Synthesis, crystal structure, and biological activity of diethyl-2-[5-(4-fluorophenyl)-7-(trifluoromethyl)pyrazolo[1,5-a] pyrimidine-3-carboxamido]pentanedioate

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The title compound, diethyl-2-[5-(4-fluorophenyl)-7-(trifluoromethyl)pyrazolo[1,5-a]pyrimidine-3-carboxamido]pentanedioate ($C_{23}H_{22}F_4N_4O_5$, *Mr*=510.45) was synthesised and structurally characterised by element analysis, IR, ^{1}H NMR and single crystal X-ray diffraction. The crystal belongs to the monoclinic system, space group C_{121} with *a*=27.001(3) Å, *b*=7.161(1) Å, *c*=12.0900 Å, α=90.00 °, β=92.379 (7) °, γ=90.00 °. In the crystal structure, the dihedral angles between the benzene ring and pyrazolopyrimidine are 53.618(162)°. Through C–H…F and C–H…O weak hydrogen bonds among molecules, the whole molecule is stacked into a three-dimensional structure. In addition, the compound possesses a moderate antituberculosis activity.

Keywords: synthesis, X-ray diffraction, biological activity

There is a critical need for the development of new drugs to treat cancer due to the recent and rapid appearance of numerous single, multiple, and extensively drug-resistant forms of the disease. L-Glutamic acid plays an important role in the biosynthesis of purine and pyrimidine bases of DNA and RNA.[1–4] It is metabolised to L-glutamine by L-glutamine synthetase and this metabolic process is essential for normal maintenance of cells. The synthesis of L-glutamine is hindered in neoplastic cells due to lower reactivity of L-glutamine synthetase. Thus antagonists of this enzyme can interfere with the metabolic role of L-glutamine and act as anticancer agents,[5]and also used as antifungals and antituberculostatics drugs.[6–8]

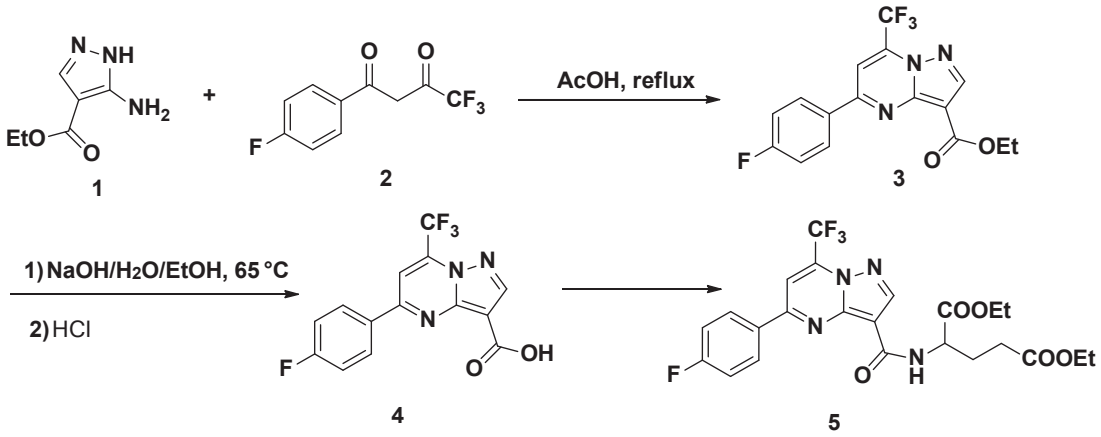
Therefore, we now report the design and synthesis of a new glutamic acid compound, diethyl 2-[5-(4-fluorophenyl)-7-(trifluoromethyl)pyrazolo[1,5-*a*]pyrimidine-3-carboxamido] pentanedioate, with its synthetic route depicted in Scheme 1. In order to confirm its structure and investigate its stereoconfiguration, a single crystal of the title compound was obtained from ethyl acetate and the molecular structure was determined by element analysis, IR, ^{1}H NMR and X-ray diffraction.

Experimental

Melting points (°C) were measured with Koeffler melting point apparatus and are uncorrected. TLC was performed on aluminium sheets precoated with silica gel (Merck, Kieselgel 60 F-254). ^{1}H NMR spectra were recorded on Bruker AV-600 in DMSO-d_6 using TMS as an internal standard (chemical shifts in ppm). Crystal data was obtained on a Bruker P4 X-diffractometer. All solvents and reagents were obtained from commercial sources and were used without purification. 4-Ethoxycarbonyl-5-amino-pyrazole **1** was purchased from Aldrich. 4,4,4-Trifluoro-1-4-fluorophenyl)butane-1,3-dione was obtained as reported.[9]

Synthesis of 5-(4-fluorophenyl)-7-(trifluoromethyl)pyrazolo[1,5-a] pyrimidine-3-carboxylic acid

A solution of ethyl 5-amino-1H-pyrazole-4-carboxylate (15.5 g, 0.1 mol) and 4,4-trifluoro-1-(4-fluorophenyl)-butane-1,3-dione (23.4 g, 0.1 mol) in acetic acid (50 mL) was heated at reflux for 6 h. After cooling to room temperature, the formed precipitate was filtered off, washed with water, and dried. The resulting ethyl carboxylate was added to a mixture of NaOH (5.6 g, 0.14 mol) in EtOH/water (1 : 3) (120 mL), and the reaction mixture was kept at 65 °C for 5 h. The mixture was cooled to room temperature and acidified with concentrated HCl until pH 1 was reached. The formed precipitate was filtered off, washed with water, and recrystallised from MeCN to give 20.6 g pure 5-(4-fluorophenyl)-7-(trifluoromethyl)pyrazolo[1,5-a]pyrimidine-3-carboxylic acid in 63.4% yield m.p. 252–253 °C; ^{1}H NMR (500 MHz): δ 2.35–2.55 (s, 1H, COOH), 7.25 (d, *J* = 8.2 Hz, 2H, ArH), 8.2 (s, 1H, ArH), 8.5 (m, 2H, ArH), 8.52 (s, 1H, ArH); ^{13}C NMR (125 MHz): δ 166.23 (COOH), 120.16 (CF3), 163.41, 2 × 116.12, 2 × 130.27, 133.73 (4-fluorophenyl), 104.21, 106.33, 132.10, 145.66, 148.31, 155.14 (pyrazolo[1,5-*a*]pyrimidine); LC/MS *m*/*z* 326 (M + 1).



Scheme 1 Synthetic route of diethyl-2-[5-(4-fluorophenyl)-7-(trifluoromethyl)pyrazolo[1,5-a]pyrimidine-3-carboxamido]pentanedioate.

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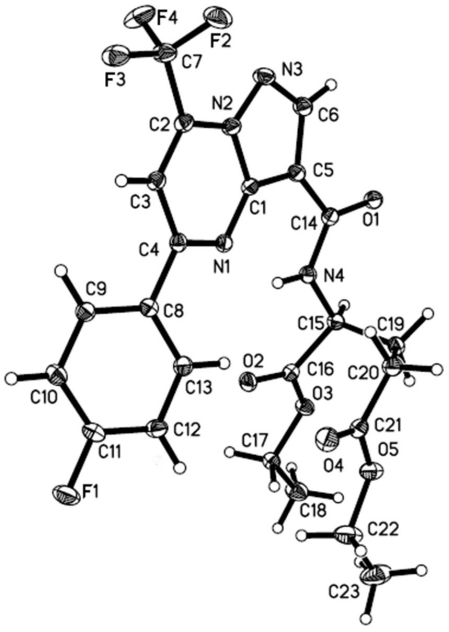

Fig. 1 The structure of $C_{23}H_{22}F_4N_4O_5$ with all non-H atom-labelling scheme and ellipsoids drawn at the 30% probability level.

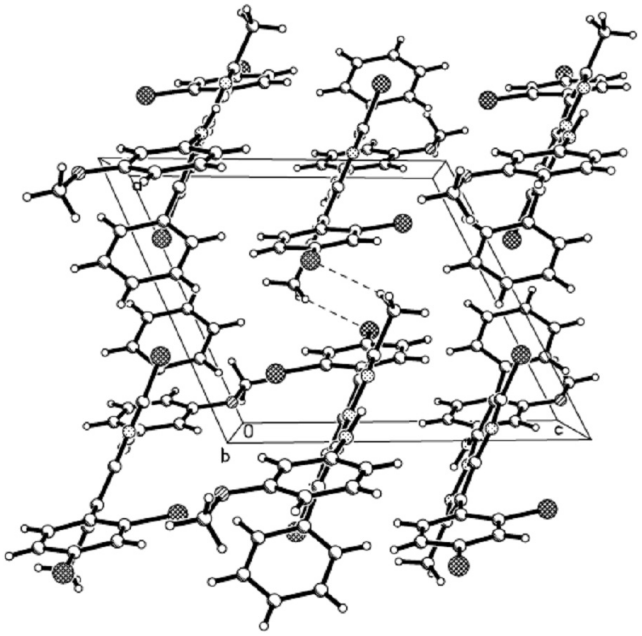

Fig. 2 A packing diagram of $C_{23}H_{22}F_4N_4O_5$.

Synthesis of diethyl-2-[5-(4-fluorophenyl)-7-(trifluoromethyl)pyrazolo [1,5-a]pyrimidine-3-carboxamido]pentanedioate

Under a nitrogen atmosphere, a mixture of 5-(4-fluorophenyl)-7-(trifluoromethyl)pyrazolo[1,5-*a*]pyrimidine-3-carboxylic acid (2.0 g, 6.15 mmol), EDCI (1.42 g, 7.38 mmol), HOBt (0.83 g, 6.15 mmol), DMAP (0.75 g, 6.15 mmol) in 30 mL dried DMF was stirred at room temperature for 0.5 h, a solution of L-glutamic acid diethyl ester hydrochloride (1.77 g, 7.38 mmol) in 30 mL of dried DMF was added. After the mixture was stirred for 12 h at room temperature, it was diluted with water (200 mL), extracted by ethyl acetate three times (100 mL × 3), and dried over anhydrous sodium sulfate filtered, and concentrated under reduced pressure to give crude product, which was purified by column chromatography to give 2.1 g of the title compound as yellow–green powder, yield: 66.90%. m.p. 154–157 °C; ^{1}H NMR (600 MHz, DMSO-d_6): δ 8.73 (s,1H, ArH), 8.54 (m, 2H, ArH), 8.51 (s,1H, –NH), 8.37 (s, 1H,ArH), 7.44 (t, J=9.0 Hz, 2H, PhH), 4.71 (m, 1H, CH), 4.20 (q, J=7.2 Hz, 2H, CH_2), 3.98 (m,2H, CH_2), 2.46 (m, 2H, CH_2), 2.23 (m, 1H, CH_2), 2.09 (m, 1H, CH_2), 1.22 (t, J=7.2 Hz, 3H, CH_3–H), 1.08 (t, J=7.2 Hz, 3H, CH_3–H). IR (KBr) ν: 3325, 3114, 3049, 2996, 2952, 1736, 1655, 1634, 1600, 1568, 1550, 1517, 1485, 1473, 1408, 1383, 1326, 1290, 1258, 1167, 1076, 1033, 845, 764, 632. Anal. calcd for $C_{23}H_{22}F_4N_4O_5$: C, 54.12; H, 4.34; N, 10.98; found: C, 54.10; H, 4.35; N, 11.03%.

Table 1 Crystal data for diethyl-2-[5-(4-fluorophenyl)-7-(trifluoromethyl) pyrazolo[1,5-a]pyrimidine-3-carboxamido]pentanedioate

Crystal size	0.14 mm × 0.06 mm × 0.12 mm
Formula	$C_{23}H_{22}F_4N_4O_5$
fw	510.45
T/K	293(2)
Crystal system	Monoclinic
Space group	C_{121}
a/Å	27.001(3)
b/Å	7.161(1)
c/Å	12.0900
α/°	90.00
β/°	92.379 (7)
γ/°	90.00
V/Å^3	2335.7 (5)
Z	4
Dc/g.cm^{-3}	1.452
F(000)	1056
GOF on F^2	1.140
Reflection/unique	13125/3735
R_1,wR_2 [I>2 (I)]	0.0531, 0.1072
R_1,wR_2 (all data)	0.0552, 0.1089

$R=\sum(||F_o|-|F_c||)/\sum|F_o|$
$wR=[\sum w(F_o^2-|F_c^2)^2/\sum w(F_o)^2]^{1/2}$

Table 2 Hydrogen bond lengths (Å) and bond angles (°)

D–H…A	d(D–H)	d(H…A)	d(D…A)	∠DHA
C(22)–H(22B)…F(4)	0.9900	2.489	3.261	134.53
C(10)–H(10)O(1)	0.9500	2.503	3.410	159.69
C(6)–H(6)…O(3)	0.9500	2.644	3.459	144.12
N(4)–H(1)…N(1)	0.83(3)	2.25(3)	2.942(3)	140(2)

Crystal data structure determination

A colourless crystal ($C_{23}H_{22}F_4N_4O_5$) with dimensions of 0.14 mm × 0.06 mm × 0.12 mm was selected for data collection which was performed on a Bruker P4 diffractometer equipped with a graphite-monochromatic CuKα radiation (λ=1.54187 Å) by using an ω scan mode at 298(2) K. A total of 10466 reflections were collected in the range of 3.3<θ<72.0° (index ranges: –33<h<32, –7<k<8, –14<l<14) and 3735 were independent (R_{int}=0.064), of which 3466 observed reflections with $I>2\sigma$ (I) were used in the structure determination and refinements. The structure was solved by direct methods with SHELXS-97 program[10] and expanded by the Fourier technique. The non-hydrogen atoms were refined anisotropically. The hydrogen atoms bound to carbon were determined with theoretical calculations and those attached to nitrogen and oxygen were determined with successive difference Fourier syntheses. The structure was refined by full-matrix Least-squares techniques on F^2 with SHELXL-97[10]. The final refinement gave the final R=0.0552 and wR=0.1072 (w=1/ [σ^2 (Fo^2)+(0.0696P) ^{2}] where P=(Fo^2+2Fc^2)/3). S=1.14, (Δ /σ) max=0.001, (Δ ρ) max=0.49 and (Δ ρ) min=–0.55 e Å^{-3}. All calculations were performed using the Crystal Structure crystallographic software package except for the refinement. Crystallographic data and experimental details of structural analyses for compound are summarised in Table 1. The hydrogen bond data of title compound is listed in Table 2. CCDC 882212.

Table 3 *In vitro* anticancer activity test[a] of diethyl-2-[5-(4-fluorophenyl)-7-(trifluoromethyl)pyrazolo[1,5-a]pyrimidine-3-carboxamido]pentanedioate on DU-145, PC-3 and COLO-205 cell lines

Compound	% Inhibition at 80 μg mL^{-1} in		
	DU-145	PC-3	COLO-205
Dethyl-2-[5-(4-fluorophenyl)-7-(trifluoromethyl)pyrazolo[1,5-a]pyrimidine-3-carboxamido]pentanedioate	84.5	70	42.5
Adriamycin	86.5	98.2	97.4

[a]Test MTT colourimetric assay in DU-145, PC-3(prostate) and COLO-205(colon) human cancer cell lines.

In vitro *anticancer activity test of the title compound on DU-145, PC-3 and COLO-205 cell lines*

The title compound exhibited significant inhibitory activity in PC-3 cell line, DU-145 cell line and COLO-205 cell line. The title compound was slightly less potent than adriamycin in DU-145 but obviously less potent than adriamycin in PC-3, COLO-205. The percentage inhibition determined is reported in Table 3.

Results and discussion

The element analysis, IR (Fig. S1 in the electronic supplementary information, ESI), ^{1}H NMR (Fig. S2, ESI) and X-ray diffraction data for the product are in good agreement with the structure of the title compound. A perspective view of the crystal structure and packing diagram are shown in Figs 1 and 2, the F(1)–C(11), O(4)–C(21) and O(5)–C(21) bonds are 1.354(2), 1.203(3) and 1.339(3) Å respectively. The bond length of O(1)–C(14) is 1.234(2) Å and belongs to the typical C=O double bond. The dihedral angle between the benzene ring and pyrazolopyrimidine is 53.618(162)°. On the other hand, there is weak intramolecular hydrogen bond N(4)–H(1)…N(1). Meanwhile, intermolecular hydrogen bond C(22)–H(22B)…F(4), N(4)–H(4)…Cl(2) and C(10)–H(10)…O(1) are also present as shown in Table 2. These interaction forces give the molecules a three-dimensional structure. In addition, preliminary bioassay indicated that the title compound possessed some anti-tuberculosis activity, and the activity data are still waiting for further analysis.

Electronic Supplementary Information

Figures S1 and S2 have been deposited in the ESI available through: stl.publisher.ingentaconnect.com/content/stl/jcr/supp-data

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