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CONVENIENT SYNTHESIS, ANTI-INFLAMMATORY, ANALGESIC AND ULCEROGENIC ACTIVITES OF SOME NEW BIS-HYDRAZONES AND PYRAZOLE DERIVATIVES

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Abstract: The reaction of acid hydrazides (**1a-c**) with 2-chloro-1-(4-chlorophenyl)ethanone (**2a**) or 2-bromo-1-(4-bromophenyl)ethanone (**2b**) afforded bis-hydrazones **6a-d**, while the reaction of **1a-c** with 2-oxo-*N*-aryl-propanehydrazonoyl chlorides (**3a,b**) furnished *N*-(aryl)propanehydrazonoyl chlorides **8a-c**. The reaction of the latter chlorides with sodium benzenesulfinate furnished sulfones **11a-c**. On the other hand, treatment of benzothiazole-2-carbohydrazide (**1c**) with the appropriate ketones yielded the corresponding hydrazones **13a,b,** while the reaction of **1c** with 2-(ethoxymethylene)malononitrile (**14**) or with 2-[bis(methylthio)methylene]malononitrile (**16**) afforded pyrazole derivatives **15** and **17**, respectively. In acute toxicity study, no mortalities were observed for the tested compounds. All the tested compounds showed significant anti-inflammatory activity, while some of them exhibited potent analgesic activity. In addition, all compounds exhibited lower ulcerogenic effects than the standard ketoprofen.

Keywords: bis-hydrazones, benzothiazole, sulfones, pyrazoles, anti-inflammatory, analgesic activity

A large number of benzothiazole derivatives have attracted continuing interest because of their varied biological activities viz. significant anticancer (1-9), and antioxidant (10, 11) antimicrobial (12-16), antitubercular (17), antimalarial (18), anticonvulsant (19), anthelmintic (20), analgesic (21), anti-inflammtory (22), and antidiabetic (23) activities. Recently, benzothiazole derivatives have been evaluated as potential amyloid-binding diagnostic agents in neurodegenerative disease (24, 25) and as selective fatty acid amide hydrolase inhibitors (26). On the other hand, many hydrazones have been reported as useful bioactive agents. Some acetyl/arylhydrazones and heteroarylhydrazones revealed a remarkable antiviral activity against HSV-1, HIV-1, *versus* influenza A_2, A_3 and Semliki Forest viruses (27-29). In addition, various compounds including pyrazole nucleus are known to possess analgesic, anti-inflammatory, antipyretic, antiarrhythmic, tranquillizing, muscle relaxant, psychoanaleptic, anticonvulsant, hypotensive, antidiabetic and monoamine oxidase inhibitory activities (30-34).

Intrigued by the above observations and in continuation of our study on the synthesis of biologically active heterocycles (35-38), we synthesized some new hydrazones incorporated to the benzothiazole moiety to investigate their anti-inflammatory and analgesic activity.

EXPERIMENTAL

Cemistry

All melting points were uncorrected and measured using an Electro-thermal IA 9100 apparatus (Shimadzu, Japan). Microanalytical data were performed by Elementar Vario EL apparatus. The IR spectra were recorded (for potassium bromide pellets) using KBr disc technique on a Perkin-Elmer 1650 Spectrophotometer. NMR experiments were conducted at ICB-NMR Service Centre (Pozzuoli, Italy), and were measured in DMSO, (shifts are referenced to the TMS signal) on a Bruker Avance-400 operating at 400 MHz, and on a JEOL-Ex-300 MHz.The ^{13}C NMR spectra were run at 75.46 MHz in deuterated dimethyl sulfoxide (DMSO-d_6) and

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chemical shifts were expressed as ppm (δ) values against TMS as an internal reference (Faculty of Science, Cairo University, Cairo, Egypt). Mass spectra were recorded on Shimadzu GCMS-QP-1000EX mass spectrometer at 70 eV (Cairo University, Cairo, Egypt). Benzothiazole-2-carbohydrazide (**1c**) (39) and 2-oxo-*N*-arylpropanehydrazonoyl chlorides (**7a,b**) (40) were prepared according to the literature procedures.

General procedure for the synthesis of compounds 6a-d

To a solution of carbohydrazides (**1a-c**) (10 mmol) in ethanol (50 mL), 2-chloro-1-(4-chlorophenyl)ethanone (**2a**) or 2-bromo-1-(4-bromophenyl)ethanone (**2b**) (5 mmol) was added. The reaction was refluxed for 7 h, and then was cooled to room temperature. The formed solid was filtered off, washed with ethanol and recrystallized from EtOH/DMF to afford compounds **6a-d**, respectively.

(Z)-N',N''-(1-(4-Chlorophenyl)ethene-1,2-diyl) bis(4-methylbenzhydrazide) (6a)

Yield 60%; m.p. 260-262°C; IR (KBr, cm^{-1}): 3450-3042 (4NH), 1733-1672 (2C=O); ^{1}H NMR (DMSO-d_6, δ, ppm): 2.34 (s, 3H, CH_3), 2.47 (s, 3H, CH_3), 7.19-9.01 (m, 13H; 12ArH + C=C*H*-), 10.40 (s, 1H, D_2O exchangeable, NH), 11.85 (s, 1H, D_2O exchangeable, NH), 12.91 (s, 1H, D_2O exchangeable, NH), 14.45 (s, 1H, D_2O exchangeable, NH); MS m/z (%): 434 (M^+, 18), 313 (17), 119 (100), 91 (30). Analysis: calcd. for $C_{24}H_{23}ClN_4O_2$ (434.92): C, 66.28; H, 5.33; N, 12.88%; found: C, 66.09; H, 5.16; N, 13.03%.

(Z)-N',N''-(1-(4-Chlorophenyl)ethene-1,2-diyl) bis(4-nitrobenzhydrazide) (6b)

Yield 66%; m.p. 247-249°C; IR (KBr, cm^{-1}): 3430-3082 (4NH), 1735-1670 (2C=O); ^{1}H NMR (DMSO-d_6, δ, ppm): 7.62-9.01 (m, 13H, ArH; 12ArH + C=C*H*-), 9.65 (s, 1H, D_2O exchangeable, NH), 11.43 (s, 1H, D_2O exchangeable, NH), 12.57 (s, 1H, D_2O exchangeable, NH), 14.14 (s, 1H, D_2O exchangeable, NH); MS m/z (%): 496 (M^+, 19), 312 (30), 136 (100), 101 (41), 75 (28). Analysis: calcd. for $C_{22}H_{17}ClN_6O_6$ (496.86): C, 53.18; H, 3.45; N, 16.91%; found: C, 53.39; H, 3.22; N, 17.17%.

(Z)-N',N''-(1-(4-Bomophenyl)ethene-1,2-diyl) bis(4-nitrobenzhydrazide) (6c)

Yield 60%; m.p. 271-273°C; IR (KBr, cm^{-1}): 3436-3087 (4NH), 1671-1654 (2C=O); ^{1}H NMR (DMSO-d_6, δ, ppm): 7.75-8.78 (m, 13H, ArH; 12ArH + C=C*H*-), 9.62 (s, 1H, D_2O exchangeable, NH), 11.56 (s, 1H, D_2O exchangeable, NH), 12.84 (s, 1H, D_2O exchangeable, NH), 14.05 (s, 1H, D_2O exchangeable, NH); MS m/z (%): 541 (M^+, 45), 182 (100), 101 (87), 75 (48). Analysis: calcd. for $C_{22}H_{17}BrN_6O_6$ (541.31): C, 48.81; H, 3.17; N, 15.53%; found: C, 48.70; H, 3.14; N, 15.73%.

(Z)-N,N'-(1-(4-Chlorophenyl)ethene-1,2-diyl)bis (benzo[d]thiazole-2-carbohydrazide) (6d)

Yield 62%; m.p. 244-246°C; IR (KBr, cm^{-1}): 3447-3131 (4NH), 1691-1652 (2C=O), 1610 (2C=N); ^{1}H NMR (DMSO-d_6, δ, ppm): 7.32-8.39 (m, 12H, ArH), 9.01 (s, 1H, C=C*H*-), 10.65 (s, 1H, D_2O exchangeable, NH), 11.83 (s, 1H, D_2O exchangeable, NH), 13.10 (s, 1H, D_2O exchangeable, NH), 14.43 (s, 1H, D_2O exchangeable, NH); MS m/z (%): 521 (M^+, 15), 490 (59), 473 (66), 356 (100), 162 (81), 135 (90). Analysis: calcd. for $C_{24}H_{17}ClN_6O_2S_2$ (521.01): C, 55.33; H, 3.29; N, 16.13; S, 12.31%; found: C, 55.21; H, 3.24; N, 16.05; S, 12.50%.

General procedure for the synthesis of N-arylpropanehydrazonoyl chlorides 8a-c

A mixture of carbohydrazides (**1a-c**) (10 mmol) and the appropriate 2-oxo-*N*-arylpropanehydrazonoyl chloride (**7a** or **7b**) (10 mmol) in absolute ethanol (50 mL) was refluxed for 9 h. The formed solid was filtered, washed with ethanol and recrystallized from EtOH/DMF to afford the corresponding hydrazonoyl chlorides **8a-c**, respectively.

(1Z,2E)-2-(2-(4-Methylbenzoyl)hydrazono)-N'-p-tolylpropanehydrazonoyl chloride (8a)

Yield 90%; m.p. 241-243°C; IR (KBr, cm^{-1}): 3268, 3215 (2NH), 1634 (C=O), 1557 (2C=N); ^{1}H NMR (DMSO-d_6, δ, ppm): 2.25 (s, 3H, CH_3), 2.36 (s, 6H, $2CH_3$), 7.08-7.85 (m, 8H, ArH), 9.92 (s, 1H, D_2O exchangeable, NH), 10.65 (s, 1H, D_2O exchangeable, NH); ^{13}C NMR (DMSO-d_6, δ, ppm): 13.45 (-CH_3), 20.05 (-CH_3), 20.79 (-CH_3), 113.69, 123.03, 128.24, 128.45, 129.26, 129.69, 130.72, 141.10, 141.28; MS m/z (%): 342 (M^+, 44), 306 (42), 119 (100), 91 (48). Analysis: calcd. for $C_{18}H_{19}ClN_4O$ (342.82): C, 63.06; H, 5.59; N, 16.34%; found: C, 63.14; H, 5.46; N, 16.29%.

(1Z,2E)-2-(2-(4-Nitrobenzoyl)hydrazono)-N'-phenylpropanehydrazonoyl chloride (8b)

Yield 90%; m.p. 241-243°C; IR (KBr, cm^{-1}): 3301, 3176 (2NH), 1665 (C=O), 1598 (2C=N); ^{1}H NMR (DMSO-d_6, δ, ppm): 2.39 (s, 3H, CH_3), 6.68-8.40 (m, 9H, ArH), 10.18 (s, 1H, D_2O exchangeable, NH), 11.12 (s, 1H, D_2O exchangeable, NH); MS m/z

(%): 359 (M^+, 18), 323 (52), 206 (24), 150 (29), 104 (38), 92 (100), 65 (65). Analysis: calcd. for $C_{16}H_{14}ClN_5O_3$ (359.77): C, 53.42; H, 3.92; N, 19.47%; found: C, 53.60; H, 3.88; N, 19.29%.

(1Z,2E)-2-(2-(Benzo[d]thiazole-2-carbonyl)hydrazono)-N'-p-tolylpropanehydrazonoyl chloride (8c)

Yield 90%; m.p. 241-243°C; IR (KBr, cm^{-1}): 3321, 3256 (2NH), 1692 (C=O), 1600 (2C=N); ^{1}H NMR (DMSO-d_6, δ, ppm): 2.25 (s, 3H, CH_3), 2.46 (s, 3H, CH_3), 7.05-8.12 (m, 8H, ArH), 10.03 (s, 1H, D_2O exchangeable, NH), 11.46 (s, 1H, D_2O exchangeable, NH); MS m/z (%): 385 (M^+, 19), 349 (28), 268 (100), 69 (28). Analysis: calcd. for $C_{18}H_{16}ClN_5OS$ (385.87): C, 56.03; H, 4.18; N, 18.15; S, 8.31%; found: C, 55.94; H, 4.25; N, 18.02; S, 8.44%.

Synthesis of sulfones 11a-c

To a solution of the appropriate propanehydrazonoyl chloride (**8a-c**) (1 mmol) in absolute ethanol (50 mL), sodium benzenesulfinate dihydrate (0.4 g, 2 mmol) was added. The mixture was refluxed for 22 h, then left to cool. The reaction mixture was poured into cold water and the solid product was filtered, washed with water, dried and finally recrystallized from EtOH/DMF to afford the corresponding sulfones **11a-c**.

4-Methyl-N'-(1-(phenylsulfonyl)-1-(2-p-tolylhydrazono)propan-2-ylidene)benzohydrazide (11a)

Yield 63%; m.p. 257-259°C; IR (KBr, cm^{-1}): 3216 (2NH), 1642 (C=O), 1522 (2C=N); ^{1}H NMR (DMSO-d_6, δ, ppm): 1.65 (s, 3H, CH_3), 1.77 (s, 3H, CH_3), 2.40 (s, 3H, CH_3), 6.12-7.20 (m, 13H, ArH), 11.55 (s, 1H, D_2O exchangeable, NH), 12.89 (s, 1H, D_2O exchangeable, NH); MS m/z (%): 448 (M^+, 74), 307 (62), 119 (100), 106 (73), 91 (50). Analysis: calcd. for $C_{24}H_{24}N_4O_3S$ (448.54): C, 64.27; H, 5.39; N, 12.49; S, 7.15%; found: C, 64.11; H, 5.36; N, 12.60; S, 7.03%.

4-Nitro-N'-(1-(2-phenylhydrazono)-1-(phenylsulfonyl)propan-2-ylidene)benzohydrazide (11b)

Yield 63%; m.p. 257-259°C; IR (KBr, cm^{-1}): 3339 (2NH), 1684 (C=O), 1596 (2C=N); ^{1}H NMR (DMSO-d_6, δ, ppm): 2.49 (s, 3H, CH_3), 6.93-8.40 (m, 14H, ArH), 11.94 (s, 1H, D_2O exchangeable, NH), 14.43 (s, 1H, D_2O exchangeable, NH); MS m/z (%): 465 (M^+, 27), 324 (36), 92 (100), 77 (58), 65 (36). Analysis: calcd. for $C_{22}H_{19}N_5O_5S$ (465.48): C, 56.77; H, 4.11; N, 15.05; S, 6.89%; found: C, 56.59; H, 3.98; N, 15.22; S, 7.03%.

N'-(1-(Phenylsulfonyl)-1-(2-p-tolylhydrazono)-propan-2-ylidene)benzo[d]thiazole-2-carbohydrazide (11c)

Yield 63%; m.p. 257-259°C; IR (KBr, cm^{-1}): 3434, 3352 (2NH), 1687 (C=O), 1587 (2C=N); ^{1}H NMR (DMSO-d_6, δ, ppm): 2.51 (s, 3H, CH_3), 5.97-7.45 (m, 14H, ArH), 11.37 (s, 1H, D_2O exchangeable, NH), 13.22 (s, 1H, D_2O exchangeable, NH); MS m/z (%): 491 (M^+, 42), 350 (33), 106 (100), 77 (48). Analysis: calcd. for $C_{24}H_{21}N_5O_3S_2$ (491.59): C, 58.64; H, 4.31; N, 14.25; S, 13.05%; found: C, 58.76; H, 4.20; N, 14.36; S, 12.93%.

Synthesis of hydrazones 13a, b

A mixture of 2-benzothiazolecarboxylic acid hydrazide (**1c**) (0.19 g, 1 mmol) and the appropriate ketone (1 mmol) in absolute ethanol (30 mL) was refluxed for 8 h then left to cool. The solid product was collected by filtration, washed with ethanol and dried. Recrystallization from the EtOH/DMF afforded the corresponding hydrazones **13a, b**.

N'-(1-Phenylethylidene)benzo[d]thiazole-2-carbohydrazide (13a)

Yield 78%; m.p. 275-277°C; IR (KBr, cm^{-1}): 3320 (NH), 1681 (C=O), 1517 (2C=N); ^{1}H NMR (DMSO-d_6, δ, ppm): 1.77 (s, 1H, CH_3), 6.53-7.48 (m, 9H, ArH), 10.87 (s, 1H, D_2O exchangeable, NH); MS m/z (%): 295 (M^+, 13), 294 (33), 135 (100), 77 (23). Analysis: calcd. for $C_{16}H_{13}N_3OS$ (295.36): C, 65.06; H, 4.44; N, 14.23; S, 10.86%; found: C, 64.92; H, 4.31; N, 14.25; S, 10.98%.

N'-(1-(4-Tolyl)ethylidene)benzo[d]thiazole-2-carbohydrazide (13b)

Yield 76%; m.p. 213-215°C; IR (KBr, cm^{-1}): 3345 (NH), 1689 (C=O), 1502 (2C=N); ^{1}H NMR (DMSO-d_6, δ, ppm): 1.75 (s, 1H, CH_3), 1.86 (s, 1H, CH_3), 6.45-7.40 (m, 8H, ArH), 11.12 (s, 1H, D_2O exchangeable, NH); ^{13}C NMR (DMSO-d_6, δ, ppm): 14.45 (-CH_3), 20.83 (-CH_3), 120.71, 122.98, 124.1, 125.84, 126.54, 127.01, 127.21, 128.76, 128.98, 134.63, 135.95, 139.72, 152.56, 155.89, 157.77; MS m/z (%): 309 (M^+, 20), 308 (47), 147 (59), 135 (100), 117 (44), 91 (36). Analysis: calcd. for $C_{17}H_{15}N_3OS$ (309.39): C, 66.00; H, 4.89; N, 13.58; S, 10.36%; found: C, 66.16; H, 4.77; N, 13.65; S, 10.49%.

General procedure for synthesis of pyrazoles 15 and 17

To a solution of hydrazide (**1c**) (0.19 g, 1 mmol) in ethanol (20 mL), 2-(ethoxymethylene)malononitrile (**14**) or 2-(bis(methylthio)methylene)malononitrile (**16**) (1 mmol) was added. The

mixture was refluxed for 10 h and then allowed to cool. The formed solid was filtered off, washed with ethanol and recrystallized from EtOH/DMF to afford the corresponding pyrazole derivatives **15** and **17**, respectively.

5-Amino-1-(benzo[d]thiazole-2-carbonyl)-1H-pyrazole-4-carbonitrile (15)

Yield 71%; m.p. 208-210°C; IR (KBr, cm^{-1}): 3391, 3291 (NH_2), 2205 (C=N), 1644 (C=O), 1507 (C=N); ^1H NMR (DMSO-d_6, δ, ppm): 5.71 (s, 2H, D_2O exchangeable, NH_2), 7.52-8.29 (m, 5H, ArH); MS m/z (%): 269 (M^+, 29), 268 (20), 220 (11), 193 (63), 135 (100), 108 (25). Analysis: calcd. for $C_{12}H_7N_5OS$ (269.28): C, 53.52; H, 2.62; N, 26.01; S, 11.91%; found: C, 53.58; H, 2.74; N, 25.88; S, 11.84%.

5-Amino-1-(benzo[d]thiazole-2-carbonyl)-3-(methylthio)-1H-pyrazole-4-carbonitrile (17)

Yield 78%; m.p. 256-258°C; IR (KBr, cm^{-1}): 3379, 3199 (NH_2), 2215 (C=N), 1706 (C=O), 1664 (C=N); ^1H NMR (DMSO-d_6, δ, ppm): 4.49 (S, 3H, CH_3), 5.80 (s, 2H, D_2O exchangeable, NH_2), 7.52-8.29 (m, 4H, ArH),; MS m/z (%): 315 (M^+, 22), 298 (6), 269 (13), 162 (92), 135 (100), 108 (34). Analysis: calcd. for $C_{13}H_9N_5OS_2$ (315.37): C, 49.51; H, 2.88; N, 22.21; S, 20.33%; found: C, 49.51; H, 2.88; N, 22.21; S, 20.33%.

PHARMACOLOGY

Animals

White adult mice Mus musculus (weighing 25–30g) and adult Wistar rats (weighing 100–150g), of either sex were used in the present study. The animals were kept under natural conditions (temperature: 21 ± 2°C; 12h/12h light-dark cycle) and were allowed free access to water and food. Experiments were carried out on groups of 5 animals each. All experimental procedures used in the present study followed the Institutional Animal Ethics Committee regulations. All experiments were performed in the morning, according to the guidelines for the care of laboratory animals (41).

Chemicals

Formaldehyde, DMSO (dimethyl sulfoxide) and acetic acid were obtained from Sigma Chemical Co. (St. Louis, MO, USA), while ketoprofen oral suspension (5 mg/mL) was obtained from a local pharmacy.

Acute toxicity and lethality test

The acute toxicity and lethality (LD_{50}) of the tested compounds was estimated in mice using the method described by Lorke (42). In the first stage of the test, three groups (n = 3) were used for each tested compound, each one of these compounds was administered orally in the form of DMSO/water suspension (1% w/v) at a dose of 10, 100, or 1000 mg/kg (n = 3) . Animals were observed continuously for the first three hours for any toxic symptoms after administrations and number of deaths within 24 h. Since no deaths occurred in any of these groups for each compound, a second stage of the test was conducted in which 1500, 2000 and 3000 mg/kg doses of each compound were administered to a fresh groups of animals (n = 1) and no death was recorded within 24 h. Thus, the oral LD_{50} in mice were found to be greater than 3000 mg/kg for each tested compound.

Anti-inflammatory activity

Anti-inflammatory activity were assessed using formalin induced rat paw edema method according to Dharmasiri (43).Wistar albino rats of either sex weighing 100-120 g were divided into 13 groups of 5 animals each. They were treated *via* oral route as follows: the 1st group was given DMSO (1%, w/v) aqueous suspension and kept as control. The 2nd group received ketoprofen (10 mg/kg body weight) as standard drug; the tested compounds, in the form of DMSO aqueous suspensions, were given at a dose of 100 mg/kg body weight to the rest of these groups. After one hour, 0.1 mL of 2% formaldehyde was injected into the footpad of the left hind paw of each rat for induction of paw edema (43). The initial paw thickness was measured for each animal using Vernier caliper before induction of edema. The increase in this thickness was determined after 30 min, 1, 2 and 3 h after formaldehyde injection. The anti-inflammatory activity was expressed as inhibition percent in paw thickness in the treated groups comparing with the control one using the formula by Adedapo (44).

Edema inhibition percent = $[(T_c - T_t) / T_c] \times 100$

where, T_c and T_t represent the average paw thickness in the control and treated groups, respectively.

Analgesic activity

Hot plate test

Analgesic activity was determined in mice by hot plate method described by Vogel and Vogel (45). The animals were divided into control, standard and tested groups of 5 mice each. DMSO aqueous solution (2%, v/v) (5 mL/kg) were orally administered to animals in the first group and served as control, ketoprofen (10 mg/kg) as standard to the

Table 1. Anti-inflammatory activity of the tested compounds (100 mg/kg *p.o.*) against formalin induced paw edema.

Treatment	Increase in paw thickness (mm)				Inhibition %			
	30 min	1 h	2 h	3 h	30 min	1 h	2 h	3 h
Control	0.52 ± 0.018	0.55 ± 0.02	0.57 ± 0.022	0.57 ± 0.022	0	0	0	0
Ketoprofen	0.12 ± 0.006 ^{c}	0.09 ± 0.005 ^{c}	0.07 ± 0.003 ^{c}	0.05 ± 0.002 ^{c}	76.92	83.64	87.72	91.23
6b	0.20 ± 0.013 ^{c***}	0.20 ± 0.013 ^{c***}	0.18 ± 0.01 ^{c***}	0.18 ± 0.01 ^{c***}	61.54	63.64	68.42	68.42
6c	0.25 ± 0.013 ^{c***}	0.23 ± 0.012 ^{c***}	0.22 ± 0.013 ^{c***}	0.22 ± 0.012 ^{c***}	51.92	58.18	61.4	61.4
8a	0.08 ± 0.005 ^{c***}	0.05 ± 0.002 ^{c***}	0.05 ± 0.002 $^{c\ ***}$	0.05 ± 0.002 ^{c}	84.62	90.91	91.23	91.23
8b	0.15 ± 0.007 ^{c**}	0.15 ± 0.006 ^{c***}	0.13 ± 0.008 ^{c***}	0.12 ± 0.007 ^{c***}	71.15	72.73	77.2	78.95
8c	0.25 ± 0.012 ^{c***}	0.25 ± 0.013 ^{c***}	0.22 ± 0.014 ^{c***}	0.22 ± 0.014 ^{c***}	51.92	54.55	61.4	61.4
11a	0.30 ± 0.017 ^{c***}	0.28 ± 0.014 ^{c***}	0.28 ± 0.014 ^{c***}	0.28 ± 0.013 ^{c***}	42.31	49.09	50.88	50.88
11c	0.15 ± 0.007 ^{c**}	0.13 ± 0.009 ^{c***}	0.12 ± 0.006 ^{c***}	0.12 ± 0.008 ^{c***}	71.15	76.36	78.95	78.95
13a	0.35 ± 0.018 ^{c***}	0.32 ± 0.016 ^{c***}	0.30 ± 0.013 ^{c***}	0.33 ± 0.014 ^{c***}	32.69	41.82	47.37	42.11
13b	0.35 ± 0.018 ^{c***}	0.35 ± 0.018 ^{c***}	0.31 ± 0.017 ^{c***}	0.31 ± 0.016 ^{c***}	32.69	36.36	45.61	45.61
15	0.30 ± 0.016 ^{c***}	0.28 ± 0.013 ^{c***}	0.26 ± 0.013 ^{c***}	0.26 ± 0.013 ^{c***}	42.31	49.09	54.39	54.39
17	0.28 ± 0.013 ^{c***}	0.22 ± 0.012 ^{c***}	0.25 ± 0.012 ^{c***}	0.25 ± 0.014 ^{c***}	46.15	60	56.14	56.14

Values with superscript letter c in the same column are significantly different from control group at $p < 0.001$, values with superscript stars are significantly different from ketoprofen * at $p < 0.05$, ** at $p < 0.01$ and *** at $p < 0.001$. Values are represented as the mean ± SE. n = 5.

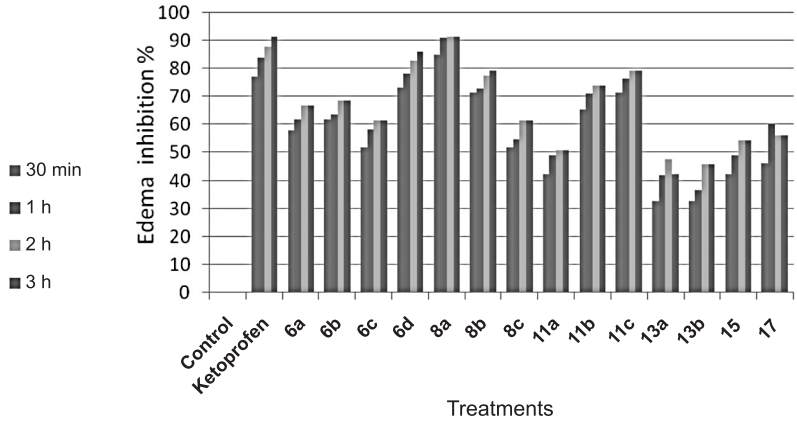


Figure 1. Anti-inflammatory activity of the tested compounds (100 mg/kg *p.o.*) against formalin induced paw edema

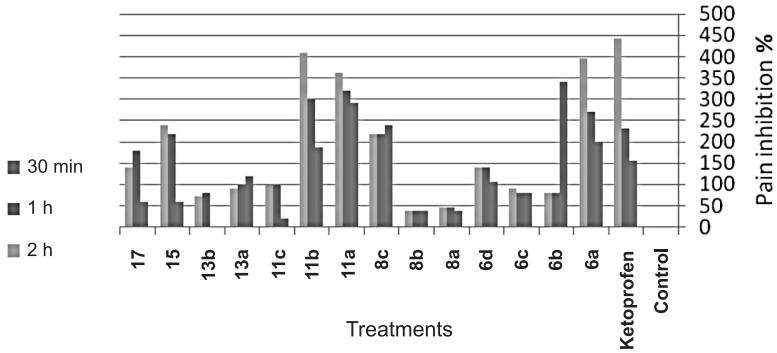


Figure 2. Analgesic activity of the tested compounds (100 mg/kg *p.o.*) against thermal stimulation using hot plate method

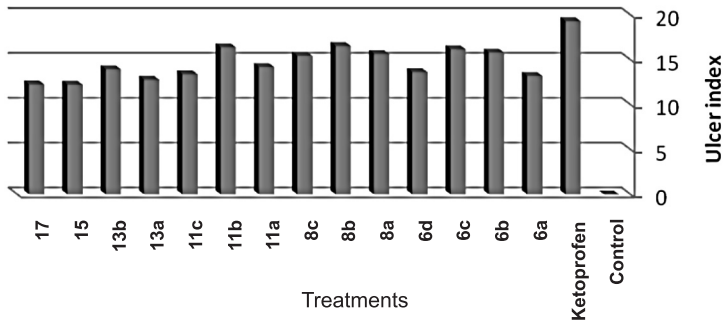
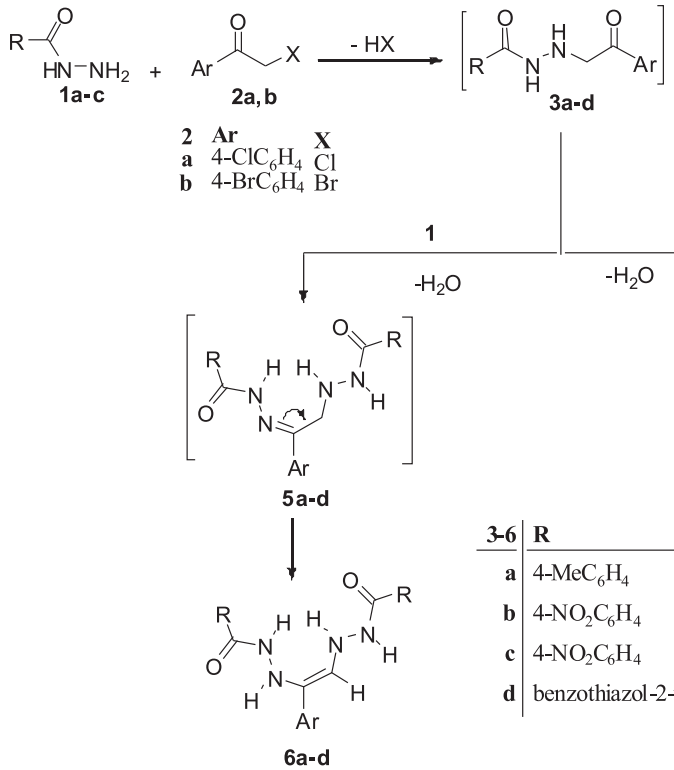


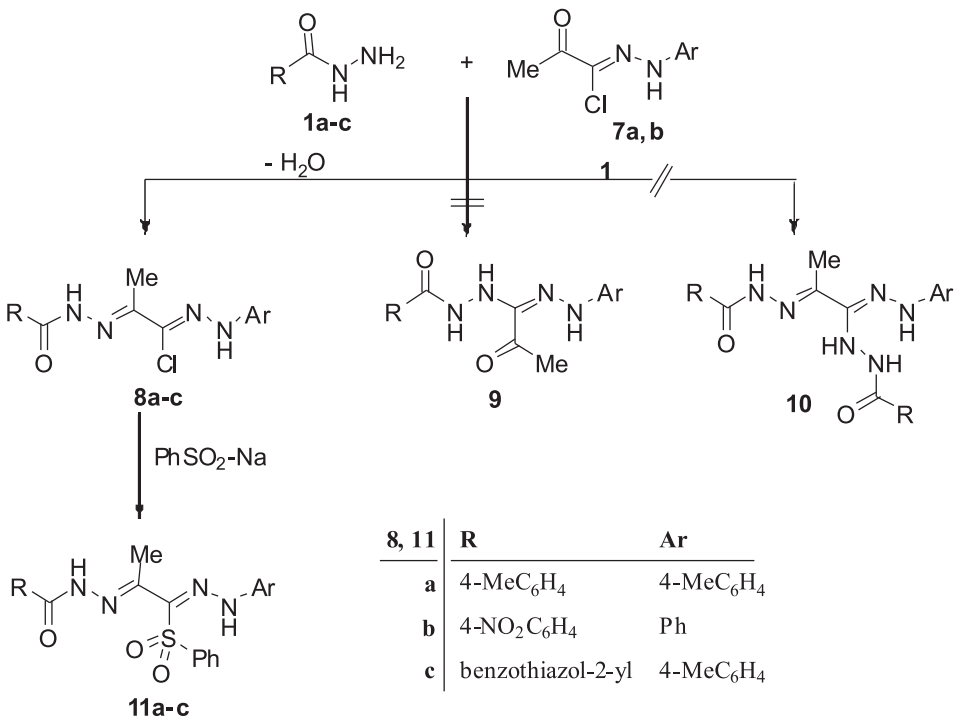
Figure 3. Ulcerogenic liabilities of the tested compounds (100 mg/kg)

second group. The tested compounds in the form of DMSO aqueous suspensions (100 mg/kg) were given to animals of the rest groups. The hot-plate (Model 7280, Ugo Basile, Italy) was maintained at 55.0 ± 0.2°C and each animal was placed separately into a glass beaker on the heated surface and the



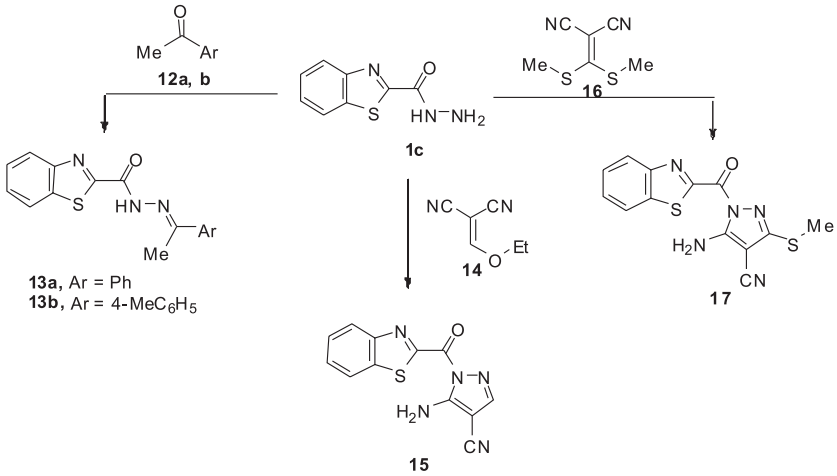
3-6	R	Ar	X
a	4-MeC_6H_4	4-ClC_6H_4	Cl
b	4-$NO_2C_6H_4$	4-ClC_6H_4	Cl
c	4-$NO_2C_6H_4$	4-BrC_6H_4	Br
d	benzothiazol-2-yl	4-ClC_6H_4	Cl

Scheme 1. Synthetic route to compounds **5a-d** and **6a-d**



8, 11	R	Ar
a	4-MeC_6H_4	4-MeC_6H_4
b	4-$NO_2C_6H_4$	Ph
c	benzothiazol-2-yl	4-MeC_6H_4

Scheme 2. Synthetic route to compounds **8a-c** and **11a-c**



Scheme 3. Synthesis of compounds **13a,b, 15** and **17**

Table 2. Analgesic activity of the tested compounds (100 mg/kg *p.o.*) against thermal stimulation using hot plate method.

Compound	Latency time (s)			Pain inhibition % (PIP)		
	30 min	1 h	2 h	30 min	1 h	2 h
Control	2.5 ± 0.08	2.5 ± 0.07	2.5 ± 0.09	0	0	0
Ketoprofen	6.4 ± 0.22	8.3 ± 0.31	13.6 ± 0.42	156	232	444
6b	11 ± 0.58 ^{c***}	4.5 ± 0.37 ^{c***}	4.5 ± 0.36 ^{c***}	340	80	80
6c	4.5 ± 0.19 ^{c***}	4.5 ± 0.19 ^{c***}	4.8 ± 0.22 ^{c***}	80	80	92
8a	3.5 ± 0.12 ^{c***}	3.7 ± 0.12 ^{c***}	3.7 ± 0.14 ^{c***}	40	48	48
8b	3.5 ± 0.11 ^{c***}	3.5 ± 0.12 ^{c***}	3.5 ± 0.11 ^{c***}	40	40	40
8c	8.5 ± 0.39 ^{c**}	8.0 ± 0.37 ^{c}	8.0 ± 0.37 ^{c***}	240	220	220
11a	11.0 ± 0.52 ^{c***}	11.37 ± 0.47 ^{c***}	12.5 ± 0.51 ^{c***}	292.86	321.11	362.96
11c	3.0 ± 0.12 ^{b***}	5.0 ± 0.23 ^{c***}	5.0 ± 0.24 ^{c***}	20	100	100
13a	5.5 ± 0.23 ^{c*}	5.0 ± 0.22 ^{c***}	4.8± 0.19 ^{c***}	120	100	92
13b	2.5 ± 0.18***	4.5 ± 0.21 ^{c***}	4.3 ± 0.22 ^{c***}	0	80	72
15	4.0 ± 0.17 ^{c***}	8.0 ± 0.35 ^{c}	8.5 ± 0.36 ^{c***}	60	220	240
17	4.0 ± 0.07 ^{c***}	7.0 ± 0.28 ^{c*}	6.0± 0.29 ^{c***}	60	180	140

Values with superscript letter b and c in the same column are significantly different from the control group at $p < 0.01$ and 0.001, respectively. Values with superscript stars are significantly different from ketoprofen * at $p < 0.05$, ** at $p < 0.01$ and *** at $p < 0.001$. Values are represented as the mean ± SE; n = 5.

time (in s) to discomfort reaction (licking paws or jumping) was recorded as a response latency time, prior to and 30, 60 and 120 min after administration in each group. A cut-off period of 15 s was considered as maximal latency to avoid injury to the paws (46). The pain inhibition percentage (PIP) (47) was calculated according to the following formula:

$$\text{Pain inhibition percentage (PIP)} = [(T_t - T_c) / T_c] \times 100$$

where T_t is drug latency time and T_c is control latency time.

Ulcerogenic liability

The ulcerogenic liability was determined in albino rats according to the standard method of Barsoum (48) and Hamza (49). Rats of either sex weighing 100-120 g were divided into thirty groups of five animals each. The animals were fasted 18 h

Table 3. Ulcerogenic liability of the synthesized compounds.

Ulcer index	Average severity	Average number of ulcers	% Incidence divided by 10	No. of animals with ulcers	Treatment
Control	0/5	0	0	0	0
Ketoprofen	5/5	10	7.24	2.06	19.30 ± 0.94
6b	5/5	10	4.28	1.53	15.81 ± 0.64*
6c	5/5	10	5.13	1.04	16.17 ± 0.71*
8a	5/5	10	4.16	1.5	15.66 ± 0.73*
8b	5/5	10	4.56	1.18	16.74 ± 0.65
8c	5/5	10	3.73	1.7	15.43 ± 0.63**
11a	5/5	10	3.4	0.8	14.2 ± 0.46**
11c	5/5	10	2.8	0.6	13.4 ± 0.53***
13a	4/5	8	3.54	1.24	12.78 ± 0.47***
13b	5/5	10	3.08	0.86	13.94 ± 0.53***
15	4/5	8	3.29	0.94	12.23 ± 0.44***
17	4/5	8	3.13	1.13	12.26 ± 0.45***

Values with superscript stars *, **, *** are significantly different from the ketoprofen value at $p < 0.05$; 0.01 and 0.001; results are represented as the mean ± SE. n = 5.

before drug administration. The animals were treated *via* oral route either with 1 mL of DMSO aqueous solution (2%, v/v) as control group, ketoprofen (10 mg/kg body weight) as standard one. The tested compounds **1**-**13** in the form of DMSO aqueous suspensions (100 mg/kg body weight) were administered each in one of the other groups. Treatment was continued once daily for 3 successive days in all groups. One hour after the last dose, the animals were sacrificed and the stomach was removed, opened along the greater curvature and rinsed with saline. The gastric mucosa were examined with a magnifying lens (10×) for the presence of lesions in the form of hemorrhages or linear breaks and erosions. The ulcer index was calculated (Table 3) and the degree of ulcerogenic effect was expressed in terms of:

1. Percentage incidence of ulcer divided by 10.
2. Average number of ulcers per stomach.
3. Average severity of ulcers.

The ulcer index is the sum of the above three values.

Statistical analysis

Results of anti-inflammatory and analgesic activity are presented as the mean ± SE (standard error). The significant difference between groups was tested using one way ANOVA followed by Dunnett's test at p = 0.05, 0.01, 0.001.

RESULTS AND DISCUSSION

Chemistry

The reaction of carbohydrazides (**1a**-**c**) with l-aryl-2-bromoethanone (**2a,b**) in refluxing ethanol afforded a single product (based on TLC). The elemental analysis and mass spectrum of the reaction product proved that the reaction proceeded in 2 : 1 molar ratio (**1a**-**c** : **2a,b**). The ^{1}H NMR spectra were characterized by the presence of four signals in the regions δ 9.62-14.05 ppm assigned to the 4 NH groups. The IR spectra showed two absorption bands in the region 1652-1735 cm^{-1} due to the two carbonyl groups in addition to the bands of 4 NH functions in the region 3042-3430 cm^{-1}. The mechanistic pathway of latter reaction is assumed to proceed *via* a preliminary formation of the non-isolable intermediates **3a**-**d** followed by its reaction with another molecule of hydrazide (**1a**-**c**) with elimination of water molecule to form the intermediate (**5a**-**d**) (Scheme 1) which was consequently tautomerized into compounds **6a**-**d** as final products. These results were in agreement with those recently reported (50).

Next, the reaction of 2-oxo-*N*-arylpropanehydrazonoyl chlorides (**3a,b**) with acid hydrazides (**1a**-**c**) in refluxing ethanol afforded, in each case, a single yellow product. IR spectra of the latter products revealed a carbonyl absorption band in the region 1634-1692 cm^{-1} in addition to the absorption bands

of 2 NH functions in the region 3268-3256 cm^{-1}. Their ^{1}H NMR spectra exhibited two D_2O exchangeable signals of 2 NH groups in the regions δ 9.92-11.46. The ^{13}C NMR spectrum of **8a** showed three signals of 3 methyl groups at δ 13.45, 20.05 and 20.79 ppm, respectively. The latter spectroscopic data of the reaction products and their satisfactory elemental analyses supported the structure *N*-arylpropanehydrazonoyl chlorides (**8a-c**) as postulated in Scheme 2. This observation is also in accordance with the results in the literature (51).

Furthermore, **8a-c** reacted with sodium benzenesulfinate in refluxing ethanol, yielding sulfones **11a-c**, respectively as shown in Scheme 2. The latter reaction was found to give a single product as evidenced by TLC analysis and its structure was established on the basis of elemental analysis and spectral data.

In addition, the reaction of benzothiazole-2-carbohydrazide **1c** with ketones **12a,b** afforded hydrazones **13a,b**. Their ^{1}H-NMR spectra showed a singlet proton at δ 1.75-1.77 ppm attributed to methyl group. On the other hand, reaction of hydrazide **1c** with 2-(methoxymethylene)malononitrile **14** or bis(methylthio) derivative **16** afforded the pyrazole derivatives **15** and **17,** respectively (Scheme 3). The IR spectra of compounds **15** and **17** showed characteristic absorption bands in the range 2215-2205 cm^{-1} due to carbonitrile group, also their IR spectra showed absorption bands in the range 3391-3199 cm^{-1} indicating the presence of amino groups.

PHARMACOLOGY

Acute toxicity

Oral administration of the tested compounds in mice in doses ranging from 1000 to 3000 mg/kg body weight caused no acute toxic symptoms and no mortalities. Thus, the oral LD_{50} in mice were found to be greater than 3000 mg/kg for each tested compound, what implies a remote risk of acute intoxication and indicates a high degree of relative safety for oral administration of these compounds.

Anti-inflammatory activity

Injection of formalin (0.1 mL 2%) into the footpad provoked marked, time-related, progressive increases in the hind paw diameters of the control, untreated rats. Although pedal inflammation (edema) was always evident within 5–10 min following formalin injection, maximal swelling and/or edema occurred approximately 90 min following administration. Acute inflammation induced by formaldehyde results from cell damage, which provokes the production of endogenous mediators, such as, histamine, serotonin, prostaglandins, and bradykinin (52). It is well known that inhibition of edema induced by formalin in rats is one of the most suitable test procedures to screen antiarthritic and anti-inflammatory agents as it closely resembles human arthritis. Arthritis induced by formalin is a model used for the evaluation of an agent with probable anti-proliferative activity (53).

All the tested synthesized compounds in oral doses of 100 mg/kg each showed significant ($p < 0.05–0.001$) anti-inflammatory activity against formalin induced paw edema (Table 1). The obtained edema inhibition percent are shown in Figure 1. Among the tested compounds **8a** showed the highest anti-inflammatory activity with edema inhibition of 91.23%, 3 h after formalin injection. High activity was exhibited by compound **8b** and **11c** with inhibition % of 71.15-78.95, 30 min to 3 h after edema induction, respectively, for both compounds. Previous studies revealed that the substitution of electron withdrawing groups in 2-aminobenzothiazole increased anti-inflammatory activity (54). Also different studies showed that many benzothiazole derivatives display anti-inflammatory activity (54-57). Some of these were investigated for their ability to inhibit human cyclooygenase enzyme-2 (58). Moderate activity was obtained by tested compound **6b** followed by lower activity for **8c** and **6c**. Compounds **11a**, **13a** and **13b** demonstrate the lowest anti-inflammatory activity between the tested compounds.

Analgesic activity

Hot-plate test: Analgesic effect was assessed in a thermal model using hot-plate test; this method was selected to evaluate centrally mediated effects of the tested compounds (59). In hot plate test, ketoprofen (10 mg/kg, *p.o.*) significantly ($p < 0.001$) increased the latency time to thermal stimulation (Table 2). The tested benzothiazole derivative **11a** was found to be the most potent in increasing the latency to thermal stimulation with increased pain inhibition percent from 292.86 to 362.96% at 30 min to 2 h after administration, respectively. This followed by the derivative **8c** with 240-220 PIP, 30 min to 2 h post administration, respectively. Compound **6b** demonstrated very high analgesic activity only after 30 min with pain inhibition 340% that was more potent than the reference drug, this activity dramatically declined after that to be 80% at 1 h and 2 h indicating bad pharmacokinetic profile for this compound. Compound **15** exhibited gradually increased analgesic activities with pain inhibition of

240%, 2h post administration. Compound **17** demonstrates moderate analgesic activity with peak PIP% of 180, 1 h post administration (Fig 2).

Ulcerogenic effect

The ulcerogenic liability for the tested compounds was determined in albino rats and the obtained data are recorded in Table 3. It has been observed that all the tested compounds possess less ulcerogenic potentialities (ulcer index of 12.23-16.74) compared to that of the standard drug ketoprofen (ulcer index of 19.30) as shown in Figure 3. Compounds **13a**, **13b**, **15** and **17** showed the least ulcerogenic liabilities compared to the other compounds.

CONCLUSION

We reported here the convenient synthesis of some bis-hydrazones. All of the tested compounds exhibited promising anti-inflammatory activity compared to ketoprofen, with marked decrease in the ulcerogenic side effects. Additionally, some of synthesized compounds, **6a**, **11a**, **11b** demonstrate analgesic activity.

Acknowledgments

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