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Reaction of lupane and oleanane triterpenoids with Lawesson's reagent

Miroslav Kvasnica [a,b], Iva Rudovska [b], Ivana Cisarova [c], Jan Sarek [b,*]

[a] Institute of Organic Chemistry and Biochemistry, Academy of Sciences, Flemingovo n. 2, 16610 Prague 6, Czech Republic
[b] Department of Organic and Nuclear Chemistry, Faculty of Science, Charles University in Prague, Hlavova 8, 12843 Prague 2, Czech Republic
[c] Department of Inorganic Chemistry, Faculty of Science, Charles University in Prague, Hlavova 8, 12843 Prague 2, Czech Republic

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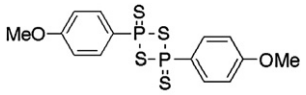
Abstract

The reactions of selected triterpenic oxo compounds with Lawesson's reagent were investigated. We examined sulfurization of some oxygen compounds and for these reactions several hindered ketones, one aldehyde, α-hydroxyketones, esters, or anhydrides were chosen. We synthesized 15 new sulfur derivatives, including thioketone **16**, dimeric sulfides **17**–**19**, and thiaderivatives **20**–**22**. We also observed unusual transformations, which afforded oxathiaphosphinines **23a**, **23b**, and dithiaphospholanes **24**. The prepared compounds failed to demonstrate any significant cytotoxic activity.

Keywords: Terpenoids; Lawesson's reagent; Ketones; Sulfur

1. Introduction

Lawesson's reagent[1] is a widely used thionation reagents.[2] It is used especially for thionation of carbonyl compounds (ketones, esters, amides, anhydrides, etc.)[3–7] and alcohols.[8,9] Unlike other thionation reagents, e.g., phosphorus pentasulfide,[10] reactions with Lawesson's reagent are useful because of their high yields, convenient handling, and especially soft thionation reactions, e.g., in the case of isoprenoids.[11,12] In some cases, the main disadvantage of this reagent is the formation of by-products or stable heterocyclic intermediates.[12]



Lawesson´s reagent (LR)

Triterpenoids are an important group of natural compounds possessing a variety of biological activities.[13] Recently, we have examined the structure–activity relationships in lupane and 18α-oleanane derivatives (betulinines).[14–18] Significant antitumor activity of several triterpenic derivatives has been found. This fact has encouraged us to prepare newly modified skeleton of betulinines. Thionation is one possible modification that could have some influence on antitumor activity in spite of lipophilicity that is an inherent feature of thionation. Herein we report on the reaction of several lupane and 18α-oleanane carbonyl derivatives with Lawesson's reagent to form new sulfur analogues. In addition, we made a cytotoxic screening of prepared compounds. Only several lupane and 18α-oleanane triterpenoids with C–S bonds have previously been published.[19]

2. Results and discussion

As starting materials for preparation of triterpenoids used for thionation reactions we used betulin (**1**) easily accessible from the birch bark (*Betula pendula*) by extraction with ethanol[14] and betulinic acid (**2**) from the bark of the plane tree (*Platanus hispanica*) isolated by MeOH extraction.[18] According to known procedures starting with betulin (**1**), we synthesized unsaturated ketone **3**,[20] ketone **4**,[21] heptanorketone **6**,[14] seco diketones **7** and **8**,[20] seco diketone **9**,[22] hydroxyketones **11** and **12**,[23] ketoacid **13**, anhydride **14**, and β-ketoester

* Corresponding author. Tel./fax: +420 221 951 332.
E-mail address: jan.sarek@gmail.com (J. Sarek).

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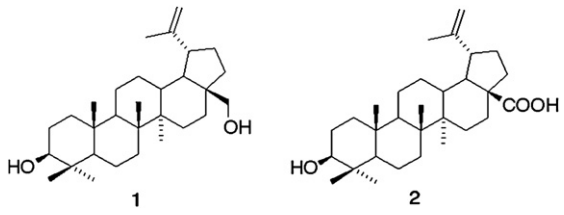
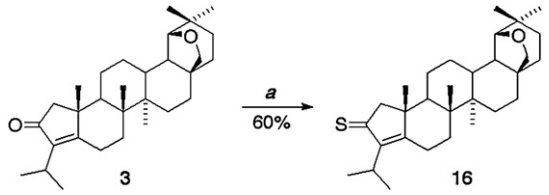
15.[14] From betulinic acid (**2**) we prepared benzyl-ester **5**[24] and unsaturated aldehyde **10**.[25]



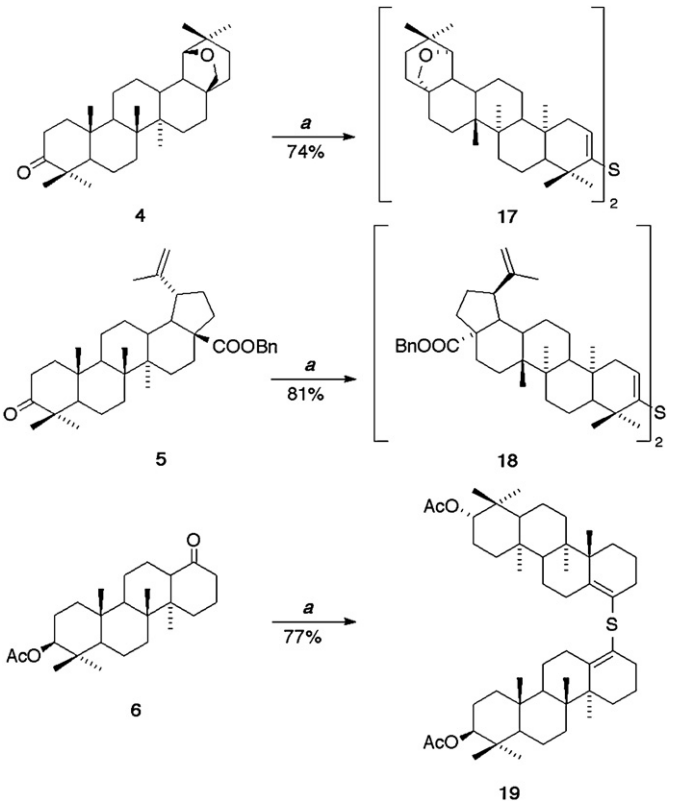
The reaction procedure for thionation was identical for all derivatives and was carried out in refluxing toluene with equimolar amount of Lawesson's reagent. Only in the case of low reactivity, toluene was replaced by *o*-xylene.

Reaction of unsaturated ketone **3** afforded only expected thioketone **16** (Scheme 1). However, 12 h reflux in *o*-xylene led to full conversion and due to the formation of by-products, the reaction had to be quenched earlier.



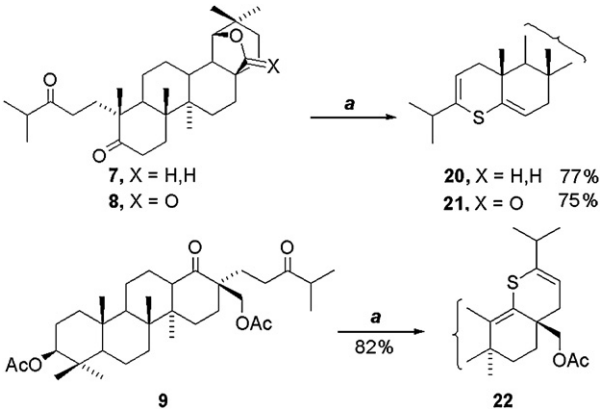
Scheme 1. *Reagents and conditions*: (a) LR, *o*-xylene, reflux, 12 h.

Opposite to ketone **3**, reaction of saturated ketones **4**–**6** gave unexpected dimer sulfides **17**–**19** (Scheme 2) in good yields (approximately 80%). The mechanism of this reaction is based on dimerization of saturated thioketones. Dimerization of these thioketones is enabled by ready formation of their enol form. This was explained previously.[12]



Scheme 2. *Reagents and conditions*: (a) LR, toluene, reflux, 5 h.

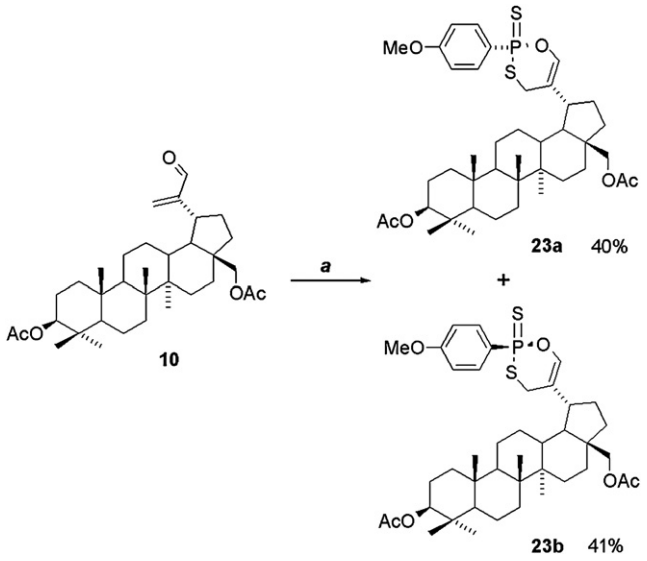
Similarly, reactions of seco diketones **7**–**9** resulted in unsaturated cyclic sulfides **20**–**22** (Scheme 3). Although the mechanism is the same as for saturated ketones **4**–**6**, no dimer products were observed. Similar reactions are used for the preparation of substituted thiophenes.[26,27] No lactone oxygen was replaced in the case of diketone **8**.



Scheme 3. *Reagents and conditions*: (a) LR, toluene, reflux, 5 h.

In previous examples, we only used ketones with no other functional groups nearby. If there is another functional group (e.g., double bond, hydroxyl group) we can expect some untypical products. This was proved for unsaturated aldehyde **10** and hydroxyketones **11** and **12**.

Reaction of aldehyde **10** with Lawesson's reagent afforded only two isomeric oxathiaphosphinines **23a** and **23b** (Scheme 4). The mechanism of formation of these products is as follows. Firstly, the phosphorus atom of the ylide attacks the carbonyl oxygen to create a P–O bond followed by migration of double bond. The last step is closing of the cycle by formation



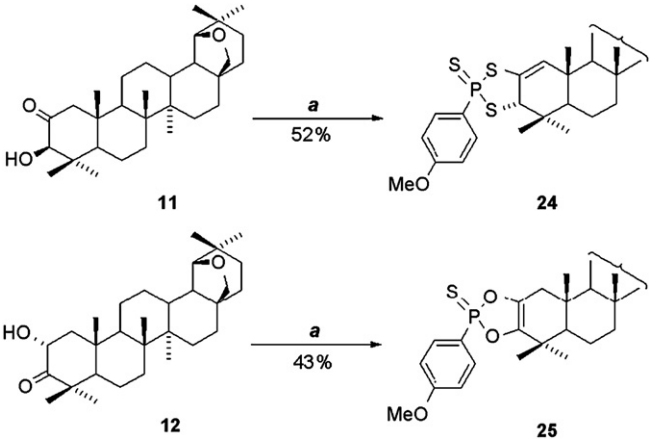
Scheme 4. *Reagents and conditions*: (a) LR, toluene, reflux, 4 h.

of a C–S bond (Scheme 5). The absolute configuration on the phosphorus atom was determined by X-ray analysis of **23b**, which has configuration *S* (Fig. 1).

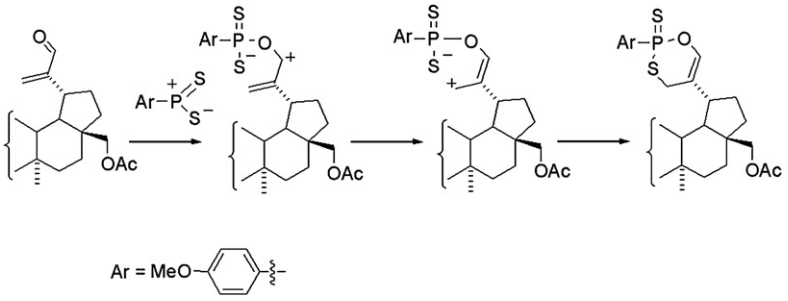
On the other hand, the reaction of hydroxyketones **11** and **12** resulted in complicated mixtures of products. Using HPLC we were able to isolate only one major product from each reaction. We obtained dithiaphospholane **24** from hydroxyketone **11**, and dioxaphospholane **25** from hydroxyketone **12** (Scheme 6). The reason why we isolated just these two compounds could be explained by the different retention times of these two derivatives in contrast to the other ones in mixture.

Reaction of ketoacid **13** did not lead to sulfur containing derivative but only to diene **26** (Scheme 7). It was found that decarboxylation and deoxygenation with migration of double bonds occurred during reaction with Lawesson's reagent. The reaction was very fast. It took only about 2 h and the yield was approximately 60%.

While in previous cases we obtained derivatives with a modified triterpenic skeleton, the reactions of anhydride **14** and β-ketoester **15** afforded only products with modified acetate groups (Scheme 7). The attempt to prepare thioanhydride resulted only in 3β-thioacetate **27** in low yield (about 45%). Starting anhydride **14** composed the rest of the reaction mixture. Prolongation of reaction time to 50 h does not lead to higher yield. In the case of β-ketoester **15**, we obtained a mixture of three compounds identified as bis(thioacetate) **28a**, 28-thioacetate **28b**, and 3β-thioacetate **28c** after 15 h. No substitution in other positions was observed in both cases. Long reaction times (>10 h) made it possible to modify acetyl groups whereas the 4 h reaction of aldehyde **10** did not afforded any thioacetate. The combination of thioacetates of β-ketoester **15** is probably caused by the low steric hindrance of the 28-acetyl group in contrast to anhydride **14**.



Scheme 6. *Reagents and conditions*: (a) LR, toluene, reflux, 5 h.



Scheme 5. Probably mechanism of formation of oxathiaphosphinines **23a** and **23b**.

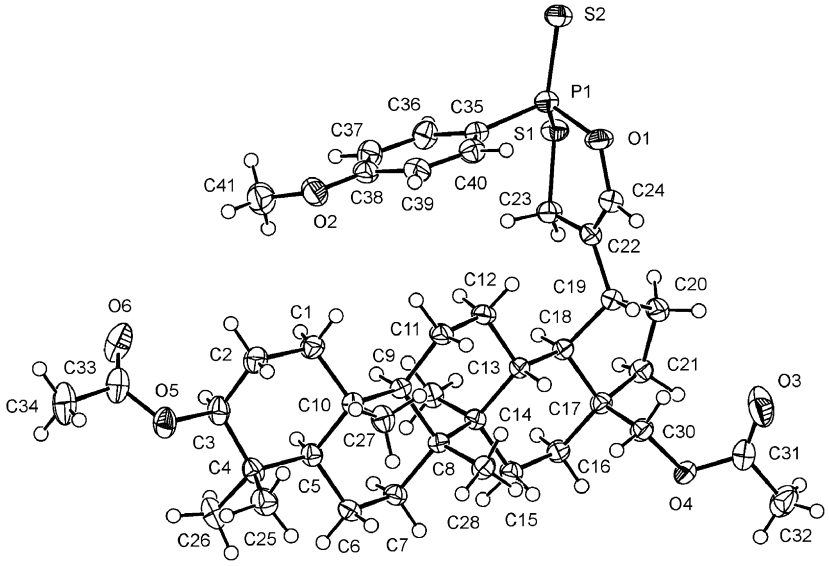
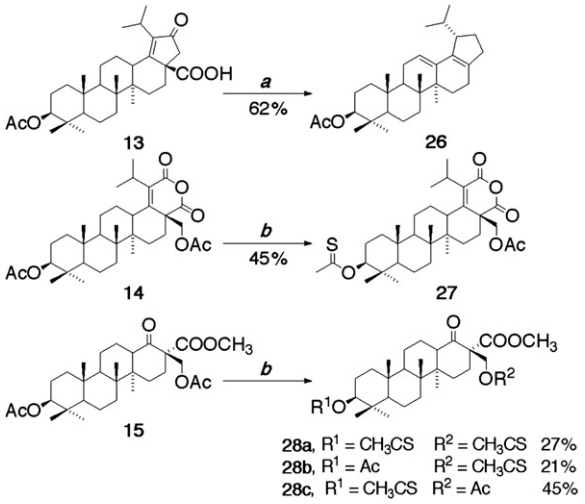


Figure 1. ORTEP drawing of oxathiaphosphinine **23b** with atom numbering scheme. Thermal ellipsoids are drawn at the 50% probability level.



Scheme 7. *Reagents and conditions*: (a) LR, toluene, reflux, 2 h; (b) LR, *o*-xylene, reflux, 50 h (for **14**) or 15 h (for **15**).

3. Conclusion

In this work we showed that Lawesson's reagent is suitable for the preparation of different sulfur derivatives of triterpenoids. Triterpenic ketones are the best starting derivatives. The limitations of this thionation are other functional groups nearby. Nevertheless, it could be a possible way to prepare triterpenic phosphorus heterocycles. Stable carbonyl derivatives did not undergo transformation with Lawesson's reaction; only acetates could be modified under this condition. Synthetic results of this work could be useful for other chemists working on the field of isoprenoid chemistry. It could be used not only for isoprenoid chemistry (reaction with α-hydroxyketones, α,β-unsaturated aldehydes or ketones, seco diketones, etc.). The preparation of new sulfur and/or phosphorus containing heterocycles is interesting for broader organic synthesis.

All prepared sulfur compounds **16**–**22**, **23a**, **23b**, **24**, **25**, **27**, **28a**–**c**, and diene **26** were characterized by spectroscopic methods and tested for in vitro antitumor activity on CEM leukemia cells. Unfortunately, the prepared triterpenoids failed to demonstrate any antitumor activity.

4. Experimental

4.1. General

Melting points were determined on Kofler block and are uncorrected. Optical rotatory power was measured on an Autopol III (Rudolph Research, Flanders, NJ) polarimeter as $CHCl_3$ solutions. ^1H, ^{13}C and ^{31}P NMR spectra were recorded on Varian UNITY *Inova* 400 (400 MHz for ^1H), using $CDCl_3$ as a solvent. Chemical shifts are expressed in parts per million with tetramethylsilane as an internal standard for ^1H spectra. ^{13}C NMR spectra are referenced to $CDCl_3$ (77.00 ppm) and ^{31}P NMR spectra are referenced to H_3PO_4 (0 ppm) as an external standard. Mass spectra (EI) were measured on INCOS 50 (Finnigan MAT) mass spectrometer. Mass spectra (FAB) were measured on ZAB-EQ (VG Analytical, Ltd., Manchester). IR spectra were recorded on Nicolet Avatar 370 FT-IR spectrometer, using $CHCl_3$ as a solvent. TLC was performed on Kieselgel 60 F_{254} (Merck) sheets; detected by UV fluorescence and spraying 10% sulfuric acid with heating to 110–200 °C. Used HPLC system consisted of High Pressure Pump Gilson (model 361), Inject Valve Rheodyne, Preparative Column (25×250 mm) with silica gel filling (Biospher 7 μm; Labio), Differential–Refractometrical Detector (Laboratorní přístroje, Praha, CR) connected with PC (software Chromulan) and Automatic Fraction Collector Gilson (model 246). Lawesson's reagent was purchased from Sigma–Aldrich.

4.2. General procedure for reaction of triterpenic carbonyl compounds with Lawesson's reagent

Lawesson's reagent (1 mmol) was added to a solution of triterpenic ketone (1 mmol) in toluene or *o*-xylene (8 mL). The reaction mixture was refluxed for several hours (mentioned in each experiment). After that, the mixture was filtered through silica gel in toluene, solvent was evaporated under reduced pressure, and crude product was chromatographed using HPLC (mobile phase hexane–ethyl acetate, ratio mentioned in each experiment).

4.2.1. Thioketone *16*

Starting with ketone **3** (250 mg, 0.57 mmol) in *o*-xylene (12 h), chromatography (phase 17:3) and crystallization from MeOH gave thioketone **16** (155 mg, 60%).

Mp 111–113 °C; $[\alpha]_D^{20}$ −117 (*c* 0.36). IR ($CHCl_3$): 1687, 1591, 1453, 1385, 1302, 1253, 1031 cm^{-1}. ^1H NMR ($CDCl_3$): δ=0.81 (s, 3H), 0.88 (s, 3H), 0.94 (s, 3H), 1.14 (s, 3H), 1.16 (s, 3H), 1.19 (d, *J*=7.2 Hz, 3H), 1.20 (d, *J*=7.2 Hz, 3H), (7×CH_3), 2.41 (dt, *J*=13.7, 4.7 Hz, 1H, H-6a), 2.62 (d, *J*=19.7 Hz, 1H, H-1a), 2.885 (d, *J*=19.5 Hz, 1H, H-6b), 2.892 (td, *J*=14.0, 3.4 Hz, 1H, H-1b), 3.41 (septet, *J*=7.2 Hz, 1H, H-4), 3.47 (d, *J*=7.9 Hz, 1H, H-28a), 3.55 (br s, 1H, H-19α), 3.79 (dd, *J*=7.8, 1.7 Hz, 1H, H-28b). MS-EI: *m/z* (%)=454 (14) [M^+], 439 (100), 396 (2), 383 (1), 367 (2), 257 (11), 245 (48), 215 (9), 205 (10), 192 (19). Anal. Calcd for $C_{30}H_{46}OS$: C, 79.23; H, 10.20; S, 7.05. Found: C, 79.15; H, 10.22; S, 6.93.

4.2.2. Dimer sulfide *17*

Starting with ketone **4** (300 mg, 0.68 mmol) in toluene (5 h), chromatography (phase 24:1) and crystallization from MeOH gave dimer sulfide **17** (220 mg, 74%).

Mp>300 °C (decomp.); $[\alpha]_D^{20}$ +52 (*c* 0.50). IR ($CHCl_3$): 1621, 1450, 1384, 1036, 968, 816, 766. ^1H NMR ($CDCl_3$): δ=0.81 (s, 6H), 0.85 (s, 6H), 0.92 (s, 6H), 0.93 (s, 6H), 1.00 (s, 6H), 1.09 (s, 6H), 1.16 (s, 6H), (14×CH_3), 2.15 (dd, *J*=17.2, 6.7 Hz, 2H, 2×H-1a), 3.46 (d, *J*=7.8 Hz, 2H, 2×H-28a), 3.56 (br s, 2H, 2×H-19α), 3.79 (d, *J*=7.8 Hz, 2H, 2×H-28b), 5.86 (dd, *J*=6.4, 1.8 Hz, 2H, 2×H-2). MS-FAB: *m/z* (%)=878 (92) [M^+], 455 (100), 423 (73). Anal. Calcd for $C_{60}H_{94}O_2S$: C, 81.94; H, 10.77; S, 3.65. Found: C, 81.71; H, 10.95; S, 3.58.

4.2.3. Dimer sulfide ***18***

Starting with ketone **5** (300 mg, 0.55 mmol) in toluene (5 h), chromatography (phase 24:1) and crystallization from mixture MeOH–$CHCl_3$ gave dimer sulfide **18** (243 mg, 81%).

Mp 170–173 °C; $[\alpha]_D^{20}$ +49 (*c* 0.28). IR ($CHCl_3$): 1717, 1641, 1456, 1375, 1153, 1128, 891. ^{1}H NMR ($CDCl_3$): δ=0.78 (s, 6H), 0.82 (s, 6H), 0.94 (s, 6H), 1.01 (s, 6H), 1.09 (s, 6H), 1.68 (s, 6H), (12×CH_3), 1.82–1.94 (m, 4H), 2.00 (dd, *J*=17.1, 6.6 Hz, 2H, 2×H-1a), 2.21 (dt, *J*=12.2, 3.7 Hz, 2H), 2.28 (td, *J*=12.4, 3.3 Hz, 2H), 2.65 (m, 2H), 3.02 (dt, *J*=11.1, 4.7 Hz, 2H), 4.59 (m, 2H, H-29a), 4.72 (br d, *J*=2.1 Hz, 2H, H-29b), 5.09 (d, *J*=12.4 Hz, 2H, 2×Ar-C*H*), 5.15 (d, *J*=12.4 Hz, 2H, Ar-C*H*), 5.70 (dd, *J*=6.4, 0.9 Hz, 2H, 2× H-2), 7.29–7.38 (m, 10H, 2×Ar-*H*). MS-FAB: *m/z* (%)=1088 (2) $[M+H]^+$, 559 (3), 527 (1), 91 (100). Anal. Calcd for $C_{74}H_{102}O_4S$: C, 81.72; H, 9.45; S, 2.95. Found: C, 81.66; H, 9.56; S, 2.88.

4.2.4. Dimer sulfide ***19***

Starting with ketone **6** (300 mg, 0.77 mmol) in toluene (5 h), chromatography (phase 24:1) and crystallization from mixture MeOH–$CHCl_3$ gave dimer sulfide **19** (230 mg, 77%).

Mp 237–238 °C; $[\alpha]_D^{20}$ −8 (*c* 0.80). IR ($CHCl_3$): 1722, 1626, 1372, 1256, 1029. ^{1}H NMR ($CDCl_3$): δ=0.84 (s, 6H), 0.85 (s, 6H), 0.87 (s, 6H), 0.88 (s, 6H), 1.11 (s, 6H), (10× CH_3), 1.96 (m, 2H, H-12α), 2.05 (s, 6H, 2×CH_3), 2.14 (m, 2H, H-17β), 2.59 (m, 2H, H-17α), 3.29 (ddd, *J*=14.4, 4.8, 2.0 Hz, 2H, H-12β), 4.47–4.52 (m, 2H, 2×H-3α). MS-FAB: *m/z* (%)=774 (3) $[M^+]$, 403 (28), 371 (61). Anal. Calcd for $C_{50}H_{78}O_4S$: C, 77.47; H, 10.14; S, 4.14. Found: C, 77.59; H, 10.22; S, 4.01.

4.2.5. Sulfide ***20***

Starting with diketone **7** (250 mg, 0.55 mmol) in toluene (5 h), chromatography (phase 19:1) and crystallization from isopropanol gave cyclic sulfide **20** (192 mg, 77%).

Mp 165–167 °C; $[\alpha]_D^{20}$ +15 (*c* 0.38). IR ($CHCl_3$): 1602, 1453, 1385, 1030. ^{1}H NMR ($CDCl_3$): δ=0.81 (s, 3H), 0.90 (s, 3H), 0.94 (s, 3H), 1.04 (s, 3H), 1.09 (s, 3H), 1.11 (d, *J*=6.8 Hz, 6H), (7×CH_3), 1.88 (d, *J*=16.8 Hz, 1H, H-1a), 2.19 (d, *J*=17.4 Hz, 1H, H-1b), 2.33 (dd, *J*=17.2, 7.0 Hz, 1H, H-7a), 2.34 (septet, *J*=6.8 Hz, 1H, H-4), 3.46 (d, *J*=7.8 Hz, 1H, H-28a), 3.54 (br s, 1H, H-19α), 3.80 (d, *J*=7.9 Hz, 1H, H-28b), 5.42 (dd, *J*=7.0, 2.0 Hz, 1H, H-2), 5.80 (dd, *J*=6.1, 2.3 Hz, 1H, H-6). MS-EI: *m/z* (%)=454 (100) $[M^+]$, 439 (4), 411 (7), 383 (2), 269 (3), 233 (18), 219 (29), 207 (19), 192 (11). Anal. Calcd for $C_{30}H_{46}OS$: C, 79.23; H, 10.20; S, 7.05. Found: C, 79.09; H, 10.11; S, 7.19.

4.2.6. Sulfide ***21***

Starting with diketone **8** (100 mg, 0.21 mmol) in toluene (5 h), chromatography (phase 19:1) and crystallization from isopropanol gave cyclic sulfide **21** (75 mg, 75%).

Mp 286–288 °C; $[\alpha]_D^{20}$ +40 (*c* 0.50). IR ($CHCl_3$): 1764, 1603, 1450, 1386, 1119, 971. ^{1}H NMR ($CDCl_3$): δ=0.85 (s, 3H), 0.97 (s, 3H), 0.98 (s, 3H), 1.04 (s, 3H), 1.08 (s, 3H), 1.108 (d, *J*=6.8 Hz, 3H), 1.110 (d, *J*=6.8 Hz, 3H), (7×CH_3), 1.88 (d, *J*=16.8 Hz, 1H, H-1a), 2.15 (d, *J*=17.6 Hz, 1H, H-1b), 2.32 (dd, *J*=16.8, 7.0 Hz, 1H, H-7a), 2.34 (septet, *J*=6.8 Hz, 1H, H-4), 3.95 (br s, 1H, H-19α), 5.41 (dd, *J*=6.4, 2.4 Hz, 1H, H-2), 5.79 (dd, *J*=6.1, 2.3 Hz, 1H, H-6). MS-EI: *m/z* (%)=468 (100) $[M^+]$, 453 (2), 425 (3), 399 (1), 267 (3), 233 (14), 219 (16), 207 (13), 189 (19). Anal. Calcd for $C_{30}H_{44}O_2S$: C, 76.87; H, 9.46; S, 6.84. Found: C, 76.77; H, 9.48; S, 6.78.

4.2.7. Sulfide ***22***

Starting with diketone **9** (500 mg, 0.90 mmol) in toluene (5 h), chromatography (phase 10:1) and crystallization from MeOH gave cyclic sulfide **22** (408 mg, 82%).

Mp 186–188 °C; $[\alpha]_D^{20}$ −96 (*c* 0.34). IR ($CHCl_3$): 1725, 1602, 1453, 1382, 1253, 1031, 975. ^{1}H NMR ($CDCl_3$): δ=0.84 (s, 3H), 0.86 (s, 3H), 0.90 (s, 3H), 0.92 (s, 3H), 1.13 (d, *J*=6.8 Hz, 6H), 1.14 (s, 3H), 2.05 (s, 3H), 2.06 (s, 3H), (9× CH_3), 2.31 (dd, *J*=16.5, 7.5 Hz, 1H, H-22a), 2.39 (septet, *J*=6.9 Hz, 1H, H-20), 2.84 (dd, *J*=15.3, 3.2 Hz, 1H, H-22b), 3.96 (d, *J*=11.1 Hz, 1H, H-28a), 4.07 (d, *J*=11.1 Hz, 1H, H-28b), 4.47–4.52 (m, 1H, H-3α), 5.49 (dd, *J*=7.6, 2.3 Hz, 1H, H-21). MS-EI: *m/z* (%)=556 (37) $[M^+]$, 541 (2), 513 (1), 496 (10), 436 (2), 293 (100), 233 (57), 219 (11), 203 (13), 189 (22). Anal. Calcd for $C_{34}H_{52}O_4S$: C, 73.34; H, 9.41; S, 5.76. Found: C, 73.21; H, 9.50; S, 5.88.

4.2.8. Reaction of unsaturated aldehyde ***10***

Starting with aldehyde **10** (300 mg, 0.56 mmol) in toluene (4 h) and chromatography (phase 6:1) gave two products.

(P-*R*) Oxathiaphosphinine **23a**: (164 mg, 40%), mp 212–213 °C (MeOH–$CHCl_3$); $[\alpha]_D^{20}$ −18 (*c* 0.54). IR ($CHCl_3$): 1724, 1651, 1595, 1463, 1258, 1115, 1030. ^{1}H NMR ($CDCl_3$): δ=0.84 (s, 3H), 0.85 (s, 6H), 0.97 (s, 3H), 1.03 (s, 3H), 2.04 (s, 3H), 2.08 (s, 3H), (7×CH_3), 2.42 (td, *J*=11.1, 6.1 Hz, 1H, H-19β), 3.33 (t, *J*=16.5 Hz, 1H, H-29a), 3.71 (dd, *J*=15.9, 12.7 Hz, 1H, H-29b), 3.85 (d, *J*=10.8 Hz, 1H, H-28b), 3.87 (s, 3H, OCH_3), 4.22 (d, *J*=10.8 Hz, 1H, H-28a), 4.49 (dd, *J*=11.1, 5.3 Hz, 1H, H-3α), 6.40 (d, *J*= 20.3 Hz, 1H, H-30), 6.99 (dd, *J*=8.7, 3.2 Hz, 2H, 2×Ar-*H*), 7.92 (dd, *J*=14.7, 8.7 Hz, 2H, 2×Ar-*H*). ^{31}P{^{1}H} NMR ($CDCl_3$): δ=80.53 (s, 1P). MS-EI: *m/z* (%)=742 (3) $[M^+]$, 727 (1), 588 (9), 556 (4), 540 (10), 496 (1), 480 (7), 465 (5), 437 (6), 407 (5), 261 (12), 215 (15), 202 (87), 189 (100). Anal. Calcd for $C_{41}H_{59}PO_6S_2$: C, 66.28; H, 8.00; S, 8.63. Found: C, 66.11; H, 8.21; S, 8.69.

(P-*S*) Oxathiaphosphinine **23b**: (170 mg, 41%), mp 229–231 °C (MeOH–$CHCl_3$); $[\alpha]_D^{20}$ −32 (*c* 0.57). IR ($CHCl_3$): 1725, 1650, 1594, 1464, 1254, 1111, 1029. ^{1}H NMR ($CDCl_3$): δ=0.80 (s, 3H), 0.84 (s, 6H), 0.85 (s, 3H), 1.00 (s, 3H), 2.05 (s, 3H), 2.07 (s, 3H), (7×CH_3), 2.40 (td, *J*=11.4, 6.0 Hz, 1H, H-19β), 3.10 (ddd, *J*=15.9, 9.9, 1.7 Hz, 1H, H-29a), 3.23 (dd, *J*=20.8, 16.2 Hz, 1H, H-29b), 3.78 (d, *J*= 11.0 Hz, 1H, H-28a), 3.85 (s, 3H, OCH_3), 4.20 (d, *J*=11.1 Hz, 1H, H-28b), 4.45–4.50 (m, 1H, H-3α), 6.47 (dd, *J*=20.6, 1.7 Hz, 1H, H-30), 6.96 (dd, *J*=8.7, 3.4 Hz, 2H, 2×Ar-*H*), 7.77 (dd, *J*=14.3, 8.9 Hz, 2H, 2×Ar-*H*). ^{31}P{^{1}H} NMR ($CDCl_3$): δ=78.70 (s, 1P). MS-EI: *m/z* (%)=742 (2) $[M^+]$,

727 (1), 588 (13), 556 (8), 540 (11), 496 (2), 480 (7), 465 (5), 437 (6), 407 (5), 261 (12), 215 (17), 202 (73), 189 (100). Anal. Calcd for $C_{41}H_{59}PO_6S_2$: C, 66.28; H, 8.00; S, 8.63. Found: C, 66.15; H, 8.18; S, 8.60.

*4.2.9. Reaction of hydroxyketone **11***

Starting with ketone **11** (300 mg, 0.66 mmol) in toluene (5 h), chromatography (phase 4:1) and crystallization from MeOH gave dithiaphospholane **24** (225 mg, 52%).

Mp 266–268 °C; $[\alpha]_D^{20}$ +21 (*c* 0.53). IR ($CHCl_3$): 1593, 1497, 1295, 1256, 1179, 1097, 1030, 830. ^{1}H NMR ($CDCl_3$): δ=0.76 (s, 3H), 0.90 (s, 3H), 0.91 (s, 3H), 1.01 (s, 3H), 1.02 (s, 3H), 1.14 (s, 3H), 1.19 (s, 3H), (7×CH_3), 3.44 (d, *J*=7.8 Hz, 1H, H-28a), 3.51 (br s, 1H, H-19α), 3.76 (dd, *J*=7.8, 0.4 Hz, 1H, H-28b), 3.87 (s, 3H, OCH_3), 4.28 (s, 1H, H-3β), 5.86 (d, *J*=4.6 Hz, 1H, H-1), 6.99 (dd, *J*=8.9, 3.4 Hz, 2H, 2×Ar-*H*), 8.07 (dd, *J*=15.0, 8.9 Hz, 2H, 2×Ar-*H*). ^{31}P{^{1}H} NMR ($CDCl_3$): δ=83.55 (s, 1P). MS-EI: *m/z* (%)=656 (100) [M^+], 623 (12), 454 (23), 421 (9), 235 (7), 215 (5), 201 (12), 191 (5). Anal. Calcd for $C_{37}H_{53}PO_2S_3$: C, 67.64; H, 8.13; S, 14.64. Found: C, 67.51; H, 8.22; S, 14.58.

*4.2.10. Reaction of hydroxyketone **12***

Starting with ketone **12** (300 mg, 0.66 mmol) in toluene (5 h), chromatography (phase 4:1) and crystallization from MeOH gave dioxaphospholane **25** (177 mg, 43%).

Mp 109–111 °C; $[\alpha]_D^{20}$ +46 (*c* 0.17). IR ($CHCl_3$): 1595, 1457, 1260, 1120, 1029, 864. ^{1}H NMR ($CDCl_3$): δ=0.80 (s, 3H), 0.93 (s, 3H), 0.94 (s, 3H), 1.02 (s, 3H), 1.05 (s, 3H), 1.10 (s, 3H), 1.15 (s, 3H), (7×CH_3), 1.99 (d, *J*=15.9 Hz, 1H, H-1a), 2.32 (d, *J*=15.9 Hz, 1H, H-1b), 3.46 (d, *J*=7.8 Hz, 1H, H-28a), 3.54 (br s, 1H, H-19α), 3.78 (d, *J*=7.5 Hz, 1H, H-28b), 3.86 (s, 3H, OCH_3), 6.96 (dd, *J*=8.7, 3.4 Hz, 2H, 2×Ar-*H*), 7.83 (dd, *J*=14.7, 8.9 Hz, 2H, 2×Ar-*H*). ^{31}P{^{1}H} NMR ($CDCl_3$): δ=103.63 (s, 1P). MS-EI: *m/z* (%)=624 (100) [M^+], 609 (32), 593 (4), 438 (1), 282 (3), 205 (3), 189 (2). Anal. Calcd for $C_{37}H_{53}O_4PS$: C, 71.12; H, 8.55; S, 5.13. Found: C, 71.01; H, 8.66; S, 4.98.

*4.2.11. Reaction of ketoacid **13***

Starting with acid **13** (500 mg, 0.98 mmol) in toluene (2 h), chromatography (phase 9:1) and crystallization from isopropanol gave diene **26** (273 mg, 62%).

Mp 228–230 °C; $[\alpha]_D^{20}$ +28 (*c* 0.36). IR ($CHCl_3$): 1723, 1602, 1465, 1371, 1257, 1028. ^{1}H NMR ($CDCl_3$): δ=0.62 (d, *J*=6.8 Hz, 3H), 0.87 (s, 3H), 0.88 (s, 3H), 0.89 (s, 3H), 0.91 (d, *J*=6.8 Hz, 3H), 0.98 (s, 3H), 1.00 (s, 3H), (7×CH_3), 1.95–2.20 (m, 5H), 2.06 (s, 3H, $COCH_3$), 2.34 (m, 1H, H-22b), 2.90 (m, 1H, H-19β), 4.49–4.54 (m, 1H, H-3α), 5.40 (dd, *J*=4.6, 3.1 Hz, 1H, H-12). MS-EI: *m/z* (%)=452 (100) [M^+], 437 (13), 409 (81), 392 (12), 377 (7), 349 (45), 267 (18), 255 (19), 239 (4), 213 (6), 202 (10), 189 (15). Anal. Calcd for $C_{31}H_{48}O_2$: C, 82.24; H, 10.69. Found: C, 82.38; H, 10.77.

*4.2.12. Reaction of anhydride **14***

Starting with anhydride **14** (500 mg, 0.88 mmol) in *o*-xylene (50 h), chromatography (phase 6:1) and crystallization from mixture MeOH–$CHCl_3$ gave thioacetate **27** (231 mg, 45%).

Mp 239–241 °C; $[\alpha]_D^{20}$ +94 (*c* 0.40). IR ($CHCl_3$): 1788, 1742, 1626, 1459, 1365, 1282, 1272, 1057, 909. ^{1}H NMR ($CDCl_3$): δ=0.87 (s, 3H), 0.92 (s, 3H), 0.93 (s, 3H), 0.94 (s, 3H), 1.12 (s, 3H), 1.14 (d, *J*=6.8 Hz, 3H), 1.31 (d, *J*=6.8 Hz, 3H), (7×CH_3), 1.77 (td, *J*=13.1, 3.4 Hz, 1H), 1.86–1.95 (m, 3H), 2.02 (s, 3H, $COCH_3$), 2.05 (m, 1H), 2.53 (td, *J*=14.5, 3.5 Hz, 1H), 2.57 (s, 3H, $COCH_3$), 2.73 (dd, *J*=12.5, 2.3 Hz, 1H, H-13β), 3.25 (septet, *J*=6.9 Hz, 1H, H-20), 3.89 (d, *J*=11.0 Hz, 1H, H-28a), 4.54 (d, *J*=11.0 Hz, 1H, H-28b), 5.15 (dd, *J*=11.9, 4.6 Hz, 1H, H-3α). MS-EI: *m/z* (%)=586 (not found) [M^+], 510 (24), 469 (100), 425 (12), 291 (6), 265 (21), 231 (5), 217 (7), 205 (64), 189 (45). Anal. Calcd for $C_{34}H_{50}O_6S$: C, 69.59; H, 8.59; S, 5.46. Found: C, 69.41; H, 8.69; S, 5.30.

*4.2.13. Reaction of β-ketoester **15***

Starting with β-ketoester **15** (500 mg, 0.97 mmol) in *o*-xylene (15 h), chromatography (phase 6:1) gave three products.

Bis(thioacetate) **28a**: (143 mg, 27%), mp 200–202 °C (MeOH–$CHCl_3$); $[\alpha]_D^{20}$ +25 (*c* 1.13). IR ($CHCl_3$): 1736, 1715, 1452, 1270, 1255, 1027. ^{1}H NMR ($CDCl_3$): δ=0.87 (s, 3H), 0.93 (s, 6H), 0.97 (s, 3H), 1.13 (s, 3H), (5×CH_3), 1.76 (td, *J*=13.3, 3.4 Hz, 1H), 1.87 (ddd, *J*=13.3, 8.1, 4.0 Hz, 1H), 1.97–2.06 (m, 2H), 2.51 (dt, *J*=15.1, 4.6 Hz, 1H), 2.56 (s, 6H, 2×$CSCH_3$), 2.60 (dd, *J*=11.9, 3.5 Hz, 1H, H-13β), 3.78 (s, 3H, OCH_3), 4.78 (d, *J*=11.3 Hz, 1H, H-28a), 4.90 (d, *J*=11.4 Hz, 1H, H-28b), 5.15 (dd, *J*=11.8, 4.6 Hz, 1H, H-3α). MS-EI: *m/z* (%)=550 (not found) [M^+], 475 (100), 431 (11), 417 (22), 399 (37), 371 (13), 339 (12), 339 (11), 271 (15), 231 (8), 205 (14), 191 (15). Anal. Calcd for $C_{30}H_{46}O_5S_2$: C, 65.42; H, 8.42; S, 11.64. Found: C, 65.29; H, 8.59; S, 11.58.

Thioacetate **28b**: (108 mg, 21%), mp 251–253 °C (MeOH–$CHCl_3$); $[\alpha]_D^{20}$ +38 (*c* 1.28). IR ($CHCl_3$): 1731 sh, 1455, 1376, 1255, 1031. ^{1}H NMR ($CDCl_3$): δ=0.846 (s, 3H), 0.854 (s, 3H), 0.90 (s, 3H), 0.96 (s, 3H), 1.12 (s, 3H), 2.05 (s, 3H), (6×CH_3), 2.50 (dt, *J*=15.2, 4.8 Hz, 1H), 2.56 (s, 3H, $CSCH_3$), 2.59 (dd, *J*=12.0, 3.6 Hz, 1H, H-13β), 3.78 (s, 3H, OCH_3), 4.48 (dd, *J*=10.8, 5.2 Hz, 1H, H-3α), 4.78 (d, *J*=11.4 Hz, 1H, H-28a), 4.90 (d, *J*=11.4 Hz, 1H, H-28b). MS-EI: *m/z* (%)=534 (not found) [M^+], 474 (8), 459 (13), 431 (12), 415 (5), 398 (9), 355 (7), 271 (100), 213 (13), 204 (14), 190 (63). Anal. Calcd for $C_{30}H_{46}O_6S$: C, 67.38; H, 8.67; S, 6.00. Found: C, 67.21; H, 8.81; S, 5.88.

Thioacetate **28c**: (165 mg, 45%), mp 194–195 °C (MeOH–$CHCl_3$); $[\alpha]_D^{20}$ +49 (*c* 0.26). IR ($CHCl_3$): 1738, 1713, 1453, 1368, 1269, 1252, 1029. ^{1}H NMR ($CDCl_3$): δ=0.87 (s, 3H), 0.93 (s, 6H), 0.95 (s, 3H), 1.14 (s, 3H), (5×CH_3), 1.76 (td, *J*=13.3, 3.4 Hz, 1H), 1.83–1.89 (m, 1H), 2.06 (s, 3H, $COCH_3$), 2.41–2.49 (m, 1H), 2.57 (s, 3H, $CSCH_3$), 2.61 (dd, *J*=11.9, 3.5 Hz, 1H, H-13β), 3.77 (s, 3H, OCH_3), 4.45 (d, *J*=11.1 Hz, 1H, H-28a), 4.56 (d, *J*=11.1 Hz, 1H, H-28b), 5.15 (dd, *J*=11.8, 4.6 Hz, 1H, H-3α). MS-EI: *m/z* (%)=534 (not found) [M^+], 459 (100), 417 (12), 399 (4), 255 (11), 231 (5), 205 (7), 191 (6). Anal.

Calcd for $C_{30}H_{46}O_6S$: C, 67.38; H, 8.67; S, 6.00. Found: C, 67.28; H, 8.77; S, 5.93.

4.3. X-ray crystallographic data[28]

Single crystals of **23b** were grown from mixture methanol–chloroform.

Crystal data for **23b**: $C_{41}H_{59}O_6PS_2$, M=742.97, Orthorhombic, $P2_12_12_1$ (No 19), a=8.9959(1) Å, b=17.9408(2) Å, c=24.2538(3) Å, V=3914.41(8) Å^3, Z=4, D_x=1.261 Mg m^{-3}. A colorless crystal of dimensions 0.15×0.17×0.60 mm was mounted on glass capillary with epoxy glue and measured at Nonius Kappa CCD diffractometer by monochromatized Mo Kα radiation (λ=0.71073 Å) at 150(2) K. An absorption was neglected (μ=0.222 mm^{-1}); a total of 62,352 measured reflections in the range h=−11 to 11, k=−23 to 23, l=−31 to 31 (θ_{max}=27.5°), from which 8957 were unique (R_{int}=0.048), 8159 observed according to the $I>2\sigma(I)$ criterion. Cell parameters from 5032 reflections (θ=1–27.5°). The structure was solved by direct methods (SIR92)[29] and refined by full-matrix least squares based on F2 (SHELXL97).[30] The hydrogen atoms were fixed into idealized positions (riding model) and assigned temperature factors either $H_{iso}(H)$=1.2 U_{eq}(pivot atom) or $H_{iso}(H)$=1.5 U_{eq}(pivot atom) for methyl moiety. The refinement converged (Δ/σ_{max}=0.000) to R=0.0382 for observed reflections and wR=0.0945, GOF=1.03 for 459 parameters and all 8957 reflections. The final difference map displayed no peaks of chemical significance ($\Delta\rho_{max}$=1.02, $\Delta\rho_{min}$=−0.34 e Å^{-3}). The absolute configuration was determined unambiguously from anomalous dispersion in agreement to the known configuration of lupane moiety (chiral parameter −0.06(5)).

Selected bond lengths (Å) and angles (°): C(19)–C(22) 1.518(3), C(22)C(23) 1.507(3), S(1)–C(23) 1.841(2), S(1)–P(1) 2.0696(7), P(1)–O(1) 1.6275(16), O(1)–C(24) 1.412 (3), C(22)–C(24) 1.321(3), S(2)–P(1) 1.9271(8), P(1)–C(35) 1.7944(19). C(19)–C(22)–C(23) 117.29(16), S(1)–C(23)–C(22) 115.31 (13), P(1)–S(1)–C(23) 95.74(7), S(1)–P(1)–O(1) 103.73(6), P(1)–O(1)–C(24) 121.93(13), O(1)–C(24)–(22) 128.37(18), C(23)–C(22)–C(24) 125.02(18), C(19)–C(22)–C(24) 117.67 (17), O(1)–P(1)–C(35) 104.52(9), S(1)–P(1)–S(2) 113.76(3), S(2)–P(1)–O(1) 110.20(6).

4.4. Cytotoxic MTT assay

Screening of cytotoxic activity was performed on highly chemosensitive T-lymphoblastic leukemia CEM cells using cytotoxic MTT assay.[31] The cells were prepared and diluted according to the expected target cell density (5000 cells/well). The cells were added by pipette (80 μL) into 96-well microtiter plates. Inoculates were allowed a pre-incubation period of 24 h at 37 °C and 5% CO_2 for stabilization. Fourfold dilutions, in 20 μL aliquots, of the intended test concentration were added at time zero to the microtiter plate wells. All tested compounds were dissolved in 10% DMSO and concentrations were examined in duplicate. Incubation of the cells with the test compounds lasted for 72 h at 37 °C, in a 5% CO_2 atmosphere at 100% humidity. At the end of the incubation period, the cells were assayed using MTT. Aliquots (10 μL) of the MTT stock solution were pipetted into each well and incubated for a further 1–4 h. After this incubation period formazan produced was dissolved by the addition of 100 μL/well of 10% aq SDS (pH=5.5), followed by a further incubation at 37 °C overnight. The optical density (OD) was measured at 540 nm with a Labsystem iEMS Reader MF. Tumor cell survival (TCS) was calculated using the following equation: TCS=($OD_{drug\text{-}exposed\ well}$/mean $OD_{control\ wells}$)×100%. The TCS_{50} value, the drug concentration lethal to 50% of the tumor cells, was calculated from appropriate dose–response curves.

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Supplementary data

^{13}C NMR data of compounds **16–22**, **23a**, **23b**, **24–27**, and **28a–c** are provided. Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2008.02.023.

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