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Novel synthesis and structural characterization of Venetoclax impurities *via* [2,3] Meisenheimer rearrangement

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Venetoclax **1**, a BCL-2 inhibitor, is extensively used in the treatment of chronic lymphocytic leukemia, small lymphocytic lymphoma, and acute myeloid leukemia. In this study, is reported the synthesis of a novel Venetoclax impurity, VHA **3**, *via* a [2,3] Meisenheimer rearrangement of VNO **2**. Upon heating, VHA **3** undergoes further rearrangement, resulting in the formation of [1,2] Meisenheimer rearranged product VHA **4**. The structures of these impurities have been confirmed using ^{1}H and ^{13}C NMR, HPLC, and mass spectrometry. This synthetic approach provides an efficient method for synthesising Venetoclax impurities VHA **3** and VHA **4**.

Keywords: Meisenheimer rearrangement, VNO (Venetoclax N-Oxide), VHA (Venetoclax hydroxylamine), *m*-CPBA

Venetoclax **1** (Fig. 1) is an orally bioavailable, B-cell lymphoma-2 (BCL-2)-selective inhibitor[1,2]. The BCL-2 protein family plays a crucial role in the process of apoptosis[3,4]. Genetically programmed apoptosis can be divided into two pathways: extrinsic and intrinsic. However, the paths are not separate, but rather interact. Proteins of the BCL-2 protein family regulate the intrinsic pathway of apoptosis[5-7]. The intrinsic pathway is triggered by cell damage. Additionally, most of the anti-cancer agents work by triggering the intrinsic pathway.

Venetoclax **1** is used for the treatment of chronic lymphocytic leukaemia (CLL), small lymphocytic lymphoma (SLL), acute myeloid leukaemia (AML) and mantle cell lymphoma (MCL) as a single agent or in combination treatment[8–10]. There are 175 clinical studies involving Venetoclax currently active or recruiting in the U.S. National Library of Medicine Clinical Trials database[11] and numerous research studies investigating the treatment potential of Venetoclax[12]. The European Medicines Agency (EMA) assessment report (EPAR) for VenclyxtoTM states that Venetoclax exhibits sensitivity to light, oxidation and slight sensitivity to UV radiation, acid, heat and a combination of heat and moisture[13].

The synthesis of safe anticancer drugs has become an essential research focus. Controlling the impurities generated during production and storage is critical for ensuring drug safety.

Our comprehensive literature review reveals that, the synthesis of Meisenheimer [2,3] rearranged product VHA **3** has not been previously reported[14]. In this work, we report the synthesis and characterization of the novel impurity VHA **3** and subsequent

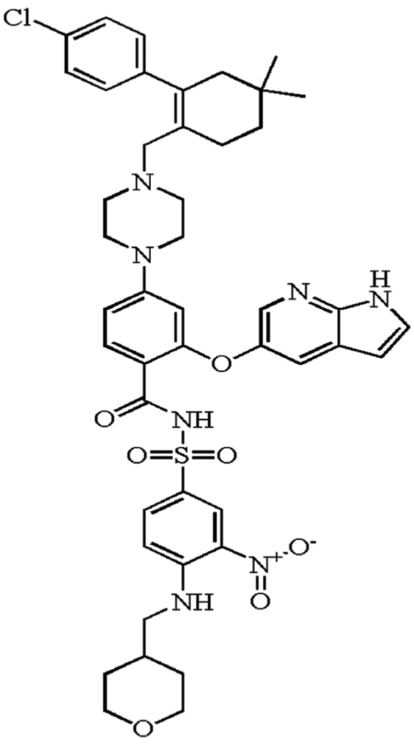


Fig. 1 — Venetoclax **1**

rearrangement of VHA **3** led to the formation of VHA **4**, a [1,2] Meisenheimer rearranged product.

Under oxidative stress, Venetoclax tends to form VNO **2**. Being a tertiary amine N-oxide, VNO **2** undergoes a Meisenheimer [2,3] rearrangement to produce the VHA **3** impurity by concerted mechanism. The Meisenheimer rearrangement of tertiary amine N-oxides, known for its [1,2] and [2,3] modes, proceeds through either a radical or a concerted mechanism. The VHA **3** undergoes rearrangement at higher reaction temperature and led to the formation of VHA **4** by concerted mechanism. These impurities were characterized using ^{1}H NMR, ^{13}C NMR, HPLC, mass spectrometry. The impurities VNO **2**, VHA **3** and VHA **4** were shown in Fig. 2.

Materials and Methods

Chemicals and reagents

All of the compounds used in the current study were synthesized in Bioneeds India Private Limited. Venetoclax N-oxide and Venetoclax hydroxyl amine impurities were synthesized and then purified using SepaBean^{TM} flash column chromatography to get desired compound. All the HPLC grade solvents, and reagents were procured from Avra, Spectochem, Sonia, *etc*.

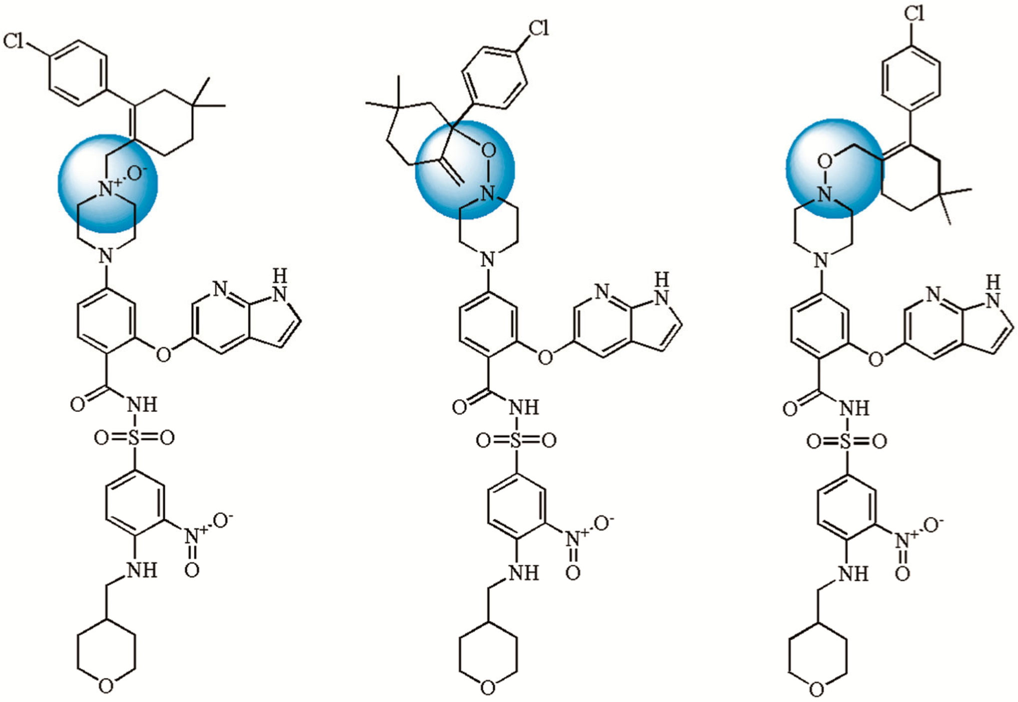
Instrumentation

HPLC

An Agilent Technologies (LC2695) HPLC system was used for the analysis of Venetoclax impurities using a PDA-2996 detector set at 220 nm. To process the HPLC data, CDH software was used. Waters XBridge Phenyl C18 column (4.6× 150 mm, 3.5 µm) (Part no: 186003335) or equivalent was used to perform the HPLC analysis. Mobile phase A was *p*H 8.3 phosphate buffer and mobile phase B was acetonitrile. The linear gradient program was set as follows: T min/B(mL/min): T0/0; T5/0; T30/ 25; T85/90; T90/100. T93/0 and T100/0 The flow rate was set at 1.0 mL/ min, and the injection volume was 10 µL. Acetonitrile was used as a diluent for the sample preparation.

LCMS

The synthesized Venetoclax impurities were analysed using Agilent technologies 6120 Quadrupole LC-MS system. The Inertsil ODS 3v 3 µm C-18 column (4.6 × 75 mm) was used for chromatographic separation. The wavelength of the UV detector was set at 270 nm. Mobile phase A is 0.1% Ammonium formate in water, and mobile phase B is HPLC grade acetonitrile. The flow rate was set at 1.0 mL/min, and



VNO (2) **VHA (3)** **VHA (4)**

Fig. 2 — Venetoclax impurities

the column oven temperature was set at 25°C. The capillary voltage was 3.5 kV. The source temperature was maintained at 100°C. The linear gradient program was set as follows: Tmin/B (%): T0/02; T02/02; T07/90; T15/90; T16/02; and T20/02.

NMR

The synthesized Venetoclax impurities (Fig. 3) were analysed by Bruker 400 MHz instrument using DMSO-d_6 as a solvent.

Experimental Section

Synthesis of the 4-(3-((1*H*-Pyrrolo[2,3-b]pyridin-5-yl)oxy)-4-(((3-nitro-4-(((tetrahydro-2*H*-pyran-4-yl) methyl)amino)phenyl)sulfonyl)carbamoyl) phenyl)-1-((4'-chloro-5,5-dimethyl-3,4,5,6-tetrahydro-[1,1'-biphenyl]-2-yl)methyl)piperazine 1-oxideVNO 2

To a stirred solution of Venetoclax (1.0 g, 1.45 mmol) in DCM (10.0 mL, 10.0 vol) was added *m*-CPBA (0.89 g, 1.72 mmol) slowly at 10−15°C for 30 min. The reaction was stirred for 60 min. After the completion of the reaction, aqueous $NaHCO_3$ solution (5.0 mL, 5.0 vol) was added at RT. The two layers were separated, and the aqueous layer was washed with DCM (5 mL). The organic layer was dried by using anhydrous sodium sulfate. The organic solvent was evaporated under vacuum to afford the crude compound, which is further subjected to column purification using 7% MeOH in DCM to get a pale-yellow solid with 93% yield. The pure compound was characterized by using different analytical techniques (Fig. 4, Fig. 5, Fig. 6 and Fig. 7).

Synthesis of the 2-(1*H*-pyrrolo[2,3-b]pyridin-5-yloxy)-4-(4-(1-(4-chloro phenyl)-5,5-dimethyl-2-methylenecyclohexyloxy)piperazin-1-yl)-N-(3-nitro-4-((tetrahydro-2*H*-pyran-4-yl) methylamino)phenylsulfonyl)benzamide VHA 3

Venetoclax N-oxide VNO **2** (1.0 g, 1.13 mmol) was taken in THF (20 mL, 20 vol) in a clean RBF. The reaction was heated to 80− 85°C for 2 h. The reaction was monitored by TLC and LCMS. After the completion of the reaction, the product was purified by column chromatography using 5% MeOH in DCM to get a yellow solid with 95% yield, which was analysed by using LCMS, ^{1}H and ^{13}C NMR (Fig. 8 and Fig. 9), HPLC and mass spectroscopy analysis.

Synthesis of the 2-(1*H*-pyrrolo[2,3-b]pyridin-5-yloxy)-4-(4-((2-(4-chloro phenyl)-4,4-dimethylcyclohex-1-enyl)methoxy)piperazin-1-yl)-N-(3-nitro-4-((tetrahydro-2*H*-pyran-4-yl) methylamino)phenylsulfonyl)benzamide VHA 4.

VHA **3** (1.0 g, 1.13 mmol) was added in *o*-Xylene (20 mL, 20 vol). The reaction mixture was heated to 100− 105°C for 6 h. The reaction was monitored by TLC and LCMS. After the completion of the reaction, the compound was purified by column chromatography using 3% MeOH: DCM to get a

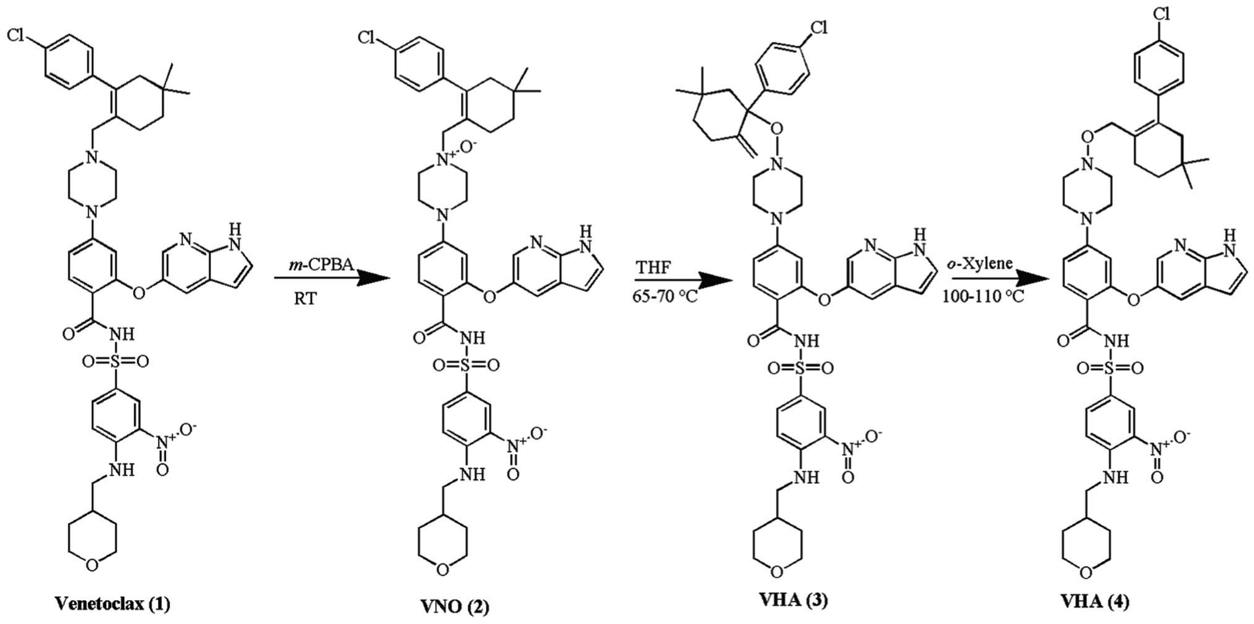


Fig. 3 — Scheme for preparation of Venetoclax impurities

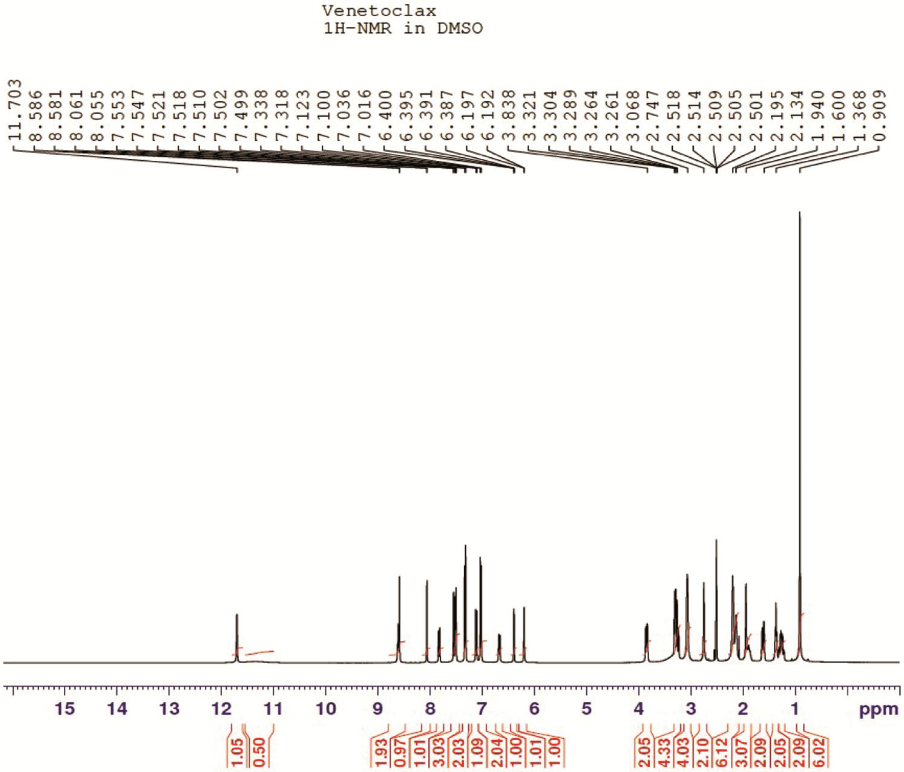


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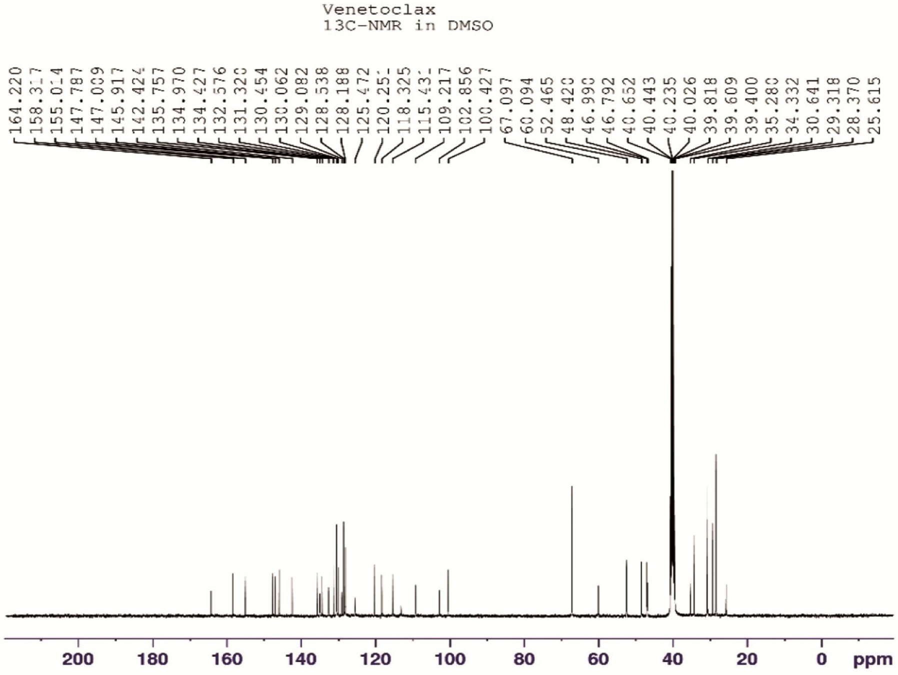
Fig. 4 — ^{1}H NMR spectrum of the Venetoclax **1**



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SFO2             400.3116012 MHz
NUC2                       1H
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PLW13              0.13741000 W

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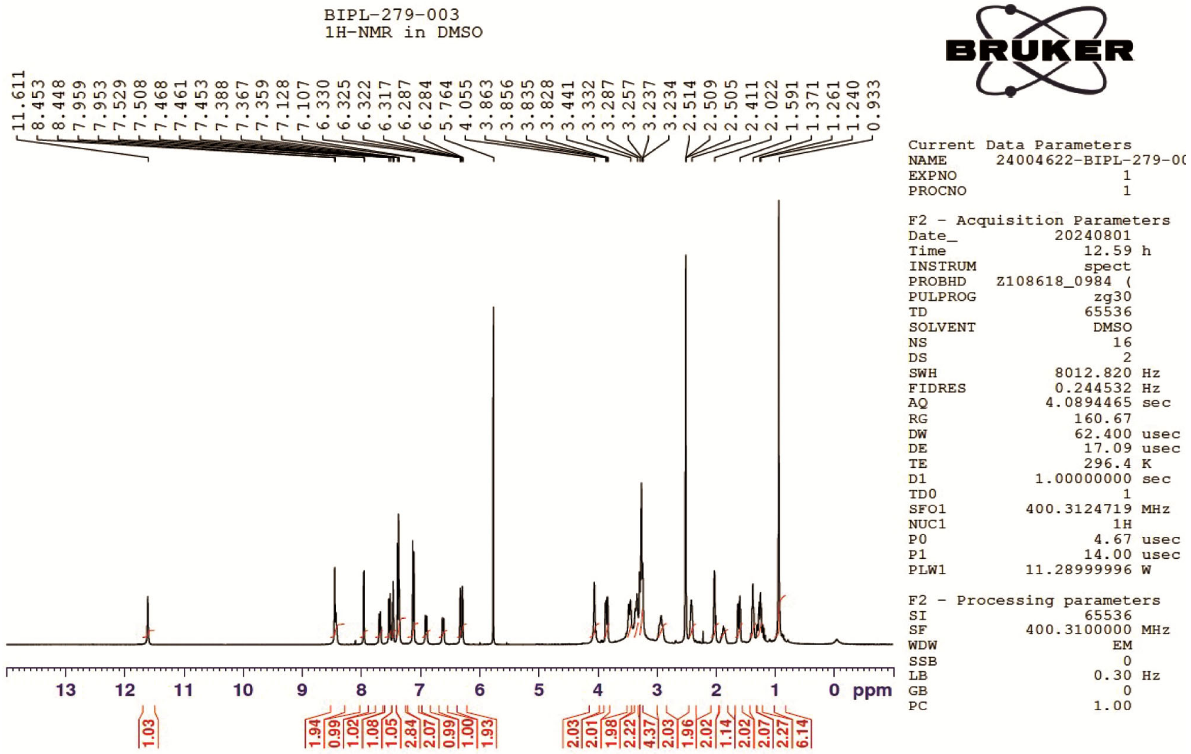
Fig. 5 — ^{13}C NMR spectrum of the Venetoclax **1**



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TE                    296.4 K
D1               1.00000000 sec
TD0                       1
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NUC1                     1H
P0                     4.67 usec
P1                    14.00 usec
PLW1            11.28999996 W

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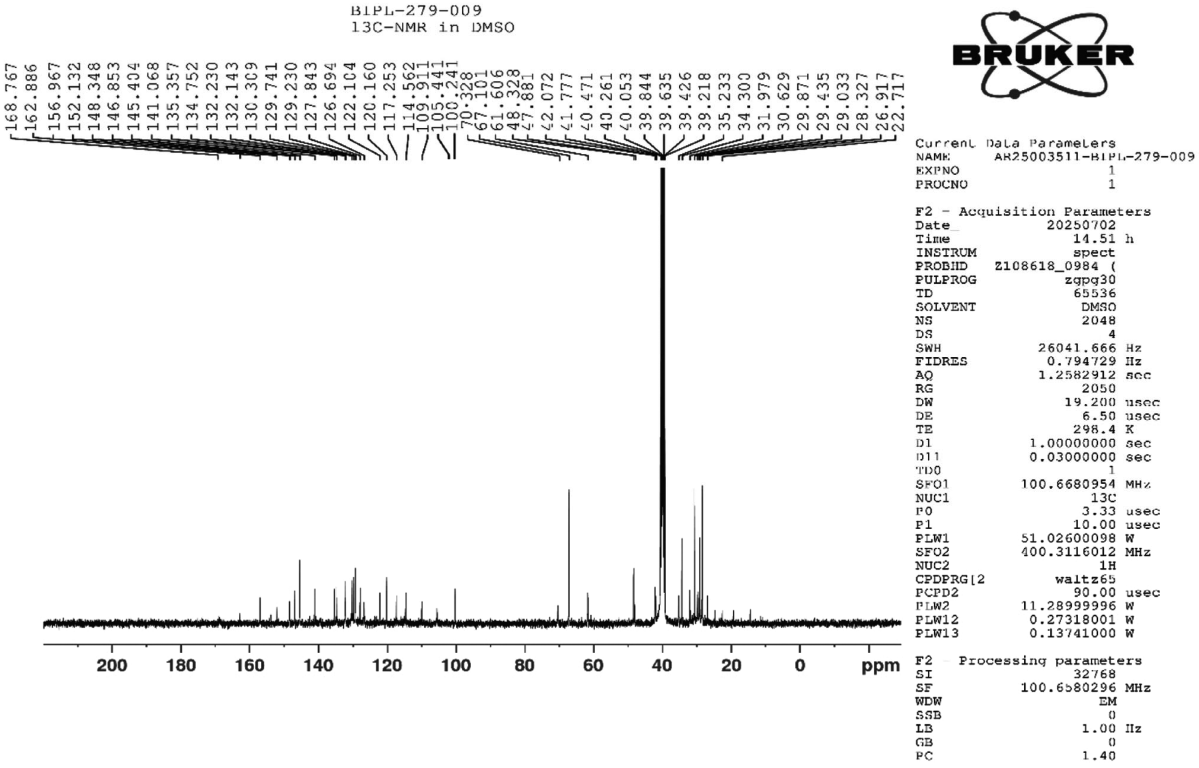
Fig. 6 — ^{1}H NMR spectrum of the VNO **2**



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NUC1                    13C
P0                     3.33 usec
P1                    10.00 usec
PLW1            51.02600098 W
SFO2            400.3116012 MHz
NUC2                     1H
CPDPRG[2            waltz65
PCPD2                 90.00 usec
PLW2            11.28999996 W
PLW12            0.27318001 W
PLW13            0.13741000 W

F2 - Processing parameters
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GB                        0
PC                     1.40
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Fig. 7 — ^{13}C NMR spectrum of the VNO **2**

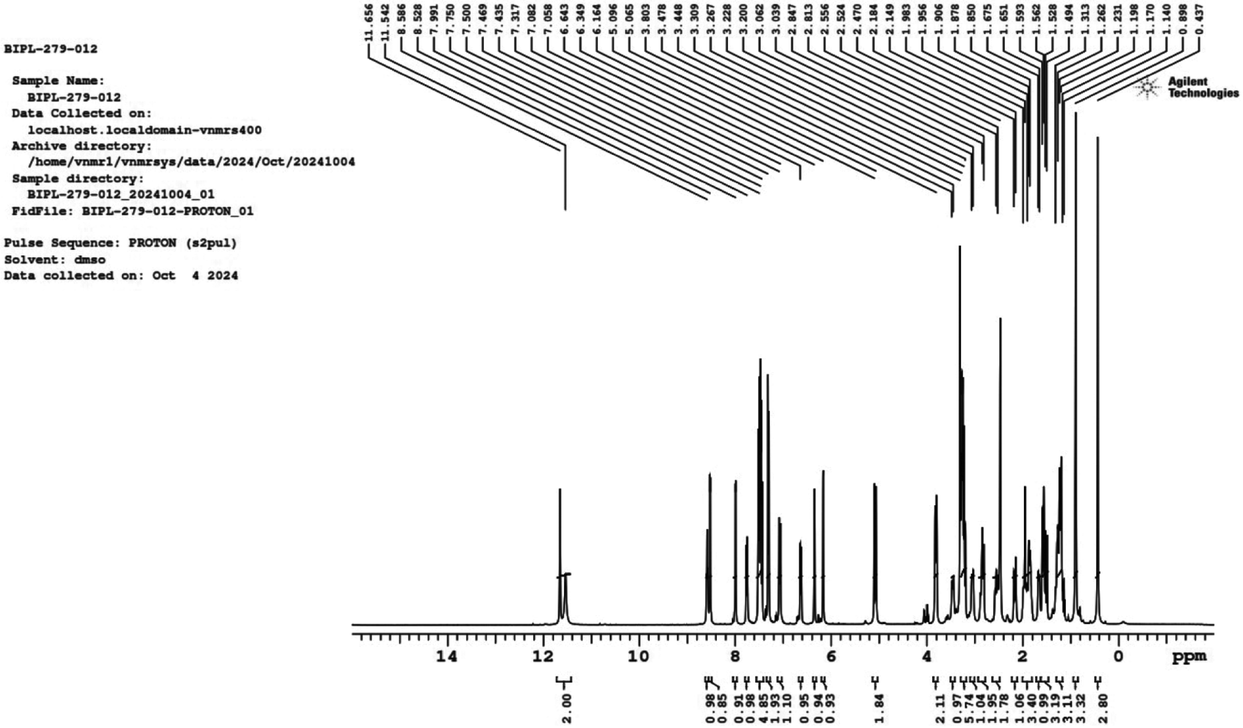


Fig. 8 — ^{1}H NMR spectrum of the VHA **3**

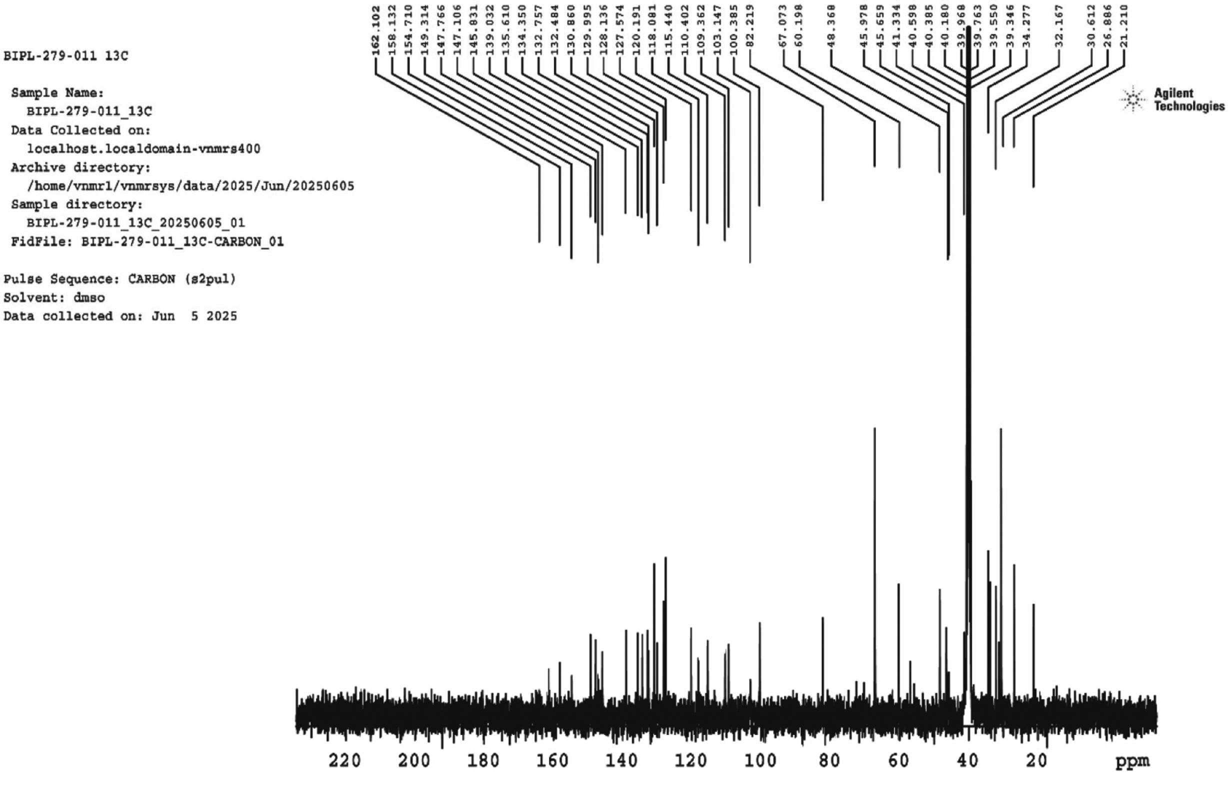


Fig. 9 — ^{13}C NMR spectrum of the VHA **3**

pale-yellow solid with 91% yield, which was analysed by using LCMS, ^{1}H and ^{13}C NMR (Fig. 10 and Fig. 11), HPLC, and mass spectroscopy.

Results and Discussion

We have synthesised VNO **2** by oxidizing Venetoclax with *m*-CPBA. The VNO **2** was rearranged to novel [2,3] Meisenheimer rearranged impurity VHA **3** using THF under reflux condition, which was further rearranged to form VHA **4** with *o*-Xylene at elevated temperature. All impurities were characterized by ^{1}H NMR, ^{13}C NMR, HPLC and mass spectrometry.

VNO **2** was synthesized by oxidizing Venetoclax with *m*-CPBA in dichloromethane[14]. The crude VNO **2** was purified by flash column chromatography using 7% MeOH: DCM as mobile phase, yielding 93% of VNO **2**. Mass analysis of VNO **2** revealed a peak at 884.37 (positive mode) and 882.26 (negative mode), with fragment peaks at 866.38 (loss of water) and 652.37 (loss of the chlorophenyldimethyl-cyclohexylenyl moiety). The ^{1}H NMR values are presented in Table 1. In ^{1}H NMR spectra, we observed a change in the chemical shift values of three $-CH_2$ groups (H-10, H-11, and H-14) attached to the nitrogen of piperazine when compared to Venetoclax **1**. The protons H-10 were shifted from δ 2.75 to 4.05. H-11 and H-14 protons were shifted from δ 2.08-2.20 to 3.33−3.36, and H-11′ and H-14′ were shifted from δ 2.08-2.20 to 3.36−3.39. There was a slight change in the chemical shift of the methylene group (H-5) from δ 2.08-2.20 to 2.41 (Fig. 6). HPLC analysis confirms the purity of VNO **2** at 40.34 RT with 98.8% purity.

In the ^{13}C NMR spectrum, we have observed a change in the chemical shifts of methylene carbons attached to the piperazine ring. The oxidation of the piperazine group of Venetoclax to its corresponding *N*-oxide results in deshielding of the carbons attached to the nitrogen of the piperazine ring. Thus, *N*-oxide formation was caused by a down shield of methylene carbon at 15, 16, and 19 carbons, which were attached directly to the piperazine ring. The carbon attached to 15-CH_2 was shifted from δ 60.1 to 70.3, 16-CH_2 was shifted from δ 52.5 to 61.2, and 19- CH2 was shifted from δ 52.5 to 61.6. We did not observe significant change in the carbon value of the aromatic ring attached to the piperazine ring. The cumulative

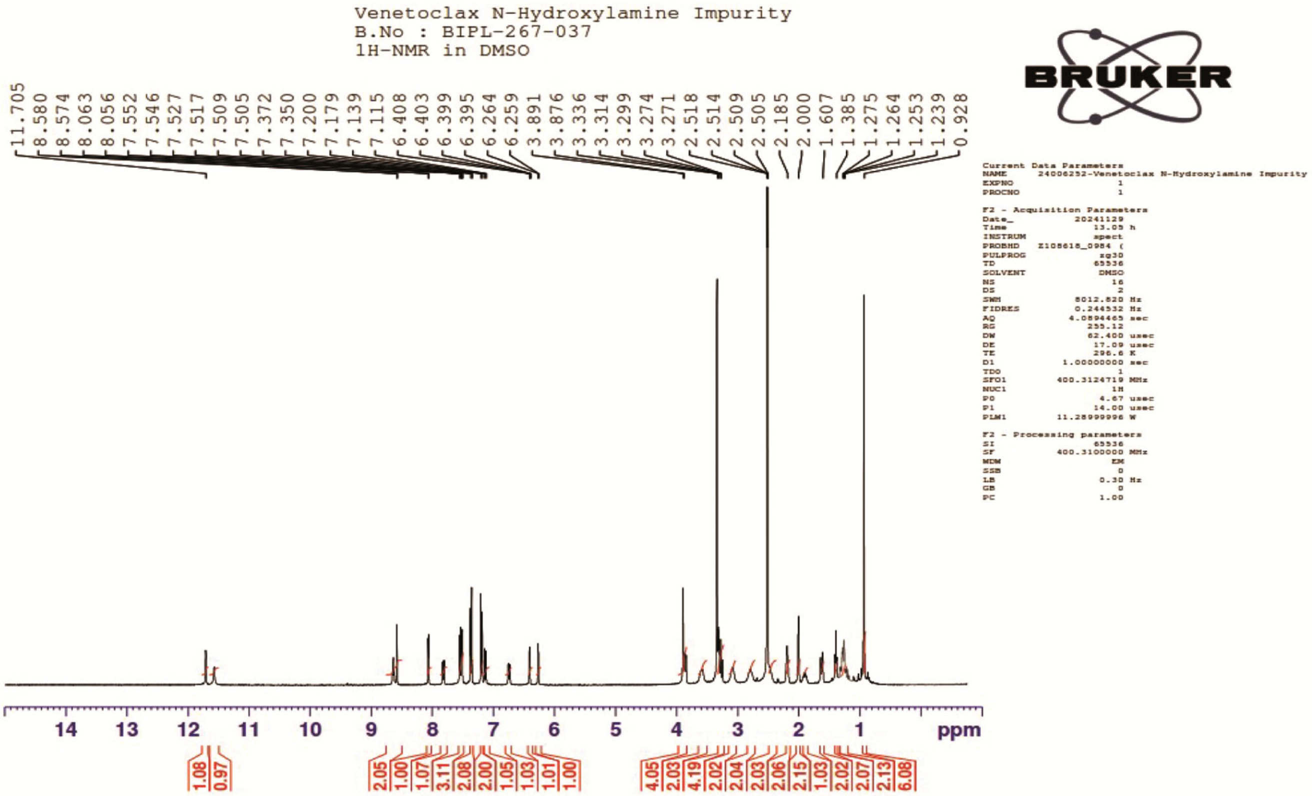


Fig. 10 — ^{1}H NMR spectrum of the VHA **4**

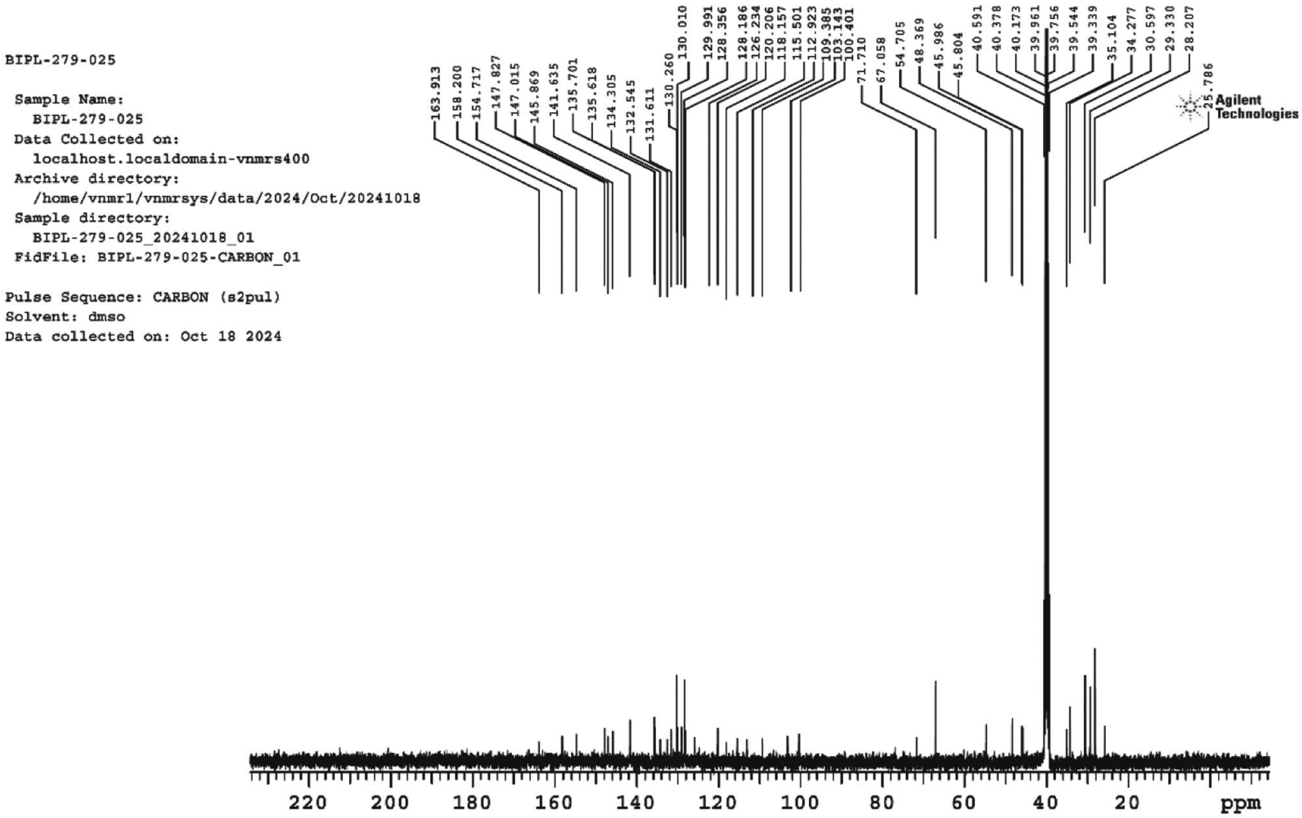


Fig. 11 — ^{13}C NMR spectrum of the VHA **4**

spectroscopic data, including both ^{1}H and ^{13}C NMR along mass spectrometric data confirmed the formation of an N-oxide at the piperazine nitrogen adjacent to the aliphatic methylene group at C-15.

The synthesized VNO **2** was treated with THF at reflux for 2 h. The VNO **2** was converted to VHA **3** up to 95%. The crude VHA **3** was purified by flash column chromatography using 5% MeOH: DCM as mobile phase. Mass spectrometry confirmed the formation of VHA **3**, with a peak at 884.37 (positive mode). Then, we characterized the pure sample using ^{1}H NMR. In the ^{1}H NMR spectrum (Fig. 8), we observed a change in the chemical shift values of three methylene protons attached to the nitrogen of the piperazine ring (H-10, H-11, and H-14). The protons H-10 were shifted from δ 4.05 to 5.06-5.10. This unusual shift of H-10 protons is due to the methylene group formation from the [2,3] Meisenheimer rearrangement of VNO **2**. H-11 and H-14 protons were shifted from δ 3.33-3.36 to 2.52-2.58, and H-11′ and H-14′ were shifted from δ 3.36-3.39 (2H) to 3.04-3.06 (1H), 3.45-3.48 (1H). This splitting of H-11′ and H-14′ could be due to the spatial orientation towards the adjacent cyclohexyl ring. These chemical shift values were different when compared with those of the VNO **2**. The HPLC analysis confirms the formation of VHA **3** at 61.07 RT and 1.08 RRT with 95% purity. After thorough analysis, it was confirmed that VNO **2** undergoes [2,3] Meisenheimer rearrangement to form VHA **3**.

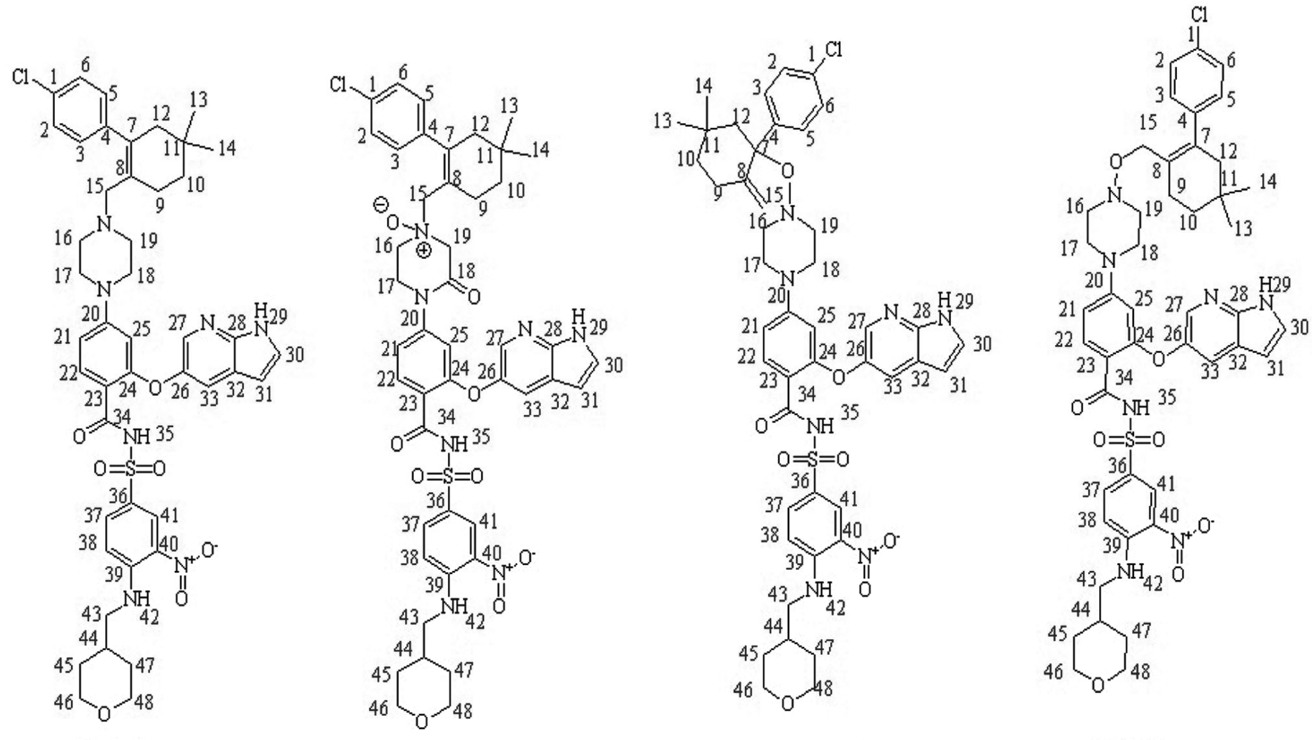
In the ^{13}C NMR spectrum of the VHA **3** impurity, a significant downfield shift was observed for the methylene carbon at δ 82.22, corresponding to the C-15 (−CH2_2) group adjacent to the piperazine ring. This chemical shift is notably higher than the corresponding signal in Venetoclax (δ 59.6) and its N-oxide derivative (VNO impurity, δ 70.0). This was due to the [2,3] Meisenheimer rearrangement of Venetoclax *N*-oxide to form the VHA impurity. In addition, the C-16 and C-19 methylene carbons, both α to the nitrogen atom, exhibited downfield shifts from δ 52.0 (in Venetoclax) to δ 60.1 and δ 52.0 (in Venetoclax) to δ 48.37 in the VHA **3** impurity.

The synthesized [2,3] Meisenheimer rearranged compound VHA **3** was treated with *o*-Xylene at 110°C for 6 h to get VHA **4**. The crude VHA **4** was purified by flash column chromatography using 3% MeOH: DCM as mobile phase, resulting in a 91%

Table 1 — ^{1}H and ^{13}C NMR assignments of VNO **2**, VHA **3** and VHA **4**

Carbon Number	Hydrogen no	No of Protons	^{1}H NMR and ^{13}C NMR chemical shift multiplicity							
			Venetoclax	Venetoclax ^{13}C	VNO (2)	VNO (2) ^{13}C	VHA (3)	VHA (3) ^{13}C	VHA (4)	VHA (4) ^{13}C
1				131.3		130.1		139		131.6
2	1	1	7.32-7.34 (d, J= 8.0 Hz)	128.5	7.36-7.39 (m)	127.7	7.50-7.52 (d, J= 8.4 Hz)	130.86	7.35-7.37 (d, J= 8.8 Hz)	128.4
3	2	1	7.02-7.04(d, J= 8.0 Hz)	130.4	7.11-7.13 (m)	129.8	7.30-7.32 (d, J= 8.4 Hz)	127.57	7.18-7.20 (d, J= 8.8 Hz)	130.2
4				142.4		144.6		145.83		141.6
5	3	1	7.02-7.04(d, J= 8.0 Hz)	130.4	7.11-7.13 (m)	129.8	7.30-7.32 (d, J= 8.4 Hz)	127.57	7.18-7.20 (d, J= 8.8 Hz)	130.2
6	4	1	7.32-7.34 (d, J= 8.0 Hz)	128.5	7.36-7.39 (m)	127.7	7.50-7.52 (d, J= 8.4 Hz)	130.86	7.35-7.37 (d, J= 8.8 Hz)	128.4
7				135		134.9		81.8		135.6
8				129.1		128.7		134.3		129
9	5	2	2.08-2.20 (m)	25.6	2.41 (brs)	22.7	1.84-1.98 (m,1H), 2.15-2.18 (m,1H)	21.2	2.18 (brs)	25.8
10	6	2	1.35-1.38(t, J= 6.2 Hz)	35.3	1.36-1.38 (m)	34.8	1.14-1.31 (m,1H), 1.49-1.59 (m,1H)	41.33	1.37-1.40 (t, J= 6.4 Hz)	35.1
11				29.3		28.6		32.16		29.3
12	7	2	1.86-1.94 (m)	46.8	2.02 (s)	45.7	1.65-1.67 (m,1H), 1-84-1.98 (m,1H)	45.98	2.00 (s)	46
13	8	3	0.91 (s)	28.4	0.93 (s)	27.9	0.90 (s)	26.88	0.93 (s)	28.2
14	9	3	0.91 (s)	28.4	0.93 (s)	27.8	0.44 (s)	26.88	0.93 (s)	28.2
15	10	2	2.75 (brs)	60.1	4.05 (brs)	70.3	5.06-5.10 (m)	82.22	3.89 (s)	71.7
16,19	11,14	2	2.08-2.20 (m)	52.5	3.33-3.36 (m)	61.2	2.52-2.58 (m)	60.19	3.07 (brs)	54.7
17,18	12,13	2	3.07 (brs)	47	3.25-3.28 (m)	47.8	2.81-2.85 (m)	60.19	2.79 (brs)	45.8
17,18	12',13'	2	3.07 (brs)	47	2.90-2.92 (m)	47.4	3.20-3.31 (m)	48.37	3.57 (brs)	45.8
16,19	11',14'	2	2.08-2.20 (m)	52.5	3.36-3.39 (m)	61.6	3.04-3.06(m,1H), 3.45-3.48 (m,1H)	48.37	2.50 (brs)	54.7
20				155		152.1		154.7		154.7
21	15	1	6.66-6.68 (m)	109.2	6.60-6.62 (d, J= 7.6 Hz)	109.9	6.62-6.64 (m)	109.36	6.72-6.75 (m)	109.4
22	16	1	7.50-7.55 (m)	132.6	7.36-7.39 (m)	132.1	7.43-7.47 (m)	132.75	7.50-7.53 (m)	132.5
23				113.1		114.5		115.44		112.9
24				158.3		156.9		158.13		158.2
25	17	1	6.19-6.20 (d, J= 2.0 Hz)	102.8	6.28-6.29 (m)	105.4	6.16-6.17 (d, J= 1.6 Hz)	103.14	6.26-6.26 (d, J= 2.0 Hz)	103.1
26				147		146.8		147.1		147
27	18	1	8.05-8.06 (d, J-2.4 Hz)	135.7	7.95-7.95 (d, J= 2.4 Hz)	141	7.99-8.00 (d, J= 2.4 Hz)	135.61	8.06-8.06 (d, J= 2.8 Hz)	135.7
28				145.9		145.4		147.76		145.9
29	19	1	11.70 (s)	NA	NA	0	11.54 (s)	NA	11.56 (s)	NA
30	20	1	7.50-7.55 (m)	128.2	7.45-7.47 (m)	126.9	7.43-7.47 (m)	128.13	7.50-7.53 (m)	128.2
31	21	1	6.39-6.40 (m)	100.4	6.32-6.33 (m)	100.24	6.35 (brs)	100.38	6.39-6.41 (m)	100.4
32				120.2		122.1		120.19		120.2
33	22	1	7.50-7.55 (m)	118.3	7.51-7.53 (d, J= 8.4 Hz)	120.1	7.43-7.47 (m)	118.08	7.55-7.55 (d, J=2.4 Hz)	118.15
34				164.2		168.7		162.1		163.9
35	23	1	11.32(s)	NA	11.61 (s)	NA	11.66 (s)	NA	11.7 (s)	NA
36				125.5		126.9		128.13		126.2
37	24	1	7.81-7.84 (m)	134.4	7.67-7.70 (m)	134.7	7.75-7.77 (m)	135.61	7.80-7.83 (m)	134.3
38	25	1	7.10-7.12 (d, J= 9.2 Hz)	115.4	6.89-6.91 (d, J= 9.2 Hz)	117.2	7.06-7.08 (m)	110.4	7.11-7.14 (d, J= 9.6 Hz)	115.5
39				147.8		148.3		149.31		147.8
40				130.1		129.2		132.4		130
41	26	1	8.58-8.62 (m)	128.2	8.43-8.45 (m)	127.2	8.52-8.53 (d, J= 2.0 Hz)	129.9	8.57-8.58 (d, J=2.4 Hz)	128.2
42	27	1	8.58-8.62 (m)	NA	8.43-8.45 (m)	NA	8.57-8.60 (t, J= 5.8 Hz)	NA	8.62-8.65 (t, J=5.6 Hz)	NA
43	28	2	3.24-3.32 (m)	48.4	3.24-3.33 (m)	47.8	3.20-3.31 (m)	45.66	3.24-3.34 (m)	48.4
44	29	1	1.86-1.94 (m)	34.3	1.85-1.88 (m)	33.8	1.84-1.98 m)	34.27	1.88-1.92 (m)	34.3
45	30	2	1.60-1.63 (m)	30.6	1.59-1.62 (m)	30.2	1.49-1.59 (m)	30.61	1.61-1.64 (m)	30.6
46	31	2	3.83-3.87 (m)	67.1	3.83-3.86 (m)	66.6	3.80-3.83 (m)	67.07	3.84-3.88 (m)	67
47	32	2	3.24-3.32 (m)	67.1	3.44-3.47 (m)	66.6	3.20-3.31 (m)	67.07	3.24-3.34 (m)	67
48	33	2	1.21-1.31(m)	30.6	1.18-1.3 (m)	30.2	1.14-1.31 (m)	30.61	1.21-1.30 (m)	30.6

Note: s-singlet, d-doublet, t-triplet, brs-broad singlet and m-multiplet



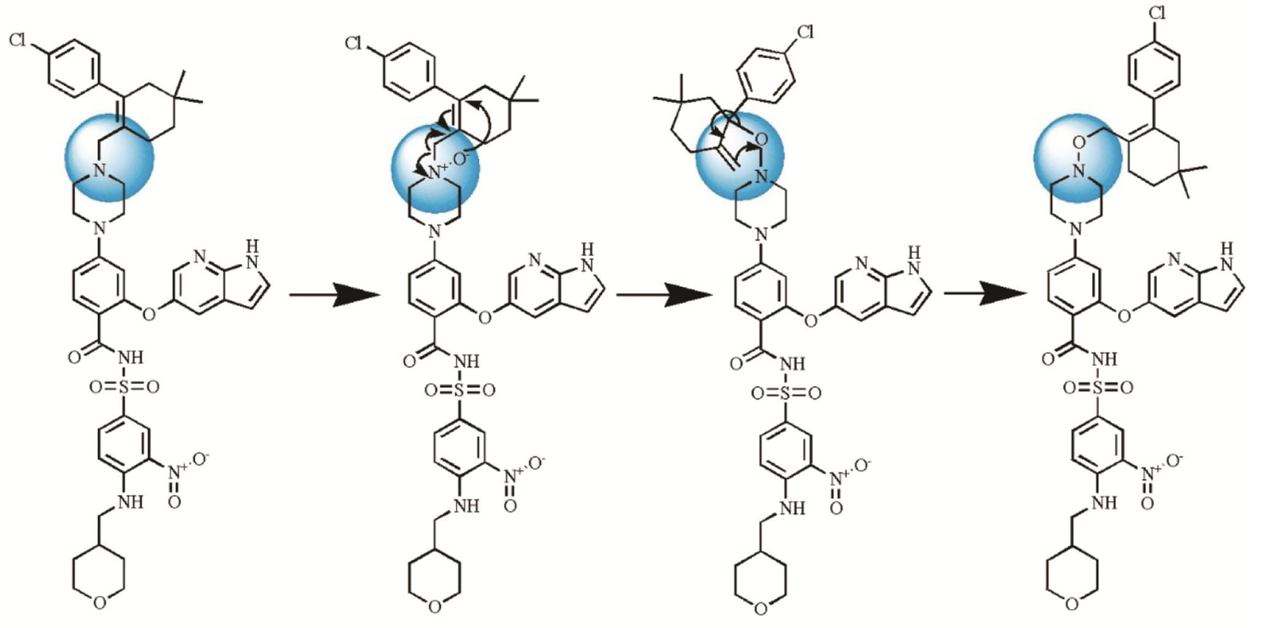
Vnetoclax VNO (2) VHA (3) VHA (4)

Fig. 12 — Proton numbering of the VNO **2**, VHA **3** and VHA **4**

yield. The mass spectrum of VHA **4** observed a peak at 884.37 in the positive mode, which confirms the compound formation. Then, we characterized the sample using ^{1}H NMR. In the ^{1}H NMR spectrum (Fig. 10), we observed a change in the chemical shift values of three methylene protons attached to the nitrogen of the piperazine ring (H-10, H-11, and H-14). Protons attached to H-10 were shifted from δ 5.06-5.10 to 3.89. H-11 and H-14 protons were shifted from δ 2.52-2.58 to 3.07, and H-11′ and H-14′ were shifted from δ 3.04-3.06 (1H), 3.45-3.48 (1H) to 2.50. These chemical shift values were different when compared with those of the VHA **3**. The HPLC analysis confirms the formation of VHA **4** at 57.96 RT and 1.02 RRT with 95% purity. Though the molecular weight of the VHA **3** and VHA **4** are same, they differ in RRT and chemical shift values in proton NMR. This confirms that the VNO **2** initially undergo [2,3] Meisenheimer rearrangement and forms VHA **3** and this further undergo [1,2] Meisenheimer rearrangement under harsh condition to afford VHA **4**. The VHA **4** was synthesised with 80-85% overall yield in 3 steps.

In the ^{13}C NMR spectrum of the VHA **4** impurity, a significant downfield shift was observed for the methylene carbon at δ 71.7, corresponding to the C-15 (−CH2_2) group adjacent to the piperazine ring. This chemical shift is notably higher than the corresponding signal in Venetoclax (δ 59.6) and its N-oxide derivative (VNO impurity, δ 70.0). This was due to the [1,2] Meisenheimer rearrangement of Venetoclax *N*-oxide to form the VHA impurity. In addition, the C-16 and C-19 methylene carbons, both α to the nitrogen atom, exhibited downfield shifts from δ 52.0 (in Venetoclax) to δ 54.7 in the VHA impurity.

The first step in the process involves the formation of an N-oxide intermediate. This is typically achieved by oxidizing the nitrogen atom of piperazine in Venetoclax **1** using *m*-CPBA. Upon oxidation, the N-oxide [VNO **2**] is formed, which then undergoes a [2,3]-sigmatropic rearrangement *via* a concerted mechanism, resulting in the Meisenheimer [2,3]-rearrangement product [VNO **3**] (Fig. 12). Subsequently, the intermediate [VHA **3**] undergoes further rearrangement through a concerted mechanism, as illustrated in Fig. 13, leading to the formation of the more stable [VHA **4**].



Venetoclax (1) **VNO (2)** **VHA (3)** **VHA (4)**

Fig. 13 — Plausible mechanism for the formation of the VHA (4)

Conclusion

In this study, we successfully synthesized a novel impurity, 2-(1*H*-pyrrolo[2,3-b]pyridin-5-yloxy)-4-(4-(1-(4-chlorophenyl)-5,5-dimethyl-2-methylenecyclohexyloxy)piperazin-1-yl)-N-(3-nitro-4-((tetrahydro-2*H*-pyran-4-yl)methylamino) phenyl sulfonyl)benzamide VHA **3** and demonstrated that its rearrangement leads to the formation of VHA **4** with quantitative yield. This comprehensive synthetic strategy provides a reliable method for preparing three Venetoclax impurities—VNO **2**, VHA **3**, and VHA **4**. These impurities were thoroughly characterized through ^{1}H NMR, ^{13}C NMR, HPLC, and mass spectrometry analysis.

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