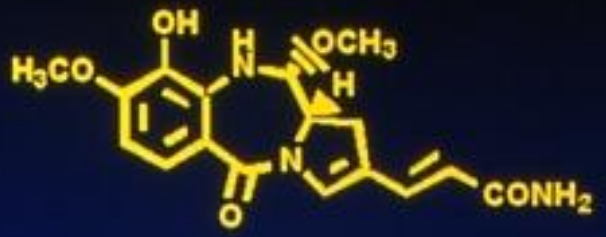
XIV convegno nazionale divisione di chimica farmaceutica

Ligandi alchilanti del solco minore del DNA: nuovi risultati nella progettazione di farmaci antitumorali

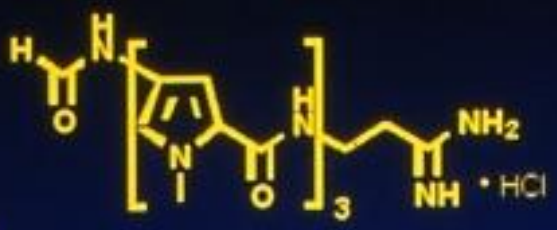
Salsomaggiore, 21-25 Settembre 1998

NATURAL COMPOUNDS



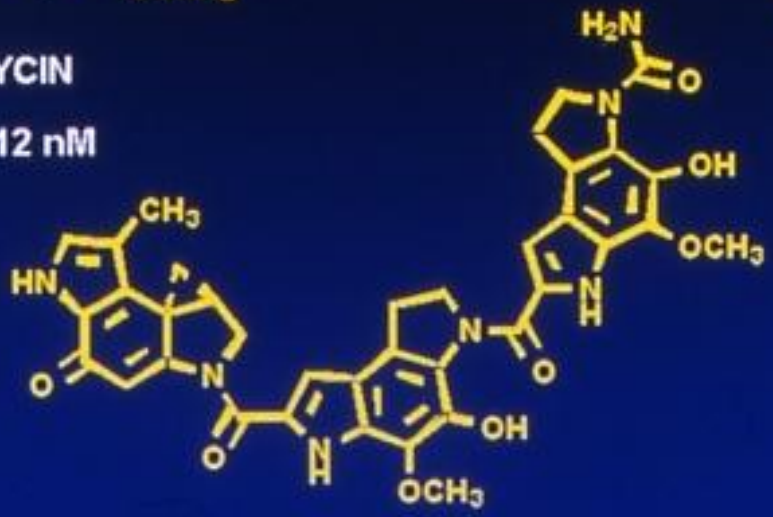
ANTHRAMYCIN

IC_{50} L1210 12 nM



DISTAMYCIN A

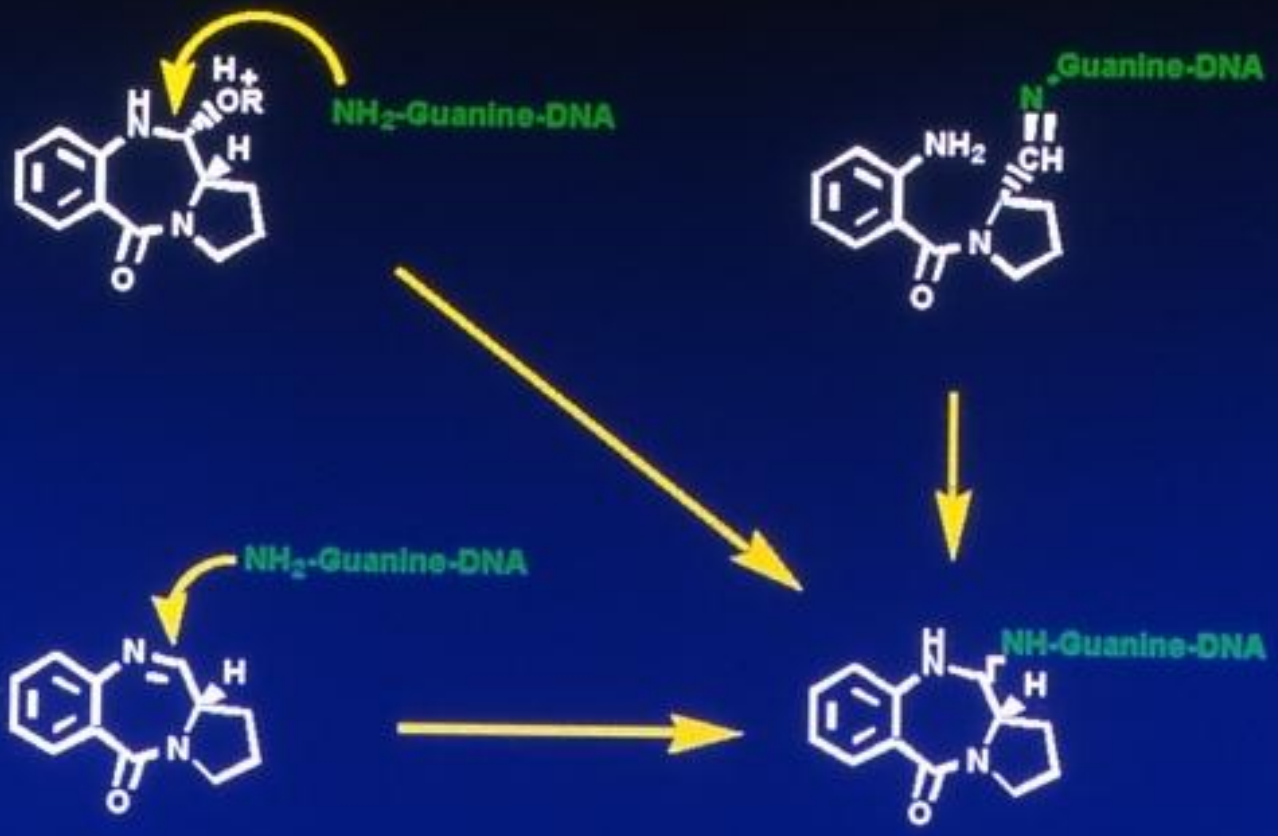
IC_{50} L1210 10μM



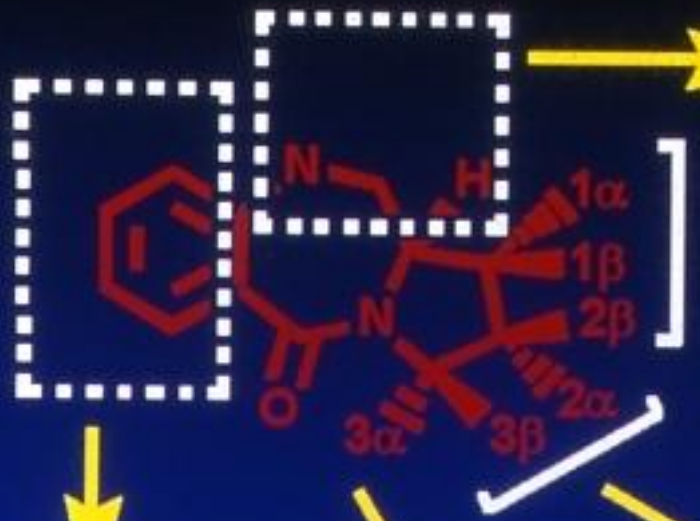
(+)-CC-1065

IC_{50} L1210 20 pM

POSSIBLE MECHANISM OF DNA ALKYLATION BY PBD's



PBD's SAR



-N10-C1 imine, carbinolamine or carbinolamine methyl ether reacting moieties
- no substituents
- configuration S at C11a

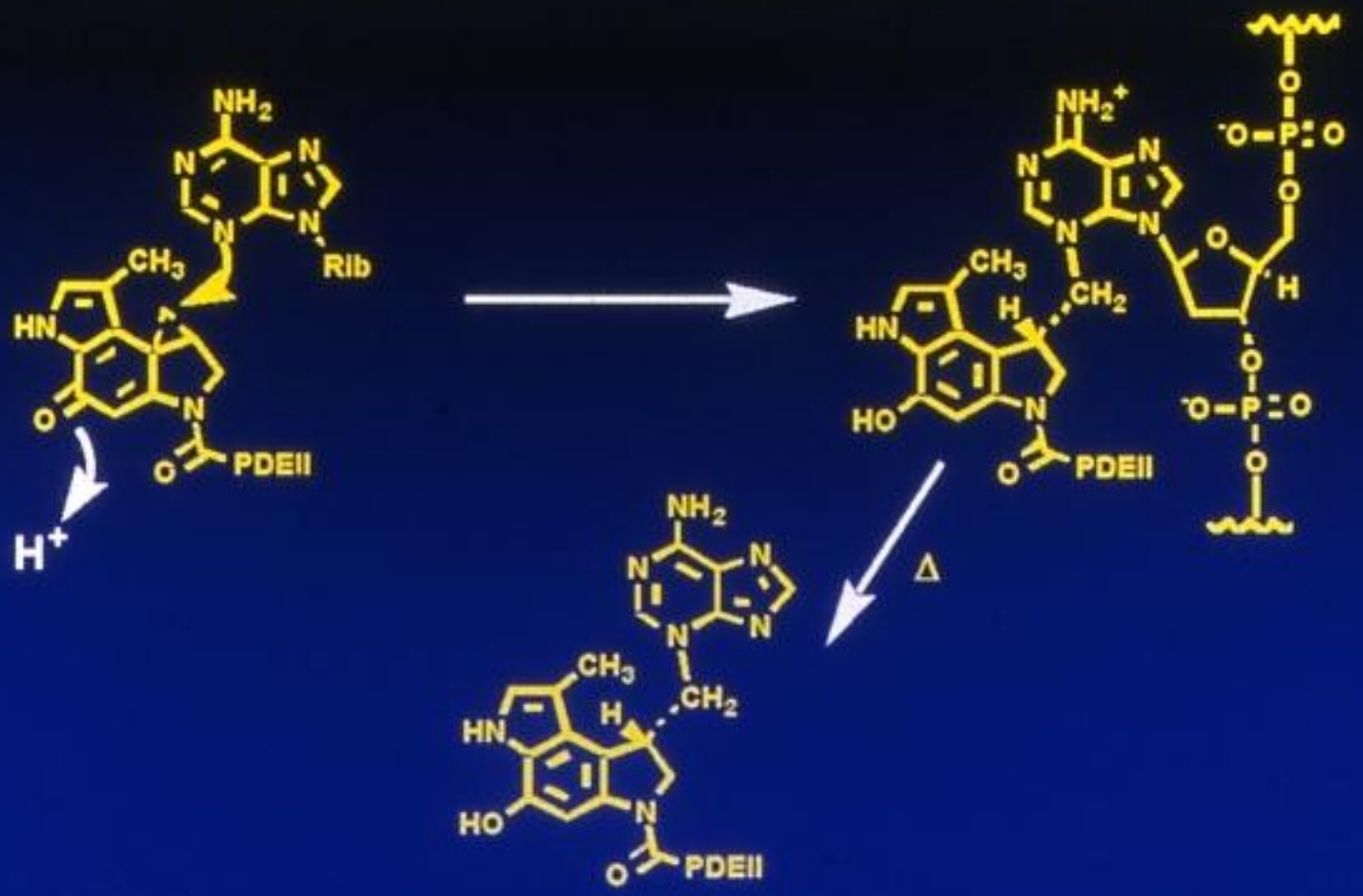
no substituents allowed

- 9-position can allow only low hindrance substituents (OH, CH_3)
- 8-position can accomodate linear chains
- 7 and 6 positions allow bulky groups, pointing out of the minor groove

no-substituents larger than OH

linear chains with or without unsaturations

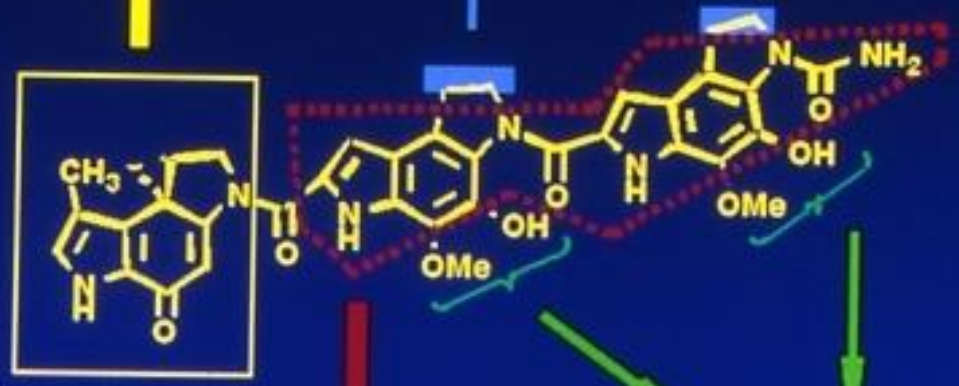
CC-1065 DNA ALKYLATION



CC-1065 SAR

Antitumor activity
Sequence specificity
DNA Bonding

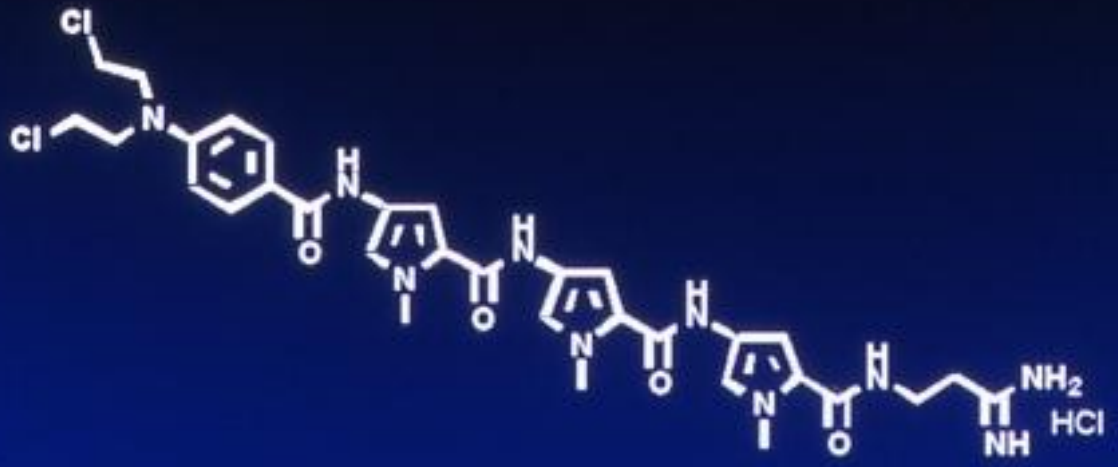
Modulation of Sequence Specificity
DNA Winding
Delayed Death



Stiffening of helix

Increase Rate of Alkylation
Increase Cytotoxic Potency

TALLIMUSTINE
(FCE 24517, BENZOYLMUSTARD DERIVATIVE OF DISTAMYCIN A)



IC_{50} = 50.3 ng/ml
O.D = 3.13 mg/K
T/C = 175

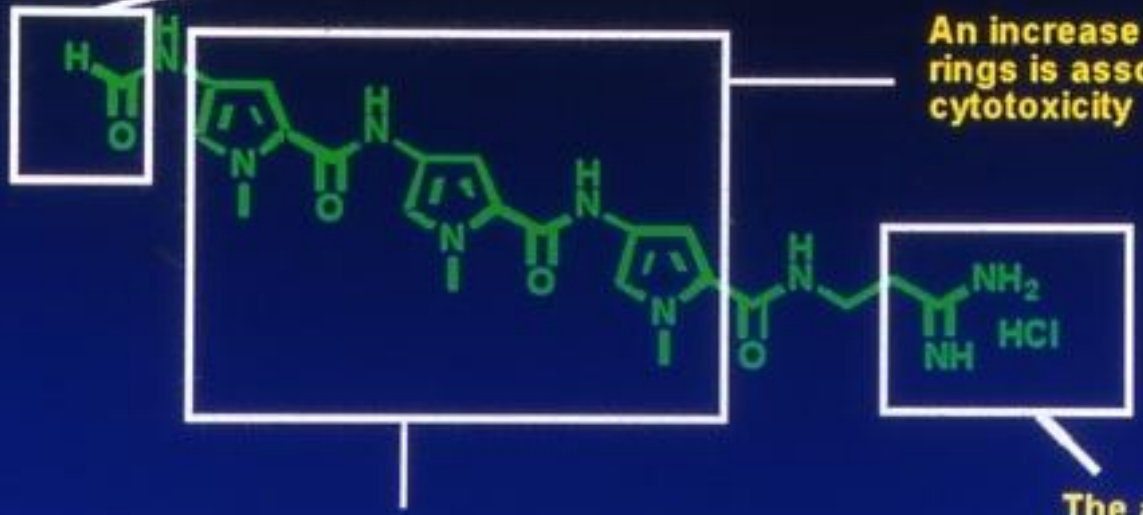
Mode of action: still under investigation

Binds to A-T rich sequences in the minor groove of B-DNA

Alkylates N-3 adenine in DNA with high sequence selectivity

DISTAMYCIN SAR

The substitution of the formyl group with an alkylating moiety lead to an increase of cytotoxicity



An increase of the number of pyrrole rings is associated with an increase ii cytotoxicity

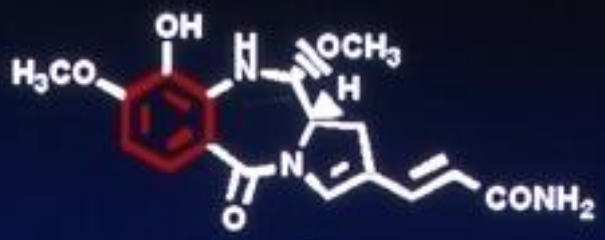
The isosteric replacement of pyrrole units with pyrazole units maintains the AT sequence selectivity, while the replacement with imidazole, furan o thiazole rings leads to an alteration of DNA sequence binding in favour of GC recognition.

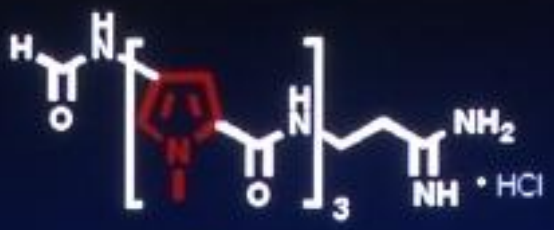
The amidino moiety can be replaced by ionizable and non-ionizable groups, with a modulation of basic strenght, lipophilicity and metabolic stability

GOAL

- Heterocycle substitution, in order to modulate sequence selectivity and potency in *in vitro* and *in vivo* assays

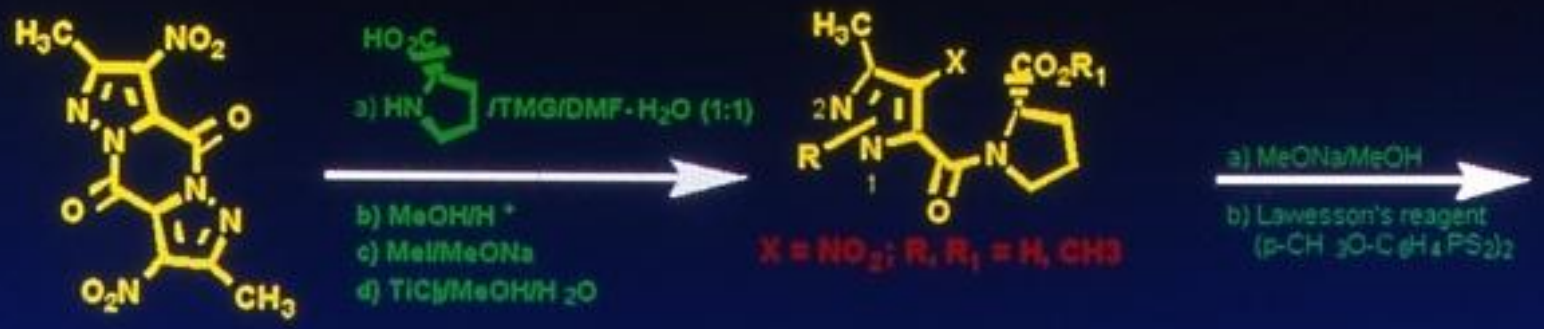
GOAL

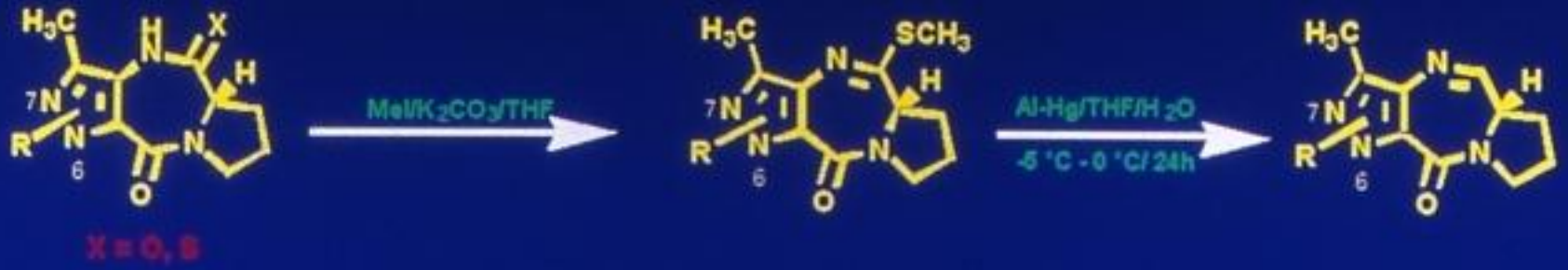




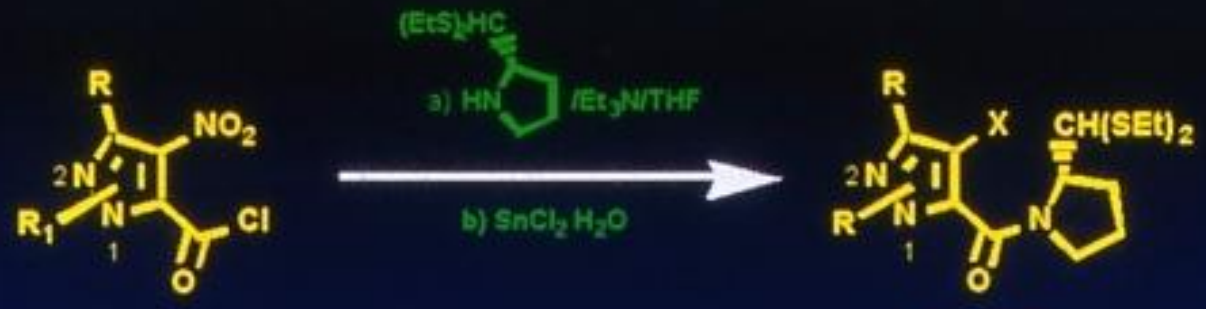
DIFFERENT HETEROCYCLES

PBD's PYRAZOLE ANALOGS

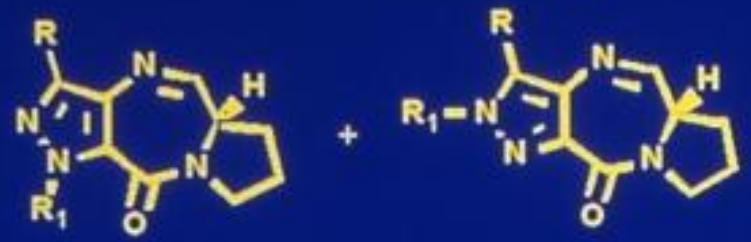




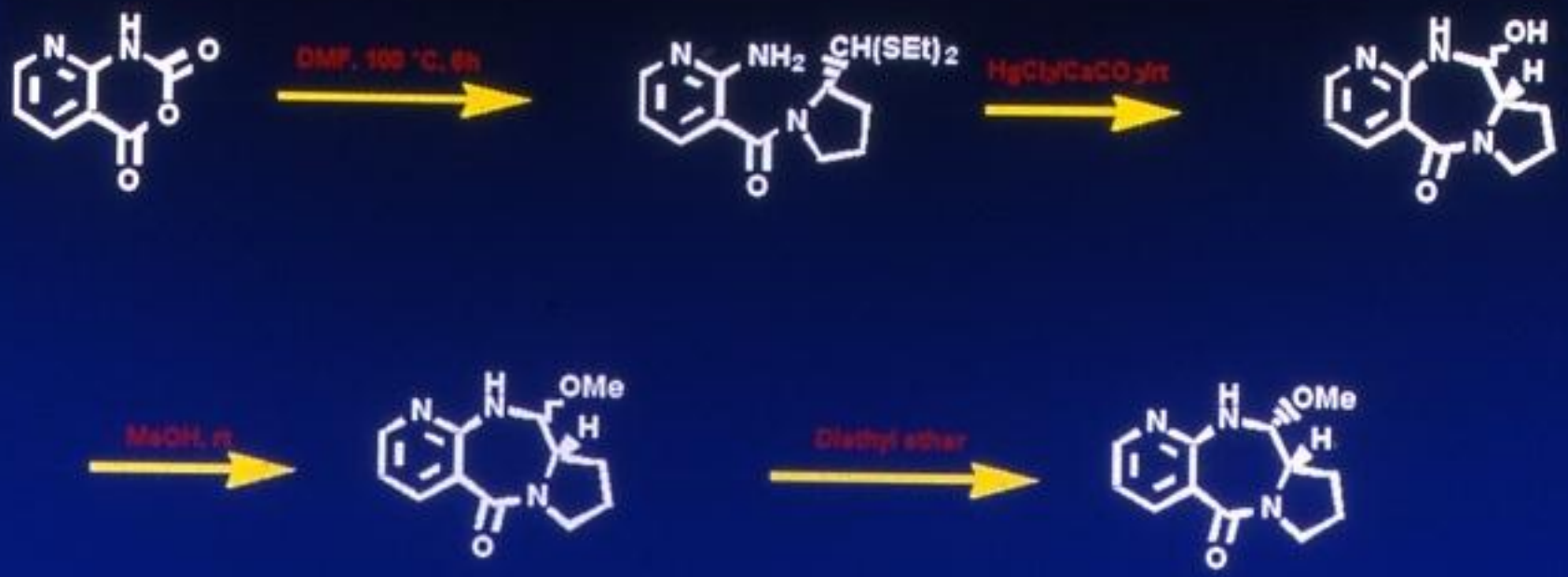
PBD's PYRAZOLE ANALOGS



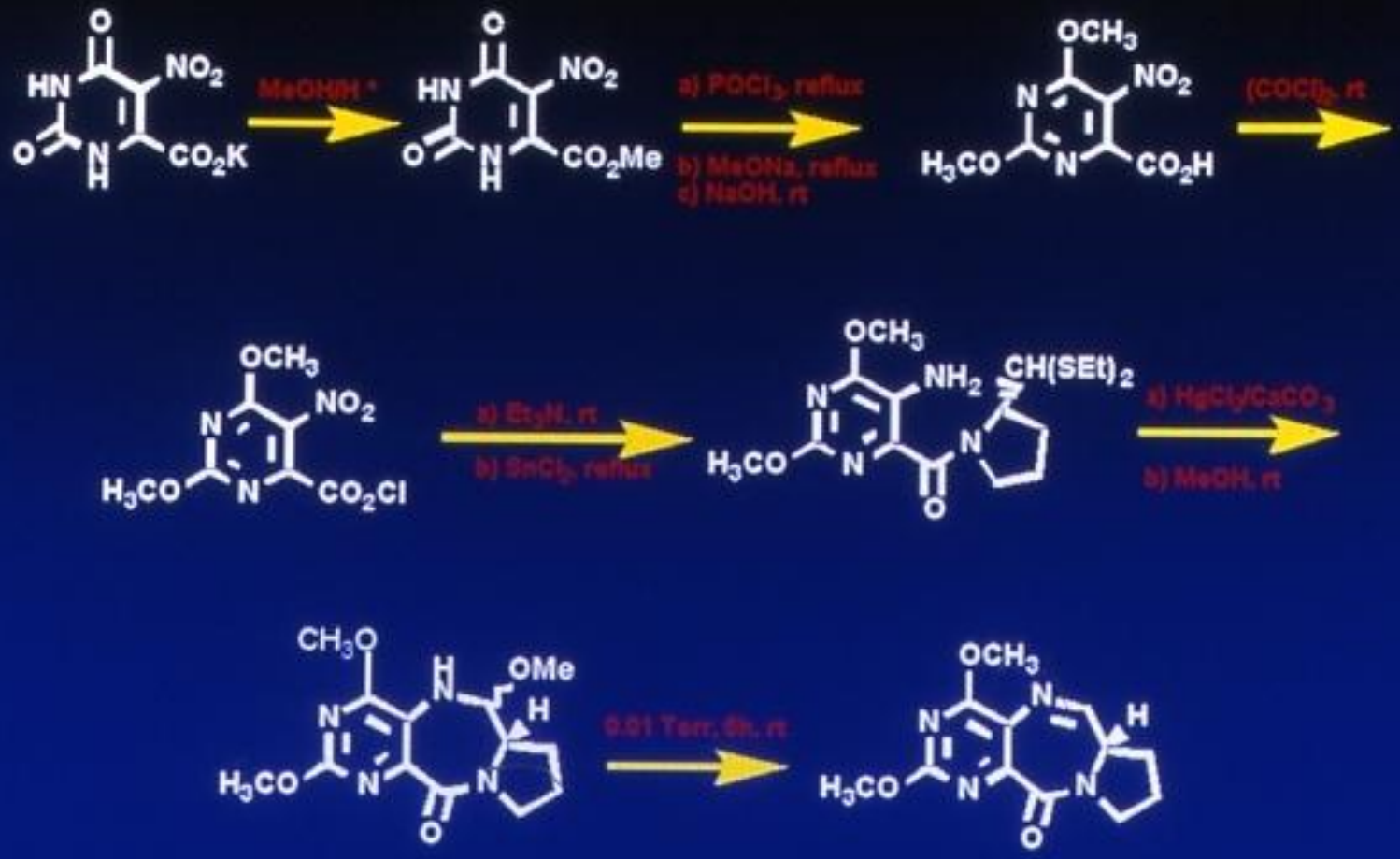
R = Me, i-Pr, MeO_2C; R_1 = Me, Et, Bn, 4-Cl-Bn, 4-MeO-Bn, 3,4-diMe-Bn



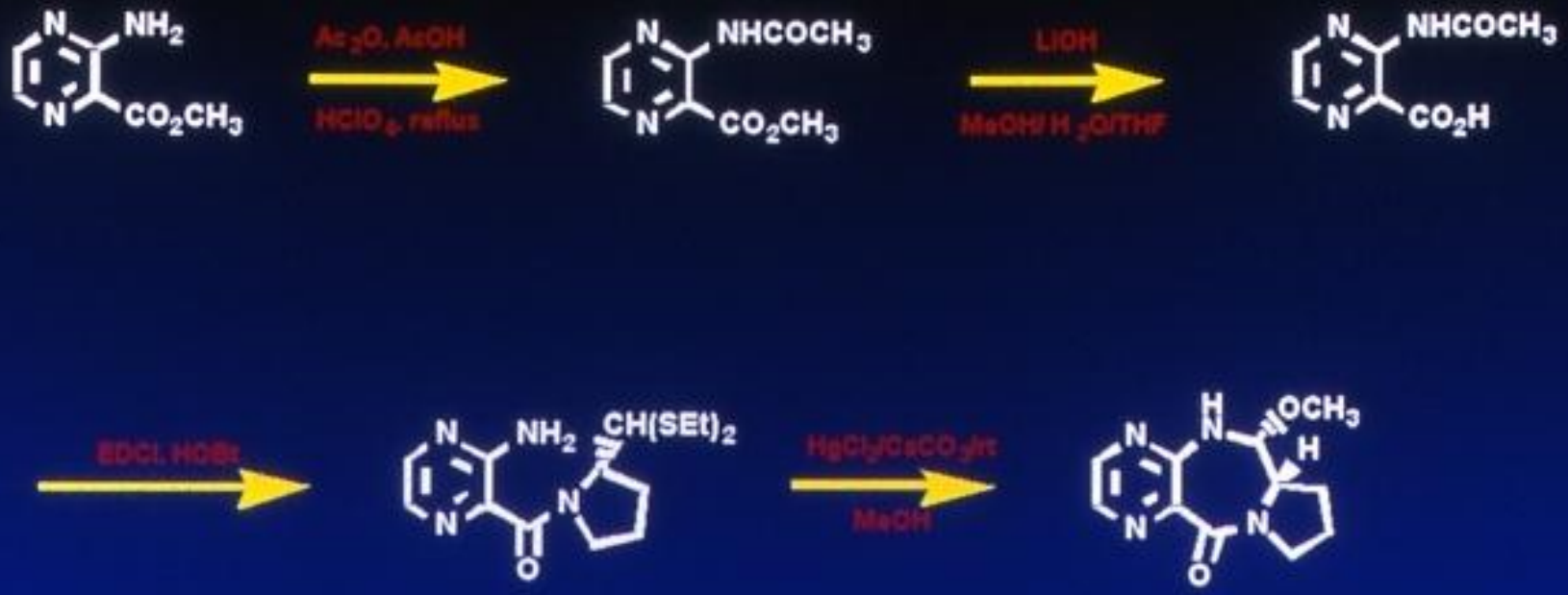
PBD's PYRIDINE ANALOG



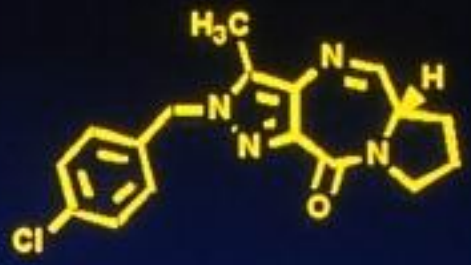
PBD's PYRIMIDINE ANALOG



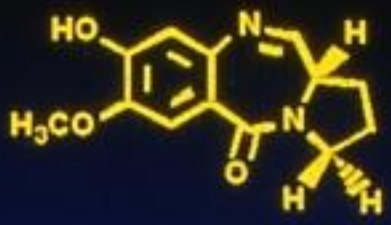
PBD's PYRAZINE ANALOGS



RESULTS

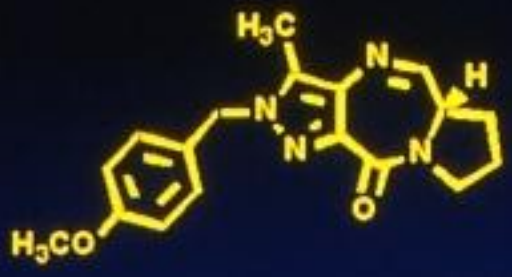


IC_{50} L1210 0.19 μM

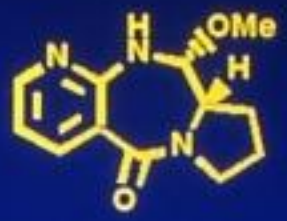


DC-81

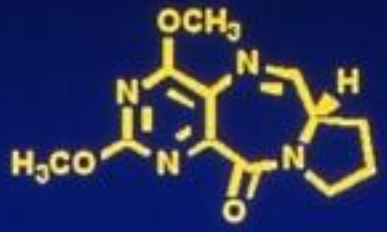
IC_{50} L1210 0.38 μM



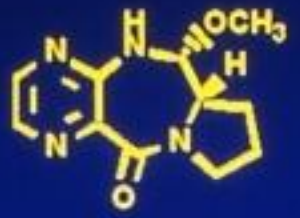
IC_{50} L1210 0.38 μM



IC_{50} L1210 31.2 μM



IC_{50} L1210 11 μM

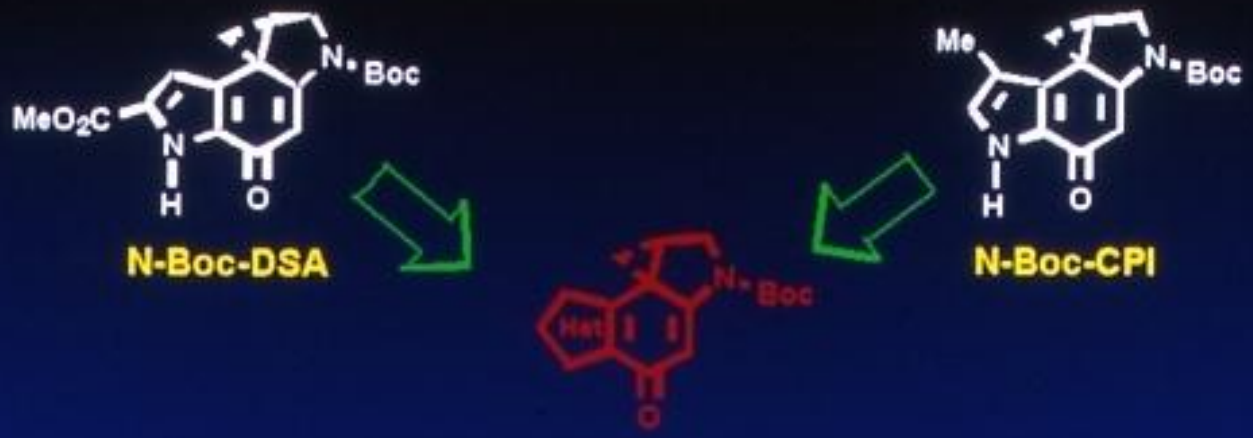


IC_{50} L1210 15 μM

CONCLUSIONS

- Substitution at N7 position increase cytotoxicity with respect to the N6 substituted compounds
- N7-benzyl or N7-substituted benzyl are the best substituents in terms of cytotoxicity
- Bulky groups at C8 position produce a decrease of cytotoxicity
- The low cytotoxicity of six-membered analogs is probably due to the greater basicity of the A ring
- Some compounds show cytotoxicity comparable to DC-81, this seems to confirm the importance of C2 chain in linking DNA

GOAL

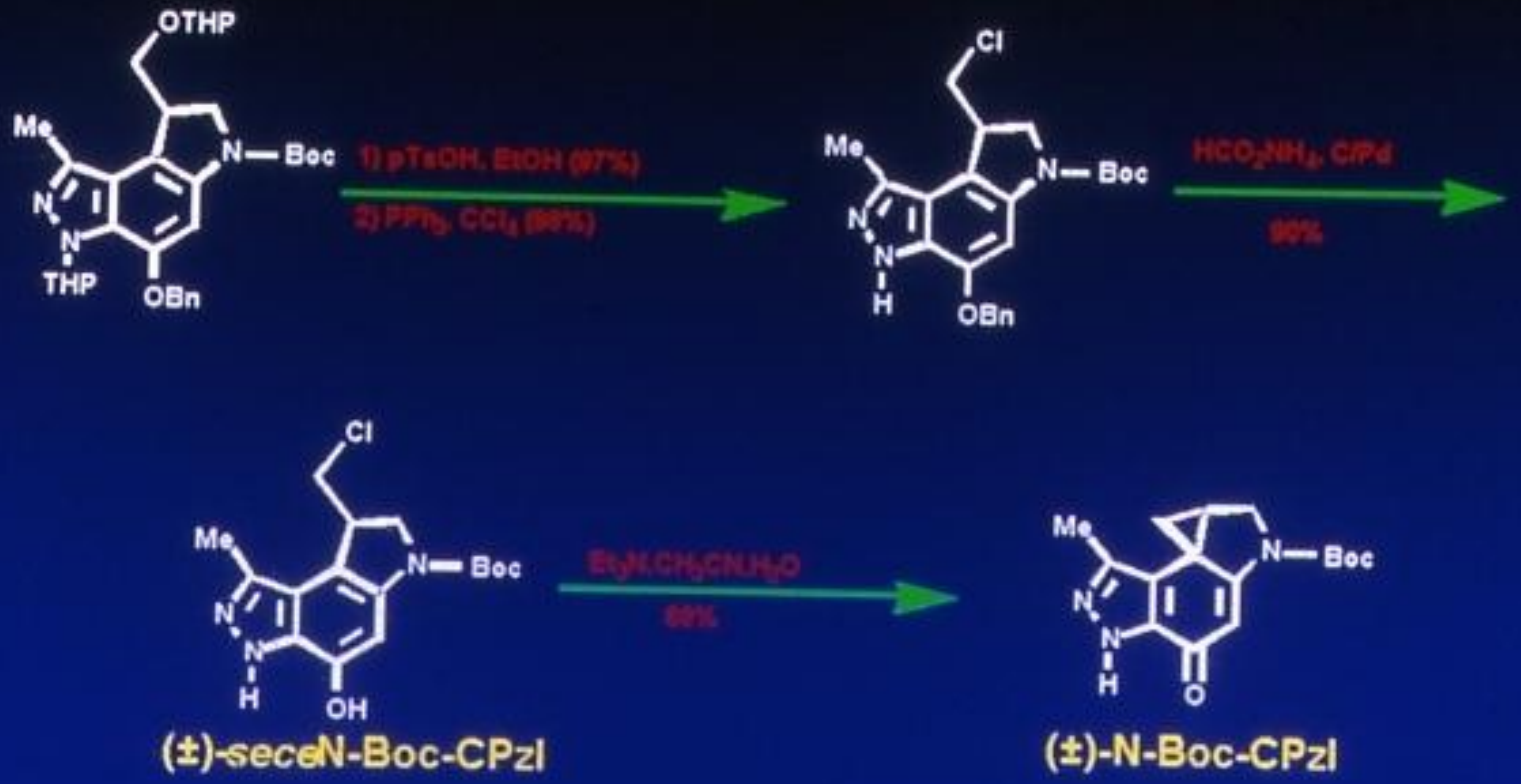


1) Studies in order to determine the sequence selectivity with different heterocycles
2) To study in more details the effect of different heterocycles on chemical reactivity of cyclopropane ring

CPzI SYNTHESIS

CPzI SYNTHESIS

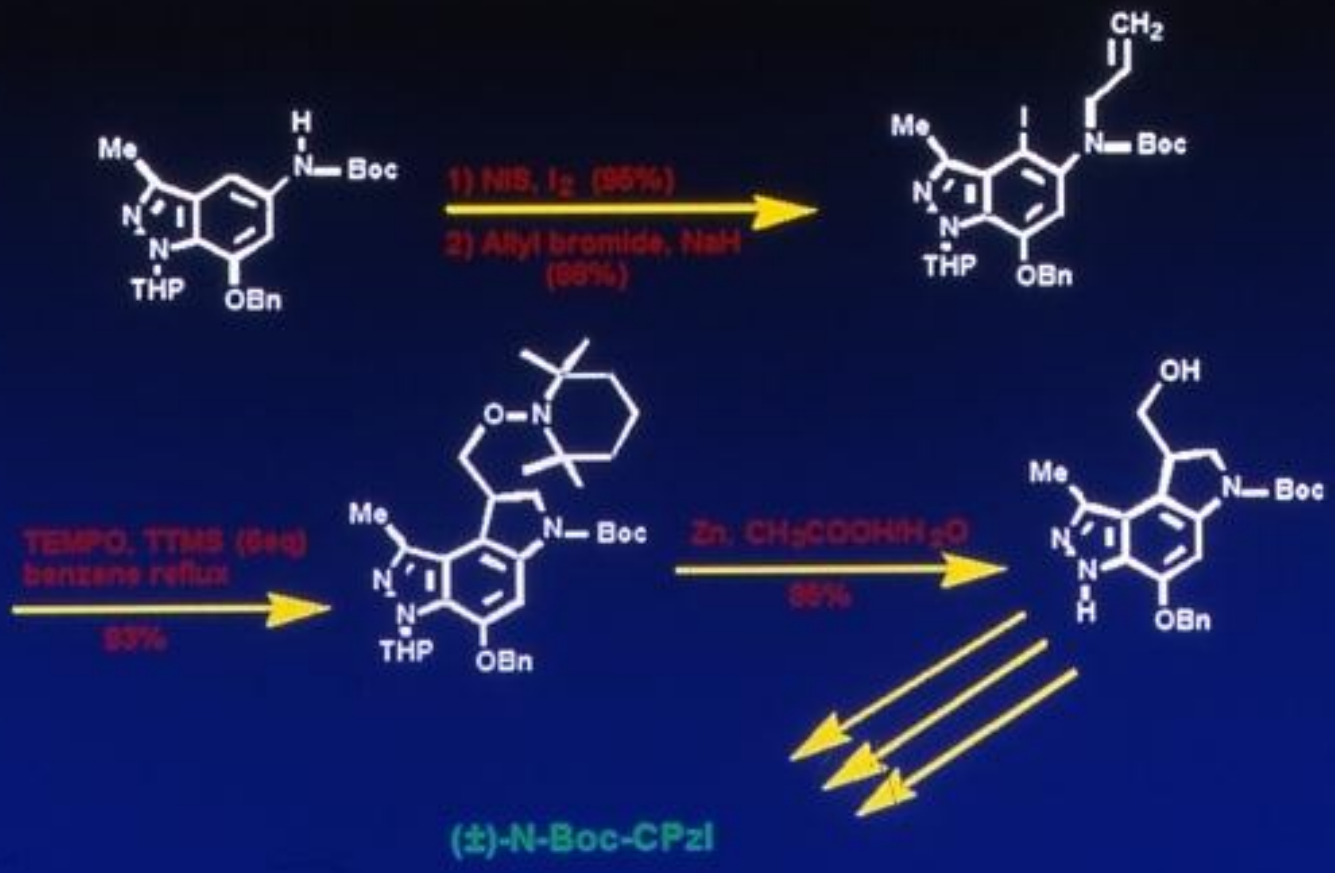
CPzI SYNTHESIS



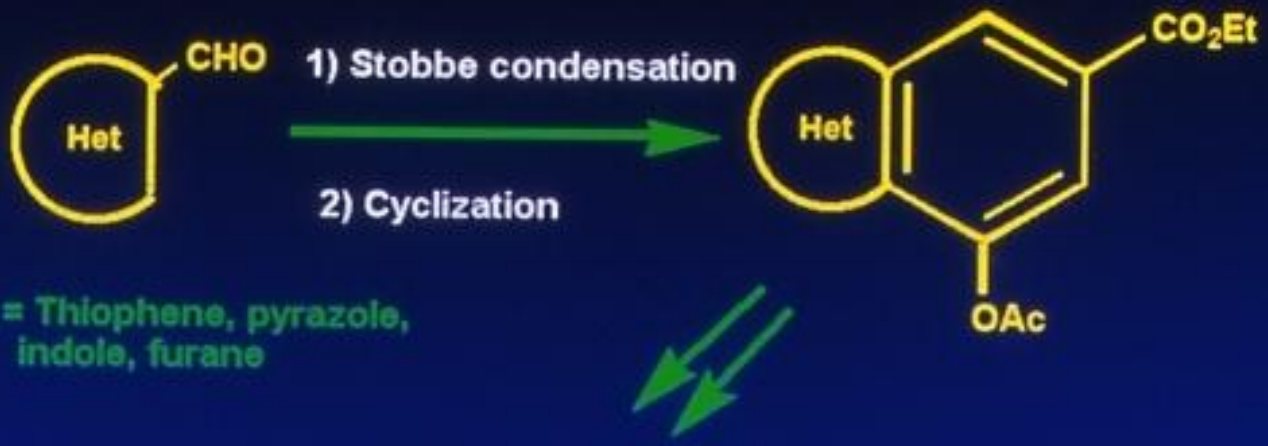
(±)-seco-N-Boc-CPzI

(±)-N-Boc-CPzI

ALTERNATIVE SYNTHESIS



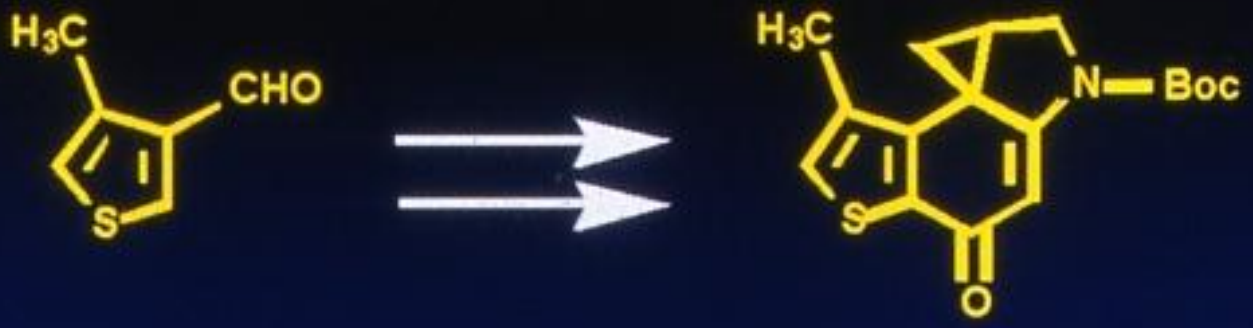
FLEXIBLE STOBBE'S APPROACH TO CPI ANALOGS



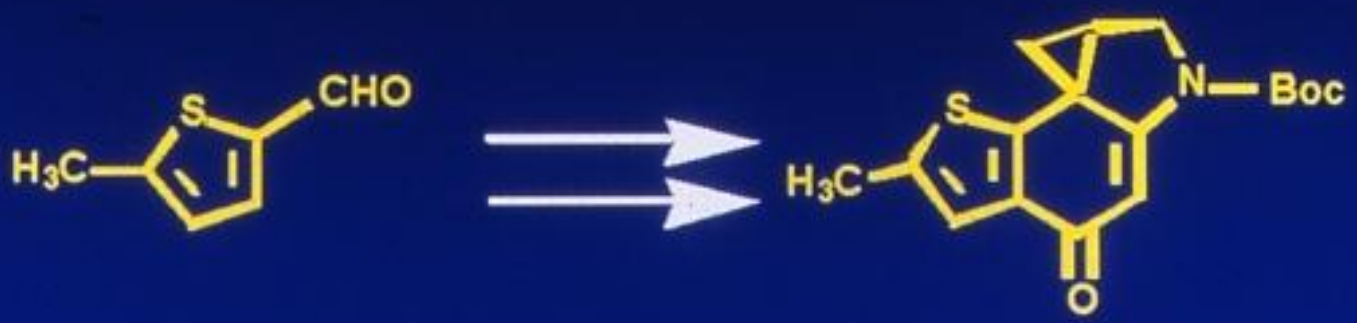
Het = Thiophene, pyrazole, indole, furane

CPI ANALOGS

THIOPHENE DERIVATIVES SYNTHESIS

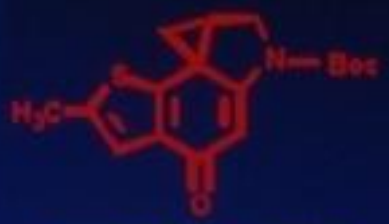


(±)-N-Boc-CTI



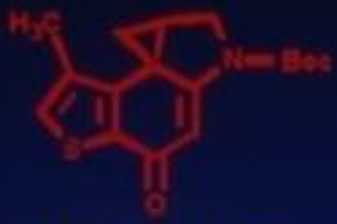
(±)-N-Boc-ICTI

RESULTS



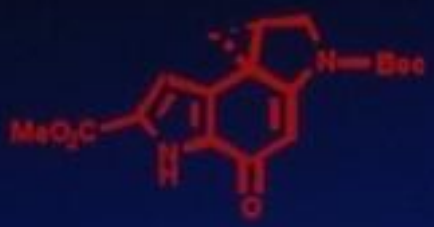
(±)-N-Boc-iCTI

IC_{50} L1210 = 14.5 nM



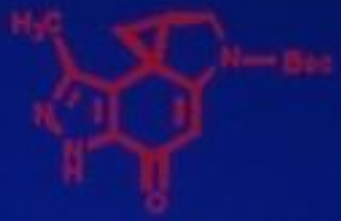
(±)-N-Boc-CTI

IC_{50} L1210 = 29 nM



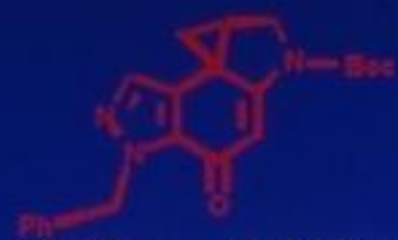
(+)-N-Boc-DSA

IC_{50} L1210 = 6 nM



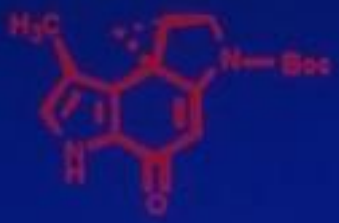
(±)-N-Boc-CPzI

IC_{50} L1210 = 370 nM



(±)-N-Boc-dMNBCPzI

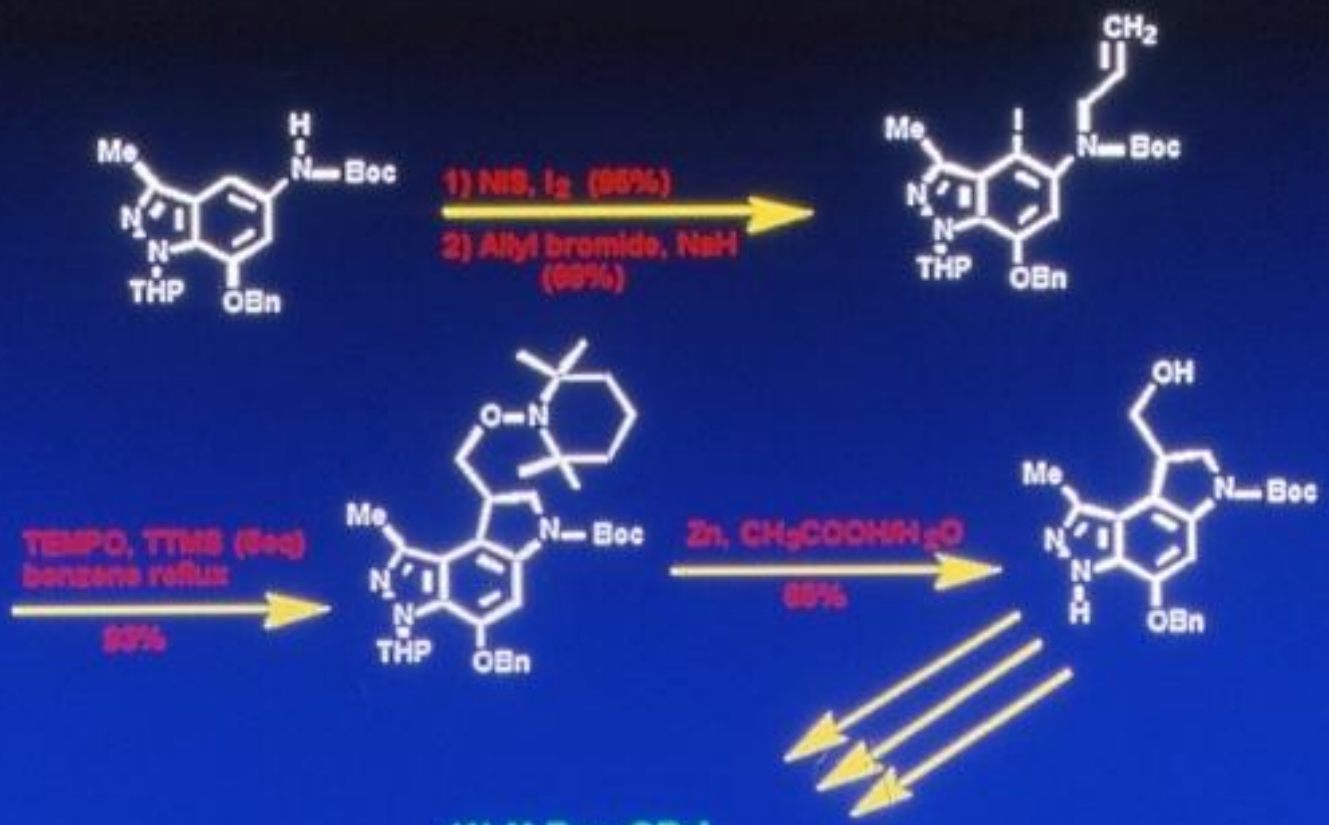
IC_{50} L1210 = 3,064 nM



(+)-N-Boc-CPI

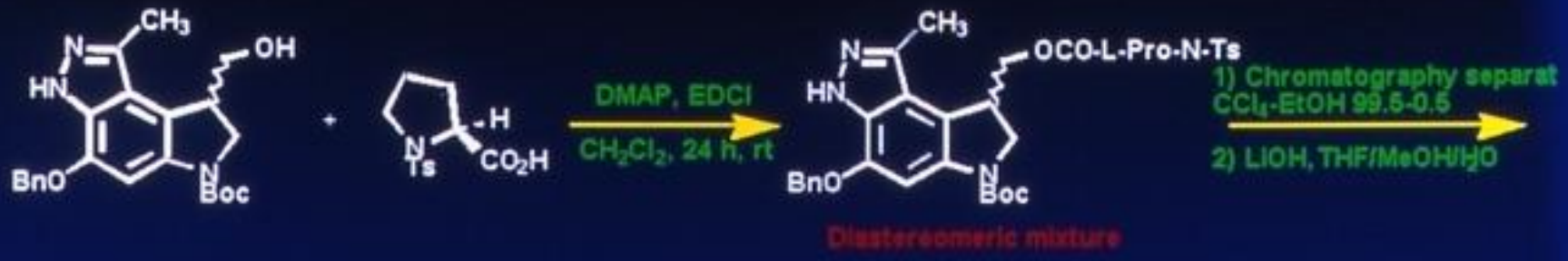
IC_{50} L1210 = 330 nM

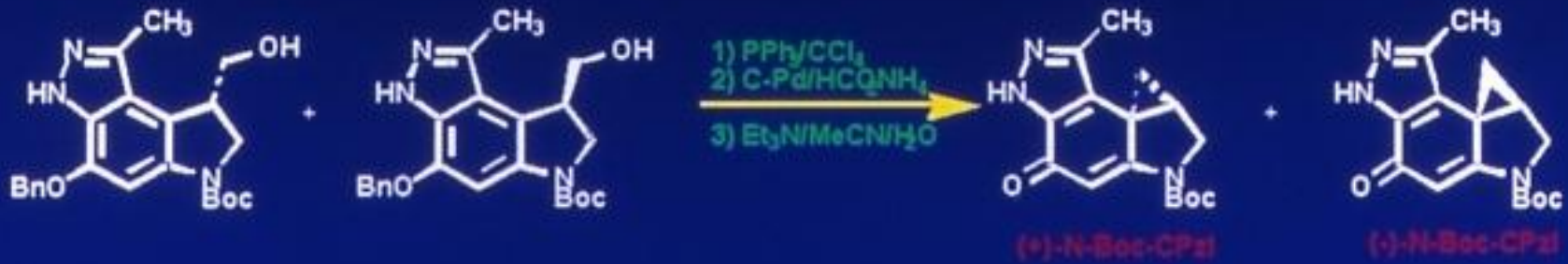
ALTERNATIVE SYNTHESIS



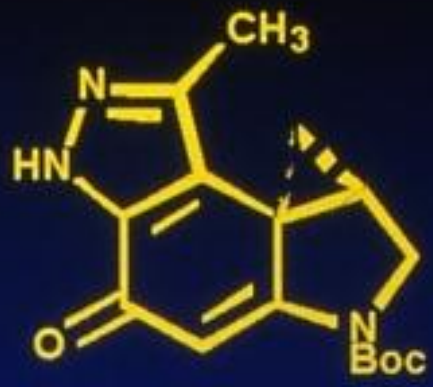
(±)-N-Boc-CPzI

ENANTIOMERIC RESOLUTION





ENANTIOMERIC DISTINCTION



(+)-N-Boc-CPzI

L1210 72h 206 nM

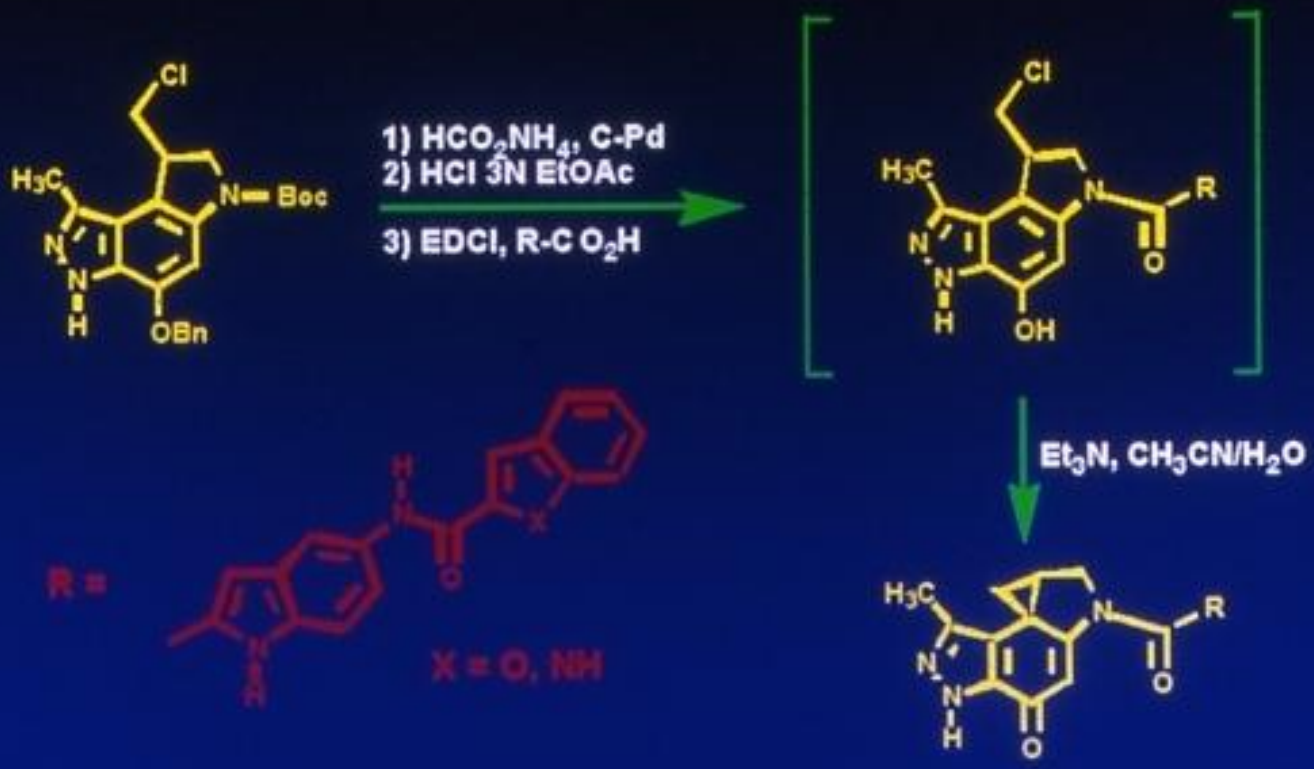
(-)-N-Boc-CPzI

L1210 72h 923 nM

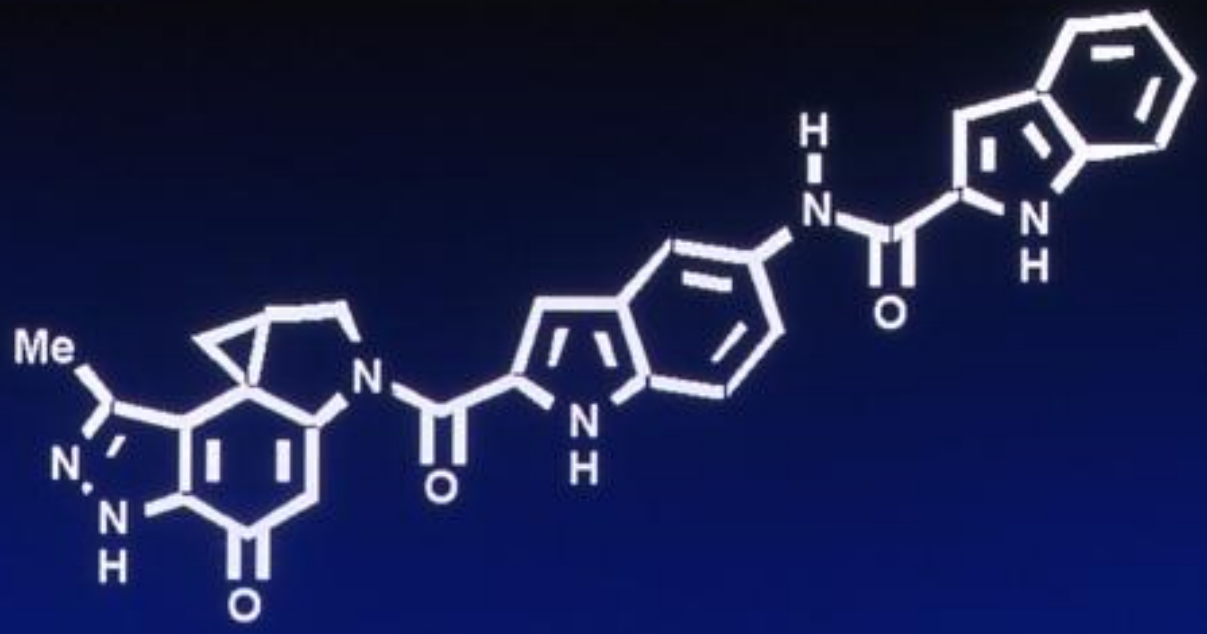
CONCLUSIONS

- A benzyl group on pyrazole nucleus is detrimental in terms of cytotoxicity
- The enantiomeric distinction has been confirmed also for N-Boc-CPzI derivative.
- The surprising potency of thiophene analogs is not clear and is still under investigation.

CC-1065 ANALOGS (CPzI)



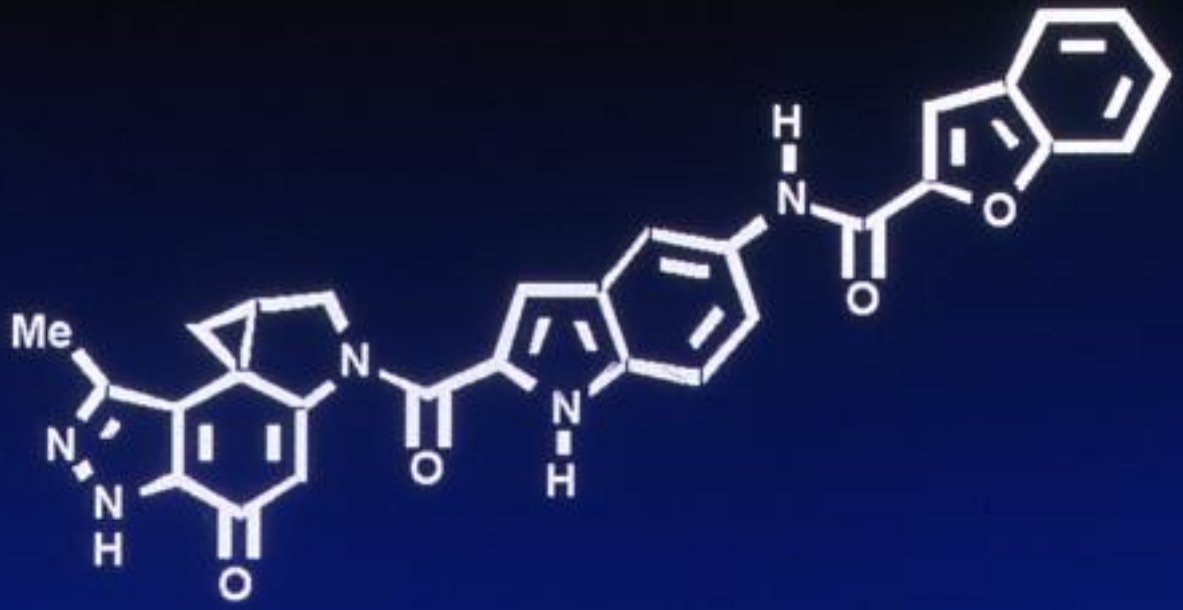
RESULTS



In vitro activity on L1210 cell lines (48 h treatment): 35 pM

In vivo activity: L1210 - O.D. 0.1 mg/Kg; T/C% 150

RESULTS



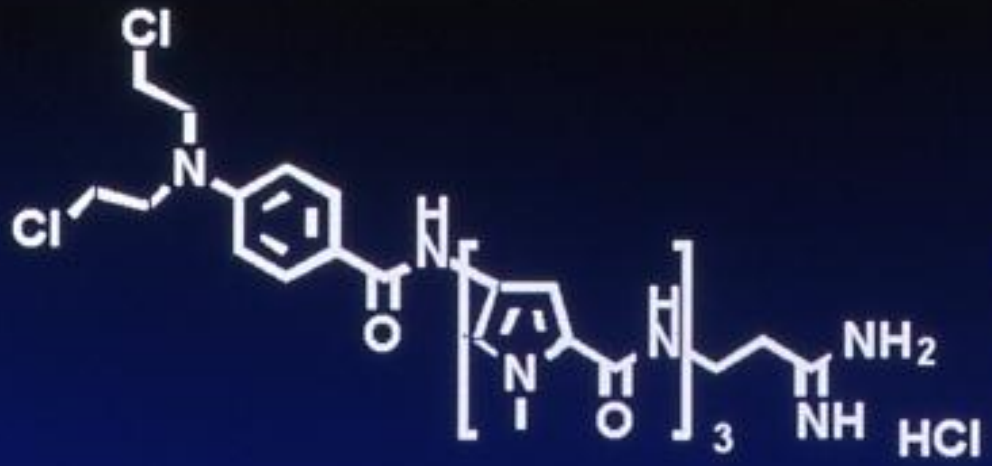
In vitro activity on L1210 cell lines (48 h treatment): 25 pM

In vivo activity: L1210 - O.D. 0.1 mg/Kg; T/C% 463

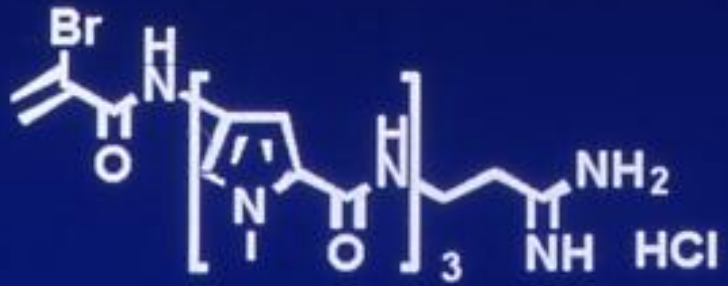
CONCLUSIONS

- The substitution of the pyrrole moiety with a pyrazole nucleus does not change sequence selectivity (high AT specificity).
- The presence of a benzofurane ring on the "carrier" confers to the CC-1065 analogs good potency and activity with respect to the $(indole)_2$-derivative. This is in agreement with the studies performed by Upjohn Company.
- These results confirm the hypothesis that the presence of a hydrophilic function (pyrazole nitrogen) confers to the drug an increase in stability in water solution and consequentely a better distribution to tumor treated animals. In fact the increase of hydrophobic characteristics of the molecule (benzyl chain) produce the opposite effect.

REFERENCE COMPOUNDS



Tallimustine
IC_{50}=50.3 ng/ml
O.D=6.25 mg/Kg
%T/C=125



IC_{50}=49.6 ng/ml
O.D=12.5 mg/Kg
%T/C=175

Tallimustine Pyrazole Derivatives

L1210 murine leukemia

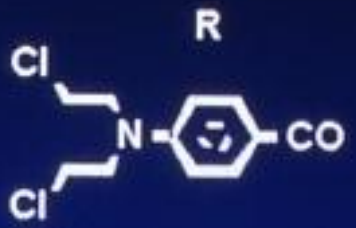
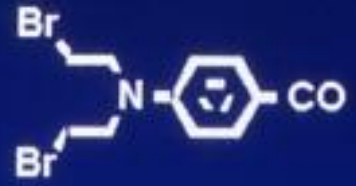
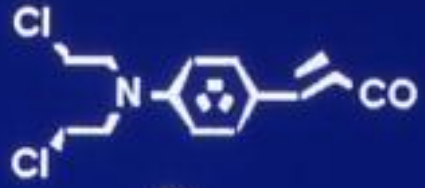
A	B	C	IC_{50} (ng/mL)	O.D. (mg/Kg)	% T/C
pyrrole	pyrrole	pyrrole	50.3	6.25	125
pyrrole	pyrrole	pyrazole	35	6.25	213
pyrrole	pyrazole	pyrazole	225	12.5	213
pyrazole	pyrrole	pyrrole	1398	12.5	218
pyrrole	pyrazole	pyrrole	78.6	n.d	n.d
pyrazole	pyrazole	pyrrole	1887	n.d	n.d
pyrazole	pyrazole	pyrazole	1325	12.5	100

Tallimustine Derivatives

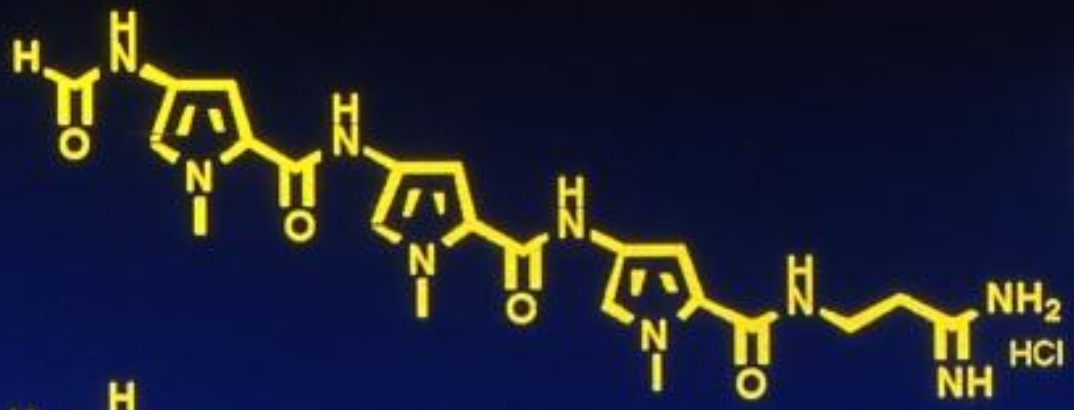
	L1210 murine leukemia		
	IC_{50} (ng/mL)	O.D. (mg/Kg)	% T/C
X=CH Y=S Z= N (thiazole) R= benzoic acid mustard	32460	n.d	n.d
X=CH Y=S Z= N (thiazole) R=α-bromoacryloyl	23820	n.d	n.d
X= N-CH_3 Y= N Z= CH (pyrazole) R= benzoic acid mustard	1398	12.5	118
X= N-CH_3 Y= N Z= CH (pyrazole) R=α-bromoacryloyl	151	30	181
X= N-CH_3 Y=CH Z= N (imidazole) R= benzoic acid mustard	1902	25	114

Tallimustine Derivatives

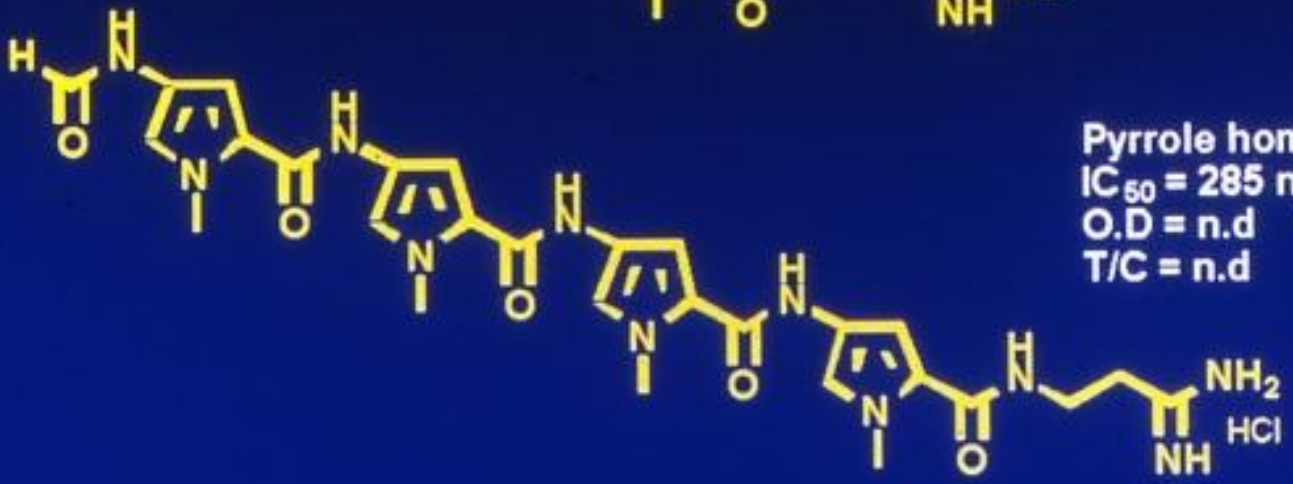
L1210 murine leukemia

R	IC_{50} (ng/mL)	O.D. (mg/Kg)	% T/C
	225	12.5	213
	6.27	1.56	750
	10.8	n.d	n.d
	46.6	n.d	n.d

RESULTS



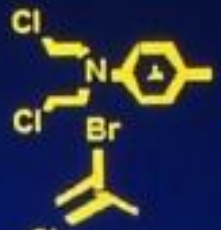
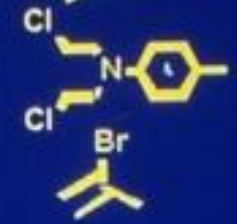
Distamycin A
IC_{50} = 5216 ng/ml
O.D = 200 mg/Kg
T/C = 113



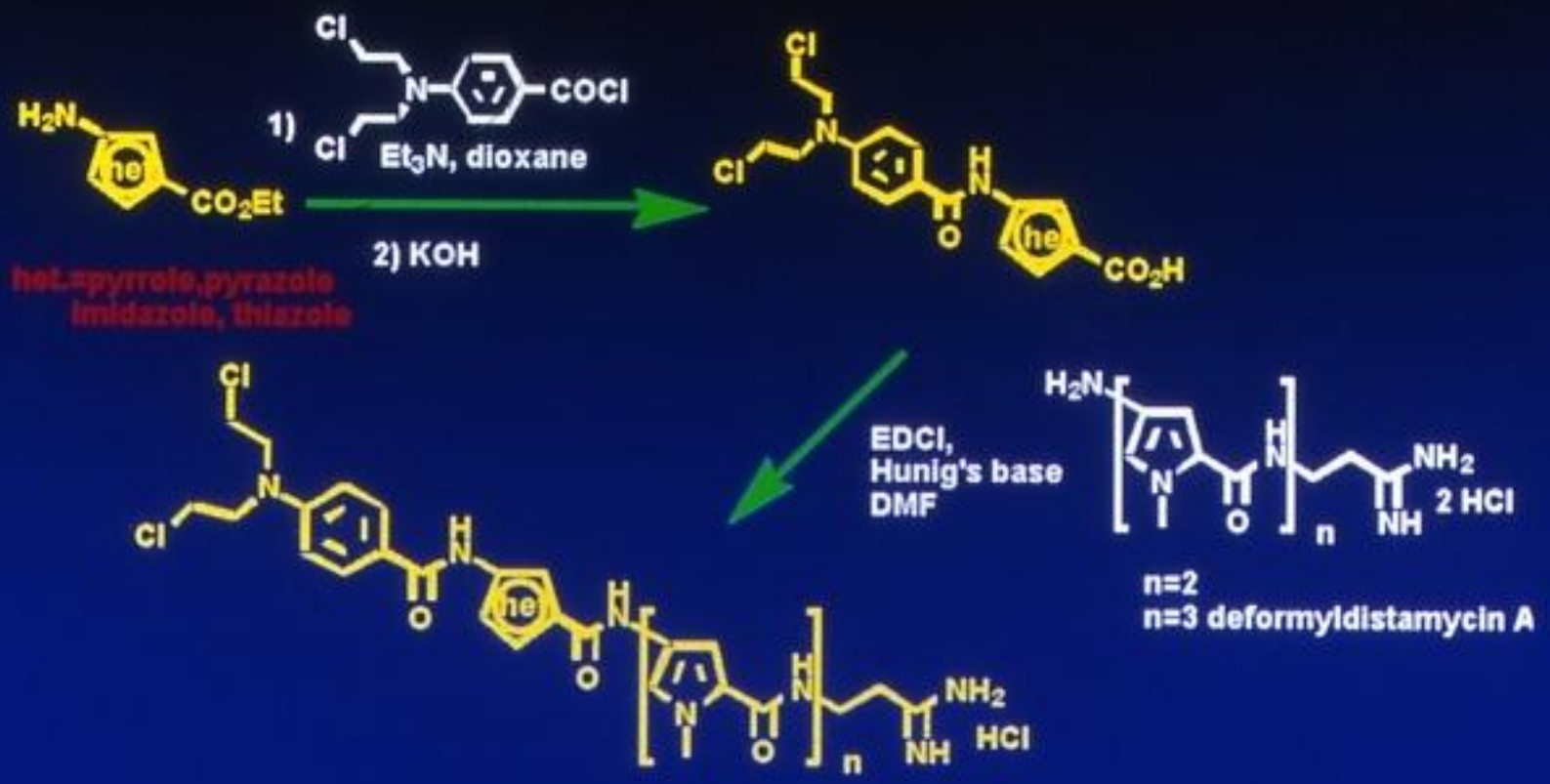
Pyrrole homologou
IC_{50} = 285 ng/ml
O.D = n.d
T/C = n.d

Distamycin Derivatives

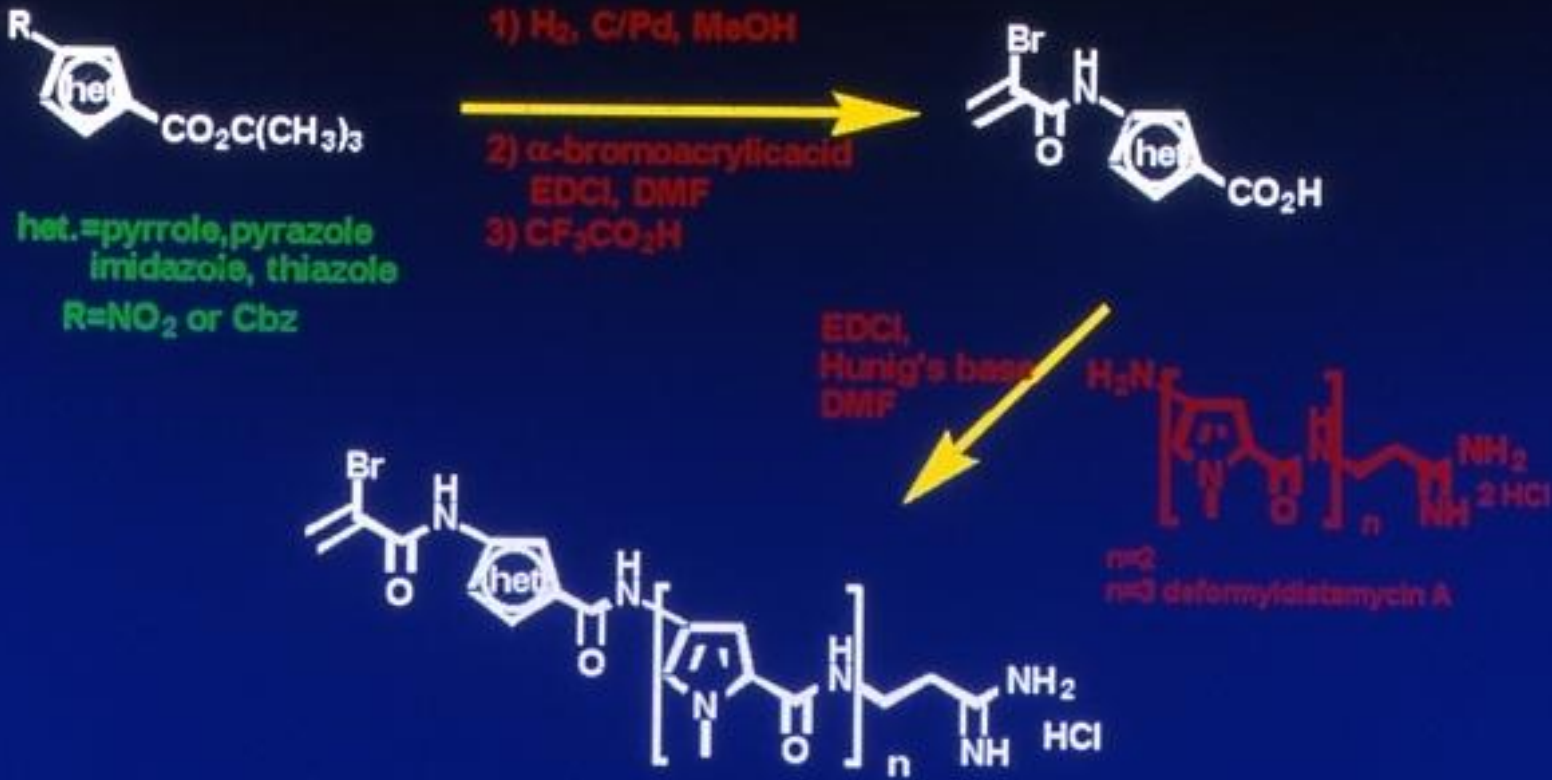
L1210 murine leukemia

R	heterocycle	IC_{50} (ng/mL)	O.D. (mg/Kg)	% T/C
	pyrrole	16.3	0.39	138
	pyrrole	4.7	3.12	206
	pyrazole	29.1	3.13	144
	pyrazole	9.9	6.25	200
	imidazole	2000	6.25	125
	imidazole	35	6.25	163

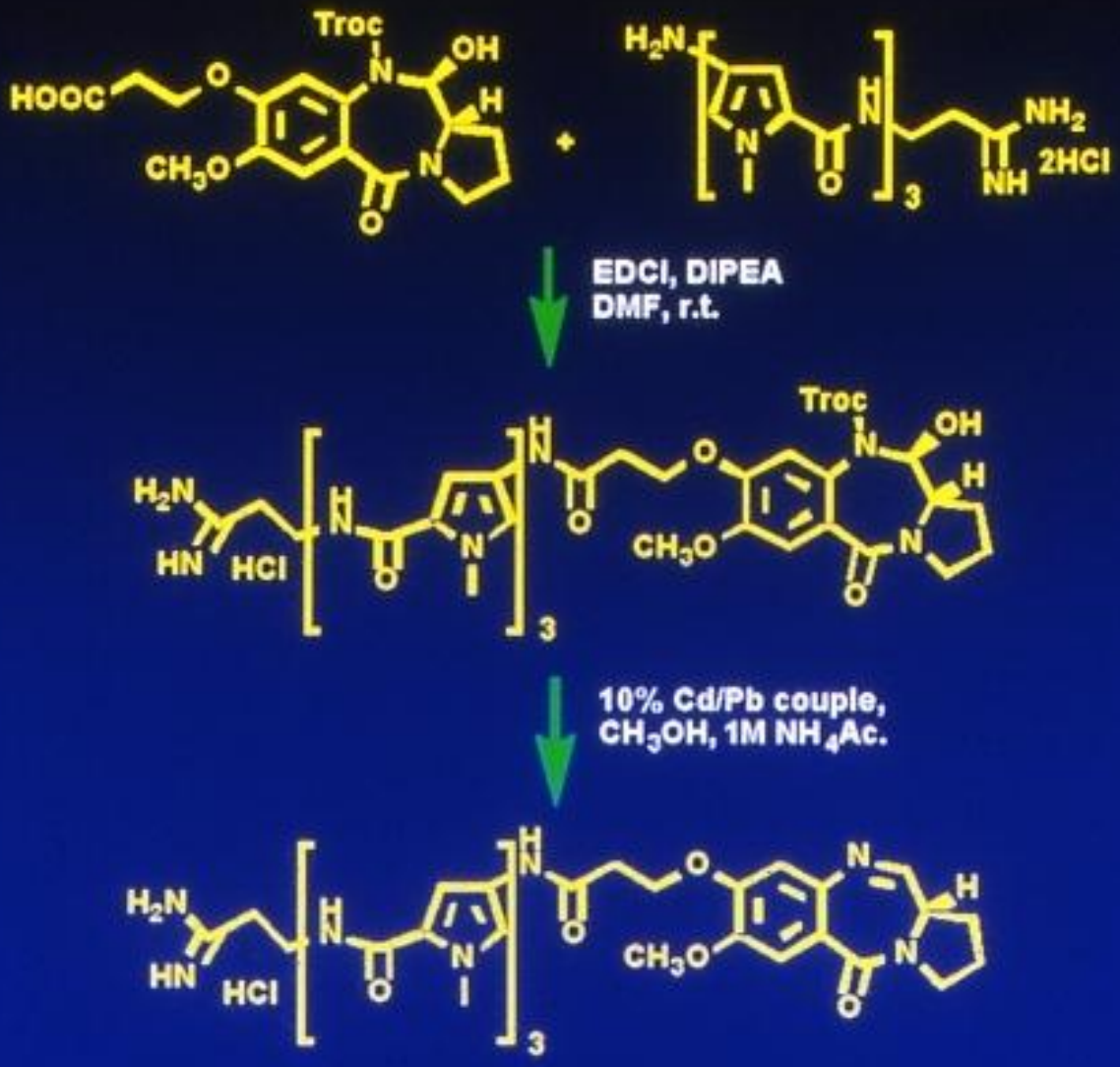
SYNTHESIS


Cl
Cl
N
COCl
1)
Cl
Et3N, dioxane
H2N
het
CO2Et
2) KOH
het.=pyrrole,pyrazole
imidazole, thiazole
Cl
Cl
N
O
H
N
het
CO2H
Cl
N
Cl
O
H
N
het
O
H
N
N
O
n
NH
NH2
HCl
EDCI,
Hunig's base
DMF
H2N
N
O
H
N
n
NH
NH2
2 HCl
n=2
n=3 deformyldistamycin A

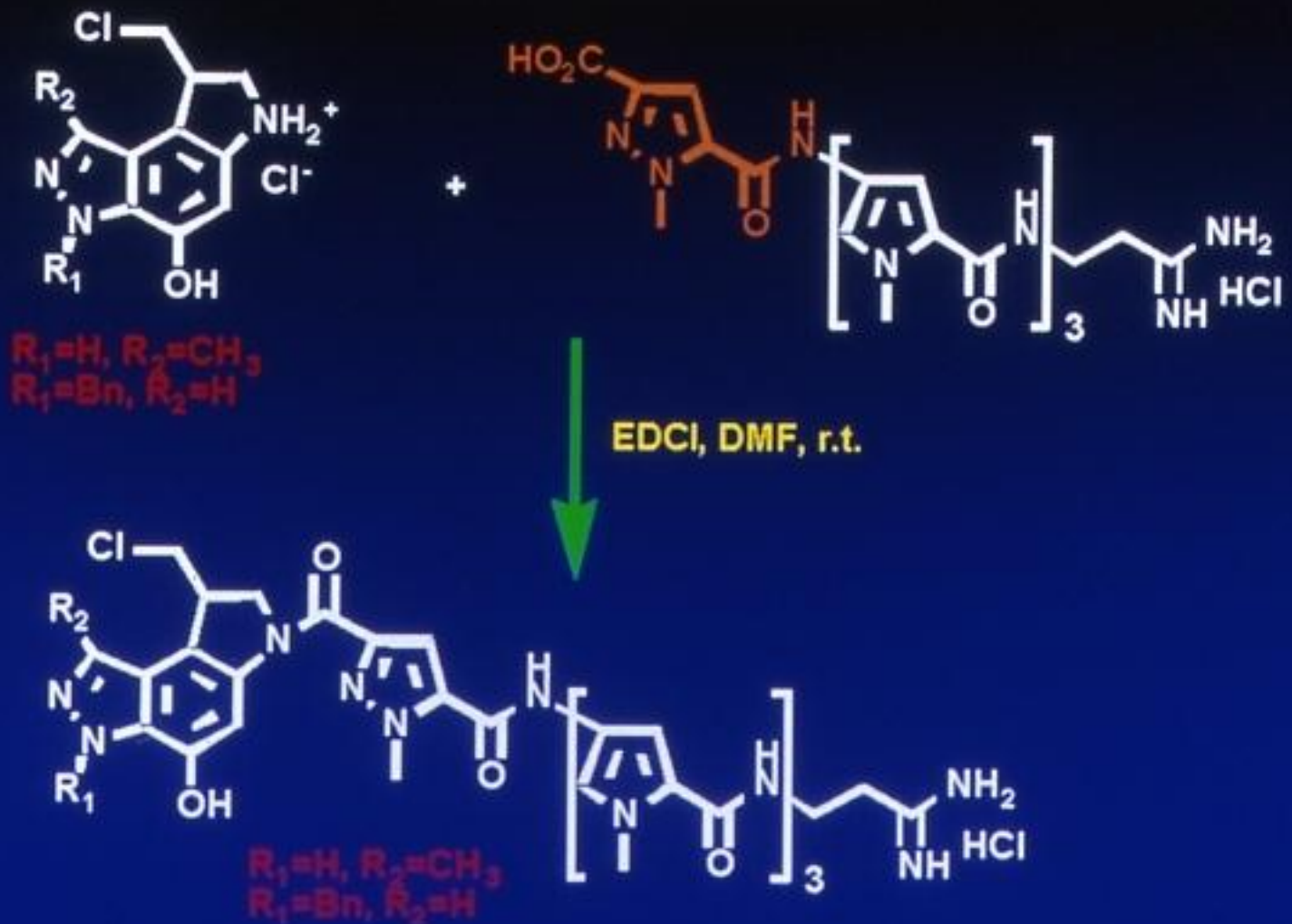
SYNTHESIS


R
het
$CO_2C(CH_3)_3$
het.=pyrrole,pyrazole
imidazole, thiazole
R=NO_2 or Cbz
1) H_2, C/Pd, MeOH
2) α-bromoacrylicacid
EDCI, DMF
3) CF_3CO_2H
Br
CO_2H
EDCI,
Hunig's base
DMF
H_2N
NH_2
2 HCl
n=2
n=3 deformyldistamycin A
HCl

HYBRID PBD-DISTAMYCIN



HYBRID CC-1065-DISTAMYCIN PYRAZOLE HOMOLOGUE



CONCLUSIONS

- For the compounds having the same oligopeptide frame the derivatives bearing an α-bromoacryloyl moiety show a better biological profile with respect to BAM counterparts
- Replacement of a pyrrole with pyrazole appears devoid effects when occuring at the amidine terminus, while appears detrimental when occuring near the BAM moiety
- Biological profile of tallimustine derivatives is a result of cooperative effects due to both by the nature of alkylating moiety and oligopeptidic fragment

ACKNOWLEDGMENTS

- Dr. Nicola Mongelli (Pharmacia Upjohn)
- Dr. Paolo Cozzi (Pharmacia Upjohn)
- Dr. Abdel Naser Zaid
- Dr. Andrea Guiotto
- Dr. Alberto Leoni
- Dr. Romeo Romagnoli
- Dr. Barbara Cacciari
- Dr. Giampiero Spalluto