Res Chem Intermed
DOI 10.1007/s11164-016-2851-x

New benzothiazole/benzoxazole-pyrazole hybrids with potential as COX inhibitors: design, synthesis and anticancer activity evaluation

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Received: 20 August 2016 / Accepted: 26 December 2016

Abstract Ten new hybrids were designed and synthesized, their chemical structures were confirmed through spectral and elemental analysis. The new hybrids were screened against lung, breast and liver cancer cell lines (A549, MCF7 and Hep3B), in addition to normal fibroblast cells. Compound **13a** was the most active and selective one on the lung cancer cell line (A549), its IC_{50} and S.I. values were 2.4 μM and 83.2, respectively. Compound **14b** was active on MCF7 with the best selectivity towards this cell line. The new derivatives were screened for their inhibitory activity against COX enzymes, the obtained results revealed that compound **13a** and **14b** were more active inhibitors for COX-2 than celecoxib. This finding encourages us to consider COX-2 inhibitory activity as a proposed mechanism for their anticancer activity.

Keywords Pyrazole · Benzothiazole · Benzoxazole · COX inhibitors · Anticancer

Introduction

Cyclooxygenase enzyme (COX) is known as prostaglandin–endoperoxide synthase (PTGS) and is responsible for the first step in the synthesis of prostaglandins (PGs) from arachidonic acid [1]. There are two isoforms of cyclooxygenases, COX-1,

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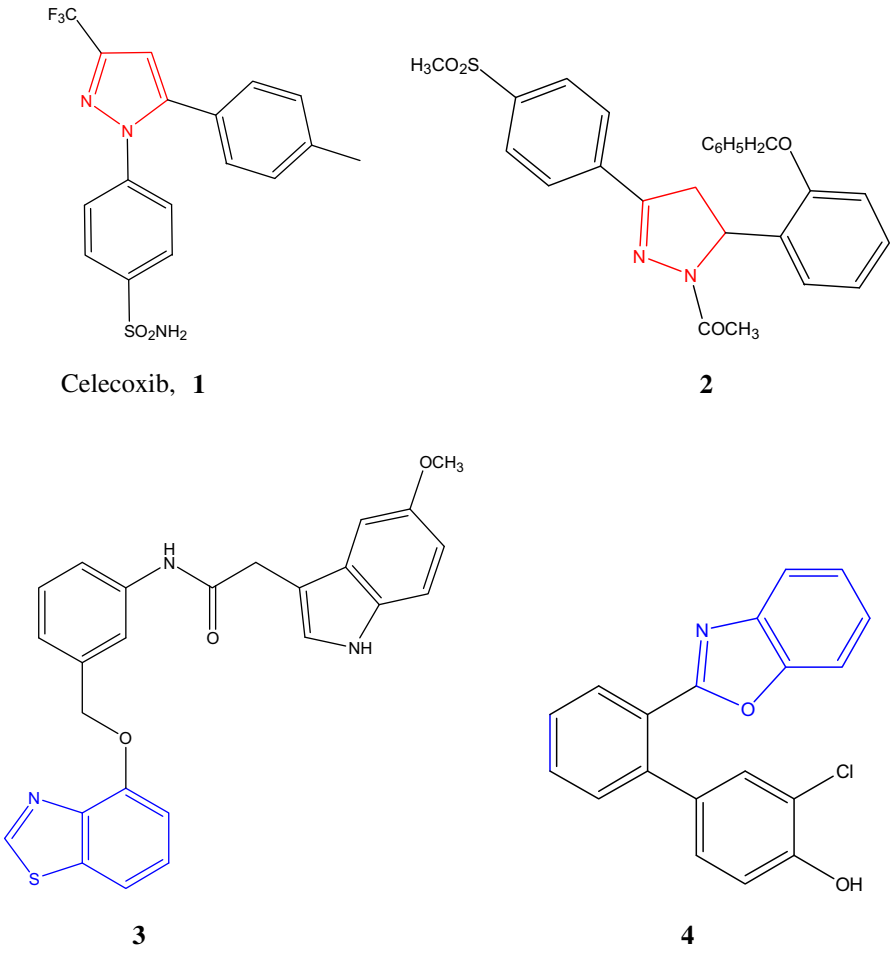
Published online: 05 January 2017



Celecoxib, **1** **2**

3 **4**

Fig. 1 Chemical structures of some known COX-2 inhibitors

which is responsible for the production of cytoprotective PGs and is considered as a housekeeping enzyme, COX-2 that plays a major role in inflammatory response [2]. COX enzymes served as molecular targets for all NSAIDs [3] and recently they have been considered as a molecular target in cancer treatment [4]. COX-2 has been reported as the most frequently evaluated anticancer and anti-inflammatory target [5], while COX-2 was highly expressed in human epithelial and solid tumors and in situ carcinoma of the bladder and breast [6]. It causes cancer through several biological pathways. It promotes angiogenesis [7], inhibits apoptosis [8], and induces VEGF [9], IL-6 [10], IL-8 [11] and TIE-2 [12]. In addition, COX-2-selective inhibitors have been proved to have antineoplastic properties [13]. Celecoxib (Fig. 1), the COX-2 selective inhibitor, has proved to prevent the formation of adenomas in patients with familial polyposis [14], also showing activity against ovarian cancer [15]. It was recently approved to act as a chemo-

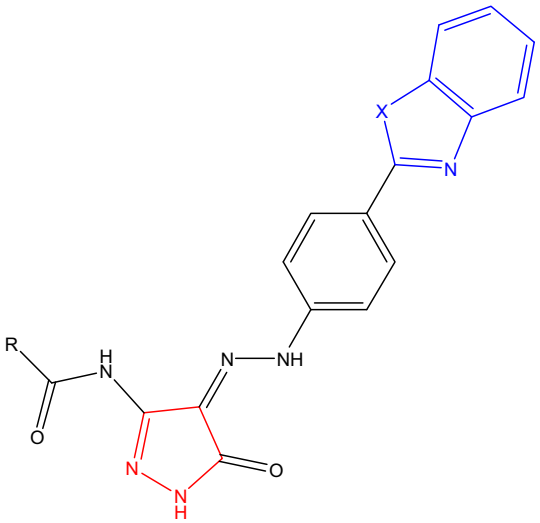


Fig. 2 The general formula of the new hybrid molecules

preventive in colon cancer and has been investigated for the treatment of other advanced cancers [16].

The pyrazole nucleus plays an important role in medicinal chemistry [17], as it has been used to develop new bioactive agents such as anticancer [18], anti-inflammatory, hypoglysimic [19] and antimicrobial [20]. In 2010, the synthesis of new pyrazoline derivatives such as coxib analogs was reported, and compound **2** (Fig. 1) showed COX-2 inhibitory activity [21]. In addition, benzothiazoles and benzoxazoles are well-known privileged scaffolds in medicinal chemistry, and are widely used for the design of new bioactive compounds due to their different pharmacological activities [22–25], especially anticancer [26]. The benzothiazole derivative (compound **3**; Fig. 1) was found to be able to decrease COX-2 expression [27]. Moreover, the benzoxazole-based derivative (compound **4**; Fig. 1) selectively inhibited COX-2, and its selectivity index was better than celecoxib [28].

These findings encouraged us to design and synthesize hybridized molecules of benzothiazole/pyrazole and benzoxazole/pyrazole to evaluate their COX inhibitory activity and also their anticancer activity. Figure 2 illustrates the general formula of the target compounds. In comparison to celecoxib, the pyrazole moiety was replaced with pyrazolinone, linked to benzothizole or benzoxazole at C4 with different substituents at C3 aiming to obtain new hybrids useful as anticancer active agents.

Methods

Chemistry

Melting points were determined using a Griffin apparatus and were uncorrected. A Shimadzu 435 spectrometer was used for recording the infrared (IR) spectra using KBr discs. ^{1}H NMR and ^{13}C NMR spectra were detected using a Bruker 400 MHz

spectrometer (Faculty of Pharmacy, Beni-Suef University, Beni-Suef, Egypt). Chemical shifts are expressed in values (ppm) and tetramethylsilane (TMS) is the internal standard. The addition of D_2O was used to confirm the exchangeable protons, J values (coupling constant) were estimated in hertz (Hz). C, H, N microanalysis was performed at the Micro-analytical Center, Cairo University, Egypt. Thin layer chromatography (TLC) was performed using Kiesel gel 0.25-mm, 60 G F 254, Merck Silica gel plates, and the running solvent system was chloroform/ethanol (9.5:0.5), while ultraviolet light was used to detect the spots. Compounds **7a** and **7b** were prepared according to previously reported procedures [29].

General procedure for preparation of compounds 8a and 8b

To an ice-cold solution of **7a** or **7b** (0.01 mol) in hydrochloric acid (2.5 mL) and distilled water (5 mL), a solution of sodium nitrite (0.90 g, 0.013 mol) in distilled water (5 mL) was added portion-wise, Then, this mixture was added portion-wise to a well-stirred ice solution of ethyl cyanoacetate (1.13g, 0.01mol) in aqueous ethanol (10 mL, 50%) containing sodium acetate (0.82 g, 0.011 mol). After completion of addition, the reaction mixture was stirred and kept in ice for 2 h and then filtered. The product was dried and recrystallized from acetic acid.

[(4-Benzoxazol-2-yl-phenyl)-hydrazono]-cyano-acetic acid ethyl ester 8a Yield 90%, yellow crystal, mp: 234-236 °C . IR (γ_{max}./cm^{-1}): 3206 (NH), 2216 (CN), 1738 (C=O). ^{1}H NMR ($CDCl_3$, 400 MHz); δ 1.38 (t, J = 7.4 Hz, 3H, $CH_2\underline{CH_3}$), 4.39 (q, J = 7.4 Hz, 2H, $\underline{CH_2}CH_3$), 7.64 (d, J_1= 7.2, 2H, phenyl C2-H & C6-H), 7.71-7.81 (m, 4H, benzoxazole), 8.27 (d, J_1= 7.2, 2H, phenyl C3-H & C5-H), 13.00 (s, 1H, NH). ^{13}C NMR ($CDCl_3$); δ 14.40, 63.20, 106.24, 112.20, 117.44, 120.60, 121.10, 125.20, 125.90, 127.80, 129.34, 138.60, 144.70, 146.40, 156.40, 164.20. EIMS (m/z); 334 ($M^{+\cdot}$, 100%); Anal. calcd. for $C_{18}H_{14}N_4O_3$ (334.33); C, 64.66, H, 4.22, N, 16.76. Found; C, 64.60, H, 4.20, N, 16.80.

[(4-Benzothiazol-2-yl-phenyl)-hydrazono]-cyano-acetic acid ethyl ester 8b Yield 90%, yellow crystal, mp: 230–232°C. IR (cm^{-1}): 3220 (NH), 2210 (CN), 1721 (C=O). ^{1}H NMR ($CDCl_3$, 400 MHz); δ 1.35 (t, J = 3.2 Hz, 3H, $CH_2\underline{CH_3}$), 4.36 (q, J = 3.2 Hz, 2H, $\underline{CH_2}CH_3$), 7.51 (d, J_1= 8, J_2= 6.4, 2H, phenyl C2-H & C6-H), 7.66 (d, J_1= 8, J_2= 6.4, 2H, phenyl C3-H & C5-H), 8.03–8.14 (m, 4H, benzothiazole), 12.96 (s, 1H, NH),). ^{13}C NMR ($CDCl_3$); δ 14.54, 62.13, 108.44, 112.20, 116.30, 117.17, 122.63, 123.16, 125.78, 127.04, 129.01, 138.21, 145.44, 147.32, 158.60, 168.22. EIMS (m/z); 350 ($M^{+\cdot}$, 100%); Anal. calcd. for $C_{18}H_{14}N_4O_2S$ (350.39): C, 61.70, H, 4.03, N, 15.99. Found; C, 61.50, H, 4.10, N, 16.00.

General procedure for preparation of compounds 9a and 9b

A mixture of compound **8a** or **8b** (0.01 mol) and hydrazine hydrate (99%) (0.011 mol) in absolute ethanol (20 mL) was heated under reflux for 12 h. The reaction mixture was evaporated under reduced pressure. The residue was washed with water, dried and recrystallized from acetic acid to yield compounds **9a** or **9b**.

5-Amino-4-[(-benzoxazol-2-yl-phenyl)-hydrazono]-2,4-dihydro-pyrazol-3-one 9a Yield 90%, yellow crystal, mp: 250-252 °C . IR (Cm^{-1}): 3360, 3280 (NH_2), 3218 (NH), 1687 (C=O). ^{1}H NMR (DMSO); δ 5.77 (s, 2H, NH_2), 7.40 (d, J = 4.4 Hz, 2H, phenyl C4-H & C5-H), 7.66–7.76 (m, 4H, benzoxazole), 8.20 (d, J = 4.4 Hz, 2H, phenyl C3′-H & C5′-H), 9.53 (s, 1H, pyrazole, NH), 12.26 (s, 1H, NH. NH–N=). ^{13}C NMR (DMSO); δ 111.16, 111.50, 117.07, 117.15, 120.00, 120.07, 122.87, 125.21, 125.69, 129.18, 142, 21, 145.30, 150.75, 162.47. EIMS (m/z); 320 ($M^{+\cdot}$, 100%); Anal. calcd. for $C_{16}H_{12}N_6O_2$ (320.31): C, 60.00, H, 3.78, N, 26.24. Found; C, 60.20, H, 3.70, N, 26.30.

5-Amino-4-[(-benzothiazol-2-yl-phenyl)-hydrazono]-2,4-dihydro-pyrazol-3-one 9b Yield 90%, yellow crystal, mp: 242-244 C . IR(Cm^{-1}): 3370, 3290(NH_2), 3176 (NH), 1676 (C=O). ^{1}H NMR (DMSO); δ 5.77 (s, 2H, NH_2), 7.47 (d, J = 4.8 Hz, J_2 = 5.2 Hz 2H, phenyl C4-H & C5-H), 7.65–7.71 (m, 2H, phenyl C3'-H & C5'-H), 8.05-8.16 (m, 4H, benzothiazole, NH_2), 9.54 (s, 1H, pyrazole, NH), 12.30 (s, 1H, NH. NH-N=). ^{13}C NMR (DMSO); δ 111.55, 117.20, 122.65, 123.17, 123.23, 125.88, 127.05, 129.04, 129.65, 134.95, 144.89, 154.20, 161.10, 167.07. EIMS (m/z); 336 ($M^{+\cdot}$, 100%); Anal. calcd. for $C_{16}H_{12}N_6OS$ (336.37): C, 57.13, H, 3.60, N, 24.98 . Found; C, 57.20, H, 3.50, N, 25.00.

General method adopted for the preparation of compounds 10a–12b

Compound **9a** or **9b** (2 mmol) was dissolved in dioxan (20 mL) and acetyl chloride, chloroacetyl chloride or benzoyl chloride (2 mmol) was added, the reaction mixture was stirred at room temperature for 24 h, solvent was evaporated under vacuum. The obtained yellowish white solid was recrystallized from acetone to give compounds **10a-12b** in a pure form, their physical and spectral data are listed below.

N-{4-[(4-Benzoxazol-ylphenyl)hyrazono]-5-oxo-4,5-dihydro-1H-pyrazol-3-yl) acetamide (10a) Yield 80%, mp: 210-212°C. IR (cm^{-1}): 3228 (NH), 3040 (CH aromatic), 2919 (CH aliph), 1684 (C=O, pyrazolone), 1646 (C=O of amide). ^{1}H NMR (DMSO, 400 MHz): δ 2.54 (s, 3H, CH_3), 3.39 (s, 1H, NH), 7.39 (dd, J_1= 8.1 Hz, J_2= 7.2 Hz, 2H, CH aromatic), 7.43 (dd, J_1= 8.1 Hz, J_2= 7.2 Hz, 2H, CH aromatic), 7.72 (s, 1H, NH), 7.78-7.81 (m, 2H, CH aromatic), 8.21-8.25 (m, 2H, CH aromatic), 8.27 (s, 1H, NH); ^{13}C NMR (DMSO): 39.27, 111.37, 120.13, 120.53, 125.42, 125.99, 129.55,130.62, 135.55, 142.35, 145.22, 152.12, 158.68, 162,24, 168.34, 175.14; Anal. Calcd. for $C_{18}H_{14}N_6O_3$ (362.34): C, 59.67; H, 3.89; N, 23.19. Found: 59.40; H, 3.75; N, 23.20.

N-{4-[(4-Benzothiazol-ylphenyl)hyrazono]-5-oxo-4,5-dihydro-1H-pyrazol-3-yl) acetamide (10b) Yield 83%, mp: 215°C. IR (cm^{-1}): 3233 (NH), 3055 (CH aromatic), 2919 (CH aliph), 1675 (C=O, pyrazolone),1647 (C=O of amide). ^{1}H NMR (DMSO, 400 MHz): δ 2.65 (s, 3H, CH_3), 3.37 (s, 1H, NH), 7.37 (dd, J_1= 8.4 Hz, J_2= 7.2 Hz, 2H, CH aromatic), 7.46 (dd, J_1= 8.4 Hz, J_2= 7.2 Hz, 2H, CH aromatic), 7.53-7.55 (m, 2H, CH aromatic), 7.57 (s, 1H, NH), 8.05 (s, 1H, NH), 8.12-8.16 (m, 2H, CH aromatic);^{13}C NMR (DMSO): 39.28, 116.34, 120.54, 122.83, 123.23, 126.03, 127.21,129.38, 134.85, 142.90, 155.25, 157.45, 159.88, 163,35,

177.24, 178.37; Anal. Calcd. $C_{18}H_{14}N_6O_2S$ (378.41): C, 57.13; H, 3.73; N, 22.21. Found: 57.30; H, 3.55; N, 22.20.

N-{4-[(4-Benzoxazol-ylphenyl)hyrazono]-5-oxo-1,5-dihydro-1H-pyrazol-3-yl) benzamide (11a) Yield 85%, mp: 220-222°C. IR (cm^{-1}): 3226, 3188 (NH), 3043 (CH aromatic), 1688 (C=O, pyrazolone), 1611 (C=O of amide). ^{1}H NMR (DMSO, 400 MHz): δ 3.21 (s, 1H, NH), 7.35 (dd, J_1= 8.4 Hz, J_2= 6.4 Hz, 2H, CH aromatic), 7.42 (dd, J_1= 8.4 Hz, J_2= 6.4 Hz, 2H, CH aromatic), 7.44-7.50 (m,, 2H, CH aromatic), 7.68 (s,1H, NH), 7.70-7.80 (m, 5H, CH aromatic), 8.15-8.25 (m, 2H, CH aromatic), 12.99 (s, 1H, NH);^{13}C NMR (DMSO):111.491, 119.60, 120.51, 121.87, 122.43, 122.85 123.15, 125.35, 125.65, 126.35, 129.42, 129.54, 141.95, 154.20, 156.42, 158.78, 161,42, 175.62, 177.73; Anal. Calcd. for $C_{23}H_{16}N_6O_3$ (424.41): C, 65.09; H, 3.80; N, 19.80; Found: 65.20; H, 3.95; N, 20.10.

N-{4-[(4-Benzothiazol-ylphenyl)hyrazono]-5-oxo-1,5-dihydro-1H-pyrazol-3-yl) benzamide (11b) Yield 87%, mp: 230-232°C. IR (cm^{-1}): 3203 (NH), 3056 (CH aromatic), 1678 (C=O, pyrazolone), 1647 (C=O of amide);.^{1}H NMR (DMSO, 400 MHz): δ 3.19 (s, 1H, NH), 7.32 (dd, J_1= 9.2 Hz, J_2= 6.8 Hz, 2H, CH aromatic), 7.44 (dd, J_1= 9.2 Hz, J_2= 6.8Hz, 2H, CH aromatic), 7.46-7.50 (m, 2H, CH aromatic), 7.70 (s, 1H, NH),7.94-8.04 (m, 5H, CH aromatic), 8.12-8.18 (m, 2H, CH aromatic), 8.22 (s, 1H, NH).^{13}C NMR (DMSO):115.45, 117.37, 120.54, 122.83, 123.23, 124.54, 124.87, 126.03, 126.49, 127.75, 129.08, 130.03, 141.95, 152.89, 153.34, 157.57, 160,41, 170.89, 175.37. Anal. Calcd. for $C_{23}H_{16}N_6O_3$ (440.48): C, 62.72; H, 3.66; N, 19.08; Found: 62.80; H, 3.55; N, 19.35.

N-{4-[(4-Benzoxazol-ylphenyl)hyrazono]-5-oxo-4,5-dihydro-1H-pyrazol-3-yl)2-chloroacetamide (12a) Yield 79%, mp: 192-194°C. IR (cm^{-1}): 3397, 3140 (NH), 3046 (CH aromatic), 2921 (CH aliph), 1684 (C=O pyrazolone),1649(C=O of amide).^{1}H NMR (DMSO, 400 MHz): δ 3.41 (s, 1H, NH), 3.87 (s, 2H, CH_2), 7.36 (dd, J_1 = 8.4 Hz, J_2 = 11.2 Hz, 2H, CH aromatic), 7.42 (dd, J_1 = 8.4 Hz, J_2 = 11.2Hz, 2H, CH aromatic), 7.69 (s, 1H, NH), 7.71-7.78 (m, 2H, CH aromatic), 8.15-8.24 (m, 2H, CH aromatic), 8.26 (s, 1H, NH). ^{13}C NMR (DMSO): 40.59, 111.37, 120.20, 120.53, 123.40, 125.40, 125.97,129.30, 129.53, 141.97, 143.51, 150.65, 162.17, 163,34, 176.14, 175.73. Anal. Calcd. for $C_{18}H_{13}ClN_6O_3$ (396.79): C, 54.49; H, 3.30; N, 21.18. Found: 54.40; H, 3.35; N, 21.10.

N-{4-[(4-Benzothiazol-ylphenyl)hyrazono]-5-oxo-4,5-dihydro-1H-pyrazol-3-yl)2-chloroacetamide (12b) Yield 85%, mp: 195-197°C. IR (cm^{-1}): 3392, 3138 (NH), 3046 (CH aromatic), 2921 (CH aliph), 1673 (C=O pyrazolone), 1648 (C=O of amide). ^{1}H NMR (DMSO, 400 MHz): δ 3.41 (s, 1H, NH), 3.88 (s, 2H, CH_2), 7.32 (dd, J_1= 8.8 Hz, J_2 = 6 Hz, 2H, CH aromatic), 7.44 (dd, J_1= 8.8 Hz, J_2 = 6 Hz, 2H, CH aromatic), 7.57 (s, 1H, NH), 7.81–7.93 (m, 2H, CH aromatic), 8.10–8.16 (m, 2H, CH aromatic), 8.18 (s, 1H, NH). ^{13}C NMR (DMSO): 40.53, 111.50, 120.54, 122.85, 123.25, 126.01, 127.20, 129.37, 130.04, 134.86, 142.88, 154.01, 166.81, 167,34, 175.12, 177.74; Anal. Calcd. for $C_{18}H_{13}ClN_6O_2S$ (412.85): C, 52.37; H, 3.17; N, 20.36. Found: 52.30; H, 3.10; N, 20.20.

General method adopted for the preparation of compounds 13a–14b

To a solution of compounds **9a** or **9b** (2.0 mmol) in absolute ethanol (30 mL), the appropriate aldehyde (2.1 mmol) and 0.5 mL glacial acetic acid was added, and the reaction mixture was refluxed for 20 h and cooled to room temperature. Then the obtained crystals were filtered, washed with water, dried and recrystallized from ethanol to give the perspective compounds listed below.

4-[(4-Benzoxazol-ylphenyl)hyrazono]-5-[(4-nitrobenzylidine) amino]-2,4-dihydro pyrazol-3-one (13a) Yield 75%, mp: 250-252°C. IR (cm^{-1}): 3229 (NH), 3050 (CH aromatic), 1685 (C=O, pyrazolone), 1648 (C=O of amide). ^{1}H NMR (DMSO, 400 MHz): δ 3.57 (s, 1H, NH), 7.36 (dd, J_1 = 8.4 Hz, J_2 = 10.8 Hz, 2H, CH aromatic), 7.42 (dd, J_1 = 8.4 Hz, J_2 = 10.8 Hz, 2H, CH aromatic), 7.69-7.74 (m, 2H, CH aromatic), 7.74 (s, 1H, NH), 7.77–7.87 (m, 2H, CH aromatic), 8.13–8.33 (m,, 4H, CH aromatic), 8.43 (s, 1H, N=CH). ^{13}C NMR (DMSO):111.38, 115.32, 115.94, 120.21, 120.54, 125.24, 125.98, 126.34, 129.54, 134.44, 134.98, 136.24, 150.92, 152.86, 158.32, 159.52, 164.48, 174.82, 175.38. Anal. Calcd. for $C_{23}H_{15}N_7O_4$ (453.41); C, 60.93; H, 3.33; N, 21.62; Found: 60.90; H, 3.50; N, 21.50.

4-[(4-Benzothiazol-ylphenyl)hyrazono]-5-[(4-nitrobenzylidine) amino]- 2,4-dihydropyrazol-3-one (13b) Yield 82%, mp: 240-242°C. IR (cm^{-1}): 3220 (NH), 3058 (CH aromatic), 1674 (C=O, pyrazolone), 1646(C=O of amide).^{1}H NMR (DMSO, 400 MHz): δ 3.58 (s, 1H, NH), 7.33 (dd, J_1 = 10 Hz, J_2 = 7.6 Hz, 2H, CH aromatic), 7.44 (dd, J_1 = 10 Hz, J_2 = 7.6Hz, 2H, CH aromatic), 7.58-7.74 (m,, 4H, CH aromatic), 7.76 (s, 1H, NH), 8.02-8.41 (m,, 4H, CH aromatic), 8.67 (s, 1H, N=CH). ^{13}C NMR (DMSO):110.54, 114.36, 114.84, 120.21, 120.54, 122.85, 126.01, 126.32, 127.20, 129.37, 134.85, 135.44, 149.82, 150.56, 157.22, 159.02, 163.24, 172.62, 174.88. Anal. Calcd. for $C_{23}H_{15}N_7O_3S$ (469.48): C, 58.84; H, 3.22; N, 20.88; Found: 58.70; H, 3.30; N, 20.90.

4-[(4-Benzoxazol-ylphenyl)hyrazono]-5-[(4-chlorobenzylidine) amino]- 2,4-dihydro pyrazol-3-one (14a) Yield 80%, mp: 235-237°C. IR (cm^{-1}): 3168 (NH), 3052 (CH aromatic), 1683 (C=O, pyrazolone), 1650(C=O of amide), ^{1}H NMR (DMSO, 400 MHz): δ 3.54 (s, 1H, NH), 7.35 (dd, J_1 = 8.4 Hz, J_2= 12.8 Hz, 2H, CH aromatic), 7.42 (dd, J_1 = 8.4 Hz, J_2= 12.8Hz, 2H, CH aromatic), 7.67–7.78 (m, 4H, CH aromatic), 7.80 (s, 1H, NH), 8.11-8.28 (m, 4H, CH aromatic), 8.30 (s, 1H, N=CH). ^{13}C NMR (DMSO):111.37, 115.16, 115.72, 119.01, 120.20, 120.53, 125.40, 125.97, 128.88, 129.53, 134.84, 141.97, 150.65, 152.62, 156.12, 160.02, 162.17, 174.12, 175.22. Anal. Calcd. for $C_{23}H_{15}ClN_6O_2$ (442.86): C, 62.38; H, 3.41; N, 19.98; Found: 62.40; H, 3.40; N, 20.10.

4-[(4-Benzothiazol-ylphenyl)hyrazono]-5-[(4-chlorobenzylidine) amino]- 2,4-dihydro pyrazol-3-one (14b) Yield 78%, mp: 237–239°C. IR (cm^{-1}): 3233 (NH), 3054 (CH aromatic), 1668 (C=O, pyrazolone), 1649 (C=O of amide). ^{1}H NMR (DMSO, 400 MHz): δ 4.31 (s, 1H, NH), 7.32 (dd, J_1 = 8.4 Hz, J_2 = 7.8 Hz, 2H, CH aromatic), 7.45 (dd, J_1 = 8.4 Hz, J_2 = 7.8 Hz, 2H, CH aromatic), 7.54–7.60 (m, 4H, CH aromatic), 7.90 (s, 1H, NH), 8.04-8.16 (m, 4H, CH aromatic), 8.72 (s, 1H,

N=CH). ^{13}C NMR (DMSO):110.30, 114.42, 115.92, 118.01, 120.55, 121.41, 123.25, 127.20, 127.35, 128.41, 131.62, 140.89, 151.55, 151.88, 155.92, 159.21, 161.75, 173.22, 174.75. Anal. Calcd. for $C_{23}H_{15}ClN_6OS$ (458.92): C, 60.19; H, 3.39; N, 18.31; Found: 60.20; H, 3.30; N, 18.20.

Pharmacological screening

Anticancer activity

Antitumor activity evaluation was performed at the Center of Genetic Engineering, Al-Azhar University, Cairo, Egypt. Reagents and chemicals were obtained from Sigma Aldrich Chemical (St. Louis, MO, U.S.A.). The tested cell lines were obtained from the American Type Culture Collection (ATCC, MN, U.S.A.) through the Tissue Culture Unit, The Egyptian Organization for Biological Products and Vaccines (Vacsera, Egypt). The synthesized target compounds were evaluated in vitro for their cytotoxic activity against three different cancer cell lines, A549, MCF7 and Hep3B, in addition to normal fibroblast cells using the Sulphorhodamine-B (SRB) assay method [30]. Cells were seeded for 24 h in a 96-well microtiter plate at a concentration of 1000–2000 cells/well, then the cells were incubated for 48 h with various concentrations (0, 6.25, 12.5, 25, 50, 100 μg/mL) of the tested compounds. Each experiment was repeated three times, and after incubation for 48 h, the cells were fixed with 10% trichloroacetic acid 150 μl/well for 1 h at 4°C, and washed by distilled water 3 times. The wells were stained for 10–30 min at room temperature with 0.4% SRB, and dissolved in 1% acetic acid 70 μl/well, then washed with acetic acid 1% to remove unbound dye until colorless drainage obtained. The plates were subjected to air-drying for 24 h and not exposed to UV. The dye was solubilized with 150 μl/well of 10 mMTrise- EDTA (PH 7.4) for 5 min on a shaker at 1600 rpm. The optical density (OD) of each well was measured at 545 nm with an ELISA micro-plate reader. Survival curves were obtained by plotting the surviving fraction against different concentrations of the tested compounds. The IC_{50} values were calculated (Table 1), using Sigmaplot software.

Colorimetric COX-1/COX-2 inhibition assay

Caymaṅs colorimetric COX (ovine) Inhibitor Screening Assay Kit (Cayman Chemical, Ann Arbor, MI, USA) was used to measure in vitro inhibition of COX-1/COX-2. IC_{50} values for each tested compound against COX-1 and COX-2 were detected in comparison to celecoxib as a reference drug (Table 2). COX-1/COX-2 enzymes were incubated at 25°C with different concentrations of the tested compounds **10a–14b**, celecoxib or vehicle for 5 min. Arachidonic acid and colorimetric substrate were added. The COX peroxidase inhibition activity of the tested compounds was detected by monitoring the production of N,N,N0,N0-tetramethyl-p-phenylenediamine (TMPD) at 590 nm according to the manufacturer's instructions [31]. The COX-inhibiting activity of the tested compounds was then calculated using the equation: % inhibition = $[1-(A_2-A_0)/(A_1-A_0)] \times 100$,

Table 1 IC_{50} values and S.I. for the newly synthesized target compounds (μM)

10aNo.	A549 IC_{50}(μM)	S.I.	MCF7 IC_{50}(μM)	S.I.	Hep3B IC_{50}(μM)	S.I.	Normal fibroblast cells IC_{50}(μM)
10a	21.27	0.17	43.23	0.08	91.83	0.04	3.80
10b	172.28	0.68	1127.56	0.10	109.64	1.07	117.47
11a	80.56	1.29	18.66	5.60	38.91	2.68	104.66
11b	183.47	0.31	617.41	0.09	36.22	1.57	57.20
12a	8.66	22.26	135.67	1.42	12.93	14.90	192.77
12b	4.99	15.32	851.82	0.08	105.94	0.72	76.46
13a	2.35	83.19	157.63	1.24	121.08	1.61	195.84
13b	6.99	5.27	231.66	0.15	81.49	0.45	36.87
14a	110.09	1.94	97.47	2.19	247.59	0.86	214.31
14b	131.99	4.32	34.39	16.59	155.35	3.67	570.62

Selectivty index (S.I.) = IC_{50} of the tested compound on normal cells/IC_{50} on the cancer cell line

Table 2 In vitro COX-1 and COX-2 inhibition of the new target compounds **10a**–**14b** and celecoxib

No.	IC_{50} (μM)[a]		
	COX-1	COX-2	COX-2 selectivity index[b]
Celebrex (reference)	7.34	1.11	6.61
10a	4.51	0.86	5.24
10b	4.27	0.76	5.62
11a	8.14	2.74	2.97
11b	6.84	1.54	4.44
12a	8.74	1.74	5.02
12b	9.11	2.45	3.72
13a	3.54	0.56	6.32
13b	7.62	1.23	6.195
14a	10.11	3.11	3.25
14b	5.34	0.74	7.22

[a] The IC_{50} value represents the compound concentration that produces 50 % inhibition of COX-1 or COX-2

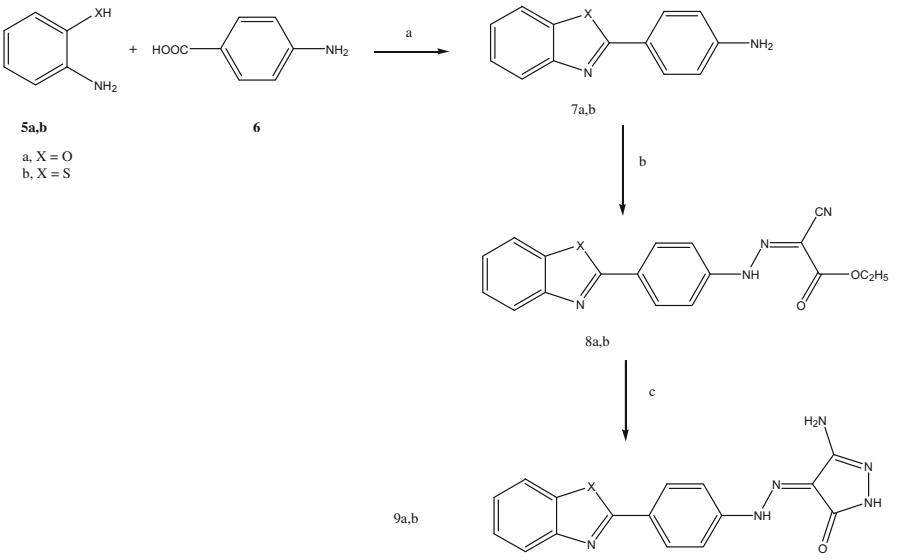
[b] Selectivty index (IC_{50} on COX-1 / IC_{50} on COX-2)

where A_0 is the absorbance of the blank, A_1 is the absorbance of the vehicle control, and A_2 is the absorbance of the test.

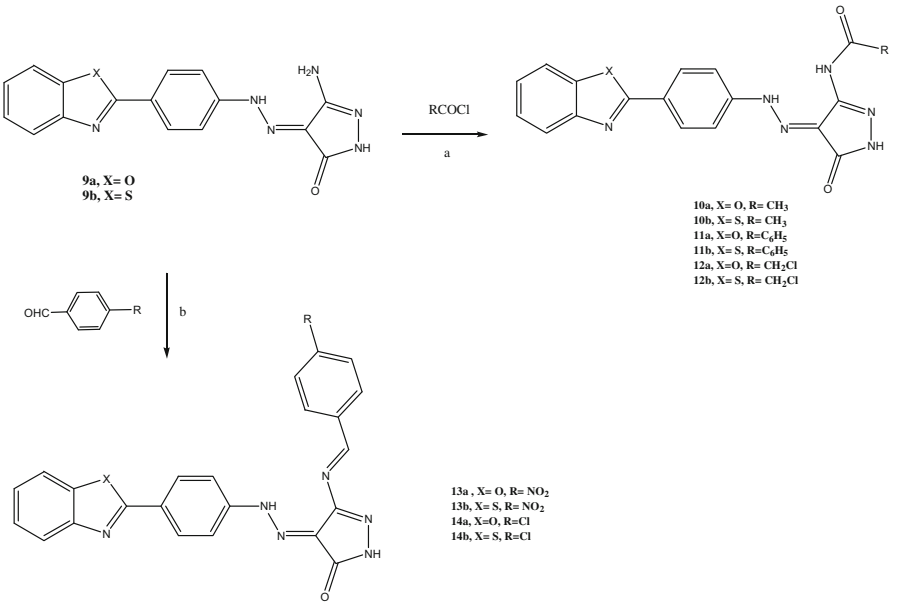
Results and discussion

Chemistry

The general synthetic pathways are illustrated in Schemes 1 and 2. Compounds **7a**and **7b** were prepared according to the previously described methods [29]. Compounds **8a** and **8b** were reacted with hydrazine hydrate to afford the starting materials **9a** and **9b** (Scheme 1). For substituting the C3 of the obtained pyrazolinone, the starting materials **9a** and **9b** were subjected to further reactions



Scheme 1 Synthesis of compounds **9a** and **9b**. Reagents and conditions: *a* Poly phosphoric acid, heating at 200 °C for 3h, cooling, Na_2CO_3. *b* HCl, $NaNO_2$. Ethyl cyanoacetate, CH_3COONa, aqueous ethanol. *c* hydrazine hydrate, absolute ethanol, reflux for 12 h, crystallization from acetic acid



Scheme 2 Synthesis of benzothizole/benzoxazole–pyrazole hybrids **10a–14b**. Reagents and conditions: *a* dioxan, acid chloride, r.t., 24h; *b* absolute ethanol, aldehyde, glacial acetic, reflux 20 h

as shown in Scheme 2. The obtained target compounds **10a–14b** were well characterized using IR, ^{1}H NMR, ^{13}C NMR and elemental microanalysis to prove their chemical structures. IR spectra of compounds **10a–12b** showed the appearance of an additional CO group, ^{1}H NMR of compounds **10a** and **10b** revealed a signal at 2.5, 2.6 characterizing their CH_3 group respectively; however, the ^{1}H NMR of compounds **11a** and **11b** showed the appearance of additional aromatic protons 6–8 ppm. The ^{1}H NMR spectra of compounds **12a** and **12b** showed signals at 3.4 and 3.8 ppm indicating CH_2Cl of these two compounds, respectively. Compounds **13a–14b** showed a singlet signal in their ^{1}H NMR spectra characterizing the N=CH proton at 8.4, 8.6, 8.3, and 8.7, respectively, in addition to the aromatic protons at 7–8 ppm. Also, ^{13}C NMR and elemental microanalysis were used as tools in structure confirmation.

Pharmacological screening

Anticancer activity

The new target compounds **10a–14b** were screened against lung, breast and liver cancer cell lines (A549, MCF7 and Hep3B), in addition to the normal fibroblast cells using the sulphorhodamine-B assay method [30]. The obtained IC_{50} values and S.I. for the tested compounds are shown in Table 1. The obtained data revealed that compounds **12a–13b** are the most active and selective towards the A549 cell line; their IC_{50} values were less than 10 μM. Compound **13a** was the most active and selective one on the A549 cell line; its IC_{50} and S.I. values were 2.4 μM and 83.2, respectively. It can be seen that substituting the two hybrids **9a** and **9b** with chloro acetyl moiety **12a** and **12b** or p-nitro Schiff base moiety **13a** and **13b** at C3 of the pyrazolinone nucleus leads to obtaining good anticancer active candidates **12a–13b** against the lung cancer cell line A549. Compound **14b** was shown to be active on MCF7 with the best selectivity towards this cancer cell line, while compound **11a** also showed good activity on the same cancer cell line but with much less selectivity than compound **14b**; thus, substituting C3 of benzothizole/pyrazole hybrid with p-chloro schiff base leads to obtaining the active and selective compound **14b** on the MCF7 cancer cell line. As for the Hep3B cancer cell line, compound **12a** was shown to be the most active and selective compound; its IC_{50} value was 13 μM with S.I. equal 14.9. Compounds **11a** and **11b** also showed activity and selectivity towards this cancer cell line.

COX inhibition assay

COX-2 represent a verified target in cancer treatment, hence the newly synthesized target compounds were screened on COX enzymes using Cayman's colorimetric COX (ovine) screening assay [31]. Their IC_{50} values expressed in μM and also their selectivity towards COX-2 are represented in Table 2. The obtained results revealed that compounds **13a** and **14b** were shown to be more active inhibitors than celecoxib; their IC_{50} values on COX-2 were 0.56, 0.75 and 1.11 μM, respectively. Also, compounds **10a** and **10b** showed lower IC_{50} values on COX-2 than celcoxib

(Table 2); however, the IC_{50} of compounf **12a** on COX-2 was 1.74 μM, which is greater than the reference drug (1.11 μM). As for the selectivity of the tested compounds towards COX-2 over COX-1, compound **14b** showed better selectivity than celcoxib and compounds **13a** and **13b** also showed a closely similar selectivity to celcoxib, and their selectivity index values towards COX-2 over COX-1 were 6.3, 6.2 and 6.6, respectively. Compound **12a** showed moderate selectivity towards COX-2 (5.02). We can say that the anticancer activity of compound **13a** (the best active compound on A549) and compound **14b** (the active and the most selective on MCF7) can go through COX-2 inhibition. Compound **13a** and **14b** are good inhibitors for COX-2 and represent hopeful selective candidates in treating A549 and MCF7 cancers, respectively.

Structure–activity relationship (SAR)

Introducing benzoxazole or benzothiazole moiety into C4 of pyrazolone was a useful strategy to get new bioactive anticancer molecules **11a–13b** and **14b**. Substituting C4 of pyrazolone with benzoxazole **12a** and **13a** or benzothiazole **12b** and **13b** lead to obtaining very potent and selective compounds on the A549 cancer cell line, as the IC_{50} value of these four new hybrids **12a–13b** was less than 10 μM and they were more selective to COX2 over COX1. Hybridizing benzoxazole with pyrazolone at C4 position afforded **11a** with good activity on both MCF7 and Hep3B; however, substituting the same position with benzothiazole **11b** leads to maintaining the same activity on Hep3B and diminished activity on MCF7. Substituting C3 of the pyrazolone/benzoxazole hybrid with *p*-nitro benzaldehyde afforded compound **13a** which showed a greater inhibitory activity towards COX-2 than coxib and showed also to be active against lung cancer (A549). Substituting C3 of pyrazolone/benzothiazole with p-chloro benzaldehyde lead to obtaining an active molecule **14b** on MCF7 with greater selectivity towards COX-2 than coxib. However, substituting pyrazolone/benzoxazole system with the same moiety **14b** leads to the loss of anticancer and COX-2 inhibitory activity. Acyl moiety at C3 position in both benzoxazole or benzothiazole/pyrazole hybrids **10a** and **10b** lead to diminished anticancer activity and poor selectivity towards cancer versus the normal cell line.

Conclusions

In this study, new hybrids of pyrazole–benzothiazole/benzoxazole were designed and successfully synthesized. The synthesized hybrids showed better activity on the A549 cell line than both MCF7 and Hep3B cancer cell lines, as four compounds of the new hybrids **12a–13b** showed activity on A549 with an IC_{50} value less than 10 μM, in addition, these compounds showed good selectivity towards this cancer cell line. Compounds **10a**, **11a** and **14b** showed activity on MCF7; their IC_{50} values were less than 50 μM and compound **14b** was the most selective one. Compounds **11a**, **11b** and **12a** were the most active on Hep3B, with $IC_{50} < 50$ μM, and compound **12a** was the most active and selective one towards this cell line. This

research work demonstrated that the anticancer active compounds **13a** and **14b** which are good inhibitors for COX-2 enzyme can be subjected for further clinical trials to be approved as promising agents to treat lung (A549) and breast (MCF7) cancer, respectively. Moreover, compound **12a** is a hopeful and selective candidate in treatment of Hep3B cancer; however, its mechanistic studies needs further exploration.

Acknowledgements Authors are grateful to Dr. Ahmed B. M. Mehany, Zoology Department, Faculty of Science, Al-Azhar university, Egypt, for his help in performing cytotoxicity for the new compounds.

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