Organic & Biomolecular Chemistry

ROYAL SOCIETY OF CHEMISTRY

PAPER

View Article Online
View Journal | View Issue

Cite this: *Org. Biomol. Chem.*, 2021, **19**, 6883

PMDTA-catalyzed multicomponent synthesis and biological activity of 2-amino-4*H*-chromenes containing a phosphonate or phosphine oxide moiety†

Ádám Tajti, [a] Kármen Emőke Szabó,[a] Nóra Popovics-Tóth, [a] Javad Iskanderov,[a] Franc Perdih, [b] László Hackler, Jr.,[c] Beáta Kari, [c] László G. Puskás [c] and Erika Bálint *[a]

A new approach for the preparation of (2-amino-3-cyano-4*H*-chromen-4-yl)phosphonate derivatives is described. The multicomponent reaction of salicylaldehydes, malononitrile and dialkyl phosphites catalyzed by pentamethyldiethylenetriamine (PMDTA) provided the bicyclic derivatives in high yields. The method developed did not require chromatographic separation, since the products could be recovered from the reaction mixture by simple filtration. Our approach made also possible condensation with secondary phosphine oxides, and this reaction has not been previously reported in the literature. The crystal structures of five derivatives were studied by single-crystal XRD analysis. The *in vitro* cytotoxicity on different cell lines and the antibacterial activity of the (2-amino-4*H*-chromen-4-yl)phosphonates synthesized were also explored. According to the IC_{50} values determined, several derivatives showed moderate or promising activity against mouse fibroblast (NIH/3T3) and human promyelocytic leukemia (HL-60) cells. Furthermore, three (2-amino-3-cyano-4*H*-chromen-4-yl)phosphine oxides were active against selected Gram-positive bacteria.

Received 22nd June 2021,
Accepted 22nd July 2021

DOI: 10.1039/d1ob01204e

rsc.li/obc

Introduction

Phosphorus-substituted heterocycles represent an important group within organophosphorus compounds. They have gained an increasingly growing interest due to their importance in synthetic, medicinal and polymer chemistry.[1–3]

In the last few decades, (2-amino-4*H*-chromen-4-yl)phosphonates have received significant attention. These compounds are analogues of 2-amino-4*H*-chromenes, which have valuable applications as pharmaceutical agents,[4] and are widely employed as cosmetics, pigments and potential biodegradable agrochemicals.[5] A few (2-amino-4*H*-chromen-4-yl) phosphonates showed a moderate antioxidant effect[6] and a modest anticancer activity against human lung adenocarcinoma (A549) and human epidermoid cancer (KB) cell lines.[7] Therefore, the introduction of a phosphonate moiety on the 2-amino-4*H*-chromene ring may also have a synergistic effect on the various biological properties of this scaffold. Consequently, the development of green and efficient methods for the synthesis of (2-amino-4*H*-chromen-4-yl)phosphonates is of great importance.

Multicomponent reactions may represent an efficient method for the one-pot synthesis of complex ring systems.[8] Due to their simplicity, high atom efficiency and the ability to provide easy access to large compound libraries, these transformations have attracted significant attention in the field of organic chemistry.[9] (2-Amino-4*H*-chromen-4-yl)phosphonates may be prepared by the three-component reaction of salicylaldehyde derivatives, CH-acidic nitriles (*e.g.* malononitrile or ethyl cyanoacetate) and dialkyl- or trialkyl phosphites.[10] Most procedures utilizing the above synthesis route were carried out in the presence of both a catalyst and a solvent. Diethylamine,[11] dibutylamine,[7] triethylamine,[12] dimethylaminopyridine,[13] imidazole,[14] ethylenediamine diacetate,[15] lithium hydroxide,[16] potassium phosphate,[17] magnesium oxide[18] or indium chloride[19] were tried out in ethanol, and

[a]*Department of Organic Chemistry and Technology, Budapest University of Technology and Economics, Budafoki út 8, 1111 Budapest, Hungary. E-mail: balint.erika@vbk.bme.hu*
[b]*Faculty of Chemistry and Chemical Technology, University of Ljubljana, Večna pot 113, 1000 Ljubljana, Slovenia*
[c]*Avidin Ltd, Alsó kikötő sor 11/D, 6726 Szeged, Hungary*

†Electronic supplementary information (ESI) available: Full experimental procedures, characterization data and ^{31}P, ^{1}H and ^{13}C NMR spectra. CCDC 2083632, 2083633, 2083634, 2083635 and 2083636. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/d1ob01204e

iron oxide,[20] iodine[21] or β-cyclodextrin[22] in water. Water may be considered as a green solvent; however, it requires an additional extraction step during the reaction workup. In a few cases, special solvents, such as PEG,[23] ionic liquids[24,25] or a mixture of urea and choline chloride,[26] were used, which also served as a catalyst. Only four solvent-free variations can be found in the literature;[27–30] however, in these procedures, special catalysts were used, or a simple catalyst was required in a large excess. The condensation of salicylaldehydes, malononitrile and trialkyl phosphites was carried out in the presence of a silica-bonded 2-HEAA-3 catalyst at room temperature[27] or applying iodine at 50 °C.[28] In other cases, dialkyl phosphites were used as phosphorus reagents, and the reactions were carried out applying ZnO nanorods at 100 °C,[29] or in the presence of a large excess (3.5 equiv.) of tetramethylguanidine catalyst at 25 °C.[30]

Although many variations have been reported on the multicomponent synthesis of (2-amino-4*H*-chromen-4-yl)phosphonates, a suitable method using a simple basic catalyst under solvent-free conditions is still required. Furthermore, an extensive literature survey has revealed that there are no reports at all on the preparation of (2-amino-4*H*-chromen-4-yl)phosphine oxides.

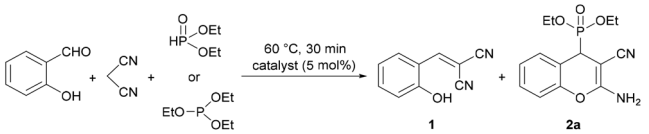
Based on the above considerations, in this paper, we report on a fast, cheap and efficient PMDTA-catalyzed solvent-free process for the synthesis of novel (2-amino-3-cyano-4*H*-chromen-4-yl)phosphonates by the domino Knoevenagel–phospha-Michael reaction of salicylaldehydes, malononitrile and dialkyl phosphites. Our approach made also possible condensation with secondary phosphine oxides, and this reaction has not been previously reported in the literature. The biological activity of the synthesized compounds was investigated in terms of antibacterial activity and *in vitro* cytotoxicity assays.

Results and discussion

Synthesis of (2-amino-3-cyano-4*H*-chromen-4-yl)phosphonates

To optimize the three-component reaction of salicylaldehyde, malononitrile and diethyl phosphite, at first, the effect of various basic catalysts was studied (Table 1). The reactions were monitored by HPLC, and the reaction mixtures were analyzed by HPLC-MS. The condensations were carried out in a molar ratio of 1 : 1 : 1 in the presence of 5 mol% of an organic base at 60 °C for 30 min in an oil bath. At first, organic bases containing one nitrogen atom were tested as catalysts (Table 1, entries 1–3). Using dipropylamine (DPA) in the absence of any solvent, the conversion was complete, and the reaction mixture contained 65% of 2-(2-hydroxybenzylidene)malononitrile (**1**) ($[M + H]^+$ = 171.1) as an intermediate formed by the Knoevenagel condensation of salicylaldehyde with malononitrile and 35% of diethyl (2-amino-3-cyano-4*H*-chromen-4-yl) phosphonate (**2a**) (Table 1, entry 1). Performing the three-component reaction in the presence of triethylamine (TEA) or dipropylethylamine (DIPEA), the ratio of intermediate **1** and product **2a** was 62 : 38 or 58 : 42, respectively (Table 1, entries 2 and 3). After that, the condensation was investigated using organic bases containing two nitrogen atoms, such as dimethylaminopridine (DMAP), 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), diazobicyclooctane (DABCO) or tetramethylethylenediamine (TMEDA) (Table 1, entries 4–7). In these reactions, the desired diethyl (2-amino-3-cyano-4*H*-chromen-4-yl) phosphonate (**2a**) was formed in 27%, 41%, 53% and 58% yield, respectively. Finally, the domino Knoevenagel–phospha-Michael reaction was carried out by the addition of pentamethyldiethylenetriamine (PMDTA) as a catalyst containing three nitrogen atoms, and the proportion of product **2a** was increased to 86% (Table 1, entry 8). As expected, the conden-

Table 1 Study of the three-component reaction of salicylaldehyde, malononitrile and diethyl or triethyl phosphite[a]



Entry	P-reagent	Catalyst [5 mol%]	Solvent	Product composition[b] [%] **1**	**2a**
1	DEP	DPA	—	65	35
2	DEP	TEA	—	62	38
3	DEP	DIPEA	—	58	42
4	DEP	DMAP	—	73	27
5	DEP	DBU	—	59	41
6	DEP	DABCO	—	47	53
7	DEP	TMEDA	—	42	58
8	DEP	PMDTA	—	14	86
9	DEP	PMDTA	EtOH	65	35
10	DEP	PMDTA	MeCN	70	30
11	TEP	PMDTA	—	100	0
12	TEP	PMDTA	H_2O	27	73

[a] The reaction was performed in the presence of salicylaldehyde (1.0 mmol), malononitrile (1.0 mmol), diethyl phosphite (1.0 mmol) and a basic catalyst (0.05 mmol). [b] Determined by HPLC analysis (256 nm).

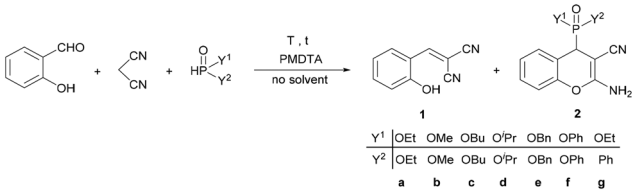
sation was significantly affected by the number of basic nitrogen atoms in the catalyst, which provided more active sites, and a few steric effects were also observed. Among the catalysts tested, PMDTA was found to be the most effective; therefore, further experiments were performed in the presence of this catalyst. To study the effect of solvent, a reaction was also performed in ethanol, which is a common solvent used in the synthesis of similar derivatives, and in acetonitrile (Table 1, entries 9 and 10). In both reactions, the proportion of the expected chromenylphosphonate (**2a**) decreased dramatically, and only 35% or 30% of product **2a** was present in the mixtures.

The presence of the solvent in the reaction carried out at 60 °C for 30 min reduced the reaction rate, thus, the solvent-free reaction allowed a higher proportion of product **2a** under the same conditions (Table 1, entries 8 *vs.* 9 and 10). Although in a few literature examples, salicylaldehyde and malononitrile were reacted with triethyl phosphite (TEP) instead of DEP as the P-reagent, according to our experience only 2-(2-hydroxybenzylidene)malononitrile (**1**) was formed, and no product **2a** in the reaction mixture was obtained at 60 °C for 30 min in the absence of any solvent (Table 1, entry 11). Therefore, under the conditions applied, the reactivities of DEP and TEP were significantly different. When the condensation was performed in water, 73% of chromenylphosphonate (**2a**) was present in the reaction mixture (Table 1, entry 12). This may be explained by the base-catalyzed hydrolysis of TEP to DEP in the presence of water, which then reacts in the condensation – a similar observation was previously described by our group in the case of Kabachnik–Fields reactions.[31]

The model reaction was further optimized with respect to the reaction time and temperature, as well as the catalyst amount; furthermore, the condensation was extended to various dialkyl phosphites and ethyl phenyl-*H*-phosphinate (Table 2).

First, the reaction time was gradually increased from 30 min to 60 min, and then, a higher temperature of 80 °C was also tried out; however, the ratio of product **2a** did not change significantly (Table 2, entries 1–4). By increasing the amount of catalyst to 10 mol% at 60 °C, a product ratio of 91% was reached after 15 min, which increased to 100% after 30 min (Table 2, entries 4 and 5). Diethyl (2-amino-3-cyano-4*H*-chromen-4-yl)phosphonate (**2a**) was isolated in a yield of 92% (Table 2, entry 6). When carrying out the domino Knoevenagel–phospha-Michael reaction with dimethyl phosphite, a reaction time of 15 min was enough for a complete conversion, and the corresponding chromenylphosphonate (**2b**) was obtained in a yield of 90% (Table 2, entry 7). Applying dibutyl- or diisopropyl phosphite as the P-reagent, a reaction temperature of 80 °C and a reaction time of 30 min were necessary to efficiently obtain products **2c** and **2d**, respectively (Table 2, entries 8–11). The condensation with dibenzyl- and diphenyl phosphite was similar to the reaction of diethyl phosphite, and at 60 °C for 30 min the proportions of the corres-

Table 2 Condensation of salicylaldehyde, malononitrile and various phosphorus reagents[a]



Entry	Y^1	Y^2	T [°C]	t [min]	PMDTA [mol%]	Product composition[b] [%]		Yield[c] [%]
						1	**2**	
1	EtO	EtO	60	30	5	14	86	—
2	EtO	EtO	60	45	5	11	89	—
3	EtO	EtO	60	60	5	8	92	—
4	EtO	EtO	80	30	5	8	92	—
5	EtO	EtO	60	15	10	9	91	—
6	EtO	EtO	60	30	10	0	100	92 (**2a**)
7	MeO	MeO	60	15	10	0	100	90 (**2b**)
8	BuO	BuO	60	30	10	21	79	—
9	BuO	BuO	80	15	10	16	84	—
10	BuO	BuO	80	30	10	1	99	92 (**2c**)
11	^{i}PrO	^{i}PrO	80	30	10	3	97	84 (**2d**)
12	BnO	BnO	60	30	10	3	97	85 (**2e**)
13	PhO	PhO	60	30	10	2	98	90 (**2f**)
14	EtO	Ph	60	15	10	3	97	86[d] (**2g**)

[a] The reaction was performed in the presence of salicylaldehyde (1.0 mmol), malononitrile (1.0 mmol), dialkyl phosphite (1.0 mmol) and PMDTA (0.05–0.1 mmol) at 60–80 °C for 15–60 min. [b] Determined by HPLC analysis (256 nm). [c] Isolated yield. [d] The product was formed as a 42 : 58 mixture of two diastereomers.

ponding (2-amino-3-cyano-4*H*-chromen-4-yl)phosphonates (**2e** and **2f**) were 97% and 98%, respectively. Finally, the three-component reaction was performed using ethyl phenyl *H*-phosphinate at 60 °C for 15 min, and the desired chromenylphosphinate (**2g**) was obtained in a ratio of 97%, and it was isolated in a yield of 86% (Table 2, entry 14). Due to the second chiral center on the phosphorus atom, compound **2g** was obtained as a mixture of two diastereomers in a ratio of 42 : 58 based on the ^{31}P NMR spectrum.

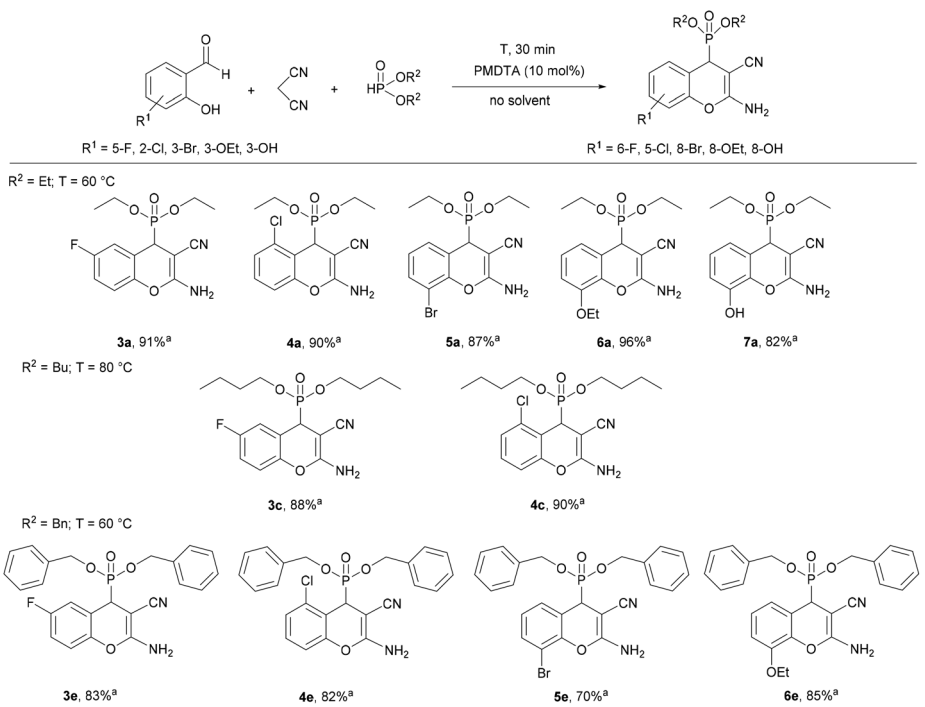
In the next part of our work, the three-component reaction of substituted salicylaldehydes, malononitrile and dialkyl phosphites was studied under the optimal conditions determined for each phosphite (Scheme 1). Using diethyl phosphite, the condensations were complete at 60 °C for 30 min, similar to the reaction of salicylaldehyde. Starting from 5-fluoro- or 2-chlorosalicylaldehyde, the desired products (**3a** or **4a**) were isolated in yields of 91% and 90%, respectively. 3-Bromosalicylaldehyde was found to be slightly less reactive at 60 °C for 30 min than salicylaldehydes bearing 5-F or 2-Cl substituents. Using 3-ethoxysalicylaldehyde, an excellent yield (96%) was obtained. According to our experiences, 3-hydroxy-substituted salicylaldehyde was less reactive as compared to other substituted salicylaldehydes, and product **7a** was isolated in a yield of 82%. After that, 5-fluoro- and 2-chloro-salicylaldehyde was reacted with malononitrile and dibutyl phosphite at 80 °C for 30 min, and the corresponding dibutyl chromenylphosphonates (**3c** and **4c**) were obtained in high yields (88% and 90%, respectively). Finally, the condensations were performed with dibenzyl phosphite at 60 °C for 30 min. As in the previous examples, starting from salicylaldehyde or 5-fluoro-, 2-chloro- or 3-ethoxysalicylaldehyde, better results were obtained (81–85% yield), while the derivative containing a bromine substituent at position eight (**6e**) was obtained in a yield of 70%. The method developed could be effectively applied for various substituted salicylaldehydes.

In contrast to the previous reports dealing with a similar topic, the multicomponent approach developed is a fast, cheap and simple solvent-free methodology for the synthesis of (2-amino-3-cyano-4*H*-chromen-4-yl)phosphonates. Besides a comprehensive optimization, we have provided exact product compositions, including the amount of the "Knoevenagel product" (**1**).

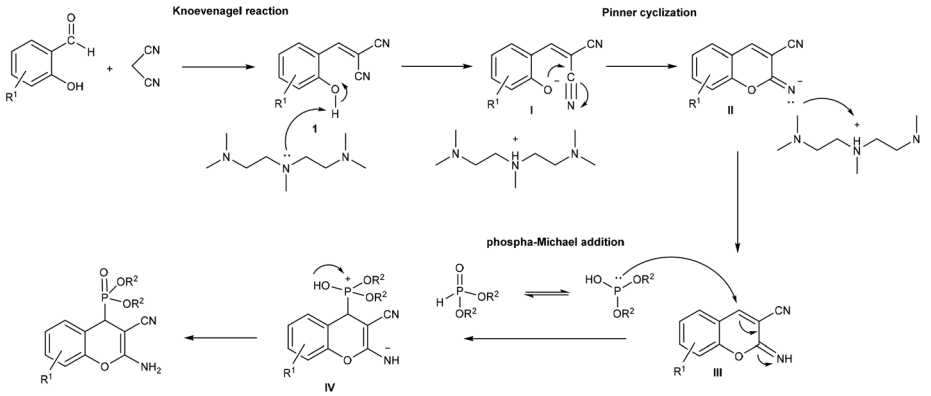
The formation of (2-amino-3-cyano-4*H*-chromen-4-yl)phosphonates can be explained by the proposed mechanism shown in Scheme 2. First, by the Knoevenagel condensation of salicylaldehyde and malononitrile in the presence of PMDTA, 2-(2-hydroxybenzylidene)malononitrile (**1**) is formed, and then, an intramolecular Pinner-like reaction (cyclization of the hydroxyl group of **1** on the cyano group) leads to iminocoumarine (**III**). Finally, the phospha-Michael addition of dialkyl phosphite, as the nucleophile, affords the phosphonium salt intermediate **IV**, which further transforms into the corresponding (2-amino-3-cyano-4*H*-chromen-4-yl)phosphonate.

Synthesis of (2-amino-3-cyano-4*H*-chromen-4-yl)phosphine oxides

As a novel extension, the PMDTA-catalyzed domino Knoevenagel–phospha-Michael reaction was investigated with secondary phosphine oxides, such as diphenyl-, di(*p*-tolyl)-, bis(3,5-dimethylphenyl)- and di(naphthalen-2-yl)phosphine



Scheme 1 Condensation of salicylaldehydes, malononitrile and dialkyl phosphites. [a]Isolated yield.



Scheme 2 Proposed mechanism for the PMDTA-catalyzed synthesis of (2-amino-3-cyano-4*H*-chromen-4-yl)phosphonates.

oxides. In these experiments, a small amount of acetonitrile as a solvent was used to overcome the heterogeneity. First, the condensation of salicylaldehyde, malononitrile and diphenylphosphine oxide was carried out in the presence of 5 mol% of PMDTA (Table 3). Stirring the reaction mixture at room temperature for 60 min, the conversion was 80%, and the mixture comprised 17% of the Knoevenagel intermediate (**1**) and 83% of the desired chromenylphosphine oxide (**8a**) (Table 3, entry 1). On increasing the reaction time to 180 min, the condensation was almost complete; however, the ratio of **1** and **8a** was 11 : 89, respectively (Table 3, entry 1). Performing the reaction at 60 °C for 10 min, a complete conversion was achieved, and the proportion of the diphenyl (2-amino-3-cyano-4*H*-chromen-4-yl)phosphine oxide (**8a**) was 92%. By increasing the reaction time to 15 min, only product **8a** was formed.

Next, the three-component reaction of various salicylaldehydes, malononitrile and secondary phosphine oxides was studied under the optimized conditions (5 mol% PMTDA, 60 °C, 15 min) (Scheme 3). It was found that all the target derivatives (**8a–12d**) could be efficiently prepared using the developed procedure. It should be noted that the purification did not require chromatographic separation, and the products could be recovered from the reaction mixture by simple filtration. Altogether 20 new (2-amino-3-cyano-4*H*-chromen-4-yl) phosphine oxides (**8a–12d**) were synthesized in high yields (82–97%). These derivatives can be considered as a new family among *O*-heterocyclic organophosphorus compounds.

The PMDTA-catalyzed multicomponent method developed is a fast, efficient and convenient methodology for the synthesis of (2-amino-3-cyano-4*H*-chromen-4-yl)phosphonates and a novel method for the preparation of (2-amino-3-cyano-4*H*-chromen-4-yl)phosphine oxides, which utilizes a cheap base catalyst, and in most cases, solvent-free conditions and short reaction time. The scope of the reaction was extended to 38 derivatives, of which 33 are new compounds.

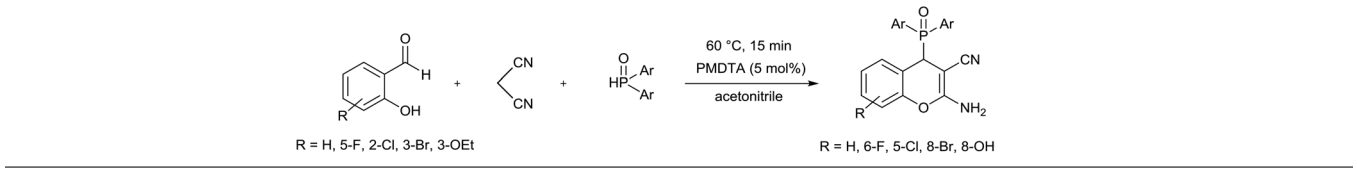
X-ray diffraction study

In addition to the spectroscopic analysis, we have determined the crystal structures of compounds **2c**, **6e** and **8a–c**. Single-crystal XRD analysis confirmed the molecular structures (Fig. 1 and Fig. S1†) and revealed the formation of intermolecular N–H···O=P and N–H···N hydrogen bonding leading to hydrogen-bonded chains or layers (Table S2†). Although the studied compounds possessed a similar chromenylphosphonate scaffold, their crystal structures showed a range of H-bonding interactions. The intermolecular N–H···O=P hydrogen

Table 3 PMDTA-catalyzed three-component reaction of salicylaldehyde, malononitrile and diphenylphosphine oxide[a]

Entry	T [°C]	t [min]	Conversion[b] [%]	Product composition[b] [%]	
				1	**8a**
1	25	60	80	17	83
2	25	180	96	11	89
3	60	10	100	8	92
4	60	15	100	0	100

[a] The reaction was performed in the presence of salicylaldehyde (1.0 mmol), malononitrile (1.0 mmol), diphenylphosphine oxide (1.0 mmol) and PMDTA (0.05 mmol) at 25–60 °C for 10–180 min. [b] Determined by HPLC analysis (256 nm).



Ar	Yield [%][a]
Ph	92 (**8a**)
4-Me-C_6H_4	95 (**8b**)
3,5-diMe-C_6H_3	89 (**8c**)
naphthalen-2-yl	85 (**8d**)

Ar	Yield [%][a]
Ph	88 (**9a**)
4-Me-C_6H_4	92 (**9b**)
3,5-diMe-C_6H_3	97 (**9c**)
naphthalen-2-yl	90 (**9d**)

Ar	Yield [%][a]
Ph	92 (**10a**)
4-Me-C_6H_4	90 (**10b**)
3,5-diMe-C_6H_3	94 (**10c**)
naphthalen-2-yl	89 (**10d**)

Ar	Yield [%][a]
Ph	86 (**11a**)
4-Me-C_6H_4	87 (**11b**)
3,5-diMe-C_6H_3	82 (**11c**)
naphthalen-2-yl	73 (**11d**)

Ar	Yield [%][a]
Ph	92 (**12a**)
4-Me-C_6H_4	90 (**12b**)
3,5-diMe-C_6H_3	88 (**12c**)
naphthalen-2-yl	82 (**12d**)

Scheme 3 Synthesis of (2-amino-3-cyano-4*H*-chromen-4-yl)phosphine oxides. [a]Isolated yield.

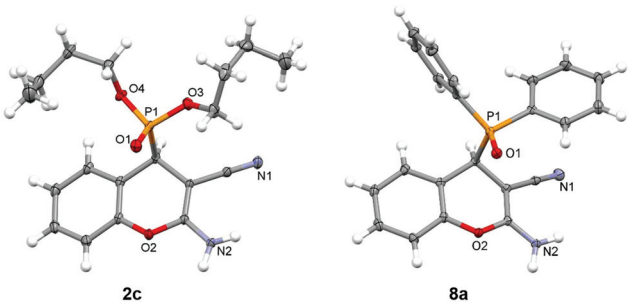


Fig. 1 Molecular structures of compounds **2c** and **8a**.

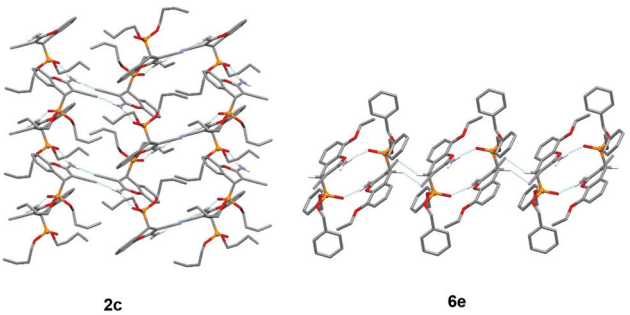


Fig. 2 Supramolecular structures of compounds **2c** and **6e**. Blue dashed lines indicate hydrogen bonds.

bonding between the amino group and the phosphonate oxygen atom was found to be a robust structural motif, since it was found in all structures under study. It was also observed that in structures **2c** and **14a–c**, the amino group as a hydrogen bond donor is involved in the formation of two interactions, while in **6e**, the amino group is involved only in one interaction.

In compound **2c**, the amino group is involved in the formation of the centrosymmetric N–H···N hydrogen bond with the cyano group and in the formation of the N–H···O=P interaction with two adjacent molecules, thus enabling the formation of hydrogen-bonded layers (Fig. 2). On the other hand, in compound **6e**, the amino group is involved only in the formation of centrosymmetric N–H···O=P interactions, and the hydrogen-bonded chain is formed through the centrosymmetric C–H···N interaction connecting the chromenyl scaffold with the cyano group of the adjacent molecule (Fig. 2).

In the structures of **8a**·CH_3CN, **8b** and **8c**, three different packing motifs were observed. In **8a**·CH_3CN and **8c**, the amino group is involved in a centrosymmetric N–H···N interaction with the cyano group of the adjacent molecule, and the hydrogen-bonded layer is formed through the N–H···O=P interaction between the amino group and the phosphonate oxygen atom of the adjacent molecule (Fig. 3). Although in **8a** and **8c**, similar hydrogen bonds are present, and layer structures are

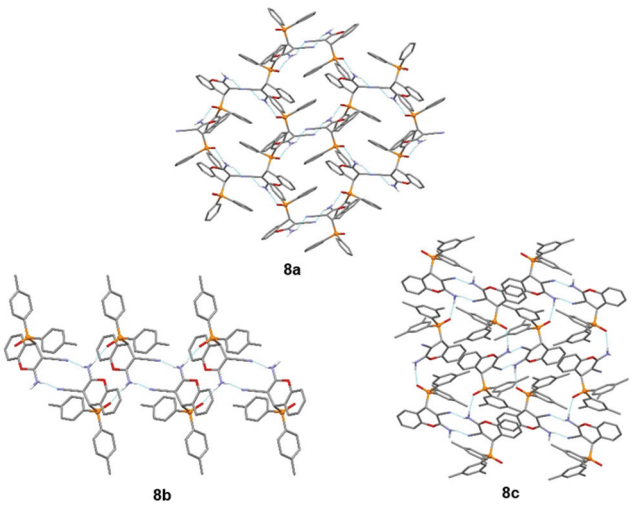


Fig. 3 Supramolecular structures of compounds **8a**, **8b** and **8c**. Blue dashed lines indicate hydrogen bonds.

formed, the supramolecular structure of **8a** possesses a honeycomb-like structure, which is not the case in **8c**. In **8b**, a hydrogen-bonded chain is formed through two centrosymmetric hydrogen bonds both formed by the amino group, namely, a

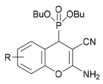
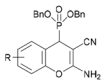
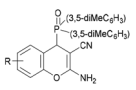
centrosymmetric N–H···N interaction with the cyano group and a centrosymmetric N–H···O═P interaction with the phosphonate oxygen atom of two adjacent molecules (Fig. 3).

Biological activity studies

The *in vitro* cytotoxicity and the antibacterial activity of the synthesized (2-amino-4*H*-chromen-4-yl)phosphonates and (2-amino-4*H*-chromen-4-yl)phosphine oxides were also investigated. The cytotoxicity evaluations were performed on three different cell lines, such as human lung adenocarcinoma (A549), mouse fibroblasts (NIH/3T3) as a healthy cell line and human promyelocytic leukemia (HL-60), using the fluorescent resazurin assay as described previously.[32] Positive controls were doxorubicin for A549 and NIH/3T3 (IC_{50} = 0.31 ± 0.24 μM and 5.65 ± 0.81 μM, respectively) and bortezomib for HL60 (IC_{50} = 7.42 ± 2.60 nM). The antibacterial activity of the compounds was tested on green fluorescent protein (GFP) producing *Bacillus subtilis* (Gram-positive) and *Escherichia coli* (Gram-negative) bacterial cells. The GFP producing bacteria are effective tools for screening the antibacterial activity, since the GFP signal measured by fluorimetry is proportional to the number of bacterial cells. Active compounds kill bacterial cells, which results in a decrease in the GFP fluorescence signal; therefore it is suitable for evaluating the antimicrobial effects of different agents. Positive controls were doxycycline and gentamicin for *Bacillus subtilis* (IC_{50} = 0.04 ± 0.01 μM and 0.49 ± 0.14 μM) and *Escherichia coli* (IC_{50} = 0.10 ± 0.02 μM and 4.23 ± 0.99 μM) bacterial cells. The IC_{50} values (50% inhibiting concentration) determined are shown in Table 4.

Among the chromenylphosphonates containing an unsubstituted backbone (**2a–c**, **2e** and **2f**), the dibenzyl (2-amino-3-cyano-4*H*-chromen-4-yl)phosphonate (**2e**) showed the highest activity. Although the anti-cancer effect of **2e** against A549 (IC_{50} = 26.46 ± 1.02 μM) and HL-60 (IC_{50} = 6.25 ± 1.06 μM) cell lines was lower than those of the reference drugs (IC_{50} = 0.31 ± 0.24 μM and 7.42 × 10^{-3} ± 2.60 × 10^{-3} μM, respectively), the activity against NIH/3T3 cells (IC_{50} = 8.73 ± 1.17 μM) was close to the value of doxorubicin (IC_{50} = 5.65 ± 0.81 μM). In addition, the diphenyl (2-amino-3-cyano-4*H*-chromen-4-yl)phosphonate (**2f**) showed a modest activity (IC_{50} = 28.18 ± 1.17 μM) against HL-60 cells. Next, the cytotoxic activities of 6-fluoro- or 5-chloro-substituted dibutyl (2-amino-3-cyano-4*H*-chromen-4-yl)phosphonates (**3c** and **4c**), as well as 6-fluoro-, 5-chloro-, 8-bromo- or 8-ethoxy-substituted dibenzyl (2-amino-3-cyano-4*H*-chromen-4-yl)phosphonates (**3e**, **4e**, **5e** and **6e**) were determined to investigate the effect of substituents on the 2-amino-4*H*-chromene backbone. The dibutyl (2-amino-3-cyano-6-fluoro-4*H*-chromen-4-yl)phosphonate (**3c**) was not active against the cell lines investigated, similar to the unsubstituted derivative (**2c**); however, the 5-chloro-substituted chromenylphosphonate butyl ester (**4c**) showed a moderate cytotoxicity (IC_{50} = 17.55 ± 1.70 μM) against HL-60 cells. The substituted dibenzyl chromenylphosphonates (**3e**, **4e**, **5e** and **6e**) were more active than the butyl esters (**3c** and **4c**). All substituted

Table 4 *In vitro* cytotoxicity and antibacterial activity of (2-amino-4*H*-chromen-4-yl)phosphonates and (2-amino-4*H*-chromen-4-yl)phosphine oxides[a]

		In vitro cytotoxicity [IC_{50}, μM]			Antibacterial activity (IC_{50}, μM)	
Compound	R	A549	NIH/3T3	HL-60	*B. subtilis*	*E. coli*
	Me (**2b**)	>30	>30	>30	>10	>10
	Et (**2a**)	>30	>30	>30	>10	>10
	Bu (**2c**)	>30	>30	>30	>10	>10
	Bn (**2e**)	26.46 ± 1.02	**8.73 ± 1.17**	**6.25 ± 1.06**	>10	>10
	Ph (**2f**)	>30	>30	28.18 ± 1.17	>10	>10
	6-F (**3c**)	>30	>30	>30	>10	>10
	5-Cl (**4c**)	>30	>30	17.55 ± 1.70	>10	>10
	6-F (**3e**)	>30	21.2 ± 1.71	**3.62 ± 1.38**	>10	>10
	5-Cl (**4e**)	>30	23.49 ± 1.09	**7.51 ± 1.02**	>10	>10
	8-Br (**5e**)	28.65 ± 1.22	**9.33 ± 1.18**	4.79 ± 1.08	>10	>10
	8-OEt (**6e**)	>30	27.99 ± 1.06	14.37 ± 1.24	>10	>10
	H (**8c**)	>30	>30	>30	8.92 ± 1.21	>10
	6-F (**9c**)	>30	>30	10.06 ± 1.25	**5.03 ± 1.28**	>10
	5-Cl (**10c**)	>30	>30	>30	**5.29 ± 1.38**	>10
	8-Br (**11c**)	>30	>30	9.8 ± 1.33	>10	>10
	8-OEt (**12c**)	>30	>30	>30	>10	>10
Doxorubicin		0.31 ± 0.24	5.65 ± 0.81	—	—	—
Bortezomib		—	—	7.42 × 10^{-3} ± 2.60 × 10^{-3}	—	—
Doxycycline		—	—	—	0.126 ± 0.029	0.10 ± 0.02
Gentamicin		—	—	—	0.115 ± 0.001	4.23 ± 0.99

[a] Data are expressed as mean ± standard deviation.

dibenzyl (2-amino-3-cyano-4*H*-chromen-4-yl)phosphonates (**3e**, **4e**, **5e** and **6e**) showed good or moderate activities against NIH/3T3 and HL-60 cells. Compounds containing a 6-fluoro (**3e**) or an 8-bromo (**5e**) substituent on the chromene ring were found to be the most active derivatives against the HL-60 cell line (IC_{50} = 3.62 ± 1.38 μM and 4.79 ± 1.08 μM, respectively).

Among the chromenylphosphine oxides, the 6-fluoro- or 5-chloro-substituted [bis(3,5-dimethylphenyl)](2-amino-3-cyano-4*H*-chromen-4-yl)phosphine oxides (**9c** and **10c**) showed a moderate activity against the HL-60 cell line (IC_{50} = 10.06 ± 1.12 μM and 9.8 ± 1.33 μM, respectively).

According to the results of the antibacterial activity studies, none of the synthesized chromenylphosphonates and chromenylphosphine oxides were active against selected *Escherichia coli* bacteria; however, the growth of *Bacillus subtilis* bacteria was reduced by the [bis(3,5-dimethylphenyl)](2-amino-3-cyano-4*H*-chromen-4-yl)phosphine oxides (**8c**, **9c** and **10c**). The IC_{50} values obtained were in the range of 5–9 μM.

The most active compounds were the dibenzyl (2-amino-3-cyano-4*H*-chromen-4-yl)phosphonates (**2e**, **3e**, **4e**, **5e** and **6e**), since they showed activity in the 8–9 μM range against NIH/3T3 cells and in the 3–7 μM range against HL-60 cells.

Conclusions

In summary, a facile and efficient PMDTA-catalyzed synthetic method was developed for the preparation of novel (2-amino-3-cyano-4*H*-chromen-4-yl)phosphonates by the domino Knoevenagel–phospha-Michael reaction. Our approach made also possible condensation with secondary phosphine oxides, and this reaction has not been reported previously in the literature. The model reaction of salicylaldehydes, malononitrile and dialkyl phosphites was optimized in detail, and then, the condensation was extended to various substituted salicylaldehydes and secondary phosphine oxides. The approach developed did not require chromatographic separation, since the products could be recovered from the reaction mixture by simple filtration. Altogether 18 (2-amino-3-cyano-4*H*-chromen-4-yl)phosphonate derivatives and 20 (2-amino-3-cyano-4*H*-chromen-4-yl)phosphine oxides were prepared in good to high yields, and fully characterized. Except five chromenylphosphonates (**2a–d** and **6a**), all of them are new compounds. The crystal structure of compounds **2c**, **6e** and **8a–c** was studied by single-crystal XRD analysis. The *in vitro* cytotoxicity and the antibacterial activity of the chromenylphosphonates and chromenylphosphine oxides synthesized were also investigated. Several chromenylphosphonates showed moderate and promising activities against the tested cell lines, especially the dibenzyl (2-amino-3-cyano-4*H*-chromen-4-yl)phosphonates, which had their IC_{50} values in the 8–9 micromolar range against NIH/3T3 cells, and in the 3–7 micromolar range against HL-60 cells. None of the prepared derivatives reduced the growth of Gram-negative bacteria; however, chromenylphosphine oxides containing 3,5-dimethylphenyl groups on the phosphorus atom were active against selected Gram-positive bacteria. Two of the latter derivatives also showed activity in the 10 micromolar range against HL-60 cells.

Author contributions

E. B. and Á. T. planned the experiments, Á. T., K. E. Sz., N. P.-T. and J. I. carried out the experiments, F. P. performed the crystal structure analysis, B. K. and L. H. J. performed the biological evaluation (screening), E. B. and L. G. P. contributed reagents/materials/analysis tools, E. B. and Á. T. wrote the paper, and L. H. J. and L. G. P. reviewed the paper. All the authors have read and agreed to the published version of the manuscript.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

The project was supported by the Hungarian Research Development and Innovation Office (FK123961), the bilateral Hungarian-Slovenian Science and Technology Cooperation project (2018-2.1.11-TÉT-SI-2018-00008) and the Slovenian Research Agency (P1-0230-0175). N. P.-T. was supported by the Servier-Beregi PhD Research Fellowship. E. B. was supported by the János Bolyai Research Scholarship of the Hungarian Academy of Sciences (BO/00278/17/7) and the ÚNKP-20-5-BME-288 New National Excellence Program of the Ministry of Human Capacities. F. P. thanks the EN-FIST Centre of Excellence, Slovenia, for the use of the Supernova diffractometer.

Notes and references

1 Phosphorus Heterocycles II, in *Topics in Heterocyclic Chemistry*, ed. R. K. Bansal, Springer, Berlin, Germany, 2010, vol. 21.
2 T. E. Ali and S. M. Abdel-Kariem, *ARKIVOC*, 2015, **2015**, 246.
3 L. Chen, X.-Y. Liu and Y.-X. Zou, *Adv. Synth. Catal.*, 2020, **362**, 1724.
4 N. Thomas and S. M. Zachariah, *Asian J. Pharm. Clin. Res.*, 2013, **6**, 11.
5 A. Pragi, A. Varun, H. S. Lamba and W. Deepak, *Int. J. Pharma Sci. Res.*, 2012, **3**, 2947.
6 M. Rajasekhar, K. U. M. Rao, C. S. Sundar, N. M. B. Reddy, S. K. Nayak and C. S. Reddy, *Chem. Pharm. Bull.*, 2012, **60**, 845.
7 R. M. N. Kalla, J.-S. Choi, J.-W. Yoo, S. J. Byeon, M. S. Heo and I. Kim, *Eur. J. Med. Chem.*, 2014, **76**, 61.
8 M. Haji, *Beilstein J. Org. Chem.*, 2016, **12**, 1269.
9 Multicomponent Reactions 1, in *Science of Synthesis*, ed. T. J. J. Müller, Thieme, Stuttgart, Germany, 2014.

10 S. U. Deshmukh, J. N. Sangshetti, S. V. Bhosale and R. P. Pawar, *ChemistrySelect*, 2021, **6**, 617.
11 M. Kulkarni, V. Pandurangi, U. Desai and P. Wadgaonkar, *C. R. Chim.*, 2012, **15**, 745.
12 P. Kour, A. Kumar, R. Sharma, R. Chib, I. Ali Khan and V. Rai, *Res. Chem. Intermed.*, 2017, **43**, 1.
13 P. Kour, A. Kumar and V. Rai, *C. R. Chim.*, 2017, **20**, 140.
14 F. Darvish, A. Abdollahzade and D. Saravani, *Res. Chem. Intermed.*, 2017, **43**, 1487.
15 S. Kolla and Y. Lee, *Tetrahedron*, 2012, **68**, 226.
16 P. Dai, G. Zha, X. Lai, W. Liu, Q. Gan and Y. Shen, *RSC Adv.*, 2014, **4**, 63420.
17 D. Gaikwad, K. Undale, T. Shaikh and D. Pore, *C. R. Chim.*, 2011, **14**, 865.
18 G. Brahmachari and S. Laskar, *Phosphorus, Sulfur Silicon Relat. Elem.*, 2014, **189**, 873.
19 P. Jayashree, G. Shanthi and P. Perumal, *Synlett*, 2009, 917–920.
20 R. Mohammadi and M. Kassaee, *J. Mol. Catal. A: Chem.*, 2013, **380**, 152.
21 M. Rajasekhar, R. K. Maheswara, C. Sundar, N. Reddy, S. Nayak and C. Reddy, *Chem. Pharm. Bull.*, 2015, **60**, 854.
22 S. Murthy, B. Madhav, V. Reddy and Y. Nageswar, *Tetrahedron Lett.*, 2010, **51**, 3649.
23 B. Das, P. Balasubramanyam, C. G. Reddy and N. Salvanna, *Helv. Chim. Acta*, 2011, **94**, 1347.
24 S. Sobhani and M. Honarmand, *J. Iran. Chem. Soc.*, 2012, **9**, 661.
25 Y. Wang, Y. Wu, Y. Wang and L. Dai, *Chin. J. Chem.*, 2012, **30**, 1709.
26 S. Krishnammagari, B. Cho and Y. Jeong, *Phosphorus, Sulfur Silicon Relat. Elem.*, 2018, **193**, 306.
27 S. Sobhani and M. Honarmand, *Catal. Lett.*, 2013, **143**, 476.
28 S. Sobhani and R. Jahanshahi, *Synth. Commun.*, 2013, **43**, 3247.
29 M. Hosseini-Sarvari and A. Roosta, *Comb. Chem. High Throughput Screening*, 2014, **17**, 47.
30 R. Kalla, S. Byeon, M. Heo and I. Kim, *Tetrahedron*, 2013, **69**, 10544.
31 G. Keglevich, E. Bálint, R. Kangyal, M. Bálint and M. Milen, *Heteroat. Chem.*, 2014, **25**, 282.
32 G. J. Szebeni, A. Balázs, I. Madarász, G. Pocz, F. Ayaydin, I. Kanizsai, R. Fajka-Boja, R. Alföldi, L. Hackler and L. G. Puskás, *Int. J. Mol. Sci.*, 2017, **18**, 2105.