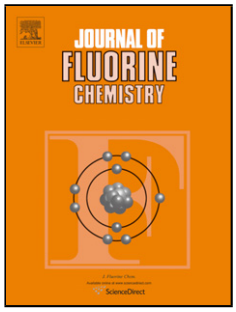

Synthesis, characterization, *in vitro* DNA photocleavage and cytotoxicity studies of 4-arylazo-1-phenyl-3-(2-thienyl)-5-hydroxy-5-trifluoromethylpyrazolines and regioisomeric 4-arylazo-1-phenyl-5(3)-(2-thienyl)-3(5)-trifluoromethylpyrazoles

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Synthesis, Characterization, *in vitro* DNA Photocleavage and Cytotoxicity studies of 4-Arylazo-1-phenyl-3-(2-thienyl)-5-hydroxy-5-trifluoromethylpyrazolines and Regioisomeric 4-Arylazo-1-phenyl-5(3)-(2-thienyl)-3(5)-trifluoromethylpyrazoles

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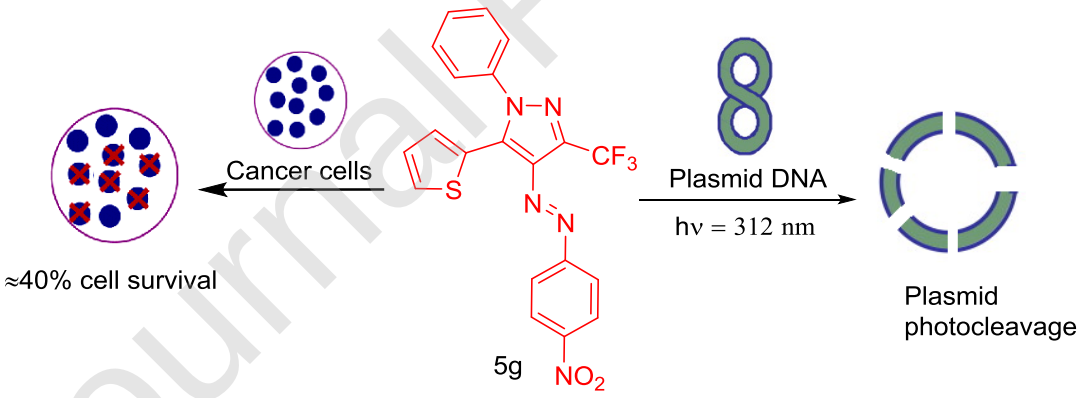
Graphical Abstract:-



Highlights

- Synthesis of 4-arylazo-1-phenyl-3-(2-thienyl)-5-hydroxy-5-trifluoromethylpyrazolines and regioisomeric 4-arylazo-1-phenyl-5(3)-(2-thienyl)-3(5)-trifluoromethylpyrazoles was accomplished.
- All the synthesized compounds were screened for their DNA photocleavage on suercoiled pBr322 plasmoid.

- Seventeen compounds were evaluated against MCF7, BT474, SBALL and MOLT4 cancer cell lines.
- 1-Phenyl-5-(2-thienyl)-4-(4-nitrophenylazo)-3-trifluoromethylpyrazole was found to be the most promising compound as photocleavage and anticancer agent.

Abstract

1-(2-Thienyl)-2-arylazo-4,4,4-trifluorobutane-1,3-diones (**4**), generated by the condensation of aryldiazonium salts (**2**) with 4,4,4-trifluoro-1-(2-thienyl)butane-1,3-dione (**3**), were used as common intermediates to synthesize regioisomeric 4-arylazo-1-phenyl-5(3)-(2-thienyl)-3(5)-trifluoromethylpyrazoles (**5** and **6**) and 4-arylazo-1-phenyl-3-(2-thienyl)-5-hydroxy-5-trifluoromethylpyrazolines (**7**) by reacting with phenylhydrazine in neutral conditions. All the synthesized compounds **4**, **5**, **6** and **7** were screened for their DNA photocleavage activity on pBR322 supercoiled DNA plasmid under UV radiation at λ_{max} 312 nm without any additive. The results indicated that 3-trifluoromethyl isomers (**5**) photocleaves plasmid DNA better than the corresponding 5-trifluoromethylpyrazoles (**6**) and 5-hydroxy5-trifluoromethylpyrazolines (**7**). 4-(4-Nitrophenylazo)-1-phenyl-5-(2-thienyl)-3-trifluoromethylpyrazole **5g**, exhibited the best potential as DNA photocleaver, cleaving the supercoiled plasmid (Form-I) into smaller fragments at 20 µM of compound with 60 µM of DNA plasmid in DMSO. Selected seventeen compounds were further screened for their cytotoxicity against four cancer cell lines namely MCF-7, BT-474, SBALL and MOLT-4 and again compound **5g** was identified as the most potent compound with an IC_{50} value of 8.6±3.8 µM against MCF-7 cell line.

Keywords:

DNA photocleavage, cytotoxicity, 4-arylazo-3-trifluoromethylpyrazoles, 4-arylazo-5-trifluoromethylpyrazole, 4-arylazo-5-hydroxy-5-trifluoromethylpyrazolines.

1. Introduction

Pyrazole derivatives have increasingly attracted attention in the recent years due to their biological and chemotherapeutic importance as antitubercular [1,2], antibacterial [3, 4,5], antidepressant [6,7], anti-inflammatory [8,9], antimicrobial [10,11], antiviral [12] antihypertensive [13] and anticancer [14,15] agents. Various reports show that pyrazole derivatives having appropriate groups at N-1, C-3, C-4 and C-5 can act as cytotoxic and DNA photocleavage agents for cancerous cells. For example pyrazole derivative bearing azo group at position-4, CAN508, (**I**) (**Figure 1**) was identified as novel anti-proliferative agent having selectivity for cancer cells [16,17]. Compounds containing azo group have also been reported to possess various biological activities like antimicrobial [18] and anti-HIV properties [19]. The excitation of electrons on azoaryl ring can lead to cleavage of carbon nitrogen bond leading to the extrusion of nitrogen and the formation of aryl radicals which act as alkylating agent capable of DNA binding and photocleavage [20,21].

Moreover, 3-(2-thienyl) derivative of pyrazole namely N-acetyl-3-(2-thienyl)-5-ferrocenyl-2-pyrazoline (**II**) was reported for *in vitro* inhibition of angiogenesis and human lung cancer growth [22] (**Figure 1**). The compounds containing thiophene ring such as *α*-terthienyl (2,2´:5,2´´-terthiophene) and its derivatives are well known singlet oxygen sensitizers and phototoxic agents [23,24,25,26].

Additionally, compounds containing fluorine are gaining interest of researchers from all around the globe [27]. Incorporation of trifluoromethyl group into the organic moieties leads to alteration of properties of the compound. A large number of fluorinated antiproliferative agents are being used for the treatment of cancer [28]. 4-[5-(4-Methylphenyl)-3-(trifluoromethyl)-1*H*-pyrazol-1-yl]benzenesulfonamide, celecoxib (**III**) is being used for the treatment of metastasis of human pancreatic cancer [29] (**Figure 1**). Thus, in view of the biological features associated with trifluoromethyl, thienyl and azo groups, it was envisaged in the present study to introduce the three pharmacophores on the pyrazole ring with a hope to get new molecules with increased potential as DNA photocleavers and cytotoxic agents.

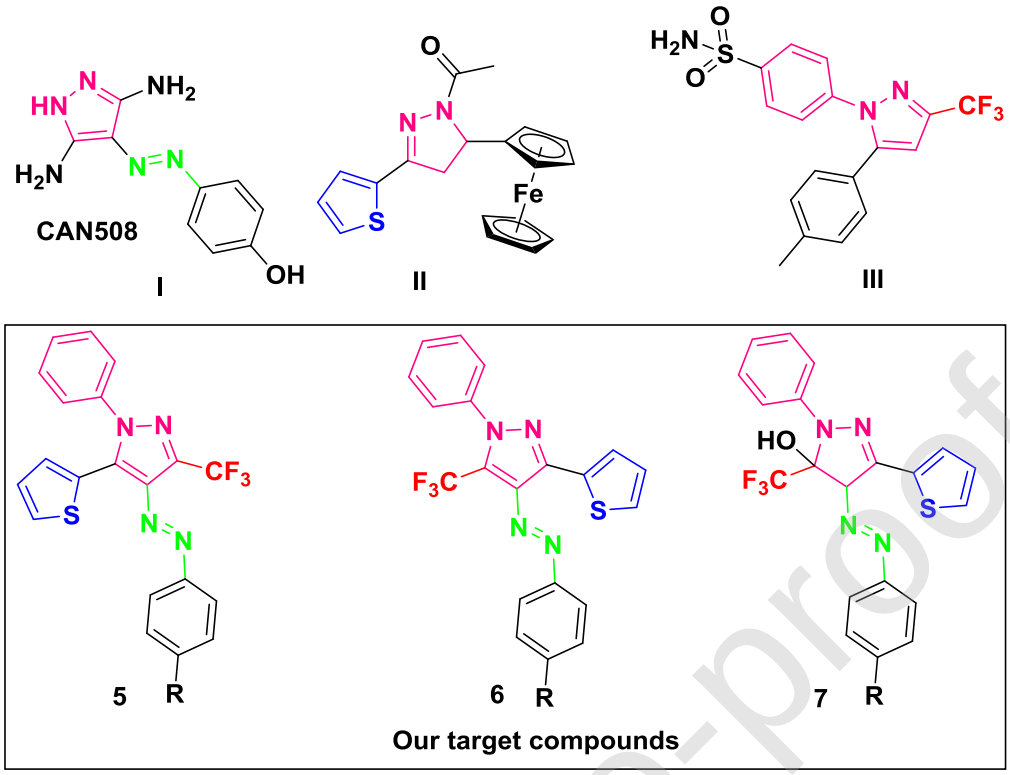


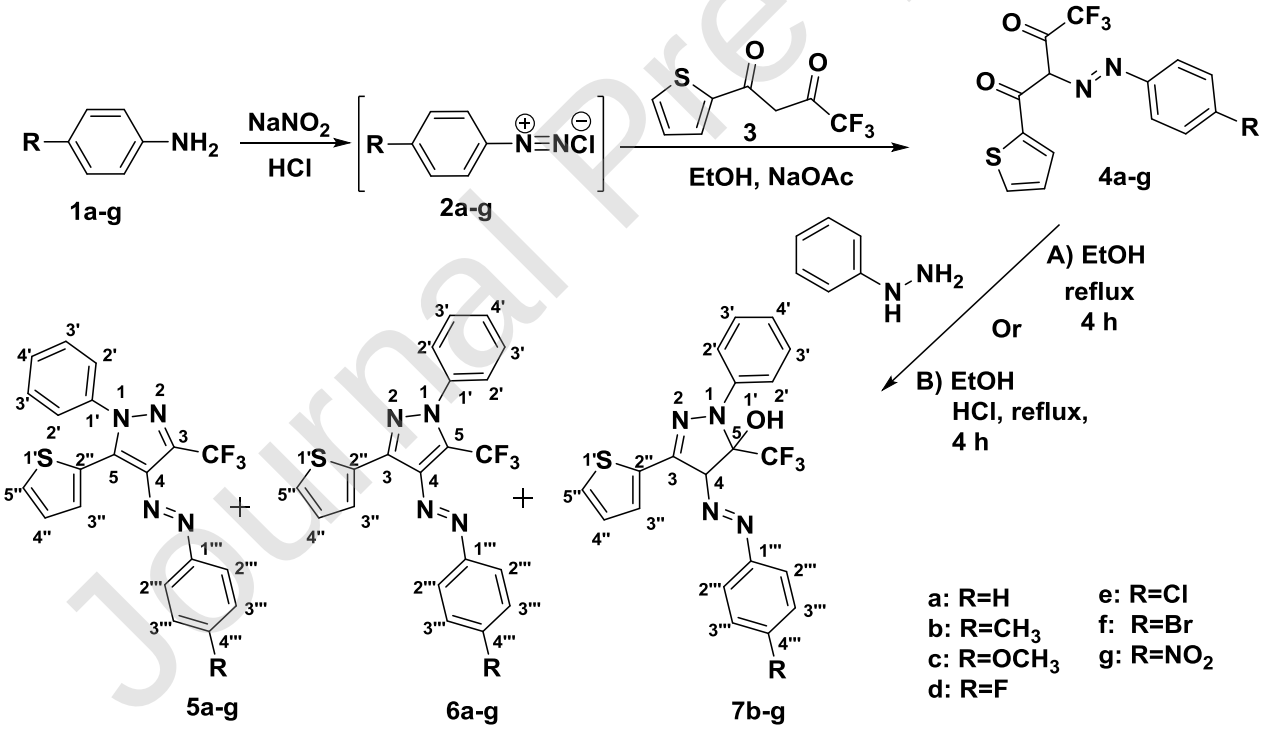
Figure 1: Examples of bioactive pyrazoles.

Therefore, in continuation of our interest towards the synthesis of thienyl/trifluoromethyl/arylazo substituted pyrazole derivatives, [**Error! Bookmark not defined.**,30,31,32,33] photocleavage [**Error! Bookmark not defined.**,34] and cytotoxicity [35] studies and need for the development of the novel anti-cancer drugs [36,37] we hereby report the design and synthesis of novel regioisomeric 4-arylazo-1-phenyl-5(3)-(2-thienyl)-3(5)-trifluoromethylpyrazoles (**5** & **6**) and 4-arylazo-1-phenyl-3-(2-thienyl)-5-hydroxy-5-trifluoromethylpyrazolines (**7**) (**Figure 1**) which was achieved by simple steps. We also evaluate the cytotoxic effects of these synthesized compounds against multiple cancer cell lines and their capability to damage the supercoiled DNA *in vitro* studies which are two independent activities utilized for the development of drugs to treat cancer [38].

2. Chemistry

2.1 Synthesis

Synthesis of the title compounds namely, 4-arylazo-1-phenyl-5(3)-(2-thienyl)-3(5)-trifluoromethylpyrazoles (**5a-g** and **6a-g**) and 4-arylazo-1-phenyl-3-(2-thienyl)-5-hydroxy-5-trifluoromethylpyrazolines (**7b-g**) is outlined in (**Scheme 1**). Initially, the key intermediates 1-(2-thienyl)-2-arylazo-4,4,4-trifluorobutane-1,3-diones (**4a-g**) were synthesized by the condensation of **2a-g** generated in situ by diazotization of corresponding anilines with 4,4,4-trifluoromethyl-1-(thiophen-2-yl)butane-1,3-dione (**3**) following the literature procedure [39] (**Scheme 1**). Subsequent reaction of **4a-g** with phenylhydrazine under neutral conditions afforded a mixture of three products which were characterized as 4-arylazo-1-phenyl-5-(2-thienyl)-3-trifluoromethylpyrazoles (**5a-g**), 4-arylazo-1-phenyl-3-(2-thienyl)-5-trifluoromethylpyrazoles (**6a-g**) and 4-arylazo-1-phenyl-3-(2-thienyl)-5-hydroxy-5-trifluoromethylpyrazolines (**7b-g**). All the three products were separated by column chromatography using 100-200 silica gel and petroleum ether : ethylacetate (98:2) as eluent. In general, 3-trifluoromethyl (**5**) isomer was found to be the major product (75%) and corresponding 5-trifluoromethylpyrazole (**6**) and its hydrated form pyrazoline (**7**) were obtained in minor amount (15% and 10% respectively).



Scheme 1: Synthesis of target compounds **5a-g**, **6a-g** and **7b-g** under neutral (A) and acidic (B) conditions.

It has been observed by us that the reaction under acidic conditions results in the formation 3-trifluoromethyl isomer as major or exclusive product [40]. Therefore, we explored the reaction of **4** with phenylhydrazine in acidic medium also. It is interesting to note that in present study no significant effect was observed on the yield of 3-trifluoromethylpyrazoles, however the yield of 5-trifluoromethylpyrazoles increased due to acid catalyzed dehydration of 5-hydroxy-5-trifluoromethylpyrazolines. The structure of the compounds **5a-g**, **6a-g** and **7b-g** was established by their elemental analysis, mass, IR and ^{1}H, ^{13}C and ^{19}F NMR spectra.

2.2 Results and discussion

The IR spectra of compounds **5** and **6** showed two absorption bands appearing at 3000-3100 cm^{-1} (for aromatic C-H stretch) and 1525-1540 cm^{-1} (for -N=N- stretch). The ^{19}F-NMR spectra of compounds **5b** and **5g** exhibited signal at δ -62.40 and -62.98 while **6b** and **6g** showed signals at -55.61 ppm and -55.81 ppm, consistent with the literature values of signals for 3-trifluoromethyl and 5-trifluoromethyl groups respectively [**Error! Bookmark not defined.**]. The IR spectra of compounds **7** displayed an additional band in the region 3260 cm^{-1} due to O-H stretch. In ^{1}H NMR compound **7** displayed a broad singlet at δ 8.1-9.7 ppm corresponding to OH (exchangeable with D_2O) besides the signals for aromatic protons. Additionally, ^{19}F NMR of compound **7g** showed a peak at δ -74.35 ppm which corresponds to literature values of 5-trifluoromethylpyrazolines [**Error! Bookmark not defined.**].

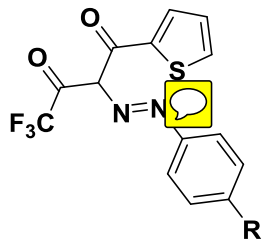
2.3 Theoretical studies: Molecular orbital energy calculation

The developments in the field of computational chemistry nowadays are quite helpful for calculating the HOMO-LUMO energy gap which is further useful to investigate the electronic transitions leading to generation of reactive chemical intermediates like free radicals. These free radical species are essential for the cleavage of DNA strands. Therefore, we performed a theoretical study by using mm+ force field energy minimization for the calculation of molecular orbital energies (HOMO and LUMO). The minimum energy conformations of compounds **4a-g**, **5a-g**, **6a-g** and **7b-g** were obtained by geometry optimization, which were used further for single point level calculations. The molecular orbital energy calculation for compounds **4a-g**, **5a-g**, **6a-g** and **7b-g** were obtained from their most stable minimum energy conformations using

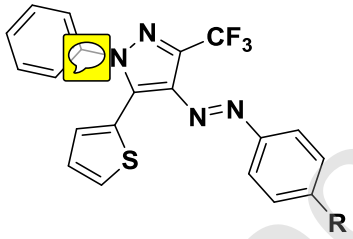
ChemBio3D ultra [41]. The energy gap between HOMO-LUMO (ΔE_{L-H}) of compounds **4**, **5**, **6** and **7** are listed in **Table 1**.

Table 1

The minimum energy and frontier orbitals energies for **4a-g, 5a-g, 6a-g** and **7b-g.**

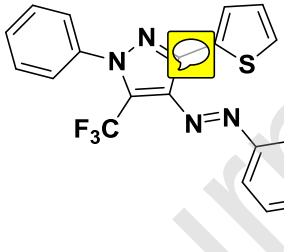


4(a-g) R= H, CH_3, OCH_3, F, Cl, Br, NO_2

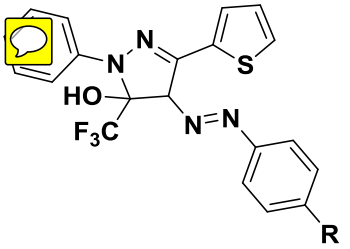


5(a-g) R= H, CH_3, OCH_3, F, Cl, Br, NO_2

Compd	E^a	LUMO[a]	HOMO[a]	ΔE_{L-H}	Compd	E^a	LUMO[a]	HOMO[a]	ΔE_{L-H}
4a	57.7328	-3.000	-3.460	0.460	**5a**	73.9795	-1.095	-3.115	2.02
4b	57.5564	-2.851	-3.385	0.534	**5b**	73.8702	-1.089	--2.970	1.881
4c	63.7486	-2.579	-3.337	0.758	**5c**	79.9100	-1.097	-2.741	1.644
4d	58.2057	-2.445	-3.305	0.860	**5d**	74.0681	-1.078	-2.630	1.552
4e	58.7020	-2.495	-3.315	0.820	**5e**	74.5686	-1.080	-2.669	1.589
4f	58.8395	-2.606	-3.326	0.720	**5f**	74.7200	-1.080	-2.765	1.685
4g	54.8972	-3.352	-5.888	2.536	**5g**	76.0220	-1.292	-5.412	4.120



6(a-g) R= H, CH_3, OCH_3, F, Cl, Br, NO_2



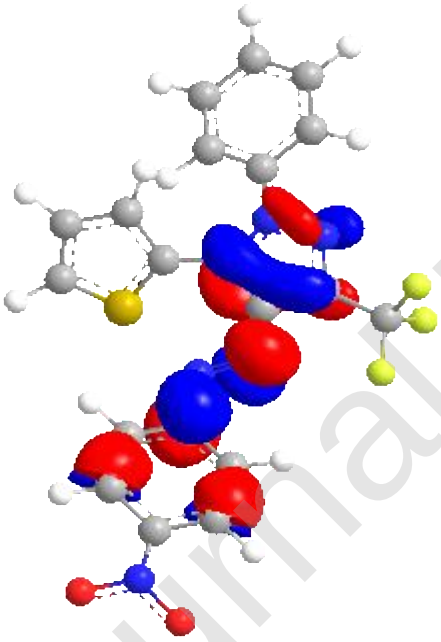
7(b-g) R= H, CH_3, OCH_3, F, Cl, Br, NO_2

Compd	E^a	LUMO[a]	HOMO[a]	ΔE_{L-H}	Compd	E^a	LUMO[a]	HOMO[a]	ΔE_{L-H}
6a	60.9890	-1.216	-3.417	2.201	**7a**	-	-	-	-
6b	60.8859	-1.209	-3.274	2.065	**7b**	37.4727	-2.897	-3.386	0.534
6c	66.9146	-1.193	-3.049	1.856	**7c**	43.5794	-2.557	-3.385	0.828
6d	61.0448	-1.172	-2.941	1.716	**7d**	37.6048	-2.382	-3.378	0.996
6e	61.5415	-1.185	-2.974	1.789	**7e**	38.1063	-2.448	-3.376	0.928
6f	61.6948	-1.168	-3.067	1.899	**7f**	38.2702	-2.594	-3.377	0.783

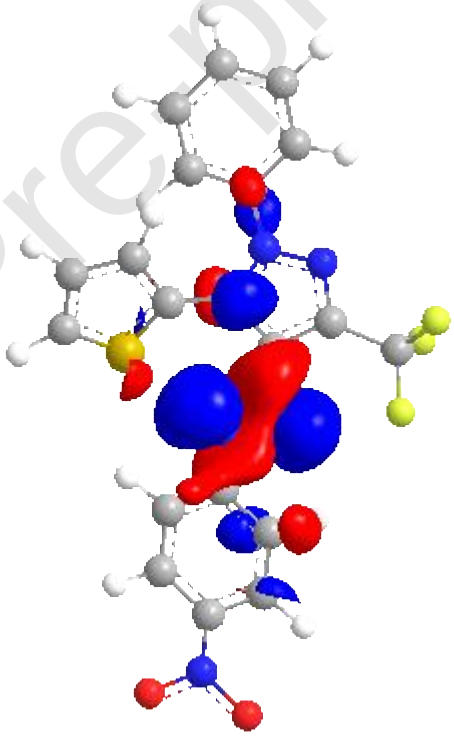
6g	63.1372	-1.636	-5.564	3.928	**7g**	41.4539	-3.326	-5.915	2.589

E[a] (minimum energy of molecule in least strained conformation, kcal/mol) and the HOMO-LUMO energies were obtained using ChemBio3D ultra.

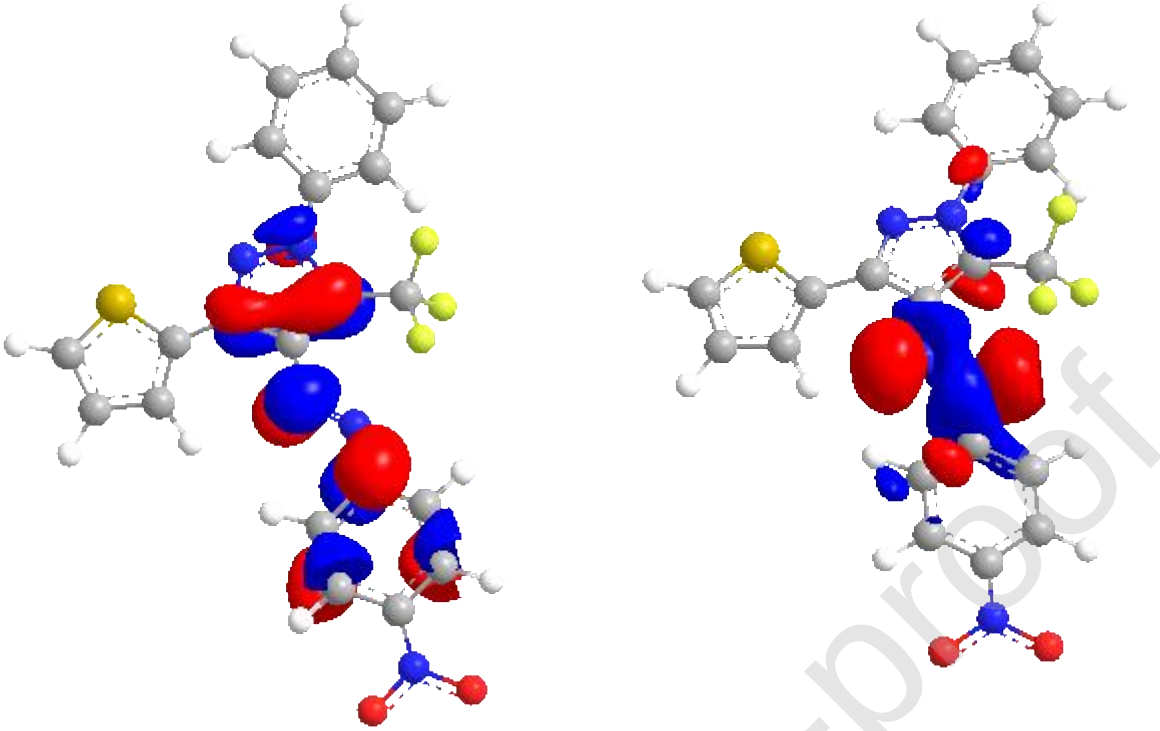
The theoretical results indicate that 3-trifluoromethylpyrazole **5g** and 5-trifluoromethylpyrazole **6g** (**R=NO_2**) were found to have drastically highest energy gap between HOMO and LUMO (ΔE_{L-H}=4.12 and 3.928 kcal/mol respectively) than other derivatives prepared in the present study (ΔE_{L-H}=0.53-2.59). The high energy gap between HOMO and LUMO for compounds **5g** and **6g** can enhance the susceptibility of bond cleavage [42] under the influence of UV light, due to formation of aryl radical. Thus, it may be predicted that compounds **5g** and **6g** may have good potential as DNA photocleavers. The ball and stick model of HOMO and LUMO of compound **5g** and **6g** are shown in **Figure 2**.



HOMO of 5g



LUMO of 5g



HOMO of 6g **LUMO of 6g**

Figure 2: HOMO and LUMO of **5g** and **6g**.

3. Biological results and discussion

There are primarily, two forms of DNA, native supercoiled circular DNA (Form I) and relaxed circular DNA (Form II). Both the DNAs (Form I and Form II) have similar molecular weight, charge but due to tight conformation, supercoiled DNA migrates faster through the gel than relaxed circular DNA which forms the basis of their separation by gel electrophoresis. Some of the potential chemical compounds are capable of cleaving one strand of supercoiled DNA (Form I) to generate a relaxed circular form (Form II) on photoirrdiation [43,44,45]. Moreover, relaxed circular DNA (Form II) may also undergo further photocleavage on binding with the compound. Efficiency of DNA photocleavage can be estimated by measuring the decrease in the intensity of Form I.

DNA photocleavage potential of **4a-g**, **5a-g**, **6a-g** and **7b-g** were analyzed by their interaction with pBR322 supercoiled plasmid DNA under UV irradiation using agarose gel electrophoresis. The DNA cleavage potential of the compounds was determined by comparing the bands appeared in control and test compounds in absence and presence of UV-irradiation. The UV-VIS

absorbance studies were carried out to determine the maximum absorbance, λ_{max}, for **4a-g**, **5a-g**, **6a-g** and **7b-g** (**Table 2**) which may be helpful in ascertaining the wavelength of UV radiation for best photocleavage results. It was evident from the absorbance data that most of the compounds showed maximum absorption wavelength in the region of 280-300 nm. Therefore, we have chosen the UV radiations of wavelength 312 nm for the photocleavage experiments.

Table 2

Absorption peaks of **4a-g**, **5a-g**, **6a-g** and **7b-g**.

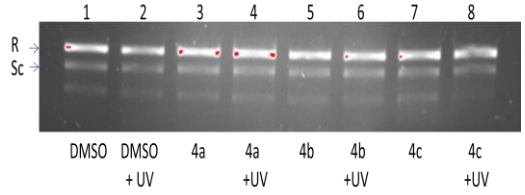
Compd	λ_{abs}/nm(logε)[a]	Compd	λ_{abs}/nm(logε)[a]	Compd	λ_{abs}/nm(logε)[a]	Compd	λ_{abs}/nm(logε)[a]
4a	276.89(0.94), 291.09(0.95)	**5a**	285.26(057)	**6a**	292.45(1.40)	**7a**	-
4b	296.81(0.89)	**5b**	285.26(0.82)	**6b**	285.98(1.10)	**7b**	287.06(0.97)
4c	276.89(2.28), 289.97(2.274)	**5c**	286.44(0.65)	**6c**	292.45(0.29)	**7c**	297.83(0.46)
4d	297.59(0.45)	**5d**	286.44(1.68)	**6d**	280.56(0.94)	**7d**	297.83(0.33)
4e	298.72(0.67)	**5e**	285.26(0.30), 260.03(0.29)	**6e**	288.08(1.24)	**7e**	274.06(0.40)
4f	306.00(0.361)	**5f**	285.26(0.82) 260.03(0.76)	**6f**	291.33(0.76)	**7f**	274.06(1.33)
4g	286.68(1.77)	**5g**	258.85(0.46) 289.90(0.36)	**6g**	295.12(0.98)	**7g**	288.08(0.72)

[a] in DMSO.

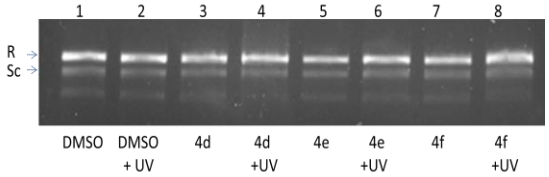
3.1 *DNA photocleavage Study*

The DNA photocleavage efficiency of compounds **4a-g**, **5a-g**, **6a-g** and **7b-g** was evaluated against pBR322 supercoiled DNA plasmid. The gel electrophoresis of pBR322 DNA, after addition of the compounds **4a-g**, **5a-g**, **6a-g** and **7b-g** is shown in **Fig. 3** (A-K).

A



B



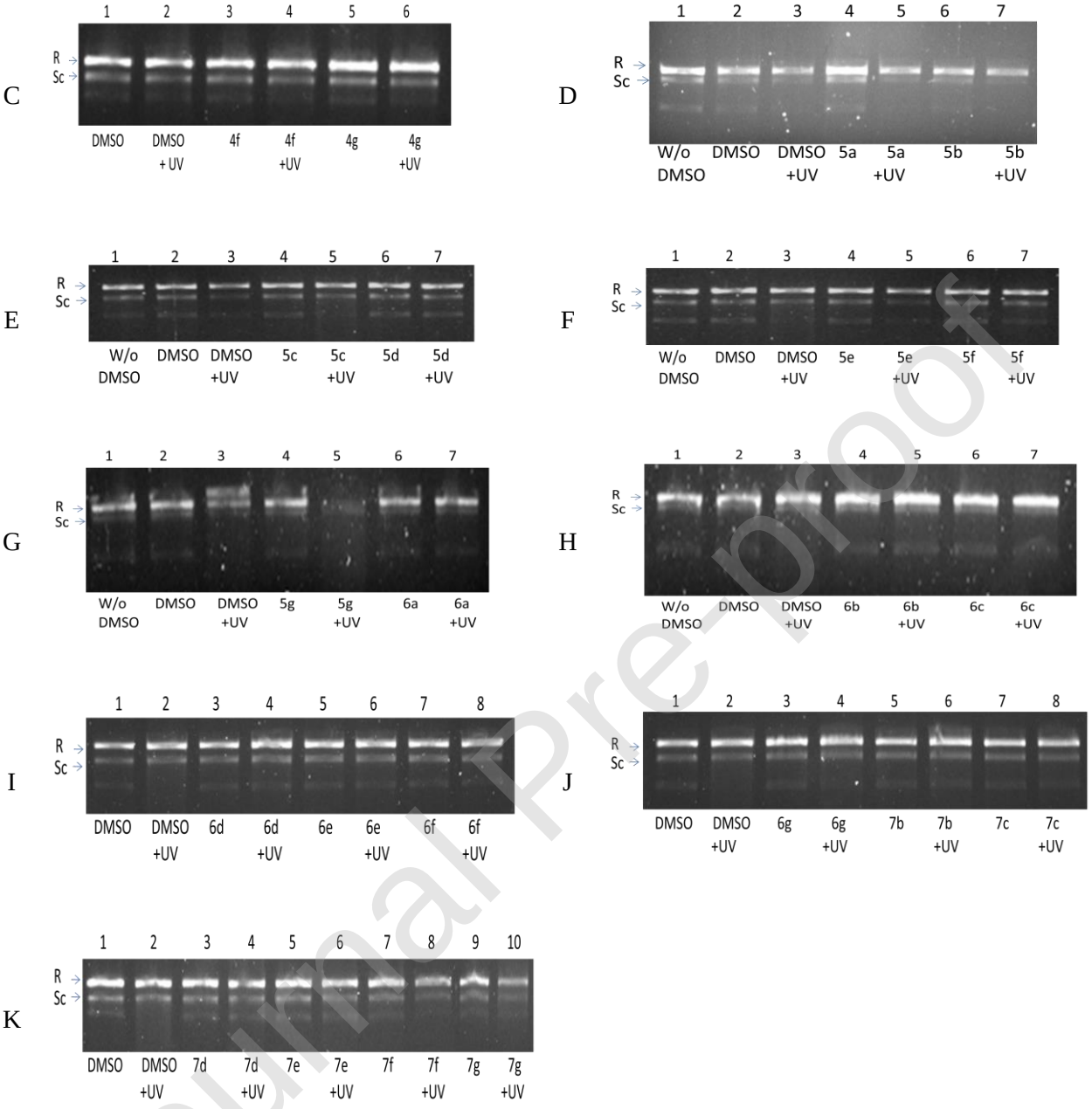


Fig.3. Ethidium bromide stained agarose (1%) gel showing the interaction of compounds **4a-g**, **5a-g**, **6a-g** and **7b-g** (20 µM) in DMSO with supercoiled pBR322 DNA (60 µM) (A-K) in 0.04 M Triseborate, 0.114% acetic acid and 50 mM EDTA, pH 8.0, λ_{irr} 312 nm, 15W, 45 min. The lanes without compound are control experiments as indicated by w/o DMSO (without DMSO), DMSO and DMSO + UV. R-Relaxed form of DNA (Form II), Sc-Supercoiled form of DNA (Form I).

In gel electrophoresis analysis (**Fig. 3**) the Lane 1 corresponds to experiments carried without compound where DNA appears as supercoiled form (Form I) having small impurity of relaxed DNA form (Form II). The control experiments involve irradiation of DNA solution in presence and absence of DMSO without the addition of compound (Lane no. 1, 2 in A-C, I-K and Lane no. 1, 2, 3 in D-H) as well as incubation of DNA and compounds in the dark (all odd no. lanes from 3 onward in A-C, I-K and all even no. lanes from 4 onward in D-H).

The gel electrophoresis of pBR322 plasmid DNA with compounds **4a-g**, have shown that these compounds do not cleave the supercoiled DNA Form I (Sc), as same intensity bands of both the forms is observed in presence or absence of UV light **Fig. 3** (gel A-C).

In gel electrophoresis of pBR322 with compounds **5a-g**, it was observed that the compounds **5a-f** could not effectively cleave the supercoiled DNA Form I (Sc) as only slight decrease in concentration of band was observed in case of (**5e**) and almost same intensity of supercoiled form in case of (**5a-c, 5f**). However, compound **5g** demonstrated maximum photocleavage potential against pBR322 DNA since it not only cleaved Form I (Sc) but also relaxed circular Form II (R) completely to smaller fragments as shown in **Fig. 3** (gel D-G).

Further, electrophoresis experiments carried out with 5-trifluoromethylpyrazoles **6a-g** demonstrated no photocleavage as no decrease in intensity of supercoiled DNA Form I was observed (**Fig. 3**, gel G, H, I and J).

Among all the 5-hydroxy-5-trifluoromethylpyrazolines **7b-g**, it was observed that compounds **7b**,**c** and **d** did not cleave the supercoiled DNA to significant extent, however **7f** and **7g** shows decrease in intensity of band corresponding to Form I (**Fig. 3,** gel J and K) indicating slight cleavage.

In summary, among all the tested compounds 3-trifluoromethylpyrazoles **5** exhibited highest DNA photocleavage activity as compared to corresponding 5-trifluoromethylpyrazoles **6** and pyrazolines **7**. Among 3-trifluoromethylpyrazoles **5,** NO_2-substituted derivative **5g** exhibited maximum photocleavage activity. Arylazodiketones **4a-g** and 5-trifluoromethylpyrazoles, **6a-g**, displayed very poor or no DNA photocleavage activity as no compound could effectively cleave the supercoiled form. Further it was observed that out of 5-hydroxy-5-

trifluoromethylpyrazolines, **7b-g**, only methoxy **7f** and nitro substituted **7g** derivatives cleaved the supercoiled form to noticeable amount. The highest DNA cleavage activity exhibited by compound **5g** can be well correlated with the theoretical calculations where the energy gap between HOMO and LUMO was highest which may contribute to ease of formation of aryl radicals and DNA cleavage.

3.2 *Cytotoxicity*

Seventeen synthesized compounds *viz* **5a, 5c-g, 6a, 6c-g** and **7c-g** were selected and evaluated for their cytotoxic evaluation against four human cancer cell lines, such as SBALL (Acute Lymphoblastic Leukemia), BT-474 (breast cancer), MOLT-4 (Acute Lymphoblastic Leukemia) and MCF-7 (breast cancer) by using MTT assay [46]. Doxorubicin was used as standard cytotoxic drug.

Initial screening of these compounds for their cytotoxic evaluation at 10 μM concentration revealed that compounds **5a**, **5g**, **6a**, **6g**, **7e** and **7g** exhibited cyototoxicity for MCF-7 cell line comparable to positive control doxorubicin (**Table 3**).

Table 3

Percentage cell survival of compounds **5a, 5c-g, 6a, 6c-g and 7c-g** at 10 μM concentration using MTT assay against four cancer cell lines.

Tested Compd.	R	% Cell Survival ± S.D.[a]			
		MCF-7	BT-474	SBALL	MOLT-4
PBS		100 ± 0.00	100 ± 0.00	100.00 ± 22.33	100.00 ± 28.44
5a	-H	58.38 ± 10.52	79.41 ± 30.84	89.65 ± 17.89	63.15 ± 14.54
5c	$-OCH_3$	70.83 ± 12.13	82.23 ± 17.86	90.20 ± 11.18	77.99 ± 46.71
5d	-F	69.66 ± 11.31	86.34 ± 15.23	92.19 ± 32.83	87.53 ± 26.86
5e	-Cl	72.57 ±16.31	83.56 ± 17.56	95.36 ± 27.36	73.20 ± 9.66
5f	-Br	74.48 ± 6.87	82.6 ± 18.76	85.30 ± 26.58	74.18 ± 17.12
5g	$-NO_2$	42.96 ± 6.49	66.69 ± 6.66	68.18 ± 16.46	58.53 ± 26.81
6a	-H	56.15 ± 19.13	74.18 ± 17.62	70.19 ± 24.61	66.31 ± 15.03
6c	$-OCH_3$	82.58 ±10.25	80.6 ± 25.67	90.99 ± 15.37	83.82 ± 39.33
6d	-F	72.88 ± 8.96	88.38 ± 21.28	91.65 ± 12.44	105.14 ± 58.48

6e	-Cl	73.13 ± 16.63	64.89 ± 31.02	90.64 ± 13.12	81.75 ± 30.40
6f	-Br	73.19 ± 9.30	83.93 ± 25.31	81.06 ± 21.55	72.67 ± 10.71
6g	$-NO_2$	55.30 ± 11.50	72.66 ± 11.04	78.47 ± 18.98	87.48 ± 32.39
7c	$-OCH_3$	63.10 ± 10.67	85.83 ± 18.90	81.42 ± 13.11	86.44 ± 47.76
7d	-F	72.48 ± 15.15	79.91 ± 25.03	94.90 ± 16.55	84.33 ± 29.47
7e	-Cl	45.91 ± 8.37	56.36 ± 20.37	64.49 ± 30.29	62.38 ± 28.73
7f	-Br	89.30 ± 6.21	69.39 ± 21.54	85.00 ± 26.22	71.54 ± 24.74
7g	$-NO_2$	81.16 ± 11.69	54.19 ± 32.68	62.13 ± 33.59	64.24 ± 14.70
Doxorubicin		41.15 ± 2.58	37.43 ± 8.30	21.90 ± 11.25	12.57 ± 5.28

[a]The activity data represents mean values ± SD of experiments conducted in triplicates at three independent times.

Based on data, it was inferred that the compounds bearing fluoro, bromo and methoxy substituents at *para* position of phenyl ring (**5c**, **5d**, **5f**, **6c**, **6d**, **6f**, **7c**, **7d** and **7f**) showed very little cytotoxicity against all the tested cancer cell lines. The compounds with unsubstituted phenyl (**5a** and **6a**) an increased cytotoxicity was observed against MCF-7 and MOLT-4 cancer cell lines with % cell survival of 56.15-58.38 and 63.15-66.31, respectively. Out of 4-chlorophenyl substituted pyrazole derivatives (**5e**, **6e** and **7e**), only pyrazoline derivative **7e** could exhibit good cytotoxicity against all cancer cells with maximum cytotoxic effect on MCF-7 cell line having less than 50% cell survival (**Table 3**). Interestingly, nitro-substituted pyrazole derivatives (**5g**, **6g** and **7g**) showed highest cytotoxicity against most of the tested cancer cell lines. It can be correlated to the fact that nitro is a strong electron withdrawing group which can create electron deficient sites within the molecule and hence can easily interact with the biological nucleophiles present in proteins or nucleic acids via nucleophilic addition, reduction or complexation, and can induce the cytotoxicity [47]. Out of these compounds (**5g**, **6g** and **7g**), **5g** was found to be the most potent compound with % cell survival of 42.96±6.49 comparable to Doxorubicin (% cell survival 41.15±2.58) against MCF-7 cell line. Compound **7e** was also found to have lowest % cell survival, nearly equal to standard drug Doxorubicin selectively for MCF-7 cell lines.

Five promising compounds **5a**, **5g**, **6a**, **7e** and **7g** were selected for further evaluation for their IC_{50} values against four cancer cell lines. The compounds **5a**, **6a** and **7e** have shown moderate

cytotoxicity and the compounds **5g** and **7g** were identified as most active compounds listed in **Table 4**. The results of cytoxicity and DNA photocleavage shows that compound **5g** could be used as potential anticancer agent by further derivatization.

Table 4

IC_{50} values of compounds **5a**, **5g**, **6a**, **7e** and **7g** against four cancer cell lines.

Compound	IC_{50}[a] value (in μM) against cancer cell line			
	MCF-7	**BT-474**	**SBALL**	**MOLT-4**
5a	11.2 ± 5.2	12.3 ± 1.3	14.6 ± 3.3	13.2 ± 2.4
5g	8.6 ± 3.8	15.4 ± 3.5	18.2 ± 2.4	11.3 ± 2.2
6a	20.2 ± 5.3	14.1 ± 2.6	13.2 ± 3.1	13.1 ± 1.3
7e	12.3 ± 6.1	18.9 ± 5.3	21.2 ± 8.7	13.6 ± 4.4
7g	10.5 ± 4.3	17.3 ± 3.6	15.3 ± 2.5	13.2 ± 2.3
Doxorubicin	0.42 ± 0.18	0.31± 0.13	0.22 ± 0.12	0.25 ± 0.08

[a] IC_{50} = half maximal concentration represents the concentration of drug able to inhibit by 50% the *in vitro* growth. Each value represents mean ± SD of three experiments.

4. Conclusion

The present work deals with design, synthesis and biological potential of trifluoromethyl-4-arylazopyrazole derivatives as DNA photocleaving and cytotoxic agents. Activity profile concluded that 3-trifluoromethylpyrazoles **5** resulted in good to excellent DNA cleavage and cytotoxic activities. Moreover, compound **5g** was identified as both good DNA photocleaving and cytotoxic agent. Measuring the cytotoxic effects of synthesized compounds against multiple cancer cell lines using a cell proliferation assay is a widely used approach [**Error! Bookmark not defined.**]. Studying DNA-photocleavage is another evidence-based approach that evaluates compound's potential to damage the DNA and fragment it. DNA fragmentation is one of the hallmarks of apoptosis (cell death) which is expected to happen in cancer (or dividing cells) upon treatment with anti-cancer drugs [**Error! Bookmark not defined.**]. Further derivatization of 5g can lead to development of compounds with enhanced cytotoxic capabilities with the potential to be developed as an anticancer drug for future.

5. Experimental

Melting point were determined in open capillaries with an electrical melting point apparatus and are uncorrected, the IR spectra of the compounds were recorded on Buck Scientific IR M-500 spectrophotometer using KBr pellets (ν_{max} in cm^{-1}), ^{1}H and ^{13}C NMR spectra on a Bruker instrument at 400 and 100 MHz, respectively, using deuteriochloroform as a solvent. Chemical shifts are expressed in δ-scale downfield from TMS as an internal standard. ^{19}F NMR spectra were run on DPX 400 at 376 MHz, using deuteriochloroform as a solvent. The internal standard for ^{19}F spectra was fluorotrichloromethane, setting the $CFCl_3$ signal at δ 0.0. Elemental and mass analyses were performed at Sophisticated Analytical Instrument Facility, Panjab University, Chandigarh, India.

5.1 General procedure for the synthesis of 1-(2-thienyl)-2-(4-substitutedphenylazo)-4,4,4-trifluorobutane-1,3-diones **4a–g**.

1-(2-Thienyl)-2-(4-substitutedphenylazo)-4,4,4-trifluorobutane-1,3-diones **4a–g** were prepared by treating diazotized arylamines with 1-(2-thienyl)-4,4,4-trifluorobutane-1,3-diones in 50% ethanol in presence of sodium acetate according to the previous literature reports**Error! Bookmark not defined.**.

5.2 General procedure for the synthesis of 1-phenyl-5-(2-thienyl)-4-(4-substitutedphenylazo)-3-trifluoromethylpyrazoles **5a-g**, 1-phenyl-3-(2-thienyl)-4-(4-substitutedphenylazo)-5-trifluoromethylpyrazoles **6a-g** and 1-phenyl-3-(2-thienyl)-4-(4-substitutedphenylazo)-5-trifluoromethyl-5-hydroxypyrazolines **7b-g.**

An ethanolic solution of phenyl hydrazine (0.108 mg, 1.0 mmol) and an appropriate 1-(2-thienyl)-2-(4-substitutedphenylazo)-4,4,4-trifluorobutane-1,3-diones **4** (1.0 mmol) was refluxed on a heating water bath for 4 hours. TLC monitoring of the reaction indicated the formation of a mixture of three products. On completion of the reaction excess of solvent was removed by distillation and the products were purified by column chromatography using silica gel mesh (size 100-200) with 2 % ethylacetate in petroleum ether as eluent. Isolated compounds in order of their elution from column chromatography were characterized as 1-phenyl-5-(2-thienyl)-4-(4-substitutedphenylazo)-3-trifluoromethylpyrazoles **5,** 1-phenyl-3-(2-thienyl)-4-(4-substitutedphenylazo)-5-trifluoromethylpyrazoles **6** and 1-phenyl-3-(2-thienyl)-4-(4-

substitutedphenylazo)-5-trifluoromethyl-5-hydroxypyrazolines **7,** respectively. Pyrazoline, **7a** was not isolated in measurable amount under these conditions.

Also, addition of few drops of HCl to the reaction mixture resulted in disappearance of pyrazolines (**7**) in all the cases.

5.2.1 1-Phenyl-5-(2-thienyl)-4-phenylazo-3-trifluoromethylpyrazole **5a**.

M. Pt. 211-215°C, Yield 68%, IR (KBr, cm^{-1}): 1705, 1643, 1443, 1381, 1134; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.09–7.14 (m, 2H, 3''-H-Thienyl, 4'''-H-Ph); 7.61 (m, 8H, 3'-H-Ph, 2'',4''-H-Thienyl, 2''',3'''-H-Ph); 7.81–7.87 (m, 3H, 2'-H, 4'-H-Ph); ^{13}C NMR (100 MHz, $CDCl_3$): 123.03, 125.92, 126.51, 127.12, 129.10, 129.53, 129.53, 129.70, 130.20, 130.60, 138.49, 138.94, 152.96; MS (EI) m/z: Found 398.96 $[M+1]^+$, require 399.08, Anal. Calcd. For $C_{20}H_{13}F_3N_4S$: C, 60.30; H, 3.29; N, 14.06 Found: C, 59.91; H, 3.06; N, 13.87 %.

5.2.2 1-Phenyl-5-(2-thienyl)-4-(4-methylphenylazo)-3-trifluoromethylpyrazole **5b**.

M. Pt. 242-246°C, Yield 59%, IR (KBr, cm^{-1}): 1597, 1497, 1450, 1319, 1126; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.43 (s, 3H, CH_3); 6.99-7.02 (m, 2H, 3''-H-Thienyl, 4'-H-Ph), 7.28-7.31 (d, 2H, *J*=8.4 Hz, 3'''-H-Ph); 7.44–7.51 (m, 6H, 2',3'-H-Ph, 2'',4''-H-Thienyl), 7.80-7.82 (d, 2H, *J*=8.4 Hz, 2'''-H-Ph); ^{13}C NMR (100 MHz, $CDCl_3$): 21.59, 122.32, 123.03, 126.53, 127.08, 127.51, 129.51, 129.53, 129.61, 129.65, 129.76, 130.00, 130.47, 133.00, 138.09, 138.99, 151.14; MS (EI) m/z: Found 413.04 $[M+1]^+$, require 413.10, Anal. Calcd. For $C_{21}H_{15}F_3N_4S$: C, 61.16; H, 3.67; N, 13.58 Found: C, 60.93; H, 3.31; N, 13.41 %.

5.2.3 1-Phenyl-5-(2-thienyl)-4-(4-methoxyphenylazo)-3-trifluoromethylpyrazole **5c**.

M. Pt. 251-254°C, Yield 63%, IR (KBr, cm^{-1}): 1597, 1497, 1311, 1250, 1134; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.88 (s, 3H), 6.99–7.02 (m, 4H, 2'',4''-H-Thienyl, 3'''-H-Ph); 7.43-7.49 (m, 6H, Ph-H, 3''-H-Thienyl); 7.87–7.91 (d, 2H, 2'''-H-Ph); ^{13}C NMR (100 MHz, $CDCl_3$): 55.63, 99.99, 114.26, 119.72, 126.51, 127.05, 127.63, 129.60, 129.86, 134.87, 137.68, 139.04, 147.46, 162.26; MS (EI) m/z: Found 429.02 $[M+1]^+$, require 429.09, Anal. Calcd. For $C_{21}H_{15}F_3N_4OS$: C, 58.87; H, 3.53; N, 13.08 Found: C, 58.43; H, 3.29; N, 12.91 %.

5.2.4 1-Phenyl-5-(2-thienyl)-4-(4-fluorophenylazo)-3-trifluoromethylpyrazole **5d**.

M. Pt. 258-262°C, Yield 61%, IR (KBr, cm^{-1}): 1705, 1636, 1497, 1450, 1227, 1134; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 6.99-7.02 (m, 2H, 2",4"-H-Thienyl); 7.17-7.20 (m, 2H, 3"'-H-Ph); 7.44-7.51 (m, 6H, Ph-H, 3"-H-Thienyl); 7.89-7.93 (m, 2H, 2"'-H-Ph); ^{13}C NMR (100 MHz, $CDCl_3$): 115.99, 116.16, 116.22, 124.97, 125.06, 125.18, 125.85, 126.46, 127.17, 127.31, 127.43, 127.63, 128.39, 129.22, 129.54, 129.71, 130.16, 130.56, 133.17, 138.51, 138.89, 149.52, 163.23; MS (EI) m/z: Found 417.03 $[M+1]^+$, require 417.07, Anal. Calcd. For $C_{20}H_{12}F_4N_4S$: C, 57.69; H, 2.90; N, 13.46 Found: C, 57.35; H, 2.54; N, 13.07 %.

5.2.5 1-Phenyl-5-(2-thienyl)-4-(4-chlorophenylazo)-3-trifluoromethylpyrazole **5e**.

M. Pt. 186-189°C, Yield 65%, IR (KBr, cm^{-1}):1697, 1643, 1474, 1389, 1134; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 6.99-7.02 (m, 2H, 2",4"-H-Thienyl); 7.44-7.52 (m, 8H, 2',3',4'-H-Ph,3"'-H-Ph, 3"-H-Thienyl), 7.82-7.86 (d, 2H, *J*=8.4 Hz, 2"'-H-Ph); ^{13}C NMR (100 MHz, $CDCl_3$): 124.24, 126.47, 127.20, 127.22, 129.22, 129.38, 129.56, 129.76, 130.30, 130.64, 134.59, 137.08, 138.80, 138.86, 151.35; MS (EI) m/z: Found 432.90/434.92 $[M+1]^+/[M+1+2]^+$ (3:1), require 433.04/435.04 (3:1), Anal. Calcd. For $C_{20}H_{12}ClF_3N_4S$: C, 55.50; H, 2.79; N, 12.94 Found: C, 55.30; H, 2.34; N, 12.53 %.

5.2.6 1-Phenyl-5-(2-thienyl)-4-(4-bromophenylazo)-3-trifluoromethylpyrazole **5f**.

M. Pt. 260-265°C, Yield 59%, IR (KBr, cm^{-1}): 1694, 1496, 1251, 1134;^{1}H NMR (400 MHz, $CDCl_3$) δ: 6.99-7.02 (m, 2H, 2",4"-H-Thienyl); 7.44–7.53 (m, 6H, 2',3',4'-H-Ph, 3"-H-Thienyl); 7.61-7.65 (d, 2H, *J*=8.4 Hz, 3"'-H-Ph); 7.75-7.78 (d, 2H, *J*=8.4 Hz, 2"'-H-Ph); ^{13}C NMR (100 MHz, $CDCl_3$): 119.53, 124.46, 125.58, 126.47, 127.21, 129.57, 129.78, 130.33, 130.65, 131.77, 132.38, 134.59, 138.85, 151.70; MS (EI) m/z: Found 476.82/478.91 $[M+1]^+/[M+1+2]^+$ (1:1), require 476.99/478.99 (1:1), Anal. Calcd. For $C_{20}H_{12}BrF_3N_4S$: C, 50.33; H, 2.53; N, 11.74 Found: C, 50.09; H, 2.21; N, 11.47 %.

5.2.7 1-Phenyl-5-(2-thienyl)-4-(4-nitrophenylazo)-3-trifluoromethylpyrazole **5g**.

M. Pt. 279-284°C, Yield 68%, IR (KBr, cm^{-1}): 1666, 1589, 1512, 1443, 1327, 1134; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 6.85-6.89 (m, 2H, 2",4"-H-Thienyl); 7.50–7.59 (m, 6H, 2',3',4'-H-Ph, 3"-H-Thienyl); 8.01-8.04 (d, 2H, *J*=8.4 Hz, 2"'-H-Ph); 8.38-8.41 (d, 2H, *J*=8.4 Hz, 3"'-H-Ph); ^{13}C NMR (100 MHz, $CDCl_3$):123.54, 124.80, 125.50, 126.43, 127.40, 129.67, 129.99, 130.92,

131.04, 138.66, 148.67, 156.07; MS (EI) m/z: Found 443.93 $[M+1]^+$, require 444.07, Anal. Calcd. For $C_{20}H_{12}BrF_3N_4S$: C, 54.18; H, 2.73; N, 15.79 Found: C, 53.91; H, 2.53; N, 14.97 %.

5.2.8 1-Phenyl-3-(2-thienyl)-4-phenylazo-5-trifluoromethylpyrazole **6a**.

M. Pt. 198-202°C, Yield 14%, IR (KBr, cm^{-1}): 1695, 1643, 1443, 1250, 1134; ^1H NMR (400 MHz, $CDCl_3$) δ: 7.12–7.14 (m, 1H, 3"-H-Thienyl); 7.41-7.43 (d, 1H, *J*=8.0 Hz, 2"-H-Thienyl); 7.54–7.56 (m, 9H, 2',3',4'-H-Ph, 4"-H-Thienyl, 2"',4"'-H-Ph); 7.97-7.99 (d, 2H, *J*=8.0 Hz, 4"'-H-Ph); ^{13}C NMR (100 MHz, $CDCl_3$): 123.09, 125.87, 126.52, 127.13, 129.16, 129.22, 129.53, 129.75, 130.14, 130.60, 138.74, 138.93, 142.80, 152.88; MS (EI) m/z: Found 399.02 $[M+1]^+$, require 399.08. Anal. Calcd. For $C_{20}H_{13}F_3N_4S$: C, 60.30; H, 3.29; N, 14.06 Found: C, 60.11; H, 3.09; N, 13.89 %.

5.2.9 1-Phenyl-3-(2-thienyl)-4-(4-methylphenylazo)-5-trifluoromethylpyrazole **6b**.

M. Pt. 224-228°C, Yield 15%, IR (KBr, cm^{-1}): 1696, 1589, 1389, 1227, 1134; ^1H NMR (400 MHz, $CDCl_3$) δ: 2.50 (s, 3H); 7.20-7.23 (q, 1H, *J*=3.0 Hz, 3"-H-Thienyl); 7.45-7.47 (d, 2H, *J*=8.0 Hz, 3"'-H-Ph); 7.60-7.68 (m, 5H, 2',3',4'-H-Ph); 7.73-7.74 (d, 1H, 4"-H-Thienyl), 7.82–7.85 (m, 3H, 2"-H-Thienyl, 2"'-H-Ph); ^{13}C NMR (100 MHz, $CDCl_3$): 21.63, 122.32, 123.03, 123.11, 125.86, 126.37, 126.53, 127.41, 127.43, 128.10, 128.30, 129.20, 129.32, 129.51, 129.68, 129.76, 129.90, 132.76, 139.59, 142.50, 151.06; MS (EI) m/z: Found 413.03 $[M+1]^+$, require 413.10, Anal. Calcd. For $C_{21}H_{15}F_3N_4S$: C, 61.16; H, 3.67; N, 13.58 Found: C, 60.96; H, 3.35; N, 13.37 %.

5.2.10 1-Phenyl-3-(2-thienyl)-4-(4-methoxyphenylazo)-5-trifluoromethylpyrazole **6c**.

M. Pt. 181-185°C, Yield 14%, IR (KBr, cm^{-1}): 1705, 1650, 1443, 1389, 1134; ^1H NMR (400 MHz, $CDCl_3$) δ: 3.91 (s, 3H), 7.02–7.06 (d, 2H, *J*=8.0 Hz, 3"'-H-Ph); 7.10-7.13 (q, 1H, *J*=8.4 Hz, 3"-H-Thienyl); 7.39-7.41 (d, *J*=8.4 Hz, 4"-H-Thienyl), 7.51–7.58 (m, 5H, 2',3',4'-H-Ph); 7.85 (m, 1H, 2"-H-Thienyl); 7.96-7.99 (d, 2H, *J*=8.0 Hz, 2"'-H-Ph); ^{13}C NMR (100 MHz, $CDCl_3$): 55.68, 114.39, 123.39, 125.10, 125.85, 126.60, 127.32, 127.38, 128.13, 129.18, 129.61, 132.85, 139.64, 142.75, 147.40, 162.69; MS (EI) m/z: Found 428.98 $[M+1]^+$, require 429.09, Anal. Calcd. For $C_{21}H_{15}F_3N_4OS$: C, 58.87; H, 3.53; N, 13.08 Found: C, 58.47; H, 3.42; N, 12.97 %.

5.2.11 1-Phenyl-3-(2-thienyl)-4-(4-fluorophenylazo)-5-trifluoromethylpyrazole **6d**.

M. Pt. 214-218°C, Yield 15%, IR (KBr, cm^{-1}): 1685, 1585, 1443, 1227, 1134; ^1H NMR (400 MHz, $CDCl_3$) δ: 7.11-7.13 (m, 1H, 3"-H-Thienyl); 7.21-7.23 (m, 2H, 3"'-H-Ph); 7.36-7.38 (d, 1H, *J*=8.4 Hz, 4"-H-Thienyl); 7.44-7.51 (m, 8H, 2',3',4'-H-Ph, 2"-H-Thienyl, 2"'-H-Ph); ^{13}C NMR (100 MHz, $CDCl_3$): 124.97, 125.81, 126.46, 127.31, 127.43, 127.63, 128.39, 129.22, 129.54, 129.71, 130.16, 130.56, 133.17, 138.51, 138.89, 149.52, 163.23; MS (EI) m/z: Found 417.02 $[M+1]^+$, require 417.07, Anal. Calcd. For $C_{20}H_{12}F_4N_4S$: C, 57.69; H, 2.90; N, 13.46 Found: C, 57.49; H, 2.62; N, 13.15 %.

5.2.12 1-Phenyl-3-(2-thienyl)-4-(4-chlorophenylazo)-5-trifluoromethylpyrazole **6e**.

M. Pt. 191-194°C, Yield 17%, IR (KBr, cm^{-1}): 1689, 1565, 1450, 1134; ^1H NMR (400 MHz, $CDCl_3$) δ: 7.16-7.22 (m, 1H, 3"-H-Thienyl), 7.64-7.58 (m, 8H, 3"'-H-Ph, 2',3',4'-H-Ph, 4"-H-Thienyl); 7.86 (m, 1H, 2"-H-Thienyl); 7.93–7.95 (d, 2H, *J*=8.0 Hz, 2"'-H-Ph); ^{13}C NMR (100 MHz, $CDCl_3$): 124.33, 125.90, 126.42, 127.49, 127.59, 127.79, 128.15, 128.54, 129.29, 129.58, 129.88, 132.58, 137.73, 139.50, 142.91, 151.29; MS (EI) m/z: Found 432.92/434.93 $[M+1]^+/[M+1+2]^+$ (3:1), require 433.04/435.04 (3:1), Anal. Calcd. For $C_{20}H_{12}ClF_3N_4S$: C, 55.50; H, 2.79; N, 12.94 Found: C, 55.32; H, 2.49; N, 12.72 %.

5.2.13 1-Phenyl-3-(2-thienyl)-4-(4-bromophenylazo)-5-trifluoromethylpyrazole **6f**.

M. Pt. 214-218°C, Yield 15%, IR (KBr, cm^{-1}): 1685, 1565, 1443, 1236, 1134; ^1H NMR (400 MHz, $CDCl_3$) δ: 7.05-7.07 (dd, 1H, *J*=8.0 Hz, 3"-H-Thienyl); 7.35–7.37 (dd, *J*=8.0 Hz, 1H, 4"-H-Thienyl); 7.46-7.50 (m, 5H, 2',3',4'-H-Ph); 7.60-7.62 (d, 2H, *J*=8.0 Hz, 3"'-H-Ph); 7.77-7.79 (m, 3H, *J*=8.4 Hz, 2"-H-Thienyl, 2"'-H-Ph); ^{13}C NMR (100 MHz, $CDCl_3$): 124.55, 125.92, 126.28, 127.51, 127.83, 128.58, 129.31, 129.91, 131.93, 132.60, 151.67; MS (EI) m/z: Found 476.87/478.89 $[M+1]^+/[M+1+2]^+$ (1:1), require 476.99/478.99 (1:1), Anal. Calcd. For $C_{20}H_{12}BrF_3N_4S$: C, 50.33; H, 2.53; N, 11.74 Found: C, 50.15; H, 2.39; N, 11.47 %.

5.2.14 1-Phenyl-3-(2-thienyl)-4-(4-nitrophenylazo)-5-trifluoromethylpyrazole **6g**.

M. Pt. 281-285°C, Yield 20%, IR (KBr, cm^{-1}): 1656, 1585, 1488, 1456, 1250, 1134; ^1H NMR (400 MHz, $CDCl_3$) δ: 7.11-7.16 (m, 1H, 3"-H-Thienyl); 7.36–7.41 (m, 1H, 4"-H-Thienyl); 7.51-

7.56 (m, 5H, 2',3',4'-H-Ph); 7.90 (m, 1H, 2''-H-Thienyl); 8.08–8.10 (d, 2H, *J*=9.0 Hz, 2'''-H-Ph); 8.40-8.43 (d, 2H, *J*=9.2 Hz, 3'''-H-Ph); ^{13}C NMR (100 MHz, $CDCl_3$): 123.57, 124.89, 125.32, 126.43, 127.40, 129.67, 130.92, 129.32, 130.09, 140.00, 162.75; ^{19}F NMR (300 MHz, $CDCl_3$) δ: -55.81; MS (EI) m/z: Found 443.95 $[M+1]^+$, require 444.07, Anal. Calcd. For $C_{20}H_{12}BrF_3N_4S$: C, 54.18; H, 2.73; N, 15.79 Found: C, 53.98; H, 2.61; N, 14.86 %.

5.2.15 1-Phenyl-3-(2-thienyl)-4-(4-methylphenylazo)-5-hydroxy-5-trifluoromethylpyrazoline **7b**.

M. Pt. 228-234°C, Yield 11%, IR (KBr, cm^{-1}): 3263, 1696, 1595, 1450, 1134; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.31 (s, 3H), 7.11-7.13 (m, 4H, 2',3'-H-Ph), 7.15-7.16 (m, 1H, 3''-H-Thienyl), 7.34-7.38 (m, 2H, 3'''-H-Ph), 7.50-7.52 (m, 1H, 4'-H-Ph), 7.55-7.57 (d, *J*=8.0Hz, 2H, 2'''-H-Ph); 8.18-8.19 (dd, 1H, 4''-H-Thienyl); 8.18-8.19 (dd, 1H, 2''-H-Thienyl); 8.24 (bs, 1H, OH); ^{13}C NMR (100 MHz, $CDCl_3$): 20.76, 99.99, 114.73, 126.6, 129.34, 129.51, 129.65, 130.03, 132.20, 134.38, 134.62, 139.55, 140.20, 141.95; MS (EI) m/z: Found 430.91 $[M+1]^+$, require 431.11, Anal. Calcd. For $C_{20}H_{12}BrF_3N_4S$: C, 58.60; H, 3.98; N, 13.02 Found: C, 58.29; H, 3.65; N, 12.99 %.

5.2.16 1-Phenyl-3-(2-thienyl)-4-(4-methoxyphenylazo)-5-hydroxy-5-trifluoromethylpyrazoline **7c**.

M. Pt. 252-256°C, Yield 12%, IR (KBr, cm^{-1}): 3250, 1705, 1651, 1453, 1134; ^{1}H NMR (400 MHz, DMSO) δ: δ: 3.89 (s, 3H, CH_3), 7.21-7.23 (m, 4H, 2',3'-H-Ph), 7.25-7.26 (m, 1H, 3''-H-Thienyl), 7.36-7.38 (m, 2H, 3'''-H-Ph), 7.52-7.54 (m, 1H, 4'-H-Ph), 7.56-7.58 (d, *J*=8.0Hz, 2H, 2'''-H-Ph); 8.20-8.22 (dd, 1H, 4''-H-Thienyl); 8.23-8.24 (dd, 1H, 2''-H-Thienyl); 8.28 (bs, 1H, OH); ^{13}C NMR (100 MHz, $CDCl_3$): 56.76, 100.99, 113.83, 125.91, 129.24, 129.31, 129.55, 131.03, 131.26, 133.42, 134.84, 140.16, 140.62, 141.68; MS (EI) m/z: Found 446.98 $[M+1]^+$, require 447.10, Anal. Calcd. For $C_{21}H_{17}F_3N_4O_2S$: C, 56.50; H, 3.84; N, 12.55 Found: C, 56.26; H, 3.55; N, 12.39 %.

5.2.17 1-Phenyl-3-(2-thienyl)-4-(4-fluorophenylazo)-5-hydroxy-5-trifluoromethylpyrazole **7d**.

M. Pt. 245-249°C, Yield 15%, IR (KBr, cm^{-1}): 3123, 1685, 1556, 1456, 1134; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.02-7.04 (m, 2H, 3'''-H-Ph); 7.17-7.19 (m, 3H, 3''-H-Thienyl, 3'-H-Ph); 7.34-7.36 (m, 2H, 2'''-H-Ph); 7.49–7.59 (m, 4H, 2',4'-H-Ph, 4-H); 7.70-7.72 (dd, 1H, 4''-H-Thienyl);

8.16-8.17(dd, 1H, *J*=7.0Hz, 2"-H-Thienyl); 8.22 (bs, 1H, OH); ^{13}C NMR (100 MHz, $CDCl_3$): 115.84, 115.92, 116.10, 116.33, 127.04, 128.38, 129.17, 129.24, 129.56, 129.79, 134.51, 134.77, 138.85, 139.26, 142.55, 148.34; 180.78; MS (EI) m/z: Found 434.89 $[M+1]^+$, require 435.08, Anal. Calcd. For $C_{20}H_{14}F_4N_4OS$: C, 55.30; H, 3.25; N, 12.90 Found: C, 55.12; H, 3.09; N, 12.81 %.

5.2.18 1-Phenyl-3-(2-thienyl)-4-(4-chlorophenylazo)-5-hydroxy-5-trifluoromethylpyrazoline **7e**.

M. Pt. 265-270°C, Yield 10%, IR (KBr, cm^{-1}): 3259, 1695, 1565, 1451, 1134; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.21-7.28 (m, 1H, 3"-H-Thienyl); 7.33-7.35 (m, 4H, 2',3'-H-Ph); 7.37-7.40 (d, 2H, *J*=9.2 Hz, 3"'-H-Ph), 7.48-7.56 (m, 3H, 4'-H-Ph, 4",2"-H-Thienyl), 8.02-8.03 (d, 2H, *J*=4.4 Hz, 2"'-H-Ph); 10.20 (bs, 1H, OH); ^{13}C NMR (100 MHz, $CDCl_3$): 112.5, 115.93, 124.35, 127.54, 127.94, 129.31, 129.61, 129.65, 129.90, 134.65, 134.99; MS (EI) m/z: Found 451.05/452.05 $[M+1]^+/[M+1+2]^+$ (3:1), require 450.94/451.87 (3:1), Anal. Calcd. For $C_{20}H_{14}ClF_3N_4OS$: C, 53.28; H, 3.13; N, 12.43 Found: C, 53.12; H, 2.97; N, 12.08 %.

5.2.19 1-Phenyl-3-(2-thienyl)-4-(4-bromophenylazo)-5-hydroxy-5-trifluoromethylpyrazoline **7f**.

M. Pt. 227-229°C; Yield 12%, IR (KBr, cm^{-1}): 3261, 1691, 1551, 1456, 1134; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.08-7.10 (m, 2H, 3'-H-Ph); 7.16-7.19 (m, 3H, 4"-H-Thienyl, 2'-H-Ph); 7.31-7.35 (d, 2H, *J*=8.4 Hz, 3"'-H-Ph); 7.54-7.60 (m, 3H, 3"-H-Thienyl, 4-H, 4'-H-Ph), 7.71-7.72 (d, 2H, J=8.4 Hz, 2"'-H-Ph); 8.16-8.17 (dd, 1H, 2"-H-Thienyl); 8.22 (bs, 1H, -OH); ^{13}C NMR (100 MHz, $CDCl_3$): 99.99, 113.81, 116.12, 116.33, 122.79, 127.17, 129.07, 129.49, 129.64, 129.96, 132.46, 134.74, 134.95, 139.25, 143.22, 143.95; 180.87; MS (EI) m/z: Found 493.81/495.93 $[M+1]^+/[M+1+2]^+$ (1:1), require 494.00/496.00 (1:1), Anal. Calcd. For $C_{20}H_{14}BrF_3N_4OS$: C, 48.50; H, 2.85; N, 11.31 Found: C, 48.39; H, 2.61; N, 11.12 %.

5.2.20 1-Phenyl-3-(2-thienyl)-4-(4-nitrophenylazo)-5-hydroxy-5-trifluoromethylpyrazoline **7g**.

M. Pt. 290-294°C, Yield 10%, IR (KBr, cm^{-1}): 3230, 1705, 1656, 1456, 1227, 1134; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.28-7.32 (d, 2H, *J*=8.4 Hz, 2"'-H-Ph); 7.46-7.48 (d, 2H, *J*=8.4 Hz, 3"'-H-Ph); 7.54-7.58 (m, 6H, 4-H, 2',3',4'-H-Ph); 8.07-8.09 (m, 1H, 3"-H-Thienyl); 8.22-8.26 (m, 1H, 4"-H-Thienyl,); 8.40-8.42 (m, 1H, 2"-H-Thienyl,); 9.73 (bs, 1H, -OH); ^{13}C NMR (100 MHz, $CDCl_3$): 100.98, 114.12, 118.99, 122.87, 124.65, 126.62, 127.12, 127.65, 128.25, 129.05,

129.42, 129.82, 131.10, 131.41, 132.34, 133.52, 135.65, 139.58, 142.93; 181.85; ^{19}F NMR (300 MHz, $CDCl_3$): -74.35; MS (EI) m/z: Found 460.10 $[M-1]^+$, require 460.08, Anal. Calcd. For $C_{20}H_{14}F_3N_5O_3S$: C, 52.06; H, 3.06; N, 15.18 Found: C, 51.89; H, 2.85; N, 14.72 %.

6. Biological activity

6.1. Materials and methods

The DMSO, ethylenediaminetetraacetic acid (EDTA), ethidium bromide (EtBr) and Bacteriophage plasmid, pBR322 DNA were purchased from Sigma Aldrich, USA. Stock solution of compounds (20 mg/ml) were prepared in DMSO and stored in brown containers in the refrigerator. All gel electrophoresis experiments were performed in TAE buffer (0.04 M Triseborate, 0.114% acetic acid and 50 mM EDTA, pH 8.0).

6.2. Photocleavage of plasmid DNA

The photoinduced DNA cleaving activities of **4a-g**, **5a-g**, **6a-g** and **7b-g** were assayed with a supercoiled, covalently closed, circular pBR322 double-stranded DNA in presence of UV light, which is a very sensitive molecular biology tool for detection of any changes in DNA. Stock solutions of test compounds were prepared by dissolving 0.01 g of compound in 0.5 ml of DMSO. The compounds **4a-g**, **5a-g**, **6a-g** and **7b-g** (0.5 µg) in DMSO were added separately to volume of 10 µl containing plasmid DNA pBR322 in 0.04 M Triseborate, 0.114% acetic acid and 50 mM EDTA at pH 8.0 buffer respectively. The same volume of DMSO used to make solutions of compounds, was added to the control. Polyethylene microcentrifuge tubes containing the reaction solutions were irradiated at room temperature for 45 min at 312 nm using an ultraviolet lamp (15 W).

Following irradiation, the reaction was stopped by removing the UV light and 5 ml of glycerol loading dye (containing 0.25% bromophenol blue in 30% glycerol) in TAE buffer was added and the resulting mixture was loaded onto a 1% agarose gel into gel cassette fitted with a comb. After solidification of gel, the comb was removed carefully and gel was placed in electrophoresis chamber containing TAE buffer. Electrophoresis was carried out for 1 h at 110 V and gel was incubated in 1% ethidium bromide (3,8-diamino-5-ethyl-6-phenylphenanthridinium bromide)

solution for 10 min. DNA in the gel was visualized by a UV transilluminator and photographs were taken with a digital photocamera.

6.3. Cytotoxic Evaluation

6.3.1. Cell culture: Human breast cancer cell lines (MCF-7 and BT-474) were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal serum albumin and 50 µg/mL of penicillin and streptomycin each. Human lymphocytic lukemia cell lines (MOLT-4 and SBALL) were cultured in Roswell Park Memorial Institute medium (RPMI) supplemented with 10% fetal serum albumin and 50 µg/mL of penicillin and streptomycin each. All cell lines were maintained in an incubator containing 5% CO_2 at 37°C.

6.3.2. Cell viability assay: Cells were seeded in a 96-well plate at a density of 1,00,000 per mL and grown overnight. Cells were treated with various compounds at a final concentration of 10 µM and incubated for 48 h. Cell viability assay was performed using a MTT cell proliferation kit from ATCC (American Type Culture Collection) (#30-1010K). In summary, 10 µL MTT reagent was added to each well, and cells were placed back in incubator for 4 h. 100 µL of detergent (from kit) was added and absorbance data was collected at 570 nm using Biotek synergy 2 spectrophotometer, Data was calculated as percentage of cell survival using the following formula:

$$\% \text{ Cell survival} = (100/A_t * A_s)$$

Where At and As are the absorbance of wells treated with test compounds and solvent control respectively.

Conflict of interest

There is no conflict of interest.

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