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Syntheses of Idarubicin Analogues Containing a Glucose or Galactose Moiety as a Glycone

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The new idarubicin analogues (**12** and **13**) with a glucose or galactoseas as a glycone were synthesized from daunomycin (**2**). (+)-4-Demethoxydaunomycinone (**6**) obtained from reaction of **2** with $AlCl_3$ was converted to 4-trifluoromethanesulfonyl daunomycinone (**7**) through reaction with trifluoromethanesulfonic anhydride. The treatment of **7** with 1,1-bis-(diphenylphospino)ferrocene/Pd$(OAc)_2$ in triethylamine/formic acid/dioxane provided the idarubicinone (**5b**). Glycosylation of 7-hydroxy group of **5b** with two kinds of tetraacetyl pyranosyl halide (**8** and **9**) by a modified Koenigs-Knorr procedure and then deacetylation using aqueous 0.1 N LiOH solution and amberlite cationic resin gave the objective materials. The *in vitro* MTT assay of the analogues (**12b** and **13a**) in comparison with idarubicin (**5a**) on peripheral blood human promyelocytic-leukemia cell line and human breast cancer cell line were also described.

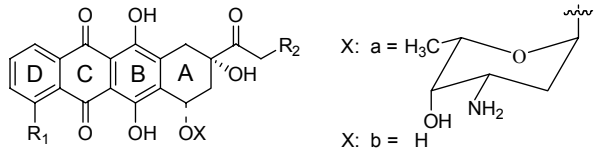
Key Words: Anthracycline, Idarubicin, Idarubicinone, Glucose, Galactose

Introduction

Anthrcycline antibiotics are well-known anticancer agents. Adriamycin (doxorubicin, **1**), daunomycin (daunorubicin, **2**) and carminomycin (**3**) are prominent members of a class of clinically important anthracycline antibiotics used most often in antitumor combination chemotherapy (Figure 1). They have been widely used in clinical therapy as major drugs in the treatment of solid tumors since the early 1970s. However, their utility is still limited due to a number of side effects, the most serious being cumulative dose-dependent cardiotoxicity.[1] Therefore, numerous synthetic efforts have been devoted to overcome these disadvantages culminating in development of artificial 4-demethyoxyadriamycin (**4**) and 4-demethoxydaunomycin (idarubicin, **5a**) to improve the pharmacological profile.[1b,2-5] Especially, its pharmacodynamic and pharmacokinetic properties were published by Hollingshead and Faulds in 1991.[6] (+)-Idarubicin (**5a**) having the possibility of oral administration has been also known to show higher drug efficacy about seven to eight times than previously published anthracycline series.[5a,7,8]

Because these compounds cannot be prepared from the fermentation method, many papers on the synthesis of **4** and **5a** have been reported. The antitumor activity of anthracyclines is mainly dependent on the chirality of A-ring with their natural absolute configurations.[9a] Therefore, much effort has been devoted to develope more efficient methods for the enantiomeric synthesis of aglycones[9] and alternating sugar moiety to other glycone.

Recent examples[10] have been reported on coupling some drugs with glucuronide that were found to have an enhanced drug efficacy. The syntheses of anthracycline analogues that are attached with glucuronide at amino group of daunosamine in doxorubicin and/or daunomycin[11] and the syntheses of idarubicinone-7-β-D-glucuronide, daunomycinone-7-D-glucuronide, and doxorubicinone-7-D-glucuronide have been also reported.[12] Related to these reports, herein we are pleased to report the synthesis and some biological properties of idarubicin analogues (**12** and **13**) that are coupled aglycone **5b** of idarubicin (**5a**) with α-D-acetoxyglucopyranosyl bromide (**8**) or α-D-acetoxy-galactopyranosyl bromide (**9**), respectively.



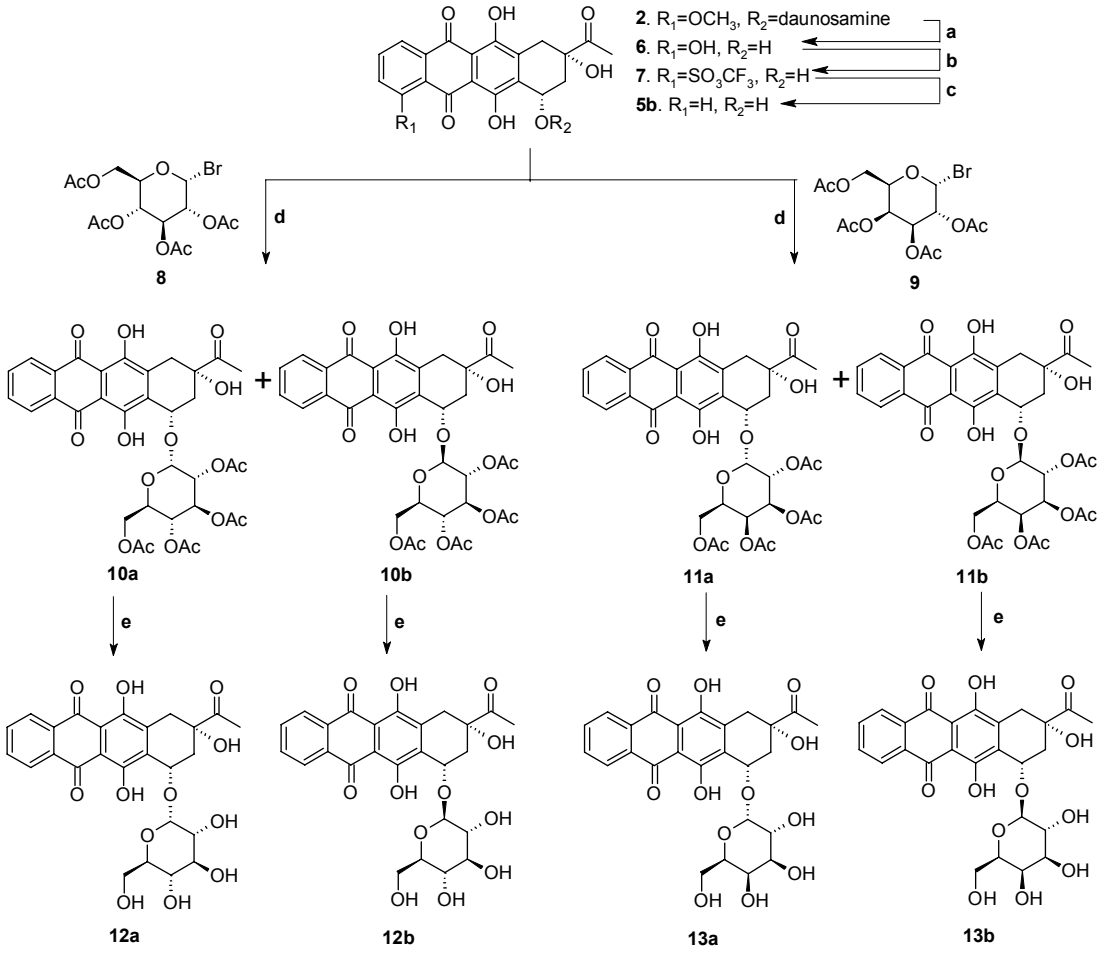
1. R_1 = OMe, R_2 = OH, X = a (Adriamycin)
2. R_1 = OMe, R_2 = H, X = a (Daunomycin)
3. R_1 = R_2 = OH, X = a (Carminomycin)
4. R_1 = H, R_2 = OH, X = a (4-Demethoxyadriamycin)
5a. R_1 = R_2 = H, X = a (Idarubicin)
5b. R_1 = R_2 = H, X = b (Idarubicinone)

Figure 1. Chemical structures of some anthracyclines.

Results and Discussion

The syntheses of aglycone of anthracylines in the previous publications require very complicated multi-steps.[13] However we endeavor to directly prepare idarubicinone (**5b**) from commercially available daunomycin (**2**) by the basis of a known procedure[14] using daunomycinone as a starting material. The target materials (**12** and **13**) were obtained from coupling of **5b** with a suitably protected pyranosyl halide **8** or **9** respectively, by modified Koenigs-Knorr glycosylation using mercury (II) bromide[15] followed by alkaline deacetylation using aqueous LiOH solution (Scheme 1).

The coupling reactions were attempted under several catalyst and conditions on the basis of our previous results.[12b] The stereochemical outcome of glycosidation was influenced by kinds of catalysts and reaction conditions. Results of the coupling of **5b** with protected pyranosyl halide **8** and **9** in different reaction con-



Scheme 1. (a) $AlCl_3$, CH_2Cl_2, oxalic acid, 24 hr; (b) Tf_2O, Pyr, DIPEA, DMAP, 0 - 5 °C, 5 min; (c) Dioxane, TEA, HCO_2H, $Pd(OAc)_2$, 40 °C, 1,1-bis(diphenylphosphino)ferrocene, 24 hr; (d) $HgBr_2$, yellow HgO, 3 Å molecular sieves/CH_2Cl_2, reflux, 15 - 32 hr; (e) 0.1 N LiOH/H_2O, MeOH/H_2O/THF(5/2/1), rt, 2 hr, amberlite cation resin.

Table 1. Summary of the characteristics of the synthesized polymers.

Entry	Glycones	Methods[a]	Products	Ratio[b]	Yields (%)[c]
1		A		Only β	20
2	**8**	B	**10a/10b**	α/β = 1:3	39
3		C		Only β	18
4		A		Only β	23
5	**9**	B	**11a/11b**	α/β = 1:3	40
6		C		-	-

[a]Reaction conditions: (A) $ZnBr_2/CH_2Cl_2$, rt or reflux; (B) yellow HgO, $HgBr_2/CH_2CH_2$, rt; (C) AgOTf/CH_2Cl_2, rt. [b]Ratio was determined by ^{1}H NMR spectroscopy. [c]Total isolated yields.

ditions are summarized in Table 1. Coupling of **5b** with protected pyranosyl bromide **8** or **9** using $ZnBr_2$ or AgOTf as a catalyst produced only β-anomer without producing α-anomer (entry 1, 3 and 4), whereas the same coupling using $HgBr_2$ which gave effective yield in various reaction conditions, afforded a mixture of α- and β-anomer with an 1:3 ratio (entry 2 and 5). However, coupling of **5b** with **9** using AgOTf was unsuccessful and some of aglycon was only obtained (entry 6).

We could isolate two stereochemical compounds for each reaction through a careful chromatography using silica gel in 10% acetone/dichloromethane as an eluent. The anomeric configurations of compound **10** and **11** were easily determined by analyzing coupling constants in ^{1}H NMR spectra between the 1'-H and 2'-H protons on the glycone moieties. The coupling constants for the 1'-H and 2'-H protons for various types of drug-sugar conjugates have been reported.[10a,16] The coupling constants ($J_{1'2'}$) of α-anomers were 2-5 Hz, whereas those of β-anomer were 6-10 Hz. Additionally, the axial anomeric proton (1'-C) of the β-anomer always resonates at a higher field than the equatorial proton of the corresponding α-anomer. Hence, we wish to identify unambiguously the anomeric 1'-H proton and the neighboring 2'-H proton, and to compare their chemical shifts and coupling constants (Table 2) with each other. In the ^{1}H NMR of **10a** and **10b**, we carefully analyzed the region of 4-6 ppm for the 2'-H, 3'-H and 4'-H. For **10a**, the signal of 2'-H was appeared at 4.95 ppm as a double-doublet with coupling constants of 3.7 Hz (1'-H, 2'-H) and 9.8 Hz (2'-H, 3'-H). For comparison, the 2'-H signal **10b**, was appeared at 4.90 ppm as a double-doublet with coupling constants of 7.1 Hz (1'-H, 2'-H) and 9.8 Hz (2'-H, 3'-H). The anomeric proton of **10a** appeared at

Table 2. ^{1}H NMR chemical shifts (δ, ppm) and coupling constants (*J*, Hz) for sugar moieties of **10** and **11** in $CDCl_3$.

Multiplicity	H-1' ($J_{1'2'}$)	H-2' ($J_{1'2'}$, $J_{2'3'}$)	H-3' ($J_{2'3'}$, $J_{3'4'}$)	H-4' ($J_{3'4'}$, $J_{4'5'}$)	H-5' ($J_{4'5'}$)
10a	6.10 (4.4)	4.95 (3.7, 9.8)	5.40 (9.8)	5.16 (9.3)	4.66 (9.8)
10b	5.17 (9.8)	4.90 (7.1, 9.8)	5.56 (9.8)	5.28 (6.8)	4.57 (9.8)
11a	6.18 (3.4)	4.98 (3.4, 10.3)	5.34 (2.4, 5.8)	5.28 (2.0, 10.8)	4.35 (4.4)
11b	5.68 (8.3)	5.06 (8.5, 10.2)	5.41 (1.8, 4.4)	5.33 (2.0, 10.3)	4.29 (m)

Table 3. MTT assay data of idarubicin analogues (**12b** and **13a**) and idarubicin (**5a**) on peripheral blood human promyelocytic leukemia cell line (HL-60) and human breast cancer cell line (MCF-7).

	HL-60			MCF-7		
	0.1[a]	1.0[a]	10.0[a]	0.1[a]	1.0[a]	10.0[a]
Control[b]		1.67 ± 0.12			1.21 ± 0.30	
5a	0.57 ± 0.13*	0.40 ± 0.08*	0.30 ± 0.01*	0.67 ± 0.10*	0.45 ± 0.09*	0.37 ± 0.13*
12b	1.63 ± 0.04	1.51 ± 0.37	1.48 ± 0.49	0.96 ± 0.32	0.97 ± 0.32	1.19 ± 0.23
13a	1.31 ± 0.63*	0.85 ± 0.37*	0.53 ± 0.20*	1.07 ± 0.38	0.64 ± 0.08*	0.45 ± 0.08*

[a]Unit; mg/mL. [b]Nontreatment group. *$P < 0.05$ compared with control. Values are means ± SE.

6.10 ppm with coupling constant of 4.4 Hz confirms α-anomer, whereas that of **10b** appeared at 5.17 ppm with coupling constant of 9.8 Hz is β-isomer. For **11a** and **11b**, the anomeric configurations of the galatopyranosyl moiety have the same tendency of the spectral data of **10a** and **10b** as shown in Table 2.

Deacetylation of the protected acetyl compounds (**10a**, **b** and **11a**, **b**) was carried out using 0.1 N LiOH solution in MeOH/THF/H_2O (5/2/1, v/v). After the reaction was completed, the resulting solution was neutralized with ion exchange resin and then the polar compound was purified with reversed-phase C_{18} column chromatography to give final products.[11,12] Deprotection of **10a** failed to provide the desired product **12a**, which is hydrolyzed to aglycone and glycone, whereas deprotection of β-anomer **10b** yielded **12b** in 29%. However, the deacetylation of **11a** gave the product **13a** in 28% yield; in case of **11b** was not able to obtain the **13b**. These results indicate that β-anomer of idarubicinone-glucose molecule was more stable than α-anomer, whereas α-anomer of idarubicinone-galactose was more stable than β-anomer due to 1,3-diaxial interactions of galactose moiety. Generally, yield of β-anomer is generally higher than that of α-anomer in the literature.[12b] However, unexpectedly, β-anomer **13b** could not be isolated due to its unstability. This result show that the stability of each anomers depend on geometric structure of the pyranosyl moiety in our experiment.

The effects of idarubicin analogues (**12b** and **13a**) were evaluated by microculture tetrazolium (MTT) assays. For that purpose, human acute myeloid leukemia (HL-60) and human breast cancer cell line (MCF-7) were utilized. The multiple concentrations of the compounds in the range of 0.1-10 mg/mL were used and their idarubicin-resistant cell lines were shown in Table 3. All analogues (**12b** and **13a**) showed lower value of resistance index against HL-60 (peripheral blood human promyelocytic leukemia cell line) than idarubicin (**5a**), but only compound **13a** exhibited significant cytotoxic activities against both cell lines at concentration of 1.0 and 10.0 mg/mL, respectively. These results indicate that the idarubicin analogue **13a** containing a galatose moiety maintains the activity inherent in the idarubicin, whereas the other idarubicin analogue **12b** causes a decreased anticancer activity.

In conclusion, we have synthesized new idarubicin analogues (**12** and **13**) that showed biological activity as potential anticancer agents. Detailed ^{1}H NMR analyses unambiguously proved the anomeric configuration of the new compounds. Further studies on the biological tests will be reported as they are completed.

Experimental Section

All reactions were carried out in oven-dried glassware equipped with tight fitting rubber septa and under a positive dry argon and nitrogen atmosphere, except for those reactions utilizing water as a solvent. Bulk grade *n*-hexane and dichloromethane were distilled before use as described in the literature.[17] Other commercially available reagents and solvents, highly pure and sure sealed, were used without further purification. All reactions were monitored with TLC (Merck pre-coated silica gel plates (Art.5554)) with fluorescent indicator at 254 and 365 nm. Gravity column chromatography and flash column chromatography were carried out on silica gel (Merck, 230 - 400 mesh). ^{1}H and ^{13}C NMR spectra were recorded on a JEOL JNM EX-400 spectrometer. Chemical shifts were internally referenced to TMS for ^{1}H or to solvent signals for ^{13}C. Infrared spectra were recorded on a Nicolet 5-DXB series FT-IR spectrophotometer. Mass spectra were obtained on a JEOL JMS DX-110/110A Tandem mass spectrometer (FAB^+, ESI). UV-VIS absorption spectra were recorded on a Hitachi-556 spectrophotometer. Optical rotations were determined using the Rudolph AUTOPOL IV apparatus with a 0-100-1.5 polarimeter sample tube. Melting points were obtained on a Buchi 510 melting point apparatus and were uncorrected.

(+)-4-Demethyldaunomycinone (6). To a solution of duno-

mycin (**2**: 10.0 g, 17.8 mmol) in dry methylene chloride (800 mL) under stirring in a nitrogen atmosphere, anhydrous aluminum chloride (23.7 g, 177.0 mmol) was added portionwise over a period of 1.5 hr. The reaction mixture was heated at reflux for 1 day, and then the solvent was distilled off. A solution of 0.1 M oxalic acid (400 mL × 2) was carefully added to the residue and the resulting mixture was stirred for two hours at room temperature to give dark red solid. The mixture was cooled and the reddish solid residue was filtered off and washed several times with water. After evaporation of $MgSO_4$-dried organic phase, crystallization with CH_2Cl_2 and *n*-hexane gave (+)-4-demethyldaunomycinone (**6**: 6.8 g, 99%, R_f = 0.66) as a red powder: mp 232 ~ 234 °C (lit[18a], 233 ~ 235 °C); $[\alpha]_D^{20}$ +57.8° (c 0.1, CH_2Cl_2); IR (KBr) 3440, 2921, 2844, 1712, 1614, 1426, 1209 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 12.33 (s, 1H, PhOH), 12.16 (s, 1H, PhOH), 12.08 (s, 1H, PhOH), 8.44 (dd, 1H, *J* = 1.7, 7.7 Hz, ArH), 7.88 (d, 1H, *J* = 7.8 Hz, ArH), 7.84 (dd, 1H, *J* = 1.7, 8.4 Hz, ArH), 5.30 (bs, 1H, C_9OH), 5.29 (bs, 1H, $C_{7eq}H$), 3.21 (d, 1H, *J* = 18.6 Hz, $C_{10eq}H$), 3.18 (d, 1H, *J* = 18.6 Hz, $C_{10ax}H$), 2.38 (s, 3H, $C_{14}H$), 2.31 (d, 1H, *J* = 15.6, $C_{8eq}H$), 2.29 (d, 1H, *J* = 15.6, $C_{8ax}H$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 121.0, 187.0, 186.6, 161.0, 155.9, 155.8, 135.7, 119.8, 118.4, 61.9, 56.7, 35.3, 33.2, 33.1, 29.6, 24.5; UV (CH_2Cl_2) λ_{max} 235, 254, 498; Mass (FAB, $[M + Na]^+$) *m/z* 407.1.

4-Demethyl-4-*para*-toluenesulfonyl-13-dioxalanyl-daunomycinone (7). To a solution of (+)-4-demethyldaunomycinone (**6**: 6.0 g, 15.7 mmol) in 320 mL of pyridine, cooled to 0 ~ 5 °C, *N*,*N*-diisopropylethylamine (10 mL, 59.1 mmol) and dimethylaminopyridine (2.3 g, 18.9 mmol) were added. To a resulting mixture, kept at 0 ~ 5 °C, trifluoromethanesulfonic anhydride (5 mL, 31.2 mmol) was added dropwise. After stirring for 30 min in the cold bath, the mixture was poured into 90 mL of 10% HCl, stirred for 30 min at room temperature and extracted with CH_2Cl_2 (400 mL × 2). The combined organic phase were washed with 0.1 M HCl (100 mL) and then with H_2O (150 mL × 2). The organic phase was evaporated to dryness and the residue was purified by column chromatography (CH_2Cl_2/Hexane/CH_3OH = 10:4:1) to give compound **7** (5.9 g, 73%, R_f = 0.49) as dark red solid: mp 162 ~ 164 °C $[\alpha]_D^{20}$ +149.3° (c 0.1, CH_2Cl_2); IR (KBr) 3424, 2946, 1732, 1605, 1409, 1350, 1100 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 13.47 (s, 1H, PhOH), 12.97 (s, 1H, PhOH), 7.90 (d, 1H, *J* = 7.3 Hz, ArH), 7.74 (t, 1H, *J* = 7.9 Hz, ArH), 7.35 (d, 1H, *J* = 8.3 Hz, ArH), 5.32 (bs, 1H, $C_{7eq}H$), 4.57 (s, 1H, C_9OH), 3.21 (d, 1H, *J* = 18.54 Hz, $C_{10eq}H$), 2.98 (d, 1H, *J* = 18.54 Hz, $C_{10ax}H$), 2.43 (s, 3H, $C_{14}H$), 2.36 (d, 1H, *J* = 14.15 Hz, $C_{8eq}H$), 2.19 (d, 1H, *J* = 14.15 Hz, $C_{8ax}H$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 211.6, 184.7, 156.9, 147.9, 136.7, 135.6, 135.5, 132.4, 129.0, 127.8, 122.1, 117.4, 110.8, 110.4, 61.7, 35.3, 33.2, 31.9, 29.6; UV (CH_2Cl_2) λ_{max} 255, 471, 499, 534; Mass (FAB, $[M + Na]^+$) *m/z* 539.0.

Idarubicinone (5b). To a mixture of triethylamine (4.0 mL, 29.7 mmol), 99% formic acid (1.0 mL, 26.5 mmol), 1,4-dioxane (20 mL) and 1,1-bis(diphenylphosphino)ferrocene (0.85 g, 1.53 mmol), was added $Pd(OAc)_2$ (71.8 mg, 0.3 mmol) consequently under nitrogen stream. The solution was heated to give a dark red color complex. Then a suspension of 4-demethyl-4-*para*-toluenesulfonyl-13-dioxalanyl-daunomycinone (**7**: 4.0 g, 6.4 mmol) in dioxane (80 mL) was slowly added dropwise and then stirred for 16 hr at room temperature. After completion of the reaction, the mixture was quenched with water, poured into CH_2Cl_2 (400 mL × 2), washed with NaOH (200 mL × 3) and H_2O (400 mL) subsequently. The organic phase was separated and concentrated to small volume. Methanol (50 mL) and 1 N HCl (50 mL) were added. The crystal was left under stirring for 30 min, then filtered and washed with H_2O (30 mL). After drying over $MgSO_4$ the residue was purified by column chromatography (CH_2Cl_2/acetone = 20:1) and then recrystallized from CH_2Cl_2 and Et_2O to obtain idarubicinone (**5b**: 1.8 g, 79%, R_f = 0.45) as a red solid: mp 178 ~ 180 °C (lit[18b], 174 ~ 178 °C); $[\alpha]_D^{20}$ +131.2° (c 0.1, CH_2Cl_2); IR (KBr) 3407, 2929, 2852, 1736, 1605, 1516, 1221 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 13.62 (s, 1H, PhOH), 13.34 (s, 1H, PhOH), 8.36 (m, 2H, ArH), 7.86 (m, 2H, ArH), 5.34 (bs, 1H, $C_{7eq}H$), 4.56 (s, 1H, C_9OH), 3.22 (dd, 1H, *J* = 2.0, 18.6 Hz, $C_{10eq}H$), 2.98 (d, 1H, *J* = 18.6 Hz, $C_{10ax}H$), 2.43 (s, 3H, $C_{14}H$), 2.36 (dd, 1H, *J* = 1.9, 15.6 Hz, $C_{8eq}H$), 2.21 (d, 1H, *J* = 15.6, $C_{8ax}H$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 210.5, 186.5, 185.3, 156.5, 156.0, 135.7, 134.6, 134.6, 133.5, 133.4, 127.1, 126.1, 110.0, 109.8, 76.2, 60.3, 35.4, 33.2, 29.7; UV (CH_2Cl_2) λ_{max} (log ε) 235, 252, 287, 484; Mass (FAB, $[M + Na]^+$) *m/z* 391.0.

(7S,9S)-(+)-Idarubicinone-7-(tetra-*O*-acetyl)-α-D-glucose (10a) and (7S,9S)-(+)-idarubicinone-7-(tetra-*O*-acetyl)-β-D-glucose (10b). A mixture of idarubicinone (**5b**: 2.0 g, 5.4 mmol), powdered molecular sieves 3 Å (2.0 g activated at 350 °C under a steam of N_2) in dry CH_2Cl_2 (400 mL) was stirred for 30 min at room temperature. Then, HgO (5.9 g, 27.1 mmol)/$HgBr_2$ (2.9 g, 8.2 mmol) as catalysts and α-D-acetoxyglucopyranosyl bromide (**8**: 3.4 g, 8.2 mmol) were added, the mixture was stirred for 18 hr in dark place. The mixture was filtered through a celite bed and washed repeatedly with dry CH_2Cl_2. The filtrate and washings combined were washed with aqueous 30% KI, saturated aqueous $NaHCO_3$, and water, dried ($MgSO_4$) and concentrated. The residue was purified by column chromatography on silica gel (CH_2Cl_2/acetone = 10:1) to give **10a** (minor, 0.4 g, 11%, R_f = 0.62) and **10b** (major, 1.0 g, 28%, R_f = 0.51) as a red power. **10a**: mp 189 - 190 °C $[\alpha]_D^{20}$ +13.9 (c 0.1, CH_2Cl_2); IR (KBr) 3424, 2917, 2848, 1740, 2848, 1740, 1618, 1413, 1230 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 13.64 (s, 1H, PhOH), 13.26 (s, 1H, PhOH), 8.32 (dd, 2H, *J* = 2.9, 8.7 Hz, ArH), 7.83 (dd, 2H, *J* = 2.2, 8.8 Hz, ArH), 6.10 (d, 1H, *J* = 4.4 Hz, $C_{1'}H$), 5.40 (t, 1H, *J* = 9.8 Hz, $C_{3'}H$), 5.31 (d, 1H, *J* = 4.9 Hz, $C_{7eq}H$), 5.16 (t, 1H, *J* = 9.3, $C_{4'}H$), 4.95 (dd, 1H, *J* = 3.9, 9.8 Hz, $C_{2'}H$), 4.66 (d, 1H, *J* = 9.8 Hz, $C_{5'}H$), 4.48 (s, 1H, C_9OH), 4.02 (m, 1H, $C_{5'}CH_2$), 3.21 (d, 1H, *J* = 19.0 Hz, $C_{10eq}H$), 2.95 (d, 1H, *J* = 19.0 Hz, $C_{10ax}H$), 2.61 (d, 1H, *J* = 15.6 Hz, $C_{8eq}H$), 2.43 (s, 3H, $C_{14}H$), 2.05 (dd, 1H, *J* = 4.4, 15.6 Hz, $C_{8ax}H$), 2.12 (s, 3H, SugarOAc), 2.09 (s, 3H, SugarOAc), 2.04 (s, 3H, SugarOAc), 1.90 (s, 3H, SugarOAc); ^{13}C NMR (100 MHz, $CDCl_3$) δ 195.5, 195.0, 178.3, 174.0, 162.2, 161.0, 156.3, 153.4, 144.1, 135.7, 126.0, 124.7, 122.5, 121.1, 119.8, 118.9, 118.4, 115.7, 114.0, 109.9, 108.3, 106.2, 103.8, 98.09, 75.8, 56.3, 34.7, 32.7, 31.9, 29.7, 24.5, 22.6; UV (CH_2Cl_2) λ_{max} (log ε) 234, 252, 483, 499; Mass (FAB, $[M + Na]^+$) *m/z* 721.2. **10b**: mp 156 - 158 °C; $[\alpha]_D^{20}$ +88.3° (c 0.1, CH_2Cl_2); IR (KBr) 3461, 2958, 2848, 1749, 1618, 1438, 1230 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 13.60 (s, 1H, PhOH), 13.28 (s, 1H, PhOH), 8.32 (dd, 2H, *J* = 3.8, 7.7 Hz, ArH), 7.83 (dd, 2H, *J* = 3.4, 8.8 Hz, ArH), 5.56 (t, 1H, *J* = 9.8 Hz, $C_{3'}H$), 5.54 (s, 1H, C_9OH), 5.30 (d,

1H, J = 3.9 Hz, $C_{7eq}H$), 5.28 (t, 1H, J = 6.8 Hz, $C_{4'}H$), 5.17 (t, 1H, J = 9.8, $C_{1'}H$), 4.90 (dd, 1H, J = 7.1, 9.8 Hz, $C_{2'}H$), 4.57 (d, 1H, J = 9.8 Hz, $C_{5'}H$), 3.81 (m, 1H, $C_{5'}CH_2$), 3.23 (d, 1H, J = 18.6 Hz, $C_{10eq}H$), 2.98 (d, 1H, J = 18.6 Hz, $C_{10ax}H$), 2.44 (s, 3H, $C_{14}CH_3$), 2.36 (dd, 1H, J = 2.0, 15.9 Hz, $C_{8eq}H$), 2.22 (s, 3H, SugarOAc), 2.12 (d, 1H, J = 18.6 Hz, $C_{8ax}H$), 2.10 (s, 3H, SugarOAc), 2.05 (s, 3H, SugarOAc), 1.96 (s, 3H, SugarOAc); ^{13}C NMR (100 MHz, $CDCl_3$) δ 211.6, 186.2, 186.0, 170.7, 170.0, 169.5, 169.3, 155.8, 135.4, 134.6, 134.3, 132.8, 126.6, 111.2, 110.7, 110.2, 110.1, 101.3, 94.9, 91.0, 72.6, 72.1, 71.5, 69.3, 67.5, 61.4, 61.1, 35.0, 32.7, 30.5, 29.3, 24.1; UV (CH_2Cl_2) λ_{max} (log ε) 235, 251, 288, 481, 496; Mass (FAB, $[M + Na]^+$) m/z 721.1.

(7S,9S)-(+)-Idarubicinone-7-(tetra-*O*-acetyl)-α-D-galactose (11a) and (7S,9S)-(+)-idarubicinone-7-(tetra-*O*-acetyl)-β-D-galactose (11b). A mixture of idarubicinone (**5b**: 2.0 g, 5.4 mmol), powdered molecular sieves 3 Å (2.0 g, activated at 350 °C under a steam of N_2) in dry CH_2Cl_2 (400 mL) was stirred for 30 min at room temperature. Then, HgO (5.9 g, 27.1 mmol)/ $HgBr_2$ (2.94 g, 8.2 mmol) as catalysts and α-D-acetoxygalactopyranosyl bromide (**9**: 3.4 g, 8.2 mmol) were added, the mixture was stirred for 15 hr in dark place. The mixture was filtered through a celite bed and washed repeatedly with dry CH_2Cl_2. The filtrate and washings combined were washed with aqueous 30% KI, saturated aqueous $NaHCO_3$, and water, dried ($MgSO_4$) and concentrated. The residue was purified by column chromatography on silica gel (CH_2Cl_2/acetone = 10:1) to give **11a** (minor, 0.3 g, 9%, R_f = 0.53) and **11b** (major, 1.2 g, 31%, R_f = 0.54) as a red power. **11a**: mp 106 - 109 °C $[\alpha]_D^{20}$ +141° (c 0.1, CH_2Cl_2); IR (KBr) 3452, 2921, 2848, 1753, 1626, 1434, 1234 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.86 (s, 1H, PhOH), 13.66 (s, 1H, PhOH), 8.43 (dd, 2H, J = 4.4, 7.8 Hz, ArH), 7.67 (dd, 2H, J = 4.4, 7.8 Hz, ArH), 6.18 (d, 1H, J = 3.4 Hz, $C_{1'}H$), 5.34 (dd, 1H, J = 2.4, 5.8 Hz, $C_{3'}H$), 5.28 (dd, 1H, J = 2.0, 10.8 Hz, $C_{4'}H$), 5.17 (bs, 1H, $C_{7eq}H$), 4.98 (dd, 1H, J = 3.4, 10.3 Hz, $C_{2'}H$), 4.72 (s, 1H, C_9OH), 4.35 (d, 1H, J = 4.4 Hz, $C_{5'}H$), 4.17 (m, 1H, $C_{5'}$-CH_2), 3.00 (d, 1H, J = 19.0 Hz, $C_{10eq}H$), 2.82 (d, 1H, J = 19.2 Hz, $C_{10ax}H$), 2.68 (d, 1H, J = 15.4 Hz, $C_{8eq}H$), 2.44 (s, 3H, $C_{14}CH_3$), 2.19 (dd, 1H, J = 3.4, 15.4 Hz, $C_{8ax}H$), 2.17 (s, 3H, SugarOAc), 2.12 (s, 3H, SugarOAc), 2.04 (s, 3H, SugarOAc), 2.00 (s, 3H, SugarOAc); ^{13}C NMR (100 MHz, $CDCl_3$) δ 211.5, 186.7, 186.6, 170.3, 170.1, 156.4, 156.1, 135.7, 134.8, 134.5, 134.5, 133.3, 133.3, 127.0, 127.0, 111.2, 110.8, 92.1, 71.6, 70.8, 70.0, 67.8, 61.7, 61.0, 35.3, 33.2, 29.6, 29.3, 24.4, 20.5, 20.6; UV (CH_2Cl_2) λ_{max} (log ε) 252, 258, 285, 484; Mass (FAB, $[M + Na]^+$) m/z 721.2. **11b**: mp 100 - 102 °C $[\alpha]_D^{20}$ + 275.0° (c 0.1, CH_2Cl_2); IR (KBr) 3465, 2925, 2852, 1753, 1626, 1430, 1238 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.51 (s, 1H, PhOH), 13.22 (s, 1H, PhOH), 8.29 (dd, 2H, J = 3.0, 8.4 Hz, ArH), 7.81 (dd, 2H, J = 3.4, 7.6 Hz, ArH), 5.68 (d, 1H, J = 8.3 Hz, $C_{1'}H$), 5.41 (dd, 1H, J = 1.8, 4.4 Hz, $C_{3'}H$), 5.33 (dd, 1H, J = 2.0, 10.3 Hz, $C_{4'}H$), 5.31 (bs, 1H, $C_{7eq}H$), 5.06 (dd, 1H, J = 8.5, 10.2 Hz, $C_{2'}H$), 4.59 (s, 1H, C_9OH), 4.29 (m, 3H, $C_{5'}H$, $C_{5'}CH_2$), 3.15 (dd, 1H, J = 2.4, 18.6 Hz, $C_{10eq}H$), 2.92 (d, 1H, J = 18.6 Hz, $C_{10ax}H$), 2.39 (s, 3H, $C_{14}CH_3$), 2.33 (dd, 1H, J = 1.9, 16.6 Hz, $C_{8eq}H$), 2.22 (d, 1H, J = 16.6 Hz, $C_{8ax}H$), 2.14 (s, 3H, SugarOAc), 2.07 (s, 3H, SugarOAc), 2.02 (s, 3H, SugarOAc), 1.97 (s, 3H, SugarOAc); ^{13}C NMR (100 MHz, $CDCl_3$) δ 212.8, 211.57, 186.2, 186.1, 185.9, 170.3, 169.9, 169.5, 169.3, 156.0, 155.8, 155.7, 136.7, 135.4, 134.6, 132.0, 126.7, 111.1, 110.6, 110.8, 101.8, 95.3, 91.6, 68.5, 68.2, 67.3, 66.9, 66.8, 65.2, 35.0, 34.4; UV (CH_2Cl_2) λ_{max} (log ε) 236, 252, 258, 286, 484; Mass (FAB, $[M + Na]^+$) m/z 721.2.

(7S,9S)-(+)-Idarubicinone-7-β-D-glucose (12b). 10b (260 mg, 0.4 mmol) was dissolved in 18 mL of MeOH/H_2O/THF (5/2/1, v/v) and 23 mL (6.0 equiv) of 0.1 N LiOH/H_2O solution at 0 °C. The deep blue solution was monitored on reversed-phase TLC (SiO_2-C_{18}, MeCN/H_2O, 1/2). After 2 hr, the reaction mixture was neutralized by adding *ca*. 1.0 g of amberlite cation exchange material (H^+ form). The amberlite resin was removed by filtration. After evaporation of MeOH and THF, red aqueous solution was transferred to a reversed-phase column packed with RP-C_{18} material and eluted with MeCN/H_2O (1:4) for purification. The solvents were removed under reduced pressure to give **12b** (64 mg, 29%, R_f = 0.61) as a red solid: mp 202 - 205 °C $[\alpha]_D^{20}$ +262.5° (c 0.1, CH_3OH); IR (KBr) 3387, 2913, 2848, 1740, 1610, 1462, 1250 cm^{-1}; ^{1}H NMR (400 MHz, MeOH-d_4) δ 12.01 (s, 1H, PhOH), 11.25 (s, 1H, PhOH), 8.37 (d, 1H, J = 8.8 Hz, ArH), 8.36 (d, 1H, J = 8.8 Hz, ArH), 7.85 (dd, 2H, J = 3.4, 7.6 Hz, ArH), 6.34 (d, 1H, J = 7.4 Hz, $C_{1'}H$), 5.71 (dd, 1H, J = 8.0, 8.3 Hz, $C_{3'}H$), 5.39 (d, 1H, J = 2.6 Hz, $C_{7eq}H$), 5.37 (t, 1H, J = 7.2, 7.4 Hz, $C_{4'}H$), 4.74 (dd, 1H, J = 6.4, 9.7 Hz, $C_{2'}H$), 5.46 (m, 3H, $C_{5'}H$, $C_{5'}CH_2$), 4.69 (s, 1H, C_9OH), 3.29 (dd, 1H, J = 1.7, 18.6 Hz, $C_{10eq}H$), 2.99 (d, 1H, J = 18.6 Hz, $C_{10ax}H$), 2.75 (d, 1H, J = 14.4 Hz, $C_{8eq}H$), 2.21 (s, 3H, $C_{14}H$), 2.15 (d, 1H, J = 14.4 Hz, $C_{8ax}H$); ^{13}C NMR (100 MHz, MeOH-d_4) δ 209.7, 208.8, 170.8, 133.0, 131.4, 130.6, 129.6, 126.3, 89.2, 89.1, 88.0, 75.9, 75.4, 75.2, 74.8, 74.1, 73.8, 73.7, 73.5, 73.3, 73.2, 71.9, 69.1, 65.3, 53.9, 30.0; UV (CH_2Cl_2) λ_{max} (log ε) 206, 218, 225, 246, 249, 494, 528; Mass (FAB, $[M + Na]^+$) m/z 553.1.

(7S,9S)-(+)-Idarubicinone-7-α-D-galactose (13a). 11a (280 mg, 0.4 mmol) was dissolved in 20 mL of MeOH/H_2O/THF (5/2/1, v/v) and 22 mL (6.0 equiv) of 0.1 N LiOH/H_2O solution at 0 °C. The deep blue solution was monitored on reversed-phase TLC (SiO_2-C_{18}, MeCN/H_2O, 1/2). After 2 hr, the reaction mixture was neutralized by adding *ca*. 1.3 g of amberlite cation exchange material (H^+ form). The amberlite resin was removed by filtration. After evaporation MeOH and THF, red aqueous solution was transferred to a reversed phase column packed with RP-C_{18} material and eluted with MeCN/H_2O (1:4) for purification. The solvents were removed under reduced pressure to give **13a** (62 mg, 28%, R_f = 0.71) as a red solid: mp 169 - 171 °C $[\alpha]_D^{20}$ +7.0° (c 0.1, CH_3OH); IR (KBr) 3419.8, 2945.7, 2855.8, 1732.1, 1605.4, 1409.3, 1200.9 cm^{-1}; ^{1}H NMR (400 MHz, MeOH-d_4) δ 12.31 (s, 1H, PhOH), 12.14 (s, 1H, PhOH), 8.33 (d, 1H, J = 8.3 Hz, ArH), 8.31 (d, 1H, J = 7.7 Hz, ArH), 7.71 (t, 1H, J = 2.9, 7.7 Hz, ArH), 7.53 (dd, 1H, J = 3.4, 8.3 Hz, ArH), 5.83 (d, 1H, J = 1.2 Hz, $C_{1'}H$), 5.78 (bs, 1H, $C_{7eq}H$), 5.47 (m, 1H, $C_{3'}H$), 5.29 (m, 1H, $C_{4'}H$), 5.17 (m, 3H, $C_{5'}H$, $C_{5'}CH_2$), 5.14 (dd, 1H, J = 2.0, 4.6 Hz, $C_{2'}H$), 4.69 (s, 1H, C_9OH), 3.36 (dd, 1H, J = 2.0 Hz, 18.3 Hz, $C_{10eq}H$), 3.32 (d, 1H, J = 18.3 Hz, $C_{10ax}H$), 2.58 (d, 1H, J = 15.8 Hz, $C_{8eq}H$), 2.53 (d, 1H, J = 15.8 Hz, $C_{8ax}H$), 2.20 (s, 3H, $C_{14}H$); ^{13}C NMR (100 MHz, MeOH-d_4) δ 211.9, 198.4, 187.1, 186.7, 161.0, 155.9, 155.8, 135.7, 135.5, 133.5, 120.8, 119.8, 118.3, 111.5, 111.1, 105.7, 105.3, 61.9, 56.7, 35.3, 33.1, 31.9, 30.1, 29.6, 29.3, 24.5; UV (CH_2Cl_2) λ_{max} (log ε) 232, 250, 284, 496; Mass (FAB, $[M + Na]^+$) m/z 553.1.

Cell culture. HL-60 (peripheral blood human promyelocytic

leukemia) cell line and MCF-7 (human breast cancer) cell line were purchased from American Type Culture Collection (Manassas, VA, USA) and maintained in RPMI 1640 (Invitrogen, Carlsbad, CA, USA) and Dulbecco's modified Eagle's medium (Invitrogen) with 10% fatal bovine serum (FBS), 2 mM L-glutamine (Sigma-Aldrich, St Louis, MO, USA), 0.9 g/L $NaHCO_3$ (Sigma-Aldrich), 25 mM HEPES (*N*-[2-hydroxyethyl]piperazine-*N*'-[2-ethanesulfonic acid]; Sigma-Aldrich), 100 U/mL penicillin (Sigma-Aldrich), and 500 μg/mL streptomycin (Sigma-Aldrich), at 37 ^{o}C, in a 5% CO_2 atmosphere.

MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) assay of cell viability. MTT assays are based on the ability of viable cells to convert MTT dye (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide), a soluble tetrazolium salt (thioazyl blue) into an insoluble formazan precipitate, which is quantitated by spectrophotometry following solubilization in dimethyl sulfoxide.[19] Briefly, subconfluent proliferating cells in 96-well plate were treated with synthesized drugs in standard growth medium for 24 hr, washed vigorously to remove the drug, and then postincubated for 48 hr in fresh, drug-free culture medium. At this time, the cells were solubilized and absorbance readings were taken using a Dynatech 96-well spectrophotometer (Beckman DU-640, Beckman Instrument, Inc., Fullerton, CA). The amount of MTT dye reduction was calculated based on the difference between absorbance at 570 and at 630 nm. Cell viability was expressed as the amount of dye reduction relative to that of the untreated control cells. Ten replicate wells were tested per assay condition, and each experiment was repeated at least 3 times.

Statistical analysis. Data represent means ± standard error (SE) of at least three separate experiments. Statistical comparisons were performed using one-way ANOVA followed by the Scheffe's test. Statistical significance of difference between groups was determined using the Student's *t* test. Differences were considered significant if the P value was less than 0.05.

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References

1. (a) Priebe, W. *Anthracycline Antibiotics, New analogues, Methods of Delivery, and Mechanisms of Actions*; ACS Symposium Series 574; American Chemical Society: Washington, DC, 1995; pp 100-114. (b) Arcamone, F. *Doxorubicin. Anticancer Antibiotics*; Medicinal Chemistry, A Series of Monographs volume 17; Academic Press: London, 1981; Vol. 17, pp 1-369. (c) Mettler, F. P.; Young, D. M.; Ward, J. M. *Cancer Res*. **1977**, *37*, 2705-2713.
2. Lown, J. W. *Anthracycline and Anthracenedione-based Anticancer Agents*; Elsevier: Amsterdam, 1988.
3. (a) Naff, M. B.; Plownan, J.; Narayanan, V. L. *Anthracycline Antibiotics* El Khadem, H. S., Ed.; Academic Press: New York, 1982; pp 1-57. (b) Grein, A.; Spalla, C.; Di Marco, A.; Canevazzi, G. *Giorn. Microbiol*. **1963**, *11*, 109-118. (c) Du Bost, M.; Ganter, P.; Maral, R.; Niner, L.; Pinnert, S.; Preudhomme, J.; Werner, G. H. C. R. *Acad. Sci*. **1963**, *257*, 1813-1815 (d) Arcamone, F.; Cassinelli, G.; Franceschi, P.; Mondelli, R.; Orezzi, P.; Penco, S. *Gazz. Chim. Ital*. **1970**, *100*, 949-989.
4. Arcamone, F.; Cassinelli, G.; Fantini, G.; Grein, A.; Orezzi, P.; Pol, C.; Spalla, C. *Biotechnol. Bioeng*. **1969**, *11*, 1101-1110.
5. (a) Arcamone, F.; Bernardi, L.; Giardino, P.; Patelli, B.; Di Marco, A.; Casazza, A. M.; Pratesi, G.; Reggiani, P. *Cancer Treat. Rep*. **1976**, *60*, 829-836. (b) Arcamone, F.; Bernald, L.; Patelli, B.; Giardino, P.; Di Marco, A.; Cassaza, A. M.; Soranzo, C.; Pratesi, G. *Experientia* **1978,** *34*, 1255-1257.
6. Hollingshead, L. M.; Faulds, D. *Drugs* **1991**, *42*, 690-719.
7. Neidle, S. *Nature* **1977**, *268*, 195-196.
8. (a) Hauser, F. M.; Hewawasam, P.; Rho, Y. S. *J. Org. Chem.* **1989**, *54*, 5110-5114. (b) Rho, Y. S.; Ko, H. K.; Sin, H. S.; Yoo, D. J. *Bull. Kor. Chem. Soc.* 1**999**, *20*, 1517-1520. (c) Parker, K. A.; Kallmerten, J. L. *Tetrahedron Lett*. **1979**, 1197-1200. (d) Keredesky, F. A. J.; Cava, M. P. *J. Am. Chem. Soc*. **1978**, *100*, 3635-3636.
9. (a) Badalassi, F.; Crotti, P.; Di Bubno, C.; D'Arata, F.; Favero, L.; Ramacciotti, A. *Tetrahedron: Asymmetry* **2001**, *12*, 3155-3161. (b) Sekine, A.; Ohshima, T.; Shibasaki, M. *Tetrahedron* **2002**, *58*, 75-82. (c) Krohn, K. *Tetrahedron* **1990**, *46*, 291-318. (d) Rho, Y. S.; Park, S. H.; Kim, W. J.; Kim, G. I.; Yoo, D. J.; Kang, H. S.;S. R. *Synthetic Comm.* **2002**, *32*, 1961-1975. (e) Rho, Y. S.; Kim, W. J.; Yoo, D. J.; Kang, H. S.; Chung, S. R. *Bull. Kor. Chem. Soc.* **2001**, *22*, 963-968. (f) Rho, Y. S.; Ko, H. K.; Kim, W. J.; Yoo, D. J.; Kang, H. S. *Bull. Kor. Chem. Soc.* **2000**, *21*, 774-778. (g) Rho, Y. S.; Choi, Y. H.; Kim, G. I.; Sin, H. S.; Yoo, D. J.; Kim, S. H.; Cheong, C. J. *Bull. Kor. Chem. Soc.* **1999**, *20*, 551-555.
10. (a) Rukhman, I.; Gutman, A. L. *Tetrahedron Lett.* **2000**, *41*, 6889-6892 and references therein. (b) Mitchell, M. B.; Whitcombe, I. W. A. *Tetrahedron Lett.* **2000**, *41*, 8829-8834.
11. Leenders, R. G .G.; Damen, E. W. P.; Bijisterveld, E. J. A.; Scheeren, H. W.; Houba, P. H. J.; Meulen-Muileman, I. H.; Boven, E.; Haisma, H. *Bioorg. Med. Chem*. **1999**, *7*, 1597-1610.
12. (a) Rho, Y. S.; Park, J. H.; Kim, G. I.; Kim, H. S.; Sin, H. S.; Suh, P. W.; Yoo, D. J. *Synthetic Comm.* **2004**, *34*, 1703-1722. (b) Rho, Y. S.; Kim, S. Y.; Kim, W. J.; Yun, Y. K.; Sin, H. S.; Yoo, D. J. *Synthetic Comm.* **2004**, *34*, 3497-3511.
13. Rho, Y. S.; Ko, H. K.; Sin, H.; Yoo, D. J. *Bull. Korean Chem. Soc*. **1999**, *20*, 1517-1520.
14. Valentina Pizzamiglio, S Fiorano(IT); Walter Cabri, Rozzano(IT); Elio Mapelli, Melzo(IT); *US patent 2004/0044189 A1*, 2004.
15. Aligiannis, N.; Pouli, N.; Marakos, P.; Skaltsounis, A.-L.; Pratsinis, H. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 3505-3507.
16. (a) Mortensen, R. W.; Corcoran, O.; Cornett, C.; Sidelmann, U. G.; Troke, J.; Lindon, J. C.; Nicholson, J. K.; Hansen, S. H. *J. Pharm. Biomed. Anal*. **2001**, *24*, 477-485; (b) Sidelmann, U. G.; Gavaghan, C.; Carless, H. A. J.; Farrant, R. D.; Lindon, J. C.; Wilson, I. D.; Nicholson, J. K. *Anal. Chem*. **1995**, *67*, 3401-3404.
17. Armerigo, W. L. F.; Perrin, D. D. *Purification of Laboratory Chemicals*, 4th Ed.; Oxford: Butterworth-Heinemann, 1996.
18. (a) Wani, M. C.; Taylor, H. L.; Wall, M. E.; Mcphail, A. T.; Onan, K. D. *J. Am. Chem. Soc*. **1975**, *97*, 5955-5956 (b) Hassall, C. H.; Broadhurst, M. J.; Thomas, G. J. *J. Chem. Soc. Perkin Trans. 1* **1982**, 2239-2248.
19. Fan, S.; Gao, M.; Meng, Q.; Laterra, J. J.; Symons, M. H. Coniglio, S. *Oncogene* **2005**, *24*, 1749-1766.