REPORT

Unconventional synthesis of nitrogenated heterocycles with potencial bioactive properties

Hortensia M Rodríguez[1], Margarita Suárez[1], ✉ Osvaldo Reyes[2], Osnieski Martín[1], Estael Ochoa[1]

[1]Organic synthesis laboratory, Faculty of Chemistry, University of Havana, Cuba, Zapata / G and Carlos Aguirre, Vedado, Plaza de la Revolución, CP 10400, Havana, Cuba
[2]Peptide Synthesis Laboratory, Center for Genetic Engineering and Biotechnology Ave 31 / 158 and 190 PO Box 6162, Cubanacán, Playa, Havana, Cuba

*I*ntroduction

In recent years, a new approach called "Green Chemistry" emerged in the field of organic chemistry, to prevent or minimize contamination from the laboratory bench up to industrial production. It purpose is to avoid residue formation, saving time and costs. One of the strategies proposed for the existing products involves designing alternative synthesis steps that do not use toxic substrates or solvents, nor do they generate toxic by products. Also, new synthesis methodologies successfully applied with unconventional power sources can be used, such as infrared and microwave (MW) radiations and ultrasound, among others.

The development of combinatorial chemistry has accelerated the discovery process for new active compounds and raised interest in the development of solid-phase synthesis (SPS) methodologies for organic molecules. All these have widened the horizons for searching for new potential drug candidates.

In this paper, we describe the synthesis of nitrogenated heterocyclic compounds with potential bioactive properties by MW-assisted organic synthesis procedures in the absence of solvents and SPS.

*R*esults and discussion

MW-assisted N-alkylation of adenine, guanine and 6-aminothiouracil.

A new MW-assisted procedure for N-alkylation of purinic bases was studied. N-alkylation of adenine (1), guanine (2) and 6-amino-2-thiouracil (3) was carried out in neutral media without solvents in a domestic MW oven, the amine groups reacting with the corresponding alkylating agents. For the 6-amino-2-thiouracil, this method was combined with phase-transfer catalysis (PTC) (Figures 1, 2, 3 and 4) [1].

The differential chemoselectivity observed for both methods of 6-amino-2-thiouracil N-alkylated derivative synthesis results from the basic media employed in the second procedure. When the reaction is carried out in the absence of a base, the alkylation selectively occurs in the primary amine group of the neutral 6-amino-2-thiouracil, in a basic medium under PTC. The reaction occurs between the anionic species, and alkylation is detected in position 1 of the anion.

To determine the possible specific effects of MW heating according to the established methodology, these compounds were synthesized under the same conditions (reaction time, recipients and temperature) in a thermostatic oil bath. In all of them the reaction was absent. This evidences the specific effect of the MW, which is essential to explain the results attained. In general, the specific effect increases by enhancing the polarity of the system. Reactions that occur in a neutral medium (Figures 1, 2 and 3) involve neutral reagents (amines and the alkylating agent). They comprise the formation of a dipole in the transitional state that is more polar than the basal state, and also stabilized by the dipole-dipole interactions affected by the MW (Figure 5).

1. Rodríguez H, Pérez R, Suárez M, Lam A, Loupy A. Alkylation of some pyrimidine and purine derivatives using microwave-assisted methods heterocycles (2001); 55(2):291-301. (Índice de impacto: 1 070).

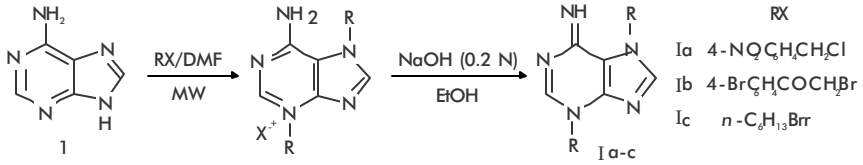

Figure 1. MW-assisted N-alkylation of adenine (1) in the absence of solvents.

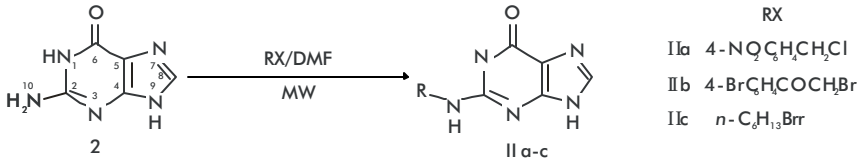

Figure 2. MW-assisted N-alkylation of guanine (2) in the absence of solvents.

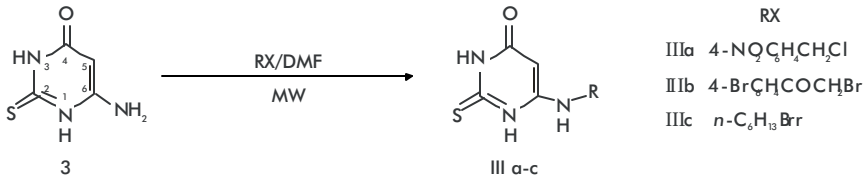

Figure 3. MW-assisted N-alkylation of 6-amino-2-thiouracil (3) in the absence of solvents.

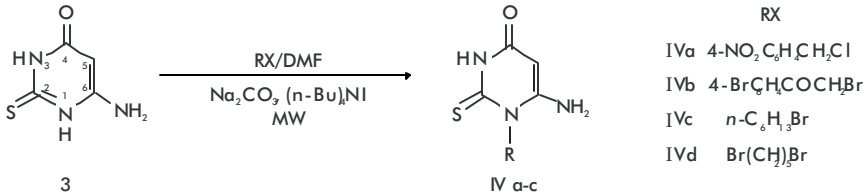

Figure 4. MW-assisted N-alkylation of 6-amino-2-thiouracil (3) under phase transfer catalysis (PTC) conditions.

✉ Autor de correspondencia

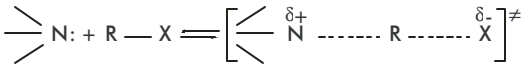

Figure 5. Formation of a dipolar transitional state in alkylation reactions.

In the MW-assisted reaction carried out under PTC, polarity rises when charged species are involved, especially with anions carrying dislocated charges.

It was necessary to include low amounts of DMF, a polar molecule highly sensitive to MW radiations, as an energy-transfer medium to achieve high temperatures in all reactions.

This new methodology fulfils the current requirements for clean chemistry (*Green Chemistry*), with a minimal environmental impact. It also allows appropiate yields of 3, 7-N-alkylated derivatives of adenine (Ia-c, 72-82%), N^2-monoalkylated guanine derivatives (IIa-c, 52-70%) and N^6 or N^1 monoalkylated 6-amino-2-thiouracil derivatives (IIIa-c, 80-84% and IVa-c, 87-95%), at time periods lower than 11 minutes.

MW-assisted synthesis of 3,4-dihydro-2-1 (1*H*) pyridones in the absence of solvents

Multicomponent reactions (MCR) are defined as the series of subreactions simultaneously and/or sequentially occurring between two reactive molecules, producing the synthesis of several by products by the direct combination of more than two reagents in a single step. It has been an interest of the Organic Synthesis Laboratory at the University of Havana for several years to develop and diversify 3,4-dihydro-2(1*H*)-pyridones (3,4-DHPs), due to their similarity to the 1,4-DHP and their potential bioactive properties.

We have previously established the methodology for synthesizing such compounds in solution, but with reactions times of 11-14 hours and yields between 55-75%.

Because these compounds are important intermediaries for synthesizing several heterocycles that hard to obtain by direct synthesis, we developed a new methodology based on MW-assisted synthesis of 3,4-DHPs (Va-i) in the absence of solvents by using a type R3C MCR in a single mode Synthewave™ 402 organic synthesizer (Figure 6) [2].

It is noteworthy, that there was no need to purify the products, obtaining yields of 81 and 91% and reaction times of 10 and 15 minutes, respectively.

To determine the possible intervention of MW-specific overheating, the compounds were synthesized under the same conditions (reaction time, flasks and temperature) in a thermostatic oil bath, and results compared. Yields obtained for these compounds in solution are less than 65% after purification, due to re-crystalization. Therefore, they were lower than the results obtained for the MW-assisted synthesis in the absence of solvents.

By using conventional heating (Δ) in the absence of solvents, yields were low (10-38%) after resenting 2 hours with compounds Va-c and 6 hours with the other. These indicate the strong effect of MW, also related to the mechanism and polarity evolution during the reaction. Results demonstrate the advantages of MW activation mode compared to traditional methods.

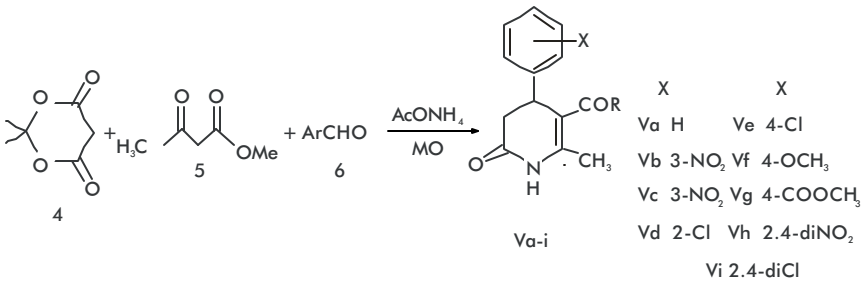

Figure 6. MW-assisted synthesis of 3,4-dihydro-2(1*H*)-pyridones (Va-i) in the absence of solvents.

To complement these results study, mechanical-quantical studies were mode on the mechanism for obtaining 3,4-DHPs (Va-i), resul-ting in an energy profile that justifies the formation of the final reaction product. This supported the analysis of the MW-specific effect according to the expressed mechanism, concluding that the excellent results in terms of high yields and short reaction time periods derive from the favoring effect of MW. This also corresponds to the mechanism and polarity evolution during the reaction.

In summary, a new method of MW-assisted synthesis devoid of solvents was designed, and nine 3,4-DHPs (Va-i) were obtained by a type R3C reaction with good yields (above 80%) and short reaction periods (between 10 and 15 minutes). This new procedure belongs to the new *Green Chemistry* approach, due to the lack of solvents, short reaction periods, while also saving time and power compared to traditional methods.

3,4-dihydro-2(1*H*)-pyridones SPS synthesis

Due to the relevance of available methodologies using SPS procedures to synthesize organic molecules with potential biological activities for further designing combinatorial libraries, we also designed a SPS methodology to obtain new 4-aryl-substituted 5-carboxi-6-methyl-3,4-DHPs (XIa-i). Wang's resin was employed as the solid support due to its ubi-quity for the synthesis of organic molecules (Figure 7).

The SPS of the 3,4-DHPs (XIa-i) was carried out in 4 steps. First, the immobilized β-cetoester VI was prepared (Figure 7). This aceto acetylation takes place when treating Wang's resin with two 2,2,6-trimethyl-1,3-dioxin-4-one equivalents in the presence of toluene as the dissolvent and under reflux conditions. The qualitative conversion in this first step of the reaction was checked by infrared spectroscopy. To determine yields, the immobilized β-cetoester VI was released/heterocycled by treating with hydrazine in ethanol for 30 minutes to obtain the 3-methyl-3-pyrazolin-5-one (VII) with an 89% yield and 91% purity by Reverse Phase-High Pressure Liquid Chromatography (RP-HPLC).

The second step consisted in obtaining the immobilized enamine by the reaction of immobilized β-cetoester VI with ammonium acetate in acetic acid as the solvent under reflux for six hours. To confirm the presence of the enamine VIII and to quantify this step, the enamine was released by treating the resin with a TFA:DCM (1:1, v/v) solution and the 3-amino-2-enebutanoic acid (XII) obtained with 82% yield and 86% purity, as determined by RP-HPLC, are shown in table 1.

2. Rodríguez H, Suárez M, Pérez R, Petit A, Loupy A. Solvent-free synthesis of 4-aryl substituted 5-alkoxycarbonyl-6-methyl-3,4-dihydropyridones under microwave irradiation. Tetrahedron Letters (2003); 44:3709-12. (Índice de impacto: 2 477).

3. Rodríguez H, Reyes O, Suárez M, Garay HE, Pérez R, Cruz LJ, Verdecia Y, Martín N, Seoane C. Solid-phase sintesis of 4-aryl substituted 5-carboxy-6-methyl-3,4-dihydropyridones. Tetrahedron Letters (2002); 43:439-41. (Índice de impacto: 2 477).

4. Rodríguez H, Martín O, Ochoa E, Suárez M, Reyes O, Garay HE, Albericio F, Martín N. Solid-Phase Synthesis and Structural Study of Substituted 1,4,5,6-Tetrahydro-6-oxopyridine-3-carboxylic Acids (p NA). QSAR and Combinatorial Sciences, [On line: 4 de julio de 2006]. (Índice de impacto: 1 826.)

The third step consisted of generating the immobilized 3,4-DHPs (XIa-i) by reacting the enamine VIII with the ilidenic (IX) intermediary, resulting from Knoevenagel's condensation between Meldrum's acid and the corresponding aromatic aldehyde. In the last synthesis step, the release of X from the resin was achieved by treating it with TFA:DCM (1:1, v/v) to obtain the substituted 5-carboxi-6-methyl-3,4-DHPs (XIa-i).

The structures of all these compounds ((VI, VII, VIII, IX and XIa-i) were corroborated by NMR-^{1}H, ^{13}C and ESI- and HPLC-mass spectrometry.

In summary, a new SPS methodology was established for synthesizing a small chemical library of new 3,4-DHPs (XIa-i) with good yields and purity percentage. The novelty of these compounds resides in the acid carboxyl group placed in position 5 of the pyridone ring.

Conclusions

In this study, MW-assisted organic synthesis techniques were applied to N-alkylated derivatives of purinic bases (adenine and guanine) and a pyrimidinic derivative (6-aminothiouracil), obtaining three N-3,7-dialkyladenines Ia-c, three N^{2}-monoalkylguanines IIa-c and three N^{6}-monoalkylthiouracils IIIa-c. To obtain the pyrimidinic derivative (6-aminothiouracil), the MW irradiation was combined as a source for activating the PTC and four N1-alkylthiouracils IVa-d were generated. Also, two new methodologies were developed to synthesize 3,4-3,4-DHPs. The first one through a type R3C reaction in the absence of solvents and under MW-irradiation as the activating source, to synthesize nine type V com-pounds; and the second one consisting of SPS in four steps that to designing and obtaining a combinatorial library of these and other analogue compounds. All these provide the basis for the search for new more effective drugs for treating cardiovascular diseases.

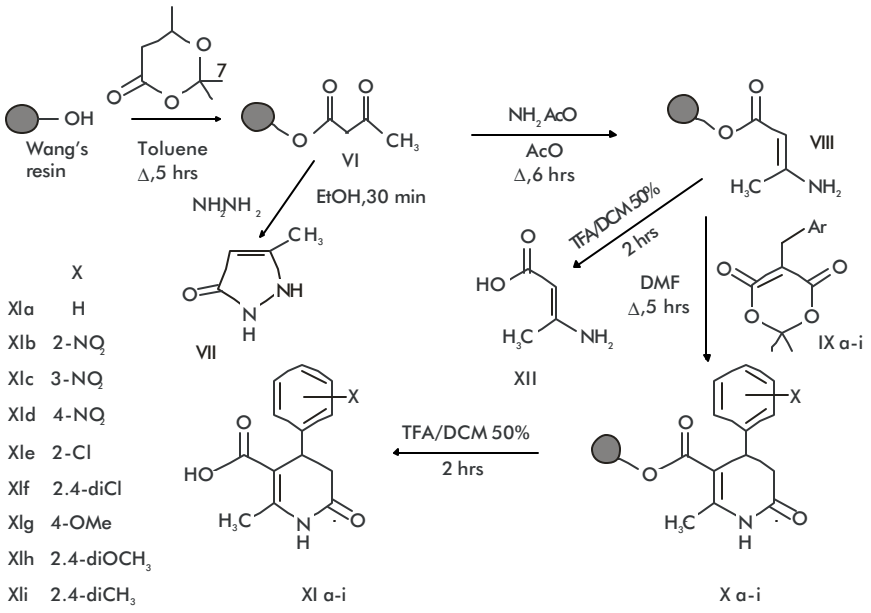

Figure 7. General diagram of 3,4-dihydro-2(*1H*)-pyridone (XIa-i) synthesis. Yields and purity of synthesized 3,4-dihydropyridones were determined by RP-HPLC.

Table 1. Results of 3,4-dihydro-2(1H)-pyridones SPS

Compound	X	Yield (%)	Purity (%)
XIa	H	82	95
XIb	2-NO_2	73	88
XIc	3-NO_2	65	85
XId	4-NO_2	85	78
XIe	2-Cl	71	95
XIf	2,4diCl	85	82
XIg	4-CH_3O	81	92
XIh	2,4-diCH_3O	75	89
XIi	2,4-diCH_3	77	91