Thiazolidine Esters: New Potent Urease Inhibitors

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Summary: A variety of esters of thiazolidine-4-caboxylic acid were synthesized and investigated for their urease inhibitory properties. A significant increase in urease inhibitory activities of these ester derivatives has been observed. The order of activity increases from methyl ester to heptyl ester but further prolongation of the alkyl chain was proved to be detrimental for receptor binding. These findings provide evidence that the nature of the alkyl chain has a significant impact on the coordination of thiazolidine esters with bi-metallic nickel center of urease. It was also observed that inhibition potentiated by lower pH and with increase in time.

Key words: Thiazolidine esters; Urease; *Bacillus pasteurii*; Jack bean.

Introduction

Urease enzyme is present throughout the plant and microbial kingdoms. Ureases activity has been reported to be a significant factor in the pathogenesis of various diseases, which is harmful for agriculture, as well as to animal and human health. Urease is mainly contributes in the on-set kidney stones disease and also involve in the pathogenesis of ammonia, pyelonephritis, urolithiasis, coma of hepatocytes, hepatic encephalopathy, and urinary tube diseases [1]. In agriculture, urease release high amount of ammonia into the atmosphere and causes significant problems to the environment and to the economy of the country. This further increases plant destruction initially by reducing the availability of essential nutrient to them and secondarily by toxicity of ammonia, which rise-up the soil pH [2]. Further it is reported to be the main reason of *Helicobacter pylori* (HP) pathologies, which at low pH promote bacterial survival of the stomach in time of colonization and, hence, perform a key part in the pathogenesis of gastric ulcer and cancer [3]. The apparent cure for controlling bacterial infections with antimicrobials, however, has mostly proven useless [4], and until now, just a few combinations of therapies have reached to clinical practice. Thus there is a dire need for novel and alternative treatment. The chance of resistance development is another valid reason for finding novel drugs for treatment of *H. pylori* infection. *H. pylori* also provide in its urease a distinctive nonmammalian target [1]. Nowadays based on urease inhibition different strategies are considered necessary for treatment of urease producing bacterial infections.

In the active site of the enzyme, Zerner demonstrated in his initial research on the archetype urease from *Cavalia ensiformis* (Jack bean), the existence of 2 Lewis acid nickel ions and a reactive amino acid [5]. Later on others researchers have verified a unique amino acid sequence in *H. pylori* urease, which shows resemblances with ureases of a lot of other microbes as well as with Jack bean urease. The data suggested that these have common ancestral gene. Therefore, they have similarities in their active sites [6].

Thiazolidine or tetrahydrothiazole is a five-membered heterocyclic compound. Thiazolidine contains nitrogen and sulphur in its basic skeleton. This class of compound has an important role in organic, bioorganic, natural product and medicinal chemistry. Therefore, alot of antimicrobial substances such as thienamicyn penicillins, narcodicins, and cephalosporins, have been prepared from thiazolidines [7-9]. Thiazolidines are α-amino acid derivatives which posses antiproliferative action [10, 11]. Antihypertensive activity of *N*-(Mercaptoacyl)-thiazolidine carboxylic acid has already been reported. Reactive oxygen species and free radicals are involved in many diseases processes [12]. Glutathione (L-glutamyl-L-cysteinyl-glycine), a thiazolidine derivative and an essential component of

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all living cell, is a natural scavanger of reactive oxygen intermediates and free radicals. Like *N*-acetyl-L-cysteine, the L-Cystein prodrugs also enhance the levels of intracellular glutathione. 1-(3-Phenylpropyl)-4-[2-(3-pyridyl)] thiazolidine-4-carbonyl piperazine, is a powerful platelet-activating factor (PAF) antagonist.

These thiazolidine esters were selected for urease inhibition studies because of the reason that understudy compounds have structural similarities with already studied urease inhibitors [13]. Herein this study we present first time, thiazolidine esters as potent urease inhibitors.

Results and Discussion

Structure-Activity Relationship (SAR): Out of fifteen thiazolidines compounds which were tested for their urease inhibitory effects, nine **1**-**9** exhibited good inhibition at micromolar levels against both sources of urease enzyme (Jack bean *and Bacillus pasteurii)* at pH = 8.2 and pH = 5.0. The IC_{50} results are shown in Table-1.

2-Heptyl (4*R*)-1,3-thiazolidine-4-carboxylate (**9**) showed potent urease inhibitory activity with IC_{50} = 0.33 and 0.30 µM, respectively, whereas standard inhibitor thiourea has an IC_{50} = 21.01 µM against the Jack bean urease, and IC_{50} = 15.66 µM against *Bacillus pasteurii* urease. The compounds **10** to **14**, which are *n*-octyl, 2-octyl, *n*-nonyl, 2-cloroethyl and 2-ethoxyethyl analogues, respectively, showed less inhibitory activity as shown in Table-2. The range of inhibitory activities was between 37.32 to 79.32 µM against both sources of ureases. After close study of the chemical structure, it was observed that these thiazolidine esters have resemblance with some already reported urease inhibitors in literature [13]. The known crystallographic structure of other similar urease enzymes from species of *Klebsiella aerogenes* would be helpful to know the urease active site interaction with thiazolidines [14, 15]. Urease active site analysis revealed that Asp362, His136, His138, His248, His274 and Lys219 amino acids, which belong to UreB subunit come into direct interaction with one water molecule, urea and with two nickel ions [1, 16]. Additionally, His322 in catalysis acts as a general base, because it is near the active site. Initial molecular docking studies shows that the carbonyl oxygen of the carboxylic acid of thiazolidine-4-carboxylic acid interact directly with the nickel ion in the bi-metallic nickel center of urease as shown in Fig. 1, and this is the major interaction responsible for activity. The inhibitory activities of thiazolidines with the urease are largely dependent on the length of pre-incubation. It was infered that an extended contact between the inhibitors and the urease is desirable in the formation of a stable enzyme inhibitor complex as shown Fig. 2and 3.

Table-1: Structure-Activity Relationship of thiazolidines.

Compound	Urease (Jack bean) IC_{50} µM at pH=8.2, SEM	Urease (Jack bean) IC_{50} µM at pH=5.0, SEM	Urease (*Bacillus pasteurii*) IC_{50} µM at pH=8.2, SEM	Urease (*Bacillus pasteurii*) IC_{50} µM at pH=5.0, SEM
1	8.50 ±0.12	7.07 ±0.50	7.25 ±1.4	5.52 ±0.10
2	3.30 ±0.17	1.79 ±0.07	1.46 ±0.02	1.31 ±0.54
3	2.01 ±0.62	1.70 ±0.77	1.94 ±0.52	1.60 ±0.83
4	2.27 ±0.62	2.11 ±0.79	2.19 ±0.32	2.0 ±0.95
5	1.49 ±0.09	0.93 ±0.02	1.23 ±0.34	1.82 ±0.01
6	1.66 ±0.013	1.03 ±0.45	1.48 ±0.86	1.0 ±0.040
7	0.571 ±0.01	0.40 ±0.003	0.45 ±0.82	0.38 ±0.02
8	0.33 ±0.043	0.29 ±0.012	0.30 ±0.002	0.24 ±0.092
9	0.39 ±0.032	0.34 ±0.60	0.37 ±0.96	0.29 ±0.067
10	39.30 ±0.02	34.99 ±0.49	34.32 ±0.049	29.07 ±0.93
11	43.39 ±0.72	40.57 ±0.55	41.22 ±0.81	39.0 ±0.73
12	65.78 ±1.12	62.20 ±0.35	63.96 ±0.04	59.42 ±0.09
13	134.01 ±0.05	121.56 ±0.12	96.75 ±0.64	92.64 ±0.60
14	201.51 ±0.65	180.99 ±0.05	198.46 ±0.02	172.01 ±0.12

Standard mean error of 3-5 assays
SEM = Standard Mean of Error.

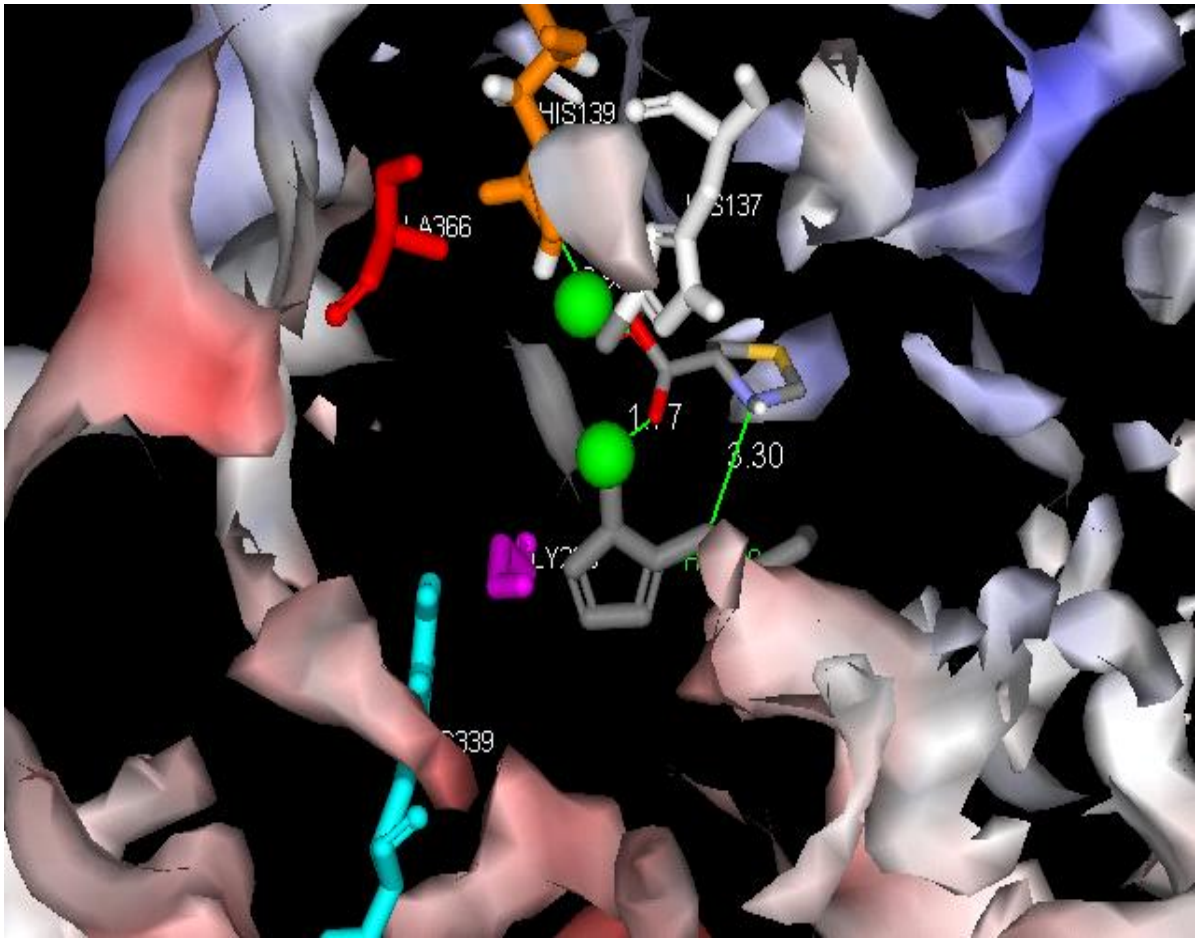
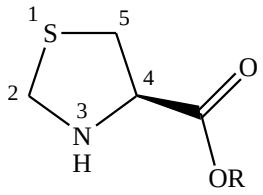


Fig. 1: Thiazolidine-4-caboxylic acid in the bi-metallic nickel (in green colour) center of urease.



S. NO	-R	% Yield
1	$-CH_3$	82
2	$-C_2H_5$	76
3	$-CH_2CH_2CH_3$	81
4	$-CH(CH_3)_2$	82
5	$-CH_2(CH_2)_2CH_3$	78
6	$-C(CH_3)_3$	76
7	$-CH_2(CH_2)_4CH_3$	82
8	$-CH_2(CH_2)_5CH_3$	75
9	$-CH(CH_3)CH_2(CH_2)_3CH_3$	73
10	$-CH_2(CH_2)_6CH_3$	75
11	$-CH(CH_3)CH_2(CH_2)_4CH_3$	61
12	$-CH_2(CH_2)_7CH_3$	75
13	$-CH_2CH_2Cl$	81

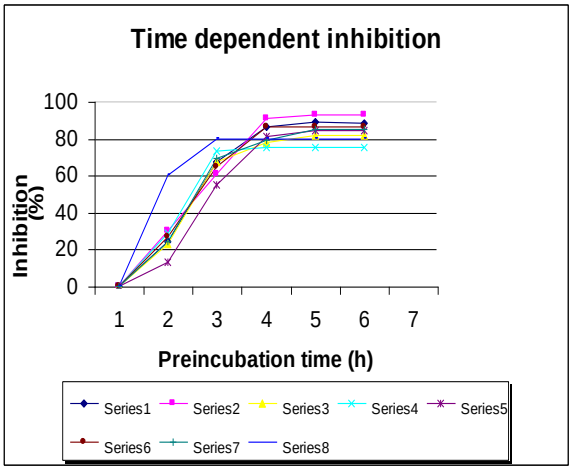


Fig. 2: Time progression of inhibition of jack bean urease activity by thiazolidines 1-7 where compound 8 is acetohydroxamic acid as control.

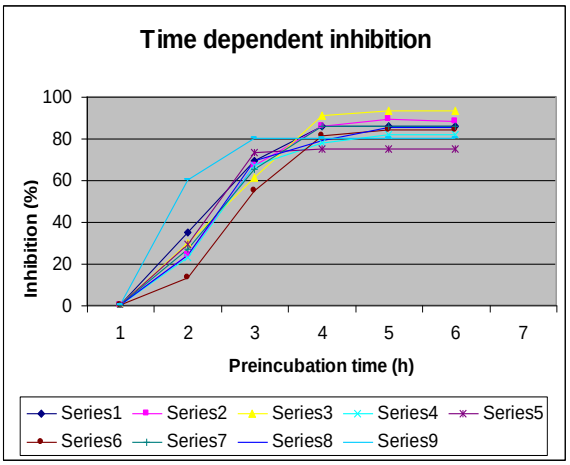


Fig. 3: Time progression of inhibition of jack bean urease activity by thiazolidine esters 8-15 where compound 9 is acetohydroxamic acid as control.

On the basis of these outcomes, it may be suggested that the thiazolidine-4-carboxylic acids act as bidentate ligands, being able to bind with the nickel atoms in a pseudotetrahedral coordination geometry through their carbonyl oxygens. The increase in inhibitory potency after esterfication may be due to increase in electron donating ability of the alkyl of the ester functionalities, as the length of alkyl chain increases, the potency increases as well. On the other hand decrease in activity in case of octyl and nonyl ester substituent may be due to steric effect as enzyme cannot favorably accommodate these bulkier analogs. All the ester analogs show enhanced activities at pH = 5.0 than pH = 8.2. This may be due to the presence of positively charged cation at lower acidic pH.

Furthermore it was also observed that the low biological activity of chlorine and ethoxy substituents may be due to the inductively electron withdrawing effect of these substituents present on aliphatic chain. These results demonstrated that all esters are excellent inhibitors against the bacterial urease (*Bacillus pasteurii*), than the plant urease (Jack bean). It was also clear from the results that all branched analogs are less active than their straight chain analogs, it may be probably because of the steric bulk of branched chain substituents. Further molecular docking, molecular dynamics simulation, STD and Transfer NOE NMR studies are in progress to develop an in-depth understanding of the mechanism of ligand binding. In conclusion thiazolidine esters were discovered as potent new of urease inhibitors.

Experimental

Standard methods were used for drying of alcohols. All other reagents and solvents were used directly and without purification, as they were of reagent grade. Then silica gel (70-230, E. Merck) was used to perform Column chromatography. Analysis of IR Spectroscopic was carry out on Shimadzu-IR-460 (in KBr) and Jasco-A-302 spectrophotometers (in $CHCl_3$ solutions) & their values are shown in $cm.^{-1}$ Analysis of ^{1}H-NMR spectroscopic was carried out on Bruker apparatus at 400 MHz and in δ (ppm) the values are reported. For internal standard TMS was taken. Analysis of EI-MS spectroscopic were carried out on Finnigan-MAT-311-*A* and in *m/z* (rel. abund. %) there values were expressed.

Urease Inhibition Assay

Twenty five (25 µl) solution of *Jack bean* and *Bacillus pasteurii* Urease was prepared as a reaction mixtures. Then in 96-well plate, reaction mixture for 30 minutes was incubated with 5 µl test compounds (**1-15**) at 30° C. Thereafter, 55 µl of buffers were incubated for 15 minutes, which consist of 100 mM urea. In last according to Weatherburn indophenol method, by measuring ammonia production the final urease activity was calculated [17]. Briefly, to each well of 96-well plate, 45 µl each of phenol reagent and 70 µl of alkali reagent were also added. Then after 50 min at 630 nm absorbance was taken by using a microplate reader (Molecular Device, USA). To minimize error chances and to confirm the result, all reactions were repeated three times. The final volume was rised to 200 µl. SoftMax Pro software of USA company (Molecular Device) was used to process the results. At pH 8.2 all the assays were carried out. The formula $100-(OD_{testwell}/OD_{control}) \times 100$ was used to calculate the percentage inhibitions. As standard inhibitor of enzyme, Thiourea was used as positive control, where $OD_{control}$ was the negative control.

IC_{50} values Determination

Against Jack bean urease and urease of *Bacillus pasteurii*, concentration of the tested compounds that are sufficient to inhibit the hydrolysis reaction up to 50%, which was calculated by controlling the effect effect of different compounds of various concentrations. Then with the help of EZ-Fit Enzyme Kinetics program of USA (*Perrella Scientific Inc., Amherst*), the IC_{50} values were measured.

Synthesis of Thiazolidine Esters

L-Cysteine hydrochloride was dissolved in water and then to this solution formaldehyde was added. At 25ºC the mixture was stirred to afford (*4R*)-1,3-thiazolidine-4-carboxylic acid. Then in a number of alcohols the (*4R*)-1,3-thiazolidine-4-carboxylic acid was suspended. To these suspensions, thionyl chloride was added dropwise to afford the corresponding ester hydrochlorides Fig. 4. Then with 5% cold aqueous solution of sodium bicarbonate the ester hydrochloride salts were treated. At final extracted with diethyl ether, and dried using sodium sulphate, which then evaporated to afford the corresponding esters.

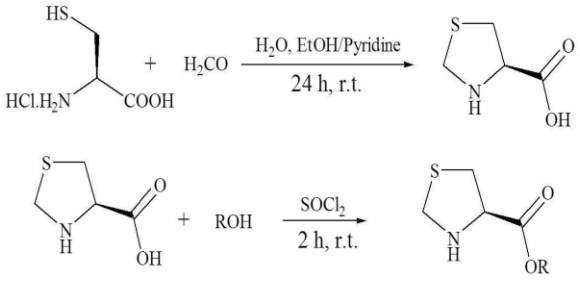


Fig. 4: Synthetic scheme of Thiazolidine Esters synthesis.

General Procedure

A mixture of (*4R*)-1,3-thiazolidine-4-carboxylic acid (0.1 g, 0.75 mM) and anhydrous alcohol (10 eqs.) was stirred at 0º C. To this suspension, thionyl chloride (0.178 g, 1.5 mM) was dropwise added and for 12 h the reaction mixture was stirred, as directed by TLC. Solvents were then evaporated at reduced pressures and crystalline product in the form of hydrochloride salt was obtained which was then dissolved in 5% ice cooled sodium bicarbonate solution. The ester was extracted with diethyl ether and dried over sodium sulfate. Dried ether extract was evaporated at reduced pressure and oily product was finally obtained.

Methyl (4R)-1,3-thiazolidine-4-carboxylate **1**

Yield 0.091 g (82%), Oil; R_f = 0.55 (hexane/ethyl acetate, 8:2); ^{1}H-NMR (400 MHz, DMSO-d$_6$): δ 4.76 (t, 1H, $J_{4\alpha,5\beta}$ = 6.6 Hz, H-4α), 4.27 (q, 2H, J = 9.5 Hz, SCH_2NH), 3.53 (s, 3H, CH_3), 3.35 (dd, 1H, $J_{5\beta,4\alpha}$ = 6.6 Hz, H-5β), 3.27 (dd, 1H, $J_{5\alpha,4\alpha}$ = 6.4 Hz, H-5β); IR (KBr) ν_{max} 3350, 1731 cm^{-1}; EI MS *m/z* (% rel. abund.) 147 (M$^+$, 77), 132 (40), 88 (100), 59 (52).

Ethyl (4R)-1,3-thiazolidine-4-carboxylate **2**

Yield 0.09 g (76%), Oil; R_f = 0.51 (hexane/ethyl acetate, 8:2); ^{1}H-NMR (400 MHz, DMSO-d$_6$): δ 4.72 (t, 1H, $J_{4\alpha,5\alpha}$ = 6.6 Hz, H-4α), 4.27 (q, 2H, J = 9.5 Hz, SCH_2NH), 3.59 (q, 2H, J = 6.8 Hz, CH_2CH_3), 3.35 (dd, 1H, $J_{5\alpha,4\alpha}$ = 6.6 Hz, H-5α), 3.27 (dd, 1H, $J_{5\beta,4\alpha}$ = 6.4 Hz, H-5β), 0.98 (t, 3H, J = 6.9 Hz, CH_3); IR (KBr) ν_{max} 3350, 1731 cm^{-1}; EI MS *m/z* (% rel. abund.): 161 (M$^+$, 77), 147 (22), 132 (44), 88 (100), 59 (28).

n-Propyl (4R)-1,3-thiazolidine-4-carboxylate **3**

Yield 0.109 g (82%), Oil; R_f = 0.52 (hexane/ethyl acetate, 8:2); ^{1}H-NMR (400 MHz, DMSO-d$_6$): δ 4.76 (t, 1H, $J_{4\alpha,5\alpha}$ = 6.6 Hz, H-4α), 4.27 (q, 2H, J = 9.5 Hz, SCH_2NH), 3.45 (t, 2H, J = 6.8 Hz, OCH_2), 3.35 (dd, 1H, $J_{5\alpha,4\alpha}$ = 6.6 Hz, H-5α), 3.27 (dd, 1H, $J_{5\beta,4\alpha}$ = 6.4 Hz, H-5β), 1.81 (m, 2H, CH_2CH_3), 0.98 (t, 3H, J = 6.9 Hz, CH_3); IR (KBr) ν_{max} 3350, 1731 cm^{-1}; EI MS *m/z* (% rel. abund.) 175 (M$^+$, 34), 132 (49), 88 (100), 59 (8).

Iso-propyl (4R)-1,3-thiazolidine-4-carboxylate **4**

Yield 0.11 g (82%), Oil; R_f = 0.57 (hexane/ethyl acetate, 8:2); ^{1}H-NMR (400 MHz, DMSO-d$_6$): δ 4.74 (t, 1H, $J_{4\alpha,5\alpha}$ = 6.6 Hz, H-4α), 4.29 (q, 2H, J = 9.5 Hz, SCH_2NH), 3.45 (m, 1H, J = 6.8 Hz, OCH), 3.35 (dd, 1H, $J_{5\alpha,4\alpha}$ = 6.6 Hz, H-5α), 3.27 (dd, 1H, $J_{5\beta,4\alpha}$ = 6.4 Hz, H-5β), 1.01 (d, 6H, J = 6.9 Hz, 2CH_3); IR (KBr) ν_{max} 3350, 1731 cm^{-1}; EI MS *m/z* (% rel. abund.) 175 (M$^+$, 39), 132 (59), 88 (100), 59 (28).

n-Butyl (4R)-1,3-thiazolidine-4-carboxylate **5**

Yield 0.14 g (78%), Oil; R_f = 0.49 (hexane/ethyl acetate, 8:2); ^{1}H-NMR (400 MHz, DMSO-d$_6$): δ 4.76 (t, 1H, $J_{4\alpha,5\alpha}$ = 6.6 Hz, H-4α), 4.27 (q, 2H, J = 9.5 Hz, SCH_2NH), 3.45 (t, 2H, J = 6.8 Hz, OCH_2), 3.35 (dd, 1H, $J_{5\alpha,4\alpha}$ = 6.6 Hz, H-5α), 3.27 (dd, 1H, $J_{5\beta,4\alpha}$ = 6.4 Hz, H-5β), 1.41-1.26 (m, 4H, 2CH_2), 0.98 (t, 3H, J = 6.9 Hz, CH_3); IR (KBr) ν_{max} 3350, 1731 cm^{-1}; EI MS *m/z* (% rel. abund.) 189 (M$^+$, 64), 132 (49), 88 (100), 59 (18).

t-Butyl (4R)-1,3-thiazolidine-4-carboxylate **6**

Yield 0.11 g (76%), Oil; R_f = 0.61 (hexane/ethyl acetate, 8:2); ^{1}H-NMR (400 MHz,

DMSO-d_6): δ 4.76 (t, 1H, $J_{4\alpha,5\alpha}$ = 6.6 Hz, H-4α), 4.27 (q, 2H, J = 9.5 Hz, SCH_2NH), 3.35 (dd, 1H, $J_{5\alpha,4\alpha}$ = 6.6 Hz, H-5α), 3.27 (dd, 1H, $J_{5\beta,4\alpha}$ = 6.4 Hz, H-5β), 1.10 (s, 9H, 3CH_3); IR (KBr) ν_{max} 3350, 1731 cm^{-1}; EI MS *m/z* (% rel. abund.) 189 (M^+, 54), 132 (29), 88 (100), 59 (8).

n-Hexyl (4R)-1,3-thiazolidine-4-carboxylate **7**

Yield 0.11 g (82%), Oil; R_f = 0.55 (hexane/ethyl acetate, 8:2); ^{1}H-NMR (400 MHz, DMSO-d_6): δ 4.75 (t, 1H, $J_{4\alpha,5\alpha}$ = 6.6 Hz, H-4α), 4.26 (q, 2H, J = 9.5 Hz, SCH_2NH), 3.53 (t, 2H, J = 6.8 Hz, OCH_2), 3.35 (dd, 1H, $J_{5\alpha,4\alpha}$ = 6.6 Hz, H-5α), 3.27 (dd, 1H, $J_{5\beta,4\alpha}$ = 6.4 Hz, H-5β), 1.23-1.56 (m, 8H, 4CH_2), 0.93 (t, 3H, J = 6.9 Hz, CH_3); IR (KBr) ν_{max} 3350, 1731 cm^{-1}; EI MS *m/z* (% rel. abund.) 217 (M^+, 57), 132 (49), 88 (100), 59 (52).

n-Heptyl (4R)-1,3-thiazolidine-4-carboxylate **8**

Yield 0.13 g (75%), Oil; R_f = 0.52 (hexane/ethyl acetate, 8:2); ^{1}H-NMR (400 MHz, DMSO-d_6): δ 4.75 (t, 1H, $J_{4\alpha,5\alpha}$ = 6.6 Hz, H-4α), 4.27 (q, 2H, J = 9.5 Hz, SCH_2NH), 3.49 (t, 2H, J = 6.8 Hz, OCH_2), 3.35 (dd, 1H, $J_{5\alpha,4\alpha}$ = 6.6 Hz, H-5α), 3.27 (dd, 1H, $J_{5\beta,4\alpha}$ = 6.4 Hz, H-5β), 1.01-1.23 (m, 10H, 5CH_2) 0.88 (t, 3H, J = 6.9 Hz, -CH_3); IR (KBr) ν_{max} 3350, 1731 cm^{-1}; EI MS *m/z* (% rel. abund.) 231 (M^+, 24), 132 (8), 88 (100), 59 (12).

2-Heptyl (4R)-1,3-thiazolidine-4-carboxylate **9**

Yield 0.13 g (76%), Oil; R_f = 0.52 (hexane/ethyl acetate, 8:2); ^{1}H-NMR (400 MHz, DMSO-d_6): δ 4.75 (t, 1H, $J_{4\alpha,5\alpha}$ = 6.6 Hz, H-4α), 4.27 (q, 2H, J = 9.5 Hz, SCH_2NH), 3.53 (m, 1H, J = 6.8 Hz, OCH), 3.35 (dd, 1H, $J_{5\alpha,4\alpha}$ = 6.6 Hz, H-5α), 3.27 (dd, 1H, $J_{5\beta,4\alpha}$ = 6.4 Hz, H-5β), 1.59 (d, 3H, J = 6.5 Hz,OCHCH_3), 1.1-1.23 (m, 8H, 5CH_2) 0.88 (t, 3H, J = 6.9 Hz, CH_3); IR (KBr) ν_{max} 3350, 1731 cm^{-1}; EI MS *m/z* (% rel. abund.) 231 (M^+, 76), 132 (6), 88 (100), 59 (31).

n-Octyl (4R)-1,3-thiazolidine-4-carboxylate **10**

Yield 0.13 g (75%), Oil; R_f = 0.52 (hexane/ethyl acetate, 8:2); ^{1}H-NMR (400 MHz, DMSO-d_6): δ 4.75 (t, 1H, $J_{4\alpha,5\alpha}$ = 6.6 Hz, H-4α), 4.27 (q, 2H, J = 9.5 Hz, SCH_2NH), 3.49 (t, 2H, J = 6.6 Hz, OCH_2), 3.34 (dd, 1H, $J_{5\alpha,4\alpha}$ = 6.6 Hz, H-5α), 3.26 (dd, 1H, $J_{5\beta,4\alpha}$ = 6.4 Hz, H-5β), 1.01-1.33 (m, 12H, 6CH_2) 0.84 (t, 3H, J = 6.9 Hz, CH_3); IR (KBr) ν_{max} 3350, 1731 cm^{-1}; EI MS *m/z* (% rel. abund.) 245 (M^+, 61), 132 (37), 88 (100), 59 (29).

2-Octyl (4R)-1,3-thiazolidine-4-carboxylate **11**

Yield 0.11 g (61%), Oil; R_f = 0.55 (hexane/ethyl acetate, 8:2); ^{1}H-NMR (400 MHz, DMSO-d_6): δ 4.75 (t, 1H, $J_{4\alpha,5\alpha}$ = 6.6 Hz, H-4α), 4.26 (q, 2H, J = 9.5 Hz, SCH_2NH), 3.53 (m, 1H, J = 6.9 Hz, OCH), 3.35 (dd, 1H, $J_{5\alpha,4\alpha}$ = 6.6 Hz, H-5α), 3.27 (dd, 1H, $J_{5\beta,4\alpha}$ = 6.4 Hz, H-5β), 1.59 (d, 3H, J = 6.5 Hz,OCHCH_3), 1.25-1.56 (m, 10H, 5CH_2), 0.91 (t, 3H, J = 6.9 Hz, CH_3); IR (KBr) ν_{max} 3350, 1731 cm^{-1}; EI MS *m/z* (% rel. abund.) 245 (M^+, 17), 132 (49), 88 (100), 59 (32).

n-Nonyl (4R)-1,3-thiazolidine-4-carboxylate **12**

Yield 0.13 g (75%), Oil; R_f = 0.52 (hexane/ethyl acetate, 8:2); ^{1}H-NMR (400 MHz, DMSO-d_6): δ 4.75 (t, 1H, $J_{4\alpha,5\alpha}$ = 6.6 Hz, H-4α), 4.27 (q, 2H, J = 9.5 Hz, SCH_2NH), 3.49 (t, 2H, J = 6.7 Hz, OCH_2), 3.34 (dd, 1H, $J_{5\alpha,4\alpha}$ = 6.6 Hz, H-5α), 3.26 (dd, 1H, $J_{5\beta,4\alpha}$ = 6.4 Hz, H-5β), 1.01-1.43 (m, 14H, 7CH_2) 0.84 (t, 3H, J = 6.9 Hz, CH_3); IR (KBr) ν_{max} 3350, 1731 cm^{-1}; EI MS *m/z* (% rel. abund.) 260 (M^+, 51), 132 (37), 88 (100), 59 (29).

2-Chloroethyl (4R)-1,3-thiazolidine-4-carboxylate **13**

Yield 0.11 g (81%), Oil; R_f = 0.49 (hexane/ethyl acetate, 8:2); ^{1}H-NMR (400 MHz, DMSO-d_6): δ 4.75 (t, 1H, $J_{4\alpha,5\alpha}$ = 6.6 Hz, H-4α), 4.27 (q, 2H, J = 9.5 Hz, SCH_2NH), 3.51 (t, 2H, J = 6.9 Hz, OCH_2), 3.35 (dd, 1H, $J_{5\alpha,4\alpha}$ = 6.6 Hz, H-5α), 3.27 (dd, 1H, $J_{5\beta,4\alpha}$ = 6.4 Hz, H-5β), 3.22 (t, 2H, J = 6.9 Hz, CH_2Cl); IR (KBr) ν_{max} 3350, 1731 cm^{-1}; EI MS *m/z* (% rel. abund.) 195 (M^+, 8), 159 (4), 132 (10), 88 (100), 59 (18).

2-Ethoxyethyl (4R)-1,3-thiazolidine-4-carboxylate **14**

Yield 0.12 g (77%), Oil; R_f = 0.51 (hexane/ethyl acetate, 8:2); ^{1}H-NMR (400 MHz, DMSO-d_6): δ 4.75 (t, 1H, $J_{4\alpha,5\alpha}$ = 6.6 Hz, H-4α), 4.27 (q, 2H, J = 9.5 Hz, SCH_2NH), 3.59 (t, 2H, J = 6.6 Hz, CO_2CH_2), 3.43 (q, 2H, J = 6.8 Hz, OCH_2), 3.35 (dd, 1H, $J_{5\alpha,4\alpha}$ = 6.6 Hz, H-5α), 3.26 (dd, 1H, $J_{5\beta,4\alpha}$ = 6.4 Hz, H-5β), 3.13 (t, 2H, J = 9.9 Hz, CH_2O), 1.08 (t, 3H, J = 6.9 Hz, CH_3); IR (KBr) ν_{max} 3350, 1731 cm^{-1}; EI MS *m/z* (% rel. abund.) 205 (M^+, 24), 132 (25), 88 (100), 59 (66).

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