Synthesis and Evaluation of Antioxidant, Anti-inflammatory and Antiulcer Activity of Conjugates of Amino Acids with Nifedipine

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A new series of novel (2*S*)-2-({2-[1,4-dihydro-3,5-bis(methoxycarbonyl)-2,6-dimethyl-4-(2-nitrophenyl)pyridin-1-yl]-2-oxoethyl}amino)-3-(4-hydroxyphenyl) propanoic acid (3a) and its analogues 3b—j has been synthesized. These compounds were evaluated for their *in vitro* antioxidant activity, anti-inflammatory activity and antiulcer activity. Compounds 3b and f exhibited significant antioxidant action comparable with that of standard. Efficacy against inflammation and ulceration was also found to be significant. The chemical structures of these compounds were confirmed on the basis of spectral data.

Key words nifedipine; amino acid; conjugate; antioxidant; anti-inflammatory; antiulcer

Gastric acid secretion is a calcium dependent process and calcium channel blockers including nifedipine have exhibited good potential to reduce gastric secretion.[1—3] The role of free radicals in inflammation of gastric mucosa and use of antioxidants for protection against ulceration has been widely suggested.[4,5] Several amino acids[6] and their conjugates with curcumin,[7] pyrrole[8] and 5*H*-dibenzazepine[9] have been reported with significant antioxidant action. Inspired with antisecretory profiles of nifedipine and antioxidant properties of amino acids and in connection with our research on the design and synthesis of biologically active and pharmacologically important new heterocycles, it was thought worthwhile to synthesize the amino acid conjugates of nifedipine with a view to obtain certain new chemical entities with potential to reduce excess gastric acid secretion as well as protect gastric mucosa against inflammation and ulceration. On the other hand, to the best of our knowledge, previously there is no report, on the synthesis of (2*S*)-2-({2-[1,4-dihydro-3,5-bis(methoxycarbonyl)-2,6-dimethyl-4-(2-nitrophenyl)pyridin-1-yl]-2-oxoethyl}amino)-3-(4-hydroxyphenyl)propanoic acid (**3a**) and its analogues **3b**—**j**.

The key intermediate dimethyl 1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)pyridine-3,5-dicarboxylate (**1**) was prepared by Hansch pyridine synthesis by treating 2-nitrobenzaldehyde with methyl acetoacetate in presence of ammonium hydroxide. Dimethyl 1-(2-chloroacetyl)-1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)pyridine-3,5-dicarboxylate (**2**) was prepared by reaction of nifedipine (1) in benzene with 3-chloroacetyl chloride in presence of triethylamine and continuous stirring. Further treatment of compound **2** with amino acids in presence of K_2CO_3 in methanol followed by reflux yielded the title compounds **3a**—**j**. The synthetic route leading to the title compounds is summarized in Chart 1. The chemical structures of all the newly synthesized compounds were confirmed by their IR, ^{1}H-NMR, elemental and mass spectra analysis. The compounds were screened for their antioxidant, anti-inflammatory and antiulcer activities.

***In-Vitro* Antioxidant Study** The free radical scavenging activity of synthesized compounds was evaluated by the method first employed by Blois[10] using 1,1-diphenyl-2-picrylhydrazyl (DPPH). To 1 ml of each compound of different concentrations (1, 2, 3, 4, 5 mg/ml) 1 ml of 0.1 mmol DPPH was added and incubated in the dark room for 35 min. The absorbance was measured at 517 nm and percentage quenching of DPPH was calculated. For all the compounds and standard half inhibition concentration (IC_{50}) was calculated and showed in Table 1. Conjugation of amino acids increased the antioxidant potential of nifedipine as evident from the lower IC_{50} value of compounds. The IC_{50} value of compound **3b** was lowest among the test compounds followed by that of **3f** indicating their good radical scavenging potential. The DPPH radical scavenging action of **3b** was found to be better than ascorbic acid (Fig. 1).

Anti-inflammatory Activity of Synthesized Compounds Acute inflammation was produced by sub plantar injection of 0.1 ml of 1% suspension of carrageenin in normal saline in the right hind paw of the rats. Paw volume was measured plethysmometrically[11] at 0 and 4 h after carrageenin injection. The animals were treated with the synthesized compounds (50 mg/kg). Saline (3 ml/kg, orally) treated animals served as control and acetyl salicylic acid (100 mg/kg, orally) was administered as standard drug. The drugs were administered simultaneously with carrageenin injection. Mean increase in paw volume was measured and reported in Table 1. All the test compounds reduced the paw volume. The anti-inflammatory activity was found to be significant ($p<0.001$) for compounds **3b**, **c** and **f** (Fig. 2).

Antiulcer Activity of Synthesized Compounds The antiulcer activity of the synthesized compounds were evaluated by pyloric ligature induced gastric ulcers in rat model using parameters including volume, pH, ulcer index, free acidity and total acididy.[12] The test compounds exhibited variable antiulcer activity. Reduction in acid secretion was more significant ($p<0.001$) in animals administered with compounds **3b**, **c** and **f**. The acid neutralizing ability of **3b** and **3f** were more significant ($p<0.001$) and comparable with omeprazole. The reduction in free acidity by **3b** and **f** was close to that of the standard. The reduction in total acidity by both compounds was significant ($p<0.001$) but less than omeprazole. The ulcer healing capacity as indicated by ulcer index was significant ($p<0.001$) for compounds **3a**, **d**, **f**, **h** and **i**. This study shows that antioxidant action complements the antisecretory properties of the compounds as evident from activities of compounds **3b** and **f**. Compound **3b** showed

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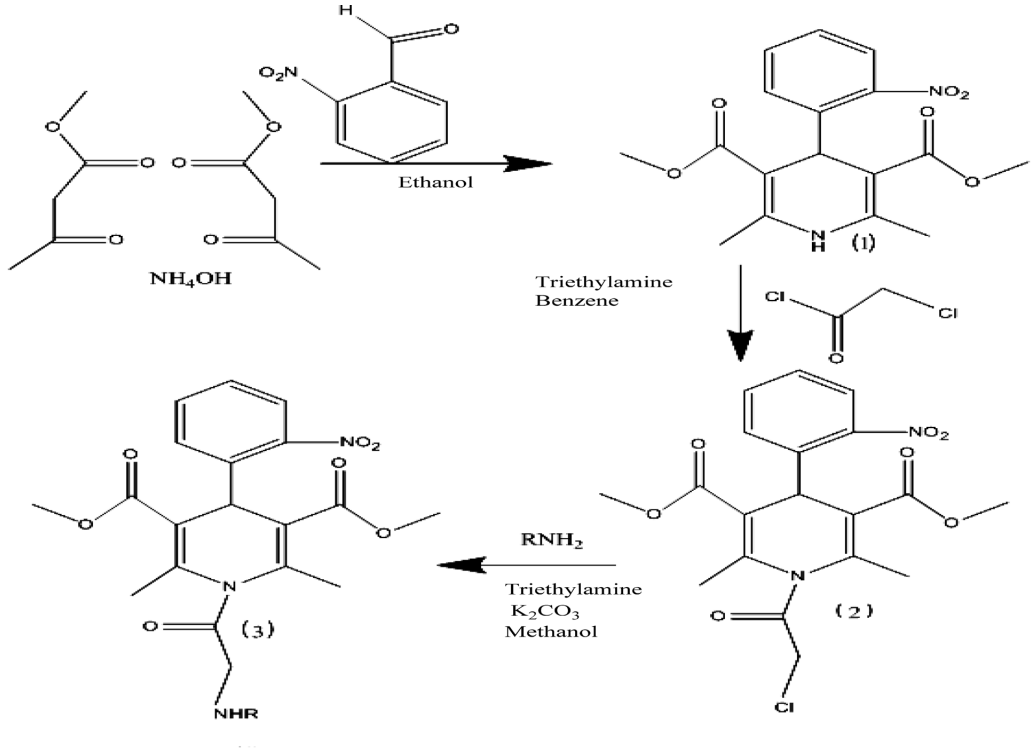


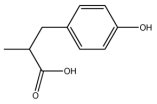
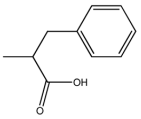
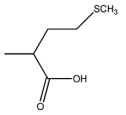
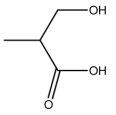
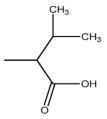
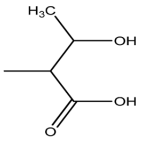
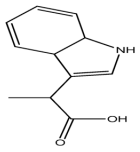
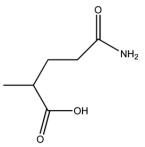
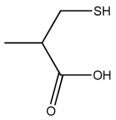
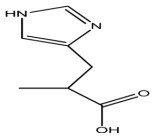
Comp.	R	Comp.	R	Comp.	R	Comp.	R
3a		3b		3c		3d	
3e		3f		3g		3h	
3i		3j					

Chart 1

better antioxidant action than **3f** but the anti-inflammatory and antiulcer actions were comparable. Thus it can be suggested that antioxidant action of **3b** has not been able to remarkably potentiate its antisecretory properties. From this study it can be proposed that conjugation of amino acids to nifedipine enhanced the antioxidant action that complemented the antisecretory action of the compounds resulting in increased anti-inflammatory and antiulcer potential. This improvement was more significant in phenyl alanine and methionine conjugates of nifedipine.

Experimental

General All reagents and solvents were used as purchased without further purification. Melting points were determined on a Sisco melting point apparatus and are uncorrected. Crude products were purified by column chromatography on silica gel of 60—120 mesh. IR spectra were obtained on a JASCO FTIR-4100 spectrometer using KBr pellet. NMR spectra were recorded on BRUKER AVANCE-II-400 MHz spectrometer for ^{1}H-NMR. The chemical shifts were reported as ppm down field using tetramethylsilane (TMS) as an internal standard. Elemental analysis was carried out with PERKIN ELMER-2400 analyser. Mass spectra were recorded on a MICRO-MASS Q-TOF MICRO spectrometer operating at 70 eV.

Typical Procedure Dimethyl 1,4-Dihydro-2,6-dimethyl-4-(2-nitrophenyl)pyridine-3,5-dicarboxylate (1) A solution of 2-nitro benzaldehyde (0.2 mol), methyl acetoacetate (0.2 mol) and ammonium hydroxide (8 ml) in ethanol (60 ml) was heated under reflux for 3 h. To the resulting mixture warm water (40 ml) was added and then allowed to cool. The separated product was filtered off, washed with 60% aqueous ethanol and recrystallised from ethanol. The purity of the compound was checked with TLC.

Dimethyl-1-(2-chloroacetyl)-1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)pyridine-3,5-dicarboxylate (2) Chloroacetyl chloride (2.2 mmol) was added drop by drop for about 30 min to the well stirred solution of **1** (2 mmol) and triethylamine (2.2 mmol) in 50 ml benzene. The reaction mixture was stirred at room temperature for about 6h. Progress of the reaction was monitored by TLC using 9 : 1 (hexane : ethyl acetate) mixture as mobile phase. After completion of reaction, the reaction mass was quenched in ice

Table 1. Antioxidant, Anti-inflammatory and Antiulcer Activity of 2(*S*)-2-({2-[1,4-Dihydro-3,5-bis(methoxycarbonyl)-2,6-dimethyl-4-(2-nitrophenyl)pyridin-1-yl]-2-oxoethyl}amino)-3-(4-hydroxyphenyl)propanoic Acid (**3a**) and Its Analogues **3b**—**j**

Group	RSA	Anti-inflammatory activity		Antiulcer activity				
	IC_{50} (μg/ml)	Increase PV	%	Volume (ml)	pH	Ulcer index	FA (meq/l)	TA (meq/l)
Cont.	—	0.5833±0.03073	—	6.4±0.3141	1.912±0.2693	16.80±1.152	46.17±3.655	50.38±2.585
Standard	50	0.1500±0.02236***	74.3	2.167±0.283***	5.050±0.3354***	2.750±0.428***	6.867±0.5858***	8.150±0.4682***
1	272	0.3124±0.02108***	33.4	4.85±1.2	2.29±0.453	14.2±0.541	39.5±0.89	45.8±0.44
3a	326	0.3333±0.02108***	42.8	4.783±0.2587	3.300±0.2582	13.083±0.5016	38.33±4.295	42.00±4.187
3b	10	0.1833±0.01667***	68.6	2.383±0.25***	4.100±0.152***	4.250±0.295***	8.817±0.8845***	15.82±1.839***
3c	180	0.3667±0.04216**	37.1	3.833±0.40***	2.962±0.3102	8.250±0.676**	39.83±3.400	43.83±3.936
3d	169	0.3500±0.02236**	39.9	5.850±0.37	2.483±0.3833	7.267±0.518***	26.33±2.290**	31.67±2.801**
3e	381	0.3167±0.04773***	45.7	4.817±0.365	2.383±0.2725	12.883±0.4191	31.00±4.017	36.33±3.621
3f	65	0.2000±0.02582***	65.1	2.233±0.2951***	4.648±0.317***	3.200±0.646***	8.383±0.5770***	11.00±0.3907***
3g	254	0.400±0.06009*	34.2	4.500±0.2569*	2.983±0.3591	11.333±0.4807	33.67±2.753	39.50±3.063
3h	90	0.353±0.03651	31.4	3.983±0.5474**	2.903±0.3795	6.283±0.426***	32.17±3.894	39.00±3.838
3i	81	0.3167±0.04773	28.5	4.533±0.5542	3.117±0.3745	5.817±0.451***	27.17±3.978**	31.67±3.499**
3j	332	0.5833±0.03073***	51.4	4.083±0.2344**	3.233±0.4863	14.100±0.663***	35.17±2.455**	38.33±2.124***

RSA: Radical (DPPH) scavenging activity. Data are represented as mean±S.E.M. Statistical analysis was done with one way analysis of variance (ANOVA). ***$p<0.001$, **$p<0.05$ and *$p<0.01$ as compared to control ($n=6$ in each group).

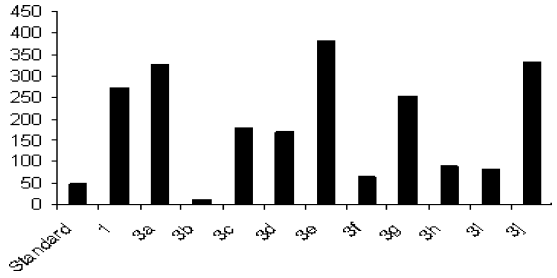


Fig. 1. IC_{50} of Compounds

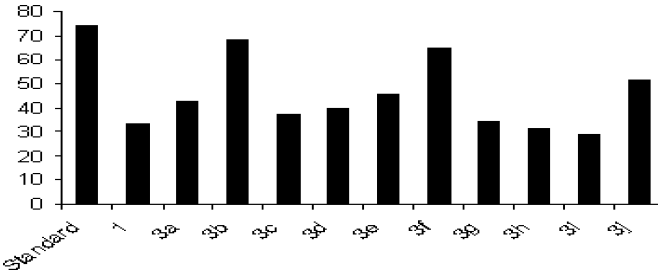


Fig. 2. Reduction in Paw Volume (%) by Compounds

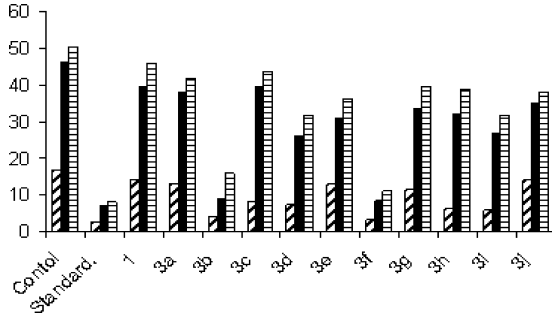


Fig. 3. Antiulcer Activity of Compounds

□: Total acidity, ■: free acidity, □: ulcer index.

cold water and extracted in diethyl ether. The ether layer was washed twice with 5% $NaHCO_3$ and twice with distilled water. Finally the ether layer was dried with anhydrous Na_2SO_4.

(2*S*)-2-({2-[1,4-Dihydro-3,5-bis(methoxycarbonyl)-2,6-dimethyl-4-(2-nitrophenyl)pyridin-1-yl]-2-oxoethyl}amino)-3-(4-hydroxyphenyl)-propanoic Acid (3a) Tyrosine (1.2 mmol) in methanol (25 ml) was neutralized with triethylamine (1.2 mmol). To this K_2CO_3 (600 mg) was added. Later the solution of **2** (1 mmol) in methanol (50 ml) was added drop by drop for 30 min. The reaction mixture was refluxed for 8 h. The progress of the reaction was monitored by TLC. The reaction mixture was then desolventized and compound was extracted in ethyl acetate. The ethyl acetate layer was washed with water and dried over anhydrous Na_2SO_4. The product was further recrystallised from ethanol. Other compounds of the series **3a**—**j** were obtained in a similar manner.

Dimethyl-1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)pyridine-3,5-dicarboxylate (**1**): Yellow solid, yield 71%, mp 172—174 °C. IR (KBr) cm^{-1}: 3339, 3089, 2985, 1689, 1125. ^{1}H-NMR ($CDCl_3$) δ: 7.2—7.6 (m, Ar-H), 6.2 (s,1H, NH), 5.2 (s, 1H, –CH–), 3.8 (s, 6H, $-CH_3$), 2.3 (s, 6H, $-CH_3$). MS *m*/*z*: 346 (M^+).

Dimethyl-1-(2-chloroacetyl)-1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-pyridine-3,5-dicarboxylate (**2**): Pale yellow solid, yield 65%, mp 154—156 °C. IR (KBr) cm^{-1}: 3076, 2974, 1711,1688, 1125. ^{1}H-NMR ($CDCl_3$) δ: 7.5—7.9 (m, Ar-H), 4.5 (s, 1H, –CH–), 3.7(s, 6H,$-CH_3$), 2.3 (s, $-CH_3$), 2.4—3.3 (m, Alk-H).

(2*S*)-2-({2-[1,4-Dihydro-3,5-bis(methoxycarbonyl)-2,6-dimethyl-4-(2-nitrophenyl)pyridin-1-yl]-2-oxoethyl}amino)-3-(4-hydroxyphenyl)propanoic Acid (**3a**): Light yellow solid, yield 64%, mp 108—110 °C. IR (KBr) cm^{-1}: 3458, 3131, 3023, 2925, 1728, 1689, 1143. ^{1}H-NMR ($CDCl_3$) δ: 11 (s, 1H, OH), 6.2—7.9 (m, Ar-H), 5.8 (s, 1H, OH), 4.7 (s, 1H, –CH–), 2.2—2.8 (m, $-CH_2-$), 3.6 (s, OH, $-CH_3$). *Anal.* Calcd for $C_{28}H_{30}N_3O_{10}$: C, 59.25; H, 5.32. Found: C, 59.08; H, 5.12. MS *m*/*z*: 567 (M^+). $[\alpha]_D^{25}$ −11.

(2*S*)-2-((3-(3,5-Bis(methoxycarbonyl)-2,6-dimethyl-4-(2-nitrophenyl)-pyridine-1(4*H*)-3-oxoacetyl)amino)-3-phenylpropanoic Acid (**3b**): Light yellow solid, yield 48%, mp 158—160 °C. IR (KBr) cm^{-1}: 3444, 3145, 3045, 2931, 1734, 1691, 1125. ^{1}H-NMR ($CDCl_3$) δ: 10.2 (s, 1H, OH), 7.3—7.9 (m, Ar-H), 4.5 (s, 1H, –CH–), 3.3—3.5 (m,$-CH_2-$), 3.78 (s, 6H, $-CH_3$), 2.2—2.7 (m, $-CH_2-$). *Anal.* Calcd for $C_{28}H_{30}N_3O_9$: C, 60.97; H, 5.48. Found: C, 61.1; H, 5.89. MS *m*/*z*: 551 (M^+). $[\alpha]_D^{25}$ −6.

(2*S*)-2-((3-(3,5-Bis(methoxycarbonyl)-2,6-dimethyl-4-(2-nitrophenyl)-pyridine-1(4*H*)-3-oxoacetyl)amino)-3-(3*R*)-hydroxybutanoic Acid (**3c**): Yellow solid, yield 44%, mp 123—125 °C. IR (KBr) cm^{-1}: 3395, 3125, 3043, 2961, 1721, 1687, 1145. ^{1}H-NMR ($CDCl_3$) δ: 10 (s, 1H, OH), 7.5—7.8 (m, Ar-H), 4.3 (s, 1H, –CH–), 3.9 (s, 1H, OH), 2.1—2.6 (m, $-CH_2-$), 3.4 (s, 6H, $-CH_3$). *Anal.* Calcd for $C_{23}H_{28}N_3O_{10}$: C, 54.65; H, 5.58. Found: C, 54.02; H, 5.11. MS *m*/*z*: 505 (M^+). $[\alpha]_D^{25}$ −62.

(2*R*)-2-((3-(3,5-Bis(methoxycarbonyl)-2,6-dimethyl-4-(2-nitrophenyl)-pyridine-1(4*H*)-3-oxoacetyl)amino)-3-hydroxypropanoic Acid (**3d**): Brownish yellow solid, yield 49%, mp 155—157 °C. IR (KBr) cm^{-1}: 3445, 3132, 1723, 1695, 2922, 1145. ^{1}H-NMR ($CDCl_3$) δ: 1.8 (s, 1H, NH), 2.1 (s, 6H, $-CH_3$), 2.3—2.8 (m, $-CH_2-$), 3.6 (s, 6H, $-CH_3-$), 3.7—4.1 (m, $-CH_2$), 4.6 (s, 1H, –CH–), 7.4—7.8 (m, Ar-H). *Anal.* Calcd for $C_{22}H_{26}N_3O_{10}$: C, 53.76; H, 5.33. Found: C, 53.86; H, 5.98. $[\alpha]_D^{25}$ 138.

(2*R*)-2-((3-(3,5-Bis(methoxycarbonyl)-2,6-dimethyl-4-(2-nitrophenyl)-pyridine-1(4*H*)-3-oxoacetyl)amino)-3-methylbutanoic Acid (**3e**): Yellow solid, yield 47%, mp 151—153 °C. IR (KBr) cm^{-1}: 3388, 3043, 2946, 1709,

1678,1132 . ^{1}H-NMR ($CDCl_3$) δ: 10.4 (s, 1H, OH), 7.6—7.8 (m, Ar-H), 4.3 (s, 1H, –CH–), 3.5 (s, 1H, OH), 2.1—2.6 (m, –CH_2–), 3.4 (s, 6H, –CH_3). *Anal.* Calcd for $C_{24}H_{30}N_3O_9$: C, 57.25; H, 6.0. Found: C, 57.25; H, 6.0. Found: C, 57.88; H, 5.69. MS *m*/*z*: 503 (M^+). $[\alpha]_D^{25}$ 42.

(2*R*)-2-((3-(3,5-Bis(methoxycarbonyl)-2,6-dimethyl-4-(2-nitrophenyl)-pyridine-1(4*H*)-3-oxoacetyl)amino)-4-(methylthio)butanoic Acid (**3f**): Deep yellow solid, yield 51%, mp 153—155 °C. IR (KBr) cm^{-1}: 3415, 3122, 3025, 2955, 7748, 1128. ^{1}H-NMR ($CDCl_3$) δ: 11 (s, 1H, OH), 7.5—7.8 (m, Ar-H), 4.6 (s, 1H, –CH), 3.8 (s, 6H, –CH_3), 2.1—2.7 (m, –CH_2–). *Anal.* Calcd for $C_{24}H_{30}N_3O_9S$: C, 53.82; H, 5.64. Found: C, 53.87; H, 5.47. $[\alpha]_D^{25}$ 85.

(2*S*)-2-((3-(3,5-Bis(methoxycarbonyl)-2,6-dimethyl-4-(2-nitrophenyl)-pyridine-1(4*H*)-3-oxoacetyl)amino)-3-(1*H*-indol-3-yl)propanoic Acid (**3g**): Brownish yellow solid, yield 56%, mp 122—124 °C. IR (KBr) cm^{-1}: 3426, 3125, 3045, 1731, 2960. ^{1}H-NMR ($CDCl_3$) δ: 2 (1H, s, NH), 2.28 (s, 6H, CH_3), 2.3—2.8 (m, –CH_2), 3.6 (s, 6H, –CH_3), 3.1—3.3 (m, –CH_2–), 4.8 (s, 1H, –CH–), 7.2—7.9 (m, Ar-H), 11 (s, 1H, OH). *Anal.* Calcd for $C_{30}H_{31}N_4O_9$: C, 61.01; H, 5.29. Found: C, 60.561; H, 5.12. MS *m*/*z*: 590 (M^+). $[\alpha]_D^{25}$ −68.

(2*S*)-2-({2-[1,4-Dihydro-3,5-bis(methoxycarbonyl)-2,6-dimethyl-4-(2-nitrophenyl)pyridin-1-yl]-2-oxoethyl}amino)-4-carbamoylbutanoic Acid (**3h**): Brown solid, yield 56%, mp 150—152 °C. IR (KBr)cm^{-1}: 3437, 3245, 3160, 3046, 2950, 1748, 1148. ^{1}H-NMR ($CDCl_3$) δ: 11 (s, 1H, OH), 7.1 (1H, NH), 7.4—7.8 (m, Ar-H), 4.7 (s, 1H, –CH), 3.8 (s, 6H, CH_3), 2.2—2.8 (m, –CH_2). *Anal.* Calcd for $C_{24}H_{29}N_4O_{10}$: C, 54.13; H, 5.48. Found: C, 54.42; H, 5.13. $[\alpha]_D^{25}$ −44.

(2*R*)-2-({2-[1,4-Dihydro-3,5-bis(methoxycarbonyl)-2,6-dimethyl-4-(2-nitrophenyl)pyridin-1-yl]-2-oxoethyl}amino)-3-mercaptopropanoic Acid (**3i**): Brown solid, yield 54%, mp 129—131 °C. IR (KBr) cm^{-1}: 3418, 3116, 3024, 2960, 1709, 1685. ^{1}H-NMR ($CDCl_3$) δ: 2 (1H, s, NH), 2.3 (s, 6H, CH_3), 2.3—2.8 (m, –CH_2), 3.8 (s, 6H, –CH_3), 4.8 (s, 1H, –CH–), 7.5—7.9 (m, Ar-H), 10.6 (s, 1H, OH). ^{1}H-NMR ($CDCl_3$) δ: *Anal.* Calcd for $C_{25}H_{31}N_4O_{11}S_2$: C, 47.91; H, 4.98. Found: C, 48.08; H, 5.32. $[\alpha]_D^{25}$ −65.

(2*S*)-2-({2-[1,4-Dihydro-3,5-bis(methoxycarbonyl)-2,6-dimethyl-4-(2-nitrophenyl)pyridin-1-yl]-2-oxoethyl}amino)-2-(1*H*-imidazol-4-yl)acetic Acid (**3j**): Deep yellow solid, yield 53%, mp 127—129 °C. IR (KBr) cm^{-1}: 3390, 3167, 3032, 2960, 1732, 1134. ^{1}H-NMR ($CDCl_3$) δ: *Anal.* Calcd for $C_{25}H_{28}N_5O_9$: C, 50.38; H, 4.73. Found: C, 50.02; H, 4.11. 567 MS *m*/*z*: 596 (M^+). $[\alpha]_D^{25}$ −3.

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