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Original article

New pyrazole derivatives containing 1,2,4-triazoles and benzoxazoles as potent antimicrobial and analgesic agents

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ABSTRACT

Azole class of compounds are well known for their excellent therapeutic properties. Present paper describes about the synthesis of three series of new 1,2,4-triazole and benzoxazole derivatives containing substituted pyrazole moiety (**11a–d**, **12a–d** and **13a–d**). The newly synthesized compounds were characterized by spectral studies and also by C, H, N analyses. All the synthesized compounds were screened for their analgesic activity by the tail flick method. The antimicrobial activity of the new derivatives was also performed by Minimum Inhibitory Concentration (MIC) by the serial dilution method. The results revealed that the compound **11c** having 2,5-dichlorothiophene substituent on pyrazole moiety and a triazole ring showed significant analgesic and antimicrobial activity.

1. Introduction

The treatment of infectious diseases still remained as an important and challenging problem due to emerging infectious diseases and increasing number of multi-drug resistant microbial pathogens. In spite of the large number of antibiotics and chemotherapeutics, the emergence of old and new antibiotic resistant bacterial strains constitutes a substantial need for the new class of potent antimicrobial agents. Heterocyclic compounds play an important role in designing a new class of structural entities of medicinal importance with new mechanisms of action. These heterocyclic compounds are well known to possess diverse pharmacological properties, viz. antimicrobial, analgesic, anti-inflammatory, anticancer, anticonvulsant and antimalarial.

The chemistry of the carbon–nitrogen double bond plays a vital role in the progress of science. Compounds with the structure of –C=N– (azomethine group) are known as Schiff bases, which are usually synthesized by the condensation of primary amines and active carbonyl groups. The chemistry of Schiff bases derived from 1,2,4-triazole analogues has been an interesting field of study for a long time. It is well known from the literature that Schiff bases derived from 1,2,4-triazoles displayed excellent biological properties. In particular, they show antibacterial, antifungal [1], antitubercular [2], antioxidant [3], anticancer [4], antimalarials, anticonvulsant, anti-inflammatory [5,6] and pesticidal properties. They are important molecules in the medicinal and pharmaceutical fields and it has been suggested that the azomethine linkage might be responsible for the biological activities displayed by Schiff bases. Therefore it was planned to synthesize hybrids of substituted 1,2,4-triazoles. Hydrazone derivatives have shown different biological properties [7], such as anti-inflammatory, analgesic [8], anticonvulsant, antituberculous, and antimicrobial activity [9]. Hydrazones are important compounds for drug design, as possible ligands for metal complexes, organocatalysis and also for the syntheses of heterocyclic compounds. Derivatives of benzoxazole have gained much importance because of its wide applications in medicinal sector. They are associated with diverse pharmacological activities such as analgesic, antituberculosis [10], antibacterial [11], antifungal [12], anti-inflammatory, antioxidant and anthelmintic activity [13].

Further, pyrazole derivatives have shown significant biological activities, such as antimicrobial [1], analgesic [14], anti-inflammatory [15] and anticancer [16]. This gave a great impetus to the search for potential pharmacologically active drugs carrying

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pyrazole substituents. The search for novel antimicrobial and analgesic agents devoid of side effects continues to be an active area of research in medicinal chemistry. Although new and more expensive drugs have been developed, their cost is beyond the common man's reach. As a consequence, these trends have emphasized the pressing need for new, more effective, cheaper and safe antimicrobial agents.

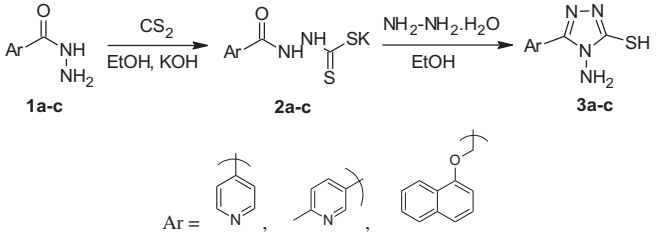
Many azole classes of compounds, mainly 1,2,4-triazoles and their derivatives constitute an important class of organic compounds with analgesic, anti-inflammatory and antimicrobial activities [17,18]. In addition, hydrazone derivatives also exhibit potent antimicrobial, analgesic and anti-inflammatory activities [17]. Small modifications in the structure of the targets will alter their biological character as well as their physiochemical properties. Keeping in view of this and in continuation of our search for biologically potent molecules [18–22], we hereby report the synthesis and biological evaluation of some new Schiff base analogous of 1,2,4-triazoles and benzoxazoles carrying substituted pyrazole moiety with the hope enhancing the biological activity.

2. Chemistry

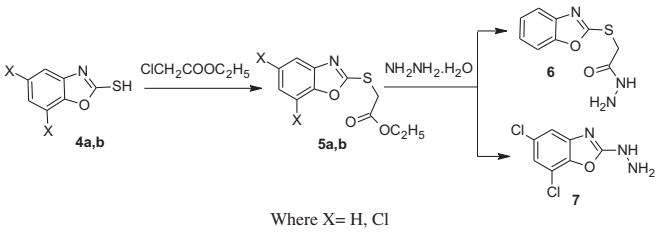
Intermediate triazoles **3a**–**c** were synthesized from corresponding acid hydrazides (**1a**–**c**) through multi-step reactions (Scheme 1) as per the reported literature [23,24]. The hydrazides were converted to potassium salt (**2a**–**c**) by reacting with carbon disulphide and potassium hydroxide in absolute ethanol. On reacting with hydrazine hydrate, the salt undergoes ring closure to yield the triazoles, **3a**–**c**.

2-(1,3-Benzoxazol-2-ylsulfanyl)-1-(hydrazinyloxy)ethanone **6** was synthesized from 1,3- benzoxazole-2-thiol (**5a**) by following the reported literature [13]. The anomalous reaction of hydrazine hydrate with ethyl[(5,7-dichloro-1,3-benzoxazol-2-yl)sulfanyl]acetate (**5b)** resulted in the formation of compound **7** [13]. The reaction sequence has been presented in Scheme 2.

Synthetic route of 3-substituted-1*H*-pyrazole-4-carbaldehydes (**10a**–**d**) has been reported in our previous work [25]. Substituted pyrazole aldehydes (**10a**–**d**) were synthesized by the Vilsmayer Haack reaction of se/micarbazones (**9a**–**d**), which in turn obtained by the reaction of semicarbazide hydrochloride with various substituted acetophenones in ethanol media [25]. The targeted Schiff bases (**11a**–**d**) were obtained in good yield by refluxing equimolar amount of 3-aryl-1-*H*-pyrazole-4-carbaldehyde (**10a**–**d**) with 4-amino-5-aryl-4*H*-1,2,4-triazole-3-thiol (**3a**–**c**) in the presence of catalytic amount of sulphuric acid using ethanol as solvent. New hydrazone derivatives (**12a**–**d** and **13a**–**d**) were synthesized by refluxing equimolar amount of 3-aryl-1-*H*-pyrazole-4-carbaldehyde (**10a**–**d**) with 2-(1,3-benzoxazol-2-ylsulfanyl) acetohydrazide (**6**)/5,7-dichloro-2-hydrazinyl-1,3-benzoxazole (**7**) in ethanol media, in the presence of catalytic amount of acetic acid. The reaction pathway has been summarized in Scheme 3.



Scheme 1. Synthetic route for the 1,2,4-triazoles.



Scheme 2. Synthetic route for the benzoxazolehydrazides.

3. Results and discussion

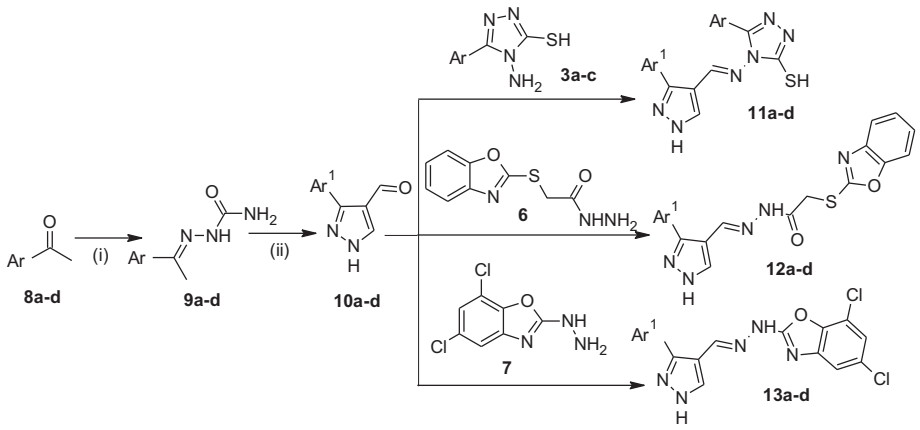
All the final derivatives **11a**–**d**, **12a**–**d** and **13a**–**d** were characterized after crystallization from appropriate solvents. IR spectrum of Schiff Base **11a** showed absorption at 3120 cm^{-1} which is due to the NH stretching. The band appeared at 1620 cm^{-1} is due to C=N stretching confirmed the structure of the compound. The ^1H NMR spectrum of **11a** showed a singlet at δ 2.50 which is due to SCH_3 protons. Two doublets at δ 7.38 ($J = 4.8$ Hz) and δ 7.65 ($J = 4.8$ Hz) are due to aromatic protons of p-thioanisyl moiety. A singlet at δ 8.21 is due to pyrazole-5H proton. Two doublets at δ 7.86 ($J = 6.7$ Hz) and δ 8.73 ($J = 6.7$ Hz) are due to aromatic protons of the pyridine ring. −N=CH protons appeared as a singlet at δ 9.52. Two singlets appeared in δ 13.81 and δ 14.38 are due to triazole SH and pyrazole-NH protons respectively further confirmed the structure. The mass spectrum of **11a** showed a molecular ion peak at $m/z = 394$ (m + 1), which is in agreement with the molecular formula $C_{18}H_{15}N_7S_2$.

IR spectrum of hydrazone **12a** showed absorption at 3215 cm^{-1} which is due to the NH stretching. Both the C=O and C=N stretching frequencies merged together and resulted in a broad band in 1652. The ^1H NMR spectrum of **12a** showed a singlet at δ 4.12 is due to CH_2 protons. Similarly multiplets at δ 7.27–7.80 are due to aromatic protons. A singlet at δ 7.84 is due to pyrazole-5H proton. The −N=CH− protons appeared as a singlet at δ 8.18. Two singlets appeared in δ 11.37 and δ 13.39 are due to −CO−NH− and pyrazole-NH protons respectively. The mass spectrum of **12a** showed a molecular ion peak at $m/z = 447$ (M + 1), which is in agreement with the molecular formula $C_{19}H_{13}Cl_2N_5O_2S_2$. Similarly the spectral values for all the compounds and C, H, N analyses are given in the experimental part and the characterization is provided in Table 1.

4. Pharmacology

4.1. Antimicrobial studies

All the newly synthesized compounds **11a-d**, **12a-d** and **13a-d** were screened for their antimicrobial activity. Antimicrobial study was assessed by Minimum Inhibitory Concentration (MIC) by the serial dilution method [26]. For this, *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa* microorganisms were used to investigate the antibacterial activity. Antifungal activity was carried out against *Candida albicans*. Ceftriaxone and Fluconazole were used as standard drugs for antibacterial and antifungal activities respectively. Several colonies of *S. aureus*, *B. subtilis*, *E. coli*, *P. aeruginosa* and *Pseudomonas albicans* were picked off a fresh isolation plate and inoculated in corresponding tubes containing 5 mL of trypticase soya broth. The broth was incubated for 6 h at 37 °C until there was visible growth. The MC Farland No.5 standard was prepared by adding 0.05 mL of 1% w/v $BaCl_2.2H_2O$ in Phosphate Buffered saline (PBS) to 9.95 mL of 1% v/v H_2SO_4 in PBS. The growth of all the four cultures was adjusted to Mc



Where, (i) Semicarbazide. HCl, Sodium Acetate, Ethanol (ii) DMF, $POCl_3$

Ar^1 = 2,4-Dichlorophenyl, 4-Thioanisyl, 2,5-Dichlorothiophene, Biphenyl

Ar = 1-Naphthyloxymethyl, Isonicotinyl, 6-Methylnicotinyl

Scheme 3. Synthetic route for the compounds **11a–d**, **12a–d** and **13a–d**.

Farland No.5 turbidity standard using sterile PBS. This gives a 108 cfu/mL suspension. The working inoculums of aforementioned four different microorganisms containing 105 cfu/mL suspension was prepared by diluting the 108 cfu/mL suspension, 103 times in trypticase soya broth.

4.1.1. Preparation of antimicrobial suspension (1 mg/mL)

Dissolved 10 mg of each compound in 10 mL of dimethyl formamide to get 1 mg/mL concentration.

4.1.2. Preparation of dilutions

In all, for each of the 12 antimicrobial compounds and standard antimicrobial i. e Ceftriaxone for antibacterial and Fluconazole for antifungal, 24 tubes of 5 mL capacity were arranged in five rows with each row containing 6 tubes. Then 1.9 mL of trypticase soya broth was added in the first tube in each row and then 1 mL in the remaining tubes. Now, 100 μL of antimicrobial suspension dissolved in dimethyl formamide was added to the first tube in each row and then after mixing the content, 1 mL was serially transferred from these tubes to the second tube in each of the rows. Then the contents of the second tube of each of the rows were mixed and transferred to the third tube in each of the rows. This serial dilution was repeated till the sixth tube in each of the rows. This provided antimicrobial concentrations of 50, 25, 12.5, 6.25, 3.125, 1.6125 μg/mL in the first to sixth tube respectively in each row. Finally, 1 mL of 10^5 cfu/mL of *S. aureus*, *B. subtilis*, *E. coli*, *P. aeruginosa* and *P. albicans* suspension was added to the first, second, third, fourth and fifth rows of tubes respectively. Along with the test samples and Ceftriaxone/Fluconazole (standard), the inoculums control (without antimicrobial compound) and broth control (without antimicrobial compound and inoculum) were maintained. All the test sample and control tubes were then incubated for 16 h at 37 °C.

4.1.3. Interpretation

After incubation, the tubes showing no visible growth were considered to be representing the MIC. The details of the results are furnished in Table 2. Inoculums control showed visible growth, whereas the broth control showed no growth.

Among the screened samples, **11c** and **11d** emerged as potent antimicrobial agents. **11c** showed excellent activity against *S. aureus* compared to the standard. **11c** showed similar activity against *B. subtilis* and *P. aeruginosa* as compared to standard drug Ceftriaxone. **11c** contains 2,5-dichlorothiophene substituent on a pyrazole ring which accounts for the enhanced activity. **11d** showed similar activity as that of standard drugs against *S. aureus*, *B. subtilis* and *P. aeruginosa*. **11d** having 2,4-dichlorophenyl moiety on pyrazole ring and the antibacterial activity is due to the presence of this group. **11b** showed similar activity as that of standard, against *P. aeruginosa* and **13c** had shown similar activity against *S. aureus* compared to Ceftriaxone. Compound **11b** has 2,4-dichlorophenyl moiety and **13c** has 2,5-dichlorothiophene

Table 1
Characterization data of the compounds **11a–d**, **12a–d** and **13a–d**.

Compounds	Ar^1	Ar	Molecular formula (Mol. wt.)	Yield (%)	M.p. (°C)
11b	2,4-Dichlorophenyl	1-Naphthyloxymethyl	$C_{23}H_{16}Cl_2N_6OS$ (495)	77	196–198
11c	2,5-Dichlorothiophene	1-Naphthyloxymethyl	$C_{21}H_{14}Cl_2N_6OS_2$ (501)	78	158–160
11d	2,4-Dichlorophenyl	6-Methylnicotinyl	$C_{18}H_{13}Cl_2N_7S$ (430)	76	156–158
12a	2,4-Dichlorophenyl	–	$C_{19}H_{17}Cl_2N_5O_2S$ (446)	90	150–152
12b	4-SCH_3-C_6H_4	–	$C_{20}H_{17}N_5O_2S_2$ (423)	86	168–170
12c	2,5-Dichlorothiophene	–	$C_{17}H_{11}Cl_2N_5O_2S_2$ (452)	85	184–186
12d	Biphenyl	–	$C_{25}H_{19}N_5O_2S$ (453)	88	196–198
13a	2,4-Dichlorophenyl	–	$C_{17}H_9Cl_4N_5O$ (441)	82	288–290
13b	4-SCH_3-C_6H_4	–	$C_{18}H_{13}Cl_2N_5OS$ (418)	85	244–246
13c	2,5-Dichlorothiophene	–	$C_{15}H_7Cl_4N_5OS$ (447)	81	284–286
13d	Biphenyl	–	$C_{23}H_{15}Cl_2N_5O$ (448)	83	264–266

Table 2
Antimicrobial activity data of the compounds **11a–d**, **12a–d** and **13a–d** in MIC (μg/mL).

Comp. No.	Antibacterial activity				Antifungal activity
	S. aureus	*B.subtilis*	*E.coli*	*P.aeruginosa*	*C. albicans*
11a	3.1250	3.1250	3.1250	6.2500	12.5000
11b	6.2500	3.1250	3.1250	1.6125	12.5000
11c	1.6125	1.6125	3.125	1.6125	6.25000
11d	3.1250	1.6125	3.1250	1.6125	6.25000
12a	12.5000	6.2500	3.125	6.2500	12.5000
12b	6.2500	12.500	6.2500	6.2500	12.5000
12c	6.2500	3.1250	3.1250	6.2500	25.0000
12d	12.5000	12.5000	25.0000	12.5000	25.0000
13a	12.5000	6.2500	12.5000	3.1250	12.5000
13b	6.2500	12.5000	12.5000	12.5000	25.0000
13c	3.1250	6.2500	3.1250	3.1250	12.5000
13d	12.5000	25.0000	12.5000	12.5000	25.0000
Standard	3.1250	1.6125	1.6125	1.6125	6.2500
Inoculum control	xxx[a]	xxx[a]	xxx[a]	xxx[a]	xxx[a]
Broth control	No growth	No growth	No growth	No growth	No growth

[a] Indicates Growth in all concentrations.

substituent respectively, which is responsible for the enhanced activity of the compounds. The remaining compounds showed moderately good activity against all of the four tested bacterial strains compared to the standard, Ceftriaxone.

Antifungal activity data revealed that compounds **11c** and **11d** had shown similar activity against the fungal stain *C. albicans* compared to the standard drug Fluconazole. Other derivatives were less active compared to standard. Among the three series, **11a–d** which contains triazole moiety showed good antimicrobial activity compared to the other two series **12a–d** and **13a–d**, which contains benzoxazole substituent.

4.2. Analgesic studies: tail flick method

This method was described by Asongalem et al. [27]. Young male Wistar strain albino rats (170–210 g body weight) were used for the study. Fourteen groups having six animals in each group were taken. Group one served as vehicle control, 0.5% CMC was given and group two served as standard received Pentazocin at a dose 2 mg/kg by i.p. route. Remaining groups received test drug at a dose level of 200 mg/kg and was given by i.p. route. Each of the preparation was given in such a manner so that the fluid intake was same in all cases. They were placed into individual restraining cages leaving the tail hanging out freely. Before testing, the animals were allowed to adapt to the cages for 30 min. The lower 5 cm portion of the tail was marked. This part of the tail was immersed in a cup of freshly filled water at 55 ± 2 °C. Within a few seconds, the rat reacts by withdrawing the tail. The reaction time was recorded using a stopwatch. After each determination, the tail was carefully dried. The reaction time was determined before and periodically after oral administration of the test substance (after 0, 30, 60 and 120 min). The cut off time of the immersion was 10 s. Percentage analgesic activity shown by the tested compounds is presented in Table 3.

Table 3
Analgesic activity (tail flick method) of compounds **11a-d, 12a-d** and **13a-d**.

Compound	% Inhibition ± SEM ($n = 6$)			
	0 min	30 min	60 min	120 min
Control	1.9 ± 0.12	4.20 ± 0.12	4.18 ± 0.13	4.20 ± 0.10
Pentazocin (Standard)	1.9 ± 0.12	2.17 ± 0.06[c]	2.18 ± 0.06[c]	2.22 ± 0.07[c]
11a	2.62 ± 0.28	2.77 ± 0.21[a]	2.83 ± 0.22[a]	2.90 ± 0.22
11b	2.85 ± 0.33	2.93 ± 0.32	2.25 ± 0.11	3.08 ± 0.33
11c	2.67 ± 0.41	2.52 ± 0.31[b]	2.28 ± 0.20[c]	2.20 ± 0.13[c]
11d	2.93 ± 0.30	3.50 ± 0.39	4.15 ± 0.32	2.98 ± 0.38
12a	2.27 ± 0.43	3.38 ± 0.36	3.45 ± 0.23	3.62 ± 0.55
12b	3.05 ± 0.33	3.15 ± 0.37	3.53 ± 0.22	3.83 ± 0.21
12c	2.55 ± 0.42	2.82 ± 0.43[a]	2.93 ± 0.44	3.06 ± 0.44
12d	3.13 ± 0.37	3.27 ± 0.39	3.43 ± 0.41	3.63 ± 0.39
13a	2.75 ± 0.31	2.92 ± 0.30	3.05 ± 0.30	3.20 ± 0.31
13b	3.25 ± 0.38	3.42 ± 0.36	3.57 ± 0.38	3.63 ± 0.37
13c	2.98 ± 0.35	3.03 ± 0.36	3.07 ± 0.36	3.10 ± 0.35
13d	2.43 ± 0.24	2.65 ± 0.26[a]	2.77 ± 0.26	2.95 ± 0.28[a]

Statistical analysis was done by one way analysis of variance (ANOVA) followed by Dunnet's test, $n = 6$. Values were compared with respect to standard drug pentazocin.
[a] $P < 0.05$ (Significant from the control).
[b] $P < 0.01$ (Significant from the control).
[c] $P < 0.001$ (Significant from the control).

Out of the newly synthesized compounds, **11c** showed significant activity at 30 min and highly significant activity at 60 and 120 min. As regards the relationships between the structure of the heterocyclic scaffold and the detected analgesic properties, it showed varied biological activity. Probably in this case the nature of the heterocyclic ring as well as the presence of different substituents causes a certain change of activity. Among the three series, **11a–d** which contains triazole moiety showed good activity compared to the other two series **12a–d** and **13a–d**. In the first series **11a–d,** compound **11c** is showing significant analgesic activity and have 2,5-dichlorothiophene moiety. From the obtained results, it is clear that the major role for analgesic activity is played by the substituent present on pyrazole and the heterocyclic rings bonded to pyrazole moiety.

5. Conclusion

Three series of new Schiff bases and hydrazones of substituted pyrazole were synthesized in reasonably good yields. The newly synthesized compounds were characterized by ^{1}H NMR, ^{13}C NMR, mass spectrometry, IR studies and elemental analyses. All the newly synthesized compounds **11a-d, 12a-d** and **13a-d** were screened for analgesic activity by tail flick method and the results showed moderately good activity compared to the standard drug Pentazocin. Among the synthesized compounds, **11c** showed significant analgesic activity and have 2,5-dichlorothiophene substituent on pyrazole ring, which accounts for the enhanced analgesic activity. The antimicrobial activity of the new derivatives was also performed and the results revealed that among the screened samples, **11c** and **11d** emerged as potent antimicrobial agents. **11c** and **11d** contains 2,5-dichlorothiophene, 2,4-dichlorophenyl substituent respectively on pyrazole ring. Among the synthesized compounds, **11a–d** which contains triazole moiety showed good analgesic and antimicrobial activity compared to **12a–d** and **13a–d**, which contains benzoxazole substituent.

As regards the relationships between the structure of the heterocyclic scaffold and the detected antimicrobial/analgesic properties, it showed varied biological activity. Probably in this case the nature of the heterocyclic ring as well as the presence of different substituents caused a certain change of activity. From the antimicrobial and analgesic screening results, one can conclude that, a combination of two different heterocyclic systems namely pyrazole and 1,2,4-triazoles has enhanced the pharmacological effect and hence they are ideally suited for further modifications to obtain more efficacious antibacterial/analgesic compounds.

6. Experimental

6.1. Chemistry

Melting points were determined by open capillary method and were uncorrected. The IR spectra (in KBr pellets) were recorded on

a JASCO FT/IR-4100 spectrophotometer. ^{1}H NMR spectra were recorded (DMSO-d_6) on a Bruker (400 MHz) using TMS as internal standard. Chemical shift values are given in δ (ppm) scales. The mass spectra were recorded on a JEOL JMS-D 300 spectrometer operating at 70 eV. Elemental analyses were performed on a Flash EA 1112 series CHNS–O Analyzer. The completion of the reaction was checked by thin layer chromatography (TLC) on silica gel coated aluminium sheets (silica gel 60 F254) obtained from Merck. Commercial grade solvents and reagents were used without further purification.

6.2. General procedure for the synthesis of 4-{[(E)-{4-[aryl]-1H-pyrazol-3-yl}methylidene] amino}-5-(substituted)-4H-1,2,4-triazole-3-thiol (**11a-d**)

An equimolar mixture of 3-aryl-1*H*-pyrazole-4-carbaldehyde, **10a-d** (0.1 mol) and 4-amino-5-substituted-4*H*-1,2,4-triazole-3-thiol, **3a-d** (0.1 mol) were dissolved in warm ethanol (20 mL). A catalytic amount of sulphuric acid was added to the reaction mixture and refluxed for 8 h, then kept at room temperature overnight. The resultant solid was filtered, washed with chilled ethanol, dried and recrystallized from ethanol–dioxane (1:2) mixture to afford compounds **3a**–**d**.

6.2.1. 4-{[(E)-{4-[4-(Methylsulfanyl)phenyl]-1H-pyrazol-3-yl} methylidene]amino}-5-(pyridin-4-yl)-4H-1,2,4-triazole-3-thiol (**11a**)

IR (KBr, υ_{max} cm^{-1}): 3120 (N–H-str), 2914 (C–H-str), 1620(C=N), 1542 (C=C); ^{1}H NMR (DMSO-d_6): δ 2.50 (s, 3H, SCH_3), 7.38 (d, 2H, J = 4.8 Hz, p-thioanisyl), 7.65 (d, 2H, J = 4.8 Hz, p-thioanisyl), 7.86 (d, 2H, J = 6.7 Hz, 4-pyridyl), 8.21 (s, 1H, pyrazole-5H), 8.73 (d, 2H, J = 6.7 Hz, 4-pyridyl), 9.52 (s, 1H, N=CH), 13.81 (s, 1H, SH), 14.38 (s, 1H, pyrazole-NH); MS: m/z = 394 (M + 1). Anal. calcd. for $C_{18}H_{15}N_7S_2$: C, 59.94; H, 3.84; N, 24.92. Found: C, 59.91; H, 3.80; N, 24.92%.

6.2.2. 4-({(E)-[4-(2,4-Dichlorophenyl)-1H-pyrazol-3-yl] methylidene}amino)-5-[(naphthalen-1-yloxy)methyl]-4H-1,2,4-triazole-3-thiol (**11b**)

IR (KBr, υ_{max} cm^{-1}): 3118 (N–H-str), 2917 (C–H-str), 1622 (C=N), 1596 (C=C); ^{1}H NMR (DMSO-d_6): δ 5.34 (s, 2H, OCH_2), 7.09–7.86 (m, 10H, Ar–H), 8.27 (s, 1H, pyrazole-5H), 8.68 (s, 1H, N=CH), 13.62 (s, 1H, SH), 14.21 (s, 1H, pyrazole-NH); ^{13}C NMR: δ 141.81, 134.28, 133.86, 133.39, 127.00, 126.29, 125.82, 125.31; MS: m/z = 496 (M + 1). Anal. calcd. for $C_{23}H_{16}Cl_2N_6OS$: C, 55.76; H, 3.26; N, 16.96. Found: C, 55.73; H, 3.24; N, 16.93%.

6.2.3. 4-({(E)-[4-(2,5-Dichlorothiophen-3-yl)-1H-pyrazol-3-yl] methylidene}amino)-5-[(naphthalene-1-yloxy)methyl]-4H-1,2,4-triazole-3-thiol (**11c**)

IR (KBr, υ_{max} cm^{-1}): 3368 (N–H-str), 2915(C–H-str), 1652 (C=N), 1571 (C=C); ^{1}H NMR (DMSO-d_6): δ 5.33 (s, 2H, OCH_2), 7.11–8.01 (m, 8H, Ar–H), 8.41 (s, 1H, pyrazole-5H), 9.76 (s, 1H, N=CH), 13.76 (s, 1H, SH), 14.05 (s, 1H, pyrazole-NH); MS: m/z = 502 (M + 1). Anal. calcd. for $C_{21}H_{14}Cl_2N_6OS_2$: C, 50.30; H, 2.81; N, 16.76. Found: C, 50.27; H, 2.79; N, 16.73%.

6.2.4. 4-({(E)-[4-(2,4-Dichlorophenyl)-1H-pyrazol-3-yl] methylidene}amino)-5-(6-methylpyridin-3-yl)-4H-1,2,4-triazole-3-thiol (**11d**)

IR (KBr, υ_{max} cm^{-1}): 3240 (N–H-str), 2913 (C–H-str), 1626(C=N), 1552 (C=C); ^{1}H NMR (DMSO-d_6): δ 2.49 (s, 3H, CH_3), 7.36–7.92 (m, 5H, Ar–H), 8.43 (s, 1H, pyrazole-5H), 8.75 (s, 1H, pyridine-2H), 9.49 (s, 1H, N=CH), 13.83 (s, 1H, SH), 14.13 (s, 1H, pyrazole-NH); MS: m/z = 431 (M + 1). Anal. calcd. for $C_{18}H_{13}Cl_2N_7S$: C, 50.24; H, 3.05; N, 22.78. Found: C, 50.21 H, 3.01; N, 22.74%.

6.3. General procedure for the synthesis of hydrazones **12a-d** and **13a-d**

An equimolar mixture of 3-aryl-1*H*-pyrazole-4-carbaldehyde, **10a-d** (0.1 mol) and corresponding benzoxazolehydrazides, **6** or **7**, (0.1 mol) were dissolved in warm ethanol (20 mL) containing glacial acetic acid (0.5 mL). The reaction mixture was refluxed for 8 h and then kept at room temperature overnight. The solids precipitated were filtered, washed with chilled ethanol, dried and recrystallized from hot ethanol to afford compounds **12a-d** and **13a-d**.

6.3.1. 2-(1,3-Benzoxazol-2-ylsulfanyl)-N′-{(E)-[3-(2,4-dichlorophenyl)-1H-pyrazol-4-yl] methylidene}acetohydrazide (**12a**)

IR (KBr, υ_{max} cm^{-1}): 3215 (N–H-str), 2971 (C–H-str), 1652 (C=O, C=N); ^{1}H NMR (DMSO-d_6): δ 4.12 (s, 2H, $-CH_2-$CO), 7.27–7.80 (m, 8H, Ar–H), 7.84 (s, 1H, pyrazole-5H), 8.18 (s, 1H, N=CH), 11.37 (s, 1H, CO–NH), 13.39 (s, 1H, pyrazole-NH); MS: m/z = 447 (M + 1). Anal. calcd. for $C_{19}H_{17}Cl_2N_5O_2S$: C, 51.13; H, 2.94; N, 15.69. Found: C, 51.09; H, 2.91; N, 15.66%.

6.3.2. 2-(1,3-Benzoxazol-2-ylsulfanyl)-N′-[(E)-{3-[4-(methylsulfanyl)phenyl]-1H-pyrazol-4-yl}methylidene] acetohydrazide (**12b**)

IR (KBr, υ_{max} cm^{-1}): 3154 (N–H-str), 2916 (C–H-str), 1659 (C=O), 1610 (C=N); ^{1}H NMR (DMSO-d_6): δ 2.45 (s, 3H, SCH_3), 4.17 (s, 2H, $-CH_2-$CO), 7.26–7.62 (m, 8H, Ar–H), 8.07 (s, 1H, pyrazole-5H), 8.20 (s, 1H, N=CH), 11.42 (s, 1H, CO–NH), 13.39 (s, 1H, pyrazole-NH); MS: m/z = 424 (M + 1). Anal. calcd. for $C_{20}H_{17}N_5O_2S_2$: C, 56.72; H, 4.05; N, 16.54. Found: C, 56.69; H, 4.03; N, 16.51%.

6.3.3. 2-(1,3-Benzoxazol-2-ylsulfanyl)-N′-{(E)-[3-(2,5-dichlorothiophen-3-yl)-1H-pyrazol-4-yl]methylidene} acetohydrazide (**12c**)

IR (KBr, υ_{max} cm^{-1}): 3163 (N–H-str), 2945 (C–H-str), 1654 (C=O, C=N); ^{1}H NMR (DMSO-d_6): δ 4.18 (s, 2H, $-CH_2-$CO), 7.25–7.60 (m, 5H, Ar–H), 8.14 (s, 1H, pyrazole-5H), 8.31 (s, 1H, N=CH), 11.46 (s, 1H, CO–NH), 13.38 (s, 1H, pyrazole-NH); MS: m/z = 453 (M + 1). Anal. calcd. for $C_{17}H_{11}Cl_2N_5O_2S_2$: C, 45.14; H, 2.45; N, 15.48. Found: C, 45.12; H, 2.43; N, 15.44%.

6.3.4. 2-(1,3-Benzoxazol-2-ylsulfanyl)-N′-[(E)-(3- biphenyl -1H-pyrazol-4-yl)methylidene] acetohydrazide (**12d**)

IR (KBr, υ_{max} cm^{-1}): 3179 (N–H-str), 2966 (C–H-str), 1656 (C=O, C=N); ^{1}H NMR (DMSO-d_6): δ 4.46 (s, 2H, $-CH_2-$CO), 7.21–7.68 (m, 13H, Ar–H), 8.07 (s, 1H, pyrazole-5H), 8.25 (s, 1H, N=CH), 11.42 (s, 1H, CO–NH), 13.32 (s, 1H, pyrazole-NH); MS: m/z = 454 (M + 1). Anal. calcd. for $C_{25}H_{19}N_5O_2S$: C, 66.21; H, 4.22; N, 15.44. Found: C, 66.17; H, 4.19; N, 15.41%.

6.3.5. 5,7-Dichloro-2-[(2E)-2-{[3-(2,4-dichlorophenyl)-1H-pyrazol-4-yl]methylidene} hydrazinyl]-1,3-benzoxazole (**13a**)

IR (KBr, υ_{max} cm^{-1}): 3132 (N–H-str), 2902 (C–H-str), 1660 (C=N), 1551 (C=C); ^{1}H NMR (DMSO-d_6): δ 7.21–7.76 (m, 5H, Ar–H), 7.93 (s, 1H, pyrazole-5H), 8.19 (s, 1H, N=CH), 12.05 (s, 1H, NH), 13.46 (s, 1H, pyrazole-NH); MS: m/z = 442 (M + 1). Anal. calcd. for $C_{17}H_9Cl_4N_5O$: C, 46.29; H, 2.06; N, 15.88. Found: C, 46.25; H, 2.03; N, 15.84%.

6.3.6. 5,7-Dichloro-2-[(2E)-2-({3-[4-(methylsulfanyl)phenyl]-1H-pyrazol-4-yl}methylidene) hydrazinyl]-1,3-benzoxazole (**13b**)

IR (KBr, υ_{max} cm^{-1}): 3126 (N–H-str), 2909 (C–H-str), 1657 (C=N), 1550 (C=C); ^{1}H NMR (DMSO-d_6): δ 2.46 (s, 3H, SCH_3), 7.25–7.57 (m, 6H, Ar–H), 8.08 (s, 1H, pyrazole-5H), 8.19 (s, 1H, N=CH), 12.12 (s, 1H, NH), 13.41 (s, 1H, pyrazole-NH); MS: m/z = 419 (M + 1).

Anal. calcd. for $C_{18}H_{13}Cl_2N_5OS$: C, 51.68; H, 3.13; N, 16.74. Found: C, 51.64; H, 3.11; N, 16.71%.

*6.3.7. 5,7-Dichloro-2-[(2E)-2-{[3-(2,5-dichlorothiophen-3-yl)-1H-pyrazol-4-yl]methylidene} hydrazinyl]-1,3-benzoxazole (**13c**)*

IR (KBr, υ_{max} cm^{-1}): 3130 (N–H–str), 2912 (C–H-str), 1621 (C=N), 1583 (C=C); ^{1}H NMR (DMSO-d_6): δ 7.24–7.37 (m, 3H, Ar–H), 8.05 (s, 1H, pyrazole-5H), 8.24 (s, 1H, N=CH), 12.14 (s, 1H, NH), 13.51 (s, 1H, pyrazole-NH); MS: m/z = 448 (M + 1). Anal. calcd. for $C_{15}H_7Cl_4N_5OS$: C, 40.29; H, 1.58; N, 15.66. Found: C, 40.26; H, 1.56; N, 15.63%.

*6.3.8. 2-[(2E)-2-{[3-(Biphenyl-4-yl)-1H-pyrazol-4-yl]methylidene} hydrazinyl]-5,7-dichloro-1,3-benzoxazole (**13d**)*

IR (KBr, υ_{max} cm^{-1}): 3110 (N–H-str), 2905 (C–H-str), 1659 (C=N), 1548 (C=C); ^{1}H NMR (DMSO-d_6): δ 7.24–8.02 (m, 11H, Ar–H), 8.07 (s, 1H, pyrazole-5H), 8.27 (s, 1H, N=CH), 12.15 (s, 1H, NH), 13.49 (s, 1H, pyrazole-NH); ^{13}C NMR (DEPT, DMSO-d_6, 100 MHz): δ 140.68, 129.53, 129.22, 128.26, 127.46, 120.93, 115.67; MS: m/z = 449 (M + 1). Anal. calcd. for $C_{23}H_{15}Cl_2N_5O$: C, 61.62; H, 3.37; N, 15.62. Found: C, 61.59; H, 3.35; N, 15.60%.

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Appendix A. Supplementary data

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