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Full Length Research Paper

Design, synthesis and evaluation of the molluscicidal activity of novel hydrocarbyl substituted salicylic acid derivatives

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Based on the molluscicidal activity of ginkgolic acids (GAs) isolated from *Ginkgo biloba* L and synthetic GA analogues, a series of long chain, substituted at 4 and 5 position, salicylic acid derivatives, including the *Z/E* isomers and their saturated derivatives, were prepared. Their molluscicidal activities against the host snail *Oncomelania hupensis* were evaluated. The acute fish toxicity against the wild-type zebrafish of saturated derivatives was tested. The results and analysis of the structural-activity relationship revealed that the *E*-isomers and their saturated derivatives were better molluscicidal agents than their respective *Z*-isomers. Molluscicidal activities were increased with the growing of the alkyl chain lengths. Acute fish toxicity test indicated that the synthesized salicylic acid derivatives were much safer than niclosamide in field use.

Key words: Hydrocarbyl substituted salicylic acid derivatives, synthesis, molluscicidal activity, *Oncomelania hupensis*, schistosomiasis, acute fish toxicity, zebrafish.

INTRODUCTION

Schistosomiasis is one of the most prevalent of the tropical infectious diseases with more than 200 million people infected globally and close to 800 million at risk (Steinmann et al., 2006). In China, zoonotic schistosomiasis, caused by *Schistosoma japonicum*, is a chronic debilitating disease in the south, with 325,824 infected in 2010 (Lei et al., 2011) and 65 million people at risk of infection (Zhou et al., 2005; McManus et al., 2010). The snail, *Oncomelania hupensis*, is the main agent responsible for the transmission of this disease. In current years, China is facing a new challenge because schistosomiasis has reemerged in some regions due to an addition in habitats suitable for the snails, including increased flood areas and large water resource development projects (Yuan et al., 2005).

Snail control can be a powerful intervention tool as it essentially removes the intermediate host from the parasite's life cycle, thus breaking transmission (Fong, 2013). Currently, the niclosamide is the only commercially available molluscicide recommended by the World Health

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Organization (WHO, 1993); however, its use meets with many complications, notably environmental toxicity and high-cost implications (McManus et al., 2009, 2010). Due to this fact, much attention has been given to more environmentally friendly chemicals of natural products to identify novel molluscidal agents (Singh et al., 2010; Pereira et al., 2011; Miyasato et al., 2012; González-Cruz and San Martín, 2013; Rodrigues et al., 2013) and then the synthesis of new chemicals analogues with increased activities is ensuing (Fadda et al., 2009; EI Shehry et al., 2010; Hajare et al., 2011).

Ginkgolic acids (GAs) are natural salicylic acid derivatives of long alkyl or alkenyl substituents at the ortho position to the carboxyl group, which represent a new group of potential molluscicidal agents that have been discovered in recent years (Pan et al., 2007). Other 6-alkylsalicylic acids have also been identified in other plant species such as the anacardic acids (Ana) isolated from the cashew (*Anacardium occidentale*). Kubo and colleagues found these compounds to have molluscicidal activities against the South American snail *Biomphalaria glabratus* (Kubo et al., 1986).

In our previous study, five monomers of GAs were separated and purified from the sarcotesta of *Ginkgo biloba L*. The order for their molluscicidal activities were as follows: $GA_{13:0}$>$GA_{15:1}$>$GA_{15:0}$>$GA_{17:1}$>$GA_{17:2}$ (Mao et al., 2007). We also study the molluscicidal effects of a series of *Z/E* isomers of GA analogues with different chain lengths (5 to 13 carbon atoms) and phenyl rings and find that the *E*-isomers are better molluscicidal agents than their respective *Z*-isomers and molluscicidal activity is found to decrease regularly with the shortening of the alkyl chain lengths (Zhang et al., 2011).

In order to further understand the relationship of the substituted position of the long chain in salicylic acid and their molluscicidal activity, a series of *Z/E* isomers and their saturated products of 4- and 5-alkylsalicylic acid derivatives with different chain lengths (7-13 carbon atoms) have been synthesized from 4- and 5-methylsalicylic acid. Their molluscicidal effects against the host snail *O. hupensis* were evaluated, and the structure-activity relationships were explored.

MATERIALS AND METHODS

Synthesis of the compounds

The unhydrolyzed mixtures of *Z* and *E* isomers 6a-6c and 18a-18c were synthesized from 5-methylsalicylic and 4-methylsalicylic acid according to the reaction sequence delineated in Schemes 1 and 2 (Hamada et al., 1991). These were separated by column chromatography on silica gel. Target compounds 9a-9c, 10a-10c, 12a-12c, 21a-21c, 22a-22c and 24a-24c were obtained by ester hydrolysis and extracted from acidic solution with dichloromethane. To the *Z* and *E* isomers, based on the spin-spin coupling constants of the double bond protons, the isomer with smaller coupling constant (J=8-12 Hz) was assigned to the *Z*-configuration, and the larger coupling constant (J= 12-16 Hz) was assigned to the *E* configuration.

EXPERIMENTAL

General

^{1}H-NMR spectra were recorded in $CDCl_3$ on a Varian Mercury Plus 400 NMR spectrometer or a Bruker Avance III 400 MHz NMR spectrometer. The chemical shifts (δ) were given in ppm and the coupling constants (J) in Hz. MS spectra were measured with a Thermo-Finnigan model LCQ Classic LC/MS/MS ion trap mass spectrometer and Agilent 1100 chromatography system coupled with an Agilent G1946D mass detector. High-resolution electrospray ionization mass spectra (HRESIMS) were measured on a Bruker Daltonics micrOTOF-QII mass spectrometer and Thermo Fisher Q Exactive Mass Spectrometer. Column chromatography (CC) was performed with silica gel 200–300 mesh (Qingdao Haiyang Chemical Co., Ltd). Different aldehydes were purchased from Shanghai Jingchun Reagent Co., Ltd. Other reagents were purchased from commercial suppliers and used as received, unless otherwise noted.

Synthesis

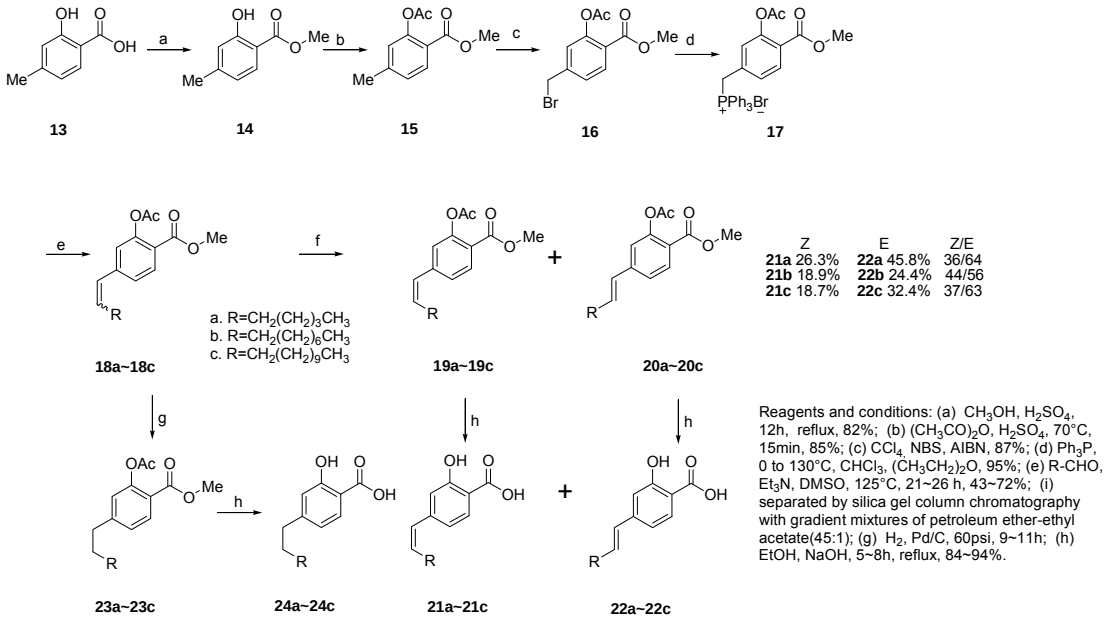
Synthesis of 5- hydrocarbyl substituted salicylic acid derivatives

Methyl 5-methylsalicylate (compound 2): A mixture of 5-Methylsalicylic acid (1, 9.5 g, 62 mmol) and methanol (165 ml) was stirred at room temperature until the solid dissolved completely. Sulfuric acid (32 ml) was then added dropwise in an ice bath. Then the mixture was heated at reflux for 12 h and was left to cool to room temperature. The reaction mixture was then poured into ice-cold water (150 ml), extracted three times with dichloromethane (150 ml), and the organic layer was washed with saturated sodium bicarbonate solution several times until no bubble emerges. The sample was then dried over Na_2SO_4 to give the desired product 2 as yellow oil (10.02 g, 97%). ^{1}H-NMR ($CDCl_3$, 400 MHz), δ 2.29 (s, 3H), 3.95 (s, 3H), 6.88 (d, J=8.5 Hz, 1H), 7.29 (d, J=8.5 Hz, 1H), 7.64 (brs, 1H), 10.57 (s, 1H).

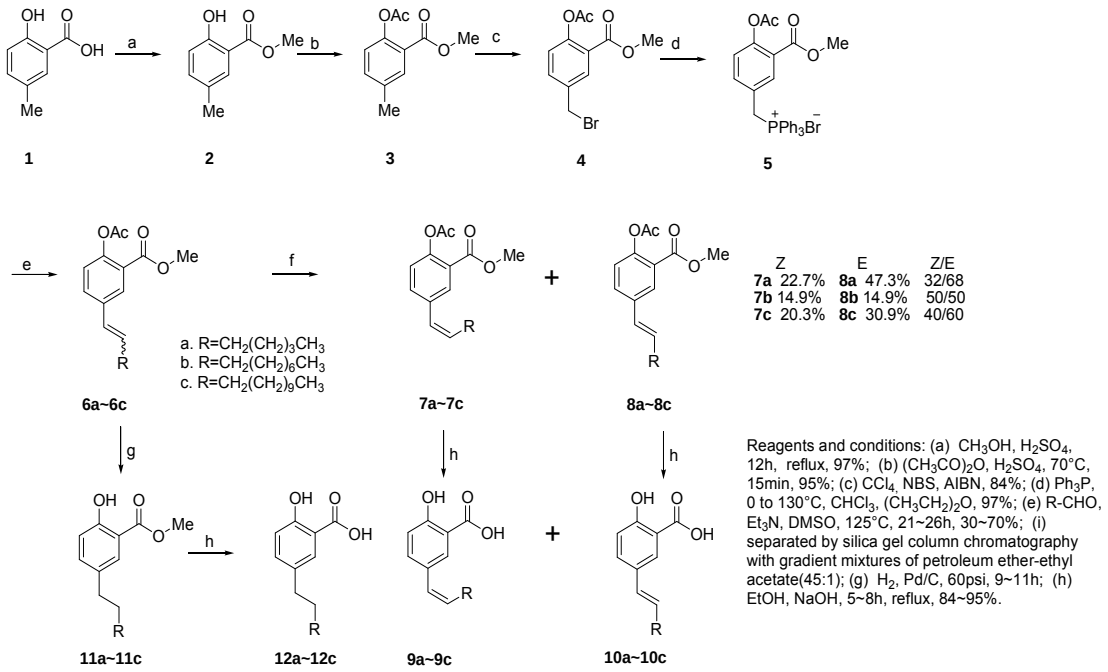
Methyl O-acetyl-5-methylsalicylate (compound 3): A mixture of acetic anhydride (34 ml) and methyl 5-methylsalicylate (10.02 g, 60 mmol), was stirred at room temperature until the solid was dissolved completely. Sulfuric acid (27 drops) was then added dropwise. After 10 min, the mixture was placed into a water bath of 70°C for 15 min and then left to cool to room temperature. The reaction mixture was then poured into ice-cold water (100 ml), extracted three times with dichloromethane (100 ml), and the organic layer was washed with saturated sodium bicarbonate solution several times until no bubble emerges. The sample was then dried over Na_2SO_4 to give the desired product 3 as yellow oil (11.93 g, 95%) (Lazar et al., 2004). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 2.34 (s, 3H), 2.38 (s, 3H), 3.87 (s, 3H), 6.99 (d, J=8.2 Hz, 1H), 7.36 (dd, J=8.2 Hz, 2.3 Hz, 1H), 7.82 (d, J=2.3 Hz, 1H).

Methyl O-acetyl-6-bromomethylsalicylate (compound 4): To a stirred solution of compound 3 (11.45 g, 55 mmol) dissolved in carbon tetrachloride (110 ml) were added a mixture of N-bromosuccinimide (11.23 g, 63 mmol) and azobisisobutyronitrile (78.5 mg, 0.05 mmol). This solution was then heated at reflux for 3 h. After cooling, the reaction mixture was filtered and the filtrate was condensed under reduced pressure to give the crude product. Chromatographic purification of the crude material (silica gel, petroleum ether- ethyl acetate = 30:1) gave the bromide 4 (13.19 g, 84%) as a white powder. This power was directly used for the next reaction without further identification.

4-Acetoxy-3-methoxycarbonylbenzyl triphenylphosphonium bromide (compound 5): A mixture of compound 4 (11.67 g, 41



Scheme 1. Synthesis of 5-hydrocarbyl substituted salicylic acid derivatives from 5-methylsalicylic acid.



Scheme 2. Synthesis of 4-hydrocarbyl substituted salicylic acid derivatives from 4-methylsalicylic acid.

mmol) and triphenylphosphine (10.74 g, 41 mmol) was added to a magnetically stirred round bottomed flask at 0°C, and then slowly heated to 130°C. After all the power melted together, the reaction mixture was cooled to 70°C. A small amount of chloroform was added and refluxed until all the solid had dissolved. Anhydrous ether (35 ml) was then added and the reaction mixture was filtered to give the phosphonium salt as a slightly yellow powder (21.7 g, 97%).

General procedure for preparation of methyl O-acetyl-5-(1-alkenyl) salicylates (compound 6a-6c): The phosphonium salt 5 (548 mg, 1 mmol) was dissolved in DMSO (5 ml). Triethylamine (0.170 ml, 1.2 mmol) and different aldehydes were added. The reaction mixture was heated to 125°C for 21 to 26 h, then allowed

to cool to ambient temperature, poured onto ice-cold water (25 ml), and extracted three times with ether (each 50 ml). The organic layer was washed with water (50 ml) and saturated brine (50 ml), and dried over Na_2SO_4. The solution was then filtered, and concentrated under reduced pressure to give the crude product as a mixture of geometric isomers.

Separation of *Z* and *E* isomers 7a-7c and 8a-8c of methyl O-acetyl-5-(1-alkenyl) salicylates: Chromatographic separation of the crude material (silica gel, petroleum ether and ethyl acetate, about 45:1) gave the Z (7a-7c) and E (8a-8c) isomers as yellow oils with a ratio of 32/68 to 50/50 (Z/E) (Scheme 1).

General procedure for preparation of methyl O-acetyl-5-(1-alkyl) salicylates (compound 11a-11c): The mixture of geometric isomers was dissolved in acetic acid. 10% Molecular quantities of 10% Pd/C were added. The reaction mixture was swung under 60 psi of H_2 for 9 to 11 h at room temperature, then filtered and the filtrate was poured onto water, and extracted three times with ethyl acetate. The organic layer was washed with saturated brine, and dried over Na_2SO_4. The solution was then filtered, and concentrated under reduced pressure to give the crude product. Chromatographic purification of the crude material (silica gel mixture with 1 to 2% weight quantities of $AgNO_3$, petroleum ether-ethyl acetate = 50:1) gave the 11a-11c.

General procedure for preparation of 5-(1-alkenyl) salicylic acids and 5-(1-alkyl) salicylic acids: Triple molecular quantities of 1 mol/L NaOH and the ester 7a-7c, 8a-8c and 11a-11c, in EtOH were heated at reflux for 5 to 8 h, respectively. After removal of the solvent, the residue was acidified to pH 2 with 1 mol/L HCl, diluted with water, and extracted three times with dichloromethane. The organic layer was washed with water and saturated brine, dried over Na_2SO_4, and concentrated to give the products as yellowish or white powders.

(*Z*)-5-(1-Heptenyl) salicylic acid (compound 9a): *(Z)*-5-(1-Heptenyl)salicylic acid (9a, 23.4 mg, 92%) was obtained as a yellowish powder from the hydrolysis of 7a (25.4 mg, 0.09 mmol) by 1 mol/L NaOH (0.27 ml, 0.27 mmol) in EtOH (0.7 ml) for 5 h. ^{1}H-NMR ($CDCl_3$, 400 MHz), δ 0.87 (t, J=6.7 Hz, 3H), 1.25 (m, 4H), 1.44 (m, 2H), 2.30 (m, 2H), 5.65 (m, 1H), 6.32 (d, J=11.5 Hz, 1H), 6.97 (d, J=8.5 Hz, 1H), 7.45 (dd, J=8.5, 2.2 Hz, 1H), 7.83 (d, J=2.2 Hz, 1H), 10.39 (brs, 0.5H); HRESIMS found: 233.1227. Calculated: 233.1178 for $C_{14}H_{17}O_3$ ([M-H]$^-$).

(*E*)-5-(1-Heptenyl) salicylic acid (compound 10a): (*E*)-5-(1-Heptenyl)salicylic acid (10a, 53.2 mg, 84%) was obtained as a yellowish powder from the hydrolysis of 7a (78.5 mg, 0.27 mmol) by 1 mol/L NaOH (0.81 ml, 0.81 mmol) in EtOH (2.2 ml) for 7 h. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 0.89 (t, J=6.5 Hz, 3H), 1.25 (m, 4H), 1.43 (m, 2H), 2.38 (m, 2H), 6.13 (m, 1H), 6.31 (d, J=16.2 Hz, 1H), 6.95 (d, J=8.8 Hz, 1H), 7.55 (dd, J=8.8, 2.2 Hz, 1H), 7.83(d, J=2.2 Hz, 1H), 10.42 (brs, 0.5H); HRESIMS found: 233.1228. Calculated: 233.1183 for $C_{14}H_{17}O_3$([M-H]$^-$).

5-(1-Heptyl) salicylic acid (compound 12a): 5-(1-Heptyl)salicylic acid (12a, 103.2 mg, 89%) was obtained as a yellowish powder from the hydrolysis of 11a (143.5 mg, 0.49 mmol) by 1 mol/L NaOH (1.48 ml, 1.48 mmol) in EtOH (3.8 ml) for 8 h. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 0.89 (t, J=6.5 Hz, 3H), 1.26 (m, 8H), 1.58 (m, 2H), 2.55 (t, J=7.4, 2H), 6.93 (d, J=8.6 Hz, 1H), 7.35 (dd, J=8.6, 2.3 Hz, 1H), 7.70 (d, J=2.3 Hz, 1H), 10.28 (brs, 1H); HRESIMS found: 235.1292. Calculated: 235.1334 for $C_{14}H_{19}O_3$ ([M-H]$^-$).

(*Z*)-5-(1-Decenyl) salicylic acid (compound 9b): (*Z*)-5-(1-Decenyl)salicylic acid (9b, 43.6 mg, 90%) was obtained as a yellowish powder from the hydrolysis of 7b (58.2 mg, 0.18 mmol) by 1 mol/L NaOH (0.6 ml, 0.6 mmol) in EtOH (1.2 ml) for 5 h. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 0.87 (t, J=6.7 Hz, 3H), 1.25 (m, 10H), 1.45 (m, 2H), 2.30 (m, 2H), 5.65 (m, 1H), 6.31 (d, J=11.5 Hz, 1H), 6.97 (d, J=8.5 Hz, 1H), 7.45 (dd, J=8.5, 2.2 Hz, 1H), 7.83 (d, J=2.2 Hz, 1H), 10.40 (brs, 0.5H); HRESIMS found: 258.1099. Calculated: 258.1620 for $C_{17}H_{22}O_2$ ([M-H_2O]$^+$).

(*E*)-5-(1-Decenyl) salicylic acid (compound 10b): *(E)*-5-(1-Decenyl)salicylic acid (10b, 44.8 mg, 93%) was obtained as a yellowish powder from the hydrolysis of 8b (58.0 mg, 0.18 mmol) by 1 mol/L NaOH (0.6 ml, 0.6 mmol) in EtOH (1.2 ml) for 6 h. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 0.87 (t, J=6.5 Hz, 3H), 1.25 (m, 10H), 1.44 (m, 2H), 2.38 (m, 2H), 6.14 (m, 1H), 6.31 (d, J=16.2 Hz, 1H), 6.97 (d, J=8.5 Hz, 1H), 7.55 (dd, J=8.5, 2.2 Hz, 1H), 7.83 (d, J=2.2 Hz, 1H), 10.40 (brs, 0.5H); HRESIMS found: 258.1099. Calculated: 258.1620 for $C_{17}H_{22}O_2$ ([M-H_2O]$^+$).

5-(1-Decyl) salicylic acid (compound 12b): 5-(1-Decyl)salicylic acid (12b, 179 mg, 94%) was obtained as a yellowish powder from the hydrolysis of 11b (229 mg, 0.7 mmol) by 1 mol/L NaOH (2.1 ml, 2.1 mmol) in EtOH (5 ml) for 7 h. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 0.87 (t, J=6.5Hz, 3H), 1.26 (m, 14H), 1.57 (m, 2H), 2.55 (t, J=7.4, 2H), 6.93 (d, J=8.6 Hz, 1H), 7.34 (dd, J=8.6, 2.3 Hz, 1H), 7.68 (d, J=2.3 Hz, 1H), 10.30 (brs, 1H); HRESIMS found: 279.0932. Calculated: 279.1960 for $C_{17}H_{27}O_3$ ([M+H]$^+$).

(*Z*)-5-(1-Tridecenyl) salicylic acid (compound 9c): (*Z*)-5-(1-Tridecenyl)salicylic acid (9c, 28.4 mg, 95%) was obtained as a white powder from the hydrolysis of 7c (35.2 mg, 0.09 mmol) by 1 mol/L NaOH (0.3 ml, 0.3 mmol) in EtOH (1.0 ml) for 5 h. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 0.87 (t, J=6.7 Hz, 3H), 1.25 (m, 16H), 1.43 (m, 2H), 2.30 (m, 2H), 5.65 (m, 1H), 6.31 (d, J=11.2 Hz, 1H), 6.97 (d, J=8.7 Hz, 1H), 7.45 (dd, J=8.7, 2.2 Hz, 1H), 7.83 (d, J=2.2 Hz, 1H), 10.40 (brs, 0.5H); HRESIMS found: 319.2630. Calculated: 319.2273 for $C_{20}H_{31}O_3$ ([M+H]$^+$).

(*E*)-5-(1-Tridecenyl) salicylic acid (compound 10c): (*E*)-5-(1-Tridecenyl)salicylic acid (10c, 45.3 mg, 89%) was obtained as a white powder from the hydrolysis of 8c (59.8 mg, 0.16 mmol) by 1 mol/L NaOH (0.5 ml, 0.5 mmol) in EtOH (1.8 ml) for 6 h. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 0.89 (t, J=6.5 Hz, 3H), 1.25 (m, 16H), 1.44 (m, 2H), 2.38 (m, 2H), 6.14 (m, 1H), 6.31 (d, J=16.2 Hz, 1H), 6.97 (d, J=8.5 Hz, 1H), 7.55 (dd, J=8.5, 2.2 Hz, 1H), 7.83 (d, J=2.2 Hz, 1H), 10.40 (brs, 0.5H); HRESIMS found: 319.2240. Calculated: 319.2273 for $C_{20}H_{31}O_3$([M+H]$^+$).

5-(1-Tridecyl) salicylic acid (compound 12c): 5-(1-Tridecyl)salicylic acid (12c, 161.3 mg, 88%) was obtained as a white powder from the hydrolysis of 11c (215.3 mg, 0.57 mmol) by 1 mol/L NaOH (1.7 ml, 1.7 mmol) in EtOH (4 ml) for 8 h. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 0.87 (t, J=6.5 Hz, 3H), 1.26 (m, 20H), 1.57 (m, 2H), 2.55 (t, J=7.4, 2H), 6.93 (d, J=8.6 Hz, 1H), 7.34 (dd, J=8.6, 2.3 Hz, 1H), 7.68 (d, J=2.3 Hz, 1H), 10.30 (brs, 1H); HRESIMS found: 321.2429. Calculated: 321.2424 for $C_{20}H_{33}O_3$ ([M+H]$^+$).

Synthesis of 4- hydrocarbyl substituted salicylic acid derivatives

The general procedure for the synthesis of 4-(1-alkenyl)salicylic acids and 4-(1-alkyl)salicylic acids was similar as the synthesis of 5-(1-alkenyl)salicylic and 5-(1-alkyl)salicylic acids. So, the main intermediates and the details of the products were listed and the process of the total synthesis are as shown in Scheme 2.

Methyl 4-methylsalicylate (compound 14): Methyl 4-methylsalicylate (14) was obtained as red oil (9.31 g, 82%) from the esterification of 4-Methylsalicylic acid (13, 10.5 g, 68 mmol) by

methanol (178 ml) and sulfuric acid (36 ml) at reflux for 12 h. ^{1}H-NMR ($CDCl_3$, 400 MHz), δ 2.34 (s, 3H), 3.92 (s, 3H), 6.69 (d, J=8.4 Hz, 1H), 6.79 (brs, 1H), 7.70 (d, J=8.4 Hz, 1H), 10.72 (s, 1H).

Methyl O-acetyl-4-methylsalicylate (compound 15): Methyl O-acetyl-4-methylsalicylate (15) was obtained as yellow oil (9.96 g, 85%) from the acetylation of methyl 4-methylsalicylate (14, 9.3 g, 56 mmol) by acetic anhydride (25 ml) and sulfuric acid (25 drops) into a water bath of 70°C for 15 min. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 2.34 (s, 3H), 2.39 (s, 3H), 3.84 (s, 3H), 6.91 (s, 1H), 7.10 (d, J=8.2 Hz, 1H), 7.91 (d, J=8.2 Hz, 1H).

(*Z*)-4-(1-Heptenyl) salicylic acid (compound 21a): (*Z*)-4-(1-Heptenyl)salicylic acid (21a, 59.3 mg, 86%) was obtained as a yellowish powder from the hydrolysis of 19a (85.4 mg, 0.29 mmol) by 1 mol/L NaOH (0.9 ml, 0.9 mmol) in EtOH (2.0 ml) for 5 h. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 0.87 (t, J=6.5 Hz, 3H), 1.28 (m, 6H), 2.23 (m, 2H), 6.36 (m, 2H), 6.91 (dd, J=8.2 Hz, 2.2 Hz, 1H), 6.93 (d, 2.2 Hz, 1H), 7.80 (d, J=8.2 Hz, 1H), 10.49 (brs, 1H); HRESIMS found: 233.1230. Calculated: 233.1178 for $C_{14}H_{17}O_3$ ([M-H]$^-$).

(*E*)-4-(1-Heptenyl) salicylic acid (compound 22a): (*E*)-4-(1-Heptenyl)salicylic acid (22a, 28.3 mg, 91%) was obtained as a yellowish powder from the hydrolysis of 20a (38.5 mg, 0.13 mmol) by 1 mol/L NaOH (0.4 ml, 0.4 mmol) in EtOH (0.9 ml) for 5 h. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 0.88 (t, J=6.5 Hz, 3H), 1.29 (m, 6H), 2.32 (m, 2H), 5.80 (m, 1H), 6.36 (d, J=12.8 Hz, 1H), 6.83 (d, J=8.3 Hz, 1H), 6.91 (brs, 1H), 7.84 (d, J= 8.3Hz, 1H), 10.49 (brs, 1H); HRESIMS found: 233.1229. Calculated: 233.1178 for $C_{14}H_{17}O_3$ ([M-H]$^-$).

4-(1-Heptyl) salicylic acid (compound 24a): 4-(1-Heptyl)salicylic acid (24a, 67.4 mg, 94%) was obtained as a yellowish powder from the hydrolysis of 23a (88.7 mg, 0.30 mmol) by 1 mol/L NaOH (0.91 ml, 0.91 mmol) in EtOH (2.1 ml) for 5 h. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 0.87 (t, J=6.5 Hz, 3H), 1.26 (m, 8H), 1.60 (m, 2H), 2.59 (t, J=7.4, 2H), 6.75 (d, J=8.3 Hz, 1H), 6.81 (brs, 1H), 7.80 (d, J=8.3 Hz, 1H), 10.41 (brs, 1H); HRESIMS found: 235.1298. Calculated: 235.1334 for $C_{14}H_{19}O_3$ ([M-H]$^-$).

***(Z)*-4-(1-Decenyl) salicylic acid (compound 21b):** *(Z)*-4-(1-Decenyl)salicylic acid (21b, 88.3 mg, 84%) was obtained as a yellowish powder from the hydrolysis of 19b (126.5 mg, 0.38 mmol) by 1 mol/L NaOH (1.2 ml, 1.2 mmol) in EtOH (2.6 ml) for 8 h. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 0.87 (t, J=6.5 Hz, 3H), 1.26 (m, 12H), 2.23 (m, 2H), 6.36 (m, 2H), 6.91 (dd, J=8.2 Hz, 1.8 Hz, 1H), 6.93 (d, 1.8 Hz, 1H), 7.80 (d, J=8.2 Hz, 1H), 10.49 (brs, 1H); HRESIMS found: 275.1710. Calculated: 275.1647 for $C_{17}H_{23}O_3$ ([M-H]$^-$).

(*E*)-4-(1-Decenyl) salicylic acid (compound 22b): *(E)*-4-(1-Decenyl)salicylic acid (22b, 83.5 mg, 88%) was obtained as a yellowish powder from the hydrolysis of 20b (114.1 mg, 0.34 mmol) by 1 mol/L NaOH (1.1 ml, 1.1 mmol) in EtOH (2.4 ml) for 5 h. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 0.87 (t, J=6.5 Hz, 3H), 1.25 (m, 12H), 1.99 (m, 2H), 5.78 (m, 1H), 6.36 (d, J=12.9 Hz, 1H), 6.82 (d, J=8.3 Hz, 1H), 6.90 (brs, 1H), 7.83 (d, J=8.3 Hz, 1H), 10.49 (brs, 1H); HRESIMS found: 277.1874. Calculated: 277.1804 for $C_{17}H_{25}O_3$ ([M+H]$^+$).

4-(1-Decyl) salicylic acid (compound 24b): 4-(1-Decyl)salicylic acid (24b, 80.9 mg, 91%) was obtained as a white powder from the hydrolysis of 23b (106.8 mg, 0.32 mmol) by 1 mol/L NaOH (0.96 ml, 0.96 mmol) in EtOH (2.2 ml) for 6 h. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 0.87 (t, J=6.5 Hz, 3H), 1.26 (m, 14H), 1.59 (m, 2H), 2.59 (t, J=7.4, 2H), 6.75 (dd, J=8.1 Hz, 1.2 Hz, 1H), 6.81 (brs, 1H), 7.80 (d, J=8.1 Hz, 1H), 10.41 (brs, 1H); HRESIMS found: 279.0930. Calculated: 279.1955 for $C_{17}H_{27}O_3$ ([M+H]$^+$).

(*Z*)-4-(1-Tridecenyl) salicylic acid (compound 21c): (*Z*)-4-(1-Tridecenyl)salicylic acid (21c, 43.9 mg, 89%) was obtained as a white powder from the hydrolysis of 19c (58.0 mg, 0.16 mmol) by 1 mol/L NaOH (0.5 ml, 0.5 mmol) in EtOH (1.7 ml) for 7 h. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 0.87 (brs, 3H), 1.26 (m, 16H), 1.46 (m, 2H), 2.22 (m, 2H), 6.36 (m, 2H), 6.91 (d, J=8.1 Hz, 1H), 6.93 (brs, 1H), 7.80 (d, J=8.1 Hz, 1H), 10.39 (brs, 1H); HRESIMS found: 319.2629. Calculated: 319.2273 for $C_{20}H_{31}O_3$ ([M+H]$^+$).

(*E*)-4-(1-Tridecenyl) salicylic acid (compound 22c): (*E*)-4-(1-Tridecenyl)salicylic acid (22c, 69.1 mg, 91%) was obtained as a white powder from the hydrolysis of 20c (89.3 mg, 0.24 mmol) by 1 mol/L NaOH (0.72 ml, 0.72 mmol) in EtOH (2.2 ml) for 6 h. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 0.87 (t, J=6.5 Hz, 3H), 1.25 (m, 18H), 1.98 (m, 2H), 5.78 (m, 1H), 6.34 (d, J=12.9 Hz, 1H), 6.82 (d, J=8.2 Hz, 1H), 6.90 (brs, 1H), 7.83 (d, J=8.2 Hz, 1H), 10.49 (brs, 1H); HRESIMS found: 319.2268. Calculated: 319.2273 for $C_{20}H_{31}O_3$([M+H]$^+$).

4-(1-Tridecyl) salicylic acid (compound 24c): 4-(1-Tridecyl)salicylic acid (24c, 165.6 mg, 86%) was obtained as a white powder from the hydrolysis of 23c (226.4 mg, 0.60 mmol) by 1 mol/L NaOH (1.8 ml, 1.8 mmol) in EtOH (3.6 ml) for 8 h. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 0.87 (t, J=6.5 Hz, 3H), 1.26 (m, 20H), 1.59 (m, 2H), 2.59 (t, J=7.4, 2H), 6.75 (d, J=8.1 Hz, 1H), 6.81 (brs, 1H), 7.80 (d, J=8.1 Hz, 1H), 10.40 (brs, 1H); HRESIMS found: 321.2428. Calculated: 321.2424 for $C_{20}H_{33}O_3$ ([M+H]$^+$).

Molluscicidal test

The *O. hupensis* specimens were collected from the endemic area of *Schistosomiasis japonica* in Anhui province, China. The test was carried out with the immersion method in accordance with WHO guide-line for laboratory molluscicidal tests (Lemma et al., 1984). The snails were placed in a dark wet and warm (about 25°C) environment for 2 days without providing food. Healthy adult snails (age: about 6 months; presence of 7 to 8 whorls) were used in all molluscicidal tests. Each compound was dissolved into a small amount of dimethyl sulfoxide (less than 0.1% final concentration) and then diluted with dechlorinated water to prepare for the solutions (50, 25, 12.5, 6.25, and 3.125 mg/L, respectively). Niclosamide (Nic) was used as a positive control (concentrations: 2, 1, 0.5, 0.25, 0.125, 0.0625 mg/L, respectively) and dechlorinated water with same concentration of dimethyl sulfoxide as the negative control. The molluscicidal test was carried out using the immersion method (WHO, 1984). Ten snails in one group were placed into beakers containing test compound at different concentrations for a period of 48 h at 25 ± 2°C. Each beaker was covered with nylon netting to prevent the snails from escaping. Snail mortality was carefully checked and all tests were carried out in duplicate. We got the test data in average of 4 dependent experiments. The LC_{10}, LC_{50} and LC_{90} values were determined using Probit analysis (Finney, 1971).

Acute fish toxicity test

The wild-type zebrafish (AB* strain), *Danio rerio*, was obtained from the Zebrafish Breeding Base of the Key Laboratory of Molecular Medicine of the Ministry of Education, Fudan University. The test was carried out with the Fish Embryo Toxicity (FET) test in accordance with the OECD Guideline for the Testing of Chemicals (Braunbeck et al., 2005; OECD, 2006). Healthy adult fishes (age: 5 months; length: 4 to 6 cm; room temperature: 25°C) were used in all toxicity tests regardless of males or females. Each compound was dissolved into a small amount of dimethyl sulfoxide (less than 0.1% final concentration) and then diluted with dechlorinated water to prepare for the solutions (50, 25, 12.5, and 6.25 mg/L,

Table 1. Molluscicidal activity (mg/L) of the hydrocarbyl substituted salicylic acid derivatives against snail *O. hupensis* under laboratory conditions and 48 h exposure.

Compound	LC_{10} (C.L.)[a]			LC_{50} (C.L.)[a]			LC_{90} (C.L.)[a]		
	a	b	c	a	b	c	a	b	c
9(*Z*)	5.04 (1.29-16.19)	4.05 (0.21-17.50)	4.33 (0.78-10.07)	20.75 (11.44-30.06)	20 (8.62-31.38)	18.21 (7.77-28.65)	39.34 (15.67-63.02)	43.32 (11.71-74.93)	35.76 (9.32-62.19)
10(*E*)	1.43 (0.52-9.26)	1.19 (0.67-5.35)	1.02 (0.40-6.77)	16.44 (7.39-25.49)	11.96 (6.95-16.98)	7.71 (7.51-7.91)	32.88 (9.98-55.77)	25.47 (11.42-39.51)	15.19 (14.66-15.71)
12	1.58 (0.68-14.24)	1.29 (0.56-9.47)	1.27 (0.19-9.84)	12.38 (7.31-17.45)	12.36 (9.89-14.83)	9.50 (7.21-11.80)	24.23 (10.59-37.87)	24.44 (17.79-31.09)	20.97 (14.14-27.80)
21(*Z*)	6.53 (4.41-17.46)	6.04 (3.68-15.76)	2.18 (0.81-11.16)	21.89 (14.46-29.31)	22.52 (11.32-33.72)	26.08 (5.23-46.92)	37.06 (19.21-54.91)	43.22 (8.83-73.72)	33.82 (2.40-119.2)
22(*E*)	1.81 (0.60-11.22)	1.34 (0.06-13.26)	1.26 (0.45-7.51)	17.80 (10.65-24.96)	17.68 (15.28-20.45)	9.22 (8.38-10.04)	34.22 (16.60-51.84)	27.83 (10.23-85.43)	17.91 (15.72-20.11)
24	1.43 (0.33-12.59)	1.26 (0.14-13.46)	1.14 (0.78-9.60)	14.27 (7.80-20.73)	11.72 (5.51-17.92)	9.33 (9.00-9.66)	26.48 (10.52-42.44)	22.27 (5.37-39.18)	21.43 (17.35-26.50)
Niclosamide		0.18 (0.02-0.34)			0.51 (0.41-0.60)			0.83 (0.59-1.07)	

Data were the average from 4 dependent experiments; [a]Confidence limit.

Table 2. Acute fish toxicity (mg/L) of the hydrocarbyl substituted salicylic acid derivatives against zebrafish under laboratory conditions and 48 h exposure.

Compound	24b	24c	12b	12c	Niclosamide
LC_{50} (C.L.)[a]	44.15 (23.97~64.32)	31.1 (28.84~33.37)	44.15 (23.97~64.32)	48.1 (7.40~193.6)	-(all died in 2 h)

[a]Confidence limit.

respectively). Niclosamide (Nic) was used as a positive control (concentrations: 2, 1, and 0.5 mg/L, respectively) and dechlorinated water with same concentration of dimethyl sulfoxide as the negative control. Seven fish in one group were placed into 500 ml beakers containing test compound at different concentrations for a period of 48 h at 25 ± 2°C. The dead fish were removed as soon as they were found dead in the beakers. Air was bubbled continuously in each beaker during the test. LC_{50} values were also calculated using Probit analysis of the mortality data.

RESULTS AND DISCUSSION

In this study, a series of *Z*/*E* isomers and their corresponding saturated derivatives of 4- and 5-hydrocarbyl substituted salicylic acid were prepared. The results of the molluscicidal assays and the representative LC_{10}, LC_{50}, and LC_{90} values with the confidence limit were shown in Table 1. The results and analysis of the structural-activity relationship revealed that the *E*-isomers and their saturated derivatives were better molluscicidal agents than their respective *Z*-isomers. Activity was found to increase regularly with the growing of the alkyl chain lengths in the following sequence: c > b > a. The compounds containing 13 carbon atoms chain, 10c, 12, 22c, and 24 had the strongest activities among all of the analogues tested. Their molluscicidal activities are similar to ginkgolic acid analogues (Zhang et al., 2011). This means that the molluscicidal activity of hydrocarbyl substituted salicylic acid derivatives is concerned with the side chain lengths rather than the substituted position and maybe concerned with the postulate of physical rather than a chemical mechanism of toxicity (De Villiers et al., 1967).

The results of the acute fish toxicity assays and the representative LC_{50} values with the confidence limit were shown in Table 2. In this test, we chose some analogues which had better molluscicidal activities to examine their acute fish toxicity. Results revealed that all of the hydrocarbyl substituted salicylic acid derivatives in the test exhibited low toxicity to zebrafish. According to the LC_{50} values, 24c showed the strongest toxicity among all of the analogues tested. In the test, the negative control had no dead fish in 48 h, while the Niclosamide group had all fish dead in 2 h even in the lowest test concentration 0.5 mg/L.

Conclusions

Synthetic 4- and 5-hydrocarbyl substituted salicylic acid derivatives represent a new class of chemical entities with promising molluscicidal activities. The compounds containing 13 carbon atoms chain that had the strongest activities among all of the analogues tested. In consideration of the price and difficulty to prepare

the 6-hydrocarbyl substituted salicylic acid derivatives, ginkgolic acid analogues, the synthesis of 4- and 5-hydrocarbyl salicylic acid derivatives were more convenient. The acute fish toxicity test indicated that the synthesized salicylic acid derivatives were much safer than niclosamide in field use.

Conflict of Interest

The authors declare no conflict of interest.

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