5th International Electronic Conference on Medicinal Chemistry

1-30 November 2019

chaired by Dr. Jean Jacques Vanden Eynde

sponsored by pharmaceuticals

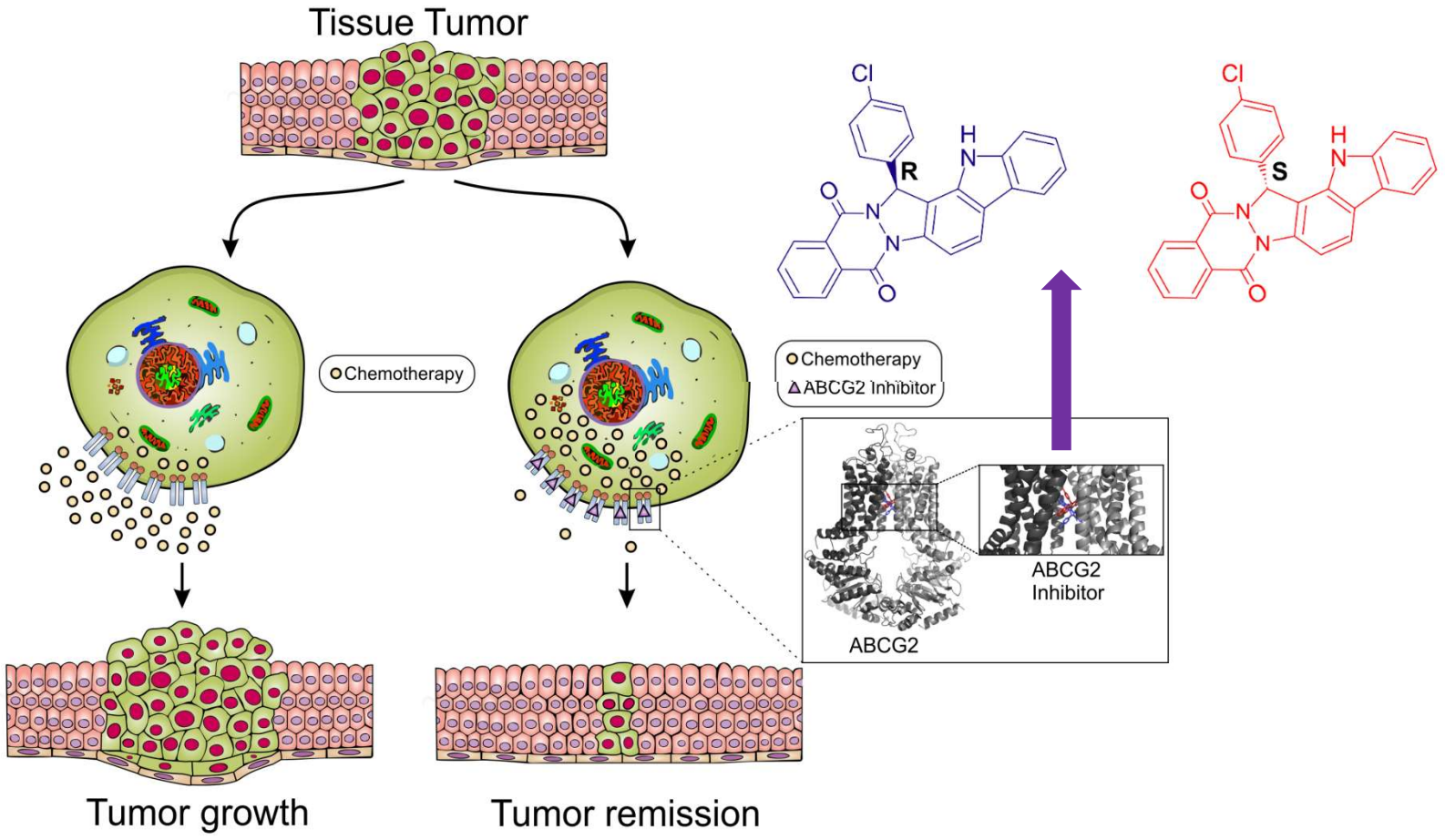
Design, Synthesis, X-ray Structure and Evaluation of Functionalized Hexacyclic Carbazoles as New Inhibitors of ABCG2 Transporter

Isadora da Silva Zanzarini [1], Esma Lamera [2], Sofiane Bouacida [2,3], Ingrid Fatima Zattoni [1], Diogo Henrique Kita [1], Zouhair Bouaziz [4], Vivian Rotuno Moure [1], Marc Le Borgne [4], Abdelmalek Bouraiou [2,*], and Glaucio Valdameri [1,5,*]

[1] Pharmaceutical Sciences Graduate Program, Laboratory of Cancer Drug Resistance, Federal University of Paraná, 80210-170 Curitiba, PR, Brazil; [2] Unité de Recherche de Chimie de l'Environnement et Moléculaire Structurale, Université des Frères Mentouri, Constantine 25000, Algeria; [3] Département des sciences de la matière, Université Oum El Bouaghi, 04000 Oum El Bouaghi, Algeria; [4] EA 4446 B2MC, Université Claude Bernard Lyon 1, 69373 Lyon, France; [5] Department of Clinical Analysis, Federal University of Paraná, 80210-170 Curitiba, PR, Brazil

* Corresponding authors: bouraiou.abdelmalek@yahoo.fr; gvaldameri@ufpr.br

Design, Synthesis, X-ray Structure and Evaluation of Functionalized Hexacyclic Carbazoles as New Inhibitors of ABCG2 Transporter

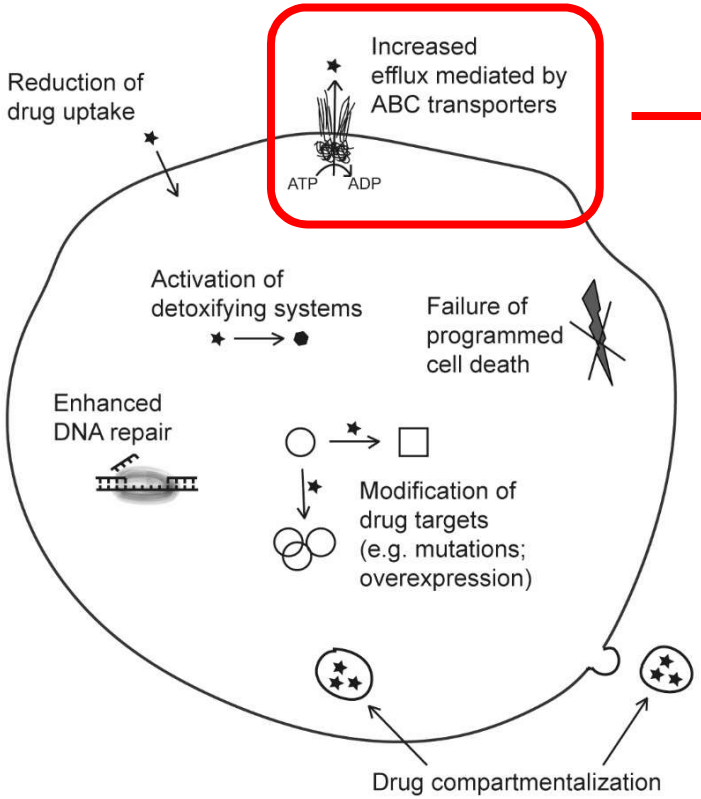


Abstract:
Cancer is one of the diseases with the highest mortality rates worldwide and the emergence of neoplasms presenting resistance to chemotherapy, also known as multidrug resistance (MDR), makes this conjuncture even worse. The overexpression of transmembrane proteins named ABC transporters is considered the main cause of this clinical condition [1]. These transporters (e.g. ABCG2) can recognize and promote the efflux of a broad spectrum of antineoplastic agents; thus, many studies have been carried out to develop compounds and evaluate their ability to inhibit this activity. Despite its pronounced relation with MDR, there are still no promising inhibitors of ABCG2 to be forwarded to clinical steps of drug development, which endorses the urgency to identify and characterize new selective inhibitors of this protein. Carbazole skeleton is a key structural motif of many biologically active compounds including natural and synthetic products [2]. Starting from the tricyclic-carbazole motif to fused tetra-, penta-, hexa- and heptacyclic carbazoles, this skeleton could enable the design of new inhibitors of ABCG2 transporter.
A one-pot method for the synthesis of novel hexacyclic carbazole derivatives from readily available starting materials using a sequential multicomponent reaction/Fisher indolization strategy is described. Then five carbazole derivatives were tested to inhibit ABCG2 activity.
1) O. Briz et al. Expert Opin Drug Metab Toxicol. 2019, 15(7):577-593. 2) S. Issa et al. J Enzyme Inhib. Med. Chem. 2019, 34(1):1321-1346.

Keywords: carbazoles; hexacyclic derivatives; multicomponent reaction; efflux pump; ABCG2; multidrug resistance

Introduction – MDR, ABC transporters and ABCG2

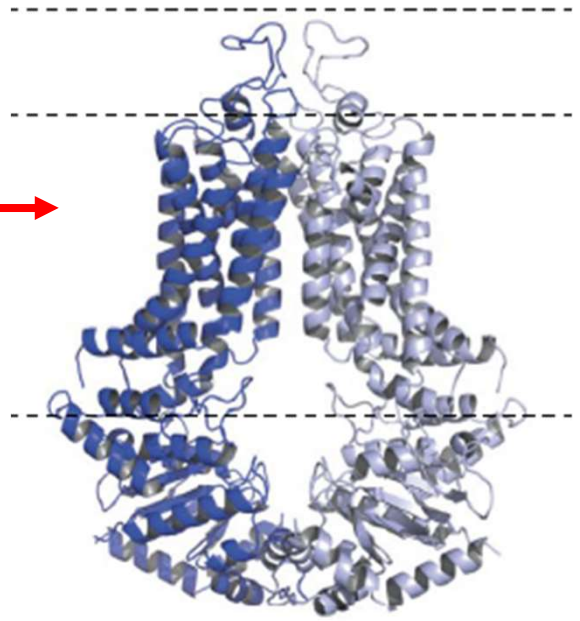
ABC transporters are a family of membrane proteins mainly responsible for Multidrug Resistance (MDR)



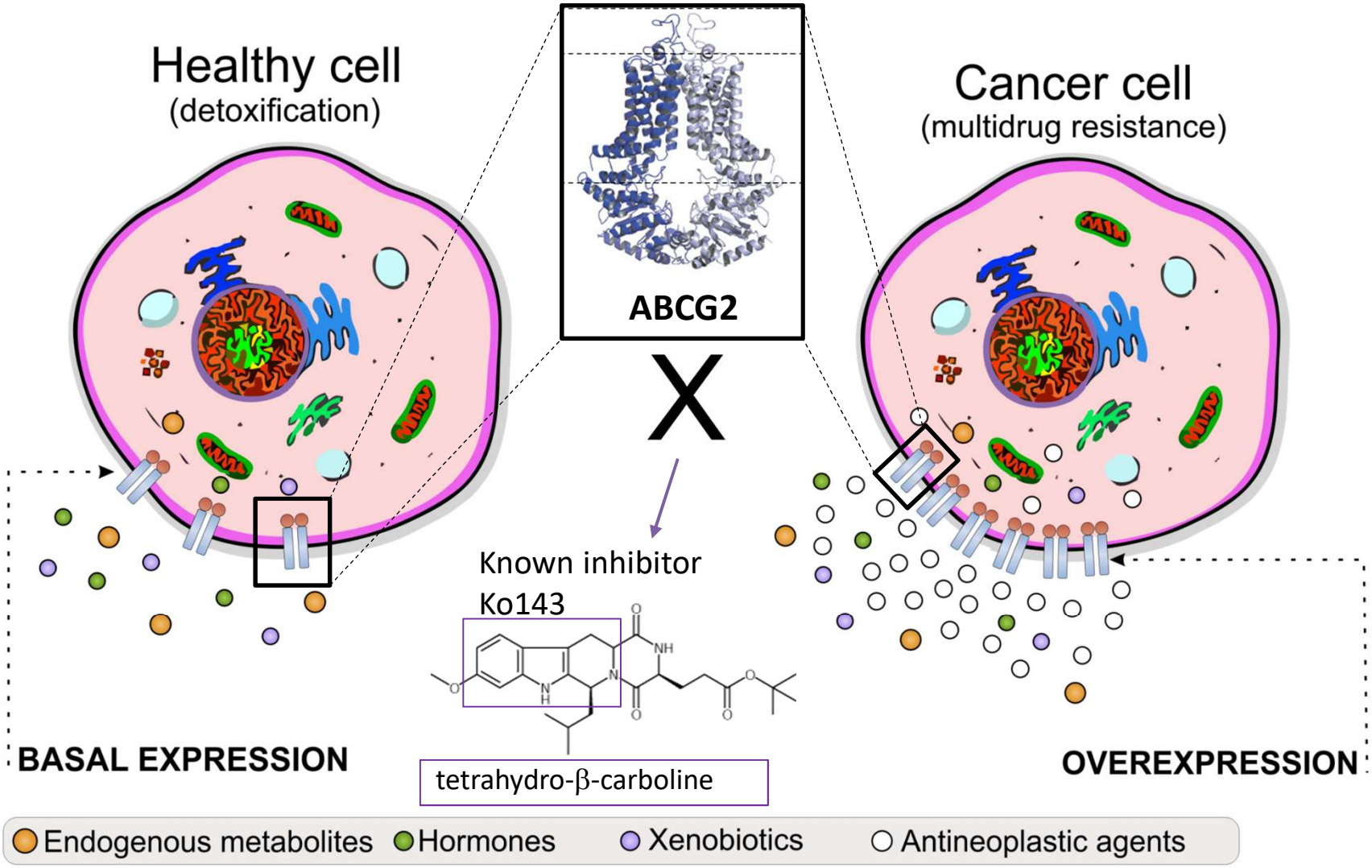
ABCG2

One of the most important

ABCG2 structure



Introduction – Role of ABCG2 in cancer

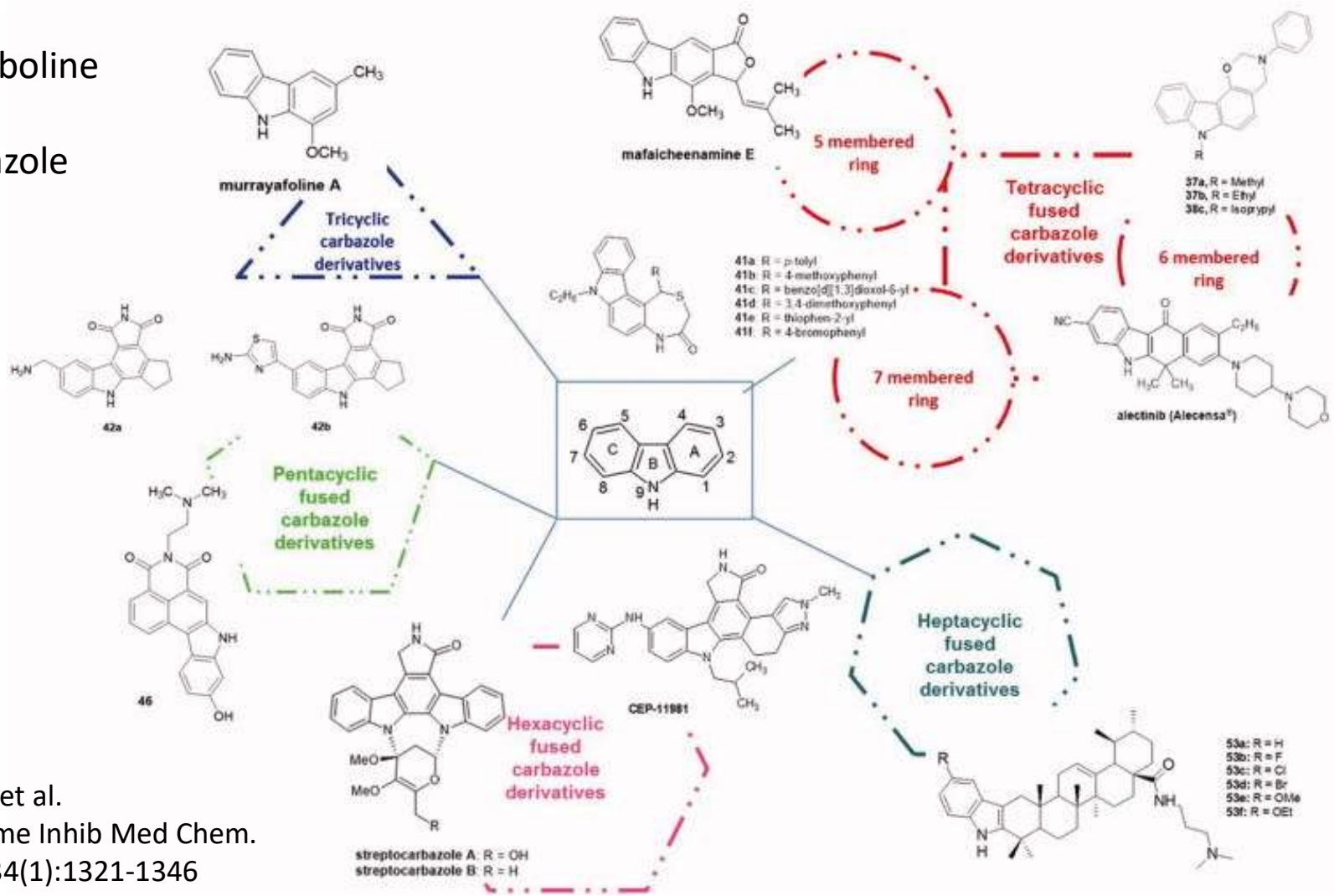


Introduction – A large molecular diversity inside carbazoles

β-carboline

…

carbazole

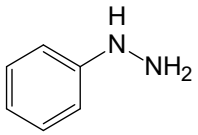


Issa S. et al.
J Enzyme Inhib Med Chem.
2019;34(1):1321-1346

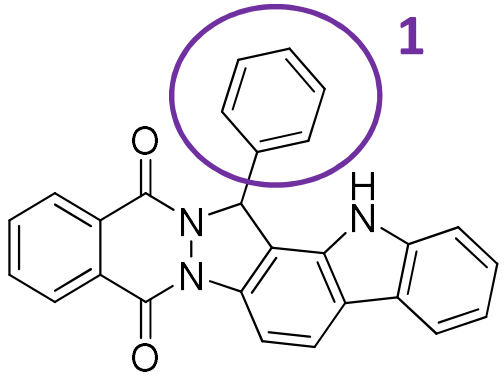
Introduction – Chemical access to a new series of hexacyclic carbazoles and testing for ABCG2 inhibitors

AIM 1:

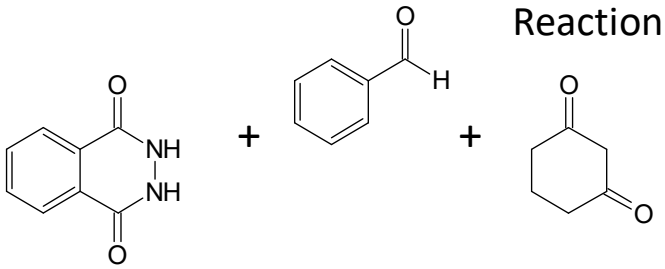
- EFFICIENT COMBINED METHOD TO SYNTHETIZE NEW CARBAZOLE DERIVATIVES
- X-RAY STUDIES
- PRELIMINARY PHARMACOMODULATION



Fischer Indolization



Multicomponent Reaction (MCR)



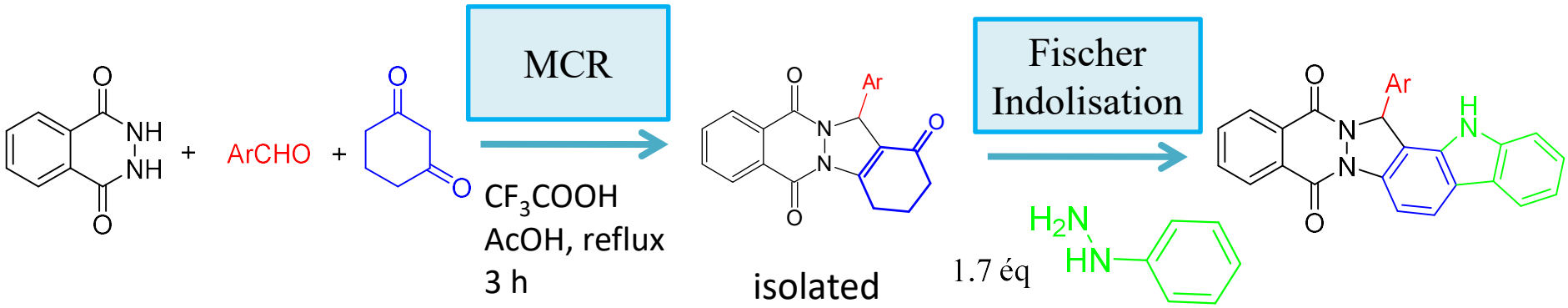
AIM 2:

- DETERMINATION OF ABCG2 INHIBITION, SELECTIVITY AND CYTOTOXICITY
- MOLECULAR INTERACTIONS/ MECHANISM OF INHIBITION
- MDR REVERSING EFFECT

Results and discussion – Chemical design of hexacyclic carbazoles

Preparation of phthalazino[2',3':1,2]pyrazolo[4,3-a]carbazole-9,14-dione derivatives

APPROACH A



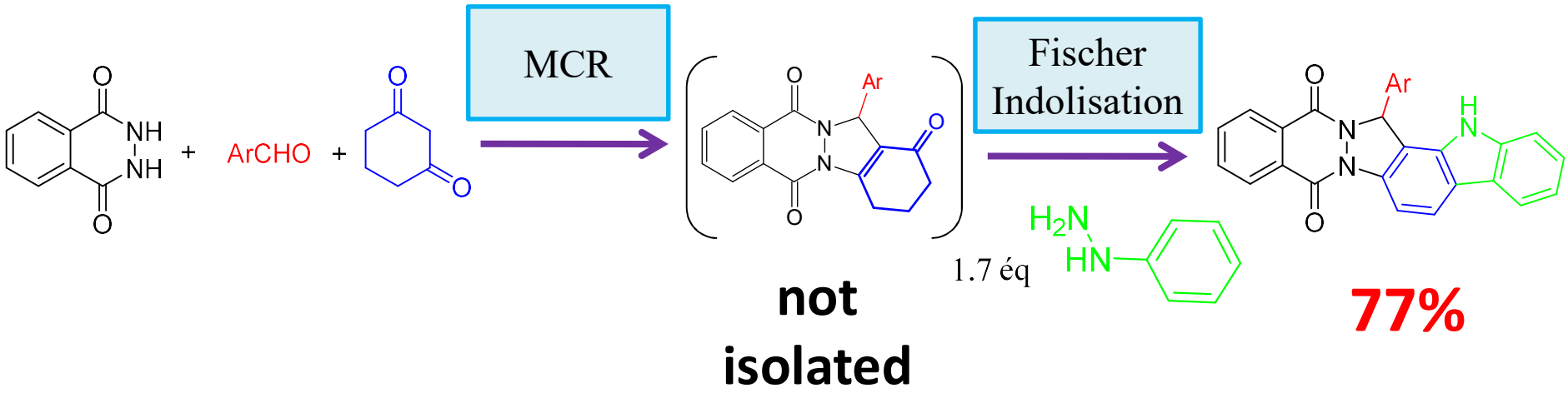
Conditions For Fischer indolization:

Entry	Solvent	Catalyst	Time	Yield (%)
1	EtOH	HCl (aq)	4 days, reflux	0
2	i-PrOH	H_2SO_4	4 days, reflux	0
3	AcOH	CF_3COOH	24 h, reflux	**40**

Results and discussion – Chemical design of hexacyclic carbazoles

Preparation of phthalazino[2',3':1,2]pyrazolo[4,3-a]carbazole-9,14-dione derivatives

APPROACH B

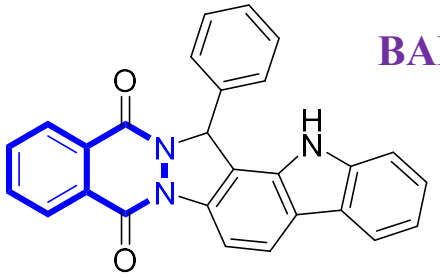


Conditions:

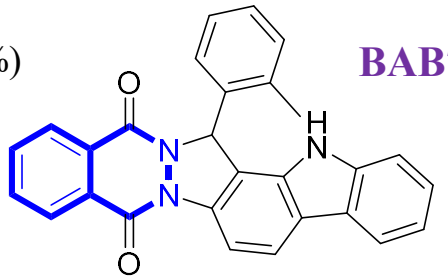
CF_3COOH AcOH, reflux 3 h	- AcOH, reflux 24 h

Results and discussion – A new library of hexacyclic carbazoles

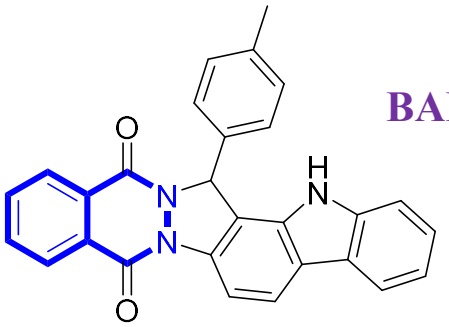
Code (yield)



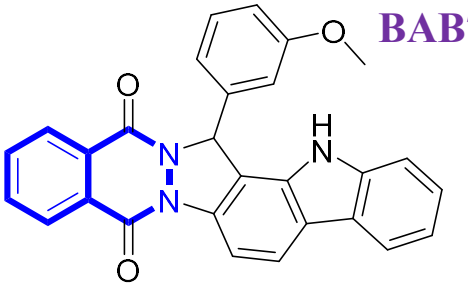
BAB67 (77%)



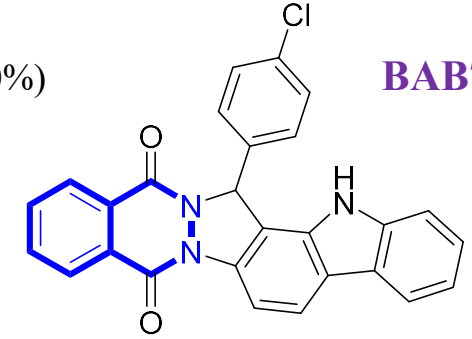
BAB77 (75%)



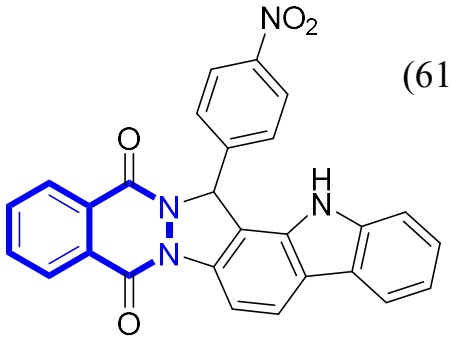
BAB74 (81%)



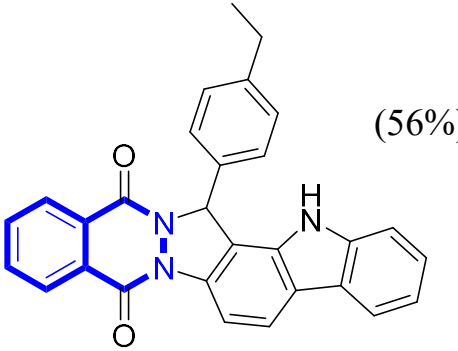
BAB72 (60%)



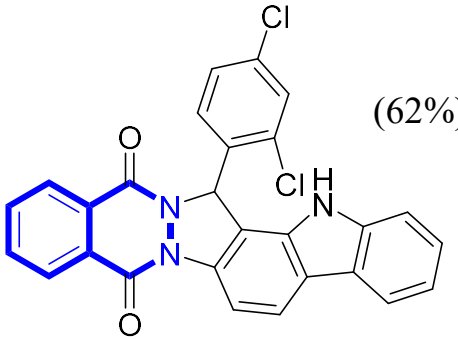
BAB75 (87%)



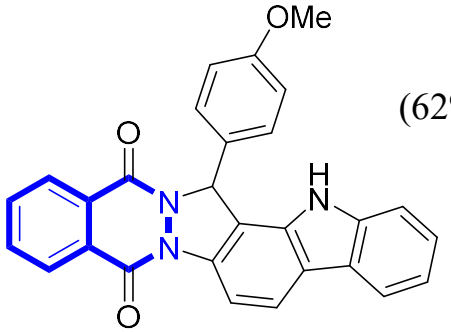
(61%)



(56%)

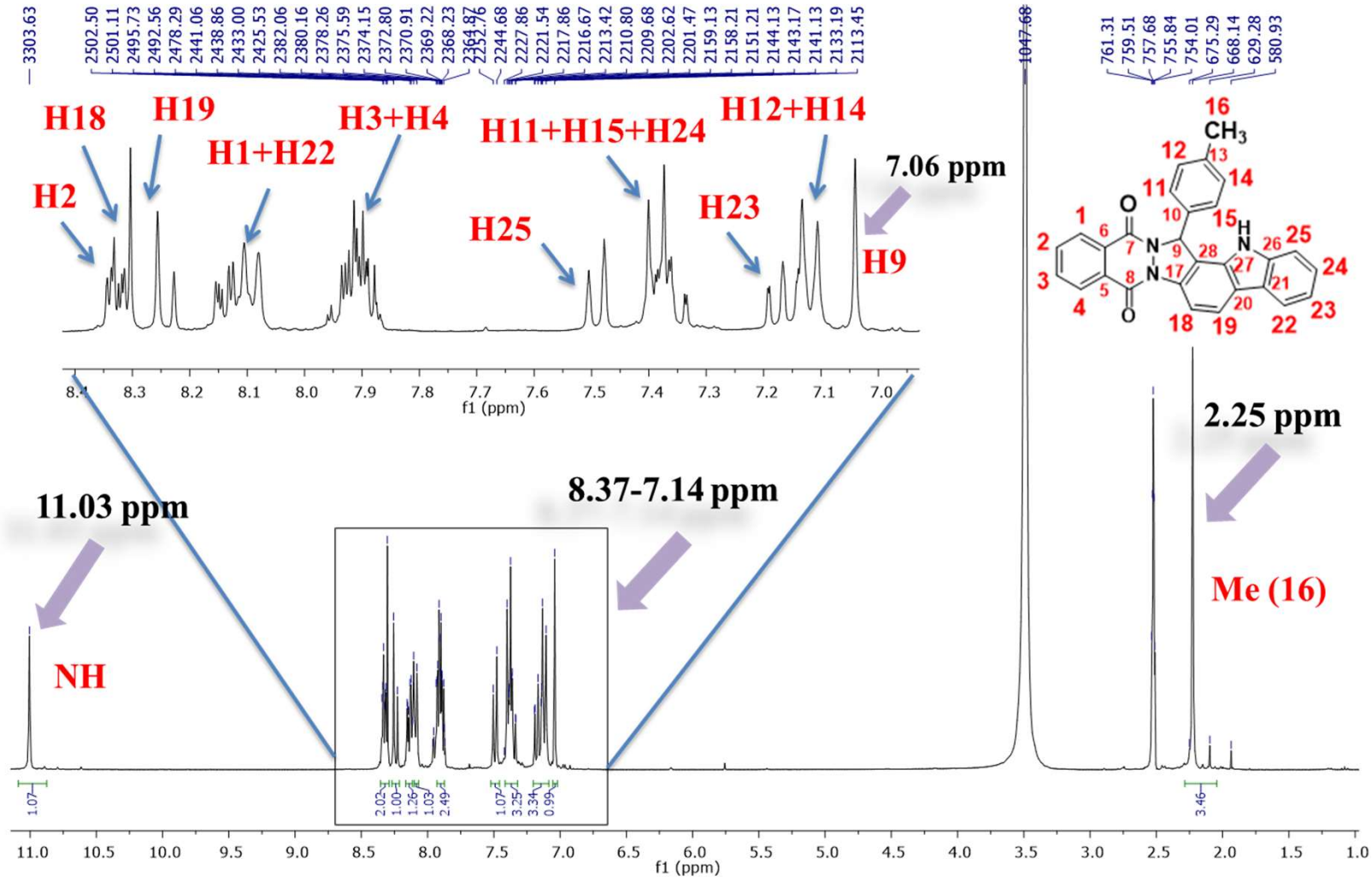


(62%)

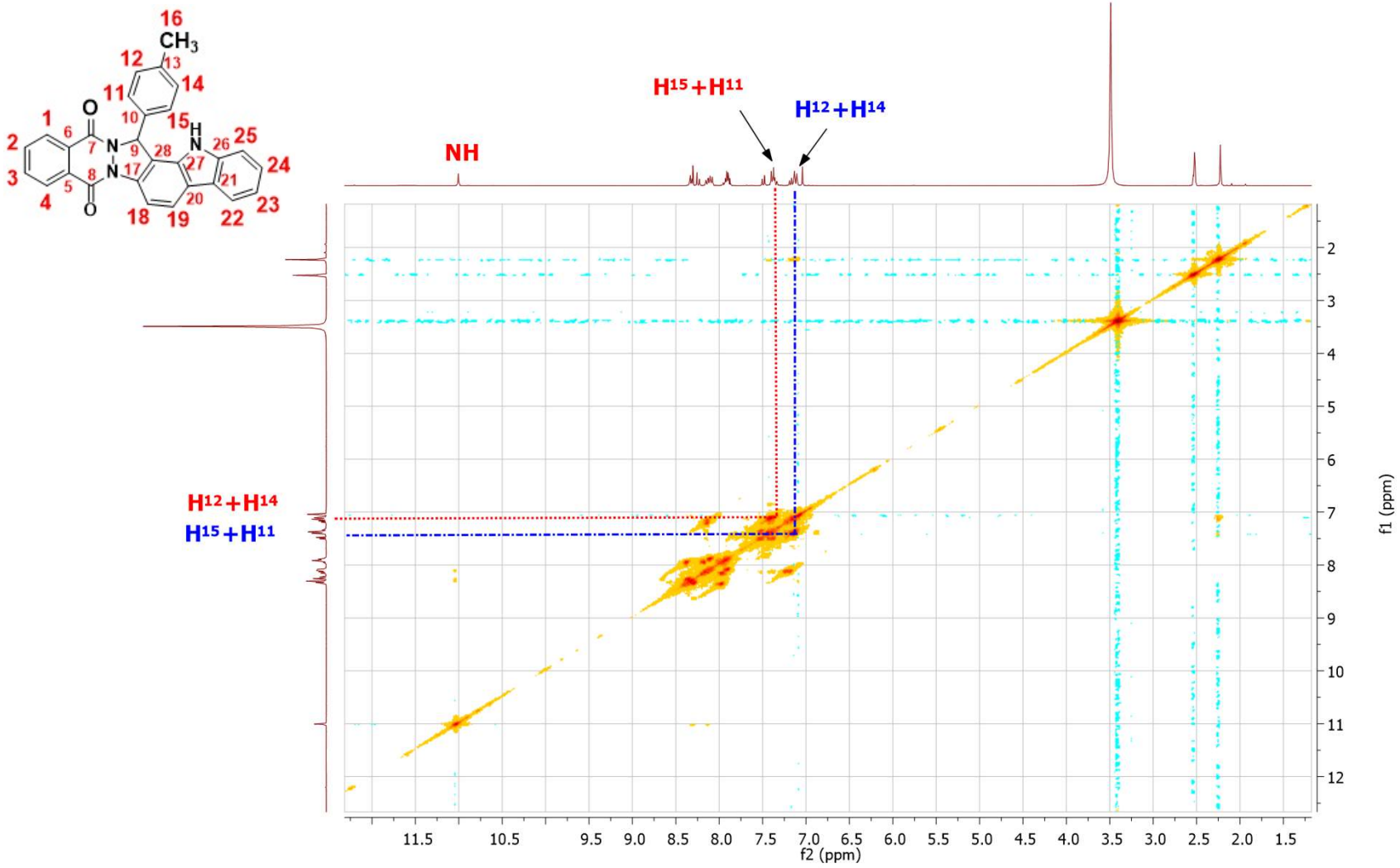


(62%)

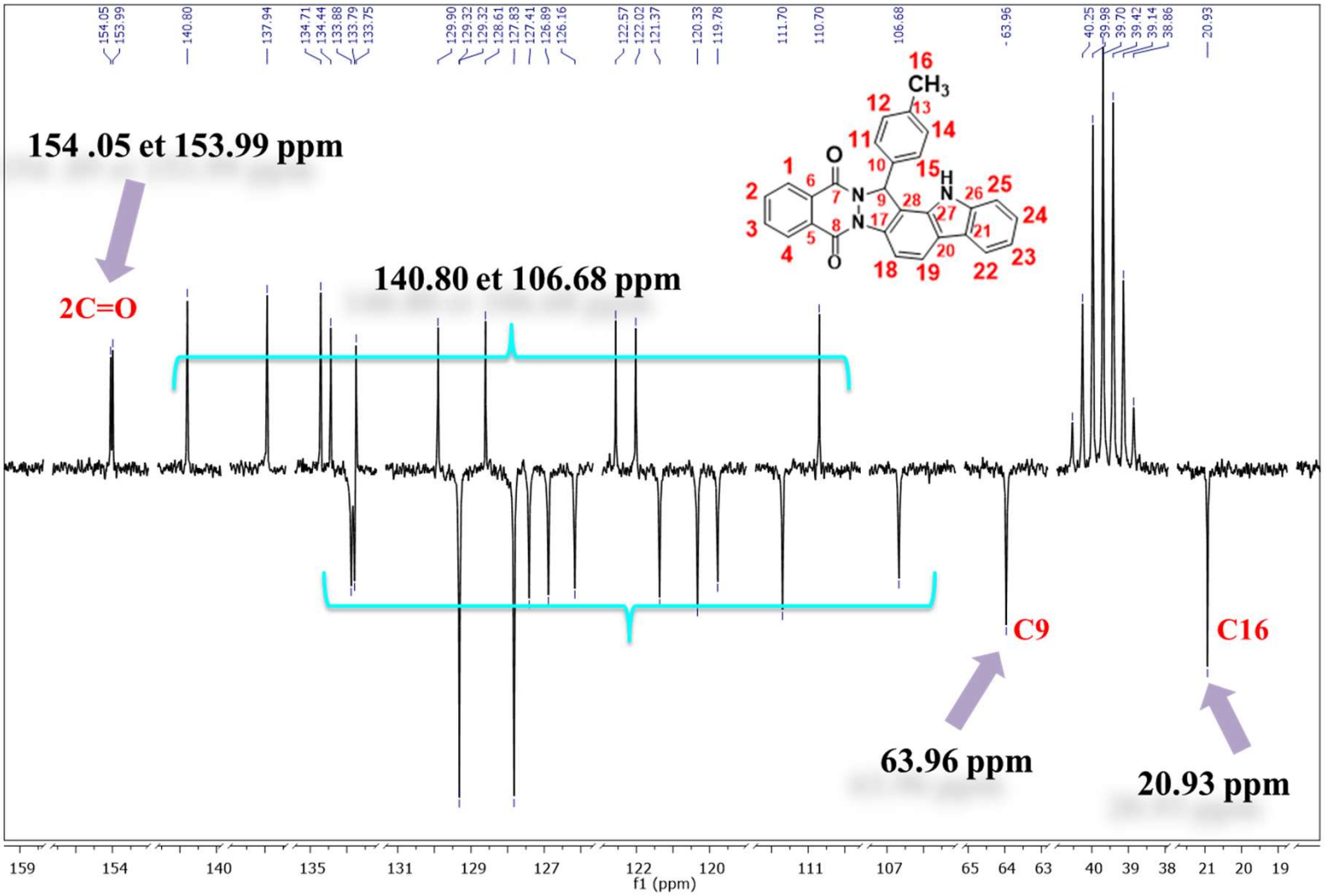
Results and discussion – 1H NMR chemical shifts of BAB74



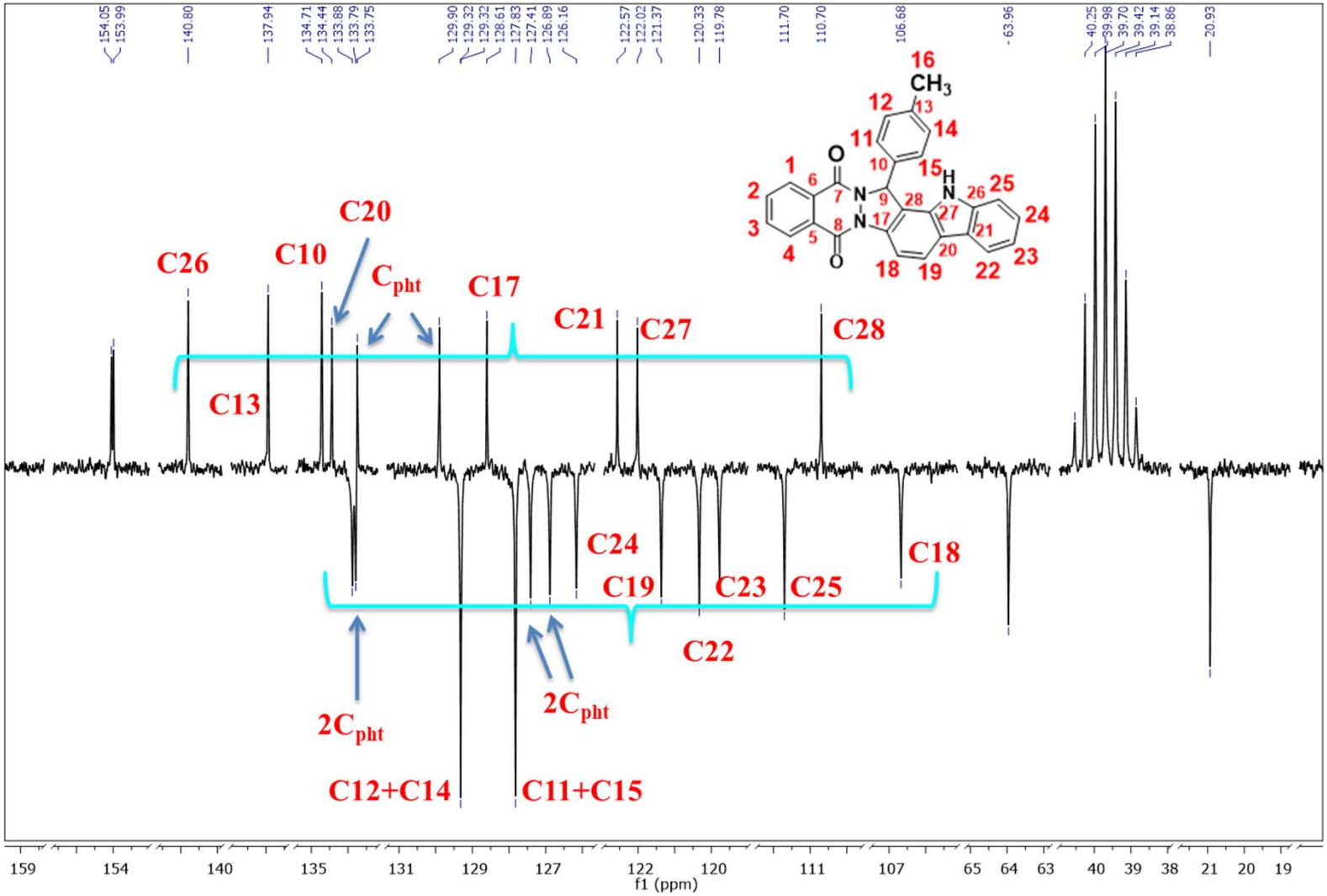
Results and discussion – COSY experiment of BAB74



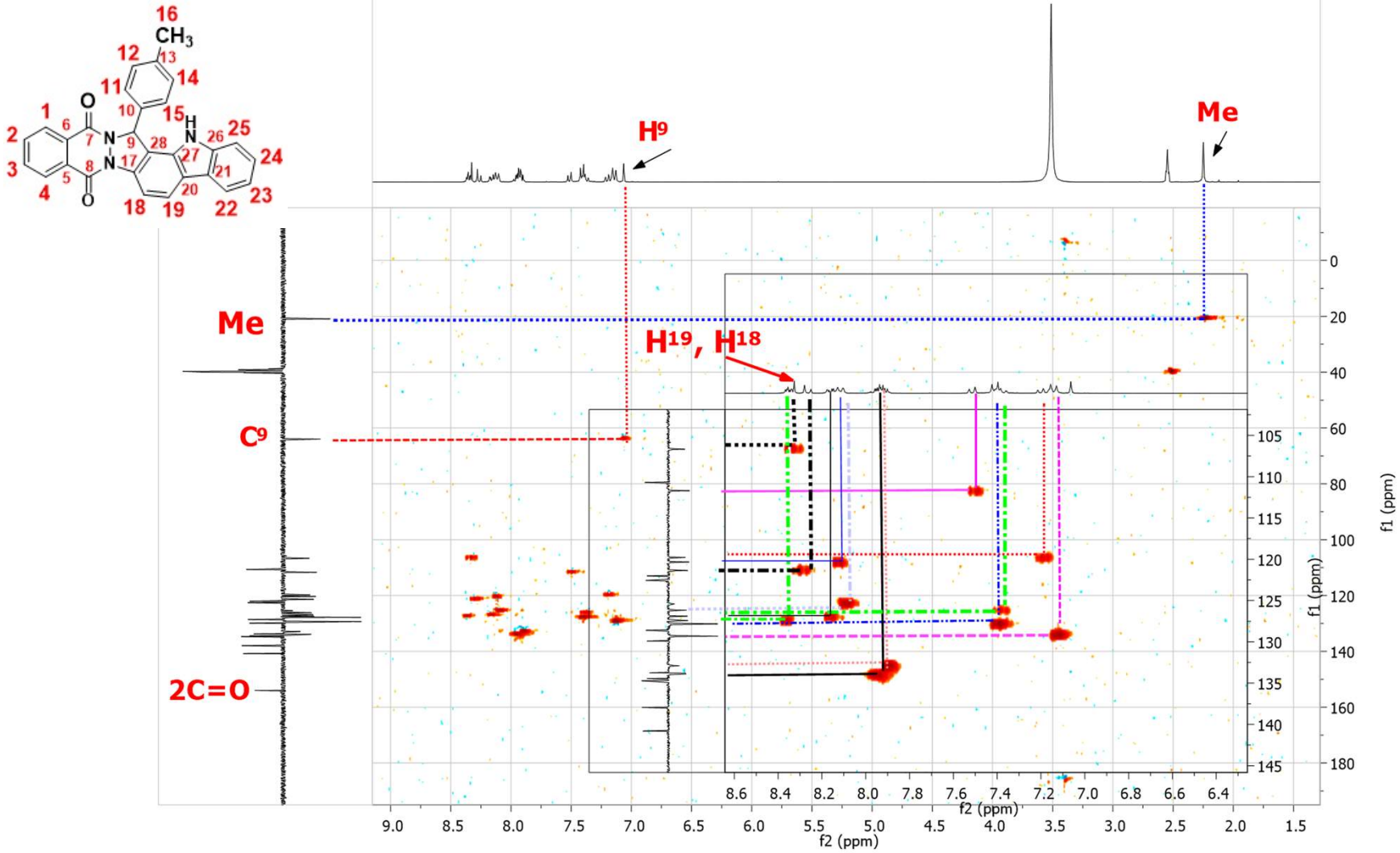
Results and discussion – 13C NMR chemical shifts of BAB74



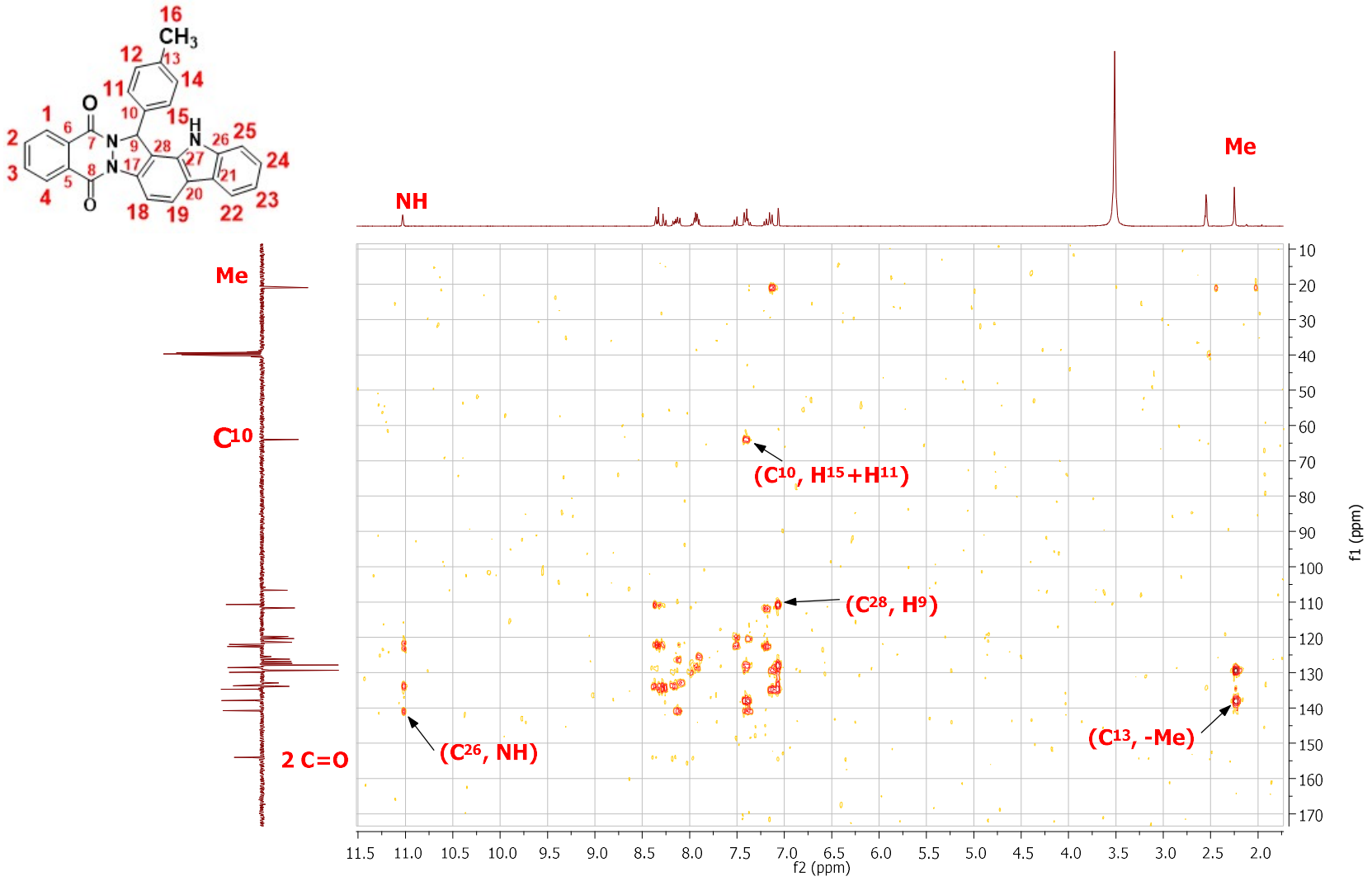
Results and discussion – 13C NMR chemical shifts of BAB74



Results and discussion – 2D HSQC experiment of BAB74



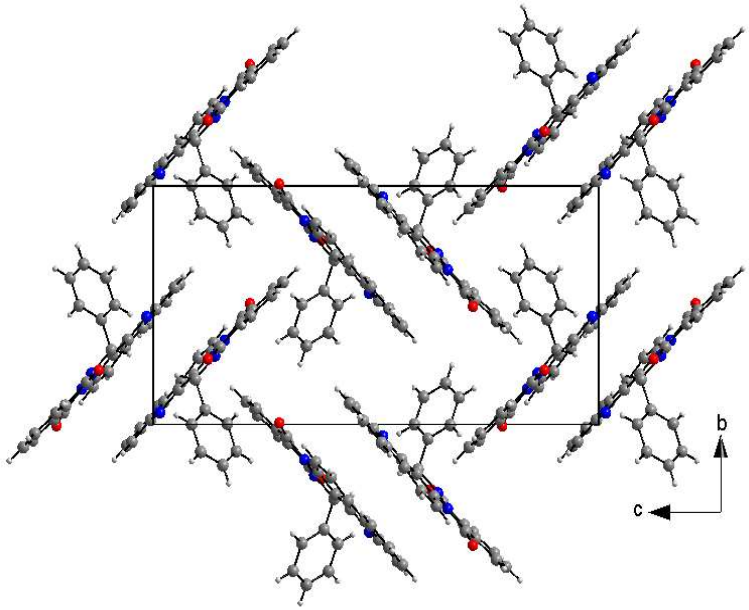
Results and discussion – 2D HMBC experiment of BAB74



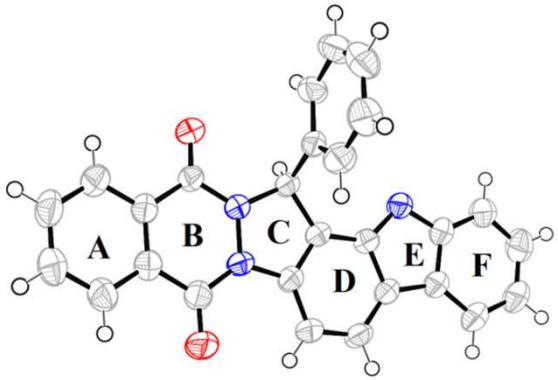
Results and discussion – X-ray studies of BAB67

Orthorhombic crystal system

a = 10.5723(6) Å; b = 10.7384(6)Å;
c = 21.6020(11)Å; α = β = γ = 90°, *Z* = 4

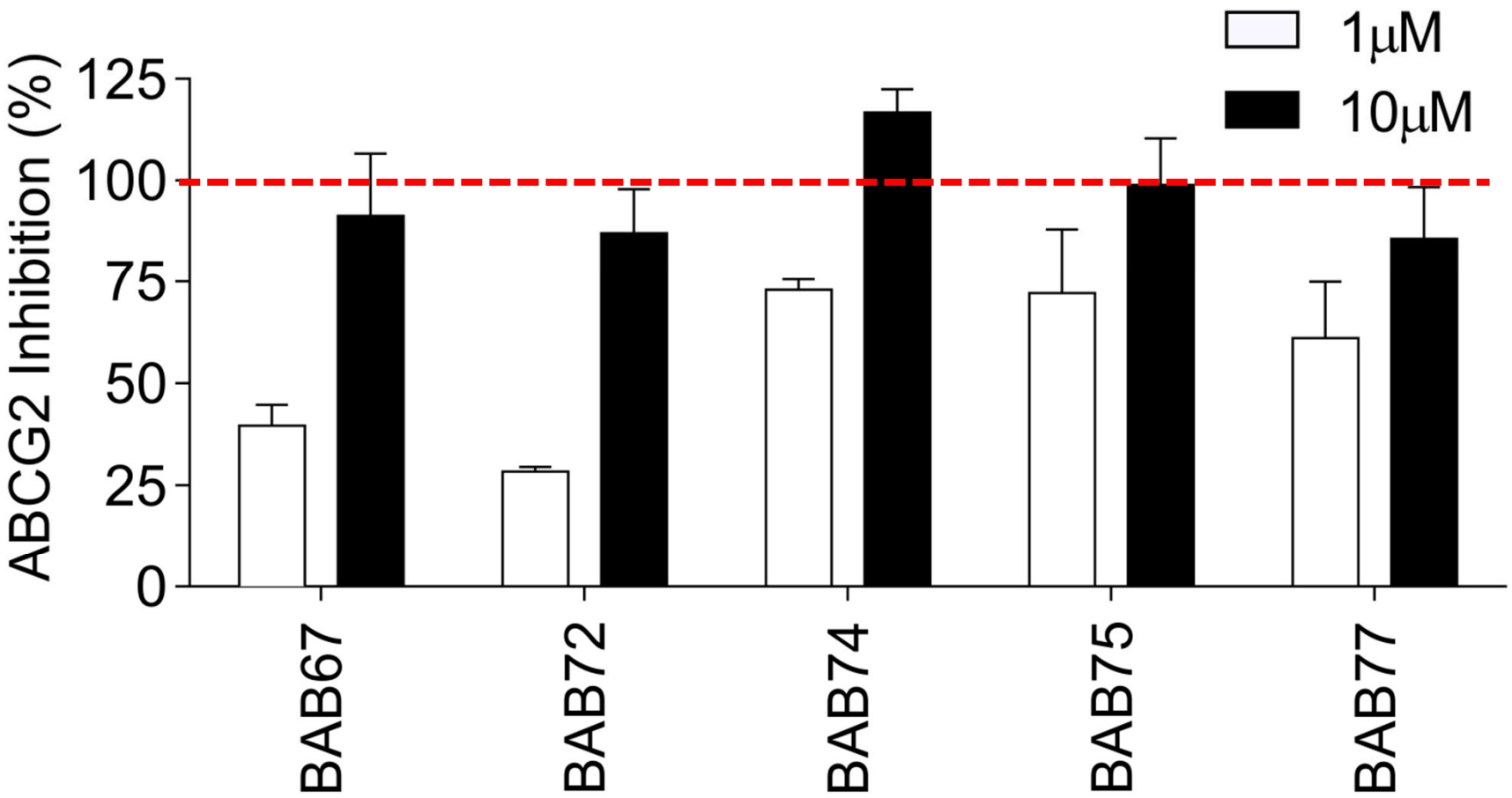


Stacking frame



ORTEP representation

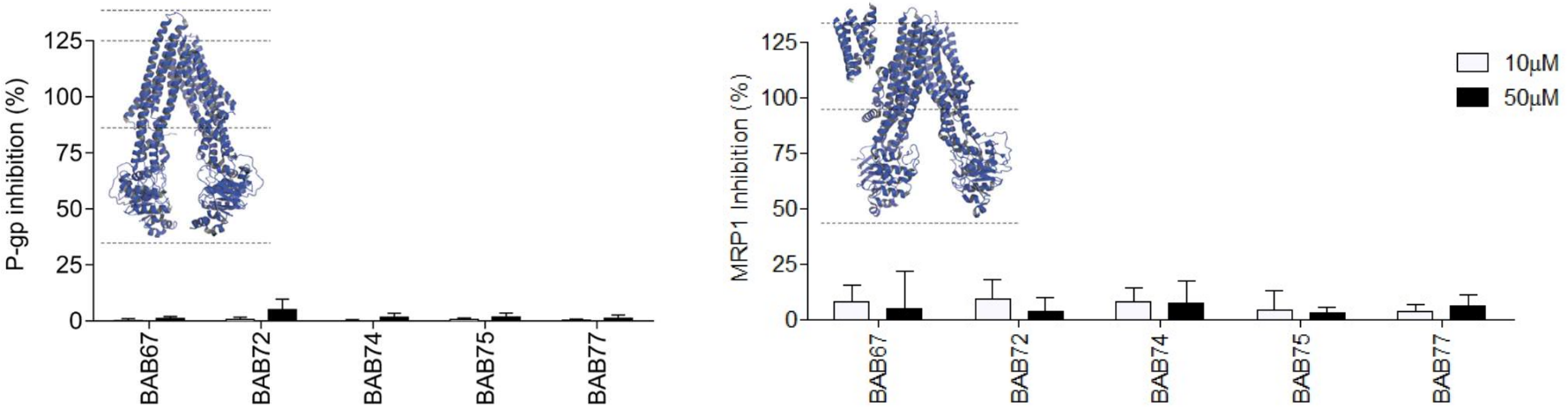
Results and discussion – ABCG2 inhibition produced by carbazoles



All the compounds were able to inhibit ABCG2, reaching total inhibition when in the highest concentration

Results and discussion – Selectivity toward ABCG2

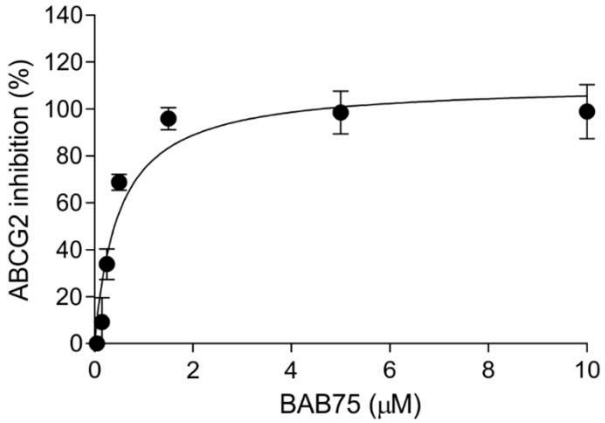
Besides ABCG2, another two ABC transporters (P-gp and MRP1) are also implicated in MDR

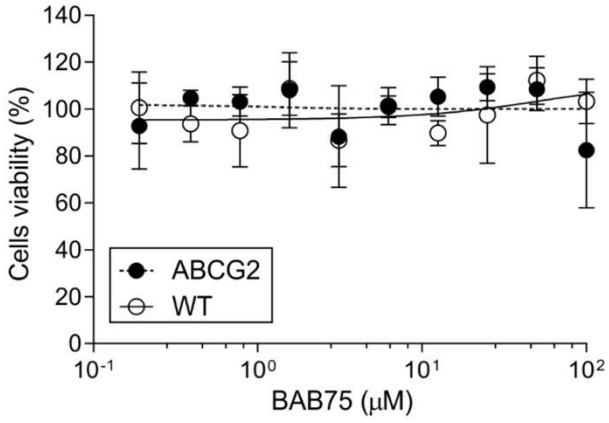


All compounds did not inhibit P-glycoprotein (P-gp) and Multidrug Resistance Protein 1 (MRP1), been selective for ABCG2

Results and discussion – ABCG2 inhibition potency & cytotoxicity

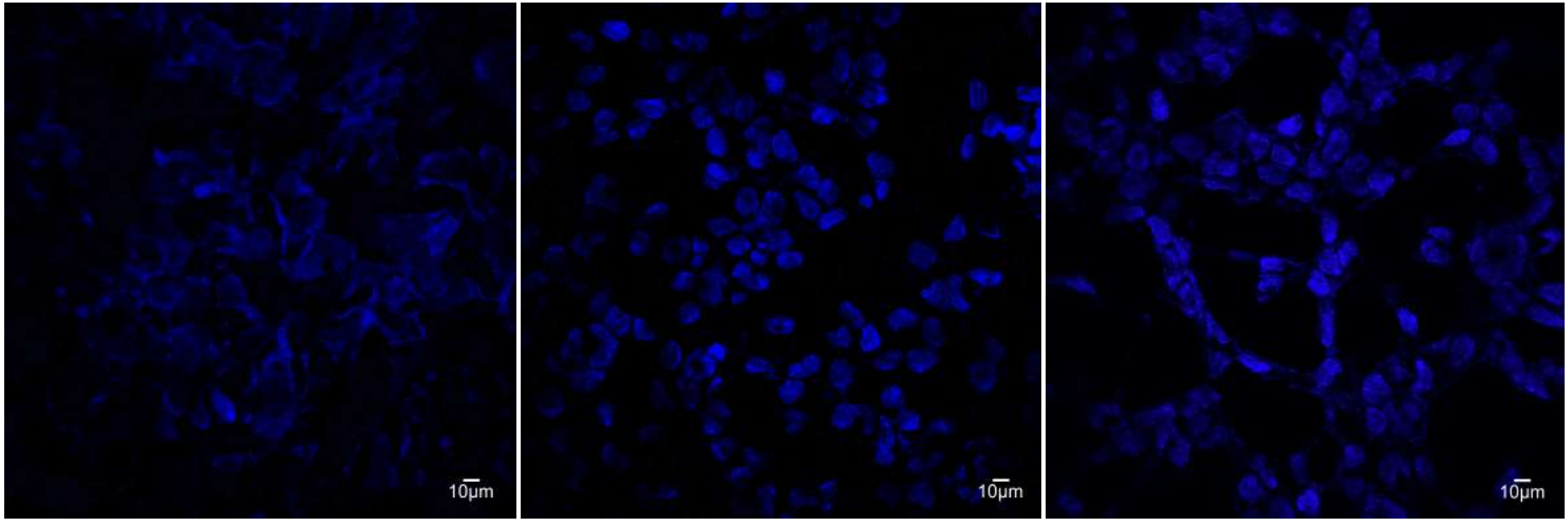
Compounds	IC_{50}±SD Inhibition (μM)	IG_{50} Cytotoxicity (μM)	Therapeutic ratio (IG_{50} / IC_{50})
BAB67	1.55±0.42	>100	>65
BAB72	3.39±1.42	>100	>29
BAB74	0.80±0.12	>100	>125
BAB75	0.49±0.20	>100	>204
BAB77	0.60±0.14	>100	>167





Based on therapeutic ratio (TR), **BAB75** was selected as the best inhibitor of the series

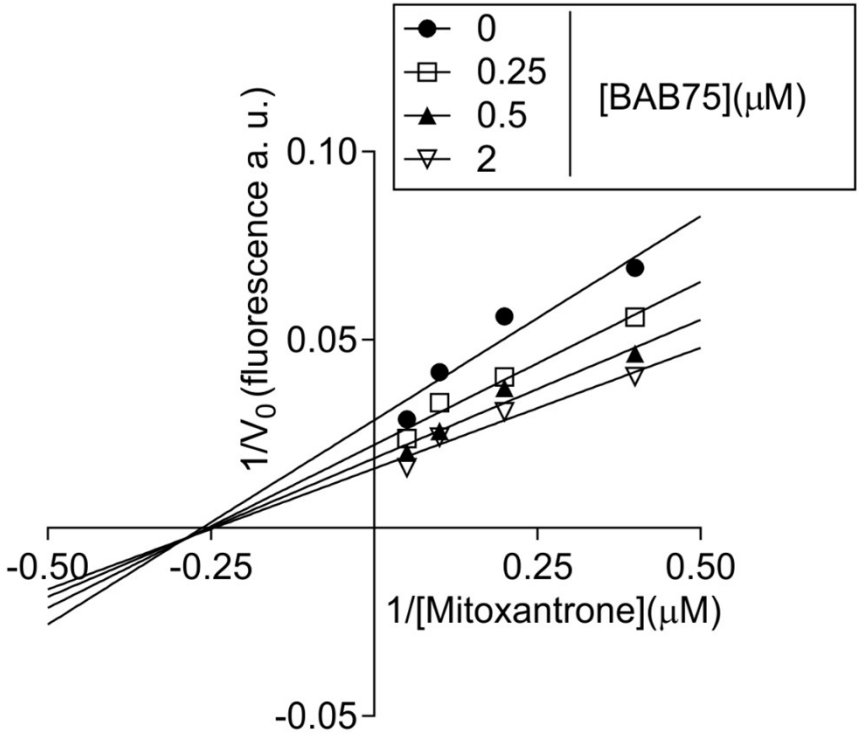
Results and discussion – Confirmation of ABCG2 inhibition using a second ABCG2 substrate (Höechst 33342)



HEK293-*ABCG2* HÖECHST (1 µM) NO INHIBITORS	HEK293-*ABCG2* HÖECHST (1 µM) Ko143 (0.5 µM)	HEK293-*ABCG2* HÖECHST (1 µM) BAB75 (10 µM)

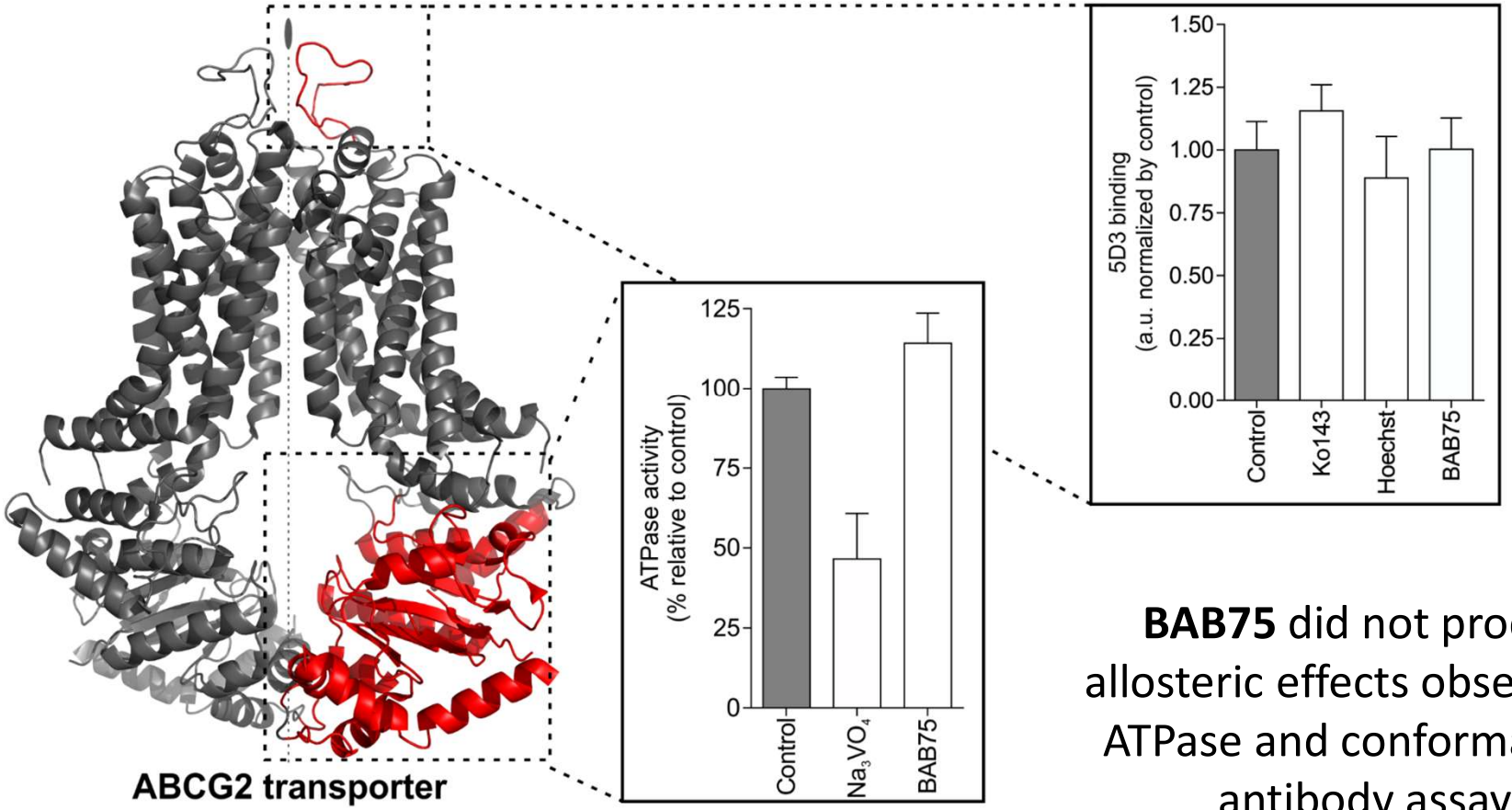
BAB75 also inhibits the efflux of Höechst 33342 mediated by ABCG2, proving to be a substrate independent type of inhibitor

Results and discussion – Type of inhibition



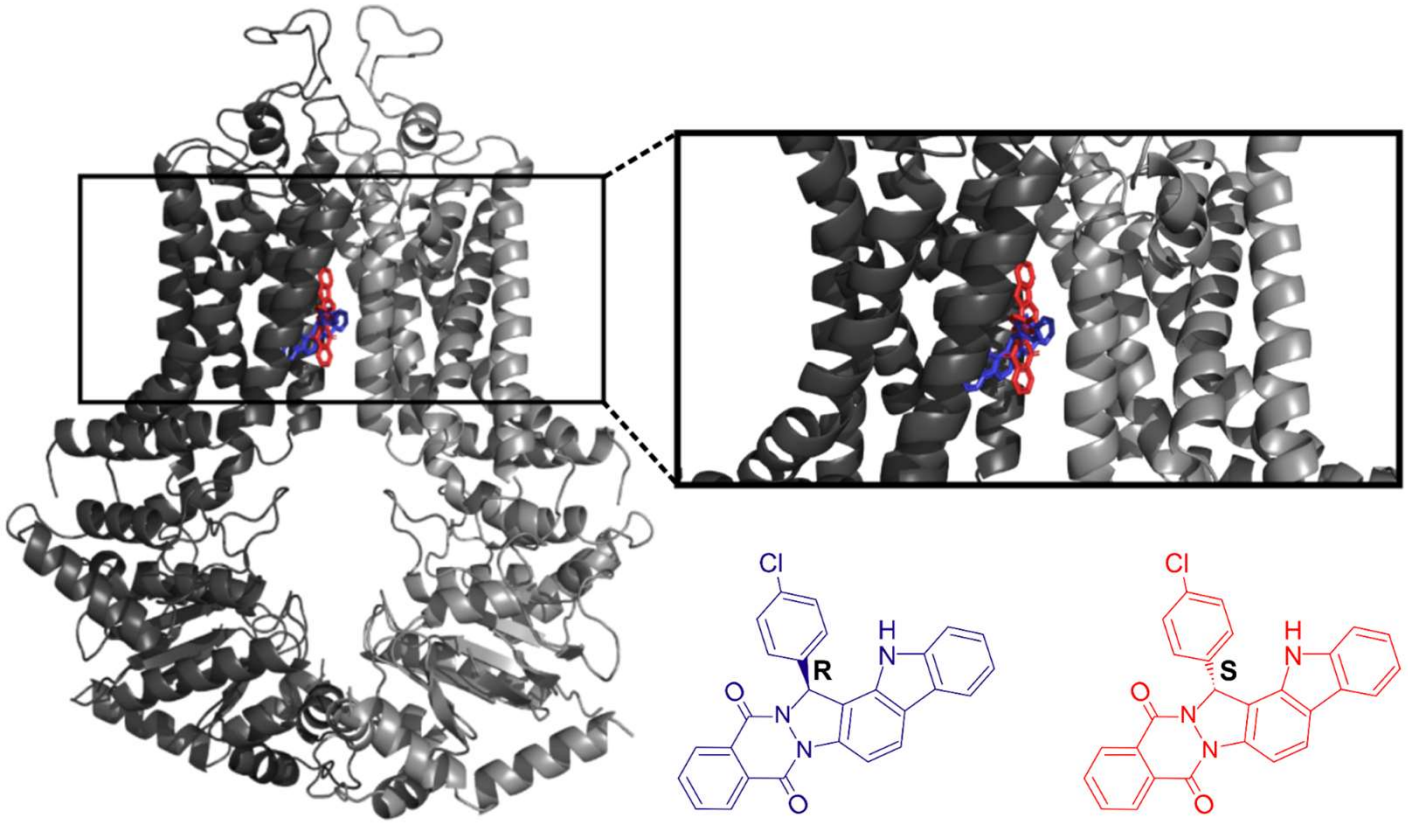
BAB75 performs a non-competitive inhibition on ABCG2-mediated mitoxantrone efflux

Results and discussion – Mechanism of inhibition



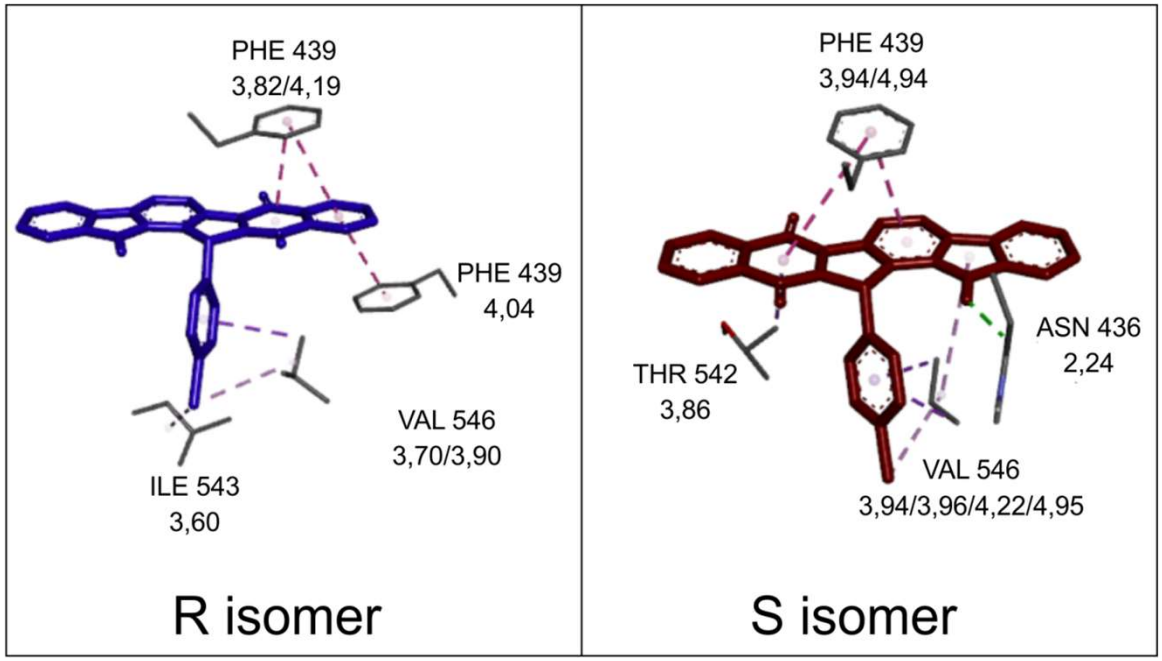
BAB75 did not produce allosteric effects observed by ATPase and conformational antibody assays

Results and discussion – Docking of BAB75 on ABCG2



The two isomers of **BAB75** bind on transmembrane drug-binding site

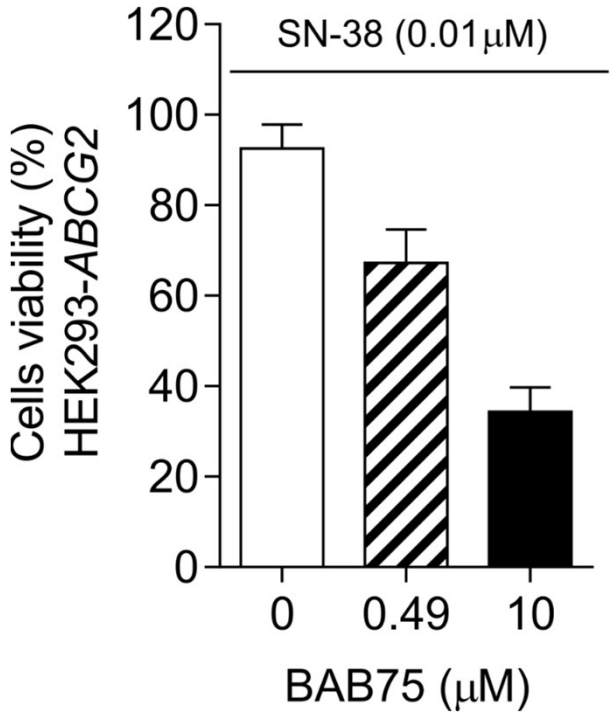
Results and discussion – Docking of BAB75 on ABCG2



-13.9 Kcal/mol **-13.5 Kcal/mol**

ABCG2 transporter is not enantioselective. Most interactions are hydrophobic, emphasizing the relevance of the aromatic core of the compound for stabilizing the interaction

Results and discussion – Chemosensitization of cells overexpressing ABCG2 to chemotherapeutic SN-38



BAB75 reverses the MDR phenotype mediated by ABCG2

Conclusions

- ✓ Carbazoles are selective ABCG2 inhibitors.
- ✓ The ABCG2 potency of inhibition (IC_{50}) was from 0.49 to 3.39 µM.
- ✓ Carbazoles are non cytotoxic, given a high therapeutic ratio (TR).
- ✓ **BAB75** was the best inhibitor (TR > 204).
- ✓ The inhibition promoted by **BAB75** was independent of the ABCG2 substrate.
- ✓ **BAB75** produced a non-competitive inhibition, did not produce effects on ATPase activity and recognition of the conformational antibody.
- ✓ Docking analysis revealed the binding site of **BAB75**.
- ✓ **BAB75** chemosensitizes cells that overexpress ABCG2.

Acknowledgments

FUNDINGS:

- CENAPD at UNICAMP-SP, Brazil for the computational resources.
- CNPq (grant number 400953/2016-1).
- Fundação Araucária (Code 006 – 09/2016).
- Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001.
- Ministère de l'Enseignement Supérieur et de la Recherche Scientifique, Algeria.