**Synthesis of glycolysis inhibitor (*E*)-3-(pyridin-3-yl)-1-(pyridin-4-yl)prop-2-en-1-one (**3PO**) and its** **inhibition of HUVEC proliferation alone or in a combination with the multi-kinase inhibitor** sunitinib

**Miroslav Murár1,Jana Horvathová2, Roman Moravčík2, Gabriela Addová3, Michal Zeman2, Andrej Boháč\*,1,4**

1 Department of Organic Chemistry, Faculty of Natural Sciences, Comenius University in Bratislava, Mlynská dolina, Ilkovičova 6, 842 15 Bratislava, Slovakia 2 Department of Animal Physiology and Ethology, Faculty of Natural Sciences, Comenius University in Bratislava, Mlynská dolina, Ilkovičova 6, 842 15 Bratislava, Slovakia and 3 Institute of chemistry, Faculty of Natural Sciences, Comenius University in Bratislava, Mlynská dolina, Ilkovičova 6, 842 15 Bratislava, Slovakia; 4 Biomagi, Ltd., Mamateyova 26, 851 04 Bratislava, Slovakia

\*Corresponding author, e-mail: andrej.bohac@fns.uniba.sk

The authors JH and AB contributed equally to writing this paper.

On behalf of all authors, the corresponding author states that there is no conflict of interest.

Contents

[Figures of 1H- and 13C-NMR spectra of 3PO 2](#_Toc515030950)

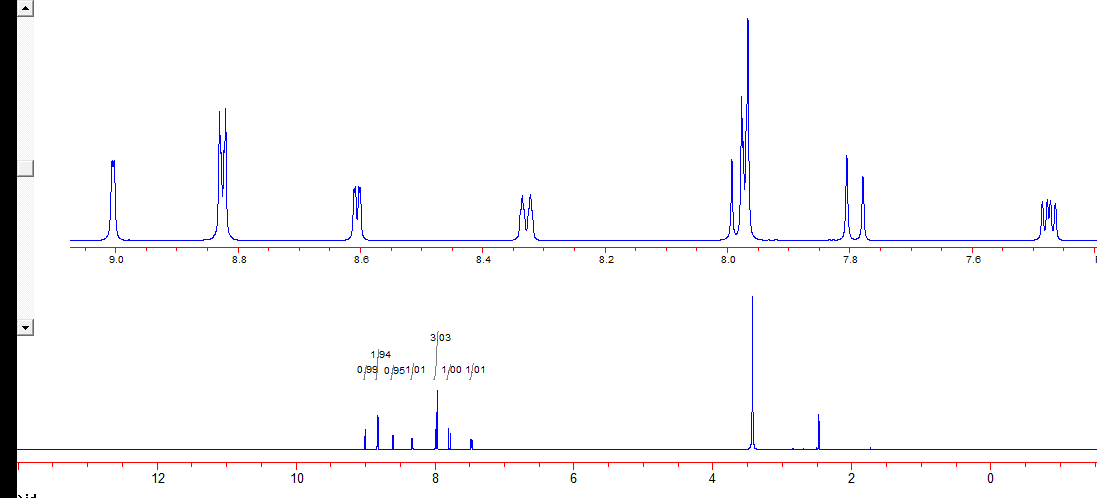
[Figures of HSQC, HMBCspectra of 3PO and its diagram of HMBC analysis 3](#_Toc515030951)

[Figures of IR and MS spectra of 3PO 4](#_Toc515030952)

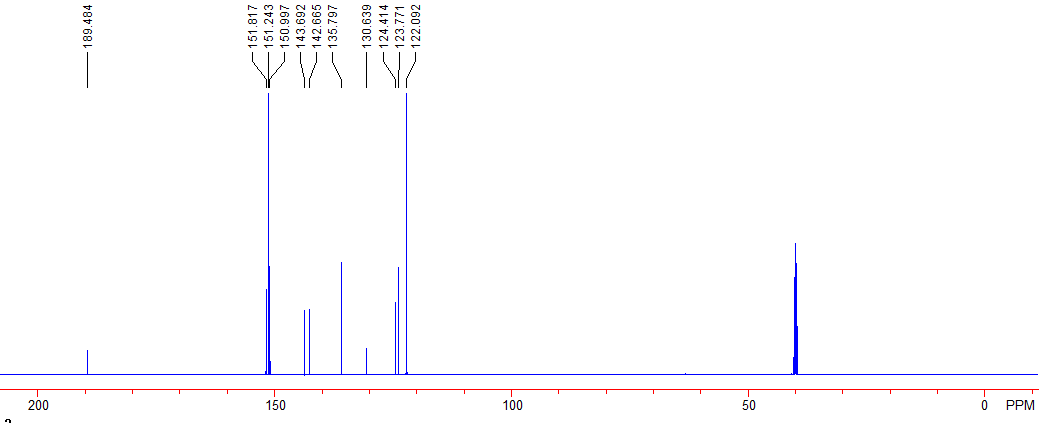
[A synthesis of 3PO in the presence of Et2NH 5](#_Toc515030953)

[A proposed enamine mechanism for the synthesis of 3PO in the presence of Et2NH 7](#_Toc515030954)

# Figures of 1H- and 13C-NMR spectra of 3PO

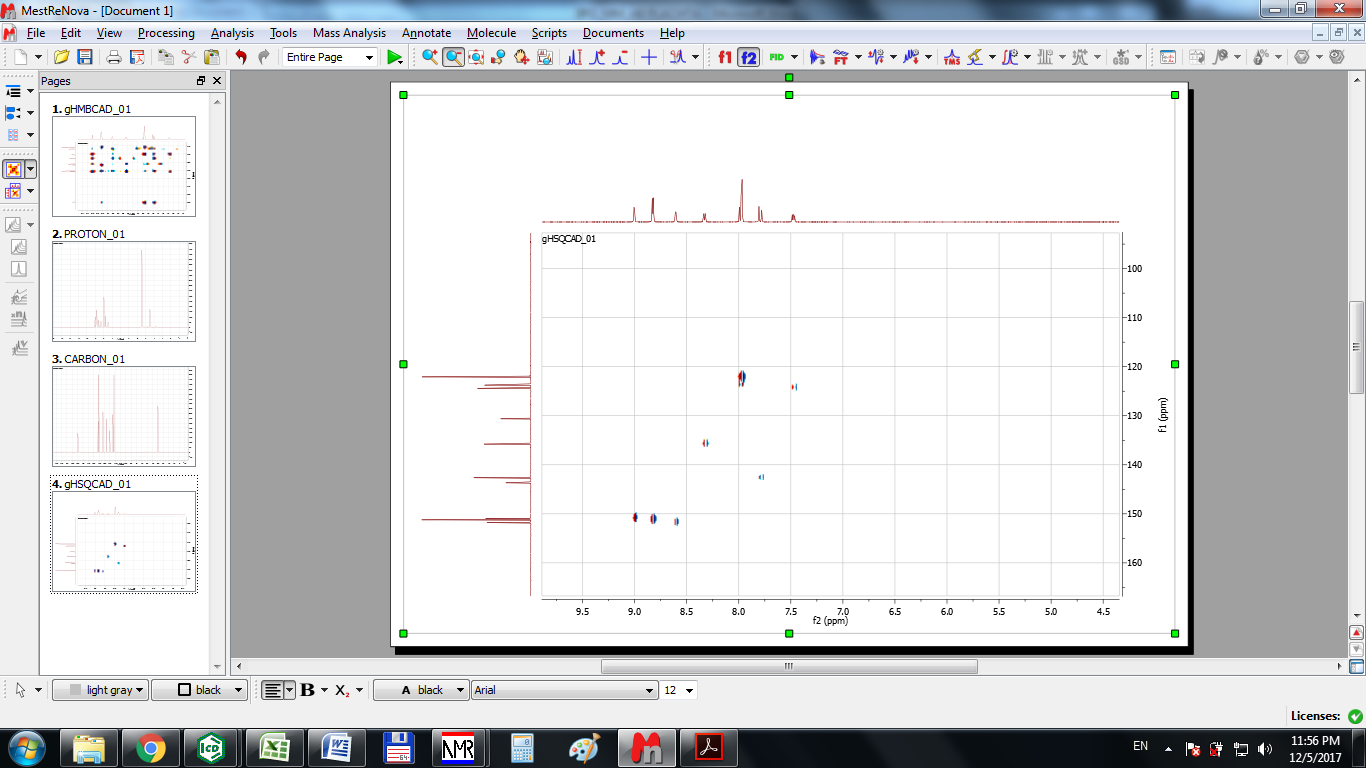


**Fig. S1** 1H-NMR(600 MHz, DMSO-*d6*) spectrum of **3PO** obtained by reaction with AcONa in refluxing AcOH and purified by crystallisation from EtOH



**Fig. S2** 13C-NMR(150 MHz, DMSO-*d6*) spectrum of **3PO**

# Figures of HSQC, HMBCspectra of 3PO and its diagram of HMBC analysis



**Fig. S3** HSQC **(**DMSO-*d6*) spectrum of **3PO**

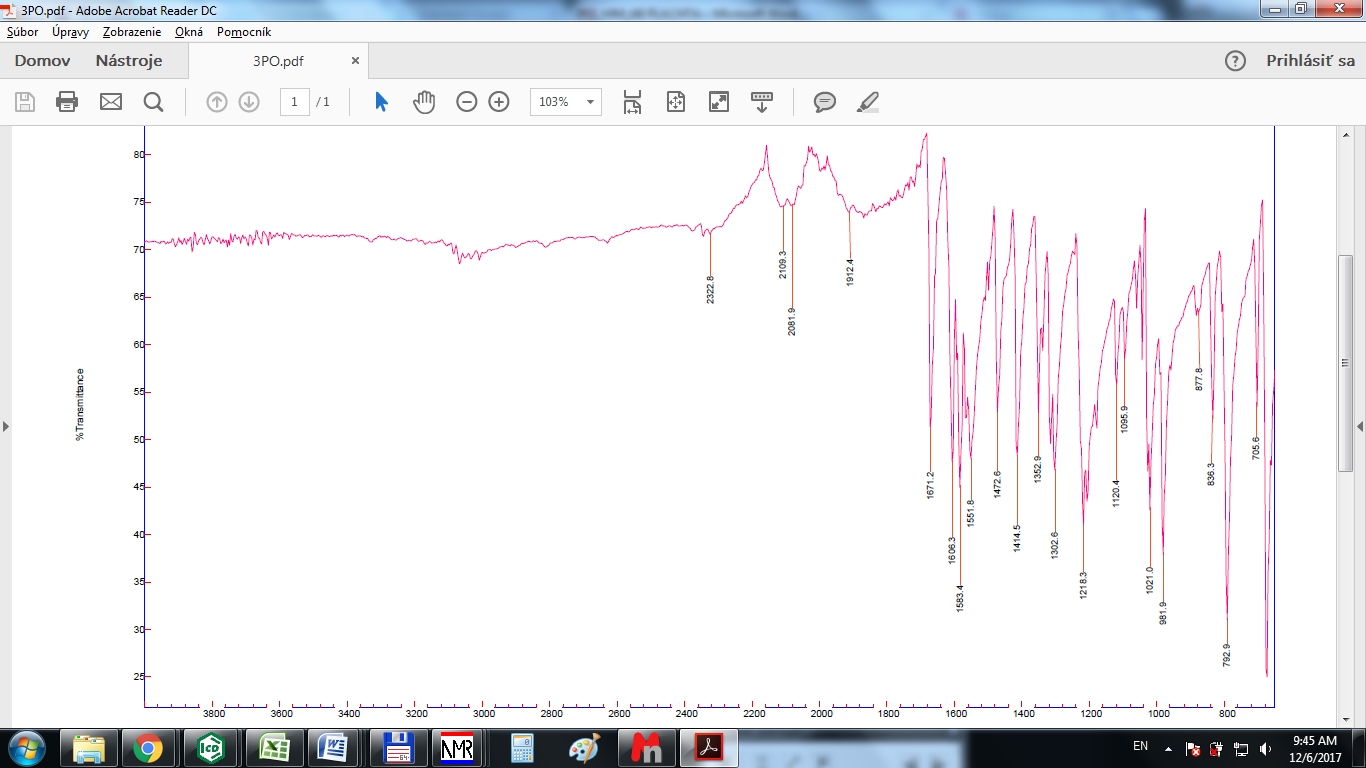


**Fig. S4** HMBC **(**DMSO-*d6*) spectrum of **3PO**

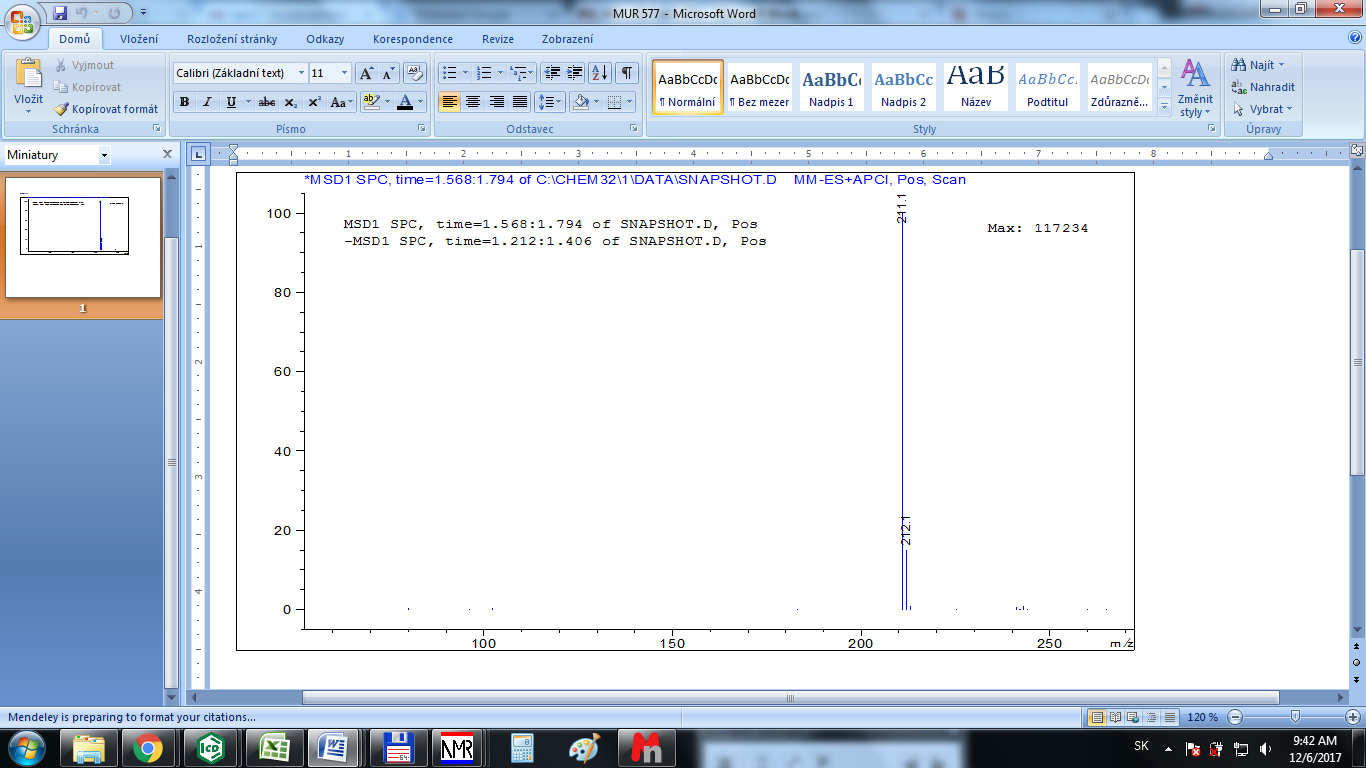


**Fig. S5** A diagram of paired interactions ofH-C signals in **3PO** from HMBC **(**DMSO-*d6*) spectrum

# Figures of IR and MS spectra of 3PO



**Fig. S6** FT IR(solid, cm-1) spectrum of **3PO**



**Fig. S7** MS (ESI m/z) spectrum of **3PO** obtained in a positive mode

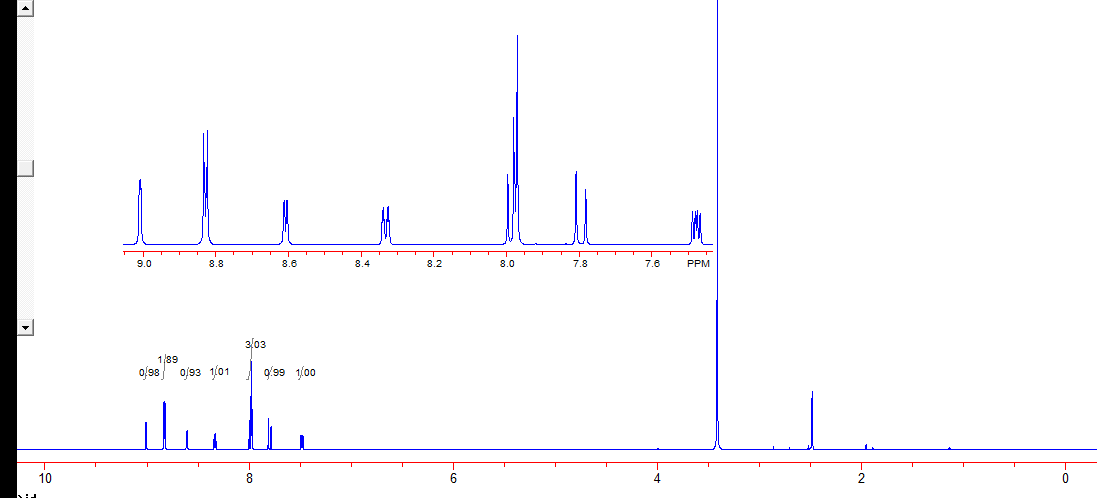
# A synthesis of 3PO in the presence of Et2NH

The synthesis of **3PO** was performed according the reaction described in the literature (Durinda et al. 1966 and 1967).



**Scheme S1** The synthesis of **3PO** from **1** and **2** by Et2NHat RT in THF

Diethylamine 171 uL (121 mg, 1.65 mmol, 1.00 mol eq) was added dropwise to a solution of 200 mg (1.65 mmol, 1.00 mol eq) pyridinylethanone **2** in 5 ml of THF (abs). The mixture was stirred at RT within 1 h. Afterwards a solution of 177 mg (1.65 mmol, 1.00 mol eq) nicotinaldehyde **1** in 5 ml of THF (abs) was added within 30 min and the reaction was stirred overnight at RT. Then TLC analysis confirmed presence of a new spot (comparable by its position with the standard of **3PO**) together with traces of starting materials **1** and **2** and four spots of more polar by-products. The mixture was cooled to 0 °C and 10 ml of 2 M HCl (aq) was added by stirring within 5 min. Then the reaction was adjusted to pH 8 by 10 % of NaOH (aq). The final mixture was extracted by EA (2 x 40 ml) and combined organic layer dried over Na2SO4, filtered and evaporated under vacuum by RVO to give 340 mg of a crude product that was further purified by FLC on Silica to yield 40.0 mg (0.19 mmol, 12 %) of product **3** (**3PO**) together with 30 mg (0.280 mmol, 17 %) of **1** and30 mg (0.247 mmol, 15 mol %) of un-reacted **2**. Obtained 1H-NMR (600 MHz, DMSO-*d6*) spectrum confirmed the structure of **3PO** (**3**). The same reaction was performed also in MeOH instead of THF. In this case the reaction was faster, no **3PO** was present and only more polar side products were produced.



**Fig. S8** The 1H-NMR (600 MHz, DMSO-*d6*) spectrum of **3PO** obtained from the reaction performed with Et2NH after FLC purification (Scheme S1)

# A proposed enamine mechanism for the synthesis of 3PO in the presence of Et2NH

For the synthesis of **3PO** in the presence of Et2NH we proposed an enamine based mechanism **(Scheme S2)** as an alternative to the Claisen-Schmidt condensation that can prefer a production of undesirable polar side products. Both reaction mechanisms can perform and this is probably a reason of a low 12 % yield of **3PO** obtained by these conditions.



**Scheme S2** Proposed mechanism for a synthesis of **3PO** in the presence of Et2NH. Pyridinylethanone **2** andEt2NH react to an enamine **2a** that can subsequently attack the nicotinaldehyde **1** to produce **3PO** precursors **3a** and/or **3b** that are more resistant to nucleophilic species usually destroying **3PO** product in a direct reaction between **1** and **2** catalysed by NaOH, K2CO3 or Et3N. Intermediates **3a** and/or **3b** are precursors of **3PO** that can be liberated on the end of the reaction after its acidic workup.

**References**

**Durinda J**, Kolena J, Szücs L, Krasnec L, Heger J (1967) Study of adrenal cortex inhibitors of the amphenone group. I. Azachalcones Ceskoslovenska farmacie 16: 14-15.

**Durinda J,** Szucs L, Krasnec L, Heger J, Springer V, Kolena J, Keleti J (1966) Chemistry and biological properties of azachalcones, Acta Facultatis Pharmaceuticae Bohemoslovenicae 12: 89-129. Chem. Abstr. 1968 68: 114494y