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Original article

# Cyclization of some carbothioamide derivatives containing antipyrine and triazole moieties and investigation of their antimicrobial activities

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ABSTRACT

Acetohydrazide derivative containing both antipyrine and triazole nuclei (**5**) was obtained starting from ethyl hydrazinecarboxylate derivative (**2**) and 4-aminoantipyrine (**1**) by three steps. The treatment of compound **5** with $CS_2$ afforded the conversion of hydrazide function into 5-mercapto-1,3,4-oxadiazole ring leading to the formation of **7**. Then, **7** gave the product containing triazolotriazine moiety (**9**) by the reaction with hydrazine hydrate. The synthesis of the compounds incorporating the 1,3,4-thiadiazole (**10a–c**), 1,2,4-triazole (**11a–c**) or 1,3-thiazole (**12**, **13**) nucleus as third heterocycle was performed by the acidic or basic treatment of compounds **6a–c** which were obtained from the reaction of **5** with several isothiocyanates, or by the condensation of **6a** with two different phenacyl bromides, respectively.

The antimicrobial activity study revealed that all the compounds showed good activities except **3–5**.


## 1. Introduction

The treatment of infectious diseases still remains an important and challenging problem due to a combination of factors including emerging infectious diseases and the increasing number of multi-drug resistant microbial pathogens. In spite of wide range of antimicrobial drugs with different mechanisms of action used to treat with microbial infections either alone or in combination and also the existence many compounds used in different phases of clinic trials, microbial infections have been becoming a worldwide problem. There is already evidence that antimicrobial resistance associated with an increase in mortality. The problem with clinically used drugs is not only the increasing microbial resistance, but also they are accompanied by toxic side effects that are often dose limiting [1–5]. Moreover, the long term use of several drugs to treat microbial infections may cause serious health problems, especially in patients with impaired liver or kidney functions.

To search and synthesize of combinational chemotherapeutic drugs with different mechanisms of action and with low side effects constitute an important part of the methods that aims to overcome the antimicrobial resistance. Beside the development of completely new agents possessing chemical characteristics that clearly differ from those of existing ones, there is another approach containing to combine two or more pharmacophores into a single molecule. Therefore, a single molecule containing more than one pharmacophore, each with different mode of action, could be beneficial for the treatment of microbial infectious. These merged pharmacophore may be addressing the active site of different targets and offer the possibility to overcome drug resistance. In addition, this approach can also reduce unwanted side effects [6–11].

Since the discovery of antipyrine as the first pyrazolin-5-one derivative used in the treatment of pain and inflammation, a great deal has been devoted for the synthesis of pyrazole derivatives as potent anti-inflammatory, analgesic, antipyretic and antimicrobial agents [12–17]. Furthermore, the synthesis of 1,2,4-triazole derivatives possessing such diverse pharmacological properties as antimicrobial [18–21], anti-inflammatory [22], analgesic [23], antitumorial [24], antihypertensive [25], anticonvulsant and antiviral activities [26] has been attracting widespread attention. With regarding to antimicrobial activity, triazole moiety resembles structurally similar to imidazole molecule. Although triazole and imidazole nuclei act by the same mechanism of action, triazoles have been possess advantages over imidazoles, which have slow metabolic rate, oral bioavailability, and less effect on human sterol

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synthesis. For these reasons imidazoles have slowly being replaced by triazole molecules. It was reported that incorporation of various halo substituents into the heterocyclic ring systems augments biological activities considerably. Furthermore, introduction a chloro-substituted benzene moiety into triazole scaffold results in increase of broad spectrum of antimicrobial activity due to the ability of halogen to act as polar hydrogen or hydroxy mimic. Substitution of hydrogen by halogen atom has been a strategy in designing molecules having biological activity [27].

Another pharmacophore heterocycle, 1,3,4-thiadiazole nucleus has been incorporated into some therapeutically important drugs, such as Acetazolam and Cefazolin [28–30]. In addition to their well documented potential antimicrobial activities, thiadiazole ring system has occupied a unique position in the design and synthesis of novel biologically active agents with remarkable analgesic and anti-inflammatory activities [31–39].

Divers pharmacological properties of the compounds containing antipyrine, triazole or thiadiazole moieties have prompted the medicinal chemists to design and synthesize hybrid molecules incorporating antipyrine and triazole scaffolds in a single molecule [40]. Moreover, the compounds including both 1,2,4-triazole and 1,3,4-thiadiazole moieties in their structures have been obtained as antimicrobial compounds in our laboratory [41–44].

Keeping this observation in view and in continuation of our study on the synthesis of biologically active compounds, this paper has presented the synthesis of new antipyrine derivatives incorporating different pharmacophores as hybrid molecules possessing antimicrobial activity.

## 2. Chemistry

The treatment of 4-aminoantipyrine (**1**) with ethyl 2-[2-(4-chlorophenyl)-1-ethoxyethylidene]hydrazinecarboxylate (**2**) resulted in the formation of 5-(4-chlorobenzyl)-4-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazole-4-yl)-2,4-dihydro-3*H*-1,2,4-triazole-3-one (**3**). The reaction of compound **3** with ethyl bromoacetate in the presence of sodium ethoxide produced ethyl [4-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl)-3-(4-chlorobenzyl)-5-oxo-4,5-dihydro-1*H*-1,2,4-triazol-1-yl]acetate (**4**). Then, this ester (**4**) was converted to the corresponding hydrazide derivative, 2-[4-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-il)-3-(4-chlorobenzyl)-5-okso-4,5-dihydro-1*H*-1,2,4-triazole-1-yl]acetohydrazide (**5**) by the reaction of **4** with hydrazine hydrate. 2-{[4-(1,5-Dimethyl-3-oxo-5-phenyl-2,3-dihydro-1*H*-pyrazole-4-yl)-3-(4-chlorobenzyl)-5-oxo-4,5-dihydro-1*H*-1,2,4-triazole-1-yl]acetyl}-*N*-(aryl)hydrazinecarbotiyoamides (**6a**–**c**) were obtained by the reaction of compound **5** with phenylisothio cyanate (for **6a**), benzylisothiocyanate (for **6b**) or 4-flouropheny lisothiocyanate (for **6c**).

The treatment of compounds **6a**–**c** with cold concentrated sulfuric acid caused to the conversion of carbothioamide structure into 1,3,4-thiadiazol rings; thus, 4-(2-phenyl-1,5-dimethyl-3-oxo-2,3-dihydro-1*H*-pyrazol-4-yl)-5-(4-chlorobenzyl)-2-{[5-(alkylamino)-1,3,4-thiadiazol-2-yl]methyl}-2,4-dihydro-3*H*-1,2,4-triazol-3-ones (**10a**–**c**) was obtained. On the other hand, the cyclisation of the same intermediates, **6a**–**c,** in the presence of 2N NaOH produced 4-(2-phenyl-1,5-dimethyl-3-oxo-2,3-dihydro-1*H*-pyrazol-4-yl)-5-(4-chlorobenzyl)-2-[(4-alkyl-5-mercapto-4*H*-1,2,4-triazol-3-yl)methyl]-2,4-dihydro-3*H*-1,2,4-triazol-3-ones (**11a**–**c**). The synthesis of 2-[3-(4-chlorobenzyl)-4-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl)-5-oxo-4,5-dihydro-1*H*-1,2,4-triazol-1-yl]-*N*′-[4-(4-chloro or 4-nitrophenyl)-3-phenyl-1,3-thiazol-2(3*H*)-ylidene] acetohydrazides (**12**, **13**) was performed by the reaction of compound **6a** with 4-chloro or 4-nitrophenacyl bromide.

The treatment of compound **5** with carbon disulfide in the presence of KOH resulted in the formation of 4-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl)-5-(4-chlorobenzyl)-2-[(5-sulfanyl-1,3,4-oxadiazol-2-yl)methyl]-2,4-dihydro-3*H*-1,2,4-triazole-3-one (**7**). Then, compound **7** was concerted to 1,5-dimethyl-4-{7-(4-chlorobenzyl)-3-thioxo-2,10-dihydro-3*H*,6*H*-bis[1,2,4]triazolo[5,1-*c*:3′,4′-*f*][1,2,4]triazin-6-yl}-2-phenyl-1,2-dihydro-3*H*-pyrazol-3-one (**9**) by the treatment with hydrazine hydrate via the formation of compound **8** that was not isolated (Fig. 1).

## 3. Results and discussion

The main aim of the present study is to synthesize and investigate the antimicrobial activities of new antipyrine derivatives containing also 1,2,4-triazole, 1,3,4-thiadiazole, 1,3,4-oxadiazole and/or 1,3-thiazole ring in the same structure.

Synthesis of the intermediate and target compounds was performed according to the reactions outlined in Fig. 1. The starting compound ethyl 2-[2-(4-chlorophenyl)-1-ethoxyethylidene] hydrazinecarboxylate (**2**) was prepared following a previously reported literature procedure [45].

In the present study, compound **4** was obtained by two methods in good yields. The $^1$H NMR spectrum of compound **4** exhibited additional signals derived from ester group were observed at 1.21 ppm ($-OCH_2\underline{CH_3}$) and 4.18 ($-O\underline{CH_2}CH_3$) ppm integrating for three protons and two protons, respectively. This group resonated at 13.88 and 61.08 ppm in the $^{13}$C NMR spectrum. When compound **4** was converted to the corresponding hydrazide (**5**) the signals derived from ester group disappeared in the $^1$H NMR spectrum of **5**, instead, new signals representing hydrazide structure appeared at 4.28 ($-NH\underline{NH_2}$) and 9.31 ($-\underline{NH}NH_2$) ppm (controlled by changing with $D_2O$) integrating for two protons and one proton, respectively.

The IR spectra of compounds **4** and **5** showed an additional peak at 1753 (for ester) or 1732 (for hydrazide) cm$^{-1}$ due to exocyclic-carbonyl function originated from ester or hydrazide structure beside the endocyclic-carbonyl peak at the position 3 of 1,2,4-triazole ring.

As different from compound **5,** $^1$H and $^{13}$C NMR spectra of compounds **6a**–**c** exhibited additional signals due to carbothioamide moiety at the related chemical shift values. In addition, compounds **6a**–**c** gave relatively stable molecular ion peak in the Mass spectra.

With the conversion of compound **5** into 4-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl)-5-(4-chlorobenzyl)-2-[(5-sulfanyl-1,3,4-oxadiazol-2-yl)methyl]-2,4-dihydro-3*H*-1,2,4-triazole-3-one (**7**), the $-NH-NH_2$ signals disappeared; instead, new signal belonging to $-SH$ proton appeared at 14.73 ppm as singlet in the $^1$H NMR spectrum of compound **7**. Furthermore, the IR spectrum of compound **7** showed no signals belonging to $-NHNH_2$ group; while new signal belonging to $-SH$ proton was recorded at 2549 cm$^{-1}$. The C-2 and C-5 carbon atoms of 1,3,4-oxadiazole ring resonated at 133.87 (C-2) and 160.01 (C-5), respectively, in the $^{13}$C NMR spectrum of compound **7**. Moreover, the elemental analysis of compound **7** was consistent with the assigned structure.

In the IR spectrum of compound **9**, only one signal at 1681 cm$^{-1}$ appeared due to carbonyl function. Moreover, in the $^1$H NMR spectrum of compound **9**, no signal derived from $-SH$ group was recorded, instead, new signals due to $-NH$ group was detected at 9.37 ppm (exchangeable with $D_2O$).

In the IR spectra of compounds **10a**–**c**, the $-NH-$ stretching band appeared at 3032–3036 cm$^{-1}$. The $-NH-$ proton resonated at about 10.39–10.42 ppm in the $^1$H NMR spectrum of compounds **10a**–**c**. The IR spectra of compounds **11a**–**c** displayed $-SH$ stretching bands at 2857–2923 cm$^{-1}$. Moreover, in the $^1$H NMR spectra of compounds **11a**–**c**, additional signal due to $-SH$ group

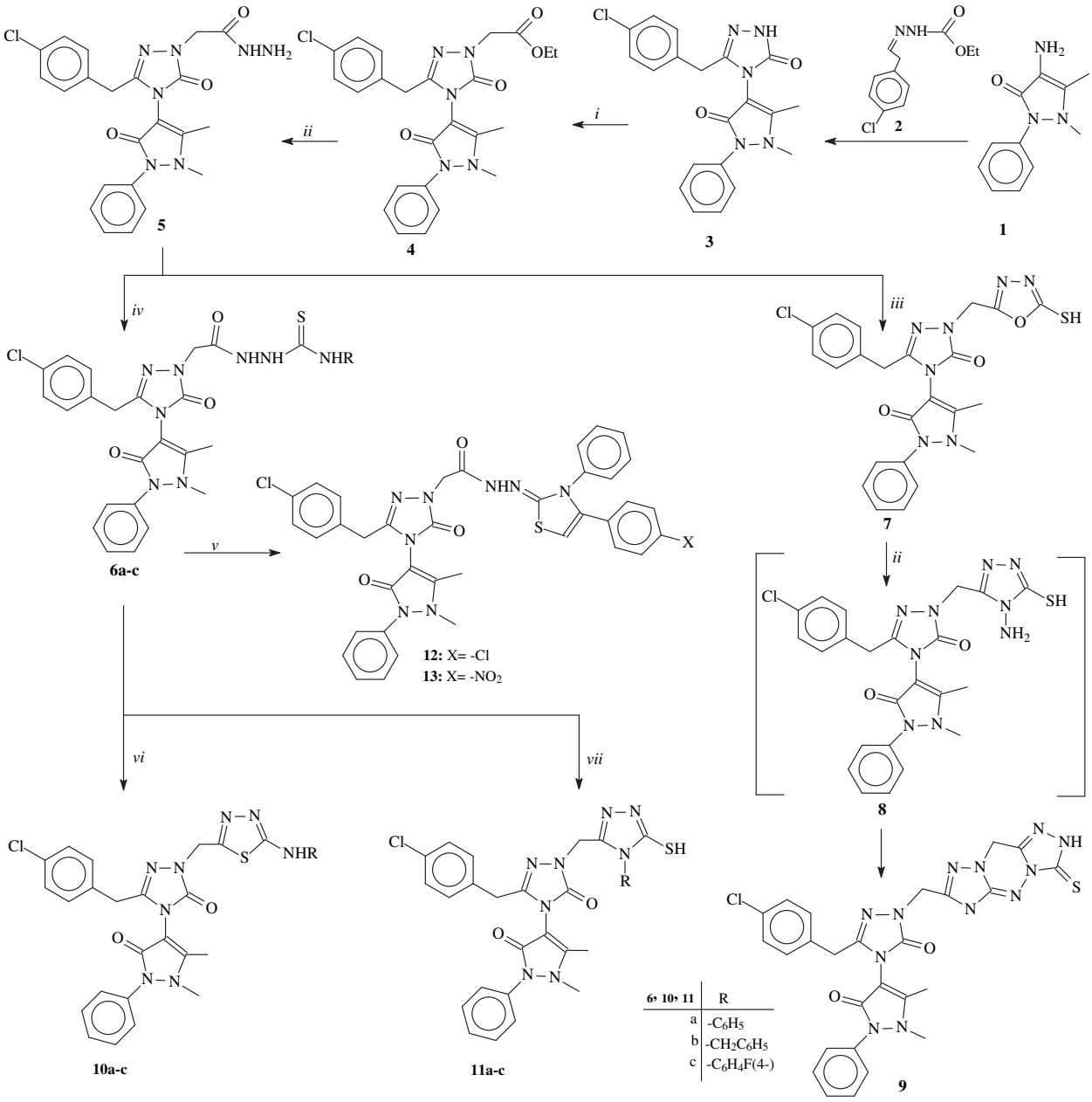


**Fig. 1.** Reactions and conditions: *i*: BrCH2COOEt, *ii*: H2NNH2, *iii*: KOH/CS2, *iv*: RNCS, *v*: C6H4COCH2X, *vi*: H2SO4, *vii*: NaOH.

was observed at 14.04–14.01 ppm (controlled with changing by $D_2O$), while the –NH– signals disappeared.

The IR and $^1H$ NMR spectra of compounds **12** and **13** showed no signal representing –SH group.

All the compounds have been tested for their antimicrobial activities. The results are presented in Table 1. All compounds, except **3**–**5**, exhibited activity against *Mycobacterium smegmatis* (Ms) that is atipic tuberculosis factor, whereas none of them was found to have antifungal activity.

According to the obtained results, it can be concluded that the conversion of carbothioamide moiety into 5-mercapto-1,3,4-oxadiazole nucleus in compound **7**, and further ring fusion into compound **9** afforded an increase in the antimicrobial and antitubercular activity. On the other hand, the cyclization of linear carbothioamide side change of **6a**–**c** into 1,3,4-thiadiazole ring caused to an increase in the activity of **10a**–**c** against enteric bacteria, *Escherichia coli* (*Ec*) *Enterobacter aerogenes* (*Ea*), *Yersinia pseudotuberculosis* (*Yp*) and Gram negative bacillus, *Pseudomonas aeruginosa* (*P*a), whereas the compounds (**10a**–**c**) displayed completely inactivity towards *Staphylococcus aureus* (*Sa*), *Enterococcus faecalis* (*Ef*) which are Gram positive cocci, and *Bacillus cereus* (*Bc*) that is Gram positive spore bacillus. When 1,3,4-thiadiazole nucleus replaced by 1,2,4-triazole ring in the structure of compounds **11a**–**c**, good antimicrobial and antitubercular activities were observed on the test microorganisms except *Candida albicans* (*Ca*), *Candida tropicalis* (*Ct*), *Aspergillus niger* (*An*) and *Saccharomyces cerevisiae* (*Sc*). Compound 12 that was obtained from the condensation of **6a** with 4-chlorophenacyl bromide displayed good activities against Ec, Yp and Ms. On the other hand, the replacement of chlorine atom in **12** by a bulky nitro group leading to **13** resulted in good activities towards all the test microorganisms except Ca, Ct, Sc and An.

**Table 1**
Screening for antimicrobial activity of the compounds (mm).

| No | Microorganisms and inhibition zone (mm) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Ec | Ea | Yp | Pa | Sa | Ef | Bc | Ms | Ca | Ct | Sc | An |
| **3** | – | – | – | – | – | – | – | – | – | – | – | – |
| **4** | – | – | – | – | – | – | – | – | – | – | – | – |
| **5** | – | – | – | – | – | – | – | – | – | – | – | – |
| **6a** | 10 | – | 8 | 8 | 8 | 8 | 10 | 10 | – | – | – | – |
| **6b** | 15 | 10 | 10 | 10 | 10 | 8 | 10 | 15 | – | – | – | – |
| **6c** | 15 | 8 | 10 | 10 | 10 | 8 | 10 | 15 | – | – | – | – |
| **7** | 35 | 30 | 30 | 30 | 30 | 28 | 30 | 38 | – | – | – | – |
| **9** | 35 | 30 | 28 | 28 | 30 | 28 | 28 | 40 | – | – | – | – |
| **10a** | 25 | 25 | 25 | 35 | – | – | – | 25 | – | – | – | – |
| **10b** | 25 | 25 | 25 | 30 | – | – | – | 25 | – | – | – | – |
| **10c** | 20 | 25 | 25 | 20 | – | – | – | 25 | – | – | – | – |
| **11a** | 25 | 25 | 30 | 30 | 25 | 20 | 30 | 34 | – | – | – | – |
| **11b** | 35 | 30 | 30 | 30 | 30 | 28 | 30 | 33 | – | – | – | – |
| **11c** | 35 | 30 | 30 | 28 | 30 | 28 | 30 | 30 | – | – | – | – |
| **12** | 15 | – | 12 | – | – | – | – | 10 | – | – | – | – |
| **13** | 15 | – | 30 | 20 | 30 | 18 | 20 | 25 | – | – | – | – |
| Amp. | 10 | 10 | 18 | 18 | 35 | 10 | 15 | | | | | |
| Strep. | | | | | | | | 35 | | | | |
| Flu | | | | | | | | | 25 | 25 | >25 | ND |

Ec: *Escherichia coli* ATCC 25922, Ea: *Enterobacter aeroginosa* ATCC 13048, Yp: *Yersinia pseudotuberculosis* ATCC 911, Pa: *Pseudomonas aeruginosa* ATCC 43288, Sa: *Staphylococcus aureus* ATCC 25923, Ef: *Enterococcus faecalis* ATCC 29212, Bc: *Bacillus cereus* 702 Roma, Ms: *Mycobacterium smegmatis* ATCC607, Ca: *Candida albicans* ATCC 60193, Ct: *Candida tropicalis* ATCC 13803, *Saccharomyces cerevisiae* RSKK 251, An: *Aspergillus niger* RSKK 4017, Amp.: Ampisilin, Flu.: Flukonazol, (–): Aktivite yok, ND: Not detected.

## 4. Experimental

### 4.1. Chemistry

All the chemicals were purchased from Fluka Chemie AG Buchs (Switzerland) are used without further purification. Melting points of the synthesized compounds are determined in open capillaries on a Büchi B-540 melting point apparatus and are uncorrected and are uncorrected. Reactions are monitored by thin-layer chromatography (TLC) on silica gel 60 F254 aluminium sheets. The mobile phase was ethanol and ethyl acetate (1:1) and detection was made using UV light. IR spectra are recorded as potassium bromide pellets using a PerkineElmer 1600 series FTIR spectrometer. $^1$H NMR and $^{13}$C NMR spectra were recorded on BRUKER AVENE II 400 MHz NMR Spectrometer (Chemical shift in ppm down field from TMS as an internal reference). The elemental analysis was performed on a Costech Elemental Combustion System CHNS-O elemental analyzer. All the compounds gave C, H and N analysis within ±0.4% of the theoretical values. The Mass spectra were obtained for compounds **3**, **6a**–**c** and **11a** at a Quattro LC-MS (70 eV) Instrument.

#### 4.1.1. 5-(4-Chlorobenzyl)-4-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazole-4-yl)-2,4-dihydro-3H-1,2,4-triazole-3-one (**3**)

*Method* 1: A mixture of compound **1** (10 mmol) and 4-aminoantipyrin (10 mmol) was heated at 110–120 °C in an oil bath for 2 h. Then, *n*-butyl acetate-diethyl ether (1:2) was added and the reaction mixture was kept overnight in cold. The solid formed was filtered off and recrystallized several times from ethyl acetate to afford the target compound.

*Method* 2: The solution of compound **1** (10 mmol) in water was refluxed with 4-aminoantipyrin (10 mmol) for 5 h (controlled with TLC). The solvent was evaporated under reduced pressure and an oily product was obtained. *n*-Butyl acetate-diethyl ether (1:2) was added into it and was kept overnight in cold. The solid formed was filtered off and recrystallized several times from ethyl acetate to afford the target compound.

Yield 78%, m.p. 270–271 °C. IR (KBr, $\upsilon$, cm$^{-1}$): 3094 (NH), 1724 and 1651 (C=O), 1623 (C=N); Anal. Calcd (%) for $C_{20}H_{18}N_5O_2Cl$: C, 60.69; H, 4.58; N, 17.69. Found: C, 60.68; H, 4.61; N, 17.49. $^1$H NMR (DMSO-$d_6$, $\delta$ ppm): 1.80 (3H, s, $CH_3$), 3.09 (3H, s, $CH_3$), 3.80 (2H, AB system, $CH_2$), 7.13 (2H, d, arH, $J$ = 8.3 Hz), 7.30–7.50 (5H, m, arH), 7.59 (2H, t, arH, $J$ = 7.8 Hz), 11.78 (1H, s, NH); $^{13}$C NMR (DMSO-$d_6$, $\delta$ ppm): 9.67 ($CH_3$), 31.25 ($CH_2$), 34.74 ($CH_3$), 100.78 (C-antipyrine), arC:[124.62 (2CH), 127.10 (CH), 128.11 (2CH), 129.06 (2CH), 130.25 (2CH), 133.96 (2C), 160.11 (C)], 131.23 (antipyrine C), 146.72 (triazole C-5), 152.59 (antipyrine C=O), 153.97 (triazole C-3), MS: *m*/*z* (%) 102 (20), 215 (100), 216 (19), 316 (13), 396 ($M^+$, 78), 398 (28), 418 (20).

#### 4.1.2. Ethyl [4-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-3-(4-chlorobenzyl) -5-oxo-4,5-dihydro-1H-1,2,4-triazol-1-yl]acetate (**4**)

*Method* 1: Compound **3** (10 mmol) was refluxed with 1 equiv. of sodium in absolute ethanol for 2 h. Then, ethyl bromoacetate (10 mmol) was added and refluxed for an additional 8 h. After evaporating the solvent under reduced pressure, a solid appeared. The crude product was recrystallized from ethanol/water (1:2) to afford compound **4**.

*Method* 2: To a solution of compound **3** (10 mmol) in ethanol, ethyl bromoacetate (10 mmol) was added and the mixture was stirred at room temperature for 1 h and refluxed for 4 h in the presence of triethylamine (10 mmol). Then, the solvent was removed under reduced pressure and a solid obtained. The crude product was recrystallized from ethanol/water (1:2) to afford compound **4.** Yield 59%, m.p. 167–168 °C. IR (KBr, $\upsilon$, cm$^{-1}$): 1753, 1724 and 1662 (3C=O), 1592 (C=N), 1207 (C–O); Anal. Calcd (%) for $C_{24}H_{24}N_5O_4Cl$: C, 59.81; H, 5.02; N, 14.53.Found: C, 59.81; H, 5.00; N, 14.28. $^1$H NMR (DMSO-$d_6$, $\delta$ ppm): 1.21 (3H, t, $CH_3$, $J$ = 7.0 Hz), 1.82 (3H, s, $CH_3$), 3.11 (3H, s, $CH_3$), 3.89 (2H, AB system, $CH_2$), 4.18 (2H, q, $CH_2$, $J$ = 7.3 Hz), 4.63 (2H, s, $CH_2$), 7.10 (2H, d, arH, $J$ = 8.3 Hz), 7.32–7.42 (5H, m, arH), 7.57 (2H, t, arH, $J$ = 7.5 Hz); $^{13}$C NMR (DMSO-$d_6$, $\delta$ ppm): 9.71 ($CH_3$), 13.88 ($CH_3$), 31.05 ($CH_2$), 34.72 ($CH_3$), 46.46 ($CH_2$), 61.08 ($CH_2$), 100.85 (antipyrine C), arC:[124.92 (2CH), 127.36 (CH), 128.30 (2CH), 129.21 (2CH), 130.32 (2CH), 131.48 (C), 133.72 (C), 133.86 (C)], 146.36 (triazole C-5), 152.35 (antipyrine C), 152.88 (triazole C-3), 159.88 (antipyrine C=O), 167.69 (C=O).

#### 4.1.3. 2-[4-(1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-il)-3-(4-chlorobenzyl)-5-oxo-4,5-dihydro-1H-1,2,4-triazole-1-yl] acetohydrazide (**5**)

A solution of compound **4** (10 mmol) in ethanol was refluxed with hydrazine hydrate (30 mmol) for 5 h (controlled with TLC). After cooling it to room temperature, acetone was added to the mixture and was kept overnight in cold. The resulting solid separated was collected by filtration and recrystallized from ethanol to afford the desired product. Yield 96%, m.p. 267–268 °C. IR (KBr, $\upsilon$, cm$^{-1}$): 3190 and 3093 (NH–$NH_2$), 1732 and 1655 (3C=O), 1596 (C=N); Anal. Calcd (%) for $C_{22}H_{22}N_7O_3Cl$: C, 56.47; H, 4.74; N, 20.95. Found: C, 56.39; H, 4.78; N, 20.93. $^1$H NMR (DMSO-$d_6$, $\delta$ ppm): 1.78 (3H, s, $CH_3$), 3.06 (3H, s, $CH_3$), 3.37 (2H, s, $CH_2$), 3.78 (2H, AB system, $CH_2$), 4.28 (2H, s, NH), 7.10 (2H, d, arH, $J$ = 8.2 Hz), 7.23–7.42 (5H, m, arH), 7.56 (2H, t, arH, $J$ = 8.2 Hz), 9.31 (1H, s, NH); $^{13}$C NMR (DMSO-$d_6$, $\delta$ ppm): 10.56 ($CH_3$), 32.14 ($CH_2$), 35.62 ($CH_3$), 39.27–40.94 (DMSO-$d_6$+$CH_2$), 101.61 (antipyrine C), arC:[125.51 (CH), 125.66 (CH), 128.01 (2CH), 129.04 (2CH), 129.99 (2CH), 131.18 (CH), 132.14 (C), 134.58 (C), 134.77 (C)], 147.65 (triazole C-5), 153.51 (antipyrine C), 154.88 (triazole C-3), 160.99 (C=O), 166.56 (antipyrine C=O).

#### 4.1.4. General method for the synthesis of compounds **6a**–**c**

A mixture of compound **5** (10 mmol) and phenylisothiocyanate (for **6a**), benzylisothiosyanate (for **6b**) or 4-fluorophenylisothiosya nate (for **6c**) (10 mmol) was allowed to reflux in ethanol for 4 h.

After evaporating the solvent under reduced pressure an oily product appeared; acetone was added into it and kept overnight in cold. This crude product was filtered off and recrystallized from ethanol to afford the desired products.

*4.1.4.1. 2-{[4-(1,5-Dimetyl-3-oxo-5-phenyl-2,3-dihydro-1H-pyrazole-4-il)-3-methyl-5-okso-4,5-dihydro-1H-1,2,4-triazole-1-yl] acetyl}-N-phenylhydrazinecarbothioamide (**6a**).* Yield 95%, m.p. 165–166 °C. IR (KBr, υ, $cm^{-1}$): 3232 (3NH), 1704 and 1651 (3C=O), 1615 (C=N), 1191 (C=S); Anal. Calcd. (%) for $C_{29}H_{27}O_3N_8SCl$: C, 57.75; H, 4.51; N, 18.50. Found: C, 57.69; H, 4.54; N, 18.46. $^1$H NMR (DMSO-$d_6$, δ ppm): 1.84 (3H, s, $CH_3$), 3.10 (3H, s, $CH_3$), 3.82 (2H, AB system, $CH_2$), 4.58 (2H, s, $CH_2$), 7.11–7.21 (3H, m, arH), 7.34–7.52 (8H, m, arH), 7.56–7.60 (3H, m, arH), 9.69 (1H, s, NH), 9.78 (1H, s, NH), 10.38 (1H, s, NH); $^{13}$C NMR (DMSO-$d_6$, δ ppm): 9.81 ($CH_3$), 31.06 ($CH_3$), 34.76 ($CH_2$), 38.05–40.58 (DMSO-$d_6$ + $CH_2$), 100.57 (antipyrine C), arC: [124.90 (2CH), 125.21 (2CH), 127.36 (2CH), 128.01 (2CH), 128.29 (2CH), 129.23 (2CH), 130.40 (2CH), 131.45 (C), 133.74 (C), 133.89 (C), 138.84 (C)], 146.10 (triazole C-5), 152.48 (antipyrine C), 153.19 (triazole C-3), 159.93 (antipyrine C=O), 166.401 (C=O), 181.98 (C=S); MS *m*/*z* (%): 176 (19), 229 (17), 357 (10), 468 (44), 490 (20), 532 (17), 585 (27), 603 ($M^+$, 56), 625 (100), 627 (45), 648 (19).

*4.1.4.2. 2-{[4-(1,5-Dimethyl-3-oxo-5-phenyl-2,3-dihydro-1H-pyrazole-4-yl)-3-(4-chlorobenzyl)-5-oxo-4,5-dihydro-1H-1,2,4-triazole-1-yl]acetyl}-N-benzylhydrazinecarbothioamide (**6b**).* Yield 87%, m.p. 140–141 °C. IR (KBr, υ, $cm^{-1}$): 3288 and 3164 (3NH), 1708 and 1652 (3C=O), 1548 (C=N), 1189 (C=S); Anal. Calcd. (%) for $C_{30}H_{29}N_8O_3SCl$: C, 58.39; N, 18.16; H, 4.74. Found: C, 58.37; N, 18.15; H, 4.88. $^1$H NMR (DMSO-$d_6$, δ ppm): 1.83 (3H, s, $CH_3$), 3.09 (3H, s, $CH_3$), 3.79 (2H, AB system, $CH_2$), 4.51 (2H, s, $CH_2$), 4.76 (2H, s, $CH_2$), 7.10 (3H, d, arH, $J$ = 8.6 Hz), 7.14–7.44 (9H, m, arH), 7.59 (2H, t, arH, $J$ = 7.5 Hz), 8.58 (1H, s, NH), 9.49 (1H, s, NH), 10.18 (1H, s, NH); $^{13}$C NMR (DMSO-$d_6$, δ ppm): 9.83 ($CH_3$), 31.17 ($CH_2$), 34.76 ($CH_3$), 38.49–40.17 (DMSO-$d_6$+$CH_2$), 46.49 ($CH_2$), 100.61 (antipyrine C), arC: [124.89 (2CH), 126.55 (CH), 126.81 (CH), 127.36 (2CH), 127.98 (2CH), 128.28 (2CH), 129.23 (2CH), 130.38 (2CH), 131.46 (C), 133.74 (C), 133.88 (C), 139.05 (C)], 146.06 (triazole C-5), 152.45 (antipyrine C), 153.17 (triazole C-3), 159.93 (antipyrine C=O), 166.40 (C=O), 181.98 (C=S); MS *m*/*z* (%): 116.78 (29), 116.90 (25), 124.70 (22), 203.67 (16), 203.98 (22), 228.94 (28), 270.32 (14), 337.97 (16), 356.08 (20), 357.15 (29), 358.09 (15), 467.94 (33), 468.19 (53), 507.99 (21), 530.25 (23), 532.26 (20), 617.21 ($M^+$, 64), 639. 03 (91), 639.16 (100), 641.11 (M + 1 + Na, 51), 642.30 (19).

*4.1.4.3. 2-{[4-(1,5-Dimethyl-3-oxo-5-phenyl-2,3-dihydro-1H-pyrazole-4-yl)-3-(4-chlorobenzyl)-5-oxo-4,5-dihydro-1H-1,2,4-triazole-1-yl]acetyl}-N-(4-fluorophenyl) hydrazinecarbothioamide (**6c**).* Yield 83%, m.p. 139–140 °C. IR (KBr, υ, $cm^{-1}$): 3233 (3NH), 1710 and 1657 (3C=O), 1590 (C=N), 1213 (C=S); Anal. Calcd. (%) for $C_{29}H_{26}N_8O_3SFCl$: C, 56.08; N, 18.04; H, 4.22. Found C, 56.19; N, 18.00; H, 4.28. $^1$H NMR (DMSO-$d_6$, δ ppm): 1.86 (3H, s, $CH_3$), 3.11 (3H, s, $CH_3$), 3.79 (2H, AB system, $CH_2$), 4.58 (2H, s, $CH_2$), 7.16–7,21 (4H, m, arH), 7.26–7.53 (6H, m, arH), 7.57 (2H, t, arH, $J$ = 7.8 Hz), 9.79 (2H, brs, 2NH), 10.39 (1H, s, NH); $^{13}$C NMR (DMSO-$d_6$, δ ppm): 9.84 ($CH_3$), 31.14 ($CH_2$), 34.77 ($CH_3$), 38.48–40.16 (DMSO-$d_6$+$CH_2$), 100.61 (antipyrine C), arC: [114.49 (2CH), 114.94 (2CH), 124.93 (2CH), 127.39 (CH), 128.31 (2CH), 129.25 (2CH), 130.43 (2CH), 131.50 (C), 133.74 (C), 133.91 (C), 135.19 (2C)], 146.14 (triazole C-5), 152.48 (antipyrine C), 153.24 (triazole C-3), 159.98 (antipyrine C=O), 162.02 (C=O), 166.49 (C=S); MS *m*/*z* (%): 107.09 (100), 115.01 (50), 122.94 (41), 124.76 (19), 152.55 (24), 154.81 (11), 178.64 (10), 229.76 (10), 468.07 (21), 490.14 (23), 508.12 (23), 603.07 (13), 621.11 ($M^+$, 33).

*4.1.5. 4-(1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-5-(4-chlorobenzyl)-2-[(5-sulfanyl-1,3,4-oxadiazol-2-yl)methyl]-2,4-dihydro-3H-1,2,4-triazole-3-one (**7**)*

Compound **5** (10 mmol) and $CS_2$ (0.6 mL, 10 mmol) were added to a solution of KOH (0.56 g, 10 mmol) in 50 mL $H_2O$ and 50 mL ethanol and the mixture was refluxed for 3 h. Then, the reaction content was acidified with conc. HCl. The precipitate was filtered off, washed with $H_2O$ and recrystallized from ethanol to afford the desired compound. Yield 88%, m.p. 232–233 °C. IR (KBr, υ, $cm^{-1}$): 2549 (SH), 1722 and 1640 (2C=O), 1615, 1588 and 1491 (3C=N); Anal. Calcd (%) for $C_{23}H_{20}N_7O_3SCl$: C, 54.17; H, 3.95; N, 19.31. Found: C, 54.23; H, 3.90; N, 19.28; $^1$H NMR (DMSO-$d_6$, δ ppm): 1.85 (3H, s, $CH_3$), 3.13 (3H, s, $CH_3$), 3.82 (2H, AB system, $CH_2$), 5.15 (2H, s, $CH_2$), 7.15 (2H, d, arH, $J$ = 8.2 Hz), 7.24–7.42 (5H, m, arH), 7.57 (2H, t, arH, $J$ = 7.8 Hz), 14.73 (1H, brs, SH); $^{13}$C NMR (DMSO-$d_6$, δ ppm): 9.91 ($CH_3$), 31.30 ($CH_2$), 34.80 ($CH_3$), 38.10–40.60 (DMSO-$d_6$ + $CH_2$), 100.13 (antipyrine C), arC: [125.40 (2CH), 127.86 (CH), 128.55 (2CH), 129.50 (2CH), 130.62 (2CH), 131.77 (C), 133.62 (2C)], 133.87 (oxadiazole C-2), 147.51 (triazole C-5), 152.32 (antipyrine C), 152.76 (triazole C-3), 159.09 (antipyrine C=O), 160.01 (oxadiazole C-5).

*4.1.6. 1,5-Dimethyl-4-{7-(4-chlorobenzyl)-3-thioxo-2,10-dihydro-3H,6H-bis[1,2,4]triazolo [5,1-c:3′,4′-f][1,2,4]triazin-6-yl}-2-phenyl-1,2-dihydro-3H-pyrazole-3-one (**9**)*

To the solution of compound **7** (10 mmol) in ethanol hydrazine hydrate (30 mmol) was added and the reaction mixture was refluxed for 4 h. On cooling it overnight in cold, a solid obtained. The crude product was filtered off, washed with $H_2O$ and recrystallized from ethanol to afford the desired compound. Yield 75%, m.p. 219–220 °C. IR (KBr, υ, $cm^{-1}$): 3195 (NH), 1681 (C=O), 1607, 1590, 1547 and 1489 (4C=N); Anal. Calcd (%) for $C_{23}H_{20}N_9OSCl$: C, 54.60; H, 3.98; N, 24.91. Found: C, 54.57; H, 3.90; N, 24.94; $^1$H NMR (DMSO-$d_6$, δ ppm): 2.01 (3H, s, $CH_3$), 2.98 (3H, s, $CH_3$), 3.35 (2H, s, $CH_2$), 3.58 (2H, s, $CH_2$), 7.20–7.38 (10H, m, arH), 7.40–7.56 (4H, m, arH), 9.37 (1H, s, NH); $^{13}$C NMR (DMSO-$d_6$, δ ppm): 11.38 ($CH_3$), 36.08 ($CH_3$), 38.27–40.77 (DMSO-$d_6$ + $CH_2$), 41.69 ($CH_2$), 106.84 (antipyrine C), arC: [124.76 (2CH), 127.50 (2CH), 128.89 (2CH), 129.88 (2CH + C), 131.36 (CH + C), 135.11 (C)], 135.55 (antipyrine C), 152.82 (triazole C-5), 158.01 (triazole C-3), 162.43 (triazole C-5), 163.45 (antipyrine C=O), 170.94 (C=S).

*4.1.7. General method for the synthesis of compounds **10a–c***

A mixture of the corresponding carbothioamide **6a–c** (10 mmol) and concentrated sulfuric acid (64 mmol) was stirred in an ice bath for 15 min. Then, the mixture was allowed to reach to room temperature. After stirring for an additional 30 min, the resulting solution was poured into ice-cold water and made alkaline to pH 8 with ammonia. The precipitated product was filtered, washed with water and recrystallized from ethanol to afford the desired product.

*4.1.7.1. 4-(2-Phenyl-1,5-dimethyl-3-oxo-2,3-dihydro-1H-pyrazole-4-yl)-5-(4-chlorobenzyl)-2-{[5-(phenylamino)-1,3,4-thiadiazol-2-yl] methyl}-2,4-dihydro-3H-1,2,4-triazole-3-one (**10a**).* Yield 89%, m.p. 229–230 °C. IR (KBr, υ, $cm^{-1}$): 3032 (NH), 1720 and 1681 (2C=O); $C_{29}H_{25}O_2N_8SCl$: C, 59.53; N, 19.15; H, 4.31. Found C, 59.48; N, 19.16; H, 4.26; $^1$H NMR (DMSO-$d_6$, δ ppm): 1.84 (3H, s, $CH_3$), 3.13 (3H, s, $CH_3$), 3.88 (2H, AB System, $CH_2$), 5.23 (2H, s, $CH_2$), 7.10 (3H, d, arH, $J$ = 8.3 Hz), 7.37–7.45 (6H, m, arH), 7.52–7.56 (5H, m, arH), 10.42 (1H, s, NH).

*4.1.7.2. 4-(2-Phenyl-1,5-dimethyl-3-oxo-2,3-dihydro-1H-pyrazole-4-yl)-5-(4-chlorobenzyl)-2-{[5-benzylamino)-1,3,4-thiadiazol-2-yl] methyl}-2,4-dihydro-3H-1,2,4-triazole-3-on (**10b**).* Yield 80%, m.p. 212–213 °C. IR (KBr, υ, $cm^{-1}$): 3034 (NH), 1709 and 1641 (2C=O); $C_{30}H_{27}O_2N_8SCl$: C, 60.14; N, 18.70; H, 4.54. Found C, 59.99; N, 18.72; H, 4.59; $^1$H NMR (DMSO-$d_6$, δ ppm): 1.81 (3H, s, $CH_3$), 3.18 (3H, s,

$CH_3$), 3.87 (2H, AB System, $CH_2$), 5.12 (2H, s, $CH_2$), 5.28 (2H, s, $CH_2$), 7.08 (3H, d, arH, $J = 8.3$ Hz), 7.37–7.45 (6H, m, arH), 7.50–7.55 (5H, m, arH), 10.39 (1H, s, NH).

*4.1.7.3. 4-(2-Phenyl-1,5-dimethyl-3-oxo-2,3-dihydro-1H-pyrazole-4-yl)-5-(4-chlorobenzyl)-2-{[5-(4-fluorophenylamino)-1,3,4-thiadiazol-2-yl]methyl}-2,4-dihydro-3H-1,2,4-triazole-3-on (**10c**).* Yield 59%, m.p. 237–239 °C. IR (KBr, $\upsilon$, $cm^{-1}$): 3036 (NH), 1709 and 1640 (2C=O); $C_{29}H_{24}O_2N_8SClF$: C, 57.76; N, 18.58; H, 4.01. Found C, 57.71; N, 18.62; H, 4.07; $^1$H NMR (DMSO-$d_6$, $\delta$ ppm): 1.83 (3H, s, $CH_3$), 3.14 (3H, s, $CH_3$), 3.88 (2H, AB System, $CH_2$), 5.24 (2H, s, $CH_2$), 7.07 (3H, d, arH, $J = 8.3$ Hz), 7.35–7.44 (6H, m, arH), 7.50–7.53 (5H, m, arH), 10.42 (1H, s, NH).

*4.1.8. General method for the synthesis of compounds **11a–c***

A solution of corresponding carbothioamide **6a–c** (10 mmol) in ethanol/water (1:1) was refluxed in the presence of 2 N NaOH for 3 h then, the resulting solution was cooled to room temperature and acidified to pH 3–4 with 37%HCl. The precipitate formed was filtered off, washed with water and recrystallized from ethanol/water (1:1) to afford the desired compound.

*4.1.8.1. 4-(2-Phenyl-1,5-dimethyl-3-oxo-2,3-dihydro-1H-pyrazol-4-yl)-5-(4-chlorobenzyl)-2-[(4-phenyl-5-mercapto-4H-1,2,4-triazol-3-yl)methyl]-2,4-dihydro-3H-1,2,4-triazol-3-one (**11a**).* Yield 76%, m.p. 247–248 °C. IR (KBr, $\upsilon$, $cm^{-1}$): 2857 (SH), 1703 and 1681 (2C=O), 1594, 1492 and 1423 (3C=N); $C_{29}H_{25}O_2N_8SCl$: C, 59.53; N, 19.15; H, 4.31. Found C, 59.49; N, 19.18; H, 4.28. $^1$H NMR (DMSO-$d_6$, $\delta$ ppm): 1.59 (3H, s, $CH_3$), 3.06 (3H, s, $CH_3$), 3.63 (2H, AB system, $CH_2$), 4.93 (2H, AB system, $CH_2$), 7.04 (2H, d, arH, $J = 8.3$ Hz), 7.29–7.61 (11H, m, arH), 14.04 (1H, s, SH); $^{13}$C NMR (DMSO-$d_6$, $\delta$ ppm): 10.51 ($CH_3$), 31.85 ($CH_2$), 35.44 ($CH_3$), 38.84–41.35 (DMSO-$d_6$+$CH_2$), 100.96 (antipyrine C), arC: [125.74 (2CH), 128.19 (3CH), 129.08 (3CH), 130.03 (3CH), 131.13 (3CH), 132.30 (C), 133.67 (C), 134.36 (C), 134.58 (C)], 147.28 (triazole C-5), 148.03 (triazole C-3), 152.45 (antipyrine C), 152.96 (triazole C-3), 160.51 (antipyrine C=O), 169.23 (C=S); MS *m/z* (%): 121 (41), 123 (44), 148 (41), 171 (22), 188 (41), 189 (19), 197 (16), 211 (13), 229 (28), 263 (22), 273 (16), 344 (16), 357 (16), 447 (11), 585 ($M^+$, 66), 607 (100), 609 (44), 610 (19), 629 (16), 663 (19).

*4.1.8.2. 4-(2-Phenyl-1,5-dimethyl-3-oxo-2,3-dihydro-1H-pyrazol-4-yl)-5-(4-chlorobenzyl)-2-[(4-benzyl-5-mercapto-4H-1,2,4-triazol-3-yl)methyl]-2,4-dihydro-3H-1,2,4-triazol-3-one (**11b**).* Yield 87%, m.p. 131–132 °C. IR (KBr, $\upsilon$, $cm^{-1}$): 2923 (SH), 1701 and 1685 (2C=O), 1592, 1490 and 1455 (3C=N); $C_{30}H_{27}O_2N_8SCl$: C, 60.14; N, 18.70; H, 4.54. Found C, 60.09; N, 18.68; H, 4.58. $^1$H NMR (DMSO-$d_6$, $\delta$ ppm): 1.62 (3H, s, $CH_3$), 3.05 (3H, s, $CH_3$), 3.78 (2H, AB system, $CH_2$), 4.92 (2H, AB system, $CH_2$), 5.25 (2H, s, $CH_2$), 7.05 (2H, d, arH, $J = 8.2$ Hz), 7.17 (2H, d, arH, $J = 7.4$ Hz), 7.21–7.43 (8H, m, arH), 7.52 (2H, t, arH, $J = 8.2$ Hz), 14.02 (1H, s, SH); $^{13}$C NMR (DMSO-$d_6$, $\delta$ ppm): 11.65 ($CH_3$), 33.26 ($CH_2$), 36.54 ($CH_3$), 39.79–42.30 (DMSO-$d_6$+$CH_2$), 48.09 ($CH_2$), 101.84 (antipyrine C), arC: [127.55 (2CH), 128.54 (2CH), 129.84 (2CH), 130.05 (2CH), 130.46 (2CH), 130.82 (CH), 131.53 (CH), 131.82 (CH), 132.60 (CH), 133.74 (C), 135.44 (C), 135.59 (C), 137.13 (C)], 149.29 (triazole C-5), 149.91 (triazole C-3), 153.93 (antipyrine C), 154.87 (triazole C-3), 160.51 (antipyrine C=O), 169.90 (C=S).

*4.1.8.3. 4-(2-Phenyl-1,5-dimethyl-3-oxo-2,3-dihydro-1H-pyrazol-4-yl)-5-(4-chlorobenzyl)-2-{[4-(4-fluorophenyl)-5-mercapto-4H-1,2,4-triazol-3-yl]methyl}-2,4-dihydro-3H-1,2,4-triazol-3-one (**11c**).* Yield 77%, m.p. 157–158 °C. IR (KBr, $\upsilon$, $cm^{-1}$): 2857 (SH), 1707 and 1682 (2C=O), 1596, 1492 and 1448 (3C=N); $C_{29}H_{24}O_2N_8SClF$: C, 57.76; N, 18.58; H, 4.01. Found C, 57.84; N, 18.51; H, 4.08. $^1$H NMR (DMSO-$d_6$, $\delta$ ppm): 1.66 (3H, s, $CH_3$), 3.07 (3H, s, $CH_3$), 3.75 (2H, AB system, $CH_2$), 5.00 (2H, AB system, $CH_2$), 7.08 (2H, d, arH, $J = 8.2$ Hz), 7.22 (2H, d, arH, $J = 8.3$ Hz), 7.24–7.46 (7H, m, arH), 7.54 (2H, t, arH, $J = 8.2$ Hz), 14.01 (1H, s, SH); $^{13}$C NMR (DMSO-$d_6$, $\delta$ ppm): 10.65 ($CH_3$), 36.27 ($CH_3$), 39.54–41.98 (DMSO-$d_6$+$CH_2$), 48.12 ($CH_2$), 101.76 (antipyrine C), arC: [127.51 (2CH), 127.98 (2CH), 129.71 (2CH), 129.89 (2CH), 130.33 (2CH), 131.02 (CH), 131.53 (CH), 131.80 (CH), 132.63 (CH), 133.52 (C), 135.45 (C), 135.60 (C)], 149.31 (triazole C-5), 150.03 (triazole C-3), 153.92 (antipyrine C), 154.75 (triazole C-3), 160.66 (antipyrine C=O), 169.87 (C=S).

*4.1.9. General method for the synthesis of compounds **12** and **13***

4-Chlorophenacylbromide (10 mmol) (for **12**) or 4-nitrophenacyl bromide (10 mmol) (for **13**) and sodium acetate (16.4 g 200 mmol) were added to the solution of compound **6** in ethanol and the reaction mixture was allowed to reflux for 8 h. Then, the mixture was cooled to room temperature, poured into ice-cold water while stirring and left overnight in cold. The formed solid was filtered, washed with water three times and recrystallized from water to afford the pure compounds.

*4.1.9.1. 2-[3-(4-Chlorobenzyl)-4-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-5-oxo-4,5-dihydro-1H-1,2,4-triazol-1-yl]-N′-[4-(4-chlorophenyl)-3-phenyl-1,3-thiazol-2(3H)-ylidene]acetohydrazide (**12**).* Yield 79%, m.p. 180–181 °C. IR (KBr, $\upsilon$, $cm^{-1}$): 3126 (NH), 1719 and 1673 (3C=O), 1603 (C=N); Anal. Calcd. (%) for $C_{37}H_{30}N_8O_3SCl_2$: C, 60.25; N, 15.19; H, 4.10. Found C, 60.19; N, 15.13; H, 4.12. $^1$H NMR (DMSO-$d_6$, $\delta$ ppm): 1.60 (3H, s, $CH_3$, 3.06 (3H, s, $CH_3$), 3.60–3.90 (4H, m, $2CH_2$) 4.11 (1H, s, CH), 6.98–7.21 (5H, m, arH), 7.23–7.79 (10H, m, arH), 7.98–8.17 (3H, m, arH), 10.68 (1H, m, NH); $^{13}$C NMR (DMSO-$d_6$, $\delta$ ppm): 9.81 ($CH_3$), 31.21 ($CH_2$), 34.69 ($CH_3$), 38.48–40.59 (DMSO-$d_6$+$CH_2$), 100.29 (antipyrine C), 116.90 (CH), arC: [125.08 (2CH), 125.19 (2CH), 126.56 (2CH), 127.54 (2CH), 128.36 (2CH), 128.94 (2CH), 129.07 (CH), 129.34 (CH), 129.67 (CH), 129.92 (CH), 130.31 (CH), 130.44 (CH), 131.60 (C), 132.17 (2C), 133.65 (C), 133.84 (C), 138.70 (C)], 146.44 (C), 146.97 (triazole C-5), 151.78 (triazole C-3), 152.21 (antipyrine C), 152.30 (N=C), 155.07 (antipyrine C=O), 159.84 (C=O).

*4.1.9.2. 2-[3-(4-Nitrobenzyl)-4-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-5-oxo-4,5-dihydro-1H-1,2,4-triazol-1-yl]-N′-[4-(4-nitrophenyl)-3-phenyl-1,3-thiazol-2(3H)-ylidene]acetohydrazide (**13**).* Yield 81%, m.p. 192–193 °C. IR (KBr, $\upsilon$, $cm^{-1}$): 3230 (NH), 1701 and 1643 (3C=O), 1592 (C=N), 1519 and 1351 ($NO_2$); Anal. Calcd (%) for $C_{37}H_{30}N_9O_5SCl$: C: 59.39, N: 16.87, H: 4.01. Found: C: 59.33, N: 16.81, H: 3.97; $^1$H NMR (DMSO-$d_6$, $\delta$ ppm): 1.79 (3H, s, $CH_3$), 3.09 (3H, s, $CH_3$), 3.38 (2H, s, DMSO + $CH_2$), 3.75 (2H, brs, $CH_2$), 4.40 (1H, brs, CH), 6.91–6.95 (2H, d, arH, $J = 8.1$ Hz), 7.05–7.22 (3H, m, arH), 7.34–7.46 (7H, m, arH), 7.56 (2H, t, arH, $J = 7.5$ Hz), 7.99 (2H, d, arH, $J = 9.1$ Hz), 8.25 (2H, d, arH, $J = 8.1$ Hz), 10.04 (1H, s, NH).

## 5. Antimicrobial activity

### 5.1. Antimicrobial activity assessment

All test microorganisms were obtained from the Hifzissihha Institute of Refik Saydam (Ankara, Turkey) and were as follows: *E. coli* ATCC35218, *E. aerogenes* ATCC13048, *Y. pseudotuberculosis* ATCC911, *P. aeruginosa* ATCC43288, *S. aureus* ATCC25923, *E. faecalis* ATCC29212, *B. cereus* 709 Roma, *M. smegmatis* ATCC607, *C. albicans* ATCC60193, *C. tropicalis* ATCC 13803, *A. niger* RSKK 4017 and *S. cerevisiae* RSKK 251. All the newly synthesized compounds were weighed and dissolved in dimethylsulphoxide to prepare extract stock solution of 5.000 μg/mL.

*5.1.1. Agar-well diffusion method*

Screening test using agar-well diffusion method [46] as adapted earlier [47] was used for all newly synthesized compounds. Each microorganism was suspended in Mueller Hinton (MH) (Difco,

Detroit, MI) broth and diluted approximately to $10^6$ colony forming unit (cfu)/mL. They were "flood-inoculated" onto the surface of MH agar and Sabouraud Dextrose Agar (SDA) (Difco, Detriot, MI) and then dried. For *C. albicans* and *C. tropicalis*, SDA were used. Five-millimeter diameter wells were cut from the agar using a sterile cork-borer, and 50 mL of the extract substances was delivered into the wells. The plates were incubated for 18 h at 35 °C. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organism. Ampicillin (10 μg), Streptomisin (10 μg) and fluconazole (5 μg) were standard drugs. Dimethyl sulfoxide and ethanol were used as solvent controls. The antimicrobial activity results are summarized in Table 1.

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## References

[1] R.K. Rawal, Y.S. Phabhakar, S.B. Kati, E. De Clercq, Bioorg. Med. Chem. 13 (2005) 6771–6776.
[2] C.G. Bonde, N.J. Gaikwad, Bioorg. Med. Chem. 12 (2004) 2151–2161.
[3] R.K. Rawal, R. Tripathi, S.B. Kati, C. Pannecouque, E. De Clercq, Bioorg. Med. Chem. 15 (2007) 1725–1731.
[4] P.P. Dixit, V.J. Patil, P.S. Nair, S. Jain, N. Sinha, S.K. Arora, Eur. J. Med. Chem. 41 (2006) 423–428.
[5] Z.A. Kaplancikli, G. Turan-Zitouni, A. Özdemir, G. Revial, Eur. J. Med. Chem. 43 (2008) 155–159.
[6] V.R. Solomon, C. Hua, H. Lee, Bioorg. Med. Chem. 18 (2010) 1563–1572.
[7] C. Hu, V.R. Solomon, G. Ulibarri, H. Lee, Bioorg. Med. Chem. 16 (2008) 7888–7893.
[8] C. Hu, V.R. Solomon, P. Cano, H. Lee, Eur. J. Med. Chem. 45 (2010) 705–709.
[9] V.V. Kouznetsov, A. Gomez-Barrio, Eur. J. Med. Chem. 44 (2009) 3091–3113.
[10] J. Adamec, R. Beckert, D. Weiss, V. Klimesova, K. Waisser, U. Mollmann, J. Kaustova, V. Buchta, Bioorg. Med. Chem. 15 (2007) 2898–2906.
[11] B. Meunier, Acc. Chem. Res. 41 (2008) 69–72.
[12] S.A.F. Rostom, I.M. El-Ashmawy, H.A. Abd El Razik, M.H. Badr, H.M.A. Ashour, Bioorg. Med. Chem. 17 (2009) 882–895.
[13] A.A. Bekhit, T. Abdel-Azneim, Bioorg. Med. Chem. 12 (2004) 1935–1945.
[14] E. Banoglu, Ç. Akoglu, S. Ünlü, E. Küpeli, E. Yesilada, M.F. Sahin, Arch. Pharm. 337 (2004) 7–14.
[15] S.C. Jain, J. Sinha, S. Bhagat, W. Errington, C.E. Olsen, Synth. Commun. 33 (2003) 563–577.
[16] A. Gürsoy, S. Demirayak, G. Capan, K. Erol, K. Vural, Eur. J. Med. Chem. 35 (2000) 359–364.
[17] S. Bondock, R. Rabie, H.A. Etman, A.A. Fadda, Eur. J. Med. Chem. 43 (2008) 2122–2129.
[18] B.S. Holla, R. Gonsalves, S. Shenoy, Il Farmaco 53 (1998) 574–578.
[19] S. Ersan, S. Nacak, R. Berkem, Il Farmaco 53 (1998) 773–776.
[20] H. Yüksek, A. Demirbaş, A. Ikizler, C.B. Johansson, C. Çelik, A.A. Ikizler, Arzn.-Forsh. Drug Res. 47 (1997) 405–409.
[21] A.A. Ikizler, F. Uçar, N. Demirbas, I. Yasa, A. Ikizler, T. Genzer, Indian J. Het. Chem. 61 (1999) 271–274.
[22] B. Tozkoparan, N. Gökhan, G. Aktay, E. Yeşilada, M. Ertan, Eur. J. Med. Chem. 34 (2000) 743–750.
[23] G. Turan-Zitouni, Z.A. Kaplancikli, K. Erol, F.S. Kilic, Il Farmaco 54 (1999) 218–223.
[24] N. Demirbas, R. Ugurluoglu, A. Demirbas, Bioorg. Med. Chem. 10 (2002) 3717–3723.
[25] H. Emilsson, H. Salender, J. Gaarder, Eur. J. Med. Chem. Chim. Ther. 21 (1985) 333–338.
[26] M. Kritsanida, A. Mouroutsou, P. Marakos, N. Pouli, S. Papakonstantinou-Garoufalias, C. Pannecouque, M. Witvouw, E. De Clercq, Il Farmaco 57 (2002) 253–257.
[27] P.K. Sahoo, E.R. Sharma, E.P. Pattanayak, Med. Chem. Res. 19 (2010) 127–135.
[28] C. Temperini, A. Cecchi, A. Scozzafava, C.T. Supuran, Bioorg. Med. Chem. 17 (2009) 1214–1221.
[29] S.S. Castle, Compr. Pharm. Ref (2008) 1–5.
[30] A.S. Crucq, C. Slegers, V. Deridder, B. Tilquin, Talanta 52 (2000) 873–877.
[31] P.X. Franklin, A.D. Pillai, P.D. Rathod, S. Yerande, M. Nivsarkar, H. Padh, K.K. Vasu, V. Sudarsanam, Eur. J. Med. Chem. 43 (2008) 129–134.
[32] A.A. Geronikaki, A.A. Lagunin, D.I. Hadjipavlou-Litina, P.T. Eleftheriou, D.A. Filimonov, V.V. Poroikov, I. Alam, A.K. Saxena, J. Med. Chem. 51 (2008) 1601–1609.
[33] S. Schenone, C. Brullo, O. Bruno, F. Bondavalli, A. Ranise, W. Filippelli, B. Rinaldi, A. Capuano, G. Falcone, Bioorg. Med. Chem. 14 (2006) 1698–1705.
[34] U. Salgın-Goksen, N. Gokhan-Kelekc, O. Goktas, Y. Koysal, E. Kılıc, S. Isık, G. Aktay, M. Ozalp, Bioorg. Med. Chem. 15 (2007) 5738–5751.
[35] C.L. Liu, Z.M. Li, B. Zhong, J. Flour. Chem. 125 (2004) 1287–1290.
[36] A. Cukurovali, I. Yilmaz, S. Gur, C. Kazaz, Eur. J. Med. Chem. 41 (2006) 201–207.
[37] A.M. Mahran, N.A. Hassan, Arch. Pharm. Res. 29 (2006) 46–49.
[38] C.J. Chen, B.A. Song, S. Yang, G.F. Xu, P.S. Bhadury, L.H. Jin, D.Y. Hu, Q.Z. Li, F. Liu, W. Xue, P. Lu, Z. Chen, Bioorg. Med. Chem. 15 (2007) 3981–3989.
[39] P. Karegoudar, M.S. Karthikeyan, D.J. Prasad, M. Mahalinga, B.S. Holla, N.S. Kumari, Eur. J. Med. Chem. 43 (2008) 261–267.
[40] G. Turan-Zitouni, M. Sıvacı, F.S. Kılıc, K. Erol, Eur. J. Med. Chem. 36 (2001) 685–689.
[41] H. Bayrak, A. Demirbaş, S. Alpay-Karaoğlu, N. Demirbaş, Eur. J. Med. Chem. 44 (2009) 1057–1066.
[42] H. Bayrak, A. Demirbas, N. Demirbas, S. Alpay-Karaoglu, Eur. J. Med. Chem. 44 (2009) 4362–4366.
[43] A. Demirbas, D. Sahin, N. Demirbas, S. Alpay-Karaoglu, Eur. J. Med. Chem. 44 (2009) 2896–2903.
[44] N. Demirbas, S. Alpay-Karaoglu, A. Demirbas, K. Sancak, Eur. J. Med. Chem. 39 (2004) 793–804.
[45] R. Milcent, C. Redeuilh, J. Het. Chem. 16 (1979) 403–407.
[46] C. Perez, M. Pauli, P. Bazerque, Acta Biologia Med. Experimentalis 15 (1990) 113–115.
[47] I. Ahmad, Z. Mehmood, F. Mohammed, J. Ethnopharmacology 62 (1998) 183–193.