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Novel thiazolinyl, thiazolidinonyl, thiadiazolyl and oxadiazolyl-benzotriazole derivatives with potential antiinflammatory activity and minimum ulcerogenic effect

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Four novel series of 1*H*-benzotriazole derivatives; containing thiazolin, thiazolidin, thiadiazole and oxadiazole moieties; namely 1-[(3,4-disubstituted thiazolin-2-ylidene)hydrazinocarbonyl]methyl-1*H*-benzotriazoles **3a**–**l**; 1-[3-substituted 5-ethoxycarbonyl-4-methyl thiazolin-2-ylidene)hydrazinocarbonyl]methyl-1*H*-benzotriazoles **4a**–**c**; 1-[(3-substituted-4-oxothiazolidin-2-ylidene)hydrazinocarbonyl]methyl-1*H*-benzotriazoles **5a**–**d**; 1-[(5-substituted aminothiadiazol-2-yl)methyl]-1*H*-benzotriazoles **6a**–**c** have been synthesized by cyclization of the key intermediates 1-[(substituted thiocarbamoylhydrazinocarbonyl)methyl]-1*H*-benzotriazoles **2a**–**d**. Furthermore 1-[(5-substituted aminooxadiazol-2-yl)methyl]-1*H*-benzotriazoles **7a**, **b** were obtained by one-pot synthesis starting from 1*H*-benzotriazol-1-acetic acid hydrazide. The antiinflammatory activity of representative compounds was evaluated *in vivo* against indomethacin as a standard using the sponge implantation model of inflammation in rats. Both non-immunological parameters such as exudate volume, total leucocyte count (TLC), and differential leucocyte count (DLC), and immunological parameters, for example neutrophil phagocytic function by reduced cytochrome C levels, and the assay of interleukin-1β (IL-1β) levels in drug-pre-treated rats, were determined. The ulcerogenic activity of compounds showing marked antiinflammatory activity was also studied. Compounds **3e**, **5b** and **5c** showed antiinflammatory activity comparable to indomethacin, and they also demonstrated minimum ulcerogenic activity.

1. Introduction

Antiinflammatory agents may act by interfering with any one of several mechanisms including both non-immunological and immunological mechanisms [1]. The literature is rich in continuing research to find new non-steroidal antiinflammatory compounds [2–6]. Furthermore, several drugs like aspirin, mefenamic acid, indomethacin, tolmetin, ibuprofen, feldene and phenylbutazone are widely employed in the symptomatic treatment of rheumatic fever, rheumatoid arthritis, oesteoarthritis, spondylitis and, to a lesser extent, gout. Most of these drugs strongly inhibit the synthesis of prostaglandin by blocking prostaglandin synthetase [7]. However, most of these drugs are not devoid of ulcerogenic effects.

In addition, various forms of pharmacological activity have been described for substituted benzotriazoles, for instance *N*-[2-(alkylamino)ethyl] benzotriazol-x-yl-acetamides or isobutyramides [8, 9] exhibited local anaesthetic activity, and their QSAR have also been studied [9]. In addition, benzotriazoles have been reported to exhibit 5-HT_{1A} receptor antagonism [10–12]. 1- and 2-[2-(4-Substituted-1-piperazinyl)ethyl or propoxy]benzotriazoles have been found to exhibit antiserotonergic, antiadrenergic and antihistaminic effects [13]. 2-[4-(Dialkylaminoalkoxy)phenyl]benzotriazoles and their N-oxides have been reported to be leukotriene-D_4 antagonists [14], and they also inhibited platelet aggregation and exerted an anti-hypercholesterolemic effect [14]. Benzotriazoles have also been reported to exert antipsychotic [15], antifungal [16], and antineoplastic [17] activities.

In a previous publication we studied the chronotropic and inotropic activity of some benzotriazole derivatives [18], while the antiinflammatory activity of benzotriazoles has also received our attention [19–21].

Motivated by the above, and in the hope of finding new leads in the field of NSAIDS that may have greater activity and lower ulcerogenic effect, and persuing our researches on benzotriazoles [18, 22, 23] we report on the synthesis, and antiinflammatory and antiulcerogenic activity of some novel thiazolinyl, thiazolidinonyl, thiadiazolyl and oxadiazolyl benzotriazoles.

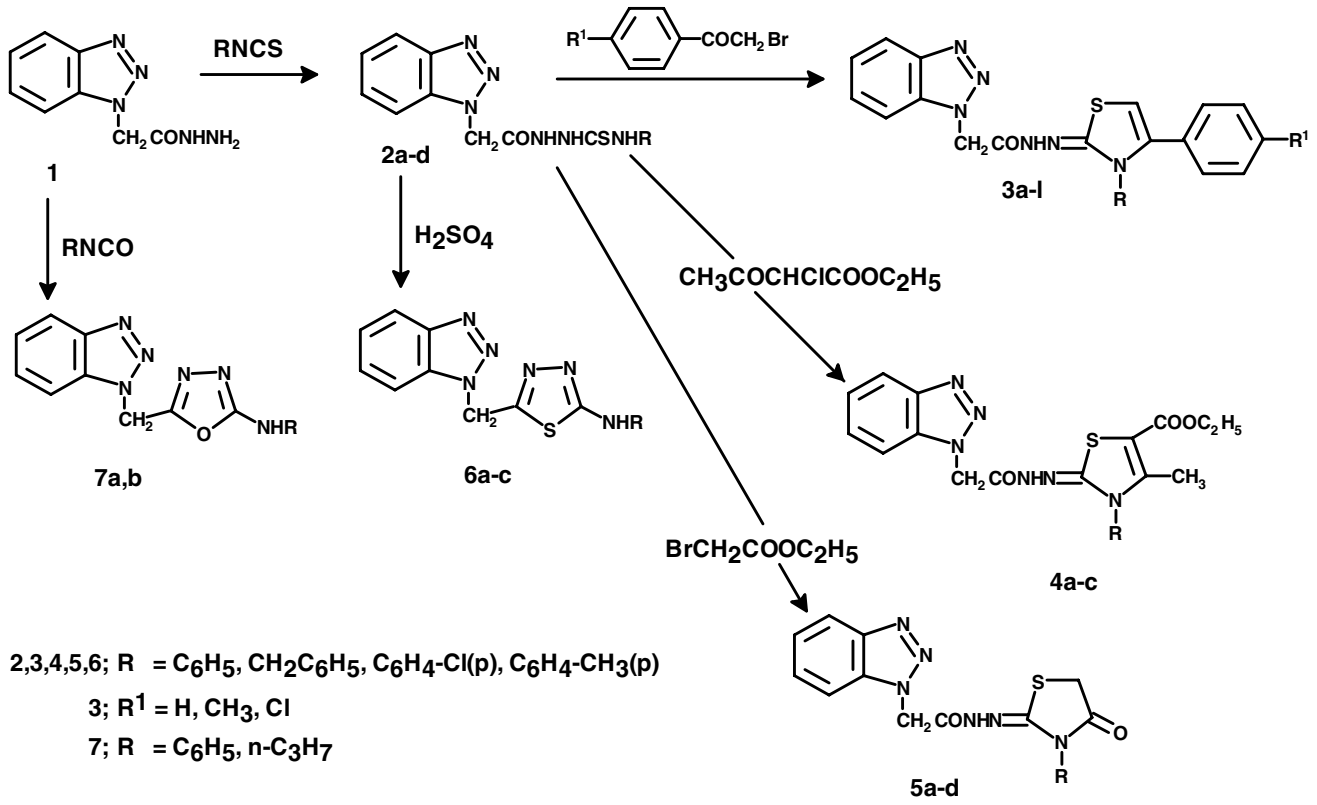
2. Investigations, results and discussion

2.1. Synthesis and characterization

For the synthesis of the target compounds the reaction sequences outlined in Scheme 1 are followed. Thus 1*H*-benzotriazole-1-acetic acid hydrazide (**1**) was reacted with substituted isothiocyanates to produce the key intermediates 1-[(substituted thiocarbamoylhydrazinocarbonyl) methyl-1*H*-benzotriazoles **2a**–**d** [22]. These were cyclized with substituted phenacyl bromides or ethyl α-chloroacetoacetate to produce the thiazolines **3a**–**l** and **4a**–**c** respectively. Cyclization of **2a**–**d** with ethyl bromoacetate produced 1-[(3-substituted-4-oxothiazolidin-2-ylidene) hydrazinocarbonyl]methyl-1*H*-benzotriazoles **5a**–**d**. On the other hand, reaction of **2a**–**c** with conc. H_2SO_4 gave 1-[(5-substituted aminothiadiazol-2-yl)methyl-1*H*-benzotriazoles **6a**–**c**. In addition, 1-[(5-substituted aminooxadiazol-2-yl)methyl-1*H*-benzotriazoles **7a**–**b** were obtained by one-pot synthesis starting from 1*H*-benzotriazol-1-acetic acid hydrazide and substituted isocyanates. The compounds prepared were characterized by IR, ^{1}H NMR and microanalyses.

The IR spectra of the thiazolines **3a**–**l** showed bands characteristic for NH at 3416–3441 cm^{-1}; C=O at 1684–1679 cm^{-1}, and C–S–C at 1268–1231 and 1049–1044 cm^{-1}. The ^{1}H NMR of **3b** showed three singlets at 2.07, 4.88 and 6.06 ppm due to NH, CH_2CO and thiazolin C_5–H respectively; while, the ^{1}H NMR of **3e** was characterized by three singlets at 1.6, 5.2 and 5.8 ppm due to NH, CH_2–CO and thiazolin-C_5–H respectively, besides the signals characteristic of other substituents at their expected chemical shifts. The IR of **4a**–**c** showed bands

Scheme



due to NH, C=O amide and C=O ester. The ^{1}H NMR of **4b** and **c** were characterized by the presence of the triplet and quartet characterizing the ester moiety at 1.2–1.23 and 4.18–4.2 ppm respectively, they also showed a singlet at 2.3–2.43 ppm due to the methyl group, besides the signals characteristic of CH_2CO and other substituents at their expected chemical shifts. The thiazolidinones **5a–d** were characterized by IR which showed two carbonyl absorption bands at 1679–1674 and 1664–1663 cm^{-1}, besides the bands due to NH and C–S–C. The ^{1}H NMR of **5c** and **5d** were characterized by two singlets due to CH_2CO and thiazoline-C_5–H_2 at 4.01–5.9 and 6.03–6.2 ppm respectively. The thiadiazoles **6a–c** were characterized by IR which showed NH at 3202–3200, 3138–3125 cm^{-1} and C–S–C of the thiadiazole ring at 1248–1243, 1073–1063 cm^1, they also showed δ NH and C=N. The ^{1}H NMR of **6c** was characterized by three singlets at 2.22, 6.33 and 10.27 ppm due to CH_3 (of p-tolyl), CH_2 and NH respectively, this besides the signals characteristic of the benzotriazole moiety at their expected chemical shifts. The oxadiazoles **7a**, **b** showed in their IR spectra, NH at 3437–3436 and 3268–3201 cm^{-1} and C–O–C of the oxadiazole ring at 1243–1228 and 1073–1064 cm^{-1}, besides the bands characteristic of δ NH and C=N. The ^{1}H NMR of **7a** was characterized by two singlets at 2.07 and 3.31 ppm due to NH and CH_2 respectively, besides the signals characteristic of the Ar–H at their expected chemical shifts.

2.2. Discussion of the pharmacological results

2.2.1. Antiinflammatory activity

The antiinflammatory activity of representative newly synthesized compounds **3e**, **3g**, **3l**, **4a**, **4b**, **5b**, **5c** and **6b** was evaluated *in vivo* using the sponge implantation model of inflammation in rats with indomethacin as a standard. Polyester sponge implantation, the animal model chosen in this study, has the merit of triggering a non-immune acute type of inflammatory response. This model was used here to assess the possibility of some of the synthesized compounds altering the course of inflammation and they have been claimed to potentially modulate inflammation. This was assessed by determining their effects on the inflammatory exudate parameters: exudate volume, TLC, DLC, reduced cytochrome C and interleukin-1 beta (IL-1β) levels. Indomethacin was used as a standard antiinflammatory drug (Tables 1 and 2).

Table 1: The effect of inflammation induced by sponge implantation, in drug pre-treated rats (mean ± S.E., n = 10) on non-immunological parameters

	Exudate volume $\bar{X}$ (± S.E.)	TLC $\bar{X}$ (± S.E.)	DLC (neutrophil %) $\bar{X}$ (± S.E.)
Inflammatory control group	0.348 (±0.013)	264.50 (±14.12)	81.30 (±2.18)
Indomethacin group	0.242 (±0.011)	132.80 (±11.36)	59.80 (±1.64)
3e	0.281 (±0.016)	168.40 (±14.62)	65.40 (±1.96)
3g	0.327 (±0.016)	286.20 (±13.82)	86.40 (±2.72)
3l	0.340 (±0.014)	258.20 (±15.42)	80.50 (±2.63)
4a	0.361 (±0.016)	271.10 (±16.17)	83.20 (±2.82)
4b	0.357 (±0.014)	262.40 (±13.22)	80.40 (±2.56)
5b	0.261 (±0.015)	142.60 (±12.84)	61.30 (±1.96)
5c	0.258 (±0.010)	148.60 (±16.22)	62.60 (±1.86)
6b	0.337 (±0.012)	258.20 (±15.54)	84.20 (±2.72)
F test	10.8434	18.7486	21.1497
LSD	0.039	40.7590	6.6214
P	<0.001	<0.001	<0.001

Exudate volume (expressed in ml); total leucocyte count (TLC) (expresed as cell/cm^3); differential leucocyte count (DLC) (expressed as exudate neutrophil %)

Table 2: The effect of inflammation induced by sponge implantation on the immunological parameters

	Unstimulated reduced cytochrome C levels $\bar{X}$ (± S.E.)	Stimulated reduced cytochrome C levels $\bar{X}$ (± S.E.)	Unstimulated IL-1β levels $\bar{X}$ (± S.E.)	LPS stimulated IL-1β levels $\bar{X}$ (± S.E.)
Inflammatory control group	1.312 (±0.017)	1.411 (±0.024)	193.60 (±13.41)	261.80 (±15.36)
Indomethacin group	0.764 (±0.012)	0.821 (±0.014)	113.50 (±4.81)	128.30 (±4.18)
3e	0.886 (±0.028)	0.916 (±0.018)	128.30 (±6.86)	148.60 (±5.28)
3g	1.416 (±0.026)	1.518 (±0.031)	190.80 (±14.22)	266.50 (±16.83)
3l	1.390 (±0.024)	1.460 (±0.032)	196.40 (±14.60)	282.20 (±16.68)
4a	1.390 (±0.019)	1.512 (±0.028)	198.80 (±14.52)	270.40 (±16.42)
4b	1.376 (±0.016)	1.490 (±0.032)	199.40 (±15.48)	274.20 (±14.32)
5b	0.812 (±0.022)	0.864 (±0.017)	122.30 (±5.16)	132.20 (±5.54)
5c	0.842 (±0.032)	0.896 (±0.019)	124.40 (±6.62)	142.40 (±5.86)
6b	1.410 (±0.021)	1.468 (±0.034)	200.80 (±16.62)	276.40 (±15.20)
F test	16.7211	146.6183	12.2765	30.0558
LSD	0.0635	0.0732	31.2811	35.9277
P	<0.001	<0.001	<0.001	<0.001

Neutrophil Phagocyte Function (expressed in nmol $O^{2.-}$/2X10^6 PMN/hour) on both unstimulated and stimulated reduced cytochrome C levels; and the assay of Interleukin-1β (IL-1β) (expressed in pg/ml) on both unstimulated IL-1β levels and LPS stimulated IL-1β levels

The neutrophil phagocytic function was determined as one of the most important indices used to assess the different neutrophilic functions.

This is because it is the ultimate and major step in neutrophilic response to acute inflammation. The main step in this function is the respiratory burst, that reflects an important facet of immune system integrity. This was evaluated by the cytochrome C reduction test, where the reduction of cytochrome C by superoxide anion will change its light absorption properties, as detected spectrophotometrically.

The superoxide radical ($O^{2.-}$) is a highly reactive intermediate produced in biological systems by one-electron reduction of oxygen. It is generated by phagocytes during the respiratory burst. It is an intermediate in the formation of H_2O_2 and HOCl which are considered powerful antimicrobial agents. The detection of $O^{2.-}$ is based on its ability to reduce cytochrome C.

2.2.1.1. Effect on exudate volume

Results presented in Table 1 revealed that pre-treatment with indomethacin significantly reduced the exudate volume in comparison to the inflammatory control group ($P < 0.001$). The mean values were 0.242 ± 0.011 and 0.348 ± 0.013 respectively.

Pre-treatment with the investigated compounds (**3e**, **5b** and **5c**) significantly reduced the exudate volume as compared to the inflammatory control group ($P < 0.001$). The mean values were 0.281 ± 0.016, 0.261 ± 0.015 and 0.258 ± 0.010 respectively, compared to a control mean value of 0.348 ± 0.013. On the other hand, pre-treatment with the investigated compounds **3g**, **3l**, **4a**, **4b** and **6b** did not significantly alter the exudate volume, compared to the inflammatory control group ($P < 0.05$). Their means were 0.327 ± 0.016, 0.340 ± 0.014, 0.361 ± 0.016, 0.357 ± 0.014 and 0.337 ± 0.012 respectively, in comparison with a control mean value of 0.348 ± 0.013.

2.2.1.2. Effect on total leucocyte cell counts (TLC)

Results presented in Table 1 show that pre-treatment with indomethacin significantly reduced the exudate TLC, as compared to the inflammatory control group ($P < 0.001$). The mean value was 132.80 ± 11.36 compared to 264.50 ± 14.12.

Pre-treatment with the probed compounds **3e**, **5b** and **5c** significantly reduced the exudate TLC as compared to the inflammatory control group ($P < 0.001$). The mean values were 168.4 ± 14.62, 142.60 ± 12.84 and 148.60 ± 16.22 respectively, compared to a control mean value of 264.50 ± 14.12. However, pre-treatment with the investigated compounds **3g**, **3l**, **4a**, **4b** and **6b** did not significantly reduce the exudate TLC, in comparison to the control group ($P < 0.05$). Their mean values were 286.20 ± 13.82, 258.20 ± 15.42, 271.10 ± 16.17, 262.40 ± 13.22 and 258.20 ± 15.54 respectively, compared to the control mean value of 264.50 ± 14.12.

2.2.1.3. Effect on differential leucocyte cell counts (DLC)

Results presented in Table 1 show that pre-treatment with indomethacin significantly reduced the exudate neutrophil % as compared to the inflammatory control group ($P < 0.001$). The mean value was 59.80 ± 1.64 compared to a control mean value of 81.30 ± 2.18.

Pre-treatment with the investigated compounds **3e**, **5b** and **5c** significantly reduced the exudate neutrophil% in comparison to the inflammatory control group ($P < 0.001$). The mean values were 65.40 ± 1.96, 61.30 ± 1.96 and 62.60 ± 1.86 respectively, compared to a control mean value of 81.30 ± 2.18. However, pre-treatment with the investigated compounds **3g**, **3l**, **4a**, **4b** and **6b** did not significantly alter the exudate neutrophile % as compared to the inflammatory control group ($P < 0.05$). The mean values were 86.40 ± 2.72, 80.50 ± 2.63, 83.20 ± 2.82, 80.40 ± 2.56 and 84.20 ± 2.72 respectively, compared to the control mean value of 81.30 ± 2.18.

2.2.1.4. Effect on neutrophil phagocyte function

The neutrophil phagocyte function was measured using the cytochrome C reduction test. In this test both the basal level of reduced cytochrome C (unstimulated), as well as its level after stimulation of the granulocytes by means of zymosan particles (stimulated) were estimated. The results were expressed as nmol $O^{2.-}$/2 X 10^6 PMN/h. Results presented in Table 2 show that pre-treatment with indomethacin significantly reduces the unstimulated reduced cytochrome C level as compared to the inflammatory control group ($P < 0.001$). The mean value was 0.764 ± 0.012 compared to a control mean value of 1.312 ± 0.017. Simi-

larly, the results show that pre-treatment with the investigated compounds **3e**, **5b** and **5c** significantly reduces cytochrome C level, compared to the inflammatory control group ($P < 0.001$). The mean values were 0.886 ± 0.028, 0.812 ± 0.022 and 0.842 ± 0.032 respectively, compared to a control value of 1.312 ± 0.017. On the other hand, pre-treatment with the investigated compounds **3g**, **3l**, **4a**, **4b** and **6b** did not significantly reduce the unstimulated reduced cytochrome C level as compared to the inflammatory control group ($P > 0.05$). Their mean values were 1.416 ± 0.026, 1.390 ± 0.024, 1.390 ± 0.019, 1.376 ± 0.016 and 1.410 ± 0.021 respectively, compared to the control mean value of 1.312 ± 0.017.

The results presented in Table 2 also show that pre-treatment with indomethacin significantly reduces the stimulated reduced cytochrome C level as compared to the inflammatory control group ($P < 0.001$). The mean value was 0.821 ± 0.014 compared to control mean value of 1.411 ± 0.024.

Pre-treatment with the investigated compounds **3e**, **5b** and **5c** significantly decreased the stimulated reduced cytochrome C level in comparison to the inflammatory control group ($P < 0.001$). Their mean values were 0.916 ± 0.018, 0.864 ± 0.017 and 0.896 ± 0.019 respectively, compared to a control mean value of 1.411 ± 0.024. On the other hand, pre-treatment with the investigated compounds **3g**, **3l**, **4a**, **4b** and **6b** did not significantly alter the stimulated reduced cytochrome C level as compared to the control group ($P < 0.05$). Their mean values were 1.518 ± 0.031, 1.460 ± 0.032, 1.512 ± 0.028, 1.490 ± 0.032 and 1.468 ± 0.034 respectively, compared to the control mean value 1.411 ± 0.024.

2.2.1.5. Effect on interleukin-1β level (IL-1β)

The assay of interleukin-1β using an IL-1β Elisa Kit was done by estimating both the unstimulated level of IL-1β (spontaneously released as well as the lipo-polysaccharide (LPS) stimulated release of IL-1β. The results were expressed in pg/ml.

The results presented in Table 2 show that pre-treatment with indomethacin significantly decreased the unstimulated IL-1β level as compared to the inflammatory control group ($P < 0.001$). The mean value was 113.50 ± 4.81 compared to the control mean value of 193.60 ± 13.41.

Pre-treatment with the investigated compounds **3e**, **5b** and **5c** significantly decreased the unstimulated IL-1β levels in comparison to the inflammatory control group ($P < 0.001$). Their mean values were 128.30 ± 6.86, 122.30 ± 5.16 and 124.40 ± 6.62 respectively, compared to a control mean value of 193.60 ± 13.41. On the other hand, pre-treatment with the investigated compounds **3g**, **3l**, **4a**, **4b**, **5b** and **6b** did not significantly alter the unstimulated IL-1β level as compared to the control group ($P < 0.05$). Their mean values were 190.80 ± 14.22, 196.40 ± 14.60, 198.80 ± 14.52, 199.40 ± 15.48 and 200.80 ± 16.62 respectively, compared to the control mean value of 193.60 ± 13.41.

Results presented in Table 2 also show that pre-treatment with indomethacin significantly decreased the LPS-stimulated IL-1β level as compared to the inflammatory control group ($P < 0.001$). The mean value was 128.30 ± 4.18 compared to a control mean value of 261.80 ± 15.36.

Pre-treatment with the investigated compounds **3e**, **5b** and **5c** significantly decreased the LPS-stimulated IL-1β level in comparison to the inflammatory control ($P < 0.001$). Their mean values were 148.60 ± 5.28, 132.20 ± 5.54 and 142.40 ± 5.86 respectively, compared to a control mean value of 261.80 ± 15.36. However, pre-treatment with the investigated compounds **3g**, **3l**, **4a**, **4b** and **6b** did not significantly alter the LPS-stimulated IL-1β levels in comparison to the inflammatory control group ($P < 0.05$). Their mean values were 266.50 ± 16.83, 282.20 ± 16.68, 270.40 ± 16.42, 274.20 ± 14.32 and 276.40 ± 15.20 respectively, compared to the control mean value 261.80 ± 15.36.

2.2.2. Ulcerogenic activity

Compounds which showed marked antiinflammatory activity (**3e**, **5b** and **5c**) were tested for their ulcerogenic activity. Phenylbutazone and indomethacin were tested as reference drugs. It was found that the incidence of ulcers with these compounds was 40%, 20% and 30% respectively as compared to the known antiinflammatory drugs phenylbutazone (60%) and indomethacin (100%) (see experimental section).

2.2.3. Conclusion

One can conclude from this study that the sponge implantation model of inflammation seemed to be a reliable method for collection of an adequate amount of exudate necessary for the evaluation of different inflammatory markers. Moreover, it can be concluded from the present study that pre-treatment with the investigated compounds **3e**, **5b** and **5c** significantly altered the exudate markers, not only, the non-immunological parameters (exudate volume, TLC and DLC), but also the immunological parameters (reduced cytochrome C and IL-1β levels). Therefore, compounds **3e**, **5b** and **5c** were capable of modulating the inflammatory response and were assumed to have *in vivo* antiinflammatory activity similar to that of indomethacin. Moreover, they showed minimum ulcerogenic activity as compared to phenylbutazone and indomethacin. These speculations remain to be further expanded in other models of experimental inflammation and confirmed by clinical trials before a final drug design is made.

3. Experimental

M.p.s were uncorrected and determined in open glass capillaries. IR spectra were measured as KBr discs on a Perkin Elmer 1430 spectrophotometer. ^{1}H NMR spectra were recorded on Varian EM-390, 90 MHz and Brucker 400 MHz spectrometers, in DMSO-d_6 or $CDCl_3$ using TMS as internal standard. The chemical shifts are given in δ ppm values and the exchangeable protons were confirmed by D_2O. The microanalyses were performed at the microanalytical unit, Faculty of Science, Cairo University, Egypt and the values were within ±0.4% of the theoretical data.

3.1. Synthesis of the compounds

3.1.1. 1-[(3,4-Disubstituted thiazolin-2-ylidene)hydrazinocarbonyl] methyl-1H-benzotriazoles **3a–l**

To a solution of substituted thiosemicarbazides **2a–d** (1 mmol) in a mixture of absolute EtOH (10 ml) and dry dioxane (5 ml), an equimolar amount of the appropriate phenacyl bromide (1 mmol) was added. The reaction mixture was heated under reflux for 5 h and allowed to reach room temperature. The PH was then adjusted to 8 by the addition of a cold saturated solution of NaAc (10 g/10 ml H_2O). The reaction mixture was allowed to stand overnight and the separated product was filtered, washed several times with H_2O, dried and recrystallized from EtOH except for compounds **3g**, **3h**, **3i**, **3l** which were crystallized from dioxane/H_2O (Table 3).

IR (KBr, cm^{-1}): 3416–3441 (NH); 1684–1679 (C=O); 1663–1662 (C=N); 1646–1629 (C=C thiazolin); 1605–1589, 1519 (C=C); 1575–1571 (δ NH); 1268–1231, 1049–1044 (C–S–C). ^{1}H NMR (**3b**), (DMSO-d_6), (δ ppm): 2.07 (s, 1 H, NH, D_2O exchangeable); 2.37 (s, 3 H, CH_3);

Table 3: 1-[(3,4-Disubstituted thiazolin-2-ylidene) hydrazinocarbonyl] methyl-1 *H*-benzotriazoles 3a–l

Compd.	R	R^1	Yield (%)	M.p. °C	Mol. formula (Mol. wt.)
3a	C_6H_5	H	90	151–152	$C_{23}H_{18}N_6OS$ (426.5)
3b	C_6H_5	CH_3	92	145–146	$C_{24}H_{20}N_6OS$ (440.5)
3c	C_6H_5	Cl	98	100–101	$C_{23}H_{17}ClN_6OS$ (461.0)
3d	$C_6H_5-CH_2-$	H	95	105–106	$C_{24}H_{20}N_6OS$ (440.5)
3e	$C_6H_5-CH_2-$	CH_3	96	203–204	$C_{25}H_{22}N_6OS$ (454.6)
3f	$C_6H_5-CH_2-$	Cl	98	115–116	$C_{24}H_{19}ClN_6OS$ (475.0)
3g	$C_6H_4-Cl(p)$	H	93	125–126	$C_{23}H_{17}ClN_6OS$ (460.9)
3h	$C_6H_4-Cl(p)$	CH_3	95	130–132	$C_{24}H_{19}ClN_6OS$ (475.0)
3i	$C_6H_4-Cl(p)$	Cl	95	215–216	$C_{23}H_{16}Cl_2N_6OS$ (495.4)
3j	$C_6H_4-CH_3(p)$	H	92	120–122	$C_{24}H_{20}N_6OS$ (440.5)
3k	$C_6H_4-CH_3(p)$	CH_3	90	125–126	$C_{25}H_{22}N_6OS$ (454.6)
3l	$C_6H_4-CH_3(p)$	Cl	91	205–206	$C_{24}H_{19}ClN_6OS$ (475.0)

4.88 (s, 2 H, CH_2CO); 6.06 (s, 1 H, thiazolin-C_5–H); 6.95–7.82 (m, 11 H, Ar–H and benzotriazole $C_{5,6}$–H); 7.87 (d, 1 H, J = 8 Hz, benzotriazole C_7–H); 7.96 (d, 1 H, J = 8 Hz, benzotriazole C_4–H). ^{1}H NMR (**3e**), ($CDCl_3$), (δ ppm): 1.6 (s, 1 H, NH, D_2O exchangeable); 2.36 (s, 3 H, CH_3); 4.8 (s, 2 H, $CH_2C_6H_5$); 5.2 (s, 2 H, CH_2CO); 5.8 (s, 1 H, thiazoline-C_5–H); 6.8–7.8 (m, 13 H, Ar–H).

3.1.2. 1-[(3-Substituted-5-ethoxycarbonyl-4-methylthiazolin-2-ylidene) hydrazinocarbonyl]methyl-1 H-benzotriazoles **4a–c**

A mixture of the appropriate thiosemicarbazides **2a–c** (1 mmol) and ethyl α-chloroacetoacetate (0.18 g, 0.3 ml, 1.1 mmol) in absolute EtOH (10 ml) and dry dioxane (5 ml) was heated under reflux for 4 h. The reaction mixture was treated according to the previously described procedure, to isolate compounds **4a–c**. They were crystallized from EtOH (Table 4).
IR (KBr, cm^{-1}) : 3478–3460, 3443, 3232–3170 (NH); 1727–1712 (C=O ester); 1700–1680 (C=O amide); 1651–1627 (C=N); 1604–1587, 1520–1519 (C=C); 1560–1552 (δ NH); 1265–1255, 1095–1093 (C–O–C); 1255–1229, 1071–1070 (C–S–C). ^{1}H NMR (**4b**), (DMSO-d_6), (δ ppm): 1.23 (t, 3 H, J = 7 Hz, CH_2CH_3); 2.08 (s, 3 H, CH_3); 2.43 (s, 2 H, $CH_2C_6H_5$); 4.18 (q, 2 H, J = 7 Hz, CH_2CH_3); 5.17, 5.5 (two d, J = 12 Hz, each 1 H, CH_2CO magnetic non-equivalent protons); 7.26 (t, 2 H, J = 6.8 Hz, $C_6H_5-C_{3,5}$–H); 7.31(d, 2 H, J = 6.8 Hz, $C_6H_5-C_{2,6}$–H); 7.34 (t, 1 H, J = 6.8 Hz, $C_6H_5-C_4$–H); 7.4 (t, 1 H, J = 8 Hz, benzotriazole-C_5–H); 7.55 (t, 1 H, J = 8 Hz, benzotriazole-C_6–H); 7.82 (d, 1 H, J = 8 Hz, benzotriazole C_7–H); 8.04 (d, 1 H, J = 8 Hz, benzotriazole C_4–H); 10.78 (s, 1 H, NH, D_2O exchangeable). ^{1}H NMR (**4c**), (DMSO-d_6), (δ ppm): 1.2 (t, 3 H, J = 7 Hz, CH_2CH_3); 2.3 (s, 3 H, CH_3); 4.2 (q, 2 H, J = 7 Hz, CH_2CH_3); 5.7 (s, 2 H, CH_2CO); 6.8–7.9 (m, 8 H, Ar–H).

3.1.3. 1-[(3-Substituted-4-oxothiazolidin-2-ylidine)hydrazinocarbonyl] methyl-1H-benzotriazoles **5a–d**

A mixture of the appropriate thiosemicarbazides **2a–d** (1 mmol) and an equimolar amount of ethyl bromoacetate (0.17 g, 0.13 ml, 1.1 mmol) in absolute EtOH (10 ml) and dry dioxane (5 ml) was heated under reflux for 5 h. The reaction mixture was then worked up as described above to isolate compounds **5a–d**, which were crystallized from EtOH (Table 5).
IR (KBr, cm^{-1}): 3558–3503 (OH enolic); 3435–3237 (NH); 1679–1674, 1664–1663 (C=O); 1664–1645 (C=N); 1610–1597, 1518–1515 (C=C); 1589–1549 (δ NH); 1279–1275, 1040–1030 (C–S–C). ^{1}H NMR (**5c**), (DMSO-d_6), (δ ppm) : 5.9 (s, 2 H, CH_2CO); 6.2 (s, 2 H, thiazolidinone-C_5-H_2); 7–8 (m, 8 H, Ar–H). ^{1}H NMR (**5d**), (DMSO-d_6), (δ ppm): 2.07 (s, 1 H, NH, D_2O exchangeable); 2.28 (s, 3 H, CH_3); 4.01 (s, 2 H, CH_2CO); 6.03 (s, 2 H, thiazolidinone C_5-H_2); 7.08, 7.21 (two d, each 2 H, J = 7Hz, C_6H_4-CH_3); 7.35 (t, 1 H, J = 8 Hz, benzotriazole C_5–H); 7.46 (t, 1 H, J = 8 Hz, benzotriazole C_6-H); 7.54 (d, 1 H, J = 8 Hz, benzotriazole C_7-H); 7.96 (d, 1 H, J = 8 Hz, benzotriazole C_4–H).

Table 4: 1-[(3-Substituted-5-ethoxycarbonyl-4-methylthiazolin-2-ylidene)hydrazinocarbonyl]methyl-1 *H*-benzotriazoles 4a–c

Compd.	R	Yield (%)	M.p. °C	Mol. formula (Mol. wt.)
4a	C_6H_5	90	294–295	$C_{21}H_{20}N_6O_3S$ (436.5)
4b	$C_6H_5-CH_2$	92	282–283	$C_{22}H_{22}N_6O_3S$ (450.5)
4c	$C_6H_4-Cl(P)$	91	276–277	$C_{21}H_{19}ClN_6O_3S$ (470.9)

Table 5: 1-[(3-Substituted-4-oxothiazolidin-2-ylidene)hydrazinocarbonyl] methyl-1 *H*-benzotriazoles 5a–d

Compd.	R	Yield (%)	M.p. °C	Mol. formula (Mol. wt.)
5a	C_6H_5	80	212–213	$C_{17}H_{14}N_6O_2S$ (366.4)
5b	$C_6H_5-CH_2-$	79	263–264	$C_{18}H_{16}N_6O_2S$ (380.4)
5c	$C_6H_4-Cl(p)$	81	256–257	$C_{17}H_{13}ClN_6O_2S$ (400.9)
5d	$C_6H_4-CH_3(p)$	69	130–131	$C_{18}H_{16}N_6O_2S$ (380.4)

Table 6: 1-[(-5-Substituted aminothiadiazol-2-yl)methyl]-1 *H*-benzotriazoles 6a–c and 1-[(5-substituted aminooxadiazol-2-yl)methyl]-1 *H*-benzotriazoles 7a, b

Compd.	R	X	Yield (%)	M.p. °C	Mol. formula (Mol. wt.)
6a	C_6H_5	S	91	255–256	$C_{15}H_{12}N_6S$ (308.4)
6b	$C_6H_4Br(p)$	S	87	285–286	$C_{15}H_{11}BrN_6S$ (387.3)
6c	$C_6H_4-CH_3(p)$	S	89	240–242	$C_{16}H_{14}N_6S$ (322.4)
7a	C_6H_5	O	60	250–251	$C_{15}H_{12}N_6O$ (292.3)
7b	n-C_3H_7	O	62	290–291	$C_{12}H_{14}N_6O$ (258.3)

3.1.4. 1-[(5-Substituted aminothiadiazol-2-yl)methyl]-1 H-benzotriazoles **6a–c**

An ice cold stirred solution of the appropriate thiosemicarbazides **2a–c** (1 mmol) in conc. H_2SO_4 (3 ml) was left for a period of 30 min at RT. The reaction mixture was carefully poured while stirring on to 6 ml conc. NH_4OH and 20 g crushed ice. The precipitate obtained was filtered, washed with H_2O until the washings were neutral, dried in vaccum and recrystallized from dioxane as pale yellow crystals (Table 6).
IR (KBr, cm^{-1}): 3202–3200, 3138–3125 (NH); 1645 (C=N); 1619–1608, 1519–1515 (C=C); 1563–1561 (δ NH); 1248–1243, 1073–1063 (C–S–C). ^{1}H-MR (**6c**), (DMSO-d_6), (δ ppm): 2.22 (s, 3 H, CH_3); 6.33 (s, 2 H, CH_2); 7.11, 7.42 (two d, each 2 H, J = 7Hz, C_6H_4–CH_3); 7.43 (t, 1 H, J = 8 Hz, benzotriazole C_5–H); 7.59 (t, 1 H, J = 8 Hz, benzotriazole C_6–H); 7.9 (d, 1 H, J = 8 Hz, benzotriazole C_7–H); 8.07 (d, 1 H, J = 8 Hz, benzotriazole C_4–H), 10.27 (s, 1 H, NH, D_2O exchangeable).

3.1.5. 1-[(5-Substituted aminooxadiazol-2-yl)methyl-1 H-benzotriazoles **7a–b**

A solution containing **1** (0.96 g, 5 mmol) and the appropriate isocyanate (5 mmol) in a mixture of absolute EtOH (10 ml) and dry dioxane (5 ml) was heated under reflux for 6 h, then cooled to RT. The separated product was filtered, washed with EtOH, dried and recrystallized from dioxane/EtOH (Table 6).
IR (KBr, cm^{-1}): 3437–3436, 3268–3201 (NH); 1624–1610 (C=N); 1561–1534 (δ NH); 1522–1513 (C=C); 1243–1228, 1166–1162, 1073–1064 (C–O–C). ^{1}H NMR (**7a**), (DMSO-d_6), (δ ppm): 2.07 (s, 1 H, NH, D_2O exchangeable); 3.31 (s, 2 H, CH_2), 6.93–7.5 (m, 7 H, Ar–H, and benzotriazole $C_{5,6}$–H); 8.3 (d, 1 H, J = 8 Hz, benzotriazole C_7–H); 8.6 (d, 1 H, J = 8 Hz, benzotriazole C_4–H).

3.2. Pharmacology

3.2.1. Antiinflammatory activity

3.2.1.1. Material and methods

One hundred male albino rats weighing 150–200 grams were used throughout this work. They were kept in the animal house under standard conditions of light and temperature with free access to food and water. The animals were randomly divided into ten groups each of ten rats as follows:
Group **I** received 1% gum acacia orally (suspending vehicle) and served as an inflammatory control group, to estimate reference values of the parameters studied.
Group **II** (indomethacin pre-treated inflammatory group) received indomethacin (indomethacin-Pharco Pharmaceuticals, Egypt) suspended in 1% gum acacia, in a dose of 10 mg/kg body weight/day orally, divided into two equal doses, for three successive days [24]. On the third day, the first dose was administered thirty minutes before sponge implantation and the second dose one hour before its removal.
Group **III** to **X** (pre-treated inflammatory groups) each being treated with the appropriate newly synthesized investigational compounds **3e**, **3g**, **3l**, **4a**, **4b**, **5b**, **5c**, and **6b** respectively. The compounds suspended in 1% gum acacia, was given in a dose of 10 mg/kg body weight/day, orally, divided into two equal doses, for three successive days, as under group **II**.

3.2.1.2. Induction of inflammation

Inflammation was induced by subcutaneous implantation of dry polyester sponge (type E_{41}-blue-Kay Metzeler, U.K.) [25]. The polyester sponge was cut to a standard size of 15 mm diameter, 4 mm thickness, and a weight of 17–17.5 mg. The sponges were sterilized in 70% (v/v) ethanol, washed thoroughly twice with distilled water and antiseptic solution (Cyteal-Pierre Fabre laboratories, France), and were then dried in an oven (Gallenkamp-Poland) at 100 °C for 1.5 h.
For implantation, the rats were anaesthetized with diethyl ether and the skin of the ventral aspect of the abdominal surface was shaved and swabbed with a 1% solution of Savlon in 70% (v/v) ethanol/water [25–27]. Two small medial incisions each of 1 cm length, were made on either side of the midline of the ventral abdominal surface, and two cavities were performed by blunt dissection, separating the dermis from the muscular layers. Lastly one dry sponge was implanted in each side, the incisions were then closed with silk sutures (Mersilk 310-sterile silk suture, Ethicon, U.K.) [25] and the rats were kept in their cages for 6 h.

3.2.1.3. Collection of the exudate

After 6 h, the animals were reanaesthetized with ether. The ventral incisions were quickly opened and the sponges were carefully dissected out to avoid local bleeding. Both sponge were gently squeeezed with a 5 ml syringe plunger and the total exudate was collected in polyethylene centrifuge tubes containing 0.1 ml of 1.5% EDTA [25]. This was used to determine non-immunological markers, namely: exudate volume, and total and differential leucocyte cell counts, and immunological markers, namely: cytochrome C reduction test and interleukin-1 beta levels.

3.2.1.4. Cell isolation and preparations

Granulocyte cell suspension was prepared using a modification of the method of Lehrer and Cline [28].

3.2.1.5. Neutrophil phagocyte function estimation [29–32]

The isolated cells were resuspended in 1 ml Hank's balanced salt solution (HBSS, free from phenol red-Gibco, England) and were adjusted to a concentration of 2×10^5 cells/ml. This was stored at −20 °C until tested for neutrophilic function. This was evaluated by the cytochrome C reduction test, where the reduction of cytochrome C by superoxide anion will change its light absorbance properties, as detected spectrophotometrically.

3.2.1.6. Interleukin-1 beta level estimation (IL-1β) [33]

The granulocyte cell layer was washed 3 times in RPMI-1640 complete medium (Grand Island Biological Company, N.Y.) containing 2 mmol glutamine, 100 units/ml penicillin, 100 g/ml streptomycin and 20 mmol N-1-hydroxyethylpiperazine-N-2-ethane sulphonic acid (HEPES buffer) (Nalge, Sybron Corporation-Rochester, N.Y.). The supernatant was aspirated and the cell pellet was vigorously tapped and resuspended in a known volume of complete RPMI medium supplemented with 10% inactivated fetal bovine serum (FBS), heat inactivated at 56 °C for 30 min. (Biofluid-Inc. Rockville, USA).
The granulocyte count was then adjusted to 2×10^6 cells/ml by complete RPMI-1640 supplemented with 10% heat inactivated FBS (RPMI-HIFBS). This constituted the granulocytes cell suspension that was stored at −20 °C until tested for IL-1β levels.

3.2.1.7. Stimulation of granulocytes by lipopolysaccharide (LPS) as a mitogen (lyophilized E-coli, Sigma-USA) [34]

100 µl of the prepared cell suspension were added to LPS (10 µg/ml) (reconstituted in 5 ml sterile complete RPMI-1640 medium and stored at −20 °C until used) in a flat-bottomed well of a microtitre plate. Another 100 µl of the cell suspension (without stimulant) were pipetted into a flat-bottomed well to serve as a control. Each sample was placed in triplicate wells to obtain enough supernatants for measuring IL-1β levels. Complete RPMI medium was added to all wells to obtain a final volume of 200 µl per well.
The cell culltures were incubated for 24 h at 37 °C, 10% CO_2 and 100% humidity in a CO_2 jar (Angelantoni Scientifica-Italy). After 24 h, the cultures were centrifuged at 700 × g for 10 min and the supernatants containing putative IL-1β were collected and stored at −20 °C until tested for the cytokine by ELISA Kit.

3.2.1.8. Statistical methods

Data are expressed as means with their corresponding standard errors. Data were evaluated by the one way analysis of variance. Then, the data were subjected to the least significant difference "LSD" test [35]. The results are given in Tables 1 and 2.

3.2.2. Ulcerogenic activity

The compounds **3e**, **5b**, **5c** were tested for their ulcerogenic activity, phenylbutazone and indomethacin being tested as reference drugs. Sixty male albino rats (150–200 g) were fasted for 12 h prior to drugs administration. Water was given ad libitum. The animals were divided into six groups of ten as follows:
Group **I** (control group) received 1% gum acacia orally.
Group **II** received phenylbutazone at a dose of 10 mg/kg/day orally.
Group **III** received indomethacin at a dose of 10 mg/kg/day orally.
Group **IV**, **V** and **VI** received compounds **3e**, **5b** and **5c** respectively at a dose of 10 mg/kg/day orally.
The drugs were administered orally in two equal doses at 0 and 12 h for three successive days. Animals were sacrificed after 6 h from the last dose. The stomach was removed. An opening at the greater curvature was made and the stomach was washed with cooled saline and inspected with a 3× magnifying lens for any evidence for hyperemia and hemorrhage, definite hemorrhagic erosion or ulcer. The percentage ulceration for each group was calculated as follows:

$$\%\ \text{Ulceration} = \frac{\text{Number of animals bearing ulcer in a group}}{\text{Total number of animals in that group}} \times 100\ [36]$$

The % ulceration for compounds **3e**, **5b** and **5c** were 40%, 20% and 30% respectively; relative to the antiinflammatory drugs phenylbutazone (60%) and indomethacin (100%).

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