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Synthesis and structural determination of 3,5-bis(2-fluorobenzylidene)-4-piperidone analogs of curcumin

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ABSTRACT

Analogs of cytotoxic compound 3,5-bis(2-fluorobenzylidene)-4-piperidone (**1**) were synthesized and their molecular structures were characterized by ^{1}H, ^{13}C NMR, ESI–MS, IR spectra and X-ray crystallography. The central ring of the piperidin-4-one ring assumes a sofa conformation with two benzylidene rings connected through *E,E* oriented groups. The compound **1** crystallized in triclinic space group $P\bar{1}$. In order to obtain potentially more efficacious compounds, three dimers of 3,5-bis(2-fluorobenzylidene)-4-piperidone were synthesized. The two molecules of **1** were conjugated together via —*N*—oxalyl—*N*— (**3**), —*N*—fumaryl—*N*— (**4**) or —*N*—DTPA—*N*— (**5**) linkers. Compound **3** crystallized in monoclinic space group $P2_1/n$.

1. Introduction

We synthesized a curcumin analog, 3,5-bis(2-fluorobenzylidene)-4-piperidone (**1**) by acid catalyzed Claisen–Schmidt condensation of 4-piperidone and 2-fluorobenzaldehyde [1]. Compound **1** is an antiproliferative compound, and is also known as EF24 [2,3]. As compared to curcumin, it possesses significantly more potent biological activity and better bioavailability profile [4]. The compound has been investigated *in vitro* and in preclinical models of cancer, including those of breast cancer [5], colon cancer [3] and pancreatic cancer (unpublished results in our laboratory). Although the exact molecular target(s) for its action is not clearly understood, the role of NFκB has been proposed as pivotal to the compound **1**'s antiproliferative effects [3–6]. The exhaustive structure activity relationship of compound **1** has not been reported, but it is possible to obtain better understanding about stereochemical features of molecular targets by elucidating molecular and crystal structure of compound **1**. In this article, we report a detailed crystallographic analysis of compound **1**. In addition, we report for the first time dimers of compound **1** for potentially enhanced activity against proliferating cells.

2. Experimental

2.1. Materials and methods

All reagents were obtained from commercial sources and used directly without further purification. The reactions were monitored by thin layer chromatography (TLC) on 250 μm silica plates. ^{1}H NMR spectra and ^{13}C NMR spectra (DMSO-*d*6 and $CDCl_3$) were recorded at 300 MHz, and 75 MHz on Mercury-VX 300, 4 nuclei autoswitchable PFG probe. The *E,E*-geometry of benzylidene double bonds was confirmed by conducting Nuclear Overhauser Enhancement (NOE) experiments using 1D-NOESY pulse sequence in V NMR software (Varian Inc., Palo Alto, CA). Spectra were referenced to the residual protonated solvents. Chemical shifts and coupling constants were reported in δ parts per million (ppm) and Hertz (Hz), respectively. Mass spectra were recorded by Finnigon Mat LCQ mass spectrometer (San Jose, CA). Samples for IR spectroscopic measurements were finely ground and prepared as KBr pellets in a Bruker IFS 66v spectrometer with a KBr beam splitter. Sixty-four scans at a spectral resolution of 1 cm^{-1} were averaged for each spectrum. The reported melting points (degree Celsius) are uncorrected.

The crystal structures of 3,5-bis(2-fluorobenzylidene)-4-piperidone (**1**) and its dimer (**3**) were determined by X-ray diffraction, measured with a CCD Bruker APEX diffractometer [7]. The structure was solved by direct methods using SHELXL [8] and refined on F^2 by full-matrix least-squares with the SHELXL [9]. Details of data collection and refinement parameters of compounds **1** and **3** are listed in Table 1. The selected bond distances and angles of

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Table 1
Crystal data and structure refinement for **1** and **3**.

Compound	**1**	**3**
Empirical formula	C_{21} H_{20} Cl F_2 N O_3	C_{40} H_{28} F_4 N_2 O_4
Formula mass	407.83	676.64
Crystal system	Triclinic	Monoclinic
Space group	$P\bar{1}$	$P2_1/n$
Unit cell dimensions		
a (Å)	8.8238 (9)	14.560 (4)
b (Å)	10.6781 (11)	11.287 (4)
c (Å)	11.9079 (12)	19.410 (5)
Volume (Å^3)	973.06 (17)	3183.9 (16)
Z	2	4
D_{calc} (Mg/m^3)	1.392	1.412
F(0 0 0)	424	1400
Absorption coefficient μ (mm^{-1})	0.237	0.107
Theta range for data collection	1.85–28.34°	2.09–26.02
Reflections collected	13658	25705
Data/restraints/parameters	4837/3/278	5860/6/471
wR(F^2 all data)	*wR2* = 0.1036	*wR2* = 0.1112
R($F_{obsd.}$ data)	*R1* = 0.0391	*R1* = 0.0423
The data completeness	0.998	0.935
Goodness-of-fit on F^2	1.038	1.003
Observed data [$I > 2\sigma(I)$]	4165	4597
Largest diff. peak and hole (e/Å3)	0.515 and −0.394	0.246 and −0.266
The minimum and maximum transmission factors	0.901 and 0.952	0.954 and 0.979

compounds **1** and **3** were listed in Tables 2a and b. The complete set of structural parameters in CIF format is available as an Electronic Supplementary Publication from the Cambridge Crystallographic Data Centre (CCDC Nos. 730847 and 730848).

2.2. Synthesis of 3,5-bis(benzylidene)-4-piperidones (*1*–*5*)

2.2.1. [3,5-Bis(2-fluorobenzylidene)-4-piperidone acetate] (*1*)

Hydrochloric acid gas (generated *in situ*) was bubbled into a solution of 4-piperidone hydrochloride monohydrate (3 gm, 19.5 mmol) in glacial acetic acid (80 ml) until a clear solution was obtained (about 15 min). 2-Fluorobenzaldehyde (6 ml, 56.5 mmol) was added and the reaction mixture was left at room temperature for 48 h. The crystals formed were filtered on a Buchner funnel, washed with absolute ethanol (50 ml) and consequently with ether (50 ml), and then dried to get compound **1** as a yellow crystalline solid (6.81 gm, 94% yield). R_f (60:40 ethyl acetate:hexanes) = 0.46. It has m.p. of 185–186 °C. ^{1}H NMR (300 MHz, DMSO-*d*6): δ 9.85 (br s, 1H, NH), 7.88 (s, 2H, C=CH), 7.54 (q, 2H, Ar—H, *J* = 8.5), 7.48 (t, 2H, Ar—H, *J* = 7.8), 7.37 (q, 4H, Ar—H, *J* = 7.0), 4.35 (s, 4H), 1.91 (s, 3H, CH_3). ^{13}C NMR (75 MHz, DMSO-*d*6): δ 181.98, 172.09, 160.37 (d, *J* = 249.1), 132.55 (d, *J* = 8.5), 131.79 (d, *J* = 3.8), 131.03 (d, *J* = 1.5), 129.89, 124.95 (d, *J* = 3.2), 121.52 (d, *J* = 13.1), 116.09 (d, *J* = 21.0), 43.85 (d, *J* = 3.1), 21.16. FT-IR (cm^{-1}): 797, 1201, 1243, 1453, 1483, 1616, 1715, 2977, 3025. ESI HRMS calculated for $C_{19}H_{16}F_2NO$ (M^+ + H—CH_3COOH) 312.12, found 312.13.

2.2.2. [3,5-Bis(2-fluorobenzylidene)-4-piperidone] (*1*)

Free base of compound **1** was generated by treating the acetate salt with 10% potassium carbonate solution. ^{1}H NMR (300 MHz, $CDCl_3$): 9.94 (s, 1H, NH), 7.90 (s, 2H, C=CH), 7.61–7.54 (m, 2H, Ar—H), 7.51 (t, 4H, Ar—H, *J* = 7.8), 7.39 (q, 2H, Ar—H, *J* = 7.2), 4.37 (s, 4H). ESI HRMS calculated for $C_{19}H_{16}F_2NO$ (M^+ + H) 312.12, found 312.13.

2.2.3. [3,5-Bis(benzylidene)-4-piperidone hydrochloride] (*2*)

Compound **2** was synthesized in a manner similar to that of compound **1**. From 1.35 g, 8.79 mmol of 4-piperidonehydrochloride monohydrate and (2.03 ml, 17.50 mmol) of benzaldehyde, the title compound of 2.05 gm, 85% yield was obtained as hydrochloride salt (m.p of 242–243 °C). ^{1}H NMR (300 MHz, DMSO-*d*6): δ 9.45 (s, 1H, NH, D_2O exchangeable), 7.90 (s, 2H, C=CH), 7.50–7.40 (m, 10H, Ar—H), 4.50 (s, 4H). ^{13}C NMR (75 MHz, DMSO-*d*6): δ 165.43, 139.92, 130.76, 130.39, 123.52, 114.15, 44.12. ESI HRMS calculated for $C_{19}H_{18}NO$ (M^+ + H) 276.13, found 276.12.

Table 2a
Selected bond lengths, angles and torsion angles for compound 1.

Atoms	*Bond lengths* (Å)	*Atoms*	*Bond lengths* (Å)
O_1—C_4	1.227 (16)	C_5—C_6	1.505 (19)
N_1—C_6	1.490 (19)	C_7—C_8	1.461 (2)
N_1—C_2	1.486 (2)	C_8—C_{13}	1.395 (2)
C_2—C_3	1.504 (19)	C_8—C_9	1.404 (2)
C_3—C_7	1.343 (2)	C_{14}—C_{15}	1.465 (2)
C_3—C_4	1.490 (19)	C_{15}—C_{16}	1.390 (2)
C_4—C_5	1.494 (2)	C_{16}—C_{17}	1.376 (2)
C_5—C_{14}	1.348 (2)	C_{15}—C_{20}	1.401 (2)
Atoms	*Angles* (°)	*Atoms*	*Angles* (°)
C_2—N_1—C_6	113.39 (11)	C_{14}—C_5—C_6	125.84 (13)
N_1—C_2—C_3	110.40 (12)	C_4—C_5—C_6	118.34 (12)
C_7—C_3—C_4	116.76 (12)	N_1—C_6—C_5	110.20 (12)
C_7—C_3—C_2	124.69 (13)	C_3—C_7—C_8	129.68 (13)
C_4—C_3—C_2	118.55 (12)	C_{13}—C_8—C_7	118.79 (13)
O_1—C_4—C_3	120.41 (13)	C_9—C_8—C_7	124.81 (13)
O_1—C_4—C_5	120.58 (13)	C_5—C_{14}—C_{15}	132.63 (14)
C_3—C_4—C_5	119.01 (12)	F_2—C_{16}—C_{17}	117.43 (14)
C_{14}—C_5—C_4	115.81 (12)	F_2—C_{16}—C_{15}	118.87 (14)
$F_{2'}$—C_{20}—C_{19}	120.1 (5)	$F_{2'}$—C_{20}—C_{15}	117.1 (15)
C_{19}—C_{20}—C_{15}	122.34 (15)	C_{13}—C_8—C_9	116.25 (13)
Atoms	*Torsion angles* (°)	*Atoms*	*Torsion angles* (°)
C_3—C_7—C_8—C_9	33.0 (2)	N_1—C_2—C_3—C_4	−33.36 (17)
C_5—C_{14}—C_{15}—C_{16}	−31.5 (3)	C_4—C_5—C_6—N_1	34.09 (16)

2.2.4. 1,2-Bis-[3,5-bis(2-fluorobenzylidene)-4-oxo-piperidin-1-yl]-ethan-1,2-dione (*3*)

To a solution of compound **1** (300 mg, 0.96 mmol) in 1,2 dichloroethane, triethylamine (0.4 ml, 2.85 mmol) was added. The reaction mixture was cooled to 0 °C on ice and oxalyl chloride (50 µl, 0.57 mmol) in 1,2 dichloroethane was added drop-wise. The reaction mixture was stirred at room temperature for 16 h. To this reaction mixture was added 10% potassium carbonate solution (10 ml) and stirring was continued for another 3 h. The organic phase was separated, dried (Na_2SO_4) and concentrated to obtain crude syrup. The residue was chromatographed on silica and pure compound **3** was eluted with 60:40 ethyl acetate in hexanes. Compound **3** was obtained as a yellow crystalline solid (244 mg, 63% yield), and was recrystallized from chloroform. R_f (60:40 ethyl acetate:hexanes) = 0.50. It has m.p. of 113–115 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.83 (s, 2H, C=CH), 7.71 (s, 2H, C=CH), 7.50–7.30 (m, 4H, Ar—H), 7.25–7.05 (m, 12H, Ar—H), 4.46 (s, 4H), 4.33 (s, 4H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 183.93, 162.35, 161.89 (d, *J* = 55.3), 159.38 (d, *J* = 55.3), 131.95 (d, *J* = 3.9), 131.78, 131.66, 131.19, 130.52, 124.48, 124.31, 122.13 (d, *J* = 14.0), 121.52 (d, *J* = 13.2), 116.32 (d, *J* = 21.8), 116.05 (d, *J* = 21.8), 46.37, 41.94. FT-IR (cm^{-1}): 1156, 1176, 1225, 1239, 1254, 1273, 1453, 1484, 1621, 1652. ESI HRMS calculated for $C_{40}H_{28}F_4N_2NaO_4$ (M^+ + Na) 699.19, found 699.13.

2.2.5. 1,4-Bis-[3,5-bis(2-fluorobenzylidene)-4-oxo-piperidin-1-yl]-2E-butene-1,4-dione (*4*)

Dimer **4** was prepared in a manner similar to that of compound **3**. Briefly, compound **1** (500 mg, 1.60 mmol) and fumaryl chloride (85 µl, 0.80 mmol) were reacted to obtain compound **4** as a yellow solid (395 mg, 70% yield) after usual work up and purification. R_f (60:40 ethyl acetate:hexanes) = 0.50. The compound showed m.p. of 145–146 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.91 (s, 2H, C=CH),

Table 2b
Selected bond lengths, angles and torsion angles for compound 3.

Atoms	*Bond lengths* (Å)	*Atoms*	*Bond lengths* (Å)
O_{1A}—C_{4A}	1.225 (2)	O_{1B}—C_{4B}	1.223 (2)
N_{1A}—C_{6A}	1.460 (2)	N_{1B}—C_{6B}	1.462 (2)
N_{1A}—C_{2A}	1.461 (2)	N_{1B}—C_{2B}	1.464 (2)
C_{2A}—C_{3A}	1.506 (3)	C_{2B}—C_{3B}	1.504 (3)
C_{3A}—C_{7A}	1.342 (3)	C_{3B}—C_{7B}	1.343 (3)
C_{3A}—C_{4A}	1.500 (3)	C_{3B}—C_{4B}	1.501 (3)
C_{4A}—C_{5A}	1.497 (3)	C_{4B}—C_{5B}	1.501 (3)
C_{5A}—C_{14A}	1.346 (3)	C_{5B}—C_{14B}	1.343 (3)
C_{5A}—C_{6A}	1.507 (3)	C_{5B}—C_{6B}	1.515 (3)
C_{7A}—C_{8A}	1.472 (3)	C_{7B}—C_{8B}	1.472 (3)
C_{8A}—C_{13A}	1.388 (3)	C_{8B}—C_{13B}	1.390 (3)
C_{8A}—C_{9A}	1.396 (3)	C_{8B}—C_{9B}	1.405 (3)
C_{14A}—C_{15A}	1.457 (3)	C_{14B}—C_{15B}	1.467 (3)
C_{15A}—C_{20A}	1.396 (3)	C_{15B}—C_{20B}	1.392 (3)
C_{15A}—C_{16A}	1.402 (3)	C_{15B}—C_{16B}	1.397 (3)
Atoms	*Angles* (°)	*Atoms*	*Angles* (°)
N_{1A}—C_1—C_3	114.99 (15)	F_{2A}—C_{20A}—C_{15A}	117.93 (17)
O_4—C_3—N_{1B}	124.83 (17)	N_{1B}—C_{2B}—C_{3B}	108.95 (15)
C_{6A}—N_{1A}—C_{2A}	113.40 (14)	C_{7B}—C_{3B}—C_{4B}	118.38 (18)
C_{7A}—C_{3A}—C_{4A}	116.96 (17)	C_{7B}—C_{3B}—C_{2B}	124.76 (17)
C_{7A}—C_{3A}—C_{2A}	124.80 (17)	C_{4B}—C_{3B}—C_{2B}	116.75 (16)
C_{4A}—C_{3A}—C_{2A}	118.17 (16)	O_{1B}—C_{4B}—C_{5B}	121.16 (17)
O_{1A}—C_{4A}—C_{5A}	121.34 (16)	O_{1B}—C_{4B}—C_{3B}	120.65 (17)
O_{1A}—C_{4A}—C_{3A}	120.93 (17)	C_{5B}—C_{4B}—C_{3B}	118.17 (17)
C_{5A}—C_{4A}—C_{3A}	117.62 (16)	C_{14B}—C_{5B}—C_{4B}	116.88 (18)
C_{14A}—C_{5A}—C_{4A}	117.59 (17)	C_{14B}—C_{5B}—C_{6B}	123.38 (17)
C_{14A}—C_{5A}—C_{6A}	124.42 (17)	C_{4B}—C_{5B}—C_{6B}	119.73 (16)
C_{4A}—C_{5A}—C_{6A}	117.97 (15)	N_{1B}—C_{6B}—C_{5B}	109.15 (15)
N_{1A}—C_{6A}—C_{5A}	109.88 (15)	C_{3B}—C_{7B}—C_{8B}	127.47 (19)
C_{3A}—C_{7A}—C_{8A}	129.08 (18)	C_{13B}—C_{8B}—C_{9B}	115.96 (17)
C_{13A}—C_{8A}—C_{7A}	119.97 (17)	C_{13B}—C_{8B}—C_{7B}	124.91 (17)
C_{9A}—C_{8A}—C_{7A}	123.92 (16)	C_{9B}—C_{8B}—C_{7B}	119.13 (18)
C_{10A}—C_{9A}—C_{8A}	121.42 (18)	C_{10B}—C_{9B}—C_{8B}	121.6 (2)
C_{5A}—C_{14A}—C_{15A}	128.59 (18)	C_{5B}—C_{14B}—C_{15B}	127.98 (19)
C_{20A}—C_{15A}—C_{16A}	114.94 (17)	C_{20B}—C_{15B}—C_{14B}	120.14 (17)
C_{20A}—C_{15A}—C_{14A}	119.73 (17)	C_{16B}—C_{15B}—C_{14B}	123.78 (17)
C_{16A}—C_{15A}—C_{14A}	125.31 (17)	C_{19B}—C_{20B}—C_{15B}	123.72 (18)
Atoms	*Torsion angles* (°)	*Atoms*	*Torsion angles* (°)
C_{3A}—C_{7A}—C_{8A}—C_{9A}	26.7 (3)	C_{3B}—C_{7B}—C_{8B}—C_{13B}	38.9 (3)
C_{5A}—C_{14A}—C_{15A}—C_{16A}	−23.3 (3)	C_{5B}—C_{14B}—C_{15B}—C_{16B}	−39.9 (3)

7.86 (s, 2H, C═CH), 7.42–7.00 (m, 16H, Ar—H), 6.95 (s, 2H, fumaryl CH═CH), 4.78 (s, 4H), 4.54 (s, 4H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 160.03, 169.17, 161.55 (d, J = 51.5), 159.10 (d, J = 51.5), 133.40 (d, J = 15.5), 131.43 (d, J = 9.2), 130.73, 130.29, 124.25 (d, J = 5.2), 124.58 (d, J = 14.1), 122.23 (d, J = 14.1), 116.32 (d, J = 21.9), 115.95 (d, J = 21.9), 47.22, 43.38. ESI HRMS calculated for $C_{42}H_{31}F_4N_2O_4$ (M^+ + H) 703.22, found 703.20.

*2.2.6. 1,7-Bis-[3,5-bis(2-fluorobenzylidene)-4-oxo-piperidin-1-yl]-1,4,7-triazaheptane-1,7-dione-1,4,7-triacetic acid (**5**)*

To a solution of compound **1** (622 mg, 2 mmol) in dry dimethylsulfoxide (DMSO) (12 ml), was added bis-[2-(2,6-dioxo-morpholin-4-yl)ethyl]-aminoacetic acid (357 mg, 1 mmol) and triethylamine (1.4 ml, 10 mmol). The reaction mixture was stirred at room temperature for 2 h. Reaction was monitored by TLC, and upon consumption of the starting material, the reaction mixture was evaporated to dryness to remove triethylamine. The solid was separated by pouring reaction mixture into water, followed by centrifugation. The solid was recrystallized from methanol and water to get the title compound as a yellow solid (890 mg, 91% yield). R_f (30:70 methanol:chloroform) = 0.30. It has m.p. of 90–92 °C. ^{1}H NMR (300 MHz, DMSO-*d*6): δ 7.70 (s, 2H, C═CH), 7.67 (s, 2H, C═CH), 7.60–7.45 (m, 8H, Ar—H), 7.40–7.20 (m, 8H, Ar—H), 4.73 (s, 4H), 4.69 (s, 4H), 3.44–3.40 [(m, 4H, $(NCOCH_2)_2$], 3.22–3.20 (m, 2H, CH_2COOH), 3.10–3.01(m, 4H, CH_2COOH), 2.48–2.45 (m, 8H, CH_2). ^{13}C NMR (75 MHz, DMSO-*d*6): δ 183.41, 170.18, 168.14, 166.86, 158.39 (d, J = 249.9), 131.93, 129.87 (d, J = 8.6), 128.90, 126.31, 122.70, 119.86 (d, J = 11.5), 113.81 (d, J = 21.6), 53.70, 52.52, 49.57, 48.23, 43.72, 40.39. ESI HRMS calculated for $C_{52}H_{49}F_4N_5NaO_{10}$ (M^+ + Na) 1002.33, found 1002.21.

2.3. X-ray crystallography

Yellow prism-shaped crystals, of dimensions (**1**) 0.44 × 0.30 × 0.22 mm and (**3**) 0.41 × 0.30 × 0.20 mm were selected for structural analysis. Intensity data for the compounds were collected using a diffractometer with a Bruker APEX ccd area detector (1) and graphite-monochromated Mo Kα radiation (λ = 0.71073 Å). Both samples were cooled to 100(2) K.

3. Results and discussion

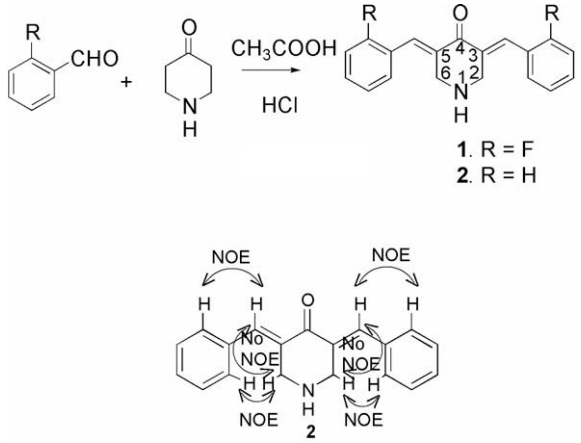
3.1. Synthesis

Acid-catalyzed Claisen–Schmidt condensation of 4-piperidone hydrochloride and substituted aromatic aldehydes afforded 3,5-bis(benzylidene)-4-piperidones **1** and **2** as their hydrochloride salts in good yields (Scheme 1). There was no significant difference in chemical shifts of these protons in ^{1}H NMR. The ^{13}C NMR of compounds **1**, **3**, **4** and **5** show the typical coupling pattern with the fluorine atoms. These compounds contain piperidine nitrogen atom which can be protonated during the electrospray ionization to give $[M + H]^+$ as base peaks in the mass spectra.

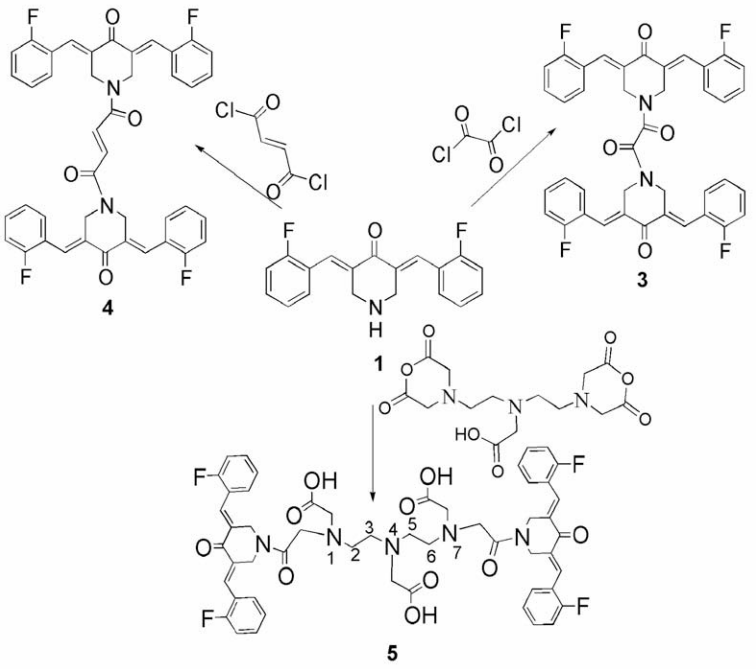
It should be noted that these compounds are potential antitumor agents, and even small differences in the structures may cause significant changes in cytotoxicity. The piperidine nitrogen is reactive towards anhydrides and acid chlorides. Several *N*-acyl derivatives of 3,5-bis(benzylidene)-4-piperidones were known in literature [10–13]. We modified secondary *N*-atom in piperidone ring of compound **1** to prepare dimers with a goal to enhance antiproliferative activity (not reported). First, we prepared —*N*—oxalyl—*N*-linked dimer (**3**) (Scheme 2). The dimer obtained showed a characteristic molecular ion peak in the ESI–MS. The second dimer (**4**) of compound **1** was prepared via —*N*—fumaryl—*N*— linker (Scheme 2). The third dimer (**5**) was obtained by treating compound **1** with DTPA dianhydride (Scheme 2). Compound **5** and was characterized by ^{1}H, ^{13}C NMR spectroscopy and mass spectrometry.

3.2. Spectroscopy

The ^{1}H NMR spectrum of these 3,5-bis(benzylidene)-4-piperidones were simple in appearance. The vinyl protons appeared at



Scheme 1. Synthesis of compounds **1**, **2** and NOE showing *E*,*E*-geometry of double bonds in compound **2**.



Scheme 2. Synthesis of compounds **3**, **4** and **5**.

δ 7.80–7.90 ppm, typical of *E* geometry [14,15]. This is due to the anisotropic effect of the carbonyl group resulting in the downfield shifting of vinyl protons. The appearance of vinyl protons at δ 6.20 ppm is an indication of *Z* conformation [16,17]. The *E,E*-geometry of double bonds was further confirmed from NOE experiments. The piperidone methylene protons were observed at δ 4.30–4.50 ppm as a singlet. The dimer (**3**) showed a pattern almost similar to that of monomer (**1**) in ^{1}H NMR except that all protons appeared as double. Compound **4** showed fumaric acid vinyl protons merging with aromatic protons in the region of δ 7.42–6.95 ppm. The dimer (**5**) showed aromatic peaks at 7.60–7.20 ppm and aliphatic CH_2 peaks at δ 3.50–2.50 ppm. All compounds (**1–5**) showed characteristic molecular ion peaks in ESI–MS spectrometry.

The infrared spectra of compounds **1** and **3** showed absorptions at 1715, 1652 and 1621 cm^{-1} indicative of the carbonyl functionality. The NH absorption at 3025 cm^{-1} was present in compound **1**. The NH absorption was absent in compound **3**, suggestive of *N*-acylation.

3.3. NOE experiments

The *E,E*-geometry of benzylidene double bonds was further confirmed by conducting NOE experiments of 3,5-bis(benzylidene)-4-piperidone (**2**). The vinyl protons at δ 7.90 ppm in 3,5-bis(benzylidene)-4-piperidone (**2**) when irradiated, showed NOE with aromatic *ortho* protons at δ 7.47 ppm and did not show any NOE with piperdine methylene protons at δ 4.50 ppm. The protons at δ 4.50 ppm demonstrated NOE with δ 7.44 aromatic *ortho* protons which are in close proximity (Scheme 1).

3.4. Crystal structures

Compound **1** crystallizes in triclinic crystal system (space group $P\bar{1}$) and compound **3** crystallizes in monoclinic crystal system (space group $P2_1/n$). The molecular structures and atomic numbering of both compounds **1** and **3** are shown in Fig. 1. In the X-ray crystal structure, the central piperidone ring was in sofa conformation and benzylidene double bonds were in *E,E*-geometry. The *E*-conformation is consistent with the previous literature reports for 3,5-bis(benzylidene)-4-piperidones as well as 2,6-bis(arylidene)cyclohexanones [18–20]. The dihedral angles between the planes passing through the benzene rings in curcumin is 19.1(3)° [21] and in compound **1**, the dihedral angles passing through the plane of C(3)—C(7)—C(8)—C(9) and C(5)—C(14)—C(15)—C(16) are 33.0(2)° and −31.5(2)°, respectively. Compared to curcumin, these compounds possess more rigid structures due to the presence of a

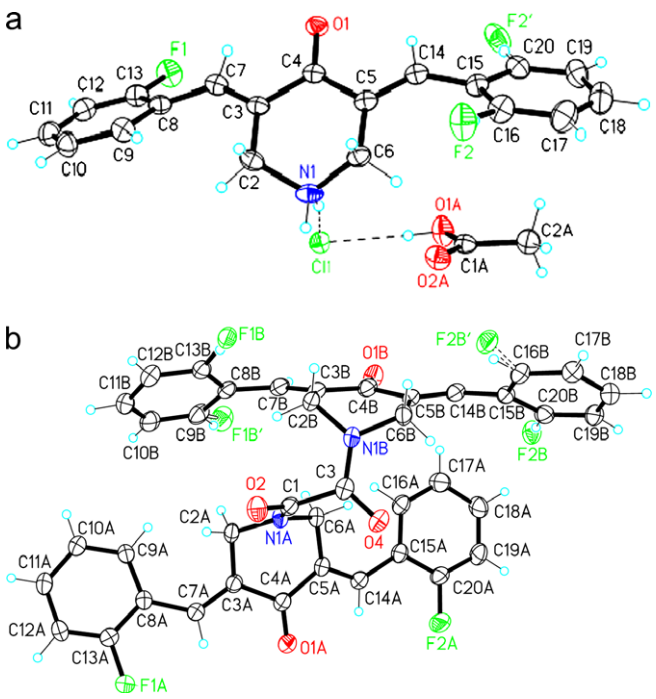


Fig. 1. The molecular structure of compounds (a) **1** and (b) **3**. Displacement ellipsoids were drawn at the 50% probability level.

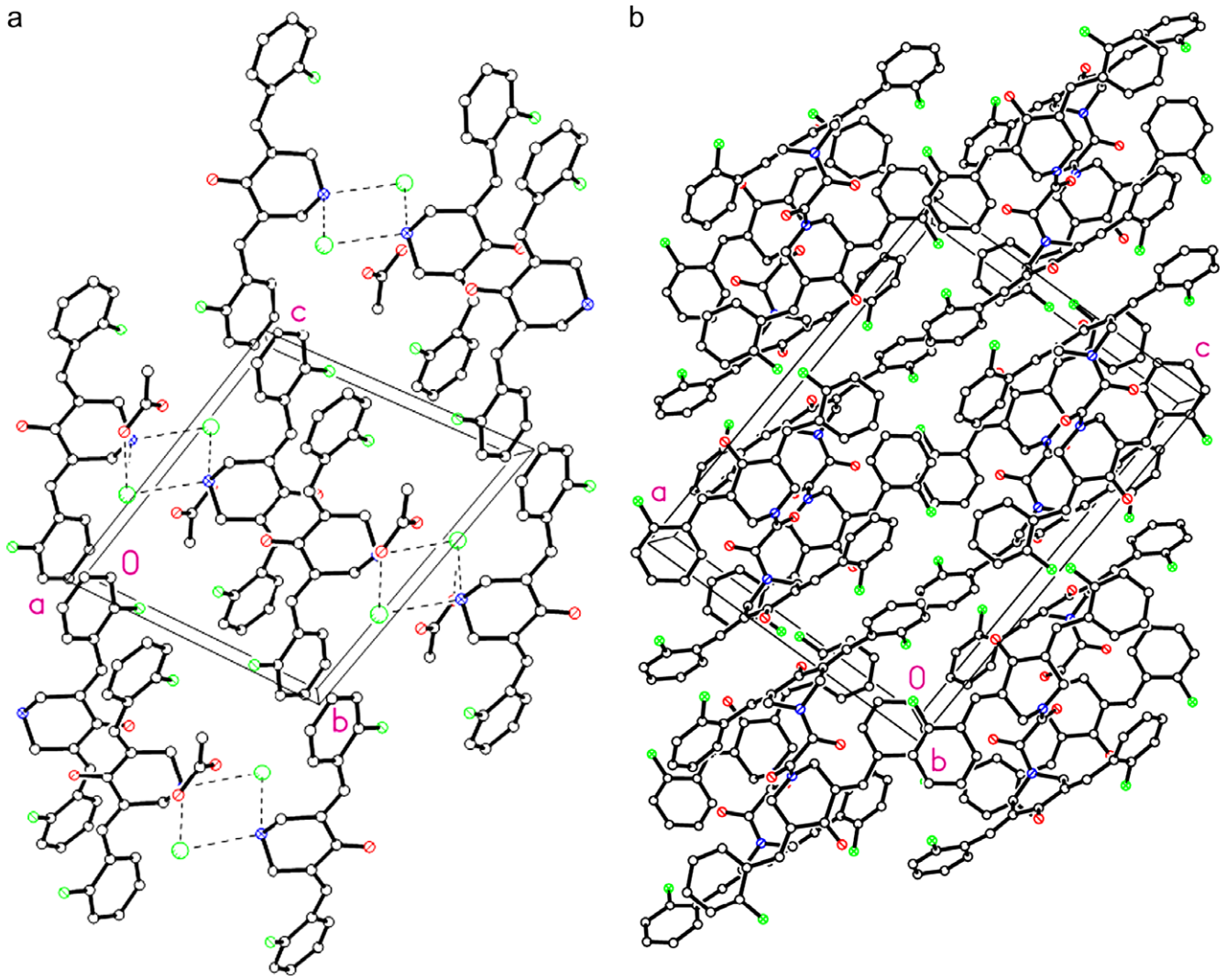


Fig. 2. Perspective view of the crystal packing of compounds (a) **1** and (b) **3**. H atoms and the lesser occupied F atoms in the structure are removed for clarity.

piperidone ring. This structural anomaly may have implications in apparently more potent antiproliferative actions of 3,5-bis(2-fluorobenzylidene)-4-piperidone analogs. As reported for similar compounds in the literature [18], the two aryl groups in **1** and **3** are twisted away from the central ring due to steric repulsions between the H atoms on C2/C6 and the phenyl rings. This is demonstrated by the angles of C(3)—C(7) —C(8), C(5)—C(14)—C(15), C(7)—C(8)—C(9) and C(14)—C(15)—C(16) which are greater than the 120° expected of sp^2 carbon atoms (Tables 2a and b). To reduce the interactions further, the two phenyl rings rotate about the C(7)—C(8) and C(14)—C(15) bonds in (**1**) and C(7B)—C(8B), C(7A)—C(8A), C(14A)—C(15A) and C(14B)—C(15B) bonds in (**3**). Hence one of the fluorine atoms on benzene ring seems disordered in the monomer (**1**) and appears in both *ortho* positions. On the other hand, in case of the dimer (**3**), both the fluorine atoms appear disordered resulting in a crystal data showing the two *ortho* positions of both benzene rings occupied by fluorine.

The 2-fluorobenzylidene group with full occupancy orientates fluorine toward the carbonyl of the central ring. The fluorine in the other aryl group is distributed between the two *ortho* positions of the ring, with the predominantly occupied site [0.910 (3), 0.090 (3)] oriented away from the carbonyl of the central ring. The two fluoro atoms on the dimer were disordered. The occupancies for F1B were refined to 0.804(3) and 0.196(3) for primed and unprimed atoms. The occupancies for F2B were refined to 0.852(3) and 0.148(3) for the primed and unprimed atoms.

Packing of atoms in compounds **1** and **3** is shown in Fig. 1. Single-crystal-X-ray analysis of **1** reveals that there are two molecules in one unit cell joined through hydrogen bonds (Fig. 2a). Compound **3** packed in layers parallel with the (101) plane (Fig. 2b).

In case of compounds **4** and **5**, we were unable to obtain crystalline products amenable to X-ray diffraction.

4. Conclusions

In summary, we synthesized and characterized bis(2-fluorobenzylidene)-4-piperidone (**1**) and its three dimers (**3**, **4** and **5**). From literature [4] and our own *in vitro* experiments (not reported), we know that compound **1** is a highly potent cytotoxic agent. The rigidity of these molecules apparent in the crystal structures provide a clue about the enhanced antiproliferative activity of 3,5-bis(2-fluorobenzylidene)-4-piperidones as compared to curcumin.

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