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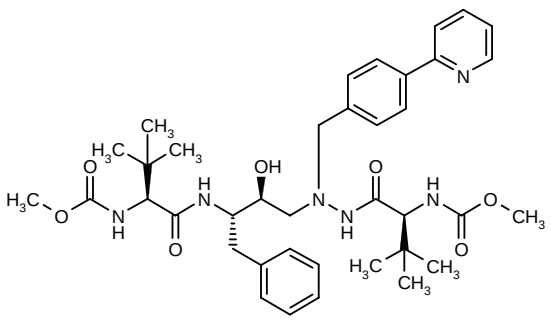
BMS-232632

Anti-HIV
HIV-1 Protease Inhibitor

CGP-73547

N'-[2(*S*)-Hydroxy-3(*S*)-[*N*-(methoxycarbonyl)-L-*tert*-leucylamino]-4-phenylbutyl]-*N*$^{\alpha}$-(methoxycarbonyl)-*N*'-[4-(2-pyridyl)benzyl]-L-*tert*-leucylhydrazide



$C_{38}H_{52}N_6O_7$ Mol wt: 704.8638

CAS: 198904-31-3

EN: 257722

Synthesis

BMS-232632 has been obtained by two related ways:

1) The reaction of 4-bromobenzaldehyde (I) with trimethylorthoformate (II) and *p*-toluenesulfonic acid in methanol gives ketal (III), which is condensed with 2-pyridylmagnesium bromide in THF, yielding 4-(2-pyridyl)benzaldehyde (IV). The reaction of (IV) with *tert*-butyl carbazate (V) in refluxing ethanol affords hydrazone (VI), which is reduced with H_2 over Pd\C in methanol to the hydrazine (VII) (1). The condensation of (VII) with the epoxide (VIII) in hot isopropanol gives the expected addition product (IX), which by treatment with HCl or formic acid results in the fully deprotected intermediate (X). Finally, this compound is condensed with *N*-(methoxycarbonyl)-L-*tert*-leucine (XI) by means of *O*-(2-oxo-1,2-dihydro-1-pyridyl)-*N*,*N*,*N*´,*N*´-tetramethyluronium tetrafluoroborate (TPTU) in dichloromethane or DMF (1, 2). Scheme 1.

2) Intermediate (IX) can also be obtained as follows: The cyclization of 4-cyanobenzoic acid methyl ester (XII) with acetylene at 15 Atm by means of cobaltocene in toluene at 180 °C gives 4-(2-pyridyl)benzoic acid methyl ester (XIII), which is saponified with NaOH in methanol to the corresponding acid (XIV). The activation of (XIV) with isobutyl chloroformate yields the mixed anhydride (XV), which is condensed with *N*-(*tert*-butoxycarbonyl)-L-phenylalaninal (XVI) and KCN in dichloromethane, affording (XVII). The reaction of (XVII) with *tert*-butyl carbazate (V) by means of acetic acid in methanol gives hydrazone (XVIII), which is reduced to hydrazine (XIX) by means of sodium cyanoborohydride in THF. The isomerization of (XIX) by means of 7-methyl-1,5,7-triazabicyclo[4.4.0]dec-5-ene (MTDE) in hot diglyme yields hydrazide (XX), which is finally reduced to intermediate (IX) with diisobutylaluminum hydride in dichloromethane\THF (3). Scheme 2.

Description

Crystals, m.p. 207-9 °C, $[\alpha]_D$ –47° (c 1, EtOH) (2).

Introduction

HIV protease, the enzyme responsible for the processing of polyproteins to structural proteins and viral enzymes, is essential for the maturation of viral particles into a fully infectious virus. This led to the selection of HIV protease as a target for AIDS therapy (4).

Currently available HIV drugs include five nucleoside reverse transcriptase inhibitors (zidovudine, didanosine, stavudine, lamivudine and zalcitabine), two nonnucleoside reverse transcriptase inhibitors (nevirapine and delavirdine) (5) and four peptidic protease inhibitors (saquinavir, indonavir, ritonavir and nelfinavir) (6). Combination of reverse transcriptase inhibitors with HIV-1 protease inhibitors has shown excellent efficacy in AIDS patients (7-10).

The search for new HIV-1 protease inhibitors continues and at least six such compounds are under clinical development: BMS-232632 (Bristol-Myers Squibb), ABT-378 (Abbott), DMP-450 (DuPont Merck), KNI-272 (Japan Energy), lasinovir (Novartis) and PNU-140690 (Pharmacia & Upjohn). The U.S. FDA recently granted

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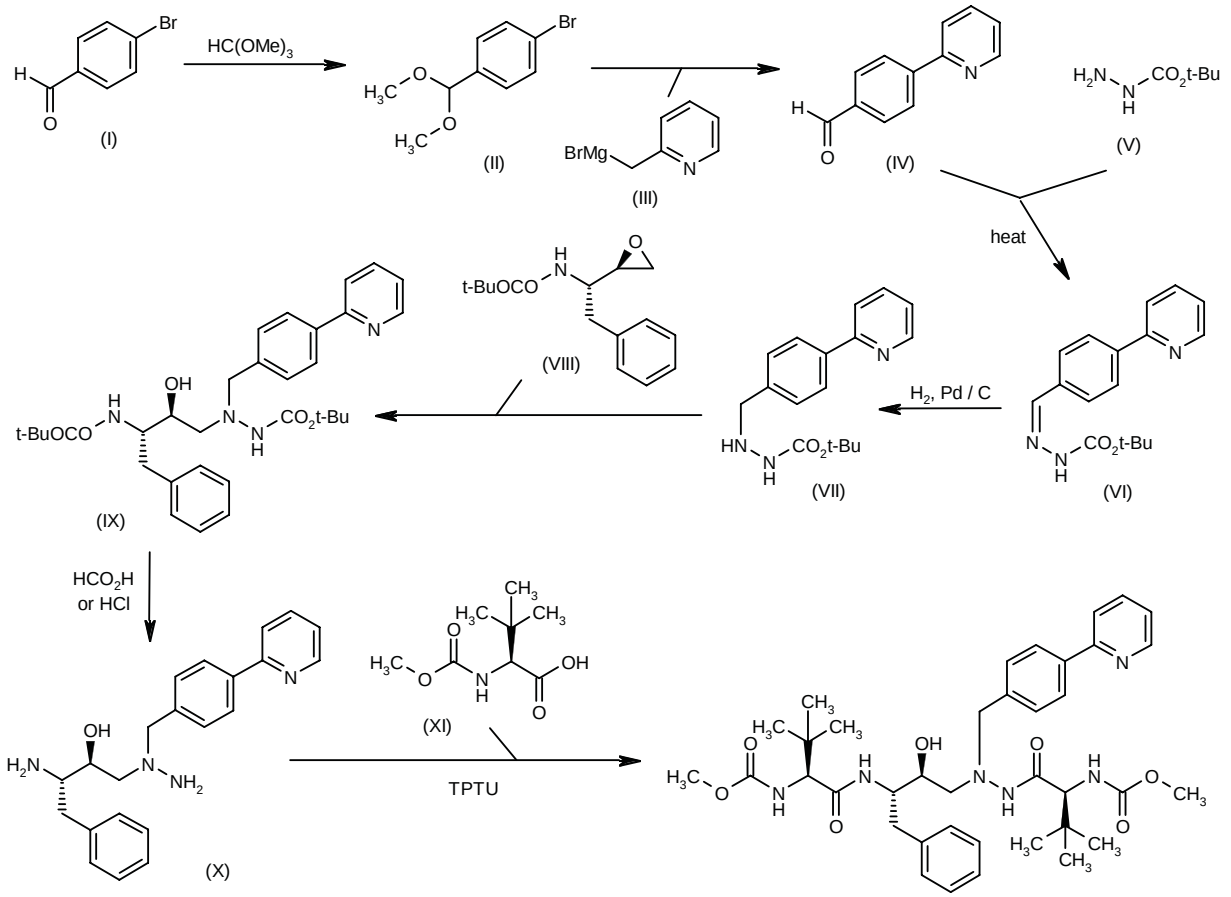
Scheme 1: Synthesis of BMS-232632



accelerated approval for Glaxo Wellcome and Vertex's amprenavir (Agenerase®), the first new protease inhibitor to obtain regulatory approval in more than 2 years (11). Table I presents the HIV protease inhibitory activity of selected compounds.

With the aim of obtaining new potent and orally active HIV protease inhibitors with activity against mutant HIV strains to overcome cross-resistance in patients, a series of aza-dipeptides with bis-aryl substituents as ligands for the P_1' pocket were synthesized. Resulting compounds showed excellent activity against wild-type and mutant HIV strains, as well as high bioavailability in mice after oral administration. One compound, CGP-73547 (BMS-232632), was selected for further development.

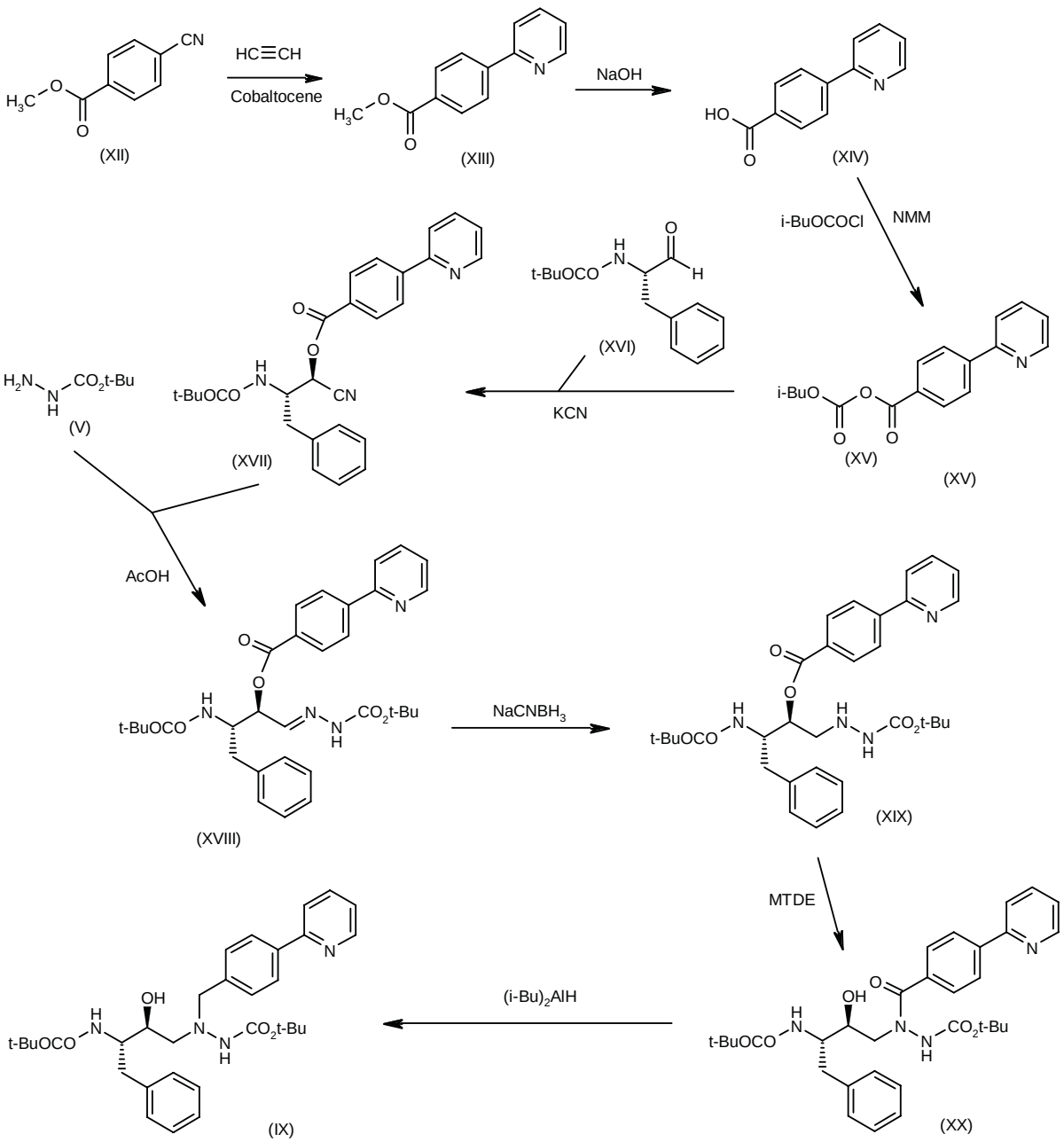
Pharmacological Actions

BMS-232632 is a potent azapeptide HIV protease inhibitor that has shown potent anti-HIV activity, with EC_{50} values of 2-5 nM and an IC_{50} against purified HIV-1 protease of 26 nM. It is, therefore, more potent than the currently approved HIV-1 protease inhibitors, even in the presence of 40% human serum (2, 12, 13).

In MT-2 human T lymphocytes infected with HIV-1/MN, BMS-232632 displayed ED_{50}/ED_{90} values of 1.4/3 nM, with more than 1000-fold selectivity over cytotoxic values (CC_{30}/CC_{90} = 21.8/31.8 μM). BMS-232632 maintained good activity against saquinavir- and indinavir-resistant HIV-1, with effective doses of 0.004-0.01 and 0.03-0.1 μM, respectively, compared to effective doses of 0.01 μM against wild-type virus (2).

Furthermore, after *in vitro* passage experiments, BMS-232632 induced resistance less readily than nelfinavir or ritonavir and, when acquired, resistance involved a novel protease mutation (N88S and, secondarily, I84V). As a result, BMS-232632-resistant viruses remained sensitive to saquinavir, while showing partial resistance to nelfinavir, indinavir, ritonavir and amprenavir. On the other hand, viruses resistant to nelfinavir, saquinavir and amprenavir remained sensitive to BMS-232632, while partial cross-resistance was observed between indinavir and ritonavir (12, 13).

Scheme 2: Synthesis of Intermediate (IX)



Synergism of BMS-232632 when used in combination with other antiretroviral drugs was evaluated in human lymphocytes infected with an HIV-1 clinical isolate. When used concomitantly with stavudine, didanosine, lamivudine, zidovudine, nelfinavir, indinavir, ritonavir, saquinavir or amprenavir, BMS-232632 yielded additive to moderately synergistic anti-HIV effects, with no enhanced cytotoxic effects at the highest concentrations tested. These results indicate that BMS-232632 can be used in a variety of combinations involving nucleoside analogs and/or other protease inhibitors (12, 14).

Pharmacokinetics and Metabolism

In a double-blind, placebo-controlled, phase I clinical study, BMS-232632 was given to healthy volunteers as single oral doses of 100, 300, 600, 900 or 1200 mg to

Table I: Pharmacological activity of selected HIV protease inhibitors launched or under development (from Prous Science MFLine database).

Compound	Activity	Parameter	Value (nM)	Material	Ref.
ABT-378	Protease Inhibition	K_i	0.0013	HIV protease	17
	Antiviral Activity	IC_{50}	17	Human MT-4 cells	17
	Antiviral Activity	IC_{50}	6.5	Human mononuclear cells	17
Amprenavir	Protease Inhibition	K_i	0.17	HIV protease	18
	Antiviral Activity	IC_{90}	46	T-cell lines	18
	Cytotoxicity	CC_{50}	≥98884	T-cell lines	18
	Cytotoxicity	CC_{50}	89000	Human MT-4 cells	19
BMS-232632	Protease Inhibition	K_i	1.0	HIV-1 protease	14
	Protease Inhibition	IC_{50}	26	HIV protease	2
	Antiviral Activity	IC_{50}	2.6[a]	Human MT-2 cells	2, 14
	Antiviral Activity	IC_{90}	3.0	Human MT-2 cells	2
	Cytotoxicity	CC_{50}	39[a]	Variety of cell cultures	14
DMP-450	Protease Inhibition	K_i	0.31	HIV protease	18
	Antiviral Activity	IC_{90}	125	T-cell lines	18
	Cytotoxicity	CC_{50}	≥93165	T-cell lines	18
Indinavir sulfate	Protease Inhibition	K_i	0.37	HIV protease	18
	Antiviral Activity	IC_{90}	50	T-cell lines	18
	Cytotoxicity	CC_{50}	≥70236	T-cell lines	18
Kynostatin-272	Protease Inhibition	K_i	0.0055	HIV-1 protease	20
	Antiviral Activity	IC_{50}	80	Human mononuclear cells	21
	Antiviral Activity	IC_{90}	270	Human mononuclear cells	21
	Cytotoxicity	CC_{50}	>>1250	Human mononuclear cells	21
Lasinavir	Protease Inhibition	IC_{50}	1.0[a]	HIV protease	22, 23
	Antiviral Activity	IC_{50}	5.2	Human MT-2 cells	22
	Antiviral Activity	IC_{90}	30	Human MT-2 cells	22
	Cytotoxicity	CC_{50}	>100000	Human MT-2 cells	22
Nelfinavir mesiltate	Protease Inhibition	K_i	1.85[a]	HIV-1 protease	24, 25
	Antiviral Activity	IC_{50}	15	Human MT-2 cells	26
	Cytotoxicity	CC_{50}	6928	Human MT-4 cells	26
PNU-140690	Protease Inhibition	K_i	0.008	HIV-1 protease	27
	Antiviral Activity	IC_{50}	75	Human mononuclear cells	28
	Antiviral Activity	IC_{90}	200	Human mononuclear cells	28
	Cytotoxicity	CC_{50}	>30000	Human mononuclear cells 28	
Ritonavir	Protease Inhibition	K_i	0.19[a]	HIV protease	17, 18
	Antiviral Activity	IC_{50}	58	Human MT-4 cells	17
	Antiviral Activity	IC_{90}	76	T-cell lines	18
	Cytotoxicity	CC_{50}	≥69352	T-cells lines	18
	Cytotoxicity	CC_{50}	37265[a]	Human MT-4 cells	26, 29, 39
Saquinavir	Protease Inhibition	K_i	0.25	HIV protease	18
	Protease Inhibition	IC_{50}	35	HIV protease	2
	Antiviral Activity	IC_{50}	8.46[a]	Human MT-2 cells	2, 25, 26
	Antiviral Activity	IC_{90}	10.2[a]	T-cell lines	2, 18
	Cytotoxicity	CC_{50}	≥74531	T-cell lines	18
	Cytotoxicity	CC_{50}	23985[a]	Human MT-4 cells	26, 31

HIV protease inhibitory activity is against purified, recombinant wild-type HIV/HIV-1 protease enzyme. Antiviral activity is against HIV/HIV-1 infected cell cultures. Cytotoxicity refers to the toxic effects of the compound on uninfected cell cultures. [a]Mean value from different studies using comparable experimental method.

determine the pharmacokinetic properties of the drug. Good absorption was observed, with peak plasma concentrations and AUC increasing in a greater-than-dose-proportional manner. The bioavailability of a capsule formulation of BMS-232632 was about 60% of that of an oral solution of the drug. After doses of 300 mg, plasma concentrations exceeded the antiviral EC_{50} values for over 24 h. At this dose, BMS-232632 was well tolerated (15, 16).

Clinical Studies

In the first clinical assessment of BMS-232632 in human subjects, clinical examination and laboratory tests were used to determine the tolerability of oral doses of 100, 300, 600, 900 and 1200 mg of the drug in healthy volunteers. Treatment-related adverse events were mild and reversible upon continuous treatment for 14 days (15, 16). Phase II studies with BMS-232632 for the treatment of HIV infection are currently under way in the U.S.

Manufacturer

Bristol-Myers Squibb Co. (US), acquired from Novartis AG (CH).

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