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Synthesis and biological evaluation of novel phane-structured diazacrowns containing γ-piperidone and pyridine rings

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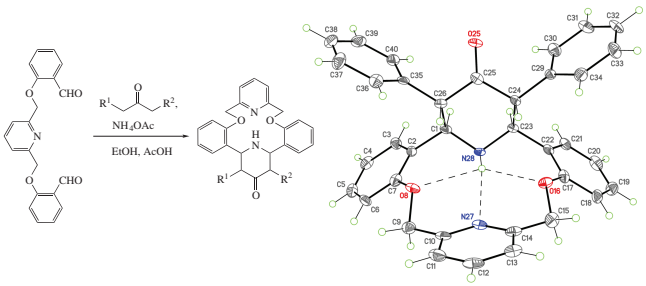
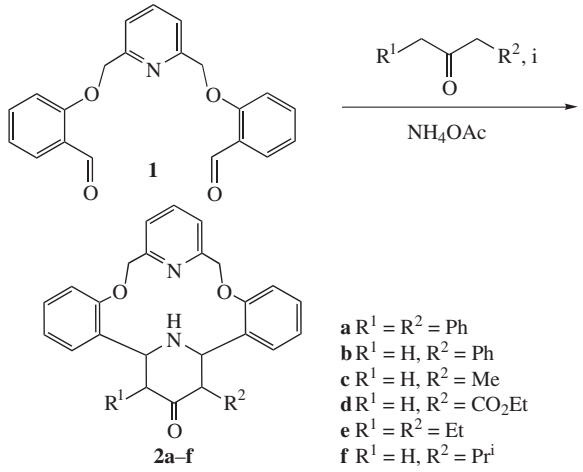
Six novel phane-structured diazacrowns containing γ-piperidone and pyridine rings were synthesized from podand 2,6-bis(2-formylphenoxymethyl)pyridine, with the γ-piperidone moiety having been constructed in the course of its domino condensation with simple ketones and ammonium acetate. The compounds were tested *in vitro* for antimicrobial and cytotoxic activity against four human cancer cell lines (Hep-G2, RD, MCF-7, Lu-1) and the Vero cell line. X-Ray structure study of one representative compound revealed its *rac*-1*RS*,23*SR*,24*RS*,26*SR* configuration.



Keywords: diazacrownophane, γ-piperidone, pyridine, cytotoxicity, antimicrobial, domino condensation.

Nowadays, many crownophanes with diversifying structure containing heterosubunit were synthesized,[1] and their chemical transformations were studied.[2,3] These azacrownophanes not only would form sustainable complexes with metal ions, but also express high cytotoxicity against several human cancer cell lines such as Hep-G2 (human hepatocellular carcinoma), Lu-1 (human lung adenocarcinoma), RD (human rhabdomyosarcoma), MCF-7 (human breast adenocarcinoma), and HeLa (human cervical cancer cells).[1(c),(e),4] Based on this successful study, we herein have obtained a new group of azacrowns containing pyridine and γ-piperidone rings (Scheme 1).



Scheme 1 *Reagents and conditions*: i, AcOH, EtOH, 60 °C, 11–20 h.

Podand 2,6-bis(2-formylphenoxymethyl)pyridine **1** was synthesized by a known method.[5] Its further multicomponent condensation with ketones and ammonium acetate taken in the ratio of 1:1:10, respectively, gave novel macroheterocycles **2a–f** containing both γ-piperidone and pyridine rings.[†] Their structures were determined by IR, ^{1}H and ^{13}C NMR spectroscopy, HRMS spectrometry and X-ray analysis (for details, see Online Supplementary Materials).

Compound **2a** was characterized by single-crystal X-ray diffraction study[‡] (Figure 1 and Online Supplementary

[†] *General procedure for the synthesis of diazacrowns* **2a–f**. 2,6-Bis(2-formylphenoxymethyl)pyridine **1** (0.25 g, 0.72 mmol) was added to a solution of ketone (1.1 mmol) and ammonium acetate (0.56 g, 7.2 mmol) in EtOH (20 ml) and AcOH (1 ml). The mixture was stirred at 60 °C (TLC monitoring). After 11–20 h, the solvent was removed at reduced pressure. Distilled water (30 ml) was added to the residue, temperature was brought to 25 °C, the mixture was neutralized with Na_2CO_3 and extracted with CH_2Cl_2 (3 × 30 ml). The combined extracts were washed with brine, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was subjected to column chromatography with an elution hexane/EtOAc = 1:1 (650 ml), the proper fractions were evaporated, and the residue was recrystallized from CH_2Cl_2 to obtain pure product **2**. For details and characterization, see Online Supplementary Materials.

[‡] The crystal of **2a**·2$CHCl_3$ ($C_{38}H_{32}N_2O_3Cl_6$, M = 777.36) is monoclinic, space group $P2_1/n$, at T = 120 K: a = 10.9906(16) Å, b = 19.149(3) Å, c = 34.090(5) Å, β = 96.159(2)°, V = 7133.1(18) Å^3, Z = 8, d_{calc} = = 1.448 g cm^{-3}, $F(000)$ = 3200, μ = 0.523 mm^{-1}. X-ray diffraction data (46553 reflections, 10332 independent reflections, R_{int} = 0.064) were collected on a Bruker APEX-II CCD diffractometer [λ(MoK_α)-radiation, graphite monochromator, ω and φ scanning mode, 2θ = 51.0°] and

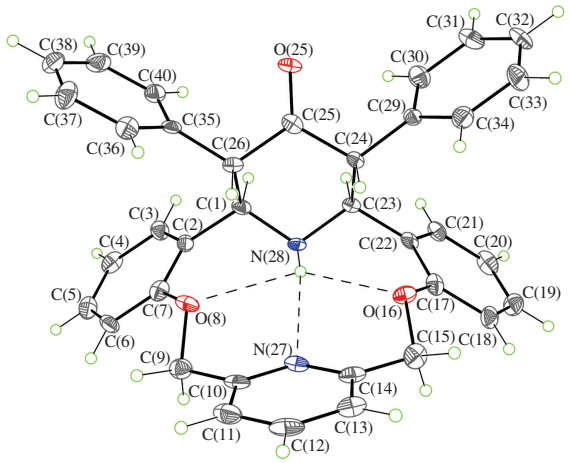


Figure 1 Molecular structure of compound **2a** (one of the two crystallographically independent molecules is presented only). The intramolecular N–H···O and N–H···N hydrogen bonds are depicted by dashed lines.

Materials). It crystallizes in the monoclinic space group $P2_1/n$ with two crystallographically independent molecules in the asymmetric unit. These two independent molecules adopt very close geometries and, mainly, are distinguished by the twist angle of one of the two phenyl substituents (C29–C34) relative to the parent piperidinone ring (Figure S2 in Online Supplementary Materials).

Overall, the molecule of **2a** possesses the idealized C_s (m) intrinsic symmetry. However, in the crystal, the geometry of **2a** is slightly distinguished from the idealized one due to the crystal packing effects. Moreover, compound **2a** crystallizes as a chloroform disolvate, *i.e.* **2a** · $2CHCl_3$. The molecule of **2a** comprises a fused pentacyclic system containing the central diaza-14-crown-2-ether macrocycle as well as the piperidinone, pyridine and two benzene rings. The diaza-14-crown-2-ether ring adopts a bowl conformation, which is stabilized by the two N–H···O and one N–H···N intramolecular hydrogen bonds (Table S1, Online Supplementary Materials). The configuration of the C(7)–O(8)–C(9)–C(10)–N(27)–C(14)–C(15)–O(16)–C(17) azapolyether chain is t–g^-–t–t–g^+–t (t = *trans*, 180°; g = *gauche*, ±60°).The piperidinone ring has a distorted (flattened from a side of the carbonyl group) *chair* conformation. The two phenyl substituents occupy the sterically more favorable equatorial positions. The dihedral angles between the basal C(1)/C(23)/C(24)/C(26) plane of the piperidinone ring and planes of the two benzene rings fused to the diaza-14-crown-2-ether moiety are 74.0(3) and 70.9(3)°. The N(28) nitrogen atom has a trigonally pyramidal configuration.

The structure of **2a** possesses four asymmetric centers at the C(1), C(23), C(24), and C(26) carbon atoms and can have potentially sixteen diastereomers. The crystal of **2a** is racemic and consists of enantiomeric pair of a single diastereomer with the *rac*-1*RS*,23*SR*,24*RS*,26*SR* relative configuration of the chiral centers. In the crystal, the molecules of **2a** form hydrogen-bonded associates with chloroform solvate molecules in the 1:2 ratio by the intermolecular C–H···O and C–H···N hydrogen bonds (Table S2, Online Supplementary Materials). The H-bonded associates are packed in stacks along the crystallographic *a* axis (Figure S3).

New compounds **2a–f** were tested *in vitro* for their cytotoxic activity against human cancer cell lines such as Hep-G2, Lu-1, RD, MCF-7 and on the Vero cell line (Table 1). The results showed that compound **2d** inhibited Hep-G2, Lu-1, RD and MCF-7 cell lines. In particular, the viability of the Lu-1 and RD cell lines decreased to 0% when incubated with 10 μg ml^{-1} (21.8 × 10^{-3} μM) of **2d**. The synthesized diazacrown **2d** was selected for determination of cytotoxicity parameter IC_{50}. It inhibited Hep-G2, Lu-1, RD and MCF-7 cell lines with IC_{50} values of 6.36 μg ml^{-1} (13.8 × 10^{-3} μM), 2.93 μg ml^{-1} (6.3 × 10^{-3} μM), 2.09 μg ml^{-1} (4.6 × 10^{-3} μM), 5.89 μg ml^{-1} (12.8 × 10^{-3} μM), respectively. In addition, compound **2d** was tested for cytotoxicity on the normal African green monkey kidney cell line (Vero cell line) and the result was negative (see Table 1).

Diazacrowns **2a–f** were also assessed for the antimicrobial activity against *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 10145), *Bacillus subtillis* (ATCC 6633), *Staphylococcus aureus* subsp. *aureus* (ATCC 25923), *Aspergillus niger* (ATCC 6275), *Fusarium oxysporum* (ATCC 7601), *Saccharomyces cerevisiae* (VTCC–Y–62) and *Candida albicans* (ATCC 10231). The initial concentration for each compound was 50 μg ml^{-1}, and the samples were diluted stepwise until positive signal of antimicrobial activity disappeared. The assessment showed that compounds **2b**,**c** had an antimicrobial activity on the *Gram*-positive bacteria *Bacillus subtillis* (ATCC 6633) and on the fungi *Aspergillus niger* (ATCC 6275) with the MIC = 100 μg ml^{-1} (see Table S1, Online Supplementary Materials).

Table 1 Cytotoxicity tests performed on compounds **2a–f** (10 μg ml^{-1}) in four cancer cell lines and Vero cell.

Compound	Cell line, cell survival (%)					Result
	Hep-G2	Lu-1	RD	MCF-7	Vero	
DMSO	100	100	100	100	100	Negative
Ellipticine	1.57±1.03	2.32±0.95	1.56±0.63	3.26±1.11	32.07±1.23	Positive
2a	96.97±1.68	98.36±0.52	85.07±1.91	99.45±0.12	99.13±0.85	Negative
2b	81.58±2.76	97.63±0.88	90.33±3.02	98.85±0.52	88.23±2.52	Negative
2c	88.47±1.67	96.42±2.51	92.79±2.55	98.72±0.36	74.67±0.95	Negative
2d	0.60±0.33	0	0	15.39±1.52	63.87±1.02	Positive
2e	95.55±1.84	99.95±0.05	97.01±1.37	98.87±0.80	87.28±2.41	Negative
2f	99.24±0.44	98.95±1.03	95.57±2.17	99.07±0.59	99.88±0.12	Negative

corrected for absorption using the SADABS program.[6] The structure was determined by direct methods and refined by full-matrix least squares technique on F^2 with anisotropic displacement parameters for non-hydrogen atoms. The crystal was found to contain four solvate chloroform molecules in the asymmetric unit. The hydrogen atoms of the NH-groups were localized in the difference-Fourier map and refined isotropically with fixed displacement parameters [U_{iso}(H) = 1.2U_{eq}(N)]. The other hydrogen atoms were placed in calculated positions and refined within riding model with fixed isotropic displacement parameters [U_{iso}(H) = 1.5U_{eq}(C) for the methyl groups and 1.2U_{eq}(C) for the other groups]. The final divergence factors were R_1 = 0.107 for 8144 independent reflections with $I > 2\sigma(I)$ and wR_2 = 0.252 for all independent reflections, S = 1.064. The calculations were carried out using the SHELXTL program.[7]

CCDC 2006963 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Center *via* https://www.ccdc.cam.ac.uk.

Table 2 Results of IC_{50} (μg ml^{-1}) tests of compound **2d**.

Compound	Cell line				
	Hep-G2	Lu-1	RD	MCF-7	Vero
Ellipticine	0.33	0.45	0.27	0.39	3.21
2d	6.36	2.93	2.09	5.89	–

In conclusion, six representatives of novel phane-structured diazacrowns were synthesized by a simple one-pot reaction. Their structure and configuration were confirmed by NMR, MS and X-ray diffraction analysis. These compounds were tested *in vitro* against the Human cancer and Vero cell lines and for antimicrobial activity. Among them, compound **2d** showed the best cytotoxicity against four cancer cell lines Hep-G2, Lu-1, RD and MCF-7 and it was not active on the Vero cell line.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2020.11.021.

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