Synthesis and Antimicrobial Screening of Different Pyrazol Derivatives

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Abstract:
*A new series of derivatives different **pyrazol derivatives** were synthesized by one pot cyclocondensation reaction of 4-(3-methyl-1-phenyl-1H-pyrazol-5-yl) piperazine-1-carbodithioic acid with substituted benzyl chloride. All the synthesized compounds were characterized by elemental analysis, FT-IR, 1H NMR and 13C NMR spectral data. All the synthesized compounds were screened against Four bacterial Amphiciline, Chlorniphenicole, Ciprofloxacine Norfloxacine and for antifungal activity against two fungal Nystain, and Grisiofulvin using the broth microdilution MIC method. The screening findings showed that several of the compounds were determined to be equipotent, meaning they were more potent than commercial medications against the majority of used strains.*

Keywords: *Pyrazole, Piperazine, Hetero cyclic, Microbial Activity*

1. Introduction

In this chapter involves the studies of Pyrazole and its derivatives on synthesis, characterization and biological evolution of some new Pyrazole containing derivatives and also including the sulfonates compounds. There is wide and depth research behind the Pyrazole derivatives of organic compounds. It has been reported the chemist or scientist from all over the globe by the past few decades. The pyrazolemotif found in a number of molecules that possessa wide range of pharmacological activity due to nitrogen atom in the ring.

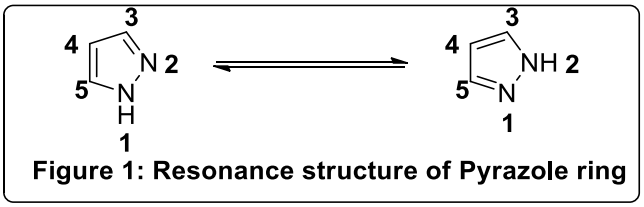


Figure 1: Resonance structure of Pyrazole ring

As early as in 1883 “LUDWIG KNORR” was discovered the antipyretic action of a pyrazole1 derivatives in man, he suggested that the name of the compound antipyrine. This continuously interested in pyrazole chemistry. Pyrazolesimple doubly unsaturated compound having two nitrogen atoms i.e. one basic nitrogen atom and other neutral nitrogen atom the aromatic nature arises from the four electrons and the unshared pair of electrons on the –NH ring nitrogen.Pyrazole is a tautomeric substance and the method of synthesis was developed by Hans von pechmann in 1898.2It is aromatic in nature with six л delocalized electron system and also a shared lone pair electron on nitrogen atom due to this it is an act as nucleophile or attacking electron in electrophilic substitution reaction and form a stable molecules.

1.1 Aims and inference of current work

In this work, we report on the synthesis and characterization of a more potent stable substitute molecule for the biological response, as well as its antimicrobial activity against gram-positive and gram-negative bacteria and fungi, as well as derivatives of piperazine-1-carbodithioate. The work's primary contribution is that it will yield a pyrazol derivative with noteworthy biological activity. The biological

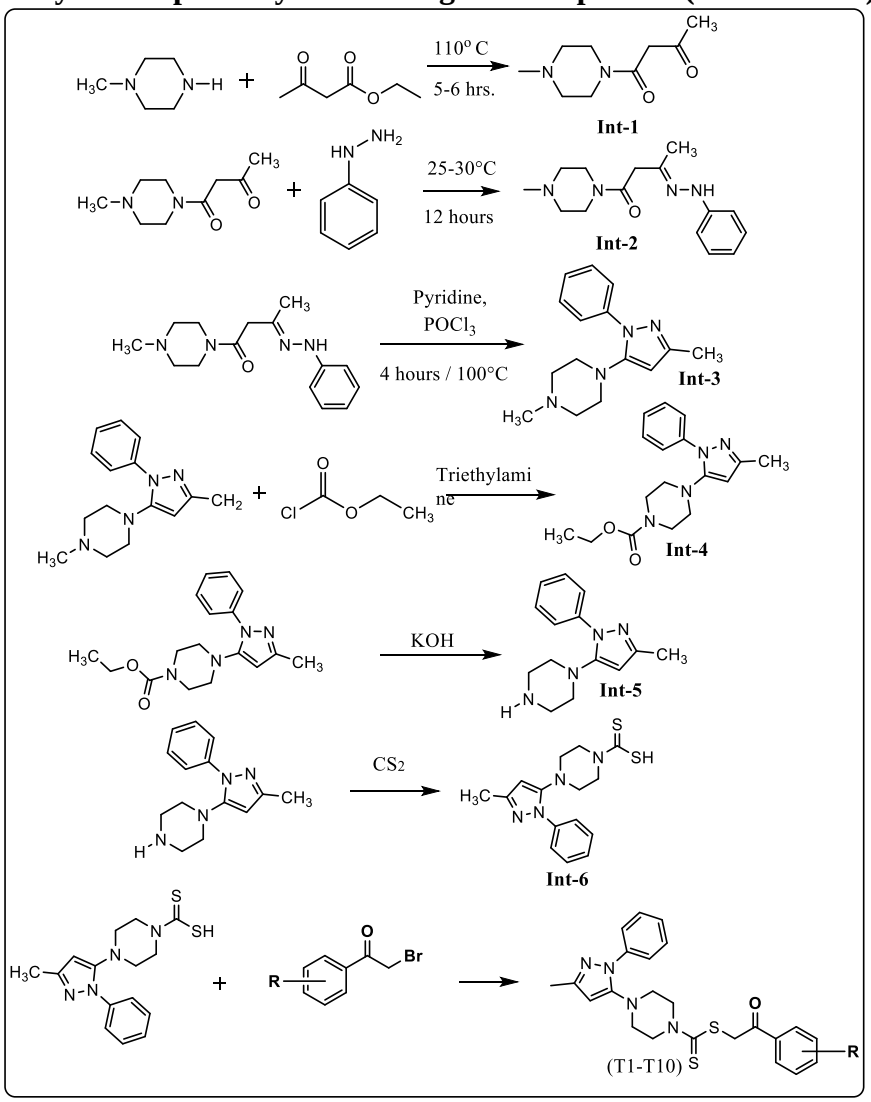
profile and importance of this class of molecules, as previously discussed, motivate us to keep working toward the synthesis of possible heterocyclic molecules.

2. Experimental section

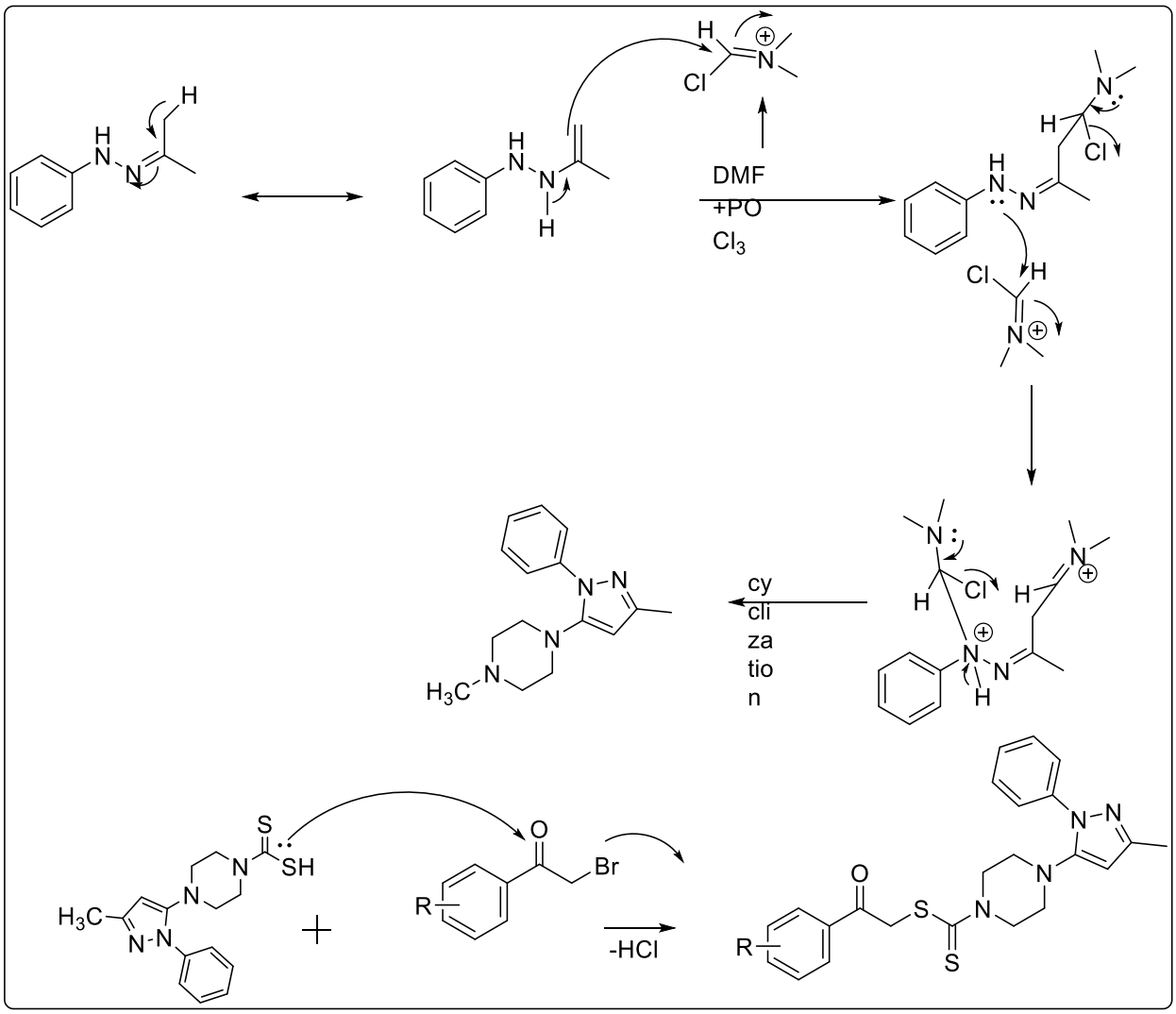
2.1 Reaction Scheme

Figure 8 depicts the synthesis pathway for the targeted compounds (T-01 to T-10), while Figure 9 depicts a likely reaction mechanism. Different pyrazol derivatives were obtained by reacting 4-(3-methyl-1-phenyl-1H-pyrazol-5-yl) piperazine-1-carbodithioic acid with substituted benzyl chloride in the final step of the synthesis.

The synthetic pathway for the targeted compounds (T-01 to T-10):



2.2 Plausible reaction mechanism



3. Material and method

Without any additional purification, all of the chemicals and solvents utilized to create the synthesized library were bought from CDH Chemical in Delhi and were of AR grade. Thin-layer chromatography (TLC) was used to track reactions using silica gel-G plates (G60 F254 (Merck)) with a 0.5 mm thickness. UV light (254 and 365 nm) was used to visualize the reactions. Melting points were measured using the open capillary method and are uncorrected. Using a DRS probe KBr pallet, IR spectra were captured using an FTIR-8400 spectrophotometer (Shimadzu, Kyoto, Japan). The produced compounds' 1H-NMR spectra were captured using a Bruker-Avance-II (400 MHz) DMSO-d6 solvent. As an internal standard, chemical shifts are represented as δ ppm downfield from TMS. A direct inlet probe on a GCMS-QP 2010 mass spectrometer (Shimadzu, Kyoto, Japan) was used to determine mass spectra. Table 1 displays the physical constants of the substances that were produced.

4. Experimental procedures

General synthesis of substitute (E)-4-((2,4-dioxo-3-(2-oxo-2-phenylethyl) thiazolidin-5-ylidene) methyl)-3-methoxyphenyl benzenesulfonate (T-01 to T-10). Use dimethylformamide as a solvent in a flask with a round neck. Next, fill the flask with potassium carbonate. Next, incorporate several phenacylbromide variations. Overnight, the reaction mixture was agitated at room temperature. After adding ice, the product was filtered, cleaned with hexane, and then recrystallized from ethanol.

Table 1: Physical parameters of (substituted, 2-oxoethyl 4-(3-methyl-1-phenyl-1H-pyrazol-5-yl) piperazine-1-carbodithioate derivatives (T1-T10)

No.	Code	Molecular formula	Substitution	Molecular Weight	Melting Point
1.	T-1	$C_{24}H_{26}N_4O_2S_2$	4-OCH_3	466.62	202-204
2.	T-2	$C_{23}H_{23}ClN_4OS_2$	4-Cl	471.03	192-194
3.	T-3	$C_{23}H_{24}N_4OS_2$	H	436.59	178-180
4.	T-4	$C_{23}H_{23}N_5O_3S_2$	4-NO_2	481.59	180-182
5.	T-5	$C_{23}H_{23}BrN_4OS_2$	4-Br	515.49	206-208
6.	T-6	$C_{24}H_{26}N_4OS_2$	4-CH_3	450.62	186-188
7.	T-7	$C_{23}H_{23}FN_4OS_2$	4-F	454.58	180-182
8.	T-8	$C_{23}H_{23}N_5O_3S_2$	3-NO_2	481.59	170-175
9.	T-9	$C_{23}H_{23}BrN_4OS_2$	3-Br	515.49	190-195
10.	T-10	$C_{23}H_{23}ClN_4OS_2$	3-Cl	471.03	180-185

5. Characterization studies

T1 2-(4-methoxyphenyl)-2-oxoethyl 4-(3-methyl-1-phenyl-1*H*-pyrazol-5-yl)piperazine-1carbodithioate
^{1}H NMR
1H NMR (400 MHz, DMSO-d6) δ 8.08 (d, J = 8.2 Hz, 2H), 7.86 (d, J = 7.9 Hz, 2H), 7.53 (t, J = 7.5 Hz, 2H), 7.36 (s, 1H), 7.13 (d, J = 8.4 Hz, 2H), 5.97 (s, 1H), 4.98 (s, 2H), 4.24 (d, J = 64.3 Hz, 4H), 3.91 (s, 3H), 2.98 (s, 4H), 2.22 (s, 3H)
IR (KBr, ν_{max}, cm^{-1})
3348.54, 3101.64, 2924.18, 2839.31, 2623.28, 2522.98, 2414.96, 2283.79, 2044.61, 1967.46, 1898.02, 1805.43, 1681.98, 1597.11, 1496.81, 1435.09, 1219.05, 1165.04, 1003.02, 925.86, 817.85, 594.10m 493.7
T-22-(4-chlorophenyl)-2-oxoethyl 4-(3-methyl-1-phenyl-1*H*-pyrazol-5-yl)piperazine-1-carbodithioate
^{1}H NMR
1H NMR (400 MHz, DMSO-d6) δ 8.07 (d, J = 8.5 Hz, 2H), 7.81 (d, J = 7.9 Hz, 2H), 7.64 (d, J = 8.5 Hz, 2H), 7.49 (t, J = 7.8 Hz, 2H), 7.32 (s, 1H), 5.92 (s, 1H), 4.95 (s, 2H), 4.26 (s, 3H), 4.12 (s, 2H), 2.94 (d, J = 5.1 Hz, 3H), 2.18 (s, 3H).
IR (KBr, ν_{max}, cm^{-1})
3726.60, 3371.68, 3063.06, 2985.91, 2885.60, 2839.31, 2769.87, 2708.15, 2569.27, 2476.68, 2306.94, 2152.63, 1936.60, 1890.30, 1689.70, 1589.40, 1496.81, 1435.09, 1280.78, 1234.48, 1157.33, 1095.60, 995.30, 925.86, 817.85, 771.55, 686.68, 540.09, 470.65

6. Conclusion

In this study, we report on the synthesis of more potent stable compounds comprising derivatives of pyrazol piperazine-1-carbodithioate as well as their characterisation and substitution. There was no requirement for filtration because the reaction was conducted cleanly and the products were produced in excellent yields without any further side product generation. We have synthesized ten molecules in total, and we have verified every structure using spectroscopy.

7. General introduction of microbial screening

Microbes, which include bacteria and archaea, are two different domains of microorganisms. They were the earliest forms of life to develop and had a significant impact on the ecology of our planet. The history of microbial activity on Earth spans almost 3.5 billion years. Prior to 2.5 billion years ago,

cyanobacteria evolved and began photosynthesis, which produced oxygen as a byproduct. This means that early microbial activity was anaerobic, meaning it took place in the absence of oxygen [1]. Microbes have persisted in being essential to many ecological processes, including decomposition and nitrogen fixation, which has an impact on the evolution of higher life forms and the formation of different ecosystems. Recent years have seen a rise in the significance of microbial study in fields like environmental science, biotechnology, and medicine as we have been able to comprehend and utilize their amazing potential for a wide range of uses.

8. Experimental section

8.1 Materials and methods

Sterile Petri dishes, sterile 96-well microtitre plates (round-bottom wells work best), filter-sterilized antibiotics, sterile diluents, test tubes, and liquid cultures of bacteria at an appropriate growth phase are the necessary materials.

8.2 The General protocol for biological screening is as follows

1. In Muller-Hinton broth, grow the test microbiological strains for 24 hours at 37°C for bacteria and 28°C for fungus.
2. Thaw and weigh the common medications nystatin, ampicillin, and streptomycin. To get a stock solution of 1000 µg/mL, dilute the antibiotics in DMSO.
3. Fill the first tube in the set of tubes with 2 mL of Muller-Hinton broth, and the other tubes with 1 mL of soup. Give the tubes the appropriate labels.
4. Fill the first tube with 1 mL of the medication stock solution. Your final concentration will be 1000 µg/mL as a result.
5. Thoroughly combine the medication with the broth. Remove 1 milliliter of the antibiotic solution and move it to the subsequent tube. The medication in the second tube has a 500 µg/mL concentration.
6. Keep serially diluting the sample until the last tube has a concentration of 62.5 µg/mL.
7. From the last tube, discard 1 mL of the antibiotic solution.
8. Reduce the cell density of the test microbiological cultures to 1–5 × 105 CFU/mL for fungus and 4-5 × 105 CFU/mL for bacteria.
9. Fill every tube—aside from the positive control tube—with 5 µL of the diluted microbial cultures.
10. For bacteria and fungi, incubate the tubes at the appropriate temperature for 24 to 48 hours.
11. Using a spectrophotometer, determine the colonies' absorbance at 600 nm after incubation.
12. The minimum inhibitory concentration (MIC) is the antibiotic's concentration at which the bacteria cannot grow visibly. This is verified by contrasting the culture's absorbance with that of the negative control tube.

The medication solution and no microbes are present in the positive control tube. This tube is used to make sure the medication is working and isn't having a harmful effect that would prevent the microbe from growing. The bacterium and no medication are present in the negative control tube. This tube is used to check on the proper growth of the bacterium.

The lowest concentration of a medication that prevents the growth of microorganisms is known as the minimum inhibitory concentration, or MIC. Each drug's absorbance value at various dilutions was contrasted with that of the bacterial positive control. For every dilution, the percentage of growth inhibition was computed by utilizing both positive and negative controls. A bacterial growth-free antibiotic's minimum dilution was defined as the minimum inhibitory concentration (MIC) value. Compound dilution (µg/mL): 125, 62.5, 500, 250, 1000, 500.

Antibiotics

1. Ampicillin (Antibacterial against gram-positive bacteria)
2. Chloramphenicol (Antibacterial against gram-positive bacteria)
3. Ciprofloxacin (Antibacterial against gram-negative bacteria)

4. Norfloxacin (Antibacterial against gram-negative bacteria)
5. Nystatin (antifungal against fungi)
6. Griseofulvin (Antifungal against fungi)
7. Gentamycin

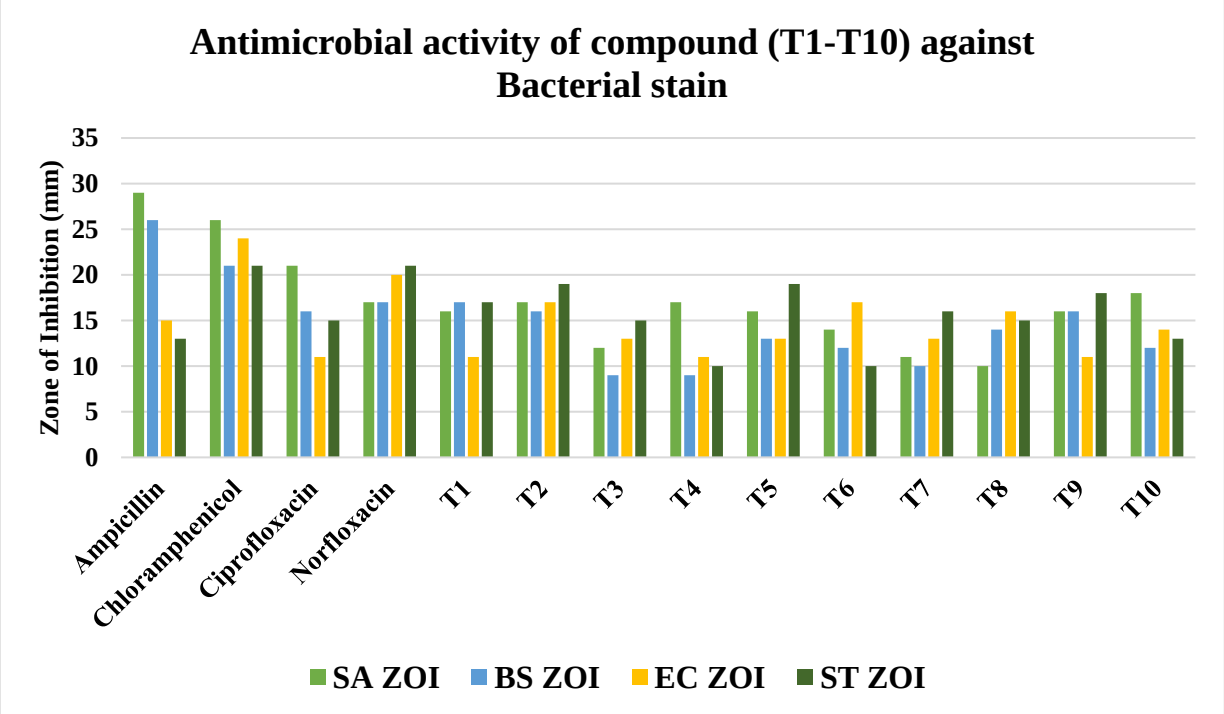
Test culture

1. Gram Negative: Escherichia Coli & Salmonella Typhimurium
2. Gram positive: Staphylococcus Aureus & Bacillus Subtilis
3. Fungi: Aspergillus Niger & Aspergillus clavatus, Candida, Albicans

8.3 Antimicrobial activity of compound (T1-T10) against Bacterial stain

Compounds	SA	SA	*BS*	*BS*	EC	EC	ST	ST
	Conc (µg/mL)	ZOI	Conc (µg/mL)	ZOI	Conc (µg/mL)	ZOI	Conc (µg/mL)	ZOI
Ampicillin	250	29	250	26	100	15	100	13
Chloramphenicol	50	26	50	21	50	24	50	21
Ciprofloxacin	50	21	50	16	25	11	25	15
Norfloxacin	10	17	100	17	10	20	10	21
T1	200	16	200	17	200	11	200	17
T2	200	17	200	16	200	17	200	19
T3	200	12	200	9	200	13	200	15
T4	200	17	200	9	200	11	200	10
T5	200	16	200	13	200	13	200	19
T6	200	14	200	12	200	17	200	10
T7	200	11	200	10	200	13	200	16
T8	200	10	200	14	200	16	200	15
T9	200	16	200	16	200	11	200	18
T10	200	18	200	12	200	14	200	13

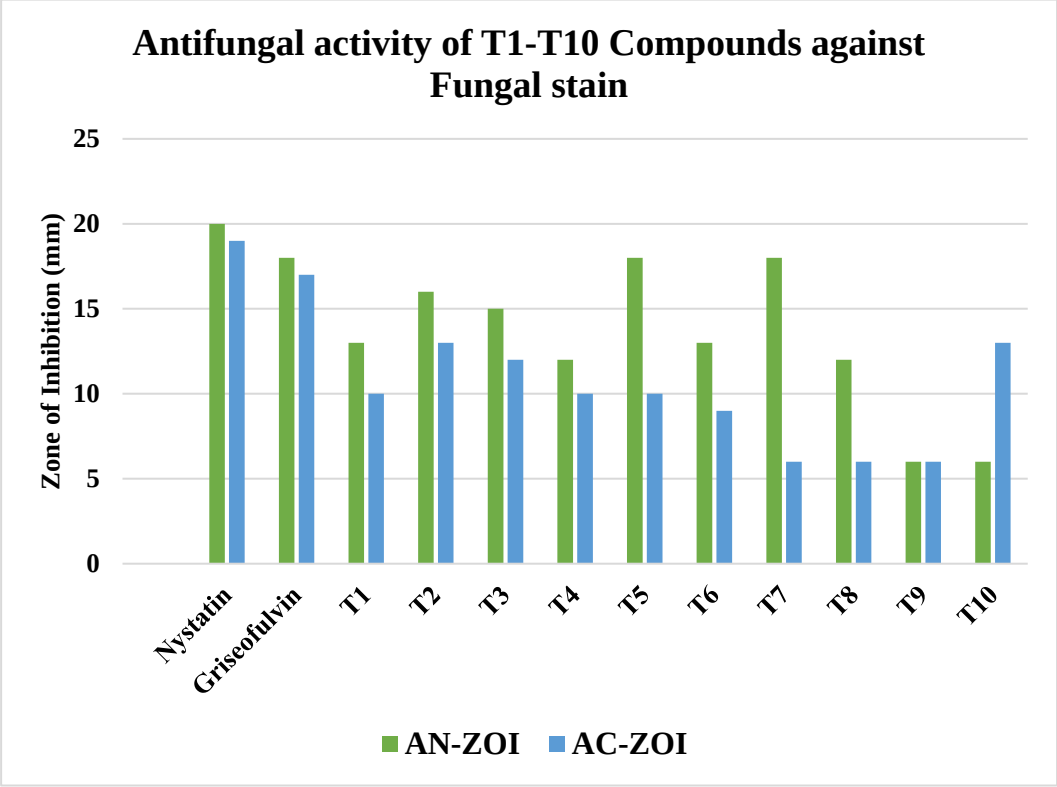
8.4 Graphical Representation of antimicrobial activity of compound (T1-T10) against Bacterial stain



8.5 Antimicrobial activity of compound (T1-T10) against Fungal stain

Compounds	AN	AN-ZOI	AC	AC-ZOI
Nystatin	100	20	100	19
Griseofulvin	100	18	100	17
T1	250	13	350	10
T2	100	16	300	13
T3	250	15	300	12
T4	200	12	250	10
T5	250	18	200	10
T6	250	13	100	9
T7	125	18	250	6
T8	250	12	350	6
T9	200	6	125	6
T10	400	6	500	13

Graphical Representation of antimicrobial activity of compound (T1-T10) against Bacterial stain



9. Conclusion

To summarise, in earlier chapters, we discussed the pyrazole as a potent microbial agent. In this chapter, we discuss their applications in relation to substituted aromatic Phenacyl bromide (T1 to T10) for their biological assessment. The synthesized compounds T1, T2, T4, and T10 were found to be effective against gram-positive bacteria; additionally, T1, T2, T6 and T9 were found to be effective against gram-negative bacteria; additionally, T2, T3 and were found to be effective as an anti-fungal agent; consequently, T1, T2 and T6 were found to be broad spectrum antibiotic molecules in comparison to other in this.

In this section of the study, we also concentrate on the biological activity of synthesized metal complexes using the ligands discussed in earlier chapters. We discover that T1, T2, T4, T6, T9 and T10 have good antibacterial and antifungal properties, and we also highlight T1 and T2, which demonstrate satisfactory results when compared to other molecules in the gram-positive, gram-negative, and fungal species.

As a result, the synthetic compounds' antibacterial results are expected, given their notable activity when compared to commercially available or standard medications. New antimicrobial agents can be designed and developed with the help of these findings.

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