New Benzimidazoles Derivates (Small Hydroxamates) Possible Inhibitors of the Matrix Metalloproteinase

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In this paper we present experimental data regarding the synthesis of eight new hydroxamates of benzimidazoles (4a-h) by hydroxylamidation reaction of corresponding esters (3a-h). The starting materials were substituted in 5-position benzimidazolthiols (1a-e). The new compounds will be tested as inhibitors of matrix metallo proteinases.

Keywords: benzimidazoles, hydroxamates compounds, ^{1}H and ^{13}C – NMR spectra

The role of matrix metalloproteinases (MMPs), zinc-dependent proteinases in pathology has been extensively described in the literature. MMP activity is regulated via a member of mechanisms including inhibition by tissue inhibitors of metalloproteinases (TIMPs). A disturbed balance of MMPs and TIMPs is found in various pathologies conditions, such as cancer, rheumatoid arthritis, cardiovascular disease and multiple sclerosis [1 - 5]. This explains the increased interest in designing and synthesized MMPs inhibitors.

The matrix metal proteinases are attractive target for the low molecular weight inhibitors-hydroxamate structures-with zinc bound by cores like the natural products.

We obtained new synthetic potential MMP-inhibitors using new benzimidazoles with the property to chelate the zinc ions.

Experimental part

Melting points have been determined in open capillaries with automated Melting Point System (Stanford Research Systems).

FT-IR spectra were measured at room temperature with a BRUKER VERTEX device with diamond optic; solid absorption spectra were determined in ATR.

The thin layer chromatography analysis (TLC) has been effected on silica 60 F_{254} MERCK plates, one-dimensional working method (chloroform/methanol 4:1 v/v eluent). The plates were detected by UV light irradiation (λ = 254 nm).

^{1}H and ^{13}C spectra have been recorded on two Varian instruments, one of them being a Gemini 300 BB (300.075 MHz for proton and 75.462 MHz for carbon) and the other being a Unity Inova 400 (399.821 MHz for proton and 100.054 for carbon). The solvent used was deuterated chloroform or DMSO with min. 98% deuterium. As a reference point we had TMS signal for proton and carbon spectra (δ = 0 ppm). The working temperature was 20° C ± 1. The notations presented in table 2 were used for assigning the chemicals shifts of the protons.

Synthesis of the benzimidazoles hydroxamates

The benzimidazoles derivatives (small hydroxamates) (4a-h) were prepared with good yield (75-85 %) by hydroxylamidation reaction of the corresponding esters (3a-h). The starting materials are (un)substituted in 5-position benzimidazolthiols (1a-e).

The general synthetic route for the compounds (4a-h) is described in the scheme 1 [6, 7].

General working method

0.033 mole 2-mercaptobenzimidazole (1a-e) suspended in 30 ml methanol; separate 0.044 mole potassium hydroxide is dissolved in 30 mL methanol and is added drop wise, at room temperature, over methanol solution of benzimidazole; it is stirred at room temperature until it dissolves completely (solution *p*H =10-13). Then, a solution from 0.049 mole of the (2a-d) solvent in 40 mL methanol is added drop wise during 1.5 h. The reaction mixture is stirred at room temperature for 5-10 h(depending on the alkylation agent). The salts are filtered, the filtrate is added drop wise during 20 min under stirring, over the hydroxylamine solution - 0.2 mole in 100 mL methanol (previous prepared). The reaction mass is left, under stirring, for 3-6 h. After that it is concentrated until solution is dry, then is diluted with water and acidulated with 2N HCl solution until *p*H = 5 – 5.5. After that it is cooled at 0-5° C for 1 hour and then filtered. The precipitate is washed with ice water, ethyl ether and hexane.There are obtained small benzimidazoles hydroxamates.

Yield = 75-85 %.

2-(1H-benzimidazol-2-yl-sulfanil)-N-hydroxy-acetamide (4a)

White crystals with m.p = 172-175° C; Yield=70%; **$C_9H_9N_3O_2S$**, M=223.24;

C% calculated/found =48.43/47.94; N% calculated/found=18.33/18.90;

^{1}H-NMR (dmso-d_6, δ ppm, *J* Hz): 12.62 (bs, 1H, OH, deuterable); 10.85 (bs, 1H, H deuterable); 9.07 (s, 1H, NH, deuterable); 7.44 (bd, 2H, H-4-7);7.12 (m, 2H, H-5-6); 3.94(s, 2H, H-10).

The protons H-4, NH-7, respectively H-5 and H-6 signals are going to be isochrones because of the proton reposition at N-1 and N-3 imidazole atoms with a frequency near by

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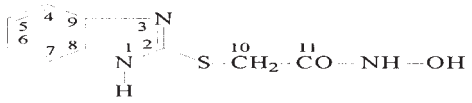
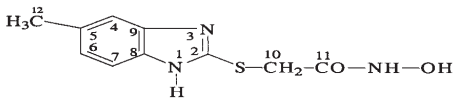
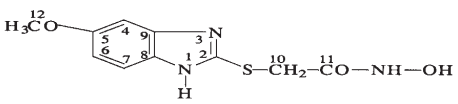
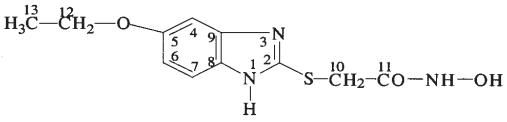
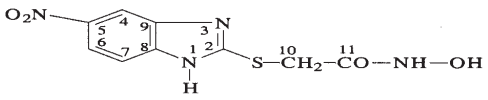
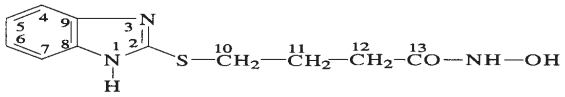
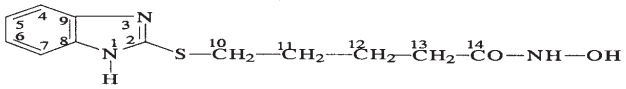
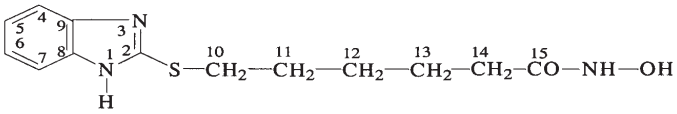
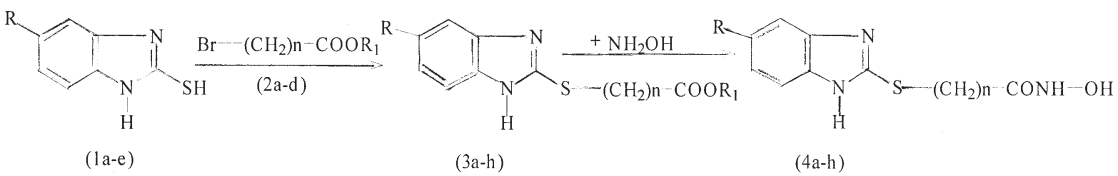
Table 1

Compound	Structure
4a	
4b	
4c	
4d	
4e	
4f	
4g	
4h	



3(a-e): R_1 = -CH_3
3(f-h): R_1 = -CH_2-CH_3 Scheme 1

the observation frequency in H-NMR. At the room temperature and observation frequency of 400 MHz, the equivalence of chemical removals of H-4 and H-7 protons is not yet reached and the adequate signal is large, near by coalescence. In the same time the proton signal H-5 and H-6 appears rather a multiplet with a fine structure.

^{13}C-NMR (dmso-d_6, δ ppm): 164.47(C-11); 149.92(C-2); 141.37(bs, C-8 and C-9); 121.63(bs, C-5 and C-6); 117.34(bs, C-4 or C-5); 110.45(bs, C-7); 32.53(C-10).

The situation with the proton fixed at N-1 or N-3 is also observed in ^{13}C spectra. The C-4 and C-7 signals are large, but further from coalescence and the C-5 and C-6 signals are beyond the coalescence and appear like a very large singlet.

FT-IR (solid in ATR, ν cm^{-1}): 3146; 3055; 3024; 2989; 2904; 2821; 2699; 2614; 1657 sh; 1631; 1511; 1402; 1355; 1319; 1266; 1225; 1152; 1116; 1057; 1007; 976; 818; 765; 736; 658; 595; 555.

Table 2

Compound	*R*	*n*
4a	-H	1
4b	$-CH_3$	1
4c	$-OCH_3$	1
4d	$-O-CH_2-CH_3$	1
4e	$-NO_2$	1
4f	-H	3
4g	-H	4
4h	-H	5

2-(5-Methyl-1H-benzimidazol-2-yl-sulfanil)-N-hydroxy-acetamide (4b)

White crystals with m.p = 150-152° C; Yield=63%; **$C_{10}H_{11}N_3O_2S$**, M=237.06;

C% calculated/found =50.63/49.87; N% calculated/found=17.72/17.89

^{1}H-NMR (dmso-d_6, δ ppm, *J* Hz): 7.56 (d, 1H, H-7, 8.4); 7.47 (d, 1H, H-4, 1.5); 7.27 (dd, 1H, H-6, 1.5, 8.4); 4.19 (s, 2H, H-10); 2.45 (s, 3H, H-13).

^{13}C-NMR (dmso-D_6, δ ppm): 163.30(C-11); 148.98(C-2); 134.62(C-8); 130.89(C-5); 126.12(C-6); 112.88(C-4); 112.70(C-7); 108.95(C-9); 33.18(C-10); 21.03(C-13).

FT-IR (solid in ATR, ν cm^{-1}): 3133; 3086; 3022; 2999; 2937; 2816; 2783; 2571; 2476; 1654; 1617; 1526; 1502; 1450; 1399; 1331; 1267; 1227; 1206; 1141; 1047; 966; 943; 887; 861; 832; 801; 760; 698; 665; 623; 594; 556; 430.

2-(5-Methoxy-1H-benzimidazol-2-yl-sulfanil)- N-hydroxy-acetamide (4c)

White crystals with m.p = 147-150° C (desc.); Yield=65%; **$C_{10}H_{11}N_3O_3S$**, M=253.27;

C% calculated/found = 48.36/48.78; N% calculated/found = 16.60/16.76

^{1}H-NMR (dmso-d_6, δ ppm, *J* Hz): 12.47 (bs, 1H, OH, deuterable); 10.84 (bs, 1H, NH, deuterable); 9.06 (s, 1H, NH, deuterable); 7.34 (vbs, 1H, H-7); 6.94 (vbs, 1H, H-4); 6.75 (dd, 1H, H-5, 2.2,8.7); 3.90 (s, 2H, H-10); 3.75 (s, 3H, H-12).

^{13}C-NMR (dmso-d_6, δ ppm): 164.55(C-11); 155.37(bs, C-2); 148.12(bs, C-5); 135.98(bs, C-9); 117.74(bs, C-8); 110.38(bs, C-7); 110.53(bs, C-6); 94.27(bs, C-4); 55.48(C-12); 32.66(C-10).

FT-IR (solid in ATR, ν cm^{-1}): 3285; 3078; 2955; 2906; 2832; 2364; 1618; 1512; 1454; 1424; 1396; 1310; 1274; 1206; 1159; 1113; 1055; 1025; 982; 949; 829; 800; 772; 725; 620; 513; 441.

2-(5-Ethoxy-1H-benzimidazol-2-yl-sulfanil)- N-hydroxy-acetamide (4d)

White crystals with m.p = 135-137° C; Yield=70%; **$C_{11}H_{13}N_3O_3S$**, M=267.30;

C% calculated/found = 49.43/48.69; N% calculated/found = 15.73/15.89

^{1}H-NMR (dmso-d_6, δ ppm, *J* Hz): 12.45 (bs, 1H, OH, deuterable); 10.82 (bs, 1H, NH, deuterable); 9.08 (s, 1H, NH, deuterable); 7.32 (vbs, 1H, H-7); 6.92 (vbs, 1H, H-4); 6.73 (dd, 1H, H-5, 2.4, 8.7); 4.00 (q, 2H, H-12, 7.0); 3.89 (s, 2H, H-10); 1.32 (t, 3H, H-13, 7.0).

By heating at 70 °C the spectra aspect is changed. The H-4 and H-7 signals are going to be more pointed and recognizable by multiplicity and intensity. The proton migration is accelerated from N-1 to N-3 and spectra becomes *fixed spectra*.

^{13}C-NMR (dmso-d_6, δ ppm): 162.31(bs, C-2); 154.49(C-2); 111.04(C-7); 63.68(C-12); 32.66(C-10); 14.47(C-13).

The other signals are very large and hard distinguishable near by coalescence

FT-IR (solid in ATR, ν cm^{-1}): 3290; 3239; 3074; 3025; 2979; 2878; 2408; 1614; 1516; 1465; 1427; 1396; 1311; 1275; 1204; 1171; 1114; 1046; 971; 909; 842; 803; 725; 621; 515; 438.

2-(5-Nitro-1H-benzimidazol-2-yl-sulfanil)- N-hydroxy-acetamide (4e)

Yellowisch crystals with m.p = 156-158° C (desc.); Yield=78%; **$C_9H_8N_4O_4S$**, M=268.24;

C% calculated/found = 40.29/40.55; N% calculated/found = 20.89/21.10;

^{1}H-NMR (dmso-d_6, δ ppm, *J* Hz): 10.50 (vbs, 2H, H deuterable); 8.28 (d, 1H, H-4, 2.1); 8.04 (dd, 1H, H-6, 2.1, 8.8); 7.58 (d, 1H, H-7); 4.02 (s, 2H, H-10).

^{13}C-NMR (dmso-d_6, δ ppm): 164.11(C-11); 155.93(C-2); 143.94(bs, C-8 or C-9); 142.20(C-5); 139.66(bs, C-9 or C-8); 117.59(C-6); 113.50 (bs, C-7); 110.39 (bs, C-4); 32.54 (C10).

FT-IR (solid in ATR, ν cm^{-1}): 3227; 3101; 2995; 2874; 2743; 1650; 1613; 1511; 1469; 1416; 1331; 1244; 1158; 1061; 980; 908; 881; 825; 790; 722; 677; 572; 544; 458; 432.

4-(1H-benzimidazol-2-yl-sulfanil)- N-hydroxy-butiramide (4f)

White crystals with m.p = 170-172° C (desc.); Yield=63%; **$C_{11}H_{13}N_3O_2S$**, M=251.30;

C% calculated/found = 52.58/52.05; N% calculated/found = 16.73/16.90;

^{1}H-NMR (dmso-d_6, δ ppm, *J* Hz): 12.50 (vbs, 1H, OH, deuterable); 10.05 (bs, 1H, H deuterable); 8.76 (s, 1H, NH deuterable); 7.43 (bd, 2H, H-4-7); 7.10 (m, 2H, H-5-6); 3.26 (t, 2H, H-10, 7.3); 2.14 (t, 2H, H-12, 7.3); 1.94 (qv, 2H, H-11, 7.3).

^{13}C-NMR (dmso-d_6, δ ppm): 168.44(C-11); 150.04(C-2); 140.50-134.20(vbs, 2C, C-8 and C-9, signal at coalescence); 121.36(s, C-5 and C-6); 114.03(bs, C-4 and C-5); 31.14(C-10); 30.74 (C-12); 25.52 (C-11).

In ^{13}C – NMR Spectra C-8 and C-9 signal was identified by the integral area. At room temperature (~ 298 K) both signals are at coalescence and invisible.

FT-IR (solid in ATR, ν cm^{-1}): 3240; 2990; 2944; 2635; 1722; 1645; 1536; 1495; 1436; 1402; 1377; 1319; 1296; 1266; 1227; 1136; 1064; 982; 928; 890; 846; 816; 736; 662; 603; 430.

5-(1H-benzimidazol-2-yl-sulfanil)-pentanoic acid hydroxyamide (4g)

White crystals with m.p = 167-168° C; Yield=55%; **$C_{12}H_{15}N_3O_2S$**, M=265.33;

C% calculated/found =54.33/53.78; N% calculated/found=15.84/16.00;

^{1}H-NMR (dmso-d_6, δ ppm, *J* Hz): 10.37 (bs, 1H, OH, deuterable); 8.69 (bs, 1H, H deuterable); 7.43 (m, 2H, H-5, H-6, syst. AA'BB'); 7.11 (m, 2H, H-4, H-7, syst. AA'BB'); 3.27 (t, 2H, H-10, 6.7); 1.99(t, 2H, H-14, 6.9); 1.78 – 1.68 (m, 4H, H-11, H-12).

^{13}C-NMR (dmso-d_6, δ ppm): 166.88(C-14); 150.18(C-2); 141.50-131.20(vbs, 2C, C-8 and C-9, signal at coalescence); 121.34(C-5, C-6); 113.47(bs, C-4, C-7); 38.69(C-10); 31.79(C-13); 28.91(C-12); 24.24(C-11).

C-8 and C-9 signals are invisible because those are at coalescence at this temperature. The value noted in NMR Spectra depends on integral area with an extension about 10 ppm.

FT-IR (solid in ATR, ν cm^{-1}): 3146; 3055; 3024; 2989; 2904; 2821; 2699; 2614; 1657 sh; 1631; 1511; 1402; 1355; 1319; 1266; 1225; 1152; 1116; 1057; 1007; 976; 818; 765; 736; 658; 595; 555.

6-(1H-benzimidazol-2-yl-sulfanil)-hexanoic acid hydroxyamide (4h)

White crystals with m.p = 168-171° C; Yield=60%; **$C_{13}H_{17}N_3O_2S$**, M=279.35;

C% calculated/found =55.91/55.07; N% calculated/found=15.05/15.20;

^{1}H-NMR (dmso-d_6, δ ppm, *J* Hz): 10.37 (bs, 1H, OH, deuterable); 8.70 (bs, 1H, H deuterable); 7.51-7.32 (m, 2H, H-4, H-7, syst. AA'BB'); 7.10 (m, 2H, H-5, H-6, syst. AA'BB'); 3.25 (t, 2H, H-10, 7.3); 1.96(t, 2H, H-14, 7.3); 1.69 (qv, 2H, H-11, 7.3); 1.52 (qv, 2H, H-13, 7.3); 1.38 (qv, 2H, H-13, 7.3).

^{13}C-NMR (dmso-d_6, δ ppm): 169.10(C-15); 150.26(C-2); 143.75 (bs, C-8 or C-9); 135.47(bs, C-8 or C-7); 121.46 (bs, C-5 or C-6); 121.13 (bs, C-5 or C-6); 117.30 (bs, C-4 or C-7); 110.26 (bs, C-4 or C-7); 32.17(); 31.06(); 29.05(C-); 27.70(); 24.70().

FT-IR (solid in ATR, ν cm^{-1}): 3250s; 2940w; 1643vs; 1534m; 1489w; 1460w; 1428m; 1398s; 1367m; 1266m; 1221w; 1122w; 1059w; 1010w; 983w; 922w; 844w; 802w; 731m; 658m; 624m; 512w; 475w; 436w.

Results and discussion

It was proposed the obtaining of new compounds (4a-h) of benzimidazole hydroxamates, in order to test the inhibitive effect on matrix-metalloproteinases.

The compounds(4a-h) were obtained from hydroxylamidation reaction of corresponding esters (3a-h). From this method 8 new compounds (4a-h) were obtained; these compounds were characterized (by melting point, IR and ^{1}H and ^{13}C – NMR spectra), and the purity was determined by TLC and elemental analysis.

Conclusions

Eight new compounds (4a-h); benzimidazoles hydroxamates were synthesized from hydroxylamidation of the esters (3a-h) with hydroxylamine. The new compounds were synthesized in order to determine the inhibitive effect on matrix metalloproteinases. The biologic studies demonstrated the inhibitive effect of the new synthesized compound on matrix metalloproteinases and showed the selective character of inhibition according to the type of enzyme inhibited and the intensity of inhibition [8, 9].

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Manuscript received: 4.10.2008