# Towards the design of β-carbolines as potential PDE4 inhibitors.

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**Summary**

The phosphodiesterases (PDEs) are enzymes that catalyze the hydrolysis of cyclic nucleotides cGMP and cAMP into their respective 5'nucleoside monophosphates. Recently, interest in the potential use of isoenzyme selective PDE inhibitors has increased for its implication in various diseases. A series of substituted β-carbolines, structurally related to DMCM, as PDE4 inhibitors was considered. This work led to some optimized compounds, which detail of synthesis will be presented. To determine the needs for the activity of these compounds, we also performed a computational study to define the binding mode of the ligands into the protein's site and so to carry out a strategy for a drug design. This finding could give details how selective inhibitors with different chemical structures bind to the similar catalytic pockets of PDEs.

**Keywords**: DMCM, synthesis, β-carboline, PDE4 inhibitors, molecular modelling.

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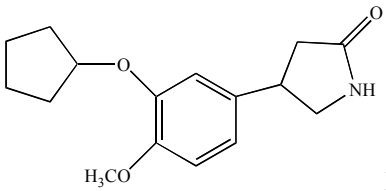
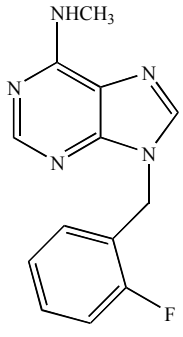
## Introduction

The hydrolysis of the second messengers adenosine-3',5'-cyclic phosphate (cAMP) and cGMP is catalyzed by a superfamily of enzymes, called phosphodiesterases (PDEs) causing their inactivation. Eleven different PDE families have been described to date (from PDE 1 to PDE 11) and these can be further divided into a number of subtypes and slice variants, which differ in their substrate preference and catalytic activity, sensitivity to endogenous activators and inhibitors, their distinct genes, and cellular and tissue distribution[1]. Within PDE families, sequence identity is more extensive, reflecting greater relatedness in their biochemical properties. PDEs are clinical target fro a range of biological disorders, such as asthma, depression, chronic obstructive pulmonary disease, retinal degradation, congestive heart failure, autoimmune disease and inflammation in general. cAMP-specific PDE is the predominant isoenzyme present in pro-inflammatory cells which are thought to be important in acute and chronic, particularly inflammatory and allergen-induced airway disorders of various origin (bronchial asthma, emphysema, COPD): for these reasons, PDE4 has become an important target for drug screening. Thus, there is remarkable interest in PDE 4 cAMP specific phosphodiesterases as PDE4 selective inhibitors have potent anti-inflammatory action and are being developed as therapeutic agents for respiratory diseases. Unfortunately a mixture of side effects has limited their therapeutic use. Rolipram, the archetypical PDE4 inhibitor, and its close analogues, show a good activity and selectivity, but their various side effects, such as emesis, nausea, gastric acid secretion or central nervous system activation, have hindered their therapeutic deployment[2,3]. Therefore, the design of novel, potent and selective new generation PDE4 inhibitors with reduced emetogenic properties represents a critical requires and is still a challenge in the pharmaceutical area. Thus, it's important to increase both the potency and the selectivity, develop new compounds featuring original chemical structures. To achieve potent and selective PDE4 inhibitors with an improvement in the therapeutic index, three ways are possible: 1) To modify rolipram's structure; 2) To start from the structure of natural substrate (cAMP)[4]; 3) To carry out new inhibitor not structurally related to rolipram.

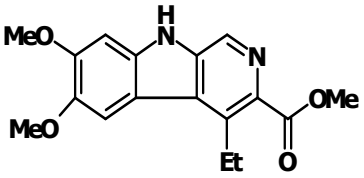



**ROLIPRAM**




**BWA78-U**

The first two approaches have been studied by different scientific groups; while the last one was still remaining not much investigate. Therefore, one way to obtain potent selective inhibitors with an improvement in the therapeutic index is to select new original chemical structure. For these reason, the purpose of our present study was to consider the third supposition. In the last few years, the attention was moved in to a methyl-6,7-dimethoxy-4-ethyl-β-carboline-3-carboxylate (DMCM) which showed a good activity as PDE 4 inhibitor[5]. So its modification led to a novel class of PDE4 inhibitors, we hope, without the unwanted side effects mentioned above.



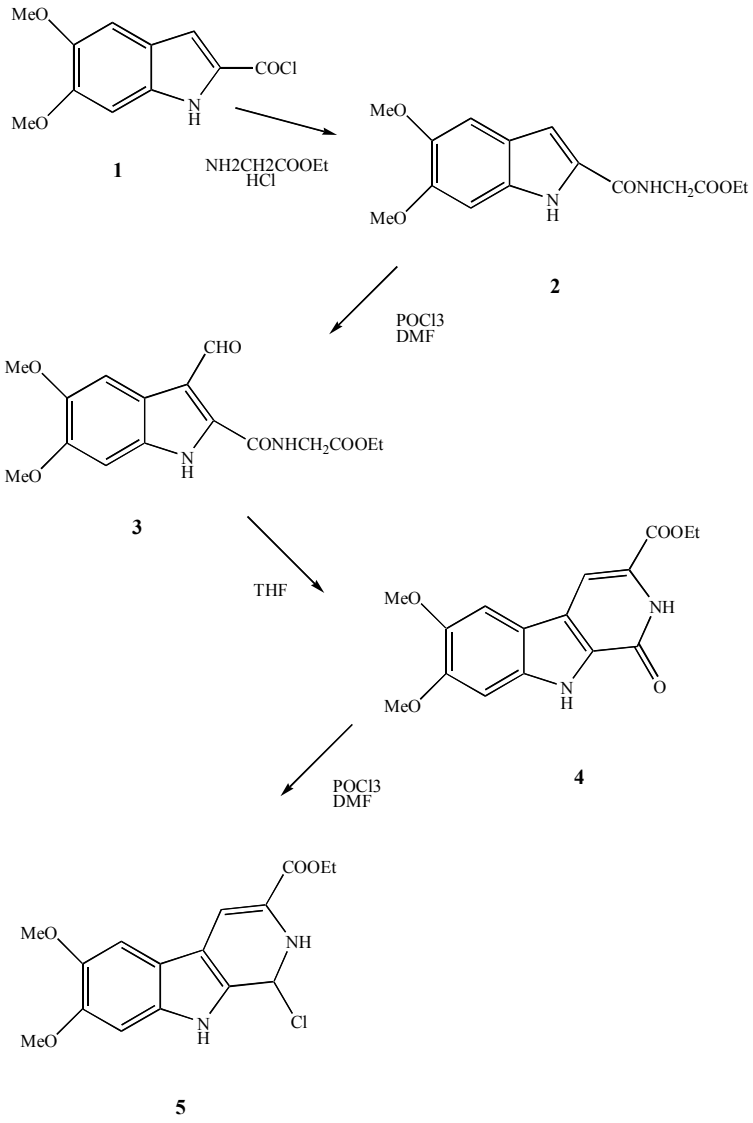

**DMCM**

Another aim of this work was to try to understand better how selective inhibitors with different chemical structures bind to the similar catalytic pockets of PDE. For this purpose we undertook a computational study of β-carbolinic derivatives, which we studied using theoretical techniques. In fact computational approaches facilitated the selection of desired compounds and accelerate, sometime, the identification of biological hits. All the synthesized compounds were tested both on the enzymatic inhibition and on the selectivity profile.

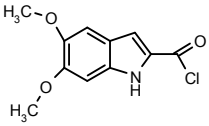
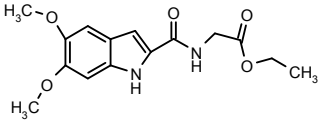
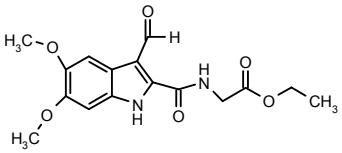
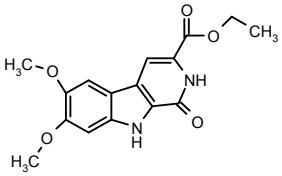
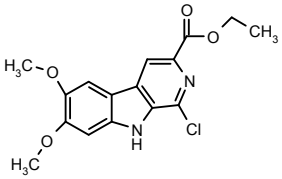
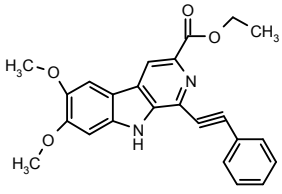
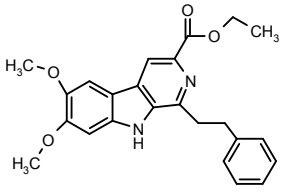
## EXPERIMENTAL

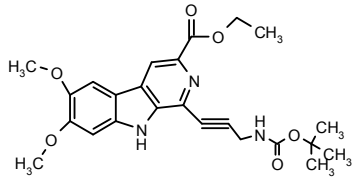
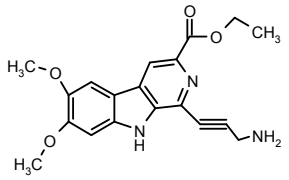
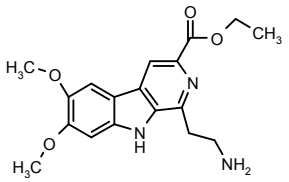
### Synthetic Chemistry

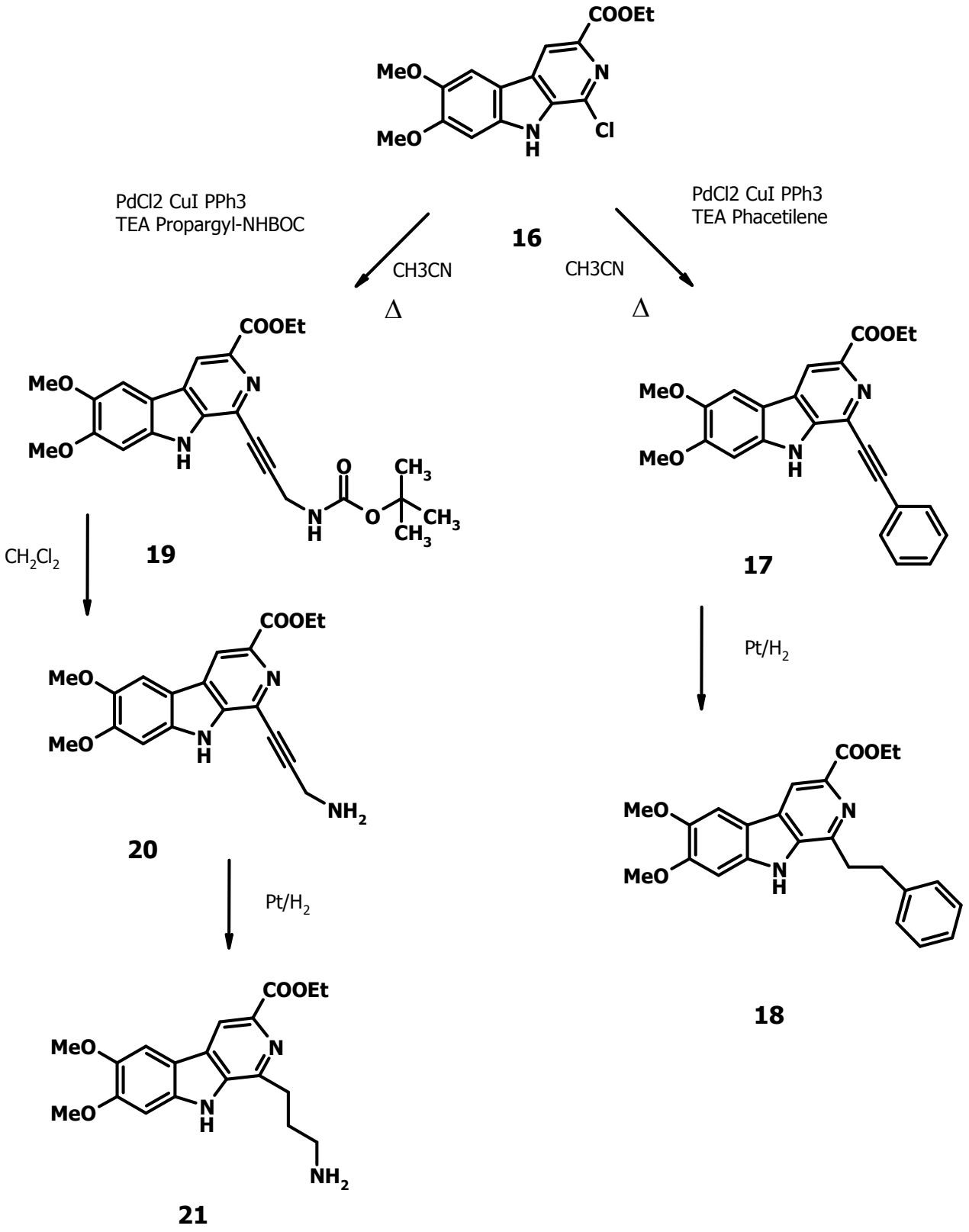
A cyclization of β-carbolinic nucleus was done using as starting point compound **1** that under appropriate reactions afforded the corresponding **4,** to which following to another one yielding to compound **5**. Substitution of methoxylic groups at the 6 and 7 position of β-carboline ring system would be expected to lose the activity against PDE4, consequently a number of derivatives were designed and synthesized with no change at these positions. In 1 position, as you can see in the compound **5**, was introduced a chloride that allow as to replace it with another's biggest substituent through the Sonogashira' reaction. In fact preliminary studies reported in literature and molecular modelling studies suggest that the pocket in which the ligand could be placed, have a lot of space to accommodate big structures. The chemical structures of all the synthesized compounds were confirmed by MS, UV, IR, $^{1}$H-NMR and elemental analyses data. Melting points (m.p.) were determined in capillary tubes on Gallenkam apparatus. MS spectra were obtained from Finnigan LCQ Deca Spectrometer. Silica gel Whatman K6F and F 254 were used in analytical thin-layer chromatography (TLC) and silica gel was also used in column chromatography. $^{1}$H-NMR spectra were recorded on a Bruker Advance 300 and 600 Mhz spectrometer.


MeO
MeO
COCl
N
H
1
NH2CH2COOEt
HCl
MeO
MeO
CONHCH2COOEt
N
H
2
POCl3
DMF
CHO
MeO
MeO
CONHCH2COOEt
N
H
3
THF
COOEt
MeO
MeO
NH
N
H
O
4
POCl3
DMF
COOEt
MeO
MeO
NH
N
H
Cl
5

We reported in the following table the characteristics of the synthesized compounds.

| Compounds | Structural formula | Empirical formula | Molecular Weight | Y% | M.P. °C |
|---|---|---|---|---|---|
| **1** |  | $C_{11}H_{10}NO_3Cl$ | 239.66 | 80 | 186-187 |
| **2** |  | $C_{15}H_{18}N_2O_5$ | 306.32 | 55 | 223-224 |
| **3** |  | $C_{16}H_{18}N_2O_6$ | 334.33 | 92 | oil |
| **4** |  | $C_{16}H_{16}N_2O_5$ | 316.32 | 85 | 191-192 |
| **5** |  | $C_{16}H_{17}N_2O_4Cl$ | 336.78 | 72 | 187-188 |
| **6** |  | $C_{24}H_{20}N_2O_4$ | 400.44 | 55 | 220-221 |
| **7** |  | $C_{24}H_{24}N_2O_4$ | 404.47 | 50 | 218-219 |
| **8** | | $C_{24}H_{27}N_3O_6$ | 453.50 | 45 | oil |

| | | | | | |
|---|---|---|---|---|---|
| |  | | | | |
| **9** |  | $C_{19}H_{19}N_3O_4$ | 353.38 | 50 | oil |
| **10** |  | $C_{18}H_{21}N_3O_4$ | 343.39 | 40 | oil |


COOEt
MeO
MeO
N
H
Cl
16
PdCl2 CuI PPh3
TEA Propargyl-NHBOC
CH3CN
Δ
PdCl2 CuI PPh3
TEA Phacetilene
CH3CN
Δ
O
CH3
CH3
CH3
19
17
CH2Cl2
Pt/H2
NH2
20
Pt/H2
18
21

In the following table we reported spectroscopic $^{1}$H-NMR data, DMSO $_{d6}$ , 300Mhz.

| **Compounds** | **$^{1}$H (ppm)** |
|---|---|
| **1** | 10.10(s,1H,NHAr), 7.38(s,1H,HAr), 6.95(s,1H,HAr), 6.80(s,1H,HAr), 3.73(s,6H,2$OCH_3$) |
| **2** | 10.10(s,1H,NHAr), 8.0(s,1H,NH), 7.38(s,1H,HAr), 6.95(s,1H,HAr), 6.80(s,1H,HAr), 4.12(q,2H,$CH_2$), 3.92(s,2H,$CH_2$), 3.73(s,6H,2$OCH_3$), 1.30(t,3H,$CH_3$) |
| **3** | 10.10(s,1H,NHAr), 9.61(s,1H,CH), 8.0(s,1H,NH), 6.90(s,1H,HAr), 4.12(q,2H,$CH_2$), 3.92(s,2H,$CH_2$), 3.73(s,6H,2$OCH_3$), 1.30(t,3H,$CH_3$) |
| **4** | 10.10(s,1H,NHAr), 8.0(s,1H,NHAr), 6.92(s,1H,HAr), 6.40(s,2H,2$CH_3$), 4.19(q,2H,$CH_2$), 3.92(s,2H,$CH_2$), 3.73(s,6H,2$OCH_3$), 1.30(t,3H,$CH_3$) |
| **5** | 10.10(s,1H,NHAr), 8.64(s,1H,HAr), 6.95(s,1H,HAr), 6.80(s,1H,HAr), 4.29(q,2H,$CH_2$), 3.73(s,6H,2$OCH_3$), 1.30(t,3H,$CH_3$) |
| **6** | 10.10(s,1H,NHAr), 8.28(s,1H,HAr), 7.42(d,2H,2HAr), 7.24(d,1H,HAr), 7.23(d,1H,2HAr), 6.95(s,1H,HAr), 6.80(s,1H,HAr), 4.29(q,2H,$CH_2$), 3.73(s,6H,2$OCH_3$), 1.30(t,3H,$CH_3$) |
| **7** | 10.10(s,1H,NHAr), 8.20(s,1H,HAr), 7.21(d,2H,2HAr), 7.12(d,1H,HAr), 7.08(d,1H,2HAr), 6.95(s,1H,HAr), 6.80(s,1H,HAr), 4.29(q,2H,$CH_2$), 3.73(s,6H,2$OCH_3$), 3.21(t,2H,$CH_2$), 2.88(t,2H,$CH_2$), 1.30(t,3H,$CH_3$) |
| **8** | 10.10(s,1H,NHAr), 8.33(s,1H,HAr), 8.0(s,1H,NH), 6.95(s,1H,HAr), 6.80(s,1H,HAr), 4.29(q,2H,$CH_2$), 3.73(s,6H,2$OCH_3$), 3.70(d,2H,$CH_2$), 1.40(m,9H,3$CH_3$), 1.30(t,3H,$CH_3$) |
| **9** | 10.10(s,1H,NHAr), 8.33(s,1H,HAr), 6.95(s,1H,HAr), 6.80(s,1H,HAr), 4.29(q,2H,$CH_2$), 3.73(s,6H,2$OCH_3$), 3.39(t,2H,$CH_2$), 2.0(s,1H,NH), 1.30(t,3H,$CH_3$) |
| **10** | 10.10(s,1H,NHAr), 8.20(s,1H,HAr), 6.95(s,1H,HAr), 6.80(s,1H,HAr), 4.29(q,2H,$CH_2$), 3.73(s,6H,2$OCH_3$), 3.14(t,2H,$CH_2$), 2.98(t,2H,$CH_2$), 2.0(s,1H,NH), 1.30(t,3H,$CH_3$) |

**Computational Chemistry**

**Experimental**

Phosphodiesterases play a critical role in maintaining the cellular level of cAMP and cGMP.

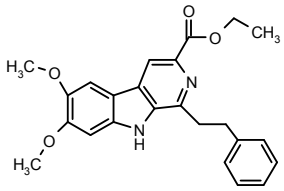
Of the 11 families of human cyclic nucleotide phosphodieterases, the PDE4 class of enzymes is selective for cAMP, while the PDE5 enzyme is selective for the cGMP. Within the PDE4 subfamily, which is comprised of four members, the PDE4B is of particular importance in the inflammatory responses of lymphocytes. Therefore, PDE4B represents a good-looking target for antinflammatory therapeutics, and even if a large number of PDE4 inhibitors are currently being

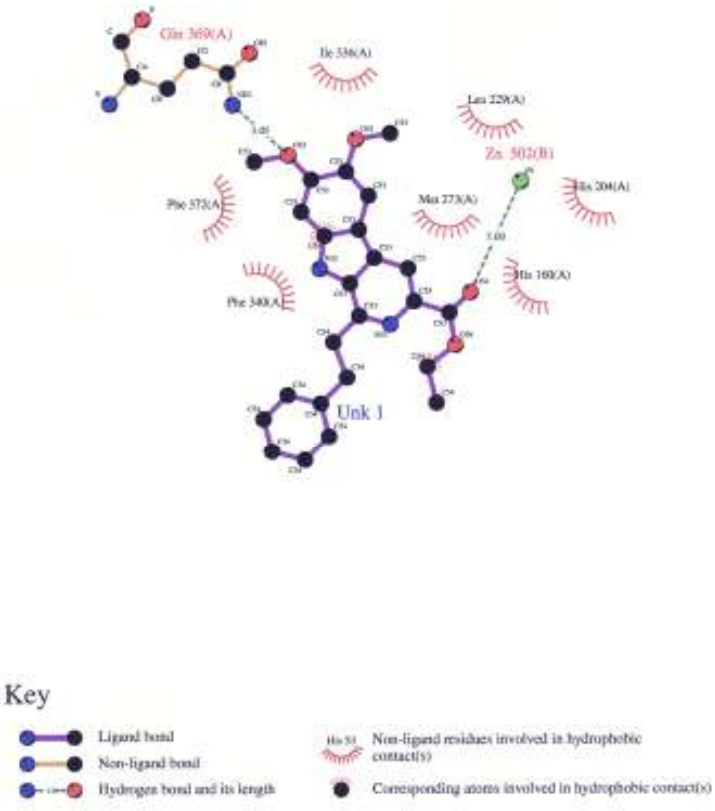
tested they showed various side effects, probably caused by the inhibition of PDE4D in the brain. In fact they show 16 helixes in common and the same folding, expect for the random loop of residues 422-434, which correspond to the 17 helix in PDE4B[6]. Thus, the design of inhibitors selective towards different PDE4 subtypes is demanding owed to the high degree of sequence and structural similarity. Trying to recognize of these PDE4 subtypes at the atomic level would greatly facilitate the design of subtype selective inhibitors with reduced side effects and enhanced pharmacological profiles.

Recently, the X-Ray Crystallographic structure of the catalytic domain of human phosphodieterase has been solved in complex with rolipram and made available on the Protein Data Bank (access code 1OYN)[7]. In order to identify how selective inhibitors with different chemical structures bind to the similar catalytic pockets of PDEs we performed a computational study to define the binding site of the protein, the position and shape of which was used in docking calculations. Studies of molecular modelling were performed using Cerius 2[8], installed on a Silicon Graphics workstation. The module LUDI in Cerius 2 was used to design inhibitors de novo, requiring only a receptor structure and a coordinate in the active site. Ligand Fit in CERIUS 2, was used to dock our series of hypothetical ligand molecules into a protein binding site. During docking, the protein was rigid while the ligand remains flexible allowing different conformations to be docked within the binding site. There were 3 key steps in this process: A SITE SEARCH performed in order to define the protein binding site; CONFORMATIONAL SEARCH generate possible ligands conformations; LIGAND FITTING[9] docks the conformations into binding site and computes docking score, which predicts ligand-protein binding affinities. Most of the molecules in the cluster form some hydrophobic contacts with the more conserved aminoacidic residues: the best value is compound **7** in the conformation number 1. In fact not always the best ligand is that with a lowest energy, but sometime, into a energy value acceptable may be not the first conformation, come out from the conformational search.

**Compound 7**

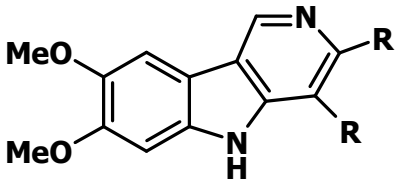
Conf. 1




Infact its two oxygen atoms of metoxilic groups form two H-bonds with Gln 369, which is the only residue absolutely conserved across PDE families, and it has some interactions with Tyr159, Met 253 and Hys 160. On these preliminary results we thought to perform some molecular modelling studies on γ-carboline to better understand if this way could be a good idea for new synthesis.

In fact the same β-carbolyne were converted by computational method in the correspondent γ and some of these showed an interesting interaction with the aminoacid involved in PDE binding through molecular simulation's studies.




γ-carboline- derivatives

These findings have led to intensive efforts in the last few years to identify highly potent PDE 4 inhibitors as promising treatment of autoimmune and inflammatory diseases.

## Discussion

Intracellular modulation of cAMP levels by phosphodiesterase type 4 (PDE 4) inhibitors represents a promising novel approach for the treatment of chronic inflammatory diseases such as asthma, COPD and rheumatoid arthritis[10].

We have focused our interest on the PDE4 which specifically hydrolyzes cAMP. PDE 4 is a cAMP-specific phosphodiesterase highly expressed in inflammatory cells and airway smooth muscle. The observation that an elevation of cAMP in these cells can suppress inflammatory effects and can induce muscle relaxation has inspired great attention in developing selective PDE4 inhibitors as therapeutic agents for asthma and other inflammatory diseases[11].

Our interest in the field of PDE inhibitors prompted us to synthesize and evaluate a series of β-carbolines compound to verify if this scaffold is an appropriate centre for the inhibition of PDE4. The β-carboline DMCM is a potent ligand of central benzodiazepine receptors, unexpectedly appeared a potent PDE4 inhibitor with μM range IC50 value.

The general purpose of this project was synthesis and computational study of new DMCM derivatives to obtain more potent compounds than DMCM, after structural optimization. Among the series of β-carboline derivatives, some of them will be selected and tested for their pharmacological properties.

Our study suggest that the potencies of β-carboline derivatives were decreased by the replacement of two methoxylic groups into 6 and 7 position in β-carboline nucleus, an huge group at 1 position should be a good start to synthesize other compound; furthermore, also changing the position of nitrogen atom from the β position to the γ position we could obtain great result as confirmed by computational studies. We have explored the influence of substituents in position 1, 2 and 3 and we also see the importance of two methoxylic groups into the precise positions. To obtain more information about the structural needs, synthesis of new β-carbolines with different sustituents at other positions is pleasing.

In conclusion, an efficient synthesis of β-carbolines by the palladium copper-catalyzed coupling has been developed. A variety of functionalized terminal participate in this process to afford the desired heterocyclic in good to excellent yields. In order to improve their pharmacological profiles, we submitted these for biological evaluation. Further researches are currently performed toward the scope and its limitations to identify much more molecules with a good selectivity and potency but with always more less side effects.

**Acknowledgments**

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