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Ampicillin prodrugs: amide conjugates from amino acids and ampicillin

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Ampicillin (**1**) is an antibacterial agent used against infections caused by both gram negative and gram positive organisms. [1]. The β-lactam antibiotics are not very effectively absorbed after oral administration. Even compounds that show appreciable activity after oral administration, such as α-amino benzyl penicillin, are by no means fully absorbed from the gastro intestinal tract [2].

The free carboxylic acid function of the β-lactam antibiotics is necessary for their antibiotic activity, and larger mammals including man, lack an esterase capable of hydrolysing simple β-lactam antibiotic ester. Rasheed et al. synthesized some ampicillin conjugates from ampicillin with N-protected amino acids and reported that synthesized compounds had better activity and intestinal absorption [3]. Therefore it was thought worthwhile to synthesize and evaluate ampicillin conjugates with N-protected amino acids having a free carboxyl group. Thus the objective of this study was to investigate: (a) whether the amide derivatives of **1** would behave as prodrug (b) to what extent the physical properties of the synthesized amide conjugates vary with structure and (c) to seek synthesized conjugates of **1** which would be non-irritant, rapidly absorbed and rapidly hydrolysed to release free drug.

A series of conjugates of **1** were prepared using N-protected amino acids (valine, threonine, leucine, tryptophan and methionine). The structures of the synthesized amide conjugates were elucidated by IR and Mass spectroscopy.

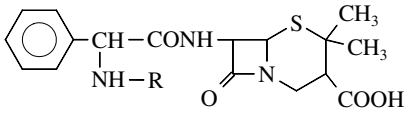
The compounds **AP1** and **AP3** showed better activity than the other synthesized compounds. A remarkable rise in intestinal absorption was observed for all the synthesized compounds except for compound AP4. The synthesized conjugates resisted hydrolysis in stimulated intestinal fluid but the rate of hydrolysis was enhanced in simulated intestinal fluid with 10% plasma (**AP1** > **AP2** > **AP5** > **AP3** > **AP4**). The high partition values are preferable for greater absorption through lipoidal cell membrane. Thus, it can be assumed that the compounds reported here behave as ideal prodrugs of **1**.

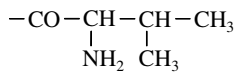
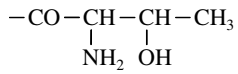
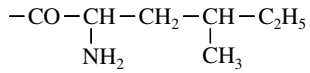
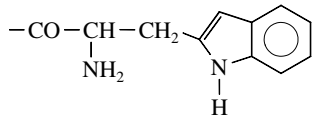
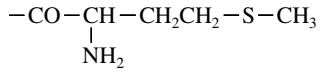
Experimental

The method reported by Toth et al. [4] was adopted to prepare t-Boc-valine, t-Boc-threonine, t-Boc-leucine, t-Boc-tryptophan, t-Boc-methionine. To the suspension of t-Boc amino acid (0.1 M), 6 ml of thionyl chloride and 2 ml of benzene were added and the mixture was refluxed for 1 h to get t-Boc amino acid chloride. The Schotten-Baumann reaction was used to get the conjugates. t-Boc amino acid chloride was made to react with **1** in the presence of methyl iso butyl ketone and potassium-2-ethyl hexanoate. The method reported by Toth et al. was followed to remove the protecting group using 3% of hydrochloric acid in methanol.

According the above procedure the following products were synthesized: Conjugate of **1** with L-valine (**AP1**), 6(α-amino iso valerionamido) phenyl amino acetyl 3,3 dimethyl-7-oxo-4-thia-1-aza-bicyclo (3,2,0) heptane-2-carboxylic acid, conjugate of **1** with L-threoine (**AP2**), 6(α-amino-β-hydroxy butyrionamido) phenyl amino acetyl 3,3 dimethyl-7-oxo-4-thia-1-aza-bicyclo (3,2,0) heptane-2-carboxylic acid, conjugate of **1** with L-leucine (**AP3**), 6(α-amino iso caprionamido) phenyl amino acetyl 3,3 dimethyl-7-oxo-4-thia-1-aza-bicyclo (3,2,0) heptane-2-carboxylic acid, conjugate of **1** with L-tryptophan (**AP4**), 6(α-amino-3-indole propionamido) phenyl amino

Table: Characteristics of the prodrugs



Compd.*	R	Yield (%)	M.P. (°C)	Partition coefficient	Plasma Protein binding (%)	Intestinal absorption after 60 min	Minimum inhibitory concentration (μg/ml)**				
							S. thypi	E. coli	S. aureus	P. aeruginosa	B. subtilis
1	–H	–	190–192	1.84	23.00	60.17	10	5	10	5	10
AP1		78	180–181	4.63	24.65	62.34	15	10	10	10	20
AP2		75	183–184	5.67	25.26	61.92	20	25	10	15	30
AP3		71	188–190	6.56	27.84	63.13	15	10	15	10	15
AP4		69	185–187	7.73	29.30	60.15	15	20	25	17	30
AP5		65	193–194	8.12	26.72	69.27	15	20	15	15	15

* All the compounds were analysed for C, H and N content. The results agreed within ±0.5% of the theoretical value. The IR spectra in KBr phase confirmed the presence of amide linkage in all the compounds. The m.p.'s were determined in open capillaries and are uncorrected. The molecular weight of compounds was determined by acidimetry. The mass spectra were also recorded

** A control set was run to ascertain sterlity of distilled water and no growth was observed

acetyl 3,3 dimethyl-7-oxo-4-thia-1-aza-bicyclo (3,2,0) heptane-2-carboxylic acid, conjugate of **1** with L-methionine (**AP5**), 6(α-amino-γ-methyl thio butyrionamido) phenyl amino acetyl 3,3 dimethyl-7-oxo-4-thia-1-aza-bicyclo (3,2,0) heptane-2-carboxylic acid. Their physical characteristics are reported in the Table.

The partition coefficient of the synthesized compounds was determined between octan-1-ol and phosphate buffer (pH 7.4) [5]. The plasma protein binding of the compounds synthesized was determined by the equilibrium dialysis method reported by Vanderbelt using cellophane membrane and saline phosphate buffer (pH 7.4) [6]. The rate of hydrolysis of the compounds was determined at 37 ± 0.5 °C in simulated intestinal fluid and simulated intestinal fluid +10% plasma [7]. The James and Norman in situ rat gut technique was used to perform the absorption studies [8]. To estimate antibacterial activity of **AP1**, **AP2**, **AP3**, **AP4**, **AP5** and **1** the minimum inhibitory concentration (MIC) against *S. typhi*, *E. coli*, *S. aureus*, *P. aeruginosa* and *B. subtillis* was determined [9].

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Received October 30, 2000
Accepted Febuary 10, 2001

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A validated gas chromatographic method for the determination of isosorbide-5-mononitrate in human plasma and its application to pharmacokinetic studies

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Isosorbide-5-mononitrate (ISMN), the main pharmacologically active metabolite of isosorbide dinitrate (ISDN), is a vasodilator agent used in the prophylaxis and treatment of angina pectoris. ISMN, unlike ISDN, does not undergo first-pass metabolism in the liver, thus its absolute bioavailability is almost 100% [1, 2]. The ISDN concentration data show a considerable variability, as expected for a drug subject to first-pass metabolism [3]. This prompts the analysis of ISMN plasma concentrations in order to study the bioequivalence of ISDN formulations.

In recent years, a number of different GC methods coupled with electron-capture detection or mass spectrometry have been employed for the determination of ISMN in human plasma [4–8]. However, either these methods were not sufficiently sensitive or require expensive instruments or sample preparation techniques. The study of pharmacokinetic parameters in humans requires a sensitive and reliable analytical method. The typical C_{max} values of ISMN after a sublingual dose of 5 mg of ISDN are approximately 35 ng/ml [9]. Therefore, the objective of this study was to develop and validate a capillary GC method suitable for the quantitation of ISMN in real human plasma samples obtained in bioequivalence and pharmacokinetic studies, which require high sensitivity and selectivity.

Typical chromatograms obtained with a drug-free plasma and a plasma sample containing 19.5 ng/ml of ISMN obtained from a subject 12 h post-administration, after a single sublingual dose of 5 mg ISDN show no peaks, due to endogenous plasma components or ISDN, interfering with ISMN or internal standard. The retention times of ISMN and internal standard were 2.63 and 4.27 min, respectively. The total run time for an assay was approximately 5 min. The assay was validated over the concentration range 2.5 to 50 ng/ml ISMN. Data were obtained through linear regression analysis of peak height ratios versus concentrations of added ISMN. A weighting factor of 1/concentration was employed. The method was linear over the concentration range 2.5 to 50 ng/ml using a 0.5-ml plasma sample. A typical calibration curve had the regression equation of $y = 0.1626 + 0.1918x$ ($r = 0.9994$). Minimum quantitation limit was 2.5 ng/ml.

Inter-run assay precision assessed as the coefficient of variation values (CV%) was found to be 5.7%, 4.4%, and 4.2% for samples containing 10, 20, and 30 ng/ml, respectively. Inter-run accuracy, assessed as the relative error, was between 98.8% to 101.0%. Recovery was based on direct comparison of peak heights. The mean recoveries for ISMN were between $96.4\% \pm 6.5\%$ to $105.0\% \pm 3.1\%$. Mean recovery of internal standard was $100.9\% \pm 3.8\%$. Slightly higher than 100% recoveries are most likely due to partial evaporation of acetonitrile during sample preparation. System reproducibility expressed as the coefficient of variation values (CV%) and based on absolute peak height, was 8.4% and 6.8% for ISMN and 9.6% for internal standard.

In conclusion, a rapid, specific, sensitive, precise and accurate GC method with electron-capture detection for the