A little about Pyrazolo[3,4-d][1,2]Diazepine: simple sustainable synthesis, NMR identification and prototropic equilibrium

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Abstract: A simple and sustainable method for the synthesis of pyrazolo [3,4-d] [1,2] diazepin-8-one, the heterocyclic isoster of 2,3-benzodiazepine, has been developed. It was found that the use of acids to cyclization of the pyrazolopyrone hydrazinolysis product leads to the formation of a condensed pyridine ring instead of 1,2-diazepine. Pyrazolodiazepinone in acidic medium also dramatically contracts the seven-membered cycle. The structure of this compound is proved by the hmbc and hsqc 2D NMR heteronuclear correlation in deuterated dimethyl sulfoxide solution in dynamic. Was studied the transformation of its tautomers. It was found that the problem for the pyrazolo [3,4-d] [1,2] diazepin-8-one molecule identification is caused by prototropic isomerization of the pyrazole ring.

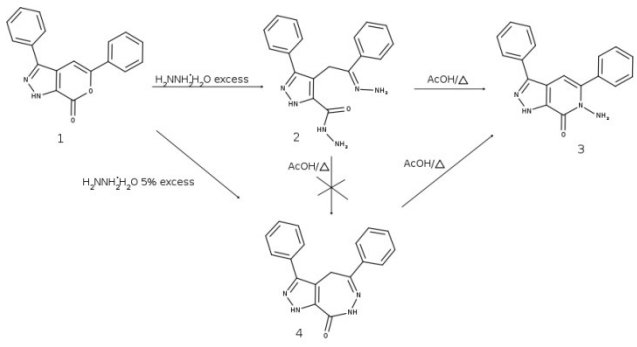
Introduction

Seven-membered heteroatomic systems are an attractive compromise between the flexibility of the skeleton, which is able to adapt to some extent to the geometric features of biological targets, and the compactness of a cyclic molecule. Condensed 1,2-diazepines, these seven-membered heterocycles, containing an effective ligand in their structure, have long been attracting our attention. In the article, we examined practical possibilities of varying their structure within the pyrazolodiazepine motif. Pyrazolodiazepines are heterocyclic compounds with a wide spectrum of biological activity. Pyrazolo[1,4]- and pyrazolo[1,5]diazepines showed anti-inflammatory, antipyretic and analgesic activity[1], they are agonists of oxytocin receptors [2]. Among them were found compounds with anxiolytic, sedative, anticonvulsant and muscle relaxant activity[3] such as anticonvulsant and anxiolytic drug Zolazepam, anxiolytic Ripazepam and antidepressant Zometapine. However, pyrazolo[3,4-d] [1,2]diazepines are still rare compounds. At the same time, as heterocyclic isosteres of 2,3-benzodiazepine, they attract by an unusual combination of subunits, because allows to expand the structural diversity of diazepine libraries.

Results and Discussion

In recent studies, we investigated the synthesis and transformations of 5,7-dihydropyrrolo [3,4-d] [1,2] diazepin-1(2H)-ones[4, 5]. Their seven-membered cycle is more stable than at 2,3-benzodiazepine. The proposed method is convenient for modifying the substituents in the pyrrolo [3,4-d] [1,2-diazepine framework and for creating new heterocyclic systems with the pyrrolo [3,4-d] [1,2] diazepine fragment. We applied the same methodology to the synthesis of pyrazole-condensed analogues of 2,3-benzodiazepine.

Several approaches to the synthesis of the skeleton of pyrazolo [3,4-d][1,2] diazepine are known. Based on our previous experience, we selected a modification of pyrazolo [3,4-d] [1,2]diazepin-8-one. Of all the known publications[6–13], the simplest and most convenient way to obtain it is the hydrazinolysis of 3,5-diphenylpyrano [3,4-c]pyrazole-8(1H)-one [11]. This approach is characterized by the simplicity of the reaction scheme, the use of inexpensive reagents, and allows a broad modification of the initial molecule. When reproducing the synthesis of 4,7-dihydro-3,5-diphenylpyrazolo[3,4-d][1,2] diazepin-8(1H)-one[10], we encountered the problem of controlling the formation of the seven-membered cycle. In our hands, the reaction of pyranopyrazole 1 with an excess of hydrazine hydrate produces 4-hydrazone hydrazide of 3 (5)-phenyl-4-phenacylpyrazole-5(3)-carboxylic acid (2). However, its further heating in ethanol - acetic acid leads to the formation of 6-amino-1,6-dihydro-3,5-diphenyl-7H-pyrazolo [3,4-c]pyridin-7-one instead of the expected diazepine 4. When replacing acetic acid with a hydrochloric or p-toluenesulfonic acid in alcohol, we also obtained only compound 3 (Scheme 1).



Scheme 1. Pyrazolopyrane recyclizations in acidic and neutral conditions.

It is known that when heating 2,3-benzodiazepines and their isosteres in acids, 1,2-diazepine cycle contracts. As was shown [14, 15], the seven-membered ring of 2,3-benzodiazepines in the presence of acids transforms into pyridine cycle with the formation of 2-aminoisoquinolines. In contrast, the 1,2-diazepine cycle successfully formes in a neutral or basic medium. After eliminating acids from the reaction scheme, we carried out the reaction of pyrone 1 with a slight excess of hydrazine hydrate in boiling ethyl cellosolve. As a result, 4,7-dihydro-3,5-diphenylpyrazolo [3,4-d][1,2]diazepin-8(1H)-one (4) was obtained after crystallization from dioxane with the yield of 64%. Upon short-term boiling of pyrazolodiazepine 4 in a mixture of ethanol-hydrochloric acid, as expected, pyrazolopyridine 3,

which is the product of the narrowing of the seven-membered ring, was formed.

Although pyrazolopyridine 3 and pyrazolodiazepine 4 have different melting points, they have the same elemental composition, and therefore the result of mass spectrometric determination of molecular weight will not be a proving evidence of the structure of compound 4. Therefore, we studied in detail the structure of pyrazolodiazepinone 4 using one- and two-dimensional 1H and 13C NMR spectroscopy (COSY, HSQC, HMBC). The probable prototropic isomerism of the pyrazole cycle and the lability of the 3,4-diazepine cycle complicate the analysis of NMR spectra (Fig. 1).

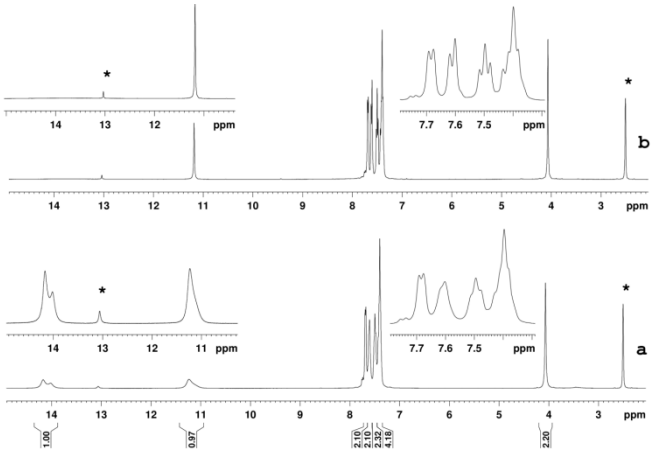


Figure 1. ^{1}H spectra of compound 4 in DMSO-d_6 before (a) and after (b) 19 days. The symbol * marks the signals of solvent (2.5 ppm) and impurities (13.1 ppm), the nature of which is being specified.

Based on the value of the integral intensity and by analogy with the previously studied compounds, we attributed the signal at 4.10 ppm with an intensity of 2H to the CH_2 group of the diazepine cycle, and the multiplet in the region of 7.3-7.7 ppm (10H) to two monosubstituted phenyl groups. This group of signals contains two characteristic doublets of ortho-protons displaced to a weak field relatively to the other signals. In the region of 11.3-14.5 ppm there are 4 wide signals of NH protons (Fig. 1a), and in pairs they correspond to an integral intensity of 1H. We suggested that the absence of expected significant signals is also associated with their broadening.

The carbon spectrum of the compound taken immediately after preparation of the solution (Fig. 2a) turned out to be uninformative. It contains signals of ^{13}C-(H) atoms of monosubstituted phenyl groups (all three groups according to symmetry), as well as a number of strongly broadened signals. The total number of signals is much smaller than can be expected according to the formula. The presence of broadened signals in the spectrum indicates a complex behavior of the compound, which is most likely associated with valence isomerism. We hypothesized that the absence of expected significant signals is also associated with their broadening.

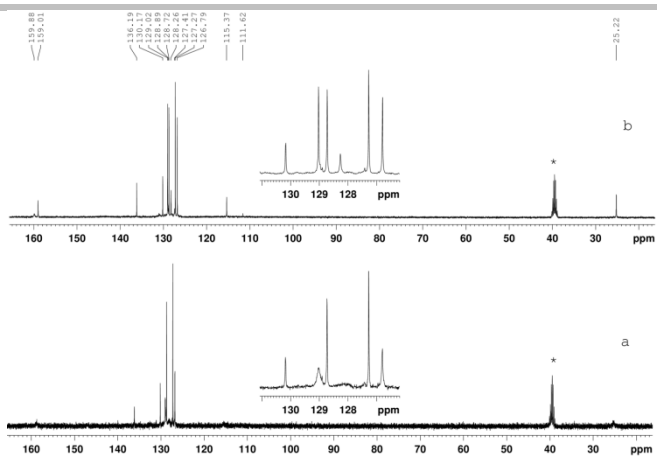


Figure 2. ^{13}C Spectrum of a solution of compound 4 in DMSO-d_6 a) a freshly prepared solution, b) after 19 days. The * symbol indicates a solvent signal.

After 19 days, the appearance of the ^{13}C spectrum changed dramatically. A series of new narrow signals appeared in it (Fig. 2b): the signal of the carbon atom of the CH_2 group in a strong field at 25 ppm, the characteristic signal of the carbonyl group at 159 ppm. The signals of aromatic carbon atoms have narrowed significantly. The absence of cross peaks from weak-field protons in the two-dimensional HSQC spectrum (Fig. 3) of a freshly prepared solution allowed us to state the above mentioned signals belong to protons bonded to heteronuclei. A pleasant surprise was that the signal at 4.1 ppm does belongs to the CH_2 group, since a wide signal like this in this region of the 1H spectrum can also be attributed to the water signal.

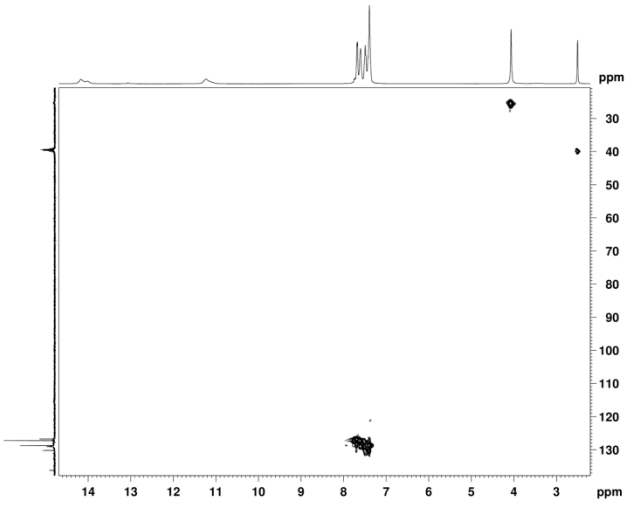


Figure 3. HSQC spectrum of a freshly prepared solution of compound 4 in DMSO-d_6.

The absence of cross peaks from weak-field protons is also observed in the two-dimensional HMBC spectrum (Fig. 4) of a freshly prepared solution. It should be noted that there are several cross peaks from the signal of the CH_2 group in regions of the carbon spectrum in which no signals are observed. Taking into account the known tendency of the HMBC method to give fictitious cross-peaks[12], we believe that in this case some of

them may be carbon atoms which are actually present but are quickly relaxing. In our opinion, the reason for the lack of a part of cross-peaks in the two-dimensional spectra of a freshly prepared solution lies in the structure of two-dimensional sequences, that is in the length of pauses. The width of weak-field signals in the initial spectrum is more than 20 Hz, the relaxation time of the corresponding groups is ≈ 0.05 s, and the pause d6 in HMBC for the constant of 10 Hz is 0.065 s. This allows the spins to relax before the necessary evolution takes place within the framework of the sequence.

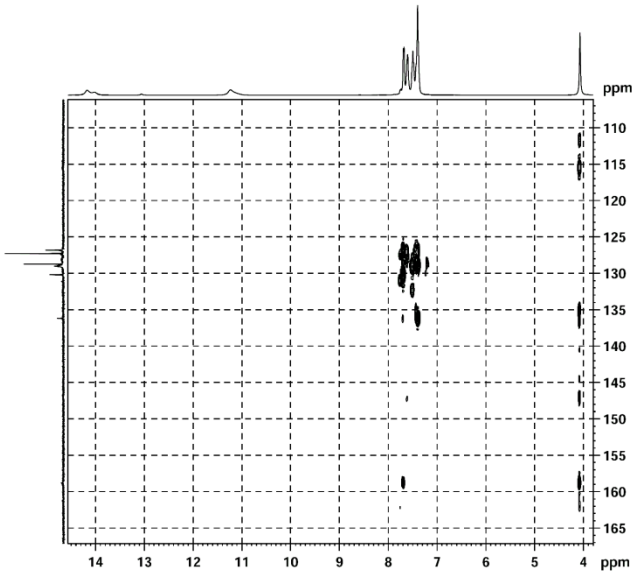


Figure 4. HMBC spectrum of a freshly prepared solution of compound 4 in DMSO-d_6.

After the settling of the solution, the appearance of the 2D spectra of diazepine 4 also changed; we obtained new cross peaks in the HSQC and HMBC spectra (Fig. 5). They correspond to the interaction of the NH proton at 11.3 ppm (7 Hz) and protons of the CH_2 group at 4.1 ppm (3 Hz) with carbon at 159 ppm.

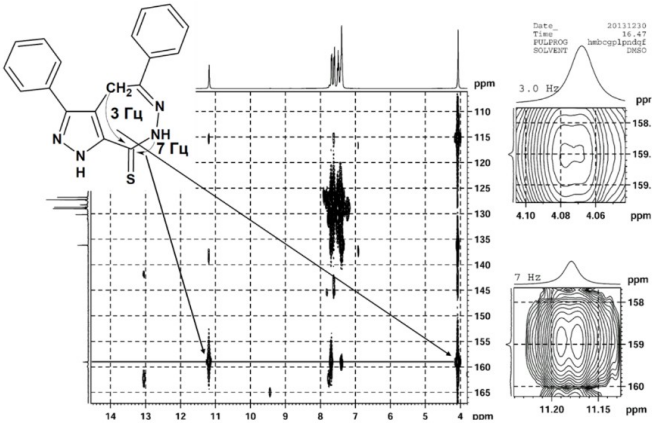


Figure 5. HMBC spectrum of a solution of compound 4 in DMSO-d_6 after 19 days of exposure. The figure shows the values of the coupling constant for the selected interactions.

We assume that the product crystallized from dioxane is initially in a lactim form -N=C-OH, in which there is a hydrogen bond between the hydrogen atom of the OH group and the nitrogen atom of the pyrazole ring (Fig. 6, a, b). Probably, the hydrogen bond stabilizes one of the pyrazole forms, which slows down the prototropic isomerism of the pyrazole ring. When the sample is kept for 19 days, an isomer with a lactam group O=C-N-H is formed (Fig. 6, c) due to the prototropic isomerism of the diazepine ring, and the hydrogen atom of that group gives a narrow signal at 11.3 ppm. At the same time, the fixation of the hydrogen atom in the pyrazole ring disappears; therefore, its signal in the spectrum broadens due to the rapid exchange between the positions in the pyrazole ring.

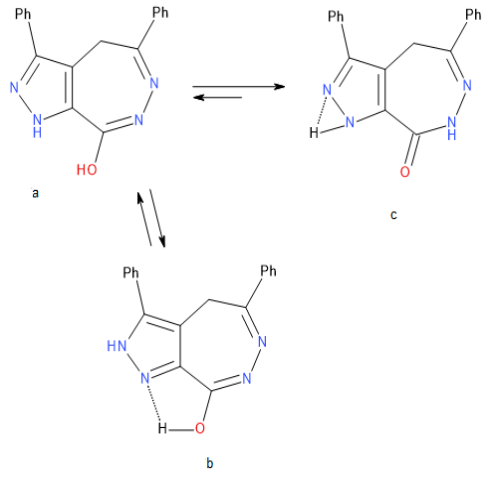


Figure 6. The proposed scheme for the pyrazolodiazepine 4 tautomerism in solution

Therefore, after crystallization from dioxane, pyrazolodiazepine 4 is in 8-hydroxy form (Fig. 6, a, b), and in the DMSO medium it is in the form of lactam (Fig. 6, c). Also, the transition is quite slow and takes several weeks.

Conclusions

As a result of the work done, a reliable preparative method for producing a highly modifiable pyrazolo [3,4-d] [1,2] diazepin-8-one was developed, the structure of this compound was established and the transformations of its tautomers were studied.

Experimental Section

All reagents and solvents were obtained from commercial suppliers and used without further purification unless otherwise specified. All reagents were weighed and handled in air at room temperature. Melting points were determined with a capillary melting point apparatus and are uncorrected. The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance II instrument (400 and 100 MHz, respectively) in DMSO-d6, internal standard was TMS.. Mass spectra were recorded on an Agilent 1100 LCMSD SL instrument (ESI). Elemental analyses were performed at the Laboratory of Organic Analysis, Institute of Organic Chemistry NAS of Ukraine of Kyiv, and their results were found to be in good agreement

(±0.4%) with the calculated values. Compounds 1 and 2 were prepared by the methods of Migliara et al. [11].

6-Amino-1,6-dihydro-3,5-diphenyl-7H-pyrazolo[3,4-c]pyridin-7-one (3).

a) To a solution of 1 g (3 mmol) of compound 2 in 40 ml of ethanol add 10 ml of hydrochloric acid and reflux for 1 hour. After evaporation in vacuo, the precipitate is washed with ice water and filtered. Recrystallized from ethanol. The same result was obtained after boiling compound 2 in 20 ml of acetic acid.
b) 1 g (2.87 mmol) of pyrazolodiazepine 4 in 20 ml of ethanol and 20 ml of hydrochloric acid is refluxed for 1 hour. After evaporation in vacuo, the residue is neutralized with sodium bicarbonate solution, the pyrazolopyridine 3 precipitate is washed with ice water and filtered. Recrystallized from ethanol. Yield 46% (a), 70% (b), white powder, mp . 289-291°C (ethanol) (m.p. 290°C (ethanol)11). Yield 46% (a), 70% (b). ^{1}H NMR spectrum, δ, ppm (J, Hz): 5.62 (2H, s, NH2); 6.77 (1H, s, CH=); 7.37-7.98 (10H, m, 2 x C6H5); 14.26 (1H, broad, NH). ^{13}C NMR spectrum, δ, ppm: δ 95.09; 99.19; 125.96; 126.44; 126.67; 127.77; 127.99; 128.15; 128.21; 128.87; 128.97; 129.69; 132.46; 132.73; 134.75; 135.78; 141.90. Mass spectrum, m/z (I_{rel}, %): 303 [M+H]+ (100), 304 [M+2H]++ (30). Found, %; C 71.25; H 4.55; N 18.83. $C_{18}H_{14}N_4O$. Calculated, %: C 71.5; H 4.7; N 18.5%.

4,7-Dihydro-3,5-diphenylpyrazolo[3,4-c] [1,2]diazepin-8(1H)-one (4). 1 g (3.47 mmol) of pyranopyrazole 1 in ethyl cellosolve (15 ml) with hydrazine hydrate (3.64 mmol) refluxed for 12 hours. After evaporation under reduced pressure, the viscous substance is treated with methanol. The precipitated crystals are filtered off and recrystallized from ethanol. Yield: 75%, white powder, mp 244-246°C (ethanol) (m.p. 253°C (ethanol)11). ^{1}H NMR spectrum, δ, ppm (J, Hz): 4.05 (2H, s, CH2); 7.29-7.96 (10H, m, 2 x C6H5); 11.10 (1H, s, NH); 14.08 (1H, broad, NH). ^{13}C NMR spectrum, δ, ppm: 25.22 (CH2); 115.36; 126.80; 127.27; 128.27; 128.74; 129.03; 130.18; 136.19; 159.00; 159,86 (C=O). Mass spectrum, m/z (I_{rel}, %): 303 [M+H]+ (100), 304 [M+2H]++ (20). Found, %: C 71.34; H 4.57; N 18.70. $C_{18}H_{14}N_4O$. Calculated, %: C 71.5; H 4.7; N 18.5.

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Keywords: pyrazolo [3,4-d][1,2] diazepine, preparation, NMR identification, tautomerism.

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