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Review Article

Organic carbamates in drug development. Part II: antimicrobial agents – recent reports

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Abstract

The continuous evolution of new microbes that are resistant to available antimicrobial agents poses a serious problem in the medical world and presents a challenge for the development of newer agents with a broadened spectrum of activity and improved pharmacological and pharmacokinetic properties. In this area, a number of organic carbamates have emerged in the recent past as potential antibacterial and antiviral agents. The carbamate residue present in such molecules either contributes as a core component or towards improvement of their pharmacological and pharmacokinetic properties. Such compounds include both natural and synthetic products. In this review, recent data on carbamate antibacterial and antiviral agents are discussed.

Introduction

In our recent review on organic carbamates in the design of anticancer agents (1), their main application was concluded to be in the development of prodrugs. However, in the field of antimicrobial agents, the carbamate residue is generally part of the core molecule, as in oxazolidinones, or incorporated into a known molecule to improve its pharmacokinetic properties, including metabolic stability, and broaden its activity profile.

The development of antimicrobial agents is a continuing process with the objective of obtaining a broader spectrum of antimicrobial activity and covering emerging new strains of viruses and bacteria that are resistant to available antimicrobial agents. In this area, various organic carbamates with promising antimicrobial activity have been reported, certain of which have found clinical application.

In this review, although not exhaustive, an attempt has been made to cover carbamate compounds showing promising antibacterial and antiviral activity reported from 1990 onwards.

Carbamates as antibacterial agents

Semisynthetic carbamate derivatives of known antibiotics

1. Macrolide carbamates

Erythromycin derivatives

Macrolide antibiotics, including erythromycin (**1**) and clarithromycin (**2**), represent an important class of antibiotics with activity against Gram-positive bacteria. As part of the ongoing research on this class of compounds to

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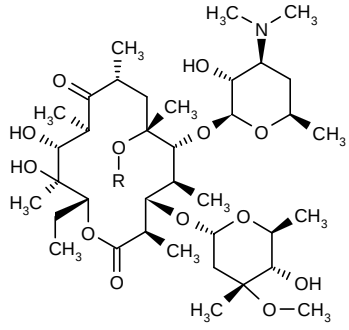
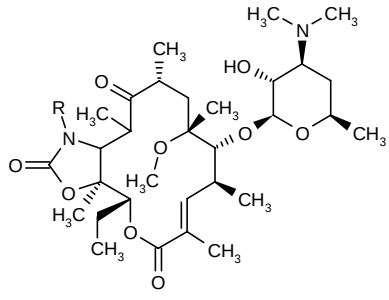
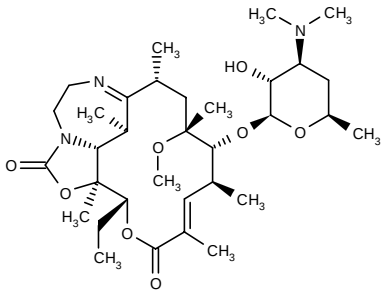


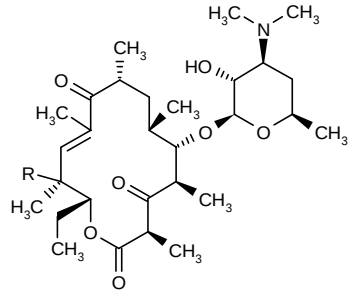
(**1**) Erythromycin A, R = H
(**2**) Clarithromycin, R = Me



(**3**) C-10α (natural)
C-10β (unnatural)

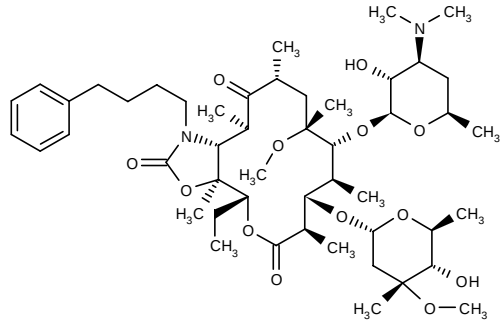


(**4**)



(**5**) Narbomycin, R = H
(**6**) Pikromycin, R = OH

achieve greater efficacy, safety, stability and a broader spectrum of activity, a number of cyclic esters of erythromycin fused at the 11,12-position were synthesized and evaluated for antibacterial activity. Arylalkyl-substituted carbamate esters (**3**; R = arylalkyl) were found to possess better antibacterial activity in *in vitro* studies. The natural C-10α analogues were found to be superior to the corresponding unnatural C-10β analogues. The bridged 9,11-iminoethylcarbamate **4** had good overall activity in the same range as the arylalkylcarbamates **3**. However, none of the derivatives showed activity better than clarithromycin in *in vivo* studies (2).

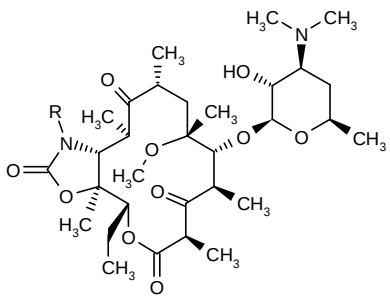


(**7**) A-66321

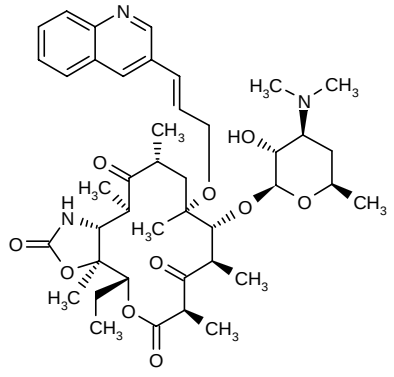
Ketolides

It was previously thought that in the macrolide structure the cladinose sugar residue at position 3 played a major role in drug resistance (3, 4) and antibacterial effect. However, replacement of the cladinose sugar by a keto group led to the ketolides narbomycin (**5**) (5) and pikromycin (**6**) (6), which showed antibacterial activity of a low order. In another study (7), the introduction of a cyclic 11,12-carbamate in macrolides produced the carbamate A-66321 (**7**), which was active against macrolide-resistant organisms.

In view of the above observations, 11,12-carbamate and carbazate derivatives of 3-ketolides were prepared which showed promising activity. The keto group was found to produce greater acid stability and to assist in binding of the ligand to the ribosomal target, without causing macrolide-lincosamide-streptogramin B (MLS_B) resistance in inducible strains. The introduction of a heteroarylalkyl chain on the carbamate nitrogen was also found to be beneficial. One of the most promising carbazates was HMR-3004 (**8**), with potent activity against multidrug-resistant *Streptococcus pneumoniae*, as well as strong activity against *Haemophilus influenzae*, *Moraxella catarrhalis,* group A streptococci and atypical bacteria, *e.g., Chlamydia, Mycoplasma* and *Legionella* (8). Unlike macrolides, HMR-3004 was not an inducer of MLS_B resistance (9, 10).



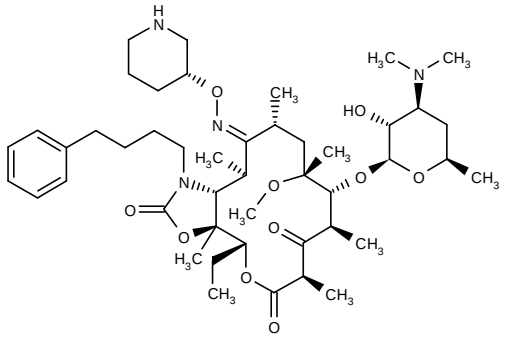
(**8**) HMR-3004, R = 4-quinolyl-$(CH_2)_3$-NH
(**9**) Telithromycin, R = 4-(3-pyridyl)-1-imidazolyl-$(CH_2)_4$



(**11**) Cethromycin

Another compound which has emerged as a potent drug is telithromycin (**9**, HMR-3647) (11, 12), the first member of the new family of the MLS_B class of antibacterial agents. Telithromycin is very active against erythromycin-susceptible and -resistant pathogens, including penicillin-resistant *S. pneumoniae* and *H. influenzae*. It has shown clinical efficacy in human respiratory tract infections (13) and was recently introduced for the treatment of community-acquired pneumonia (CAP), acute exacerbation of chronic bronchitis (AECB), acute maxillary sinusitis (AMS) and pharyngitis/tonsillitis. It has a low potential for inducing cross-resistance among other MLS_B macrolides and also shows favorable pharmacokinetics following oral administration; it is well absorbed, achieves good plasma levels and is highly concentrated in pulmonary tissues and white blood cells. Telithromycin is therefore a well-tolerated once-daily oral therapy for the treatment of respiratory infections.

Both ketolides, HMR-3004 and telithromycin, are active against *Toxoplasma gondii in vitro* and in murine models of infection (14). In a further modification of the ketolides, 9-oximino derivatives were synthesized (15). The 9-[3(*R*)-piperidinyl]oxime 11,12-carbamate (**10**) displayed improved antibacterial activity against *S. pneumoniae* and *Streptococcus pyogenes* resistant to erythromycin.



(**10**)

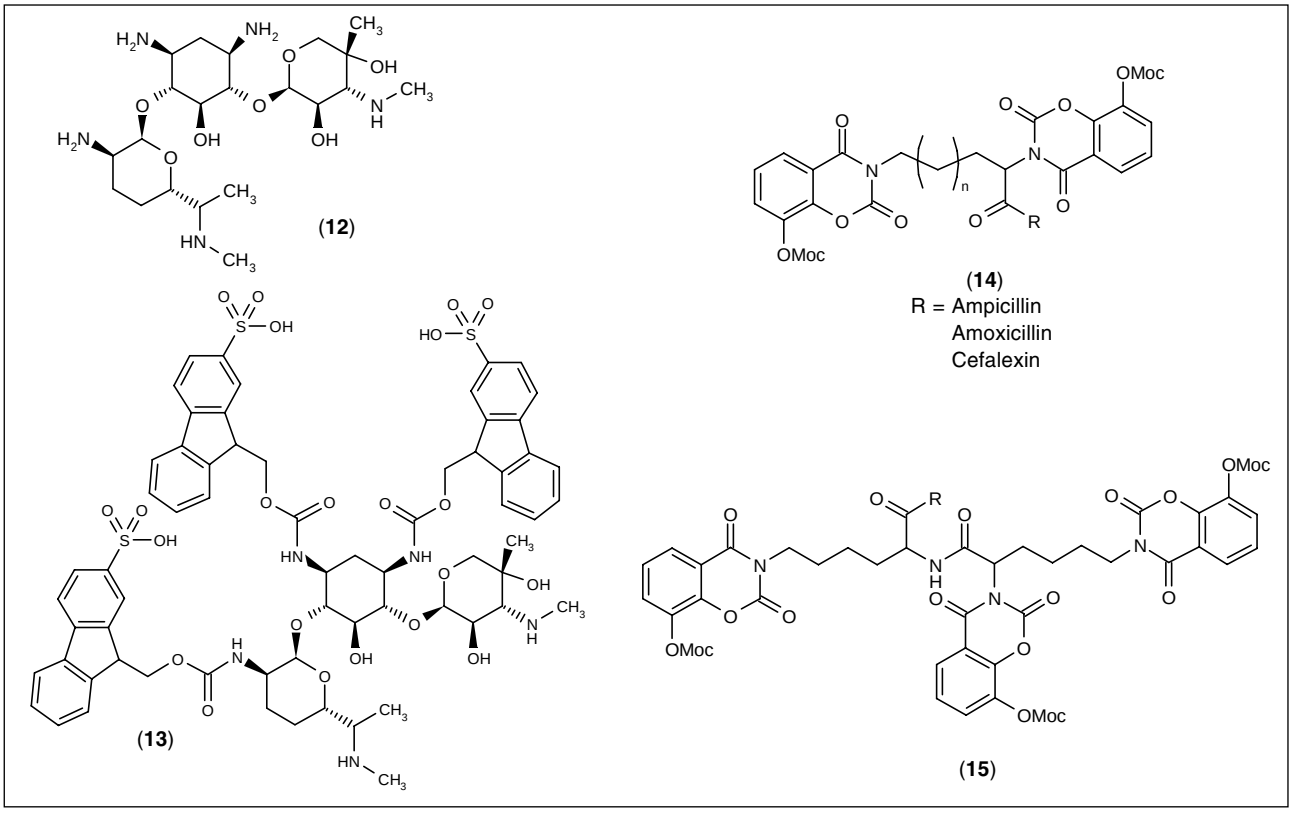
A new ketolide with a broad antibacterial spectrum, including activity against penicillin- and macrolide-resistant Gram-positive bacteria, is cethromycin (**11**, ABT-773) (16). Cethromycin is active against common respiratory tract pathogens such as *S. pneumoniae, H. influenzae* and *M. catarrhalis*. It is the most active antimicrobial tested against *S. pneumoniae*. Cethromycin and azithromycin are equivalent in activity against *H. influenzae* and *M. catarrhalis,* and more active than either clarithromycin or erythromycin (17). Cethromycin showed tighter ribosome binding than erythromycin and also accumulated in macrolide-sensitive *S. pneumoniae* at a higher rate, inhibiting protein synthesis in this microorganism (18).

2. Gentamicin carbamates

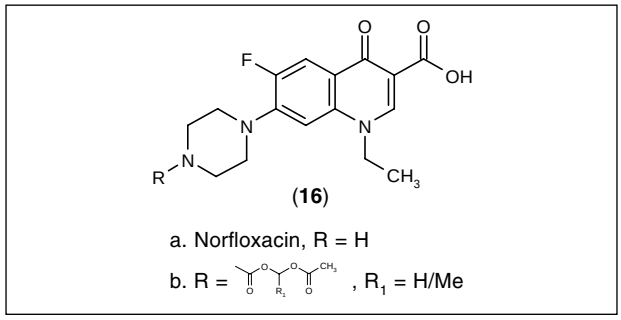
N-[(2-Sulfo)-9-fluorenylmethoxycarbonyl]$_3$-gentamicin C_1 (FMS_3-gentamicin, **13**) was prepared as a prodrug of gentamicin (**12**) to increase its half-life (19). Gentamicin as such is rapidly eliminated from the body through glomerular filtration in the kidneys. It was observed that when the amino groups present in gentamicin are linked to 3 or more (2-sulfo)-9-fluorenylmethoxycarbonyl (FMS) groups, its affinity towards human serum albumin (HSA) increases. FMS_3-gentamicin associates with HSA with a K_a value of $1.31 \pm 0.2 \times 10^5\ M^{-1}$. Although the FMS_3-gentamicin complex has less than 1% the antibacterial potential of gentamicin, upon incubation at pH 8.5 and 37 °C, it undergoes slow hydrolysis to release gentamicin ($t_{1/2}$ = 8.0 ± 0.2 h) and generate antibacterial activity. In rats, intravenous or subcutaneous administration of FMS_3-gentamicin maintained a prolonged pharmacokinetic profile, with peak and trough concentrations of active gentamicin sustained over 4-5 times longer than after administration of gentamicin itself.

3. Carbamate siderophores as β-lactam conjugates

Carbamate siderophores attached to β-lactam antibiotics improve their penetration, thereby enhancing their



antibacterial activity. Thus, compounds **14** and **15**, prepared by conjugation of bis- and tris-catecholate hydroxamates as siderophores, showed high *in vitro* activity against Gram-negative bacteria, especially *Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae, Serratia marcescens* and *Stenotrophomonas maltophilia* (20). These compounds use active iron uptake routes to penetrate the bacterial outer membrane barrier for enhanced antibacterial activity.



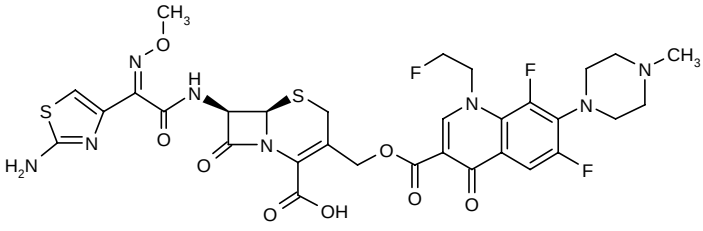
4. Norfloxacin carbamates

Norfloxacin (**16a**) is a synthetic second-generation quinolone antibacterial agent with broad-spectrum activity against Gram-positive and Gram-negative bacteria. Due to its poor oral absorption (35-40%) and disagreeable taste, molecular modifications have been attempted. (Acyloxy)alkylcarbamate derivatives (**16b**) have been reported by Alexander *et al.* (21). The prodrugs prepared were rapidly hydrolyzed by rat and dog serum esterases, but slowly in human serum. Their bioavailability was also poor due to their low water solubility.

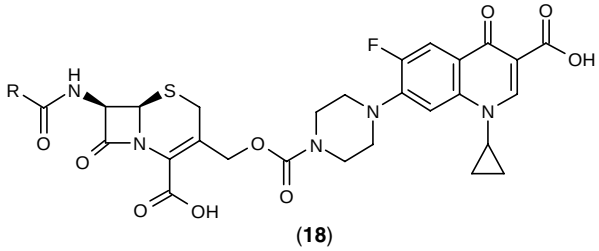
5. β-Lactam-quinolone conjugates

Cephalosporins conjugated to an antibacterial quinolone at the 3'-position through a carbamate link have been developed as dual-action antibacterial agents. The design of such antibacterial compounds aims at combining the complementary behavior of the two antibacterial classes, quinolones being active against β-lactam-resistant strains and cephalosporins having more potent activity against streptococci. One such compound is Ro-23-9424 (**17**) (22), which has reached clinical trials.

In order to develop an antibacterial agent with better solubility, superior pharmacokinetics and enhanced stability, carbamate-linked cephalosporin-quinolone conjugates of structure **18** have also been prepared (23). In mice, Ro-24-4383 (**18a**) was found to be more effective than cefotaxime against infections due to *P. aeruginosa* or *Staphylococcus* spp., and better than ciprofloxacin against *S. pneumoniae*. It could not be ascertained whether the antibacterial activity of these conjugates was due to the intact molecule or their hydrolyzed cephalosporin and quinolone components.

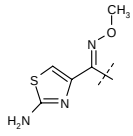


(**17**) Ro-23-9424



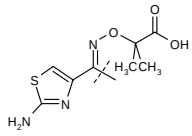
(**18**)

a. Ro-24-4383, R =

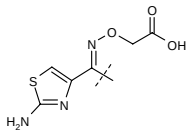


b. R = CH_2OPh

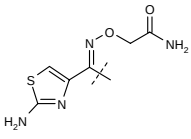
c. R=



d. R =



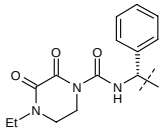
e. R =



f. R = H

g. R = CH2CN

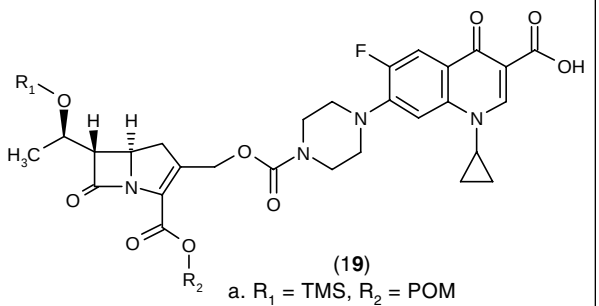
h. R =



i. R =

In a similar approach, penems and carbapenems were linked to a quinolone at the 2'-position through a carbamate moiety for the preparation of broad-spectrum antibacterial compounds (24). Of the carbapenem derivatives **19** and **20**, optically active **20** exhibited excellent activity against Gram-negative pathogens, including good antipseudomonal potency, and high activity against the Gram-positive pathogens *Staphylococcus aureus*, *S. pneumoniae* and *S. pyogenes*.

The carbamate-linked penem **21** showed weaker activity against anaerobes but much better activity against Gram-negative and Gram-positive organisms, including *P. aeruginosa,* due to the contribution of the β-lactam and quinolone antibacterial moieties.

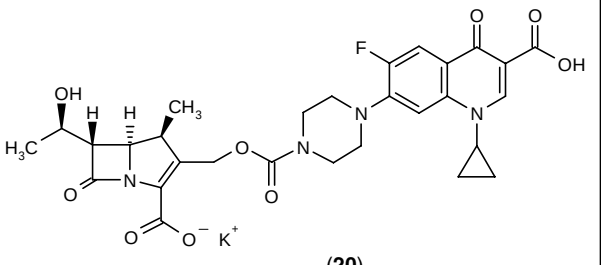


(**19**)

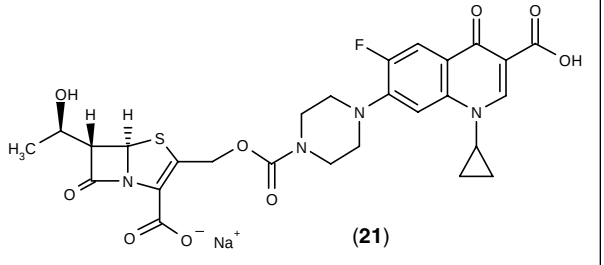
a. R_1 = TMS, R_2 = POM

b. R_1 = H, R_2 = POM

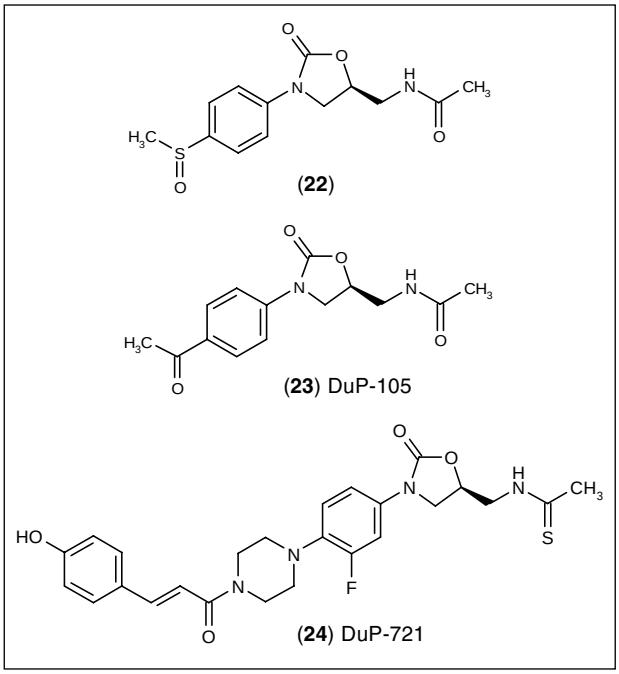
c. R_1 = H, R_2 = Na

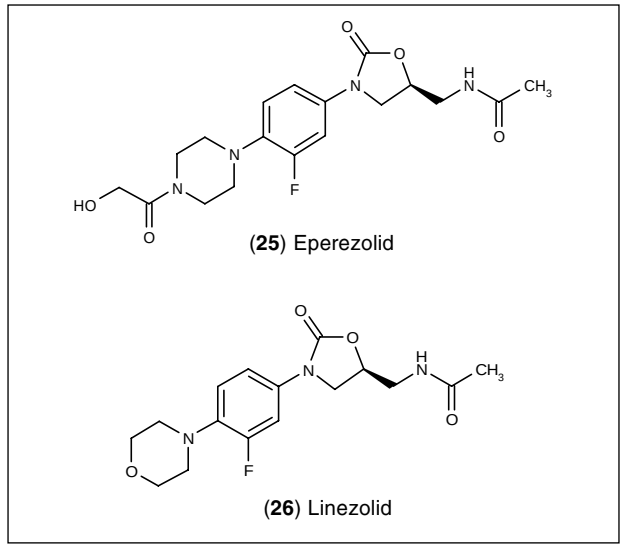


(**20**)



(**21**)





6. Oxazolidinone antibacterial agents

The antibacterial activity of oxazolidinones was discovered through random screening (25-28). Two compounds, DuP-105 (**22**) and DuP-721 (**23**), showed *in vitro* activity against staphylococci, streptococci, enterococci, anaerobic bacteria and mycobacteria (29). However, the development of these compounds was discontinued in phase I clinical trials (30).

Oxazolidinones were found to inhibit protein synthesis, but not RNA or DNA synthesis, which suggested that the inhibition occurs at an early phase in the initiation stage of protein synthesis (31, 32).

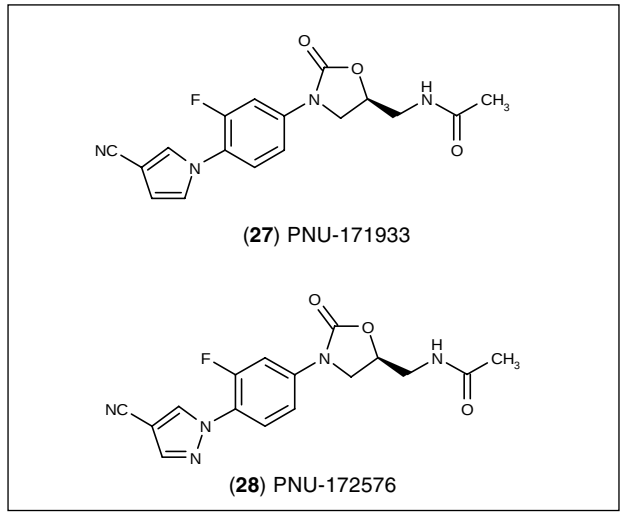
In order to optimize the biological profile, structure-activity relationship studies were undertaken by Lohray *et al.* (33) and Gregory *et al.* (34) using substitutions at the aryl group and position 5 of 3-aryl-2-oxazolidinones. The piperazine analogue **24** showed potent activity against a wide range of Gram-positive bacteria.

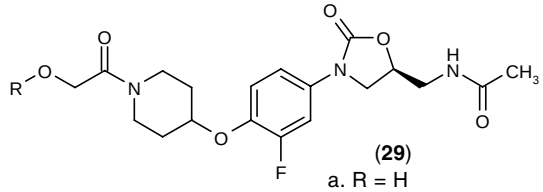
Some dependence on the nature of A and B groups was observed, acetamide substitution in B generally yielding compounds with better activity.

In further modification studies, eperezolid (**25**, U-100592) and linezolid (**26**, U-100766) emerged as promising agents with reduced toxicity (35). These two antibacterial agents are not cross-resistant with vancomycin against enterococci or with penicillin against pneumococci. They elicit bacteriostatic activity against staphylococci and enterococci, but are bactericidal against streptococci. Their *in vivo* activity against Gram-positive organisms is similar to vancomycin (36). Linezolid (Zyvox®) is currently marketed for the treatment of multidrug-resistant Gram-positive infections such as nosocomial and community-acquired pneumonia and skin infections.

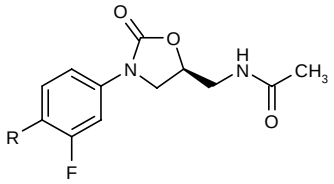
In order to increase the Gram-positive activity of linezolid and also to broaden its spectrum to include Gram-negative pathogens such as *H. influenzae* and *M. catarrhalis*, further modification of oxazolidinones was attempted, leading to the discovery of the (pyrrolophenyl)- and (pyrazolylphenyl)oxazolidinones PNU-171933) (**27**) and PNU-172576 (**28**). Both compounds showed potent antibacterial activity *in vitro* against Gram-positive and Gram-negative pathogens and were very effective *in vivo* against *S. aureus* and *S. pneumoniae* (37).

Recently, Weidner-Wells *et al.* (38, 39) replaced the morpholino group of linezolid with piperidinyloxy, pyrrolidinyloxy and azetidinyloxy groups. In contrast to linezolid, none of these agents exhibited significant activity against Gram-negative organisms such as *E. coli*. Two of the most active piperidinyloxy-substituted derivatives (**29**) were only 2-4-fold less potent than linezolid against

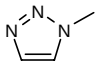


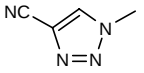


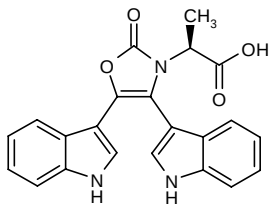
(**29**)
a. R = H
b. R = CH_2Ph



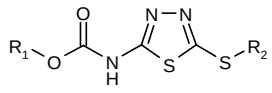
(**30**) R = 

(**31**) R = 

(**32**) R = 



(**33**)



(**34**)

Gram-positive pathogens. The effect on antibacterial activity of various substituents at the piperidine nitrogen was evaluated. Diverse functional groups were tolerated on the piperidinyl nitrogen, although bulky substituents had a detrimental effect on antibacterial activity. The most potent compound with an α-hydroxy acetamide substituent (**29a**) showed *in vivo* efficacy against *S. aureus* infection.

Replacement of the morpholino residue of linezolid with an azolyl moiety such as pyrrole, pyrazole, imidazole, triazole or tetrazole resulted in the preparation of compounds with good activity against both Gram-positive and Gram-negative bacteria (40). The pyrrole-substituted analogue **30** and the 1*H*-1,2,3-triazolyl analogue **31** were active against *H. influenzae* and *M. catarrhalis*. Substitution on the azolyl moiety suggested a strong relationship between activity and electronic character of the ring. Thus, aldehyde, aldoxime and cyanoazoles were generally more active against Gram-positive and Gram-negative pathogens. In particular, the 3-cyanopyrrole (**27**), 4-cyanopyrazole (**28**) and 4-cyano-1*H*-1,2,3-triazole compounds (**32**) had MIC values against *S. aureus* of ≤ 0.5-1 μg/ml, and of 2-4 μg/ml against *H. influenzae* and *M. catarrhalis*. These analogues were also effective against *S. aureus* and *S. pneumoniae* in mouse models of infection, with ED_{50} values in the range of 1.2-1.9 mg/kg *versus* 2.8-4.0 mg/kg for eperezolid (37).

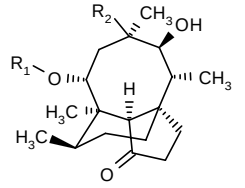
The 3-*N*-substituted-4,5-bis(3-indolyl)oxazol-2-ones were inactive against the Gram-negative microorganism *E. coli* and the fungus *Candida albicans,* and only the compound **33** exerted growth-inhibitory effects *in vitro* against the Gram-positive bacterium *S. chartreusis* (41).

7. 1,3,4-Thiadiazole carbamates

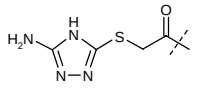
Based on the previously reported antimicrobial activity of 1,3,4-thiadiazole compounds (42-44), carbamate derivatives of 5-thio-1,3,4-thiadiazoles (**34**) were investigated for their antimicrobial activity (45). All the thiadiazole analogues were found to be less active compared to ampicillin. Most analogues that exhibited activity against Gram-negative bacteria were selective for *P. aeruginosa*, the most potent being the alkylthio analogues.

8. Mutilin carbamates

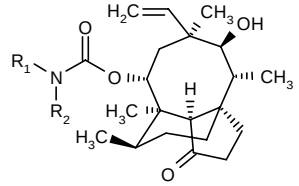
The antibiotic pleuromutilin (**35**) was discovered in 1951 (46), but remained underexploited until its semisynthetic analogues tiamulin (**36**) and TDM-85530 (**37**), with improved antibacterial activity, were developed. The former was developed for veterinary use, while the latter was discontinued in phase I clinical trials (47).



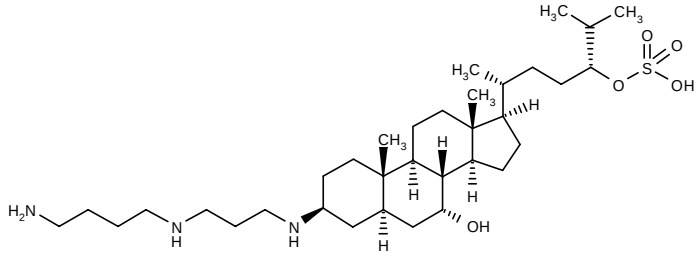
(**35**) Pleuromutilin, $R_1 = COCH_2OH$, $R_2 = CH{=}CH_2$
(**36**) Tiamulin, $R_1 = COCH_2SCH_2CH_2N(Et)_2$, $R_2 = CH{=}CH_2$

(**37**) TDM-85530, R =  $R_2 = CH_2CH_3$

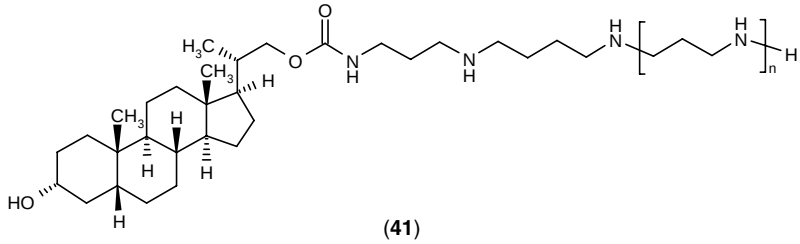
(**38**) Mutilin, $R_1 = H$, $R_2 = CH{=}CH_2$



(**39**)
a. $R_1 = SO_2Ph$, $R_2 = H$
b. R_1 = 4-MeO-PhCO, $R_2 = H$



(**40**) Squalamine



(**41**)

Pleuromutilin exerts its antibacterial activity by inhibiting bacterial protein synthesis through an interaction with the prokaryotic ribosome (48). This mode of action has the advantage of endowing these compounds with no clinically relevant cross-resistance with other classes of antibacterial agents. Structure-activity relationship studies with mutilin antibiotics underscored the importance of the C-14 side-chain (49).

In order to expand the antibacterial profile of mutilin (**38**) antibiotics, novel C-14 ether, amide, urea and carbamate derivatives were prepared (50). Of these, ether, amide and acylurea derivatives showed reduced activity, whereas carbamates (**39**) demonstrated potent activity against Gram-positive and Gram-negative bacteria, including methicillin-resistant *S. aureus* and macrolide-resistant *S. pneumoniae*.

The *N*-arylsulfonyl (**39a**) and *N*-aroyl (**39b**) carbamates displayed excellent broad-spectrum antibacterial activity. The *N*-aroyl carbamate possessed potent *in vitro* activity against *S. aureus, S. pneumoniae* and *H. influenzae*.

9. Polyamine carbamates of sterols

The steroid backbone has been found to play an important role in antimicrobial activity. Some sterol polyamine conjugates and squalamine (**40**) analogues exhibit antimicrobial activity (51-53). Based on these observations, spermidine (**41**; n = 0) and spermine derivatives (**41**; n = 1) were synthesized (54) and evaluated for their antimicrobial activity. *In vitro* evaluation of these carbamate derivatives suggested that: 1) 5β-conjugates were more active than 5α-conjugates; and 2) a hydroxyl or fluoro substitution at C-7α increased the antibacterial activity.

Carbamates as antiviral agents

Development of HIV protease inhibitors as antiviral agents

The most dreaded and widespread viral disease to which human beings are exposed today is the acquired immunodeficiency syndrome, or AIDS. The causative agent of AIDS is the human immunodeficiency virus, or HIV. This virus encodes a homodimeric aspartyl protease that is required for viral replication (55, 56). Inhibition of this enzyme results in the production of morphologically altered, noninfectious viral particles. Therefore, inhibition of HIV-1 protease has become a major target in the development of drugs for AIDS (57).

For the design of drugs to inhibit HIV protease, the most common approach had been the use of structures (58) that mimic the transition state (59, 60) from the cleavage of the enzyme's natural substrate. In the recent past, several drugs, namely ritonavir (61), saquinavir mesilate (60), nelfinavir mesilate (62) and amprenavir (63), have been introduced. However, the need for newer protease inhibitors continues because of drawbacks of existing drugs, such as dose-limiting and potentially long-term idiosyncratic toxicities, complex dosing patterns in combination with other antiviral agents and the emergence of drug resistance (64). Different classes of compounds have been developed in an attempt to identify protease inhibitors, as discussed below.

1. Hydroxyethylamine class

The role of HIV protease in the development of AIDS was identified through site-directed mutagenesis studies (55, 56, 65). Structural studies of retroviral protease

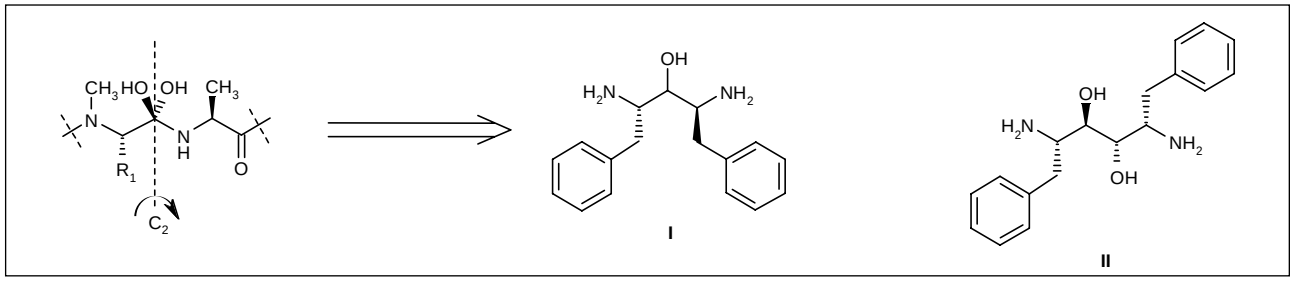
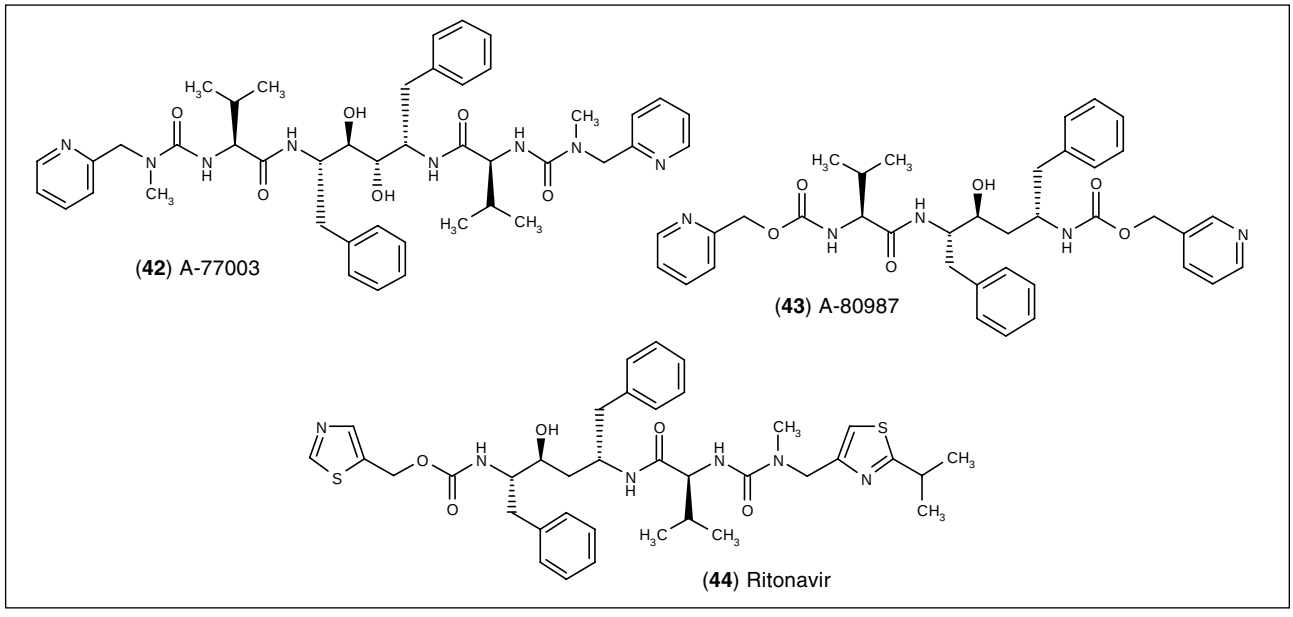


Fig. 1.



(including HIV protease) have shown it to be a C_2-symmetric homodimer with a single active site. Each monomeric unit having the conserved catalytic triads (Asp-Thr-Gly) is common to the aspartyl protease class. Based on this C_2-symmetric structure of HIV protease, inhibitors were designed. Cleavage of the asymmetric dipeptide substrate around the C_2 axis, bisecting the carbon-nitrogen single bond, was presumed to lead to symmetric or pseudosymmetric core diamines (**I** and **II**; Fig. 1) (66, 67). X-ray studies of a complex structure of HIV protease with the inhibitor Boc-Phe-ψ[(*S*)-CH(OH)CH_2NH]-Phe-Gln-Phe-NH_2 (68) showed the binding sites S_1, S_2, S_3 and S_1', S_2', S_3' on the substrate for the ligand binding sites P_1, P_2, P_3 and P_1', P_2', P_3', which refers to substituents on either side of the inhibitor from the center of symmetry (Fig. 2). Major work on the development of HIV protease inhibitors is based on modifications of the ligand binding units and the dipeptide chain towards optimization of the anti-HIV activity.

Structure-activity relationship studies with **I** and **II** led to the identification of A-77003 (**42**) (66, 69), which showed promising anti-HIV activity (70), though displaying low oral bioavailability. Further structural modification produced A-80987 (**43**) (71), with improved oral bioavailability.

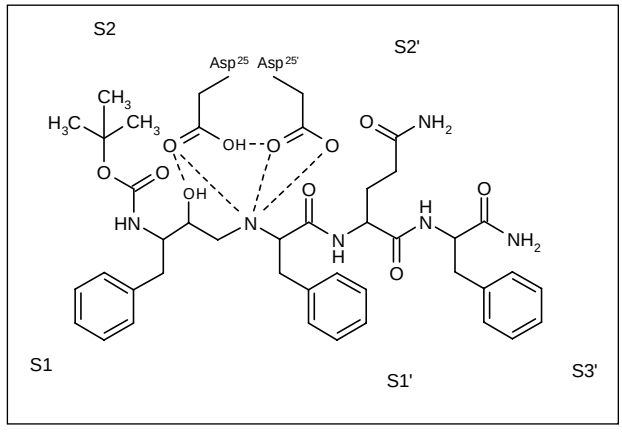
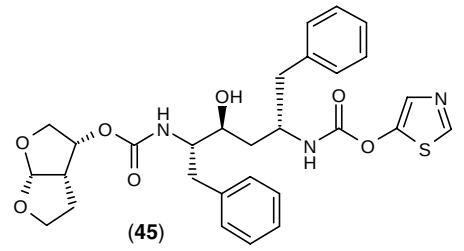
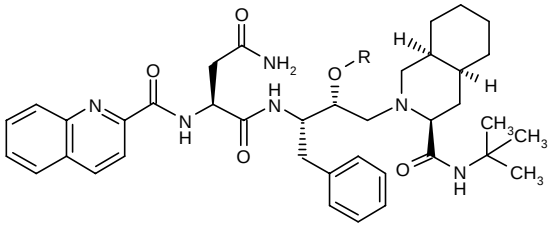


Fig. 2.

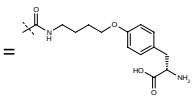
However, both of these inhibitors had the disadvantage of a short half-life due to *N*-oxidation of the pyridyl groups (72). To overcome this problem, replacement of the pyridyl group was carried out (61) and led to ritonavir (**44,** ABT-538), with a substantially reduced rate of metabolism and high bioavailability.

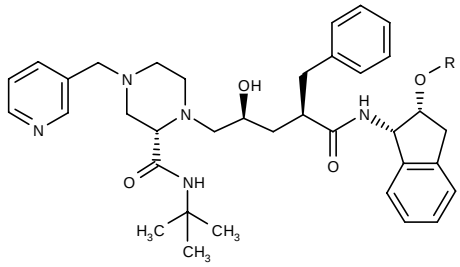


(**45**)

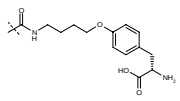


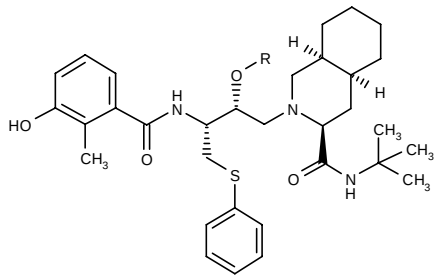
(**46**)

a. Saquinavir, R = H
b. Saquinavir carbamate, R = 

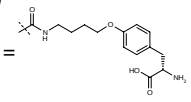


(**47**)

a. Indinavir, R = H
b. Indinavir carbamate, R = 



(**48**)

a. Nelfinavir, R = H
b. Nelfinavir carbamate, R = 

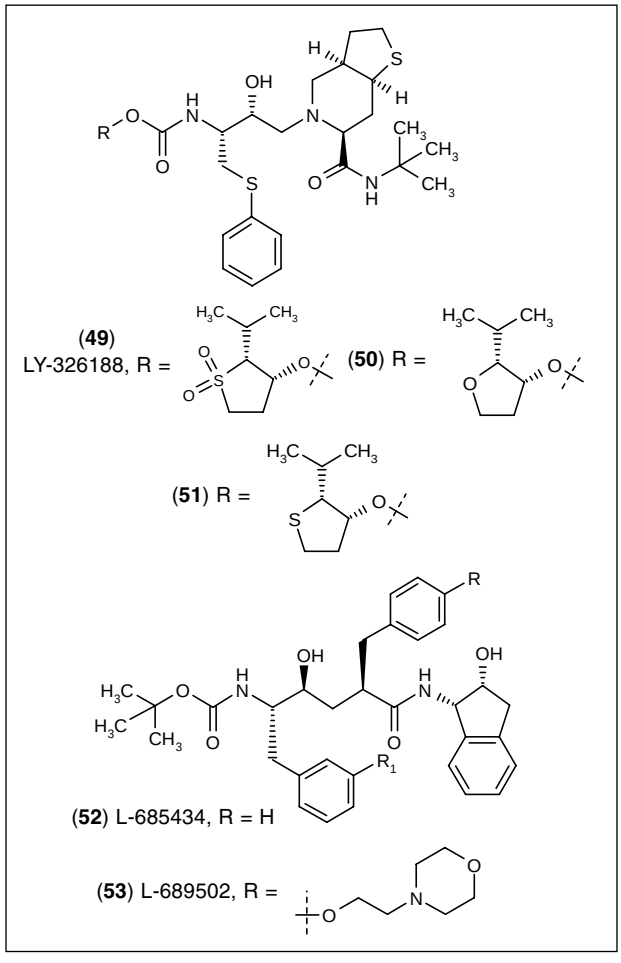
Phase I/II clinical trials with ritonavir revealed rapid (73, 74) and profound reductions in circulating viral RNA concomitant with a large increase in CD4 cell levels (75, 76). Phase III trials showed a significant increase in life span and led to its introduction for the treatment of HIV infection.

In a structural modification of ritonavir, the thiazole unit was replaced with conformationally constricted furofurans, exemplified by **45** (77). A number of compounds showed improved *in vitro* activity in MT-4 cells as compared to ritonavir. However, the *in vivo* activity of these compounds was reduced due to their poor bioavailability. In general, the (3*R*)-furofuran was found to be a better ligand than the corresponding (3*S*)-furofuran.

Further modification of the P_2/P_2' ligands resulted in the development of the highly potent protease inhibitors saquinavir (**46a**), indinavir (**47a**) and nelfinavir (**48a**), which have received FDA approval for the treatment of AIDS. These agents are not carbamates, and their carbamate derivatives, **46b**, **47b** and **48b**, were prepared as prodrugs (78) and showed high chemical stability but low anti-HIV activity.

In order to modify the highly potent but modestly absorbed antiviral compound saquinavir (**46a**) (79), a small heterocyclic P_2 ligand was introduced (80, 81) and the *cis*-decahydroisoquinoline residue was replaced by *cis*-octahydrothienopyridine for P_1' (82). The most active compound of this series was LY-326188 (**49**), which exhibited 2-fold higher antiviral activity than saquinavir. The corresponding tetrahydrofuran (**50**) and thiofuran compounds (**51**) were less active (82).

The introduction of a novel hydroxyindan moiety as the P_2' ligand produced the potent HIV-1 protease inhibitor L-685434 (**52**) (83). Due to its poor aqueous



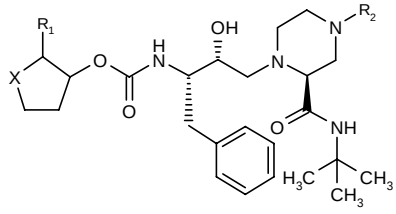
(**49**)
LY-326188, R =

(**50**) R =

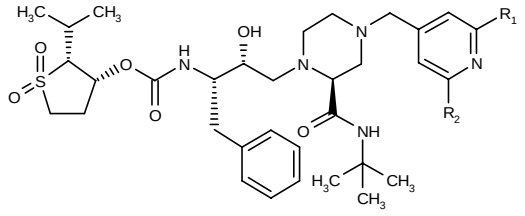
(**51**) R =

(**52**) L-685434, R = H

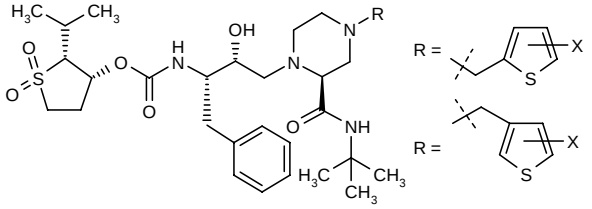
(**53**) L-689502, R =



(**54**)



(**55**)
a. L-738872, R_1 = Me, R_2 = Cl
b. R_1 = Me, R_2 = H
c. R_1 = Et, R_2 = H



(**56**)

solubility, however, compound **52** showed little cell penetration. Computer-assisted molecular modeling (76) suggested the possibility of incorporating polar substituents on the P_1 and P_1' ligands. Thus, the synthesis of compounds having polar groups substituted on the P_1, P_1' phenyl residues was carried out (84). The most potent compound of the series was L-689502 (**53**). The high value for the ratio of IC_{50} to CIC (0.0375) shown by **53** is consistent with increased penetration into HIV-infected lymphocytes. At concentrations of 12-50 nM, it completely prevented the spread of the virus in human H9T lymphoid cells.

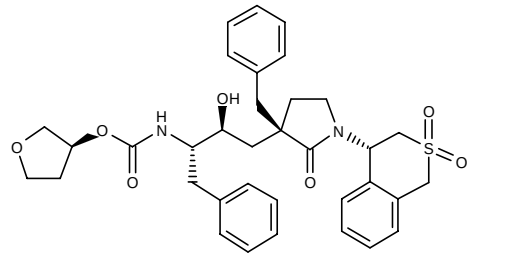
Based on the structure of the noncarbamate protease inhibitor indinavir (**47**) (85) inhibitors of prototype **54** were designed (86) having the piperazine carboxamide unit at the *C*-terminus instead of the *N*-terminus as in **47**. Structure-activity relationship studies revealed that the urethane moiety having a (3*R*)-hydroxy substituent was more tightly bound to the enzyme than that with 3*S* stereochemistry. 1,1-Dioxotetrahydrothienyl compounds (**54**; X = SO_2, R_1 = isopropyl) were found to be better than the corresponding tetrahydrofuran derivatives (**54**; X = O, R_1 = H). The most active compound was L-738872 (**55a**; CIC_{95} = 12.50 nM), with better pharmacokinetics compared to indinavir.

A number of other compounds in this series bearing substituted piperazines as P_3' showed potent inhibitory activity (87, 88). In a study of 2-alkyl and 2,6-dialkylpyridine analogues of **55a** (88), 2-methyl and 2-ethyl compounds (**55b,c**) showed an extended half-life. In a further modification, the pyridyl substituent was replaced by a mono or bicyclic thienylmethyl residue as the P_3' ligand. All compounds in this series (**56**) exhibited nanomolar to subnanomolar inhibitory potency towards HIV protease and high activity (CIC_{95} = 25-100 nM) in inhibiting viral spread. The incorporation of bicyclic thienothiophene moieties led to extremely effective inhibition of the spread of virus in a whole-cell assay at concentrations as low as 6 nM. However, due to their limited water solubility, these compounds exhibited a poor oral absorption profile (87).

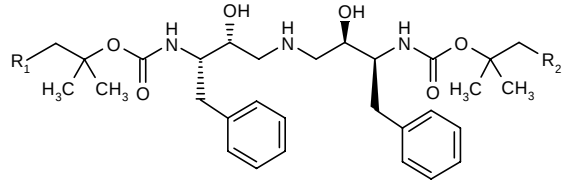
The short half-life of the piperazine class of compounds was found to be due to their extensive metabolism, including *N*-dealkylation of the arylmethylene linked to the piperazine 4-nitrogen. In an attempt to inhibit metabolism, a series of cycloalkylpiperazines was synthesized (89). Smaller sized cycloalkyl rings such as cyclopropyl and cyclobutyl showed higher potency against the enzyme. These compounds also possessed desirable pharmacokinetic profiles.

In a different approach, the stability of the amide bond was increased through lactam ring formation, as in L-700497 (**57**; IC_{50} = 1.8 nM) (90). In still another modification, aminodiol derivatives were developed as HIV protease inhibitors and the Boc derivative BMS-182193 (**58a**) showed significant activity (91).

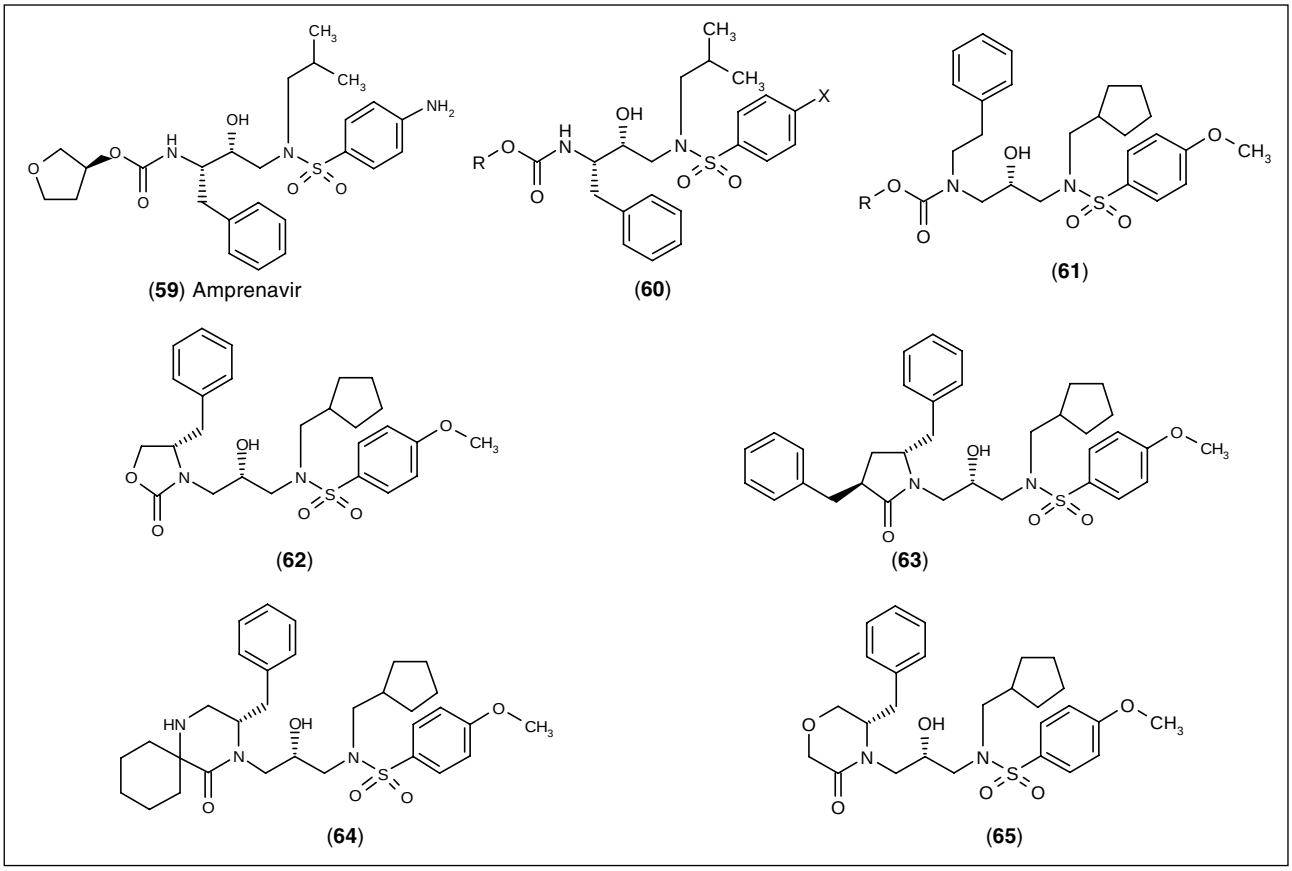
Modification of the Boc group to the corresponding hydroxy derivatives (**58b,c**) produced more potent protease inhibitors (92). In order to achieve better bioavailability, a longer half-life and overcome drug resistance



(**57**) L-700497



(**58**)
a. BMS-182193, R_1 = R_2 = H
b. R_1 = R_2 = OH
c. R_1 = H, R_2 = OH



problems, attempts were made to prepare nonpeptide molecules having similarity to the above dipeptides; a sulfone functionality was introduced in place of carboxyl. Thus, incorporation of a 3(*S*)-tetrahydrofuran ligand by Vertex in a sulfonamide-based isostere afforded the very potent and orally active amprenavir (**59**, VX-478), which has received FDA approval. The crystal structure of HIV-1 protease in complex with amprenavir (93) showed effective binding to the enzyme.

The antiviral activity of amprenavir is specific for HIV and it is > 5,000-fold more selective for viral than for human aspartyl protease (94, 95). The resistance profile of amprenavir appears to be different from that of other protease inhibitors such as saquinavir and indinavir (63). The concentration of amprenavir producing 50% inhibition (IC_{50}) of HIV-1 in MT-4 cells (T-cell line) and peripheral blood lymphocytes was 0.084 and 0.08 µmol/l, respectively (96).

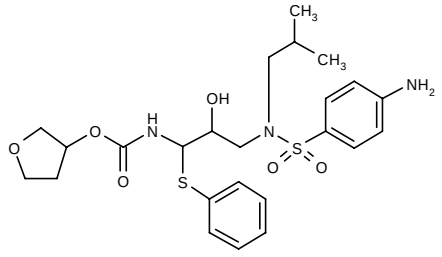
Amprenavir is administered orally to patients at a dose of 1200 mg twice daily. It is also used in combination with other antiviral agents (52). Its elimination half-life is 9 h. The side effects most frequently observed with the use of amprenavir are rash, diarrhea and headache. Recently, the development of amprenavir as monotherapy was terminated because of only moderate results in clinical trials (97).

In a modification of the amprenavir structure, high-affinity P_2 (R_1) ligands such as tetrahydrothienyl, 1,1-dioxotetrahydrothienyl, furofuran and furopyran were introduced in the (*R*)-(hydroxyethyl)sulfonamide-based isostere **60**. This led to a series of active compounds (98). The carbamate ester of 3(*S*)-hydroxytetrahydrothiophene (**60**; R = tetrahydrothien-3(*S*)-yl, X = OMe) exhibited enzyme-inhibitory potency (K_i) of 2.5 nM and prevented the spread of HIV-1 in MT-4 cells with an IC_{50} of 47 nM. The corresponding 1,1-dioxothienyl compound was even better (K_i = 1.4 nM; IC_{50} = 19 nM). A high order of activity was observed with a furofuran substituent as P_2 (K_i = 1.1 nM; IC_{50} = 1.4 nM). In general, methoxy compounds (X = OMe) were better than the corresponding amino compounds (X = NH_2).

Attempts to inhibit metabolism also included rigidification of the P_1/P_2 region in amprenavir (61). These compounds (**61**) were less active than amprenavir.

Various other conformationally restricted protease inhibitors related to amprenavir (**62-65**) were synthesized by Salituro *et al.* (99). The most potent compound was **63**, with equivalent enzyme-inhibitory potency to amprenavir (K_i = 0.5 *vs.* 0.6 nM).

Bioisosteres of amprenavir were prepared by replacing the methylene of the benzyl group with sulfur atoms (100). However, such a modification, as in prototype **66**,



(**66**)

drastically reduced the enzyme-inhibitory activity and protection against infection in MT-4 cell cultures.

2. Difluorostatone-type carbamates

Screening the protease library, it was discovered that the difluorostatone-type compound **67** possessed protease-inhibitory activity. Modification of **67** resulted in an extremely efficient inhibitor (**68**; R = R_1 = H).

Structure-activity relationship studies suggested the importance of the ketone and amide functionalities for inhibition of HIV-1 protease (101). Compound **68** exhibited potent inhibition of purified HIV-1 protease *in vitro* (K_i = 1 nM), as well as inhibition of HIV-1 replication in various T-cell lines at micromolar concentrations. However, **68** was found to be cytotoxic at concentrations 5-10 times greater than the inhibitory concentration. Modification of the phenylalanyl-type difluorostatone (R = H) to the *O*-benzyltyrosyl analogue (R = OCH_2Ph) was found to be extremely favorable (102), resulting in increased inhibition of replication, as well as a better therapeutic index. In this series, introduction of an *N*-methyl group at the carboxy terminus (R = OCH_2Ph, R_1 = Me) produced an extremely potent inhibitor (K_i = 0.4 nM) with an IC_{50}/EC_{50} value of > 300.

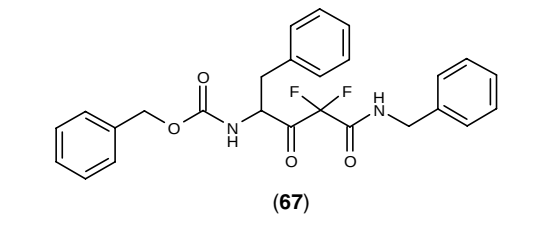
Compounds with high antiviral activity also emereged when the benzylamine carboxy terminus was replaced by an (*R*)-valinol ether carboxy terminus (**69**) (103).

3. Antisense oligonucleotide carbamates

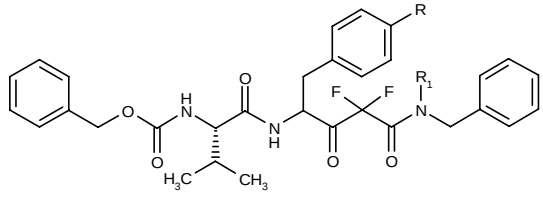
Antisense oligonucleotides with the ability to inhibit viral replication and cell proliferation have been developed (104-108). However, one of the main problems in this approach is the low stability of antisense oligonucleotides. To overcome this problem, various modifications of the intermediate linkages have been attempted. The other requirement is the selective hybridization of such oligonucleotides with their target mRNAs.

In one such approach, thiono triester-modified antisense oligonucleotides were designed as antiviral agents (109). It has been observed that lipophilic groups attached to oligonucleotides result in enhanced pharmacological properties (110). Furthermore, the nature and position of the lipophilic group incorporated influence the ability of the modified oligonucleotides to cross cell membranes or to hybridize effectively to target mRNA. Therefore, the introduction of nonionic internucleotide linkages and lipophilic groups was undertaken. A series of phosphorothioate oligonucleotide linked with a lipophilic group were synthesized and evaluated *in vitro* for inhibition of human cytomegalovirus (HCMV). From this series, oligonucleotides conjugated with a 6-(3-cholesteryloxycarboxyamino)hexyl residue as a lipophilic group (**70**) showed a higher degree and increased rate of cellular association. In addition, they exhibited potent anti-HCMV activity (> 90% inhibition of DNA replication at 0.05 μM), as well as enhanced nuclease resistance and cellular association.

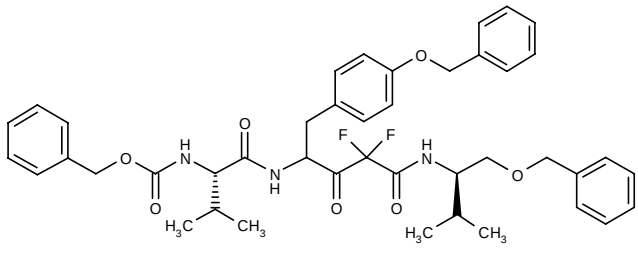
In another study (111), novel heterodimers of type **71** and **72** containing an acyclic nucleoside and a carbamate linkage were incorporated into oligonucleotides. A study



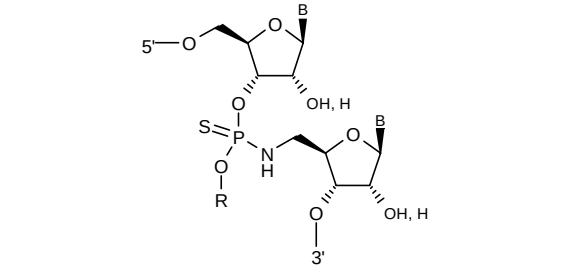
(**67**)



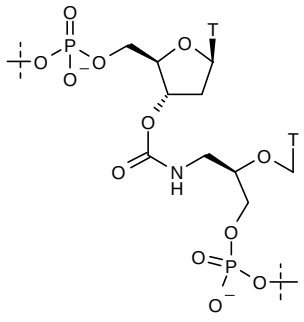
(**68**)



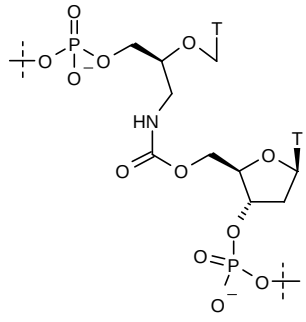
(**69**)



(**70**)



(**71**)



(**72**)

showed that such modified nucleotides formed duplexes with the complementary strands and had better base pair recognition properties than the native oligonucleotides. However, no antiviral activity has been reported for these compounds.

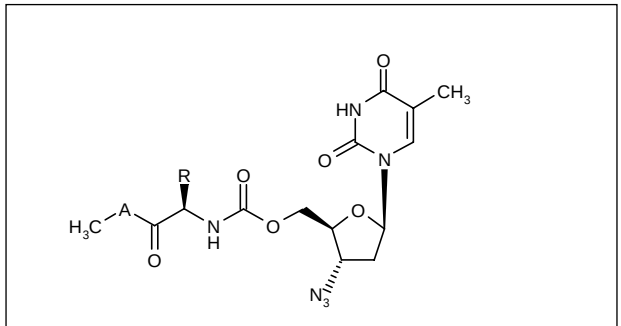
Carbamate esters of the anti-HIV nucleoside AZT (**73**) (112) were synthesized as prodrugs. These phosphoramidate monoesters of AZT bearing aliphatic amino acid methyl esters or methyl amides showed antiviral activity at 0.05-30 μM but no toxicity at 100 μM. They also showed greater stability to hydrolysis and the formation of the cytotoxic material AZT-MP.

ω-Hydroxyalkylcarbamate esters (**74**) have also been reported by Vlieghe *et al.* (113) as prodrugs of AZT. However, the corresponding carbonates were found to be better antiviral agents.

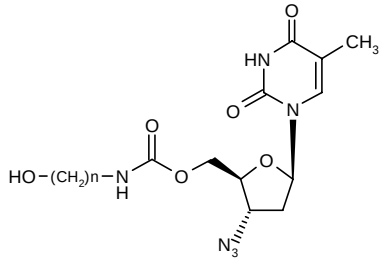
4. Carbamates affecting dimerization interface of HIV-1 protease

Dimerization of the HIV-1 protease generates a catalytic center as well as a substrate binding pocket. The dimerization interface has a 4-stranded β-sheet (114). Targeting the β-sheet portion by agents that would block the assembly of the homodimer or disrupt the dimeric interface leads to loss of biological activity. Thus, peptide units were designed to interact with *C*- and *N*-terminal regions of the HIV-1 protease (115-118). These studies suggested that short peptides are able to interrupt homodimer formation by noncovalent dissociative inhibition.

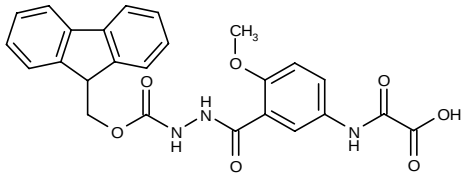
In a recent report (119), the design of a nonpeptide β-strand-mimetic carbamate (**75**), was described. It was found to inhibit dimerization of HIV-1 protease not only through a dissociative mechanism, but also partially through an active site-directed mechanism. However, the inhibitory activity of the compound was poor as compared to peptide dimerization inhibitors and further modification



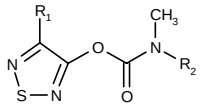
(**73**) (A =O, NH)



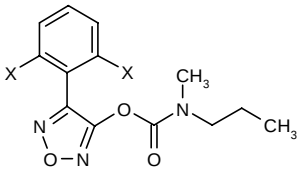
(**74**)



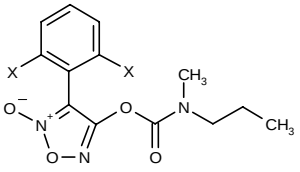
(**75**)



(**76**)
a. R_1 = 2,6-$(Cl)_2$-Ph, R_2 = Pr



(**77**)



(**78**)

will be necessary to develop nonpeptide dimerization inhibitors.

5. Thia- and oxadiazole derivatives

From a random screening of compounds having herbicidal activity, a series of 1,2,5-thiadiazoles (**76**) (120) were identified as HIV-1 inhibitors. Structure-activity relationship studies revealed the importance of an aromatic ring at C-4 and the carbamate residue at C-3 for inhibitory activity.

The introduction of a chlorine atom at the 2- or 2,6-position of the phenyl group led to an increase in activity. 4-(2,6-Dichlorophenyl)-1,2,5-thiadiazol-3-yl-*N*-methyl-*N*-propylcarbamate (**76a**) exhibited the most potent activity, with EC_{50} and CC_{50} values of 0.013 and 131 μM, respectively, in MT-4 cells.

In a study on the 4-aryl-1,2,5-oxadiazol-3-ylcarbamates (**77**) (121) and their corresponding *N*-oxides (**78**), the introduction of a chlorine atom increased the activity, as observed with thiadiazoles (120). Removal of the *N*-oxide also significantly increased activity. Compound **78** (X = Cl) was the most active compound in the 1,2,5-oxadiazole series (EC_{50} = 0.12 μM).

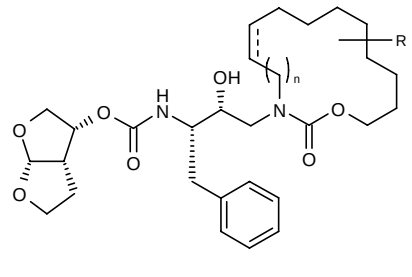
6. Cyclourethane-derived HIV protease inhibitors

Macrocyclic urethanes incorporating an (*R*)-hydroxyethylamine isostere were reported by Gosh *et al.* (122). The X-ray structure of saquinavir bound to HIV protease revealed that a 14-16-membered cyclourethane would fit into S_1'-S_2' binding sites of the HIV-1 protease. Thus, unsaturated cyclourethanes and corresponding saturated cyclourethanes (**79**) were prepared and evaluated for anti-HIV activity. Cyclic urethanes showed better inhibitory activity compared to acyclic compounds. Saturated urethanes were found to be better than the corresponding unsaturated; cyclourethane (**80**) was the most potent inhibitor.

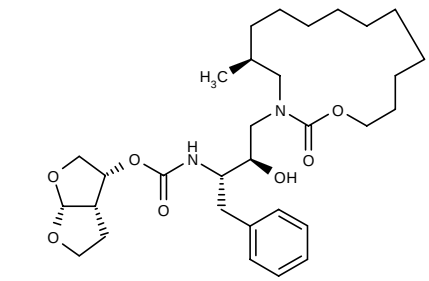
7. Miscellaneous compounds

Small peptidic aldehydes as inhibitors of human rhinovirus 3C protease

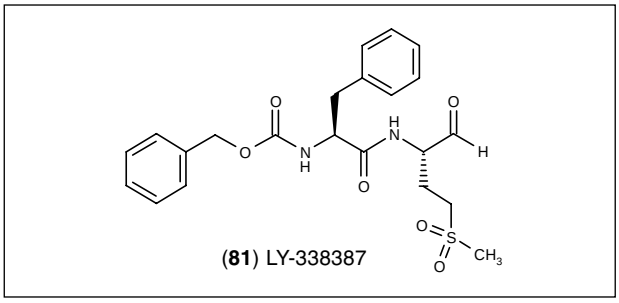
The human rhinoviruses (HRVs) are considered to be the major cause of the common cold (123). A polyprotein produced by HRV is cleared by the 3C protease or its precursor 3CD, resulting in the production of a viral protein that assembles into infectious virions.



(**79**)



(**80**)


(**81**) LY-338387

In order to develop inhibitors of HRVs, small peptide aldehydes were designed to mimic the substrate for HRV 3C protease. In this series, the most potent compound was LY-338387 (**81**). It is a reversible inhibitor of $3C^{pro}$ with a K_i of 0.47 μM. It showed good tissue culture activity (IC_{50} = 3.4 μM) without cytotoxicity (TC_{50} > 224 μM) (124).

5-Oxohexahydropyrrolo[3,2-*b*]pyrroles as inhibitors of human cytomegalovirus protease

Human herpesviruses encode a serine protease which is essential for viral replication (125). X-ray structures of serine proteases of HCMV, herpes simplex virus type 1 (HSV-1), HSV-2 and varicella-zoster virus (VZV) revealed that these enzymes belong to a novel class of serine proteases having an active site composed of the His-His-Ser triad. Based on the substrate requirement of the consensus sequence of HCMV protease (**82**), pyrrolidine-5,5-*trans*-lactams (5-oxohexahydropyrrolo[3,2-*b*]-pyrroles) (**83**) were designed as inhibitors of HCMV protease (126). Structure-activity relationship studies showed that the optimum bulk requirement and stereochemistry at C-6 is α-methyl for S_1.

To define the chirality of the preferred substituents at S_1, compound **83** was resolved (127) into its enantiomers (*SRS*) and (*RSR*). Only the *SRS* (**84**) was found to be active against HCMV δ Ala protease (IC_{50} = 79 μM). Increasing the size of the substituent at S'_1 from acyl to isopropyl or *t*-butyl caused a loss in potency. However, the bicyclic dansyl-(*S*)-proline analogue (**85**) was found to be significantly more active (IC_{50} = 0.54 μM). The corresponding cyclopropyl dansyl-(*S*)-proline (**86**) was the most active in this series (IC_{50} = 0.34 μM).

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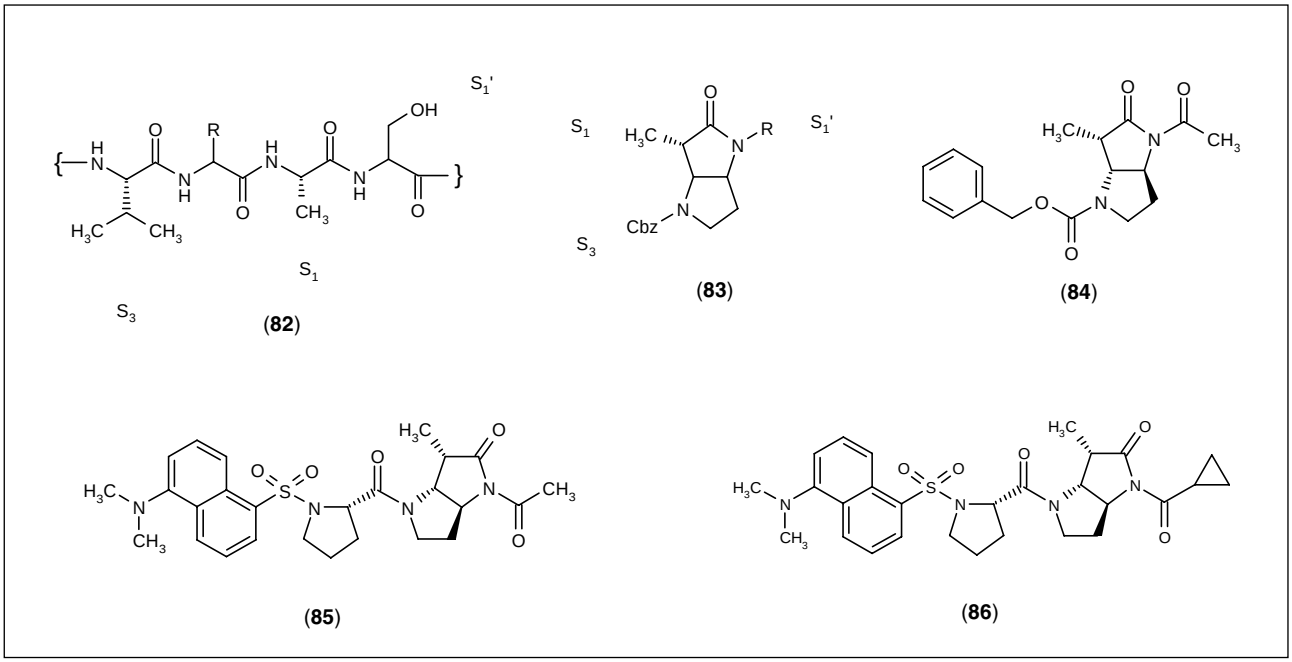
(**82**)

(**83**)

(**84**)

(**85**)

(**86**)

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