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BIGINELLI REACTION: COMPARATIVE STUDY OF FRUIT JUICES, OPTIMIZATION STRATEGIES, *IN-SILICO* ANALYSIS AND PHARMACOLOGICAL ACTIVITY

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ABSTRACT

This research article explores the influence of fruit juices on the efficiency of the biginelli reaction, a versatile multicomponent synthesis for diverse dihydropyrimidinones. A comparative analysis of fruit juices as green reaction media assessed their impact on reaction rates, yields, and selectivity. Biginelli reactions were carried out in fruit juices using equimolar mixture of thiourea /urea, ethyl acetoacetate and aromatic aldehyde under microwave irradiation. The reaction progressed within minutes, monitored by TLC, and confirmed through Proton NMR spectroscopy. Synthesized derivatives were evaluated for binding affinity towards protein (PDB ID: 1HD2) by molecular docking. Compounds 1a and 2a exhibited high yields, 81% and 71% respectively. Molecular docking revealed good binding affinity for compounds 2a, 1a, and 3a. Compound 2a demonstrated remarkable antioxidant activity with IC_{50} values; 4.18(µg/ml) and 2.54(µg/ml) for DPPH and Nitric oxide radical scavenging assays. Compound 2a could be considered as useful scaffold for further development to potent dihydropyrimidinone derivatives for enhanced antioxidant activity.

Keywords: Biginelli reaction; Dihydropyrimidinones; Anti-oxidant activity; Molecular docking; Proton NMR; Ethyl acetoacetate.

INTRODUCTION

Heterocyclic compounds, consisting of various elements such as nitrogen, oxygen, and sulfur, represent a crucial category of organic compounds pivotal in pharmaceuticals, agrochemicals, and veterinary products [1,2]. The synthesis and application of heterocyclic compounds are integral to the understanding of cellular metabolism, underscoring their significance across diverse life forms[3]. Nitrogen-containing heterocyclic derivatives represent a significant class of compounds in pharmaceutical chemistry, contributing to our understanding of biological and pharmaceutical perspectives while aiding in the

comprehension of vital life processes[4]. Multicomponent reactions (MCRs), a vital synthetic tool, allow for the creation of diverse biologically active compounds with specific stereochemistry, further enhancing the importance of these compounds in drug development[5].

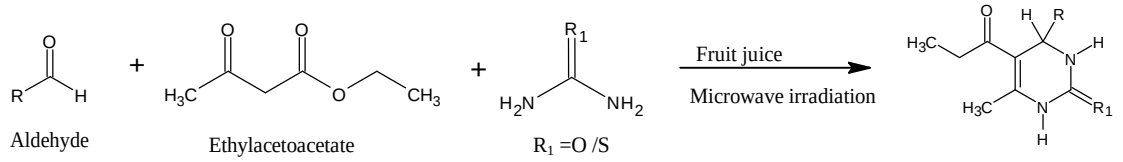
Notably, the Biginelli Reaction stands as a prime example, generating functionalized 3,4-dihydro-2(H)-pyrimidinones (DHPMs), where DHPM acts as a critical building block essential for pharmaceutical production, particularly in combating viral infections. This reaction, catalyzed by Bronsted and Lewis acids like copper (II) trifluoroacetate hydrate and boron trifluoride on various solid phases, emphasizes the versatility and significance of heterocyclic compounds in drug synthesis[6].

The pursuit of sustainable and environmentally conscious methodologies in organic synthesis has led to the exploration of microwave-assisted reactions and alternative reaction media. Microwave-associated reactions offer compelling advantages over traditional methods, showcasing accelerated reaction rates, higher yields, and improved purity through selective heating[6]. Concurrently, the use of fruit juices as reaction media has emerged as an eco-friendly and cost-effective alternative, drawing attention for its natural properties and environmental compatibility[8].

This study aims to explore the incorporation of these innovative methods. Molecular docking studies are used to determine the interaction of two molecules and to find the best orientation of ligand which would form a complex with overall minimum energy[9]. The results are analyzed by a statistical scoring function which converts interacting energy into numerical values called as the docking score[10].

The incorporation of microwave-associated reactions and the utilization of fruit juices as reaction media offers a promising avenue for sustainable and innovative organic synthesis, reinforcing the current focus on green chemistry practices. This study seeks to investigate and analyze the potential benefits and limitations of these methods, aiming to contribute to the advancement of environmentally conscious methodologies in the field of organic synthesis.

Our aim was to explore the use of fruit juices as reaction media in the biginelli reaction, comparing their efficacy along with various reagents to determine which combination results in the highest product yield.



Aldehyde + Ethylacetoacetate + R_1 =O /S → DHPM

MATERIAL AND METHODS

Materials

All the chemicals employed in the synthesis (Benzaldehyde, Vanillin, 4-nitro benzaldehyde, Urea, Thoi-urea, Ethyl acetoacetate) were obtained from SD Fine-Chem limited, Qualikems Fine Pvt. Limited and Merck Chemicals. All other chemicals were of analytical grade purchased from local suppliers. The microwave employed for microwave irradiation was of domestic grade (Make: Whirlpool, model AVM562, 800W). The spectroscopical analysis and pharmacological activity was performed at Lifesciences Lab, Hyderabad.

Collection of juices

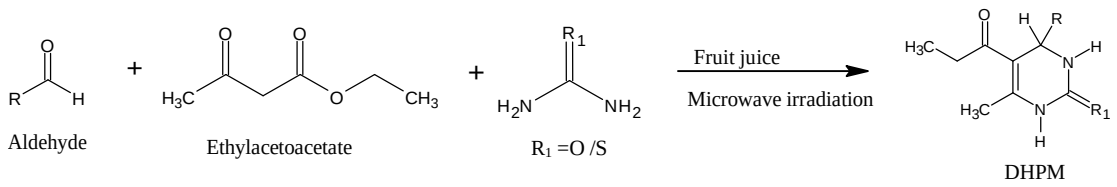
Fresh fruits were obtained from the local market and their juices were extracted and filtered using a Whatman filter paper. After filtering, the fruit juices were used directly for the reaction without adding any foreign chemicals into it. The employed fruit juices are pomegranate (pH 2.93-3.20) pineapple (pH 2.51-3.91).

General method for the synthesis of DHPM

In a 100 ml borosilicate conical flask 0.01 mole of urea/thiourea, 0.01 mole of ethyl acetoacetate and 0.01 mole of desired aldehyde were taken. Then 10 ml of desired fruit juice was added to this reaction mixture. The reaction mixture was irradiated at 180W under microwave condition with successive cooling and stirring of the reaction mixture at room temperature after every 1 min of MW irradiation.

The progress of the reaction was continuously monitored by TLC. After completion of reaction (which was indicated by TLC) the reaction mixture was cooled down to room temperature after which the solid crude product was slowly precipitated out of the reaction mixture. The crude product was recrystallized from hot ethanol to get pure DHPM as whitish/yellowish solid powder. The obtained DHPMs were characterized by melting point and NMR spectroscopy.

The benzaldehyde, vanillin and 4- nitro benzaldehyde was employed individually for one-pot multi component condensation reaction with urea/thiourea and ethyl acetoacetate at room temperature. The fruit juices namely pomegranate juice, lemon juice and pineapple juice were individually used as the reaction medium for performing these multi component condensation reactions (Biginelli reaction)[11].



Aldehyde + Ethylacetoacetate + R_1 =O /S → DHPM

Molecular docking

Protein Downloading: Open the Protein Databank www.wwpdb.org - search for protein (PDB ID: 1HD2). Download in PDB format. Open downloaded protein in Visualizer. Delete heteroatoms, ligands molecules, and water. The ligands were drawn in Chem Sketch. The molecule thus obtained were saved in PDB format.

Molecular docking was successfully conducted using AutoDock Vina in PyRx. PyRx was launched, and a new project was created. The receptor and ligand structures were imported, with the search space defined either automatically or manually. The docking parameters were configured to meet specific requirements, and the docking process was initiated. Progress was displayed by PyRx throughout the procedure. Once completed, the results were analyzed in the "Results" tab, allowing the visualization of docking poses, the evaluation of binding energies, and the examination of other pertinent data. Finally, the results were saved in the preferred file format, completing a thorough assessment of the interaction between the protein and ligand[12].

Pharmacological Activity

Anti-oxidant activity was performed using two methods

- DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging method
- Nitric oxide scavenging method

DPPH radical scavenging method

The DPPH solution was prepared using a suitable solvent. The Vitamin C standard solutions were prepared in an appropriate solvent, such as distilled water or phosphate-buffered saline (PBS). Equal volumes (e.g., 1 mL) of the Vitamin C standard solutions and the DPPH solution were taken in separate test tubes. Control tubes were prepared with only the DPPH solution and another with only the solvent (blank). The reaction mixture was incubated at room temperature for a specific period, typically 30 minutes to 1 hour. The radical scavenging activity of different samples was compared based on their inhibitory percentages. The radical scavenging activity was calculated by the formula given below.

DPPH scavenging effect (%)/% Inhibition=A0-A1/A0 × 100

Where A0=The absorbance of control, A1=The absorbance of sample.

Nitric oxide scavenging method

The Griess reagent was prepared. The gallic acid standard solutions with different concentrations (e.g., 10-100 µg/mL) were prepared in an appropriate solvent, such as distilled water or phosphate-buffered saline (PBS). Equal volumes (e.g., 1 ml) of the gallic acid standard solutions and sodium nitroprusside (SNP) solution were taken in separate test tubes. Control tubes were prepared with only the SNP solution and another with only the solvent (blank). The reaction mixture was incubated at a specific temperature, typically 25-37°C, for a defined time period (e.g., 30 minutes to 2 hours). An equal volume of the Griess reagent was added to all the reaction tubes, including the controls. Color development was allowed. The NO radical scavenging activity of different samples was compared based on their inhibitory percentages. The radical scavenging activity was calculated by the formula given below.

Percentage (%) of nitric oxide radical scavenging assay = [(A0-A1)/A0] × 100

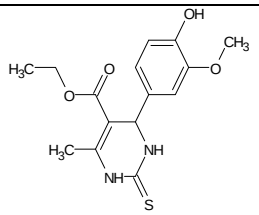
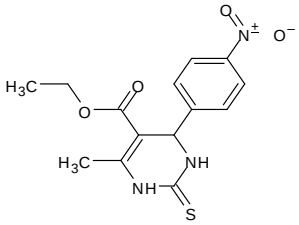
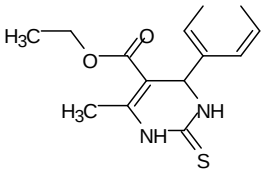
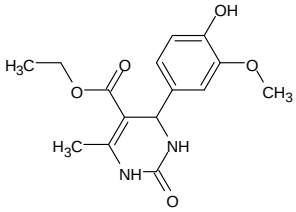
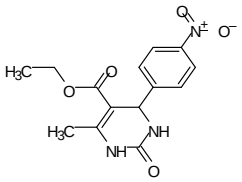
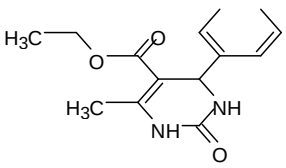
Where A0 = absorbance of control, and A1= absorbance of the treated sample

Table-1. REACTION DATA SUMMARY

Compound code	Aldehyde	Reagent	Fruit Juice	Time (mins)	Yield	Melting point(C°)
1a	Vanillin	Thiourea	Pomegranate	8-9	71%	80
1b	Vanillin	Thiourea	pineapple	7-8	68%	83
2a	4-Nitro benzaldehyde	Thiourea	pomegranate	8-9	81%	82
2b	4-Nitro benzaldehyde	Thiourea	pineapple	7-8	14%	85
3a	Benzaldehyde	Thiourea	pomegranate	8-9	31%	84
3b	Benzaldehyde	Thiourea	pineapple	7-8	13%	83
4a	Vanillin	Urea	pomegranate	8-9	57%	78
4b	Vanillin	Urea	pineapple	7-8	53%	85
5a	4-Nitro benzaldehyde	Urea	pomegranate	8-9	50%	87
5b	4-Nitro benzaldehyde	Urea	pineapple	7-8	21%	95
6a	Benzaldehyde	Urea	pomegranate	8-9	30%	86
6b	Benzaldehyde	Urea	pineapple	7-8	20%	88

In this study, the components obtained earlier were depicted using ChemSketch to visualize their molecular structures. Subsequently, their respective IUPAC names, molecular formulas, and molecular weights were determined. The melting points of compounds were determined using capillary tubes. These components were then subjected to molecular docking analysis to assess their binding affinity with the targeted protein.

Table 2. COMPOUND CHARACTERISTICS

Compound	Structure	IUPAC name	Molecular formula	Molecular weight (g/mol)
1		Ethyl-4-(4-hydroxy-3 methoxy phenyl) - 6 methyl -2 thioxo 1,2,3,4 tetrahydropyrimidine-5 carboxylate	$C_{15}H_{18}N_2O_4S$	322.37
2		Ethyl-6-methyl-4-(4nitrophenyl) - 2-sulfadiene-1,2,3,4 tetrahydropyrimidine-5 carboxylate	$C_{14}H_{15}N_3O_4S$	321.36
3		Ethyl-6-methyl-4-phenyl-2 sulfanilidine 1,2,3,4 tetrahydropyrimidine-5 carboxylate	$C_{14}H_{16}N_2O_2S$	276.36
4		Ethyl-4-(4-hydroxy-3 methoxy phenyl) - 6 methyl -2 oxo 1,2,3,4 tetrahydropyrimidine-5 carboxylate	$C_{15}H_{18}N_2O_5$	306.31
5		Ethyl-6-methyl-4-(4nitrophenyl)-2-oxo-1,2,3,4 tetrahydropyrimidine-5 carboxylate	$C_{14}H_{15}N_3O_5$	305.29
6		Ethyl- - 6 methyl -2 oxo-4phenyl-1,2,3,4 tetrahydropyrimidine-5 carboxylate	$C_{14}H_{16}N_2O_3$	260.29

In order to assess the progress of the reaction and analyze the conformation of the products, Thin Layer Chromatography (TLC) was employed. The results of the TLC analysis are illustrated in Figure 1.

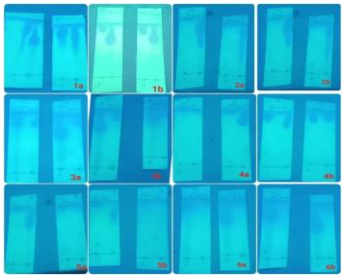


Figure 1. TLC of different reactions indicating their progress

The reactions have exhibited serious dependence on the acidity of fruit juice and the electronic nature of the aromatic aldehyde. It was worth to note that for any particular aldehyde, the reaction was fastest in pomegranate juice that has minimum pH.

NMR spectroscopy:

NMR spectroscopy was employed to discern and authenticate the synthesized compounds 1a, 2a, and 3a. The decision to focus on these specific compounds was primarily driven by their superior yields compared to the other derivatives synthesized in the study

Compound 1a:

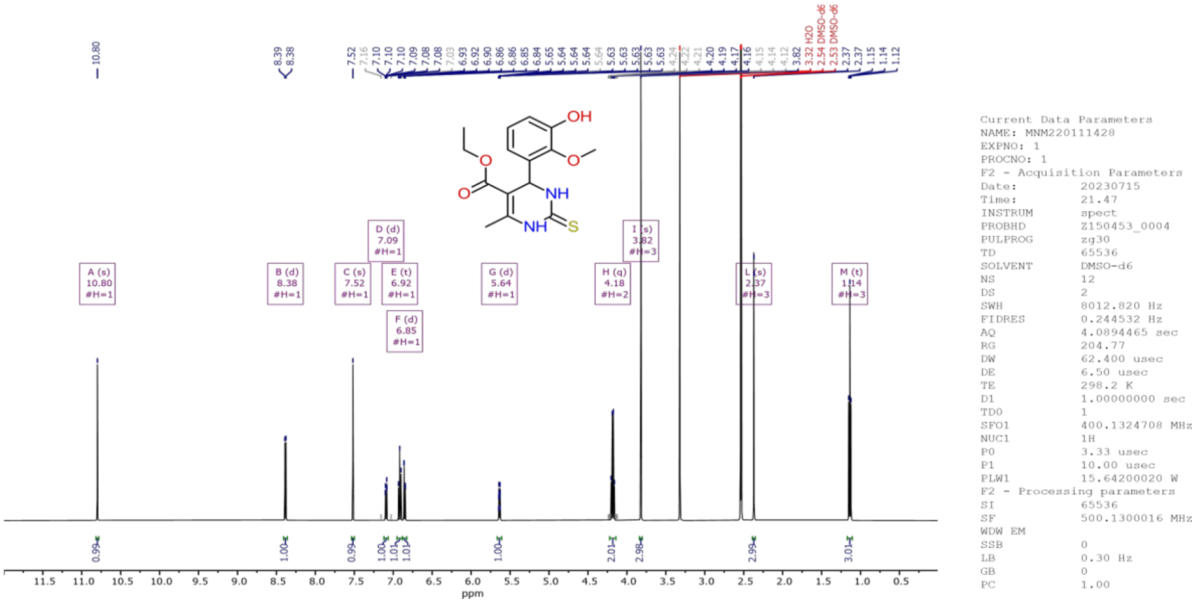


Figure 2. Proton NMR of 1a

Compound 1a: ^{1}H NMR (500 MHz, DMSO-d6) δ 10.799 (s, 1H), 8.384 (d, J = 6.639 Hz, 1H), 7.517 (s, 1H), 7.092 (d, J = 8.021, 0.885, 0.885 Hz, 1H), 6.917 (t, J = 8.128, 8.128 Hz, 1H), 6.853 (d, J = 8.342, 1.254 Hz, 1H), 5.637 (d, J = 6.642, 0.968, 0.968, 0.931 Hz, 1H), 4.181 (q, J = 7.219, 7.219, 7.172 Hz, 2H), 3.821 (s, 3H), 2.370 (s, 3H), 1.138 (t, J = 7.097, 7.097 Hz, 3H).
Compound 2a:

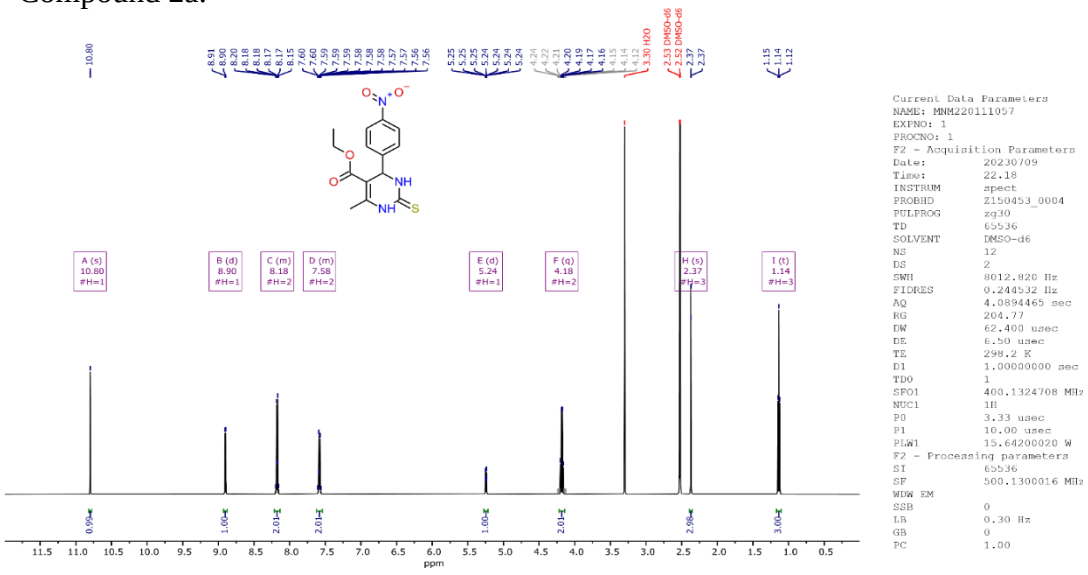


Figure 3. Proton NMR of 2a

Compound 2a: ^{1}H NMR (500 MHz, DMSO-d6) δ 10.799 (s, 1H), 8.902 (d, J = 6.410 Hz, 1H), 8.215 – 8.136 (m, 2H), 7.622 – 7.542 (m, 2H), 5.244 (d, 1H), 4.181 (q, J = 7.212, 7.212, 7.154 Hz, 2H), 2.370 (s, 3H), 1.138 (t, J = 7.097, 7.097 Hz, 3H).

Compound 3a:

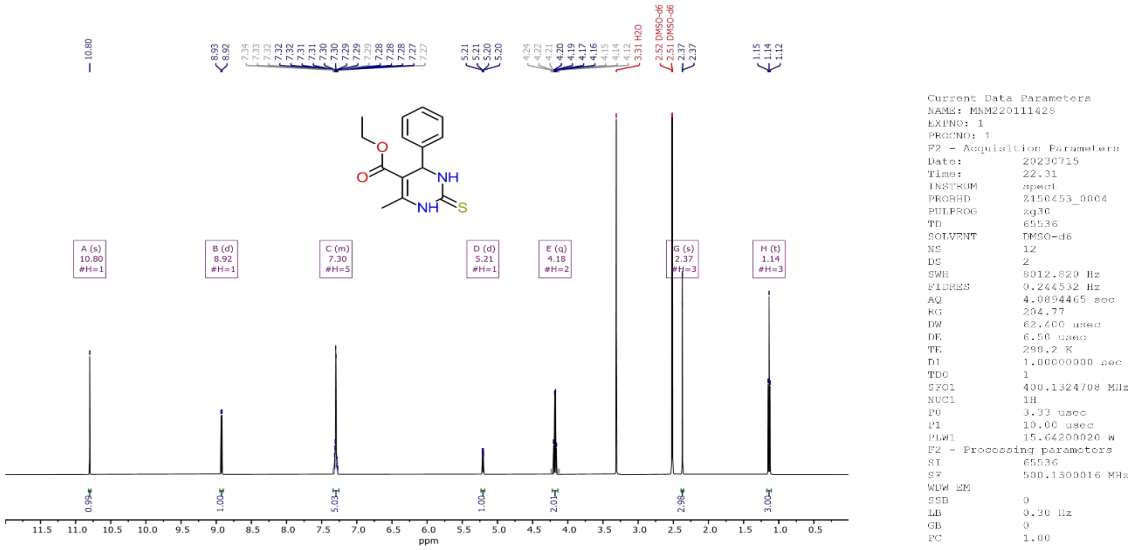


Figure 4. Proton NMR of 3a

Compound 3a: ^{1}H NMR (500 MHz, DMSO-d6) δ 10.799 (s, 1H), 8.925 (d, J = 6.410 Hz, 1H), 7.338 – 7.256 (m, 5H), 5.207 (d, J = 6.650, 1.158 Hz, 1H), 4.181 (q, J = 7.218, 7.218, 7.171Hz, 2H), 2.370 (s, 3H), 1.138 (t, J = 7.097, 7.097 Hz, 3H

Molecular docking scores:

Table 3 presents the docking scores of compounds 1a to 6a, along with the corresponding interacting hydrogen-bonding amino acids. The docking scores are indicative of the binding affinities of each compound to the target protein.

Table 3. MOLECULAR DOCKING SCORES

Compound code	Docking score	Interacting H-bonding amino acids
1a	-5.6	Ser98, Glu100
2a	-5.8	---
3a	-5.5	Asn86
4a	-5.4	Glu100
5a	-5.3	Asn86
6a	-5.4	Asn86

The findings from the study demonstrate that compounds 2a, 1a, and 3a exhibit notable binding affinity with the targeted protein. The comprehensive analysis revealed significant 2D interactions and 3D docking poses for each of these compounds.

Compound 1

Ethyl-4-(4-hydroxy-3 methoxy phenyl) - 6 methyl -2 thioxo 1,2,3,4 tetrahydropyrimidine5 carboxylate showed good hydrogen bonding with amino acids SER and GLU and Pi-alkyl interactions with the amino acids LEU,ILE, LYS, PRO and vanderwaals interaction with amino acids ASP, VAL, THR, ASN, GLY of the protein (PDB ID: 1HD2)

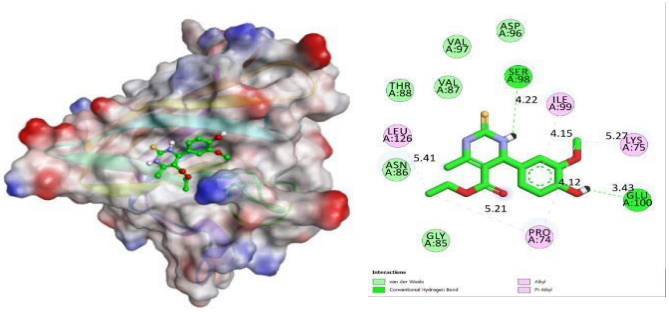


Figure 5. 3-D Docking poses and 2-D interactions of compound 1 with protein (1HD2)

Compound 2

Ethyl-6-methyl-4-(4-nitrophenyl) - 2-sulfadiene-1,2,3,4 tetrahydropyrimidine-5 carboxylate showed Pi-alkyl interactions with the amino acids LYS, ILE, PRO and vanderwaals interaction with amino acids LEU, ASN, THR, VAL, GLY, ASN, GLU, SER and no hydrogen bonding with the protein (PDB ID: 1HD2)

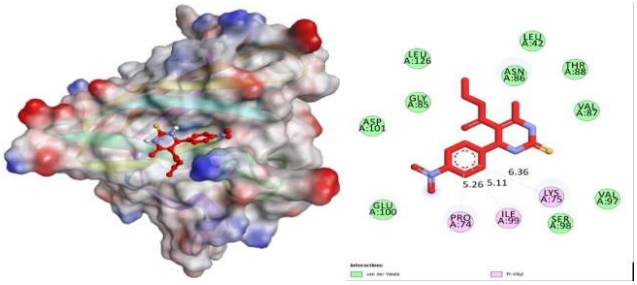


Figure 6. 3-D Docking poses and 2-D interactions of compound 2 with protein (1HD2)

Compound 3

Ethyl-6-methyl-4-phenyl-2 sulfanilidine 1,2,3,4 tetrahydropyrimidine-5 carboxylate showed good hydrogen bonding with amino acid ASN and Pi-alkyl interactions with the amino acids VAL, ILE, LYS, PRO and vanderwaals interaction with amino acids ASP, GLU, SER, THR, LEU, GLY of the protein (PDB ID: 2C9B)

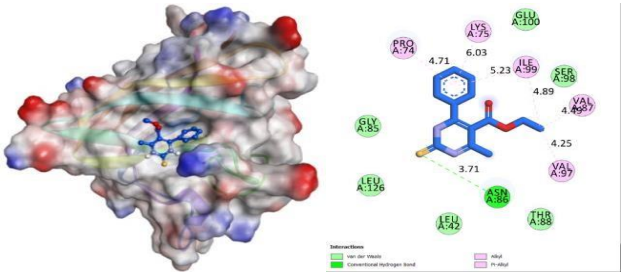


Figure 7. 3-D Docking poses and 2-D interactions of compound 3 with protein (1HD2)

Compound 4

Ethyl-4-(4-hydroxy-3 methoxy phenyl) - 6 methyl -2 oxo 1,2,3,4 tetrahydropyrimidine-5 carboxylate showed good hydrogen bonding with amino acid GLU and Pi-alkyl interactions with the amino acids LEU, ILE, LYS, PRO and vanderwaals interaction with amino acids GLY, LEU, ASN, THR, ASP of the protein (PDB ID: 1HD2)

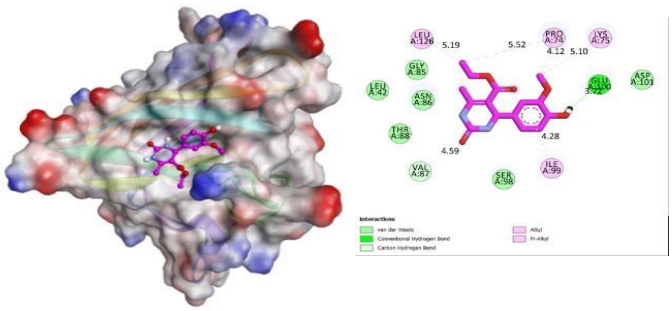


Figure 8. 3-D Docking poses and 2-D interactions of compound 4 with protein (1HD2)

Compound 5

Ethyl-6-methyl-4-(4-nitrophenyl)-2-oxo-1,2,3,4 tetrahydropyrimidine-5 carboxylate showed good hydrogen bonding with amino acid ASN and Pi-alkyl interactions with the amino acids LEU, LYS, PRO and vanderwaals interaction with amino acids ASP, GLY, LEU, THR, VAL, GLU, ASP of the protein (PDB ID: 1HD2)

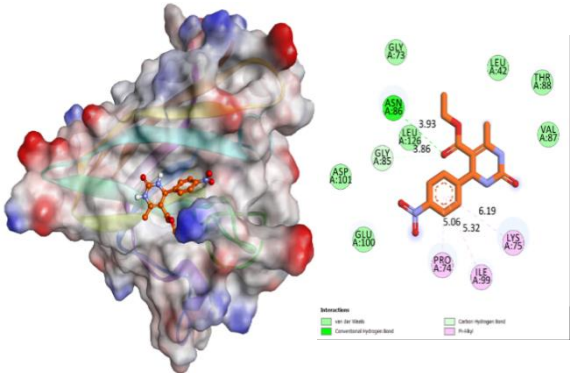


Figure 9. 3-D Docking poses and 2-D interactions of compound 5 with protein (1HD2)

Compound 6

Ethyl- - 6 methyl -2 oxo-4-phenyl-1,2,3,4 tetrahydropyrimidine-5 carboxylate showed good hydrogen bonding with amino acid ASN and Pi-alkyl interactions with the amino acids VAL, ILE, LYS, PRO and van der Waals interaction with amino acids LEU, GLY, THR of the protein (PDB ID: 1HD2)

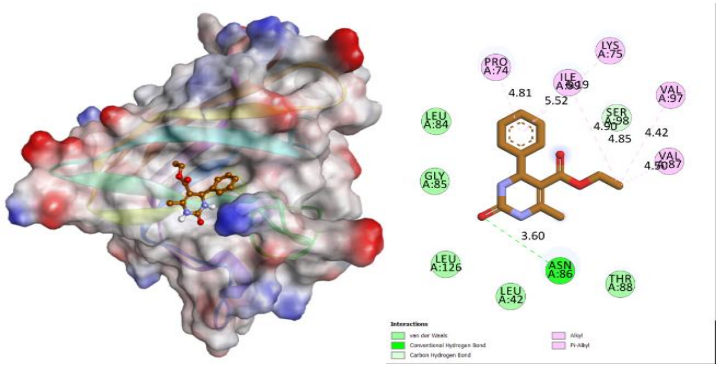


Figure 10. 3-D Docking poses and 2-D interactions of compound 6 with protein (1HD2)

Anti-oxidant activity:

Compounds exhibiting favourable docking scores were selected for further evaluation of their pharmacological activity, specifically their anti-oxidant potential. The assessment of antioxidant activity aimed to elucidate the compounds' capacity to counteract oxidative stress and evaluate their potency in this regard. To determine their anti-oxidant activity, two distinct types of tests were conducted: DPPH radical scavenging activity and Nitric oxide radical scavenging activity.

DPPH radical scavenging activity

The DPPH radical scavenging activity test is a widely used method to measure the ability of compounds to neutralize free radicals, particularly the stable radical 1,1-diphenyl-2picrylhydrazyl (DPPH). The results of this test provide insights into the compounds' efficiency in quenching DPPH radicals and thereby inhibiting oxidative damage.

The evaluation of DPPH radical scavenging activity was performed on compounds 1a, 2a, 3a, and Vitamin C as the standard. The results, summarized in Table 5, indicate that compound **2a** exhibited superior antioxidant activity compared to compounds 1a and 3a, as well as the standard (Vitamin C).

Table-4 DPPH RADICAL SCAVENGING ACTIVITY

Compound code	Dose (µg/ml)	Percentage inhibition ±SEM	IC_{50} (µg/ml)
1a	1	21.41±0.114	5.84
	2.5	45.30±0.152	
	5	81.78±0.156	
S2a	1	10.24±0.123	4.18
	2.5	29.51±0.056	
	5	57.55±0.653	
3a	1	10.24±0.123	4.49
	2.5	25.35±0.108	
	5	55.59±0.066	
Standard (Vit-C)	1	10.82±0.133	3.63
	2.5	37.11±0.139	
	5	72.54±0.105	

Nitric oxide radical scavenging activity

The nitric oxide radical scavenging activity test evaluates the compounds' capability to counteract nitric oxide radicals. Nitric oxide is a key molecule involved in various physiological processes, but excessive production can lead to oxidative stress and cellular damage. The test provides crucial information regarding the compounds' potential to mitigate nitric oxide-mediated oxidative stress

In the study, the assessment of Nitric oxide radical scavenging activity was conducted on compounds 1a, 2a, and 3a, along with the standard compound gallic acid. The results, as presented in Table 6, unveiled that compound **2a** exhibited superior outcomes compared to compounds 1a and 3a and even surpassed the performance of the standard gallic acid.

Table-5 NITRIC OXIDE RADICAL SCAVENGING ACTIVITY

Compound code	Dose (µg/ml)	Percentage inhibition± SEM	IC_{50} (µg/ml)
1a	1	29.10±0.141	3.15
	2.5	37.01±0.167	
	5	75.56±0.104	
2a	1	30.11±0.163	2.54
	2.5	47.73±0.150	
	5	78.69±0.093	
3a	1	22.91±0.095	5.88
	2.5	48.40±0.104	
	5	77.71±0.092	
Standard (Gallic acid)	1	34.38±0.501	0.63
	2.5	41.01±0.161	
	5	56.47±0.152	

By performing these anti-oxidant tests, the study aims to comprehensively understand the pharmacological activity of the selected compounds and assess their potency as anti-oxidants. The results obtained showed **2a** compounds with significant anti-oxidant properties, thus paving the way for potential therapeutic applications in conditions associated with oxidative stress and free radical-induced damage.

RESULTS

Synthesis

In the course of our experiments, various compounds were synthesized by utilizing distinct reagents in combination with a range of fruit juices as reaction media. The outcomes highlight the efficacy of fruit mediums and the potential significance of these compounds for further investigation of their docking scores and pharmacological activity

DISCUSSION

Pomegranate juice consistently outperforms pineapple juice as a catalyst in biginelli reactions due to its inherent acidity (pH 2.8 to 3.2), facilitating protonation and cyclocondensation. The acidity, coupled with diverse organic compounds like polyphenols and flavonoids, enhances catalytic activity. Vanillin yields higher than 4-nitrobenzaldehyde and benzaldehyde due to its electron-donating nature. Thiourea, with its sulfur atom, shows superior performance over urea in catalyzing the reaction. Pomegranate juice's

high sugar content and potassium ions create a favorable reaction environment, potentially enhancing enzyme catalysis. In summary, pomegranate juice, with its acidic nature, organic composition, and mineral content, stands out as a highly promising catalyst for biginelli reactions compared to pineapple juice.

CONCLUSION

From these discussions, it has been concluded that the Biginelli reaction represents an environmentally friendly and economically viable approach. This achievement was realized through the utilization of diverse reagents and fruit juices. Among the synthesized compounds, compound 2a has emerged as an exceptionally promising scaffold for subsequent development. Its capability to serve as a cornerstone for the generation of more potent dihydropyrimidine derivatives, precisely tailored to exhibit enhanced antioxidant activity, holds substantial promise for further advancement. These findings present exciting prospects for designing and synthesizing novel therapeutic agents with advantageous applications across various medical and interdisciplinary domains.

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