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Original article

Hydrogenative desulphurization of thienopyrrolizinones: An easy and selective access to (*Z*)-phenethylidenepyrrolizinones with in vitro cytotoxic activity

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ABSTRACT

Attempts in view to dearomatize some previously reported tripentones with potent antineoplastic activities led in thiophene series to an unexpected hydrogenative desulphurization reaction. The resulting (*Z*)-phenethylidenepyrrolizinones were tested in vitro over human epidermoid carcinoma KB cell line. The results of this biological evaluation indicated that the tricyclic core of our model can be cleaved with a partial respect of the activity.

1. Introduction

We have previously reported the synthesis and the biological evaluation of an original series of arylthienopyrrolizinones named by us "tripentones" [1]. Some members of this new family including compound MR22388 **1**, exhibit an *in vitro* cytotoxic activity in the nanomolar range associated to an anti-tubulin effect and to a cyclin-dependent kinase (CDK) inhibition (Fig. 1) [2,3].

In order to better understand both the SAR and the cellular mechanism of action in this series, we undertook recently some pharmacomodulations of MR22388 aiming at diversifying the nature of the aromatic ring by replacing its thiophene either by a pyrrole or a pyrazole ring or at evaluating the enlargement of the pyrrolizine moiety into a pyrrolopyrazinone system [4–7]. In order to complete this study, we wish to describe herein our attempts to partially dearomatize our thienopyrrolizinones in order to potentially enhance their cytotoxicity, taking for model some tetrahydro-5*H*-pyrrolo[2,1-*a*]isoindolones whose potent CDK1,2,4,6 inhibitory activities were recently reported (Fig. 2) [8,9].

2. Chemistry

The previously reported chemical sequence leading to 3-(3-hydroxy-4-methoxy)phenyl-8*H*-thieno[2,3-*b*]pyrrolizin-8-one **1** was achieved in seven steps starting from (3-benzyloxy-4-methoxy)phenyl-acetonitrile **2** (Scheme 1) [3].

Very few examples of dearomatization of pyrrolizinone into pyrrolizidinone are described in literature. To the best of our knowledge, only one article reported it in 1960 [10]. This reaction took place in two steps. The first one consisted in rhodium-on-alumina hydrogenation of both the pyrrole nucleus of **10** into pyrrolidine and of its carbonyl group into a hydroxyl one. A second step was needed to re-oxidize, according to the Oppenauer's conditions, the alcohol **11** into the expected pyrrolizinone **12** (Scheme 2).

Concerning the tripentone series for which no hydrogenation attempts were hitherto undertaken, we ran several reactions using H_2 in the presence of various catalysts on charcoal like Pd, Rh or PtO_2, in a Paar apparatus under atmospheric pressure. However, all our attempts achieved either starting from **1** or from its protected *O*-benzyl derivative **9** failed to reduce the aromatic rings of the tripentone system. The starting material was recovered intact in each case except for the PtO_2 hydrogenation where an *O*-debenzylation of **9** took place partially yielding **1**.

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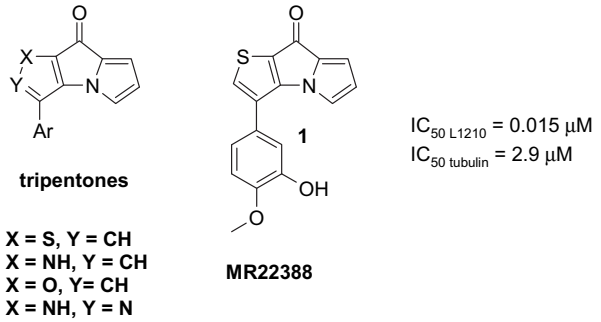


Fig. 1. Structure of tripentones and IC_{50} values for compound MR22388.

The well-known potent catalyst-poisoning properties of the thiophene ring were invoked to explain these failures [11]. Taking that reactivity into account, we attempted the reduction of one of the heterocyclic isosters of MR22386 we previously described, the pyrrole tripentone **13** [6]. Under treatment with H_2 in the presence of catalytic amount of palladium–charcoal in MeOH under atmospheric pressure, the latter furnished successfully in good yield the corresponding partially dearomatized tripentone **14** (Scheme 3). During this reaction, only the pyrrolizine ring of **13** was reduced into a tetrahydro one, its *N*-benzyl pyrrole ring, as well as its carbonyl group remained intact. However, in the same time the *O*-benzyl protecting group of **13** was selectively cleaved to liberate the free hydroxyl, while its *N*-benzyl group remained unreactive.

Since it was also well established that Raney Nickel was less poisoned by thiophene in determinate solvent, **1** was then hydrogenated in the presence of this catalyst, in MeOH, under atmospheric pressure and at room temperature. In these conditions a hydrogenative desulphurization occurred leading to the phenethylidenepyrrolizinone **15**. The latter was obtained in 67% yield selectively in its *Z* form (Scheme 4). Surprisingly, no hydrogenation of the alkene moiety of **15** was observed under those conditions, probably due to its conjugation with the phenyl ring.

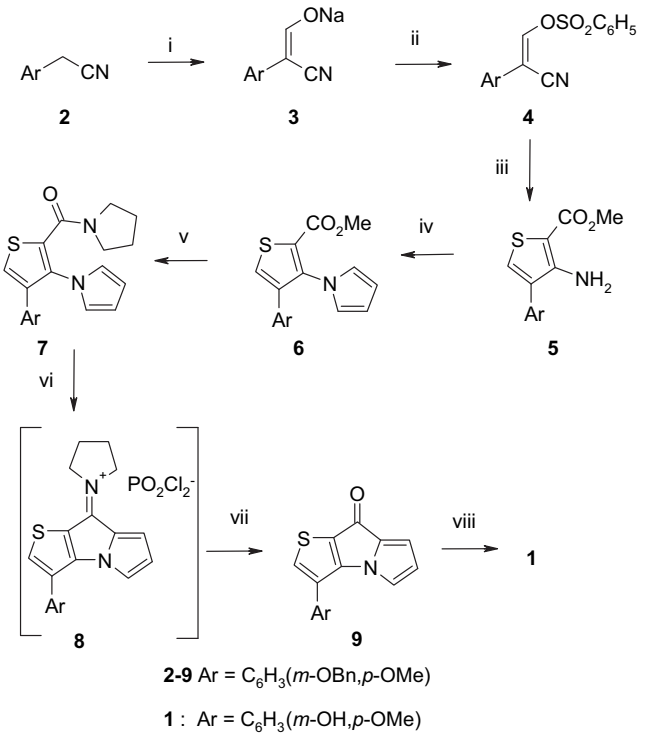
Structure of **15** was assigned on the basis of ^{2}D NMR spectra, completed by NOE experiments. Specifically, irradiation of protons H2 led to an NOE with the *cis* methyl group, without effect on H2″ and H6″ protons of the *trans* phenyl ring. These observations were consistent with a *Z* relative configuration for **15** (Fig. 3).

This unexpected reaction led us to exemplify it starting from other thienopyrrolizinones **16**–**18**, we previously described [2,3]. In all cases, a Raney Nickel desulphurization took place leading to **19**–**22** (Scheme 4). The *p*-MeO and *p*-Cl phenethylidene derivatives **19**, **20** were again selectively formed in their *Z* relative configuration. The 3-methyltripentone **18** conduced in the same conditions to a mixture of the isopropylidene **21** and isopropylpyrrolizinone **22**. The absence of any aromatic ring conjugated with the alkene of **21** implied a higher reactivity of this one to hydrogenation.

3. Biology

Compounds synthesized in this work were tested for their in vitro cytotoxicity against KB cells at 10^{-5} and 10^{-6} M

Fig. 2. Structure of expected tetrahydropyrrolizinones and reference tetrahydropyrrolo-isoindolones.



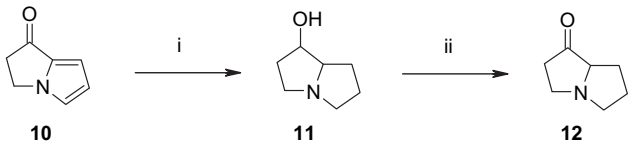
Scheme 1. Reagents: (i) HCO_2Et, MeONa, MeOH; (ii) benzenesulfonyl chloride, DMF; (iii) $HSCH_2CO_2Me$, MeONa, MeOH; (iv) 2,5-dimethoxyTHF, 4-chloropyridine hydrochloride, dioxane; (v) pyrrolidine; (vi) $POCl_3$; (vii) 2.5 M NaOH; (viii) 33% HBr in AcOH.

concentrations in triplicate, using taxotere at 2.5×10^{-10} M as reference. The results are expressed in terms of inhibition percentage at each concentrations and IC_{50} were measured for percentage greater than 80% at 10^{-6} M (Table 1).

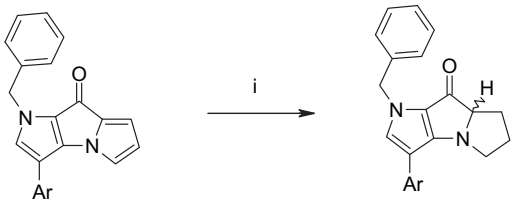
Our lead compound MR22388 (**1**) exhibited an IC_{50} of 5.7 nM against KB cells, confirming its cytotoxicity already observed against a large panel of cell lines [3]. During the course of our study, it has been proven that the replacement of the thiophene ring by various isosteres had a great influence on the activity of this series [3,5,6]. The hydrogenative desulphurization of **1** conduced for the first time to a novel phenethylidenepyrrolizinone **15** with a good cytotoxicity over human KB cells ($IC_{50} = 70$ nM). According to the initial SAR highlighted for the MR22388 series, replacement of the *m*-OH, *p*-OMe phenyl groups of **15** by other substituents dramatically decreases the activity. This moiety appears thus closely implied again in the biological activity of **15** in a similar manner as established for some antimitotic drugs including, for example, combretastatin A4, anthracenone (**23**) and naphthothiophenone (**24**), whose analogy with **15** must be pointed out (Fig. 4) [12–15]. This result gives us some important information in order to explore new developments of our series.

4. Conclusion

Even if these results do not improve the antineoplastic activity inherent to some tripentones, they allowed us to work out an original method of desulphurisation in thiophene series leading to



Scheme 2. Dearomatization of pyrrolizinone **10** into pyrrolizidinone **12**. Reagents: (i) H_2 (42 psi), Rh/alumina, AcOH; (ii) $Al[OC(CH_3)_3]_3$, toluene, cyclohexanone.



Scheme 3. Synthesis of compound **14**. Reagents: (i) $H_{2\ atmP}$, Pd/C, MeOH.

novel phenethylidenepyrrolizinones. This work furthermore opens novel possibilities to diversify the tripentone structure towards new biologically active series that partially conserve the cytotoxicity. These compounds must be considered as new hits whose pharmacomodulation is currently under investigation.

5. Experimental protocols

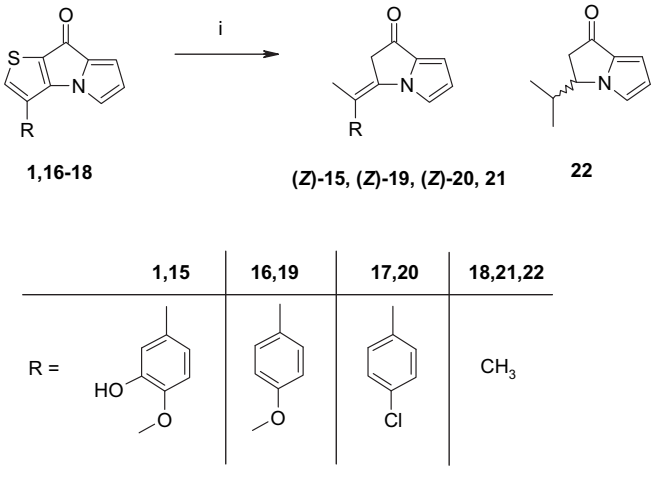
5.1. General

Melting points were determined on a Kofler melting point apparatus and are uncorrected. IR spectra were recorded on a Genesis series FTIR spectrometer using KBr pellets. The ^1H (400 MHz) and ^{13}C (100 MHz) NMR spectra were obtained on a Jeol Lambda 400 spectrometer using DMSO-d_6 or $CDCl_3$ as solvent and TMS as internal standard. The chemical shifts (δ) are reported in ppm, and the coupling constants are in hertz. NOE experiments were recorded on a Bruker AC 400 spectrometer and the samples were degassed by bubbling nitrogen through the solution. Electron impact mass spectra (EIMS) were obtained using a Jeol JMS GCMate spectrometer. Reactions were monitored by thin-layer chromatography (TLC) using 0.2 mm Polygram Sil silica gel G/UV 254 precoated plates with visualization by irradiation with a short-wavelength UV light. Silica gel flash chromatography was performed using 63–200 mM Kieselgel Merck 60 silica gel.

5.2. Chemistry

5.2.1. 1-Benzyl-3-(3-hydroxy-4-methoxyphenyl)-5,6,7,7a-tetrahydropyrrolo[2,3-b]pyrrolizin-8(1H)-one (**14**)

A solution of 150 mg (0.33 mmol) of **13** in methanol (50 mL) was hydrogenated at room temperature and atmospheric pressure over Pd/C for 90 min. The solution was filtered from catalyst through filter paper, then through a small pad of Celite and dried over $MgSO_4$. Filtration and evaporation afforded 80 mg of **14** as an orange solid. Yield: 73%. MP: 120 °C. IR (cm^{-1}): 3272, 2936, 2901, 2835, 1651, 1563, 1418, 1257, 700. HRMS (m/z): 374.16448 (calc: 374.16302). ^1H NMR ($CDCl_3$) δ: 7.32 (m, 5H, H_{arom}), 7.05 (s, 1H, H_2), 7.04 (d, 1H, $J = 1.9$ Hz, $H_{2'}$), 6.98 (dd, 1H, $J = 1.9$ Hz, $J = 8.3$ Hz, $H_{6'}$), 6.83 (d, 1H, $J = 8.3$ Hz, $H_{5'}$), 5.20 (d, 1H, $J = 14.5$ Hz, NCHPh), 5.07 (d, 1H, $J = 14.5$ Hz, NCHPh), 4.33 (m, 1H, H_{7a}), 3.89 (s, 3H, OCH_3), 3.31 (m, 1H, H_7), 2.94 (m, 1H, H_7), 2.17 (m, 1H, H_5), 1.90 (m, 3H, $2H_6$ and



Scheme 4. Synthesis of compounds **15**, **19**–**22**. Reagents: (i) $H_{2\ atmP}$, Raney Ni, MeOH.

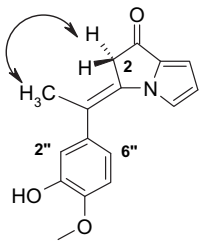
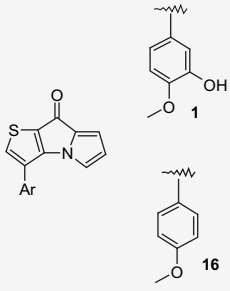
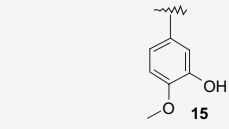
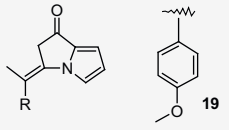
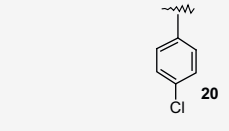
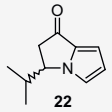


Fig. 3. NOE correlations for **15**.

Table 1
Percentage of inhibition of KB cells growing at various concentrations and IC_{50} for taxotere and compounds **1**, **15**, **19**, **20**, **22**.

Cmpd	10^{-5} M (%)	10^{-6} M (%)	$IC_{50} \pm SD$ (nM)
Taxotere 2.5×10^{-10} M	–	–	0.4
 **1**	86	86	5.7 ± 1.15
16	84	92	50 ± 10
 **15**	95	91	70 ± 5.2
 **19**	78	1	–
 **20**	0	0	–
 **22**	0	0	–

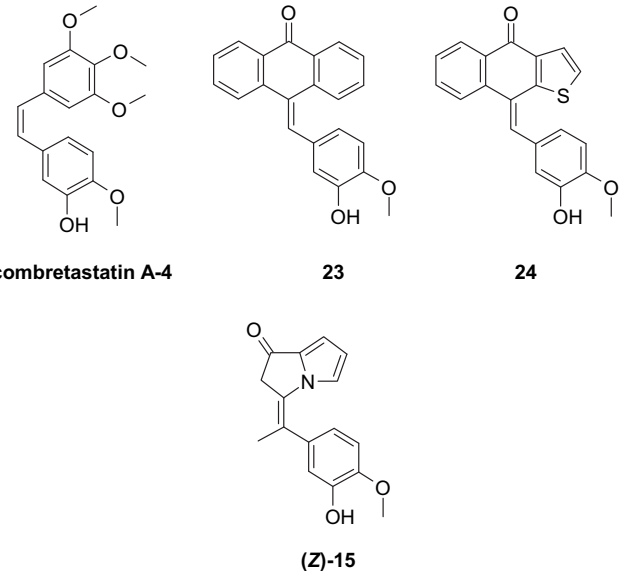


Fig. 4. Structure of combretastatin A-4 and compounds **23**, **24**; analogy with compound **15**.

H_5). ^{13}C NMR ($CDCl_3$) δ: 188.29 (C8), 164.00 (C3a), 145.86, 145.08, 136.69, 132.90, 128.86, 128.18, 128.12, 126.35, 122.66, 117.70, 112.29, 111.67, 110.97, 78.89 (C7a), 56.03 (OCH_3), 51.56 (CH_2), 50.89 (C5), 27.77 (C7), 27.03 (C6).

*5.2.2. (3Z)-3-[1-(3-Hydroxy-4-methoxyphenyl)ethylidene]-2,3-dihydro-1H-pyrrolizin-1-one (**15**)*

A solution of 200 mg (0.67 mmol) of **1** in methanol (100 mL) was hydrogenated at room temperature and atmospheric pressure over Ni-Raney for 2 h. The solution was filtered from catalyst through filter paper, then through a small pad of Celite and dried over $MgSO_4$. The solvent was removed under reduced pressure and the residue was purified by column chromatography on silica gel (EtOAc/cyclohexane 1:3) to give 120 mg of **15** as a white solid. Yield: 67%. MP: 170 °C. IR (cm^{-1}): 3295, 3143, 3002, 2937, 1688, 1537, 1509, 1360, 1296, 1258, 1145, 1029, 819, 748, 662. HRMS (*m*/*z*): 269.10484 (calc: 269.10518). ^1H NMR ($CDCl_3$) δ: 6.92 (d, 1H, $J = 8.3$ Hz, H5″), 6.83 (d, 1H, $J = 2.0$ Hz, H2″), 6.75 (dd, 1H, $J = 2.0$ Hz, $J = 8.3$ Hz, H6″), 6.69 (d, 1H, $J = 3.9$ Hz, H5), 6.25 (dd, 1H, $J = 2.7$ Hz, $J = 3.9$ Hz, H6), 6.16 (d, 1H, $J = 2.7$ Hz, H7), 5.70 (s, 1H, OH), 3.95 (s, 3H, OCH_3), 3.63 (s, 2H, 2H2), 2.05 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$) δ: 186.50 (C1), 146.34 (C3″), 146.27 (C4″), 134.51 (C7a), 133.47 (C1″), 126.26 (C3), 122.28 (C7), 119.61 (C6″), 118.21 (C1′), 116.68 (C6), 114.21 (C2″), 111.27 (C5″), 107.95 (C5), 56.00 (OCH_3), 43.44 (C2), 22.41 (CH_3).

*5.2.3. (3Z)-3-[1-(4-Methoxyphenyl)ethylidene]-2,3-dihydro-1H-pyrrolizin-1-one (**19**)*

Using the same procedure described for **15** and starting from a solution of 100 mg (35.5 mmol) of **16** in MeOH (30 mL), 35 mg of **19** was obtained as a brown solid. Yield: 39%. MP: 118 °C. IR (cm^{-1}): 1682, 1507, 1360, 1308, 1241, 1035, 839, 816, 759. HRMS (*m*/*z*): 253.11018 (calc: 253.11027). ^1H NMR ($CDCl_3$) δ: 7.19 (d, 2H, $J = 8.8$ Hz, H3″ and H5″), 6.98 (d, 2H, $J = 8.8$ Hz, H2″ and H6″), 6.69 (d, 1H, $J = 3.9$ Hz, H5), 6.24 (dd, 1H, $J = 2.7$ Hz, $J = 3.9$ Hz, H6), 6.09 (d, 1H, $J = 2.7$ Hz, H7), 3.87 (s, 3H, OCH_3), 3.64 (s, 2H, 2H2), 2.07 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$) δ: 186.53 (C1), 159.23 (C4″), 134.51 (C7a), 134.52 (C1″), 129.16 (C3″ and C5″), 126.33 (C3), 122.13 (C7), 118.28 (C1′), 116.66 (C6), 114.69 (C2″ and C6″), 107.92 (C5), 55.28 (OCH_3), 43.42 (C2), 22.5 (CH_3).

*5.2.4. (3Z)-3-[1-(4-Chlorophenyl)ethylidene]-2,3-dihydro-1H-pyrrolizin-1-one (**20**)*

Using the same procedure described for **15** and starting from a solution of 100 mg (34.9 mmol) of **17** in MeOH (30 mL), 30 mg of **20** was obtained as a brown solid. Yield: 33%. MP: 158 °C. IR (cm^{-1}): 1708, 1360, 1276, 1076, 737. HRMS (*m*/*z*): 257.06131 (calc: 257.06073). ^1H NMR ($CDCl_3$) δ: 7.37 (d, 2H, $J = 8.8$ Hz, H3″ and H5″), 7.15 (d, 2H, $J = 8.8$ Hz, H2″ and H6″), 6.63 (d, 1H, $J = 3.9$ Hz, H5), 6.19 (dd, 1H, $J = 2.7$ Hz, $J = 3.9$ Hz, H6), 6.00 (d, 1H, $J = 2.7$ Hz, H7), 3.57 (s, 2H, 2H2), 2.01 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$) δ: 186.53 (C1), 159.23 (C4″), 134.51 (C7a), 134.52 (C1″), 129.16 (C3″ and C5″), 126.33 (C3), 122.13 (C7), 118.28 (C1′), 116.66 (C6), 114.69 (C2″ and C6″), 107.92 (C5), 55.28 (OCH_3), 43.42 (C2), 22.5 (CH_3).

*5.2.5. 3-Isopropylidene-2,3-dihydro-1H-pyrrolizin-1-one (**21**) and 3-isopropyl-2,3-dihydro-1H-pyrrolizin-1-one (**22**)*

Using the same procedure described for **15** and starting from a solution of 100 mg (52.8 mmol) of **18** in MeOH (30 mL), 35 mg of **21** and 30 mg of **22** were obtained as a yellow solid and a brown oil respectively.

21: Yield: 41%. MP: 96 °C. IR (cm^{-1}): 1683, 1356, 1302, 1106, 1030, 748. HRMS (*m*/*z*): 161.08397 (calc: 161.08405). ^1H NMR ($CDCl_3$) δ: 7.43 (d, 1H, $J = 2.9$ Hz, H7), 6.78 (d, 1H, $J = 3.9$ Hz, H5), 6.56 (dd, 1H, $J = 2.9$ Hz, $J = 3.9$ Hz, H6), 3.50 (s, 2H, 2H2), 2.06 (s, 3H, CH_3), 1.85 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$) δ: 186.53 (C1), 159.23 (C4″), 134.51 (C7a), 134.52 (C1″), 129.16 (C3″ and C5″), 126.33 (C3), 122.13 (C7), 118.28 (C1′), 116.66 (C6), 114.69 (C2″ and C6″), 107.92 (C5), 55.28 (OCH_3), 43.42 (C2), 22.5 (CH_3).

22: Yield: 35%. IR (cm^{-1}): 1687, 1526, 1366, 1304, 1071, 741. HRMS (*m*/*z*): 163.09995 (calc: 163.09972). ^1H NMR ($CDCl_3$) δ: 7.04 (d, 1H, $J = 2.9$ Hz, H7), 6.69 (d, 1H, $J = 3.9$ Hz, H5), 6.51 (dd, 1H, $J = 2.9$ Hz, $J = 3.9$ Hz, H6), 4.49 (dt, 1H, $J = 7.8$ Hz, $J = 3.5$ Hz, $J = 3.5$ Hz, H3), 3.03 (dd, 1H, $J = 18.1$ Hz, $J = 7.8$ Hz, H2a), 2.75 (dd, 1H, $J = 18.1$ Hz, $J = 3.5$ Hz, H2b), 2.25 (m, 1H, CH), 0.97 (d, 3H, $J = 6.8$ Hz, CH_3), 0.74 (d, 3H, $J = 6.8$ Hz, CH_3). ^{13}C NMR ($CDCl_3$) δ: 188.67 (C1), 132.52 (C7a), 121.27 (C7), 115.80 (C5), 106.17 (C6), 58.65 (C3), 40.72 (C2), 31.63 (CH), 17.31 (CH_3), 14.72 (CH_3).

5.3. Cell culture and proliferation assay

The human epidermoid carcinoma KB cell lines were obtained from ECACC (Salisbury, UK) and grown in D-MEM medium supplemented with 10% fetal calf serum (Invitrogen), in the presence of penicilline, streptomycine and fungizone in 75 cm^3 flask under 5% CO_2. Cells were plated in 96-well tissue culture microplates at a density of 650 cells/well in 200 µl medium and treated 24 h later with compounds dissolved in DMSO with compound concentrations ranged 0.5 nM to 10 µM using a Biomek 3000 automate (Beckman). Controls received the same volume of DMSO (1% final volume). After 72 h exposure MTS reagent (Promega) was added and incubated for 3 h at 37 °C: the absorbance was monitored at 490 nm and results expressed as the inhibition of cell proliferation calculated as the ratio [(OD490 treated/OD490 control) × 100]. For IC_{50} determinations (50% inhibition of cell proliferation) experiments were performed in separate duplicate.

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