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RESEARCH ARTICLE

Synthesis and Biological Evaluation of Some Pyrazole Derivatives, Containing (Thio) Semicarbazide, as Dual Anti-Inflammatory Antimicrobial Agents

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Abstract: ***Background*:** Several series of pyrazole derivatives containing (thio) semicarbazide (**4a-4h**, **5a-5l**, **6a-6f**, **7a-7c**) were designed and synthesized to screen dual inflammatory and antimicrobial activities.

***Methods*:** The products were characterized by ^{1}H NMR, ^{13}C NMR and HRMS. *In vitro* LPS-induced TNF-α model and *in vivo* xylene-induced ear-edema model were used to evaluate their anti-inflammatory activity. Their *in vitro* antimicrobial activities were evaluated using a serial dilution method against several gram-positive strains, gram-negative strains and a fungi strain.

***Results*:** Bioassays indicated that most of the compounds markedly inhibited the expression of TNF-α at the concentration of 20 μg/mL Compounds **5i**, **6b**, and **7b** had comparable *in vivo* anti-inflammatory activity to the reference drug dexamethasone at the dose of 50 mg/kg. In addition, several compounds showed antimicrobial activity against different strains, and compounds **5g** and **5h** exhibited potent inhibitory activities with the MIC value of 8 μg/mL against the *Streptococcus pneumoniae CMCC 31968* and *Staphylococcus aureus CMCC 25923*, respectively. Compound **7b**, which exhibited both anti-inflammatory and antimicrobial activities, should be studied as it is or after derivatization.

***Conclusion*:** It can be concluded that pyrazoles, with (thio)-semicarbazone moieties, have the potential to be developed into new anti-inflammatory agents.

Keywords: Anti-inflammatory, antimicrobial, pyrazole, (thio) semicarbazide, TNF-α, dexamethasone.

1. INTRODUCTION

Inflammation is part of the complex biological response of body tissues in response to damage resulting from microbial pathogen infection, chemical stimuli, and physical trauma [1, 2]. Although acute inflammation is required as a defense mechanism, persistent inflammation is harmful and should be suppressed. Dysregulation of inflammation plays a crucial role in chronic inflammation that can contribute to diseases such as arthritis, heart attacks, and Alzheimer's disease [3]. Furthermore, inflammatory responses play vital roles in different stages of tumor development [4].

Recently, the incidence of inflammation due to bacterial infections is on the rise. Many patients with inflammatory conditions require treatment with antimicrobial agents [5]. The co-administration of multiple drugs for the treatment of inflammatory conditions, associated with some microbial infections, may result in additional health problems, espe cially in patients with impaired liver or kidney function [6]. As an alternative, monotherapy using an anti-inflammatory drug with antimicrobial properties would be better from pharmacoeconomics and safety aspects; this will enhance patient compliance and lower the risk of adverse effects.

Pyrazoles are significant double nitrogen, five-membered, heterocyclic compounds. Since the discovery and approval of celecoxib as an anti-inflammatory agent in the 1990s, many medicinal chemists have focused on the design and synthesis of pyrazole derivatives, particularly their anti-inflammatory activity [7-10]. Pyrazoles can form hydrogen bonds and can readily bind with various enzymes and receptors, allowing them to exhibit diverse pharmacological activities such as antimicrobial [11-13], anticancer [14], antidepressant [15], and anticonvulsant activities [16]. (Thio) semicarbazones have also been extensively investigated for their uses in medicinal chemistry. The compounds containing (thio) semicarbazone were reported to have a broad

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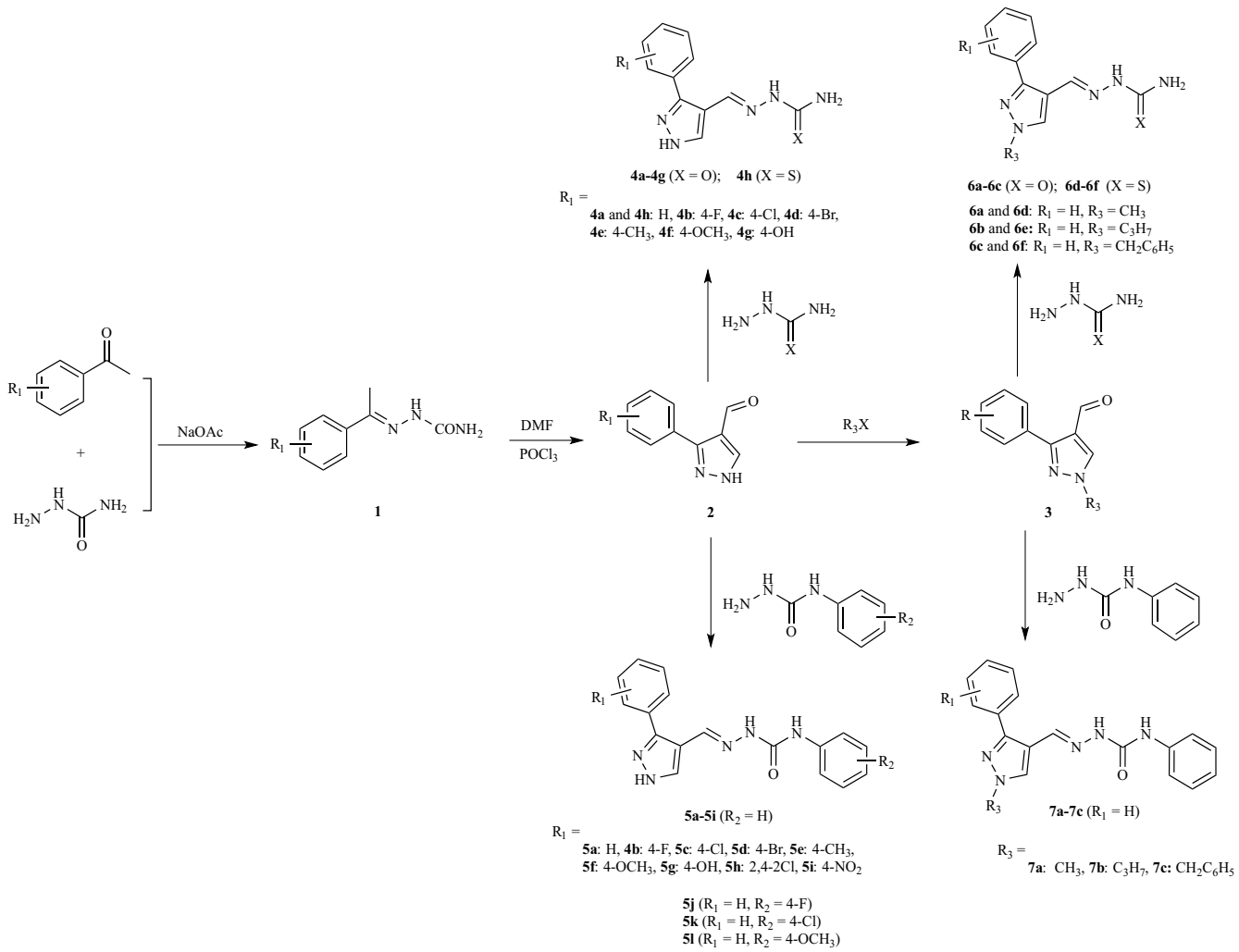


Scheme 1. The synthesis route of target compounds **4a-4h**, **5a-5l**, **6a-6f**, and **7a-7c**.

application in drug development for the treatment of cancer [17] and bacterial infections [18], especially as anti-inflammatory and analgesic agents [19, 20].

Based on these findings, the objective of this study was to combine the (thio) semicarbazide group with the pyrazole moiety to form a skeleton that has the potential to act as a dual anti-inflammatory antimicrobial agent. In this study, twenty-nine 3, 4-substituted pyrazole derivatives were synthesized and their anti-inflammatory and antimicrobial activities were tested.

2. CHEMISTRY

According to the designed structures, twenty-nine pyrazole derivatives were divided into four series, which were prepared from 2 types of intermediates **2** and **3**. The compounds were synthesized according to the sequence shown in Scheme **1**. Firstly, the synthesis of acetophenonehydrazones (**1**) was carried out at 50 °C by reacting the substituted acetophenone with semicarbazide hydrochloride, in the presence of NaOAc and alcohol. Under Vilsmeier-Haack (DMF-$POCl_3$) conditions, compound **1** was transformed into the corresponding 4-carboxaldehyde functionalized pyrazoles (**2**), which then reacted with semicarbazide, thiosemicarbazide and N-phenylhydrazinecarboxamide to produce compounds **4a-4g**, **4h**, and **5a-5l**. On the other hand, compound **2** was alkylated by haloalkane to give the *N*-substituted-4-carboxaldehyde pyrazoles (**3**), which was then reacted with semicarbazide, thiosemicarbazide, and *N*-phenylhydrazineca rboxamide to produce compounds **6a-6c**, **6d-6f**, and **7a-7c** (Scheme **1**). The structure of the desired products was confirmed by ^{1}H NMR, ^{13}C NMR, and mass spectrometry.

Compound **4a** was used as an example of the structure conformation. In the ^{1}H-NMR spectrum, one singlet, due to the NH_2 of semicarbazide, was observed at 6.33 ppm. The aromatic protons of the benzene ring and pyrazole ring revealed peaks in the 7.41-7.94 ppm range. The absorption peak of C-H in imine was found at 8.16 ppm. The absorption peak of NH in the amide was observed at 9.95 ppm as a singlet. The absorption peak in the hydrogen spectrum was in complete conformity with the hydrogen signal in the structure. The ^{13}C NMR spectra also gave accurate information about the structure of compound **4a**, which has 10 types of carbons in different chemical environments. Moreover, the high-resolution mass spectrometry of **4a** displayed an [M + H]+ signal at m/z 230.1034, corresponding to its molecular weight of 230.1036.

3. EXPERIMENTAL PROTOCOLS

3.1. Chemistry

Melting points were determined in open capillary-tubes and were uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded using an AV-300 spectrometer (Bruker, Switzerland), and all chemical shifts were given in parts per million relative to tetramethylsilane. High resolution mass spectra were measured on an MALDI-TOF/TOF mass spectrometer (Bruker Daltonik, Germany). All the reagents and solvents were purchased from Aladdin (Shanghai, China) or Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China), and were used as received.

3.1.1. Synthesis of 2-(1-Phenylethylidene) Hydrazine-1-Carboxamides (1)

A solution of semicarbazide hydrochloride (9 mmol) and sodium acetate (18 mmol) in 20 mL of water was heated to 50 °C. After dissolving, 10 mL of alcohol solution containing acetophenone (9 mmol) was added dropwise, and then stirred at 50 °C for 3-8 h until the reaction completed. After cooling the mixture, the deposit was filtered and recrystallized in ethanol to obtain compounds **1**.

3.1.2. Synthesis of 3-Phenyl-1H-Pyrazole-4-Carbaldehydes (2)

To a cooled (0-5°C) solution of dimethyl formamide (55 mmol), $POCl_3$ (18 mmol)was added dropwise and stirred for 5 min. Then compound **1** (4.5 mmol) was added in six portions at intervals and stirred at 70°C for 5 h. The resulting mixture was poured into 20 mL of ice-water and then adjusted to PH of 6-7 using 20% aqueous sodium hydroxide. After staying over-night below 5°C, precipitates were formed, filtered, and dried to yield compounds **2**.

3.1.3. Synthesis of N-Substituted-3-Phenyl-1H-Pyrazole-4-Carbaldehydes (3)

To a solution of compound **2** (5mmol) in 20 mL of acetonitrile, K_2CO_3 (6mmol) and dimethyl sulphate or bromopropane or benzyl chloride (6 mmol) was added.The mixture was stirred and refluxed for 3-5 h. Upon completion, the solvent was removed, and the residue was extracted with dichloromethane (30 mL × 3). The combined organic layers were dried over anhydrous $MgSO_4$ and concentrated in vacuo to obtain a white crude solid of **3**, which was directly used in the next step without purification.

3.1.4. Synthesis of 2-((3-Phenyl-1H-Pyrazol-4-yl) Methylene) Hydrazine-1-Carboxamides (4a-4g)

To a solution of compound **2** (0.03 mol) in 10mL of 50% alcohol, hydrazinecarboxamide hydrochloride (0.03 mol) was added and then refluxed for 1 h. After cooling, the precipitation was filtered and recrystallized with 50% ethanol to give the pure compounds **4a-4g**.

3.1.5. Synthesis of 2-((3-Phenyl-1H-Pyrazol-4-yl) Methylene) Hydrazine-1-Carboxamide (4h)

To a solution of compound **2** (R_1 = H, 0.03 mol) in 10mL absolute alcohol, thiosemicarbazide (0.03 mol) was added and stirred for 2 h at room temperature. After cooling, the precipitation was filtered and recrystallized with ethanol to give the compound **4h**.

3.1.6. Synthesis of N-Phenyl-2-((3-Phenyl-1H-Pyrazol-4-yl) Methylene) Hydrazine-1- Carboxamides(5a-5l)

To a solution of compound **2** (0.03 mol) in 10mL absolute alcohol, *N*-phenylhydrazinecarboxamide (0.03 mol) was added and refluxed for 2 h. After cooling, the precipitation was filtered and recrystallized with ethanol to give the compound **5a-5l**.

3.1.7. Synthesis of 2-((1-Substituted-3-Phenyl-1H-Pyrazol-4-yl) Methylene) Hydrazine-1- Carboxamides (6a-6c)

To a solution of compound **3** (0.01 mol) in 10mL of 50% alcohol, hydrazinecarboxamide hydrochloride (0.01 mol) was added and then refluxed for 1 h. After cooling, the solvent was evaporated in *vacu*o, followed by the purification of the resulting residue by silica gel column chromatography (dichloromethane/methanol = 60/1) to generate a pure solid **6a-6c**.

3.1.8. Synthesis of 2-((1-Methyl-3-Phenyl-1H-Pyrazol-4-yl) Methylene) Hydrazine-1- Carbothioamides(6d-6f)

To a solution of compound **3** (R_1 = H, 0.01 mol) in 10mL absolute alcohol, thiosemicarbazide (0.01 mol) was added and stirred for 4 h at room temperature. After cooling, the precipitation was filtered and recrystallized with ethanol to give the compound **6q-6f**.

3.1.9. Synthesis of 2-((1-Substituted-3-Phenyl-1H-Pyrazol-4-yl) Methylene)-N-Phenylhydrazine-1-Carboxamides (7a-7c)

To a solution of compound **3** (0.01 mol) in 10mL absolute alcohol, *N*-phenylhydrazinecarboxamide (0.01 mol) was added and refluxed for 2 h. After cooling, the precipitation was filtered and recrystallized with ethanol to give the compound **7a-7c**.

3.1.9.1. 1-((3-Phenyl-1H-Pyrazol-4-yl) Methylidene) Semicarbazide (4a)

Mp 216-218°C, yield 82.1%. ^{1}H-NMR (DMSO-d_6, 300MHz): δ 6.33 (s, 2H, NH_2), 7.41-7.57 (m, 6H, Ph-H, N-NH), 7.94 (s, 1H, N-CH), 8.16 (s, 1H, CH=N), 9.95 (s, 1H, CONH). ^{13}C-NMR (DMSO-d_6,75MHz): δ 157.19, 145.19, 134.25, 133.54, 131.38, 129.23, 128.73, 128.47, 114.92. ESI-HRMS calcd for $C11H12N5O^+$ ($[M + H]^+$): 230.1036; found: 230.1034.

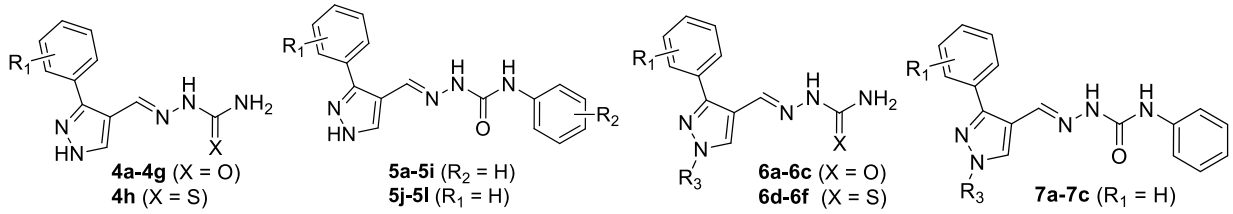
3.1.9.2. 1-((3-(4-Fluorophenyl)-1H-Pyrazol-4-yl) Methylidene) Semicarbazide (4b)

Mp 196-200 ºC, yield 53.7%. ^{1}H-NMR (DMSO-d_6, 300MHz): δ 6.58 (s, 2H, NH_2), 7.29-7.62 (m, 4H, Ph-H), 7.92 (s, 1H, N-CH), 8.17 (s, 1H, CH=N), 10.00 (s, 1H, CONH). ^{13}C-NMR (DMSO-d_6,75MHz): δ 162.51 (d, $^1J_{c\text{-}f}$ = 243.8 Hz), 157.28, 144.80, 133.60, 130.61 (d, $^3J_{c\text{-}f}$ = 8.3 Hz), 128.24, 128.21, 116.11 (d, $^2J_{c\text{-}f}$ = 21.5 Hz), 114.87. ESI-HRMS calcd for $C11H11FN5O^+$ ($[M + H]^+$): 248.0942; found: 248.0935.

3.1.9.3. 1-((3-(4-Chlorophenyl)-1H-Pyrazol-4-yl) Methylidene) Semicarbazide (4c)

Mp 178-181°C, yield 66.8%. ^{1}H-NMR (DMSO-d_6, 300MHz): δ 5.09 (s, 2H, NH_2), 7.57 (q, 4H, J = 8.6 Hz,

Table 1. Inhibitory activity (MIC, μg/mL) of compounds 4a-4h、5a-5l、6a-6f、7a-7c against different bacterial and fungal strains.



Compound	R_1-	R_2-	R_3-	Gram-positive Strains						Gram-negative Strains				Fungj
				26003[a]	25923[b]	32067[c]	31968[d]	29212[e]	63501[f]	25922[g]	44568[h]	27853[i]	10104[j]	98001[k]
4a	H	—	—	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128
4b	F	—	—	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128
4c	Cl	—	—	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128
4d	Br	—	—	128	64	>128	>128	128	128	>128	>128	>128	>128	>128
4e	CH_3	—	—	>128	128	>128	>128	>128	128	>128	>128	>128	>128	>128
4f	OCH_3	—	—	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128
4g	OH	—	—	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128
4h	H	—	—	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128
5a	H	H	—	128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128
5b	F	H	—	128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128
5c	Cl	H	—	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128
5d	Br	H	—	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128
5e	CH_3	H	—	128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128
5f	OCH_3	H	—	128	64	>128	>128	>128	>128	>128	>128	>128	>128	>128
5g	OH	H	—	>128	>128	>128	8	>128	>128	>128	>128	>128	>128	>128
5h	2,4-2Cl	H	—	128	8	32	>128	32	128	>128	>128	>128	>128	>128
5i	NO_2	H	—	128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128
5j	H	F	—	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128
5k	H	Cl	—	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128
5l	H	OCH_3	—	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128
6a	H	—	CH_3	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128
6b	H	—	C_3H_7	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128
6c	H	—	$PhCH_2$-	128	128	>128	>128	>128	128	>128	>128	>128	>128	>128
6d	H	—	CH_3	128	64	>128	128	>128	128	>128	128	128	64	>128
6e	H	—	C_3H_7	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128
6f	H	—	$PhCH_2$-	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128
7a	H	—	CH_3	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128
7b	H	—	C_3H_7	128	64	>128	>128	64	128	128	128	128	64	64

(Table 1) contd…

Compound	R_1-	R_2-	R_3-	Gram-positive Strains						Gram-negative Strains				Fungj
				26003[a]	25923[b]	32067[c]	31968[d]	29212[e]	63501[f]	25922[g]	44568[h]	27853[i]	10104[j]	98001[k]
7c	H	—	$PhCH_2$-	128	64	>128	>128	128	128	>128	>128	128	128	128
Penicillin	—	—	—	0.25	0.25	>128	>128	128	128	128	>128	>128	32	16
Norfloxacin	—	—	—	0.25	0.25	>128	>128	1	2	0.25	0.25	2	4	1
Gatifloxacin	—	—	—	0.25	0.25	>128	>128	1	2	0.25	0.25	2	2	1
Moxifloxacin	—	—	—	0.25	0.25	>128	>128	1	2	0.25	0.25	2	4	0.5
Oxacillin	—	—	—	0.25	0.25	>128	>128	128	>128	128	>128	>128	128	128

[a]*Staphylococcus aureus* CMCC(B)26003; [b]*Staphylococcus aureus* CMCC 25923; [c] *Streptococcus pyogenes* CMCC 32067; [d]*Streptococcus pneumoniae* CMCC 31968 [e]*Enterococcus faecalis* CMCC 29212; [f]*Bacillus subtilis* CMCC 63501; [g]*Escherichia coli* CMCC 25922; [h]*Escherichia coli* CMCC 44568;[i]*Pseudomonas aeruginosa* CMCC 27853; [j]*Pseudomonas aeruginosa* CMCC 10104; [k]*Candida albicans* CMCC 98001.

Ph-H), 7.94 (s, 1H, N-CH), 8.18 (s, 1H, CH=N), 9.97 (s, 1H, CONH). ^{13}C-NMR (DMSO-d_6,75MHz): δ157.20, 144.76, 133.46, 133.39, 133.33, 130.77, 130.20, 129.19, 115.09. ESI-HRMS calcd for $C11H11ClN5O^+$ ($[M + H]^+$): 264.0647; found: 264.0642.

3.1.9.4. 1-((3-(4-Bromophenyl)-1H-Pyrazol-4-yl) Methylidene) Semicarbazide (4d)

Mp 200-203°C, yield 54.3%. ^{1}H-NMR (DMSO-d_6, 300MHz): δ 5.04 (s, 2H, NH_2), 7.53 (d, 2H, J = 8.5 Hz, Ph-H), 7.68 (d, 2H, J = 8.5 Hz, Ph-H), 7.94 (s, 1H, N-CH), 8.18 (s, 1H, CH=N), 9.94 (s, 1H, CONH). ^{13}C-NMR (DMSO-d_6,75MHz): δ157.17, 144.83, 133.39, 133.27, 132.11, 131.14, 130.47, 121.99, 115.10. ESI-HRMS calcd for $C11H11BrN5O^+$ ($[M + H]^+$): 308.0141; found: 308.0132.

3.1.9.5. 1-((3-(4-Methylphenyl)-1H-pyrazol-4-yl) Methylidene) Semicarbazide (4e)

Mp 243-246°C, yield 44.2%. ^{1}H-NMR (DMSO-d_6, 300MHz): δ 2.37 (s, 3H, CH_3), 6.29 (s, 2H, NH_2), 7.30 (d, 2H, J = 7.9 Hz, Ph-H), 7.44 (d, 2H, J = 7.9 Hz, Ph-H), 7.93 (s, 1H, N-CH), 8.12 (s, 1H, CH=N), 9.91 (s, 1H, CONH). ^{13}C-NMR (DMSO-d_6,75MHz): δ157.21, 138.26, 133.70, 129.80, 129.73, 128.96, 128.33, 120.13, 114.70, 21.27. ESI-HRMS calcd for $C12H14N5O^+$ ($[M + H]^+$): 244.1193; found: 244.1199.

3.1.9.6. 1-((3-(4-Methoxylphenyl)-1H-Pyrazol-4-yl) Methylidene) Semicarbazide (4f)

Mp 239-241°C, yield 67.8%. ^{1}H-NMR (DMSO-d_6, 300MHz): δ 3.82 (s, 3H, OCH_3), 6.25 (s, 2H, NH_2), 7.06 (d, 2H, J = 8.4 Hz, Ph-H), 7.49 (d, 2H, J = 8.4 Hz, Ph-H), 7.92 (s, 1H, N-CH), 8.09 (s, 1H, CH=N), 9.90 (s, 1H, CONH). ^{13}C-NMR (DMSO-d_6,75MHz): δ159.84, 157.23, 144.51, 134.77, 133.80, 129.79, 123.49, 114.70, 114.44, 55.73. ESI-HRMS calcd for $C12H14N5O2^+$ ($[M + H]^+$): 260.1142; found: 260.1134.

3.1.9.7. 1-((3-(4-Hydroxyphenyl)-1H-Pyrazol-4-yl) Methylidene) Semicarbazide (4g)

Mp 266-272°C, yield 54.2%. ^{1}H-NMR (DMSO-d_6, 300MHz): δ 6.32 (s, 2H, NH_2), 6.89 (d, 2H, J = 8.5 Hz, Ph-H), 7.36 (d, 2H, J = 8.5 Hz, Ph-H), 7.90 (s, 1H, N-CH), 8.06 (s, 1H, CH=N), 9.91 (s, 1H, CONH). ^{13}C-NMR (DMSO-d_6,75MHz): δ158.23, 157.28, 144.54, 135.12, 134.07, 129.81, 121.55, 116.07, 114.14. ESI-HRMS calcd for $C11H12N5O2^+$ ($[M + H]^+$): 246.0986; found: 246.0995.

3.1.9.8. 1-((3-Phenyl-1H-Pyrazol-4-yl) Methylidene) Thiosemicarbazide (4h)

Mp 216-219°C, yield 64.4%. ^{1}H-NMR (DMSO-d_6, 300MHz): δ7.43-7.56 (m, 6H, Ph-H, N-NH), 7.67 (s, 1H, NH_2), 7.97 (s, 1H, NH_2), 8.18 (s, 1H, N-CH), 8.23 (s, 1H, CH=N), 11.16 (s, 1H, NH). ^{13}C-NMR (DMSO-d_6,75MHz): δ177.54, 137.14, 134.70, 130.90, 129.34, 129.23, 129.04, 128.49, 114.27. ESI-HRMS calcd for $C11H12N5S^+$ ($[M + H]^+$): 246.0808; found: 246.0815.

3.1.9.9. N-Phenyl-2-((3-Phenyl-1H-Pyrazol-4-yl) Methylene) Hydrazinecarboxamide (5a)

Mp 200-223°C, yield 54%. ^{1}H-NMR (DMSO-d_6, 300MHz): δ 6.99-7.63 (m, 10H, Ph-H), 8.05 (s, 1H, N-CH), 8.37 (s, 1H, CONH), 8.63 (s, 1H, CH=N), 10.45 (s, 1H, CONH), 13.28 (s, 1H, pyrazol-NH). ^{13}C-NMR (DMSO-d_6,75MHz): δ153.34, 139.58, 135.02, 133.99, 133.64,129.28, 128.96, 128.83, 128.82, 128.58, 122.77, 119.89, 114.53. ESI-HRMS calcd for $C17H16N5O^+$ ($[M + H]^+$): 306.1349; found: 306.1345.

3.1.9.10. N-Phenyl-2-((3-(4-Fluorophenyl)-1H-Pyrazol-4-yl) Methylene)Hydrazinecarboxamide(5b)

Mp 226-230°C, yield 60.8%. ^{1}H-NMR (DMSO-d_6, 300MHz): δ 6.99-7.67 (m, 9H, Ph-H), 8.03 (s, 1H, N-CH), 8.34 (s, 1H, CONH), 8.55 (s, 1H, CH=N), 10.39 (s, 1H, CONH). ^{13}C-NMR (DMSO-d_6,75MHz): δ162.58 (d, $^1J_{c\text{-}f}$ = 244.1 Hz), 153.32, 139.56, 135.54, 134.88, 134.08, 130.72 ($^3J_{c\text{-}f}$ = 8.3 Hz), 128.97, 128.35, 122.78, 119.85, 116.16 ($^2J_{c\text{-}f}$ = 21.5 Hz), 114.54. ESI-HRMS calcd for $C17H15FN5O^+$ ($[M + H]^+$): 324.1255; found: 324.1265.

3.1.9.11. N-Phenyl-2-((3-(4-Chlorophenyl)-1H-Pyrazol-4-yl) Methylene) Hydrazinecarboxamide(5c)

Mp 207-210°C, yield 79.1%. ^{1}H-NMR (DMSO-d_6, 300MHz): δ 7.00 (t, 1H, J = 7.3 Hz, Ph-H), 7.29 (t, 2H, J =

7.9 Hz, Ph-H), 7.55-7.65 (m, 6H, Ph-H), 8.05 (s, 1H, N-CH), 8.36 (s, 1H, CONH), 8.57 (s, 1H, CH=N), 10.49 (s, 1H, CONH). ^{13}C-NMR (DMSO-d_6,75MHz): δ153.28, 139.55, 134.83, 133.87, 133.46, 130.35, 129.21, 129.17, 128.99, 128.92, 122.75, 119.74, 114.68. ESI-HRMS calcd for $C17H15ClN5O^+$ ($[M + H]^+$): 340.0960; found: 340.0953.

3.1.9.12. N-Phenyl-2-((3-(4-Bromophenyl)-1H-Pyrazol-4-yl) Methylene) Hydrazinecarboxamide (5d)

Mp 220-223°C, yield 46.4%. ^{1}H-NMR (DMSO-d_6, 300MHz): δ 7.01 (t, 1H, J = 7.3 Hz, Ph-H), 7.30 (t, 2H, J = 7.8 Hz, Ph-H), 7.55-7.73 (m, 6H, Ph-H), 8.03 (s, 1H, N-CH), 8.37 (s, 1H, CONH), 8.52 (s, 1H, CH=N), 10.45 (s, 1H, CONH), 13.31 (s, 1H, pyrazol-NH). ^{13}C-NMR (DMSO-d_6,75MHz): δ153.29, 139.49, 134.75, 132.14, 130.66, 129.09, 129.02, 122.80, 122.13, 121.83, 119.77, 118.58, 114.69. ESI-HRMS calcd for $C17H15BrN5O^+$ ($[M + H]^+$): 384.0454; found: 384.0467.

3.1.9.13. N-Phenyl-2-((3-(4-Methylphenyl)-1H-Pyrazol-4-yl) Methylene) Hydrazinecarboxamide(5e)

Mp 200-201°C, yield 30.9%. ^{1}H-NMR (DMSO-d_6, 300MHz): δ2.38 (s, 3H, CH_3), 7.01 (t, 1H, J = 7.3 Hz, Ph-H), 7.27-7.60 (m, 8H, Ph-H), 8.04 (s, 1H, N-CH), 8.30 (s, 1H, CONH), 8.56 (s, 1H, CH=N), 10.41 (s, 1H, CONH), 13.25 (s, 1H, pyrazol-NH). ^{13}C-NMR (DMSO-d_6,75MHz): δ153.35, 139.56, 135.11, 129.85, 129.83, 129.02, 128.97, 128.49, 128.42, 122.76, 119.82, 118.59, 114.33, 21.29. ESI-HRMS calcd for $C18H18N5O^+$ ($[M + H]^+$): 320.1506; found: 320.1497.

3.1.9.14. N-Phenyl-2-((3-(4-Methoxylphenyl)-1H-Pyrazol-4-yl) Methylene) Hydrazinecarboxamide(5f)

Mp 193°C, yield 72.3%. ^{1}H-NMR (DMSO-d_6, 300MHz): δ3.82 (s, 3H, CH_3), 7.01 (t, 1H, J = 7.3 Hz, Ph-H), 7.08 (d, 2H, J = 8.6 Hz, Ph-H), 7.29 (t, 2H, J = 7.8 Hz, Ph-H), 7.52 (d, 2H, J = 8.6 Hz, Ph-H), 7.59 (d, 2H, J = 8.2 Hz, Ph-H), 8.02 (s, 1H, N-CH), 8.27 (s, 1H, CONH), 8.55 (s, 1H, CH=N), 10.38 (s, 1H, CONH). ^{13}C-NMR (DMSO-d_6,75MHz): δ159.90, 153.35, 139.57, 135.29, 135.22, 129.99, 129.93, 129.08, 128.96, 122.76, 119.83, 114.72, 114.07, 55.73. ESI-HRMS calcd for $C18H18N5O2^+$ ($[M + H]^+$): 336.1455; found: 336.1459.

3.1.9.15. N-Phenyl-2-((3-(4-Hydroxyphenyl)-1H-Pyrazol-4-yl) Methylene) Hydrazinecarboxamide(5g)

Mp 237-240°C, yield 63.5%. ^{1}H-NMR (DMSO-d_6, 300MHz): δ 6.91 (d, 2H, J = 8.2 Hz, Ph-H), 7.00 (t, 1H, J = 7.3 Hz, Ph-H), 7.29 (t, 2H, J = 7.6 Hz, Ph-H), 7.40 (d, 2H, J = 8.2 Hz, Ph-H), 7.62 (d, 2H, J = 7.9 Hz, Ph-H), 8.01 (s, 1H, N-CH), 8.26 (s, 1H, CONH), 8.61 (s, 1H, CH=N), 9.87 (s, 1H, OH), 10.39 (s, 1H, CONH), 13.16 (s, 1H, pyrazol-NH). ^{13}C-NMR (DMSO-d_6,75MHz): δ158.31, 153.38, 139.60, 135.44, 135.38, 129.94, 129.82, 128.97, 122.74, 122.66, 119.86, 116.09, 113.81. ESI-HRMS calcd for $C17H16N5O2^+$ ($[M + H]^+$): 322.1299; found: 322.1304.

3.1.9.16. N-Phenyl-2-((3-(2,4-Dichlorophenyl)-1H-Pyrazol-4-yl) Methylene) Hydrazinecarboxamide(5h)

Mp 198-201°C, yield 50.4%. ^{1}H-NMR (DMSO-d_6, 300MHz): δ 7.01 (t, 1H, J = 7.0 Hz, Ph-H), 7.29 (t, 2H, J = 7.7 Hz, Ph-H), 7.43-7.79 (m, 6H, Ph-H, N-CH), 7.99 (s, 1H, CONH), 8.39 (s, 1H, CH=N), 10.45 (s, 1H, CONH), 13.39 (s, 1H, pyrazol-NH). ^{13}C-NMR (DMSO-d_6,75MHz): δ153.10, 146.41, 146.34, 139.20, 134.58, 134.52, 134.43, 134.00, 130.00, 129.46, 129.11, 127.87, 122.86, 119.51, 115.93. ESI-HRMS calcd for $C17H14Cl2N5O^+$ ($[M + H]^+$): 374.0570; found: 374.0566.

3.1.9.17. N-Phenyl-2-((3-(4-Nitrophenyl)-1H-Pyrazol-4-yl) Methylene) Hydrazinecarboxamide(5i)

Mp 233-236°C, yield 45%. ^{1}H-NMR (DMSO-d_6, 300MHz): δ 7.01 (t, 1H, J = 7.2 Hz, Ph-H), 7.28 (t, 2H, J = 7.7 Hz, Ph-H), 7.56 (d, 2H, J = 8.0 Hz, Ph-H), 7.93 (d, 2H, J = 8.5 Hz, Ph-H), 8.11 (s, 1H, N-CH), 8.34 (d, 2H, J = 8.5 Hz, Ph-H), 8.48 (s, 1H, CONH), 8.57 (s, 1H, CH=N), 10.54 (s, 1H, CONH). ^{13}C-NMR (DMSO-d_6,75MHz): δ153.26, 147.31, 139.48, 139.34, 134.42, 129.53, 129.04, 128.98, 124.32, 124.24, 122.83, 119.79, 115.59. ESI-HRMS calcd for $C17H15N6O3^+$ ($[M + H]^+$): 351.1200; found: 351.1200.

3.1.9.18. N-(4-Fluorophenyl)-2-((3-Phenyl-1H-Pyrazol-4-yl) Methylene) Hydrazinecarboxamide (5j)

Mp 236-240°C, yield 41.4%. ^{1}H-NMR (DMSO-d_6, 300MHz): δ 7.13 (t, 2H, J = 8.9 Hz, Ph-H), 7.45-7.65 (m, 7H, Ph-H), 8.01 (s, 1H, N-CH), 8.35 (s, 1H, CONH), 8.69 (s, 1H, CH=N), 10.42 (s, 1H, CONH), 13.18 (s, 1H, pyrazol-NH). ^{13}C-NMR (DMSO-d_6,75MHz): δ158.15 (d, $^1J_{c\text{-}f}$ = 237.0 Hz), 153.48, 135.96 ($^4J_{c\text{-}f}$ = 2.3 Hz), 135.05, 129.29, 128.84, 128.80, 128.55, 128.49, 121.79 ($^3J_{c\text{-}f}$ = 7.7 Hz), 121.42, 115.43 ($^2J_{c\text{-}f}$ = 22.0 Hz), 114.56. ESI-HRMS calcd for $C17H15FN5O^+$ ($[M + H]^+$): 324.1255; found: 324.1259.

3.1.9.19. N-(4-Chlorophenyl)-2-((3-Phenyl-1H-Pyrazol-4-yl) Methylene) Hydrazinecarboxamide (5k)

Mp 237-240°C, yield 78.2%. ^{1}H-NMR (DMSO-d_6, 300MHz): δ 7.34 (t, 2H, J = 8.7 Hz, Ph-H), 7.48-7.59 (m, 5H, Ph-H), 7.67 (t, 2H, J = 8.8 Hz, Ph-H), 8.05 (s, 1H, N-CH), 8.36 (s, 1H, CONH), 8.78 (s, 1H, CH=N), 10.50 (s, 1H, CONH), 13.43 (s, 1H, pyrazol-NH). ^{13}C-NMR (DMSO-d_6,75MHz): δ153.28, 138.68, 135.30, 129.36, 129.30, 128.85, 128.79, 128.74, 128.56, 128.50, 126.34, 121.42, 114.49. ESI-HRMS calcd for $C17H15ClN5O^+$ ($[M + H]^+$): 340.0960; found: 340.0952.

3.1.9.20 N-(4-Methoxyphenyl)-2-((3-Phenyl-1H-Pyrazol-4-yl) Methylene) Hydrazinecarboxamide (5l)

Mp 210-212°C, yield 61.6%. ^{1}H-NMR (DMSO-d_6, 300MHz): δ 3.74 (s, 3H, CH_3), 6.88 (d, 2H, J = 8.9 Hz, Ph-H), 7.45-7.61 (m, 7H, Ph-H), 8.05 (s, 1H, N-CH), 8.34 (s, 1H, CONH), 8.49 (s, 1H, CH=N), 10.33 (s, 1H, CONH), 13.05 (s, 1H, pyrazol-NH). ^{13}C-NMR (DMSO-d_6,75MHz): δ155.29, 153.59, 134.68, 132.61, 131.45, 129.27, 129.18, 128.87, 128.81, 128.56, 121.81, 114.63, 114.15, 55.64. ESI-HRMS calcd for $C18H18N5O2^+$ ($[M + H]^+$): 336.1455; found: 336.1451.

3.1.9.21. 1-((1-Methyl-3-Phenyl-1H-Pyrazol-4-yl) Methylidene) Semicarbazide (6a)

Mp 221-222°C, yield 46.2%. ^{1}H-NMR (DMSO-d_6, 300MHz): δ3.72 (s, 3H, NCH_3), 6.23 (s, 2H, NH_2), 7.44-7.56

(m, 5H, Ph-H), 7.57 (s, 1H, N-CH), 7.99 (s, 1H, CH=N), 9.83 (s, 1H, NH). ^{13}C-NMR (DMSO-d_6,75MHz): δ156.01, 140.87, 135.49, 131.84, 129.24, 128.47, 128.27, 127.93, 115.72, 36.39. ESI-HRMS calcd for $C_{12}H_{14}N_5O^+$ ([M + H]$^+$): 244.1193; found: 244.1189.

3.1.9.22. 1-((3-Phenyl-1-Propyl-1H-Pyrazol-4-yl) Methylidene) Semicarbazide (6b)

Mp156-161°C, yield 56.3%. ^{1}H-NMR (DMSO-d_6, 300MHz): δ 0.89 (t, 3H, J = 7.3 Hz, CH_3), 1.82-1.89 (m, 2H, CH_2), 4.11 (t, 2H, J = 6.7 Hz, NCH_2), 6.27 (s, 2H, NH_2), 7.39-7.58 (m, 5H, Ph-H), 7.94 (s, 1H, N-CH), 8.27 (s, 1H, CH=N), 9.93 (s, 1H, NH). ^{13}C-NMR (DMSO-d_6,75MHz): δ157.19, 149.37, 133.55, 133.47, 130.22, 128.98, 128.42, 128.26, 115.29, 53.76, 23.46, 11.43. ESI-HRMS calcd for $C_{14}H_{18}N_5O^+$ ([M + H]$^+$): 272.1506; found: 272.1498.

3.1.9.23. 1-((1-Benzyl-3-Phenyl-1H-Pyrazol-4-yl) Methylidene) Semicarbazide (6c)

Mp 143-144°C, yield 66.0%. ^{1}H-NMR (DMSO-d_6, 300MHz): δ 5.39 (s, 2H, CH_2), 5.77 (s, 2H, NH_2), 7.32-7.57 (m, 10H, Ph-H), 7.92 (s, 1H, N-CH), 8.37 (s, 1H, CH=N), 9.96 (s, 1H, NH). ^{13}C-NMR (DMSO-d_6,75MHz): δ157.15, 149.85, 137.44, 133.42, 133.32, 130.54, 129.12, 129.02, 128.48, 128.39, 128.31, 128.20, 115.95, 55.78. ESI-HRMS calcd for $C_{18}H_{18}N_5O^+$ ([M + H]$^+$): 320.1506; found: 320.1511.

3.1.9.24. 1-((1-Methyl-3-Phenyl-1H-Pyrazol-4-yl) Methylidene) Thiosemicarbazide (6d)

Mp 168°C, yield 45.2%. ^{1}H-NMR (DMSO-d_6, 300MHz): δ 3.90 (s, 3H, CH_3), 7.40-7.57 (m, 5H, Ph-H), 7.61 (s, 1H, NH_2), 8.07 (s, 1H, NH_2), 8.17 (s, 1H, N-CH), 8.32 (s, 1H, CH=N), 11.19 (s, 1H, NH). ^{13}C-NMR (DMSO-d_6,75MHz): δ177.74, 150.33, 136.71, 133.12, 131.64, 129.06, 128.47, 128.41, 115.03, 55.87.ESI-HRMS calcd for $C_{12}H_{14}N_5S^+$ ([M + H]$^+$): 260.0964; found: 260.0960.

3.1.9.25. 1-((3-Phenyl-1-Propyl-1H-Pyrazol-4-yl) Methylidene) Thiosemicarbazide (6e)

Mp 179-180°C, yield 60.3%. ^{1}H-NMR (DMSO-d_6, 300MHz): δ 0.88 (t, 3H, J = 7.3 Hz, CH_3), 1.82-1.89 (m, 2H, CH_2), 4.11 (t, 2H, J = 6.8 Hz, NCH_2), 7.40-7.58 (m, 5H, Ph-H), 7.65 (s, 1H, NH_2), 8.09 (s, 1H, NH_2), 8.19 (s, 1H, N-CH), 8.38 (s, 1H, CH=N), 11.20 (s, 1H, NH). ^{13}C-NMR (DMSO-d_6,75MHz): δ177.74, 150.19, 136.74, 133.24, 130.75, 129.04, 128.54, 128.43, 114.78, 53.89, 23.40, 11.43. ESI-HRMS calcd for $C_{14}H_{18}N_5S^+$ ([M + H]$^+$): 288.1277; found: 288.1273.

3.1.9.26. 1-((1-Benzyl-3-Phenyl-1H-Pyrazol-4-yl) Methylidene) Thiosemicarbazide (6f)

Mp 146-147°C, yield 46.5%. ^{1}H-NMR (DMSO-d_6, 300MHz): δ 5.40 (s, 2H, CH_2), 7.68 (s, 1H, NH_2), 7.33-7.58 (m, 10H, Ph-H), 8.10 (s, 1H, NH_2), 8.18 (s, 1H, N-CH), 8.47 (s, 1H, CH=N), 11.21 (s, 1H, NH). ^{13}C-NMR (DMSO-d_6,75MHz): δ177.77, 150.62, 137.27, 136.61, 133.01, 131.05, 129.15, 129.07, 128.56, 128.49, 128.37, 128.23, 115.43, 55.90. ESI-HRMS calcd for $C_{18}H_{18}N_5S^+$ ([M + H]$^+$): 336.1277; found: 336.1274.

3.1.9.27. N-Phenyl-2-((1-Methyl-3-Phenyl-1H-Pyrazol-4-yl) Methylene) Hydrazinecarboxamide (7a)

Mp 187-191°C, yield 68.2%. ^{1}H-NMR (DMSO-d_6, 300MHz): δ 3.94 (s, 3H, NCH_3), 7.01 (t, 1H, J = 7.2 Hz, Ph-H), 7.30 (t, 2H, J = 7.5 Hz, Ph-H), 7.42-7.60 (m, 7H, Ph-H), 8.03 (s, 1H, N-CH), 8.40 (s, 1H, CONH), 8.55 (s, 1H, CH=N), 10.43 (s, 1H, CONH). ^{13}C-NMR (DMSO-d_6,75MHz): δ153.30, 149.83, 139.53, 134.83, 133.40, 131.70, 129.06, 129.00, 128.50, 128.40, 122.80, 119.79, 115.19, 55.36.ESI-HRMS calcd for $C_{18}H_{18}N_5O^+$ ([M + H]$^+$): 320.1506; found: 320.1510.

3.1.9.28. N-Phenyl-2-((1-Propyl-3-Phenyl-1H-Pyrazol-4-yl) Methylene) Hydrazinecarboxamide (7b)

Mp 163-164°C, yield 57.2%. ^{1}H-NMR (DMSO-d_6, 300MHz): δ0.91 (t, 3H, J = 7.3 Hz, CH_3), 1.82-1.92 (m, 2H, CH_2), 4.14 (t, 2H, J = 6.9 Hz, NCH_2), 7.01 (t, 1H, J = 7.3 Hz, Ph-H), 7.27-7.61 (m, 9H, Ph-H), 8.04 (s, 1H, N-CH), 8.44 (s, 1H, CONH), 8.58 (s, 1H, CH=N), 10.45 (s, 1H, CONH). ^{13}C-NMR (DMSO-d_6,75MHz): δ153.32, 149.76, 139.52, 134.95, 133.49, 130.81, 129.05, 129.00, 128.54, 128.38, 122.81, 119.83, 114.88, 53.84, 23.55, 11.44. ESI-HRMS calcd for $C_{20}H_{22}N_5O^+$ ([M + H]$^+$): 348.1819; found: 348.1814.

3.1.9.29. N-Phenyl-1-((1-Benzyl-3-Phenyl-1H-Pyrazol-4-yl) Methylidene) Semicarbazide (7c)

Mp 201-203°C, yield 56.5 %. ^{1}H-NMR (DMSO-d_6, 300MHz): δ 5.43 (s, 2H, NCH_2), 7.01 (t, 1H, J = 6.5 Hz, Ph-H), 7.30-7.59 (m, 14H, Ph-H), 8.05 (s, 1H, N-CH), 8.54 (s, 1H, CONH), 8.59 (s, 1H, CH=N), 10.46 (s, 1H, CONH). ^{13}C-NMR (DMSO-d_6,75MHz): δ 153.31, 150.23, 139.51, 137.44, 134.80, 133.26, 131.18, 129.14, 129.08, 128.99, 128.60, 128.51, 128.32, 128.14, 122.82, 119.87, 115.52, 55.81. ESI-HRMS calcd for $C_{24}H_{22}N_5O^+$ ([M + H]$^+$): 396.1819; found: 396.1819.

3.2. Pharmacology

3.2.1. Cell Line and Reagents

Mouse RAW264.7 macrophage cell line was obtained from the China Cell Bank (Beijing, China). Fetal bovine serum was purchasedfrom Biological Industries (Kibbutz Beit-Haemek, Israel). LPS waspurchased from Sigma (St. Louis, MO, USA), and was dissolved in PBS. DXMS and compounds were dissolved in DMSO for *in vitro* experiments.

3.2.2. Cell Treatment and ELISA Assay (TNF-α)

Mouse RAW264.7 macrophages were cultured in RPMI 1640 medium (Zhejiang Tianhang Biotechnology Co.,Ltd.) supplemented with 10% FBS, 100 U/mL penicillin, and 100 μg/mL streptomycin at 37 C with 5% CO_2. Cells were pre-treated with test compounds (20 μg/mL), DXMS (20 μg/mL) or vehicle control for 4 h, then treated with LPS (1μg/ml) for 24 h. After treatment, the supernatant was separated and used

for the determination of levels of TNF-α by ELISA using mouse TNF-α ELISA Kits Biolegend (San diego, CA, USA).

3.2.3. Xylene-induced Ear-edema Model

In this screening, selected compounds were evaluated to evaluate their anti-inflammatory activity *via in vivo* inhibition assay of xylene-induced ear edema in mice [21, 22]. Compounds and DXMS were dissolved in DMSO and administered to mice intraperitoneally at a dose of 50 mg/kg (0.05 mL/20 g body weight). Control group received vehicle only. Thirty minutes after administration, mice were used in the xylene-induced ear-edema test (20 μL xylene was daubed by a micropipette on the surface of the right ear of each mouse). After 30 min, mice were sacrificed, and a cylindrical tissue plug (7 mm diameter) was excised from both ears of mice. Edema was quantified by measuring the difference in weight between the plugs from the right and left ears. Anti-inflammatory activity was expressed as the percent reduction in edema in comparison with the control group. The DXMS was tested in parallel as a reference drug.

3.2.4. Evaluation of Anti-bacterial Activity In Vitro

The anti-bacterial activity *in vitro* against six gram-positive strains (*S. aureus* (CMCC(B) 26003 and CMCC 25923), *S. pyogenes* CMCC 32067, *S. pneumoniae* CMCC 31968, *Enterococcusfaecalis* CMCC 29212, and Bacillus subtilis CMCC 63501), four gram-negative strains (*E. coli* (CMCC 25922 and CMCC 44568) and *P. aeruginosa* (CMCC 27853 and CMCC 10104)), and a Fungistrain (Candida albicans CMCC 98001) was evaluated using a two-fold serial dilution technique [23]. Test bacteria were grown to mid-log phase in Mueller-Hinton broth (MHB) or Tryptone Soya Broth (TSB) and diluted 1000-fold in the same medium. The bacteria of 10^5 CFU/mL were inoculated into MHB or TSB and dispensed at 200 μL/well in a 96-well microtiter plate. Penicillin, norfloxacin, gatifloxacin, moxifloxacin, and oxacillin were used as positive controls. The inhibition activities of test compounds and positive drugs were expressed as MIC value, which was defined as the concentration of a test sample that completely inhibited bacteria growth during 24 h incubation at 37 °C. Bacteria growth was determined by measuring the absorption at 630 nm using a microtiter enzyme-linked immunosorbent assay (ELISA) reader. All experiments were carried out three times in parallel.

4. RESULTS AND DISCUSSION

4.1. Anti-inflammatory Activity

4.1.1. In Vitro Inhibition LPS-induced TNF-a Release

Lipopolysaccharide (LPS), an endotoxin of gram-negative bacterial cell walls, is thought to be a major risk factor in initiating the inflammatory processes by stimulating the release of inflammatory cytokines, such as tumor necrosis factor (TNF-α) [24]. The LPS-induced TNF-α release model was considered to be the effective screening method for new anti-inflammatory agents *in vitro*. In the current study, compounds (**4a-4h**, **5a-5l**, **6a-f**, and **7a-7c**) were evaluated for their anti-inflammatory activity based on their ability to inhibit TNF-α release in LPS-stimulated RAW264.7 mouse macrophages. The cells were seeded and pretreated with the test compounds or dexamethasone (DXMS) at a concentration 20 μg/mL for 4 h before treatment with LPS (1 μg/mL) for 24 h at 37 °C. Cell-free supernatants were collected and analyzed for levels of TNF-α using an ELISA kit. The screen results of target compounds and positive control DXMS are shown in Fig. (**1**).

As shown in Fig. (**1**), most of the tested compounds significantly inhibited LPS-induced TNF-α generation *in vitro*.

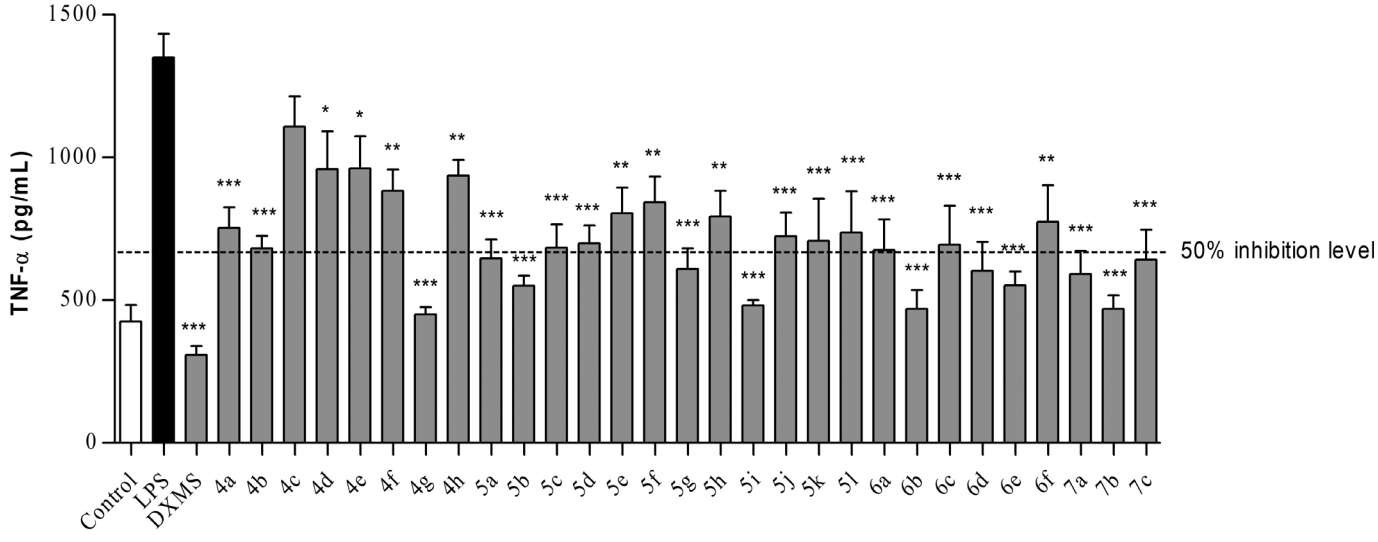


Fig. (1). Anti-inflammatory activities of the compounds **4a-4h**, **5a-5l**, **6a-f**, and **7a-7c** in the LPS-induced TNF-a release test in RAW264.7 macrophage. The results are expressed as the level of TNF-α. Each bar represents mean ± SEM of three parallel experiments. Values are significant at $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ as compared to the LPS group (all comparisons were made by ANOVA followed by Dunnett's test).

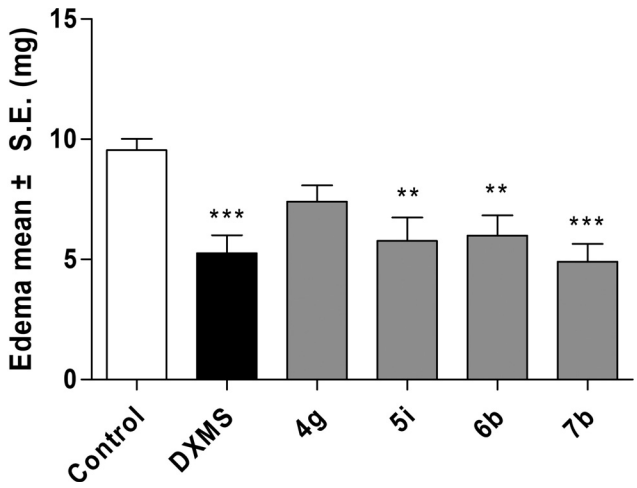


Fig. (2). Anti-inflammatory activities of the compounds **4g**, **5i**, **6b**, and **7b** in the xylene-induced ear-edema test in mice. The test compounds and DXMS were administered *i.e.* at a dose of 50 mg/kg, and edema was quantified after30 min. ** $p<0.01$ and *** $p<0.001$compared with the control group (all comparisons were made by ANOVA followed by Dunnett's test).

Among these compounds, **4g**, **5a**, **5b**, **5g**, **5i**, **6b**, **6d**, **6e**, **7a**, **7b**, and **7c** exhibited strong inhibition (inhibitory rates>50%). Compounds **4g**, **5i**, **6b**, and **7b** showed 66.6%, 64.3%, 65.2%, and 65.2% inhibition, respectively. The positive control drug DXMS exhibited an inhibition rate of 77.2% at the same concentration (20 μg/mL).

The series of compounds **4a-4g** showed relatively weaker anti-inflammatory activity than the other three series of compounds. SARs of this series indicated that the fluorine and hydroxyl moieties could significantly increase the cytokine-inhibitory ability but other substituents such as Cl, Br, CH_3, and OCH_3 groups markedly decrease the inhibitory ability. The replacement of =O with =S in compound **4a**yielded compound **4h**, which did not show markedly improved cytokine-inhibitory ability. The introduction of aryl groups at the terminal *N* atom in series **4** produced compounds **5a-5l**. In this series, compounds **5a**, **5b**, **5g**, and **5i**, containing hydrogen, fluorine, hydroxyl, and nitro groups, respectively, exhibited better inhibitory ability compared to the others. This was similar to the results obtained for series **4**. In addition, the introduction of substituents at the R_2 position lowered the anti-inflammatory activity, such as in compounds **5j**-**5l**. The introduction of alkyl groups to the pyrazole ring in the compounds **4a** and **4h** produced compounds **6a-6c** and **6d-6f**, respectively. From scheme **1**, it can be observed that this modification enhanced the cytokine-inhibitory ability, and the compounds with methyl and propyl groups (**6a**, **6b**, **6d**, **6e**) showed better inhibitory activity than the benzyl-substituted compounds (**6c** and **6f**). Similarly, the introduction of alkyl groups at the pyrazole ring in compound **5a** gave compounds **7a-7c**, in which compound **7b**, with a propyl group, exhibited the best activity.

4.1.2. In Vivo Inhibition of Xylene-induced Ear Edema

Xylene-induced edema leads to an acute inflammatory response and is known to cause severe vasodilation and edematousskin changes, partially associated with phospholipase A2 (PLA2) [25, 26]. Xylene treatment in mice increases the release of inflammatory mediators, such as histamine, serotonin, bradykinin, and TNF-α. Based on the *in vitro* inhibitory effects of our test compounds toward TNF-α expression, the *in vivo* anti-inflammatory activity of four compounds, **4g**, **5i**, **6b**, and **7b**, were evaluated using thexylene-induced ear edema mouse model. In this test, dimethyl sulfoxide (DMSO) was used as the vehicle, and DXMS was used as the positive control. Anti-inflammatory activity was defined by the ability of each compound to prevent edema. As shown in Fig. (**2**), all four tested compounds and DXMS inhibited the ear edema, though the edema after treatment with compound **4g** was not significantly different from that in the control group. Compound **7b** inhibited edema by 48.63 %, which was more effective than DXMS at inhibiting xylene-induced ear edema (44.86 %). The *in vivo* activities of the tested compounds did not correlate with previous *in vitro* data. This may be due to the different bioavailability and pharmacokinetic profiles of these compounds. Compound **7b**, which has more lipophilic groups, showed better membrane permeability and higher *in vivo* activity.

4.2. Antimicrobial Activity

All test target compounds were evaluated for their *in vitro* anti-bacterial activity using a serial dilution method to obtain the minimum inhibitory concentration (MIC) against several gram-positive and gram-negative bacterial and fungal strains and fungi strain. Penicillin, Norfloxacin, Gatifloxacin, Moxifloxacin, and Oxacillin were used as positive control drugs.

The results of antimicrobial screening are described as MIC values and listed in Table **1**. Eleven of the tested compounds showed antimicrobial activity against different strains with MIC values ranging from 8 to 128 μg/mL.Compounds **4d**, **5a**, **5b**, **5e**, **5f**, **5g**, **5h**, **6c**, and **6d** exhibited moderate inhibition activity against gram-positive strains. Compounds **5g** and **5h** exhibited the most potent inhibitory activities, with an MIC value of 8 μg/mL against the *Streptococcus pneumoniae CMCC 31968* and *Staphylococcus aureus CMCC 25923*, respectively. Compounds **6d**, **7b** and **7c** inhibited more than seven bacterial strains, including gram-negative

strains, and were considered as broad-spectrum antimicrobial compounds. Additionally, compounds **7b** and **7c** showed inhibitory activities against the fungi *Candida albicans CMCC 98001*.

CONCLUSION

It can be concluded that pyrazoles, with (thio)-semicarbazone moieties, have the potential to be developed into new anti-inflammatory agents. A majority of synthesized compounds exhibited potent TNF-α inhibitory activity, and **5i**, **6b**, and **7b** were shown to have comparable *in vivo* anti-inflammatory activity to the reference drug, DXMS. On the other hand, compounds **6d**, **7b**,and **7c** displayed broad-spectrum antimicrobial activity against several gram-positive and gram-negative bacteria. Compound **7b** is worth investigating further because it exhibited both anti-inflammatory and antimicrobial activities and can be used as a lead compound for the development of a new class of dual anti-inflammatory-antimicrobial agents.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

After review of the procedure and aim of this study, local ethical committee approval was obtained.

HUMAN AND ANIMAL RIGHTS

No humans were used in this study. All Procedures involving animals and their care were conducted in accordance with the Guide for the Care and Use of Laboratory Animals, 8th Edition, National Academies Press, Washington DC.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The data that support the findings of this study are available from the corresponding author, [A.L. Pereora-Suarez], upon reasonable request.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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