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RESEARCH ARTICLE

Synthesis and carbonic anhydrase inhibitory properties of novel 1,4-dihydropyrimidinone substituted diarylureas

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Abstract

A new series of 1,4-dihydropyrimidinone (DHPM) substituted diaryl urea and thiourea derivatives were synthesized and their inhibitory effects on the activity of purified human carbonic anhydrase (hCA) I and II were evaluated. 4-Nitrophenyl-1,4-DHPM was prepared with dimedone, nitrobenzaldehyde and urea or thiourea and nitro group was reduced to amine derivative. The compound was reacted with isocyanates and isothiocyanates to get the final products. The results showed that all the synthesized compounds inhibited the carbonic anhydrase isoenzyme activity; **4c** ($IC_{50} = 66.23\ \mu M$ for hCA I) and **4f** ($IC_{50} = 63.09\ \mu M$ for hCA II) have the most inhibitory effect. The synthesized compounds are very bulky to be able to bind near the zinc ion and they much more probably bind as the coumarins and activators.

Keywords

Carbonic anhydrase, dihydropyrimidinone, enzyme inhibitor, urea

History

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Introduction

Carbonic anhydrases (CAs, EC 4.2.1.1) are widespread zinc metalloenzymes that catalyse the reversible hydration of carbon dioxide (CO_2) to bicarbonate (HCO_3^-) and a proton (H^+) with water[1]. CAs are ubiquitous enzymes present in prokaryotes and eukaryotes which are encoded by four evolutionarily unrelated gene families (the α-, β-, γ- and ξ-CAs)[2,3]. There are 16 isozymes which are characterized and many of them are involved in critical physiological processes[4]. In humans, CAs are found in a variety of tissues such as kidneys, lungs, eyes, skins, the nervous systems and the gastrointestinal tract[5]. The different isozymes are found in different parts of the cell and CA I and II are localized in the cytosol[6]. Biological activities of this metalloenzyme family have several medicinal applications such as treatment of glaucoma, diuretics, in the management of several neurological disorders, whereas several agents are in clinical evaluations as antiobesity or antidrugs[7].

In recent years, much attention has been focused on the synthesis of 1,4-dihydropyrimidinone (DHPM) due to their significant biological activities. The compounds have various therapeutic and pharmacological properties such as calcium channel modulators, antihypertensive agents, α_{1a}-adrenergic receptor antagonists[8,9], antiviral, antitumour, antibacterial and anti-inflammatory activities[10,11]. The DHPM core is also found in many natural products and marine alkaloids and have been found to be potent HIV gp-120CD_4 inhibitors[12].

Multicomponent reactions are important for various reactions in medicinal and organic chemistry[13]. The simple and direct method for the synthesis of DHPMs first reported by Biginelli[14] in 1893 was using an aldehyde, a β-ketoester and urea (or thiourea) under strongly acidic conditions, but the reaction suffered from drawbacks such as long reaction time, low yields, etc. For this transformation, several methods were improved such as using zirconium hydrogen phosphate[15], alumina sulphuric acid (ASA)[16] and heteropoly acids[17].

Ureido-substituted benzenesulphanilamides show very interesting profile for the inhibition of several human CAs (hCAs) such as hCAs I and II (cytosolic isoforms) and hCAs IX and XII (transmembrane, tumour-associated enzymes). They mentioned that the compounds have excellent inhibitory effects for all these isoforms due to the urea moiety[18]. CA IX is highly expressed in breast malignancies, and CA IX and CA XII are variably expressed in breast cancer cell lines. Human breast cancers provided definitive evidence of CA IX as an independent poor prognostic biomarker for distant metatheses and survival. Lou et al.[19] have studied CA inhibitory activities *in vitro* on 4T1 mouse metastatic breast cancer cells.

In this study, a new series of 1,4-dihydropyrimidine substituted diaryl urea and thiourea derivatives were synthesized and their inhibitory effects on the activity of purified hCA I and II were evaluated.

Materials and methods

1,4-Dihydropyrimidine substituted urea and thiourea derivatives shown in Scheme 1 were synthesized and their effect was examined on CA I and II. 4-Nitrophenyl-1,4-DHPM was prepared with dimedone, nitrobenzaldehyde and urea or thiourea by ASA acid catalyst. The compound was reduced to amine derivative with tin (II) chloride in ethanol. The amine containing dihyropyrimidinone compound was reacted with isocyanates and isothiocyanates to get the products (**4a–i**) at high yields.

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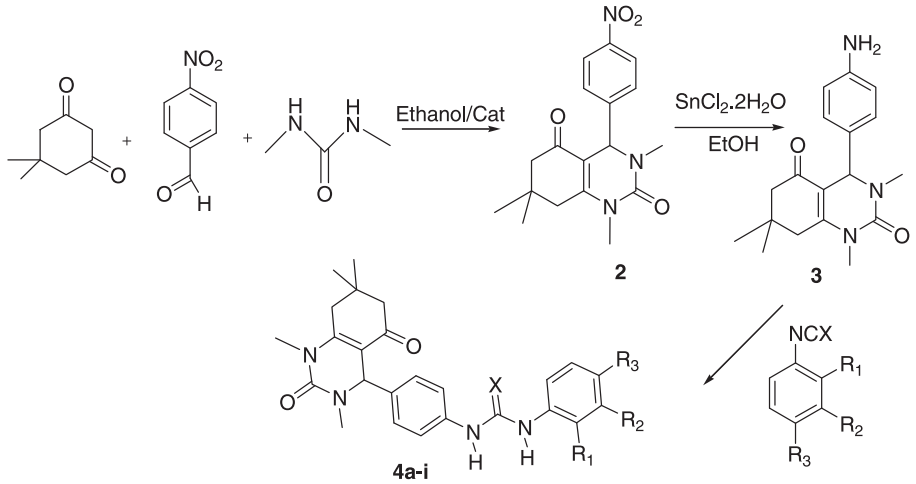
Scheme 1. Synthesis of 1,4-dihydropyrimidine substituted urea and thiourea derivatives.



	R_1	R_2	R_3	X
4a	H	H	H	O
4b	H	H	OCH_3	O
4c	H	H	F	O
4d	H	H	H	S
4e	H	H	NO_2	S
4f	H	CF_3	H	S
4g	H	OCH_3	H	S
4h	Cl	H	Cl	S
4i	H	F	H	S

The prepared compounds were characterized by ^{1}H-, ^{13}C-NMR, infrared (IR) and elemental analysis. The hydrogens attached to the nitrogen resonances between 8.00 and 10.40 ppm and were indicated from the ^{1}H-NMR spectra. The signals for aromatic hydrogens are between 6.50 and 8.50 ppm. The hydrogen next to the phenyl ring was observed around 5.00 ppm. From the ^{13}C-NMR spectra, ketone and urea carbonyl are seen between 200 and 150 ppm, respectively. In the IR spectra of compounds, it was possible to observe the absorptions between 3250 and 3450 cm^{-1} relating to NH stretching and absorptions in 1650–1750 cm^{-1} from urea carbonyl moiety stretching.

General

All starting materials and reagents were purchased from commercial suppliers. Reactions were monitored by TLC and TLC plates (Fluka, Taufkirchen, Germany) visualized with short-wave UV fluorescence ($k = 254$ nm). Melting points were taken on a Yanagimoto Barnstead Electrothermal (Surrey, UK) micro-melting point apparatus and were uncorrected. IR spectra were measured on a SHIMADZU Prestige-21 (200 VCE) (Kyoto, Japan) spectrometer. ^{1}H- and ^{13}C-NMR spectra were measured on spectrometer at Varian Infinity Plus 300 and at 75 Hz (California), respectively. ^{1}H- and ^{13}C-chemical shifts are referenced to the internal deuterated solvent. The elemental analysis was carried out with a Leco CHNS-932 (St. Joseph, Michigan) instrument. Flash column chromatography was performed using Merck silica gel 60 (230–400 mesh ASTM) (Darmstadt, Germany).

*General procedure 1: synthesis of 1,4-DHPM (**2**)*

A mixture of 4-nitrobenzaldehyde (3 mmol), dimedone (3 mmol), urea or thiourea (4.5 mmol) and ASA catalyst (7% mmol) in ethanol were finely mixed together in a test tube at 90 °C for one hour. After cooling, the reaction mixture was poured onto crushed ice (50 g) and stirred for 10 min. The precipitate was filtered under suction and washed with cold water (20 mL) to remove excess urea. Then, the solid was dissolved in ethanol and filtered to remove the catalyst and purified further by recrystallization (hot ethanol).

1,3,7,7-Tetramethyl-4-(4-nitrophenyl)-3,4,7,8-tetrahydroquinazoline-2,5(1H,6H)-dione (**2**): yield 88%, m.p. 145.5 °C; ^{1}H-NMR (DMSO-d_6; 300 MHz): 8.16(2H,d), 7.45(2H,d), 5.56(H,s), 3.25(3H,s), 2.97(3H,s), 2.40(2H,d,d), 2.20(2H,d,d), 1.10(3H,s) and 1.00(3H,s); ^{13}C-NMR(DMSO-d_6; 75 MHz):194.04, 153.43, 153.05, 146.28, 132.84, 127.35, 123.40, 118.87, 110.01, 58.42, 49.59, 40.31, 35.14, 33.03, 31.00 and 28.93; and IR (KBr, υ, cm^{-1}): 3042, 2964, 2875, 1690 and 1580.

*General procedure 2: reduction of nitro group (**3**)*

Compound **2** (3.24 mmol) and $SnCl_2$ (16.22 mmol) in ethanol (30 mL) were stirred under reflux for 4 h. When the reaction was completed, the solvent was evaporated under reduced pressure and the reaction mixture was diluted with water (15 mL), adjusted to pH = 10 with 1 M sodium hydroxide solution and extracted with ethyl acetate (3×25 mL^2). The organic phase was dried over $MgSO_4$ and filtered and purified.

4-(4-Aminophenyl)-1,3,7,7-tetramethyl-3,4,7,8-tetrahydroquinazoline-2,5(1H,6H)-dione (**3**): recrystallized from ether to give yellow crystals. Yield 75%, m.p. 180.6 °C; ^{1}H-NMR (300 MHz, $CDCl_3$, δ, ppm): 7.37(2H,d), 6.98(2H,d), 5.38(H,s), 4.47 (2H,s), 3.25(3H,s), 2.97(3H,s), 2.40(2H,d,d), 2.20(2H,d,d), 1.10(3H,s) and 1.00(3H,s); ^{13}C-NMR(75 MHz, $CDCl_3$, δ, ppm): 194.04, 153.43, 153.05, 128.28, 127.84, 127.35, 123.40, 118.87, 110.01, 58.42, 49.59, 40.31, 35.14, 33.03, 31.00 and 28.93 and IR (KBr, υ, cm^{-1}): 3022, 2962, 2870, 1628 and 1582.

*General procedure 3: synthesis of 1,4-DHPM substituted urea and thiourea derivatives (**4a–i**)*

A solution of 4-(4-aminophenyl)-1,3,7,7-tetramethyl-3,4,7,8-tetrahydroquinazoline-2,5(1H,6H)-dione (**3**) (0.644 mmol) and isocyanate or isothiocyanate derivatives (0.704 mmol) in toluene (20 mL) were stirred at 60 °C for 20 h. When the reaction was completed, the solvent was evaporated under reduced pressure and recrystallized from ether.

1-Phenyl-3-(4-(1,3,7,7-tetramethyl-5-oxo-1,2,3,4,5,6,7,8-octahydroquinazolin-4-yl)phenyl)urea (**4a**): yield: 82%, m.p: 150.6 °C; ^{1}H-NMR($CDCl_3$-d_1; 300 MHz): 7.90(H,s), 7.60(H,s), 7.45(2H,d), 7.40(H,d), 7.38(H,t), 7.35(2H,d), 7.30(H,d), 7.25 (H,t), 7.10(H,t), 5.25(H,s), 3.30(3H,s), 2.80(3H,s), 2.40(2H,d,d), 2.20(2H,d,d), 1.10(3H,s) and 0.98(3H,s); ^{13}C-NMR($CDCl_3$-d_1; 75MHz): 195.53, 153.75, 153.49, 153.40, 139.14, 138.922, 134.99, 129.25, 127.32, 123.18, 120.33, 119.57, 110.91, 58.59, 49.78, 40.38, 34.90, 33.18, 31.13, 28.87 and 28.61 and IR(KBr, υ, cm^{-1}): 3340, 2956, 1662, 1595. Anal. calcd for $C_{25}H_{30}N_4O_2$: C, 71.74; H, 7.22 and N, 13.39. Found: C, 71.62; H, 7.47 and N, 13.72.

1-(4-Methoxyphenyl)-3-(4-(1,3,7,7-tetramethyl-5-oxo-1,2,3,4, 5,6,7,8-octahydroquina zolin-4-yl)phenyl)urea (**4b**): yield: 85%, m.p.: 134.2 °C; ^{1}H-NMR($CDCl_3$-d_1) (300 MHz): 8.01(H,s), 7.62(H,s), 7.20(2H,d), 7.15(2H,d), 6.85(2H,d), 6.70(2H,d), 5.22(H,s), 3.80(3H,s), 3.30(3H,s), 2.80(3H,s), 2.40(2H,d,d), 2.20(2H,d,d), 1.10(3H,s) and 0.98(3H,s); ^{13}C-NMR($CDCl_3$-d_1; 75 MHz): 195.49, 160.47, 153.73, 153.44, 153.41, 140.44, 139.99, 138.82, 135.10, 129.87, 127.32, 120.09, 110.92, 58.58, 55.45, 49.76, 40.37, 34.87, 33.14, 31.08, 28.84 and 28.58 and IR(KBr, υ, cm^{-1}): 3307, 2954, 1595, and 1539. Anal. calcd for $C_{26}H_{32}N_4O_3$: C, 66.62; H, 7.19 and N, 12.49. Found: C, 67.12; H, 7.57 and N, 12.82.

1-(4-Fluorophenyl)-3-(4-(1,3,7,7-tetramethyl-5-oxo-1,2,3,4,5,6, 7,8-octahydroquinazolin-4-yl)phenyl)urea (**4c**): yield: 90%, m.p.: 164.7 °C; ^{1}H-NMR($CDCl_3$-d_1; 300 MHz): 7.80(H,s), 7.42(H,s), 7.35(2H,d), 7.30(2H,d), 7.10(2H,d), 6.92(2H,d), 5.20(H,s), 3.20(3H,s), 2.80(3H,s), 2.42(2H,d,d), 2.20(2H,d,d), 1.10(3H,s) and 0.98(3H,s); ^{13}C-NMR($CDCl_3$-d_1; 75 MHz): 195.49, 165.47, 154.73, 153.44, 153.41, 145.44, 140.99, 138.82, 137.10, 129.87, 127.32, 122.09, 110.92, 58.58, 55.45, 49.76, 40.37, 34.87, 33.14, 31.08, 28.84 and 28.58 and IR(KBr, υ, cm^{-1}): 3340, 2960, 1602 and 1504. Anal. calcd for $C_{25}H_{29}FN_4O_2$: C, 68.79; H, 6.70 and N, 12.83. Found: C, 68.62; H, 6.37 and N, 12.52.

1-Phenyl-3-(4-(1,3,7,7-tetramethyl-5-oxo-1,2,3,4,5,6,7,8-octahydroquinazolin-4-yl)-phenyl)thiourea (**4d**): yield: 78%, m.p.: 211.7 °C, ^{1}H-NMR (DMSO-d_6; 300 MHz): 9.76(H,s), 9.73(H,s), 7.45(2H,d), 7.40(H,d), 7.38(H,t), 7.35(H,t), 7.30(H,d), 7.25(H,t), 7.10(H,t), 5.20(H,s), 3.30(3H,s), 2.85(3H,s), 2.40(2H,d,d), 2.20(2H,d,d), 1.10(3H,s) and 0.98(3H,s); ^{13}C-NMR(DMSO-d_6; 75 MHz): 193.54, 180.03, 153.54, 153.15, 140.04, 139.50, 137.49, 128.99, 127.26, 124.99, 124.23, 124.05, 110.06, 65.69, 58.09, 49.71, 34.69, 33.02, 30.89, 28.85 and 28.58 and IR (KBr, υ, cm^{-1}): 3332, 3188, 1672 and 1593. Anal. calcd for $C_{25}H_{30}N_4OS$: C, 69.09; H, 6.96; N, 12.89 and S, 7.38. Found: C, 69.61; H, 7.37; N, 13.51 and S, 7.63.

1-(4-Nitrophenyl)-3-(4-(1,3,7,7-tetramethyl-5-oxo-1,2,3,4,5,6, 7,8-octahydroquinazolin-4-yl)phenyl)thiourea (**4e**): yield: 88%, m.p.: 209.8 °C; ^{1}H-NMR (DMSO-d_6; 300 MHz): 10.40(H,s), 10.20(H,s), 8.20(2H,d), 7.80(2H,d), 7.40(2H,s), 7.20(2H,d), 5.22(H,s), 3.20(3H,s), 2.80(3H,s), 2.60(2H,d,d), 2.10(2H,d,d), 1.10(3H,s) and 0.98(3H,s); ^{13}C-NMR(DMSO-d_6; 75 MHz): 193.66, 179.80, 153.81, 153.17, 146.91, 142.91, 139.04, 138.23, 127.36, 125.05, 124.17, 122.17, 109.98, 79.64, 58.04, 49.68, 34.70, 33.00, 30.91, 28.85 and 28.46 and IR(KBr, υ, cm^{-1}): 3319, 3186, 1620 and 1506. Anal. calcd for $C_{25}H_{29}N_5O_3S$: C, 62.61; H, 6.09; N, 14.60 and S, 6.69. Found: C, 62.02; H, 6.27; N, 13.92 and S, 6.18.

1-(4-(1,3,7,7-Tetramethyl-5-oxo-1,2,3,4,5,6,7,8-octahydroquinazolin-4-yl)phenyl)-3-(3-(trifluoromethyl)phenyl)thiourea (**4f**): yield: 80%, m.p.: 210.1 °C; ^{1}H-NMR (DMSO-d_6; 300 MHz): 10.01(H,s), 10.08(H,s), 7.85(H,s), 7.70(H,d), 7.52(H,t), 7.45(H,d), 7.38(2H,d), 7.20(2H,d), 5.20(H,s),3.20(3H,s), 2.80(3H,s), 2.60(2H,d,d), 2.10(2H,d,d) and 1.10(3H,s); ^{13}C-NMR (DMSO-d_6; 75 MHz): 194.54, 183.03, 154.54, 153.15, 142.04, 140.50, 138.49, 129.99, 128.26, 124.99, 124.83, 124.75, 110.06, 65.69, 58.09, 49.71, 34.69, 33.02, 30.89, 28.85 and 28.58 and IR(KBr, υ, cm^{-1}): 3320, 3170, 1615 and 1555. Anal. calcd for $C_{26}H_{29}F_3N_4OS$: C, 62.13: H, 5.82; N, 11.15 and S, 6.38. Found: C, 62.52; H, 6.23; N, 12.02 and S, 6.78.

1-(3-Methoxyphenyl)-3-(4-(1,3,7,7-tetramethyl-5-oxo-1,2,3,4, 5,6,7,8-octahydroquina zolin-4-yl)phenyl)thiourea (**4g**): yield: 82%, m.p.: 210.2 °C; ^{1}H-NMR (DMSO-d_6; 300 MHz): 9.78 (H,s), 9.81(H,s), 7.40(2H,d), 7.30(H,s), 7.28(H,t), 7.20(2H,d), 6.98(H,d), 6.82(H,d), 5.20(H,s), 3.20(3H,s), 2.80(3H,s), 2.42 (2H,d,d), 2.20(2H,d,d), 1.10(3H,s) and 0.98(3H,s); ^{13}C-NMR (DMSO-d_6; 75 MHz): 193.53, 179.83, 159.91, 153.52, 153.17, 141.14, 139.48, 137.56, 129.75, 127.24, 124.11, 116.12, 110.08, 58.11, 55.65, 49.73, 34.69, 33.01, 30.88, 28.87 and 28.55 and IR(KBr, υ, cm^{-1}): 3161, 2960, 1660 and 1635. Anal. calcd for $C_{26}H_{32}N_4O_2S$: C, 67.21; H, 6.94; N, 12.06 and S, 6.90. Found: C, 67.62; H, 7.27; N, 12.52 and S, 7.45.

1-(2,4-Dichlorophenyl)-3-(4-(1,3,7,7-tetramethyl-5-oxo-1,2,3,4, 5,6,7,8-octahydroquinazolin-4-yl)phenyl)thiourea (**4h**): yield: 78%, m.p.: 210.9 °C; ^{1}H-NMR (DMSO-d_6; 300 MHz): 10.01 (H,s), 10.10(H,s), 7.62(H,s), 7.42(H,s), 7.40(2H,d), 7.33(H,s), 7.20(2H,d), 5.20(H,s), 3.20(3H,s), 2.80(3H,s), 2.42(2H,d,d), 2.20(2H,d,d), 1.10(3H,s) and 0.98(3H,s); ^{13}C-NMR(DMSO-d_6; 75 MHz): 193.65, 180.11, 153.89, 153.21, 142.81, 139.03, 138.17, 134.11, 129.59, 128.89, 127.53, 124.44, 123.94, 122.12, 109.94, 58.01, 49.71, 34.72, 33.02, 30.91, 28.84 and 28.49 and IR(KBr, υ, cm^{-1}): 3188, 2960, 1660 and 1635. Anal. calcd for $C_{25}H_{28}Cl_2N_4OS$: C, 59.64; H, 5.61; N, 11.13 and S, 6.37. Found: C, 59.34; H, 5.67; N, 11.52 and S, 5.98.

1-(3-Fluorophenyl)-3-(4-(1,3,7,7-tetramethyl-5-oxo-1,2,3,4,5,6, 7,8-octahydroquinazolin-4-yl)phenyl)thiourea (**4i**): yield: 80%, m.p.: 184.2 °C; ^{1}H-NMR (DMSO-d_6; 300 MHz): 10.01(H,s), 9.98(H,s), 7.52(H,d), 7.40(2H,d), 7.32(H,t), 7.22(2H,d), 7.20 (H,s), 6.98(H,d), 5.20(H,s), 3.20(3H,s), 2.80(3H,s), 2.42(2H,d,d), 2.20(2H,d,d) and 1.10(3H,s); ^{13}C-NMR(DMSO-d_6; 75 MHz): 193.66, 180.00, 164.04, 160.84, 153.86, 153.21, 141.91, 139.30,

137.86, 130.57, 127.41, 124.32, 119.57, 110.41, 58.01, 52.95, 49.69, 34.71, 33.01, 30.91, 28.84 and 28.48 and IR(KBr, υ, cm^{-1}): 3329, 2960, 1670 and 1591. Anal. calcd for $C_{25}H_{29}FN_4OS$: C, 66.34; H, 6.46; N, 12.38 and S, 7.08. Found: C, 66.65; H, 6.67; N, 12.82 and S, 7.30.

Preparation of haemolysate and purification from red blood cells

Blood samples (25 mL) were taken from healthy human volunteers. They were anticoagulated with acid-citrate-dextrose, centrifuged at 2000 *g* for 20 min at 4 °C and the supernatant was removed. The packed erythrocytes were washed three times with 0.9% NaCl and then haemolysed in cold water. The ghosts and any intact cells were removed by centrifugation at 2000 *g* for 25 min at 4 °C and the pH of the haemolysate was adjusted to pH 8.5 with solid Tris-base. The 25 mL haemolysate was applied to an affinity column containing L-tyrosine-sulphonamide-sepharose-4B[20] equilibrated with 25 mM Tris–HCl/0.1 M Na_2SO_4 (pH 8.5). The affinity gel was washed with 50 mL of 25 mM Tris–HCl/22 mM Na_2SO_4 (pH 8.5). The hCA isozymes were then eluted with 0.1 M NaCl/25 mM Na_2HPO_4 (pH 6.3) and 0.1 M CH_3COONa/0.5 M $NaClO_4$ (pH 5.6), which recovered hCA I and II, respectively. Fractions of 3 mL were collected and their absorbance measured at 280 nm.

CA enzyme assay

CA 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]. The assay solution was 0.5 M Na_2CO_3/0.1 M $NaHCO_3$ (pH 10.0) and phenol red was added as the pH indicator. CO_2-hydratase activity (enzyme units) was calculated using the equation t_0-t_c/t_c where t_0 and t_c are the times for pH change of the nonenzymatic and the enzymatic reactions, respectively.

In vitro inhibition studies

For the inhibition studies of sulphanilamide, different concentrations of these compounds were added to the enzyme. Activity percentage values of CA for different concentrations of each sulphanilamide were determined by regression analysis using Microsoft Office 2000 Excel (New York, NY). CA enzyme activity without a synthesized compounds solution was accepted as 100% activity.

Results and discussion

For evaluation, the physiologically relevant human CA isozyme hCA I and II inhibitory activity, several new diaryl urea and thiourea compounds were subjected to CA inhibition assay with CO_2 as substrate.

Sulphonamides are coordinated to the zinc (II) ion within the hCA active site, whereas its organic scaffold fills the entire enzyme cavity, making an extensive series of van der Waals and polar interactions with amino acid residues both at the bottom, middle and entrance of the active site cavity[22]. Coumarins/thiocoumarins may possess various tautomeric forms, such as the zwitterionic benzo(thio)pyrylium phenoxides, which may bind within the CA active site similarly to phenols, i.e. by enchoring to the zinc-bound water molecule/hydroxide ion[23]. Coumarins cannot bind enzyme effectively in the restricted space near Zn^{2+} ion due to its bulky pendant group and exhibit unusual binding mode not interacting with the metal ion of the enzyme[24,25]. The synthesized compounds are very bulky to be able to bind near the zinc ion and they much more probably bind as the coumarins and activators.

Table 1. The IC_{50} (μM) values of compounds (**4a–i**).

Compounds	hCA I	hCA II
4a	135.26	153.01
4b	153.15	156.96
4c	66.23	142.11
4d	136.11	88.94
4e	197.70	155.28
4f	105.86	63.09
4g	164.13	169.71
4h	132.19	151.09
4i	130.30	89.21

The results showed that all the compounds (**4a–i**) inhibited enzyme activity. The inhibition constants of the synthesized compounds against CAs are given in Table 1. The following structure–activity relationship observations can be drawn from the data.

(i) The slow cytosolic isoform hCA I was weakly inhibited by the 1,4-DHPM substituted diaryl urea and thiourea derivatives with inhibition constants in the range 66.23–197.70 μM. The best hCA I inhibitor among the newly synthesized and investigated compounds was 4-flouro substituted derivative (**4c**). Fluorinated molecule can bind to a hydrolytic enzyme as a covalent adduct with an active site nucleophile, effectively binding as a transition state analogue[26]. It is obviously clear that bulky groups (such as nitro and methoxy) on the phenyl ring affect inhibition due to steric effect and IC_{50} values are higher than 150 μM.

(ii) The second off target isoform, hCA II, which is in fact the physiologically dominant cytosolic isozyme, was also weakly inhibited by all the compounds, with inhibition constants in the region of 63.09–169.71 μM. The best hCA II inhibitor among the newly synthesized and investigated compounds was 4-(triflouro substituted) phenyl derivative (**4f**). From the values given in Table 1, flouro substituted urea derivatives showed more inhibitory effect than nitro, chloro, methoxy and unsubstituted ureas. Flourophenyl sulphamate adducts were reported that the sulphomates possess a rather variable binding pattern within the hCA II active site[26,27].

The clinically used sulphonamides are low micromolar to low nanomolar inhibitors, whereas the compounds in this study are millimolar CAIs. For hCA II, the clinical used sulphonamides are stronger inhibitory effect than the synthesized compounds[7].

Enzyme inhibition studies are important issue for drug design and biochemical applications[28–39]. The results showed that 1,4-dihydropyrimidine substituted urea and thiourea derivatives inhibited the hCA I and II enzyme activities. The compounds have weak inhibitory effects and may be taken for further evaluation *in vivo* studies.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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