Tetrahedron 68 (2012) 4628–4634

Contents lists available at SciVerse ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

Synthesis and electrochemical evaluation of substituted imidazo[4,5-*d*]pyrrolo[3,2-*f*][1,3] diazepine scaffolds

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ARTICLE INFO

Article history:
Received 25 January 2012
Received in revised form 5 April 2012
Accepted 6 April 2012
Available online 20 April 2012

Keywords:
Cyanoformimidoyl imidazole
Cyanoacetamide
Imidazo-pyrrolodiazepine
Cyclic voltammetry
Reduction potential

ABSTRACT

Substituted 4-(2,5-dihydro-1*H*-pyrrol-3-yl)-1*H*-imidazoles were prepared from 5-amino-1-aryl-4-cyanoformimidoylimidazoles and cyanoacetamide, under mild experimental conditions. The pyrrolyl-imidazoles were cyclized to the corresponding 7,8-dihydroimidazo[4,5-*b*]pyrrolo[3,4-*d*]pyridines by reflux in ethanol, with catalysis by DBU. The same pyrrolyl-imidazoles were reacted with orthoesters, at room temperature and in the presence of sulfuric acid, to generate 3,7-dihydro-8*H*-imidazo[4,5-*d*]pyrrolo[3,2-*f*]diazepines in very good yield. Electrochemical studies of the imidazo[4,5-*d*]pyrrolo[3,2-*f*][1,3] diazepine derivatives were carried out. The reduction potential of 7-ethyl-3-(4-methoxyphenyl)-8-oxo-7,8-dihydro-3*H*-imidazo[4,5-*d*]pyrrolo[3,2-*f*][1,3] diazepine-9-carbonitrile was in the adequate range for presenting bioreduction properties.

1. Introduction

The imidazole ring is present in the structure of several bioactive natural products[1] and of a broad range of medicinally useful agents.[2] The synthesis of substituted imidazoles has been widely reported in the literature[3] and varying the position and nature of the substituents allowed the preparation of a variety of derivatives. The synthesis of 4/5-heterylimidazoles, although less common, has also been described. According to the literature reports, it is usually difficult to use the available cross-coupling methods to link the imidazole to the heterocyclic ring. The best approach is still to construct the heterocyclic unit from an appropriate substituent in the imidazole moiety, or to build the imidazole ring from convenient precursors, already incorporating the heterocycle. The synthesis of 4-pyridinyl[4] and 4-pyrimidinyl[4,5] imidazoles has been reported, and also of imidazoles where the heterocycle is linked to a linear chain in the 4 or 5 position.[6] Recently, Hosmane[7] et al. reported the use of 4,4′-biimidazole, prepared by a synthetic approach previously developed in our research group, as a new precursor for the 5:7:5-fused biimidazolediazepine ring system. These compounds showed potent anti-cancer properties[8] and the analogy with the pyrrolo imidazodiazepine scaffold reported in this work suggests that this new tricyclic systems may also present angiogenic activity.

In general, electron transfer (ET) and oxidative stress have been increasingly implicated in the action of drugs, in particular as anti-infective and anti-cancer agents.[9] Many bioactive substances, or their metabolites, incorporate ET groups, so electrochemistry can provide valuable insight concerning the mode of action of drugs. Significantly, a large number of physiologically active substances possess redox potential values greater than about −0.5 V versus NHE (−0.744 V vs SCE), in the physiological active range. They can accept electrons from biological donors or suffer metabolic changes, providing easily reduced derivatives.[9,10] The redox potentials shift greatly in aprotic media compared to water and are normally more negative (for reductions) than those measured in water. Therefore the values of redox potential of physiologically active substances in aprotic media are usually higher than −1.10 V versus SCE.[10]

A few studies[11–13] established a relationship between the reduction potential of anti-cancer agents and their cytotoxicity against several tumor cell lines – the more delocalized the electronic system, implying higher reduction potentials, the greater the cytotoxic activity.

These results suggest that a preliminary screening of the potential anti-cancer activity of synthetic compounds can be made using their reduction potential. With this aim the electrochemical behavior of the imidazo[4,5-*d*]pyrrolo[3,2-*f*]diazepine derivatives was studied and DMSO was used as solvent in order to mimic the hydrophobic cell environment.

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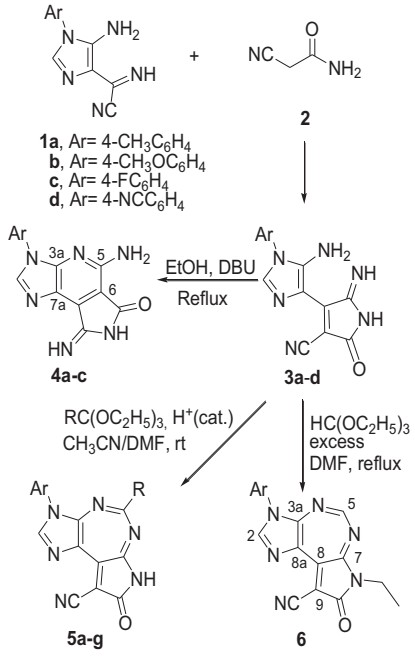
doi:10.1016/j.tet.2012.04.030

2. Results and discussion

2.1. Synthesis

In our research group, 5-amino-4-cyanoformimidoyl imidazoles **1** have been used as versatile precursors for the synthesis of fused heterocycles incorporating the imidazole unit.[14] The reaction of imidazoles **1** with carbon acids (malononitrile,[15] methyl cyanoacetate,[16] benzoylacetonitrile, and phenylsulfonylacetonitrile[17]) indicated that the addition occurred selectively to the carbon atom of the cyanoformimidoyl substituent. Ammonia or HCN were eliminated from the adduct, depending on whether the reaction was carried out in the absence or in the presence of DBU, respectively. Intramolecular cyclization led to the formation of highly substituted imidazo[4,5-*b*]pyridines, isolated in excellent yield.

This work reports the reaction of 5-amino-4-cyanoformimidoyl imidazole **1** with cyanoacetamide **2** (1.5–2 molar equiv) using ethanol/acetonitrile or ethanol/DMF as solvent (Scheme 1, Table 1, entries 1–4). The use of solvent mixtures was critical for the formation of product **3**. Imidazole **1** was soluble in acetonitrile (**1a**, **1c**) or DMF (**1b**, **1d**) and ethanol was essential for the reaction as in its absence no evolution was detected. The 4-pyrrolylimidazole **3** was the only isolated product that gradually precipitated from a deep red solution and was filtered after 3–10 days at room temperature. To rationalize the reactivity of cyanoacetamide in this Knoevenagel-type condensation, imidazole **1a** was combined with 2 molar equiv of cyanoacetamide in DMSO-d_6 and the reaction was followed by H^1 NMR. After 10 days at 20 °C, the signals for the product were completely absent in the spectrum. The reagents were then combined in deuterated methanol using 3 molar equiv of cyanoacetamide and the reaction was followed by ^{1}H NMR over a 24 h period. A signal at δ 3.58 ppm, assigned to the methylene group of cyanoacetamide, was present in the spectrum as a triplet (J=2.7 Hz), evidencing long range coupling with the two amide protons.



Scheme 1. Synthesis of compounds **3**–**6**.

The C–H signal for product **3a** (δ 7.58 ppm) was visible in the spectrum after 10 min at room temperature and a single product was slowly formed as only 8% of **3a** was generated after 24 h at 20 °C. The low rate of reaction may result from the formation of an equilibrium mixture between the reagents and adduct **7**, which is slowly displaced as product **3** precipitates from solution (Scheme 2). The formation of compound **3** requires the elimination of ammonia from intermediate **7**, leading to structure **8**. Compound **8** was never isolated nor identified, as it promptly evolved to the 4-pyrrolyl-imidazole **3** by intramolecular cyclization. The ammonia generated during the reaction could be responsible for the cyclization step, as previous work showed that, for similar structures bearing a cyano and a carbamoyl group on adjacent alkene carbon atoms, a pyrrole ring was always formed upon addition of base.[18] Acetic acid or trifluoroacetic acid were added to the reaction mixture (Table 1, entries 1 and 3) in order to trap ammonia as the reaction proceeded, but compound **3** was again the only product isolated in moderate yield.

The structure of compound **3** was supported mainly by ^{1}H and ^{13}C NMR data. The signal for the C–H proton in the δ 7.7–7.8 ppm region and the corresponding carbon around δ 138 ppm were considered indicative of the presence of a substituted imidazole ring. The two signals around δ 10.2 and δ 9.3 ppm were assigned to the imino and pyrrolo protons and a broad singlet in the δ 9.35–9.64 ppm region, integrating for two protons, was associated to the amino group.

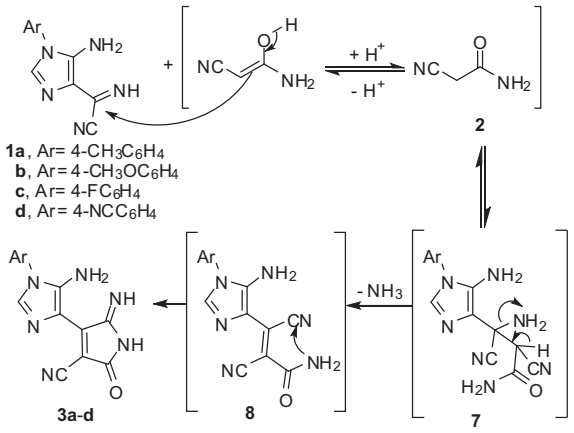
This value was considered unusually high, when compared with the chemical shift of the amino group in an analogous structure **9** (around δ 7.2 ppm) (Fig. 1). The electron-withdrawing effect of the substituent on C-4 of both compounds must be comparable, as the chemical shift for the imidazole proton on C-2 has the same value for **3** and **9** (δ 7.7 ppm). The striking difference in the chemical shift of the amino substituent on C-5 can only be explained by the vicinity of the imino double bond. Considering that the pyrrole substituent is slightly twisted in relation to the plane of the imidazole ring, the protons of the amino group can be experiencing the deshielding anisotropic effect of the π bond, pushing the chemical shift of these protons to an unexpected low field. The imino and carbonyl carbon atoms were associated with the two signals around δ 168 ppm and δ 160 ppm and a single peak at δ 115.9, assigned to the cyano group, further confirms the presence of the cyclic pyrrole substituent. In the infrared spectrum, the carbonyl stretching vibration corresponds to an intense band between 1710 and 1729 cm^{-1}. According to the literature[18] this high value, compared with what was expected for the carbonyl group of the conjugated linear amide, is typical of the pyrrolone structure.

The functional groups present in the pyrrolyl-imidazole **3** prompted us to induce intramolecular cyclization. The reaction occurred when a suspension of **3a**–**c** in ethanol was refluxed in the presence of a catalytic amount of DBU (Scheme 1, Table 1, entries 5–7). The deep red suspension was converted to a dark green solution, with formation of a greenish-yellow precipitate identified as the 7,8-dihydroimidazo[4,5-*b*]pyrrolo[3,4-*d*]pyridin-6(3*H*)-one **4**. The synthesis of a similar structure (isolated in 7% yield) was previously reported in the literature, and the pyrrole ring was generated upon cyclization of an amide and ester functions in positions 6 and 7 of a substituted imidazo-pyridone.[19]

Compound **3** was also reacted with 5 equiv of orthoesters (orthoformate, orthoacetate, orthopropionate, and orthobenzoate) in acetonitrile and in the presence of acid catalysis. Cyclization occurred between the imidate carbon and the imino nitrogen, generating the fused diazepine ring of compounds **5a**–**g** (Scheme 1, Table 1, entries 8–14). When compound **3b** was combined with an excess of triethyl orthoformate and the mixture was refluxed in DMF for 2 h, the same diazepine moiety was generated, but the N–H proton on the pyrrole unit was replaced by the ethyl group leading to compound **6**. The use of triethyl orthoformate as an alkylating reagent was reported in a previous work,[20] when 1,2,4-benzothiadiazine 1,1-dioxide and triethyl orthoformate were heated at 150 °C for 3 h. In this work, the alkylation was also due to the large excess of orthoformate present in the reaction mixture and not caused by ethanol, released during the synthesis. This was

Table 1
Yield and reaction conditions for the synthesis of compounds **3**–**5**

Entry	Ar	R	Reaction conditions	Product (Y%)
1	4-$H_3CC_6H_4$	—	**1a**+**2** (2 equiv), EtOH/MeCN (3:1), 3 d, rt	**3a** (91%)
		—	**1a**+**2** (2 equiv), EtOH/MeCN (3:1), AcOH (40 μL, 0.7 mmol), 5 h, rt	**3a** (70%)
2	4-$H_3COC_6H_4$	—	**1b**+**2** (1.5 equiv), EtOH/DMF (2:1), 5 d, rt	**3b** (83%)
3	4-FC_6H_4	—	**1c**+**2** (2 equiv), EtOH/MeCN (4:1), 5 d, rt	**3c** (77%)
		—	**1c**+**2** (2 equiv), EtOH/MeCN (3:1), TFA (10 μL, 0.13 mmol), 5 h, rt	**3c** (65%)
4	4-NCC_6H_4	—	**1d**+**2** (1.5 equiv), EtOH/DMF (2:1), 10 d, rt	**3d** (94%)
5	4-$H_3CC_6H_4$	—	**3a**, EtOH, DBU (cat.), 2 h, reflux	**4a** (81%)
6	4-$H_3COC_6H_4$	—	**3b**, EtOH, DBU (cat.), 2 h, reflux	**4b** (91%)
7	4-FC_6H_4		**3c**, EtOH, DBU (cat.), 2 h, reflux	**4c** (89%)
8	4-$H_3CC_6H_4$	H	**3a**, $HC(OEt)_3$ (5 equiv), MeCN, H_2SO_4 (cat), 5 min, rt	**5a** (83%)
9	4-$H_3COC_6H_4$	H	**3b**, $HC(OEt)_3$ (5 equiv), MeCN, H_2SO_4 (cat), 20 min, rt	**5b** (88%)
10	4-FC_6H_4	H	**3c**, $HC(OEt)_3$ (5 equiv), MeCN, H_2SO_4 (cat), 10 min, rt	**5c** (85%)
11	4-NCC_6H_4	H	**3d**, $HC(OEt)_3$ (5 equiv), MeCN, H_2SO_4 (cat), 30 min, rt	**5d** (65%)
12	4-$H_3CC_6H_4$	Me	**3a**, $MeC(OEt)_3$ (5 equiv), MeCN, H_2SO_4 (cat), 6 d, rt	**5e** (82%)
13	4-$H_3CC_6H_4$	Et	**3a**, $EtC(OEt)_3$ (5 equiv), MeCN, H_2SO_4 (cat), 2 h, rt	**5f** (82%)
14	4-$H_3CC_6H_4$	Ph	**3a**, $PhC(OEt)_3$ (5 equiv), MeCN, H_2SO_4 (cat), 6 d, rt	**5g** (82%)



Scheme 2. A plausible mechanism for the formation of 4-pyrrolyl-imidazoles **3**.

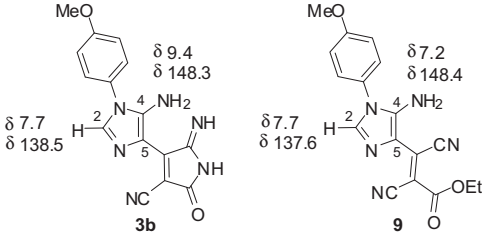


Fig. 1. ^1H and ^{13}C NMR values for compound **3b** and **9**.

confirmed by refluxing a solution of compound **5b** in DMF and triethyl orthoformate for 2 h. Product **6** was isolated in 66% yield after removal of the solvent and addition of ethanol. When the same experimental procedure was reproduced, using DMF and ethanol, the starting material **5b** was recovered in 70% yield. To our knowledge, there is no previous record for the synthesis or isolation of this type of fused tricyclic structure. Substituted imidazodiazepines are well known compounds since the discovery of pentostatin, a potent inhibitor of adenosine deaminase used in the treatment of leukemia.[21] The severe toxicity, poor oral absorption, and rapid metabolism of this compound, resulted in an eager search for analogous structures with reduced side effects.[22]

The fused tricyclic structure of compounds **4** and **5** was supported by NMR correlation techniques. The extended aromatic system was confirmed by the chemical shift of the C–H imidazole proton (δ 8.6–8.7 ppm for compounds **4** and δ 8.9–9.0 ppm for compounds **5**) and that of the corresponding carbon (around δ 145 ppm for compounds **4** and δ 147 ppm for compounds **5**). In the ^{13}C NMR of compounds **4**, the signals for the pyrrole carbon atoms are broad and could not be identified in **4a** and **4c**. This suggests extensive tautomerism of the N–H protons associated with this ring. The presence of the diazepine ring in compounds **5** was carefully confirmed through the 3-bonds H–C correlation of the proton on C-5 (around δ 8.5 ppm) with the imino carbon in the pyrrole ring (C-7, around δ 160 ppm) and the imidazole carbon C-3a (around δ 145 ppm). The H–N correlation spectrum (HMBC) of compound **4a**[23] showed that C2–H (δ 8.97 ppm) was in the vicinity of an imino (δ 251.6 ppm) and an amino (δ 184.9 ppm) nitrogen atom. The proton C5–H (δ 8.51 ppm) in compound **5a** interacted with two different imino nitrogen atoms (δ 255.9 ppm and δ 263.7 ppm). This data was in good agreement with the values reported in the literature for the ^{15}N NMR of substituted purine derivatives.[24]

2.2. Electrochemistry

In order to get information regarding the potential bioactivity of the imidazo[4,5-*d*]pyrrolo[3,2-*f*][1,3] diazepine derivatives, their electrochemical properties were analyzed by cyclic voltammetry. Cyclic voltammograms of compounds **5a**–**f** and **6** were recorded in DMSO containing 0.10 M tetrabutylammonium tetrafluoroborate (nBu$_4$NBF$_4$) as supporting electrolyte at different scan rates (0.020–0.400 V s^{-1}), using a glassy carbon electrode, at room temperature under argon atmosphere. The relevant electrochemical data for all compounds are summarized in Table 2, where the potentials are registered against saturated calomel electrode (SCE).

With the exception of compound **6**, that presented a more complex cyclic voltammogram, the electrochemical behavior of all

Table 2
Cyclic voltammetric data for reduction of compounds **5a**–**f**, **6** at a Glassy Carbon Electrode obtained at a scan rate of 0.10 V s^{-1}. A 1.0 mM solution of each compound in DMSO containing 0.10 M nBu$_4$NBF$_4$ was used. Potentials are given versus SCE

Compound	E_p (I′c)/V	E_p (Ic)/V	E_p (IIc)/V
5a	—	−1.484	−2.107
5b	—	−1.478	−2.108
5c	—	−1.441	−2.001
5d	—	−1.417	−2.013
5e	—	−1.517	−2.140
5f	—	−1.502	−2.032
6	−0.744	−1.470	−2.290

compounds was similar, suggesting an analogous reduction mechanism. The voltammograms obtained for compound **5b**, typical of the whole series, and for compound **6** are presented in Fig. 2.

Reduction of compounds **5a**–**f** occurred in two voltammetric waves. The first one appears as a reversible wave (labeled Ic and Ia, Fig. 2a), whereas the second reduction wave can be considered irreversible (labeled IIc). The first reduction peak potentials of the compounds range from −1.417 V up to −1.517 versus SCE and the second peak covers a wider range (140 mV) and varies from −2.001 V to −2.140 versus SCE. Peak currents obtained for both electrochemical processes of all the compounds increase linearly with the square root of the scan rate, suggesting diffusion-controlled processes.

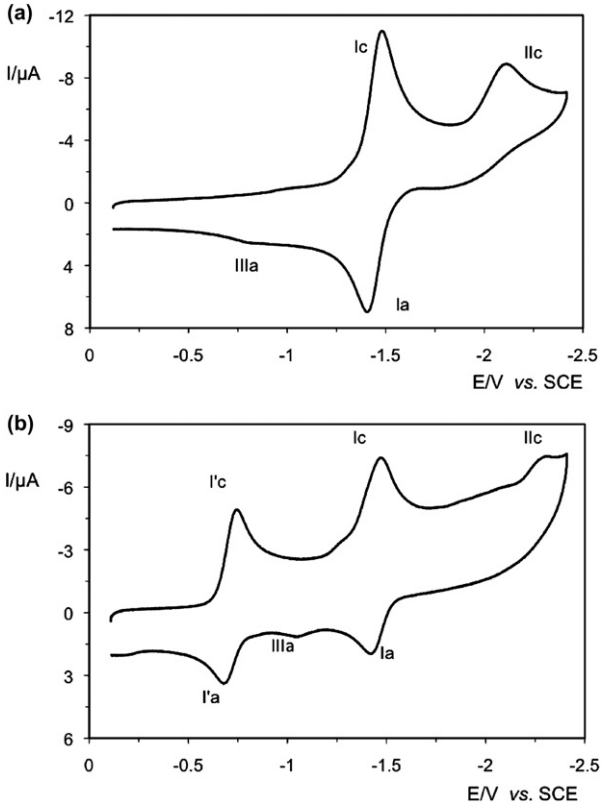
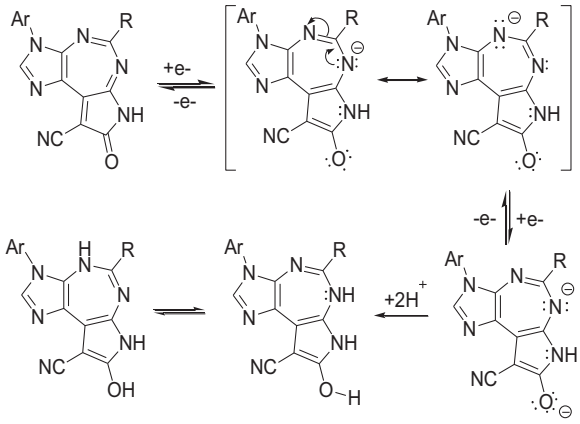


Fig. 2. Cyclic voltammograms of a 1.0 mM solution of (a) compound **5b** and (b) compound **6**, in DMSO containing 0.1 M nBu$_4$NBF$_4$, at a glassy carbon electrode; scan rate of 0.10 V s^{-1}.

The first peak of the voltammograms (Ic and Ia) corresponds to a reversible Nernstian one electron transfer (E_p(Ic) is independent of the scan rate and I_p(Ia)/I_p(Ic) equals 1).[25] The data for the second reduction peak suggests that the second electron transfer is followed by other processes (E_p(IIc) varies with scan rate and an anodic peak is absent in the reverse scan).[25] Moreover, on the reverse scan, a small oxidation wave (IIIa) at ca. −0.8 V versus SCE was observed. This wave vanished when the potential scan was reversed after the first reduction peak, indicating that it corresponds to the oxidation of a product formed on the second reduction process. The height of this peak is different for each compound suggesting that the amount of product formed is probably related to the rate of the chemical reaction coupled to the second electron transfer.

Based on previous studies regarding reduction of quinones in non aqueous media[11] we propose a reduction mechanism that involves the carbonyl group of the lactam ring (Scheme 3).

The first electron uptake (wave Ic, Fig. 2a) leads to a stable radical anion, that is, highly stabilized on the seven-membered ring, in particular on the second nitrogen atom. The radical anion



Scheme 3. Proposed mechanism for the redox process of imidazo[4,5-*d*]pyrrolo[3,2-*f*][1,3] diazepines **5a**–**f**.

then suffers a second electron transfer, at a more negative potential (wave IIc, Fig. 2a), generating a dianion that seems to be unstable under these conditions. It can be protonated by residual water from the solvent or from a nearby molecule of the fused tricyclic compound, with an acidic proton on the lactam nitrogen. Protonation can occur on the oxygen atom and on one of the nitrogen atoms of the diazepine ring leading to different products. The substituents may affect the stability of the compound and/or the kinetics of the chemical reaction.

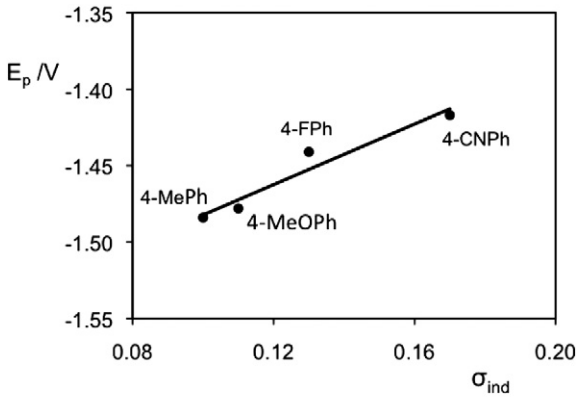
The cyclic voltammogram of compound **6** (Fig. 2b) presents two major peaks (Table 2). The first peak, at −0.744 V versus SCE, corresponds to a reversible process (I′c and I′a) and the second one, at −1.470 V versus SCE (Ic and Ia) can be considered a quasi-reversible process.[25] The replacement of a hydrogen atom (compound **5b**) by an ethyl group (compound **6**) at the lactam nitrogen generates a compound, that is, more easily reduced. The only structural difference between compounds **6** and **5b** is the presence of an ethyl group instead of the hydrogen atom and this must be responsible for the observed differences in peak potentials.

The amide moiety in compound **5b** allows the formation of a stable dimer, through two intermolecular hydrogen bonds. Compound **6** can only exist as the monomeric species. The more negative reduction potential obtained for compound **5b** can be assigned to this stabilization. The reduction of compound **6** is easier, and occurs at a less negative reduction potential. This observation is in agreement with a similar situation reported in the literature and sustained by theoretical calculations.[26] Further work is currently being developed on the synthesis and electrochemical analysis of different *N*-substituted imidazo-pyrrolodiazepines in order to support this mechanistic proposal.

Previous studies[27] identified correlations between redox potentials of organic molecules and Hammett substituent constants. These correlations are associated to the fact that electrochemical processes involve addition or removal of electrons from an organic framework and therefore should be related with the ability of substituents to withdraw or donate electrons to that framework.[27] In this work, a quantitative measure of the effect of the aryl substituent on the imidazole ring over the electrochemical reduction of compounds **5a**–**d** was also established. The first reduction peak potentials obtained by cyclic voltammetry were plotted versus the Hammett-constants considering inductive/field effect,[28] as shown by Fig. 3. The peak reduction potentials vary linearly with Hammett-constant (r=0.97) according to the equation:

$$E_p = 0.991\sigma_{ind} - 1.58$$

This correlation suggests that the reduction potentials of compounds **5** are affected primarily by the inductive effect of the aryl



Compound	Substituent	σ_{ind}	E_p (Ic)/V *vs*. SCE
5a	4-$H_3CC_6H_4$	0.10	-1.484
5b	4-$CH_3OC_6H_4$	0.11	-1.478
5c	4-F C_6H_4	0.13	-1.441
5d	4-NCC_6H_4	0.17	-1.417

Fig. 3. Correlation of first reduction peak potential, E_p (Ic), of compounds **5a**–**d** obtained by cyclic voltammetry with the calculated σ_{ind} Hammett-constants[28] of the aryl substituent.

substituent on the imidazole moiety and, as expected, the introduction of electron-withdrawing substituents facilitates the reduction.

In summary, the peak potential of the synthesized compounds (Table 2) partially reflects the influence of substituents R and Ar on their redox properties.

(A) The replacement of a hydrogen atom on the C2 of the diazepine ring by an ethyl or methyl group (compounds **5a**, **5f**, and **5e**) leads to a decrease in the reduction potential, therefore making it more difficult to reduce.

(B) Electron-withdrawing groups (Ar=4-NCPh, 4-FPh) on the imidazole ring facilitate the reduction of compounds (higher reduction potential) whereas electron-donating groups (Ar=4-H_3CPh, 4-H_3COPh) have the reverse effect (Fig. 3). This may be the result of extended stabilization of the radical anion on the aryl substituent.

The first reduction peak potential can be used as a descriptor of the ease of reduction of these compounds that can be ranked as follows: **6**>**5d**>**5c**>**5b**>**5a**>**5f**>**5e**.

3. Conclusion

Easily accessible pyrrolyl-imidazoles **3** were used as precursors of fused imidazo-pyrrolo-pyridines **4** and imidazo-pyrrolo-diazepines **5**. These compounds, which are not easily prepared by other methods, were generated under mild reaction conditions and isolated in very good yields. Considering that redox potentials provide information on the feasibility of electron transfer in vivo and that physiologically active substances should possess a redox potential in the range −0.7−−1.1 V versus SCE in aprotic media,[10] compound **6** may be a promising anti-cancer agent, analogous to 5:7:5-fused diimidazodiazepine recently recognized as highly active.

4. Experimental section

4.1. General

All compounds were fully characterized by elemental analysis and spectroscopic data. The NMR spectra were recorded at 300 MHz for ^{1}H and 75 MHz for ^{13}C or at 400 MHz for ^{1}H and 100 MHz for ^{13}C, including the ^{1}H–^{13}C correlation spectra (HMQC and HMBC). Deuterated DMSO was used as solvent. The chemical shifts are expressed in δ (parts per million) and the coupling constants (J) in hertz (Hz). IR spectra were recorded on a FT-IR using Nujol mulls and NaCl cells. The reactions were monitored by thin layer chromatography (TLC) using silica gel. The melting points were determined on a melting point apparatus and are uncorrected.

Electrochemical measurements were made in methyl sulfoxide (DMSO, extra dry, 99.7%) used without further purification. Tetra-*n*-butylammonium tetrafluoroborate (nBu$_4$NBF$_4$, 98%) kept for 24 h in a vacuum oven at 70–80 °C to remove traces of water was used as supporting electrolyte. A conventional two-compartment three-electrode glass cell, a glassy carbon working electrode (3 mm diameter), together with a platinum counter-electrode were used. The pseudo-reference electrode consisted of a silver wire immersed in DMSO containing nBu$_4$NBF$_4$ (0.1 M) and the ferrocene/ferrocenium cation couple was used as internal standard. All potentials presented are referenced to the saturated calomel electrode (SCE). Solutions of the tested compounds (1 mM) in DMSO containing 0.1 M of nBu$_4$NBF$_4$ were prepared just before electrochemical experiments. Prior to the measurements, solutions were bubbled with argon to eliminate dissolved oxygen and the working electrode was cleaned with an aqueous suspension of 0.05 μm alumina on a polishing pad, rinsed and wiped dry. The cyclic voltammograms were recorded at scan rates in the range 0.020–0.400 V s^{-1} using various starting and switching potentials.

4.2. General procedure for the synthesis of 4-[5-amino-1-(aryl)-1*H*-imidazol-4-yl]-5-imino-2-oxo-2,5-dihydro-1*H*-pyrrole-3-carbonitrile 3

Method A: A solution of 5-amino-1-aryl-4-(cyanoformimidoyl) imidazole **1a**–**d** (0.80–1.80 mmol) in EtOH (10–20 mL) and MeCN (3–6 mL) or DMF (3–5 mL) was combined with cyanoacetamide (1.5–2.0 molar equiv) and the mixture was stirred at room temperature for 3–10 days. A red solid suspension separated from a deep red solution and was filtered and washed with Et$_2$O and EtOH. Structure **3** was assigned to the products on the basis of elemental analysis, ^{1}H NMR and ^{13}C NMR spectroscopy.

Method B: A solution of 5-amino-1-(4-methylphenyl)-4-(cyanoformimidoyl)imidazole **1a** or **1c** (0.80 mmol) in EtOH (10 mL) and MeCN (3 mL) was combined with cyanoacetamide (2.0 molar equiv) and (40 μL, 0.7 mmol) of acetic acid (for **1a**) or (10 μL, 0.13 mmol) of trifluoroacetic acid (for **1c**). The mixture was stirred at room temperature for 19 h (**1a**) or 5 h (**1c**). A red solid suspension separated from a deep red solution and was filtered and washed with Et$_2$O and EtOH. The isolated product was identified as **3a** (70%) or **3c** (65%).

*4.2.1. [5-Amino-1-(4-methylphenyl)-1H-imidazol-4-yl]-5-imino-2-oxo-2,5-dihydro-1H-pyrrole-3-carbonitrile (**3a**).* Red solid (215 mg, 0.73 mmol, 91%). Mp 271–273 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.24 (s, 1H), 9.37 (s, 2H), 9.34 (s, 1H), 7.74 (s, 1H), 7.38 (s, 5H), 2.39 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 167.5, 160.0, 147.9, 147.2, 138.9, 138.2, 130.8, 130.5, 125.7, 115.9, 115.0, 85.7, 20.7; IR (Nujol mull) 3322, 3237, 2216, 1724, 1633, 1578. Anal. Calcd for $C_{15}H_{12}N_6O.0.1H_2O$: C, 61.26; H, 4.18; N, 28.58, found: C, 60.95; H, 4.32; N, 28.54.

*4.2.2. 4-[5-Amino-1-(4-methoxyphenyl)-1H-imidazol-4-yl]-5-imino-2-oxo-2,5-dihydro-1H-pyrrole-3-carbonitrile (**3b**).* Red solid (210 mg, 0.67 mmol, 84%). Mp 267–268 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.22 (s, 1H), 9.35 (s, 2H), 9.31 (s, 1H), 7.71 (s, 1H), 7.43 (d, J=9.0 Hz, 2H), 7.13 (d, J=9.0 Hz, 2H), 3.82 (s, 3H); ^{13}C NMR

(75 MHz, DMSO-d_6) δ 168.0, 160.0, 159.7, 148.3, 147.2, 138.5, 127.6, 126.0, 116.0, 115.2, 114.9, 85.5, 55.6; IR (Nujol mull) 3357, 3235, 2215, 1729, 1706, 1629, 1608, 1578. Anal. Calcd for $C_{15}H_{12}N_6O_2.0.2H_2O$: C, 57.76; H, 4.00; N, 26.94, found: C, 57.62; H, 4.12; N, 26.69.

*4.2.3. 4-[5-Amino-1-(4-fluorophenyl)-1H-imidazol-4-yl]-5-imino-2-oxo-2,5-dihydro-1H-pyrrole-3-carbonitrile (**3c**).* Red solid (185 mg, 0.62 mmol, 77%). Mp 274–276 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.24 (s, 1H), 9.38 (br s, 3H), 7.76 (s, 1H), 7.59 (dd, J_1=9.0 Hz, J_2=4.8 Hz, 2H), 7.45 (t, J=8.7 Hz, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 168.0, 162.2 (d, J=244.88 Hz), 160.0, 148.1, 147.2, 138.2, 128.7 (d, J=9.15 Hz), 127.8 (d, J=3.15 Hz), 116.9 (d, J=23.18 Hz), 115.9, 114.8, 85.8; IR (Nujol mull) 3310, 3253, 2211, 2188, 1710, 1628, 1590. Anal. Calcd for $C_{14}H_9N_6OF.0.33$ DMF: C, 56.24; H, 3.52; N, 27.73, found: C, 55.85; H, 3.50; N, 28.13.

*4.2.4. 4-[5-Amino-1-(4-cyanophenyl)-1H-imidazol-4-yl]-5-imino-2-oxo-2,5-dihydro-1H-pyrrole-3-carbonitrile (**3d**).* Red solid (190 mg, 0.63 mmol, 79%). Mp >350 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.64 (br s, 4H), 8.11 (d, J=8.7 Hz, 2H), 7.85 (s, 1H), 7.76 (d, J=8.7 Hz, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 167.9, 159.9, 147.4, 147.3, 137.51, 137.48, 134.2, 126.8, 118.1, 115.7, 114.9, 111.6, 86.6; IR (Nujol mull) 3246, 3220, 2230, 2204, 1715, 1662, 1589. Anal. Calcd for $C_{15}H_9N_7O$ DMF. $0.25H_2O$: C, 56.76; H, 4.37; N, 29.42, found: C, 56.55; H, 4.08; N, 29.57.

4.3. General procedure for the synthesis of 5-amino-8-imino-3-(aryl)-7,8-dihydroimidazo[4,5-*b*]pyrrolo[3,4-*d*]pyridin-6(3*H*)-one 4

A solution of **3a**–**c** (0.89 mmol) in EtOH (10 mL) with a catalytic amount of DBU was refluxed for 2 h. A turbidity gradually developed leading eventually to a greenish-yellow solid, which was filtered and washed with Et_2O and EtOH. Structure **4** was assigned to the products on the basis of elemental analysis, ^{1}H NMR and ^{13}C NMR spectroscopy.

*4.3.1. 5-Amino-8-imino-3-(4-methylphenyl)-7,8-dihydroimidazo [4,5-b]pyrrolo[3,4-d]pyridin-6(3H)-one (**4a**).* Greenish-yellow solid (210 mg, 0.72 mmol, 81%). Mp >350 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.68 (br, 1H), 9.12 (br, 1H), 8.66 (s, 1H), 7.69 (d, J=9.0 Hz, 2H), 7.37 (d, J=9.0 Hz, 2H), 6.79 (br, 2H), 2.39 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 160.8, 152.8, 150.9, 144.9, 137.4, 132.4, 131.2 (br), 129.8, 123.8, 122.5, 20.6; IR (Nujol mull) 3279, 3155, 1714, 1661 cm^{-1}. Anal. Calcd for $C_{15}H_{12}N_6O.0.6\ C_2H_5OH$: C, 60.82; H, 4.91; N, 26.27, found: C, 60.59; H, 4.95; N, 26.17.

*4.3.2. 5-Amino-8-imino-3-(4-methoxyphenyl)-7,8-dihydroimidazo [4,5-b]pyrrolo[3,4-d]pyridin-6(3H)-one (**4b**).* Greenish-yellow solid (250 mg, 0.81 mmol, 91%). Mp >350 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.5–9.0 (br s, 2H), 8.60 (s, 1H), 7.69 (d, J=9.0 Hz, 2H), 7.13 (d, J=9.0 Hz, 2H), 6.78 (s, 2H), 3.82 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.6, 160.5, 158.7, 152.8, 151.1, 145.1, 131.3, 127.7, 125.7, 122.4, 114.5, 103.2, 55.5; IR (Nujol mull) 2262, 2190, 2140, 1660, 1640, 1595, 1569 cm^{-1}. Anal. Calcd for $C_{15}H_{12}N_6O_2$: C, 58.44; H, 3.92; N, 27.26, found: C, 58.27; H, 4.06; N, 27.02.

*4.3.3. 5-Amino-3-(4-fluorophenyl)-8-imino-7,8-dihydroimidazo[4,5-b]pyrrolo[3,4-d]pyridin-6(3H)-one (**4c**).* Yellow solid (233 mg, 0.79 mmol, 89%). Mp 334–335 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.62 (br s, 1H), 9.14 (br s, 1H), 8.68 (s, 1H), 7.87 (dd, J_1=9.0 Hz, J_2=4.8 Hz, 2H), 7.44 (t, J=9.0 Hz, 2H), 6.82 (br s, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 161.2 (d, J=243.5 Hz), 152.9, 150.9, 144.9, 131.3, 126.3 (d, J=8.63 Hz), 122.4, 116.3 (d, J=22.88 Hz); IR (Nujol mull) 3339, 3216, 1716, 1664 cm^{-1}. Anal. Calcd for $C_{14}H_9N_6OF.0.1H_2O$: C, 56.40; H, 3.11; N, 28.20, found: C, 56.31; H, 3.48; N, 27.81.

4.4. General procedure for the synthesis of 3-(4-aryl)-8-oxo-7,8-dihydro-3*H*-imidazo[4,5-*d*]pyrrolo[3,2-*f*][1,3] diazepine-9-carbonitrile 5a–d

Triethyl orthoformate (1.70 mmol) was added to a solution of **3a**–**d** (0.34 mmol) in MeCN (5 mL). Sulfuric acid (5 μL) was added and the reaction mixture was stirred at rt for 5–20 min. The solid was filtered and washed with Et_2O and EtOH. Structure **5** was assigned to the products on the basis of elemental analysis, ^{1}H NMR and ^{13}C NMR spectroscopy.

*4.4.1. 3-(4-Methylphenyl)-8-oxo-7,8-dihydro-3H-imidazo[4,5-d]pyrrolo[3,2-f][1,3] diazepine-9-carbonitrile (**5a**).* Orange solid (85 mg, 0.28 mmol, 83%). Mp >350 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.80 (br, 1H), 8.98 (s, 1H), 8.52 (s, 1H), 7.56 (d, J=8.1 Hz, 2H), 7.42 (d, J=8.1 Hz, 2H), 2.41 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 168.3, 160.3, 150.0, 147.2, 146.2, 138.9, 138.1, 131.2, 129.8, 129.3, 126.0, 113.7, 85.8, 20.7; IR (Nujol mull) 2220, 1704, 1630, 1592 cm^{-1}. Anal. Calcd for $C_{16}H_{10}N_6O.0.1H_2O$: C, 63.20; H, 3.38; N, 27.64, found: C, 63.03; H, 3.71; N, 27.59.

*4.4.2. 3-(4-Methoxyphenyl)-8-oxo-7,8-dihydro-3H-imidazo[4,5-d] pyrrolo[3,2-f][1,3] diazepine-9-carbonitrile (**5b**).* Yellow solid (95 mg, 0.30 mmol, 88%). Mp >350 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.80 (s, 1H), 8.97 (s, 1H), 8.53 (s, 1H), 7.60 (d, J=9.0 Hz, 2H), 7.16 (d, J=9.0 Hz, 2H), 3.85 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 168.4, 160.3, 159.7, 150.0, 147.9, 146.3, 138.1, 129.2, 127.6, 126.5, 114.5, 113.7, 85.7, 55.6; IR (Nujol mull) 2218, 1704, 1630, 1592 cm^{-1}. Anal. Calcd for $C_{16}H_{10}N_6O_2$: C, 60.38; H, 3.17; N, 26.40, found: C, 59.89; H, 3.36; N, 26.49.

*4.4.3. 3-(4-Fluorophenyl)-8-oxo-7,8-dihydro-3H-imidazo[4,5-d]pyrrolo[3,2-f][1,3] diazepine-9-carbonitrile (**5c**).* Yellow solid (88 mg, 0.29 mmol, 85%). Mp >350 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.84 (s, 1H), 9.01 (s, 1H), 8.54 (s, 1H), 7.76 (dd, J_1=8.7 Hz, J_2=4.8 Hz, 2H), 7.50 (t, J=8.7 Hz, 2H), ^{13}C NMR (75 MHz, DMSO-d_6) δ 168.3, 162.1 (d, J=245.0 Hz), 160.5, 150.1, 147.7, 146.2, 138.1, 130.06 (d, J=3.0 Hz), 129.2, 128.6 (d, J=9.0 Hz), 116.3 (d, J=23.0 Hz), 113.6, 86.0; IR (Nujol mull) 2227, 1731, 1630, 1589 cm^{-1}. Anal. Calcd for $C_{15}H_7N_6OF$: C, 58.83; H, 2.30; N, 27.44, found: C, 58.65; H, 2.45; N, 27.32.

*4.4.4. 3-(4-Cyanophenyl)-8-oxo-7,8-dihydro-3H-imidazo[4,5-d]pyrrolo[3,2-f][1,3] diazepine-9-carbonitrile (**5d**).* Yellow solid (95 mg, 0.30 mmol, 89%). Mp >350 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.90 (br s, 1H), 9.10 (s, 1H), 8.56 (s, 1H), 8.16 (d, J=9.0 Hz), 7.98 (d, J=9.0 Hz, 2H), ^{13}C NMR (100 MHz, DMSO-d_6) δ 168.3, 160.6, 150.4, 147.3, 145.8, 138.1, 137.5, 133.6, 129.5, 127.0, 118.2, 113.6, 111.7, 86.4; IR (Nujol mull) 2227, 1731, 1630, 1589 cm^{-1}. Anal. Calcd for $C_{16}H_7N_7O$: C, 61.34; H, 2.25; N, 31.30, found: C, 61.27; H, 2.56; N, 30.97.

4.5. Synthesis of 5-alkyl/aryl-3-(4-methylphenyl)-8-oxo-7,8-dihydro-3*H*-imidazo[4,5-*d*]pyrrolo[3,2-*f*][1,3] diazepine-9-carbonitrile (5e–g)

The appropriate orthoester (1.70 mmol) was added to a solution of **3a** (0.34 mmol) in MeCN (5 mL). Sulfuric acid (5 μL) was added and the mixture was stirred at rt for 2 h – 6 days. The solid was filtered and washed with Et_2O and EtOH. Structure **5e**–**g** was assigned to the product on the basis of elemental analysis, ^{1}H NMR and ^{13}C NMR spectroscopy.

*4.5.1. 5-Methyl-3-(4-methylphenyl)-8-oxo-7,8-dihydro-3H-imidazo [4,5-d]pyrrolo[3,2-f][1,3] diazepine-9-carbonitrile (**5e**).* Orange solid (70 mg, 0.22 mmol, 65%). Mp >350 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.53 (br, 1H), 8.87 (s, 1H), 7.56 (d, J=8.4 Hz, 2H), 7.41 (d, J=8.4 Hz, 2H), 2.60 (s, 3H), 2.41(s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 168.4, 160.7, 159.0, 147.1, 145.5, 138.7, 137.7, 131.4, 129.7, 128.3, 125.8, 113.8, 84.9, 30.3, 20.7; IR (Nujol mull) 2225, 1735, 1662, 1601, 1556 cm^{-1}. Anal. Calcd for $C_{17}H_{12}N_6O.0.1H_2O$: C, 64.18; H, 3.83; N, 26.42, found: C, 64.01; H, 3.98; N, 26.32.

*4.5.2. 5-Ethyl-3-(4-methylphenyl)-8-oxo-7,8-dihydro-3H-imidazo [4,5-d]pyrrolo[3,2-f][1,3] diazepine-9-carbonitrile (**5f**).* Orange solid (90 mg, 0.28 mmol, 82%); mp 336–337 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.51 (br s, 1H), 8.93 (s, 1H), 7.60 (d, J=8.4 Hz, 2H), 7.41 (d, J=8.4 Hz, 2H), 2.88 (q, J=7.2 Hz, 2H), 2.41 (s, 3H), 1.17 (t, J=7.2 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 168.4, 164.2, 159.1, 146.9, 145.4, 138.5, 138.5, 131.4, 129.6, 128.2, 125.6, 113.8, 85.1, 35.7, 20.7, 12.1; IR (Nujol mull) 2224, 1731, 1641, 1613, 1592 cm^{-1}. Anal. Calcd for $C_{18}H_{14}N_6O$: C, 65.44; H, 4.27; N, 25.44, found: C, 65.10; H, 4.33; N, 25.53.

*4.5.3. 3-(4-Methylphenyl)-8-oxo-5-phenyl-7,8-dihydro-3H-imidazo [4,5-d]pyrrolo[3,2-f][1,3]diazepine-9-carbonitrile (**5g**).* Orange solid (110 mg, 0.28 mmol, 83%); mp 332–334 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.68 (s, 1H), 9.00 (s, 1H), 8.26 (dd, J_1=7.5 Hz, J_2=2.1 Hz, 2H), 7.67 (d, J=8.1 Hz, 2H), 7.50–7.30 (m, 5H), 2.45 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 168.4, 159.4, 154.6, 147.7, 145.6, 138.7, 138.5, 137.9, 131.6, 131.4, 129.8, 129.2, 129.0, 128.7, 125.7, 113.9, 85.5, 20.8; IR (Nujol mull) 2228, 1727, 1633, 1590 cm^{-1}. Anal. Calcd for $C_{22}H_{14}N_6O.H_2O$, 0.2 NH_3: C, 66.09; H, 4.19; N, 21.72, found: C, 66.11; H, 4.06; N, 21.79.

4.6. Synthesis of 7-ethyl-3-(4-methoxyphenyl)-8-oxo-7,8-dihydro-3*H*-imidazo[4,5-*d*]pyrrolo[3,2-*f*][1,3] diazepine-9-carbonitrile (6)

Triethyl orthoformate (1 mL) was added to a solution of **3b** (0.26 mmol) in dimethylformamide (3 mL). The mixture was refluxed for 2 h and the solvent was removed under reduced pressure. Addition of ethanol led to a cream solid, which was filtered and washed with diethyl ether and ethanol. The isolated product was identified as **6**. Cream solid (75 mg, 0.22 mmol, 84%); mp 258–260 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.01 (s, 1H), 8.66 (s, 1H), 7.61 (d, J=9.0 Hz, 2H), 7.17 (d, J=9.0 Hz, 2H), 4.03 (q, J=7.2 Hz, 2H), 3.85 (s, 3H), 1.23 (t, J=7.2 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 166.7, 159.7, 157.3, 149.5, 148.2, 146.5, 137.9, 129.5, 127.6, 126.4, 114.6, 113.7, 84.0, 55.6, 34.9, 13.3; IR (Nujol mull) 2217, 1716, 1625, 1523 cm^{-1}. Anal. Calcd for $C_{18}H_{14}N_6O_2$. 0.4H_2O: C, 61.15; H, 4.22. Found: C, 61.43; H, 4.29.

Acknowledgements

The authors gratefully acknowledge the financial support by the University of Minho and Fundação para a Ciência e Tecnologia through the Portuguese NMR network (RNRMN), the Project F-COMP-01-0124-FEDER-022716 (ref. FCT PEst-C/QUI/UI0686/2011) FEDER-COMPETE and the post-doc grant awarded to Dr. M.Z. (SFRH/BPD/27029/2006).

Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2012.04.030. These data include MOL files and InChiKeys of the most important compounds described in this article.

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28. ACD/Labs software, version 8.0 for Microsoft Windows, licence No. 33377.