ANADOLU UNIVERSITY
INSTITUTE OF HEALTH SCIENCES

European Journal of Life Sciences 2022; 1: 20-27

Research Article

Design, synthesis and *in vitro* COX inhibitory profiles of a new series of tetrazole-based hydrazones

Mehlika Dilek Altıntop[1], Belgin Sever[1], Halide Edip Temel[2], Zafer Asım Kaplancıklı[1], Ahmet Özdemir[1]

[1]Anadolu University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Eskişehir, Turkey.
[2]Anadolu University, Faculty of Pharmacy, Department of Biochemistry, Eskişehir, Turkey.

✉ Mehlika Dilek Altıntop
mdaltintop@anadolu.edu.tr

https://doi.org/10.55971/EJLS.1095818

Received: 03.30.2022
Accepted: 04.20.2022
Available online: 06.06.2022

ABSTRACT

Inhibition of cyclooxygenases (COXs) by selective and nonselective inhibitors is a favorable approach for pharmacologic intervention in a variety of disorders such as cancer. For this purpose, a new class of tetrazole-hydrazone hybrids (**1-12**) was designed. A facile and efficient three-step procedure was applied for the preparation of compounds **1-12**, which were tested for their inhibitory activities towards cyclooxygenases (COXs) by means of an *in vitro* colorimetric inhibitor screening method. The most potent and selective COX-1 inhibitors were determined as 2-[(1-methyl-1*H*-tetrazol-5-yl)thio]-*N*'-(4-(piperidin-1-yl)benzylidene) acetohydrazide (**1**) (40.88±2.79%) and 2-[(1-methyl-1*H*-tetrazol-5-yl)thio]-*N*'-(4-(morpholin-4-yl)benzylidene)acetohydrazide (**2**) (39.80±2.78%), whereas the most potent and selective COX-2 inhibitor was found as 2-[(1-phenyl-1*H*-tetrazol-5-yl)thio]-*N*'-(4-(pyrrolidin-1-yl) benzylidene)acetohydrazide (**10**) (42.38±1.16%). In general, 1-methyl-1*H*-tetrazole moiety resulted in selective COX-1 inhibition, whereas 1-phenyl-1*H*-tetrazole moiety gave rise to preferential COX-2 inhibition.

Keywords: Cyclooxygenase, hydrazone, synthesis, tetrazole

1. INTRODUCTION

Prostaglandins (PGs) and thromboxane A_2 (TXA_2) belong to a class of lipid signaling molecules derived from arachidonic acid (AA) [1]. Biosynthesis of PGs and TXA_2 is catalyzed by two cyclooxygenase (COX) isoforms, COX-1 and COX-2 [1,2], which have similar structural and catalytic properties [3]. However, both enzymes differ in terms of their regulation of expression, tissue distribution, and pharmacological functions [3,4]. COX-1 is constitutively expressed in many tissues (e.g., thrombocytes, gastrointestinal tract, lung, kidney, liver, brain, spleen and prostate), where COX-1-derived prostanoids participate in homeostatic functions. Under normal physiological conditions, COX-1-derived prostanoids play a fundamental role in the maintenance of platelet function, renal perfusion, and cytoprotective levels of PGs in the gastric mucosa [3-6]. On the contrary, COX-2 is frequently referred to as the inducible isoform, responsible for enhanced PG production in response to inflammatory and mitogenic stimuli [4-7]. The up-regulation and overexpression of COX-2 lead to inflammation, loss of apoptosis, uncontrolled cell proliferation, metastasis, and angiogenesis finally resulting in cancer [8].

Today, the concept of "constitutive" and "inducible" isoforms has been challenged [4] by mounting evidence pointing out that COX-2 is also constitutively expressed in some tissues [9]

and COX-1 is up-regulated in various pathological conditions including thrombosis, atherosclerosis, and tumorigenesis [10].

Inhibition of COXs by a variety of selective and nonselective inhibitors is a promising approach for pharmacologic intervention in many pathological conditions [11,12].

Hydrazides-hydrazones are not only useful intermediates for the synthesis of various heterocyclic compounds but also frequently occurring motifs in drug molecules [13-16] due to their diverse pharmacological activities such as anti-inflammatory, analgesic, antidepressant, anticonvulsant, antitumor, antimycobacterial, and antimalarial activities [13-21]. In particular, the anti-inflammatory potency of hydrazones is well-documented and some of them exert their action through the inhibition of COXs [17-21].

Tetrazole scaffold is reported to be among the top 25 most commonly utilized nitrogen heterocycles in pharmaceutical agents [22]. The tetrazole ring is a well-known bioisostere of the carboxylic acid group [23] and therefore this core is frequently used for the design of promising COX inhibitors for the management of inflammation [24,25].

Prompted by aforementioned data [17-25], the facile and efficient synthesis of a novel series of tetrazole-based hydrazones (**1-12**) was performed and *in vitro* studies were conducted to assess their inhibitory effects on COXs.

2. MATERIALS AND METHODS

2.1. Chemicals and Equipments

All chemicals were purchased from commercial suppliers and were used without further purification. The melting points (M.p.) of the compounds were detected on a MP90 digital melting point apparatus (Mettler Toledo, Ohio, USA) and are uncorrected. Rotavapor® R-100 (Büchi, Switzerland) was used for the evaporation of the solvents. Nuclear Magnetic Resonance (NMR, ^{1}H and ^{13}C) spectra were recorded on a Bruker spectrometer (Bruker, Billerica, MA, USA). Chemical shifts were reported in parts per million (ppm) and the coupling constants (*J*) were expressed in Hertz (Hz). and High Resolution Mass Spectrometry (HRMS) spectra were obtained from a Shimadzu LCMS-IT-TOF system (Shimadzu, Kyoto, Japan).

2.2. General procedure for the preparation of ethyl 2-[(1-methyl/phenyl-1*H*-tetrazol-5-yl)thio] acetate

A mixture of 5-mercapto-1-methyl/phenyl-1*H*-tetrazole (0.05 mol) and ethyl chloroacetate (0.05 mol) in the presence of potassium carbonate (0.05 mol) in acetone was refluxed for 10 h. Upon completion of the reaction, the solvent was evaporated. The crude product was solved in water and then extracted with diethyl ether. After extraction, the solvent was evaporated [26,27].

2.3. General procedure for the preparation of 2-[(1-methyl/phenyl-1*H*-tetrazol-5-yl)thio)] acetohydrazide

A mixture of the appropriate ester (0.05 mol) and hydrazine hydrate (0.1 mol) in ethanol was stirred at room temperature for 3 h [26,27]. Upon completion of the reaction, the precipitate was collected by filtration and dried.

2.4. General procedure for the preparation of 2-[(1-methyl/phenyl-1*H*-tetrazol-5-yl)thio]-*N*'-(4-substituted benzylidene) acetohydrazides (1-12)

A mixture of the appropriate hydrazide (0.01 mol) and aromatic aldehydes (0.01 mol) was refluxed in ethanol for 7 h. Upon completion of the reaction, the precipitate was collected by filtration and dried. The product was crystallized from ethanol.

2.4.1. 2-[(1-Methyl-1H-tetrazol-5-yl)thio]-N'-(4-(piperidin-1-yl)benzylidene)acetohydrazide (1)

M.p.: 138.4 °C. Yield: 84%.

^{1}H NMR (300 MHz, DMSO-d_6): 1.57 (brs, 6H), 3.25 (brs, 4H), 3.98 (s, 3H), 4.14 and 4.57 (2s, 2H), 6.95 (d, *J*= 8.91 Hz, 2H), 7.49 (dd, *J*= 2.37 Hz, 2.49 Hz, 8.94 Hz, 2H), 7.89 and 8.03 (2s, 1H), 11.51 (s, 1H).

^{13}C NMR (75 MHz, DMSO-d_6): 24.39 (CH_2), 25.42 ($2CH_2$), 34.13 (CH_3), 36.37 (CH_2), 48.81 (CH_2),

48.87 (CH_2), 114.97 (CH), 115.09 (CH), 123.40 (C), 128.64 (CH), 128.93 (CH), 145.05 (CH), 148.20 (C), 152.84 (C), 167.96 (C).

HRMS (ESI) (*m/z*): $[M+H]^+$ calcd. for $C_{16}H_{21}N_7OS$: 360.1601, found: 360.1596.

2.4.2. 2-[(1-Methyl-1H-tetrazol-5-yl)thio]-N'-(4-(morpholin-4-yl)benzylidene)acetohydrazide (2)

M.p.: 199.2 °C. Yield: 90%.

^{1}H NMR (300 MHz, DMSO-d_6): 3.19-3.21 (m, 4H), 3.72-3.75 (m, 4H), 3.98 (s, 3H), 4.15 and 4.58 (2s, 2H), 6.98 (d, *J*= 8.79 Hz, 2H), 7.54 (dd, *J*= 2.43 Hz, 2.58 Hz, 8.90 Hz, 2H), 7.92 and 8.06 (2s, 1H), 11.54 (s, 1H).

^{13}C NMR (75 MHz, DMSO-d_6): 34.11 (CH_3), 36.34 (CH_2), 47.86 (CH_2), 47.92 (CH_2), 66.40 ($2CH_2$), 114.74 (CH), 114.83 (CH), 124.57 (C), 128.56 (CH), 128.84 (CH), 144.88 (CH), 148.04 (C), 152.64 (C), 168.05 (C).

HRMS (ESI) (*m/z*): $[M+H]^+$ calcd. for $C_{15}H_{19}N_7O_2S$: 362.1394, found: 362.1387.

2.4.3. 2-[(1-Methyl-1H-tetrazol-5-yl)thio]-N'-(4-(4-methylpiperazin-1-yl)benzylidene)acetohydrazide (3)

M.p.: 139.2 °C. Yield: 88%.

^{1}H NMR (300 MHz, DMSO-d_6): 2.21 (s, 3H), 2.41-2.43 (m, 4H), 3.21-3.24 (m, 4H), 3.98 (s, 3H), 4.15 and 4.57 (2s, 2H), 6.97 (d, *J*= 8.76 Hz, 2H), 7.52 (dd, *J*= 3.12 Hz, 3.15 Hz, 8.84 Hz, 2H), 7.90 and 8.04 (2s, 1H), 11.52 (s, 1H).

^{13}C NMR (75 MHz, DMSO-d_6): 34.13 (CH_3), 36.35 (CH_2), 46.21 (CH_3), 47.51 (CH_2), 47.57 (CH_2), 54.87 ($2CH_2$), 114.89 (CH), 114.98 (CH), 124.08 (C), 128.57 (CH), 128.85 (CH), 144.96 (CH), 148.10 (C), 152.53 (C), 168.01 (C).

HRMS (ESI) (*m/z*): $[M+H]^+$ calcd. for $C_{16}H_{22}N_8OS$: 375.1710, found: 375.1712.

2.4.4. 2-[(1-Methyl-1H-tetrazol-5-yl)thio]-N'-(4-(pyrrolidin-1-yl)benzylidene)acetohydrazide (4)

M.p.: 203.4 °C. Yield: 82%.

^{1}H NMR (300 MHz, DMSO-d_6): 1.93-1.97 (m, 4H), 3.25-3.29 (m, 4H), 3.98 (s, 3H), 4.13 and 4.56 (2s, 2H), 6.56 (d, *J*= 8.76 Hz, 2H), 7.48 (dd, *J*= 3.09 Hz, 3.12 Hz, 8.75 Hz, 2H), 7.87 and 8.01 (2s, 1H), 11.42 (s, 1H).

^{13}C NMR (75 MHz, DMSO-d_6): 25.41 ($2CH_2$), 34.10 (CH_3), 36.40 (CH_2), 47.69 ($2CH_2$), 112.00 (2CH), 120.94 (C), 128.84 (CH), 129.13 (CH), 145.63 (CH), 148.81 (C), 149.32 (C), 167.77 (C).

HRMS (ESI) (*m/z*): $[M+H]^+$ calcd. for $C_{15}H_{19}N_7OS$: 346.1445, found: 346.1428.

2.4.5. 2-[(1-Methyl-1H-tetrazol-5-yl)thio]-N'-(4-(1H-imidazol-1-yl)benzylidene)acetohydrazide (5)

M.p.: 191.1 °C. Yield: 88%.

^{1}H NMR (300 MHz, DMSO-d_6): 3.99 (s, 3H), 4.20 and 4.63 (2s, 2H), 7.14 (brs, 1H), 7.76 (d, *J*= 8.76 Hz, 2H), 7.82-7.85 (m, 3H), 8.06 and 8.23 (2s, 1H), 8.35 (brs, 1H), 11.81 and 11.85 (2s, 1H).

^{13}C NMR (75 MHz, DMSO-d_6): 34.13 (CH_3), 36.19 (CH_2), 118.26 (CH), 120.79 (2CH), 128.86 (CH), 129.08 (CH), 130.63 (CH), 132.76 (C), 136.02 (CH), 138.29 (C), 143.45 (CH), 146.72 (C), 168.60 (C).

HRMS (ESI) (*m/z*): $[M+H]^+$ calcd. for $C_{14}H_{14}N_8OS$: 343.1084, found: 343.1074.

2.4.6. 2-[(1-Methyl-1H-tetrazol-5-yl)thio]-N'-(4-(1H-1,2,4-triazol-1-yl)benzylidene)acetohydrazide (6)

M.p.: 257.9 °C. Yield: 86%.

^{1}H NMR (300 MHz, DMSO-d_6): 3.99 (s, 3H), 4.20 and 4.64 (2s, 2H), 7.88 (d, *J*= 8.64 Hz, 2H), 7.96 (d, *J*= 8.73 Hz, 2H), 8.07 and 8.24 (2s, 1H), 8.27 (s, 1H), 9.37 (s, 1H), 11.84 (s, 1H).

^{13}C NMR (75 MHz, DMSO-d_6): 34.14 (CH_3), 36.17 (CH_2), 120.00 (2CH), 128.78 (CH), 129.00 (CH), 133.67 (C), 137.99 (C), 142.96 (CH), 143.36 (CH), 146.54 (CH), 153.07 (C), 168.62 (C).

HRMS (ESI) (*m/z*): $[M+H]^+$ calcd. for $C_{13}H_{13}N_9OS$: 344.1037, found: 344.1028.

2.4.7. 2-[(1-Phenyl-1H-tetrazol-5-yl)thio]-N'-(4-(piperidin-1-yl)benzylidene)acetohydrazide (7)

M.p.: 189.4 °C. Yield: 83%.

^{1}H NMR (300 MHz, DMSO-d_6): 1.56 (brs, 6H), 3.24 (brs, 4H), 4.28 and 4.69 (2s, 2H), 6.94 (dd, *J*= 2.37 Hz, 2.64 Hz, 8.99 Hz, 2H), 7.50 (dd, *J*= 3.63 Hz, 3.66 Hz, 8.75 Hz, 2H), 7.65-7.68 (m, 5H), 7.91 and 8.06 (2s, 1H), 11.55 and 11.59 (2s, 1H).

^{13}C NMR (75 MHz, DMSO-d_6): 24.38 (CH_2), 25.42 ($2CH_2$), 36.72 (CH_2), 48.87 ($2CH_2$), 114.96 (CH), 115.08 (CH), 123.39 (C), 124.91 (2CH), 128.63 (CH), 128.94 (CH), 130.53 (2CH), 131.09 (CH), 133.57 (C), 145.16 (CH), 148.24 (C), 152.84 (C), 167.71 (C).

HRMS (ESI) (*m/z*): $[M+H]^+$ calcd. for $C_{21}H_{23}N_7OS$: 422.1758, found: 422.1756.

2.4.8. 2-[(1-Phenyl-1H-tetrazol-5-yl)thio]-N'-(4-(morpholin-4-yl)benzylidene)acetohydrazide (8)

M.p.: 251.6 °C. Yield: 89%.

^{1}H NMR (300 MHz, DMSO-d_6): 3.20 (brs, 4H), 3.74 (brs, 4H), 4.27 and 4.69 (2s, 2H), 6.99 (d, *J*= 8.25 Hz, 2H), 7.54 (d, *J*= 6.90 Hz, 2H), 7.69 (brs, 5H), 7.92 and 8.07 (2s, 1H), 11.58 and 11.62 (2s, 1H).

^{13}C NMR (75 MHz, DMSO-d_6): 36.68 (CH_2), 47.91 ($2CH_2$), 66.40 ($2CH_2$), 114.75 (CH), 114.84 (CH), 124.55 (C), 124.95 (2CH), 128.56 (CH), 128.84 (CH), 130.54 (2CH), 131.12 (CH), 133.57 (C), 144.99 (CH), 148.24 (C), 152.84 (C), 167.71 (C).

HRMS (ESI) (*m/z*): $[M+H]^+$ calcd. for $C_{20}H_{21}N_7O_2S$: 424.1550, found: 424.1551.

2.4.9. 2-[(1-Phenyl-1H-tetrazol-5-yl)thio]-N'-(4-(4-methylpiperazin-1-yl)benzylidene)acetohydrazide (9)

M.p.: 209.2 °C. Yield: 87%.

^{1}H NMR (300 MHz, DMSO-d_6): 2.21 (s, 3H), 2.43 (brs, 4H), 3.21-3.23 (m, 4H), 4.27 and 4.69 (2s, 2H), 6.97 (dd, *J*= 2.34 Hz, 2.43 Hz, 8.87 Hz, 2H), 7.52 (dd, *J*= 2.97 Hz, 8.76 Hz, 2H), 7.69-7.71 (m, 5H), 7.91 and 8.06 (2s, 1H), 11.56 and 11.61 (2s, 1H).

^{13}C NMR (75 MHz, DMSO-d_6): 36.67 (CH_2), 46.20 (CH_3), 47.55 ($2CH_2$), 54.85 ($2CH_2$), 114.89 (CH), 114.99 (CH), 124.07 (C), 124.94 (2CH), 128.57 (CH), 128.86 (CH), 130.54 (2CH), 131.12 (CH), 133.56 (C), 145.06 (CH), 148.12 (C), 152.54 (C), 167.76 (C).

HRMS (ESI) (*m/z*): $[M+H]^+$ calcd. for $C_{21}H_{24}N_8OS$: 437.1867, found: 437.1884.

2.4.10. 2-[(1-Phenyl-1H-tetrazol-5-yl)thio]-N'-(4-(pyrrolidin-1-yl)benzylidene)acetohydrazide (10)

M.p.: 215.1 °C. Yield: 81%.

^{1}H NMR (300 MHz, DMSO-d_6): 1.95 (brs, 4H), 3.27 (brs, 4H), 4.26 and 4.68 (2s, 2H), 6.57 (d, *J*= 8.67 Hz, 2H), 7.48 (d, *J*= 8.67 Hz, 2H), 7.69 (brs, 5H), 7.88 and 8.03 (2s, 1H), 11.47 and 11.50 (2s, 1H).

^{13}C NMR (75 MHz, DMSO-d_6): 25.41 ($2CH_2$), 36.73 (CH_2), 47.68 ($2CH_2$), 112.01 (2CH), 120.92 (C), 124.94 (2CH), 128.83 (CH), 129.14 (CH), 130.53 (2CH), 131.10 (CH), 133.57 (C), 145.72 (CH), 148.83 (C), 149.33 (C), 167.53 (C).

HRMS (ESI) (*m/z*): $[M+H]^+$ calcd. for $C_{20}H_{21}N_7OS$: 408.1601, found: 408.1604.

2.4.11. 2-[(1-Phenyl-1H-tetrazol-5-yl)thio]-N'-(4-(1H-imidazol-1-yl)benzylidene)acetohydrazide (11)

M.P.: 236.4 °C. Yield: 86%.

^{1}H NMR (300 MHz, DMSO-d_6): 4.32 and 4.75 (2s, 2H), 7.14 (brs, 1H), 7.65-7.71 (m, 5H), 7.76 (d, *J*= 8.70 Hz, 2H), 7.84 (d, *J*= 8.70 Hz, 3H), 8.07 and 8.25 (2s, 1H), 8.36 (brs, 1H), 11.86 and 11.92 (2s, 1H).

^{13}C NMR (75 MHz, DMSO-d_6): 36.54 (CH_2), 118.29 (CH), 120.80 (2CH), 124.92 (2CH), 128.84 (CH), 129.08 (CH), 130.55 (2CH), 131.13 (CH), 132.75 (CH), 132.83 (C), 133.55 (C), 136.02 (CH), 138.30 (C), 143.56 (CH), 146.75 (C), 168.35 (C).

HRMS (ESI) (*m/z*): $[M+H]^+$ calcd. for $C_{19}H_{16}N_8OS$: 405.1241, found: 405.1250.

2.4.12. 2-[(1-Phenyl-1H-tetrazol-5-yl)thio]-N'-(4-(1H-1,2,4-triazol-1-yl)benzylidene)acetohydrazide (12)

M.p.: 240.6 °C. Yield: 84%.

^{1}H NMR (300 MHz, DMSO-d_6): 4.32 and 4.75 (2s, 2H), 7.67-7.70 (m, 5H), 7.89 (d, *J*= 8.73 Hz, 2H), 7.96 (d, *J*= 8.49 Hz, 2H), 8.09 and 8.26 (2s, 1H), 8.28 (s, 1H), 9.38 (s, 1H), 11.88 (s, 1H).

^{13}C NMR (75 MHz, DMSO-d_6): 36.48 (CH_2), 120.02 (2CH), 124.94 (2CH), 128.77 (CH), 129.01 (CH), 130.56 (2CH), 131.14 (CH), 133.55 (C), 133.66 (C), 138.01 (C), 142.96 (CH), 143.46 (CH), 146.58 (CH), 153.07 (C), 168.36 (C).

HRMS (ESI) (*m/z*): $[M+H]^+$ calcd. for $C_{18}H_{15}N_9OS$: 406.1193, found: 406.1195.7

2.5. Biochemistry

2.5.1. Determination of COX inhibitory potency

COX colorimetric inhibitor screening assay kit (item no: 701050), which includes both ovine COX-1 and human recombinant COX-2, is used to measure the peroxidase component of COXs. In this method, the peroxidase activity is detected colorimetrically *via* monitoring the appearance of oxidized *N,N,N',N'*-tetramethyl-*p*-phenylenediamine (TMPD) at 590 nm.

In the current work, this kit was applied to determine the inhibitory activities of compounds **1-12** (at 100 μM) towards both COXs according to the manufacturer's instructions (Cayman, Ann Arbor, MI, USA). All measurements were performed in triplicate and the data were expressed as mean ± SD. SC-560 (at 1 μM) was used as a selective COX-1 inhibitor, whilst rofecoxib (at 10 μM) was used as a selective COX-2 inhibitor.

3. RESULTS AND DISCUSSION

3.1. Chemistry

The synthesis of the hitherto unreported compounds (**1-12**) was performed as depicted in Figure 1. Ethyl 2-[(1-methyl/phenyl-1*H*-tetrazol-5-yl)thio]acetate was prepared *via* the treatment of 5-mercapto-1-methyl/phenyl-1*H*-tetrazole with ethyl chloroacetate in the presence of potassium carbonate. The reaction of this ester with hydrazine hydrate yielded 2-[(1-methyl/phenyl-1*H*-tetrazol-5-yl)thio)] acetohydrazide, which underwent a subsequent nucleophilic addition-elimination reaction with aromatic aldehydes affording compounds **1-12**. Their structures were confirmed by ^{1}H and ^{13}C NMR, HRMS data. In the ^{1}H NMR spectra of compounds **1-12**, the signal due to CH=N proton was detected in the region 7.87-8.26 ppm as two singlets. The signal due to N-H proton appeared in the region 11.42-11.92 ppm as a singlet or two singlets. The

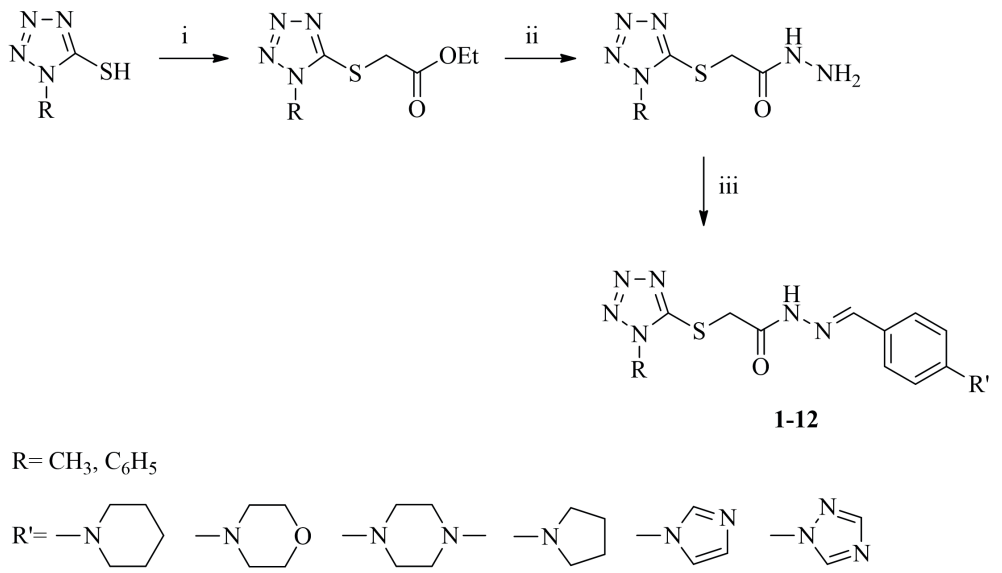


Figure 1. The synthetic route for the preparation of compounds **1-12**. Reagents and conditions: (i) $ClCH_2COOEt$, K_2CO_3, acetone, reflux, 10h; (ii) $NH_2NH_2.H_2O$, ethanol, rt, 3h; (iii) ArCHO, ethanol, reflux, 7h.

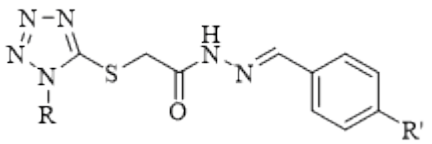
signal due to the S-CH_2 protons was observed as two singlets in the region 4.13-4.75 ppm. In the ^{1}H NMR spectra of compounds **1-6**, the protons of the methyl group attached to the *N*1 atom of the tetrazole ring gave rise to a singlet peak at 3.98-3.99 ppm. In the ^{13}C NMR spectra of compounds **1-12**, the signal due to the CH=N carbon was observed at 143.45-146.58 ppm, whereas the signal due to the C=O carbon was detected in the region 167.53-168.62 ppm. The S-CH_2 carbon gave rise to a singlet peak at 36.17-36.73 ppm. In the ^{13}C NMR spectra of compounds **1-6**, the methyl carbon attached to the *N*1 atom of the tetrazole ring gave rise to a singlet peak at 34.10-34.14 ppm. The HRMS data were also in agreement with the proposed structures of compounds **1-12**.

3.2. *In vitro* COX inhibition

A colorimetric assay was employed to assess the COX inhibitory profiles of compounds **1-12** (Table 1). Compounds **7** and **11** did not show any inhibitory activity towards COXs. Compounds **3**, **8**, **9** and **12** showed inhibitory activity towards both COXs. Among them, compound **3** preferentially inhibited COX-1, whilst compounds **8** and **9** preferentially inhibited COX-2. Compound **12** showed nonselective COX inhibitory activity and its inhibitory effects on COX-1 and COX-2 were found to be insignificant. The 1,2,4-triazole ring at the 4th position of the benzylidene motif caused a diminution in COX inhibitory potency.

The most potent and selective COX-1 inhibitors were found as compounds **1** (40.88±2.79%) and **2** (39.80±2.78%) carrying 1-methyl-1*H*-tetrazole moiety as compared to SC-560 (97.36±2.62%). These compounds did not show any inhibitory activity towards COX-2. The replacement of the methyl group of compound **1** with the phenyl moiety of compound **7** resulted in the loss of COX-1 inhibitory activity as well as COX-2 inhibitory potency. The replacement of the methyl group of compound **2** with the phenyl moiety of compound **8** led to a decrease in COX-1 inhibitory potency

Table 1. COX inhibition (%) caused by compounds **1-12** and reference agents



Compound (100 µM)	R	R'	Inhibition%	
			COX-1	COX-2
1	CH_3	Piperidin-1-yl	40.88±2.79	----
2	CH_3	Morpholin-4-yl	39.80±2.78	----
3	CH_3	4-Methylpiperazin-1-yl	27.97±2.69	12.45±1.22
4	CH_3	Pyrrolidin-1-yl	35.36±3.93	----
5	CH_3	1*H*-Imidazol-1-yl	33.41±3.09	----
6	CH_3	1*H*-1,2,4-Triazol-1-yl	19.55±1.96	----
7	C_6H_5	Piperidin-1-yl	----	----
8	C_6H_5	Morpholin-4-yl	14.37±2.31	30.26±0.76
9	C_6H_5	4-Methylpiperazin-1-yl	21.77±1.58	38.82±0.24
10	C_6H_5	Pyrrolidin-1-yl	----	42.38±1.16
11	C_6H_5	1*H*-Imidazol-1-yl	----	----
12	C_6H_5	1*H*-1,2,4-Triazol-1-yl	9.86±3.06	12.46±1.46
SC-560 (1 µM)	-	-	97.36±2.62	ND
Rofecoxib (10 µM)	-	-	ND	98.36±1.86

ND: Not determined.

(14.37±2.31%) and a substantial increase in COX-2 inhibitory activity (30.26±0.76%).

The most potent COX-2 inhibitors were found as compounds **10** (42.38±1.16%) and **9** (38.82±0.24%) carrying 1-phenyl-1*H*-tetrazole moiety as compared to rofecoxib (98.36±1.86%). Compound **9** also inhibited COX-1 (21.77±1.58%), whilst compound **10** did not show any inhibitory potency on COX-1.

In general, 1-methyl-1*H*-tetrazole moiety gave rise to selective COX-1 inhibition, whereas 1-phenyl-1*H*-tetrazole moiety led to preferential COX-2 inhibition. It can be concluded that the substituent at the 1st position of the tetrazole ring is of great importance for selectivity. The replacement of the alkyl (methyl) group with the bulky aryl moiety shifted the selective inhibition from COX-1 to COX-2.

4. CONCLUSION

In this paper, we conducted a simple and efficient protocol for the preparation of a series of tetrazole-based hydrazones (**1-12**), which were investigated for their inhibitory effects on COXs at 100 μM using an *in vitro* colorimetric assay. Based on *in vitro* experimental data, compounds **1** (40.88±2.79%) and **2** (39.80±2.78%) carrying 1-methyl-1*H*-tetrazole moiety were found as the most potent and selective COX-1 inhibitors. On the other hand, compound **10** (42.38±1.16%) carrying 1-phenyl-1*H*-tetrazole moiety was determined as the most potent and selective COX-2 inhibitor in this series. This work could represent a rational guideline for further structural modifications to generate a new class of tetrazole-based hydrazones endowed with selective COX-1 or COX-2 inhibitory activity.

Ethical approval

Not applicable, because this article does not contain any studies with human or animal subjects.

Author contribution

Concept: MDA, ZAK and AÖ; Design: MDA and ZAK; Materials: MDA, BS, HET, ZAK and AÖ; Data Collection and/or Processing: MDA, BS and HET; Analysis and/or Interpretation: MDA and HET; Literature Search: MDA; Writing: MDA; Critical Reviews: MDA, BS, HET, ZAK and AÖ.

Source of funding

This study was supported by Anadolu University Scientific Research Projects Commission under the grant no: 2005S019.

Conflict of interest

The authors declare that there is no conflict of interest.

REFERENCES

1. Rouzer CA, Marnett LJ. Structural and chemical biology of the interaction of cyclooxygenase with substrates and non-steroidal anti-inflammatory drugs. Chem Rev. 2020;120:7592-7641. https://dx.doi.org/10.1021/acs.chemrev.0c00215
2. Brock TG, McNish RW, Peters-Golden M. Arachidonic acid is preferentially metabolized by cyclooxygenase-2 to prostacyclin and prostaglandin E2. J Biol Chem. 1999;274(17):11660-11666. https://doi.org/10.1074/jbc.274.17.11660
3. Mazaleuskaya LL, Ricciotti E. Druggable prostanoid pathway. Adv Exp Med Biol. 2020;1274:29-54. https://doi.org/10.1007/978-3-030-50621-6_3
4. Pannunzio A, Coluccia M. Cyclooxygenase-1 (COX-1) and COX-1 inhibitors in cancer: A review of oncology and medicinal chemistry literature. Pharmaceuticals (Basel). 2018;11(4):101. https://doi.org/10.3390/ph11040101
5. Sharma V, Bhatia P, Alam O, et al. Recent advancement in the discovery and development of COX-2 inhibitors: Insight into biological activities and SAR studies (2008-2019). Bioorg Chem. 2019;89:103007. https://doi.org/10.1016/j.bioorg.2019.103007

6. Zidar N, Odar K, Glavac D, Jerse M, Zupanc T, Stajer D. Cyclooxygenase in normal human tissues – is COX-1 really a constitutive isoform, and COX-2 an inducible isoform? J Cell Mol Med. 2009;13:3753-3763. https://doi.org/10.1111/j.1582-4934.2008.00430.x

7. Yu T, Lao X, Zheng H. Influencing COX-2 activity by COX related pathways in inflammation and cancer. Mini Rev Med Chem. 2016;16:1230-1243. https://doi.org/10.2174/1389557516666160505115743

8. Gandhi J, Khera L, Gaur N, Paul C, Kaul R. Role of modulator of inflammation cyclooxygenase-2 in gammaherpesvirus mediated tumorigenesis. Front Microbiol. 2017;8:538. https://doi.org/10.3389/fmicb.2017.00538

9. Caiazzo E, Ialenti A, Cicala C. The relatively selective cyclooxygenase-2 inhibitor nimesulide: What's going on? Eur J Pharmacol. 2019;848:105-111. https://doi.org/10.1016/j.ejphar.2019.01.044

10. Vitale P, Tacconelli S, Perrone MG, et al. Synthesis, pharmacological characterization, and docking analysis of a novel family of diarylisoxazoles as highly selective cyclooxygenase-1 (COX-1) inhibitors. J Med Chem. 2013;56(11):4277-4299. https://doi.org/10.1021/jm301905a

11. Perrone MG, Scilimati A, Simone L, Vitale P. Selective COX-1 inhibition: A therapeutic target to be reconsidered. Curr Med Chem. 2010;17(32):3769-3805. https://doi.org/10.2174/092986710793205408

12. Carullo G, Galligano F, Aiello F. Structure-activity relationships for the synthesis of selective cyclooxygenase 2 inhibitors: An overview (2009-2016). Medchemcomm. 2016;8(3):492-500. https://doi.org/10.1039/c6md00569a

13. Kumar P, Narasimhan B. Hydrazides/hydrazones as antimicrobial and anticancer agents in the new millennium. Mini-Rev Med Chem. 2013;13:971-987. https://doi.org/10.2174/1389557511313070003

14. Rollas S, Küçükgüzel SG. Biological activities of hydrazone derivatives. Molecules. 2007;12(8):1910-1939. https://doi.org/10.3390/12081910

15. Narang R, Narasimhan B, Sharma S. A review on biological activities and chemical synthesis of hydrazide derivatives. Curr Med Chem. 2012;19(4):569-612. https://10.2174/092986712798918789

16. Mali SN, Thorat BR, Gupta DR, Pandey A. Mini-review of the importance of hydrazides and their derivatives—Synthesis and biological activity. Eng Proc. 2021;11:21. https://doi.org/10.3390/ASEC2021-11157

17. Ju Z, Su M, Hong J, et al. Design of balanced COX inhibitors based on anti-inflammatory and/or COX-2 inhibitory ascidian metabolites. Eur J Med Chem. 2019;180:86-98. https://doi.org/10.1016/j.ejmech.2019.07.016

18. Abdelgawad MA, Labib MB, Abdel-Latif M. Pyrazole-hydrazone derivatives as anti-inflammatory agents: Design, synthesis, biological evaluation, COX-1,2/5-LOX inhibition and docking study. Bioorg Chem. 2017;74:212-220. https://doi.org/10.1016/j.bioorg.2017.08.014

19. Gorantla V, Gundla R, Jadav SS, et al. Molecular hybrid design, synthesis and biological evaluation of *N*-phenyl sulfonamide linked *N*-acyl hydrazone derivatives functioning as COX-2 inhibitors: new anti-inflammatory, anti-oxidant and anti-bacterial agents. New J Chem. 2017;41:13516-13532. https://doi.org/10.1039/c7nj03332j

20. Mohammed KO, Nissan YM. Synthesis, molecular docking, and biological evaluation of some novel hydrazones and pyrazole derivatives as anti-inflammatory agents. Chem Biol Drug Des. 2014;84(4):473-488. https://doi.org/10.1111/cbdd.12336

21. El-Sayed MA, Abdel-Aziz NI, Abdel-Aziz AA, El-Azab AS, Asiri YA, Eltahir KE. Design, synthesis, and biological evaluation of substituted hydrazone and pyrazole derivatives as selective COX-2 inhibitors: Molecular docking study. Bioorg Med Chem. 2011;19(11):3416-3424. https://doi.org/10.1016/j.bmc.2011.04.027

22. Xiong Q, Dong S, Chen Y, Liu X, Feng X. Asymmetric synthesis of tetrazole and dihydroisoquinoline derivatives by isocyanide-based multicomponent reactions. Nat Commun. 2019;10(1):2116. https://doi.org/10.1038/s41467-019-09904-5

23. Lamie PF, Philoppes JN, Azouz AA, Safwat NM. Novel tetrazole and cyanamide derivatives as inhibitors of cyclooxygenase-2 enzyme: design, synthesis, anti-inflammatory evaluation, ulcerogenic liability and docking study. J Enzyme Inhib Med Chem. 2017;32:805-820. https://doi.org/10.1080/14756366.2017.1326110

24. Labib MB, Fayez AM, El-Nahass ES, Awadallah M, Halim PA. Novel tetrazole-based selective COX-2 inhibitors: Design, synthesis, anti-inflammatory activity, evaluation of PGE2, TNF-α, IL-6 and histopathological study. Bioorg Chem. 2020;104:104308. https://doi.org/10.1016/j.bioorg.2020.104308

25. Wei CX, Bian M, Gong GH. Tetrazolium compounds: synthesis and applications in medicine. Molecules. 2015;20(4):5528-5553. https://doi.org/10.3390/molecules20045528

26. Altıntop MD, Kaplancıklı ZA, Akalın Çiftçi G, Demirel R. Synthesis and biological evaluation of thiazoline derivatives as new antimicrobial and anticancer agents. Eur J Med Chem. 2014;74:264-277. https://doi.org/10.1016/j.ejmech.2013.12.060

27. Altıntop MD, Özdemir A, Turan-Zitouni G, et al. Synthesis and biological evaluation of some hydrazone derivatives as new anticandidal and anticancer agents. Eur J Med Chem. 2012;58:299-307. https://doi.org/10.1016/j.ejmech.2012.10.011