Synthesis, crystal structure and biological activity of 5-(4-fluorophenyl)-*N*,*N*-dimethyl-7-(trifluoromethyl)pyrazolo[1,5-*a*]pyrimidine-3-carboxamide

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The title compound, 5-(4-fluorophenyl)-*N*,*N*-dimethyl-7-(trifluoromethyl)pyrazolo[1,5-*a*]pyrimidine-3-carboxamide, has been synthesised by condensation of dimethylamine with 5-(4-fluorophenyl)-7-(trifluoromethyl)pyrazolo[1,5-*a*]pyrimidine-3-carboxylic acid. This intermediate was prepared from ethyl 5-amino-1*H*-pyrazole-4-carboxylate by cyclisation with 4,4,4-trifluoro-1-(4-fluorophenyl)butane-1,3-dione and then saponification with sodium hydroxide. The crystal structure of the title compound was determined. The compound possesses distinct effective inhibition on the proliferation of some cancer cell lines.

Keywords: synthesis, X-ray diffraction, biological activity, anticancer agents

Pyrazolopyrimidines have attracted the attention of organic chemists due to their biological and chemotherapeutic importance.[1] Among them, pyrazolo[1,5-*a*]pyrimidines are of interest as potential bioactive molecules. They are known to exhibit a wide range of biological activities as, for example, anticancer agents,[2–4] antimicrobial agents,[5] antiepileptic agents,[6] anxiolytics,[7] antidepressants,[8] agents for treatment of sleep disorders[9] and oncolytics.[10]

Our interest in fused pyrimidine heterocycles is a result of the known biological activity of these systems reported in the literature. In order to find novel bioactive compounds, several new kinds of pyrazolopyrimidines have been synthesised in our laboratory.[11,12] We now report the synthesis of a new pyrazolo[1,5-*a*]pyrimidine, 5-(4-fluorophenyl)-*N*,*N*-dimethyl-7-(trifluoromethyl)pyrazolo[1,5-*a*]pyrimidine-3-carboxamide, by a three-step synthesis. In order to confirm its structure and investigate its stereoconfiguration, a single crystal of the title compound was obtained and the molecular structure was determined by elemental analysis, IR spectroscopy, ^{1}H NMR spectroscopy and X-ray diffraction. The biological tests suggested the compound displayed distinct effective inhibition on the proliferation of cancer cell lines. The synthetic procedure for the title compound is shown in Scheme 1.

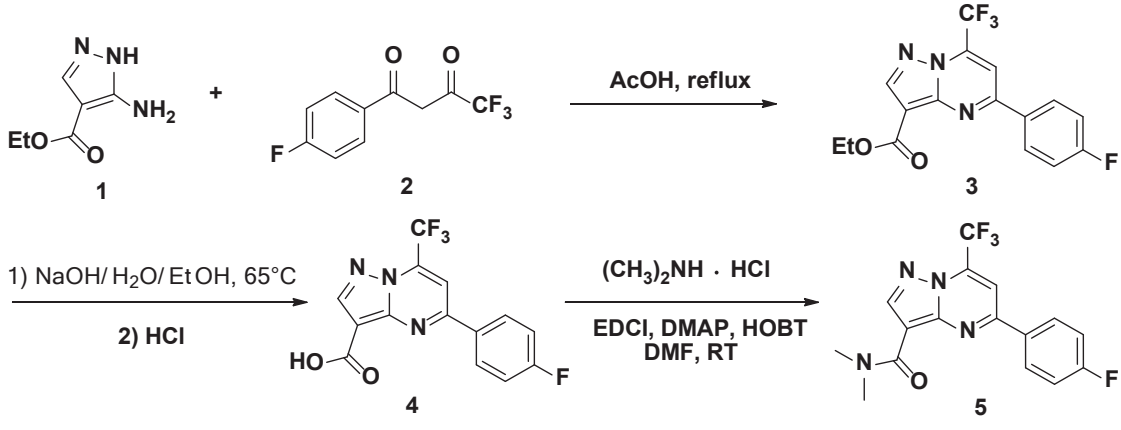
Experimental

Melting points (°C) were measured with a Koffler melting point apparatus and are uncorrected. Thin-layer chromatography (TLC) was performed on aluminium sheets pre-coated with silica gel (Merck, Kiesel gel 60 F-254). ^{1}H NMR spectra were recorded on a Bruker AV-600 (or 500) in DMSO-d_6 using TMS as an internal standard (chemical shifts in ppm). IR spectra were recorded in KBr pellets on a PerkinElmer Spectrum One FTIR spectrometer. Crystal data were obtained on a Bruker P4 X-diffractometer. Elemental analysis was carried out on a Carlo Erba 1108 analyser. All solvents and reagents were obtained from commercial sources and were used without purification. Ethyl 5-aminopyrazole-4-carboxylate **1** was purchased from Aldrich and 4,4,4-trifluoro-1-(4-fluorophenyl)butane-1,3-dione was obtained as reported.[13]

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

A solution of ethyl 5-amino-1*H*-pyrazole-4-carboxylate (**1**) (15.5 g, 0.1 mol) and 4,4,4-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 **3** was filtered off, washed with water and dried: m.p. 167–169 °C; IR (KBr, cm^{-1}): 2965, 1697, 1634, 1594, 1570, 1466, 1397, 1327, 1198, 1171, 1027, 848, 778; ^{1}H NMR (600 MHz, DMSO-d_6): δ 8.75(s, 1H, ArH), 8.48 (m, 2H, ArH), 8.37 (s, 1H, ArH), 7.45 (m, 2H, ArH), 4.34 (q, J = 7.2 Hz, 2H, CH_2), 1.36 (t, J = 7.2 Hz, 3H, CH_3).

The resulting ethyl carboxylate **3** 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,



Scheme 1 Synthetic route of the title compound.

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and recrystallised from MeCN to give: 20.6 g pure 5-(4-fluorophenyl)-7-(trifluoromethyl)pyrazolo[1,5-*a*]pyrimidine-3-carboxylic acid (**4**) in 63.4% yield (two steps); m.p. 252–253 °C; IR (KBr, cm^{-1}): 3446, 2928, 2856, 1672, 1655, 1601, 1564, 1457, 1406, 1337, 1235, 1200, 1161, 841, 558; ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.52 (s, 1H, ArH), 8.50 (m, 2H, ArH), 8.20 (s, 1H, ArH), 7.25 (d, J = 8.2 Hz, 2H, ArH), 2.35–2.55 (s, 1H, COOH); LC/MS *m/z*: 326 (M + 1). Anal. calcd for $C_{14}H_7F_4N_3O_2$: C 51.70, H 2.17, N 12.92; found C 51.61, H 2.21, N 12.83%.

Synthesis of 5-(4-fluorophenyl)-N,N-dimethyl-7-(trifluoromethyl) pyrazolo[1,5-a]pyrimidine-3-carboxamide (**5**)

Under an N_2 atmosphere, a mixture of 5-(4-fluorophenyl)-7-(trifluoromethyl)pyrazolo[1,5-*a*]pyrimidine-3-carboxylic acid (**4**) (1.63 g, 5.00 mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCl) (1.16 g, 6.00 mmol), 1-hydroxybenzotriazole (HOBT) (0.68 g, 5.00 mmol) and 4-dimethylaminopyridine (DMAP) (0.61 g, 5.00 mmol) in 50 mL dried *N,N*-dimethylformamide (DMF) was stirred at room temperature for 0.5 h and a solution of dimethylamine hydrochloride (0.49 g, 6.00 mmol) in 20 mL of dried DMF was added. After the mixture was stirred for 10 h at room temperature, it was was diluted with water (100 mL), extracted by ethyl acetate three times (80 mL × 3), and dried over anhydrous sodium sulfate. It was then filtered off and concentrated *in vacuo* to give crude product, which was purified by column chromatography to give 1.22 g of the title compound as: Yellow powder, yield: 69.1%; m.p. 170–173 °C; IR (KBr, cm^{-1}): 3055, 2933, 1632, 1618, 1569, 1533, 1514, 1413, 1396, 1339, 1325, 1258, 1233, 1199, 1173, 1119, 838, 773, 760, 685; ^{1}H NMR (600 MHz, DMSO-d_6): 8.54 (s, 1H, ArH), 8.41 (m, 2H, ArH), 8.26 (s, 1H, ArH), 7.42 (m, 2H, ArH), 3.14 (s, 3H, CH_3), 3.06 (s, 3H, CH_3). Anal. calcd for $C_{16}H_{12}F_4N_4O$: C 54.55, H 3.43, N 15.90; found C 54.46, H 3.45, N 15.83%

Crystal data structure determination

The white powder of the title **5** compound was dissolved in ethanol/acetone mixed solvents = 1:1 (v/v). After slowly evaporating the solvents for several days, some single crystals suitable for X-ray analysis were obtained. A yellow crystal ($C_{16}H_{12}F_4N_4O$) with dimensions of 0.20 × 0.18 × 0.10 mm was selected for data collection which was performed on a Bruker P4 diffractometer equipped with a graphite-monochromatic Cu *K*α radiation (λ = 0.71073 Å) by using an ω scan mode at 298(2) K. A total of 6565 reflections were collected in the range of 2.5 < θ < 25.0° (index ranges: –9 < h <9, –11 < k < 11, –11 < l < 12) and 2808 were independent (R_{int} = 0.090), of which 864 observed reflections with $I > 2\sigma$ (I) were used in the structure determination and refinements. The structure was solved by direct methods with the SHELXS-97 program[14] 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 the full-matrix least-squares techniques on F^2 with SHELXL-97.[14] The final refinement gave the final R = 0.053 and wR = 0.134 (w = 1/ [σ^2 (Fo^2) + (0.020P) ^{2}]) where P = (Fo^2 + 2Fc^2)/3), S = 1.05, (Δ/σ) max = 0.002, (Δρ) max = 0.24 and (Δρ) min = –0.28e/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 **5** are summarised in Table 1. The hydrogen bond data of title compound is listed in Table 2. CCDC1433227 contains the supplementary crystallographic data for this paper. The data can be obtained free of charge from the Cambridge Crystallographic Data Centre *via* www.ccdc.cam.ac.uk/data_request/cif

In vitro *anticancer activity test of the title compound on BGC-823 and HepG-2 cell lines*

The named compound **5** was evaluated for its *in vitro* cytotoxic activity against two cancer cell lines (BGC-823 and HepG-2) by the MTT-based assay using sorafenib tosylate as a positive control (Table 3). Biological activity determination results indicated that the title compound exhibited significant inhibitory activity in the BGC-823 cell line and HepG-2 cell lines. The title compound was slightly less potent than sorafenib tosylate in the BGC-823 cell line but obviously less potent than sorafenib tosylate in the HepG-2 cell line. The percentage inhibition determined is reported in Table 3. Further structure optimisation may result in more active anticancer compounds.

Table 1 Crystal data for the title compound[a] **5**

Crystal size	0.20 × 0.18 × 0.10 mm
Formula	$C_{16}H_{12}F_4N_4O$
fw	352.30
T/K	293(2)
Crystal system	*Triclinic*
Space group	*P* –1
a/Å	8.1533(16)
b/Å	9.775(2)
c/Å	10.112(2)
α/°	95.02(3)
β/°	91.66(3)
γ/°	93.43(3)
V/Å^3	800.9(3)
Z	2
Dc/g cm^{-3}	1.461
F(000)	360
GOF on F^2	1.050
Reflection/unique	6565/2808
R_1,wR_2[I> 2 (I)]	0.0609, 0.1101
R_1,wR_2 (all data)	0.1394, 0.1335

[a] $R_1 = \sum(||F_o| - |F_c||)/\sum|F_o|$ and $wR_2 = [\sum w(F_o^2 - F_c^2)^2/\sum w(F_o^2)^2]^{1/2}$.

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

D–H···A	d(D–H)	d(H···A)	d(D···A)	∠DHA
C(14)-F(4)...F(4)	1.378	2.919	3.482	102.25
C(13)-H(13)...O(1)	0.930	2.405	3.314	165.86

Table 3 *In vitro* anticancer activity test[a] of 5-(4-fluorophenyl)-*N,N*-dimethyl-7-(trifluoromethyl)pyrazolo[1,5-*a*]pyrimidine-3-carboxamide (**5**) on BGC-823 and HepG-2 cell lines

Compound	% inhibition at 40 mg mL^{-1} in	
	BGC-823	HepG-2
5-(4-Fluorophenyl)-*N,N*-dimethyl-7-(trifluoromethyl)pyrazolo[1,5-*a*]pyrimidine-3-carboxamide	74.9	38.5
Sorafenib tosylate	81.4	61.9

[a]Test MTT colourimetric assay in BGC-823 and HepG-2 human cancer cell lines.

Results and discussion

The named compound **5** was prepared according to Scheme 1. The elemental analyses, IR (Fig. S1), ^{1}H NMR (Fig. S2) 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(4)–C(14), N(2)–N(3) and N(4)–C(10) bonds are 1.379(4), 1.376(4) and 1.334(4) Å respectively. The bond length of O(1)–C(3) is 1.247(4)Å which is typical for a C=O double bond. The dihedral angle between the benzene ring and pyrazolopyrimidine is 9.98°. Weak intermolecular hydrogen bonds C(14)–F(4)…F(4) and C(13)–H(13)…O(1) are also present as shown in Table 2. These interactions are responsible for maintaining the three-dimensional crystal structure.

In addition, a preliminary bioassay indicated that the title compound possessed some anticancer activity and the activity

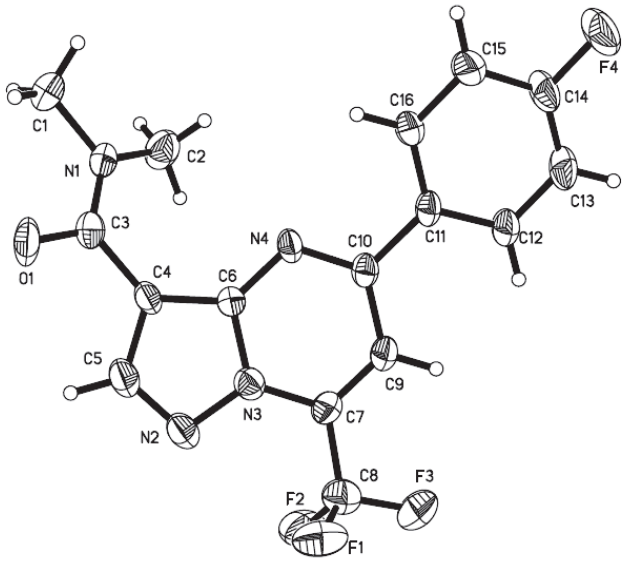

Fig. 1 The structure of $C_{16}H_{12}F_4N_4O$ (**5**) with all non-H atoms labelled and ellipsoids drawn at the 30% probability level.

data are still waiting for further analysis. Further studies on structural optimisation and biological activities of these derivatives are underway in our laboratory and will be reported in the future.

Electronic Supplementary Information

The ESI (IR and ^{1}H NMR spectra) is available through: stl.publisher.ingentaconnect.com/content/stl/jcr/supp-data

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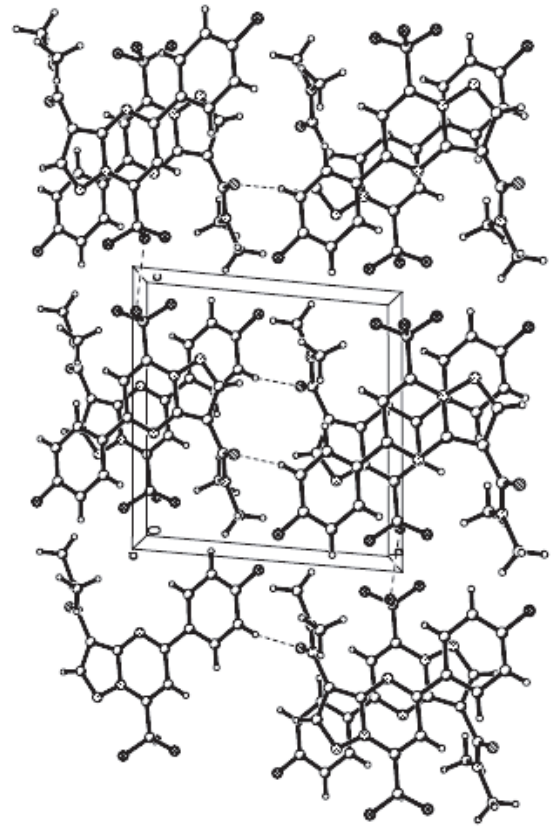

Fig. 2 Packing diagram of $C_{16}H_{12}F_4N_4O$ (**5**).

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