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Synthesis, characterisation and *in vitro* evaluation of antioxidant and anti-inflammatory activity of some novel 5-aryl-7-[(1e)-aryl substituted]-1h, 3h-pyrido [2, 3-d] pyrimidine-2, 4-dione derivatives

Rama Rao Nadendla and KNV Chenchu Lakshmi

Abstract
Novel 5-aryl -7-[(1E)-aryl substituted]-1H, 3H-pyrido [2, 3-d] pyrimidine-2, 4-dione derivatives (1p-15p) were synthesized in two steps. Step 1 by reacting various aromatic aldehydes with aliphatic ketone i.e acetone under strong basic conditions by conventional method to form1, 5-diaryl substituted-(E, E)-1, 4-pentadien-3-one derivatives which were condensed with 6-amino uracil to form 5-Aryl -7-[(1e)-Aryl Substituted]-1H, 3H-Pyrido [2, 3-D] Pyrimidine-2, 4-Dione Derivatives. The purity and progress of reaction was assessed by TLC and melting point. Synthesised compounds were characterised by various spectroscopic methods such as IR, H1 &C13 NMR. The synthesised pyridopyrimidine were evaluated for their *In vitro* antioxidant and anti-inflammatory activities by DPPH assay and heat haemolysis method respectively. The results indicate that pyridopyrimidine showed dose dependent free radical scavenging activity in DPPH method and in RBC stabilisation method. 12p and 13p compounds showed higher free radical scavenging inhibition and compounds 1p, 9p, 10p, 11p, 12p and 14p exbhited good stabilisation of RBC membrane nearer to standard drug. The study provides a good scope for further development of novel targeted molecules.

Keywords: pyridopyrimidine, DPPH method, heat haemolysis method

1. Introduction
The heterocyclic fusion of pyrimidine and pyridine rings resulted in formation of pyridopyrimidines, the structural analogs of biogenic quinazolines and pteridines. Pyridopyrimidines and related fused heterocycles are of interest as potential bioactive molecules. Also, due to the presence of pyridopyrimidine moiety in some important drugs, interest in the construction of such molecules has been aroused. In the last few years, an enormous number of papers and reviews have been reported dealing with the chemistry and applications of this class of compounds [1, 2].
Pyrido [2,3-d] pyrimidines are the most abundance isomer in the literature and hence, this scaffold is associated with a wide range of biological activities, such as molluscicidal agents against Biomphalaria alexandrina snails, [3] anticancer, [4-6] antimicrobial, [7-10] anti-inflammatory and analgesic, [11-13] antiviral, [14, 15] antihypertensive, potent inhibitor of dihydrofolate reductase (DHFR) [16] which is an important target site in most of the parasitic diseases, Tyrosine kinase inhibitor, [17, 18] Cyclin-Dependent Kinase 4 (CDK4) inhibitor, [19] antihistaminic, calcium channel antagonist, antileishmanial, diarrhea, and diuretic activities. [20]

2. Materials and Methods
Melting points were determined in open glass capillaries using Tempo (600) melting point apparatus and were uncorrected. IR spectra (KBr discs) Bruker analysers were confirmed by Shimadzu FT-IR Spectrophotometer using KBr pellets technique, Model No.8400S (Japan). 1H and 13C NMR spectra were recorded on Bruker 400 MHz NMR spectrometer (Switzerland) using DMSO as solvent. T.L.C. was run on silica gel G plates using ethyl acetate: n-hexane (5:5) as developing solvent to assess the progress of reaction and purity of the compounds. All other chemicals used in the present study were of analytical grade.

2.1 Drugs and Chemicals
6 – Aminouracil (Alfa Aesar – L03332), Benzaldehyde (LOBA-B.NO-L107581308), Acetone (LOBA-LL17391207), P- Dimethylaminobenzaldehyde (LOBA-B.NO-G329509),

P- Chlorobenzaldehyde (LOBA-B.NO-S39291202), 2,4-Dichlorobenzaldehyde (KEMPHASOLB.NO13402L007), Sodium hydroxide pellets (LOBA- 0590000500), Anisaldehyde (LOBA – LL1053120), 3,4 – Dimethoxybenzaldehyde (SD FINE CHEM LIMITED – 76142k01), 3,4,5 – Trimethoxybenzaldehyde (SISCO – T-8380376), Salicylaldehyde (SD FINE CHEM LIMITED-H042/0103/0404/13), Vanillin (CDH LABORATORY–0110.9), Syringaldehyde (SD FINE CHEM LIMITED-B123/1601/2702/13), 3 – Bromobenzaldehyde (OTTO KEMI-B2065), 2 – Nitrobenzaldehyde (LOBA-0493200025), 4-Cyanobenzaldehyde (LOBA), Naphthaldehyde (LOBA), Thiophene-2-aldehyde, Methanol (SD FINE-CHEMLIMITED- B.NO-IOZA-0502-0409-13), Charcoal (QUALINGENS-BNO-17335406-S), Ethanol (CSS-B.NO-110605), Silica gel (S42171204), DPPH (HI-MEDIA-B.NO-0000171982) Agar agar (LOBA-B.NO-V0324/1), Peptone (HIMEDIA – RM667-500G), Beef extract (HIMEDIA – RM002-500G), Sodium chloride (SD FINE CHEM LIMITED-B.NO-H12A/1611/2301/13), Ascorbic acid (LOBA-B.NO-SL44911205), DMSO ((LOBA-B.NO-LMO4231309), Aspirin, Ethyl acetate (LOBA-LL14051205).

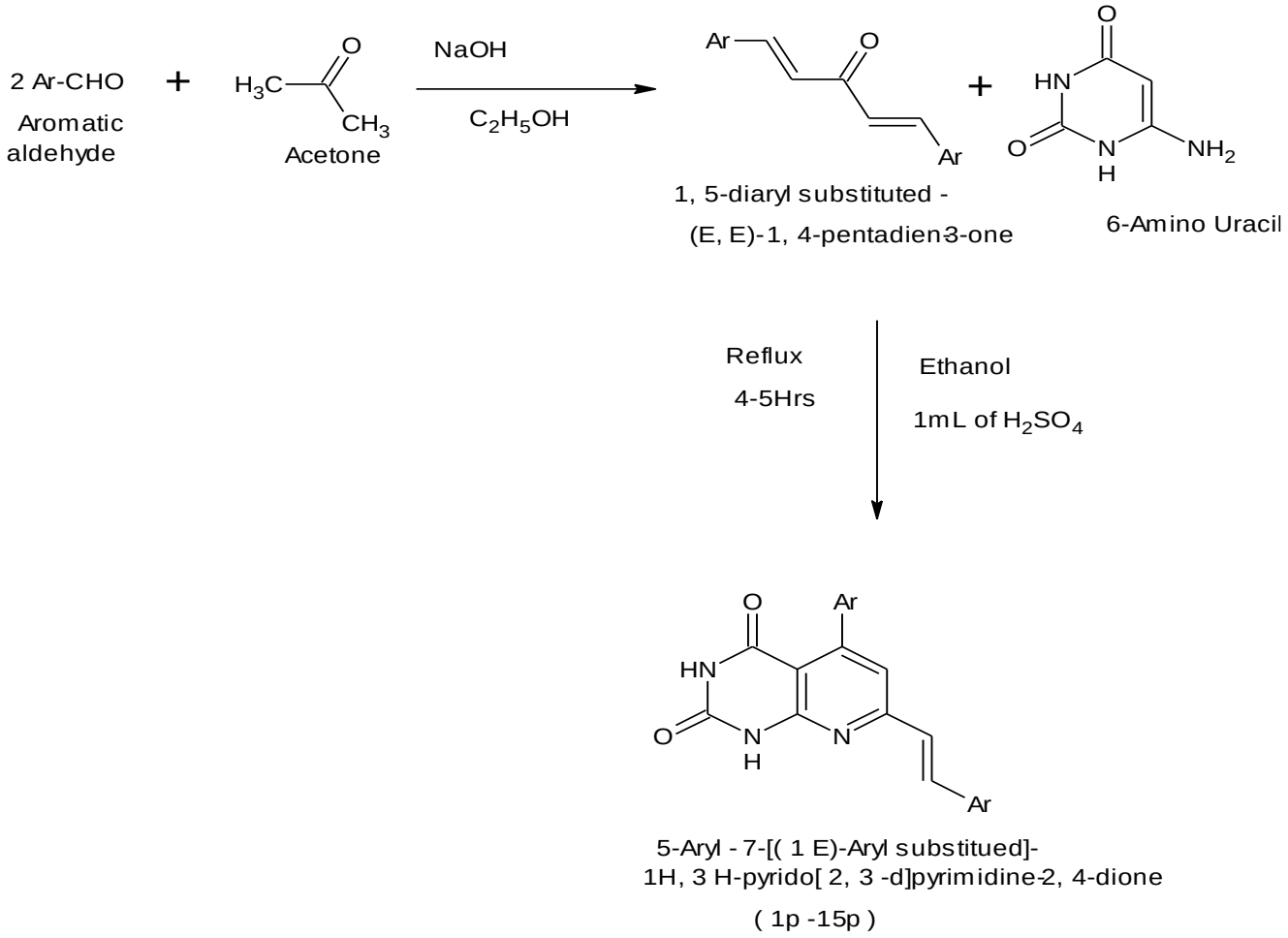
2.2 General procedure

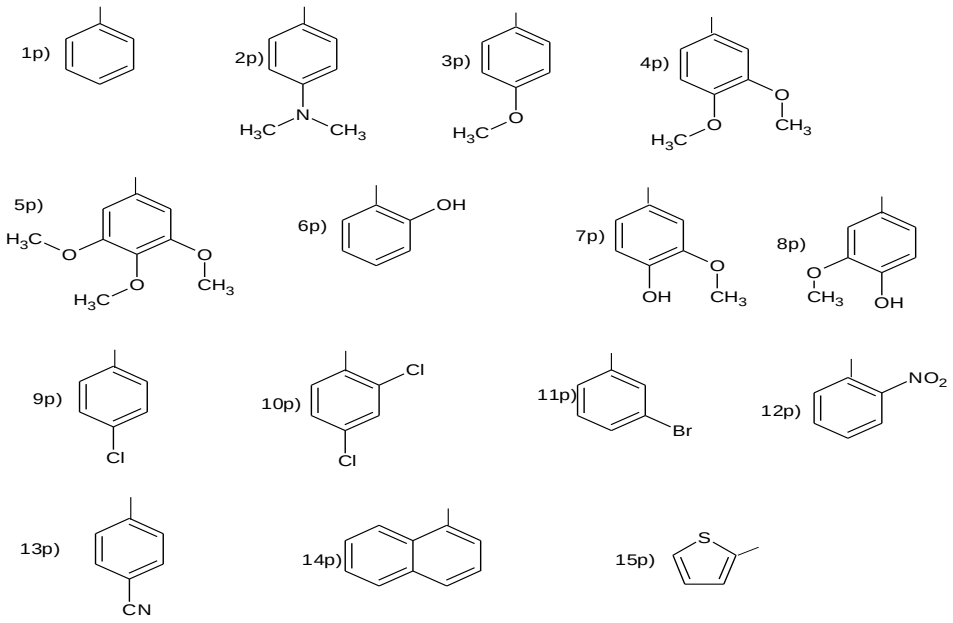
STEP 1: Procedure for Synthesis of 1, 5-diaryl substituted-(E, E)-1, 4-pentadien-3-one derivatives

2.5gm of sodium hydroxide pellets were dissolved in 25ml of water, then to the solution 15ml of methanol was added and the mixture was cooled down in the stream of cold water. The mixture was added to the 100ml Erlen Mayer flask containing 25mMol of aromatic aldehydes and 13mMol of acetone. The mixture was stirred for 15min; the precipitation formed was filtered off with suction and washed thoroughly with cold water. The product was dried in the air and recrystallized by using methanol.

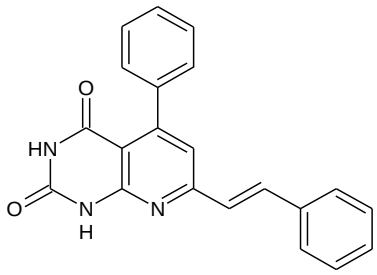
STEP 2: Procedure for synthesis of 5-Aryl -7-[(1E)-Aryl substituted]-1H, 3H-pyrido [2, 3-d] pyrimidine-2, 4-dione derivatives (1p-15p)

Step 1 product was dissolved in ethanol and equimolar quantity of the 6-amino uracil was added to the mixture. To the reaction mixture 1 mL of conc. H_2SO_4 was added and refluxed for 3-4 hours. After reflux the mixture was added to the cold water; precipitate formed was filtered and recrystallized by using ethanol. The purity and progress of reaction was confirmed by thin layer chromatography, melting point and column chromatography. The synthesized compounds were characterized by IR, proton NMR and C^{13}NMR spectroscopic techniques. The procedure was illustrated in scheme-1.

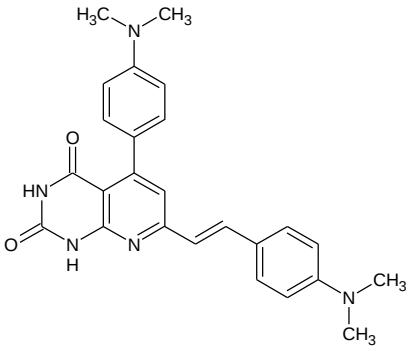




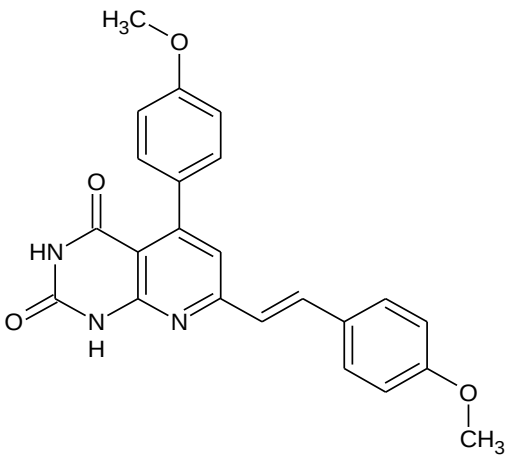
Scheme 1: synthesis of 5-Aryl -7-[(1E)-Aryl substituted]-1H, 3H-pyrido [2, 3-d] pyrimidine-2, 4-dione derivatives



Compound 1p: IUPAC Name: 5-phenyl -7-[(1E)-2-phenylethenyl]-1H,3H-pyrido[2,3-d] pyrimidine-2,4-dione, Molecular Formula:$C_{21}H_{15}N_3O_2$, Molecular weight: 341.37g/mol, Melting Point: Estimated = 280^0c, Observed = 270^0c, Elemental composition: C%-73.89H%4.43O%9.37N%12.31, Lipinski's rule of five: [Y], %yield: 67%, R_F value:0.6 (Ethyl acetate: Hexane 60:40), Solubility : soluble in DMSO, IR:3028-2915 (C=CH stretching), 2318 (overtone band), 1703, 1645 (C=O), 1593(C=N), 1490-1444 (C=C). H^1 NMR of Compound 1p: 12.476 (S, NH), 11.198(S, NH), 7.669, 7.651, 7.378, 7.375, 7.359, 7.356, 7.340, 7.337, 7.111, 7.109, 7.092, 7.090, 7.073, 7.072(m, Ar-H), 6.941, 6.921(m, C=C), 52. 13, 2.508, 2.504(Solvent Peak) C^{13}NMR of Compound 1p**:** 162.68 (C=O), 154.95, 141.47, 130.97, 130.32, 122.16, 120.36, 120.15, 110.78(C=C) &Aromatic carbons 40.22, 40.01, 39.80, 39.59, 39.38, 39.17, 38.96.

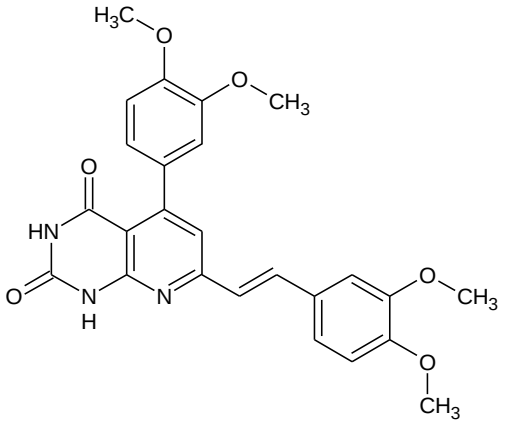


Compound 2p: IUPAC Name: 5-[4-(dimethylamino)phenyl]-7-[(1E)-2-[4-(dimethylamino) phenyl]ethenyl]-1H,3H-pyrido[2,3-d]pyrimidine-2,4-dione, Molecular Formula: $C_{25}H_{25}N_5O_2$, Molecular Weight : 427.51g/mol, Melting Point : Estimated= 290^0C, Observed = 285^0C, Elemental Composition: C% 70.24, H%5.89, O%7.49, N%16.38 Lipinski's rule of five: [N], %yield:70%, R_F value:0.65 (Ethyl acetate: Hexane 60:40),Solubility: soluble in DMSO IR:3745(NH stretching), 3058-2743(CH Stretching), 1714,1673(C=O), 1540(C=N), 1382-1481(C=C). H^1 NMR :11.675(S,NH), 9.969(S,NH), 8.370, 8.133, 8.123, 8.111, 7.982, 7.968, 7.958, 7.923, 7.902, 7.882, 7.723, 7.705, 7.675, 7.582, 7.572, 7.560, 7.535, 7.519, 7.499, 7.482, 7.464, 7.446, 7.362, 7.120, 7.100, (m, Ar-H) 6.888, 6.868(m, C=C), 3.854, 3.393, 2.514, 2.510.

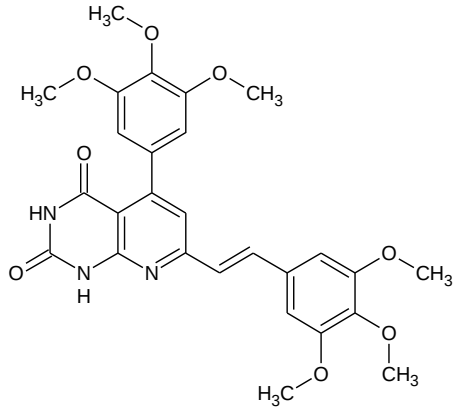


Compound 3p: IUPAC Name: 5-(4-methoxyphenyl)-7-[(1E)-2-(4-methoxyphenyl)ethenyl]-1H,3H-pyrido[2,3-d]pyrimidine-2,4-dione, Molecular formula: $C_{23}H_{19}N_3O_4$, Molecular weight:401.422 g/mol, Melting point: Estimated= 290^0C,Observed= 280^0C,Elemental composition:C (68.82%), H (4.77%), N (10.47%), O (15.94%),Lipinski's rule of five: [✓], %yield:70%, R_F value:0.65, Solubility: soluble in DMSO IR:3744(NH stretching), 3153 (C=CH), 1702(C=O), 1591(C=N), 1455(C=C),1292(COC). H^1 NMR: 11.795 (S,NH), 10.054(S,NH), 8.421, 8.135, 8.115, 8.053, 7.978, 7.965, 7.956, 7.919, 7.898, 7.878, 7.837, 7.816, 7.795, 7.722, 7.699, 7.678, 7.581, 7.571, 7.562, 7.546, 7.528, 7.515, 7.496,

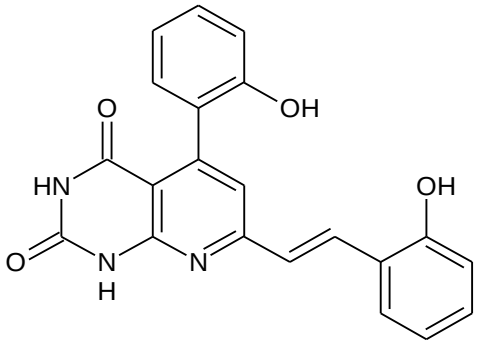
7.475, 7.456, 7.438, 7.054, 7.034(m, Ar-H) (m, C=C), 3.875, 3.826, 3.578, 3.346(S, OCH3), 2.513, 2.509. C^{13} NMR: 163.92(C=O), 163.45(C=O), 154.49, 153.74, 149.58, 149.54, 148.95, 148.88, 129.92, 128.88, 128.10, 125.22, 125.14, 124.27, 119.95, 116.16, 115.92(C=C) & Aromatic carbons, 66.87, 63.78(OCH3), 40.18, 39.97, 39.76, 39.55, 39.34, 39.14, 38.93.



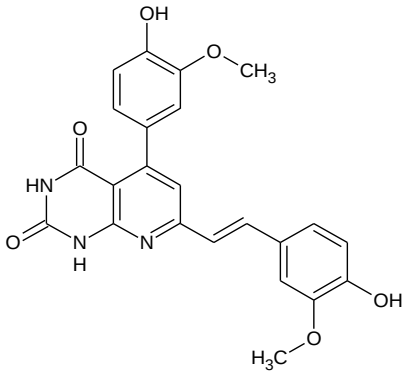
Compound 4p: IUPAC Name: 5-(3,4-dimethoxyphenyl)-7-[(1E)-2-(3,4-dimethoxyphenyl) ethenyl]-1H,3H-pyrido[2,3-d]pyrimidine-2,4-dione, Molecular Formula: C25H23N3O6, Molecular Weight: 427.51g/mol, Melting Point: Estimated= 290 ^{0}C,Observed= 274 ^{0}C, Elemental Composition: C (65.07%), H (5.02%), N (9.11%), O (20.8%), Lipinski's Rule of Five: [✓], %yield:70%, R_F value:0.65, Solubility: soluble in DMSO IR:3743(NH stretching) 3000-2834(CH stretching), 2314 (overtone band) 1701, 1642 (C=O), 1584 (C=N), 1247(COC). H^1 NMR: 11.675(S,NH), 9.969(S,NH), 8.370, 8.133, 8.123, 8.111, 7.982, 7.968, 7.958, 7.923, 7.902, 7.882, 7.723, 7.705, 7.675, 7.582, 7.572, 7.560, 7.535, 7.519, 7.499, 7.482, 7.464, 7.446, 7.362, 7.120, 7.100(m, Ar-H), 6.888, 6.868(m, C=C), 3.854, 3.393 (OCH3), 2.514, 2.510. C^{13}NMR: 161.16 (C=O), 157.41(C=O), 143.00, 142.11, 131.75, 130.25, 130.14, 128.30, 127.18, 126.93, 125.04, 123.58**,** 114.49, 114.44, 114.21, 113.75, 113.38, 113.17(C=C) & Aromatic carbons**,** 65.33**,** 55.34, 55.31, 55.26, 55.12(OCH3)**,** 40.17, 39.96, 39.75, 39.53, 39.33, 39.12, 38.91



Compound 5p: IUPAC Name: 5-(3,4,5-trimethoxyphenyl)-7-[(1E)-2-(3,4,5 trimethoxyphenyl)ethenyl]-1H,3H-pyrido[2,3-d]pyrimidine-2,4-dione, Molecular formula:C27H27N3O8, Molecular weight: 521.526 g/mol, Melting Point: Estimated=280℃, Observed=275℃, Elemental Composition: C (62.18%), H (5.22%), N (8.06%), O (24.54%), Lipinski's rule of five: [N], %yield:70%, R_F value:0.65 (Ethyl acetate: Hexane 60:40) Solubility: soluble in DMSO, IR: 3744 (NH Stretching) 3106, 2940, 2835 (CH stretching), 2315 (overtone band) 1702, 1640 (C=O),1585(C=N),1231(COC). H^1 NMR : 11.722(S,NH), 10.034(S,NH), 8.421, 8.135, 8.115, 8.053, 7.978, 7.965, 7.956, 7.919, 7.898, 7.878, 7.837, 7.816, 7.795, 7.722, 7.699, 7.678, 7.581, 7.571, 7.562, 7.546, 7.528, 7.515, 7.496, 7.475, 7.456, 7.438, 7.054, 7.034(m, Ar-H) (m, C=C), 3.875, 3.826, 3.758, 3.346, 2.513, 2.509. C^{13}NMR: 161.16(C=O), 157.41, 143.00, 142.11, 131.75, 130.25, 130.14, 128.30, 127.18, 126.93, 125.04, 123.58, 114.49, 114.44, 114.21, 113.75, 113.38, 113.17 (C=C) & Aromatic carbons, 65.33, 55.34, 55.31, 55.26, 55.12, 54.96, 54.90, 54.87(OCH3), 40.17, 39.96, 39.75, 39.53, 39.33, 39.12, 38.91.

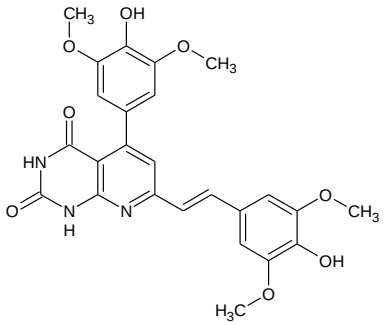


Compound 6p: IUPAC Name:5-(2-hydroxyphenyl)-7-[(1E)-2-(2-hydroxyphenyl)ethenyl]-1H,3H-pyrido[2,3-d]pyrimidine-2,4-dione, Molecular Formula:C21H15N3O4, Molecular Weight:373.368 g/mol, Melting Point: Estimated=290 ℃,Observed=280 ℃, Elemental Composition: C (67.56%), H (4.05%), N (11.25%), O (17.14%), Lipinski's Rule of Five: [✓], %Yield: 70%, R_F Value:0.65 (Ethyl acetate: Heaxane 60:40), Solubility: soluble in DMSO IR : 3744 (NH stretching) 3449-3398(OH stretching) 3075,2882,2888,2803(CH stretching), 2314 (overtone band), 1700(C=O), 1624(C=C, weak), 1575(C=N), 1164(CO stretching). H^1 NMR : 11.818(S,NH), 11.737(S,NH), 11.019, 10.979, 7.347, 7.328, 7.297, 7.288, 7.284, 7.268, 7.265, 7.249,7.245, 7.213, 7.182, 7.179, 7.164, 7.160, 7.145, 7.142, 7.101, 7.085, 7.060, 7.042, 7.040(m, Ar-H) (m, C=C), 4.157, 4.146, 3.514, 3.457, 2.509, 2.504. C^{13}NMR : 166.26, (C=O)161.84, 161.68, 160.79, 147.50(C-OH), 133.64, 133.25, 132.11, 131.76, 131.55, 131.28, 131.13, 129.95, 128.98, 128.59, 128.44, 128.14, 127.83, 127.11, 126.47, 126.37, 126.03, 125.66, 125.40, 124.40, 114.51, 114.35(C=C) &Aromatic carbons, 40.22, 40.01, 39.80, 39.59, 39.38, 39.17, 38.96

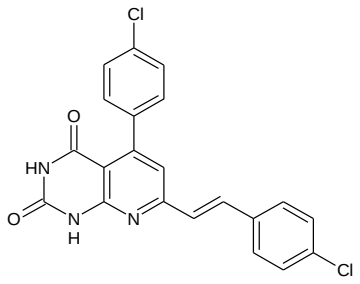


Compound 7p: IUPAC Name: 5-(4-hydroxy-3-

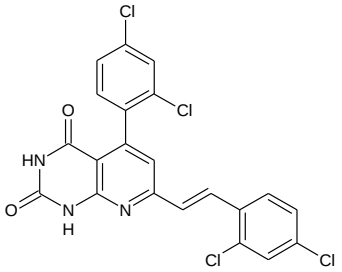
methoxyphenyl)-7-[(1E)-2-(4-hydroxy-3-methoxyphenyl) ethenyl]-1H,3H-pyrido[2,3-d]pyrimidine-2,4-dione, Molecular Formula: C23H19N3O6, Molecular Weight: 433.42 g/mol, Melting Point: Estimated = 310℃, Observed=290 ℃, Elemental Composition: C (63.74%), H (4.42%), N (9.7%), O (22.15%) Lipinski's Rule of Five: [✓], %Yield: 68%, R_F Value: 0.65 (Ethyl acetate: Heaxane 60:40) Solubility: soluble in DMSO IR: 3742 (NH stretching), 3327(OH stretching) 3170, 3009 (CH stretching), 2314(overtone band), 1708(C=O), 1630(C=C, weak), 1588 (C=N), 1272(COC),1155(CO stretching). H^1 NMR: 10.453 (S, NH), 10.425 (S,NH), 9.776, 7.436, 7.432, 7.416, 7.412, 7.394, 7.390, 6.973, 6.954, 6.720, 6.626, 6.605(m, Ar-H), 6.482, 6.463(m, C=C), 5.248, 3.845. C^{13}NMR : 162.16(C=O), 150.59(C-OH), 150.05(C-OH), 149.55, 149.44, 148.59, 148.43, 140.05, 139.01, 138.39, 137.66, 133.84, 133.35, 133.03, 131.04, 131.00, 130.85, 129.79, 129.67, 129.39, 129.22, 129.11, 128.35, 128.29, 128.18, 126.71, 125.12, 124.73, 124.69, 124.59, 123.77, 111.01(C=C)&Aromatic carbons, 64.36, 51.83(OCH3), 40.18, 39.97, 39.76, 39.55, 39.34, 39.13, 39.93



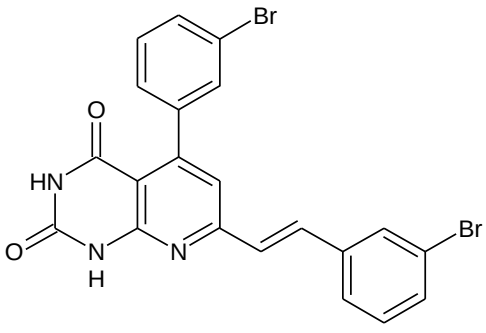
Compound 8p: IUPAC Name: 5-(4-hydroxy-3,5-dimethoxyphenyl)-7-[(1E)-2-(4-hydroxy-3,5-dimethoxy phenyl) ethenyl]- 1H, 3H – pyrido [2,3-d] pyrimidine -2,4-dione, Molecular Formula: C25H23N3O8, Molecular Weight : 493.472 g/mol, Melting Point : Estimated = 320℃, Observed = 316℃, Elemental Composition : C (60.85%), H (4.7%), N (8.52%), O (25.94%), Lipinski's Rule of Five: [✓], %Yield: R_F Value: 0.65 (Ethyl acetate :Hexane 60:40) Solubility: soluble in DMSO IR: 3743(NH stretching), 3388 (OH stretching), 3172,3070,2845(CH stretching), 2314(overtone band), 1739, 1692 (C=O), 1622(C=C, weak), 1592 (C=N), 1277 (COC),1163(CO stretching). H^1 NMR: 11.818, 11.737(S,NH), 11.019, 10.979(S,NH), 7.347, 7.328, 7.297, 7.288, 7.284, 7.268, 7.265, 7.249, 7.245, 7.213, 7.182, 7.179, 7.164, 7.160, 7.145, 7.142, 7.101, 7.085, 7.060(m, Ar-H), 7.042, 7.040(m, C=C), 4.157(S,OH), 4.146, 3.154, 3.457, 2.509, 2.504, 3.656(OCH3), 2.512, 2.508, 2.504. C^{13}NMR: 166.98(C=O), 164.19, 156.25, 155.08, 150.93(C-OH), 150.12(C-OH), 149.17, 147.78, 147.17, 142.55, 138.79, 122.79(C=C) & Aromatic carbons.



Compound 9p: IUPAC Name: 5-(4-chlorophenyl)-7-[(1E)-2-(4-chlorophenyl)ethenyl]-1H,3H-pyrido[2,3-d]pyrimidine-2,4-dione. Molecular formula: C21H13Cl2N3O2, Molecular Weight: 410.25 g/mol, Melting Point: Estimated = 300℃, Observed = 290℃, Elemental Composition: C (61.48%), H (3.19%), Cl (17.28%), N (10.24%), O (7.8%), Lipinski's Rule of Five: [N], %Yield:, R_F Value:0.65 (Ethyl acetate: Hexane 60:40) Solubility: soluble in DMSO IR: 3743 (NH stretching), 3031, 2888(CH stretching), 2313 (over tone band), 1701(C=O), 1620(C=C, weak), 1559(C=N),622(C-Cl stretching). H^1 NMR: 11.875 (S,NH), 10.621(S,NH), 8.083, 8.064, 7.615, 7.597, 7.337, 7.317, 7.298, 7.114, 7.095, 7.076, 7.057, 7.035, 7.016, 6.998, 6.939, 6.920, 6.894(m, Ar-H), 6.875, 6.787(m, C=C), 2.513, 2.509, 2.504. C^{13}NMR: 162.94(C=O), 156.44, 151.53, 136.43, 129.81, 128.99, 128.35, 125.55, 124.23, 123.03, 121.79, 116.64, 114.73(C=C) & Aromatic carbons, 49.65, 49.44, 49.22, 49.01, 48.80, 48.58, 48.37

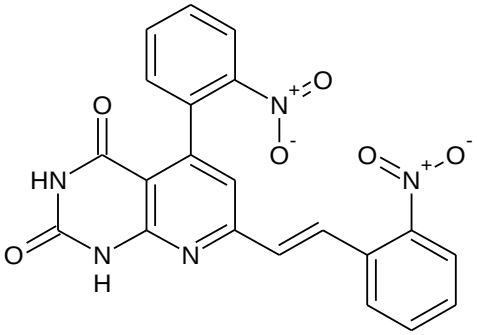


Compound 10p : IUPAC Name : 5-(2,4-dichlorophenyl)- 7-[(1E)-2-(2,4-dichlorophenyl) ethenyl]- 1H,3H-pyrido [2,3-d]pyrimidine-2,4-dione. Molecular Formula: C21H11Cl4N3O2, Molecular Weight: 479.14 g/mol, Melting Point: Estimated = 280℃, Observed = 270℃, Elemental Composition: C (52.64%), H (2.31%), Cl (29.59%), N (8.77%), O (6.68%), Lipinski's Rule of Five: [N], %Yield: 70%, R_F Value :0.65 (Ethyl acetate: Hexane 60:40) Solubility: Soluble in DMSO IR: 3743 (NH stretching), 3075 (CH stretching, broad),2314(over tone band), 1702 (C=O), 1637 (C=C, weak), 1558(C=N), 667(C- Cl stretching). H^1 NMR: 11.894(S, NH), 10.621(S,NH), 8.083, 8.064, 7.615, 7.597, 7.337, 7.317, 7.298, 7.114, 7.095, 7.076, 7.057, 7.035, 7.016, 6.998, 6.939, 6.920, 6.894(m, Ar-H), 6.875, 6.787(m, C=C), 2.513, 2.509, 2.504. C^{13} NMR: 163.94(C=O), 156.44, 130.44, 129.81, 128.99, 128.35, 125.55, 124.24, 124.21, 123.02, 122.59, 121.79, 114.73(C=C)&Aromatic carbons, 49.65, 49.43, 49.22, 49.01, 48.79, 48.58, 48.37

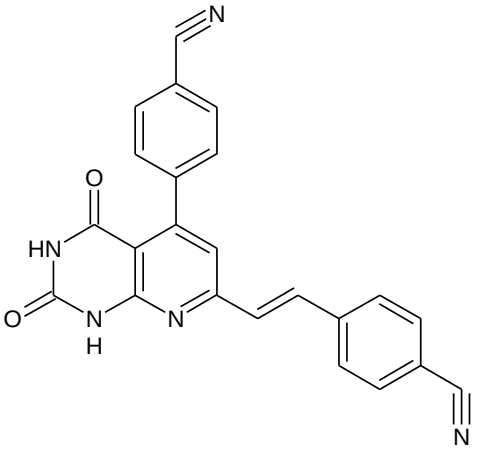


Compound 11p: IUPAC Name:5-(3-bromophenyl)-7-[(1E)-2-(3-bromophenyl)ethenyl]-1H,3H-pyrido[2,3-d]pyrimidine-2,4-dione, Molecular Formula: C21H13Br2N3O2, Molecular Weight: 499.162 g/mol, Melting Point: Estimated= 320 ℃,Observed=300 ℃, Elemental Composition: C (50.53%), H

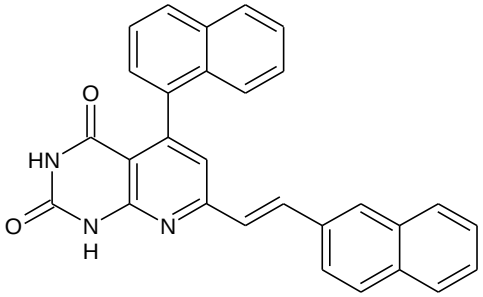
(2.63%), Br (32.02%), N (8.42%), O (6.41%), Lipinski's Rule of Five: [N], %Yield:, R_F Value:0.65 (Ethyl acetate: Hexane 60:40) Solubility: soluble in DMSO IR : 3742 (NH stretching), 3061(CH stretching, broad), 2314(over tone band), 1701(C=O),1633 (C=C, weak), 1558(C=N),567(C-Br stretching). H^1 NMR: 11.89(S,NH), 10.621(S,NH), 8.083, 8.064, 7.615, 7.597, 7.337, 7.317, 7.298, 7.114, 7.095, 7.076, 7.057, 7.035, 7.016, 6.998, 6.939, 6.920, 6.894(m, Ar-H), 6.875, 6.787(m, C=C), 2.513, 2.509, 2.504. C^{13} NMR: 161.13(C=O), 160.22, 154.83, 153.45,139.18, 139.06, 130.34, 126.55, 112.82, 112.01, 110.23, 102.16(C=C)&Aromatic carbons, 40.18, 39.97, 39.76, 39.56, 39.35, 39.14, 38.93.



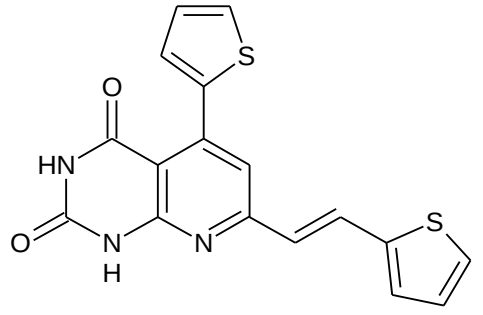
Compound 12p: IUPAC Name: 5-(3-nitrophenyl)-7-[(1E)-2-(3-nitrophenyl)ethenyl]-1H,3H-pyrido[2,3-d]pyrimidine-2,4-dione, Molecular Formula: C21H13N5O6, Molecular Weight:431.364 g/mol, Melting Point: Estimated=290℃,Observed=280℃, Elemental Composition: C (58.47%), H (3.04%), N (16.24%), O (22.25%), Lipinski's Rule of Five: [✓], %Yield:, R_F Value:0.65 (Ethyl acetate: Hexane 60:40), Solubility: soluble in DMSO IR: 3742 (NH stretching), 3060(CH stretching, broad), 2315 (over tone band), 1701(C=O),1633 (C=C, weak), 1598 (C=N), 1513-1563 & 1395-1337 (NO stretching) H^1 NMR : 11.780(S,NH), 10.084(S,NH), 8.421, 8.135, 8.115, 8.053, 7.978, 7.965, 7.956, 7.919, 7.898, 7.878, 7.837, 7.816, 7.795, 7.722, 7.699, 7.678, 7.581, 7.571, 7.562, 7.546, 7.528, 7.515, 7.496, 7.475, 7.456, 7.438(m, Ar-H), 7.054, 7.034(m, C=C), 2.513, 2.509.



Compound 13p: IUPAC Name:4-{7-[(1E)-2-(4-cyanophenyl)ethenyl]-2,4-dioxo-1H,3H-pyrido[2,3-d]pyrimidin-5-yl}benzonitrile, Molecular Formula:C23H13N5O2, Molecular Weight: 391.39 g/mol, Melting Point: Estimated = 310℃,Observed =310℃, **Elemental Composition:** C (70.58%), H (3.35%), N (17.89%), O (8.18%), Lipinski's Rule of Five: [N], %yield:75%, R_F Value:0.65 (Ethyl acetate: Hexane 60:40), Solubility: soluble in DMSO IR:3742(NH stretching), 3163,2906(CH stretching, broad),2314(overtone band), 2228(CN), 1707(C=O), 1655(C=C, weak),1595(C=N). H^1 NMR: 11.737(S,NH), 11.098(S,NH), 7.901, 7.897, 7.881, 7.876, 7.338, 7.330, 7.312, 7.269, 7.177, 7.081, 7.025, 6.858, 6.758(m, Ar-H), 6.731, 6.650(m, C=C), 2.513, 2.510.



Compound 14p: IUPAC Name: 5-(1,2,3,4-tetrahydronaphthalen-1-yl)-7-[(1E)-2-(1,2,3,4-tetrahydronaphthalen-2-yl)ethenyl]-1H,3H-pyrido[2,3-d]pyrimidine-2,4-dione, Molecular Formula : C29H27N3O2, Molecular Weight:449.554 g/mol, Melting Point: Estimated = 290℃, Observed = 276℃, Elemental composition : C (77.48%), H (6.05%), N (9.35%), O (7.12%), Lipinski's Rule of Five: [N], %Yield: 79%, R_F Value: 0.65 (Ethyl acetate: Hexane 60:40) Solubility: soluble in DMSO IR: 3743(NH stretching), 3135, 3043(CH stretching, broad),2313(over tone band) 1703 (C=O), 1591(C=N). H^1 NMR: 11.737 (S, NH), 11.098(S,NH), 7.901, 7.897, 7.881, 7.876, 7.338, 7.330, 7.312, 7.269, 7.177, 7.081, 7.025, 6.858, 6.758(m, Ar-H), 6.731, 6.650(m, C=C)



Compound 15p: IUPAC Name:5-(thiophen-2-yl)-7-[(1E)-2-(thiophen-2-yl)ethenyl]-1H,3H-pyrido[2,3-d]pyrimidine-2,4-dione, Molecular Formula: C17H11N3O2S2, Molecular Weight: 353.41 g/mol, Melting Point : Estimated= 270℃, Observed=264℃, Elemental Composition: C (57.78%), H (3.14%), N (11.89%), O (9.05%), S (18.14%), Lipinski's Rule of Five: [✓], %Yield: 82%, R_F Value:0.65 (Ethyl acetate: Hexane 60:40) Solubility: soluble in DMSO IR: 3743(NH stretching), 3164 (CH stretching, broad),2314 (overtone band) 1703 (C=O), 1554(C=N), 1039 (C=S). H^1 NMR : 11.361 (S,NH), 10.280(S,NH), 7.936, 7.918, 7.819, 7.799, 7.716, 7.711, 7.596, 7.578, 7.560, 7.513, 7.494, 7.476, 7.449, 7.444, 7.428(m, Ar-H), 7.028, 6.819(m, C=C), 2.510.C^{13}NMR : 165.98(C=O), 152.81, 152.48, 142.86, 131.16, 130.96, 129.40, 129.32, 129.27, 129.12, 126.41, 125.10, 122.40, 122.00, 121.67, 119.34, 115.21, 113.38, 112.56(C=C)&Aromatic carbons, 40.19, 39.98, 39.78, 39.57, 39.36, 39.15, 38.94

3. In vitro evaluation studies

3.1 Anti-Oxidant Activity [21, 22]:

Method: DPPH free radical scavenging assay: The percentage of antioxidant activity (AA %) of each substance was assessed by DPPH free radical assay. The samples were reacted with the stable DPPH radical in an methanol solution. The reaction mixture consisted of adding 1 mL of test samples (1p-15p) in methanol, 1 mL of DPPH radical solution in methanol. When DPPH reacts with an antioxidant compound, which can donate hydrogen, it is reduced. The changes in colour (from deep violet to light yellow) were read [Absorbance (Abs)] at 517 nm after 100 min of reaction using a UV-VIS spectrophotometer (DU 800; Beckman Coulter, Fullerton, CA, USA). The mixture of methanol (1 mL) and sample (Ascorbic acid) (1 mL) serve as blank. The control solution was prepared by mixing methanol (1 mL) and DPPH radical solution (1 mL). The scavenging activity percentage (AA %) was determined by

$$\%\text{Inhibition} = \frac{\text{O.D of } control - \text{O.D of test}}{\text{O.D of control}}$$

% Inhibition at different concentrations were tabulated in table-1.

Table 1: Effect of Free radical scavenging activity of pyridopyrimidine compounds 1p-15p at different concentrations

Compounds	% Inhibition			
	2.5 µg/ml	5 µg/ml	7.5 µg/ml	10 µg/ml
1	71.3	71.9	76.60	73.93
2	69.41	67.4	67.1	65.0
3	25.2	21.7	21.7	6.95
4	27.82	25.2	40.0	38.2
5	37.3	43.4	47.8	45.2
6	45.2	38.2	44.34	46.95
7	48.6	45.2	47.82	53.9
8	37.3	23.4	12.17	9.56
9	46.0	47.8	53.04	52.17
10	51.3	47.8	52.17	47.8
11	47.8	55.6	49.56	49.56
12	59.1	56.5	52.17	47.8
13	55.6	56.52	56.52	57.2
14	46.08	43.47	42.60	32.17
15	32.17	32.17	30.43	32.17
Ascorbic acid	37.39	66.0	53.04	25.2

3.2 Anti Inflammatory Activity [23, 24]

Method: RBC Heat Hemolysis method, The human red blood cell (HRBC) membrane Stabilization method procedure: The blood was collected from healthy human volunteer who had not taken any NSAIDS for 2 weeks prior to the experiment. Then blood transferred to the centrifuge tubes and centrifuged at 3,000 rpm for 10min and was washed three times with equal volume of normal saline solution. The volume of blood measured and reconstituted as 5%v/v suspension with normal saline solution. Various concentrations (100,250 and 500µg/ml) of compounds were prepared using methanol and to each concentration 3 ml of HRBC suspension was added. These suspensions were incubated in water bath at 56^0c for 30 min, after incubation centrifuge tubes were cooled under running tap water. Then reaction mixture was centrifuged at 2,500 rpm for 5 min and the haemoglobin content of the supernatant solution was estimated on UV spectrophotometer at 560 nm. Aspirin (100, 250 and 500 µg/ml) was used as standard and control was prepared by HRBC suspension.the results were tabulated in table-2.

$$\text{The \%inhibition of Haemolysis} = \frac{\text{O.D of control} - \text{O.D of test}}{\text{O.D of control}} \times 100$$

Table 2: Effect of pyridopyrimidine on RBC Membrane stabilization activity

Compound	% inhibition		
	100µg/ml	250µg/ml	500µg/ml
1p	76.6	73.3	74.3
2p	86.3	66	64.6
3p	86.6	75.6	74
4p	81.3	51.3	36.6
5p	75.3	54	18
6p	78	76	81.3
7p	73	83.3	66
8p	81.6	87.6	78.3
9p	84	87.6	83
10p	78	78	76
11p	69.6	72.3	85.3
12p	80	60	88.6
13p	67	78	49.3
14p	73	84.6	81
15p	84.3	82.6	80
Std(Aspirin)	75	84.2	89.6

4. Discussion

4.1 Synthesis of pyridopyrimidine

The compounds designed were synthesised by conventional method and their characterisation was done by spectroscopic methods. The spectroscopic features IR data reveal the presence of NH (3400 Cm^{-1} to 3500Cm^{-1}), CH (2900-3100 Cm^{-1}) stretching C=O (1700 Cm^{-1}), C=N (1550-1490 Cm^{-1}). Proton NMR reveals the presence of two NH singlet's between 10-12ppm, unsaturated HC=CH at 6.9-7.0ppm and multiplet for aromatic proton between 6.0-9.0ppm. C^{13} NMR reveals the presence of carbonyl group between 155-165ppm, aromatic carbon peaks at a range of 110-155ppm.

4.1.1 Antioxidant activity

The 2, 2-diphenyl-1-picrylhydrazyl (DPPH) method is a preferred method because it is fast, easy and reliable and does not require a special reaction and device. Ascorbic acid showed dose dependent antioxidant activity in DPPH assay. Pyridopyrimidine showed dose dependent free radical scavenging activity in DPPH method similar to standard. 1p, 2p, 12p and 13p compounds showed higher % inhibition when compared with other compounds.

4.1.2 Anti-inflammatory activity

Aspirin showed dose dependent red blood cell (RBC) membrane stabilisation activity in hypo saline induced RBC membrane haemolysis assay. Synthesised pyridopyrimidine compounds also showed dose dependent membrane stabilisation activity. Among all the com pounds 1p, 9p, 10p, 11p, 12p and 14p compounds results are nearer to standard drug.

5. Conclusion

A series of new novel 5-aryl -7-[(1E)-aryl substituted]-1H, 3H-pyrido [2, 3-d] pyrimidine-2, 4-dione derivatives (1p-15p) were prepared by conventional method and evaluated for their antioxidant activity by DPPH free radical scavenging assay and anti-inflammatory activity by heat haemolysis method for

which the mechanisms underlying this process remain to be fully elucidated. It is intended that the results from these studies will assist in elucidating their precise mechanism of action and provide an approach for further optimization and development to get new leads in the treatment of microbiall infections.

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7. References

1. Dongre RS, Bhat AR, Meshram JS. Anticancer activity of assorted annulated pyrimidine: a comprehensive review. Am J Pharm Tech Res. 2014; 4(1):138-55.
2. Crawford TD, Ndubaku CO, Chen H, Boggs JW, Bravo BJ, DeLa Torre K, *et al*. Discovery of selective 4-amino-pyridopyrimidine inhibitors of MAP4K4 using fragment-based lead identification and optimization. Journal of medicinal chemistry. 2014; 57(8):3484-93.
3. Palop JA, Plano D, Moreno E, Sanmartín C. Novel quinazoline and pyrido [2, 3-d] pyrimidine derivatives and their hydroselenite salts as antitumoral agents. Arkivoc. 2014; 2:187-206.
4. Gineinah MM, Nasr MNA, Badr SMI, El-Husseiny WM. Med. Chem. Res., 2013, 22, 3943-3952. AMF. Elgohary AM, Green EE. Efficient synthesis of some pyrido [2, 3-d]-pyrimidin-4 (3H)-one derivatives via iodine catalyst in aqueous media and evaluation the synthesized compounds as anticancer. Sci. J. Chem. 2013; 1(1):1-6.
5. Maheta JG, Gol RM, Barot VM. Synthesis of novel (2H) indazole scaffold as an antimicrobial and anti-tubercular agent. Chemistry & Biology Interface. 2016; 6(1).
6. Arikkatt SD, Mathew BV, Joseph J, Chandran M, Bhat AR, Krishnakumar K. Pyrimidine derivatives and its biological potential–a review. Int. J Org. Bioorg. Chem. 2014; 4(1):1-5.
7. Aly HM, Saleh NM. Utility of a pyrimidine thione derivative in the synthesis of new fused pyrimido [4, 5d] pyrimidine, pyrido [2, 3-d] pyrimidine and different types of thienopyrimidine derivatives. Int. J Adv. Res. 2014; 2(4):694-702.
8. Mohamed NR, Abdelhalim MM, Khadrawy YA, Elmegeed GA, Abdel-Salam OM. One-pot three-component synthesis of novel heterocyclic steroids as a central antioxidant and anti-inflammatory agents. Steroids. 2012; 77(13):1469-76.
9. DeGoey DA, Betebenner DA, Grampovnik DJ, Liu D, Pratt JK, Tufano MD, *et al*. Discovery of pyrido [2, 3-d] pyrimidine-based inhibitors of HCV NS5A. Bioorganic & medicinal chemistry letters. 2013; 23(12):3627-30.
10. Reyes-Díaz I, Gómez-Jeria JS. Quantum-chemical modeling of the hepatitis C virus replicon inhibitory potency and cytotoxicity of some pyrido [2, 3-d] pyrimidine analogues. J Comput. Methods Drug Des. 2013; 3:11-21.
11. Farghaly TA, Abbas IM, Abdalla MM, Mahgoub RO. Synthesis of new pentaheterocyclic ring systems as anti-androgene, anti-HCV and anti-H1N1 agents. Arkivoc. 2012; 6:57-70.
12. Blankley CJ, Bennett LR, Fleming RW, Smith RD, Tessman DK, Kaplan HR. Antihypertensive activity of 6-arylpyrido [2, 3-d] pyrimidin-7-amine derivatives. 2. 7-Acyl amide analogs. Journal of medicinal chemistry. 1983; 26(3):403-11.
13. Gangjee A, Vasudevan A, Queener SF, Kisliuk RL. 6-Substituted 2, 4-Diamino-5-methylpyrido [2, 3-d] pyrimidines as Inhibitors of Dihydrofolate Reductases from Pneumocystis carinii and Toxoplasma gondii and as Antitumor Agents. Journal of medicinal chemistry. 1995; 38(10):1778-85.
14. Gangjee A, Vasudevan A, Queener SF, Kisliuk RL. 2, 4-Diamino-5-deaza-6-substituted pyrido [2, 3-d] pyrimidine antifolates as potent and selective nonclassical inhibitors of dihydrofolate reductases. Journal of medicinal chemistry. 1996; 39(7):1438-46.
15. Guo XN, Zhong L, Tan JZ, Li J, Luo XM, Jiang HL, *et al*. In vitro pharmacological characterization of TKI-28, a broad-spectrum tyrosine kinase inhibitor with anti-tumor and anti-angiogenic effects. Cancer biology & therapy. 2005; 4(10):1125-32.
16. Kammasud N, Boonyarat C, Sanphanya K, Utsintong M, Tsunoda S, Sakurai H, *et al*. 5-Substituted pyrido [2, 3-d] pyrimidine, an inhibitor against three receptor tyrosine kinases. Bioorganic & medicinal chemistry letters. 2009; 19(3):745-50.
17. Quintela J, Peinador C, Botana L, Estévez M, Riguera R. Synthesis and antihistaminic activity of 2-guanadino-3-cyanopyridines and pyrido [2, 3-d]-pyrimidines. Bioorganic & medicinal chemistry. 1997; 5(8):1543-53.
18. Pastor A, Alajarin R, Vaquero JJ, Alvarez-Builla J, de Casa-Juana MF, Sunkel C, *et al*. Synthesis and structure of new pyrido [2, 3-d] pyrimidine derivatives with calcium channel antagonist activity. Tetrahedron. 1994; 50(27):8085-98.
19. Agarwal A, Goyal N, Chauhan PM, Gupta S. Dihydropyrido [2, 3-d] pyrimidines as a new class of antileishmanial agents. Bioorganic & medicinal chemistry. 2005; 13(24):6678-84.
20. Kots AY, Choi BK, Estrella-Jimenez ME, Warren CA, Gilbertson SR, Guerrant RL, *et al*. Pyridopyrimidine derivatives as inhibitors of cyclic nucleotide synthesis: Application for treatment of diarrhea. Proceedings of the National Academy of Sciences. 2008; 105(24):8440-5.
21. Ferrali M, Signorini C, Ciccoli L, Comporti M. Iron release and membrane damage in erythrocytes exposed to oxidizing agents, phenylhydrazine, divicine and isouramil. Biochemical journal. 1992; 285(1):295-301.
22. Shinde UA, Kulkarni KR, Phadke AS, Nair AM, Mungantiwar AA, Dikshit VJ, *et al*. Mast cell stabilizing and lipoxygenase inhibitory activity of *Cedrus deodara* (Roxb.) Loud. wood oil.
23. Ahmad M, Saeed F, Noor Jahan M. Evaluation of insecticidal and antioxidant activity of selected medicinal plants. Journal of Pharmacognosy and Photochemistry. 2013; 1(2):153-8.
24. Koleva II, Van Beek TA, Linssen JP, Groot AD, Evstatieva LN. Screening of plant extracts for antioxidant activity: a comparative study on three testing methods. Phytochemical Analysis: An International Journal of Plant Chemical and Biochemical Techniques. 2002; 13(1):8-17.