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Synthesis and Evaluation *in vitro* Effects of Some Macrocyclic Thiacrown Ethers on Erythrocyte Carbonic Anhydrase I and II

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A new series of macrocyclic thiacrown ethers (**A1-6**) were synthesized. These compounds were investigated as erythrocyte carbonic anhydrase I and II, which had been purified by Sepharose-4B-L-tyrosine-sulfonamide affinity gel. These ethers showed inhibition effect for human carbonic anhydrase I and interestingly, behaved as an activator for human carbonic anhydrase II. IC_{50} values of the compound that caused inhibition for human carbonic anhydrase I were determined by means of activity percentage diagrams. IC_{50} values for macrocyclic thiacrown ethers (**A1**), (**A2**), (**A3**), (**A4**), (**A5**) and (**A6**) were determined as 1.22, 1.61, 2.11, 1.66, 0.84 and 1.45 mM respectively. Thus macrocyclic thiacrown ether (**A5**) was by far the most effective inhibitor.

Key Words: Carbonic anhydrase, Enzyme inhibitor, Macro cyclic thiacrown ethers.

INTRODUCTION

The metalloenzyme carbonic anhydrase (CA, EC 4.2.1.1) catalyzes a very simple but critically important physiological reaction *i.e.*, the involvement of the carbonic anhydrase enzyme family, which catalyzes the physiological hydration of CO_2 to yield bicarbonate and a proton, in many physiological/pathological processes open up widespread opportunities for the development of diverse, specific inhibitors for clinical application[1,2].

The formation of thiacrown macrocyclics containing a carbonyl group has also been reported[3-6]. The synthesis of macrocyclic ether-esters compounds[7], a wide variety of polyether-diester compounds including ether-esters[8-14], thioether-esters[10,12,14,15] and ether thialesters[10], was prepared by Bradshaw, Izattand Christensen by coupling of either dibasic acid salts and α,ω-dihalo compounds or dibasic acid chlorides and α,ω-dihydroxy compounds. Two macrocyclic polyether-monoester compounds have been reported by Matsushima *et al.*[16] in a moderate yield. Edema and his colleagues [17,18] have realized diketo functionalized thiacrown ethers in 38-57 % yields[19,20].

In the present study we have synthesized some macrocyclic thiacrown ethers for evaluation as potential inhibitors of human carbonic anhydrase I and human carbonic anhydrase II.

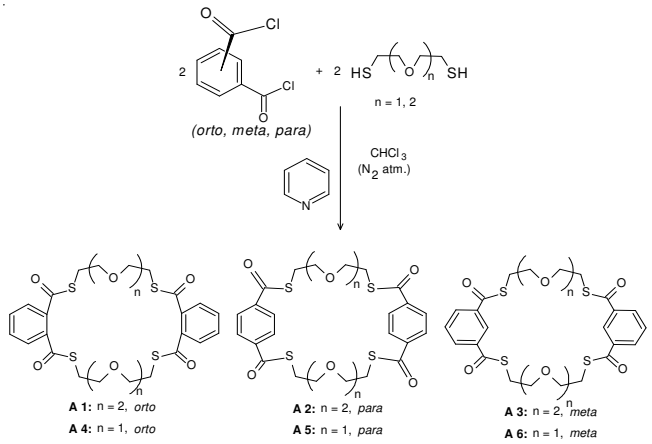
EXPERIMENTAL

Sepharose 4B, L-tyrosine, sulphonamide, protein assay reagents and chemicals for electrophoresis were obtained from Sigma Chem. Co. All other chemicals used were of analytical grade and obtained from either Sigma or Merck.

Synthesis and chemical characterization: In this study a new series of new macrocyclics thiacrown ethers were synthesized from di achly phthaloyl dichloride and 2,2'-(ethylenedioxy)diethanethiol and 2-mercaptoethyl ether. The compounds prepared with pyridine as base on nitrogen atmosphere in chloroform[18]. The reactions are given in **Scheme-I**, the yields and melting points of the compounds are given in Table-1. All compounds characterized by H NMR, FT-IR and GC-MS IRand MS. In the infrared spectra of compounds, it was possible to observe the absorption 680 cm^{-1} relating to C-S-C stretch, absorptions in between 1700-1720 cm^{-1} relating to O=C-S stretch and absorptions in between 1630-1640 cm^{-1} relating to aromatic C-C stretch. The ^1H NMR spectra for all the synthesized all compounds show signals between 2.4 and 2.45 ppm relating to hydrogens attached to the (C-S-C=O). The signals for aromatic hydrogens are between 6.55 and 8.15 ppm.

Melting points were taken on a Elektrotermal 9200 melting point apparatus. IR spectra were measured on a Perkin Elmer Spektrum 100 FT-IR spectrometer. ^1H NMR spectra were measured on spectrometer at Varian 400 MHz. Mass spectra were obtained using Shimadzu GSMS-QP2010 spectrometer. Column chromatography was performed using Merck silica gel 60 (230-400 mesh ASTM). Solvents were dried following standard methods. All chemicals was purchased from Merck, Alfa Easer, Sigma-Aldrich and Fluka.

Synthesis of thiacrown ethers: The *o*-phthaloyl dichloride, *p*-phthaloyl dichloride, *m*-phthaloyl dichloride (10 mmol), 2,2'-(ethylenedioxy) diethanethiol or 2-mercaptoethyl ether (10 mmol) and pyridine (20 mmol) were dissolved in chloroform. These solutions were stirred and heated under reflux for 24 h on N_2 atmosphere. The solutions were evaporated then purified by chromatography on silica gel with *n*-hexane-chloroform as eluant.



Compound	n	m.p. (ºC)	Yield (%)
A 1	2	85-86	48
A 2	1	77-78	78
A 3	2	80-81	68
A 4	1	75-76	44
A 5	2	68-69	52
A 6	1	75-76	46

Scheme-I

Spectral data

7,8,10,11,13,14,23,24,26,27,29,30-Dodecahydrodibenzo [i,w][1,4,15,18,7,12,21,26]tetraoxa tetrathiacyclooctacosine-5,16,21,32-tetrone (A1): Yield 48 %; m.p. 85-86 ºC; ^{1}H NMR ($CDCl_3$, 400 MHz) δ/ppm: 2.62 ppm (2H, t, C-S-C=O), 3.6 ppm (2H, s, C-O-C) 4.25 ppm (4H, t, -C-C-S-C=O), 8.0 (H, d, o-benzo), 8.9 (H, d, p-benzo); FT-IR (γ cm^{-1}) 680 (C-S-C stretch), 1719 (O=C-S stretch), 1635 (aromatic C=C stretch); GS-MS (m/z, M^+) :625,80.

6,9,22,25-tetraoxa-3,12,19,28-tetrathiatricyclo [28.2.2.2^{14,17}] hexatriaconta1(32),14,16, 30,33,35 heksaen-2,13,18,29-tetrone (A2): Yield 78 %; m.p. 77-78 ºC; ^{1}H NMR ($CDCl_3$, 400 MHz) δ/ppm: 2.45 ppm (2H, t, C-S-C=O), 3,20 ppm (4H, t, -C-C-S-C=O), 3.50 ppm (2H, s, C-O-C) 7.80 (H, s, benzo); FT-IR (γ cm^{-1}) 680 (C-S-C stretch), 1716 (O=C-S stretch), 1634 (aromatic C=C stretch); GS-MS (m/z, M^+): 624,80.

6,9,23,26-tetraoxa-3,12,20,29-tetrathiatricyclo [29.3.1.1^{14,18}]hexatriaconta-1(35),14(36),15, 17, 31, 33- hexaene-2,13,19,30-tetrone (A3): Yield 68 %; m.p. 80-81 ºC; ^{1}H NMR ($CDCl_3$, 400 MHz) δ/ppm: 2.42 ppm (2H, t, C-S-C=O), 3.4 ppm (2H, s, C-O-C) 4.1 ppm (4H, t, -C-C-S-C=O), 7.25 (H, s, o-benzo), 8 (H, d, p-benzo) 8.9 (H, d, p-benzo); FT-IR (γ cm^{-1}) 680 (C-S-C stretch), 1722(O=C-S stretch), 1635 (aromatic C=C stretch); GS-MS (m/z, M^+) :624,30.

7,8,10,11,20,21,23,24-octahydrodibenzo [f,q][1,12,4,9, 15,20]dioxatetrathia-cyclodocosine-5,13,18,26-tetrone (A4): Yield 44 %; m.p. 75-76 ºC; ^{1}H NMR ($CDCl_3$, 400 MHz) δ/ppm: 3.45 ppm (2H, t, C-S-C=O), 4.20 ppm (2H, s, C-O-C), 7.40 (H, d, o-benzo), 7.60 (H, d, p-benzo); FT-IR (γ cm^{-1}) 680 (C-S-C stretch), 1715 (O=C-S stretch), 1635 (aromatic C=C stretch); GS-MS (m/z, M^+) :624,30.

6,19-dioxa-3,9,16,22-tetrathiatricyclo [22.2.2.2^{11,14}] triaconta-1(26),11,13,24,27,29- hexaene- 2,10,15,23-tetrone (A5): Yield 52 %; m.p. 68-69 ºC; ^{1}H NMR ($CDCl_3$, 400 MHz) δ/ppm: 3.35 ppm (2H, t, C-S-C=O), 4.20 ppm (2H, s, C-O-C), 7.80 (H, s, benzo); FT-IR (γ cm^{-1}) 680 (C-S-C stretch), 1718(O=C-S stretch), 1633 (aromatic C=C stretch); GS-MS (m/z, M^+) :536,70.

6,20-dioxa-3,9,17,23-tetrathiatricyclo[23.3.1.1^{11,15}] triaconta-1(29),11(30),12,14,25,27-hexaene-2,10,16,2-tetrone (A6): Yield 46 %; m.p. 75-76 ºC; ^{1}H NMR ($CDCl_3$, 400 MHz) δ/ppm:.80 ppm (2H, t, C-S-C=O), 4.10 ppm (2H, s, C-O-C), 7.25 (H, s, o-benzo), 7.80 (H, d, p-benzo) 8.30 (H, d, p-benzo); FT-IR (γ cm^{-1}) 680 (C-S-C stretch), 1720 (O=C-S stretch), 1634 (aromatic C=C stretch); GS-MS (m/z, M^+) :536,70.

Carbonic anhydrase enzyme assay: Carbonic anhydrase activity was measured by the Maren method which is based on determination of the time required for the pH to decrease from 10.0 to 7.4 due to CO_2 hydration[21,22]. Human CA I and II were purified from red blood cells according to the method of Ozensoy *et al.*[23,24].

***In vitro* inhibition studies:** For the inhibition studies of ethers different concentrations of these compounds were added to the enzyme activity. Activity % values of carbonic anhydrase for different concentrations of each compound were determined by regression analysis using Microsoft Office 2000 Excel. Carbonic anhydrase enzyme activity without a compound solution was accepted as 100 % activity. For the compounds having an inhibition affect, the inhibitor concentration causing up to 50 % inhibition (IC_{50} values) was determined from the graphs.

RESULTS AND DISCUSSION

In this study, carbonic anhydrase I and II isoenzymes from human erythrocytes were purified by a simple one step procedure by using Sepharose 4B-L-tirozin-sulfanilamide affinity column. The activity of the eluents was determined. The inhibitory affects of some macro cyclic thiacrown ethers on human cytosolic carbonic anhydrase I and II activity were investigated. Different inhibition effects of the applied these compounds were obtained and showed in Table-1. Compound (5) has been shown to be the strongest inhibitor against the hCA I activity while all compounds cause the activation on hCA II activity. We have determined the IC_{50} values of 0.84-2.11 mM for the inhibition of hCA I activity.

TABLE-1
IC_{50} VALUES OF THIOCROWN ETHERS ON hCA I AND hCA II

A1	1.22 mM	activated
A2	1.61 mM	activated
A3	2.11 mM	activated
A4	1.66 mM	activated
A5	0.84 mM	activated
A6	1.45 mM	activated

Human carbonic anhydrase-I and human carbonic anhydrase-II enzyme active sites is similar to a certain extent. Interaction of these compounds is a contradiction reveals a completely different way. This situation can be explained by differences in amino acid sequences of isoenzymes. The same compounds also interact with different groups can be considered outside the enzyme active site. However, this issue must be more powerful to use expressions necessary to determine the mechanisms of inhibition. These compounds work done by us for the first time in this study the effects on isoenzymes, no data was found in the literature for comparison. However, when compared with sulphonamides, hCA-I against whom a much lower interest were found. The synthesized compounds to inhibit only one isoenzyme are an extremely important finding. Because of this situation just think that the contribution of the design of isoenzymes-specific compounds.

Declaration of interest: The authors report no conflicts of interest.

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