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Mendeleev Commun., 2011, **21**, 239–241
Mendeleev Communications

1-Hydroxy-8-methyl-1,4,8-triazaspiro[4.5]decan-2-one and its (±)-3-methyl homologue: regioselective synthesis and *in vivo* evaluation as adjuvants in leukemia chemotherapy

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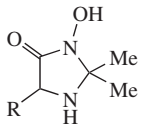
DOI: 10.1016/j.mencom.2011.09.002

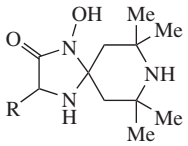
Condensation of glycine and DL-alanine hydroxamic acids with 1-methyl-4-piperidone regioselectively gave the corresponding titled spiro piperidine hydroxyimidazolidinones capable of enhancing sensitivity of P388 and L1210 leukemias in mice models to the known anticancer drugs cisplatin and cyclophosphamide.

Development of new chemotherapeutic compounds capable of reducing the side effects of anticancer drugs by decreasing the therapeutic doses while maintaining the cytostatic effect of the latter is an urgent task in medicinal chemistry.[1] Hydroxamic acids (HAs) show anticancer and antimetastatic activity in tumour models[2] and act as inhibitors of metalloenzymes involved in pathophysiological response reactions of tumour cells to cytotoxic agents.[3,4] Furthermore, HAs are donors of nitric oxide (NO)[4] that enhances the anticancer activity of a number of cytostatics.[1(a),5]

We have shown previously that reactions of glycine hydroxamic acid (GlyHA) and DL-alanine hydroxamic acid (DL-AlaHA) with acetone or triacetonamine occur as regioselective N,N′-type cyclocondensations to give mono- (**1**,[6] **2**[7]) and spirobicyclic (**3**, **4**) HAs.[8] The structures of cyclic hydroxamic acids (CHAs) **1**–**3** were confirmed by X-ray diffraction analysis.[6–8]



1 R = H
(±)-**2** R = Me

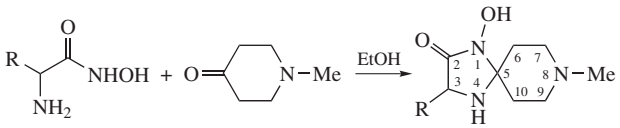


3 R = H
(±)-**4** R = Me

It has also been shown that chemotherapy of an experimental P388 leukemia with non-toxic acids **3** or **4** (LD_{50} > 1 g kg^{-1}) combined with antitumor drugs of various types increases the *in vivo* antileukemic activity of these cytostatics by 30–55% in comparison with their individual inhibitory activity.[8]

The purposes of this work included: (i) synthesis of less sterically hindered analogues of acids **3**, **4** by cyclocondensation of GlyHA or DL-AlaHA with 1-methyl-4-piperidone (MPD); (ii) *in vivo* assay of the resulting compounds as adjuvants of cytostatic agents in leukemia models and plotting the structure–activity relationship in a series of spirobicyclic HAs incorporating the spiro piperidine moiety that is also contained in the structures of a number of inhibitors of response of tumour cells to the exposure to DNA-damaging antitumour agents.[1(b)]

Reactions of GlyHA and DL-AlaHA with MPD occur, as in the case of acetone[6,7] and triacetonamine,[8] as N,N′-type regioselective cyclocondensation to give CHAs **5** and **6**, respectively (Scheme 1).[†] The reactions were carried out in EtOH (Δ, 1 h) in the



GlyHA (R = H)
DL-AlaHA (R = Me)

MPD

5 R = H
(±)-**6** R = Me

Scheme 1

[†] IR spectra of samples pressed in KBr pellets were recorded on a Specord-82M spectrophotometer in the 400–4000 cm^{-1} range. NMR spectra were measured on a Bruker Avance III-500 spectrometer operating at 500.20 MHz (^{1}H) and 125.775 MHz (^{13}C). The starting compounds, *N*-hydroxyaminoacetamide (GlyHA) and *N*-hydroxy-DL-2-amino-2-methylacetamide (DL-AlaHA) were synthesized according to known procedures.[12] Melting points were determined using a Boetius RNMK hot stage micromelting apparatus. Elemental analyses (C, H, N) were obtained on a Vario MicroCube Elementar.

General procedure for the synthesis of **5**, **6**. A suspension of GlyHA (or DL-AlaHA) (30 mmol) and 1-methyl-4-piperidone (MPD, 36 mmol) in 75 cm^3 of anhydrous ethanol was refluxed with stirring for 1 h. Then the mixture was filtered and the solvent was evaporated *in vacuo* almost to dryness. Then MeCN (50 cm^3) was added to the residue and the resulting mixture was kept overnight with cooling. The precipitate thus obtained was filtered off and purified by recrystallisation from MeCN (or dioxane), then dried *in vacuo* (1 Torr) to yield acid **5** (or **6**) as a white solid. Compounds **5** and **6** are well soluble in H_2O, DMSO, MeOH and slightly soluble in MeCN, dioxane, benzene and $CHCl_3$ at room temperature.

1-Hydroxy-8-methyl-1,4,8-triazaspiro[4.5]decan-2-one **5**: yield 79%, mp 173–174 °C (MeCN). ^{1}H NMR (CD_3OD) δ: 1.58 (ddd, 2H, α-H_A, *J* 13.2, 2.7 and 2.5 Hz), 2.17 (td, 2H, α-H_B, *J* 13.2 and 4.6 Hz), 2.30 (s, 3H, NMe), 2.37 (ddd, 2H, β-H_C, *J* 12.0, 2.7 and 13.0 Hz), 2.83 (ddd, 2H, β-H_D, *J* 12.0, 2.5 and 4.6 Hz), 3.36 (s, 2H, NCH_2CO). ^{1}H NMR ([$^2H_6$]DMSO) δ: 1.35 (br. d, 2H, α-H_A, *J* 13.0 Hz), 1.87 (td, 2H, α-H_B, *J* 12.9 and 4.5 Hz), 2.10 (td, 2H, β-H_C, *J* 13.1 and 2.0 Hz), 2.12 (s, 3H, NMe), 2.61 (ddd, 2H, β-H, *J* 10.9, 2.2 and 4.0 Hz), 3.00 (br. t, 1H, NH, $^3J_{H(5)}$ 7.6 Hz), 3.12 (d, 2H, NCH_2CO, $^3J_{NH}$ 7.7 Hz), 9.52 (br. s, 1H, OH). ^{1}H NMR ($CDCl_3$) δ: 1.52 (br. d, 2H, α-H_A, *J* 12.2 Hz), 2.30 (br. td, 2H, α-H_B, *J* 13.1 and 3.8 Hz), 2.35 (s, 3H, NMe), 2.38 (br. td, 2H, β-H_C, *J* 13.1 and 2.0 Hz), 2.88 (br. dd, 2H, β-H_D, *J* 11.2 and 2.1 Hz), 3.38 (s, 2H, NCH_2CO). ^{13}C NMR (CD_3OD) δ: 32.50 (tm, 2C, *C*H_2CH$_2$NMe, ^1J 128.3 Hz), 45.50 (qm, NMe, ^1J 135.8 Hz), 45.78 [dd, C(O)*C*H_2NH, ^1J 143.4 and 144.6 Hz], 52.20 (br. tm, 2C, *C*H_2NMe, ^1J 135.8 Hz), 78.25 (m, NCN), 170.94 (t, C=O, ^2J 5.0 Hz). IR (KBr, ν/cm^{-1}): 3306 (m, NH),

absence of any catalyst to afford products **5** and **6** in high yields (79% and 82%, respectively) similarly to the synthesis of compounds **3** (EtOH, Δ, 1 h, 75%), **4** (80%),[8] **2** (excess ketone, Δ, 3 h, 83%)[7] and **1** [MeOH + 2 equiv. $Me_2C(O)$, Δ, 3 h, 57%],[6] and unlike the acid-catalysed cyclocondensation between α-amino acid amides and cyclic ketones that occur in solution in low yields.[9]

The structure of the resulting CHAs **5** and **6** was confirmed by elemental analyses and IR, ^{1}H and ^{13}C NMR spectra† (*cf.* the data for compounds **1**–**4**[6–8]). The broadened bands in the region of 2750–2250 cm^{-1} (with absorption maxima at 2520 and 2605 cm^{-1}, respectively) in the IR spectra (KBr) and the positive test reactions with $FeCl_3$ confirm that compounds **5**, **6** contain an OH group, which excludes the formation of a six-membered hydroxamate (–CO–NH–OR) by the possible N,O-type alternative cyclocondensation. The chemical shifts of the C(5) atoms in cyclic acids **5** (δ_C 78.3) and **6** (δ_C 76.9) in the ^{13}C NMR spectra (CD_3OD) are similar to those of the respective atoms in acids **3** (DMSO-d_6, δ_C 79.6) and **4** (CD_3OD, δ_C 80.3);[8] they match the tetrahedral configuration of spirocyclic carbon[6–8] but not trigonal carbon of the C=N group ($\delta_C > 120$)[10] in the possible corresponding acyclic azomethine form. The presence of the NH group signal in the ^{1}H NMR spectra [DMSO-d_6, δ_{NH} 3.00 (**5**), 2.72 (**6**)]† also contradicts the formation of products **5** and **6** in the azomethine form.

Ring–chain tautomerism of cyclic products, which is slow on the NMR time scale, is not observed due to the absence of signals of azomethine forms in the ^{1}H and ^{13}C NMR spectra of compounds **5** and **6**.†

The hydroxynitrone form (HO–C=N→O) of acids **5**, **6** corresponding to the prototropic equilibrium with the hydroxyamide form (O=C–N–OH) that is fast on NMR time scale, is not observed in solution to any noticeable extent.[11] This follows from a comparison of the chemical shifts of carbonyl carbon atoms in the ^{13}C NMR spectra (CD_3OD) of acids **5** ($\delta_{C=O}$ 170.9) and **6** ($\delta_{C=O}$ 172.7),† similarly to acids **3** (DMSO-d_6, $\delta_{C=O}$, 171.2) and **4** (CD_3OD, $\delta_{C=O}$ 174.4),[8] and the corresponding model compounds with fixed tautomeric forms, namely, 3-methoxy-1,2,2,5,5-pentamethylimidazolidin-4-one (O=C–N–OMe, MeOH, δ_C 171.1) and its methoxynitrone form (MeO–C=N→O, MeOH, δ_C 156.7).[11]

A comparative *in vivo* study of the adjuvant capability of non-toxic homologues **5** and **6** on experimental tumor models, leukemias P388 and L1210, was carried out‡ in combination with clinically used anticancer drugs cisplatin (CSP) or cyclophosphamide (CPA)[1] administered in therapeutic lower doses.

A study on the P388 leukemia model showed that combined use of acids **5** or **6** with cisplatin results in an increase in the antileukemic activity of CSP by 140% (Figure 1) or 68%, respectively (Table S1, Online Supplementary Materials), in comparison with the inhibitory activity of the cytostatic when administered alone in the same subtherapeutic dose.

Using the L1210 leukemia model, it was found efficient to combine acids **5** or **6** with CPA, *i.e.*, DNA-alkylating agent, which leads to an increase in the antileukemic activity of this cytostatic

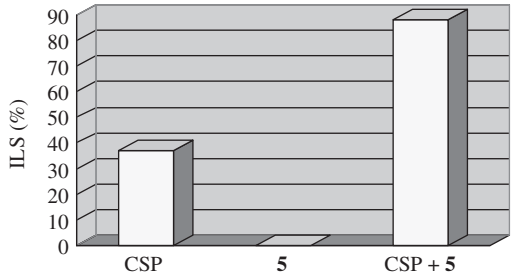


Figure 1 Increase in sensitivity of P388 leukemia model to treatment with CSP and acid **5** *in vivo*. Column CSP: single dose, 0.5 mg kg^{-1}, times of administration, 1, 3, 5 and 7 days, ILS (increase in the average life span), 37%. Column **5**: 400 mg kg^{-1}, 1–7 days, 0%. Column CSP + **5**: the same doses and times, 88%.

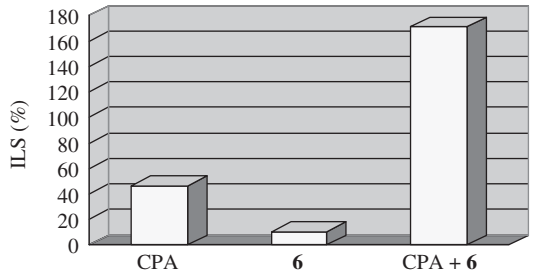


Figure 2 Increase in sensitivity of L1210 leukemia model to treatment with CPA and acid **6** *in vivo*. Column CPA: single dose, 20 mg kg^{-1}, times of administration, 1 and 6 days, ILS, 46%. Column **6**: 400 mg kg^{-1}, 1–7 days, 10%. Column CPA + **6**: the same doses and times, 171%.

3257 (m, NH), 2991 (w, CH), 2960 (m, CH), 2936 (m, CH), 2902 (w, CH), 2840 (m, CH), 2750–2250 (br. m, 2520$_{max}$, OH), 1694 (vs, C=O), 1573 (w), 1459 (m), 1440 (m), 1360 (s), 1301 (m), 1275 (m), 1253 (w), 1185 (m), 1171 (w), 1123 (w), 1083 (m), 1063 (m), 1041 (w), 1015 (w), 992 (m), 953 (w), 891 (m), 851 (w), 792 (m), 691 (w), 627 (w), 576 (w), 565 (w), 480 (w), 441 (m). Found (%): C, 51.68; H, 8.39; N, 22.56. Calc. for $C_8H_{15}N_3O_2$ (%): C, 51.87; H, 8.16; N, 22.69.

(±)-1-Hydroxy-3,8-dimethyl-1,4,8-triazaspiro[4.5]decan-2-one **6**: yield 82%, mp 172–173 °C (dioxane). ^{1}H NMR (CD_3OD) δ: 1.32 (d, 3H, CH*Me*, ^3J 7.0 Hz), 1.57 (ddd, 1H, α-H, *J* 13.5, 2.5 and 5.5 Hz), 1.61 (ddd, 1H, α-H, *J* 14.0, 2.5 and 5.5 Hz), 2.10 (td, 1H, α-H, *J* 13.0 and 4.5 Hz), 2.32 (td, 1H, α-H, *J* 13.0 and 4.5 Hz), 2.32 (s, 3H, NMe), 2.40 (td, 1H, β-H, *J* 12.5 and 2.5 Hz), 2.45 (td, 1H, β-H, *J* 12.5 and 2.5 Hz), 2.87 (m, 2H, β-H), 3.50 (q, 1H, C*H*Me, ^3J 7.0 Hz). ^{1}H NMR ([$^2H_6$]DMSO) δ: 1.14 (d, 3H, CH*Me*, ^3J 7.0 Hz), 1.33 (ddd, 1H, α-H, *J* 12.5, 2.5 and 5.0 Hz), 1.40 (ddd, 1H, α-H, *J* 13.0, 3.0 and 5.5 Hz), 1.81 (td, 1H, α-H, *J* 12.5 and 4.5 Hz), 1.99 (td, 1H, α-H, *J* 13.0 and 4.5 Hz), 2.13 (s, 3H, NMe), 2.14 (m, 1H, β-H, *J* 2.5 Hz), 2.16 (m, 1H, β-H, *J* 3.0 Hz), 2.62 (m, 2H, β-H), 2.72 (d, 1H, NH, $^3J_{CHMe}$ 8.5 Hz), 3.27 (m, 1H, C*H*Me), 9.50 (br. s, 1H, OH). ^{1}H NMR ($CDCl_3$) δ: 1.32 (d, 3H, CH*Me*, ^3J 7.0 Hz), 1.50 (dd, 1H, α-H, *J* 13.0 and 2.5 Hz), 1.58 (br. d, 1H, α-H, *J* 12.5 Hz), 2.14 (td, 1H, α-H, *J* 13.0 and 4.0 Hz), 2.39 (s, 3H, NMe), 2.43 (td, 2H, β-H, *J* 12.5 and 2.5 Hz), 2.48 (m, 1H, α-H, *J* 13.5 and 3.5 Hz), 2.90 (m, 2H, β-H), 3.43 (q, 1H, C*H*Me, ^3J 7.0 Hz). ^{13}C NMR (CD_3OD) δ: 17.52 (dq, CH*Me*, ^1J 125.2 Hz, ^2J 3.7 Hz), 31.35 (tm, 1C, CH_2CH_2NMe, ^1J 130.8 Hz), 34.84 (tm, 1C, CH_2CH_2NMe, ^1J 128.3 Hz), 45.47 (qm, NMe, ^1J 135.4 Hz), 52.01 (tm, 1C, CH_2NMe, ^1J 134.6 Hz), 52.31 (dq, CHMe, ^1J 140.8 Hz, ^2J 5.0 Hz), 52.48 (tm, 1C, CH_2NMe, ^1J 138.3 Hz), 76.89 (m, NCN), 172.68 (dq, C=O, ^2J 5.0 Hz, ^3J 3.8 Hz). IR (KBr, ν/cm^{-1}): 3325 (w, NH), 3284 (m, NH), 3155 (w, NH), 3000 (w, CH), 2957 (m, CH), 2936 (m, CH), 2883 (m, CH), 2861 (m, CH), 2815 (m, CH), 2790 (m, CH), 2750–2300 (br., 2605$_{max}$, OH), 1700 (vs, C=O), 1465 (m), 1457 (m), 1445 (m), 1425 (w), 1363 (s), 1332 (w), 1304 (w), 1275 (m), 1242 (m), 1188 (m), 1174 (w), 1117 (m), 1108 (w), 1069 (m), 1043 (w), 1009 (m), 981 (m), 947 (w), 885 (m), 845 (w), 811 (w), 785 (m), 755 (w), 627 (w), 591 (w), 492 (m), 441 (m). Found (%): C, 54.01; H, 8.66; N, 20.87. Calc. for $C_9H_{17}N_3O_2$ (%): C, 54.25; H, 8.60; N, 21.09.

‡ The study of the antileukemic activity of acids **5** and **6** was carried out using the P388 and L1210 leukemia tumor models on BDF_1 hybrid male mice with a weight of 22–24 g. (Mice were housed in a vivarium and provided with water and food *ad libitum*.) The transplantation of P388 (10^6 cells) and L1210 (10^5 cells) leukemias was performed intraperitoneally according to a standard procedure.[13] Mice were injected intraperitoneally with aqueous solutions of compounds **5** and **6**. The single doses, the times and the results of administration are given in Figures 1, 2 and Tables S1, S2 (Online Supplementary Materials). The overall toxicity (LD_{50}) was determined on BDF hybrid mice with a single injection of aqueous solutions of acids **5** and **6**. The antileukemic activity was assessed from the increase in the average life span [ILS (%) = $100(T/T_c - 1)$] where T and T_c are the measured average life span (in days) of mice of the treated and control groups, respectively. Each group comprised eight animals. The survived mice were sacrificed after ether narcosis on day 60 after tumor transplantation.

by 25% and 270% (Figure 2), respectively (Table S2, Online Supplementary materials).

Hence, glycine-derived achiral acid **5** increases the inhibitory activity of CSP more efficiently than alanine-derived racemic acid **6** in the P388 leucosis model. On the other hand, acid **6** enhances the antileukemic activity of CPA much more efficiently than acid **5** in the L1210 lymphoblastic leucosis model.

Thus, spatially less-hindered and probably more lipophilic acids **5**, **6** are more efficient adjuvants of antitumour agents than their analogues **3**, **4**,[8] which is probably due to an improvement in the transport of their molecules through biomembranes. Taking into account the data obtained in another study,[8] it can also be concluded that reactions of GlyHA and DL-AlaHA with γ-piperidones, like with simple ketones,[6,7] under neutral conditions in solution occur as general N,N′-type regioselective cyclocondensation to give spirocyclic HAs, incorporating a pharmacologically potential 1,4,8-triazaspiro[4.5]decan-2-one framework,[9] in high yields.

Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.mencom.2011.09.002.

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Received: 11th March 2011; Com. 11/3695