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Design, synthesis, and anti-tumor activity of (2-*O*-alkyloxime-3-phenyl)-propionyl-1-*O*-acetylbritannilactone esters

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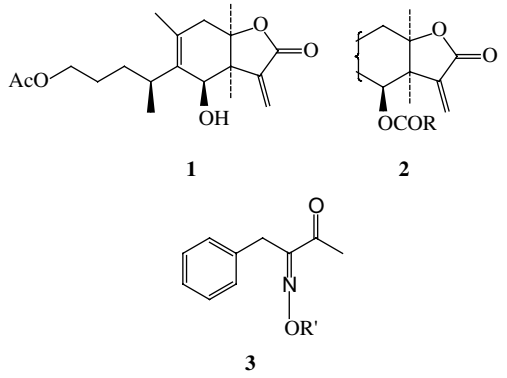
Abstract—The extracts of *Inula britannica* have anti-inflammatory, anti-bacterial, anti-hepatitic, and anti-tumor activities. Various sesquiterpene lactones with cytotoxic properties including 1-*O*-acetylbritannilactone (**1**) have been isolated from this Chinese medicinal plant. Eight derivatives of 1-*O*-acetylbritannilactone, (2-*O*-alkyloxime-3-phenyl)-propionyl-1-*O*-acetylbritannilactone esters were designed and synthesized. Four of these compounds were tested to show inhibitory activity on the growth of human leukemia HL-60 and cancer Bel-7402 cell lines.

1. Introduction

Inula britannica is a wild plant found in Eastern Asia. In traditional Chinese medicine, both *I. britannica* and *Inula japonica* are called 'Xuanfuhua'. The flowers from these plants have been used for the treatment of digestive disorders, bronchitis, and inflammation. The extract of *I. britannica* has been reported to have anti-inflammatory, anti-bacterial, anti-hepatitic, and anti-tumor activities.[1]



1 **2**

3

Figure 1. Structures of compounds (**1**), (**2**), and (**3**).

The phytochemical composition of *I. britannica* has been studied,[1–6] and several sesquiterpene lactones, which show cancer cytotoxic properties have been isolated.[2–6] 1-*O*-Acetylbritannilactone (**1**) (Fig. 1) is one of the α,β-unsaturated sesquiterpene lactones present. It has been reported that the bioactivity of the structure element was caused by the reaction of α-methylene-γ-lactone with nucleophiles, such as a cysteine sulfhydryl group in protein, by a Micheal addition reaction.[7,8] The differences in bioactivity between individual unsaturated sesquiterpene lactone are not only due to the numbers of alkylating reagent structures, but also due to lipophilicity, molecular geometry, and the chemical environment.[8]

1.1. Design of the target molecule

When the structures of several natural, unsaturated sesquiterpene lactones such as Multiradition,[9] Eupaformasanin,[10] Eupahyssopion,[11] are analyzed and compared, the results show that it is the hydroxyl group, on the same side as the α-methylene group, that is conjugated as an ester and is responsible for the bioactivity of the structure. To date, numerous prodrug strategies that could improve the aqueous solubility and pharmacological functions have been tried. Some of them have reported the synthesis of prodrug esters with amino acid moieties.[12–14] It was demonstrated that 1,6-*O*,*O*-diacetylbritannilactone decreased cell growth in breast, prostate, and colorectal cell lines (IC_{50} 200 nM to 2 μM), induced G2-M cell cycle arrest, and is 10 times more

Keywords: 1-*O*-Acetylbritannilactone; *Inula britannica*; Cytotoxicity; Human HL-60; Bel-7402 cell; Oximino derivatives.
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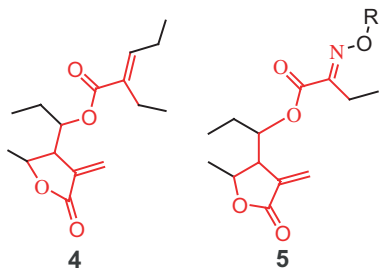


Figure 2. Isosteric structure of designed molecular and natural products.

active than 1-*O*-acetylbritannilactone.[6] Some aryl sulfonyl proline derivatives of 1-*O*-acetylbritannilactone, such as phenylsulfonyl proline, display potent cytotoxicity in human HL-60 cells (IC_{50} = 4.6 μg/mL).[15] It may be possible to determine if the acylation of the hydroxy group can improve bioactivity. The structure (**2**) may be summed up as shown in Figure 1.

The study shows further that acylation of the hydroxy group often contains a carbon–carbon double bond, mostly as an α,β-unsaturated carboxylic ester.[16–20] Based on bioisosteric theory we designed an α-alkyloxime-3-phenylpropionyl (**3**) as an acyl group (Fig. 1). The similarity in the structure between the fragment of the designed molecule (**5**) with that of the natural products (**4**) is represented in Figure 2. The oximino group is a stable functional group frequently employed in modern drugs and may be transformed into a α-ketone acid, which is a metabolic intermediate compound found in organisms. We therefore introduced an *O*-alkoxyimino group into 1-*O*-acetylbritannilactone. (2-*O*-Butyloxime-3-phenyl)-propionyl-1-*O*-acetylbritannilactone ester and its crystal structure were reported recently. The compound has cancer cytotoxicity with a IC_{50} value of 4.3 μg/mL toward HL-60.[21]

In order to improve bioactivity, we designed a series chain, (2-*O*-alkyloxime-1-phenyl)-propionic acid, and introduced it into the structure of 1-*O*-acetylbritannilactone (**1**) to transform the 6-hydroxy group into its esters (**12**) and to try to influence its activity.

2. Results and discussion

2.1. Chemistry

(2-*O*-Alkyloxime-1-phenyl)-propionic acid (**10**) was synthesized and the acylation of the hydroxyl group of compound (**1**) was accomplished as shown in Scheme 1. The key intermediate, ethyl 2-hydroxyimino-3-phenylpropionate (**8**) was obtained by nitrosation of diethyl benzylmalonate (**6**) with ethyl nitrate in sodium ethoxide at 0 °C, or nitrosation of ethyl benzylacetylacetate **7** with ethyl nitrite in sulfuric acid solution at 0 °C. Compound (**8**) was reacted with bromoalkane in acetone using anhydrous K_2CO_3 as the base at 30–35 °C to form ethyl 2-alkoxyimino-3-phenylpropionate (**9**). Compound (**10**), (2-*O*-alkyloxime-1-phenyl)-propionic acid, was prepared by hydrolysis of (**9**) in NaOH solution in a solvent mixture of water and ethanol. Finally, 1-*O*-acetylbritannilactone (**1**) was reacted with 2-*O*-alkyloxime-3-phenylpropionyl chloride (**11**), which was obtained by the reaction of (**10**) with thionyl chloride and refluxed for 2 h, to give the target compound (2-*O*-alkyloxime-3-phenyl)-propionyl-1-*O*-acetylbritannilactone ester (**12**).

2.2. Structure analysis

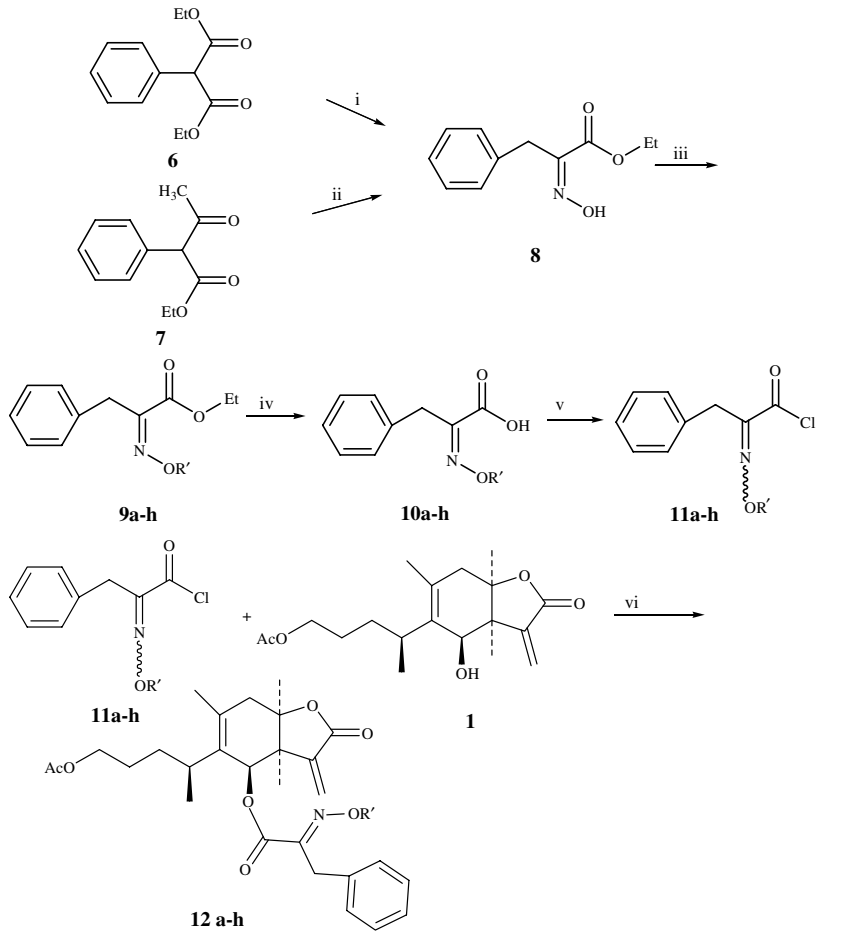
The structures of all target compounds are confirmed by elemental analysis, IR, NMR, and MS. There are typical peaks belonging to the parent in 1730, 1660, and 815 cm^{-1}, and peaks belonging to the side chain at about 1765 cm^{-1} (C=O) and 1630 cm^{-1} (C=N) in IR. Mass spectra of target compounds gave two fragment ion peaks at *m*/*z* 212 and 96 resulting from the retro-Diels–Alder reaction of the parent ion. They also give characteristic ion peaks at *m*/*z* 39, 51, 65, 77, 91, 117, and 145 from the side chain. For all target compounds typical peaks of about 7.10 (m, 5H), 6.30 (d, J = 2.3 Hz, 1H), 5.80 (d, J = 2.2 Hz, 1H), 5.10 (d, J = 1.8 Hz, 1H), 4.70 (m, 1H), 4.16 (t, 2H), 3.85 (s, 2H), 3.30 (m, 1H), 2.65 (m, 1H), 2.45 (dd, J = 2.2 Hz, 1H), 2.25 (ddd, J = 3.4 Hz, 1H), 1.99 (s, 3H), 1.77 (s, 3H), 1.55 (m, 2H), 1.25 (m, 2H), 0.95 (d, J = 3.1 Hz, 3H) ppm occur in ^1H NMR. The structures of compounds (**12e**) and (**12g**) were confirmed further by ^{13}C NMR. The ^1H–^1H COSY NMR of compound (**7g**) shows *cis* coupling H8 and H7 and H7and H6, which are identical with the literature.[6] The results indicate that the absolute configuration of the parent compound (**1**) does not change after forming its derivatives.

2.3. X-ray crystal structure analysis of 1-*O*-acetylbritannilactone 1 and its derivative 12e

Single crystals of 1-*O*-acetylbritannilactone (**1**) suitable for X-ray analysis were obtained from a CH_2Cl_2 solution. The structure of (**1**) is depicted in Figure 3. As anticipated, the structure of (**1**) coincided with the results obtained from other methods (NMR, MS, IR). It is an α,β-unsaturated sesquiterpene lactone containing a six-membered ring, which adopted a slightly twisted boat conformation and was fused by a planar five-membered ring. The dihedral angle between these two rings is 58.9°. The length of the double bond C_7=C_8 in the six-membered ring is 1.329(2) Å, while the bond distances of C_{13}=C_{15} and C_{14}=O_5, which are both connected to the five-membered ring, are 1.319(3) and 1.207(3) Å, respectively. The bond of C(12)–O(3) is at the opposite of the planar five-membered ring and long alkyl chain connected to the C_7 atom and can reduce the steric obstacle.

The crystals of (2-*O*-butyloxime-3-phenyl)-propionyl-1-*O*-acetylbritannilactone ester (**12e**) were obtained from a CH_2Cl_2 solution. The structure is depicted in Figure 4. The stereochemistry of oxime ether group is an *E*-configuration.

Although most of the bond lengths and angles of the bicyclic structure of these two compounds are nearly equal, and steric structure of the lactone fragment is



Scheme 1. Reagents and conditions: (i) EtONO/NaOEt, 0 °C; (ii) EtONO/80%H_2SO_4, 0–5 °C; (iii) bromoalkane, K_2CO_3/acetone, 30–35 °C; (iv) NaOH, H_2O/EtOH, H_3O^+; (v) $SOCl_2$, CH_2Cl_2; (vi) DMAP/CH_2Cl_2.

similar, some differences occur between both structures as can be seen by comparing the X-ray diffraction data of (**1**) with those of (**12e**). The angle of C(17)–C(13)–C(12) in (**12e**) is 2° larger than that of C(15)–C(13)–C(14) in (**1**). And the angle of C(17)–C(13)–C(14) in (**12e**) is about 2° less than that of C(15)–C(13)–C(11) in (**1**). These data suggest that an α-methylene group of compound (**12e**) moved toward the six-membered ring by the introduction of a side chain. The angle of C(13)–C(14)–C(15) in (**12e**) is about 3° less than that of C(13)–C(11)–C(12) in (**1**). And the angle of O(3)–C(15)–C(8) in (**12e**) is about 4° less than that of O(3)–C(12)–C(7) in (**1**). The results reveal that a six-membered ring of compound (**12e**) is closed to the lactone ring by the formation of an ester.

2.4. Anti-cancer activity

After target compounds were obtained, anti-cancer activities of four selected derivatives (**12b**), (**12d**), (**12e**), and (**12g**) were compared with that of the parent compound (**1**) in the two cancer cell lines HL-60 and Bel-7402. The cytotoxicities of these compounds are compared in Table 1. From the results we can see that the cytostatic activity of compound (**12**) is much higher than that of (**1**). Compound (**12g**) has the highest activity, with IC_{50} of 0.22 μg/mL against Bel-7402 and 3.8 μg/mL against HL-60. But the parent compound showed only 15.6 and 18.4 μg/mL for the above two cells, respectively. We suggest that the improvement of bioactivity is a comprehensive effect of many factors, but in this case perhaps the most important reason is the introduction of the modifying group (2-*O*-alkyloxime-1-phenyl)-propionyl into the target compound. Apparently, this side chain has the effect of enhancing the bioactivity of the parent compound. From the data of the crystal structure, although the cyclic difference between compounds (**1**) and (**12e**) is very small, some change of the bond angles in compound (**12e**) show that the bridge rings are more closer to each other. In fact, the introduction of *O*-alkoxyimino group into the hydroxy in compound (**1**) stabilizes the α,β-unsaturated sesquiterpene lactone. These effects aid to raise the bioavailability of compound (**12**). This is perhaps the second reason why compounds (**12**) have higher anti-cancer activity than the parent compound (**1**).

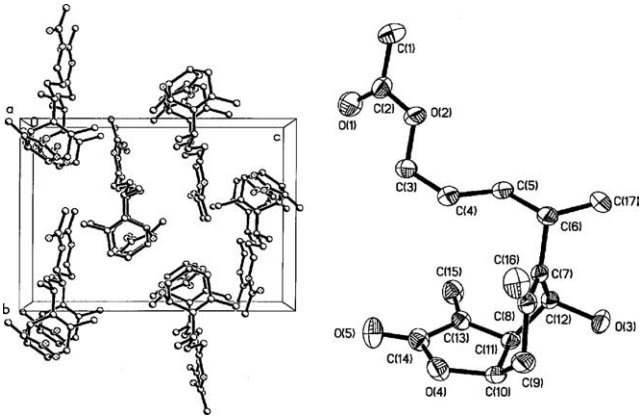


Figure 3. Structure of 1-*O*-acetylbritannilactone **1**. Selected bond distances (Å) and bond angles (°): O(1)–C(2) 195(3), O(2)–C(2) 1.331(2), O(2)–C(3) 1.444(2), O(3)–C(12) 1.441(2), O(4)–C(14) 1.334 (2), O(4)–C(10) 1.465(2), O(5)–C(14) 1.207(3), C(1)–C(2) 1.485(3); C(3)–C(4) 1.498(3); C(4)–C(5) 1.522(3), C(5)–C(6) 1.523(3), C(6)–C(7) 1.521(2), C(6)–C(17) 1.528(3), C(7)–C(8) 1.329(2), C(7)–C(12) 1.515(2), C(8)–C(16) 1.503(3), C(8)–C(9) 1.508(3), C(9)–C(10) 1.510(3), C(10)–C(11) 1.533(3), C(11)–C(13) 1.501(3), C(11)–C(12) 1.528(3), C(13)–C(15) 1.319(3), C(13)–C(14) 1.471(3). C(2)–O(2)–C(3) 116.03(16), C(14)–O(4)–C(10) 112.15(15), O(1)–C(2)–O(2) 122.5(2), O(1)–C(2)–C(1) 125.7(2), O(2)–C(2)–C(1) 111.8(2), O(2)–C(3)–C(4) 107.69(17), C(3)–C(4)–C(5) 114.48(18), C(4)–C(5)–C(6) 113.45(16), C(7)–C(6)–C(5) 112.60 (15), C(7)–C(6)–C(17) 111.90(16), C(5)–C(6)–C(17) 111.00(17), C(8)–C(7)–C(12) 117.59(15), C(8)–C(7)–C(6) 125.74(16), C(12)–C(7)–C(6) 116.56(15), C(7)–C(8)–C(16) 126.03(18), C(7)–C(8)–C(9) 119.13(17), C(16)–C(8)–C(9) 114.82(18), C(8)–C(9)–C(10) 113.31(16), O(4)–C(10)–C(9) 107.82(16), O(4)–C(10)–C(11) 106.31 (15), C(9)–C(10)–C(11) 114.59(16), C(13)–C(11)–C(12) 113.52(15), C(13)–C(11)–C(10) 103.28(16), C(12)–C(11)–C(10) 113.25(15), O(3)–C(12)–C(7) 111.45(14), O(3)–C(12)–C(11) 108.03(14), C(7)–C(12)–C(11) 113.21(14), C(15)–C(13)–C(14) 122.1(2), C(15)–C(13)–C(11) 129.4(2), C(14)–C(13)–C(11) 108.51(16), O(5)–C(14)–O(4) 121.6(2), O(5)–C(14)–C(13) 128.9(2), O(4)–C(14)–C(13) 109.52(17).

3. Experimental

The solvents and reagents were analytical grade and purified by standard procedures prior to use.[22] 1-*O*-Acetylbritannilactone (**6**) was isolated from the flowers of *I. britannica var. Chinensis*.[6] FT-IR spectra were recorded on a 170SX (Nicolet) spectrometer at room temperature. Elemental analysis was carried out on a Perkin–Elmer 240C analyzer. The mass spectrum (FAB MS) analysis was carried out on a HP 5989A using a NBA matrix. NMR spectra were measured in $CDCl_3$ using a multinuclear FT-NMR spectrometer ARX300 (Bruker). The X-ray diffractions were conducted on a Bruker Smart 1000 diffractometer. The preliminary screenings of bioactivity were performed at the Beijing Institute of Materia Medica, Chinese Academy of Sciences.

3.1. Ethyl α-hydroxyiminophenylpropionate (8)

Ethyl α-benzylacetylacetate (0.1 mol) and ethyl nitrite (0.105 mol) were added to the 0.1 mol solution of sodium ethoxide in ethanol by stirring at 0–5 °C in an ice-water bath. The reaction continued for 2 h. Then the mixture was put into a refrigerator overnight and condensed to remove ethanol. Ice-water (150 mL) was added to the residue and the pH of the solution was adjusted to 5 with 0.5 mol HCl. The solution was extracted with ether (3 × 50 mL) and the organic phase was dried over anhydrous $MgSO_4$. The ether was removed under reduced pressure (70 mmHg) to give 15.5 g of yellow solid product (yield 75%), mp 56 °C.

3.2. General procedure for ethyl 2-alkyloxyimino-3-phenylpropionate (9a–h)

Methyl iodine or bromoketone (0.08 mol) were added, dropwise with stirring, to a suspended solution of ethyl α-hydroxyiminophenylpropionate (0.05 mol) and anhydrous K_2CO_3 powder (0.55 mol) in acetone (50 mL). The reaction was carried out at 30 °C for 2 h, solids were removed by filtration and the solids were washed with acetone. The filtrate was concentrated under reduced pressure to obtain the title products. Table 2 lists the melting points and yields of compounds (**9a**–**h**).

3.3. General procedure for (2-*O*-alkyloxime-3-phenyl)-propionic acid (10a–h)

The mixture of ethyl α-methoxyiminophenylpropionate (0.05 mol) and 2 N NaOH (60 mL) (ethanol 15 mL, water 45 mL) was stirred at 90 °C for 3 h. The reaction solution was poured into 150 mL water with stirring and acidified using dilute HCl to pH 1.5. The aqueous solution was extracted with ether, and the combined organic extracts were dried over anhydrous $MgSO_4$ and concentrated under reduced pressure (0.5 Pa). The product was obtained by the recrystallization of the residue from ethanol–water, it has mp 42 °C, and the yield is 84%. Table 3 lists melting points and yields of all compounds (**10a**–**h**).

3.4. General procedure for (2-*O*-alkyloxime-3-phenyl)-propionyl chloride 11a–h

To the above compound (0.0164 mol in anhydrous benzene), 15 mL 0.082 mol sulfonyl chloride was added with stirring. The mixture was refluxed for 2 h and concentrated under reduced pressure (0.85 Pa) to remove solvent and excess sulfonyl chloride. Another 15 mL anhydrous benzene was added to the residue and the above processes were repeated until all sulfonyl chloride was removed. The yield of the product was 75–88%.

3.5. General procedure for (2-*O*-alkyloxime-3-phenyl)-propionyl-1-*O*-acetylbritannilactone ester 12a–h

DMAP (0.03 mmol) was added to an anhydrous CH_2Cl_2 solution of 1-*O*-acetylbritannilactone (50 mg, 0.16 mmol) and 3 mL anhydrous Et_3N, and the mixture was stirred at 0 to −5 °C. Then (2-*O*-butyloxime-3-phenyl)-propionic chloride (7.2 mg, 0.3 mmol), in anhydrous CH_2Cl_2 (10 mL), was added, dropwise for 25 min. The reaction mixture was stirred, at room temperature, for 12 h. The reaction solution was washed with water (15 mL), 5% Na_2CO_3 (20 mL) and saturated NaCl (20 mL). The solution was dried over anhydrous $MgSO_4$ overnight and filtered, and the filtrate was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (column ∅ 2.5 × 20 cm)

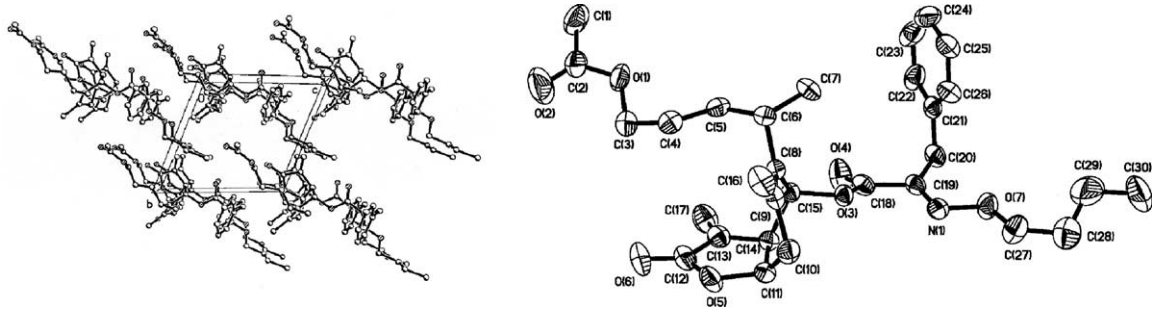


Figure 4. Structure of 2-butylimino-3-phenylpropionic acid ester **12e**. Selected bond distances (Å) and bond angles (°): C(1)–C(2) 1.492(11), C(2)–O(2) 1.182(9), C(2)–O(1) 1.313(7), C(3)–O(1) 1.458(7), C(3)–C(4) 1.510(8), C(4)–C(5) 1.525(8), C(5)–C(6) 1.544(7), C(6)–C(8) 1.541(5), C(6)–C(7) 1.560(7), C(8)–C(9) 1.334(6), C(8)–C(15) 1.529(5), C(9)–C(16) 1.495(6), C(9)–C(10) 1.526(6), C(10)–C(11) 1.520(8), C(11)–O(5) 1.483(6), C(11)–C(14) 1.545(7), C(12)–O(6) 1.219(6), C(12)–O(5) 1.350(7), C(12)–C(13) 1.467(7), C(13)–C(17) 1.324(8), C(13)–C(14) 1.498(7), C(14)–C(15) 1.547(6), C(15)–O(3) 1.473(5), C(18)–O(4) 1.203(6), C(18)–O(3) 1.360(5), C(18)–C(19) 1.503(7), C(19)–N(1) 1.279(6), C(19)–C(20) 1.509(6), C(20)–C(21) 1.525(7), C(21)–C(22) 1.383(7), C(21)–C(26) 1.398(8), C(22)–C(23) 1.393(13), C(23)–C(24) 1.351(13), C(24)–C(25) 1.389(13), C(25)–C(26) 1.382(8), C(27)–O(7) 1.438(7), C(27)–C(28) 1.520(11), C(28)–C(29) 1.354(16), C(29)–C(30) 1.593(18), N(1)–O(7) 1.411(5). O(2)–C(2)–O(1) 122.0(7), O(2)–C(2)–C(1) 125.3(6), O(1)–C(2)–C(1) 112.6(6), O(1)–C(3)–C(4) 108.3(5), C(3)–C(4)–C(5) 114.4(5), C(4)–C(5)–C(6) 112.1(4), C(8)–C(6)–C(5) 111.3(3), C(8)–C(6)–C(7) 111.0(4), C(5)–C(6)–C(7) 110.7(4), C(9)–C(8)–C(15) 117.9(3), C(9)–C(8)–C(6) 126.0(4), C(15)–C(8)–C(6) 116.0(3), C(8)–C(9)–C(16) 126.7(4), C(8)–C(9)–C(10) 119.7(4), C(16)–C(9)–C(10) 113.6(4), C(11)–C(10)–C(9) 112.1(3), O(5)–C(11)–C(10) 107.3(4), O(5)–C(11)–C(14) 105.5(4), C(10)–C(11)–C(14) 114.6(4), O(6)–C(12)–O(5) 121.5(5), O(6)–C(12)–C(13) 128.6(5), O(5)–C(12)–C(13) 109.9(5), C(17)–C(13)–C(12) 124.3(5), C(17)–C(13)–C(14) 127.2(5), C(12)–C(13)–C(14) 108.5(4), C(13)–C(14)–C(11) 103.9(4), C(13)–C(14)–C(15) 110.8(3), C(11)–C(14)–C(15) 113.8(4), O(3)–C(15)–C(8) 107.5(3), O(3)–C(15)–C(14) 109.6(3), C(8)–C(15)–C(14) 113.4(3), O(4)–C(18)–O(3) 123.8(4), O(4)–C(18)–C(19) 122.2(4), O(3)–C(18)–C(19) 114.0(4), N(1)–C(19)–C(18) 117.1(4), N(1)–C(19)–C(20) 127.9(5), C(18)–C(19)–C(20) 114.9(4), C(19)–C(20)–C(21) 110.1(4), C(22)–C(21)–C(26) 117.9(5), C(22)–C(21)–C(20) 121.7(5), C(26)–C(21)–C(20) 120.4(4), C(21)–C(22)–C(23) 121.1(7), C(24)–C(23)–C(22) 120.5(6), C(23)–C(24)–C(25) 119.6(7), C(26)–C(25)–C(24) 120.4(8), C(25)–C(26)–C(21) 120.4(6), O(7)–C(27)–C(28) 107.0(6), C(29)–C(28)–C(27) 111.4(10), C(28)–C(29)–C(30) 112.7(9), C(19)–N(1)–O(7) 10.8(4), C(2)–O(1)–C(3) 118.6(5), C(18)–O(3)–C(15) 115.6(3), C(12)–O(5)–C(11) 111.6(4), N(1)–O(7)–C(27) 110.3(4).

Table 1. The activity of some compounds against different cell bioassays (ED_{50} μg/mL)

Cell model	Compound	Concentration (μg/mL)	% Inhibition	ED_{50}
HL-60[a]	**12b**	5.00	13.2	
	12d	5.00	26.9	
	12e	5.00		4.3
	12g	0.10		3.8
	1	5.00		18.4
Bel-7402[b]	**12b**	5.00	20.3	
	12d	5.00	19.5	
	12e	5.00		7.2
	12g	0.01		0.2
	12h	5.00	16.0	
	1	5.00		15.6

[a] Promyelocytic leukemia.
[b] Hepatocellular carcinoma.

using benzene and ethyl acetate (3:1) as eluent to yield (**7**) as a bright yellow amorphous powder.

3.6. (2-*O*-Methyloxime-3-phenyl) propionyl-1-*O*-acetyl-britannilactone ester (12a)

75% Yield from **1**, mp 49–51 °C; $[\alpha]_D^{20}$ −177.5 (*c* 0.002, CH_2Cl_2); IR (KBr pellet) ν_{max}/cm^{-1}: 3030 (Ph), 1734 (C=O), 1663 (C=C), 1764 (C=O), 1636 (C=N); ^1H NMR (300 MHz, $CDCl_3$): δ 7.18–7.02 (m, 5H), 6.30 (d, *J* = 2.3 Hz, 1H), 5.88 (d, *J* = 2.2 Hz, 1H), 5.08 (d, *J* = 1.8 Hz, 1H), 4.71 (m, 1H), 4.16 (t, 2H), 3.90 (s, 3H), 3.85 (s, 2H), 3.33 (m, 1H), 2.64 (m, 1H), 2.46 (dd, *J* = 2.2 Hz, 1H), 2.26 (ddd, *J* = 3.4 Hz, 1H), 1.99 (s, 3H), 1.77 (s, 3H), 1.55 (m, 2H), 1.25 (m, 2H), 0.95 (d, *J* = 3.1 Hz, 3H); *m/z* 272, 215, 212, 202, 189, 143, 117, 91, 77, 65, 51, 39, 31; Anal. Calcd for $C_{27}H_{33}NO_7$: C, 67.06; H, 6.88; N, 2.90. Found: C, 67.07; H, 6.89; N, 2.87.

Table 2. Melting point and yield of compound **9a**–**h**

Compounds	Mp (°C)	Yield (%)
Ethyl α-methoxyiminophenylpropionate	25–28	75
Ethyl α-ethoxyiminophenylpropionate	37–40	85
Ethyl α-propoxyiminophenylpropionate	48–50	74
Ethyl α-*i*-propoxyiminophenylpropionate	46–49	80
Ethyl α-butoxyiminophenylpropionate	53–56	78
Ethyl α-dodecanoxyiminophenylpropionate	62–65	72
Ethyl α-benzyloxyiminophenylpropionate	66–68	85
Ethyl α-cyclohexyloxyiminophenylpropionate	61–64	75

3.7. (2-*O*-Ethyloxime-3-phenyl)-propionyl-1-*O*-acetyl-britannilactone ester (12b)

78% Yield from **1**, mp 50–52 °C; $[\alpha]_D^{20}$ −152.3 (*c* 0.002, CH_2Cl_2); IR (KBr pellet) ν_{max}/cm^{-1}: 3030 (Ph), 1734 (C=O), 1662 (C=C), 1766 (C=O), 1634 (C=N). ^1H NMR (300 MHz, $CDCl_3$): δ 7.14–7.02 (m, 5H), 6.26 (d, *J* = 2.2 Hz, 1H), 5.84 (d, *J* = 2.1 Hz, 1H), 5.04 (d, *J* = 1.8 Hz, 1H), 4.75 (m, 1H), 4.23 (t, 2H), 3.85 (q, 2H), 3.82 (s, 2H), 3.37 (m, 1H), 2.58 (m, 1H), 2.46 (dd, *J* = 2.2 Hz, 1H), 2.26 (ddd, *J* = 3.2 Hz, 1H), 1.93 (s, 3H), 1.71 (s, 3H), 1.53 (m, 2H), 1.27 (m, 2H), 0.98 (t, *J* = 3.1 Hz, 3H), 0.87 (t, 3H); *m/z* 272, 215, 212, 202, 189, 143, 117, 91, 77, 65, 51, 39, 29. Anal. Calcd for $C_{28}H_{35}NO_7$: C, 67.59; H, 7.09; N, 2.81. Found: C, 67.61; H, 7.04; N, 2.80.

Table 3. Melting points and yields of compound **10a–h**

Compounds	Mp (°C)	Yield (%)
α-Methoxyiminophenylpropionic acid	35	84
α-Ethoxyiminophenylpropionic acid	46–48	85
α-Propoxyiminophenylpropionic acid	55–57	85
α-*i*-Propoxyiminophenylpropionic acid	54–56	75
α-Butoxyiminophenylpropionic acid	64	80
α-Dodecanoxyiminophenylpropionic acid	86	79
α-Benzyloxyiminophenylpropionic acid	80	85
α-Cyclohexyloxyiminophenylpropionic acid	75–77	70

3.8. (2-*O*-Propyloxime-3-phenyl)-propionyl-1-*O*-acetylbritannilactone ester (12c)

80% Yield from **1**, mp 51–53 °C; $[\alpha]_D^{20}$ −100.4 (*c* 0.002, CH_2Cl_2); IR (KBr pellet) ν_{max}/cm^{-1}: 3030 (Ph), 1735 (C=O), 1662 (C=C), 1766 (C=O), 1637 (C=N); ^1H NMR (300 MHz, $CDCl_3$): δ 7.21–7.05 (m, 5H), 6.36 (d, J = 2.1 Hz, 1H), 5.90 (d, J = 2.1 Hz, 1H), 5.09 (d, J = 1.8 Hz, 1H), 4.71 (m, 1H), 4.25 (t, 2H), 3.93 (t, 2H), 3.83 (s, 2H), 3.40 (m, 1H), 2.50 (m, 1H), 2.45 (dd, J = 2.2 Hz, 1H), 2.22 (ddd, J = 3.3 Hz, 1H), 2.02 (s, 3H), 1.78 (s, 3H), 1.57 (m, 2H), 1.48 (m, 2H), 1.23 (m, 2H), 0.94 (d, J = 3.0 Hz, 3H), 0.77 (t, 3H); *m*/*z* 272, 215, 212, 202, 189, 143, 117, 96, 91, 77, 65, 51, 42, 39; Anal. Calcd for $C_{29}H_{37}NO_7$: C, 68.08; H, 7.29; N, 2.74. Found: C, 68.11; H, 7.30; N, 2.73.

3.9. (2-*O*-*i*-Propyloxime-3-phenyl)-propionyl-1-*O*-acetylbritannilactone ester (12d)

75% Yield from **1**, mp 50–53 °C; $[\alpha]_D^{20}$ −101.4 (*c* 0.002, CH_2Cl_2); IR (KBr pellet) ν_{max}/cm^{-1}: 3032 (Ph), 1735 (C=O), 1663 (C=C), 1766 (C=O), 1636 (C=N); ^1H NMR (300 MHz, $CDCl_3$): δ 7.20–7.03 (m, 5H), 6.33 (d, J = 2.3 Hz, 1H), 5.88 (d, J = 2.2 Hz, 1H), 5.06 (d, J = 1.8 Hz, 1H), 4.75 (m, 1H), 4.16 (t, 2H), 3.84 (q, 1H), 3.79 (s, 2H), 3.40 (m, 1H), 2.60 (m, 1H), 2.43 (dd, J = 2.2 Hz, 1H), 2.12 (ddd, J = 3.3 Hz, 1H), 2.00 (s, 3H), 1.76 (s, 3H), 1.53 (m, 2H), 1.27 (m, 2H), 1.00 (d, J = 3.0 Hz, 3H), 0.92 (d, 6H); *m*/*z* 272, 215, 212, 202, 189, 143, 117, 96, 91, 77, 65, 51, 42, 39; Anal. Calcd for $C_{29}H_{37}NO_7$: C, 68.08; H, 7.29; N, 2.74. Found: C, 68.07; H, 7.25; N, 2.75.

3.10. (2-*O*-*n*-Butyloxime-3-phenyl)-propionyl-1-*O*-acetylbritannilactone ester (12e)

80% Yield from **1**, mp 53–55 °C; $[\alpha]_D^{20}$ −59.5 (*c* 0.002, CH_2Cl_2); IR (KBr pellet): ν_{max}/cm^{-1} 3029 (Ph), 1767 (CO), 1734 (CO), 1663 (C=C), 1632 (C=N); ^1H NMR (300 MHz, $CDCl_3$) δ 7.25 (m, 5H), 6.35 (d, J = 2.7 Hz, 1H), 5.91 (d, J = 2.1 Hz, 1H), 5.29 (d, J = 1.8 Hz, 2H), 4.75 (m, 1H), 4.30 (t, 2H), 3.95 (m, 2H), 3.90 (m, 2H), 3.81 (m, 2H), 3.38 (m, 1H), 2.60 (m, 1H), 2.42 (m, 2H), 2.02 (m, 3H), 1.77 (s, 3H), 1.66 (m, 2H), 1.36 (m, 4H), 0.95 (m, 2H), 0.73 (d, J = 3.1 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.35, 17.84, 18.85, 19.95, 20.01, 26.02, 30.63 (2C), 32.52, 34.04 (2C), 42.11, 63.62, 69.63, 74.15, 75.21, 75.24, 78.42, 124.29, 126.08, 128.14 (2C), 131.01, 133.94 (2C), 135.78, 149.38, 162.60, 168.77, 170.40; *m*/*z* 272, 215, 212, 202, 189, 143, 117, 96, 91, 77, 65, 58, 56, 51, 42, 39; Anal. Calcd for $C_{30}H_{39}NO_7$: C, 68.55; H, 7.48; N, 2.66. Found: C, 68.59; H, 7.51; N, 2.61.

3.11. (2-*O*-*n*-Dodecanyloxime-3-phenyl)-propionyl-1-*O*-acetylbritannilactone ester (12f)

65% Yield from **1**, mp 108–111 °C; $[\alpha]_D^{20}$ −67.4 (*c* 0.002, CH_2Cl_2); IR (KBr pellet) ν_{max}/cm^{-1}: 3033 (Ph), 1761 (CO), 1725 (CO), 1663 (C=C), 1632 (C=N); ^1H NMR (300 MHz, $CDCl_3$) δ 7.26–7.06 (m, 5H), 6.32 (d, J = 2.4 Hz, 1H), 5.90 (d, J = 2.3 Hz, 1H), 5.08 (d, J = 1.8 Hz, 1H), 4.72 (m, 1H), 4.36 (t, 2H), 3.92 (t, 2H), 3.72 (s, 2H), 3.44 (m, 1H), 2.55 (m, 1H), 2.46 (dd, J = 2.2 Hz, 1H), 2.16 (ddd, J = 3.3 Hz, 1H), 2.04 (s, 3H), 1.80 (s, 3H), 1.58 (m, 2H), 1.35 (m, 2H), 1.04–1.23 (m, 20H), 0.87 (d, J = 3.0 Hz, 3H), 0.80 (d, J = 3.1 Hz, 3H). Anal. Calcd for $C_{38}H_{55}NO_7$: C, 71.55; H, 8.69; N, 2.20. Found: C, 71.52; H, 8.67; N, 2.12.

3.12. (2-*O*-Benzyloximene-3-phenyl)-propionyl-1-*O*-acetylbritannilactone ester (12g)

76% Yield from **1**, mp 72–74 °C. $[\alpha]_D^{20}$ −83.9 (*c* 0.002, CH_2Cl_2). IR (KBr pellet) ν_{max}/cm^{-1}: 3030 (Ph), 1767 (CO), 1738 (CO), 1663 (C=C), 1638 (C=N). ^1H NMR (300 MHz, $CDCl_3$) δ 7.26 (m, 5H), 7.21–7.06 (m, 5H), 6.35 (d, J = 2.4 Hz, 1H), 5.91 (d, J = 2.3 Hz, 1H), 5.12 (s, 2H), 4.84 (d, J = 1.8 Hz, 1H), 4.73 (m, 1H), 4.36 (t, 2H), 3.78 (s, 2H), 3.36 (m, 1H), 2.62 (m, 1H), 2.51 (dd, J = 2.2 Hz, 1H), 2.14 (ddd, J = 3.3 Hz, 1H), 2.03 (s, 3H), 1.79 (s, 3H), 1.58 (m, 2H), 1.23 (m, 2H), 0.71 (d, J = 3.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 17.79, 19.96, 25.97, 30.57, 30.98, 32.42, 33.88, 42.11, 63.51, 69.63, 74.13, 75.30, 75.34, 78.42, 124.24, 126.08, 126.77 (2C), 127.34, 128.14 (2C), 130.14 (2C), 133.96 (2C), 135.70, 137.87, 140.94, 142.2, 150.14, 162.33, 168.66, 170.29. Anal. Calcd for $C_{33}H_{37}NO_7$: C, 70.82; H, 6.66; N, 2.50. Found: C, 70.84; H, 6.65; N, 2.52.

3.13. (2-*O*-Cyclohexyloximene-3-phenyl)-propionyl-1-*O*-acetylbritannilactone ester (12h)

77% Yield from **1**, mp 66–70 °C; $[\alpha]_D^{20}$ −80.5 (*c* 0.002, CH_2Cl_2); IR (KBr pellet) ν_{max}/cm^{-1}: 2936 (Ph), 1766 (CO), 1735 (CO), 1663 (C=C), 1638 (C=N); ^1H NMR (300 MHz, $CDCl_3$) δ 7.21–7.06 (m, 5H), 6.36 (d, J = 2.2 Hz, 1H), 5.91 (d, J = 2.1 Hz, 1H), 5.10 (d, J = 1.9 Hz, 1H), 4.70 (m, 1H), 4.32 (t, 2H), 3.72 (s, 2H), 3.40 (m, 1H), 3.31 (m, 1H), 2.45 (m, 1H), 2.51 (dd, J = 2.3 Hz, 1H), 2.14 (ddd, J = 3.1, Hz, 1H), 2.03 (s, 3H), 1.78 (s, 3H), 1.72 (m, 2H), 1.62 (m, 2H), 1.50 (m, 2H), 1.48–1.37 (m, 6H), 0.77 (d, J = 3.0 Hz, 3H); Anal. Calcd for $C_{32}H_{41}NO_7$: C, 69.67; H, 7.49; N, 2.54. Found: C, 69.69; H, 7.44; N, 2.51.

3.14. X-ray crystal structure analysis of compound (1) and (12e)

Single crystals of 1-*O*-acetylbritannilactone (**1**) and (2-*O*-butyloxime-3-phenyl)-propionyl-1-*O*-acetylbritanni-

lactone ester (**12e**), were obtained by slow evaporation from CH_2Cl_2 solution in the form of a prism needle and triclinic needle crystals. The suitable crystals were selected for the crystallographic study. All diffraction measurements were performed at a temperature of 298(2) K using a Bruker Smart 1000 diffractometer and graphite mono chromated MoKα radiation ($\lambda = 0.71073$ Å). The structure was solved using direct methods and refined by full matrix least-squares on F^2.

3.15. Crystal data of 1-*O*-acetylbritannilactone (1)

$C_{17}H_{24}O_5$, 308.36, orthorhombic, space group $P_{2(1)2(1)2(1)}$, $a = 8.0017(7)$, $b = 12.3653(10)$, $c = 16.8794(14)$ Å, $V = 1670.1(2)$ Å^3, $T = 298(2)$ K, $Z = 4$, $D_C = 1.226$ mg m^{-3}, $F(0\,0\,0) = 664$, absorption coefficient = 0.089 mm^{-1}, crystal size = $0.20 \times 0.25 \times 0.30$ mm, 6995 reflection measured, 2949 unique ($R_{int} = 0.0241$), which were used in all calculations. The final wR (F^2) was 0.0837 (all data).

3.16. Crystal data of (2-*O*-butyloxime-1-phenyl)-propionyl-1-*O*-acetylbritannilactone ester (12e)

$C_{30}H_{39}NO_7$, 525.62, triclinic, space group P_1, $a = 9.185(4)$, $b = 9.470(4)$, $c = 9.840(4)$ Å, $V = 759.4(5)$ Å^3, $T = 298(2)$ K, $Z = 1$, $D_C = 1.149$ mg m^{-3}, $F(0\,0\,0) = 282$, absorption coefficient = 0.081 mm^{-1}, crystal size = $0.20 \times 0.25 \times 0.30$ mm, 2686 reflection measured, 2686 unique which were used in all calculations. The final wR (F^2) was 0.1584 (all data).

3.17. Bioactivity assay

The anti-cancer activity test was evaluated using the following methods. Anti-cancer activity in vitro was evaluated using a system based on the tetrazolium salt (MTT), which was reduced by living cells to yield a soluble formazan product that could be assayed colorimetrically. Stock solutions of compounds were freshly prepared in 10% (v/v) DMSO, and diluted to the required concentration with culture before use. Tumor cells (HL-60, Bel-7402) were grown in RPMI 1640 medium supplemented with 10% freshly inactivated fetal calf serum (FCS) and antibiotics. Cells harvested from the exponential phase (2×10^{-5} per mL) were seeded into a 96-well plate, the compounds studied were then added in a concentration gradient, and the final concentrations were maintained at 1000, 100, 10, 1, and 0.1 μmol/L, respectively. The plate was maintained at 37 °C in a humidified atmosphere of 5% CO_2 and incubated for 48 h. MTT solution of an appropriate concentration (1 mg/L) was then added to each well. After incubation for 4 h at 37 °C, acid–isopropanol (100 μL of 0.04 N HCl in isopropanol) was added to all wells and mixed thoroughly to dissolve the dark blue crystals. Measurement of the absorbance of the solutions, related to the number of live cells, was carried out using a Bio-Rad Model 450 Microplate reader at 570 nm. IC_{50} values were calculated from curves constructed by plotting the suppression ratios (%) versus compound concentration (M).

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