Original Article

Saccharomyces cerevisiae Catalyzed Synthesis and Evaluation of Pyrazoles

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ABSTRACT

Background: Biocatalysis in organic solvents has several benefits over aqueous solvents, including solubility of organic substrates, ease of workup, separation of the product, and, in certain cases, reusability of biocatalysts. **Materials and Methods:** A simple, effective, and environmentally friendly technique for synthesizing pyrazoles has been established, which involves the cyclo condensation of chalcones and isonicotinic acid hydrazide in organic solvents using a relatively inexpensive catalyst baker's yeast (*Saccharomyces cerevisiae*). Molecular properties prediction and docking studies were also performed. **Results:** The predicted molecular properties suggest that the molecules are safe for oral consumption and their docking studies on 2BVC-*Mycobacterium tuberculosis* glutamine synthetase show their favorable ligand binding nature. The structural assignments were validated based on spectrum data. The compounds were evaluated for their anti-tubercular, *in vitro* anti-inflammatory, and anti-oxidant effects. The analysis reveals that synthesized derivatives of pyrazoles possessing methoxy, nitro, hydroxyl, fluoro, and chloro substitution through the phenyl ring help the molecule to increase its pharmacological activities. However, the substituted thiophene ring in the side chain also facilitates the biological action of the molecules. **Conclusion:** Anti-inflammatory, anti-oxidant, and anti-tubercular properties are due to the presence of the pyrazole ring.

Keywords: Pyrazoles, Chalcones, *Saccharomyces cerevisiae*, *In vitro* Anti-inflammatory, Antioxidant, Anti-tubercular.

INTRODUCTION

Pyrazoles are appealing medicinal development targets due to their broad variety of biological activities.[1-3] NSAIDs are used to treat a wide variety of conditions, including rheumatoid arthritis, respiratory tract infections, fever, soft tissue lesions, and oral cavity lesions. Celecoxib 1 (Pyrazole nucleus COX-2 inhibitor) is a non-addictive anti-inflammatory and analgesic. (Figure 1). COX-2 is selectively inhibited by a diaryl heterocyclic template in this type of diaryl heterocyclic model. Chalcone-based molecules, on the other hand, have bioactive properties such as those listed above, as well as anti-inflammatory and antibiotic properties. Pain and inflammation-related conditions are currently treated with NSAIDs containing thiophenes, such as tiaprofenic acid 2 and tenidap 3. (Figure 1).[4-8] The inclusion of pyrazole-containing derivatives in new anti-TB drugs could be extremely beneficial. According to the study, MABA's assay of newly developed pyrazole dérivatives was found to be effective against MTB H37Rv when tested *in vitro* (ATCC 27294).[9] Biocatalysis has several advantages, including chemically soluble substrates, easy processing, and product separation. We used baker's yeast as a biocatalyst to make pyrazoles from chalcones and isonicotinic acid hydrazide. For example, baker's yeast can be used as a biocatalyst in the addition of Michael's reaction, the Aldol reaction, and the Henry reaction.[10-15] The toxicity of

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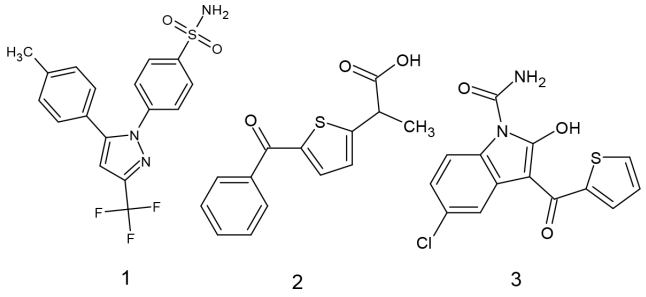


Figure 1: COX-2 inhibitors that comprise the pyrazole nucleus.

the noble compounds was also predicted using RO5 analysis and a preclinical evaluation.[16-17]

EXPERIMENTAL WORK

Computational studies

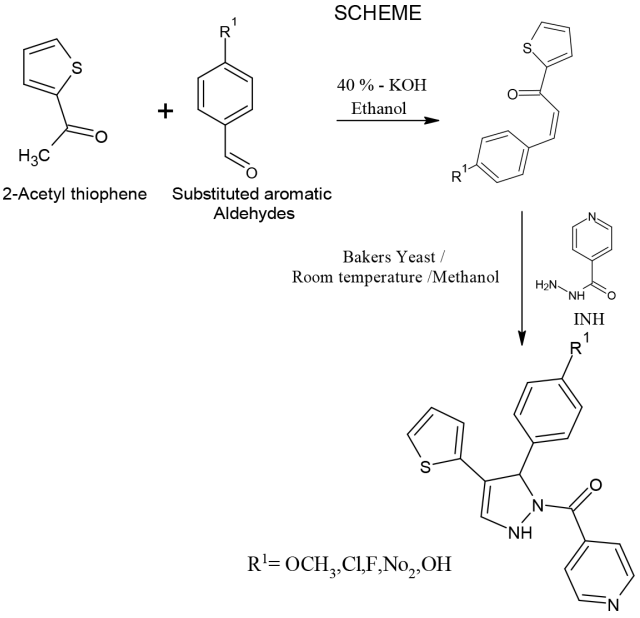
ADME properties were predicted for the substances. We used the TPSA to calculate the percentage of absorbance. Percent ABS = 109 (0.345xTPSA). Toxicological endpoints can be predicted using LAZAR (long-term toxicity, reproductive toxicity, and carcinogenicity).[18] When assessing drug conformity, OSIRIS, for example, considers aspects of the drug's structure as well as the risk of undesirable side effects. (www.cbligand.org/BBB/) was used to predict the blood-brain barrier. The molecular simulation program Autodock 4.2.6 was used to investigate the docking of receptor compounds (PDB ID: 2BVC- Mycobacterium tuberculosis glutamine synthetase).[19]

Synthesis of 2-Acetyl-Thiophene based Chalcones

Substituted aromatic aldehydes and 2-acetyl thiophene (0.01mol) were needed to make chalcones in ethanol (30 ml) with 40% aqueous KOH (15 ml). Diluting with hydrochloric acid in the mixtures resulted in acidification. It was then purified by column chromatography on silica gel using hexane/ethyl acetate (9:1) to provide chalcones.

Preparation of Pyrazoles

To dissolve the yeast (1g) along with isonicotinic acid hydrazide (0.01 mole) and chalcones (0.01 mole), we used about 15 mL of methanol. A magnetic stirrer was used to keep the reaction mixture moving at room temperature. The reaction could be tracked using thin-layer chromatography as it progressed. After the 32-hr reaction was completed, the catalyst was removed from the silica bed and rinsed with methanol solution (50 mL). Condensing the reaction mixture in an enclosed chamber under extreme pressure and vacuuming it up produced crude products. With the help of ethanol, the recrystallization process was carried out.



In-vitro Anti-inflammatory Activity

Heat-induced hemolysis

In the experiment, 1 ml of 100-500 µg/ml samples were used along with a 1 ml of 10% RBC suspension serving as the control. Before being placed in a test tube, it was mixed with saline solution. Ibuprofen is one of the most commonly prescribed medications and is used as a standard drug. The centrifuge tubes were removed after 30 min of cooling in a water bath set to 56°C. An absorbance measurement was taken at a wavelength of 560 nm after centrifuging the reaction mixture for five minutes at a speed of 2500 rpm. All samples were tested three times to ensure the best results.[20-21] To Figure out the percentage of hemolysis is being inhibited, the following formula used. Percentage inhibition = ((Abs control –Abs sample) / Abs control) × 100.

Inhibition of Albumin Denaturation

To lower the pH, 2 ml of 100-500 µg/ml DMSO and 0.2 percent bovine albumin fraction were added to the reaction mixture, along with a trace amount of 1N HCL. The toxicity levels were assessed after a 20-min incubation period and à subsequent 20-min heat treatment. The Model 371 UV Visible Spectrophotometer is made by Elico India Ltd. It was repeated three times during the experiment. Saline and a 1% bovine albumin solution (0.2 milliliters each) were used as controls in experiments.[22-23] Percentage inhibition = ((Abs control –Abs sample) / Abs control) × 100.

DPPH Assay

Using 2,2-diphenyl-1-picrylhydrazyl, the ability of pyrazole derivatives to scavenge free radicals was

Table 1: Molecular properties parameters important for good bioavailability.

Property	(M)	(C)	(F)	(N)	(H)
Formula	$C_{20}H_{17}N_3O_2S$	$C_{19}H_{14}ClN_3OS$	$C_{19}H_{14}FN_3OS$	$C_{19}H_{14}N_4O_3S$	$C_{19}H_{15}N_3O_2S$
Molecular weight	363.43 g/mol	367.85 g/mol	351.40 g/mol	378.40 g/mol	349.41 g/mol
Number of rotatable bonds	5	4	4	5	4
Number of Hydrogen bond acceptors	4	3	4	5	4
Number of Hydrogen bond donors	0	0	0	0	1
Molar Refractivity	109.10	107.61	102.56	111.43	104.63

Table 2: Pharmacokinetic parameters important for good oral bioavailability of pyrazoles.

Compound code	ClogP [a]	TPSA [b]	Blood-brain barrier prediction [c]	% ABS
M	1.58	82.7	-0.002	80.46
C	2.25	73.47	-0.015	83.65
F	1.75	73.47	0.001	83.65
N	0.73	119.2	-0.037	67.87
H	1.3	93.7	-0.047	76.67

Note: a: Calculated by ChemDraw Ultra 12.0, b: Calculated by Molinspration tool, c: Predicted at https://www.cbligand.org/BBB/index.php

Table 3: Toxicity prediction of titled pyrazole derivatives.

Compound code	Mutagenic	Tumorigenic	Irritant	Reproductive effect
1	No	Yes	No	Yes
2	No	Yes	No	Yes
3	No	Yes	No	Yes
4	No	Yes	No	Yes
5	No	Yes	No	Yes

investigated (DPPH). In concentrations ranging from 10-50 µg/ml, DMSO is available. This experiment includes an ascorbic acid positive control. After 30 min of mixing in a dark room with equal volumes of the drug solution and DPPH solution, triplicates of the 517 nm absorbance were measured.[24-25] The following equation was used to calculate the effect of DPPH on scavenging: Scavenging effect (%) = (Control−sample O.D./control O.D.)×100.

Nitric oxide scavenging activity

To compare the results, pyrazole derivatives were incubated with sodium nitroprusside (SNP) concentrations of 1, 2, or 5 mM. Phosphate buffer 0.1 M (pH 7.4) was used to make all of the solutions, which were then poured into test tubes. The light activates the NO+ molecule in SNP. The nitrite levels were measured after 30 min of incubation. For the measurements, Greiss' reagent contained sulphanilamide (1%) and naphthyl ethylenediamine (5% phosphoric acid solution) (0.10). After 10 min of incubation at room temperature, the absorbance at 546 nm was measured three times.[26-27] Scavenging effect (%) = (Control−sample O.D./control O.D.)×100. The statistical analysis was performed using GraphPad Prism 5.

Anti-tubercular Activity

MABA (Microplate Almar Blue Analysis) can be used to test antimycobacterial activity against M. tuberculosis (H37 RV strain: ATCC 27294). This non-lethal, heat-resistant reagent exemplifies the BACTEC radiometric approach. The medium's stability is improved by incubating the test wells in 200 µl of sterile deionized water in each of the plate's 96 wells. For the 96-well plate, 100 µl dilutions in middle brook 7H9 broth are prepared. This study used a concentration range of 100-0.8 µg/ml. The plates are kept in a paraffin-wrapped container at 37°C for five days. The plate is stained with Alamar blue staining solution and left to dry at room temperature for 24 hr. The introduction of a blue and pink tint was a significant step forward.[28]

RESULTS AND DISCUSSION

All of the synthesized molecules in Table 1 could be good candidates for orally active agents with high bioavailability, according to Lipinski's rule of five (Table 1). The pharmacokinetic parameters in Table 2 are required for good oral bioavailability. They do not affect the brain due to their low blood-brain barrier permeability. Due to the presence of unsubstituted and lipophilic halogen or electron-donating groups, the phenyl nucleus of these derivatives had a very small surface area (TPSA). Because they are hydrophilic, their TPSA scores indicate that they are more likely to cross the blood-brain barrier. ABS was found to be responsible for 66% to 76% of all named thiophenes. Based on their toxicity, all compounds are predicted to have tumorigenic and reproductive effects (Table 3).

Spectral Characterization

M: [5-(4-methoxyphenyl)-4-(thiophen-2-yl)-2, 5-dihydro-1H-pyrazol-1-yl] (pyridin-4-yl) methanone

Yellow crystals. Yield 82%. m.p: 85°C. R*f* = 0.34. IR (ν_{max}, cm^{-1}): 3431.73, 3078.47, 2925.97, 2849.03, 1889.55, 1646.38, 1461.54, 1016.03. ^{1}H –NMR (500 MHz, $CDCl_3$): δ_H 7.526-7.507 (1H, q, J 9.5 Hz), 7.237-7.219 (1H, q J 9Hz, Ar-H), 4.265 (3H, s, OCH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ_C Carbonyl carbon-184.15; alkene-aromatic-152.692, 151.240, 140.351, and 135.056; Alkene-aromatic-129.958, 129.725, 129.632, 129.034; O-CH_3 -40.576- 39.485; allylic next to double bond-22.745, 21.523. MS (ESI, m/z): 363.42. Anal. calcd for $C_{20}H_{17}N_3O_2S$ (363.43): C (66.10%), H (4.71%), N (11.56%), O (8.80%), S (8.82%), found: C (66.20%), H (4.65%), N (11.50%), O (8.70%), S (8.90%).

C: [5-(4-chlorophenyl)-4-(thiophen-2-yl)-2,5-dihydro-1H-pyrazol-1-yl] (pyridin-4-yl) methanone

Yellowish orange crystals. Yield 80%. m.p: 105°C; R*f* = 0.51. IR (ν_{max}, cm^{-1}):3434.07 (Amine N-H stretch 3400-3500), 3078.55, 2924.64, 2856.12, 1645.76, 1217.27, 813.84. ^{1}H-NMR (500 MHz, $CDCl_3$): δ_H 7.538-7.520 (1H, q, J 9 Hz), 7.253-7.235 (1H, q, J 9 Hz), 4.284 (2H, s, R-CH_2- Cl). ^{13}C NMR (75 MHz, $CDCl_3$): δ_C Carbonyl Carbon-169.825, alkene- aromatic-150.174, 137.321, 123.297, 114.048, (O-CH_3 -40.482 to 39.480, Chloro-C- 34.549. MS (ESI m/z): 289.76 $[M+H]^+$. Anal. calcd for $C_{19}H_{14}ClN_3OS$ (367.85): C (62.04%), H (3.84%), Cl (9.64%) N (11.42%), O (4.35%), and S (8.72%), found: C (62.21 %), H (3.72 %), Cl (9.68 %) N (11.31%), O (4.34 %), and S (8.70%).

F: [5-(4-fluorophenyl)-4-(thiophen-2-yl)-2,5-dihydro-1 H-pyrazol-1-yl] (pyridin-4-yl) methanone

Orange red crystals. Yield 76%. m.p: 115°C. R*f* = 0.59. IR (ν_{max}, cm^{-1}): 3364.13, 3079.32, 2925.23, 1647.79, 1341.75, 826.92. ^{1}H-NMR (500 MHz, $CDCl_3$): δ_H 8.743-8.660 (1H, m, J 9 Hz), 10.025 (1H,s), 11.777(1H,s), 4.284 (2H, s, R-CH_2-F). ^{13}C NMR (75 MHz, $CDCl_3$): δ_C carbonyl Carbon-173.142, alkene- aromatic- 169.621, 161.860, 153.418, 149.614, 148.983, 133.317, 129.721, 129.018, 126.558, 123.654, 119.298, O-CH_3 - 40.471 to 39.470, allylic next to double bond-22.894, fluoro-C-22.793). MS (ESI m/z):351.40 $[M+H]^+$. Anal. calcd for $C_{19}H_{14}FN_3OS$ (351.40): C (64.94%), H (4.02%), F (5.41%), N (11.96%), O (4.55%), S (9.12%), found: C (64.88%), H (4.14%), F (5.32%), N (11.82%), O (4.48%), S (9.02%).

N: [5-(4-nitrophenyl)-4-(thiophen-2-yl)-2,5-dihydro-1H-pyrazol-1-yl] (pyridin-4-yl) methanone

Yellowish orange crystals. Yield 74%. m.p: 140°C. R*f* = 0.50. IR (ν_{max}, cm^{-1}): 3091.18, 2924.52, 1647.76, 1342.14, 1513.26, 830.73. ^{1}H-NMR (500 MHz, $CDCl_3$): δ_H 8.379-8.375 (1H, d, J 2 Hz), 8.213-8.191 (1H, q, J 11 Hz), 8.049-8.027 (1H, q, J 11 Hz), 7.893-7.887 (1H, d, J 3 Hz), 7.763(1H, s), 7.543-7.444 (1H, m, 49.5 Hz), 7.318(1H, s), 7.124 (1H, s). ^{13}C NMR (75 MHz, $CDCl_3$): δ_C Carbonyl Carbon-169.877, Alkene-aromatic- 150.158, 137.647, 123.120, 114.079, O-CH_3 - 40.494, 40.327, 40.160, 39.993, 39.826, 39.659, 39.492, allylic next to double bond- 34.486. MS (ESI m/z): 378.39 $[M+H]^+$. Anal. Calcd for $C_{19}H_{14}N_4O_3S$ (378.40): C (60.31%), H (3.73%), N (14.81%), O (12.68%), S (8.47%), found: C (60.24%), H (3.61%), N (14.68%), O (12.54%), S (8.40%).

H: [5-(4-hydroxyphenyl)-4-(thiophen-2-yl)-2,5-dihydro-1H-pyrazol-1-yl] (pyridin-4-yl) methanone

Yellowish crystals. Yield 68%. m.p: 130°C. R*f* = 0.38. IR (ν_{max}, cm^{-1}): 3243.60, 2922.27, 2821.62, 1642.16, 1350.37, 817.86. ^{1}H-NMR (500 MHz, $CDCl_3$): δ_H 7.526-7.507 (1H, q, J 9.5 Hz), 7.237-7.219 (1H, q J 9Hz), 4.265 (1H, s, OH). ^{13}C NMR (75 MHz, $CDCl_3$): δ_C Carbonyl Carbon-173.18, Alkene- aromatic- 149.589, 149.062, 148.937, 134.14, 126.022, 118.789. MS (ESI m/z): 349.40 [M+H] +; Anal. Calcd for $C_{19}H_{15}N_3O_2S$ (349.41): C (65.31%), H (4.33%), N (12.03%), O (9.16%) S (9.18%), Found: C (65.18 %), H (4.21%), N (11.91%), O (9.02%) S (9.12%).

Biocatalysts (Baker's Yeast) were used to synthesize a wide range of compounds. To identify and characterize all derivatives, physical methods such as melting point, thin layer chromatography, and spectral analysis such as UV, IR, and NMR were used (^{1}H and ^{13}C). Tables 1-4 show the predicted molecular properties, pharmacokinetics, and toxicity studies for each designed moiety. These substances, in my opinion, are safe for oral consumption. Table 4 shows the docking results for molecules generated by autodock. For protein 2BVC-*Mycobacterium tuberculosis* glutamine synthetase, Compound M docked with a score of -8.20 kcal/mol. This enzyme has the potential to improve bacterial nitrogen metabolism. Figure 2 depicts a two-dimensional

Table 4: Docking scores of synthesized pyrazole molecules by Autodock.

Sl. No	Receptor	Docking scores in Kcal/mol				
		M	C	F	N	H
1	2BVC	-8.20	-6.86	-6.07	-4.49	-5.03

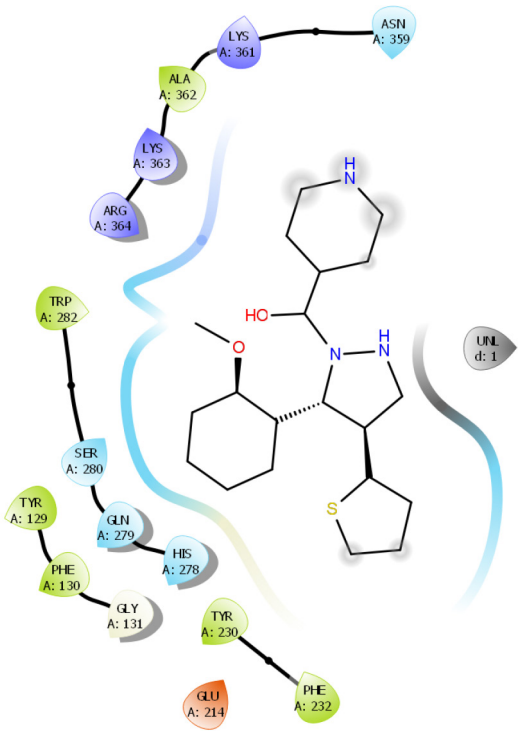


Figure 2: 2D interaction between Compound M and amino acids of 2BVC.

(2D) interaction between an amino acid and compound M. Nonpolar, polar, and basic residues are separated by only six angstroms. This compound is expected to interact with several hydrophobic residues, including Phe 130, Tyr 129, Phe 130, Tyr 230, Phe232, Trp232, and Ala 362, due to its binding location on 2BVC near the lipid-water interface. Arg 364 could also be found near the lipid-water interface, which would explain its presence. All of the following conditions must be met for ligand binding activity to occur:

In vitro anti-inflammatory activity of the drug is compared to that of the standard drug Ibuprofen in Figures 2-5. Sample M inhibited heat-induced hemolysis by 96.34 and, Sample C by 95.46%, Sample F by 95.70%, Sample N by 96.82%, and Sample H by 97.00% when tested at 500 g/ml. According to the study, all of the compounds had better activity at a concentration of 500 g/ml. Ibuprofen has 97.56% activity. The IC_{50} values for the various compounds tested for the M, C, F, N, H were 0.2020, 0.4890, 1.803, 1.767, 1.165 ug/ml, respectively.

Sample-M showed 74.13% inhibition, Sample-C 68.96, Sample-F 70.68%, Sample-N 77.58%, and Sample-H 79.31% at 500 µg/ml and ibuprofen with 76.55% in an Albumin Denaturation Studies (Figures 3 and 4). The IC_{50} values for the various compounds tested for the M, C, F, N, H, and ascorbic acid were 136.4, 194.5, 322.4, 182.1, and 190.5 µg/ml respectively.

All of the substances studied have high anti-oxidant activity, according to the DPPH scavenging experiment. Figure 5 depicts the results. The most effective inhibitors were M (69.51%), C (68.940%), and F (50.14%), while N (68.37%) and H (70.37%) were the standard ascorbic acid (58.11%). Nitric oxide scavenging activity percentage for compounds M (34.340%), C (40.420%), and F (58.660%), while N (25.220%) and H (45.280%) and standard ascorbic acid (58.22%). IC_{50} values for the various compounds tested for the M, C, F, N, H, and ascorbic acid were 170.6, 114.8, 34.17, 1039,67.38,28.84 ug/ml respectively. All the compounds showed moderate nitric oxide scavenging activity than standard ascorbic acid.

The pyrazole-based agent appears to be the most effective scavenger. Concentration boosts activity. An aromatic system may have aided electron-withdrawing groups to absorb free radicals, like reactive oxygen and nitrogen. The ability of the compounds to scavenge free radicals is due to the NH proton of the pyrazole

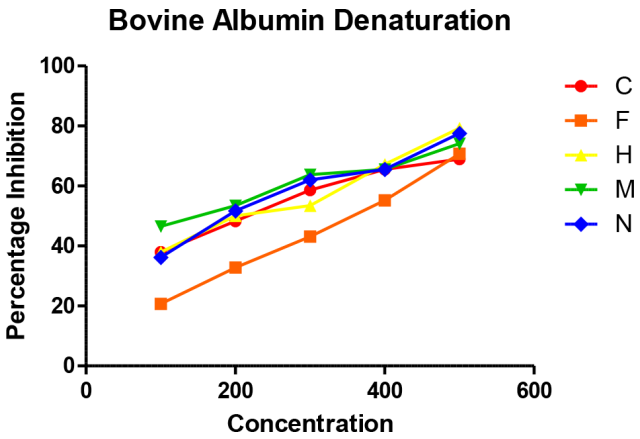


Figure 3: Bovine albumin denaturation activity of pyrazole derivatives.

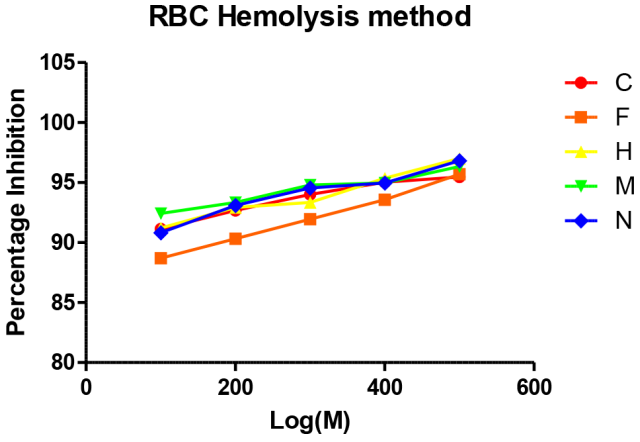


Figure 4: Heat-induced Haemolysis activity of pyrazole Derivatives.

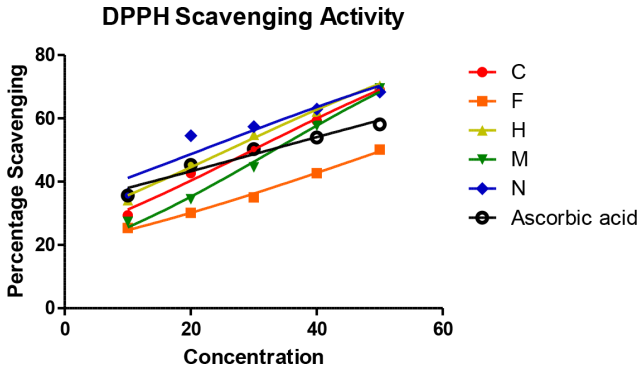


Figure 5: DPPH scavenging activity of pyrazole derivatives.

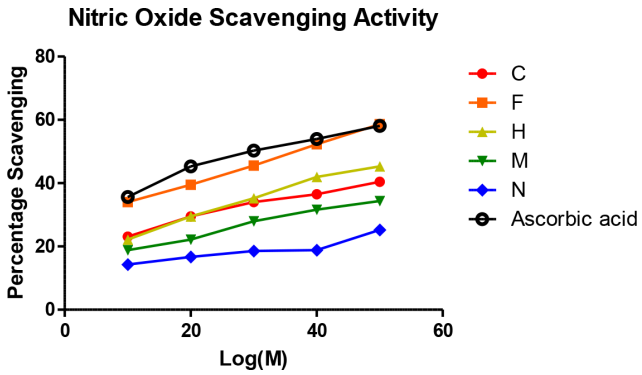


Figure 6: Nitric oxide scavenging activity of pyrazole derivatives.

Table 5: MIC values for the anti-tubercular activity of pyrazoles.

Samples	100 µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml	3.12 µg/ml	1.6 µg/ml	0.8 µg/ml
Nitro	S	S	S	R	R	R	R	R
Methoxy	R	R	R	R	R	R	R	R
Fluoro	R	R	R	R	R	R	R	R
Chloro	R	R	R	R	R	R	R	R
Hydroxy	S	R	R	R	R	R	R	R

Note: S – Sensitive; R- Resistant; Standard values for the Anti-Tb test which was performed. Pyrazinamide-3.125µg/ml; Ciprofloxacin-3.125µg/ml; Streptomycin-6.25µg/ml.

moiety. Nitric oxide release measurements are not up to the mark concerning the standard (Figure 6).

When compared to standard concentrations of Pyrazinamide, Ciprofloxacin, and Streptomycin, a 25 µg/ml concentration of Compound N was found to be effective against a mycobacterium strain. Our compounds activity is quite low when compared to experimental standards (Table 5 and Figures 7 and 8).

The substitutions N1, C3, and Can be particularly useful in the study of pyrazole. Substituting electron-withdrawing substitutes into the molecule accomplishes

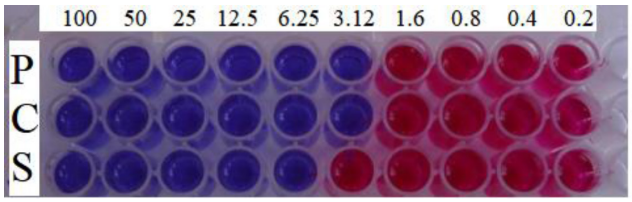


Figure 7: Standard drug photograph MIC values of pyrazoles on MABA Assay.

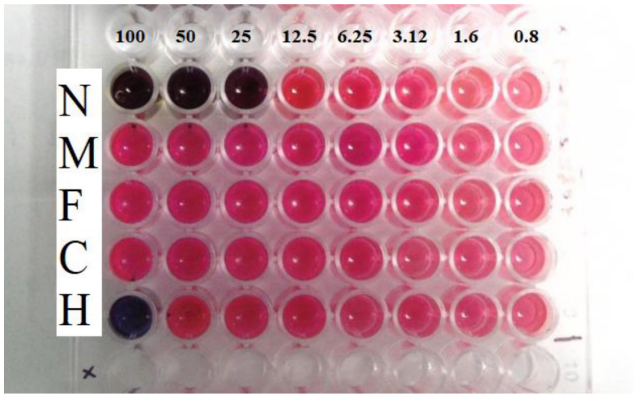


Figure 8: Photograph of MIC values of pyrazoles on MABA Assay.

this. By incorporating electron-withdrawing groups like nitrogen on the ortho position of aromatic rings, these compounds have been shown to significantly improve their reductive properties. Compound N has very mild inhibition regarding the MIC values for the anti-tubercular activity of compounds.

CONCLUSION

Basic research looked into the Physiochemical, toxicological, docking investigations, synthesis, and biological screening of new pyrazole derivatives. In moderate conditions, baking yeast produces pyrazoles from isonicotinic acid hydrazide and chalcone with better yields. Methanol can be used to make large amounts of the resulting compounds. When potassium hydroxide is mixed with substituted thiophenes and benzaldehydes in water, this reaction produces chalcones. Using baker's yeast and an organic solvent, the pyrazole can be synthesized from chalcones with excellent to moderate yields, depending on the yield. The project's main objectives are to cut costs while also protecting the environment. FTIR, ^{1}H-NMR, ^{13}C-NMR, and Mass Spectroscopy were used to confirm novel pyrazole derivatives. Anti-inflammatory, anti-oxidant, and anti-tubercular properties are due to the presence of the Pyrazole ring.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

NSAIDs: Non-steroidal anti-inflammatory drugs; **COX:** Cyclooxygenase; **MABA:** Microplate Alamar blue assay; **TB:** Tuberculosis; **MTB:** Mycobacterium tuberculosis; **RO5:** Rule of five; **ADME:** Absorption, Distribution, Metabolism, Excretion; **TPSA:** Total polar surface area; **PDB:** Protein data bank; **KOH:** Potassium hydroxide; **RBC:** Red blood cells; **DMSO:** Dimethyl sulfoxide; **HCL:** Hydrochloric acid; **UV:** Ultra-violet; **DPPH:**2,2-diphenyl-1-picrylhydrazyl; **O.D:** Optical density; **SNP:** Sodium nitro prusside; **ABS:** Absorbance; **m.p:** Melting point; **R_f:** Retention factor; **IR:** Infrared Spectroscopy; **^{1}H–NMR:** Proton nuclear magnetic resonance; **$CDCl_3$:** Deuterated chloroform; **^{13}C NMR:** Carbon-13 nuclear magnetic resonance; **MS:** Mass spectrometry; **ESI:** Electrospray Ionisation; **MIC:** Minimum inhibitory concentrations.

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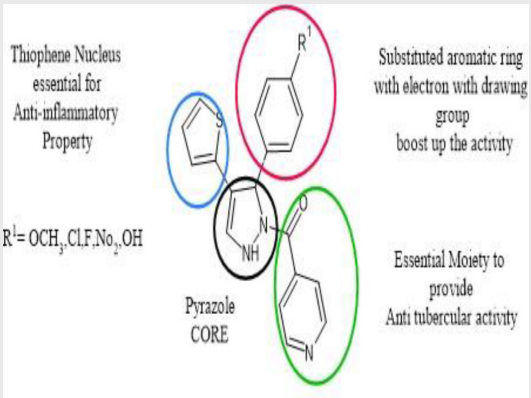
PICTORIAL ABSTRACT



SUMMARY

Baker's yeast is used as a catalyst in the cyclo condensation of chalcones and isonicotinic acid hydrazide in organic solvents. Predictions of molecular characteristics and docking studies were also carried out to forecast their biological effects. Based on the spectrum data, the structural assignments were confirmed. They were tested for anti-tubercular, *in vitro* anti-inflammatory, and antioxidant properties. According to the study results, synthetic derivatives of pyrazoles with phenyl ring substitutions such as methoxy or nitro or hydroxy or fluoro or chloro enhances the molecule's pharmacological activity.

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