Mongolian Academy of Sciences

Mongolian Journal of Chemistry

The Institute of Chemistry & Chemical Technology

Natural products as sources of new fungicides (I): synthesis and antifungal activity of Kakuol derivatives against phytopathogenic fungi

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ARTICLE INFO: Received 26 July 2014; revised 10 November 2014; accepted 26 November 2014

Abstract: In order to establish an advanced structural-activity relationship (SAR) and to explore the feasibility of kakuol analogues with better anti-fungi activity, a series of 2-hydroxy-4,5-methylenedioxyaryl ketones were conveniently synthesized by the Friedel-Crafts acyl reaction, etherification reaction, reduction reaction and oximation reaction. Their structures characterized by ^{1}H and ^{13}C NMR and HRMS methods. Their *in vitro* antifungal activities were assayed. Most of the derivatives showed a remarkable *in vitro* activity, and some of them appeared significantly more effective than a commercial fungicide hymexazol as positive control. In particular compounds **2h** and **2i**, were found active with a IC_{50} value of 3.1 mg/ml and 2.9 mg/ml against *Glomerella cingulate*, which suggested that 2-hydroxy-4,5-methylenedioxyaryl ketones might be a promising candidates in the development of new antifungal compounds. Compounds **2e**, **5** and **6** also exhibited high antifungal activities on a wide range of organisms, which might be considered as leading compounds in the development of new antifungal compounds.

Keywords: 2-hydroxy-4,5-(methyl-enedioxy)propiophenone, kakuol dervatives, antifungal activity, structure-activity relationship

INTRODUCTION

Crops are facing a tremendous damage from pests, diseases and weed damages which results in direct economic losses including reduction in grain yield and quality [1]. Although these losses may be attenuated by the use of disease-tolerant cultivars, crop rotation, or sanification practices, the use of fungicides will remain the predominant method of crop protection for the foreseeable future. Despite the availability of effective fungicides [2], new fungicides with novel chemical structures which have higher potency and broader spectrum of activity against resistant fungal strains have attracted great attentions recently.

Recent studies have demonstrated that kakuol and its derivatives can be seen as important intermediates for the synthesis of some agrochemicals and pharmaceuticals. For example, kakuol (1-[2-hydroxy-4, 5 - (methylenedioxy)phenyl]propan - 1 - one, Fig. 1) isolated from the rhizomes of *Asarum sieboldii* (Miq.) Maek, exhibited antifungal activity against some important plant pathogens such as *Colletotrichum orbiculare*, *Rhizoctonia solani*, *Botrytis cinerea*, *Phytophthora capsici* and *Cladosporium cucumerinum* [3], *Asarum sieboldii* isolated from flower buds of *Magnolia fargesii* had been reported to have antifungal and insecticidal activity [4, 5]. Several analogous propiophenones, such as 2-methoxy-3,4-(methylenedioxy)propiophenone [6] methylkakuol and 2,4,5-trimethoxypropiophenone [7, 8], isolated from *Asarum, Anethum* and *Piper species,* respectively, yet their antifungal activity have not been reported. In 2010, Loana Musso et al. reported of a structure-activity relationship (SAR) kakuol derivatives, and found that the compounds bearing a C=C bond conjugated to the C=O group was beneficial to their antifungal activity [9]. Some data related to structure-activity relationship (SAR) kakuol and methylkakuol have been reported in a recent patent and studies [10-12]. These facts justify for urther studies on this class of molecules to identify the essential structural requirements for their antifungal activity. The urther study about the synthesis and the determination of a structure-activity relationship (SAR) for a series of kakuol analogues still needed.

In Loana's study, they prepared 2-hydroxy-4,5-methylenedioxyaryl ketones by two steps which involving Grignard reaction, but with a low yields (from 5% - 40%). Based on their study, in our work the synthesis method has been improved, the reaction time has been shortened, and the yield has been

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DOI: http://doi.dx.org/10.5564/mjc.v15i0.331

raised. Several new compounds have been prepared by one step using Friedel-Crafts acyl reaction. In this study, we prepared a series of alkyl and benzyl kakuol derivatives **2a**-**2n**, **3**,**4**, **5** and **6**, moreover evaluated *in vitro* antifungal activities against several phytopathogens. Herein, we report the interesting findings of these kakuol derivatives.

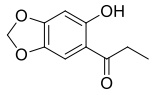
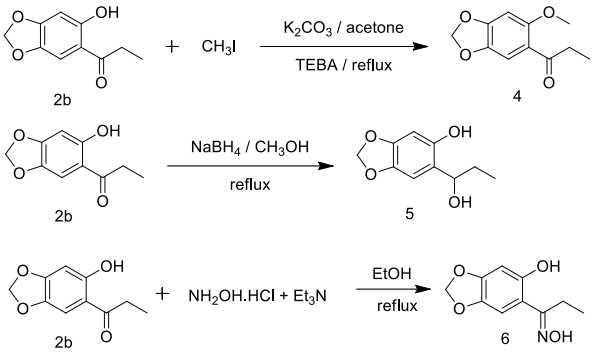


Fig. 1. Structure of kakuol (2b)



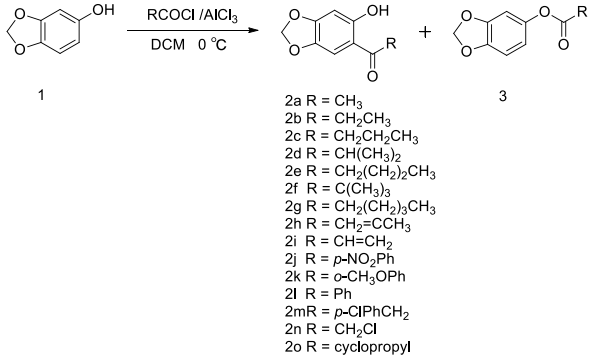
Scheme 2. Synthesis of Compounds 4, 5, and 6

EXPERIMENTAL

Materials and methods: The melting points of the products were determined on an XT-4 apparatus (Beijing Tech Instrument Co., Beijing, China) and are uncorrected. The IR spectra were recorded on a Bruker VECTOR22 spectrometer in film or KBr discs. ^{1}H and ^{13}C NMR spectra were taken on a Varian Mercury 400 MHz (400 MHz for ^{1}H and 100 MHz for ^{13}C, respectively) or a Bruker Avance 500 MHz (500 MHz for ^{1}H and 125 MHz for ^{13}C, respectively) spectrometer in $CDCl_3$ solution using TMS as an internal standard (d given in ppm, J in Hz). MS spectra were taken on a Bruker Daltonics esquire 6000 (ESI-ION TRAP) or on a Waters ESI-Q-TOF Ultima Global mass spectrometer. Thin-layer chromatography (TLC) was performed with silica gel plates using silica gel 60 GF254 and column chromatography (CC) was carried out on silica gel (200-300 mesh) (Qingdao Haiyang Chemical Co. Ltd. Qingdao, Shandong province, China).

Synthetic procedures: All the solvents were of analytical grade, alkyl or benzyl acyl chlorides and sesamol were commercial reagents. Target compounds (**2a**- **2n**) were synthesized by the Friedel-Crafts acyl reactions of sesamol with the corresponding benzyl acyl chloride or alkyl acyl chloride (Scheme 1).

General Synthetic Procedure for Compounds **2a**-**2n**.



Scheme 1. Synthesis of 2-Hydroxy-4,5-methylenedioxyaryl Ketones 2a - 2n

[13] To a three-necked flask (50 ml) equipped with a condenser, an addition funnel, an HCl trap and a stopper with nitrogen flow, $AlCl_3$ (1.5 mmol) and dry CH_2Cl_2 (5 ml) were added. The flask was then cooled with ice bath, and a solution of acyl chloride (1.2 mmol) in CH_2Cl_2 (5 ml) slowly added via the addition funnel, until all the solid $AlCl_3$ had dissolved. A solution of phenol (1 mmol) in CH_2Cl_2 was then added dropwise through the addition funnel, to avoid excessive evolution of gaseous HCl. The ice bath was then removed, and the mixture was refluxed for 2 hours, then cooled to room temperature. The reaction was poured into a beaker containing ice (5 g) and concentrated HCl (5 ml), and stirred 30 min. The organic layer was extracted with ethyl acetate (3 x 10 ml), washed with saturated $NaHCO_3$, brine and dried over Na_2SO_4 and concentrated under vacuum. The concentrated mixture was subjected to silica gel column chromatography and eluted by the mixture of petroleum ether and ethyl acetate (10:1 ~ 15:1, v/v) gradient elution to give title compounds.

Synthetic Procedure for Compound **4**. [14] To a solution of **2b** (195 mg, 1 mmol) in acetone (5 ml), K_2CO_3 (165 mg, 1.2 mmol) and MeI (0.3 ml, 5 mmol) were added, and the mixture was refluxed for 15 h. The mixture was filtered, then the solution was removed in vacuo, the residue was extracted by ethyl acetate. The organic layer was washed with H_2O and brine, the solution was dried (Na_2SO_4), and solvent was removed in vacuo. The crude material was purified by FCC (petroleum ether and ethyl acetate 15 :1) to give 4 (108 mg, 56 %).

Synthetic Procedure for Compound **5**. [15] To a solution of **2b** (195 mg, 1 mmol) in MeOH (5 ml), This solution was chilled to 0°C, and sodium borotetrahydride (0.05 g, 1.2 mmol) was added portion-wise over 30 mins. Upon completion by TLC the reaction was quenched by the slow addition of ice. The resulting mixture was slowly evaporated to remove the methanol. The resulting solution was taken up in 10 ml of ethyl acetate and washed with 1M HCl. The organic layer was then washed three times with water and once with brine. The organic layer was dried over sodium sulfate and concentrated in vacuo to give a brown viscous oil. The crude material was purified by FCC (petroleum ether and ethyl acetate 5 :1) to give 5 (176 mg, 90 %).

Synthetic Procedure for Compound **6** [16]. To a

suspension of $NH_2OH{\cdot}HCl$ (69 mg, 1 mmol) and AcONa (163 mg, 1.2 mmol) in MeOH (10 ml), **2b** (195 mg, 1 mmol) was added, and the mixture was refluxed for 6 h. After the addition of a second portion of $NH_2OH{\cdot}HCl$ (69 mg, 1 mmol) and AcONa (163 mg, 1.2 mmol), the mixture was refluxed for 8 h. The solvent was evaporated, and the crude material was dissolved in AcOEt. The organic phase was washed with H_2O, dried (Na_2SO_4), and the solvent was removed in vacuo. The crude material was purified by FCC (petroleum ether /AcOEt 10 : 1) to give **6** (169 mg, 81 %).

Antifungal bioassay: The tested phytopathogenic fungi (*Glomerella cingulate*, *Botrytis cinerea*, *FusaHum graminearum Sehw. Curvularia lunata*, *and Fusarium oxysporum f. sp. vasinfectum*) were provided by College of Sciences, Northwest A&F University. Cultures of the test fungi were maintained on PDA slants at 4°C and were subcultured in Petri dishes prior to testing.

The *in vitro* antifungal activity of the test compounds was evaluated by using mycelial growth inhibitory rate method [17]. Briefly, the media (100 ml) incorporating test samples were inoculated at the center of agar discs of the test fungi (4 mm diameter). Three replicate plates for each fungus were incubated at 26 (±2)°C for all test fungi. Control plates containing media mixed with acetone (1 ml) were included. After incubation for 72 h, the mycelial growth of fungi (mm) in both treated (T) and control (C) Petri dishes was measured diametrically in three different directions (decussation method) till the fungal growth in the control dishes was almost complete. The percentage of growth inhibition (I) was calculated using the following formula.

I (%) = [(C-T) ⁄C] x 100.

Statistical analysis: The experimental data of the antifungal activities were analyzed using SPSS 13.0 for Windows (SPSS, Chicago, IL,USA).

Analytical data: 1-(6-hydroxy-1,3-benzodioxol-5-yl) ethan-1-one (2a) White crystal, yield 71.0%, m.p. 112 -113 °C. IR (KBr) ν_{max} (per cm): 2918, 1630, 1483-1364, 1031, 921. ^{1}H-NMR (400 MHz, $CDCl_3$): 13.09 (s, OH), 7.21 (s, 1 arom. H), 6.54 (s, 1 arom. H), 6.07 (s, 2H, OCH_2O), 2.50 (t, 3H, Me). ^{13}C NMR (100 MHz, $CDCl_3$) δ 202.6, 160.6, 154.5, 141.3, 112.1, 108.4 , 101.2, 98.26, 26.52. HR-EI-MS: *m/z* $[M+H]^+$ 180.0415 (calcd for $C_9H_8O_4$, 181.0512).

1-(6-hydroxy-1,3-benzodioxol-5-yl)propan-1-one (2b) Yellow crystal, yield 73.5%, m.p. 110-111 °C. IR (KBr) ν_{max} (per cm): 2917, 1628, 1500-1372, 1036, 943. ^{1}H-NMR (400 MHz, $CDCl_3$): 13.08 (s, OH), 7.07 (s, 1 arom. H), 6.43 (s, 1 arom. H), 6.01 (s, 2H, OCH_2O), 2.81 (t, 2H, CH_2Me), 1.01 (t, 3H, Me). ^{13}C NMR (100 MHz, $CDCl_3$) δ 202.6, 161.6, 154.5, 141.3, 112.1, 108.5, 101.2, 98.2, 35.3, 26.5. HR-EI-MS: *m/z* $[M+H]^+$ 194.0517 (calcd for $C_{10}H_{10}O_4$, 194.0625).

1-(6-hydroxy-1,3-benzodioxol-5-yl)butan-1-one (2c) White crystal, yield 60.0%, m.p. 83-85 °C. IR (KBr) ν_{max} (per cm): 2897, 1619, 1452-1326, 1030, 934. ^{1}H-NMR (400 MHz, $CDCl_3$): 13.17 (s, OH), 7.08 (s, 1 arom. H), 6.44 (s, 1 arom. H), 5.97 (s, 2H, OCH_2O), 2.88 (q, 2H, CH_2Me), 1.69-1.79 (m, 2H, CH_2), 1.21 (t, 3H, Me). ^{13}C NMR (100 MHz, $CDCl_3$) δ 204.2, 162.1, 154.1, 140.3, 111.1, 106.5, 101.8, 98.7, 40.5, 18.1,13.8. HR-EI -MS: *m/z* $[M+H]^+$ 208.0723 (calcd for $C_{11}H_{12}O_4$, 208.2121).

1-(6-hydroxy-1,3-benzodioxol-5-yl)-2-methylpropan-1-one (2d) Light yellow crystal, yield 50.0%, m.p. 79-80 °C. IR (KBr) ν_{max} (per cm): 2919, 1619, 1476-1378, 1031, 942. ^{1}H-NMR (400 MHz, $CDCl_3$): 13.32 (s, OH), 7.12 (s, 1 arom. H), 6.46 (s, 1 arom. H), 5.98 (s, 2H, OCH_2O), 3.37-3.12 (m, 1H, CH), 1.21-1.25 (m, 6H, Me_2). ^{13}C NMR (100 MHz, $CDCl_3$) δ 208.2, 162.1, 154.1, 140.4, 110.6, 106.4, 101.8, 98.9, 34.7, 29.6, 19.2. HR-EI-MS: *m/z* $[M+H]^+$ 208.0411 (calcd for $C_{11}H_{12}O_4$, 208.2055).

1-(6-hydroxy-1,3-benzodioxol-5-yl)pentan-1-one (2e) White crystal, yield 55.0%, m.p. 55-56 °C. IR (KBr) ν_{max} (per cm): 2950, 1619, 1477-1381, 1167, 1029, 920. ^{1}H-NMR (400 MHz, $CDCl_3$): 13.16 (s, OH), 7.08 (s, 1 arom. H), 6.44 (s, 1 arom. H), 5.97 (s, 2H, OCH_2O), 2.84 (t, 2H, CH_2Me), 1.65-1.75 (m, 2H, CH_2), 1.37-1.48 (m, 2H, CH_2), 0.96 (t, 3H, Me). ^{13}C NMR (100 MHz, $CDCl_3$) δ 204.4, 162.1, 154.1, 140.3, 111.8, 106.6, 101.8, 98.7, 37.9, 36.8, 22.4, 13.8. HR-EI-MS: *m/z* $[M+H]^+$ 223.0821 (calcd for $C_{11}H_{12}O_4$, 202.2236).

1-(6-hydroxy-1,3-benzodioxol-5-yl)-2,2-dimethylpropan-1-one (2f) Light yellow crystal, yield 47.0%, m.p. 88-90 °C. IR (KBr) ν_{max} (per cm): 2913, 1602, 1483-1423, 1033, 941. ^{1}H-NMR (400 MHz, $CDCl_3$): 13.62 (s, OH), 7.37 (s, 1 arom. H), 6.46 (s, 1 arom. H), 5.97 (s, 2H, OCH_2O), 1.41(s, 9H, Me_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 209.7, 163.6, 153.2, 139.4, 109.6, 107.6, 101.7,99.2, 43.9, 28.6. HR-EI-MS: *m/z* $[M+H]^+$ 223.2151 (calcd for $C_{11}H_{12}O_4$, 202.0742).

1-(6-hydroxy-1,3-benzodioxol-5-yl)hexan-1-one (2g) White crystal, yield 45.0%, m.p. 54-55 °C. IR (KBr) ν_{max} (per cm): 2953, 1621, 1484-1401, 1041, 926. ^{1}H-NMR (400 MHz, $CDCl_3$): 13.16 (s, OH), 7.28 (s, 1 arom. H), 7.06 (s, 1 arom. H), 5.96 (s, 2H, OCH_2O), 2.81 (t, 2H, CH_2Me), 1.73 (m, 2H, CH_2), 1.66-1.75 (m, 2H, CH_2), 1.33-1.39 (m, 2H, CH_2), 0.91 (t, 3H, Me). ^{13}C NMR (100 MHz, $CDCl_3$) δ 204.3, 162.1, 154.1, 140.2, 111.7, 106.5, 101.7, 98.6, 38.1, 31.1, 24.3, 22.3, 13.8. HR-EI-MS: *m/z* $[M+H]^+$ 236.1019 (calcd for $C_{13}H_{16}O_4$, 236.2653).

1-(6-Hydroxy-1,3-benzodioxol-5-yl)-2-methylprop-2-en-1-one (2h) Yellow crystal, yield 55.0%, m.p. 54 - 55°C. IR (KBr) ν_{max} (per cm): 2914, 1625, 1477-1340, 1034, 931. ^{1}H-NMR (400 MHz, $CDCl_3$): 12.87 (s, OH), 7.12 (s, 1 arom. H), 6.97 (s, 1 arom. H), 6.30 (s,1H, CH=C$\underline{H}_2$), 5.99 (s, 2H, OCH_2O), 5.75 (s,1H, CH=CH_2), 2.02 (s, 3H, Me). ^{13}C NMR (100 MHz, $CDCl_3$) δ 201.4, 162.1, 154.6, 142.7, 140.1, 120.9, 110.7, 109.3, 101.8, 98.8, 19.7. HR-EI-MS: *m/z* $[M+H]^+$ 206.0622 (calcd for

$C_{11}H_{10}O_4$, 206.1937).

1-(6-Hydroxy-1,3-benzodioxol-5-yl)prop-2-en-1-one (2i) Yellow crystal, yield 65.0%, m.p. 116 -118 °C. IR (KBr) ν_{max} (per cm): 2935, 1605, 1482-1360, 1040, 935. ^{1}H-NMR (500 MHz, $CDCl_3$): 13.49 (s, OH), 7.18 (s, 1 arom. H), 7.10 (dd, 1H, CH=CH_2), 6.61 (s, 1 arom. H), 6.40 (dd, 1 H, CH=CH_2), 6.06 (s, 2H, OCH_2O), 5.79 (dd, 1 H, CH=CH_2). ^{13}C NMR (100 MHz, $CDCl_3$) δ 190.2, 158.6, 157.4, 143.2, 134.5, 130.2, 113.7, 106.3, 102.1, 98.1. HR-EI-MS: *m/z* $[M+H]^+$ 192.0426 (calcd for, $C_{10}H_8O_4$, 192.0428).

(6-hydroxy-1,3-benzodioxol-5-yl)(4'-nitrophenyl) methanone (2j) Yellow crystal, yield 40.0%, m.p. 194-193 °C. IR (KBr) ν_{max} (per cm): 1736, 1591, 1464-1319, 1025, 923. ^{1}H-NMR (400 MHz, DMSO-d_6): 12.79 (s, OH), 8.42 (q, 2 arom. H), 7.94 (q, 2 arom. H), 6.92(s, 1 arom. H), 6.59 (s, 1 arom. H), 6.08 (s, 2H, OCH_2O). ^{13}C NMR (100 MHz, DMSO-d_6) δ 205.8, 163.6, 155.6, 149.3, 143.8, 140.8, 129.5, 123.5, 111.19, 109.11, 109.1, 102.6, 98.3, 47.5. HR-EI-MS: *m/z* $[M+H]^+$ 287.04 17(calcd for $C_{14}H_9NO_6$, 287.2285).

(6-hydroxy-1,3-benzodioxol-5-yl)(2'-methoxyphenyl) methanone (2k) Yellow crystal, yield 35.0%, m.p. 117 -119 °C. IR (KBr) ν_{max} (per cm): 2919, 1599, 1470-1333, 1025, 923. ^{1}H-NMR (400 MHz, $CDCl_3$): 13.01 (s, OH), 7.43-7.47 (m, 1 arom. H), 7.25-7.26 (m, 1 arom. H), 7.04-7.06 (m, 1 arom. H), 6.99-7.04 (m, 1 arom. H), 6.65 (s, 1 arom. H), 6.50 (s, 1 arom. H), 5.94 (s, 2H, OCH_2O). ^{13}C NMR (100 MHz, $CDCl_3$) δ 199.5, 162.8, 156.1, 154.6, 140.2, 131.4, 128.5, 127.9, 120.5, 120.5, 113.1, 109.9, 101.8, 98.43, 57.7. HR-EI-MS: *m/z* $[M+H]^+$ 273.2545 (calcd for $C_{15}H_{12}O_5$, 272.0625).

(6-hydroxy-1,3-benzodioxol-5-yl)(phenyl) methanone (2l) Yellow crystal, yield 40.0%, m.p. 108-110 °C. IR (KBr) ν_{max} (per cm): 1604, 1474-1340, 1034, 935. ^{1}H-NMR (400 MHz, DMSO-d_6): 13.00 (s, OH), 7.63-7.26 (m, 7H, arom. H), 6.94 (s, 1 arom. H), 6.55 (s, 1 arom. H), 5.89 (s, 2H, OCH_2O). ^{13}C NMR (100 MHz, DMSO-d_6) δ 194.1, 163.3, 154.5, 140.2, 138.2, 130.4, 128.3, 111.6, 109.9, 101.9, 98.8. HR-EI-MS: *m/z* $[M+H]^+$ 242.2268 (calcd for $C_{14}H_9ClO_4$,242.0579).

(4'-chlorophenyl)(6-hydroxy-1,3-benzodioxol-5-yl) methanone (2m) Yellow crystal, yield 40.0%, m.p. 128-130 °C. IR (KBr) ν_{max} (per cm): 2595, 1626, 1454-1357, 1030, 930. ^{1}H-NMR (400 MHz, DMSO-d_6): 12.94 (s, OH), 7.30-7.33 (m, 2 arom. H), 7.17-7.20 (m, 2 arom. H), 6.45 (s, 1 arom. H), 5.98 (s, 2H, OCH_2O). ^{13}C NMR (100 MHz, DMSO-d_6) δ 204.4, 162.8, 155.1, 140.6, 132.6, 130.6, 129.7, 128.9, 111.4, 106.5, 101.9, 98.9, 44.2. HR-EI-MS: *m/z* $[M+H]^+$ 276.0185 (calcd for $C_{14}H_9ClO_4$, 276.6913).

2-chloro-1-(6-hydroxy-1,3-benzodioxol-5-yl) ethanone (2n) Yellow crystal, yield 61.0%, m.p. 113-115 °C. IR (KBr) ν_{max} (per cm): 2921, 1634, 1481-1393, 1023, 922. ^{1}H-NMR (400 MHz, $CDCl_3$): 12.48 (s, OH), 7.01 (s, 1 arom. H), 6.48 (s, 1 arom. H), 6.01 (s, 2H,OCH_2O), 4.54 (s, 2H,CH_2Cl). ^{13}C NMR (100 MHz, $CDCl_3$) δ 194.1, 163.1, 155.3, 140.8, 109.7, 105.8, 102.1, 98.9, 44.7. HR-EI-MS: *m/z* $[M+H]^+$ 214.0412 (calcd for $C_9H_8O_4$, 214.6098).

Cyclopropyl (6-hydroxy-1,3-benzodioxol-5-yl) methanone (2o) Yellow crystal, yield 50.0%, m.p. 85-87 °C. IR (KBr) ν_{max} (per cm): 3081, 1621, 1487-1381, 1030, 924. ^{1}H-NMR (400 MHz, $CDCl_3$): 13.27 (s, OH), 7.30 (s, 1 arom. H), 6.44 (s, 1 arom. H), 5.98 (s, 2H, OCH_2O), 2.43-2.48 (m, 1H, CH), 1.23-1.28 (m, 1H, CH_2), 1.01-1.08 (m, 1H, CH_2). ^{13}C NMR (100 MHz, $CDCl_3$) δ 203.1, 161.7, 154.1, 140.5, 112.6, 106.7, 101.7, 98.6, 16.1, 11.5. HR-EI-MS: *m/z* $[M+H]^+$ 206.0579 (calcd for $C_9H_8O_4$, 206.1947).

1-(6-Methoxy-1,3-benzodioxol-5-yl)propan-1-one (4) Yellow crystal, yield 56.0%, m.p. 88-89 °C. IR (KBr) ν_{max} (per cm): 2984, 1623, 1467-1407, 1028, 889. ^{1}H-NMR (400 MHz, DMSO-d_6): 7.31 (s, 1 arom. H), 6.52 (s, 1 arom. H), 5.97 (s, 2H, OCH_2O), 3.85 (s, 3H, Me), 2.94 (q, 2H, $MeCH_2$), 1.13 (t, 2H, $MeCH_2$). ^{13}C NMR (100 MHz, DMSO-d_6) δ 199.6, 155.8, 152.1, 141.2, 120.1, 107.8, 102.1, 96.5, 65.1, 36.3, 8.8. HR-EI-MS: *m/z* $[M+H]^+$ 208.0738 (calcd for $C_{11}H_{12}O_4$, 208.0741).

6-(1-hydroxypropyl) -1,3- benzo dioxol-5-ol (5) White crystal. Yield 48.0%, m.p. 52-54 °C. IR (KBr) ν_{max} (per cm): 3295, 2920, 1503-1396. 1041, 927. ^{1}H-NMR (400 MHz, $CDCl_3$): 13.01 (s, OH), 6.80 (s, 1 arom. H), 6.50 (s, 1 arom. H), 5.90 (s, 2H, OCH_2O), 4.75 (t, 1H, CH), 1.83 (m, 2H, CH_2), 0.93 (t, 3H, Me). ^{13}C NMR (100 MHz, $CDCl_3$) δ 151.1, 146.9, 141.1, 125.2, 106.9, 101.1, 95.5, 71.7, 30.3, 10.4. HR-EI-MS: *m/z* $[M+H]^+$ 196.0719 (calcd for $C_{10}H_{12}O_4$, 196.1953).

6-[(1E)-N-Hydroxypropanimidoyl]-1,3-benzodioxol-5-ol (6) White crystal, yield 81.0%, m.p. 173-174 °C. IR (KBr) ν_{max} (per cm): 3366, 2897, 1637, 1495-1398, 1020, 928. ^{1}H-NMR (400 MHz, DMSO-d_6): 11.92 (s, 1 arom. OH), 11.29 (s, 1H, N-OH), 7.04 (s, 1 arom. H), 6.51 (s, 1 arom. H), 5.98 (s, 2H, OCH_2O), 2.76 (q, 2H, CH_2), 1.07 (t, 3H, Me). ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.2, 154.5, 148.5, 140.3, 109.7, 106.2, 101.3, 98.4, 17.9, 11.1. HR-EI-MS: *m/z* $[M+H]^+$ 209.1986 (calcd for $C_{10}H_{11}NO_4$, 209.0688).

RESULTS AND DISCUSSION

Chemistry: As shown in Scheme 1, kakuol and the series of 2-hydroxyaryl ketones could be readily obtained in a single step by the Friedel-Crafts acylation reaction of sesamol with the corresponding benzyl acyl chloride or alkyl acyl chloride in the presence of dry $AlCl_3$ in good yields. Commercially unavailable acyl chlorides were prepared according to reported methods.

Specifically, as stated in experimental section, benzylated products and some alkylated products with large steric hindrance group such as **2f**, **2j**, **2k** and **2m**, were obtained by increasing reaction temperature and time in order to get a reasonable yield, the best results were obtained for **2b** at 65 °C for 5 h in yield of 73 % and for **2a** at 60 °C for 4 h in yield of 71 %. The progress of the reactions was monitored by TLC.

In some cases, for the compounds **2a-o** besides the

desired ortho-acyl phenols, we observed the formation of the very small amount of corresponding esters **3**, as analyzed by TLC. The presence of these compounds in the reaction mixture made the purification of desired ortho-acyl phenols very difficult and in most cases, a further purification by crystallization after flash chromatography was needed.

Compound **4** was obtained from kakuol（**2b**）by methylation with MeI and K_2CO_3 in acetone [18] (Scheme 2). The synthesis of compound **5** was preparing in 90 % yield by refluxing a solution of **2b** in methanol with $NaBH_4$ [19] for 1 h and **6** was performed by reacting kakuol (**2b**) with $NH_2OH{\cdot}HCl$ [15] and AcONa in MeOH.

Evaluation of antifungal activity: The *in vitro* antifungal activities of all synthetic kakuol derivatives against five phytopathogenic fungi: *Glomerella cingulate*, *Botrytis cinerea*, *FusaHum graminearum* Sehw., *Curvularia lunata* and *Fusarium oxysporum f. sp. Vasinfectum*, were evaluated by mycelial rate growth assay, and the results are listed in Table 1.

As reported in the Table 1, the natural product compound **2b** showed a broad spectrum of activity and led to a good reduction of mycelial growth on *Botrytis cinerea* (*ca.* 70.82 % at 100 mg/ml). While it was less efficient on *Curvularia lunata* (28.03 % at 100 mg/ml) and *FusaHum graminearum* (25.0 % at 100 mg/ml). Among ketones derivatives **2a**-**2o**, with aliphatic chains, compounds **2a** -**2g** showed moderate to strong antifungal activities against the test phytopathogenic fungi, while **2e** exerted strong activities (*Curvularia lunata* 86.03 %; *Botrytis cinerea* 75.02 % at 100 mg/ml). Compound **2o** with cyclopropyl as side chain had strong antifungal activities against all the test phytopathogenic fungi. The same result was observed as we introduced Cl in the side chain (**2n**). The results revealed that the antifungal activity increased as the alkyl side chains getting longer cyclopropyl group is detrimental to the activity.

On the other hand, the introduction of unsaturated aliphatic chain, gave compound **2h** and **2i** with increased activity. In fact, they almost completely inhibited the mycelial growth of the tested pathogens at 100 mg/ml. The results listed in the Table 1 showed that the introduction of a Me group at the α-position of the C=O group (**2h**) generally did not affect the activity compared to compound **2i**. This finding is consistent with Loana Musso's research results. Thus, we can assume that α,β- unsaturated ketone, plays an important role.

So we increased the unsaturated carbon chain by introducing benzene ring as side chain hopefully to find more active compounds. However, compound **2l** led to a substantial decrease of antifungal activity against the tested organisms, **2k** and **2m** with electron -donating group at the benzene ring, we saw an increase of antifungal activities. While those with electron-withdrawing group (**2j**), even had no obvious inhibitory action on *Fusarium oxysporum f. sp. Vasinfectum*. Thus, we suggested that aromatic ring is not crucial.

Introducting the methoxy group to the free phenol group, ether **4** presented values of mycelial growth inhibition comparable to those of kakuol (**2b**) with the exception for *Fusarium oxysporum f. sp. vasinfectum* (36 % at 100 mg/ml; Table 1). This result suggested that the presence of the OH group may not crucial for the activity.

Transforming the C=O group of kakuol (**2b**) into an alcohol, **5**, led to an increase of activity against the tested organisms, except for *Fusarium oxysporum f. sp. vasinfectum* (36.07 % at 100 mg/ml; Table 1). Moreover, transforming the C=O into an oxime, **6**, led to a significant increase of antifungal activity against the tested organisms. This results suggested that the C=O group is important, but in some situations, is replaceable.

In order to test and verify Loana Musso's finding, we also using ChemDraw Ultra Vers. 5.0 calculating log *P* (logarithm of partition coefficient in octan-1-ol/H2O) to reveal the relevance of lipophilicity, the lipophilicity values of the synthetic compounds, [20] and [21]. As showed in Table 2, compounds **2h** (log *P* 1.77), and **2i** (log *P* 1.42) showed remarkable higher antifungal activities than the more liphophilic **2c** (log *P* 1.81), **2d** (log *P* 2.66), **2f** (log *P* 2.66), and **2g** (log *P* 2.64), while **2e** (log *P* 2.22) showed a relative high antifungal activities, compounds **2o** (log *P* 1.46), **5** (log *P* 1.65) and **6** (log *P* 1.78) exhibited higher antifungal activities than the natural occurred product **2b** on a wide range of organisms. The introduction of a benzene rings (i.e., **2l**, log *P* 2.63; **2m**, log *P* 3.19) led to a decrease of antifungal activity. This result showed that the most active compounds have a log *P* value in the range of 1.42 – 1.80 (compounds **2h**, **2i**, **2o**, and **5**), the only exception being compound **2e** that maintained a moderate antifungal activity, despite its quite high log *P* value (2.22).

Among the tested compounds, **2h** and **2i** appeared to be two most promising candidates. For this reason, they were tested at lower concentrations in order to evaluate their median inhibitory concentration (IC_{50}) for the most sensitive organisms (*Glomerella cingulate*, *FusaHum graminearum Sehw.*, and *Fusarium oxysporum f. sp. vasinfectum*). The IC_{50} values for compound **2h** and **2i** against *Glomerella cingulate* were 3.1 mg/ml and 2.9 mg/ml; A slightly higher IC_{50} value (**2h**, 6.9 mg/ml; **2i**, 6.2 mg/ml) was found against the *Fusarium oxysporum f. sp. vasinfectum*, whereas *FusaHum graminearum Sehw.* were found less sensitive (IC_{50} values of 15.2 mg/ml and 14.9 mg/ml resp). Taken together, a pharmacophore figure of this class of kakuol derivates using SAR data is given in Figure 2.

Table 1. Growth Inhibition [%] induced by Compounds 1-6 （conc. 100 mg/mL）

Compd.	*Glomerella cingulate*	*Botrytis cinerea*	*FusaHum graminarum Sehw.*	*Curvularia lunata*	*Fusarium oxysporum f. sp. vasinfectum*
1	25.02	28.33	11.52	10.04	8.92
2a	32.57	73.36	33.33	28.06	44.45
2b	35.00	70.82	25.00	28.03	48.95
2c	35.00	73.33	26.02	44.44	48.92
2d	42.51	73.35	25.04	48.05	45.85
2e	67.57	75.02	62.50	86.03	51.12
2f	35.05	60.81	22.00	32.04	26.75
2g	37.50	37.52	15.65	12.03	22.21
2h	100.00	95.04	100.00	96.05	97.82
2i	100.00	98.60	100.00	100.00	100.00
2j	20.03	26.32	8.85	7.26	-
2k	35.05	46.03	25.05	28.15	24.42
2l	22.25	15.50	23.45	18.60	8.82
2m	22.75	59.23	20.82	26.73	28.00
2n	22.25	30.00	27.71	37.25	21.28
2o	68.25	78.94	66.19	76.74	80.00
4	40.93	58.00	22.96	30.05	36.07
5	52.33	73.35	50.15	43.35	36.45
6	54.50	75.45	64.22	53.37	64.55
Thiabendazole	77.35	100.00	100.00	60.00	100.00

Table 2. The lipophilicity values of the compounds 1–6

Compd.	**1**	**2a**	**2b**	**2c**	**2d**	**2e**	**2f**	**2g**	**2h**	**2i**
log P	1.42	0.74	1.39	1.81	1.96	2.22	2.66	2.64	1.77	1.42
Compd.	2j	2k	2l	2m	2n	2o	4	5	6	
log P	1.41	2.51	2.63	3.19	1.26	1.46	1.65	1.65	1.78	

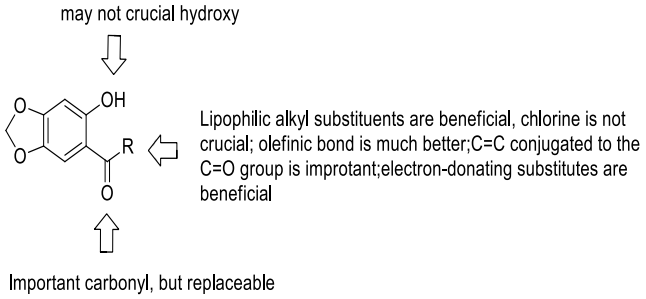


Fig 2. Pharmacophore Figure of Kakuol Derivates

CONCLUSIONS

A series of kakuol derivatives were prepared and their *in vitro* antifungal activities against phytopathogens were evaluated. Among the synthetic derivatives, some compounds such as **2h** and **2i** were found to exert broad-spectrum antifungal activities, which might be promising leads for agricultural fungicides, compounds **2e**, **5** and **6** also exhibited higher antifungal activities than kakuol on a wide range of organisms, which might be considered as leading compounds in the development of new antifungal compounds and there are more works to be in progress.

ACKNOWLEDGMENTS

This manuscript was financially supported by Northwest A & F University, College of Science.

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