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A new salt of dyclonine (DYC): synthesis, crystal structure, luminescent properties, thermal and biological activities

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Abstract A new organic salt of dyclonine (DYC) with *p*-toluenesulfonic acid (*p*-TSA) has been synthesized and characterized by elemental analysis, single-crystal X-ray diffraction, IR spectra, UV spectra, and hot stage microscopy. The structure determination shows that the asymmetric unit of the salt consisting of two entire ions of DYC^+ and *p*-TSA^-, and that they are not symmetric. The salt is primarily stabilized by a strong N–H···O hydrogen bonding interaction between DYC^+ and *p*-TSA^-. In addition, the luminescent properties and biological activities are discussed.

Keywords Synthesis · Crystal structure · Dyclonine · *p*-Toluenesulfonic acid · Biological activities

Introduction

Local anaesthetic (LA) drugs of general formula lip–CO–hydr (lip = lipophilic end, mostly phenyl ring; CO = negatively charged linkage, commonly ester or amide; hydr = hydrophilic group, tertiary or secondary amine) are well known for the formation of polymorphs and solvates [1, 2]. LA compounds are commonly separated into two main groups, the ester type LA with a short duration of anaesthesia and the amide type LA with a longer duration of action [3], whereas

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DYC belongs to a different group of local anesthetics classified as ketones [4]. DYC is exclusively used for topical anesthesia, delivering the effects of analgesia and anti-infection at the same time, it is effective during bronchoscopy or awake endotracheal intubation [5]. It is used for oral pain and used to anesthetize mouths for patients who have sensitivity. It is also useful as a mouthwash or gargle in suppressing the gag reflex. In addition, DYC also has bactericidal and fungicidal properties, used topically as the hydrochloride salt. Dyclonine hydrochloride (DYC HCL), chemically denoted as 3-piperidino 4′-butoxypropiophenone hydrochloride, is a well-known anesthetic/analgesic agent for topical use on the mucous membranes of the mouth and throat [6]. It is a topical anesthetic which is said to have a wide margin of safety, good anesthetic properties, and a low sensitizing potential [4]. The crystal structure of DYC HCL has been reported [7]. In addition to its anesthetic/analgesic properties, DYC HCL is known to possess antimicrobial activity [8].

There appears to have been very little attention given in the past to the study of the antimicrobial properties of agents intended for topical anesthesia. That such concomitant action would be of definite advantage is apparent, not only from the standpoint of prevention and control of infection but also for formulation purposes. In this work, we applied the crystal engineering strategy on the DYC with *p*-TSA and obtained a salt. This salt was characterized by single-crystal X-ray diffraction, IR spectroscopy, elemental analysis, UV, and hot stage microscopy (HSM). Its luminescence properties and biological activities are discussed.

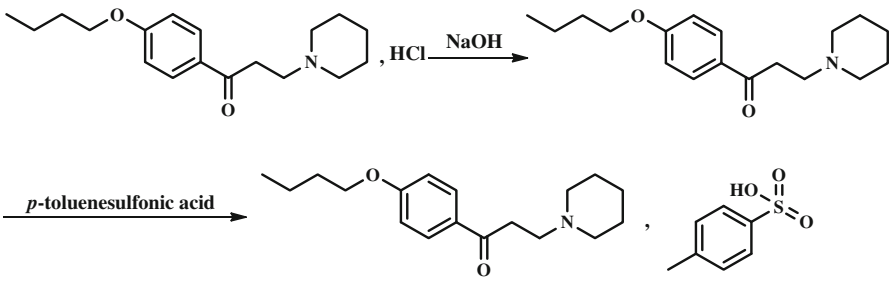
Experimental

Materials and measurements

All chemicals were of analytical grade and solvents were purified by conventional methods. Melting points were determined using an X4 digital microscopic melting point apparatus and are uncorrected. Elemental analyses for C, H, N and S were performed on a Perkin-Elmer 240 analyzer. UV–Vis spectra were recorded on a UV-6000PC spectrometer at room temperature in H_2O solution. Infrared spectra were recorded on a SHIMADZU IR prestige-21 FTIR-8400S spectrometer in the spectral range 4,000–400 cm^{-1}, with the samples in the form of potassium bromide pellets. Fluorescence spectroscopy data for salt **1** were recorded on a F-2700 FL spectrophotometer. Hot stage microscopy for salt **1** was performed on a LEICA DM750P microscope using a Mettler-Toledo FP82HT hot stage (Scheme 1).

Synthesis of DYC

The compound DYC was synthesized by the following reaction: DYC HCL was added to a solution of NaOH in H_2O at r.t.. The mixture was stirred for 10 min, the water phase was extracted three times with EtOAc. The organic phase was dried



Scheme 1 The synthetic scheme of salt **1**

over $MgSO_4$, and concentrated. A light yellow liquid was obtained after the solvent were removed under reduced pressure (yield 87 %).

Synthesis of salt **1**

The preparation of salt **1** was conducted in solution crystallization experiments. Salt **1** was obtained by the following procedure: a 1:1 stoichiometric amount of DYC and *p*-TSA were added to a 20 ml stirring EtOAc solution at a temperature of 40 °C, the resulted solution stired for 10 min before being left to evaporate at room temperature. Colorless club-shaped crystals of **1** were obtained within 3 days (yield 86 %). m.p. 134–137 °C. Elemental analysis for $C_{25}H_{35}NO_5S$, Anal. Calcd. (%): C, 65.05; N, 3.03; H, 7.64; S, 6.95. Found: C, 65.18; N, 3.24; H, 7.30; S, 6.87. UV (nm): 221, 282. IR (KBr pellet, cm^{-1}): 2,955, 2,871, 2,678, 2,546, 1,670, 1,601, 1,574, 1,457, 1,425, 1,316, 1,261, 1,227, 1,177, 1,148, 1,116, 1,039, 1,028, 1,007, 965, 800, 679. (The IR spectra of salt **1** and DYC HCL are shown in Fig. S1.)

X-ray crystallographic study

The single-crystal X-ray diffraction data of salt **1** was collected at 296 K with graphite-monochromated MoKα radiation ($\lambda = 0.071073$ nm), a Rigaku SCXmini diffractometer with the ω-scan technique were used [9]. The lattice parameters were integrated using vector analysis and refined from the diffraction matrix, and the absorption correction was carried out by using the Bruker SADABS program with the multi-scan method. A summary of crystallographic data, data collection, and refinement parameters for salt **1** are given in Table 1. Their structures were solved by full-matrix least-squares methods on all F^2 data, using the SHELXS-97 and SHELXL-97 programs [10] for structure solution and structure refinement, respectively. Reliability factors were defined as $R1 = \Sigma(|F_o| - |F_c|)/\Sigma|F_o|$ and the function as minimized was $R_w = [\Sigma_w(F_o^2 - F_c{}^2)^2/w(F_o)^4]^{1/2}$, where, in the least-squares calculation, the unit weight was used. All non-hydrogen atoms were refined anisotropically, and hydrogen atoms were inserted at their calculated positions and fixed at these positions [11]. The molecular graphics were prepared by using the DIAMOND program [12] and mercury [13]. CCDC references number 943026 contains the supplementary crystallographic data in CIF format for salt **1** reported in

Table 1 Crystal data and structure refinement for salt **1**

Compound	Salt **1**
Formula	$C_{25}H_{35}NO_5S$
Formula weight	461.61
Crystal system	Triclinic
Space group	*P*-1
a(Å)	7.5053(10)
b(Å)	14.1987(19)
c(Å)	23.819(3)
α(°)	88.029(2)
β(°)	83.134(2)
γ(°)	78.761(2)
V(Å$^{3)}$	2,471.6(6)
Z	4
D_c(Mg m^{-3})	1.240
T(K)	296(2)
μ(mm^{-1})	0.166
Cryst dimensions	0.40 × 0.20 × 0.20
No. of reflns collected	17,350
No. of unique reflns	8,490
No. of params	581
Goodness-of-fit on F^2	1.000
R_1, wR_2 ($(I > 2\sigma(I))$	0.0983, 0.2153
R_1, wR_2 (all data)	0.0687, 0.1973
CCDC No.	943,026

this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Results and discussion

Structure description

Salt **1** crystallizes as colorless club-shaped crystals. The structure determination shows that **1** forms a 1:1 (DYC^+:*p*-TSA^-) salt in the Triclinic *P*-1 space group with $Z = 4$ (Fig. 1a). The asymmetric unit (ASU) consisting of two entire ions of DYC^+ and *p*-TSA^-, for they are not symmetric. They can be described as two hydrogen-bonded dimer units consisting of a DYC^+ ion connected by N–H···O [N1–H1···O5 = 1.84 Å; N2–H2A···O8 = 1.82 Å] hydrogen bonds to a *p*-TSA^- ion (Fig. 1a). Every two dimers are buckled together to form a double-hook structure when viewed along the *a* axis (Fig. 1b). The double-hook structures are further stacked by many hydrogen bonds (Table 2) and C–H···π (Fig. 1d) intermolecular interactions in the adjacent double-hook units, into an interlaced three-dimensional structure (Fig. 1c).

UV–Vis spectrum

In the UV–Vis spectrum of salt **1** (or DYC HCL) in H_2O solution, two intense bands at 221.0 (or 220.5) and 282.0 (or 282.5) nm are attributed to the DYC $\pi-\pi^*$ and $n-\pi^*$ transitions, for the acids in the two salts are not the same (Fig. S1).

Luminescence properties

Although there is a similarity between the UV–Vis spectrum of salt **1** and DYC HCL, their solid-state emission spectra have a great difference. The emission spectrum for salt **1** showed a broad emission maximum at 432 nm but there is no obvious peaks in excitation spectrum in the range of 250–400 nm, while for DYC HCL, no obvious peaks were observed in its solid-state emission and excitation spectra. The emission peak (432 nm) of salt **1** is probably due to $\pi-\pi^*$ transitions of *p*-TSA because no similar peak appears for DYC HCL (Fig. S2).

Evaluation of antifungal activities

The synthesized salt **1** were tested for antifungal activity against *Candida albicanes* (ATCC 10231) and *Aspergillus niger* (ATCC 16404) by the disc diffusion method [14] at different concentrations using DMSO (dimethylsulphooxide) as solvent control, while nutrient agar was employed as culture medium. After 72 h of incubation at 28 °C, the zone of inhibition was measured in mm. A standard antibiotic Nystatin was used as control to compare antifungal activities of salt **1** and the results are represented in Table 3.

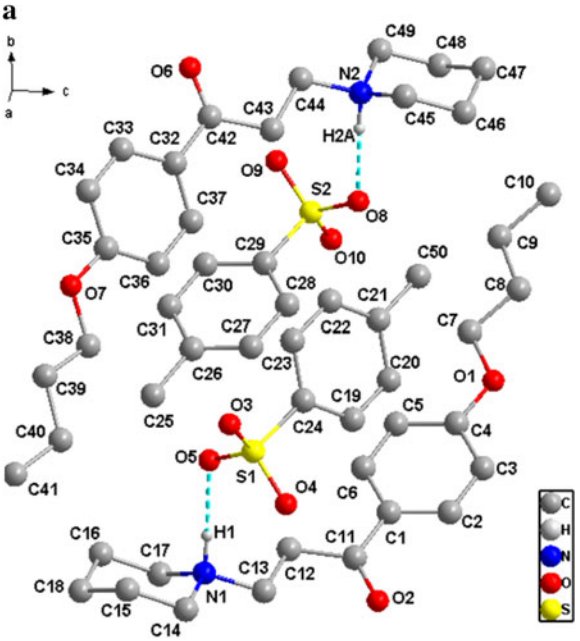


Fig. 1 **a** Molecular structure of salt **1**. **b** The crystal packing of salt **1** viewed along the *a* axis showing hydrogen bonding. **c** 3D Pack structure of salt **1** along the *b* axis showing hydrogen bonding. **d** View of six edge-to-face C–H···π stacking interactions in salt **1**. Some hydrogens have been omitted for clarity. (Color figure online)

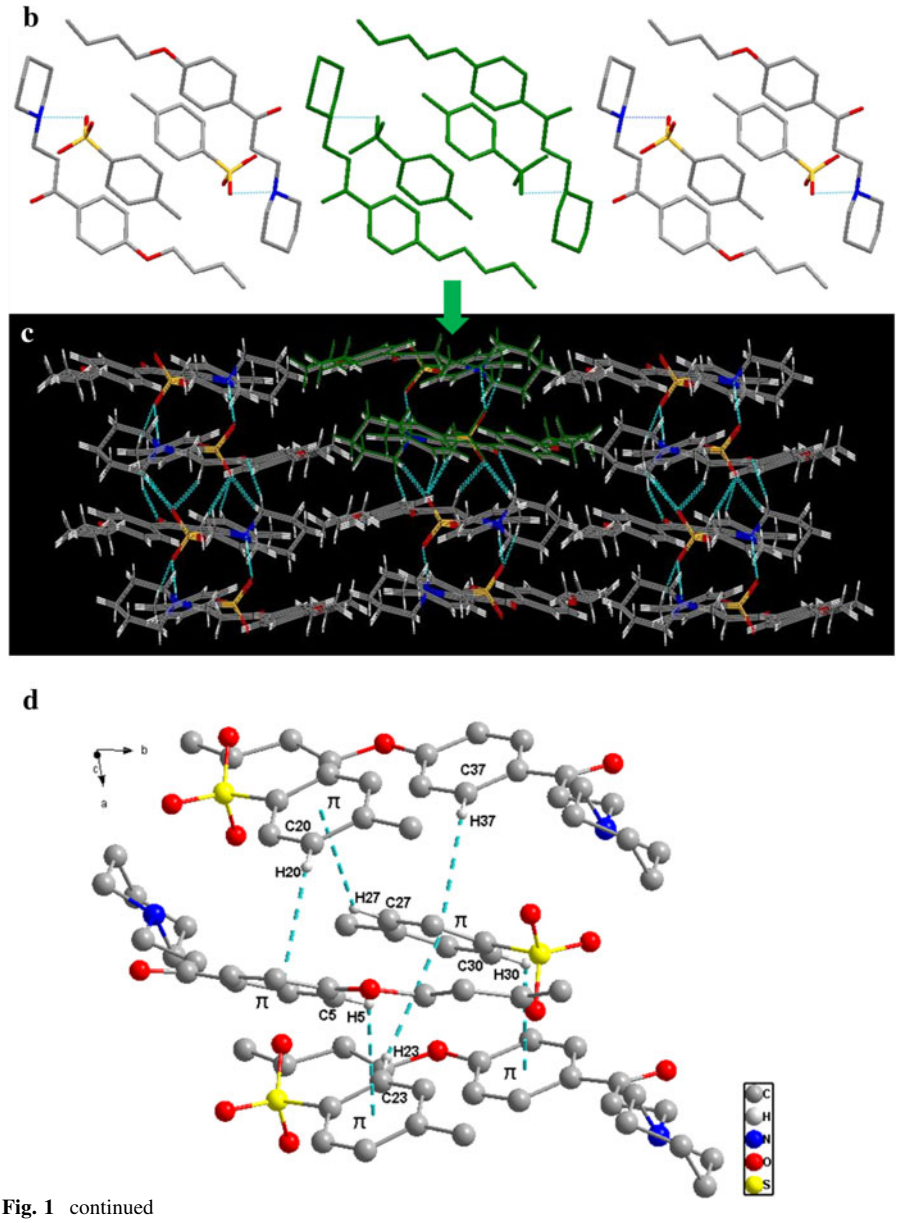


Fig. 1 continued

The antifungal activities of different concentrations of salt **1** were tested. From the data obtained in Table 2, it is clear that antifungal activities of salt **1** were increased gradually with the enhanced concentrations, but it was found to be inactive against *Aspergillus niger* up to 200 μg disk^{-1}. We conclude that salt **1** showed highly biological activity against *Candida albicans* and moderately activity

Table 2 The hydrogen bonding geometry and π-stacking interaction for salt **1**

D–H···A	D–H	H···A	D···A	∠DHA
N1–H1···O5[a]	0.91	1.84	2.750(3)	177
N2–H2A···O8[b]	0.91	1.82	2.735(3)	178
C12–H12B···O3	0.97	2.58	3.402(4)	143
C17–H17A···O6	0.97	2.39	3.332(4)	164
C17–H17B···O3	0.97	2.59	3.535(4)	165
C22–H22···O10[c]	0.93	2.54	3.380(4)	150
C25–H25A···O3[d]	0.96	2.55	3.428(5)	151
C43–H43B···O10	0.97	2.48	3.446(4)	176
C45–H45A···O10	0.97	2.46	3.362(4)	154
C45–H45B···O2	0.97	2.51	3.466(4)	168
C5–H5···π	0.93	3.06	3.532(3)	113
C20–H20···π	0.93	2.91	3.558(6)	128
C23–H23···π	0.93	3.18	3.829(8)	129
C27–H27···π	0.93	3.04	3.724(4)	132
C30–H30···π	0.93	3.09	3.579(6)	115
C37–H37···π	0.93	2.99	3.578(6)	122

For the intermolecular C–H···π interactions, the meaning of "A" indicate the centroid of the phenyl ring

[a] −1 + x, y, z

[b] 1 + x, y, z

[c] x, 1 + y, z

[d] x,−1 + y, z

Table 3 Antifungal activities of salt **1** in terms of diameter of inhibition zone in mm

Compounds	Concentrations (μg/disk)	Inhibition zones (mm)	
		Candida albicans	*Aspergillus niger*
Salt **1**	12.5	7.5	–
	25	8.1	–
	50	10.8	–
	100	13.7	–
	200	14.4	10
	400	15.3	15.1
	800	18.1	18.3
	1,000	18.5	11
Nystatin	1.25	8.4	–
	2.5	9.1	–
	5	14.4	12.8
	10	20.5	21.8
	20	20.9	23.9
	40	21.6	25.6

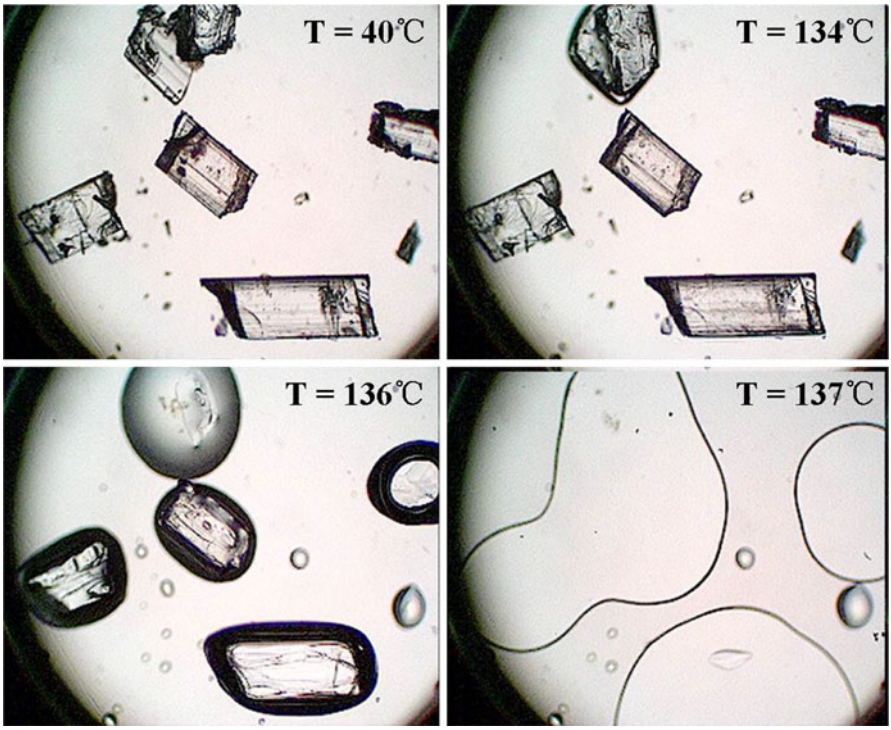


Fig. 2 Hot stage microscopy for salt **1**

against *Aspergillus niger* but lower than the reference Nystatin; these activities may attributed to piperidine rings in DYC.

Hot stage microscopy (HSM)

The hot stage microscopy for salt **1** is shown in Fig. 2. During the heating procedure, the big club-shaped crystals of salt **1** begin to be crushed into small crystals and then melt in a temperature range of 134–137 °C.

Conclusions

We have investigated the detailed crystal formation of DYC with *p*-TSA through a solution reaction. A thorough structural investigation of the resulting salt reveals that the N–H···O hydrogen bonding interactions between DYC^+ and $p\text{-}TSA^-$ play a key role in forming the elementary structure of the salt. The emission spectrum for salt **1** showed a broad emission maximum at 432 nm but there is no obvious peak in the excitation spectrum. From the data obtained in evaluation of antifungal activities, it is clear that salt **1** showed high biological activity against *Candida*

albicans and moderate activity against *Aspergillus niger*, and that these activities may be attributed to piperidine rings in DYC.

Supplementary material

Crystallographic data for the structures reported in this article have been deposited with the Cambridge Crystallographic Data Center as supplementary publication No. CCDC 943026. Copies of the data can be obtained free of charge via www.ccdc.cam.ac.uk (or from the Cambridge Crystallographic Centre, 12 Union Road, Cambridge CB21EZ, UK; Fax: t44 1223 336033; Email: deposit@ccdc.cam.ac.uk).

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