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2-Diazoindoles: building blocks for the synthesis of antineoplastic agents

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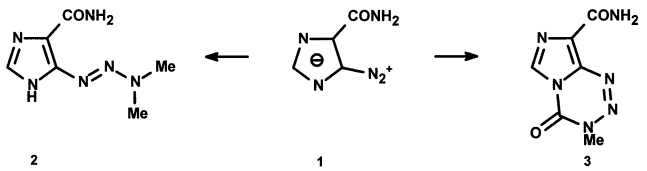
Abstract

2-Diazoindoles were prepared by diazotization of the corresponding 2-aminoindoles followed by neutralisation. 2-Diazoindoles were utilised for the synthesis of 2-triazenoindoles, indolo[2,1-d][1,2,3,5]tetrazines and indolo[2,1-c][1,2,4]triazines. Most of these compounds exhibited in vitro antiproliferative activity. © 2002 Éditions scientifiques et médicales Elsevier SAS. All rights reserved.

Keywords: Diazoindoles; Aminoindoles; Antineoplastic

For a long time diazoazoles have been very important key intermediates for the synthesis of molecules of biological interest. Among them, the most important was 5-diazoimidazole-4-carboxamide (**1**) which was the precursor of dacarbazine, 5-(3,3-dimethyl-1-triazenyl)-imidazole-4-carboxamide (**2**), the only triazene derivative used in anticancer chemotherapy and the most active drug available for treatment of malignant melanoma and Hodgkin tumours resistant to MOPP therapy, a combination of mechlorethamine, oncovine® (vincristine), prednisone and procarbazine [1,2] (Scheme 1).

Compound **1** was also the precursor of temozolomide (**3**), an imidazo-tetrazinone derivative which is now in the market with the trade name temodal® and is used against malignant melanoma, mycosis fungoides, and brain tumours [3–6].



2 1 3

Scheme 1.

3-Diazopyrroles (**4**) were utilised for the synthesis of 3-triazenopyrroles (**5**), which showed in vitro antileukaemic activity with IC_{50} in the low μM range [7]. Benzocondensation on the pyrrole ring led to the 3-triazenoindoles (**6**), which resulted 20–40 times more active in in vitro antileukaemic assays [8] (Scheme 2).

2-Diazopyrroles (**7**) were employed for the synthesis of 2-triazenopyrroles (**8**) which showed to be cytotoxic against leukaemic, lymphoma and carcinoma cell lines, with IC_{50} 3.9–21 μM, and to inhibit Cox-B2 and VSV with EC_{50} 10–50 μM [9].

Compounds **7** reacted with alkyl- or aryl-isocyanates to give the pyrrolo[2,1-d] [1,2,3,5]tetrazinone derivatives (**9**) which hold the deaza skeleton of temozolomide [10], and showed potent antiproliferative activity with IC_{50} 0.09–56 μM [11] (Scheme 3).

From compounds **7**, by reaction with methylene active compounds, it was possible to isolate derivatives of the pyrrolo[2,1-c][1,2,4]triazine ring system (**10**) [12]. These derivatives showed good antiproliferative activity with IC_{50} in the range 5.5–88 μM [13].

Having in mind the promising biological activities showed by the compounds obtained from diazopyrroles and considering the increase in antiproliferative activity achieved by the benzocondensation as in the case of 3-triazenoindoles, we thought to use also 2-diazoindoles as building blocks for the synthesis of derivatives with antineoplastic activity.

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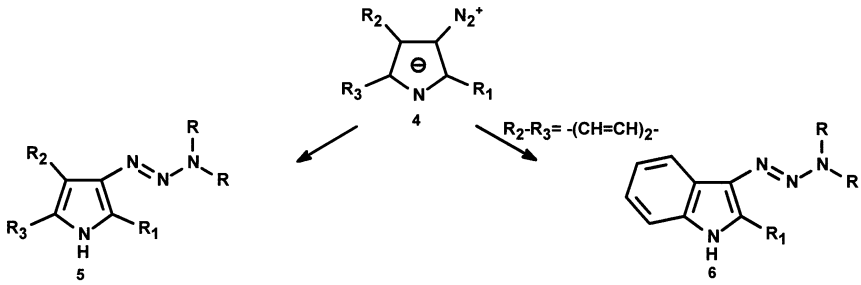


Scheme 2.

But 2-diazoindoles were unknown, probably because 2-aminoindoles are not easily available since they are unstable, difficult to handle and autooxidise extremely rapidly [14].

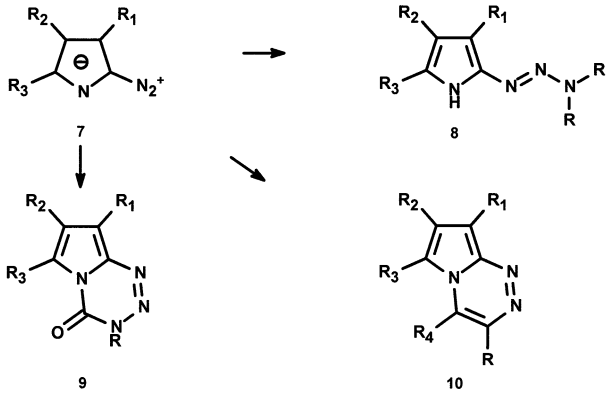
However, as protonation studies carried out on aminopyrroles [15] contributed to the understanding of the behaviour of these derivatives toward electrophiles and to the isolation of 2-diazopyrroles [16], we prepared some 2- and 3-aminoindoles in order to study their tautomerism and their behaviour towards protonation, which should have provided informations about the feasibility of the diazotization reaction of 2-aminoindoles leading to the unknown 2-diazoindoles. In fact, an evaluation of the behaviour of aminoindoles against protonation allowed us to be confident on the feasibility of the diazotization reaction, at least for derivatives bearing electron-withdrawing substituents, and suggested weakly acid reaction conditions. In fact 3-aminoindoles, that undergo very easily diazotization to give either diazonium salts and diazo compounds, are protonated at the exocyclic nitrogen both in DMSO/TFA and pure TFA. 2-Aminoindoles, instead, to give exocyclic protonation need an electron withdrawing group at the 3 position [17] (Scheme 4).

Thus 2-diazoindoles (**12**) were obtained by diazotization of the corresponding 2-aminoindoles (**11**) under the same reaction conditions employed in the preparation of the 2-diazopyrroles, i.e. with sodium nitrite in acetic acid at 0 °C. Strict control of the temperature is crucial to achieve reasonable yields (50–60%). 2-Diazoindoles are, as expected, light sensitive and can be stored only at −20 °C under nitrogen (Scheme 5).

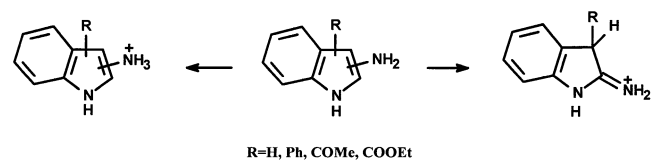
The structure of the 2-diazoindoles was confirmed by analytical and spectral data (IR, ^{1}H and ^{13}C NMR). In fact the presence of the diazo group was confirmed by IR spectroscopy, the only method that gives direct diagnostic information about the diazo function, which appeared as a sharp and strong band at 2117–2157 cm^{-1}.

Regarding the structure of the 2-diazoindoles, it would be expected that **a** would be the major dipolar canonical structure contributing to the resonance hybrid of the 2-diazoindoles and this expectation is supported by the ^{13}C NMR spectra which exhibited patterns comparable to those of other substituted 1H-indoles [18–21], and very similar to the ^{13}C NMR data of 3-ethoxycarbonylindole-2-diazonium chloride. On the other hand, a significant contribute of the canonical structures **c** and **d**, in which the location of the negative charge on the *ipso* carbon and on the benzene positions is unlikely. The structure **b** with the negative charge on the 3-position might provide some contribution to the resonance hybrid (Scheme 6).

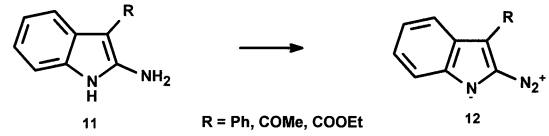
In order to confirm the structure assignment based on the NMR analysis, we run semiempirical molecular orbital calculation by using Vamp (V 6.5) software, supplied by Oxford Molecular. The structure of the diazo compounds and of the corresponding diazonium



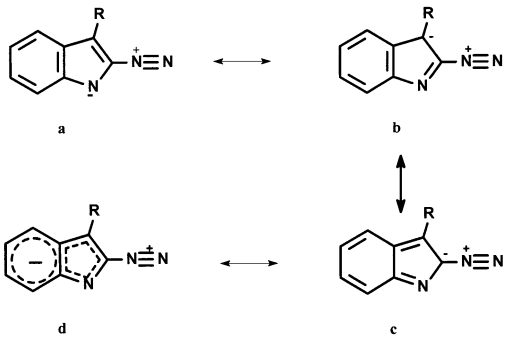
Scheme 3.



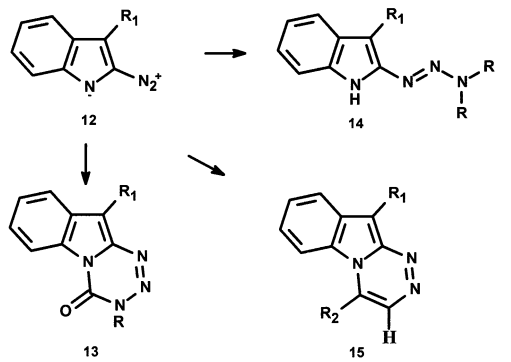
Scheme 4.



Scheme 5.



Scheme 6.



Scheme 7.

cations were fully optimised in vacuo by SCF calculation using at the beginning different Hamiltonians (AM1, PM3, MNDO) methods. All the methods gave good results in evaluating the interatomic distances, from which the bond orders could be derived, however, the best results in term of charge were obtained by using the PM3 Hamiltonian. Data obtained further supported a major contribute of structure **a** with the negative charge located on N-1, but also a substantial contribute from structure **b**.

Solvent effect was simulated using the PM3 method, which allowed also a neural net estimate of the ^{13}C chemical shifts [22]. Data obtained in the solvent were in agreement with those obtained in vacuo either for charges and interatomic distances. Moreover, we found a good linear correlation between the theoretical and experimental ^{13}C chemical shifts values ($r^2 = 0.88$ for the diazo and 0.92 for the diazonium species; the r^2 value was increased to 0.94 and 0.97, respectively, if the value obtained for C-2 is excluded). Evidently, the algorithm is not suitable for evaluating the solvent effect in the case of carbon more directly involved in this particular mesoionic structure.

Therefore, the structure of 2-diazoindoles differs from those of diazopyrroles and 3-diazoindoles in which the negative charge is mainly located on the *ipso* carbon [16,23,24].

Once obtained in preparative yields, the 2-diazoindoles (**12**) were employed for the synthesis of derivatives of the new ring system indolo[2,1-d][1,2,3,5]-tetrazinone (**13**), which exhibited in vitro antiproliferative activity against several human tumour cell lines with IC_{50} in the range 0.08–93 μM [25] (Scheme 7).

Compounds **12**, by reaction with secondary amines, gave the 2-triazenoindoles (**14**), and one of them showed moderate activity with IC_{50} in the range 2.3–99 μM [26].

Reaction of compounds **12** with methylene active compounds led to derivatives of the new ring system indolo[2,1-c][1,2,4]triazine (**15**), which are currently under screening to evaluate their antineoplastic activity [27].

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