SYNTHESIS AND CHARACTERIZATION OF 4-NITROPHENYLHYDRAZONE-*N*-ACYLHYDRAZONES (HAH) USING A GREEN MECHANOCHEMICAL PROTOCOL

Humberto Aguiar Dias Neto; José Maurício dos Santos Filho

*Laboratório de Planejamento e Síntese Aplicados à Química Medicinal-**SintMed**®, Universidade Federal de Pernambuco, Recife, PE, Brazil*
humbertoadneto@hotmail.com

Introduction

Chronic inflammatory diseases like asthma, rheumatoid arthritis and psoriasis are complex phenomena triggered by genetic and environmental factors. The molecular mechanisms that initiate these inflammatory conditions are complex and associated to specific genetic factors regulating the transcription of targeting genes, usually increasing their transcription's rate and, therefore, the formation of messenger RNA (mRMN) and proteins. Many of these transcription factors are cell-specific and regulate cellular processes. Other, however, are ubiquitous, and their activity can be modulated by external signals. This is the case of the nuclear factor *kappa* B (NF-*k*B), which regulates several enzymes associated to the inflammation, as well as inflammatory genes, *e.g.*, the tumor necrosis factor *a* (TNF *a*) [1].
Once *N*-Acylhydrazones (NAH) has been recognised as effective anti-inflammatory compounds [2], ***SintMed***® has developed a new concept for their synthesis in association to hydrazone moieties, using mechanochemistry, a green, efficient and stereoselective methodology, giving access to a new series of compounds, which will be evaluated as anti-inflammatory agents. The inhibition of the *in vivo* nitric oxide and TNF *a* production was the elected experimental model for our studies, avoiding *in vivo* experiments, therefore, saving on costs and preserving the animal lives.

Methodology

All intermediates and target molecules were characterised by infrared spectroscopy (IV), using a Tensor27 spectroscope from Bruker. Hydrogen magnetic resonance (^{1}H NMR) analyses were performed at 300 and 400 MHz. Melting points (m. p.) were measured using a

digital melting point apparatus and are uncorrected. The planned substituted hydrazone-*N*-acylhydrazones (HAH) were obtained by reacting 4-nitrophenyl cetohydrazone with substituted arylhydrazides under grindstone conditions. The first substrate arises from the Japp-Klingermann reaction [3], which promotes the condensation between the diazonium salt of 4-nitroaniline and ethyl acetoacetate. The substituted aryl hydrazides come from the corresponding aryl carboxylic acids in two steps. Applying the grindstone methodology developed at ***SintMed***® [4], both reactants are mixed together in a mortar with 4 to 6 drops of acetic acid as catalyst. Grinding the mixture for times varying from 40 to 50 minutes, the reaction's completion was monitored by thin layer chromatography. The crude products present high purity after drying in a desiccator under vacuum, which was established by ^{1}H NMR analysis. In addition, the HAH derivatives were isolated exclusively as *E*-isomers, confirming the robustness of the method. This steroselectivity is fundamental for the biological studies, once the evaluation of diastereomeric mixtures can lead to the misinterpretation of the outcomes.

Results and discussion

The grindstone synthesis of hydrazone-*N*-acylhydrazones was found to be successful, once the products could be prepared without organic solvents, avoiding tedious work up and purification. Initially, a series of 10 unpublished HAH derivatives **1-10** could be isolated as pure *E*-isomers in good to excellent yields, supporting the importance of this synthetic method to the investigations carried out at the ***SintMed***®. All compounds were fully characterised. The general data for the derivatives are given as follows, disclosing each substituent used at the aryl hydrazide moiety.

Compound 1 (4-aminophenyl): yield 90%, m. p. 302.1-302.7 °C, IV (KBr, $\tilde{\nu}$, cm^{-1}) 3192, 3139 (N–H), 3064, 3026 (C–H Ar), 1648 (C=O), 1599 (C=C), 1568 (C=N);

Compound 2 (3,5-dinitrophenyl): yield 92%, m. p. 276.2-276.9 °C, IV (KBr, $\tilde{\nu}$, cm^{-1}) 3253, 3196 (N–H), 3103 (C–H Ar), 1661 (C=O), 1644 (C=C), 1607 (C=N);

Compound 3 (phenyl): yield 90%, m. p. 294.5-195.9 °C, IV (KBr, $\tilde{\nu}$, cm^{-1}) 3222, 3197, (N–H), 3063, 3026 (C–H Ar), 1654 (C=O), 1610 (C=C), 1597 (C=N);

Compound 4 (2-hydroxyphenyl): yield 93%, m. p. 305.7-306.5 °C, IV (KBr, $\tilde{\nu}$, cm^{-1}) 3308 (O–H), 3243, 3211, 3160 (N–H), 3079 (C–H Ar), 1646 (C=O), 1599 (C=C), 1555 (C=N);

Compound 5 (2-aminophenyl): yield 91%, m. p. 272.0-274.3 °C, IV (KBr, $\tilde{\nu}$, cm^{-1}) 3431, 3409, 3325, 3227 (N–H), 3072 (C–H Ar), 1644 (s, C=O), 1598 (C=C), 1568 (C=N);

Compound 6 (4-hydroxyphenyl): yield 92%, m. p. 307.5-308.6 °C, IV (KBr, $\tilde{\nu}$, cm^{-1}) 3386 (O–H), 3216, 3134 (N–H), 3060, 3022 (C–H Ar), 1654 (C=O), 1609 (C=C), 1594 (C=N);

Compound 7 (4-chlorophenyl): yield 91%, m. p. 292.9-294.7 °C, IV (KBr, $\tilde{\nu}$, cm^{-1}) 3291, 3226 (N–H), 3062 (C–H Ar), 1677 (C=O), 1635 (C=C), 1596 (C=N);

Compound 8 (piperonyl): yield 77%, m. p. 305.2-306.0 °C, IV (KBr, $\tilde{\nu}$, cm^{-1}) 3222, 3198 (N–H), 1664 (C=O), 1643 (C=C), 1596 (C=N);

Compound 9 (4-dimethylaminophenyl): yield 90%, m. p. 311.5-312.9 °C, IV (KBr, $\tilde{\nu}$, cm^{-1}) 3190 (N–H), 3062 (C–H Ar), 1651 (C=O), 1608 (C=C), 1569 (C=N);

Compound 10 (vanilinyl): yield 92%, m. p. 300.2-301.8 °C, IV (KBr, $\tilde{\nu}$, cm^{-1}) 3486 (O–H), 3390, 3217, 3192 (N–H), 3063 (C–H Ar), 1655 (C=O), 1596 (C=C), 1568 (C=N).

These compounds are potential anti-inflammatory agents, and the investigation of their biological activities should take place in the next weeks.

Conclusions

The planned synthesis of compounds **1-10** using the grindstone methodology is extremely efficient, leading to novel HAH derivatives with potential anti-inflammatory activity. All described compounds are characterised, so that the further synthesis of other analogues can be easily achieved. The method's stereoselectivity is efficient based on the NMR analysis, which confirms the presence exclusively of the *E*-isomers.

Key words: Hydrazone-*N*-acylhydrazones; grindstone chemistry; anti-inflammatory activity.

References

[1] Epstein, F.H., *New Engl. J. Med.*, **1997**, 336, 1066-1071.

[2] Kajal, A. *et al.*, *Int. J. Med. Chem.* **2014**, 2014, 1–11.

[3] Japp, F. R. and Klingemann, F., *Ber. Deuts. Chem. Ges.*, **1887**, 20, 2942–2944.

[4] dos Santos Filho, J. M. and Pinheiro, S. M. , *Green Chemistry*, **2017**, 19, 2212-2224.

Chronic inflammatory diseases such as asthma and rheumatoid arthritis affect millions of people around the world and represent a burden to these populations. The treatment with corticoids and immunosuppressive therapy allows the diseases' control, but not the cure, usually with severe side effects. The external regulation of some genetic factors, such as NF-*k*B, can lead to the control of these diseases. Since *N*-acylhydrazones have demonstrated anti-inflammatory activity, acting by different molecular mechanisms, the research group with the ***SintMed***® has designed and synthesised a new class of hydrazone-*N*-acylhydrazones (HAH), in order to investigate their anti-inflammatory and immunomodulatory properties. Following a new green, stereoselective and highly efficient synthetic protocol developed by our group, the targeting compounds were prepared using the grindstone methodology, which has proved to be appropriate to our purposes. This new approach for the synthesis of *N*-acylhydrazones has overcome the difficulties faced when the traditional methodology is applied, mainly due to the nature of the Japp-Klingermann product used as one of the reactants, which bears an acid sensitive vinylogous keto group.