Original Article

In-silico Studies, Synthesis and Antioxidant Studies of Different Substituted 7-Phenyl-5-(Thiophen-2-Yl)-2-Thioxo-2,3-Dihydropyrido[2,3-D]Pyrimidine-4(1h)-Ones

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ABSTRACT

Background: Free radicals and ROS which are formed in normal physiological conditions, damages the cells if it is not eliminated by endogenous system which causes oxidative stress. Pyridopyrimidines are important molecule in heterocyclic chemistry, gaining interest because of their biological and pharmacological activities, especially for their potential anti-tumor, antibacterial and tyrosine kinase inhibitors, among other pharmacological properties along with anti-oxidant properties. **Materials and Methods:** In the current study, novel pyridopyrimidines derivatives i.e different substituted 7-phenyl-5-(thiophen-2-yl)-2-thioxo-2,3-dihydropyrido[2,3-d]pyrimidine-4(1H)-one(3a-p) were synthesized to develop potent anti-oxidant agent. Based the *in-silico* studies, especially depending on the docking score, 8 compounds were selected for the synthesis and evaluated for their anti-oxidant potency. Selected compounds were synthesized using mercapto-4-hydroxy-6-amino pyrimidine (1) and substituted α, β- unsaturated ketones(2a-p). **Results:** Synthesized compounds were characterized by IR, NMR and Mass spectra. *In-vitro* anti-oxidant studies were performed for 8 best scored compounds by DPPH assay and nitric oxide inhibition assay. From the *in-vitro* result, compound 3j, 3a, and 3o are considered as promising molecules. **Conclusion:** The promising activity may be because of presence of electron releasing group (3a- p-OH, 3o- p-NH_2) and electron withdrawing group (3j-p-CF_3) which can be considered as lead molecules for the further discovery.

Keywords: Pyridopyrimidines, Thiophene, *In-silico*, Synthesis, Anti-oxidant.

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INTRODUCTION

Free radicals and reactive oxygen species (ROS), which are produced in normal physiological circumstances but become destructive when not eliminated by endogenous systems, which causes oxidative stress.[1] Despite the fact that organisms have cellular defence machinery to prevent oxidative stress, some pathogenic conditions make it impossible to eliminate all excessive intracellular ROS.[2] In fact, oxidative stress is caused by a discrepancy between reactive oxygen species production and endogenous antioxidant mechanisms. ROS are a major source of oxidative stress, which has a significant pathological role in human diseases and disorders such as cancer, cardiovascular disease, neural disorders, alzheimer's disease, mild cognitive impairment, parkinson's disease, alcohol-induced liver disease, ulcerative colitis, ageing and atherosclerosis. ROS in excess are hazardous because they induce biomolecular oxidation, which causes cell death and oxidative stress, they attack specific biomolecules in the body, causing extensive cellular damage such as nucleic acid strand scission, polypeptide modification, and lipid peroxidation.[3] Superoxide anions, hydroxyl

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radicals, and hydrogen peroxides are oxygen-derived free radicals that are cytotoxic and cause tissue damage.[4] Antioxidants can obstruct oxidation by interacting with free radicals, chelating catalytic metals, and functioning as oxygen scavengers. Antioxidant supplements, on the other hand, may be utilized to assist the human body in reducing oxidative damage.[5] Due to the high cost of natural antioxidants, medicinal chemists develop and employ synthetic antioxidants such as propyl gallate (PG), butylated hydroxyl anisole (BHA), and butylated hydroxyl toluene (BHT).

Pyridopyrimidines are important molecule in heterocyclic chemistry, gaining interest because of their biological and pharmacological activities, especially for their potential anti-tumor, antibacterial and tyrosine kinase inhibitors, among other pharmacological properties along with anti-oxidant properties. As free radicals and ROS are responsible for inducing many diseases and which are generated by the involvement of some of the enzymes like Heme oxygenase-1 (HO1) (5), cytochrome P450 (CP450), lipoxygenase (LO), myeloperoxidase (MP), NADPH oxidase (NO) and xanthine oxidase (XO) during the metabolism of arachidonic acid and their inhibitiors breaks the ROS production cycle with the consequent reduction of the oxidative stress and maintenance of redox homeostasis.[6] Hence we planned to design novel pyridopyrimidines derivatives i.e different substituted 7-phenyl-5-(thiophen-2-yl)-2-thioxo-2,3-dihydropyrido[2,3-d]pyrimidine-4(1H)-one is an attempt to develop an active anti-oxidant agent with improved activity which might inhibit any one of these enzymes by reducing oxidative stress.

MATERIALS AND METHODS

In-silico Studies

In-silico studies were carried out to determine the drug likeness of the molecule. Lipinski's rules of five, ADMET and physicochemical properties, bioactivity score of the molecules and molecular docking to determine the best molecules with preferred orientation.[7-8]

Drug Likeness Evaluation of Target Pyridopyrimidines(3a-p)

Determination of Lipinski's rule of Five and physico-chemical parameters of 3a-p

Lipinski's rule of 5 is mainly used to verify molecular properties which are essential and related to PK properties of the drug molecule using Molinspiration software.[9-13]

Analysis of Electronic Parameters and ADME Properties of 3a-p

It is mainly by using Qikprop of Schrodinger 2018-3 suite device Maestro 11.7.012. Qikprop is a rapid, precise, accessible to predict ADME properties.[14-15]

Evaluation of Toxicity of 3a-p

In the process of drug development, toxicity assessment is one of the method to determine the safety profile of the molecule.[16] *In-silico* toxicity profiling of the molecule will help in the drug development. *In-silico* toxicity prediction was mainly done using admetSAR database which is freely available.[17]

Molecular Docking Studies of 3a-p

Molecular docking was performed by utilizing Glide module of Schrodinger 2018-3 suite device Maestro 11.7.012 by targeting heme-oxygenase, Cytochrome P450, Xanthine Oxidase.[18] Crystal structures with good resolution of all the target proteins were taken from PDB. (PDB ID: 2DY5, 3NV5 and 3B9J).[19-20]

Synthesis of Target Pyridopyrimidines (3a-p)

All of the reactions were conducted in a controlled laboratory environment. Himedia, CDH, and Sigma Aldrich provided laboratory grade reagents and analytical grade solvents were used for all of the synthetic work. Recrystallization with appropriate solvents was used to purify the products. Digital melting point apparatus was used to determine melting points, which are uncorrected. IR, NMR, and mass spectroscopy were used to characterize the synthesized compounds. The Alpha Bruker IR spectrometer has been used to record IR spectra on KBr discs (cm^{-1}). ^{1}H-NMR spectra were obtained on a BrukerAvance-II 300MHz NMR spectrometer using DMSO-d6 as the solvent and TMS as the internal standard. Chemical shifts are expressed in parts per million (ppm) relative to TMS (=0). SYNAPT-G2 LC-MS spectrometer was used to record the mass spectra.

Synthesis of 2-mercapto-4-hydroxy-6-amino pyrimidine(1)

Equimolar quantities of thiourea (0.01 mol) and ethyl cyanoacetate (0.01 mol) in presence of sodium ethoxide with 20 ml of ethanol was refluxed for 7-8hr. The solution is then cooled and acidified with HCl. The resultant precipitate is filtered, dried and recrystallized from DMF (Figure 1).[21-22]

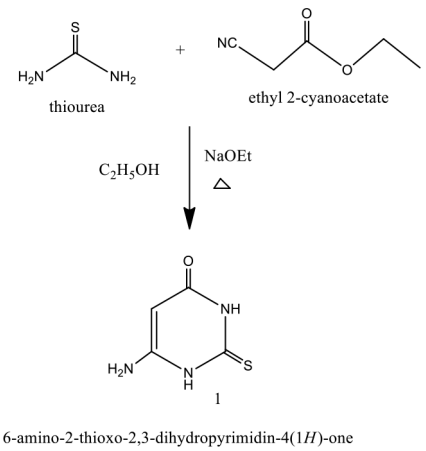

Figure 1: Synthesis of 2-mercapto-4-hydroxy-6-amino pyrimidine(1).

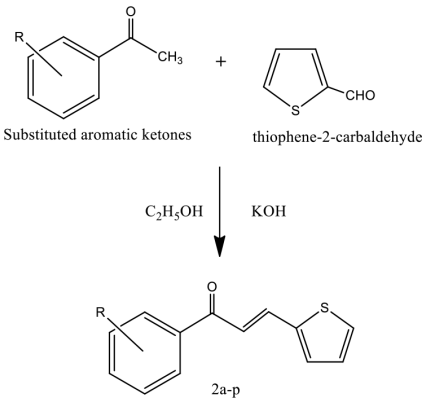

Figure 2: Synthesis of substituted α-β unsaturated ketones (2a-p).

Synthesis of Substituted α-β Unsaturated Ketones (2a-p)

Equimolar quantities of substituted acetophenones (0.01 mol) and thiophene-2-carbaldehyde (0.01 mol) in presence of KOH with 20 ml of ethanol was refluxed for 6-7hr. The solution is then cooled and acidified with HCl. The resultant precipitate is poured into ice and filtered, washed with water, dried and recrystallized from ethanol (Figure 2).[23-25]

Synthesis of Different Substituted 7-phenyl-5-(thiophen-2-yl)-2-thioxo-2,3-dihydropyrido[2,3-d] pyrimidine-4(1h)-one(3a-p)

Equimolar mixture of 2-mercapto-4-hydroxy-6-amino pyrimidine(1) and substituted α-β unsaturated ketones (2a-p) is refluxed with DMF for 8-12 hr. The solution is then cooled and acidified with HCl. The resultant precipitate is poured into ice and filtered, washed with water, dried and recrystallized using DMF (Figure 3).[26-28]

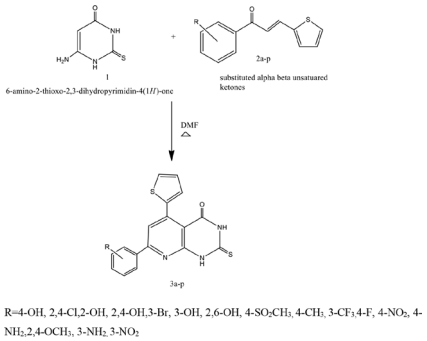

Figure 3: Synthesis of different substituted 7-phenyl-5-(thiophen-2-yl)-2-thioxo-2,3-dihydropyrido[2,3-d]pyrimidine-4(1h)-one(3a-p).

Spectral Characterization of Synthesized Derivatives

7-(4-hydroxyphenyl)-5-(thiophen-2-yl)-2-thioxo-2,3-dihydropyrido[2,3-d]pyrimidin-4(1H)-one (3a); yellow crystals, Yield: 84%, M.P. 332-334°C; IR(KBr, cm^{-1}): 3336, 3231(2NH), 3342(OH), 1656(C=C), 1792(C=O), 1640(C=N), 784(C=S), 3123(aromatic C-H); ^1H NMR(DMSO, δ ppm): 12.12, 13.03(2s, 2H, 2NH), 7.64(s, 1H, H of C_6 pyridine), 8.23(d, 1H,CH), 7.94(d, 1H, CH), 7.54(d, 1H, CH), 7.38(d, 1H, CH), 5.23(s, 1H, OH of 4-hydroxy phenyl), 7.16(t, 1H, thienyl proton), 7.41(d, 1H, thienyl proton),7.76(d, 1H, thienyl proton); Mass (LC-MS, m/z): 353.03(M^+); Elemental Analysis: C-57.77, H-3.14, N-11.89, O-9.05, S-18.15.

7-(2,4-dichlorophenyl)-2-sulfanylidene-5-(thiophen-2-yl)-2,3-dihydropyrido[2,3-d]pyrimidin-4(1H)-one (3b); brownish yellow crystals, Yield: 78%, M.P. 324-326°C; IR(KBr, cm^{-1}): 3334, 3232(2NH), 1654(C=C), 1789(C=O), 1640(C=N), 778(C=S), 3128(aromatic C-H), 820(C-Cl); ^1H NMR(DMSO, δ ppm): 12.08, 13.01(2s, 2H, 2NH), 7.66(s, 1H, H of C_6 pyridine), 8.18(s, 1H,CH), 7.94(d, 1H, CH), 7.74(d, 1H, CH), 7.12(t, 1H, thienyl proton), 7.41(d, 1H, thienyl proton),7.75(d, 1H, thienyl proton); Mass (LC-MS, m/z): 408.03(M^+2), 406.02(M^+); Elemental Analysis:C-50.25, H,--2.23-,Cl-17.45,N-10.34, O-3.94, S,15.78.

7-(2-hydroxyphenyl)-5-(thiophen-2-yl)-2-thioxo-2,3-dihydropyrido[2,3-d]pyrimidin-4(1H)-one(3c); yellow crystals, Yield: 76%, M.P. 338-340°C; IR(KBr, cm^{-1}): 3329, 3227(2NH), 3346(OH), 1652(C=C), 1788(C=O), 1634(C=N), 784(C=S), 3129(aromatic C-H); ^1H NMR(DMSO, δ ppm): 12.12, 13.03(2s, 2H, 2NH), 7.64(s, 1H, H of C_6 pyridine), 8.21(d, 1H,CH), 7.82(t, 1H, CH), 7.68(t, 1H, CH), 7.48(d, 1H, CH), 5.28(s, 1H, OH of 2-hydroxy phenyl), 7.18(t, 1H, thienyl proton), 7.44(d, 1H, thienyl proton),7.72(d, 1H, thienyl proton); Mass (LC-MS, m/z): 353.03(M^+); Elemental Analysis: C-57.77, H-3.14, N-11.89, O-9.05, S-18.15.

7-(2,4-dihydroxyphenyl)-5-(thiophen-2-yl)-2-thioxo-2,3-dihydropyrido[2,3-d]pyrimidin-4(1H)-one(3d); yellow crystals, Yield: 72%, M.P. 342-344°C; IR(KBr, cm^{-1}): 3326, 3221(2NH), 3343(OH), 1648(C=C), 1768(C=O), 1624(C=N), 783(C=S), 3127(aromatic C-H); ^{1}H NMR(DMSO, δ ppm): 12.23, 13.17(2s, 2H, 2NH), 7.58(s, 1H, H of C_6 pyridine), 8.34(s, 1H,CH), 7.87(d, 1H, CH), 7.74(d, 1H, CH), 5.28, 5.73(s, 1H, 2,4-OH of 4-hydroxy phenyl), 7.23(t, 1H, thienyl proton), 7.46(d, 1H, thienyl proton),7.74(d, 1H, thienyl proton); Mass (LC-MS, m/z): 369.03(M^+); Elemental Analysis: C-55.27, H-3.00, N-11.37, O-12.99, S-17.36.

5-(thiophen-2-yl)-2-thioxo-7-(3-(trifluoromethyl) phenyl)-2,3-dihydropyrido[2,3-d]pyrimidin-4(1H)-one (3j); brownish crystals, Yield: 87%, M.P. 334-336°C; IR(KBr, cm^{-1}): 3319, 3215(2NH), 1645(C=C), 1749(C=O), 1618(C=N), 782(C=S), 3220(aromatic C-H), 1346(C-F); ^{1}H NMR(DMSO, δ ppm): 12.23, 13.09(2s, 2H, 2NH), 7.51(s, 1H, H of C_6 pyridine), 8.32(s, 1H,CH), 7.94(d, 1H, CH), 7.77(t, 1H, CH), 7.64(d, 1H, CH), 7.12(t, 1H, thienylproton), 7.39(d, 1H, thienyl proton),7.67(d, 1H, thienyl proton); Mass (LC-MS, m/z): 405.4(M^+); Elemental Analysis: C-53.33, H-2.49, F-14.06, N-10.36,O-3.95, S-15.82.

7-(4-aminophenyl)-2-sulfanylidene-5-(thiophen-2-yl)-2,3-dihydropyrido[2,3-d]pyrimidin-4(1H)-one(3m); yellow crystals, Yield: 88%, M.P. 328-330°C; IR(KBr, cm^{-1}): 3340, 3238(2NH), 3421(NH of amine), 1646(C=C), 1785(C=O), 1638(C=N), 785(C=S), 3132(aromatic C-H); ^{1}H NMR(DMSO, δ ppm): 12.24, 13.12(2s, 2H, 2NH), 4.94(s, 2H, NH_2), 7.57(s, 1H, H of C_6 pyridine), 8.19(d, 1H,CH), 7.98(d, 1H, CH), 7.74(d, 1H, CH), 7.68(d, 1H, CH), 7.23(t, 1H, thienyl proton), 7.48(d, 1H, thienyl proton),7.69(d, 1H, thienyl proton); Mass (LC-MS, m/z): 352.39(M^+); Elemental Analysis: C-57.93, H-3.43, N-15.90, O- 4.54, S-18.20.

7-(2,4-dimethoxyphenyl)-2-sulfanylidene-5-(thiophen-2-yl)-2,3-dihydropyrido[2,3-d]pyrimidin-4(1H)-one(3n); white crystals, Yield: 70%, M.P. 336-338°C; IR(KBr, cm^{-1}): 3338, 3231(2NH), 1650(C=C), 1764(C=O), 1632(C=N), 784(C=S), 3125(aromatic C-H), 2835(OCH_3); ^{1}H NMR(DMSO, δ ppm): 12.24, 13.24(2s, 2H, 2NH), 7.49(s, 1H, H of C_6 pyridine), 8.18(s, 1H,CH), 7.92(d, 1H, CH), 7.76(d, 1H, CH), 3.78, 3.38(s, 3H, 2,4-OCH_3), 7.19(t, 1H, thienyl proton), 7.42(d, 1H, thienyl proton),7.66(d, 1H, thienyl proton); Mass (LC-MS, m/z): 352.4(M^+); Elemental Analysis: C- 57.41, H-3.80, N-10.57,O-12.08, S-16.13.

7-(3-aminophenyl)-2-sulfanylidene-5-(thiophen-2-yl)-2,3-dihydropyrido[2,3-d]pyrimidin-4(1H)-one(3o); yellow crystals, Yield: 85%, M.P. 324-326°C; IR(KBr, cm^{-1}): 3339, 3228(2NH), 3418(NH of amine), 1640(C=C), 1782(C=O), 1642(C=N), 785(C=S), 3130(aromatic C-H); ^{1}H NMR(DMSO, δ ppm): 12.24, 13.16(2s, 2H, 2NH), 4.95(s, 2H, NH_2) 7.54(s, 1H, H of C_6 pyridine), 8.21(d, 1H,CH), 7.94(d, 1H, CH), 7.81(d, 1H, CH), 7.68(d, 1H, CH), 7.28(t, 1H, thienyl proton), 7.50(d, 1H, thienyl proton),7.76(d, 1H, thienyl proton); Mass(LC-MS, m/z): 352.4(M^+); Elemental Analysis: C- 57.93, H-3.43; N- 15.90, O-4.54, S- 18.20.

In-vitro Antioxidant Study

DPPH (1,1-diphenyl-2-picryl-hydrazyl) free Radical Scavenging Assay

DPPH is a stable diamagnetic molecule that accepts a hydrogen radical or an electron. Because of the reduction in the presence of an antioxidant molecule, the colour changes from purple to yellow this is noticeable visually. Therefore, DPPH is frequently utilized as a substrate to assess anti-oxidant activity of a molecule.[29-30] In a 96-well microtiter plate, the assay was performed. 100µL DMSO solution of each test sample and a standard Ascorbic acid in different concentration (10 - 50g/mL) id added to different wells. An equal amount of DMSO was taken as control.[31-32]

$$\text{A DPPH scavenging effect (percentage)} = A_0 - A_1 / A_0 \times 100.$$

A_0represents the absorbance of the control response. A_1 was the absorbance when a test or standard sample was present.

Nitric Oxide Inhibition Assay

The test was done in a 96-well microtiter plate. 100µL of different concentration of test sample solution (10-50g/mL) in DMSO and standard ascorbic acid is added in to wells. Both test and standard samples treated with 50µL of sodium nitroprusside in phosphate buffered saline. The same reaction mixture with an equal amount of DMSO is taken as control.[33] After 15 min of incubation, reaction mixture is treated with 50 µL of Griess reagent (0. 1% N-(1-naphthyl) ethylenediamine dihydrochloride, 2% H_3PO_4 and 1% sulfanilamide). At 540nm, the absorbance of these solutions was measured. The following formula is used to compute the percentage of nitrite radical scavenging activity.[34-35]

$$\text{Scavenged nitric oxide (percentage)} = A_0 - A_1 / A_0 \times 100$$

RESULTS AND DISCUSSION

Based on the calculated values in the determination of Lipinski's rule of five, it was inferred that all the compounds successfully satisfied all the parameters of Lipinski's rule of five. Molecular weight and Log *p* are

the important parameter, mainly correlated to passive intestinal absorption. All the compounds were found to have the molecular weight less than 500 Dalton's and Log *p* values ranges from 2.34-4.75 (less than 5). So that all the compounds are expected to show good intestinal absorption. It is predicted that compound 3b will be highly lipophilic and 3hr will be least lipophilic. So, the permeability of 3b through cell membrane will be greater compared other compounds. Total number of hydrogen bond acceptors and donors are the good oral bioavailability predictors. Number of hydrogen bond acceptors and donors in all the screened compounds were within the permitted range i.e., not more than 10 and 5 respectively (Table 1). tPSA is related to hydrogen bonding potential of compound and good descriptor to characterize drug absorption, bioavailability, caco-2 permeability and BBB penetration. For all the screened compounds tPSA is found to be in the range of 61.55-107.37, which is within the permitted range i.e< 140 Å, where 3l and 3p found to have nearest values to standard. Number of rotatable bonds ranges from 2-4, in all the screened compounds which are found to be moderately flexible. Out of screened compounds 3b, 3h, 3i, 3j, 3k, 3m, 3o and 3p and standard drug do not have any reactive functional groups, whereas 3a, 3c, 3d, 3e, 3f, 3g, 3l, and 3n found to have a reactive FG, but all the compounds fall within the recommended range so it is expected that there will not be any decomposition or toxicity problems *in-vivo*. All the tested compounds found to have zero dipole, indicates molecules are non-polar in nature. Measure of total polarizability of molecule describing the steric effects. Polarizability of all tested compounds are within the recommended range and it is ranging from 35.636-40.914 (Table 2). Volume of all the compounds is found to be within the recommended range, an increase of the molecular volume, generally increases the residence time in the vascular system. Values for SASA, FOSA and FISA of all the tested compounds are within the range. In-case of FISA values are on the lower side indicates they possess very less hydrophilic component of the SASA i.e, SASA on N, O, and H on hetero atoms. In the systemic circulation, solubility is one of the important parameters to reach desired concentration, Conformation-independent predicted aqueous solubility of all the compounds is within the range except 3b, 3e and 3j (Table 3). Predicted apparent Caco-2 cell permeability of compounds showed 3b, 3e, 3j, 3k, 3l and 3n has excellent permeability in turn expected to have excellent intestinal permeability 3a, 3c, 3f, 3i and 3o is expected to have good intestinal permeability where as 3d, 3g, 3h, 3m and 3p has got lesser permeability, but values for all compounds found to be better than standard ascorbic acid. Predicted values for brain/blood partition coefficient for all the screened compounds fall into the recommended range. MDCK cells are considered to be a good mimic for the BBB. Most of the derivatives

Table 1: Determination of Lipinski's rule of five of 3a-p.

Compound	Mol. Wt	Log *p*	nON	nOHNH	nviolation
3a	353.413	2.99	5	3	0
3b	406.304	4.75	4	2	0
3c	353.413	3.20	5	3	0
3d	369.412	2.70	6	4	0
3e	416.309	4.25	4	2	0
3f	353.413	2.97	5	3	0
3g	369.412	2.94	6	4	0
3h	415.499	2.34	6	2	0
3i	351.44	3.92	4	2	0
3j	405.412	4.34	4	2	0
3k	355.404	3.63	4	2	0
3l	382.411	3.43	7	2	0
3m	352.428	2.54	5	4	0
3n	397.466	3.51	6	2	0
3o	352.44	2.52	5	4	0
3p	382.411	3.40	7	2	0
Ascorbic Acid	176.126	-1.40	6	4	0

Table 2: Determination of physicochemical parameters of 3a-p.

Comp.	tPSA	nrotb	#rtvFG	dipole	polarizability
Range	<140Å^2	<10	0 – 2	1.0 – 12.5	13.0 – 70.0
3a	81.87	2	1	0	35.985
3b	61.66	2	0	0	38.242
3c	81.77	2	1	0	36.005
3d	102.00	2	1	0	35.9
3e	61.55	2	1	0	37.774
3f	81.77	2	1	0	36.007
3g	102.00	2	1	0	35.636
3h	95.69	3	0	0	40.914
3i	61.55	2	0	0	37.44
3j	61.55	3	0	0	38.894
3k	61.55	2	0	0	35.876
3l	107.37	3	1	0	37.885
3m	87.57	2	0	0	37.209
3n	80.01	4	1	0	35.726
3o	87.57	2	0	0	37.316
3p	107.37	3	0	0	40.426
Ascorbic Acid	107.22	2	0	0	11.760

Table 3: Determination of electronic parameters of 3a-p.

Compound	Volume	SASA	FOSA	FISA	CIQPlogS
Range	500.0 – 2000.0	300.0 – 1000.0	0.0 – 750.0	7.0 – 330.0	–6.5 – 0.5
3a	1023.57	604.653	0	149.27	-5.435
3b	1060.488	606.591	0	84.473	-7.034
3c	1020.827	600.211	0	133.765	-5.435
3d	1044.195	613.074	0	188.423	-5.387
3e	1053.699	621.354	0	94.702	-7.07
3f	1024.031	605.168	0	149.39	-5.435
3g	1035.433	607.308	0	181.29	-5.387
3h	1140.893	648.025	81.289	162.385	-5.701
3i	1033.155	594.635	88.27	82.483	-5.922
3j	1072.052	615.243	0.703	83.723	-7.016
3k	989.955	572.305	0	83.535	-6.002
3l	1074.752	631.944	0	192.155	-6.004
3m	1036.631	612.775	0	159.971	-5.427
3n	1003.839	679.552	182.87	98.606	-5.427
3o	1046.535	579.431	0	144.465	-6.175
3p	1164.126	601.406	0	179.411	-6.116
Ascorbic Acid	539.547	348.025	84.949	244.905	-1.062

expected to show excellent MDCK cell permeability than the standard ascorbic acid, in which 3b, 3e, 3j and 3k showed much greater result so expected to have excellent BBB permeability whereas 3d, 3g, 3l and 3p found to have lesser permeability compared to others. Based on the values all the compounds are expected to have good skin permeability and binding to human serum albumin. With regard to prediction of human oral absorption most of the compounds have high oral absorption, few molecules like 3b, 3e, 3j and 3n predicted to have low oral absorption where as standard ascorbic acid medium oral absorption (Table 4). Ames toxicity studies are mainly performed to determine whether "the compound is mutagenic or not". It is one of the extensively used method that employ bacteria to analyze whether a particular chemical is able to produce mutations in the DNA of that bacteria. Out of screened compounds 3l and 3p are found to be Ames toxic with probability of 75%, it was may be because of the presence of -NO_2 group. Remaining all the compounds along with standards were Non-ames toxic, with almost similar probability. All the derivatives were found non carcinogenic with higher probability, so all the compounds are predicted to be non-carcinogenic. All the compounds were found to fall in category III of Acute oral toxicity which means all the compounds are found to have good LD_{50} values i.e>500mg and <5000mg, where as standard drug comes under category IV. Rat and fish toxicity values i.e LD_{50} mol/kg and pLC_{50} mg/L is found for all the compounds (Table 5). In the molecular docking studies, all 16 compounds were docked against 3 different targets, out of which best docked compounds were identified as 3a, 3b and 3j for 2DY5, 3a, 3m, 3n for 3NV5 and 3c, 3d and 3o for 3B9J based on their docking score, better docking score was observed for the test compounds than the standard drug, in case of 2DY5 and 3NV5 ((Table 6). In the interaction of 3a with 2DY5 we have observed solvent exposure on the thiophene ring (Figure 4). In case of 3b, interacted with 2DY5, we have seen a halogen bond between 2-Cl substituent on 7th phenyl ring with an water molecule, we have also observed a hydrogen bonding between NH(3rd position in the pyridopyrimidine ring) with HIS25 (Figure 5). In case 3j with 2DY5, solvent exposure was seen on the pyridopyrimidine ring, hydrogen bond was also observed between NH(3rd position in the pyridopyrimidine ring) and HIS25 (Figure 6). In case of 3NV5, interacted with 3a, we observed solvent exposure on S present in the 2nd position of pyridopyrimidine ring as well as on the 7th phenyl ring, hydrogen bonding was also seen between the 4th - OH substituent present on the 7th phenyl group with TYR76 (Figure 7). In case of 3m, we have seen solvent exposure on S present in the 2nd position of pyridopyrimidine ring, hydrogen bonding was also seen between the 4th - NH_2 substituent present on the 7th phenyl group with TYR76 (Figure 8). In case of 3NV5, interacted with 3n, we observed solvent exposure on the

Table 4: Determination of ADME parameters of 3a-p.

Compound	QPPCaco (nm/sec)	QPlogBB	QPPMDK	QPlogKp	QPlogKhsa	Human Oral Absorption
Range	<25 poor, >500 great	–3.0 – 1.2	<25 poor, >500 great	–8.0 – –1.0	–1.5 – 1.5	1, 2 or 3 for low, medium, or high
3a	380.519	-0.805	903.635	-2.838	0.17	3
3b	1566.188	0.298	10000	-2.022	0.619	1
3c	533.83	-0.64	1261.746	-2.505	0.167	3
3d	161.838	-1.269	346.983	-3.563	0	3
3e	1252.689	-0.019	8695.591	-1.95	0.477	1
3f	379.522	-0.808	901.65	-2.839	0.171	3
3g	189.115	-1.202	372.497	-3.399	-0.018	3
3h	285.762	-0.887	597.66	-3.327	0.093	3
3i	1635.737	0.005	3892.932	-1.878	0.548	3
3j	1592.057	0.266	10000	-1.939	0.647	1
3k	1598.598	0.127	6923.775	-1.837	0.438	3
3l	149.173	-1.284	328.198	-3.683	0.313	3
3m	301.227	-0.89	628.601	-3.11	0.22	3
3n	1150.325	-0.418	2966.566	-2.008	0.404	1
3o	422.606	-0.634	906.157	-2.887	0.163	3
3p	197.036	-1	394.867	-3.575	0.374	3
Ascorbic Acid	47.148	-1.723	18.219	-5.393	-0.942	2

Table 5: Evaluation of toxicity of 3a-p.

Comp. code	AMES toxicity		Carcinogenicity		Acute Oral Toxicity		Rat acute toxicity LD_{50} mol/kg	Fish toxicity pLC_{50} mg/L
	Result	Probability	Result	Probability	Result	Probability		
3a	-	0.6654	-	0.8694	III	0.6316	2.2217	1.8146
3b	-	0.6373	-	0.8890	III	0.6006	2.2927	1.4757
3c	-	0.6896	-	0.8706	III	0.5753	2.3536	1.6524
3d	-	0.7208	-	0.8210	III	0.5704	2.3479	1.5705
3e	-	0.6142	-	0.9041	III	0.6313	2.2984	1.5796
3f	-	0.6654	-	0.8694	III	0.6315	2.2217	1.8146
3g	-	0.7208	-	0.8210	III	0.5704	2.3479	1.5705
3h	-	0.6701	-	0.7433	III	0.6066	2.4075	2.0593
3i	-	0.6109	-	0.9715	III	0.6807	2.1725	1.8212
3j	-	0.5895	-	0.8866	III	0.5742	2.5066	1.5791
3k	-	0.6058	-	0.8953	III	0.5972	2.3857	1.5530
3l	+	0.7503	-	0.7381	III	0.5730	2.4594	1.5960
3m	-	0.5973	-	0.8904	III	0.7125	2.2270	1.8359
3n	-	0.6199	-	0.8700	III	0.5528	2.3329	1.4762
3o	-	0.5973	-	0.8904	III	0.7125	2.2270	1.8359
3p	+	0.7503	-	0.7381	III	0.6212	2.4594	1.5960
Ascorbic Acid	-	0.9400	-	0.8589	IV	0.5871	1.3059	1.5598

Table 6: Molecular docking studies of 3a-p.

Compounds	2DY5	3NV5	3B9J
3a	-6.625	-6.093	-2.782
3b	-6.724	-5.546	-4.357
3c	-4.484	-4.15	-4.931
3d	-4.81	-2.639	-5.946
3e	-5.483	-3.887	-1.496
3f	-4.842	-4.203	-4.869
3g	-4.508	-3.658	-2.36
3h	-4.386	-4.019	-1.704
3i	-6.416	-2.759	-1.936
3j	-7.478	-4.217	-2.37
3k	-6.333	-3.836	-1.232
3l	-5.534	-4.1	-3.926
3m	-5.523	-6.011	-1.486
3n	-5.931	-5.58	-4.54
3o	-3.7	-5.564	-7.039
3p	-4.658	-3.746	-2.025
Ascorbic Acid	-4.233	-3.979	-7.344

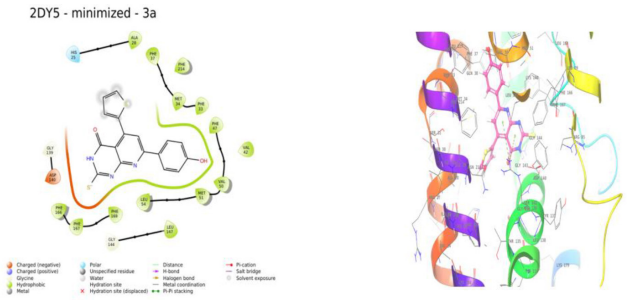

Figure 4: Interaction of 3a with 2DY5.

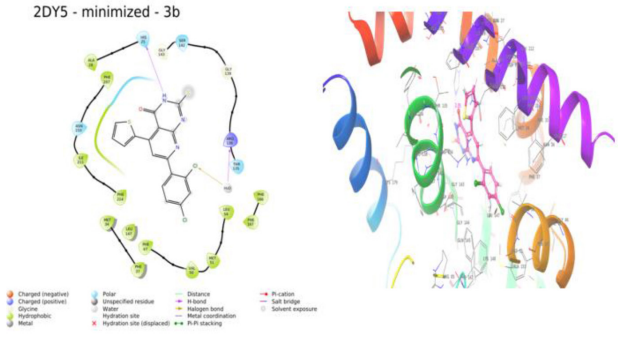

Figure 5: Interaction of 3b with 2DY5

pyridopyrimidine ring and 7th phenyl ring, hydrogen bonding was seen between the NH of 3rd position and HIS 362, O atom present in 4th position and ALA 365, salt bridge was seen between Sulphur of 2nd position and ARG206 (Figure 9). In case of 3B9J, 3o interacted with different amino acid residues of the protein,

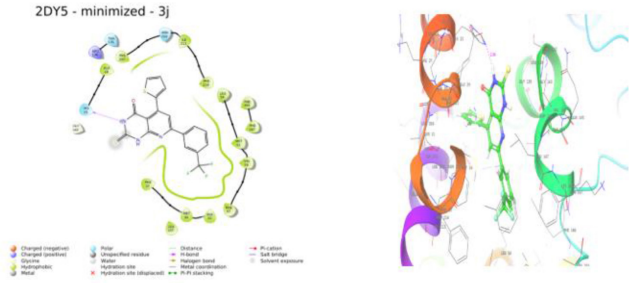

Figure 6: Interaction of 3j with 2DY5.

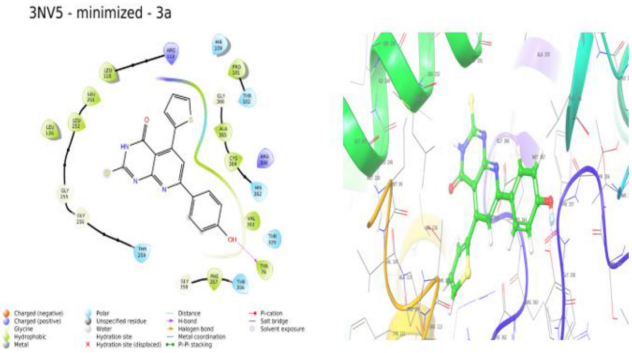

Figure 7: Interaction of 3a with 3NV5.

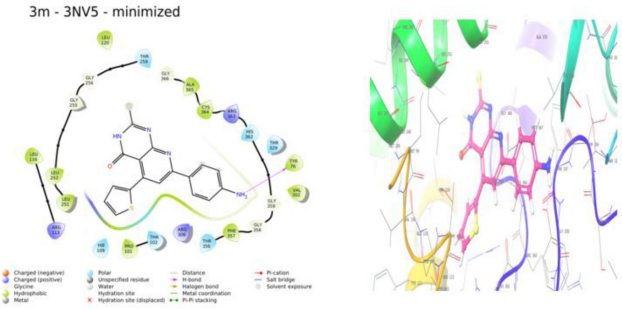

Figure 8: Interaction of 3m with 3NV5.

interaction was seen between the S present at the second position and GLY260 and ASN261, oxygen present at the 4th position was interacted by forming a hydrogen bond with a water molecule, pi-pi stacking was seen between the 7th phenyl group substituted with amino group and LYS256, where as amino group formed hydrogen bonding with GLU267 (Figure 10). In case of 3d, we have seen 3 different hydrogen bonding interaction with the protein, Hydrogen present on 3rd nitrogen, and hydroxyl group present on the 7th phenyl group interacted with GLU267, LEU257 and LEU404 (Figure 11). Based the *in-silico* studies, especially depending on the docking score, 8 compounds were selected for the synthesis to determine its *anti-oxidant* properties. In the synthesis, 6-amino thiouracil (1) was synthesized using thiourea and ethyl cyanoacetate using

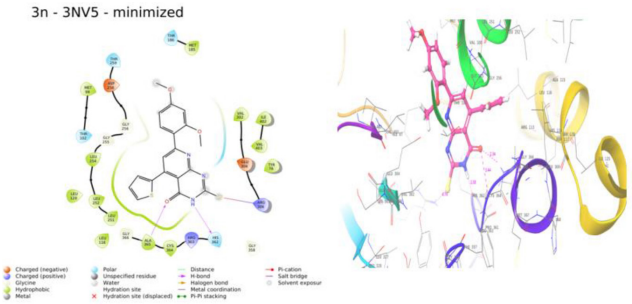

Figure 9: Interaction of 3n with 3NV5.

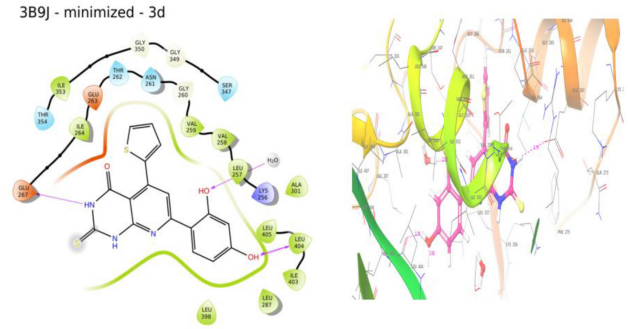

Figure 10: Interaction of 3d with 3B9J.

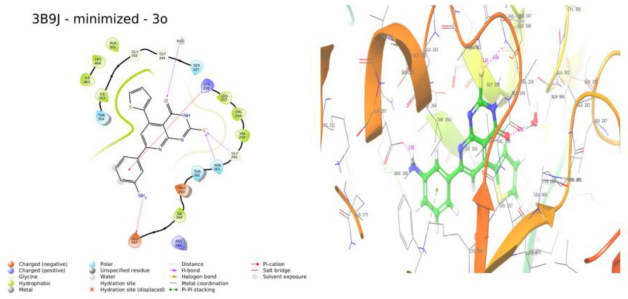

Figure 11: Interaction of 3o with 3B9J.

Table 7: Physicochemical properties of synthesized compounds(3a-p).

Compound	R	Molecular Formula	Molecular weight	Melting point(°C)
3a	4-OH	$C_{17}H_{11}N_3O_2S_2$	353.42	332-334
3b	2,4-Cl	$C_{17}H_9C_{l2}N_3OS_2$	406.31	324-326
3c	2-OH	$C_{17}H_{11}N_3O_2S_2$	353.42	338-340
3d	2,4-OH	$C_{17}H_{11}N_3O_3S_2$	369.42	342-344
3j	3-CF_3	$C_{18}H_{10}F_3N_3OS_2$	405.42	334-336
3m	4-NH_2	$C_{17}H_{12}N_4OS_2$	352.43	328-330
3n	2,4-OCH_3	$C_{17}H_{15}N_3O_3S_2$	397.47	336-338
3o	3- NH_2	$C_{17}H_{12}N_4OS_2$	352.43	324-326

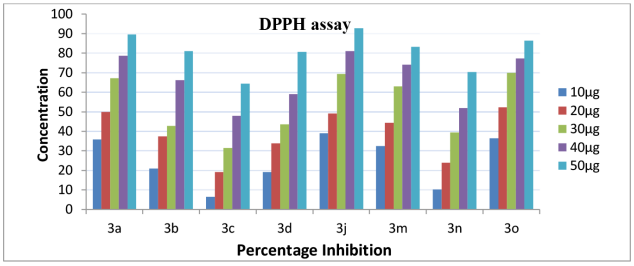

Figure 12: Percentage inhibition of compounds by DPPH assay.

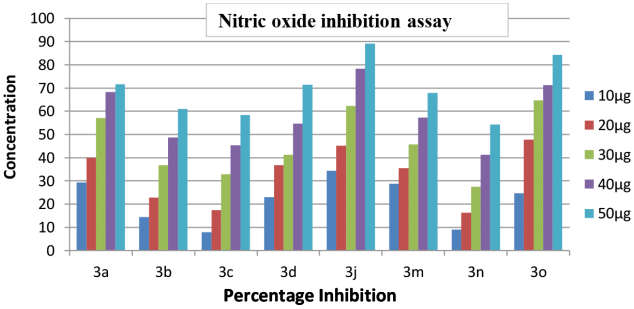

Figure 13: Percentage inhibition of compounds by Nitric oxide inhibition assay.

sodium ethoxide in ethonolic media. α-β unsaturated compounds (2a-p) were synthesized by thiophene-2-carboxaldehyde and different substituted acetophenones (a-p). Further 6-amino thiouracil (1) and α-β unsaturated compounds(2a-p) reacted in DMF to yield targeted 7-phenyl-5-(thiophen-2-yl)-2-thioxo-2,3-dihydropyrido[2,3-d]pyrimidine-4(1H)-one with different substitution (Figure 3). Physicochemical properties of the compounds were given (Table 7). In the spectral data of compounds, peaks for important functional groups were observed for two different -NH group of pyrimidine ring, C=N, C=C, C=O, C=S peaks were also appeared. In ^1H spectral data, two peaks were observed in the downfield for two different deshielded protons of two -NH group of pyrimidine ring, A singlet was observed for all the compounds for the proton of C_6 pyridine ring. Some of the important thienyl protons were also observed. In mass spectrum, M^+ ion peak was observed for all the respective compounds.

In-vitro anti-oxidant studies were performed for 8 best scored compounds by DPPH assay and nitric oxide inhibition assay (Figure 12 and 13). Out of all the tested compounds, compound 3j exhibited maximum inhibition, a better inhibition was observed than standard ascorbic acid. Compound 3a has shown slightly higher activity than standard whereas activity of 3o is slightly lower and comparable with standard ascorbic acid. IC_{50} of 3j, 3a and 3o is 22.03, 22.91 and 18.44µg/ml respectively which are lesser than standard IC_{50} values. In case of nitric oxide inhibition assay, 3j exhibited excellent inhibition which is slightly higher than the standard ascorbic acid, where as 3o was also

Table 8: Percentage inhibition of compounds by DPPH assay.

Compounds	Percentage inhibition						
Concentration	10µg/ml	20µg/ml	30µg/ml	40µg/ml	50µg/ml	IC_{50}	R^2
3a	35.85	49.97	67.16	78.76	89.63	22.91	0.948
3b	20.92	37.42	42.8	66.21	80.99	30.52	0.983
3c	6.52	19.19	31.47	47.98	64.29	41.65	0.982
3d	19.19	33.78	43.57	59.11	80.61	31.99	0.989
3j	38.96	49.07	69.37	80.99	92.78	22.03	0.945
3m	32.38	44.39	63.08	74.17	83.29	25.28	0.954
3n	10.32	23.84	39.36	51.87	70.34	33.8	0.994
3o	36.42	52.31	69.87	77.19	86.31	18.44	0.969
STD	36.54	41.96	54.77	78.8	87.34	25.06	0.950

Table 9: Percentage inhibition of compounds by Nitric oxide inhibition assay.

Compounds	Percentage inhibition						
Concentration	10µg/ml	20µg/ml	30µg/ml	40µg/ml	50µg/ml	IC_{50}/ml	R^2
3a	29.36	39.91	57.05	68.19	71.69	29.02	0.937
3b	14.39	22.87	36.74	48.75	61.08	41.06	0.997
3c	7.81	17.49	32.87	45.32	58.37	44.20	0.99
3d	23.01	36.74	41.27	54.6	71.41	34.35	0.968
3j	34.28	45.21	62.34	78.29	89.21	24.09	0.964
3m	28.74	35.42	45.78	57.29	67.89	33.69	0.949
3n	8.97	16.21	27.49	37.89	46.23	48.34	0.986
3o	24.59	47.81	64.75	71.29	84.26	25.74	0.958
STD	27.34	42.9	65.7	77.9	86.4	24.99	0.970

shown promising activity which was slightly lower than standard. IC_{50} of 3j and 3o is 24.09 and 25.74 (Table 8 and 9). From the *in-vitro* result, compound 3j, 3a, and 3o are considered as promising molecules.

CONCLUSION

The main objective of this study was to study the anti-oxidant potency of different substituted 7-phenyl-5-(thiophen-2-yl)-2-thioxo-2,3-dihydro-pyrido[2,3-d] pyrimidine-4(1H)-one, Based on the docking score, best scored molecules were selected and synthesized. Anti-oxidant potential of synthesized compounds were determined by the well-known assay's. From the *in-vitro* data, compound 3j, 3a, and 3o are considered as promising molecules i.e p-OH, p-CF_3 and p-NH_2 substituted compounds. The promising activity may be because of presence of electron releasing group (3a- p-OH, 3o- p-NH_2) and electron withdrawing group (3j- p-CF_3) which can be considered as lead molecules for the further discovery.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

ROS: Reactive Oxygen Species; **IR:** Infra-Red; **NMR:** Nuclear Magnetic Resonance; **PDB:** Protein Data Bank; **PK:** Pharmacokinetics; **tPSA:** Topological Polar Surface Area; **SASA:** Solvent Accessible Surface Area; **FOSA:** A hydrophobic component of SASA; **FISA:** A hydrophilic component of SASA; **MDCK cells:** Madin-Darby Canine Kidney cells; **LD_{50}:** Lethal Dose 50.

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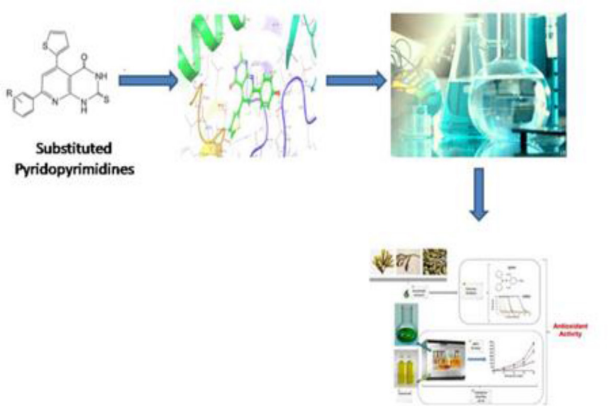
PICTORIAL ABSTRACT



SUMMARY

Novel substituted pyridopyrimidinesi.e 7-phenyl-5-(thiophen-2-yl)-2-thioxo-2,3-dihydropyrido[2,3-d] pyrimidine-(1H)-ones were considered for the evaluation of their anti-oxidant potential because of their wide biological application. *In-silico* studies were carried out along with molecular docking was performed to filter the best compounds. Best compounds with good docking score were selected for synthesis and evaluation. Anti-oxidant studies were carried out by DPPH free radical scavenging assay and Nitric oxide inhibition assay. Most of the compounds showed good oral bioavaiabity (lipinsk's rule of 5), ADMET properties, electronic parameters in the *in-silico* evaluation. Based on the docking score, 8 compounds were selected for synthesis and its anti-oxidant properties were determined. From the *in-vitro* data, compound 3j, 3a, and 3o are considered as promising molecules i.e p-OH, p-CF_3 and p-NH_2 substituted compounds. The promising activity may be because of presence of electron releasing group (3a- p-OH, 3o- p-NH_2) and electron withdrawing group (3j- p-CF_3) which can be considered as lead molecules for the further discovery.

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