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Synthesis and crystal structures of *cis*- and *trans*-1-(3′-*N*, *N*-dimethylthiocarbamoyl-4′-methoxy)-2-(3″, 4″, 5″-trimethoxyphenyl) ethene

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The combretastatin A-4 analogue *cis*-1-(3′-*N*,*N*-dimethylthiocarbamoyl-4′-methoxy)-2-(3″,4″, 5″-trimethoxyphenyl) ethene (**1**) and its trans stereoisomer (**2**) were synthesized. The molecular structures of these compounds were obtained by single-crystal X-ray diffraction. Crystallization of **1** occurs in the centrosymmetric monoclinic space group *C*2/*c* (No. 15) with $a = 21.468(5)$, $b = 7.932(1)$, $c = 23.949(3)$; and $\beta = 100.75(1)^\circ$ and $Z = 8$. Crystallization of **2** occurs in the centrosymmetric monoclinic space group $P2_1/n$ (No. 14) with $a = 11.7825(7)$, $b = 11.562(1)$, $c = 14.911(1)$ and $\beta = 93.294(6)$; and $Z = 4$. Details of the synthesis and the structural characterization of the title compounds are presented and discussed.

KEY WORDS: combretastatin; stilbene; thiocarbamate.

Introduction

The use of vascular targeting agents is a relatively recent approach to the treatment of solid tumors.[1,2] This approach to cancer therapy takes advantage of the observation that tumors that enlarge beyond a certain mass can no longer be supported by one main blood vessel, and therefore become vascularized. Disruption of these new tumor-associated capillaries (neovascalature) prevents the blood flow necessary to feed the tumor, resulting in tumor cell starvation, the build-up of toxins, and massive necrotic cell death.

One of the best characterized small molecule vascular targeting agents is Combretastatin A-4 (CA4) (**3**).[3–5] While this compound is known to be an extremely effective inhibitor of the assembly of tubulin into microtubules,[6] its potential as a vascular targeting agent was only revealed after its formulation into the water-soluble prodrug, disodium phosphate Combretastatin A-4 (CA4DP) (**4**).[7] Although the prodrug **4** is inactive towards tubulin, it is dephosphorylated in the neovasculature of the tumor to reveal the parent tubulin polymerization inhibiting drug **3**. The resulting disruption of the microtubule skeleton of the endothelial cells in the neovasculature causes them to lose their characteristic flat shape and become bloated, thereby occluding the narrow capillaries. The loss of blood flow to the tumor mass results in extensive necrotic cell death.[8]

The amine analogue (**5**) of CA4 is also a very active tubulin polymerization inhibitor.[9] Formulated as the serine amide **6** this compound also shows significant promise as a vascular targeting

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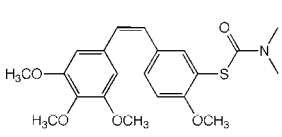
agent for the treatment of cancer.[10] As is true for the CA4DP prodrug of CA4, compound 6 is inactive until converted to the parent drug **5**. However, the activation chemistry for the two prodrugs is very different (dephosphorylation for **4** and **3** compared to deacylation for **6** and **5**). This prompted us to investigate the thiol analogue (**7**) of compounds **3** and **5**, and to study the formulation of this novel compound into reductively activated prodrugs. In the course of this work a crystalline derivatives of compound **7** (its *N,N*-dimethylcarbamate **1**) and its trans stereoisomer (derivative **2**) were isolated and structurally characterized in order to unequivocally confirm their molecular structure and to gather information for our molecular recognition studies.



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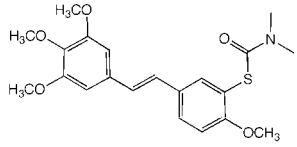

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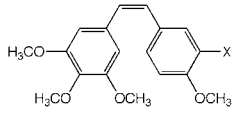

3 X = OH
4 X = OPO_3Na_2
5 X = NH_2
6 X = $NHCH(CO_2H)CH_2OH$
7 X = SH

Experimental

Synthesis

3-(N,N-dimethylthiocarbamoyloxy)-4-methoxybenzaldehyde. A solution of *N,N*-dimethylthiocarbamoyl chloride (4.07 g, 0.0329 mmol) in THF was added dropwise at 0°C to a solution of 3-hydroxy-4-methoxybenzaldehyde (5.03 g, 0.0329 mol) and potassium hydroxide (1.84 g, 0.0329 mol) in water (22 mL). The resulting white precipitate was filtered, washed with water and dried by vacuum. Yield 6.72 g (85%) of 3-(*N,N*-dimethylthiocarbamoyloxy)-4-methoxybenzaldehyde. ^{1}H NMR (300 MHz, $CDCl_3$, δ relative to residual $CHCl_3$ at 7.26 ppm): δ 9.86 (1H, s; CHO), 7.77 (1H, dd, $J = 8.5$ and 2.0 Hz; ArH), 7.57 (1H, d, $J = 2.0$ Hz; ArH), 7.06 (1H, d, $J = 8.5$ Hz; ArH), 3.90 (3H, s; OCH_3), 3.45 (3H, s; $N(CH_3)$), and 3.35 (3H, s; $N(CH_3)$).

3-(N,N-dimethylcarbamoylthio)-4-methoxybenzaldehyde. A solution of 3-(*N,N*-dimethylcarbamoylthio)-4-methoxybenzaldehyde (4.93 g, 0.0206 mol) in 200 mL of diphenyl ether was heated to 250°C under argon for 2 h. The reaction mixture was cooled to room temperature and added to 1 L of hexanes. The precipitate that formed was collected by suction filtration, washed with hexanes, and dried under vacuum. Yield 3.77 g (77%) of 3-(*N,N*-dimethylcarbamoylthio)-4-methoxybenzaldehyde as a light brown solid. ^{1}H NMR (300 MHz, $CDCl_3$, δ relative to residual $CHCl_3$ at 7.26 ppm): δ 9.87 (1H, s; CHO), 7.99 (1H, d, $J = 2.1$ Hz; ArH), 7.94 (1H, dd, $J = 2.1$ and 8.5 Hz; ArH), 7.06 (1H, d, $J = 8.5$ Hz; ArH), 3.95(3H, s; OCH_3), 3.08 (6H, 2 broad s; $N(CH_3)_2$).

3, 4, 5-Trimethoxybenzyl triphenylphosphonium bromide. To solution of CBr_4 (22.9 g, 0.069 mol) in anhydrous acetone (170 mL) at 0°C under argon, 3,4,5-trimethoxybenzyl alcohol (10 g, 0.050 mol) and triphenylphosphine (17.99 g, 0.068 mol) were added. After the reaction was stirred at room temperature overnight the solvent was removed under reduced pressure to obtain a crude sample of 3,4,5-trimethoxybenzyl bromide as a brown gel. That crude product was immediately dissolved in toluene (200 mL), triphenylphosphine (14.54 g, 0.055 mol) was added, and the reaction was refluxed for 2 h. The reaction was then allowed to mix overnight, resulting in the formation of a sticky orange precipitate. The solvent was removed under reduced pressure and the residue dried under vacuum. The crude product was recrystalized from ethanol, and the resulting white crystals dried under vacuum. Yield 17.64 g (66.8%) of 3,4,5-trimethoxybenzyl triphenylphosphonium bromide.

(E) and (Z)-1-(3′-N, N-dimethylthiocarbamoyl-4′-methoxy)-2-(3″,4″,5″-trimethoxyphenyl) ethene. 3,4,5-trimethoxybenzyl triphenylphosphonium bromide (4.37 g, 8.35 mol) was added to approximately 170 mL of anhydrous THF under argon and stirred vigorously at −20°C. A 2.0 M solution of *n*-BuLi in cyclohexane (0.535 g, 4.18 mL 8.36 mmol) was then added dropwise to the solution over a period of 15 minutes. The resulting blood-red solution was allowed to warm to RT and 3-(*N*,*N*-dimethylcarbamoylthio)-4-methoxybenzaldehyde (1 g, 4.18 mmol) in 30 mL of anhydrous THF was added dropwise over 20 minutes, during which time the solution became dark orange in color. The reaction was followed by monitoring the disappearance of the aldehyde by thin layer chromatography (40% EtOAc/60% hexanes, approx. 1.5 h). When the reaction reached completion it was quenched by the careful addition of 210 mL ice-cold water and the organic products extracted (4 extractions using 250 mL of diethyl ether). The ether extracts were then washed with 200 mL of ice-cold water and dried over sodium sulfate. Removal of the solvent in vacuo resulted in the isolation of a yellow gel. ^{1}H NMR analysis of this crude product revealed the presence of a 2:1 mixture of the Z and E isomers of the product. The crude mixture was subjected to flash chromatography (Biotage Flash40S system, 40 g silica gel, 40% EtOAc/60% hexanes), resulting in the isolation of white and yellow crystalline solids, corresponding to E and Z isomers respectively, which were recrystalized from methanol. Yields were 0.71 g (42%) of Z-1-(3′-*N*,*N*-dimethylthiocarbamoyl-4′-methoxy)-2-(3″,4″,5″-trimethoxyphenyl) ethene and 0.34 g (21%) of E-1-(3′-*N*,*N*-dimethylthiocarbamoyl-4′-methoxy)-2-(3″,4″,5″-trimethoxyphenyl) ethene.

^{1}H NMR Z-isomer (300 MHz, $CDCl_3$, δ relative to residual $CHCl_3$ at 7.26 ppm): δ 7.44 (1H, d, $J = 2.3$ Hz; ArH), 7.31 (1H, dd, $J =$ 8.6 and 2.3 Hz; ArH), 6.82 (1H, d, $J = 8.6$ Hz; ArH), 6.51 (2H, s; ArH), 6.45 (1H, d, 1H, $J = 12.3$ Hz; vinylic H), 6.40 (1H, d, $J = 12.3$ Hz; vinylic H), 3.84 (3H, s; OCH_3), 3.82 (3H, s; OCH_3), 3.70 (6H, s; OCH_3), 3.03 (6H, broad s; $N(CH_3)_2$).

^{13}C NMR Z-isomer (75 MHz, $CDCl_3$, δ relative to $CHCl_3$ at 77 ppm): δ 14.21, 21.07, 36.96, 56.00, 56.23, 60.41, 60.91, 105.83, 111.11, 116.56, 128.60, 129.21, 129.98, 132.31, 132.65, 137.12, 138.53, 152.99, 159.32, and 165.93.

^{1}H NMR E-isomer (300 MHz, $CDCl_3$, δ relative to residual $CHCl_3$ at 7.26 ppm): δ 7.64 (1H, d, $J = 2.3$ Hz; ArH), 7.51 (1H, dd, $J = 8.6$ and 2.3 Hz; ArH), 6.94 (1H, d, $J = 8.6$ Hz; ArH), 6.93 (1H, d, $J = 16.1$ Hz; vinylic H), 6.89 (1H, d, $J = 16.1$ Hz; vinylic H), 6.69 (2H, s; ArH), 3.90 (6H, s; OCH_3), 3.89 (3H, s; OCH_3), 3.85 (3H, s; OCH_3), 3.11 (6H, broad s, $N(CH_3)_2$).

^{13}C NMR E-isomer (75 MHz, $CDCl_3$, δ relative to $CHCl_3$ at 77 ppm): δ 37.06, 56.13, 56.31, 60.99, 103.41, 111.66, 117.26, 127.02, 127.36, 129.50, 130.52, 133.30, 136.05, 137.77, 153.40, and 159.59.

X-ray. A summary of crystal data is presented in Table 1. Diffracted intensities were collected on an Enraf-Nonius CAD4 diffractometer using graphite-monochromated Mo Kα- X-radiation. Final unit cell dimensions were determined from the setting angles of 25 reflections for **1** and **2**. The data were corrected for Lorentz and polarization effects.

Both structures were solved by direct methods which gave the positions of all non-hydrogen atoms using SHELXTL ver. 6.12.[11] Refinements were made by full-matrix least squares on all F^2 data using SHELXL. Anisotropic thermal parameters were included for all non-hydrogen atoms. All hydrogen atoms were included in calculated positions and allowed to ride on their parent carbon atom with fixed isotropic thermal parameters [$U_{iso}(H) = 1.2U_{iso}$(parent)].

Results and discussion

The synthesis of the two title compounds was relatively straightforward. A thermal Newmann-Kwart rearrangement[12] efficiently transformed isovanillin into the desired protected thiol. Wittig reaction of this aldehyde

Table 1. Crystal Data and Structure Refinement

	1	**2**
CCDC deposit no.	247276	247137
Color/Shape	Colorless/prism	Colorless/prism
Crystal size (mm)	0.31 × 0.20 × 0.13	0.59 × 0.52 × 0.34
Empirical formula	$C_{21}H_{25}NO_5S$	$C_{21}H_{25}NO_5S$
Formula weight	403.48	403.48
Temperature (K)	298(2)	298(2)
Crystal system	Monoclinic	Monoclinic
Space Group	C2/c	$P2_1/n$
a (Å)	21.468(5)	11.7825(7)
b (Å)	7.932(1)	11.562(1)
c (Å)	23.949(3)	14.911(1)
β (°)	100.75(1)	93.294(6)
V (Å^3)	4006(1)	2027.8(3)
Z	8	4
D_c (Mg M^{-3})	1.338	1.322
μ (Mo Kα) (mm^{-1})	0.194	0.192
Diffractometer/scan	Enraf-Nonius CAD4/ω	Enraf-Nonius CAD4/ω
$\Delta\theta$ for data collection (°)	1.73–24.98	2.14–24.93
Reflections measured	3885	4028
h, k, l limits	$-7 \le h \le 25, 0 \le k \le 9, -28 \le l \le 27$	$-13 \le h \le 13, -2 \le k \le 13, -2 \le l \le 17$
Unique Reflections	3520 ($R_{int} = 0.0123$)	3527 ($R_{int} = 0.0081$)
Observed reflections	2491 [$I > 2\sigma(I)$]	2105 [$I > 2\sigma(I)$]
Data/restraints/parameters	3520/0/254	3527/0/253
Goodness of fit on F^2	1.028	1.011
Final R indices [$I > 2\sigma(I)$]	$R1 = 0.0480$, $wR^2 = 0.1044$	$R1 = 0.0558$, $wR^2 = 0.11127$
R indices (all data)	$R1 = 0.0744$, $wR^2 = 0.1134$	$R1 = 0.1002$, $wR^2 = 0.1281$
Largest diff. peak (eÅ^{-3})	0.307	0.194

with 3,4,5-trimethoxybenzyl triphenylphosphonium bromide afforded a 2:1 mixture of the Z and E stilbene isomers which could be readily separated by column chromatography.

Thermal ellipsoid plots of compounds **1** and **2** are shown in Figs. 1 and 2 respectively and selected geometric parameters are presented in Table 2. The geometric parameters of the two structures match up very closely, for example the S─$C_{(ring)}$ bond length are identical at 1.771(3) Å, and the C─$O_{(carbonyl)}$ bond lengths are nearly identical at 1.213(3) Å for **1** and 1.212(3) for **2**.

The only bond lengths that show a significant deviation are those associated with the alkene double bond, which is 1.332(4) for **1** and 1.313(4) for **2**. Smaller differences are noticed in the $C_{(alkene)}$–$C_{(ring)}$ bond lengths with C6─C11 being 1.476(4) Å for **1** and the same bond being 1.466(6) Å for **2**. Similarly the C12─C13 bond length for **1** is 1.480(4) Å and for **2** it is 1.464(4) Å. The longer bond lengths for the *cis* isomer are likely due to

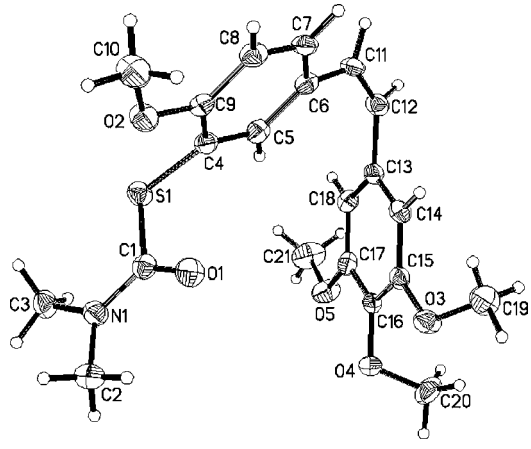

Fig. 1. A view of the crystallographic orientation of **1**. Thermal ellipsoids are drawn at the 50% probability level.

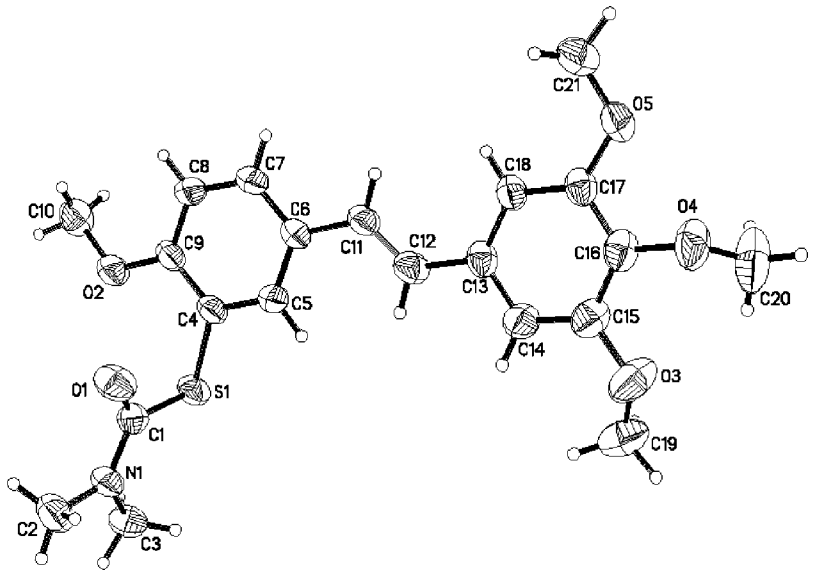

Fig. 2. A view of the crystallographic orientation of **2**. Thermal ellipsoids are drawn at the 50% probability level.

Table 2. Selected Geometric Parameters for **1** and **2** in (Å) and (°)

Selected Geometric Parameters for **1**			
S(1)—C(4)	1.771(3)	S(1)—C(1)	1.816(3)
O(1)—C(1)	1.213(3)	N(1)—C(1)	1.347(3)
N(1)—C(3)	1.459(3)	N(1)—C(2)	1.462(3)
C(6)—C(11)	1.476(4)	C(11)—C(12)	1.332(4)
C(12)—C(13)	1.480(4)		
C(4)—S(1)—C(1)	100.7(1)	C(9)—O(2)—C(10)	117.0(2)
C(1)—N(1)—C(3)	124.1(2)	C(1)—N(1)—C(2)	118.7(2)
C(3)—N(1)—C(2)	117.0(2)	O(1)—C(1)—N(1)	125.1(3)
O(1)—C(1)—S(1)	121.6(2)	N(1)—C(1)—S(1)	113.3(2)
C(11)—C(12)—C(13)	126.5(2)	C(12)—C(11)—C(6)	128.7(2)
C7—C6—C11—C12	152.34(0.27)	C6—C11—C12—C13	−9.80(0.45)
C18—C13—C12—C11	122.78(0.29)	C12—C11—C6—C5	−35.13(0.41)
Selected Geometric Parameters for **2** in (Å) and (°)			
S(1)—C(4)	1.771(3)	S(1)—C(1)	1.793(3)
O(1)—C(1)	1.212(3)	N(1)—C(1)	1.346(4)
N(1)—C(3)	1.448(4)	N(1)—C(2)	1.457(4)
C(6)—C(11)	1.466(4)	C(11)—C(12)	1.313(4)
C(12)—C(13)	1.464(4)		
C(4)—S(1)—C(1)	99.84(14)	C(9)—O(2)—C(10)	117.7(2)
C(1)—N(1)—C(3)	124.7(3)	C(1)—N(1)—C(2)	118.4(3)
C(3)—N(1)—C(2)	116.8(3)	O(1)—C(1)—N(1)	123.5(3)
O(1)—C(1)—S(1)	122.0(2)	N(1)—C(1)—S(1)	114.5(2)
C(11)—C(12)—C(13)	129.1(3)	C(12)—C(11)—C(6)	125.9(3)
C7—C6—C11—C12	−177.78(0.31)	C12—C11—C6—C5	2.27(0.50)
C6—C11—C12—C13	−176.84(0.30)	C11—C12—C13—C18	5.51(0.54)

steric repulsions of the rings that are now on the same side of the double bond. These steric forces also likely cause the major difference between the two structures which is the ring tilt about the double bond. In the structure of **1**, which consists of a *cis* arrangement of the rings across the double bond, the planes of the two rings are 71.2° to each other, while for **2**, the *trans* structure they are much closer to coplanar at only 6.7°. The torsion angle reflects this twist as well, for **1** the angle C6—C11—C12—C13 is 9.8 (5)° and in **2** the corresponding angle (C6—C11—C12—C13) is 3.2 (3)°. The twist is more clearly seen by looking at the torsion angles $C_{(alkene)}$–$C_{(alkene)}$–$C_{(ring)}$–$C_{(ring)}$, for 1 these angles range about from 27° to 57° from coplanar. The angles are much more acute for 2 with a range from about 2.2° to 5.5° from coplanar.

Acknowledgments

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Supplementary material CCDC-247276 and CCDC-247137 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge at www.ccdc.cam.ac.uk/conts/retrieving.html [or from the Cambridge Crystallographic Data Centre (CCDC), 12 Union Road, Cambridge CB2 1EZ,UK; fax +44(0)1223–336033; e-mail: deposit@ccdc.ccam.ac.uk].

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