NOVEL BIOACTIVE 1,2,4-OXADIAZOLE NATURAL PRODUCT ANALOGS. SYNTHESIS, STRUCTURAL ANALYSIS AND POTENTIAL ANTITUMOR ACTIVITY

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The synthesis of new 1,2,4-oxadiazoles derivatives is reported. The main target was 3-tert-butyl-5-[4-(4,4-dimethyl-4,5-dihydrooxazol-2-yl)phenyl]-1,2,4-oxadiazole (5), which was synthesized starting from 1,2,4-oxadiazol nitrile 1 in three steps. The target molecule 5 was investigated for antitumor activities in vitro, towards a panel of 12 cell lines using a monolayer cell survival and proliferation assay. The structural assignments were corroborated in four cases by X-ray structure analysis.

INTRODUCTION

1,2,4-Oxadiazoles represent an interesting motif in the development of synthetic and pharmacological chemistry. Furthermore, this heterocyclic unit can be found in biologically active compounds. 1,2,4-Oxadiazoles have been described in the literature as bioisosteres for amides and esters, with a high hydrolytic and metabolic stability.

The 1,2,4-oxadiazole unit has been identified in the core of some natural products. Two examples are furnished by the 3-substituted indole alkaloids, phidianidines A and B, reported by Carbone et al., as selective inhibitors of the dopamine transporter DAT and partial agonists of the μ opioid receptor. These molecules were isolated from the aeolid opisthobranch Phidiana militaris. A further example is quisqualic acid, isolated from the seeds of Quisqualis indica and Q. fructus. This derivative has been reported to be a strong agonist for AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptors and group I metabotropic glutamate receptors.

The oxadiazole ring system is also present in the molecules of some drugs that are available on the pharmaceutical market (Fig. 2). Because the oxadiazole ring system can act as a bioisostere for amide and carboxylic acid moiety, it can usefully be introduced into bioactive molecules. For example, several peptides that possess biological activities were found to play diverse roles in biotransformation; some can act as hormones, others as enzyme inhibitors or neurotransmitters. However, they are rapidly hydrolyzed by peptidase enzymes, which make their pharmaceutical application problematic. One solution to this problem is to replace the amide bond by the oxadiazole ring system.
The other substituent that we wished to insert into our molecules is the 2-oxazoline system, a five-membered heterocyclic system with significant applications in the synthesis of organic compounds and in the field of drug discovery and development. These bioactive molecules are usually generated by heating N-acyl derivatives of β-hydroxylamines, or by reacting them with thionyl chloride, sulfuric acid, or phosphorus pentoxide. One can also condense carboxylic acids with β-hydroxylamines at high temperatures under strongly acidic conditions. Other preparation protocols include the use of iminoether hydrochlorides, nitriles and isocyanides. Vorbrüggen et al. reported a one-pot synthesis starting from readily available carboxylic acids using Ph₃P/CCl₄ as an activating agent. Finally, carboxylate esters can be directly transformed into 2-oxazolines using lanthanum chloride as catalyst. This class of heterocyclic compounds has received much attention because many compounds possessing the 2-oxazoline motif have shown biological activity (Fig. 3).

2-Indolyloxazolines are potential candidates as oral anticancer agents because of their potent inhibition of tubulin polymerization. Another
example is D-fluviabactin, which, in a model of iron overload in chronically transfused thalassemia patients, has been shown to efficiently sequester and remove iron from animals. Deflazacor (commercially available as Dezacor, Flantadin and Lantadin) is a corticosteroid derivative used as an inflammatory agent. L-161, 240 has been reported as a strong antibacterial agent, with a minimal inhibitory concentration comparable to those of other clinically relevant antibiotics, such as ampicillin or rifampicin. Finally, several 2-oxazolines are potent pesticides. In addition, 2-oxazolines have also been described as potential prodrug precursors of carboxylic acids.

RESULTS AND DISCUSSION

In our previous work we described the synthesis of several 1,2,4-oxadiazole derivatives starting from various benzoic acids. As a precursor for several 1,2,4-oxadiazol derivatives, 4-[3-(tert-buty1)-1,2,4-oxadiazol-5-yl]benzonitrile (1) was generated from commercially available 4-cyanobenzoic acid and tert-buty1-amidoxime (Scheme 1). Activation of the 4-cyanobenzoic acid with CDI (1,1'-carbonyldiimidazole) and further acylation of the tert-buty1-amidoxime in DMF as solvent furnished the O-acylamidoxime, which was not isolated; on heating to 120 °C for several hours, it underwent cyclisation with the elimination of one molecule of water, delivering the nitrile 1 in 76% yield after purification.

Compound 1 was fully characterized. The 1H NMR data in CDCl3 reveal the aromatic protons at 8.25 ppm and 7.80 ppm along with nine protons of the tert-butyl group at 1.43 ppm as a sharp singlet. The MS data include the molecular peak m/z = 227.1 (M+) and two main fragments (M+ - 15, M+ - 97). The solid-state structure of 1 was established by X-ray diffraction analysis (Fig. 4); the molecule 1 crystallizes with imposed mirror symmetry in the monoclinic space group P21/m, whereby only the methyl group at C4 and two hydrogens of the methyl group at C3 lie outside the mirror plane.

Starting from 4-[3-(tert-butyl)-1,2,4-oxadiazol-5-yl]benzonitrile (1) we wished to generate 4-[3-(tert-butyl)-1,2,4-oxadiazol-5-yl]benzoic acid (2). The acid derivative is one of the intermediates in the synthesis of the main target product, 3-tert-butyl-5-[4-(4,4-dimethyl-4,5-dihydrooxazol-2-yl)phenyl]-1,2,4-oxadiazole (5). Scheme 2 shows the general approach for the synthesis of 5.

The hydrolysis of nitriles is one of the main synthetic routes for building amides and carboxylic acids. Because of the limited reactivity of nitriles, their transformation in most cases requires harsh reaction conditions, such as strongly acidic or basic, generating as intermediate the corresponding amide, which in most cases is hydrolyzed (Scheme 3).

Scheme 1 – One pot synthesis of 4-[3-(tert-butyl)-1,2,4-oxadiazol-5-yl]benzonitrile (1) using the amidoxime route; i. 1,1 eq. CDI in DMF, 30 minutes; ii. 1,1 eq. CDI in DMF, 120°C, 4h.

Fig. 4 – Molecular structure of 4-[3-(tert-butyl)-1,2,4-oxadiazol-5-yl]benzonitrile (1). Atoms are drawn as 50% thermal ellipsoids. Selected bond lengths [Å] and angles [°]: C1-N1 1.3074(17), C1-N2 1.3871(15), N1-O1 1.4163(14), C5-O 1.3437(15), C5-N2 1.3013(17), C12-N3 1.1438(18), C9-C12-N3 179.16(14).
From the carboxylic acid 2, the methyl ester derivative, methyl 4-[3-(tert-butyl)-1,2,4-oxadiazol-5-yl]benzoate (3), was generated rapidly and quantitatively by reaction with diazomethane (1 N in diethyl ether).

The solid-state structure of 3 was established by X-ray diffraction analysis and is shown in Fig. 5. Similarly to 1, 3 crystallized in the monoclinic space group $P2_1/m$ with imposed mirror symmetry, although the ester group is slightly disordered to both sides of the mirror plane.

In order to introduce the oxazoline motif into the 1,2,4-oxadiazol system, we first had to generate the amide intermediate 4-[3-(tert-butyl)-1,2,4-oxadiazol-5-yl]-N-(1-hydroxy-2-methylpropan-2-yl)benzamide (4) from the acid 2 (Scheme 4). The acyl chloride derivative was generated in situ by reacting the acid with SOCl$_2$ at room temperature and acid chloride was used further (without purification, as a solution in DCM) in the highly exothermic reaction with 2-amino-2-methyl-1-propanol at low temperature. Control of the temperature is crucial because at temperatures above 0°C several byproducts are generated and the purification is more difficult.

The amide 4 was cyclized to the corresponding oxazoline 5 by the dropwise addition of thionyl chloride at 0 °C. After treatment with aqueous NaOH and extraction with diethyl ether, the oxazoline 5 was isolated as a white solid in good yield (86%).

The solid-state structure of 5 was established by X-ray diffraction analysis (Fig. 6). The compound crystallizes without imposed symmetry in the monoclinic space group $P2_1/c$. The ring systems display typical bond lengths and angles. The oxadiazole and the central ring are essentially parallel (interplanar angle 1°), whereas the oxazole ring is rotated by 11.5° with respect to the central ring. This rotation is sufficiently small that the molecule still displays approximate mirror symmetry (r.m.s. deviation 0.2 Å).
Scheme 4 – Synthesis of the amido derivative 4 via the acyl chloride route.

Fig. 6 – Molecular structure of 3-tert-butyl-5-[4-(4,4-dimethyl-4,5-dihydrooxazol-2-yl]phenyl]-1,2,4-oxadiazole (5). Atoms are drawn as 50% thermal ellipsoids. Selected bond lengths [Å]: N1-C1 1.3039(14), N2-C1 1.3876(12), O1-N1 1.4194(11), O1-C6 1.3453(12), N2-C6 1.2982(13), O2-C13 1.3621(12), O2-C14 1.4498(12), N3-C13 1.2680(14), N3-C15 1.4834(12).

Scheme 5 – Proposed synthetic route for 3-tert-butyl-5-[4-((4,4-dimethyl-4,5-dihydrooxazol-2-yl)methyl]phenyl]-1,2,4-oxadiazole (9).

In our attempt to synthesize 3-tert-butyl-5-[4-((4,4-dimethyl-4,5-dihydrooxazol-2-yl)methyl]phenyl]-1,2,4-oxadiazole 9, a longer chain oxazoline derivative, we failed to generate the amide derivative 8 (Scheme 5). In a first step the cyanide compound 2-[4-(3-tert-butyl)-1,2,4-oxadiazol-5-yl]phenyl]acetonitrile 6 was generated from 4-(cyanomethyl)benzoic acid. The reaction followed the general synthetic protocol but the yield and purity for 6 were dramatically lower. All attempts to purify the compound failed and we were forced to use it despite 40% impurities.

We managed to synthesize and isolate the acid derivative 2-[4-(3-tert-butyl)-1,2,4-oxadiazol-5-yl]phenyl]acetic acid 7 using the same saponification method as for compound 2.

The solid-state structure of 7 was established by X-ray diffraction analysis and shown in Fig. 7. The compound crystallizes in the orthorhombic space group Pbcn without imposed symmetry. Molecules are connected by the well-known carboxylic acid dimer motif across inversion centers.

Fig. 7 – Molecular structure of ethyl 2-[4-(3-tert-butyl)-1,2,4-oxadiazol-5-yl]phenyl]acetic acid (7). Atoms are drawn as 50% thermal ellipsoids. Selected bond lengths [Å]: N1-C1 1.3840(14), N2-C1 1.3038(13), O1-N2 1.4256(11), O1-C6 1.3520(12), N1-C6 1.3002(13), O2-C14 1.2159(13), C1-C14 1.3195(13).
The formation of the amide derivative 8 was not successful. The reaction was repeated 3 times with minor modifications of the protocol (lower temperature, shorter time), but all attempts failed. The reaction mixture changed color from colorless to black and no signs of the amide derivative were detected. Other synthetic routes are under study.

**ANTI-TUMOR ACTIVITY**

*In vitro* anti-tumor activity of one compound was assessed using a monolayer cell survival and proliferation assay in a panel of 12 cell lines, comprising colon, gastric, lung, ovarian, pancreatic, prostate, renal and uterus cancer, as well as melanoma. Concentration-dependent activity was detected for compound 5 across all cell lines tested. By exhibiting a geometric mean IC_{50} value of 17. µM (Fig. 8), IC_{50} values for the individual cell lines were in the range from 11.9 µM (UXF 1138, uterus cancer) and 24.2 µM (MEXF 462, melanoma). Overall, good potency was found for compound 5 towards all cell lines tested.

**EXPERIMENTAL**

All reagents were purchased from commercial sources (Sigma-Aldrich or Acros) and used without further purification. Solvents were of analytical grade.

**General Procedure**

*Synthesis of 4-(3-tert-butyl-1,2,4-oxadiazol-5-yl)benzonitrile (1)*

A solution of 4-cyanobenzoic acid (50.0 g, 0.340 mol) in DMF (500 mL) was treated with a solution of CDI (1,1'-carbonyldimidazole) (60.55 g, 0.374 mol) in DMF (400 mL). After 30 min stirring at room temperature, a solution of tert-butylamidoxime (43.38 g, 0.374 mol) in DMF (200 mL) was added, and the reaction mixture was stirred for 1 h at room temperature. A second portion of CDI (60.55 g, 0.374 mol) dissolved in DMF (400 mL) was added and the mixture was heated to reflux for 5 h. The mixture was cooled to room temperature and poured into a water-ice mixture. The solid thus formed was filtered off, washed with water, dried and flash chromatographed with ethyl acetate/hexane. Yield: 76% (58.65 g, 0.258 mol). Crystals suitable for X-ray diffraction analysis were formed by slow evaporation of a chloroform solution at room temperature.

MS: m/z = 227.1 (M+, 65), 212.1 (40), 130.1 (100); δ_{H} (CDCl_{3}, 200 MHz): 8.32 – 8.19 (m, 2H), 7.97 – 7.78 (m, 2H), 1.43 (s, 9H); δ_{C} (CDCl_{3}, 50 MHz): 178.48 (Cq, N-C-O), 174.96 (Cq, N-C-N), 132.76 (CH, 2 C), 128.58(CH, 2 C), 127.89 (Cq), 119.89 (Cq, -CN), 111.82 (Cq, -C-CN) 32.62 (Cq, -C(CH_{3})_{3}), 28.43 (CH_{3}, 3 C, (CH_{3})_{3}C-).

**Synthesis of 4-(3-tert-butyl-1,2,4-oxadiazol-5-yl)benzoic acid (2)**

A suspension of 10 g of 4-(3-tert-butyl-1,2,4-oxadiazol-5-yl)benzonitrile (44.00 mmol) and 8.8 g of NaOH (220.00 mmol) in H_{2}O (150 mL) was heated to reflux for 3 h. The mixture was cooled to room temperature and the pH was adjusted 2 using 2 N HCl. The solid thus formed was filtered off, washed with plenty of water (100 mL) and dried. Yield: 56% (5.59 g, 24.64 mmol).

MS: EI m/z = 246.1 (M+, 30), 231.1 (35), 149.1 (100); δ_{H} (CDCl_{3}, 200 MHz): 8.26 (s, 4H), 1.45 (s, 9H); δ_{C} (CDCl_{3}, 50 MHz): 179.13 (Cq, N-C-O), 165.67 (Cq, -COOH), 158.45 (Cq, N-C-N), 131.41 (Cq, -C-COOH), 129.25 (Cq), 128.82 (CH, 4 C), 34.76 (Cq, N-C-N), 132.76 (CH, 2 C), 128.58(CH, 2 C), 127.89 (Cq), 119.89 (Cq, -CN), 111.82 (Cq, -C-CN) 32.62 (Cq, -C(CH_{3})_{3}), 28.43 (CH_{3}, 3 C, (CH_{3})_{3}C-).

**Synthesis of methyl 4-(3-tert-butyl-1,2,4-oxadiazol-5-yl)benzoate (3)**

A solution of 1.0 g (4.06 mmol) of 4-(3-tert-butyl-1,2,4-oxadiazol-5-yl)benzoic acid in 50 mL of diethyl ether was cooled in an ice bath and a 2.5 N solution of diazomethane in diethyl ether was added dropwise until the white solid disappeared and the gas evolution stopped. The solvent was removed under high vacuum to afford a white solid. No further purification was necessary. Yield: 1.02 g (39.43 mmol, 97%). Crystals suitable for X-ray diffraction analysis were formed by slow evaporation of a dichloromethane solution at room temperature.

MS: EI m/z = 260.1 (M+, 30), 245.1 (30), 163.1 (100); δ_{H} (CDCl_{3}, 200 MHz): 8.27 –8.13 (m, 4H), 3.96 (s, 3H), 1.44 (s, 9H); δ_{C} (CDCl_{3}, 50 MHz): 178.61 (Cq, N-C-O), 174.23 (Cq, -COOMe), 166.09 (Cq, N-C-N), 133.45 (Cq, -C-COOH), 130.14 (CH, 2 C), 128.30 (Cq), 128.05 (CH, 2 C), 52.47 (Cq, CH_{3}), 32.57 (Cq, -C(CH_{3})_{3}), 28.47 (CH_{3}, 3 C, (CH_{3})_{3}C-).
Synthesis of 4-(3-tert-butyl-1,2,4-oxadiazol-5-yl)-N-(1-hydroxy-2-methylprop-2-yl)benzamide (4)

A mixture of 5 g of 4-(3-tert-butyl-1,2,4-oxadiazol-5-yl)benzoic acid (20.3 mmol) and 4.42 mL of SOCl2 (7.24 g, 60.91 mmol) was stirred at ambient temperature under an argon atmosphere for 3 h. The excess SOCl2 was removed by distillation and the residue, 4-(3-tert-butyl-1,2,4-oxadiazol-5-yl)benzoyl chloride, was dissolved in DCM (25 mL) and added dropwise to a solution containing 3.61 g of 2-amino-2-(CH3)2C-). The reaction mixture was then poured into 100 mL of ethereal solution. The precipitate was separated by precipitation. The filtrate was concentrated by evaporation and the residue was crystallized from EtO. Yield: 76% (4.89 g, 15.42 mmol).

MS (ESI): 318.13 [100, M+H+], 340.00 [60, M+Na+], 566.85 [55, 2xM+Na+] δH (CDCl3, 400 MHz): 8.13 – 8.06 (m, 2H), 7.82 – 7.77 (m, 2H), 6.37 (bs, 1H, NH), 4.33 (bs, 1H, OH), 3.66 (s, 2H, CH2), 1.38 (s, 6H, 1.36 (s, 9H); δC (CDCl3, 100 MHz): 178.57 (Cq, N-C-O), 174.09 (Cq, NH-CO), 167.09 (Cq, N-C-N), 138.26 (Cq, -C-CON), 128.75 (CH, 2C), 127.56 (CH), 127.13 (Cq, -C-CONH), 70.38 (Cq, 70.38 (Cq, 3q, -CH2-OH), 31.54 (Cq, -C(CH3)3), 28.45 (CH3, 3 C, (CH3)3C-), 24.49 (CH2, 2C, (CH3)2C-).

Synthesis of 3-tert-butyl-5-[4-(4,4-dimethyl-4,5-dihydrooxazol-2-yl)phenyl]-1,2,4-oxadiazole (5)

15 mL of SOCl2 was added dropwise to 4 g of the amide (12.6 mmol) under argon atmosphere. After the solution was stirred for 2 h, 15 mL of methanol was added to destroy the excess of SOCl2. The reaction mixture was then poured into 100 mL of KOH (4 N) solution and twice extracted with EtO2 (2 x 100 mL). The ethereal layer was separated, dried over Na2SO4 and evaporated. Yield: 86% (3.24 g, 10.86 mmol). Single crystals suitable for X-ray diffraction analysis were generated by slow diffusion of hexane into a concentrated DCM solution.

MS (ESI): 300.17 [100, M+H+], 301.17 [20, M+H+] δH (CDCl3, 400 MHz): 8.11 – 8.06 (m, 2H), 8.02 – 7.97 (m, 2H), 4.06 (s, 2H, CH2), 1.35 (s, 9H), 1.32 (s, 6H); δC (CDCl3, 100 MHz): 178.43 (Cq, N-C-O), 174.41 (Cq, N-CO), 161.07 (Cq, N-C-N), 131.64 (Cq, -C-CON), 128.73 (CH, 2C), 127.88 (CH, 2C), 126.64 (Cq, 79.27 (CH2, O-CH2-C), 70.38 (Cq), 32.48 (Cq, -C(CH3)3), 28.46 (CH2, 3 C, (CH3)3C-), 28.34(CH3, 2 C, (CH3)2C-).

Synthesis of 2-[4-(3-tert-butyl-1,2,4-oxadiazol-5-yl)phenyl]acetonitrile (6)

A solution of 4-(cyanomethyl)benzoic acid (27.3 g, 0.17 mol) in DMF (250 mL) was treated with a solution of CDI (1,1'-carbonyldiimidazole) (30.2 g, 0.187 mol) in DMF (200 mL). After 30 min stirring at room temperature, a solution of tert-butylamidoxime (21.7 g, 0.187 mol) in DMF (200 mL) was added, and the reaction mixture was stirred for 1 h at room temperature. A second portion of CDI (27.3 g, 0.17 mol) dissolved in DMF (200 mL) was added and the mixture was heated to reflux for 6 h. The mixture was cooled to room temperature and poured into a water-ice mixture. The solid thus formed was filtered off, washed with water, dried and flash chromatographed with ethyl acetate/hexane. Yield: 70% (17.13 g, 0.12 mol). The product is approximately 60% pure according to the spectral data.

X-Ray structure determinations

All compounds crystallized solvent-free. Crystals were mounted in inert oil on glass fibres and transferred to the cold gas stream of a suitable cryostream to obtain the appropriate crystals for data collection. Space group: P21/c. Crystallographic data have been deposited at the Cambridge Crystallographic Data Centre as supplementary material (CCDC 787219).

In vitro antitumor activity towards human tumor cell lines

Antitumor activity of the compounds was tested in a monolayer cell survival and proliferation assay using human tumor cell lines.

Ten out of the twelve cell lines as tested were established at Oncotest from patient-derived human tumor xenografts passed subcutaneously in nude mice. The origin of the donor xenografts was described. The cell line 22RV1 was supplied by ATCC ((Rockville, MD), HT-29 was kindly provided by the National Cancer Institute (Bethesda, MA, USA). Cells were cultured in RPMI 1640 medium, supplemented with 10% fetal calf serum and 0.1 mg/mL gentamicin under standard conditions (37 °C, 5% CO2). Authenticity of all cell lines was proven by STR analysis at the DSMZ (Braunschweig, Germany).

A modified propidium iodide staining assay was used to assess the compounds’ activity toward human tumor cell lines. Briefly, cells were harvested from exponential phase cultures by trypsinization, counted and plated in 96-well flat-bottom microtiter plates at a cell density dependent on
the cell line (4,000–20,000 cells/well). After 24 h recovery period to allow the cells to adhere and resume exponential growth, compounds were added at 10 concentrations in half-log increments and left for further 4 days. The inhibition of proliferation was determined by measuring the DNA content using an aqueous propidium iodide solution (7 µg/mL). Fluorescence was measured using the Enspire Multimode-Plate Reader (excitation λ = 530 nm, emission λ = 620 nm), providing a direct relationship to the total viable cell number. In each experiment, all data points were determined in duplicates. Anti-tumor activity was reported as the absolute IC₅₀ value, which reflects the concentration of the test compound that achieves test/control values of 50%. Calculation was done by 4 parameter non-linear curve fit (Oncotest Data Warehouse Software). The overall potency of a compound was reflected by the geometric mean IC₅₀ values of all individual IC₅₀ values.

CONCLUSIONS

The 1,2,4-oxadiazole derivative 3-tert-butyl-5-[4-(4,4-dimethyl-4,5-dihydrooxazol-2-yl)phenyl]-1,2,4-oxadiazole 5 was synthesized starting from 1,2,4-oxadiazole nitrile 1 in three steps. All intermediates were isolated and characterized in moderate to good yields. Compound 5 was tested for anti-tumor activity in vitro towards a panel of 12 cell lines using a monolayer cell survival and proliferation assay. With an IC₅₀ value of 17.3 µM, compound 5 was mildly active. Attempts to generate a longer chain to the oxazoline substituent have failed so far. Taking into account the bioactive natural products (quisqualic acid and phidianidines A and B), it would be of great pharmacological interest to construct new derivatives of 1,2,4-oxadiazole with efficient biological transport in cells by using natural transporters such as amino-acids, peptides or sugars and to reduce/remove the secondary effects, to minimize the toxicity, and to increase the selectivity. All derivatives were obtained in high purity (at least 96% based on ¹H-NMR) and good to high yields (75-96%). The structural assignments were corroborated in four cases by X-ray structure analysis.

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Table 1
Crystallographic data

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<td>Dm (Mg m⁻³)</td>
<td>1.311</td>
<td>1.296</td>
<td>1.265</td>
<td>1.283</td>
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<tr>
<td>μ (mm⁻¹)</td>
<td>0.09</td>
<td>0.09</td>
<td>0.07</td>
<td>0.09</td>
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<tr>
<td>F(000)</td>
<td>240</td>
<td>276</td>
<td>640</td>
<td>1104</td>
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<tr>
<td>2θmax</td>
<td>62</td>
<td>52.6</td>
<td>152</td>
<td>55.8</td>
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<tr>
<td>Transmissions</td>
<td>No abs. corr.</td>
<td>1,000.0, 972</td>
<td>1,000.0, 647</td>
<td>1,000.0, 956</td>
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<tr>
<td>Recl. measured</td>
<td>15358</td>
<td>23572</td>
<td>21038</td>
<td>76664</td>
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<tr>
<td>Recl. indep.</td>
<td>1482</td>
<td>1493</td>
<td>3266</td>
<td>3204</td>
</tr>
<tr>
<td>Rmerge</td>
<td>0.027</td>
<td>0.060</td>
<td>0.931</td>
<td>0.076</td>
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<td>Parameters</td>
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<td>127</td>
<td>204</td>
<td>179</td>
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<td>Restraints</td>
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<td>7</td>
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<td>0</td>
</tr>
<tr>
<td>wR²(F², all refl.)</td>
<td>0.110</td>
<td>0.088</td>
<td>0.092</td>
<td>0.060</td>
</tr>
<tr>
<td>R(F²,F')</td>
<td>0.038</td>
<td>0.033</td>
<td>0.034</td>
<td>0.032</td>
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<tr>
<td>S</td>
<td>1.07</td>
<td>0.82</td>
<td>1.05</td>
<td>0.80</td>
</tr>
<tr>
<td>max. Δρ (e Å⁻³)</td>
<td>0.40</td>
<td>0.19</td>
<td>0.22</td>
<td>0.17</td>
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</table>
REFERENCES


