Synthesis and carbonic anhydrase inhibitory properties of novel uracil derivatives

ARTICLE *in* BIOORGANIC & MEDICINAL CHEMISTRY LETTERS · MAY 2015

Impact Factor: 2.42 · DOI: 10.1016/j.bmcl.2015.05.073 · Source: PubMed

4 AUTHORS, INCLUDING:

Murat Güney
Ataturk University
13 PUBLICATIONS **103** CITATIONS
SEE PROFILE

Murat Senturk
Ataturk University
58 PUBLICATIONS **1,225** CITATIONS
SEE PROFILE

Deniz Ekinci
Ondokuz Mayıs Üniversitesi
63 PUBLICATIONS **1,380** CITATIONS
SEE PROFILE

Bioorganic & Medicinal Chemistry Letters 25 (2015) 3261–3263

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis and carbonic anhydrase inhibitory properties of novel uracil derivatives

Murat Güney [a,*], Hüseyin Çavdar [b], Murat Şentürk [a,*], Deniz Ekinci [c]

[a] Ağrı İbrahim Çeçen University, Department of Chemistry, 04100 Ağrı, Turkey
[b] Dumlupinar University, Education Faculty, 43100 Kutahya, Turkey
[c] Ondokuz Mayıs University, Department of Agricultural Biotechnology, 55139 Samsun, Turkey

ARTICLE INFO

Article history:
Received 15 April 2015
Revised 22 May 2015
Accepted 24 May 2015
Available online 30 May 2015

Keywords:
Carbonic anhydrase
Hydroxyl
Uracil
Enzyme inhibitor

ABSTRACT

Carbonic anhydrase (CA) inhibitors are valuable molecules based on several therapeutic applications, including antiglaucoma activity. In the present study, inhibition of two human cytosolic carbonic anhydrase isozymes I and II with some uracil derivatives (**3**–**9**) were investigated. Compounds **3**–**9** showed K_I values in the range of 10.83–464 μM for hCA I and of 28.88–778.5 μM against hCA II, respectively. Kinetic investigations showed that similarly to classical CA inhibitors, all investigated natural compounds act as competitive inhibitors with 4-NPA as substrate. Uracil derivatives investigated here are promising agents which may be used as lead molecules in order to derivative novel carbonic anhydrase inhibitors that might be useful in medical applications.

Uracil (U) which is a common and naturally occurring pyrimidine derivative is one of the four nucleobases in RNA.[1] Uracil undergoes two tautomeric forms at pH 7.0. This amide-imidic acid tautomeric shifts from lactam which is the amide tautomer to the imidic acid tautomer is referred to as the lactim structure (Fig. 1). Uracil is a weak acid.[2]

In drug discovery studies, uracils are very important structures because of wide range of biological activities and synthetic accessibility and ability to confer drug like properties to the compound libraries appended on them at N1, N3, C5 and C6 positions.[3a] As well as antiviral and anti-tumour properties which is two most reported activities of uracil analogues;[3b,c] they also possess herbicidal, insecticidal and bactericidal actions.[4]

5-Fluorouracil (5-FU) is antimetabolite of the pyrimidine analogue type and a well-known anti-tumour agent which has been widely used in the treatment of solid tumours such as colon or breast cancers.[5] Because 5-FU is similar in shape to uracil, but does not perform the same chemistry as uracil, the drug inhibits RNA replication enzymes, thereby eliminating RNA synthesis and stopping the growth of cancerous cells. Although 5-FU has had clinical success as a single agent, it has been modified by different ways to synthesize its derivatives which may improve its therapeutic index

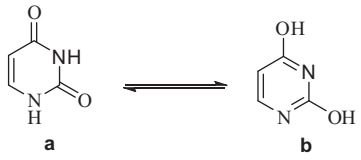


Figure 1. Tautomeric forms of uracil: lactam–lactim.

because of its well-known side effects such as short half-life, wide distribution, low selectivity, and various toxic side effects.[2]

Some researcher prepared N1-mono and N1, N3-bis-substituted 5-fluoro, 5-bromo, 5-iodouracils.[6] Uracils were converted to the corresponding benzyl halides in the presence of K_2CO_3 the target pyrimidines as mixtures of mono- and bis-derivatives that were separated by treatment with KOH solution. Some biologically activities as toxicity, anti-tumour, and antibacterial properties of synthesized compounds were investigated. Chemotherapy tests showed that the a large number of the studied compounds had a statistically important antitumour activity. In addition to 5-fluoro- and 5-iodouracils exhibited more pronounced anti-tumour properties than 5-bromouracil derivatives.[6]

The carbonic anhydrases (CA; carbonate hydrolase, EC 4.2.1.1) are a ubiquitous family of zinc-containing enzymes, which classically participate in the maintenance of pH homeostasis in the human body, catalyzing the reversible hydration of carbon dioxide in a two-step reaction to yield bicarbonate and protons.[7] Isoforms

* Corresponding authors. Tel.: +90 472 215 9994; fax: +90 4722156554.
E-mail addresses: mguney@agri.edu.tr (M. Güney), msenturk@agri.edu.tr (M. Şentürk).

http://dx.doi.org/10.1016/j.bmcl.2015.05.073
0960-894X/

Table 1
hCA I and II inhibition data some compounds, by an esterase assay with 4-nitrophenylacetate as substrate[12b]

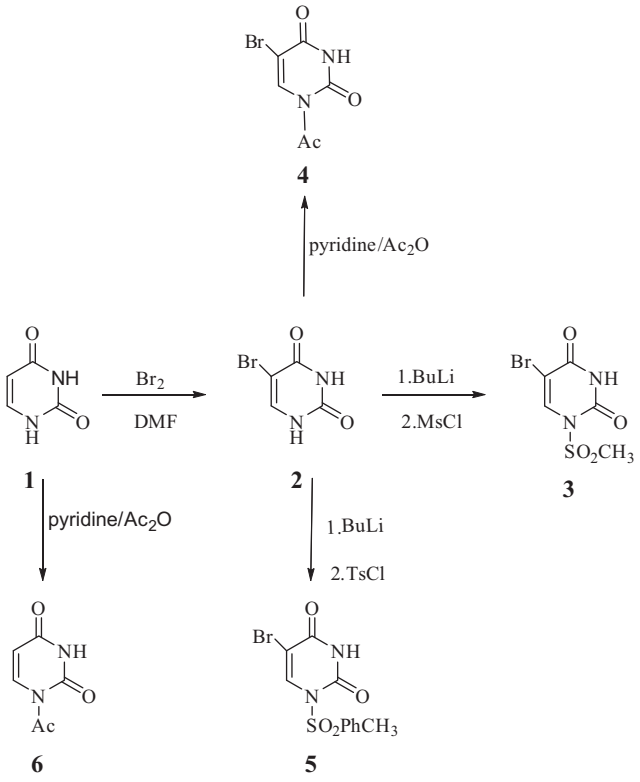
Compound	K_I^* (μM)	
	hCA I	hCA II
3	428 ± 2.42	645 ± 3
4	464 ± 3.24	778.5 ± 5.3
5	10.83 ± 0.76	28.88 ± 0.92
7	57.76 ± 0.28	NE
8	316.2 ± 5.32	166.4 ± 2.46
9	49.51 ± 0.35	NE
10[a]	795	7.7
11[a]	4003	9.9
12[a]	10.2	5.5
Acetazolamide[b]	36.2	0.37

[a] From Ref. 6a.
[b] From Ref. 14d NE: no-effect.
* Mean from at least three determinations. Errors in the range of 3–8% of the reported value (data not shown).

of carbonic anhydrase are found in several of tissues where they participate in many important biological processes such as acid-base balance, respiration, carbon dioxide and ion transport, bone resorption, ureagenesis, gluconeogenesis, lipogenesis and electrolyte secretion. Many CA isozymes involved in these processes are important therapeutic targets with the potential to be inhibited/activated for the treatment of a range of disorders such as edema, glaucoma, obesity, cancer, epilepsy and osteoporosis.[2–4]

Some research groups recently investigated the interaction of 12 mammalian CA isozymes with phenol and several types of phenolic compounds and determined the inhibitory action of the phenols.[6–8] In this study we extend these researches to series of phenolic compounds, some of which are widely used as antioxidant food additives or as drugs and prodrugs. Among the various natural or unnatural phenolic compounds with antioxidant properties, these compounds are very active in quenching reactive oxygen species.[9a] They are reported to possess anticancer, anti-carcinogenic, antimutagenic, antibacterial, antiviral or anti-inflammatory activities.[10] Phenol, phenolic compounds and hydroxybenzoic acid derivatives are widely used as prodrugs or drugs. Salicylic acid is known for its ability to ease aches, pains, and reduce fevers. These medicinal properties, particularly fever relief, have been known since ancient times, and it was used as an anti-inflammatory drug.[9,10b]

In the present study we have purified CA I and II (hCA I and hCA II) from human erythrocytes and examined the in vitro inhibition effects of above mentioned uracil derivatives **3**–**9** on these enzymes (Figs. 1, 2 and Table 1), using the esterase activity of hCA I and II, with 4-NPA as substrate. Uracil derivatives **3**–**5**[13] were synthesized from commercially available uracil (**1**). For this purpose uracil **1** was converted to the brom uracil **2**[14a] by using 1.1 equiv bromine in DMF at 120 °C. Condensation of brom uracil **2** with methanesulfonyl chloride, *p*-toluenesulfonyl chloride and acetic anhydride to afford uracil derivatives **3** which has antitumour activity,[14a] **4**[14b] and **5**[14c] in comparable yield, respectively. Also uracil (**1**) was converted to uracil derivative **6**[14d] by using



Scheme 1. Compounds **1**–**6** synthesis scheme.

pyridine and acetic anhydride. The other tested compounds **7**–**12** were purchased from sigma aldrich (Fig. 2, Scheme 1).

(i) Against the slow cytosolic isozyme hCA I, compounds **3**, **4**, and **8** behave as weak inhibitors, with K_i values in the range of 316.2–464 μM, similarly to the structurally related compounds **10** and **11** (K_Is of 795 and 4003 μM). It is interesting that compound **5** was much better hCA I inhibitor as compared to the corresponding compounds **7** and **9** from which it was derived. Kinetic investigations (Lineweaver–Burk plots, data not shown) indicate that similarly to sulfonamides and inorganic anions,[9–12] all the investigated natural compounds act as competitive inhibitors with 4-NPA as substrate.[12a]

(ii) A better inhibitory activity has been observed with compounds **10**–**12** investigated here for the inhibition of the rapid cytosolic isozyme hCA II (Table 1). Structure–activity relationship (SAR) is thus quite sharp for this small series of hydroxylic compounds: compounds **10**, **11** and **12** are effective leads, with two mono or di hydroxy moieties is already a submicromolar hCA II inhibitor. This effect is maintained when different groups are present in the *meta*

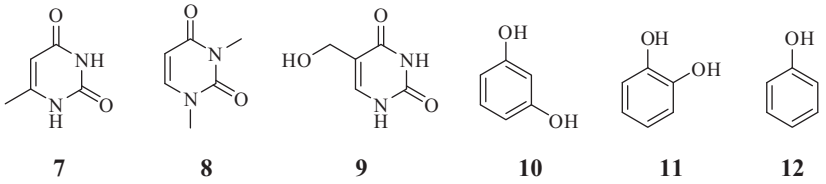


Figure 2. Structure of tested compounds.

position to the phenol OH moiety, such as in resorcinol. The best hCA II inhibitor in this series of derivatives were compound **5** with a K_I of 28.88 μM.[11]

In a recent study it was reported that catechol and resorcinol[10a] act as a CAI inhibitor, and could represent the starting point for a new class of inhibitors that may have advantages for patients with sulfonamide allergies.[11] The sulfonamide zinc-binding group is thus superior to the thiol one (from the thioxolone hydrolysis product) for generating CA inhibitors with a varied and sometimes isozyme-selective inhibition profile against the mammalian enzymes. However, it is still important to explore further classes of potent CAIs in order to detect compounds with different inhibition profiles.

Compounds **3**–**12** used in this study affect the activity of CA isozymes due to the presence of the different functional groups (OH, Br, mesityl, tosyl, and acetate) present in their scaffold. Therefore, our findings indicate another class of possible CAIs of interest, in addition to the well-known inhibitors, the phenols/biphenyl diphenols bearing bulky *ortho* moieties in their molecules. Some hydroxyl compounds investigated here exhibited effective hCA I and II inhibitory activity, in the low micromolar range, by the esterase method which usually gives K_I-s an order of magnitude higher as compared to the CO_2 hydrase assay.[15] These findings point out that substituted hydroxyl compounds may be used as leads for generating potent CAIs eventually targeting other isoforms.

Acknowledgments

This study was financed by Agri Ibrahim Cecen University Scientific Research Council, (project no: Agri BAP-2010/K-09) for (MG) and by TUBITAK (The Scientific and Technological Research Council of Turkey) (Project no: 114Z731) for (MS). The authors are grateful to Prof. Dr. Claudiu T. Supuran, University of Florence, for various valuable studies on carbonic anhydrase inhibitors.

References and notes

1. Martins, Z.; Botta, O.; Fogel, M. L.; Sephton, M. A.; Glavin, D. P.; Watson, J. S.; Dworkin, J. P.; Schwartz, A. W.; Ehrenfreund, P. *Earth Planet. Sci. Lett.* **2008**, *270*, 130.
2. Pałasz, A.; Ciez, D. *Eur. J. Med. Chem.* **2014**. http://dx.doi.org/10.1016/j.ejmech.2014.10.008.
3. (a) Newkome, G. R.; Pandler, W. W. *Contemporary Heterocyclic Chemistry*; Wiley: New York, 1982; (b) Parker, W. B. *Chem. Rev.* **2009**, *109*, 2880; (c) Pathak, T. *Chem. Rev.* **2002**, *102*, 1623.
4. (a) Soladino, R.; Crestini, C.; Palamara, A. T.; Danti, M. C.; Manetti, F.; Corelli, F.; Garaci, E.; Botta, M. *J. Med. Chem.* **2001**, *44*, 4554; (b) Zhi, C.; Long, Z.; Manikowski, A.; Bron, N. C.; Tarantino, P. M.; Holm, K.; Dix, E. J.; Wright, G. E.; Foster, K. A.; Butler, M. M.; LaMarr, W. A.; Skow, D. J.; Motorina, I.; Lamothe, S.; Storer, R. *J. Med. Chem.* **2005**, *48*, 7063; (c) Fustero, S.; Piera, J.; Sanz-Cervera, J. F.; Catalan, S.; Ramirez, A. C. *Org. Lett.* **2004**, *6*, 1417.
5. Smith, N. F.; Figg, W. D.; Sparreboom, A. *Drug Dev. Res.* **2004**, *62*, 233.
6. (a) Arutyunyan, A. A.; Mamyan, S. S.; Stepanyan, H. M.; Paronikyan, R. V. *Pharm. Chem. J.* **2013**, *47*, 303; (b) Korkmaz, N.; Obaidi, O. A.; Senturk, M.; Astley, D.; Ekinci, D.; Supuran, C. T. *J. Enzyme Inhib. Med. Chem.* **2015**, *30*, 75; (c) Ekinci, D.; Fidan, I.; Durdagi, S.; Kaban, S.; Supuran, C. T. *J. Enzyme Inhib. Med. Chem.* **2013**, *28*, 370; (d) Koz, O.; Ekinci, D.; Perrone, A.; Piacente, S.; Alankus-Caliskan, O.; Bedir, E.; Supuran, C. T. *J. Enzyme Inhib. Med. Chem.* **2013**, *28*, 412; (e) Ekinci, D.; Karagoz, L.; Ekinci, D.; Senturk, M.; Supuran, C. T. *J. Enzyme Inhib. Med. Chem.* **2013**, *28*, 283; (f) Ekinci, D.; Kurbanoglu, N. I.; Salamci, E.; Senturk, M.; Supuran, C. T. *J. Enzyme Inhib. Med. Chem.* **2012**, *27*, 845.
7. (a) Maren, T. H. *Annu. Rev. Physiol.* **1988**, *50*, 695; (b) Supuran, C. T. *Nat. Rev. Drug Disc.* **2008**, *7*, 168.
8. Hilvo, M.; Baranauskiene, L.; Salzano, A. M.; Scaloni, A.; Matulis, D.; Innocenti, A.; Scozzafava, A.; Monti, S. M.; Di Fiore, A.; De Simone, G.; Lindfors, M.; Jänis, J.; Valjakka, J.; Pastoreková, S.; Pastorek, J.; Kulomaa, M. S.; Nordlund, H. R.; Supuran, C. T.; Parkkila, S. *J. Biol. Chem.* **2008**, *283*, 27799.
9. (a) Supuran, C. T.; Scozzafava, A. *Bioorg. Med. Chem.* **2007**, *15*, 4336; (b) Supuran, C. T.; Scozzafava, A. *Expert Opin. Ther. Pat.* **2002**, *12*, 217; (c) Senturk, M.; Gulcin, I.; Dastan, A.; Kufrevioglu, O. I.; Supuran, C. T. *Bioorg. Med. Chem.* **2009**, *17*, 3207; (d) Ekinci, D.; Senturk, M.; Kufrevioglu, O. I. *Expert Opin. Ther. Pat.* **1831**, *2011*, 21.
10. (a) Innocenti, A.; Vullo, D.; Scozzafava, A.; Supuran, C. T. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 1583; (b) Innocenti, A.; Hilvo, M.; Scozzafava, A.; Parkkila, S.; Supuran, C. T. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 3593; (c) Sarikaya, S. B. O.; Topal, F.; Senturk, M.; Gulcin, I.; Supuran, C. T. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 4259.
11. (a) Ekinci, D.; Cavdar, H.; Talaz, O.; Senturk, M.; Supuran, C. T. *Bioorg. Med. Chem.* **2010**, *18*, 3559; (b) Alp, C.; Ekinci, D.; Gultekin, M. S.; Senturk, M.; Sahin, E.; Kufrevioglu, O. I. *Bioorg. Med. Chem.* **2010**, *18*, 4468; (c) Ekinci, D.; al-Rashida, M.; Abbas, G.; Senturk, M.; Supuran, C. T. *J. Enzyme Inhib. Med. Chem.* **2012**, *27*, 744.
12. (a) Nair, S. K.; Ludwig, P. A.; Christianson, D. W. *J. Am. Chem. Soc.* **1994**, *116*, 3659; (b) Verpoorte, J. A.; Mehta, S.; Edsall, J. T. *J. Biol. Chem.* **1967**, *242*, 4221; (c) Wilbur, K. M.; Anderson, N. G. *J. Biol. Chem.* **1976**, *176*, 147; (d) Lineweaver, H.; Burk, D. *J. Am. Chem. Soc.* **1934**, *57*, 685.
13. *Detailed synthetic procedures for the preparation of all derivatives can be found in:* Synthesis of 5-bromo-1*H*-pyrimidine-2,4-dione (**2**): to a magnetically stirred suspension of 1*H*-pyrimidine-2,4-dione (**1**) (1.00 g, 8.92 mmol) in 5 mL of DMF at 120 °C was added dropwise bromine (1.57 g, 9.81 mmol) over a period of 5 min. After being stirred for 4 h, reaction mixture was cooled to room temperature. CH_2Cl_2 was added into the reaction mixture and the obtained solid was filtered and identified as 5-bromo-1*H*-pyrimidine-2,4-dione (**2**) (1.50 g, 88%). Colorless crystals, mp >300 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.48 (bs, 1H), 11.20 (bs, 1H), 7.88 (d, *J* = 6.2 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 160.80, 151.53, 142.88, 95.14.
*5-Bromo-1-methanesulfonyl-1H-pyrimidine-2,4-dione (**3**):* A 2.5 M solution of *n*-BuLi in hexanes (0.63 mL, 1.57 mmol) was added dropwise to a solution of monobromide **2** (300 mg, 1.57 mmol) in dry THF (10 mL) at 0 °C and the resulting mixture was stirred 30 min. at the same temperature. Methanesulfonyl chloride (0.12 mL, 1.57 mmol) was added portion wise. The mixture was stirred 10 min at 0 °C and was stirred at room temperature 3 h. The resulting suspension was treated with CH_2Cl_2, filtered and product **3** was obtained (240 mg, 57%). White crystals, mp 237–239 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.32 (s, 1H, NH), 8.07 (s, 1H), 3.68 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 159.75, 148.52, 137.59, 98.69, 42.18.
*1-Acetyl-5-bromo-1H-pyrimidine-2,4-dione (**4**):* To the monobromide **2** (100 mg, 0.52 mmol)) a solution of acetic anhydride (190 mg, 1.86 mmol) and pyridine (3 mL) was added. The reaction mixture was kept under nitrogen at room temperature for 24 h. The excess acetic anhydride and the solvent were removed under vacuum and the ^{1}H NMR analysis of residue indicated the formation of the acetate **4** (122 mg, 100%). Recrystallization from CH_2Cl_2/*n*-hexane (1:3), white crystals, mp 174–175 °C. ^{1}H NMR (400 MHz, acetone-d_6): δ 10.74 (bs, 1H, NH), 8.48 (s, 1H, CH), 2.68 (s, 3H, CH_3) ^{13}C NMR (100 MHz, acetone-d_6): δ 169.24, 158.70, 149.15, 137.01, 100.22, 26.18. IR (KBr, cm^{-1}): 3070, 2857, 1708, 1619, 1406, 1362, 1263, 1171, 1037, 976, 855.
*5-Bromo-1-(toluene-4-sulfonyl)-1H-pyrimidine-2,4-dione (**5**):* A 2.5 M solution of *n*-BuLi in hexanes (0.21 mL, 0.52 mmol) was added dropwise to a solution of monobromide **2** (100 mg, 0.52 mmol) in dry THF (10 mL) at 0 °C and the resulting mixture was stirred 30 min. at the same temperature and *p*-toluenesulfonyl chloride (100 mg, 0.52 mmol) was added portion wise. The mixture was stirred 10 min at 0 °C and was stirred at room temperature 20 h. The crude was quenched with water (50 mL), extracted with EtOAc (3 × 30 mL). The combined organic extracts were dried over $MgSO_4$, concentrated in vacuo. The crude product was purified by crystallization from hot methanol to give 5-bromo-1-(toluene-4-sulfonyl)-1*H*-pyrimidine-2,4-dione (**5**) (120 mg,% 66). White crystals, mp 252–254 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.16 (bs, 1H, NH), 8.38 (d, *J* = 0.7, 1H, CH), 7.96 (d, *J* = 7.9 Hz, 2H, TsH) 7.47 (d, *J* = 7.9 Hz, 2H, TsH), 2.41 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 159.63, 147.37, 147.24, 137.46, 133.51, 130.54, 129.92, 99.51, 21.90. IR (KBr, cm^{-1}): 3148, 3053, 2913, 2845,1756, 1682, 1376, 1253, 1173, 1065, 998, 811.
*1-Acetyl-1H-pyrimidine-2,4-dione (**6**):* The reaction was carried out according to the above mentioned procedure by using 100 mg (0.89 mmol) of **1**, pyridine (3 mL), and acetic anhydride (320 mg, 3.13 mmol). After 48 h, the solvent was evaporated and the ^{1}H NMR analysis of a residue indicated the formation of the acetate **6** (137 mg, 100%). Recrystallization from CH_2Cl_2/*n*-hexane (1:3), white crystals, mp 190–192 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.70 (bs, 1H, NH), 8.23 (d, *J* = 8.4, 1H, H_6), 5.89 (dd, *J* = 8.4, *J* = 1.83 Hz, 1H, H_5), 2.76 (s, 3H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.56, 162.51, 149.36, 137.60, 104.98, 27.27. IR (KBr, cm^{-1}): 3014, 2823, 1730, 1683, 1630, 1440, 1397, 1222, 1107, 830.
14. (a) Glavaš-Obrovac, L.; Karner, I.; Pavlak, M.; Radačić, M.; Kasnar-Samprec, J.; Žinić, B. *Nucleosides Nucleotides Nucleic* **2005**, *24*, 557; (b) Fytelson, M.; Johnson, B. T. *J. Am. Chem. Soc.* **1942**, *64*, 306; (c) Višnjevac, A.; Žinić, M.; Luić, M.; Žiher, D.; Kajfež Novak, T.; Žinić, B. *Tetrahedron* **2007**, *63*, 86; (d) Spector, B. L.; Keller, B. E. *J. Biol. Chem.* **1958**, *232*, 185.
15. Detailed procedures for enzyme purification can be found in: (a) Ekinci, D.; Cavdar, H.; Durdagi, S.; Talaz, O.; Senturk, M.; Supuran, C. T. *Eur. J. Med. Chem.* **2012**, *49*, 68; (b) Ekinci, D.; Ceyhun, S. B.; Senturk, M.; Erdem, D.; Kufrevioglu, O. I.; Supuran, C. T. *Bioorg. Med. Chem.* **2011**, *19*, 744; (c) Alp, C.; Ozsoy, S.; Alcan Alp, N.; Erdem, D.; Gultekin, M. S.; Kufrevioglu, O. I.; Senturk, M.; Supuran, C. T. *J. Enz. Inhib. Med. Chem.* **2012**, *27*, 818; (d) Ozdemir, Z. O.; Senturk, M.; Ekinci, D. *J. Enz. Inhib. Med. Chem.* **2013**, *28*, 316.