Synthesis and Antimicrobial Activity Evaluation of Pyrazolone Incorporated Azo Derivatives of 4-cyano/4-carbomoylthiophene-2-carboxylates

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ABSTRACT:

A series of Pyrazolone incorporated azo derivatives of 4-cyano/4-carbomoyl-thiophene-2-carboxylates were successfully synthesized, characterized and evaluated for their antimicrobial activities. The compounds showed excellent to moderate antimicrobial activities against Gram negative bacteria *Escherichia coli* and Gram-positive bacteria *Staphylococcus aureus* and antifungal activity against *Aspergillus niger* and *Candida albicans* with MIC 50 µg/mL on comparison with standard drug Gentamicin (20 µg/mL) and Fluconazole (20 µg/mL).

Keywords- Pyrazolone, Thiophene-2-carboxylate, Azo compounds, Antimicrobial activity

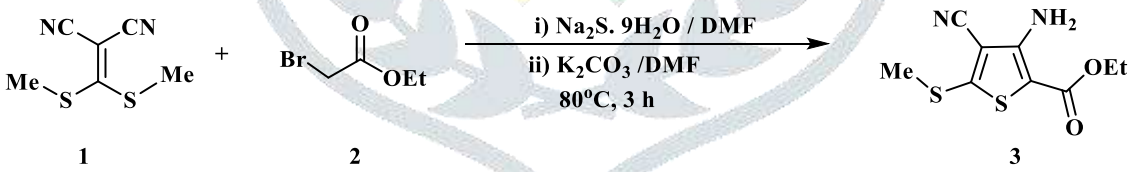
I. INTRODUCTION:

Pyrazole derivatives are the most active classes of compounds that show broad spectrum of pharmacological activities like antimicrobial [1-4, 15, 16], anticonvulsant [1, 5, 6], anticancer [7], cardiovascular [8], analgesic [9, 15, 16], anti-inflammatory [10, 11, 15, 16], antitubercular [12, 13, 15, 16], antipyretic, anticancer, antimalarial [15, 16], mice antinociceptive activity [17] etc. In recent years, many drugs have been developed from pyrazole derivatives for example, *Celecoxib* (anti-inflammatory and COX-2 inhibitor) [18]; *Rimonabant* (as a cannabinoid receptor and anti-obesity) [18]; *Fomepizole* (alcohol dehydrogenase inhibitor) and *Sildenafil* (phosphodiesterase-2 inhibitor) [18]. These active Schiff bases of 4-acyl-5-pyrazolones showed applications as intermediates in analytical, agricultural, biological, and pharmaceutical chemistry [19]. The pyrazoles are also used as intermediates for the synthesis of heterocyclic azopyrazole disperse, acid, and reactive dyes [20]. Azo molecules are popular for their therapeutic uses and also involved in a number of biological reactions such as inhibition of DNA, RNA and protein synthesis, carcinogenesis, and nitrogen fixation [21]. Azo compounds were reported to show a variety of biological activities including antibacterial, antifungal, pesticidal, antiviral and anti-inflammatory activities [22]. Thiophene based pyrazole derivatives has many significant biological activities such as antiproliferative activity [23], antitumor [24, 26], antimicrobial, anti-inflammatory [25]. Thiophene based

azo dyes showed generally red to blue colour with a high extinction coefficient in comparison with aniline-based azo dyes. 2-aminothiophene and its benzo analogs are very useful compounds as intermediates in the dyestuff industry and find a wide range of pharmaceutical applications [27]. Azodyes prepared from antipyrine showed color and dyeing properties on polyester fabrics [28]. Thiophene incorporated pyrazolones obtained via diazo coupling of diazonium salt of 3-substituted-2-amino-4,5,6,7-tetrahydrobenzo[b]thiophenes with 3-methyl-1*H*-pyrazol-5(4*H*)-one, 3-methyl-1-phenyl-1*H*-pyrazol-5(4*H*)-one or 3-amino-1*H*-pyrazol-5(4*H*)-one also showed good dyeing properties such as color measurements and fastness properties in comparison with aniline-based azo dyes [28]. (3,5-dimethyl-phenyl-1H-pyrazol-4-yl)-(substituted-benzothiazol-2-yl)-diazenes were derived from substituted 2-aminobenzothiazole, acetyl acetone and various phenyl hydrazines showed antifungal activity [29]. This literature survey encouraged us for the synthesis of pyrazole incorporated azo derivatives of 4-cyano/4-carbomoyl thiophene-2-carboxylate.

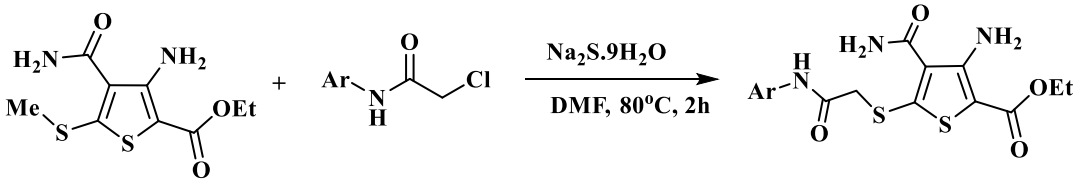
II. MATERIALS AND METHODS:

Synthesis of ethyl 3-(2-(3-methyl-1*H*-pyrazol-4(5*H*)-yl-5-ol)diazenyl)-5-(methylthio)-4-cyanothiophene-2-carboxylates **8(a-h)** was carried out using 3-amino-4-cyano-5(aryl substituted methylthio)thiophene-2-carboxylates **3, 4, 6(a-f)** and **7.** The synthesis of compounds **3, 4, 6(a-f)**, **7** and **10** was carried out using literature methods [30-35]. The compound **3 (**1 mmol**)** were diazotized in Conc. HCl and water mixture (1:1) with equimolar quantity of sodium nitrite ($NaNO_2$) followed by portion wise addition of the ice-cold solution of pyrazolone **7** in pyridine 2 mL. After stirring the mixture for 3 h, yellow solid separated as product **8a**. ***(Scheme 2)***.



1 2 3

Scheme 1: Synthesis of ethyl 3-amino-4-cyano-5-(methylthio)thiophene-2-carboxylate, 3 [30]



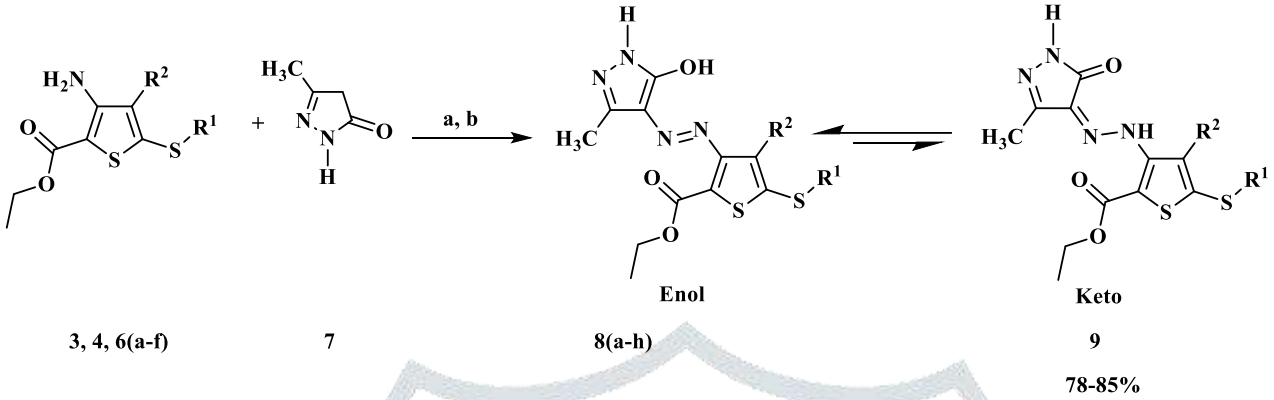
4 5a-f 6a-f

Ar = C_6H_5, 4-$MeOC_6H_4$, 4-ClC_6H_4, 4-BrC_6H_4, 3-$NO_2C_6H_4$, 4-$NO_2C_6H_4$

Scheme 2: Synthesis of ethyl 5- ((4-substituted phenylcarbamoyl) methylthio)-3-amino- 4-carbamoylthiophene-2-carboxylate derivatives, 6a-f [30]

The ethyl 3-amino-4-cyano-5-(methylthio)thiophene-2-carboxylate, **3 (**1 mmol**)** was diazotized in conc. HCl and water mixture (1:1) with equimolar quantity of sodium nitrite ($NaNO_2$). The ice-cold solution of

5-methyl-2,4-dihydro-3H-pyrazol-3-one **7** in 2 mL pyridine was prepared and added portion wise addition of the ice-cold solution of diazotized compound **3**. The reaction mixture continuously stirred for 3 h at room temperature and yellow solid separated was collected as product **8a**. The same procedure was employed for the synthesis of compounds **8b-h** from the compounds **4** and **6a-f.** ***(Scheme 3)***.



3, 4, 6(a-f) **7** **8(a-h)** **9**

78-85%

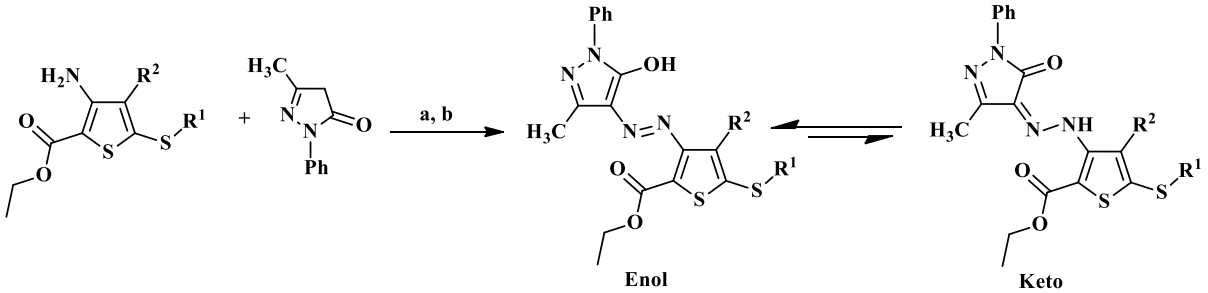
Scheme 3: Synthesis of ethyl 3-(2-(3-methyl-1*H*-pyrazol-4(5*H*)-yl-5-ol)diazenyl)-5-(methylthio)-4-carbomoylthiophene-2-carboxylates, 8(a-h)

Reaction Conditions: a) $NaNO_2$/ Conc. HCl, 0-5ºC, stir 15 min; b) Comp.3, 4, 6(a-f), pyridine/H_2O, 0-5ºC, stir 3-6 h

TABLE I

Compounds 8(a-h) Practical Yield

Comp. No.	R^1	R^2	Comp. No.	R^1	R^2	% yield
3	SMe	CN	**8a**	SMe	CN	**78.0**
4	SMe	$CONH_2$	**8b**	SMe	$CONH_2$	**79.0**
6a	CH_2CONH-C_6H_5	$CONH_2$	**8c**	CH_2CONH-C_6H_5	$CONH_2$	**79.3**
6b	CH_2CONH-4-$MeOC_6H_4$	$CONH_2$	**8d**	CH_2CONH-4-$MeOC_6H_4$	$CONH_2$	**85.0**
6c	CH_2CONH-4-ClC_6H_4	$CONH_2$	**8e**	CH_2CONH-4-ClC_6H_4	$CONH_2$	**80.0**
6d	CH_2CONH-4-BrC_6H_4	$CONH_2$	**8f**	CH_2CONH-4-BrC_6H_4	$CONH_2$	**83.0**
6e	CH_2CONH-3-$O_2NC_6H_5$	$CONH_2$	**8g**	CH_2CONH-3-$O_2NC_6H_5$	$CONH_2$	**81.0**



3, 4, 6(a-f) **10** **11(a-h)** **12**

75-83%

Scheme 4: Synthesis of ethyl 3-(2-(3-methyl-1-phenyl-1*H*-pyrazol-4(5*H*)-yl-5-ol)diazenyl)-5 (methylthio)-4-carbomoylthiophene-2-carboxylates, 11(a-h)

Reaction Conditions: a) $NaNO_2$/Conc.HCl, 0-5ºC, stir 15 min; b) Comp.3, 4, (a-f), pyridine/H_2O, 0-5ºC, stir 3-6 h

TABLE III

Compounds 11(a-h) Practical Yield

Comp. No.	R^1	R^2	Comp. No.	R^1	R^2	% yield
3	SMe	CN	**11a**	SMe	CN	**74.6**
4	SMe	$CONH_2$	**11b**	SMe	$CONH_2$	**78.3**
6a	$CH_2CONH\text{-}C_6H_5$	$CONH_2$	**11c**	$CH_2CONH\text{-}C_6H_5$	$CONH_2$	**77.6**
6b	$CH_2CONH\text{-}4\text{-}MeOC_6H_4$	$CONH_2$	**11d**	$CH_2CONH\text{-}4\text{-}MeOC_6H_4$	$CONH_2$	**79.0**
6c	$CH_2CONH\text{-}4\text{-}ClC_6H_4$	$CONH_2$	**11e**	$CH_2CONH\text{-}4\text{-}ClC_6H_4$	$CONH_2$	**77.0**
6d	$CH_2CONH\text{-}4\text{-}BrC_6H_4$	$CONH_2$	**11f**	$CH_2CONH\text{-}4\text{-}BrC_6H_4$	$CONH_2$	**80.0**
6e	$CH_2CONH\text{-}3\text{-}O_2NC_6H_5$	$CONH_2$	**11g**	$CH_2CONH\text{-}3\text{-}O_2NC_6H_5$	$CONH_2$	**83.0**
6f	$CH_2CONH\text{-}4\text{-}O_2NC_6H_5$	$CONH_2$	**11h**	$CH_2CONH\text{-}4\text{-}O_2NC_6H_5$	$CONH_2$	**81.0**

Similarly, synthesis of ethyl 3-(2-(3-methyl-1-phenyl-1*H*-pyrazol-4(5*H*)-yl-5-ol)diazenyl)-5 (methylthio)-4-cyano OR 4-carbomoylthiophene-2-carboxylates, **11(a-h)** was carried out using 3-methyl-1-phenyl-1H-pyrazol-5(4H)-one **10** and 3-amino-4-cyano-5-(aryl substituted methylthio) thiophene-2-carboxylates **3, 4, 6(a-f)**. The structure of the compound **11a** was confirmed by IR, ^{1}H NMR and ^{13}C NMR.

III. RESULTS AND DISCUSSION:

The structure of the synthesized compounds **8(a-h)** and **11(a-h)** were confirmed by IR, ^{1}H NMR and ^{13}C NMR. The structure of the compound **8a** was confirmed by IR, ^{1}H NMR and ^{13}C NMR. The IR spectrum of **8a** displayed stretching frequencies could be assigned as 3441 (OH), 3336, 3062 (=CH), 2985 (ArCH), 2218 (CN), 1732(2CO), 1674 (CO) cm^{-1}. IR spectrum of **8c** displayed stretching frequencies assigned as 3441 (OH), 3340 (NH, NH_2), 3062 (=CH), 2985 (ArCH), 1732(C=O), 1678 (C=O) cm^{-1}. ^{1}H NMR spectrum of compound **8d** showed singlet at δ 1.31-1.34 (CH_3), singlet at δ 2.31 (pyrazole CH_3), singlet at δ 4.03 (SCH_2), singlet at δ 3.45 ($ArOCH_3$), singlet at δ 6.62 (pyrazolone NH), singlet at δ 7.78 (NH), singlet at δ 10.18 ppm (OH) groups. All the four aromatic protons of were appeared in their respective region at δ 6.89-6.91 and at δ 7.45-7.47 ppm as doublets. The ^{13}C NMR spectrum of compound **8d** showed δ 14.87 (pyrazolone CH_3), 40.91 (SCH_2), 55.65 (OCH_3), 60.25 (OCH_2), 97.98 (thiophene C_3 and C_4), 114.44 (phenyl C_3 and C_5), 121.37 (phenyl C_2 and C_6), 125.39 (phenyl C_1), 132.10 (hydrazone, C=N), 145.57 (pyrazoline, C=N), 154.07 (thiophene C_1 and C_5), 156.03 (phenyl C_4), 163.01 (pyrazoline, C=O), 165.04 (ester, C=O), 165.44 (amide, C=O) as there characteristic peaks.The IR spectrum of **11a** displayed stretching frequencies as 3441 (OH), 3336 (NH, NH_2), 3201 (=CH), 2981, 2920 (ArCH), 2218 (CN), 1670 (C=O) cm^{-1}. Similarly, IR spectrum of **11b** displayed stretching frequencies could br assigned as 3441 (OH), 3336 (NH, NH_2), 3066 (=CH), 2981, 2920 (ArCH), 1732 (C=O), 1670 (C=O), 1618 cm^{-1}. ^{1}H NMR spectrum of compound **11c** showed singlet at δ 1.31-1.34 (CH_3), singlet at δ 2.38 (pyrazole CH_3), singlet at δ 4.21 (SCH_2), singlet at δ 6.62 (NH_2), singlet at δ 7.78 (NH), singlet at δ 10.76 ppm (OH) groups. All the five aromatic protons phenyl ring of pyrazole were appeared in their respective region at δ 7.23-7.27 ppm as multiplets. The ^{13}C NMR spectrum of compound **11c** showed δ ppm: 14.72 (ester CH_3), 17.59 (SCH_3), 18.28 (pyrazolone CH_3), 40.43 (SCH_2), 61.88 (OCH_2), 117.27 (phenyl, C_2, C_6 and C_3, C_5), 118.38 (thiophene C_2 and C_5), 122.37 (phenyl C_4), 125.39 (phenyl C_1), 129.41 (thiophene C_3 and C_4), 129.41

(hydrazone, C=N), 146.95 (pyrazoline, C=N), 153.84 (pyrazoline, C=O), 155.56 (ester, C=O), 159.57 (amide, C=O) ppm as their characteristic peaks.

IV. ANTIMICROBIAL ASSAY:

The antimicrobial assay evaluation of the recently synthesized 3-aminothiophene-2-carboxylate derivatives was done using agar well plate method [36,37]. The antibacterial and antifungal assays were performed in Muller-Hinton broth and Crazek Dox broth [36,37]. The standard strains used for the antimicrobial assay was procured from Microbial Culture Collection, Pune, India.

Antimicrobial evaluation was performed using the bacteria reseeded in Muller-Hinton broth for 24 hr at 37ºC and fungi reseeded in CrazekDox broth for 48 hr at 25ºC. The antibacterial activity of tested samples was studied in triplicate against gram positive bacteria *Staphylococcus aureus* (ATCC 29737) and gram-negative bacteria *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853). The same samples were tested for antifungal activity in triplicate against *Candida albicans* (MTCC 277) and *Aspergillus niger* (MCIM 545). The compounds were dissolved in DMSO at desired concentrations of 200, 100, 50 µg/ mL. DMSO was loaded as negative control. Gentamicin (10 µg/ mL) and Fluconazole (20 µg/ mL) were used as standards for evaluating the antibacterial and antifungal activity. The zone of inhibition (mm) was determined from the diameter of the zone of inhibition using calliper. The lowest concentration that showed invisible growth after spot subculture was considered as Minimum Inhibitory Concentration (MIC µg/mL) value for each sample after 24 hr incubation period at 37ºC. (MIC µg/ mL) value for each sample was determined using MH agar plates by pouring the molten agar in unique sized petri dishes as per National Committee for Clinical Laboratory Standards (NCCLS, M7-A5, January 2000) [36, 37].

Compounds **8a, 8b, 8c, 8d** and **8e** with chloro (Cl), methoxy (OCH_3) functionalities showed moderate antibacterial activity against Gram positive bacteria *Staphylococcus aureus* with MIC 100µg/mL. The compounds **8e** and **8h** containing chloro (Cl) and para nitro (p-NO_2) group showed excellent antibacterial activity against Gram negative bacteria *Escherichia coli* and Gram-positive bacteria *Staphylococcus aureus* with MIC 50 µg/mL when compared with standard drug Gentamicin (20 µg/mL). The compounds **8a**, **8c**, **8d**,**8e** showed moderate antibacterial activity against Gram-negative *Pseudomonas aeruginosa* (ATCC 27853). Similarly, compounds **8e** and **8h** containing chloro (Cl) and para nitro (p-NO_2) group showed excellent antifungal activity against *Aspergillus niger* and *Candida albicans* with MIC 50 µg/mL on comparison with standard drug Fluconazole (20 µg/mL). Compounds **8a, 8b, 8c, 8d, 8e** and **8h** containing chloro, methoxy and nitro groups showed moderate antifungal activities against *Aspergillus niger* and *Candida albicans* with MIC 100 µg/mL on comparison with standard drug Fluconazole (20 µg/mL).

(**TABLE III**)

TABLE III

Antimicrobial Activity of Compounds- *Zone of Inhibition (mm)*

Comp. No.	Conc. (µg/ml)	*Zone of Inhibition (mm)*				
		S. aureus ATCC 25923	***E. coli*** ATCC 25922	***P. aeruginosa*** ATCC 27853	***A. niger*** MCIM 745	***C. albicans*** MTCC 277
	200	15.2	**18.4**	15.3	15.4	**16.5**
8a	**100**	**15.0**	18.2	**15.1**	**15.2**	16.3
	50	14.8	17.8	14.6	14.9	16.2
	200	15.6	**12.8**	**11.6**	15.6	15.3
8b	**100**	**15.2**	12.6	11.3	**15.1**	**15**
	50	14.7	12.3	11.1	14.7	14.9
	200	**13.5**	15.5	15.3	**15.2**	15.5
8c	**100**	13.3	**15.1**	**15**	14.9	**15.1**
	50	13.2	14.6	14.6	14.5	14.6
	200	**11.6**	15.6	**12.8**	15.3	15.4
8d	**100**	11.3	**15.3**	12.5	**15**	**15.1**
	50	11.1	14.7	12	14.9	14.7
	200	21.6	15.3	24.8	15.4	15.3
8e	**100**	21.3	**15.0**	24.5	**15.2**	**15.1**
	50	**21.0**	14.6	**24.3**	14.9	14.9
	200	15.5	**16.5**	15.5	22.8	15.6
8h	**100**	**15.1**	16.3	**15.1**	22.6	**15.2**
	50	14.6	16.2	14.6	**22.3**	14.7
	200	**12.9**	15.4	15.3	**13.5**	**18.4**
11a	**100**	12.6	**15.2**	**15**	13.3	18.2
	50	12.2	14.9	14.6	13.2	17.8
	200	**14.7**	**12.9**	**18.2**	**12.8**	15.3
11b	**100**	14.5	12.6	18	12.6	**15.1**
	50	14.1	12.2	17.8	12.3	14.9
	200	**11.6**	15.5	15.5	**16.5**	**12.9**
11c	**100**	11.3	**15.1**	**15.1**	16.3	12.6
	50	11.1	14.6	14.6	16.2	12.2
	200	**13.8**	**15.3**	**15.2**	15.6	15.6
11d	**100**	13.6	14.8	14.9	**15.2**	**15.2**
	50	13.2	14.3	14.5	14.7	14.7
	200	15.5	15.4	**14.8**	15.3	24.8
11e	**100**	**15.1**	**15.2**	14.4	**15**	24.5
	50	14.6	14.9	14	14.6	**24.3**
	200	20.7	24.2	15.5	15.6	15.6
11g	**100**	20.3	23.9	**15.1**	**15.2**	**15.2**
	50	**20.2**	**23.6**	14.6	14.7	14.7
DMSO		**12**	**14**	**12.5**	**10**	**10.5**
Gentamicin		**22**	**28**	**20**	**-----**	**-----**
Fluconazole		**-----**	**-----**	**-----**	**20**	**24**

TABLE IV:
Antimicrobial activity of compounds- *Minimum Inhibitory Concentration (MIC*-µg/ml)

Comp. No.	*Minimum Inhibitory Concentration (MIC*-µg/ml)				
	S. aureus **ATCC 25923**	***E. coli*** **ATCC 25922**	***P. aeruginosa*** **ATCC 27853**	***A. niger*** **MCIM 745**	***C.albicans*** **MTCC 277**
8a	100	200	100	100	200
8b	100	200	200	100	100
8c	200	100	100	200	100
8d	200	100	200	100	100
8e	**50**	100	100	**50**	100
8h	100	200	**50**	100	**50**
11a	200	100	100	200	200
11b	200	200	200	200	100
11c	200	100	100	200	200
11d	200	200	200	100	100
11e	100	100	200	**50**	**50**
11g	**50**	**50**	100	100	100
Gentamicin	**20**	**20**	**20**	-----	-----
Fluconazole	-----	-----	-----	**20**	**20**
MIC in µg / mL)= 50 µg / mL: excellent activity; 100 µg / mL: moderate activity; 200 µg / mL: slight activity					

Compound **11g** containing meta nitro (m-NO_2) group showed excellent antibacterial activity against Gram negative bacteria *Escherichia coli;* compound **11h** with para nitro (p-NO_2) group showed excellent antibacterial activity against Gram negative bacteria *Pseudomonas aureginosa* and Gram positive bacteria *Staphylococcus aureus* with MIC 50 µg/mL. Similarly, compound **11e** containing m-chloro (m-Cl) group showed excellent antifungal activities against *Aspergillus niger* and *Candida albicans* with MIC 50 µg/mL on comparison with standard drug Fluconazole (20 µg/mL). Compounds **11b, 11d** and **11g** containing methylthio (SCH_3), methoxy (OCH_3) and nitro (NO_2) groups showed moderate antifungal activities against *Aspergillus niger* and *Candida albicans* with MIC 100 µg/mL on comparison with standard drug Fluconazole (20 µg/mL). (**TABLE III** and **IV**)

V. STATISTICAL ANALYSIS:

The standard deviation value was calculated using ANOVA method and expressed in terms of ± SD. It has been observed that differences below 0.0001 levels ($p \leq 0.0001$) were considered as statistically significant.

VI. EXPERIMENTAL:

Synthesis of ethyl 3-(2-(3-methyl-1*H*-pyrazol-4(5*H*)-yl-5-ol)diazenyl)-5-(methylthio)-4-cyanothiophene-2-carboxylate, 8a

A well stirred solution of ethyl 3-amino-4-cyano-5-(methylthio)thiophene-2-carboxylate **81** (0.001 mol, 0.25 g) in of conc. HCl (0.5 mL) and H_2O (1 mL) was cooled in an ice-bath and diazotized with the solution of $NaNO_2$ (0.001 mol, 0.1 g) in H_2O (1 mL). The cold diazonium solution was added slowly to a well

stirred solution of 3-methyl-1*H*-pyrazol-5(4*H*)-one, **19** (0.1 g, 0.001 mol) in pyridine (5 mL). The reaction mixture was stirred for another 2 h. The crude product was filtered off, dried well and recrystallized from the Ethanol:DMF (7:3) to give **8a**.

Yellow powder; Yield: 0.225g, 71%; M.P. 156–158°C; IR (Platinum ATR, ν_{max}cm^{-1}): 3441 (OH), 3148 (NH), 3055 (Ar CH), 2225 (-CN), 1676, 1601, 1546,1518, 1497, 1442, 1356, 941, 831, 750; ^{1}H NMR (500MHz, $CDCl_3$): δ 1.31-1.34 (s, J = 7 Hz, 3H, CH_3), 2.31 (s, 3H, pyrazoline CH_3), 2.59 (s, 3H, SCH_3), 4.22-4.26 (s, J = 7 & 14 Hz, 2H, OCH_2), 7.68 (s, 1H, pyrazoline NH), 10.24 (s, 1H, OH). *Anal. calcd. for* $C_{13}H_{13}N_5O_3S_2$ (Mol. Wt.: 351.4): C, 44.43; H, 3.73; N, 19.93; *found*: C, 44.39; H, 3.82; N, 19.88.

Similar procedure was used for the synthesis of compounds 8(b-h).

Ethyl 3-(2-(3-methyl-1*H*-pyrazol-4(5*H*)-yl-5-ol)diazenyl)-5-(methylthio)-4-carbamoyl thiophene-2-carboxylate, 8b

Yellow powder; Yield: 0.282 g, 75%; M.P. 160–162°C; IR (Platinum ATR, ν_{max}cm^{-1}): 3415 (OH), 3382, 3338, 3309, 3205 (NH, NH_2), 1702, (pyrazolone amide CO), 1668 (ester CO), 1633 (amide CO), 1513 (N=N); ^{1}H NMR (500MHz, $CDCl_3$): δ 1.31-1.34 (s, J = 7 Hz, 3H, CH_3), 2.31 (s, 3H, pyrazoline CH_3), 2.59 (s, 3H, SCH_3), 4.22-4.26 (s, J = 7 & 14 Hz, 2H, OCH_2), 6.97 (s, 2H, NH_2), 7.76 (s, 1H, pyrazoline NH), 10.23 (s, 1H, OH). *Anal. calcd. for* $C_{13}H_{15}N_5O_4S_2$ (Mol. Wt.: 369.42): C, 42.27; H, 4.09; N, 18.96; *found*: C, 42.37; H, 4.19; N, 18.73.

Ethyl 3-(2-(3-methyl-1*H*-pyrazol-4(5*H*)-yl-5-ol)diazenyl)-5-((phenylcarbamoyl) methylthio)-4-carbomoylthiophene-2-carboxylate, 8c

Yellow powder; Yield: 0.380 g, 76%; M.P. 208–210°C; IR (Platinum ATR, ν_{max}cm^{-1}): 3439 (OH), 3379, 3337, 3310, 3208 (NH, NH_2), 1705, (pyrazolone amide CO), 1671 (ester CO), 1638 (amide CO), 1512 (N=N); ^{1}H NMR (500MHz, DMSO-d_6): δ 1.31-1.34 (s, J = 7 Hz, 3H, CH_3), 2.31 (s, 3H, pyrazoline CH_3), 2.59 (s, 3H, SCH_3), 4.22-4.26 (s, J = 7 & 14 Hz, 2H, OCH_2), 6.97 (s, 2H, NH_2), 7.15-7.16 (s, 1H, ArH), 7.26-7.33 (s, 2H, ArH), 7.55-7.56 (s, 2H, ArH), 7.76 (s, 1H, pyrazoline NH), 10.23 (s, 1H, OH); *Anal. calcd. for* $C_{20}H_{20}N_6O_5S_2$ (Mol. Wt.: 488.54): C, 59.66; H, 4.74; N, 14.65; *found*: C, 59.69; H, 4.81; N, 14.74.

Ethyl 3-(2-(3-methyl-1*H*-pyrazol-4(5*H*)-yl-5-ol)diazenyl)-5-((4-methoxyphenylcarbamoyl) methylthio)-4-carbomoylthiophene-2-carboxylate, 8d

Pale Yellow powder; Yield: 0.418g, 79%; M.P.196–198°C; IR (Platinum ATR, ν_{max}cm^{-1}): 3435 (OH), 3374, 3329, 3309, 3211 (NH, NH_2), 1710, (pyrazolone amide CO), 1674 (ester CO), 1639 (amide CO), 1512 (N=N); ^{1}H NMR (500MHz, $CDCl_3$): δ 1.31-1.34 (s, J = 7 Hz, 3H, CH_3), 2.09 (s, 3H, pyrazoline CH_3) 4.03 (s, 2H, SCH_2), 3.45 (s, 3H, $ArOCH_3$), 4.18-4.22 (q, J = 7 & 14 Hz, 2H, OCH_2), 6.62 (s, 2H, NH_2), 6.89-6.91 (d, J = 7.8 Hz, 2H, ArH), 7.45-7.47 (d, J = 7.8 Hz, 2H, ArH) 7.78 (s, 2H, NH),10.18 (s, 1H, OH); ^{13}C-NMR (125 MHz, DMSO-*d*6) ppm: 14.87 (pyrazolone CH_3), 40.91 (SCH_2), 55.65 (OCH_3), 60.25 (OCH_2), 97.98 (thiophene C_3 and C_4), 114.44 (phenyl C_3 and C_5), 121.37 (phenyl C_2 and C_6), 125.39 (phenyl C_1), 132.10 (hydrazone, C=N), 145.57 (pyrazoline, C=N), 154.07 (thiophene C_1 and C_5), 156.03 (phenyl C_4), 163.01 (pyrazoline, C=O), 165.04 (ester, C=O), 165.44 (amide, C=O); *Anal. calcd. for* $C_{21}H_{22}N_6O_6S_2$ (Mol. Wt.: 518.57): C, 48.64; H, 4.28; N, 16.21; *found*: C, 48.7; H, 4.32; N, 16.19.

Ethyl 3-(2-(3-methyl-1*H*-pyrazol-4(5*H*)-yl-5-ol)diazenyl)-5-((4-chlorophenylcarba-moyl) methylthio)-4-carbomoylthiophene-2-carboxylate, 8e

Yellow powder; Yield: 0.417 g, 78%; M.P. 154–156°C; IR (Platinum ATR, ν_{max}cm^{-1}): 3421 (OH), 3371, 3327, 3307, 3213 (NH, NH_2), 1707, (pyrazolone amide CO), 1672 (ester CO), 1638 (amide CO), 1512 (N=N); ^{1}H NMR (500MHz, $CDCl_3$): δ 1.31-1.34 (t, J = 7 Hz, 3H, CH_3), 2.31 (s, 3H, pyrazoline CH_3) 3.96 (s, 2H, SCH_2), 4.27-4.33 (q, J = 7 & 14 Hz, 2H, OCH_2), 6.96 (s, 2H, NH_2), 7.45-7.49 (d, J = 8 Hz, 2H, ArH), 7.69-7.71 (d, J = 8 Hz, 2H, ArH), 7.99 (s, 1H, pyrazoline NH), 8.41 (s, 1H, NH=N), 13.88 (s, 1H, OH). *Anal. calcd. for* $C_{20}H_{19}ClN_6O_5S_2$ (Mol. Wt.: 522.99): C, 45.93; H, 3.66; N, 16.07; *found*: C, 46.41; H, 3.46; N, 16.27

Ethyl 3-(2-(3-methyl-1*H*-pyrazol-4(5*H*)-yl-5-ol)diazenyl)-5-((4-bromophenylcarbamoyl) methylthio)-4-carbomoylthiophene-2-carboxylate, 8f

Yellow powder; Yield: 0.463g, 80%; M.P. 192–194°C; IR (Platinum ATR, ν_{max}cm^{-1}): 3441 (OH), 3368, 3317, 3300, 3213 (NH, NH_2), 3055 (aromatic CH), 1713 (pyrazolone amide CO), 1680(ester CO), 1640 (amide CO), 1512 (N=N); ^{1}H NMR (500MHz, $CDCl_3$): δ 1.32-1.36 (t, J = 7.1 Hz, 3H, CH_3), 2.32 (s, 3H, pyrazoline CH_3) 3.98 (s, 2H, SCH_2), 4.29-4.33 (q, J = 7.1 & 14 Hz,, 2H, OCH_2), 6.97 (s, 2H, NH_2), 7.41-7.44 (d, J = 7.9 Hz, 2H, ArH), 7.68-7.70 (d, J = 7.9 Hz, 2H, ArH), 7.94 -7.96 (s, 1H, NH), 8.43 (s, 1H, NH=N), 13.93 (s, 1H, OH). *Anal. calcd. for* $C_{20}H_{19}BrN_6O_5S_2$ (Mol. Wt.: 567.44): C, 42.33; H, 3.37; N, 14.81; *found*: C, 42.41; H, 3.73; N, 14.74.

Ethyl 3-(2-(3-methyl-1*H*-pyrazol-4(5*H*)-yl-5-ol)diazenyl)-5-((3-nitrophenylcarbamoyl) methylthio)-4-carbomoylthiophene-2-carboxylate, 8g

Faint yellow powder; Yield: 0.440 g, 81%; M.P. 204–206°C; IR (Platinum ATR, ν_{max}cm^{-1}): 3442 (OH), 3368, 3317, 3300, 3213 (NH, NH_2), 1709, (pyrazolone amide CO), 1674 (ester CO), 1634 (amide CO), 1508 (N=N); ^{1}H NMR (500MHz, $CDCl_3$): δ 1.32-1.35 (t, J = 7.1 Hz, 3H, CH_3), 2.33 (s, 3H, pyrazoline CH_3), 3.99 (s, 2H, SCH_2), 4.27-435 (q, J = 7.1 & 14 Hz, 2H, OCH_2), 6.99 (s, 2H, NH_2), 7.55-7.56 (m, J = 8.5 Hz, 1H, ArH), 7.91-7.93 (m, J = 7.1 and 1.9 Hz, 1H, ArH), 7.98 (s, 1H, NH), 8.15-8.19 (m, J = 7.1 and 1.9 Hz, 1H, ArH), 8.44 (s, 1H, NH=N), 8.56-8.59 (m, J = 1.9 Hz,1H, ArH), 14.23 (s, 1H, OH). *Anal. calcd. for* $C_{20}H_{19}N_7O_7S_2$ (Mol. Wt.: 533.54): C, 45.02; H, 3.59; N, 18.38; *found*: C, 45.79; H, 3.69; N, 18.07.

Ethyl 3-(2-(3-methyl-1*H*-pyrazol-4(5*H*)-yl-5-ol)diazenyl)-5-((4-Nitrophenylcarbamo yl)methylthio)-4-carbomoylthiophene-2-carboxylate, 8h

Yellow powder; Yield: 0.436, 80.2%; M.P. 174–176°C; IR (Platinum ATR, ν_{max}cm^{-1}): 3415 (OH), 3368, 3317, 3300, 3213 (3NH, NH_2), 1709, (pyrazolone amide CO), 1674 (ester CO), 1634 (amide CO), 1508 (N=N); ^{1}H NMR (500MHz, $CDCl_3$): δ 1.32-1.36 (t, J = 7.1 Hz,3H, CH_3), 2.34 (s, 3H, pyrazoline CH_3) 3.96 (s, 2H, SCH_2), 4.30-4.35 (q, J = 7.1 & 14 Hz, 2H, OCH_2), 6.98 (s, 2H, NH_2), 7.91-7.97 (d, J = 8.5 Hz, 2H, ArH), 8.13 (s, 1H, NH), 8.25- 8.27 (d, J = 8.5 Hz, 2H, ArH), 8.51 (s, 1H, NH=N), 14.11 (s, 1H, OH). *Anal. calcd. for* $C_{20}H_{19}N_7O_7S_2$ (Mol. Wt.: 533.54): C, 45.02; H, 3.59; N, 18.38; *found*: C, 45.92; H, 3.49; N, 18.15.

Synthesis of ethyl 3-(2-(3-methyl-1-phenyl-1*H*-pyrazol-4(5*H*)-yl-5-ol)diazenyl)-5-(methylthio)-4-cyano thiophene-2-carboxylate, 11a

A well stirred solution of ethyl 3-amino-4-cyano-5-(methylthio)thiophene-2-carboxylate **4** (0.001 mol, 0.25 g) in 0.5 mL of conc. HCl and 1 mL of H_2O was cooled in an ice bath and diazotized with the solution of $NaNO_2$ (0.001 mol, 0.1 g) in 1mL H_2O. The cold diazonium salt solution was added slowly to a well stirred solution of 3-methyl-1-phenyl-1*H*-pyrazol-5(4*H*)-one, **10** (0.1 g, 0.001 mol) in pyridine (5 mL).The reaction mixture was stirred for another 2 h. The crude product was filtered off, dried well and recrystallized from the Ethanol: DMF (7:3) to give **11a**.

Similar procedure was used for the synthesis of compounds 11(b-h).

Ethyl 3-(2-(3-methyl-1-phenyl-1*H*-pyrazol-4(5*H*)-yl-5-ol)diazenyl)-5-(methylthio)- 4-cyano thiophene-2-carboxylate, 11a

Yellow powder; Yield: 0.183 g, 74.6 %; M.P. 172-174 ºC; IR (Platinum ATR, ν_{max} cm^{-1}): 3441 (OH), 3366 (NH, hydrazone), 2218 (CN), 1706, 1656 (2C=O), 1496 (N=N); ^{1}H NMR (500 MHz, DMSO-*d*6): δ ppm: 1.32-1.35 (t, *J* = 7.1 Hz, 3H, CH_3), 2.60 (s, 3H, pyrazoline CH_3), 2.78 (s, 3H, SCH_3), 4.35-4.40 (q, *J* = 7.1 & 14 Hz, 2H, OCH_2), 7.43 (d, *J* = 8.0 and 1.5 Hz,1H, ArH), 7.45-7.46 (d, *J* = 8.0 Hz, 2H, ArH), 7.47-7.49 (d, *J* = 8.0 and 1.5 Hz, 2H, ArH), 10.75 (s, 1H, OH); *Anal. calcd. for* $C_{19}H_{17}N_5O_3S_2$ (Mol. Wt.: 427.5): C, 45.02; H, 3.59; N, 18.38; *found*: C, 45.92; H, 3.49; N, 18.15.

Ethyl 3-(2-(3-methyl-1-phenyl-1*H*-pyrazol-4(5*H*)-yl-5-ol)diazenyl)-5-(methylthio)- 4-carbamoyl thiophene-2-carboxylate, 11b

Yellow powder; Yield: 0.2 g, 78.3%; M.P. 222-224 ºC; IR (Platinum ATR, ν_{max} cm^{-1}): 3441 (OH), 3366 (NH), 1706, 1656 (2C=O), 1496 (N=N); ^{1}H NMR (500 MHz, DMSO-*d*6): δ ppm: 1.32-1.35 (t, *J* = 7.1 Hz, 3H, CH_3), 2.60-2.62 (s, 3H, pyrazoline CH_3), 2.78 (s, 3H, SCH_3), 4.35-4.40 (q, *J* = 7.1 & 14 Hz, 2H, OCH_2), 6.62 (s, 2H, NH_2), 7.43 (d, *J* = 8.0 and 1.5 Hz,1H, ArH), 7.45-7.46 (d, *J* = 8.0 Hz, 2H, ArH), 7.47-7.49 (d, *J* = 8.0 and 1.5 Hz, 2H, ArH), 10.72 (s, 1H, OH); *Anal. calcd. for* $C_{19}H_{19}N_5O_4S_2$ (Mol. Wt.: 445.52): C, 51.22; H, 4.30; N, 15.72; *found:* C, 51.32; H, 4.39; N, 15.62.

Ethyl 3-(2-(3-methyl-1-phenyl-1H-pyrazol-4-yl-5-ol)diazenyl)-5-((phenylcarbamoyl) methylthio)-4-carbamoylthiophene-2-carboxylate, 11c

Yellow powder; Yield: 0.253 g, 77.6%; M.P. 180-182 ºC; IR (Platinum ATR, ν_{max} cm^{-1}): 3440 (OH), 3366 (NH, hydrazone), 1706, 1656 (2C=O), 1496 (N=N); ^{1}H NMR (500 MHz, DMSO-*d*6): δ ppm: 1.32-1.35 (t, *J* = 7.2 Hz, 3H, CH_3), 2.24 (s, 3H, pyrazoline CH_3), 4.21 (s, 2H, SCH_2), 4.35-4.40 (q, *J* = 7.2 & 14 Hz, 2H, OCH_2), 6.61 (s, 2H, NH_2), 7.19-7.25 (s, 1H, pyrazoline ArH), 7.43-7.46 (d, *J* = 8.0 Hz, 2H, pyrazoline ArH), 7.47-7.49 (d, *J* = 8.0 Hz, 2H, pyrazoline ArH), 7.61 (d, *J* = 7.8 Hz, 1H, ArH), 7.78 (s, 1H, NH), 7.82-7.84 (d, *J* = 7.8 Hz, 2H, ArH), 7.90-7.91 (d, *J* = 7.8 Hz, 2H, ArH), 10.76 (s, 1H, OH); *Anal. calcd. for* $C_{26}H_{24}N_6O_5S_2$(Mol. Wt.: 564.64): C, 55.31; H, 4.28; N, 14.88; *found*: C, 55.40; H, 4.23; N, 14.77.

Ethyl 3-(2-(3-methyl-1-phenyl-1H-pyrazol-4(5H)-yl-5-ol)diazenyl)-5-((4-methoxy phenylcarbamoyl) methylthio)-4-carbamoylthiophene-2-carboxylate, 11d

Yellow powder; Yield: 0.27 g, 79%; M.P. 174-176 ºC; IR (Platinum ATR, ν_{max} cm^{-1}): 3442 (OH), 3366 (NH, hydrazone), 1706, 1656 (2C=O), 1496 (N=N); ^{1}H NMR (500 MHz, DMSO-*d*6): δ ppm: 1.22-1.25 (t, *J* = 7.1 Hz, 3H, CH_3), 2.38 (s, 3H, pyrazoline CH_3), 3.72 (s, 3H, OCH_3), 4.01-4.03 (s, 2H, SCH_2), 4.20 (q, *J* = 7.1 & 14 Hz, 2H, OCH_2), 6.62 (s, 2H, NH_2), 6.89 – 6.91 (d, *J* = 8.2 Hz, 2H, ArH), 7.21 (s, 1H, tautameric NH), 7.45 – 7.47 (m, 5H, pyrazolone ArH), 7.78 (d, *J* = 8.2 Hz, 2H, ArH), 7.83 (s, 1H, NH), 10.18 (s, 1H, OH); ^{13}C-NMR (125 MHz, DMSO-*d*6) δ ppm: 14.72 (ester CH_3), 17.59 (SCH_3), 18.28 (pyrazolone CH_3), 40.43 (SCH_2), 61.88 (OCH_2), 117.27 (phenyl, C_2 , C_6 and C_3 , C_5), 118.38 (thiophene C_2 and C_5), 122.37 (phenyl C_4), 125.39 (phenyl C_1), 129.41 (thiophene C_3 and C_4), 129.41 (hydrazone, C=N), 146.95 (pyrazoline, C=N), 153.84 (pyrazoline, C=O), 155.56 (ester, C=O), 159.57 (amide, C=O);*Anal. calcd. for* $C_{27}H_{26}N_6O_6S_2$ (Mol. Wt.: 594.66): C, 54.53; H, 4.41; N, 14.13; *found*: C, 54.49; H, 4.46; N, 14.17.

Ethyl 3-(2-(3-methyl-1-phenyl-1H-pyrazol-4(5H)-yl-5-ol)diazenyl)-5-((4-chloro phenylcarbamoyl) methylthio)-4-carbamoylthiophene-2-carboxylate, 11e

Yellow powder; Yield: 0.265 g, 77%; M.P. 168-170 ºC; IR (Platinum ATR, ν_{max} cm^{-1}): 3443 (OH), 3366 (NH, hydrazone), 1706, 1656 (2C=O), 1496 (N=N); ^{1}H NMR (500 MHz, DMSO-*d*6): δ ppm: 1.22-1.25 (t, *J* = 7.1 Hz, 3H, CH_3), 2.38 (s, 3H, pyrazoline CH_3), 4.09 (s, 2H, SCH_2), 4.22-4.26 (q, *J* = 7.1 & 14 Hz, 2H, OCH_2), 6.68 (s, 2H, NH_2), 6.93 – 6.95 (d, *J* = 8.5 Hz, 2H, ArH), 7.45 – 7.47 (m, 5H, pyrazolone ArH), 7.79-7.81 (d, *J* = 8.5 Hz, 2H, ArH), 7.85 (s, 1H, NH), 10.21 (s, 1H, OH); *Anal. calcd. for* $C_{26}H_{23}ClN_6O_5S_2$ (Mol. Wt.: 599.08): C, 52.13; H, 3.87; N, 14.03; *found*: C, 52.25 ; H, 3.91; N, 14.12.

Ethyl 3-(2-(3-methyl-1-phenyl-1H-pyrazol-4(5H)-yl-5-ol)diazenyl)-5-((4-bromo phenyl carbamoyl) methylthio)-4-carbamoylthiophene-2-carboxylate, 11f

Yellow powder; Yield: 0.276 g, 80%; M.P. 180-182 ºC; IR (Platinum ATR, ν_{max} cm^{-1}): 3441 (OH), 3366 (NH, hydrazone), 1706, 1656 (2C=O), 1496 (N=N); ^{1}H NMR (500 MHz, DMSO-*d*6): δ ppm: 1.22-1.25 (t, *J* = 7.1 Hz, 3H, CH_3), 2.38 (s, 3H, pyrazoline CH_3), 4.09 (s, 2H, SCH_2), 4.22-4.26 (q, *J* = 7.1 & 14 Hz, 2H, OCH_2), 6.68 (s, 2H, NH_2), 6.93 – 6.95 (d, *J* = 8.5 Hz, 2H, ArH), 7.45 – 7.47 (m, 5H, pyrazolone ArH), 7.79-7.81 (d, *J* = 8.5 Hz, 2H, ArH), 7.85 (s, 1H, NH), 10.21 (s, 1H, OH); *Anal. calcd. for* $C_{26}H_{23}BrN_6O_5S_2$ (Mol. Wt.: 643.53): C, 48.53; H, 3.60; N, 13.06; *found*: C, 48.49; H, 3.54; N, 13.12.

Ethyl 3-(2-(3-methyl-1-phenyl-1*H*-pyrazol-4(5*H*)-yl-5-ol)diazenyl)-5-((4-nitrophenyl carbamoyl)methylthio)-4-carbamoylthiophene-2-carboxylate, 11g

Yellow powder; Yield: 0.292 g, 83%; M.P. 216-218 ºC; IR (Platinum ATR, ν_{max} cm^{-1}): 3445(OH), 3366 (NH, hydrazone), 1706, 1656 (2C=O), 1496 (N=N); ^{1}H NMR (500 MHz, DMSO-*d*6): δ ppm: 1.32-1.35 (t, *J* = 7.1 Hz, 3H, CH_3), 2.08 (s, 3H, pyrazoline CH_3), 4.09 (s, 2H, SCH_2), 4.32-4.36 (q, *J* = 7.1 & 14 Hz, 2H, OCH_2), 6.68 (s, 2H, NH_2), 7.45 – 7.47 (m, 5H, pyrazolone ArH), 7.85 (s, 1H, NH), 8.37-8.39 (d, *J* = 9.5 Hz, 2H, ArH), 8.44 – 8.45 (d, *J* = 9.5 Hz, 2H, ArH), 10.76 (s, 1H, OH); *Anal. calcd. for* $C_{26}H_{23}N_7O_7S_2$ (Mol. Wt.: 609.63): C, 51.22; H, 3.80; N, 16.08; *found*: C, 51.16; H, 3.89; N, 16.10.

Ethyl3-(2-(3-methyl-1-phenyl-1*H*-pyrazol-4(5*H*)-yl-5-ol)diazenyl)-5-((3-nitrophenyl carbamoyl) methylthio)-4-carbamoylthiophene-2-carboxylate, 11h

Yellow powder; Yield: 0.284 g, 81%; M.P. 210-212 ºC; IR (Platinum ATR, ν_{max} cm^{-1}): 3443 (OH), 3366 (NH, hydrazone), 1706, 1656 (2C=O), 1496 (N=N); ^{1}H NMR (500 MHz, DMSO-*d*6): δ ppm: 1.30-1.34 (t, *J* = 7.1 Hz, 3H, CH_3), 2.09 (s, 3H, pyrazoline CH_3), 4.10 (s, 2H, SCH_2), 4.33-4.37 (q, *J* = 7.1 & 14 Hz, 2H, OCH_2), 6.60 (s, 2H, NH_2), 7.63 – 6.95 (d, *J* = 8.0 and 1.5 Hz, 1H, pyrazolone ArH), 7.80 – 7.82 (d, *J* = 8.0 Hz, 2H, pyrazolone ArH), 7.84-7.85 (d, *J* = 8.0 and 1.5 Hz, 2H, pyrazolone ArH), 7.87(s, 1H, NH), 8.20-8.22 (d, *J* = 9.0 and 1.9 Hz, 1H, ArH), 8.24-8.26 (d, *J* = 9.0 and 1.9 Hz, 2H, ArH), 8.27 (d, *J* = 9.0 and 1.9 Hz, 1H, ArH), 10.76 (s, 1H, OH); *Anal. calcd. for* $C_{26}H_{23}N_7O_7S_2$ (Mol. Wt.: 609.63): C, 51.22; H, 3.80; N, 16.08; *found*: C, 51.20; H, 3.85; N, 16.12.

VII. CONCLUSIONS

The series of ethyl 3-(2-(3-methyl-1*H*-pyrazol-4(5*H*)-yl-5-ol)diazenyl)-5-(methylthio)-4-cyano OR 4-carbomoylthiophene-2-carboxylates, **8(a-h)** and ethyl 3-(2-(3-methyl-1-phenyl-1*H*-pyrazol-4(5*H*)-yl-5-ol)diazenyl)-5-(5-(methylthio)-4-cyano OR 4-carbomoylthiophene-2-carboxylates, **11(a-h)** is synthesized from 4-cyano/4-carbomoyl thiophene-2-carboxylates. Some of the compounds showed excellent to moderate antimicrobial activities against germs.

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