Bioorganic Chemistry 103 (2020) 104171

Contents lists available at ScienceDirect

Bioorganic Chemistry

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

Synthesis of nitrogen, phosphorus, selenium and sulfur-containing heterocyclic compounds – Determination of their carbonic anhydrase, acetylcholinesterase, butyrylcholinesterase and α-glycosidase inhibition properties

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ARTICLE INFO

Keywords:
Pyrrole
Carbonic anhydrase
Enzyme inhibition
α-Glycosidase
Acetylcholinesterase, Butyrylcholinesterase

ABSTRACT

Sulfur-containing pyrroles (**1–3**), tris(2-pyridyl)phosphine(selenide) sulfide (**4–5**) and 4-benzyl-6-(thiophen-2-yl)pyrimidin-2-amine (**6**) were synthesized and characterized by elemental analysis, IR and NMR spectra. In this study, the synthesized compounds of nitrogen, phosphorus, selenium and sulfur-containing heterocyclic compounds (**1–6**) were evaluated against the human erythrocyte carbonic anhydrase I, and II isoenzymes, acetylcholinesterase (AChE), butyrylcholinesterase (BChE), and α-glycosidase enzymes. The synthesized heterocyclic compounds showed IC_{50} values in range of 33.32–60.79 nM against hCA I, and 37.05–66.64 nM against hCA II closely associated with various physiological and pathological processes. On the other hand, IC_{50} values were found in range of 13.13–22.21 nM against AChE, 0.54–31.22 nM against BChE, and 13.51–26.55 nM against α-glycosidase as a hydrolytic enzyme. As a result, nitrogen, phosphorus, selenium and sulfur-containing heterocyclic compounds (**1–6**) demonstrated potent inhibition profiles against indicated metabolic enzymes. Therefore, we believe that these results may contribute to the development of new drugs particularly in the treatment of some global disorders including glaucoma, Alzheimer's disease and diabetes.

1. Introduction

Pyrroles are widely used in medicinal chemistry and drug discovery. Pyrrole derivatives are found in varieties of biological context as part of co-factors and natural products. Common naturally produced molecules containing pyrrole nucleus include Vitamin B12, bile pigments like bilirubin and biliverdin, porphyrins of haeme, chlorophyll, chlorines, bacteriochlorines and porphyrinogens [1]. The attention to the functionalized pyrroles is now growing because some of them exhibit a large spectrum of bioactivities including carbonic anhydrase isoenzyme inhibition effects [2–4].

The electron-rich nitrogen heterocycles, for instance pyrroles, pyridines, pyrimidines, are not only able to readily accept or donate a proton, but they can also easily establish diverse weak interactions. Some of these intermolecular processes such as like hydrogen bonding formation, dipole-dipole interactions, hydrophobic effects, van der Waals forces and π-stacking interactions of nitrogen compounds have increased their importance in the field of medicinal chemistry and allows them to bind with a variety of enzymes and receptors in biological targets with high affinity due to their improved solubility [5].

Pyrrole-containing analogs are considered as a potential source of biologically active compounds that contains a significant set of

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https://doi.org/10.1016/j.bioorg.2020.104171
Received 7 April 2020; Received in revised form 13 July 2020; Accepted 25 August 2020
Available online 26 August 2020

advantageous properties and can be found in many natural products. The marketed drugs containing a pyrrole ring system are known to have many biological properties such as antipsychotic, β-adrenergic antagonist, anxiolytic, anticancer, antibacterial, antifungal, antiprotozoal, antimalarial and many more [6a]. Pyrrole derivatives, pyrrolizines, constitute a class of pyrrolic compound, which can serve as promising scaffolds for anticancer drugs. For instance, mitomycin C has antitumor activities against a wide variety of cancer types [6b]. Pyrroles decorated with sulfur-containing functionalities are a rarity. Pyrroles bearing thioester functions (S-alkyl, $R^1C(O)SR^2$) are amongst the best known, courtesy of the critical role that thioesterase-appended pyrroles play in the biosynthesis of secondary metabolites containing pyrroles [7a,7b].

Carbonic anhydrases (CAs, E.C.4.2.1.1) are zinc-containing metalloenzymes. They catalyzes the reversibly conversion of carbon dioxide (CO_2) and water to bicarbonate (HCO_3^-) and proton (H^+) ions [8–10]. In the mammal, when CO_2 in the blood plasma passes into red blood cells through diffusion, it is rapidly converted into carbonic acid with the CA enzyme. CAs are found in both eukaryotic and prokaryotic cells and encoded by eight distinct gene families including α-, β-, γ-, δ-, ζ-, η-, θ-, and *t*-CAs [11–13]. However, only the α-CA family is found in mammals [14–16]. The human carbonic anhydrases (hCAs) are belonging to the α-subfamily of CAs. This subfamily divided into sixteen diverse isoforms. They have different catalytic activity, amino acid sequence, kinetic properties, cellular and tissue distributions, biochemical properties, subcellular localization and sensitivity to inhibitors or activators [17–19]. α-CAs are grouped as cytoplasmic isoenzymes including CAs I, II, III, VII and XIII, membrane bound CAs including IV, IX, XII, XIV and XV. CA IV is a glycosylphosphatidylinositol (GPI)-anchored protein. CA V is mitochondrial isoenzyme. CA VI is secretory CA isoform. Lastly, CAs VIII, X and XI are CA-related proteins (CARPs). However, CARPs have not performed CO_2 hydration activity and physiological function [20–22]. CA inhibitors (CAIs) show numerous bioactivities linked to many global diseases such as glaucoma, obesity, cancer, epilepsy and osteoporosis [23–26]. Due to these important physiological functions, numerous studies have been performed on CAs. Both CA I, and CA II are the most studied and examined isoenzymes [27–29]. CA I is expressed in erythrocytes and the gastrointestinal tract, on the other hand, CA II is expressed in almost all tissues mainly including eye, erythrocytes, gastrointestinal tract, bone osteoclasts, kidney, brain, testis and lung [30,31]. CA II isoenzyme has a single chain protein including 295 amino acids with nearly 29 kDa molecular weight [32–34]. Despite significant and increasing improvements in CAIs, there are many difficulties such as high levels of isoenzymes in tissues and CA inhibitors selectivity [35–37].

Dementia is one of the fastest spreading diseases in the world. Alzheimer's disease (AD) leads to emotional trouble and escorted by abnormality in cholinergic neurotransmission of the central nervous system [38–41]. The pathophysiology of AD is relevant to the depletion of the neurotransmitter acetylcholine (ACh), associated with AChE activity. AChE (E.C.3.1.1.7) hydrolyzes the neurotransmitter ACh [42–45]. Butyrylcholinesterase (BChE, E.C.3.1.1.8) created inside liver, consider as a backup for the analogous AChE. Also, BChE had a crucial role in the cholinergic mechanism, and its inhibition improved the cognitive functions. Both cholinergic enzymes share some structural similarities, containing the presence of a catalytic center. The searches of new cholinesterase inhibitors sound a significant strategy to familiarize new drug candidates against AD and related dementias [46–50].

Diabetes mellitus (DM) was known as a popular metabolic degenerative disorder, is characterized by a high level of blood glucose. Antidiabetic treatments diagnose that determining blood glucose amounts are effective therapy for diabetes [51–53]. α-Glycosidases are found on the brushy surface of the small intestine and responsible for the breakdown of complex carbohydrate polymers [54–56]. Also, they divide disaccharides and oligosaccharides into monosaccharides, which easily absorbed from the intestinal walls. α-Glycosidase inhibitor delayed or blocked the digestion mechanisms of carbohydrates, but does not cause malabsorption [57–59].

In the present work, we have studied a series of recently synthesized, earlier unknown nitrogen, sulfur, selenium and phosphorus polyfunctional compounds, including pyrrolecarbodithioates, aminopyrimidine, tris(2-pyridyl)phosphinesulfide and –selenide as potential functional biological active compounds. Mechanism of their action was investigated and relationship between structure and efficiency of their biological activity was estimated. Especially, in the light of this information, the aim of this study was to investigate the inhibition impacts of nitrogen, phosphorus, selenium and sulfur-containing heterocyclic compounds (**1–6**) on hCA I, and hCA II isoenzymes, AChE, BChE, and α-glycosidase enzymes. Also, their inhibition profiles were compared to the standard compounds like acetazolamide, tacrine and acarbose as a clinical used inhibitors.

2. Material and methods

2.1. Chemistry

2.1.1. General information

IR spectra were recorded in KBr pellets or film on a Bruker JFS-25 spectrometer in the 400–4000 cm^{-1} region. ^1H, ^{13}C, ^{15}N, ^{31}P and ^{77}Se NMR spectra were recorded at room temperature on a Bruker-DPX-400 instrument with an operating frequency of 400.13 (^1H), 100.62 (^{13}C), 40.5 (^{15}N), 162.0 (^{31}P), 77.0 (^{77}Se) MHz, the solvent is DMSO-d_6 and $CDCl_3$. ^1H and ^{13}C NMR chemical shifts were recorded relative to residual solvent signals ($CDCl_3$, δ 7.27 and 77.0 ppm, respectively, or DMSO, δ 2.50 and 39.5 ppm, respectively). Elemental analysis was performed on a Flash EA 1112 Series analyzer.

2.1.2. Ethyl 5-phenyl-1H-pyrrole-2-carbodithioate (1)

A mixture of 2-phenylpyrrole (1.43 g, 10 mmol) and $KOH{\cdot}5H_2O$ (1.30 g, 20 mmol) in DMSO (20 mL) is stirred for 30 min and carbon disulfide (1.52 g, 20 mmol) is added. The reaction mixture is allowed to stand at room temperature for 2 h and then ethyl iodide (1.56 g, 10 mmol) is added. The reaction mixture is stirred for 2 h, then diluted with water (40 mL) and extracted with diethyl ether. After removing the solvent the residue is passed through column (Al_2O_3, hexane-diethyl ether, 1:1) to afford: 0.2 g of diethyl disulfide; 0.7 g (24%) of ethyl 5-phenyl-1*H*-pyrrole-1-carbodithioate and 1.46 g (59%) of target ethyl 5-phenyl-1*H*-pyrrole-2-carbodithioate as yellow crystals, mp 67–68 °C. ^1H NMR (400 MHz, DMSO-d_6): 11.79 (br s, 1H, NH), 7.93–7.91 (m, 2H, H_o Ph), 7.44–7.42 (m, 2H, H_m Ph), 7.34–7.33 (m, 1H, H_p Ph), 7.19 (dd, J = 3.6, 2.4 Hz, H-3 pyrrole), 6.79 (dd, J = 3.6, 2.6 Hz, H-4 pyrrole), 3.33 (q, J = 7.5 Hz, 2H, SCH_2), 1.30 (t, J = 7.5 Hz, Me). ^{13}C NMR (101 MHz, DMSO-d_6): 206.0 (C=S), 141.8 (C-*i*), 140.5 (C-5), 130.8 (C-2), 129.0 (C-*m*), 128.6 (C-*p*), 126.3 (C-*o*), 115.0 (C-4), 110.6 (C-3), 28.6 (SCH_2), 13.5 (Me).

2.1.3. 2-[(Ethylsulfanyl)(4,5,6,7-tetrahydro-1H-indol-2-yl)methylene] malononitrile (2)

Malonodinitrile (0.99 g, 15 mmol), $KOH{\cdot}5H_2O$ (0.98 g, 15 mmol) and DMSO (50 mL) are stirred at room temperature for 0.5 h, then ethyl 4,5,6,7-tetyrahydroindole-carbodithioare (2.25 g, 10 mmol) is added and the mixture is heated at 108–110 °C for 1.5 h. The reaction mixture is diluted with water (1:3), the crystals formed are filtered off and dried to afford 2.26 g (88%) of 2-[(ethylsulfanyl)(4,5,6,7-tetrahydro-1*H*-indol-2-yl)methylene]malononitrile, mp 138–139 °C (methanol). ^1H (DMSO-d_6): 11.81 (br s, 1H, NH), 6.93 (d, J = 1.8 Hz, 1H, H-3), 3.05 (q, J = 7.4 Hz, 2H, SCH_2), 2.67–2.66 (m, 2H, CH_2-7), 2.50–2.49 (m, 2H, CH_2-4), 1.74–1.73 (m, 2H, CH_2-6), 1.69–1.68 (m, 2H, CH_2-5), 1.18 (t, J = 7.4 Hz, 3H, Me). ^{13}C ($CDCl_3$-d_3): 159.3 (=<u>C</u>-SEt), 141.6 (C-5), 126.1 (C-2), 124.8 (C-4), 120.8 (C-3), 116.6 (CN), 115.5 (CN), 68.2 [=<u>C</u>$(CN)_2$)], 31.2 (SCH_2), 23.5 (CH_2-7), 23.0 (CH_2-5), 22.7 (CH_2-6), 22.4 (CH_2-4), 14.6 (SCH_2<u>Me</u>).

2.1.4. 3-Imino-1-ethylsulfanyl-5,6,7,8-tetrahydro-3H-pyrrolo[1,2–a] indole-2-carboxamide (3)

Solution of 2-cyano-3-(ethylsulfanyl)-3-(4,5,6,7-tetrahydro-1*H*-indol-2-yl)acrylamide (0.55 g, 2 mmol) in methanol (10 mL) is heated in the presence of triethylamine (2–3 drops) for 10 h and cooled to room temperature. The crystals formed are filtered off, washed with diethyl ether and dried to afford 3-imino-1-ethylsulfanyl-5,6,7,8-tetrahydro-3*H*-pyrrolo[1,2–a]indole-2-carboxamide (0.25 g, 46%), mp 190–192 °C. ^{1}H NMR ($CDCl_3$-d_3): 8.51 (br s, 1H, $CONH_2$), 7.76 (br s, 1H, =NH), 6.16 (s, 1H, pyrrole), 5.42 (br s, 1H, $CONH_2$), 3.19 (q, J = 7.2 Hz, SCH_2), 2.72–2.70 (m, 2H, CH_2-7), 2.47–2.45 (m, 2H, CH_2-4), 1.90–1.88 (m, 2H, CH_2-5), 1.78–1.76 (m, 2H, CH_2-6), 1.43 (t, J = 7.2 Hz, Me). ^{13}C NMR ($CDCl_3$-d_3): 165.15 (CO), 157.79 (C-1), 155.58 (C-3), 131.21 (C-4), 129.45 (C-7), 125.93 (C-5), 113.91 (C-6), 112.76 (C-2), 26.2 (SCH_2), 22.9, 22.7, 22.2, 22.1 (CH_2-4–7), 14.3 (SCH_2Me).

2.1.5. Tris(2-pyridyl)phosphine sulfide (4)

Tris(2-pyridyl)phosphine (0.521 g, 2 mmol) was dissolved in dichloromethane (10 mL) and elemental selenium was added (0.155 g, 2.01 mmol) at room temperature. The reaction mixture was stirred. The reaction was completed in 2 h with higher yield. The solution was filtered and solvent removed. The white crystalline product was crystallized from ethanol, and dried on air. The purity of the tri(2-pyridyl) phosphine selenide was checked by IR, ^{1}H, ^{13}C, ^{31}P, ^{77}Se NMR spectra and melting point. Yield 95%, m.p. 175 °C; lit. 176–178 °C (EtOH). Found: C, 52.3; H, 3.5; N, 12.5; P, 9.1; Se, 22.9%. Anal. Calcd. for: $C_{15}H_{12}N_3PSe$. C, 52.3; H, 3.5; N, 12.2; P, 9.0; Se, 22.9%. ^{1}H NMR (400 MHz, $CDCl_3$), δ ppm: 7.31 (dddd 3H, $^{3}J_{HH}$ = 7.8 Hz, $^{3}J_{HH}$ = 4.7 Hz, $^{3}J_{HH}$ = 3.1 Hz, $^{4}J_{HH}$ = 1.2 Hz, H^5 Py), 7.79 tdd (3H, H^4-Py, $^{3}J_{HH}$ = 7.8 Hz, $^{3}J_{HH}$ = 4.7 Hz, $^{4}J_{HH}$ = 1.8 Hz), 8.30 ddd (3H, H^3-Py, $^{3}J_{HH}$ = 7.8 Hz, $^{3}J_{HP}$ = 6.8 Hz, $^{4}J_{HH}$ = 1.2 Hz), 8.69 br.d (3H, H^6-Py, $^{3}J_{HH}$ = 4.8 Hz). ^{13}C NMR (100.62 MHz, $CDCl_3$): δ = 124.88 (C^5-Py, $^{4}J_{PC}$ = 3.2 Hz), 129.22 (C^3-Py, $^{2}J_{PC}$ = 26.0 Hz), 136.17 (C^4-Py, $^{3}J_{PC}$ = 10.5 Hz), 149.86 (C^6-Py, $^{3}J_{PC}$ = 19.0 Hz), 154.12 (C^2-Py, $^{1}J_{PC}$ = 106.0 Hz). ^{31}P NMR (161.98 MHz, $CDCl_3$): δ = 30.13 ppm, ($^{1}J_{PSe}$ = 732.6 Hz). ^{77}Se NMR (77.0 MHz, $CDCl_3$): δ = -307.2 ppm, ($^{1}J_{PSe}$ = 746.5 Hz). IR (KBr pellet, cm^{-1}): 3063, 1566, 1449, 1420, 1282, 1124, 1050, 985, 772, 734, 560, 505, 450.

2.1.6. Tris(2-pyridyl)phosphine selenide (5)

Tris(2-pyridyl)phosphine (0.521 g, 2 mmol) was dissolved in dichloromethane (10 mL) and elemental sulfur was added (0.064 g, 2.01 mmol) at room temperature. The reaction mixture was stirred. The reaction was completed in 2 h with higher yield. The solution was filtered and solvent removed. The white crystalline product was crystallized from isopropanol, and dried on air. The purity of the tri(2-pyridyl) phosphine sulfide was checked by IR, ^{1}H, ^{13}C, ^{31}P NMR spectra and melting point. Yield 95%, m.p. 158–160 °C (isoPrOH); Yield 95%, lit. 160 °C ($CHCl_3$) [2]. Found: C, 60.7; H, 4.0; N, 14.3; P, 19.1; S, 10.9%. Anal. Calcd. for: $C_{15}H_{12}N_3PS$. C, 60.6; H, 4.1; N, 14.1; P, 19.4; S, 10.8%. ^{1}H NMR (400 MHz, $CDCl_3$), δ ppm: 7.32 (dddd 3H, $^{3}J_{HH}$ = 7.8 Hz, $^{3}J_{HH}$ = 4.8 Hz, $^{4}J_{HH}$ = 1.2 Hz, $^{5}J_{HH}$ = 2.9 Hz, H^5 Py), 7.77 tdd (3H, H^4-Py, $^{3}J_{HH}$ = 7.8 Hz, $^{4}J_{PH}$ = 4.6 Hz, $^{4}J_{HH}$ = 1.7 Hz), 8.25 ddd (3H, H^3–Py, $^{3}J_{HH}$ = 7.8 Hz, $^{3}J_{HP}$ = 6.5 Hz, $^{4}J_{HH}$ = 1.0 Hz), 8.70 br. d (3H, H^6-Py, $^{3}J_{HH}$ = 4.8 Hz). ^{13}C NMR (100.62 MHz, $CDCl_3$), δ: 124.90 (C^5- Py, $^{4}J_{PC}$ = 3.3 Hz), 128.56 (C^3– Py, $^{2}J_{PC}$ = 25.0 Hz), 136.04 (C^4-Py, $^{3}J_{PC}$ = 10.2 Hz), 149.87 (C^6-Py, $^{3}J_{PC}$ = 19.3 Hz), 155.05 (C^2– Py, $^{1}J_{PC}$ = 114.6 Hz). ^{31}P NMR (161.98 MHz, $CDCl_3$): δ = 34.8 ppm. IR (KBr pellet, cm^{-1}): 3037, 2986, 1571, 1449, 1419, 1279, 1235, 1208, 1161, 1051, 1131, 1085, 1042, 987, 774, 742, 731, 661, 614, 549, 519, 473, 438, 394.

2.1.7. 4-Benzyl-6-(thiophen-2-yl)pyrimidin-2-amine (6)

A mixture of 2-acethylthiophene (0.631 g, 5.0 mmol), phenylacetylene (0.510 g, 5.0 mmol), and KOBut (0.673 g, 6.0 mmol) in DMSO (10 mL) was heated (100 °C) and stirred at 100 °C for 30 min. After cooling (70 °C), H_2O (0.090 g, 5.0 mmol) and $(NH_2)_2C$ = NH·HCl (0.573 g, 6.0 mmol) were added to the reaction mixture and stirred at 70 °C for 0.5 h. Then KOH·0·.5H_2O (0.325 mg, 5.0 mmol) was added and the mixture was stirred at 70 °C for 30 min. The reaction mixture, after cooling at room temperature, was diluted with H_2O (15 mL), neutralized with NH_4Cl and was extracted with $CHCl_3$ (10 mL × 4). The organic extract was washed with H_2O (5 mL × 3) and dried ($MgSO_4$). $CHCl_3$ was evaporated in vacuum and the residue was purified by column chromatography (Al_2O_3, eluent C_6H_6/Et_2O with gradient from 1:0 to 10:1). 4-Benzyl-6-(thiophen-2-yl)pyrimidin-2-amine was isolated as a cream solid (0.401 g, 30% yield); m.p. 180–185 °C; elemental analysis calcd (%) for $C_{15}H_{13}N_3S$ (267.35): C 67.39%; H 4.90%; N 15.72%; S 11.99%; found: C 67.37%; H 4.91%; N 15.62%; S 11.94%. IR (film): ν_{max} 3485, 3289, 3166, 3100, 3083, 3028, 2920, 1622, 1573, 1556, 1548, 1518, 1494, 1459, 1446, 1416, 1366, 1342, 1227, 1194, 1172, 1074, 1044, 1030, 908, 862, 839, 826, 791, 736, 728, 707, 622, 608, 577, 568, 520 cm^{-1}; ^{1}H NMR (400.1 MHz, $CDCl_3$): δ 3.93 (s, 2H, $C\underline{H}_2Ph$), 5.05 (br.s, 2H, NH_2), 6.72 (s, 1H, H^5), 7.06 (dd, J = 3.8, 5.0 Hz, 1H, H^9), 7.20–7.42 (m, 5H, $H^{o,m,p}$), 7.41 (d, J = 5.0 Hz, 1H, H^{10}), 7.57 (d, J = 3.8 Hz, 1H, H^8) ppm; ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 44.2 (CH_2Ph), 105.4 (C^5), 126.8 (C^p), 127.0 (C^8), 128.1 (C^9), 128.7 (C^m), 129.2 (C^{10}), 129.3 (C^o), 138.0 (C^i), 143.0 (C^7), 160.3 (C^6), 163.2 (C^4), 170.9 (C^2) ppm; ^{15}N NMR (40.5 MHz, $CDCl_3$): δ –136.6 (N^3), –148.3 (N^1), –305.6 (NH_2) ppm.

2.2. Biochemical studies

2.2.1. Carbonic anhydrase purification studies

In this work, both hCA I, and II isoenzymes were purified by Sepharose-4B-L-Tyrosine-sulfanilamide affinity chromatography [60,61]. Sepharose-4B-L-Tyrosine-sulfanilamide was used as an affinity matrix for selective retention of both hCA isoenzymes [62–64]. Both hCA isoenzymes activity was spectrophotometrically determined according to previous method described in details [65,66]. p-Nitrophenylacetate (PNA) was used as substrate and transformed to p-nitrophenolate ions (PNP) [67]. One CA isoenzyme unit is accepted as the amount of CA, which had absorbance change at 348 nm of PNA to PNP over a period of 3 min at 25 °C [68,69].

After affinity chromatography, the tubes containing the enzyme were identified at 280 nm [70]. The protein quantity was spectrophotometrically determined at 595 nm according to the Bradford method [71]. During the purification steps, bovine serum albumin was used as the standard protein [72]. Purity control of CA isozymes was determined the two different acrylamides concentration. It was carried out in 10 and 3% acrylamides for the running and the stacking gel, respectively, containing 0.1% sodium dodecyl sulphate (SDS) according to Laemmli procedure [73] as described in previous studies [74,75].

2.2.2. Cholinesterases assays

AChE from electric eel (*Electrophorus electricus*) and BChE from equine serum inhibitory effects of nitrogen, phosphorus, selenium and sulfur-containing heterocyclic compounds (**1–6**) were measured by slightly modifying the colorimetric Ellman's method [76] as described previously [77,78]. Acetylthiocholine iodide (AChI) and butyrylthiocholine iodide (BChI) were used as substrates of the both enzymatic reactions. Additionally, 5,5-dithiobis(2-nitro-benzoic acid) (DTNB) was used as common substrate for the determination of the AChE and BChE activities. Briefly, 1 mL of Tris/HCl buffer (pH 8.0, 1.0 M) and 10 µL of sample solution at different concentrations were dissolved in deionized water. Then, an aliquot AChE or BChE enzymes (50 µL) was mixed and incubated at room temperature for 10 min. After incubation period, an aliquot of DTNB (50 µL, 0.5 mM) was added. Then, the reaction was allowed to start by the addition of 50 µL of AChI or BChI (10 mM). The breakdown of these substrates was monitored spectrophotometrically by yellow color formation of 5-thio-2-

nitrobenzoate anion as the result of the reaction of DTNB with thiocholine from hydrolysis of AChI or BChI with absorption at a wavelength of 412 nm [79,80].

2.2.3. α-Glycosidase assay

The inhibitory effect of the nitrogen, phosphorus, selenium and sulfur-containing heterocyclic compounds (**1–6**) on α-glycosidase enzyme, which obtained from *Saccharomyces cerevisiae*, was carried out using the p-nitrophenyl-D-glycopyranoside (p-NPG) substrate according to the analysis of Tao et al. [81] as described previously [82,83]. Briefly, 200 μL of phosphate buffer was mixed with 40 μL of homogenate solution in same buffer solution (0.15 EU/mL, pH 7.4). After preincubation, 50 μL of p-NPG in phosphate buffer (5 mM, pH 7.4) was added and incubated again at 30 °C. Absorbances were measured spectrophotometrically at 405 nm according to previous studies [84].

2.2.4. Determination of inhibitor parameter

Metabolic enzymes inhibition by the nitrogen, phosphorus, selenium and sulfur-containing heterocyclic compounds (**1–6**) was measured for each substrate. Control activity was taken as 100%. The sulfur-containing heterocyclic compounds concentrations causing 50% inhibition (IC_{50}) values were calculated from activity (%)-Nitrogen, phosphorus, selenium and sulfur-containing heterocyclic compounds concentration graph as described previously in details [85–87]. The K_i values were determined from a series of experiments using three different nitrogen, phosphorus, selenium and sulfur-containing heterocyclic compounds (**1–6**) and substrates at five different concentrations to construct Lineweaver–Burk curves as described previously [88].

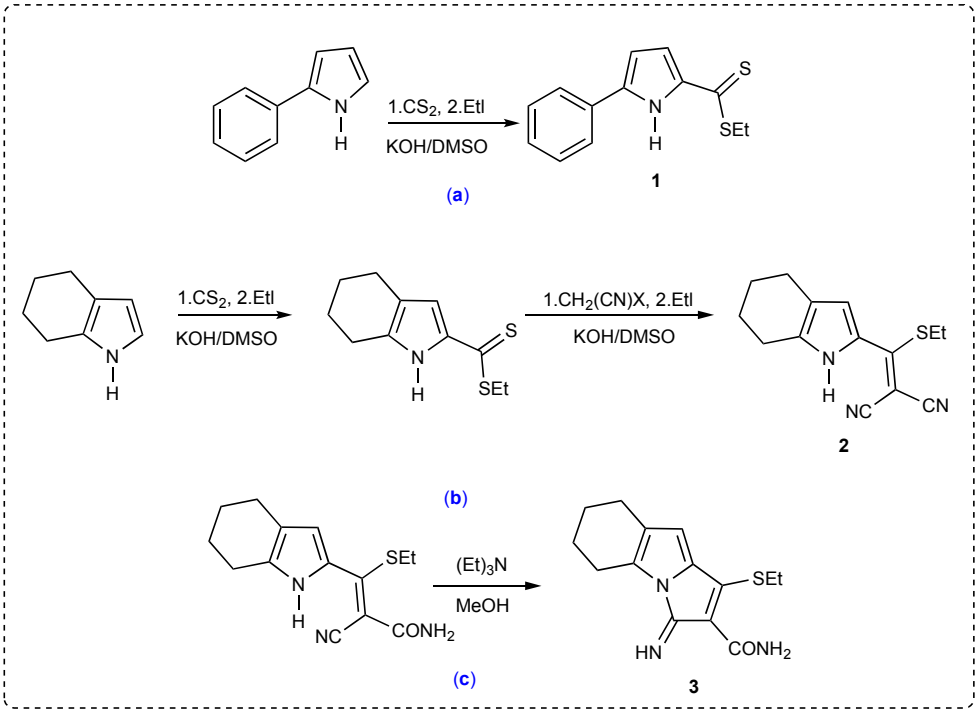
3. Results and discussion

All compounds are synthesized by original straightforward methods from available starting materials. When selecting the compounds for the study, we consider that if some of them would show promising performance characteristics, the synthetic procedures might be easily scaled up. We have developed the methodology of synthesis of sulfur-containing pyrroles, including dithioesters and compounds with alkylthio groups, on the base of reaction of pyrroles with carbon disulfide (Scheme 1a) and transformations of pyrrole-2-carboditioates thus formed in functionalized 2-vinylpyrroles with alkylthio groups (Scheme 1b) or products of their intramolecular cyclization, 1-alkylthio-3-imino-3*H*-pyrrolizines (Scheme 1c) [89].

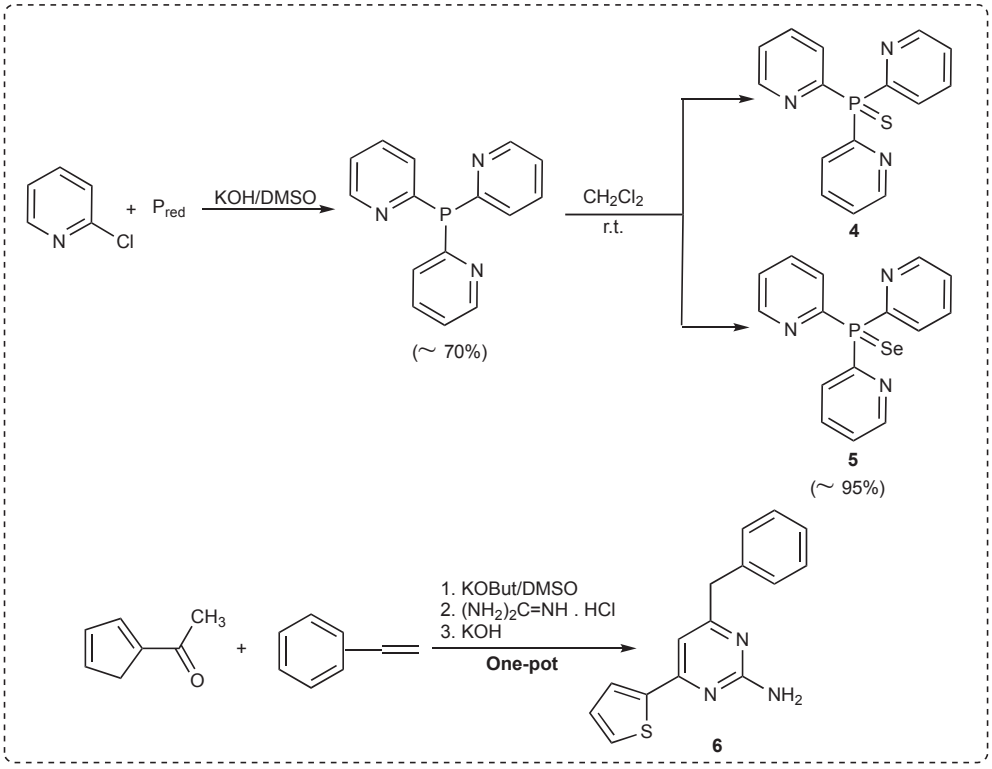
5-Phenylpyrrole-2-carbodithioate (**1**) was chosen taking into account the known sensitivity of pyrroles to oxidizers, on the one hand, and physiological properties, especially the antioxidant activity of sulfide and thiocarbonyl fragments, on the other hand. For the same reasons, pyrrole compounds **2** (2,2-dicyanoethenyl-4,5,6,7-tetrahydroindole) and **3** (3-imino-1-ethylsulfanyl-5,6,7,8-tetrahydro-3H-pyrrolo[1,2–a]indole-2-carboxamide) were selected as containing divalent sulfur atoms, functional groups, and exo- and endocyclic double bonds sensitive to radical attack. Tris(2-pyridyl)phosphine sulfide and selenide was synthesized tris(2-pyridyl)phosphine and elemental sulfur and selenium. Tris(2-pyridyl)phosphine was prepared according to the our literature procedure from red phosphorus and 2-chloropyridine in superbasic system KOH/DMSO (Scheme 2) [90]. Tris-(2-pyridyl)phosphine sulfide (**4**) and tris-(2-pyridyl)phosphine selenide (**5**) were supposed to be antioxidants of combined action owing to the high ability of oxidizing the P═S and P = Se bonds. In addition, the choice of these compounds is due to the fact that they possess a high physiological activity, suppressing various pathological processes occurring in living organisms.

4-Benzyl-6-(thiophen-2-yl)pyrimidin-2-amine was prepared from 2-acethylthiophene, phenylacetylene and $(NH_2)_2C$ = NH·HCl [13] (Scheme 2) [91]. At the same time It was expected that the thiophene-aminopyrimidine ensemble (4-benzyl-6-(thiophen-2-yl)pyrimidin-2-amine) (**6**) would be able to exhibit physiological properties due to its potentially high and multifaceted chemical activity.

CA isozymes are a broad family of enzymes involved the some physiological and pathological processes. Especially, involvement of physiologically relevant CA I, and II isoenzymes in many crucial physiological and biological processes such as regulation of the acid-base homeostasis made them act as worthy drug targets in epilepsy, glaucoma, and cerebral edema [92]. So, recent advances in the use of CA inhibitors as new antiobesity, anticancer and anti-infective drugs have



Scheme 1. The synthesis route of compounds **1–3.**



Scheme 2. The synthesis route of compounds 4–6.

Table 1
The half maximal inhibition concentration (IC_{50} values, nM) of some nitrogen, phosphorus, selenium and sulfur-containing heterocyclic compounds (**1–6**) against carbonic anhydrase I, and II isoenzymes (hCA I and II), acetylcholinesterase (AChE) and α-glycosidase (α-Gly) enzymes.

Compounds	hCA I	r^2	hCA II	r^2	AChE	r^2	BChE	r^2	α-Gly	r^2
1	48.46	0.9205	37.05	0.9609	13.13	0.9895	27.18	0.9761	26.55	0.9400
2	45.0	93.03	38.5	0.9833	17.34	0.9752	28.40	0.9611	19.85	0.9555
3	59.74	0.9629	51.72	0.9424	18.09	0.9508	27.07	0.9936	17.91	0.9384
4	33.32	0.9459	37.66	0.9956	14.44	0.9739	24.93	0.9810	13.51	0.9375
5	56.34	0.9810	53.72	0.9352	19.8	0.9435	31.22	0.9922	18.88	0.9315
6	60.79	0.9424	66.64	0.9339	22.21	0.9316	0.54	0.9383	24.06	0.9575
AZA*	25.80	0.9887	23.70	0.9825	–	–	–	–	–	–
TAC*	–	–	–	–	5.57	0.9937	4.67	0.9579	–	–
ACR*	–	–	–	–	–	–	–	–	22,800	–

*Acetazolamide (AZA) was used as a standard inhibitor for both hCA I, and II isoenzymes. Tacrine (TAC) was used as a standard inhibitor for AChE and BChE enzymes. Acarbose (ACR) was used as a standard inhibitor for α-glycosidase and taken from reference of [81].

been widely emphasized in recent years, as well as diuretics and anti-glaucoma medications [93,94]. In this study, we investigated the effects of nitrogen, phosphorus, selenium and sulfur-containing heterocyclic compounds (**1–6**) on hCA I, and II isoenzymes purified from human red blood cells using by Sepharose-4B-L-Tyrosine affinity column chromatography. Also, for controlling of both isoenzymes purities, SDS-polyacrylamide gel electrophoresis (PAGE) was performed [95] and single band observed for each purified hCA isoenzyme. Purification results of hCA I, and hCA II isoenzymes were summarized in Table 1. As for bot isoenzymes, nitrogen, phosphorus, selenium and sulfur-containing heterocyclic compounds (**1–6**) showed IC_{50} values in the low nanomolar range. IC_{50} value refers to the binding affinity of the inhibitor to the enzyme [96]. The CA I is a physiologically relevant isoenzyme, found at the highest level in erythrocytes and is also expressed in normal colorectal mucosa and encoded by the CA1 gene in humans [97]. It is expressed in normal colorectal mucosa and exists at the highest level in red blood cells [98]. Therefore, nitrogen, phosphorus, selenium and sulfur-containing heterocyclic compounds (**1–6**) demonstrated high affinity and low nanomolar inhibition levels against both isoenzymes. IC_{50} values of nitrogen, phosphorus, selenium and sulfur-containing heterocyclic compounds (**1–6**) were found between 33.32 and 60.79 nM for hCA I, and 37.05–66.64 nM for hCA II, which includes the primary Na-carrying mechanism into the eyes and regulate the intraocular pressure (Table 1). Lover IC_{50} value indicates that the inhibitor is bound to the enzyme with strong affinity [99,100]. So, a close look at the inhibitors of CA II is very important for treatment of glaucoma [101,102]. Also, Among them, tris(2-pyridyl)phosphine selenide (**4**) had strong inhibition effect against hCA I isoenzyme (IC_{50}: 33.32 nM, r^2: 0.9459), and the physiologically relevant hCA II isoform (IC_{50}: 37.05 nM, r^2: 0.9956). On the other hand, acetazolamide (AZA), which used as the reference inhibitor, had IC_{50} values were found as 25.80 nM (r^2: 0.9887) and 23.70 nM (r^2: 0.9825) for hCA I and hCA II isoenzymes, respectively. These results clearly showed that nitrogen, phosphorus, selenium and sulfur-containing heterocyclic compounds (**1–6**) had strong of similar inhibitory effect to AZA against both hCA isoenzymes (Table 1). AZA, dorzolamide, methazolamide, dichlorphenamide, ethoxzolamide, and sulthiame are examples of CA inhibitors that are clinically used for the treatment of glaucoma, obesity

and epilepsy [103]. It is well known CA II inhibitors are directly bound to the zinc metal in the center of active site of hCA II. Generally, at the bottom catalytic active site of both hCA is a cone-shaped cavity that is partitioned into two conserved environments, hydrophilic and hydrophobic walls, with a Zn^{2+} ion. The Zn^{2+} ion is coordinated as tetrahedral with putative three histidine residues (His94, His96, and His119) and a solvent molecule (H_2O). It can bind to hydroxide ion (−OH) followed by reaction with CO_2 to yield HCO_3^- ions. In this form, CAIs should include zinc-binding group, which can successfully coordinate with the binding site Zn^{2+} ion. Zinc-binding group is coupled with variable chemical moieties that known as tail. This tail facilitates the interactions with specific amino acid residues within the active site of CA. Although the CA binding site is highly conserved among the different isoenzymes, there is variability in the polarity and hydrophobicity of its peripheral part [104].

AChE and BChE inhibition properties of novel nitrogen, phosphorus, selenium and sulfur-containing heterocyclic compounds (**1–6**) were determined according to the Ellman's procedure [76] as previously described [105]. Novel nitrogen, phosphorus, selenium and sulfur-containing heterocyclic compounds (**1–6**) are the potent derivatives with IC_{50} values in ranging between 13.13 and 22.21 nM for AChE (Table 1) and 0.54 to 31.22 nM for BChE, whereas, TAC had IC_{50} value of 5.57 nM against AChE (r^2: 0.9937) and 5.57 nM against BChE (r^2: 0.9579). All studied sulfur-containing heterocyclic compounds demonstrated powerful inhibition against cholinergic enzyme of AChE, but ethyl 5-phenyl-1*H*-pyrrole-2-carbodithioate (**1**) showed the best inhibition effect against AChE (IC_{50}: 13.13 nM; r^2: 0.9895) (Table 1). It is well known that pyrrole and fused pyrrole compounds possess a wide spectrum of pharmacological activities mainly acting as anticancer agents [106]. On the other hand, when the inhibition results for BChE were examined, it was clearly observed that 4-benzyl-6-(thiophen-2-yl) pyrimidin-2-amine (**6**) was the best inhibitor with IC_{50} value of 0.54 nM, which 8.65 times greater than tacrine (IC_{50}: 4.67 nM; r^2: 0.9579). Compound **6** is the strongest against BChE. Based on these results, it can be said that BChE is the more prevalent enzyme within age and in AD, in detriment of AChE.

Finally, α-glycosidase was much more inhibited by all tested nitrogen, phosphorus, selenium and sulfur-containing heterocyclic compounds (**1–6**) (IC_{50}: 13.51–26.55 nM) (Table 1). The results showed that all nitrogen, phosphorus, selenium and sulfur-containing heterocyclic compounds (**1–6**) had effective α-glycosidase inhibition effects than that of acarbose (IC_{50}: 22800 nM) as standard α-glycosidase inhibitor [81]. Also, when the results were evaluated, tris(2-pyridyl) phosphine selenide (**4**) was found to be the best inhibitor for α-glycosidase (IC_{50}:13.51 nM; r^2: 0.9375). In fact, nowadays, it is well established that there is a connection between diabetes and AD; in fact, a common pathophysiology between both diseases has been previously suggested [107a]. In this study, there is epidemiological and basic science evidence, which suggest a possible shared pathophysiology between T2DM and AD. Also, it was hypothesized that AD might be type 3 diabetes (T3DM), but definitive biochemical mechanisms remain unknown [107a]. Currently, there is a rapid increasing studies pointing toward insulin deficiency and insulin resistance as mediators of AD-type neurodegeneration. However, this new fluctuation of information is full of contradictory and unsolved concepts related to the potential contributions of type T2DM, obesity, metabolic syndrome, and to AD pathogenesis [107b].

Brain diabetes has been associated with cognitive impairment and alteration of acetylcholine as neurotransmitter responsible for cognitive functionality. Amyloid formation is one of the most evident pathological events in both diseases in pancreas of diabetic patients, and Aβ in the brain of Alzheimer's patients [108a]. In fact, many authors claim that antidiabetic agents (targeting, for instance, α-glucosidase) could be useful for preventing or even treating AD [108b]. I think that correlation between both diseases within the introduction section can improve interest towards the family of compounds presented herein.

4. Conclusions

In conclusion, the inhibitory effects of some nitrogen, phosphorus, selenium and sulfur-containing heterocyclic compounds on hCA I, and II isoenzymes and AChE, BChE and α-glycosidase enzymes were evaluated together. The sulfur-containing heterocyclic compounds we used in our study showed inhibition effects on hCA I, and II isoenzymes and AChE, BChE and α-glycosidase enzymes activities at low nanomolar concentrations. We believe that these results may be useful in the synthesis of new CA isoenzyme inhibitors and in the development of drugs for the treatment of some diseases.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

S.A would like to extend his sincere appreciation to the Researchers Supporting Project (RSP-2019/59), King Saud University, Saudi Arabia.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2020.104171.

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