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SPECTROSCOPIC, COMPUTIONAL AND BIOLOGICAL EVALUATION OF NOVEL PYRAZOLE CARRYING PYRAZOLINE DERIVATIVES

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ABSTRACT
A new series of pyrazoline carrying pyrazole derivatives weresynthesizedand characterized by FT-IR, ^{1}H-NMR, ^{13}C-NMR and analytical data. All the synthesized compounds were screened for *in-silico*molecular docking analysis with *Hu*AR receptor. In addition, the *In-vitro* antioxidant and antibacterial activities were also performed. Compounds **4a**, **4e,** and**4f**exhibited significant antibacterial activity. Compounds **4e** and**4b** showed moderate radical scavenging activity.

KEYWORDS
Pyrazole, Pyrazoline, Docking studies, Antioxidant, Antibacterial activity.

INTRODUCTION
Developing a lead molecule and potent drug against the known targets has been challenging work in medicinal chemistry. Among the heterocyclic compounds pyrazole and pyrazoline nucleus stands unique due to its potency to inhibit many chronical diseases. Pyrazole and its derivatives have attracted the attention of chemists because of their wide range of pharmacological activity exhibited by them such asantibacterial [I],anti-inflammatory[II], analgesic [III], anticancer [IV], anti-convulsant [V], and anti-depressant activities [VI]. Similarly, the pyrazoline derivatives also exhibit a wide variety of biological activities [VII, IX]. These observations prompted us to synthesize some novel pyrazoline derivatives incorporated with active pharmacophores in a single molecular framework and to evaluate their biological activities. So in this paper, we report the synthesis of pyrazoline carrying pyrazole moiety by the reaction of various chalcones carrying pyrazole with hydrazine hydrate in the presence of acetic/propionic acid. All the synthesized compounds were tested for their *in-vitro*antioxidant and antibacterial activities. Further docking studies were carried out to evaluate the potency of the newly synthesized compounds as Human Androgen Receptor Modulators.

EXPERIMENTAL

MATERIALS AND METHOD

All the reagents and solvents were purchased from Sigma-Aldrich or Hi-Media and used after distillation/ recrystallization. ^{1}H NMR, ^{13}C-NMR spectra were recorded on Bruker Avance II NMR spectrometer operating at 400MHz and all the chemical shift values were reported in parts per million (ppm) relative to tetramethylsilane (TMS). Mass spectra were acquired on a SHIMADZU LCMS-8030 mass spectrometer. Melting points of the synthesized compounds were determined in open capillary tubes in Innovative DTC-967A digital melting point apparatus. SHIMADZU FT-IR 157 spectrophotometer was used for recording IR spectra. C H N analysis was performed with Vario-EI Elementar-III model analyzer.

General procedure for the synthesis of 1-(thiophene-2-yl)-3-(3-methyl-5-aryloxy-1-phenyl-1H-pyrazol-4-yl) prop-2-en-1-one (3 a-c)

To a mixture of 3-methyl-5-aryloxy-1-phenyl-1H-pyrazole-4-carbaldehyde (**1 a-c**) (10 mmol) and acetyl thiophene(**2**) (10 mmol) in ethanol (25 mL), potassium hydroxide (0.5g, 10 mmol) in ethanol was added dropwise under ice bath maintaining the temperature of the reaction mixture at 0-5 ^{o}C and the mixture was stirred for 4 hours. After the completion of the reaction (monitored by TLC), the solid product separated was filtered, washed with water, dried and recrystallized from ethanol: dimethylformamide mixture.

General procedure for the synthesis of 1-(5-(thiophene-2-yl)-3-methyl-5-aryloxy-1-phenyl-3,4-dihydro-1H, 2H-[3,4-bipyrazol]-2-yl)ethan/propan-1-one (4 a-f)

3-(3-Methyl-5-aryloxy-1-phenyl-1H-pyrazol-4-yl)-1-(thiophen-2-yl) prop-2-en-1-one (**3 a-c**)(10 mmol), hydrazine hydrate and acetic/ propionic acid(10 mmol) was taken in a round-bottomed flask and stirred at 80 ^{0}C for 17-24 hours. The resulting mixture was poured into ice-cold water (50 g) and allowed to stand at room temperature for 2 hours. The precipitate that separated was filtered, dried and recrystallized from ethanol: dimethylformamide mixture.

1-(5-(5-(p-Chlorophenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-3-(thiophen-2-yl)-4,5-dihydropyrazol-1-yl)ethanone (4a)

M.P: 149-152^{0}C, Yield: 68 %, CHN Analysis: Calc.C, 62.95; H, 4.44; N, 11.75, Found: C, 62.85; H, 4.48; N, 11.65, IR (υ_{max}, cm-^{1}): 1668 (C=O) , 1588 (C=N),3042 (C-H),1540 (C-O-C), 2844 (Ar-H),1650(C=C), ^{1}H NMR: 400 MHz, $CDCl_3$: δ , 2.1 (s, 3H, CO-CH_3 of pyrazoline), δ, 2.38 (s, 3H, -CH_3 pyrazole), δ, 3.2 (dd, J_{AX}= 5.1 Hz, J_{AB}=17.4 Hz, 1H, pyrazoline -CH_2), δ, 3.4 (dd , J_{BX}= 12.2 Hz, J_{BA}=17.4 Hz, 1H, pyrazoline -CH- chiral proton), δ, 5.42 (dd, J_{XA}=5.1 Hz, J_{XB}=12.4 Hz, 1H, H of pyrazoline CH_2), δ, 6.6 (m, 1H thiophene -4H), δ, 6.62(d, J=7.4 Hz, 2H ortho protons of p-chloro phenyl), δ, 6.76 (d, J= 3.6, 1H thiophene -2H), δ, 7.2(d, J= 7.4 Hz, 2H meta protons of p-chloro phenyl), δ, 7.4 (t, 1H, phenyl), δ, 7.6 (t, 2H, phenyl) δ, 7.82(m, 3H, 2H of phenyl and 1H of thiophene), ^{13}C NMR 100 MHz, $CDCl_3$: δ, 13.1 (CH_3), δ, 22.21 (CH_3,pyrazole ring), δ, 38.61 (CH_2,pyrazoline ring), δ, 48.68 (-CH, pyrazoline ring), δ, 155.46 (C=N), δ, 169.42 (C=O), Mass: m/z,477/479(M^+ +1)/ (M^+ + 3) (M.F. $C_{25}H_{21}ClN_4O_2S$).

1-(5-(5-(p-Chlorophenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-3-(thiophen-2-yl)-4,5-dihydropyrazol-1-yl)propan-1-one (4b)

M.P: 160-162^{0}C, Yield: 72%, CHN Analysis: Calc.C, 63.60; H, 4.72; N, 11.41. Found: C, 63.70; H, 4.82; N, 11.46, IR (υ_{max}, cm-^{1}): 1648 (C=O), 1482 (C=N),2940 (C-H),1300 (C-O-C), 2812 (Ar-H),1540(C=C),^{1}H NMR: 400 MHz, $CDCl_3$: δ, 1.1 (t, 3H, -CH_3 propyl), δ, 1.8 (q, 1H, of –CH_2-Propyl), δ, 2.1 (q, 1H, of –CH_2-Propyl), δ , 2.41 (s, 3H, Pyrazole –CH_3), δ , 3.14 (dd, J_{AX}= 5 Hz, J_{AB}=17.4 Hz, 1H, pyrazoline -CH_2), δ , 3.4 (dd , J_{BX}= 12.42 Hz, J_{BA}=17.4 Hz, 1H, 1H, pyrazoline -CH- chiral proton), δ, 5.24 (dd, J_{XA}=5 Hz, J_{XB}=12.56 Hz, 1H, H of pyrazoline CH_2), δ, 6.38(m, 1H thiophene-4H), δ, 6.5(d, J=7.1 Hz, 2H ortho protons of p-chloro phenyl), δ, 6.8(d, J= 3.9 Hz, 1H thiophene-3H), δ, 7.1(d, J= 7.2 Hz, 2H meta protons of p-chloro phenyl), δ, 7.42 (t, 1H, phenyl), δ, 7.7 (t, 2H, phenyl) δ, 8.1(m, 3H, 2H of phenyl and 1H of thiophene), ^{13}C NMR 100MHz, $CDCl_3$: δ, 11.68 (CH_3), δ, 14.42 (CH_2-), δ, 23.71 (CH_3,pyrazole ring), δ, 39.21 (CH_2,pyrazoline ring), δ, 49.19 (-CH, pyrazoline ring), δ, 156.6 (C=N), δ, 167.2 (C=O), Mass: m/z,492/494 (M^+ +1))/ (M^+ + 3) (M.F. $C_{26}H_{23}ClN_4O_2S$).

1-(5-(3-Methyl-5-(naphthalen-2-yloxy)-1-phenyl-1H-pyrazol-4-yl)-3-(thiophen-2-yl)-4,5-dihydropyrazol-1-yl)ethanone (4c)

M.P: 178-180 ^{0}C, Yield: 74 %, CHN Analysis: Calc. C, 70.71; H, 4.91; N, 11.37, Found: C, 70.61; H, 5.01; N, 11.28, IR (υ_{max}, cm-^{1}): 1672 (C=O) , 1544 (C=N),2978 (C-H),1470 (C-O-C), 2846 (Ar-H),1450(C=C), ^{1}H NMR: 400 MHz, $CDCl_3$: δ, 1.82 (s, 3H, CO-CH_3 of pyrazoline), δ, 2.47 (s, 3H, Pyrazole –CH_3), δ , 3.1 (dd, J_{AX}= 5.04 Hz, J_{AB}=17.28 Hz, 1H, pyrazoline -CH_2), δ , 3.6 (dd , J_{BX}= 12.28 Hz, J_{BA}=17.32 Hz, 1H, pyrazoline –CH- chiral proton), δ, 5.3 (dd, J_{XA}=5 Hz, J_{XB}=12.24 Hz, 1H, H of pyrazoline CH_2), 6.8-7.9(m, 15H, Ar-H of β- Napthyl, thiophene and phenyl), ^{13}C NMR 100 MHz, $CDCl_3$: δ, 13.2 (CH_3), δ, 23.8 (CH_3,pyrazole ring), δ, 38.1 (CH_2,pyrazoline ring), δ, 49.7 (-CH, pyrazoline ring), δ, 157.7(C=N), δ, 168.8 (C=O), Mass: m/z,493 (M^+ +1) (M.F. $C_{29}H_{24}N_4O_2S$).

1-(5-(3-Methyl-5-(naphthalen-2-yloxy)-1-phenyl-1H-pyrazol-4-yl)-3-(thiophen-2-yl)-4,5-dihydropyrazol-1-yl) propan-1-one (4d)

M.P: 172-175^{0}C, Yield: 77%, CHN Analysis: Calc.C, 71.12; H, 5.17; N, 11.06, Found: C, 71.22; H, 5.18; N, 11.18, IR (υ_{max}, cm-^{1}): 1670 (C=O), 1528 (C=N),3040 (C-H),1454 (C-O-C), 2874 (Ar-H),1356(C=C), ^{1}H NMR: 400 MHz, $CDCl_3$: δ , 1.15 (t, 3H, -CH_3-propyl), δ, 1.94 (q, 1H, of –CH_2 of Propyl), δ, 2.17 (q, 1H, of –CH_2 of Propyl),δ , 2.48 (s, 3H, Pyrazole –CH_3), δ , 3.3 (dd, J_{AX}= 5.04 Hz, J_{AB}=17.28 Hz, 1H, pyrazoline-CH_2), δ , 3.5 (dd , J_{BX}= 12.32 Hz, J_{BA}=17.28 Hz, 1H, pyrazoline –CH-chiral proton), δ, 5.41 (dd, J_{XA}=5 Hz, J_{XB}=12.28 Hz, 1H, H of pyrazoline CH_2), 6.89-7.71(m, 15H, thiophene and phenyl protons), ^{13}C NMR 100 MHz, $CDCl_3$: δ, 11.8 (CH_3), δ, 12.6 (CH_2-), δ, 23.8 (CH_3,pyrazole ring), δ, 40.21 (CH_2,pyrazoline ring), δ, 48.8 (-CH, pyrazoline ring), δ, 158.4 (C=N), δ, 167.3 (C=O), Mass: m/z,507 (M^+ +1) (M.F. $C_{30}H_{26}N_4O_2S$).

1-(5-(5-(2,4-Dichlorophenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-3-(thiophen-2-yl)-4,5-dihydropyrazol-1-yl)ethanone (4e)

M.P:147-148^{0}C, Yield: 88 %,CHN Analysis: Calc.C, 58.71; H, 3.94; N, 10.96, Found: C, 58.74; H, 3.96; N, 10.94, IR (υ_{max}, cm-^{1}): 1650 (C=O), 1544 (C=N),2942 (C-H),1448 (C-

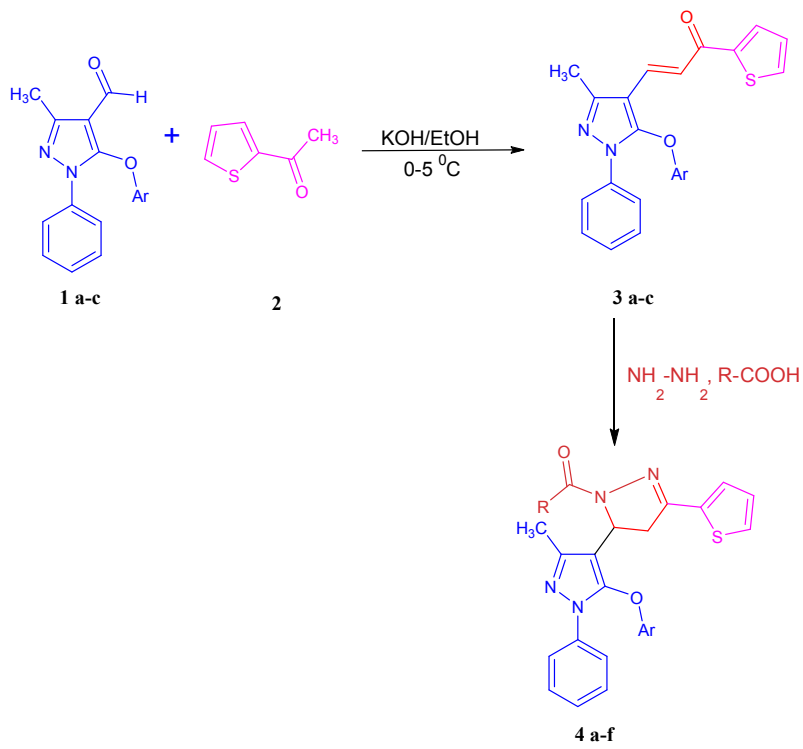
O-C), 2980(Ar-H),1556 (C=C), ^{1}H NMR: 400 MHz, δ , 2.1 (s, 3H, CO-CH_3 of pyrazoline), δ, 2.4 (s, 3H, Pyrazole –CH_3), δ , 3.3 (dd, J_{AX}= 5.6 Hz, J_{AB}=17.4 Hz, 1H, pyrazoline –CH_2), δ , 3.5 (dd, J_{BX}= 12.2 Hz, J_{BA}=17.44 Hz, 1H, pyrazoline –CH- chiral proton), δ, 5.4 (dd, J_{XA}=5.6 Hz, J_{XB}=12.44 Hz, 1H, H of pyrazoline CH_2), δ, 6.44-6.72(m, 11H, Ar-H of 2, 4-dichloro phenyl, thiophene and phenyl), ^{13}C NMR 100 MHz, $CDCl_3$: δ, 12.8 (CH_3), δ, 23.7 (CH_3,pyrazole ring), δ, 39.1 (CH_2,pyrazoline ring), δ, 48.7 (-CH, pyrazoline ring), δ, 157.9(C=N), δ, 174.6 (C=O), Mass: m/z,512/514/516 (M^+ +1) (M^+ +3) (M^+ +5) (M.F. $C_{25}H_{20}Cl_2N_4O_2S$).

1-(5-(5-(2,4-Dichlorophenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-3-(thiophen-2-yl)-4,5-dihydropyrazol-1-yl)propan-1-one (4f)

M.P: 158-160^{0}C, Yield: 87%,CHN Analysis: Calc.C, 59.43; H, 4.22; N, 10.66, Found: C, 59.44; H, 4.28; N, 10.68, IR (υ_{max}, cm-^{1}): 1648 (C=O), 1554 (C=N),3040 (C-H),1460(C-O-C), 2818 (Ar-H),1542(C=C), ^{1}H NMR: 400 MHz, $CDCl_3$: δ , 1.2 (t, 3H, -CH_3-propyl), δ, 1.98 (q, 1H, of –CH_2-Propyl), δ, 2.26 (q, 1H, of –CH_2-Propyl),δ , 2.38 (s, 3H, Pyrazole –CH_3), δ , 3.4 (dd, J_{AX}= 5.4 Hz, J_{AB}=17.4 Hz, 1H, pyrazoline –CH_2), δ , 3.6 (dd, J_{BX}= 12.32 Hz, J_{BA}=17.44 Hz, 1H, pyrazoline –CH- chiral proton), δ, 5.4 (dd, J_{XA}=5.44 Hz, J_{XB}=12.28 Hz, 1H, H of pyrazoline CH_2), δ, 6.44-7.7(m, 11H, Ar-H of 2, 4-dichloro phenyl, thiophene and phenyl), ^{13}C NMR 100 MHz, $CDCl_3$: δ, 12.8 (CH_3), δ, 12.7 (CH_2-), δ, 24.2 (CH_3, pyrazole ring), δ, 42.1 (CH_2,pyrazoline ring), δ, 48.7 (-CH, pyrazoline ring), δ, 158.4 (C=N), δ, 172.4 (C=O), Mass: m/z,526/528/530 (M^+ +1) (M^+ +3) (M^+ +5) (M.F. $C_{26}H_{22}Cl_2N_4O_3$).

RESULTS AND DISCUSSION

The synthesis of compound **1a-c** to **4a-c** is as shown in **Scheme 1**. 1-(Thiophene-2-yl)-3-(3-methyl-5-aryloxy-1-phenyl-1H-pyrazol-4-yl) prop-2-en-1-one **3 a-c**was synthesized by the reaction of substituted pyrazole aldehyde **1 a-c** with 2-acetyl thiophene **2** in alcohol medium employing potassium hydroxide (KOH) as the catalyst at 0-5 ^{0}C.When these propenones**3 a-c** were treated with hydrazine hydrate in presence of aliphatic acid, gave 1-acetyl/propanoyl -3-thiophene-5-(5-aryloxy-3-methyl-1-phenyl-1H-pyrazol-4-yl)-2-pyrazolines **4 a-f** ingood yield.



Ar = p-chloro phenyl, β-naphthyl,2, 4 dichloro phenyl, R=CH_3, -C_2H_5

Scheme 1: 1-Acetyl/propanoyl -3-thiophene-5-(5-aryloxy-3-methyl-1-phenyl-1H-pyrazol-4-yl)-2-pyrazolines (**4 a-f**)

Table 1: Characterization data of 1-acetyl/propanoyl-3-(thiophene-2-yl)-5-(5- aryloxy-3-methyl-1-phenyl- 1H-pyrazol-4-yl)-2-pyrazolines (**4a-f**)

Compound	Ar	R	M.P (^{0}C) (Mol. Wt)	Yield (%)	Colour and crystal nature
4a	p-chlorophenyl	CH_3	149-152 (476)	68	White amorphous micro crystals
4b	p-chloro phenyl	CH_2-CH_3	160-162 (491)	72	Cream white amorphous crystals
4c	β- naphthyl	CH_3	178-180 (492)	74	Brown amorphous microcrystals
4d	β- naphthyl	CH_2-CH_3	172-175 (506)	77	Buff colouredamorphous micro crystals
4e	2,4-di-chloro phenyl	CH_3	147-148 (511)	88	Light brown crystalline solid
4f	2,4-di-chloro phenyl	CH_2-CH_3	158-160 (525)	87	Cream white micro crystals

Molecular Docking

The three-dimensional ligand structures of **(4a-f)**were prepared, protonation states were assigned, and low-energy three-dimensional conformations were generated with CORINA [X].

The ligand (pyrazole) was extracted and ligand defined for the binding site using GOLD Suite [XI].

The *in silico* analysis was undertaken for compounds (**4 a-f**) with *Hu*AR. Compounds **4 a-f** (except **4a**) makes a good interaction with the human androgen receptor through hydrogen bonds and the results are summarized in **Table. 2**. Among the set of tested compounds, compounds **4a (**22.96**)**and **4b (**19.01**)** showed the highest chem score values, whereas compound **4d** had the least chem score value (8.43). The morenumber of hydrogen bonds (two bonds) interaction with *HuAR*were found with **4c, 4d**and**4e.** From the results obtained by the studies, pyrazoline hybrid carrying mono chloro substituted atom in the aryloxy moiety (**4a**and **4b**) showed minimum binding interaction with amino acids compared to those carrying β-napthyloxy and 2,4 di-chlorophenxoy moiety.

Table. 2*In Silico* Docking Results for compounds 4 a-f with *HuAR*

Compound No	Chem Score	Number of Hydrogen Interaction	Amino acid residues of *HuAR* in interaction with docked compounds
4a	22.96	-	-
4b	19.01	1	Thr200
4c	13.70	2	Thr200
4d	8.43	2	Thr200, Leu34
4e	14.26	2	Thr200
4f	14.53	1	Thr200

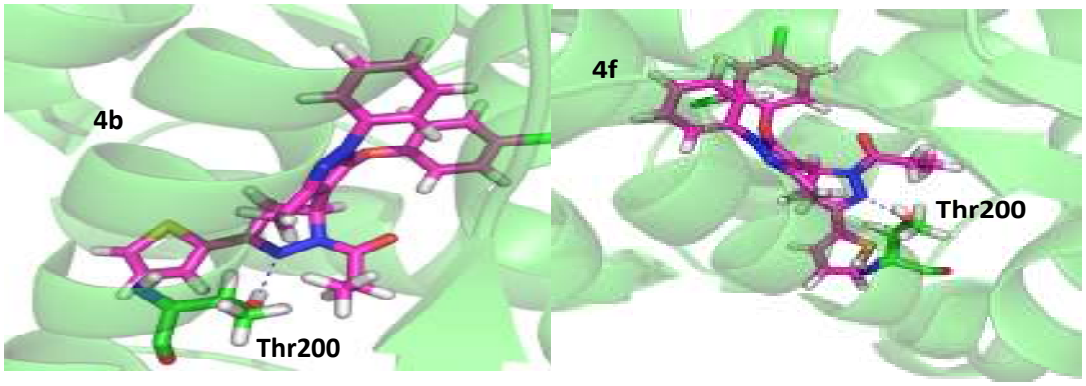


Figure 1:4b and **4f** (magenta stick model) docked with *Hu*AR (green ribbon model), **4b** and **4f**are interacting with Thr200 (green stick model. Blue dotted lines suggest hydrogen bonding.

ANTIBACTERIAL STUDIES

Bacterial strains were purchased from National collection of industrial microorganisms, Pune, India. Newly synthesized compounds were tested for their antibacterial activity against Gram-positive bacteria *Staphylococcus aureus* (NCIM - 5021), *Bacillus subtilis* (NCIM 2197)and Gram-negative bacteria *Escherichia coli* (NCIM-2931)*, Pseudomonas aeruginosa* (NCIM-2036) by using cup plate method. [XII, XIII] Ciprofloxacin was used as the reference drug. Compounds **4a, 4e,** and **4f** exhibited significant antibacterial activity against *Bacillus subtilis*, *Staphylococcus aureus,*and Pseudomonas aeruginosa. Rest of the compounds displayed comparatively moderate activity (**Fig. 2).**

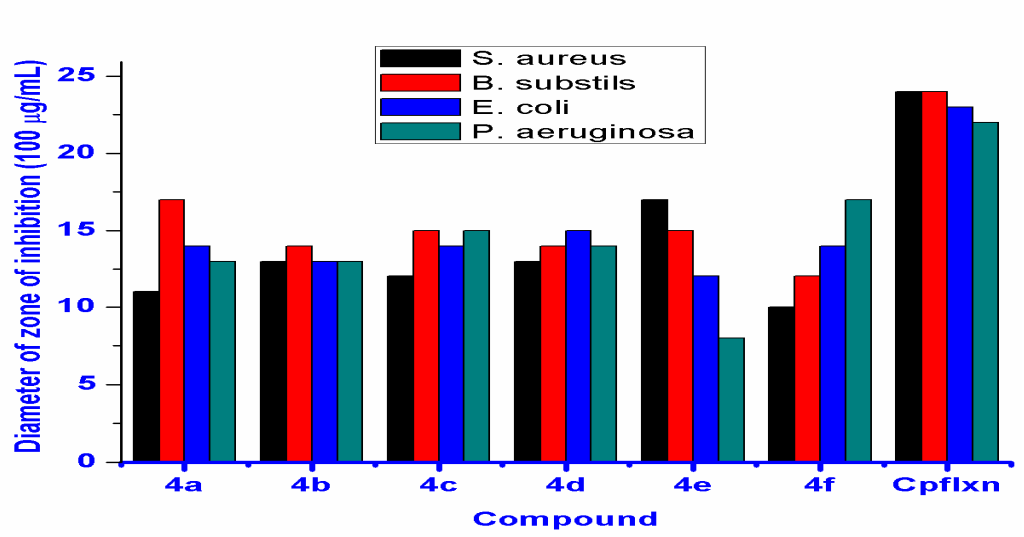


Figure 2: Antibacterial activity of pyrazoline carrying pyrazole **(4 a-f)**

ANTIOXIDANT STUDIES

Free radical scavenging activity of the synthesized compounds (**4 a-f**) was carried out as per the reported procedure.[XIV] The absorbance of stable DPPH radical was measured at 517 nm. Radical scavenging activity was expressed as the inhibition percentage and was calculated using the equation of DPPH radical scavenging activity and the results are presented in **Fig. 3.**

$$\text{DPPH radical scavenging activiy (\%)} = \frac{(\text{Abc} - \text{Abs})}{(\text{Abc})} \times 100$$

Where Abc is the absorbance of DPPH radical + methanol; Abs is the absorbance of DPPH radical + test sample/standard BHA.

Antioxidant study results showed that pyrazoline hybrids are moderate DPPH radical scavengers. Particularly pyrazoline hybrids **4e** (44.08) and **4b** (40.0) carrying 2, 4 - dichlorophenoxy and 4-chlorophenoxy in the pyrazole ring exhibited moderate radical scavenging activity among the tested compounds.

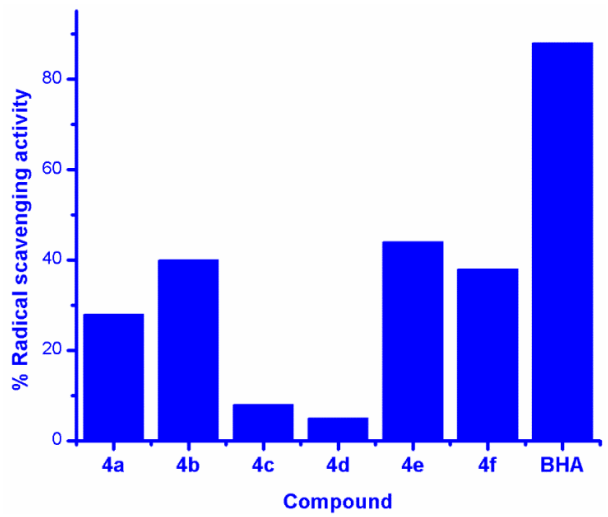


Figure 3: DPPH scavenging activity of DPPH free radical scavenging activity of pyrazoline carrying pyrazole **(4 a-f).**

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