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Brucella suis histidinol dehydrogenase: Synthesis and inhibition studies of substituted N-L-histidinylphenylsulfonyl hydrazide

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Abstract
Histidinol dehydrogenase (HDH, EC EC1.1.1.23) catalyses the final step in the biosynthesis of histidine and constitutes an attractive novel target for the development of new agents against the pathogenous, bacteria *Brucella suis*. A small library of new HDH inhibitors based on the L-histidinylphenylsulfonyl hydrazide scaffold has been synthesized and their inhibitory activity investigated. The obtained results demonstrate that modification of the group between the histidinyl moiety and the phenyl ring constitutes an important structural factor for the design of effective HDH inhibitors.

Keywords: *Brucella suis, histidinol dehydrogenase, enzyme inhibitors, histidinylphenylsulfonyl hydrazide*

Introduction

Brucella sp. is the causative agent of brucellosis, the most important anthropozoonotic disease worldwide [1–3]. This extremely infectious pathogen is traditionally considered as a biological warfare agent classified in the category B [4]. It is responsible for a highly disabling and incapacitating disease that, without treatment, is lethal in 5 to 10% of the cases. There is currently no vaccine available for humans, and even if antibiotic treatment is actually efficient, occurrence of resistant strains is easy. However, human brucellosis remains a threat because curing of the disease is long and persistent forms may appear [5].

Brucella sp. is a facultative intracellular pathogen that multiplies inside the macrophages of its host [6], The "virulome" of this bacteria has been defined as the whole set of genes required for its virulence, i.e. involved in the invasion of the host by the pathogen and in the adaptation to the environment provided by the host [7]. Among them, the gene *hisD* (BR0252) encoding the enzyme histidinol dehydrogenase, was recently identified [7–8].

Histidinol dehydrogenase (HDH, EC1.1.1.23) is a dimeric metalloenzyme containing one Zn^{2+} ion in each subunit, which catalyzes the last two steps in the biosynthesis of L-histidine: sequential NAD-dependent oxidations of L-histidinol lo L-histidine, *via* L-histidinal. This enzyme is present only in bacteria and in plants. To date, histidinol dehydrogenases have been cloned and characterized from only two species of bacteria, *Salmonella typhimurium* and *Escherichia coli* [9–11].

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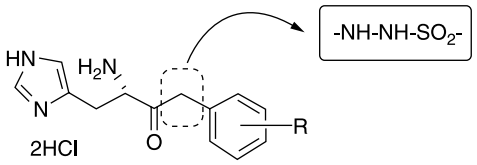


Figure 1. Chemical structure of new *Brucella suis* HDH inhibitors.

The histidine biosynthesis pathway being absent in mammalian cells, this metalloenzyme represents a selective and promising target for the development of new antibacterial agents avoiding secondary effects of potential inhibitors on the host. Therefore, the potential attractiveness of HDH as a target for antibacterial agents encourages the development of HDH inhibitors.

Recently our group succeeded in cloning and overexpression of the *B. suis* HDH, and purification of the protein. We designed and developed a series of effective inhibitors which exhibited excellent inhibition profiles with inhibition constants (IC_{50}) in the nanomolar range [12]. We also observed that bulky groups R were advantageous for obtaining increased inhibitory activities [12].

Starting from these previously reported compounds as lead molecules, we report here the effect of replacing the methylene group by a sulfonyl hydrazide moiety. Several reasons led us to incorporate such moiety in our HDH targeting compounds: (i) the easy preparation and subsequent derivatization of such products leading to a chemical diversity necessary when such new targets are considered; (ii) the presence of such moiety in some compounds of biological interest recently described in literature [13–14] (Figure 1).

Materials and methods

Chemistry

All reagents and solvents were of commercial quality and used without further purification. All reactions were carried out under an inert nitrogen atmosphere. TLC analyses were performed on silica gel 60 F_{254} plates (Merck Art.1.05554). Spots were visualized under 254 nm UV illumination, or by ninhydrin solution spraying. Melting points were determined on a Büchi Melting Point 510 and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on Bruker DRX-400 spectrometer using DMSO-d_6 as solvent and tetramethylsilane as internal standard. For ^{1}H NMR spectra, chemical shifts are expressed in δ (ppm) downfield from tetramethylsilane, and coupling constants ($\mathcal{J}$) are expressed in Hertz. Electron Ionization mass spectra were recorded in positive or negative mode on a Water MicroMass ZQ.

*Preparation of Nα-(tert-butoxycarbonyl)-Nτ-methoxytrityl-L-histidine hydrazide (**2**).* To a solution of methyl N_α -(tert-butoxycarbonyl- N_τ-methoxytritryl-L-histidinate 1 (1eq.) (prepared as previously described [12]) in methanol was added 10 eq. of hydrazine monohydrate and the solution was stirred at room temperature. The reaction was monitored by TLC until complete consumption of the starting material. The crude product was then co-evaporated with toluene to give yellowish oil which was used in the next step without further purification. ^{1}H NMR (DMSO- d_6, 400 MHz) 7.37 (s, 6H), 7.22 (s, 1H), 7.00 (dd, $\mathcal{J}$ = 30.4, 6.9 Hz, 8H), 6.74–6.6 (s, 1H),4.13 (s, 1H), 3.75 (s, 3H), 3.37 (dd, $\mathcal{J}$ = 13.3, 6.5 Hz, IH), 2.71 (td, $\mathcal{J}$ = 22.7, 14.1, 14.12 Hz, 2H), 1.31 (s, 9H). ^{13}C NMR (DMSO-d_6, 101MHz) 170.74, 158.58, 154.95, 142.67, 137.45, 136.95, 134.05, 130.60, 129.08, 128.00, 127.78, 118.92, 113.28, 77.68, 73.84, 54.93, 52.88, 30.88, 28.09; MS ESI$^+$ *m/z* 542.26 $(M + H)^+$ 564.24. ESI$^-$ *m/z* 541.19 $(M - H)^-$, 576.20 $(M + Cl)^-$.

Synthesis of substituted N-L-histidinyl-phenylsulfonylhydrazide. General procedure. To 1eq. of Nα-(*tert*-butoxycarbonyl)-1-methoxytrityl-L-histidine hydrazide **2** in distilled pyridine was added 1eq. of the corresponding substituted phenylsulfonylchloride.

The reaction was stirred for two hours. Upon completion, the solvent was evaporated under reduced pressure. The crude product was purified on silica gel (CH_2Cl_2/ Methanol: 95/5) to afford the expected compound as a white powder. The different compounds were deprotected under acidic conditions (HCl solution in dioxane 4M) to remove both the methoxytrityl and the *tert*-butoxycarbonyl groups.

*N-L-histidinyl-4-methylphenylsulfonyl hydrazide (**3a**).* mp 141–143°C; ^{1}H NMR (DMSO-d_6, 400MHz) 14.67 (s, 1H), 10.70 (s, 1H), 10.11 (d, $\mathcal{J}$ = 1.5 Hz, 1H), 9.10 (d, $\mathcal{J}$ = 0.97 Hz, 1H), 8.66 (s, 2H), 7.62 (d, $\mathcal{J}$ = 8.2 Hz, 2H), 7.44 (s, 1H), 7.37 (d, $\mathcal{J}$ = 8.1 Hz, 2H), 4.21 (s, 1H), 3.13 (dd, $\mathcal{J}$ = 6.3, 3.01 Hz, 2H), 2.38 (s, 3H). ^{13}C NMR (DMSO-d_6, 101MHz) 166.68, 143.36, 135.96, 133.83, 129.41, 127.41, 126.16, 117.58, 49.53, 26.09, 21.03; MS ESI$^+$ *m/z* 324 $(M + H)^+$, 364.22 $(M + K)^+$. ESI$^-$ *m/z* 322.15 $(M - H)^-$, 358.14 $(M + Cl)^-$.

*N-L-histidinyl-4-tert-butylphenylsulfonyl hydrazide (**3b**).* ^{1}H NMR (DMSO-d_6, 400MHz) 14.69 (s, 1H), 10.64 (d, lH, $\mathcal{J}$ = 0.6Hz), 10.10 (d, lH, $\mathcal{J}$ = 0.6Hz), 9.10 (d, lH, $\mathcal{J}$ = 1.3Hz), 8.70 (s, 2H), 7.67 (d, 2H, $\mathcal{J}$ = 8.7Hz), 7.60 (d, 2H, $\mathcal{J}$ = 8.8Hz), 7.45 (d, 1H, $\mathcal{J}$ = 1.2Hz), 4.20 (t, 1H, $\mathcal{J}$ = 6.8Hz), 3.15 (d, 2H, $\mathcal{J}$ = 6.9Hz), 1.30 (s, 9H). ^{I3}C NMR

(DMSO-d_6, 101MHz) 166.92, 156.03, 136.12, 133.91, 127.21, 126.23, 125.84, 117.61, 49.66, 34.83, 30.75, 26.11; MS ESI$^+$ *m/z* 366.18 $(M + H)^+$. ESI$^+$ *m/z* 364.16 $(M - H)^-$, 400.09 $(M + Cl)^-$, 765.18 $(2M + Cl)^-$.

*N-L-histidinyl-4-methoxyphenylsulfonyl hydrazide (**3c**)*. mp 187–189°C; ^{1}H NMR (DMSO-d_6, 400MHz) 14.73 (s, 1H), 10.69 (d, 1H, $\mathcal{J} = 1.6$Hz), 10.00 (d, 1H, $\mathcal{J} = 2.1$Hz), 9.11 (d, 1H, $\mathcal{J} = 1.2$Hz), 8.68 (s, 2H), 7.64 (d, 2H, $\mathcal{J} = 8.9$Hz), 7.48 (d, 1H, $\mathcal{J} = 0.5$Hz), 7.08 (d, 2H, $\mathcal{J} = 9.0$Hz), 4.22 (s, IH), 3.84 (s, 3H), 3.14 (d, 2H, $\mathcal{J} = 7.0$Hz). ^{13}C NMR (DMSO-d_6, 101MHz) 166.63, 162.66, 133.79, 130.22, 129.66, 126.24, 117.57, 114.17, 55.68, 49.51, 26.14; MS ESI$^+$ *m/z* 340.24 $(M + H)^+$. ESI$^-$ *m/z* 338.20 $(M - H)^-$, 374.19 $(M + Cl)^-$, 713.23 $(2M + Cl)^-$.

*N-L-histidinyl-biphenyl-4-ylsulfonyl hydrazide (**3d**)*. mp 220°C (decomposition); ^{1}H NMR (DMSO-d_6. 400MHz) 14.73 (s, 1H), 10.78 (s, 1H), 10.25 (s, 1H), 9.11 (d, 1H, $\mathcal{J} = 1.0$Hz), 8.71 (m, 2H), 7.81 (m, 6H), 7.48 (m, 4H), 4.24 (t, 1H, $\mathcal{J} = 6.8$Hz), 3.17 (d, 2H, $\mathcal{J} = 6.8$Hz). ^{13}C NMR (DMSO-d_6, 101 MHz) 166.82, 144.38, 138.34, 137.64, 133.86, 129.07, 128.51, 128.03, 127.11, 127.03, 126.29, 117.59, 49.57, 26.16; MS ESI$^+$ *m/z* 386.13 $(M + H)^+$, 771.24 $(2M + H)^+$. ESI$^-$ *m/z* 384.10 $(M - H)^-$, 419.96 $(M + Cl)^-$, 805.20 $(2M + Cl)^-$.

*N-L-histidinyl-pentafluorophenylsulfonyl hydrazide (**3e**)*. mp 215°C (decomposition); ^{1}H NMR (DMSO-d_6, 400MHz) 14.55 (s, 1H), 11.36 (s, 1H), 9.07 (d, $\mathcal{J} = 1.2$ Hz, 1H), 8.66 (s, 1H), 7.48 (s, 1H), 4.26 (t, $\mathcal{J} = 6.5$ Hz, 1H), 3.23 (dd, $\mathcal{J} = 6.1$, 3.3 Hz, 1H). ^{13}C NMR (DMSO-d_6, 101MHz) 167.52,133.95, 126.01, 117.88, 49.88, 25.99; MS ESI$^+$*m/z* 400.09 $(M + H)^+$. ESI$^-$ *m/z* 398.11 $(M - H)^-$, 434.17 $(M + Cl)^-$, 833.17 $(2M + CL)^-$.

*N-L-histidinyl-4-cyanophenylsulfonyl hydrazide (**3f**)*. mp 205°C (decomposition); ^{1}H NMR (DMSO-d_6, 400MHz) 14.70 (s, 1H), 10.96 (s, 1H), 10.59 (s, 1H), 9.09 (d, 1H, $\mathcal{J} = 1.3$Hz), 8.71 (s, 2H), 8.07 (d, 2H, $\mathcal{J} = 8.6$Hz), 7.91 (d, 2H, $\mathcal{J} = 8.6$Hz), 7.48 (d, 1H, $\mathcal{J} = 0.9$Hz), 4.24 (t, 1H, $\mathcal{J} = 6.8$Hz), 3.15 (d, 2H, $\mathcal{J} = 6.8$Hz). ^{13}C NMR (DMSO-d_6, 101MHz) 166.91, 143.19, 133.84, 133.13, 128.20, 126.16, 117.74, 117.65, 115.27, 49.54, 26.06; MS ESI$^+$*m/z* 335.20 $(M + H)^+$. ESI$^-$ *m/z* 333.20 $(M - H)^-$, 369.13 $(M + Cl)^-$, 667.29 $(2M - H)^-$, 703.18 $(2M + Cl)^-$.

*N-L-histidinyl-4-trifluoromethylphenylsulfonyl hydrazide (**3g**)*. mp 195°C (decomposition); ^{1}H NMR (DMSO-d_6, 400MHz) 14.71 (s, 1H), 10.90 (s, 1H), 10.53 (s, 1H), 9.10 (d, 1H, $\mathcal{J} = 1.3$Hz), 8.72 (s, 2H), 7.97 (s, 4H), 7.48 (d, 1H, $\mathcal{J} = 1.0$Hz), 4.23 (t, 1H, $\mathcal{J} = 6.8$Hz), 3.17 (d, 2H, $\mathcal{J} = 6.7$Hz). ^{13}C NMR (DMSO-d_6, 101MHz) 166.97, 143.12 (d, $\mathcal{J}_{C-F} =$ 1.32 Hz), 134.17 (s,1C), 133.85, 132.51 (dd, $\mathcal{J}_{C-F} =$ 64.18, 31.94 Hz), 128.40, 126.217, 117.68, 49.60, 26.06; MS ESI$^+$*m/z* 378.16 $(M + H)^+$. ESI$^-$ *m/z* 376.24 $(M - H)^-$, 789.10 $(2M + Cl)^-$.

*N-L-histidinyl-4-nitrophenylsulfonyl hydrazide (**3h**)*. mp 205°C(decomposition); ^{1}H NMR (DMSO-d_6, 400MHz)14.56 (s, 1H), 10.97 (s, 1H), 10.65 (s, 1H), 9.07 (d, 1H, $\mathcal{J} = 1.1$Hz), 8.66 (s, 2H), 8.40 (m, 2H), 8.02 (m, 2H), 7.46 (d, 1H, $\mathcal{J} = 0.7$Hz), 4.22 (t, 1H, $\mathcal{J} = 6.9$Hz), 3.14 (d, 2H, $\mathcal{J} = 6.9$Hz). ^{13}C NMR (DMSO-d_6, 101MHz) 166.92, 149.81, 144.64, 133.87, 129.14, 126.16, 124.26, 117.66, 49.55, 26.04; MS ESI$^+$*m/z* 355.14 $(M + H)^+$, ESI$^-$ *m/z* 353.16 $(M - H)^-$, 389.22 $(M + Cl)^-$, 707.16 $(2M - H)^-$, 734.17$(2M + Cl)^-$.

*N-L-histidinyl-4-fluorophenylsulfonyl hydrazide (**3i**)*. mp 222–224°C; ^{1}H NMR (DMSO-d_6, 400MHz) 14.70 (s, 1H), 10.81 (s, 1H), 10.27 (s, 1H), 9.10 (d, 1H, $\mathcal{J} = 1.3$Hz), 8.69 (s, 2H), 7.80 (dd, 2H, $\mathcal{J} = 5.2$Hz, 8.9Hz), 7.47 (d, 1H, $\mathcal{J} = 0.9$Hz), 7.42 (t, 2H, $\mathcal{J} = 8.9$Hz), 4.23 (t, 1H, $\mathcal{J} = 6.8$Hz), 3.14 (d, 2H, $\mathcal{J} = 6.9$Hz). ^{13}C NMR (DMSO-d_6 101 MHz) 166.76, 164.53 (d, $\mathcal{J}_{C-F} = 251.1$ Hz), 135.19, 133.84, 130.56 (d, $\mathcal{J}_{C-F} = 9.4$ Hz), 126.21, 117.60, 116.19 (d, $\mathcal{J}_{C-F} = 22.9$ Hz), 49.55, 26.10; MS ESI$^+$*m/z* 328.16 $(M + H)^+$, 655.20 $(2M + H)^+$. ESI$^-$ *m/z* 326.14 $(M - H)^-$, 362.14 $(M + Cl)^-$, 689.14 $(2M + Cl)^-$.

*N-L-histidinyl-4-chlorophenylsulfonyl hydrazide (**3j**)*. mp 210°C (decomposition); ^{1}H NMR (DMSO-d_6, 400MHz) 14.71 (s, 1H), 10.84 (s, 1H), 10.34 (s, 1H), 9.10 (s, 1H), 8.72 (s, 2H), 7.75 (d, $\mathcal{J} = 8.4$ Hz, 2H), 7.65 (d, $\mathcal{J} = 8.4$ Hz, 2H), 7.48 (s, 1H), 4.23 (t, $\mathcal{J} = 6.4$ Hz, 1H), 3.16 (d, $\mathcal{J} = 6.5$ Hz, 2H). ^{13}C NMR (DMSO-d_6, 101MHz) 166.80, 137.91, 137.84, 133.79, 129.36, 129.14, 126.17, 117.62, 49.54, 26.08; MS ESI$^+$*m/z* 344.14 $(M + H)^+$. ESI$^-$ *m/z* 342.16 $(M - H)^-$, 378.09 $(M + Cl)^-$, 723.09 $(2M + Cl)^-$.

*N-L-histidinyl-4-bromophenylsulfonyl hydrazide (**3k**)*. mp 93–94°C; ^{1}H NMR (DMSO-d_6, 400MHz) 14.60 (s, 1H), 10.80 (s, 1H), 10.34 (s, 1H), 9.08 (d, $\mathcal{J} = 0.8$ Hz, 1H), 8.65 (s, 2H), 7.80 (d, $\mathcal{J} = 8.6$ Hz, 2H), 7.67 (d, $\mathcal{J} = 8.64$ Hz, 2H), 7.46 (s, 1H), 4.20

(t, $\mathcal{J} = 6.6$ Hz, 1H), 3.14 (d, $\mathcal{J} = 6.8$ Hz, 2H). ^{13}C NMR (DMSO-d_6, 101MHz) 166.80, 138.26, 133.93, 132.07, 129.40, 127.03, 126.22, 117.63, 49.59, 26.06; MS ESI$^+$ *m/z* 390.05 $(M + H)^+$. ESI$^-$ *m/z* 386.02 $(M - H)^-$, 424.1 $(M + Cl)^-$, 810.91 $(2M + Cl)^-$.

*N-L-histidinyl-4-iodophenylsulfonyl hydrazide (**3l**)*. mp 139–141°C; ^{1}H NMR (DMSO-d_6, 400MHz) 14.65 (s, 1H), 10.79 (s, 1H), 10.30 (s, 1H), 9.09 (d, $\mathcal{J} = 1.1$ Hz, 1H), 8.69 (s, 2H), 7.97 (d, $\mathcal{J} = 8.5$ Hz, 2H), 7.50 (d, $\mathcal{J} = 8.5$ Hz, 2H), 7.47 (s, 1H), 4.22 (t, $\mathcal{J} = 6.6$ Hz, 1H), 3.15 (d, $\mathcal{J} = 6.7$ Hz, 2H). ^{13}C NMR (DMSO-d_6, 101 MHz) 166.82, 138.65, 137.86, 133.85, 129.03, 126.17, 117.65, 101.59, 49.57, 26.08; MS ESI$^+$ *m/z* 436.03 $(M + H)^+$. ESI$^-$ *m/z* 434.05 $(M - H)^-$, 469.99 $(M + Cl)^-$.

*N-L-histidinyl-2-naphthysulfonyl hydrazide (**3m**)*. mp 220°C (decomposition); ^{1}H NMR (DMSO-d_6 400MHz) 14.69 (s, 1H), 10.81 (s, 1H), 10.31 (s, 1H), 9.09 (d, $\mathcal{J} = 1.2$ Hz, 1H), 8.65 (s, 2H), 8.44 (d, $\mathcal{J} = 1.8$ Hz, 1H), 8.20 (d, $\mathcal{J} = 8.1$ Hz, 1H), 8.11 (d, $\mathcal{J} = 8.8$ Hz, 1H), 8.04 (d, $\mathcal{J} = 8.1$ Hz, 1H), 7.80 (dd, $\mathcal{J} = 8.7$, 1.9 Hz, 1H), 7.68 (dddd, $\mathcal{J} = 20.9$, 8.1, 6.9, 1.3 Hz, 2H), 7.44 (d, $\mathcal{J} = 0.8$ Hz, 1H), 4.22 (t, $\mathcal{J} = 6.5$ Hz, 1H), 3.13 (ddd, $\mathcal{J} = 36.6$, 15.6, 6.7 Hz, 2H). ^{13}C NMR (DMSO-d_6, 101MHz) 166.86, 136.20, 134.46, 133.85, 131.56, 129.34, 129.02, 128.87, 128.44, 127.73, 127.39, 126.16, 122.92, 117.67, 49.64, 26.06; MS ESI$^+$ *m/z* 360.19 $(M + H)^+$, 719.31 $(2M + H)^+$. ESI$^-$ *m/z* 358.21 $(M - H)^-$, 394.14 $(M + Cl)^-$, 753.28 $(2M + Cl)^-$.

Enzyme assays

The activity and specificity of HDH were measured by monitoring the reduction of NAD^+ to NADH directly at 340 nm ($\varepsilon_M = 6200\ M^{-1}.cm^{-1}$) as previously described [15]. The enzymatic activity was studied at 30°C in the presence of 0.5 mM histidinol, 5 mM NAD^+ and 0.5 mM $MnCl_2$ in 50 mM sodium glycine buffer at pH = 9.2. For kinetic studies, experiments were carried out with 150 mM sodium glycine (pH 9.2) and 2 mM NAD^+. The K_m for the substrate was determined by varying the concentration of histidinol from 10 to 50 μM. Activity (1 unit) is defined as the amount of HDH producing 1 μmol of NADH per min in the reaction. To perform IC_{50} determinations of the different inhibitors, the latter were added at various concentrations, ranging from 1 μM to 400 μM, and preincubated for 5 min at 30°C with the enzyme solution prior to the initiation of the reaction. The enzyme concentration in the assay system was 4.5×10^{-11} M.

Results and discussion

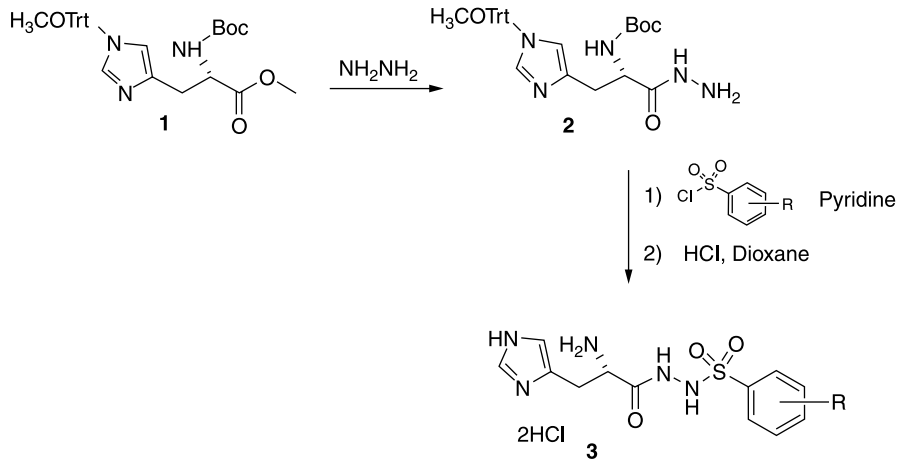
Chemistry

Substituted histidinyl phenylsulfonylhydrazide derivatives **3** were readily prepared according to the synthetic pathway depicted in Scheme 1. The precursor **1** was synthesized in four steps starting from L-histidine as previously described [12]. Reaction of compound **1** with hydrazine hydrate afforded the corresponding hydrazide **2** which was reacted with various substituted phenylsulfonyl chloride in the presence of pyridine to yield, after acidic treatment the corresponding hydrazides **3**.

All the synthesized compounds **3** listed in Figure 2 were fully characterized by ^{1}H-NMR, ^{13}C-NMR and mass spectral data.

Brucella suis HDH inhibitory activity

All the newly synthesized compounds were assayed for their inhibitory activity against the purified *B. suis* HDH. Inhibitory data are presented in Table I. The results show a decreased affinity for **3** compared to the



Scheme 1. The synthetic pathway of **2**–**3**.

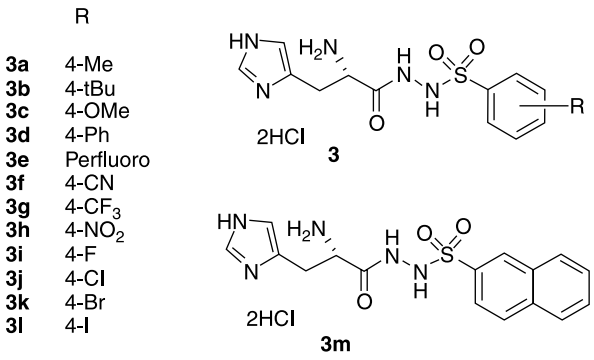


Figure 2. Structure of inhibitors series **3**.

previously reported derivatives [12], with IC_{50} within the range of 25 to more than 400 μM. The most active derivatives in this series were compounds **3d** and **3k** with an IC_{50} of 25μM and 70μM respectively. Additionally, some compounds such as **3e**, **3i**, **3j**, **3h** were devoid of any inhibitory activity for concentration ≥400 μM.

The following SAR should be noted from data of Table I: among the compound series, the activity order was **3d** > **3k** > **3c** > **3a** > **3f**, which might reflect the importance of the substituent bulkiness in the position 4 of the phenyl ring.

Compared with the previously reported series [12], compounds **3** are much less effective, indicating that the nature and the length of the linker between the histidinyl moiety and the phenyl ring is an important factor which can be modulated for the design and the discovery of new potential inhibitors.

In conclusion, a small library of substituted L-histidinyl phenylsulfonylhydrazide was synthesized as potential *B. suis* histidinol dehydrogenase inhibitors. The introduction of a hydrazinosulfonyl scaffold was shown to influence potencies of inhibitors. From these data, compound **3d** demonstrated the best inhibitor activity. Nevertheless, this series of compounds remains less active compared to the one previously described. Informations derived from this study indicate that the linker between the histidinyl moiety and the phenyl ring constitute an important structural feature which could be subject to further modifications in order to design more potent inhibitors.

Table I. Inhibition of *B. suis* histidinol dehydrogenase with compounds **3a** to **3m**.

Compounds	HDH (*Brucella suis*) IC_{50} (μM)[a]
3a	160
3b	375
3c	135
3d	25
3e	>400
3f	190
3g	200
3h	>400
3i	>400
3j	>400
3k	70
3l	200
3m	140

[a]The values are the means of three independent assays. Variations were in the range of 5–10% of the shown data.

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