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Greener synthesis and DNA photocleavage activity of 1, 5-Diaryl-3-trifluoromethylpyrazole derivatives: †

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Abstract: 1,5-Diaryl-3-trifluoromethypyrazole derivatives have acquired much attention in the past few years due to their good biological potential. In the present communication, some 1,5-diaryl-3-trifluoromethylpyrazoles were synthesized by one-pot solid phase reaction. The DNA photocleavage study of the synthesized compounds was performed using agarose gel electrophoresis method. To have better insight of how our ligands interacted with the DNA, molecular docking simulations through Autodock Vina were also performed. All the compounds have shown good binding with the DNA. It has been found that pyrazoles containing phenyl rings at both positions (1 and 5) possessing electron releasing as well as electron withdrawing groups enhance the DNA photocleavage potential. As the synthesized derivatives shown the promising DNA photocleavage activity, so they may serve the basis of some bioactive heterocycles in the future.

Keywords: Trifluoromethypyrazole; Computational study; DNA photocleavage activity

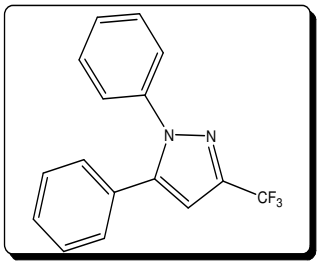
1. Introduction

Pyrazole and its derivatives are well known for their excellent biological properties [1-4]. In view of the importance of pyrazole nucleus, recently several synthetic routes have been reported in the literature. However, regardless of their potential synthetic utility, many of the reported methodologies suffer from certain drawbacks like harsh reaction conditions, complex product isolation procedures, application of hazardous organic solvents etc. [5, 6]. Therefore, the development of cost effective and eco-friendly chemical protocols for the preparation of biologically important heterocycles constitutes a major challenge for chemists in organic synthesis.

2. Materials and Methods

2.1 Synthesis of 1,5-diaryl-3-trifluoromethylpyrazoles (3a-l)

Most of the common chemicals used in the present study were purchased from commercial suppliers and were used without further purification. Melting points were taken on LabIndia visual melting range apparatus and are reported as such. ^{1}H NMR and ^{13}C NMR spectra were taken on Bruker Nuclear Magnetic Resonance (NMR) spectrometer instrument of 300 MHz and 75 MHz, using tetramethylsilane (TMS) as an internal standard in $CDCl_3$. However, β-diketones bearing trifluoromethyl **1** and aryl hyrazines **2** were prepared according to the well known procedure [7-9]. Compounds **1** (β-diketones bearing trifluoromethyl) (1 mmol), arylhydrazines **2** (1 mmol) and *p*-toluenesulphonic acid (2.5 mmol) were ground vigorously in a mortar using pestle. The contents were then transferred into a 250 ml conical flask and heated to about 50°C for 10-20 min. A yellow colored solid thus obtained was washed with water, filtered, dried and recrystallized from ethanol.



(3a)

Yield: 68 %;
m.p. 80°C (Lit. m.p. 79 °C);
^{1}H NMR ($CDCl_3$, 300 MHz,): δ = 6.75 (s, 1H, 4-H), 7.23-7.34 (m, 10 H, Ph'-H & Ph''-H)

Figure 1 *1,5-Diphenyl-3-trifluoromethylpyrazole*

2.2 DNA Photocleavage activity

DNA photocleavage experiment was performed by taking 10μl solution of *P*BR322 DNA in TE (*Tris* 10mM, EDTA 0.01mM, pH 8.0) buffer along with 40μg of synthesized compounds. The sample solutions held in caps of polyethylene microcentrifuge tubes were placed directly on the surface of a trans-illuminator (8000 m W/cm) at 360 nm and were irradiated for 30 min at room temperature. After UV-irradiation, samples were further incubated at 37° C for 1 hr. Irradiated samples were then mixed with 6x loading dye constituted from 0.25% bromophenol blue and 30% glycerol. The samples were then analyzed by electrophoresis on a 0.8% agarose horizontal slab gel in *Tris*-Acetate EDTA buffer (40 mM Tris, 20 mM acetic acid, 1 mM EDTA, pH: 8.0). Untreated plasmid DNA was maintained as a control in each run of gel electrophoresis which was carried out at 5V/cm for 2 hr. Gel was stained with ethidium bromide (1μg/ml) and photographed under UV light [10]

2.3 Docking studies

Molecular docking is a computational method to generate and score the probable protein-ligand complexes calculated on the basis of binding affinities. This technique is successfully used for discriminating compounds on the basis of their ability to fit in the binding sites of the protein [11,12]. The co-crystal complex structure of aB-DNA Dodecamer (PDB ID: 1BNA) was employed for the molecular docking studies.

The molecular docking simulations in case of 1,5-diaryl-3-trifluoromethylpyrazoles (**3a-l**) were performed using AutodockVina [13]. Autodock tools of Molecular Graphic Laboratory (MGL) were employed to generate the complete pdbqt files of receptor of ligands and receptors. Receptor generation and optimization was done in the following steps: (i) addition of polar hydrogens to the receptors, (ii) removing the water of crystallization from the complex, (iii) computaion of the Gasteiger Charges, and (iv) location of the grid box (Discovery Studio was employed to identify the coordinates of the centre of internal ligand in the binding site). The site of the Grid Box is given in Table 1. For the ligand files, PDB files drawn in Marvin sketch of Chemaxon were loaded in Autodock tools and converted to pdbqt files.

For initiating the molecular docking in AutodockVina, one configuration file is prepared which contain the information about the position of receptor file, ligand, coordinates of grid box and the size of the grid box which was set to 30 X 30 X 30 points.

Table 1. Position of the GRID box center in the protein molecule

PDB code	X, Y, Z coordination (Å)		
	X	Y	Z
1BNA	**5.7**	**-3.8**	**29.8**

3. Results and Discussion

3.1. Chemistry

Viewing a wide range of pharmaceutical activities of pyrazole and its derivatives, it was decided to synthesize some 1,5-diaryl-3-trifluoromethyl substitution based pyrazoles for biological interest. In the present communication, we disclosed the p-TSA (para-toluene sulphonic acid) mediated synthesis of 1, 5-diaryl and 3-trifluoromethyl based pyrazoles from the reaction of 1,3-dicarbonyl compounds with different hydrazines. General method for the synthesis of 1,5-diaryl-3-trifluoromethylpyrazoles (**3a-l**) is outlined in Figure 2, Table 2, which was accomplished by a grinding technique.

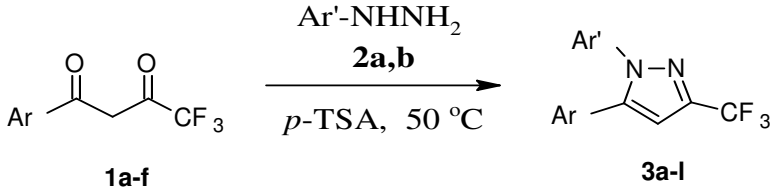


Figure 2 General method for synthesis of 1,5-diaryl-3-trifluoromethylpyrazoles (**3a-l**)

Table 2 1,5-diaryl-3-trifluoromethylpyrazole derivatives (**3a-l**)

Compound	Ar	Ar′	Compound	Ar	Ar′
3a	C_6H_5	C_6H_5	**3g**	C_6H_5	4-$NO_2C_6H_4$
3b	4-$OCH_3C_6H_4$	C_6H_5	**3h**	4-$NO_2C_6H_4$	4-$NO_2C_6H_4$
3c	4-$NO_2C_6H_4$	C_6H_5	**3i**	4-BrC_6H_4	4-$NO_2C_6H_4$
3d	4-BrC_6H_4	C_6H_5	**3j**	4-ClC_6H_4	4-$NO_2C_6H_4$
3e	4-ClC_6H_4	C_6H_5	**3k**	4-$OCH_3C_6H_4$	4-$NO_2C_6H_4$
3f	4-FC_6H_4	C_6H_5	**3l**	4-FC_6H_4	4-$NO_2C_6H_4$

It has been observed that condensation of β-diketones with hydrazines in the presence of p-TSA furnish the desired product rapidly (within 10-20 min). The known products were characterized by comparing their melting points with those reported in the literature [14]. A sharp singlet near δ 6.8 in ^{1}H NMR spectra confirms the formation of pyrazole nucleus. Furthermore, A sharp signal in ^{19}F NMR spectrum at δ -62.9 ppm also provides a firm evidence in support of the structure of 1,5-diaryl-3-triflouromethylpyrazoles (**3a-l**).

3.2. DNA Photocleavage study

The DNA photocleavage study of the synthesized compounds (**3a-j**) was performed using agarose gel electrophoresis method and results are presented in Figure 3. The decrease in the intensity of plasmid DNA in case of compounds **3a-j** in (Lane 1-10) as compared to control (Lane C) indicated the cleavage of DNA forms. Further, pyrazoles containing phenyl rings at both positions (1 and 5) possessing electron releasing as well as electron donating groups enhance the DNA photocleavage potential.

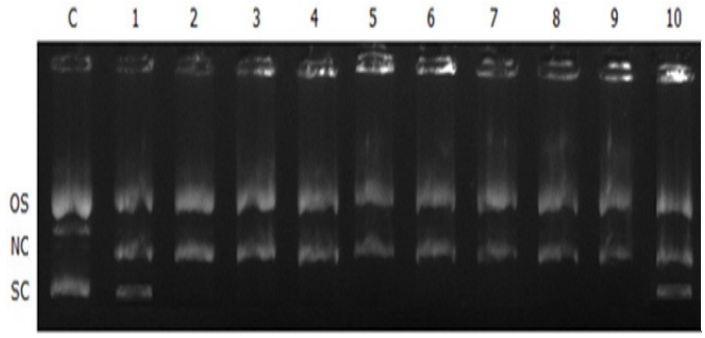


Figure 3 DNA Photocleavage study of 1,5-Diaryl-3-trifluoromethypyrazoles (**3a-j**) Lane C: Control plasmid DNA + UV + DMSO, Lane 1: DNA + 40μg **3a**, Lane 2: DNA + 40μg **3b,** Lane 3: DNA + 40μg **3c,** Lane 4: DNA + 40μg **3d,** Lane 5: DNA + 40μg **3e,** Lane 6: DNA + 40μg **3f,** Lane 7: DNA + 40μg **3g,** Lane 8: DNA + 40μg **3h,** Lane 9: DNA + 40μg **3i,** Lane 10: DNA + 40μg **3j**.

3.3 Docking study

With an aim to achieve some more support to our proposition and to find some indepth relationship with experimental *in vitro* DNA photcleavage activity, a versatile docking method was developed by using software Autodock Vina. All the compounds have shown good binding with the DNA, showing the binding energy in between -7.0 to -8.0 KJ/mol. The binding pose of one derivative **3a** with the DNA molecule is presented in the Figure 4.

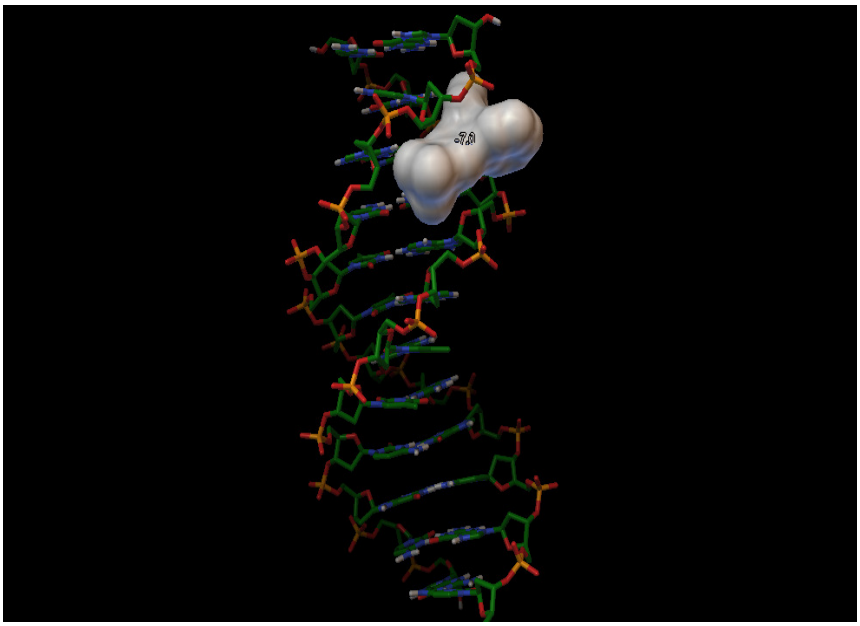


Figure 4 Molecular docked model of compound **3a** with DNA [dodecamer duplex of sequence d(CGCGAATTCGCG)2 (PDB ID: 1BNA)]

4. Conclusion

The present methodology overcomes the formation of unwanted isomeric byproducts, low yields, high temperature, and dry solvents. All the compounds have shown the promising DNA photocleavage activity and thus may serve the basis of some bioactive heterocycles in the future.

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Author Contributions: Vinod Kumar and Girish Kumar Gupta conceived and designed the experiments; Girish Kumar Gupta performed the experiments; Vinod Kumar and Girish Kumar Gupta analyzed the data; Vipin Saini contributed reagents/materials/analysis tools; Girish Kumar Gupta wrote the paper.

Conflicts of Interest: The authors declare no conflict of interest.

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