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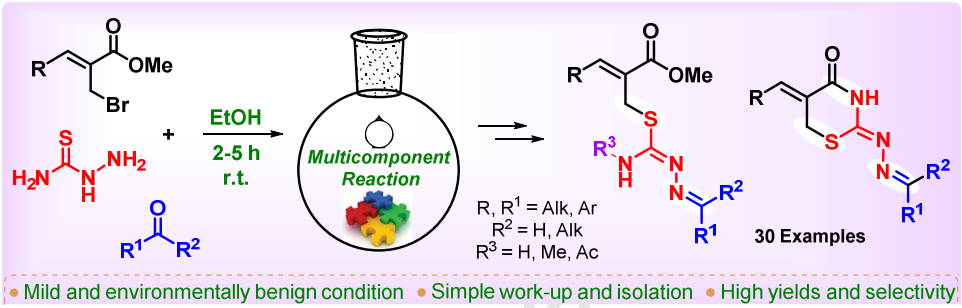

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Synthesis of isothiosemicarbazones of potential antitumoral activity through a multicomponent reaction involving allylic bromides, carbonyl compounds and thiosemicarbazide

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ABSTRACT

A convenient route for the synthesis of isothiosemicarbazones and 2-hydrazono-1,3-thiazin-4-ones through a multicomponent reaction featuring allylic bromides, carbonyl compounds and thiosemicarbazides is described. The transformations proceed under mild and environmentally benign conditions with high yields and stereoselectivity. All novel compounds were obtained in high purity without the need for chromatography stages. Different functional groups are well tolerated, including halogen, alkoxy, nitro and unsaturated groups. This simple protocol offers straightforward access to a wide range of highly functionalized substances with pharmacologically privileged structures. All compounds were fully characterized and the structural assignment of key products was unequivocally confirmed by X-ray diffraction analysis. Selected isothiosemicarbazones were screened against a triple-negative breast cancer cell line and some compounds were shown to reduce the number of live tumoral cells (41-54%).

1. Introduction

The growing concern with regard to environmental protection has brought about the paradigm of green chemistry. This approach favors the development of new methodologies that are not only high yielding but also environmentally benign and highly efficient. In principle, these goals can be achieved with the appropriate selection of starting materials and reaction conditions, as well as a re-evaluation of the work-up, separation and purification procedures [1].

The use of environmentally acceptable reagents and solvents coupled with a reduction in the number of synthetic steps are requirements to develop more sustainable synthetic methods. In this context, multicomponent reactions (MCRs) and domino processes have become popular tools among synthetic chemists, in which several steps of bond formation are performed in a single reaction flask without the need for the separation or isolation of intermediates to give complex molecular structures from simple and readily available substrates [2]. Besides saving time, energy and resources by avoiding purification stages between individual steps within a multistep synthesis, MCRs are generally atom-economic transformations that can be carried out at room temperature in non-toxic solvents with reduced generation of waste, in accordance with the principles of green chemistry. Due to the advantages of MCRs over traditional multi-step synthesis in creating structural diversity efficiently, they have been successfully applied in programs devoted to drug discovery conducted by pharmaceutical companies.

Functionalized allylic bromides derived from the Morita-Baylis-Hillman (MBH) reaction [3] are versatile building blocks widely used in modern synthetic organic chemistry. Due to the highly electrophilic nature of allylic bromides, combined with straightforward methods for their preparation [4], these molecules are extremely useful substrates for nucleophilic displacement with a range of C-, O-, N-, P-, S-, and Se-nucleophiles [5,6]. In the particular case of reactions involving allylic bromides and 1,3-bidentate nucleophiles, such as thioureas and thiosemicarbazides, the resulting products are isothiouronium salts **A**, isothiosemicarbazides **B** and cyclic analogues **C**, which display pronounced antitumor activity in leukemia and melanoma models [7] (Fig. 1).

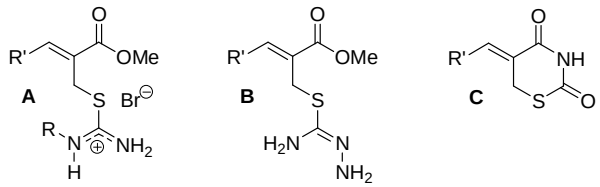

Fig. 1. Structures of isothiouronium salts **A**, isothiosemicarbazides **B** and 1,3-thiazines **C** of biological importance.

In fact, thiosemicarbazones [8] (**I**) as well as isothiosemicarbazones [9] (**II** and **III**) have attracted the attention of medicinal chemists due to their wide variety of biological activity, including anticancer, antimicrobial, and antioxidant

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properties, among others (Fig. 2). However, the preparation of isothiosemicarbazones usually involves high temperatures [9], acid catalysts [9b] and/or multistep synthesis [9b-e], eventually requiring the isolation and purification of products through tedious work-up or chromatography [9c-e]. Therefore, milder and greener methods to build up large collections of isothiosemicarbazones and analogues for biological screenings are in high demand.

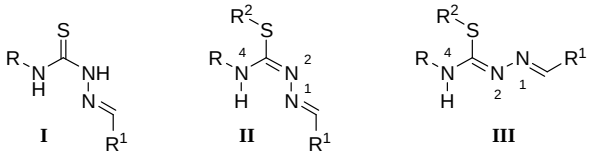


Fig. 2. Representation of thiosemicarbazones (**I**) and isothiosemicarbazones (**II**, **III**).

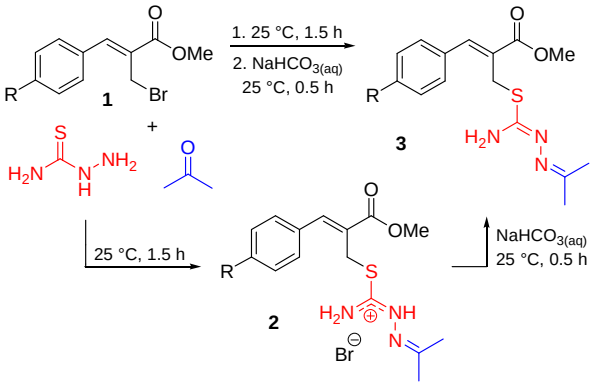
Herein, we present a straightforward method for the synthesis of functionalized isothiosemicarbazones through a novel multicomponent reaction (MCR), involving an allylic bromide **1**, a carbonyl compound and thiosemicarbazide, which is carried out in alcoholic medium at room temperature with no need for a catalyst or additive.

2. Results and discussion

2.1. MCR with acetone

Initially, the synthesis of isothiosemicarbazone salts **2** was performed in a single reaction step, employing the MCR from allylic bromides **1**, thiosemicarbazide and acetone (used in excess to act simultaneously as the carbonyl reagent and the solvent) at room temperature and in the absence of any catalyst or additive (Table 1). Isothiosemicarbazone salts **2a-c** were cleanly obtained in high yields (97-78%) and short reaction times regardless of the substitution pattern (R) at the aromatic ring. The use of an equivalent amount of acetone combined with ethanol, 2-propanol or acetonitrile as the solvent led to similar results. Salts **2** are stable crystalline solids that can be stored for months without significant decomposition. However, basic treatment with aqueous bicarbonate readily converted salts **2** into the neutral isothiosemicarbazones **3**, with good overall yields (84-73% from bromides **1**) and high purity without any further purification.

Table 1
Tricomponent reaction of allylic bromides **1**, isothiosemicarbazide and acetone.

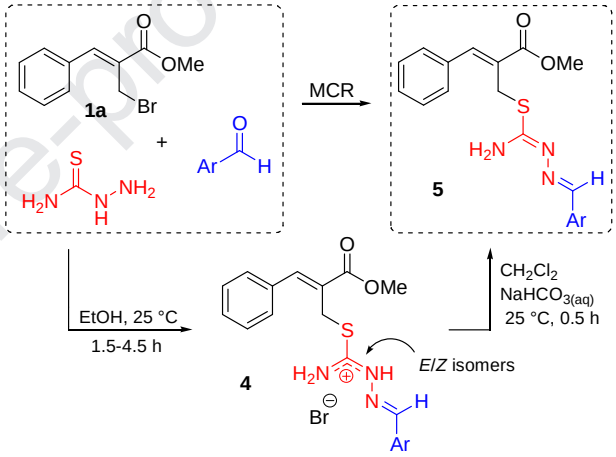


#	Compound	R	Yield 2 (%)[a]	Yield 3 (%)[a]
1	**a**	H	78	94
2	**b**	CH_3O	97	75
3	**c**	NO_2	88	95

[a] Yields of isolated products.

2.2. MCR with aldehydes

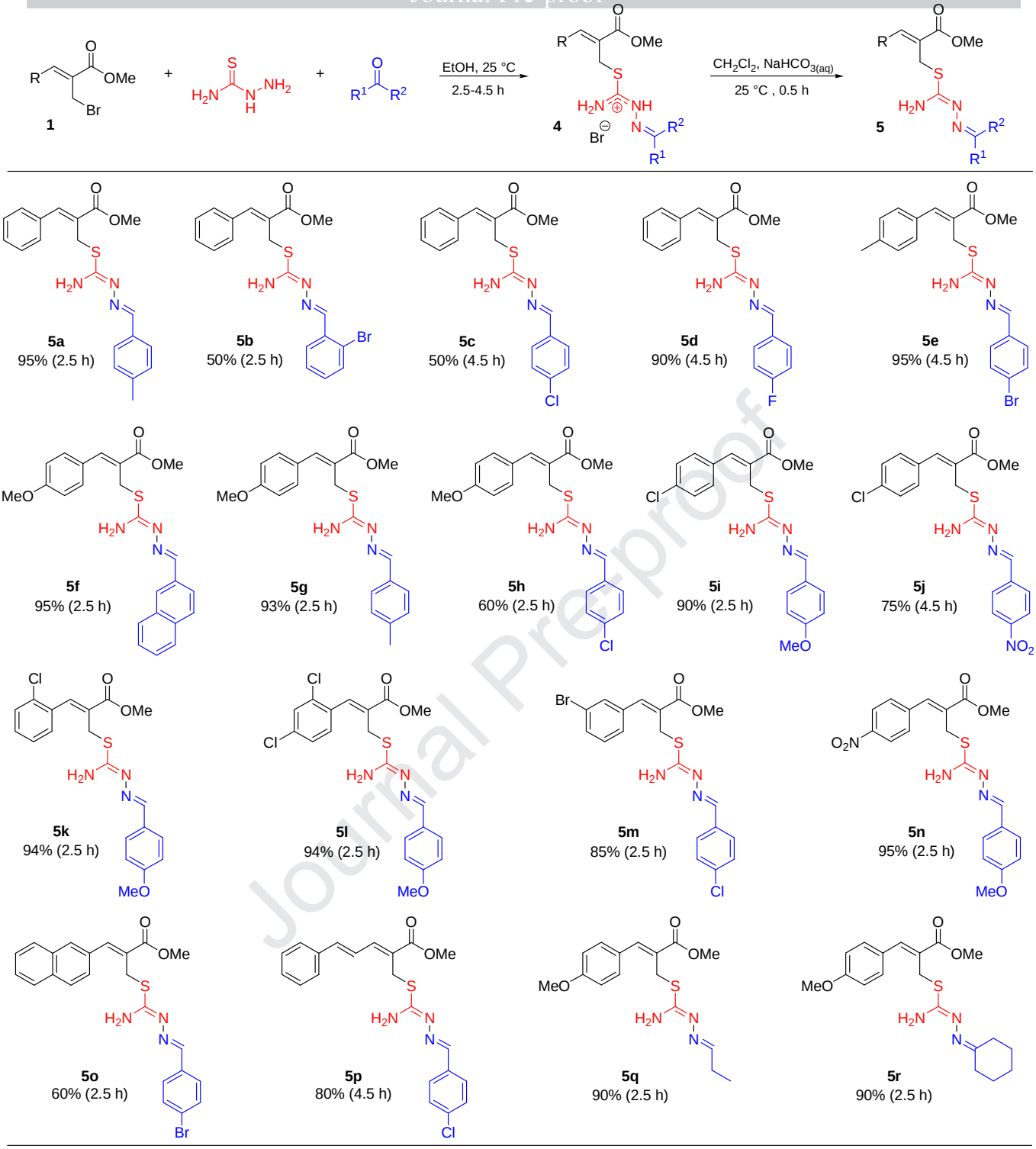
To expand the structural diversity to other carbonyl compounds, a representative series of aldehydes was employed in this novel MCR involving allylic bromides **1** and thiosemicarbazide (Scheme 1). Ethanol was chosen as the solvent, rather than 2-propanol or acetonitrile, due to its wide availability, low cost and environmental considerations [10]. However, in most of the reactions, the expected isothiosemicarbazone salts **4** were obtained as a mixture of isomers (varying from 2:1 to 10:1), which are possibly related to the pair of *E*/*Z* diastereoisomers around the NC=NN double bond. It is well known that N^4-unsubstituted isothiosemicarbazones predominantly have the configuration in which the nitrogen atoms at the extremities (N^1 and N^4) lie *cis* to each other about the C=N^2 bond (i.e., *E*-configuration), due to the presence of intramolecular hydrogen bonding (see structure **II** in Fig. 2). Conversely, the isomer of *Z*-configuration (structure **III** in Fig. 2) may coexist in solution if the solvent is sufficiently polar to break down the intramolecular N-H---N interaction [9a,11].



Scheme 1. Optimization of the conditions for the MCR.

Attempts to obtain a single diastereoisomer **4** by varying the reaction time (2-72 h), the solvent (2-propanol, acetonitrile) or the order of addition of the reagents did not lead to any improvement in the isomeric ratio. In addition, we were not able to achieve a satisfactory separation of isomers by applying chromatography or recrystallization techniques.

Since the salt **4** could not be obtained as a sole isomer in pure form, attention was turned to the preparation of the corresponding isothiosemicarbazones **5** (Scheme 1). The direct neutralization of an aqueous (or ethanolic) solution of the salt **4** with sodium bicarbonate or hydroxide gave the expected isothiosemicarbazone **5** contaminated with non-characterized side products. However, the use of a less polar solvent, such as dichloromethane, in an aqueous biphasic system was found to be crucial for the neutralization. Thus, the addition of saturated bicarbonate to a stirred suspension of **4** in CH_2Cl_2 followed by an aqueous work-up led to the isothiosemicarbazones **5** in good to excellent yields (95-50%), as a sole isomer in each case (Scheme 2). Ethyl acetate was able to replace CH_2Cl_2 as a more environmentally benign solvent, although with less satisfactory results relating to yield and selectivity. A wide range of allylic bromides **1** was employed, including aryl-substituted precursors as well as an alkenyl [R = (*E*)-styryl, **5p**] analogue.



Scheme 2. Scope of the MCR involving allylic bromides **1**, carbonyl compounds and thiosemicarbazide (yields were not optimized).

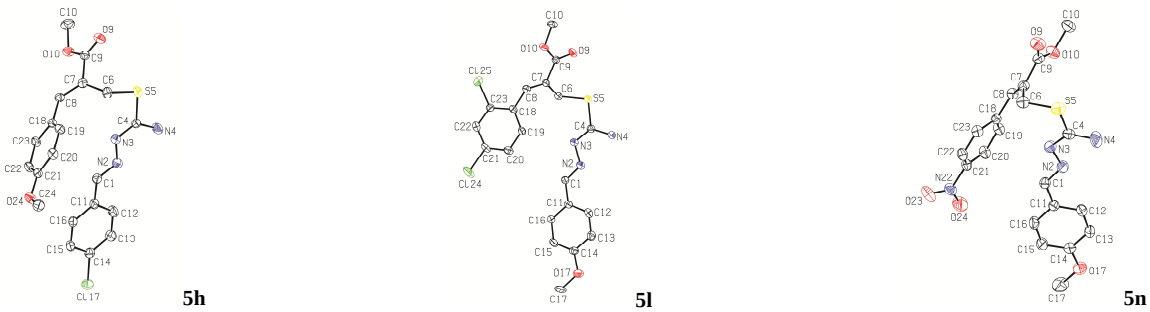


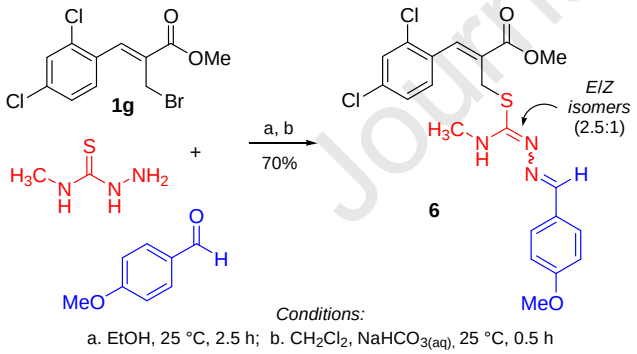
Fig. 3. ORTEP plots of isothiosemicarbazones **5h**, **5l**, and **5n**; ellipsoids are drawn at the 50% probability level. Hydrogen atoms were omitted for clarity.

The carbonyl counterpart was also shown to be versatile, since aldehyde (aromatic and aliphatic) and ketone react equally well (Scheme 2). The effect of the substitution pattern on the aromatic ring (R, R^1) was also explored and no significant difference in the reactivity was found for substrates bearing electron-releasing or electron-withdrawing groups. Remarkably, this one-pot transformation tolerates a variety of functional groups, including halogen (fluorine, chlorine, bromine), alkoxy, nitro and conjugated unsaturated groups, thus evidencing the potential synthetic applicability and the mildness of the reaction conditions involved in the process. The complete diastereoselectivity observed for the two C=N unsaturated bonds formed during the MCR should also be noted.

Isothiosemicarbazones **5h,l,n** were slowly crystallized in ethanol/ethyl acetate (1:1 v/v), which allowed for unequivocal structural elucidation by single crystal X-ray crystallographic analysis (Fig. 3 and Supporting Information).

2.3. Reaction profile and synthetic applications

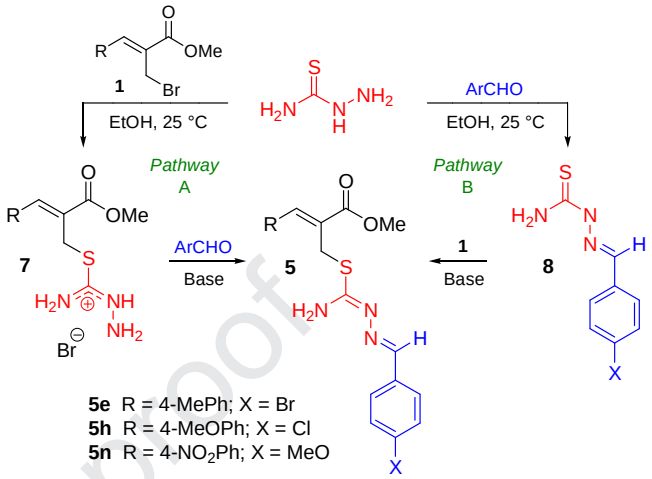
Encouraged by the significant results achieved with the MCR featuring thiosemicarbazide, we moved our attention to a substituted dinucleophile, 4-methylthiosemicarbazide (Scheme 3). In this case, the reaction proceeded well and the expected tricomponent product **6** was obtained in 70% yield, although the diastereoselectivity was not as high as those observed for the isothiosemicarbazones **5** originating from the parent thiosemicarbazide. This distinct behavior is not entirely unexpected considering the known tendency of N^4-substituted isothiosemicarbazones to coexist in solution as mixtures of *E*/*Z* diastereoisomers [9c,11], but attempts to separate them were unsuccessful.



Scheme 3. MCR with 4-methylthiosemicarbazide.

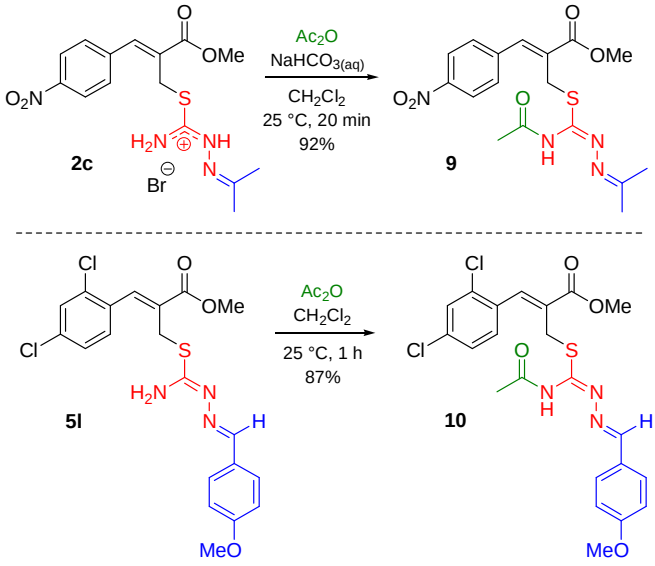
To gain a more in-depth insight into the MCR profile, the two possible two-step pathways (nucleophilic displacement and condensation) available for generating the isothiosemicarbazones **5** were investigated in detail (Scheme 4). In pathway A, the transformation commenced with the S_N2-type reaction between thiosemicarbazide and allylic bromides **1** in ethanol at rt for 0.5-1 h to give quantitatively (>90%) the isothiosemicarbazide salts **7**, which were subsequently treated with an aldehyde for 1 h to furnish the multicomponent adducts **5** in high yields. In pathway B, the steps were inverted: first, thiosemicarbazide condensed with an aldehyde, under the same conditions employed for pathway A. In this case, the formation of the expected thiosemicarbazones **8** in reasonable amounts (>70% conversion) was only achieved after 1-2 h. The second step involved the subsequent S_N2 reaction between **8** and allylic bromide **1** for 1 h to deliver the tricomponent product **5**.

These results indicate that pathway A is preferable to B because the first step in pathway A (formation of salt **7**) is more favorable than that in pathway B (formation of thiosemicarbazone **8**), while the second step is equally efficient in the two pathways. Nonetheless, due to the comparable facility that these pathways take place, it is reasonable to expect that they are equally important in terms of the outcome of the MCR.



Scheme 4. Proposed mechanistic pathways for the MCR.

Despite the presence of multiple functionalities in isothiosemicarbazones **2** and **5**, it is possible to develop chemoselective transformations providing that the reaction conditions are correctly modulated. Thus, the selective N^4-acetylation of isothiosemicarbazone salt **2c** was readily achieved in a mild basic medium using Ac_2O in a biphasic organic/aqueous system [12] to furnish **9** in high yield (Scheme 5). On the other hand, alkaline conditions did not work well for the acetylation of isothiosemicarbazone **5l** and led to a complex mixture of acetylated products. In this case, selective acetylation of **5l** was successfully performed in the absence of base to give **10** cleanly.



Scheme 5. Chemoselective acetylation of isothiosemicarbazones.

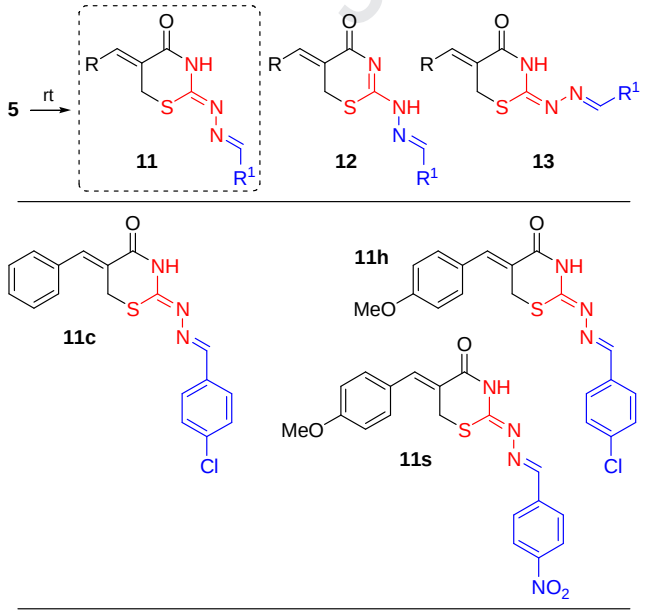
During the studies to determine the scope of this novel MCR, we observed that some isothiosemicarbazones **5** were prone to

spontaneous cyclization, generating the corresponding 2-hydrazono-1,3-thiazin-4-ones **11** (Scheme 6). The synthesis and reactivity of the 1,3-thiazin-4-one framework [12,13] has recently been reviewed [14], highlighting the importance of this class of S-heterocycles in medicinal chemistry [7d,14].

In the case of thiazinone **11c**, it was isolated as a crystalline solid in good yield (70%), although the NMR spectra (^{1}H and ^{13}C) indicated the presence of two isomers (**11** and, possibly, **12** or **13**) coexisting in solution (Scheme 6). On the other hand, thiazinone **11h** was obtained as a sole isomer, although in low isolated yield (4%) due to difficulties associated with separating the cyclic compound **11** from its precursor **5**. Surprisingly, thiazinone **11s** was obtained directly from the MCR with allylic bromide **1b**, 4-nitrobenzaldehyde and isothiosemicarbazide in excellent yield (96%), without isolating the elusive isothiosemicarbazone intermediate. These results indicate that the propensity of isothiosemicarbazones **5** to cyclize to the corresponding thiazinones **11** is dependent on the electronic factors exerted by the substituents presented in the aryl rings.

The structural determination of 2-hydrazono-1,3-thiazin-4-ones **11c** and **11h** was unequivocally assigned by X-ray diffraction analysis (Fig. 4), thus confirming the proposed stereochemistry (see the crystallographic data in the Supporting Information).

It is important to compare our results with those recently described by Ablajan and cols [15], where the MCR involving isothiosemicarbazide, carbonyl compounds and ethyl 2-chloroacetoacetate catalyzed by anhydrous sodium acetate under reflux in ethanol led to the formation of thiazoles in high yields (Scheme 7). In our case, the MCR employing allylic bromides **1**, isothiosemicarbazide and carbonyl compounds in ethanol at rt (with no catalyst or additive) gave rise to the isothiosemicarbazones **5**, which subsequently cyclized to a 6-membered ring (*i.e.*, 1,3-thiazinones **11**) instead of a thiazole or other isomeric 5-membered ring, as described elsewhere [15,16]. Therefore, the MCR described herein expands the scope of allylic bromides **1** as building blocks for the synthesis of sulfur-containing heterocycles as pharmacologically privileged structures.



Scheme 6. Cyclization of isothiosemicarbazones **5** to thiazinones **11**.

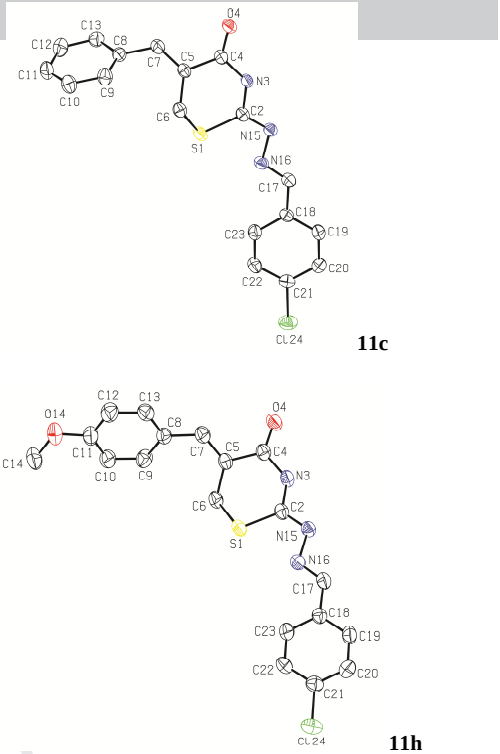
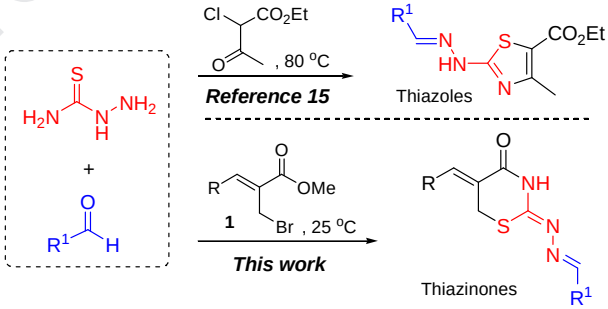


Fig. 4. ORTEP plots of 1,3-thiazin-4-ones **11c** and **11h**; ellipsoids are drawn at the 50% probability level. Hydrogen atoms were omitted for clarity.



Scheme 7. Previous work on MCR involving isothiosemicarbazide.

2.4. Antitumor activity of isothiosemicarbazones

Due to the pronounced antitumor activity of related thiosemicarbazides and analogues [7], it was important to verify whether the novel isothiosemicarbazones **3** and **5** present cytotoxicity toward a tumor cell line. Therefore, the cell viability of triple-negative breast cancer cell line MDA-MB-231, which is related to an aggressive type of cancer [17], was evaluated by the MTT assay [18], after 24 h of exposure to isothiosemicarbazone **3** or **5** (50 µM).

As can be seen in Table 2, five out of the twenty isothiosemicarbazones screened (**5e,g,m,n,p**; respectively, entries 7, 9, 15, 16, 18) led to significant reduction (41-54%) in the number of live tumoral cells. On the other hand, all isothiosemicarbazones presented low cytotoxicity for HUVEC, a non-tumoral cell line. While these results are of a preliminary nature, they strongly suggest that these novel isothiosemicarbazones **5** undergo distinct interactions with non-tumoral and tumoral cell lines, which is a desirable property for the future development of selective anticancer agents.

Table 2
Cellular viability (in %) of selected isothiosemicarbazones **3** and **5**.[a]

Entry	Compound[b]	MDA-MB-231[c]	HUVEC[d]
1	**3a**	102 ± 7.8	98 ± 2.4
2	**3b**	76 ± 10.8	90 ± 2.1
3	**3c**	58 ± 9.0	95 ± 8.6
4	**5a**	67 ± 3.2	82 ± 6.1
5	**5b**	59 ± 4.7	72 ± 4.2
6	**5d**	76 ± 10.4	80 ± 3.7
7	**5e**	43 ± 1.0	82 ± 4.6
8	**5f**	94 ± 17.4	98 ± 4.4
9	**5g**	54 ± 5.4	84 ± 2.5
10	**5h**	63 ± 9.3	83 ± 4.2
11	**5i**	55 ± 6.1	87 ± 4.7
12	**5j**	75 ± 3.9	80 ± 2.0
13	**5k**	60 ± 10.3	77 ± 8.1
14	**5l**	63 ± 10.0	90 ± 8.0
15	**5m**	47 ± 8.4	80 ± 5.7
16	**5n**	41 ± 7.5	87 ± 3.2
17	**5o**	85 ± 5.5	107 ± 1.6
18	**5p**	54 ± 8.9	86 ± 3.3
19	**5q**	82 ± 9.7	88 ± 5.6
20	**5r**	99 ± 15.2	100 ± 5.4

[a] Results expressed as the mean ± SD, n = 3.
[b] Concentration tested = 50 µM.
[c] MDA-MB-231: triple-negative breast cancer cell line
[d] HUVEC: vascular endothelium cell line

3. Conclusion

In conclusion, isothiosemicarbazones **3** and **5** were readily prepared using multicomponent synthesis from accessible starting materials, such as aldehydes or ketones, thiosemicarbazide and allylic bromides **1**, through the use of environmentally benign conditions (ethanol as the solvent at room temperature) without extra additives. This one-pot transformation tolerates a variety of functional groups, thus evidencing its potential synthetic applicability. Besides the twenty-two isothiosemicarbazones **3**, **5** and **6** synthesized by this method, the three isothiosemicarbazone salts **2** and the two acetylated derivatives **9** and **10** are novel compounds that were obtained in good to excellent yields (95-50%) and high stereoselectivity without requiring further purification stages in most cases. The somewhat unexpected cyclization of selected isothiosemicarbazones **5** to give the 2-hydrazono-1,3-thiazin-4-one framework **11** expanded the scope of this MCR, allowing access to a class of S-heterocycles under mild conditions. Finally, compounds **5e,g,m,n,p** showed cytotoxic activity toward an aggressive breast cancer cell line, thus highlighting the anticipated pharmacological importance of this class of compounds. Further studies on the chemical and biological properties of these novel isothiosemicarbazones and thiazinones are actively in progress and will be reported in due course.

4. Experimental section

4.1. General information

All chemicals were of reagent grade and were used as received. Melting points were determined using a hot plate apparatus and are uncorrected. Infrared spectra were acquired with a FT-IR spectrometer (range 4000 e 400 cm^{-1}) using KBr for solids and film for liquid samples. ^{1}H NMR spectra were recorded at 400 MHz or at 200 MHz and ^{13}C {^{1}H} NMR spectra (fully decoupled) were recorded at 100 MHz or at 50 MHz. Splitting patterns are designated as s (singlet), bs (broad singlet), d (doublet), dd (doublet of doublet), t (triplet), q (quartet), sx (sextet), m (multiplet). Coupling constants (*J*) are measured in Hertz (Hz). Chemical shifts were recorded in parts per million (ppm, δ) relative to solvent ($CDCl_3$ at 7.26 ppm or DMSO-d_6 at 2.48 ppm for ^{1}H NMR, and $CDCl_3$ at 77.16 ppm or DMSO-d_6 at 39.52 ppm for ^{13}C NMR) as the internal standard. Elemental analyses were conducted in a CHNS microanalyzer and the analytical results were within ± 0.4% of the theoretical values. The ESI-QTOF mass spectrometer was operated in the positive ion mode at 4.5 kV and at a desolvation temperature of 180 °C. The standard electrospray ion (ESI) source was used to generate the ions. The instrument was calibrated in the range m/z 50-3000 using a calibration standard (low concentration tuning mix solution) and data were processed with the aid of computer software.

Allylic bromides **1** were prepared from Morita-Baylis-Hillman adducts according to the described methods [4a] and their physical and spectral data were consistent with the expected structures and the related literature [4a,d,19]. The allylic bromides **1a** (R = phenyl), **1d** (R = 4-methylphenyl), **1e** (R = 4-chlorophenyl), and **1h** (R = 3-bromophenyl) were obtained as mixtures of diastereoisomers (*Z*:*E* 9-19:1) and were used as such. Isothiosemicarbazide salts **7** were prepared and characterized as previously described [7b,c]. Thiosemicarbazones **8** were prepared as follows: To a stirred solution of thiosemicarbazide (1.0 mmol) in 1.0 mL of ethanol at 25 °C was added the appropriate aldehyde (1.0 mmol). After stirring for 1 h, the insoluble solid formed was resolubilized by gently warming the reaction mixture and the final solution was allowed to slowly crystallize at rt. The crystalline thiosemicarbazone **8** formed was collected by filtration and dried. Their physical and spectral data were consistent with the expected structures and the related literature [20].

*4.2. Typical procedure for the preparation of isothiosemicarbazone salts **2***

To a stirred solution of allylic bromide **1** (1.0 mmol) in 3.0 mL of acetone at 25 °C was added thiosemicarbazide (1.05 mmol). After stirring for 1.5 h, the final mixture was concentrated under reduced pressure and the solid formed was crushed with ethyl ether/CH_2Cl_2 (4:1) and filtered to obtain the expected products **2** in pure form.

*4.2.1. (4E,8Z)-5-Amino-3,4-diaza-8-methoxycarbonyl-2-methyl-9-phenyl-6-thianona-2,4,8-triene hydrobromide (**2a**)*

Yield: 78% (300 mg); white solid, mp 143.0-144.0 °C. IR (KBr) ν_{max}/cm^{-1}: 3276, 3094, 2917, 1709, 1640, 1579, 1450, 1277, 1203. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.02 (s, 3H), 2.16 (s, 3H), 3.88 (s, 3H), 4.14 (s, 2H), 7.35-7.48 (m, 5H), 7.97 (s, 1H), 8.77 (bs, 1H), 9.80 (bs, 1H), 12.28 (bs, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.2 (CH_3), 25.1 (CH_3), 28.0 (CH_2), 53.5 (OCH_3), 125.0 (C), 129.3 (2 × CH), 129.5 (2 × CH), 130.4 (CH), 133.5 (C), 145.7 (=CH), 160.8 (C), 168.5 (C), 171.3 (C). Anal. calcd for $C_{15}H_{20}BrN_3O_2S$ (%): C, 46.64; H, 5.22; N, 10.88; S, 8.30. Found: C, 46.28; H, 5.10; N, 10.58; S, 8.47.

*4.2.2. (4E,8Z)-5-Amino-3,4-diaza-8-methoxycarbonyl-9-(4-methoxyphenyl)-2-methyl-6-thianona-2,4,8-triene hydrobromide (**2b**)*

Yield 97% (402 mg); white solid, mp 156.0-158.0 °C. IR (KBr) ν_{max}/cm^{-1}: 3263, 3092, 2947, 2841, 1717, 1634, 1601, 1509, 1436, 1262, 1173. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.04 (s,

3H), 2.19 (s, 3H), 3.85 (s, 3H), 3.88 (s, 3H), 4.15 (s, 2H), 6.99 (d, J = 9.0 Hz, 2H), 7.38 (d, J = 9.0 Hz, 2H), 7.90 (s, 1H), 8.91 (bs, 1H), 9.86 (bs, 1H), 12.37 (bs, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.2 (CH_3), 25.1 (CH_3), 28.3 (CH_2), 53.2 (OCH_3), 55.5 (OCH_3), 114.8 (2 × CH), 121.9 (C), 125.8 (C), 132.0 (2 × CH), 145.3 (=CH), 160.6 (C), 161.4 (C), 168.8 (C), 171.4 (C). Anal. calcd for $C_{16}H_{22}BrN_3O_3S$ (%): C, 46.16; H, 5.33; N, 10.09. Found: C, 46.08; H, 5.55; N, 10.10.

*4.2.3. (4E,8Z)-5-Amino-3,4-diaza-8-methoxycarbonyl-2-methyl-9-(4-nitrophenyl)-6-thianona-2,4,8-triene hydrobromide (**2c**)*

Yield 88% (378 mg); yellowish solid, mp 177.5-179.0 °C. IR (KBr) ν_{max}/cm^{-1}: 3274, 3167, 3065, 2955, 1721, 1638, 1577, 1507, 1434, 1344, 1252. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.06 (s, 3H), 2.19 (s, 3H), 3.94 (s, 3H), 4.15 (s, 2H), 7.60 (d, J = 8.6 Hz, 2H), 8.02 (s, 1H), 8.34 (d, J = 8.6 Hz, 2H), 12.27 (bs, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.0 (CH_3), 25.2 (CH_3), 28.1 (CH_2), 53.6 (OCH_3), 124.5 (2 × CH), 128.0 (C), 130.2 (2 × CH), 139.9 (C), 143.1 (=CH), 148.3 (C), 161.0 (C), 167.1 (C), 171.0 (C). Anal. calcd for $C_{15}H_{19}BrN_4O_4S$ (%): C, 41.77; H, 4.44; N, 12.99; S, 7.43. Found: C, 41.39; H, 4.49; N, 12.77; S, 7.33.

*4.3. Typical procedure for the preparation of isothiosemicarbazones **3***

To a stirred solution of allylic bromide **1** (1.0 mmol) in 3.0 mL of acetone at 25 °C was added thiosemicarbazide (1.0 mmol). After stirring for 1.5 h, satd $NaHCO_3$ (3.0 mL) was added to the mixture and the resulting suspension was allowed to stir for 0.5 h. The final mixture was diluted with dichloromethane, washed twice with water, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The resulting isothiosemicarbazones **3a-c** were obtained in high purity without any further purification step.

*4.3.1. (4E,8Z)-5-Amino-3,4-diaza-8-methoxycarbonyl-2-methyl-9-phenyl-6-thianona-2,4,8-triene (**3a**)*

Yield 94% (287 mg); 7*Z*:7*E* 9:1; yellow oil. IR (neat) ν_{max}/cm^{-1}: 3449, 3343, 3059, 2992, 2949, 1713, 1632, 1595, 1269, 761, 700. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.94 (s, 3H), 1.98 (s, 3H), 3.85 (s, 3H), 4.22 (s, 2H), 5.47 (bs, 2H), 7.35-7.50 (m, 5H), 7.82 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 18.1 (CH_3), 25.2 (CH_3), 27.7 (CH_2), 52.5 (OCH_3), 127.3 (C), 128.8 (2 × CH), 129.3 (CH), 129.8 (2 × CH), 134.4 (C), 142.6 (=CH), 157.3 (C), 162.2 (C), 167.9 (C=0). HRMS (ESI+): m/z calcd for $C_{15}H_{20}N_3O_2S$ $[M+H]^+$: 306.1271, found: 306.1266.

*4.3.2. (4E,8Z)-5-Amino-3,4-diaza-8-methoxycarbonyl-9-(4-methoxyphenyl)-2-methyl-6-thianona-2,4,8-triene (**3b**)*

Yield 75% (251 mg); yellow oil. IR (neat) ν_{max}/cm^{-1}: 3449, 3341, 3000, 2951, 1707, 1603, 1511, 1258, 1179, 836. ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.98 (s, 3H), 1.99 (s, 3H), 3.83 (s, 3H), 3.84 (s, 3H), 4.25 (s, 2H), 5.46 (bs, 2H), 6.92 (d, J = 8.9 Hz, 2H), 7.49 (d, J = 8.9 Hz, 2H), 7.77 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 18.2 (CH_3), 25.3 (CH_3), 28.0 (CH_2), 52.5 (OCH_3), 55.5 (OCH_3), 114.4 (2 × CH), 124.8 (C), 127.1 (C), 132.0 (2 × CH), 142.6 (=CH), 157.6 (C), 160.7 (C), 162.3 (C), 168.3 (C=O). HRMS (ESI+): m/z calcd for $C_{16}H_{22}N_3O_3S$ $[M+H]^+$: 336.1376, found: 336.1373.

*4.3.3. (4E,8Z)-5-Amino-3,4-diaza-8-methoxycarbonyl-2-methyl-9-(4-nitrophenyl)-6-thianona-2,4,8-triene (**3c**)*

Yield 95% (333 mg); yellow solid, mp 115.0-117.0 °C. IR (neat) ν_{max}/cm^{-1}: 3451, 3341, 3076, 2994, 2947, 1703, 1634, 1595, 1518, 1342, 1256, 848. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.86 (s, 3H), 1.98 (s, 3H), 3.88 (s, 3H), 4.19 (s, 2H), 5.33 (bs, 2H), 7.62 (d, J = 8.6 Hz, 2H), 7.80 (s, 1H), 8.23 (d, J = 8.6 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 18.0 (CH_3), 25.1 (CH_3), 27.1 (CH_2), 52.8 (OCH_3), 123.8 (2 × CH), 130.4 (2 × CH), 131.2 (C), 139.3 (=CH), 141.0 (C), 147.6 (C), 156.4 (C), 162.4 (C), 167.0 (C=O). HRMS (ESI+): m/z calcd for $C_{15}H_{19}N_4O_4S$ $[M+H]^+$: 351.1122, found: 351.1124.

*4.4. Typical procedure for the preparation of isothiosemicarbazones **5** and **6***

To a stirred solution of allylic bromide **1** (1.0 mmol) in 3.0 mL of ethanol (or acetonitrile, for the alkyl-substituted product **5q**) at 25 °C were added the carbonyl compound (aldehyde or ketone, 1.0 mmol) and thiosemicarbazide (1.0 mmol) or 4-methylthiosemicarbazide (for **6**). After stirring for 1.5-4.5 h, the final mixture was concentrated under reduced pressure and the solid formed was crushed with ethyl ether and decanted to give the isothiosemicarbazone salt **4**. To this solid was added 2.0 mL of dichloromethane followed by satd $NaHCO_3$ (3.0 mL). After stirring for 0.5 h, the final mixture was diluted with dichloromethane, washed twice with water, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The resulting isothiosemicarbazones **5a-r** and **6** were obtained in high purity without any further purification step. The isothiosemicarbazones **5a-e,i,j,m** were obtained as mixtures of diastereoisomers (7*Z*:7*E* = 9-20:1) with the same isomeric ratio observed for the starting allylic bromides (**1a,d,e,h**).

*4.4.1. (1E,3E,7Z)-4-Amino-2,3-diaza-7-methoxycarbonyl-1-(4-methylphenyl)-8-phenyl-5-thiaocta-1,3,7-triene (**5a**)*

Time = 2.5 h; yield 95% (350 mg); 7*Z*:7*E* = 9:1; yellow oil. IR (neat) ν_{max}/cm^{-1}: 3453, 3341, 3155, 3025, 2951, 1711, 1609, 1587, 1530, 1271, 816, 732. ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.38 (s, 3H), 3.87 (s, 3H), 4.27 (s, 2H), 5.80 (bs, 2H), 7.19 (d, J = 7.6 Hz, 2H), 7.37-7.47 (m, 5H), 7.60 (d, J = 7.6 Hz, 2H), 7.83 (s, 1H), 8.12 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 21.6 (CH_3), 27.7 (CH_2), 52.5 (OCH_3), 127.6 (C), 127.8 (2 × CH), 128.8 (2 × CH), 129.3 (CH), 129.4 (2 × CH), 129.8 (2 × CH), 132.4 (C), 134.6 (C), 140.3 (C), 142.5 (=CH), 155.0 (HC=N), 161.0 (C), 168.0 (C=O). HRMS (ESI+): m/z calcd for $C_{20}H_{22}N_3O_2S$ $[M+H]^+$: 368.1427, found: 368.1430.

*4.4.2. (1E,3E,7Z)-4-Amino-2,3-diaza-1-(2-bromophenyl)-7-methoxycarbonyl-8-phenyl-5-thiaocta-1,3,7-triene (**5b**)*

Time = 2.5 h; yield 50% (216 mg); 7*Z*:7*E* = 9:1; yellow solid, mp 114.8-116.8 °C. IR (neat) ν_{max}/cm^{-1}: 3404, 3288, 3172, 2943, 1709, 1601, 1524, 1265, 1212, 751. ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.88 (s, 3H), 4.28 (s, 2H), 5.91 (bs, 2H), 7.18-7.50 (m, 8H), 7.56 (d, J = 8.0, 1H), 7.84 (s, 1H), 8.02 (d, J = 8.0, 1H), 8.60 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 27.8 (CH_2), 52.6 (OCH_3), 124.9 (C), 127.4 (CH), 127.5 (C), 128.1 (CH), 128.9 (2 × CH), 129.5 (CH), 129.8 (2 × CH), 131.1 (CH), 133.3 (CH), 134.1 (C), 134.4 (C), 142.8 (=CH), 153.7 (HC=N), 162.5 (C), 168.0 (C=O). HRMS (ESI+): m/z calcd for $C_{19}H_{19}BrN_3O_2S$ $[M+H]^+$: 434.0356, found: 434.0355.

*4.4.3 (1E,3E,7Z)-4-Amino-2,3-diaza-1-(4-chlorophenyl)-7-methoxycarbonyl-8-phenyl-5-thiaocta-1,3,7-triene (**5c**)*

Time = 4.5 h; yield 50% (424 mg); 7*Z*:7*E* = 9:1; yellow oil. IR (neat) ν_{max}/cm^{-1}: 3451, 3357, 2949, 1709, 1611, 1524, 1269, 1087, 826, 787. ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.85 (s, 3H), 4.28 (s, 2H), 5.95 (bs, 2H), 7.32 (d, J = 8.4 Hz, 2H), 7.36-7.50 (m, 5H), 7.61 (d, J = 8.4 Hz, 2H), 7.83 (s, 1H), 8.07 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 27.8 (CH_2), 52.6 (OCH_3), 127.6 (C), 128.9 (2 × CH), 129.0 (4 × CH), 129.4 (CH), 129.8 (2 × CH), 133.7 (C), 134.6 (C), 135.9 (C), 142.6 (=CH), 153.6 (HC=N),

161.9 (C), 168.0 (C=O). HRMS (ESI+): m/z calcd for $C_{19}H_{19}ClN_3O_2$ $[M+H]^+$: 338.0881, found: 338.0887.

*4.4.4. (1E,3E,7Z)-4-Amino-2,3-diaza-1-(4-fluorophenyl)-7-methoxycarbonyl-8-phenyl-5-thiaocta-1,3,7-triene (**5d**)*

Time = 4.5 h; yield 90% (334 mg); 7*Z*:7*E* = 9:1; yellow oil. IR (neat) ν_{max}/cm^{-1}: 3455, 3343, 2951, 1709, 1601, 1507, 1228, 836, 734. ^{1}H NMR (200 MHz, $CDCl_3$): δ 3.88 (s, 3H), 4.26 (s, 2H), 5.81 (bs, 2H), 7.07 (t, *J* = 8.6 Hz, 2H), 7.37-7.47 (m, 5H), 7.68 (dd, *J* = 8.6, 5.5 Hz, 2H), 7.83 (s, 1H), 8.09 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 27.7 (CH_2), 52.6 (OCH_3), 115.8 (d, *J* = 32.0 Hz, 2 × CH), 127.6 (C), 128.8 (2 × CH), 129.3 (CH), 129.6 (d, *J* = 9.0 Hz, 2 × CH), 129.8 (2 × CH), 131.4 (d, *J* = 2.0 Hz, C), 134.6 (C), 142.6 (=CH), 153.6 (HC=N), 161.5 (C), 163.9 (d, *J* = 248.0 Hz, CF), 168.0 (C=O). HRMS (ESI+): m/z calcd for $C_{19}H_{19}FN_3O_2S$ $[M+H]^+$: 372.11765, found: 372.11768.

*4.4.5. (1E,3E,7Z)-4-Amino-2,3-diaza-1-(4-bromophenyl)-7-methoxycarbonyl-8-(4-methylphenyl)-5-thiaocta-1,3,7-triene (**5e**)*

Time = 4.5 h; yield 95% (424 mg); 7*Z*:7*E* = 9:1; yellow solid, mp 62.6-65.6 °C. IR (neat) ν_{max}/cm^{-1}: 3437, 3288, 3027, 2949, 1703, 1640, 1607, 1528, 1269, 812, 728. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.37 (s, 3H), 3.86 (s, 3H), 4.26 (s, 2H), 5.91 (bs, 2H), 7.22 (d, *J* = 7.8 Hz, 2H), 7.39 (d, *J* = 7.8 Hz, 2H), 7.50 (d, *J* = 8.4 Hz, 2H), 7.56 (d, *J* = 8.4 Hz, 2H), 7.81 (s, 1H), 8.10 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 21.6 (CH_3), 27.9 (CH_2), 52.6 (OCH_3), 124.2 (C), 126.5 (C), 129.2 (2 × CH), 129.6 (2 × CH), 130.0 (2 × CH), 131.7 (C), 131.9 (2 × CH), 134.2 (C), 139.8 (C), 142.8 (=CH), 153.6 (HC=N), 162.2 (C), 168.2 (C=O). HRMS (ESI+): m/z calcd for $C_{20}H_{21}BrN_3O_2S$ $[M+H]^+$: 448.0512, found: 448.0517.

*4.4.6. (1E,3E,7Z)-4-Amino-2,3-diaza-7-methoxycarbonyl-8-(4-methoxyphenyl)-1-(2-naphthyl)-5-thiaocta-1,3,7-triene (**5f**)*

Time = 2.5 h; yield 95% (411 mg); yellow solid, mp 138.0-140.0 °C. IR (neat) ν_{max}/cm^{-1}: 3465, 3319, 3055, 2953, 1693, 1605, 1534, 1432, 1256, 1179, 820. ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.80 (s, 3H), 3.86 (s, 3H), 4.34 (s, 2H), 5.97 (bs, 2H), 6.94 (d, *J* = 8.8 Hz, 2H), 7.48-7.51 (m, 4H), 7.80-7.87 (m, 4H), 7.96 (s, 1H), 8.00-8.03 (m, 1H), 8.36 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 28.0 (CH_2), 52.5 (OCH_3), 55.4 (OCH_3), 114.4 (2 × CH), 123.5 (CH), 125.0 (C), 126.5 (CH), 126.9 (CH), 127.1 (CH), 127.9 (C), 128.36 (CH), 128.45 (CH), 129.5 (CH), 131.9 (2 × CH), 132.9 (C), 133.3 (C), 134.4 (C), 142.4 (=CH), 154.9 (HC=N), 160.6 (C), 161.8 (C), 168.3 (C=O). HRMS (ESI+): m/z calcd for $C_{24}H_{24}N_3O_3S$ $[M+H]^+$: 434.1533, found: 434.1528.

*4.4.7. (1E,3E,7Z)-4-Amino-2,3-diaza-7-methoxycarbonyl-8-(4-methoxyphenyl)-1-(4-methylphenyl)-5-thiaocta-1,3,7-triene (**5g**)*

Time = 2.5 h; yield 93% (370 mg); yellow oil. IR (neat) ν_{max}/cm^{-1}: 3461, 3347, 3045, 2947, 1701, 1605, 1589, 1520, 1267, 824, 749. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.36 (s, 3H), 3.80 (s, 3H), 3.84 (s, 3H), 4.29 (s, 2H), 5.91 (bs, 2H), 6.92 (d, *J* = 8.8 Hz, 2H), 7.17 (d, *J* = 7.8 Hz, 2H), 7.48 (d, *J* = 8.8 Hz, 2H), 7.60 (d, *J* = 7.8 Hz, 2H), 7.78 (s, 1H), 8.20 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 21.5 (CH_3), 27.9 (CH_2), 52.4 (OCH_3), 55.3 (OCH_3), 114.3 (2 × CH), 124.9 (C), 127.0 (C), 127.7 (2 × CH), 129.3 (2 × CH), 131.8 (2 × CH), 132.4 (C), 140.3 (C), 142.4 (=CH), 154.8 (HC=N), 160.6 (C), 161.2 (C), 168.2 (C=O). HRMS (ESI+): m/z calcd for $C_{21}H_{24}N_3O_3S$ $[M+H]^+$: 398.1533, found: 398.1531.

*4.4.8. (1E,3E,7Z)-4-Amino-2,3-diaza-1-(4-chlorophenyl)-7-methoxycarbonyl-8-(4-methoxyphenyl)-5-thiaocta-1,3,7-triene (**5h**)*

Time = 2.5 h; yield 60% (250 mg); yellow solid, mp 116.4-118.4 °C. IR (neat) ν_{max}/cm^{-1}: 3447, 3327, 2947, 1701, 1607, 1528, 1301, 1256, 836. ^{1}H NMR (200 MHz, $CDCl_3$): δ 3.76 (s, 3H), 3.80 (s, 3H), 4.25 (s, 2H), 5.87 (bs, 2H), 6.88 (d, *J* = 8.8 Hz, 2H), 7.28 (d, *J* = 8.6 Hz, 2H), 7.42 (d, *J* = 8.8 Hz, 2H), 7.58 (d, *J* = 8.6 Hz, 2H), 7.73 (s, 1H), 8.09 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 28.0 (CH_2), 52.5 (OCH_3), 55.4 (OCH_3), 114.4 (2 × CH), 125.0 (C), 127.1 (C), 128.9 (4 × CH), 131.9 (2 × CH), 133.8 (C), 135.8 (C), 142.5 (=CH), 153.5 (HC=N), 160.7 (C), 162.1 (C), 168.3 (C=O). HRMS (ESI+): m/z calcd for $C_{20}H_{21}ClN_3O_3S$ $[M+H]^+$: 418.09867, found: 418.09871.

*4.4.9. (1E,3E,7Z)-4-Amino-2,3-diaza-8-(4-chlorophenyl)-7-methoxycarbonyl-1-(4-methoxyphenyl)-5-thiaocta-1,3,7-triene (**5i**)*

Time = 2.5 h; yield 90% (376 mg); 7*Z*:7*E* = 15:1; yellow solid, mp 113.0-115.2 °C. IR (neat) ν_{max}/cm^{-1}: 3471, 3359, 3000, 2945, 1705, 1609, 1585, 1242, 1079, 828. ^{1}H NMR (200 MHz, $CDCl_3$): δ 3.83 (s, 3H), 3.86 (s, 3H), 4.25 (s, 2H), 5.68 (bs, 2H), 6.90 (d, *J* = 9.1 Hz, 2H), 7.34-7.45 (m, 4H), 7.65 (d, *J* = 9.1 Hz, 2H), 7.74 (s, 1H), 8.02 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 27.5 (CH_2), 52.6 (OCH_3), 55.5 (OCH_3), 114.2 (2 × CH), 127.9 (C), 128.4 (C), 129.1 (2 × CH), 129.4 (2 × CH), 131.2 (2 × CH), 133.2 (C), 135.3 (C), 141.0 (=CH), 154.8 (HC=N), 160.1 (C), 161.4 (C), 167.8 (C=O). HRMS (ESI+): m/z calcd for $C_{20}H_{21}ClN_3O_3S$ $[M+H]^+$: 418.0987, found: 418.0986.

*4.4.10. (1E,3E,7Z)-4-Amino-2,3-diaza-8-(4-chlorophenyl)-7-methoxycarbonyl-1-(4-nitrophenyl)-5-thiaocta-1,3,7-triene (**5j**)*

Time = 4.5 h; yield 75% (325 mg); 7*Z*:7*E* = 15:1; yellow solid, mp 126.0-128.0 °C. IR (neat) ν_{max}/cm^{-1}: 3492, 3365, 3064, 2951, 1709, 1658, 1591, 1507, 1336, 1285, 838. ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.87 (s, 3H), 4.26 (s, 2H), 5.89 (bs, 2H), 7.39 (s, 4H), 7.76 (s, 1H), 7.83 (d, *J* = 8.6 Hz, 2H), 8.01 (s, 1H), 8.23 (d, *J* = 8.6 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 27.4 (CH_2), 52.7 (OCH_3), 124.0 (2 × CH), 128.2 (2 × CH + C), 129.1 (2 × CH), 131.0 (2 × CH), 133.1 (C), 135.3 (C), 141.1 (C), 141.2 (=CH), 148.4 (C), 151.9 (HC=N), 163.3 (C), 167.6 (C=O). HRMS (ESI+): m/z calcd for $C_{19}H_{18}ClN_4O_4S$ $[M+H]^+$: 433.0732, found: 433.0734.

*4.4.11. (1E,3E,7Z)-4-Amino-2,3-diaza-8-(2-chlorophenyl)-7-methoxycarbonyl-1-(4-methoxyphenyl)-5-thiaocta-1,3,7-triene (**5k**)*

Time = 2.5 h; yield 94% (393 mg); yellow solid, mp 35.0-37.0 °C. IR (neat ν_{max}/cm^{-1}: 3449, 3335, 3053, 2953, 1695, 1605, 1509, 1301, 1248, 834, 753. ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.82 (s, 3H), 3.87 (s, 3H), 4.12 (s, 2H), 5.81 (bs, 2H), 6.89 (d, *J* = 8.8 Hz, 2H), 7.26-7.30 (m, 2H), 7.37-7.45 (m, 2H), 7.62 (d, *J* = 8.8 Hz, 2H), 7.88 (s, 1H), 8.02 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 27.6 (CH_2), 52.6 (OCH_3), 55.4 (OCH_3), 114.1 (2 × CH), 127.0 (CH), 127.8 (C), 129.3 (2 × CH), 129.8 (CH), 130.0 (C), 130.2 (CH), 130.4 (CH), 133.3 (C), 134.2 (C), 139.2 (=CH), 154.5 (HC=N), 160.2 (C), 161.3 (C), 167.4 (C=O). HRMS (ESI+): m/z calcd for $C_{20}H_{21}ClN_3O_3S$ $[M+H]^+$: 418.0987, found: 418.0989.

*4.4.12. (1E,3E,7Z)-4-Amino-2,3-diaza-8-(2,4-dichlorophenyl)-7-methoxycarbonyl-1-(4-methoxyphenyl)-5-thiaocta-1,3,7-triene (**5l**)*

Time = 2.5 h; yield 94% (425 mg); yellow solid, mp 122.7-124.7 °C. IR (neat) ν_{max}/cm^{-1}: 3445, 3333, 3006, 2953, 1693, 1607, 1587, 1509, 1289, 1252, 834. ^{1}H NMR (200 MHz, $CDCl_3$):

δ3.84 (s, 3H), 3.88 (s, 3H), 4.12 (s, 2H), 5.65 (bs, 2H), 6.91 (d, *J* = 8.3 Hz, 2H), 7.26-7.42 (m, 3H), 7.65 (d, *J* = 8.3 Hz, 2H), 7.79 (s, 1H), 7.94 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ27.4 (CH_2), 52.6 (OCH_3), 55.4 (OCH_3), 114.2 (2 × CH), 127.3 (CH), 127.8 (C), 129.4 (2 × CH), 129.6 (CH), 130.7 (C), 131.3 (CH), 132.1 (C), 135.0 (C), 135.3 (C), 137.7 (=CH), 154.5 (HC=N), 159.6 (C), 161.3 (C), 167.2 (C=O). HRMS (ESI+): m/z calcd for $C_{20}H_{20}Cl_2N_3O_3S$ $[M+H]^+$: 452.0597, found: 452.0593.

*4.4.13. (1E,3E,7Z)-4-Amino-2,3-diaza-8-(3-bromophenyl)-1-(4-chlorophenyl)-7-methoxycarbonyl-5-thiaocta-1,3,7-triene (**5m**)*

Time = 2.5 h; yield 85% (397 mg); 7*Z*:7*E* = 20:1; yellow solid, mp 40.0-42.0 °C. IR (neat) ν_{max}/cm^{-1}: 3451, 3339, 3059, 2949, 1709, 1611, 1587, 1524, 1289, 787. ^{1}H NMR (200 MHz, $CDCl_3$): δ3.82 (s, 3H), 4.21 (s, 2H), 5.69 (bs, 2H), 7.18-7.46 (m, 6H), 7.58 (d, *J* = 8.6 Hz, 2H), 7.67 (s, 1H), 7.99 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ27.2 (CH_2), 52.6 (OCH_3), 122.8 (C), 128.1 (C), 128.82 (2 × CH), 128.85 (2 × CH), 129.1 (CH), 130.2 (CH), 132.0 (CH), 132.4 (CH), 133.6 (C), 135.7 (C), 136.6 (C), 140.4 (=CH), 153.4 (HC=N), 161.2 (C), 167.5 (C=O). HRMS (ESI+): m/z calcd for $C_{19}H_{18}BrClN_3O_2S$ $[M+H]^+$: 467.9965, found: 467.9967.

*4.4.14. (1E,3E,7Z)-4-Amino-2,3-diaza-7-methoxycarbonyl-1-(4-methoxyphenyl)-8-(4-nitrophenyl)-5-thiaocta-1,3,7-triene (**5n**)*

Time = 2.5 h; yield 95% (407 mg); yellow solid, mp 120.0-122.0 °C. IR (neat) ν_{max}/cm^{-1}: 3463, 3347, 3074, 2955, 1705, 1605, 1511, 1350, 1250, 830. ^{1}H NMR (200 MHz, $CDCl_3$): δ 3.83 (s, 3H), 3.88 (s, 3H), 4.24 (s, 2H), 5.57 (bs, 2H), 6.89 (d, *J* = 8.9 Hz, 2H), 7.56-7.63 (m, 4H), 7.78 (s, 1H), 7.91 (s, 1H), 8.23 (d, *J* = 8.6 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ27.2 (CH_2), 52.8 (OCH_3), 55.5 (OCH_3), 114.3 (2 × CH), 124.0 (2 × CH), 127.7 (C), 129.4 (2 × CH), 130.5 (2 × CH), 131.6 (C), 139.1 (=CH), 141.4 (C), 147.8 (C), 154.8 (HC=N), 159.3 (C), 161.6 (C), 167.2 (C=O). HRMS (ESI+): m/z calcd for $C_{20}H_{21}N_4O_5S$ $[M+H]^+$: 429.1227, found: 429.1228.

*4.4.15. (1E,3E,7Z)-4-Amino-2,3-diaza-1-(4-bromophenyl)-7-methoxycarbonyl-8-(2-naphthyl)-5-thiaocta-1,3,7-triene (**5o**)*

Time = 2.5 h; yield 60% (289 mg); yellow solid, mp 133.3-135.0 °C. IR (neat) ν_{max}/cm^{-1}: 3465, 3319, 3055, 2953, 1693, 1605, 1589, 1534, 1256, 820, 749. ^{1}H NMR (400 MHz, $CDCl_3$): δ3.90 (s, 3H), 4.38 (s, 2H), 5.80 (bs, 2H), 7.37 (d, *J* = 8.4 Hz, 2H), 7.45-7.57 (m, 5H), 7.74 (s, 1H), 7.82-7.87 (m, 3H), 7.96 (s, 1H), 7.98 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ27.7 (CH_2), 52.6 (OCH_3), 124.2 (C), 126.7 (CH), 126.9 (CH), 127.2 (CH), 127.7 (CH), 127.9 (C), 128.4 (CH), 128.8 (CH), 129.1 (2 × CH), 130.0 (CH), 131.8 (2 × CH), 132.2 (C), 133.2 (C), 133.4 (C), 134.0 (C), 142.4 (=CH), 153.6 (HC=N), 161.7 (C), 168.1 (C=O). HRMS (ESI+): m/z calcd for $C_{23}H_{21}BrN_3O_2S$ $[M+H]^+$: 484.0513, found: 484.0514.

*4.4.16. (1E,3E,7Z,9E)-4-Amino-2,3-diaza-1-(4-chlorophenyl)-7-methoxycarbonyl-10-phenyl-5-thiadeca-1,3,7,9-tetraene (**5p**)*

Time = 4.5 h; yield 80% (331 mg); yellow solid, mp 141.7-142.7 °C. IR (neat) ν_{max}/cm^{-1}: 3455, 3298, 2945, 1689, 1605, 1591, 1515, 1289, 834, 751. ^{1}H NMR (200 MHz, $CDCl_3$): δ3.83 (s, 3H), 4.27 (s, 2H), 5.60 (bs, 2H), 6.84-6.99 (m, 1H), 7.26-7.51 (m, 11H), 8.26 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 26.4 (CH_2), 52.2 (OCH_3), 124.6 (CH), 127.0 (C), 127.6 (2 × CH), 128.8 (4 × CH), 128.9 (2 × CH), 129.2 (CH), 133.6 (C), 135.8 (C), 136.4 (C), 141.0 (=CH), 141.3 (=CH), 153.3 (HC=N), 161.7 (C), 167.8 (C=O). HRMS (ESI+): m/z calcd for $C_{21}H_{21}ClN_3O_2S$ $[M+H]^+$: 414.1038, found: 414.1040.

*4.4.17. (3E,5E,9Z)-6-Amino-4,5-diaza-9-methoxycarbonyl-10-(4-methoxyphenyl)-7-thiadeca-3,5,9-triene (**5q**)*

Time = 2.5 h; yield 90% (302 mg); yellow oil. IR (neat) ν_{max}/cm^{-1}: 3443, 3339, 3174, 2968, 1707, 1632, 1603, 1511, 1258, 1179, 836, 732. ^{1}H NMR (200 MHz, $CDCl_3$): δ1.10 (t, *J* = 7.2 Hz, 3H), 2.23-2.38 (m, 2H), 3.84 (s, 6H), 4.21 (s, 2H), 5.66 (bs, 2H), 6.92 (d, *J* = 8.4 Hz, 2H), 7.47 (d, *J* = 8.4 Hz, 2H), 7.66 (t, *J* = 5.0 Hz, 1H), 7.76 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 10.8 (CH_3), 26.2 (CH_2), 28.0 (CH_2), 52.4 (OCH_3), 55.4 (OCH_3), 114.3 (2 × CH), 124.9 (C), 127.0 (C), 131.9 (2 × CH), 142.4 (=CH), 160.1 (C), 160.3 (HC=N), 160.7 (C), 168.2 (C=O). HRMS (ESI+): m/z calcd for $C_{16}H_{22}N_3O_3S$ $[M+H]^+$: 336.1376, found: 336.1374.

*4.4.18. (2E,6Z)-3-Amino-1,2-diaza-1-(cyclohexylidene)-6-methoxycarbonyl-7-(4-methoxyphenyl)-4-thiahepta-2,6-diene (**5r**)*

Time = 2.5 h; yield 90% (337 mg); yellow oil. IR (neat) ν_{max}/cm^{-1}: 3449, 3341, 3000, 2933, 1707, 1603, 1511, 1258, 1179, 836, 732. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.58-1.61 (m, 4H), 1.67-1.71 (m, 2H), 2.28-2.32 (m, 2H), 2.61 (bs, 2H), 3.82 (s, 3H), 3.83 (s, 3H), 4.23 (s, 2H), 5.52 (bs, 2H), 6.91 (d, *J* = 8.6 Hz, 2H), 7.49 (d, *J* = 8.6 Hz, 2H), 7.76 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 26.2 (CH_2), 26.6 (CH_2), 27.7 (CH_2), 27.9 (CH_2), 28.1 (CH_2), 36.0 (CH_2), 52.4 (OCH_3), 55.4 (OCH_3), 114.3 (2 × CH), 124.7 (C), 127.0 (C), 132.0 (2 × CH), 142.4 (=CH), 158.0 (C), 160.6 (C), 167.7 (C), 168.2 (C=O). HRMS (ESI+): m/z calcd for $C_{19}H_{26}N_3O_3S$ $[M+H]^+$: 376.1689, found: 376.1687.

*4.4.19. (1E,3E,7Z)-2,3-Diaza-8-(2,4-dichlorophenyl)-7-methoxycarbonyl-1-(4-methoxyphenyl)-4-methylamino-5-thiaocta-1,3,7-triene (**6**)*

Obtained as a mixture of isomers (3*E*:3*Z* = 2.5:1). Time = 2.5 h; yield 70% (325 mg); yellow solid, mp 117.1-120.2 °C. IR (neat) ν_{max}/cm^{-1}: 3368, 3009, 2939, 1707, 1605, 1552, 1265, 1089, 822. ^{1}H NMR (400 MHz, $CDCl_3$): Data for the major isomer: δ 2.94 (d, *J* = 5.2 Hz, 3H), 3.83 (s, 3H), 3.88 (s, 3H), 4.20 (s, 2H), 6.38 (d, *J* = 5.2 Hz, 1H), 6.90 (d, *J* = 8.6 Hz, 2H), 7.26-7.29 (m, 1H), 7.40-7.48 (m, 2H), 7.62 (d, *J* = 8.6 Hz, 2H), 7.82 (s, 1H), 8.22 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): Data for the major isomer: δ 27.1 (CH_2), 29.9 (CH_3), 52.6 (OCH_3), 55.4 (OCH_3), 114.1 (2 × CH), 127.3-135.2 (C_{Ar} + CH_{Ar}), 137.7 (=CH), 152.7 (HC=N), 161.0 (C), 162.0 (C), 167.1 (C=O). HRMS (ESI+): m/z calcd for $C_{21}H_{22}Cl_2N_3O_3S$ $[M+H]^+$: 466.0753, found: 466.0754.

*4.5. Preparation of (4E,8Z)-5-acetylamino-3,4-diaza-8-methoxycarbonyl-2-methyl-9-(4-nitrophenyl)-6-thianona-2,4,8-triene (**9**)*

To a stirred suspension of the isothiosemicarbazone salt **2c** (1.0 mmol) in 3.0 mL of CH_2Cl_2 at 25 °C was added acetic anhydride (3.0 mmol) followed by satd $NaHCO_3$ (3.0 mL). After stirring for 20 min, the final mixture was diluted with CH_2Cl_2 and the organic extract was washed with H_2O and brine, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The resulting residue was purified by column chromatography (7:3 hexane/ethyl acetate) to give the corresponding acetylated isothiosemicarbazone **9**. Yield 92% (360 mg); yellowish solid, mp 151.5-153.0 °C. IR (KBr) ν_{max}/cm^{-1}: 3322, 3288, 3112, 2953, 1717, 1634, 1545, 1511, 1344, 1206. ^{1}H NMR (200 MHz, $CDCl_3$): δ1.94 (s, 3H), 2.03 (s, 3H), 2.15 (s, 3H), 3.85 (s, 3H), 4.09 (s, 2H), 7.66 (d, *J* = 8.6 Hz, 2H), 7.85 (s, 1H), 8.22 (d, *J* = 8.6 Hz, 2H), 9.70 (bs, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ18.6 (CH_3), 24.0 (CH_3), 25.3 (CH_3), 27.9 (CH_2), 52.9 (OCH_3), 124.0 (2 × CH), 130.49 (C), 130.54 (2 × CH),

140.4 (=CH), 141.2 (C), 147.8 (C), 152.6 (C), 165.9 (C), 166.9 (C), 167.6 (C). HRMS (ESI+): m/z calcd for $C_{17}H_{20}N_4O_5SNa$ $[M+Na]^+$: 415.1047, found: 415.1044.

*4.6. Preparation of (1E,3E,7Z)-4-acetylamino-2,3-diaza-8-(2,4-dichlorophenyl)-7-methoxycarbonyl-1-(4-methoxyphenyl)-5-thiaocta-1,3,7-triene (**10**)*

To a stirred solution of isothiosemicarbazone **5l** (1.0 mmol) in 3.0 mL of dichloromethane at 25 °C was added acetic anhydride (1.5 mmol). After stirring for 1 h, the final mixture was concentrated under reduced pressure and the solid formed was crushed with ethyl ether and filtered to obtain the expected acetylated isothiosemicarbazone **10** in pure form. Yield 87% (429 mg); yellow solid, mp 147.0-149.0 °C. IR (neat) ν_{max}/cm^{-1}: 3335, 3072, 2960, 1724, 1701, 1611, 1536, 1236, 832. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.19 (s, 3H), 3.86 (s, 6H), 4.10 (s, 2H), 6.94 (d, J = 8.8 Hz, 2H), 7.28 (dd, J = 8.4, 2.0 Hz, 1H), 7.41 (d, J = 2.0 Hz, 1H), 7.52 (d, J = 8.4 Hz, 1H), 7.65 (d, J = 8.8 Hz, 2H), 7.84 (s, 1H), 7.91 (s, 1H), 9.76 (bs, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 24.0 (CH_3), 27.5 (CH_2), 52.6 (OCH_3), 55.5 (OCH_3), 114.3 (2 × CH), 126.6 (C), 127.3 (CH), 129.6 (CH), 129.9 (2 × CH), 130.0 (C), 131.3 (CH), 132.3 (C), 135.1 (C), 135.2 (C), 138.2 (=CH), 154.9 (C), 156.8 (HC=N), 162.0 (C), 166.9 (C=O), 167.3 (C=O). HRMS (ESI+): m/z calcd for $C_{22}H_{22}Cl_2N_3O_4S$ $[M+H]^+$: 494.0703, found: 494.0704.

*4.7. Cyclization to 2-hydrazono-1,3-thiazin-4-ones **11***

Isothiosemicarbazones **5c** and **5h** were left standing at room temperature for weeks to give crystals of the corresponding 2-hydrazono-1,3-thiazin-4-ones **11c** and **11h**. 2-Hydrazono-1,3-thiazin-4-one **11s** was obtained directly through the work-up of the corresponding MCR reaction from 4-methoxy-substituted allylic bromide **1b**, thiosemicarbazide and 4-nitrobenzaldehyde in ethanol as described in section 4.4.

*4.7.1. (2Z,5Z)-5-Benzylidene-2-[(E)-4-chlorobenzylidene]hydrazono-1,3-thiazin-4-one (**11c**)*

Obtained as a 3:1 mixture of isomers. Yield 70% (247 mg); yellow solid, mp 185.1-186.0 °C. IR (neat) ν_{max}/cm^{-1}: 3439, 2925, 2851, 1679, 1626, 1573, 1489, 1289, 1087, 822. ^{1}H NMR (400 MHz, $CDCl_3$): Data for the major isomer: δ 3.96 (s, 2H), 7.34-7.48 (m, 7H), 7.68-7.71 (m, 2H), 7.78 (s, 1H), 8.47 (s, 1H), 9.67 (s, NH); Data for the minor isomer: δ 4.04 (s, 2H), 7.34-7.48 (m, 7H), 7.71-7.73 (m, 2H), 7.88 (s, 1H), 8.36 (s, 1H), 9.78 (s, NH). ^{13}C NMR (100 MHz, $CDCl_3$): Data for the major isomer: δ 24.9 (CH_2), 125.9 (C), 129.0-134.3 (C_{Ar} + CH_{Ar}), 136.8 (C), 139.3 (=CH), 140.3 (C), 157.1 (HC=N), 163.7 (C), 166.2 (C). HRMS (ESI+): m/z calcd for $C_{18}H_{14}ClN_3OS$ $[M+H]^+$: 356.0619, found: 356.0613.

*4.7.2. (2Z,5Z)-2-[(E)-4-Chlorobenzylidene]hydrazono-5-(4-methoxybenzylidene)-1,3-thiazin-4-one (**11h**)*

Yield 4% (15 mg); yellow solid, 178.9-180.0 °C. IR (neat) ν_{max}/cm^{-1}: 3449, 3172, 2931, 2837, 1677, 1603, 1573, 1509, 1256, 1173, 1030, 824. ^{1}H NMR (200 MHz, $CDCl_3$): δ 3.86 (s, 3H), 4.00 (s, 2H), 7.00 (d, J = 8.6 Hz, 2H), 7.33 (d, J = 8.3 Hz, 2H), 7.40 (d, J = 8.6 Hz, 2H), 7.70 (d, J = 8.3 Hz, 2H), 7.73 (s, 1H), 8.33(s, 1H), 8.41 (bs, 1H). HRMS (ESI+): m/z calcd for $C_{19}H_{16}ClN_3O_2S$ $[M+H]^+$: 386.0725, found: 386.0727.

*4.7.3. (2Z,5Z)-5-(4-Methoxybenzylidene)-2-[(E)-4-nitrobenzylidene]hydrazono-1,3-thiazin-4-one (**11s**)*

Yield 96% (380 mg); yellow solid, mp 244.1-246.5 °C. IR (neat) ν_{max}/cm^{-1}: 3447, 3117, 2931, 2839, 1689, 1597, 1511, 1340, 1256, 1034, 842. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.81 (s, 3H), 4.11 (s, 2H), 7.04 (d, J = 8.6 Hz, 2H), 7.48 (d, J = 8.6 Hz, 2H), 7.53 (s, 1H), 8.01 (d, J = 8.4 Hz, 2H), 8.31 (d, J = 8.4 Hz, 2H), 8.54 (s, 1H), 11.47 (bs, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 24.1 (CH_2), 55.2 (OCH_3), 114.3 (2 × CH), 124.0 (2 × CH), 124.1 (C), 126.3 (C), 128.5 (2 × CH), 131.5 (2 × CH), 136.9 (C), 140.4 (=CH), 148.1 (C), 154.3 (HC=N), 160.0 (C), 161.8 (C), 165.8 (C). HRMS (ESI+): m/z calcd for $C_{19}H_{16}N_4O_4S$ $[M+H]^+$: 397.0965, found: 397.0967.

4.8. Cytotoxicity assay

To evaluate the potential antitumoral activity of the isothiosemicarbazones **3** and **5**, the MTT [3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide] method was used to determine the cell viability of MDA-MB-231(Human Caucasian Breast Adenocarcinoma Cells) and HUVEC (Human Umbilical Vein Endothelial Cells) lines. Therefore, cell lines (1×10^4/well) were seeded in 96-well plates and incubated for 24 h with 50 μM of the isothiosemicarbazone. After incubation, the old culture medium was replaced with fresh culture medium with 5 mg/mL of MTT, followed by incubation for 2 h at 37 °C. MTT-formazan crystals were dissolved in DMSO and the absorbance was measured at 540 nm, using a micro-well system reader. As control group of each cell line, cells were incubated without the compounds and considered as 100% cell viability. Results where expressed as the mean ± SD of three experiments.

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Appendix A. Supplementary data

Supplementary data for this article (^{1}H and ^{13}C NMR spectra for all novel compounds) can be found online at https://doi.org/ Crystallographic data for **5h** (CCDC 1987926), **5l** (CCDC 1987929), **5n** (CCDC 1987928), **11c** (CCDC 1987930), and **11h** (CCDC 1987927) have been deposited at the Cambridge Crystallographic Database Centre and can be found at http://www.ccdc.ac.uk

References

[1] (a) P.T. Anastas, Aldrichim. Acta 48 (2015) 3-10;
(b) R.S. Varma, Green Chem. 16 (2014) 2027-2041;
(c) R.A. Sheldon, Chem. Soc. Rev. 41 (2012) 1437-1451.

[2] (a) M.M. Heravi, V. Zadsirjan, M. Dehghani, T. Ahmadi, Tetrahedron 74 (2018) 3391-3457;
(b) Y. Hayashi, Chem. Sci. 7 (2016) 866-880;
(c) R.C. Cioc, E. Ruijter, R.V.A. Orru, Green Chem. 16 (2014) 2958-2975;
(d) B. Ganem, Acc. Chem. Res. 42 (2009) 463-472.

[3] (a) D. Basavaiah, B.S. Reddy, S.S. Badsara, Chem. Rev. 110 (2010) 5447-5674;

(b) Y. Wei, M. Shi, Chem. Rev. 113 (2013) 6659-6690;
(c) T.N. Reddy, V.J. Rao, Tetrahedron Lett. 59 (2018) 2859-2875.

[4] (a) M. Ferreira, L. Fernandes, M.M. Sá, J. Braz. Chem. Soc. 20 (2009) 564-568;
(b) M.D. Ramos, L. Fernandes, M.M. Sá, Tetrahedron 62 (2006) 11652-11656;
(c) B. Das, J. Banerjee, N.A. Ravindranath, Tetrahedron 60 (2004) 8357-8361;
(d) R. Buchholz, H.M.R. Hoffmann, Helv. Chim. Acta. 74 (1991) 1213-1221.

[5] (a) P. Suman, A. Patel, L. Solano, G. Jampana, Z.S. Gardner, C.M. Holt, S.C. Jonnalagadda, Tetrahedron 73 (2017) 4214-4226;
(b) M. Bhaktadoss, A. Devaraj, D. Kannan, Eur. J. Org. Chem. (2014) 1505-1513;
(c) K.H. Kim, J.W. Lim, J. Lee, M.J. Go, J.N. Kim, Adv. Synth. Catal. 356 (2014) 3363-3369;
(d) A. Santhoshi, B. Mahendar, S. Mattapally, P.S. Sadhu, S.K. Banerjee, V.J. Rao, Bioorg. Med. Chem. Lett. 24 (2014) 1952-1957;
(e) C.H. Lim, S.H. Kim, K.H. Kim, J.N. Kim, Tetrahedron Lett. 54 (2013) 2476-2479.

[6] (a) M. Ferreira, T.B. Bisol, H.P. Conceição, T.V.C. Russo, A.J. Bortoluzzi, M.M. Sá, Synthesis 49 (2017) 667-676;
(b) B.G. Batista, D.F.D. Lana, G.P. Silveira, M.M. Sá, M. Ferreira, T.V.C. Russo, R.F.S. Canto, F.A.R. Barbosa, A.L. Braga, T.F.A. Kaminski, L.F.S. Oliveira, M.M. Machado, W. Lopes, M.H. Vainstein, M.L. Teixeira, S.F. Andrade, A.M. Fuentefria, ChemistrySelect 2 (2017) 11926-11932;
(c) M. Ferreira, M.M. Sá, Adv. Synth. Catal. 357 (2015) 829-833;
(d) M.M. Sá, L. Meier, Heteroatom Chem. 24 (2013) 384-391;
(e) M.M. Sá, L. Fernandes, M. Ferreira, A.J. Bortoluzzi, Tetrahedron Lett. 49 (2008) 1228-1232.

[7] (a) L.S. Assunção, I.F. Kretzer, J.A.S. Restrepo, L.J. Mello Jr, A.H. Silva, E.M. Oliveira, M. Ferreira, M.M. Sá, T.B. Creczynski-Pasa, Biochim. Biophys. Acta 1863 (2019) 1332-1342;
(b) M. Ferreira, L.S. Assunção, A.H. Silva, F.B. Filippin-Monteiro, T.B. Creczynski-Pasa, M.M. Sá, Eur. J. Med. Chem. 129 (2017) 151-158;
(c) J. Cisilotto, M. Ferreira, F.B. Filippin-Monteiro, A.J. Bortoluzzi, M.M. Sá, T.B. Creczynski-Pasa, Anticancer Agents Med. Chem. 15 (2015) 353-362;
(d) M. Ferreira, L.S. Assunção, F.B. Filippin-Monteiro, T.B. Creczynski-Pasa, M.M. Sá, Eur. J. Med. Chem. 70 (2013) 411-418.

[8] (a) F. Ali, K.M. Khan, U. Salar, M. Taha, N.H. Ismail, A. Wadood, M. Riaz, S. Perveen, Eur. J. Med. Chem. 138 (2017) 255-272;
(b) D.T. Nguyen, T.H. Le, T.T.T Bui, Eur. J. Med. Chem. 60 (2013) 199-207;
(c) H.-J. Zhang, Y. Qian, D.-D. Zhu, X.-G. Yang, H.-L. Zhu, Eur. J. Med. Chem. 46 (2011) 4702-4708;
(d) A. J. Kesel, Eur. J. Med. Chem. 46 (2011) 1656-1664;
(e) R. Trondl, L.S. Flocke, C.R. Kowol, P. Heffeter, U. Jungwirth, G.E. Mair, R. Steinborn, E.A. Enyedy, M.A. Jakupec, W. Berger, B.K. Keppler, Mol. Pharm. 85 (2014) 451-459;
(f) G.A. Gazieva, L.V. Anikina, S.A. Pukhov, T.B. Karpova, Y.V. Nelyubina, A.N. Kravchenko, Mol. Divers. 20 (2016) 837-846.

[9] (a) M.T. Cocco, C. Congiu, V. Onnis, M.L. Pellerano, A.D. Logu, Bioorg. Med. Chem. 10 (2002) 501-506;
(b) E. Maccioni, M.C. Cardia, L. Bonsignore, A. Plumitallo, M.L. Pellerano, A.D. Logu, Il Farmaco 57 (2002) 809-817;
(c) M.C. Cardia, M. Begala, A. Delogu, E. Maccioni, A. Plumitallo, Il Farmaco 55 (2000) 93-98;
(d) E. Novotná, K. Waisser, J. Kunes, K. Palát, L. Skálová, B. Szotáková, V. Buchta, J. Stolaríková, V. Ulmann, M. Pávová, J. Weber, J. Komrsková, P. Hasková, I. Vokrál, V. Wsól, Arch. Pharm. Chem. Life Sci. 350 (2017) e1700020;
(e) H. Hammoud, K. Elhabazi, R. Quillet, I. Bertin, V. Utard, E. Laboureyras, J.-J. Bourguignon, F. Bihel, G. Simonnet, F. Simonin, M. Schmitt, ACS Chem. Neurosci. 9 (2018) 2599-2609.

[10] D. Prat, A. Wells, J. Hayler, H. Sneddon, C.R. McElroy, S. Abou-Shehada, P.J. Dunn, Green Chem. 18 (2016) 288-296.

[11] (a) C. Yamazaki, Can. J. Chem. 53 (1975) 610-615;
(b) C. Yamazaki, J. Org. Chem. 46 (1981) 3956-3959.

[12] M.M. Sá, M. Ferreira, A.J. Bortoluzzi, L. Fernandes, S. Cunha, Arkivoc xi (2010) 303-321.

[13] (a) Y.I. Ryabukhin, O.B. Korzhavina, K.F. Suzdalev, Adv. Heterocycl. Chem. 66 (1996) 131-191;
(b) A.A. Hassan, N.K. Mohamed, A.A. Aly, H.N. Tawfeek, H. Hopf, S. Bräse, M. Nieger, Mol. Divers. 23 (2019) 821-828.

[14] (a) E.V. Nosova, G.N. Lipunova, V.N. Charushin, O.N. Chupakhin, Mini-Rev. Med. Chem. 19 (2019) 999-1014;
(b) L.J. Silverberg, Q.J. Moyer, Arkivoc i (2019) 139-227.

[15] M. Xiabing, K. Ablajan, M. Obul, M. Seydimemet, R. Ruzi, L. Wenbo, Tetrahedron 76 (2016) 2349-2353.

[16] (a) L. Fliri, G. Partl, T. Gelbrich, S. Nerdinger, K. Wurst, H. Schottenberger, Heterocycl. 101 (2020) 593-610;
(b) C. Yamazaki, Bull. Chem. Soc. Jap. 53 (1980) 3289-3294.

[17] K.J. Chavez, S.V. Garimella, S. Lipkowitz, Breast Dis. 32 (2010) 35-48.

[18] T. Mosmann, J. Immunol. Methods 65 (1983) 55-63.

[19] (a) H.M.R. Hoffmann, J.J. Rabe, J. Org. Chem. 50 (1985) 3849-3859;
(b) A. Foucaud, F.E. Guemmout, Bull. Soc. Chim. Fr. (1989) 403-408;
(c) A. Gruiec, A. Foucaud, New J. Chem. 15 (1991) 943-947;
(d) L. Fernandes, A.J. Bortoluzzi, M.M. Sá, Tetrahedron 60 (2004) 9983-9989;
(e) P.V. Ramachandran, D.R. Nicponski, Chem. Commun. 50 (2014) 15216-15219.

[20] M.S. Alam, L. Liu, Y.-E. Lee, D.-U. Lee, Chem. Pharm. Bull. 59 (2011) 568-573.

HIGHLIGHTS

→ Isothiosemicarbazones were readily prepared through multicomponent synthesis

→ The transformations proceed under mild conditions with high yields and selectivity

→ Unexpected cyclization to the 1,3-thiazinone framework expanded the scope of the MCR

→ All compounds were obtained in high purity without the need for chromatography stages

→ Isothiosemicarbazones showed cytotoxic activity toward aggressive breast cancer cells

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Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

none