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Synthesis, reactivity and biological evaluation of novel halogenated tripentones

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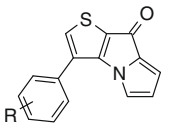
ABSTRACT

We describe herein the synthesis and the biological evaluation of a novel series of a potent anticancer agents: the tripentones. For the first time, a halogen atom was introduced in high yields on the pyrrole ring of the tricycle. This synthesis and the reactivity of the novel halogenated tripentones in metallo-catalysed cross-coupling reactions will be described in that article. Finally their influence on biological activity will be discussed.

1. Introduction

Anticancer researches at the laboratory have conduced to the discovery of the thienopyrrolizinones family (Fig. 1) or tripentones.[1] Many works have been carried out in that series that gave us crucial information and led to the discovery of MR22388 **8b** a potent anticancer agent.[2] However if **8b** appeared to possess low nanomolar activity against various cell lines its development has been confronted to a lack of bioavailability. We then decided to explore novel chemical modulations of the tricycle. Different analogs have been produced and evaluated replacing the thiophene ring by a pyrrole, furane, pyrazole or benzene one.[3]

If the biological activity is closely related to the presence of the thiophene and the 4-methoxy-3-hydroxyphenyl substituent very little information are available concerning the influence of substitution on the right hand part of the molecule and more precisely on the pyrrole ring.[4]



8a R=*p*OMemOBn
8b R=*p*OMemOH
8c R=pOMe

Figure 1.

Conserving the requirements for activity, our goal was first to explore the chemical access to these structures but also to rationalize their influence on activity.

Furthermore this substitution could be a good opportunity for us to introduce groups that improve the hydrosolubility of that particular series.

2. Chemistry

The general route towards the tripentone **8a**, the benzylated analog of MR22388 proceeded in an eight step synthesis from the corresponding arylacetonitrile **1** (Scheme 1).[1]

Crucial intermediates could be identified as the thiophenic *o*-aminoester **4a**. It could be obtained in three steps from **1a**, and involved in a Clauson-Kaas reaction[5] in order to introduce the pyrrole ring. The ring closure procedure developed in that series is using a Vilsmeier–Haack mechanism and therefore requests the synthesis of carboxamide **6a**.

This protected tripentone **8a** will be one of our starting materials for the novel pharmacomodulation. We will see that in many cases, a final deprotection reaction will be needed to liberate the phenolic group. Such a transformation will lead to novel analogs of MR22388 whose biological activity could be assessed.

Various routes could be employed to substitute the pyrrole moiety of the tripentone (Fig. 2). Indeed we could either introduce the substituent from the cyclised tripentones and beneficiate from the reactivity of the pyrrole ring to substitute the final tricyclic system (route A) or introduce it during the preparation of the pyrrole ring (route B) according to a modified Clauson-Kaas procedure. Results exposed in that paper will be focused on route A.

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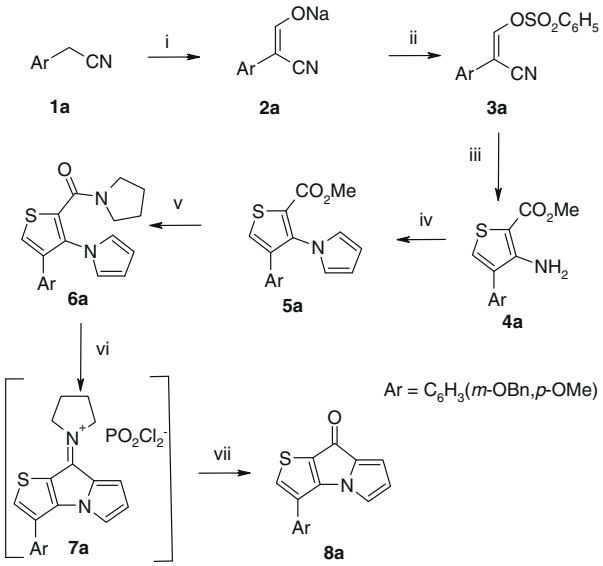
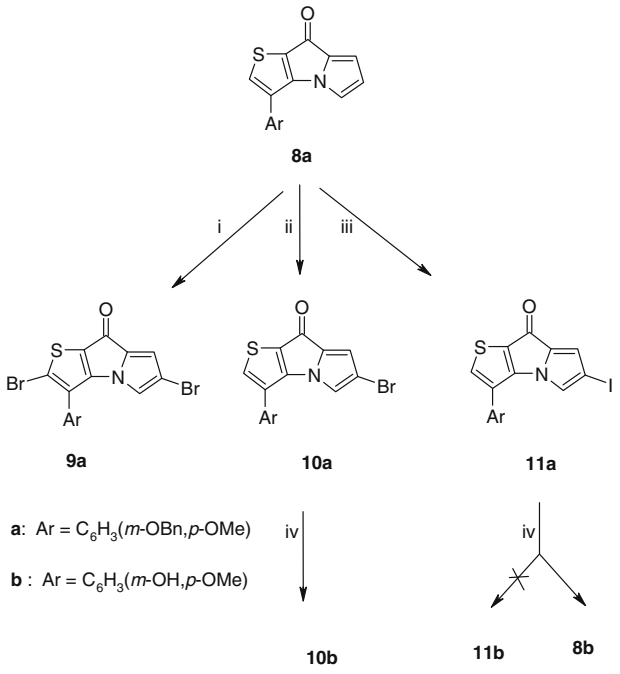


Scheme 1. Reagents and conditions: (i) NaH, HCO_2Et, THF; (ii) $PhSO_2Cl$, NEt_3, toluene; (iii) $HSCH_2CO_2Me$, MeONa, MeOH; (iv) 2,5-dimethoxyTHF, 4-chloropyridine HCl, dioxane, 100 °C; (v) pyrrolidine, 87 °C; (vi) $POCl_3$, 70 °C; (vii) 10% NaOH, 50 °C.



Scheme 2. Reagents and conditions: (i) Br_2, $CHCl_3$, rt; (ii) NBS, THF, −84 °C to rt, 80%; (iii) KOH, I_2, DMF, rt, 90%; (iv) (a) 33% HBr in AcOH, 25 °C; (b) NaOH 1 N, MeOH, 25 °C, 55%.

The first substituent we decided to introduce was a halogen atom. It could be a source of multiple coupling reactions and therefore chemical diversity. Furthermore it should be easily introduce beneficiating from the reactivity of the pyrrole ring towards electrophilic aromatic substitution.

We decided to study the condition of electrophilic halogenations of tripentone **8a**. It appears that this type of compound is really activated towards electrophilic bromination. The use of one equivalent of bromine at room temperature conduced exclusively to a mixture of mono- and di-bromo tripentone on position 2 and 6, both of the activated position of the tricycle. Looking for milder conditions our best results were obtained when *N*-bromosuccinimide was used at −84 °C. Compound **10a** was exclusively obtained in 80% yield. It appears that position 6 is the most reactive towards electrophilic substitution (Scheme 2). The position of the bromination was determined by a NMR study but also according to the X-ray structure of **10a** (Fig. 3).

We could compare this result with the reactivity of corresponding substituted pyrrole ring. It is well established that when the 2 position of a pyrrole ring is occupied by an electron-withdrawing group, electrophilic attack occurs more readily at the 4 position than at the normally more reactive 5 position.[6]

We then tried to replace *N*-bromosuccinimide by *N*-iodosuccinimide in order to introduce an iodine atom. Unfortunately this reaction conserved exclusively the starting material, even upon heating. Iodination could however be easily realized with 90% yield using a mixture of I_2 and KOH to afford the isomer **11a**.[7] A solution of hydrobromic acid was used to afford compound **10b** in good yields. Interestingly, under the same conditions **11a** conduced in high yields to the dehalogenated product **8b**.

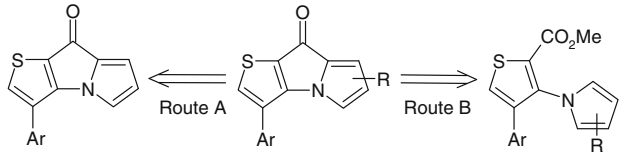


Figure 2.

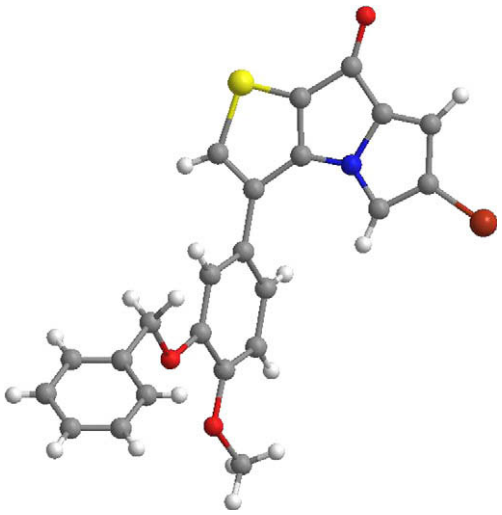
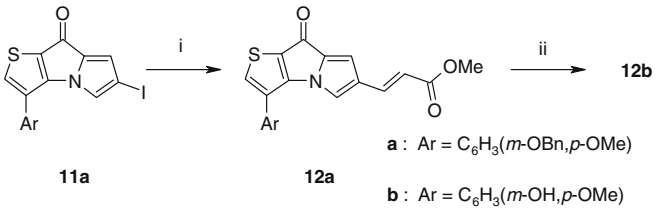


Figure 3.

The reactivity of these important intermediates was then evaluated in various metallo catalyzed cross-coupling reactions. Our first attempts were to introduce alkyl chain using a Heck coupling reaction.[8] Starting from compound **11a** and methyl acrylate the novel compound **12a** was obtained under microwave irradiation (Scheme 3). The corresponding bromotripentone **10a** remains inactive under this condition. Compound **12a** was deprotected according to the standard reaction procedure.

The reactivity of the tripentone **10a** was evaluated under Buchwald[9] conditions in order to introduce an aromatic amine instead of the halogen atom. Compound **13a** was obtained when **10a** was reacted with *p*-aminoanisole. If unreactive towards the Heck coupling, the bromo isomer **10a** appears to be a good coupling partner in the Suzuki–Miyaura cross-coupling reaction.[10] The best condi-



Scheme 3. Reagents and conditions: (i) CH_2CHCO_2Me, $PdOAc_2$, PPh_3, TEA, dioxane, 50%; (ii) (a) 33% HBr in AcOH, 25 °C; (b) NaOH 1 N, MeOH, 25 °C, 40%.

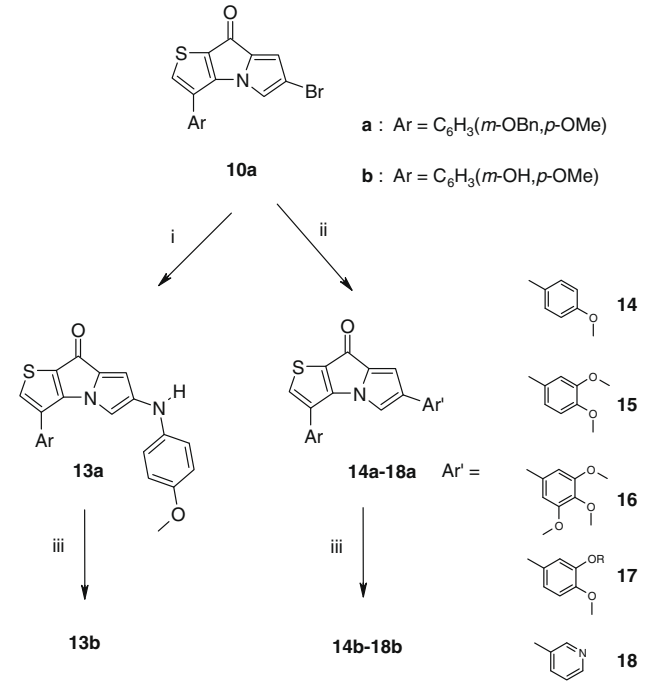
tions found in that particular series were the use of $Pd(PPh_3)_4$ as a catalyst and K_3PO_4 as a base in DMF. Under these conditions a series of novel tripentones arylated on position 6 **14a**–**18a** was synthesized with good to excellent yields, before to be deprotected (Scheme 4).

3. Pharmacological results

The influence of these novel substitutions on the cytotoxic activity of our tripentones was firstly evaluated in vitro on KB-cells and reported in Table 1.

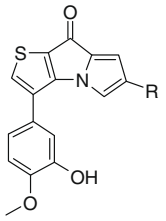
These results gave us some crucial information in order to details our SAR study and more particularly the influence of a novel substituent on the pyrrole ring. It appears clearly that small substituent on that position, like bromo- and iodo- compounds conserved an interesting cyctotoxicity. On the other hand its replacement by a larger chain and an even bigger aromatic ring caused an important decrease of the activity.

In conclusion, we have described here the synthesis and the antiproliferative activities of novel pyrrolo[2,3-*b*]pyrrolizinones, which have gave crucial information in the development of novel tripentones compounds. Finally a novel and convergent synthesis was reported through Suzuki cross-coupling and will be applied to afford more diversity on these new structures.



Scheme 4. Reagents and conditions: (i) $CH_3OC_6H_4NH_2$, Cs_2CO_3, BINAP, Pd_2dba_3, dioxane, 70%; (ii) $ArB(OH)_2$, $Pd(PPh_3)_4$, K_3PO_4, DMF, 60–80%; (iii) (a) 33% HBr in AcOH, 25 °C; (b) NaOH 1 N, MeOH, 25 °C, 50–90%.

Table 1
Cytotoxicity results for compounds **1**–**18b**



Compds	R	% Inhib 10^{-5} M	% Inhib 10^{-6} M	IC_{50}, nM L1210
1a	H	86	86	4.0
10b	Br	94	94	17.0
12b	$(CH)_2CO_2Me$	1	3	ND[b]
14b	C_6H_4OMe	98	94	113
15b	C_6H_3di-OMe	97	15	ND[b]
16b	C_6H_2tri-OMe	35	NT[a]	ND[b]
17b	C_6H_3*p*OM*e*m*O*H	90	15	ND[b]
18b	mC_5NH_4	64	20	ND[b]

[a] NT: not tested.
[b] ND: not determined.

4. Experimental

4.1. General

All commercial solvents and reagents were used as-received except THF, which was distilled over Na/benzophenone under Argon. Flash chromatography was realized on silica gel (SDS AAC 60, 70–200) or on neutral alumina gel (Merck 90, 63–200). IR spectra were recorded on KBr disks with a Perkin–Elmer BX FTIR apparatus. ^1H and ^{13}C NMR spectra were recorded, respectively, at 400 and 100 MHz with a Jeol Lambda 400 NMR spectrometer. Chemical shifts δ are reported in parts per million with the solvent resonance as the internal standard; coupling constants J are given in hertz. Multiplicity is given as follows: s (singlet), d (doublet), t (triplet), q (quartet), quint. (quintet), sept. (septet), m (multiplet). The microwave reactions were performed using a Biotage Initiator Microwave oven using 2–5 mL sealed vials. Temperature was measured with an IR-sensor and reaction times are given as hold times. LC/MS (ESI) analyses were realized with a Waters alliance 2695 as separating module using the following gradient: A (95%)/B (5%) to A (5%)/B (95%) in 10 min. This ratio was hold during 3 min before return to initial conditions in 1 min. Initial conditions were then maintained for 5 min (A: H_2O, B: CH_3CN; each containing HCOOH: 0.1%; Column: C18 Xterra MSC118/2.1_50 mm). MS detection was performed with a Micromass ZMD 2000 by positive ESI. EIMS and HRMS (EI) were performed at 70 eV with a JEOL JMS GCMate. Melting points were determined on Kofler melting point apparatus.

4.2. Halogenation procedure

4.2.1. 3-(3-Benzyloxy-4-methoxyphenyl)-6-bromo-thieno[2,3-*b*]pyrrolizin-8-one (10a)

A solution of 100 mg (0.26 mmol) of the tripentone **8a** in 5 mL anhydrous THF was cooled to −84 °C. 46 mg (0.26 mmol, 1 equiv) of *N*-bromosuccinimide was slowly added and the reaction mixture was stirred at −85 °C for 1.5 h and 24 h at rt. The solvent was removed under vacuum and the residue diluted in 150 mL water. The solution was extracted with Et_2O (2 × 100 mL). The combined organic phases were washed with aqueous NaCl (2 × 100 mL), dried ($MgSO_4$) and evaporated. This residue was purified by silica gel chromatography, eluting by cyclohexane/ethyl acetate (1:2) to furnish the tripentone **10a** as an orange solid

(97 mg, 80%). Mp 110 °C. IR (KBr): ν = 3429, 3119, 3065, 2924, 1693(CO), 1524, 1503, 1439, 1413, 1377, 1275, 1255, 1155, 1139, 1023, 922, 771, 742, 697, 580 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 7.38 (m, 6H, H_{phenyl} + H_2), 7.03 (dd, 1H, ^4J = 1.9 Hz, ^3J = 8.0 Hz, $H_{6'}$), 7.01 (d, 1H, ^3J = 8.0 Hz, $H_{5'}$), 6.96 (d, 1H, ^4J = 1.9 Hz, $H_{2'}$), 6.65 (d, 1H, ^4J = 0.9 Hz, H_5), 6.63 (d, 1H, ^4J = 0.9 Hz, H_7), 5.19 (s, 2H, OCH_2Ph), 3.99 (s, 3H, OCH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 173.31, 159.93, 150.42, 148.56, 136.50, 135.16, 119.70, 116.68, 114.00, 112.08, 100.34, 71.33, 56.14. MS (EI^+) *m/z*: 466.9 (M+1, 31), 464.9 (M−1, 31).

4.2.2. 3-(3-Benzyloxy-4-methoxyphenyl)-6-iodo-thieno[2,3-*b*]pyrrolizin-8-one (11a)

To a solution of 200 mg (0.52 mmol, 1 equiv) of the tripentone (**8a**) in 10 mL DMF was added 87 mg (1.55 mmol, 3 equiv) of KOH. The reaction mixture was cooled down to 0 °C and 262 mg (1.03 mmol, 2 equiv) of iodine was added. The reaction mixture was stirred at rt for 12 h before to be diluted with 50 mL of a saturated solution of $Na_2S_2O_3$. The combined organic phases were washed with aqueous NaCl (2 × 100 mL), dried ($MgSO_4$) and evaporated. This residue was purified by silica gel chromatography, eluting by cyclohexane/ethyl acetate (1:2) to furnish the tripentone **11a** as an orange solid (180 mg, 90%). Mp 157 °C. IR (KBr): ν = 2958, 2924, 2869, 2836, 1682(CO), 1520, 1498, 1436, 1413, 1376, 1254, 1240, 1141, 1010, 912, 802, 760, 699, 586, 581 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 7.36 (m, 6H, H_{phenyl} + H_2), 7.01 (m, 3H, $H_{6'}$ + $H_{5'}$ + $H_{2'}$), 6.74 (s, 1H, H_5), 6.72 (s, 1H, H_7), 5.18 (s, 2H, OCH_2Ph), 3.97 (s, 3H, OCH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 172.9, 150.8, 150.4, 148.6, 136.7, 136.5, 134.7, 129.6, 128.7, 128.6, 128.2, 127.3, 124.4, 124.1, 121.2, 121.0, 113.9, 112.0, 71.35. MS (EI^+) *m/z*: 512.8 (M, 38), 386.0 (27).

4.3. Typical metallo-catalyzed reaction

4.3.1. 3-[3-(3-Benzyloxy-4-methoxyphenyl)-8-oxo-8*H*-thieno[2,3-*b*]pyrrolizin-6-yl]-acrylic acid methyl ester (12a)

A solution of palladium acetate (9.7 × 10^{-6} mol, 0.05 equiv), triphenylphosphine (1.9 × 10^{-5} mol, 1 equiv) and triethylamine (1.9 × 10^{-4} mol, 1 equiv) in dioxane was stirred under argon for 5 min. 100 mg (1.9 × 10^{-4} mol, 1 equiv) of tripentone **11a** was then added and the reaction mixture was stirred for 10 min. Methyl acrylate (9.7 × 10^{-4} mol, 5 equiv) was then added and the reaction mixture was heated under reflux for 12 h. 150 mL water was then added and the reaction mixture was extracted with ethyl acetate (2 × 100 mL). The combined organic layers were washed with brine (2 × 100 mL), dried ($MgSO_4$) and evaporated to give a yellow oil which was purified by silica gel chromatography, eluting by cyclohexane/ethyl acetate (4:1) to furnish tripentone **12a** as a yellow solid in 54% yield. Mp 130 °C. IR (KBr): ν = 2922, 2851, 1693(CO), 1635(CO), 1475, 1260, 11,65, 1116, 1024 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 7.38 (m, 7H, H_{arom} + H_2 + H_{ethyl}), 7.04 (dd, 1H, ^4J = 1.7 Hz, ^3J = 8.0 Hz, $H_{6'}$), 7.00 (d, 1H, ^3J = 8.0 Hz, $H_{5'}$), 6.97 (d, 1H, ^4J = 1.7 Hz, $H_{2'}$), 6.85 (s, 1H, H_5); 6.73 (s, 1H, H_7), 6.05 (d, 1H, J = 15.8 Hz, H_{ethyl}), 5.21 (s, 2H, OCH_2), 3.98 (s, 3H, OCH_3), 3.77 (s, 3H, OCH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.01, 167.63, 150.45, 148.49, 145.08, 137.11, 136.94, 136.53, 135.18, 129.74, 128.77, 128.31, 127.13, 124.98, 124.36, 121.25, 121.07, 114.93, 113.69, 112.53, 112.02, 71.12, 56.13, 51.56. MS (EI^+) *m/z*: 471.2 (M, 5), 91.1 (100). LC–MS (ESI): t_R = 11.72 min; *m/z* $[M+H]^+$: 472.01.

4.3.2. 3-(3-Benzyloxy-4-methoxyphenyl)-6-(4-methoxyphenylamino)-thieno[2,3-*b*]pyrrolizin-8-one (13a)

To a mixture of *p*-anisidine (53 mg, 0.43 mmol), $Pd(OAc)_2$ (1.93 mg, 8.86 × 10^{-6} mol), Cs_2CO_3 (279 mg, 0.86 mmol) and 4,5-bis(diphenylphosphino)-9,9-dimethylxanthene (Xantphos, 7.4 mg, 0.013 mmol) was added tripentone **10a** (200 mg, 0.43 mmol) in anhydrous 1,4-dioxane (1.5 mL). The tube was sealed and irradiated at 160 °C for 40 min. After cooling to room temperature, the reaction mixture was diluted with CH_2Cl_2, filtered and concentrated. This residue was purified by silica gel chromatography, eluting by cyclohexane/ethyl acetate (2:1) to furnish 80 mg (36%) of the desired product as a yellow solid. Mp 134 °C. IR (KBr): ν = 3106 (NH), 2922, 2801, 2357, 1637 (CO), 1553, 1538, 1522, 1473, 1456, 1342, 1286, 1282, 1176, 766, 631 cm^{-1}.^1H NMR (400 MHz, $CDCl_3$): δ = 7.41d, 1H, J = 2.96 Hz, H_{arom}), 7.36 (s, 1H, H_2), 7.35 (d, 2H, J = 1.96 Hz, H_{arom}), 7.22 (d, 1H, J = 3.92 Hz, H_{arom}), 7.10 (d, 2H, J = 8.79 Hz, $H_{2''}$ + $H_{6''}$), 6.95 (s, 1H, NH), 6.77 (d, 2H, J = 8.79 Hz, $H_{3''}$ + $H_{5''}$), 6.71 (m, 3H, $H_{2'}$, $H_{5'}$, H_{arom}), 6.62 (dd, 1H, ^4J = 1.96 Hz, ^3J = 8.8 Hz, $H_{6'}$), 6.60 (d, 1H, J = 1.96 Hz, H_5), 6.35 (d, 1H, J = 1,96 Hz, H_7), 4.80 (s, 2H, OCH_2), 3.84 (s, 3H, OCH_3), 3.72 (s, 3H, OCH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 157.34, 156.17, 148.96, 147.70, 139.90, 138.58, 136.88, 135.88, 130.41, 129.38, 128.76, 128.44, 127.70, 126.97, 126.75, 126.71, 126.42, 122.16, 121.55, 120.85, 120.71, 120.65, 114.88, 113.84, 112.15, 111.53, 96.79, 70.24, 55.84, 55.32. LC–MS (ESI): t_R = 12.03 min; *m/z* $[M+H]^+$: 509.17.

4.3.3. 3-(3-Benzyloxy-4-methoxyphenyl)-6-(4-methoxyphenyl)-thieno[2,3-*b*]pyrrolizin-8-one (14a)

A solution of tripentone **10b** (50 mg, 0.11 mmol) in DMF (2 mL) under argon was added to a mixture of $Pd(PPh_3)_4$ (5.3 × 10^{-3} mmol, 5%), 4-methoxyphenylboronic acid (0.21 mmol, 2 equiv) and K_3PO_4 (0.27 mmol, 2.5 equiv). The mixture was then irradiated at 120 °C for 100 min using a Biotage microwave oven. The mixture was then diluted with 50 mL water and extracted with diethyl ether (2 × 75 mL). The combined organic layers were washed with brine (2 × 100 mL), dried ($MgSO_4$) and evaporated to give a yellow oil which was purified by silica gel chromatography, eluted by cyclohexane/ethyl acetate (3:1) to furnish tripentone **14a** as a red solid in 75% yield. Mp 134 °C. IR (KBr): ν = 3429, 2924, 2853, 1673(CO), 1526, 1505, 1455, 1367, 1259, 1136, 1080, 1023, 801, 743, 696 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 7.32 (m, 5H, H_{arom}), 6.98 (m, 2H, $H_{5'}$ + $H_{6'}$), 6.75 (d, 2H, ^4J = 3.8 Hz, $H_{2''}$ + $H_{6''}$), 6.42 (d, 1H, ^4J = 1.9 Hz, $H_{2'}$), 6.30 (s, 1H, H_5 or H_7), 6.09 (s, 1H, H_5 or H_7), 5.90 (d, 2H, ^4J = 3.8 Hz, $H_{3''}$ + $H_{5''}$), 4.82 (s, 2H, OCH_2Ph), 3.93 (s, 3H, OCH_3). 3.57 (s, 3H, OCH_3). MS (EI^+) *m/z* 368.4 (15), 236.2 (19), 152.2 (18), 111.1 (47), 97.1 (91), 83.4 (100). LC–MS (ESI): t_R = 12.75 min; *m/z* $[M+H]^+$: 494.75.

4.3.4. 3-(3-Benzyloxy-4-methoxyphenyl)-6-(3,4-dimethoxyphenyl)-thieno[2,3-*b*]pyrrolizin-8-one (15a)

This compound was obtained from **10a** as described for **14a** as a red solid and directly engaged in the debenzylation step.

4.3.5. 3-(3-Benzyloxy-4-methoxyphenyl)-6-(3,4,5-trimethoxyphenyl)-thieno[2,3-*b*]pyrrolizin-8-one (16a)

This compound was obtained from **10a** as described for **14a** as a red solid in 58% yield. Mp 148 °C. IR (KBr): ν = 3078, 2928, 1673 (CO), 1585, 1470, 1416, 1379, 1327, 1250, 1120, 1004, 844, 802, 740, 697 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 7.31 (s, 2H, $H_{2''}$ + $H_{6''}$), 7.22 (m, 6H, $H_{arom,}$ + H_2), 6.95 (m, 3H, $H_{2'}$, $H_{5'}$, $H_{6'}$), 6.64 (s, 2H, H_5 + H_7), 5.19 (s, 2H, OCH_2), 3.97 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 3.83 (s, 6H, OCH_3).^{13}C NMR (100 MHz, $CDCl_3$): δ = 174.0, 153.6, 150.8, 150.4, 148.6, 137.4, 136.5, 136.4, 134.5, 128.6, 127.2, 126.8, 124.9, 121.1, 116.6, 113.9, 113.3, 112.1, 104.7, 102.8, 71.4, 65.8, 60.9, 56.3. LC–MS (ESI): t_R = 13.67 min; *m/z* $[M+H]^+$: 534.57.

4.3.6. 3,6-Bis-(3-benzyloxy-4-methoxy-phenyl)-thieno[2,3-*b*]pyrrolizin-8-one (17a)

This compound was obtained from **10a** as described for **14a** as an orange solid in 46% yield. Mp 155 °C. IR (KBr): ν = 3094, 3030, 2090,

2833, 1678 (CO), 1605, 1562, 1454, 1372, 1259,1215, 1022, 967, 747 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 7.32 (m, 1H, H_{arom} + H_2), 7.09 (dd, 1H, ^4J = 1.96 Hz, ^3J = 7.79 Hz, $H_{6'}$), 7.04 (d, 1H, ^4J = 1.96 Hz, $H_{2'}$), 7.01 (d, 1H, ^3J = 7.79 Hz, $H_{5'}$), 6.90 (dd, 1H, ^4J = 1.96 Hz, ^3J = 7.79 Hz, $H_{6''}$), 6.87 (d, 1H, ^4J = 1.96 Hz, $H_{2''}$), 6.65 (d, 1H, ^3J = 7.79 Hz, $H_{5''}$), 6.47 (d, 2H, J = 2.91 Hz, H_5 + H_7), 5.19 (s, 2H, OCH_2), 5.14 (s, 2H, OCH_2), 3.98 (s, 3H, OCH_3), 3.89 (s, 3H, OCH_3).^{13}C NMR (100 MHz, $CDCl_3$): δ = 174.1, 150.8, 150.2, 150.1, 148.7, 148.1, 143.4, 136.8, 136.3, 134.9, 129.6, 129.3, 128.7, 128.5, 128.4, 127.8, 127.7, 127.3, 127.1, 126.9, 126.4, 124.7, 120.9, 118.0, 116.3, 113.6, 113.1, 112.0, 111.8, 111.6, 106.5, 102.8, 71.2, 70.7, 56.7, 56.0. MS (EI^+) *m/z* 599.3 (100), 509.2 (53), 480.2 (39).

4.3.7. 3-(3-Benzyloxy-4-methoxyphenyl)-6-pyridin-3-yl-thieno[2,3-*b*]pyrrolizin-8-one (18a)

This compound was obtained from **10a** as described for **14a** as a yellow solid in 40% yield. Mp 140 °C. IR (KBr): ν = 2956, 2922, 2853, 1682 (CO), 1620, 1579, 1562, 1504, 1462, 1312, 1225, 931, 606 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 8.49 (m, 1H, $H_{pyridine}$), 7.83 (m, 2H, $H_{pyridine}$), 7.55 (m, 2H, $H_{pyridine}$ + $H_{5'}$), 7.32 (m, 6H, $H_{aromatic}$ + H_2), 7.14 (d, 1H, ^4J = 1.96 Hz, $H_{2'}$), 7.05 (dd, 1H, ^3J = 1.96 Hz, ^4J = 8,8 Hz, $H_{6'}$), 6.92 (s, 1H, H_5 or H_7), 6.89 (s, 1H, H_7 or H_5), 5.20 (s, 2H, OCH_2), 3.99 (s, 3H, OCH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 147.13, 146.97, 136.10, 133.10, 132.28, 131.10, 130.72, 128.23, 127.63, 127.03, 125,26, 125,51, 125,13, 120.23, 118.81, 117.32, 116.28, 113.90, 71.02, 55.50. LC–MS (ESI): t_R = 10.05 min; *m/z* $[M+H]^+$: 465.04.

4.4. O-Debenzylation procedure

4.4.1. 3-(3-Hydroxy-4-methoxyphenyl)-6-bromo-thieno[2,3-*b*]pyrrolizin-8-one (10b)

A solution of **10a** (0.04 g, 0.086 mmol) in a 33% solution of hydrobromic acid in glacial acetic acid (15 mL) was stirred at room temperature for 1 h. After cooling, the reaction mixture was diluted with water (50 mL) and the resulting precipitate was extracted with ethyl acetate (3 × 20 mL). Then, the organic layers were combined, washed with water (2 × 100 mL), dried ($MgSO_4$) and evaporated to give a dark red solid. The residue was diluted in methanol (10 mL) and a molar aqueous solution of NaOH is added (5 mL) before to be stirred for 1 h. The reaction mixture was concentrated under *vacuum*, diluted with water, acidified with aqueous 1 M HCl and extracted with ethyl acetate (2 × 100 mL). The organic layers were combined, washed with water (2 × 100 mL), dried ($MgSO_4$) and evaporated to give a dark red solid which was purified by silica gel chromatography, eluting by cyclohexane/ethyl acetate (3:2) to furnish thienopyrrolizinone **10b** as a red solid (12 mg, 24%). Mp 154 °C. IR (KBr): ν = 3411, 2924, 2853, 1678(CO), 1526, 1436, 1282, 1220, 1022, 922, 776, 581 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 7.52 (s, 1H, H_2), 7.25 (m, 3H, $H_{2'}$, $H_{5'}$, $H_{6'}$), 6.81 (s, 1H, H_5), 6.65 (s, 1H, H_7), 5.76 (bl, 1H, OH), 3.97 (s, 3H, OCH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 173.8, 151.3, 154.2, 147.6, 146.5, 144.9, 135.2, 132.7, 129.9, 126.5, 125.6, 120.2, 117.1, 114.5, 111.4. 56.5. HRMS (EI): calcd ($M^{+\cdot}$) for $C_{16}H_{10}BrNO_3S$: 374.95642, found: 374.956402.

4.4.2. 3-[3-(3-Hydroxy-4-methoxyphenyl)-8-oxo-8*H*-thieno[2,3-*b*]pyrrolizin-6-yl]-acrylic acid methyl ester (12b)

This compound was obtained from **12a** as described for **10b** as an orange solid in 61% yield. Mp 153 °C. IR (KBr): ν = 3134, 2934, 2895, 1694(CO), 1635(CO), 1476, 1243, 1100, 1067 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 7,55 (d, 1H, J = 15.8 Hz), 7.48 (dd, 1H, ^4J = 1.7 Hz, ^3J = 8.0 Hz, $H_{6'}$), 6.99 (d, 1H, ^3J = 8.0 Hz, $H_{5'}$), 6.98 (d, 1H, ^4J = 1.7 Hz, $H_{2'}$), 6.87 (s, 1H, H_5), 6.31 (s, 1H, H_7), 6.06 (d, 1H, J = 15.8 Hz, H_{ethyl}), 5.39 (br s, 1H, OH), 3.99 (s, 3H, OCH_3), 3.76 (s, 3H, OCH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 173.31, 167.85, 156.72, 147.56, 146.81, 146.50, 135.46, 133.85, 131.17, 130.55, 125.70, 125.18, 120.29, 119.40, 113.05, 112.70, 109.68, 108.00, 55.94, 50.14. LC–MS (ESI): t_R = 9.72 min; *m/z* $[M+H]^+$: 382.03. HRMS (EI): calcd ($M^{+\cdot}$) for $C_{20}H_{15}NO_5S$: 381.06707, found: 381.06861.

4.4.3. 3-(3-Hydroxy-4-methoxyphenyl)-6-(4-methoxyphenylamino)-thieno[2,3-*b*]pyrrolizin-8-one (13b)

This compound was obtained from **13a** as described for **10b** as a yellow solid in 43% yield. Mp 145 °C. IR (KBr): ν = 3390 (OH), 3108 (NH), 2929, 2836, 2360, 2343, 1646 (CO), 1600, 1538, 1512, 1482, 1439, 1387, 1299, 1271, 1243, 1174, 821, 799, 731 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 7.27 (s, 1H, H_2), 7.13 (d, 2H, J = 8.79 Hz, $H_{2''}$ + $H_{6''}$), 6.95 (s, 1H, NH), 6.87 (d, 1H, J = 1,96 Hz, H_5), 6.77 (d, 2H, J = 8.79 Hz, $H_{3''}$ + $H_{5''}$), 6.74 (d, 1H, ^3J = 8.8 Hz, $H_{5'}$), 6.67 (d, 1H, ^4J = 1.96 Hz, $H_{2'}$), 6.58(d, 1H, J = 1,96 Hz, H_7), 6.45 (dd, 1H, ^4J = 1.96 Hz, ^3J = 8.8 Hz, $H_{6'}$), 3.82 (s, 3H, OCH_3), 3.75 (s, 3H, OCH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 157.44, 157.17, 154.86, 147.80, 145.68, 137.78, 135.88, 133.41, 129.48, 128.76, 128.44, 127.70, 126.87, 126.75, 126.63, 122.36, 120.96, 120.66, 114.88, 112.15, 111.53, 56.74, 56.22. LC–MS (ESI): t_R = 9.52 min; *m/z* $[M+H]^+$: 419.23. HRMS (EI): calcd ($M^{+\cdot}$) for $C_{23}H_{18}N_2O_4S$: 418.0987, found: 418.09924.

4.4.4. 3-(3-Hydroxy-4-methoxyphenyl)-6-(4-methoxyphenyl)-thieno[2,3-*b*]pyrrolizin-8-one (14b)

This compound was obtained from **14a** as described for **10b** as a red solid in 60% yield. Mp 122 °C. IR (KBr): ν = 3422, 2961, 2926, 2855, 1668 (CO), 1563, 1502, 1438, 1381, 1279, 1248, 1131, 1086, 1041, 883, 803, 785, 758, 603 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 7.46 (s, 1H, H_2), 7.31 (d, 2H, ^4J = 8.7 Hz, $H_{2''}$ + $H_{6''}$), 7.18 (s, 1H, H_5 or H_7), 7.11 (d, 1H, ^4J = 2.2 Hz, $H_{2'}$), 7.04 (s, 1H, H_5 or H_7), 6.98 (d, 1H, ^3J = 7.0 Hz, $H_{5'}$), 6.94 (m, 1H, $H_{6'}$), 6.88 (d, 2H, ^4J = 8.7 Hz, $H_{3''}$ + $H_{5''}$), 5.7 (bl, 1H, OH), 3.98 (s, 3H, OCH_3), 3.96 (s, 3H, OCH_3).^{13}C NMR (100 MHz, $CDCl_3$): δ = 158.5, 150.7, 149.6, 146.1, 136.5, 134.4, 132.6, 132.5, 129.5; 129.4, 126.2, 125.6, 122.2, 120.0, 119.8, 116.5, 114.2, 113.1, 110.9. LC–MS (ESI): t_R = 10.83 min; *m/z* $[M+H]^+$: 404.19. HRMS (EI): calcd ($M^{+\cdot}$) for $C_{23}H_{17}NO_4S$: 403.08781, found: 403.08626.

4.4.5. 6-(3,4-Dimethoxyphenyl)-3-(3-hydroxy-4-methoxyphenyl)-thieno[2,3-*b*]pyrrolizin-8-one (15b)

This compound was obtained from **15a** as described for **10b** as a red solid in 40% yield. Mp 190 °C. IR (KBr): ν = 2955, 2932, 2854, 1688 (CO), 1567, 1508, 1477, 1436, 1256, 1224, 1135, 1032, 958, 803, 771, 762, 587 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 7.46 (s, 1H, H_2), 7.12 (d, 1H, ^4J = 1.9 Hz, $H_{2'}$ or $H_{2''}$), 7.03 (dd, 1H, ^3J = 8.2 Hz, ^4J = 1.9 Hz, $H_{6'}$ or $H_{6''}$), 6.99 (d, 1H, ^4J = 1.9 Hz, H_5 or H_7), 6.96 (d, 1H, ^3J = 8.2 Hz, $H_{5'}$ or $H_{5''}$), 6.94 (dd, 1H, ^3J = 8.2 Hz, ^4J = 1.9 Hz, $H_{6'}$ or $H_{6''}$), 6.90 (d, 1H, ^4J = 1.9 Hz, H_5 or H_7), 6.88 (d, 1H, ^4J = 1.9 Hz, $H_{2'}$ or $H_{2''}$). 6.84 (d, 1H, ^3J = 8.2 Hz, $H_{5'}$or $H_{5''}$), 5.8 (bl, 1H, OH), 3.98 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 119.8, 117.6, 116.6, 114.2, 113.1, 111.7, 111.0, 109.1, 56.1, 56.0, 41.4. MS (EI^+) *m/z*: 433.1 (M, 60), 418.1 (32), 368.3 (46), 341.2 (25), 326.2 (100). LC–MS (ESI): t_R = 10.30 min; *m/z* $[M+H]^+$: 433.80. HRMS (EI): calcd ($M^{+\cdot}$) for $C_{24}H_{19}NO_5S$: 433.09837, found: 433.09656.

4.4.6. 3-(3-Hydroxy-4-methoxyphenyl)-6-(3,4,5-trimethoxyphenyl)-thieno[2,3-*b*]pyrrolizin-8-one (16b)

This compound was obtained from **16a** as described for **10b** as a red solid in 60% yield. Mp 156 °C. IR (KBr): ν = 3328, 2927, 2853, 2360, 2341, 1614 (CO), 1509, 1463, 1348, 1264, 1112, 1025, 803, 670 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 7.39 (s, 2H, $H_{2''}$ + $H_{6''}$), 7.30 (s, 1H, H_2), 7.11 (d, ^4J = 1,96 Hz, 1H, $H_{2'}$), 7.03 (m, 2H, $H_{5'}$, $H_{6'}$), 6.72 (s, 2H, H_5 + H_7), 5.4 (br s, 1H, OH), 3.98 (s, 3H, OCH_3),

3.84 (s, 3H, OCH_3), 3.82 (s, 6H, OCH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.2, 154.2, 152.4, 150.6, 149.9, 138.7, 137.5, 136.1, 134.2, 128.9, 128.6, 127.4, 126.6, 125.4, 124.2, 122.8, 121.3, 117.1, 113.7, 112.1, 104.6, 60.87, 55.97, 55.96. LC–MS (ESI): t_R = 10.52 min; *m/z* $[M+H]^+$: 464.72. HRMS (EI): calcd ($M^{+\cdot}$) for $C_{25}H_{21}NO_6$ S: 463.10892, found: 463.10785.

4.4.7. 3,6-Bis-(3-hydroxy-4-methoxyphenyl)-thieno[2,3-*b*]pyrrolizin-8-one (17b)

This compound was obtained from **17a** as described for **10b** as a yellow solid in 48% yield. Mp 142 °C. IR (KBr): ν = 3429, 3379, 2957, 2843, 2360, 1657(CO); 1532, 1511, 1485, 1466,1378, 1288,1267, 1176,974, 865 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 7.42 (s, 1H, H_2), 7.22 (dd, 1H, ^4J = 1.96 Hz, ^3J = 7.79 Hz, $H_{6'}$), 7.17 (d, 1H, ^4J = 1.96 Hz, $H_{2'}$), 7.04 (d, 1H, ^3J = 7.79 Hz, $H_{5'}$), 6.97 (dd, 1H, ^4J = 1.96 Hz, ^3J = 7.79 Hz, $H_{6''}$), 6.87 (d, 1H, ^4J = 1.96 Hz, $H_{2''}$), 6.65 (d, 1H, ^3J = 7.79 Hz, $H_{5''}$), 6.45 (d, 2H, J = 2.91 Hz, H_5 + H_7), 3.93 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3). ^{13}C NMR (100 MHz, $CDCl_3$): 158.1, 157.6, 147.3, 146.8, 145.7, 145.1, 137.4, 132.3, 130.7, 128.2, 125.9, 125.0, 120.4, 120.2, 120.0, 119.1, 117.2, 116.9, 115.6, 109.4, 55.7, 55.5. LC–MS (ESI): t_R = 9.73 min; *m/z* $[M-H]^-$: 418.97. HRMS (EI): calcd ($M^{+\cdot}$) for $C_{23}H_{17}NO_5S$: 419.08274, found: 419.08299.

4.4.8. 3-(3-Hydroxy-4-methoxyphenyl)-6-pyridin-3-yl-thieno[2,3-*b*]pyrrolizin-8-one (18b)

This compound was obtained from **18a** as described for **10b** as a yellow solid in 33% yield. Mp 152 °C. IR (KBr): 3114, 2965, 2932, 2883, 1673 (CO), 1641, 1539, 1421, 1151, 1014, 792, 707 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 8.58 (m, 1H, $H_{pyridine}$), 7.86 (m, 2H, $H_{pyridine}$), 7.58 (m, 2H, $H_{pyridine}$ + $H_{5'}$), 7.53 (s, 1H, H_2), 7.17 (d, 1H, ^4J = 1.96 Hz, $H_{2'}$), 7.09 (dd, 1H, ^3J = 1.96 Hz, ^4J = 8,8 Hz, $H_{6'}$), 6.95 (s, 1H, H_5 or H_7), 6.89 (s, 1H, H_7 or H_5), 5.4 (br s, 1H, OH), 3,98 (s, 3H, OCH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 147.59; 147.24; 146.28; 134.78; 132.78; 132.21; 128.62;128.52; 127.48; 127.32; 123.62; 119.80; 116.96; 114.26; 111.77; 111.00; 56.34. LC–MS (ESI): t_R = 7.75 min; *m/z* $[M+H]^+$: 375.97. HRMS (EI): calcd ($M^{+\cdot}$) for $C_{21}H_{14}N_2O_3S$: 374.07249, found: 374.07362.

4.5. Pharmacology

4.5.1. Cell culture

The human cell lines KB (month epidermoid carcinoma), HCT116 and HCT15 (colon adenocarcinoma), Vero (monkey kidney) and MRC5 (human fetal lung) were purchased from ECACC (Salisbury, UK) and HL60 (promyeocytic leukaemia) cells from ATCC. MCF7 (breast adenocarcinoma) were given by Dr Matthias Kassack (Bonn University, Germany). KB, Vero and MRC5 cells were grown in D-MEM medium supplemented with 10% fetal calf serum, in the presence of penicilline, streptomycine and fungizone in 75 cm^2 flask under 5% CO_2, whereas HCT116, HCT15, MCF7, HL60 were grown in RPMI medium. Resistant MCF7 and HL60 cells were obtained by prolonged treatment with doxorubicine.

4.5.2. Cell proliferation assay

Cells were plated in 96-well tissue culture plates in 200 μl medium and treated 24 h later with compounds dissolved in DMSO with compound concentrations ranged 0.05 nM to 1 μM using a Biomek 3000 (Beckman). Controls received the same volume of DMSO (1% final volume). After 72 h exposure to the drug, 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 $[(1-(OD_{490}\ \text{treated}/OD_{490}\ \text{control}))\times 100]$. For IC_{50} determinations (50% inhibition of cell proliferation) experiments were performed in separate duplicate.

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