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Graphical Abstract

Series of new pyrazole derivatives hybridized with five-membered heterocyclic moieties were evaluated for dual activity as antimalarial-antileishmanial agents. Six compounds displayed dual activity against malaria and leishmaniasis.

New heterocyclic hybrids of pyrazole and its bioisosteres: Design, synthesis and biological evaluation as dual acting antimalarial-antileishmanial agents

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ABSTRACT

A new series of pyrazole derivatives were synthesized by hybridization with five-membered heterocyclic moieties such as thiazoles, thiazolidinones, 1,3,4-thiadiazoles and pyrazolines. The compounds were evaluated for their *in vivo* antimalarial activity against *Plasmodium berghei* infected mice and the most active derivatives were further examined for their *in vitro* antimalarial activity against chloroquine resistant (RKL9) strain of *P. falciparum*. Compounds **2c**, **2d**, **4b**, **4c**, **4d**, **5a**, **6c**, **8c** and **9b** had more than 90% parasite suppression activity of that found with the antimalarial reference standard drug, chloroquine phosphate and had lower IC_{50} values than chloroquine. Compounds **4b** and **9b** were the most active derivatives, and their activities were 5-fold higher than chloroquine. All the newly synthesized compounds were evaluated for their *in vitro* antileishmanial activity against *Leishmania aethiopica* promastigotes and antiamastigote. The results showed that compounds **2c**, **2d**, **3d**, **4b**, **4c**, **4d** and **5a** had lower or similar IC_{50} values than the reference standard drugs, amphotericin B and miltefosine. Compound **3d** had the highest antileishmanial activity. Collectively, compounds **2c**, **2d**, **4b**, **4c, 4d** and **5a** exhibited dual activity against malaria and leishmaniasis and were safe and well tolerated by the experimental animals orally up to 300 mg/ kg and parenterally up to 100 mg/ kg.

Keywords

Pyrazole, thiazole, thiazolidinone, 1,3,4-thiadiazole, antimalarial, antileishmanial,.

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1. Introduction

Malaria and leishmaniasis are dangerous tropical protozoal diseases that continue to increase in prevalence and cause morbidity all over the world. Despite attempts to control malaria over the last few decades, its prevalence has increased in developing countries [1]. Leishmaniasis is very common disease in Africa. Globally, it has been estimated that there are about 12-15 million people currently infected with leishmaniasis and it is predicted that this number will increase by about 2 million annually as a result of new infected cases [2]. Current drugs used for the treatment of both diseases are effective, but resistance and side effects have been reported almost for all the current therapeutic agents [2].

Pyrazole derivatives are very interesting compounds that have been reported to possess versatile biological activities, including; anticancer [3, 4], anti-inflammatory [5, 6], antiviral [7, 8] and antimicrobial [9, 10] activities. Furthermore, several reports showed that pyrazole derivatives **A**[11], **B**[12], and **C**[13] had antimalarial activity, whereas compounds **D**[14], **E**[15], and **F**[16] exhibited antileishmanial activity (Figure 1).

There is high interest in utilizing different heterocyclic skeletons for the development of dual therapeutic drugs [12, 17]. It has been reported that some five-membered heterocyclic ring systems, such asthiazolidin-4-one **G** [18], thiazole **H** [19], 1,3,4-thiadiazole **I** [20] and pyrazoline **J** [21] (Figure 2), have beneficial biological activities against malaria and leishmaniasis. As a continuation of our research toward the discovery of new antimalarial and/or antileishmanial agents [12, 22-24]; we designed, synthesized some pyrazole hybrids with these five-membered bioisosteres **I** and **II** (Figure 3) and evaluated the compounds for their dual activity as antimalarial-antileishmanial agents. The target compounds were designed to be attached to different moieties to impart variable electronic and lipophilic properties with the aim of understanding the effects of these properties on the antimalarial and/or antileishmanial activities. Nitro-heterocyclic compounds were found to be effective against various bacterial and parasitic infections due to nitro-reductases mediated activation within the pathogen that produce toxic effects on bacteria and parasites [25]. Therefore, a nitro group was added as one of the substituents to increase the antiprotozoal activities of the targeted compounds.

2. Chemistry

The target compounds were synthesized as outlined in Schemes 1 and 2. In scheme 1, the starting compounds 1,3-diaryl-1*H*-pyrazole-4-carboxaldehydes **(1a-d)** were synthesized by reacting their hydrazones with Vilsmeir-Haack reagent, as described earlier [26-28]. The 1,3-diaryl-1*H*-pyrazole-4-carboxaldehydes **(1a-d)** were then reacted with phenylthiosemicarbazide to obtain the key intermediate *N*-phenylhydrazinecarbothioamide derivatives **(2a-d)** [29-31], which were cyclized with acetic anhydride to give diacetyl-1,3,4-thiadiazole derivatives **(3a-d)**[30, 32], or with ethylbromoacetate to afford 3-aryl-4-thiazolidinone derivatives **(4a-d)** [30, 32, 33], or with 4-bromophenacyl bromide to yield the corresponding 3,4-diarylthiazole derivatives **(5a-d)**[7, 34].

In scheme 2, the key intermediates 3-(1,3-diaryl-1*H*-pyrazol-4-yl)-1-phenylprop-2-en-1-ones (**6a-c)** were afforded from the condensation of the starting pyrazole aldehydes **1a, 1b** and **1c** with acetophenonein alcoholic solution of potassium hydroxide [35, 36]. The obtained pyrazoline derivatives intermediates **(6a-c)** were then reacted with phenylhydrazine hydrochloride in acidic medium to afford 1,3-diaryl-4-(1,3-diphenyl-4,5-dihydro-1*H*-pyrazol-5-yl)pyrazoles **7a-c** [35, 37] or with hydrazine hydrate in ethanol, acetic acid and propanoic acid to give 1,3-diaryl-4-(3-phenyl-4,5-dihydro-1*H*-pyrazol-5-yl)pyrazoles (**8a-c)** [35, 38, 39], 1,3-diaryl-4-(1-acetyl-3-phenyl-4,5-dihydro-1*H*-pyrazol-5-yl)pyrazoles (**9a-c)** [35, 40] and 1,3-diaryl-4-(3-phenyl-1-propanoyl-4,5-dihydro-1*H*-pyrazol-5-yl)pyrazoles (**10a-c)** [41], respectively.

3. Results and discussion

3.1. Analytical results

The IR spectra of the starting pyrazole 4-carboxaldehydes **(1a-d)** showed a characteristic peak at 1669-1688 cm^{-1}, which was attributed to the aldehydic C=O group. Their ^{1}H-NMR spectra displayed two singlet that were attributed to pyrazole-C_5-H and the aldehydic proton. The ^{1}H-NMR spectra of carbothioamide derivatives **(2a-d)** showed the disappearance of the aldehydic proton and the existence of two deuterium exchangeable singlet, which were attributed to CH=N-NH-CS-NH-phenyl. The ^{1}H-NMR spectra of thiadiazole, thiazolidinone and thiazoline derivatives **(3a-d)**, **(4a-d)** and **(5a-d)** lacked deuterium exchangeable singlet, which is characteristic for the two NH of thiosemicarbazone moiety. Compounds **(3a-d)** showed a singlet of thiadiazole-C_5 proton at 7.08-7.17 ppm, while compounds **(4a-d)** and **(5a-d)** showed one singlet at 4.11-4.15 ppm and a singlet at 6.70-6.77 ppm, which were assigned for thiazolidine-C_5 proton and thiazoline-C_5 proton, respectively. The ^{1}H-NMR spectra of chalcones **(6a-c)** showed two doublets that appeared in the range of 7.59-7.98 ppm; each was integrated for one proton and was assigned to the vinylic protons. The *E* configuration was obtained as the coupling constant of the vinylic protons and was equal to 15.3 Hz [42].

The IR spectra of all pyrazoline derivatives **(7-10 a-c)** lacked the carbonyl peak in the corresponding chalcones, which verified their synthesis. ^{1}H-NMR spectra of target pyrazoline derivatives showed the characteristic peaks for pyrazoline-C_4 and C_5 protons, at their expected chemical shifts. ^{1}H-NMR spectra of compounds **(8a, b)** showed broad singlet at 7.51-7.55 ppm, which is deuterium exchangeable and was assigned for pyrazoline NH proton, whereas NH proton in compound **8c** appeared as doublet, due to coupling with pyrazoline-C_5 proton. The ^{1}H-NMR spectra of compounds **(9a-c)** showed singlet representing the $COCH_3$group. In compounds **(10a-c),** characteristic triplet and quartet peaks of propanoyl protons were found.

3.2. Biological screening

3.2.1. In vivo antimalarial activity testing against P. berghei

Results for the *in vivo* anti-malarial activity of the synthesized compounds are shown in Table 1. All the compounds showed parasitaemia level lower than the negative control (< 50% of that found in the control), indicating that they have significant suppressive effect

against *P. berghei*. Compounds **2c**, **2d**, **4b**, **4c**, **4d**, **5a**, **6c**, **8c** and **9b** exhibited the highest activity with percent suppression > 90%.

Pyrazolethiosemicarbazone derivatives (**2c** and **2d**) had high antimalarial activity, and the activity was increased upon cyclization into 3-aryl-4-thiazolidinone (**4b, 4c** and **4d**). Also, the antimalarial activity of pyrazolethiosemicarbazone derivatives (**2a** and **2b**) was significantly increased upon cyclization into 4,5-diarylthiazole (**5a** and **5b**). The low dissolution rate of some derivatives of 3-phenyl-2,3-dihydrothiazole (**5c** and **5d**) may explain their lower percent suppression compared to their similar analogs. The presence of three atoms spacer between the pyrazole ring and its bioisosteres in the cyclized derivatives exhibited better antimalarial activity than the directly attached ones. For instance, compounds obtained from the hybridization between pyrazole and 4-thiazolidinone (**4b**, **4c** and **4d)** or thiazole moiety (**5a)** showed better antimalarial activity than the hybridization with 1,3,4-thiadiazole (**3a-d)** or pyrazoline moieties except in **(8c** and **9b)**.

It was clear that most of the highly active antimalarial compounds were bearing a nitro group (**2c**, **2d**, **4c**, **4d**, **6c**, **8c**), which highlight the importance of nitro compounds in treatment of some infectious diseases. Compound **8c** was almost as active as its chalcone **6c**. This probably can be attributed to the presence of nitro groups in both compounds that might be responsible for activity and masked any minor difference due to synthesis. Hybridization between pyrazole and pyrazoline ring possessed a significant antimalarial activity and this activity varied according to the substitution at position 1. The 1*H*-pyrazoline derivatives (**8a-c)** showed good activity, which was slightly increased when *H* was replaced by phenyl (**7a-c)**, with the exception of **8c,** which was more active than **7c**. The 1- acetylpyrazoline derivatives (**9a** and **9b)** showed higher activity than unsubstituted pyrazoline (**8a** and **8b)** and phenylpyrazoline (**7a-c)** compounds. Compound **9c** was less active compared to **9a** and **9b**, which might be due to the low solubility of this compound and likely subsequent low absorption. The 1-propanoylpyrazoline derivatives (**10a-c)** showed low antimalarial activity compared with their parent unsubstituted compounds. Generally, hybridization of pyrazoles with other bioisosteres increased the antimalarial activity more than compounds lacking hybridization **(2a-d)** and **(6a-c)**.

3.2.2. In vitro antimalarial activity testing against P. falciparum

The most active compounds **2c**, **2d**, **4b**, **4c**, **4d**, **5a**, **6c**, **8c** and **9b** that showed the highest *in vivo* percent suppression against *P. berghei*, were then evaluated for their *in vitro* anti-plasmodial activity against chloroquine resistant (RKL9) strains of *P. falciparum* using the standard method of Trager and Jensen [43]. Results revealed that all selected compounds had greater activity than chloroquine phosphate (IC_{50} = 0.1920 μM) against chloroquine resistant (RKL9) strain of *P. falciparum*. Compounds **4b** and **9b** were the most potent against RKL9 strains (Table 2). This finding was rationalized by performing molecular docking for these compounds on *P. falciparum* dihydrofolate reductase (*Pf*-DHFR) active site.

3.2.3. In vitro antileishmanial activity on L. aethiopica promastigotes

A quantitative colorimetric assay using the oxidation-reduction indicator Alamar blue® was developed to measure cytotoxicity of the synthesized compounds against the protozoan parasite *L. aethiopica*. Alamar blue assay was used to determine the viability of promastigotes and evaluate the antileishmanial activity of the synthesized compounds. Compounds **2c**, **2d**,

3d, **4b**, **4c**, **4d** and **5a** showed higher activity than the reference standard drugs amphotericin B and miltefosine, whereas compounds **1a**, **1c**, **1d**, **2b**, **5b**, **6a** and **7c** showed lower antileishmanial activity compared to both standard drugs. Other compounds were found to be less active than amphotericin B but more potent than miltefosine (Table 3). The antileishmanial activity of the most active compounds was rationalized through molecular docking of these compounds in the active site of *Leishmania major* pteridine reductase crystal structure *Lm*-PTR1.

The hybridization between pyrazole and 3-aryl-4-thiazolidinone **(4b**, **4c**, **4d)** or 3,4-diarylthiazole **(5a)**, through three atoms spacers, showed high inhibitory effect on the promastigotes, with IC_{50} values ranging from 0.0142- 0.0341 µg/ml. Some carbothioamide derivatives **(2c** and **2d)** also showed high inhibitory effect. The nitrated carbothioamide derivatives (**2c** and **2d**) and their cyclized 4-thiazolidinone derivatives (**4c** and **4d**) showed higher antileishmanial activity than the non-nitrated compounds. This may be explained by the presence of nitro group that may contribute the activity of some trypanosomes, especially in *Leishmania*.

The hybridization with 1,3,4-thiadiazole, compounds **3a**, **3b** and **3c,** resulted in lower inhibitory effect on the promastigotes compared to amphotericin B. This may be due to the direct attachment of the heterocyclic ring to the parent pyrazole ring. An exceptionally high antileishmanial activity was found for compound **3d**. This high activity was further explored using molecular docking to PTR1 active site.

The attachment of pyrazoline ring directly to the parent pyrazole resulted in pronounced antileishmanial activity. For example, unsubstituted pyrazoline derivatives **8a-c** possessed better antileishmanial activity than their phenyl-substituted derivatives **7a-c**. Similarly, 1-propanoylpyrazoline derivatives **10a-c** had better antileishmanial activity compared to their phenyl substituted ones **7a-c** and the 1-acetylpyrazoline derivatives (**9a** and **9b**). The higher antileishmanial activity of 1-acetylpyrazoline derivative **9c** (IC50 = 1.0882 µg / ml) relative to all other pyrazoline derivatives may be explained by its strong binding affinities with receptor active site, good solubility and the presence of nitro group in its structure. Most of the 1,3-diarylpyrazole-4-carboxaldehyde derivatives (**1a**, **1c** and **1d**) showed pronounced antileishmanial activity but their activities were lower than the standard drug miltefosine. However, the condensation of these aldehydes into carbothioamides **2a-d** and chalcones **6a-c** resulted in increased antileishmanial activity.

2.2.4. In vitro antileishmanial activity on L. aethiopica amastigotes

The nitrophenylpyrazolylthiadiazole **3d** and the nitrophenylpyrazolylthiazolidinone **4d** had lower IC_{50} values than amphotericin B and miltefosine against *L. aethiopica* amastigotes. The nitrophenylpyrazolylthiosemicarbazones **2c** and **2d,** as well as nitrophenylpyrazolylthiazolidinone **4c** were less active than amphotericin B but were more potent than miltefosine (Table 4). The thiazoline derivative **5a** was found to be equipotent to miltefosine against *L. aethiopica* amastigotes.

3.2.5. In vivo acute toxicity testing

The most active antimalarial and antileishmanial compounds, **2c**, **2d**, **3d**, **4b**, **4c**, **4d**, **5a**, **6c**, **8c** and **9b**, were tested for their toxicity in mice. The experimental mice did not have

any toxicity signs after treatment with test compounds. There was no significant difference in the weight of the mice and no death cases was recorded during 3 days of observation post administration of the test compounds (data not shown). The test compounds were well tolerated by the experimental animals orally up to 300 mg/kg. Moreover, these compounds were tested for their toxicity through the parenteral route and the results revealed that all the selected test compounds were nontoxic up to 100 mg/ kg.

3.3. Molecular docking

In the current study, molecular docking was performed in order to rationalize the obtained biological results. The interactions of the synthesized compounds with the active site of target macromolecules were investigated to study the mode of binding and their orientations and related to their antimalarial and/ or antileishmanial activity. Two characteristic proteins were used: *Plasmodium falciparum* dihydrofolate reductase *Pf*-DHFR (PDB ID: 1J3I) and *Leishmania major* pteridine reductase *Lm*-PTR (PDB ID: 2BFM).

3.3.1. Antimalarial docking study

The binding site of *P. falciparum* wild-type dihydrofolate reductase *Pf*-DHFR (PDB ID: 1J3I) was explored computationally, which provides some insights in the discovery of novel antimalarial drugs and necessary structural requirement for antifolates in antimalarial chemotherapy [44]. The interaction of *Pf*-DHFR with its co-crystallized ligand 6,6-dimethyl-1-[3-(2,4,5-trichlorophenoxy)propoxy]-1,6-dihydro-1,3,5-triazine-2,4-diamine (WR99210) showed hydrogen bond interaction with Ile 14, Asp 54 and Ile 164, in addition to hydrophobic interactions with other amino acid residues such as Cys 15, Ala 16, Met 55 and Phe 58 (Figure 4). Docking results for compound **4b** showed that thiazolidinone carbonyl moiety exhibited hydrogen bond interaction with Arg 122 (Figure 5). On the other hand, compound **9b** revealed hydrogen bondg interaction between the pyrazoline moiety and Ser 108 (Figure 6).

3.3.2. Antileishmanial docking study

The binding site of *Leishmania major* pteridine reductase *Lm*-PTR1 (PDB ID: 2BFM) was explored computationally, which provides discernment of the novel antileishmanial drugs. PTR1 represents a target for the development of improved therapies for infections caused by this protozoan [45]. The determination of three dimensional co-crystal structure of *Lm*-PTR1 complex with 5-[(3,4,5-trimethoxyphenyl)methyl]pyrimidine-2,4-diamine (trimethoprim, TOP) showed hydrogen bond interaction with Tyr 194, a water molecule is trapped between the diaminopyrimidine, which led to a hydrogen bond with TOP N_2, and Ser 111, as well as hydrophobic interactions with Phe 113 and other amino acid residues (Figure 7). Leishmanial docking results for compound **3d** showed hydrogen bond interaction between acetamide carbonyl moiety and Arg 17, with scoring of interactions equal to 54% which was the best scoring result among all tested compounds (Figure 8). Compound **4c** formed hydrogen bonding interaction between side chain nitrogen and Tyr 194, in addition to other hydrophobic interactions with Phe 113 and Arg 17 (Figure 9).

4. Conclusion

The objectives of the present study were to design, synthesize and investigate the antimalarial and antileishmanial activities of some pyrazole hybrids with five-membered bioisosteres to synthesize new structure leads serving as dual antimalarial-antileishmanial agents. Results of the *in vivo* antimalarial screening in mice infected with chloroquine sensitive *P. berghei* revealed that compounds **2c**, **2d**, **4b**, **4c**,**4d**, **5a**, **6c**, **8c** and **9b** exhibited parasitemia suppression > 90%. These compounds were further tested for their *in vitro* antimalarial activity against chloroquine resistant (RKL9) strain of *P. falciparum*. These compounds showed IC_{50} values better than the standard drug chloroquine phosphate. Compounds **4b** and **9b** were the most active and were 5-fold more active than chloroquine. These findings were supported by the docking results obtained for these molecules, which demonstrated that these compounds established hydrogen bond interactions with some amino acid residues in the pocket of *Pf*-DHFR, in addition to some hydrophobic interactions with good scoring results.

The *in vitro* antipromastigote activity demonstrated that compounds **2c**, **2d**, **3d**, **4b**, **4c**, **4d** and **5a** had IC_{50} better than both standard drugs miltefosine and amphotericin B deoxycholate, which indicates their high antileishmanial activity against *L. aethiopica*. Compound **3d** was 3-fold more active than amphotericin and 225 folds more active than miltefosine. Furthermore, compounds **3d** and **4d** showed lower IC_{50} values than amphotericin B and miltefosine against *L. aethiopica* amastigotes. These results were rationalized through molecular docking. These compounds showed hydrogen bonding with some amino acid residues in *Lm*-PTR1 active site, which showed good binding profile. Toxicity studies for the most active compounds indicated their safety orally and parenterally up to 300 and 100 mg/ kg, respectively. In conclusion, compounds **2c**, **2d**, **4b**, **4c**, **4d** and **5a** demonstrated dual activity against both malaria and leishmania and represent fruitful scaffolds for the development of dual acting antileishmanial-antimalarial agents.

5. Experimental:

5.1. Chemistry

Melting points were determined in open-glass capillaries using a Griffin melting point apparatus and are all uncorrected. Infrared spectra (IR) were recorded on Perkin-Elmer 1430 infrared spectrophotometer. ^{1}H-NMR and ^{13}C-NMR were scanned on Jeol-400 MHz NMR-spectrometer (DMSO-d6) and chemical shifts are given in δ (ppm) downfield from tetramethylsilane (TMS) as internal standard. Microanalyses were performed on Vario El Fab-Nr elemental analyzer. Following up of the reactions as performed by thin-layer chromatography (TLC) on silica gel (60 GF254) coated glass plates and the spots were visualized by exposure to iodine vapors or UV-lamp at λ 254 nm for few seconds.

5.1.1. General method for preparation of 1,3-diaryl-1H-pyrazole-4-carboxaldehydes 1a-d

Few drops of glacial acetic acid, p-substituted phenylhydrazine hydrochloride (25 mmole) and anhydrous sodium acetate (2.05 g, 25 mmole) were added to a solution of p-substituted acetophenone (25 mmole) in ethanol (10 ml). The reaction mixture was heated under reflux for 2-5 h, and then allowed to cool to room temperature. The separated solid product was filtered, washed with ethanol and dried. $POCl_3$ (7.20 g, 4.3 ml, 25 mmole) was

added to dry dimethylformamide (19.38 g, 20.4 ml, 265 mmole) in ice bath drop wise over a period of 30 minutes, stirred for 45 minutes, then the previously prepared hydrazone was added drop wise at 0 °C, stirred and left to reach room temperature The reaction mixture was then heated at 70–80 °C for 3-5 h. The reaction mixture was poured onto crushed ice and boiled. The precipitate obtained was then filtered, washed with water, dried and crystallized from ethanol.

1,3-Di(4-methylphenyl)-1H-pyrazole-4-carboxaldehyde ***1a***

The product was obtained as a yellow solid. Yield 85%; m.p. 130°C; IR (cm^{-1}): 1670 (C=O), 1519 (C=N); ^{1}H-NMR (DMSO-d_6, δ ppm): 2.36, 2.38 (2s, each 3H, $2CH_3$), 7.31 (d, *J* = 8 Hz, 2H, C-C_6H_4-CH_3-$C_{3,5}$-H), 7.36 (d, *J* = 7.8 Hz, 2H, N-C_6H_4-CH_3-$C_{3,5}$-H), 7.82 (d, *J* = 7.8 Hz, 2H, N-C_6H_4-CH_3-$C_{2,6}$-H), 7.86 (d, *J* = 8 Hz, 2H, C-C_6H_4-CH_3-$C_{2,6}$-H), 9.21 (s, 1H, pyrazole C_5-H); 9.96 (s, 1H, CHO). ^{13}C-NMR (DMSO-d_6, δ ppm):21.02, 21.42 (2 CH_3), 119.61 (N-methylphenyl-$C_{2,6}$), 122.45 (pyrazole-C_4), 129.02 (C-methylphenyl-C_1), 129.08 (C-methylphenyl-$C_{2,6}$), 129.62 (C-methylphenyl-$C_{3,5}$), 130.56 (N-methylphenyl-$C_{3,5}$), 134.91 (pyrazole-C_5), 136.91 (C-methylphenyl-C_4), 137.70 (N-methylphenyl-C_4), 139.18 (N-methylphenyl-C_1), 153.08 (pyrazole-C_3), 185.08 (C=O); Elemental analysis: Calcd for $C_{18}H_{16}N_2O$: C 78.24, H 5.84; N 10.14, found C78.51, H 5.64, N 10.32.

1-(4-Bromophenyl)-3-(4-methylphenyl)-1H-pyrazole-4-carboxaldehyde ***1b***

The compound was obtained as a yellow solid. Yield 91%; m.p. 138-140°C; IR (cm^{-1}): 1669 (C=O), 1520 (C=N), 725 (C-Br); ^{1}H-NMR (DMSO-d_6, δ ppm): 2.37 (s, 3H, CH_3), 7.31 (d, *J* = 7.65 Hz, 2H, C-C_6H_4-CH_3-$C_{3,5}$-H), 7.75 (d, *J* = 8 Hz, 2H, C_6H_4-Br-$C_{2,6}$-H), 7.81 (d, *J* = 8 Hz, 2H, C_6H_4-Br-$C_{3,5}$-H), 7.95 (d, *J* = 7.65 Hz, 2H, C-C_6H_4-CH_3-$C_{2,6}$-H), 9.30 (s, 1H, pyrazole C_5-H), 9.97 (s, 1H, CHO); EIMS m/z (% relative abundance): 342 (100) ($M^{+\bullet}$+2), 341 (55), 340 (100) ($M^{+\bullet}$), 399 (34), 325 (42), 232 (11), 157 (12), 155 (13), 130 (14), 116 (15), 115 (15), 91 (26), 90 (11), 89 (16), 80 (34), 77 (11), 76 (22), 75 (19), 65 (17), 64 (17), 63 (13); Elemental analysis Calcd for $C_{17}H_{13}BrN_2O$: C 59.84, H 3.84, N 8.21, found C 60.02, H 3.58, N 8.44.

1-(4-Methylphenyl)-3-(4-nitrophenyl)-1H-pyrazole-4-carboxaldehyde ***1c***

The compound was obtained as a yellow solid. Yield 80%; m.p. 150-152°C; IR (cm^{-1}): 1679 (C=O), 1601 (C=N), 1516, 1345 (NO_2); ^{1}H-NMR (DMSO-d_6, δ ppm): 2.38 (s, 3H, CH_3), 7.40 (d, *J* = 8.6 Hz, 2H, C_6H_4-CH_3-$C_{3,5}$-H), 7.88 (d, *J* = 8.6 Hz, 2H, C_6H_4-CH_3-$C_{2,6}$-H), 8.27 (d, *J* = 7.8 Hz, 2H, C_6H_4-NO_2-$C_{2,6}$-H), 8.37 (d, *J*= 7.8 Hz, 2H, C_6H_4-NO_2-$C_{3,5}$-H), 9.37 (s, 1H, pyrazole C_5-H), 10.01 (s, 1H, CHO); Elemental analysis Calcd for $C_{17}H_{13}N_3O_3$: C 66.44, H 4.26, N 13.67, found C 66.40, H 4.38, N 13.39.

1-(4-Bromophenyl)-3-(4-nitrophenyl)-1H-pyrazole-4-carboxaldehyde ***1d***

The compound was obtained as a yellow solid. Yield 40%; m.p. 198-200°C; IR (cm^{-1}): 1688(C=O), 1602 (C=N), 1525, 1344 (NO_2), 713(C-Br); ^{1}H-NMR (DMSO-d_6, δ ppm): 7.80 (d, *J* = 8.3 Hz, 2H, C_6H_4-Br-$C_{2,6}$-H), 7.99 (d, *J* = 8.3Hz, 2H, C_6H_4-Br-$C_{3,5}$-H), 8.27 (d, *J* = 8.6 Hz, 2H, C_6H_4-NO_2-$C_{2,6}$-H), 8.36 (d, *J* = 8.6 Hz, 2H, C_6H_4-NO_2-$C_{3,5}$-H), 9.46 (s, 1H, pyrazoleC_5-H), 10.03 (s, 1H, CHO); Elemental analysis Calcd for $C_{16}H_{10}BrN_3O_3$: C 51.63, H 2.71, N 11.29; found C 51.82, H 2.93, N 11.32.

5.1.2. General method for preparation of2-[(1,3-diaryl-1H-pyrazol-4-yl)methylene]-N-phenylhydrazinecarbothioamides 2a-d

An equivalent amount of phenylthiosemicarbazide (0.67 g, 4 mmole) was added to a suspension of the appropriate 1,3-diaryl-1*H*-pyrazole-4-carboxaldehyde **1a-d** (4 mmole) in absolute ethanol (15 ml). The reaction mixture was heated under reflux for 2-4 h and allowed to cool to room temperature. The separated solid product was filtered, washed with ethanol and crystallized from proper solvent(s).

2-[(1,3-Di(4-methylphenyl)-1H-pyrazol-4-yl)methylene]-N-phenylhydrazine-carbothioamide ***2a***

The product was crystallized from DMF:ethanol (1:5) as a white solid. Yield 99%; m.p. 194-196°C; IR (cm^{-1}): 3330 (NH), 1596 (C=N), 1542, 1268, 1204, 957 (NCS amide I, II, III and IV bands, respectively); ^{1}H-NMR (DMSO-d_6, δ ppm): 2.41, 2.42 (2s, each 3H, $2CH_3$), 7.27 (t, *J* = 9 Hz, 1H, phenyl-C_4-H), 7.36-7.47 (m, 6H, phenyl-$C_{2,3,5,6}$-H and C-C_6H_4-CH_3-$C_{3,5}$-H), 7.62- 7.66 (m, 4H, N-C_6H_4-CH_3-H), 7.83 (d, *J* = 8.25 Hz, 2H, C-C_6H_4-CH_3-$C_{2,6}$-H), 8.37 (s, 1H, N=CH), 9.20 (s, 1H, pyrazole-C_5-H), 9.81, 11.75 (2s, each 1H, N-NH and CS-NH, D_2O exchangeable); Elemental analysis Calcd for $C_{25}H_{23}N_5S$: C 70.56, H 5.45, N 16.46, found C 69.98, H 5.63; N 16.12.

2-[(1-(4-Bromophenyl)-3-(4-methylphenyl)-1H-pyrazol-4-yl)methylene]-N-phenylhydrazine-carbothioamide ***2b***

The product was crystallized from DMF:ethanol (1:5) as an off white solid. Yield 98%; m.p. 258-260°C; IR (cm^{-1}): 3433 (NH), 1623 (C=N), 1535, 1296, 1214, 954 (NCS amide I, II, III and IV bands, respectively), 729 (C-Br); ^{1}H-NMR (DMSO-d_6, δ ppm): 2.38 (s, 3H, CH_3), 7.22 (t, *J* = 7.35 Hz, 1H, phenyl-C_4-H), 7.34 (d, *J* = 7.8 Hz, 2H, C_6H_4-CH_3-$C_{3,5}$-H), 7.42 (t, *J* = 7 Hz, 2H, phenyl-$C_{3,5}$-H), 7.59 (d, *J* = 7.95Hz, 2H, C_6H_4-Br-$C_{2,6}$-H), 7.60 (d, *J* = 7.95 Hz, 2H, C_6H_4-Br-$C_{3,5}$-H), 7.76 (d, *J* = 8.7 Hz, 2H, C_6H_4-CH_3-$C_{2,6}$-H), 7.87 (d, *J* = 8.4 Hz, 2H, phenyl-$C_{2,6}$-H), 8.32 (s, 1H, N=CH), 9.23 (s, 1H, pyrazole-C_5-H), 9.75, 11.74 (2s, each 1H, N-NH and CS-NH, D_2O exchangeable); EIMS m/z (% relative abundance): 491 (72) ($M^{+\bullet}$+2), 489 (58) ($M^{+\bullet}$), 129 (100); Elemental analysis Calcd for $C_{24}H_{20}BrN_5S$: C 58.78, H 4.11, N 14.28, found C 59.12, H 3.98, N 13.95.

2-[(1-(4-Methylphenyl)-3-(4-nitrophenyl)-1H-pyrazol-4-yl)methylene]-N-phenyl-hydrazinecarbothioamide ***2c***

The product was crystallized from DMF:H_2O (9:1) as a yellow solid. Yield 85%; m.p. 270-272°C; IR (cm^{-1}): 3322 (NH), 1596 (C=N), 1550, 1333, 1200, 960 (NCS amide I, II, III and IV bands, respectively), 1550, 1333 (NO_2); ^{1}H-NMR (DMSO-d_6, δ ppm): 2.38 (s, 3H, CH_3), 7.22 (t, *J* = 7.95 Hz, 1H, phenyl-C_4-H), 7.36-7.41 (m, 4H, phenyl-$C_{3,5}$-H and C_6H_4-CH_3-$C_{3,5}$-H), 7.58 (d, *J* = 7.5 Hz, 2H, phenyl-$C_{2,6}$-H), 7.82 (d, *J* = 8.4 Hz, 2H, C_6H_4-CH_3-$C_{2,6}$-H),

8.02 (d, *J* = 8.85 Hz, 2H, C_6H_4-NO_2-$C_{2,6}$-H), 8.36 (d, *J* = 8.85 Hz, 2H, C_6H_4-NO_2-$C_{3,5}$-H), 8.38 (s, 1H, N=CH), 9.23 (s, 1H, pyrazole-C_5-H), 9.78, 11.75 (2s, each 1H, N-NH and CS-NH, D_2O exchangeable); ^{13}C-NMR (DMSO-d_6, δ ppm): 20.48 (CH_3), 117.46 (pyrazole-C_4), 118.74 (N-methylphenyl-$C_{2,6}$), 123.90 (NO_2-phenyl-$C_{3,5}$), 125.32 (NO_2-phenyl-$C_{2,6}$), 128.18 (phenyl-$C_{2,6}$), 128.51 (phenyl-C_4), 129.15 (phenyl-$C_{3,5}$), 130.07 (N-methylphenyl-$C_{3,5}$), 135.69 (pyrazole-C_5), 135.15 (methylphenyl-C_4), 136.97 (phenyl-C_1), 138.61 (NO_2-phenyl-C_1), 138.75 (HC=N), 147.14 (NO_2-phenyl-C_4), 148.94 (pyrazole-C_3), 175.31 (C=S); Elemental analysis Calcd for $C_{24}H_{20}N_6O_2S$: C 63.14, H 4.42, N 18.41, found C 63.22, H 4.63, N 18.68.

2-[(1-(4-Bromophenyl)-3-(4-nitrophenyl)-1H-pyrazol-4-yl)methylene]-N-phenyl-hydrazinecarbothioamide ***2d***

The product was crystallized from dioxane:H_2O (9:1) as a yellow solid. Yield 98%; m.p. > 300°C; IR (cm^{-1}): 3439 (NH), 1598 (C=N), 1549, 1254, 1108, 960 (NCS amide I, II, III and IV bands, respectively), 1549, 1337 (NO_2), 705 (C-Br); ^{1}H-NMR (DMSO-d_6, δ ppm): 7.22 (t, *J* = 6.6 Hz, 1H, phenyl-C_4-H), 7.38 (t, *J* = 7.8 Hz, 2H, phenyl-$C_{3,5}$-H), 7.58 (d, *J* = 8.55 Hz, 2H, C_6H_4-Br-$C_{2,6}$-H), 7.79 (d, *J* = 8.55 Hz, 2H, C_6H_4-Br-$C_{3,5}$-H), 7.91 (d, *J* = 8.4 Hz, 2H, phenyl-$C_{2,6}$-H), 8.02 (d, *J* = 8.25 Hz, 2H, C_6H_4-NO_2-$C_{2,6}$-H), 8.36 (d, *J* = 8.25 Hz, 2H, C_6H_4-NO_2-$C_{3,5}$-H), 8.37 (s, 1H, N=CH), 9.30 (s, 1H, pyrazole-C_5-H), 9.76, 11.78 (2s, each 1H, N-NH and CS-NH, D_2O exchangeable); Elemental analysis Calcd for $C_{23}H_{17}BrN_6O_2S$: C 52.98, H 3.29, N 16.12, found C 53.22, H 3.02, N 15.88.

5.1.3. General method for preparation ofN-[4-acetyl-5-(1,3-diaryl-1H-pyrazol-4-yl)-4,5-dihydro-1,3,4-thiadiazol-2-yl]-N-phenylacetamides 3a-d

A solution of the selected thiosemicarbazone **2a-d** (1 mmole) in acetic anhydride (5 ml) was heated under reflux for 3-5 h. The reaction mixture was left to attain room temperature, cold water (10 ml) was then added and the mixture was stirred for 30 min to decompose excess acetic anhydride. The separated solid product was filtered, washed with water, dried and crystallized from the proper solvent.

N-{4-Acetyl-5-[1,3-di(4-methylphenyl)-1H-pyrazol-4-yl]-4,5-dihydro-1,3,4-thiadiazol-2-yl}-N-phenylacetamide ***3a***

The product was crystallized from ethanol:H_2O (3:1) as a yellow solid. Yield 80%; m.p. of 152-154°C; IR (cm^{-1}): 1678, 1657 (C=O), 1593 (C=N), 1298, 1064 (C-S-C); ^{1}H-NMR (DMSO-d_6, δ ppm): 1.87, 1.88 (2s, each 3H, 2$COCH_3$), 2.35, 2.38 (2s, each 3H, 2CH_3), 7.08 (s, 1H, thiadiazole C_5-H), 7.31 (d, *J* = 8.1 Hz, 2H, C-C_6H_4-CH_3-$C_{3,5}$-H), 7.32 (d, *J* = 8.4 Hz, 2H, N-C_6H_4-CH_3-$C_{3,5}$-H), 7.50- 7.54 (m, 5H, phenyl-H), 7.62 (d, *J* = 8.4 Hz, 2H, N-C_6H_4-CH_3-$C_{2,6}$-H), 7.76 (d, *J* = 8.4 Hz, 2H, C-C_6H_4-CH_3-$C_{2,6}$-H), 8.22 (s, 1H, pyrazole C_5-H); Elemental analysis Calcd for $C_{29}H_{27}N_5O_2S$: C 68.35, H 5.34, N 13.74, found C 68.52, H 5.64, N 13.98.

*N-{4-Acetyl-5-[1-(4-bromophenyl)-3-(4-methylphenyl)-1H-pyrazol-4-yl]-4,5-dihydro-1,3,4-thiadiazol-2-yl}-N-phenylacetamide****3b***

The product was crystallized from ethanol:H_2O (3:1) obtained as a yellow solid. Yield 78%; m.p. 190-192°C; IR (cm^{-1}): 1688, 1662 (C=O), 1592 (C=N), 1233, 1068 (C-S-C), 703 (C-Br); ^{1}H-NMR (DMSO-d_6, δ ppm): 1.85, 1.88 (2s, each 3H, 2$COCH_3$), 2.38 (s, 3H, CH_3),

7.09 (s, 1H, thiadiazole C_5-H), 7.33 (d, *J* = 7.95 Hz, 2H, C_6H_4-CH_3-$C_{3,5}$-H), 7.67 (d, *J* = 8.85 Hz, 2H, C_6H_4-Br-$C_{2,6}$-H), 7.46-7.57 (m, 5H, phenyl-H), 7.70 (d, *J* = 8.85 Hz, 2H, C_6H_4-Br-$C_{3,5}$-H), 7.88 (d, *J* = 7.95 Hz, 2H, C_6H_4-CH_3-$C_{2,6}$-H), 8.33 (s, 1H, pyrazole C_5-H); EIMS m/z (% relative abundance): 576 (2) ($M^{+\bullet}$+3), 575 (4) ($M^{+\bullet}$+2); Elemental analysis Calcd for $C_{28}H_{24}BrN_5O_2S$: C 58.54, H 4.21, N 12.19, found C 58.81, H 4.50, N 11.84.

N-{4-Acetyl-5-[1-(4-methylphenyl)-3-(4-nitrophenyl)-1H-pyrazol-4-yl]-4,5-dihydro-1,3,4-thiadiazol-2-yl}-N-phenylacetamide ***3c***

The product was crystallized from dioxane:H_2O (9:1) as a yellow solid. Yield 60%; m.p. 198-200°C; IR (cm^{-1}): 1690, 1664 (C=O), 1597 (C=N), 1545, 1339 (NO_2), 1230, 1075 (C-S-C); ^{1}H-NMR (DMSO-d_6, δ ppm): 1.85, 1.87 (2s, each 3H, 2$COCH_3$), 2.36 (s, 3H, CH_3), 7.17 (s, 1H, thiadiazole C_5-H), 7.34 (d, *J* = 8.65 Hz, 2H, C_6H_4-CH_3-$C_{3,5}$-H), 7.51-7.54 (m, 5H, phenyl-H), 7.80 (d, *J* = 8.65 Hz, 2H, C_6H_4-CH_3-$C_{2,6}$-H), 8.06 (d, *J* = 8.85 Hz, 2H, C_6H_4-NO_2-$C_{2,6}$-H), 8.34 (d, *J* = 8.85 Hz, 2H, C_6H_4-NO_2-$C_{3,5}$-H), 8.40 (s, 1H, pyrazole C_5-H); ^{13}C-NMR (DMSO-d_6, δ ppm): 21.01 (methylphenyl-CH_3), 21.88 (thiadiazole-CO-$\underline{CH_3}$), 24.00 (thiadiazole-N-CO-$\underline{CH_3}$), 66.89 (thiadiazole-C_5), 119.18 (methylphenyl-$C_{2,6}$), 123.76 (pyrazole-C_4), 124.44 (NO_2-phenyl-$C_{3,5}$), 128.22 (pyrazole-C_5), 129.23 (NO_2-phenyl-$C_{2,6}$), 129.46 (phenyl-$C_{2,6}$), 129.70 (phenyl-C_4),130.14 (phenyl-$C_{3,5}$), 130.48 (methylphenyl-$C_{3,5}$), 137.04 (methylphenyl-C_4), 137.28 (methylphenyl-C_1), 139.58 (phenyl-C_1), 139.91 (NO_2-phenyl-C_1), 146.80 (NO_2-phenyl-C_4), 147.45 (thiadiazole-C_2), 149.17 (pyrazole-C_3), 168.47 (thiadiazole-C=O), 171.07 (thiadiazole-N-C=O); Elemental analysis Calcd for $C_{28}H_{24}N_6O_4S$: C 62.21, H 4.47, N 15.55, found C 61.92, H 4.22, N 15.84.

N-{4-Acetyl-5-[1-(4-bromophenyl)-3-(4-nitrophenyl)-1H-pyrazol-4-yl]-4,5-dihydro-1,3,4-thiadiazol-2-yl}-N-phenylacetamide ***3d***

The product was crystallized from dioxane:H_2O (9:1) as a yellow solid. Yield 94%; m.p. 222-224°C; IR (cm^{-1}): 1686, 1660 (C=O), 1599 (C=N), 1540, 1343 (NO_2), 1230, 1111 (C-S-C), 705 (C-Br); ^{1}H-NMR (DMSO-d_6, δ ppm):1.85, 1.87 (2s, each 3H, 2$COCH_3$), 7.17 (s, 1H, thiadiazole C_5-H), 7.45-7.55 (m, 5H, phenyl-H), 7.74 (d, *J* = 8.85 Hz, 2H, C_6H_4-Br-$C_{2,6}$-H), 7.92 (d, *J* = 8.85 Hz, 2H, C_6H_4-Br-$C_{2,6}$-H), 8.07 (d, *J* = 8.85 Hz, 2H, C_6H_4-NO_2-$C_{2,6}$-H), 8.34 (d, *J* = 8.85 Hz, 2H, C_6H_4-NO_2-$C_{3,5}$-H), 8.50 (s, 1H, pyrazole C_5-H); Elemental analysis Calcd for $C_{27}H_{21}BrN_6O_4S$: C 53.56, H 3.50, N 13.88, found C 53.28, H 3.81, N 14.20.

5.1.4. General method for preparation of 2-{[(1,3-diaryl-1H-pyrazol-4-yl)methylene]-hydrazono}-3-phenylthiazolidin-4-ones 4a-d

A mixture of the selected thiosemicarbazone **2a-d** (1 mmole), ethyl bromoacetate (0.09 ml, 0.14 g, 1 mmole), anhydrous sodium acetate (0.08 g, 1 mmole) in absolute ethanol (12 ml) was refluxed for 3-8 h. The obtained precipitate was collected by filtration, washed with water, dried, and crystallized from a proper solvent(s).

2-{[(1,3-Di(4-methylphenyl)-1H-pyrazol-4-yl)methylene]hydrazono}-3-phenylthiazolidin-4-one ***4a***

The product was crystallized from dioxane:H_2O (9:1) as a white solid. Yield 84%; m.p. 222-224°C; IR (cm^{-1}): 1721 (C=O), 1615(C=N), 1245, 1116 (C-S-C); ^{1}H-NMR (DMSO-d_6, δ

ppm): 2.36 (s, 6H, $2CH_3$), 4.11 (s, 2H, thiazole-C_5-H), 7.29 (d, *J* = 7.95 Hz, 2H, C-C_6H_4-CH_3-$C_{3,5}$-H), 7.32- 7.47 (m, 5H, N-phenyl-H); 7.50 (d, *J* = 7.65 Hz, 2H, N-C_6H_4-CH_3-$C_{3,5}$-H); 7.73 (d, *J* = 7.65 Hz, 2H, N-C_6H_4-CH_3-$C_{2,6}$-H); 7.82 (d, *J* = 7.95 Hz, 2H, C-C_6H_4-CH_3-$C_{2,6}$-H); 8.25 (s, 1H, N=C-H); 8.82 (s, 1H, pyrazole-C_5-H); Elemental analysis Calcd for $C_{27}H_{23}N_5OS$: C 69.65, H 4.98, N 15.04, found C 69.48, H 5.16, N 14.84.

2-{[(1-(4-Bromophenyl)-3-(4-methylphenyl)-1H-pyrazol-4-yl)methylene]-hydrazono}-3-phenylthiazolidin-4-one ***4b***

The product was crystallized from DMF:H_2O (9:1) as a white solid. Yield 86%; m.p. 222-224°C; IR (cm^{-1}): 1728 (C=O), 1607 (C=N), 1237, 1069 (C-S-C), 825 (C-Br); ^{1}H-NMR (DMSO-d_6, δ ppm): 2.35, 2.37 (2s, 3H, CH_3, *E* and *Z* isomers), 4.11, 4.15 (2s, 2H, thiazole-C_5-H, *E* and *Z* isomers), 7.29-7.95 (m, 13H, aromatic H), 8.21, 8.24 (2s, 1H, HC=N, *E* and *Z* isomers), 7.76, 8.93 (2s, 1H, pyrazole-C_5-H, *E* and *Z* isomers); EIMS m/z (% relative abundance): 531 (100) ($M^{+\bullet}$+2), 529 (97) ($M^{+\bullet}$); Elemental analysis Calcd for $C_{26}H_{20}BrN_5OS$: C 58.87, H 3.80, N13.20, found C 59.16, H 4.08, N 13.54.

2-{[(1-(4-Methylphenyl)-3-(4-nitrophenyl)-1H-pyrazol-4-yl)methylene]-hydrazono}-3-phenylthiazolidin-4-one ***4c***

The product was crystallized from dioxane:H_2O (9:1) as a white solid. Yield of 81%; m.p. 266-268°C; IR (cm^{-1}): 1720 (C=O), 1617 (C=N), 1525, 1338 (NO_2), 1239, 1114 (C-S-C); ^{1}H-NMR (DMSO-d_6, δ ppm): 2.37 (s, 3H, CH_3), 4.12 (s, 2H, thiazole-C_5-H), 7.38 (d, *J* = 8.7 Hz, 2H,C_6H_4-CH_3-$C_{3,5}$-H), 7.50-7.52 (m, 5H, N-phenyl-H), 7.82 (d, *J* = 8.7 Hz, 2H, C_6H_4-CH_3-$C_{2,6}$-H), 8.27 (d, *J* = 9 Hz, 2H, C_6H_4-NO_2-$C_{2,6}$-H), 8.34 (d, *J* = 9 Hz, 2H, C_6H_4-NO_2-$C_{3,5}$-H), 8.37 (s, 1H, HC=N), 8.91 (s, 1H, pyrazole-C_5-H); ^{13}C-NMR (DMSO-d_6, δ ppm): 20.50 (CH_3), 34.30 (thiazolidinone-C_5), 117.46 (pyrazole-C_4), 118.76 (methylphenyl-$C_{2,6}$), 123.93 (NO_2-phenyl-$C_{3,5}$), 125.31 (NO_2-phenyl-$C_{2,6}$), 128.16 (phenyl-$C_{2,6}$), 128.50 (phenyl-C_4), 128.94 (pyrazole-C_5), 129.16 (phenyl-$C_{3,5}$), 130.05 (methylphenyl-$C_{3,5}$), 135.13 (methylphenyl-C_4), 136.57 (methylphenyl-C_1), 136.98 (phenyl-C_1), 138.76 (NO_2-phenyl-C_1), 147.17 (NO_2-phenyl-C_4), 148.96 (CH=N), 154.73 (pyrazole-C_3), 155.68 (thiazolidinone-C_2), 175.33 (thiazolidinone-C_4); Elemental analysis Calcd for $C_{26}H_{20}N_6O_3S$: C 62.89, H 4.06, N 16.93, found C 62.66, H 3.84, N 17.12.

2-{[(1-(4-Bromophenyl)-3-(4-nitrophenyl)-1H-pyrazol-4-yl)methylene]-hydrazono}-3-phenylthiazolidin-4-one ***4d***

The product was crystallized from dioxane:H_2O (9:1) as a white solid. Yield 93%; m.p. 242-244°C; IR (cm^{-1}): 1725 (C=O), 1624 (C=N), 1528, 1338 (NO_2), 1241, 1109 (C-S-C), 756 (C-Br); ^{1}H-NMR (DMSO-d_6, δ ppm): 4.12 (s, 2H, thiazole-C_5-H), 7.36-7.59 (m, 5H, N-phenyl-H), 7.76 (d, *J* = 9 Hz, 2H, C_6H_4-Br-$C_{2,6}$-H), 7.92 (d, *J* = 9 Hz, 2H, C_6H_4-Br-$C_{3,5}$-H), 8.02 (d, *J* = 9 Hz, 2H, C_6H_4-NO_2-$C_{2,6}$-H), 8.25 (d, *J* = 9 Hz, 2H, C_6H_4-NO_2-$C_{3,5}$-H), 8.37 (s, 1H, N=C-H), 9.00 (s, 1H, pyrazole-C_5-H); Elemental analysis Calcd for $C_{25}H_{17}BrN_6O_3S$: C 53.48, H 3.05, N 14.97, found C 53.68, H 3.35, N 14.72.

5.1.5. General method for preparation 2-{[(1,3-diaryl-1H-pyrazol-4-yl)methylene]-hydrazono}-4-(4-bromophenyl)-3-phenyl-2,3-dihydrothiazoles 5a-d

To a suspension of the selected thiosemicarbazone**2a-d** (1 mmole) in absolute ethanol (8 ml), 4-bromophenacyl bromide (0.28 g, 1 mmole) and anhydrous sodium acetate (0.08 g, 1 mmole) were added. The reaction mixture was heated under reflux for 2–4 h then allowed to cool. The obtained precipitate was filtered, washed with ethanol, dried, and crystallized from DMF/ H_2O (9:1).

4-(4-Bromophenyl)-2-{[(1,3-di(4-methylphenyl)-1H-pyrazol-4-yl)methylene]-hydrazono}-3-phenyl-2,3-dihydrothiazole ***5a***

The product was obtained as a white solid. Yield 98%; m.p. 208-210°C; IR (cm^{-1}): 1594(C=N), 1217, 1068 (C-S-C), 730 (C-Br); ^{1}H-NMR (DMSO-d_6, δ ppm): 2.34-2.39 (m, 6H, $2CH_3$, *E* and *Z* isomers), 6.70, 6.77 (2s, 1H, thiazole-C_5-H, *E* and *Z* isomers), 7.10, 7.15 (2d, *J* = 8.7 Hz , 2H, C_6H_4-Br-$C_{2,6}$-H), 7.24-7.54 (m, 13.5H, phenyl-H, C_6H_4-Br-$C_{3,5}$-H, C-C_6H_4-CH_3-$C_{3,5}$-H, N-C_6H_4-CH_3-H and pyrazole-C_5-H, *E* and *Z* isomers), 7.70, 7.83 (2d, *J* = 8.4 Hz , 2H, C-C_6H_4-CH_3-$C_{2,6}$-H), 8.13, 8.47 (2s, 1H, HC=N, *E* and *Z* isomers), 8.75 (s, 0.5H, pyrazole-C_5-H, *E* and *Z* isomers); Elemental analysis Calcd for $C_{33}H_{26}BrN_5S$: C 65.56, H 4.33, N 11.58, found C 65.81, H 4.02, N 11.83.

4-(4-Bromophenyl)-2-{[(1-(4-bromophenyl)-3-(4-methylphenyl)-1H-pyrazol-4-yl)methylene]hydrazono}-3-phenyl-2,3-dihydrothiazole ***5b***

The product was obtained as a white solid. Yield 87%; m.p. 200-202°C; IR (cm^{-1}): 1589 (C=N), 1214, 1070 (C-S-C), 729 (C-Br).; ^{1}H-NMR (DMSO-d_6, δ ppm): 2.36, 2.38 (2s, 3H, CH_3, *E* and *Z* isomers), 6.70, 6.77 (2s, 1H, thiazole-C_5-H, *E* and *Z* isomers), 7.06, 7.16 (2d, *J* = 8.4 Hz, 2H, thiazole-C_6H_4-Br-$C_{2,6}$-H), 7.24- 7.46 (m, 7H, phenyl-H and C_6H_4-CH_3-$C_{3,5}$-H), 7.51-7.57 (m, 4H, pyrazole-C_6H_4-Br-H), 7.70, 7.72 (2d, *J* = 8.55 Hz, 2H, thiazole-C_6H_4-Br-$C_{3,5}$-H), 7.77, 7.93 (2d, *J* = 9 Hz, 2H, C_6H_4-CH_3-$C_{2,6}$-H), 8.12, 8.49 (2s, 1H, N=C-H, *E* and *Z* isomers), 7.48, 8.49 (2s, 1H, pyrazole-C_5-H, *E* and *Z* isomers); Elemental analysis Calcd for $C_{32}H_{23}Br_2N_5S$: C 57.41, H 3.46, N 10.46, found C 57.74, H 3.72, N 10.64.

4-(4-Bromophenyl)-2-{[(1-(4-methylphenyl)-3-(4-nitrophenyl)-1H-pyrazol-4-yl)methylene]hydrazono}-3-phenyl-2,3-dihydrothiazole ***5c***

The product was obtained as a white solid. Yield 60%; m.p. 236-238°C; IR (cm^{-1}): 1597(C=N), 1553, 1336 (NO_2), 1215, 1070 (C-S-C), 719(C-Br); ^{1}H-NMR (DMSO-d_6, δ ppm): 2.37, 2.40 (2s, 3H, CH_3, *E* and *Z* isomers), 6.71, 6.79 (2s, 1H, thiazole-C_5-H, *E* and *Z* isomers), 7.11, 7.16 (2d, *J* = 8.4 Hz, 2H, thiazole-C_6H_4-Br-$C_{2,6}$-H), 7.26-7.39 and 7.45-7.52 (2m, 9H, phenyl-H and C_6H_4-CH_3-H), 7.83, 7.96 (2d, *J* = 8.4 Hz, 2H, thiazole-C_6H_4-Br-$C_{3,5}$-H), 8.26 (d, *J* = 8.8 Hz, 2H, C_6H_5-NO_2-$C_{2,6}$-H), 8.34 (d, *J* = 8.8 Hz, 2H, C_6H_5-NO_2-$C_{3,5}$-H), 7.43, 8.55 (2s, 1H, HC=N, *E* and *Z* isomers), 7.63, 8.83 (2s, 1H, pyrazole-C_5-H, *E* and *Z* isomers); ^{13}C-NMR (DMSO-d_6, δ ppm): 20.46, 20.47 (CH_3), 102.34, 102.70 (thiazole-C_5), 114.56, 117.27 (pyrazole-C_4), 118.12, 118.15 (methylphenyl-$C_{2,6}$), 118.56, 118.71 (Br-phenyl-C_4), 121.73, 121.89 (phenyl-C_4), 123.46, 123.88 (phenyl-$C_{2,6}$), 127.98, 128.46 (NO_2-phenyl-$C_{3.5}$), 128.65, 128.96 (NO_2-phenyl-$C_{2,6}$), 129.51, 129.63 (Br-phenyl-$C_{2,6}$), 129.96, 130.12 (Br-phenyl-C_1), 130.18, 130.30 (phenyl-$C_{3,5}$), 131.04, 131.22 (methylphenyl-$C_{3,5}$), 136.18, 136.50 (pyrazole-C_5), 136.71, 136.86 (methylphenyl-C_4), 137.32, 137.68 (methylphenyl-C_1), 138.38, 139.02 (Br-phenyl-$C_{3,5}$), 144.13, 144.44 (NO_2-phenyl-C_1), 145.67, 145.93 (phenyl-C_1), 146.85, 147.11 (thiazole-C_4), 148.79, 149.78 (NO_2-phenyl-C_4), 152.03, 152.07 (CH=N),

153.52, 153.64 (pyrazole-C_3), 168.87, 171.19 (thiazole-C_2); Elemental analysis Calcd for $C_{32}H_{23}BrN_6O_2S$: C 60.48, H 3.65, N 13.22, found C 60.81, H 3.38, N 13.58.

4-(4-Bromophenyl)-2-{[(1-(4-bromophenyl)-3-(4-nitrophenyl)-1H-pyrazol-4-yl)methylene]hydrazono}-3-phenyl-2,3-dihydrothiazole ***5d***

The product was obtained as a white solid. Yield 77%; m.p. 270-272°C; IR (cm^{-1}): 1617 (C=N), 1550, 1335 (NO_2), 1210, 1068 (C-S-C), 717 (C-Br); ^{1}H-NMR (DMSO-d_6, δ ppm): 6.71 (s, 1H, thiazole-C_5-H), 7.11 (d, *J* = 8.4 Hz, 2H, thiazole-C_4-C_6H_4-Br-$C_{2,6}$-H), 7.26-7.39 (m, 5H, phenyl-H), 7.44 (d, *J* = 8.4 Hz, 2H, thiazole-C_4-C_6H_4-Br-$C_{3,5}$-H), 7.76 (d, *J* = 8.7 Hz, 2H, pyrazole-C_1-C_6H_4-Br-$C_{2,6}$-H), 7.94 (d, *J* = 8.7 Hz, 2H, pyrazole-C_1-C_6H_4-Br-$C_{3,5}$-H), 8.23 (s, 1H, HC=N), 8.24 (d, *J* = 7.95 Hz, 2H, C_6H_4-NO_2-$C_{2,6}$-H), 8.35 (d, *J* = 7.95 Hz, 2H, C_6H_4-NO_2-$C_{3,5}$-H), 8.92 (s, 1H, pyrazole-C_5-H); EIMS m/z (% relative abundance): 700 (35) ($M^{+\bullet}$+2), 80 (100); Elemental analysis Calcd for $C_{31}H_{20}Br_2N_6O_2S$: C 53.16, H 2.88, N 12.00, found C 52.82, H 3.22, N 11.80.

5.1.6. Synthesis of 3-(1,3-diaryl-1H-pyrazol-4-yl)-1-phenylprop-2-en-1-ones 6a-c

A suspension of the appropriate 1,3-diaryl-1*H*-pyrazole-4-carboxaldehyde **1a**,**b**,**d** (10 mmole) in dioxane:ethanol (3 ml:7 ml) was added portion wise to a mixture of acetophenone (1.23 g, 1.2 ml, 10 mmole) in 3% alcoholic KOH (20 ml). The reaction mixture was stirred for 24 h at RT. The yellow precipitate formed was then filtered, washed with ethanol, dried and crystallized from proper solvent.

3-[1,3-Di(4-methylphenyl)-1H-pyrazol-4-yl]-1-phenylprop-2-en-1-one ***6a***

The product was crystallized from ethanol as a yellow solid. Yield 72%; m.p. 174°C; IR (cm^{-1}): 1645 (C=O), 1581 (C=N); ^{1}H-NMR (DMSO-d_6, δ ppm): 2.37, 2.39 (2s, each 3H, $2CH_3$), 7.36 (d, *J* = 8.4 Hz, 2H, C-C_6H_4-CH_3-$C_{3,5}$-H), 7.37 (d, *J* = 7.95 Hz, 2H, N-C_6H_4-CH_3-$C_{3,5}$-H), 7.75 (d, *J* = 7.95 Hz, 2H, N-C_6H_4-CH_3-$C_{2,6}$-H), 7.54-7.65 (m, 3H, phenyl-$C_{3,4,5}$-H), 7.69 (d, *J* = 15.3 Hz, 1H, CH=CH-CO), 7.82 (d, *J* = 8.4 Hz, 2H, C-C_6H_4-CH_3-$C_{2,6}$-H), 7.84 (d, *J* = 15.3 Hz, 1H, CH=CH-CO), 8.08 (d, *J* = 8.7 Hz, 2H, phenyl-$C_{2,6}$-H), 9.37 (s, 1H, pyrazole-C_5-H); ^{13}C-NMR (DMSO-d_6, δ ppm): 21.01, 21.40 (2 CH_3), 118.07 (pyrazole-C_4), 119.01 (N-methylphenyl-$C_{2,6}$), 121.57 (CH=CH-CO), 128.75 (C-methylphenyl-$C_{2,6}$), 128.84 (phenyl-$C_{2,6}$), 128.95 (C-methylphenyl-C_1), 129.26 (phenyl-$C_{3,5}$), 129.72 (pyrazole-C_5), 129.92 (C-methylphenyl-$C_{3,5}$), 130.54 (N-methylphenyl-$C_{3,5}$), 133.47 (C-methylphenyl-C_4), 135.02 (phenyl-C_4), 137.01 (N-methylphenyl-C_4), 137.33 (N-methylphenyl-C_1), 138.29 (phenyl-C_1), 138.64 (CH=CH-CO), 153.31 (pyrazole-C_3), 189.25 (C=O); Elemental analysis Calcd for $C_{26}H_{22}N_2O$: C 82.51, H 5.86, N 7.40, found C 82.18, H 5.50, N 7.08.

3-[1-(4-Bromophenyl)-3-(4-methylphenyl)-1H-pyrazol-4-yl]-1-phenylprop-2-en-1-one ***6b***

The product was crystallized from ethanol as a white solid. Yield 78%; m.p. 176°C; IR (cm^{-1}): 1663 (C=O), 1603 (C=N), 698 (C-Br); ^{1}H-NMR (DMSO-d_6, δ ppm): 2.40 (s, 3H, CH_3), 7.37 (d, *J* = 7.8 Hz, 2H, C_6H_4-CH_3-$C_{3,5}$-H), 7.56 (d, *J* = 8.4 Hz, 2H, C_6H_4-Br-$C_{2,6}$-H), 7.59-7.73 (m, 4H, phenyl-$C_{3,4,5}$-H and CH=CH-CO), 7.76-7.87 (m, 3H, phenyl-$C_{2,6}$-H and CH=CH-CO), 7.91 (d, *J* = 8.4 Hz, 2H, C_6H_4-Br-$C_{3,5}$-H), 8.07 (d, *J* = 7.8 Hz, 2H, C_6H_4-CH_3-$C_{2,6}$-H), 9.43 (s, 1H, pyrazole-C_5-H); EIMS m/z (% relative abundance): 444(20) ($M^{+\bullet}$+2), 442

(20) ($M^{+\bullet}$), 337 (100); Elemental analysis Calcd for $C_{25}H_{19}BrN_2O$: C 67.73, H 4.32, N 6.32, found C 67.50, H 4.54, N 6.68.

3-(1-(4-Bromophenyl)-3-(4-nitrophenyl)-1H-pyrazol-4-yl)-1-phenylprop-2-en-1-one ***6c***

The product was crystallized fromethanol:H_2O (9:1) as a yellow solid. Yield 98%; m.p. 236-238°C; IR (cm^{-1}): 1665 (C=O), 1603(C=N), 1537, 1340 (NO_2), 691 (C-Br); ^{1}H-NMR (DMSO-d_6, δ ppm): 7.63- 7.74 (m, 3H, phenyl-$C_{3,4,5}$-H),7.82-7.89 (m, 3H, C_6H_4-Br-$C_{2,6}$-H and CH=CH-CO), 7.94-7.98 (m, 3H, C_6H_4-Br-$C_{3,5}$-H and CH=CH-CO), 8.01 (d, *J* = 9 Hz, 2H, C_6H_4-NO_2-$C_{2,6}$-H), 8.12 (d, *J* = 9.3 Hz, 2H, phenyl-$C_{2,6}$-H), 8.44 (d, *J* = 9.3 Hz, 2H, C_6H_4-NO_2-$C_{3,5}$-H), 9.50 (s, 1H, pyrazole-C_5-H); Elemental analysis Calcd for $C_{24}H_{16}BrN_3O_3$: C 60.77, H 3.40, N 8.86, found C 60.98, H 3.62, N 9.04.

5.1.7. General method for preparation of 1,3-diaryl-4-(1,3-diphenyl-4,5-dihydro-1H-pyrazol-5-yl)pyrazoles 7a-c

A mixture of the appropriate 3-(1,3-diaryl-1*H*-pyrazol-4-yl)-1-phenylprop-2-en-1-one **6a-c** (1 mmole), phenylhydrazineHCl (0.14 g, 1 mmole) and anhydrous sodium acetate (0.08 g, 1 mmole) in ethanol: glacial acetic acid (12 ml: 1 ml) was heated under reflux for 18- 48 h then allowed to cool. The obtained precipitate was filtered, washed with ethanol then with H_2O, dried and crystallized from proper solvent.

1,3-Di(4-methylphenyl)-4-(1,3-diphenyl-4,5-dihydro-1H-pyrazol-5-yl)pyrazole ***7a***

The product was crystallized from ethanol:H_2O (3:1) as a white solid. Yield 43%; m.p. 120-122°C; IR (cm^{-1}): 1592 (C=N); ^{1}H-NMR (DMSO-d_6, δ ppm): 2.50, 2.51 (2s, each 3H, $2CH_3$), 3.42-3.48 (m, 2H, pyrazoline C_4-H), 4.35 (t, *J*= 5.06 Hz, pyrazoline-C_5-H), 6.98 (t, *J* = 7.02 Hz, 1H, N-phenyl-C_4-H), 7.07-7.28 (m, 6H, N-phenyl-H and C-methylphenyl-$C_{3,5}$-H), 7.32-7.49 (m, 7H, C-methylphenyl-H and C-phenyl-$C_{3,4,5}$-H), 7.68-7.77 (m, 4H, C-methylphenyl-$C_{2,6}$-H and C-phenyl-$C_{2,6}$-H), 8.27 (s, 1H, pyrazole-C_5-H); Elemental analysis Calcd for $C_{32}H_{26}N_4$: C 82.38, H 5.62, N 12.01; found C 82.12, H 5.80, N 11.78.

1-(4-Bromophenyl)-3-(4-methylphenyl)-4-(1,3-diphenyl-4,5-dihydro-1H-pyrazol-5-yl)pyrazole ***7b***

The product was crystallized from ethanol:H_2O (3:1) as a white solid. Yield 33%; m.p. 194-196°C; IR (cm^{-1}): 1632 (C=N), 689 (C-Br); ^{1}H-NMR (DMSO-d_6, δ ppm): 2.38 (s, 1H, CH_3),3.29 (distorted dd, pyrazoline C_4-H), 4.06 (dd, *J* = 16, 12 Hz, pyrazoline-C_4-H), 5.50 (dd, *J* = 12, 7.6 Hz, pyrazoline-C_5-H), 6.73 (t, *J* = 7.4 Hz, 1H, pyrazoline-N-phenyl-C_4-H), 6.98 (d, *J* = 7.4 Hz, 2H, pyrazoline-N-phenyl-$C_{2,6}$-H), 7.15 (t, *J* = 7.4 Hz, 2H, pyrazoline-N-phenyl-$C_{3,5}$-H), 7.33 (d, *J* = 8 Hz, 2H, C_6H_4-CH_3-$C_{3,5}$-H), 7.38 (t, *J* = 7.4 Hz, 1H, pyrazoline-C_3-phenyl-C_4-H), 7.44 (t, *J* = 7.4 Hz, 2H, pyrazoline-C_3-phenyl-$C_{3,5}$-H), 7.62 (d, *J* = 8.9 Hz, 2H, C_6H_4-Br-$C_{2,6}$-H), 7.69 (d, *J* = 8 Hz, 2H, C_6H_4-CH_3-$C_{2,6}$-H), 7.76 (d, *J* = 7.4 Hz, 2H, pyrazoline-C_3-phenyl-$C_{2,6}$-H), 7.83 (d, *J* = 8.9 Hz, 2H, C_6H_4-Br-$C_{3,5}$-H), 8.39 (s, 1H, pyrazole-C_5-H); ^{13}C-NMR (DMSO-d_6, δ ppm): 18.99 (CH_3), 40.00 (pyrazoline-C_4, under DMSO), 56.50 (pyrazoline-C_5), 113.73 (N-phenyl-$C_{2,6}$), 118.98 (pyrazole-C_4), 119.45 (N-phenyl-C_4), 120.54 (bromophenyl-$C_{2,6}$), 123.70 (bromophenyl-C_4), 126.25 (methylphenyl-$C_{2,6}$), 127.65

(pyrazole-C_5), 128.33 (C-phenyl-$C_{2,6}$), 129.06 (C-phenyl-$C_{3,5}$), 129.20 (methylphenyl- C_1), 129.32 (N-phenyl-$C_{3,5}$), 129.83 (methylphenyl-$C_{3,5}$), 130.06 (C-phenyl-C_4), 132.73 (bromophenyl-$C_{3,5}$), 132.77 (methylphenyl-C_4), 138.22 (C-phenyl-C_1), 138.90 (bromophenyl-C_1), 145.07 (N-phenyl-C_1), 148.12 (pyrazole-C_3), 150.56 (pyrazoline-C_3); Elemental analysis Calcd for $C_{31}H_{23}BrN_4$: C 70.06, H 4.36, N 10.54; found C 69.78, H 4.48, N 10.22.

1-(4-Bromophenyl)-3-(4-nitrophenyl)-4-(1,3-diphenyl-4,5-dihydro-1H-pyrazol-5-yl)pyrazole ***7c***

The product was crystallized from dioxane:H_2O (9:1) as a white solid. Yield 50%; m.p. 218-220°C; IR (cm^{-1}): 1599 (C=N), 1539, 1340 (NO_2), 691 (C-Br); ^{1}H-NMR (DMSO-d_6, δ ppm): 3.43-3.46 (m, 2H, pyrazoline-C_4-H), 4.35 (t, *J* = 4.5 Hz, pyrazoline-C_5-H), 7.17 (t, *J* = 7.2 Hz, 1H, N-phenyl-C_4-H), 7.44 (t, *J* = 7.2 Hz, 2H, N-phenyl-$C_{2,6}$-H), 7.61-7.99 (m, 11H, C-phenyl-H, C_6H_4-Br-H and N-phenyl-$C_{3,5}$-H), 8.33 (d, *J* = 8.04 Hz, 2H, C_6H_4-NO_2-$C_{2,6}$-H), 8.42 (d, *J* = 8.04 Hz, 2H, C_6H_4-NO_2-$C_{3.5}$-H), 8.48 (s, 1H, pyrazole-C_5-H); Elemental analysis Calcd for $C_{30}H_{20}BrN_5O_2$: C 64.07, H 3.58, N 12.45, found C 64.30, H 3.50, N 12.72.

5.1.8. General method for preparation of 1,3-diaryl-4-(3-phenyl-4,5-dihydro-1H-pyrazol-5-yl)pyrazoles 8a-c

A mixture of the appropriate α, β-unsaturated ketone **6a-c** (1 mmole) and $NH_2NH_2.H_2O$ 99% (0.2 g, 0.19 ml, 4 mmole) in ethanol (10 ml) was heated under reflux for 15-30 minutes then allowed to cool down. The white precipitate obtained after cooling was filtered, washed with ethanol, dried and crystallized from ethanol.

1,3-Di(4-methylphenyl)-4-(3-phenyl-4,5-dihydro-1H-pyrazol-5-yl)pyrazole ***8a***

The product was obtained as a white solid. Yield 89%; m.p. 146-148°C; IR (cm^{-1}): 3322 (NH), 1609 (C=N); ^{1}H-NMR (DMSO-d_6, δ ppm): 2.33, 2.35 (2s, each 3H, $2CH_3$), 2.97 (dd, *J* =16.2, 10.8 Hz, pyrazoline C_4-H), 3.46 (dd, *J*=16.2, 10.8 Hz, pyrazoline C_4-H), 4.96 (t, *J* = 10.8 Hz, pyrazoline C_5-H), 7.28- 7.34 (m, 4H, C-C_6H_4-CH_3-$C_{3,5}$-H and N-C_6H_4-CH_3-$C_{3,5}$-H) 7.36- 7.41 (m, 3H, phenyl-$C_{3,4,5}$-H), 7.55 (br d, 1H, pyrazoline-NH, D_2O exchangeable), 7.62- 7.66 (m, 4H, N-C_6H_4-CH_3-$C_{2,6}$-H and phenyl-$C_{2,6}$-H), 7.76 (d, *J* = 8.4 Hz, 2H, N-C_6H_4-CH_3-$C_{2,6}$-H), 8.49 (s, 1H, pyrazole C_5-H); ^{13}C-NMR (DMSO-d_6, δ ppm):20.96, 21.38 ($2CH_3$), 40.00 (pyrazoline-C_4, under DMSO), 55.80 (pyrazoline-C_5), 118.59 (N-methylphenyl-$C_{2,6}$), 123.29 (pyrazole-C_4), 126.02 (C-methylphenyl-$C_{2,6}$), 127.56 (pyrazole-C_5), 128.37 (phenyl-$C_{2,6}$), 128.65 (C-methylphenyl-C_1),129.01 (phenyl-$C_{3,5}$), 129.70 (C-methylphenyl-$C_{3,5}$), 130.45 (N-methylphenyl-$C_{3,5}$), 130.74 (phenyl-C_4), 133.78 (C-methylphenyl-C_4), 135.96 (N-methylphenyl-C_4), 137.74 (phenyl-C_1), 137.86 (N-methylphenyl-C_1), 149.83 (pyrazole-C_3), 150.58 (pyrazoline-C_3); Elemental analysis Calcd for $C_{26}H_{24}N_4$: C 79.56, H 6.16, N 14.27; found C 79.30, H 5.92, N 13.84.

1-(4-Bromophenyl)-3-(4-methylphenyl)-4-(3-phenyl-4,5-dihydro-1H-pyrazol-5-yl)pyrazole ***8b***

The product was obtained as a white solid. Yield 87%; m.p. 156-158°C; IR (cm^{-1}): 3317 (NH), 1589, (C=N), 727 (C-Br); ^{1}H-NMR (DMSO-d_6, δ ppm): 2.36 (s, 3H, CH_3), 2.97 (dd, *J* = 16.2, 10.8 Hz, pyrazoline C_4-H), 3.47 (dd, *J* = 16.2, 10.8 Hz, pyrazoline C_4-H), 4.97 (t, *J* = 10.8 Hz, pyrazoline C_5-H), 7.28- 7.41 (m, 5H, C_6H_4-CH_3-$C_{3,5}$-H and phenyl-$C_{3,4,5}$-H), 7.51 (br

d, 1H, pyrazoline-NH, D_2O exchangeable), 7.62- 7.69 (m, 6H, C_6H_4-Br-H and phenyl-$C_{2,6}$-H), 7.87 (d, *J* = 8.7 Hz, 2H, C_6H_4-CH_3-$C_{2,6}$-H), 8.58 (s, 1H, pyrazole C_5-H); EIMS m/z (% relative abundance): 458 (4) ($M^{+\bullet}$+2), 456 (100) ($M^{+\bullet}$); Elemental analysis Calcd for $C_{25}H_{21}BrN_4$: C 65.65, H 4.63, N 12.25, found C 65.26, H 4.84, N 11.90.

1-(4-Bromophenyl)-3-(4-nitrophenyl)-4-(3-phenyl-4,5-dihydro-1H-pyrazol-5-yl)pyrazole ***8c***

The product was obtained as a white solid. Yield 60%; m.p. 204-206°C; IR (cm^{-1}): 3313 (NH), 1597 (C=N), 1543, 1342 (NO_2), 719 (C-Br); ^{1}H-NMR (DMSO-d_6, δ ppm): 2.98 (dd, *J* = 16.5, 10.8 Hz, pyrazoline C_4-H), 3.56 (dd, *J* = 16.5, 10.8 Hz, pyrazoline C_4-H), 5.08 (td, *J* = 10.8, 3.3 Hz, pyrazoline C_5-H), 7.30- 7.42 (m, 3H, phenyl-$C_{3,4,5}$-H), 7.60 (d, *J* = 3.6 Hz, 1H, pyrazoline-NH, D_2O exchangeable), 7.62- 7.65 (m, 2H, phenyl-$C_{2,6}$-H), 7.71 (d, *J* = 9 Hz, 2H, C_6H_4-Br-$C_{2,6}$-H), 7.92 (d, *J* = 9 Hz, 2H, C_6H_4-Br-$C_{3,5}$-H), 8.08 (d, *J* = 9 Hz, 2H, C_6H_4-NO_2-$C_{2,6}$-H), 8.32 (d, *J* = 9 Hz, 2H, C_6H_4-NO_2-$C_{3,5}$-H), 8.70 (s, 1H, pyrazole C_5-H); Elemental analysis Calcd for $C_{24}H_{18}BrN_5O_2$: C 59.03, H 3.72, N 14.34; found C 58.88, H 3.56, N 14.71.

5.1.9. General method for preparation of 4-(1-acetyl-3-phenyl-4,5-dihydro-1H-pyrazol-5-yl)-1,3-diarylpyrazoles9a-cand1,3-diaryl-4-(3-phenyl-1-propanoyl-4,5-dihydro-1H-pyrazol-5-yl)pyrazoles 10a-c

A mixture of the appropriate α, β-unsaturated ketone **6a-c** (1 mmole) and $NH_2NH_2.H_2O$ 99% (0.2 g, 0.19 ml, 4 mmole) in the appropriate carboxylic acid (acetic acid or propionic acid) (10 ml) was heated under reflux for 4-8 h then allowed to cool. The obtained precipitate was filtered, washed with ethanol and crystallized from the proper solvent.

4-(1-Acetyl-3-phenyl-4,5-dihydro-1H-pyrazol-5-yl)-1,3-di-(4-methylphenyl)-pyrazole ***9a***

The product was crystallized from ethanol as a white solid. Yield 64 %; m.p. 196-198 °C; IR (cm^{-1}): 1661 (C=O), 1617 (C=N); ^{1}H-NMR (DMSO-d_6, δ ppm): 2.32 (s, 6H, $2CH_3$), 2.36 (s, 3H, $COCH_3$), 3.20 (dd, *J* = 18, 5.2 Hz, pyrazoline C_4-H), 3.89 (dd, *J* = 18, 12 Hz, pyrazoline C_4-H), 5.68 (dd, J = 12, 5.2 Hz, pyrazoline C_5-H), 7.26 (d, J = 6.9 Hz, 2H, C-C_6H_4-CH_3-$C_{3,5}$-H), 7.28 (d, *J* = 6.3 Hz, 2H, N-C_6H_4-CH_3-$C_{3,5}$-H), 7.44-7.48 (m, 3H, phenyl-$C_{3,4,5}$H), 7.67 (d, *J* = 8.4 Hz, 2H, N-C_6H_4-CH_3-$C_{2,6}$-H), 7.74- 7.77 (m, 4H, phenyl-$C_{2,6}$-H and C-C_6H_4-CH_3-$C_{2,6}$-H), 8.28 (s, 1H, pyrazole C_5-H); ^{13}C-NMR (DMSO-d_6, δ ppm): 20.39, 20.82 ($2CH_3$), 21.91 ($CO\underline{CH_3}$), 42.18 (pyrazoline-C_4); 51.84 (pyrazoline-C_5), 118.02 (N-methylphenyl-$C_{2,6}$), 123.06 (pyrazole-C_4), 126.01 (pyrazole-C_5), 126.12 (C-methylphenyl-C_1), 126.56 (C-methylphenyl-$C_{2,6}$), 127.82 (phenyl-$C_{2,3,5,6}$), 128.63 (C-methylphenyl-$C_{3,5}$),129.05 (phenyl-C_4), 129.71 (C-methylphenyl-C_4), 130.11 (N-methylphenyl-$C_{3,5}$), 131.25 (N-methylphenyl-C_4), 135.35 (phenyl-C_1), 137. 16 (N-methylphenyl-C_1), 148.98 (pyrazole-C_3), 153.84 (pyrazoline-C_3), 167.54 (C=O); Elemental analysis Calcd for $C_{28}H_{26}N_4O$: C 77.39, H 6.03, N 12.89, found C 77.02, H 6.36, N 12.58.

4-(1-Acetyl-3-phenyl-4,5-dihydro-1H-pyrazol-5-yl)-1-(4-bromophenyl)-3-(4-methylphenyl)pyrazole ***9b***

The product was crystallized from ethanol as a white solid. Yield 73%; m.p. 232-234°C; IR (cm^{-1}): 1665 (C=O), 1592 (C=N), 689 (C-Br); ^{1}H-NMR (DMSO-d_6, δ ppm): 2.31 (s, 3H,

$COCH_3$), 2.36 (s, 3H, CH_3), 3.20 (dd, *J* = 18, 5.2 Hz, pyrazoline C_4-H), 3.89 (dd, *J* = 18, 12 Hz, pyrazoline C_4-H), 5.68 (dd, *J* = 12, 5.2 Hz, pyrazoline C_5-H), 7.29 (d, *J* = 8.1 Hz, C_6H_4-CH_3-$C_{3,5}$-H), 7.44- 7.48 (m, 3H, phenyl- $C_{3,4,5}$-H), 7.64 (d, *J* = 8.7 Hz, C_6H_4-Br-$C_{2,6}$-H), 7.68 (d, *J* = 8.1 Hz, 2H, C_6H_4-Br- $C_{3,5}$-H), 7.74-7.77 (m, 2H, phenyl- $C_{2,6}$-H), 7.86 (d, *J* = 8.7 Hz, C_6H_4-CH_3-$C_{2,6}$-H), 8.37 (s, 1H, pyrazole C_5-H); EIMS m/z (% relative abundance): 500 (6) ($M^{+\bullet}$+2), 498 (5) ($M^{+\bullet}$), 80 (100); Elemental analysis Calcd for $C_{27}H_{23}BrN_4O$: C 64.94, H 4.64, N 11.22, found C 65.20, H 4.32, N 11.64.

4-(1-Acetyl-3-phenyl-4,5-dihydro-1H-pyrazol-5-yl)-1-(4-Bromo-phenyl)-3-(4-nitrophenyl)pyrazole ***9c***

The product was crystallized fromdioxane:ethanol (5:1) as a white solid. Yield 73%; m.p. > 300°C; IR (cm^{-1}): 1659 (C=O), 1696, 1499 (C=N), 1553, 1339 (NO_2), 714 (C-Br); ^{1}H-NMR (DMSO-d_6, δ ppm): 2.37 (s, 3H, $COCH_3$), 3.35 (distorted dd, pyrazoline C_4-H), 4.01 (dd, *J* = 18, 11.85 Hz, pyrazoline C_4-H), 5.81 (dd, *J* = 11.85, 5.2 Hz, pyrazoline C_5-H), 7.47-7.54 (m, 3H, phenyl-$C_{3,4,5}$-H), 7.74 (d, *J* = 9 Hz, 2H, C_6H_4-Br-$C_{2,6}$-H), 7.80- 7.84 (m, 2H, phenyl-$C_{2,6}$-H), 7.95 (d, *J* = 9 Hz, 2H, C_6H_4-Br- $C_{3,5}$-H), 8.16 (d, *J* = 8.85 Hz, 2H, C_6H_4-NO_2-$C_{2,6}$-H), 8.38 (d, *J* = 8.85 Hz, 2H, C_6H_4-NO_2- $C_{3,5}$-H), 8.57 (s, 1H, pyrazole C_5-H); Elemental analysis Calcd for $C_{26}H_{20}BrN_5O_3$: C 58.88, H 3.80, N 13.20, found C 59.06, H 3.84, N 13.06.

1,3-Di(4-methylphenyl)-4-(3-phenyl-1-prpanoyl-4,5-dihydro-1H-pyrazol-5-yl)pyrazole ***10a***

The product was crystallized from ethanol as a white solid. Yield 75%; m.p. 202-204°C; IR (cm^{-1}): 1659 (C=O), 1596 (C=N); ^{1}H-NMR (DMSO-d_6, δ ppm): 1.06 (t, *J* = 7.65 Hz, 3H, CH_2-$\underline{CH_3}$), 2.31, 2.36 (2s, each 3H, $2CH_3$), 2.74 (q, *J* = 7.65 Hz, 2H, $\underline{CH_2}$-CH_3), 3.19 (dd, *J* = 18, 5.1 Hz, pyrazoline C_4-H), 3.87 (dd, *J* = 18, 12 Hz, pyrazoline C_4-H), 5.66 (dd, *J* = 12, 5.1 Hz, pyrazoline C_5-H), 7.26 (d, *J* = 7.8 Hz, 2H, C-C_6H_4-CH_3-$C_{3,5}$-H), 7.28 (d, *J* = 7.2 Hz, 2H, N-C_6H_4-CH_3-$C_{3,5}$-H), 7.44-7.46 (m, 3H, phenyl-$C_{3,4,5}$-H), 7.67 (d, *J* = 7.2 Hz, 2H, N-C_6H_4-CH_3-$C_{2,6}$-H), 7.74- 7.77 (m, 4H, phenyl- $C_{2,6}$-H and C-C_6H_4-CH_3-$C_{2,6}$-H), 8.26 (s, 1H, pyrazole C_5-H); ^{13}C-NMR (DMSO-d_6, δ ppm): 8.85 (CO-CH_2-$\underline{CH_3}$), 20.34, 20.82 ($2CH_3$), 26.90 (CO-$\underline{CH_2}$-CH_3), 42.00 (pyrazoline-C_4), 51.95 (pyrazoline-C_5), 118.02 (N-methylphenyl-$C_{2,6}$), 123.23 (pyrazole-C_4), 125.97 (pyrazole-C_5), 126.04 (C-methylphenyl-C_1), 126.56 (C-methylphenyl-$C_{2,6}$), 127.87 (phenyl-$C_{2,6}$), 128.63 (phenyl-$C_{3,5}$), 129.09 (C-methylphenyl-$C_{3,5}$), 129.76 (N-methylphenyl-$C_{3,5}$), 130.06 (phenyl-C_4), 130.11 (C-methylphenyl-C_4), 131.34 (N-methylphenyl-C_4), 135.36 (phenyl-C_1), 137.18 (N-methylphenyl-C_1), 149.10 (pyrazole-C_3), 153.71 (pyrazoline-C_3), 170.82 (C=O); Elemental analysis Calcd for $C_{29}H_{28}N_4O$: C 77.65, H 6.29, N 12.49, found C 77.28, H 6.02, N 12.78.

1-(4-Bromophenyl)-3-(4-methylphenyl)-4-(3-phenyl-1-propanoyl-4,5-dihydro-1H-pyrazol-5-yl)pyrazole ***10b***

The product crystallized fromdioxane:ethanol (5:1) as a white solid. Yield 87%; m.p. 238-240°C; IR (cm^{-1}): 1660 (C=O), 1591 (C=N), 691 (C-Br); ^{1}H-NMR (DMSO-d_6, δ ppm): 1.07 (t, *J* = 7.5 Hz, 3H, CH_2-$\underline{CH_3}$), 2.36 (s, 3H, CH_3), 2.74 (q, *J* = 7.5 Hz, 2H, $\underline{CH_2}$-CH_3), 3.19 (dd, *J* = 18, 5.4 Hz, pyrazoline C_4-H), 3.88 (dd, *J* = 18, 12 Hz, pyrazoline C_4-H), 5.66 (dd, *J* = 12, 5.4 Hz, pyrazoline C_5-H), 7.29 (d, *J* = 7.5 Hz, C_6H_4-CH_3-$C_{3,5}$-H), 7.45-7.47 (m, 3H, phenyl-$C_{3,4,5}$-H), 7.64 (d, *J* = 9 Hz, 2H, C_6H_4-Br-$C_{2,6}$-H), 7.68 (d, *J* = 8.1 Hz, C_6H_4-CH_3-$C_{2,6}$-

H), 7.74- 7.77 (m, 2H, phenyl-$C_{2,6}$-H), 7.85 (d, *J* = 9 Hz, 2H, C_6H_4-Br-$C_{3,5}$-H), 8.36 (s, 1H, pyrazole C_5-H); EIMS m/z (% relative abundance): 514 (2) ($M^{+\bullet}$+2), 64 (100); Elemental analysis Calcd for $C_{28}H_{25}BrN_4O$: C 65.50, H 4.91, N 10.91, found C 65.44, H 5.21, N 11.26.

1-(4-Bromophenyl)-3-(4-nitrophenyl)-4-(3-phenyl-1-propanoyl-4,5-dihydro-1H-pyrazol-5-yl)pyrazole ***10c***

The product crystallized fromdioxane:ethanol (5:1) as a white solid. Yield 54%; m.p. > 300°C; IR (cm^{-1}):1656 (C=O), 1596 (C=N), 1552, 1339 (NO_2), 690 (C-Br); ^{1}H-NMR (DMSO-d_6, δ ppm): 1.11 (t, *J* = 7.65 Hz, 3H, CH_2-$\underline{CH_3}$), 2.79 (q, *J* = 7.65 Hz, 2H, $\underline{CH_2}$-CH_3), 3.28 (distorted dd, pyrazoline C_4-H), 3.99 (dd, *J* = 17.7, 11.1 Hz, pyrazoline C_4-H), 5.79 (dd, *J* = 11.1, 4.8 Hz, pyrazoline C_5-H), 7.50-7.55 (m, 3H, phenyl-$C_{3,4,5}$-H), 7.73 (d, *J* = 9.15 Hz, 2H, C_6H_4-Br-$C_{2,6}$-H), 7.79- 7.83 (m, 2H, phenyl-$C_{2,6}$-H), 7.93 (d, *J* = 9.15 Hz, 2H, C_6H_4-Br- $C_{3,5}$-H), 8.16 (d, *J* = 9 Hz, 2H, C_6H_4-NO_2-$C_{2,6}$-H), 8.38 (d, *J* = 9 Hz, 2H, C_6H_4-NO_2- $C_{3,5}$-H), 8.54 (s, 1H, pyrazole C_5-H); Elemental analysis Calcd for $C_{27}H_{22}BrN_5O_3$: C 59.57, H 4.07, N 12.86, found: C 59.22, H 3.89, N 12.48.

5.2. Biological Screening

5.2.1. In vivo antimalarial activity testing against P. berghei

Swiss albino mice of both sexes, weighing 24-38 g and 4-6 weeks of age, were used in the antimalarial activity test. The animals were acclimatized for a period of 7 days at room temperature (23-25 ^oC) and relative humidity of 60-65%. The animals were housed in standard cages and maintained on standard pelleted diet and water. *In vivo* antimalarial activity test of the synthesized compounds was performed using a 4-day standard suppressive test using *P. berghei* ANKA strain infected mice as described by Fidock *et al* [46]. This is the most widely used test in which the efficacy of a compound is assessed by comparison of blood parasitemia level and mouse survival time in treated and untreated mice [47]. On day 0, the test mice were injected intravenously with 0.2 ml of 2 x 10^7 parasitized erythrocytes infected with *P. berghei* ANKA strain. These parasite erythrocytes were obtained from the blood of a donor mouse with approximately 20-30% parasitemia and the blood was diluted with normal saline. After 2 h of injection, the infected mice were weighed and randomly divided into 37 groups of five mice per cage. Groups 1-35 (35 cages) received the synthesized compounds orally, each at 0.048 mmol/kg/day dose levels and served as treatment groups. Group 36 received the drug vehicle (7% Tween, 3% ethanol in distilled water) and served as a negative control, while Group 37 received chloroquine phosphate at dose level of 25 mg/kg/day (0.048 mmol/kg/day) and served as a positive control. On days 1 to 3, the treatment groups were treated with the same single dose of the synthesized compound at 24 h intervals. On day 4 (i.e. 24 h after the last dose), blood smear from all test animals was prepared using Giemsa stain. Level of parasitemia was determined microscopically by counting 5 fields of approximately 100 erythrocytes per field. The difference between the mean value for the negative control group (taken as 100%) and those of the experimental groups was calculated and expressed as percent suppression or activity. Chloroquine treated mice were completely cured of the parasite.The survival time for each test mouse was recorded and the mean survival time was calculated in

comparison with that of the negative group. Percentage parasitemia and percentage suppression were calculated using the following formulae:

$$\% \text{ parasitemia} = \frac{\text{Number of infected RBC}}{\text{Number of total RBC}} \times 100$$

$$\% \, Suppression = \frac{Parasitemia \ in \ negative \ control - parasitemia \ in \ treatment \ group}{Parasitemia \ in \ negative \ control} \times 100$$

5.2.2. In vitro antimalarial activity against P. falciparum

The most active compounds, **2c, 2d, 4b, 4c, 4d, 5a, 6c, 8c and 9b**, were further examined for their antiplasmodial activities against chloroquine resistant (RKL9) *P. falciparum* strain. The *P. falciparum* strain RKL9 was maintained in a continuous culture using the standard method described by Trager and Jensen[43]. The assay was carried out in 96 well microtitre flat-bottomed tissue culture plates incubated at 37 ^{o}C for 24 h. Two-fold serial dilutions of test compounds and chloroquine diphosphate were examined for their effect on schizont maturation. The initial culture was maintained in small vials with 10% haematocrit, i.e. 10 ml erythrocytes containing 1.0% ring stage parasite in 100ml complete media. The assay culture volume was 100 ml per well. Number of parasites for the assay was adjusted to 1–1.5% by dilution with fresh human B (+) RBC. Compounds were dissolved in ethanol and further diluted with RPMI 1640 medium (thefinal ethanol concentration did not exceed 0.5%, which did not affect parasite growth). Chloroquine diphosphate was dissolved in aqueous medium. Test was done in duplicate wellsfor each dose of the drugs. Solvent control culture containing the same concentrations of thesolvent as present in the test wells was done with RPMI-1640 containing 10% AB (+) serum. Parasite growth was found to be unaffected at the solvent concentrations used. Growth of theparasites from duplicate wells of each concentration was monitored in Giemsa stained blood smears by counting number of schizont per 100 asexual parasites. Percent schizont maturation inhibition was calculated using the formula:

$$1 - \frac{Nt}{Nc} \times 100$$

Where, Nt and Nc represent the number of schizonts in the test and control well respectively.

5.2.3. In vitro antileishmanial activity on L. aethiopica promastigotes

All the compounds, dissolved in DMSO, were evaluated for their antileishmanial activity to a final concentration of 1 mg/ml. The final concentration of DMSO did not exceed 0.1 % so that it did not have any effect on parasite. Both test and standard solutions were serially diluted to appropriate concentrations using fresh complete media [20, 48]. The test compounds were prepared by three-fold serial dilutions (starting from 10 to 0.04 µg/ml). Amphotericin B deoxycholate and miltefosine were used as positive controls for comparison of the antileishmanial activity of the test compounds and were used in three-fold serial dilutions. Promastigote forms of *L. aethiopica* were used for the assay. A 100 µl of culture

media containing 3 x 10^6 promastigotes of *L. aethiopica* were seeded in each well of a 96 well flat bottom plate. Various dilutions of test compounds (10, 3.33, 1.11, 0.37, 0.12, 0.04 μg/ml) were added to the parasites. The assay was done in duplicates. Wells containing only the parasites, media and DMSO were served as negative control. The plates were then kept at room temperature (21 ± 1 °C). After 24 h, 10 μl of Alamar blue (12.5 mg resazurin dissolved in 100 ml of distilled water) [49] was added to each of the wells. Absorbance of the resulting mixture was measured after 48 h at 540 and 630 nm using a plate reader. Alamar blue works through the conversion of resazurin (7-hydroxy-3*H*-phenoxazine-3-one-10-oxide), the active ingredient of Alamar blue® (blue and non-fluorescent), to resorufin (pink and highly fluorescent) through reduction reactions of metabolically active cells. The amount of fluorescence produced is proportional to the number of living cells[50, 51].

5.2.4. In vitro antileishmanial activity on L. aethiopica amastigotes

The test compounds were serially diluted in a 96-well microtitre plate to a final test concentration of 0.04 to 10 μg/ml in 50 μl culture medium and 50 μl suspensions containing 2 × 10^7 cells/ml axenic amastigotes were added to each well. The plate contents were then incubated in humidified atmosphere at 31°C under a 5% CO_2 for 72 h. After 68 h of incubation, a 10 μl of fluorochrome resazurin solution (12.5 μg dissolved in 100 ml of PBS (pH=7.2)) was added to each well and the fluorescence intensity was measured after a total incubation time of 72 h using 37 Victor3 Multilabel Counter at excitation wavelength of 530 nm and emission wavelength of 590 nm. The EC_{50} value for each extract was evaluated from sigmoidal dose–response curves using computer software Graph pad prism 3.0 and the results were expressed as mean ± SD of triplicate experiments with each test concentration measured in duplicate. Assays with standard anti-leishmanial drugs and negative controls (medium alone and 1% DMSO) were also performed to have reference values. The background fluorescence intensity of each extract and reference drug was measured [52].

5.2.5. In vivo acute toxicity testing

The most active compounds, which showed promising dual antileishmanial and antimalarial activities, were tested for their oral acute toxicity in mice. Eleven groups of mice, each group consisting of six male mice (26-32 g) were used for testing acute toxicity [53].The mice in each group were fasted overnight and weighed prior to test. The compounds were prepared in suspension form in aqueous vehicle containing 1% gum acacia. Mice in group one to ten were given 25, 50, 100, 200 and 300 mg/kg/day of the synthesized compounds and the eleventh group was treated orally with the vehicle (control group) at a maximum dose of 1 ml/100 g of body weight. The mortality percentage in each group was recorded after 24 h [24, 37]. Additionally, the test compounds were investigated for their parenteral acute toxicity in groups of six mice each as reported earlier [12]. The compounds, or their vehicle, propylene glycol (control), were given by intraperitoneal injection in doses of 10, 25, 50, 75, 100 mg/kg. The survival percentage was followed up to seven days [54].

5.3. Molecular docking

All computer-aided docking studies were carried out using Molecular Operating Environment (MOE Dock 2008) software [55]. The crystal structure of *Pf*-DHFR with the bound (WR99210) (PDB ID: 1J3I) and *Lm*-PTR1 with the bound TOP (PDB ID: 2BFM) were

downloaded from the protein data bank. Target compounds were constructed using the builder interface of the MOE program and all hydrogens were added. Conformational analyses were done through energy minimization using Force Field MMFF94x. The active sites of both proteins were generated using the MOE- Alpha Site Finder, and then ligands were docked within this active site using MOE Dock. MOE was also used to calculate the best score between the ligands and the enzymes' binding sites. Scoring is determined as a total of two specific scoring functions: alpha hydrogen bonding and London forces. Thirty conformers of the ligand were retained with highest and best score by default. The obtained conformers that showed the best ligand-enzyme interactions were selected.

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Figure captions

Figure 1: Structures of lead antimalarial or antileishmanial pyrazoles

Figure 2: Structures of lead antimalarial or antileishmanial agents containing five-membered rings

Figure 3: General formulae of the target compounds

Figure 4: 3D View from a molecular modeling study, of the minimum-energy structure of the complex of WR99210 docked in *Pf*-DHFR (PDB ID: 1J3I). Viewed using Molecular Operating Environment (MOE) module.

Figure 5: 3D View from a molecular modeling study, of the minimum-energy structure of compound 4b docked in *Pf*-DHFR (PDB ID: 1J3I). Viewed using Molecular Operating Environment (MOE) module.

Figure 6: 3D View from a molecular modeling study, of the minimum-energy structure of compound 9b docked in *Pf*-DHFR (PDB ID: 1J3I). Viewed using Molecular Operating Environment (MOE) module.

Figure 7: 3D View from a molecular modeling study, of the minimum-energy structure of the complex of TOP docked in *Lm*-PTR (PDB ID: 2BFM). Viewed using Molecular Operating Environment (MOE) module.

Figure 8: 3D View from a molecular modeling study, of the minimum-energy structure of compound 3d docked in *Lm*-PTR (PDB ID: 2BFM). Viewed using Molecular Operating Environment (MOE) module.

Figure 9: 3D View from a molecular modeling study, of the minimum-energy structure of compound 4c docked in *Lm*-PTR (PDB ID: 2BFM). Viewed using Molecular Operating Environment (MOE) module.

Table 1: *In vivo* antimalarial activity of test compounds against *P. berghei* at dose 48.4 µM/ kg/day

Comp. No.	R^1	R^2	% Parasitemia*	% Suppression	Mean survival time (days)
1a	CH_3	CH_3	33±0.4	**61.62**	8.62
1b	CH_3	Br	30±0.8	**65.11**	8.40
1c	NO_2	CH_3	32±0.6	**62.79**	7.28
1d	NO_2	Br	35±0.8	**59.30**	8.71
2a	CH_3	CH_3	34±1.0	**60.47**	9.23
2b	CH_3	Br	30±1.6	**65.12**	8.84
2c	NO_2	CH_3	5±0.8	**94.19**	14.66
2d	NO_2	Br	5±0.4	**94.19**	14.84
3a	CH_3	CH_3	14±1.2	**83.72**	12.22
3b	CH_3	Br	12±0.4	**86.05**	12.68
3c	NO_2	CH_3	18±0.2	**79.07**	10.40
3d	NO_2	Br	12±0.4	**86.05**	16.86
4a	CH_3	CH_3	10±1.6	**88.37**	10.64
4b	CH_3	Br	4±0.2	**95.35**	16.86

4c	NO_2	CH_3	3±0.4	**96.51**	16.62
4d	NO_2	Br	2±0.6	**97.67**	16.44
5a	CH_3	CH_3	2±0.8	**97.67**	16.84
5b	CH_3	Br	14±2.8	**83.72**	11.22
5c	NO_2	CH_3	22±0.6	**74.42**	12.12
5d	NO_2	Br	28±0.2	**67.44**	12.26
6a	CH_3	CH_3	26±1.6	**69.77**	11.60
6b	CH_3	Br	22±1.4	**74.42**	11.80
6c	NO_2	Br	3±0.2	**96.51**	16.22
7a	CH_3	CH_3	26±1.2	**69.77**	10.82
7b	CH_3	Br	22±1.6	**74.42**	10.64
7c	NO_2	Br	32±2.8	**62.79**	9.84
8a	CH_3	CH_3	30±0.4	**65.12**	7.62
8b	CH_3	Br	28±0.8	**67.44**	7.88
8c	NO_2	Br	4±0.4	**95.35**	17.60
9a	CH_3	CH_3	18±1.4	**79.07**	10.08
9b	CH_3	Br	3±0.6	**96.51**	16.40
9c	NO_2	Br	26±1.2	**69.77**	6.62

10a	CH_3	CH_3	38±0.6	**55.81**	8.84
10b	CH_3	Br	42±1.8	**51.16**	6.86
10c	NO_2	Br	34±0.2	**60.47**	6.82
Control	…….	…….	86±1.2	**0.00**	5.24
Chloroquine phosphate	…….	…….	0.0	100	ND**

* Values are M± SD, P< 0.05 ** ND: No death recorded during experimental period

Table 2: *In vitro* anti-plasmodial activity against chloroquine-resistant (RKL9) strain of *P. falciparum*.

Comp. No.	IC_{50}, µM±SD*
2c	0.0378±0.006
2d	0.0384±0.002
4b	0.0364±0.004
4c	0.0402±0.002
4d	0.0392±0.012
5a	0.0418±0.008
6c	0.0388±0.010
8c	0.0420±0.006
9b	0.0368±0.008
Chloroquine	0.1920±0.003

*Results of two separate determinations.

Table 3: Antipromastigote activity of the test compounds and reference standards in µg/ml

Comp. No.	R^1	R^2	IC_{50}*
1a	CH_3	CH_3	4.0622±0.12
1b	CH_3	Br	3.1124±0.22
1c	NO_2	CH_3	3.6442±0.14
1d	NO_2	Br	4.8.412±0.18
2a	CH_3	CH_3	3.0122±0.11
2b	CH_3	Br	3.6228±0.28
2c	NO_2	CH_3	0.0241±0.24
2d	NO_2	Br	0.0214±0.16
3a	CH_3	CH_3	0.8622±0.03
3b	CH_3	Br	0.4682±0.05
3c	NO_2	CH_3	0.8461±0.02
3d	NO_2	Br	0.0142±.004
4a	CH_3	CH_3	0.8822±0.008
4b	CH_3	Br	0.0341±.004
4c	NO_2	CH_3	0.0201±0.005

4d	NO_2	Br	0.0224±0.006
5a	CH_3	CH_3	0.0311±0.004
5b	CH_3	Br	4.0621±0.12
5c	NO_2	CH_3	3.1124±0.11
5d	NO_2	Br	2.9022±0.23
6a	CH_3	CH_3	4.0011±0.16
6b	CH_3	Br	1.0288±0.086
6c	NO_2	Br	1.0831±0.11
7a	CH_3	CH_3	3.0882±0.32
7b	CH_3	Br	2.9142±0.14
7c	NO_2	Br	3.8482±0.18
8a	CH_3	CH_3	2.8441±0.28
8b	CH_3	Br	2.0122±0.24
8c	NO_2	Br	2.124±0.12
9a	CH_3	CH_3	3.0481±0.31
9b	CH_3	Br	3.0221±0.34
9c	NO_2	Br	1.0882±0.12
10a	CH_3	CH_3	1.6028±0.26

10b	CH_3	Br	2.0421±0.42
10c	NO_2	Br	2.1224±0.22
Miltefosine	…….	…….	3.1921±14
Amphotericin B deoxycholate	…….	…….	0.0472±0.002

*IC_{50}: values indicate the effective concentration of a compound required to achieve 50 %
growth inhibition in μg/ml

Table 4: Antiamastigote activity of the test compounds and reference standards in µg/ml

Comp. No.	R^1	R^2	IC_{50}*
1a	CH_3	CH_3	1.97±0.08
1b	CH_3	Br	1.82±0.14
1c	NO_2	CH_3	1.56±0.24
1d	NO_2	Br	2.03±0.34
2a	CH_3	CH_3	2.26±0.11
2b	CH_3	Br	2.41±0.16
2c	NO_2	CH_3	0.23±0.03
2d	NO_2	Br	0.21±0.06
3a	CH_3	CH_3	0.82±0.02
3b	CH_3	Br	0.74±0.04
3c	NO_2	CH_3	1.21±0.16
3d	NO_2	Br	0.13±0.02
4a	CH_3	CH_3	0.58±0.04
4b	CH_3	Br	0.32±0.02
4c	NO_2	CH_3	0.28±0.04

4d	NO_2	Br	0.19±0.02
5a	CH_3	CH_3	0.30±0.04
5b	CH_3	Br	2.21±0.09
5c	NO_2	CH_3	1.78±0.11
5d	NO_2	Br	1.07±0.18
6a	CH_3	CH_3	2.88±0.22
6b	CH_3	Br	1.01±0.08
6c	NO_2	Br	1.22±0.14
7a	CH_3	CH_3	1.78±0.16
7b	CH_3	Br	1.24±0.26
7c	NO_2	Br	2.01±0.34
8a	CH_3	CH_3	2.37±0.22
8b	CH_3	Br	1.88±0.32
8c	NO_2	Br	1.11±0.18
9a	CH_3	CH_3	2.31±0.36
9b	CH_3	Br	2.77±0.12
9c	NO_2	Br	0.89±0.04
10a	CH_3	CH_3	1.02±0.32

10b	CH_3	Br	1.79±0.14
10c	NO_2	Br	1.83±0.22
Miltefosine	…….	…….	0.3±0.04
Amphotericin B deoxycholate	…….	…….	0.2±0.02

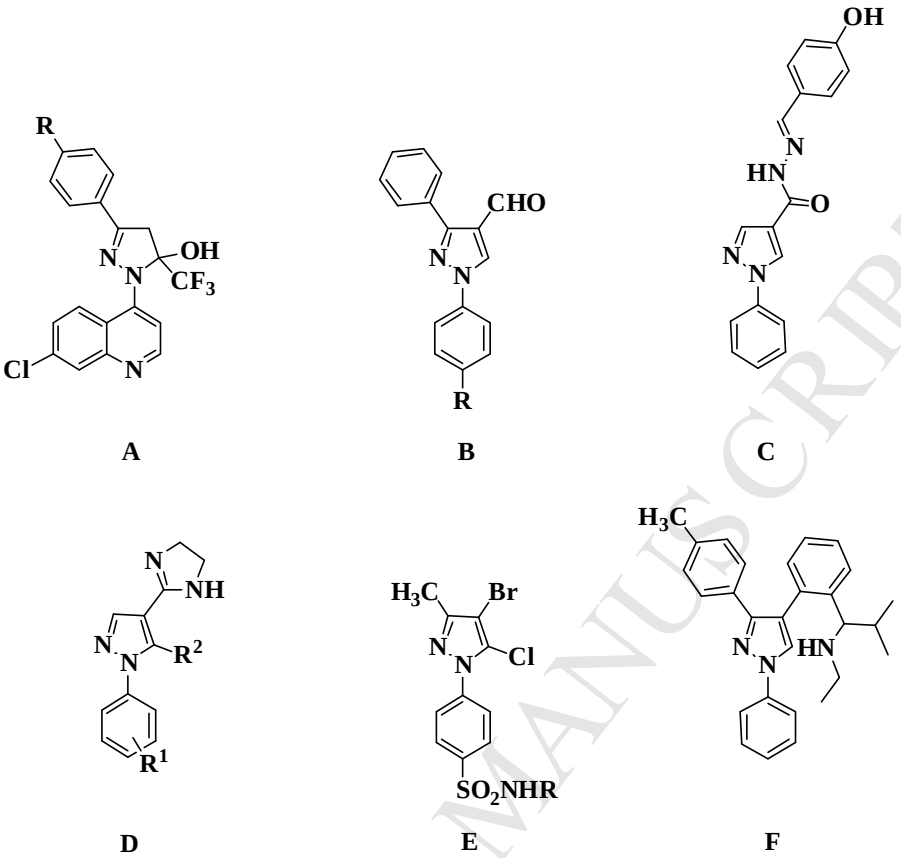


Figure 1: Structures of lead antimalarial or antileishmanial pyrazoles

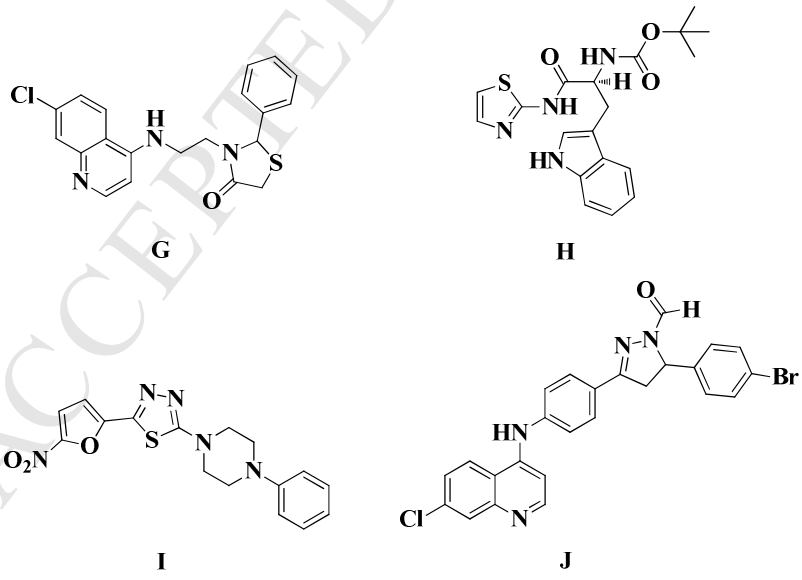


Figure 2: Structures of lead antimalarial or antileishmanial agents containing five-membered rings

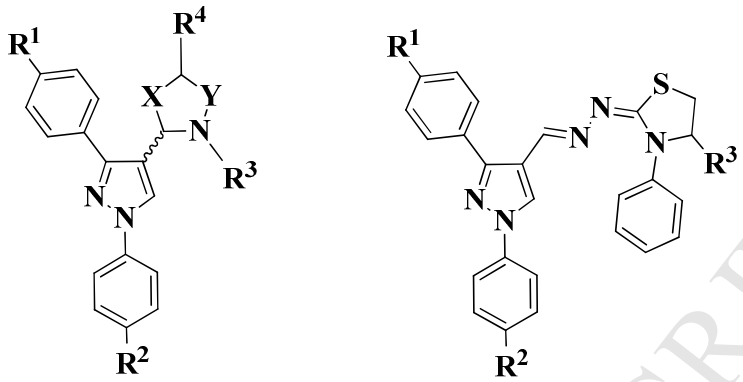


Figure 3: General formulae of the target compounds

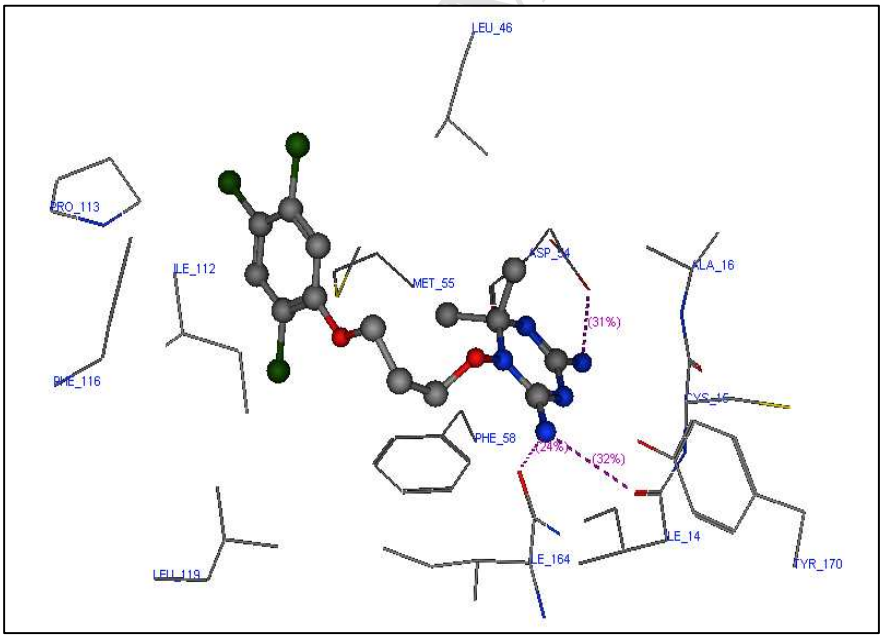


Figure 4: 3D View from a molecular modeling study, of the minimum-energy structure of the complex of WR99210 docked in *Pf*-DHFR (PDB ID: 1J3I). Viewed using Molecular Operating Environment (MOE) module.

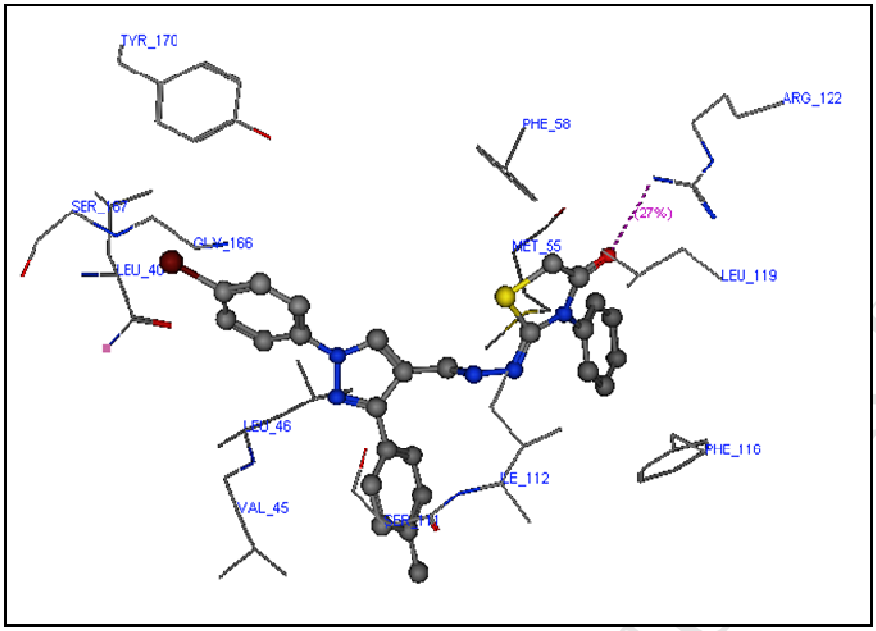


Figure 5: 3D View from a molecular modeling study, of the minimum-energy structure of compound 4b docked in *Pf*-DHFR (PDB ID: 1J3I). Viewed using Molecular Operating Environment (MOE) module.

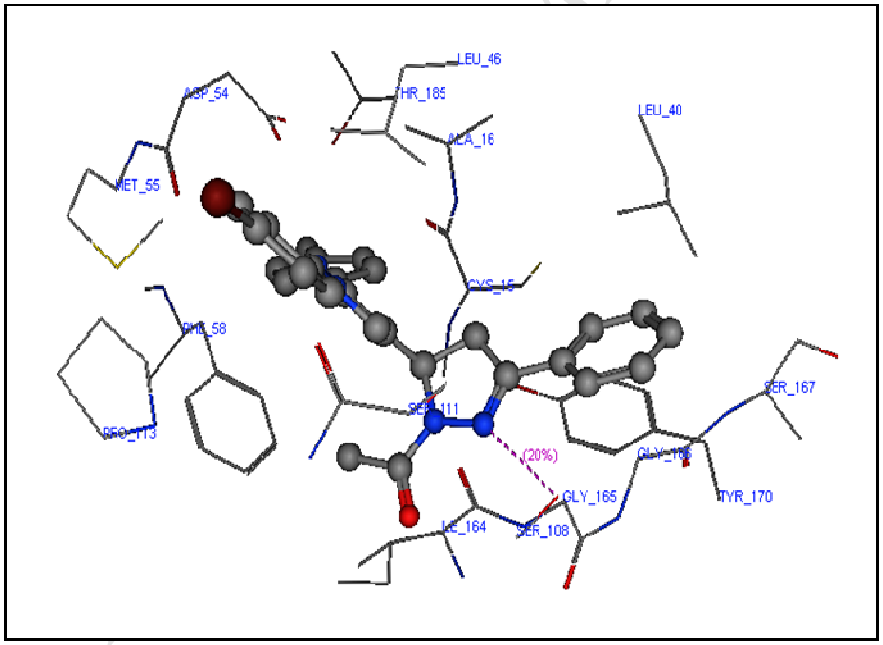


Figure 6: 3D View from a molecular modeling study, of the minimum-energy structure of compound 9b docked in *Pf*-DHFR (PDB ID: 1J3I). Viewed using Molecular Operating Environment (MOE) module.

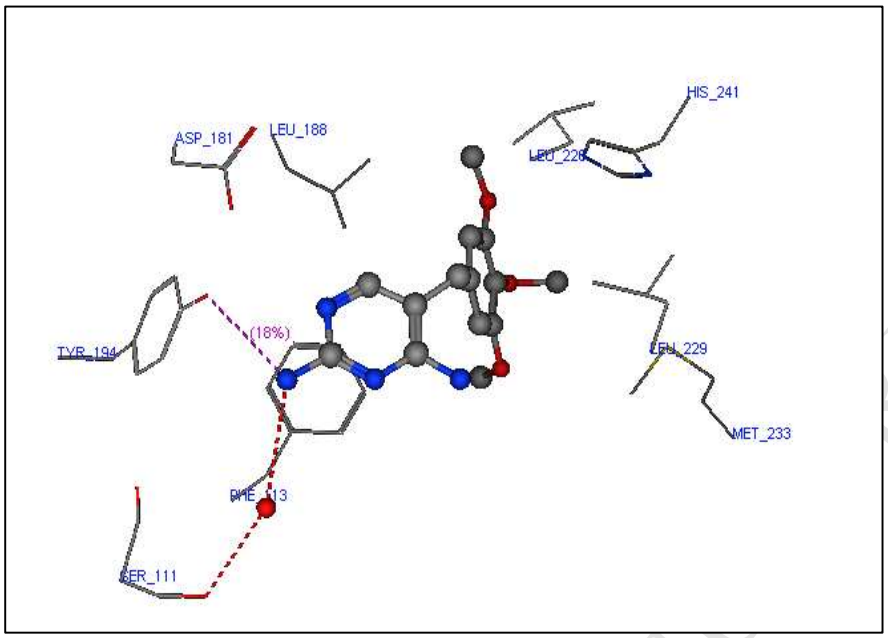


Figure 7: 3D View from a molecular modeling study, of the minimum-energy structure of the complex of TOP docked in *Lm*-PTR (PDB ID: 2BFM). Viewed using Molecular Operating Environment (MOE) module.

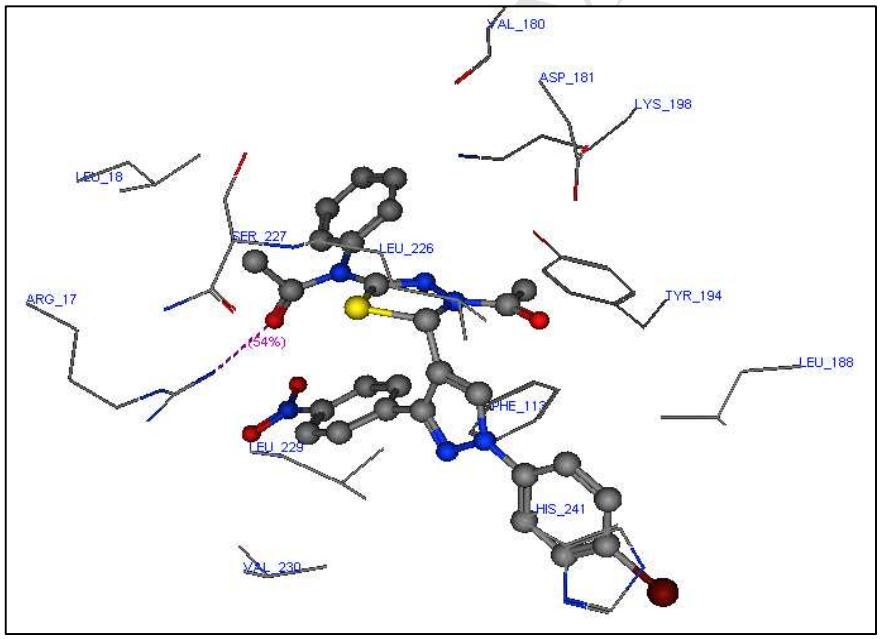


Figure 8: 3D View from a molecular modeling study, of the minimum-energy structure of compound 3d docked in *Lm*-PTR (PDB ID: 2BFM). Viewed using Molecular Operating Environment (MOE) module.

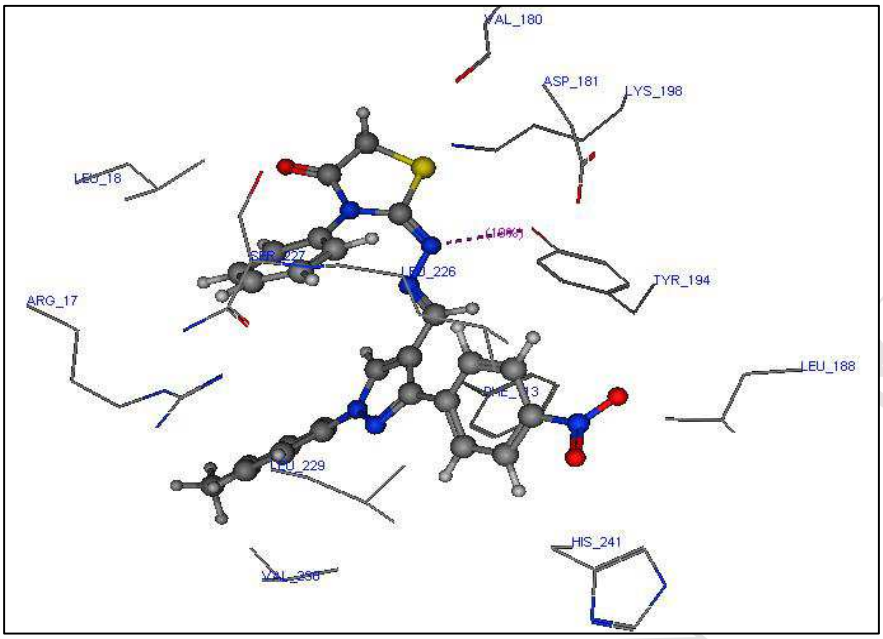
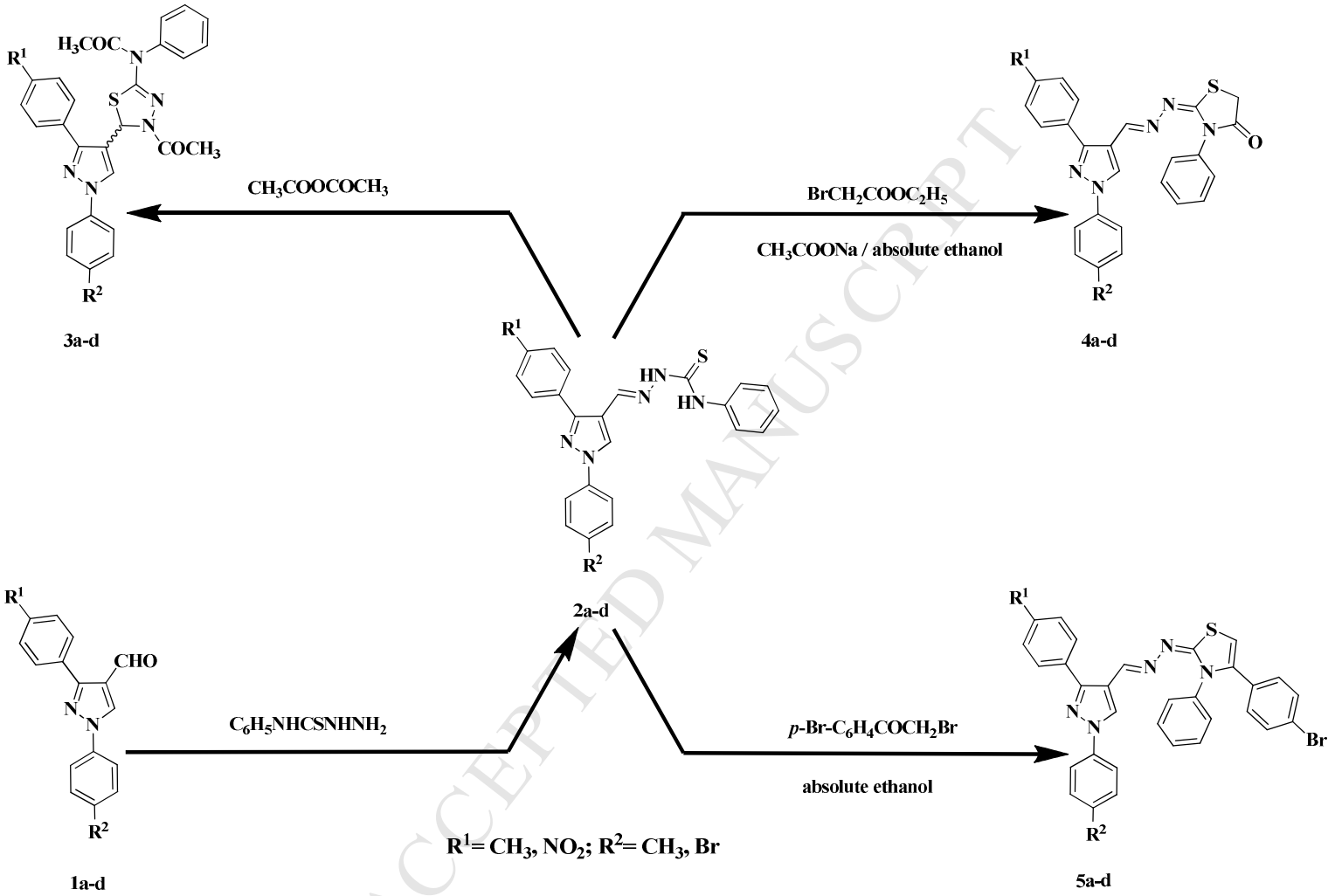
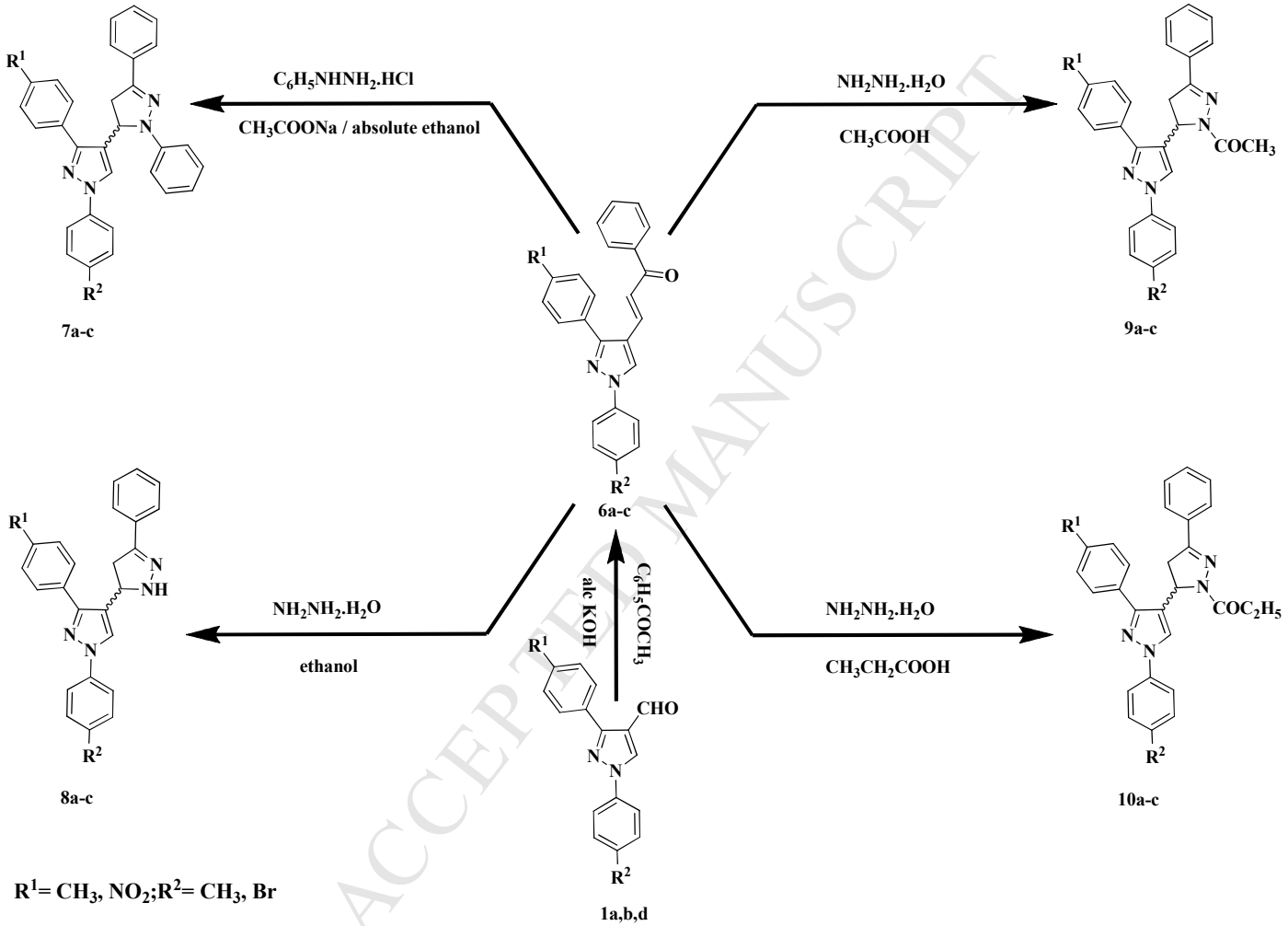


Figure 9: 3D View from a molecular modeling study, of the minimum-energy structure of compound 4c docked in *Lm*-PTR (PDB ID: 2BFM). Viewed using Molecular Operating Environment (MOE) module.



Scheme 1



Scheme 2

Highlights

- Series of pyrazoles hybridized with 5-membered heterocycles were synthesized.
- Compounds **4b** and **9b** were 5 folds more active than chloroquine against *P. falciparum*.
- Seven Compounds showed antileishmanial activity better than amphotericin B.
- Six compounds showed dual activity against malaria and leishmaniasis.

Supplementary Data

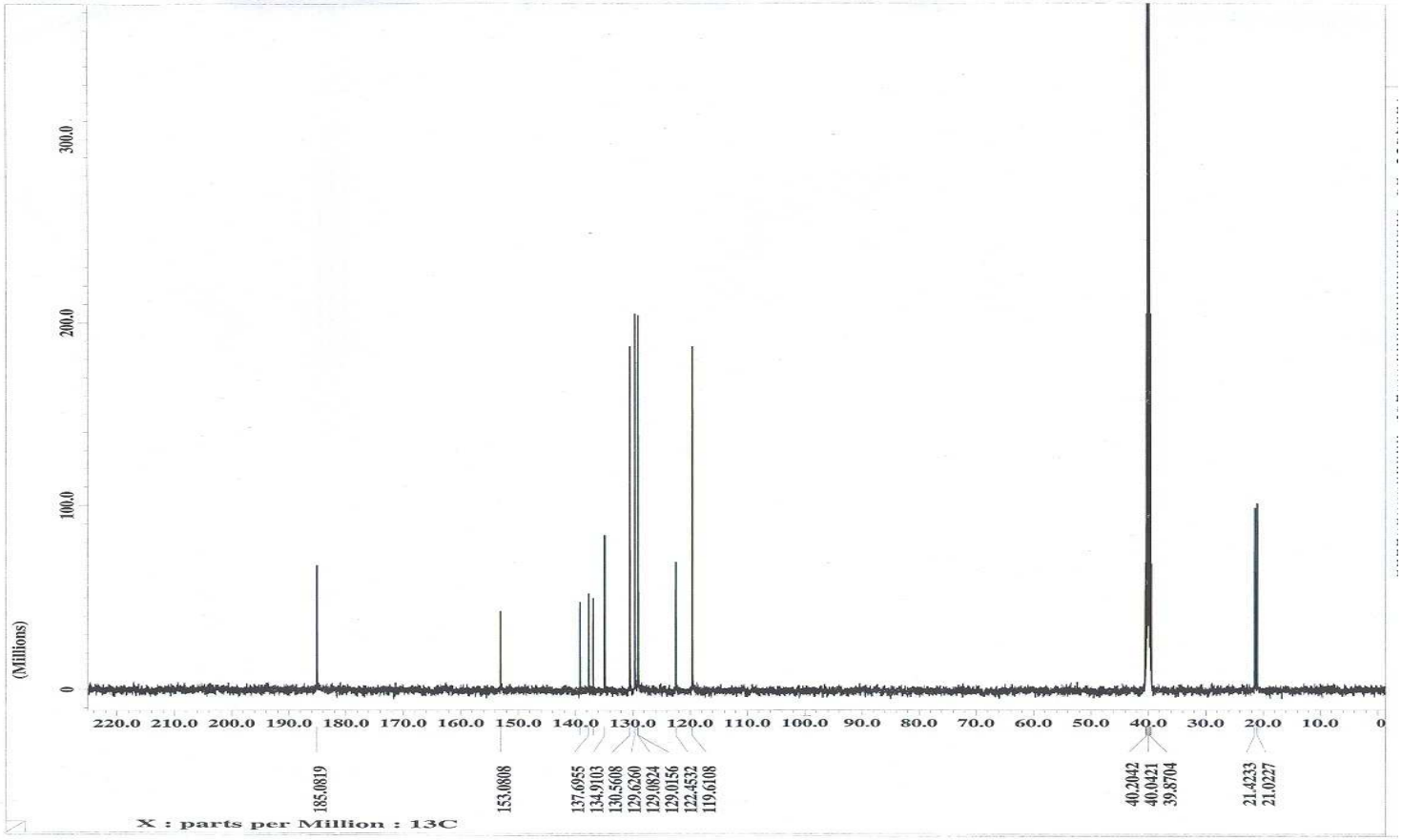


Figure 1: ^{13}C-NMR of compound 1a

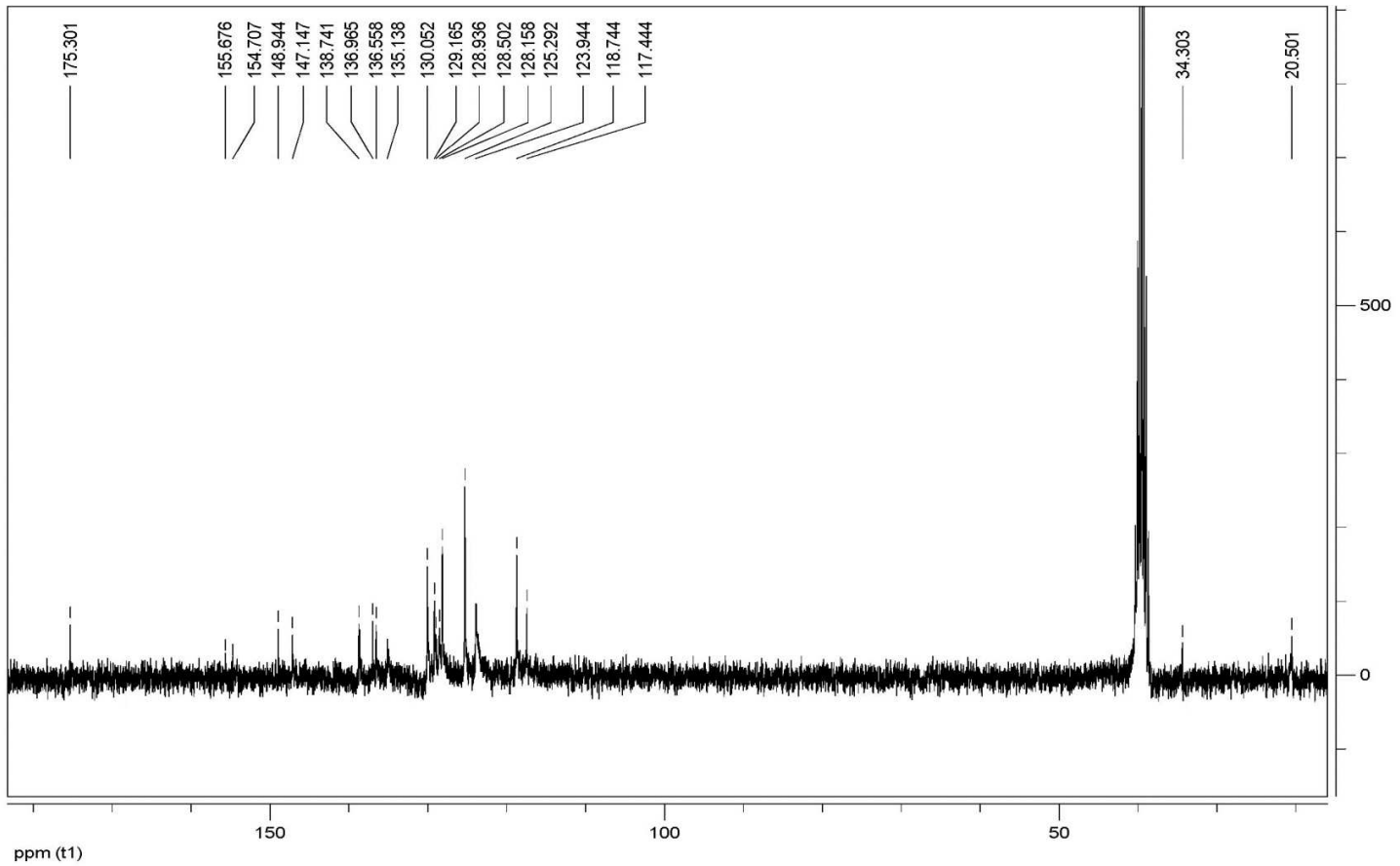


Figure 2: ^{13}C-NMR of compound 4c

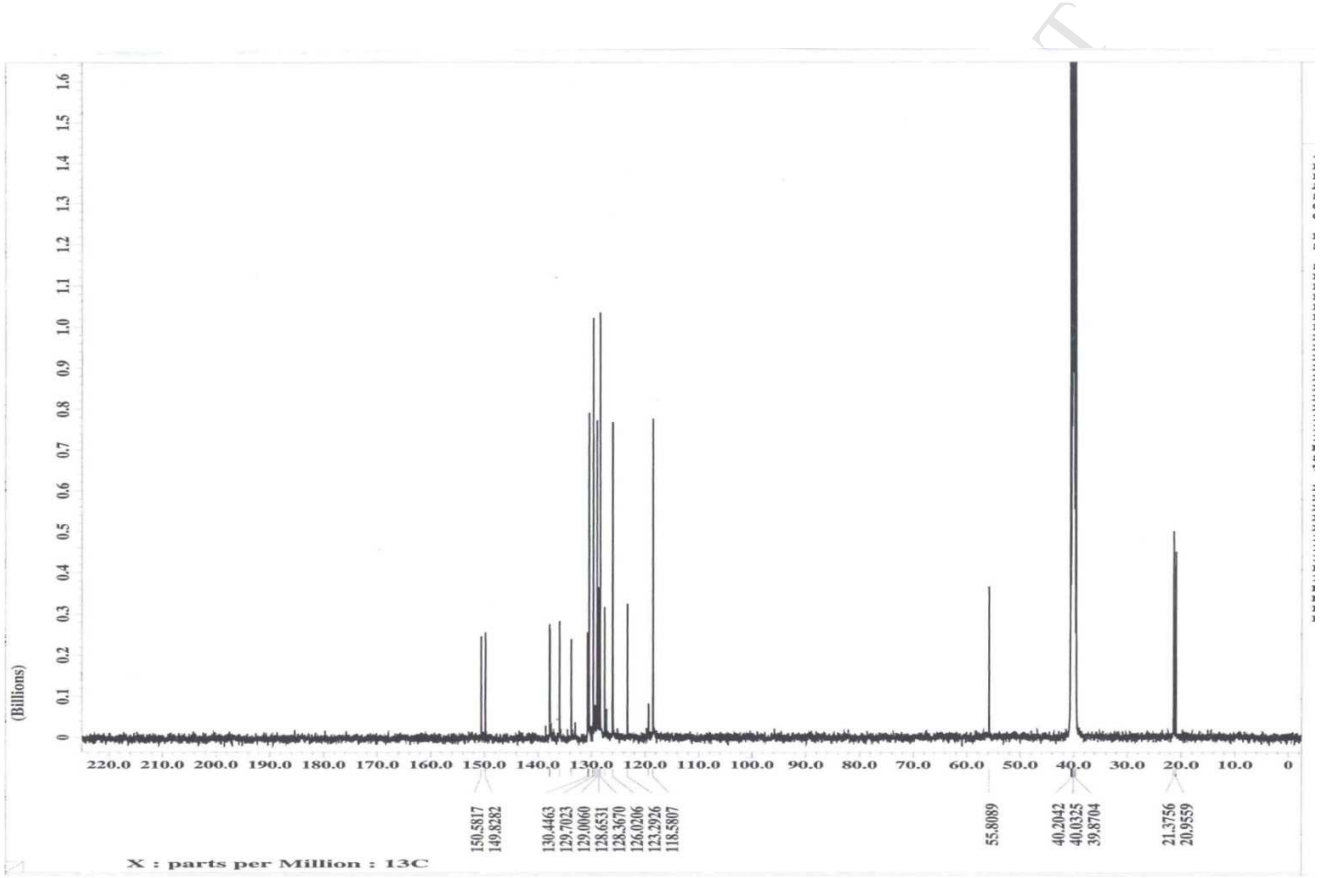


Figure 3: ^{13}C-NMR of compound 8a

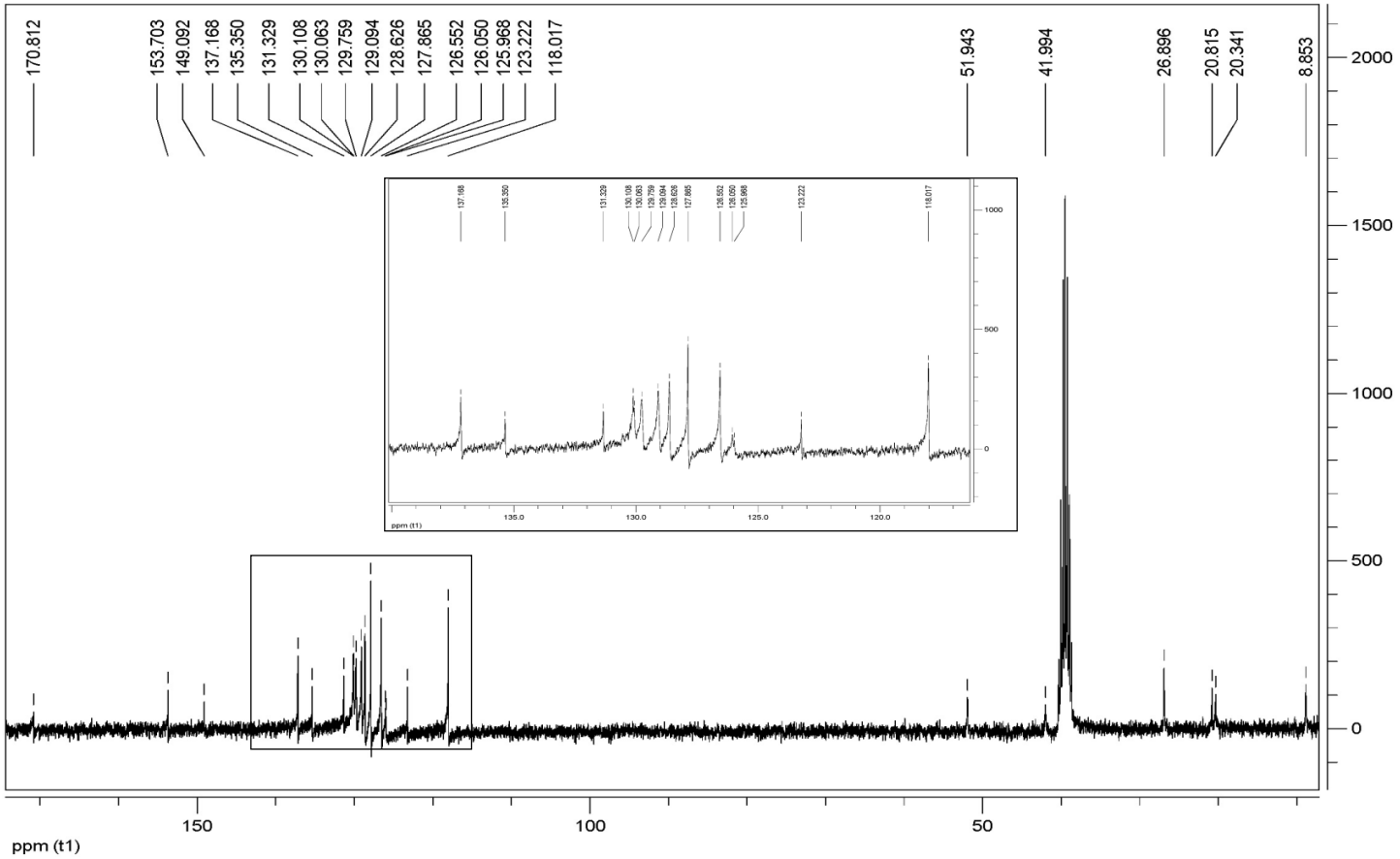


Figure 4: ^{13}C-NMR of compound 10a

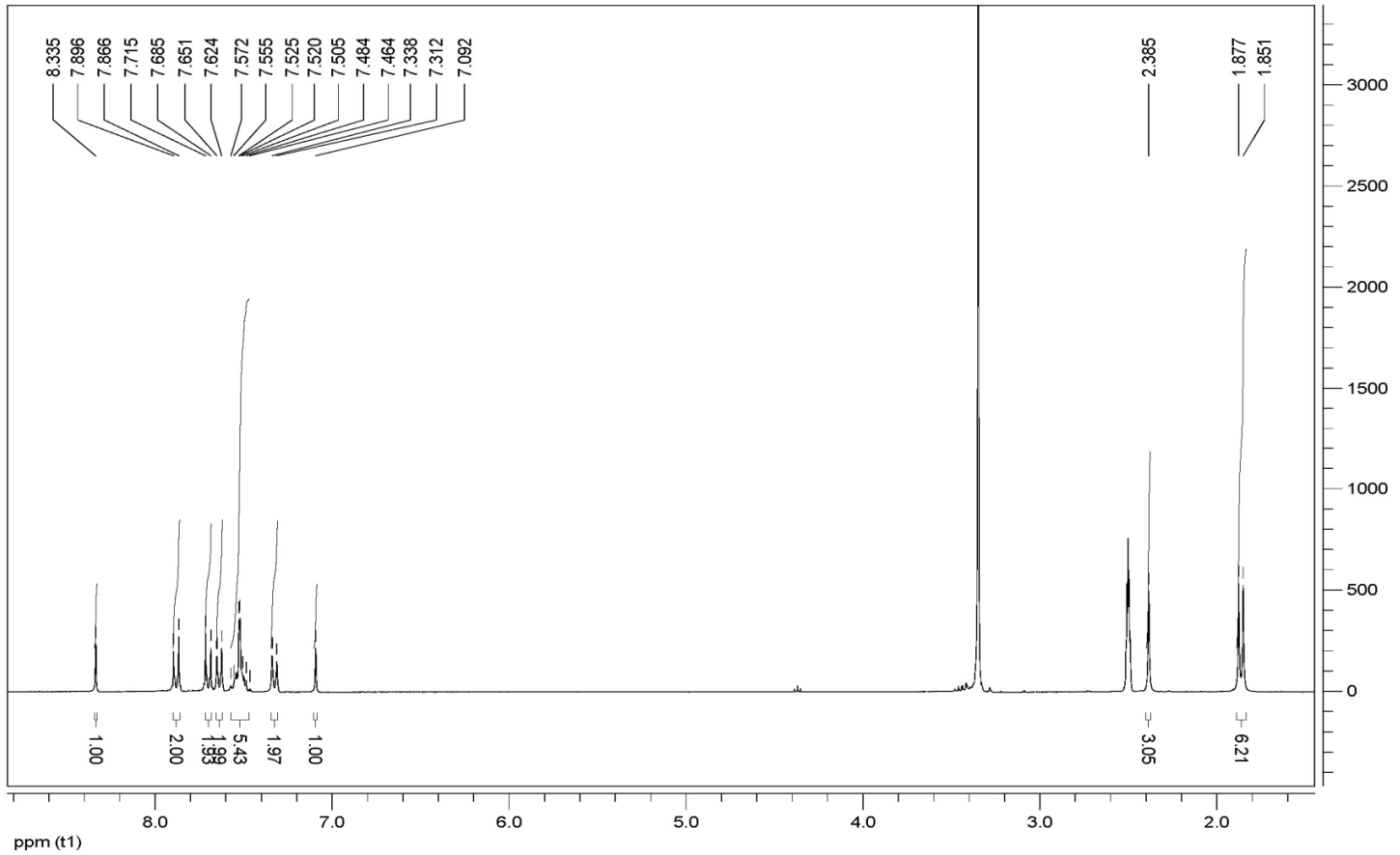


Figure 5: ^{1}H-NMR of compound 3b

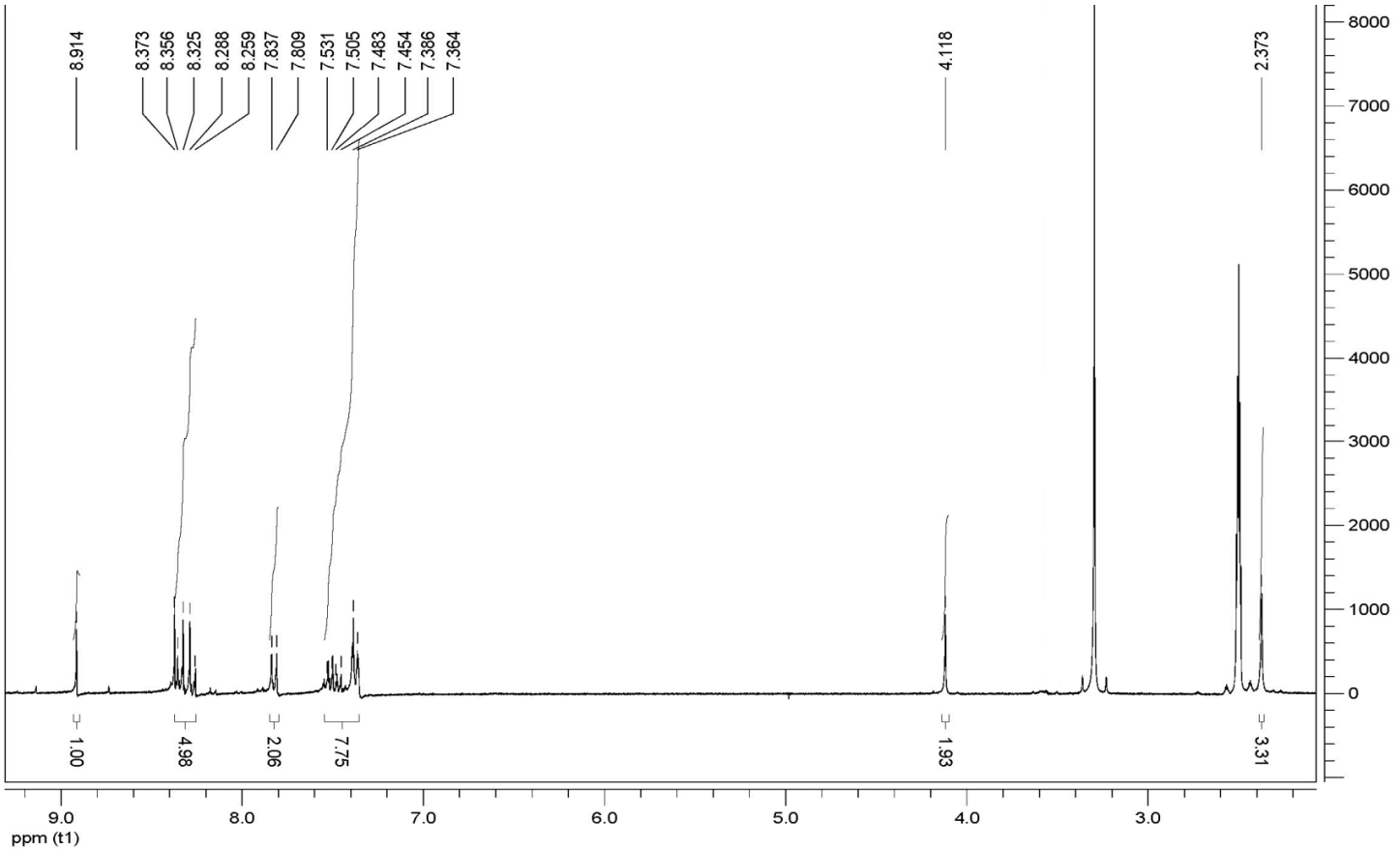


Figure 6: ^{1}H-NMR of compound 4c

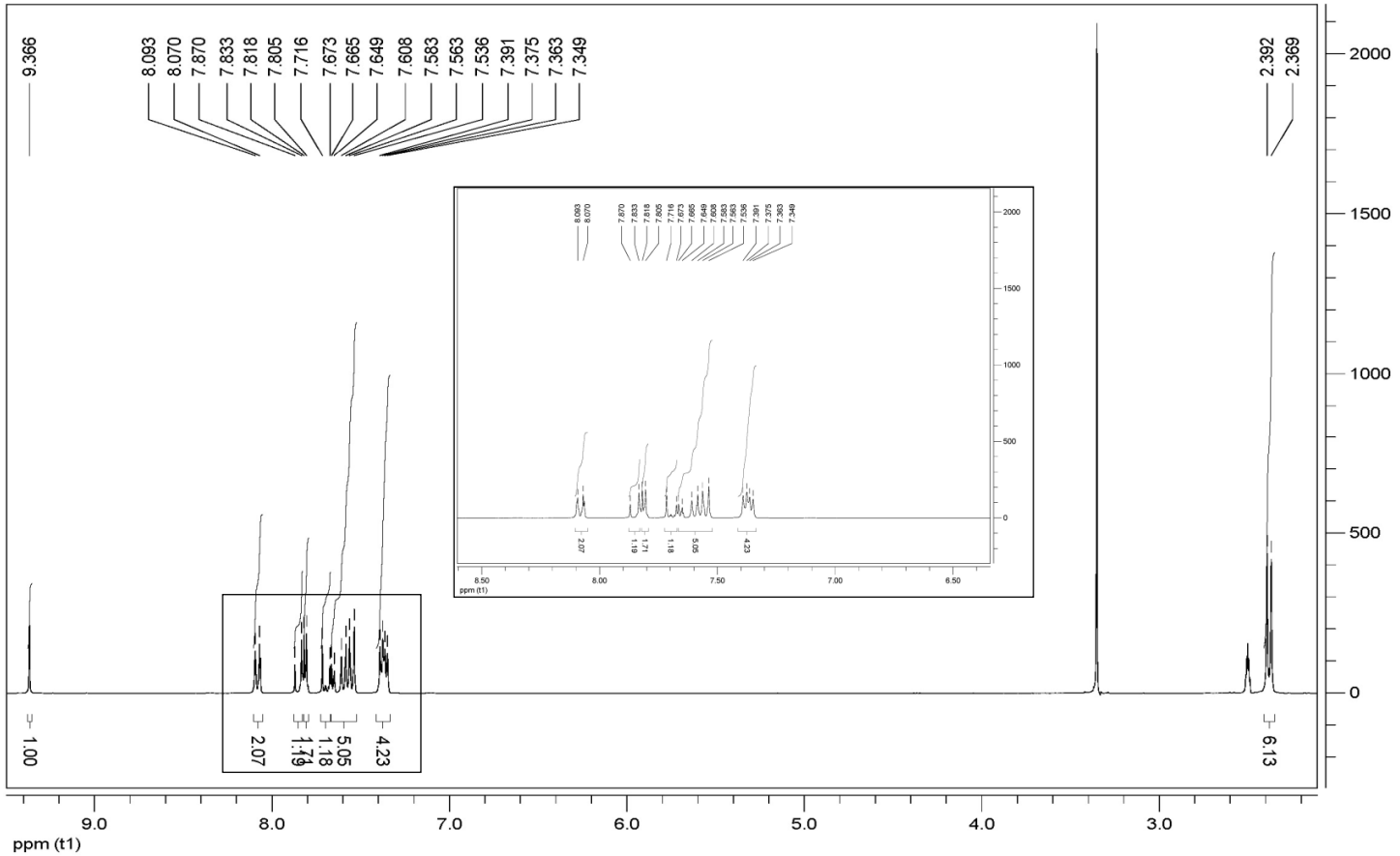


Figure 7: ^{1}H-NMR of compound 6a

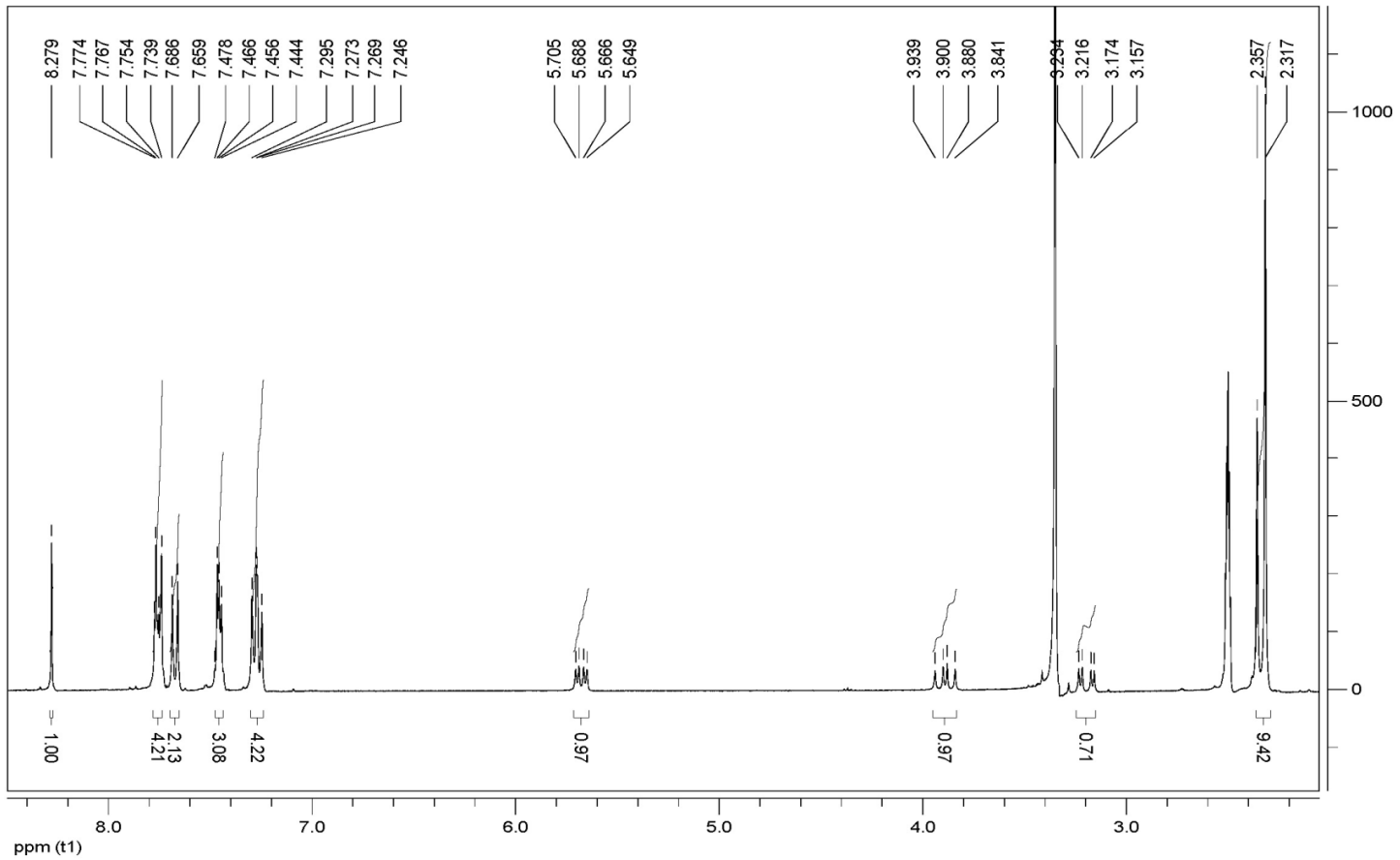


Figure 8: ^{1}H-NMR of compound 9a