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ORIGINAL RESEARCH

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Green synthesis, molecular docking, anti-oxidant and anti-inflammatory activities of α-aminophosphonates

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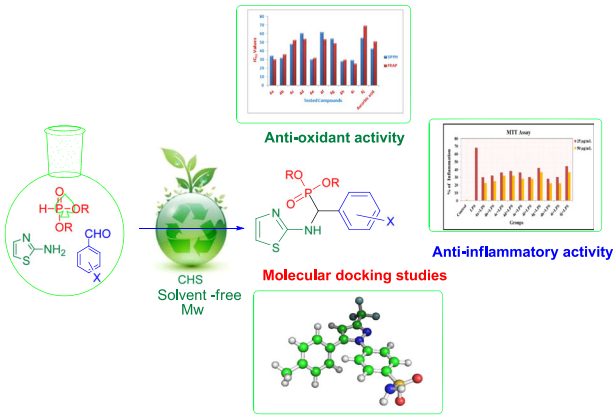
Abstract

The naturally viable α-amino acid surrogates have been synthesised via Kabachnik–Fields reaction by the condensation of 2-aminothiazole with various aldehydes and dialkyl phosphites in the presence of Caffeine hydrogen sulfate (CHS) as eco-friendly and reusable catalyst under microwave irradiation and solvent-free conditions. The title compounds were characterised by IR, ^{1}H, ^{13}C, ^{31}P NMR and mass spectral data. All the synthesised (**4a–j**) compounds were screened for their insilico and in vitro studies. The results revealed that, out of all the titled compounds **4a**, **4e**, **4h** and **4i** have exhibited significant activity in terms of antioxidant and anti-inflammatory activity. In addition, molecular docking studies were also carried out against **Cox-2** with celocoxib as the standard.

Graphical Abstract



Supplementary information The online version of this article (https://doi.org/10.1007/s00044-019-02411-8) contains supplementary material, which is available to authorised users.

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Keywords α-aminophosphonates · Caffeine hydrogen sulfate · Antioxidant · Anti-inflammatory activity · Molecular docking studies

Introduction

Synthesis of organophosphorus compounds has been over emphasised due to their eminent role in biology and pharmaceutical areas (Esalah and Husein 2008). Among them α-aminophosphonates popped up abundantly due to their isosteric structure with α-amino acids (Kafarski and Lejczak 1991). Owing to this, α-aminophosphonates have formed diverse applications as antibiotics (Lajczak et al. 1986), anti-thrombotics (Meyer and Barlett 1998), inhibitors of HIV protease (Stowasser et al. 1992; Ananda et al. 2017), anti-cancer agents (Bhattacharya et al. 2013), anti-inflammatory (Damiche and Chafaa 2017), anti-tubercular (Mulla et al. 2014), catalytic antibodies, (Hirschmann et al. 1994; Smith et al. 1994), anti-oxidants (Gundluru et al. 2016), anti-microbials (Sreekanth et al. 2018), plant growth regulators (Maier 1990; Forlani et al. 2013), herbicides (Chea et al. 2016), anti-viral agents (Xie et al. 2017; Xu et al. 2006) and carriers of hydrophilic organic molecules across the phospholipid membranes (Antipin et al. 1999).

Nowadays to manage the inflammation caused by the response of tissues to destructive pathogens, we are using non-steroidal remedies, which are alternative to the steroidal therapeutics are being used. Even though non-steroidal anti-inflammatory drugs are acting fine on inflammation, they have some drawbacks such as gastrointestinal irritation and bleeding. These problems prompted the researchers to synthesise new compounds having anti-inflammation properties with reduced side effects.

Five-membered heterocyclic compounds bearing both sulfur and nitrogen atoms are privileged essential organic structural scaffolds in compounds used as medicine. Due to their high potency towards therapeutic properties, tremendous efforts have been to synthesis thiazole bearing derivatives. These structural motifs are having numerous therapeutic properties such as anti-inflammatory (Ugwu et al. 2018), antihypertensive α-blocking (Bakr et al. 2008), anti-leishmanial (Sharma et al. 2013), neuroprotective agents in Parkinson's and Alzheimer's diseases (Anzini et al. 2010), Some of these organic structural scaffolds are used in radiodiagnostics to measure the size of the tumours in radiotherapy (Tzanopoulou et al. 2010), along with transition metals like Re and Tc. Recently it has been found that this structural motif combined with pyrimidine is having good herbicidal activities (Zuo et al. 2016), are also found use in semiconducting materials and organic light-emitting diodes (Kudrjasova et al. 2014).

Synthesis of α-amino phosphonates by the Kabachnik–Fields reaction, a vast number of catalytic protocols were established such as uncatalyzed conditions (Tibhe et al. 2010), Metal triflates (Qian and Huang 1998; Firouzabadi et al. 2004), $InCl_3$ (Ranu et al. 1999), SmI_2 (Xu et al. 2003), pentafluorophenylammoniumtriflate (PFPAT) (Malamiri and Khaksar 2014), $Mg(ClO_4)_2$ (Bhagat and Chakraborti 2007), $BiCl_3$ (Zhan and Li 2005), $LiClO_4$ (Azizi and Saidi 2003) have been employed. Most of the catalysts gave satisfactory to high yields, but they showed disadvantages like cost expensive, toxic and hygroscopic nature of catalysts, requirement of stoichiometric quantities of catalysts and long reaction times. Development of metal free, environmentally benign and economically feasible synthetic protocols for several organic transformations is the need of the hour. In this context, an elegant corrosive and recyclable catalyst called Caffeine hydrogen sulfate (CHS) (Shalini et al. 2018; Agarwal et al. 2019) has been used for the synthesis of α-(2-aminothiazole) alkylphosphonates by the reaction of 2-aminothiazole with various aldehydes and dialkyl phosphites under MW irradiation conditions. The MW irradiation (Gundluru et al. 2016; Kandula et al. 2018) offered new method to energising the reaction mixture as it involves the direct transfer of energy to the substrate molecules and will enhance the rate of the reaction, by rapid kinetic excitation of molecules and increases the product yield. This serves an ideal platform for the one-pot three components of Kabachniks–Fields reaction. In addition, in vitro antioxidant, anti-inflammatory and molecular docking studies were also carried out for the synthesised compounds (**4a–j**) to establish their bio activities.

Experimental

All the chemicals, reagents and solvents were procured from Aldrich S.D. Fine Chem. Ltd, Boisar, India and Qualigens, Mumbai, India and used without further purification. MWI was carried out in a microwave oven, catalyst system (CATA-4R). The purity and progress of the reaction was confirmed by thin layer chromatography on pre-coated silica gel plates purchased from Merck. Visualization is done under UV light chamber. Melting points were determined in an open capillary tube on EZ Melt Automated Melting Point Apparatus. IR spectra were recorded as neat samples on Bruker Alpha-Eco ATR-FTIR

interferometer with single-reflection sampling module equipped with Zn–Se crystal; the data were expressed in reciprocal centimetres (cm^{-1}). ^{1}H, ^{13}C and ^{31}P NMR spectra were recorded on Jeol Resonance spectrometer operating frequency at 400, 100 and 161.9 MHz respectively. NMR data were recorded in $CDCl_3$ and tetramethylsilane was used as a standard for both ^{1}H and ^{13}C-NMR and 85% H_3PO_4 is used as a standard for ^{31}P NMR. The high-resolution mass spectra are recorded on micromass Q-TOF micromass spectrometer using electrospray ionisation.

DPPH anti-oxidant assay

In-vitro antioxidant activity of newly synthesised compounds (**4a–j**) was performed by DPPH method (Matsubara et al. 1991), which determines the free radical inhibitory ability of synthesised compounds by scavenging the very stable 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical in methanol. Stock solution of DPPH (1.3 mg/mL) in methanol was prepared. Stock solution of DPPH 100 µL was added to 3.0 mL of methanol and absorbance was recorded at 516 nm. The various concentrations of synthesised compounds (25, 50, 75 and 100 µL) in methanol were prepared. All sample solutions 1.0 mL each is diluted with 3.0 mL with methanol and 100 µL of stock solution of DPPH was added. Test tubes were kept for 30 min in dark to complete the reaction. After 30 min, absorbance of each test tube was recorded at 516 nm on UV-VIS spectrophotometer against methanol as a blank. The effective concentration of sample required to scavenge DPPH radical by 50% (IC_{50} value) was obtained by linear regression analysis of dose-response curve plotting between percentage inhibition and concentrations. The DPPH free radical scavenging activity was calculated using the following formula:

$$\text{DPPH Scavenging}\ (\%) = \frac{(A_{cont} - A_{test})}{A_{cont}} \times 100$$

Where; Control is absorbance of a DPPH solution without compound; Test is the absorbance of the test compound with DPPH. The degree of discoloration indicates the free radical scavenging efficiency of the compound. Ascorbic acid was used as the standard free radical scavenger reference compound.

Ferric reducing antioxidant power assay

The reactive principle of chemicals in which iron reacts with a colorimetric probe to produce a blue product was used to quantitate Ferric reducing antioxidant power assay (Raquel et al. 2000). An aliquots of different concentration of test compounds (25, 50, 75 and 100 µL) was mixed with 90 µL water and 900 µL ferric reducing antioxidant power (FRAP) reagent (2.5 mL of 20 mmol/L of 2, 4, 6-tri(2-pyridinyl)-1,3,5-triazine), in 40 mmol/L of HCl, 2.5 mL of 20 mmol/L of ferric chloride, 25 mL of 0.3 mol/L of acetate buffer (pH 3.6) and incubate at 37 °C for 30 min. After incubation, the absorbance values were recorded at 593 nm with ultraviolet-visible (UV-vis) spectrophotometer. The antioxidant activity was expressed as the amount of extract required to reduce 1 mmol of ferrous ions.

$$\text{FRAP Scavenging}\ (\%) = \frac{(A_{cont} - A_{test})}{A_{cont}} \times 100$$

Where; control is absorbance of a FRAP solution without compound; test is the absorbance of the test compound with FRAP. The degree of discoloration indicates the free radical scavenging efficiency of the compound. Ascorbic acid was used as the standard free radical scavenger reference compound.

Cell line studies

Maintenance of cell lines

RAW 264.7 murine macrophage cell lines were procured from NCCS Pune. RAW macrophages were plated into T75 flasks and cultured in Dulbecco's Modified Eagle Medium (DMEM) with high glucose, stable glutamine and sodium pyruvate, supplemented with 10% foetal bovine serum heat-inactivated (65 °C for 20 min) and 1% penicillin-streptomycin antibiotics (100 IU/mL penicillin and 100 µg/mL streptomycin) at 37 °C in 5% CO_2 atmosphere.

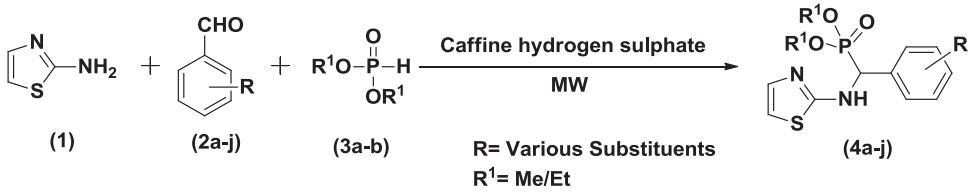
Toxicity studies

The study was conducted in accordance with OECD guidelines (Testing of Chemical Number 423). Healthy RAW 264.7 murine macrophage cell lines were used in this investigation. Cell lines were treated with different concentrations (i.e., 100 µg/mL and 200 µg/mL) of title compounds and observed for 48 h from the time of administration. The Median non-toxic concentration (MNTC) and cytotoxic concentration (CC50) were determined (Fig. 3). The minimum concentration of the test compounds which has not shown any toxic effect on healthy RAW 264.7 cell lines were selected for further anti-inflammatory analysis.

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assay

Cell viability was measured based on the formation of purple formazan, which is metabolised from colourless MTT (Sigma-Aldrich) by mitochondrial dehydrogenases, enzymes that are only active in live cells. RAW 264.7 cells

Scheme 1 Synthesis of α-amino phosphonates



(5×10^5cells/ml) were seeded in a 96-well plate. The cells were pre-treated with various concentrations of synthesised compounds for 1 h and then stimulated by 10 µg/ml LPS for 24 h. Following the incubation with title compounds and LPS, the cultured media was replaced with fresh media and the cells were incubated with 0.5 mg/ml MTT solution for 3 h. The supernatant was then discarded and the formazan blue, which was formed in the cells, was dissolved with dimethyl sulfoxide (Sigma-Aldrich). The optical density was measured at 540 nm with an ELISA plate reader. Percentage of inflammation was calculated by subtracting the percentage of cell viability over control cells (Park et al. 2009).

Determination of nitrite production

Nitrite accumulation (NO_2) in cell culture media was determined by the Griess method (Pekarova et al. 2009). Briefly, 1×10^6 cells were seeded in a T75 flask, allowed to adhere overnight, and then treated as previously mentioned in MTT assay. At the end of the different incubations in the CO_2 incubator at 37 °C with 5% CO_2, the different cell supernatants were collected, the samples (1 mL) were mixed with an equal volume of Griess reagent (1 mL of 1:1 0.1% naphthyl-ethylenediamine and 1% sulfanilamide in 5% phosphoric acid) in a tube, and incubated in the dark for 10 min at room temperature. Then the absorbance of the reaction mixture was measured at 540 nm on a microplate reader (Thermo Scientific Multiskan EX). The NO_2 concentration was determined using a sodium nitrite ($NaNO_2$) standard curve (working range: 0.1–6.25 µM). Each treatment was carried out in triplicates and the final results were expressed as µmol NO_2 -/mg protein.

Statistical analysis

All experiments were repeated at least three times. Results are expressed as the means ± standard deviation (SD) of three experiments. Statistical calculations were performed using prism Graph Pad 6.0.

Results and discussion

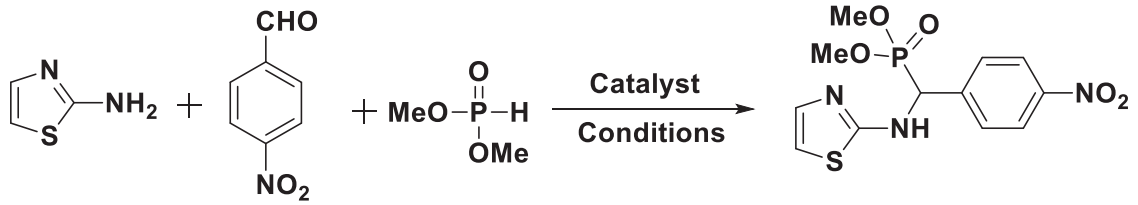
An elegant one-pot three-component Kabachnik–Fields reaction of 2-aminothiazole (**1**), various aldehydes (**2a**) and dialkyl phosphite (**3a**–**b**) in presence of caffeine hydrogen sulfate (CHS) as a simple reusable catalyst under MW irradiation conditions has been accomplished (Scheme 1). The synthesis involves by the simple workup procedures and offers moderate to excellent product yields.

To optimise the experimental conditions required for this reaction, initially the reaction is run with 2-aminothiazole (**1**), 4-nitrobenzaldehyde (**2a**) and dimethyl phosphite (**3a**) at ambient temperature under neat conditions in the absence of catalyst for 10 h (Table 1). There was no reaction (Table 1, Entry 1) and followed by increased the temperature to 100 °C for 10 h. The reaction, when run with several solid catalysts like $AlCl_3$, $FeCl_3$, p-TSA, $ZnCl_2$, $NiBr_2$, $CrCl_3$, $CeCl_3.7H_2O$, $Pd(OAc)_2$ (Table 1, Entry 2–9) by varying the temperature from ambient to 100 °C furnished a mixture of 60% of product and some amount of reactants and imine. To our delight, later when CHS (Table 1, Entry-10) is used as a catalyst the reaction proceeded even at ambient temperature and offered 70% product in just 0.5 h. Later there was no enhancement of yield of the product, even after extended the time and raising the temperature to 100 °C (Table 1).

To enhance the yield of the Kabachnik–Fields product (**4a**) the concentration of CHS catalyst is varied (Table 2, Entry 1-4). We have noticed 5 mol% of CHS is sufficient to get a 93 % yield of the product (Table 2, Entry 5). Further increasing quantity of the catalyst there is no notable improvement in the yield of the product. The reaction is screened to check the effect of the solvent on outcome of the product with 5 mol% of catalyst by using various solvents polar and non-polar solvents (Table 2, Entry 5–12). Out of all solvents, the reaction in methanol (Table 2, Entry 9) offered 93% of yield of the product, but it is a time-consuming process (20 min), but the same reaction in solvent-free condition offered the product within short time (10 min).

To optimise microwave irradiation for the reaction of 2-aminothiazole (**1**), 4-nitrobenzaldehyde (**2a**) and dimethyl phosphite (**3a**) were reactants with 5 mol% of CHS as a catalyst, we have employed microwave protocol from 100 to 600 W and the results are listed in Table 3. The reaction at 400 W afforded excellent conversion in less time and offered 97% yield (Table 3, Entry 3). The reaction at 500 W (Table 3, Entry 4) affords low conversion, due to the decomposition of the substance in the reaction mixture.

Table 1 Optimisation of catalyst for the synthesis of compound (**4a**)



1 2a 3 4a

Entry	Catalyst (mol %)	Time (h)	Temperature (°C)	Yield (%)[a]
1	Catalyst-free	24	100	nr[b]
2	$AlCl_3$ (10)	10	80	25
3	$FeCl_3$ (10)	10	80	32
4	p-TSA (10)	10	60	50
5	$ZnCl_2$ (10)	10	100	45
6	$NiBr_2$ (10)	8	80	58
7	$CrCl_3$ (10)	10	80	46
8	$CeCl_3.7H_2O$ (10)	3	80	40
9	$Pd(OAc)_2$ (10)	1	80	55
10	CHS (10)	0.5	rt	70

[a]Isolated yields

[b]No reaction

Table 2 Optimisation of the amount of catalyst and effect of solvent (**4a**)[a]

Entry	Catalyst (mol %)	Time (min)	Solvent	Yield (%)[b]
1	1	40	Solvent-free	60
2	3	40	Solvent-free	75
3	5	10	Solvent-free	93
4	7	15	Solvent-free	93
5	5	25	Toluene	90
6	5	25	THF	90
7	5	30	Ethyl acetate	90
8	5	25	DCM	90
9	5	20	Methanol	93
10	5	25	Ethanol	90
11	5	30	Acetonitrile	85
12	5	20	DMF	89

[a]Reaction conditions: 2-aminothiazole (1) (1.0 mmol), 4-nitrobenzaldehyde (1.0 mmol), and dimethyl phosphite (3) (1.0 mmol) in the presence of 5 mol% of CHS as catalyst under solvent-free conditions

[b]Isolated yields

Hence 400 W microwave irradiation is optimised for the reaction for it effective completion.

After optimisation of experimental conditions, the scope of the Kabachiniks-Fields reaction with 5 mol% of caffeine

Table 3 Effect of microwave energy in watts

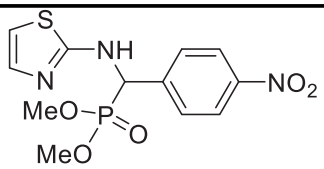
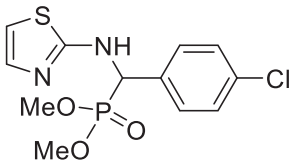
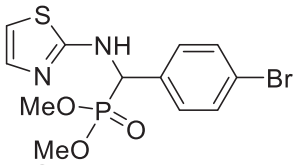
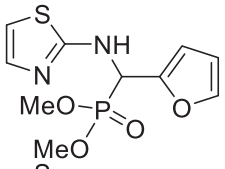
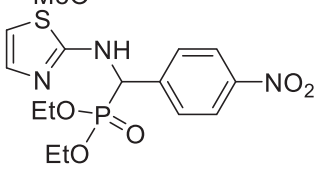
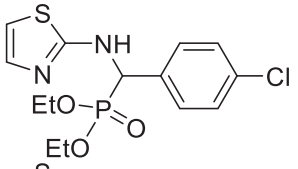
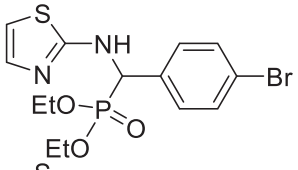
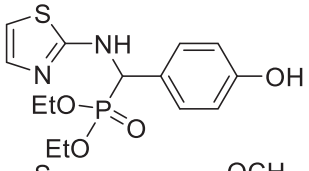
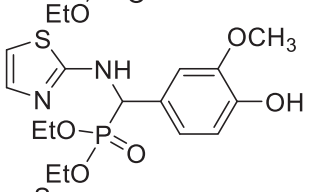
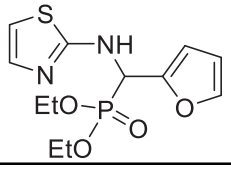
S.No.	Power (watts)	Time (min)	Yield (%)[a]
1	180	15	89
2	300	15	93
3	400	5	98
4	500	5	70

[a]Isolated yields

hydrogen sulfate as the catalyst, the reaction of 2-aminothiazole and commercially available aldehydes contains both electron withdrawing as well as electron donating groups and dialkyl phosphites was studied and the results are presented in Table 4.

On the basis of previous reports and literature, a reasonable mechanism has been proposed for this reaction (Scheme 2). The aldehyde is activated initially by the catalyst CHS and carbonyl carbon (**2**) renders to more electrophilic, this facilitates the nucleophilic addition of the amine (**1**) leads to form an imine. Further imine is activated by CHS by co-ordinating with the imine nitrogen and drawing π-electrons of the imine. Consequently the imine carbon present in Schiff's base becomes electrophilic. This situation facilitates the nucleophilic addition of phosphate (**3**) to the imine leads to the formation aminophosphonates (**4**) with the release of CHS.

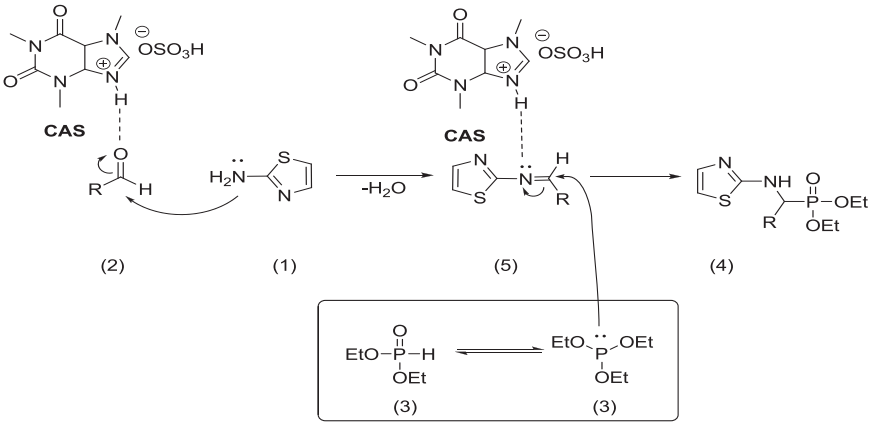
Table 4 Synthesis of the α-substituted thiazolyamine methyl phosphonates (**4a–j**)[a]

Entry	Structure of the product	Conventional		Microwave	
		Time (min)	Yield[b] (%)	Time (min)	Yield[b] (%)
4a		15	80	5	98
4b		15	65	10	94
4c		15	60	8	95
4d		10	60	6	90
4e		10	75	5	97
4f		8	75	10	95
4g		8	68	6	93
4h		17	50	15	90
4i		20	60	10	90
4j		10	73	6	92

[a]Reaction conditions: 2-aminothiazole (**1**) (1.0 mmol), various substituted aromatic aldehydes (**2a–j**) (1.0 mmol), and dialkyl phosphite (methyl and ethyl) (**3**) (1.0 mmol) in the presence of 5 mol% of CHS as catalyst under solvent-free conditions

[b]Isolated yields

Scheme 2 Possible reaction mechanism for the synthesis of α-aminophosphonates



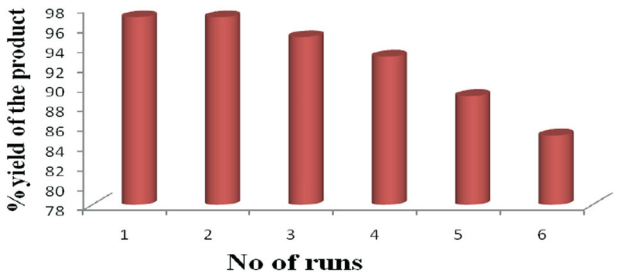


Fig. 1 recyclability of catalyst

Reusability of catalyst

Reusability of catalyst is very important from the economical and industrial point of view. To analyse the activity and reusability of the CHS, we studied this reaction by examining up to six cycles shown in Fig. 1. The activity of catalyst decreased in the subsequent runs. After each run, we have recovered the catalyst by the addition of DCM to the reaction mixture and the insoluble catalyst was recovered by centrifugation, it was washed twice with DCM and dried in oven at 60 °C for next use.

The chemical structures of the all the synthesised compounds (**4a**–**j**) were characterised by IR, ^{1}H-NMR, ^{13}C-NMR and ^{31}P NMR spectroscopy. In FT-IR spectra the following stretching frequencies were observed, (a) -NH stretching frequencies at 3285–3250 cm^{-1}, (b) –P = O stretching vibration at 1280–1240 cm^{-1}, (c) -P-O-C stretching vibrations at 1048–1006 cm^{-1}, (d) –P–C stretching vibration at 780–740 cm^{-1}. In ^{1}H-NMR spectra the chemical shift for N–H proton was found in the range between 6.25–5.60 ppm. The signals for P–CH appeared in the range from 5.50 to 4.70 ppm as a doublet. ^{13}C-NMR chemical shift appeared in the range from 13.20–168.30 ppm. ^{31}P NMR chemical shifts appeared in the region of 23.78–21.10 ppm.

General procedure for the synthesis of compound (4a)

A mixture of 2-aminothiazole (1 mmol), 4-nitrobenzaldehyde (1 mmol), dimethyl phosphite (1.0 mmol) and CHS (5 mol %) were charged in 25 ml conical flask and reaction was monitored at 400 W in microwave. The progress of the reaction was monitored by TLC analysis by using ethyl acetate: hexane (8:2) as eluents. After completion of reaction, the resulting reaction mixture was treated with DCM and filtered to remove the catalyst for reuse. After separation of the catalyst, it was washed with 20 ml of DCM and dried under oven for reuse. Further the filtrate was quenched with 10 ml of water followed by 5 ml of brine solution dried over anhydrous magnesium sulfate and concentrated by using rotary evaporator. Later the pure products obtained were purified by recrystallisation from ethanol. The same procedure is adopted for synthesis of remaining compounds.

Spectral characterisation

Dimethyl ((4-nitrophenyl)(thiazol-2-ylamino)methyl) phosphonate 4a

White solid; Yield 97%, Mp; 125–127 °C; FT-IR (cm^{-1}): ν_{max} 3282 (NH), 1274 (P = O), 1018 (P-O-C), 785 (P-C); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm): 8.29 (1H, d, J = 12.0 Hz, Ar-H), 8.12 (2H, d, J = 8.0 Hz, Ar-H), 7.97 (1H, d, J = 8.0 Hz, Ar-H), 6.98 (1H, d, J = 4.0 Hz, Ar-H), 6.98 (1H, d, J = 8.0 Hz, Ar-H), 5.73 (1H, d, J = 24.0 Hz, P-CH), 3.82 (3H, d, J = 12.0 Hz, -OC$\underline{H}_3$), 3.61 (3H, d, J = 12.0 Hz, -OC$\underline{H}_3$); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ (ppm): 161.55 (C-4), 145.92 (C-11), 136.30 (C-8), 133.67 (C-6), 132.67 (C-13 & C-9), 123.46 (C-12 & C-10), 113.29 (C-7), 68.99 (C-1), 54.03 (d, J = 7.0 Hz, C-21), 53.69 (d, J = 8.0 Hz, C-22); ^{31}P-NMR ($CDCl_3$, 161.8 MHz) δ (ppm): 23.78 Hz; HRMS (ESI) m/z Calcd. for $C_{12}H_{14}N_3O_5PS$ $[M + H]^+$ 344.3018 found 344.3010.

Dimethyl ((4-chlorophenyl)(thiazol-2-ylamino)methyl) phosphonate 4b

White solid; Yield: 94%, Mp; 128–130 °C; IR (cm^{-1}): ν_{max} 3283 (NH), 1277 (P = O), 1013 (P-O-C), 749 (P-C); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm): 7.38 (2 H, d, J = 8.0 Hz, Ar-H), 7.37 (2H, d, J = 8.0 Hz, Ar-H), 7.09 (1H, d, J = 4.0 Hz, Ar-H), 6.89 (1H, d, J = 4.0 Hz, Ar-H), -NH is exchanged with D_2O, 4.90 (1H, dd, J^1 = 8.0 Hz & J^2 = 4.0 Hz, -PCH), 4.33 (3H, d, J = 8.0 Hz, -OC$\underline{H_2}$CH$_3$), 4.21 (3H, d, J = 8.0 Hz, -OC$\underline{H_2}$CH$_3$); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ (ppm): 163.21 (C-4), 136.1 (C-6), 135.70 (C-7), 132.90 (C-17), 128.25 (C-15 & C-19), 125.25 (C-16 & C-18), 113.01 (C-7), 65.60 (d, J = 10.0 Hz C-1), 53.40 (d, J = 15.0 Hz, C-14& C-13): ^{31}P-NMR ($CDCl_3$, 161.8 MHz) δ (ppm): 21.15; HRMS (ESI) m/z Calcd. for $C_{12}H_{14}ClN_2O_3PS$ 333.7468 $[M + H]^+$ found 333.7460.

Dimethyl ((4-bromophenyl)(thiazol-2-ylamino)methyl) phosphonate 4c

White solid; yield: 95%, Mp; 128–130 °C; IR (cm^{-1}): ν_{max} 3284 (NH), 1277 (P = O), 1013 (P-O-C), 749 (P-C); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm): 7.55 (2 H, s, J = 8.0 Hz, Ar-H), 7.48 (2H, d, J = 8.0 Hz, Ar-H), 7.17 (1H, d, J = 4.0 Hz, Ar-H), 6.98 (1H, d, J = 4.0 Hz, Ar-H) –NH is exchanged with D_2O, 5.20 Hz (1 H, d, J = 4.0 Hz, P-CH), 3.80 (3H, d, J = 4.0 Hz, -OC$\underline{H_3}$), 3.75 (3H, d, J = 4.0 Hz, -OC$\underline{H_3}$); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ (ppm): 165.20 (C-4), 140.20 (C-11), 136.05 (C-8), 132.20 (C-6), 129.20 (C-13 & C-9), 125.50 (C-12 & C-10), 114.48 (C-7), 63.76 (d, J = 10.0 Hz, C-1), 53.04 (d, J = 5.0 Hz C-13), 49.56 (C-14); ^{31}P-NMR ($CDCl_3$, 161.8 MHz) δ (ppm): 22.10; HRMS (ESI) m/z Calcd. for $C_{12}H_{14}BrN_2O_3PS$ 378.2008 $[M + H]^+$ found 378. 2010.

Dimethyl (furan-2-yl(thiazol-2-ylamino)methyl) phosphonate 4d

Brown solid; yield: 90%, Mp; 110-112 °C; FT-IR (cm^{-1}): ν_{max} 3278 (NH), 1256 (P = O), 1019 (P-O-C), 764 (P-C); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm): 7.09 (1H, d, J = 12.0 Hz, Ar-H), 6.71 (1H, d, J = 12.0 Hz, Ar-H), 6.48 (1H, d, J = 8.0 Hz, Ar-H), 6.26 (1H, s, J = 8.0 Hz, Ar-H), 6.12 (1H, d, J = 4.0 Hz, Ar-H), -NH is exchanged with D_2O, 5.47 (1H, d, J = 20.0 Hz, P-CH), 3.79 (3H, d, J = 8.0 Hz, -OC$\underline{H_3}$), 3.55 (3H, d, J = 8.0 Hz, -OC$\underline{H_3}$); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ (ppm): 168.69 (C-4), 152.20 (C-9), 139.38 (C-12), 138.65 (C-6), 115.02 (C-7) 108.92 (C-11), 107.78 (C-10), 63.40 (C-1), 54.14 (C-17), 48.91 (C-18); ^{31}P-NMR ($CDCl_3$, 161.8 MHz) δ (ppm): 22.95; HRMS (ESI) m/z Calcd. for $C_{10}H_{13}N_2O_4PS$ 289.2658 $[M + H]^+$ found 289.2663.

Diethyl ((4-nitrophenyl)(thiazol-2-ylamino)methyl) phosphonate 4e

White solid; yield: 97%, Mp; 120-122 °C: FT-IR (cm^{-1}): ν_{max} 3284 (NH), 1242 (P = O), 1117 (P-O-C), 774 (P-C); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm): 8.22 (2H, d, J = 12.0 Hz, Ar-H), 7.69 (2H, d, J = 8.0 Hz, Ar-H), 7.17 (1H, s, J = 12.0 Hz, Ar-H), 6.97 (1H, d, J = 12.0 Hz, Ar-H), 6.05 (1H, s, -NH), 4.90 (1H, t, J^1 = 16.0 Hz & J^2 = 4.0 Hz, P-CH), 4.23-3.84 (4H, m, -OC$\underline{H_2}$CH$_3$), 1.34 (3H, t, J^1 = 4.0 Hz & J^2 = 8.0 Hz, -OCH$_2$C$\underline{H_3}$), 1.19 (3H, t, J^1 = 4.0Hz & J^2 = 8.0 Hz, -OCH$_2$C$\underline{H_3}$); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ (ppm): 159.04 (C-4), 144.42 (C-11), 133.36 (C-6), 129.04 (C-13 & C-9), 119.29 (C-12 & C-10), 114.83 (C-7), 63.79 (C-4), 63.66 (t, J^1 = 7.0 Hz & J^2 = 28.0 Hz, C-1), 55.70 (C-18), 54.26 (C-19), 16.30 (d, J = 14.0 Hz, C-20) 14.65 (d, J = 20.0 Hz, C-24); ^{31}P-NMR ($CDCl_3$, 161.8 MHz) δ (ppm): 22.97. HRMS (ESI) m/z Calcd. for $C_{14}H_{19}N_3O_5PS$ $[M + H]^+$ 372.3558 found 372.3570.

Diethyl ((4-chlorophenyl)(thiazol-2-ylamino)methyl) phosphonate 4f

Yellow solid; yield: 95%, Mp; 120–122 °C (Boughabaa et al. 2018).

Diethyl ((4-bromophenyl)(thiazol-2-ylamino)methyl) phosphonate 4g

Brown solid; yield: 93%, Mp; 131–133 °C (Boughabaa et al. 2018).

Diethyl((4-hydroxyphenyl)(thiazol-2-ylamino)methyl) phosphonate 4h

White solid; yield: 90%, Mp; 128–130 °C; FT-IR (cm^{-1}): ν_{max} 3487 (-OH), 3290 (NH), 1267 (P = O), 1112 (P-O-C), 797 (P-C); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm): 7.63 (2H, d, J = 8.0 Hz, Ar-H), 7.40 (2H, d, J = 8.0 Hz, Ar-H), 7.09 (1H, s, Ar-H), 6.91 (1H, s, Ar-H), 6.21(1H, s, -NH), 5.49 (1H, s, -OH), 4.85 (1H, dd, J^1 = 8.0 Hz & J^2 = 8.0 Hz, -PCH), 4.19-3.73 (4H, m, -OC$\underline{H_2}$CH$_3$), 1.27 (3H, t, J^1 = 8.0 Hz & J^2 = 8.0 Hz, -OCH$_2$C$\underline{H_3}$), 1.12 (3H, t, J^1 = 8.0 Hz & J^2 = 8.0 Hz, -OCH$_2$C$\underline{H_3}$); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ (ppm): 160.91 (C-4), 155.45 (C-11), 137.89 (C-6), 131.46 (d, J = 7.2 Hz, C-5 & C-9), 124.03 (d, J = 6.25 Hz), 113.01 (C-12 & C-10), 109.42 (C-7), 69.83 (C-1), 64.10 (d, J = 6.25 Hz, C-18), 63.38 (d, J = 6.5 Hz, C-19), 16.33 (d, J = 6.25 Hz, C-20)), 14.13 (d, J = 26.25, C-21); ^{31}P-NMR ($CDCl_3$, 161.8 MHz) δ (ppm); 21.00. HRMS (ESI) m/z Calcd. for $C_{14}H_{19}N_2O_4PS$ 343.3578 $[M + H]^+$ found 343.3574.

Diethyl((4-hydroxy-3-methoxyphenyl)(thiazol-2-ylamino) methyl)phosphonate 4i

Yellow solid; Yield: 90%; Mp: 125-127 °C; IR (cm^{-1}): $\nu_{max.}$ 3436 (-OH), 3312 (-NH), 1269 (P = O), 1023 (P-O-C), 756 (P-C); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm): 7.42 (1H, d, J = 4.0 Hz, Ar-H), 6.79 (1 H, d, J = 8.0 Hz, Ar-H), 6.56 (1H, s, Ar-H), 6.85 (1H, d, J = 4.0 Hz, Ar-H), –NH and –OH is exchanged with D_2O, 4.75 (1H, d, J = 8.0 Hz, P-CH), 4.16-3.81 (4 H, m, -OC$\underline{H_2}$CH$_3$), 3.93 (3H, s, -OCH$_3$), 1.30 (3H, t, J^1 = 8.0 Hz & J^2 = 8.0 Hz, -OCH$_2$C$\underline{H_3}$), 1.18 (3H, t, J^1 = 8.0 Hz & J^2 = 8.0 Hz, -OCH$_2$C$\underline{H_3}$); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ (ppm): 158.23 (C-2), 148.21 (C-8), 144.34 (C-9), 140.90 (C-4), 131.83 (C-6), 130.09 (C-11), 122.47 (C-11), 116.23 (C-07), 113.42 (C-10), 68.03 (d, J = 37.51 Hz, C-15) 62.40 (d, J = 61.2 Hz, C-22), 57.36 (C-24), 16.98 (d, J = 25.2 Hz, C-21), 14.78 (C-23); ^{31}P-NMR ($CDCl_3$, 161.8 MHz) δ (ppm): 21.78; HRMS (ESI) m/z Calcd. for $C_{15}H_{21}N_2O_5PS$ 373.3838 $[M + H]^+$ 373.3830.

Diethyl (furan-2-yl(thiazol-2-ylamino)methyl)phosphonate 4j

Brick red solid; yield: 92%, Mp; 112–114 °C; FT-IR (cm $^{-1}$): $\nu_{max.}$ 3283 (NH), 1277 (P = O), 1013 (P-O-C), 749 (P-C). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm):7.63 (1H, d, J = 4.0 Hz Ar-H), 7.52 (1H, d, J = 8.0 Hz, Ar-H), 6.68 (1H, d, J = 12.0 Hz, Ar-H), 6.40 (1H, d, J = 8.0 Hz, Ar-H), 5.67 (1H, s, -NH), 5.10 (1H, d, J = 12.0 Hz, P-CH), 4.17-4.06 (4H, m, P-OC$\underline{H_2}$CH$_3$), 1.32-1.26 (6H, m, P-OCH$_2$C$\underline{H_3}$); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ (ppm): 165.00 (C-4), 150.01 (C-5), 145.10 (C-12), 138.59 (C-6), 110.74 (C-7), 109.00 (C-11), 108.00 (C-11) 66.59 (d, J = 9.0 Hz, C-11), 54.16 (C-17), 54.09 (C-17), 16.14 (d, J = 24.0 Hz, C-17), 13.20 (d, J = 24.0 Hz, C-14); ^{31}P-NMR ($CDCl_3$, 161.8 MHz) δ (ppm): 21.10. HRMS (ESI) m/z Calcd. for $C_{12}H_{17}N_2O_4PS$ $[M + H]^+$ 317.3198 found 317. 3190.

Biological activity

DPPH radical scavenging activity

The antioxidant activity of all the synthesised compounds was expressed as IC_{50} (inhibitory concentration, 50%). In the case of dialkyl((substituted phenyl)(thiazol-2ylamino) methyl) phosphonates (**4a–j**) DPPH radical scavenging activity was measured with respect to ascorbic acid (IC_{50} 42.82 µg/mL) as a control (Table 5). Out of **4a–j** compound **4h** showed the remarkable DPPH radical scavenging activity (IC_{50} of 28.06 µg/mL) when compared with that of standard. The other compounds exhibited DPPH radical scavenging activity in the following order **4i** (IC_{50} 29.48 ± 0.24 µg/mL) > **4e** (IC_{50} 30.22 ± .0.44 µg/mL) > **4b** (IC_{50} 32.06 ± 0.46 µg/mL) > **4a** (IC_{50} 34.26 ± 0.16 µg/mL) > **4c** (IC_{50} 48.32 ± 0.42 µg/mL) > **4g** (IC_{50} 54.26 ± 0.24 µg/mL) > **4j** (IC_{50} 55.32 ± 0.24 µg/mL) > **4f** (IC_{50} 61.96 ± 0.36 µg/mL) **4d** (IC_{50} 60.42 ± 0.22 µg/mL) (Fig. 2).

Table 5 The in vitro free radical scavenging activity of α-aminophosphonates (**4a–j**)

Entry	DPPH radical scavenging activity[a]	FRAP radical scavenging activity[a]
4a	34.26 ± 1.16	30.16 ± 1.38
4b	32.06 ± 1.46	36.12 ± 1.20
4c	48.32 ± 1.42	52.62 ± 1.42
4d	60.42 ± 1.22	54.02 ± 1.20
4e	30.22 ± 1.44	32.02 ± 1.64
4f	61.96 ± 1.36	53.46 ± 1.06
4g	54.26 ± 1.21	48.84 ± 1.36
4h	28.06 ± 1.28	29.86 ± 1.66
4i	29.48 ± 1.45	25.44 ± 1.14
4j	55.32 ± 1.56	69.12 ± 1.12
Ascorbic acid	42.82 ± 1.26	51.02 ± 1.66

[a]IC_{50} (µg/mL)

Ferric ion reducing antioxidant power assay

The reducing power assay of the all the titled compounds (**4a–j**) was measured in IC_{50} values and ascorbic acid was taken as a standard with its IC_{50} value as 51.02 µg/mL. Majority of the synthesised compounds showed higher anti-oxidant activity than the control (Table 5). The compound **4i** showed remarkable anti-oxidant activity with its IC_{50} value as 25.44 µg/mL. The remaining compounds displayed FRAP scavenging activity in the following order **4h** (IC_{50} 29.86 ± 0.66 µg/mL) > **4a** (IC_{50} 30.16 ± 0.38 µg/mL) > **4e** (IC_{50} 32.02 ± .0.64 µg/mL) > **4b** (IC_{50} 53.46 ± 0.20 µg/mL) > **4g** (IC_{50} 48.84 ± 0.36 µg/mL) > **4c** (IC_{50} 52.62 ± 0.42 µg/mL) > **4f** (IC_{50} 53.46 ± 0.20 µg/mL) **4d** (IC_{50} 54.02 ± 0.20 µg/mL) > **4j** (IC_{50} 69.12 ± 0.12 µg/mL) (Fig. 2).

Cytotoxic activity

Prior to the evaluation of anti-inflammatory effect of title compounds, cytotoxicity was determined using RAW 264.7 cell lines. The cytotoxicity (cell death) was not observed for the test compounds up to 200 µg/mL. The minimum lowest concentrations such as 25 µg/mL and 50 µg/mL were taken into consideration to test the anti-inflammatory effect against LPS-induced inflammatory cell death in RAW 264.7 cell lines respectively. The cytotoxicity of test compounds was shown in Fig. 3.

In order to determine the anti-inflammatory effect of synthesised compounds, MTT assays were performed in RAW 264.7 cell lines. Cells were treated with test

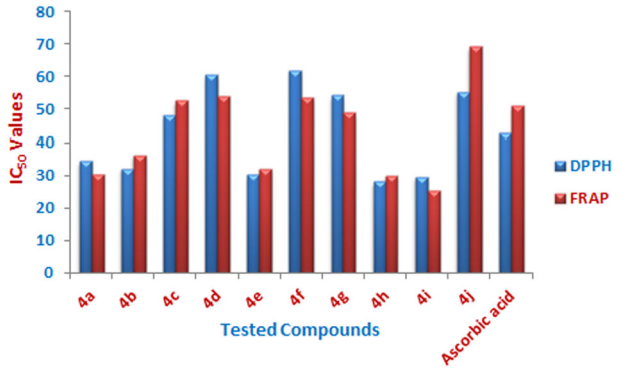


Fig. 2 Anti-oxidant activity of titled compounds

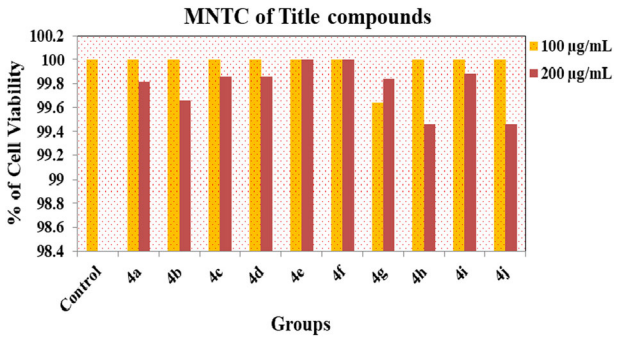


Fig. 3 Cytotoxic effect of title compounds during the treatment with different concentration in RAW 264.7 macrophage cell lines MTT Assay

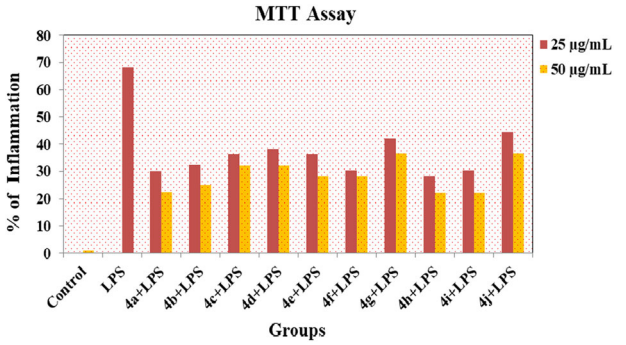


Fig. 4 Anti-inflammatory effects of title compounds with different concentrations during the pre-treatment in LPS-induced inflammation in RAW 264.7 macrophage cell lines

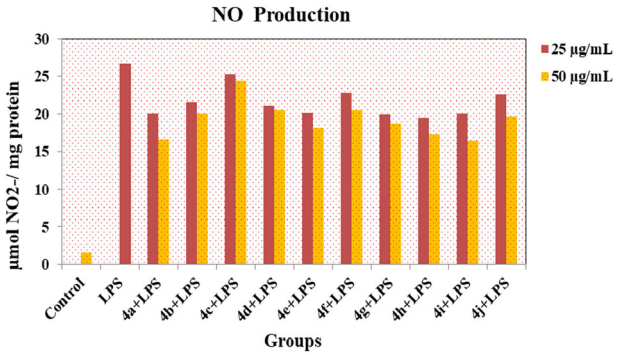


Fig. 5 Inhibition of NO production by title compounds with different concentrations in LPS-stimulated RAW 264.7 macrophages

compounds with different concentrations (25 µg/mL and 50 µg/mL) followed by LPS (10 µg/mL). As demonstrated in Fig. 4, all the test compounds exhibited strong anti-inflammatory activity against LPS-stimulated inflammation in RAW 264.7 cell lines. Higher than 50% of inflammation was observed during the treatment with LPG at 10 µg/mL. Whereas significant reduction in cell death was observed during the pre-treatment with tested compounds. The percentage of inflammation was reduced on dose dependent manner during the treatment with synthesised compounds. Among all the title compounds, **4a**, **4b**, **4e**, **4h** and **4i** have shown strong anti-inflammatory activity against LPS-induced cell death in RAW 264.7 cell lines.

NO production

In order to provide strength of the synthesised compounds as to be anti-inflammatory agents the effect of synthesised compounds on NO production stimulated by LPS was also investigated using RAW 264.7 cell lines. The addition of LPS to RAW 264.7 cells resulted in an increase in NO production levels (Fig. 5). However, on pre-treatment with synthesised compounds significantly suppress LPS-induced NO production in a concentration-dependent manner. Concomitant to the previous report, out of all title compounds **4a**, **4b**, **4e**, **4h** and **4i** have shown strong inhibition against LPS-induced NO production in RAW 264.7 cell lines. Significantly reduced levels of NO production were observed in all compounds during pre-treatment.

In Silico studies

Molecular docking

The structure of 3LN1 (COX-2) and Celicoxib (CEL) were taken from Protein Data Bank. The molecules were loaded into MOE working environment followed by protonation and energy minimisation using MMFF94x force field at a cut off value of 0.05. Chem sketch Module has been used for structure drawing of the title compounds; energy minimisation was done with universal force field (UFF) and converted into pdb format as required for docking. Alpha triangle placement methodology was used for effective docking of compounds and the poses are generated by superposition of triplets of receptor site points and ligand atom triplets. For each compound, ten conformations were

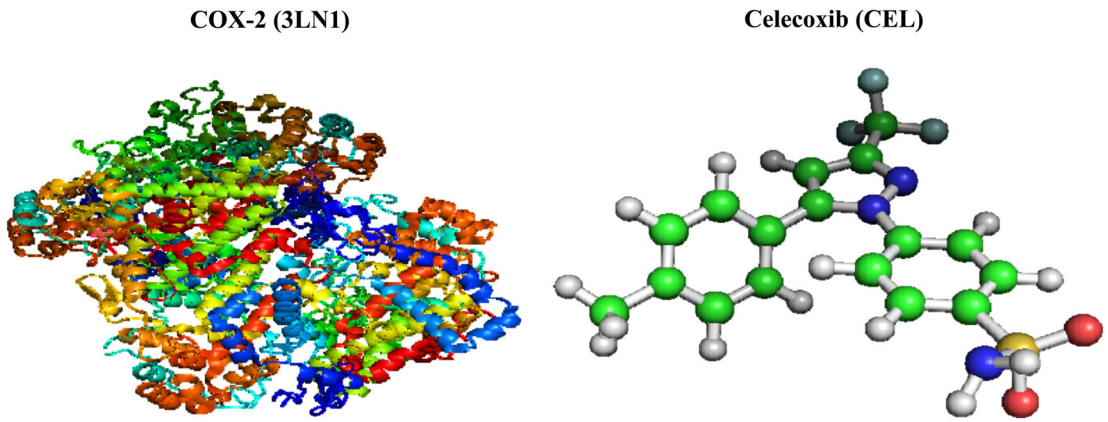


Fig. 6 Structures of COX-2 and Celecoxib

generated and they were refined and rescored. Interactions of the ligands with target protein were analysed using PyMol Visualizer (Vilar et al. 2008). The structures of 3LN1 and CEL were mentioned in Fig. 6.

All the ten title compounds were docked into the active site of the enzyme COX-2 (PDB ID: 3LN1) which formed high binding energies than the reference compound celecoxib except **4d** (Table 6). Compounds **4a**, **4e**, **4h**, **4i** and **4g** have shown highest binding energies with COX-2 enzyme than the rest of the title compounds. All the title compounds were actively fitted in the active site of the target gene, COX-2. Asp, Gly, Glu and Lys were the key residues of COX-2, which are involved in bond formation during the docking with title compounds. Out of all the title compounds, compound **4f** have formed hydrophobic interaction with COX-2. The protein-ligand interactions of best lead titled compounds were shown in Fig. 7.

Structure activity relation (SAR studies)

Most of the compounds such as **4a**, **4b**, **4e**, **4h** and **4i** showed better free radical scavenging activity than the control in both **DPPH** and **FRAP** assays. Out of all the titled compounds **4i** and **4j** showed remarkable anti-oxidant activity. Their high-antioxidant activity may be attributed, to the presence of hydroxy and methoxy groups present in the aromatic ring.

Further in **MTT** assay, out of all the titled compounds **4a**, **4h** and **4i** (25 µg/mL & 50 µg/mL) showed that remarkable decrease in percentage of anti-inflammation in RAW 264.7 macrophage cell lines with respect to LPS as a standard. Again we carried out nitric oxide production in cell lines same results were repeated in the compounds **4a**, **4h** and **4i**. This may be, due to the presence of nitro, methoxy and hydroxy groups in the aromatic ring.

Exploration of structure–bioactivity relationship of the titled compounds (**4a–j**) reveals that even though the basic core moiety of α-aminophosphonate remains same in all compounds, different substituent's present in the phenyl ring and at the α-carbon excert significant effect on their biological and physical properties. The binding models signifying that the titled compounds are held in the active site by a combination of various hydrogen bonding interactions. When compare with standard celecoxib all the compounds exhibited good binding energies. Out of all the compounds compound **4e** with three hydrogen bonds showed highest binding energy (−8.5 K cal/mol). In the compound **4e**, hydrogen bonding interactions are present between –NH, and -NO_2 groups with Glu 332, Phe 566 and Lys 293 amino acid residues with bond distance 2.5, 2.0 and 2.1 respectively. Whereas the other active compounds such as **4a**, **4b**, **4c**, **4d**, **4f**, **4g**, **4h** and **4j** showed better interactions than that of the reference with amino acids *viz* Asp 333, Lys 253, Asn 567, Gly 310, Gln 313, Asn 333, Phe 556 through H-bonding with bond lengths of 2.1, 2.2, 2.3, 2.5, 2.7, 2.8 and 2.9 by using –NH, -P = O, -OH and –OCH_3 as the active sites present on aminophosphonates. The nitro substituted compounds **4a** & **4e** at the para position of the phenyl ring showed strong binding energies between these two compound **4e** exhibited higher binding energy due to the presence of ethyl group on phosphorous.

Conclusion

In conclusion, we have accomplish synthesis of dialkyl ((substituted phenyl)(thiazol-2ylamino) methyl)phosphonates (**4a–j**) by using one-pot three-component Kabachniks–Fields reaction by reacting 2-aminothiazole, different aldehydes and dialkylphosphate in presence of environmentally benign and reusable catalyst called caffeine hydrogen sulfate (CHS) as a low expensive green catalyst in solvent-free conditions under microwave irradiation. The compounds such as **4a**, **4e**, **4h** and **4i** have been established as potent anti-oxidant and anti-

Table 6 Bonding characterisation of synthesised compounds (**4a–j**) and Celecoxib, (Reference drug) against COX-2

Compound	Binding energy (K cal mol^{-1})	Binding interaction	Bond length (A°)	Bond angle (°)	Bond type
Celecoxib	−7.4	Arg 293 CA .....OS	2.5	108.1	H- acc
4a	−8.2	Asp 333 CA .....HN	2.2	87.7	H- don
		Asp 333 CA ....HN	2.5	103.2	H- don
		Lys 253 CB .....OP	2.2	113.7	H- acc
		Asn 567 CB ....OP	2.6	84.8	H- acc
4b	−7.9	Gly 310 CA .....OP	2.8	110.7	H- acc
		Gly 310 CA ....OP	2.0	98.4	H- acc
		Gln 313 CA .....OP	2.1	76.8	H- acc
4c	−7.7	Gly 310 CA ....OP	2.0	113.4	H- acc
		Gln 313 CZ .....OP	2.2	97.8	H- acc
4d	−7.3	Gln 256 CA ....OC	2.2	90.3	H- acc
		Lys 253 CA ....HN	2.8	121.7	H- don
4e	−8.5	Glu 332 CB ....HN	2.5	131.7	H- don
		Phe 566 CZ ....ON	2.0	101.8	H- acc
		Lys 293 CZ ....ON	2.1	108.3	H- acc
4f	−7.6	Asn 567 CB .....HN	2.8	127.3	H- don
4g	−7.8	Glu 332 CA ....HN	2.2	101.8	H- don
		Asn 333 CZ ....HN	2.7	91.8	H- don
4h	−7.9	Asn 567 CA ....HN	2.7	78.1	H- don
		Phe 556 CB .....OH	2.2	99.3	H- acc
		Glu 332 CA ....HN	2.5	121.7	H- don
		Gln 336 CA ....OP	2.9	108.4	H- acc
4i	−8.3	Glu 267 CA ....HN	2.8	178.1	H- don
		Glu 267 CA ....HN	2.7	100.3	H- don
		Lys 253 CA ....OP	2.2	121.8	H- acc
		Asn 567 CZ .....HN	2.5	136.6	H- don
		Gln 336 CA ....OC	2.5	84.7	H- acc
		Gln 336 CA ....OC	2.0	89.1	H- acc
		Tyr 341 CA ....HO	2.0	136.6	H- don
4j	−7.5	Asn 567 CA ....OC	2.7	141.6	H- acc
		Asn 567 CB ....OC	2.4	88.4	H- acc
		Asn 567 CZ ....OP	2.3	121.7	H- acc
		Glu 267 CA ....HN	2.3	91.7	H- don
		Glu 267 CA ....HN	2.1	130.3	H- don

inflammatory agents through in vitro studies. According to the molecular docking studies among all the synthesise compounds **4a**, **4e** and **4i** showed good binding energies **−8.2**, **−8.5** and **−8.3** respectively. On the basis of the findings, further investigations are being carried out to develop best anti-oxidant and anti-inflammatory compounds by fine tuning the structure of the compounds with respect to their bioactivity.

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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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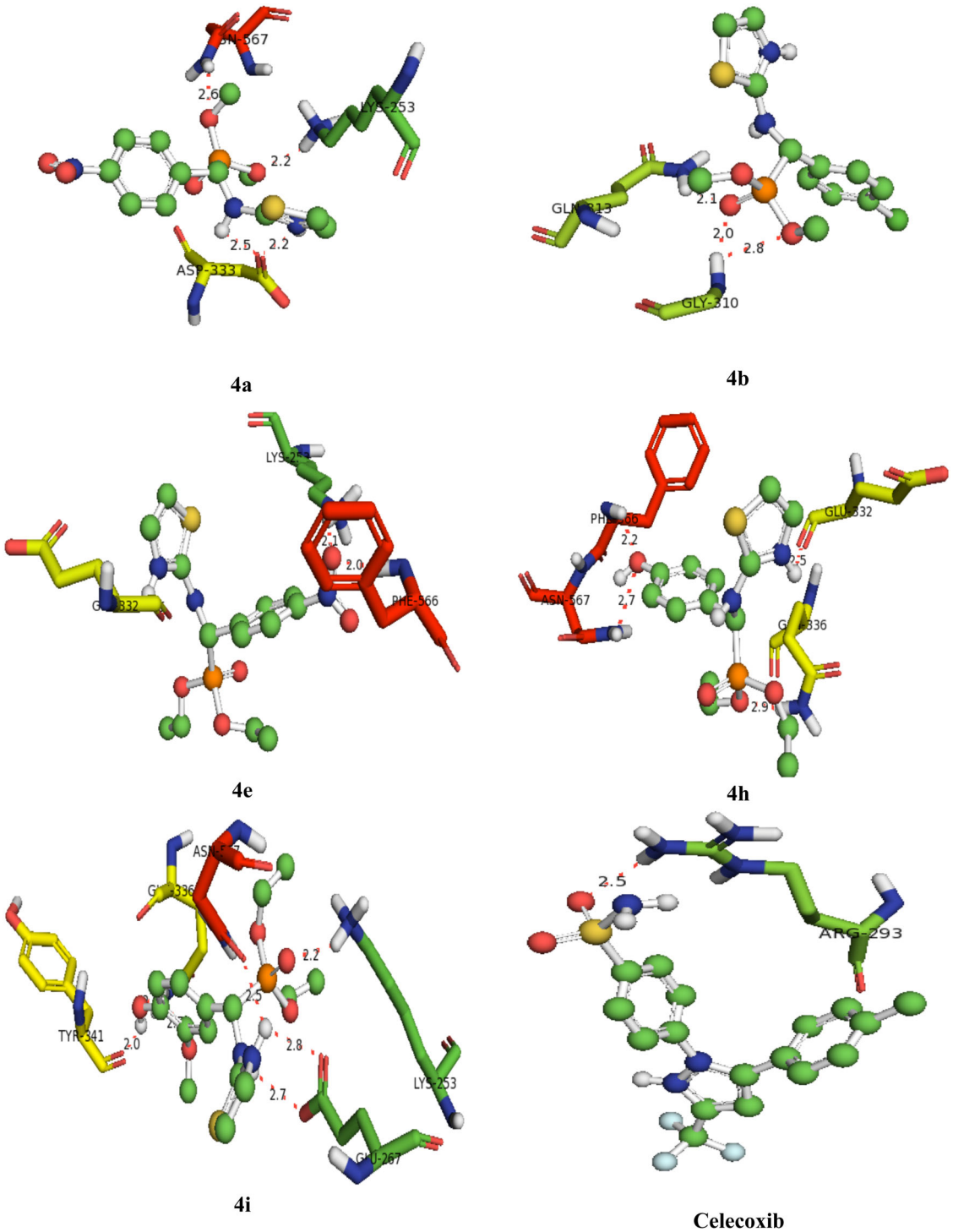

Fig. 7 Bonding interactions of the title lead compounds and standard with COX-2

References

Agarwal S, Kidwai M, Nath M (2019) A facile and green pathway for one pot multi component synthesis of functionalized spiroxy indoles using caffeinium hydrogen sulfate as a catalyst. Chem Sel 4:2135–2139

Ananda K, Kasumbwe K, Ramesh M, Gengan RM (2017) Catalytic synthesis of α-amino chromone phosphonates and their antimicrobial, toxicity and potential HIV-1 RT inhibitors based on silico screening. J Photochem Photobio B Biol 166:136–147

Antipin IS, Stoikov II, Konovalov AI (1999) α-Aminophosphonates: effective carriers for the membrane transport of bio relevant species. Phosphorus Sulfur Silicon 144:347–350

Anzini M, Chelini A, Mancini A, Cappelli A, Frosini M, Ricci L, Valoti M, Magistretti J, Castelli L, Giordani A, Makovec F, Vomero S (2010) Synthesis and biological evaluation of amidine,

guanidine, and thiourea derivatives of 2-amino-(6-trifluoromethoxy)benzothiazole as neuroprotective agents potentially useful in brain diseases. J Med Chem 53:734–744

Azizi N, Saidi MR (2003) Lithium perchlorate-catalyzed three-component coupling: a facile and general method for the synthesis of α-aminophosphonates under solvent-free conditions. Eur J Org Chem 23:4630–4633

Bakr F, Wahab A, Mohamed SF, Amr EEA, Abdalla MM (2008) Synthesis and reactions of thiosemicarbazides, triazoles, and Schiff bases as antihypertensive α-blocking agents. Mon Chem 139:1083–1090

Bhagat S, Chakraborti AK (2007) An extremely efficient three-component reaction of aldehydes/ketones, amines, and phosphites (Kabachnik-Fields Reaction) for the synthesis of α-aminophosphonates catalyzed by magnesium Perchlorate. J Org Chem 72:1263–1270

Bhattacharya AK, Raut DS, Rana KC, Polanki IK, Khan MS, Iram SE (2013) Diversity-oriented synthesis of α-aminophosphonates: a new class of potential anticancer agents. J Med Chem 66:146–152

Boughabaa S, Bouacidab S, Aoufa Z, Bechiric O, Aouf NE (2018) H6P2W18O62.14H2O Catalyzed synthesis, spectral characterization and X-ray study of α-aminophosphonates containing aminothiazole moiety. Cur Org Chem 22:1–7

Chea JY, Xu XY, Tang ZL, Gu YC, Shi DQ (2016) Synthesis and herbicidal activity evaluation of novel α-amino phosphonate derivatives containing a uracil moiety. Bioorg Med Chem Lett 26:1310–1313

Damiche R, Chafaa S (2017) Synthesis of new bioactive aminophosphonates and study of their antioxidant, anti-inflammatory and antibacterial activities as well the assessment of their toxicological activity. J Mol Struct 1130:1009–1017

Esalah J, Husein MM (2008) Removal of heavy metals from aqueous solutions by precipitation-filtration using novel organophosphorus ligands. Sep Sci Tec 43:3461–3475

Firouzabadi H, Iranpoor N, Sobhani S (2004) Metal Triflate-Catalyzed One-Pot synthesis of α-Aminophosphonates from carbonyl compounds in the absence of solvent. Synthesis 16:2692–2696

Forlani G, Berlicki L, Duo M, Dziedziola G, Giberti S, Bertazzini M, Kafarski P (2013) Synthesis and evaluation of effective inhibitors of plant δ 1-pyrroline-5-carboxylate reductase. J Agric Food Chem 61:6792–6798

Gundluru M, Sarva S, Reddy KMK, Netala VR, Vijaya T, Reddy CS (2016) Phosphosulfonic acid-catalyzed green synthesis and bioassay of α-aryl-α'-1,3,4-thiadiazolyl aminophosphonates. Heteroat Chem 27:269–278

Hirschmann R, Smith III AB, Taylor CM, Benkovic PA, Taylor SD, Yager KM, Sprengler PA, Benkovic S (1994) Peptide synthesis catalyzed by an antibody containing a binding site for variable amino acids. Science 265:234–237

Kafarski P, Lejczak B (1991) Biological activity of aminophosphonic acids. Phosphorus Sulfur Silicon 63:193–215

Kandula MKR, Sadik SM, Peddanna K, Reddy NB, Sravya G, Reddy CS (2018) Microwave assisted synthesis and anti-microbial activity of new diethyl ((dialkoxyphosphoryl) (2-hydroxyphenyl) methyl) phosphoramidates. Phosphorous Sulfur Silicon 193:329–334

Kudrjasova J, Herckens R, Penxten H, Adriaensens P, Lutsen L, Vanderzande D, Maes W (2014) Direct arylation as a versatile tool towards thiazolo-[5,4-d]thiazole-based semi conducting materials. Org Biomol Chem 12:4663–4672

Lajczak B, Kafarski P, Sztajer H, Mastalerz P (1986) Antibacterial activity of phosphono dipeptides related to alafosfalin. J Med Chem 29:2212–2217

Maier L (1990) Organic phosphorus compounds 91.1 synthesis and properties of 1-amino-2-arylethylphosphonic and -phosphinic acids as well as -phosphine oxides. Phosphorous Sulfur Silicon 53:43–67

Malamiri F, Khaksar S (2014) Pentafluorophenylammonium triflate (PFPAT): A new organo catalyst for the one-pot three-component synthesis of α-aminophosphonates. J Chem Sci 126:807–801

Matsubara N, Fuchimoto S, Iwagaki H, Nonaka Y, Kimura T, Kashino H, Edamatsu R, Hiramatsu M, Orita K (1991) The possible involvement of free radical scavenging properties in the action of cytokines. Res Commun Chem Pathol Pharm 71:239–242

Meyer JH, Barlett PA (1998) Macrocyclic inhibitors of penicillopepsin. 1. Design, synthesis, and evaluation of an inhibitor bridged between P1 and P3. J Am Chem Soc 120:4600–4609

Mulla SAR, Pathan MY, Chavan SS, Gample SP, Sarkar D (2014) Highly efficient one-pot multi-component synthesis of α-aminophosphonates and bis-α-aminophosphonates catalyzed by heterogeneous reusable silica supported dodeca tungsto phosphoric acid (DTP/SiO2) at ambient temperature and their anti-tubercular evaluation against Mycobactrium Tuberculosis. RSC Adv 4:7666–7672

Park JS, Park EM, kim DH, Jung K, Jung JS, Lee EJ, Hyun JW, Kang JL, Kim HS (2009) Anti- inflammatory mechanism of ginseng saponins in activated microglia. J Neuroimmunol 209:40–49

Pekarova M, Kralova J, Kubala L, Ciz M, Papezikova I, Macickova T, Pecivova J, Nosal R, Lojek A (2009) Carvedilol and adrenergic agonists suppress the lipopolysaccharide induced NO production in RAW 264.7 macrophages via the adrenergic receptors. J Physiol Pharm 60:143–150

Qian C, Huang T (1998) One-Pot synthesis of α-Aminophosphonates from aldehydes using lanthanide triflate as a catalyst. Org Chem 63:4125–4128

Ranu BC, Hajra A, Jana U (1999) General procedure for the synthesis of α-amino phosphonates from aldehydes and ketones using Indium (III) chloride as a catalyst. Org Lett 8:1141–1143

Raquel P, Bravo L, Saura Calixto F (2000) Antioxidant activity of dietary polyphenols As determined by a modified ferric reducing/antioxidant power assay. J Agric Food Chem 48: 3396–3402

Shalini A, Roona P, Mazaahir K, Mahendra N (2018) Caffeinium hydrogen sulfate: a green solid acid catalyst for selective One-Pot domino knoevenagel michael-transformations. Chem Sel 3:10909–10914

Sharma PC, Sinhmar A, Sharma A, Rajak H, Pathak DP (2013) Medicinal significance of benzothiazole scaffold: an insight view. J Enzym Inhib Med Chem 28:240–266

Smith III AB, Taylor CM, Benkovic SJ, Hirschmann R (1994) Peptide bond formation via catalytic antibodies: synthesis of a novel phosphonate diester hapten. Tetahedron Lett 35:6853–6856

Sreekanth T, Mohan G, Santhisudha S, Nadiveedhi MR, Murali. S, Rajasekhar A, Chippada AR, Reddy CS (2018) Meglumine sulfate-catalyzed one-pot green synthesis and antioxidant activity of α-aminophosphonates. Synth Commun 49:563–575

Stowasser B, Budt KH, Peyman A, Ruppert D (1992) New hybrid transition slate analog inhibitors of HIV protease with peripheric C_2-Symmetry. Tetahedron Lett 33:6625–6628

Tibhe GD, Lagunas-Rivera S, Vargas-Diaz E, Garcia-Barradas O, Ordonz M (2010) Un-catalyzed One-Pot diastereo selective synthesis of α-Aminophosphonates under solvent-Free conditions. Eur J Org Chem 14:6573–6581

Tzanopoulou S, Sagnou M, Petsotas MP, Gourni E, Loudos G, Xanthopoulos S, Lafkas D, Kiaris H, Varvarigou A, Pirmettis IC, Papadopoulos M, Pelecanou M (2010) Evaluation of Re and ^{99m}Tc Complexes of 2-(40-Aminophenyl)benzothiazole as potential breast cancer Radiopharmaceuticals. J Med Chem 53:4633–4641

Vilar S, Cozza G, Moro S (2008) Medicinal chemistry and the molecular operating environment (MOE): application of QSAR

and molecular docking to drug discovery. Curr Top Med Chem 8:1555–1572

Xie D, Zhang A, Liu D, Yin L, Wan J, Zeng S, Hu D (2017) Synthesis and antiviral activity of novel α-aminophosphonates containing 6-fluorobenzothiazole moiety. Phosphorous Sulphur Silicon 192:1061–1067

Xu F, Luo Y, Deng M, Shen Q (2003) One-Pot synthesis of α-amino phosphonates using samarium diiodide as a catalyst Precursor. Eur J Org Chem 24:4728–4730

Xu Y, Yan K, Song B, Xu G, Yang S, Xue W, Hu D, Lu P, Ouyang G, Jin L, Chen Z (2006) Synthesis and antiviral bioactivities of α-aminophosphonates containing alkoxy ethyl moieties. Molecules 11:666–676

Ugwu DI, Okoro UC, Ukoha PO, Gupta A, Okafor SN (2018) Novel anti-inflammatory and analgesic agents: synthesis, molecular docking and in vivo studies. J Enzyme Inhib Med Chem 33:405–415

Zhan ZP, Li JP (2005) Bismuth(III) chloride–catalyzed three-component coupling: synthesis of α-Amino phosphonates. Synth Commun 35:2501–2508

Zuo Y, Wu Q, Su SW, Niu CW, Xi Z, Yang GF (2016) Synthesis, Herbicidal activity and QSAR of novel N-Benzothiazolyl-pyrimidine-2,4-diones as protoporphyrinogen oxidase inhibitors. J Agric Food Chem 64:552–562