Synthesis and crystal structure of (4bRS,9bRS)-5-(2,4-dimethoxyphenyl)-4b,9b-7,7-dimethyldihydroxy-4b,5,6,7,8,9b-hexahydroindeno[1,2-*b*] indole-9,10-dione

Gricela Lobo[a], Elimar Zuleta[a], Katiuska Charris[a], Mario V. Capparelli[b], Alexander Briceño[c], Jorge Angel[d] and Jaime Charris[a]*

[a]*Laboratorio de Síntesis Orgánica, Facultad de Farmacia, Universidad Central de Venezuela, Apartado 47206, Los Chaguaramos, 1041-A Caracas, Venezuela*

[b]*Unidad de Estructura Molecular, Fundación Instituto de Estudios Avanzados (IDEA), Apartado 17606, Caracas 1015-A, Venezuela*

[c]*Laboratorio de Síntesis y Caracterización de Nuevos Materiales, Instituto Venezolano de Investigaciones Científicas (IVIC) Apartado 21827, Caracas, 1020-A, Venezuela*

[d]*Laboratorio de Síntesis Orgánica y Diseño de Fármacos, Dpto. de Química, Facultad Experimental de Ciencias, Universidad del Zulia, Maracaibo, Venezuela*

A highly regiospecific synthesis and crystal structure of (4b*RS*,9b*RS*)-5-(2,4-dimethoxyphenyl)-7,7-dimethyl-4b,9b-dihydroxy-4b,5,6,7,8,9b-hexahydroindeno[1,2-*b*]indole-9,10-dione is reported. It was tested *in vitro* against six human tumour cell lines and two nontumourogenic cell lines. Their *in vitro* activity against *Mycobacterium tuberculosis* is also reported. In general, it was found to possess a marginal activity.

Keywords: indeno[1,2-*b*]indole, ninhydrin, regiospecific, crystal structure

Despite recent advances in molecular biology and the progress in combinatorial synthetic methodology, the rate of introduction of new pharmaceutical products has decreased markedly over the past two decades. Structural diversity in a focused collection of potential therapeutics is believed to increase the positive hit rate. Most pharmaceutical products in use are still small synthetic organic molecules that often contain a heterocyclic ring.[1,2] However, the range of easily accessible and suitably functionalised heterocyclic building blocks for the synthesis of structurally diverse libraries is rather limited. The development of new, rapid, and clean synthetic routes toward focused libraries of such compounds is therefore of great importance to both medicinal and synthetic chemists.

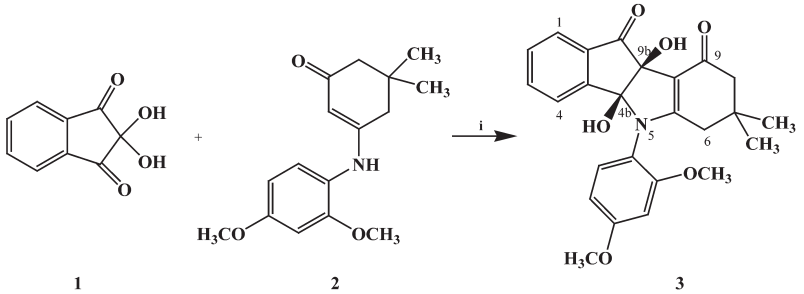
Polyhydroxylated alkaloids as indenoindoles are interesting heterocycles that can act as powerful and selective inhibitors of glycosidases and exhibit activities as powerful lipid peroxidation inhibitors,[3] potassium channel openers,[4] DNA intercalators and topoisomerase II inhibitors,[5] estrogenic agents,[6] or inhibitors of protein kinase CK2,[7] indicating a growing interest in this class of compounds.

Among the existing procedures for the preparation of indenoindoles, the Fischer indolisation starting with an indanone, via the respective phenylhydrazones, serves as the most common method.[8,9] Recently, two new syntheses by transformation and reduction of 2-nitrobenylidenephtalide, generated either by intramolecular cyclisation of 2-(2-nitrophenylethyl) benzoic acid[10] or by reaction of a phthalidyl-phosphonium bromide with 2-nitrobenzaldehyde,[5] and cyclisation of the resulting amino compounds, have been published. The formation of *vic*-dihydroxy-indenoindolones by the reaction of ninhydrine **1** with aliphatic, and aromatic amines, or alicyclic, and cyclic enaminones has been reported elsewhere.[11–14]

As part of our investigation into the synthesis of heterocyclic compounds with potential antimalarial, and anticancer activities,[15] we report here the synthesis of (4bRS,9bRS)-5-(2,4-dimethoxyphenyl)-7,7-dimethyl-4b,9b-dihydroxy-4b,5,6,7,8,9b-hexahydroindeno-[1,2-*b*]-indole-9,10-dione, and the study by X-ray diffraction analysis.

The synthesis of 3-(2,4-dimethoxyphenylamino)-5,5-dimethylcyclohex-2-enone **2** was achieved according to literature procedures by refluxing 5,5-dimethylcyclohexane-1,3-dione with aromatic amine and a catalytic amount of *p*-toluensulfonic acid in toluene and removal of water as an azeotrope with a Dean–Stark water trap,[16] to prepare the *vic*-dihydroxy-indenoindiole **3**, a solution of equimolar amounts of corresponding enaminone **2** and ninhydrin **1** in chloroform, stirred at room temperature for 24 h. TLC (EtAc:Hx 1:1) showed, that only one compound was produced (Scheme 1). Spectroscopic data (^{1}H and ^{13}C NMR) revealed that this was a cyclisation product with two ^{1}H resonances due to OH functionalities at 5.76 and 6.99 ppm and two ^{13}C resonances at 83.66 and 96.32 ppm.

The X-ray analysis confirmed the molecular structure of **3** (Fig. 1). The relevant bond lengths and angles are given in Table 1.



Scheme 1 Synthesis of (4bRS,9bRS)-5-(2,4-dimethoxypheny)l-7,7-dimethyl-(4b,9b)-dihydroxy-4b,5,6,7,8,9b-hexahydroindeno[1,2-b]indole-9,10-dione **3**.

* Correspondent. E-mail: jaime.charris@ucv.ve

Table 1 Selected bond lengths (Å) and angles (°) for **3**

C1–C2	1.574(2)	C1–C11	1.513(2)
C1–N1	1.4991(18)	C1–O1	1.3852(17)
C2–C3	1.494(2)	C2–C9	1.544(2)
C2–O2	1.4161(18)	C3–C4	1.411(2)
C3–C8	1.367(2)	C4–C5	1.512(2)
C4–O3	1.2547(19)	C5–C6	1.533(2)
C6–C7	1.540(2)	C7–C8	1.483(2)
C8–N1	1.3468(18)	C9–C10	1.472(2)
C9–O4	1.210(2)	C16–N1	1.4261(19)
C2–C1–C11	105.36(12)	C2–C1–N1	102.43(11)
C2–C1–O1	117.13(11)	C11–C1–O1	109.87(11)
C11–C1–N1	110.10(11)	O11–C1–N1	111.53(12)
C1–C2–C3	103.73(11)	C1–C2–C9	104.09(11)
C1–C2–O2	111.87(12)	C3–C2–C9	111.92(12)
C3–C2–O2	114.15(12)	C9–C2–O2	110.45(12)
C2–C3–C4	126.75(13)	C2–C3–C8	110.02(12)
C4–C3–C8	121.22(14)	C3–C4–C5	116.56(14)
C3–C4–O3	122.66(15)	C5–C4–O3	120.59(14)
C4–C5–C6	116.07(13)	C5–C6–C7	109.57(13)
C5–C6–C22	109.21(14)	C5–C6–C23	109.86(14)
C7–C6–C22	108.77(14)	C7–C6–C23	110.23(13)
C22–C6–C23	109.17(14)	C6–C7–C8	110.94(13)
C3–C8–C7	124.21(13)	C3–C8–N1	112.33(13)
C7–C8–N1	123.35(13)	C2–C9–C10	108.32(13)
C2–C9–O4	124.35(15)	C10–C9–O4	127.31(15)
C9–C10–C11	110.72(14)	C9–C10–C15	128.44(16)
C1–C11–C10	111.29(13)	C1–C11–C12	127.75(15)
C1–N1–C8	111.29(11)	C1–N1–C16	123.03(11)
C8–N1–C16	125.60(12)		

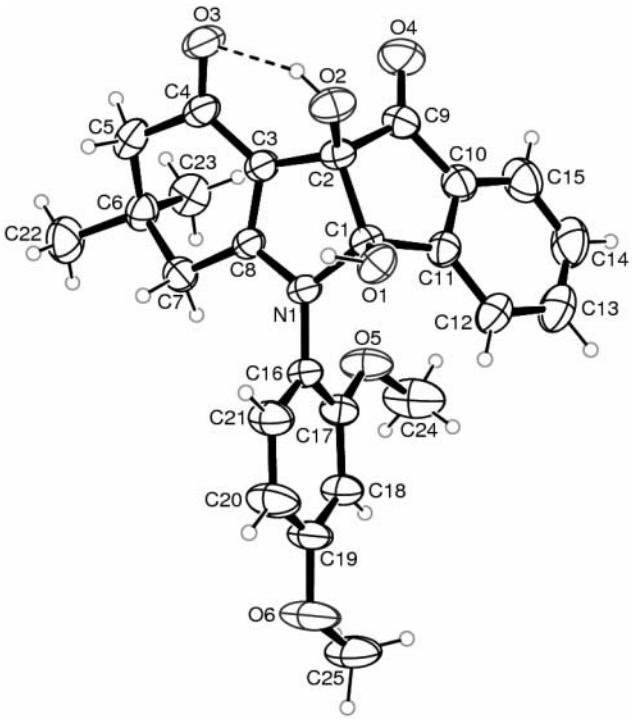


Fig. 1 Molecular structure of compound **3** showing the atomic numbering. The displacement ellipsoids are drawn at 50% probability. A dashed line indicates an intramolecular hydrogen bond.

The molecule displays two chiral centres (C4b and C9b) with a *cis* ring fusion. Therefore, since the space group is centrosymmetric, the crystal consists of an equimolar mixture of the RR and SS configurations. The two phenyl rings are quite planar (r.m.s. deviations: 0.0051 and 0.0071 Å) and the two 5-membered rings are approximately planar (r.m.s. deviations: 0.019 and 0.020 Å). The tetracyclic system is V-shaped, with the two 5-membered rings making a dihedral angle of 65.20(8)°, while the N-bonded phenyl ring is perpendicular to the heterocycle [dihedral angle 88.16(8)°]. The C3–C4–C5–C6–C7–C8 ring displays a conformation intermediate between boat and sofa [C3 and C6 are at 0.147(2) Å and 0.600(2) Å from the C4, C5, C7, C8 mean plane; the puckering parameters[17] are: q_2 = 0.4301(16) Å, q_3 = -0.1918(17) Å, ϕ_2 = 1.8(3)°, Q = 0.4709(17) Å)]. The molecule forms an O–H···O(keto) intramolecular hydrogen bond. In addition, in the crystal structure there are intermolecular hydrogen bonds of the types O–H···O(keto) and (possible) weaker C–H···O(hydroxyl) and C–H···O(methoxy) (Table 2), which link the molecules to form a three-dimensional network.

Table 2 Possible hydrogen bonds for **3** (Å and °)

D-H···A	D-H	H···A	D···A	DHA
O1–H1···O3^{i}	0.93(2)	1.86(2)	2.7912(17)	174(2)
O2–H2···O3	0.86(2)	2.12(2)	2.8886(19)	149(2)
C7–H7A···O6^{ii}	0.97	2.53	3.474(2)	164.5
C23–H23C···O1^{iii}	0.96	2.59	3.511(2)	161.6

Symmetry codes: i) $-x+1, -y, -z$; ii) $-x+1, -y, -z+1$; iii) $x+1, y, z$

Compound **3** was investigated for its *in vitro* cytotoxic activity against 3T3, BALB/3T3 clone A31 embryonic mouse fibroblast cells; Vero, normal African green monkey kidney epithelial cells; H460, human large cell lung cancer; DU145, human prostate carcinoma; MCF-7, human breast adenocarcinoma; M-14, human melanoma; HT-29, human colon adenocarcinoma; K562, human chronic myelogenous leukemia cells using previously reported methodology,[18–20] (GI_{50} > 250 μg mL^{-1}) GI_{50} is the concentration at which **3** inhibits the growth of cells by 50%. Evaluation of the antimicobacterial activity *in vitro* against sensitive MTB H37Rv strain and multidrug-resistant (MDR-MTB) clinical isolated was performed using the TEMA method.[21] (MIC value >25 μg mL^{-1}) MIC is defined as the lowest drug concentration that prevents the change in colour.

Experimental

Melting point was determined on a Thomas micro hot stage apparatus and is uncorrected. IR spectra was determined as KBr pellet on a Shimadzu model 470 spectrophotometer. The ^{1}H NMR, ^{13}C NMR spectra were recorded using a Jeol Eclipse 270 (270 MHz/67.9 MHz) spectrometer using DMSO-d_6, and are reported in ppm downfield from the residual DMSO. Elemental analyses was performed on a Perkin Elmer 2400 CHN analyser, result was within ± 0.4% of the predicted values. Chemical reagents were obtained from Aldrich Chemical Co, USA. All solvents were distilled and dried in the usual manner.

(4bRS,9bRS)-5-(2,4-dimethoxypheny)l-7,7-dimethyl-(4b,9b)-dihydroxy-4b,5,6,7,8,9b-hexahydroindeno[1,2-b]indole-9,10-dione **3**: Enaminone **2** 0.29g (1.35 mmol) and **1** 0.2g (1.12 mmol) were dissolved in chloroform 5 mL and stirred at room temperature (24 h). The solvent was evaporated *in vacuo*, the solid was isolated by suction, washed with diethyl ether and recrystallised off ethanol to afford the title compound, yield 87%; mp. 239–240 °C; IR (KBr) cm^{-1}: 1718 (CO), 3200 (OH). ^{1}H NMR DMSO-d_6: δ 0.79(s, 3H, CH_3), 0.94(s, 3H, CH_3), 1.88(s, 2H, H_6), 2.01(d, 2H, H_8, J = 4.5 Hz), 3.15(s, 3H, OCH_3), 3.80(s, 3H, OCH_3), 5.76(s, 1H, OH), 6.57(d, 1H, $H_{3'}$, J = 2.7 Hz), 6.61(d, 1H, H_4, J = 7.4Hz), 6.68(dd, 1H, $H_{5'}$, J = 2.7, 8.6 Hz), 6.98 (s, 1H, OH), 7.46–7.57(m, 3H, $H_{2,3,6'}$), 7.69(dd, 1H, H_1, J = 1.2, 8.2 Hz). ^{13}C NMR: 28.4(2), 33.5, 36.7, 51.9, 55.4, 55.9, 83.7, 96.3, 99.4, 104.6, 105.3, 117.2, 123.4, 125.0, 130.1, 132.3, 134.8, 135.0, 148.4, 157.3, 161.1, 165.7, 189.2, 198.4. Anal. Calcd for $C_{25}H_{25}NO_6$: C, 68.95; H, 5.79; N, 3.22. Found: C, 69.03; H, 5.78; N, 3.27%.

X-ray crystallography: Crystals of **3** suitable for X-ray diffraction were obtained by slow evaporation of a solution in ethanol. Crystal data, intensity data collection parameters and final refinement results are summarised in Table **3**.

Table 3 Crystal data, intensity data collection parameters and final refinement results for **3**

CCDC deposit No.	CCDC 804490
Crystal data	
Formula	$C_{25}H_{25}NO_6$
MW	435.46
Colour	yellow
Morphology	prism
Specimen size (mm)	0.45x0.36x0.25
T (K)	298(2)
a (Å)	9.480(3)
b (Å)	18.563(4)
c (Å)	12.368(3)
β (°)	94.249(6)
V (Å^3)	2170.5(9)
Crystal system	monoclinic
Space group (No.)	$P2_1/n$ (No. 14)
Z	4
D_c (g cm^{-3})	1.333
F(000)	920
μ(Mo–Kα) (mm^{-1})	0.095
θ range (°) for cell	2.8–26.3
No. reflections for cell	705
Data collection	
θ range (°)	2.0–26.5
h range	–9, 11
k range	–21, 21
l range	–14, 14
Mean Δ/I for checks (%)	<0.1
No. reflections measured	24569
No. reflections unique	4190
No. reflections I>2σ(I)	3544
Abs. correction	multi–scan
Trans. coefficient (T_{min}, T_{max})	0.925–0.958
R_{int}	0.0224
Refinement (last cycle)	
Weighting scheme (a,b)	0.0613, 0.5730
No. parameters refined	301
R^1 [I>2σ(I)]	0.0454
R^1 (all data)	0.0546
wR^2 [I>2σ(I)]	0.1134
wR^2 (all data)	0.1214
S (g.o.f.) (all data)	1.059
Δ/σ max.	0.001
Δ/σ mean	<0.0005
$\Delta\rho_r$ (min., max.) (e Å^{-3})	–0.21, 0.42

Diffraction data were measured on a Rigaku AFC-7S diffractometer with a Mercury CCD detector using graphite-monochromated Mo-Kα radiation (λ = 0.71070 Å). The structure was solved by direct methods and refined on F^2 by full-matrix least-squares, using all reflections and weights $w = [\sigma^2(F_o^2) + (a\,P)^2 + b\,P]^{-1}$, with $P = (F_o^2 + 2\,F_c^2)/3$. The C-bonded H atoms were placed in calculated positions and refined using a riding atom model with fixed C–H distances (0.93 Å for CH, 0.97 Å for CH_2, 0.96 Å for CH_3), and with $U_{iso} = p\,U_{eq}$(parent atom) (p = 1.2 for CH and CH_2, 1.5 for CH_3). The O-bonded H atoms were located in difference Fourier syntheses and refined isotropically.

The following computer programs were used: data collection, data reduction, cell refinement and absorption correction, CRYSTALCLEAR;[22] structure solution, SHELXS-97;[23] structure refinement, SHELXL-97;[23] geometrical calculations, PLATON;[24] molecular graphics, ORTEP-3.[25] The structure solution, the refinement and the drawings were carried out with the aid of the WinGX[26] suite of programs.

Comprehensive crystallographic data (excluding structure factors) for the structural analysis of **3** have been deposited with the Cambridge Crystallographic Data Centre. Copies of the data (CIF file) and can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK, fax: +44-(0)1223-336033, or from www.ccdc.cam.ac.uk/data_request/cif, quoting deposition No. CCDC 804490.

We thank the IIF and CDCH-UCV (grants IIF.01-2009, PG. 06-7548-2009/1), and CYTED-RIDIMEDCHAG programmes for financial support.

Received 14 December 2010; accepted 12 March 2011
Paper 1000478 doi: 10.3184/174751911X13015834294266
Published online: 3 May 2011

References

1 L. Schreiber, *Science*, 2000, **287**, 1964.
2 S. Teague, A. Davis, P. Leeson and T. Oprea, *Angew. Chem. Int. Ed*., 1999, **38**, 3743.
3 D. Brown, P. Graupner, M. Sainsbury and H. Shertzer, *Tetrahedron*, 1991, **47**, 4383.
4 J. Butera, S. Antane, B. Hirth, J. Lennox, J. Sheldon, N. Norton, D. Warga and T. Argentieri, *Bioorg. Med. Chem. Lett*., 2001, **11**, 2093.
5 C. Bal, B. Baldeyrou, F. Moz, A. Lansiaux, P. Colson, L. Kraus-Berthier, S. Leonce, A. Pierre, M. Boussard, A. Rousseau, M. Wierzbicki and C. Bailly, *Biochem. Pharmacol*., 2004, **68**, 1911.
6 C. Miller, M. Collini and B. Tran, US 6107292, 2000.
7 H. Hemmerling, C. Götz and J. Jose, WO 2008040547, 2008.
8 B. Robinson, *The Fischer indole synthesis*; John Wiley, Chichester, 1982.
9 J. Wang, Q. Ji, J. Xu, X. Wu and Y. Xie, *Synth. Commun*., 2005, **35**, 581.
10 F. Robredo, M. Treus, J. Estevez, L. Castedo and R. Estevez, *Synlett*, 2002, 999.
11 D. Black, M. Bowyer, G. Condie, D. Craig and N. Kumar, *Tetrahedron*, 1994, **50**, 10983.
12 J. Azizian, F. Hatamjafari, A. Karimi and M. Shaabanzadeh, *Synthesis*, 2006, 765.
13 H. Hemmerling, A. Merschenz-Quack and H. Wunderlich, *Z. Naturforsch*., 2004, **59b**, 1143.
14 H. Hemmerling and G. Reiss, *Synthesis*, 2009, 985.
15 R. Ferrer, G. Lobo, N. Gamboa, J. Rodrigues, C. Abramjuk, K. Jung, M. Lein and J. Charris, *Sci. Pharm*., 2009, **77**, 725.
16 A. Yapi, M. Mustofa, A. Valentin, O. Chavignon, J. Teulade, M. Mallie, J. Chapat and Y. Blache, *Chem. Pharm. Bull*., 2000, **48**, 1886.
17 D. Cremer and J.A. Pople, *J. Am. Chem. Soc*., 1975, **97**, 1354.
18 M. Boyd and K. Paull, *Drug Dev. Res*., 1995, **34**, 91.
19 M. Grever, S. Schepartz and B. Chabner, *Sem. Oncol*., 1992, **19**, 622.
20 P. Skehan, R. Storeng, D. Scudiero, A. Monks, J. McMahom, D. Vistica, J. Warren, H. Bokesch, S. Kenney and M. Boyd, *J. Nat. Cancer Inst*., 1990, **82**, 1107.
21 L. Caviedes, J. Delgado and R. Gilman, *J. Clin. Microbiol*., 2002, **40**, 1873.
22 Rigaku/MSC, CrystalClear version 1.3.6. Rigaku/MSC, Inc., The Woodlands, TX, USA, 2005.
23 G.M. Sheldrick, *Acta Crystallogr.*, 2008, **A64**, 112.
24 A.L. Spek, *J. Appl. Crystallogr*., 2003, **36**, 7.
25 L.J. Farrugia, *J. Appl. Crystallogr.*, 1997, **30**, 565.
26 L.J. Farrugia, *J. Appl. Crystallogr*., 1999, **32**, 837.