*molbank*

MDPI

*Communication*

# 6-Imino-1,2,3,4,8,9,10,11-octahydropyrido[1,2-*a*]pyrido imidazo[4,5-*f*]benzimidazole-13-one: Synthesis and Cytotoxicity Evaluation

**Darren Conboy and Fawaz Aldabbagh ***

Department of Pharmacy, School of Life Sciences, Pharmacy and Chemistry, Kingston University, Penrhyn Road, Kingston upon Thames KT1 2EE, UK; k1743478@kingston.ac.uk

* Correspondence: f.aldabbagh@kingston.ac.uk

Received: 23 February 2020; Accepted: 4 March 2020; Published: 5 March 2020

check for updates

**Abstract:** The first report of an iminoquinone of imidazo[4,5-*f*]benzimidazole is described. The 2D-NOESY spectrum of 1,2,3,4,8,9,10,11-octahydropyrido[1,2-*a*]pyrido[1′,2′:1,2]imidazo benzimidazol-6-amine was used to confirm the location of the imine moiety at the C-6 position of the title compound. Cytotoxicity data from the National Cancer Institute are included.

**Keywords:** imidazobenzimidazoles; Frémy oxidation; nitrogen heterocycles; quinone

## 1. Introduction

NAD(P)H:quinone oxidoreductase 1 (NQO1), also known as DT-diaphorase, is an enzyme responsible for detoxification and bioreduction of quinone prodrugs to give hydroquinone [1]. Imidazobenzimidazoles can exist in either the 4,5-*f* or 5,4-*f* arrangement, and the Skibo group was the first to report anti-tumour activity for five- and six-membered ring-fused imidazo[4,5-*f*]benzimidazolequinones (e.g., **1**, Figure 1) [2,3]. Computational docking into the NQO1 active site rationalized differential cytotoxicity of imidazo[4,5-*f*]benzimidazolequinones [3]. Our group provided synthetic routes to enable efficient access to both 4,5-*f* and 5,4-*f* imidazobenzimidazolequinones [4–6].

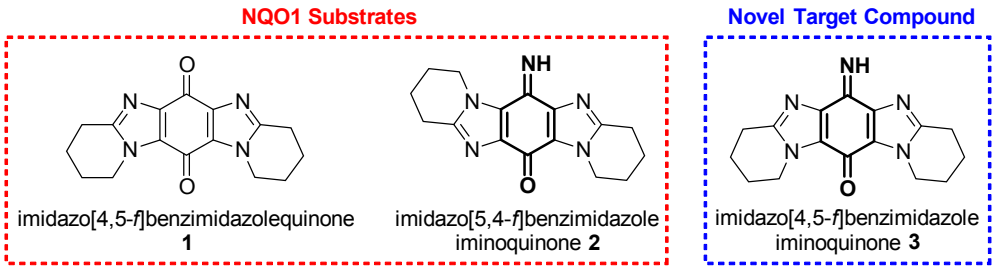


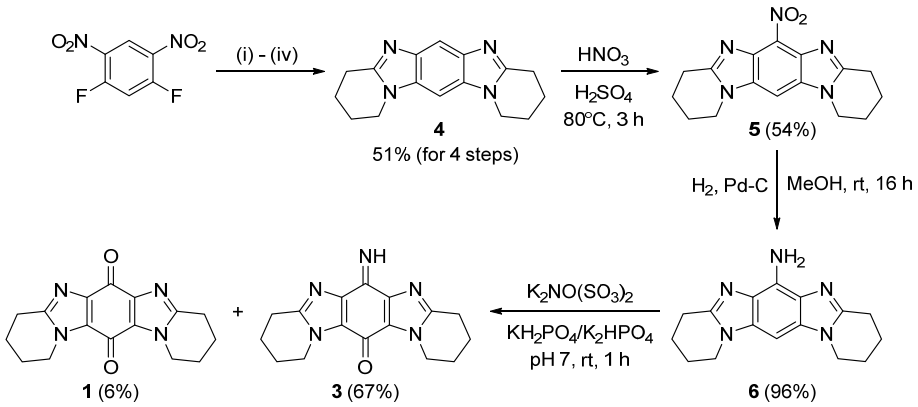

**Figure 1.** Rational drug design: targeting of iminoquinone **3** based on reported NAD(P)H:quinone oxidoreductase 1 (NQO1) substrates.

Iminoquinone **2** was unexpectedly isolated en route to the imidazo[5,4-*f*]benzimidazolequinone (see below) [4], and found to have exceptional and variable cytotoxicity against the 60 human cell lines of the developmental therapeutics program (DTP) at the National Cancer Institute (NCI) [6]. NCI COMPARE analysis and computational docking of **2** gave moderate compatibility to human NQO1. We now disclose novel isomeric iminoquinone **3**, synthesis, spectroscopic characterization, and cytotoxicity at DTP-NCI.

## 2. Results and Discussion

### *2.1. Synthesis*

The multi-step synthesis described by Fagan and Aldabbagh for imidazo[5,4-*f*] benzimidazolequinones was modified to give 6-imino-1,2,3,4,8,9,10,11-octahydropyrido pyrido[1′,2′:1,2]imidazo[4,5-*f*]benzimidazole-13-one (**3**) (Scheme 1) [5]. The first four steps furnished imidazo[4,5-*f*]benzimidazole **4**, in an overall yield of 51% without the characterization of reaction intermediates. Nitric and sulfuric acid (1:1 mixture) were used in the nitration of **4** to give 6-nitroimidazo[4,5-*f*]benzimidazole **5** in a 54% yield, alongside trace amounts of the 13-nitro and dinitro isomers, which were discarded after column chromatography. Atmospheric-pressure catalytic hydrogenation gave the novel aromatic amine 1,2,3,4,8,9,10,11-octahydropyrido[1,2-*a*]pyrido[1′,2′:1,2]imidazo[4,5-*f*]benzimidazol-6-amine (**6**), in an almost quantitative yield of 96%. The regioselectivity of the nitration/reduction to give **6** was determined from the 2D NOESY spectrum with through-space $^{1}$H-$^{1}$H coupling of the aromatic 13-H to 1-$CH_2$ and 11-$CH_2$ of **6** (Figure 2).




**Scheme 1.** Synthesis of imidazo[4,5-*f*]benzimidazole(imino)quinone **3**: (**i**) piperidine, $NaHCO_3$, THF, rt, 1 h; (**ii**) $H_2$ (balloon), Pd-C, EtOAc, rt, 16 h; (**iii**) $Ac_2O$, AcOH, 80 °C, 30 min; (**iv**) Oxone ($KHSO_5 \cdot 0.5KHSO_4 \cdot 0.5K_2SO_4$), AcOH, 40 °C, 7 h.

Iminoquinone **2** was previously obtained by Frémy salt ($K_2NO(SO_3)_2$) oxidation of the aromatic amine precursor at pH 4 [4]. Under the same buffered conditions, amine **6** gave only the undesired quinone **1**, as a result of acidic hydrolysis. Carrying out the Frémy oxidation at neutral pH, however, formed the desired iminoquinone **3** in 61% yield, along with quinone **1**, separated by column chromatography in 8% yield. For isolated **3**, the triplet at 4.31 ppm in the $^{1}$H NMR, and the peaks at 151.3, 142.4 and 130.2 ppm in the $^{13}$C NMR arise from the presence of minor quantities of quinone **1** [4], which formed upon acidic hydrolysis of the iminoquinone on silica gel using 'conventional' column chromatography (see NMR spectra in Supplementary Materials). Deactivating the silica with triethylamine prior to elution gave spectroscopically pure compound **3**, in an improved yield of 67%, along with quinone **1** in a 6% yield. The imine group of **3** was observed, with N-*H* at ~11 ppm in the $^{1}$H NMR spectrum, and *C*=N at 157 ppm in the $^{13}$C NMR spectrum, in accordance with reported data for iminoquinone **2** [4]. It is noteworthy that the non-equivalence of $CH_2$ signals for the fused pyrido-rings of **3** are not observed in the NMR spectra, presumably due to the greater rates of exchange of the N-H signals in comparison to the NMR frequency difference of the proton resonances in the separate environments. Further investigations were limited by the sparing solubility of **3** in $CDCl_3$, with the NMR spectra taken at the compound's solubility limit (see Supplementary Materials).

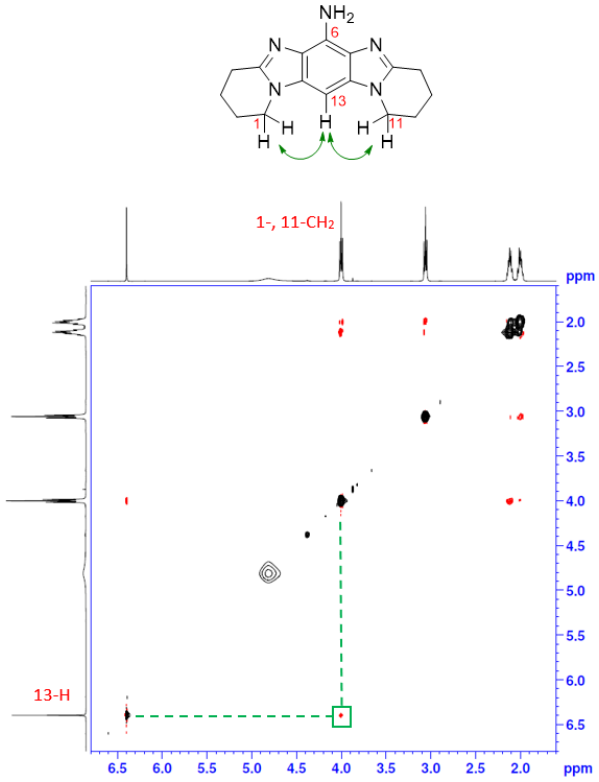



**Figure 2.** NOESY spectrum of aromatic amine **6**, with highlighted through-space $^{1}$H-$^{1}$H coupling.

### *2.2. Cytotoxicity*

The cytotoxicity of iminoquinone **3** was evaluated at the DTP-NCI, USA [2,6,7]. One-dose testing at 10 μM concentration against the 60-cancer cell line panel showed negligible cytotoxicity (see Supplementary Materials). This is in contrast to isomeric iminoquinone **2**, which displayed significant cytotoxicity against several solid tumour cell lines [6], and was selected for subsequent five dose testing. Given the low toxicity, no further biochemical assays and computational docking studies were carried out on iminoquinone **3**.

## 3. Materials and Methods

### *3.1. Materials and Measurements*

Piperidine (Sigma Aldrich, 99%, St. Louis, MO, USA), $NaHCO_3$ (Fisher Scientific, ≥99.7%, Waltham, MA, USA), 1,5-difluoro-2,4-dinitrobenzene (Sigma Aldrich, 97%), Pd-C (Sigma Aldrich, 10 wt% loading), EtOAc (VWR, 99.9%), $Ac_2O$ (ACROS Organics™, 99+%), AcOH (VWR, glacial), Oxone (Sigma Aldrich, $KHSO_5{\cdot}0.5KHSO_4{\cdot}0.5K_2SO_4$), $Na_2CO_3$ (Fisher Scientific, 99.5%), $CH_2Cl_2$ (Fisher Scientific, 99.8%), $HNO_3$ (Fisher Scientific, 70%), $H_2SO_4$ (Fisher Scientific, ≥95%), MeOH (VWR, ≥99.8%) and potassium nitrosodisulfonate (Sigma Aldrich) were used as received. THF was freshly distilled over Na and benzophenone prior to use. Thin layer chromatography (TLC) was performed on TLC silica gel 60 $F_{254}$ plates. 'Conventional' flash column chromatography was carried out on silica gel (Apollo Scientific 60/40−63 μm), and deactivated by making a slurry using $Et_3N$ in EtOAc (2% *v*/*v*), for the purification of title compound **3**. Melting points were measured on a Stuart Scientific melting point apparatus SMP1. IR spectra were recorded using a PerkinElmer Spec 1 with ATR (Perkin-Elmer, Waltham, MA, USA) attached. NMR spectra were recorded using a Bruker Avance II 400 MHz spectrometer (Bruker Corporation, Billerica, MA, USA). The chemical shifts are in ppm, relative to tetramethylsilane. $^{13}$C NMR spectra at 100 MHz are with complete proton decoupling. NMR assignments are supported by distortionless enhancement by polarization transfer (DEPT). The HRMS

spectrum of iminoquinone **3** was obtained at the National University of Ireland Galway, using an ESI time-of-flight mass spectrometer (TOFMS) on a Waters LCT Mass Spectrometry instrument, while the HRMS spectrum of aromatic amine **6** was obtained at the National Mass Spectrometry Facility at Swansea University using a Waters Xevo G2-S mass spectrometer (Waters, Milford, MA, USA) with an atmospheric solids analysis probe (ASAP). The precision of all accurate mass measurements was better than 5 ppm.

### *3.2. Synthetic Procedures*

#### 3.2.1. Synthesis of 1,2,3,4,8,9,10,11-Octahydropyrido[1,2-*a*]pyrido[1′,2′:1,2]imidazo[4,5-*f*] benzimidazole (**4**)

Piperidine (7.50 mL, 75.9 mmol), $NaHCO_3$ (4.210 g, 50.1 mmol) and 1,5-difluoro-2,4-dinitrobenzene (2.042 g, 10.0 mmol) in THF (80 mL) were stirred at room temperature for 1 h. Water (200 mL) was added and the precipitate was collected, washed with water and dried. The yellow solid was stirred with Pd-C (0.206 g) in EtOAc (100 mL) under an atmosphere of $H_2$ (1 atm; balloon) for 16 h. Upon filtering the solution through Celite and evaporating the filtrate to dryness, $Ac_2O$ (9.45 mL, 0.100 mol) and AcOH (100 mL) were added, and the solution was stirred at 80 °C for 30 min. The mixture was evaporated, $NaHCO_3$ (5% aq, 150 mL) was added, and the mixture was stirred for 1 h. The precipitate was collected, washed with water, and re-dissolved in AcOH (80 mL). Oxone (18.225 g, 59.3 mmol) was added, and the mixture was stirred at 40 °C for 7 h. The solution was evaporated, $H_2O$ (100 mL) was added, neutralized with solid $Na_2CO_3$, and extracted with $CH_2Cl_2$ (3 × 100 mL). The organic extracts were dried ($MgSO_4$) and evaporated to dryness to give the title compound **4** (1.358 g, 51%), as an off-white solid; m.p. decomp. >264 °C; lit. m.p. decomp. 266–270 °C [4]; spectral data was consistent with the literature (Supplementary Materials) [4].

#### 3.2.2. Synthesis of 6-Nitro-1,2,3,4,8,9,10,11-octahydropyrido[1,2-*a*]pyrido[1′,2′:1,2]imidazo[4,5-*f*] benzimidazole (**5**)

Imidazo[4,5-*f*]benzimidazole **4** (0.963 g, 3.6 mmol), $HNO_3$ (40 mL) and $H_2SO_4$ (40 mL) were stirred at 80 °C for 3 h. The mixture was diluted with $H_2O$ (300 mL), neutralized with solid $Na_2CO_3$, and extracted with $CH_2Cl_2$ (4 × 100 mL). The organic extracts were dried ($MgSO_4$), evaporated, and purified by column chromatography using gradient elution of EtOAc/MeOH to give the title compound **5** (0.608 g, 54%) as a yellow solid; $R_f$ 0.29 (9:1 EtOAc/MeOH); m.p. decomp. >238 °C; lit. m.p. decomp. 230–232 °C [3]; spectral data was consistent with the literature (Supplementary Materials) [3].

#### 3.2.3. Syntheis of 1,2,3,4,8,9,10,11-Octahydropyrido[1,2-*a*]pyrido[1′,2′:1,2]imidazo[4,5-*f*] benzimidazol-6-amine (**6**)

6-Nitroimidazo[4,5-*f*]benzimidazole **5** (0.502 g, 1.6 mmol), and Pd-C (50 mg) in MeOH (100 mL) were stirred under $H_2$ at room temperature for 16 h. The mixture was filtered through Celite and evaporated to dryness to give the title compound **6** (0.432 g, 96%) as an off-white solid; m.p. decomp. >215 °C; $\nu_{max}$ (neat, $cm^{-1}$) 3421, 3308 (both $NH_2$), 3237, 3205, 2951, 2936, 2877, 2864, 2831, 1694, 1683, 1630, 1596, 1532, 1513, 1484, 1456, 1439, 1426, 1385, 1345, 1324, 1283, 1255, 1218, 1164; $^1$H NMR (400 MHz, $CDCl_3$) δ: 6.40 (s, 1H), 5.04–4.56 (br.s, disappears with $D_2O$, 2H, $NH_2$), 4.00 (t, $J$ = 6.1 Hz, 4H, 1,11-$CH_2$), 3.06 (t, $J$ = 6.4 Hz, 4H, 4,8-$CH_2$), 2.16–2.07 (m, 4H), 2.04–1.95 (m, 4H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 148.9, 133.0, 127.7, 126.4 (all C), 76.1 (CH), 42.6, 25.6, 22.8, 20.9 (all $CH_2$); HRMS (ASAP) $m/z$ $[M + H]^+$ found 282.1717, $C_{16}H_{20}N_5$ requires 282.1719.

#### 3.2.4. Frémy Oxidation of **6** to Give Title Compound **3**

Potassium nitrosodisulfonate (1.012 g, 3.77 mmol) in phosphate buffer (0.2 M, pH 7, 80 mL) was added to amine **6** (0.352 g, 1.25 mmol) in the same buffer (40 mL), and stirred at room temperature for 1 h. The mixture was extracted with $CH_2Cl_2$ (4 × 90 mL), and the organic extracts were dried ($MgSO_4$),

evaporated, and purified by column chromatography using a gradient elution of EtOAc/MeOH/$Et_3N$ to give *1,2,3,4,8,9,10,11-octahydropyrido[1,2-a]pyrido[1′,2′:1,2]imidazo[4,5-f]benzimidazol-6,13-dione* (**1**) (22 mg, 6%); orange solid; $R_f$ 0.32 (9:1 EtOAc/MeOH); the spectral data and melting point were consistent with the literature [4]. Further elution gave *6-imino-1,2,3,4,8,9,10,11-octahydropyrido[1,2-a]pyrido[1′,2′:1,2] imidazo[4,5-f]benzimidazole-13-one* (**3**) (0.248 g, 67%); yellow solid; $R_f$ 0.21 (9:1 EtOAc/MeOH); m.p. decomp. >225 °C; $\nu_{max}$ (neat, $cm^{-1}$) 2946, 1637 (C=O), 1482, 1422, 1304, 1264, 1166, 1021; $^1H$ NMR (400 MHz, $CDCl_3$) δ: 11.32–10.60 (br.s, disappears with $D_2O$, 1H, NH), 4.34 (t, $J$ = 5.9 Hz, 4H, 1,11-$CH_2$), 2.99 (t, $J$ = 6.3 Hz, 4H, 4,8-$CH_2$), 2.10–1.92 (m, 8H); $^{13}C$ NMR (100 MHz, $CDCl_3$) δ: 169.4 (C=O), 157.1 (C=N), 150.7, 142.3, 127.0 (all C), 45.3, 25.0, 22.4, 19.8 (all $CH_2$); HRMS (ESI) $m/z$ $[M + H]^+$ found 296.1506, $C_{16}H_{18}N_5O$ requires 296.1511.

**Supplementary Materials:** The following are available online, $^1H$ NMR spectrum of 1,2,3,4,8,9,10,11-octahydropyrido[1,2-*a*]pyrido[1′,2′:1,2]imidazo[4,5-*f*]benzimidazole (**4**), $^1H$ NMR spectrum of 6-nitro-1,2,3,4,8,9,10,11-octahydropyrido[1,2-*a*]pyrido[1′,2′:1,2]imidazo[4,5-*f*]benzimidazole (**5**), $^1H$ NMR, $^{13}C$ NMR and $^1H$-$^1H$ NOESY spectra of 1,2,3,4,8,9,10,11-octahydropyrido[1,2-*a*]pyrido[1′,2′:1,2]imidazo[4,5-*f*]benzimidazol-6-amine (**6**), $^1H$ NMR and $^{13}C$ NMR spectra of 6-imino-1,2,3,4,8,9,10,11-octahydropyrido[1,2-*a*]pyrido[1′,2′:1,2]imidazo benzimidazole-13-one (**3**) after purification by 'conventional' flash column chromatography on silica gel and after purification by flash column chromatography on silica gel deactivated with $Et_3N$, and DTP-NCI-60 mean growth percent graph for 6-imino-1,2,3,4,8,9,10,11-octahydropyridopyrido[1′,2′:1,2]imidazo[4,5-*f*]benzimidazole-13-one (**3**).

**Author Contributions:** D.C. was the only experimentalist, who obtained and analysed all data. F.A. directed the research, reviewed the data, and wrote the manuscript with D.C. All authors have read and agreed to the published version of the manuscript.

**Funding:** There is no external funding for this research.

**Acknowledgments:** We thank Kingston University for a PhD Scholarship for D.C.

**Conflicts of Interest:** The authors declare no conflict of interest.

## References

1. Zhang, K.; Chen, D.; Ma, K.; Wu, X.; Hao, H.; Jiang, S. NAD(P)H:quinone oxidoreductase 1 (NQO1) as a therapeutic and diagnostic target in cancer. *J. Med. Chem.* **2018**, *61*, 6983–7003. [CrossRef] [PubMed]
2. Schulz, W.G.; Skibo, E.B. Inhibitors of topoisomerase II based on the benzodiimidazole and dipyrroloimidazobenzimidazole ring systems: Controlling DT-diaphorase reductive inactivation with steric bulk. *J. Med. Chem.* **2000**, *43*, 629–638. [CrossRef]
3. Suleman, A.; Skibo, E.B. A comprehensive study of the active site residues of DT-diaphorase: Rational design of benzimidazolediones as DT-diaphorase substrates. *J. Med. Chem.* **2002**, *45*, 1211–1220. [CrossRef]
4. Fagan, V.; Bonham, S.; Carty, M.P.; Aldabbagh, F. One-pot double intramolecular homolytic aromatic substitution routes to dialicyclic ring fused imidazobenzimidazolequinones and preliminary analysis of anticancer activity. *Org. Biomol. Chem.* **2010**, *8*, 3149–3156. [CrossRef] [PubMed]
5. Fagan, V.; Bonham, S.; McArdle, P.; Carty, M.P.; Aldabbagh, F. Synthesis and toxicity of new ring-fused imidazo[5,4-*f*]benzimidazolequinones and mechanism using amine *N*-oxide cyclizations. *Eur. J. Org. Chem.* **2012**, 1967–1975. [CrossRef]
6. Fagan, V.; Bonham, S.; Carty, M.P.; Saenz-Méndez, P.; Eriksson, L.A.; Aldabbagh, F. COMPARE analysis of the toxicity of an iminoquinone derivative of the imidazo[5,4-*f*]benzimidazoles with NAD(P)H:quinone oxidoreductase 1 (NQO1) activity and computational docking of quinones as NQO1 substrates. *Bioorg. Med. Chem.* **2012**, *20*, 3223–3232. [CrossRef] [PubMed]
7. Sweeney, M.; Coyle, R.; Kavanagh, P.; Berezin, A.A.; Lo Re, D.; Zissimou, G.A.; Koutentis, P.A.; Carty, M.P.; Aldabbagh, F. Discovery of anti-cancer activity for benzo[1,2,4]triazin-7-ones: Very strong correlation to pleurotin and thioredoxin reductase inhibition. *Bioorg. Med. Chem.* **2016**, *24*, 3565–3570. [CrossRef] [PubMed]