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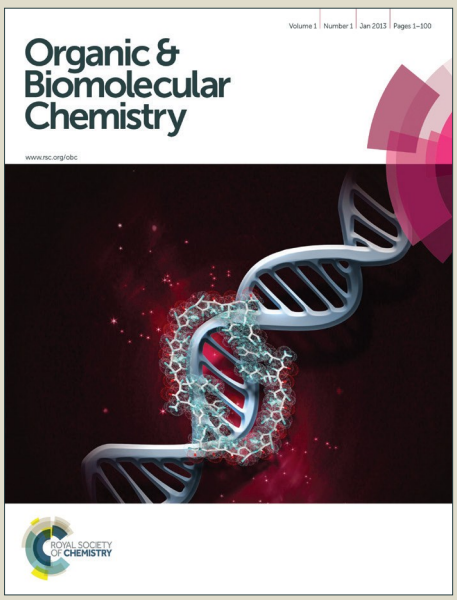

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Synthesis of 3,3'-Carbonyl-bis(chromones) and their Activity as Mammalian Alkaline Phosphatase Inhibitors

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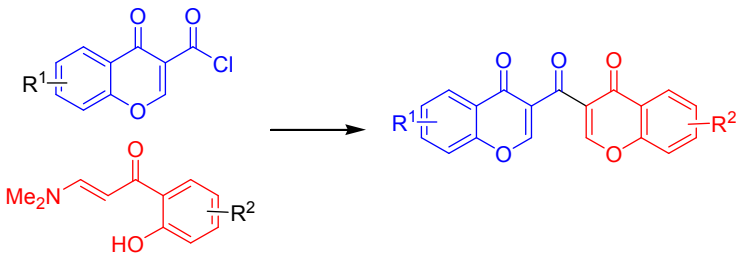
Abstract: Hitherto unknown 3,3'-carbonyl-bis(chromones) **8**, dimeric chromones bridged by a carbonyl group, were prepared by reaction of chromone-3-carboxylic acid chloride with 3-(dimethylamino)-1-(2-hydroxyphenyl)-2-propen-1-ones **9**. The method is generally applicable for the synthesis of novel symmetrical or non-symmetrical products which were found to inhibit mammalian alkaline phosphatases.



Introduction

Since the sixties of the past century various studies related to the dimerization of chromones have been carried out.[1-3] This interest stems from the structural similarity of bichromones and biflavones. Flavone analogues are widely abundant among the ingredients of pharmacologically valuable plants, such as *Aloe barbadensis*, *Stellera chamaejasme* L, *Agathis robusta* etc.[4-10] A number of syntheses of chromones, [30] 2, 2`- and 3, 3`- bis-chromones have been reported.[1, 3, 11, 12,32] In addition, the total synthesis of naturally occurring biflavones, such as chamaejasmine or 3,3'-biapigenin, has been studied in recent years.[4, 5, 13-15] Figure 1 shows a number of known bis-chromone derivatives **1**-**7** containing different types of linkages between the two heterocyclic moieties.[2, 16-21] For example, Cromolyn sodium **1** is a well-known drug used for the treatment of allergic bronchial astma.[20] Chromonyl trimers were also obtained in some studies.[21] We report the first synthesis of hitherto unknown symmetrical and non-symmetrical 3,3‘-carbonyl-bis(chromones), dimeric chromones bridged by a carbonyl group, which were shown to act as promising inhibitors of mammalian alkaline phosphatases.

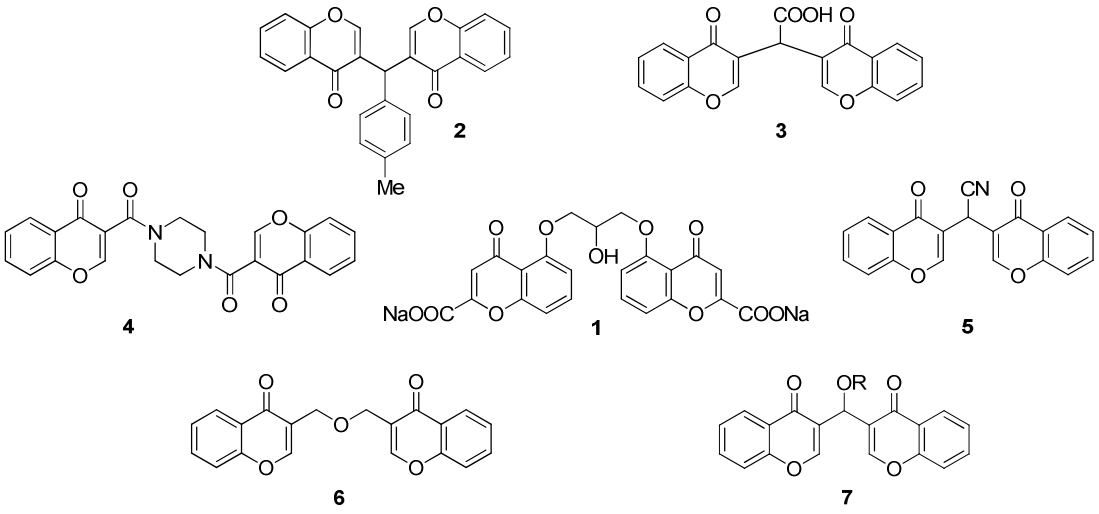


Figure 1. Some examples of known *bis*-chromones.[2, 16-21]

Results and Discussion

Synthesis. The synthesis of unstituted 3,3‘-carbonyl-bis(chromone) **8** was realized as follows. Commercially available o-hydroxyacetophenone was transformed to enaminone derivative **9** by

known procedure.[8,22] The Vilsmeier reaction of compound **9** with dimethylformamide and phosphorus oxychloride furnished, following a known procedure,[18] chromone-3-carbaldehyde which was oxidized to give chromone-3-carboxylic acid **10**.[23] Acid **10** was converted *in situ* to acid chloride **11**[19] and the subsequent addition of enaminone **9** directly afforded by a novel one-pot reaction the desired 3,3'-carbonyl-bis(chromone) **8a** (Path A, Figure 2). The formation of the product can be explained by Michael addition of the nucleophilic enamine to the carbonyl group of **11** and subsequent ring closure. The scope was studied and derivatives **8a-h** were isolated in good to very good yields (Table 1). However, the overall yields did not exceed 85% because of the formation of amides **12a-e** as side products. An attempt to synthesize the sterically hindered 3,3'-carbonyl-bis(chromone) **8i** was unsuccessful. In this reaction, by-product **12e** and chromone 4*H*-naphtho[1,2-b]pyran-4-one **13** were isolated in 19 and 63 % yields, respectively.

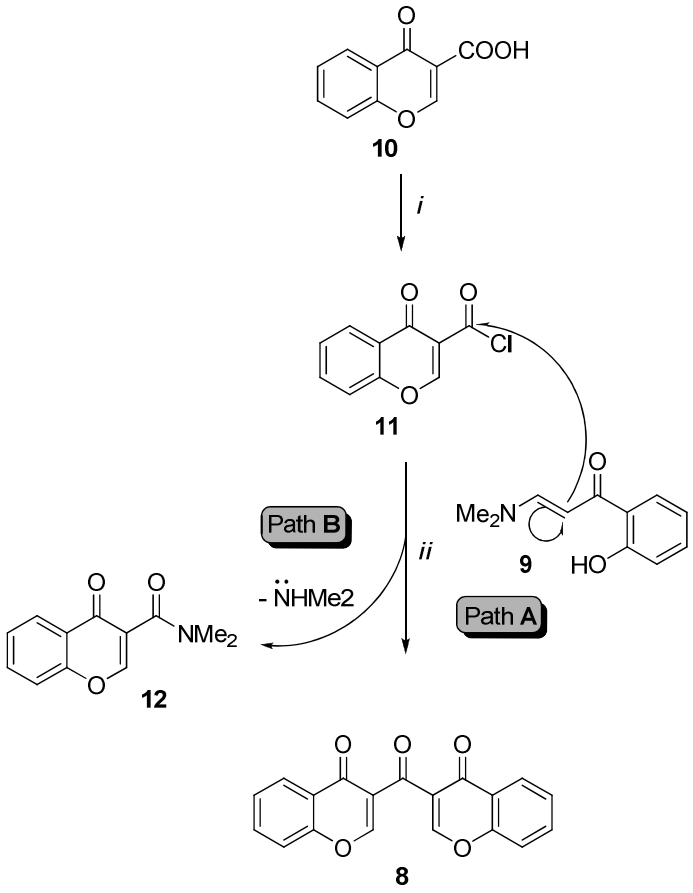
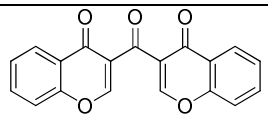
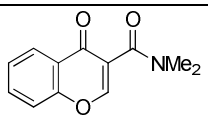
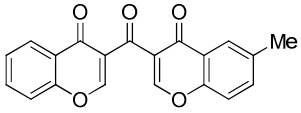
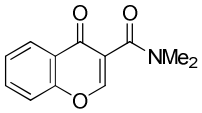
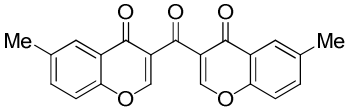
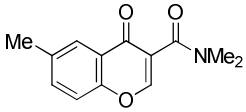
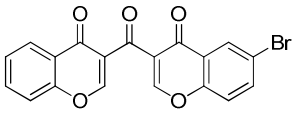
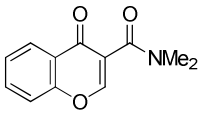
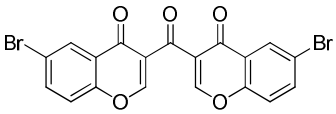
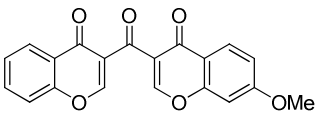
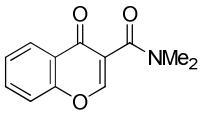
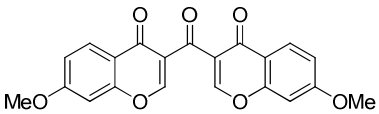
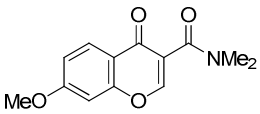
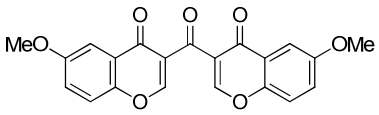
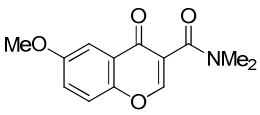
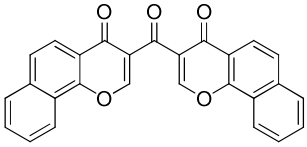
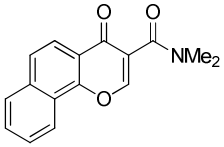
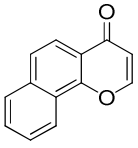


Figure 2. Possible mechanism of the formation of carbonyl dichromone **8a**. *Reagents and conditions:* (i) **10** (1 equiv.), oxalyl chloride (2 equiv.), DMF, DCM, 20 °C, 3.5 h;[19] (ii) **11** (1 equiv.), **9** (1 equiv.), pyridine (3 equiv.), DCM, 0 °C → reflux, 10 h.

Table 1. Target products **8** and by-products **12**.

3,3'-carbonyl-bis(chromone) **8**	Yield, %	By-product **12**	Yield, %
 **8a**	62	 **12a**	15
 **8b**	67	 **12a**	7
 **8c**	61	 **12b**	10
 **8d**	74	 **12a**	4
 **8e**	85	*A*	0
 **8f**	69	 **12a**	8
 **8g**	63	 **12c**	28
 **8h**	55	 **12d**	5
 **8i**	0	 **12e**, 19 %	 **13**, 63 %

[a] No by-product was isolated

The characteristic peak of the central quaternary carbon atom in the ^{13}C NMR spectrum of products **8** lies in the region between 185 and 189 ppm. This signal has a small intensity, due to the big relaxation time of the corresponding carbon center. Due to the low solubility of a number of derivatives of **8** the measurement of ^{13}C NMR spectra required a lot of time. In all cases a molecular ion was detected by mass spectrometry. Structures of 3,3'-carbonyl-bis(chromones) **8a** and **8h** were unambiguously confirmed by X-ray crystallography (Figures 3 and 4).

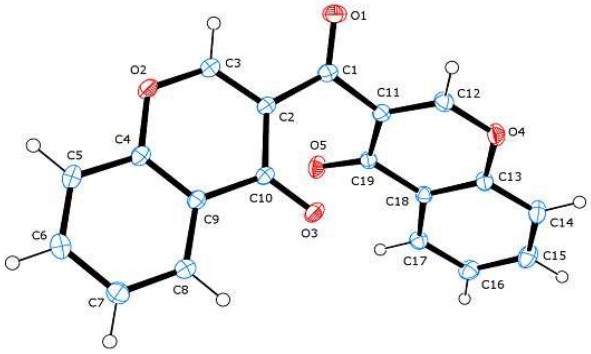


Figure 3. Crystal structure of **8a**

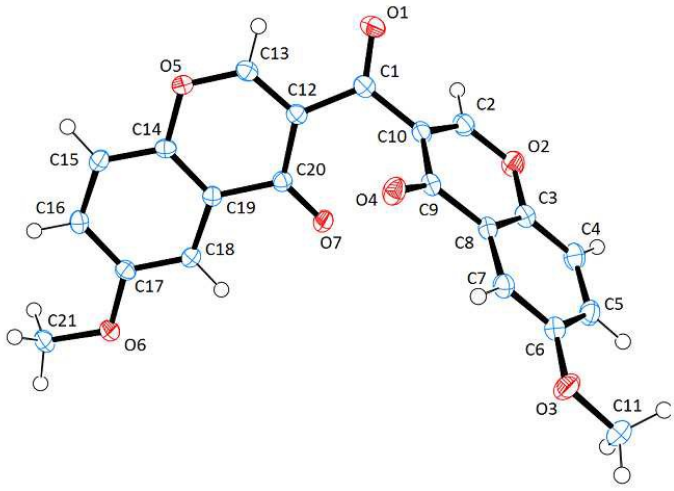


Figure 4. Crystal structure of **8h**

Alkaline Phosphatase Inhibition Studies and Structure-activity relationships. Alkaline phosphatases (APs; EC 3.1.3.1) is a class of homodimeric enzymes containing two Zn and one Mg ions at their catalytic site which play an important role in enzymatic activity.[24] The enzymes are found in many organisms from bacteria to man and are responsible for the hydrolysis of monoesters of phosphoric acid and transphosphorylation reactions. While comparing, the mammalian APs are membrane bound having higher Km values and specific activity. These mammalian APs also display lower heat stability and are inhibited by peptides and L-amino acids through an uncompetitive mechanism.[25] The APs isozyme family is divided into two sub-groups: the tissue-specific alkaline phosphatases and tissue-nonspecific alkaline phosphatases (TNAP). The tissue specific APs are represented at restricted areas such as placenta, intestine and germ cells whereas the tissue non-specific isozymes show predominance at bone, liver and kidney tissues. The hydrolysis of extracellular inorganic pyrophosphate (PPi), a potent inhibitor of calcification, to inorganic phosphate is the main physiological role of TNAP in the bone tissues.[26]

High concentration of tissue non-specific enzyme is responsible for the regulation of mineralization. An inverse concerted relationship of TNAP and PPi is necessary for normal bone formation.[27] On the other hand unwanted deposition of hydroxyapatite along with other forms of calcium phosphate in soft tissues results in overexpression of TNAP and hypercalcification in smooth muscle cells of kidney and vessels. It provokes a causative action for such severe disorders as end-stage renal disease, idiopathic infantile arterial calcification, ankylosis, osteoarthritis and diabetes.[28] Therefore potent inhibitors of TNAP could be useful therapeutic agents in the treatment of human atherosclerotic lesions. The tissue specific intestinal alkaline phosphatase (IAP) is located at the brush boarder of the intestinal epithelium and enriched in surfactant-like particles. These IAP play a pivotal role in intestinal absorption of the lipid/ nutrients across the cell membrane via its association with surfactant-like particles. The level of IAP increases after fatty meal. Thus, IAP has a central role in intestinal homeostasis and its activity could be increased through diet. This is especially true in pathological situations such as inflammatory bowel diseases in which the involvement of commensal bacteria is suspected and the level of IAP is too low to detoxify a sufficient amount of bacterial lipopolysaccharide.[29] Because of a high homology between tissue-specific IAP and TNAP, very few selective inhibitors of IAP have been reported.[30]

All the synthesized compounds were tested for their inhibitory potential against alkaline phosphatases i.e tissue non-specific alkaline phosphatase from bovine kidney (b-TNAP) and tissue specific alkaline phosphatase from calf intestine (c-IAP). The results obtained revealed that all of the investigated derivatives exhibited significant inhibition against both enzymes. However, some

of the test compounds were observed more selective on c-IAP. Compounds **8b**, **8c**, **8d** and **8e** were found equally potent on b-TNAP and c-IAP. The other compounds have shown a marked preference for c-IAP over b-TNAP. It was observed that compounds having more non-polar character showed less or even no inhibition against b-TNAP. When the number of carbon and bromine is increased the activity of the compound was decreased as it was reflected in inhibitory potencies of **8a**, **8b**, **8c**, **8d**, **8e**, **8f**, **8g** and **8h**.

Among all the investigated compounds, **8d** was found to be the most potent inhibitor of b-TNAP with the inhibitory value of IC_{50} = 2.47±0.003 μM. It showed 9 folds higher inhibitory potential than the reference compound Levamisole with IC_{50} value of 19.21±0.001 μM. But the compound **12e** was identified as selective bovine tissue non-specific alkaline phosphatase (b-TNAP) inhibitor (Table 2) with the inhibitory value of IC_{50} = 6.61±0.89 μM. It showed 3 folds higher potential than the reference compound. The detailed study of its structure justified that the activity of this compound might be due to the attachment of benzene and carboxamide directly to the chromene core ring. When the activity of this compound was compared with other derivatives containing two chromene rings, it was clearly observed that compound having one 4H-chromen-4-one ring are more active against b-TNAP than those containing two 4H-chromen-4-one rings.

The compounds **8a**, **8f**, **8g** and **8h** were found to be selective inhibitors of c-IAP. It is suggested that, compounds containing more carbon number showed higher response towards c–IAP and less active at b-TNAP. The comprehensive study of compound structures further justified that the attachment of methoxy group at the benzene ring increase the non-polar character of the compound. With the increase in non-polar character of compounds the selectivity index toward c-IAP increases. Compound **8a** with no methoxy substitution showed less selectivity against c-IAP as compared to the compound **8g** containing two methoxy groups. But when the activity of two compounds **8g** and **8h** was observed against c-IAP, it was seen that change in the substitution position affect the inhibitory potential. The substitution at 7-position (**8g**) exhibited more inhibitory potential as compared to the substitution at 6-position (**8h**). Compound **8g** was found to be the most potent inhibitor against c-IAP with inhibitory value of IC_{50} = 0.653±0.003 μM. It was more than 120 folds potent as compared to reference inhibitor L-Phenylalanine with IC_{50} value of 80.21±0.001μM.

Table 2. Bovine tissue non-specific alkaline phosphatase (b-TNAP) and calf intestinal alkaline phosphatase (c-IAP) inhibition data for the synthesized compounds.

Compound	Log P simulation*	b-TNAP	c-IAP
		IC_{50} [a](μM) ± SEM or (% inhibition)[b]	IC_{50} [a](μM)± SEM or (% inhibition)[b]
8a	3.01	33.1%[a]	23.6±1.34[b]

8b	3.44	26.5±2.45[b]	12.9±0.87[b]
8c	3.86	6.74±1.98[b]	9.31±0.02[b]
8d	3.79	2.47±0.003[b]	28.2±1.45[b]
8e	4.58	9.25±0.88[b]	23.3±1.54[b]
8f	3.04	38.1%[a]	2.93±0.33[b]
8g	3.08	37.1%[a]	0.653±0.003[b]
8h	3.08	48.2%[a]	6.34±0.33[b]
12e	1.87	6.61±0.89[b]	21.34%[a]
Levamisole	2.08	19.21±0.001[a]	
L-Phenyl alanine	-1.23	-------	80.21±0.001[a]

* Log P values were obtained by on-line calculator molinspiration.com: http://www.molinspiration.com/cgi-bin/properties. [a] The IC_{50} is the concentration at which 50% of the enzyme activity is inhibited. [b] The % inhibition of the enzyme activity caused by 0.2 mM of the tested compound.

Relationship of partition constant (Log P) value of compound with alkaline phosphatase activity

From the Log P values (Table 2), it was observed that biological activity of compounds not only depends on the Log P value but also on the substitution groups. The size and position of the substitution group at benzene ring also suggests the "true target interaction" of compound to the specific enzyme.

The compound **8a** has less log P value (Log P = 3.01) in comparison to the other bis-chromone derivatives and lipophilic in nature so it showed no response to b-TNAP. When the ring is mono or double derivatized with an methyl group i.e. electron donating group , the lipophilic character of the compound increases i.e for mono substituted compound **8b** it is 3.44 whereas when double derivatization its value increase to 3.86. The activity against c-IAP enhanced more in the double derivatization compound **8c.** In compound **8d**, there is the monosubstitution of bromine group. As it is electron withdrawing group but less electronegative as compared to other members of halogens, when it attached to the benzene ring it do not strongly deactivated ring but it might be the reason that due to the attachment of electron withdrawing group this compound exhibited more potency towards b-TNAP. But this was very interesting to see that when the compound was double derivatized **8e**, the effect was less. It might be the reason that as bromine is less electronegative and has less tendency to withdraw electrons from benzene ring, if attached on both rings it nullifies its effect and so the lipophilic behavior of the compound increase and it's also showed improvement of inhibition against c-IAP. This Change of properties could be caused not by electron effects but by big atomic size of Br.

In contrast, compounds substituted with methoxygroup (**8f**, **8g**, **8h**) appeared as stronger inhibitors of c-IAP. In compounds **8f** the ring is monosubstituted with methoxygroup. **8g** and **8h** the ring is di substituted with methoxygroup. The electron effect of Methoxy group is similar to the bromine. But to the resonance effect of the benzene when it attached to the benzene ring it pushes slightly electrons to the system. So there might be the reason that even with double derivatization of log P value decreases and thus an inverse relation was observed because of the substitution effect. This fact follows a conclusion about a significant inhibitive selectivity of a certain derivative **8** towards b-TNAP or c-IAP depending on its substitution mode.

Conclusions

In this work a convenient procedure for the synthesis of hitherto unknown 3,3'-carbonyl-bis(chromones) **8** with good yields was reported. These compounds were found to be inhibitors of mammalian Alkaline phosphatase.

Experimental session

General information

NMR spectra were recorded on Bruker Avance 250 (250 MHz), Brucker Avance 300 (300 MHz) and Brucker Avance 500 (500 MHz). Chemical shifts (ppm) are given relative to solvent: references for $CDCl_3$ were 7.26 ppm (^{1}H-NMR) and 77.16 ppm (^{13}C-NMR); references for DMSO-d_6 were 2.54 ppm (^{1}H-NMR) and 39.50 ppm (^{13}C-NMR). Multiplets were assigned as s (singlet), d (doublet), t (triplet), q (quartet), p (pentet), m (multiplet), br s (broad singlet). All measurements were carried out at room temperature unless otherwise stated. IR spectra were recorded on a Nicolet 6700 FT-IR spectrometer (ATR). A wavelength is given in cm^{-1}. Abbreviations: s = strong; m = middle; w = weak. Melting points were measured on Stanford Research Systems or Micro-Hot-Stage GalenTM III Cambridge Instruments. Abbreviation: Mp. Melting points are uncorrected. Mass spectra were obtained on a Hewlett-Packard HP GC / MS 5890 / 5972 instrument (EI, 70 eV) by GC inlet, on a MX-1321 and Finnigan MAT 95 XP instruments (EI, 70 eV) by direct inlet. The data are given as mass units per charge (*m/z*). Column chromatography was performed on silica gel (63 – 200 mesh, Merck). Chemical yields refer to pure isolated substances. Starting compounds enamino ketone, chromone-3-carbaldehyde and chromone-3-carboxylic acid were synthesized according to classic procedures. Column chromatography was performed on silica gel (63 – 200 mesh, Merck). Chemical yields refer to pure isolated substances.

Alkaline Phosphatase Inhibition Assay

A chemiluminescent substrate, CDP-star (disodium 2-chloro-5-(4-methoxyspiro[1,2-dioxetane-3,2′-(5-chlorotricyclo[3.3.1.13.7]decan])-4-yl]-1-phenyl phosphate), was used for the determination of alkaline phosphatase activity of a compound against bovine kidney alkaline phosphatase (TNAP) enzyme and calf intestine alkaline phosphatase (IAP). The conditions for the assay were optimized with the slight modifications in previously used spectrophotometric method.[31] The assay buffer which contained 8 M DEA (pH 9.8), 2.5 mM $MgCl_2$ and 0.05 mM $ZnCl_2$ was used. Initial screening was performed at a concentration of 0.2 mM of the tested compounds. The total volume of 50 µL contained 10 µL of tested compound (0.2 mM with final DMSO 1% (v/v)), followed by the addition of 20 µL of TNAP (1:800 times diluted (0.8 units/mL) enzyme in assay buffer) or 20 µL of IAP (1:800 times diluted (1 unit/mL) enzyme in assay buffer). The mixture was pre-incubated for 3-5 minutes at 37 ^{o}C and luminescence was measured as pre-read using microplate reader (BioTek FLx800, Instruments, Inc. USA). Then, 20 µL of CDP-star (final concentration of 110 µM) was added to initiate the reaction and the assay mixture was incubated again for 15 min at 37 ^{o}C. The change in the luminescence was measured as after-read. The activity of each compound was compared with total activity control (without any inhibitor). Levamisole (2 mM per well) and L-phenylalanine (4 mM per well) were used as a positive control against tissue-nonspecific alkaline phosphatase (TNAP) and calf intestinal alkaline phosphatase (IAP), respectively. For potentially active compounds, full concentration inhibition curves were produced. The compounds which exhibited over 50% inhibition of either the tissue-nonspecific alkaline phosphatase (TNAP) activity or calf intestinal alkaline phosphatase (IAP) activity were further evaluated for determination of inhibition constants (IC_{50} values). For this purpose 6 to 8 serial dilutions of each compound (200 µM to 20 nM) were prepared in assay buffer and their dose response curves were obtained by assaying each inhibitor concentration against both APs using the above mentioned reaction conditions. All experiments were repeated three times in triplicate. The Cheng Prusoff equation was used to calculate the IC_{50} values, determined by the non-linear curve fitting program PRISM 5.0 (GraphPad, San Diego, California, USA).

Principal synthesis of 8

An appropriate chromone-3-carboxylic acid **10** (1.5 g, 1 equiv.) was dissolved in 40 ml of the extra dry DCM in a pre-dried Shlenk flask under the flow of the inert gas. Thereafter the oxalyl chloride (1.4 ml, 2 equiv.) was added. The Shlenk flask was closed with a reflux condenser equipped with a bubble counter. 3 Drops of DMF were added to start the reaction. The reaction mixture was stirred 3.5 h at room temperature. After 3.5 h the solvent together with an excess of the oxalyl chloride were distilled under reduced pressure. Next, the appropriate 3-(dimethylamino)-1-(2-hydroxyphenyl)-2-propen-1-one **9** (1 equiv.) was added to the Shlenk flask under the flow of the inert gas. The reaction mixture was dissolved in a new portion of extra dry DCM (40 ml) and

situated on an ice bath. Then pyridine (3 equiv.) was added. After 1 h stirring at 0 ˚C the ice bath was removed. The reaction mixture was refluxed for the next 9 h. Thereafter it was concentrated and submitted to the column chromatography (For 3,3'-carbonyl-bis(chromones) **8**: $R_f \approx 0.8$, Heptane : Ethyl acetate, 15:1; for by-product **12**: $R_f \approx 0.15$, Heptane : Ethyl acetate, 1:5).

3,3'-Carbonylbis(4*H*-chromen-4-one) 8a.

White crystals, yield 62 %. Mp 112-113 °C. ^{1}H NMR (300.13 MHz, DMSO-d_6): δ= 7.55 (t, 2H, ^3J = 7.5, Ar), 7.76 (d, 2H, ^3J = 8.4, Ar), 7.89 (t, 2H, ^3J = 7.5, Ar), 8.04 (d, 2H, ^3J = 7.8), 8.82 (s, 2H, Ar). ^{13}C NMR (75.47 MHz, DMSO-d_6): δ = 118.66 (2 *C*H), 124.09, 125.10 (4 C), 125.52, 126.37, 134.98 (6 *C*H), 155.58 (2 C), 159.99 (2 *C*H), 173.87 (2 C), 187.20 (C). IR (ATR, cm^{-1}): $\tilde{\nu}$ = 1641 (m, C=O), 1613 (m, C=O), 1102 (m, C-O-C). MS (GC, 70eV): *m/z* (%) = 319 (M^{+1}, 20), 318 (M^+, 100), 290 (19), 289 (37), 262 (28), 261 (11), 205 (13), 173 (33), 142 (17), 121 (64), 120 (17), 92 (34), 89 (18), 76 (14), 64 (12), 63 (23), 53 (25). HRMS (ESI): calcd for $C_{19}H_{10}O_5$ 318.0523, found 318.0521.

6-Methyl-3-(4-oxo-4*H*-chromene-3-carbonyl)-4*H*-chromen-4-one 8b.

White crystals, yield 67 %. Mp 189-190 °C. ^{1}H NMR (300.13 MHz, $CDCl_3$): δ= 2.41 (s, 3H, C*H*$_3$), 7.36-7.44 (m, 2H, Ar), 7.47-7.52 (m, 2H, Ar), 7.69 (ddd, 1H, ^3J = 8.7, ^3J =7.2, ^4J =1.7, Ar), 7.95 (d, 1H, ^3J =1.4, Ar), 8.18 (dd, 1H, ^3J =8.0, ^4J =1.6, Ar), 8.39 (s, 1H, Ar), 8.40 (s, 1H, Ar). ^{13}C NMR (75.47 MHz, $CDCl_3$): δ = 21.05 (*C*H$_3$), 118.10, 118.33 (2 *C*H), 124.57, 124.94, 125.85 (3 C), 125.96, 126.09 (2 *C*H), 126.12 (C), 126.62 (*C*H), 134.36, 135.55 (2 *C*H), 136.26, 154.55, 156.25 (3 C), 158.89 (2 *C*H), 174.82, 174.94, 188.39 (3 C). IR (ATR, cm^{-1}): $\tilde{\nu}$ = 1644 (s, C=O), 1611 (s, C=O), 1562 (m, C=O), 1109 (m, C-O-C). MS (GC, 70eV): *m/z* (%) = 333 (M^{+1}, 21), 318 (M^+, 100), 304 (18), 303 (32), 276 (31), 275 (10), 232 (12), 187 (15), 173 (17), 135 (20), 134 (11), 1221 (26), 92 (12), 89 (10), 78 (10), 77 (18), 63 (12), 53 (18). HRMS (ESI): calcd for $C_{20}H_{12}O_5$ 332.0679, found 332.0678.

3,3'-Carbonylbis(6-methyl-4*H*-chromen-4-one) 8c.

White crystals, yield 61 %. Mp 224-225 °C. ^{1}H NMR (300.13 MHz, $CDCl_3$): δ= 2.41 (s, 6H, 2 C*H*$_3$), 7.39 (d, 2H, ^3J = 8.6, Ar), 7.49 (dd, 2H, ^3J = 8.6, ^4J = 1.7, Ar), 7.96 (d, 2H, ^4J = 1.7, Ar), 8.38 (s, 2H, Ar). ^{13}C NMR (62.90 MHz, DMSO-d_6): δ = 21.05 (2 *C*H$_3$), 118.07 (2 *C*H), 124.59, 125.90 (4 C), 126.00, 135.50 (4 *C*H), 136.18, 154.53 (4 C), 158.84 (2 *C*H), 174.94 (2 C), 188.50 (C). IR (ATR, cm^{-1}): $\tilde{\nu}$ = 1645 (s, C=O), 1616 (s, C=O), 1312 (m, C-O-C). MS (GC, 70eV): *m/z* (%) = 347 (M^{+1}, 22), 346 (M^+, 100), 345 (M^{-1}, 12), 318 (18), 317 (24), 303 (15), 290 (26), 246 (11), 187 (28), 135 (31), 134 (11), 78 (10), 77 (16), 53 (11). HRMS (ESI): calcd for $C_{21}H_{14}O_5$ 346.0836, found 346.0833.

6-Bromo-3-(4-oxo-4*H*-chromene-3-carbonyl)-4*H*-chromen-4-one 8d.

White crystals, yield 74 %. Mp 116-117 °C. ^{1}H NMR (300.13 MHz, DMSO-d_6): δ= 7.50-7.59 (m, 1H, Ar), 7.76 (d, 2H, ^3J = 9.0, Ar), 7.88 (ddd, 1H, ^3J = 8.7, 7.2, ^4J = 1.7, Ar), 8.03 (dd, 2H, ^3J = 9.0, ^4J = 2.4,

Ar), 8.09 (d, 1H, ^4J = 2.4, Ar), 8.84 (s, 2H, Ar). ^{13}C NMR (62.90 MHz, DMSO-d_6): δ = 118.68 (*C*H), 118.85 (C), 121.39 (*C*H), 124.07, 124.78, 125.16 (3 C), 125.52 (*C*H), 125.61 (C), 126.43, 127.57, 135.03, 137.57 (4 *C*H), 154.59, 155.56 (2 C), 160.15, 160.36 (2 *C*H), 172.77, 173.86, 186.81 (3 C). IR (ATR, cm^{-1}): $\widetilde{\nu}$ = 1649 (s, C=O), 1607 (m, C=O), 1558 (m, C=O), 1308 (m, C-O-C), 771 (m, C-Br). MS (GC, 70eV): *m/z* (%) = 399 (M^{+3}, 19), 398 (M^{+2}, 98), 397 (M^{+1}, 30), 396 (M^+, 100), 370 (18), 369 (29), 367 (25), 342 (25), 341 (11), 340 (25), 289 (15), 253 (13), 251 (13), 205 (14), 201 (17), 199 (20), 198 (12), 173 (43), 172 (11), 170 (14), 142 (12), 121 (46), 120 (18), 92 (27), 89 (14), 88 (13), 75 (15), 74 (11), 64 (11), 63 (33), 62 (12), 53 (32). HRMS (ESI): calcd for $C_{19}H_9O_5Br$ 395.9628, found 395.9633.

3,3'-Carbonylbis(6-bromo-4*H*-chromen-4-one) 8e.

White crystals, yield 85 %. Mp 274-275 °C. ^{1}H NMR (250.13 MHz, DMSO-d_6): δ= 7.78 (d, 2H, ^3J = 8.8, Ar), 8.01-8.13 (m, 4H, Ar), 8.87 (s, 2H, Ar). ^{13}C NMR was not characterized due to the low solubility of the sample. IR (ATR, cm^{-1}): $\widetilde{\nu}$ = 1641 (m, C=O), 1604 (m, C=O), 1285 (s, C-O-C), 600 (m, C-Br). MS (GC, 70eV): *m/z* (%) = 478 (M^{+4}, 45), 477 (M^{+3}, 24), 476 (M^{+2}, 100), 475 (M^{+1}, 22), 474 (M^+, 52), 450 (11), 449 (13), 448 (21), 447 (19), 446 (11), 445 (11), 422 (10), 420 (20), 418 (10), 376 (11), 369 (11), 367 (11), 253 (36), 251 (39), 222 (10), 201 (38), 200 (15), 199 (35), 198 (14), 172 (16), 170 (18), 88 (14), 75 (16), 74 (12, 63 (26), 62 (12), 53 (34). HRMS (ESI): calcd for $C_{19}H_8O_5Br_2$ 473.8733, found 473.8735; calcd for $C_{19}H_8O_5Br^{81}Br$ 475.8713, found 475.8717; calcd for $C_{19}H_8O_5{}^{81}Br_2$ 477.8692, found 477.8701.

7-Methoxy-3-(4-oxo-4*H*-chromene-3-carbonyl)-4*H*-chromen-4-one 8f.

White crystals, yield 69 %. Mp 187-188 °C. ^{1}H NMR (300.13 MHz, DMSO-d_6): δ= 3.93 (s, 3H, -OC*H*$_3$), 7.11 (dd, 1H, ^3J = 8.9, ^4J = 2.2, Ar), 7.26 (d, 1H, ^4J = 2.2, Ar), 7.55 (t, 1H, ^3J = 7.2, Ar), 7.75 (d, 1H, ^3J = 8.0, Ar), 7.87 (d, 1H, ^3J = 7.2, Ar), 7.92 (d, 1H, ^3J = 8.9, Ar), 8.04 (dd, 1H, ^3J =8.0, ^4J = 1.2, Ar), 8.75 (s, 1H, Ar), 8.79 (s, 1H, Ar). ^{13}C NMR (62.90 MHz, DMSO-d_6): δ = 56.29 (*C*H$_3$), 101.19, 115.37 (2 *C*H), 117.66 (C), 118.64 (*C*H), 124.10, 125.06, 125.28 (3 C), 125.53, 126.34, 126.95, 134.95 (4 *C*H), 155.58, 157.43 (2 C), 159.63, 159.75 (2 *C*H), 164.32, 173.10, 173.88, 187.39 (4 C). IR (ATR, cm^{-1}): $\widetilde{\nu}$ = 1644 (s, C=O), 1612 (s, C=O), 1556 (m, C=O), 1256 (m, C-OCH$_3$), 1102 (s, C-O-C). MS (GC, 70eV): *m/z* (%) = 349 (M^{+1}, 22), 348 (M^+, 100), 320 (15), 319 (33), 305 (10), 292 (28), 277 (12), 203 (12), 173 (16), 151 (27), 122 (10), 121 (24), 92 (15), 63 (16), 53 (16). HRMS (ESI): calcd for $C_{20}H_{12}O_6$ 348.0628, found 348.0622.

3,3'-carbonylbis(7-methoxy-4H-chromen-4-one) 8g.

White crystals, yield 63 %. Mp 221-222 °C. ^{1}H NMR (250.13 MHz, DMSO-d_6): δ= 3.92 (s, 6H, 2 C*H*$_3$), 7.11 (dd, 2H, ^3J =8.9, ^4J =2.3, Ar), 7.25 (d, 2H, ^4J =2.3, Ar), 7.92 (d, 2H, ^3J =8.9, Ar), 8.72 (s, 2H, Ar). ^{13}C NMR (62.90 MHz, DMSO-d_6): δ = 56.30 (2 *C*H$_3$), 101.18, 115.35 (4 *C*H), 117.67, 125.25 (4 C), 126.97

(2 *C*H), 157.44 (2 C), 159.41 (2 *C*H), 164.30, 173.11 (4 C), 187.58 (C). IR (ATR, cm^{-1}): $\tilde{\nu}$ = 1611 (s, C=O), 1437 (s, C-H), 1259 (m, C-OCH_3), 1204 (m, C-O-C), 827 (C-H). MS (GC, 70eV): *m/z* (%) = 379 (M^{+1}, 23), 378 (M^+, 100), 350 (16), 349 (33), 335 (16), 322 (25), 307 (27), 279 (10), 203 (24), 151 (41), 107 (10), 79 (10), 63 (12), 53 (10). HRMS (ESI): calcd for $C_{21}H_{14}O_7$ 378.0734, found 378.0736.

3,3'-carbonylbis(6-methoxy-4H-chromen-4-one) 8h.

White crystals, yield 55 %. Mp 221-222 °C. ^{1}H NMR (300.13 MHz, DMSO-d_6): δ= 3.84 (s, 6H, 2 O*CH*$_3$), 7.38 (d, 2H, ^3J = 2.9, Ar), 7.45 (dd, 2H, ^3J = 9.1, Ar, ^4J = 2.9, Ar), 7.71 (d, 2H, ^3J = 9.1, Ar), 8.77 (s, 2H, Ar). ^{13}C NMR (62.90 MHz, DMSO-d_6): δ = 55.82 (2 O*C*H_3), 105.44, 120.28, 123.78 (6 *C*H), 124.45, 124.90, 150.28, 157.11 (8 C), 159.60 (2 *C*H), 173.56 (2 C), 187.54 (C). IR (ATR, cm^{-1}): $\tilde{\nu}$ = 1637 (m, C=O), 1595 (m, C=O), 1481 (s, C-H), 1276 (s, C-OCH_3), 1114 (m, C-O-C), 889 (m, C-O-C-), 820 (s, C-H). MS (GC, 70eV): *m/z* (%) = 379 (M^+, 22), 378 (M^+, 100), 377 (13), 363 (12), 350 (13), 349 (32), 335 (20), 307 (18), 203 (33), 151 (21), 107 (11), 53 (10). HRMS (ESI): calcd for $C_{21}H_{14}O_7$ 378.0734, found 378.0730.

***N*,*N*-Dimethyl-4-oxo-4*H*-chromene-3-carboxamide 12a**

White crystals, yield 15 % as a by-product in the synthesis of **8a**, 7 % as a by-product in the synthesis of **8b**, 4 % as a by-product in the synthesis of **8d**, 8 % as a by-product in the synthesis of **8f**. Mp 195-196 °C.

^{1}H NMR (300.13 MHz, DMSO-d_6): δ= 2.89 (s, 3H, NC*H*$_3$), 2.97 (s, 3H, NC*H*$_3$), 7.53 (ddd, 1H, ^3J = 8.1, ^3J = 7.2, ^4J = 1.1, Ar), 7.69 (dd, 1H, ^3J = 8.5, ^4J = 0.6, Ar), 7.84 (ddd, 1H, ^3J = 8.7, ^3J = 7.2, ^4J = 1.7, Ar), 8.09 (dd, 1H, ^3J =8.0, ^4J = 1.4, Ar), 8.52 (s, 1H, Ar). ^{13}C NMR (75.47 MHz, DMSO-d_6): δ = 34.76, 37.81 (2 N*C*H_3), 118.58 (*C*H), 122.84, 123.68 (2 C), 125.24, 125.90, 134.62 (3 *C*H), 155.75 (C), 156.08 (*C*H), 163.52, 173.31 (2 C). IR (ATR, cm^{-1}): $\tilde{\nu}$ = 2925 (w, N-CH_3), 1615, 1601 (s, C=O), 1462 (s, CH_2-H), 1315 (C-N), 818, 758 (s, C-H, Ar). MS (GC, 70eV): *m/z* (%) = 217 (M^+, 30), 174 (16), 173 (54), 147 (12), 146 (65), 123 (72), 121 (100), 120 (17), 104 (17), 93 (11), 92 (12), 89 (19), 65 (11), 63 (19), 53 (30), 44 (37), 42 (15). HRMS (ESI): calcd for $C_{12}H_{11}NO_3$ 217.0748, found 217.0739.

***N*,*N*,6-Trimethyl-4-oxo-4*H*-chromene-3-carboxamide 12b**

White crystals, yield 10 % as a by-product in the synthesis of **8c**. Mp 118-119 °C. ^{1}H NMR (300.13 MHz, DMSO-d_6): δ= 2.43 (s, 3H, C*H*$_3$), 2.88 (s, 3H, NC*H*$_3$), 2.97 (s, 3H, NC*H*$_3$), 7.58 (d, 1H, ^3J = 8.6, Ar), 7.65 (dd, 1H, ^3J = 8.6, ^4J = 2.0, Ar), 7.87 (d, 1H, ^4J = 0.9, Ar), 8.48 (s, 1H, Ar). ^{13}C NMR (62.90 MHz, DMSO-d_6): δ = 20.40, 34.45, 37.78 (3 *C*H_3), 118.35 (*C*H), 122.68, 123.36 (2 C), 124.45 (*C*H), 135.56 (C), 135.63 (*C*H), 154.02 (C), 155.93 (*C*H), 163.65, 173.22 (2 C). IR (ATR, cm^{-1}): $\tilde{\nu}$ = 3078, 2924 (w, N-CH_3), 1650, 1639 (s, C=O), 1440, 1383 (s, CH_3), 1236 (m, C-N), 1132 (CH_3), 1020 (m, C-N). MS (GC, 70eV): *m/z* (%) = 231 (M^+, 21), 188 (11), 187 (34), 160 (48), 135 (100), 134 (16), 123 (42), 77 (22), 53 (16), 44 (20). HRMS (ESI): calcd for $C_{13}H_{13}NO_3$ 231.0890, found 231.0889.

7-Methoxy-*N*,*N*-dimethyl-4-oxo-4*H*-chromene-3-carboxamide 12c

White crystals, yield 28 % as a by-product in the synthesis of **8g**. Mp 137-138 °C. ^{1}H NMR (300.13 MHz, DMSO-d_6): δ= 2.88 (s, 3H, NC*H*$_3$), 2.96 (s, 3H, NC*H*$_3$), 3.90 (s, 3H, OC*H*$_3$), 7.09 (dd, 1H, ^{3}J = 8.9, ^{4}J = 2.4, Ar), 7.2 (d, 1H, ^{4}J = 2.4, Ar), 7.98 (d, 1H, ^{3}J = 8.9, Ar), 8.43 (s, 1H, Ar). ^{13}C NMR (62.90 MHz, DMSO-d_6): δ = 34.43, 37.81, 56.19 (3 CH_3), 100.88, 115.14 (2 *C*H), 117.37, 122.74 (2 C), 126.68, 155.48 (2 *C*H), 157.61, 163.64, 164.08, 172.58 (4 C). IR (ATR, cm^{-1}): $\tilde{\nu}$ = 2933 (w, N-CH_3), 1623, 1606 (s, C=O), 1436 (m, CH_2-H), 1269 (m, C-OCH_3), 1238, 1208, 1084 (m, C-N). MS (GC, 70eV): *m/z* (%) = 247 (M^+, 46), 203 (23), 176 (63), 151 (67), 134 (10), 124 (14), 123 (100), 78 (20), 44 (25). HRMS (ESI): calcd for $C_{13}H_{13}NO_4$ 247.0853, found 247.0845.

6-Methoxy-*N*,*N*-dimethyl-4-oxo-4*H*-chromene-3-carboxamide 12d

White crystals, yield 5 % as a by-product in the synthesis of **8h**. Mp 127-128 °C. ^{1}H NMR (300.13 MHz, DMSO-d_6): δ= 2.88 (s, 3H, NC*H*$_3$), 2.98 (s, 3H, NC*H*$_3$), 3.86 (s, 3H, OC*H*$_3$), 7.39-7.45 (m, 2H, Ar), 7.65 (dd, 1H, ^{3}J = 8.2, ^{4}J = 1.4, Ar), 8.48 (s, 1H, Ar). ^{13}C NMR (62.90 MHz, DMSO-d_6): δ = 34.47, 37.79, 55.77 (3 *C*H_3), 104.91, 120.18 (2 *C*H), 122.03 (C), 123.71 (*C*H), 124.40, 150.49 (2 C), 155.84 (*C*H), 156.83, 163.70, 172.97 (3 C). IR (ATR, cm^{-1}): $\tilde{\nu}$ = 2926 (w, N-CH_3), 1616, 1603 (s, C=O), 1479 (s, CH_2-H), 1319 (m, C-N), 1261 (C-OCH_3), 1108, 1077, 1024 (m, C-N). MS (GC, 70eV): *m/z* (%) = 247 (M^+, 13), 203 (24), 176 (27), 151 (100), 150 (12), 53 (14), 44 (18). HRMS (ESI): calcd for $C_{13}H_{13}NO_4$ 247.0839, found 247.0836.

***N*,*N*-Dimethyl-4-oxo-4*H*-benzo[*h*]chromene-3-carboxamide 12e**

White crystals, yield 19 % as a by-product in the synthesis of **8i**. Mp 127-128 °C. ^{1}H NMR (300.13 MHz, DMSO-d_6): δ= 2.93 (s, 3H, NC*H*$_3$), 3.00 (s, 3H, NC*H*$_3$), 7.75-7.86 (m, 2H, Ar), 7.95 (d, 1H, ^{3}J = 8.7, Ar), 8.01 (d, 1H, ^{3}J = 8.7, Ar), 8.11 (d, 1H, ^{3}J = 7.7, Ar), 8.45 (d, 1H, ^{3}J = 7.7, Ar), 8.70 (s, 1H, Ar). ^{13}C NMR (75.47 MHz, DMSO-d_6): δ = 34.45, 37.79 (2 *C*H_3), 120.07 (*C*H), 120.11 (C), 121.88 (*C*H), 123.22, 124.14 (2 C), 125.76, 127.88, 128.28, 129.87 (4 *C*H), 135.36, 153.13 (2 C), 155.14 (*C*H), 163.46, 173.07 (2 C). IR (ATR, cm^{-1}): $\tilde{\nu}$ = 3440 (w b, C-N), 2930 (w, CH_3), 1635, 1622 (s, C=O), 1397 (s, CH_3), 1094 (m, C-O-C), 768 (s, C-H). MS (GC, 70eV): *m/z* (%) = 267 (M^+, 17), 223 (12), 196 (35), 172 (12), 171 (100), 170 (24), 144 (17), 139 (22), 123 (10), 115 (14), 114 (19), 53 (12), 44 (11). HRMS (ESI): calcd for $C_{16}H_{13}NO_3$ 267.0890, found 267.0889.

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