Organic Chemistry
Current Research

Hamad Elgazwy et al., Organic Chem Curr Res 2012, 1:5
DOI: 10.4172/2161-0401.1000112

Research Article Open Access

Synthesis, Biological Evaluation of Some 2,3-dihydropyrazoles and Thiazoles as Anti-inflammatory and Antibacterial Agents

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Abstract

The present article describes the synthesis of two novel series of 5-(benzofuran-2-yl)-3-(4-(piperidin-1-yl)phenyl)-2,3-dihydropyrazole-1-carbothioamide 6 and 1-(4-(3-(benzofuran-2-yl)-2-(4-arylthiazol-2-yl)-2,5-dihydro-1H-pyrazol-5-yl)phenyl)piperidine 8a,b. All the newly synthesized target compounds (3, 4, 5, 6 8a and 8b) were screened for their *in vivo* anti-inflammatory (AI) activity using carrageenan-induced rat paw edema assay and *in vitro* antibacterial activity against two Gram-positive and two Gram-negative bacteria. All six compounds (3, 4, 5, 6, 8a, and 8b) showed consistently excellent AI activity (≥ 70% inhibition), at 3 and 4 h after the carrageenan injection, comparable to that of standard drug indomethacin (78%) whereas the remaining twelve compounds have shown significant activity with 57–75% inhibition after 3 h and 56–63% inhibition after 4 h. All the tested compounds showed moderate antibacterial properties.

Keywords: 2,3-dihydropyrazole; Thiazoles; Anti-inflammatory activity; Antibacterial activity

Introduction

The development of an effective therapeutic agent for the management of inflammation has undergone continual evolution leading to the emergence of more efficacious classes of drugs. Since the discovery of aspirin, much efforts have been devoted to the development of non-steroidal anti-inflammatory drugs (NSAIDs), which are among the most widely prescribed medication in clinical practice despite their well be documented renal and gastrointestinal (GI) side effects. Conventional NSAIDs exert non-selective inhibition [1] of COX enzymes, the agents which catalyze the rate-limiting step in the formation of prostanoids from arachidonic acid. Such indiscriminate inhibition of COX-1 as well as COX-2 has been blamed for high incidence of GI irritation or, in the worst case, development of life threatening GI ulcers and bleeding in long term users of NSAIDs. Consequently, a second generation of NSAIDs has been developed which selectively inhibit COX-2. Being selective COX-2 inhibitors, these are expected to achieve the same anti-inflammatory efficacy as traditional NSAIDs but minimize the risk of unwanted GI complications. Though the selective COX-2 inhibitors have minimal toxicity in the gastrointestinal tract, these agents can produce severe side effects in renal, hepatic, and cardiovascular systems. The recent withdrawal of valdecoxib [2] and rofecoxib [3] has focused attention on the adverse cardiovascular effects of selective COX-2 inhibitors. Thus, search for novel anti-inflammatory drugs with minimal GI side effects and high safety margin is still warranted.

Pyrazole and its derivatives are shown to possess important biological and pharmaceutical activities [4,5] such as antimicrobial [6,7], antiviral [8,9], anxiolytic [10,11] and anti-inflammatory [7,12]. Pyrazole moiety makes the core structure of various drugs such as difenamizole (Kameyama et al. [13,14] celecoxib [15] tepoxalin [16] etc. Besides this, there are several reports in the literature on the anti-inflammatory [17-20] and antimicrobial properties [21-23] of pyrazoles. However studies investigating the potential of pyrazole derivatives as dual antimicrobial–anti-inflammatory agents have only recently been initiated [24-27]. Thiazoles and their derivatives are also known to exhibit antimicrobial [28,29] as well as anti-inflammatory activity [30-32]. Since the combination of pharmacophores on the same scaffold is a well be established approach to the synthesis of more potent drugs [33,34], we decided to incorporate pyrazole moiety and thiazole ring in the same molecule while retaining the benzene sulfonamide group in an effort to synthesize new compounds with dual anti-inflammatory–antibacterial potential.

Motivated by these findings coupled with our ongoing program in the field of pyrazoles and other heterocyclic compounds [35-41] as anti-inflammatory agents, it was decided to synthesize two novel series of thiosemicarbazones 3 and thiazolylhydrazinomethylidenepyrazoles 5 with a potential to act as dual anti-inflammatory–antibacterial agents with minimal GI side effects and high safety margin.

Results and Discussion

Chemistry

Chalcones, aromatic ketones and enones acting as the precursor for flavonoids such as Quercetin, are known for their anticancer effects. Although, parent chalcones consist of two aromatic rings joined by a three-carbon α,β-unsaturated carbonyl system, various synthetic compounds possessing heterocyclic rings like pyrazole, indole etc. are well known and proved to be effective anticancer agents. In addition to their use as anticancer agents in cancer cell lines, heterocyclic analogues are reported to be effective even against resistant cell lines. In this connection, we hereby highlight the potential of various heterocyclic

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Received November 13, 2012; **Accepted** November 22, 2012; **Published** November 26, 2012

Citation: Hamad Elgazwy AS, Nassar E, Zaki MY (2012) Synthesis, Biological Evaluation of Some 2,3-dihydropyrazoles and Thiazoles as Anti-inflammatory and Antibacterial Agents. Organic Chem Curr Res 1:112. doi:10.4172/2161-0401.1000112

chalcone analogues as anticancer agents with a brief summary about therapeutic potential of chalcones, mechanism of anticancer action of various chalcone analogues, and current and future prospects related to the chalcones-derived anticancer research. Furthermore, some key points regarding chalcone analogues have been reviewed by analyzing their medicinal properties [42].

Chalcones (1,3-diphenyl-2-propen-1-one) and especially chalcones bearing oxygenated function on the aromatic rings are the precursors of all the flavonoids [43]. They are biologically active molecules found in human diet as they are accumulated in many plants and vegetables [44]. During the biosynthesis, chalcones are cyclized stereo specifically into the corresponding chroman-4-one, also called flavanone, by the enzyme chalcone isomerase (CHI, EC 5.5.1.6) [43,45]. Chalcones are also easily cyclized in slightly acidic conditions whereas the corresponding flavanones are opened in basic media [46]. Scheme 1 displays this relationship for the most abundant chalcone in plant 2_,4_,6_,4-tetrahydroxychalcone and the corresponding flavanone naringenin. Chalcones bearing non-natural substituents have been synthesized during the recent years in order to develop drugs active against cancer [47,48], malaria [49], leishmaniase [50] tuberculosis [51] and cardiovascular diseases [52] or for their properties to modulate the regulation of biochemical pathways like NO [53,54] or tyrosine kinase [55]. Chalcones are usually synthesized using the Claisen–Schmidt reaction in basic medium in polar solvent and purified by separation as the reaction led very often to a complex mixture [46]. These conditions have also been used for the creation of a combinatorial library of chalcones [56]. Improved conditions using either organo-lithium bases in a polar solvent [57] or solid catalyst have been described recently [58].

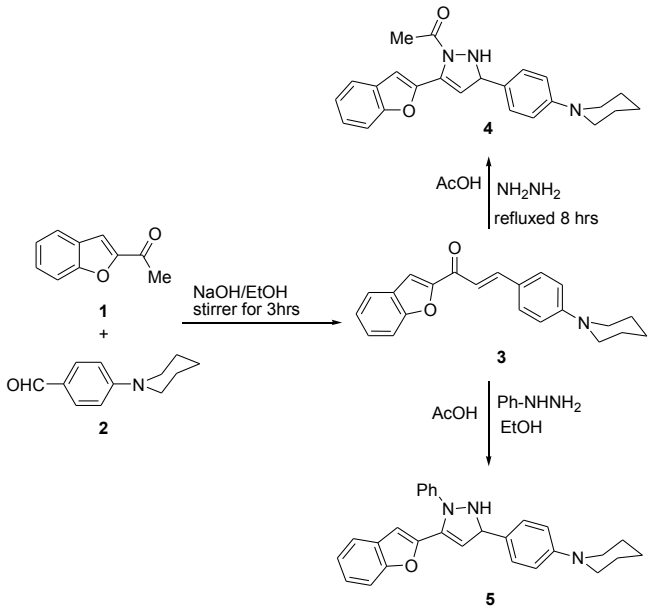
Chalcones are key precursors in the synthesis of various flavonoids as they can be transformed easily in flavanones by cyclization in acidic medium, in flavones or aurones by oxidative cyclization in presence of hydrogen peroxide in basic medium (the so called Algar–Flynn–Oyamada reaction) [59] or other oxidants [60]. However, few new methodologies for the synthesis of chalcone have been described recently [61,62]. The condensation reaction of 2-acetylbenzofuran (1) with 4-piperidinylbenzaldehyde (2) in ethanolic in the presence of NaOH solution afforded the propenone (chalcone) 3 (Scheme 1) through the knowledge reaction, as shown in scheme 1. The structure of chalcone 3 was established through spectroscopic and elemental analysis data, where its IR spectrum showed a strong peak at 1648 cm^{-1} characteristic for conjugated carbonyl group and the ^1H NMR spectrum exhibited each of the olefinic double bond protons as two doublet signals at δ=6.70 and 7.52 ppm. The chalcone 3 was reacted with hydrazine hydrate in refluxing acetic acid to give pyrazoline derivative 4. IR spectrum of 4 exhibited absorption bands at 1728 and 1611 cm^{-1} characteristic for C=O and C=N respectively. The ^1H NMR spectrum showed a singlet at δ=2.25 due to the acetyl CH_3 protons. Reaction of 3 with phenyl hydrazine in ethanol afforded the pyrazole derivative 5.

The reaction of α-haloketones with a carbothioamide 6 has been the most important method for the arylthiazoles 8a-d synthesis ever since it was introduced by Hantzsch and Weber [63]. Corresponding to the Hantzsch thiazole synthesis, the present synthesis of thiazolylpyrazoles 6 consists of the condensation of α-haloketones 7a-d with carbothioamide 6 in refluxing ethanol in the presence of triethyl amine, as outlined in Scheme 2.

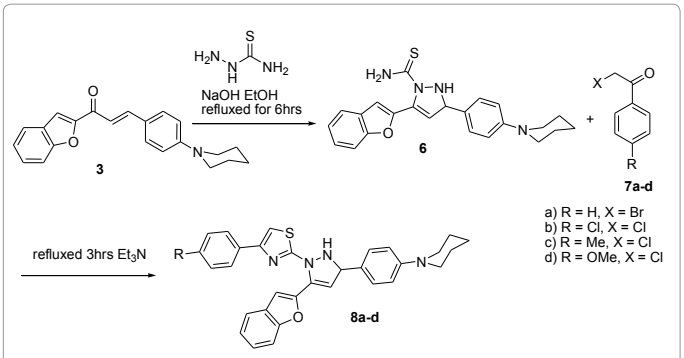
Accordingly pyrazoles (4) [27,40,41] were treated with thiosemicarbazide in the presence of catalytic amount of acetic acid to afford the corresponding carbothioamides 6 which on subsequent reaction with various α-haloketones 7a-d afforded the target thiazolylpyrazoles 8a-b. Spectral data (^1H NMR, ^{13}C NMR, IR and GC/mass) of the newly synthesized compounds were in full agreement with the proposed structures. In general, the characteristic signals in ^1H NMR of target carbothioamide 11.41–11.43, δ 8.26–8.32 and δ 7.77–7.82 corresponding to SH, NH and =NH protons indicating that in solution C=S-NH_2 moiety may exist in its tautomeric form (HS–C=NH). Another exchangeable singlet integrating for two protons in the range of δ 7.46–7.49 was ascribed to NH_2 of SO_2NH_2 group. The ^1H NMR spectra of thiazolylpyrazoles 8a-d displayed an exchangeable singlet in the range of δ 11.99–12.05 corresponding to NH besides a singlet for NH_2 of SO_2NH_2 group in the range of δ 7.44–7.49. A singlet in the range of δ 7.20–7.40 is attributed to C5-proton of thiazole ring. In some of the compounds, this C5-proton of thiazole is merged with other aromatic protons. The structure of 5 was confirmed through its spectral and elemental analysis data. The treatment of chalcone 3 with thiosemicabazide in refluxing ethanol, in the presence of NaOH gave the pyrazole-1-carbothioamides 6 which was reacted with α-haloketones 7a-d in the presence of triethyl amine gave the thiazolylpyrazoles 8a-d respectively (Scheme 2). IR spectrum of 6 showed three stretching absorption bands at 3286, 1669 and 1602 cm^{-1} corresponding to NH_2, C=S and C=N respectively. The ^1H NMR gave a singlet at δ=2.31ppm due to the NH_2 group. The structures of compounds 8a-d were also confirmed by spectral as well as elemental analysis data.

Biological evaluation

***In vivo* anti-inflammatory activity:** Anti-inflammatory drugs are among the most frequently prescribed preparation because of their analgesic, antipyretic and anti-inflammatory effects. They are widely used for the treatment of the symptoms of acute and chronic inflammatory diseases such as osteoarthritis and rheumatoid arthritis. This reagent reduced pain and swelling of joints by blocking the production of prostaglandins from arachidonic acid. Traditional non-steroidal anti-inflammatory drugs (NSAIDs) constitute one of the largest groups of pharmaceuticals with a world market in excess



Scheme 1: Synthesis of target pyrazoles, 4 and 5.



Scheme 2: Synthesis of target 1-carbothioamides 6 and thiazolylpyrazoles 8a-d.

of $ 15 billion per annum. Over the years, it has become increasingly apparent that they are multipurpose analgesic. With the isolation of salicyclic acid in 1829, from the folk remedy willow bark, NSAIDs have become an important part for the treatment of pain (at low doses) and inflammation (at high doses). All the newly synthesized thioamides 6 and thiazolylpyrazoles 8a-d were evaluated for their *in vivo* anti-inflammatory activity by carrageenan induced rat paw edema method (Winter et al.) [64]. The protocol of animal experiments has been approved by the Institutional Animal Ethics Committee (IAEC). Each test compound was dosed orally (50 mg/kg body weight) 30 min prior to induction of inflammation by carrageenan injection. Indomethacin was used as a reference anti-inflammatory drug at a dose of 10 mg/kg, i.p. The anti-inflammatory activity was then calculated at hourly intervals 1–4 h after induction and presented in table 1 as the mean paw volume (ml) as well as the percentage anti-inflammatory activity (AI%). The anti-inflammatory activity of the synthesized compounds 3, 6, 8b, 12a and b, 18a and b and Ibuprofen as a reference drug was screened using 100 mg/kg dose by carrageenan-induced hind paw edema model, [21,22] at different time intervals (Table 1). All screened compounds showed significant reduction in rat paw edema reflecting their anti-inflammatory activity. Notably, the percent inhibition reached its maximum value at the fourth hour, with compounds 12d, 12e and 18b showing to maximum potency at 4 h interval.

Among eighty compounds (3 4, 5, 6 and 8a-d) tested, five compounds (6 and 8a-d) showed consistently excellent AI activity (≥ 70% inhibition) 3 and 4 h after the carrageenan injection comparable to that of standard drug indomethacin (78%), whereas the remaining three compounds have shown significant activity with 50–70% inhibition after 3 h and 50–60% inhibition after 4 h. In general, compounds containing a halogen substituent showed better activity as compared to non-halogen-containing compounds. For instance, three of the six compounds containing chlorine (Cl) as one of the substituents (8a-d) showed excellent activity (≥ 70% inhibition) when measured 3 h after the carrageenan injection. The best compound in each series (8b) in terms of AI activity after 4 h contains a chloro (Cl) substituent at position-4 of the phenyl ring that is attached to the C-3 of the pyrazole moiety. In general, thioamides 6 showed better AI activity as compared to the thiazoles derived from them. Thus, incorporation of thioamide part of the thiosemicarbazones 6 into a thiazole nucleus (8a-d) neither has a beneficial effect nor a detrimental effect on the AI activity. Thioamides (6) showed excellent AI activity comparable to that of the standard drug indomethacin. Out of four compounds (3 and 8d) containing a methoxy substituent, five compounds (6 and 8a-d) showed excellent AI activity (≥ 70% inhibition) after 4 h. These results are in accordance with observations [28,29] claiming that the compounds with Cl (or F) and methoxy substituents show higher activity. Consistently, excellent AI activity up to 4 h suggests that the compounds do not get easily metabolized in the system.

***In vitro* antibacterial activity:** The agar plate diffusion technique [31] was applied to the newly synthesized compounds (3, 4, 5, 6, 8a, 8b, 8c and 8d) to evaluate their *in vitro* bactericidal activity against Gram positive bacteria (*Staphylococcus aureus*) and Gram negative bacteria (*Escherichia coli and Pseudomonas aeroginosa*) and for their fungicidal activity against (*Fusarium, Aspergillus niger, and Candida albicans*) (Table 2). It was found that all compounds except 4 and 5 showed a good bactericidal activity against *Staphylococcus aureus*. *Escherichia coli* and *Pseudomonas aeroginosa* were found to be sensitive to compounds 8a, 8b, 8c and 8d respectively. Most of compounds showed significant fungicidal activity especially against *Aspergillus niger* and *Fusarium oxysporium*. Compounds 8a-d has the most potent activity similar to that of antifugal drug Nystin against *Fusarium oxysporium*.

All the target compounds were evaluated for their *in vitro* antibacterial activity using agar well diffusion method [65] against *Staphylococcus aureus* (MTCC 96) and *Bacillus subtilis* (MTCC 121) representing Gram-positive bacteria, and *Escherichia coli* (MTCC 1652) and *Pseudomonas aeruginosa* (MTCC 741) representing Gram-negative bacteria (Table 2). Ciprofloxacin was used as the reference drug. Antibacterial activity, indicated by an inhibition zone surrounding the well containing the compounds, was recorded if the zone of inhibition was greater than 8 mm. MIC of various compounds against bacterial strains was tested through a macrodilution tube method as recommended by National Committee for Clinical Laboratory Standards (NCCLS) [65,16] (Table 2).

Experimental protocols: Melting points were determined in open capillaries in electrical apparatus and are uncorrected. Elemental analysis was carried out in the microanalytical laboratory, Cairo University, Cairo, Egypt, where the IR spectra were recorded on a Buck Scientific IR M500 instrument. The ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker instrument at 300 MHz and 75.5 MHz, respectively. The δ values are given in ppm relative to TMS as internal standard (for ^{1}H and ^{13}C NMR). Mass spectra (Gc/MS) were recorded on a JEOL-Accu TOF JMS-T100LC Mass spectrometer having a DART (direct analysis in real time) source in ES^{+} mode. Exchangeable (ex) protons were detected by disappearance of peaks upon D_2O addition. The purity of the compounds was checked by ^{1}H NMR. Iodine or UV lamp was used as a visualizing agent for thin layer chromatography (TLC) and reactions were routinely followed by (TLC).

(E)-1-(Benzofuran-2-yl)-3-(4-(piperidin-1-yl)phenyl)prop-2-en-1-one (3)

To a solution of 2-acetylbenzofuran (**1**) (1.6 g, 10 mmol) and 4-piperidinylbenzaldehyde (**2**) (1.89 g, 10 mmol) in ethanol (30 mL), was added 10% aqueous sodium hydroxide (10 mL) portion-wise at room temperature for 10 min. The resulting reaction mixture was stirred for 3 h, leave it in referesh for 24 h , whereupon a solid material separated out that was filtered, washed with water, dried and crystallized from EtOH/DMF to afford the target chalcone **3** as orange needles solid material in excellent yield (85%). M.P. 178-9°C; IR (KBr):: V =3099 (CH Ar), 1648 (C=O), 1578 (CH olefinic) cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6): δ=1.40-1.56 (m, 6H, piperidine H), 2.98-3.26 (m, 4H, piperidine H), 6.70-6.72 (1H, d, J=14 Hz, -CO-CH=), 6.88-6.92 (d, 2H, Ar H), 7.52-7.54 (1H, d, J=14 Hz, =CH-Ar), 7.61-7.82 (m, 6H, Ar H), 8.16 (s, 1H, benzofurane H) ppm; MS (70 eV): m/z=331 (M^{+},100), 330 (68), 274 (26), 247 (28), 214 (56), 186 (27), 145 (87), 117

compound	Inhibition % of edema Volume of edema (ml)[b] and %AI[c]			
	1 h	**2 h**	**3 h**	**4 h**
ref	0.33c ± 0.12	2.18de ± 0.45	2.30d ± 0.96	2.60e ± 2.21
Indomethacin	0.34 ± 0.02** (31)[c]	0.38 ± 0.07** (59)	0.42 ± 0.09** (65)	0.48 ± 0.05** (79)
3	4.72c ± 0.08** (30)[c]	21.86c ± 0.86** (45)[c]	33.69c ± 1.24** (57)[c]	47.73c ± 2.21** (63)[c]
4	1.25d ± 0.03** (33)[c]	15.28bc ± 1.02** (42)[c]	21.01e ± 0.75** (55)[c]	38.26 e ± 0.93** (62)[c]
5	6.27c ± 0.21** (35)[c]	16.64b ± 1.55** (48)[c]	26.07d ± 1.04** (56)[c]	42.24d ± 1.08** (61)[c]
6	5.47a ± 3.25** (39)[c]	13.63a ± 3.57** (56)[c]	18.26a ± 4.52** (54)[c]	43.05a ± 3.67** (77)[c]
8a	9.15b ± 2.42** (32)[c]	11.15a ± 4.25** (51)[c]	24.78b ± 2.65** (65)[c]	36.24a ± 4.22** (78)[c]
8b	3.32e ± 0.01** (31)[c]	12.58e ± 0.22** (59)[c]	25.87e ± 0.88** (63)[c]	35.71e ± 1.35** (78)[c]
8c	2.37e ± 0.01** (25)[c]	13.01d ± 0.85** (50)[c]	26.50d ±1.24** (62)[c]	36.32b ± 2.52** (78)[c]
8d	2.15b ± 2.42** (33)[c]	13.15a ± 4.25** (51)[c]	25.78b ± 2.65** (69)[c]	37.24a ± 4.22** (78)[c]
LSD at 5%	**5.50**	**4.12**	**4.53**	**3.58**

[a]Dose levels: test compounds (50 mg/kg body wt.), indomethacin (10 mg/kg body wt.)
[b]Values are expressed as mean ± SEM and analyzed by ANOVA
[c]Values in parentheses (percentage anti-inflammatory activity, AI%)
*Significantly different compared to respective control values, $P<0.05$
**Significantly different compared to respective control values, $P<0.01$

Table 1: Inhibitory effect *in vivo* anti-inflammatory activity of compounds 3, 6, 8b, 12a and b, 21a and b and Ibuprofen on carrageenan- induced edema of the hind paw in rats.

compounds	Diameter of growth of inhibition zone (mm)[b]			Minimum inhibitory concentration (MIC) (µg/ml)		
	Staphylococcus aureus	***Escherichia coli***	***Pseudomonas aeroginosa***	***Candida albicans***	***Aspergillus niger***	***Fusarium oxysporium***
Ciprofloxacin	++++	++++	++++	-	-	-
Nystin	-	-	-	++++	++++	++++
3	++	-	++	-	-	++
4	++	-	++	++	+++	-
5	++	++	-	-	+++	++
6	+++	-	-	++	+++	+++
8a	-	-	-	++	+++	++++
8b	++	-	++	++	+++	-
8c	++	+++	++	-	+++	++
8d	++	+++	-	-	+++	-

– No activity
[a]Concentration 4.0 mg/ml
[b]Values, including diameter of the well (8 mm), are means of three replicates
[a] The activities are based on the diameters of zones of inhibition in mm. One mL of stock solution (5.0 µg/mL in DMF) was applied in each hole of each paper disk.
+: < 15 mm; ++: 15-24 mm; +++: 25-34 mm; ++++: 35-44 mm, etc.

Table 2: Bactericidal and Fungicidal Activity *in vitro* activity of newly synthesized Compounds and Ciprofloxacin and Nystin[a]

(7). Anal. Calcd for $C_{22}H_{21}NO_2$: C, 79.73; H, 6.39; N, 4.23; Found: C, 79.13; H, 5.88; N, 4.87.

1-(3-(benzofuran-2-yl)-5-(4-(piperidin-1-yl)phenyl)-1H-pyrazol-2(5H)-yl)ethanone (4)

To a solution of chalcone 3 (0.33 g, 1.0 mmol) in acetic acid (30 mL), hydrazine hydrate (99%, 0.5 mL) was added. The reaction mixture was refluxed for 8 h then left to cool and the solid mass separated out was filtered off, washed with ethanol and recrystallized from ethanol to give compound **4** as brownish red crystals in 73% yield, mp 159-0°C; IR (KBr): $\overline{V}$=3099 (CH Ar), 1654(C=O), 1611 (C=N) cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6): δ=1.44-1.46 (m, 1H, pyrazol H), 1.56-1.62 (m, 6H, piperidine), 2.25 (s, 3H, CH_3), 2.44-2.46 (m, 1H, pyrazol), 3.04-3.20 (m, 4H, piperidine), 3.78-3.84 (m, 1H, pyrazol), 6.81-6. 83 (d, 2H, Ar H), 6.98-7.66 (m, 6H, Ar H), 7.97 (s,1H, benzofuran H) ppm; MS (70 eV): *m/z*=389 (M^++2, 1.0), 388 (M^++1, 5.0), 387(M^+, 13), 316 (4), 244(100), 229 (93), 186 (56), 117 (13), 84 (15). Anal. Calcd for $C_{24}H_{25}N_3O_2$: C, 74.39; H, 6.50; N, 10.80; Found: C, 74.83; H, 6.98; N, 10.12.

1-(4-(3-(benzofuran-2-yl)-2-phenyl-2,5-dihydro-1H-pyrazol-5-yl)phenyl)piperidine (5)

This reaction was carried out by the same procedure described in the synthesis of compound 4 by using phenyl hydrazine (0.1 g, 1.0 mmol) and ethanol instead of hydrazine hydrate and acetic acid, respectively to give compound **5** as orange red crystals in 70% yield, mp 160-2°C; IR (KBr): $\overline{V}$=3100 (CH Ar), 1580 (C=N) cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6): δ=1.45-1.48 (m, 1H, pyrazol H), 1.56-1.60 (m, 6H, piperidine), 2.44-2.46 (m, 1H, pyrazol H), 3.03-3.22 (m, 4H, piperidine), 3.82-3.87 (m, 1H, pyrazol), 6.74-6. 83 (d, 2H, Ar H), 7.00-7.72 (m, 11H, Ar H), 8.15 (s,1H, benzofuran H) ppm; MS (70 eV): *m/*

z=422 (M^+ +1, 0.5), 421 (M^+, 1.3), 361(5), 330 (6), 84 (100). Anal. Calcd for $C_{28}H_{27}N_3O$: C, 79.78; H, 6.46; N, 9.97; Found: C, 79.06; H, 5.95; N, 9.32.

3-(benzofuran-2-yl)-5-(4-(piperidin-1-yl)phenyl)-1H-pyrazole-2(5H)-carbothioamide (6)

To a suspension of chalcone 3 (3.3 g, 10 mmol) and sodium hydroxide (1.25 g, 25 mmol) in ethanol (50 mL), thiosemicarbazide (1.3 g, 12 mmol) was added. The mixture was refluxed for 6 h, then left to cool and the formed solid product was filtered off, washed with ethanol, dried, and crystallized from ethanol to afford compound 6 as pale brown crystals in 74% yield, mp 186-7°C; IR (KBr): $\overline{V}$=3286 (NH_2), 2988 (CH Ar), 1602 (C=N), 1232 (C=S) cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6): δ=1.22-1.28 (m, 1H, pyrazol H), 1.52-1.58 (m, 6H, piperidine), 2.18-2.22(m, 1H, pyrazol), 2.31(s, D_2O-exchangeable, 2H, NH_2), 3.16-3.26 (m, 4H, piperidine), 4.09-4.12 (m, 1H, pyrazol), 6.85-6.95 (d, 2H, Ar H), 6.98-7.63 (m, 6H, Ar H), 8.11 (s,1H, benzofuran H) ppm; MS (70 eV): *m*/*z*=404(M^+, 0.4), 343 (100), 287 (6), 259 (5), 231 (7), 117 (8). Anal. Calcd for $C_{23}H_{24}N_4OS$: C, 68.29; H, 5.98; N, 13.85; S, 7.93; Found: C, 67.43; H, 5.20; N, 13.21; S, 7.54.

General procedure for the synthesis of thiazoles 8a-d: To a solution of 3-(benzofuran-2-yl)-5-(4-(piperidin-1-yl)phenyl)-1H-pyrazole-2(5H)-carbothioamide 6 (400 mg, 1.0 mmol) in ethanol (30 mL) was added α-haloketones 7a-d (1.0 mol) followed by a sodium acetate (1.0 mmol) or triethyl amine (0.1 mmol). The resulting reaction mixture was refluxed for 6 h, cooled to room temperature, whereupon a solid material was separated out, which was filtered to afford crude material that was crystallized from ethanol, to yield the target, target thiazolylpyrazoles 8a-d as solid material and recrystallization from ethanol to give 8a-d in excellent yield.

1-(4-(3-(benzofuran-2-yl)-2-(4-(4-phenyl)thiazol-2-yl)-2,5-dihydro-1H-pyrazol-5-yl)phenyl)piperidine (8a)

Pale grey crystals, yield 75%, mp 145-7°C; IR (KBr): $\overline{V}$=3060 (CH Ar), 1672 (C=N), 1600 (C=N) cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6): δ=1.20-1.32 (m, 1H, pyrazol H), 1.48-1.54 (m, 6H, piperidine), 2.40-2.43 (m, 1H, pyrazol), 2.96-3.10 (m, 1H, pyrazol), 3.28-3.34 (m, 4H, piperidine), 6.85-6.90 (d, 2H, Ar H), 6.96 (s, 1H, thiazol H), 6.98-7.59 (m, 11H, Ar H), 7.8 (s, 1H, benzofuran H) ppm; MS (70 eV): *m*/*z*=504 (M^+, 2.0), 419 (4), 343 (36), 331 (100), 227 (6), 173(21), 160 (20), 117 (22), 84 (11). Anal. Calcd for $C_{31}H_{28}N_4OS$: C, 73.78; H, 5.59; N, 11.10; S, 6.35; Found: C, 74.64; H, 5.06; N, 10.56; S, 6.98.

1-(4-(3-(benzofuran-2-yl)-2-(4-(4-chlorophenyl)thiazol-2-yl)-2,5-dihydro-1H-pyrazol-5 yl)phenyl)piperidine (8b)

Pale crystals, yield 70%, mp 160-2°C; IR (KBr): $\overline{V}$=2927 (CH, Ar), 1671 (C=N), 1594 (C=N) cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6): δ=1.19-1.30 (m, 1H, pyrazol), 1.45-1.58 (m, 6H, piperidine), 2.38-2.46 (m, 1H, pyrazol), 3.27-3.31 (m, 1H, pyrazol), 3.34-3.36 (m, 4H, piperidine) 6.90-6.97 (d, 2H, Ar H),7.13-7.68 (m, 10H, Ar H), 7.98(s, 1H, thiazol), 8.12 (s, 1H, benzofurane) ppm; MS (70 eV): *m*/*z*=539 (M^+, .05), 536 (0.37), 421 (30), 352 (45), 317 (100), 222 (48), 117 (18), 84 (15). Anal. Calcd for $C_{31}H_{27}ClN_4OS$: C, 69.07; H, 5.05; N, 10.39; S, 5.95; Found: C, 68.43; H, 5.72; N, 9.58; S, 5.43.

1-(4-(3-(benzofuran-2-yl)-2-(4-p-tolylthiazol-2-yl)-2,5-dihydro-1H-pyrazol-5-yl)phenyl)piperidine (8c)

Gray crystals, yield 80%, mp 210-2°C; IR (KBr): $\overline{V}$ =2925 (CH, Ar), 1677 (C=N), 1605 (C=N) cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6): δ=1.46-1.52 (t, 6H, piperidine), 2.35 (s, 3H, Me), 2.72 (t, 4H, piperidine), 6.67 (s, 1H, HC=C), 6.92 (s, 1H, pyrazol), 6.95 (s, 1H, benzofurane) 7.07 (d, 2H, 7.15-7.78 (m, 12H, Ar H), 8.05 (s, 1H, thiazol), 8.20 (s, 1H, NH) ppm; MS (70 eV): m/e: 518.21 (100.0%), 519.22 (35.0%), 520.22 (6.4%), 520.21 (5.1%), 519.21 (2.3%), 521.21 (1.6%). Anal. Calcd for $C_{32}H_{30}N_4OS$: C, 74.10; H, 5.83; N, 10.80; S, 6.18; Found: C, 74.13; H, 5.82; N, 10.80; S, 6.19.

1-(4-(3-(benzofuran-2-yl)-2-(4-(4-methoxyphenyl)thiazol-2-yl)-2,5-dihydro-1H-pyrazol-5-yl)phenyl)piperidine (8d)

Gray crystals, yield 85%, mp 222-2°C; IR (KBr): $\overline{V}$=2929 (CH, Ar), 1679 (C=N), 1610 (C=N) cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6): δ=1.48-1.54 (t, 6H, piperidine), 3.85 (s, 3H, OMe), 2.72-2.74 (m, 4H, piperidine), 6.67 (s, 1H, HC=C), 6.97 (s, 1H, pyrazol), 6.95 (s, 1H, benzofurane) 7.15-7.78 (m, 12H, Ar H), 8.05 (s, 1H, thiazol), 8.25 (s, 1H, NH) ppm; MS (70 eV): m/e: 534.21 (100.0%), 535.21 (37.0%), 536.22 (5.9%), 536.20 (4.5%), 537.21 (1.7%), 536.21 (1.2%). Anal. Calcd for $C_{32}H_{30}N_4O_2S$: C, 71.88; H, 5.66; N, 10.48; S, 6.00; Found: C, 68.43; H, 5.72; N, 10.58; S, 6.03.

Pharmacological assay

Carrageenan-induced rat paw edema assay: In this method, rats were divided in nine groups of six each. The animals were pretreated with drugs 60 minutes before injection of carrageenan (0.1 ml of 1%). Carrageenan was injected into the sub plantar tissue of left hind paw of each rat. Swellings of carrageenan-injected foot were measured at zero, one, two, three and four hours using Plethysmometer (UGO Basile, Italy) [66,16]. The right hind paw was injected with 0.1 ml of vehicle. The animals received the standard drug Ibuprofen (20 mg/kg, p.o.) which served as reference standard.

Statistical analysis: The results were expressed as mean ± SEM. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Dunnett's test. $p<0.05$ was considered statistically significant.

Male Wistar albino rats weighing 200–250 g were used throughout the study. They were kept in the animal house under standard conditions of light and temperature with free access to food and water. Food was withdrawn 12 h before and during experimental hours. The animals were randomly divided into groups each consisting of six rats. One group of six rats was kept as control and received tween 80 (95:5). Another group received the standard drug indomethacin at a dose of 10 mg/kg body weight ip. Other groups of rats were administered the test compounds at a dose of 50 mg/kg body weight orally. A mark was made on the left hind paw just beyond the tibiotarsal articulation, so that, every time the paw was dipped up to the fixed mark, a constant paw volume was ensured. Paw volumes were measured using a plethysmometer (model 7140, Ugo Basile, Italy). Thirty minutes after administration of test compounds and standard drug, 0.1 ml of 1% w/v of carrageenan suspension in normal saline was injected into subplanter region of the left hind paw of all the animals. The initial paw volume was measured within 30 s of the injection and remeasured again 1, 2, 3, and 4 h after administration of carrageenan. The edema was expressed as an increase in the volume of paw, and the percentage of edema inhibition for each rat and each group was obtained as follows:

$$\%\text{Inhibition} = (V_t - V_o)\ \text{control} - (V_t - V_o)\ \text{tested compound} / (V_t - V_o)\ \text{control} \times 100$$

Where V_t=volume of edema at specific time interval and V_0=volume of edema at zero time interval.

***In vitro* antibacterial assay:** The antibacterial activity of newly synthesized compounds was evaluated *in vitro* by agar–well diffusion method [67]. All the microbial cultures were adjusted to 0.5 McFarland standards, which are visually comparable to a microbial suspension of ~1.5 ± 10^8 cfu/ml (McFaraland, 1907) [68] 20 ml of Mueller–Hinton agar media was poured into each Petri plate, and plates were swabbed with 100 µl inocula of the test microorganisms, and kept for 15 min for adsorption. Using sterile cork borer of 8 mm diameter, wells were bored into the seeded agar plates, and these were loaded with a 100 µl volume with concentration of 4 mg/ml of each compound reconstituted in the dimethylsulfoxide (DMSO). All the plates were incubated at 37°C for 24 h. Antibacterial activity of twenty synthetic compounds was evaluated by measuring the zone of growth inhibition against the test organisms with zone reader (HiAntibiotic zone scale). DMSO was used as a negative control whereas ciprofloxacin was used as a positive control. The experiments were performed in triplicates. The antibacterial activity of the compounds was compared with ciprofloxacin as standard. Minimum inhibitory concentration (MIC) of the newly synthesized compounds against tested bacteria was determined using macrodilution tube method as recommended by NCCLS [69]. Applying the agar plate diffusion technique [59] some of the newly synthesized compounds were screened *in vitro* for antimicrobial activity against representative Gram positive bacteria (Staphylococcus aureus, Bacillus subtilis), Gram negative bacteria (Escherichia coli, Pseudomonas aeruginosa), yeast, (Candida albicans), and fungi, (Aspergillus niger). In this method a standard 5-mm diameter sterilized filter paper disc impregnated with the compound (0.3 mg/0.1 ml of dimethyl-formamide) was placed on an agar plate seeded with thetested organism. The plates were incubated for 24 h at 37°C for bacteria and 28°C for fungi. The zone of inhibition of bacterial and fungal growth around the disc was observed. The screened results given in table 2 revealed that the synthesized compounds showed high or moderate antimicrobial activity against tested Gram positive bacteria staphylococcus.

Conclusion

Eighty new compounds including chalcone (3), thioamides (6), pyrazoles (4 and 5) and four thiazolylpyrazoles (8a-d) were synthesized and evaluated for their *in vivo* anti-inflammatory activity and *in vitro* antibacterial activity. In general thioamide (6) showed better AI activity as compared to the thiazolylpyrazoles (8a-d) derived from them indicating that the thiazole ring, derived from thiocarboxamide part of thioamides (6), is capable of retaining the AI activity and advantageous. Two pyrazole derivatives (4 and 5) showed excellent AI activity (≥ 70% inhibition) 3 h as well as 4 h after the carrageenan injection that is comparable to the standard drug indomethacin. However, none of the compounds was found to be superior over the reference drug. Though the tested compounds failed to give encouraging results in terms of their antibacterial properties, the AI activity results are promising and demand further investigations in this area.

Acknowledgments

The work was financed by a grant (Project 55455/2011) from National Natural Science Foundation of Egypt. The authors would like to express their gratitude and thanks Dr. Amira M Gamal-Eldeen at National Cancer Institute, Cairo, Egypt for doing the antitumor testing of the new compounds. Our sincere acknowledgments to the Sophisticated Analytical Instrument Facility, Central Drug Research Institute, Ain Shams University, Faculty of Science for the IR and GC/Mass spectra facilities.

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