Design, Synthesis, and Antioxidant Evaluation of Gallic Acid-Based Pyrimidine Derivatives

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ABSTRACT

N-(4-(4-aminophenyl)-6-phenylpyrimidin-2-yl)-3,4,5-trihydroxybenzamide (**IIIa-g**) was obtained from pyrimidine and 3,4,5-trihydroxybenzoyl chloride through a multi-step reaction sequence. Aminoacetophenone and different aromatic aldehydes are reacted to obtain benzaldehyde chalcones (**Ia-g**). Treatment of these chalcone with guanidine nitrate in the presence of an alkali yields 4-(4-aminophenyl)-6-phenylpyrimidin-2-amine (**Ia-g**) which are aminopyrimidines. Condensation of **II** (3,4,5-trihydroxybenzoyl chloride) and aminopyrimidines (Ia-g) in presence of acetone produces N-(4-(4-aminophenyl)-6-phenylpyrimidin-2-yl)-3,4,5-trihydroxybenzamide (**IIIa-g**). According to elemental analysis, IR, ^{1}H-NMR, ^{13}C-NMR and mass spectra of the newly synthesized compounds, their structures were confirmed. Moreover, the products were assessed for their anti oxidant and anti-bacterial properties.

INTRODUCTION

Chalcones (1,3-diaryl-2 propen-1-ones) are a part of the family of flavonoids. Their chemical structure includes two aromatic rings linked by a three carbon a,b unsaturated carbonyl system [1]. Synthetic and natural derivatives of chalcones exhibited noteworthy biological activities which included a favorable safety profile and marked promise for antioxidant, anti-inflammatory, anticancer, and antiinfective agent drug development [2].

Chalcone belongs to the most prominent class of secondary metabolites in plants. These metabolites fend for plant defense systems by battling reactive oxygen species which could severely cause molecular damage as well as damage by microorganisms, insects, and animals [3]. Also, chalcone can be synthesized chemically in the lab by means of Claisen Schmidt or aldol condensation reaction [4]. There are reports that chalcone can exert multiple benefits such as anti-inflammatory, antibacterial, antifungal, antidiabetic, and even anticancer activities. Furthermore, it enhances vision, memory, alleviates discomfort in the joints and muscles, optimizes liver and kidney functions, aids in sleep, prevents cancer, boosts immunity, and improves the health of skin and hair [5].

Gallic acids a naturally occurring low molecular weight triphenolic compound is widely found in the plant kingdom as free or as part of tannins. Salts and esters of GA are termed gallates and represent a large family of plant secondary polyphenolic metabolites.

Derivatives of gallic acid have been identified in several phytomedicines with various biological and pharmacological actions, such as radical scavenging and modulation of intracellular signaling cascades and apoptosis in tumor cells. The different potential uses offered by this uncomplicated polyphenol are made possible by the delicate balance of its antioxidant and prooxidant properties.

Gallic acid (GA) is a naturally occurring polyphenolic compound which contains three phenol rings and can be found in a variety of plants. They exist in two states, as free acids or chemically bound in polyphenolic form to the acids as tannins. Salts and esters of GA are called gallates, a type of octadecanoic acid derivatives with a vast variety of secondary polyphenolic compounds.

Antioxidant molecules All synthetic or natural chemical agents able to counter the damage that is due to reactive oxygen species (ROS) are classified as antioxidant molecules. These free radicals are generated by the body through normal oxygen use. The free radicals spoil the cell and contribute to human diseases including cardiovascular diseases, aging, diabetes and cancer [6]. Antioxidant agents, such as vitamin C (ascorbic acid), vitamin E (α-tocopherol), tripeptide glutathione (GSH), carotenoids, and flavonoids are some examples [7].

As regards the living organism, the N-containing heterocycles are the prevailing class as they include most parts. Pyrimidines are one of the significant classes of all living organisms and among N-containing heterocycles due to biological activity. The pyrimidines are electron-rich nuclei with contents of the low-nitrogen in the heterocycle. The pyrimidine ring commonly contributes to improve the pharmacokinetic/pharmacodynamic characteristics of the drug due to its ability to interact with a number of targets by providing hydrogen bonds effectively and as bioisosteres of phenyl and other aromatic π systems [8].

Pyrimidine derivatives are well known to possess a variety of biological properties, like antibacterial [9], anti-inflammatory [10,11], antifungal [12], antileishmanial [13], anticancer [14,15], analgesic [16], anticonvulsant [17], antihypertensive [18], insecticidal [19], antidiabetic [20], antiviral [21], anthelmintic [22], antitubercular [23], larvicidal [24-26], and antioxidant [27].

Based on the above evidence, chalcones, gallic acid and Pyrimidine derivatives exhibits significant biological activities. In the present work, a series of gallic acid substituted pyrimidine derivatives were designed and synthesized and their antioxidant activity were evaluated.

EXPERIMENTAL SECTION

Materials and methods

General

Melting points were determined using arson digital melting point apparatus and values obtained are uncorrected roughly nowadays. Precoated silica gel plates typically silica gel 60 F254 were used for thin layer chromatography and spots were visualized under UV light with iodine vapours subsequently. IR spectra were recorded rather quickly on a Bruker Alpha FTIR Spectrometer using pellets made of KBr.1H NMR spectra were recorded painstakingly on

BRUKER AV III 500MHz FT-NMR Spectrometer mostly in DMSO or CDCl3 with TMS as internal standard. Mass spectra were acquired rather quickly using an apex mass spectrophotometer under somewhat unusual operating conditions. Chemicals used were of analytical grade and sourced from suppliers like SD Fine or Sigma-Aldrich generally for research purposes obviously.

Chemistry

For the synthesis of title compounds, the reaction sequence outlined in scheme, were followed.

Step 1:

Synthesis of 1-(4-aminophenyl)-3-phenylprop-2-en-1-one (Ia-g):

To a solution of 4-amino acetophenone (0.05 mol) in ethanol (15 mL), Aromatic aldehyde (0.05 mol) was added. To this mixture aqueous potassium hydroxide (40%) was poured gradually with constant stirring and continues the stirring for 2 h. After adding, the mixture of potassium salt of chalcone was kept for overnight at room temperature. The potassium salt of chalcone was separated by ice-cold hydrochloric acid (10%, 30 mL). The separated solid was filtered and washed with ice-cold water till the washing was neutral to litmus. Recrystallized the compound with ethanol and dried at room temperature.

Synthesis of 4-(4-aminophenyl)-6-phenylpyrimidin-2-amine (2a-g):

A mixture of 1-(4-aminophenyl)-3-phenylprop-2-en-1-one (0.05 mol) dissolved in alcohol (25 ml) , guanidine nitrate (0.05 mol) and solution of sodium methoxide 25% (0.02 mol Na in 50 mL ethanol), was added and refluxed for 6-7 h. After the completion of reaction, the resultant mixture was cooled to room temperature. Separated product was filtered, washed with water, dried and crystallized from methanol.

Step 2:

Synthesis of 3,4,5-trihydroxybenzoyl chloride (II) :

A solution of thionyl chloride (0.3 mol) was added dropwise to a solution of gallic acid (0.25 mol). The reaction mixture was continued under stirring for 4 h and then heated for 30 min in an water bath. The progress of reaction was monitored by TLC. After cooling the excess of acid was removed under reduced pressure and the solid residue obtained was added to ice cold water, the

solid obtained was filtered off, washed it with cold water until netural pH, dried and recrystallised from ethanol.

Step 3:

N-(4-(4-aminophenyl)-6-phenylpyrimidin-2-yl)-3,4,5-trihydroxybenzamide (IIIa-g):

Compound Ia-g (0.005 mole) and 3,4,5-trihydroxybenzoyl chloride II (0.005 mole) and KOH (10 ml) in acetone (80 ml) was added. The reaction mixture was heated under reflux for 4-5 h, reaction progress was monitored by T.L.C. After completion of the reaction the mixture was poured onto crushed ice. The resulting solid was filtered off and washed with water, dried and recrystallized from ethanol.

IIIa: N-(4-(4-aminophenyl)-6-(4-nitrophenyl)pyrimidin-2-yl)-3,4,5 trihydroxybenzamide

IR (KBr) (cm-1): 1662 (C=O str), 1500.21(C=N str), 2987.4 (C-H str), 3333.2 (NH str), 3447.2 (OH str), 779.1(C-H ben), 2762.51 (C-H str),1625.13 (C=C str); **1H NMR (DMSO), δ ppm:** 9.1-9.19 δ (1H,s,NH), 6.51-6.93 δ (4H, m,Ar-H), 8.07-8.35 δ (4H, m, Ar-H), 7.10 δ (1H,s,CH), 6.22 δ (2H, s, NH2), 5.64-5.87 δ (3H,s,OH); **Anal. Calcd for $C_{23}H_{17}N_5O_6$:** C, 60.13; H, 3.73; N, 15.24; O, 20.90. Found: C, 60.02; H, 3.67; N, 15.14; O, 20.84.

IIIb: N-(4-(4-aminophenyl)-6-(4-chlorophenyl)pyrimidin-2-yl)-3,4,5-trihydroxybenzamide

IR (KBr) (cm-1): 1628 (C=O str), 1557.21(C=N str), 779.1(C-H ben), 2762.51 (C-H str, CHO), 1647.13(C=O str, CHO), 2990.45 (Ar-C-H str); **1H NMR (DMSO), δ ppm:** 9.10 δ (1H,s,NH), 6.63-6.79 δ (4H, m,Ar-H), 8.15-8.31 δ (4H, m, Ar-H), 7.22 δ (1H,s,CH), 6.30-6.38 δ (2H, s, NH2), 5.50-5.62 δ(3H,s,OH); **Anal. Calcd for $C_{23}H_{17}ClN_4O_4$:** C, 61.54; H, 3.82; Cl, 7.90; N, 12.48; O, 14.26. Found: C, 61.72; H, 3.30; Cl, 7.23; N, 12.28; O, 14.33.

IIIc: N-(4-(4-aminophenyl)-6-(3-fluorophenyl)pyrimidin-2-yl)-3,4,5-trihydroxybenzamide

IR (KBr) (cm-1): 1601.09 (C=C), 1653.61 (C=O str), 1508.21(C=N str), 779.1(C-H ben), 3320 (NH str), 3450 (OH str), 2925.24 (Ar-CH str); **1H NMR (DMSO), δ ppm:** 9.23 δ (1H,s,NH), 6.68-6.70 δ (4H, m,Ar-H), 8.14-8.20 δ (4H, m, Ar-H), 7.21 δ (1H,s,CH), 6.09 δ (2H, s, NH2), 5.55-5.72 δ(3H,s,OH); **Anal. Calcd for $C_{23}H_{17}FN_4O_4$:** C, 63.89; H, 3.96; F, 4.39; N, 12.96; O, 14.80. Found: C, 62.99; H, 3.21; F, 4.57; N, 12.23; O, 14.58.

IIId:N-(4-(4-aminophenyl)-6-(4-methoxyphenyl)pyrimidin-2-yl)-3,4,5-trihydroxybenzamide

IR (KBr) (cm-1): 1660.21 (C=O str), 1557.21(C=N str), 779.1(C-H ben), 1604.02 (C=C str), 1489.08 (C=N), 3320.12 (NH str),3456.34 (OH str), 2924.15 (Ar-C-H str); 1H NMR (DMSO), δ ppm: 9.34 δ (1H,s,NH), 6.76-6.94 δ (4H, m,Ar-H), 7.43-7.85 δ (4H, m, Ar-H), 7.05 δ (1H,s,CH), 5.51-5.60 δ(3H,s,OH); 6.11 δ (2H, s, NH2). Anal. Calcd for $C_{24}H_{20}N_4O_5$: C, 64.86; H, 4.54; N, 12.61; O, 18.00. Found: C, 64.54; H, 4.44; N, 12.68; O, 19.00.

IIIe:N-(4-(4-aminophenyl)-6-(4-(dimethylamino)phenyl)pyrimidin-2-yl)-3,4,5-trihydroxybenzamide

IR (KBr) (cm-1): 1683.92 (C=O str), 1551.32 (C=N str), 779.1(C-H ben), 3435 (OH str), 3340.13(NH str), 2954.96 (Ar-C-H str); **1H NMR (DMSO), δ ppm:** 9.33 δ (1H,s,NH), 6.65-6.82 δ (4H, m,Ar-H), 7.87-8.05 δ (4H, m, Ar-H), 7.32 δ (1H,s,CH), 5.41-4.60 δ(3H,s,OH); 6.20 δ (2H, s, NH2), 3.23-3.28 (6H, s,CH3). **Anal. Calcd for $C_{25}H_{23}N_5O_4$:** C, 65.63; H, 5.07; N, 15.31; O, 13.99. Found: C, 65.28; H, 5.33; N, 15.01; O, 14.92.

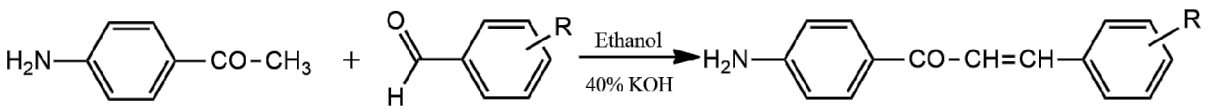
IIIf: N-(4-(4-aminophenyl)-6-(3-bromophenyl)pyrimidin-2-yl)-3,4,5-trihydroxybenzamide

IR (KBr) (cm-1): 1653.61 (C=O str), 1520.76(C=N str), 1601.09 (C=C str), 782.1(C-H ben), 3455 (OH str), 3250.13(NH str), 2925.86 (Ar-C-H str); **1H NMR (DMSO), δ ppm:** 9.20-9.35 δ (1H,s,NH), 6.01-6.12 δ (2H, s, NH2), 6.58-7.13 δ (4H, m,Ar-H), 7.41-7.68 δ (2H, m, Ar-H), 7.98-8.15 δ (4H, m, Ar-H), 7.15 δ (1H,s,CH), 5.64-5.87 δ(3H,s,OH); 6.41 δ (2H, s, NH2). **Anal. Calcd for $C_{23}H_{17}BrN_4O_4$:** C, 56.00; H, 3.47; Br, 16.20; N, 11.36; O, 12.97. Found: C, 56.25; H, 3.83; Br, 16.27; N, 11.66; O, 11.48.

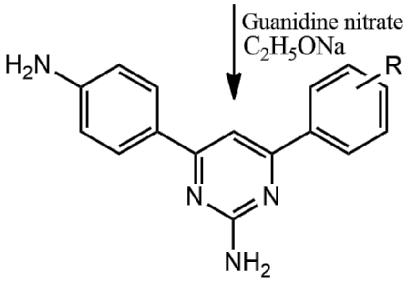
IIIg: N-(4-(4-aminophenyl)-6-(3-nitrophenyl)pyrimidin-2-yl)-3,4,5-trihydroxybenzamide

IR (KBr) (cm-1): 1658.15 (C=O str), 1516.29(C=N str), 1598.91 (C=C str), 779.1(C-H ben), 3350 (NH str), 3434.04 (OH str), 2940.21 (Ar-C-H str); **1H NMR (DMSO), δ ppm:** 9.12-9.15 δ (1H,s,NH), 6.22 δ (2H, s, NH2), 6.58-7.13 δ (4H, m,Ar-H), 7.37-7.55 δ (2H, m, Ar-H), 7.97-8.22 δ (4H, m, Ar-H), 7.77 δ (1H,s,CH), 5.34-5.47 δ(3H,s,OH). **Anal. Calcd for $C_{23}H_{17}N_5O_6$:** C, 60.13; H, 3.73; N, 15.24; O, 20.90. Found: C, 60.22; H, 3.27; N, 15.20; O, 20.58.

Step: 1



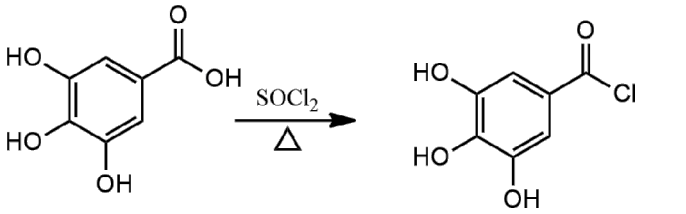
1-(4-aminophenyl)-3-phenylprop-2-en-1-one



4-(4-aminophenyl)-6-phenylpyrimidin-2-amine

Ia-Ig

Step: 2

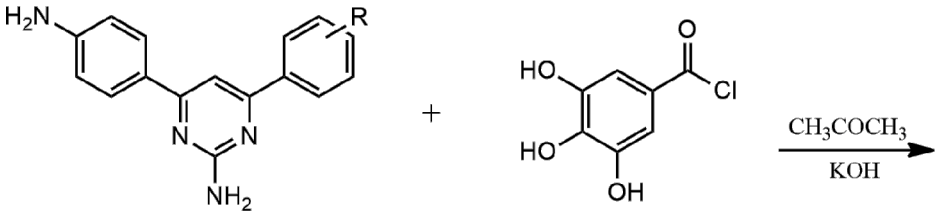


3,4,5-trihydroxybenzoic acid

3,4,5-trihydroxybenzoyl chloride

II

Step: 3

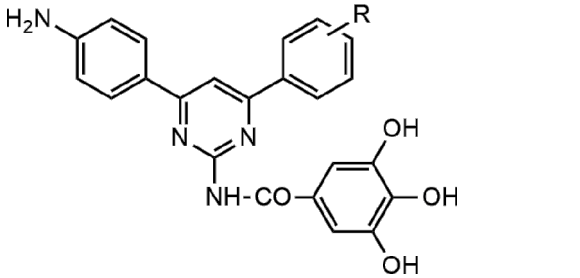


4-(4-aminophenyl)-6-phenylpyrimidin-2-amine

Ia-Ig

3,4,5-trihydroxybenzoyl chloride

II



N-(4-(4-aminophenyl)-6-phenylpyrimidin-2-yl)-3,4,5-trihydroxybenzamide

IIIa-g

R = a, 4-NO_2; b, 4-Cl; c, 3-F; d, 4-OCH_3; e, 4-$N(CH_3)_2$; f, 3-Br: g, 3-NO_2

Scheme: Conventional Synthetic route for N-(4-(4-aminophenyl)-6-phenylpyrimidin-2-yl)-3,4,5-trihydroxybenzamide (IIIa-g).

IN VITRO ANTIOXIDANT ACTIVITY

DPPH free radical - scavenging activity:

DPPH free radical scavenging capability was assessed according largely to method described by Altarejos [28] pretty much verbatim. DPPH methanolic solution 1.0 mL at 0.1 mM was mixed with sample solution 3.0 mL at varying concentrations between 10 and 320 µg/ml. Reaction mixture was incubated at room temperature in dark for thirty minutes and absorbance was recorded at five seventeen nanometers against blank. Assays were carried out thrice for each sample in a quite rigorous manner obviously. Radical scavenging activity of ascorbic acid was determined as a standard reference quite thoroughly alongside other tests. IC50 values of ascorbic acid and test samples were determined by measuring concentration required for scavenging fifty percent of free radicals. Activity was expressed as inhibition percentage of DPPH radical following equation one pretty closely.

$$I\ \% = [(Ac-As)/Ac] \times 100 \rightarrow 1$$

Where, Ac and As are the absorbance of the control and of the test/standard sample respectively.

Table 1: Reduction by DPPH stable free radical of N-(4-(4-aminophenyl)-6-phenylpyrimidin-2-yl)-3, 4, 5-trihydroxybenzamide (3a-3g).

	MEAN±SD (SEM)*							
Con (µg/ml)	**Ascorbic Acid**	**IIIa**	**IIIb**	**IIIc**	**IIId**	**IIIe**	**IIIf**	**IIIg**
10	0.0023 ± 0.0005 (0.00033)	0.0093±0.0005 (0.00033)	0.0043 ± 0.00057 (0.00033)	0.0153±0.00057 (0.00033)	0.0063 ±0.00057(0.00033)	0.0073±0.00057(0.0033)	0.0136 ±0.00057(0.00033)	0.0093 ±0.00057(0.00033)
20	0.0050±0.0(0.0)	0.0113 ±0.00057(0.00033)	0.0076 ±0.00057(0.00033)	0.0183 ±0.00057(0.00033)	0.0023 ±0.00057(0.00033)	0.0143 ±0.00057(0.00033)	0.0126 ±0.00057(0.00033)	0.015 ±0.00057(0.00033)
40	0.0063 ±0.0046(0.0026)	0.0143 ±0.00057(0.00033)	0.012 ±0.00057(0.00033)	0.024 ± 0.0(0.0)	0.0090± 0.00057(0.00033)	0.022 ±0.00057(0.00033)	0.011 ±0.00057(0.00033)	0.0210 ±0.00057(0.00033)
80	0.0143 ±0.00057 (0.00033)	0.0180 ±0.00173(0.001)	0.015 ±0.00057(0.00033)	0.0330 ±0.0010(0.00057)	0.017 ±0.00057(0.00033)	0.025 ±0.00057(0.00033)	0.020 ±0.00057(0.00033)	0.024 ±0.00057(0.00033)
160	0.0080 ±0.0 (0.0)	0.023 ±0.00057(0.00033)	0.012 ±0.0011(0.0006)	0.04 ±0.00057(0.00033)	0.021 ±0.00057(0.00033)	0.013 ±0.00057(0.00033)	0.005 ±0.0010(0.00057)	0.027 ±0.00057(0.00033)
320	0.0026 ±0.0011(0.00066)	0.027 ±0.00057(0.00033)	0.016 ±0.00057(0.00033)	0.04 ±0.00057(0.00033)	0.010 ±0.0010(0.00057)	0.011 ± 0.00115 (0.00066)	0.023 ±0.00057(0.00033)	0.030 ±0.00057(0.00033)

Table 2: Effect of N-(4-(4-aminophenyl)-6-phenylpyrimidin-2-yl)-3, 4, 5-trihydroxybenzamide (IIIa-g) at 100 µM concentration and ferric induced lipid peroxidation in rat brain homogenate.

Con (µg/ml)	% Inhibition							
	Ascorbic Acid	IIIa	IIIb	IIIc	IIId	IIIe	IIIf	IIIg
10	89.22	50	76.11	19	70	62	24.44	52
20	72.22	40	57.77	16.66	87.22	20.55	30	20
40	67	24	33.33	33.33	50	22.22	38.88	21
80	20.55	0	16.66	83.33	5.55	38.88	11.11	33.33
160	55.55	27.77	33.33	10	16.66	27.77	72.22	50
320	87.55	54	11.11	11	44.44	38.88	27.77	66.66

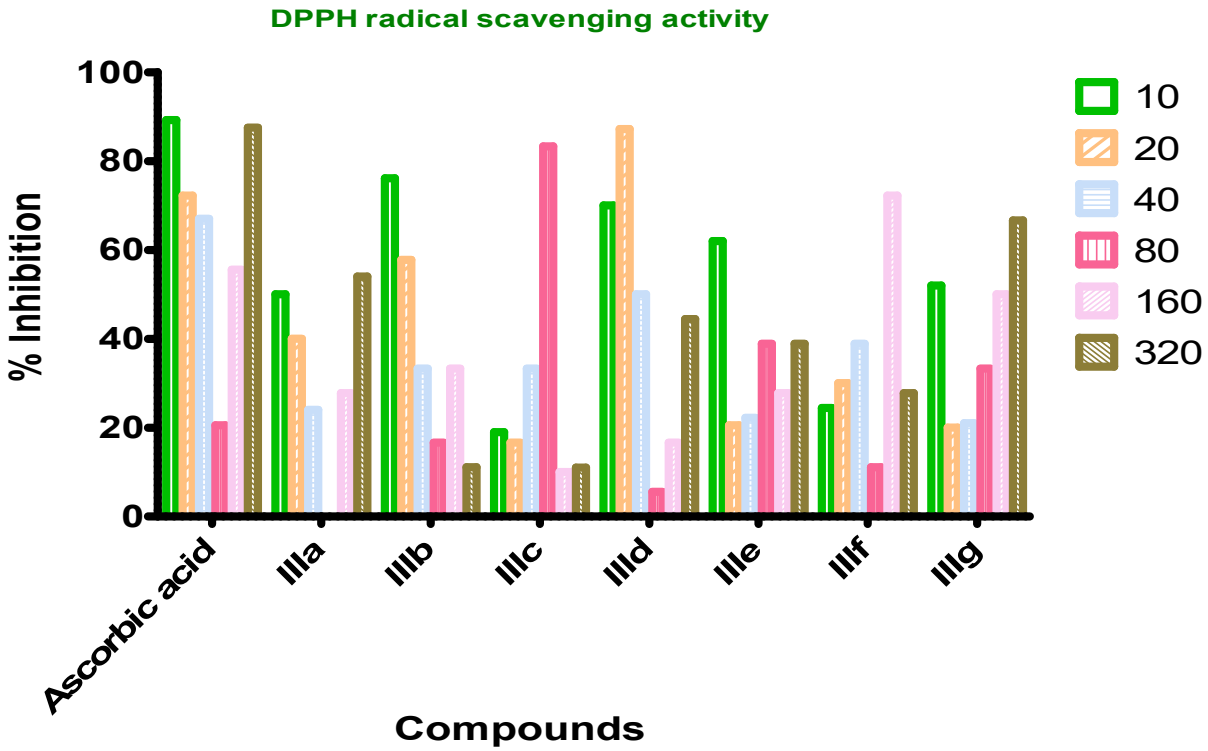


Fig. 1: % Inhibition of compounds IIIa-g in DPPH method

Ferrous (Fe2+) induced lipid peroxidation in rat brain homogenate:

Lipid peroxidation plays a crucial role in various morbid conditions and toxicities induced by certain pharmaceuticals quite frequently. Compounds inhibiting lipid peroxidation by interfering with chain reaction of peroxidation and scavenging reactive free radicals mediating tissue damage could be hugely therapeutically important [29]. Ferrous Fe2+ triggers lipid peroxidation via sundry mechanisms such as generating hydroxyl radical or decomposing lipid peroxides rapidly forming perferryl species [30]. Lipid peroxidation was kickstarted by introducing ferrous chloride at a concentration of 100 μM into rat brain homogenate mixture 2 mL total. Reaction was halted abruptly after twenty minutes by swiftly adding extremely cold reagent containing TCA TBA BHT and HCl in 2 milliliters. Samples were cooled pretty quickly after being heated in a boiling water bath for thirty minutes and absorbance of supernatant was measured at 532 nm. Ethanol served as solvent in above studies and α-tocopherol was utilized as standard meanwhile. Trials were performed thrice under highly controlled conditions. Lipid peroxidation inhibition percent was figured out using a certain equation subsequently.

$$\% \text{ Scavenged} = [(A_{blank} - A_{sample})/A_{blank}] \times 100$$

Where A sample is the absorbance of the sample solution and A blank is the absorbance of the blank without sample.

The lipid peroxidation in the ferrous induced model in rat brain homogenate was tested by test compound **(IIIa-g)** at 100 μM concentration is given Table 3 and their % of inhibition graph is shown in the Fig. 2.

Table 3: Effect of N-[3-(substituted phenyl)-1-oxo-2-propenyl] alanine (2a-2h) at 100 μM concentration and ferric induced lipid peroxidation in rat brain homogenate.

Compound	R	% inhibition
IIIa	4-NO_2	79.7
IIIb	4-Cl	44.3
IIIc	3-F	80
IIId	4-OCH_3	83
IIIe	4-$N(CH_3)_2$	80.7
IIIf	3-Br	75.1
IIIg	3-NO_2	68.7
Ascorbic acid		85.7

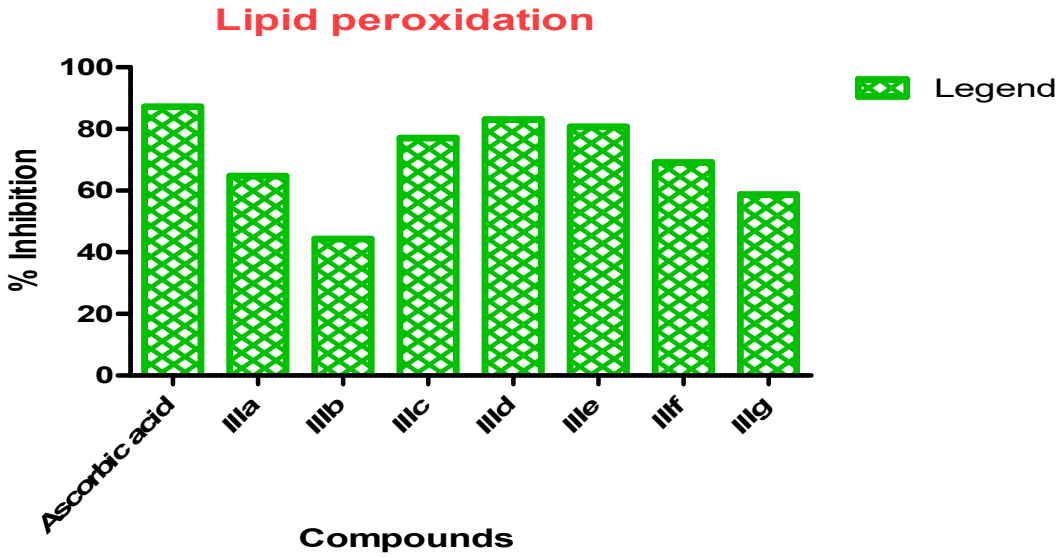


Fig. 2: % Inhibition of compounds IIIa-g in liquid peroxidation method

RESULTS AND DISCUSSION:

A series of titled compounds, i.e. N-(4-(4-aminophenyl)-6-phenylpyrimidin-2-yl)-3, 4, 5-trihydroxybenzamide have been synthesized using appropriate synthetic procedures, as per the scheme given in the methodology.

The structure of the synthesized compounds was confirmed by spectrophotometrical method.. The IR spectra of compounds (IIIa-g) exhibited a characteristic strong absorption at 2924-2940 cm-1 attributable to the C-H str of the aromatic ring. The compounds showed characteristic bands in the range 3440-3450 cm-1 O-H str of gallic acid, N-H str of amide and amine at 3320 cm-1. The pyrimidine ring characteristic absorption bands showed in the range of 1659 cm-1 (C=C str), 1520.19 (C=N Str), and 1693.03 (C=O str).

The synthesized compounds, N-(4-(4-aminophenyl)-6-phenylpyrimidin-2-yl)-3,4,5-trihydroxybenzamides, were successfully prepared following the designed synthetic route. The structural integrity and functional group confirmations were established through elemental analysis,^{1}H NMR, MASS and infrared (IR) spectroscopic analysis, providing crucial insights into the chemical features of the final products.

The IR spectral data of the synthesized derivatives (IIIa–g) consistently exhibited key absorption bands that corroborate the presence of expected functional groups. The C–H stretching vibrations of the aromatic ring were observed in the 2924–2940 cm^{-1} range, which is typical for substituted aromatic systems. Additionally, the broad and intense absorption bands appearing in the 3440–3450 cm^{-1} region can be attributed to the O–H stretching vibrations of the gallic acid moiety. This indicates the retention of hydroxyl groups, which are essential for the biological relevance of gallic acid derivatives. Moreover, the presence of amide and amine functionalities was confirmed by the N–H stretching absorption near 3320 cm^{-1}, a hallmark of primary amines and amide linkages. These bands further validate the successful incorporation of the amide bridge connecting the pyrimidine and gallic acid substructures. Importantly, characteristic absorption bands corresponding to the pyrimidine ring were also observed. These include a band at 1659 cm^{-1}, indicative of C=C stretching, a band at 1520.19 cm^{-1} for C=N stretching, and a C=O stretching band at 1693.03 cm^{-1}. These values are in agreement with the expected vibrational modes of substituted pyrimidines and further substantiate the formation of the desired heterocyclic framework.

The synthesized N-(4-(4-aminophenyl)-6-phenylpyrimidin-2-yl)-3,4,5-trihydroxybenzamides derivatives evaluated for their antioxidant potential using the DPPH free radical scavenging assay. The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical is a stable free radical widely used as a reliable method to assess the free radical scavenging ability of phenolic and polyphenolic compounds. All tested derivatives exhibited varying degrees of antioxidant activity, which can be attributed to the presence of the gallic acid moiety, a well-known phenolic antioxidant. Notably, specific substitutions on the pyrimidine ring influenced the extent of activity. The derivatives containing 4-methoxy, 3-fluoro, and 4-chloro substituents demonstrated the most significant reduction in DPPH radical absorbance, indicating enhanced antioxidant potential (Table 2).

Among these, the 4-methoxy derivative emerged as the most potent, exhibiting 87% radical scavenging activity at a concentration of 20 µg/mL, suggesting that electron-donating groups can significantly enhance antioxidant properties, likely by stabilizing the resultant phenoxyl radicals through resonance. The 3-fluoro derivative also showed substantial activity, reaching 83% scavenging at 80 µg/mL, indicating that electron-withdrawing groups, while slightly less

effective than methoxy, still promote notable activity, potentially by affecting the electron density across the molecule and improving radical stabilization.

The 4-chloro-substituted compound displayed moderate but consistent antioxidant behavior, reinforcing the role of halogen substitution in modulating the electronic environment of the aromatic system. These results highlight the influence of both electron-donating and electron-withdrawing groups on antioxidant capacity, with a general trend that substitution can fine-tune the radical-scavenging efficiency.

Lipid peroxidation is an important pathophysiological event in illness and drug toxicities. Compounds that inhibit lipid peroxidation by interfering with the chain reaction of peroxidation and by scavenging reactive free radical mediated tissue damage could be of great therapeutic importance. Fig 2 showed the inhibition of Ferrous (Fe2+) induced lipid peroxidation in rat brain homogenate. Among the substituted compounds, the 4-methoxy derivatives showed the highest activity (83%). The substitution with electron withdrawing groups like 3-flouro (80%) and 4-NO2 (79.7%) exhibited good activity at 100 µM. the other electron donating groups like 4-N $(CH_3)_2$ showed the highest activity (83%) (Table 3).

On the other hand, derivatives bearing electron-withdrawing groups such as 3-fluoro (80%) and 4-nitro (79.7%) also showed significant inhibition, indicating that these substituents may contribute to antioxidant activity through different electronic mechanisms, possibly stabilizing intermediate radical species or enhancing metal-chelation properties that interfere with Fe^{2+}-induced oxidative cascades.

An important and intriguing observation from this study was that all synthesized compounds exhibited notable DPPH and nitric oxide (NO) scavenging activity, indicating a consistent antioxidant potential across the series. Particularly compelling was the performance of non-phenolic compounds bearing electron-donating substituent's such as methoxy and dimethylamino groups. These derivatives not only demonstrated strong DPPH radical scavenging but also showed significant inhibition of Fe^{2+}-induced lipid peroxidation, highlighting their multifaceted antioxidant mechanisms.

This finding suggests that the phenolic group, although traditionally associated with antioxidant activity, may not be the sole contributor in these compounds. Instead, the **overall** pharmacophore, including the pyrimidine core and nature of the substituents, plays a pivotal role in determining the antioxidant efficacy. The presence of electron-withdrawing groups such as fluoro and nitro also enhanced activity, likely through electronic modulation and radical stabilization. Therefore, it can be concluded that both electron-donating and electron-withdrawing substituents, depending on their position and interaction with the molecular scaffold, significantly influence antioxidant performance—pointing toward a more complex structure-activity relationship than previously considered for phenolic antioxidants.

CONCLUSION

The present study successfully synthesized and characterized a novel series of N-(4-(4-aminophenyl)-6-phenylpyrimidin-2-yl)-3,4,5-trihydroxybenzamides, which demonstrated significant antioxidant activity across multiple in vitro models, including DPPH radical scavenging, NO scavenging, and lipid peroxidation inhibition assays. Among the derivatives, compounds substituted with electron-donating (e.g., 4-methoxy, 4-dimethylamino) and electron-withdrawing groups (e.g., 3-fluoro, 4-nitro) exhibited enhanced activity, highlighting the influence of substituent electronics on biological performance.

Importantly, the observation that non-phenolic analogs retained strong antioxidant potential suggests that antioxidant activity is governed not solely by phenolic hydroxyl groups, but by the overall pharmacophore architecture, including the pyrimidine core and aryl substitutions. These findings provide a foundation for further structural optimization and the development of new antioxidant agents with potential therapeutic applications in oxidative stress-related disorders.

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