COMMUNICATION

A C G publications

Org. Commun. 12:x (2019) xx-xx

organic communications

Microvawe assisted synthesis of N-(methyl and methoxy) benzylidene-4-fluoroaniline derivatives and their carbonic anhydrase I and II inhibition properties

Hulya Celik[1*] and Müslüm Kuzu[2,3]

[1]*Agri Ibrahim Cecen University, Faculty of Pharmacy, Department of Pharmaceutical Technology, Agri, Türkiye*

[2]*Agri Ibrahim Cecen University, Faculty of Science, Department of Biochemistry, Agri, Türkiye*

[3]*Karabuk University, Faculty of Health Sciences, Department of Nutrition and Dietetics, Karabuk, Türkiye*

(Received July, 10, 2019; Revised September 15, 2019; Accepted October 23, 2019)

Abstract: In this study, some Schiff base derivatives (**6a-f**) were synthesized using a microwave method. The synthesized compounds were characterized by 1D, 2D NMR and mass spectral data. Their inhibitory effects were studied on carbonic anhydrase isozymes (hCA I and II), purified from human erythrocyte cells by Sepharose-4B-L-tyrosine-sulfanilamide affinity chromatography and the compounds *N*-3-Methylbenzylidene-4-fluoroaniline (**6b),** *N*-4-Methylbenzylidene-4-fluoroaniline (**6c)**, *N*-2-Methoxybenzylidene-4-fluoroaniline (**6d**) showed moderate activity on hCAII in the range of IC_{50} values.

Keywords: Schiff base; carbonic anhydrase; microbial; N-benzylideneaniline.

1. Introduction

Schiff bases, synthesized by Hugo Schiff for the first time in 1864, are the most common compounds used in organic chemistry. Aldehyde- or ketone-like compounds in which the carbonyl group is replaced by an azomethine (anil or imine) group are called Schiff bases.[2] Schiff bases are used in diverse applications and fields, including the design of medical compounds, pharmacology and pharmaceuticals, organic chemistry, paints, plastics, the perfume industry, catalysis and materials science, enzymatic reactions, biochemistry, photochromism, measurement of the intensity of radiation, optical computers, the electronics industry, image systems, physicochemistry, and as spectrophotometric reagents used in analytical chemistry.[3,4]

* Corresponding author: E-Mail: hycelik@agri.edu.tr, Phone: + 05459391196.

The article was published by ACG Publications
www.acgpubs.org/organic-communications October-December 2019 EISSN:1307-6175
DOI: http://doi.org/10.25135/acg.oc.66.19.07.1327

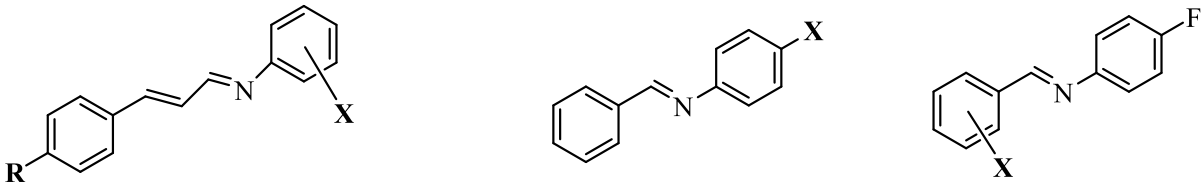
In biological systems, Schiff bases have shown antiproliferative[5], antipyretic[6], antibacterial[7], antifungal[8], antimicrobial[9,10], anticonvulsant[11,12], anti-HIV[13,14], antiinflammatory[15], antitumor[16], antiviral[17], and anticancer[18,19] activities that are worth examining further.

2. Background

Microwave technology was first used by Gedye in 1986 in organic synthesis.[20] Microwave reactions under solvent-free conditions allow for the direct and fast heating of materials with low cost, less pollution, and faster reactions while offering high efficiency and ease of use.[21,22]

Carbonic anhydrase (CA, EC 4.2.1.1) is a metalloenzyme including Zn^{2+} in the active region, found in all living species, which catalyzes the reversible conversion of carbon dioxide to bicarbonate and H^+.[23] This reaction plays a critical role in many physiological processes associated with metabolic pathways, including carbon dioxide.[24] Therefore, the regulation of enzyme activity has emerged as an approach in the treatment of diseases such as neuropathic pain,[25] glaucoma, edema, idiopathic intracranial hypertension,[26] cerebral ischemia,[27] cancer,[28] and epilepsy.[29] The synthesis and discovery of new carbonic anhydrase inhibitors are therefore of great importance. Previous studies have reported that some Schiff bases have an effect on carbonic anhydrase enzyme activity.[30] For this reason, the effects of synthesized Schiff base derivatives on the activities of CA-I and CA-II enzymes purified from human erythrocytes were investigated. In addition, in vitro antibacterial properties of the compounds were also determined.[30]

We have also recently synthesized, eight different Schiff bases with structure **1** derived from cinnamaldehyde and p-methoxycinamaldehyde by microwave irradiation and studied their inhibitory activities on hCA-I and hCA-II isoenzymes. We determined most of compounds to have stronger inhibitory effect comparable with acetazolamide, the used reference and mild antibacterial activity. [32]



1: R=CH_3, OCH_3; X=F,Cl, Br **2: X**=, F, NO_2 **3: X**=, F, Cl, Br

Figure 1. Representative Schiff bases synthesized by our research group in our previous papers

In our previous study, nine N-benzylidene-4-fluoro(nitro)anilines with structures **2** were synthesized and their effects on carbonic anhydrase isoenzymes (hCA-I and hCA-II) were investigated. These study showed that the two compounds have a strong inhibitory effect on the hCA-I and hCA-II isoenzymes.[33] Furthermore, nine Schiff bases derived halogen substituted benzaldehydes and 4-fluoroaniline with structures **3** were synthesized and the data of *in vitro* antibacterial poteintials are reported.[34]

3. Experimental

3.1. Chemical material and apparatus

2-Methylbenzaldehyde (**4a**), 3-methylbenzaldehyde (**4b**), 4-methylbenzaldehyde (**4c**), 2-methoxybenzaldehyde (**4d**), 3-methoxybenzaldehyde (**4e**), 4-methoxybenzaldehyde (**4f**), and 4-fluoroaniline (**5**) are commercially available (Merck, Sigma-Aldrich) and were used without further purification. All other chemicals were of analytical grade and obtained from Merck. Reactions were monitored via thin-layer chromatography (TLC). ^{1}H NMR and ^{13}C NMR spectra were recorded on a 400 (100) MHz Varian spectrometer using $CDCl_3$. Melting points were determined on a capillary melting apparatus (BUCHI 530) and are uncorrected. An MD 20 DB-DG Vestel microwave oven was used (230 V-50 Hz, 1050 W).

*3.2. General synthesis of Schiff bases **6a-f***

4-Fluoroaniline (**5**) (1 mmol) was added to 2-methylbenzaldehyde (**4a**), 3-methylbenzaldehyde (**4b**), 4-methylbenzaldehyde (**4c**), 2-methoxybenzaldehyde (**4d**), 3-methoxybenzaldehyde (**4e**), or 4-methoxybenzaldehyde (**4f**) (1 mmol), and then the reaction mixture was exposed to microwave radiation at 900 W. The progress of the reaction was monitored by TLC (runner phase: n-hexane and ethyl acetate, 4:1). It was determined that the reactions were completed in 5 minutes for all aromatic aniline derivatives.
^{1}H NMR and ^{13}C NMR spectra of the residue showed the formation of compounds (**6a-f**) as sole compounds.

*(E/Z)- N-2-Methylbenzylidene-4-fluoroaniline (**6a**):* Yield: 92%, yellow solid, m.p. 106-108 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04,8.02 (two, s, N=CH, *J* = 7.7 Hz), 7.39 (t, 1xArH, *J* = 7.5 Hz), 7.23 (t, 1xArH, *J* = 7.1 Hz), 6.90-6.74 (m, 4xArH), 6.62 (dd, 2xArH, *J* = 8.8, 4.5 Hz), 2.63 (s, Me). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.3, 157.9, 156.0, 141.9, 141.3, 132.9, 131.9, 131.6, 131.1, 128.6, 127.8, 126.5, 125.9, 116.6 (d, J_{CF}=8 Hz), 115.7(d, J_{CF}=22 Hz), 22.1. HRMS(MH^+) calcd. for $C_{14}H_{13}FN$ (**6a**): *m/z:* 214.1032; found *m/z:* 214.1043.

*N-3-Methylbenzylidene-4-fluoroaniline (**6b**)*[35]: Yield: 93%, colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.37(s, N=CH), 7.72 (s, 1xArH), 7.63 (d, *J* = 7.5 Hz, 1H), 7.34 (t, 1xArH, *J* = 7.5 Hz), 7.27 (d, *J* = 7.5 Hz, 1H), 7.17 (dd, 2xArH, *J* = 8.9, 5.0 Hz), 7.05 (t, 2xArH, J_{HFC}= 8.6 Hz). 2.40 (s, Me) ^{13}C NMR (101 MHz, $CDCl_3$) δ 161.2 (d, *J* = 244 Hz), 160.4 (CFN), 148.5,139.0, 136.0, 132.3, 129.2, 128.2, 126.6, 122.2, 115.7, 21.2. HRMS(MH^+) calcd. for $C_{14}H_{13}FN$ (**6b**): *m/z:* 214.1032; found *m/z:* 214.1043.

*N-4-Methylbenzylidene-4-fluoroaniline (**6c**):* Yield: 94%, yellow solid, m.p. 70 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.34 (s, N=CH), 7.75 (d, 2xArH, *J* = 8.0 Hz), 7.23 (d, 2xArH, *J* = 7.9 Hz), 7.15 (dd, 2xArH, *J* = 8.8, 5.0 Hz), 7.03 (t, 2xArH, $^{3}J_{HF}$ = 8.6 Hz), 2.37 (s, CH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ 161.4 (d, J_{CF} = 210 Hz) 160.3 (CH=N), 148.3, 142.1, 133.5, 129.3, 129.0, 122.4, 115.7, 21.6. ^{1}H and ^{13}C NMR data is agreement with data given in the literature.[36] HRMS(MH^+) calcd. for $C_{14}H_{13}F$ N (**6c**): *m/z:* 214.1032; found 214.1047.

*N-2-Methoxybenzylidene-4-fluoroaniline (**6d**)*[35]: Yield: 93%, colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.90 (s, N=CH), 8.12 (d, 1xArH, *J* = 8.0 Hz), 7.43 (t, 1xArH, *J* = 7.8 Hz), 7.20 (dd, 2xArH, *J* = 8.7, 5.0 Hz), 7.04 (dd, 1xArH, *J* = 16.2, 7.9 Hz), 6.94 (d, 1xArH, *J* = 8.3 Hz), 3.88 (s, OCH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ 161.1(d, J_{CF} = 274 Hz), 159.5 (CH=N), 159.3, 156.6, 148.7, 132.8, 127.3, 125.0, 124.8, 122.9, 120.8, 115.9, 111.2, 55.6 (OCH_3). HRMS(MH^+) calcd. for $C_{14}H_{13}FNO$ (**6d**): *m/z*: 230.0981; found *m/z:* 230.0994.

*N-3-Methoxybenzylidene-4-fluoroaniline (**6e**)*[37]*:* Yield: 92%, colorless oil.^{1}H NMR (400 MHz, $CDCl_3$) δ 8.38 (s, N=CH), 7.49 (d, 1xArH, *J* = 1.1 Hz), 7.41-7.32 (m, 2xArH), 7.18 (dd, 2xArH, *J* = 8.9, 5.0 Hz), 7.09-7.01 (m, 3xArH), 3.86 (s, OCH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ 161.6, (d, J_{CF} = 208 Hz), 160.6 (CH=N), 148.4, 138.0, 129.7, 122.1, 118.6, 116.2, 112.3, 56.4. HRMS(MH^+) calcd. for $C_{14}H_{13}FNO$ (**6e**): *m/z:* 230.0981; found *m/z:* 230.0992.

*N-4-Methoxybenzylidene-4-fluoroaniline (**6f**):* Yield: 91%, m.p. 74 °C, yellow solid, Lit[38]. m.p.70-72 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.35 (s, N=CH), 7.83 (d, 2xArH, *J* = 8.7 Hz), 7.17 (dd, 2xArH, *J* = 8.9, 5.0 Hz), 7.06 (t, 2xArH, $^{3}J_{HF}$ = 8.7 Hz), 6.98 (d, 2xArH, *J* = 8.8 Hz), 3.86 (s, OCH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ 161.1(d, J_{CF}=260 Hz), 159.5(CH=N), 148.3, 130.5, 122.9, 114.4, 55.3. HRMS(MH^+) calcd. for $C_{14}H_{13}FNO$ (**6f**): *m/z:* 230.0981; *m/z:* found 230.0995.

3.3. Purification of carbonic anhydrase and kinetic studies

Human erythrocytes were hemolyzed with iced distilled water and centrifuged at 4 °C and 20000 x g for 30 minutes. The obtained supernatant was applied to a Sepharose 4B-L-tyrosine-sulfanilamide affinity column in accordance with a previously described procedure, and the CA-I and CA-II enzymes were separately eluted.[39] The esterase activity of the CA enzyme was used in enzyme activity measurements. The activity measurements were performed spectrophotometrically at 348 nm according to the method described by Verpoorte and colleagues.[40] In order to investigate the in vitro effects of the synthesized substances on enzyme activity, measurements were made for at least five different compound concentrations and saturated substrate concentrations. The concentration amount that caused 50% inhibition (IC_{50}) for each compound was determined by drawing a % activity-compound concentration graph by taking 100% for the control measurement as in previous studies.[41-43]

3.4. In vitro antibacterial activity

Studies of antibacecteral activities of **6a-f** are given in supporting information of this article.

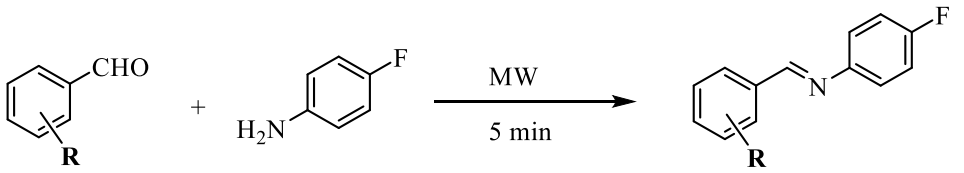
4. Present Study

In vitro antibacterial activities of the synthesized compounds were tested using the agar-well diffusion assay (AWDA) as previously described[44] at the Antimikrop R & D and Biocidal Analysis Center (Ankara, Turkey). The detail of strains and method are given in supporting information.

In this study, we present facile synthesis of six Schiff bases (**6a-f**) derived from 4-fluoroaniline and methyl or methoxy substituted benzaldehydes and their inhibitory effect on the hCA-I and hCA-II isoenzymes (Scheme 1).

As shown in Scheme 1, the preparation Schiff bases (6a-f) were readily and efficiently prepared via micowve irradiation. All ^{1}H NMR, ^{13}C NMR and HRMS data of the prepared compounds were good agreement with the structures.

In their preparation and characterization, the in vitro effects of synthesized compounds **6a-f** on CA-I and CA-II activities were investigated. For this purpose, activity measurements were conducted at five different concentrations of the compounds and a saturated substrate concentration. According to the results, an activity % versus compound concentration graph was drawn. IC_{50} values were calculated with the help of the obtained graph and the results are summarized in Table 1.[45]



	R
4a	2-CH_3
4b	3-CH_3
4c	4-CH_3
4d	2-OCH_3
4e	3-OCH_3
4f	4-OCH_3

5

	R
6a	2-CH_3
6b	3-CH_3
6c	4-CH_3
6d	2-OCH_3
6e	3-OCH_3
6f	4-OCH_3

Scheme 1. The synthesis route of compounds **6a-f**

Accordingly, all substances showed inhibition effects on both CA-I and CA-II. However, the inhibition effect of all substances was higher for the CA-II enzyme (Table 1, Figures S1 and S2).

On the other hand, among the compounds of interest, **6b** showed the strongest inhibitory effect on both CA-I and CA-II. As is understood from the results, the different groups in the molecular structure

and the different positions of the same groups cause these compounds to act differently on the CA-I and CA-II enzyme activities.

It has been reported that CA inhibition can be used in the treatment of diseases such as edema, cancer, infectious diseases, seizures, and glaucoma, and many CA inhibitor classes and inhibition mechanisms have been determined for this purpose.[26] In particular, it has been reported that CA-I is involved in brain and retinal edema while CA-II is involved in pathological conditions such as epilepsy, edema, and glaucoma, and inhibition of these enzymes can be a treatment method.[42] In previous studies, some Schiff base derivatives were synthesized and their inhibitory properties were reported in terms of CA enzyme activity.[30] In this study, we have determined the CA inhibitor properties of some new Schiff base derivatives, which can be synthesized by an easy, inexpensive, and quick method. The *in vitro* antibacterial activities of the synthesized compounds were evaluated and none of the compounds showed biological activity against tested microbial strains (see supporting information Table S1).

Table 1. IC_{50} values of the compounds against hCA-I and hCA-II

Compounds	**hCA-I**		**hCA-II**	
	IC_{50} (µM)	***r*²**	**IC_{50} (µM)**	***r*²**
6a	1165	0.97	746.27	1
6b	7.43	0.97	3.41	0.92
6c	27.73	0.86	8.77	0.98
6d	16.5	0.95	5.73	0.97
6e	43.32	0.99	21	0.94
6f	35.92	0.99	14.44	0.96
Acetazolamide	1.17	0.94	0.98	0.97

As a conclusion, Schiff bases have extensive biological applications in the pharmaceutical field. Microwave irradiation provides significant improvement over existing conventional procedures and reveal a novel method that with short reaction times, excellent efficiency and without formation of undesirable side products for the synthesis of Schiff bases **6a-f**. In addition, their effects on CA-I and CA-II enzymes were determined. The results showed that the compound **6b** showed the strongest inhibitory effect on both CA-I and CA-II.

Acknowledgments

The authors thanks Agri Ibrahim Cecen University and Department of Chemistry at Ataturk University for their use of facilities in research. They especially also thanks Barış Anıl for his taking NMR spectra, Dr. Haydar Kilic for his taking HRMS and Neslihan Celebioglu for her critical reading.

Supporting Information

Supporting information accompanies this paper at http://www.acgpubs.org/journal/organic-communications

ORCID

Hülya Çelik: 0000-0003-0805-0523
Müslüm Kuzu: 0000-0002-1375-7673

References

[1] Schiff, H. Untersuchungen Uber Salicin Derivate. *Ann. Chem.Pharm.* **1864**, *131*, 118-119.

[2] da Silva, C.M.; da Silva, D.L.; Modolo, L.V.; Alves, R.B.; de Resende, M.A.; Martins, C.V.B.; de Fatima, A. Schiff bases: a short review of their antimicrobial activities. *J. Adv. Res*. **2011**, *2*, 1–8.

[3] Dubey, S.N.; Vaid, B. K. Synthesis and Reactivity in Inorganic and Metal-Organic Chemistry. Rani, A., Kumar, M.; Khare, R.; Tuli,H.S. Schiff bases as an antimicrobial agent: A review. *J. Biol.Chem. Sci. (JBCS)*. **2015**, *1*, 62-69.

[4] Kajal, A.; Bala, S.; Kamboj, S.; Sharma, N.; Saini V. Schiff Bases: A Versatile Pharmacophore.. *J. Catal.* **2013**,Article ID *893512*, 14 pages.

[5] Sztanke, K.; Maziarka, A.; Osinka, A.; Sztanke, M. An insight into synthetic Schiff bases revealing antiproliferative activities in vitro. *Bioorg. Med. Chem.* **2013,** *21*, 3648-3666.

[6] Murtaza, S.; Akhtar, M.S.; Kanwal, F.; Abbas, A.; Ashiq S.; Shamim, S. Synthesis and biological evaluation of Schiff bases of 4-aminophenazone as an anti-inflammatory, analgesic and antipyretic agent. *J. Saudi Chem. Soc.* **2017**, *21*, 359-372.

[7] Pandeya, S. N.; Sriram, D. Synthesis, antibacterial, antifungal and anti-HIV activities of norfloxacin Mannich bases. *Acta. Pharm. Turc*. **1998**, 40, 33-38.

[8] Zhao, Q. J.; Song, Y.; Hu, H. G.; Yu, S. C.; Wu, Q. Y. Design, synthesis and antifungal activity of novel triazole derivatives. *Chinese Chem. Lett.* **2007**, *18*, 670-672.

[9] [9] Kumar, S.; Niranjan, M. S.; Chaluvaraju, K. C.; Jamakhandi, C. M.; Kadadevar, D. Synthesis and Antimicrobial Study of Some Schiff Bases of Sulfonamides. *J. Curr. Pharm. Res.* **2010**, 1, 39-42.

[10] Invidiata, F. P.; Furno, G.; Simoni, D.; Lampronti, I.; Musiu, C.; Milia, C.; Scintu, F.; La Colla, P. 3,6-Disubstituted 1,2,4-triazolo[3,4-b][1,3,4]thiadiazoles: synthesis and evaluation for antimicrobial and antiviral activity. *Farmaco* **1997**, *52*, 259-261.

[11] Chen, J.; Sun, X. Y.; Chai, K. Y.; Lee, J. S.; Song, M. S.; Quan, Z. S. Synthesis and anticonvulsant evaluation of 4-(4-alkoxylphenyl)-3-ethyl4H-1,2,4-triazoles as open-chain analogues of 7-alkoxyl-4,5dihydro[1,2,4]triazolo[4,3-a]quinolones. *Bioorg. Med. Chem.* **2015**, *15*, 6775-6781.

[12] Varma, M.; Pandeya, S. N.; Singh, K. N.; Stables J. P. Anticonvulsant activity of Schiff bases of isatin derivatives. *Acta. Pharm.* **2004**, *54*, 49-56.

[13] Pandeya, S. N.; Sriram, D.; Nath, G.; De Clercq, E. Synthesis, antibacterial, antifungal and anti-HIV activities of norfloxacin Mannich bases. *Eur. J. Med. Chem*. **2000**, *35*, 249-255.

[14] Akhtar, T.; Hameed, S.; Al-Masoudi, N. A.; Khan, K. M. Synthesis and anti-HIV activity of new chiral 1,2,4-triazoles and 1,3,4-thiadiazoles. *Heteroatom Chem.* **2007**, 18, 316-322.

[15] Turan-Zitouni, G.; Kaplancikli, Z. A.; Ozdemir, A.; Chevallet, P.; Kandilci, H. B.; Gumusel, B. Studies on 1,2,4- Triazole Derivatives as Potential Anti- Inflammatory Agents. *Arch. Pharm.* **2007**, 340, 586-590.

[16] Hodnett, E. M.; William, J.; Dunn, W. J. Structure-antitumor activity correlation of some Schiff bases. *J. Med. Chem*. **1970**, *13*, 768-770.

[17] Holla, B. S.; Veerendra, B.; Shivananda, M. K.; Boja, P. Synthesis characterization and anticancer activity studies on some Mannich bases derived from 1,2,4-triazoles. *Eur. J. Med. Chem.* **2003**, *38*, 759-767.

[18] Varma, R. S.; Nobles, W. L. Antiviral, antibacterial, and antifungal activities of isatin n-Mannich bases. *J. Pharm. Sci*. **1975**, *64*, 881-882.

[19] Bekircan, O.; Kahveci, B.; Kucuk, M. Synthesis and anticancer evaluation of some new unsymmetrical 3,5-diaryl-4H-1,2,4-triazole derivatives. *Turk. J. Chem*. **2006**, *30*, 29-40.

[20] Jacob, J. Microwave assisted reactions in organic chemistry: A review of recent advances. *Int. J. Chem.* **2012**, *4*, 29-43.

[21] Kappe, C. O.; Stadler, A. *Microwaves Organic and Medicinal Chemistry* (Book). 1st. ed. Werlag-WCH, Weinheim, **2006**

[22] Kappe, C.O.; Dallinger, D. The impact of microwave synthesis on drug discovery. *Nat. Rev. Drug Discov*. **2006**, *5*, 51-63.

[23] Kuzu, M.; Ozkaya, A.; Sahin, Z.; Dağ, U.; Comakli, V.; Demirdag, R. In vivo effects of naringenin and lead on rat erythrocyte carbonic anhydrase enzyme. *Turk. J. Pharm. Sci.* **2017**, *14*, 9-12.

[24] Tanini, D.; Capperucci, A.; Supuran, C.T.; Angeli, A. Sulfur, selenium and tellurium containing amines act as effective carbonic anhydrase activators. *Bioorg. Chem.* **2019**, *87*, 516-522.

[25] Angeli, A.; di Cesare Mannelli, L.; Lucarini, E.; Peat, T.S.; Ghelardini, C.; Supuran C.T. Design, synthesis and X-ray crystallography of selenides bearing benzenesulfonamide moiety with neuropathic pain modulating effects. *Eur. J. Med. Chem.* **2018**, *154*, 210-219.

[26] Supuran, C. T. Advances in structure-based drug discovery of carbonic anhydrase inhibitors. *Drug Discov.* **2017**, *12*, 61-88.

[27] Di Cesare Mannelli L.; Micheli, L.; Carta, F.; Cozzi, A.; Ghelardini, C.; Supuran, C.T. Carbonic anhydrase inhibition for the management of cerebral ischemia: in vivo evaluation of sulfonamide and coumarin inhibitors. *J Enzyme Inhib. Med. Chem.* **2016**, *31*, 894-899.
[28] Supuran, C.T.; Winum, J.Y. Carbonic anhydrase IX inhibitors in cancer therapy: an update. *Future Med. Chem*. **2015**, *7*,1407-1414.
[29] Aggarwal, M.; Kondeti, B.; McKenna, R. Anticonvulsant/antiepileptic carbonic anhydrase inhibitors: a patent review. *Ther. Pat.* **2013**, *23*, 717-724.
[30] Alyar, S.; Sen, C.; Alyar, H.; Adem, S.; Kalkanci, A.; Ozdemir, U. O. Synthesis, characterization, antimicrobial activity, carbonic anhydrase enzyme inhibitor effects, and computational studies on new Schiff bases of sulfa drugs and their Pd(II), Cu(II) complexes. *J. Mol. Struct.* **2018**, *1171*, 214-222.
[31] Celik, H.; Maman, M.; Babagil, A. hCA isoenzyme inhibitory activities of Schiff bases derived from cinnamaldehyde and p-methoxycinnamaldehyde. *Fresenius Environ. Bull.* **2018**, *27*, 4469-4482.
[32] Celik, H.; Maman, M.; Babagil, A. Cinnamaldehyde and pmethoxycinnamaldehyde derived Schiff bases antibacterial activities. *Int. J. Sci. Eng. Res.* **2018**, *9*, 640-651.
[33] Celik, H.; Babagil, A. Microwave-assisted synthesis of N-benzylidene-4-fluoroaniline and N-benzylidene 4-Nitroaniline and their inhibitory activities on hCA isoenzymes. *Int. J. Sec. Metabol.* **2019**, *6*, 38-48.
[34] Celik, H. Microwave assisted synthesis and antibacterial activities of some n-benzylideneaniline halogen derivatives. *Fresenius Environ. Bull.* **2019**, *28*, 5367-5381.
[35] Landge, S. M.; Atanassova, V.; Thimmaiah, M.; Török, B. Microwave-assisted oxidative coupling of amines to imines on solid acid catalysts. *Tetrahedron Lett.* **2007,** *48*, 5161 – 5164
[36] Murray, A. T.; King, R.; Donnelly, J. V. G.; Dowley, M. J. H.; Tuna, F.; Sells, D.; John, M. P.; Carbery, D. R. Symbiotic Transition-Metal and Organocatalysis for Catalytic Ambient Amine Oxidation and Alkene Reduction Reactions. *ChemCatChem* **2016**, *8*, 510-514.
[37] Miglani, S.; Mishra, M.; Chawla, P. The rapid synthesis of Schiff-bases without solvent under microwave irradiation and their antimicrobial activity. *Der Pharm. Chem.* **2012**, *4*, 2265-2269.
[38] Alcaide, B.; Mardomingo, C. L.; Plumet, J.; Cativiela, C.; Mayoral, J. A. Orbital control in the 1,3-dipolar cycloaddition of benzonitrile oxide to benzylideneanilines. *Canadian J. Chem.* **1987**, *65*, 2050-2056.
[39] Kuzu, M.; Comakli, V.; Akkemik, E.; Ciftci, M.; Kufrevioğlu, O. I. Inhibitory properties of some heavy metals on carbonic anhydrase I and II isozymes activities purified from Van Lake fish (Chalcalburnus Tarichi) gill. *Fish Physiol. Biochem.* **2018**, *44*, 1119-1125.
[40] Verpoorte, J.; Mehta, S.; Edsall, J. T. Esterase activities of human carbonic anhydrases B and C. *Biol. Chem.* **1967**, 242, 4221-4229.
[41] Akyol, H.; Kuzu, M. In vitro effects of some heavy metal ions on cytosolic thioredoxin reductase purified from rainbow trout gill tissues. *Fresenius Environ. Bull.* **2016**, *27*, 4677-4683
[42] Biçer, A.; Taslimi, P.; Yakalı, G.; Gülçin, I.; Gültekin, M. S.; Cin, G. T. Synthesis, characterization, crystal structure of novel bis-thiomethylcyclohexanone derivatives and their inhibitory properties against some metabolic enzymes. *Bioorg. Chem.* **2019**, *82*, 393-404.
[43] Wzgarda-Raj, K.; Rybarczyk-Pirek, A. J.; Wojtulewski, S.; Pindelska, E.; Palusiak, M. *Struct. Chem.* **2019**, *30*, 827-833.
[44] Holder, I. A.; Boyce, S.T. Agar well diffusion assay testing of bacterial susceptibility to various antimicrobials in concentrations non-toxic for human cells in culture. *Burns* **1994**, *20*, 426-429.
[45] Cetin A.; Türkan, F.; Taslimi, P.; Gulcin, I. Synthesis and characterization of novel substituted thiophene derivatives and discovery of their carbonic anhydrase and acetylcholinesterase inhibition effects. *J. Biochem. Mol. Toxicol.* **2019,** *33*, e22261, 8 pages.