MICROWAVE ASSISTED SOLVENT-FREE MANNICH BASES: SYNTHESIS, CHARACTERIZATION AND EFFECTS OF THESE COMPOUNDS ON hCA I AND hCA II ISOZYMES

Bülent Büyükkidan¹, Nurgün Büyükkidan¹, Metin Bülbül², Melek Yılmaz¹,
Evren Derrun Arslanbay¹, Ekrem Tunca²

¹Department of Chemistry, Faculty of Arts and Sciences, Kütahya Dumlupınar University, 43100 Kütahya, Turkey
²Department of Biochemistry, Faculty of Arts and Sciences, Kütahya Dumlupınar University, 43100 Kütahya, Turkey
bulent.buyukkidan@dpu.edu.tr

Mannich bases (2a–d) of aromatic amines were synthesized by using a conventional microwave technique under solvent-free conditions and characterized by IR and NMR (¹H and ¹³C) and elemental analysis. The inhibitory effects of the synthesized Mannich bases were examined in vitro by using hydratase and esterase assays on carbonic anhydride I and II isozymes (hCA, EC 4.2.1.1) purified from human erythrocyte cells. Acetazolamide was used as the control compound. The values of IC₅₀, the half-maximum inhibitory concentration, were found for hydratase and esterase activities. Only two compounds (2b and 2d) exhibited poor hCA I and hCA II inhibition effects on esterase activity. In contrast, compounds 2a and 2c could be used as carbonic anhydrase activators as a result of the increased esterase activity of hCA I and hCA II isozymes.

Keywords: activator; carbonic anhydrase; glaucoma; isozyme

1. INTRODUCTION

Carbonic anhydrase enzymes (CAs, EC 4.2.1.1) control pH in most tissues, including erythrocytes [1–3]. Recently, CAs have been extensively studied due to their potential applications in the prevention and treatment of a large number of diseases [4–6]. Sixteen isozymes have been described in the literature as members of the α-CA family [7–9]. Several of these isozymes (hCA I, hCA II and hCA IV) are found in human eyes and cause gradual loss of vision due to an elevation of intraocular
pressure (IOP), called glaucoma [10–13]. Since CA inhibitors lower the IOP, these compounds have been used for glaucoma treatment [14, 15].

Reactions involving amines and amino acid derivatives have been the most researched studies on the complexing of zinc, the active site of CAs [16–22]. The Mannich reaction involves condensation of ammonia, primary amines or secondary amines with formaldehyde and compounds containing at least one active hydrogen atom. This work would be a good example of the synthesis of Mannich compounds from amine derivatives by the Mannich reaction. Recently, chemists have been interested in reducing the environmental pollution caused by chemical solvents and developing eco-compatible synthetic methods [23–25]. Mannich bases have a wide range of pharmacological uses and exhibit important antifungal, antibacterial, antitumoural and anti-inflammatory effects [26, 27] and antituberculous properties [29].

We present an economical, environmentally friendly, solvent-free, one-step method for the reactions of four novel Mannich compounds (2a–d) using microwave irradiation (Fig. 1). Furthermore, we have investigated the inhibition effect of these compounds on human carbonic anhydrase (hCA I and hCA II) isozymes.

The structures of compounds 2a–d were assigned by elemental analysis and IR and NMR (1H and 13C) spectroscopy. In addition, the inhibitory effects of 2a–d were investigated, using acetazolamide (AAZ) as control compound.

2. EXPERIMENTAL

2.1. Materials

The reagents were used without purification. NMR spectra of the solution in CDCl₃ were recorded on a Bruker Avance DPX-300 instrument. A Leco CHNS-932 instrument was used for elemental analysis and IR studies were performed by Bruker Optics and Vertex 70 FT-IR. Reactions were carried out using a microwave oven manufactured by BEKO (1200 W, 2450 MHz).

2.2. Synthesis of N-(2-fluorophenyl)-N-o-tolylmethanediamine (2a) and N-(4-fluorophenyl)-N-o-tolylmethanediamine (2b)

Formaldehyde (4 mmol) was added dropwise to a solvent-free mixture of 2-methylphenylamine (4 mmol) and fluoro-substituted phenylamine (2-fluorophenylamine for 2a and 4-fluorophenylamine for 2b) (4 mmol) and the mixture irradiated by microwave at 200 W. Ethanol was used to wash the solid mixture and the residue was dried in air.

2a: Reaction time 45 min; (77%); m.p. 153 ºC; 1H NMR (300 MHz, CDCl₃) δ ppm: 6.81–6.74 (m, 8H, ArH), 4.78 (s, 2H, N–CH₂), 2.21 (s, 3H, ArCH₃); 13C NMR (300 MHz, CDCl₃) δ ppm: 152.12, 140.21, 132.35, 129.36, 127.28, 126.14, 119.96, 117.37, 114.76 and 110.32 (aromatic carbons), 70.46 (CH₂), 13.87 (CH₃); IR (cm⁻¹): 3469, 3374 (NH), 3069 (Ar–CH₃), 2968, 2831 (aliphatic –CH), 1628, 1608, 1470, 1458 (C=C); Anal. Calcd. %, for C₁₄H₁₃FN₂ (Mᵣ = 230.28): C 73.07, H 6.60, N 12.16; found: C 73.07, H 6.61, N 12.21.

2b: Reaction time 55 min; (72%); m.p. 142 ºC; 1H NMR (300 MHz, CDCl₃) δ ppm: 6.89–6.81 (m, 8H, ArH), 4.81 (s, 2H, N–CH₂), 2.22 (s, 3H, ArCH₃); 13C NMR (300 MHz, CDCl₃) δ ppm: 511.91, 148.23, 145.72, 128.76, 124.65, 119.32, 115.01, 108.45 (aromatic carbons), 69.21 (CH₂), 24.61 (CH₃); IR (cm⁻¹): 3375, 3270 (NH), 3070 (CH), 2933, 2882 (CH₁), 1638, 1603, 1502, 1451 (C=C); Anal. Calcd. %, for C₁₄H₁₃FN₂ (Mᵣ = 230.28): C 73.02, H 6.57, N 12.16; found: C 73.05, H 6.61, N 12.23.

2.3. Synthesis of N,N'-bis(2,3-dimethylphenyl)methanediamine (2c)

Formaldehyde (4 mmol) was added dropwise to the 2,3-dimethylphenylamine (8 mmol). The solvent-free reaction mixture was irradiated by microwave at 200 W. Ethanol was used to wash the solid mixture and the residue dried in air.

2c: Reaction time 50 min; (82%); m.p. 138 ºC; 1H NMR (300 MHz, CDCl₃) δ ppm: 6.54–6.48 (m, 6H, ArH), 4.72 (s, 2H, N–CH₂), 2.74 (s, 6H, ArCH₃), 2.21 (s, 6H, CH₂); 13C NMR (300 MHz, CDCl₃) δ ppm: 144.92, 139.52, 128.79, 121.54, 119.85, 111.63 (aromatic carbons), 75.88 (CH₂), 21.74 (CH₃), 12.62 (CH₃); IR (cm⁻¹): 3435, 3375 (NH), 3069 (Ar–CH₃), 2932, 2879 (aliphatic –CH), 1646, 1603, 1504, 1435 (C=C); Anal. Calcd. %, for C₂₀H₂₂N₂ (Mᵣ = 254.18): C 80.27, H 8.72, N 11.01; found: C 80.32, H 8.61, N 10.79.

2.4. Synthesis of 2-((2-fluorobenzyl)aminomethyl)-4,5-dimethylphenol (2d)

Formaldehyde (4 mmol) was added dropwise to a solvent-free mixture of 3,4-dimethylphenol (4 mmol) and (2-fluorophenyl)methanamine (4 mmol). The reaction mixture was irradiated by microwave at 200 W. Ethanol was used to wash the solid mixture and the residue dried in air.
2d: Reaction time 45 min; (70 %); m.p. 143 °C; \(^{1}H\) NMR (300 MHz, C\text{D}Cl\text{3}) \(\delta\) ppm: 7.46 (s, 1H, OH), 7.29–6.69 (m, 6H, ArH), 5.31 (s, 2H, CH\text{2}), 4.57 (s, 2H, N–CH\text{2}), 2.24 (s, 3H, CH\text{3}), 2.22 (s, 3H, CH\text{3}); \(^{13}C\) NMR (300 MHz, C\text{D}Cl\text{3}) \(\delta\) ppm: 156.83, 154.40, 151.96, 137.16, 136.40, 127.44, 124.56, 123.84, 123.76, 121.44, 117.77, 115.83 (aromatic carbons), 77.09 (CH\text{2}), 76.78 (CH\text{2}), 19.65.74 (CH\text{3}), 19.90 (CH\text{2}); IR (cm\(^{-1}\)): 3463 (O–H), 3372 (NH), 3023 (Ar–CH), 2937, 2836 (aliphatic–CH), 1612, 1577, 1493, 1451 (C=C); Anal. Calcd. %, for C\text{16}H\text{18}FNO (\(M_r = 259.32\)): C 74.11, H 7.00, N 5.40; found: C 74.18, H 7.26, N 5.63.

2.5. Purification of hCA I and hCA II isozymes

The experimental procedure for the purification of hCA I and hCA II isozymes from erythrocytes has been described in detail in our previous studies [30–32]. Briefly, the erythrocyte hemolysate, adjusted to pH 8.7, was loaded onto an affinity column of Sepharose®4B-tyrosine-p-aminobenzene sulfonamide. After removal of unbound proteins and impurities, hCA I and hCA II were eluted using the following buffers: 1.0 M NaCl/25.0 mM Na\text{2}HPO\text{4} (pH 6.3) and 0.1 M NaCH\text{3}COO/0.5 M NaClO\text{4} (pH 5.6), respectively [33]. Quantification of the purified enzymes [34] and SDS-PAGE analysis [35] were performed.

2.6. Examination of the inhibitory effects of hCA I and hCA II isozymes on the hydratase and esterase activity of the obtained compounds

Solids (1 %), which are clinically used locally for the treatment of glaucoma [36], were prepared and the inhibitory effects on the hydratase and esterase activities of the hCA I and II isozymes investigated. Hydratase and esterase activity measurements were performed at five different concentrations of the appropriate inhibitor for hCA I and hCA II isozymes purified from human erythrocytes [30–32, 37–39]. The IC\text{50} values were obtained in vitro for the synthesized compounds (2a–d), with AAZ as the control.

3. RESULTS AND DISCUSSION

In this work, four compounds (2a–d) were synthesized by the reaction of various aromatic amines with formaldehyde in the absence of solvent under microwave irradiation.

The newly synthesized Mannich bases 2a–d are shown in Figure 1.

![Syntheses of 2a–d](image.png)

Fig.1. Syntheses of 2a–d

3.1. NMR (\(^{1}H\) and \(^{13}C\)) spectra of compounds 2a–d.

NMR (\(^{1}H\) and \(^{13}C\)) studies were carried out at room temperature in C\text{D}Cl\text{3} solution (Figs. S1–4). The \(^{1}H\) NMR spectra of 2a–d displayed peaks in the aromatic region in the range 7.29–6.48 ppm. All compounds showed a singlet for methylene (CH\text{2}) protons with an intensity of two hydrogens at 4.78, 4.81, 4.72 and 4.57 ppm, respectively. These singlets support the Mannich reaction between the amines and formaldehyde. A singlet with an intensity of three hydrogens for each CH\text{3} proton was observed at 2.21 and 2.22 ppm for compounds 2a and 2b, respectively.
Two singlets of six-proton intensity were found in the \(^1\)H NMR spectrum of 2c at 2.74 and 2.21 ppm, corresponding to the four methyl groups. Compound 2d showed a singlet with an intensity of one hydrogen at 7.46 ppm for the OH proton. Also, three other singlets were observed for CH\(_2\) and the two different CH\(_3\) protons of compound 2d at 5.31, 2.24 and 2.22 ppm, respectively. In addition, three singlets were observed in the NMR spectrum of compound 2d at 5.31 ppm for CH\(_2\) and at 2.24 and 2.22 ppm for the two different CH\(_3\) protons.

Aromatic carbon atoms were observed in the range 159.11–108.45 ppm in the \(^{13}\)C NMR spectra of 2a–d (Figs. S1–4). Methylene (CH\(_2\)) carbon atoms confirming the Mannich reaction were observed at 70.46, 69.21, 75.88 and 76.78 ppm for the compounds 2a–d, respectively. The carbon atom of the methyl (CH\(_3\)) groups in compounds 2a and 2b resonated at 13.87 and 24.61 ppm, respectively. Compound 2c showed signals at 21.74 and 12.62 ppm for the two CH\(_3\) carbon atoms. In the \(^{13}\)C NMR spectrum of 2d, a signal was observed at 77.09 ppm for the carbon atom of the benzyl CH\(_2\) group.

3.2. FT-IR measurements

The IR spectra of all compounds (2a–d) displayed \(\nu\)(N–H) stretching vibration bands in the range 3469–3270 cm\(^{-1}\) (Table 1). All compounds exhibited aromatic \(\nu\)(C–H) bands in the range of 3070–3018 cm\(^{-1}\). Aliphatic \(\nu\)(C–H) bands for compounds 2a–d were found in the range 2968–2932 and 2882–2831 cm\(^{-1}\). A broad band was obtained at 3463 cm\(^{-1}\) consistent with the \(\nu\)(O–H) stretching vibration in 2d. All compounds exhibited stretching vibration in the range 1646–1435 cm\(^{-1}\) for C=C groups.

| Assignment | \(\nu\)(O–H) (cm\(^{-1}\)) | \(\nu\)(N–H) (cm\(^{-1}\)) | \(\nu\)(C–H)\(_{aliph.}\) (cm\(^{-1}\)) | \(\nu\)(C–C) (cm\(^{-1}\)) |
|------------|-----------------|-----------------|-----------------|-----------------|
| 2a         | –               | 3469 (w)        | 3018 (w)        | 2968 (w)        |
|            |                 | 3374 (w)        | 1628 (w)        | 1608 (w)        |
| 2b         | –               | 3375 (w)        | 3070 (w)        | 2933 (w)        |
|            |                 | 3270 (w)        | 1638 (w)        | 1603 (w)        |
| 2c         | –               | 3435 (w)        | 3069 (w)        | 2882 (w)        |
|            |                 | 3375 (w)        | 1502 (s)        | 1451 (w)        |
| 2d         | 3463 (br)       | 3372 (w)        | 3023 (w)        | 2937 (w)        |
|            |                 |                 | 1646 (w)        | 1603 (w)        |
|            |                 |                 | 1504 (s)        | 1435 (w)        |
|            |                 |                 | 2879 (m)        | 1435 (w)        |
|            |                 |                 | 1504 (s)        | 1435 (w)        |
|            |                 |                 | 1493 (s)        | 1451 (w)        |

w: weak, br: broad, m: medium, s: strong

3.3. In vitro inhibition studies

After purification of hCA I and hCA II, the effects of the compounds on the hydratase and esterase activities of the isozymes were investigated in vitro. The synthesized compounds did not affect the hydratase activity of hCA I and hCA II. This indicates that 2a–d did not interact with the zinc ion at the active site of the enzyme [40]. When the esterase activities of the isozymes were examined, an interesting situation arose. Compounds 2a and 2c were observed to enhance the esterase activity of hCA I and hCA II; however, 2b and 2d inhibited the esterase activities of these isozymes. The inhibitory effects on the esterase activity of 2b (448.35 ± 2.01 and 392.38 ± 1.87 \(\mu\)M) and 2d (291.60 ± 1.18 and 320.20 ± 1.18 \(\mu\)M) were considerably weaker than that of AAZ (0.43 ± 0.05 and 0.32 ± 0.08 \(\mu\)M), which was the control compound (Table 2). However, this study is important as it adds to the literature new CA activators (2a and 2c) and inhibitors (2b and 2d). Also, the effects on isozymes of the compounds studied in this work will shed light on other works in this area.

| Compounds | hCA I \(^{bc}\) | hCA II \(^{bc}\) |
|-----------|----------------|----------------|
| AAZ*      | 0.43 ± 0.05    | 0.32 ± 0.08    |
| 2a        | Activated      | Activated      |
| 2b        | 448.35 ± 2.01  | 392.38 ± 1.87  |
| 2c        | Activated      | Activated      |
| 2d        | 291.60 ± 1.18  | 320.20 ± 1.18  |

\(^{*}\)AAZ was used as reference compound
\(^{bc}\)Mean ± standard error, from three different assays
\(^{c}\)\(p < 0.0001\) for all analyses
4. CONCLUSIONS

In this work, four new compounds (2a–d) were prepared in the absence of solvent under microwave conditions. This one-step method was quite clean, efficient, economical and practical. The structures of the compounds obtained were clarified by FT-IR and NMR (1H and 13C) spectroscopy and elemental analysis. All the results were found to be in harmony with the suggested structures (Fig.1). The inhibitory effects of the synthesized compounds on hCA I and hCA II isozymes were assessed by hydratase and esterase activity assays, and IC₅₀ values were also determined. The synthesized compounds did not show inhibitory effects on the hydratase activity of hCA I and hCA II. Only compounds 2b and 2d were determined to have a weak inhibitory effect on the esterase activity of hCA I and hCA II. However, it was observed that compounds 2a and 2c increased the esterase activity of hCA I and hCA II isozymes. For this reason, these compounds (2a and 2c) may be good CA activators.

Acknowledgments. This work was funded by Grant Number 2008-1 of the Kutahya Dumlupinar University Research Institute.

REFERENCES

[1] J. Lehtonen, B. Shen, M. Vihinen, A. Casini, A. Scozzafava, C. T. Supuran, A. K. Parkkila, J. Saarnio, A. J. Kivelä, A. Waheed, W. S. Sly, S. Parkkila, Characterization of CA XIII, a novel member of the carbonic anhydrase isozyme family, J. Biol. Chem. 279, 2719–2727 (2004). DOI: 10.1074/jbc.M308984200

[2] S. Beydemir, I. Gulcin, Effects of melatonin on carbonic anhydrase from human erythrocytes in vitro and from rat erythrocytes in vivo, J. Enzym. Inhib. Med. Ch. 19, 193–197 (2004). DOI: https://doi.org/10.1080/14756360310001656736.

[3] D. Neri, C. T. Supuran, Interfering with pH regulation in tumours as a therapeutic strategy, Nat. Rev. Drug Discov. 10, 767–777 (2011). DOI: https://doi.org/10.1038/nrd3554.

[4] C. T. Supuran, A. Scozzafava, A. Cassini, Carbonic anhydrase inhibitors, Med. Res. Rev. 23, 146–189 (2003). DOI: https://doi.org/10.1002.med.10025

[5] A. Scozzafava, T. Owa, A. Mastrolorenzo, C. T. Supuran, Anticancer and antiviral sulfonamides, Curr. Med. Chem. 10, 925–953 (2003). DOI: https://doi.org/10.2174/0929867033457647.

[6] A. Casini, J. Antel, F. Abbate, A. Scozzafava, S. David, H. Waldeck, S. Schafer, C. T. Supuran, Carbonic anhydrase inhibitors: SAR and X-ray crystallographic study for the interaction of sugar sulfonamides/sulfamides with isozymes I, II and IV, Bioorg. Med. Chem. Lett. 13, 841–845 (2003). DOI: https://doi.org/10.1016/S0960-894X(03)00029-5.

[7] M. Guney, A. Coskun, F. Topal, A. Dastan, I. Gulcin, C. T. Supuran, Oxidation of cyanobenzocycloheptatrienes: Synthesis, photooxygennation reaction and carbonic anhydrase isoenzymes inhibition properties of some new benzotriazole derivatives, Bioorg. Med. Chem. 22, 3537–3543 (2014). DOI: https://doi.org/10.1016/j.bmc.2014.04.007.

[8] W. S. Sly, P. Y. Hu, Human carbonic anhydrases and carbonic anhydrase deficiencies, Annu. Rev. Biochem. 64, 375–401 (1995). DOI: https://doi.org/10.1146/annurev.bi.64.070195.002111.

[9] J. C. Montgomery, P. J. Venta, R. L. Eddy, Y. S. Fukushima, T. B. Shows, R. E. Tashian, Characterization of the human gene for a newly discovered carbonic anhydrase, CA VII, and its localization to chromosome 16, Genomics. 11, 835–848 (1991). DOI: https://doi.org/10.1016/0888-7543(91)90006-Z.

[10] A. Di Fiore, A. Scozzafava, J. Y. Winum, J. L. Montero, C. Pedone, C. T. Supuran, Carbonic anhydrase inhibitors: Binding of an antiglaucoma glycosyl-sulfanilamide derivative to human isozyme II and its consequences for the drug design of enzyme inhibitors incorporating sugar moieties, Bioorg. Med. Chem. Lett. 17, 1726–1731 (2007). DOI: https://doi.org/10.1016/j.bmcl.2006.12.099.

[11] A. Innocenti, D. Vullo, J. Pastorek, A. Scozzafava, S. Pastorekova, I. Nishimori, C. T. Supuran, Carbonic anhydrase inhibitors. Inhibition of transmembrane isozymes XII (cancer-associated) and XIV with anions, Bioorg. Med. Chem. Lett. 17, 1532–1537 (2007). DOI: https://doi.org/10.1016/j.bmcl.2006.12.113.

[12] J. Y. Winum, A. Thiry, K. E. Cheikh, J. M. Dogné, J. L. Montero, D. Vullo, A. Scozzafava, B. Masereel, C. T. Supuran, Carbonic anhydrase inhibitors. Inhibition of isoforms I, II, IV, VA, VII, IX, and XIV with sulfonamides incorporating fructopyranose-thioureido tails, Bioorg. Med. Chem. Lett. 17, 2685–2691 (2007). DOI: https://doi.org/10.1016/j.bmcl.2007.03.008.

[13] C. Yenikaya, M. Saru, M. Bülbül, H. İkikmen, H. Celik H. O. Büyükgüngör, Synthesis, characterization and antiglaucoma activity of a novel proton transfer compound and a mixed-ligand Zn(II) complex, Biorgan. Med. Chem. 18, 930–938 (2010). DOI: https://doi.org/10.1016/j.bmcl.2009.11.031.

[14] B. L. Wilkinson, L. F. Bornaghi, T. A. Houston, A. Innocenti, D. Vullo, C. T. Supuran, S. A. Poulsen, Inhibition of membrane-associated carbonic anhydrase isozymes IX, XII and XIV with a library of glycoconjugate benzenesulfonamides, Bioorg. Med. Chem. Lett. 17, 987–992 (2007). DOI: https://doi.org/10.1016/j.bmcl.2006.11.046.

[15] M. A. Santos, S. Marques, D. Vullo, A. Innocenti, A. Scozzafava, C. T. Supuran, Carbonic anhydrase inhibitors: Inhibition of cytosolic/tumor-associated isoforms I, II, and IX with imimidicidicarboxylates/hydroxamates also incorporating benzenesulfonamide moieties Bioorg. Med. Chem. Lett. 17, 1538–1543 (2007). DOI: https://doi.org/10.1016/j.bmcl.2006.12.107.

[16] C. T. Supuran, Carbonic anhydrases: novel therapeutic applications for inhibitors and activators, Nat. Rev. Drug Discov. 7, 168–181 (2008). DOI: 10.1038/nrd2467.

[17] C. Temperini, A. Scozzafava, D. Vullo, C. T. Supuran, Carbonic anhydrase activators. Activation of isoforms I, II, IV, VA, VII, and XIV with L- and D-phenylalanine and crystallographic analysis of their adducts with iso- zyme II: Stereospecific recognition within the active site.
of an enzyme and its consequences for the drug design, J. Med. Chem. 49, 3019–3027 (2006).
DOI: https://doi.org/10.1021/jm0603320.

[18] C. Temperini, A. Innocenti, A. Scozzafava, A. Mastrolorenzo, C. T. Supuran, Carbonic anhydrase activators: L-adrenaline plugs the active site entrance of isozyme II, activating better isozymes I, IV, VA, VII, and XIV, Bioorg. Med. Chem. Lett. 17, 628–635 (2007).
DOI: https://doi.org/10.1016/j.bmcl.2006.11.027.

[19] S. Parkkila, D. Vullo, L. Puccetti, A. K. Parkkila, A. Scozzafava, C. T. Supuran, Carbonic anhydrase activators: activation of isozyme XIII with amino acids and amines, Bioorg. Med. Chem. Lett. 16, 3955–3959 (2006).
DOI: https://doi.org/10.1016/j.bmcl.2006.05.023.

[20] D. Vullo, I. Nishimori, A. Innocenti, A. Scozzafava, C. T. Supuran, Carbonic anhydrase activators: An activation study of the human mitochondrial isoforms VA and VB with amino acids and amines, Bioorg. Med. Chem. Lett. 17, 1336–1340 (2007).
DOI: https://doi.org/10.1016/j.bmcl.2006.11.075.

[21] D. Vullo, A. Innocenti, I. Nishimori, A. Scozzafava, K. Kaila, C. T. Supuran, Carbonic anhydrase activators: activation of the human isozyme VII (cytosolic) and XIV (transmembrane) with amino acids and amines, Bioorg. Med. Chem. Lett. 17, 4107–4112 (2007).
DOI: https://doi.org/10.1016/j.bmcl.2007.05.052.

[22] I. Nishimori, S. Onishi, D. Vullo, A. Innocenti, A. Scozzafava, C. T. Supuran, Carbonic anhydrase activators: the first activation study of the human secretory isozyme VI with amino acids and amines, Bioorg. Chem. Med. 15, 5351–5357 (2007).
DOI: https://doi.org/10.1016/j.bmc.2007.03.004.

[23] M. S. Singh, S. Chowdhury, Recent developments in solvent-free multicomponent reactions: a perfect synergy for eco-compatible organic synthesis, RSC. Adv. 2, 4547–4592 (2012).
DOI: 10.1039/C2RA01056A.

[24] A. Kumar, S. A. Sharma, A grinding-induced catalyst- and solvent-free synthesis of highly functionalized 1,4-dihydropyridines via a domino multicomponent reaction, Green Chem. 13, 2017–2020 (2011).
DOI: 10.1039/C1GC15223H.

[25] B. Büyükkıdan, S. Özer, Synthesis and characterization of Ni(II) and Cu(II) complexes derived from novel phe-nolic Mannich bases, Turk. J. Chem. 37, 101–110 (2013).
DOI: 10.3906/kim-1203-67.

[26] M. Ashok, B. S. Holla, B. Poojary, Convenient one pot synthesis and antimicrobial evaluation of some novel Mannich bases carrying 4-methylthiobenzyl moiety, Eur. J. Med. Chem. 42, 1095–1101 (2007).
DOI: https://doi.org/10.1016/j.ejmech.2007.01.015.

[27] B. S. Holla, K. N. Poojary, R. B. Sooryanarayana, M. K. Shivananda, New bis-aaminocaproatizoles and bis-triazolothiadiazoles as possible anticaner agents, Eur. J. Med. Chem. 37, 511–517 (2002).
DOI: https://doi.org/10.1016/S0221-1382(01)0038-2.

[28] M. Amir, K. Shikha, Synthesis and anti-inflammatory, analgesic, ulcerogenic and lipid peroxidation activities of some new 2-(2,6-dichloroanilino)phenyl]acetic acid derivatives, Eur. J. Med. Chem. 39, 535–545 (2004).
DOI: https://doi.org/10.1016/j.ejmech.2004.02.008.

[29] K. Walczak, A. Gondela, J. Suwiński, Synthesis and anti-tuberculosis activity of N-aryl-C-nitroazoles, Eur. J. Med. Chem. 39, 849–853 (2004).
DOI: https://doi.org/10.1016/j.ejmech.2004.06.014.

[30] C. Tempeiri, A. Innocenti, A. Scozzafava, A. Mastrolorenzo, C. T. Supuran, Carbonic anhydrase activators: L-adrenaline plugs the active site entrance of isozyme II, activating better isozymes I, IV, VA, VII, and XIV, Bioorg. Med. Chem. Lett. 17, 628–635 (2007).
DOI: https://doi.org/10.1016/j.bmcl.2006.11.027.

[31] S. Parkkila, D. Vullo, L. Puccetti, A. K. Parkkila, A. Scozzafava, C. T. Supuran, Carbonic anhydrase activators: activation of isozyme XIII with amino acids and amines, Bioorg. Med. Chem. Lett. 16, 3955–3959 (2006).
DOI: https://doi.org/10.1016/j.bmcl.2006.05.023.

[20] D. Vullo, I. Nishimori, A. Innocenti, A. Scozzafava, C. T. Supuran, Carbonic anhydrase activators: An activation study of the human mitochondrial isoforms VA and VB with amino acids and amines, Bioorg. Med. Chem. Lett. 17, 1336–1340 (2007).
DOI: https://doi.org/10.1016/j.bmcl.2006.11.075.

[21] D. Vullo, A. Innocenti, I. Nishimori, A. Scozzafava, K. Kaila, C. T. Supuran, Carbonic anhydrase activators: activation of the human isozyme VII (cytosolic) and XIV (transmembrane) with amino acids and amines, Bioorg. Med. Chem. Lett. 17, 4107–4112 (2007).
DOI: https://doi.org/10.1016/j.bmcl.2007.05.052.

[22] I. Nishimori, S. Onishi, D. Vullo, A. Innocenti, A. Scozzafava, C. T. Supuran, Carbonic anhydrase activators: the first activation study of the human secretory isozyme VI with amino acids and amines, Bioorg. Chem. Med. 15, 5351–5357 (2007).
DOI: https://doi.org/10.1016/j.bmc.2007.03.004.

[23] M. S. Singh, S. Chowdhury, Recent developments in solvent-free multicomponent reactions: a perfect synergy for eco-compatible organic synthesis, RSC. Adv. 2, 4547–4592 (2012).
DOI: 10.1039/C2RA01056A.

[24] A. Kumar, S. A. Sharma, A grinding-induced catalyst- and solvent-free synthesis of highly functionalized 1,4-dihydropyridines via a domino multicomponent reaction, Green Chem. 13, 2017–2020 (2011).
DOI: 10.1039/C1GC15223H.

[25] B. Büyükkıdan, S. Özer, Synthesis and characterization of Ni(II) and Cu(II) complexes derived from novel phe-nolic Mannich bases, Turk. J. Chem. 37, 101–110 (2013).
DOI: 10.3906/kim-1203-67.

[26] M. Ashok, B. S. Holla, B. Poojary, Convenient one pot synthesis and antimicrobial evaluation of some novel Mannich bases carrying 4-methylthiobenzyl moiety, Eur. J. Med. Chem. 42, 1095–1101 (2007).
DOI: https://doi.org/10.1016/j.ejmech.2007.01.015.

[27] B. S. Holla, K. N. Poojary, R. B. Sooryanarayana, M. K. Shivananda, New bis-aaminocaproatizoles and bis-triazolothiadiazoles as possible anticancer agents, Eur. J. Med. Chem. 37, 511–517 (2002).
DOI: https://doi.org/10.1016/S0221-1382(01)0038-2.

[28] M. Amir, K. Shikha, Synthesis and anti-inflammatory, analgesic, ulcerogenic and lipid peroxidation activities of some new 2-[2,6-dichloroanilino]phenyl]acetic acid derivatives, Eur. J. Med. Chem. 39, 535–545 (2004).
DOI: https://doi.org/10.1016/j.ejmech.2004.02.008.