

*SupraChem2009*



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# Functionalized macrocycles as enzyme inhibitors and antioxidants in biological systems

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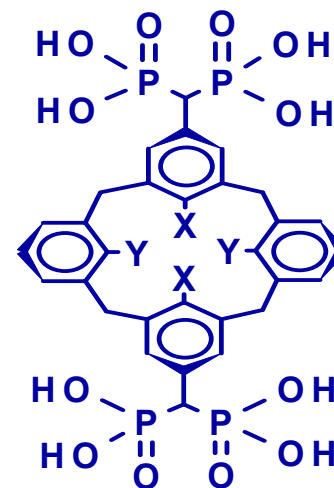
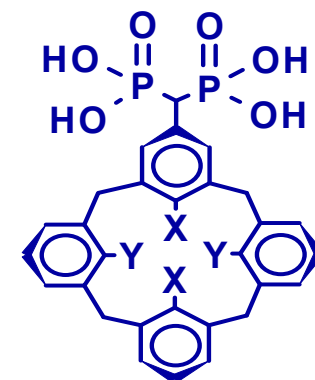
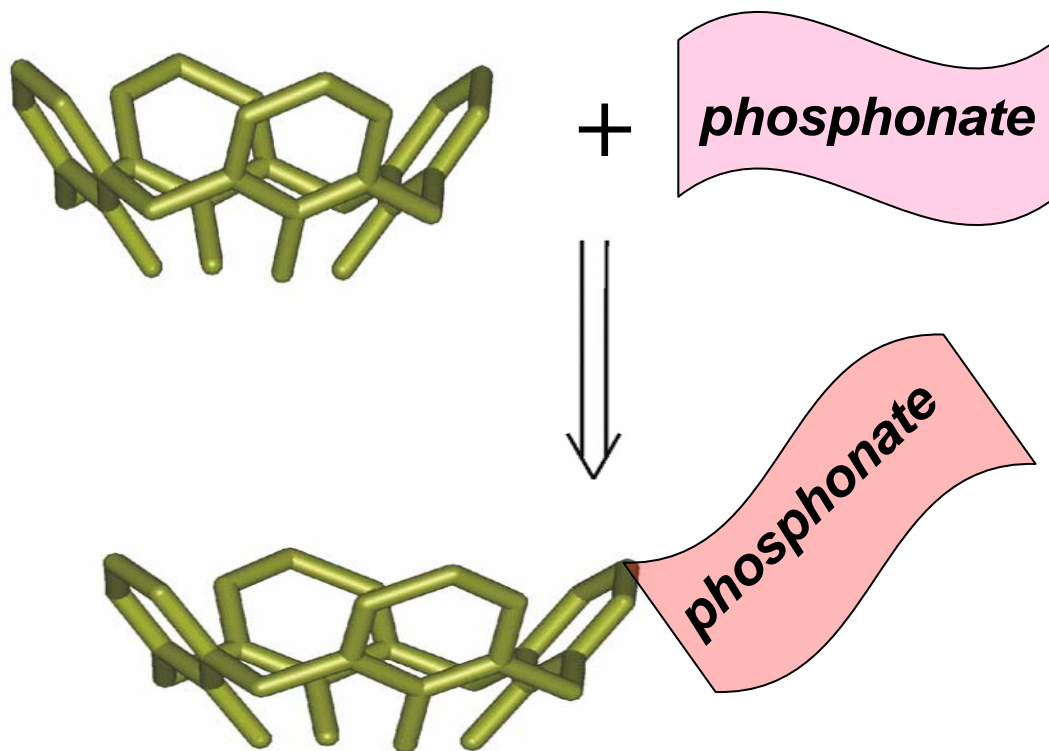
**SupraChem2009, Katsivel**

## **Plan of presentation**

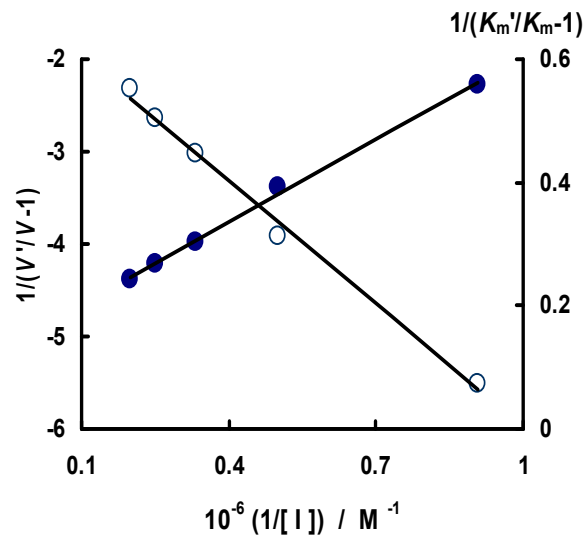
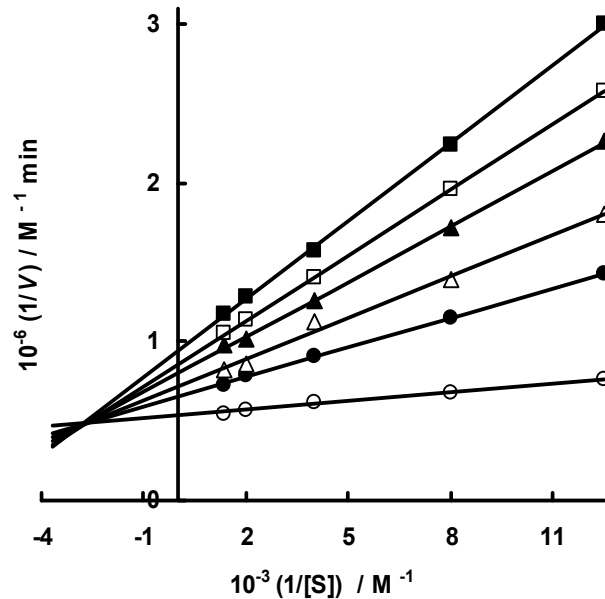
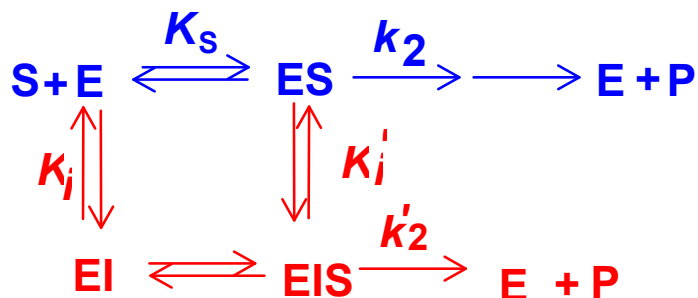
- **Study of complexation of phosphorylated calix[4]arenes and thiocalix[4]arenes with some phosphatases.**
- **Resorcinarene derivatives as potent antioxidant agents in model biological systems.**

# Calixarenes as molecular platform for preorganizing bioisosteric groups

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# Kinetics of calf intestine alkaline phosphatase inhibition by calix[4]arene methylenebisphosphonates



*Lineweaver–Burk plots of alkaline phosphatase inhibition by calix[4]arene bismethylenebisphosphonate (0, 1, 2, 3, 4, 5 μM).*

$$\frac{1}{(V'/V - 1)} = \frac{K_i'}{(k_2'/k_2 - 1)[I]} + \frac{1}{k_2'/k_2 - 1}$$

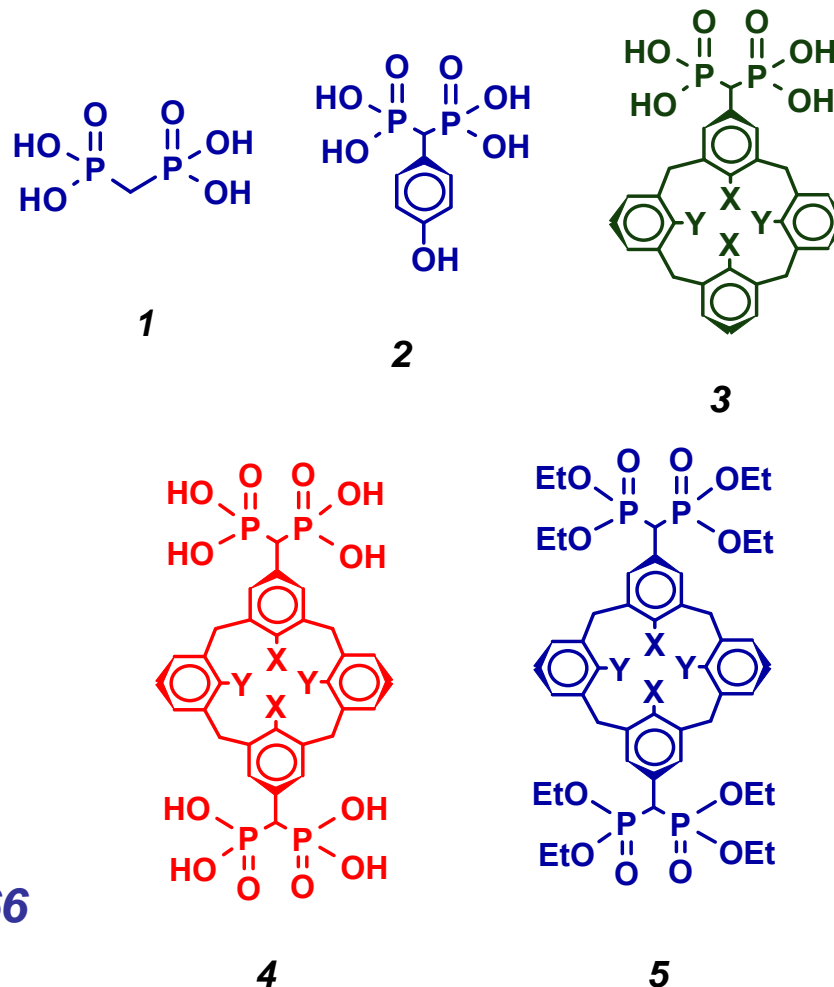
$$\frac{1}{(K_m'/K_m - 1)} = \frac{1}{K_i'/K_i - 1} + \frac{K_i'}{[I](K_i'/K_i - 1)}$$

# Role of macrocyclic platform in the inhibiting activity of methylenebisphosphonate derivatives

Table. The inhibition constants for calixarene methylenebisphosphonic acids 3,4 and model compounds 1,2,5

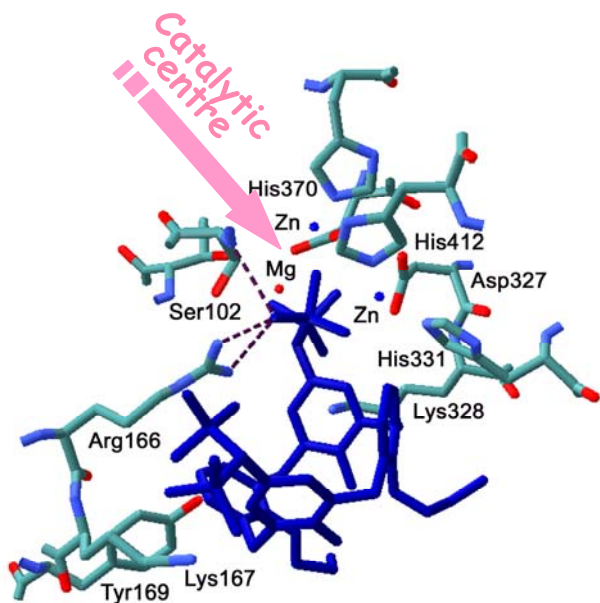
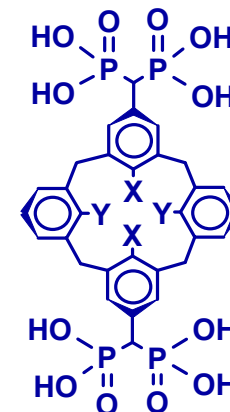
| Inhibitor | $K_i$ ( $\mu\text{M}$ ) | $K_i'$ ( $\mu\text{M}$ ) |
|-----------|-------------------------|--------------------------|
| 1         | $67 \pm 5$              | $750 \pm 100$            |
| 2         | $22 \pm 4$              | $290 \pm 110$            |
| 3         | 2.5                     | 46                       |
| 4         | 0.38                    | 2.8                      |
| 5         | $820 \pm 180$           | $8500 \pm 2100$          |

Calf intestine alkaline phosphatase,  
0.1 M Tris-HCl buffer, pH 9;  $K_m=36\pm 9 \mu\text{M}$ ;  $k_2'/k_2=0.3$



Org. Biomol. Chem., 2004, 2, 3162-3166

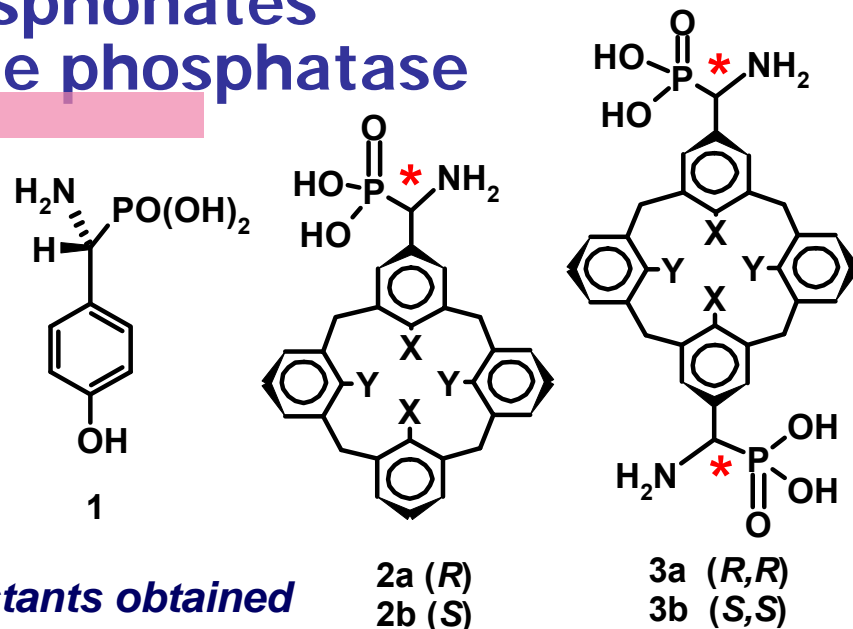
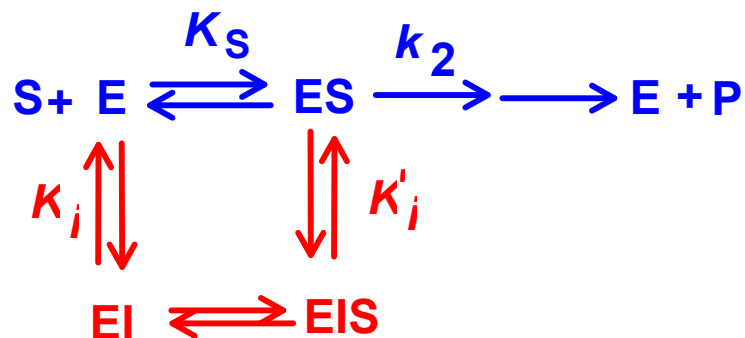
# Alkaline phosphatase as target for calix[4]arene bismethylenebisphosphonate



| Source                | $K_i$ ( $\mu\text{M}$ ) | $K_i'$ ( $\mu\text{M}$ ) |
|-----------------------|-------------------------|--------------------------|
| <i>Calf intestine</i> | 0.38                    | 2.8                      |
| <i>Bovine mucosa</i>  | $0.40 \pm 0.04$         | $5.2 \pm 0.8$            |
| <i>Bovine kidney</i>  | $12 \pm 3$              | $330 \pm 130$            |
| <i>Human placenta</i> | $81 \pm 14$             | $2100 \pm 440$           |
| <i>Shrimp</i>         | $4.9 \pm 0.4$           | $140 \pm 50$             |
| <i>E. coli</i>        | $190 \pm 70$            | $1400 \pm 1200$          |

*Ukr. Biochem. J.*, 2007, 78, 26-33.

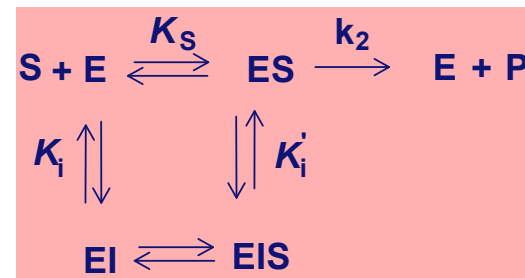
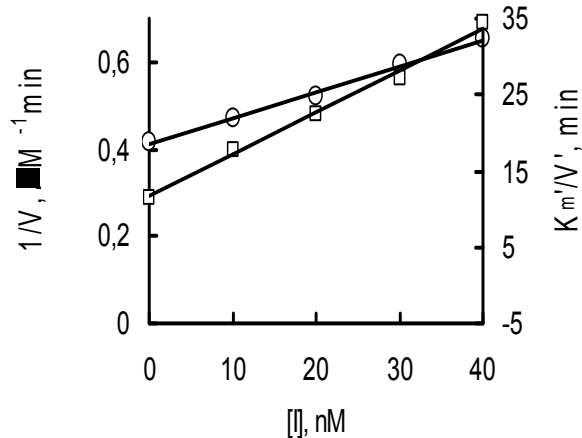
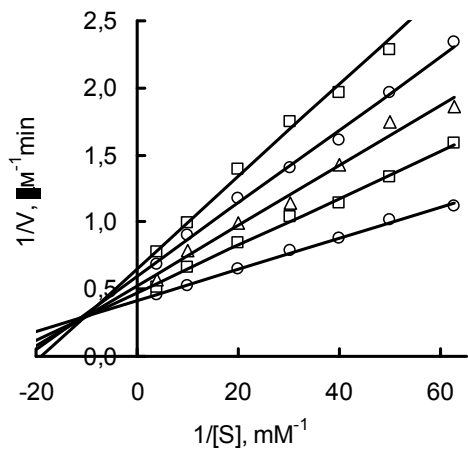
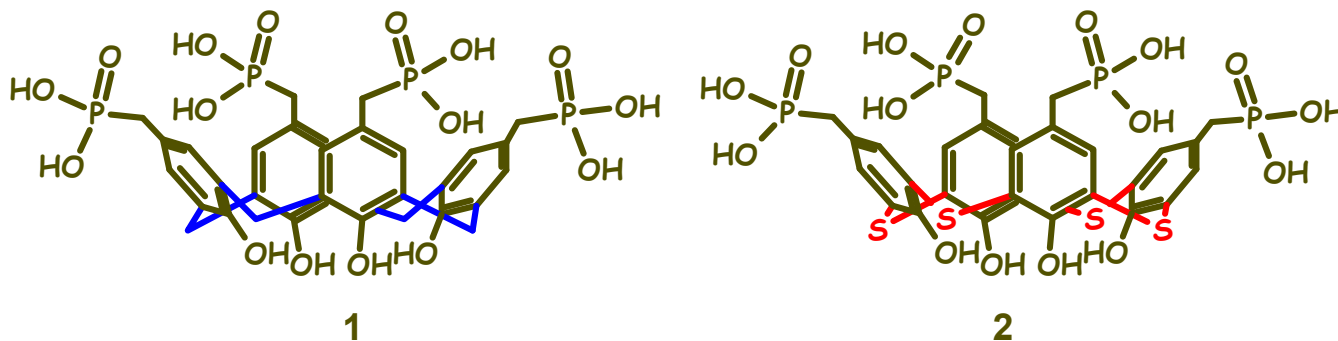
# Stereoselectivity of complexation of calix[4]arene aminophosphonates with porcine kidney alkaline phosphatase



## Mixed-type kinetics and values of constants obtained

| Inhibitor | $K_i$ (PKAP)  | $K'_i$ (PKAP)  | Relative binding | $\Delta G$ (kcal/mol) | Difference in free energy |
|-----------|---------------|----------------|------------------|-----------------------|---------------------------|
| 1         | $580 \pm 110$ | $6800 \pm 840$ |                  | -4.4                  |                           |
| 2a (R)    | $73 \pm 13$   | $540 \pm 90$   |                  | 5.6                   |                           |
| 2b (S)    | $32 \pm 5$    | $780 \pm 100$  |                  | -6.1                  |                           |
| 3a (RR)   | $1.7 \pm 3$   | $130 \pm 30$   | 50               | -7.8                  | -2.3                      |
| 3b (SS)   | $86 \pm 11$   | $610 \pm 210$  | 1                | -5.5                  | 0                         |

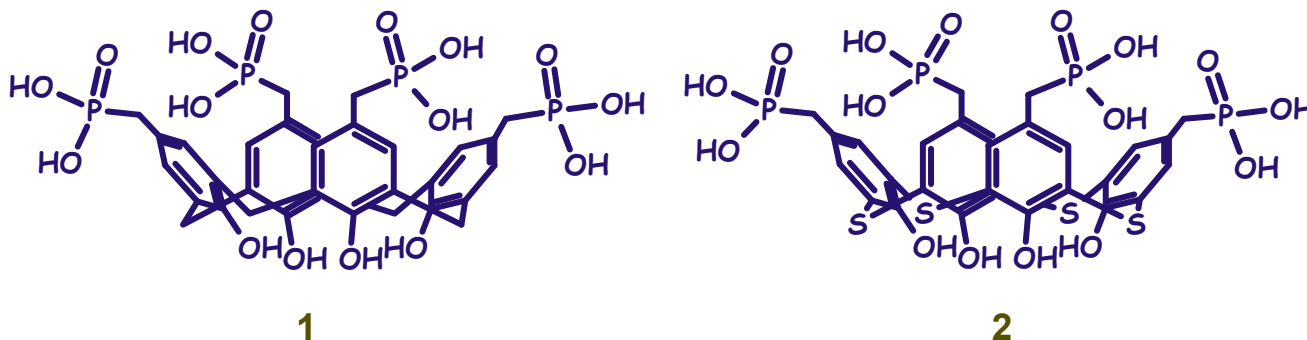
# Use of thiacalix[4]arene as molecular scaffold in the design of new alkaline phosphatase inhibitors



**Lineweaver–Burk plots and secondary representation of experiments performed: kinetics of inhibition of bovine intestine alkaline phosphatase by compounds 1, 2 corresponds to the mixed-type inhibition**

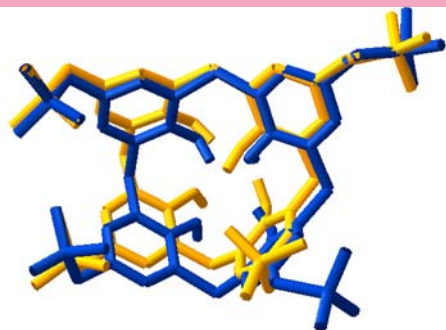
# Thiacalix[4]aren tetrakis(methylphosphonate) 2 shows stronger inhibiting effects than its calix[4]arene analogue 1

| Inhibitor (enzyme) | Inhibition mechanism | $K_i$                           | $K_i'$                          |
|--------------------|----------------------|---------------------------------|---------------------------------|
| 1 (HPAP)           | mixed                | $0,94 \pm 0,19$ mM              | $3,77 \pm 0,37$ mM              |
| 2 (HPAP)           | mixed                | $0,059 \pm 0,001$ mM            | $0,27 \pm 0,03$ mM              |
| 1 (BIAP)           | mixed                | $70 \pm 4$ nM                   | $920 \pm 220$ nM                |
| <b>2 (BIAP)</b>    | <b>mixed</b>         | <b><math>21 \pm 1</math> nM</b> | <b><math>75 \pm 7</math> nM</b> |
| 1 (SAP)            | competitive          | $33 \pm 3$ $\mu$ M              | -                               |
| 2 (SAP)            | competitive          | $3,2 \pm 0,2$ $\mu$ M           | -                               |

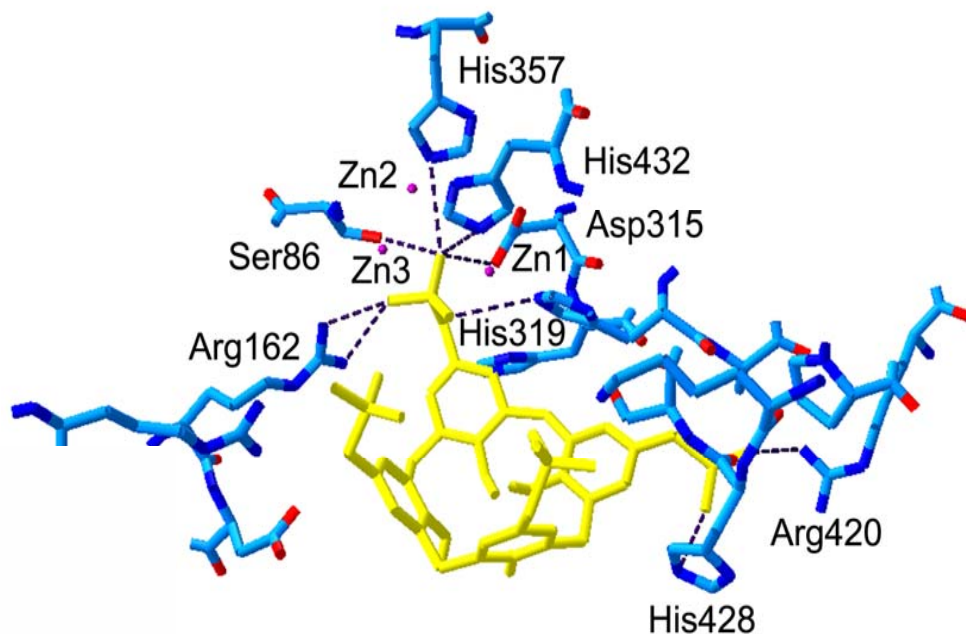


# Mechanisms of complexations of thiacalixarene and calixarene tetrakis(methylphosphonates) with alkaline phosphatase

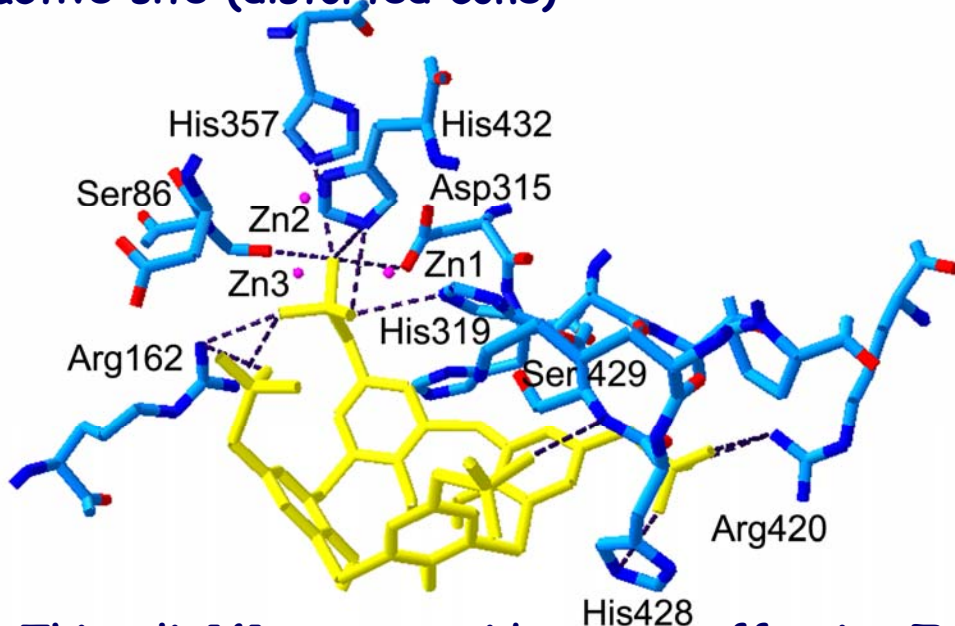
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Calculated geometries of calixarene and thiacalixarene inhibitors in enzyme active site (distorted cone)



Location of calix[4]arene in enzyme active site



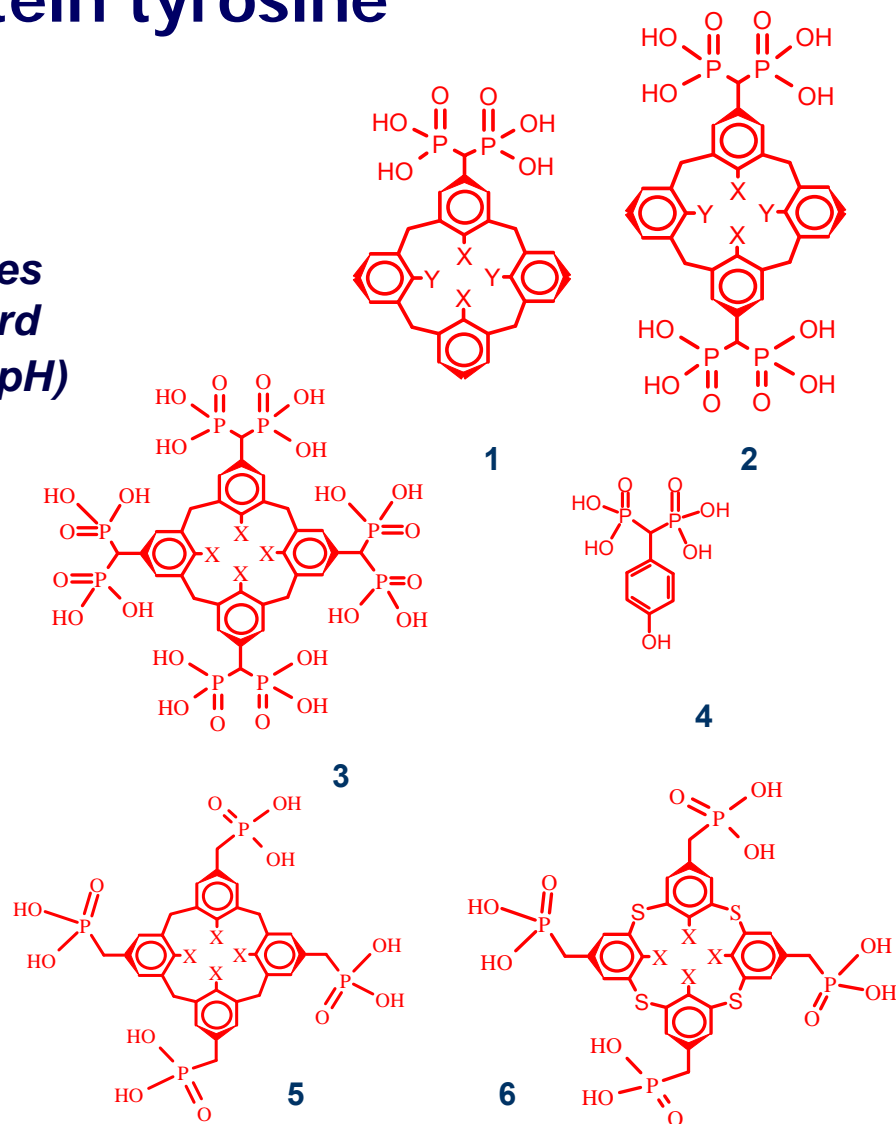
Thiacalix[4]arene provides more effective Zn-ion chelation and forms more hydrogen bonds

# Importance of the macrocyclic scaffold and phosphonate part in the complexation of inhibitors with Yersinia protein tyrosine phosphatase

**Table. Inhibiting activities of phosphonates clustered by a macrocyclic scaffold toward Yersinia protein tyrosine phosphatase (YopH)**

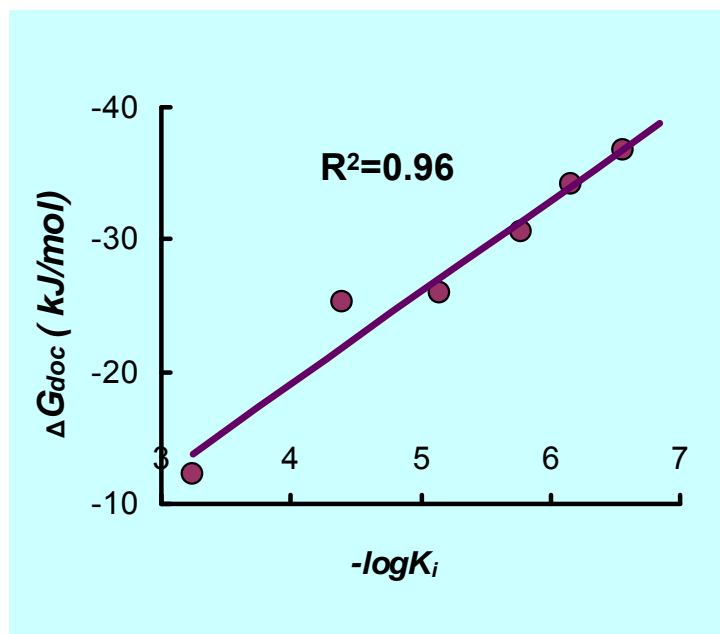
| Inhibitor | Relative potency* |
|-----------|-------------------|
| 1         | 80                |
| 2         | 400               |
| 3         | 14                |
| 4         | 1                 |
| 5         | 600               |
| 6         | 2500              |

\* Under assay conditions: 0.1 M BISTRIS buffer, pH 6.5, 30°C

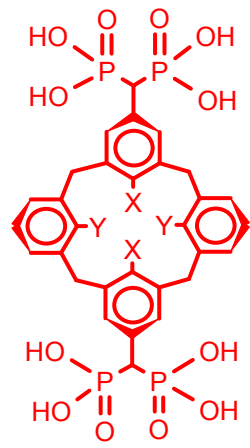
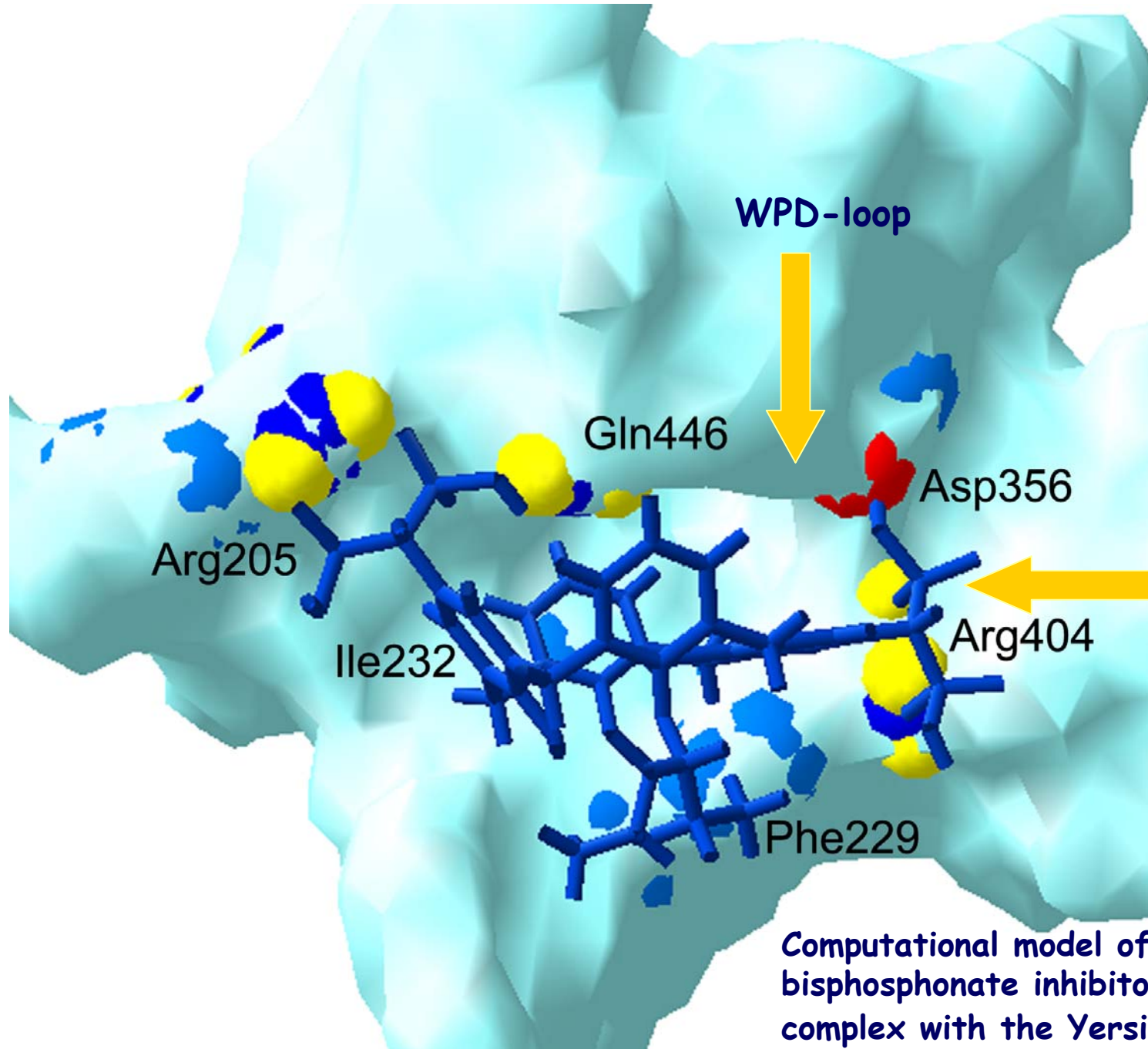


**Ki=0.22±0.03 μM**

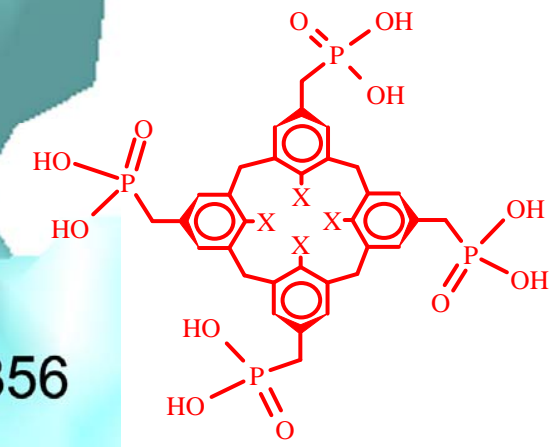
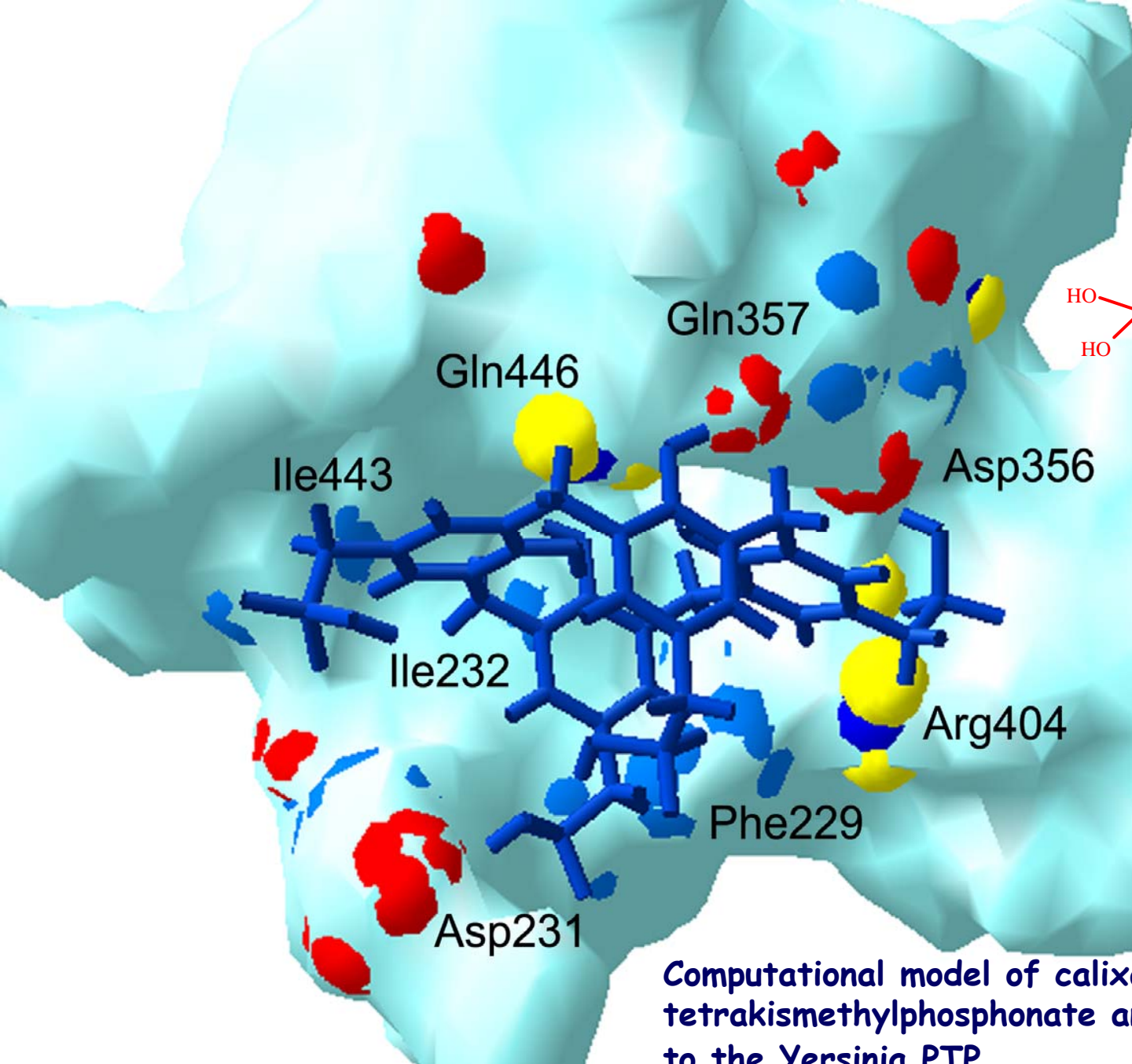
# Correlation between the experimental activities and predicted free energies of docking by QXP/FLO+ for Yersinia protein tyrosine phosphatase inhibitors



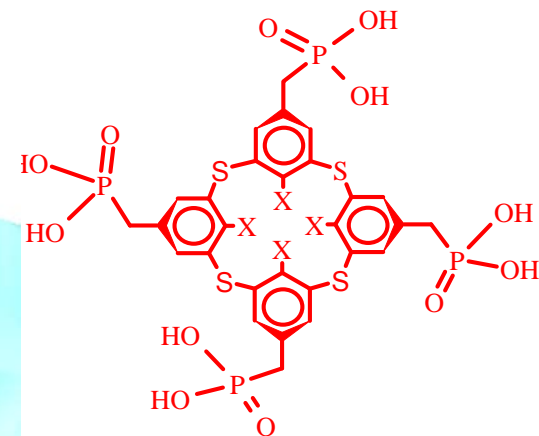
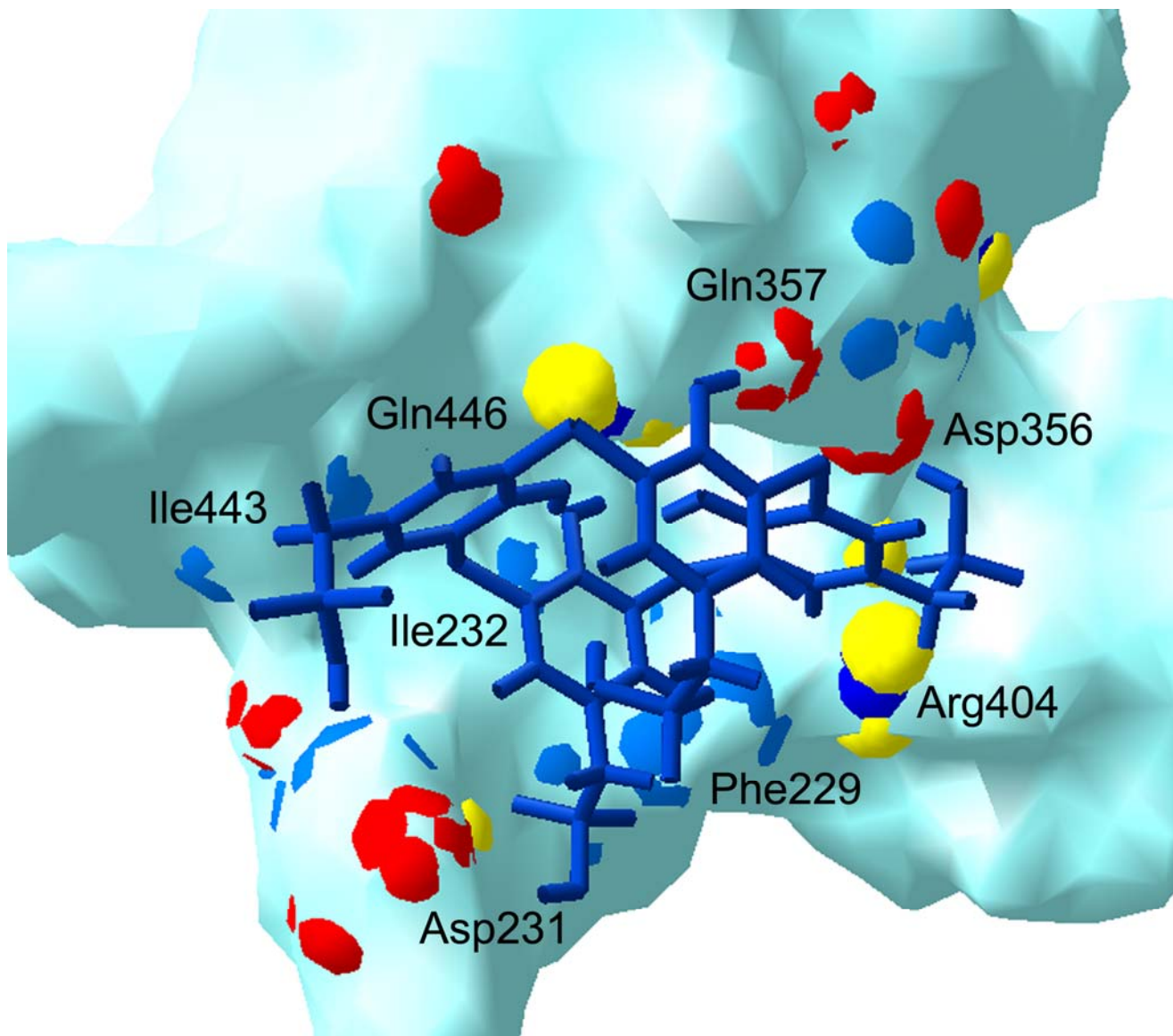
| Energies               | The correlation coefficient ( $r^2$ ) between the calculated energy and experimental activity |
|------------------------|---|
| Free energy of binding | 0.96  |
| Contact energy         | 0.76  |
| Hydrogen bond energy   | 0.15  |



Computational model of macrocyclic bisphosphonate inhibitor bounded in complex with the *Yersinia* PTP



Computational model of calixarene tetrakis(methylphosphonate) anchored to the Yersinia PTP

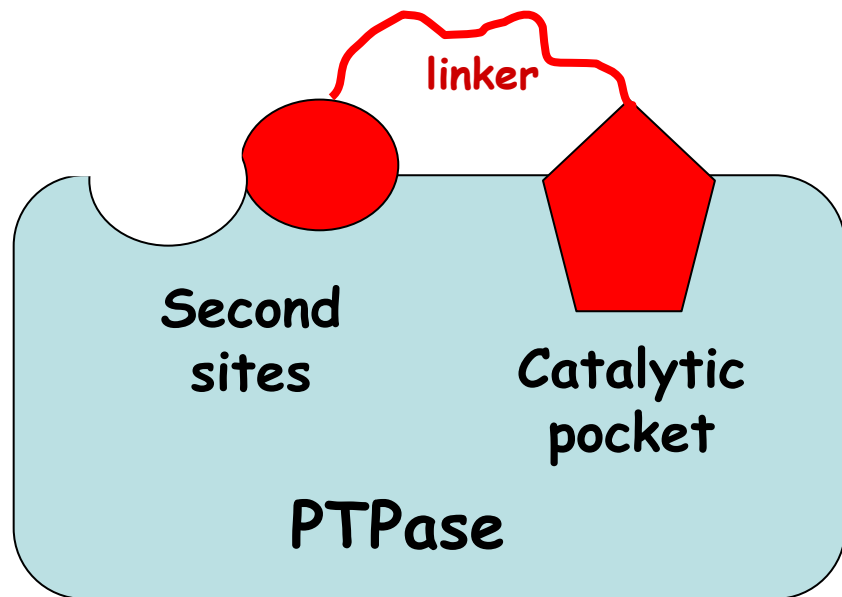


**Complexation mode of thiacalixarene inhibitor bounded in complex with the Yersinia PTP**

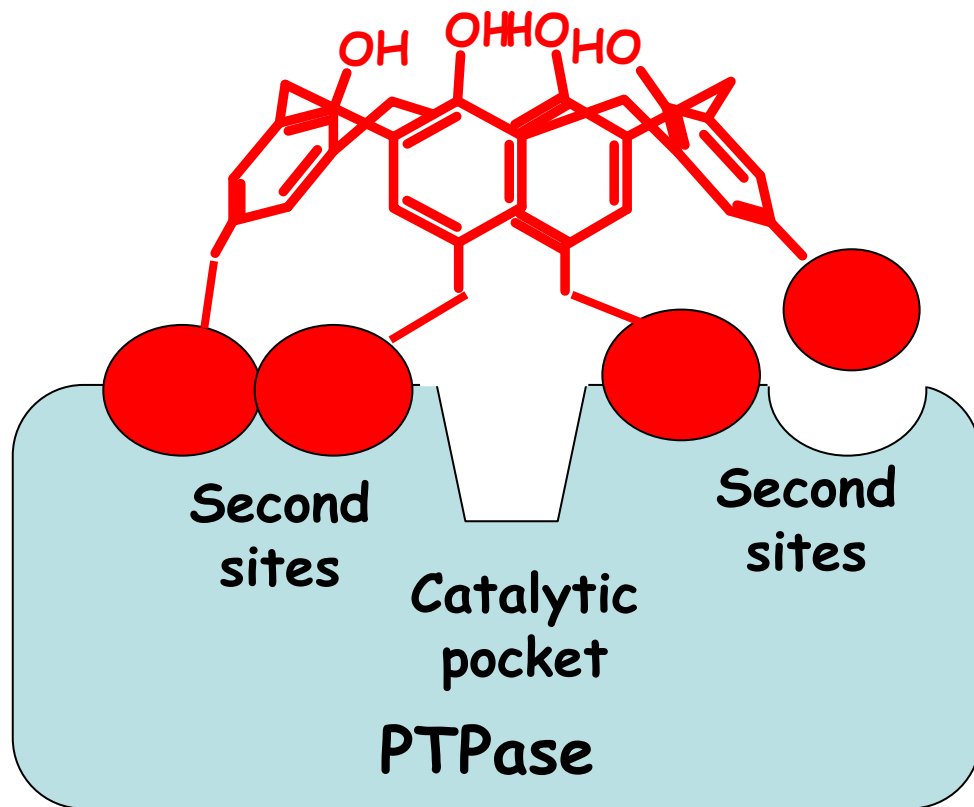
## Calixarene inhibitor

Inhibitor

linker

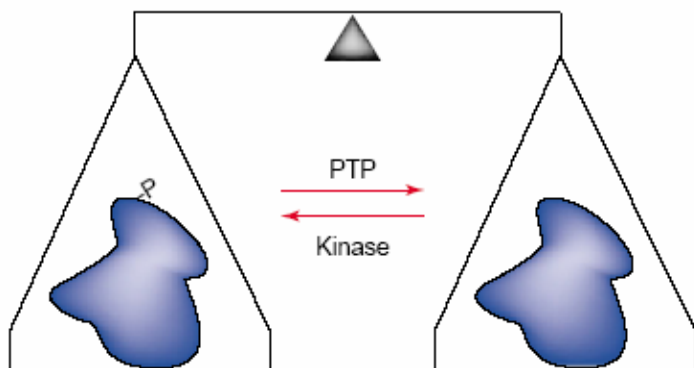


The known approach of searching of PTP inhibitors: binding in the catalytic site and the adjacent second site

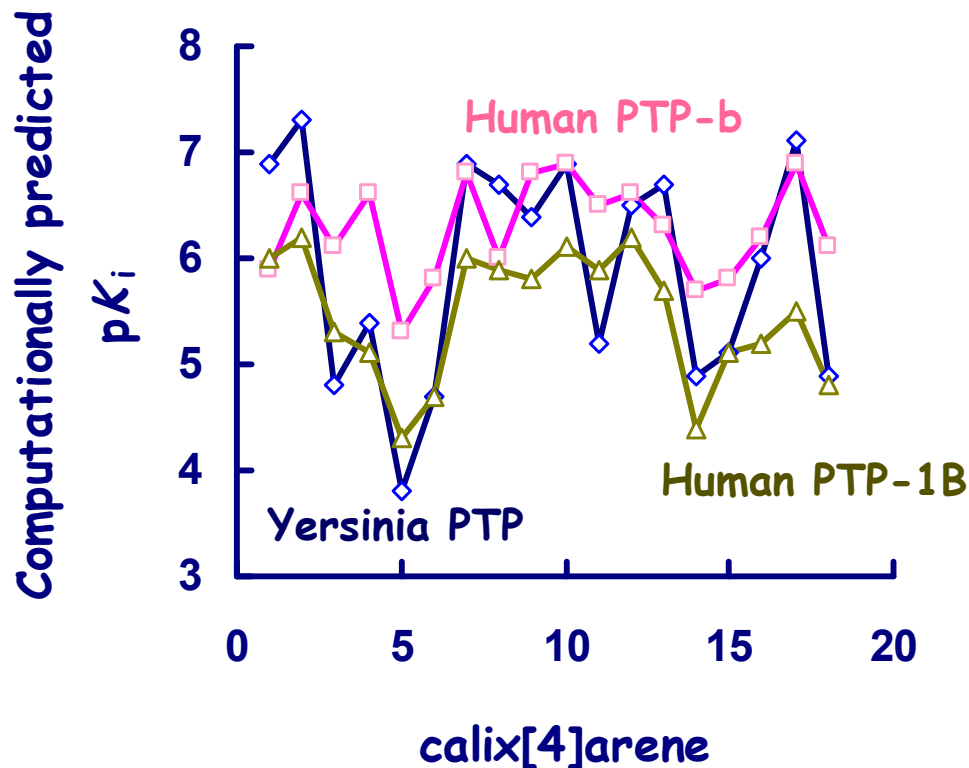


The common binding mode for calixarene inhibitors covering the active site

# Computational modelling of calixarenes for targeting the therapeutically relevant b-PTP and PTP1B



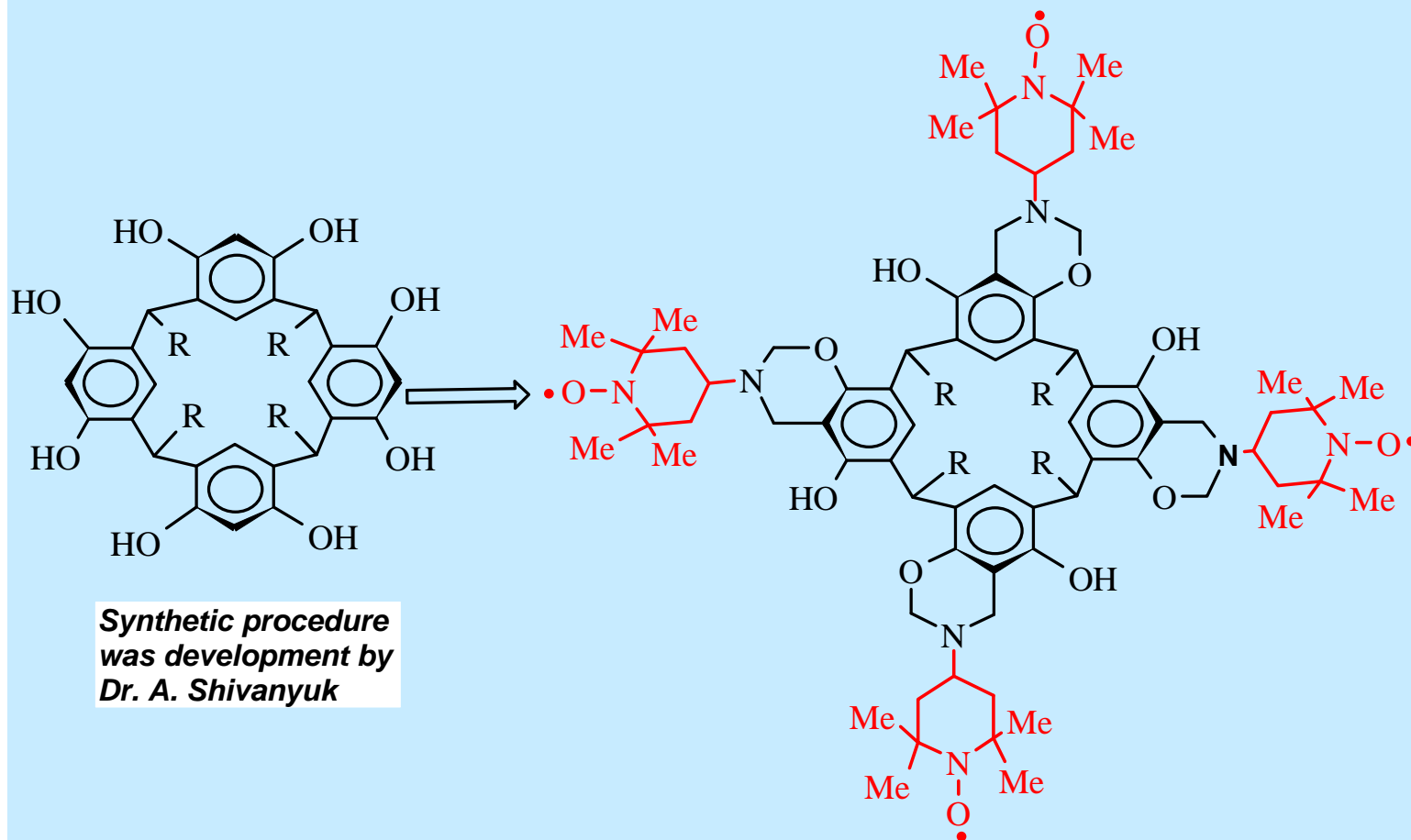
The attractiveness of PTPs as targets for bioactive compounds



Human PTP-1B and PTP-b are implicated in a growing of obesity, diabetes, inflammation and other deseases

# Resorcinarene tetranitroxides as antioxidants in model systems. How does the close spacing of resorcine and TEMPO fragments influence antiradical and antioxidant activities of macrocyclic compounds?

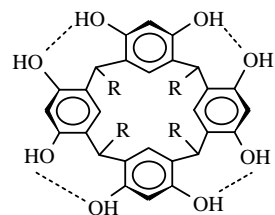
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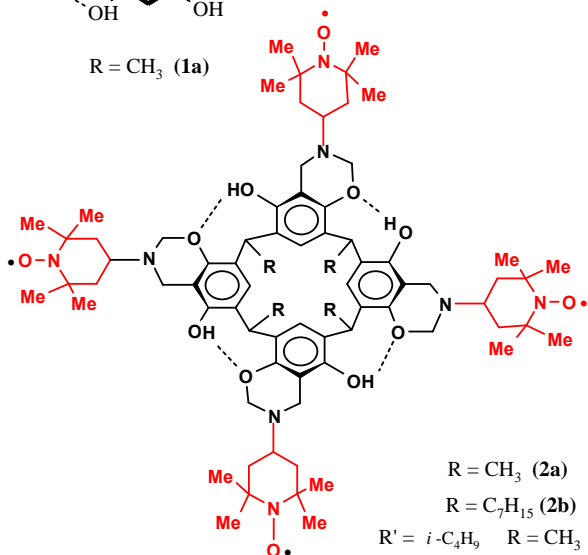
# Strong antiradical activities of resorcinarenes

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**Table 1. DPPH scavenging ability of the macrocycles in term of stoichiometry is more than two orders of magnitude higher than of resorcin**



R = CH<sub>3</sub> (1a)



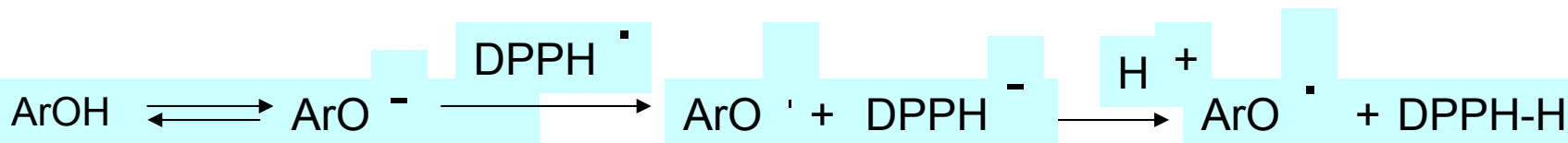
R = CH<sub>3</sub> (2a)

R = C<sub>7</sub>H<sub>15</sub> (2b)

R' = *i*-C<sub>4</sub>H<sub>9</sub>, R = CH<sub>3</sub> (2c)

| Compound        | ECR <sub>50</sub> <sup>*</sup> |
|-----------------|--------------------------------|
| 2a              | 0.19±0.04                      |
| 2b              | 0.21±0.03                      |
| 2c              | 0.18±0.02                      |
| 1a              | 0.29±0.04                      |
| 4-Hydroxy-TEMPO | 57±8                           |
| Resorcinol      | 64±17                          |

*\*The ECR50 were calculate from the plots of the percentage DPPH transformation against the ratio [Scavenger]/[DPPH].*



# Superoxide dismutase-like activity of resorcinarene tetranitroxides

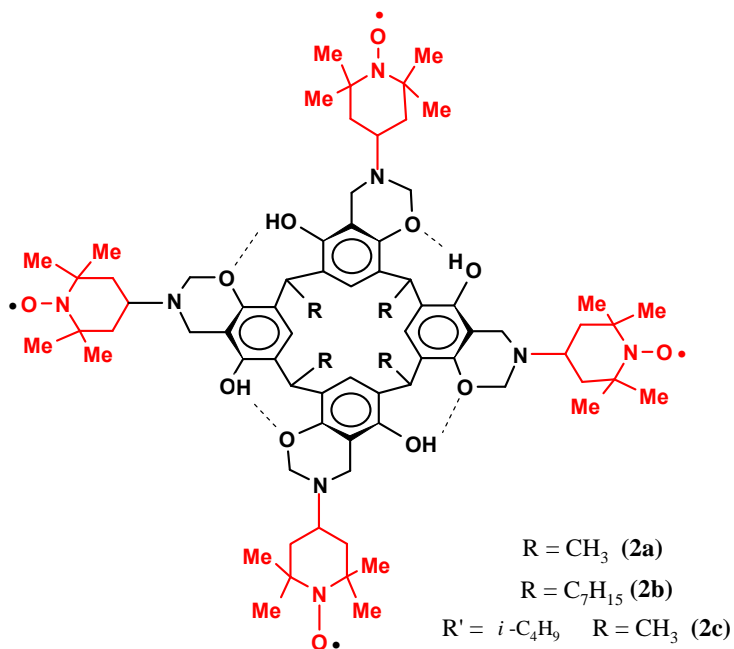
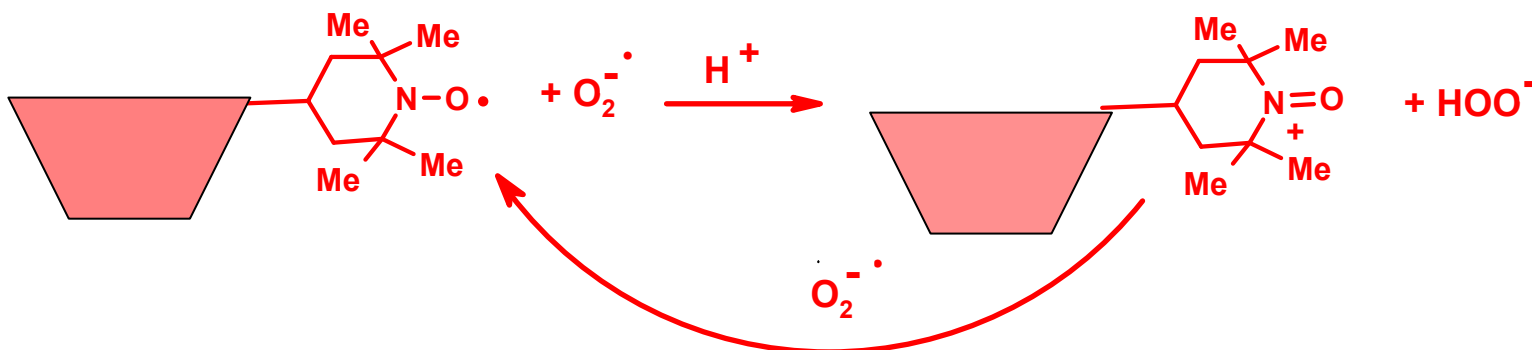


Table. Apparent second order rate constants for the reaction of the nitroxyl radicals with superoxide:

| Compound        | $(k_2 \cdot 10^{-6}), \text{M}^{-1}\text{c}^{-1}$ |
|-----------------|---|
| 2a              | $2.2 \pm 0.5$                                     |
| 2b              | $0.10 \pm 0.07$                                   |
| 2c              | n.d.  |
| 4-Hydroxy-TEMPO | $0.49 \pm 0.19$                                   |



# Resorcinarene nitroxide tetraradicals effectively suppress linoleic acid peroxidation

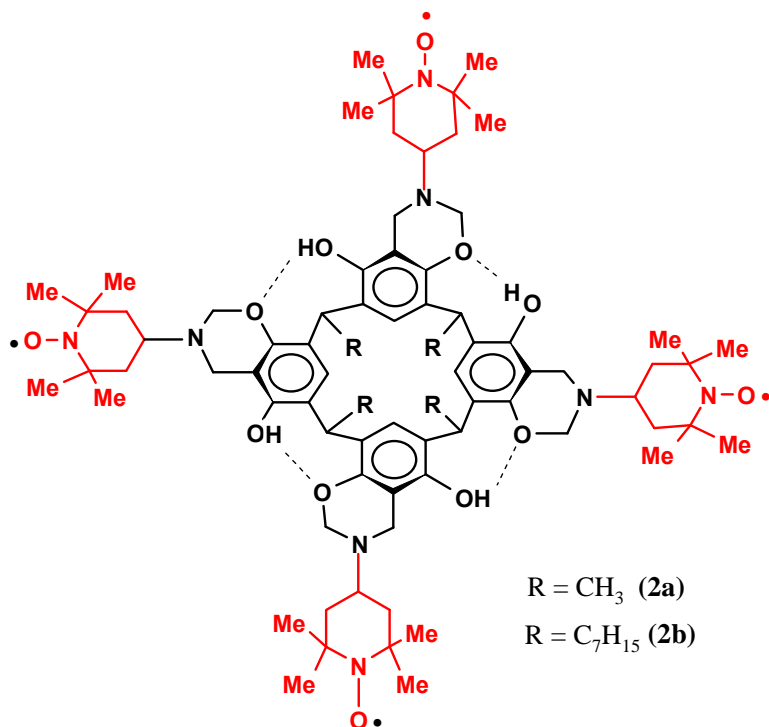


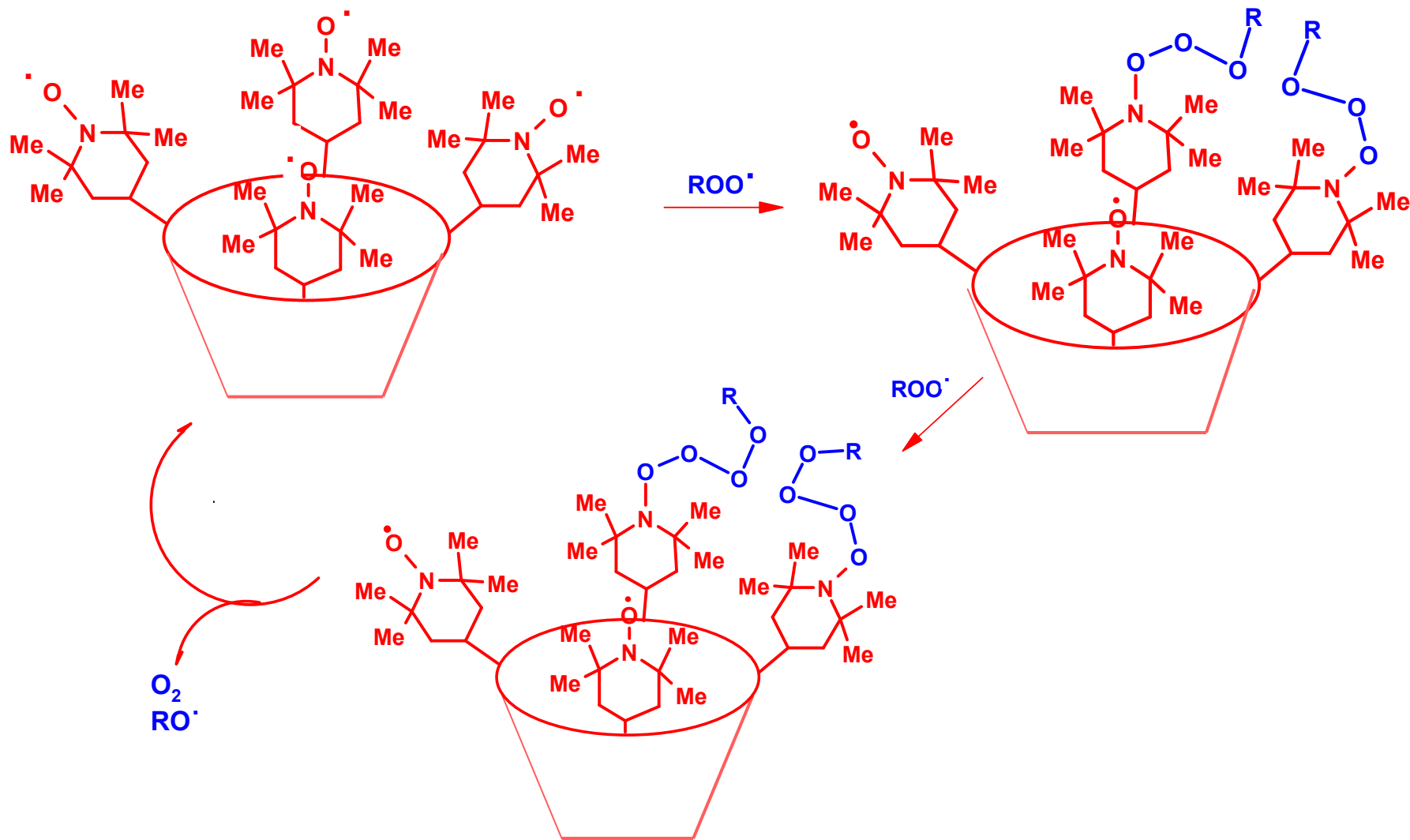
Table. Relative antioxidant efficiency values (RAE) and stoichiometric factors (n) of resorcinarene derivatives and model compounds

| Compound        | RAE      | n       |
|-----------------|----------|---------|
| 2a              | 17±4     | 30±9    |
| 2b              | 16±5     | 31±5    |
| 1a              | 1.7±0.8  | 4.9±0.8 |
| 4-Hydroxy-TEMPO | 1.4±0.4  | 2.8±0.6 |
| Resorcinol      | 0.4±0.04 | 2.4±0.2 |
| Trolox C        | 1        | 2       |

(The screening test to determine the peroxy radical-trapping efficiency of compounds tested includes examine of influence of inhibitor on conjugated dienes formation from linoleic acid in micelles in presence of 2,2'-azobis(2-amidinopropane) as the initiator. Trolox C was used as reference inhibitor)

# Proposed mechanism of peroxy radicals break-down catalyzed by resorcinarene tetranitroxides

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- Upper rim substituted calix[4]arenes can be employed as molecular scaffolds for the construction of effective inhibitors of some phosphatases.
- The bioisosteric phosphonate groups as well as macrocyclic fragment of the inhibitor take part in the complexation of these compounds with the enzymes.
- A resorcinarene oxazines bearing TEMPO fragments at the wide rim exhibit a strong antiradical and antioxidant properties in model systems.
- Structural variations at the wide and narrow rim of hydrogen bonded resorcinarenes may result in new highly active compounds that may be applicable as multifunctional protectors against free radical damage in cells.

## Acknowledgements

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Dr. O.V. Muzychka

Prof. V.P. Kukhar

Institute of Organic Chemistry, NAS of Ukraine  
(Synthesis)

Dr. S.A. Cherenok

Dr. A.B. Drapailo

Prof. V.I. Kalchenko

ChemBio Center

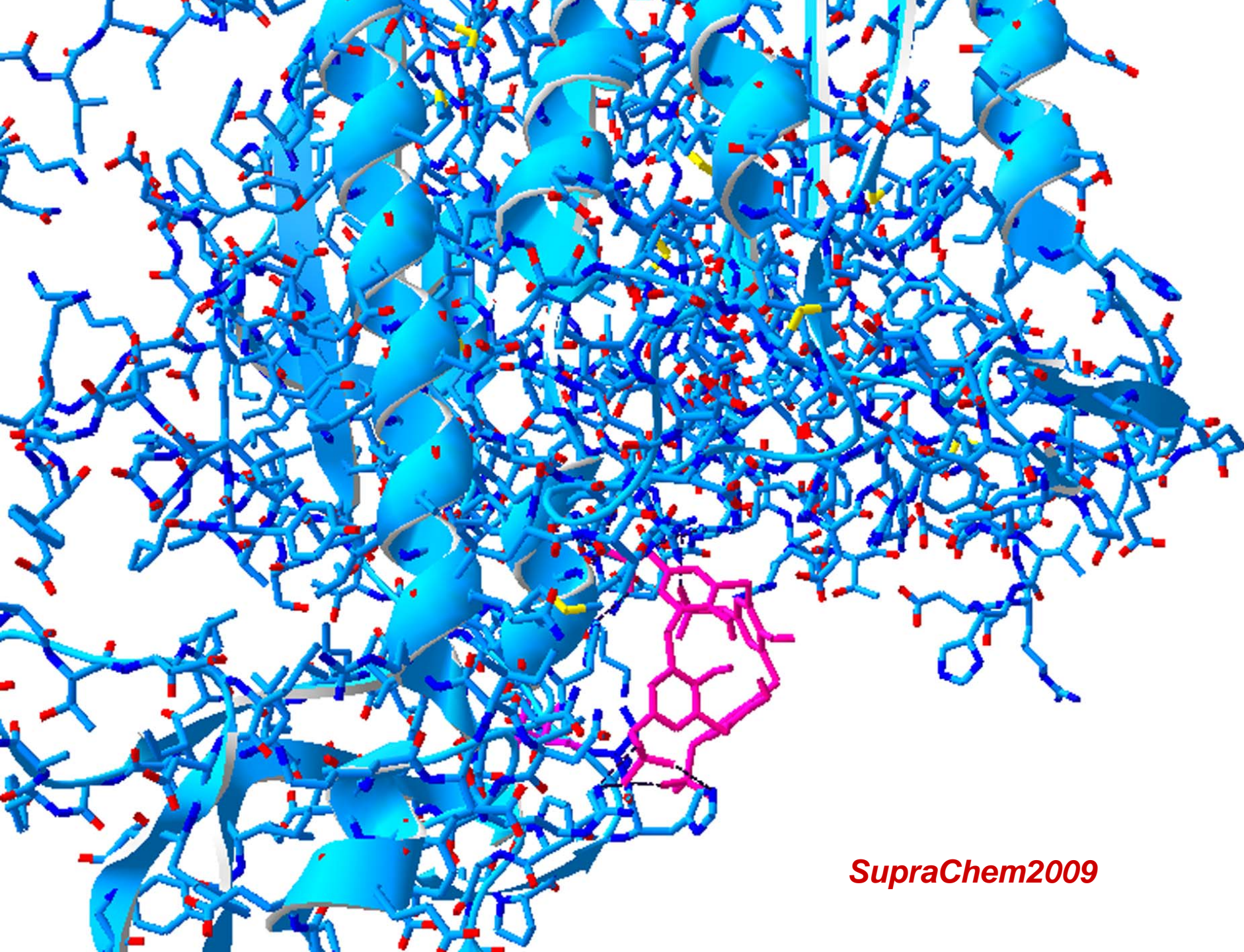
(Synthesis)

Dr. A.M. Shivanyuk

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