, *J* = 9.0 Hz, 1H, Ar-H), 7.07 (d, *J* = 8.0 Hz, 2H, Ar-H), 6.83 (d, *J* = 4.0 Hz, 1H, Ar-H), 5.48 (s, 2H, -O-CH_2-), 3.77 (s, 3H, -OCH_3), 2.48 (s, 3H, H_3C-Ar), 2.05 (s, 3H, H_3C-CO-Ar); ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ 203.69, 167.35, 163.13, 159.32, 137.50, 136.70, 135.46, 127.32, 120.12, 113.41, 113.24, 68.89, 54.83, 31.93, 22.30; EI-MS m/z: 382.32 (M^+, 61.80), 246.20 (100.00).

VI. Synthesis of 6-methyl-2-methoxy-5-acetyl-4- (4-trifluoromethylphenyl) pyrimidine 5a

2-Hydroxypyrimidine intermediate **3b** (10 mmol, 2.96 g) and triethylamine (10 mmol, 1.4 mL) were added into a 100 mL two-necked flask in ice bath, followed by a slow addition of 20 mL (0.22 mol) phosphorus oxychloride. Then, the mixture was refluxed for 12 h in an oil bath under an argon atmosphere. The reaction was monitored by TLC. When the reaction was finished, the reaction mixture was slowly poured into ice water and extracted with ether (3 × 20 mL). The organic extracts were combined, successively washed with saturated aqueous Na_2CO_3 solution (3 × 100 mL) and saturated aqueous NaCl solution (3 × 100 mL),

dried with anhydrous sodium sulfate, and concentrated *in vacuo* till dryness. The residue was purified by column chromatography on silica gel (eluent: petroleum ether/ethyl acetate, 1:20, v/v) to give the intermediate 6-methyl-2-chloro-5-acetyl-4-(4-trifluoromethylphenyl) pyrimidine as white solid 75 % yield.

Methanol (20 mL) and sodium (10 mmol, 0.23 g) were added into a 100 mL round bottom flask, stirred until the sodium was completely dissolved. Then, 6-methyl-2-chloro-5-acetyl-4- (4-trifluoromethylphenyl) pyrimidine (10 mmol, 3.1 g) was added and stirred at room temperature for 12 h. The reaction was traced by TLC. After the reaction finished, the solution was filtered and the solvent was removed. Subsequently, the product was dissolved in 100 mL dichloromethane and was washed with a saturated aqueous NaCl solution (3 × 100 mL). Then, the organic phase was dried with anhydrous $NaSO_4$, and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel by using ethyl acetate/petroleum ether (3:20, v/v) as eluent to give intermediate **5a** as a white solid 70 % yield.

VII. Synthesis of DPPP compounds 6a-6g

Intermediate **5a** (1.5 mmol, 0.47 g) and methanol (5.75 mL) were added into a 50 mL flask, and 3 mL 20 % aqueous sodium hydroxide solution was added after complete dissolution. Substituted benzaldehydes (1.5 mmol) was dissolved in methanol (1 mL) and added dropwise to the flask. The mixture was stirred at 25 ºC, and the reaction was traced by TLC. After the reaction finished, 100 mL water was added and extracted with CH_2Cl_2 (3 × 20 mL). The combined organic phase was washed with saturated saline solution (3 × 100 mL), dried with anhydrous sodium sulfate, and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel using petroleum ether/ethyl acetate (20:3, v/v) as eluent to give the products **6a-6g** yields of 50~83 %.

(i). (*E*)-3-(2-methoxy-4-methyl- 6-(4-(trifluoromethyl) phenyl)pyrimidin- 5-yl)-1-phenylprop-2-en-1-one (6a): Yellow oil in 77 % yield. ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 7.80 (d, *J* = 8.0 Hz, 2H, ArH), 7.64 (d, *J* =7.8 Hz, 2H, ArH), 7.37-7.33 (m, 5H, ArH), 7.20 (d, *J* = 7.9 Hz, 1H, -CH=CH-), 6.68 (d, *J* = 7.3 Hz, 1H, -CH=CH-), 4.13 (s, 3H, -OCH_3), 2.53 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ 195.90, 168.83, 164.49, 163.75, 146.64, 140.70, 133.56, 129.31, 128.90, 128.39, 127.58, 125.73, 125.43, 124.96, 122.25, 55.03, 22.79; EI-MS m/z: 398.37 (M^+, 84.67), 307.25 (100.00).

(ii). (*E*)-3-(2-methoxy-4-methyl- 6-(4-(trifluoromethyl)phenyl)pyrimidin-5-yl)-1-(4-methoxyphenyl)prop-2-en- 1-one (6b): Yellow oil 80 % yield. ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 7.82 (d, *J* = 8.0 Hz, 2H, ArH), 7.63 (d, *J* = 8.0 Hz, 2H, ArH), 7.29 (d, *J* = 8.0 Hz, 2H, ArH), 7.15 (d, *J* = 8.0 Hz, -CH=CH-), 6.84 (d, *J* = 8.0 Hz, 2H, ArH), 6.59 (d, *J* = 6.8 Hz, 1H, -CH=CH-), 4.12 (s, 3H, -OCH_3), 3.80 (s, 3H, -OCH_3), 2.52 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ 195.84, 168.68, 164.40, 163.49, 162.18, 146.73, 140.73, 130.30, 127.68, 125.89, 124.97, 119.55, 114.36, 55.22, 54.93, 22.70; EI-MS m/z: 428.40 (M^+, 48.24), 121.12 (100.00).

(iii). (*E*)-3-(2-methoxy-4-methyl-6- (4-(trifluoromethyl) phenyl) pyrimidin- 5-yl)-1-(*p*-tolyl) prop-2-en-1-one (6c): Yellow oil 83 % yield. ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 7.82 (d, *J* = 8.0 Hz, 2H, ArH), 7.63 (d, *J* = 8.0 Hz, 2H, ArH), 7.25 (d, *J* = 8.0 Hz, 2H, ArH), 7.19 (d, *J* = 8.0 Hz, 1H, -CH=CH-), 7.12 (d, *J* = 8.0 Hz, 2H, ArH), 6.67 (d, *J* = 7.4 Hz, 1H, -CH=CH-), 4.12 (s, 3H, $-OCH_3$), 2.52 (s, 3H, CH_3), 2.32 (s, 3H, Ar-CH_3); ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ 195.81, 168.65, 164.37, 163.54, 146.76, 141.85, 140.68, 129.53, 129.20, 128.34, 127.60, 126.59, 125.75, 125.29, 124.90, 122.19, 119.48, 54.83, 22.62, 21.22; EI-MS m/z: 412.34 (M^+, 100.00).

(iv). (*E*)-1-(4-fluorophenyl) -3-(2-methoxy-4-methyl- 6- (4-(trifluoromethyl) phenyl) pyrimidin-5-yl)prop-2-en-1- one (6d): White solid 75 % yield. Mp.: 149.3 °C~ 150.1 °C. ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 7.79 (d, *J* = 8.0 Hz, 2H, ArH), 7.64 (d, *J* = 8.0 Hz, 2H, ArH), 7.28 (t, *J* = 8.0 Hz, 2H, ArH), 7.14 (d, *J* = 8.0 Hz, 1H, -CH=CH-), 7.00 (t, *J* = 8.0 Hz, 2H, ArH), 6.57 (d, *J* = 7.4 Hz, 1H, -CH=CH-), 4.13 (s, 3H, $-OCH_3$), 2.52 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ 195.66, 168.96, 165.62, 164.55, 163.82, 163.11, 145.12, 140.7-4, 130.47, 129.85, 125.72, 122.28, 116.33, 116.11, 55.14, 22.86; EI-MS m/z: 416.32 (M^+, 80.07), 307.22 (100.00).

(v). (*E*)-1-(3-fluorophenyl)- 3-(2-methoxy-4-methyl- 6-(4-(trifluoromethyl) phenyl) pyrimidin-5-yl)prop-2-en-1- one (6e): Yellow oil 74 % yield. ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ: 7.78 (d, *J* = 8.0 Hz, 2H, ArH), 7.65 (d, *J* = 8.0 Hz, 2H, ArH), 7.31 (s, 1H, ArH), 7.11 (d, *J* = 7.8 Hz, 1H, -CH=CH-), 7.14-7.00 (m, 3H, ArH), 6.60 (d, *J* = 7.8 Hz, 1H, -CH=CH-), 4.13 (s, 3H, $-OCH_3$), 2.53 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ 195.47, 169.00, 164.55, 164.01, 163.92, 161.55, 144.67, 140.68, 135.86, 129.35, 125.52, 122.24, 118.07, 117.86, 114.62, 114.40, 55.10, 55.08, 22.81; EI-MS m/z: 416.29 (M^+, 100.00).

(vi). (*E*)-1-(2-fluorophenyl)- 3-(2-methoxy-4-methyl-6- (4-(trifluoromethyl) phenyl) pyrimidin-5-yl)prop-2-en-1- one (6f): Yellow oil 76 % yield. ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 7.78 (d, *J* = 8.0 Hz, 2H, ArH), 7.65 (d, *J* = 8.0 Hz, 2H, ArH), 7.38-7.29 (m, 3H, ArH), 7.10-7.01 (m, 2H, ArH and -CH=CH-), 6.70 (d, *J* = 7.4 Hz, 1H, -CH=CH-), 4.13 (s, 3H, $-OCH_3$), 2.54 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ 195.75, 169.02, 164.53, 164.04, 162.51, 159.97, 140.78, 138.64, 129.56, 125.46, 121.72, 116.25, 116.03, 55.10, 55.07, 22.84; EI-MS m/z: 416.32 (M^+, 90.42), 307.18 (100.00).

(vii). (*E*)-1-(2,4-dichlorophenyl)-3-(2-methoxy-4-methyl-6-(4-(trifluoromethyl) phenyl) pyrimidin-5-yl)prop-2-en-1-one (6g): Yellow oil 50 % yield. ^{1}H NMR (600 MHz, $CDCl_3$, ppm) δ: 7.77 (d, *J* = 12.0 Hz, 2H, ArH), 7.66 (d, *J* = 12.0 Hz, 2H, ArH), 7.50 (d, *J* = 11.8 Hz, 1H, ArH), 7.30 (s, 1H, ArH), 7.20 (d, *J* = 12.0 Hz, 1H, -CH=CH-), 7.16 (d, *J* = 12.0 Hz, 1H, ArH), 6.53 (d, *J* = 12.0 Hz, 1H, -CH=CH-), 4.14 (s, 3H, $-OCH_3$), 2.55 (s, 3H, CH_3); ^{13}C NMR (150 MHz, $CDCl_3$, ppm) δ: 195.35, 169.22, 164.54, 164.13, 140.60, 132.12, 131.90, 130.48, 129.93, 129.49, 128.29, 127.55, 126.29, 125.51, 125.49, 125.30, 124.49, 122.68, 120.87, 55.13, 22.88; EI-MS m/z: 466.36 (M^+, 5.03), 431.29 (100.00).

VIII. Evaluation of herbicidal activity

The inhibition rate of the growth of target compounds on the root and stem of barnyardgrass (*E. crus-galli*) and rape (*B. napus*) was determined in Petri dish bioassay at 100 mg/L, 10 mg/L and 1 mg/L concentrations (13). Each compound (2 mg) was dissolved in 100 μL DMF and emulsified with a drop of Tween-80, and the solution was diluted with water to 1000 mg/L concentrations, named concentrated solution. It was further diluted to of 100, 10 and 1 mg/L concentrations for experimental use. The solutions of commercial herbicide 2,4-D were also prepared as the positive control, and water was used as blank control. Test solutions (9 mL) were added into each Petri dish (9-cm dia) containing two layers of filter paper and 9 mL water was added in blank control. Ten seeds of barnyardgrass (*E. crus-galli*) or rape (*B. napus*) were sown on filter paper in each Petri dish. Thereafter the, the Petri dishes were placed in dark for 3-days in an incubator at 28 °C with 60 % relative humidity. In next 5-days, germinating seeds were kept in 12 h of light/darkness in the incubator at same temperature and relative humidity conditions. At the end of this test, 3-plants from each Petri dish with the longest root and stem were measured, and the mean lengths were calculated for each treatment. These values were used to calculate the inhibition rate (X) as under (1):

$$X = \frac{\bar{l}_1 - \bar{l}_2}{\bar{l}_1} \times 100\ \%$$

Where $\bar{l}_1$: Mean length of root/stem in control treatment, and $\bar{l}_2$: Mean length of root/stem in tested compound. Positive values indicate, an inhibition in root/stem elongation, while negative values indicate stimulatory growth effects. Data of shoot and root inhibitory rates were subjected to one-way Anova. Means were compared by the least significant difference test ($p < 0.05$).

RESULTS AND DISCUSSION

Synthesized compounds

This work reports for the first time, the synthesis of 11-pyrimidine derivatives, 6-with a CAPBE structure and 7-with the DPPP skeleton (Figure 1). These compounds were synthesized from acetylacetone, *p*-chlorobenzaldehyde/ *p*-trifluoromethyl benzaldehyde and urea based on a Biginelli reaction, an oxidation reaction and nucleophilic substitution/aldol condensation reactions (20). Their structures were confirmed by ^{1}H NMR, ^{13}C NMR and Mass spectra characterization analyses. For the Biginelli three-component reaction, withdrawal of a weak electron chloro (-Cl) group and substitution by benzaldehyde proved more beneficial to the yield of product, than the trifluoromethyl (-CF_3)-substituted one (**2a** *vs* **2b**), while the electronic effect did not distinct the influence of the subsequent oxidation reaction. In the nucleophilic substitution reaction, the electron-donating group substituted benzyl bromide resulted in higher product yields (**4e**, **4f** *vs* **4a**~**4d**), which may be because they are more likely to produce more stable benzyl carbocations in the reaction process. For the aldol condensation reaction between intermediate **5a** and substituted benzaldehyde, the influence of electron effect of substituted benzaldehyde is more obvious than that of steric

hindrance. For example, electron-donating group methoxyl (-OCH_3) and methyl (-CH_3) substituted benzaldehyde led to higher product yields (**6b**, **6c** *vs* **6d~6g**), while the product yields of 2-, 3- and 4-fluoro substituted benzaldehyde were almost the same (**6d~6f**).

Herbicidal activity

To determine the selective inhibitory effects on plant growth, the pyrimidine derivatives were tested against the monocot barnyardgrass and the dicot rape (23). These model plant species were selected due to their fast and uniform seed germination, which allowed homogeneous root and shoot growth (13,25). Although all pyrimidine derivatives inhibited the seedlings growth, but the CAPBE compound **4b** (with 2,4-fluoro substitution in the benzyl ring) was the only one to completely inhibited the seed germination of both target plants at 100 mg/L (Figure 2). Its herbicidal activity on barnyardgrass roots was comparable to 2,4-D. It is interesting to note that the CAPBE structure by itself represented in compound **4d** and its benzyl substituted derivatives bearing the 4-F (compound **4a**), 4-NO_2 (compound **4c**) and 4-OCF_3 groups (compound **4e**) showed the strongest herbicidal effects on barnyardgrass roots, while substitution 4-OCH_3 (compound **4f**) exerted the same sublethal phytotoxicity (about 60 %) on both barnyardgrass and rape roots. At 100 mg/L, compounds **4a** and **4c-4e** inhibited the barnyardgrass roots by 60-70 %, while, the inhibition in rape root growth was less than 50 %. It was also noticed that the roots were the primary site of action for CAPBE compounds **4a**, **4d** and **4f** showed consistent inhibitory dose-responses that were absent in shoots (1,18). The DPPP derivatives, were growth inhibitory to both target species, except **6a** and **6d** compounds which at low concentrations slightly (< 10 %) stimulated the root and shoot growth, respectively. It is interesting to note that the DPPP molecules had certain selective phytotoxicity on broadleaf species. These at 100 mg/L reduced rape shoot and root elongation by 60-90 %, while inhibition of barnyardgrass (*E. crus-galli*) was < 40 % (Figure 3).

The presence of cinnamoyl group, which is part of basic chalcone structure, could be involved in this effect. Compounds such as the *trans*-cinnamaldehyde and chalcones derivatives containing the cinnamoyl moiety also exerted more severe phytotoxicity on dicots than monocots (6). Compound **6a** was the most active on rape shoot and root growth. Hence, the substitutions done at carbons 2, 3 or 4 of the B-ring belonging to the cinnamoyl moiety of the DPPP compounds did not enhance the herbicidal activity. This was also observed in chalcone derivatives with the B-ring substituted with wide range of functional groups, which were less phytotoxic than the unsubstituted chalcone structure (12). Structure-activity relationship analysis revealed that the inhibitory effects on barnyardgrass root and shoot of CAPBE compounds were superior to DPPP compounds, while the inhibitory effects on rape root and shoot of DPPP compounds are better than CAPBE ones. For the two series of pyrimidine derivatives, electronic effect had some influences on their herbicidal activities

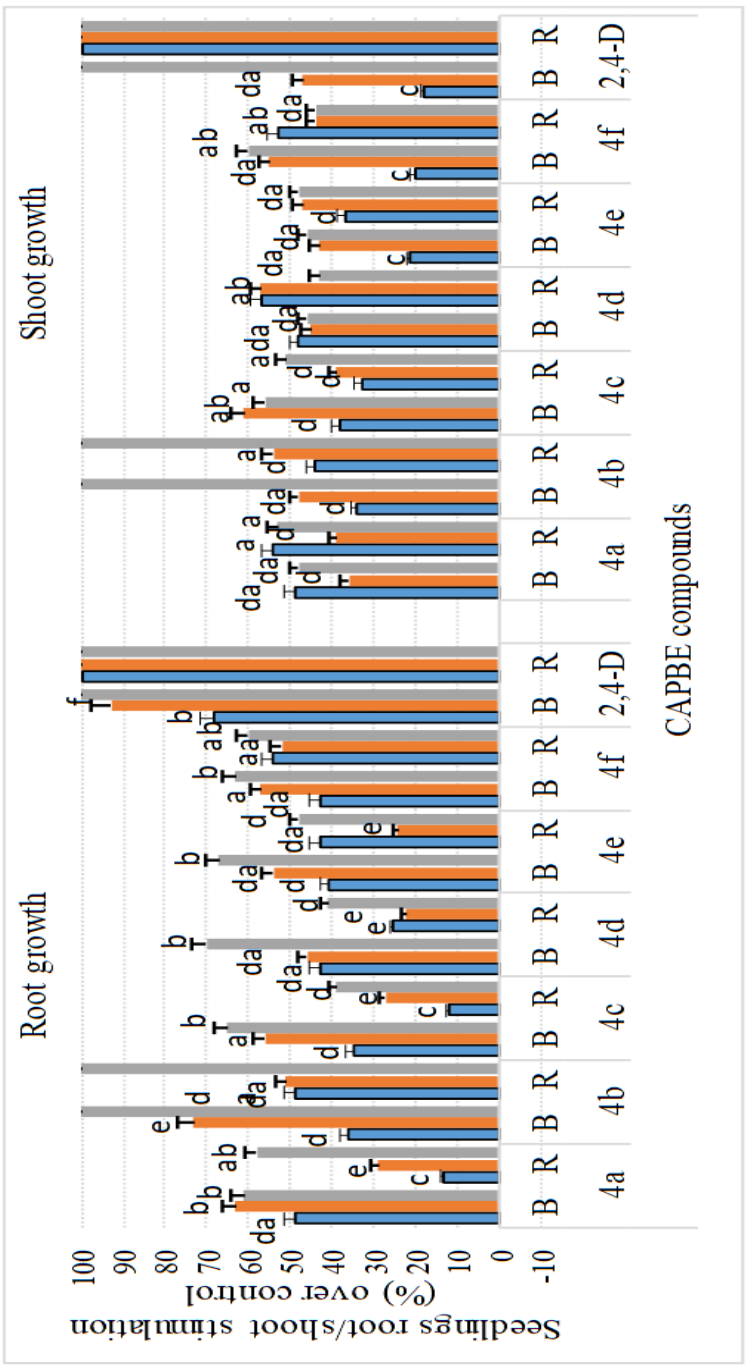

Figure 2. Herbicidal activity of chloride aryl pyrimidine benzyl ether (CAPBE) molecules on shoot and root growth of barnyardgrass (B) and rape (R). Different letters in Bars indicate significant differences among shoot or root inhibition rates at the 0.05 level (LSD test). Data are mean values ± SD (n = 3). Compounds tested concentrations were : mg/ml (■), 10 mg/ml (■) and 100 mg/ml (■).

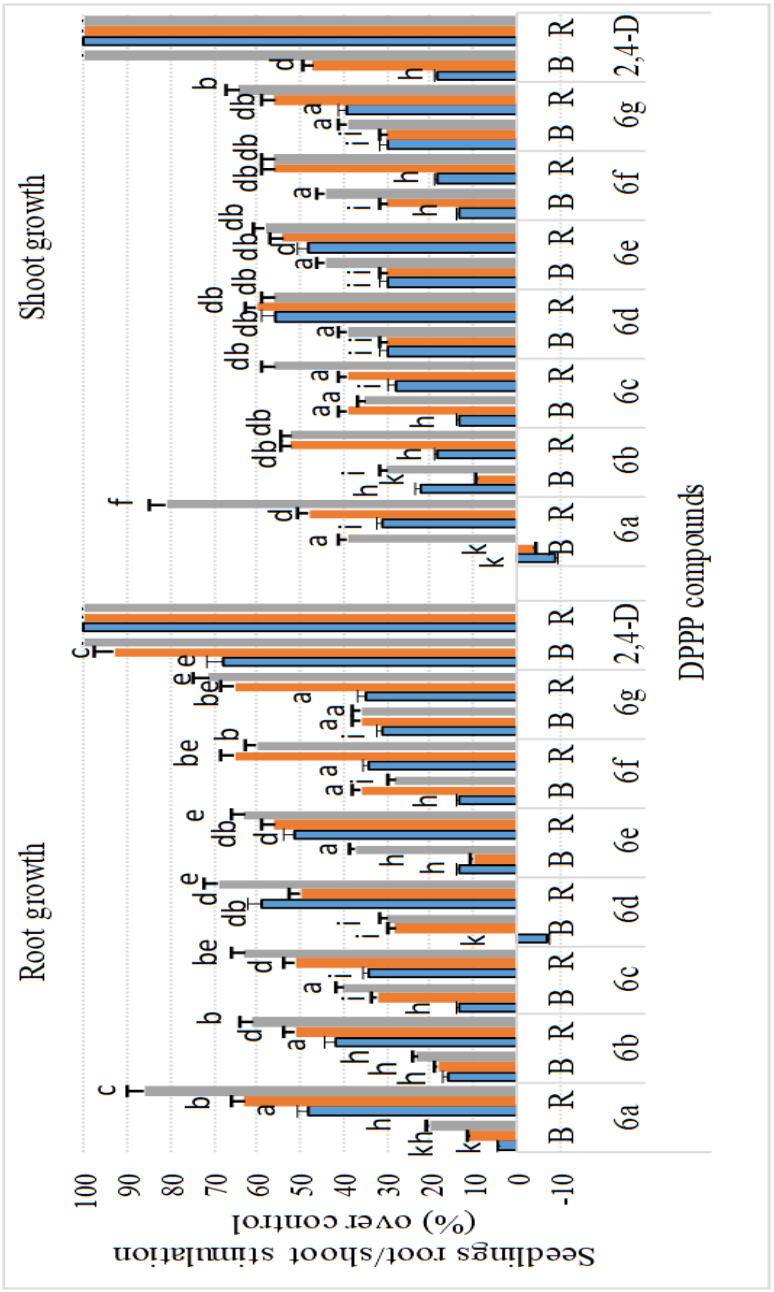

Figure 3. Herbicidal activity of diphenyl pyrimidine propenone (DPPP) molecules on shoot and root growth of (B) and rape (R). Different letters in Bars indicate significant differences among shoot or root inhibition rates at the 0.05 level (LSD test). Data are mean values ± SD (n = 3). Compounds test concentrations were : 1 mg/ml (■), 10 mg/ml (■) and 100 mg/ml (■).

especially for compound **4b**, which may be due to the small atomic radius and high electronegativity peculiarities of fluorine. For DPPP compounds, the steric effect exhibited more effects on their herbicidal activities at 1 mg/L concentration than other concentrations (compounds **6d**~**6f**).

CONCLUSIONS

We tested the phytotoxic activity of synthetic compounds based on CAPBE and DPPP skeletons and presented synthesis method for both compounds types that have hybrid pyrimidine-ether and pyrimidine-chalcone structures, respectively. They were designed by combining the allelochemical structures by active substructure splicing and bioisosterism methods. The CAPBE and DPPP cores were selective herbicidal against monocot and dicot species, respectively. Nevertheless, most compounds exerted moderate activity. Compounds 4b and 6a deserve further investigations as herbicidal lead molecules.

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DECLARATION

We declare that all authors of this Ms. have made substantial contributions. We did not exclude any author who substantially contributed to this Ms. We have followed our ethical norms established by our respective institutions.

CONFLICT OF INTEREST

The authors announce that they have no conflict of interest.

ETHICAL APPROVAL

The authors declare that the study was carried out following scientific ethics and conduct. However, this study did not involve any use of animals, hence no ethical approval has been obtained from the concerned committee.

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PUBLISHER NOTE