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Synthesis and characterization of acylated combretastatin-A4 analogs

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ABSTRACT

A series of variously substituted 3,4-diphenyl-3-butene-2-ones **(6a-6l)** have been synthesized by condensation of phenylacetone and substituted aryl aldehydes. These α,β-unsaturated ketones possessing *cis*-stilbene core are structural analogs of combretastatin-A4, an effective anti-tumor agent. All the compounds have been characterized by ^{1}H NMR, IR and mass spectroscopy and elemental analysis. The *cis*-configuration of the compounds in this series was ascertained by single crystal X-ray diffraction study of the representative compound **(6e)**.

KEYWORDS

Phenyl acetone;
α,β-Unsaturated ketones;
Combretastatin-A4;
X-ray diffraction.

1. INTRODUCTION

Combretastatins, isolated from the African willow tree *Combretum caffrum* Kuntze (Combretaceae), are known to inhibit the growth of cancer cells (murine P388 lymphocytic leukemia)[1]. The most potent compound of this class of natural products is Combretastatin-A4 (CA-4, **(1)**, Figure 1), which strongly inhibits the polymerization of tubulin by binding to the colchicines site[2]. CA-4 exhibits potent cytotoxicity against a broad spectrum of human cancer cell lines. This compound also elicits irreversible vascular shutdown within solid tumors, leaving normal vasculature intact, thereby acting as an anti-angiogenic agent[3]. There is a significant interest in the design of combretastatin analogs that have more beneficial pharmacological properties. As a result, a large number of combretastatin analogs are being synthesized and investigated for anti-cancer activity[4]. A water-soluble phosphate pro-drug derivative of CA-4P **(2)**[5] was developed, and it is presently undergoing phase II clinical trials. An amino acid pro-drug form of CA-4 **(3)**, or AVE-8062, having favorable water solubility, has entered into phase I clinical trials[6].

The anticancer potential of combretastatin has been widely studied, but it is still important to contribute, for an ample knowledge, on the biological effects of related compounds. Our efforts have been aimed at synthesizing selected stilbene-based combretastatin analogs and evaluating the cytotoxic potential of these compounds, thus giving a platform for the correlation between structure and activity using different cell models[7].

In continuation of our efforts, we have synthesized a series of α,β-unsaturated ketones **(6a-6l)**, which contain the *cis*-stilbene core present in the combretastatins (It is well known that the combretastatins contain a core *cis*-stilbene nucleus which is necessary for a good fit into the colchicine binding site in tubulin[8]. The *cis*-configuration of combretastatins is responsible for its anti-tumor activity). The assessment of ability of these compounds to inhibit the growth of L1210 and B16 cells (murine leukemia and melanoma cell lines) is presently

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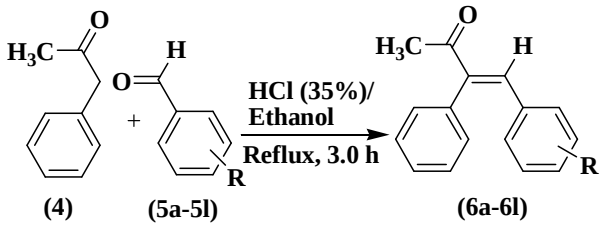
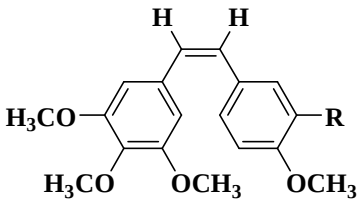


(4) (5a-5l) (6a-6l)

SCHEME 1: Synthetic pathway of combretastatin analogues (6a-6l)



(1), R = -OH, cis-combretastain A4 or CA-4
(2), R = -OPO_3
(3), R = -$NHCOCH(NH_2)CH_2OH$, AVE-8062

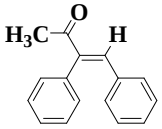
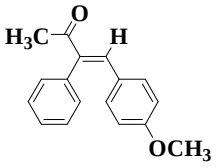
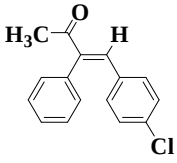
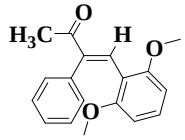
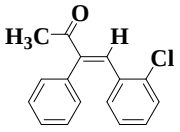
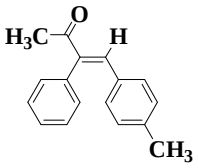
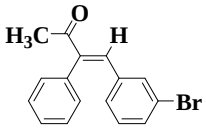
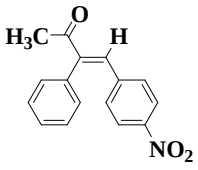
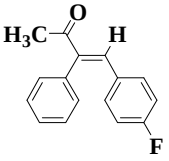
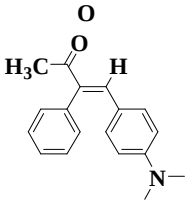
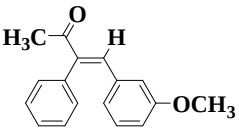
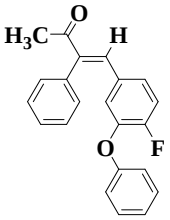
Figure 1: Structures of combretastatin-A4 (1), CA-4P (2), an amino analog of CA-4 (AVE-8062) (3)

under progress. Even though such enone-containing molecules can potentially undergo Michael reactions with biological nucleophiles such as glutathione[9], they are worthy of further investigation because α,β-enone analogues of CA-4, have been found to exhibit potent cytotoxicity against cancer cells in culture[10]. In this communication, we wish to report synthesis, characterization and geometry of the molecules about stilbene double bond. The *cis*-geometry of the compounds in this series was confirmed by single crystal X-ray diffraction study of the representative compound **(6e)**.

2. RESULTS AND DISCUSSION

Twelve combretastatin A4 analogs have been synthesized as depicted in SCHEME 1. Phenyl acetone[11] **(4)** was treated with appropriate aryl aldehyde **(5a-5l)** in ethanol in presence of concentrated hydrochloric acid under reflux conditions to obtain desired α,β-unsaturated ketones in good yield. The crude compounds were purified by column chromatography using silica gel. All the compounds were characterized by ^{1}H NMR, IR and mass spectroscopy and elemental analysis. The IR spectra of the compounds showed characteristic band for carbonyl group in the region 1650-1677 cm^{-1}. The ^{1}H NMR spectra were recorded in $CDCl_3$ which

TABLE 1: Structures of the combretastatin A4 analogs (6a-6l)

Entry	Structure	Entry	Structure
(6a)		(6g)	
(6b)		(6h)	
(6c)		(6i)	
(6d)		(6j)	
(6e)		(6k)	
(6f)		(6l)	

showed the presence of three proton singlet for methyl protons of acetyl group at around δ 2.26-2.46 ppm. The characteristic singlet for olefin proton appeared in the downfield region at δ 7.42-7.85 ppm. The elemental analysis of all the compounds was in agreement with their theoretical value. The structures of the compounds are presented in TABLE 1.

The orientation of the two phenyl rings about carbon-carbon double bond is vital from a practical standpoint because two forms (cis- and trans-) of combretas tatin have different biological activity. The knowledge of the geometric form of the combretastatin analogs is primarily important for drug-colchicine interaction. The geometry of the two phenyl rings about the carbon-

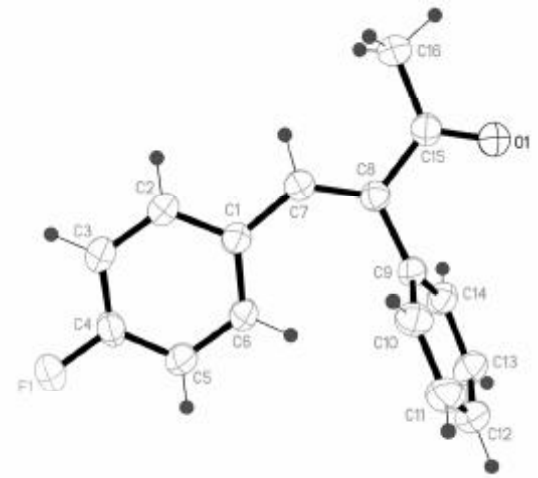


Figure 2: Molecular structure of the compound (6e) determined by X-ray crystallography

carbon double bond in these compounds was exclusively found to be *cis* as ascertained from the single crystal X-ray diffraction study of the model compound **(6e)**. Suitable single crystal for X-ray analysis of the compound **(6e)** was obtained by slow evaporation from ethanol. Figure 2 depicts the molecular structure of the compound **(6e)** which clearly indicates that phenyl ring attached to C8 is slightly rotated about C8-C9 bond owing to the repulsion between lone pair electrons on carbonyl oxygen and π-electron cloud on phenyl ring. Indeed, two rings attached to C7 and C8 are on same side of the double bond.

3. EXPERIMENTAL

All melting points were uncorrected and are in °C. FT-IR spectra were recorded on a Perkin Elmer Spectrum 100 FT-IR spectrophotometer with DRS (Diffuse Reflectance Sampler). ^{1}H NMR spectra were recorded on Varian AS-400 FT NMR spectrometer, and the chemical shifts are expressed in δ ppm using TMS as an internal standard. Microanalysis for C, H, N and S were performed on Thermofignnin Elemental analyzer. Mass spectra were recorded on API 2000 (Applied Biosystems), ion source (ESI/APCI) spectrometer. The X-ray diffraction study was undertaken using Nonius Kappa CCD diffractometer. All the reactions were monitored by thin layer chromatography (TLC). TLC was performed on F_{254}, 0.25 mm silica gel plates (Merck). Plates were eluted with appropriate solvent systems, and the developed plates were analyzed under UV 254nm. Column chromatography was performed using silica gel with particle size 100-200 mesh.

General method of synthesis of novel combretastatin analogs (6a-6l)

Phenyl acetone[11] (4) (0.5 g, 3.7 mmol) was dissolved in ethanol (5.0 ml). To the clear solution obtained was added appropriate aryl aldehyde (7.4 mmol) at room temperature. The reaction mixture was stirred for 10 min to obtain clear solution. Hydrochloric acid (35%, 0.5 ml) was then added drop wise to the reaction mixture and refluxed for 2-3 h. The progress of the reaction was monitored by TLC. Ethanol was removed under vacuum and the crude compound obtained was purified by column chromatography using n-hexane-ethyl acetate (9:1) as eluent system.

3,4-Diphenylbut-3-en-2-one (6a)

Yield, 68%; Colorless oil; ^{1}H NMR ($CDCl_3$): δ 2.30 (s, 3H, CH_3), δ 7.01(m, 2H, phenyl protons), δ 7.14-.33 (m, 8H, phenyl protons), δ7.64 (s, 1H, olefinic proton); IR (KBr) ν_{max} cm^{-1}: 3064, 1718, 1651, 1493, 1450, 1317, 1234, 1176, 759, 702; Anal. Calcd. for $C_{16}H_{14}O$: C, 86.45; H, 6.35. Found: C, 86.44; H, 6.36; MS: m/z 223 (M^++1).

4-(4-Chlorophenyl)-3-phenylbut-3-en-2-one (6b)

Yield, 72%; White crystalline solid; mp125^{0}C; ^{1}H NMR ($CDCl_3$): δ 2.29 (s, 3H, CH_3), δ 6.93 (d, 5.6Hz, 2H, phenyl protons), δ 7.11 (d, 5.6Hz, 2H, phenyl protons), δ 7.14 (dd, 1.6 and 2.4Hz, 2H, phenyl protons), δ7.38-7.41(m, 3H, phenyl protons), δ 7.57 (s, 1H, olefinic proton); IR (KBr) ν_{max} cm^{-1}: 3298,3059,1654, 1621, 1591,1488,1434,1352,1303,1235,1170, 1088, 1007, 824, 701; Anal. Calcd. for $C_{16}H_{13}ClO$: C, 74.85; H, 5.10. Found: C, 74.81; H, 5.15; MS: m/z 257 (M^++1).

4-(2-Chlorophenyl)-3-phenylbut-3-en-2-one (6c)

Yield, 77%; Colorless oil; ^{1}H NMR ($CDCl_3$): δ 2.39 (s, 3H, CH_3), 6.70 (d, 8.0Hz, 1H, phenyl proton), δ 6.83 (m, 1H, phenyl proton), δ 7.08(m, 3H, phenyl protons), δ7.27(m, 3H, phenyl protons), δ 7.33 (d, 8.0Hz, 1H, phenyl proton), δ 7.85 (s, 1H, olefinic proton); IR (KBr) ν_{max} cm^{-1}: 3054, 2929, 1677, 1593, 1440, 1233, 756, 700; Anal. Calcd. for $C_{16}H_{13}ClO$: C, 74.85; H, 5.10. Found: C, 74.86; H, 5.14; MS:

m/z 257 (M^++1).

4-(3-Bromophenyl)-3-phenylbut-3-en-2-one (6d)

Yield, 65%; Colorless oil; ^{1}H NMR ($CDCl_3$): δ2.31(s, 3H, CH_3), δ6.92 (m, 1H, phenyl proton), δ 6.99 (m,1H, phenyl proton), δ 7.14-7.43 (m, 7H, phenyl protons), δ 7.54 (s, 1H, olefinic proton); IR (KBr) ν_{max} cm^{-1}: 3063, 2997, 1961, 1658, 1428, 1390, 1279, 1234, 1166, 1072, 895, 700; Anal. Calcd. for $C_{16}H_{13}BrO$: C, 63.81; H, 4.35. Found: C, 63.74; H, 4.33; MS: m/z 303 (M^++2).

4-(4-Fluorophenyl)-3-phenylbut-3-en-2-one (6e)

Yield, 80%; Colourless crystalline solid; mp 83^{0}C; ^{1}H NMR ($CDCl_3$): δ 2.29 (s, 3H, CH_3), δ 6.82 (m, 2H, phenyl protons), δ 7.00 (m, 2H, phenyl protons), δ7.16 (m, 2H, phenyl protons), δ 7.40 (m, 3H, phenyl protons), δ 7.61 (s, 1H, olefinic proton); IR (KBr) ν_{max} cm^{-1}: 3290, 3054, 1655, 1596, 1508, 1312, 1235, 1163, 1013, 908, 835, 702; Anal. Calcd. for $C_{16}H_{13}FO$: C, 79.98; H, 5.45. Found: C, 80.01; H, 5.44; MS: m/z 241 (M^++1).

4-(3-Methoxyphenyl)-3-phenylbut-3-en-2-one (6f)

Yield, 73%; Colorless oil; ^{1}H NMR ($CDCl_3$): δ 2.30 (s, 3H, CH_3), δ 3.44 (s, 3H, OCH_3), δ6.46 (t, 2.0Hz, 1H, phenyl proton), δ6.72 (m, 2H, phenyl protons), δ 7.09 (m, 1H, phenyl proton), 7.16 (m, 3H, phenyl protons), δ 7.31 (m, 2H, phenyl protons), δ 7.60 (s, 1H, olefinic proton); IR (KBr) ν_{max} cm^{-1}: 3061, 2995, 1715, 1657, 1596, 1485, 1433, 1391, 1290, 1159, 948, 794; Anal. Calcd. for $C_{17}H_{16}O_2$: C, 80.93; H, 6.39. Found: C, 80.90; H, 6.43; MS: m/z 253 (M^++1).

4-(4-Methoxyphenyl)-3-phenylbut-3-en-2-one (6g)

Yield, 82%; Pale yellow solid; mp 48^{0}C; ^{1}H NMR ($CDCl_3$): δ 2.28 (s, 3H, CH_3), δ 3.74 (s, 3H, OCH_3), δ 6.67 (d, 8.6Hz, 2H, phenyl protons), δ 6.97 (d, 8.6Hz, 2H, phenyl protons), δ7.18 (m, 2H, phenyl protons), δ 7.38 (m, 3H, phenyl protons), δ 7.62 (s, 1H, olefinic proton); IR (KBr) ν_{max} cm^{-1}: 2927, 2835, 1650, 1599, 1509, 1355, 1308, 1255, 1169, 1032, 829, 700; Anal. Calcd. for $C_{17}H_{16}O_2$: C, 80.93; H, 6.39. Found: C, 80.89; H, 6.41; MS: m/z 253 (M^++1).

4-(2,6-Dimethoxyphenyl)-3-phenylbut-3-en-2-one (6h)

Yield, 68%; White solid; mp 102^{0}C; ^{1}H NMR ($CDCl_3$): δ 2.46 (s, 3H, CH_3), δ 3.47(s, 6H, OCH_3), δ 6.37 (d, 8.4Hz, 2H, phenyl protons), δ 7.00 (m, 2H, phenyl protons), δ 7.14 (m, 4H, phenyl protons), 7.61(s, 1H, olefinic proton); IR (KBr) ν_{max} cm^{-1}: 3308, 2994, 2841, 1664, 1595, 1474, 1304, 1260, 1235, 1109, 1022, 778, 663; Anal. Calcd. for $C_{18}H_{18}O_3$: C, 76.57; H, 6.43. Found: C, 76.60; H, 6.41; MS: m/z 283 (M^++1).

3-Phenyl-4-(4-methylphenyl)but-3-en-2-one (6i)

Yield, 70%; Off white solid; mp 58^{0}C; ^{1}H NMR ($CDCl_3$): δ 2.26 (s, 3H, CH_3), δ 2.29(s, 3H, CH_3), δ 6.90 (m, 4H, phenyl protons), δ 7.15 (dd, 1.6 and 2.0Hz, 2H, phenyl protons), δ 7.36 (m, 3H, phenyl protons), δ 7.62 (s, 1H, olefinic proton); IR (KBr) ν_{max} cm^{-1}: 3297, 3053, 2924, 1719, 1654, 1623, 1492, 1391, 1315, 1236, 1182, 1009, 814, 699; Anal. Calcd. for $C_{17}H_{16}O$: C, 86.40; H, 6.82. Found: C, 86.44; H, 6.85; MS: m/z 239 (M^++1).

4-(4-Nitrophenyl)-3-phenylbut-3-en-2-one (6j)

Yield, 81%; Off white solid; mp 110^{0}C; ^{1}H NMR ($CDCl_3$): δ 2.31 (s, 3H, CH_3), δ 7.16 (m, 4H, phenyl protons), δ 7.41 (m, 3H, phenyl protons), δ 7.62 (s, 1H, olefinic proton), δ 7.99 (d, 8.8Hz, 2H, phenyl protons); IR (KBr) ν_{max} cm^{-1}: 3112, 3058, 1673, 1661, 1590, 1517, 1350, 1230, 861, 703; Anal. Calcd. for $C_{16}H_{13}NO_3$: C, 71.90; H, 4.90; N, 5.24. Found: C, 71.92; H, 4.86; N, 5.27; MS: m/z 268 (M^++1).

4-(4-(N,N-Dimethylamino)phenyl)-3-phenylbut-3-en-2-one (6k)

Yield, 64%; Semisolid; ^{1}H NMR ($CDCl_3$): δ 2.26 (s, 3H, CH_3), δ 2.93 (s, 6H, N- CH_3), δ6.44 (d, 8.8Hz, 2H, phenyl protons), δ 6.69 (d, 8.6Hz, 2H, phenyl protons), δ7.37 (m, 3H, phenyl protons), δ 7.62 (s, 1H, olefinic proton), δ 7.73 (d, 8.6Hz, 2H, phenyl protons); IR (KBr) ν_{max} cm^{-1}: 2914, 2819, 2740, 1898, 1669, 1597, 1526, 1440, 1370, 1317, 1234, 1165, 1067, 1004, 944, 816, 726, 703; Anal. Calcd. for $C_{18}H_{19}NO$: C, 81.47; H, 7.22. Found: C, 81.49; H, 7.18; MS: m/z 266 (M^++1).

4-(4-Fluoro-3-phenoxyphenyl)-3-phenylbut-3-en-2-one (6l)

Yield, 69%; White solid; mp 69^{0}C; ^{1}H NMR ($CDCl_3$): δ 2.15(s, 3H, CH_3), δ 6.52 (dd, 5.6 and 2.0Hz, 1H, phenyl proton), δ 6.70 (m, 3H, phenyl protons), δ 6.86

(m, 1H, phenyl proton), δ 6.97 (m, 3H, phenyl protons), δ 7.16 (m, 5H, phenyl protons), δ 7.42 (s, 1H, olefinic proton); IR (KBr) ν_{max} cm^{-1}: 3062, 2928, 1715, 1656, 1589, 1510, 1492, 1393, 1273, 1213, 1117, 968, 906, 805, 767, 697; Anal. Calcd. for $C_{22}H_{17}FO_2$: C, 79.50; H, 5.16. Found: C, 79.52; H, 5.20; MS: m/z 333 (M^++1).

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