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Crystal structure and anti-inflammatory activity of (3*E*,5*E*)-3-(2-fluorobenzylidene)-1-((4-acetamidophenyl)sulfonyl)-5-(pyridin-3-ylmethylene)piperidin-4-one-methanol-hydrate (2/1/1), C₅₃H₅₀F₂N₆O₁₀S₂

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Abstract

C₅₃H₅₀F₂N₆O₁₀S₂, monoclinic, *P*₂/*n* (no. 14), *a* = 15.088(5) Å, *b* = 23.560(9) Å, *c* = 15.401(6) Å, β = 112.254(5)°, *V* = 5067(3) Å³, *Z* = 4, *R*_{gt}(*F*) = 0.0509, *wR*_{ref}(*F*²) = 0.1336, *T* = 298(2) K.

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The molecular structure is shown in the figure. Table 1 contains crystallographic data and Table 2 contains the list of the atoms including atomic coordinates and displacement parameters.

Source of material

2-Fluorobenzaldehyde (1.24 g, 10.0 mmol), 3-pyridinecarboxaldehyde (1.07 g, 10.0 mmol) and 4-piperidone hydrate hydrochloride (1.35 g, 10.0 mmol), were mixed in a solution of acetic acid (20 mL). Dry HCl gas was passed through this mixture for 30 min. After stirring at room temperature for about 24 h [monitored by thin-layer chromatography (TLC)], the precipitate was collected and washed with cold acetone. The precipitate was added to 100 mL water; an aqueous Na₂CO₃

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Table 1: Data collection and handling.

Crystal:	Yellow block
Size:	0.15 × 0.12 × 0.10 mm
Wavelength:	Mo Kα radiation (0.71073 Å)
μ:	0.18 mm ^{−1}
Diffractometer, scan mode:	SuperNova, ω
θ _{max} , completeness:	27.5°, 99%
<i>N</i> (<i>hkl</i>) _{measured} , <i>N</i> (<i>hkl</i>) _{unique} , <i>R</i> _{int} :	28143, 11167, 0.047
Criterion for <i>I</i> _{obs} , <i>N</i> (<i>hkl</i>) _{gt} :	<i>I</i> _{obs} > 2 σ(<i>I</i> _{obs}), 6078
<i>N</i> (<i>param</i>) _{refined} :	664
Programs:	CrysAlis ^{PRO} [1], SHELX [2, 3]

Table 2: Fractional atomic coordinates and isotropic or equivalent isotropic displacement parameters (Å²).

Atom	<i>x</i>	<i>y</i>	<i>z</i>	<i>U</i> _{iso} ^{*/U} _{eq}
C1	0.97818(14)	0.15423(9)	0.39036(17)	0.0486(6)
H1A	1.004799	0.161477	0.343126	0.058*
H1B	1.019458	0.127037	0.434582	0.058*
C2	0.97799(14)	0.20896(9)	0.44160(15)	0.0429(5)
C3	0.89913(14)	0.21956(9)	0.47478(17)	0.0464(5)
C4	0.81519(14)	0.18021(9)	0.44418(15)	0.0428(5)
C5	0.83011(15)	0.12332(9)	0.40706(17)	0.0516(6)
H5A	0.865535	0.098701	0.459147	0.062*
H5B	0.768453	0.105847	0.372906	0.062*
C6	1.04463(15)	0.24978(9)	0.45864(16)	0.0483(6)
H6	1.032340	0.282366	0.486278	0.058*
C7	1.13302(15)	0.25156(10)	0.44107(17)	0.0512(6)
C8	1.1754(2)	0.30298(12)	0.4388(2)	0.0818(9)
C9	1.2606(2)	0.30923(14)	0.4279(3)	0.1062(12)
H9	1.286065	0.345068	0.427067	0.127*
C10	1.3072(2)	0.26164(15)	0.4184(2)	0.0928(10)
H10	1.364881	0.264750	0.409958	0.111*
C11	1.26974(18)	0.20974(13)	0.4212(2)	0.0789(9)
H11	1.302142	0.177347	0.415134	0.095*
C12	1.18477(16)	0.20458(11)	0.43294(19)	0.0625(7)
H12	1.160906	0.168493	0.435549	0.075*
C13	0.73203(14)	0.19992(10)	0.44364(15)	0.0487(6)
H13	0.732895	0.237089	0.464274	0.058*
C14	0.63944(15)	0.17069(10)	0.41479(16)	0.0496(6)
C15	0.55659(16)	0.20101(11)	0.36297(19)	0.0621(7)
H15	0.563576	0.238500	0.347759	0.075*
C16	0.46006(18)	0.12636(13)	0.3595(2)	0.0700(8)
H16	0.399008	0.110863	0.341355	0.084*
C17	0.53654(18)	0.09324(12)	0.41103(19)	0.0657(7)

Table 2 (continued)

Atom	x	y	z	U_{iso}^*/U_{eq}
H17	0.527216	0.056451	0.427786	0.079*
C18	0.62758(16)	0.11539(11)	0.43758(17)	0.0568(6)
H18	0.680617	0.093174	0.470638	0.068*
C19	0.78102(14)	0.21005(9)	0.21268(15)	0.0442(5)
C20	0.84062(15)	0.25315(10)	0.20470(17)	0.0541(6)
H20	0.903330	0.245040	0.211851	0.065*
C21	0.80656(15)	0.30754(10)	0.18627(17)	0.0548(6)
H21	0.846771	0.336200	0.181320	0.066*
C22	0.71303(14)	0.32068(9)	0.17484(15)	0.0450(5)
C23	0.65428(14)	0.27787(10)	0.18448(16)	0.0489(6)
H23	0.591882	0.286038	0.178340	0.059*
C24	0.68874(14)	0.22324(9)	0.20318(16)	0.0485(6)
H24	0.649035	0.194700	0.209525	0.058*
C25	0.59699(17)	0.40152(11)	0.13364(16)	0.0531(6)
C26	0.59411(18)	0.46398(11)	0.11424(19)	0.0684(7)
H26A	0.601818	0.470263	0.055914	0.103*
H26B	0.644983	0.482509	0.164008	0.103*
H26C	0.533689	0.479161	0.110387	0.103*
C27	0.48584(14)	0.52801(9)	0.32655(16)	0.0482(6)
H27A	0.525733	0.555952	0.370180	0.058*
H27B	0.513508	0.520602	0.280162	0.058*
C28	0.48671(14)	0.47356(9)	0.37946(15)	0.0422(5)
C29	0.40569(15)	0.46202(9)	0.41027(16)	0.0475(6)
C30	0.32475(15)	0.50320(9)	0.38465(15)	0.0445(5)
C31	0.33812(16)	0.55927(9)	0.34260(16)	0.0504(6)
H31A	0.275955	0.576048	0.308088	0.060*
H31B	0.373735	0.585153	0.392630	0.060*
C32	0.55540(15)	0.43366(9)	0.40054(15)	0.0475(6)
H32	0.542534	0.401185	0.428047	0.057*
C33	0.64581(15)	0.43222(9)	0.38809(15)	0.0463(5)
C34	0.69202(18)	0.38031(10)	0.39275(17)	0.0580(6)
C35	0.77977(19)	0.37374(12)	0.38917(18)	0.0652(7)
H35	0.806916	0.337937	0.392847	0.078*
C36	0.82739(19)	0.42167(13)	0.37994(18)	0.0695(8)
H36	0.887820	0.418484	0.377447	0.083*
C37	0.78652(17)	0.47386(11)	0.37440(18)	0.0628(7)
H37	0.819230	0.505957	0.367665	0.075*
C38	0.69760(15)	0.47964(10)	0.37866(16)	0.0514(6)
H38	0.671303	0.515665	0.375218	0.062*
C39	0.24452(15)	0.48643(10)	0.39359(16)	0.0511(6)
H39	0.246290	0.450306	0.418527	0.061*
C40	0.15351(15)	0.51695(10)	0.36962(16)	0.0488(6)
C41	0.06843(17)	0.48632(11)	0.33016(18)	0.0625(7)
H41	0.072745	0.447542	0.321135	0.075*
C42	−0.02342(18)	0.56388(13)	0.3229(2)	0.0692(8)
H42	−0.083632	0.580222	0.306932	0.083*
C43	0.05577(17)	0.59765(11)	0.36404(18)	0.0623(7)
H43	0.049126	0.635625	0.376893	0.075*
C44	0.14484(16)	0.57419(10)	0.38578(17)	0.0548(6)
H44	0.199297	0.596647	0.411320	0.066*
C45	0.28154(14)	0.46559(9)	0.16693(15)	0.0449(5)
C46	0.33941(15)	0.41873(10)	0.17018(16)	0.0491(6)
H46	0.401104	0.423887	0.171738	0.059*
C47	0.30459(15)	0.36499(10)	0.17106(16)	0.0516(6)
H47	0.343575	0.333955	0.173603	0.062*
C48	0.21210(15)	0.35574(10)	0.16824(16)	0.0505(6)

Table 2 (continued)

Atom	x	y	z	U_{iso}^*/U_{eq}
C49	0.15540(15)	0.40318(11)	0.16609(17)	0.0566(6)
H49	0.093837	0.398223	0.164990	0.068*
C50	0.19014(15)	0.45693(10)	0.16559(16)	0.0521(6)
H50	0.151775	0.488055	0.164335	0.063*
C51	0.0938(2)	0.28025(13)	0.1597(2)	0.0835(9)
C52	0.0826(2)	0.21698(13)	0.1502(3)	0.1094(12)
H52A	0.116388	0.199493	0.209992	0.164*
H52B	0.108278	0.203645	0.105658	0.164*
H52C	0.015898	0.207337	0.128945	0.164*
C53	0.8690(3)	0.44374(17)	0.0881(3)	0.1349(16)
H53A	0.878386	0.479811	0.063821	0.202*
H53B	0.822749	0.422088	0.038805	0.202*
H53C	0.928621	0.423496	0.111873	0.202*
F1	1.12899(14)	0.35016(7)	0.44754(19)	0.1410(9)
F2	0.64508(11)	0.33292(6)	0.40313(13)	0.0896(5)
N1	0.88272(11)	0.12931(7)	0.34497(13)	0.0463(5)
N2	0.46826(13)	0.17974(10)	0.33394(16)	0.0693(6)
N3	0.68449(12)	0.37711(8)	0.15384(15)	0.0536(5)
H3	0.724785	0.401955	0.161330	0.080*
N4	0.38959(12)	0.55156(7)	0.27936(13)	0.0441(4)
N5	−0.01847(14)	0.50905(11)	0.30461(16)	0.0704(6)
N6	0.18053(13)	0.30004(9)	0.16414(16)	0.0617(6)
H6A	0.213154	0.273420	0.158875	0.092*
O1	0.90128(11)	0.26020(7)	0.52570(12)	0.0628(5)
O2	0.74164(11)	0.10352(7)	0.20780(12)	0.0683(5)
O3	0.89140(11)	0.13353(7)	0.18982(12)	0.0655(5)
O4	0.52587(12)	0.37452(8)	0.12825(14)	0.0758(6)
O5	0.40497(11)	0.41958(7)	0.45555(13)	0.0656(5)
O6	0.24555(11)	0.57241(7)	0.14147(11)	0.0619(5)
O7	0.39125(11)	0.53481(7)	0.12321(12)	0.0629(5)
O8	0.03128(15)	0.31082(10)	0.1641(2)	0.1393(11)
O9	0.81749(11)	0.28359(7)	0.66190(14)	0.0796(6)
H9A	0.859197	0.289500	0.717291	0.119*
H9B	0.845429	0.275090	0.625092	0.119*
O10	0.83559(15)	0.45229(11)	0.16088(18)	0.0994(7)
H10A	0.875470	0.470354	0.203313	0.149*
S1	0.82334(4)	0.14006(2)	0.23271(4)	0.05086(17)
S2	0.32634(4)	0.53468(2)	0.17088(4)	0.04829(16)

solution was then added until the pH value was adjusted to about 7. The precipitate was filtered, subsequently washed by water, and purified on silica gel by column chromatography using methanol/petroleum ether/EtOAc (1:10:10, v/v/v) as the eluent to afford a yellow intermediate. Next, the intermediate (0.59 g, 2.0 mmol), and 4-acetamidobenzenesulfonyl chloride (0.51 g, 2.2 mmol) were dissolved in a solution of dichloromethane (50 mL). Two drops of pyridine were added to the mixture. After stirring for about 6 h at room temperature, the precipitate was collected, washed with water and recrystallized from dichloromethane/methanol (1:1, v/v) to yield light yellow crystals of title compound.

The anti-inflammatory activities of title compound were evaluated by inhibition of LPS-induced NO secretion on

mouse RAW264.7 macrophages. Pyrrolidine dithiocarbamate (PDTC) was used as a reference standard. In preliminary experiments, the title compound had no significant toxicity at 6.0 M on RAW264.7 cells. RAW264.7 cells were pretreated with 30 M of PDTC or with 6.0 M of title compound for 2.0 h. After treatment with LPS (1.0 g/mL) for 22 h, the collected culture media were centrifuged at 1000 rpm for 10 min. The expression levels of NO secretion in the media were determined by ELISA with an ELISA kit (eBioScience, San Diego, CA). The experiment was carried out in triplicate.

Experimental details

The H atoms of water, methanol (O—H) and acetylamino group (N—H) were located in a difference Fourier map and included as riding atoms, with $d(O-H) = 0.85 \text{ \AA}$, $U_{iso}(H) = 1.5U_{eq}(O, \text{ water})$, and $d(O-H) = 0.82 \text{ \AA}$, $U_{iso}(H) = 1.5U_{eq}(O, \text{ methanol})$, and $d(N-H) = 0.82 \text{ \AA}$, $U_{iso}(H) = 1.5U_{eq}(N, \text{ acetylamino})$. Other H atoms were placed in idealized positions and treated as riding on their parent atoms, with $d(C-H) = 0.96 \text{ \AA}$ (methyl), $U_{iso}(H) = 1.5U_{eq}(C)$, and $d(C-H) = 0.97 \text{ \AA}$ (methylene), $U_{iso}(H) = 1.2U_{eq}(C)$, and $d(C-H) = 0.93 \text{ \AA}$ (aromatic), $U_{iso}(H) = 1.2U_{eq}(C)$.

Comment

In order to overcome low bioactivities, poor aqueous solubility and false positive problem of curcumin [4], a series of novel curcumin analogues have been reported, such as 3*E*, 5*E*-3,5-bis(arylidene)-4-piperidone derivatives (BAPs). BAPs contain two α,β -unsaturated keto groups, and show greater predilection or sequential interaction for bio-thiols in tumors rather than normal cell [5, 6]. In previous study, some BAPs were reported as better antitumor and anti-inflammatory agents [7–9]. Some strong electron-withdrawing substituent groups (Such as $-NO_2$, $-CN$, $-CF_3$) were assigned to both sides of BAPs. Their antitumor and anti-inflammatory activity were improved to different extent. Some crystal structure and bioactivity of 4-((*E*)-5-(2-fluorobenzylidene)-1-((4-fluorophenyl)sulfonyl)-4-oxopiperidin-3-ylidene)methyl) benzonitrile (BAP-1), (3*E*,5*E*)-3,5-bis(4-cyanobenzylidene)-1-((4-fluorophenyl)sulfonyl)piperidin-4-one (BAP-2), (3*E*,5*E*)-3,5-bis(3-nitrobenzylidene)-1-((4-(trifluoromethyl) phenyl) sulfonyl)piperidin-4-one (BAP-3) were reported [10–12].

When changing one side of BAPs to 3-pyridine substituents, while another side of BAPs is 2-F group, dissymmetric BAPs could be generated [8]. By adding the *N*-phenylsulfonyl moiety [13], the desired and improved antitumor activities and anti-inflammatory activity of BAPs could be found. In this study, we report the crystal structure and anti-inflammatory activity of (3*E*,5*E*)-3-(2-fluorobenzylidene)-1-((4-acetamidophenyl)sulfonyl)-5-(pyridin-3-ylmethylene) piperidin-4-one.

Single-crystal structure analysis reveals that there are two BAP molecules, a methanol and a water molecule in the asymmetric unit of the title crystal structure (*cf.* the figure). Bond lengths and angles are all in the expected ranges. The central 3,5-bis(arylidene)-4-piperidone scaffold is lengthened on both sides in a linear fashion, with the pyridyl and arylidene substituents adopting the *E* stereochemistry of the olefinic double bonds [14, 15]. The dihedral angles between the pyridyl and central piperidone rings of the symmetry independent molecules are $57.5(3)^\circ$ and $50.1(4)^\circ$, while the dihedral angles between arylidene and central piperidone are $24.1(3)^\circ$ and $22.5(2)^\circ$, respectively. In addition, the *N*-phenylsulfonyl substituents stretch in the direction of the carbonyl group of central piperidone, giving an overall shape of an “organic clip” [16]. The dihedral angles between *N*-phenylsulfonyl group and piperidone ring are $38.7(3)^\circ$ and $27.6(4)^\circ$, respectively.

According to the literature, the production of pro-inflammatory cytokines, such as express rate of NO production, TNF- α and IL-6, can indicate the degree of inflammation [8, 17, 18]. In our study, the effect of title compound on NO production in mouse RAW264.7 cells induced by LPS was examined by ELISA. PDTC was used as a reference standard. After treatment with PDTC, the express rate for NO production in RAW264.7 cells was $68.32 \pm 2.69\%$. After treatment with title compound, the express rate of NO production was significantly decreased, reaching $48.29 \pm 0.71\%$. The result showed that title compound displayed potential inhibitory effects on LPS-induced NO secretion compared to PDTC.

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