Synthesis and Antitubercular Activity of New L-serinyl Hydrazone Derivatives

Alessandra Campbell Pinheiro[a], Carlos Roland Kaiser[b], Thaís Cristina Mendonça Nogueira[a,b], Samir Aquino Carvalho[a,b], Edson Ferreira da Silva[a], Larisse de Oliveira Feitosa[a], Maria das Graças Müller de Oliveira Henriques[a], André Luís Peixoto Candéa[a], Maria Cristina Silva Lourenço[c] and Marcus Vinícius Nora de Souza[a,b]*

[a]*Fundação Oswaldo Cruz, Instituto de Tecnologia em Fármacos-Far Manguinhos, Fundação Oswaldo Cruz, 21041-250, Rio de Janeiro, RJ, Brazil*

[b]*Universidade Federal do Rio de Janeiro, Instituto de Química, Departamento de Química Orgânica, CP 68563, 21945-970- Rio de Janeiro, RJ, Brazil*

[c]*Fundação Oswaldo Cruz, Instituto de Pesquisas Clínicas Evandro Chagas, Departamento de Bacteriologia, Av. 21040-360, Rio de Janeiro, RJ, Brazil*

Abstract: A series of 32 L-serinyl hydrazone derivatives have been synthesized and evaluated for their *in vitro* antibacterial activity against *Mycobacterium tuberculosis* $H_{37}Rv$, being also evaluated their cell viabilities in non infected and infected macrophages with *Mycobacterium bovis* Bacillus Calmette-Guerin (BCG). The compounds **8c**, **8e**, **8h** and **8i**, were non-cytotoxic and exhibited an important minimum inhibitory concentration (MIC) activity between 25 and 100μg/mL, which can be compared with that of the tuberculostatic drug D-cicloserine (5-20μg/mL).

Keywords: BCG, D-cycloserine, hydrazone, L-cycloserine, L-serine, *Mycobacterium*, tuberculosis.

INTRODUCTION

Multidrug-resistant tuberculosis (MDR-TB) refers to organisms that are resistant to at least two of the first-line drugs, isoniazid and rifampin. There are approximately half a million new cases of MDR-TB worldwide every year [1]. More recently, extensively drug resistant tuberculosis (XDR-TB) has been emerged. These bacteria are also resistant to three or more of the second-line treatment drugs. XDR-TB is considered a great public health problem, due to be resistant to the drugs routinely used to treat tuberculosis infections [2-5].

It is estimated that MDR-TB is responsible for about 3.3% of all new tuberculosis cases every year, and XDR-TB cases, extremely difficult to treat, have been confirmed in more than 58 countries [1]. Considering that, preventing MDR and XDR-TB from spreading is essential. Unfortunately, few treatment options have been available for XDR TB, which are less effective when compared to traditional antibiotic therapies for TB.

Due to the high impact of MDR and XDR in TB treatment, new drugs and strategies to limit the spread of tuberculosis are much needed. In this context, the aim of this article is to describe the synthesis and biological activity of series of L-cicloserine analogs.

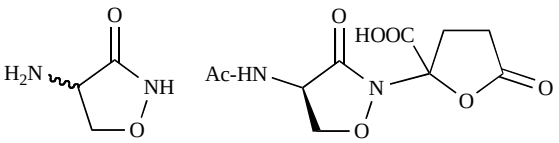
***Address correspondence to this author at the FioCruz - Fundação Oswaldo Cruz, Instituto de Tecnologia em Fármacos – Far Manguinhos, Rua Sizenando Nabuco, 100, Manguinhos, 21041-250 Rio de Janeiro, RJ, Brazil; Tel: +552139772404; Fax: +552125602518;
E-mail: marcos_souza@far.fiocruz.br

Our interest in the anti-TB profile of cicloserine analogs was supported by the structure of L-cicloserine **1a**, featured in the natural product lactivicin **2**, the first non-β-lactam broad-spectrum antibacterial agent with a mode of action similar to that of β-lactams [6] (Fig. **1**).

L-cicloserine is also the (*S*)-enantiomer of D-cicloserine **1b**, a broad–spectrum antibiotic classified as a second line drug for the treatment of tuberculosis. This drug inhibits *M. tuberculosis* in concentrations of 5 to 20 μg/mL, with no cross-resistance observed (Fig. **2**) [7-8].

In either the natural D- or synthetic L-isomer form, cicloserine acts as an essentially irreversible inhibitor of alanine racemase, responsible for the interconversion of alanine enantiomers, and thus represents the first step involved in bacterial cell wall biosynthesis [9-10].

Despite these findings, a very limited number of both D- and L-cycloserine analogs have been reported to date.



L-cycloserine, **1a** Lactivicin, **2**

D-cycloserine, **1b**

Fig. (1). D, L-cycloserine and the derivative Lactivicin.

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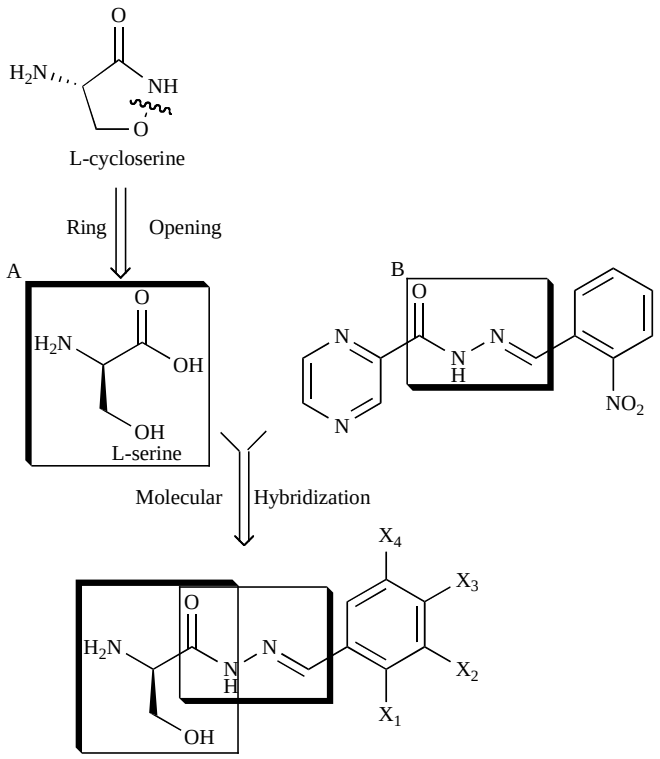


Fig. (2). Design concept of *N*-acylhydrazones, L-serine derivatives.

In our continuous search of new potent and safe antitubercular agents, we decided to synthesize a new class of L-serinyl hydrazone derivatives as attractive L-cycloserine analogues, designed by molecular hybridization. *N*-acylhydrazones are described with a wide range of pharmacological activities, such as antibacterial agents [11-14]. For example, the pirazinamide derivative **B** prepared by our group, exhibiting a minimum inhibitory concentration (MIC) of 50μg/mL, which is four times more potent than the first line drug pyrazinamide [15].

The design concept of these compounds attempts to introduce the pharmacophoric *N*-acylhydrazone subunit of **B** into the core structure of L-serine **A**, an acyclic L-cicloserine analog (Fig. **2**).

RESULTS AND DISCUSSION

Chemistry

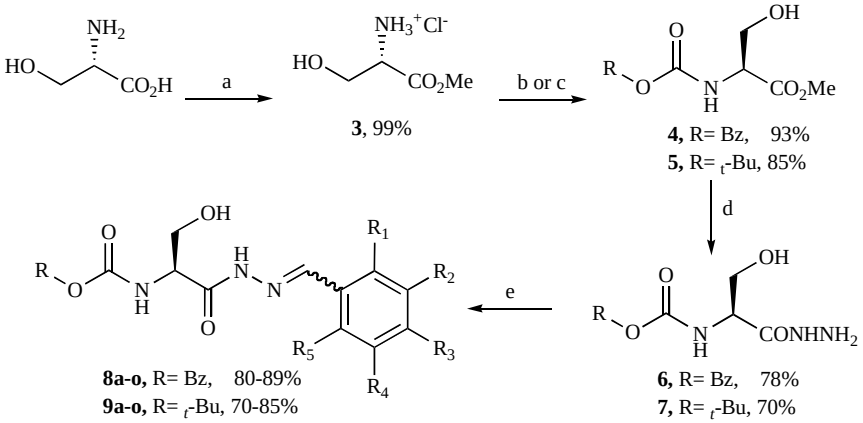
The synthetic route used for the preparation of the (*S*) title compounds is outlined in the Scheme **1**. L-serine amino acid was employed as starting material, and *t*-butoxycarbonyl (BOC) and benzyloxycarbonyl (Cbz) protecting groups were included in the core structure **2** due to an intrinsic instability observed in non-protected derivatives. This modification could also result in better pharmacodymamic profiles.

From L-serine, compounds **8** and **9** were obtained by esterification, leading **3**, followed by *N*-protection using CbzCl or $(BOC)_2O$ in order to furnish **4** or **5** in 93 and 85% yield, respectively. These compounds were converted into their hydrazine derivatives using 80% aqueous hydrazine hydrate. After washing with cold ethanol the pure product **6** was obtained in 78% yield. In order to obtain **7** the crude product was column chromatographed on silica gel, affording the desired compound in 70% yield. Finally, after condensation reactions of these compounds with substituted aldehydes, the desired derivatives **8a-o** and **9a-o** were obtained in 70-89% yield (Table **1**-**2**).

All these compounds were identified by spectral data. The ^1H NMR spectra of the final compounds (**8a-o** and **9a-o**) were consistent with two geometric isomers at C=N bond level, or only one compound, exhibiting two different conformations, and getting different levels of stability. An additional NMR study was performed at room temperature and at 60^0C, using compound **8b**, randomized chosen. Both experiments resulted in very similar spectra, suggesting the existence of two geometric isomers (*E* and *Z*) of each **8a-o** and **9a-o** compound obtained. Comparing both spectra (Fig. **3**) it is possible to observe a coalescence of labile NH protons uniquely.

However, only *E*-isomers **8b**, **8n** and **8o** have been confirmed by X-ray crystallography [16-18].

It's possible to note in NMR spectra nearly 1:1 isomeric proportion for non-hydroxyl/methoxyl substituted derivatives (**8a-f** and **9a-f**). In other RMN spectra, relative to **8g-o** and **9g-o** compounds, mainly one compound has been formed, being defined as an *E*-isomer after X-ray crystallography results.



Scheme 1. Reagents and conditions: (a) MeOH, $SOCl_2$, rt, 24h; (b) CbzCl, $NaHCO_3$, H_2O, rt, 3h; (c) $(BOC)_2O$, Et_3N, THF, rt, 24h; (d) $N_2H_4.H_2O$ (80%), EtOH, rt, 24h; (e) EtOH, ArCHO, 80^0C, 3-48h.

Table 1. Physical and structural properties of benzyl (1*S*)-2-[2-(substituted-benzylidene)hydrazino]-1-(hydroxymethyl)-2-oxoethylcarbamate (8a-o)

Compound	Substituents					Yield (%)
	R_1	R_2	R_3	R_4	R_5	
8a	H	H	H	H	H	85
8b	NO_2	H	H	H	H	80
8c	H	NO_2	H	H	H	80
8d	H	H	NO_2	H	H	82
8e	H	CN	H	H	H	82
8f	H	H	CN	H	H	80
8g	OH	H	H	H	H	89
8h	OH	OH	H	H	H	81
8i	OH	H	OH	H	H	85
8j	OH	H	H	OH	H	87
8k	OH	OCH_3	H	H	H	80
8l	H	OH	OH	H	H	80
8m	H	OCH_3	OCH_3	H	H	83
8n	OH	H	OCH_3	H	H	80
8o	OCH_3	H	H	H	H	80

Table 2. Physical and structural properties of *t*-Butyl (1*S*)-2-[2-(substituted-benzylidene)hydrazino]-1-(hydroxymethyl)-2-oxoethylcarbamate (9a-o)

Compound	Substituents					Yield (%)
	R_1	R_2	R_3	R_4	R_5	
9a	H	H	H	H	H	82
9b	NO_2	H	H	H	H	85
9c	H	NO_2	H	H	H	78
9d	H	H	NO_2	H	H	80
9e	H	CN	H	H	H	78
9f	H	H	CN	H	H	70
9g	OH	H	H	H	H	76
9h	OH	OH	H	H	H	85
9i	OH	H	OH	H	H	74
9j	OH	H	H	OH	H	79
9k	OH	OCH_3	H	H	H	80
9l	H	OH	OH	H	H	85
9m	H	OCH_3	OCH_3	H	H	82
9n	OH	H	OCH_3	H	H	84
9o	OCH_3	H	H	H	H	75

Fig. (3). NMR spectra of **8b** at 20^0C (A) and at 60^0C (B). The labile *E* and *Z* protons of NHN subunit are continued line marked; not labile *E* and *Z* protons, exemplified by N=CH, are broken line marked.

Antimycobacterial Activity

The cellular viability of all synthesized compounds was previously evaluated by Mosman's MTT microcultured tetrazolium assay, and all compounds were considered not cytotoxic to the host cells, since they did not kill more than 10% of the cells at the concentration tested (50 μg/mL).

The antimycobacterial activities of all synthesized compounds were assessed against *M. tuberculosis* ATTC 27294 using the Microplate Alamar Blue Assay (MABA) (Tables **3**-**5**). This methodology is nontoxic, uses thermally stable reagent, and shows good correlation with proportional and BACTEC radiometric methods.

Firstly, for the *N*-benzyloxycarbonyl-L-serinylhydrazone (**8a-o**) derivatives it was observed that the most active compound was **8c** (MIC=25μg/mL), which included a nitro group in the *meta* position of the aromatic hydrazone subunit. This activity can be compared to the *in vitro* activity of D-cicloserine (MIC=5-20 μg/mL).

The antimycobacterial activity decreases when the nitro group of **8c** is replaced by the cyano group (**8e**, MIC=50μg/mL), indicating the importance of a nitro group in this position for monosubstituted derivatives.

The presence of hydroxyl groups in *orto* position of the aromatic ring seems to indicate its relevance to the biological activity in the disubstituted derivatives **8h** (MIC= 50μg/mL), **8i** (MIC=100μg/mL) and **8k** (MIC=100μg/mL) (Table **3**).

In order to evaluate the contribution of benzyloxycarbonyl protecting group (Cbz) to the pharmacophoric character of these compounds to the action over *M. tuberculosis*, we performed the investigation of the tuberculostatic profile of *N*-*t*-butyloxycarbonyl-L-serinylhydrazone (**9a-o**) analogues. It was observed that the presence of Cbz in the structure of **8a-o** seems to be important to the biological activity of this class of compounds, since compounds of **9a-o** series did not remain active against *M. tuberculosis* (Table **4**).

Furthermore, compounds **10a** and **10b** were synthesized from the *N*-Cbz-protected derivatives **8c** and **8e**, respectively, with the aim of confirm the importance of Cbz and the oxazolidinone nucleus for the biological activity of these derivatives. The compounds **10a** and **10b** obtained, after a standard cyclization reaction using NaH in room temperature, showed decreased activity related to its starting materials (Table **5**).

Also, analogous non-protected compound **11a** is not active against *M. tuberculosis*, ratifying the importance of Cbz protecting group to the biological activity observed to the most active compounds synthesized, **8c** (Fig. **4**).

The biological activity observed for *N*-Cbz protected-L-serinylhydrazone derivatives could be related to clog*P* values obtained to these derivatives, indicating that lipophilicity is an important parameter for anti-TB activity in these series.

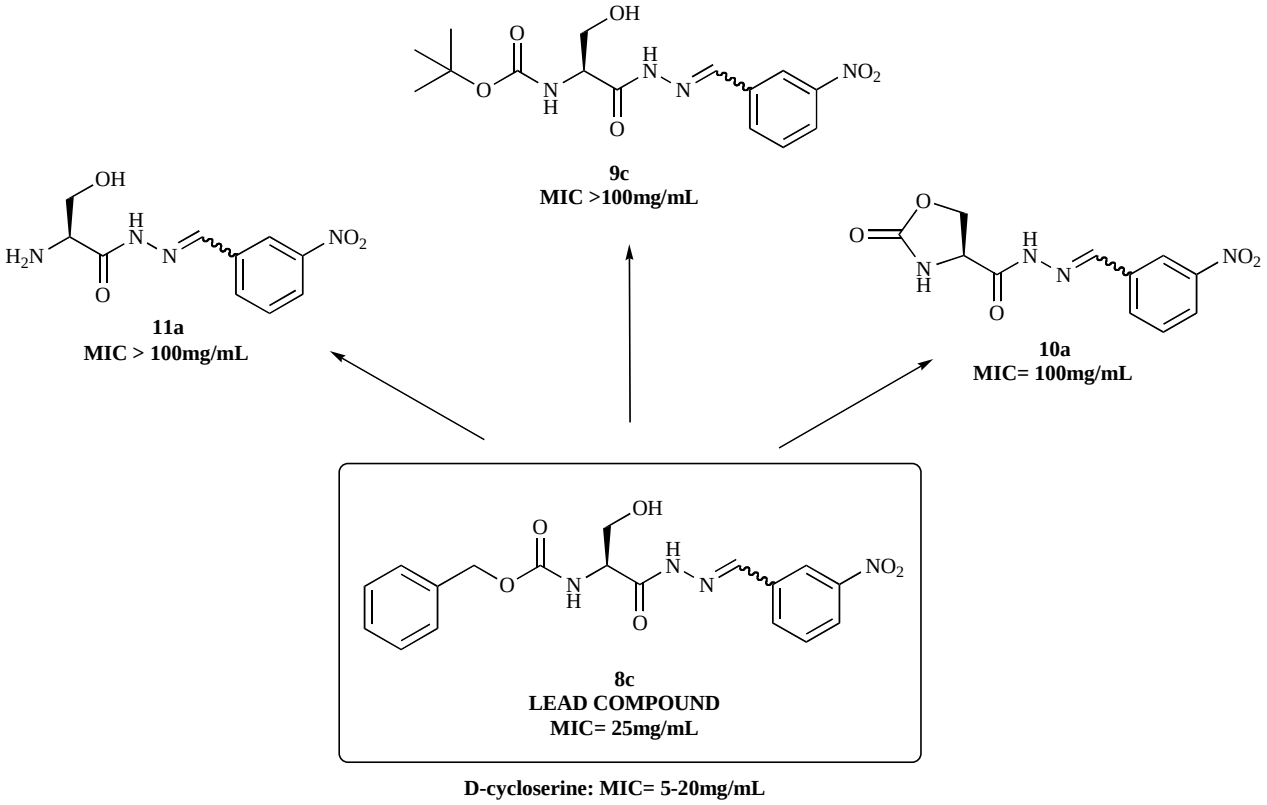
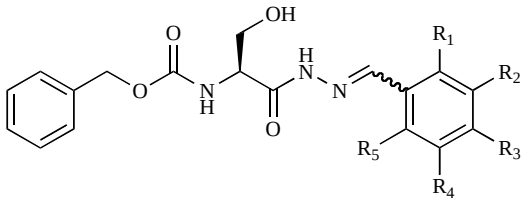


Fig. (4). Biological activities of **8c** and its analogous compounds **9c**, **10a** and **11a**.

Table 3. The *in vitro* activity of compounds 8a-o against *M. tuberculosis* H37Rv strain (ATCC 27294, susceptible both to rifampin and isoniazid) and clog*P* calculations



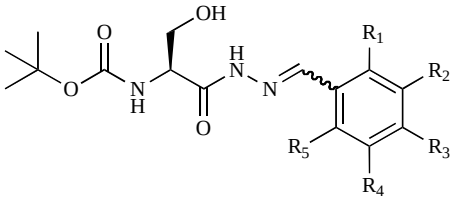
Compound	Substituents					MIC[a] (μg/mL)	clog *P*[b]
	R_1	R_2	R_3	R_4	R_5		
8a	H	H	H	H	H	>100	3.770
8b	NO_2	H	H	H	H	>100	3.681
8c	H	NO_2	H	H	H	25	3.705
8d	H	H	NO_2	H	H	>100	3.729
8e	H	CN	H	H	H	50	3.501
8f	H	H	CN	H	H	>100	3.525
8g	OH	H	H	H	H	>100	3.711
8h	OH	OH	H	H	H	50	3.007
8i	OH	H	OH	H	H	100	3.207
8j	OH	H	H	OH	H	>100	3.207
8k	OH	OCH_3	H	H	H	100	3.314
8l	H	OH	OH	H	H	>100	2.802

Table 3. contd….

Compound	Substituents					MIC[a] (μg/mL)	clog *P*[b]
	R_1	R_2	R_3	R_4	R_5		
8m	H	OCH_3	OCH_3	H	H	>100	3.417
8n	OH	H	OCH_3	H	H	>100	3.743
8o	OCH_3	H	H	H	H	>100	3.779

[a] Minimum inhibitory concentration
[b] Calculated using online www.molinspiration.com website

Table 4. The *in vitro* activity of compounds 9a-o against *M. tuberculosis* H37Rv strain (ATCC 27294, susceptible both to rifampin and isoniazid) and clog*P* calculations



Compound	Substituents					MIC[a] (μg/mL)	clog *P*[b]
	R_1	R_2	R_3	R_4	R_5		
9a	H	H	H	H	H	>100	3.361
9b	NO_2	H	H	H	H	>100	3.272
9c	H	NO_2	H	H	H	>100	3.296
9d	H	H	NO_2	H	H	>100	3.320
9e	H	CN	H	H	H	>100	3.092
9f	H	H	CN	H	H	>100	3.116
9g	OH	H	H	H	H	>100	3.301
9h	OH	OH	H	H	H	>100	2.598
9i	OH	H	OH	H	H	>100	2.798
9j	OH	H	H	OH	H	>100	2.798
9k	OH	OCH_3	H	H	H	>100	2.905
9l	H	OH	OH	H	H	>100	2.393
9m	H	OCH_3	OCH_3	H	H	>100	3.008
9n	OH	H	OCH_3	H	H	>100	3.334
9o	OCH_3	H	H	H	H	>100	3.370

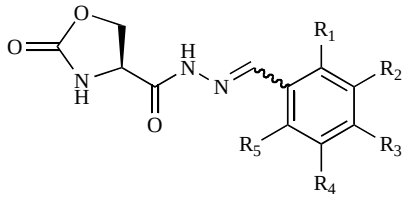
[a] Minimum inhibitory concentration
[b] Calculated using online www.molinspiration.com website

Cell Viability Assay

Cellular viability in the presence and absence of test compounds (**8c**, **8e**, **8h**, **8i**, **8k** and **10a**) was determined by Mosman's MTT (3-(4,5-dimethylthiazol-2yl)-2,5-dimethyl tetrazolium bromide; Merck) microcultured tetrazolium assay. The results were represented as percentage cell viability (Table **6**). This table shows that the compounds **8c**, **8e**, **8h** and **8i**, with 100% of cell viability, were not toxic to the host cells in the minimum concentration tested. These compounds were selected to be tested in macrophages infected with BCG (Table **7**).

This test is able to evaluate the action of these compounds over macrophages that exhibit a modified metabolism after infection. After this test the derivatives **8c**, **8e**, **8h** and **8i** were not cytotoxic, due to they did not kill more than 10% of the cells at the maximum concentration tested.

Table 5. The *in vitro* activity of compounds 10a-b and 11a against *M. tuberculosis* H37Rv strain (ATCC 27294, susceptible both to rifampin and isoniazid) and clog*P* calculations



Compound	Substituents					MIC[a] (μg/mL)	clog P[b]
	R_1	R_2	R_3	R_4	R_5		
10a	H	NO_2	H	H	H	**100**	**0.805**
10b	H	CN	H	H	H	**>100**	**0.601**
11a	H	NO_2	H	H	H	**>100**	**-0.447**

[a] Minimum inhibitory concentration
[b] Calculated using online www.molinspiration.com website

Table 6. Data of the cellular viability for a macrophage cell line J774 (ATCC TIB-67™) by Mosman's assay

Compound	10μg/mL	50μg/mL	100 μg/mL
8c	100%	100%	100%
8e	100%	100%	100%
8h	100%	100%	100%
8i	100%	100%	100%
8k	91%	91%	78%
10a	93%	93%	91%

Table 7. Data of the cellular viability for a macrophage cell line J774 (ATCC TIB-67™) infected with BCG by Mosman's assay

Compound	10μg/mL	50μg/mL	100 μg/mL
8c	100%	100%	100%
8e	100%	100%	96%
8h	100%	100%	100%
8i	100%	100%	100%

CONCLUSION

The synthesis of thirty-two compounds, including thirty benzyl/*t*-butyl (1*S*)-2-[2-(substituted-benzylidene)hydrazino] -1-(hydroxymethyl)-2-oxoethylcarbamate (**8a-o**, **9a-o**) and two (4S)-*N*'-(substituted-phenyl)methylidene)-2-oxo-1,3-oxazolidine-4-carbohydrazide (**10a-b**) was performed in good yields (50-89%). Also, 30 new compounds (**8b-c**, **8e-o**, **9a-o** and **10a-b**) were identified.

All of these compounds were submitted to antimycobacterial activity evaluation. The compounds **8c**, **8e**, **8h**, **8i**, **8k** and **10a** exhibited activity between 25 and 100μg/mL, being compared to the tuberculostatic drug D-cicloserine (5-20μg/mL). After cell viability evaluation, it was found that **8c**, **8e**, **8h** and **8i** were not cytotoxic in non infected or infected macrophages with *M. bovis* Bacillus Calmette-Guerin (BCG). Further x-ray crystallography studies are under way in our laboratory.

MATERIALS AND METHODS

General Procedures. Melting points were determined on a Buchi apparatus and are uncorrected. Infrared spectra were recorded on a Thermo Nicolet Nexus 670 spectrometer as potassium bromide pellets and frequencies are expressed in cm^{-1}. Mass spectra (ESI assay in solution of ammonium chloride) were recorded on Micromass ZQ Waters mass spectrometer. NMR spectra were recorded on a Bruker Avance 400 operating at 400.00 MHz (^1H) and 100.0 MHz (^{13}C) and Bruker Avance 500 spectrometer operating at 500.00 MHz (^1H) and 125.0 MHz (^{13}C), in deuterated dimethylsulfoxide. Chemical shifts are reported in ppm (δ) relative to tetramethylsilane and *J*-coupling in Hertz (Hz). Proton and carbon spectra were typically obtained at room temperature.

Synthesis of methyl (2*S*)-2-amino-3-hydroxypropanoate hydrochloride **3**

To a stirred solution of thionyl chloride (69.5 mL, 0.95 mol) in methanol (400mL) at 0^0C was added L-serine (20g, 0.19 mol), and the reaction mixture was stirred for 24 h at room temperature. The reaction mixture was concentrated under reduced pressure to give **3** (147.2g) in quantitative yield.

Synthesis of methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-hydroxypropanoate **4**

To a reaction mixture containing (2*S*)-2-amino-3-hydroxypropanoate hydrochloride **3** (13.2 g, 0.11 mol), water (100mL), diethyl ether (75 mL) and sodium bicarbonate (48 g, 0.55 mol) at 0°C was added dropwise benzyl chloroformate (21 mL, 0.15 mol). After 2 h at 0°C and 1 h at room

temperature, the reaction mixture was quenched with pyridine (20mL), and water was added (30 mL). The organic layer was washed with HCl (2.5 N, 20mL), dried over anhydrous sodium sulfate and concentrated under reduced pressure. The residue was chromatographed using a gradient 10 to 30% ethylacetate in hexane, affording **4** as colorless oil in 80% yield.

Synthesis of methyl (2*S*)-2-[(*tert*-butoxycarbonyl)amino] -3-hydroxypropanoate **5**

To a reaction mixture containing (2*S*)-2-amino-3-hydroxypropanoate hydrochloride **3** (3 g, 19.3 mmol) in anhydrous THF (40 mL) at room temperature was added triethylamine (3.2 mL, 23.2 mmol) and $(BOC)_2O$ (6.32 g, 29 mmol). The reaction mixture was stirred for 24 hours at room temperature, quenched with water (40 mL) and extracted with ethyl acetate. The combined organic phases were dried over anhydrous sodium sulfate and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel using a gradient 0 to 50% ethylacetate in hexane, affording **5** as a colorless oil in 85% yield.

Synthesis of benzyl (1S)-2-hydrazino-1-(hydroxymethyl) -2-oxoethylcarbamate **6**

To a stirred solution of **4** (0.3 g, 1.17 mmol) in ethanol (10 mL) was added $N_2H_4{\cdot}H_2O$ (80%, 5.5 mmol), and the reaction mixture was stirred for 24 hours at room temperature. The reaction mixture was concentrated under reduced pressure, and the residue was purified by washing with cold ethanol (3 x 10 mL), leading the pure derivative **6** in 78% yield.

Synthesis of *tert*-butyl (1*S*)-2-hydrazino-1-(hydroxymethyl)-2-oxoethylcarbamate **7**

To a stirred solution of **5** (0.3 g, 1.37 mmol) in ethanol (10 mL) at room temperature was added $N_2H_4{\cdot}H_2O$ (80%, 5.5 mmol). The reaction mixture was stirred for 24 hours at room temperature and concentrated under reduced pressure. The residue was column chromatographed on silica gel using a gradient 0 to 5% metanol in chloroform, affording **7** as a white solid in 70% yield.

General procedure for the synthesis of benzyl (1*S*)-2-[2-(substituted-benzylidene)hydrazino]-1-(hydroxymethyl)-2-oxoethylcarbamate (**8a-o**) and *tert*-butyl (1*S*)-2-[2-(substituted-benzylidene)hydrazino]-1-(hydroxymethyl)-2-oxoethylcarbamate (**9a-o**)

To a stirred solution of **6** or **7** (1.0 mmol) in ethanol (10 mL) at room temperature was added the appropriate benzaldehyde (1.05 mmol), and the reaction mixture was stirred for 4 hours at 80^0C. The reaction mixture was concentrated under reduced pressure, and the residue was purified by washing with cold ethanol (3 x 10 mL), affording the derivatives **8a-o** or **9a-o** in 70-90% yield.

General procedure for the synthesis of (4S)-*N*'-[(substituted-phenyl)methylidene]-2-oxo-1,3-oxazolidine-4-carbohydrazide **10a** and **10b**.

To a stirred solution of **8c** or **8e** (1.0 mmol) in THF (10 mL) at room temperature was added sodium hydride (60% dispersion in mineral oil; 5mmol), and the reaction mixture was stirred for 20 minutes at room temperature. The reaction mixture was concentrated, CH_2Cl_2 was added, washed with aqueous saturated NH_4Cl solution, brine and dried over Na_2SO_4. The organic layer was concentrated and the residue was purified by column chromatography on silica gel using a gradient 0 to 1% methanol in dichloromethane, affording the derivatives **8a-o** or **9a-o** in 70-90% yield.

Analytical data for compounds **3**, **4**, **5**, **6**, **7**, **8a-o, 9a-o** and **10a-b**

Methyl (2*S*)-2-amino-3-hydroxypropanoate hydrochloride **3**

Yield: 99%. m.p. 163.1^0C. ^{1}H-NMR (400MHz, DMSO-d_6) δ (ppm): 8.60 (3H; s; NH_3^+); 5.64 (1H; s; OH); 4.08 (1H; t; *J* = 3.6; CH); 3.82 (2H; m; CH_2); 3.73 (3H; s; CH_3). ^{13}C-NMR (100MHz, DMSO-d_6) δ (ppm): 168.5; 59.5; 54.4; 52.8. IR (cm^{-1}; KBr) ν_{max} 3362; 1743. MS/ESI (m/z $[M+H-HCl]^+$): 120.0

Methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-hydroxypropanoate **4**

Yield: 93%. ^{1}H-NMR (400MHz, $CDCl_3$) δ (ppm): 7.36 (5H; m; Ph); 5.75 (1H; d; *J*= 6.3; NH); 5.12 (2H; s; CH_2Ph); 4.45 (1H; m; CH); 4.00 (1H; dd; *J* = 11.2 and *J* = 3.2; CH'OH); 3.91 (1H; dd; *J* = 11.2 and *J* = 3.0; CHOH); 3.78 (3H; s; CH_3). ^{13}C-NMR (100MHz, $CDCl_3$) δ (ppm): 171.8; 157.0; 136.7; 129.2; 128.9; 128.7; 67.8; 63.7; 56.7; 53.3. IR (cm^{-1}, KBr) ν_{max} 3310; 1722; 1689. MS/ESI (m/z $[M-H]^-$): 252.2

Methyl (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-hydroxypropanoate **5**

Yield: 85%. ^{1}H-NMR (500MHz, $CDCl_3$) δ (ppm): 5.61 (1H; d; *J*= 6.8; NH); 4.38 (1H; m; CH); 3.96 (1H; dd; *J* = 11.2 and *J* = 2.9, CH'OH); 3.87 (1H; dd; *J* = 11.2 and *J* = 3.4; CHOH); 3.78 (3H; s; CH_3); 1.45 (9H; s; $(CH_3)_3$C). ^{13}C-NMR (125MHz, $CDCl_3$) δ (ppm): 171.5; 155.8; 80.3; 63.3; 55.7; 52.6; 28.3. IR (cm^{-1}; KBr) ν_{max} 3300; 1746; 1694. MS/ESI (m/z $[M+Na]^+$): 242.0.

Benzyl (1S)-2-hydrazino-1-(hydroxymethyl)-2-oxoethylcarbamate **6**

Yield: 78%. m.p. 176^0C. ^{1}H-NMR (500MHz, DMSO-d_6) δ (ppm): 9.10 (1H; s; $NHNH_2$); 7.32 (5H; m; $PhCH_2$); 7.15 (1H; d; *J*= 8.2; NHCH); 5.02 (2H; s; CH_2Ph); 4.85 (1H; t; *J*= 5.6; OH); 4.20 (2H; s; $NHNH_2$); 4.01 (1H; m; CH); 3.60-3.40 (2H; m; CH_2OH). ^{13}C-NMR (125MHz, DMSO-d_6) δ (ppm): 169.4; 155.8; 137.0; 128.3; 127.8; 127.7; 65.5; 61.7; 56.0. IR (cm^{-1}; KBr) ν_{max} 3286; 1692; 1653. MS/ESI (m/z $[M+Na]^+$): 276.0

Tert-butyl (1*S*)-2-hydrazino-1-(hydroxymethyl)-2-oxoethylcarbamate **7**

Yield: 70%. m.p. 130^0C. ^{1}H-NMR (500MHz, DMSO-d_6) δ (ppm): 9.02 (1H; s; $NHNH_2$); 6.58 (1H; d; *J*= 8.2; NHCH); 4.81 (1H; t; *J*= 5.6; OH); 4.19 (2H; s; $NHNH_2$); 3.93 (1H; m; CH); 3.60-3.40 (2H; m; CH_2OH); 1.37 (9H; s; $(CH_3)_3$C). ^{13}C-NMR (125MHz, DMSO-d_6) δ (ppm): 169.7; 155.1; 78.1; 61.9; 55.5; 28.2. IR (cm^{-1}; KBr) ν_{max} 3281; 1699; 1668. MS/ESI (m/z $[M-H]^-$): 218.1

Benzyl(1*S*)-2-[(2*E/Z*)-2-benzylidenehydrazino]-1-(hydroxymethyl)-2-oxoethylcarbamate **8a**

Yield: 85%. m.p. 157^{0}C. ^{1}H-NMR (400MHz, DMSO-d_6) δ (ppm): 11.50 and 11.42 (1H; s; NHN; (*E/Z*)-isomer); 8.25 and 7.99 (1H; s; N=CH; (*E/Z*)-isomer); 7.68 (2H; m; H2 and H6); 7.50-7.20 (9H; m; NHCH, PhCH$_2$, H3, H4 and H5); 5.05 and 5.04 (2H; s; CH$_2$Ph; (*E/Z*)-isomer); 5.03 (m) and 4.86 (t; *J*= 5.7), (1H; OH; (*E/Z*)-isomer); 5.03 and 4.15 (1H; m; CH; (*E/Z*)-isomer); 3.80-3.60 (2H; m; CH$_2$OH). ^{13}C-NMR (100MHz, DMSO-d_6) δ (ppm): 171.3; 166.9; 155.9; 147.0; 143.3; 137.0; 136.9; 134.2; 134.1; 130.0; 129.8; 128.8, 128.3; 127.8; 127.7; 127.0; 126.8; 65.5; 65.4; 61.5; 61.1; 56.4; 54.6. IR (cm^{-1}; KBr) ν_{max} 3387; 1678. MS/ESI: [M-H]: 340.3

Benzyl (1*S*)-1-(hydroxymethyl)-2-[(2*E/Z*)-2-(2-nitrobenzylidene)hydrazino]-2-oxoethylcarbamate **8b**

Yield: 80%. m.p. 155^{0}C. ^{1}H-NMR (500MHz, DMSO-d_6) δ (ppm): 11.88 and 11.71 (1H; s; NHN; (*E/Z*)-isomer); 8.66 and 8.39 (1H; s; N=CH; (*E/Z*)-isomer); 8.07 (2H; m; H3 and H6); 7.80 (1H; m; H5); 7.67 (1H; m; H4); 7.44 (d, *J*= 7.8) and 7.34 (m), (1H; NHCH; (*E/Z*)-isomer); 7.38-7.30 (5H; m; Ph); 5.05 and 5.04 (2H; s; CH$_2$Ph; (*E/Z*)-isomer); 5.03 (m) and 4.89 (t; *J*= 5.9), (1H; OH; (*E/Z*)-isomer); 5.03 and 4.15 (1H; m; CH; (*E/Z*)-isomer); 3.80-3.60 (2H; m; CH$_2$OH). ^{13}C-NMR (125MHz, DMSO-d_6) δ (ppm): 171.7; 167.4; 156.0; 148.2; 148.1; 142.4; 138.8; 137.0; 136.9; 133.8; 133.6; 130.6; 130.5; 128.7; 128.4; 128.0; 127.8; 127.7; 124.7; 124.6; 65.6; 65.4; 61.4; 61.1; 56.5; 54.4. IR (cm^{-1}; KBr) ν_{max} 3392; 1694; 1672; 1555; 1342. MS/ESI: [M-H]: 385.3

Benzyl (1*S*)-1-(hydroxymethyl)-2-[(2*E/Z*)-2-(3-nitrobenzylidene)hydrazino]-2-oxoethylcarbamate **8c**

Yield: 80%. m.p. 175^{0}C. ^{1}H-NMR (500MHz, DMSO-d_6) δ (ppm): 11.79 and 11.67 (1H; s; NHN; (*E/Z*)-isomer); 8.52 and 8.49 (1H; s; H2; (*E/Z*)-isomer); 8.37 and 8.12 (1H; s; N=CH; (*E/Z*)-isomer); 8.25 (1H; m; H4 or H6); 8.16 (d; *J*= 7.4) and 8.13 (m), (1H, H4 or H6; (*E/Z*)-isomer); 7.75 (1H; m; H5); 7.45 (d; *J*= 7.3) and 7.36 (m), (1H; NHCH; (*E/Z*)-isomer); 7.38-7.30 (5H; m; Ph); 5.05 (2H; s; CH$_2$Ph); 5.05 (m) and 4.91 (t; *J*= 5.9), (1H; OH; (*E/Z*)-isomer); 5.05 and 4.17 (1H; m; CH; (*E/Z*)-isomer); 3.80-3.60 (2H; m; CH$_2$OH). ^{13}C-NMR (125MHz, DMSO-d_6) δ (ppm): 171.7; 167.4; 155.9; 148.3; 148.2; 144.6; 141.2; 137.1; 136.9; 136.2; 136.0; 133.3; 132.8; 130.4; 128.4; 128.3; 127.8; 127.7; 124.2; 124.1; 121.2; 121.0; 65.6; 65.4; 61.5; 61.1; 56.4; 54.4. IR (cm^{-1}; KBr) ν_{max} 3392; 1694; 1674; 1553; 1342. MS/ESI: [M-H]: 385.3

Benzyl (1*S*)-1-(hydroxymethyl)-2-[(2*E/Z*)-2-(4-nitrobenzylidene)hydrazino]-2-oxoethylcarbamate **8d**

Yield: 82%. m.p. 190^{0}C. ^{1}H-NMR (500MHz, DMSO-d_6) δ (ppm): 11.87 and 11.76 (1H; s; NHN; (*E/Z*)-isomer); 8.34-8.09 (3H; m; N=CH, H3 and H5); 7.98-7.95 (2H; m; H2 and H6); 7.50 (d; *J*= 7.7) and 7.36 (m), (1H; NHCH; (*E/Z*)-isomer); 7.40-7.30 (5H; m; Ph); 5.05 (2H; s; CH$_2$Ph); 5.04 (m) and 4.93 (t; *J*= 6.3), (1H; OH; (*E/Z*)-isomer); 5.04 and 4.16 (1H; m; CH; (*E/Z*)-isomer); 3.80-3.60 (2H; m; CH$_2$OH). ^{13}C-NMR (125MHz, DMSO-d_6) δ (ppm): 171.8; 167.4; 155.9; 155.8; 147.7; 147.6; 144.4; 140.9; 140.5; 140.3; 136.9; 136.8; 128.2; 127.9; 127.7; 127.6; 124.0; 65.5; 65.3; 61.3; 61.0; 56.3; 54.4. IR (cm^{-1}; KBr) ν_{max} 1694; 1680; 1553; 1348. MS/ESI: [M-H]: 385.2

Benzyl (1*S*)-2-[(2*E/Z*)-2-(3-cyanobenzylidene)hydrazino]-1-(hydroxymethyl)-2-oxoethylcarbamate **8e**

Yield: 82%. m.p. 155^{0}C. ^{1}H-NMR (400MHz, DMSO-d_6) δ (ppm): 11.74 and 11.60 (1H; s; NHN; (*E/Z*)-isomer); 8.30-8.00 (3H; m; N=CH, H2 and H6); 7.87 (1H; m; H4 or H5); 7.63 (1H; m; H4 or H5); 7.42 (d; *J*= 7.8) and 7.40-7.25 (m), (1H; NHCH; (*E/Z*)-isomer); 7.40-7.25 (5H; m; Ph); 5.04 (2H; s; CH$_2$Ph); 5.04 (m) and 4.90 (t; *J*= 6.1), (1H; OH; (*E/Z*)-isomer); 5.04 and 4.15 (1H; m; CH; (*E/Z*)-isomer); 3.80-3.55 (2H; m; CH$_2$OH). ^{13}C-NMR (100MHz, DMSO-d_6) δ (ppm): 171.7; 167.4; 156.0; 144.7; 141.1; 137.1; 136.9; 135.6; 135.5; 133.2; 133.0; 131.3; 130.9; 130.8; 130.2; 130.1; 128.4; 128.3; 127.9; 127.8; 127.7; 118.5; 118.4; 112.0; 65.6; 65.4; 61.5; 61.1; 56.4; 54.5. IR (cm^{-1}; KBr) ν_{max} 3291; 2232; 1676. MS/ESI: [M-H]: 365.3

Benzyl (1*S*)-2-[(2*E/Z*)-2-(4-cyanobenzylidene)hydrazino]-1-(hydroxymethyl)-2-oxoethylcarbamate **8f**

Yield: 80%. m.p. 110^{0}C. ^{1}H-NMR (500MHz, DMSO-d_6) δ (ppm): 11.81 and 11.70 (1H; s; NHN; (*E/Z*)-isomer); 8.30 and 8.03 (1H; s; N=CH; (*E/Z*)-isomer); 7.93-7.85 (4H; m; H2, H3, H5 and H6); 7.48 (d, *J*= 7.8) and 7.40-7.30 (m), (1H; NHCH; (*E/Z*)-isomer); 7.40-7.30 (5H; m; Ph); 5.04 (2H; s; CH$_2$Ph); 5.04 (m) and 4.92 (dd; *J*=6.3 and *J*=5.4), (1H; OH; (*E/Z*)-isomer); 5.04 and 4.15 (1H; m; CH; (*E/Z*)-isomer); 3.80-3.50 (2H; m; CH$_2$OH). ^{13}C-NMR (125MHz, DMSO-d_6) δ (ppm): 171.8; 167.5; 156.0; 145.0; 141.4; 138.8; 138.6; 137.1; 137.0; 132.8, 128.4, 128.2, 127.9, 127.8, 127.6, 127.5; 126.8; 118.8; 118.7; 111.9; 111.7; 65.6; 65.4; 61.5; 61.1; 56.5; 54.6. IR (cm^{-1}; KBr) ν_{max} 3293; 2229; 1673. MS/ESI: [M-H]: 365.3

Benzyl (1*S*)-2-[(2*E*)-2-(2-hydroxybenzylidene)hydrazino]-1-(hydroxymethyl)-2-oxoethylcarbamate **8g**

Yield: 89%. m.p.: 160^{0}C. ^{1}H-NMR (500MHz, DMSO-d_6) δ (ppm): 11.79 (1H; s; NHN); 11.12 (1H; s; Ph−OH); 8.46 (1H; s; N=CH; (*E*)-isomer); 7.52 (1H; dd; *J*= 7.8 and *J*= 1.5; H6); 7.48 (1H; d; *J*= 7.8; NHCH); 7.40-7.20 (6H; m; Ph and (H4 or H5)); 6.93-6.84 (2H; m; H3 and (H4 or H5)); 5.05 (3H; m; CH$_2$Ph and OH); 4.13 (1H; m; CH); 3.80-3.60 (2H; m; CH$_2$OH). ^{13}C-NMR (125MHz, DMSO-d_6) δ (ppm): 171.6; 157.8; 156.4; 141.2; 137.4; 131.9, 131.6, 129.8, 128.8, 128.3; 128.2; 126.7; 119.9; 119.1; 116.6; 65.9; 61.5; 55.0. IR (cm^{-1}; KBr) ν_{max} 3312; 1681. MS/ESI: [M-H]: 356.3

Benzyl (1*S*)-2-[(2*E*)-2-(2,3-dihydroxybenzylidene)hydrazino]-1-(hydroxymethyl)-2-oxoethylcarbamate **8h**

Yield: 81%. m.p. 150^{0}C. ^{1}H-NMR (500MHz, DMSO-d_6) δ (ppm): 11.76 (1H; s; NHN); 10.95 (1H; s; C1−OH or C2−OH); 9.21 (1H; s; C1−OH or C2−OH); 8.41 (1H; s; N=CH; (*E*)-isomer); 7.44 (1H; d; *J*= 7.4; NHCH); 7.40-7.20 (5H; m; Ph); 6.94 (1H; d; *J*= 7.8; H6); 6.85-6.80 (1H; m; H4); 6.73 (1H; t; *J*= 7.8; H5); 5.04 (3H; m; CH$_2$Ph and OH); 4.15 (1H; m; CH); 3.75-3.55 (2H; m; CH$_2$OH). ^{13}C-NMR (125MHz, DMSO-d_6) δ (ppm): 171.1; 156.0; 145.6; 145.2; 141.6; 136.9; 128.4; 127.9; 127.8; 127.7; 120.0; 119.2; 117.4; 116.5; 65.4; 61.4; 56.3. IR (cm^{-1}; KBr) ν_{max} 3270; 1676. MS/ESI: [M-H]: 372.3

Benzyl (1*S*)-2-[(2*E*)-2-(2,4-dihydroxybenzylidene)hydrazino]-1-(hydroxymethyl)-2-oxoethylcarbamate **8i**

Yield: 85%. m.p. 166^{0}C. ^{1}H-NMR (500MHz, DMSO-d_6) δ (ppm): 11.60 (1H; s; NHN); 11.30 (1H; s; C1−OH or C3−OH); 10.00 (1H; s; C1−OH or C3−OH); 8.31 (1H; s; N=CH; (*E*)-isomer); 7.45 (1H; d; *J*= 7.8; H6); 7.40-7.30 (5H; m; Ph); 7.29 (1H; d; *J*= 8.5; NHCH); 6.37-6.27 (2H; m; H3 and H5); 5.05 (3H; m; CH_2Ph and OH); 4.10 (1H; m; CH); 3.75-3.55 (2H; m; CH_2OH). ^{13}C-NMR (125MHz, DMSO-d_6) δ (ppm): 171.1; 161.1; 158.4; 156.4; 142.2; 137.4; 131.7, 128.8, 128.4, 128.3; 128.2; 128.1; 110.9; 108.1; 103.0; 65.8; 61.4; 54.9. IR (cm^{-1}; KBr) ν_{max} 3412; 3312; 1677. MS/ESI: [M-H]: 372.3

Benzyl (1*S*)-2-[(2*E*)-2-(2,5-dihydroxybenzylidene)hydrazino]-1-(hydroxymethyl)-2-oxoethylcarbamate **8j**

Yield: 87%. m.p. 145^{0}C. ^{1}H-NMR (500MHz, DMSO-d_6) δ (ppm): 11.64 (1H; s; NHN); 10.23 (1H; s; C1−OH or C4−OH); 8.97 (1H; s; C1−OH or C4−OH); 8.37 (1H; s; N=CH; (*E*)-isomer); 7.40-7.30 (5H; m; Ph); 7.40 (1H; d; *J*= 8.3; NHCH); 6.93 (1H; d; *J*= 2.0; H6); 6.75-6.65 (2H; m; H3 and H4); 5.05 (2H; s; CH_2Ph); 5.10-5.00 (2H; m; OH and CH); 3.75-3.55 (2H; m; CH_2OH). ^{13}C-NMR (125MHz, DMSO-d_6) δ (ppm): 171.0; 155.9; 150.0; 149.4; 141.0; 136.9; 128.4; 127.9; 127.8; 127.7; 120.4; 118.9; 117.0; 113.7; 111.1; 65.4; 61.1; 54.4. IR (cm^{-1}; KBr) ν_{max} 3327; 1664. MS/ESI: [M-H]: 372.3

Benzyl(1*S*)-2-[(2*E*)-2-(2-hydroxy-3-methoxybenzylidene)hydrazino]-1-(hydroxymethyl)-2-oxoethylcarbamate **8k**

Yield: 80%. m.p. 140^{0}C. ^{1}H-NMR (500MHz, DMSO-d_6) δ (ppm): 11.72 (1H; s; NHN); 10.80 (1H; s; C1−OH); 8.46 (1H; s; N=CH; (*E*)-isomer); 7.42 (1H; d, *J*= 7.8; NHCH); 7.40-7.30 (5H; m; Ph); 7.10 (1H; dd; *J*= 8.0 and *J*= 1.1; H6); 7.02 (1H; d; *J*= 8.0; H4); 6.85 (1H; t; *J*= 8.0; H5); 5.04 (2H; s; CH_2Ph); 5.10-4.95 (1H; m; OH); 4.13 (1H; m; CH); 3.80 (3H; s; CH_3); 3.70-3.60 (2H; m; CH_2OH). ^{13}C-NMR (125MHz, DMSO-d_6) δ (ppm): 171.1; 156.0; 147.9; 145.9; 140.7; 137.0; 128.4; 127.8; 127.7; 127.6; 120.7; 119.2; 118.8; 113.8; 65.4; 61.0; 55.9; 54.5. IR (cm^{-1}; KBr) ν_{max} 3350; 1681. MS/ESI: [M-H]: 386.4

Benzyl (1*S*)-2-[(2*E*)-2-(3,4-dihydroxybenzylidene)hydrazino]-1-(hydroxymethyl)-2-oxoethylcarbamate **8l**

Yield: 80%. m.p. 105^{0}C. ^{1}H-NMR (500MHz, DMSO-d_6) δ (ppm): 11.24 (1H; s; NHN); 9.30 (2H; s; C2−OH and C3−OH); 8.04 (1H; s; N=CH; (*E*)-isomer); 7.40-7.30 (6H; m; Ph and NHCH); 7.18 (1H; d; *J*= 1.6; H2); 6.89 (1H; m; H6); 6.78 (1H; s; H5); 5.04 (2H; s; CH_2Ph); 5.10-4.95 (1H; m; OH); 4.10 (1H; m; CH); 3.75-3.55 (2H; m; CH_2OH). ^{13}C-NMR (125MHz, DMSO-d_6) δ (ppm): 171.3; 156.4; 148.4; 147.9, 146.2; 137.4; 128.8; 128.3; 128.2; 127.5; 126.1; 126.0; 121.0; 116.0; 113.1; 66.0; 62.1; 56.8. IR (cm^{-1}; KBr) ν_{max} 3382; 1686. MS/ESI: [M-H]: 372.3

Benzyl (1*S*)-2-[(2*E*)-2-(3,4-dimethoxybenzylidene)hydrazino]-1-(hydroxymethyl)-2-oxoethylcarbamate **8m**

Yield: 83%. m.p. 150^{0}C. ^{1}H-NMR (500MHz, DMSO-d_6) δ (ppm): 11.38 (1H; s; NHN); 8.16 (1H; s; N=CH; (*E*)-isomer); 7.40-7.25 (7H; m; Ph, NHCH and H2); 7.17 (1H; m; H6); 7.01 (1H; m; H5); 5.04 (2H; s; CH_2Ph); 5.05-4.95 (2H; m; OH and CH); 3.80 (6H; s, C2−OCH_3 and C3−OCH_3); 3.80-3.55 (2H; m; CH_2OH). ^{13}C-NMR (125MHz, DMSO-d_6) δ (ppm): 171.1; 155.9; 150.5; 149.0; 143.3; 137.1; 128.4, 128.3, 127.8, 127.7; 127.0; 126.9; 121.0; 111.5; 108.7; 65.4; 61.0; 55.6; 54.5. IR (cm^{-1}; KBr) ν_{max} 3291; 3203; 1728; 1666. MS/ESI: [M-H]: 400.3

Benzyl (1*S*)-2-[(2*E*)-2-(2-hydroxy-4-methoxybenzylidene)hydrazino]-1-(hydroxymethyl)-2-oxoethylcarbamate **8n**

Yield: 80%. m.p. 162^{0}C. ^{1}H-NMR (500MHz, DMSO-d_6) δ (ppm): 11.68 (1H; s; NHN); 11.45 (1H; s; C1−OH); 8.36 (1H; s; N=CH; (*E*)-isomer); 7.47 (1H; d; *J*= 7.8; H6); 7.40 (1H; d; *J*= 8.4; NHCH); 7.38-7.25 (5H; m; Ph); 6.55-6.40 (2H; m; H3 and H5); 5.04 (2H; s; CH_2Ph); 5.10-5.00 (1H; m; OH); 4.11 (1H; m; CH); 3.76 (3H; s; CH_3); 3.70-3.55 (2H; m; CH_2OH). ^{13}C-NMR (125MHz, DMSO-d_6) δ (ppm): 171.2; 162.5; 158.3; 156.4; 141.7; 137.4; 131.5, 128.8, 128.7, 128.3, 128.2; 127.6; 112.1; 106.9; 101.6; 66.0; 61.9; 55.8; 54.9. IR (cm^{-1}; KBr) ν_{max} 3329; 1678. MS/ESI: [M-H]: 386.3

Benzyl (1*S*)-1-(hydroxymethyl)-2-[(2*E*)-2-(2-methoxybenzylidene)hydrazino]-2-oxoethylcarbamate **8o**

Yield: 80%. m.p. 180^{0}C. ^{1}H-NMR (400MHz, DMSO-d_6) δ (ppm): 11.48 (1H; s; NHN); 8.59 (1H; s; N=CH; (*E*)-isomer); 7.83 (1H; d; J= 7.1; H5); 7.45-7.25 (6H; m; Ph e H3); 7.45-7.25 (1H; m; NHCH); 7.10 (1H; s; H2); 7.00 (1H; m; H4); 5.05 (2H; s; CH_2Ph); 5.01 (1H; m; CH); 4.97 (1H; t; *J*= 5.8; OH); 3.86 (3H; s; CH_3); 3.80-3.55 (2H; m; CH_2OH). ^{13}C-NMR (100MHz, DMSO- d_6) δ (ppm): 171.2; 157.7; 155.9; 142.5; 137.0; 131.5;131.3; 128.3; 127.7; 127.6; 125.4; 122.1; 120.7; 111.8; 65.5; 61.5; 56.4; 55.7. IR (cm-1; KBr) ν_{max} 3262; 1692. MS/ESI: [M+Na]: 370.2.

Tert-butyl (1*S*)-2-[(2*E*/*Z*)-2-benzylidenehydrazino]-1-(hydroxymethyl)-2- oxoethylcarbamate **9a**

Yield: 82%. m.p. 165^{0}C. ^{1}H-NMR (400MHz, DMSO-d_6) δ (ppm): 11.44 and 11.35 (1H; s; NHN; (*E*/*Z*)-isomer); 8.24 and 7.98 (1H; s; N=CH; (*E*/*Z*)-isomer); 7.68 (2H; m; H2 and H6); 7.42 (3H; m; H3, H4 and H5); 6.78 (d; *J*= 7.5) and 6.63 (d; *J*= 8.2), (1H; NHCH; (*E*/*Z*)-isomer); 4.93 (m) and 4.80 (t; *J*= 5.8), (1H; OH; (*E*/*Z*)-isomer); 4.93 and 4.05 (1H; m; CH; (*E*/*Z*)-isomer); 3.80-3.55 (2H; m; CH_2OH); 1,38 (9H; s; $(CH_3)_3$C). ^{13}C-NMR (100MHz, DMSO-d_6) δ (ppm): 171.6; 167.2; 155.2; 146.7; 143.1; 134.2; 134.1; 130.0; 129.8; 128.8; 127.0; 126.8; 78.2; 78.0; 61.6; 61.2; 56.0; 54.1; 28.2.

IR (cm^{-1}; KBr) ν_{max} 3370; 1684. MS/ESI: [M-H]: 306.3

Tert-butyl (1*S*)-1-(hydroxymethyl)-2-[(2*E*/*Z*)-2-(2-nitrobenzylidene)hydrazino]-2-oxoethylcarbamate **9b**

Yield: 85%. m.p. 155^{0}C. ^{1}H-NMR (500MHz, DMSO-d_6) δ (ppm): 11.82 and 11.65 (1H; s; NHN; (*E*/*Z*)-isomer); 8.65 and 8.38 (1H; s; N=CH; (*E*/*Z*)-isomer); 8.06 (2H; m; H3 and H6); 7.80 (1H; m; H5); 7.66 (1H; m; H4); 6.85 (d; *J*= 7.6) and 6.70 (d; *J*= 8.4), (1H; NHCH; (*E*/*Z*)-isomer); 4.97 (t; *J*= 5.5) and 4.82 (t; *J*= 6.0), (1H; OH; (*E*/*Z*)-isomer); 4.91 and 4.04 (1H; m; CH; (*E*/*Z*)-isomer); 3.75-3.55 (2H; m; CH_2OH); 1.39 and 1.38 (9H; s; $(CH_3)_3$C; (*E*/*Z*)-isomer). ^{13}C-NMR (125MHz, DMSO-d_6) δ (ppm): 172.0; 167.7; 155.3; 155.2; 148.2; 148.0; 142.1; 138.6; 133.7; 133.6; 130.6; 130.4; 128.7; 128,0; 127.9; 124.7; 124.6; 78.3; 78.1; 61.5; 61.2;

56.1; 54.0; 28.2. IR (cm^{-1}; KBr) ν_{max} 3238; 1705; 1524; 1363. MS/ESI: [M-H]: 351.3

Tert-butyl (1*S*)-1-(hydroxymethyl)-2-[(2*E/Z*)-2-(3-nitrobenzylidene)hydrazino]-2-oxoethylcarbamate **9c**

Yield: 78%. m.p. 172^{0}C. ^{1}H-NMR (500MHz, DMSO-d_6) δ (ppm): 11.72 and 11.59 (1H; s; NHN; (*E/Z*)-isomer); 8.52 and 8.47 (1H; s; H2; (*E/Z*)-isomer); 8.36 and 8.11 (1H; s; N=CH; (*E/Z*)-isomer); 8.24 (1H; m; H4 or H6); 8.16 (d; *J*= 7.8) and 8.12 (m), (1H; H4 or H6; (*E/Z*)-isomer); 7.73 (1H; m; H5); 6.85 (d; *J*= 7.4) and 6.72 (d; *J*= 8.8), (1H; N<u>H</u>CH; (*E/Z*)-isomer); 4.97 (m) and 4.83 (t; *J*= 5.9), (1H; m; OH; (*E/Z*)-isomer); 4.97 and 4.07 (1H; m; CH; (*E/Z*)-isomer); 3.75-3.55 (2H; m; C<u>H</u>$_2$OH); 1.39 and 1.38 (9H; s; $(CH_3)_3C$; (*E/Z*)-isomer). ^{13}C-NMR (125MHz, DMSO-d_6) δ (ppm): 172.0; 167.7; 155.3; 155.2; 148.3; 148.2; 144.3; 141.0; 136.2; 136.0; 133.2; 132.8; 130.4, 124.2, 124.1; 121.1; 121.0; 78.3; 78.1; 61.6; 61.2; 56.0; 54.0; 28.2. IR (cm^{-1}; KBr) ν_{max} 3374; 1682; 1558; 1350. MS/ESI: [M-H]: 351.2

Tert-butyl (1*S*)-1-(hydroxymethyl)-2-[(2*E/Z*)-2-(4-nitrobenzylidene)hydrazino]-2-oxoethylcarbamate **9d**

Yield: 80%. m.p. 150^{0}C. ^{1}H-NMR (500MHz, DMSO-d_6) δ (ppm): 11.76 and 11.65 (1H; s; NHN; (*E/Z*)-isomer); 8.35 and 8.09 (1H; s; N=CH; (*E/Z*)-isomer); 8.27 (2H; m; H3 and H5); 7.95 (2H; m; H2 and H6); 6.84 (d; *J*= 7.3) and 6.72 (d; *J*= 8.0), (1H; N<u>H</u>CH; (*E/Z*)-isomer); 4.98 (m) and 4.83 (t; *J*= 6.0), (1H; OH; (*E/Z*)-isomer); 4.98 and 4.07 (1H; m; CH; (*E/Z*)-isomer); 3.75-3.55 (2H; m; C<u>H</u>$_2$OH), 1.38 (9H; s; $(CH_3)_3C$). ^{13}C-NMR (125MHz, DMSO-d_6) δ (ppm): 172.0; 167.7; 155.2; 147.8; 147.7; 144.3; 140.8; 140.6; 140.5; 127.9; 127.7; 124.0; 78.3; 78.1; 61.5; 61.2; 56.0; 54.0; 28.1. IR (cm^{-1}; KBr) ν_{max} 3370; 1684; 1562; 1344. MS/ESI: [M-H]: 351.3

Tert-butyl (1*S*)-2-[(2*E/Z*)-2-(3-cyanobenzylidene)hydrazino]-1-(hydroxymethyl)-2-oxoethylcarbamate **9e**

Yield: 78%. m.p. 167^{0}C. ^{1}H-NMR (400MHz, DMSO-d_6) δ (ppm): 11.67 and 11.53 (1H; s; NHN; (*E/Z*)-isomer); 8.30-8.00 (3H; m; N=CH, H2 and H6); 7.86 (1H; m; H4); 7.64 (1H; m; H5); 6.82 (d; *J*= 7.6) and 6.74 (d; *J*= 8.3), (1H; N<u>H</u>CH; (*E/Z*)-isomer); 4.98 (m) and 4.81 (t; *J*= 6.1), (1H; OH; (*E/Z*)-isomer); 4.98 and 4.05 (1H; m; CH; (*E/Z*)-isomer); 3.75-3.55 (2H; m; C<u>H</u>$_2$OH); 1.38 (9H; s; $(CH_3)_3C$). ^{13}C-NMR (100MHz, DMSO-d_6) δ (ppm): 171.9; 167.6; 155.3; 155.2; 144.5; 140.9; 135.6; 135.5; 133.1; 132.9; 131.2; 130.9; 130.7, 130.1; 130.0; 118.4; 112.0; 78.3; 78.0; 61.6; 61.2; 56.0; 54.0; 28.2. IR (cm^{-1}; KBr) ν_{max} 3346; 1685; 2234. MS/ESI: [M-H]: 331.3

Tert-butyl (1*S*)-2-[(2*E/Z*)-2-(4-cyanobenzylidene)hydrazino]-1-(hydroxymethyl)-2-oxoethylcarbamate **9f**

Yield: 70%. m.p. 161^{0}C. ^{1}H-NMR (500MHz, DMSO-d_6) δ (ppm): 11.71 and 11.60 (1H; s; NHN; (*E/Z*)-isomer); 8.29 and 8.02 (1H; s; N=CH; (*E/Z*)-isomer); 7.90-7.80 (4H; m; H2, H3, H5 and H6); 6.84 (d; *J*= 7.4) and 6.73 (d; *J*= 8.3), (1H; N<u>H</u>CH; (*E/Z*)-isomer); 4.95 (m) and 4.82 (t; *J*= 6.4), (1H; OH; (*E/Z*)-isomer); 4.95 and 4.06 (1H; m; CH; (*E/Z*)-isomer); 3.70-3.55 (2H; m; C<u>H</u>$_2$OH); 1.38 (9H, s; $(CH_3)_3C$). ^{13}C-NMR (125MHz, DMSO-d_6) δ (ppm): 172.0; 167.7; 155.3; 155.2; 144.8; 141.2; 138.8; 138.6; 132.7; 127.6; 127.4; 118.7; 118.6; 111.8; 111.6; 78.3; 78.1; 61.5; 61.2; 56.0; 54.0; 28.2. IR (cm^{-1}; KBr) ν_{max} 3375; 1678; 2228. MS/ESI: [M-H]: 331.3

Tert-butyl (1*S*)-2-[(2*E*)-2-(2-hydroxybenzylidene)hydrazino]-1-(hydroxymethyl)-2-oxoethylcarbamate **9g**

Yield: 76%. m.p. 183^{0}C. ^{1}H-NMR (500MHz, DMSO-d_6) δ (ppm): 11.70 (1H; s; NHN); 11.13 (1H; s; Ph−O<u>H</u>); 8.44 (1H; s; N=CH, (*E*)-isomer); 7.50 (1H; d; *J*= 7.4; H6); 7.28 (1H; m; H4 or H5); 6.93-6.86 (2H; m; H3 and (H4 or H5)); 6.84 (1H; d; *J*= 7.8; N<u>H</u>CH); 4.99 (1H; s; OH); 4.05 (1H; m; CH); 3.70-3.55 (2H; m; C<u>H</u>$_2$OH); 1.39 (9H; s; $(CH_3)_3C$). ^{13}C-NMR (125MHz, DMSO-d_6) δ (ppm): 171.8; 157.8; 156.8; 141.2; 131.8; 129.8; 119.9; 119.1; 116.6; 78.8; 61.6; 56.4; 28.6. IR (cm^{-1}; KBr) ν_{max} 3366; 1692; 1678. MS/ESI: [M-H]: 322.3

Tert-butyl (1*S*)-2-[(2*E*)-2-(2,3-dihydroxybenzylidene) hydrazino]-1-(hydroxymethyl)-2-oxoethylcarbamate **9h**

Yield: 85%. m.p. 176^{0}C. ^{1}H-NMR (500MHz, DMSO-d_6) δ (ppm): 11.70 (1H; s; NHN); 10.96 (1H; s; C1−OH or C2−OH); 9.19 (1H; s; C1−OH or C2−OH); 8.40 (1H; s; N=CH; (*E*)-isomer); 6.93 (1H; d; *J*= 7.8; H6); 6.90-6.80 (1H; m; H4); 6.90-6.80 (1H; m; N<u>H</u>CH); 6.72 (1H; t; *J*= 7.8; H5); 4.99 (1H; s; OH); 4.05 (1H; m; CH); 3.70-3.55 (2H; m; C<u>H</u>$_2$OH); 1.39 (9H; s; $(CH_3)_3C$). ^{13}C-NMR (125MHz, DMSO-d_6) δ (ppm): 171.3; 155.2; 145.9; 145.5; 141.5; 120.7; 119.1; 117.3; 116.5; 78.1; 61.5; 53.9; 28.2. IR (cm^{-1}; KBr) ν_{max} 3350; 1680. MS/ESI: [M-H]: 338.3

Tert-butyl (1*S*)-2-[(2*E*)-2-(2,4-dihydroxybenzylidene) hydrazino]-1-(hydroxymethyl)-2-oxoethylcarbamate **9i**

Yield: 74%. m.p. 150^{0}C. ^{1}H-NMR (500MHz, DMSO-d_6) δ (ppm): 11.50 (1H; s; NHN); 11.30 (1H; s; C1−OH or C3−OH); 9.92 (1H; s; C1−OH ou C3−OH); 8.30 (1H; s; N=CH; (*E*)-isomer); 7.26 (1H; d; *J*= 8.4; H6); 6.80 (1H; d; *J*= 7.7; N<u>H</u>CH); 6.35-6.30 (1H; m; H5); 6.29 (1H; s; H3); 4.95 (1H; s; OH); 4.02 (1H; m; CH); 3.70-3.50 (2H; m; C<u>H</u>$_2$OH); 1.39 (9H; s; $(CH_3)_3C$). ^{13}C-NMR (125MHz, DMSO-d_6) δ (ppm): 170.8; 160.3; 157.9; 155.2; 141.8; 128.0; 110.4; 107.6; 102.3; 78.0; 61.1; 53.9; 28.2. IR (cm^{-1}; KBr) ν_{max} 3200; 1678. MS/ESI: [M-H]: 338.3

Tert-butyl (1*S*)-2-[(2*E*)-2-(2,5-dihydroxybenzylidene) hydrazino]-1-(hydroxymethyl)-2-oxoethylcarbamate **9j**

Yield: 79%. m.p. 148^{0}C. ^{1}H-NMR (500MHz, DMSO-d_6) δ (ppm): 11.58 (1H; s; NHN); 10.24 (1H; s; C1−OH or C4−OH); 8.95 (1H; s; C1−OH or C4−OH); 8.36 (1H; s; N=CH; (*E*)-isomer); 6.92 (1H; d; *J*= 2.0; H6); 6.81 (1H; d; *J*= 7.8; N<u>H</u>CH); 6.75-6.65 (2H; m; H3 and H4); 4.97 (1H; m; OH); 4.02 (1H; m; CH); 3.70-3.50 (2H; m; C<u>H</u>$_2$OH); 1.39 (9H; s; $(CH_3)_3C$). ^{13}C-NMR (125MHz, DMSO-d_6) δ (ppm): 171.7; 155.7; 150.4; 149.8; 141.2; 120.9; 119.1; 117.5; 111.5; 78.6; 61.7; 54.4; 28.6. IR (cm^{-1}; KBr) ν_{max} 3358; 1678. MS/ESI: [M-H]: 338.3

Tert-butyl (1*S*)-2-[(2*E*)-2-(2-hydroxy-3-methoxybenzylidene)hydrazino]-1-(hydroxymethyl)-2-oxoethylcarbamate **9k**

Yield: 80%. m.p.: 125^{0}C. ^{1}H-NMR (500MHz, DMSO-d_6) δ (ppm): 11.69 (1H; s; NHN); 10.81 (1H; s; C1−OH); 8.45 (1H; s; N=CH; (*E*)-isomer); 7.10 (1H; dd; *J*= 7.8 and *J*= 1.0; H6); 7.01 (1H; dd; *J*= 8.0 and *J*= 1.0; H4); 6.90-6.78 (2H; m; H5 and N<u>H</u>CH); 4.99 (1H; s; OH); 4.04 (1H; m; CH);

3.80 (3H; s; CH_3O); 3.70-3.55 (2H; m; $C\underline{H}_2OH$); 1.39 (9H; s; $(CH_3)_3C$). ^{13}C-NMR (125MHz, DMSO-d_6) δ (ppm): 171.3; 155.2; 147.9; 145.9; 140.5; 120.7; 119.0; 117.6, 112.8; 78.1; 61.2; 55.8; 54.0; 28.2.

IR (cm^{-1}; KBr) ν_{max} 3358; 1684. MS/ESI: [M-H]: 352.4

Tert-butyl (1*S*)-2-[(2*E*)-2-(3,4-dihydroxybenzylidene) hydrazino]-1-(hydroxymethyl)-2-oxoethylcarbamate **9l**

Yield: 85%. m.p. 138^{0}C. ^{1}H-NMR (500MHz, DMSO-d_6) δ (ppm): 11.20 (1H; s; NHN); 9.40 (1H; s; C2−OH or C3−OH); 9.26 (1H; s; C2−OH or C3−OH); 8.03 (1H; s; N=CH; (*E*)-isomer); 7.17 (1H; d; *J*= 1.5; H2); 6.90 (1H; s; H6); 6.77 (1H; s; H5); 6.73 (1H; d; *J*= 8.3; N$\underline{H}$CH); 4.95 (1H; m; OH); 4.01 (1H; m; CH); 3.70-3.50 (2H; m; $C\underline{H}_2OH$); 1.39 (9H; s; $(CH_3)_3C$). ^{13}C-NMR (125MHz, DMSO-d_6) δ (ppm): 171.6; 155.7; 148.2; 147.7; 144.3; 129.1; 120.5; 116.0; 113.0; 78.6; 62.2; 54.5; 28.6. IR (cm^{-1}; KBr) ν_{max} 3281; 1672. MS/ESI: [M-H]: 338.3

Tert-butyl (1*S*)-2-[(2*E*)-2-(3,4-dimethoxybenzylidene) hydrazino]-1-(hydroxymethyl)-2-oxoethylcarbamate **9m**

Yield: 82%. m.p. 120^{0}C. ^{1}H-NMR (500MHz, DMSO-d_6) δ (ppm): 11.32 (1H; s; NHN); 8.14 (1H; s; N=CH; (*E*)-isomer); 7.29 (1H; s; H2); 7.17 (1H; m; H6); 7.00 (1H; m; H5); 6.76 (1H; d; *J*= 7.3; N$\underline{H}$CH); 4.93 (1H; m; OH); 4.02 (1H; m; CH); 3.80 (6H; s; C2−OCH_3 and C3−OCH_3); 3.75-3.55 (2H; m; $C\underline{H}_2OH$); 1.39 (9H; s; $(CH_3)_3C$). ^{13}C-NMR (125MHz, DMSO-d_6) δ (ppm): 171.8; 155.7; 150.9; 149.5; 143.6; 127.4; 121.4; 111.9; 108.7; 78.5; 61.6; 56.0; 55.9; 54.6; 28.6. IR (cm^{-1}; KBr): 3227 (O-H); 1671 (COCH and COO). MS/ESI: [M-H]: 366.4

Tert-butyl (1*S*)-2-[(2*E*)-2-(2-hydroxy-4-methoxybenzylidene)hydrazino]-1-(hydroxymethyl)-2-oxoethylcarbamate **9n**

Yield: 84%. m.p. 182^{0}C. ^{1}H-NMR (500MHz, DMSO-d_6) δ (ppm): 11.60 (1H; s; NHN); 11.46 (1H; s; C1−OH); 8.35 (1H; s; N=CH; (*E*)-isomer); 7.39 (1H; d; *J*= 8.4; H6); 6.84 (1H; d; *J*= 7.4; N$\underline{H}$CH); 6.55-6.40 (2H; m; H3 and H5); 4.98 (1H; m; OH); 4.02 (1H; m; CH); 3.76 (3H; s; CH_3O); 3.70-3.50 (2H; m; $C\underline{H}_2OH$); 1.39 (9H; s; $(CH_3)_3C$). ^{13}C-NMR (125MHz, DMSO-d_6) δ (ppm): 171.5; 162.5; 158.3; 155.7; 141.8; 128.3; 112.1; 106.9; 101.4; 78.5; 61.6; 56.3; 55.6; 28.6. IR (cm^{-1}; KBr) ν_{max} 3375; 1677. MS/ESI: [M-H]: 352.4

Tert-butyl (1*S*)-1-(hydroxymethyl)-2-[(2*E*)-2-(2-methoxybenzylidene)hydrazino]-2-oxoethylcarbamate **9o**

Yield: 75%. m.p. 115^{0}C. ^{1}H-NMR (500MHz, DMSO-d6) δ (ppm): 11.46 (1H; s; NHN); 8.58 (1H; s; N=CH; (*E*)-isomer); 7.82 (1H; d; J= 7.8; H5); 7.39 (1H; m; H3); 7.10 (1H; s; H2); 7.00 (1H; m; H4); 6.75 (1H; d; J= 7.8; NHCH); 4.94 (1H; m; OH); 4.94 (1H; m; CH); 3.86 (3H; s; CH_3O); 3.75-3.55 (2H; m; CH_2OH); 1.39 (9H; s; (CH3)3C−). ^{13}C-NMR (125MHz, DMSO-d6) δ (ppm): 171.9; 158.2; 155.7; 142.8; 132.0; 125.9; 122.7; 121.2; 112.2; 78.7; 62.1; 56.5; 56.1; 28.6. IR (cm-1; KBr) ν_{max} 3321; 1670; . MS/ESI: [M-H]: 336.3

(4S)-*N*'-[(*E*)-(3-nitrophenyl)methylidene]-2-oxo-1,3-oxazolidine-4-carbohydrazide **10a**

Yield: 55%. m.p. 190^{0}C. ^{1}H-NMR (500MHz, DMSO-d_6) δ (ppm): 11.91 (1H; s; −NHN=); 8.47 (1H; m; H1); 8.12 (1H; s; −N=CH−; (*E*)-isomer); 8.25 (1H; m; H3 or H5); 8.16 (1H; m; H3 or H5); 8.04 (1H; s; NHCH); 7.75-7.65 (1H; m; H4); 5.11 (1H; ; dd; J= 12.0 and J= 6.4; CH); (1H; t; J= 12.0; CHH'); 4.35-4.25 (1H; m; CHH'). ^{13}C-NMR (125MHz, DMSO-d_6) δ (ppm): 171.6; 158.8; 148.2; 145.8; 135.8; 133.3; 130.5; 124.5; 121.6; 66.8; 53.3. IR (cm-1; KBr) ν_{max} 1767; 1689. MS/ESI: [M-H]: 277.3

(4S)-*N*'-[(*E*)- (3-cyanophenyl)methylidene]-2-oxo-1,3-oxazolidine-4-carbohydrazide **10b**

Yield: 50%. m.p. 195^{0}C. ^{1}H-NMR (500MHz, DMSO-d_6) δ (ppm): 11.84 (1H; s; NHN); 8,02 (1H; s; N=CH; (*E*)-isomer); 8.20-7.95 (3H; m; H1, H5 and NHCH); 7.86 (1H; d; *J*= 7.8; H3); 7.64 (1H; m; H4); 5.11 (1H; dd; *J*= 9.8 and *J*= 4.9; CH); 4.71 (1H; t; *J*= 9,8 CHH'); 4.28 (1H; m; CHH'). ^{13}C-NMR (100MHz, DMSO-d_6) δ (ppm): 171.7 159.0; 146.0; 135.3, 133.5, 131.6; 130.2; 118.5; 112.0; 66.9; 53.3. IR (cm^{-1}; KBr) ν_{max} 2227; 1746; 1694. MS/ESI: [M-H]: 257.3

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