# INHIBITION OF CARBONIC ANHYDRASE ISOZYMES I AND II WITH THIOPHOSPHORYLIC COMPOUNDS CONTAINING 4-CARBOXYBENZENE-SULFONAMIDO MOIETIES [1]

Andrea Scozzafava[1], Fabrizio Briganti[1], Claudiu T. Supuran[1]*, Ioan Fenesan[2], Rodica Popescu[2], Viorica Muresan[2] and Sorin Farcas[2]

[1] Università degli Studi, Laboratorio di Chimica Inorganica e Bioinorganica, Via Gino Capponi 7, I-50121, Firenze, Italy
[2] Institute of Chemistry "Raluca Ripan", Laboratory of Element-organic Compounds, Cluj-Napoca, Roumania

**Abstract:** A series of P(V) compounds possessing 4-carboxybenzenesulfonamido moieties in their molecule was studied for inhibition of the zinc enzyme carbonic anhydrase (CA), isozymes I and II. The studied derivatives inhibit both isozymes for the 4-nitrophenylacetate hydrolysis reaction catalyzed by the enzymes, and change the spectrum of Co(II)-substituted CA, proving a direct interaction between the inhibitor and the metal center within the protein. Structure-activity correlations are also discussed for the new inhibitors.

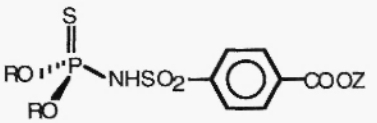
## Introduction

Inhibition of the zinc enzyme carbonic anhydrase (CA, EC 4.2.1.1) with P(V) derivatives has been recently reported.[2,3] Derivatives of types **1-4** behaved as weak inhibitors for both $CO_2$ hydration as well as 4-nitrophenyl acetate hydrolysis reactions catalyzed by CAs, but only compounds **1** were tested for inhibition of the last reaction.[2,3]

A-NH-CH(Z)-$PO_3H_2$ $R_2P(=S)NHSO_2Ar$ $RP(=X)(NHSO_2Ar)_2$ $RP(=S)(NR'_2)(NHSO_2Ar)$

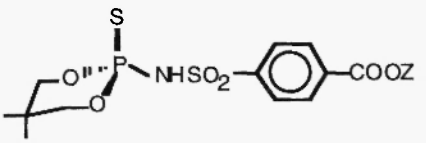
A = $PhCH_2OCO$: COOMe
Z = H, Me, *i*-Pr, $PhCH_2$
R, R' = alkyl, cycloalkyl, Ph; X = O,S
Ar = 4-substituted-phenyl

**1** **2** **3** **4**




**5** **a** R = Et, Z = H
**b** R = Et, Z = Me

**6** **a** R = Ph, Z = H
**b** R = Ph, Z = Me




**7** **a** Z = H
**b** Z = Me

Taking into account our recent report[3] that compounds **2-4** act as CA II inhibitors, as well as the fact that inhibitors possessing substituted sulfonamido moieties were relatively little studied,[2-5] we decided to investigate the interaction of compounds of type 5-7 with different CA isozymes. In this paper we report the synthesis, characterization and enzymatic inhibition studies with the P(V) derivatives **5-7**. These new compounds were characterized by standard procedures and were investigated for inhibition of two isozymes, CA I and CA II, as well as for their interaction with Co(II)-substituted CA. The main conclusion of this study is that this class of inhibitors binds to the metal center within CA active site.

## Materials and Methods

IR spectra were recorded with a Specord 75IR (Carl Zeiss, Jena) instrument in KBr pellets, $^1$H-NMR spectra in DMSO-$d_6$ as solvent, with a Varian Gemini 300FT spectrometer operating at 7 T. Chemical shifts are expressed as δ values, relative to $Me_4Si$ as internal standard. Mass spectra (MS) were obtained on a MAT 311 spectrometer operating at 70 eV, with the electron emmission of 100 μA and the ion source temperature

of 150°C. Elemental analysis were obtained by combustion with a Carlo Erba apparatus.

Inhibitors **5b-7b** were prepared from the methyl ester of 4-carboxybenzenesulfonamide[6] (from Aldrich) and the appropriate thiophosphoryl chloride.[7-9] Compounds **5a-7a** were obtained by hydrolysis of the corresponding esters **5b-7b**.

Plasmids of human CA I and CA II were expressed in the *lon Escherichia coli* strain SG20043, and the enzymes were purified thereafter by affinity chromatograhy, as described by Forsman et al.[10] Enzyme concentrations were determined spectrophotometrically, at 280 nm, considering $\varepsilon = 54{,}700\ M^{-1}\ cm^{-1}$ and the molecular weight of 30,000, or at 450 nm for the Co(II)-substituted enzyme ($\varepsilon = 250\ M^{-1}\ cm^{-1}$, at pH 6.0).[11,12] All buffers used in the kinetic measurements were brought to an ionic strength $\mu = 0.1$, by addition of $K_2SO_4$. Cobalt(II)-CA II was prepared by the method of Hunt et al.[13], by removing zinc from the native enzyme in the presence of 50 mM pyridine-2,6-dicarboxylic acid, followed by dialysis against metal-free Tris-$H_2SO_4$ buffer, and addition of the stoichiometric amount of Co(II) salt.

Initial rates of 4-nitrophenyl acetate hydrolysis were monitored spectrophotometrically, at 400 nm and 25°C, with a Cary 3 apparatus interfaced with an IBM compatible PC, accordingly to the method of Pocker and Stone.[14] Solutions of substrate were prepared in anhydrous acetonitrile; the substrate concentrations varied between $10^{-2}$ and $10^{-4}$ M. A molar absorption coefficient $\varepsilon = 18400\ M^{-1}.cm^{-1}$ was used for the 4-nitrophenolate formed by hydrolysis, in the conditions of the experiments (pH 7.80), as reported by Pocker and Stone.[14] Non-enzymatic hydrolysis rates were always subtracted from the observed rates. Duplicate experiments were done for each inhibitor, and the values reported throughout the paper are the averages of such results.

The acid-base behavior of derivatives **5a,b** was investigated by potentiometric titration, in order to determine their $pK_a$ values. A Metrolim (Switzerland) glass electrode was used and the measurements were done in aqueous methanol solution (MeOH-water 1:9, v/v).

*General procedure for the preparation of compounds* **5b-7b**

An amount of 20 mMoles of thiophosphoryl chloride **8** (diethoxy-[6], diphenoxy-[7] and 2,2-dimethyl-1,3-propylenedioxythiophosphoryl chlorides[8], respectively) and 20 mMoles of the sodium salt of 4-carboxymethyl-benzenesulfonamide **9**, were placed in a reaction flask equiped with mechanical stirrer and protected from moisture, and 20 mL of DMF were added. The mixture was stirred at 80-90 °C for 3 hours, then the cold reaction mixture was taken up in 100 mL of saturated aqueous $NaHCO_3$ solution, and the undissolved material filtered off (this consisted of unreacted starting compounds). Compounds **5b-7b** were precipitated by addition of concentrated HCl to the filtrate. Pure compounds were obtained by recrystallization from hexane/1-propanol (3/1, v/v). Yields were in the range of 30-40%.

*General procedure for the preparation of compounds* **5a-7a**

10 mMoles of methylesters **5b-7b** were treated with 10 mL of 25% aqueous NaOH solution and heated at 90 °C for 3 hours. The cooled mixture was treated with concentrated HCl and the obtained precipitates were filtered off. Recrystallization as above afforded pure derivatives **5a-7a**.

Compound **5a**: m.p. 191-192°C; IR(KBr), $cm^{-1}$: 625 (P=S), 1175 ($SO_2$ sym.), 1385 ($SO_2$ asym), 1690 (COOH), 3160 (NH); $^1$H-NMR (DMSO-$d_6$), δ, ppm: 1.10 (t, 6H, 2Me), 3.85 (m, 4H, $2CH_2$), 7.90 (d (AB), 2H, ArH), 8.10 (d (AB), 2H, ArH), $J_{AB}$ = 8 Hz. MS, m/z: 289 (M-$SO_2$, 48 %). Analysis, found: C, 37.46; H, 4.62; N, 4.09; P, 8.62%. $C_{11}H_{16}NO_6PS_2$ (353) requires: C, 37.39; H, 4.56; N, 3.96; P, 8.76 %. $pK_{a1}$ = 3.95; $pK_{a2}$ = 6.99.

Compound **5b**: m.p. 105-106°C; IR(KBr), $cm^{-1}$: 635 (P=S), 1176 ($SO_2$ sym.), 1380 ($SO_2$ asym), 1715 (COOMe), 3160 (NH); $^1$H-NMR (DMSO-$d_6$), δ, ppm: 1.20 (t, 6H, 2Me), 3.65 (s, 3H, Me from COOMe), 4.10 (m, 4H, $2CH_2$), 7.90 (d (AB), 2H, ArH), 8.10 (d (AB), 2H, ArH), $J_{AB}$ = 8 Hz. MS, m/z: 303 (M-$SO_2$, 46 %). Analysis, found: C, 39.40; H, 5.01; N, 3.92; P, 8.53%. $C_{12}H_{18}NO_6PS_2$ (367) requires: C, 39.22; H, 4.94; N, 3.81; P, 8.43 %. $pK_a$ = 4.09.

Compound **6a**: m.p. 188-189°C; IR(KBr), $cm^{-1}$: 625 (P=S), 1175 ($SO_2$ sym.), 1385 ($SO_2$ asym), 1690 (COOH), 3160 (NH); $^1$H-NMR (DMSO-$d_6$), δ, ppm: 7.10-7.30 (m, 10H, 2Ph), 7.90 (d (AB), 2H, ArH), 8.10 (d (AB), 2H, ArH), $J_{AB}$ = 8 Hz. MS, m/z: 339 (M-PhSH, 25 %). Analysis, found: C, 50.49; H, 3.35; N, 3.42; P, 6.99%. $C_{19}H_{16}NO_6PS_2$ (449) requires: C, 50.78; H, 3.59; N, 3.12; P, 6.89 %.

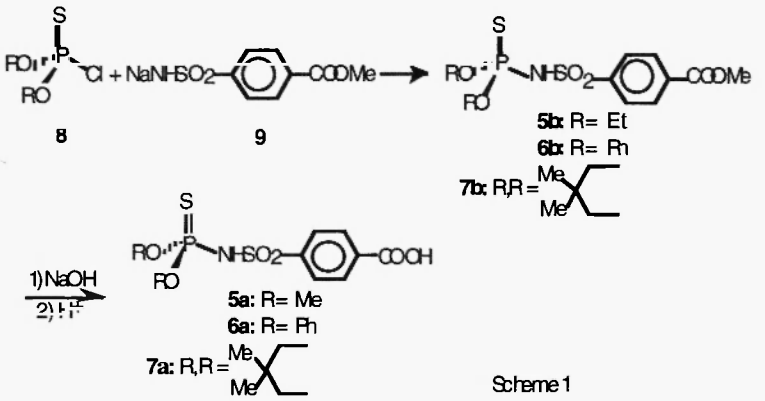
Compound **6b**: m.p. 138-139°C; IR(KBr), $cm^{-1}$: 640 (P=S), 1176 ($SO_2$ sym.), 1375 ($SO_2$ asym), 1715 (COOMe), 3160 (NH); $^1$H-NMR (DMSO-$d_6$), δ, ppm: 3.65 (s, 3H, Me from COOMe), 7.10-7.30 (m, 10H, 2Ph), 7.90 (d (AB), 2H, ArH), 8.10 (d (AB), 2H, ArH), $J_{AB}$ = 8 Hz. MS, m/z: 353 (M-PhSH, 100 %). Analysis, found: C, 52.01; H, 4.03; N, 3.26; P, 6.39%. $C_{20}H_{18}NO_6PS_2$ (463) requires: C, 51.83; H, 3.91; N, 3.02; P, 6.68 %.

Compound **7a**: m.p. 225 °C (dec.); IR(KBr), $cm^{-1}$: 625 (P=S), 1175 ($SO_2$ sym.), 1385 ($SO_2$ asym), 1690 (COOH), 3160 (NH); $^1$H-NMR (DMSO-$d_6$), δ, ppm: 0.95 (s, 6H, 2 Me), 3.90-4.00 (m, 4H, $2CH_2$), 7.90 (d (AB), 2H, ArH), 8.10 (d (AB), 2H, ArH), $J_{AB}$ = 8 Hz. MS, m/z: 301 (M- $SO_2$, 10 %). Analysis, found: C, 39.60; H, 3.92; N, 4.10; P, 8.09%. $C_{12}H_{16}NO_6PS_2$ (365) requires: C, 39.44; H, 4.14; N, 3.83; P, 8.42 %.

Compound **7b**: m.p. 225 °C (dec.); IR(KBr), $cm^{-1}$: 625 (P=S), 1175 ($SO_2$ sym.), 1385 ($SO_2$ asym), 1715 (COOMe), 3160 (NH); $^1$H-NMR (DMSO-$d_6$), δ, ppm: 0.95 (s, 6H, 2 Me from the 5,5-dimethyl-1,2,3-dioxaphosphorinanic ring), 3.65 (s,3H, Me from COOMe), 3.90-4.00 (m, 4H, $2CH_2$), 7.90 (d (AB), 2H, ArH), 8.10 (d (AB), 2H, ArH), $J_{AB}$ = 8 Hz. MS, m/z: 315 (M- $SO_2$, 100 %). Analysis, found: C, 41.05; H, 5.06; N, 3.88; P, 7.89%. $C_{13}H_{18}NO_6PS_2$ (365) requires: C,41.15; H, 4.78; N, 3.69; P, 8.16 %.

## Results and Discussion

Derivatives **5b-7b** were prepared by the nucleophilic substitution of the chlorine atom from thiophosphoryl chlorides **8** with the sodium salt of 4-carboxymethyl-benzenesulfonamide **9**, at 80-90°C, working in highly polar aprotic solvents.[15,16] Methyl esters **5b-7b** were thereafter hydrolyzed in alkaline medium and derivatives **5a-7a** were obtained after addition of hydrochloric acid (Scheme 1).




Scheme 1

The new derivatives **5-7** were characterized by standard procedures (IR and $^1$H-NMR spectroscopy, mass spectrometry and elemental analysis - see *Materials and Methods* for details). These data confirmed the proposed structures of the new compounds. Thus, in the IR spectra of all derivatives **5-7** the P=S vibrations were detected at 625-640 $cm^{-1}$, the two $SO_2$ vibrations in the range 1175-1180 $cm^{-1}$, and 1385-1390 $cm^{-1}$, respectively, the NH stretching vibration in the region 3100 - 3200 $cm^{-1}$, , whereas the ν(C=O) band was detected at 1690 $cm^{-1}$ for the carboxylic acids **5a-7a**, and 1715 $cm^{-1}$ for the methyl esters **5b-7b**. No S-H stretching vibrations were observed in the region 2200 - 2500 $cm^{-1}$ in the IR spectra of these derivatives.[15,16]

In the $^1$H-NMR spectra of the new compounds the signals of each moiety was detected in its normal range,[16,17] whereas in their mass spectra it is to note that molecular ions were not present, but fragmentation processes typical for this kind of P(V) occurred.[18-21] Thus, for derivatives **5** and **7**, $SO_2$ elimination was observed (which occurs after an intramolecular transposition, and then the M-$SO_2$ ion is detected).[18,19] For the diphenoxy-derivatives **6**, another specific behavior[20,21] was evidenced: after a thione-thiol isomerisation, the corresponding molecular ions eliminate a thiophenol molecule, and the M-PhSH ions are obtained. Elemental analysis data for the new derivatives were within ± 0.4 % of the theoretical values calculated for the proposed formulae.

Inhibition of isozymes CA I and II with the synthesized derivatives **5-7** is shown in Table I.

As seen from the above data, all P(V) derivatives behave as stronger inhibitors than the sulfonamides from which they derive (**9** and **10**), towards both investigated CA isozymes. Methyl esters were more active than

the corresponding free acids. On the other hand, the other moieties substituting the phosphorus atom greatly influenced the activity. Thus, the diethoxy derivatives **5** were more active than the compounds **7** containing the 5,5-dimethyl-1,2,3-dioxaphosphorinanic ring, which in turn were more active than the diphenoxy-substituted inhibitors **6**. This is obviously correlated with the volume of these groups substituting the phosphorus atom: the more compact they are, the corresponding compounds inhibit more strongly the two CA isozymes. As for other classes of compounds interacting with CA,[4,5,22] it seems that the most effective inhibitors in this series of P(V) derivatives should contain both hydrophobic as well as hydrophylic moieties in their molecule, which assure a favorable interaction with amino acid side chains within the active site, stabilizing the enzyme-inhibitor adducts. On the other hand, CA II is more susceptible to inhibition by this class of compounds, as compared to CA I.

Table I: CA inhibition with compounds **5-7**. For comparison, data for 4-carboxybenzenesulfonamide **10** and its methyl ester **9** are also included. $IC_{50}$ represents the molarity of inhibitor producing a 50% decrease of enzyme specific activity for 4-nitrophenyl acetate hydrolysis.

| Inhibitor | $IC_{50}$ (μM) | |
|---|---|---|
| | CA I[a] | CA II[b] |
| **5a** | 74 | 4.5 |
| **5b** | 55 | 1.6 |
| **6a** | 110 | 10.5 |
| **6b** | 98 | 8.7 |
| **7a** | 87 | 8.1 |
| **7b** | 62 | 3.9 |
| **9** | 119 | 18 |
| **10** | 145 | 29 |

[a][CA I] = 8 μM, in 10 mM Hepes buffer, pH 7.8;
[b][CA II] = 1.3 μM, in 10 mM Hepes buffer, pH 7.8.

In the previous report regarding the interaction of CA II with P(V) derivatives,[3] it was shown that the structurally-related derivatives **2-4** act as non-competitive inhibitors with the physiological substrate of the enzyme, $CO_2$, and we hypothesized that they are probably directly bound to the Zn(II) ion within th enzyme active site, similarly to the unsubstituted sulfonamides. A direct confirmation of the above hypothesis was obtained in the present work, by studying the interaction of compounds of type **2-7,10** with Co(II)-substituted CA II.

The Co(II) ion is a good spectroscopic probe,[12,23-25] and Co(II)CA has very characteristic electronic, NMR and EPR spectra, which are highly sensitive to the environment around the metal ion, constituting an easy approach for studying the interaction of this enzyme with inhibitors, activators or substrates.[23-26]

In Table II the electronic spectral data for adducts of Co(II)CA II with inhibitors derivatives of P(V), of type **2-7**, (and the sulfonamide **10** for comparison) are shown.

As shown by earlier studies of Bertini's group,[12,23-25] four bands are seen in the electronic spectrum of Co(II)CA II in the region 400-750 nm, which are highly pH dependent and sensitive to the environment around the metal ion. Unsubstituted sulfonamides, such as acetazolamide (5-acetylamino-1,3,4-thiadiazole-2-sulfonamide, a clinically used CA inhibitor[4]) which directly bind in ionized form to the metal ion,[4,27] lead to intense electronic spectra, characterized primarily by molar absorptivities over 300 $M^{-1}.cm^{-1}$ and the shift of the two bands from 616.5 and 640 nm in the spectrum of the pure enzyme, to wavelengths under 600 nm.[12] This is just the type of spectrum seen for the adduct of Co(II)CA II with 4-carboxy-benzenesulfonamide **10**, shown in Table II. It was in fact envisageable that this simple aromatic sulfonamide would bind in a similar way to heterocyclic derivatives such as acetazolamide or other of its congeners for which the crystal structures were recently reported.[28,29] Thus, the P(V) derivatives of type **2-4** previously reported as CA inhibitors,[3] as well as **5-7** for which this action was reported here, seem to bind in a similar manner to the unsubstituted sulfonamide **10**, as judged from the similarities of the electronic spectra of these adducts (Table II). Practically the first two bands in these electronic spectra (those around 520 and 545 nm) underwent slight modifications in the spectra of the P(V) adducts as compared to the spectra of the pure enzyme (except for the fact that they are much more intense), whereas the last two bands undergo major changes: shifts under 600

Table II: Electronic spectral data (in the range 400-750 nm) for adducts of Co(II)CA II with inhibitors **2-7** and **10**. Enzyme concentrations were in the range 0.5-1.2 mM and pH values specified in each case. Inhibitor concentrations were in the range 1.0-1.5 mM.

| Adduct | pH | Band position ,nm (molar absorptivity [$M^{-1}$ x $cm^{-1}$]) |
|---|---|---|
| pure enzyme | 6.0 | 520 (180); 550 (250); 616.5 (135); 640 (100) |
| pure enzyme | 8.0 | 520 (280); 550 (380); 616.5 (280); 640 (260) |
| **2** (R=EtO; Ar=4-$H_2N$-$C_6H_4$) | 7.6 | 520 (275); 575 (380); 600 sh (300) |
| **3** (R=EtO; Ar=4-$MeC_6H_4$; X=S) | 7.6 | 520 (290); 575 (350); 595 sh (270) |
| **4** (R=Ph; R'=Et; Ar=4-$MeC_6H_4$) | 7.6 | 519 (290); 574 (335); 597 sh (280) |
| **5a** | 7.6 | 521 (320); 545 (200); 572 (500); 595 sh (450) |
| **5b** | 7.6 | 520 (300); 545 (210); 570 (520); 597.5 sh (445) |
| **5b** | 5.8 | 519 (200); 547 (210); 570 (380); 596 sh (250) |
| **6b** | 7.6 | 520 (290); 545 (210); 570 (510); 595 sh (450) |
| **7b** | 7.6 | 520 (310); 547 (220); 570 (500); 600 sh (470) |
| **10** | 7.6 | 519 (350); 545 (210); 575 (520); 595 sh (500) |

nm and an even greater intensification. The nature of the substitution at the carboxyl moiety in derivatives **5-7** seems not to influence this binding, as the methyl esters **5b-7b** and the free acid **5a** had quite similar spectra in their adducts with the cobalt-enzyme. For the most active derivative, **5b**, the spectrum was also recorded at different pH values, showing that binding does not change significantly in the pH range 5.5 - 8.0. As the $pK_a$ of this compound is 4.09, it is clear that in the pH range in which the binding was studied, this compound is in the anionic form $(RO)_2P(=S)N^-$—Ar.

The increased CA inhibitory properties of the phosphoryl-substituted sulfonamides of type **2-7** is probably also due to the acidifying effect of the (thio)phosphorylic group upon the $SO_2NH$ proton. As a consequence, the formation of the conjugated base, responsible for binding to the enzyme is favoured.

In conclusion, this study reports the synthesis, characterization and CA inhibitory properties of P(V) derivatives possessing carboxybenzenesulfonamide moieties in their molecules. The obtained derivatives are stronger inhibitors than the unsubstituted sulfonamides from which they were prepared and they bind in ionized form to the metal ion within the enzyme active site, as shown by spectroscopic measurements on the Co(II)-substituted CA.

**Acknowledgments**: This research was financed in part by the EU grant ERB CIPD CT 940051.

## References

1. This paper is Part 39 of the series *Carbonic Anhydrase Inhibitors*.
2. G.Osapay and A. Csiba, *Eur.J.Med.Chem.*, **1993**, 28, 355-361.
3. C.T.Supuran, V.Muresan, R.Popescu and I.Fenesan, *Main Group Met.Chem.*,**1995**, *18*, 629-632.
4. C.T.Supuran, in "*Carbonic anhydrase and modulation of physiologic and pathologic processes in the organism*", I.Puscas Ed., Helicon, Timisoara, **1994**, pp. 29-111.
5. C.T.Supuran, I Saramet and M.D. Banciu, *Rev.Roum.Chim.*, **1995**, *40*, 1227-1232.
6. Y.M.Beasley, B.G.Overell, V.Petrov and O.S.Stephenson, *J.Pharm.Pharmacol.*, **1958**, *10*, 696-710.
7. N.N.Melnikov, Y.A.Mandelbaum and P.G.Zaks, *Zh.Obshch.Khim.*, **1959**, *29*, 522-526.
8. L.Almasi, A.Hantz and I.Felmery, *Rev.Chimie (Bucharest)*, **1959**, *6*, 332-334.
9. R.S.Edmundson, *Tetrahedron*, **1967**, *23*, 1701-1711.
10. C.Forsman, G.Behravan, A.Osterman and B.H.Jonsson, *Acta Chem.Scand.B*, **1988**, *42*, 314-318.
11. Z.Liang, Y.Xue, G.Behravan, B.H.Jonsson and S.Lindskog, *Eur.J.Biochem.*, **1993**, *211*, 821-827.
12. I.Bertini, G.Canti, C.Luchinat and A.Scozzafava, *J.Am.Chem.Soc.*, **1978**, *100*, 4873-4877.
13. J.B.Hunt, M.J.Rhee and C.B.Storm, *Anal.Biochem.*, **1977**, *79*, 614-617.
14. Y.Poker and J.T.Stone, *Biochemistry*, **1967**, *6*, 668-679.
15. L.Almasi, R.Popescu and R.Grecu, *Tetrahedron*, **1977**, *33*, 1327-1332.
16. I.Fenesan, V.Muresan, A.Barabas and M.Bodan, *Rev.Roum.Chim.*, **1984**, *29*, 761-768.

17. A.Barabas, R.Popescu, V.Muresan and L.Almasi, *Org.Magn.Resonance*, **1977**, *10*, 35-38.
18. Z.Moldovan, S.Nicoara, M.Culea, O.Cozar, I.Fenesan, P.Vegh and J.J.Rios, *J.Molec.Struct.*, **1995**, *348*, 393-396.
19. R.Popescu, I.Opreanu, N.Palibroda and Z.Moldovan, *Studia Univ. Babes-Bolyai, Chemia*, **1989**, *34*, 93.
20. Z.Moldovan, M.Culea, N.Palibroda, R.Popescu, V.Muresan and O.Musat, *Sci.Total Environm.*, **1993**, *132*, 147-154.
21. R.G.Cooks and A.F.Gerrard, *J.Chem.Soc., B*, **1968**, 1327-1329.
22. C.T.Supuran and B.W.Clare, *Eur.J.Med.Chem.*, **1995**, *30*, 687-696.
23. I.Bertini, C.Luchinat and A.Scozzafava, *Struct.Bonding*, **1982**, *48*, 45-97.
24. I.Bertini and C.Luchinat, *Acc.Chem.Res.*, **1983**, *16*, 272-282.
25. I.Bertini, C.Luchinat, R.Pierattelli and A.V.Vila, *Inorg.Chem.*, **1992**, *31*, 3975-3979.
26. F.Briganti, R.Pierattelli, A.Scozzafava and C.T.Supuran, *Eur.J.Med.Chem.*, submitted.
27. A.Liljas, K.Hakansson, B.H.Jonsson and Y.Xue, *Eur.J.Biochem.*, **1994**, *219*, 1-10.
28. J.Vidgren, A.Liljas and N.P.C.Walker, *Int.J.Biol.Macromol.*, **1990**, *12*, 342-344.
29. J.Vidgren, A.Svensson and A.Liljas, *Int.J.Biol.Macromol.*, **1993**, *15*, 97-100.

**Received: April 19, 1996 - Accepted: May 15, 1996 -**
**Received in revised camera-ready format: May 17, 1996**