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Ampicillin prodrugs: amide conjugates from aminoacids, peptide 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]. Despite of the outstanding clinical success of the β-lactam antibiotics, ineffective absorption of these compounds, particularly following oral administration has continually plagued investigators in this field. Even compounds that show appreciable activity after oral administration, such as α-amino benzyl penicillin (ampicillin) are by no means fully absorbed from the gastro intestinal tract [2]. Therefore it was thought worthwhile to synthesize and evaluate ampicillin amide conjugates with amino acids and peptide having a free carboxyl function. Thus the objective of this study was to investigate; (a) whether amide derivatives of **1** would behave as prodrug (b) to what extent the physical properties of the prodrug vary with structure (c) to what degree the *in vitro* cleavage rates vary with structure and (d) to seek prodrug derivatives of **1** which would be non-irritant, readily absorbed in the GIT and rapidly hydrolysed to release free drug.

A series of conjugates of **1** were prepared using N-protected amino acids (glycine, alanine, phenylalanine, histidine, glycylglycine). The structures of the synthesized conjugates were elucidated by IR and Mass spectroscopy.

A remarkable rise in acitivity was observed for AD2 and AD3 compounds. The prodrugs resisted hydrolysis in simulated intestinal fluid but the rate of hydrolysis was enhanced in 10% plasma and simulated intestinal fluid (AD2 > AD1 > AD5 > AD3 > AD4). The high partition values are preferable for greater absorption through lipoidal cell membrane. Thus, it would be fair to assume that compounds reported here would behave as ideal prodrugs of **1.**

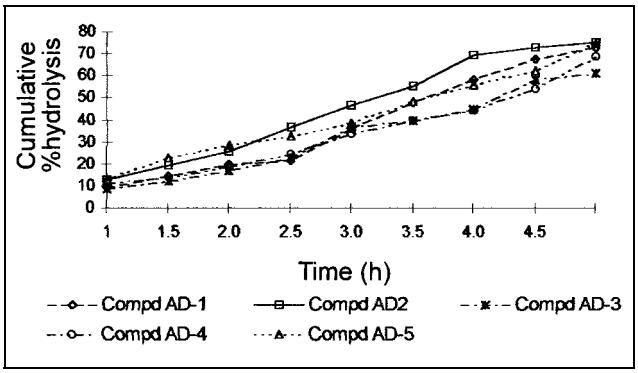
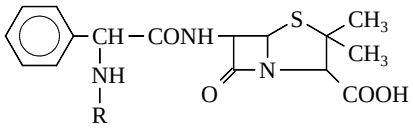


Fig.: *In vitro* hydrolysis of synthesized compounds in simulated intestinal fluid + 10% Plasma

Experimental

The method reported by Toth and Williaum [3] was adopted to prepare t-Boc-phenylalanine, t-Boc-glycine, t-Boc-alanine, t-Boc-histidine, t-Boc-glycylcine. A suspension of t-Boc aminoacid (0.1 M) and 6 ml of thionyl chloride and 2 ml of benzene was added and the mixture was refluxed for 1 h to get t-Boc aminoacid chloride which was made to react with **1** in the presence of methyl iso butyl ketone and potassium-2-ethyl hexanoate to get the conjugates. The reported method [3] was followed to remove the protecting group using 3% of hydrochloric acid in methanol.

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. aureus	*E. coli*	*K. pneumoniae*	*S. typhi*
1	–H		190–192	1.85	24.00	60.5	10	5	5	10
AD1	$-CO-CH_2NH_2$	76	193–194	5.25	25.36	61.2	15	40	20	20
AD2	$-CO-\underset{\underset{NH_2}{|}}{CH}-CH_3$	74	180–181	4.55	24.92	62.29	10	10	15	40
AD3	$-CO-\underset{\underset{NH_2}{|}}{CH}-CH_2-C_6H_5$	72	182–183	5.66	26.96	59.5	15	10	10	15
AD4	$-CO-\underset{\underset{NH_2}{|}}{CH}-CH_2-C_3H_3N_2$	71	185–186	7.33	27.50	63.24	25	20	10	10
AD5	$-CO-CH_2NHCOCH_2NH_2$	69	189–190	10.11	25.50	76.29	15	10	15	20

* 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 sterility of distilled water and no growth was observed

According this procedure the following products were synthesized: conjugate of **1** with L-glycine (AD1), 6(α-amino acetamido) phenyl amino acetyl 3,3 dimethyl-7-oxo-4-thia-1-aza-bicyclo (3,2,0) heptane-2-carboxylic acid, conjugate of **1** with β-alanine (AD2), 6(α-amino propionamido) 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-phenyl alanine (AD3), 6(α-amino β-phenyl propionamido) 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-histidine (AD4), 6(α-amino-β-imidazole propionamido) phenyl amino acetyl 3,3 dimethyl-7-oxo-4-thia-1-aza-bicyclo (3,2,0) heptane-2-carboxylic acid, conjugate of **1** with glycylglycine (AD5), 6(*N*-glycylglycinamido) 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) [4]. The plasma protein binding studies of the synthesized compounds was determined by the equilibrium dialysis method reported by Vander belt using a cellophane membrane and saline phosphate buffer (pH-7.4) [5]. The rate of hydrolysis of the compounds was determined at 37 °C in simulated intestinal fluid (pH-7.4) [6]. The results are shown in the Fig. The antibacterial activity of AD1, AD2, AD3, AD4, AD5 and **1** was determined as minimum inhibitory concentration (MIC) against S. aureus, E. coli, K. pneumoniae and S. Typhi [7]. The James and Norman in situ rat gut technique was used to perform the absorption studies of the synthesized compounds [8].

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Intramolekulare ANRORC-Reaktion ausgehend von Pyrano[4,3-*d*]pyrimidinen – eine neue Methode zur Synthese von Thieno[2,3-*d*]pyrimidinen

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Funktionalisierte Thienopyrimidine sind durch ein breites pharmakologisches Wirkungsspektrum gekennzeichnet [1–3]. So zeigen Thieno[2,3-*d*]pyrimidine, die in 2- und 4-Position mit Aryl- oder Alkylgruppen substituiert sind, unter anderem analgetische, entzündungshemmende, blutzuckersenkende Eigenschaften [1, 2, 4]. Verschiedene 2- oder 4-Aryl-thieno[2,3-*d*]pyrimidine, die in 5-Position mit einer Amino- und in 6-Position mit einer Carbonsäureestergruppe funktionalisiert sind, besitzen antibakterielle Eigenschaften [1, 5–7].
Thieno[2,3-*d*]pyrimidine mit einer Carbonsäureester- oder einer anderen Acceptorgruppe in 6-Position können durch Umsetzung funktionalisierter Pyrimidinthione mit acceptorsubstituierten Halogenmethanen erhalten werden. Hierbei erfolgt eine Cyclisierung der zunächst gebildeten Thioether unter Anellierung eines Thiophenringes [1, 2, 4, 8]. Eine elegante Synthesevariante für die als Ausgangsstoffe benötigten Pyrimidinthione geht von Diaryl-dithiazoliumsalzen **1** aus [9–11]. Die **1** sind durch Oxidation aromatischer Thioamide leicht zugänglich [10]. Bei Einwirkung acceptorsubstituierter Acetonitrile auf **1** erfolgt Ringöffnung und Schwefel-Extrusion, so daß die Thioamide **2** gebildet werden. Die **2** werden in der Regel nicht isoliert, sie unterliegen Cyclisierungsreaktionen [9, 10, 12, 13].
Das Cyclisierungsverhalten der aus Dithiazoliumsalzen **1*** mit Malodinitril oder Cyanessigsäureethylester erhaltenen Thioamide **2** wird durch die funktionelle Ausrüstung des Arylsubstituenten geprägt. Ist Ar ein unsubstituierter- oder substituierter [10] Phenylrest, so erfolgt eine cyclisierende Addition des Thioamid-Schwefel-Atoms an die Nitrilgruppe, so daß – via Iminothiazine – Pyrimidinthione **4** erhalten werden [9, 10]. Die **4** können mit acceptorsubstituierten Halogenmethanen wie z. B. Chloressigsäuremethylester zu den Thioethern **6** umgesetzt werden. Durch Thorpe-Dieckmann-Cyclisierung der **6** sind die substituierten Thieno[2,3-*d*]pyrimidine **8** zugänglich.
Ist Ar eine o-Hydroxyphenyl-Gruppe, so ist es nicht möglich, Pyrimidinthione zu erhalten. Statt dessen werden unter Eliminierung von Schwefelwasserstoff Benzoxazine **3** gebildet [12], die anschließend in Pyrano[4,3-*d*]pyrimidine **5** überführt werden können [13].
Die Pyrano[4,3-*d*]pyrimidine **5** weisen mit ihrer in den Pyranring eingebundenen cyclischen Imidsäureester- bzw. Lactonstruktur eine elektrophile Gruppe auf. Somit sollte es möglich sein, dieses Strukturelement für weiterführende Ringumwandlungsreaktionen zu nutzen. Daher wurden Versuche zur Darstellung von Thienopyrimidinen unternommen.
Durch Umsetzung der **5** mit Chloressigsäuremethylester wurden die Thioether **7** in guten Ausbeuten (76 bzw. 87%) erhalten. Diese Thioether sollten nach Deprotonierung der aktiven Methylengruppe, gefolgt von einem nucleophilen Angriff des Carbanions auf das 5-ständige (Imino)carbonyl-C-Atom des Heterocylcus in einer intramolekularen ANRORC-Reaktion (addition of a nucleophile, ring opening and ring closure) [14] zu Thieno[2,3-