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ORIGINAL ARTICLE

Design, synthesis, and characterization of 2,2-bis(2,4-dinitrophenyl)-2-(phosphonatomethylamino)acetate as a herbicidal and biological active agent

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Abstract The present study was designed to synthesize the bioactive molecule 2,2-bis(2,4-dinitrophenyl)-2-(phosphonatomethylamino)acetate (**1**), having excellent applications in the field of plant protection as a herbicide. Structure of newly synthesized molecule **1** was confirmed by using the elemental analysis, mass spectrometric, NMR, UV-visible, and FTIR spectroscopic techniques. To obtain better structural insights of molecule **1**, 3D molecular modeling was performed using the GAMESS programme. Microbial activities of **1** were checked against the pathogenic strains *Aspergillus fumigatus* (NCIM 902) and *Salmonella typhimurium* (NCIM 2501). Molecule **1** has shown excellent activities against fungal strain *A. fumigates* (35 μg/l) and bacterial strain *S. typhimurium* (25 μg/l). To check the medicinal significance of molecule **1**, interactions with bovine serum albumin (BSA) protein were checked. The calculated value of binding constant of molecule **1**–BSA complex was $1.4 \times 10^6\ M^{-1}$, which were similar to most effective drugs like salicylic acid. More significantly, as compared to herbicide glyphosate, molecule **1** has exhibited excellent herbicidal activities, in pre- and post-experiments on three weeds; barnyard grass (*Echinochloa Crus*), red spranglitop (*Leptochloa filiformis*), and yellow nuts (*Cyperus Esculenfus*). Further, effects of molecule **1** on plant growth-promoting rhizobacterial (PGPR) strains were checked. More interestingly, as compared to glyphosate, molecule **1** has shown least adverse effects on soil PGPR strains including the *Rhizobium leguminosarum* (NCIM 2749), *Pseudomonas fluorescens* (NCIM 5096), and *Pseudomonas putida* (NCIM 2847).

Keywords Glyphosate · L-fluoro-2,4-dinitrobenzene · Herbicide · Inhibitory properties · PGPR strains · Biodegradation

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Introduction

Glyphosate [N-(phosphonomethyl)glycine] is a nonselective, post-emergent herbicide used for the control of wide range of weeds. It was commercialized since 1974. It has become most popular herbicide due to economic aspects having sales worth around US$6.5 billion, use in transgenic crops, and use in glyphosate resistance crops [10, 13]. It has considered as the herbicide of century. Glyphosate targets the 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) [10, 13].

Beside of the great story of success of the glyphosate during its several decades of use over vast areas, some significant adverse effects of the herbicide have been established, like, long half-life period [10, 13, 14, 28], toxicity to earthworms [12, 66] and soil microorganisms [6, 40, 56, 57].

Non-effective behavior of glyphosate on few weeds at recommended dose level has been observed during last few years [10, 17, 41]. It has found that, including other recommended

herbicides, glyphosate was non-effective against barnyard grass (*Echinochloa Crus*), red sprangletop (*Leptochloa filiformis*), and yellow nuts (*Cyperus esculentus*) [11, 58, 61].

Same time, it was noteworthy that glyphosate can form stable complexes with soil's metal ions [19, 23, 59, 60, 62]. Stable complexation causes the depletion of important metal ions of soils, and these metal ions are very important for plant growth [28–33, 36, 37, 50, 60]. Due to strong interactions through OH (two P-OH and one of the COOH), glyphosate can adsorb on soil surface and humic substances over wide range of pH, which increases its life time because of the formation of covalent bonding [26, 43, 48, 49]. It is a well-known fact that these OH groups can allow the complexation with metal ions and adsorption on soil surface at agricultural pH range 5.5–8.0 [19, 23, 59, 60, 62]. Recent studies have revealed that in the presence of heavy metal ions, toxicity of glyphosate increased having severe adverse effects upon the soil's microorganisms [14, 56, 60].

Current study was design to synthesize a new derivative of glyphosate through the blocking of free O groups (P-OH) of glyphosate using the 1-chloro-2,4-dinitrobenzene (DNB) group. Also, the herbicidal activities of newly synthesized glyphosate derivative were performed on three weeds namely, barnyard grass (*Echinochloa Crus*), red sprangletop (*L. filiformis*), and yellow nuts (*C. esculentus*). These three weeds have caused severe crop damage from the last 10 years in our local area, and all the herbicides available in the market were futile against theses weeds.

As mentioned above, glyphosate has adverse effects against soil microorganisms either in free form or in bound (metal complex) form [14, 56, 60]. Hence, we have checked the effects of molecule **1** against the plant growth-promoting rhizobacterial (PGPR) strains.

Moreover, many amino acid and nitro derivatives have been reported to have antimicrobial activities [15, 18, 21, 22, 24, 25, 44, 52, 55, 63]. Therefore, the synthesized molecule **1** was also investigated for its potential as antimicrobial agent.

Overall, we were able to synthesize an efficient herbicidal molecule **1** having excellent herbicidal activities, very good bioactive agent, and more significantly least toxic to the PGPR strains. Hence, it could be the future's plant protecting agent with excellent herbicidal activities.

Material and methods

Chemistry

Materials and reagents

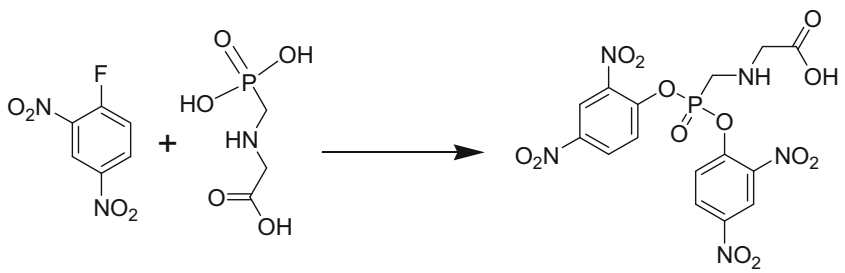
All the reagents and solvents were of analytical grade and used without purification. Glyphosate (of purity 95%) of technical grade was gifted by Gautami Ltd. Hyderabad (India) and used without any purification. Plant growth-promoting rhizobacterial (PGPR) strains (*Rhizobium leguminosarum* (NCIM 2749), *Pseudomonas fluorescens* (NCIM 5096), and *Pseudomonas putida* (NCIM 2847)) and pathogenic strains (*Aspergillus fumigatus* (NCIM 902) and *Salmonella typhimurium* (NCIM 2501)) were purchased from NCL, Pune, India.

Instrumentation

UV-visible analyses of glyphosate (dissolved in water) and molecule **1** (dissolved in MeOH) were performed on a Shimadzu-1800s UV-visible spectrometer (cubed 1 cm length) over wavelength range of 200–800 nm. FTIR analyses of the glyphosate and molecule **1** were performed on a Shimadzu-8400s spectrophotometer using the potassium bromide (KBr) pellets. NMR analyses of glyphosate (dissolved in D_2O) and dissolved **1** (dissolved in $CDCl_3$) were performed on NMR spectrophotometer (Bruker Avance III, 400 MHz) by taking TMS as reference material. Mass analyses of the molecule **1** were performed on mass spectrophotometer (Waters, Q-TOF Micromass). Elemental analyses (CHNO) of molecule **1** were performed on Thermo Scientific (FLASH 2000) CHN elemental analyzer.

Synthesis of molecule **1**

The synthetic route of 2,2-bis(2,4-dinitrophenyl)-2-(phosphonatomethylamino)acetate (molecule **1**) was depicted in Scheme 1. One mole of aqueous solution of glyphosate (10 mL of 0.001 mol (1.69 mg)) and 2 mol of aqueous solution of DNP (10 mL of 0.002 mol (3.98 mg)) was taken in a round bottom flask (RBF) and the resulting reaction mixture



Scheme 1 Scheme representing the conversion of glyphosate into 2,2-bis(2,4-dinitrophenyl)-2-(phosphonatomethylamino)acetate (molecule (**1**))

was stirred at 120 rpm for 1 h at room temperature (RT) at pH ~12 (pH maintained by using NaOH). Reaction progress was monitored by thin-layer chromatographic (TLC) method. The solvent system of TLC study was chloroform: methanol mixture (8:2). Pale-colored product was purified using the column chromatography, eluted with chloroform: methanol mixture 8:2. Eluted product was allowed for solidification through slow evaporation of solvent at RT. Solid product was dried overnight at 45 °C and allowed for spectral, herbicidal, and biological activities. The observed melting point of molecule **1** was 105 ± 2 °C.

Determination of stability of molecule **1** *in aqueous solution*

Stability of molecule **1** (dissolved in H_2O) was determined by using the UV-visible spectrometric technique at 357 and 405 nm. Here, 0.001 g of molecule **1** was dissolved in 25 mL of water and kept for 96 h. UV-visible analysis was noticed after 0, 1, 6, 12, 24, 48, 72, and 96 h, by taking water as a blank. Similar study was repeated by taking different solvents including the acidic water (ph = 3), basic water (ph = 10), DMSO, MeOH, EtOH, and $CHCl_3$.

Computational studies of molecule **1**

An attempt to gain a better insight on the molecular structure of molecule **1**, geometric optimization, and conformational analysis was performed by the use of Merck Molecular Force Field 94 (MMFF94) program [16]. All the calculations refer to isolated molecules in vacuum. To calculate the abovementioned parameters, executable programme file of GAMESS (Cambridge Software ChemBio3D Ultra 14.0.) programme were run on PC [8, 20, 27, 35].

Agriculture

Herbicidal activities

All the experiments were performed under natural conditions at Village Babehar, Distric Una, Himachal Pradesh (India), located at the 31° 49′ N, 76° 28′ E geographic coordinates. Effects of molecule **1** on three weeds; barnyard grass (*Echinochloa Crus*), red sprangletop (*L. filiformis*), and yellow nuts (*C. esculentus*), were carried out during rainy session (July–August) by taking glyphosate as control.

The recommended application doses of N-(phospho nomethyl)glycine (glyphosate) were used as mentioned by Kutman et al. [37]. The doses of glyphosate used in the first experiment were 0.7, 1.5, and 3.0 mM, respectively. Same dose levels (0.7, 1.5, and 3.0 mM) of molecule **1** were used. Experiments were performed by dividing the whole field four major parts. In the first experiment, glyphosate was sprayed immediately after seed planting (preemergence treatment), and in the second experiment, glyphosate was sprayed after the expansion of the first true leaf (postemergence treatment). In the third and fourth experiments, molecule **1** was sprayed instead of glyphosate. Each treatment was performed in triplicates.

To interpret the results quantitatively (weights of weeds, stem, and roots length of weeds), randomly, ten weeds from each experiments were taken, where sampling was done diagonally with a distance of ~10 cm. Sampling were triplicated and results were noticed in terms of factor ± means (here, factor = weight of weeds, stem and root lengths of weeds).

Effect of molecule **1** *on PGPR strains*

Effects of molecule **1** on most common and most efficient PGPR strains (*R. leguminosarum* (NCIM 2749), *P. fluorescens* (NCIM 5096), and *P. putida* (NCIM 2847)) were assayed on nutrient broth [34, 44, 47, 65]. The homogenous suspensions poured into petri dishes. After solidification of the agar in petri plates, bacteria (10^5 CFU mL^{-1}) was added to the plates. Paper discs of different concentrations (50, 100, 150, and 250 mM (prepared in deionized water)) of glyphosate/ molecule **1** were applied using a sterilized forceps. After incubation for 48 h in an incubator at 37 °C, the inhibition zone diameters were measured and expressed in millimeter. Further, the minimum inhibitory concentration (MIC) values of glyphosate/molecule **1** against different strains were performed by serial dilution method. All the experiments were performed in triplicates.

Biology

Antimicrobial assays

In vitro antibacterial studies were carried out by agar disc diffusion method against *S. typhimurium* strain (NCIM 2501) [38, 54]. Nutrient broth (NB) plates were swabbed with 24-h-old broth culture of test bacteria. Sterile paper discs (5 mm) were put into each petri plate. Different concentrations of water-dissolved molecule **1** (50, 100, 150, and 250 μg/L) were added into the discs by dipping individual disc into solution containing test tubes. The plates were incubated at 37 °C for 24 h. After appropriate incubation, the diameter of zone of inhibition of each disc was measured.

Antifungal assay

In vitro antifungal studies were carried out by potato dextrose agar medium disc diffusion method against *A. fumigatus* strain (NCIM 902). The fungus was cultured in potato dextrose agar medium. Potato dextrose agar medium (prepared from potato 150 g, dextrose 5 g, and agar 2 g in 200 mL of distilled water) was poured in the sterilized petri dishes and allowed to

solidify. The dishes were inoculated with a spore suspension of 10^6 spores/mL of medium. The molecule **1** to be tested was dissolved in water to a final concentration of 50, 100, 150, and 250 μg/L, and soaked in filter paper discs. These discs were placed on the already seeded plates and incubated at 37 °C for 96 h. After 96 h, the inhibition zone appeared around the discs in each plate and was measured.

MIC determination

The MIC denotes the lowest drug concentration that prevents the visible growth of tests microorganisms. MIC (μg/L) values were evaluated by using the serial double dilution method in the appropriate medium which is inoculated with a standardized number of microorganisms.

Molecule **1** *to bovine serum albumin protein interactions*

Qualitative and quantitative comparative analysis was performed by UV visible spectroscopic method as described by Abdi et al. [1]. Studies were performed with aqueous solutions of bovine serum albumin (BSA) protein in the absence and presence of molecule **1** (dissolved in water) at pH 7.2 by keeping the concentration of BSA constant (0.05 mM), while varying the concentration of the product (0.010, 0.0075, 0.0050, 0.0025, 0.00125 mM), in the range of 230–400 nm. The binding constants were calculated as theory/formulae reported by Abdi et al. [1]. To calculate the binding constant (k), the double reciprocal plot of $1/(A\text{-}A_0)$ versus 1/(ligand concentration) was found linear and the binding constant (k) determined from the ratio of the intercept to the slope.

Results and discussion

Chemistry

Synthesis and characterization of molecule **1**

Synthesis of molecule **1** has been depicted in Scheme 1, where 2 mol of DNB get attached with 1 mol of glyphosate through the O of P–OH. At pH ~10–12, glyphosate has negative change on O of P–OH and it can react with molecules like DNB [19, 23, 59, 60, 62]. At pH ~3.5–9.5, glyphosate reacted with DNB and 4-chloro-3,5-dinitrobenzotrifluoride through the N (having negative charge), and it was considered as the most convenient method to determine glyphosate and its metabolite (aminomethyl)-phosphonic acid through the derivatization pathway [7, 39, 51]. In the present study, from UV-visible analysis, it was noticed that glyphosate itself has no sharp absorption maximum (Fig. 1), but once glyphosate formed complex with DNB, the maximum absorption

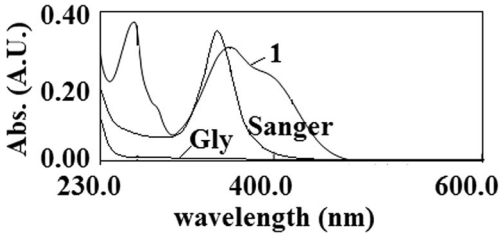


Fig. 1 UV-vis spectra of glyphosate, Sanger reagent, and molecule **1** taken in water

wavelength observed between 340 and 350 nm including bands at 265 and 405 nm.

In comparisons of the IR spectrum of glyphosate and molecule **1**, shifting in the frequencies (~10–25 cm^{-1}) was noticed as compared to IR spectrum of glyphosate (supplementary S2 and Fig. 2) [23, 60, 62]. Additionally, the sharp bands of stretching frequencies of alkenes (of rings) and nitro groups of DNB were observed in region of 1650–1450 cm^{-1} in the IR spectrum of molecule **1**. It was noticed that vibrations [ν(P = O); 1225 cm^{-1}] were shifted to greater extend (1290 cm^{-1}) due to attachment of two rings directly to O = P–O bonds. These rings were causing conjugation and leads to increase in P = O bond strength (as shown in supplementary S3). These changes and appearance of new bands have indicated the formation of pure product named as molecule **1**.

Detailed NMR analyses (^{1}H-NMR and ^{1}H-^{1}H COSY NMR) of molecule **1** were performed. In ^{1}H-NMR study, it was noticed that diasterotopic protons of the glyphosate (of CH_2CO_2 group) appeared as doublets at 4.70 ppm, and singlet at 3.88 ppm disappeared (Fig. 3 and supplementary S1) [23, 60]. In the present study, the shifting (towards low value) of the abovementioned signals has confirmed the attachment of two DNB groups at oxygen of the glyphosate of –P–OH group. The methylene protons of the glyphosate of CH_2PO_3 group appeared as doublets of doublets at 3.13 and 3.10 ppm, which shifted to 2.70 (J = 15.44 Hz) and 2.79 ppm (J = 15.44 Hz) due to spatial arrangement of rings of DNB. Additionally, the triplet at 2.56 ppm (J = 3.98 Hz) was

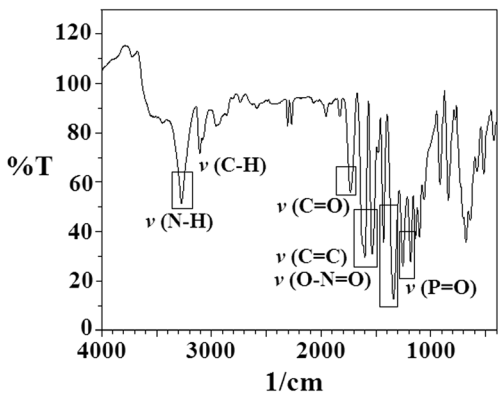


Fig. 2 FTIR spectrum of molecule **1**

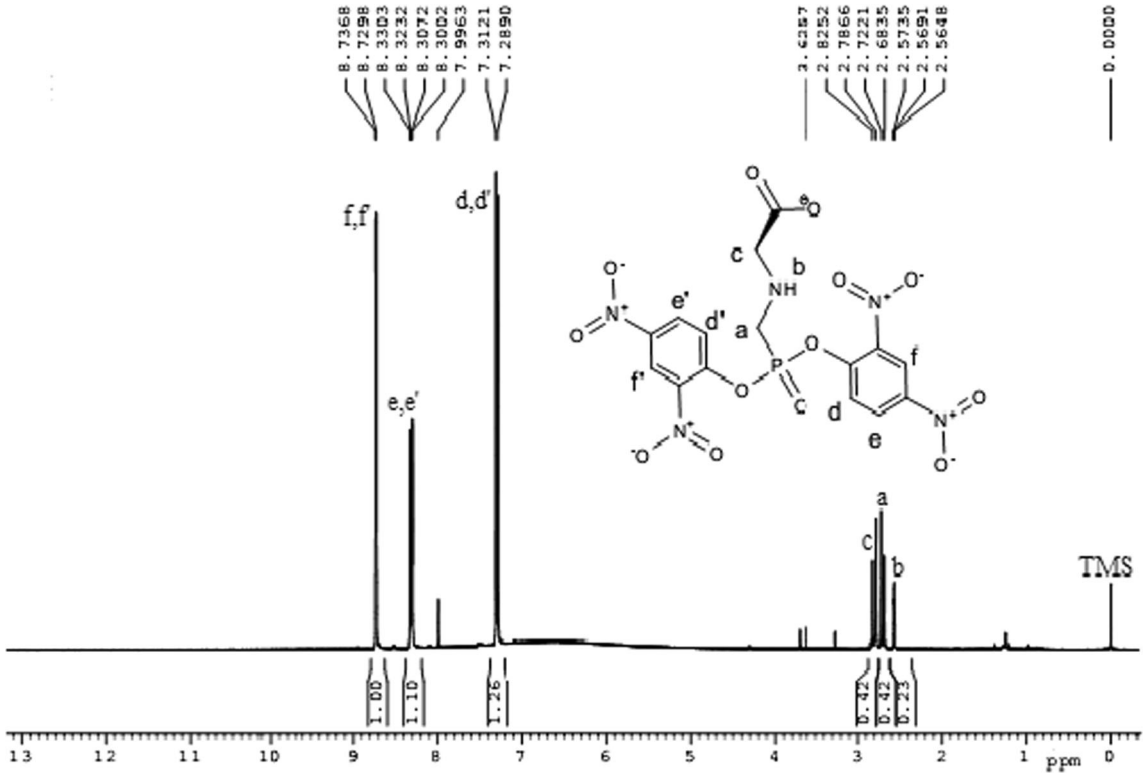


Fig. 3 ^{1}H–NMR spectrum of molecule **1**

assigned to proton of amine which was splitted by nearby protons as per $n + 1$ rule. Signals at 7.29 ppm (doublet) (J = 9.24 Hz), 8.32 ppm (quartet) (J = 12.04 Hz), and 8.73 ppm (doublet) (J = 2.81 Hz) were assigned to ring protons of DNB. In case of ^{1}H-^{1}H COSY analysis, there were very good correlations w.r.t. diagonally as well as proton-to-proton splitting observed which has shown the purity of molecule **1** (Majumdar et al. [42]) (Fig. 4).

Mass spectrum of molecule **1** has exhibited the peak at *m/z* 496.5 which corresponds to the $[C_{15}H_{12}N_5O_{13}P]$ ion called [M + 2] peak (detailed fragmentations was explained in Figs. 5 and 6). Mass fragmentations were almost similar to the studies performed by various authors in recent past [7, 39, 51]. Additionally, the above results were supported by elemental analysis (CHNO); calculated (C, 36.16; H, 1.82; N, 14.06; O, 41.75) and observed (C, 37.08; H, 1.87; N, 14.16, O, 41.52). The mass and elemental analyses were in very good agreement with UV-visible, IR, and NMR analyses. The melting point (105 ± 2 °C) was sharp indicating the purity of molecule **1**. Hence, all the elemental, spectrometric, and spectroscopic studies have revealed that the most probable structure of molecule **1** was as that of mentioned in Scheme 1.

Computational studies of molecule **1**

Since, our trials to obtain a single crystal of the molecule **1** was unsuccessful so far, and in order to gain a better understanding of geometrical structure of **1**, the molecular modeling studies have been done by means of GAMESS (Cambridge Software ChemBio3D Ultra 14.0 PC-program package). Some selected bond lengths and angles are listed in Table 1; the optimized structures, with atom labeling scheme, of molecule **1** are represented in Fig. 7. The bond angles and bond lengths of glyphosate were taken from literature [27]. It was observed that two rings

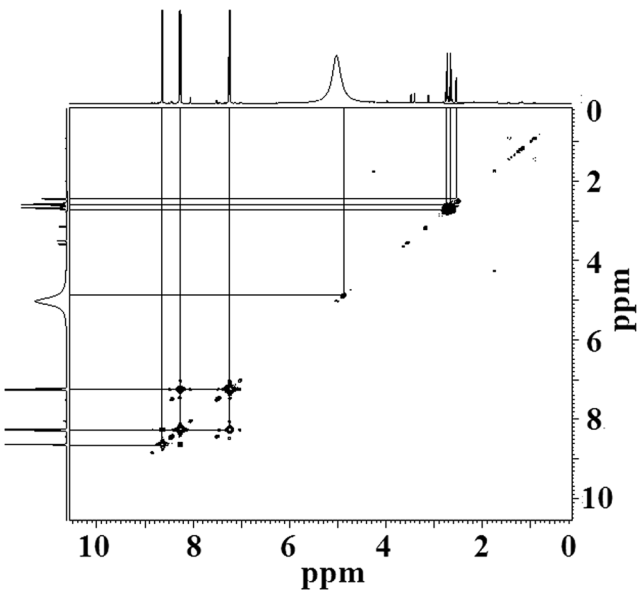


Fig. 4 ^{1}H,^{1}H-COSY spectrum of molecule **1**

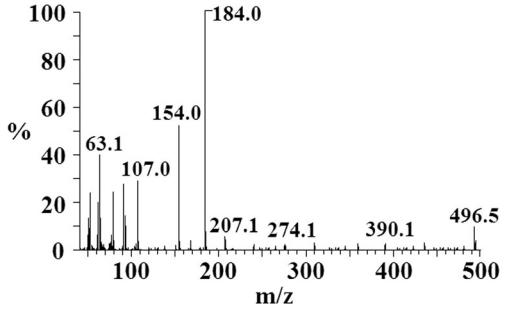


Fig. 5 GC-MS spectrum of product or molecule **1**

attached to P–O bonds were perpendicular to each other with an angle approximately 135°. One NO_2 group of first ring has effects on the second ring of molecule **1**. 3D molecular study showed that with the attachment of two rings to glyphosate, the bond lengths of glyphosate have been increased around all atoms except N(1)–C(3) and C(4)–N(1) bonds. Bond angles of glyphosate have been decreased around the all atoms except C(1)–P(3)–O(8). The maximum change among the bond length was observed around P(6)–O(9), P(6)–O(8), and P(6)–C(4), which was expected due to the conjugation effect (Supplementary S 3), caused by nitro group present at para position of molecule **1**. The slight increase in bond angle around C(4)–P(6)–O(8) was due to steric effect. From Table 1 and Fig. 7, it is clear that the bond lengths

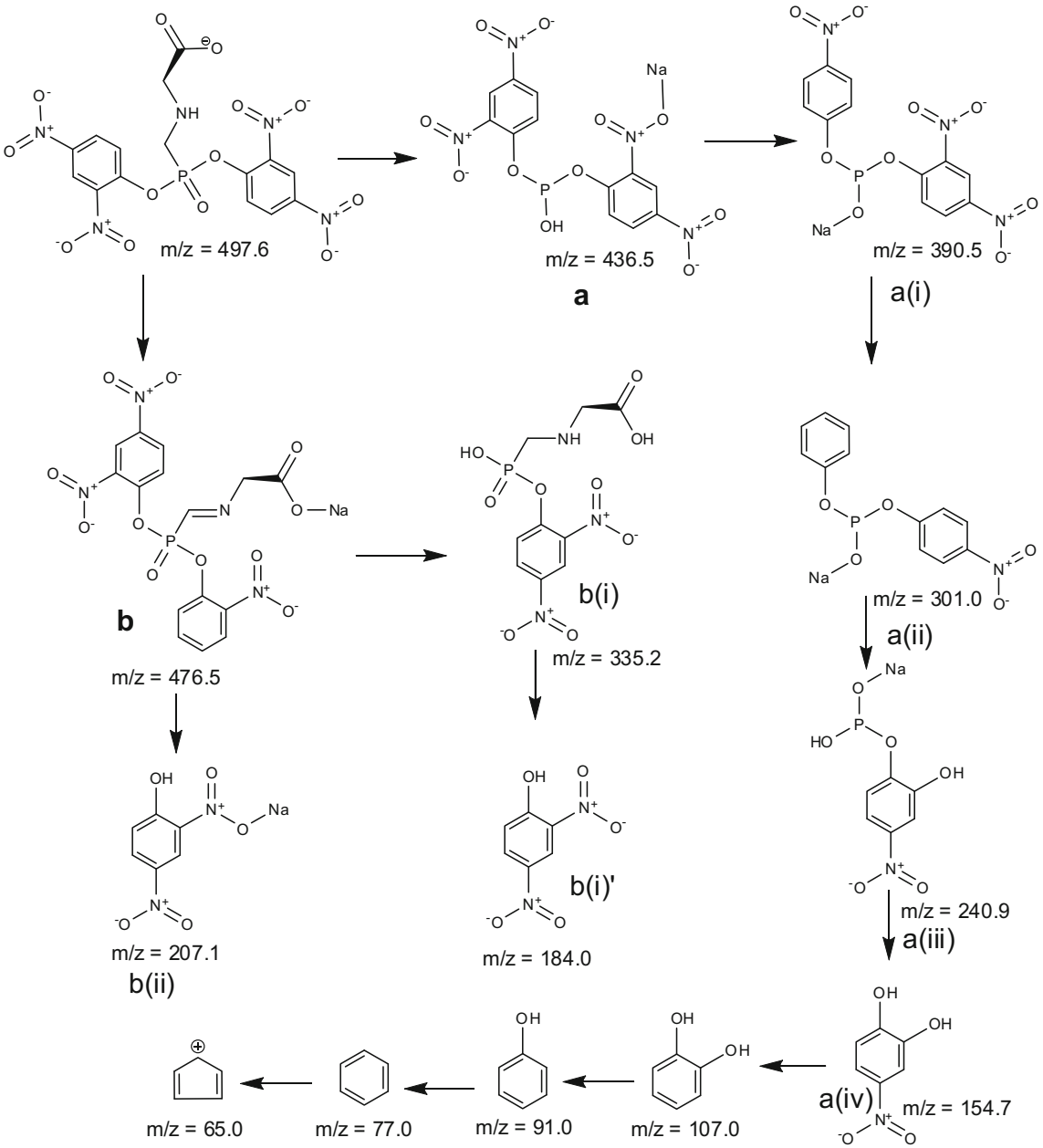


Fig. 6 Mass fragmentation sequence of molecule **1**

Table 1 Geometric parameters (bond length and bond angles) of glyphosate and molecule **1** obtained from 3D molecular optimization

Bond length (angstrom)			Bond angle (degrees)		
Bond Gly		Molecule **1**	Bond Gly		Molecule **1**
C(5)-O(11)	1.308	1.410	O(11)-C(5)-O(10)	125.5	119.174
C(5)-O(10)	1.201	1.208	O(11)-C(5)-C(3)	124.3	119.162
C(3)-C(5)	1.507	1.509	O(10)-C(5)-C(3)	122.9	122.501
N(1)-C(3)	1.479	1.455	C(3)-N(1)-C(4)	114.4	107.700
C(4)-N(1)	1.491	1.453	N(1)-C(4)-P(6)	112.2	109.474
P(6)-C(4)	1.816	1.856	C(4)-P(6)-O(7)	109.9	109.474
P(6)-O(7)	1.501	1.734	C(4)-P(6)-O(9)	106.0	102.598
P(6)-O(9)	1.568	1.615	C(4)-P(6)-O(8)	104.2	104.601
O(8)-P(6)	1.568	1.615	O(7)-P(6)-O(9)	111.4	102.602
			O(7)-P(6)-O(8)	118.2	102.602
			O(9)-P(6)-O(8)	105.7	102.602

*Gly = Glyphosate, and Comp 1 = molecule **1**.*

and angles are found to be within the normal ranges obtained from the crystal structure data of few phosphate ester-based derivatives, studied in recent past [8, 20, 27, 45]. The obtained results were in a good agreement with the experimental results and hence strongly support them. Recently, organophosphate-based series of 2-(substituted phenoxyacetoxy) alkyl-5,5-dimethyl-1,3,2-dioxapho sphinan-2-ones was synthesized by Wang et al. [64]. They found that synthesized complexes were 70–100% active against seven weeds excluding weeds under current study. It was observed that the herbicidal and microbial activities of new molecule were very similar to series s-triazine molecules synthesized by Zhao et al. [65], but the weeds and pathogenic strains used by Zhao et al. [65] were different from the current study.

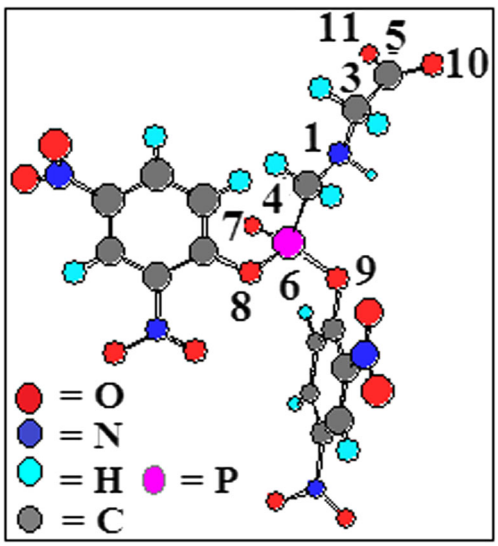


Fig. 7 3D molecular structure of molecule **1**

Stability of molecule **1**

In stability point of view, UV-visible decomposition experiments under aqueous solution were performed. It was observed that molecule **1** was stable in aqueous solution after 96 h and only very small change in the absorbance (i.e., ~3–8%) analyzed at 357 and 405 nm. Same study was performed by taking the different solvents including the acidic water (ph = 3), basic water (ph = 10), DMSO, MeOH, EtOH, and $CHCl_3$. A 96-h experiment was performed with different solvents, where maximum decomposition was noticed in case of acidic water (32%) followed by DMSO (18%), MeOH (12%), EtOH (10%) basic water (9%), and $CHCl_3$ (8%), respectively.

Agriculture

Herbicidal activities

The effects of molecule **1** on three grasses; barnyard grass (*Echinochloa Crus*), red sprangletop (*L. filiformis*), and yellow nuts (*C. esculentus*) were checked by taking glyphosate as a control. Figure 8 depicted that at dose levels 0.7, 1.5, and 3.0 mM, molecule **1** was the excellent herbicide than glyphosate under both treatments (i.e., preemergence and postemergence treatments). It was observed that Red Sprangletop (*L. filiformis*) was totally damaged with the applications of glyphosate and molecule **1**, so the quantitative analysis of Red Sprangletop (*L. filiformis*) was not included in Table 2. It was noticed that molecule **1** was far more effective than glyphosate at low (0.7 mM) and high (3.0 mM) dose levels in both experiments, i.e., preemergence treatment and postemergence treatment.

Quantitatively, the weight and lengths of roots and stems of two weeds were measured of 1-month-aged weeds (Table 2 and Supplementary S4). Sharp decrease among the weights and lengths of roots and stems of weeds were observed with the applications of molecule **1** as compared to glyphosate. Overall, it observed that molecule **1** was far more active than glyphosate.

Effect of molecule **1** *on PGPR strains*

The in vitro inhibitory effects of molecule **1** on three PGPR strains were performed to check the comparative adverse effects of molecule **1** and glyphosate. Recent studies revealed that all the strains under study have significant PGPR activities [2, 4, 5, 9, 40, 46, 53]. In the current study, up to concentration level 50 mM, non-adverse effects of molecule **1** were noticed to the PGPR strains (Table 3 and Fig. 9). In other words, molecule **1** has shown inhibition to PGPR strains after 50 mM as exhibited in Fig. 8. As per the MIC values, the order of adverse effect of molecule **1** on different PGPR strains at

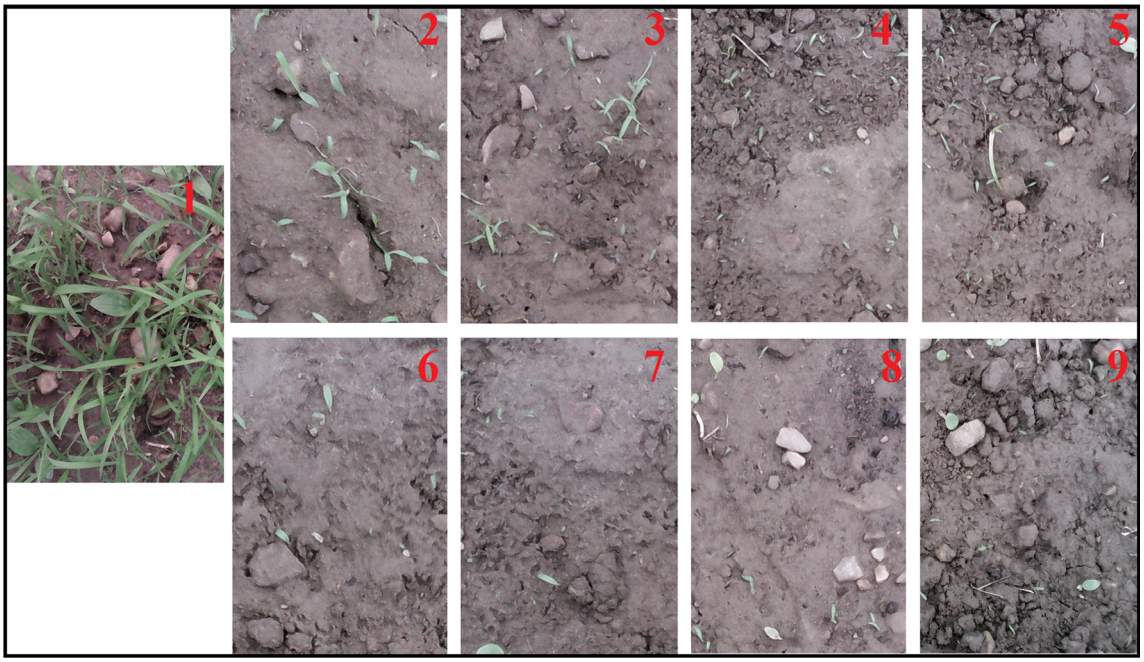


Fig. 8 Herbicidal activities at low concentration levels of molecule **1** and high level of glyphosate on three weeds under study after the 15th days of application. Therefore, low and high concentrations were 0.7 mM and 3.0 mM. (*1*) is a positive control where no herbicide added. *2* and *3* are the pre- and post-experiments at low dose level after 20 days of the spray of glyphosate. *4* and *5* are the pre and post experiments at high dose level after 20 days of the spray of glyphosate. *6* and *7* are the pre and post experiments at low dose level after 20 days of the spray of molecule **1**. *8* and *9* are the pre- and post-experiments at high dose level after 20 days of the spray of molecule **1**)

different concentrations was as follows: *R. leguminosarum* (55 ± 3 mM) > > *P. putida* (120 ± 4 mM) > *P. fluorescens* (150 ± 5 mM). These results were very consistent as per the review given by [40]. He has concluded that the phosphorus contains herbicides (e.g., glyphosate) can stimulate soil microbial growth [40]. Biodegradation of nitro group-based herbicides can easily do in the presence of PGPR by converting the nitro group into amine group [18, 22, 25, 63].

Recent studies have revealed that herbicides not only adversely affect the important soil microbial communities including rhizobacteria and their functional activities but also the growing plants [2, 3, 5, 9, 40, 46, 53]. Globally, the greater concern is therefore, as to how to minimize or reduce the effects of herbicides so that the consequential impact of these chemicals on the PGPR microorganisms involved in nutrient cycling, vis-a-vis the productivity of crops could be saved. It

Table 2 Weight and length measurement of two grasses after 30 days of experiment; Z = Barnyardgrass (*Echinochloa Crus*), and D = Yellow nuts (*Cyperus esculentus*)

Molecule	Treatment	Dose	Root length (cm) ± SD		Stem length (cm) ± SD		Root Wt. (g) ± SD		Stem Wt. (g) ± SD	
			Z	D	Z	D	Z	D	Z	D
Control			10.0 ± 1.2	8.00 ± 1.0	28.00 ± 3.1	30.10 ± 2.4	0.315 ± 0.04	0.395 ± 0.07	1.891 ± 0.07	2.501 ± 0.09
Gly	Pre	High	6.80 ± 0.9	6.00 ± 0.8	17.70 ± 1.4	24.40 ± 1.8	0.293 ± 0.02	0.183 ± 0.02	0.727 ± 0.05	0.721 ± 0.04
		Low	8.60 ± 0.8	7.90 ± 1.0	19.50 ± 1.5	24.80 ± 2.0	0.412 ± 0.03	0.262 ± 0.04	0.987 ± 0.06	0.957 ± 0.06
	Post	High	6.30 ± 0.6	4.80 ± 0.5	17.50 ± 1.4	22.30 ± 1.7	0.178 ± 0.02	0.194 ± 0.04	0.663 ± 0.04	1.037 ± 0.05
		Low	7.50 ± 0.5	6.20 ± 0.7	17.90 ± 1.6	23.20 ± 1.7	0.221 ± 0.03	0.276 ± 0.03	0.845 ± 0.06	1.325 ± 0.08
Molecule **1**	Pre	High	5.40 ± 0.4	4.00 ± 0.4	14.20 ± 0.9	14.40 ± 0.8	0.106 ± 0.04	0.043 ± 0.02	0.255 ± 0.05	0.166 ± 0.08
		Low	6.30 ± 0.8	4.80 ± 0.8	15.40 ± 1.3	15.20 ± 1.5	0.075 ± 0.03	0.019 ± 0.00	0.115 ± 0.06	0.085 ± 0.02
	Post	High	5.00 ± 0.7	5.00 ± 0.6	12.30 ± 1.1	18.80 ± 1.2	0.025 ± 0.00	0.069 ± 0.03	0.096 ± 0.04	0.195 ± 0.01
		Low	5.60 ± 0.7	5.70 ± 0.9	13.90 ± 1.4	19.20 ± 1.3	0.069 ± 0.02	0.125 ± 0.08	0.167 ± 0.07	0.425 ± 0.03

Z and D are two grass species as in images; high dose level = 3.0 mM and low dose level = 0.7 mM

Gly glyphosate, *Z* barnyard grass (*Echinochloa Crus*), *D* yellow nuts (*Cyperus esculentus*)

Table 3 Effect of molecule **1** on PGPR and pathogenic strains

Effect of molecule **1** on PGPR and pathogenic strains				
PGPR strains (MIC in mM)[a]			Pathogenic strains (MIC in μg/l)[a]	
Rhizobium leguminosarum (1)	*Pseudomonas fluorescens* (2)	*Pseudomonas putida* (3)	*Salmonella typhimurium* (1)	*Aspergillus fumigatus* (2)
55 ± 3	150 ± 5	120 ± 4	25 ± 2 (>52)[b]	35 ± 3 (>60)[c]

a mean of triplicates, control; *b* chloramphenicol (for bacteria); *c* ketoconazole (for fungi)

has been noticed that most common herbicides including the clodinafop, quizalafop-p-ethyl, metribuzin, acetochlor, terbuthylazine, atrazine, and glyphosate have adverse effects on plant growth-promoting strains [2, 5, 6, 9, 40, 46, 53]. Hence, in current study, the synthesis of molecule **1** was the most exciting achievement, because, as like other herbicides it has no adverse effect on PGPR strains.

Biology

*Effects of molecule***1** *on pathogenic strains*

Various author have noticed that many amino acid and nitro derivatives have medicinal values including the antimicrobial and antifungal activities [15, 18, 21, 22, 24, 25, 44, 52, 63]. Hence, biological study of present work was the additional work because of presence of amino acids and nitro groups in the molecule **1**. In vitro antimicrobial activities of molecule **1** were carried out on two pathogenic strains. Out of these two strains, *S. typhimurium* was a bacterial strain and *A. fumigates* was a fungal strain. Recent studies have revealed that *A. fumigatus* was the most common and dangerous fungus which can cause severe diseases among the humans and animals [38]. Bacteria *S. typhimurium* was the causative agents of typhoid fever and diarrheal diseases in humans, and responsible for an estimated 40 million cases of systemic typhoid fever worldwide each year [54]. In current study, as compared to glyphosate, molecule **1** has shown excellent antifungal

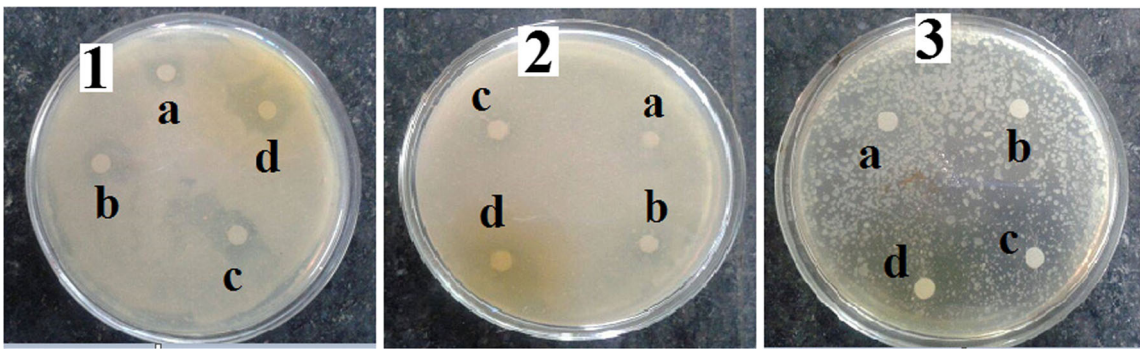


Fig. 9 Effect of molecule **1** on PGPR strains; inhibitory activities of molecule **1** against PGPR strains; *Rhizobium leguminosarum* (*1*), *Pseudomonas fluorescens* (*2*), and *Pseudomonas putida* (*3*) at 50, 100, 150, and 250 mM

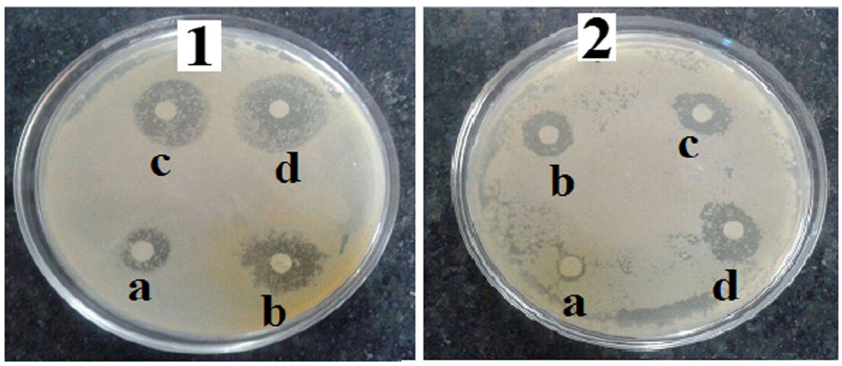


Fig. 10 Effect of molecule **1** on pathogenic strains; antimicrobial activities of molecule 1 against bacterial and fungal strain: *Salmonella typhimurium* (*1*) and *Aspergillus fumigatus* (*2*) at 50, 100, 150, and 250 μg/l.

(35 μg/L) and antibacterial (25 μg/L) activities against the *A. fumigatus* strain and *S. typhimurium* strain respectively (Table 3 and Fig. 10).

Molecule **1** *to bovine serum albumin protein interactions*

After obtaining the very good antimicrobial activities, to obtain the better insight about molecule **1** as a good medicinal agent the binding constant of the molecule **1**–BSA complex was calculated. The calculated value of binding constant of molecule **1**–BSA complex was $1.4 \times 10^6\ M^{-1}$ (Supplementary Fig. S5). In literature, the binding constants ranging from $10^6\ M^{-1}$ to $10^8\ M^{-1}$ has been considered as strong binding with BSA [1], which indicates strong interactions with human/animal proteins. The strong binding constant represents the good clinical aspects of drugs. Moreover, the presence of amino, carboxyl, phosphate and nitro groups make it more effective antimicrobial agent as various drugs in recent past have been investigated with the same functional groups [15, 21, 24, 44, 52].

Conclusion

In the present work, we design and discover a new molecule with potential herbicidal and microbial activities. The preliminary results have shown that molecule **1** exhibited excellent herbicidal activities against three grass species. Additionally, synthesized molecule has shown excellent antimicrobial activities. This study may be a new insight towards the synthesis of potential herbicidal and microbial active derivative of glyphosate.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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