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Synthesis of the HIF-2α Translation Inhibitor Compound **76** *via* a Japp-Klingemann coupling

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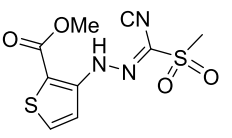
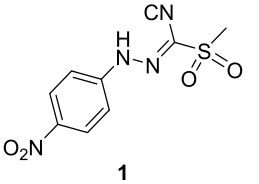
Abstract. The Iliopoulos laboratory previously reported the discovery of compound **76**, a small molecule HIF2α inhibitor. HIF2α activation is known to play a critical role in both von Hippel-Lindau (VHL) disease-related tumors and sporadic renal cell carcinoma (RCC). We required a facile and scalable synthetic route to confirm the structure, to investigate the biological properties of compound **76**, and to perform structure activity relationship optimization studies. Herein, we report the straightforward synthesis of compound **76** and a preliminary investigation into the scope of the methods developed.

Patients with von Hippel-Lindau (VHL) disease possess a germline mutation in the VHL tumor suppressor gene that confers a life-time risk of developing renal cell carcinoma (RCC), central nervous system hemangioblastomas, pheochromocytomas and pancreatic neuroendocrine tumors [1]. The VHL protein targets the Hypoxia Inducible Factors 1 and 2 (HIF1α and HIF2α) for proteasomal degradation in cells exposed to a normal range of oxygen concentration. However, low oxygen concentration (hypoxia) or loss-of-function VHL mutations lead to HIF1α/2α stabilization and transactivation of HIF-target genes. Collectively, the expression of HIF1α/2α target genes contributes to oncogenic processes, such as angiogenesis, erythropoiesis, reprogramming of metabolism, cell proliferation, and metastasis [2]. In RCC, it has been shown that HIF2α acts as an oncogene, while HIF1α is a tumor suppressor gene [3]. There are currently no drugs available to treat VHL disease. The inhibition of HIF2α by a small molecule drug is a novel therapeutic strategy for the treatment of VHL disease and HIF2α-driven tumors. The Iliopoulos laboratory conducted a high throughput screen (HTS) to discover specific HIF2α inhibitors [4]. The screen was based on a human, VHL-deficient, RCC cell line stably infected with a HIF2α-driven luciferase reporter. From this screen, a small molecule HIF2α inhibitor called 'compound **76**' was identified (**Figure 1**). Compound **76** operated by enhancing the binding of iron regulatory protein 1 (IRP1) to an iron regulatory element (IRE) in the 5′-UTR of HIF2α, but not HIF1α, mRNA, thereby specifically repressing HIF2α translation. Furthermore, treatment of VHL-/- zebrafish embryos with compound **76** rescued the RCC-reminiscent pronephric abnormalities, highlighting its potential as a tool to study VHL disease and RCC [5].

Figure 1. Structures of compound **76** and **1**.



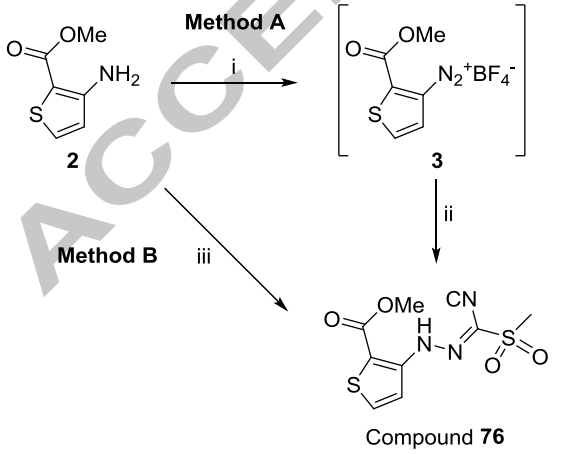
Compound **76**



1

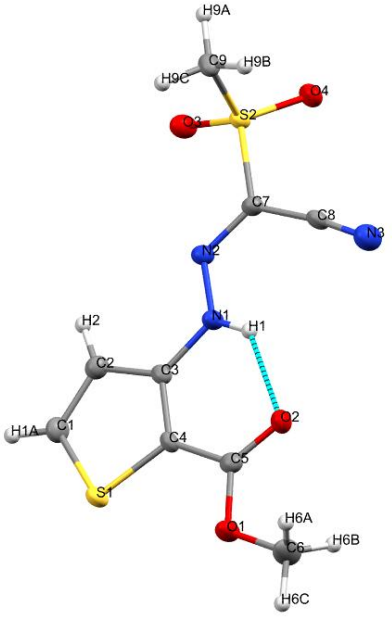
Structurally, compound **76** is a thiophene substituted at the 2-position by a methyl ester and at the 3-position by a cyano(methanesulfonyl)-hydrazine group. The cyano(methanesulfonyl)-hydrazine moiety is very uncommon; the only other occurrence of this group in the literature was the nitrophenyl derivative **1** reported in 1953 by Hunig and Boes [6]. Although compound **76** is commercially available and is included in HTS screening collections [4], there were no published syntheses of compound **76**. To confirm the structure of compound **76**, and to further explore its biology, we required a synthesis and a route that would allow for the synthesis of new analogs. The syntheses of aryl hydrazones typically have been achieved by two ways: (i) the condensation of an aryl hydrazine with a ketone or aldehydes; or (ii) *via* a Japp-Klingemann coupling [7]. There were very few examples of the syntheses and use of unstable α-oxo sulfones [8], which would allow us to form compound **76** from a starting aryl hydrazine. Therefore, we investigated the use of a Japp-Klingemann coupling with a sulfonylacetonitrile moiety. To our delight, we were able to synthesize compound **76** in moderate yields using the two different synthetic methods outlined in **Scheme 1**.

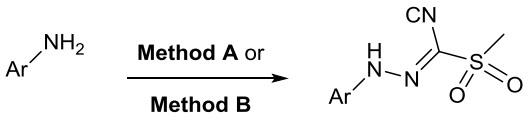
Scheme 1. Reagents and conditions: (i) $NaNO_2$, HBF_4 (aq.), MeCN, 0 ^oC; (ii) (methylsulfonyl)acetonitrile then DIPEA, 0 ^oC to RT (62% yield); (iii) (methylsulfonyl)acetonitrile, ^{t}BuONO, TsOH, MeCN, RT (40% yield).



Treatment of the commercially available methyl 3-amino-2-thiophene carboxylate (**2**) with sodium nitrite and tetrafluoroboric acid in acetonitrile at 0 ^oC gave the intermediate diazonium salt **3**. Then, i*n situ* treatment of **3** with (methylsulfonyl)acetonitrile and *N*,*N*-diisopropylethylamine (DIPEA) gave compound **76** in 62% yield (Method A). An alternative one-pot procedure involved the treatment of **2** with *tert*-butyl nitrite and a catalytic amount of *p*-toluenesulfonic acid and gave compound **76** in a moderate 40% yield (Method B). The ^{1}H-NMR, ^{13}C-NMR, and mass spectral data of the products were consistent with the structure of compound **76**. Finally, the structure of compound **76** was confirmed by single crystal X-ray analysis (**Figure 2**) [9]. A single crystal was obtained by the vapour diffusion method from dimethylformamide and water. A key intramolecular hydrogen-bonding interaction [N(1)- H(1)···O(2), 2.04 Å] can be seen between the hydrazone N-H and the carbonyl oxygen of the ester, which forms a stabilized six-membered ring and gives the molecule a planar conformation. In addition, the *E*-configuration of the hydrazone was confirmed.

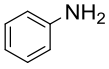
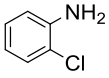
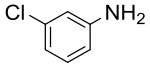
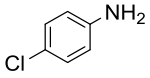
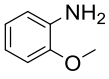
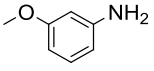
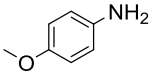
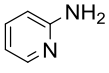
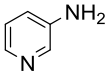
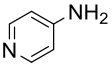
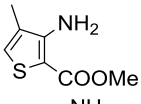
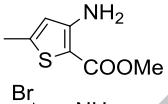
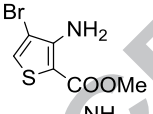
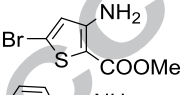
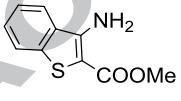
Figure 2. X-ray crystal structure confirms the structure of compound **76**. An intramolecular hydrogen-bonding interaction is denoted by the light blue line.





4 5

Table 1. Effect of substitutions on the arylamine.

#	4	Product	Yield (%)[a]
1		**5a**	82, 45
2		**5b**	37, 38
3		**5c**	32, 17
4		**5d**	52, 31
5		**5e**	43, 40
6		**5f**	47, 12
7		**5g**	47, 21
8		**5h**	0, 0[b]
9		**5i**	21, 0[b]
10		**5j**	0, 0[b]
11		**5k**	64, 49
12		**5l**	51, 51
13		**5m**	43, 37
14		**5n**	54, 42
15		**5o**	75, 23

[a] Isolated yields (Method A, Method B).
[b] No product detected on LC-MS after 24 hrs.

Investigation of the scope of the two methods was of interest to us, as we plan to synthesize analogs of compound **76** for structure activity relationship studies. Several substituted arylamine and aminothiophene substrates were explored, as shown in **Table 1** [11]. Method A proved to be the more robust synthetic procedure, providing the phenyl product **5a** in excellent yield and the different chloro- and methoxy-substituted products **5b-5g** in moderate to good yields. Method B generally gave lower yields, and this was most likely due to the products not precipitating as efficiently from the reaction as they did in Method A. Therefore, additional aqueous work-up, extraction and trituration or flash chromatography generally led to lower overall yields. In addition, three different amino-pyridine analogs were investigated, and only the reaction of 3-aminopyridine, utilizing Method A, gave the expected product **5i**, albeit in low yield. We initially hoped that Method B would work better with the aminopyridine derivatives, because both the pyridine-2-diazonium and pyridine-4-diazonium ions formed using Method A are likely to be unstable under the aqueous conditions and react to give the corresponding pyridone. Unfortunately, the one-pot, non-aqueous method did not result in the desired products either. By contrast, we were pleased to find that all four substituted thiophenes (products **5k**–**5n**) and the benzothiophene (product **5o**) were obtained in moderate to good yields, by both methods, following filtration with no further purification.

In conclusion, we developed a general and flexible synthesis of compound **76** to allow further investigation into its potential as a novel HIF2α suppressor and therapeutic for VHL disease and HIF2α-driven cancers, including RCC. Further work on structural-activity relationship and optimization of drug-like properties is forthcoming.

Acknowledgements. This work was supported by NIH 1R01CA215431 (to OI). Crystallographic data (excluding structure factors) for the structures in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication nos. CCDC 1884171. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK, (fax: +44-(0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).

Appendix A. Supplementary data. General procedures and analytical data for all new compounds is reported in the Supplementary data.

References

1. Iliopoulos O., *Front Horm Res*. **2001**, *28*, 131.
2. Keith B., Johnson R. S., Simon M. C. *Nat Rev Cancer* **2011**, *12*, 9.
3. Shen C., Beroukhim .R, Schumacher S. E., Zhou J., Chang M., Signoretti S., Kaelin W. G. *Cancer Discov.* **2012**, *1*, 222.
4. Zimmer, M., Ebert, B. L., Neil, C., Brenner, K., Papaioannou, I., Melas, A., Tolliday, N., Lamb, J., Pantopoulos, K., Golub, T., Iliopoulos, O. *Molecular cell* **2008**, *32*, 838.
5. Noonan, H. R., Metelo, A. M., Kamei, C. N., Peterson, R. T., Drummond, I. A., Iliopoulos, O. *Disease models and mechanisms* **2016**, *9*, 873.
6. Hunig, S., Boes, O. *Justus Liebigs Annalen der Chemie* **1953**, *579*, 28.
7. Phillips R. R. *Organic Reactions* (New York) **1959**, *10*, 143.
8. Schank, K.; Frisch, A.; Zwanenburg, B. *J. Org. Chem*. **1983**, *48*, 4580.
9. **X-Ray Data**. A crystal of compound **76** mounted on a diffractometer was collected data at 100 K. The intensities of the reflections were collected by means of a Bruker APEX II CCD diffractometer (MoKα radiation, λ=0.71073 Å), and equipped with an Oxford Cryosystems nitrogen flow apparatus. The collection method involved 0.5° scans in ω at 28° in 2θ. Data integration down to 0.78 Å resolution was carried out using SAINT V8.37A6 with reflection spot size optimization. Absorption corrections were made with the program SADABS.[10] The structure was solved by the Intrinsic Phasing methods and refined by least-squares methods again F2 using SHELXT-2014[11] and SHELXL-2014[12] with OLEX 2 interface[13]. Non-hydrogen atoms were refined anisotropically, and hydrogen atoms were allowed to ride on the respective atoms. Crystal data as well as details of data collection and refinement are summarized in Table 1, geometric parameters are shown in Table 2, and hydrogen-bond parameters are listed in Table 3. The Ortep plots produced with SHELXL-2014 program, and the other drawings were produced with Accelrys DS Visualizer 2.0[14].
10. Bruker AXS APEX3, Bruker AXS, Madison, Wisconsin, **2016**.
11. Sheldrick, G. M. *Acta Cryst.* **2015**. A71, 3.
12. Sheldrick, G. M. *Acta Cryst*. **2015**, C71, 3.
13. Dolomanov, O. V.; Bourhis, L. J.; Gildea, R. J.; Howard, J. A. K.; Puschmann, H. *J. Appl. Cryst.* **2009**, 42, 339.
14. Accelrys DS Visualizer v2.0.1, *Accelrys Software. Inc.*, **2007**.

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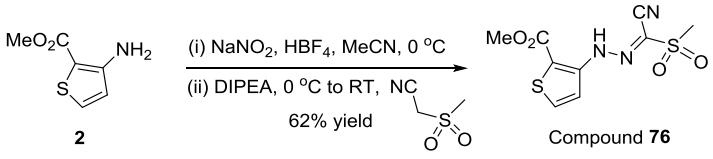

- Structure activity relationship studies were performed
- A straightforward synthesis of compound 76 was developed
- The structure of compound 76 was confirmed by single crystal X-ray