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BIOLOGICAL EVALUATION OF AZA-HETEROCYCLIC DERIVATIVES AND THEIR INTERMEDIATES

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ABSTRACT

The objective of present study was to carry out biological evaluation of aza-heterocyclic derivatives including methoxy, phenoxy, alkyl and aryl derivatives of pyrazoles and their intermediates i.e. diketones. All of the compounds were evaluated to find their potential as antimicrobial, antioxidant and antitumor agents. Antimicrobial screening was done by finding the bactericidal and fungicidal activity of the test compounds using agar well diffusion and agar tube dilution methods. Antioxidant potential was examined by DPPH free radical scavenging and DNA damage assays. Whereas antitumor findings were based on the potato disc tumor assy. Among all the tested compounds, 2-(5-(4-hydroxyphenyl)-1-phenyl-1H-pyrazol-3-yl)phenol (IX), a pyrazole having three phenyl groups has shown the highest antimicrobial, antitumor, and DNA protective activity while the compound 1-(2-hydroxyphenyl)-3-(2 methoxyphenyl)propane-1,3-dione (I) has shown the best antioxidant potential. Data obtained from these experiments indicated the possible biological activities of these compounds and enabled us to compare these compounds as antimicrobial, antioxidant and antitumor agents. On the basis of these findings, efforts for the pharmacological applications of these heterocyclic compounds can be initiated.

KEY WORDS: Heterocyclic derivatives, pyrazoles, intermediates of pyrazoles, diketones, biological evaluation.

INTRODUCTION

The search for novel biologically active substances is always of paramount significance and medicinal chemistry is serving as one of the chief props for the production of bioactive compounds (Behl and Moosmann, 2002, Padmaja *et al.*, 2009). Among different classes of biologically active agents, heterocyclic compounds occupy a distinct place as these organic compounds are widely distributed in nature, many of which are of fundamental importance to living system including nucleic acid bases, derivatives of pyrimidine and purine ring systems, as being crucial to the mechanism of replication. Chlorophyll and heme, derivatives of porphyrin ring system, are the components required for the photosynthesis and oxygen transport in the higher plants and in animals, respectively (Gilchrist *et al.*, 1997). Heterocyclic compounds are commonly used as pharmaceuticals, agrochemicals and as veterinary products (Czarnik, 1996). Pyrazoles are aza-heterocyclic (nitrogen containing) five membered ring structures and represent one of the most active classes of the heterocyclic compounds possessing a broad range of biological activities i.e. antimicrobial activity (Kalluraya, *et al.*, 2007), which came into account since the discovery of the natural pyrazole C-glycoside pyrazofurin showing broad spectrum antimicrobial activities (Bekhit, *et al.*, 2008). Pyrazomycine and Formycine are both antibiotics (Brown, 1998). They also exhibit anti-inflammatory, antipyretic activity e.g. apazone and amidopyrine (Young, 1975). Antipyrine is the one of the earliest synthetic drugs and is named after its antipyretic properties (knorr, 1895). Phenazone is used to relieve headache and malase and comparable in effects to aspirin but is much more soluble (Nollers, 1970). Some therapeutically useful compounds such as phenylbutazone, oxyphenbutazone, antipyrine and aminopyrine have analgesic and muscles relaxant action (Anderson *et al.*, 1964). Besides that Pyrazoles and its derivatives also exhibit anti-diabetic, antioxidant (Dannahardt, 2000), antitumor and cytotoxic activities (Farag *et al.*, 2008). Pyrazoles also find numerous applications in agriculture and industry as many pyrazolones are employed in coloured photography (Mess, 1954). Many analytical reagents are derivatives of pyrazoles containing different substituent such as picrolinic acid (Wiley, 1964). In continuation of our interest to discover new drugs, biological screening of methoxy, phenoxy, alkyl and phenyl derivatives of pyrazoles and their intermediates was carried out using antimicrobial antioxidant and antitumor assay, which had not been evaluated before.

MATERIALS AND METHODS

Chemistry

All the test materials listed in the Table 1 were synthesized by using the reported procedure (Ahmed *et al.*, 1997; Ahmed *et al.*, 1996; Ahmed *et al.*, 1990; Haq *et al.*, 2007; Ikram *et al.*, 2009). Structures and name of all the tested compounds are given in Table 1.

Biological studies

Following bioassays were performed to find the biological activities of the test compounds.

Antibacterial Assay

Synthesized heterocyclic compounds were screened for their bactericidal potential by the agar well diffusion method (Olaleye, 2007) against six strains of bacteria, standard antibiotics used in this experiment as control were Cefixime and Roxithromycine. Diameter of the inhibition zone around each well was measured. Experiment was run in triplicates and mean value was taken as antibacterial activity.

Antifungal Assay

Antifungal activity of the test samples against six fungal strains was examined. The agar tube dilution method was applied for screening antifungal activity (Choudhary *et al.*, 1995). Dimethylsulfoxide (DMSO) was used as negative control while reference antifungal drug (Terbinafine) was run as positive controls. The tubes were incubated at 28°C for 7 days. Fungal growth was determined by measuring the linear growth (mm) in the medium and growth inhibition was calculated with reference to the negative control.

Percentage inhibition of fungal growth

$$= \frac{\text{Linear growth in negative control} - \text{Linear growth in test sample (mm)}}{\text{Linear growth in negative control}} \times 100$$

Antioxidant Assay

DPPH free radical scavenging assay

DPPH free radical scavenging assay was performed by reported method (Gyamfi *et al.*, 1999) to check the radical scavenging activity of test samples. In caped vials 200 µL of each stock solution (1500, 750 and 325ppm), methanol in case of negative control, and ascorbic acid in case of positive control was mixed with 2.8mL of 3.2% DPPH. After incubation of 30 minutes in dark, the reduction in the number of DPPH free radicals was measured using a spectrophotometer adjusted at 517nm. The percentage scavenging was calculated by the following equation:

$$\% \text{ scavenging} = \frac{\text{absorbance of control- absorbance of test sample}}{\text{Absorbance of control}} \times 100$$

OH radical induced DNA damage assay

Determination of antioxidant and prooxidant activity of the test samples was carried out according to the reported method (Tian and Hua, 2005). Plasmid DNA, pBR322 was dissolved in 50mM phosphate buffer (pH 7.4) to get concentration of 0.5µg/3µL. Three microliters of diluted plasmid DNA was transferred to the microeppendroff tube followed by 5µL of test compounds (3000ppm, 300ppm, and 30ppm), three microliters of 2mM $FeSO_4$ and 4µL of 30% H_2O_2 were added successively. Plasmid pBR322 with $FeSO_4$ and H_2O_2 was used as a positive control for DNA damage, pBR322 DNA alone in phosphate buffer served as the negative control. The reaction mixture was incubated in dark at 37^{o}C for one hour. After incubation 3µL of bromophenol blue (loading dye) was added to each reaction mixture and then the samples were loaded on 0.9% agrose gel wells containing TBE buffer and ethidium bromide. Each reaction mixture with controls was run horizontally in 1X TBE buffer at 100 Volts for 1 hour in an electrophoresis apparatus. The gels were photographed under UV light.

Antitumor Assay

The potato disc method was used to test antitumor activity of synthesized organic compounds as reported earlier (Ferrigini *et al.,* 1982) In short, 48-h old single colony culture of Agrobacterium tumefaciens (At-10) was used as tumor inducing agent on potato discs. Test samples were evaluated for their antitumor activity at five different concentrations i.e. 1000, 100, 10, 1, 0.1 µg/ml of DMSO. The specificity drug applied here was Rifampicine (10mg/ml). Under sterilized conditions 8×5 mm potato discs were prepared from surface sterilized potato. Discs (8-10) were placed on each plate containing 20 ml of 1.5% plane agar. Each disc was treated with inoculum (test sample and AT-10 culture), plates were incubated at 28°C for 21 days then stained with Lugol's solution (10% KI and 5% I_2). Dissecting microscope was used to count the number of tumors. Each experiment was carried out in triplicate, ANOVA and Duncan's Multiple Range Test was applied to analyze data statistically. Percentage tumor inhibition was calculated using following formula.

Percentage of inhibition=

$$\frac{\text{No of tumors in negative control - No of tumors with test samples}}{\text{No of tumors in negative control}} \times 100$$

RESULTS AND DISCUSSIONS

Antibacterial Assay

All of the synthesized heterocyclic compounds were tested for their antibacterial potential. Results are given in Table 2, which reveal that all the heterocyclic compounds showed significant antibacterial activity except the XII, XIII, XIV and VIII which did not show bactericidal activity against any bacterial strain. Highest antibacterial activity was shown by the compound IX against Gram positive bacteria (inhibitory zone > 20mm) and significant activity against the Gram negative bacteria (inhibitory zone ≤ 19mm), with MIC values ranging from 0.01-0.2 ppm. It could be due to the presence of phenyl groups attached to pyrazole ring. It agrees with the results presented previously (Manojkumar *et al.*, 2009). Compounds having carbonyl groups (I and II) showed good antibacterial activity against three Gram positive bacterial strains i.e. *S. aureus, M. leutus, B. subtilus,* and two Gram negative strains i.e. *B. bronchisepticha, S. setubal,* with MIC values 0.2-0.4ppm and 0.2-0.9ppm respectively. Compounds having the methyl group substituted to the pyrazole ring showed moderate level of antibacterial activity as shown by the compounds V, VI, VII and XI with MIC values 1-0.8ppm. Results of the present study correlate with earlier studies (Padmaja *et al.*, 2009).

Antifungal Assay

All the test compounds were screened for their antifungal activity against six fungal strains Results indicate that all of the compounds showed antifungal activity. Significant antifungal activity was shown by the compounds IX, I, IV and VII and against all the six fungal strains. Highest antifungal activity was shown by the compound IX (phenyl derivative of pyrazole) against the *A. flavus* (86.4%), it also showed significant activity against rest of the five strains with percentage inhibition ranging from 60-80%. Lowest antifungal activity was shown by the III and VIII against the *mucar specie.* While rest of the compounds showed moderate level of activity. Results are summarized in Table 3.

This antifungal activity may be attributed to the presence of methoxy, alkyl and phenyl groups to the heterocyclic centers as reported earlier by Babasaheb *et al.*, 2009, according to that study it was observed that compounds substituted with electron-releasing groups (OCH_3 > CH_3) show increased antimicrobial activity because they render the ring electron rich, which is a requirement for the activity. It was also reported that substituent position in the phenyl ring has also been proved to be biologically relevant apart from active moiety

(Babasaheb *et al.,* 2009). None of the compounds showed stimulatory effect i.e. promoted linear growth of fungi rather than inhibiting it. Similar kind of results were obtained by the experiments performed earlier (Padmaja *et al.,* 2009).

DPPH Free Radical Scavenging Assay

Newly synthesized heterocyclic derivatives were subjected to DPPH free radical scavenging assay to determine their antioxidant and prooxidant activity. Results are given in Table 4. Significant activity was shown by the compounds I, II and XV at all the three concentrations. Moderate activity was shown by the compounds VIII, IX and XI. The rest of the compounds showed low level of activity. Furthermore the antioxidant activity was concentration dependent.

XVI showed the pro-oxidant activity i.e. it has enhanced the free radical formation at 100ppm as compared to control. Highest antioxidant activity was shown by the compound I, with lowest value of IC50 (2.9ppm) and lowest activity was shown by the compound VII with IC_{50} value more than 100ppm. Overall results indicate that the compounds having diketonic group has shown good antioxidant activity i.e. compounds I and II. In the earlier studies it was reported that antioxidant activity of the compounds is in fact related to their electron or hydrogen radical releasing ability to DPPH, making it stable diamagnetic molecule. (Babasaheb *et al.,* 2009). Here this requirement is being fulfilled by the compounds having carbonyl groups (I and II). While compounds having phenyl group attached to them have shown moderate level of antioxidant activity. Presence of methyl and phenyl groups in the pyrazoles enhance their antioxidant activity (Ikeda *et al.,* 2002).

OH Radical Induced DNA Damage Assay

Antioxidant and prooxidant effects of the synthesized compounds were also investigated by using a free radical-induced plasmid pBR322 DNA break systems in vitro. This assay was carried out at the three concentrations of the compounds 1000ppm, 100ppm, and 10ppm. Fenton reaction is used to produce Hydroxyl radicals in vitro. In this assay supercoiled plasmid DNA (SC) is broken into two forms open circular (OC) and linear form (Linear) by the attack of hydroxyl radical generated from the Fenton reaction (Tian and Hua, 2005). Control run in the assay showed changes from SC DNA to the OC form indicating single stranded cleavage of DNA treated with H_2O_2 in the presence of $FeSO_4$ (lane X). To elucidate the antioxidant and prooxidant effects of the synthesized compounds, brightness and thickness of SC and OC bands was examined. Heterocyclic compounds were found to be

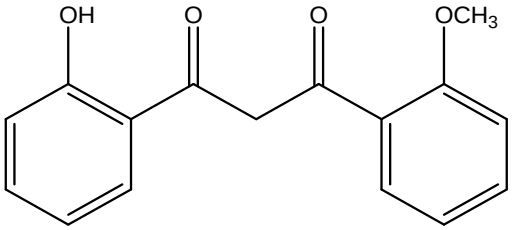
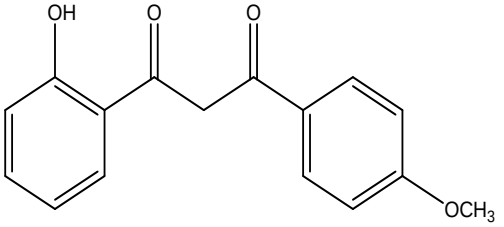
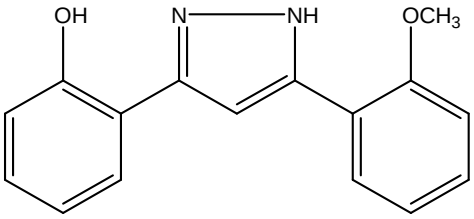
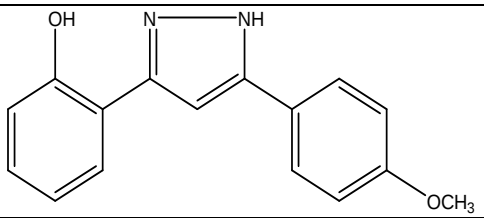
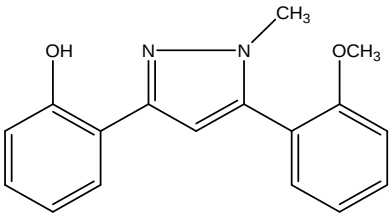
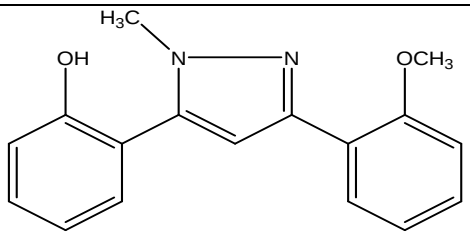
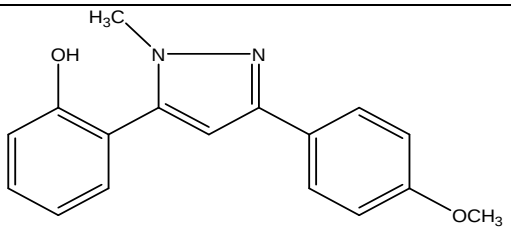
active against the DNA damage caused by the free radical. Compounds XI, IV, VIII, and IX showed DNA protection at all the three concentrations, while rest of the heterocyclic compounds were more plasmid DNA protective at 1000ppm as compared to 10ppm effectively inhibiting single strand cleavage of DNA in concentration dependant manner, reported earlier (Dong-Seong *et al.*, 2001). Results are given in Table 5.

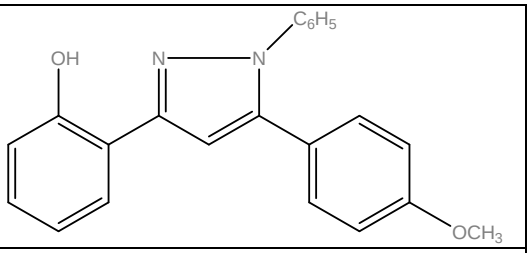
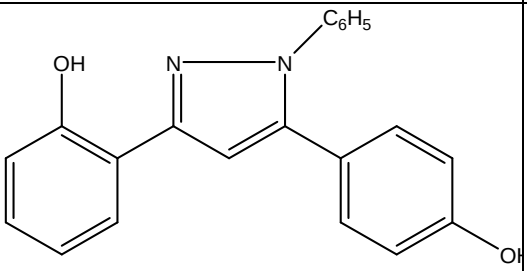
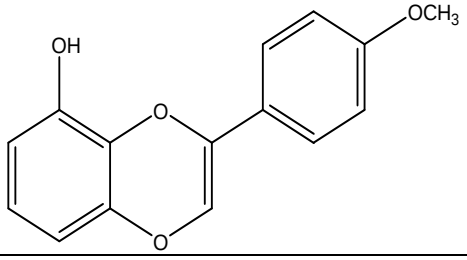
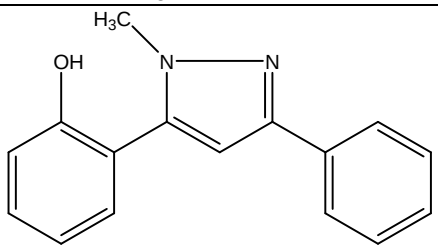
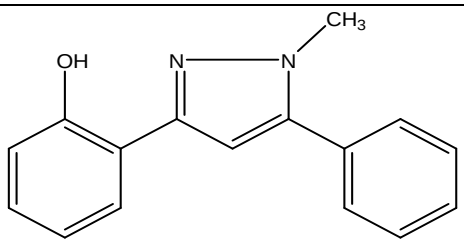
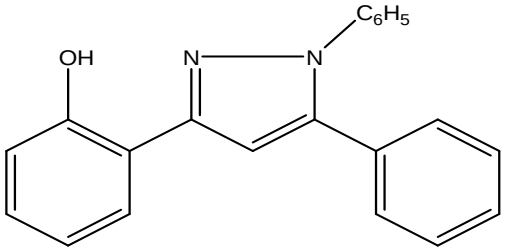
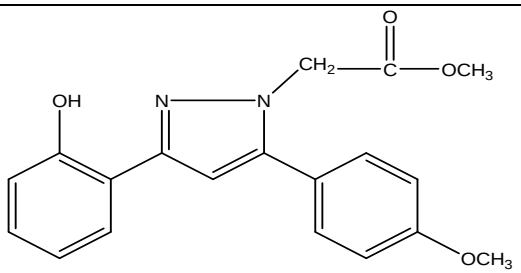
In the above study it was also observed from the gel reading patterns that the compounds XIV and VI had accelerated Fenton reaction by showing more damage at 1000ppm as compared to 10ppm resulting in a thicker band of OC than SC at 1000ppm (Fig 1). Thus indicating a pro-oxidant behavior. It is known that some chemopreventive agents acting as antioxidants at some concentrations become prooxidant at other concentrations therefore selection of dose is very important in the application of antioxidants or prooxidants. Balance of the two activities i.e. free radical-scavenging activity and reducing power on iron ions is responsible for the antioxidant effect of the test compounds. It seems that the predomination of reducing power on iron ions over free radicals scavenging activity can result in the prooxidant effect on DNA damage. Fenton reaction was also proved to be possible in vivo (Meneghini *et al.*, 1997).

Antitumor Assay

In the present study potato disc tumor assay was carried out to check the antitumor activity of the synthesized heterocyclic compounds. Results given in Table 6 indicate that all of the heterocyclic compounds showed antitumor activity in concentration dependent manner. However highest antitumor activity was shown by the IX, with IC_{50} 0.09ppm while lowest antitumor activity was shown by the compound III. Increased antitumor activities are related to an electron withdrawal from the ring increasing stability of the resulting structure (Hodnett and Dunn, 1970). Furthermore the compounds II and I having carbonyl group showed good antitumor activity and some previous studies also support the same results. It was also reported that m-alkyl substitution showed double the activity of p-alky (Ahmad *et al.*, 2008). Compounds carrying the acetyl or ester moieties are less active in tumor inhibition as the compound XIV carrying the ester linkage did not show significant antitumor activity (Ahmad *et al.*, 2008). It appears that the results of crown gall tumor bioassay could be very beneficial for the screening of potential antitumor compounds and our compounds like IX, XV and II are potential candidates for this activity.

Table. 1 Structure and name of Heterocyclic compounds

S.No	Structure	Name
I.		1-(2-hydroxyphenyl)-3-(2 methoxyphenyl) propane-1,3-dione
II.		1-(2-hydroxyphenyl)-3-(4-methoxyphenyl)propane-1,3-dione
III.		2-(5-(2-methoxyphenyl)-1H-pyrazol-3-yl)phenol
IV.		2-(5-(4-methoxyphenyl)-1H-pyrazol-3-yl)phenol
V.		2-(5-(2-methoxyphenyl)-1-methyl-1H-pyrazol-3-yl)phenol
VI.		2-(3-(2-methoxyphenyl)-1-methyl-1H-pyrazol-5-yl)phenol
VII.		2-(3-(4-methoxyphenyl)-1-methyl-1H-pyrazol-5-yl)phenol

VIII.		2-(5-(4-methoxyphenyl)-1-phenyl-1H-pyrazol-3-yl)phenol
IX.		2-(5-(4-hydroxyphenyl)-1-phenyl-1H-pyrazol-3-yl)phenol
X.		3-(4 methoxyphenyl)benzo[b][1,4] dioxin-5-ol
XI.		2-(1-methyl-3-phenyl-1H-pyrazol-5-yl)phenol
XII.		2-(1-methyl-5-phenyl-1H-pyrazol-3-yl)phenol
XIII.		2-(1,5-diphenyl-1H-pyrazol-3-yl)phenol
XIV.		methyl 2-(3-(2-hydroxyphenyl)-5-(4-methoxyphenyl)-1H-pyrazol-1-yl)acetate

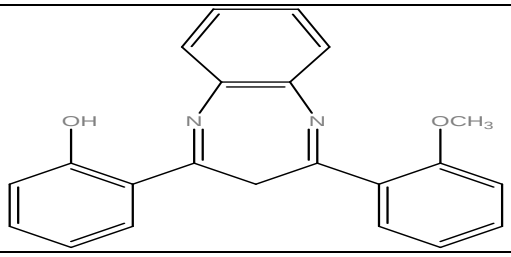
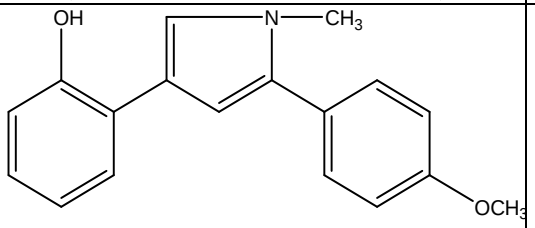
XV.		2-((1E,4E)-4-(2-methoxyphenyl)-3H-benzo[b][1,4]diazepin-2-yl)phenol
XVI.		2-(5-methoxyphenyl)-1-methyl-1H-pyrrol-3-yl)phenol

Table 3: Effect of the heterocyclic compounds on percentage inhibition of linear growth of six fungal strains.

	Compound	Percentage inhibition %					
		Mucor.sp.	***A. niger***	***F.moniliformes***	***A.fumigatus***	***A. flavus***	***F. solani***
1	VII	62.6	61.70	53.6	62.2	71.4	75
2	XVI	26	52.1	46.3	60	34.2	46
3	XII	59.3	46.8	44.5	22.2	69.6	53.5
4	XIII	74	6.38	32.7	44.4	22.8	52
5	V	6.66	36.17	32.7	56.6	29.2	37.6
6	XI	32	13.82	36.3	60	68.5	39.2
7	VI	61.3	20.2	41.6	44	34.2	32.1
8	IV	56.5	54.25	54.5	60	67.8	51.4
9	III	8.66	10.6	24.5	46.6	35.7	50.7
10	I	60	29.7	73	63.3	67.1	65.7
11	XIV	20	17.02	43	33.3	37.6	47.6
12	VIII	10	1.06	23	5.5	35	26.5
13	X	49.3	8.51	51.6	45.5	35	49.2
14	IX	66.6	60	63.6	70	86.4	78
15	II	45.3	37.2	50.5	40	56.4	64.2
16	XV	38.6	25.5	46.3	37.7	36.4	50.7

A. Niger = *Aspergillus Niger*, ***A. fumigatus*** = *Aspergillus fumigatus*, ***A. flavus*** = *Aspergillus flavus*, ***F. moniliformes*** = *Fusarium moniliformes*, ***F. solani*** = *Fusarium solani*, ***Mucor.sp*** = *Mucor speci.*

Table 2: Antibacterial activity of the 16 synthetic heterocyclic compounds

s. no	sample name	Mean zone of Inhibition (in mm ± S.D)+MIC (mg/ml)					
		M. leuteus	***S. aureus***	***B.subtilus***	***B. b***	***S. setubal***	**E. coloi**
1	VII	17.75±0.25 0.4mg/ml	11.3±0.3 0.8mg/ml	13±0.8 0.4mg/ml	14±0 0.4mg/ml	16±0.28 .6mg/ml	NIL
2	XVI	NIL	14.5±0.5 0.8mg/ml	NIL	NIL	NIL	NIL
3	XII	NIL	NIL	NIL	NIL	NIL	NIL
4	XIII	NIL	NIL	NIL	NIL	NIL	NIL
5	V	NIL	10 1mg/ml	10±0.1 1mg/ml	9±0 1mg/ml	10 1mg/ml	NIL
6	XI	12.2±0.6 0.8mg/ml	15±1 0.8mg/ml	13.6±1 o.8mg/ml	14±1 0.8mg/ml	14.1±.0.20.8mg/ml	NIL
7	VI	12±0 0.9mg/ml	14 0.8mg/ml	10±0.5 1mg/ml	12.5±0.5 0.9mg/ml	12.5±.0.5 0.8mg/ml	NIL
8	IV	16.5±0.5 0.2mg/ml	15 0.6mg/ml	14±1 0.4mg/ml	16.5±0.5 0.09mg/ml	17.1±.0.3 0.4mg/ml	NIL
9	III	NIL	NIL	NIL	NIL	NIL	NIL
10	I	14±0.4 0.2mg/ml	17.6±0.7 0.4mg/ml	14±0.1 0.2mg/ml	18.7±0.7 0.2mg/ml	18±.0.3 0.2mg/ml	NIL
11	XIV	NIL	NIL	NIL	NIL	NIL	NIL
12	VIII	NIL	NIL	NIL	NIL	NIL	NIL
13	X	NIL	10±0 1mg/ml	NIL	NIL	NIL	NIL
14	IX	20.2±0.3 0.03mg/ml	21±1 0.01mg/ml	19±0.7 0.2mg/ml	19±0.6 0.09mg/ml	19±.1 0.2mg/ml	NIL
15	II	16.4±0.4 0.2mg/ml	12.3±0 0.9mg/ml	12.2±0.3 .9mg/ml	17±0.5 0.2mg/ml	16±.0.5 0.6mg/ml	NIL
16	XV	15.8±.0.3 0.6mg/ml	14.3±0 0.8mg/ml	NIL	NIL	10 1mg/ml	NIL
17	**Roxithromycin**	21.7±.0.47	28.75±1.0	25.7±0.4	21.5±0.3	16±0.91	18.4±0.28
18	**Cefixime**	31.6±0.23	33±0.91	30.9±0.51	32±0.5	31.1±0.42	31.2±0.14

S. aureus= *Staphylococcus aureus*, ***S. Setubal***= *Salmonella Setubal*, ***B. subtilis***= *Bacillus subtilis*, ***M. luteus***= *Micrococcus luteus*, ***E. coli***= *Escherichia coli*, *B.b =Bordetella bronchiseptica*

MIC= Minimum inhibitory concentration

Table 4: Percentage DPPH free radical scavenging activity

S.no	Sample code	Percentage scavenging %			IC_{50} ppm
		100ppm	50ppm	25ppm	
1	VII	3.6	0.014	-5.06	>100
2	XVI	-1.1	2.2	5.3	_
3	XII	9.9	6.4	-3.41	>100
4	XIII	10.5	11	12	-
5	V	11.5	14	16	-
6	XI	18.38	13.5	11.5	>100
7	VI	11.3	13	19	-
8	IV	12.79	16	19.2	-
9	III	11.6	9.74	2.23	>100
10	I	84.7	77	71.2	2.9>
11	XIV	11.4	13.41	20.12	-
12	VIII	26.33	17	15.3	>100
13	X	10.38	8.36	5.9	>100
14	IX	26.1	20.52	17	>100
15	II	69	64	49.5	23
16	XV	50	37	26	100

Table 5: Comparison of DNA damage/ DNA protection activity of the heterocyclic compounds.

S. no.	Compound Name	Comparison of DNA damage/protection activity of compounds at respective concentration		
		1000ppm	100ppm	10ppm
1	VII	+++	++	+
2	XVI	++	++	+
3	XII	+++	+++	++
4	XIII	+++	++	+
5	V	+++	++	+
6	XI	+++	+++	+++
7	VI	-	++	+++
8	IV	+++	+++	+++
9	III	+++	++	++
10	I	+++	++	-
11	XIV	-	++	+++
12	VIII	+++	+++	+++
13	X	+++	++	++
14	IX	+++	+++	+++
15	II	+++	+++	++
16	XV	++	++	++

\- *DNA damage activity*

\+ *Little DNA protection activity*

++ *Moderate DNA protection activity*

+++ *Extensive DNA protection activity*

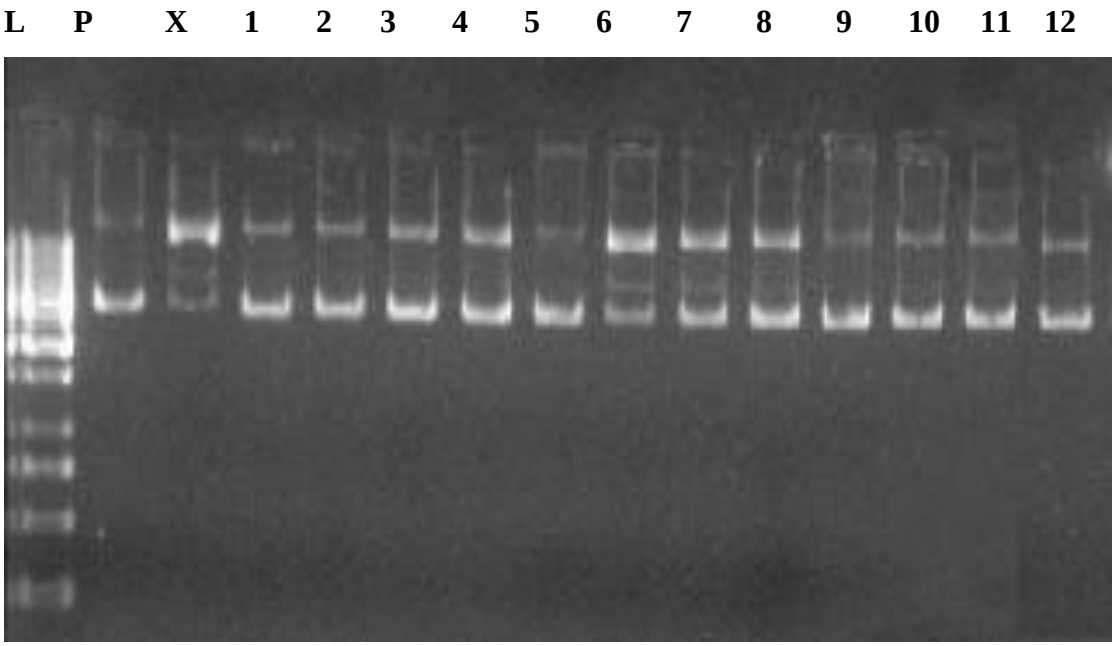


Fig 1: Effect of compounds XI, VI and IV on pBR322 Plasmid DNA.

L DNA Ladder (1Kb)

P pBR322 Plasmid

X pBR322 Plasmid treated with $FeSO_4$ and H_2O_2 (positive control)

Lane 1 Control for the damage effect of compound on DNA; plasmid + 1000ppm ofXI

Lane 2 plasmid + 1000ppm of XI + $FeSO_4$ + H_2O_2

Lane 3 plasmid + 100ppm of XI + $FeSO_4$ + H_2O_2

Lane 4 plasmid + 10ppm of XI + $FeSO_4$ + H_2O_2

Lane 5 Control for the damage effect of compound on DNA; plasmid+1000ppm of VI

Lane 6 plasmid +1000ppm of VI +$FeSO_4$ + H_2O_2

Lane 7 plasmid + 100ppm of VI+ $FeSO_4$ + H_2O_2

Lane 8 plasmid + 10ppm of VI+$FeSO_4$ + H_2O_2

Lane 9 Control for the damage effect of compound on DNA; plasmid+1000ppm Of IV

Lane 10 plasmid + 1000ppm of IV+ $FeSO_4$ + H_2O_2

Lane 1 plasmid + 100ppm of IV + $FeSO_4$ + H_2O_2

Lane 12 plasmid + 10ppm of IV+ $FeSO_4$ + H_2O_2

Table 6: Percentage tumor inhibition by 16 heterocyclic compounds (potato disc antitumor assay)

		Percentage inhibition					IC50 (ppm)
S#	compounds	1000ppm	100ppm	10ppm	1ppm	0.1ppm	
1	VII	71.52	40.64	38	27	21	1.479
2	XVI	62.8	40	28.4	23.4	0.8	1.686
3	RXII	73.2	61.6	47	29	20.8	0.58
4	XIII	54.8	53.2	50	34	28.8	1.45
5	V	61.4	41.2	33.2	29.6	3	1.65
6	XI	64	48.4	47.2	41.6	34	1.47
7	VI	57	43.5	29.1	4.7	-9.2	5.66
8	IV	80.5	64.8	57.2	27.1	8.3	1.52
9	III	38.3	31.3	20.1	14.5	1.9	2.27
10	I	76.2	55.9	43	31	11	1.36
11	XIV	62.8	56.2	34	15.9	-1.05	1.21
12	VIII	52.6	36.6	19	12	5	7.10
13	X	74.6	51.3	50	29.3	24.6	1.12
14	IX	88	80.65	70.3	60	51.6	0.09
15	II	84.3	74.7	70	40.9	25	0.21
16	XV	85.5	79.9	70.9	65.9	32.3	0.70

**More than 20% tumor inhibition is significant.*

CONCLUSION

Finally it could be concluded from present study that these heterocyclic compounds can be potent antimicrobial and antitumor agents but their DNA damaging activity at higher concentration had put us to ponder on its potency. So heterocyclic compounds could be further optimized by latest development in research field to use them more effectively. Paricularly highly significant antibacterial and antitumor activities indicate their potential to be used as antibiotics and anticancerous agents.These compounds can be screened further for other pharmacological activities to be used as potential drug candidates. Moreover their mechanism of action and structure activity relationship (SAR) can also be studied.

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