Crystal Growth, Single Crystal Structure, and Biological Activity of Thiazolo-Pyridine Dicarboxylic Acid Derivatives

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1. INTRODUCTION

Ring-fused 2-pyridone structural fragment can be found in many natural products and biologically active compounds, and its derivatives have shown potential as anticancer drugs, antibacterial agents, or protein–protein interaction inhibitors. Recently, ring-fused 2-pyridones started to attract much attention as easily accessible molecular fluorophores for application in luminescent materials. Such fluorescent compounds can be easily prepared by condensation of citric acid with α-heteroatom-containing β-aminos. For example, multistep condensation of citric acid and L-cysteine leads to formation of the fluorescent derivative 5-oxo-2,3-dihydro-5H-[1,3]thiazolo[3,2-a]pyridine-3,7-dicarboxylic acid (TPDCA / C9H7NO5S), which was identified as a main source of molecular fluorescence in luminescent gels, biodegradable polymers, and carbon dots or applied in a low-cost material for chloride sensing in the diagnosis of cystic fibrosis. Bicyclic 2-pyridone derivatives, termed pilicides, are compounds that inhibit the formation of virulence-associated organelles termed pili. Pilicides are also considered a promising alternative or complementary therapeutic to the monoclonal antibodies in cancer therapy. In the study published by Horvath et al. the authors demonstrated that ring-fused 2-pyridones triggered fibrilization of a key protein in Parkinson’s disease, α-synuclein. In a recent paper by Kulén et al., the methyl sulfonamide substituents were shown to enhance the pharmacokinetic properties of bicyclic 2-pyridone-based Chlamydia trachomatis inhibitors.

In this study, using a single crystal X-ray diffraction method, we investigated the crystal structures of four novel derivatives with general formulas 2(C9H7NO5S)·H2O, (C9H7NO5S)Na(PO2H2), and (C9H5NO5S)·(NH4)2(H2O) prepared by combining TPDCA with water, N-methyl-2-pyrrolidone (NMP), Na(PO2H2), and ammonia solution, respectively. Antibacterial and cytotoxic properties of the novel compounds were also evaluated.
Table 1. Experimental Details

| parameter       | (1)                          | (2)                          | (3)                          | (4)                          |
|-----------------|------------------------------|------------------------------|------------------------------|------------------------------|
| crystal data    |                              |                              |                              |                              |
| chemical formula| 2(C$_6$H$_7$NO$_5$S)H$_2$O    | (C$_6$H$_7$NO$_5$S)$_2$C$_5$H$_9$NO | (C$_6$H$_7$NO$_5$S)Na(PO$_2$H$_2$) | C$_6$H$_7$NO$_5$S(NH$_4$)$_2$H$_2$O |
| $M_r$           | 500.4                        | 340.4                        | 329.2                        | 293.3                        |
| crystal system, space group | monoclinic, P2$_1$   | monoclinic, P2$_1$/c         | triclinic, P$\bar{1}$        | triclinic, P$\bar{1}$       |
| temperature (K) | 293                          | 293                          | 150                          | 293                          |
| $a$, $b$, $c$ (Å) | 6.2294 (8), 9.8153 (16), 17.133 (3) | 14.7840 (9), 6.8291 (4), 16.0750 (8) | 6.2307 (3), 9.0432 (4), 11.7429 (5) | 97.642 (2), 91.975 (2), 103.694 (2) |
| $\alpha$, $\beta$, $\gamma$ (°) | 90, 92.880 (5), 90 | 90, 106.460 (2), 90 | 97.642 (2), 91.975 (2), 103.694 (2) | 97.642 (2), 91.975 (2), 103.694 (2) |
| $V$ (Å$^3$)     | 1046.2 (3)                   | 1556.44 (15)                 | 633.64 (5)                   | 624.01 (6)                   |
| $Z$             | 2                            | Mo $\text{K}a$               | Mo $\text{K}a$               | Mo $\text{K}a$               |
| radiation type  |                               |                               |                               |                               |
| $\mu$ (mm$^{-1}$)| 0.32                         | 0.24                         | 0.45                         | 0.29                         |
| crystal size (mm)| 0.18 × 0.07 × 0.02           | 0.24 × 0.09 × 0.08           | 0.16 × 0.1 × 0.08            | 0.22 × 0.08 × 0.05           |

Data collection

| diffractometer  | D8 Venture diffractometer     | D8 Venture diffractometer     | D8 Venture diffractometer     | D8 Venture diffractometer     |
|-----------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|
| absorption correction | Gaussian processed with Jana2006 | Gaussian processed with Jana2006 | Gaussian processed with SADABS | Gaussian processed with SADABS |
| $T_{min}$, $T_{max}$ | 0.96, 0.99                    | 0.967, 0.985                  | 0.704, 0.749                  | 0.92, 0.98                    |
| no. of measured, and independent reflections | 21,038, 4325                  | 21,605, 3314                  | 8781, 3842                    | 9883, 3089                    |
| no. of observed reflections | 4212 [1 > 2$\sigma$(I)]    | 2606 [1 > 2$\sigma$(I)]      | 3689 [1 > 2$\sigma$(I)]      | 2144 [1 > 3$\sigma$(I)]      |
| $R_{int}$          | 0.045                         | 0.057                         | 0.02                         | 0.033                         |
| $\langle \sin \theta/\lambda \rangle_{max}$ (Å$^{-1}$) | 0.628                         | 0.635                         | 0.715                         | 0.667                         |

Refinement

| $R(F^2 > 2\sigma(F^2))$, wR(F$^2$), S | 0.019, 0.077, 1.09 | 0.042, 0.132, 1.14 | 0.043, 0.108, 1.19 | 0.035, 0.112, 1.12 |
| no. of reflections | 4325                        | 3314                        | 3842                        | 3089                        |
| no. of parameters | 318                         | 218                         | 196                         | 185                         |
| no. of restraints | 7                            | 2                           | 2                           | 5                           |
| $\Delta$fl/\sigma_{fl}$ ($e$ Å$^{-3}$) | 0.13, −0.12 | 0.19, −0.16 | 0.37, −0.23 | 0.30, −0.19 |

*The H atoms were treated by a mixture of independent and constrained refinement.*

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The chemical formula is $2(C_9H_7NO_5S)H_2O$. With anisotropic ADPs, the residual factors converged to the value $R(F) = 0.0663$ and $\text{wR}(F^2) = 0.1497$ ($\text{GOF} = 1.54$) for 298 refined parameters. The CH hydrogen atoms were placed in calculated positions with $U_{iso}(H) = 1.2 \ U_{eq}(C)$, whereas the OH hydrogen atoms were determined from the Fourier difference maps with $U_{iso}(H) = 1.2 \ U_{eq}(O)$. The C–H and COO–H distances were constrained/restrained to 0.96 Å. This led to the final chemical formula $(C_9H_7NO_5S)C_5H_9NO$. By refining the extinction parameter, the residual factors converged to the values given in Table 1. The refined atomic positions and anisotropic ADPs are given in Tables S3 and S4, respectively.

2.1.3. Structure Refinement of Compound (3). The structure was determined using the space group $P\bar{1}$. The heaviest atoms were located using the superprol program. With isotropic ADPs, the residual factors converged to the value $R(F) = 0.1693$ and $\text{wR}(F^2) = 0.2754$ ($\text{GOF} = 3.06$) for 82 refined parameters and 3689 observed reflections. At this stage of the refinement, the chemical formula is $(C_9H_7NO_5S)Na(PO_2H_2)$. With anisotropic ADPs, the residual factors converged to the value $R(F) = 0.063$ and $\text{wR}(F^2) = 0.1807$ ($\text{GOF} = 2.04$) for 182 refined parameters. The CH hydrogen atoms were placed in calculated positions with $U_{iso}(H) = 1.2 \ U_{eq}(C)$, whereas the OH hydrogen atoms were determined from the Fourier difference maps with $U_{iso}(H) = 1.2 \ U_{eq}(O)$. The C–H and COO–H distances were constrained/restrained to 0.96 Å. This led to the final chemical formula $(C_9H_7NO_5S)Na(PO_2H_2)$. By refining the extinction parameter, the residual factors converged to the values given in Table 1. The refined atomic positions and anisotropic ADPs are given in Tables S5 and S6, respectively.

2.1.4. Structure Refinement of Compound (4). The structure was determined using the space group $P\bar{1}$. The C, O, N, and S atoms were located using the superprol program.
With isotropic ADPs, the residual factors converged to the value $R(F) = 0.1149$ and $wR(F^2) = 0.2934$ ($GOF = 3.48$) for 77 refined parameters and 3089 observed reflections. At this stage of the refinement, the chemical formula is $(C_9H_7NO_5S)_2(NH_4)_2(H_2O)$. With anisotropic ADPs, the residual factors converged to the value $R(F) = 0.067$ and $wR(F^2) = 0.1983$ ($GOF = 2.41$) for 172 refined parameters. The CH hydrogen atoms were placed in calculated positions with $U_{iso}(H) = 1.2$ $U_{eq}(C)$, whereas the OH and NH hydrogen atoms were determined from the Fourier difference maps with $U_{iso}(H) = 1.2$ $U_{eq}(O)$ or $N$). The C–H, COO–H and N–H distances were set to 0.96, 0.96 ± σ and 0.87 ± σ Å, respectively. Two restraints were applied to the water molecule. The O–H distances and the H–O–H angle were set to 0.96 Å and 104.45°, respectively. This led to the final chemical formula $(C_9H_7NO_5S)_2(NH_4)_2(H_2O)$. By refining the extinction parameter, the residual factors converged to the values given in Table 1. The refined atomic positions and anisotropic ADPs are given in Tables S7 and S8, respectively. Further details on the refinement, the chemical formula is (C9H5NO5S)(NH4)2(H2O). By re

down the $a$ axis, is given in Figure 2a. In L1, the five-membered rings have a substantially flattened envelope conformation with a dihedral angle of 21.38° between the C2C1S1 fragment and the C2N1C7S1 plane (Figure 1). The puckering parameters $Q = 0.226 (2)$ Å and $\varphi = 43.2 (5)^\circ$ and the pseudorotation parameters $P = 212.6 (3)^\circ$ and $\text{Tau}(M) = 22.2 (1)^\circ$ for the reference bond S1–C1 suggest an envelope conformation on C1. The interatomic S1–C1 and S1–C7 distances of 1.810 (3) Å and 1.734 (2) Å are typical of the S-Csp² and S-Csp³ bonds, respectively. The bicyclic S1NIC2–C8 system can be regarded as planar since the dihedral angle between the six- and five-membered rings is only 4.01°. The carboxyl group C8O2O3H is almost co-planar with the bicyclic fragment with a dihedral angle of 6.01°. The carboxyl group C9O4O5H is almost perpendicular to the bicyclic fragment with a dihedral angle of 74.58°.

Comparing to L1, in L2, the five-membered rings have a less-flattened envelope conformation with a dihedral angle of 31.59° between the C11C10S2 fragment and the C11N2C16S2 plane (Figure 1). The puckering parameters $Q = 0.3323 (18)$ Å and $\varphi = 216.9 (3)^\circ$ and the pseudorotation parameters $P = 25.7 (2)^\circ$ and $\text{Tau}(M) = 31.6 (1)^\circ$ for the reference bond S2–C10 suggest an envelope conformation on C10. The bicyclic S2N2C11–C17 fragment can be regarded as approximately planar since the dihedral angle between the six- and five-membered rings is 6.02°. The carboxyl group C17O7O8H is rotated by 21.54° from its attached ring. The carboxyl group C18O9O10H is almost perpendicular to the thiazolopyridine group with a dihedral angle of 80.68°. This is very similar to the dihedral angle of 83° observed in the unsolvated TPDCA structure. The crystal structure of (1) is stabilized by moderate and weak hydrogen bonds. Selected bond lengths and angles are given in Table 2. For clarity reasons, different colors have been used to distinguish between the different types of hydrogen bonds. The blue lines correspond to the O–H···O hydrogen bonds involving water molecules and carboxyl H and O atoms and amide keto O atoms of neighboring molecules (Figure 2c). The red and green lines correspond to the O–H···O and C–H···O hydrogen bonds, respectively, that do not involve water molecules (Figure 2d). These hydrogen bonds link the different molecules to form a 3D framework. Furthermore, symmetry equivalent L2 ligands interact via C–O···–C (orange) interactions [O9-Cg5i = 3.5427 (18) Å, C18-O9-Cg5 angle = 146.35 (12)^°, Cg5 is the centroid of the six-membered ring (N2C12-C16), symmetry code (i): $−x,−1/2+y,−z$] (Figure 2b). The additional π–π stacking interactions between L1 and L2 ligands are too weak to be taken in consideration [Cg2-Cg5i = 5.1111 (15) Å, interplanar distance = 3.3750 (8) Å, slippage = 4.0738 (8) Å, $\alpha = 10.4812 (7)^\circ$, $\beta = 17.45 (10)^\circ$, $\gamma = 37.15 (2)^\circ$, Cg2 and Cg5 are the centroids of the six-membered rings in the ligands L1 and L2, respectively, symmetry code (ii): $1+x,y,z$] (Figure 2b).

The structure of (2) contains bent hydrogen bonds. Selected bond lengths and angles are given in Table 3. Each TPDCA molecule is interconnected to two other TPDCA molecules and one NMP molecule through COO–H···O=C hydrogen bonds, forming one-dimensional infinite chains along the [001] direction (see blue and green dashed line in Figure 4 and Figure S1). Furthermore, the NMP molecules of neighboring chains interact through C=C–O···–C interactions [C10-O6–Cg2i = 3.665 (2) Å, Cg4ii is the centroid of the NMP ring (N2C10-C13), symmetry code (ii): $1−x,−1/2+y,1/2−z$] (see brown dashed line in Figure 4). The offset π–π interactions between TPDCA molecules [Cg3–Cg3i = 4.192 (1) Å, interplanar distance = 3.502 (1) Å, slippage = 2.304 (1) Å, $\alpha = 0.03 (7)^\circ$, Cg3 is the centroid of...
the six-membered ring (N1C3-C7), symmetry code (i): 2 - x, 1 - y, -z] and between the NMP molecules [Cg4-Cg5 = 5.546 (2) Å, interplanar distance = 3.875 (1) Å, slippage = 3.958 (1) Å, \( \alpha = 0.03 \) (7)\(^\circ\), Cg4 is the centroid of the NMP ring (N2C10-C13), symmetry code (i): 1 - x, 2 - y, -z] were not taken into consideration since they are too weak (see the yellow and pink dashed lines in Figure S1).

Table 2. List of Detected Hydrogen Bonds in (1)

| donor | hydrogen | acceptor | D–H distance | H···A distance | D–A distance | D–H···A angle |
|-------|----------|----------|---------------|----------------|---------------|---------------|
| C10   | H1C10    | O6       | 0.96          | 2.42           | 3.350(3)      | 163.58        |
| C13   | H1C13    | O9       | 0.96          | 2.48           | 3.316(3)      | 144.84        |
| C15   | H1C15    | O4       | 0.96          | 2.45           | 3.391(3)      | 168.29        |
| O3    | H1O3     | O9       | 0.96(2)       | 1.80(2)        | 2.716(3)      | 160(3)        |
| O5    | H1O5     | O1       | 0.96(18)      | 1.657(17)      | 2.610(2)      | 172(2)        |
| O10   | H1O10    | O6       | 0.96(17)      | 1.585(19)      | 2.521(2)      | 163�(17)      |
| O8    | H1O8     | O11      | 0.96(19)      | 1.64(2)        | 2.592(2)      | 169(2)        |
| O11   | H1O11    | O1       | 0.96(19)      | 1.927(18)      | 2.834(2)      | 156.6(17)     |
| O11   | H2O11    | O7       | 0.96(16)      | 1.991(14)      | 2.933(3)      | 166.6(18)     |
2.2.3. Crystal Structure of Compound (3). Compound (3) crystallizes in the triclinic space group P-1 with one TPDCA and one sodium hypophosphite salt molecules in the asymmetric unit (Figure 5). The structure is layered parallel to the (010) plane (Figure 6). Within a single layer, the TPDCA molecules are linked through sodium bridges in addition to the existence of offset π–π interactions (see pink dashed lines in Figure 6). Each molecule of (3) is linked to two other molecules through one weak C=H−O=C and one moderate COO−H···O−Na hydrogen bonds (see blue dashed lines in Figure 7). Two additional intramolecular hydrogen bonds were also observed (see green dashed lines in Figure 7). Selected bond lengths and angles are given in Table 4. N2H4 interacts with four different TPDCA molecules through moderate N−H···O hydrogen bonds (see green dashed lines in Figure 9b). These two types of hydrogen bonds allow the formation of layers parallel to the (001) plane. The π−π interactions between the layers are too weak to be taken in consideration, and the structure is bi-dimensional \([Cg2-Cg2i = 4.9487 (10) \text{Å}, \text{interplanar distance} = 3.351 (1) \text{Å}, \text{slippage} = 3.642 (1) \text{Å}, \alpha = 0^\circ, \text{Cg} \text{ is the centroid of the six-membered ring (N1C3-C7), symmetry code (i): } 1 - x, 1 - y, 2 - z \text{ (Figure 6)}] \) exist only within a single layer and not between the layers, which confirms that the structure is bi-dimensional.

2.2.4. Crystal Structure of Compound (4). Compound (4) crystallizes in the triclinic space group P1 with two ammonium cations, one TPDCA, and one water molecule in the asymmetric unit (Figure 8). Selected hydrogen bond lengths and angles are given in Table 5. N2H4 interacts with four different TPDCA molecules (see blue dashed lines in Figure 9a), whereas N3H4 interacts with three TPDCA molecules through moderate N−H···O hydrogen bonds (see green dashed lines in Figure 9b). The water molecule also interacts with two different TPDCA molecules through moderate O−H···O hydrogen bonds (see red dashed lines in Figure 9b). These two types of hydrogen bonds allow the formation of layers parallel to the (001) plane. The π−π interactions between the layers are too weak to be taken in consideration, and the structure is bi-dimensional \([Cg2-Cg2i = 4.9487 (10) \text{Å}, \text{interplanar distance} = 3.351 (1) \text{Å}, \text{slippage} = 3.642 (1) \text{Å}, \alpha = 0^\circ, \text{Cg} \text{ is the centroid of the six-membered ring (N1C3-C7), symmetry code (i): } 1 - x, 1 - y, 2 - z \text{ (Figure 6)}] \) exist only within a single layer and not between the layers, which confirms that the structure is bi-dimensional.

2.2.5. Comparison of TPDCA Ligands in C_{9}H_{7}NO_{5}S, (1), (2), (3), and (4). The structures of the TPDCA Ligands in the four compounds are very similar (see details in Table 6 and Figure S2). The five-membered rings have a substantially flattened envelope conformation with dihedral angles between the C2C1S1 fragments and the C2N1C7S1 planes varying from 21.38 to 37.33°. The interatomic S1-C1 and S1-C7 distances that vary from 1.810 (3) Å to 1.821 (2) Å and from 1.734 (2) Å to 1.750 (2) Å are typical of the S-Csp^3 and S-Csp^2 bonds, respectively,11,22,23,26 The bond configurations at the N1 atoms are planar trigonal with the sum of the N1 bond angles almost 360°. The bicyclic S1N1C2-C8 systems can be regarded as planar since the dihedral angles between the six- and five-membered rings are less than 11°. The carboxyl groups C8O2O3 are slightly twisted from the bicyclic fragment with dihedral angles between 4.45 and 21.54°. The carboxyl groups C8O2O3 are slightly twisted from the bicyclic fragment with dihedral angles between 4.45 and 21.54°.

Table 3. List of Detected Hydrogen Bonds in (2)

| donor | hydrogen | acceptor | D−H distance | H−A distance | D−A distance | D−H−A angle |
|-------|----------|----------|---------------|--------------|--------------|--------------|
| O2    | H1O2     | O1       | 0.96(2)       | 1.67(2)      | 2.603(2)     | 164(2)       |
| O5    | H1O5     | O6       | 0.956(18)     | 1.614(19)    | 2.555(3)     | 167(2)       |

Figure 5. View of the molecular structure of (3), showing 70% probability displacement ellipsoids. H atoms are shown as small spheres of arbitrary radii.

Figure 6. View of the layered structure of (3) along the a axis. The pink dashed lines correspond to π−π interactions between the six-membered rings. Cg2 is the centroid of the six-membered ring.

Figure 7. View of the hydrogen bonds in (3). The blue and green dashed lines correspond to inter- and intramolecular interactions, respectively.

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been approved and are used in cancer chemotherapy today.28 It has low solubility, so numerous analogues have been developed and two of them, topotecan and irinotecan, have been helpful in removing infectious bacteria from the wastewater thus can be widely utilized for wastewater treatments. Out of three tested compounds, only (1) showed some cytotoxicity against leukemia HL-60 cells.

### 3. CONCLUSIONS

Four new TPDCA derivatives: 2(C₉H₇NO₅S)H₂O (1), (C₉H₇NO₅S)C₅H₉NO (2), (C₉H₇NO₅S)Na(PO₂H₂) (3) and (C₉H₇NO₅S)(NH₄)₂(H₂O) (4) were synthesized, and their crystal structures were determined. Different types of hydrogen bonds were observed in these compounds, which led to a three-dimensional network in (1), an infinite chain along [001] in (2), an infinite two dimensional network in the (010) plane in (3), and an infinite two dimensional network in the (001) plane in (4). Furthermore, very weak π–π interactions between the six-membered rings were observed in compound (3) and C=O···π interactions were observed in (1) and (2). The antimicrobial test showed significant activity of derivatives (1) and (3) against E. coli and S. aureus. These compounds can be helpful in removing infectious bacteria from the wastewater thus can be widely utilized for wastewater treatments. Out of three tested compounds, only (1) showed some cytotoxicity against leukemia HL-60 cells.

### 4. EXPERIMENTAL SECTION

#### 4.1. Synthetic Procedures

#### 4.1.1. Synthesis of 2-(C₉H₇NO₅S)H₂O (1). The unsolvated TPDCA ligand used for the synthesis was obtained following the procedure reported,17 whereas the crystals of the solvated TPDCA molecule were obtained during the preparation of the TPDCA-Co(HPO₄)₂ compound. TPDCA (0.12 g), H₃PO₃ (0.082 g), and Co(CH₃COO)₂·4H₂O (0.1245 g) were mixed together in 10 mL of distilled water. The mixture was then stirred for 1 h and left under the fume hood at room temperature. After 1

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**Table 4. List of Detected Hydrogen Bonds in (3)**

| donor | hydrogen | acceptor | D–H distance | H–A distance | D–A distance | D–H–A angle |
|-------|----------|----------|--------------|--------------|--------------|--------------|
| O3    | H1O3     | O7       | 0.960(18)    | 1.602(17)    | 2.539(18)    | 164.3(16)    |
| O5    | H1O5     | O6       | 0.960(13)    | 1.554(12)    | 2.510(16)    | 174.1(18)    |
| C1    | H2C1     | O1       | 0.96         | 2.35         | 3.221(2)     | 150.84       |
| C4    | H1C4     | O3       | 0.96         | 2.34         | 2.688(2)     | 100.80       |

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**Table 5. List of Detected Hydrogen Bonds in (4)**

| donor | hydrogen | acceptor | D–H distance | H–A distance | D–A distance | D–H–A angle |
|-------|----------|----------|--------------|--------------|--------------|--------------|
| N3    | H1N3     | O4       | 0.870(11)    | 1.946(10)    | 2.803(3)     | 168.1(8)     |
| N3    | H2N3     | O6       | 0.87         | 1.91         | 2.781(2)     | 174.69       |
| N3    | H3N3     | O2       | 0.87         | 2.02         | 2.8860(19)   | 170.84       |
| N3    | H4N3     | O3       | 0.87         | 2.17         | 3.039(2)     | 174.06       |
| N2    | H1N2     | O3       | 0.870(10)    | 2.210(10)    | 2.976(2)     | 146.7(7)     |
| N2    | H2N2     | O1       | 0.87         | 2.01         | 2.8612(18)   | 167.36       |
| N2    | H3N2     | O5       | 0.87         | 1.98         | 2.833(2)     | 165.90       |
| N2    | H4N2     | O1       | 0.87         | 1.98         | 2.828(2)     | 163.39       |
| O6    | H1O6     | O3       | 0.953(6)     | 1.790(7)     | 2.7261(19)   | 166.7(18)    |
| O6    | H2O6     | O5       | 0.953(15)    | 1.778(17)    | 2.680(2)     | 156.8(14)    |

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Unfortunately, cancer cells often develop resistance to chemotherapeutics and new synthetic compounds are being tested for their possible anticancer properties in order to find novel lead structures. In the present study, the cytotoxic activity of compounds (1–3) was evaluated in leukemia HL-60 cell line, using MTT assay. As shown in Figure 11, only compound (1) "(C₉H₇NO₅S)·H₂O" decreased the metabolic activity of HL-60 cells by 50% at 158.5 ± 12.5 μM concentration. This structure can be further modified in order to obtain more cytotoxic analogues.

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**Figure 8.** Molecular structure of (4) showing 70% probability displacement ellipsoids. H atoms are shown as small spheres of arbitrary radii.

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**Figure 10.** (a) and (b) the inhibition zones of (1) and (3) against Gram-positive S. aureus. These compounds can be helpful in removing infectious bacteria from the wastewater thus can be widely utilized for wastewater treatments. Out of three tested compounds, only (1) showed some cytotoxicity against leukemia HL-60 cells.

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**Figure 11.** The antimicrobial test showed significant activity of derivatives (1) and (3) against E. coli and S. aureus. These compounds can be helpful in removing infectious bacteria from the wastewater thus can be widely utilized for wastewater treatments. Out of three tested compounds, only (1) showed some cytotoxicity against leukemia HL-60 cells.
week, several yellow crystals were formed. The solution was then filtered and the crystals were washed with acetone. The single-crystal XRD analyses performed on seven different crystals indicated that all of them correspond to (1). This batch was used for biological tests. The filtered solution was also evaporated for one more week. This led to a mixture of naked eye distinguishable large yellow and orange needles of (1) (~80%) and TPDCA (~20%), respectively.

4.1.2. Synthesis of \((\text{C}_9\text{H}_7\text{NO}_5\text{S})\text{C}_5\text{H}_9\text{NO}\) (2). This compound was prepared using wet chemistry from stoichiometric mixtures of TPDCA (0.12 g) and sodium hypophosphite NaH\(_2\)PO\(_2\)H\(_2\)O (0.088 g, Aldrich, 99.99%). TPDCA was dissolved in 5 mL of N-Methyl-2-pyrrolidone (NMP) to

Table 6. Comparison of the Crystallographic Data of C\(_9\)H\(_7\)NO\(_5\)S, (1), (2), (3), and (4)

| compounds           | C\(_9\)H\(_7\)NO\(_5\)S (1) | ligand1 (2) | ligand1 (3) | ligand1 (4) | ligand1 (1) | ligand1 (1) |
|---------------------|-----------------------------|-------------|-------------|-------------|-------------|-------------|
| dihedral angle      | 37.33(1)                    | 36.86(3)    | 34.76(1)    | 31.28       | 21.38(2)    | 31.59(1)    |
| between C2C1S1 and C2N1C7S1 planes (°) | 0.3950(14) & 217.9(2) | 0.3870(16) & 214.8(2) | 0.3681(14) & 216.8(2) | 0.3304(16) & 214.9(3) | 0.226(2) & 43.2(5) | 0.3323(18) & 216.9(3) |
| puckering parameters Q (Å) & φ(°) of the 5-membered ring of TPDCA | 26.6(1) & 37.4(1) | 23.0(2) & 36.4(1) | 25.6(1) & 34.8(1) | 23.0(2) & 31.0(1) | 212.6(3) & 22.2(1) | 25.7(2) & 31.6(1) |
| pseudorotation parameters P (°) & Tau(M) (°) of the 5-membered ring of TPDCA | envelope on C1 | envelope on C1 | envelope on C1 | envelope on C1 | envelope on C1 | envelope on C1 |
| conformation of the 5-membered ring of TPDCA | 1.818(2) | 1.820(2) | 1.821(2) | 1.814(2) | 1.810(3) | 1.810(3) |
| distance S1-C1 (Å) | 1.816(2) | 1.742(2) | 1.750(2) | 1.7391(14) | 1.734(2) | 1.734(2) |
| S2-C10 (Å) | 1.812(3) | 1.812(3) | 1.812(3) | 1.812(3) | 1.812(3) | 1.812(3) |
| sum of the N1 bond angles in (°) | 359.45(1) | 359.97(2) | 359.86(1) | 359.56(15) | 359.73(3) | 359.83(3) |
| dihedral angle between the six- and five-membered rings (°) | 5.82(1) | 10.10(2) | 5.67(1) | 5.10(3) | 4.01(2) | 6.02(1) |
| dihedral angle between the carboxyl group C8O2O3 and the bicyclic fragment (°) | 10.32(2) | 4.45(1) | 12.5(3) | 8.65(1) | 6.01(1) | 21.54(3) |
| and the bicyclic fragment (°) | 82.99(1) | 79.73(1) | 78.592 | 86.74(3) | 74.58(2) | 80.68(1) |
| references | 11 | this work | this work | this work | this work | this work |

Figure 9. View of the hydrogen bonds in (4). (a, b) Blue, green, and red dashed lines correspond to the interactions involving N2H\(_4\), N3H\(_4\), and H\(_2\)O, respectively. (c) View along the a axis of the layers parallel to (001) plane. The pink dashed lines correspond to the distance Cg2-Cg2i between the six-membered rings of successive layers.
form the clear solution A, which was stirred at 50 °C for 30 min. NaH₂PO₄, H₂O was dissolved in 10 mL of H₂O forming the solution B. Then, solution B was added to solution A dropwise and left stirring at 50 °C for one additional hour. The resulting clear solution was left still at room temperature. After 1 week, few tiny yellow crystals were formed. The solution was then filtered, and the crystals were washed with acetone. The single-crystal XRD analyses performed on several crystals indicated that all of them correspond to (2). This batch was used for biological tests. The filtered solution was also evaporated for one more week. This led to a mixture of large yellow and orange needles of (2) (~90%) and TPDCA (~10%), respectively.

4.1.3. Synthesis of (C₉H₇NO₅S)Na(PO₂H₂)(3). This compound was prepared exactly following the procedure above. Only NMP was replaced by dimethylformamide (DMF). After 1 week, several large yellow crystals were formed. The solution was then filtered, and the crystals were washed with acetone. The single-crystal XRD analyses performed on several crystals indicated that all of them correspond to (3). This batch was used for biological tests. The filtered solution was also evaporated for one more week. This led to a mixture of large yellow and orange needles of (3) (~80%) and TPDCA (~20%), respectively.

4.1.4. Synthesis of (C₆H₄NO₅S(NH₂)₂(H₂O))(4). TPDCA (0.10 g) was dissolved in 0.2 mL of distilled water and 0.450 mL ammonia solution at 50 °C in a glass tube. Then, the water content was slowly evaporated to leave block crystals of (4) (72%) at the bottom of the tube.

4.2. Single-Crystal X-Ray Diffraction Measurements. Suitable (1), (2), (3), and (4) single crystals for X-ray diffraction were selected on the basis of the size and the sharpness of the diffraction spots. The data collections were carried out on a D8 Venture diffractometer using Mo Kα radiation. Data processing and all refinements were performed with the APEX3 and Jana2006 program package, respectively. For (1) and (2), Gaussian-type absorption corrections were applied and the shape was determined with the video microscope; however, for (3) and (4), multiscan absorption corrections using SADABS were applied. For data collection details, see Table 1.

4.3. Microorganisms and Inoculum Preparation. The antimicrobial properties of compounds (1) and (3) were evaluated using two types of bacteria (Gram-positive and Gram-negative). The bacterial strains used in the present study were isolated from the treated sewage effluent of Qatar foundation treatment plant and identified by using the 16S RNA ribosomal method (unpublished data). For inoculation preparation, a single bacterial colony was picked from nutrient agar and streaked on nutrient agar, transferred into 10 mL of nutrient broth and placed overnight in incubator shaker at 37 °C with a shaking speed of 100 rpm. The bacterial cell density was measured at an optical density (OD) of 600 nm using a spectrophotometer. Each inoculum prepared would contain approximately 10⁷ cfu/mL of bacteria. Bacterial sensitivity to compounds (1) and (3) is performed by employing the agar well diffusion method. Three millimeter-diameter holes were made in the agar plates using 50 mL disposable pipette, and different amounts varying from 5 to 20 mg of this material were placed carefully in the holes. Ampicillin (10 μg/mL) was used as a standard antibiotic. The plates were overlaid with a mixture of each bacteria with 2 mL of molten 1.5% (wt/vol) noble agar (Sigma-Aldrich) at proximately 37 °C. Finally, the plates were incubated at 37 °C for 24 h and the average diameter of the inhibition zone surrounding the holes was examined.

4.4. Cell Culture and Cell Metabolic Activity – MTT Assay. Caucasian promyelocytic leukemia (HL-60) was obtained from the European Collection of Cell Cultures (ECACC). Cells were maintained in an RPMI 1640 + Glutamax medium (Gibco/Life Technologies, Carlsbad, CA, USA), supplemented with 10% fetal bovine serum (FBS), streptomycin, and penicillin at 37 °C in a 5% CO₂-humidified atmosphere. The influence of the compounds on cell metabolic activity was investigated using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyl tetrazolium bromide) assay. In brief, HL-60, cells were seeded in 24-well plates (TPP Techno Plastic Products AG, Switzerland) at a concentration of 8 × 10⁵/mL and incubated for 20 h at 37 °C. The cells were treated with a range of concentrations of the compounds. Compound (1) was dissolved in DMSO, while compounds (2) and (3) were dissolved in water. Cells treated with a vehicle were used as a control. Then, the cells were incubated for an additional 48 h at 37 °C. MTT solution (100 μL; 5 mg/1 mL in PBS) was added to each well, followed by 1 h of incubation. The plates were centrifuged (3000 rpm, 5 min), and the supernatant was discarded. DMSO (1 mL) was added to each well to dissolve the formazan product. The absorbance was measured at 560 nm using a FlexStation 3 Multi-Mode spectrophotometer. Each inoculum prepared would contain approximately 10⁷ cfu/mL of bacteria. Bacterial sensitivity to compounds (1) and (3) was determined by the MTT assay.
Microplate Reader (Molecular Devices, LLC). Assays were performed twice in triplicate for each dose. Data analysis was performed using Prism 5.0 (GraphPad Software Inc., San Diego, CA, USA). Results from two independent experiments in triplicate were expressed as mean ± SEM.

■ ASSOCIATED CONTENT

* Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.0c01769.

Atomic positions and anisotropic displacement parameters (Tables S1-S8), interactions between TPDCA and NMP in compound (2) (Figure S1), comparison of TPDCA ligands in C₉H₇NO₅S, (1), (2), (3), and (4) (Figure S2), and synthesis and characterization processes for compounds 1–3 (Figure S3) (PDF)

Crystallographic data of compound (1) (CIF)

Crystallographic data of compound (2) (CIF)

Crystallographic data of compound (3) (CIF)

Crystallographic data of compound (4) (CIF)

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

TPDCA, 5-oxo-2,3-dihydro-5H-[1,3]-thiazolo[3,2-a]pyridine-3,7-dicarboxylic acid; (1), [rac-(3R)-3-(dihydroxymethyl)-5-hydroxy-3,5,6,7,8,8a-hexahydro-2H-thiazolo[3,2-a]pyridin-7-yl]methylendiol; [rac-(3S)-(3-(dihydroxymethyl)-5-hydroxy-3,5,6,7,8,8a-hexahydro-2H-thiazolo[3,2-a]pyridin-7-yl]-methanediohydroxide: 2(C₉H₇NO₅S)H₂O; (2), 1-methylypyrrolidin-2-ol; [rac-(3S)-5-hydroxy-7-(3-hydroxydioxiran-3-yl)-3,5,6,7,8,8a-hexahydro-2H-thiazolo[3,2-a]pyridin-3-yl]-methanediohydroxide: (C₉H₇NO₅S)Na(PO₂H₂); (3), trihydroxy-[4-(3S)-3,7-bis[hydroxyl(sodiooxy) methyl]-3,5,6,7,8,8a-hexahydro -2H-thiazolo[3,2-a]pyridin-7-yl]-methylendiohydroxide: (C₉H₇NO₅S)C₅H₉NO; NMP, 3,5,6,7,8,8a-hexahydro-2H-thiazolo[3,2-a]pyridin-7-yl)methyl-2-pyrrolidone; ADPs, atomic displacement parameters

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