An Unequivocal Synthesis of 2-Aryl Substituted 3-Amino-2,4,5,7-tetrahydro-6H-pyrazolo[3,4-b] pyridin-6-ones

Elisabeth Bou-Petit,[a] Elsa Picas,[a] Cristina Puigjaner,[b] Merce` Font-Bardia,[c] Nabı´ Ferrer,[a] Julia` Sempere,[a] Raimon Puig de la Bellacasa,[a] Xavier Batllori,[a] Jordi Teixido´ ,[a] Roger Estrada-Tejedor,[a] Santiago Ramon y Cajal,[d] and Jose´ I. Borrell*[a]

[a] E. Bou-Petit, E. Picas, N. Ferrer, Prof. J. Sempere, Dr. R. Puig de la Bellacasa, Dr. X. Batllori, Prof. J. Teixidj, Dr. R. Estrada-Tejedor, Prof. J. I. Borrell IQS School of Engineering Universitat Ramon Llull Via Augusta, 390, E-08017 Barcelona, Spain E-mail: j.i.borrell@iqs.url.edu

[b] Dr. C. Puigjaner Unitat de Polimorfisme i Calorimetria Centres CientificotHcnics Universitat de Barcelona Baldiri Reixac 10, 08028 Barcelona, Spain

[c] Dr. M. Font-Bardia Unitat de Difraccij de Raigs X Centres CientificotHcnics Universitat de Barcelona Llu&s Sol8 i Sabar&s 1–3, 08028 Barcelona, Spain

[d] Prof. S. Ramon y Cajal Departamento de Patolog&a Hospital Universitario Valle de Hebrjn Universidad Autjnoma de Barcelona Passeig Vall d'Hebron 119–129, 08035 Barcelona, Spain

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ABSTRACT:

The reaction between pyridones (1) and substituted hydrazines 4 can afford two different regioisomeric pyrazolo[3,4-b]pyridin- 6-ones 2 and 3 depending on the initial substitution of the methoxy group and the direction of the cyclization. In the case of phenylhydrazine 4 (R3 = Ph), we have clearly shown that the treatment of pyridones 1a–d with 4 (R3 = Ph) in MeOH at temperatures below 1408C yields, independently of the nature and position of the substituents present in the pyridone ring, the open intermediates 7a–d. When the reaction is carried at 1408C under microwave irradiation, the corresponding 2-aryl substituted pyrazolo[3,4-b]pyridines 3a–d are always formed. We have experimentally determined, using DSC techniques, the activation energies of the two steps involved in the formation of 3: a) substitution of the methoxy group present in pyridines 1 with phenylhydrazine 4 (R3 = Ph) to afford intermediates 7 and b) cyclization of intermediates 7 to yield pyrazolopyridines 3. The results obtained, 15 and 42 kcal·mol@1 respectively, are in agreement with the experimental findings.

INTRODUCTION

As a part of our ongoing research in the area of tyrosine kinase inhibitors, we were interested in 3-amino-2,4,5,7-tetrahydro-6Hpyrazolo[3,4-b]pyridin-6-ones[1] as an scaffold for the synthesis of combinatorial libraries. Such structures can be obtained by cyclization of 2-methoxy-6-oxo-1,4,5,6-tetrahydropyridin-3-carbonitriles (1), synthesized by reaction of an a,b-unsaturated ester and malononitrile in NaOMe/MeOH,[2] with hydrazine[3] or substituted hydrazines. In this later case, the reaction can lead to two different positional isomers (2 or 3) depending on the reaction path. If the substitution of the methoxy group present in 1 takes place with the NH group of the substituted hydrazine the subsequent cyclization onto the cyano group would afford 2 (path A). On the contrary, if the initial attack is carried out by the NH2 group the cyclization would yield 3 (path B) (Scheme 1).
In principle, the initial substitution should be governed by the relative nucleophilicity of the two nitrogen atoms present in NH2-NH-R3, provided that the steric effects are negligible. Consequently, if R3 is a donor group the nucleophilic character of the nitrogen bonded to R3 should be increased, thus favoring the attack of the NH group, leading to the formation of isomer 2. On the contrary, if R3 is an electron-withdrawing group the initial substitution should proceed through the NH2 nitrogen, thus leading to isomer 3.
In the case of phenyl substituted hydrazines the prediction[4] of the pKa values indicates that the NH2 group should be more nucleophilic (pKa=27.8) in comparison with the NH group (pKa=21.7). Consequently, it would be reasonable to expect that the reaction could proceed affording isomer 3 as stated above.
According to our design for potential activity as tyrosine kinase inhibitors, we were interested in 2-aryl substituted structures 3. A literature search revealed that there is not a single example of such kind of structures, and only a work by Rodrigues-Santos et al. published in 2011 in which they claimed that the reaction with phenylhydrazine 4 (R3 = Ph) (or substituted phenylhydrazines) only affords isomer 2.[5] Consequently, we decided to revise the reaction between pyridones 1 and phenylhydrazine 4 (R3 = Ph). The present paper deals with the results obtained in such study.
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RESULTS AND DISCUSSION

We selected a set of a,b-unsaturated esters 5a–d to synthesize the corresponding 2-methoxy-6-oxo-1,4,5,6 tetrahydropyridin- 3-carbonitriles 1a–d in 40–78% yield, upon treatment with malononitrile (6) in NaOMe/MeOH (Scheme 2), to study the effect of the substituents R1 and R2 in the subsequent reaction with phenylhydrazine 4 (R3 = Ph).

First, we decided to reproduce the experimental conditions used by Rodrigues-Santos et al.[5] using pyridone 1a and phenylhydrazine 4 (R3 = Ph). It is necessary to point out that they described that the reaction affords pyrazolopyridines 2 both with conventional heating and microwave irradiation using a domestic microwave oven (2450 MHz). Surprisingly, when we treated 1a with phenylhydrazine 4 (R3 = Ph) using the conventional heating methodology (at reflux for 24 h using MeOH as solvent) a product was isolated in 74% yield whose structure was different from 2 or 3. In particular, the presence in the IR spectrum of a C/N stretching band at 2256 cm@1 revealed that the cyclization was not achieved. Moreover, in the 1H-NMR spectrum the signal corresponding to the methoxy group of 1a (3.94 ppm) had disappeared, the N@H of the lactam group appeared as a singlet at 10.32 ppm while a second N2-H singlet appeared at 9.11 ppm and a doublet, corresponding to the a-cyano C@H, was observed at 4.69 ppm. These evidences clearly indicate that the isolated product was the reaction intermediate 7a (R1=H, R2=Ph) (Figure 1) which had not completed the cyclization.

Our hypothesis was confirmed using NOESY spectroscopy (Figure 2) where the correlation between N1-H/N2-H groups is clearly observed as well as the correlation between H12 of the phenyl group and the N2-H.

Such result led us to study such reaction in deep by using one or two equivalents of phenylhydrazine 4 (R3 = Ph) in several solvents under different temperature and time conditions. Moreover, two different work-up methods were tested (i. e. filtration and CH2Cl2 extraction) to isolate the final product. The results are summarized in Table 1.

As it is clearly shown in Table 1, the corresponding isomer 2a was not observed in any case. A factor with a great influence on the reaction is the temperature, thus for temperatures below 1408C the cyclization reaction did not take place and intermediate 7a was obtained in all cases.

However, when the reaction is carried out at 1408C under microwave irradiation in MeOH using two equivalents of phenylhydrazine 4 (R3 = Ph) the cyclization takes place and 3a is obtained. The structure of 3a and the product purity were established using the 1H-NMR spectra. Thus, the signal appearing at 9.11 ppm (N@H) in the case of the intermediate 7a disappears and a singlet corresponding to the NH2 group (2H) is observed at 5.28 ppm. The structural assignment was confirmed by NOESY spectroscopy, where correlation between NH2 group and the phenyl groups are observed. Moreover, in the IR spectrum the band previously appearing at 2256 cm-1 corresponding to the C/N stretching band of intermediate 7a disappears.

The cyclization of a sample of the isolated intermediate 7a was carried out in order to confirm the predicted reaction mechanism. A solution of 7a in methanol was heated at 1408C for 30 minutes under microwave irradiation to achieve cyclization into isomer 3a.

The results obtained confirmed our hypothesis that the initial substitution should be governed by the relative nucleophilicity of the two nitrogen atoms present in NH2-NH-R3 Consequently, in the case of phenylhydrazine 4 (R3 = phenyl) the initial substitution proceeds through the NH2 nitrogen, thus leading to isomer 3.

However, this result is contrary to those described by Rodrigues-Santos et al.[5] who claimed that isomers 2 are always obtained. In order to cast light on such incongruence, we decided to extend the study to pyridones 1b–d to determine the possible influence of the nature and position of substituents R1 and R2.

We started the study using pyridone 1d (R1=H, R2=p-MeO-C6H4) used as model compound by Rodrigues-Santos et al.[5] The reaction was carried out using conventional heating (24 h at reflux in MeOH) with phenylhydrazine 4 (R3 = Ph) (2:1 molar excess). The reaction afforded intermediate 7d (R1=H, R2=p-MeO-C6H4) in 70% yield as in the case of pyridone 1a (see above). The structure was confirmed by IR and 1H-NMR (see supporting information). Particularly revealing was the NOESY spectrum (see supporting information) which shows the same kind of correlations observed in 7a. Cyclization of intermediate 7d as starting material was achieved upon heating in MeOH at 1408C under microwave irradiation and the cyclized compound 3d was obtained in 97% yield.

Finally, in order to definitively confirm the structure of 3d, single crystals were grown by vapor diffusion of water into 3 mL of a MeOH solution of 5 mg of 3d. The crystal structure was determined by single crystal X-ray diffraction. 3d crystallizes in monoclinic centrosymmetric space group P21/n. The ORTEP diagram and atomic numbering are given in Figure 3. Crystallographic data are summarized in supporting information.

The resulting structure clearly shows the presence of the phenyl substituent in position C2 of the pyrazole ring, thus confirming that the isomer obtained is 3d.

Further exploration of the synthesis of 3d showed that it can be directly obtained using a 2:1 molar ratio of 4 (R3 = Ph) with respect to pyridone 1d under microwave irradiation at 1408C for 30 min in 85% yield.

To establish the general applicability of this synthetic methodology, we extended the reaction to two other pyridones: 1b (R1=Ph, R2=H) and 1c (R1=Me, R2=H). When the reactions with 2 equivalents of phenylhydrazine 4 (R3 = Ph) were carried out at room temperature in MeOH the corresponding open intermediates 7b and 7c were obtained in 81% and 60% yield, respectively. The same reactions carried out at 1408C under microwave irradiation afforded 3b (48%) and 3c (58 %), respectively (see supporting information).

Consequently, we can summarize the results obtained as follows (Scheme 3): a) when the reaction between pyridones 1 and phenylhydrazine 4 (R3 = Ph) is carried out in MeOH (or other solvents) below

1408C, intermediates 7 are formed in 60–80% yield; b) such intermediates can be converted in the corresponding 2-aryl substituted 3-amino-2,4,5,7-tetrahydro-6H-pyrazolo[3,4-b]pyridin-6-ones 3 by heating in MeOH under microwave irradiation at 1408C in good yields (50-90 %); and c) pyrazolopyridines 3 can be directly obtained using pyridones 1 and phenylhydrazine 4 (R3 = Ph) heated in MeOH under microwave irradiation at 1408C in 40–60% yield. In no case we have even detected the regioisomeric pyrazolo[3,4-b]pyridines 2.[5]

Finally, to understand the high thermal level needed for the cyclization of intermediates 7, we decided to determine the activation energies of the two steps involved in the formation of pyrazolopyridines 3: a) substitution of the methoxy group present in pyridones 1 with phenylhydrazine 4 (R3 = Ph) to afford intermediates 7; and b) cyclization of intermediates 7 to yield pyrazolopyridines 3.

Preliminary ab-initio calculations using Gaussian 09 already indicated that the second barrier is higher than the first one but the values obtained were both too high. Consequently, we decided to determine experimentally such barriers using differential scanning calorimetry (DSC) techniques. Thus, a 1:2 mixture of 1c and phenylhydrazine 4 (R3 = Ph) in MeOH was introduced in a medium pressure stainless steel crucible and heated from 408C to 1608C at different heating rates under a nitrogen stream. The activation energy was then determined using the kinetic methods of Ozawa[6] and Kissinger[7] to afford 15.6:1.6 kcal·mol@1 and 14.3:1.6 kcal·mol@1, respectively.

Similarly, intermediate 7c was heated in absence of solvent in a standard aluminium crucible with a pierced lid from 1208C to 2208C at different heating rates under a nitrogen stream. The activation energy was determined using the kinetic methods of Ozawa,[6] Kissinger[7] and Kissinger-Akahira-Sunose.[8] The results obtained were 42.4:2.3, 42.8:2.4 and 40.6:0.2 kcal·mol-1, respectively. The 1H-NMR spectrum of the contents of the standard aluminum crucible showed the complete transformation of intermediate 7c to pyrazolopyridine 3c (see supporting information).

These results are clearly compatible with our findings and show why the cyclization step must proceed at 1408C. In fact, the experimental activation energies obtained are in agreement with the observations of Rodrı´guez et al.[9] who established that reactions with activation energies below 20 kcal·mol@1 occur easily by conventional heating, while reactions with activation energies above 30 kcal·mol@1 cannot be performed under conventional heating or need highly polar solvents under microwave irradiation.

Consequently, we believe that Rodrigues-Santos et al.[5] mistook intermediates 7 for isomers 2 probably because the reaction temperature reached by their microwave for home-use was not high enough to allow the reaction to complete.

A literature search has revealed, in one side, that there are almost no examples of 2-aryl substituted pyrazolo[3,4-b] pyridines referable to 3 and, on the other side, that the reactions between cyano groups and NH2-Ph groups (the mechanistic step needed for the cyclization step affording compounds 3) are normally carried out using acid catalysis.

To sum up, the formation of compounds 3 cannot be qualified as an obvious reaction because it requires heating at 1408C in MeOH under microwave irradiation due to the very high activation energy involved in such process (around 42 kcal·mol@1).

CONCLUSIONS

We have clearly shown that the treatment of pyridones 1a–d with phenylhydrazine 4 (R3 = Ph) in MeOH at temperatures below 1408C yields, independently of the nature and position of the substituents present in the pyridine ring, the corresponding open intermediates 7 a–d. When the reaction is carried at 1408C under microwave irradiation, the corresponding pyrazolo [3,4-b]pyridines 3a–d are always formed. In no case we have even detected the regioisomeric pyrazolo[3,4-b]pyridines 2.[5] We have experimentally determined that the activation energy of the cyclization step from intermediates 7 to pyrazolo[3,4-b] pyridines 3 is around 42 kcal·mol@1. Such barrier is overcome due to the overheating of the intermediate 7 solution in MeOH at 1408C under microwave irradiation at 11 bar. We are currently routinely using this methodology for the production of libraries of pyrazolo[3,4-b]pyridines 3 and extending the study to methyl substituted hydrazine 4 (R3 = Me).

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Keywords: Activation energies · Cyclization · DSC · Microwave assisted synthesis · Pyrazolo[3,4-b]pyridin-6-ones

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Legends to figures

Scheme 1. Possible reaction pathways for the formation of pyrazolo[3,4-b] pyridin-6-ones 2 and 3.

Figure. 1 Structure of reaction intermediates 7.

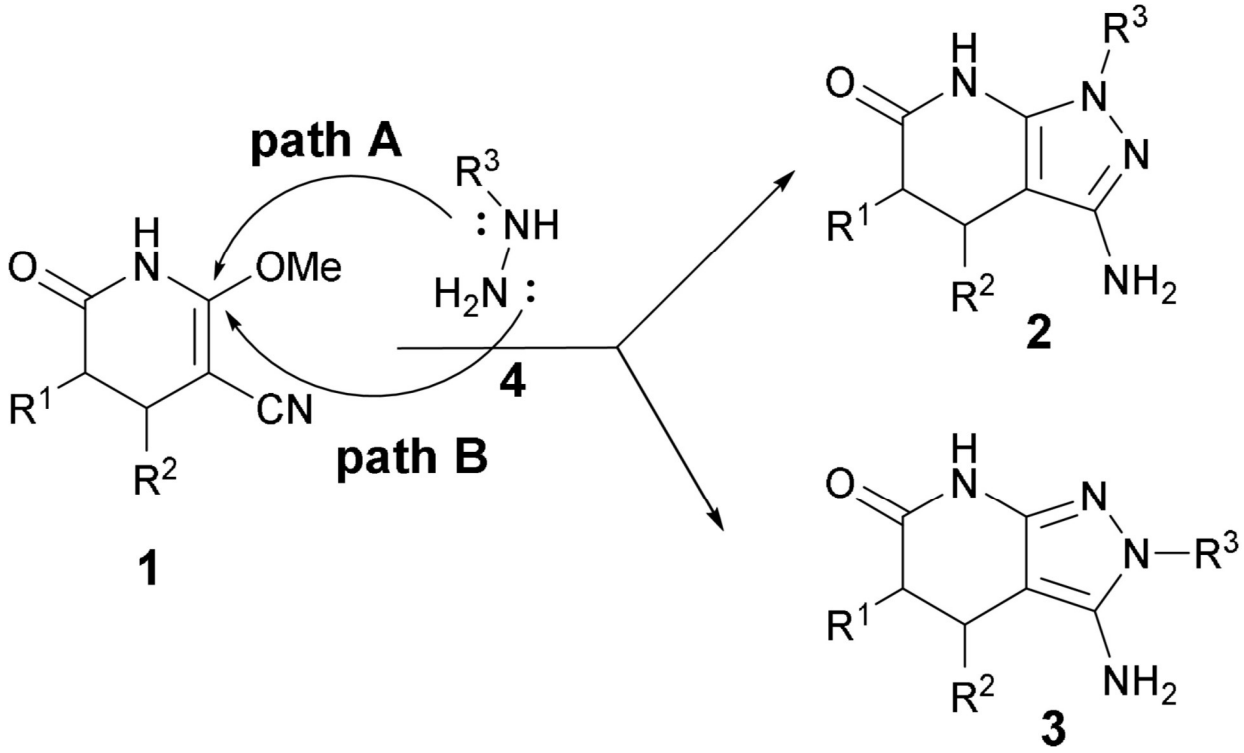
Scheme 2. Synthesis of 2-methoxy-6-oxo-1,4,5,6 tetrahydropyridin-3-carbonitriles 1a–d.

Figure. 2 NOESY spectrum of intermediate 7a.

Figure. 3 ORTEP diagram and atomic numbering of 3d.

Scheme 3. Conversion of pyridones 1 in open intermediates 7 or pyrazolopyridines 3 depending on the reaction temperature.

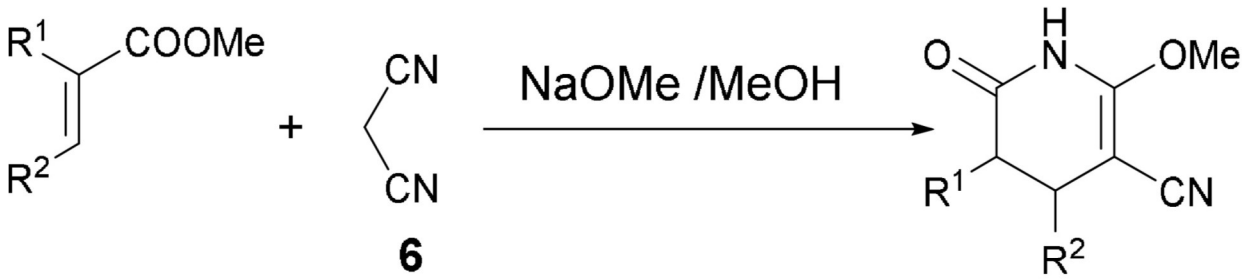
SCHEME 1



252 **SCHEME 2**

253

254



6

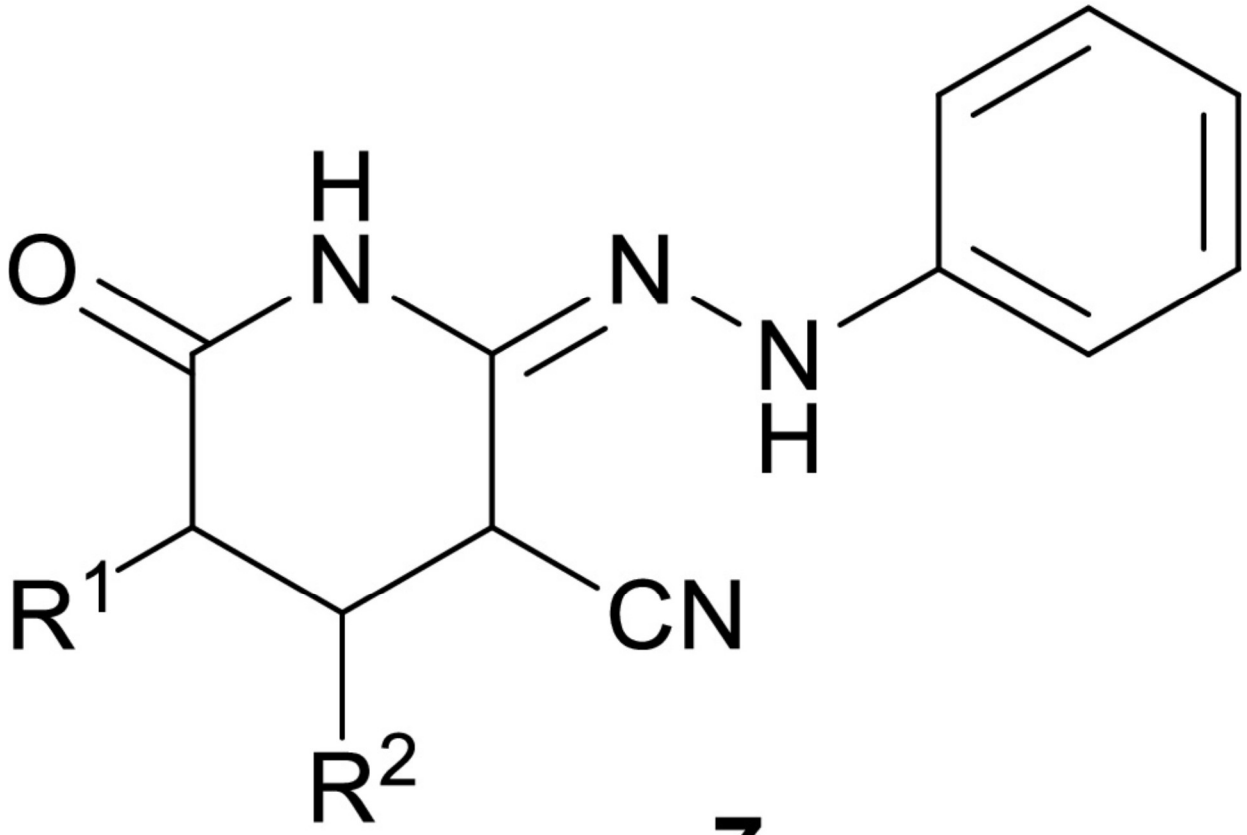
5a	R^1 = H, R^2 = Ph	**1a**
5b	R^1 = Ph, R^2 = H	**1b**
5c	R^1 = Me, R^2 = H	**1c**
5d	R^1 = H, R^2 = *p*-MeO-C_6H_4	**1d**

255

256

257

FIGURE 1



7

FIGURE 2

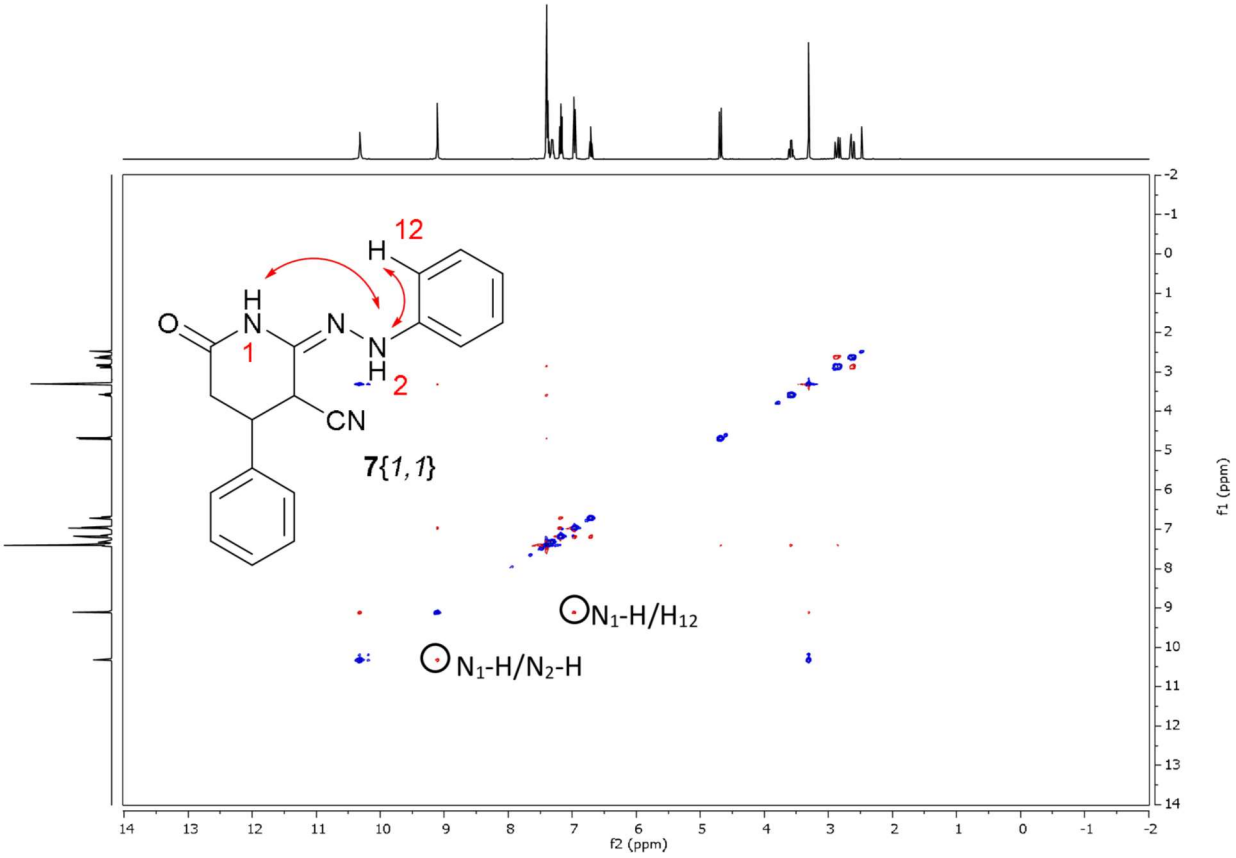
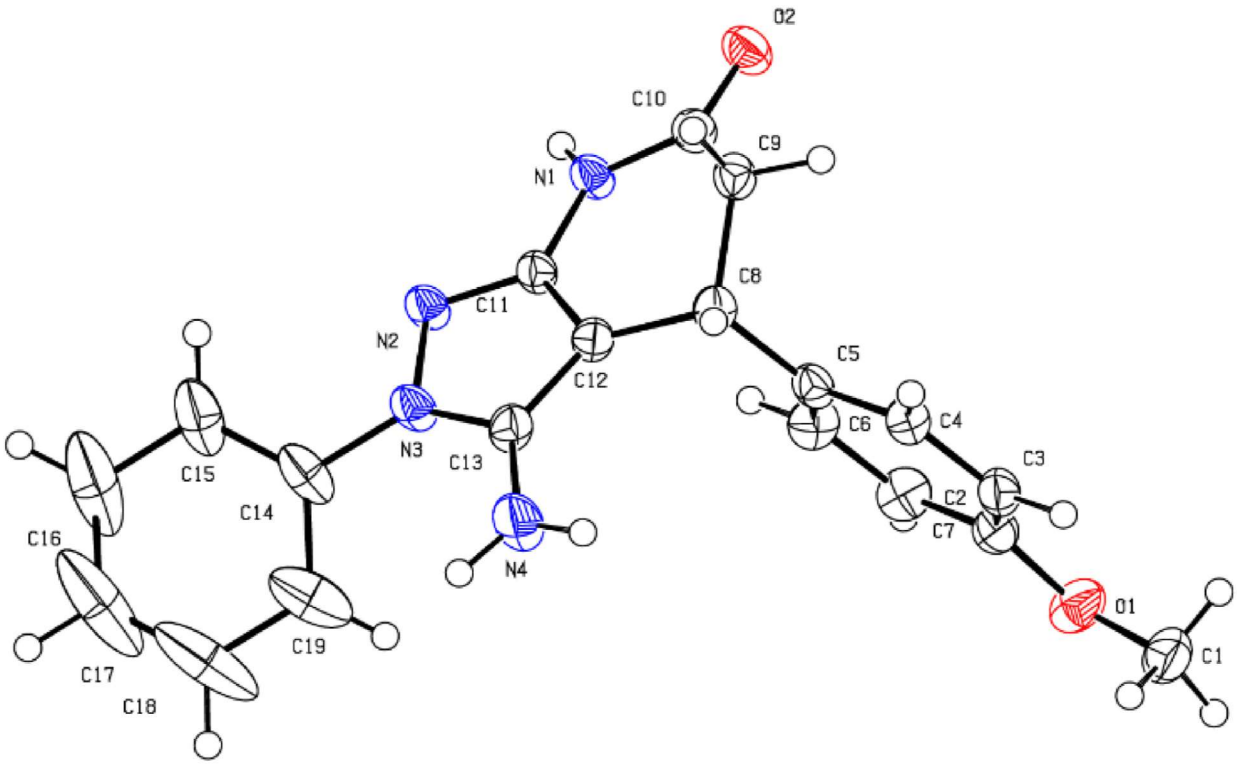


FIGURE 3


O2
C10
C9
N1
C8
C11
N2
C12
C5
C6
C4
C3
C2
C7
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N3
C13
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C15
C14
C16
C17
C18
C19

SCHEME 3

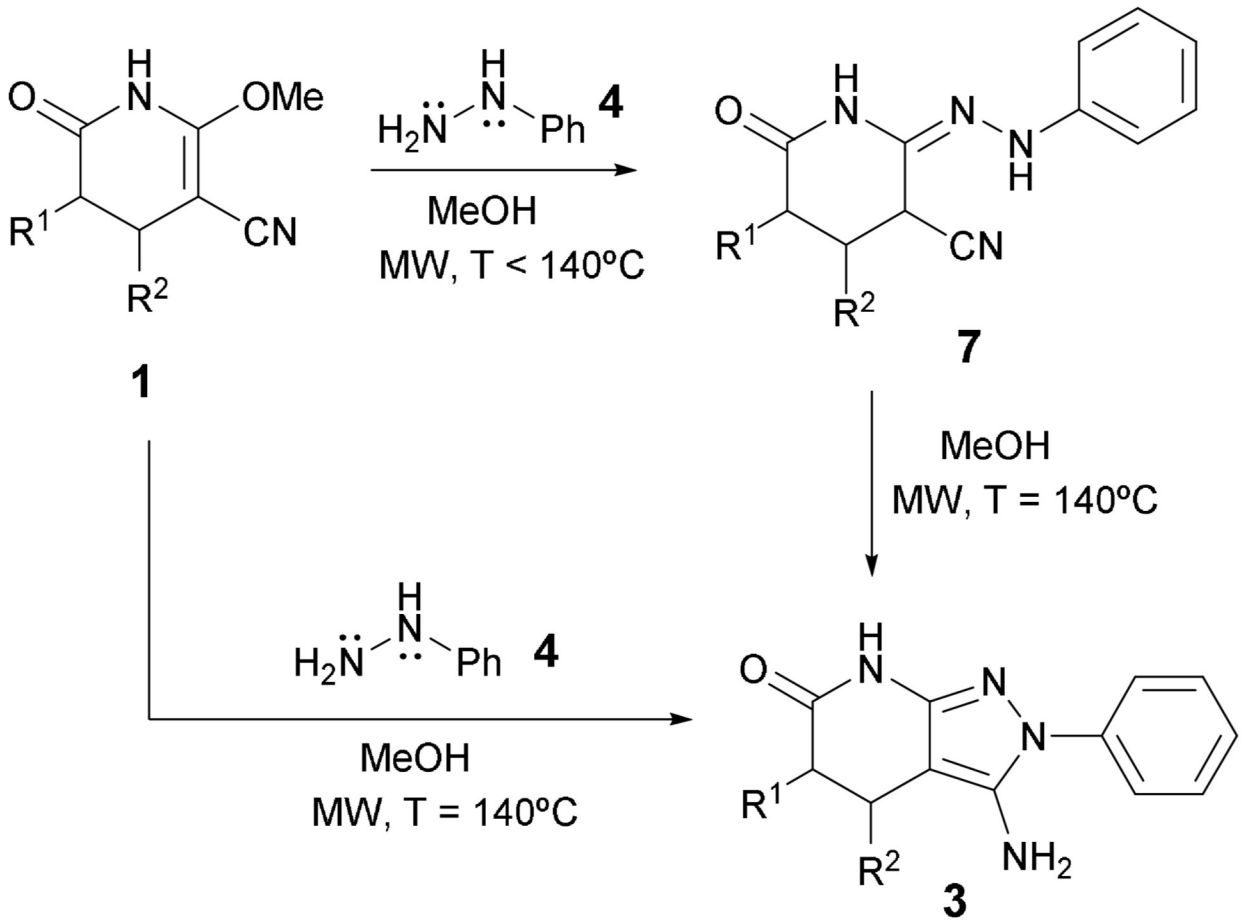
OMe
CN
R1
R2
1
4
MeOH
MW, T < 140ºC
7
MeOH
MW, T = 140ºC
4
MeOH
MW, T = 140ºC
NH2
3

Table 1 Experimental conditions tested for the treatment of 1a with phenylhydrazine 4 (R3 = Ph)

Solvent	Equiv. of **4a**	T (°C)	T (h)	Work-up[b]	Result (Yield)[c]
MeOH	2	140 mw[a]	0.5	1	**3a** (50%)
	2	140 mw	0.5	2	**3a** (47%)
	2	reflux	24	1	**7a** (70%)
	1	140 mw	0.5	2	**7a** + **3a**
	1	140 mw	0.5	1	**7a** + **3a**
	2	reflux	24	2	**7a** (74%)
	2	60 mw	0.5	1	**7a** (23%)
Solvent free	1	140 mw	0.25	2	3a (39%)
	1	60 mw	0.25	2	7a (27%)
THF	1	60 mw	0.25	1	**7a** (7%)
	1	140 mw	0.25	1	**7a** impure
	1	140 mw	0.5	1	**7a** (9%)
	2	140 mw	0.25	1	**7a** (3%)
	2	reflux	24	1	**7a** (25%)
CH_2Cl_2	2	140 mw	0.5	1	**7a** (15%)

[a] microwave irradiation. [b] Work-up: (1) filtration, (2) CH_2Cl_2 extraction. [c] isolated compound, ^{1}H-NMR spectra of mother liquors present only signals corresponding to **7a** or **3a**, depending on the experiment, and excess of phenylhydrazine 4 (R^3 = Ph).