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Design, Synthesis, Characterisation and Pharmacological Evaluation of Some Novel Substituted Pyrimidine 2-one Derivatives

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Abstract

2,3-dihydropyrimidin-2(1H)-one derivatives were known to undergo cyclo-condensation of benzaldehyde, urea and ethyl acetoacetate and evaluate their antioxidant and *in vitro* anti-inflammatory activity. Dihydropyrimidinones are mainly synthesized by Biginelli reaction, which involves cyclo condensation of benzaldehyde, urea and ethyl acetoacetate. The prepared compounds were characterized by noting their melting point, thin layer chromatography ultraviolet spectroscopy, infrared spectroscopy, nuclear magnetic resonance and Mass spectroscopy and were scrutinized for its *in vitro* anti-inflammatory activity and antioxidant activity by *in vitro* cell culture studies. The melting point, thin layer chromatography and ultraviolet spectroscopy of the synthesized compounds were found to be pure and identified chemically. The molecular structure and molecular mass of compounds were confirmed by Infrared, nuclear magnetic resonance and Mass spectroscopy. The IC_{50} value of compound 1 and compound 2 by hydrogen peroxide method was found to be 30.25 µg/ml and 26.06 µg/ml respectively. The IC_{50} value of compound 1 and compound 2 by DPPH assay method was found to be 27.872 µg/ml and 25.467 µg/ml respectively. Synthesized compounds also show anti-inflammatory activity. The result obtained in this research work is clearly indicating that the synthesized molecules possess both antioxidant activity and anti-inflammatory activity.

***Keywords:** Anti-inflammatory activity; Biginelli reaction; Dihydropyrimidinones; Docking; DPPH*

Introduction

Dihydropyrimidinone is a heterocyclic moiety having two N-atoms at positions 1 and 3. They are pyrimidine spinoffs having a ketone group. Dihydropyrimidinones are a series of highly valuable small molecules possessing versatile pharmaceutical properties [1]. Dihydropyrimidine core unit containing alkaloids have been isolated from marine sources, like batzelladine which also having different pharmacological properties. These pyrimidinone derivatives are reported to have diverse pharmacological activities such as anti-inflammatory [2], anticonvulsant, analgesic, sedative, anti-depressant, antipyretic, antioxidant [3], antitumor activity and antihypertensive activities [4,5]. The research was to design and synthesize 2,3-dihydropyrimidin-2(1H)-one derivatives and evaluate their antioxidant and *in vitro* anti-inflammatory activity (SCHEME 1).

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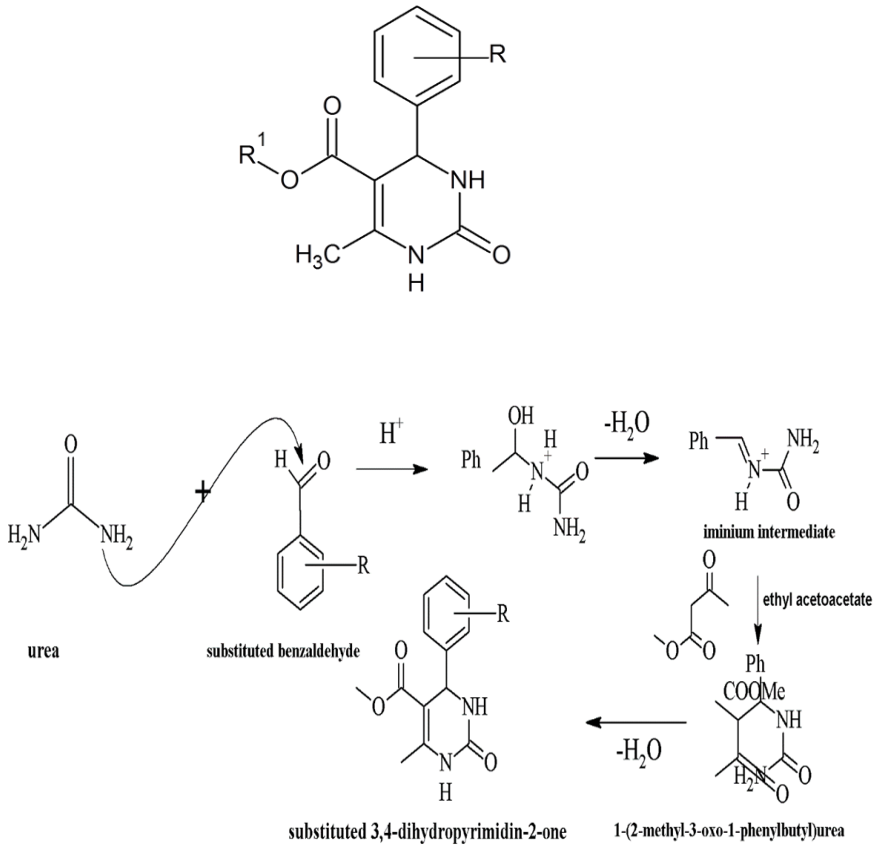
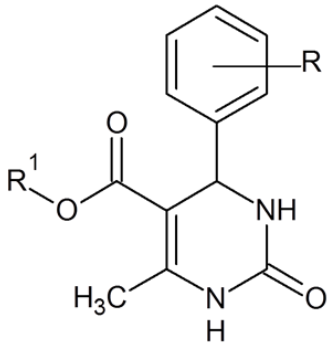


SCHEME 1. **Synthesis of substituted pyrimidone derivatives.**

Experimental Section

Synthesis of 2,3, -dihydropyrimidin-2(1H)-one derivatives

Two 2, 3 dihydropyrimidin-2-(1H)-one derivatives were selected. The docking analysis reveals the good activity against the selected anti-inflammatory targets [6-10]. The main mechanism for these ligand syntheses was based on aldol condensation. Ethyl acetoacetate reacted with benzaldehyde in the presence of acid catalyst to form an intermediate and the corresponding intermediate was reacted with urea to form 3, 4-dihydropyrimidin-2-(1H)-one.



R=C_3H_7, NO_2, R_1=CH_3CH_2COO

In vitro anti-inflammatory activity

Determination of *in vitro* anti-inflammatory effect on cultured Raw 264.7 cell lines by COX-2 inhibitory assay

The *in vitro* anti-inflammatory activity of 3,4-dihydropyrimidine-2-(1H)-one analogues was evaluated using LPS stimulated Raw 264.7 macrophage cell line [11,12]. Different concentrations (25,50,100 µg/mL) of synthesized compounds and control

were tested for *in vitro* anti-inflammatory study and inhibition of COX-2 level was determined. Percentage inhibition of COX-2 level by synthesized molecule is shown in table.

***In vitro* antioxidant activity**

3,4–dihydropyrimidin-2-(1H)-one analogues were analyzed by DPPH and hydrogen peroxide free radical scavenging activity (TABLES 1-8). Standard drug used here is ascorbic acid [13].

TABLE 1. **Docking Score for the synthesized compounds using Argus lab software.**

Compound code	Docking score (kcal/mol)
CS1	-10.3878
CS2	-9.8562
Diclofenac	-10.2105

TABLE 2. **Docking Score for the synthesized compounds using autodock software.**

Compound code	Docking score (kcal/mol)
CS1	-7.478
CS2	-6.616
Diclofenac	-7.375

TABLE 3. **Structural elucidation of the compounds.**

Compound code	Molecular formula	MW (g/mol)	No. of HBA	No. of HBD	C Log P	No of rotable bonds	TPSA (Å^2)
CS1	$C_{17}H_{22}N_2O_3$	302.37	5	2	3.88	5	67.43
CS2	$C_{14}H_{15}N_3O_5$	305.29	8	2	2.31	5	113.2

TABLE 4. **Physical characterization of the synthesized compounds.**

Compound code	Molecular formula	MW (g/mol)	Melting point (°C)	Percentage yield
CS1	$C_{17}H_{22}N_2O_3$	302.37	210-215	78.41%
CS2	$C_{14}H_{15}N_3O_5$	305.29	210-212	70.6%

TABLE 5. **Physical property of synthesized compounds.**

Properties	CS1	CS2
Nature	Powder form	Powder form
Odour	Pleasant odour	No odour
Colour	Yellow	Pale white
State	Solid state	Solid state

TABLE 6. **Solubility nature of the synthesized compounds.**

Compound code	Soluble	Partially soluble	Insoluble
CS1	DMSO, DMF	Chloroform, Hexane, Methanol	Water
CS2	DMSO, DMF	Chloroform, Hexane	Water

TABLE 7. **IUPAC name of the synthesized compounds.**

Compound code	Structure	IUPAC name	Solvent system	R_f Value
CS1		Ethyl 4-(4-isopropylphenyl)-6-methyl-2-oxo-3,4-dihydro-1H-pyrimidine-5-carboxylate	Hexane: Ethyl acetate (7:3)	0.43
CS2		Ethyl 4-[3-nitrophenyl]-6-methyl-2-oxo-3,4-dihydro-1H-pyrimidine-5-carboxylate	Hexane: Ethyl Acetate (7:3)	0.57

TABLE 8. **IR spectral studies of the synthesized compounds.**

Compound code	Chemical structure	IR (cm^{-1})
CS1		2960.73 cm^{-1} (alkane C-H str) 3116.97 cm^{-1} (aromatic C-H str) 3331.07 cm^{-1} (N-H str) 1691.57 cm^{-1} (C=O str) 1438.90 cm^{-1} (aromatic C=C str)
CS2		2964.59 cm^{-1} (alkane CH str) 3315.54 cm^{-1} (NH str) 3103.46 cm^{-1} (aromatic CH str) 1691.57 cm^{-1} (C=O str) 1521.84 cm^{-1} (N=O str) 1220.94 cm^{-1} (CN str)

Hydrogen peroxide free radical scavenging activity

Percentage inhibition of the synthesized compound was found out by hydrogen peroxide free radical scavenging activity technique and the results were shown in the TABLE 9.

Graphical representation of the inhibitory activity of the synthesized derivatives

This graph was schemed against concentration on X-axis and percentage inhibition on Y-axis [14-16]. On increasing the concentration of the sample percentage inhibition also increases; as a result, free radical scavenging activity increases. IC_{50} value of the derivatives was resoluted using Microsoft Excel.

TABLE 9. **Antioxidant activity with standard deviation.**

Compound code	Percentage inhibition ± SD				
	10 µg/mL	**20 µg/mL**	**30 µg/mL**	**40 µg/mL**	**50 µg/mL**
CS1	22.5 ± 0.025	33.8 ± 0.052	53.2 ± 0.045	65.2 ± 0.033	73.6 ± 0.009
CS2	28.5 ± 0.038	42.5 ± 0.051	57.1 ± 0.021	67.8 ± 0.011	78.9 ± 0.023
Ascorbic acid	32.6 ± 0.014	48.4 ± 0.023	59.5 ± 0.012	74.2 ± 0.007	81.5 ± 0.006

Comparing the antioxidant activity of standard drug and synthesized compound by hydrogen peroxide free radical assay method

It was perceived that the maximum hydrogen peroxide scavenging activity was showed by CS2 (IC_{50}=26.06 µg/mL). CS2 exhibited more scavenging property compared to CS1.

2, 2-diphenyl-1-picryl hydrazyl free radical assay

In this method the presence of an antioxidant species the DPPH undergoes reduction. The synthesized analogues have hydrogen donating power, which diminish the color of DPPH [17-20]. The results obtained from the DPPH assay of synthesized compound showing significant antioxidant activity when compared to reference standard (TABLE 10).

TABLE 10. **Antioxidant activity without standard deviation.**

Compound code	Percentage inhibition ± SD				
	10 µg/mL	**20 µg/mL**	**30 µg/mL**	**40 µg/mL**	**50 µg/mL**
CS1	20.906 ± 0.124	40.887 ± 0.007	49.476 ± 0.175	68.286 ± 0.03382	88.694 ± 0.003
CS2	15.973 ± 0.005	40.849 ± 0.005	60.296 ± 0.0004	67 ± 0.004	96.475 ± 0.0008
Ascorbic acid	18.322 ± 0.004	38.674 ± 0.012	56.743 ± 0.003	65.461 ± 0.004	91.592 ± 0.016

DPPH free radical assay

The IC_{50} value of synthesized compound by DPPH assay method is shown in the graph [21-24]. It shows that IC50 value of the synthesized compound CS1 and CS2 was found to be 27.872 µg/mL and 25.467 µg/mL respectively. IC_{50} value of standard ascorbic acid was found to be 27.636 µg/mL. The experimental results uphold that the synthesized derivatives have the ability to prevent the oxidation [25,26].

Graphical depiction of the inhibitory activity of the synthesized derivatives

This graph was plotted against concentration Vs percentage inhibition [27]. When the concentration increases, percentage inhibition also increases. It indicates that the synthesized compounds possess free radical activity. IC_{50} value of the compound was determined using Microsoft Excel (TABLE 11).

***In silico* molecular modelling**

A series of Dihydropyrimidinone analogues were chosen and performed *insilico* modelling docking studies [28,29], Lipinski's rule of five analysis. Softwares like ChemSketch, Molinspiration, Arguslab and Schrodinger are used for molecular modelling. Studies were performed by using 5C29 COX-2 selective receptors. The higher docking score

derivatives are selected for further synthesis and study. The docking score of the ligands were compared with the reference standard shown in TABLE 11.

TABLE 11. **Anti-inflammatory activity.**

Compound code	Percentage inhibition		
	25 µg/mL	**50 µg/mL**	**100 µg/mL**
CS1	26.51473 ± 0.1322	30.23902 ± 0.1255	44.52474 ± 0.0998
CS2	32.35131 ± 0.1217	35.13063 ± 0.1167	45.85881 ± 0.0974
Diclofenac	78.4924 ± 0.0973	88.48619 ± 0.0521	93.21547 ± 0.0307

Results, Discussion and Conclusion

2,3-Dihydropyrimidin-2-(1H)-ones are therapeutically beneficial scaffolds possessing remarkable biological activities. The current research work was based on the anti-inflammatory and antioxidant activities of 3, 4-dihydropyrimidin-2-(1H)-one analogues. The selected analogues were subjected to *in silico* molecular modelling using selective COX-2 anti-inflammatory (5c29) receptor. For further clarification of these compounds, they were subjected to drug likeness analysis using Lipinski Rule of Five. The derivatives were synthesized by Biginelli reaction, which involves cyclo-condensation of benzaldehyde, urea and ethyl acetoacetate. The yield of the synthesized compounds was found to be substantial. The synthesized new series of compounds were authenticated by melting point, TLC and spectral analysis. IR spectra of the compound was analyzed and thus the synthesized compounds were found to be pure and chemically recognized.

The molecular structure and molecular mass of the derivatives were confirmed by NMR and Mass spectroscopy respectively. These analogues were screened for *in vitro* antioxidant (1,1-diphenyl 2-picryl hydrazyl and H_2O_2 free radical scavenging) activity and anti-inflammatory activity by Raw 264.7 cell lines. From the study, it was discerned that CS2 showed reasonably better antioxidant and anti-inflammatory activity than CS1. In 3, 4-dihydropyrimidin-2-(1H)-one derivatives, the derivative with electron withdrawing nitro group in the third position showed better antioxidant and anti-inflammatory properties than isopropyl group substituted derivative. The results of this research unveiled that these synthesized analogues be likely to have moderate activity against COX-2 mediated diseases, thereby it may decrease pain and inflammation because of its antioxidant and anti-inflammatory activity.

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