ChemPubSoc Europe
DOI: 10.1002/cmdc.201900385
CHEMMEDCHEM Full Papers

A Focused Library of NO-Donor Compounds with Potent Antiproliferative Activity Based on Green Multicomponent Reactions

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Cancer is the second leading cause of death worldwide. Herein, a strategy to quickly and efficiently identify novel lead compounds to develop anticancer agents, using green multicomponent reactions followed by antiproliferative activity and structure–activity relationship studies, is described. A second-generation focused library of nitric oxide-releasing compounds was prepared by microwave-assisted Passerini and Ugi reactions. Nearly all compounds displayed potent antiproliferative activities against a panel of human solid tumor cell lines, with 1-phenyl-1-[(*tert*-butylamino)carbonyl]methyl 3-[(3-phenylsulfonyl-[1,2,5]oxadiazol-4-yl N^2-oxide)oxy]benzoate (**4 k**) and *N*-[1-(*tert*-butylaminocarbonyl)-1-phenylmethyl]-*N*-(4-methylphenyl)-3-(3-phenylsulfonyl-[1,2,5]oxadiazol-4-yl N^2-oxide)oxyphenyl carboxamide (**6 d**) exhibiting the strongest activity on SW1573 lung cell line (GI_{50} = 110 and 21 nM) with selectivity indices of 70 and 470, respectively. Preliminary mechanistic studies suggest a relationship between NO release and antiproliferative activity. Our strategy allowed the rapid identification of at least two molecules as future candidates for the development of potent antitumor drugs.

Introduction

As World Health Organization Cancer Fact Sheets reported, cancer is the second leading cause of death worldwide, accounting for 9.6 million deaths in 2018.[1] The consequences of premature deaths are substantial, in terms of the economic burden due to lost productivity and the costs associated with therapy and treatment.[2] In this context, new, more active and safer drugs to decrease their incidence are of great relevance to our society. To this end, there is a high demand for efficient synthetic methodologies amenable to the exploration of chemical space in search for new chemotypes with improved antitumor activities.

One of the most promising strategies relies on the use of multicomponent reactions (MCRs). They provide access to structurally complex and functionally diverse sets of compounds in a single synthetic step, minimizing synthetic efforts, time and the formation of by-products. These properties make them powerful synthetic tools to explore large regions of chemical space under the guidance of green chemistry principles.[3–6] Among the MCRs, the Passerini and Ugi reactions, two isocyanide-based multicomponent reactions (iMCRs)[7] have been used widely in medicinal chemistry.[8–10] The Passerini reaction is a three-component reaction (3-CR) combining an aldehyde, a carboxylic acid and an isocyanide (also called isonitrile) to form an α-acyloxyamide derivative. The Ugi reaction is a four-component reaction (4-CR) and it incorporates an amine to the aldehyde, carboxylic acid and isonitrile cocktail to deliver bis-amide structures (Figure 1 A). Due to their modular nature, both iMCRs are well suited to construct complexity with functional diversity, and importantly, they do it with excellent atom-economy (a measure of how many reactant atoms incorporate into the reaction product).[11] The syntheses of crixivan (indinavir sulfate), an inhibitor of the human immunodeficiency virus (HIV) protease, and anesthetics like lidocaine and bupivacaine, constitute paradigmatic examples of the synthetic use of these reactions in medicinal chemistry programs.[12] In recent years, different synthetic approaches aiming to improve the chemical and sustainable performance of these iMCRs

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https://doi.org/10.1002/cmdc.201900385.

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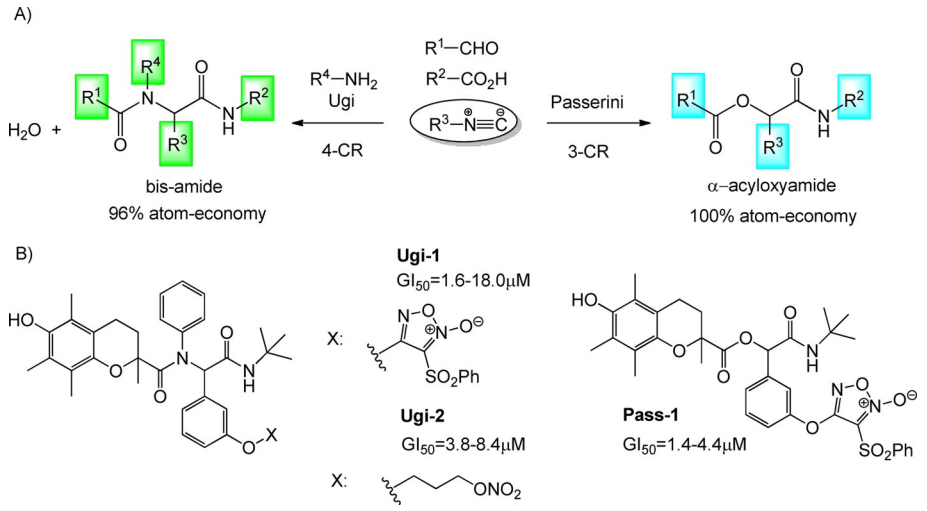

Figure 1. A) Isocyanide-based multicomponent reactions: Passerini and Ugi reactions. B) Structures of tocopherol-mimetic NO-donor hybrid compounds with antiproliferative activities.

have been described.[6] In this context, the use of environmentally friendly solvents and alternative activation methods to the conventional heating have been studied. Reports from different labs have shown the advantages of using eco-friendly solvents, such as water or ionic liquids, and solvent-free conditions (neat).[13–18] Although most iMCRs proceed under mild conditions (ambient temperature and atmospheric pressure), they demand long reaction times. Nevertheless, the use of microwave irradiation (MW) or ultrasound (US) not only allow for a substantial decrease the reaction time,[19–23] but also allow a better experimental fit to the green chemistry principles. As part of an ongoing research project aimed at the design and synthesis of novel active NO-donor compounds, we report herein the construction of a small focused library of these structures using green-oriented iMCRs (i.e., performed under 'on water'[24] or solvent-free conditions and continued microwave or ultrasound irradiation), and the study of their cytotoxicity against six human solid tumor cell lines. Some tocopherol–NO-donor hybrids previously obtained showed similar GI_{50} values when compared with the standard anticancer drugs cisplatin and etoposide (Figure 1).[25] Compounds from **Pass-1** and **Ugi-1** reactions (Figure 1 B) incorporate a furoxan system (1,2,5-oxadiazole *N*-oxide) as NO-donor, while **Ugi-2** products contain an organic nitrate. Particularly, in T-47D (breast) and WiDr (colon) drug-resistant cell lines, tocopherol analogue **Pass-1** was four to six times more active than cisplatin and etoposide, respectively.

Nitric oxide (NO), a ubiquitous free radical, was described as a signaling molecule in the cardiovascular system by the winners of the Nobel Prize: Furchgott, Ignarro, and Murad. Since then, a broad variety of biological processes in which it participates,[26,27] along with its application in the area of drug discovery, have been described.[28] Recently, the role of NO in cancer has been studied and it has been determined that at high concentrations, this free radical acts as an antineoplastic agent. In this context, NO-donor hybrid compounds have been extensively investigated.[29,30] There is a wide variety of chemical structures capable of releasing NO, such as organic nitrates and 1,2,5-oxadiazole *N*-oxides (furoxans),[31] which have been used as precursors for the synthesis of new potential anticancer agents. Therefore, the development of new and effective NO-donor hybrid molecules remains a current challenge in medicinal chemistry, especially in the design of new probes to manipulate biochemical pathways involving this free radical.[32]

In the present work, we developed a new NO-donor library through the aforementioned multicomponent reactions with optimized yields under green chemistry conditions. Nitrooxyl or furoxanyl groups were incorporated into the carboxylic acid component to evaluate whether the position of NO-donor moiety plays a fundamental role in the biological activity compared to the previous series and, thus, to explore the chemical space in search of new chemotypes with improved antitumor activity.

Results and Discussion

Chemistry: library construction

We started the library construction searching for experimental conditions for the eco-friendly Passerini reaction of aldehyde **1 a**,[33] carboxylic acid **2 a** (Figure 2) and *tert*-butylisonitrile (**3 a**) to give α-acyloxyamide **4 a** (Table 1, entries 1–11). Aldehyde **1 a** was used as the Boc-protected phenol instead of the unprotected derivative, as the latter decomposes rapidly. The use of green solvents such as water or ethanol, either at room tem-

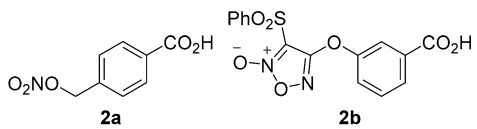

Figure 2. Carboxylic acid components that allow the incorporation of an organic nitrate or furoxan as NO-releasing moiety into Passerini and Ugi final products in the present work.[34,35]

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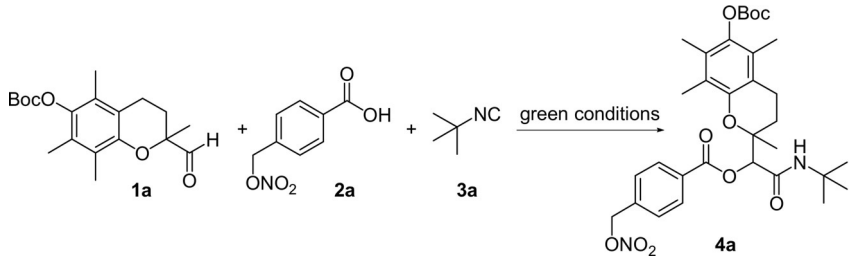
Table 1. Optimization of reaction conditions for the synthesis of NO donors via Passerini 3-CR.



Entry	Conditions	Yield [%]
1	EtOH, RT, 6 h	81
2	EtOH, MW[a], 60 °C, 30 min	76
3	H_2O, RT, 6 h	84
4	H_2O, RT, 24 h	91
5	SF[b], MW, 60 °C, 15 min	87
6	SF, MW, 60 °C, 30 min	93
7	SF, MW, 60 °C, 60 min	93
8	SF, US[c], 6 h	93
9	SF, US, 30 min	46
10	SF, conventional heating, 60 °C, 30 min	92
11	CH_2Cl_2, RT, 6 h	93

[a] MW: microwave heating. [b] SF: solvent-free. [c] US: ultrasound.

perature or under microwave irradiation (MW, closed vessel, 60 °C) was productive, delivering adduct **4a** in good yields (Table 1, entries 1–4). It was also observed that the reaction could be performed under solvent-free conditions (SF), using MW irradiation, ultrasound (US) or conventional heating as the activation sources (Table 1, entries 5–10). We decided to run the reactions at 60 °C because previous studies on the MW-activated Passerini reaction by our group had shown that it was the best temperature for the reaction.[15] Although in all the assayed conditions adduct **4a** was obtained in good to excellent yields, we chose the conditions of entry 6 as our standardized conditions: neat with MW irradiation in a closed vessel for 30 min at 60 °C. It should be noted that the α-acyloxyamide **4a** could be also obtained in excellent yield (93 %, entry 11) if the reaction was performed under classical conditions, at room temperature in CH_2Cl_2, a recognized non-eco-friendly solvent (Table 1, entry 11). Because of these non-eco-friendly properties, these last conditions were not considered.

Once a set of optimized conditions were found, we studied the diversity generation power of the reaction by selective variation of the isocyanide and the carboxylic acid modules (Figure 3). In all cases, good to excellent yields of adducts α-acyloxyamides **4** were obtained, with acid **2a** (Figure 2, organic nitrate derivative) affording adducts **4a–e** in higher average yield than **2b** (Figure 2, furoxan derivative) afforded the corresponding **4f–k**. In the case of compounds **4e** and **4j**, as 4-methoxyphenyl isocyanide has a solid-oil consistency that did not allow a homogeneous mixing with the rest of the solid reagents under solvent-free conditions, the reactions were carried out using ethanol as a green solvent.

With the purpose of preparing a series of NO-donors by the Ugi reaction, we carried out the reaction between aldehyde **1a**, carboxylic acid **2a**, *tert*-butylisocyanide **3a** and *p*-toluidine **5**, under different green conditions (Table 2, entries 1–5). The optimized experimental conditions for the Passerini reaction failed to deliver the Ugi adduct **6a** as the main reaction product (Table 2, entry 1). Variable mixtures of Ugi **6a** and Passerini **4a** adducts were obtained when the reaction was performed under different experimental conditions (Table 2, entries 2–5). Fortunately, an acceptable ratio of **6a**/**4a**: 1.8/1 (91 % yield) could be obtained using EtOH and MW irradiation (closed vessel, 30 min, 60 °C; entry 3). These conditions were used to obtain the rest of Ugi adducts **6b–g** (entries 6–11). Interestingly, this fact opened a synthetic avenue for the direct access to both series of compounds using a unique set of reactants under a unique set of reaction conditions. In this regard, good values were observed[37,38] when the "mass efficiency of the reaction" (RME) was calculated for these reactions as shown in Equation (1):

$$\mathrm{RME} = \left(\frac{\text{mass of isolated products of interest}}{\text{total mass of reagents}}\right) \times 100 \qquad (1)$$

This means that under the standardized conditions, a significant amount of the reactants is transformed into either of the two adducts. It is worth mentioning that we did not observe the Ugi product when pentanal (**1c**) was used as the aldehyde source. In this case, the MCR delivered the Passerini adduct **4o** as the only reaction product (54 %) (Table 2, entries 11).

To design these new Ugi-adducts, the results of the antiproliferative activity of the Passerini series were taken into account. Therefore, it was resolved to work only with *tert*-butylisocyanide because the introduction of changes at this level did not produce large changes in activity. Instead, the influence of the aldehyde component was explored, using aldehydes that incorporate a tocopherol-like substructure or commercially avail-

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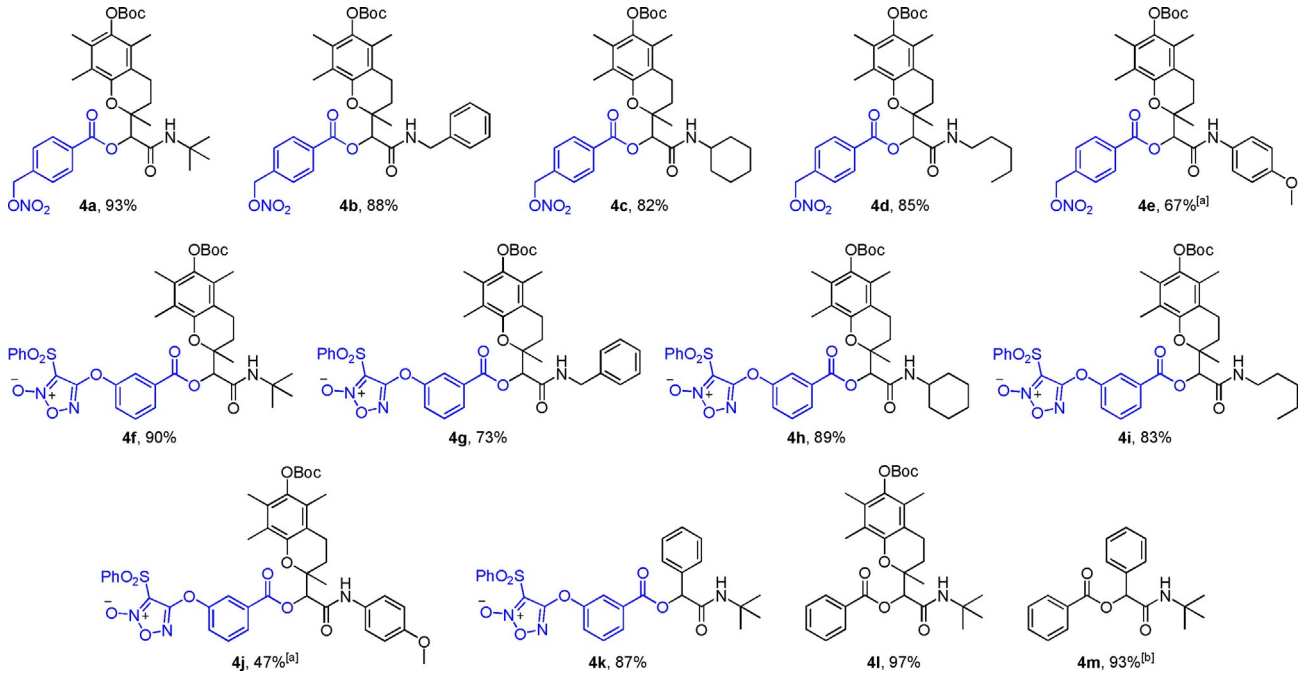
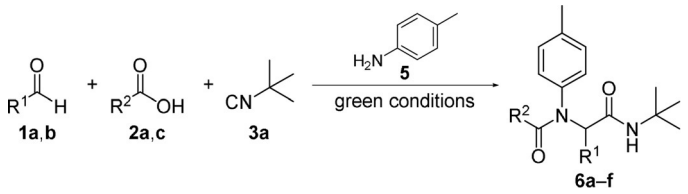
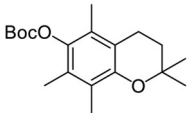
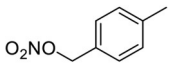
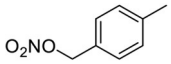
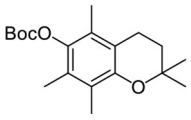
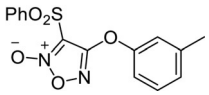
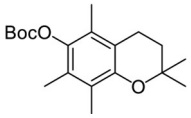


Figure 3. Scope and yields of Passerini 3-CR products described in Table 1. [a] EtOH, MW, 60 °C, 30 min. [b] Solvent-free, RT, 1 h.[36]

Table 2. Optimization of reaction conditions for the synthesis of NO donors via Ugi 4-CR and scope of the reaction under optimized green conditions.



Entry	R^1 (aldehyde)	R^2 (carboxylic acid)	Conditions	Product (Yield)	RME [%][a]
Optimization					
1			SF, MW, 60 °C, 30 min	**6a** (38%), **4a** (63%)	91
2			SF, US, 6 h	**6a** (46%), **4a** (33%)	72
3			EtOH, MW, 60 °C, 30 min	**6a** (59%), **4a** (32%)	83
4			buffer pH 5.5, US, 6 h	**6a** (53%), **4a** (52%)	94
5			buffer pH 5.5, RT, 24 h	**6a** (46%), **4a** (33%)	70
Scope					
6				**6b** (45%), **4n** (7%)	49
7				**6c** (48%), **4f** (15%)	60
8			EtOH, MW, 60 °C, 30 min	**6d** (63%), **4k** (15%)	74
9				**6e** (0%), **4o** (54%)	45
10				**6f** (32%), **4l** (33%)	59
11				**6g** (57%), **4m** (26%)	72

[a] Reaction mass efficiency: RME = [(mass of the isolated products)/(total mass of reagents) × 100].

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able simple aromatic or aliphatic ones. The carboxylic acid component contains the NO-donor group (Figure 2) or not in order to study structure–activity relationship. In this series, changes at the amine component were not explored.

Finally, we carried out the phenolic deprotection of adducts **4a**, **4f**, **4g**, **4h**, **4l**, **6a**, **6c** and **6f** to increase the functional diversity of the library for structure–activity relationship (SAR) studies. Given the presence of acid-labile groups in these molecules, we performed the deprotection using 2,2,2-trifluoroethanol,[39,40] an environmentally friendly solvent, either under reflux conditions (12 h), or under MW irradiation (15 min, 100 °C). Under these conditions, the corresponding phenolic compounds **7a–e**, **8a–c** (Figure 4) were obtained in modest to good yields (25–70%). Because antiproliferative activities (Table 3) were almost the same (> 100 μM), from the series of Passerini nitrooxy derivatives we selected only one compound (**4a**) to be deprotected to render **7a**. Regarding the furoxan derivatives, considering that antiproliferative activity was better than nitrooxy derivatives (GI_{50} values between 0.097 to 100 μM), we performed the deprotection reaction for three available compounds **4f**, **4g** and **4h** in order to see if changes in the amide side chain give differences in antiproliferative activities. After analyzing antiproliferative activity results for compounds **4f–j** and **7b–d**, we considered it unnecessary to further study this modification on compound structures. All the Ugi derivatives containing a Boc-protected phenol were deprotected to obtain derivatives **8a–c**.

Antiproliferative activity

The in vitro antiproliferative activity of the synthesized compounds was determined against six human solid tumor cells, A549 (lung), HBL-100 (breast), HeLa (cervix), SW1573 (lung), T-47D (breast) and WiDr (colon), using the sulforhodamine B assay.[25] The results were expressed as GI_{50}, which is the drug concentration resulting in a 50% decrease in cellular net growth relative to values of untreated control cells. Standard anticancer drugs cisplatin and etoposide were used as positive controls. Results are summarized in Table 3 for the **4a–o**, **7a–e** Passerini and **6a–g**, **8a–c** Ugi products as well as the starting components **1a**, **2a** and **2b**.

Results of the antiproliferative activity against six human solid tumor cells indicated that compounds **4k**, **4o**, **6d**, and **8b** exhibited noticeable activities against most tested cell lines as revealed form their GI_{50} values (Table 3). In this context, compound **4k** exhibited remarkable anticancer activity with GI_{50} values between 0.11 to 3.5 μM against all the tested cell lines, with especially high activity against breast cancer cells HBL-100 and alveolar cell carcinoma SW1573 (300 nM and 110 nM, respectively). Therefore, **4k** was almost 6–8 times more potent than cisplatin and etoposide for HBL-100 cells, almost 30 times more potent than cisplatin and almost 140 times more potent than etoposide for SW1573 cells. Its analogue **4o**, which comes from pentanal instead of benzaldehyde as aldehyde component in the corresponding Passerini reaction, showed a notable inhibition on cell proliferation with high sensitivity toward SW1573 cells (140 nM). Among the Ugi derivatives, compound **6d** showed notable anticancer activity with excellent sensitivity toward SW1573 cells (21 nM), quite more potent than the positive controls, cisplatin and etoposide. Meanwhile, compound **8b** revealed remarkable anticancer activity on the same order as compound **4k** with GI_{50} values between 0.24 to 5.8 μM against all the tested cell lines and sensitivity against HBL-100 and SW1573 cell lines. On the other hand, as summarized in Table 3, compounds **2a**, **4m**, **4a–e** do not show antiproliferative activity against the panel of cancer cells tested. These initial results suggested that the presence of the furoxan but not the organic nitrates as NO-donor moiety might play a crucial role in antiproliferative activity.

Results depicted in Table 3 also revealed that compounds **1a**, **2b**, **4l**, **4n**, **6a**, **6b**, **6g**, **6f**, **7a**, **7c**, **7d**, **7e**, **8a** and **8c** showed similar activity against all cell lines with GI_{50} ranging

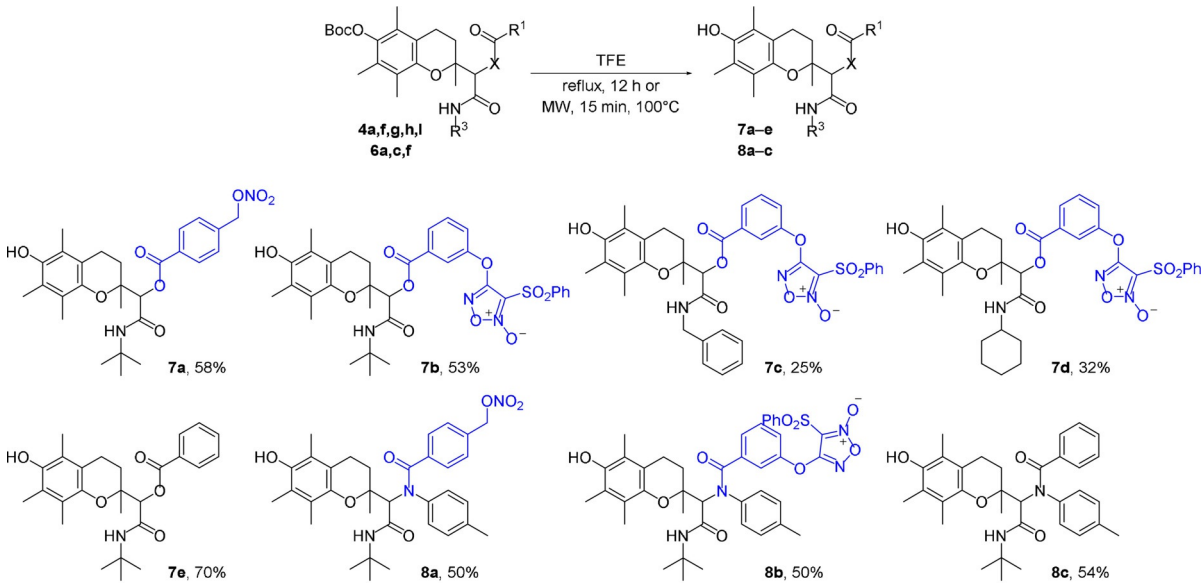

Figure 4. Synthesis and yields of hybrid NO-donor tocopherol mimetics **7a–e** and **8a–c**.

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Table 3. Antiproliferative activity (GI_{50} [μM])[a] of compounds obtained via Passerini and Ugi reactions against human solid tumor cells.

Compound	Cell line (type)					
	A549 (lung)	HBL-100 (breast)	HeLa (cervix)	SW1573 (lung)	T-47D (breast)	WiDr (colon)
Starting materials						
1a	71±16	18±8.4	17±5.5	16±8.3	79±17	64±33
2a	>100	>100	96±5.0	>100	>100	>100
2b	9.7±3.7	2.9±0.9	4.6±2.0	2.0±0.7	16±2.5	20±1.1
Passerini products						
4a	>100	>100	>100	>100	>100	>100
4b	>100	>100	>100	>100	>100	>100
4c	>100	>100	>100	>100	>100	>100
4d	>100	>100	>100	>100	>100	>100
4e	95±7.2	>100	>100	>100	>100	>100
4f	15±5.2	>100	4.8±1.1	0.17±0.01	49±10	82±25
4g	89±14	19±0.01	25±3.6	0.26±0.01	>100	>100
4h	87±23	12±6.3	32±7.4	0.28±0.01	>100	>100
4i	39±11	3.1±0.2	10±2.5	0.097±0.02	>100	>100
4j	93±12	93±9.4	71±18	0.32±0.05	>100	>100
4k	2.5±0.6	0.3±0.04	1.7±0.07	0.11±0.01	2.1±0.06	3.5±0.4
4l	18±4.4	24±19	14±2.7	32±3.1	75±26	66±15
4m	>100	>100	>100	>100	>100	>100
4n	30±2.6	21±4.9	17±0.9	7.4±0.7	3.6±0.5	12±0.01
4o	2.5±0.3	1.9±0.5	3.1±0.2	0.14±0.05	2.1±0.4	2.2±0.3
7a	5.1±1.2	22±2.9	4.7±1.0	6.0±1.0	17±2.6	42±9.8
7b	2.3±0.06	2.4±0.1	2.2±0.2	0.4±0.03	3.0±0.5	3.5±1.0
7c	12±0.3	4.2±1.0	6.9±1.1	3.4±0.4	22±6.1	31±6.0
7d	13±1.0	9.6±2.6	13±3.0	3.4±0.5	30±4.1	42±1.5
7e	4.5±0.4	13±1.6	8.3±0.9	3.9±0.5	12±1.1	17±4.5
Ugi products						
6a	6.3±1.9	66±18	14±0.1	14±8.3	15±4.4	84±23
6b	9.3±0.5	10±2.5	5.9±0.9	1.7±0.1	17±1.5	15±4.2
6c	89±14	2.6±0.7	15±2.3	0.16±0.02	>100	>100
6d	2.3±0.5	0.23±0.02	1.8±0.3	0.021±0.013	1.9±0.3	3.0±0.6
6f	3.2±0.9	3.5±0.6	3.0±0.5	3.9±0.9	4.1±0.9	6.0±1.3
6g	24±7.8	33±3.7	19±6.9	21±8.5	32±8.0	33±6.2
8a	2.5±0.8	8.1±1.9	3.7±0.9	5.5±0.6	8.1±1.0	29±4.3
8b	2.3±0.5	0.31±0.03	1.3±0.1	0.24±0.003	3.0±0.2	5.8±1.2
8c	2.4±0.1	3.7±0.4	2.0±0.3	1.8±0.06	2.9±0.5	3.8±0.3
Positive controls						
cisplatin	2.1±0.6	1.9±0.3	2.0±0.3	3.0±0.4	15±2.3	26±5.3
etoposide	0.7±0.2	2.3±0.9	3.0±0.9	15±1.5	22±5.5	23±3.1

[a] Values are the mean±SD of at least three independent experiments.

from 2.0 to 84 μM. However, compounds **4g**, **4h**, **4i**, **4j** and **6c** do not show activity against drug-resistant cell lines T-47D and WiDr, but showed variable activity against the other cell lines. Interestingly, compound **4f** showed a wide range of GI_{50} values from 170 nM against alveolar cell carcinoma SW1573, to more than 100 μM against HBL-100.

Moreover, as lung cancer is the most common cause of cancer-related death (1.76 million deaths), it is very interesting that most of the synthesized compounds (**4k**, **4o**, **4h**, **4i**, **4j**, **4f**, **4g**, **6d**, **6c**, **7b**, **8b**) exhibited sub-micromolar antiproliferative activity against SW1573 cancerous cells.

Taking the GI_{50} values for comparison, the following points were observed regarding the SAR of the newly synthesized compounds in relation to their anticancer activity: a) The incorporation of a nitrooxyl group as pharmacophore resulted in moderate enhancement of anticancer activity, as can be seen by comparing **4m** vs. **4n** and **6g** vs. **6b**; b) Replacement of the phenyl substituent in compounds **4m, 4n, 4k, 6g, 6b, 6d** with the chromane ring (tocopherol-mimetic moiety) resulted in a similar anticancer activity or a moderate increase thereof (compare GI_{50} of **4m** with **7e, 4n** with **7a, 4k** with **7b**, **6g** with **8c, 6b** with **8a**, **6d** with **8b**); c) Replacement of the *tert*-butyl group in compound **4a** with other alkyl, aryl or cycloalkyl group resulted in retention of inactivity (see GI_{50} of **4b–e**); d) The incorporation of a furoxanyl group as pharmacophore resulted in a noticeable improvement of anticancer activity as can be seen by comparing **4m** vs. **4k** and **6g** vs. **6d**; e) Replacement of the *tert*-butyl group in compound **4f** with other alkyl, aryl or cycloalkyl group resulted in retention of anticancer activity (see GI_{50} of **4g–i**); e) Deprotection of the phenolic group of the chromane ring (**4l** vs. **7e**, **4h** vs. **7d**, **6f** vs. **8c**, **4f** vs. **7b**, **4g** vs. **7c**) resulted in a poor enhancement of the antiproliferative activity, except in the case of **4a** and **7a**. In general, Passerini and Ugi derivatives containing the phenylsulfonylfuroxan substructure as NO-releasing group showed the best antitumor activity in the six-cell panel.

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Selectivity index

As a preliminary assessment of the safety profile of selected compounds, a cell proliferation study was performed in human keratinocyte HaCaT cells with 1–100 μM of the test compounds using the sulforhodamine B assay. The selectivity index (SI) was calculated for these compounds by dividing the GI_{50} for HaCaT by the GI_{50} for each tumor cell lines.

Results presented in Table 4 revealed that all selected compounds were selective toward cancerous cells, showing a selectivity index range of 2.2–471.4. Moreover, most of these compounds exhibited a remarkable selectivity toward SW1573 cancerous cells.

Table 4. Cytotoxicity of selected Passerini-3C and Ugi-4C derivatives against non-tumorigenic HaCaT human cells, along with selectivity indexes.

Compd	GI_{50} [μM][a]	SI[b]					
		A549	HBL-100	HeLa	SW1573	T-47D	WiDr
4k	7.7	3.1	25.7	4.5	70	3.7	2.2
6b	34.1	3.7	3.4	5.8	20	2.0	2.3
6d	9.9	4.3	43	5.5	471.4	5.2	3.3
7a	>100	>19.6	>4.5	>16.7	>5.9	>5.9	>2.4
7b	10.4	4.5	4.3	4.7	26	3.5	2.9
8a	>100	>40	>12.3	>27	>18.2	>12.3	>3.4
8b	7.9	3.4	25.5	6.1	32.9	2.6	1.36
8c	14.4	6.0	3.9	7.2	8.0	4.9	3.8

[a] Values are the mean of three independent experiments, with a SD less than 10% in all cases. [b] Selectivity index: $[GI_{50}(HaCaT)]/[GI_{50}(tumor\ cell\ line)]$.

In particular, the Passerini **4k** and Ugi **6d** compounds, containing a furoxan moiety as NO-donor, displayed an SI of 70 and 471.4 for SW1573 cells and a SI of 25.7 and 43 for HBL-100 cells, respectively. The significantly lower antiproliferative activity of the tocopherol-nitrooxy hybrids derivatives **7a** and **8a** in normal HaCaT cells is also noteworthy.

Assessment of NO release

Among nitric oxide releasing moieties, furoxan has attracted considerable attention due to its stability under ambient conditions and ability to produce large fluxes of NO by a thiol-dependent mechanism, thereby eliciting the cytotoxic effects associated with NO. Unlike organic nitrates, furoxans do not promote tolerance under continuous therapy.[31,41] Organic nitrates are the oldest and structurally simplest NO-donor with an important role in cardiovascular therapy but also have demonstrated anticancer properties in vitro and in vivo.[42] Many efforts have been made to fully elucidate the mechanism of NO generation by organic nitrates. In this context, enzymatic biotransformations of organic nitrates into NO have been proposed, including glutathione-S-transferases (GSTs), cytochrome P450 reductase, xanthine oxidoreductase, and aldehyde dehydrogenase-2 (ALDH2).[29,30,43–45]

Selected compounds **4k**, **7a**, **7b**, **6b**, **6d**, **8a** and **8b,** containing a NO-donor group that exhibited excellent anticancer activity, were evaluated for their ability to release NO in the presence of cancer cells, as a first approximation to their mechanism of action. As shown in Figure 5A, the intracellular NO production capability of these compounds was determined and presented as that of nitrite in the HeLa cell lysates (exposed to 100 μM of each compound) using a Griess assay.

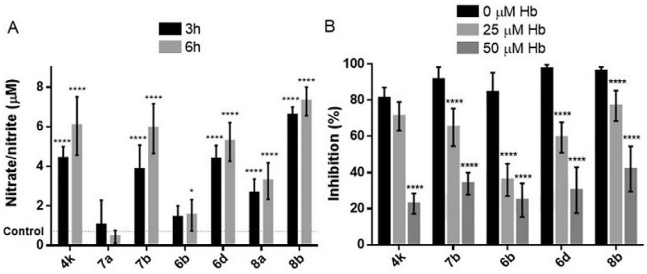

Figure 5. A) Levels of NO produced by selected NO-donor compounds in HeLa cells. Results are the mean ± SD of three independent experiments. B) Effects of hemoglobin on the antiproliferative effect of selected NO-donor compounds. HeLa cells were pretreated with the indicated concentrations of hemoglobin (0, 25, or 50 μM) for 1 h and treated with 25 μM compounds tested for 48 h. The results are expressed as the percentage of cell growth inhibition relative to control cells. Data are the mean ± SD obtained from three determinations. Statistical analysis: two-way ANOVA followed by Bonferroni multiple comparison test; $*p \leq 0.05$, $****p \leq 0.0001$.

Compounds could produce various levels of nitrite at the time point of 3 or 6 h in human cervix cancer HeLa cells, among which, the most active compound **4k**, **7b, 6d** and **8b** released the highest concentration of nitrite. It was observed that organic nitrate derivatives (**7a**, **6b** and **8a)** released less nitrite than their furoxan analogues. Furthermore, for the same selected compounds, the effect of NO on their antiproliferative activity against HeLa cells was examined. After incubation with a NO scavenger (Hb, hemoglobin), HeLa cells were treated with 25 μM of selected NO-donor compounds for 48 h. Compounds **7a** and **8a** showed no change in their antiproliferative activity in the presence of Hb (data not shown). However, as seen in Figure 5B, the other compounds in the absence of Hb markedly inhibit the growth of HeLa cells, but this antiproliferative effect decreased by pretreatment with Hb in a dose-dependent manner. These results indicated that the potent antiproliferative activity against cancerous cells of these compounds may be partially attributed to the release of nitric oxide.

Conclusions

A small library of hybrid NO-donor compounds has been assembled using Passerini 3-CR and Ugi 4-CR coupling reactions. Simple starting materials were assembled in a single synthetic step to deliver the corresponding Passerini adducts **4** or Ugi adducts **6** in good to excellent yield. The iMCRs were optimized to be performed under eco-friendly conditions, either by using MW irradiation under neat conditions or in green sol-

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vents. The main advantages of this protocol are: i) experimental simplicity, ii) mild conditions, iii) short reaction times, iv) clean reactions, and v) good to excellent yields. All synthesized molecules were evaluated against six human solid tumor cell lines. Most of them were capable of inhibiting cell growth, with the furoxans a much more promising series of compounds, in contrast to their nitrate ester analogues. Our results indicate that the Passerini derivatives **4k**, **4o** and **7b**, and Ugi derivatives **6d** and **8b**, each containing a furoxan system as NO-donor, are potent anticancer agents, exhibiting GI_{50} values against all cells in the range 0.021–5.8 μM, with the compound **6d** being the most potent. This was almost 8 times more potent than cisplatin and etoposide against resistant cell lines T-47D and WiDr, but moreover, it was extremely potent against alveolar cell carcinoma SW1573, being 140 times more active than cisplatin and 700 times more potent than etoposide. Regarding the Passerini derivatives, compound **4k** was almost 7 times more active than cisplatin and etoposide against breast cancer cells HBL-100, and almost 30 times more potent than cisplatin and almost 140 times more potent than etoposide against SW1573 cancer cells. On the other hand, these two molecules (**4k** and **6d**) exhibited lower potency against non-cancerous human keratinocytes (HaCaT), indicating a selectivity against cancerous cells. Further studies showed that **4k** and **6d** were able to release NO in the presence of HeLa cells, and the antiproliferative activities of both compounds declined with increasing concentrations of scavenger (Hemoglobin), suggesting a potential role of NO in their anticancer mechanism. It is worth mentioning that although **7b** and **8b** present biological activities similar to those of **4k** and **6d**, the latter can be synthesized from readily available starting materials and in better yields.

In summary, our strategy has identified two small hybrid molecules as future candidates for the development of potent antitumor drugs. Future studies should get a deeper insight into their antiproliferative mechanism, evaluate the antiproliferative activity of isolated isomers, and determine their in vivo activity, in particular, in a mouse model of lung alveolar carcinoma.

Experimental Section

Materials

All starting materials were commercially available research grade chemicals and used without further purification. RPMI 1640 medium was purchased from Flow Laboratories (Irvine, UK). MEM media, fetal calf serum (FCS), Fetal Bovine Serum (FBS) and GlutaMAX were from Gibco (Grand Island, NY). Trichloroacetic acid (TCA) and glutamine were from Merck (Darmstadt, Germany). DMEM media was from Capricorn Scientific (Ebsdorfergrund, Germany). Penicillin G, streptomycin, DMSO, sulforhodamine B (SRB) were from Sigma (St Louis, MO).

All solvents were distilled prior to use. Analytical TLC was performed on silica gel 60F-254 plates and visualized with UV light (254 nm) and/or *p*-anisaldehyde in acidic ethanolic solution. Column chromatography was performed using silica gel (SAI, 63–200 μm). Microwave-assisted reactions were conducted in sealed glass vessels (capacity 10 mL) using a CEM Discover microwave reactor. Ultrasound-assisted reactions were carried out in round-bottom flasks using an ultrasonic bath (Ultrasons-H, J.P. Selecta). Proton and carbon NMR spectra were recorded on a Bruker DPX-400 spectrometer. The chemical shifts are expressed in ppm relative to tetramethylsilane as internal standard and coupling constants are expressed in Hertz, followed by multiplicity indicated as s: singlet, d: doublet, t: triplet, q: quartet or combination, bs: broad singlet, or m: multiplet. Mass spectra were determined on a Shimadzu DI-2010. High-resolution mass spectra were recorded with a Micromass Autospec mass spectrometer. Melting points were determined by open glass capillary method on a melting point apparatus Electrothermal 9100.

Compounds **1a**,[33] **2a**,[34] **2b**,[35] and **4m**[36] were synthesized according to literature methods. All other materials were obtained from commercial suppliers and used as received.

Experimental procedures

General procedure for Passerini products (4a–l). Method A (microwave-assisted): A glass tube suitable for microwaves was charged sequentially with the aldehyde (0.2 mmol), the acid (0.2 mmol) and the isocyanide (0.2 mmol), under solvent-free conditions or in EtOH (1 mL). The sealed tube was heated 15–60 min at 60 °C under microwave irradiation. The reaction mixture was allowed to cool to room temperature and quenched with saturated solution of $NaHCO_3$. The aqueous mixture was extracted with EtOAc. The organic layer was washed with brine, dried with Na_2SO_4, filtered and the solvent evaporated under reduced pressure. The crude product was purified by flash column chromatography (SiO_2, hexanes/EtOAc). **Method B (ultrasound-assisted)**: A round-bottom flask was charged sequentially with the aldehyde (0.2 mmol), the acid (0.2 mmol) and the isocyanide (0.2 mmol), then placed in an ultrasound bath preheated to 60 °C. It was exposed for 30 min or 6 h at 720 W and 50–60 Hz. The reaction mixture was allowed to cool to room temperature, quenched with a saturated solution of $NaHCO_3$, and extracted with EtOAc. Work-up was continued as in Method A. **Method C (conventional heating)**: A round-bottom flask was charged sequentially with the aldehyde (0.2 mmol), the acid (0.2 mmol) and the isocyanide (0.2 mmol); then placed on a heating plate preheated to 60 °C and stirred for 30 min. The reaction mixture was allowed to cool to room temperature, quenched with a saturated solution of $NaHCO_3$, and extracted with EtOAc. Work-up was continued as in Method A. **Method D (room temperature)**: A round-bottom flask previously filled with H_2O, EtOH or CH_2Cl_2 (1 mL) was charged sequentially with the aldehyde (0.2 mmol), the acid (0.2 mmol) and the isocyanide (0.2 mmol). Then, it was stirred for 6 or 24 h. The reaction mixture was treated with a saturated solution of $NaHCO_3$ and extracted with EtOAc. Work-up was continued as in Method A.

1-[6-(*tert*-butoxycarbonyl)oxy-2,5,7,8-tetramethylchroman-2-yl]-1-[(*tert*-butylamino)carbonyl]methyl 4-(nitrooxymethyl)benzoate (4a): This compound was obtained by methods A, B, C or D. The crude product was purified by flash column chromatography (SiO_2, hexane/EtOAc, 8:2) to give the desired product (**4a**) as a white solid (46–93 %, 2:1 diastereomeric mixture); mp: 80–82 °C; major diastereomer: ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 8.03$ (d, $J = 8.1$ Hz, 2H), 7.44–7.41 (m, 2H), 5.86 (s, 1H), 5.41 (s, 2H), 5.11 (s, 1H), 2.72–2.52 (m, 2H), 2.07 (s, 3H), 2.04–2.02 (m, 1H), 2.01 (s, 3H), 1.97 (s, 3H), 1.89–1.82 (m, 1H), 1.48 (s, 9H), 1.40 (s, 3H), 1.30 ppm (s, 9H); minor diastereomer: ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 8.03$ (d, $J =$ 8.1 Hz, 2H), 7.44–7.41 (m, 2H), 5.98 (s, 1H), 5.41 (s, 2H), 5.26 (s,

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1 H), 2.72–2.52 (m, 2 H), 2.04–2.02 (m, 1 H), 2.00 (s, 3 H), 1.98 (s, 3 H), 1.95 (s, 3 H), 1.89–1.82 (m, 1 H), 1.48 (s, 9 H), 1.33 (s, 3 H), 1.26 ppm (s, 9 H); diastereomeric mixture: ^{13}C NMR (100 MHz, $CDCl_3$): δ = 165.9, 165.7, 164.8, 164.5, 152.2, 125.1, 148.0, 147.9, 141.8, 141.7, 137.9, 137.8, 130.4, 130.3, 128.7, 128.6, 127.8, 127.5, 125.9, 125.8, 122.7, 122.5, 117.6, 117.5, 83.0, 82.9, 76.5, 76.1, 73.6, 73.5, 51.7, 51.5, 28.7, 28.6, 27.7, 20.9, 20.5, 20.1, 19.9, 12.8, 12.7, 12.4, 12.1, 11.9 ppm; MS (EI, 70 eV): *m/z* (%) 614 (M^+, 5), 514 (51), 316 (18), 205 (41), 135 (92), 57 (100); HRMS *m/z* calcd for $C_{32}H_{42}N_2O_{10}$: 614.2839 $[M+H]^+$, found: 614.2827.

1-(benzylamino)carbonyl-1-[6-(*tert*-butoxycarbonyl)oxy-2,5,7,8-tetramethylchroman-2-yl]methyl 4-(nitrooxymethyl)benzoate (4 b): This compound was obtained by method A. The crude product was purified by flash column chromatography (SiO_2, hexane/EtOAc, 8:2) to give the desired product (**4 b**) as a white solid (88 %, 2:1 diastereomeric mixture); mp: 72–74 °C; major diastereomer: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.03–7.99 (m, 2 H), 7.41–7.39 (m, 2 H), 7.21–7.11 (m, 5 H), 6.39 (bs, 1 H), 5.39 (s, 2 H), 5.28 (s, 1 H), 4.41 (s, 2 H), 2.70–2.53 (m, 2 H), 2.06–2.02 (m, 1 H), 1.96 (s, 3 H), 1.94 (s, 3 H), 1.91–1.87 (m, 1 H), 1.76 (s, 3 H), 1.47 (s, 9 H), 1.40 ppm (s, 3 H); minor diastereomer: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.03–7.99(m, 2 H), 7.41–7.39 (m, 2 H), 7.21–7.11 (m,5 H), 6.44 (bs, 1 H), 5.41 (s, 1 H), 5.39 (s, 2 H), 4.40 (s, 2 H), 2.70–2.53 (m, 2 H), 2.06–2.02 (m, 1 H), 1.97 (s, 3 H), 1.95 (s, 3 H), 1.91–1.87 (m, 1 H), 1.73 (s, 3 H), 1.48 (s, 9 H), 1.36 ppm (s, 3 H); diastereomeric mixture: ^{13}C NMR (100 MHz, $CDCl_3$): δ = 167.0, 166.7, 164.9, 164.6, 152.2, 152.1, 147.9, 147.7, 141.8, 141.7, 138.0, 137.9, 137.7, 130.4, 130.2, 130.1, 128.8, 128.7, 128.6, 128.0, 127.9, 127.7, 127.6, 127.5, 125.9, 125.7, 122.9, 122.6, 117.5, 117.4, 83.0, 82.9, 78.5, 76.6, 76.0, 76.1, 73.6, 73.5, 43.7, 43.5, 28.6, 27.9, 27.7, 20.9, 20.7, 20.0, 19.9, 12.7, 11.9, 11.8 ppm; MS (EI, 70 eV): *m/z* (%) 548 (M^+−Boc, 7), 503 (76), 351 (14), 205 (90), 135 (88), 91 (63); HRMS: *m/z* calcd for $C_{35}H_{40}N_2O_{10}Na$: 671.2581 $[M+Na]^+$, found: 671.2590.

1-[6-(*tert*-butoxycarbonyl)oxy-2,5,7,8-tetramethylchroman-2-yl]-1-[(cyclohexylamino)carbonyl]methyl 4-(nitrooxymethyl)benzoate (4 c): This compound was obtained by method A. The crude product was purified by flash column chromatography (SiO_2, hexane/EtOAc, 8:2) to give the desired product (**4 c**) as a white solid (82 %, 2:1 diastereomeric mixture); mp: 143–145 °C; major diastereomer: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.02 (d, J = 8.1 Hz, 2 H), 7.44–7.41 (m, 2 H), 5.97 (d, J = 8.2 Hz, 1 H), 5.41 (s, 2 H), 5.22 (s, 1 H), 3.77–3.70 (m, 1 H), 2.60–2.53 (m, 2 H), 2.09–2.07 (m, 1 H), 2.05 (s, 3 H), 2.01 (s, 3 H), 1.97 (s, 3 H), 1.90–1.85 (m, 1 H), 1.65–1.61 (m, 2 H), 1.54–1.52 (m, 2 H), 1.48 (s, 9 H), 1.40 (s, 3 H), 1.31–1.19 (m, 4 H), 1.10–1.05 ppm (m, 2 H); minor diastereomer: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.03 (d, J = 5 Hz, 2 H), 7.44–7.41 (m, 2 H), 6.00 (d, J = 8.2 Hz, 1 H), 5.42 (s, 2 H), 5.34 (s, 1 H), 3.77–3.70 (m, 1 H), 2.72–2.56 (m, 2 H), 2.09–2.07 (m, 1 H), 2.00 (s, 3 H), 1.96 (s, 3 H), 1.92 (s, 3 H), 1.90–1.85 (m, 1 H), 1.65–1.61 (m, 2 H), 1.54–1.52 (m, 2 H), 1.48 (s, 9 H), 1.35 (s, 3 H), 1.31–1.19 (m, 4 H), 1.10–1.05 ppm (m, 2 H); diastereomeric mixture: ^{13}C NMR (100 MHz, $CDCl_3$): δ = 165.9, 165.7, 164.9, 164.5, 152.2, 152.1, 148.0, 147.9, 141.8, 141.7, 137.9, 137.8, 130.4, 130.3, 128.7, 128.6, 127.8, 127.5, 126.0, 125.7, 122.8, 122.5, 117.6, 117.5, 83.0, 82.9, 76.0, 75.9, 73.6, 73.5, 48.7, 48.3, 33.2, 33.1, 33.0, 32.9, 29.4, 29.1, 28.6, 28.0, 27.7, 25.5, 25.4, 25.0, 24.9, 24.8, 24.7, 20.9, 20.4, 20.0, 19.9, 12.8, 12.7, 12.3, 12.1, 11.9, 11.4 ppm; MS (EI, 70 eV): *m/z* (%) 640 (M^+, 1), 595 (2), 540 (18), 493 (100), 205 (78), 135 (81), 83 (15), 57 (37); HRMS *m/z* calcd for $C_{34}H_{44}N_2O_{10}$: 640.2996 $[M+H]^+$, found: 640.2969.

1-[6-(*tert*-butoxycarbonyl)oxy-2,5,7,8-tetramethylchroman-2-yl]-1-[(pentylamino)carbonyl]methyl 4-(nitrooxymethyl)benzoate (4 d): This compound was obtained by method A. The crude product was purified by flash column chromatography (SiO_2, hexane/EtOAc, 8:2) to give the desired product (**4 d**) as a colorless oil (85 %, 2:1 diastereomeric mixture) which solidifies when stored cold. Major diastereomer: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.03–8.01 (m, 2 H), 7.44–7.41 (m, 2 H), 6.06 (bs, 1 H), 5.41 (s, 2 H), 5.24 (s, 1 H), 3.24–3.20 (m, 2 H), 2.72–2.54 (m, 2 H), 2.09–2.04 (m, 1 H), 2.02 (s, 3 H), 2.01 (s, 3 H), 1.97 (s, 3 H), 1.90–1.86 (m, 1 H), 1.48 (s, 9 H), 1.46–1.43 (m, 2 H), 1.41 (s, 3 H), 1.23–1.13 (m, 4 H), 0.80–0.73 ppm (m, 3 H); minor diastereomer: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.03–8.01 (m, 2 H), 7.44–7.41 (m, 2 H), 6.14 (bs, 1 H), 5.41 (s, 2 H), 5.38 (s, 1 H), 3.24–3.20 (m, 2 H), 2.72–2.54 (m, 2 H), 2.09–2.04 (m, 1 H), 1.99 (s, 3 H), 1.97 (s, 3 H), 1.91 (s, 3 H), 1.90–1.86 (m, 1 H), 1.48 (s, 9 H), 1.46–1.43 (m, 2 H), 1.36 (s, 3 H), 1.23–1.13 (m, 4 H), 0.80–0.73 ppm (m, 3 H); diastereomeric mixture: ^{13}C NMR (100 MHz, $CDCl_3$): δ = 166.8, 166.7, 164.9, 164.5, 152.2, 152.1, 148.0, 147.9, 141.8, 141.7, 138.0, 137.9, 130.4, 130.3, 128.7, 128.6, 127.6, 127.8, 127.6, 126.0, 125.8, 122.8, 122.5, 117.5, 117.4, 83.0, 82.9, 76.0, 75.9, 73.6, 73.5, 39.6, 39.4, 29.3, 29.2, 29.1, 29.0, 28.5, 28.0, 27.7, 22.3, 22.2, 20.9,20.5, 20.0, 19.9, 13.9, 13.8, 12.7, 12.6, 12.0, 11.9, 11.8 ppm; MS (EI, 70 eV): *m/z* (%) 628 (M^+, 1), 583 (1), 528 (10), 481 (100), 205 (76), 135 (40), 71 (5), 57 (27); HRMS *m/z* calcd for $C_{33}H_{44}N_2O_{10}$: 628.2996 $[M+H]^+$, found: 628.2980.

1-[6-(*tert*-butoxycarbonyl)oxy-2,5,7,8-tetramethylchroman-2-yl]-1-[(4-methoxyphenyl)aminocarbonyl]methyl 4-(nitrooxymethyl)-benzoate (4 e): This compound was obtained by method A. The crude product was purified by flash column chromatography (SiO_2, hexane/EtOAc, 8:2) to give the desired product (**4 e**) as a brownish solid (67 %, 2:1 diastereomeric mixture); mp: 70–72 °C; major diastereomer: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.06–8.04 (m, 2 H), 7.71 (bs, 1 H), 7.44–7.42 (m, 2 H), 7.35–7.31 (m, 2 H), 6.77–6.75 (m, 2 H), 5.41 (s, 2 H), 5.34 (s, 1 H), 3.70 (s, 3 H), 2.72–2.57 (m, 2 H), 2.13–2.07 (m, 1 H), 2.04 (s, 3 H), 2.02 (s, 3 H), 1.98 (s, 3 H), 1.95–1.90 (m, 1 H), 1.48 (s, 9 H), 1.47 ppm (s, 3 H); minor diastereomer: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.06–8.04 (m, 2 H), 7.81 (bs, 1 H), 7.44–7.42 (m, 2 H), 7.35–7.31 (m, 2 H), 6.77–6.75 (m, 2 H), 5.49 (s, 1 H), 5.41 (s, 2 H), 6.69 (s, 3 H), 2.72–2.57 (m, 2 H), 2.13–2.07 (m, 1 H), 2.01 (s, 3 H), 1.99 (s, 3 H), 1.98 (s, 3 H), 1.95–1.90 (m, 1 H), 1.48 (s, 9 H), 1.41 ppm (s, 3 H); diastereomeric mixture: ^{13}C NMR (100 MHz, $CDCl_3$): δ = 164.9, 164.8, 164.7, 164.6, 156.7, 156.6, 152.2, 152.1, 147.9, 147.7, 142.0, 141.9, 138.2, 138.0, 130.5, 130.4, 130.2, 130.1, 129.9, 128.7, 128.6, 128.0, 127.7, 126.1, 125.9, 122.8, 122.7, 121.8, 121.7, 117.6, 117.5, 114.2, 114.1, 83.1, 83.0, 76.4, 76.3, 76.2, 73.6, 73.5, 55.5, 28.9, 27.7, 20.9, 20.6, 20.0, 19.9, 12.8, 12.3, 12.1, 11.9 ppm; MS (EI, 70 eV): *m/z* (%) 619 (M^+−NO_2, 1), 564 (M^+−Boc, 3), 556 (22), 519 (40), 385 (6), 205 (100), 135 (72), 105 (24), 57 (19); HRMS: *m/z* calcd for $C_{35}H_{40}N_2O_{11}Na$: 687.2530 $[M+Na]^+$, found: 687.2526.

1-[6-(*tert*-butoxycarbonyl)oxy-2,5,7,8-tetramethylchroman-2-yl]-1-[(*tert*-butylamino)carbonyl]methyl 3-(3-phenylsulfonyl-[1,2,5]oxadiazol-4-yl N^2-oxide)oxybenzoate (4 f): This compound was obtained by method A. The crude product was purified by flash column chromatography (SiO_2, hexane/EtOAc, 7:3) to give the desired product (**4 f**) as a white solid (90 %, 2:1 diastereomeric mixture); mp: 90–92 °C; major diastereomer: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.15 (d, J = 5 Hz, 2 H), 8.08–8.04 (m, 2 H), 7.83 (t, J = 7.5 Hz, 1 H), 7.22–7.68 (m, 2 H), 7.62–7.60 (m, 2 H), 5.98 (bs, 1 H), 5.22 (s, 1 H), 2.83–2.61 (m, 2 H), 2.27–2.21 (m, 1 H), 2.18 (s, 3 H), 2.13 (s, 3 H), 2.09 (s, 3 H), 2.01–1.93 (m, 1 H), 1.60 (s, 9 H), 1.52 (s, 3 H), 1.42 ppm (s, 3 H); minor diastereomer: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.15 (d, J = 5 Hz, 2 H), 8.08–8.04 (m, 2 H), 7.83 (t, J = 7.5 Hz, 1 H), 7.22–7.68 (m, 2 H), 7.62–7.60 (m, 2 H), 6.12 (bs, 1 H), 5.38 (s, 1 H), 2.83–2.61 (m, 2 H), 2.27–2.21 (m, 1 H), 2.11 (s, 3 H), 2.10 (s, 3 H), 2.06 (s, 3 H), 2.01–1.93 (m, 1 H), 1.60 (s, 9 H), 1.44 (s, 3 H), 1.38 ppm (s, 3 H); diastereo-

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meric mixture: ^{13}C NMR (100 MHz, $CDCl_3$): δ = 165.7, 165.5, 164.1, 163.8, 158.1, 152.6, 152.2, 147.8, 147.7, 141.8, 141.7, 137.9, 135.9, 131.7, 130.4, 130.3, 129.9, 128.7, 128.1, 127.9, 127.6, 126.0, 125.8, 125.0, 124.9, 122.7, 122.4, 121.3, 121.2, 117.6, 117.5, 83.0, 82.9, 78.6, 76.1, 76.0, 51.8, 51.6, 29.1, 28.9, 28.8, 28.7, 27.7, 21.1, 20.9, 20.0, 19.9, 14.2, 14.1, 12.8, 12.7, 12.4, 12.1, 11.9 ppm; MS (EI, 70 eV): *m/z* (%) 679 (M^+−Boc, 3), 455 (39), 316 (19), 260 (11), 205 (27), 120 (100), 57 (37); HRMS: *m/z* calcd for $C_{39}H_{45}N_3O_{12}SNa$: 802.2622 [M+Na]$^+$, found: 802.2628.

1-(benzylamino)carbonyl-1-[6-(*tert*-butoxycarbonyl)oxy-2,5,7,8-tetramethylchroman-2-yl]methyl 3-(3-phenylsulfonyl-[1,2,5]oxadiazol-4-yl *N*^{2}-oxide)oxybenzoate (4g): This compound was obtained by method A. The crude product was purified by flash column chromatography (SiO_2, hexane/EtOAc, 7:3) to give the desired product (4g) as a white solid (73%, 2:1 diastereomeric mixture); mp: 68–70 °C; major diastereomer: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.03–8.01 (m, 2H), 7.96–7.92 (m, 2H), 7.71 (t, J = 7.5 Hz, 1H), 7.59–7.55 (m, 2H), 7.49–7.47 (m, 2H), 7.23–7.11 (m, 5H), 6.40 (bs, 1H), 5.28 (s, 1H), 4.44–4.41 (m, 2H), 2.71–2.54 (m, 2H), 2.09–2.00 (m, 1H), 1.96 (s, 3H), 1.94 (s, 3H), 1.92–1.87 (m, 1H), 1.75 (s, 3H), 1.47 (s, 9H), 1.41 ppm (s, 3H); minor diastereomer: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.03–8.01 (m, 2H), 7.96–7.92 (m, 2H), 7.71 (t, J = 7.5 Hz, 1H), 7.59–7.55 (m, 2H), 7.49–7.47 (m, 2H), 7.23–7.11 (m, 5H), 6.45 (bs, 1H), 5.42 (s, 1H), 4.44–4.41 (m, 2H), 2.71–2.54 (m, 2H), 2.09–2.00 (m, 1H), 1.98 (s, 3H), 1.95 (s, 3H), 1.92–1.87 (m, 1H), 1.74 (s, 3H), 1.47 (s, 9H), 1.36 ppm (s, 3H); diastereomeric mixture: ^{13}C NMR (100 MHz, $CDCl_3$): δ = 166.7, 166.5, 164.2, 164.0, 158.1, 152.6, 152.2, 147.8, 147.6, 141.9, 141.8, 137.8, 137.6, 136.0, 131.6, 131.5, 130.4, 130.3, 129.9, 128.8, 128.7, 128.2, 128.0, 127.9, 127.7, 127.6, 126.0, 125.8, 125.1, 125.0, 122.9, 122.6, 121.3, 121.2, 117.4, 83.0, 82.9, 78.6, 76.7, 76.1, 75.9, 43.8, 43.6, 29.1, 28.7, 27.7, 20.9, 20.7, 19.9, 19.8, 12.7, 12.0, 11.9, 11.8 ppm; MS (EI, 70 eV): *m/z* (%) 629 (2), 489 (23), 350 (28), 205 (15), 121 (100), 91 (56); HRMS: *m/z* calcd for $C_{42}H_{43}N_3O_{12}SNa$: 836.2465 [M+Na]$^+$, found: 836.2438.

1-[6-(*tert*-butoxycarbonyl)oxy-2,5,7,8-tetramethylchroman-2-yl]-1-[(cyclohexylamino)carbonyl]methyl 3-(3-phenylsulfonyl-[1,2,5]oxadiazol-4-yl *N*^{2}-oxide)oxybenzoate (4h): This compound was obtained by method A. The crude product was purified by flash column chromatography (SiO_2, hexane/EtOAc, 7:3) to give the desired product (**4h**) as a white solid (89%, 2:1 diastereomeric mixture); mp: 72–74 °C; major diastereomer: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.04–8.02 (m, 2H), 7.95–7.92 (m, 2H), 7.73–7.69 (m, 1H), 7.60–7.56 (m, 2H), 7.51–7.48 (m, 2H), 6.00 (d, J 8.5 Hz, 1H), 5.21 (s, 1H), 3.78–3.69 (m, 1H), 2.71–2.53 (m, 2H), 2.05 (s, 3H), 2.02 (s, 3H), 1.97 (s, 3H), 1.89–1.85 (m, 2H), 1.65–1.62 (m, 2H), 1.56–1.52(m, 2H), 1.48 (s, 9H), 1.40 (s, 3H), 1.30–1.18 (m, 2H), 1.12–1.02 ppm (m, 4H); minor diastereomer: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.04–8.02 (m, 2H), 7.95–7.92 (m, 2H), 7.73–7.69 (m, 1H), 7.60–7.56 (m, 2H), 7.51–7.48 (m, 2H), 6.03 (d, J 8.5 Hz, 1H), 5.35 (s, 1H), 3.78–3.69 (m, 1H), 2.71–2.53 (m, 2H), 1.99 (s, 3H), 1.97 (s, 3H), 1.92 (s, 3H), 1.89–1.85 (m, 2H), 1.65–1.62 (m, 2H), 1.56–1.52(m, 2H), 1.48 (s, 9H), 1.34 (s, 3H), 1.30–1.18 (m, 2H), 1.12–1.02 ppm (m, 4H); diastereomeric mixture: ^{13}C NMR (100 MHz, $CDCl_3$): δ = 165.7, 165.6, 164.2, 163.9, 158.1, 156.6, 156.5, 152.2, 152.1, 147.9, 147.8, 141.9, 141.7, 137.9, 137.8, 136.0, 135.9, 131.7, 131.6, 130.5, 130.3, 129.9, 128.7, 128.1, 128.0, 127.9, 127.6, 126.0, 125.8, 125.0, 124.9, 122.8, 122.4, 121.3, 121.2, 117.5, 117.4, 83.0, 82.9, 78.5, 76.6, 76.0, 48.8, 48.4, 33.2, 33.1, 33.0, 32.9, 29.1, 28.7, 27.7, 25.5, 25.4, 25.0, 24.8, 24.7, 22.7, 22.6, 20.9, 20.4, 20.0, 19.9, 12.8, 12.7, 12.4, 12.1, 11.9, 11.4 ppm; MS (EI, 70 eV): *m/z* (%) 621 (M^+−Boc-Cyclohexyl, 2), 481 (M^+−Boc-furoxanyl, 15), 360 (15), 342 (26), 205 (36), 121 (100), 83 (33), 77 (70), 56 (20); HRMS: *m/z* calcd for $C_{41}H_{47}N_3O_{12}SNa$: 828.2778 [M+Na]$^+$, found: 828.2784.

1-[6-(*tert*-butoxycarbonyl)oxy-2,5,7,8-tetramethylchroman-2-yl]-1-(pentylaminocarbonyl)methyl 3-(3-phenylsulfonyl-[1,2,5]oxadiazol-4-yl *N*^{2}-oxide)oxybenzoate (4i): This compound was obtained by method A. The crude product was purified by flash column chromatography (SiO_2, hexane/EtOAc, 7:3) to give the desired product (**4i**) as a colorless oil (88%, 2:1 diastereomeric mixture), which solidifies when stored cold. Major diastereomer: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.04–8.02 (m, 2H), 7.97–7.92 (m, 2H), 7.73–7.69 (m, 1H), 7.59–7.56 (m, 2H), 7.51–7.48 (m, 2H), 6.08 (bs, 1H), 5.24 (s, 1H), 3.27–3.19 (m, 2H), 2.76–2.55 (m, 2H), 2.09–2.04 (m, 1H), 2.05 (s, 3H), 2.02 (s, 3H), 1.97 (s, 3H), 1.89–1.86 (m, 1H), 1.48 (s, 9H), 1.45–1.42 (m, 2H), 1.41 (s, 3H), 1.23–1.18 (m, 4H), 0.80–0.76 ppm (m, 3H); minor diastereomer: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.04–8.02 (m, 2H), 7.97–7.92 (m, 2H), 7.73–7.69 (m, 1H), 7.59–7.56 (m, 2H), 7.51–7.48 (m, 2H), 6.17 (bs, 1H), 5.39 (s, 1H), 3.27–3.19 (m, 2H), 2.76–2.55 (m, 2H), 2.09–2.04 (m, 1H), 1.99 (s, 3H), 1.97 (s, 3H), 1.90 (s, 3H), 1.89–1.86 (m, 1H), 1.48 (s, 9H), 1.45–1.42 (m, 2H), 1.35 (s, 3H), 1.23–1.18 (m, 4H), 0.80–0.76 ppm (m, 3H); diastereomeric mixture: ^{13}C NMR (100 MHz, $CDCl_3$): δ = 166.6, 166.5, 164.2, 163.8, 158.1, 152.6, 152.5, 152.2, 152.1, 147.9, 147.8, 141.9, 141.8, 137.9, 137.8, 136.0, 135.9, 131.6, 130.5, 130.4, 129.9, 128.7, 128.1, 127.9, 127.6, 126.1, 125.8, 125.1, 125.0, 122.8, 122.5, 121.3, 121.2, 117.5, 83.0, 82.9, 78.5, 76.7, 76.0, 75.9, 39.7, 39.5, 29.2, 29.1, 29.0, 28.0, 27.7, 22.3, 20.9, 20.5, 20.0, 19.9, 13.9, 13.8, 12.8, 12.7, 12.1, 11.9, 11.8 ppm; MS (EI, 70 eV): *m/z* (%) 693 (M^+−Boc, 1), 609 (2), 469 (32), 330 (44), 205 (22), 121 (100), 77 (78), 71 (3); HRMS: *m/z* calcd for $C_{40}H_{47}N_3O_{12}SNa$: 816.2778 [M+Na]$^+$, found: 816.2777.

1-[6-(*tert*-butoxycarbonyl)oxy-2,5,7,8-tetramethylchroman-2-yl]-1-((4-methoxyphenyl)aminocarbonyl)methyl 3-(3-phenylsulfonyl-[1,2,5]oxadiazol-4-yl *N*^{2}-oxide)oxybenzoate (4j): This compound was obtained by method A. The crude product was purified by flash column chromatography (SiO_2, hexane/EtOAc, 7:3) to give the desired product (**4j**) as a brownish solid (47%, 2:1 diastereomeric mixture); mp: 79–81 °C; major diastereomer: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.04–8.02 (m, 2H), 8.00–7.95 (m, 2H), 7.72 (bs, 1H), 7.71–7.69 (m, 1H), 7.59–7.55 (m, 2H), 7.52–7.50 (m, 2H), 7.36–7.31 (m, 2H), 6.78–6.75 (m, 2H), 5.34 (s, 1H), 3.71 (s, 3H), 2.74–2.55 (m, 2H), 2.13–2.09 (m, 1H), 2.05 (s, 3H), 2.02 (s, 3H), 1.99 (s, 3H), 1.97–1.92 (m, 1H), 1.49 (s, 9H), 1.48 ppm (s, 3H); minor diastereomer: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.04–8.02 (m, 2H), 8.00–7.95 (m, 2H), 7.83 (bs, 1H), 7.71–7.69 (m, 1H), 7.59–7.55 (m, 2H), 7.52–7.50 (m, 2H), 7.36–7.31 (m, 2H), 6.78–6.75 (m, 2H), 5.51 (s, 1H), 3.70 (s, 3H), 2.74–2.55 (m, 2H), 2.13–2.09 (m, 1H), 2.01 (s, 3H), 2.00 (s, 3H), 1.98 (s, 3H), 1.97–1.92 (m, 1H), 1.49 (s, 9H), 1.41 ppm (s, 3H); diastereomeric mixture: ^{13}C NMR (100 MHz, $CDCl_3$): δ = 164.6, 164.2, 163.9, 158.0, 156.7, 152.2, 147.6, 142.0, 141.9, 137.8, 136.0, 131.5, 131.4, 130.5, 130.4, 130.1, 129.9, 128.2, 128.1, 126.2, 125.9, 125.2, 125.1, 122.8, 122.6, 121.7, 121.6, 121.4, 121.3, 117.5, 114.2, 83.1, 83.0, 76.5, 76.4, 76.1, 55.5, 29.0, 27.7, 20.9, 20.0, 19.9, 12.8, 12.3, 12.1, 11.9 ppm; MS (EI, 70 eV): *m/z* (%) 505 (2), 468 (2), 469 (32), 330 (44), 205 (15), 121 (41), 108 (15), 77 (100); HRMS: *m/z* calcd for $C_{42}H_{43}N_3O_{13}SNa$: 852.2414 [M+Na]$^+$, found: 852.2407.

1-phenyl-1-(*tert*-butylaminocarbonyl)methyl 3-(3-phenylsulfonyl-[1,2,5]oxadiazol-4-yl *N*^{2}-oxide)oxybenzoate (4k): This compound was obtained by method A. The crude product was purified by flash column chromatography (SiO_2, hexane/EtOAc, 7:3) to give the desired product (**4k**) as a white solid (87%); mp: 172–174 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.13–8.10 (m, 2H), 8.08–8.06 (m, 1H), 8.03–8.02 (m, 1H), 7.83–7.79 (m, 1H), 7.69–7.65 (m, 2H), 7.60–7.59

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(m, 2H), 7.55–7.53 (m, 2H), 7.46–7.41 (m, 3H), 6.20 (s, 1H), 5.85 (s, 1H), 1.38 ppm (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 166.9, 163.8, 158.0, 152.5, 137.7, 136.0, 135.5, 131.6, 130.4, 129.9, 129.2, 128.9, 128.7, 128.1, 127.6, 125.0, 121.2, 110.7, 76.5, 51.8, 28.7 ppm; HRMS: *m/z* calcd for $C_{27}H_{25}N_3O_8SNa$: 574.1260 [*M*+Na]$^+$, found: 574.1261.

1-(6-(*tert*-butoxycarbonyl)oxy-2,5,7,8-tetramethylchroman-2-yl)-1-(*tert*-butylaminocarbonyl)methyl benzoate (4l): This compound was obtained by method A. The crude product was purified by flash column chromatography (SiO_2, hexane/EtOAc, 85:15) to give the desired product (**4l**) as a colorless oil (97%, 2:1 diastereomeric mixture), which solidifies when stored cold. Major diastereomer: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.11–8.09 (m, 2H), 7.65–7.59 (m, 1H), 7.52–7.47 (m, 2H), 5.96 (s, 1H), 5.21 (s, 1H), 2.82–2.62 (m, 2H), 2.17 (s, 3H), 2.11 (s, 3H), 2.06 (s, 3H), 2.00–1.90 (m, 2H), 1.58 (s, 9H), 1.49 (s, 3H), 1.39 ppm (s, 9H); minor diastereomer: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.11–8.09 (m, 2H), 7.65–7.59 (m, 1H), 7.52–7.47 (m, 2H), 6.11 (s, 1H), 5.36 (s, 1H), 2.82–2.62 (m, 2H), 2.09 (s, 3H), 2.07 (s, 3H), 2.03 (s, 3H), 2.00–1.90 (m, 2H), 1.58 (s, 9H), 1.45 (s, 3H), 1.36 ppm (s, 9H); diastereomeric mixture: ^{13}C NMR (100 MHz, $CDCl_3$): δ = 166.2, 165.9, 165.4, 165.1, 152.3, 152.2, 148.0, 147.9, 141.7, 141.6, 133.5, 133.4, 129.9, 129.8, 129.5, 129.4, 128.6, 125.5, 127.7, 127.4, 125.9, 125.7, 122.8, 122.5, 117.6, 117.5, 83.0, 82.9, 76.4, 76.1, 51.7, 51.4, 28.7, 28.6, 27.7, 20.8, 20.4, 20.1, 19.9, 12.8, 12.7, 12.4, 12.1, 11.9 ppm; HRMS: *m/z* calcd for $C_{31}H_{41}NO_7Na$: 562.2781 [*M*+Na]$^+$, found: 562.2781.

1-phenyl-1-(*tert*-butylaminocarbonyl)methyl 4-(nitrooxymethyl)-benzoate (4n): This compound was obtained as secondary product on the preparation of the Ugi product **6b**, as a white solid (7%); mp: 106–108 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.14 (d, *J* = 8 Hz, 2H), 7.54–7.51 (m, 5H), 7.43–7.39 (m, 2H), 6.22 (s, 1H), 5.90 (s, 1H), 5.51 (s, 2H), 1.38 ppm (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 167.1, 164.4, 138.0, 135.7, 130.3, 129.6, 129.1, 129.0, 128.9, 128.7, 127.6, 127.5, 76.3, 73.5, 51.7, 28.7 ppm; HRMS: *m/z* calcd for $C_{20}H_{22}N_2O_6Na$: 409.1376 [*M*+Na]$^+$, found: 409.1379.

1-pentyl-1-(*tert*-butylaminocarbonyl)methyl 3-(3-phenylsulfonyl-[1,2,5]oxadiazol-4-yl *N*^{2}-oxide)oxybenzoate (4o): This compound was obtained as secondary product on the preparation of the Ugi product **6e**, as a white solid (54%); mp: 95–97 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.06–8.03 (m, 1H), 7.99–7.93 (m, 2H), 7.76–7.70 (m, 1H), 7.62–7.55 (m, 2H), 7.53–7.51 (m, 1H), 7.17–7.06 (m, 1H), 6.91–6.88 (m, 1H), 5.73 (bs, 1H), 5.21–5.17 (m, 1H), 1.93–1.87 (m, 2H), 1.29 (s, 9H), 1.28–1.17 (m, 4H), 0.84 ppm (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 168.6, 164.1, 158.1, 152.6, 137.8, 136.0, 130.4, 129.9, 129.8, 129.6, 128.6, 127.9, 124.9, 121.1, 110.7, 75.5, 60.6, 28.8, 28.7, 27.0, 22.4, 13.9 ppm; HRMS: *m/z* calcd for $C_{25}H_{29}N_3O_8SNa$: 554.1573 [*M*+Na]$^+$, found: 554.1573.

General procedure for Ugi products (6a–6f). Method A (microwave-assisted): A glass tube suitable for microwaves was charged sequentially with the aldehyde (0.2 mmol), *p*-toluidine (0.2 mmol), the carboxylic acid (0.2 mmol) and *tert*-butylisonitrile (0.2 mmol), under solvent-free conditions or in EtOH (1 mL). The sealed tube was heated for 30 min at 60 °C under microwave irradiation. The reaction mixture was allowed to cool to room temperature, quenched with saturated solution of $NaHCO_3$ and extracted with EtOAc. The combined organic layers were washed with brine, dried with Na_2SO_4, filtered and the solvent was evaporated under reduced pressure. The crude product was purified by flash column chromatography (SiO_2, hexanes/EtOAc). **Method B (ultrasound-assisted)**: A round-bottom flask was charged sequentially with the aldehyde (0.2 mmol), *p*-toluidine (0.2 mmol), the carboxylic acid (0.2 mmol) and *tert*-butylisonitrile (0.2 mmol) under solvent-free conditions or in buffer pH 5.5 (1 mL); then placed in an ultrasound bath preheated to 60 °C. The reaction was exposed for 6 h at 720 W and 50–60 Hz. The reaction mixture was allowed to cool, treated with a saturated solution of $NaHCO_3$, and extracted with EtOAc. Work-up was continued as in Passerini reaction Method A.

***N*-(1-(*tert*-butylaminocarbonyl)-1-(6-*tert*-butoxycarbonyloxy-2,5,7,8-tetramethylchroman-2-yl)methyl)-*N*-(4-methylphenyl)-4-(nitrooxymethyl)phenyl carboxamide (6a)**: This compound was obtained by method A or B. The crude product was purified by flash column chromatography (SiO_2, hexane/EtOAc, 7:3) to give the desired product (**6a**) as a white solid (38–59%, 2:1 diastereomeric mixture); mp: 78–80 °C; major diastereomer: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.21–7.19 (m, 2H), 7.10–7.04 (m, 4H), 6.81–6.80 (m, 2H), 6.73 (bs, 1H), 5.69 (s, 1H), 5.20 (s, 2H), 2.70–2.53 (m, 2H), 2.16 (s, 3H), 2.14–2.12 (m, 1H), 2.03 (s, 3H), 2.01 (s, 3H), 1.97 (s, 3H), 1.96–1.94 (m, 1H), 1.50 (s, 9H), 1.37 (s, 3H), 1.08 ppm (s, 9H); minor diastereomer: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.25–7.24 (m, 2H), 7.10–7.04 (m, 4H), 6.94–6.89 (m, 2H), 6.83 (bs, 1H), 5.51 (s, 1H), 5.23 (s, 2H), 2.70–2.53 (m, 2H), 2.18 (s, 3H), 2.14–2.12 (m, 1H), 2.00 (s, 3H), 1.96–1.94 (m, 1H), 1.91 (s, 3H), 1.83 (s, 3H), 1.48 (s, 9H), 1.35 (s, 3H), 1.14 ppm (s, 9H); diastereomeric mixture: ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.7, 170.3, 165.4, 165.1, 151.2, 151.1, 146.6, 146.3, 140.9, 140.8, 138.8, 138.6, 137.5, 135.4, 136.6, 136.4, 131.9, 130.1, 130.0, 129.0, 128.3, 127.9, 127.8, 127.7, 127.0, 126.7, 125.2, 124.9, 121.5, 121.3, 116.9, 116.6, 82.1, 81.9, 77.8, 77.5, 73.1, 73.0, 67.2, 67.1, 50.3, 50.1, 28.7, 28.4, 28.0, 27.7, 27.6, 27.5, 27.4, 26.7, 19.9, 19.2, 19.0, 11.9, 11.8, 11.7, 11.5, 10.9, 10.8 ppm; MS (EI, 70 eV): *m/z* (%) 703 (M$^+$, 5), 658(11), 603(4), 558(32), 295(14), 205(50), 135(100), 91(21), 57(77); HRMS *m/z* calcd for $C_{39}H_{49}N_3O_9$: 703.3469 [*M*+H]$^+$, found: 703.3518.

***N*-[1-(*tert*-butylamino)carbonyl]-1-(phenylmethyl)-*N*-(4-methylphenyl)-4-(nitrooxymethyl)phenyl carboxamide (6b)**: This compound was obtained by method A. The crude product was purified by flash column chromatography (SiO_2, hexane/EtOAc, 8:2) to give the desired product (**6b**) as a white solid (45%); mp: 128–130 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.37 (d, *J* = 8 Hz, 2H), 7.28–7.23 (m, 5H), 7.17 (d, *J* 8 Hz, 2H), 6.89–6.81 (m, 4H), 6.07 (s, 1H), 5.74 (s, 1H), 5.32 (s, 2H), 2.18 (s, 3H), 1.38 ppm (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.4, 168.5, 138.3, 137.4, 137.1, 134.9, 133.1, 130.1, 129.9, 129.1, 129.0, 128.5, 128.4, 128.1, 74.1, 67.3, 51.7, 28.7, 21.0 ppm; HRMS: *m/z* calcd for $C_{27}H_{29}N_3O_5Na$: 498.2005 [*M*+Na]$^+$, found: 498.2003.

***N*-[1-(*tert*-butylamino)carbonyl]-1-(6-*tert*-butoxycarbonyloxy-2,5,7,8-tetramethylchroman-2-yl) methyl)-*N*-(4-methylphenyl)-3-[3-(phenylsulfonyl)-[1,2,5]oxadiazol-4-yl *N*^{2}-oxide]oxyphenyl carboxamide (6c)**: This compound was obtained by method A. The crude product was purified by flash column chromatography (SiO_2, hexane/EtOAc, 7:3) to give the desired product (**6c**) as a white solid (48%, 2:1 diastereomeric mixture); mp: 77–79 °C; major diastereomer: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.08–8.05 (m, 2H), 7.83–7.76 (m, 1H), 7.69–7.63 (m, 2H), 7.25–7.03 (m, 6H), 6.90 (d, *J* 8 Hz, 2H), 6.83 (s, 1H), 5.77 (s, 1H), 2.81–2.65 (m, 2H), 2.23 (s, 3H), 2.12 (s, 3H), 2.11 (s, 3H), 2.08–2.03 (m, 2H), 1.90 (s, 3H), 1.59 (s, 9H), 1.47 (s, 3H), 1.17 ppm (s, 9H); minor diastereomer: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.08–8.05 (m, 2H), 7.83–7.76 (m, 1H), 7.69–7.63 (m, 2H), 7.25–7.03 (m, 6H), 6.98 (d, *J* 8 Hz, 2H), 6.94 (s, 1H), 5.58 (s, 1H), 2.81–2.65 (m, 2H), 2.25 (s, 3H), 2.10 (s, 3H), 2.08–2.03 (m, 2H), 2.06 (s, 3H), 2.00 (s, 3H), 1.57 (s, 9H), 1.44 (s, 3H), 1.24 ppm (s, 9H); diastereomeric mixture: ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.7, 166.1, 157.9, 152.2, 151.7, 147.6, 141.9, 138.5, 137.9, 135.8, 129.8, 129.4, 129.3, 129.0, 128.6, 127.7, 126.6, 126.3, 122.3, 120.5, 120.0, 117.9, 110.6, 83.1, 78.5, 68.4, 51.4, 51.2, 29.3,

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28.6, 28.5, 27.7, 26.9, 22.7, 21.0, 20.9, 14.1, 12.9, 12.8, 11.9 ppm; HRMS: *m/z* calcd for $C_{46}H_{52}N_4O_{11}SNa$: 891.3251 $[M+Na]^+$, found: 891.3255

***N*-[1-(*tert*-butylaminocarbonyl)-1-phenylmethyl]-*N*-(4-methylphenyl)-3-[3-(phenylsulfonyl)-[1,2,5]oxadiazol-4-yl N^2-oxide]oxyphenyl carboxamide (6d)**: This compound was obtained by method A. The crude product was purified by flash column chromatography (SiO_2, hexane/EtOAc, 8:2) to give the desired product (**6d**) as a white solid (63%); mp: 170–171 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.06–8.04 (m, 2H), 7.82–7.78 (m, 1H), 7.68–7.64 (m, 2H), 7.29–7.13 (m, 9H), 6.88–6.79 (m, 4H), 6.06 (s, 1H), 5.67 (s, 1H), 2.14 (s, 3H), 1.38 ppm (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 169.3, 168.4, 157.9, 151.8, 138.3, 138.0, 137.9, 137.3, 135.9, 134.8, 130.2, 129.9, 129.8, 129.3, 129.2, 128.6, 128.5, 128.4, 126.9, 120.7, 120.2, 110.6, 67.2, 51.7, 28.7, 21.0 ppm; HRMS: *m/z* calcd for $C_{34}H_{32}N_4O_7SNa$: 663.1889 $[M+Na]^+$, found: 663.1891.

***N*-[1-(*tert*-butylaminocarbonyl)-1-(6-*tert*-butoxycarbonyloxy-2,5,7,8-tetramethylchroman-2-yl)methyl]-*N*-(4-methylphenyl)-phenylcarboxamide (6f)**: This compound was obtained by method A. The crude product was purified by flash column chromatography (SiO_2, hexane/EtOAc, 7:3) to give the desired product (**6f**) as a white amorphous solid (32%, 2:1 diastereomeric mixture). Major diastereomer: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.31–7.25 (m, 1H), 7.20–7.08 (m, 6H), 6.89–6.87 (m, 2H), 6.82 (s, 1H), 5.81 (s, 1H), 2.79–2.66 (m, 2H), 2.23 (s, 3H), 2.13–2.08 (m, 2H), 2.12 (s, 3H), 2.10 (s, 3H), 1.93 (s, 3H), 1.58 (s, 9H), 1.46 (s, 3H), 1.16 ppm (s, 9H); minor diastereomer: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.31–7.25 (m, 1H), 7.20–7.08 (m, 6H), 6.97–6.95 (m, 2H), 6.94 (s, 1H), 5.64 (s, 1H), 2.79–2.66 (m, 2H), 2.25 (s, 3H), 2.13–2.08 (m, 2H), 2.09 (s, 3H), 2.06 (s, 3H), 2.01 (s, 3H), 1.57 (s, 9H), 1.45 (s, 3H), 1.21 ppm (s, 9H); diastereomeric mixture ^{13}C NMR (100 MHz, $CDCl_3$): δ = 172.6, 172.2, 166.6, 166.3, 152.3, 152.2, 147.7, 147.4, 141.8, 137.3, 137.1, 136.5, 136.4, 130.1, 129.1, 128.8, 128.3, 127.6, 127.5, 126.2, 125.9, 122.6, 122.4, 118.0, 117.7, 83.1, 83.0, 78.9, 78.6, 51.3, 51.1, 28.6, 28.5, 27.7, 27.6, 21.0, 20.3, 20.0, 12.9, 12.8, 12.5, 11.9, 11.8 ppm; HRMS: *m/z* calcd for $C_{38}H_{48}N_2O_6Na$: 651.3410 $[M+Na]^+$, found: 651.3409

***N*-[1-(*tert*-butylaminocarbonyl)-1-phenylmethyl]-*N*-(4-methylphenyl)-phenylcarboxamide (6g)**: This compound was obtained by method A. The crude product was purified by flash column chromatography (SiO_2, hexane/EtOAc, 8:2) to give the desired product (**6g**) as a white solid (57%); mp: 157–159 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.35–7.25 (m, 7H), 7.21–7.13 (m, 3H), 6.90–6.88 (m, 2H), 6.83–6.81 (m, 2H), 6.07 (s, 1H), 5.86 (s, 1H), 2.18 (s, 3H), 1.39 ppm (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 171.2, 168.7, 138.8, 136.8, 136.2, 135.1, 130.0, 129.8, 129.3, 129.0, 128.6, 128.4, 128.2, 127.6, 67.4, 51.6, 28.7, 21.0 ppm; HRMS: *m/z* calcd for $C_{26}H_{28}N_2O_2Na$: 423.2048 $[M+Na]^+$, found: 423.2051.

General procedure for the phenolic group deprotection (7a–e, 8a–c): Method A (conventional heating): Compound **4a**, **4f**, **4l**, **6a**, **6c** or **6f** (0.07 mmol) was dissolved in 2,2,2-trifluoroethanol (TFE, 0.5 mL) and heated at reflux for 12 h. The reaction mixture was allowed to cool to room temperature, and solvent was evaporated under reduced pressure. The crude product was purified by column chromatography (SiO_2, hexanes/EtOAc). **Method B (microwave-assisted)**: Compound **4g** or **4h** was charged in a glass tube suitable for microwaves (0.07 mmol) and dissolved in 2,2,2-trifluoroethanol (TFE, 0.5 mL). The sealed tube was heated for 15 min at 100 °C under microwave irradiation. The reaction mixture was allowed to cool to room temperature, and solvent was evaporated under reduced pressure. The crude product was purified by column chromatography (SiO_2, hexanes/EtOAc).

1-(*tert*-butylaminocarbonyl)-1-(6-hydroxy-2,5,7,8-tetramethylchroman-2-yl)methyl 4-(nitrooxymethyl)benzoate (7a): This compound was obtained from **4a** by the method A. The crude product was purified by flash column chromatography (SiO_2, hexane/EtOAc, 8:2) to give the desired product (**7a**) as a white solid (58%, 2:1 diastereomeric mixture); mp: 69–71 °C; major diastereomer: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.15 (d, J = 8 Hz, 2H), 7.53–7.50 (m, 2H), 5.95 (s, 1H), 5.51 (s, 2H), 5.20 (s, 1H), 4.46 (s, 1H), 2.85–2.56 (m, 2H), 2.19 (s, 6H), 2.12 (s, 3H), 1.98–1.89 (m, 2H), 1.50 (s, 3H), 1.40 ppm (s, 9H); minor diastereomer: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.15 (d, J = 8 Hz, 2H), 7.53–7.50 (m, 2H), 6.15 (s, 1H), 5.51 (s, 2H), 5.39 (s, 1H), 4.41 (s, 1H), 2.85–2.56 (m, 2H), 2.16 (s, 6H), 2.14 (s, 3H), 2.03 (s, 3H), 1.98–1.89 (m, 2H), 1.42 (s, 3H), 1.36 ppm (s, 9H); diastereomeric mixture: ^{13}C NMR (100 MHz, $CDCl_3$): 166.1, 165.8, 164.9, 164.5, 145.6, 145.4, 144.0, 143.9, 137.8, 137.7, 130.5, 130.4, 128.7, 128.6, 122.3, 121.9, 121.7, 121.4, 119.0, 118.8, 117.5, 117.4, 77.9, 75.9, 75.7, 75.5,73.7, 73.6, 51.7, 51.4, 29.3, 28.7, 28.6, 28.0, 20.8, 20.6, 20.2, 20.1, 12.5, 12.3, 12.0, 11.3 ppm; MS (EI, 70 eV): *m/z* (%) 514 (M^+, 6), 467 (100), 317 (13), 205 (82), 133 (90), 57 (41); HRMS *m/z* calcd for $C_{27}H_{34}N_2O_8$: 514.2315 $[M+H]^+$, found: 514.2329.

1-(*tert*-butylaminocarbonyl)-1-(6-hydroxy-2,5,7,8-tetramethylchroman-2-yl)methyl 3-[((3-phenylsulfonyl-[1,2,5]oxadiazol-4-yl N^2-oxide)oxy]benzoate (7b): This compound was obtained from **4f** by method A. The crude product was purified by flash column chromatography (SiO_2, hexane/EtOAc, 7:3) to give the desired product (**7b**) as a white solid (53%, 2:1 diastereomeric mixture); mp: 91–93 °C; major diastereomer: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.14–8.12 (m, 2H), 8.08–8.05 (m, 1H), 8.01–7.99 (m, 1H), 7.84–7.80 (m, 1H), 7.70–7.66 (m, 2H), 7.60–7.57 (m, 2H), 5.96 (s, 1H), 5.20 (s, 1H), 4.46 (s, 1H), 2.85–2.57 (m, 2H), 2.18 (s, 3H), 2.13 (s, 3H), 2.07 (s, 3H), 1.98–1.91 (m, 2H), 1.50 (s, 3H), 1.40 ppm (s, 9H); minor diastereomer: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.14–8.12 (m, 2H), 8.08–8.05 (m, 1H), 8.01–7.99 (m, 1H), 7.84–7.80 (m, 1H), 7.70–7.66 (m, 2H), 7.60–7.57 (m, 2H), 6.15 (s, 1H), 5.38 (s, 1H), 4.41 (s, 1H), 2.85–2.57 (m, 2H), 2.19 (s, 3H), 2.16 (s, 3H), 2.14 (s, 3H), 1.98–1.91 (m, 2H), 1.42 (s, 3H), 1.36 ppm (s, 9H); diastereomeric mixture: ^{13}C NMR (100 MHz, $CDCl_3$): δ = 165.9, 165.7, 164.2, 163.8, 158.1, 152.4, 145.6, 145.5, 144.0, 143.9, 137.8, 136.0, 131.7, 130.4, 130.3, 129.9, 128.7, 128.2, 128.1, 125.0, 124.9, 122.3, 121.9, 121.7, 121.3, 119.0, 118.8, 117.5, 117.4, 110.7, 78.3, 76.1, 75.6, 75.5, 51.8, 51.5, 29.4, 28.7, 28.6, 28.0, 21.1, 21.0, 20.2, 20.1, 12.5, 12.3, 12.1, 11.3 ppm; MS (EI, 70 eV): *m/z* (%) 595 (5), 454 (20), 316 (17), 205 (9), 121 (100), 77 (48), 57 (24); HRMS: *m/z* calcd for $C_{34}H_{37}N_3O_{10}SNa$: 702.2097 $[M+Na]^+$, found: 702.2093.

1-[(benzylamino)carbonyl]-1-(6-hydroxy-2,5,7,8-tetramethylchroman-2-yl)methyl 3-[(3-phenylsulfonyl-[1,2,5]oxadiazol-4-yl N^2-oxide)oxy]benzoate (7c): This compound was obtained from **4g** by the method B. The crude product was purified by flash column chromatography (SiO_2, hexane/EtOAc, 7:3) to give the desired product (**7c**) as a white solid (25%, 1:1 diastereomeric mixture); mp: 67–69 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.14–8.12 (m, 4H), 8.08–8.03 (m, 2H), 7.99–7.98 (m, 2H), 7.84–7.79 (m, 2H), 7.71–7.65 (m, 4H), 7.60–7.58 (m, 4H), 7.32–7.22 (m, 10H), 6.57 (t, J 5.9 Hz, 1H), 6.49 (t, J 5.9 Hz, 1H), 5.55 (s, 1H), 5,39 (s, 1H), 4.56–4.50 (m, 4H), 4.37 (s, 1H), 4.34 (s, 1H), 2.81–2.60 (m, 2H), 2.14 (s, 3H), 2.13 (s, 3H), 2.12 (s, 3H), 2.11 (s, 3H), 2.09–1.95 (m, 2H), 1.87 (s, 3H), 1.86 (s, 3H), 1,51 (s, 3H), 1.46 ppm (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 172.3, 172.2, 166.9, 166.7, 158.1, 158.0, 152.6, 152.5, 143.9, 143.7, 141.8, 141.6, 137.8, 137.6, 136.0, 135.0, 133.5, 133.3, 130.5, 130.0, 129.9, 128.8, 128.7, 128.6, 128.4, 128.3, 128.2, 128.0, 127.9, 127.8, 127.7, 127.6, 127.5, 125.1, 125.0, 122.4, 122.0, 121.3, 121.2, 119.0, 118.9, 117.4, 117.3, 110.7, 110.6, 78.3, 77.2, 75.6, 75.5,

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43.9, 43.6, 29.3, 28.2, 21.1, 21.0, 20.9, 20.1, 20.0, 14.2, 14.1, 12.2, 11.8, 11.4, 11.3 ppm; HRMS: *m/z* calcd for $C_{37}H_{35}N_3O_{10}SNa$: 736.1941 $[M+Na]^+$, found: 736.1953.

1-[(cyclohexylamino)carbonyl]-1-(6-hydroxy-2,5,7,8-tetramethylchroman-2-yl)methyl 3-[(3-phenylsulfonyl-[1,2,5]oxadiazol-4-yl *N*²-oxide)oxy]benzoate (7d): This compound was obtained from **4h** by the method B. The crude product was purified by flash column chromatography (SiO_2, hexane/EtOAc, 7:3) to give the desired product (**7d**) as a white solid (32%, 1:1 diastereomeric mixture); mp: 69–71 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.15–8.12 (m, 4H), 8.02–7.95 (m, 2H), 7.84–7.80 (m, 2H), 7.72–7.67 (m, 4H), 7.62–7.57 (m, 6H), 6.15 (d, J = 8.6 Hz, 1H), 6.10 (d, J = 8.6 Hz, 1H), 5.46 (s, 1H), 5.31 (s, 1H), 4.41 (s, 1H), 4.35 (s, 1H), 3.88–3.80 (m, 2H), 2.84–2.59 (m, 4H), 2.20 (s, 3H), 2.19 (s, 3H), 2.17 (s, 3H), 2.16 (s, 3H), 2.14 (s, 3H), 2.13 (s, 3H), 2.07–2.04 (m, 2H), 1.99–1.95 (m, 2H), 1.77–1.71 (m, 4H), 1.66–1.60 (m, 8H), 1.50 (s, 3H), 1.44 (s, 3H), 1.39–1.33 (m, 4H), 1.20–1.14 ppm (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 171.2, 171.1, 165.9, 165.7, 158.2, 158.1, 152.5, 152.4, 145.6, 144.1, 143.9, 137.8, 137.7, 136.0, 135.9, 131.1, 131.7, 130.4, 130.3, 129.9, 129.2, 129.1, 128.7, 128.4, 128.3, 128.2, 128.1, 125.0, 124.9, 121.3, 121.2, 119.0, 118.8, 117.5, 117.4, 110.8, 110.7, 78.2, 76.8, 76.2, 76.1, 48.8, 48.3, 33.2, 33.1, 32.9, 32.8, 28.2, 28.1, 25.5, 25.4, 25.0, 24.7, 21.0, 20.7, 20.2, 20.1, 14.2, 14.1, 12.4, 12.3, 12.2, 12.0, 11.3 ppm; HRMS: *m/z* calcd for $C_{36}H_{39}N_3O_{10}SNa$: 728.2254 $[M+Na]^+$, found: 728.2249.

1-(*tert*-butylamino)carbonyl-1-(6-hydroxy-2,5,7,8-tetramethylchroman-2-yl)methyl benzoate (7e): This compound was obtained from **4l** by method A. The crude product was purified by flash column chromatography (SiO_2, hexane/EtOAc, 8:2) to give the desired product (**7e**) as a yellowish solid (70%, 2:1 diastereomeric mixture); mp: 152–154 °C; major diastereomer: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.13–8.10 (m, 2H), 7.65–7.60 (m, 1H), 7.51–7.46 (m, 2H), 5.96 (s, 1H), 5.21 (s, 1H), 2.86–2.58 (m, 2H), 2.19 (s, 6H), 2.12 (s, 3H), 1.98–1.91 (m, 2H), 1.50 (s, 3H), 1.40 ppm (s, 9H); minor diastereomer: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.13–8.10 (m, 2H), 7.65–7.60 (m, 1H), 7.51–7.46 (m, 2H), 6.16 (s, 1H), 5.40 (s, 1H), 2.86–2.58 (m, 2H), 2.16 (s, 3H), 2.14 (s, 3H), 2.03 (s, 3H), 1.98–1.91 (m, 2H), 1.43 (s, 3H), 1.36 ppm (s, 9H); diastereomeric mixture: ^{13}C NMR (100 MHz, $CDCl_3$): δ = 166.4, 166.1, 165.5, 165.1, 145.6, 145.4, 144.1, 144.0, 133.5, 133.4, 129.9, 129.8, 129.5, 129.4, 128.6, 128.5, 122.4, 121.9, 121.7, 121.4, 119.1, 118.8, 117.4, 117.3, 77.7, 77.3, 75.9, 75.6, 51.6, 51.4, 29.2, 28.7, 28.6, 28.1, 20.8, 20.5, 20.3, 20.1, 12.5, 12.3, 12.2, 12.0, 11.4, 11.3 ppm; HRMS: *m/z* calcd for $C_{26}H_{33}N_2O_5Na$: 462.2256 $[M+Na]^+$, found: 462.2258.

***N*-[1-(*tert*-butylaminocarbonyl)-1-(6-hydroxy-2,5,7,8-tetramethylchroman-2-yl)methyl]-*N*-(4-methylphenyl)-4-[(nitrooxy)methyl]phenylcarboxamide (8a)**: This compound was obtained from **6a** by method A. The crude product was purified by flash column chromatography (SiO_2, hexane/EtOAc, 7:3) to give the desired product (**8a**) as a white solid (58%, 2:1 diastereomeric mixture); mp: 76–78 °C; major diastereomer: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.36–7.28 (m, 2H), 7.22–7.12 (m, 4H), 7.05 (s, 1H), 6.91–6.89 (m, 2H), 5.79 (s, 1H), 5.29 (s, 2H), 4.53 (s, 1H), 2.83–2.67 (m, 2H), 2.25 (s, 3H), 2.21 (s, 3H), 2.21 (s, 3H), 2.18 (s, 3H), 2.11–2.04 (m, 2H), 1.94 (s, 3H), 1.46 (s, 3H), 1.17 ppm (s, 9H); minor diastereomer: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.36–7.28 (m, 2H), 7.22–7.12 (m, 4H), 7.20 (s, 1H), 6.99–6.97 (m, 2H), 5.63 (s, 1H), 5.32 (s, 2H), 4.47 (s, 1H), 2.83–2.67 (m, 2H), 2.28 (s, 3H), 2.14 (s, 3H), 2.11–2.04 (m, 2H), 2.02 (s, 3H), 1.75 (s, 3H), 1.44 (s, 3H), 1.21 ppm (s, 9H); diastereomeric mixture: ^{13}C NMR (100 MHz, $CDCl_3$): δ = 166.5, 166.3, 162.3, 162.1, 152.3, 152.1, 145.7, 143.8, 143.4, 137.6, 132.8, 130.1, 129.2, 128.8, 128.1, 122.0, 121.6, 119.2, 118.9, 117.7, 117.4, 78.3, 78.1, 74.1, 51.3, 28.8, 28.5, 21.0, 20.8, 20.5, 20.4, 20.2, 13.0, 12.6, 12.4, 12.3, 11.4, 11.3 ppm; MS (EI, 70 eV): *m/z* (%) 603 (M^+, 6), 556 (52), 514 (19), 205 (100), 133 (81), 91 (21), 57 (26); HRMS *m/z* calcd for $C_{34}H_{41}N_3O_7$ 603.2945 $[M+H]^+$, found: 603.2934.

***N*-[1-(*tert*-butylaminocarboyl)-1-(6-hydroxy-2,5,7,8-tetramethylchroman-2-yl)methyl]-*N*-(4-methylphenyl)-3-(3-phenylsulfonyl-[1,2,5]oxadiazol-4-yl *N*²-oxide)oxyphenyl carboxamide (8b)**: This compound was obtained from **6c** by the general procedure. The crude product was purified by flash column chromatography (SiO_2, hexane/EtOAc, 7:3) to give the desired product (**8b**) as a white solid (50%, 2:1 diastereomeric mixture); mp: 60–62 °C; major diastereomer: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.09–8.04 (m, 2H), 7.84–7.75 (m, 1H), 7.69–7.62 (m, 2H), 7.26–6.97 (m, 8H), 6.90 (s, 1H), 5.76 (s, 1H), 4.48 (s, 1H), 2.82–2.66 (m, 2H), 2.23 (s, 3H), 2.20 (s, 3H), 2.18 (s, 3H), 2.10–2.04 (m, 2H), 1.92 (s, 3H), 1.46 (s, 3H), 1.17 ppm (s, 9H); minor diastereomeric: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.09–8.04 (m, 2H), 7.84–7.75 (m, 1H), 7.69–7.62 (m, 2H), 7.26–6.97 (m, 8H), 6.88 (s, 1H), 5.60 (s, 1H), 4.43 (s, 1H), 2.82–2.66 (m, 2H), 2.25 (s, 3H), 2.18 (s, 3H), 2.14 (s, 3H), 2.10–2.04 (m, 2H), 2.00 (s, 3H), 1.43 (s, 3H), 1.22 ppm (s, 9H); diastereomeric mixture: ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.7, 166.2, 158.0, 151.6, 145.7, 143.7, 138.5, 137.9, 137.8, 136.7, 165.9, 130.9, 129.8, 129.3, 129.0, 128.6, 126.7, 121.9, 121.7, 120.5, 120.1, 117.6, 110.6, 78.1, 77.2, 68.3, 30.1, 29.3, 28.8, 28.5, 20.9, 20.8, 20.1, 19.2, 12.9, 12.3, 11.4 ppm; HRMS: *m/z* calcd for $C_{41}H_{44}N_4O_9SNa$: 791.2727 $[M+Na]^+$, found: 791.2722.

***N*-[1-(*tert*-butylaminocarbonyl)-1-(6-hydroxy-2,5,7,8-tetramethylchroman-2-yl)methyl]-*N*-(4-methylphenyl)phenylcarboxamide (8c)**: This compound was obtained from **6f** by method A. The crude product was purified by flash column chromatography (SiO_2, hexane/EtOAc, 7:3) to give the desired product (**8c**) as a white solid (54%, 2:1 diastereomeric mixture); mp: 80–82 °C; major diastereomer: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.32–7.21 (m, 3H), 7.17–7.09 (m, 4H), 7.08 (s, 1H), 6.90 (d, J = 8 Hz, 2H), 5.82 (s, 1H), 2.82–2.66 (m, 2H), 2.24 (s, 3H), 2.21 (s, 3H), 2.18 (s, 3H), 2.11–2.05 (m, 2H), 1.95 (s, 3H), 1.46 (s, 3H), 1.17 ppm (s, 9H); minor diastereomer: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.32–7.21 (m, 3H), 7.17–7.09 (m, 4H), 7.04 (s, 1H), 6.98 (d, J = 8 Hz, 2H), 5.66 (s, 1H), 2.82–2.66 (m, 2H), 2.27 (s, 3H), 2.18 (s, 3H), 2.14 (s, 3H), 2.11–2.05 (m, 2H), 2.03 (s, 3H), 1.45 (s, 3H), 1.20 ppm (s, 9H); diastereomeric mixture: ^{13}C NMR (100 MHz, $CDCl_3$): δ = 172.5, 171.7, 166.7, 166.4, 145.6, 137.2, 136.4, 136.1, 134.0, 133.6, 131.2, 130.2, 129.1, 129.0, 128.8, 127.5, 122.0, 121.6, 119.2, 119.0, 117.7, 78.1, 68.2, 51.2, 51.0, 31.7, 28.5, 22.7, 21.0, 20.8, 20.2, 13.0, 12.3, 11.4, 11.3 ppm; HRMS: *m/z* calcd for $C_{33}H_{40}N_2O_4Na$: 551.2886 $[M+Na]^+$, found: 551.2888.

Cell culture

Human breast cancer cell line T-47D (ATCC, HTB-133) was grown in RPMI 1640 supplemented with 10% (*v/v*) heat-inactivated FCS. The human solid tumor cell lines A549, HBL-100, HeLa, SW1573, and WiDr were a kind gift from Prof. G. J. Peters (VU Medical Center, Amsterdam, The Netherlands). Cells were cultured in RPMI 1640 supplemented with 5% heat-inactivated FCS and 2 mM L-glutamine. All cells were routinely propagated in 25 cm² tissue culture flasks at 37 °C in a 5% CO_2, 95% humidified air incubator until reaching approximately 70% confluence. Cells were subsequently trypsinized, adjusted for concentration, and used for different experimental settings. For all described assays, cells were cultured for less than twenty passages.

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Antiproliferative activity

All the synthesized compounds were screened for antiproliferative activity against A549, HBL-100, HeLa, SW1573, T-47D and WiDr cell lines using the SRB assay.[24] Exponentially growing cells were trypsinized and resuspended in antibiotic-containing medium (100 units penicillin G and 0.1 mg of streptomycin per mL). Single cell suspensions displaying >97% viability as determined by trypan blue dye exclusion were subsequently counted. After counting, dilutions were made to give the appropriate cell densities for inoculation onto 96-well microtiter plates. Cells were inoculated at densities of 2500 (A549, HBL-100, HeLa and SW1573) and 5000 (T-47D and WiDr) cells per well, in a final volume of 100 μL, based on their doubling times. Compounds were initially dissolved in DMSO at 40 mM and tested in triplicate at different dilutions in the range of 1 to 100 μM. Control cells were exposed to an equivalent concentration of DMSO (0.25% *v/v*, negative control). The drug treatment started on day 1 after plating. Drug incubation times were 48 h, after which cells were precipitated with 25 μL ice-cold TCA (50% *w/v*) and fixed for 60 min at 4 °C. Then the SRB assay was performed. The optical density (OD) of each well was measured at 530 nm, using a BioTek PowerWave XS Absorbance Microplate Reader. Values were corrected for background OD from wells only containing medium. Standard anticancer drugs cisplatin and etoposide were used as positive controls.

HaCaT cells were seeded in a 96-well plate at densities of 10×10^3 per well with Dulbecco's modified Eagle medium, supplemented with 10% FBS and 1% penicillin-streptomycin, and allowed to attach for 24 h in a humidified 5% CO_2 atmosphere at 37 °C. HaCaT cells were kindly provided by Virology Section, Facultad de Ciencias, UdelaR (Montevideo, Uruguay). Afterward, the culture milieu was removed and compounds solubilized in DMSO were added at the desired final concentrations (0.78–100.0 μM) diluted in fresh culture medium (200 μL final volume) in triplicate. Controls with medium and 0.25% DMSO were included in each experiment. The cells were further incubated at 37 °C and 5% CO_2 for 48 h. After compound incubation, the culture medium remaining in the multi-well plate was discarded, each well was washed with 200 μL of PBS at room temperature, cells were fixed and the SRB assay was performed. The GI_{50} was determined as the concentration that decreases absorbance 50% compared with the control (1% DMSO) as determined by linear regression analysis. Each assay was reproduced at least three times.

Nitric oxide-releasing activity

The HeLa (cervical adenocarcinoma) cell line was cultured in MEM, supplemented with 10% FBS and 1% penicillin-streptomycin. Cell cultures were maintained under a humidified 5% CO_2 atmosphere at 37 °C. Cells were seeded in a 96-well plate at densities of 10×10^3 per well and allowed to attach for 24 h in a humidified 5% CO_2 atmosphere at 37 °C. Afterward, the culture milieu was removed and the solubilized compounds in DMSO were added at the desired final concentrations (100 μM) diluted in fresh culture medium (200 μL final volume) in triplicate. Controls with medium and 0.25% DMSO were included in each experiment. The cells were further incubated at 37 °C and 5% CO_2, and the NO production was measured as the nitrite content in the culture medium at 3 h and 6 h by the Griess reaction. To develop this assay, 50 μL of culture medium was transferred to a new multi-well plate. At the same time, a reference curve with $NaNO_2$ was performed at serial dilutions between 0 and 100 μM in 50 μL of culture medium. Then, 50 μL of 1% sulfanilamide solution in 5% phosphoric acid was added to each well and incubated for 10 min protected from light. Then 50 μL of 0.1% *N*-1-naphthylethylendiamine dihydrochloride in water was added and the plates were incubated for another 10 min in the dark. The absorbance was measured at 540 nm using a microplate spectrophotometer (Varioskan Flash Microplate spectrophotometer; Thermo Fisher, Vantaa, Finland). NO levels were determined in at least three independent experiments using the nitrate standard curve. Statistical analysis was carried out using two-way analysis of variance (ANOVA).

Antiproliferative activity in the presence of hemoglobin

To investigate the contribution of NO to the antiproliferative activity of the studied compounds, we tested their effect on HeLa cancer cell growth in the absence and presence of Hb. Cells were seeded into a 96-well plate and the next day, they were pretreated with Hb (0, 25, or 100 μM) for 1 h and then treated with 25 μM of the selected compounds for 48 h. Later, the culture medium was discarded, and cells were fixed with a MeOH/AcOH solution, followed by the SRB assay. Statistical analysis was carried out using two-way analysis of variance (ANOVA).

Acknowledgements

This work was supported by Agencia Nacional de Investigación e Innovación (ANII-Fondo Clemente Estable, FCE-2-2011-1-5717), PEDECIBA-Química, Uruguay. We thank ANII for the scholarship to M.I. and L.C.

Conflict of interest

The authors declare no conflict of interest.

Keywords: antiproliferative agents · green chemistry · microwave-assisted synthesis · multicomponent reactions · solvent-free

[1] *Cancer*, WHO Cancer Fact Sheets, September 12, 2018, World Health Organization: http://www.who.int/news-room/fact-sheets/detail/cancer (accessed March 27, 2019).
[2] P. Hanly, I. Soerjomataram, L. Sharp, *Int. J. Cancer* **2015**, *136*, E136–E145.
[3] E. Ruijter, R. Scheffelaar, R. V. A. Orru, *Angew. Chem. Int. Ed.* **2011**, *50*, 6234–6246; *Angew. Chem.* **2011**, *123*, 6358–6371.
[4] J. M. Fortunak, *Future Med. Chem.* **2009**, *1*, 571–575.
[5] H. Sneddon, *Future Med. Chem.* **2014**, *6*, 1373–1376.
[6] R. C. Cioc, E. Ruijter, R. V. A. Orru, *Green Chem.* **2014**, *16*, 2958–2975.
[7] A. Domling, I. Ugim, *Angew. Chem. Int. Ed.* **2000**, *39*, 3168–3210; *Angew. Chem.* **2000**, *112*, 3300–3344.
[8] I. Akritopoulou-Zanze, *Curr. Opin. Chem. Biol.* **2008**, *12*, 324–331.
[9] T. Zarganes-Tzitzikas, A. Dömling, *Org. Chem. Front.* **2014**, *1*, 834–837.
[10] T. Zarganes-Tzitzikas, A. L. Chandgude, A. Dömling, *Chem. Rec.* **2015**, *15*, 981–996.
[11] B. M. Trost, *Science* **1991**, *254*, 1471–1477.
[12] L. Weber, *Curr. Med. Chem.* **2002**, *9*, 2085–2093.
[13] M. C. Pirrung, K. Das Sarma, *J. Am. Chem. Soc.* **2004**, *126*, 444–445.
[14] M. C. Pirrung, K. Das Sarma, *Tetrahedron* **2005**, *61*, 11456–11472.
[15] M. Ingold, G. V. López, W. Porcal, *ACS Sustainable Chem. Eng.* **2014**, *2*, 1093–1097.
[16] X. Fan, Y. Li, X. Zhang, G. Qu, J. Wang, *Can. J. Chem.* **2006**, *84*, 794–799.
[17] N. Liu, S. Cao, J. Wu, J. L. Yu, L. Shen, X. Feng, X. H. Qian, *Tetrahedron* **2008**, *64*, 3966–3974.

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[18] D. Koszelewski, W. Szymanski, J. Krysiak, R. Ostaszewski, *Synth. Commun.* **2008**, *38*, 1120 – 1127.
[19] A. F. S. Barreto, O. E. Vercillo, C. K. Z. Andrade, *J. Braz. Chem. Soc.* **2011**, *22*, 462 – 467.
[20] T. Bousquet, M. Jida, M. Soueidan, R. Deprez-Poulain, F. Agbossou-Niedercorn, L. Pelinski, *Tetrahedron Lett.* **2012**, *53*, 306 – 308.
[21] C. Hulme, S. Chappeta, J. Dietrich, *Tetrahedron Lett.* **2009**, *50*, 4054 – 4057.
[22] J. Tye, M. Whittaker, *Org. Biomol. Chem.* **2004**, *2*, 813 – 815.
[23] C. Cui, C. Zhu, X. J. Du, Z. P. Wang, Z. M. Li, W. Zhao, *Green Chem.* **2012**, *14*, 3157 – 3163.
[24] S. Narayan, J. Muldoon, M. G. Finn, V. V. Fokin, H. C. Kolb, K. B. Sharpless, *Angew. Chem. Int. Ed.* **2005**, *44*, 3275 – 3279; *Angew. Chem.* **2005**, *117*, 3339 – 3343.
[25] M. Ingold, R. Dapueto, S. Victoria, G. Galliussi, C. Batthyány, M. Bollati-Fogolín, D. Tejedor, F. García-Tellado, J. M. Padrón, W. Porcal, G. V. López, *Eur. J. Med. Chem.* **2018**, *143*, 1888 – 1902.
[26] N. S. Bryan, *Future Sci. OA* **2015**, *1*, FSO48.
[27] A. Horton, I. T. Schiefer, *Nitric Oxide* **2019**, *84*, 69 – 78.
[28] R. A. M. Serafim, F. G. Pernichelle, E. I. Ferreira, *Expert Opin. Drug Discovery* **2017**, *12*, 941 – 953.
[29] S. Huerta, *Future Sci. OA* **2015**, *1*, FSO44.
[30] Z. Huang, J. Fu, Y. Zhang, *J. Med. Chem.* **2017**, *60*, 7617 – 7635.
[31] L. L. Fershtat, N. N. Makhova, *ChemMedChem* **2017**, *12*, 622 – 638.
[32] R. A. M. Serafim, M. C. Primi, G. H. Trossini, E. I. Ferreira, *Curr. Med. Chem.* **2012**, *19*, 386 – 405.
[33] J. Rodríguez-Duarte, R. Dapueto, G. Galliussi, L. Turell, A. Kamaid, N. K. H. Khoo, F. J. Schopfer, B. A. Freeman, C. Escande, C. Batthyány, G. Ferrer-Sueta, G. V. López, *Sci. Rep.* **2018**, *8*, 12784.
[34] S. Endres, A. Hacker, E. Noack, G. Kojda, J. Lehmann, *Eur. J. Med. Chem.* **1999**, *34*, 895 – 901.
[35] L. A. Dutra, L. de Almeida, T. G. Passalacqua, J. S. Reis, F. A. Torres, I. Martinez, R. G. Peccinini, C. M. Chin, K. Chegaev, S. Guglielmo, R. Fruttero, M. A. Graminha, J. L. dos Santos, *Antimicrob. Agents Chemother.* **2014**, *58*, 4837 – 4847.
[36] M. Ingold, R. Dapueto, G. V. López, W. Porcal, *Educación Química* **2016**, *27*, 15.
[37] R. C. McElroy, A. Constantinou, L. C. Jones, L. C. Summerton, J. H. Clark, *Green Chem.* **2015**, *17*, 3111 – 3121.
[38] M. Ingold, L. Colella, R. Dapueto, G. V. López, W. Porcal, *World J. Chem. Educ.* **2017**, *5*, 153 – 157.
[39] J. Choy, S. Jaime-Figueroa, T. Lara-Jaime, *Tetrahedron Lett.* **2010**, *51*, 2244 – 2246.
[40] A. Dandia, R. Singh, J. Joshi, S. Kumari, *Mini-Rev. Org. Chem.* **2014**, *11*, 462 – 476.
[41] O. N. Burov, M. E. Kletskii, N. S. Fedik, A. V. Lisovin, S. V. Kurbatov, *Chem. Heterocycl. Compd.* **2015**, *51*, 951 – 960.
[42] B. J. McDonald, B. M. Bennett, *Can. J. Physiol. Pharmacol.* **1990**, *68*, 1552 – 1557.
[43] H. Li, X. Liu, H. Cui, Y.-R. Chen, A. J. Cardounel, J. L. Zweier, *J. Biol. Chem.* **2006**, *281*, 12546 – 12554.
[44] H. Li, H. Cui, X. Liu, J. L. Zweier, *J. Biol. Chem.* **2005**, *280*, 16594 – 16600.
[45] Z. Chen, J. Zhang, J. S. Stamler, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 8306 – 8311.

Manuscript received: June 26, 2019
Revised manuscript received: July 25, 2019
Accepted manuscript online: July 29, 2019
Version of record online: August 23, 2019

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