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Polymorphic and solvate structures of ethyl ester and carboxylic acid derivatives of WIN 61893 analogue and their stability in solution†

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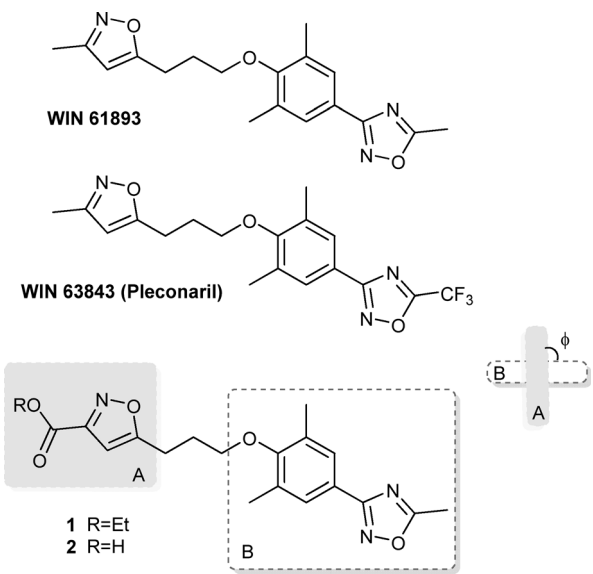
3-Ethyl ester- (**1**) and 3-carboxylic acid-isoxazole (**2**) derivatives of an antiviral drug analogue WIN 61893 were synthesized and characterized by X-ray crystallography and NMR spectroscopy. Crystallization experiments afforded two polymorphic structures for the ethyl ester derivative and two solvate structures for the carboxylic acid derivative based on their ability to form intermolecular hydrogen bonding interactions with the solvent molecules. The conformations of the derivatives depended greatly on the orientation of the planar isoxazole and phenyl-oxadiazole ring systems with respect to one another and were found to take up perpendicular, linear or tilted conformations. The carboxylic acid derivative was furthermore observed to undergo isoxazole ring cleavage by decarboxylation in DMSO solution and transforming into a β-keto nitrile ring-opening product over several days, whereas, the isoxazole ring of the ethyl ester derivative was not affected.

Introduction

Enteroviruses cause a variety of human illnesses related to common respiratory infections, rash or mild fever to serious or life-threatening infections including meningitis, myocarditis, encephalitis, and paralytic poliomyelitis.[1,2] Rhinoviruses in the enterovirus genus, in particular, are the predominant cause of the common cold in humans. Therefore, the development of antiviral drugs designed to inhibit or interfere with the viral replication process have been pursued over the past decades and still continues to be a current topic.[1,3,4]

One such family of potential antiviral drugs designed to target the early events (attachment, entry and uncoating) of viral replication are the "WIN compounds" named after the developer Sterling-Winthrop (Scheme 1).[1,3] The antiviral drug candidate development – after a few promising lead structures – led to WIN 63843 analogue or better known as Pleconaril, which showed a drastic decrease in the metabolic degradation of the molecule and a broad range of antiviral activity against enteroviruses in comparison to the other WIN analogues.[5,6] Structural studies,[7–10] of several WIN analogues including Pleconaril with human rhinoviruses (*e.g.* HRV14 and HRV16) showed the drug molecules to bind specifically into an interior hydrophobic pocket within the virus capsid causing a conformational change in the virus that eventually



Scheme 1 Chemical structures of WIN 61893, WIN 63843 (Pleconaril) and derivatives **1** and **2**.

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† Electronic supplementary information (ESI) available: Crystallographic data for **1**-form I, **1**-form II, **2**-EtOH and **2**-DMSO, respectively. Synthesis scheme, ^{1}H and ^{13}C NMR spectra of the intermediates (**4**–**5**) and products (**1**–**2**), crystallographic data and details, ^{1}H and ^{13}C NMR spectra and details of the ring opening product (**3**) are included in the ESI. CCDC 999254–999257. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c4ce01152j

blocks the replication and infectivity of the virus.[1,3,5,11] Thus, the structural and conformational properties of the WIN analogues seem to be crucial for their mechanism of action and, therefore, it is important to elucidate their structural characteristics in detail.

Previously, three different polymorphic structures have been reported for Pleconaril, two of which the single crystal structure is known (forms I and III).[12,13] In these two polymorphic structures the Pleconaril molecules were described to pack either as a network of dimers (form I) or as a three-dimensional weakly hydrogen-bonded network of monomers (form III).[13] In comparison to the structure of the prior WIN 61893 analogue, the oxadiazole ring methyl group was substituted with a trifluoromethyl group in the Pleconaril derivative (Scheme 1).[14]

Our aim was to functionalize the isoxazole ring 3-position with an anchoring carboxylate group in order to produce a potentially better analogue to the inherent palmitic acid that is commonly found in the hydrophobic pocket of enterovirus capsid. Here, we focused on the structural and conformational properties of the modified WIN 61893 compound. Therefore, 3-(ethyl ester)- (**1**) and 3-(carboxylic acid)-isoxazole (**2**) derivatives of WIN 61893 compound were prepared and characterized (Scheme 1). Their structural and conformational properties were studied in detail by single crystal X-ray diffraction analysis revealing the importance of the hydrogen bonding ability of the carboxylate group on the crystal packing. Solution stability was furthermore investigated by NMR spectroscopy, which showed the isoxazole ring of the carboxylic acid derivative (**2**) susceptible to undergo ring opening by decarboxylation under certain reaction conditions.

Experimental

Materials and methods

All materials were commercial unless otherwise mentioned. Ethyl chlorooximidoacetate was prepared according to literature procedures.[15] DMF was distilled over 4 Å molecular sieves and stored under nitrogen over 3 Å molecular sieves. K_2CO_3 was dried in an oven at 120 °C and stored in a desiccator. ^{1}H and ^{13}C NMR spectra were recorded with Bruker Avance 300, 400 or 500 MHz spectrometers and chemical shifts were calibrated to the residual proton or carbon resonance of the solvent. Accurate HRMS spectra were measured with Micromass LCT ESI-TOF mass spectrometer using Leucine Enkephalin as the internal calibration. Melting points were determined with Stuart SMP3 melting point apparatus in open capillaries. Synthesis scheme is shown in the ESI† (Scheme S1).

Synthesis of WIN 61893 derivatives[16]

3,5-Dimethyl-4-(pent-4-yn-1-yloxy)benzonitrile (4). A mixture of 3,5-dimethyl-4-hydroxy-benzonitrile (1.0 g, 6.93 mmol), anhydrous and finely divided K_2CO_3 (1.9 g, 13.7 mmol) and KI (0.12 g, 0.72 mmol) was suspended in dry DMF (15 mL) with vigorous stirring under nitrogen atmosphere. 5-Chloro-1-pentyne (1.05 mL, 9.91 mmol) was then added and the reaction mixture was heated to 75 °C and allowed to stir overnight. The resulting yellow suspension was filtered by suction through a pad of Hyflo Super® and solvent was removed by rotavapor. The yellow residue was dissolved in ethyl acetate and the organic layer was washed once with water and once with brine. The organic layer was separated, dried with anhydrous Na_2SO_4 and solvent was removed by rotavapor. Purification by flash column chromatography on silica (mesh 0.063–0.200 mm) with ethyl acetate–hexane 2 : 8 provided the title compound as a white solid after recrystallization from hexane. Yield 1.3 g (89%). m.p. 48–49 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ_H = 7.32 (q, 2H, J = 0.59 Hz, ArH), 3.91 (t, 2H, J = 6.10 Hz, CH_2O), 2.48 (td, 2H, J = 6.88 and 2.68 Hz, CH_2), 2.30 (s, 6H, $ArCH_3$), 2.02 (t, 2H, J = 6.52 Hz, CH_2–C≡), 1.99 (t, 1H, J = 2.66 Hz, ≡CH) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz): δ_C = 159.7, 132.8, 132.6, 119.0, 107.4, 83.2, 70.4, 69.1, 29.0, 16.2, 15.10 ppm. HRMS (ESI-TOF) m/z: 236.1037 [M + Na^+], required 236.1046.

3-(3,5-Dimethyl-4-(pent-4-yn-1-yloxy)phenyl)-5-methyl-1,2,4-oxadiazole (5). A mixture of **4** (0.50 g, 2.35 mmol), anhydrous and finely divided K_2CO_3 (1.6 g, 11.9 mmol) and hydroxylamine hydrochloride (0.83 g, 11.9 mmol) in absolute ethanol (10 mL) was refluxed with vigorous stirring for 17 hours. The hot reaction mixture was filtered by suction and washed thoroughly with ethanol. Solvent was removed by rotavapor yielding crude amidoxime intermediate, which was redissolved in dry pyridine (2.5 mL) with stirring. Acetyl chloride (0.335 mL, 4.71 mmol) was added carefully to maintain a gentle reflux and the yellow reaction mixture was then refluxed for 2 hours. The resulting dark brown mixture was allowed to cool to RT, diluted with water and extracted two times with dichloromethane. The combined organic layers were washed successively with water and brine, and the separated organic layer was dried with Na_2SO_4. Solvent was removed by rotavapor and the crude mixture was passed through a silica column (mesh 0.063–0.200 mm) with ethyl acetate–hexane 3 : 7, which provided the title compound as yellow oil. Yield 0.20 g (31%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ_H = 7.72 (q, 2H, J = 0.63 Hz, ArH), 3.91 (t, 2H, J = 6.09 Hz, CH_2O), 2.64 (s, 3H, CH_3), 2.50 (td, 2H, J = 4.29 and 2.69 Hz, CH_2), 2.33 (s, 6H, $ArCH_3$), 2.02 (t, 2H, J = 6.52 Hz, CH_2–C≡), 1.99 (t, 1H, J = 2.66 Hz, ≡CH) ppm. Product was used directly without further analyses.

Ethyl 5-(3-(2,6-dimethyl-4-(5-methyl-1,2,4-oxadiazol-3-yl)-phenoxy)propyl)isoxazole-3-carboxylate (1). Oxadiazole **5** (0.39 g, 1.44 mmol) was dissolved in dry DMF (10 mL) at RT under nitrogen atmosphere. A solution of ethyl chlorooximidoacetate[15] (0.66 g, 4.33 mmol) in DMF (5 mL) was added slowly over 20 minutes with stirring. The reaction mixture was allowed to stir at RT for 30 minutes and then heated to 90 °C. A solution of triethylamine (0.605 mL) in DMF (5 mL) was then added slowly over 30 minutes and the resulting yellow reaction mixture was allowed to stir at 90 °C

for one day. After cooling to RT, the reaction mixture was diluted with water and extracted three times with ethyl acetate. The combined organic layers were washed with water, aqueous 10% $NaHSO_4$ solution and brine. The separated organic layer was dried with Na_2SO_4 and solvent was removed by rotavapor. Flash chromatographic purification on silica (mesh 0.063–0.200 mm) with gradient ethyl acetate–hexane 1 : 3 → 1 : 1 gave the title compound as a white solid. Yield 0.26 g (46%). m.p. 95–96 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ_H = 7.73 (s, 2H, ArH), 6.49 (s, 1H, C = CH), 4.44 (q, 2H, J = 7.15 Hz, CH_2OCO), 3.87 (t, 2H, J = 6.04 Hz, CH_2O), 3.13 (t, 2H, J = 15.1 Hz, CH_2), 2.64 (s, 3H, CH_3), 2.31 (s, 6H, $ArCH_3$), 2.25 (q, 2H, J = 6.83 Hz, CH_2), 1.42 (t, 3H, J = 7.14 Hz, CH_3) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz): δ_C = 176.3, 174.6, 168.1, 160.1, 158.1, 156.5, 131.6, 128.1, 122.3, 101.8, 70.4, 62.1, 28.2, 23.6, 16.3, 14.1, 12.4 ppm. HRMS (ESI-TOF) m/z: 408.1542 [M + Na^+], required 408.1530.

5-(3-(2,6-Dimethyl-4-(5-methyl-1,2,4-oxadiazol-3-yl)-phenoxy)-propyl)isoxazole-3-carboxylic acid (2). A solution of **1** (80 mg, 0.21 mmol) and NaOH (14 mg, 0.35 mmol) in ethanol–water mixture (1 : 1, 20 mL) was refluxed with stirring for 1 hour. Ethanol was removed by rotavapor. The remaining aqueous solution was washed with diethyl ether and acidified by the addition of 6 M hydrochloric acid. The precipitated white solid was extracted with ethyl acetate and the combined organic layers were washed with water and brine. The separated organic layer was then dried with Na_2SO_4 and solvent was removed by rotavapor. Recrystallization of the crude product from methanol afforded the title compound as a white solid. Yield 62 mg (84%). m.p. 144–146 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ_H = 7.73 (s, 2H, ArH), 6.55 (s, 1H, C = CH), 3.88 (t, 2H, J = 5.88 Hz, CH_2O), 3.16 (t, 2H, J = 15.0 Hz, CH_2), 2.65 (s, 3H, CH_3), 2.32 (s, 6H, $ArCH_3$), 2.26 (q, 2H, J = 6.86 Hz, CH_2) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz): δ_C = 176.6, 175.5, 168.2, 161.7, 158.3, 155.9, 131.7, 128.3, 122.4, 102.1, 70.5, 28.4, 23.8, 16.5, 12.5 ppm. HRMS (ESI-TOF) m/z: 713.2590 [2 M − H^-], required 713.2577.

X-ray crystallography

Data were recorded with AGILENT SuperNova diffractometer using a micro-focus X-ray source and multilayer optics monochromatized MoK_α [$\lambda(MoK_\alpha)$ = 0.71073 Å; 50 kV, 0.8 mA] radiation. All data were collected at a temperature of 170(2) K. Data reduction and analytical absorption correction by Clark and Reid[17] were made with Crysalis^{PRO}.[18] The structures were solved by direct methods (SHELXS-97 (ref. 19)) or by charge flipping (Superflip[20]) integrated in the program of Olex2,[21] and refinements based on F^2 were made by full-matrix least-squares techniques (SHELXL-97 (ref. 19)). Hydrogen atoms were calculated to their idealized positions with isotropic temperature factors (1.2 or 1.5 times the C/O temperature factor) and refined as riding atoms. See Table S1 (ESI†) for complete crystallographic data and structural refinement parameters. CCDC numbers 999254–999257 contain the supplementary crystallographic data for this paper.

Crystal data of structure 1-form I. $C_{20}H_{23}N_3O_5$, M = 385.41, monoclinic, a = 7.8644(2), b = 23.7196(5), c = 10.4242(2) Å, β = 96.213(3)°, V = 1933.11(7) Å^3, T = 170(2) K, space group $P2_1/n$, Z = 4, μ(Mo Kα) = 0.096 mm^{-1}, 11 637 reflections collected, 5318 unique (R_{int} = 0.0202) which were used in all calculations. The final wR_2 was 0.1328 (all data) and R_1 was 0.0530 (>2σ(I)), CCDC number 999254.

Crystal data of structure 1-form II. $C_{20}H_{23}N_3O_5$, M = 385.41, monoclinic, a = 18.6194(5), b = 8.3392(2), c = 12.7669(3) Å, β = 100.331(3)°, V = 1950.19(8) Å^3, T = 170(2) K, space group $P2_1/c$, Z = 4, μ(Mo Kα) = 0.096 mm^{-1}, 10 497 reflections collected, 5164 unique (R_{int} = 0.0420) which were used in all calculations. The final wR_2 was 0.1674 (all data) and R_1 was 0.0596 (>2σ(I)), CCDC number 999255.

Crystal data of structure 2-EtOH. $C_{40}H_{50}N_6O_{12}$, M = 806.86, monoclinic, a = 8.3029(3), b = 45.516(2), c = 11.0152(4) Å, β = 103.692(4)°, V = 4044.5(3) Å^3, T = 170(2) K, space group $P2_1/c$, Z = 4, μ(Mo Kα) = 0.099 mm^{-1}, 18 378 reflections collected, 9215 unique (R_{int} = 0.0267) which were used in all calculations. The final wR_2 was 0.1408 (all data) and R_1 was 0.0593 (>2σ(I)), CCDC number 999256.

Crystal data of structure 2-DMSO. $C_{20}H_{25}N_3O_6S$, M = 435.49, monoclinic, a = 13.7775(3), b = 7.2131(1), c = 21.4071(3) Å, β = 90.490(2)°, V = 2127.32(6) Å^3, T = 170(2) K, space group $P2_1/c$, Z = 4, μ(Mo Kα) = 0.194 mm^{-1}, 11 563 reflections collected, 5806 unique (R_{int} = 0.0182) which were used in all calculations. The final wR_2 was 0.1245 (all data) and R_1 was 0.0446 (>2σ(I)), CCDC number 999257.

Solution stability experiments

The stability of derivatives **1** and **2** in solution was investigated by NMR spectroscopy. Samples were prepared in DMSO-d_6 and $CDCl_3$ solutions. ^{1}H NMR spectra were recorded repeatedly with a Bruker 400 MHz spectrometer at 30 °C during several weeks. In case isoxazole ring opening was observed, ^{13}C NMR spectrum was also measured of the final ring-opening product for a complete structure analysis.

Results and discussion

Synthesis and crystallization

WIN 61893 analogues having either ethyl ester (**1**) or carboxylic acid (**2**) functionality at the 3-position of the isoxazole ring were synthesized according to previously published procedures using 3,5-dimethylphenol as the aromatic unit (Scheme 1 and S1†).[16] Crystallization experiments gave two polymorphic structures for the ethyl ester derivative **1** by slow evaporation at room temperature from CH_2Cl_2-hexane (**1**-form I) or from hot methanol (**1**-form II) solutions. Form I could also be obtained by slow vapor diffusion with water from either methanol or ethanol solutions of derivative **1**. Carboxylic acid derivative **2**, on the other hand, crystallized as two different solvate structures (**2**-EtOH and **2**-DMSO). Ethanol-solvate (**2**-EtOH) was obtained by slow evaporation at room temperature from hot ethanol solution, whereas, DMSO-solvate (**2**-DMSO) crystallized by slow vapor diffusion

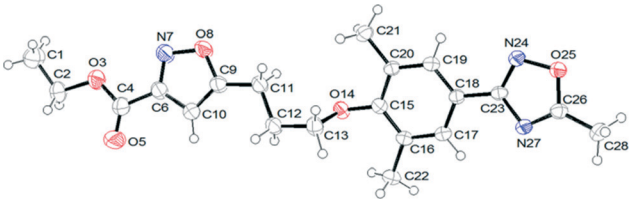


Fig. 1 A representative Ortep figure of WIN 61893 analogue (derivative **1**, structure **1**-form II) with 50% ellipsoid probability and a numbering scheme used for all structures.

with water from DMSO solution (Table S1†). All structures crystallized in a monoclinic space group, $P2_1/n$ or $P2_1/c$, with one molecule in the asymmetric unit, except for structure 2-EtOH, which contained two molecules in the asymmetric unit (2-EtOH-a and 2-EtOH-b).

Structural characteristics

All three rings (isoxazole, phenyl and oxadiazole) in the WIN 61893 derivatives **1** and **2** structures have a planar conformation as expected. The skeleton of the derivatives can be viewed as consisting of three different structural units: carboxylic acid or ester functionalized isoxazole ring [plane A, defined as O3, C4, O5, C6, N7, O8, C9, C10 and C11] at one end, which is connected by a propyloxy unit [C11–C12–C13–O14] to a conjugated phenyl-oxadiazole ring system [plane B, defined as O14, C15, C16, C17, C18, C19, C20, C21, C22, C23, N24, O25, C26, N27 and C28] at the other end (Scheme 1, Fig. 1). As such, the conformation of the molecule depends to a great extent on the orientation of these structural units with respect to one another (Table 1). In all of the structures of **1** and **2** the connecting propyloxy unit is in a *gauche* conformation with a torsion angle (C11–C12–C13–O14) in the range of 59.7–67.6°. The set of five torsion angles determined for the propyloxy unit (Table 1) shows the different conformational properties between the four structures, but the orientation of the structural units can also be simply viewed as the angle (ϕ, Scheme 1) between planes A and B, which shows the derivatives **1** and **2** to take up three different conformations: perpendicular (1-form I, ϕ = 60.8°), linear (**1**-form II and 2-EtOH-b, ϕ = 14.2 and 5.9°) and tilted (2-EtOH-a and 2-DMSO, ϕ = 37.7 and ϕ = 33.7°) conformations.

Comparison of the derivatives **1** and **2** structures with the known polymorphic structures of Pleconaril – form I and form III – reveals similar conformational features in the main WIN 61893 framework.[13] Likewise, the propyloxy unit is found in a *gauche* conformation in both of the Pleconaril forms I and III (63.6–67.9°) structures. However, only linear conformation between planes A and B is observed for both of the Pleconaril polymorphs (form I: ϕ = 17.2° and form III: ϕ = 9.9°).[13]

Crystal packing interactions

Although the structural characteristics discussed above are very similar for both derivatives **1** and **2**, the differences between the structures come into play when considering the crystal packing interactions that the molecules are able to form. The pivotal difference between the derivatives **1** and **2** is the ester or the carboxylic acid functionality of the isoxazole ring, and namely the ability of this functional group to form hydrogen-bonding interactions. Therefore, polymorphic structures (**1**-form I and **1**-form II) are observed with the ethyl ester derivative **1**, while the carboxylic acid derivative **2** more readily forms solvate structures (2-EtOH and 2-DMSO) by hydrogen bonding to the solvent molecules included in the crystal lattice.

When looking at the packing interactions of the ethyl ester derivative **1**, the conformation of the molecule – perpendicular or linear – seems to have a major role in the crystal packing of the two polymorphs (Fig. 2, Table 2). A common descriptor in both structures, however, is the formation of an inversion dimer by two opposite facing molecules. In **1**-form I, the inversion dimer is held together by aromatic C_{13}–H$\cdots\pi$ (distance to centroid of 3.30 Å) interactions between the parallel propyloxy and phenyl units, respectively (Fig. 2a, top). The ethyl ester functionalized isoxazole unit (plane A), which is in an almost perpendicular orientation to the phenyl-oxadiazole unit (plane B), conveniently forms $N_7\cdots$H–C_{11} (2.67 Å) and O=$C_4\cdots O_{14}$ (3.17 Å) interactions, respectively, with the propyloxy unit of the neighboring dimer in the diagonal direction. The carbonyl oxygen furthermore forms $O_5\cdots$H–$C_{13/22}$ (2.62 Å) interactions to the propyloxy unit and the phenyl ring methyl group of the second neighboring dimer, which altogether translates into a zigzag layer assembly of infinite arrays of molecules in the crystal lattice (Fig. 2a, middle and bottom).

In **1**-form II, on the other hand, the inversion dimers are connected by intermolecular C_{13}–H$\cdots O_3$ (2.67 Å) interactions

Table 1 Geometrical parameters of the WIN 61893 derivatives **1** and **2** structures

Torsion angles (°)	1-Form I	1-Form II	2-EtOH-a/b	2-DMSO
O8–C9–C11–C12	73.1	177.0	171.6, 164.1	−168.5
C9–C11–C12–C13	−179.0	−173.1	173.8, −177.2	−169.9
C11–C12–C13–O14[a]	−67.4 (g)	−63.3 (g)	63.7 (g), −67.6 (g)	−59.7 (g)
C12–C13–O14–C15	176.0	174.3	−166.7, 163.4	164.3
C13–O14–C15–C16	82.7	82.3	−95.7, 86.9	80.4
Planes A and B angle, ϕ (°)[b]	60.8 (P)	14.2 (L)	37.7 (T), 5.9 (L)	33.7 (T)

[a] Conformation of the propyloxy unit, *gauche* = g. [b] Planes A and B angle: $\phi > 60°$ noted as perpendicular (P), $\phi > 20°$ noted as tilted (T) and $\phi < 20°$ noted as linear (L) orientation with respect to one another.

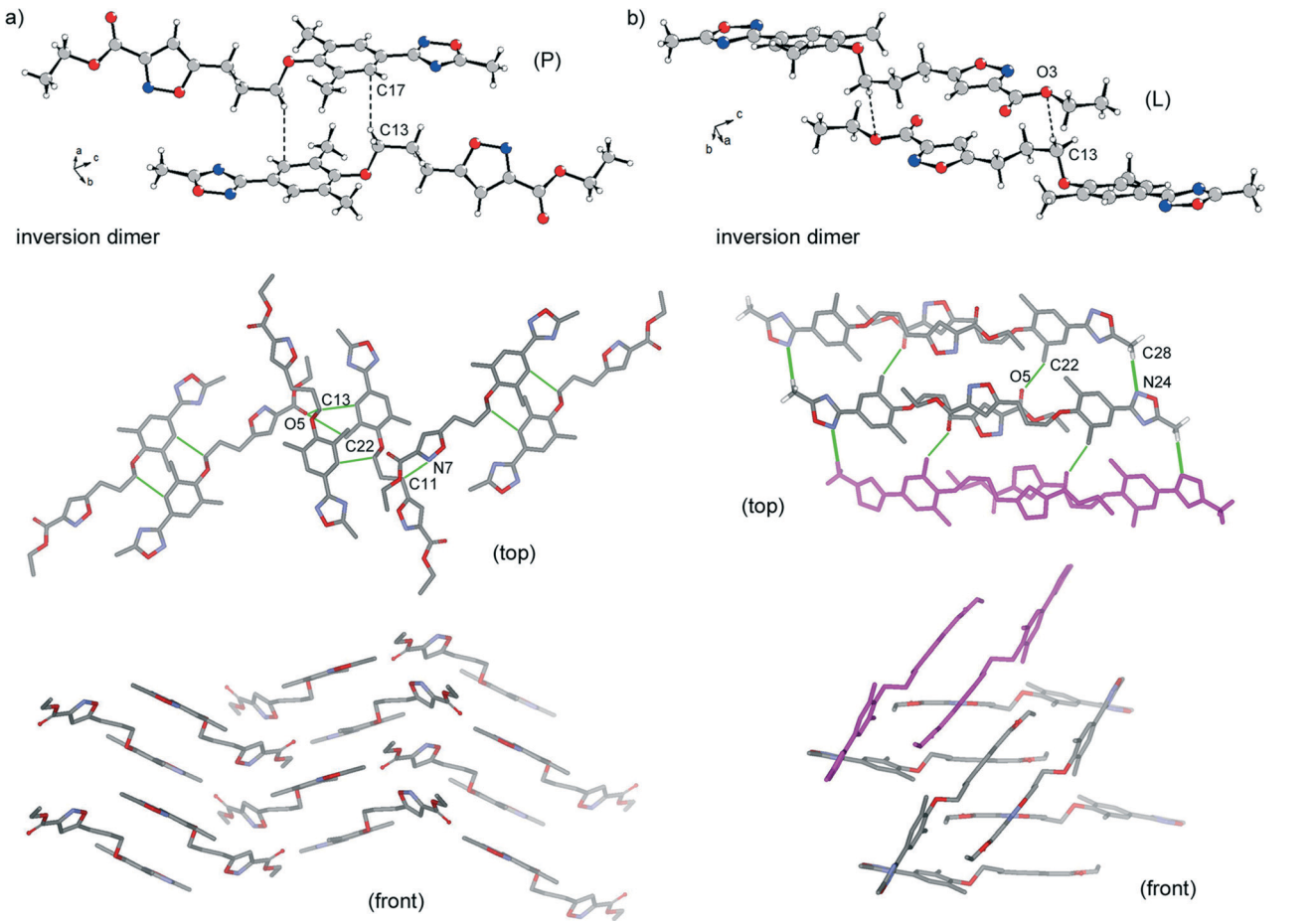


Fig. 2 Inversion dimers and crystal packing of the polymorphic structures of derivative **1**: a) **1**-form I and b) **1**-form II. Dashed black or solid green lines highlight the selected intermolecular interactions. Hydrogen atoms have been omitted for clarity when applicable.

between the parallel propyloxy unit and the isoxazole ester group (Fig. 2b, top). In this case, derivative **1** is in a linear conformation and the neighboring dimers interact most favorably in the diagonal direction by forming intermolecular C_{28}–H···N_{24} (2.64 Å) interactions between two oxadiazole groups, C_{22}–H···O_5 (2.34 Å) interactions between the phenyl ring methyl and carbonyl groups, and C_{22}–H···$C_{11/22}$ (distance of 2.40–2.87 Å) interactions between the propyloxy unit and the phenyl ring methyl groups, which lead into a diagonal criss-cross type of packing in the crystal lattice (Fig. 2b, middle and bottom).

As expected, in the solvate structures of derivative **2**, the hydrogen bonding interactions with the solvent molecules play a major role in the crystal packing (Fig. 3). As seen in the EtOH-solvate structure 2-EtOH, a solvent mediated dimer is formed of two ethanol molecules and of two derivatives **2** molecules of different conformations – tilted (2-EtOH-a) and linear (2-EtOH-b). The dimer is held together by intermolecular hydrogen bonded network of the $R_4^4(14)$ motif, in which two types of hydrogen bonds are formed between the solvent ethanol and isoxazole nitrogen (O_{EtOH}–H···N_7, distance of 2.05–2.06 Å) or carboxylic acid oxygen (O_3–H···O_{EtOH}, distance of 1.74–1.78 Å), respectively (Fig. 3a, top). The dimer interacts with the respective conformational inversion counterparts by forming intermolecular $C_{10a/b}$–H···$N_{24a/b}$ (distance of 2.67–2.73 Å) interactions between the acidic isoxazole hydrogen and the oxadiazole nitrogen, C_{12b}–H···O_{25b} (2.69 Å) interactions between the propyloxy unit and the oxadiazole oxygen, and $O_{5a/b}$···H–$C_{19a,21a/b}$ (distance of 2.46–2.55 Å) interactions between the carbonyl oxygen and the phenyl ring methyl groups (Fig. 3a, top). The conformational difference of the structural isomers is seen as the number of formed intermolecular interactions with the respective inversion counterparts, which in the case of the linear isomer is four interactions; whereas, the tilted isomer is only able to make two of the above mentioned intermolecular interactions. The assemblies of four molecules then layer diagonally (highlighted with magenta color), which creates once again a zigzag type of layer packing in the crystal lattice (Fig. 3a, middle and bottom).

For the DMSO-solvate structure 2-DMSO, a different kind of packing of derivative **2** molecules into the crystal lattice is observed. Since DMSO can only function as a hydrogen bond acceptor, only one type of hydrogen bonding interaction of O_3–H···O_{DMSO} (1.73 Å) is formed between the carboxylic acid group and the DMSO solvent molecule. Contrary to the other structures, the inversion molecules stack in an alternating fashion *via* intermolecular aromatic π···H–C_{21} (distance to

Table 2 Selected hydrogen bond parameters of the WIN 61893 derivatives **1** and **2** structures

	1-Form I			**1**-Form II	
Interaction	D–H···A (Å)	υ (°)	Interaction	D–H···A (Å)	υ (°)
C_{22}–H···O_5	2.62	150.0	C_{13}–H···O_3	2.67	160.4
C_{13}–H···O_5	2.62	150.3	C_{22}–H···O_5	2.34	164.9
C_{11}–H···N_7	2.67	167.6	C_{28}–H···N_{24}	2.66	169.2
C_{13}–H···Ct[a]	3.30	149.2	C_{11}–H···C_{22}	2.87	164.9
C_{13}–H···C_{17}	2.73	125.2	C_{22}–H···C_{22}	2.40	130.0
C_{22}–H···C_{22}	2.86	113.4			
O═C_4···O_{14}	3.17	—			
	2-EtOH			**2**-DMSO	
Interaction	D–H···A (Å)	υ (°)	Interaction	D–H···A (Å)	υ (°)
O_3–H···O_{EtOH}	1.78	172.7	O_3–H···O_{DMSO}	1.73	177.1
	1.74	171.7	$C_{DMSO\text{-}103}$–H···O_5	2.52	137.7
O_{EtOH}–H···N_7	2.05	177.3	$C_{DMSO\text{-}103}$–H···O_8	2.57	149.2
	2.06	168.7	C_{28}–H···O_3	2.59	153.4
C_{21}–H···O_5	2.46	153.2	C_{28}–H···N_7	2.72	133.3
	2.50	153.5	C_{21}–H···Ct[a]	2.82	141.1
C_{19B}–H···O_{5B}	2.55	150.5	C_{11}–H···C_{18}	2.87	160.1
C_{12B}–H···O_{25B}	2.70	134.5	C_{11}–H···Ct[a]	3.40	165.9
C_{10}–H···N_{24}	2.73	163.8	C_{13}–H···C_{19}	2.74	159.5
	2.67	178.8	C_{13}–H···Ct[a]	3.51	152.7
			$C_{DMSO\text{-}102}$–H···O_{DMSO}	2.71	123.5
			$C_{DMSO\text{-}103}$–H···O_{DMSO}	2.63	154.3

[a] Ct = aromatic ring centroid (C15–C16–C17–C18–C19–C20).

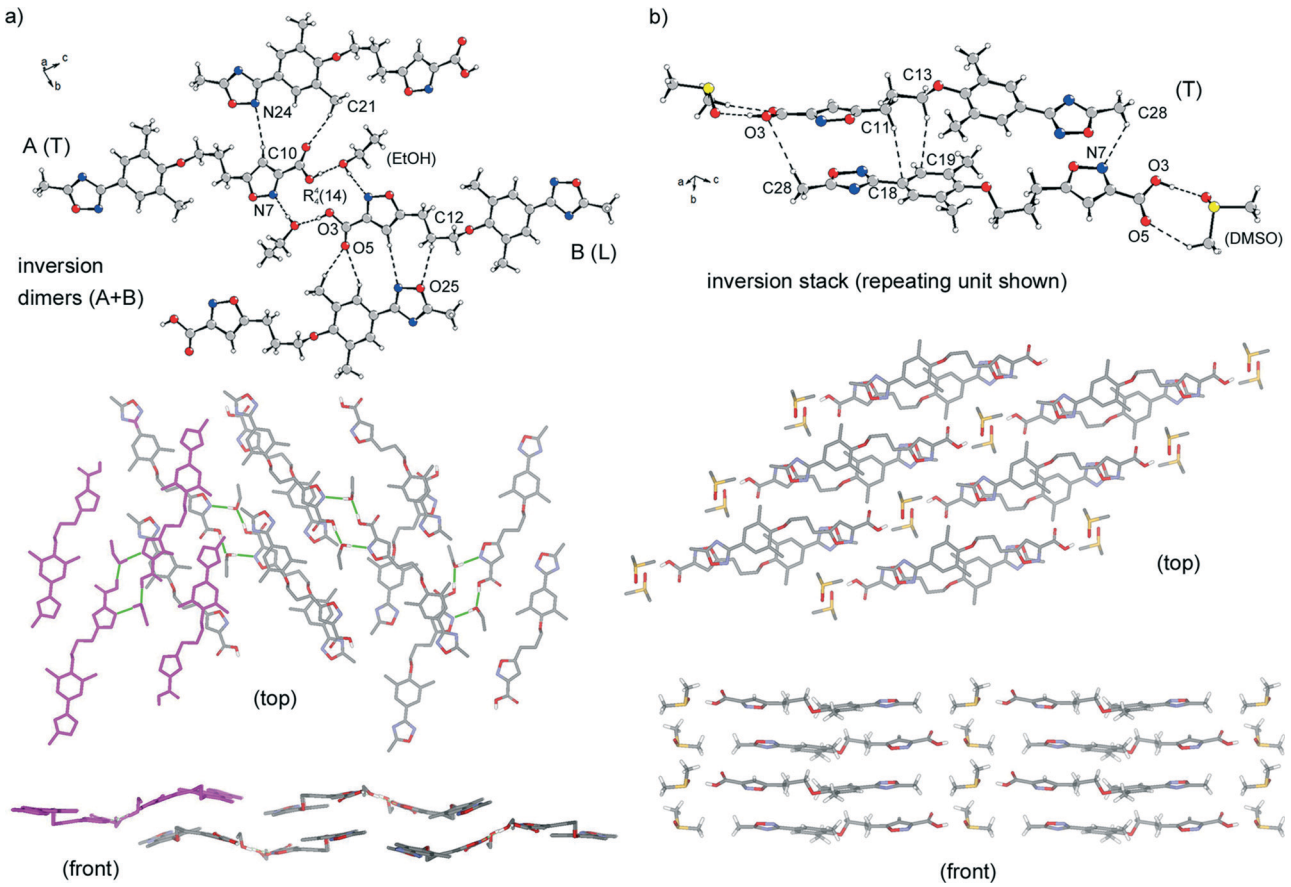


Fig. 3 Inversion dimer (a) and inversion stack (b) of the solvate structures of derivative **2** with crystal packing shown for: a) **2**-EtOH and b) **2**-DMSO. Dashed black or solid green lines highlight the selected intermolecular interactions. Hydrogen atoms have been omitted for clarity when applicable.

centroid of 2.82 Å), C_{28}–H···O_3 (2.59 Å), C_{28}–H···N_7 (2.72 Å) and C_{28}–H···C_4 (2.90 Å) interactions, rather than forming separate inversion dimers as seen with structures **1**-form I, **1**-form II and **2**-EtOH (Fig. 3b, top). The inversion stacks assemble in the crystal lattice in a brick wall pattern, in which the neighboring inversion stacks are slightly shifted to one another (Fig. 3b, middle). The channels that are formed between the neighboring stacks are filled with solvent DMSO molecules that coordinate with one another *via* $C_{102/103}$–H···O_{DMSO} (distance of 2.63–2.71 Å) interactions between the DMSO methyl and carbonyl groups, and by C_{103}–H···$O_{5/8}$ (distance of 2.52–2.57 Å) interactions between the DMSO methyl group and the carbonyl and isoxazole oxygens of the neighboring derivatives **2** molecules (Fig. 3b, bottom).

While the conformational features of the main WIN 61893 framework of derivatives **1** and **2** structures were found to be very similar to Pleconaril forms I and III structures, there are differences seen in the crystal packing, which seem to greatly depend on the characteristic intermolecular interactions formed by the carboxylate or the trifluoromethyl functionalities, as well as, on the preferred orientation of the planes A and B with respect to one another.

In the case of Pleconaril forms I and III structures, only linear conformation was observed, which allows the molecules to pack in a highly parallel layers by C–H···O, C–H···N, and C–H···F hydrogen bonding and π–π stacking interactions[13] in comparison to the more complex zigzag, diagonal criss-cross and brick wall type of networks that were observed with derivatives **1** and **2** structures having linear, tilted and perpendicular conformations. Furthermore, the key feature in the formation of hydrogen bonded structural motifs (inversion dimer of form I and catemer synthon of form III) in the Pleconaril structures was shown to be due to the C–H···O and C–H···N hydrogen bonds formed primarily by the oxadiazole ring with the neighboring oxadiazole (form I) or isoxazole (form III) rings.[13] Although similar motifs were also seen in derivatives **1** and **2** structures, it should be emphasized that the carboxylate functionality additionally plays a significant role in the formation of the structural motifs and hydrogen bonding interactions as described above. Therefore, the similarities in the structures of derivatives **1** and **2** and Pleconaril seem to arise from the interactions formed by the shared WIN 61893 framework, whereas, the structural differences are brought upon the specific hydrogen bond forming abilities of the carboxylate or trifluoromethyl functionalities, and consequently due to the multitude of conformations of derivatives **1** and **2** in comparison to Pleconaril.

Solution stability

The isoxazole unit of WIN 61893 derivatives **1** and **2** structures not only convey the essential structural difference between the two derivatives, but as a structural motif the heterocyclic isoxazole ring has interesting properties on its own, which play a key role in the stability of the derivatives **1** and **2** in solution. As a conjugated heterocycle, the isoxazole ring has typical properties of an aromatic system, but in addition it contains a weak nitrogen–oxygen bond, which is susceptible to undergo ring cleavage when exposed to certain reaction conditions.[22,23] The most typical ring cleavage products of the isoxazole ring include, for example, 1,3-dicarbonyl, enaminoketone, γ-amino alcohol, α,β-unsaturated oxime, β-hydroxy nitrile and β-hydroxy ketone compounds depending on the nature and number of substituents in the isoxazole ring.[22] Therefore, the stability of the derivatives **1** and **2** were studied by ^{1}H NMR spectroscopy in polar aprotic DMSO-d_6 and in non-polar $CDCl_3$ solutions.

Coincidentally, it was observed that the carboxylic acid derivative **2** underwent slow isoxazole ring cleavage within a few weeks at room temperature in the polar aprotic DMSO-d_6 solution, whereas, it was stable in the non-polar $CDCl_3$ solution (Fig. 4 and S4†). The ethyl ester derivative **1**, on the other hand, did not show any ring cleavage and remained stable in both solvents even after several weeks at room temperature (Fig. S3 and S5†). In order to track down the ring-opening product of derivative **2**, the NMR spectra was carefully analysed after the isoxazole ring opening had completely taken place. From the ^{1}H NMR spectra it was apparent that after one week in DMSO-d_6 solution the isoxazole ring proton H-10 of derivative **2** at 6.70 ppm began to disappear and a new singlet peak was formed at 4.09 ppm, which became a single H-10 resonance after several weeks (Fig. 4). Also, all the propyloxy unit protons H-11 through H-13 shifted upfield due to the isoxazole ring opening (Fig. 4, Table S2†). It was considered that the carboxylic acid group was responsible

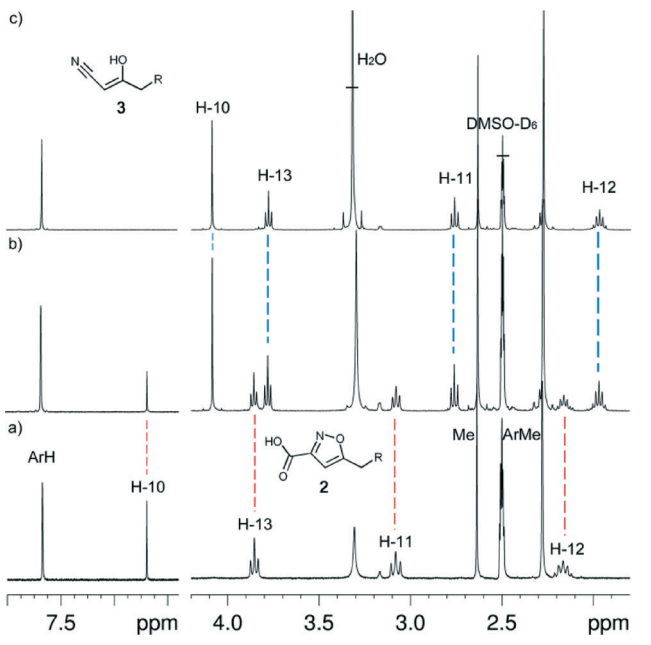
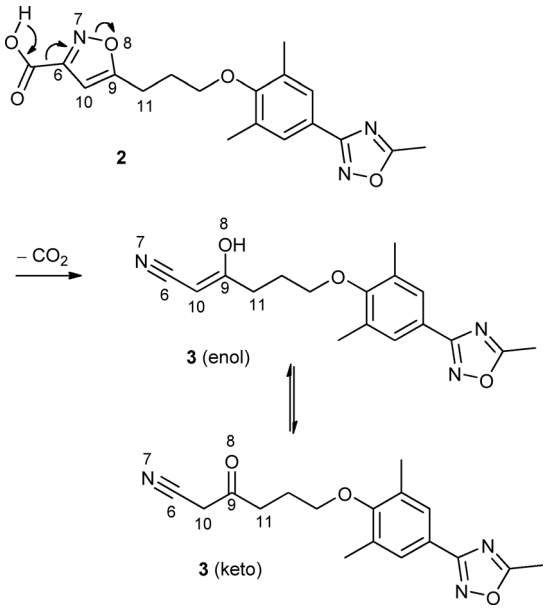


Fig. 4 Time dependent ^{1}H NMR spectra of WIN 61893 carboxylic acid derivative **2** in DMSO-d_6 at 30 °C showing the isoxazole ring cleavage into β-keto nitrile derivative **3** over several days: a) freshly prepared solution, b) after 8 days and c) after 5 weeks. Molecular formula is shown only up to carbon C-11 for clarity (see Scheme 2).



Scheme 2 Decarboxylation of 3-carboxyisoxazole group of derivative **2** into a β-keto nitrile or α-cyanoenol tautomer are shown for derivative **3**.

for the isoxazole ring cleavage since no ring opening was observed with the ethyl ester derivative **1**. Therefore, subsequent decarboxylation and ring opening of the 3-carboxyisoxazole ring of derivate **2** into a β-keto nitrile derivative **3** was suggested[23,24] and supported by ^{13}C NMR spectrum (Fig. S6†) as the upfield shift of carbon C-6 from 158.3 to 115.5 ppm corresponding the resonance of the cyano group, and as the downfield shift of carbon C-9 from 161.7 to 199.5 ppm associated with the carbonyl group formation (Scheme 2, Table S2†). In addition, the loss of aromaticity upon the isoxazole ring opening was seen as a drastic upfield shift of carbon C-10 from 102.1 to 37.9 ppm.

The stability of the isoxazole ring of the WIN 61893 derivatives **1** and **2** in solution is a key issue when considering these compounds as possible antiviral agents and their distribution and metabolism in biological media, as well as, mechanism of action. Isoxazole unit containing biologically active compounds have been shown to undergo metabolic processes resulting in the aforementioned isoxazole ring-opening products.[25] 3-Carboxyisoxazole and 3-unsubstituted isoxazole rings in particular were shown to convert into either β-keto nitrile or the tautomer α-cyanoenol ring-opening products during metabolic biotransformation processes.[24,25]

Conclusions

In conclusion, WIN 61893 analogues having either ethyl ester or carboxylic acid functionality at the 3-position in the isoxazole ring were prepared and crystallized. Two polymorphic structures of the ethyl ester derivative were obtained, whereas, the carboxylic acid derivative crystallized as two solvate structures. In all of the structures, the conformation of the WIN 61893 skeleton was mainly defined by the orientation of the isoxazole ring with respect to the planar phenyloxadiazole unit taking either perpendicular, linear or tilted conformations. The propyloxy unit between the planar ring structures was found in a *gauche* orientation. The stability of the ethyl ester and carboxylic acid derivatives were studied in solution, which revealed that the carboxylic acid derivative was susceptible to undergo isoxazole ring cleavage in DMSO solution by decarboxylation yielding a ring opening product β-keto nitrile derivative.

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