

Synthesis and Evaluation of Some Novel Pyrazoles for Antibacterial and Antioxidant Activity

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Abstract

A series of novel pyrazoles were synthesized starting from 4-fluoro-3-chloroaniline by condensation with aromatic aldehydes to give chalcones which were cyclized with hydrazine to obtain pyrazoles. The chlorine was replaced with various aromatic amines to obtain the final derivatives. Evaluation of antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* highlights the importance of methyl substitution and requirement of high lipophilicity. Ferric ion reduction studies indicate the requirement of electron releasing aromatic substitutions for enhanced antioxidant activity.

Key words : Pyrazoles, *Staphylococcus aureus*, *Escherichia coli*, Ferric ion, antioxidant activity.

Introduction

Every individual or human being desires to live a healthy and respectful life. However, human suffering has become synonymous with the rapid advancements made in technology and travel. Rapid growth in population with great mobility of people for economic gains has led to a spread of infectious diseases across regions. Reports state that there are 17 infectious diseases, which include

the likes of leprosy, tuberculosis, malaria, leishmaniasis etc., to be endemic in 149 countries, with over one billion people being exposed to them¹. Tropical countries are prone to certain common infections like malaria, dengue, chikungunya, filariasis, tuberculosis, leprosy, leishmaniasis, typhoid etc². An area of concern is the development of resistance to antibiotics. Some of the well documented drug resistant strains are methicillin resistant *Staphylococcus aureus* (MRSA), *Escherichia*

coli ST131 and *Klebsiella* ST258³. Extremely stressful life and the enormous level of pollution that one is exposed to has affected the health of the common man. Free radicals are extensively produced in the body due to various chemo stimuli. These reactive oxygenated species are known to damage various tissues in the body. The role of antioxidants in scavenging these free radicals is well known⁴. Suitably substituted pyrazole heterocycles are important drug candidates possessing a variety of pharmacological activities. Literature reports indicate that pyrazole derivatives are active as antibacterials^{5,6}, antifungal⁷, antitubercular⁸, xanthine oxidase inhibitors⁹, antiinflammatory agents as COX inhibitors¹⁰, anticancer¹¹ among others.

Material and Methods

Chemistry:

The general chemicals and reagents were procured from Loba or SD Fine chem and of laboratory reagent grade. Fluoro chloroaniline was procured from a bulk drug supplier at Hyderabad. Wherever necessary, the chemicals were purified before use by distillation or recrystallization. The melting points were determined by open capillary method and are uncorrected. Infrared (IR) spectra were recorded using KBr pellets on a Shimadzu 8400S FTIR. Proton NMR were recorded on a Bruker 300 MHz instrument and with reference to internal standard tetramethyl silane (TMS).

Synthesis of Fluoro chloroacetanilide (2)

In a 100 mL round bottomed flask was

placed 7.3 g (0.05 mole) of 4-fluoro-3-chloroaniline (**1**), 25 mL acetic acid and 15 mL of acetic anhydride. The mixture was heated on a water bath for 2h, cooled and poured into 100 g of crushed ice with rapid stirring. A white solid that separated was filtered and dried over air and then in an oven at 105 °C for 1 hour. The product (**2**) was recrystallized from ethanol.

Melting range: 114 – 118 °C; FTIR (KBr, V_{max} , cm^{-1}): 824 (C – Cl *str*), 1214 (C – F *str*), 1618 (N – H *def*), 1735 (C = O *str*), 3213 (N – H *str*). ¹H NMR in CDCl₃ (values in δ ppm): 2.15(3H, s, COCH₃), 4.59(1H, s, ArNH), 6.41-6.50(1H, m, Ar-H), 6.64-6.73(1H; dd, Ar-H), 6.87-6.96(1H; app t, Ar-H).

Synthesis of Chalcone (4a and 4b)

In a 250 mL round bottomed flask was dissolved 4.7 g (0.025 mol) of 4-fluoro-3-chloroacetanilide (**2**) in 50 mL of rectified spirit. To the solution was added drop wise 20 mL of 30% w/v KOH solution and the resulting mixture magnetically stirred for 2h. To this was added 0.025 mole of the aldehyde (3a -3b) and the reaction refluxed between 10 -14h, cooled and poured into 100 mL of ice cold water and transferred to a separating funnel and extracted twice, each 25 mL portions of ethyl acetate to remove any unreacted aldehyde. The aqueous layer acidified to a pH of 6 with dilute hydrochloric acid during which the time a solid separated. The solution filtered and the product (4a – 4b) dried in an oven at 105 °C for 1h, recrystallized with ethanol.

1 – Phenyl – N – (3 – chloro – 4 – fluorophenyl) propenamide 4a

Melting range: 110 – 112 °C; FTIR (KBr, V_{max} , cm^{-1}): 1604 (N – H *def*), 1630 (Ar C = C *str*), 1742 (C = O *str*), 3375, 3475 (N – H *str*). ^1H NMR in CDCl_3 (values in δ ppm): 6.41-6.43(1H, d, vinylic-H), 6.74-6.76(1H; d, vinylic-H), 6.97-7.84 (8H; m, Ar-H), 8.04 (1H, s, ArNH).

1 – (4 – Methoxyphenyl) – N – (3 – chloro – 4 – fluorophenyl) propenamide 4b

Melting range: 141 – 142 °C; FTIR (KBr, V_{max} , cm^{-1}): 947 (C-F *str*), 1055 (C-O *str*), 1593 (Ar C = C *str*), 1693 (N-H *def*), 1734 (C = O *str*), 3377 (N – H *str*). ^1H NMR in CDCl_3 (values in δ ppm): 3.91 (3H, s, $-\text{OCH}_3$), 6.38-6.40(1H, d, vinylic-H), 6.64-6.66(1H; d, vinylic-H), 6.90-7.68 (7H; Ar-H), 8.40 (1H, s, ArNH),

Construction of pyrazole ring

In a 100 mL round bottomed flask was dissolved the chalcone, 4a or 4b (0.02 mol) in 25 mL of ethanol and added 5 mL hydrazine hydrate. The resulting solution was refluxed for 6 – 10h, cooled and poured into crushed ice. The solid that separated was filtered. The reaction was monitored by TLC. The crude product was recrystallized from ethanol to obtain pyrazole intermediates 5a – 5b.

N-(3 – Chloro – 4 – fluorophenyl) – 3 – amino – 5 – phenyl pyrazole 5a

Melting range: 103 – 105 °C; FTIR

(KBr, V_{max} , cm^{-1}): 841 (C-Cl *str*), 1041 (C-F *str*), 1204 (C-O *str*), 1586 (Ar C = C *str*), 1690 (N-H *def*). ^1H NMR in CDCl_3 (values in δ ppm): 4.91 (1H, s, amine NH), 5.91 (1H, s, Pyrazole NH), 6.60-7.80 (9H; Ar-H), 7.98 (1H, s, amine NH).

N-(3 – Chloro – 4 – fluorophenyl) – 3 – amino – 5 – (4 – methoxyphenyl) pyrazole 5b

Melting range: 113 – 115 °C; FTIR (KBr, V_{max} , cm^{-1}): 823 (C-Cl *str*), 1051 (C-F *str*), 1195 (C-O *str*), 1591 (Ar C = C *str*), 1695 (N-H *def*). ^1H NMR in CDCl_3 (values in δ ppm): 3.86 (3H, s, $-\text{OCH}_3$), 6.01 (1H, s, Pyrazole NH), 6.64-7.68 (8H; Ar-H), 7.95 (1H, s, amine NH).

General procedure for substitution of chlorine.

In a dry 25 mL 2-necked round bottom flask fitted with a condenser and a nitrogen inlet assembly was placed 5a or 5b (0.001 mol). To this was added 10 mL of dry solvent, 4 equivalents of anhydrous potassium carbonate, 1-4 equivalents of 6 (a-j), 1-2.5 mol% cuprous oxide or cuprous iodide as catalyst. The entire assembly was flushed twice with dry nitrogen and the reaction mixture was refluxed for 16 – 24h under nitrogen atmosphere. The reaction monitored by TLC. After the completion of reaction, the mixture was cooled and poured into 100 mL of ice cold water, extracted with 3 x 20 mL portions of ethyl acetate. The combined organic layer was dried over anhydrous sodium sulphate and evaporated in vacuum to obtain the desired products (7a-j & 8a -j). Purification

done by column chromatography on silacgel # 80/120, gradient elution with hexane: ethyl acetate as solvent system.

3 – [N – (4 – fluoro – 3 – phenylamino) phenyl]amino – 5 – phenylpyrazole.7a

Melting range: 112 – 114 °C; FTIR (KBr, V_{max} , cm^{-1}): 1001 (C – F *str*): 1369 (C – N *str*), 1626 (N – H *def*), 1637 (Ar C = C *str*), 3412 (N – H *str*). ^1H NMR in CDCl_3 (values in δ ppm): 6.99 (1H, s, Ar-NH), 7.09 – 8.05 (14H; m, Ar-H), 8.27 (1H; s, NH), 8.41 (1H; s, NH).

3 – [N – (4 – fluoro – 3 – (2-chlorophenyl) amino)phenyl]amino – 5 – phenylpyrazole.7b

Melting range: 124 – 126 °C; FTIR (KBr, V_{max} , cm^{-1}): 980 (C – F *str*): 1371 (C – N *str*), 1636 (N – H *def*), (Ar 3401 (N – H *str*).

3 – [N – (4 – fluoro – 3 – (2-nitrophenyl) amino)phenyl]amino – 5 – phenylpyrazole.7c

Melting range: 150 – 152 °C; FTIR (KBr, V_{max} , cm^{-1}): 1045 (C – F *str*): 1385 (C – N *str*), 1430 (NO_2 *asym.str*), 1543 (NO_2 *sym.str*), 1626 (N – H *def*), 3385 (N – H *str*).

3 – [N – (4 – fluoro – 3 – (2-methylphenyl) amino)phenyl]amino – 5 – phenylpyrazole.7d

Melting range: 160 – 162 °C; FTIR (KBr, V_{max} , cm^{-1}): 1103 (C – F *str*), 1608 (N – H *def*), 2960 (C – H *str*), 3299 (N – H *str*).

3 – [N – (4 – fluoro – 3 – (3-chlorophenyl) amino)phenyl]amino – 5 – phenylpyrazole.7e

Melting range: 210 – 212 °C; FTIR (KBr, V_{max} , cm^{-1}): 1115 (C – F *str*), 1645 (N – H *def*), 2960 (C – H *str*), 3301 (N – H *str*).
3 – [N – (4 – fluoro – 3 – (3-nitrophenyl) amino)phenyl]amino – 5 – phenylpyrazole.7f

Melting range: 200 – 202 °C; FTIR (KBr, V_{max} , cm^{-1}): 1110 (C – F *str*), 1445 (NO_2 *asym.str*) 1551 (NO_2 *sym.str*), 1608 (N – H *def*), 2967 (C – H *str*), 3250 (N – H *str*).

3 – [N – (4 – fluoro – 3 – (3-methylphenyl) amino)phenyl]amino – 5 – phenylpyrazole.7g

Melting range: 208 – 210 °C; FTIR (KBr, V_{max} , cm^{-1}): 980 (C – F *str*), 1665 (N – H *def*), 2942 (C – H *str*), 3345 (N – H *str*).

3 – [N – (4 – fluoro – 3 – (4-chlorophenyl) amino)phenyl]amino – 5 – phenylpyrazole.7h

Melting range: sticky mass; FTIR (KBr, V_{max} , cm^{-1}): 1080 (C – F *str*), 1635 (N – H *def*), 2954 (C – H *str*), 3345 (N – H *str*).

3 – [N – (4 – fluoro – 3 – (4-nitrophenyl) amino)phenyl]amino – 5 – phenylpyrazole.7i

Melting point: 150 °C; FTIR (KBr, V_{max} , cm^{-1}): 1070 (C – F *str*), 1390 (NO_2 *asym.str*), 1516 (NO_2 *sym.str*), 1647 (N – H *def*), 3475 (N – H *str*).

3 – [N – (4 – fluoro – 3 – (4-methylphenyl) amino)phenyl]amino – 5 – phenylpyrazole.7j

Melting range: sticky mass; FTIR (KBr, V_{max} , cm^{-1}): 1074 (C – F *str*), 1645 (N – H *def*), 2995 (C – H *str*), 3331 (N – H *str*).

3 – [N – (4 – fluoro – 3 – phenylamino) phenyl]amino – 5 – (4 – methoxyphenyl) pyrazole.8a

Melting point: 180 °C; FTIR (KBr, V_{max} , cm^{-1}): 1008 (C – F *str*), 1130 (C–O *str*), 1647 (N – H *def*), 3475 (N – H *str*). ^1H NMR in CDCl_3 (values in δ ppm): 3.90 (3H, s, OCH₃), 6.82 - 6.84 (2H, d, Ar-H), 7.29 – 7.37 (4H; m, Ar-H), 7.70 – 7.76 (4H; m, Ar-H), 7.88 – 7.92 (4H; m, Ar-H), 7.99 (1H; s, Ar-NH), 8.27 (1H; s, NH), 8.41 (1H; s, NH).

3 – [N – (4 – fluoro – 3 – (2-chlorophenyl) amino)phenyl]amino– 5 – (4 – methoxyphenyl) pyrazole.8b

Melting point: 195 °C; FTIR (KBr, V_{max} , cm^{-1}): 1035 (C – F *str*), 1095 (C –O *str*), 1647 (N – H *def*), 3475 (N – H *str*).

3 – [N – (4 – fluoro – 3 – (2-nitrophenyl) amino)phenyl]amino– 5 – (4 – methoxyphenyl) pyrazole.8c

Melting point: 220 °C; 1035 (C – F *str*), 1095 (C –O *str*), 1386 (NO₂ *asym.str*), 1535 (NO₂ *sym.str*), 1647 (N – H *def*), 3475 (N – H *str*).

3 – [N – (4 – fluoro – 3 – (2-methylphenyl) amino)phenyl]amino– 5 – (4 – methoxyphenyl) pyrazole.8d

Melting range: 110 – 112 °C; FTIR

(KBr, V_{max} , cm^{-1}): 1008 (C – F *str*), 1140 (C – O *str*), 1640 (N – H *def*), 3358 (N – H *str*).

3 – [N – (4 – fluoro – 3 – (3-chlorophenyl) amino)phenyl]amino– 5 – (4 – methoxyphenyl) pyrazole.8e

Melting range: low melting; FTIR (KBr, V_{max} , cm^{-1}): 1005 (C – F *str*), 1121 (C – O *str*), 1610 (N – H *def*), 3300 (N – H *str*).

3 – [N – (4 – fluoro – 3 – (3-nitrophenyl) amino)phenyl]amino– 5 – (4 – methoxyphenyl) pyrazole.8f

Melting range: 163 – 165 °C; FTIR (KBr, V_{max} , cm^{-1}): 1021 (C – F *str*), 1087 (C – O *str*), 1412 (NO₂ *asym.str*), 1541 (NO₂ *sym.str*), 1635 (N – H *def*), 3387 (N – H *str*).

3 – [N – (4 – fluoro – 3 – (3-methylphenyl) amino)phenyl]amino– 5 – (4 – methoxyphenyl) pyrazole.8g

Melting range: 93 - 95 °C; FTIR (KBr, V_{max} , cm^{-1}): 1005 (C – F *str*), 1087 (C – O *str*), 1605 (N – H *def*), 3289 (N – H *str*).

3 – [N – (4 – fluoro – 3 – (4-chlorophenyl) amino)phenyl]amino– 5 – (4 – methoxyphenyl) pyrazole.8h

Melting range: 135 °C; FTIR (KBr, V_{max} , cm^{-1}): 1021 (C – F *str*), 1104 (C –O *str*), 1687 (N – H *def*), 3357 (N – H *str*).

3 – [N – (4 – fluoro – 3 – (4-nitrophenyl) amino)phenyl]amino– 5 – (4 – methoxyphenyl) pyrazole.8i

Melting range: 136 – 138 °C; FTIR (KBr, V_{max} , cm^{-1}): 1047 (C – F *str*), 1115 (C – O *str*), 1335 (NO₂ *asym.str*), 1535 (NO₂ *sym.str*), 1637 (N – H *def*), 3410 (N – H *str*).

3 – [N – (4 – fluoro – 3 – (4-methylphenyl) amino)phenyl]amino– 5 – (4 – methoxyphenyl) pyrazole.8j

Melting range: 162 – 166 °C; FTIR (KBr, V_{max} , cm^{-1}): 1001 (C – F *str*), 1098 (C – O *str*), 1641 (N – H *def*), 3365 (N – H *str*). ¹H NMR in CDCl₃ (values in δ ppm): 1.52 (3H, s, Ar-CH₃), 3.77 (1H; s, NH), 4.18 (3H, s, OCH₃), 5.30 (1H; s, NH), 6.61 - 6.84 (6H, m, Ar-H), 7.17- 7.25 (6H; m, Ar-H), 8.02 (1H; s, NH)

Antibacterial activity :

The newly synthesized compounds were screened for their antibacterial activity *in vitro* against *Staphylococcus aureus* ATCC 6538 and *Escherichia coli* k12 by Kirby-Bauer disc diffusion method¹². The inoculums were prepared in 10 ml peptone water (peptone 10g, NaCl 5g and water q.s 1000 ml, adjusted to pH 7.2 and sterilized at 15 psi for 15 mins) and incubated at 37 p C for 3-4h. The turbidity of the suspension adjusted to Mc.Farland standard by dilution with isotonic saline. Mueller Hinton agar media poured into the petriplates, sterilized at 121 p C, 20 psi for 11 minutes and the top layer streaked with the inoculum using a cotton swab. 5 mm paper discs (Whatman #1) impregnated with a solution (prepared in DMSO) equivalent to 50/100 μ g of sample or standard ampicillin were placed in the petriplates and incubated for 24h. The zone of inhibition measured and the values

recorded as a mean of 6 readings (table 1).

Ferric ion Reduction (Antioxidant) Activity¹³:

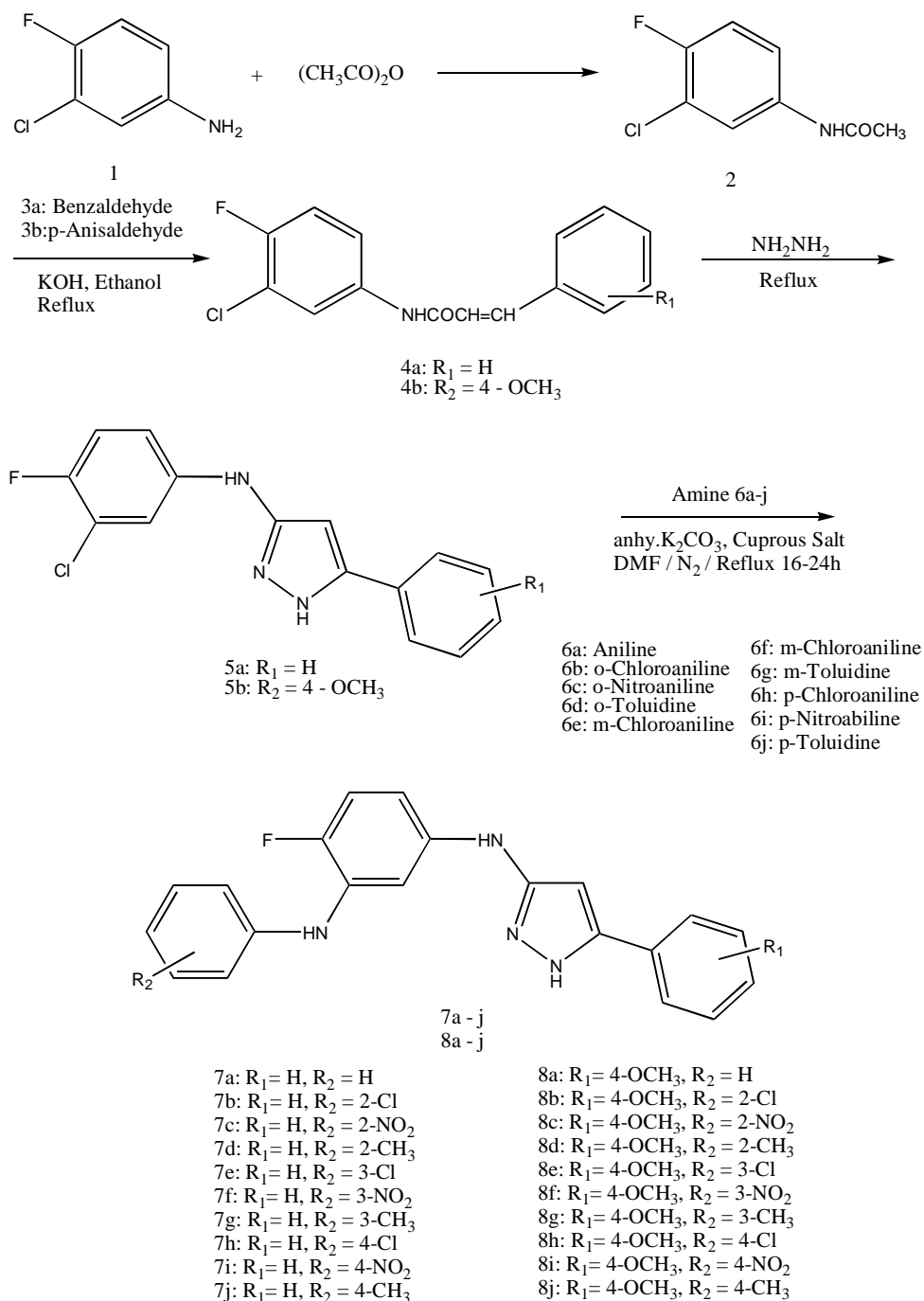
In a screw capped test tube were placed 1 mL of 1 millimolar ferric ammonium sulphate solution, 1 mL of test (1 millimolar in acetone) or standard (1 millimolar sodium dithionate) or blank (acetone), 2.5 mL of 1,10-phenanthroline solution (180 mg in 1000 mL) and added 0.5 mL of acetone to make a final volume of 5 mL. Each of the test tubes mentioned above was allowed to stand at room temperature for 15 min and the absorbance recorded at 510 nm using a double beam spectrophotometer. The readings recorded were auto corrected for blank absorbance. Each test or standard reading was recorded in triplicate and the values given as mean (table 2). Absorbance due to standard was considered equivalent to 100% reduction of all the ferric ions present. The antioxidant activity was calculated using the formula:

$$\% \text{ Anti-oxidant activity} = [A_T / A_S] \times 100$$

Where A_T is the absorbance of test and A_S is absorbance of standard.

Computational studies :

Structures of test molecules were drawn by molecular editor at web based molinspiration.com and corresponding ‘Smiles’ for each molecule determined. The physicochemical parameters: calculated log P (cLogP), total polar surface area (TPSA), hydrogen bonding acceptors (OHNH), number of rotatable bonds (RB), volume of molecule for each molecule found using the ‘smiles.’ (Table 3).



Scheme 1: Synthesis of substituted pyrazoles from 4-fluoro-3-chloroaniline.

Table 1. Anti bacterial activity of newly synthesized compounds, zone of inhibition in mm

Compound No	<i>Staphylococcus aureus</i>		<i>Escherichia coli</i>	
	50 µg	100 µg	50 µg	100 µg
7a	8	12	6	10
7b	9	11	6	8
7c	6	8	9	15
7d	13	16	6	9
7e	7	10	7	10
7f	8	12	9	16
7g	14	16	6	10
7h	9	10	6	11
7i	7	9	9	14
7j	15	18	8	12
8a	8	12	8	10
8b	7	9	6	9
8c	9	10	8	10
8d	10	12	8	9
8e	9	11	7	9
8f	8	12	8	10
8g	9	12	8	9
8h	7	10	6	8
8i	7	9	8	12
8j	9	12	8	10
Ampicillin	16	21	14	18

Results and Discussion

All the synthesized compounds were characterized by IR and proton NMR techniques. They were evaluated for anti bacterial activity against *Staphylococcus aureus* and *Escherichia coli* (table 1) and antioxidant activity. Data indicated that the test compounds were better active against *Staphylococcus aureus* than *Escherichia coli*. Compounds 7d, 7g, 7j and 8d were relatively more effective against the gram positive *Staphylococcus aureus* than other test molecules. The presence of electron

releasing CH₃ group at the para position of the aromatic ring may be responsible for its enhanced effectiveness against the microorganism. Data from computational study (table 3) showed higher cLogP values for these molecules indicating higher lipophilicity and could lead to better permeability for these compounds across the bacterial cell membrane. At a dose of 100 µg, series 7 compounds were better than series 8 compounds against *Escherichia coli*. This clearly indicated that OCH₃ substitution on the aromatic ring was not favourable for the activity. Results from ferric ion reduction

Table 2. Ferric ion reduction activity (antioxidant activity)

Sample code	AbsorbanceAt 510 nm	AntioxidantActivity (%)
7a	0.237	34.5
7b	0.250	36.4
7c	0.208	30.3
7d	0.252	36.7
7e	0.243	35.4
7f	0.217	31.6
7g	0.303	44.2
7h	0.243	35.4
7i	0.212	30.9
7j	0.313	45.6
8a	0.276	40.2
8b	0.285	41.5
8c	0.277	40.4
8d	0.333	48.5
8e	0.340	49.6
8f	0.315	45.9
8g	0.465	67.8
8h	0.242	35.3
8i	0.22	32.4
8j	0.393	57.3
Standard*	0.686	100

*Ferric ammonium sulphate

activity indicated that the compounds possessed mild to moderate anti oxidant activity and is more pronounced in series 8. This can be attributed to the electron rich nature of such molecules which makes it suitable for reducing the ferric ions to ferrous ions. Activity is substantially diminished when nitro substitutions are present on either of the benzene rings. The effect is more pronounced when these electron withdrawing groups are especially at ortho or para positions. By way

of resonance, they withdraw electrons available on the amine nitrogen thereby greatly reducing its ability to quench ferric ions. Chloro substitutions have not had much effect on the activity. This could be possibly attributed to the inductive effect playing along with the resonance effect. Halogens when present on the aromatic ring are known to inductively withdraw electrons but by resonance donate electrons. From the data, we can infer that electron withdrawing groups decrease the

Table 3. Computational Data from Molinspiration Studies

Comp.Code	CLogP	TPSA	OHNH	RB	Vol
7a	6.19	52.74	3	5	308.83
7b	6.82	52.74	3	5	322.37
7c	6.10	98.56	3	6	332.16
7d	6.59	52.74	3	5	325.39
7e	6.85	52.74	3	5	322.37
7f	6.13	98.56	3	6	332.17
7g	6.62	52.74	3	5	325.40
7h	6.87	52.74	3	5	322.37
7i	6.15	98.56	3	6	332.17
7j	6.64	52.74	3	5	325.40
8a	6.25	61.97	3	6	334.38
8b	6.88	61.97	3	6	347.92
8c	6.16	107.8	3	7	357.71
8d	6.65	61.97	3	6	350.94
8e	6.90	61.97	3	6	347.92
8f	6.18	107.8	3	7	357.71
8g	6.67	61.97	3	6	350.94
8h	6.93	61.97	3	6	347.92
8i	6.21	107.8	3	7	357.71
8j	6.70	61.97	3	6	350.94

ferric ion reduction capability and electron releasing groups increase the ferric ion reduction capability.

Conclusion

Suitably substituted pyrazoles are effective candidates for anti bacterial and antioxidant activity. The presence of a methyl substitution at the para position enhanced lipophilicity, thereby increasing permeation across bacterial cell membrane resulting in higher activity against *Staph aureus*. The presence of OCH₃ group may not be significant in enhancing antibacterial activity against *E.Coli*. Electron releasing groups suitably placed on

the aromatic ring enhance antioxidant activity whereas electron withdrawing groups decrease activity.

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