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Article

Synthesis of Novel PETT Analogues: 3,4-Dimethoxy Phenyl Ethyl 1,3,5-Triazinyl Thiourea Derivatives and their Antibacterial and Anti-HIV Studies

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Derivados de 3,4-dimetóxi-fenil-etil-1,3,5-triazinil tioureias (**8a-o** e **9a-o**) foram preparados pela condensação de 2,4,6-tricloro-1,3,5-s-triazina (**1**) com 4-hidroxi cumarina (**2**), 3,4-dimetoxi-fenil-etil tioureia (**4**) e várias ureias/tioureias substituídas (**6a-o/7a-o**), sendo posteriormente testadas com relação a atividade anti-bacteriana e anti-HIV frente a diferentes microorganismos. As estruturas dos compostos sintetizados foram estabelecidas usando-se ^{1}H RMN, IV e análise elementar.

3,4-Dimethoxy phenyl ethyl-1,3,5-triazinyl thiourea derivatives (**8a-o** and **9a-o**) were prepared by condensation of 2,4,6-trichloro-1,3,5-s-triazine (**1**) with 4-hydroxy coumarin (**2**), 3,4-dimethoxy phenyl ethyl thiourea (4) and various substituted phenyl urea/thiourea (**6a-o/7a-o**) and tested for their antibacterial and anti-HIV activities against different microorganisms. The structures of novel synthesized compounds have been established on the basis of ^{1}H NMR, IR and Elemental Analysis.

Keywords: PETT analogues, 3,4-dimethoxyphenylethyl-1,3,5-triazinyl thiourea, antibacterial, anti-HIV

Introduction

Nitrogen containing heterocycles play an important role, not only for life science industry but also in many other industrial fields related to special and fine chemistry. Among them 1,3,5-triazines represent a widely used lead structure with multitude of interesting applications in numerous fields.[1]

Several derivatives of s-triazine show antimicrobial,[2] antibacterial,[3] and herbicidal activities.[4] Some are also used for the treatment of HIV infection.[5,6] Several workers investigated the s-triazine nucleus in the scope of potential therapeutic agents for diseases due to bacteria, malaria, and cancer.[7] The above literature survey led us to consider the s-triazine nucleus as a possible scaffold.

Coumarin derivatives have revealed new biological activities with interesting potential in therapeutic applications besides their traditional employment as anticoagulants (anti-vitamin-K activity) and suntan agents (photosensitizing action of furocoumarins). They have yielded important results as antibiotics (Novobiocin and analogues),[8] anti-AIDS agents (Calanolides)[9] and antitumor drugs (Gelparvarin).[10] Some of these drugs derived from 4-hydroxycoumarin have been thoroughly investigated.[11]

Phenylethylamine derivatives possess a broad spectrum of biological activity and fulfill an important function in animal metabolism.[12] Synthetic beta-phenylethylamines are the key substances in the synthesis of a number of isoquinoline alkaloids: 1-benzylisoquinolines, N-benzylisoquinolines, aporphines and diisoquinolines.[13] Recently, these compounds are used in the synthesis of anti-HIV compounds as their thiourea derivatives. We have synthesized novel 3,4-dimethoxy phenyl ethyl thiourea derivatives.

Over the last few years, the thiourea moiety has been of interest to design molecules designed as receptor antagonists, as natural product mimics or as synthetic intermediates to amidines or guanidines.[14]

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Thiourea not only confers antibacterial, antitubercular or antileprotic activity, but has also been reported to possess antifungal as well as antiviral properties.[15]

Recently, the PETT[16] type of [reverse transcriptase (RT)] inhibitors has been described. The representative compound of this series is trovirdine, a potent RT inhibitor, which, however, has low oral bioavailability.[17]

Diphenyl urea derivatives find their wide clinical application in the therapy of functional diseases. Diphenyl urea derivatives are widely used particularly in pharmaceutical chemistry. Urea derivatives possess wide therapeutic activities, *i.e.* antithyroidal,[18] hypnotic and anaesthetic,[19] antibacterial,[20] diuretic[21] and anthelmintics.

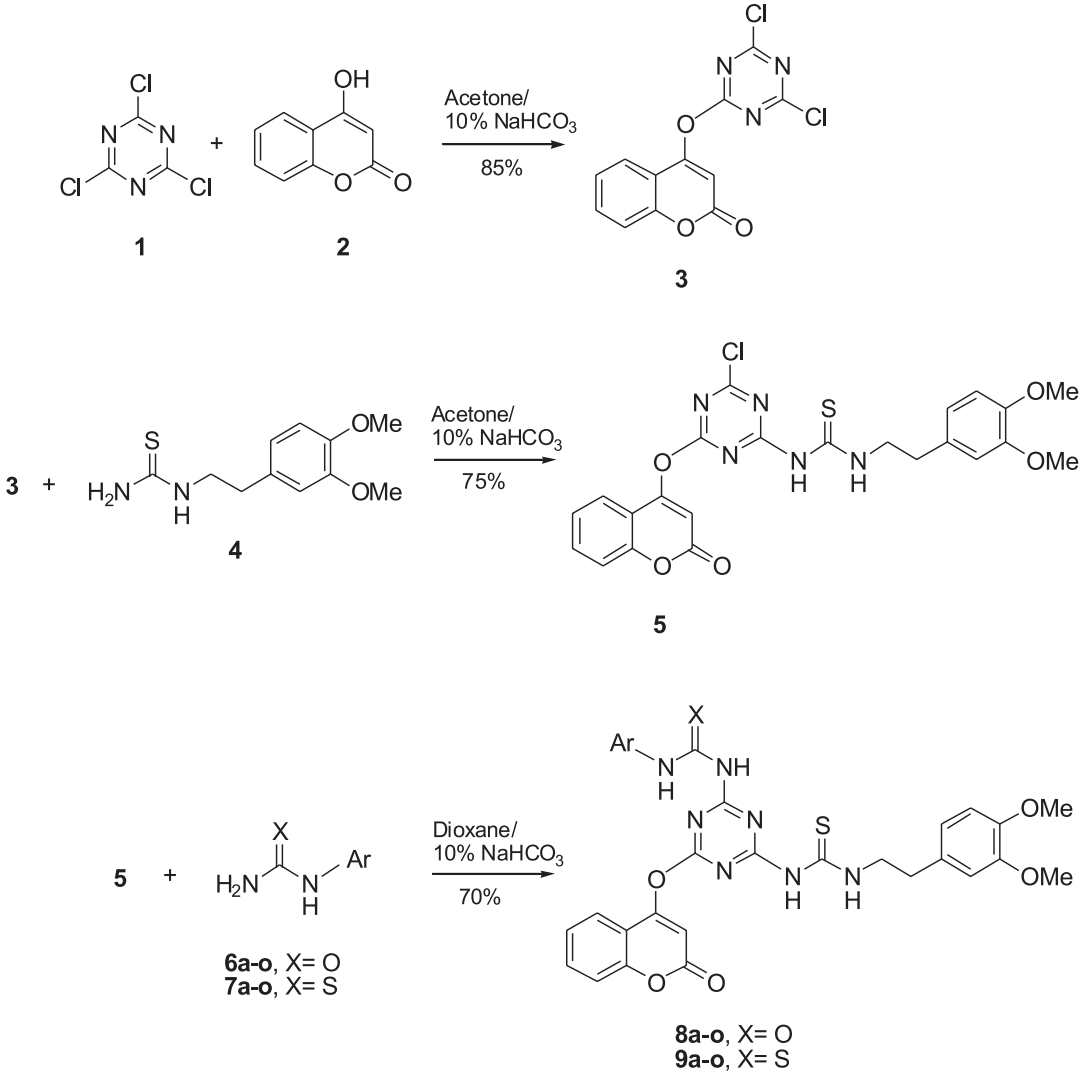
Some specific HIV-1 RT inhibitory have been described including urea analogues[22,23] of PETT (Phenyl Ethyl Thiazolyl Thiourea) derivatives and the series includes derivatives with an ethyl linker and conformationally restricted analogues.

These pharmacological properties of coumarins drew our interest in synthesizing several new compounds featuring heterocyclic rings, *i.e.* s-triazine (**1**) in a 4-hydroxycoumarin (**2**), which was then condensed with 3,4-dimethoxy phenyl ethyl thiourea (**3**) and various phenyl ureas, various phenyl thioureas (**6a-o/7a-o**) with the aim to obtain more potent pharmacologically active compounds (**8a-o/9a-o**).

Results and Discussion

3,4-Dimethoxy phenyl ethyl-1,3,5-triazinyl thiourea derivatives (**8a-o/9a-o**) were prepared by the condensation of 2,4,6-trichloro-1,3,5-s-triazine (**1**) with 4-hydroxy-coumarin (**2**), 3,4-dimethoxy phenyl ethyl thiourea (**3**) and various substituted phenyl thiourea/urea (**6a-o/7a-o**).

We have synthesized 30 compounds and all the 30 compounds along with their parent compound s-triazine



Scheme 1.

Table 1. Characterization data of **8a**-**o** and **9a**-**o**

Compound	Ar	X	Molecular Formula	mp / (°C)	Yield / (%)[a]
8a	C_6H_5	O	$C_{30}H_{27}N_7O_6S$	97-100	70
8b	$CH_2C_6H_5$	O	$C_{31}H_{29}N_7O_6S$	150-155	53
8c	$C_{10}H_7$	O	$C_{34}H_{29}N_7O_6S$	195-200	76
8d	2-$OCH_3C_6H_4$	O	$C_{31}H_{29}N_7O_7S$	210-215	67
8e	4-$OCH_3C_6H_4$	O	$C_{31}H_{29}N_7O_7S$	115-120	79
8f	2-$CH_3C_6H_4$	O	$C_{31}H_{29}N_7O_6S$	230-232	52
8g	3-$CH_3C_6H_4$	O	$C_{31}H_{29}N_7O_6S$	225-227	63
8h	4-$CH_3C_6H_4$	O	$C_{31}H_{29}N_7O_6S$	170-172	69
8i	2-$NO_2C_6H_4$	O	$C_{30}H_{26}N_8O_8S$	176-178	59
8j	3-$NO_2C_6H_4$	O	$C_{30}H_{26}N_8O_8S$	215-219 (d.)	54
8k	4-$NO_2C_6H_4$	O	$C_{30}H_{26}N_8O_8S$	202-205	51
8l	2-ClC_6H_4	O	$C_{30}H_{26}N_7O_6SCl$	140-145	60
8m	3-ClC_6H_4	O	$C_{30}H_{26}N_7O_6SCl$	255 (d.)	73
8n	4-ClC_6H_4	O	$C_{30}H_{26}N_7O_6SCl$	163-165	73
8o	4-FC_6H_4	O	$C_{30}H_{26}N_7O_6SF$	240-245 (d.)	56
9a	C_6H_5	S	$C_{30}H_{27}N_7O_5S_2$	115-117	72
9b	$CH_2C_6H_5$	S	$C_{31}H_{29}N_7O_5S_2$	132-135	69
9c	$C_{10}H_7$	S	$C_{34}H_{29}N_7O_5S_2$	145-150	50
9d	2-$OCH_3C_6H_4$	S	$C_{31}H_{29}N_7O_6S_2$	225-230 (d.)	53
9e	4-OCH_3C_6H4	S	$C_{31}H_{29}N_7O_6S_2$	178-180	66
9f	2-CH_3C_6H4	S	$C_{31}H_{29}N_7O_5S_2$	250-255 (d.)	72
9g	3-CH_3C_6H4	S	$C_{31}H_{29}N_7O_5S_2$	175-177	63
9h	4-CH_3C_6H4	S	$C_{31}H_{29}N_7O_5S_2$	130-132	75
9i	2-$NO_2C_6H_4$	S	$C_{30}H_{26}N_8O_7S_2$	>300	57
9j	3-$NO_2C_6H_4$	S	$C_{30}H_{26}N_8O_7S_2$	230-232	68
9k	4-$NO_2C_6H_4$	S	$C_{30}H_{26}N_8O_7S_2$	260-263 (d.)	59
9l	2-ClC_6H_4	S	$C_{30}H_{26}N_7O_5S_2Cl$	210-215	55
9m	3-ClC_6H_4	S	$C_{30}H_{26}N_7O_5S_2Cl$	180-183	60
9n	4-ClC_6H_4	S	$C_{30}H_{26}N_7O_5S_2Cl$	110-112	67
9o	4-FC_6H_4	S	$C_{30}H_{26}N_7O_5S_2F$	150-152	56

[a]yields of purified compounds.

and intermediates were subjected to antibacterial as well as anti-HIV activity evaluation. The antibacterial activity was determined using the disc diffusion method[24,25] by measuring the inhibition zones in mm. The compounds were screened for their HIV-1 and HIV-2 inhibitory activities described earlier.[26] This series contains three types of chemical linkages; ether linkage with 4-hydroxycoumarin, thioureido linkage with 3,4-dimethoxy phenyl ethyl thiourea and thioureido/ureido linkage with substituted phenyl ring.

In the case of ether linkage with 4-hydroxy coumarin as well as thioureido linkage with thiourea and ureido linkage with phenyl urea much deviation of activity from the parent molecule has been observed.

All three linkages show increased biological activity as compared with their parent molecule. All compounds showed comparable activity with the standard drugs (Table 2). **8e** showed maximum zone of inhibition (14 mm) against *E. coli* and *B. subtilis* while **8g** (zone of inhibition 17 mm) against *S. typhi* and **8f** (16 mm) against *S. aureus* showed excellent antibacterial activity.

As shown above s-triazine shows moderate activity against *E. coli, S. aureus, S. typhi* and *B. subtilis*.

Five compounds *viz*. (**9a**, **9m**, **9f**, **9g** and **9h** mentioned in Table 2) have exhibited maximum zone of inhibition against *B. subtilis*, *S. aureus*, *E. coli* and *S. typhi*. Their zone of inhibitions is given in the Table 2. Thus it is observed that the compound with substituents like methoxy, methyl and halo in the aromatic group enhanced the antibacterial activity.

Recently, a number of investigators have reported on nonnucleoside inhibitors of human immunodeficiency virus type 1 and 2 (HIV-1 and HIV-2) reverse transcriptase (RT) that may have potential for therapeutic use in AIDS chemotherapy. These derivatives exhibit no selectivity for HIV-1 and HIV-2 although these derivatives contain phenyl ethyl triazinyl thiourea lead structure. All compounds in this series showed poor activity against both HIV-1 and HIV-2 as their selectivity index is <1 (Table 3).

In this series we have synthesized another PETT type compounds by replacing thiazole with triazine. Antiviral activity of the compound of this series was assessed by measuring the protection provided by the inhibitor against cytopathic effect (CPE) of viral infection. **9k** was found moderately active against both type of HIV-1 (IIIB) and HIV-2 (ROD) as their selectivity index was 3 for both HIV-1 and HIV-2 while **9c**, **9h** and **9n** have selectivity index =9,

Table 2. Antibacterial activity of **8a-o** and **9a-o**

Compound	Ar	Antibacterial Activity[a]			
		E. coli	*S. aureus*	*S. typhi*	*B. subtilis*
8a	C_6H_5	13	12	11	08
8b	$CH_2C_6H_5$	12	11	10	10
8c	$C_{10}H_7$	11	-	10	-
8d	2-$OCH_3C_6H_4$	11	14	10	-
8e	4-$OCH_3C_6H_4$	14	12	11	14
8f	2-$CH_3C_6H_4$	13	16	12	11
8g	3-$CH_3C_6H_4$	12	09	17	-
8h	4-$CH_3C_6H_4$	-	10	15	10
8i	2-$NO_2C_6H_4$	-	09	-	09
8j	3-$NO_2C_6H_4$	09	-	11	08
8k	4-$NO_2C_6H_4$	-	08	-	-
8l	2-ClC_6H_4	10	-	12	-
8m	3-ClC_6H_4	-	15	15	10
8n	4-ClC_6H_4	12	11	-	09
8o	4-FC_6H_4	-	11	17	10
9a	C_6H_5	11	14	13	17
9b	$CH_2C_6H_5$	13	14	10	-
9c	$C_{10}H_7$	-	11	-	10
9d	2-$OCH_3C_6H_4$	11	09	10	08
9e	4-$OCH_3C_6H_4$	13	12	11	13
9f	2-$CH_3C_6H_4$	12	15	11	-
9g	3-$CH_3C_6H_4$	14	10	13	-
9h	4-$CH_3C_6H_4$	-	10	15	10
9i	2-$NO_2C_6H_4$	10	-	-	10
9j	3-$NO_2C_6H_4$	-	10	12	-
9k	4-$NO_2C_6H_4$	12	-	10	08
9l	2-ClC_6H_4	-	-	12	-
9m	3-ClC_6H_4	-	15	-	-
9n	4-ClC_6H_4	12	-	-	12
9o	4-FC_6H_4	10	09	14	12
Standard Drugs	Tetracycline	15	19	24	21
	Chloramphenicol	18	25	24	20

[a] (Zone of inhibition in mm) measured at 50 μg mL^{-1} concentration; -: not active.

=3 and =11 against HIV-1. Other compounds of the series were poorly active as their antivirally effective concentration (EC_{50}) and cytotoxic concentration (CC_{50}) was the same and their SI also <1 (Table 3).

Conclusions

The synthesis and anti-HIV activity studies of 2-(coumarinyl-4-oxy)-4-(3,4-dimethoxy phenyl ethyl thioureido)-6-(aryl thioureido/ureido)-s-triazine has been described. The antimicrobial screening of the series reveal the following points: hyper conjugation of methyl group and electron donating effect of para-methoxy group favor the increase in activity of lead molecule. The structural variations such as methyl group at *ortho*, *meta*, and *para* position to the ureido linkage against all microorganisms resulted in a increased of the antibacterial activity of parent compound due to the crowding effect (hyper conjugation) of methyl group. The halogen substituents increase the compound activity.

Only minor change in antibacterial activity of both series was observed by changing urea derivatives with thiourea derivatives or *vice-versa*.

In the case of anti-HIV activity, data show that compounds of both series have poor potency and/or selectivity. This may be related to the presence of bulky groups on triazine heterocyclic ring. Also, the presence of an ether linkage with 4-hydroxycoumarin results in overcrowding thereby disturbing the overall planarity of the coumarin system.

Experimental

General

All melting points are uncorrected. ^{1}H-NMR spectra were obtained using a Verian-300 model spectrophotometer and were recorded at 300 MHz in DMSO-d_6. Chemical shifts are reported in ppm relative to the residual signal of the solvent. IR spectra were recorded on a FT "BOMMEN"

Table 3 Anti-HIV activity of some selected compounds

Compound	strain	EC_{50} / (μg mL^{-1}) [a]	CC_{50} / (μg mL^{-1}) [b]	SI[c]
8h	III B	>125	>125	×1
		>125	>125	×1
	ROD	>125	>125	×1
9b	III B	>22	=22	<1
		>25	>25	×1
	ROD	>114	=114	<1
9c	III B	=8.46	=74.9	=9
		>56.6	=56.6	<1
	ROD	>56.7	=56.7	<1
9e	III B	>125	>125	×1
		>113	=113	<1
	ROD	>125	>125	×1
9h	III B	=23.9	=67.5	=3
		>52.4	=52.4	<1
	ROD	>47.2	=47.2	<1
9i	III B	>125	>125	×1
		>93.4	=93.4	<1
	ROD	>108	=108	<1
9k	III B	=14.5	=53.8	=4
		=18.7	=49.4	=3
	ROD	=19.3	=50.8	=3
9n	III B	=1.57	=17.6	=11
		=2.25	=17.8	=8
	ROD	>17.5	=17.5	<1

[a] EC_{50}, Antivirally Effective Concentration; [b] CC_{50}, Cytotoxic Concentration of compound that reduces the viability of mock infected cell by 50% as determined by the MTT method; [c] Selectivity Index, *i.e.*, CC_{50}/EC_{50} ratio.

spectrophotometer using potassium bromide pellets. Elemental analyses were done on "Haraeus Rapid Analyser". The signals of the compounds **(8a-o** and **9a-o)** were sharp and coupling constants were determined. The data of 3,4-dimethoxy phenyl ethyl-1,3,5-triazinyl thiourea derivatives **(8a-o** and **9a-o)** are presented here.

*2-(Coumarinyl-4-oxy)-4,6-dichloro-s-triazine (**3**)*

To a stirred solution of cyanuric chloride (0.1 mol, 18.4g.) in acetone (100 mL) at 0-5 °C, the solution of 4-hydroxy coumarin (0.1 mol, 16.2g.) in 10% $NaHCO_3$ (90 mL) was added drop wise in two hours. The progress of reaction was monitored by TLC using acetone:toluene (10:1) as eluent. After completion of reaction, the stirring was stopped and the solution was treated with crushed ice. The product obtained was filtered and dried. The crude product was purified by recrystallization from acetone to give the title compound (**1**); yield 85%, mp 208-210 °C; Found N, 13.35. $C_{12}H_5N_3O_3Cl_2$ requires N, 13.55.

*2-(Coumarinyl- 4-oxy)-4-(3, 4-dimethoxy phenyl ethyl thioureido)-6-chloro-s-triazine (**5**)*

3,4-Dimethoxy phenyl ethyl thiourea (0.05 mol, 12.0 g) dissolved in acetone:water (10:2, 60 mL) was slowly added to well-stirred slurry of 2-(Coumarinyl-4-oxy)-4,6-dichloro-s-triazine (0.05 mol, 15.5g.) in acetone (90 mL), maintaining the temperature at 45 °C. The pH was adjusted to neutral by the addition of 10% $NaHCO_3$ solution. The progress of reaction was monitored by TLC using acetone:toluene (10:1) as eluent. The temperature was gradually raised to 50 °C during two hours and further maintained for two hours. After completion of reaction, the solution was poured in ice-cold water. The solid product obtained was filtered and dried. The crude product was purified by recrystallization from absolute alcohol to give the title compound (**5**); yield 75%, mp 145-150 °C; Found N, 13.41. $C_{22}H_{20}N_5O_5Cl$ requires N, 13.63.

*General procedure for preparation of 2-(coumarinyl-4-oxy)-4-(3,4-dimethoxy phenyl ethyl thioureido)-6-(aryl ureido/thioureido)-s-triazine (**8a-o/9a-o**)*

A mixture of 2-(coumarinyl-4-oxy)-4-(3,4-dimethoxy phenyl ethyl thioureido)-6-chloro-s-triazine (0.005 mol) and aryl urea/thiourea (0.005 mol) in dioxane (50 mL) was refluxed on heating mantle with stirring at reflux temperature for four hours. The pH was adjusted neutral by the addition of 10% $NaHCO_3$ solution. After the completion of reaction, the content was added to cold-water. The product obtained was purified by recrystallization from absolute alcohol. The physical and analytical data of novel compounds of this series are given in Table 1.

*2-(Coumarinyl-4-oxy)-4-(3,4-dimethoxy phenyl ethyl thioureido)-6-(phenyl ureido)-s-triazine (**8a**)*

IR $\nu_{(max)}$/cm^{-1}: Thiourea C=S str. 1539 N-H str. 3345 N-H def. 1604; Urea C=O str. 1550 N-H str. 2800; Coumarin C=O str. (δ lactone) 1729 C=C str. (α,β-unsat.) 1697; s-Triazine C-N str. 806; Ether C-O-C str. (asym.) 1234 C-O-C str. (sym.) 1026; Methylene C-H str. 2935 C-H def. 1454. ^{1}H-NMR: δ 2.82 (t, 2H, *J* 7.2Hz, -CH$_2$), 3.20 (t, 2H, *J* 6.4Hz, -CH$_2$), 8.90 (s, 1H, Ar-NH), 9.42 (s, 1H, CS-NH), {6.69 (s, 1H, Ar-NH), 9.72 (br. s, 1H, Ar-NH)} D$_2$O exchangeable, 6.80 (s, 1H-C=CH), 3.76 (s, 6H, 2x-OCH$_3$), 6.61 (dd, 1H, *J* 2.2Hz, *J* 8.3Hz, Ar-H), 6.73 (dd, 1H, *J* 1.9Hz, *J* 8.2Hz, Ar-H), 7.40-8.0 (m, 10H, Ar-H).

*2-(Coumarinyl-4-oxy)-4-(3,4-dimethoxy phenyl ethyl thioureido)-6-(benzyl ureido)-s-triazine (**8b**)*

^{1}H NMR: δ 2.82 (t, 2H, *J* 7.1Hz, -CH$_2$), 3.20 (t, 2H, *J* 6.9Hz, -CH$_2$), 8.90 (s, 1H, Ar-NH), 9.42 (s, 1H, CS-NH), {6.69 (s, 1H, Ar-NH) 9.72 (br. s, 1H, Ar-NH)} D$_2$O exchangeable, 6.80 (s, 1H-C=CH), 3.76 (s, 6H, 2x-OCH$_3$), 6.61 (dd, 1H, *J* 2.0Hz, *J* 8.2Hz, Ar-H), 6.73 (dd, 1H, *J* 1.95Hz, *J* 7.98Hz, Ar-H), 7.40-8.0 (m, 10H, Ar-H).

*2-(Coumarinyl-4-oxy)-4-(3,4-dimethoxy phenyl ethyl thioureido)-6-(1-naphthyl ureido)-s-triazine (**8c**)*

^{1}H NMR: δ 2.82 (t, 2H, *J* 6.9Hz, -CH$_2$), 3.20 (t, 2H, *J* 6.77Hz, -CH$_2$), 8.90 (s, 1H, Ar-NH), 9.42 (s, 1H, CS-NH), {6.69 (s, 1H, Ar-NH) 9.72 (br. s, 1H, Ar-NH)} D$_2$O exchangeable, 6.80 (s, 1H-C=CH), 3.76 (s, 6H, 2x-OCH$_3$), 6.61 (dd, 1H, *J* 1.92Hz, *J* 8.11Hz, Ar-H), 6.73 (dd, 1H, *J* 2.1Hz, *J* 8.22Hz, Ar-H), 7.40-8.0 (m, 10H, Ar-H).

*2-(Coumarinyl-4-oxy)-4-(3,4-dimethoxy phenyl ethyl thioureido)-6-(2-methoxy ureido)-s-triazine (**8d**)*

^{1}H NMR: δ 2.75 (t, 2H, *J* 7.12Hz, -CH$_2$), 3.35 (t, 2H, *J* 6.98Hz, -CH$_2$), 8.88 (s, 1H, Ar-NH), 9.45 (s, 1H, CS-NH), {6.67 (s, 1H, Ar-NH) 9.70 (br. s, 1H, Ar-NH)} D$_2$O exchangeable, 6.85 (s, 1H-C=CH), 3.85 (s, 6H, 2x-OCH$_3$), 3.35 (s, 6H, -OCH$_3$), 6.69 (dd, 1H, *J* 1.96Hz, *J* 7.98Hz, Ar-H), 6.74 (dd, 1H, *J* 2.22Hz, *J* 8.18Hz, Ar-H), 6.92 (d, 2H, *J* 8.3Hz, Ar-H), 7.40 (d, 2H, *J* 8.35Hz, Ar-H), 7.60-8.20 (m, 5H, Ar-H).

*2-(Coumarinyl-4-oxy)-4-(3,4-dimethoxy phenyl ethyl thioureido)-6-(4-methoxy ureido)-s-triazine (**8e**)*

IR ν_{max}/cm^{-1}: Thiourea C=S str. 1539 N-H str. 3300 N-H def. 1604; Urea C=O str. 1554 N-H str. 2850; Coumarin C=O str. (δ lactone) 1728 C=C str. (α,β-unsat.) 1697; s-Triazine C-N str. 806; Ether C-O-C str. (asym.) 1238 C-O-C str. (sym.) 1026; Methylene C-H str. 2940 C-H def. 1454. ^{1}H-NMR: δ 2.75 (t, 2H, *J* 6.98Hz, -CH$_2$), 3.35 (t, 2H, *J* 6.69Hz, -CH$_2$), 8.88 (s, 1H, Ar-NH), 9.45 (s, 1H, CS-NH), {6.67 (s, 1H, Ar-NH) 9.70 (br. s, 1H, Ar-NH)} D$_2$O exchangeable, 6.85 (s, 1H-C=CH), 3.85 (s, 6H, 2x-OCH$_3$), 3.35 (s, 6H, -OCH$_3$), 6.69 (dd, 1H, *J* 2.08Hz, *J* 8.23Hz, Ar-H), 6.74 (dd, 1H, *J* 1.99Hz, *J* 8.15Hz, Ar-H), 6.92 (d, 2H, *J* 8.26Hz, Ar-H), 7.40 (d, 2H, *J* 8.18Hz, Ar-H), 7.60-8.20 (m, 5H, Ar-H).

*2-(Coumarinyl-4-oxy)-4-(3,4-dimethoxy phenyl ethyl thioureido)-6-(2-tolyl ureido)-s-triazine (**8f**)*

^{1}H NMR: δ 2.71 (t, 2H, *J* 7.17Hz, -CH$_2$), 3.34 (t, 2H, *J* 6.77Hz, -CH$_2$), 8.90 (s, 1H, Ar-NH), 9.51 (s, 1H, CS-NH), {8.12 (s, 1H, Ar-NH) 8.46 (br. s, 1H, Ar-NH)} D$_2$O exchangeable, 6.73 (s, 1H-C=CH), 3.70 (s, 6H, 2x-OCH$_3$), 2.45 (s, 3H, -CH$_3$), 6.76 (dd, 1H, *J* 1.98Hz, *J* 7.89Hz, Ar-H), 6.80 (dd, 1H, *J* 1.93Hz, *J* 7.85Hz, Ar-H), 7.20 (d, 2H, *J* 8.4Hz, Ar-H), 7.40 (d, 2H, *J* 8.35Hz, Ar-H), 7.60-8.10 (m, 5H, Ar-H).

*2-(Coumarinyl-4-oxy)-4-(3,4-dimethoxy phenyl ethyl thioureido)-6-(3-tolyl ureido)-s-triazine (**8g**)*

^{1}H NMR: δ 2.71 (t, 2H, *J* 7.22Hz, -CH$_2$), 3.34 (t, 2H, *J* 6.79Hz, -CH$_2$), 8.90 (s, 1H, Ar-NH), 9.51 (s, 1H, CS-NH), {8.12 (s, 1H, Ar-NH) 8.46 (br. s, 1H, Ar-NH)} D$_2$O exchangeable, 6.73 (s, 1H-C=CH), 3.70 (s, 6H, 2x-OCH$_3$), 2.45 (s, 3H, -CH$_3$), 6.76 (dd, 1H, *J* 2.06Hz, *J* 8.09Hz, Ar-H), 6.80 (dd, 1H, *J* 2.12Hz, *J* 8.15Hz, Ar-H), 7.20 (d, 2H, *J* 8.36Hz, Ar-H), 7.40 (d, 2H, *J* 8.28Hz, Ar-H), 7.60-8.10 (m, 5H, Ar-H).

*2-(Coumarinyl-4-oxy)-4-(3,4-dimethoxy phenyl ethyl thioureido)-6-(4-tolyl ureido)-s-triazine (**8h**)*

^{1}H NMR: δ 2.71 (t, 2H, *J* 7.12Hz, -CH$_2$), 3.34 (t, 2H, *J* 6.99Hz, -CH$_2$), 8.90 (s, 1H, Ar-NH), 9.51 (s, 1H, CS-NH), {8.12 (s, 1H, Ar-NH) 8.46 (br. s, 1H, Ar-NH)} D$_2$O exchangeable, 6.73 (s, 1H-C=CH), 3.70 (s, 6H, 2x-OCH$_3$), 2.45 (s, 3H, -CH$_3$), 6.76 (dd, 1H, *J* 2.16Hz, *J* 8.29Hz, Ar-H), 6.80 (dd, 1H, *J* 2.09Hz, *J* 8.19Hz, Ar-H), 7.20 (d, 2H, *J* 8.24Hz, Ar-H), 7.40 (d, 2H, *J* 8.19Hz, Ar-H), 7.60-8.10 (m, 5H, Ar-H).

*2-(Coumarinyl-4-oxy)-4-(3,4-dimethoxy phenyl ethyl thioureido)-6-(2-nitrophenyl ureido)-s-triazines (**8i**)*

^{1}H NMR: δ 2.82 (t, 2H, *J* 6.92Hz, -CH$_2$), 3.20 (t, 2H, *J* 6.72Hz, -CH$_2$), 8.90 (s, 1H, Ar-NH), 9.42 (s, 1H, CS-NH), {6.69 (s, 1H, Ar-NH) 9.72 (br. s, 1H, Ar-NH)} D$_2$O exchangeable, 6.80 (s, 1H-C=CH), 3.76 (s, 6H, 2x-OCH$_3$), 6.61 (dd, 1H, *J* 1.98Hz, *J* 7.99Hz, Ar-H), 6.73 (dd, 1H, *J* 2.12Hz, *J* 8.16Hz, Ar-H), 7.40-8.0 (m, 10H, Ar-H).

*2-(Coumarinyl-4-oxy)-4-(3,4-dimethoxy phenyl ethyl thioureido)-6-(3-nitrophenyl ureido)-s-triazine (**8j**)*

^{1}H NMR: δ 2.82 (t, 2H, *J* 7.22Hz, -C$\underline{H}_2$), 3.20 (t, 2H, *J* 6.78Hz, -C$\underline{H}_2$), 8.90 (s, 1H, Ar-N$\underline{H}$), 9.42 (s, 1H, CS-N$\underline{H}$), {6.69 (s, 1H, Ar-N$\underline{H}$) 9.72 (br. s, 1H, Ar-N$\underline{H}$)} D_2O exchangeable, 6.80 (s, 1H-C=C$\underline{H}$), 3.76 (s, 6H, 2x-OC$\underline{H}_3$), 6.61 (dd, 1H, *J* 2.18Hz, *J* 8.20Hz, Ar-$\underline{H}$), 6.73 (dd, 1H, *J* 2.32Hz, *J* 8.26Hz, Ar-$\underline{H}$), 7.40-8.0 (m, 10H, Ar-$\underline{H}$).

*2-(Coumarinyl-4-oxy)-4-(3,4-dimethoxy phenyl ethyl thioureido)-6-(4-nitrophenyl ureido)-s-triazine (**8k**)*

^{1}H NMR: δ 2.82 (t, 2H, *J* 7.29Hz, -C$\underline{H}_2$), 3.20 (t, 2H, *J* 6.65Hz, -C$\underline{H}_2$), 8.90 (s, 1H, Ar-N$\underline{H}$), 9.42 (s, 1H, CS-N$\underline{H}$), {6.69 (s, 1H, Ar-N$\underline{H}$) 9.72 (br. s, 1H, Ar-N$\underline{H}$)} D_2O exchangeable, 6.80 (s, 1H-C=C$\underline{H}$), 3.76 (s, 6H, 2x-OC$\underline{H}_3$), 6.61 (dd, 1H, *J* 1.9Hz, *J* 7.88Hz, Ar-$\underline{H}$), 6.73 (dd, 1H, *J* 1.96Hz, *J* 7.97Hz, Ar-$\underline{H}$), 7.40-8.0 (m, 10H, Ar-$\underline{H}$).

*2-(Coumarinyl-4-oxy)-4-(3,4-dimethoxy phenyl ethyl thioureido)-6-(2-chlorophenyl ureido)-s-triazine (**8l**)*

^{1}H NMR: δ 2.82 (t, 2H, *J* 7.12Hz, -C$\underline{H}_2$), 3.20 (t, 2H, *J* 6.89Hz, -C$\underline{H}_2$), 8.90 (s, 1H, Ar-N$\underline{H}$), 9.42 (s, 1H, CS-N$\underline{H}$), {6.69 (s, 1H, Ar-N$\underline{H}$) 9.72 (br. s, 1H, Ar-N$\underline{H}$)} D_2O exchangeable, 6.80 (s, 1H-C=C$\underline{H}$), 3.76 (s, 6H, 2x-OC$\underline{H}_3$), 6.61 (dd, 1H, *J* 2.01Hz, *J* 7.87Hz, Ar-$\underline{H}$), 6.73 (dd, 1H, *J* 2.03Hz, *J* 7.76Hz, Ar-$\underline{H}$), 7.40-8.0 (m, 10H, Ar-$\underline{H}$).

*2-(Coumarinyl-4-oxy)-4-(3,4-dimethoxy phenyl ethyl thioureido)-6-(3-chlorophenyl ureido)-s-triazine (**8m**)*

^{1}H NMR: δ 2.82 (t, 2H, *J* 7.18Hz, -C$\underline{H}_2$), 3.20 (t, 2H, *J* 6.72Hz, -C$\underline{H}_2$), 8.90 (s, 1H, Ar-N$\underline{H}$), 9.42 (s, 1H, CS-N$\underline{H}$), {6.69 (s, 1H, Ar-N$\underline{H}$) 9.72 (br. s, 1H, Ar-N$\underline{H}$)} D_2O exchangeable, 6.80 (s, 1H-C=C$\underline{H}$), 3.76 (s, 6H, 2x-OC$\underline{H}_3$), 6.61 (dd, 1H, *J* 2.13Hz, *J* 8.23Hz, Ar-$\underline{H}$), 6.73 (dd, 1H, *J* 2.07Hz, *J* 8.19Hz, Ar-$\underline{H}$), 7.40-8.0 (m, 10H, Ar-$\underline{H}$).

*2-(Coumarinyl-4-oxy)-4-(3,4-dimethoxy phenyl ethyl thioureido)-6-(4-chlorophenyl ureido)-s-triazine (**8n**)*

^{1}H NMR: δ 2.82 (t, 2H, *J* 7.05Hz, -C$\underline{H}_2$), 3.20 (t, 2H, *J* 6.75Hz, -C$\underline{H}_2$), 8.90 (s, 1H, Ar-N$\underline{H}$), 9.42 (s, 1H, CS-N$\underline{H}$), {6.69 (s, 1H, Ar-N$\underline{H}$) 9.72 (br. s, 1H, Ar-N$\underline{H}$)} D_2O exchangeable, 6.80 (s, 1H-C=C$\underline{H}$), 3.76 (s, 6H, 2x-OC$\underline{H}_3$), 6.61 (dd, 1H, *J* 1.93Hz, *J* 8.04Hz, Ar-$\underline{H}$), 6.73 (dd, 1H, *J* 1.98Hz, *J* 8.17Hz, Ar-$\underline{H}$), 7.40-8.0 (m, 10H, Ar-$\underline{H}$).

*2-(Coumarinyl-4-oxy)-4-(3,4-dimethoxy phenyl ethyl thioureido)-6-(4-flourophenyl ureido)-s-triazine (**8o**)*

^{1}H NMR: δ 2.82 (t, 2H, *J* 7.14Hz, -C$\underline{H}_2$), 3.20 (t, 2H, *J* 6.8Hz, -C$\underline{H}_2$), 8.90 (s, 1H, Ar-N$\underline{H}$), 9.42 (s, 1H, CS-N$\underline{H}$), {6.69 (s, 1H, Ar-N$\underline{H}$) 9.72 (br. s, 1H, Ar-N$\underline{H}$)} D_2O exchangeable, 6.80 (s, 1H-C=C$\underline{H}$), 3.76 (s, 6H, 2x-OC$\underline{H}_3$), 6.61 (dd, 1H, *J* 2.09Hz, *J* 8.23Hz, Ar-$\underline{H}$), 6.73 (dd, 1H, *J* 2.01Hz, *J* 8.11Hz, Ar-$\underline{H}$), 7.40-8.0 (m, 10H, Ar-$\underline{H}$).

*2-(Coumarinyl-4-oxy)-4-(3,4-dimethoxy phenyl ethyl thioureido)-6-(phenyl thioureido)-s-triazine (**9a**)*

IR ν_{max}/cm^{-1}: Thiourea C=S str. 1542 N-H str. 3350 N-H def. 1607; Coumarin C=O str. (δ lactone) 1731 C=C str. (α,β-unsat.) 1685; s-Triazine C-N str. 806; Ether C-O-C str. (asym.) 1234 C-O-C str. (sym.) 1026; Methylene C-H str. 2935 C-H def. 1454. ^{1}H-NMR: δ 2.72 (t, 2H, *J* 7.23Hz, -C$\underline{H}_2$), 3.34 (t, 2H, *J* 6.99Hz, -C$\underline{H}_2$), 8.89 (s, 1H, Ar-N$\underline{H}$), 9.48 (s, 1H, CS-N$\underline{H}$), {8.12 (s, 1H, Ar-N$\underline{H}$) 8.44 (br. s, 1H, Ar-N$\underline{H}$)} D_2O exchangeable, 6.83 (s, 1H-C=C$\underline{H}$), 3.69 (s, 6H, 2x-OC$\underline{H}_3$), 6.60 (d, 1H, *J* 8.12Hz, Ar-$\underline{H}$), 6.71 (d, 1H, *J* 8.20Hz, Ar-$\underline{H}$), 7.41-8.05 (m, 10H, Ar-$\underline{H}$).

*2-(Coumarinyl-4-oxy)-4-(3,4-dimethoxy phenyl ethyl thioureido)-6-(benzyl thioureido)-s-triazine (**9b**)*

^{1}H NMR: δ 2.71 (t, 2H, *J* 7.06Hz, -C$\underline{H}_2$), 3.34 (t, 2H, *J* 6.98Hz, -C$\underline{H}_2$), 8.90 (s, 1H, Ar-N$\underline{H}$), 9.51 (s, 1H, CS-N$\underline{H}$), {8.12 (s, 1H, Ar-N$\underline{H}$) 8.46 (br. s, 1H, Ar-N$\underline{H}$)} D_2O exchangeable, 6.73 (s, 1H-C=C$\underline{H}$), 3.70 (s, 6H, 2x-OC$\underline{H}_3$), 2.45 (s, 3H, -C$\underline{H}_3$), 6.76 (dd, 1H, *J* 2.10Hz, *J* 8.21Hz, Ar-$\underline{H}$), 6.80 (dd, 1H, *J* 2.02Hz, *J* 8.16Hz, Ar-$\underline{H}$), 7.20 (d, 2H, *J* 7.79Hz, Ar-$\underline{H}$), 7.40 (d, 2H, *J* 8.27Hz, Ar-$\underline{H}$), 7.60-8.10 (m, 5H, Ar-$\underline{H}$).

*2-(Coumarinyl-4-oxy)-4-(3,4-dimethoxy phenyl ethyl thioureido)-6-(1-naphthyl thioureido)-s-triazine (**9c**)*

^{1}H NMR: δ 2.72 (t, 2H, 6.92Hz, -C$\underline{H}_2$), 3.34 (t, 2H, 6.6Hz, -C$\underline{H}_2$), 8.89 (s, 1H, Ar-N$\underline{H}$), 9.48 (s, 1H, CS-N$\underline{H}$), {8.12 (s, 1H, Ar-N$\underline{H}$) 8.44 (br. s, 1H, Ar-N$\underline{H}$)} D_2O exchangeable, 6.83 (s, 1H-C=C$\underline{H}$), 3.69 (s, 6H, 2x-OC$\underline{H}_3$), 6.60 (d, 1H, *J* 7.89Hz, Ar-$\underline{H}$), 6.71 (d, 1H, *J* 7.98Hz, Ar-$\underline{H}$), 7.41-8.05 (m, 10H, Ar-$\underline{H}$).

*2-(Coumarinyl-4-oxy)-4-(3,4-dimethoxy phenyl ethyl thioureido)-6-(2-methoxy thioureido)-s-triazine (**9d**)*

^{1}H NMR: δ 2.75 (t, 2H, *J* 7.19Hz, -C$\underline{H}_2$), 3.35 (t, 2H, *J* 6.92Hz, -C$\underline{H}_2$), 8.88 (s, 1H, Ar-N$\underline{H}$), 9.45 (s, 1H, CS-N$\underline{H}$), {6.67 (s, 1H, Ar-N$\underline{H}$) 9.70 (br. s, 1H, Ar-N$\underline{H}$)} D_2O exchangeable, 6.85 (s, 1H-C=C$\underline{H}$), 3.85 (s, 6H, 2x-OC$\underline{H}_3$), 3.35 (s, 6H, -OC$\underline{H}_3$), 6.69 (dd, 1H, *J* 1.96Hz, *J* 8.21Hz, Ar-$\underline{H}$), 6.74 (dd, 1H, *J* 1.92Hz, *J* 8.11Hz, Ar-$\underline{H}$), 6.92 (d, 2H, *J* 8.29Hz, Ar-$\underline{H}$), 7.40 (d, 2H, *J* 8.12Hz, Ar-$\underline{H}$), 7.60-8.20 (m, 5H, Ar-$\underline{H}$).

*2-(Coumarinyl-4-oxy)-4-(3,4-dimethoxy phenyl ethyl thioureido)-6-(4-methoxy thioureido)-s-triazine (**9e**)*

^{1}H NMR: δ 2.75 (t, 2H, *J* 6.98Hz, -C$\underline{H}_2$), 3.35 (t, 2H, 6.73Hz, -C$\underline{H}_2$), 8.88 (s, 1H, Ar-N$\underline{H}$), 9.45 (s, 1H, CS-N$\underline{H}$),

{6.67 (s, 1H, Ar-NH) 9.70 (br. s, 1H, Ar-NH)} D_2O exchangeable, 6.85 (s, 1H-C=CH), 3.85 (s, 6H, 2x-OCH_3), 3.35 (s, 6H, -OCH_3), 6.69 (dd, 1H, *J* 1.95Hz, *J* 8.13Hz, Ar-H), 6.74 (dd, 1H, *J* 2.02Hz, *J* 8.26Hz, Ar-H), 6.92 (d, 2H, *J* 7.76Hz, Ar-H), 7.40 (d, 2H, *J* 7.89Hz, Ar-H), 7.60-8.20 (m, 5H, Ar-H).

*2-(Coumarinyl-4-oxy)-4-(3,4-dimethoxy phenyl ethyl thioureido)-6-(2-tolyl thioureido)-s-triazine (**9f**)*

^{1}H NMR: δ 2.71 (t, 2H, *J* 7.22Hz, -CH_2), 3.34 (t, 2H, *J* 6.84Hz, -CH_2), 8.90 (s, 1H, Ar-NH), 9.51 (s, 1H, CS-NH), {8.12 (s, 1H, Ar-NH) 8.46 (br. s, 1H, Ar-NH)} D_2O exchangeable, 6.73 (s, 1H-C=CH), 3.70 (s, 6H, 2x-OCH_3), 2.45 (s, 3H, -CH_3), 6.76 (dd, 1H, *J* 2.19Hz, *J* 8.32Hz, Ar-H), 6.80 (dd, 1H, *J* 2.17Hz, *J* 8.29Hz, Ar-H), 7.20 (d, 2H, *J* 7.77Hz, Ar-H), 7.40 (d, 2H, *J* 8.05Hz, Ar-H), 7.60-8.10 (m, 5H, Ar-H).

*2-(Coumarinyl-4-oxy)-4-(3,4-dimethoxy phenyl ethyl thioureido)-6-(3-tolyl thioureido)-s-triazine (**9g**)*

^{1}H NMR: δ 2.71 (t, 2H, *J* 7.11Hz, -CH_2), 3.34 (t, 2H, *J* 6.92Hz, -CH_2), 8.90 (s, 1H, Ar-NH), 9.51 (s, 1H, CS-NH), {8.12 (s, 1H, Ar-NH) 8.46 (br. s, 1H, Ar-NH)} D_2O exchangeable, 6.73 (s, 1H-C=CH), 3.70 (s, 6H, 2x-OCH_3), 2.45 (s, 3H, -CH_3), 6.76 (dd, 1H, *J* 2.06Hz, *J* 8.12Hz, Ar-H), 6.80 (dd, 1H, *J* 2.22Hz, *J* 8.35Hz, Ar-H), 7.20 (d, 2H, *J* 7.82Hz, Ar-H), 7.40 (d, 2H, *J* 8.14Hz, Ar-H), 7.60-8.10 (m, 5H, Ar-H).

*2-(Coumarinyl-4-oxy)-4-(3,4-dimethoxy phenyl ethyl thioureido)-6-(4-tolyl thioureido)-s-triazine (**9h**)*

IR ν_{max}/cm^{-1}: Thiourea C=S str. 1539 N-H str. 3350 N-H def. 1593; Coumarin C=O str. (δ lactone) 1730 C=C str. (α,β-unsat.) 1681; s-Triazine C-N str. 806; Ether C-O-C str. (asym.) 1234 C-O-C str. (sym.) 1026; Methylene C-H str. 2940 C-H def. 1440; Methyl C-H def. 1382. ^{1}H-NMR: δ 2.71 (t, 2H, *J* 7.28Hz, -CH_2), 3.34 (t, 2H, *J* 6.94Hz, -CH_2), 8.90 (s, 1H, Ar-NH), 9.51 (s, 1H, CS-NH), {8.12 (s, 1H, Ar-NH) 8.46 (br. s, 1H, Ar-NH)} D_2O exchangeable, 6.73 (s, 1H-C=CH), 3.70 (s, 6H, 2x-OCH_3), 2.45 (s, 3H, -CH_3), 6.76 (dd, 1H, *J* 2.23Hz, *J* 8.36Hz, Ar-H), 6.80 (dd, 1H, *J* 2.02Hz, *J* 8.18Hz, Ar-H), 7.20 (d, 2H, *J* 7.59Hz, Ar-H), 7.40 (d, 2H, *J* 7.83Hz, Ar-H), 7.60-8.10 (m, 5H, Ar-H).

*2-(Coumarinyl-4-oxy)-4-(3,4-dimethoxy phenyl ethyl thioureido)-6-(2-nitrophenyl thioureido)-s-triazine (**9i**)*

^{1}H NMR: δ 2.72 (t, 2H, *J* 7.09Hz, -CH_2), 3.34 (t, 2H, *J* 6.72Hz, -CH_2), 8.89 (s, 1H, Ar-NH), 9.48 (s, 1H, CS-NH), {8.12 (s, 1H, Ar-NH) 8.44 (br. s, 1H, Ar-NH)} D_2O exchangeable, 6.83 (s, 1H-C=CH), 3.69 (s, 6H, 2x-OCH_3), 6.60 (d, 1H, *J* 7.82Hz, Ar-H), 6.71 (d, 1H, *J* 7.99Hz, Ar-H), 7.41-8.05 (m, 10H, Ar-H).

*2-(Coumarinyl-4-oxy)-4-(3,4-dimethoxy phenyl ethyl thioureido)-6-(3-nitrophenyl thioureido)-s-triazine (**9j**)*

^{1}H NMR: δ 2.72 (t, 2H, *J* 7.23Hz, -CH_2), 3.34 (t, 2H, *J* 6.98Hz, -CH_2), 8.89 (s, 1H, Ar-NH), 9.48 (s, 1H, CS-NH), {8.12 (s, 1H, Ar-NH) 8.44 (br. s, 1H, Ar-NH)} D_2O exchangeable, 6.83 (s, 1H-C=CH), 3.69 (s, 6H, 2x-OCH_3), 6.60 (d, *J* 7.76Hz, 1H, Ar-H), 6.71 (d, 1H, *J* 8.12Hz, Ar-H), 7.41-8.05 (m, 10H, Ar-H).

*2-(Coumarinyl-4-oxy)-4-(3,4-dimethoxy phenyl ethyl thioureido)-6-(4-nitrophenyl thioureido)-s-triazine (**9k**)*

^{1}H NMR: δ 2.72 (t, 2H, *J* 7.09Hz, -CH_2), 3.34 (t, 2H, *J* 6.76Hz, -CH_2), 8.89 (s, 1H, Ar-NH), 9.48 (s, 1H, CS-NH), {8.12 (s, 1H, Ar-NH) 8.44 (br. s, 1H, Ar-NH)} D_2O exchangeable, 6.83 (s, 1H-C=CH), 3.69 (s, 6H, 2x-OCH_3), 6.60 (d, 1H, *J* 7.82Hz, Ar-H), 6.71 (d, 1H, *J* 8.06Hz, Ar-H), 7.41-8.05 (m, 10H, Ar-H).

*2-(Coumarinyl-4-oxy)-4-(3,4-dimethoxy phenyl ethyl thioureido)-6-(2-chlorophenyl thioureido)-s-triazine (**9l**)*

^{1}H NMR: δ 2.72 (t, 2H, *J* 7.17Hz, -CH_2), 3.34 (t, 2H, *J* 6.97Hz, -CH_2), 8.89 (s, 1H, Ar-NH), 9.48 (s, 1H, CS-NH), {8.12 (s, 1H, Ar-NH) 8.44 (br. s, 1H, Ar-NH)} D_2O exchangeable, 6.83 (s, 1H-C=CH), 3.69 (s, 6H, 2x-OCH_3), 6.60 (d, 1H, *J* 7.93Hz, Ar-H), 6.71 (d, 1H, *J* 8.18Hz, Ar-H), 7.41-8.05 (m, 10H, Ar-H).

*2-(Coumarinyl-4-oxy)-4-(3,4-dimethoxy phenyl ethyl thioureido)-6-(3-chlorophenyl thioureido)-s-triazine (**9m**)*

^{1}H NMR: δ 2.72 (t, 2H, *J* 7.13Hz, -CH_2), 3.34 (t, 2H, *J* 6.87Hz, -CH_2), 8.89 (s, 1H, Ar-NH), 9.48 (s, 1H, CS-NH), {8.12 (s, 1H, Ar-NH) 8.44 (br. s, 1H, Ar-NH)} D_2O exchangeable, 6.83 (s, 1H-C=CH), 3.69 (s, 6H, 2x-OCH_3), 6.60 (d, 1H, *J* 7.88Hz, Ar-H), 6.71 (d, 1H, *J* 8.13Hz, Ar-H), 7.41-8.05 (m, 10H, Ar-H).

*2-(Coumarinyl-4-oxy)-4-(3,4-dimethoxy phenyl ethyl thioureido)-6-(4-chlorophenyl thioureido)-s-triazine (**9n**)*

^{1}H NMR: δ 2.72 (t, 2H, *J* 7.28Hz, -CH_2), 3.34 (t, 2H, *J* 6.92Hz, -CH_2), 8.89 (s, 1H, Ar-NH), 9.48 (s, 1H, CS-NH), {8.12 (s, 1H, Ar-NH) 8.44 (br. s, 1H, Ar-NH)} D_2O exchangeable, 6.83 (s, 1H-C=CH), 3.69 (s, 6H, 2x-OCH_3), 6.60 (d, 1H, *J* 7.87Hz, Ar-H), 6.71 (d, 1H, *J* 8.12Hz, Ar-H), 7.41-8.05 (m, 10H, Ar-H).

*2-(Coumarinyl-4-oxy)-4-(3,4-dimethoxy phenyl ethyl thioureido)-6-(4-flourophenyl thioureido)-s-triazine (**9o**)*

^{1}H NMR: δ 2.72 (t, 2H, *J* 7.13Hz, -CH_2), 3.34 (t, 2H,

J 6.99Hz, -C<u>H</u>$_2$), 8.89 (s, 1H, Ar-N<u>H</u>), 9.48 (s, 1H, CS-N<u>H</u>), {8.12 (s, 1H, Ar-N<u>H</u>) 8.44 (br. s, 1H, Ar-N<u>H</u>)} D_2O exchangeable, 6.83 (s, 1H-C=C<u>H</u>), 3.69 (s, 6H, 2x-OC<u>H</u>$_3$), 6.60 (d, 1H, *J* 7.92Hz, Ar-<u>H</u>), 6.71 (d, 1H, *J* 8.23Hz, Ar-<u>H</u>), 7.41-8.05 (m, 10H, Ar-<u>H</u>).

In Vitro antibacterial assay

The antibacterial activity test of the newly synthesized compounds have been carried out using the disc diffusion method. This consists in introducing the germ (*E. coli*, *S. aureus*, *S. typhi*, *B. subtilis)* at a concentration of 10^6 CFC /mL on the surface of a Mueller-Hinton gelose plate. Then sterile filter paper discs (Diameter 6 mm) impregnated with the product were applied on the plates. The samples were kept at a temperature of 40 °C during 2 h to permit the diffusion of the product on the gelose. After 24 h of incubation at 37 °C, the diameter of the inhibition zones.

In Vitro antiviral assay

Evaluation of the antiviral activity of the compounds HIV-1 strain III_B and HIV-2 strain (ROD) in MT-4 cells were performed using the MTT assay as previously described.[26] Stock solution (10× final concentration) of test compound was added in 25 µL volumes to two series of triplicate wells to allow simultaneous evaluation of their effects on mock- and HIV-infected cells at the beginning of each experiment. Serial 5-fold dilution of test compounds were made directly in flat-bottomed 96-well microtiter trays using a Biomek 2000 robot (Beckman Instruments, Fullerton, CA). Untreated control HIV- and mock-infected cell samples were included for each sample.

HIV-1(III_B) or HIV-2 (ROD) stock (50 µL) at 100-300 $CCID_{50}$ (50% cell culture infectious dose) or culture medium was microtiter tray wells. Mock-infected cells were used to evaluate the effect of test compound on uninfected cells in order to assess the cytotoxicity of the test compound. Exponentially growing MT-4 cells were centrifuged for 5 min at 1000 rpm, and the supernatant was discarded. The MT-4 cells were resuspended at 6×10^5 cells mL^{-1}, and an amount of 50 µL volume was transferred to the microtiter tray wells. Five days after infection, the viability of mock- and HIV-infected cells were examined spectrophotometrically by the MTT assay.

The MTT assay is based on the reduction of yellow 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Across Organics, Geel, Belgium) by mitochondrial dehydrogenase of metabolically active cells to a blue-purple formazan that can be measured spectrophotometrically. The absorbances were read in an eight-channel computer-controlled photometer (Multiscan Ascent Reader, Labsystem, Helsinki, Finland) at two wavelength (540 and 690 nm). All data were calculated using the median OD (optical density) value of three wells. The 50% cytotoxic concentration (CC_{50}) was defined as the concentration of the test compound that reduced the absorbance (OD_{540}) of the mock-infected control sample by 50%. The concentration achieving 50% protection from the cytopathic effect of the virus in infected cell was defined as the 50% effective concentration (EC_{50}).

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Supplementary Information

Elemental analyses data of compounds **8a-o** and **9a-o** are listed in Table S1 at http://jbcs.sbq.org.br, as PDF file.

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