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Synthesis, characterization, herbicidal activities and *in silico* studies of some highly functionalized pyrimidine derivatives

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Abstract
Phenylpyrimidine derivatives are a subclass of pyrimidine derivatives that have been extensively studied for their herbicidal activity. These compounds contain a phenyl group attached to the pyrimidine ring. The herbicidal activity of phenylpyrimidine derivatives is a result of their ability to inhibit specific enzymes involved in plant growth and development, leading to growth inhibition and death of the weed. A series of highly functionalized pyrimidine derivatives were synthesized. All of these compounds were confirmed by ^{1}H NMR, ^{13}C NMR, elemental, FT-IR, and mass spectrum analysis. Their herbicidal activities were screened *in vitro* against the sterilized seeds of *Raphanus sativus* at different concentrations. The compounds showed noticeable pre-emergent herbicidal activities. The herbicidal activity results of the phenylpyrimidine-5-carboxylate derivative were found to be better than its -chloro and -methoxy derivatives but were below par with standard pendimethalin. *In silico* studies were carried out to predict the toxicity and herbicide-likeness using PROTOX-II software and SwissADME predictor. The compounds fared well in both *in vitro* and *in silico* studies providing ample scope for further modifications and study of phenylpyrimidine-5-carboxylate derivatives as a potent herbicide.

Keywords: Synthesis, herbicidal activity, phenylpyrimidine derivatives, *in-silico*

1. Introduction
The realm of heterocyclic chemistry stands out as the most intricate and varied segment of organic chemistry. It represents a swiftly expanding field due to the extensive utilization of these compounds in pharmaceuticals, agriculture, and industry, as highlighted by studies [5, 6]. Nitrogen-containing heterocycles are prevalent primarily because they are widely present in nucleic acids. These heterocyclic compounds with nitrogen in nucleic acids fall into two principal categories: purines and pyrimidines.
Pyrimidines are organic compounds with a six-membered heterocyclic aromatic structure, featuring two nitrogen atoms within the benzene ring [3]. Pyrimidine derivatives exhibit diverse biological activities, making them attractive for medicinal chemistry research and drug development.
Phenylpyrimidine derivatives are a subclass of pyrimidine derivatives that have been extensively studied for their herbicidal activity. These compounds possess a phenyl ring attached to a pyrimidine ring, conferring unique properties and potential pharmacological applications. Over the past two decades, there has been a growing emphasis among scientists on exploring the wide range of biological activities exhibited by pyrimidine derivatives [7]. The herbicidal activity of phenylpyrimidine derivatives is a result of their ability to inhibit specific enzymes involved in plant growth and development, leading to growth inhibition and death of the weed.
In the current research, a series of phenylpyrimidine derivatives were synthesized, and their herbicidal activity was examined. Additionally, the study included several *in-silico* investigations to further explore the properties and potential activities of these synthesized compounds.

2. Experimental
The chemicals utilized were of analytical reagent grade, ensuring the highest level of purity, and were utilized as they were, without requiring additional purification. The reagents used were benzaldehyde (assay>99%, Merck), aniline (assay>99%, Molychem),

p-Methoxybenzaldehyde (assay>99%, Molychem), *p*-chlorobenzaldehyde (assay>99%, Molychem), acetophenone (assay>99%, HiMedia) were used. The medium of reaction was absolute ethanol (Merck).

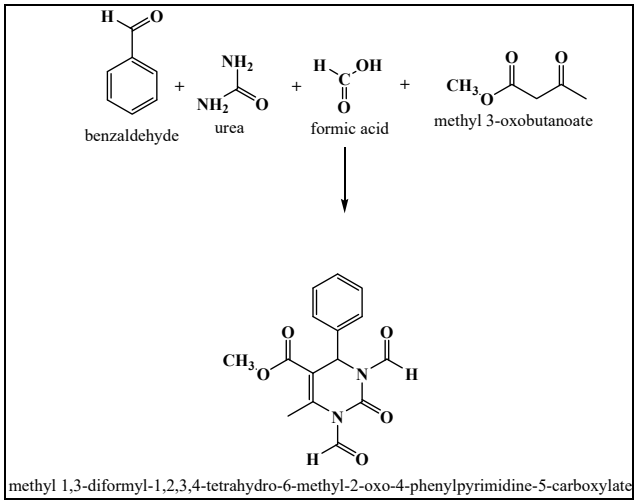
2.1 Synthesis

A mixture of benzaldehyde (10 mmol), formic acid (10 mmol, 1.16 mL), urea (10 mmol, 1.015 mL), and methyl 3-oxobutanoate (10 mmol, 1.276 g) was placed in a round-bottom flask. The reaction proceeded under reflux at 60 °C with continuous stirring using a magnetic stirrer. Thin-layer chromatography (TLC) was utilized to monitor the progress of the reaction, using hexane and ethyl acetate at 20:80 respectively and once the reaction reached completion, it was quenched by pouring the reaction mixture onto crushed ice. Subsequently, the resulting precipitate was isolated by filtration and washed with cold water two times. The crude product was subjected to recrystallization using ethanol to obtain the purified product.

The crude product (L_1A) was further purified by recrystallization. The same process described above was repeated for other derivatives but with different reagents. In this case, *p*-chlorobenzaldehyde (product L_1B) and *p*-methoxybenzaldehyde (product L_1C) were used instead of aniline. Schemes 1-3 represent the target synthesis products.

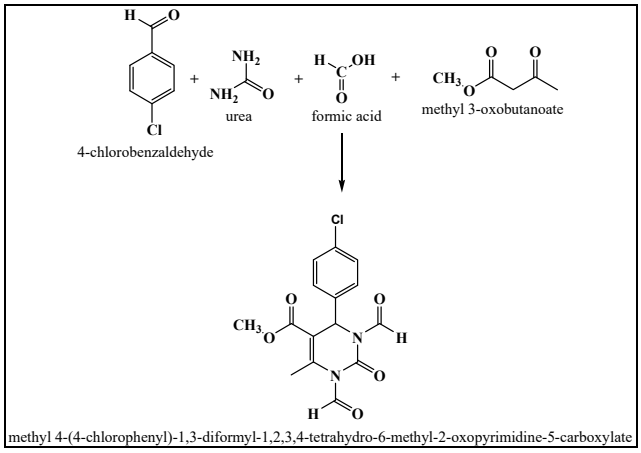
The formation of phenylpyrimidine derivatives is represented in Scheme 4.1-4.3, and their NMR spectral data has been represented as follows

$C_{15}H_{14}N_2O_5$ (L_1A) : ^{1}H NMR (400 MHz, DMSO-D6) δ 9.41 (d, J = 2.1 Hz, 1H), 7.95 (dd, J = 3.5, 2.0 Hz, 1H), 7.55 – 7.46 (m, 2H), 7.42 (td, J = 5.1, 2.7 Hz, 3H), 5.32 (d, J = 3.4 Hz, 1H), 3.71 (s, 3H), 2.43 (s, 3H); ^{13}C NMR (101 MHz, DMSO-D6) δ 166.38, 152.72, 149.23, 145.21, 133.40, 129.81, 129.11, 129.00, 127.84, 126.71, 99.52, 54.32, 51.34, 18.37.



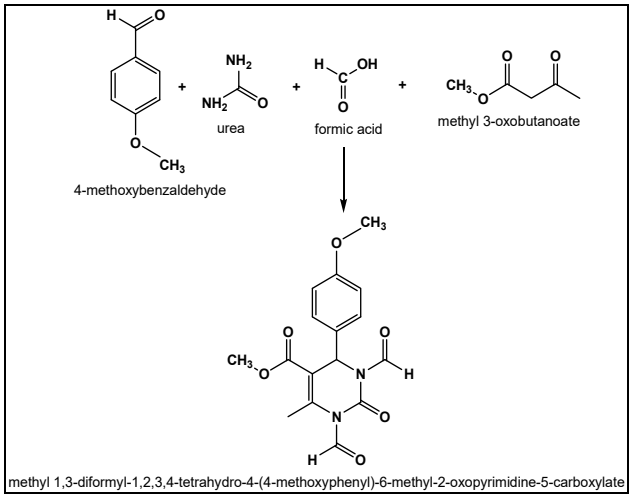
Scheme 1: Synthesis of methyl 1,3-diformyl-1,2,3,4-tetrahydro-6-methyl-2-oxo-4-phenylpyrimidine-5-carboxylate

$C_{15}H_{13}ClN_2O_5$ (L_1B): ^{1}H NMR (400 MHz, DMSO-D6) δ 9.25 (d, J = 2.0 Hz, 1H), 7.77 (dd, J = 3.6, 2.1 Hz, 1H), 7.38 – 7.32 (m, 2H), 7.24 – 7.18 (m, 2H), 5.11 (d, J = 3.4 Hz, 1H), 3.49 (s, 3H), 2.22 (s, 3H); ^{13}C NMR (101 MHz, DMSO-D6) δ 192.19, 165.74, 152.02, 149.06, 143.64, 131.87, 131.22, 131.05, 129.41, 129.30, 128.78, 128.49, 128.16, 98.61, 53.28, 50.87, 17.89.



Scheme 2: Synthesis of methyl 4-(4-chlorophenyl)-1,3-diformyl-1,2,3,4-tetrahydro-6-methyl-2-oxopyrimidine-5-carboxylate

$C_{16}H_{16}N_2O_6$ (L_1C): ^{1}H NMR (400 MHz, DMSO-D6) δ 9.15 (d, J = 2.0 Hz, 1H), 7.66 (dd, J = 3.5, 2.1 Hz, 1H), 7.13 – 7.07 (m, 2H), 6.86 – 6.80 (m, 2H), 5.05 (d, J = 3.4 Hz, 1H), 3.68 (s, 3H), 3.48 (s, 3H), 2.20 (s, 3H); ^{13}C NMR (101 MHz, DMSO-D6) δ 166.40, 158.99, 152.71, 148.91, 137.38, 127.87, 114.30, 99.79, 55.59, 53.70, 51.32, 18.34.



Scheme 3: Synthesis of methyl 1,3-diformyl-1,2,3,4-tetrahydro-4-(4-methoxyphenyl)-6-methyl-2-oxopyrimidine-5-carboxylate

2.2 Physical and Spectral Measurement

The compound's melting point was determined using the Decibel DB-3135H melting point apparatus, its molar conductivity was assessed using the Systronic conductivity TDS meter 308, and elemental analysis for carbon (C), hydrogen (H), and nitrogen (N) was conducted using the Elementar Analysensysteme Germany (Vario Micro Cube). The magnetic molar susceptibilities were measured using Quincke's Tube and the Digital Gaussmeter (DGM-102) at room temperature. The compound's molecular weight was determined using Mass Spectrometry through the Xevo G2-XS QTof Mass Spectrophotometer, ^{1}H-NMR, and ^{13}C-NMR were obtained using the Jeol JNM-ECZ 400S instrument (at frequencies of 400 MHz and 100 MHz, respectively), and its FTIR analysis was conducted using the Nicolet iS50 FTIR Tri-detector.

2.2 Herbicidal Activity

The method followed by [10] with slight modifications was carried out to determine the herbicidal activity of the synthesized compounds dissolved in distilled water with 5% Tween-20 solution.

Petri dish bioassays were conducted to assess the impact of various extract components on the germination, root, and shoot growth of radish (*Raphanus sativus*) seeds. The seeds, sourced from Pantnagar, underwent sterilization using 95% ethanol for 15 seconds to prevent potential contamination from bacteria or fungi. For evaluating the herbicidal activity of the extract, Petri dishes containing germination paper and 10 *Raphanus sativus* seeds each were treated with 3mL of different concentrations (25, 50, 75, and 100 µg/mL). A 5% Tween-20 solution in distilled water served as the negative control, while a Pendimethalin solution in water acted as the positive control. Each treatment was triplicated using three Petri plates, and the experiment was conducted at a room temperature of 25-28 °C. Five days post-treatment, the germination rate, as well as root and shoot lengths, were measured. Assessment of seed inhibition and germination occurred at 24, 48, 72, 96, and 120 hours after treatment.

To calculate % inhibition of germination of the herbicidal activity, the following equation was used:

$$\% \text{ Inhibition } = \frac{\text{Germination in control - Germination sample}}{\text{Germination in control}} \times 100$$

2.3 ADMET and Toxicology Studies

Virtual screening was employed to assess the physicochemical attributes and appraise the pharmacokinetics (drug-likeness) of chosen compounds, utilizing the SwissADME tool [9, 4]. Simultaneously, PROTOX-II software was utilized to determine oral toxicity and bioavailability [2]. For the necessary SMILES format essential for SwissADME and PROTOX-II, ChemDraw software was utilized [1].

3. Results and Discussion

3.1 IR Spectral Data

The FT-IR spectra of the samples were acquired following thorough dehydration in a hot air oven to eliminate any water molecule peaks and ensure accurate readings. The spectra corresponding values have been shown in the Table 1.

Table 1: Corresponding values of FT-IR spectra.

Compound	ν(N-H)	ν(C=O)	ν(C-N)	ν(C=C)
L_1A	3370	~1641(s)	~1287 (s)	900-1100
L_1B	3379	1649 (sh)	1280 (sh)	900-1100
L_1C	3374	~1656(s)	~1279 (s)	900-1100

All three compounds (L_1A, L_1B, L_1C) showed N-H characteristic stretching at around 3376cm^{-1} and C=O stretching at around 1650cm^{-1}. This shows the presence of both amine and carbonyl groups in all three, with little difference. This difference could be due to amine group substitution, which is observed at 736cm^{-1} (C-Cl stretch) and 1029cm^{-1} (C-O-C stretch) in the case of L_1B & L_1C respectively. Thus, the spectral analysis validates the presence of both an amine group and a ketone group in all three samples. Additionally, in L_1B, a C-Cl group is confirmed, and in L_1C, a C-OCH_3 group is identified.

3.2 NMR Analysis

All the synthesized compounds showed striking similarity with expected ^{1}H and ^{13}C spectra. In ^{13}C spectra, all of the compounds showed a carbonyl peak around 160-170 ppm corresponding to the carbonyl carbon in pyrimidine. In ^{1}H spectra, the range between 4.95-4.75 ppm and 3.39-3.60 ppm corresponds to β and α carbon (next to the carbonyl group) respectively. The values between 3.13 - 3.36 ppm correspond to the N-H stretch. Hence, all the compounds are phenylpyrimidine derivatives. Moreover, the chemical shift of 3.39 (s,3H) is characteristic of the O-CH_3 bond, which is observed for L_1C (a methoxy derivative).

3.3 UV-Vis Spectral Analysis

The UV-Vis spectra, with the corresponding λ_{max} values listed in Table 2. Different measurements indicate the creation of three unique compounds with a redshift as the substituent group size increases. Table 2 presents the elemental composition, highlighting a significant resemblance between the computed and observed values.

Table 2: CHN Elemental Analysis data and UV-Vis Spectral values

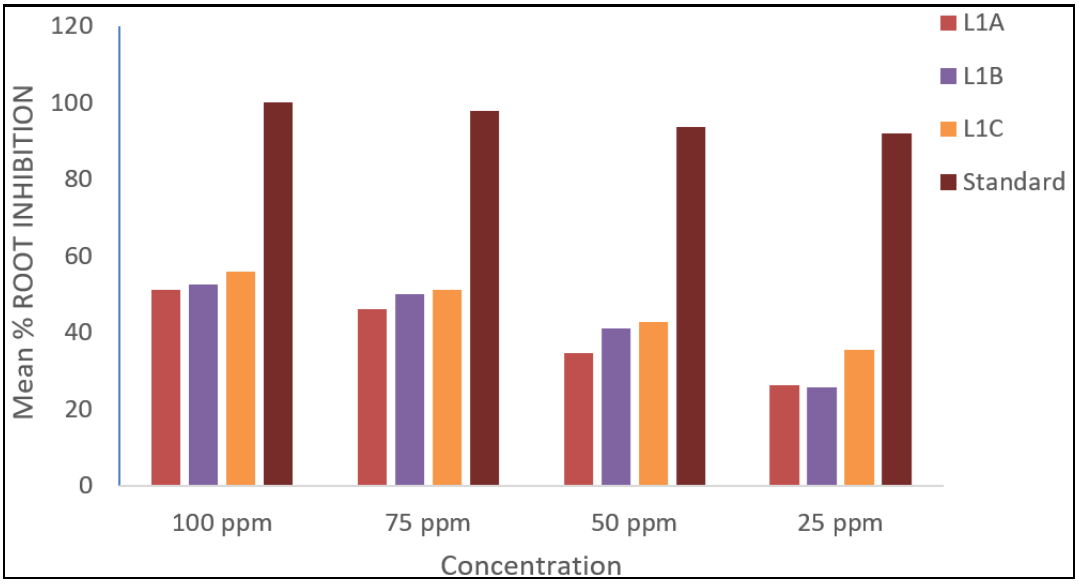
Compound	C	H	N	λ_{max}
L_1A	59.60 (58.69)	4.67 (4.35)	9.27 (8.65)	260
L_1B	53.50 (53.11)	3.89 (2.7)	8.32 (7.71)	285
L_1C	78.82 (78.43)	6.134 (5.93)	4.67 (4.326)	315

3.4 Herbicidal activity

The assessment of herbicidal activity, measured in terms of both % inhibition of root germination and % inhibition of shoot germination, was conducted across a concentration range of 25-100 ppm. Three distinct analyses were performed, encompassing % seed germination (Table 5), % root inhibition (Table 3), and % shoot inhibition (Table 4). In all the cases of % inhibition of root germination of % inhibition of shoot germination, it was observed that for all three compounds with the decrease in the concentration of the compounds, there was a decrease in the % inhibition. The order of root inhibition and shoot inhibition observed was: Pendimethalin > L_1C > L_1A > L_1B.

Table 3: Percentage of seed inhibition of phenylpyrimidine derivatives

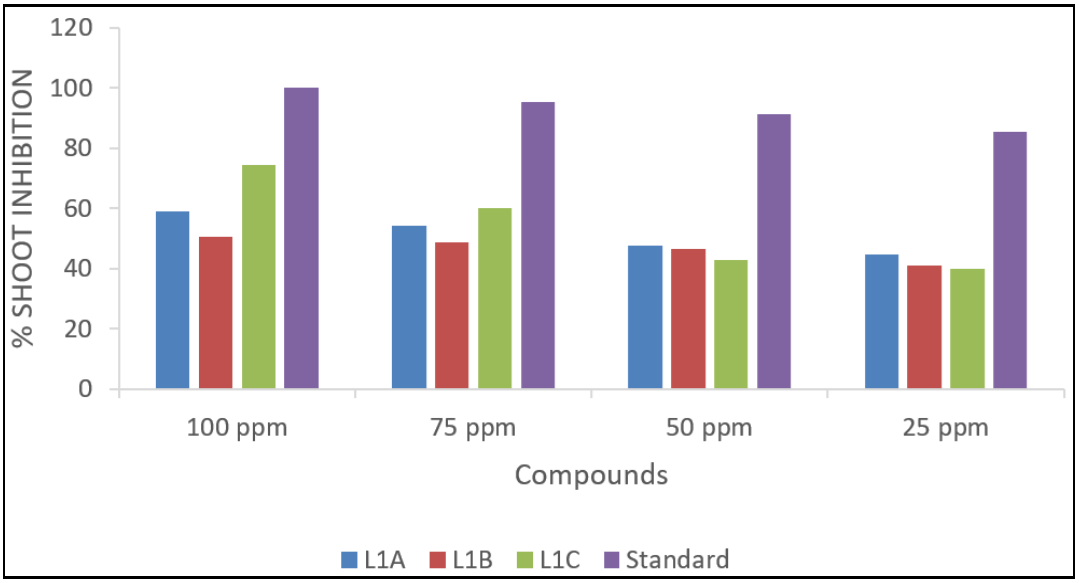
Herbicidal Activity: Mean % Root Inhibition						
SR No.	Root Inhibition	100 ppm	75 ppm	50 ppm	25 ppm	IC_{50}
1	L_1A	51.145	46.154	34.537	26.329	43.370
2	L_1B	52.654	49.905	41.026	25.623	49.820
3	L_1C	55.846	51.079	42.872	35.407	39.560
4	Standard	99.970	95.395	91.356	85.369	56.52



Herbicidal Activity: Mean % Root Inhibition

Table 4: Percentage seed inhibition of phenylpyrimidine derivatives

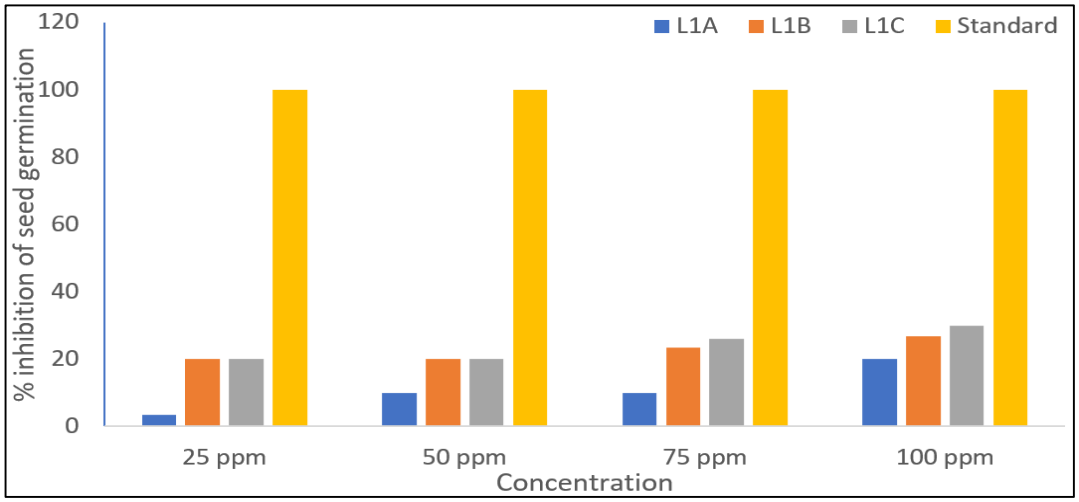
Herbicidal Activity: % Shoot Inhibition						
Sr. No.	**Shoot Inhibition**	**100 ppm**	**75 ppm**	**50 ppm**	**25 ppm**	**IC_{50}**
1	L_1A	59.048	54.286	47.619	44.762	64.770
2	L_1B	50.476	48.571	46.667	40.952	47.360
3	L_1C	74.286	60.000	42.857	40.000	71.640
4	Standard	99.970	97.890	93.720	91.920	61.88



Herbicidal Activity: % Shoot Inhibition

Table 5: Percentage seed inhibition of phenylpyrimidine derivatives

S. No	**Compound**	**% inhibition seed germination activity**			
		25 ppm	**50 ppm**	**75 ppm**	**100 ppm**
1	L_1A	3.33±5.77	10.00±0.00	10.00±0.00	20.00±0.00
2	L_1B	20.00±0.00	20.00±0.00	23.33±5.77	26.67±5.77
3	L_1C	20.00±0.00	20.00±0.00	26.00±6.67	30.00±0.33
4	Standard	100	100	100	100



% inhibition of seed germination activity

3.5 ADMET and Toxicity Studies

The compounds underwent *in-silico* Absorption, Distribution, Metabolism, Excretion (ADME), and toxicity studies. These computational analyses were employed to predict and evaluate the pharmacokinetic properties, as well as potential adverse effects or toxicity, providing valuable insights into the compounds' bioavailability and safety profiles, as detailed in Table 6. Furthermore, the compounds were deemed favourable in terms of drug-likeness, complying with Lipinski's rule of five [8], thereby demonstrating their potential for effective utilization in pharmaceutical applications.

Table 6: *In-silico* ADME and toxicity studies of the synthesized compounds

Compound	M.wt	H-bond donors	H-bond acceptors	Log P	Drug-Likeliness	LD_{50}	Toxicity Class
L_1A	302	0	21	2.37	Yes	2495	5
L_1B	336	0	20	3.03	Yes	2973	5
L_1C	332	0	24	2.38	Yes	6200	6

4. Conclusion

The synthesis of phenylpyrimidine derivative (L_1A) and its –chloro (L_1B) and -methoxy (L_1C) derivatives was successfully revealed and characterized using a range of spectroscopic and analytical techniques. The herbicidal activity of L_1A, L_1B and L_1C against seeds of *Raphanus sativus* at four different concentrations (100 µg/ml, 50 µg/ml, 75 µg/ml, and 25 µg/ml). Pendimethalin was used as a positive control (standard), which showed maximum % inhibition of seed germination. Water was used as a negative control, which shows the minimum % inhibition of seed germination. L_1C showed maximum % inhibition of seed germination at the highest concentration and the order of herbicidal activity was found as: $L_1C > L_1A > L_1B$. The order of root inhibition and shoot inhibition observed was: Pendimethalin > L_1C > L_1A > L_1B. Dose-dependent inhibition activity was observed in all three compounds, with a minimal effect at lower concentrations and maximization at higher concentrations. SwissADME and PROTOX-II results showed that the compounds were least toxic with L_1A and L_1B belonging to toxicity class V and L_1C to class VI with a higher LD_{50} dose value compared to the standard. The compounds fared well in both *in vitro* and *in silico* studies providing ample scope for further modifications and study of phenylpyrimidine-5-carboxylate derivatives as a potent herbicide.

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