DESIGN, SYNTHESIS AND BIOLOGICAL EVALUATION OF NON-PEPTIDIC INHIBITORS OF FARNESYLTRANSFERASE

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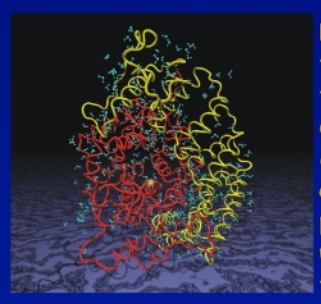
INTRODUCTION

Ras proteins are plasma membrane-bound GTP-binding proteins which are involved in the transmission of signals from extracellular stimuli to the nucleus. Mutations in ras genes, that lead to uncontrolled cell growth, have been identified in human cancers especially those of pancreas, colon, lung and bladder [1]. These oncogenic proteins are irreversibly complexed with GTP and this discovery has induced many researchers to explore the role of Ras-induced cellular transformations in order to find novel anticancer drugs.



Ras proteins are initially synthesized in the cytoplasma, where they undergo farnesylation as the first step of the post-translational modifications. Farnesylation of the Cysteine unit of the so-called CA$_1$A$_2$X box is followed by the proteolytic cleavage of the three terminal aminoacids (AAX) and by methyl esterification at the new C terminal Cysteine residue by a protein methyltransferase [2]. The last step is the acylation, with palmitic acid, of Cysteine residue located upstream of the farnesylated Cysteine.

This palmitoylation increases the binding of Ras proteins to the cell membrane. In this sequence, the determinant step is the farnesylation which is catalyzed by farnesyltransferase (Ftase). Several studies showed that the FTase inhibition blocks Ras-induced biological transformations, whereas the other modifications are not essential for the Ras proteins activity [3]. Therefore, inhibition of this enzyme represents a potential target for the development of anticancer agents.

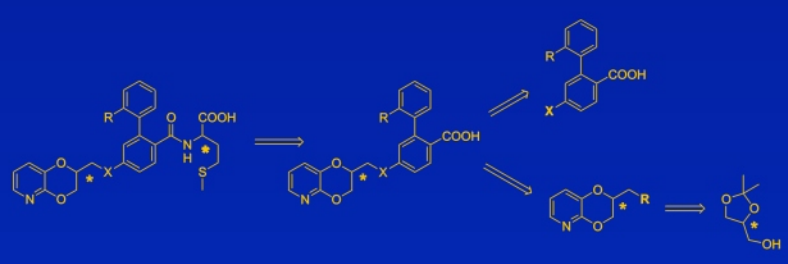
AIM OF THE WORK

Among many classes of Farnesyltransferase inhibitors (FTIs) reported in the literature our attention was focused on a series of peptidomimetics inhibitors that bear a diphenyl group in lieu of the A$_1$A$_2$ amino acids and where the sulphydryl group is substituted by a pyridine ring.
Aim of this work was to verify the effects of the following structural modifications:

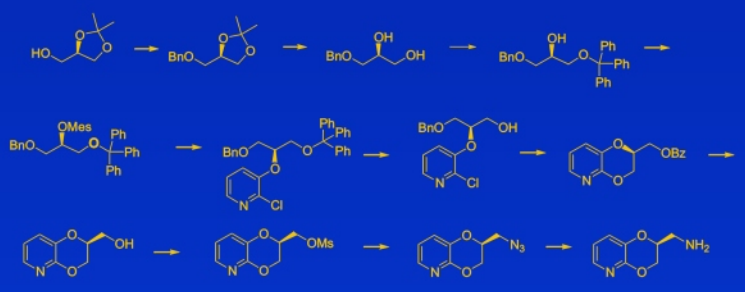
- increasing the electronrichness of pyrido system including it in a pyridodioxane moiety that reduces the overall flexibility.
- introducing a second chiral center to highlight the stereoselectivity of the binding site.
- changing the pyridodioxane with a benzodioxane moiety to verify the need of the nitrogen atom.
- modifying the terminal aminoacid (X) to assess the need of the thiomethyl function.

On these bases, the compounds 1 - 40 were developed.

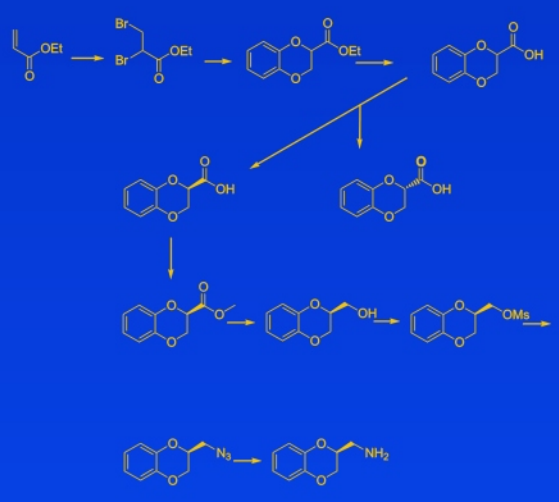
DISCONNECTION APPROACH



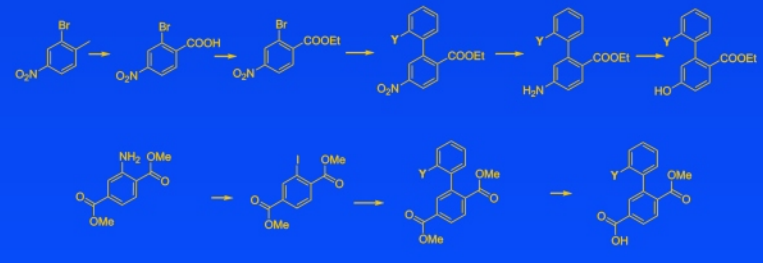
We used (S)- and (R)-glycerol acetonide as building blocks to synthesize the enantiomers of the mesyloxymethyl- and aminomethylpyridodioxane intermediates.

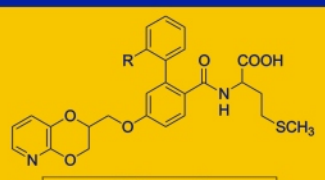


The preparation of both the enantiomers of the mesyloxymethyl and aminomethylbenzodioxane intermediates was based on the resolution of 1,4-benzodioxan-2-carboxilic acid with (+)-dehydroabietylamine[4]



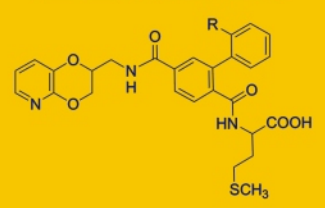
The reaction of these intermediates with the appropriate biphenyl derivatives (as shown below) and the subsequent condensation with the aminoacids yielded, after hydrolysis, the compounds **1 -40**:



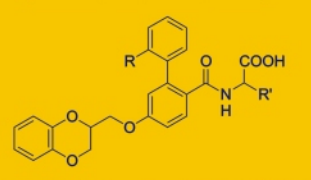


Inhibition of farnesyltransferase @ 50 nM

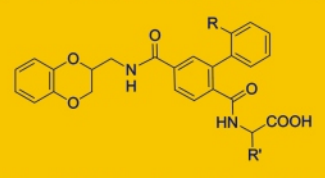
Compound	R	Isomer	% inhib.
1	Me	SS	10 %
2	Me	RR	16 %
3	Me	SR	11 %
4	Me	RS	12 %
5	OMe	SS	14 %
6	OMe	RR	13 %
7	OMe	SR	9 %
8	OMe	RS	12 %



Compound	R	Isomer	% inhib.
9	Me	SS	18 %
10	Me	RR	15 %
11	Me	SR	16 %
12	Me	RS	100 %
13	OMe	SS	60 %
14	OMe	RR	18 %
15	OMe	SR	65 %
16	OMe	RS	100 %



Compound	R	R'	Isomer	% inhib.
17	Me	H	S	14 %
18	Me	H	R	11 %
19	Me	$CH_2CH(CH_3)_2$	RS	25 %
20	Me	$CH_2CH(CH_3)_2$	SS	12 %
21	Me	$CH_2CH_2SCH_3$	RS	60 %
22	Me	$CH_2CH_2SCH_3$	SS	47 %
23	OMe	H	R	12 %
24	OMe	H	S	8 %
25	OMe	$CH_2CH(CH_3)_2$	SS	33 %
26	OMe	$CH_2CH(CH_3)_2$	RS	60 %
27	OMe	$CH_2CH_2SCH_3$	SS	90 %
28	OMe	$CH_2CH_2SCH_3$	RS	100 %



Compound	R	R'	Isomer	% inhib.
29	Me	H	S	60 %
30	Me	H	R	11 %
31	Me	$CH_2CH(CH_3)_2$	RS	42 %
32	Me	$CH_2CH(CH_3)_2$	SS	13 %
33	Me	$CH_2CH_2SCH_3$	RS	60 %
34	Me	$CH_2CH_2SCH_3$	SS	86 %
35	OMe	H	R	9 %
36	OMe	H	S	10 %
37	OMe	$CH_2CH(CH_3)_2$	SS	76 %
38	OMe	$CH_2CH(CH_3)_2$	RS	11 %
39	OMe	$CH_2CH_2SCH_3$	SS	44 %
40	OMe	$CH_2CH_2SCH_3$	RS	64 %

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[1] J. L. Bos, Cancer Res. **1990**, 50, 1352.
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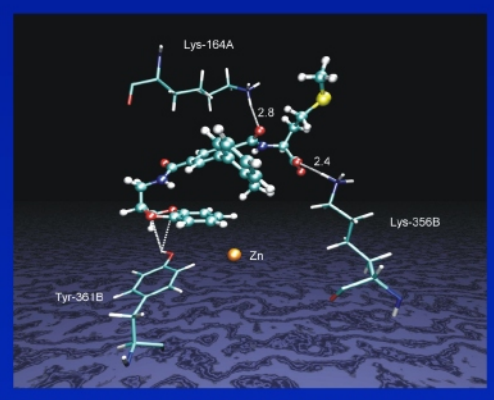
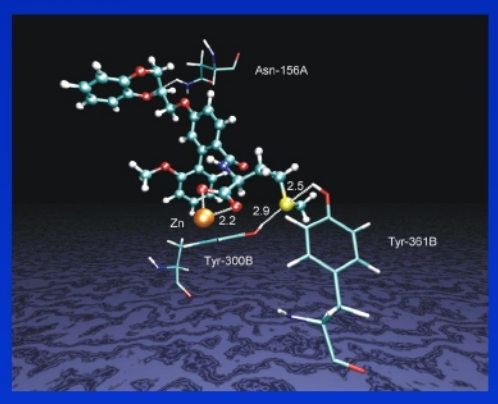
[3] a) J. W. Leitner, T. Kline, K. Carel, M. Goalstone, B. Draznin, Endocrinology **1997**, 138, 2211. b) L. Gutierrez, A. I. Magree, G. J. Marshall, J. F. Hancock, EMBO J. **1989**, 1093. c) T. Dudler, M. H. Gelb, J. Biol. Chem.**1996**, 271, 11541.
[4] C. Bolchi, L. Fumagalli, B. Moroni, M. Pallavicini, E. Valoti Tetrahedron: Asymetry **2003**, 14, in press.

DOCKING ANALYSIS

The docking analysis indicates that these inhibitors can assume two different binding modes depending on the group that interacts with the zinc ion: the benzo/pirydo dioxane in the first case or the carboxylate in the second one.



The comparison between the docking results and the inhibition values allow to hypothesise that the pyridodioxane derivatives prefer to bind the enzyme according to first mode (pyrydo nitrogen atom interacts with the Zn^{++} ion), while the carboxyl group directly interacts with the Zn^{++} ion in the benzodioxane derivatives.



These hypothesis is supported by the following considerations:

- In the pyridodioxanes only the amido derivatives are active, underlining the relevance of distance between diphenyl and pyrido moieties and the mutual disposition..
- On the opposite, the activity of benzodioxane derivatives seem not dependent on the linker between the two ring systems, suggesting that the benzodioxane is not involved in critical interaction with Ftase.

CONCLUSIONS

The analysis of the enzymatic inhibition data allows to draw the following conclusions:

- The methoxyl derivatives are always more active than the methyl ones.
- The sostitution of methionine with leucine resulted in a slightly decreased activity; the glycine derivatives are inactive.
- In the benzodioxane derivatives, the activity is not influenced by the linker structure (-CH_2-O- or -CH_2-NH-CO-).
- The configurations of chiral centres don't play a pivotal role, maybe due to the large dimension of the enzyme cavity.
- The pyridine is not essential for the activity and can be replaced with a phenyl ring.

The last conclusion is the most important as it confirms that the ability to coordinate the metal is not a critical prerequisite to obtain active ligands.