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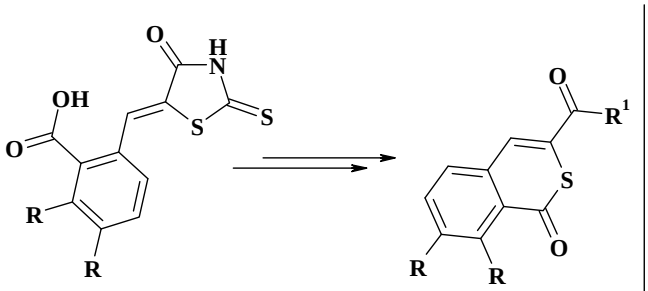

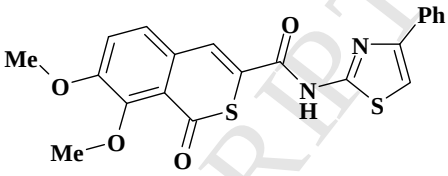

		GI_{50}, µM
RPMI-8226	(Leukemia)	**1.80**
NCI-H322M	(NSC Lung Cancer)	**1.28**
SNB-75	(CNS Cancer)	**1.54**
MALME-3M	(Melanoma)	**1.60**
TK-10	(Renal Cancer)	**1.43**

Isothiocoumarin-3-carboxylic acid derivatives: synthesis, anticancer and antitrypanosomal activity evaluation

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Abstract

A series of new isothiocoumarin-3-carboxylic acids derivatives had been obtained based on the 5-arylidenerhodanines hydrolysis. Anticancer activity screening allowed identification of 7,8-dimethoxy-1-oxo-1*H*-isothiochromene-3-carboxylic acid (4-phenylthiazol-2-yl)-amide (**30**) with the highest level of antimitotic activity (GI_{50} *NCI-H322M*/NSC Lung Cancer = 1.28 μM). Evaluation of the antitrypanosomal activity against *Trypanosoma brucei brucei* showed that investigated compounds did not exhibit significant antiparasitic effects. Additionally, the most pharmacologically attractive compounds were non-toxic and well tolerated by the experimental animals.

1. Introduction

(Iso)coumarins and dihydroquinolines as representatives of biological active compounds are promising scaffolds in the search of new drug-like small molecules [1-3] including those showing antitumor [4] and antitrypanosomal activity [5]. In the contrast information on isothiocoumarin (1-oxo-1*H*-2-benzothiopyrane or 1-oxo-1*H*-isothiochromene) derivatives which can be considered as bioisosters of the above mentioned compounds is rather scarce both concerning the synthetic protocols and biological activities [6,7]. Although 1-oxo-1*H*-2-benzothiopyrane is simple hetero-bicyclic system, the methods of its synthesis are limited, difficult in performance and often ineffective [8]. In the synthesis of mentioned heterocyclic derivatives methyl-, 2-aroylmethyl- [9], carboxymethyl- and 2-cyanomethylbenzoates [10]; 2-diphenyldithiochloroformates [11]; homophthalic acids [12]; S-(substituted-phenyl)-3-oxobutanethioates or benzylthiolate/benzenethiols [13] for example are used as starting reactants.

The efficient approach for isothiocoumarin derivatives obtaining including isothiocoumarin-3-carboxylic acids is based on the 5-ylidene-4-thiazolidinones hydrolysis with further intermediates (3-substituted-2-mercaptoacrylic acids) cyclization described in the early 50-ies [14-16] and improved in 2000 years [17-19]. Starting substances are easily accessible 5-arylidene-2-thioxothiazolidine-4-ones (5-arylidenerhodanines) with carboxylic group in *ortho* position of benzene ring. At the base hydrolysis of mentioned rhodanines two-step reaction occurs in which intermediates of the hydrolysis undergo heterocyclization with the formation of the 1-oxo-1*H*-2-benzothiopyran-3-carboxylic acids. This approach is efficient for not only isothiocoumarins synthesis, but also for the obtaining of 3-mercaptocoumarins [20,21], ketocinchonic acids, 2-indole-carboxylic acids etc. structure of which depends on the nature of substituent in the *ortho*- position of aryl(heter)ylidene fragment [22,23] (Scheme 1).

Scheme 1

The same protocol is described for the isocoumarin-3-carboxylic acids as well as dihydroisoquinoline-carboxylic acids synthesis as intermediates in the reaction of hippuric acid azlactones base hydrolysis yielding yohimbine alkaloids [3,24]. Moreover mentioned α-mercapto-β-substituted acrylic acids, as well as 5-ylidenethiazolidinones used therein deserve special attention as multifunctional reactants for various heterocyclic derivatives synthesis [25-29].

The aim of our work was the search of new possible anticancer and anti-trypanosomal agents among isothiocoumarin-3-carboxylic acids derivatives. The argument of the possibility of simultaneous anticancer and antitrypanosomal activities identification is the data about chemopreventive action of known antitrypanosomal drugs [30].

2. Results and discussion

2.1. Chemistry

Following our previous research in the thiazolidinone field [31-35] and proceeding from the above mentioned approach a series of isothiocoumarin-3-carboxylic acid derivatives had been obtained. The starting 5-arylidenerhodanines were prepared through condensation of 2-formylbenzoic acid or opianic (6-formyl-2,3-dimethoxybenzoic) acid with rhodanine (acetic acid medium in the presence of sodium acetate). Mentioned thiazolidinones derivatives are unstable in strongly alkaline medium via hydrolysis to the 3-substituted-2-mercapto-acrylic acids. In case of compounds with substituent in *ortho* position the two-step reaction occurs in which intermediates of the hydrolysis undergo heterocyclization with the formation of the 1-oxo-1*H*-2-benzothiopyran-3-carboxylic acids (Scheme 1). The heterocyclization reaction was performed in the 30% sodium hydroxide water solution and followed by acidification with hydrochloric acid the target **1** and **2** were obtained in high yield (Scheme 2). Aiming isothiocoumarin-3-carboxylic acid functionalization the latter were converted with thionyl chloride to give corresponding acid

chlorides [17]. For the target compounds (**3-39**) synthesis isothiocoumarin-3-carboxylic acids chlorides were utilized in the acylation reactions of aliphatic alcohols, amines, and amino acids.

Scheme 2

The increasing of the pharmacological activity caused by the introduction of the substituent in the (iso)coumarin core C-3 position defined the modification direction [36]. The data characterizing synthesized compounds are presented in the experimental part. Analytical and spectral data (^{1}H NMR, ^{13}C NMR) confirmed the structure of the synthesized compounds. In the ^{1}H NMR spectra the isothiocoumarin fragment is characterized by the singlet of =CH group in the 4th position at 7.10-8.30 ppm, and the subspectrum of two triplets and two doublets at 7.00-8.30 ppm (R = H) or by two doublets at ~7.30-7.70 and ~7.40-7.90 ppm (R = OMe).

2.2. Anticancer activity

Newly synthesized compounds were selected by the National Cancer Institute (NCI) Developmental Therapeutic Program (www.dtp.nci.nih.gov) for the *in vitro* cell line screening to investigate their anticancer activity. Anticancer assays were performed according to the US NCI protocol, which was described elsewhere [37-39]. The compounds were first evaluated at one dose primary assay towards three cell lines (panel consisting of three types of human cancers: breast (*MCF7*), lung (*NCI-H460*) and CNS (*SF-268*) – concentration 10^{-4}M) or approximately 60 cell lines (concentration 10^{-5}M) for compounds **14 (743078), 30 (748183), 9 (748491)** and **29 (748231)** (here and hereafter in brackets: NCS – NCI code). In the screening protocol, each cell line was inoculated and preincubated for 24–48 h on a microtiter plate. Test agents were then added at a single concentration and the culture was incubated for further 48 h. End point determinations were made with a protein binding dye, sulforhodamine B (SRB). Results for each

test agent were reported as the percent growth (GP) of the treated cells when compared to the untreated control cells. The screening results are shown in Table 1.

Table 1

For the compound **14** the average index of cell growth (GP_{mean}) was 86.7%, however it should be noted high selectivity to the *NCI-H572* (Non-Small Cell Lung Cancer) – GP = -11.00%, *UO-31* (renal cancer) – GP = -62.11%, and to the *CCRF-CEM* (Leukemia) – GP = -27.96%. The most prominent results were observed for **30**: GP_{mean} = 23.84% and the most sensitive cell lines were as follows: *A549/ATCC* (Non-Small Cell Lung Cancer) GP = -50.81%; *SF-539* (CNS Cancer) GP = -47.97%; *SNB-75* (CNS Cancer) GP = -43.79%; *OVCAR-3* (Ovarian Cancer) GP = -67.02%; *UO-31* (Renal Cancer) GP = -65.51%; *CAKI-1* (Renal Cancer) GP = -70.76%. Negative values of GP indicated the cytotoxicity of tested compounds. In contrast structurally related 1-oxo-1*H*-isothiochromene-3-carboxylic acid (4-phenyl-thiazol-2-yl)-amide (**29**) did not possess any anticancer effect (GP_{mean} = 98.97%), as well as [(1-oxo-1H-isothiochromene-3-carbonyl)-amino]-acetic acid (**9**) (GP_{mean} = 100.94%).

For the other derivatives, the prescreening assay realized on three cell lines identified the compounds **7, 19, 24, 31, 30** with the highest selectivity towards the NCI-H460 cell line. Data analysis showed an increase of biological activity when there is: *i*) substitution of chlorine atom in the amide fragment (**25**) with fluorine (**24**), *ii*) moving trifluoromethyl moiety in amide fragments from position 2 (**26**) in position 3 (**19**), and *iii*) the absence of methoxy-groups in isothiocoumarin fragment (comparison of **31** and **32**).

The most active compounds in the one dose assay (**7**, **19**, **24**, **30** and **31**) as well as **8**, **15**, **16**, **23**, **36**, **37**, were further selected for an advanced assay against a panel of approximately 60 tumor cell lines (cell lines were derived from nine different cancer types: leukemia, melanoma, lung, colon, CNS, ovarian, renal, prostate and breast cancers) at concentrations 10-fold diluted

(100μM, 10μM, 1μM, 0.1μM and 0.01μM). The percentage of growth was evaluated spectrophotometrically versus controls not treated with test agents after 48-h exposure and using the SRB protein assay to estimate cell viability or growth. Three antitumor activity dose-response parameters were calculated for each cell line: GI_{50} – molar concentration of the compound that inhibits 50% net cell growth; TGI - molar concentration of the compound leading to the total inhibition; and LC_{50} - molar concentration of the compound leading to 50% net cell death (presented in negative logarithm). Furthermore, a mean graph midpoints (MG_MID) were calculated for each of the parameters, giving an average activity parameter over all cell lines for the tested compound. For the MG_MID calculation, insensitive cell lines were included with the highest concentration tested (Table 2).

Table 2

Obtained data indicate in general low to moderate anticancer activity, the highest activity was observed for aromatic amides **19** and **24**. Comparison of anticancer profiles of isopropyl esters **7** and **8** showed a slight loss of activity in the absence of methoxy-groups. Secondary amides were characterized by somewhat less antitumor activity in comparison with esters. The introduction of morpholine fragment (**36**) leaded to the complete loss of activity (comparison of **36** and **37**). Likewise esters compound **37** revealed slight selectivity of action to melanoma and lung cancer cell lines. Worth mentioning was the antitumor effect of **31** from a series of heterocyclic amides of isothicoumarin-3-carboxylic acids. Against the backdrop of slight total activity **31** revealed the highest antimitotic effect among all tested compounds against leukemia line SR (pGI_{50}= 6.47) and selectivity towards CNS cancer cell lines. Moreover, 7,8-dimethoxy-1-oxo-1*H*-isothiochromene-3-carboxylic acid (4-phenylthiazol-2-yl)-amide (**30**) showed the highest anticancer activity among all tested compounds. These findings are also interesting in the

context of a comparison with the related heterocyclic amides activities (**28** and **29**) that do not possess anti-tumor activity.

Aiming to investigate the possible mechanisms of cell growth inhibition we carried out the COMPARE-analysis, namely comparison of selectivity patterns (mean graph fingerprints according DTP protocol) of tested compounds with standard anticancer agents, NCI active synthetic compounds and natural extracts (http://dtp.nci.nih.gov/docs/compare/compare.html). It is based on the comparison of the patterns of differential growth inhibition for cultured cell lines and can potentially gain insight into the mechanism of the cytotoxic action. Unfortunately no significant correlations were detected.

2.3. Antitrypanosomal activity

Series of isothiocoumarin-3-carboxylic acids derivatives (**1**, **5**, **9**, **17-19**, **21-23**, **25**, **26**, **29**, **30**, **32**, **35)** had been tested for their antitrypanosomal activity against *Trypanosoma brucei brucei* (*Tbb*) and the results are shown in Table 3.

Table 3

Bloodstream forms of *Tbb* strain 90-13 were cultured in HMI9 medium supplemented with 10% FCS at 37°C under an atmosphere of 5% CO_2 [40]. In all experiments, log-phase cell cultures were harvested by centrifugation at 3000 x g and immediately used. Drug assays were based on the conversion of a redox-sensitive dye (resazurin) to a fluorescent product by viable cells [41]. Compounds were tested at concentrations of 10 and 1 μg/mL. Investigated compounds did not exhibit significant antitrypanosomal effects except several substances, namely 1-oxo-1*H*-isothiochromene-3-carboxylic acid naphthalen-1-ylamide (**22**) and 7,8-dimethoxy-1-oxo-1*H*-isothiochromene-3-carboxylic acid (4-sulfamoyl-phenyl)-amide (**17**) that inhibited growth of *Tbb* bloodstream forms by more than 50% at 10μg/mL (table 3). In the spirit of such results little

information is available regarding the antiprotozoal effects of not only isothiocoumarins but coumarins and isocoumarins as well. 3-Aryl-4-hydroxycoumarins had been reported to show low to moderate antitrypanosomal activity against *Trypanosoma cruzi* [42]; 4-oxo-1,4-dihydroquinoline-3-carboxamides exhibited antitrypanosomal activity against *Tbb* and *Trypanosoma brucei rhodesience* [5].

2.4. Acute toxicity in vivo

The most active of the synthesized compounds were evaluated for their approximate LD_{50} in mice [43,44]. The stock solutions of the compounds used in this study were prepared immediately before usage and increasing amounts of substances (100-1000 mg/kg) were injected intraperitoneally. The LD_{50} were calculated according to Litchfield and Wilcoxon [44]. The results indicated that tested compounds were non-toxic and well tolerated by the experimental animals as demonstrated by their LD_{50} (870±112.0 mg/kg (**17**), 750±31.0 mg/kg (**19**), 410±48.5 mg/kg (**22**), 515±61.4 mg/kg (**30**)).

3. Conclusions

In the presented paper the series of isothiocoumarin-3-carboxylic acids derivatives had been obtained based on the 5-arylidenerhodanines hydrolysis. Anticancer activity screening allowed establishing the influence of carboxylic group modification and led to the 7,8-dimethoxy-1-oxo-1*H*-isothiochromene-3-carboxylic acid (4-phenylthiazol-2-yl)-amide identification with the highest level of antimitotic activity. Antitrypanosomal activity evaluation showed that investigated compounds did not exhibit significant antiparasitic effects. Worth mentioning is that the pharmacologically attractive tested compounds were non-toxic and well tolerated by mice.

4. Experimental

4.1. Materials and methods

Melting points were measured in open capillary tubes on a BŰCHI B-545 melting point apparatus and are uncorrected. The elemental analyses (C, H, N) were performed using the Perkin-Elmer 2400 CHN analyzer. Analysis results indicated by the symbols of the elements or functions were within ±0.4% of the theoretical values. The ^{1}H-NMR spectra were recorded on Varian Gemini 400 MHz spectrometer and ^{13}C NMR spectra on Varian Mercury-400 100MHz spectrometer in DMSO-d_6 using tetramethylsilane (TMS) as an internal standard. Chemical shifts are reported in ppm units with the use of δ scale. Mass spectra were obtained using electrospray (ES) ionization techniques on an Agilent 1100 series LCMS instrument.

4.2. Chemistry

*General procedure for synthesis of 1-oxo-1H-isothiochromene-3-carboxylic acids (**1**, **2**)*
Appropriate 5-arylidenerhodanine (20 mmol) was added to 30% NaOH aqueous solution (13 mL) and the mixture was boiled for 0.5 h. After cooling the reaction mixture was poured into conc. HCl (40 mL) and ice (100 g). The precipitate was filtered off and purified by recrystallization (BuOH).

*1-Oxo-1H-isothiochromene-3-carboxylic acid (**1**).* Yield 78%, mp 258-260^{o}C, lit 260-262^{o}C [6]. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.77 (t, 1H, *J* = 8.4 Hz, 6-H), 7.90 (t, 1H, *J* = 8.0 Hz, 7-H), 7.99 (d, 1H, *J* = 8.0 Hz, 5-H); 8.20 (d, 1H, *J* = 8.8 Hz, 8-H), 8.21 (s, 1H, 4-H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 186.1, 163.7, 136.4, 134.6, 132.7, 131.4, 130.2, 129.0, 128.3, 125.7. EI-MS (m/z): 206 (M^{+}). Calcd. for $C_{10}H_6O_3S$: C, 58.24; H, 2.93; Found: C, 58.40; H, 3.05%

*7,8-Dimethoxy-1-oxo-1H-isothiochromene-3-carboxylic acid (**2**).* Yield 80%, mp 252-254^{o}C, lit 257-258^{o}C [14]. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.82 (s, 3H, OCH_3), 3.97 (s, 3H, OCH_3), 7.38 (d, 1H, *J* = 8.7 Hz, 6-H), 7.48 (d, 1H, *J* = 8.7 Hz, 5-H), 7.89 (s, 1H, 4-H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 183.7, 163.7, 155.3, 147.7, 130.6, 129.6, 128.5, 126.8, 123.3, 118.7, 60.9, 56.5. EI-MS (m/z): 266 (M^{+}). Calcd. for $C_{12}H_{10}O_5S$: C, 54.13; H, 3.79; Found: C, 54.30; H, 3.85%.

*General procedure for synthesis of 1-oxo-1H-isothiochromene-3-carboxylic acid esters (**3-8**)*

The mixture of appropriate acid (**1** or **2**) (13 mmol) and thionyl chloride (30 mL) was boiled for 0.5 h. The mixture was cooled, and hexane (60 mL) was added, the precipitated acid chloride was filtered off and washed with hexane. The mixture of obtained acid chloride (8 mmol) and appropriate alcohol (20 mL) was boiled for 1 h. The obtained precipitate was filtered off and purified by recrystallization [17].

*1-Oxo-1H-isothiochromene-3-carboxylic acid methyl ester (**3**).* Yield 81%, mp 133-135°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.93 (s, 3H, CH_3), 7.80 (t, 1H, *J* = 8.2 Hz, 6-H), 7.92 (t, 1H, *J* = 8.2 Hz, 7-H), 8.03 (d, 1H, *J* = 7.6 Hz, 5-H), 8.21 (d, 1H, *J* = 8.0 Hz, 8-H), 8.29 (s, 1H, 4-H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 185.7, 162.7, 136.1, 135.3, 133.4, 132.3, 129.3, 128.8, 128.0, 125.6, 53.8. Calcd. for $C_{11}H_8O_3S$: C, 59.99; H, 3.66; Found: C, 60.10; H, 3.80%.

*7,8-Dimethoxy-1-Oxo-1H-isothiochromene-3-carboxylic acid methyl ester (**4**).* Yield 86%, mp 150-153°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.77 (s, 3H, $COOCH_3$), 3.89 (s, 3H, OCH_3), 3.96 (s, 3H, OCH_3), 7.62 (d, 1H, *J* = 8.7 Hz, 6-H), 7.76 (d, 1H, *J* = 8.8 Hz, 5-H), 8.10 (s, 1H, 4-H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 184.0, 162.9, 155.7, 148.0, 130.6, 129.8, 128.3, 126.7, 122.9, 118.5, 60.8, 56.6, 53.1. Calcd. for $C_{13}H_{12}O_5S$: C, 55.71; H, 4.32; Found: C, 55.83; H, 4.49%.

*1-Oxo-1H-isothiochromene-3-carboxylic acid ethyl ester (**5**).* Yield 78%, mp 120-122°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.40 (t, 3H, *J* = 7.2 Hz, OCH_2CH_3), 4.39 (q, 2H, *J* = 7.2 Hz, OCH_2CH_3), 7.80 (t, 1H, *J* = 8.4 Hz, 6-H), 7.92 (t, 1H, *J* = 7.6 Hz, 7-H), 8.03 (d, 1H, *J* = 8.0 Hz, 5-H), 8.21 (d, 1H, *J* = 7.6 Hz, 8-H), 8.26 (s, 1H, 4-H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 185.6, 161.9, 136.1, 134.8, 133.0, 131.7, 129.0, 128.9, 128.7, 125.7, 62.4, 14.4. Calcd. for $C_{12}H_{10}O_3S$: C, 61.52; H, 4.30; Found: C, 61.65; H, 4.50%.

*7,8-Dimethoxy-1-oxo-1H-isothiochromene-3-carboxylic acid ethyl ester (**6**).* Yield 86%, mp 144-146°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.33 (t, 3H *J* = 7.1 Hz, OCH_2CH_3), 3.76 (s, 3H, OCH_3), 3.96 (s, 3H, OCH_3), 4.34 (q, 2H, *J* = 6.8 Hz, OCH_2CH_3), 7.68 (d, 1H, *J* = 8.5 Hz, 6-H), 7.84 (d, 1H, *J* = 8.6 Hz, 5-H), 8.13 (s, 1H, 4-H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 183.6, 162.6, 156.1, 147.9, 130.7, 130.6, 129.7, 125.3, 123.5, 120.0, 62.8, 61.4, 57.2, 14.7. Calcd. for $C_{14}H_{14}O_5S$: C, 57.13; H, 4.79; Found: C, 57.30; H, 43.85%.

*1-Oxo-1H-isothiochromene-3-carboxylic acid isopropyl ester (**7**).* Yield 77%, mp 90-92°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.32 (d, 6H, *J* = 6.2 Hz, $CH(CH_3)_2$), 5.20 (m, 1H, CH), 7.79 (t,

1H, *J* = 8.2 Hz, 6-H), 7.91 (t, 1H, *J* = 7.6 Hz, 7-H), 8.05 (d, 1H, *J* = 8,0 Hz, 5-H), 8.24 (d, 1H, *J* = 7.6 Hz, 8-H), 8.27 (s, 1H, 4-H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 185.3, 162.9, 136.1, 134.8, 133.1, 132.4, 129.2, 128.6, 128.0, 125.2, 70.6, 22.1. Calcd. for $C_{13}H_{12}O_3S$: C, 62.88; H, 4.87; Found: C, 62.95; H, 4.55%.

*7,8-Dimethoxy-1-oxo-1H-isothiochromene-3-carboxylic acid isopropyl ester (**8**).* Yield 80%, mp 126-128^{o}C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.32 (d, 6H, *J* = 6.4 Hz, CH$(CH_3)_2$), 3.75 (s, 3H, OCH_3), 3.95 (s, 3H, OCH_3), 5.13 (m, 1H, CH), 7.69 (d, 1H, *J* = 8.7 Hz, 6-H), 7.86 (d, 1H, *J* = 8.7 Hz, 5-H), 8.12 (s, 1H, 4-H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 183.7, 162.1, 156.0, 147.9, 130.7, 130.6, 129.6, 125.7, 123.5, 120.0, 70.7, 61.4, 57.2, 22.2. Calcd. for$C_{15}H_{16}O_5S$: C, 58.43; H, 5.23; Found: C, 58.30; H, 5.05%.

*General procedure for synthesis of 3-(1-oxo-1H-isothiochromene-3-carbonyl-amino)-carboxylic acids (**9-11**)*

The mixture of the acid chloride (5 mmol) and appropriate amino acid (10 mmol) in acetic acid medium (8 mL) was boiled for 1 h. After cooling the resulting precipitate was filtered, washed with water and recrystallized [25].

*[(1-Oxo-1H-isothiochromene-3-carbonyl)-amino]-acetic acid (**9**).* Yield 77%, mp 122-124^{o}C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.95 (d, 2H, *J* = 5.6 Hz, CH_2), 7.08 (t, 1H, *J* = 7.6 Hz, 6-H), 7.89 (d, 1H, *J* = 7.6 Hz, 5-H), 7.97 (t, 1H, *J* = 7.6 Hz, 7-H), 8.17 (s, 1H, 4-H), 8.20 (d, 1H, *J* = 8.0 Hz, 8-H), 9.24 (br.s, 1H, NH), 12.75 (br.s, 1H, COOH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 187.0, 171.4, 163.3, 136.9, 135.6, 133.8, 132.8, 131.8, 128.8, 125.9, 124.6, 42.0. Calcd. for $C_{12}H_9NO_4S$: C, 54.75; H, 3.45; N,5.32; Found: C, 54.90; H, 3.65; N, 5.48%.

*[(7,8-Dimethoxy-1-oxo-1H-isothiochromene-3-carbonyl)-amino]-acetic acid (**10**).* Yield 69%, mp 221-223^{o}C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.77 (s, 3H, OCH_3), 3.96 (s, 3H, OCH_3), 3.89 (d, 2H, *J* = 5.6 Hz, CH_2), 7.57 (d, 1H, *J* = 8.2 Hz, H_6), 7.61 (d, 1H, *J* = 8.2 Hz, H_5), 7.96 (s, 1H, H_4), 8.90 (t, 1H, *J* = 5.6 Hz, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 184.1, 172.3, 164.1, 156.1, 147.9, 131.5, 129.9, 128.5, 127.1, 123.8,119.0, 61.0, 56.8, 42.2. Calcd. for $C_{14}H_{13}NO_6S$: C, 52.01; H, 4.05; N, 4.33; Found: C, 51.90; H, 3.95; N, 4.48%.

*3-[(7,8-Dimethoxy-1-oxo-1H-isothiochromene-3-carbonyl)-amino]-propionic acid (**11**).* Yield 56%, mp 214-216^{o}C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.77 (s, 3H, OCH_3), 3.95 (s, 3H,

OCH_3), 3.45 (m, 4H, CH_2CH_2), 7.53 (d, 1H, *J* = 8.8 Hz, 6-H), 7.59 (d, 1H, *J* = 8.8 Hz, 5-H), 7.89 (s, 1H, 4-H), 8.62 (t, 1H, *J* = 4.2 Hz, NH), 12.26 (br.s, 1H, COOH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 183.5, 171.9, 163.3, 155.8, 148.0, 130.7, 129.8, 128.6, 126.9, 123.6, 118.9, 60.6, 60.0, 56.6, 42.5. Calcd. for $C_{15}H_{15}NO_6S$: C, 53.41; H, 4.48; N, 4.15; Found: C, 53.52; H, 4.60; N, 4.03%.

*General procedure for synthesis of 1-oxo-1H-isothiochromene-3-carboxylic acid amides (**12-39**)*
To the solution of appropriate amine (20 mmol) in a/h dioxane (30 mL) the solution of appropriate isothiocoumarin-3-carboxylic acid chloride (10 mmol) in a/h dioxane (20 mL) was added. The mixture was refluxed for 1 h. Resulting precipitate was filtered, washed with water and recrystallized from appropriate solvent. When the precipitate wasn't formed the reaction mixture was poured into the water [25].

*1-Oxo-1H-isothiochromene-3-carboxylic acid phenylamide (**12**).* Yield 81%, mp 193-195^{o}C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.13 (t, 1H, *J* = 7.6 Hz, 6-H), 7.35 (t, 2H, *J* = 7.6 Hz, arom.), 7.72 (d, 2H, *J* = 8.0 Hz, arom.), 7.77 (t, 1H, *J*= 8.0 Hz, 7-H), 7.88-7.96 (m, 2H, arom.), 8.23 (d, 1H, *J* = 8.4 Hz, 8-H), 8.28 (s, 1H, 4-H), 10.51 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 186.4, 161.3, 138.6, 136.7, 134.8, 134.7, 132.3, 130.9, 128.8, 125.6, 124.4, 120.8. Calcd. for $C_{16}H_{11}NO_2S$: C, 68.31; H, 3.94; N, 4.98; Found: C, 68.50; H, 4.12; N, 5.05%.

*7,8-Dimethoxy-1-oxo-1H-isothiochromene-3-carboxylic acid phenylamide (**13**).* Yield 92%, mp 208-210^{o}C. ^{1}HNMR (400 MHz, DMSO-d_6): δ 3.77 (s, 3H, OCH_3), 3.96 (s, 3H, OCH_3), 7.11-7.15 (m, 2H, arom.), 7.38-7.41 (m, 2H, arom.), 7.70-7.73 (m, 3H, arom.), 8.19 (s, 1H, 4-H), 10.50 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 184.3, 161.6, 155.1, 147.6, 138.6, 130.9, 130.8, 129.4, 129.1, 125.0, 124.6, 122.9, 120.9, 119.7, 61.1, 56.8. Calcd. for $C_{18}H_{15}NO_4S$: C, 63.33; H, 4.43; N, 4.10; Found: C, 63.50; H, 4.55; N, 4.30%.

*1-Oxo-1H-isothiochromene-3-carboxylic acid (4-hydroxyphenyl)-amide (**14**).* Yield 70%, mp >240^{o}C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.74 (d, 2H, *J* = 9.0 Hz, arom.), 7.45 (d, 2H, *J* = 8.9 Hz, arom.), 7.65 (m, 1H, arom.), 7.78-7.85 (br.s, 2H, arom.), 8.13 (s, 1H, 4-H), 8.21 (d, 1H, *J* = 8.0 Hz, 8-H), 8.80 (br.s, 1H, OH), 9.96 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 187.1, 161.4, 155.2, 137.0, 135.6, 135.0, 132.8, 131.7, 130.2, 128.8, 125.9, 124.5, 123.2, 115.9. Calcd. for $C_{16}H_{11}NO_3S$: C, 64.63; H, 3.73; N, 4.71; Found: C, 64.50; H, 3.85; N, 4.95%.

*7,8-Dimethoxy-1-oxo-1H-isothiochromene-3-carboxylic acid (4-hydroxyphenyl)-amide (**15**).* Yield 80%, mp 215-216°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.76 (s, 3H, OCH_3), 3.95 (s, 3H, OCH_3), 6.76 (d, 2H, *J* = 8.7 Hz, arom.), 7.47 (d, 2H, *J* = 8.5 Hz, arom.), 7.67 (d, 1H, *J* = 8.6 Hz, 6-H), 7.72 (d, 1H, *J* = 8.6 Hz, 5-H), 8.12 (s, 1H, 4-H), 10.28 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 184.7, 161.4, 155.3, 154.8, 147.9, 131.6, 131.2, 130.4, 129.6, 124.7, 123.2, 123.0, 120.1, 115.8, 61.4, 57.1. Calcd. for $C_{18}H_{15}NO_5S$: C, 60.49; H, 4.23; N, 3.92; Found: C, 60.30; H, 4.35; N, 3.75%.

*1-Oxo-1H-isothiochromene-3-carboxylic acid (4-sulfamoylphenyl)-amide (**16**).* Yield 94%, mp >240°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.20 (s, 2H, NH_2), 7.76-7.81 (m, 1H, arom.), 7.82 (d, 2H, *J* = 9.2 Hz, arom.), 7.90 (d, 2H, *J* = 9.2 Hz, arom.), 7.90-7.95 (m, 2H, arom.), 8.23 (d, 1H, *J* = 8.0 Hz, 8-H), 8.31 (s, 1H, 4-H), 10.82 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 186.6, 162.0, 141.5, 139.8, 136.4, 135.4, 133.8, 132.7, 131.8, 128.6, 127.0, 125.7, 125.5, 120.6. Calcd. for $C_{16}H_{12}N_2O_4S_2$: C, 53.32; H, 3.36; N, 7.77; Found: C, 53.55; H, 3.25; N, 7.90%

*7,8-Dimethoxy-1-oxo-1H-isothiochromene-3-carboxylic acid (4-sulfamoylphenyl)-amide (**17**).* Yield 90%, mp >240°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.79 (s, 3H, OCH_3), 3.97 (s, 3H, OCH_3), 7.17 (s, 2H, NH_2), 7.64 (br.s, 2H, 5-H, 6-H), 7.80 (d, 2H, *J* = 8.8 Hz, arom.), 7.87 (d, 2H, *J* = 8.8 Hz, arom.), 8.15 (s, 1H, 4-H), 10.63 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 184.5, 162.3, 155.7, 147.9, 141.9, 139.9, 130.9, 130.7, 129.9, 127.3, 126.0, 123.3, 120.8, 120.1, 61.5, 57.1. Calcd. for $C_{18}H_{16}N_2O_6S_2$: C, 51.42; H, 3.84; N, 6.66; Found: C, 51.55; H, 3.95; N, 6.55%.

*1-Oxo-1H-isothiochromene-3-carboxylic acid (3-trifluoromethylphenyl)-amide (**18**).* Yield 60%, mp 195-197°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.36 (d, 1H, *J* = 8.6 Hz, arom.), 7.50 (t, 1H, *J* = 8.2 Hz, 6-H), 7.70 (m, 1H, 7-H), 7.84 (m, 2H, arom.), 8.11 (m, 2H, arom.), 8.20 (s, 1H, 4-H), 8.24 (d, 1H, *J* = 8.0 Hz, 8-H), 10.52 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 186.5, 162.0, 139.3, 136.4, 135.4, 133.7, 132.7, 131.8, 130.5, 129.8 (q, *J* = 272.0 Hz), 128.6, 127.3 (q, *J* = 32.0 Hz), 125.7, 125.4, 124.4, 121.1 (q, *J* = 3.4 Hz), 117.0 (q, *J* = 3.0 Hz). Calcd. for $C_{17}H_{10}F_3NO_2S$: C, 58.45; H, 2.89; N, 4.01; Found: C, 58.60; H, 3.05; N, 4.20%.

*7,8-Dimethoxy-1-oxo-1H-isothiochromene-3-carboxylic acid (3-trifluoromethylphenyl)-amide (**19**).* Yield 70%, mp 210-212°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.76 (s, 3H, OCH_3), 3.96 (s, 3H, OCH_3), 7.49 (d, 1H, *J* = 7.2 Hz, arom.), 7.63 (t, 1H, J = 7.9 Hz, arom.), 7.69 (d, 1H, *J* = 8.8

Hz, 6-H), 7.73 (d, 1H, *J* = 8.8 Hz, 5-H), 8.00 (d, 2H, *J* = 7.4 Hz, arom.), 8.14 (s, 1H, arom.), 8.21 (s, 1H, 4-H), 10.77 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 184.4, 162.3, 155.7, 148.0, 139.8, 131.0, 130.8, 130.7, 130.1 (q, *J* = 32.3 Hz), 129.9, 125.9, 125.1 (q, *J* = 273.0 Hz), 124.6, 123.3, 121.2 (q, *J* = 4.7 Hz), 120.1, 117.2 (q, *J* = 3.7 Hz), 61.44, 57.13. Calcd. for $C_{19}H_{14}F_3NO_4S$: C, 55.74; H, 3.45; N, 3.42; Found: C, 55.90; H, 3.65; N, 3.12%.

*4-[(1-Oxo-1H-isothiochromene-3-carbonyl)-amino]-benzoic acid ethyl ester (**20**).* Yield 70%, mp 194-196^{o}C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.33 (t, 3H, *J* = 7.0 Hz, OCH_2CH_3), 4.29 (q, 2H, *J* = 7.0 Hz, OCH_2CH_3), 7.80-7.90 (m, 1H, 6-H), 7.94-8.03 (m, 2H, arom.), 8.23 (d, 1H, *J* = 7.8 Hz, 8-H), 8.34 (s, 1H, 4-H), 7.89 (d, 2H, *J* = 9.0 Hz, arom.), 8.00 (d, 2H, *J* = 9.0 Hz, arom.), 10.94 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 186.9, 165.9, 162.3, 143.2, 136.7, 136.0, 134.1, 133.0, 132.1, 130.8, 128.9, 125.9, 125.8, 120.5, 61.3, 14.9. Calcd. for $C_{19}H_{15}NO_4S$: C, 54.58; H, 4.28; N, 3.96; Found: C, 54.70; H, 4.12; N, 4.15%.

*4-[(7,8-Dimethoxy-1-oxo-1H-isothiochromene-3-carbonyl)-amino]-benzoic acid ethyl ester (**21**).* Yield 85%, mp 219-221^{o}C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.33 (t, 3H, *J* = 6.8 Hz, OCH_2CH_3), 3.76 (s, 3H, OCH_3), 3.96 (s, 3H, OCH_3), 4.30 (q, 2H, *J* = 6.8 Hz, OCH_2CH_3), 7.69-7.74 (m, 2H, 5-H, 6-H), 7.86 (d, 2H, *J* = 8.5 Hz, arom.), 7.98 (d, 2H, *J* = 8.6 Hz, arom.), 8.22 (s, 1H, 4-H), 10.74 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 184.4, 165.9, 162.3, 155.7, 147.9, 143.4, 131.0, 130.9, 130.8, 129.9, 126.0, 125.7, 123.3, 120.4, 120.1, 61.4, 61.2, 57.1, 14.9. Calcd. for $C_{21}H_{19}NO_6S$: C, 61.01; H, 4.63; N, 3.39; Found: C, 60.85; H, 4.55; N, 3.24%.

*1-Oxo-1H-isothiochromene-3-carboxylic acid naphthalen-1-ylamide (**22**).* Yield 87%, mp 199-201^{o}C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.54-7.64 (m, 4H, arom.), 7.81-7.86 (m, 1H, arom.), 7.90 (d, 1H, *J* = 8.2 Hz, arom.), 7.98-8.07 (m, 4H, arom.), 8.24 (d, 1H, *J* = 8.3 Hz, 8-H), 8.53 (s, 1H, 4-H), 10.85 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 186.5, 162.2, 136.8, 134.7, 134.4, 134.1, 133.1, 132.4, 131.0, 129.3, 128.8, 128.4, 126.9, 126.3, 126.2, 125.7, 124.8, 124.0, 123.5. Calcd. for $C_{20}H_{13}NO_2S$: C, 72.49; H, 4.23; N, 3.95; Found: C, 72.60; H, 4.38; N, 3.75%.

*7,8-Dimethoxy-1-oxo-1H-isothiochromene-3-carboxylic acid naphthalen-1-ylamide (**23**).* Yield 81%, mp >240^{o}C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.81 (s, 3H, OCH_3), 3.98 (s, 3H, OCH_3), 7.50-7.62 (m, 4H, arom.), 7.72 (br.s, 2H, arom.), 7.88 (d, 2H, *J* = 7.9 Hz, arom.), 7.98-8.05 (m, 2H, arom.), 8.33 (s, 1H, 4-H), 10.53 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 184.6, 162.9, 155.5, 147.9, 134.4, 133.5, 131.2, 130.9, 129.8, 129.6, 128.8, 127.4, 127.0, 126.9, 126.3,

125.6, 124.5, 123.9, 123.4, 120.1, 61.5, 57.1. Calcd. for $C_{22}H_{17}NO_4S$: C, 67.50; H, 4.38; N, 3.58; Found: C, 67.72; H, 4.25; N, 3.40%.

*7,8-Dimethoxy-1-oxo-1H-isothiochromene-3-carboxylic acid (4-fluorophenyl)-amide (**24**).* Yield 77%, mp >240°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.79 (s, 3H, OCH_3), 3.97 (s, 3H, OCH_3), 7.11 (t, *J* = 8.8 Hz, 2H, arom.), 7.63 (br.s, 2H, 5-H, 6-H), 7.70-7.74 (m, 2H, arom.), 8.10 (s, 1H, 4-H), 10.40 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): 184.0, 161.2, 159.2 (d, *J* = 221.0 Hz), 155.0, 135.0, 131.3, 131.0, 129.0, 124.6, 122.5 (d, *J* = 7.5 Hz), 119.1, 115.3 (d, *J* = 22.1 Hz), 60.9, 56.6. Calcd. for $C_{18}H_{14}FNO_4S$: C, 60.16; H, 3.93; N, 3.90; Found: C, 60.30; H, 4.10; N, 4.05%.

*7,8-Dimethoxy-1-oxo-1H-isothiochromene-3-carboxylic acid (4-chlorophenyl)-amide (**25**).* Yield 62%, mp 231-233°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.79 (s, 3H, OCH_3), 3.97 (s, 3H, OCH_3), 7.34 (d, 2H, *J* = 9.2 Hz, arom.), 7.63 (s, 2H, 5-H, 6-H), 7.74 (d, 2H, *J* = 9.2 Hz, arom.), 8.10 (s, 1H, 4-H), 10.45 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 185.2, 161.8, 155.3, 154.9, 147.8, 131.8, 131.4, 130.5, 129.7, 125.0, 123.2, 123.0, 119.7, 115.8, 61.0, 57.2. Calcd. for $C_{18}H_{14}ClNO_4S$: C, 57.53; H, 3.75; N, 3.73; Found: C, 57.60; H, 3.90; N, 3.95%.

*7,8-Dimethoxy-1-oxo-1H-isothiochromene-3-carboxylic acid (2-trifluoromethylphenyl)-amide (**26**).* Yield 65%, mp 199-201°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.77 (s, 3H, OCH_3), 3.96 (s, 3H, OCH_3), 7.50-7.62 (m, 2H, arom.), 7.64-7.85 (m, 4H, arom.), 8.15 (s, 1H, 4-H), 10.40 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 184.0, 162.7, 155.3, 147.6, 135.1, 133.7, 131.6, 130.64, 129.9, 129.5, 128.4, 127.0 (q, *J* = 30 Hz), 127.0 (q, *J* = 3.0 Hz), 126.7, 125.4, 123.0, 119.8, 61.1, 56.8. Calcd. for $C_{19}H_{14}F_3NO_4S$: C, 55.74; H, 3.45; N, 3.42; Found: C, 55.85; H, 3.60; N, 3.20%.

*1-Oxo-1H-isothiochromene-3-carboxylic acid thiazol-2-ylamide (**27**).* Yield 70%, mp >240°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.25 (br.s, 1H, thiaz.), 7.56 (d, 1H, *J* = 3.6 Hz, thiaz.), 7.80 (m, 1H, arom.), 7.96 (m, 2H, 5-H, 6-H), 8.20 (d, 1H, *J* = 7.8 Hz, 8-H), 8.46 (s, 1H, 4-H), 13.27 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 187.3, 137.0, 135.5, 133.1, 131.9, 129.0, 126.4, 125.9, 113.8. Calcd. for $C_{13}H_8N_2O_2S_2$: C, 54.15; H, 2.80; N, 9.72; Found: C, 54.30; H, 3.00; N, 9.90%.

*7,8-Dimethoxy-1-oxo-1H-isothiochromene-3-carboxylic acid thiazol-2-ylamide (**28**).* Yield 80%, mp >240°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.79 (s, 3H, OCH_3), 3.96 (s, 3H, OCH_3), 7.09 (d,

1H, *J*= 3.4 Hz, thiaz.), 7.45 (d, 1H, *J* = 3.5 Hz, thiaz.), 7.58 (s, 2H, 5-H, 6-H), 8.32 (s, 1H, 4-H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 184.2, 155.2, 147.7, 131.0, 129.5, 126.4, 123.3, 119.0, 113.3, 60.9, 56.6. Calcd. for $C_{15}H_{12}N_2O_4S_2$: C, 51.71; H, 3.47; N, 8.04; Found: C, 51.80; H, 3.55; N, 7.95%.

*1-Oxo-1H-isothiochromene-3-carboxylic acid (4-phenylthiazol-2-yl)-amide (**29**).* Yield 75%, mp >240^{o}C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.37 (m, 1H, arom.), 7.47 (t, 2H, *J* = 7.0 Hz, arom.), 7.73 (br.s, 1H, arom.), 7.83-7.86 (m, 1H, arom.), 7.93-8.05 (m, 4H, arom.), 8.23 (d, 1H, *J* = 7.7 Hz, 8-H), 8.65 (s, 1H, 4-H), 13.23 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 186.8, 136.7, 135.8, 133.3, 132.4, 129.5, 129.0, 128.7, 127.3, 126.5, 126.00, 109.7. Calcd. for $C_{19}H_{12}N_2O_2S_2$: C, 62.62; H, 3.32; N, 7.69; Found: C, 62.50; H, 3.10; N, 7.50%.

*7,8-Dimethoxy-1-oxo-1H-isothiochromene-3-carboxylic acid (4-phenylthiazol-2-yl)-amide (**30**).* Yield 75%, mp >240^{o}C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.78 (s, 3H, OCH_3), 3.96 (s, 3H, OCH_3), 7.35 (t, 1H, *J* = 7.0 Hz, arom.), 7.45 (t, 2H, *J* = 7.3 Hz, arom.), 7.64 (s, 1H, arom.), 7.68 (t, 2H, *J* = 8.7 Hz, arom.), 7.93 (d, 2H, *J* = 7.4 Hz, arom.), 8.47 (s, 1H, 4-H), 12.86 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 184.3, 161.9, 158.7, 155.9, 149.9, 148.0, 134.8, 130.9, 130.2, 129.4, 128.6, 127.6, 126.5, 123.3, 120.0, 109.5, 61.4, 57.1. Calcd. for $C_{21}H_{16}N_2O_4S_2$: C, 59.42; H, 3.80; N, 6.60; Found: C, 59.50; H, 3.90; N, 6.50%.

*1-Oxo-1H-isothiochromene-3-carboxylic acid (5-methylisoxazol-3-yl)-amide (**31**).* Yield 87%, mp 232-234^{o}C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.43 (s, 3H, Me), 6.71 (s, 1H, =CH isoxaz.), 7.84 (t, 1H, *J* = 7.8 Hz, 6-H), 7.93 (t, 1H, *J* = 7.8 Hz, 7-H), 8.02 (d, 1H, *J* = 8.0 Hz, 5-H), 8.23 (d, 1H, *J* = 8.0 Hz, 8-H), 8.50 (s, 1H, 4-H), 11.77 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 186.5, 170.3, 161.6, 158.3, 136.3, 135.4, 132.9, 132.5, 132.0, 128.7, 126.6, 125.6, 97.1, 12.5. Calcd. for $C_{14}H_{10}N_2O_3S$: C, 58.73; H, 3.52; N, 9.78; Found: C, 58.90; H, 3.65; N, 9.95%.

*7,8-Dimethoxy-1-oxo-1H-isothiochromene-3-carboxylic acid (5-methylisoxazol-3-yl)-amide (**32**).* Yield 87%, mp 234-236^{o}C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.43 (s, 3H, Me), 3.78 (s, 3H, OCH_3), 3.96 (s, 3H, OCH_3), 6.68 (s, 1H, =CH isoxaz.), 7.55 (d, 1H, *J* = 8.8 Hz, 6-H), 7.60 (d, 1H, *J* = 8.8 Hz, 5-H), 8.31 (s, 1H, 4-H), 11.45 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 184.4, 170.5, 161.9, 158.7, 155.8, 147.9, 130.9, 130.1, 129.4, 127.2, 123.3, 120.1, 97.4, 61.5, 57.2, 12.8. Calcd. for $C_{16}H_{14}N_2O_5S$: C, 55.48; H, 4.07; N, 8.09; Found: C, 55.55; H, 4.19; N, 8.21%.

*1-Oxo-1H-isothiochromene-3-carboxylic acid (1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-amide (**33**).* Yield 93%, mp 179-181°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.20 (s, 3H, Me), 3.15 (s, 3H, Me), 7.28 (t, 1H, *J* = 7.6 Hz, 6-H), 7.40 (d, 2H, *J* = 8.2 Hz, arom.), 7.46 (t, 2H, *J* = 8.2 Hz, arom.), 7.70 (t, 1H, *J* = 7.8 Hz, 7-H), 7.80-7.87 (m, 2H, arom.), 8.22 (d, 1H, *J* = 8.4 Hz, 8-H), 8.33 (s, 1H, 4-H), 9.90 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 186.6, 161.8, 161.8, 152.7, 136.7, 135.3, 134.7, 133.7, 132.3, 131.0, 129.2, 128.8, 126.5, 125.6, 125.0, 123.9, 107.3, 36.3, 11.7. Calcd. for $C_{21}H_{17}N_3O_2S$: C, 64.44; H, 4.38; N, 10.73; Found: C, 64.55; H, 4.45; N, 10.90%.

*7,8-Dimethoxy-1-oxo-1H-isothiochromene-3-carboxylic acid (1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-amide (**34**).* Yield 93%, mp 220-221°C. ^{1}HNMR (400 MHz, DMSO-d_6): δ 2.17 (s, 3H, Me), 3.34 (s, 3H, Me), 3.76 (s, 3H, OCH_3), 3.95 (s, 3H, OCH_3), 7.34 (t, 1H, *J* = 8.6 Hz, arom.), 7.37 (d, 2H, *J* = 8.2 Hz, arom.), 7.52 (t, 2H, *J* = 8.2 Hz, arom.), 7.63 (d, 1H, *J* = 8.8 Hz, 6-H), 7.72 (d, 2H, *J* = 8.8 Hz, 5-H), 8.20 (s, 1H, 4-H), 9.85 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 184.2, 161.9, 155.0, 152.8, 147.7, 135.3, 131.0, 130.4, 129.2, 129.0, 126.4, 125.1, 123.8, 123.2, 119.1, 107.5, 60.9, 56.7, 36.4, 11.7. Calcd. for $C_{23}H_{21}N_3O_5S$: C, 61.19; H, 4.69; N, 9.31; Found: C, 61.30; H, 4.80; N, 9.20%.

*3-(Morpholine-4-carbonyl)-isothiochromen-1-one (**35**).* Yield 70%, mp 119-121°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.60-3.70 (m, 8H, 4 x CH_2), 7.46 (br.s, 1H, 6-H), 7.66 (t, 1H, *J* = 8.2 Hz, 7-H), 7.78-7.87 (m, 2H, 4-H, 5-H), 8.20 (d, 1H, *J* = 8.2 Hz, 8-H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 185.9, 164.0, 137.3, 135.5, 133.1, 132.4, 131.1, 128.0, 125.7, 122.6, 66.7, 48.1, 43.0. ^{13}C NMR (100 MHz, DMSO-d_6): δ 185.9, 164.0, 137.3, 135.5, 133.1, 132.4, 131.1, 128.0, 125.7, 122.6, 66.7, 48.1, 43.1. Calcd. for $C_{14}H_{13}NO_3S$: C, 61.07; H, 4.76; N, 5.09; Found: C, 61.15; H, 4.60; N, 4.90%.

*7,8-Dimethoxy-3-(morpholine-4-carbonyl)-isothiochromen-1-one (**36**).* Yield 80%, mp 188-190°C. ^{1}HNMR (400 MHz, DMSO-d_6): δ 3.60-3.70 (m, 8H, 4 x CH_2), 3.79 (s, 3H, OCH_3), 3.98 (s, 3H, OCH_3), 7.30 (s, 1H, 4-H), 7.50 (s, 2H, 5-H, 6-H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 183.4, 164.2, 154.8, 147.7, 131.5, 129.8, 129.0, 123.0, 122.6, 120.2, 66.7, 61.4, 57.1, 46.9, 43.9. Calcd. for $C_{16}H_{17}NO_5S$: C, 57.30; H, 5.11; N, 4.18; Found: C, 57.45; H, 5.20; N, 4.25%.

*3-(Azepane-1-carbonyl)-7,8-dimethoxy-isothiochromen-1-one (**37**).* Yield 60%, mp 101-103°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.55 (br.s, 4H, 2 x CH_2), 1.69 (br.s, 4H, 2 x CH_2), 3.53 (br.s, 4H, 2 x CH_2), 3.74 (s, 3H, OCH_3), 3.92 (s, 3H, OCH_3), 7.40 (s, 1H, 4-H), 7.64 (d, 1H, *J* = 8.8 Hz, 6-H), 7.68 (d, 1H, *J* = 8.7 Hz, 5-H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 182.9, 164.7, 154.2, 147.3, 131.5, 131.0, 128.5, 122.0, 121.1, 120.0, 61.1, 56.8, 49.6, 46.1, 29.2, 27.5, 26.9, 26.1. Calcd. for $C_{18}H_{21}NO_4S$: C, 62.23; H, 6.09; N, 4.03; Found: C, 62.30; H, 6.20; N, 4.15%.

*1-Oxo-1H-isothiochromene-3-carboxylic acid (2-hydroxyethyl)-amide (**38**).* Yield 62%, mp 178-180°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.34 (q, 2H, CH_2), 3.55 (q, 2H, CH_2), 4.81 (br.s, 1H, OH), 7.80 (t, 1H, *J* = 7.8 Hz, 6-H), 7.88 (d, 1H, *J* = 7.3 Hz, 5-H), 7.97 (t, 1H, *J* = 7.8 Hz, 7-H), 8.16 (s, 1H, 4-H), 8.20 (d, 1H, *J* = 8.2 Hz, 8-H), 8.85 (br.s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 185.5, 162.0, 136.3, 134.6, 133.0, 131.8, 129.2, 129.0, 128.6, 125.7, 60.0, 42.9. Calcd. for $C_{12}H_{11}NO_3S$: C, 57.82; H, 4.45; N, 5.62; Found: C, 57.95; H, 4.60; N, 5.70%.

*7,8-Dimethoxy-1-oxo-1H-isothiochromene-3-carboxylic acid (2-hydroxyethyl)-amide (**39**).* Yield 60%, mp 182-184°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.30 (q, 2H, CH_2), 3.50 (q, 2H, CH_2), 3.75 (s, 3H, OCH_3), 3.94 (s, 3H, OCH_3), 4.76 (t, 1H, *J* = 4.2 Hz, OH), 7.59 (d, 1H, *J* = 8.7 Hz, 6-H), 7.68 (d, 2H, *J* = 8.7 Hz, 5-H), 7.98 (s, 1H, 4-H), 8.65 (t, 1H, *J* = 4.2 Hz, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 184.3, 162.5, 154.7, 147.6, 131.3, 128.7, 123.6, 123.1, 119.0, 60.8, 60.0, 56.5, 42.7. Calcd. for $C_{14}H_{15}NO_5S$: C, 54.36; H, 4.89; N, 4.53; Found: C, 54.50; H, 5.00; N, 4.60%.

4.3. Primary anticancer assay

Primary anticancer assay was performed on a panel of approximately sixty human tumor cell lines derived from nine neoplastic diseases, in accordance with the protocol of the Drug Evaluation Branch, National Cancer Institute, Bethesda [38-40]. Tested compounds were added to the culture at a single concentration (10^{-5} M) and the cultures were incubated for 48 h. End point determinations were made with a protein binding dye, sulforhodamine B (SRB). Results for each tested compound were reported as the percent of growth of the treated cells when compared to the untreated control cells. The percentage growth was evaluated spectrophotometrically versus controls not treated with test agents. The cytotoxic and/or growth

inhibitory effects of the most active selected compounds were tested *in vitro* against the full panel of human tumor cell lines at concentrations ranging from 10^{-4} to 10^{-8} M. 48-h Continuous drug exposure protocol was followed and an SRB protein assay was used to estimate cell viability or growth.

Using absorbance measurements [time zero (*Tz*), control growth in the absence of drug (*C*), and test growth in the presence of drug (*Ti*)], the percentage growth was calculated for each drug concentration. Percentage growth inhibition was calculated as:

$[(Ti - Tz) / (C - Tz)] \times 100$ for concentrations for which $Ti \geq Tz$,

$[(Ti - Tz) / Tz] \times 100$ for concentrations for which $Ti < Tz$.

Dose response parameters (GI_{50}, TGI, LC_{50}) were calculated for each compound. Growth inhibition of 50% (GI_{50}) was calculated from $[(Ti - Tz)/(C - Tz)] \times 100 = 50$, which is the drug concentration resulting in a 50% lower net protein increase in the treated cells (measured by SRB staining) as compared to the net protein increase seen in the control cells. The drug concentration resulting in total growth inhibition (TGI) was calculated from $Ti = Tz$. The LC_{50} (concentration of drug resulting in a 50% reduction in the measured protein at the end of the drug treatment as compared to that at the beginning) indicating a net loss of cells following treatment was calculated from $[(Ti - Tz)/ Tz] \times 100 = -50$. Values were calculated for each of these parameters if the level of activity was reached; however, if the effect was not reached or was excessive, the value for that parameter was expressed as more or less than the maximum or minimum concentration tested. The lowest values were obtained with the most sensitive cell lines.

4.4. Antitrypanosomal activity assay

Bloodstream forms of *Trypanosoma brucei brucei* strain 90-13 were cultured in HMI9 medium supplemented with 10% FCS at 37°C under an atmosphere of 5% CO_2 [41]. In all experiments, log-phase parasite cultures were harvested by centrifugation at 3,000 x *g* and immediately used. Drug assays were based on the conversion of a redox-sensitive dye (resazurin)

to a fluorescent product by viable cells [42]. Drug stock solutions were prepared in pure DMSO. *T. brucei* bloodstream forms (10^5 cells/mL) were cultured in 96-well plates either in the absence or in the presence of different concentrations of inhibitors in a final volume of 200 µL. After a 72-h incubation, resazurin solution was added in each well at the final concentration of 45 µM and fluorescence was measured at 530 nm and 590 nm absorbance after a further 4-h incubation. The percentage of inhibition of parasite growth rate was calculated by comparing the fluorescence of parasites maintained in the presence of drug to that in the absence of drug. DMSO was used as control.

4.4. Acute toxicity in vivo

The experiments were conducted on white male mice weighing 23-25 g. Compounds were dissolved in saline solution (0.9% NaCl) with l-2 drops of Polysorbate 80 **(**Tween-80®). After dissolution they were administered to mice via intraperitoneal route. The LD_{50} was evaluated for 4 or 5 different doses each on 6 animals and calculated by the Litchfield-Wilcoxon method [43].

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Scheme and table captions:

Scheme 1. Utilization of 5-ylidene-4-azolidinones in isothiocoumarins and related cores synthesis.

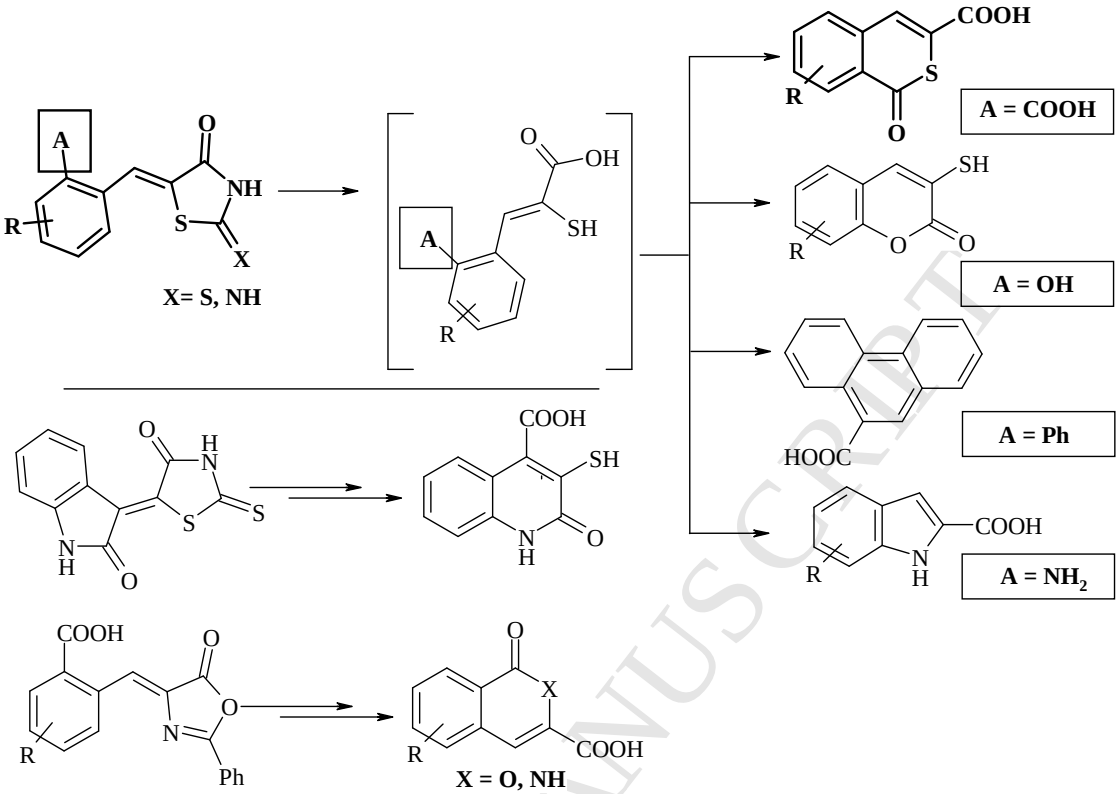
Scheme 2. Synthesis of target isothiocoumarin-3-carboxylic acids derivatives. Reagents, conditions: (*i*) 1. 30% NaOH (water solution), reflux 0.5h, 2. HCl conc.; (*ii*) 1. $SOCl_2$, reflux 0.5h, 2. alcohol, reflux 1.0 h (**3**-**8**) or amino acid, AcOH, reflux 1.0 h (**9**-**11**) or amine, dioxane, reflux 1.0 h (**12**-**39**).

Table 1. Anticancer activity (one dose primary assay) of synthesized compounds.

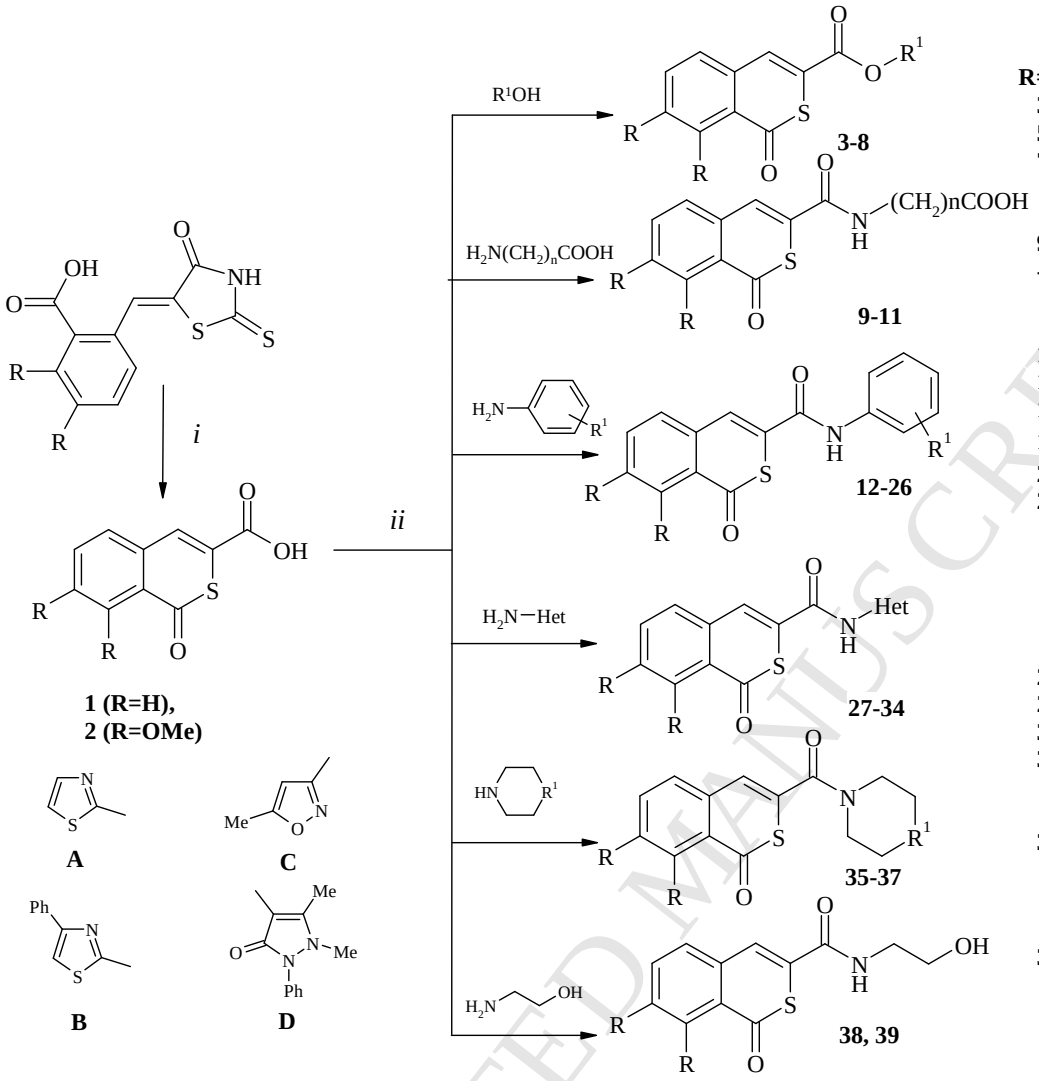
Table 2. Cytotoxic activity of the compounds (10^{-4}–10^{-8} M) towards 60 cancer cell lines.

Table 3. Antitrypanosomal activity screening on the *Tbb* bloodstream forms at 10 and 1 μg/mL.

Scheme 1.



Scheme 2.



R=H	R=OMe	R^1
3	4	Me
5	6	Et
7	8	*i*-Pr
		n
9	10	1
-	11	2
		R^1
12	13	H
14	15	4-OH
16	17	4-SO_2NH_2
18	19	3-CF_3
20	21	4-COOEt
22	23	R^1+C_6H_4 = 1-Naph
-	24	4-F
-	25	4-Cl
-	26	2-CF_3
		Het
27	28	A
29	30	B
31	32	C
33	34	D
		R^1
35	36	O
-	37	$(CH_2)_2$
38	39	

Table 1.

Compound, (NCS)	GP, % (cell lines)			Compound, (NCS)	GP, % (cell lines)		
	NCI-H460	*MCF7*	*SF-268*		*NCI-H460*	*MCF7*	*SF-268*
4 (729945)	82	108	113	25 (733391)	93	80	91
7 (**729563**)	**13**	**87**	**69**	26 (729562)	90	99	107
10 (733573)	109	109	112	**27** (**735622**)	93	91	90
12 (735600)	75	78	83	28 (731911)	94	95	98
17 (733575)	97	72	74	**31** (**729564**)	**31**	**85**	**86**
19 (**731904**)	**4**	**45**	**49**	32 (733381)	70	52	51
21 (729561)	103	105	117	33 (729565)	52	107	106
24 (733574)	11	55	60	35 (735623)	109	114	111

Table 2.

Compound, NCS	MG_MID (delta, range)			Cancer types	Most sensitive cell lines (pGI_{50}/pTGI)
	pGI_{50}	pTGI	pLC_{50}		
7 **729563**	4.27 (0.56, 0.82)	4.03 (0.28, 0.31)	4.00 (0.02, 0.02)	Ovarian Cancer Renal Cancer NSC Lung Cancer Melanoma Breast Cancer	*IGROV-1* (4.68/4.00) *RXF 393* (4.82/4.17) *EKVX* (4.72/4.28) *SK-MEL-5* (4.61/4.31) *UACC-257* (4.71/4.18) *NCI/ADR-RES* (4.57/4.16) *BT-549* (4.61/4.20)
8 **735670**	4.22 (0.48, 0.70)	4.01 (0.33, 0.34)	4.00 (0.0, 0.0)	NSC Lung Cancer CNS Cancer Renal Cancer	*HOP 92* (4.54/4.07) *SNB* (4.63/4.27) *RXF 393* (4.70/4.34)
15 **735601**	4.15 (1.31, 1.46)	4.01 (0.36, 0.37)	4.00 (0.0, 0.0)	Leukemia NSC Lung cancer Colon Cancer Breast Cancer	*CCRF-CEM* (5.25/4.00) *SR* (5.46/4.37) *HOP-92* (4.75/4.02) *KM12* (4.58/4.00) *MDA-MB-435* (4.73/4.11)
16 **735624**	4.01 (0.49, 0.50)	4.00 (0.0, 0.0)	4.00 (0.0, 0.0)	Leukemia	*SR*(4.50/4.00)
19 **731904**	4.83 (0.86, 1.69)	4.38 (0.89, 1.27)	4.15 (0.62, 0.74)	Leukemia NSC Lung cancer Colon Cancer CNS Cancer Melanoma Renal Cancer Prostate Cancer Breast Cancer	*CCRF-CEM* (4.92/4.04) *MOLT-4* (5.25/4.59) *RPMI-8226* (5.69/5.24) *HOP-62* (5.27/4.79) *HOP-92* (5.52/5.00) *NCI-H23* (5.19/4.73) *NCI-H460* (5.27/4.00) *HCT-116* (5.47/4.84) *KM12* (5.02/4.00) *U251* (5.50/4.96) *LOX IMVI* (5.16/4.66) *M14* (5.11/4.70) *786-O* (5.47/4.91) *PC-3* (5.33/4.71) *NCI/ADR-RES* (5.074/4.66) *BT-549* (5.61/5.15) *MDA-MD-231/ATCC*(5.68/5.27)
23 **735620**	4.01 (0.68, 0.69)	4.00 (0.16, 0.16)	4.00 (0.0, 0.0)	NSC Lung Cancer	*HOP-92*(4.69/4.16)
24 **733574**	4.52 (0.74, 1.26)	4.14 (0.55, 0.69)	4.01 (0.22, 0.23)	NSC Lung Cancer Colon Cancer CNS Cancer	*A549/ATCC* (4.73/4.00) *HOP-69* (5.17/4.64) *NCI-H522* (4.74/4.15) *HCC-2998* (4.85/4.31) *HCT-116* (4.85/4.39) *U-251* (5.26/4.69) *SF-295* (4.74/4.29)

				Melanoma	*UACC-62* (4.71/4.28)
				Ovarian Cancer	*OVCAR-5* (4.73/4.21)
					OVCAR-8 (4.91/4.17)
				Renal Cancer	*786-0* (4.94/4.38)
					ACHN (5.15/4.57)
					RXF-393 (4.97/4.47)
					TK-10 (4.82/4.48)
				Breast Cancer	*NCI/ADR-RES* (4.90/4.00)
30 **748183**	5.12 (0.77, 1.89)	4.26 (1.23, 1.49)	4.06 (1.08, 1.14)	Leukemia	*SR* (5.74/4.00)
				NSC Lung Cancer	*NCI-H322M* (5.89/5.25)
				CNS Cancer	*SF-539* (5.75/5.44)
					SNB-75 (5.81/5.45)
					U251 (5.70/5.35)
				Melanoma	*MALME-3M* (5.80/5.08)
				Ovarian Cancer	*OVCAR-3* (5.72/5.43)
					OVCAR-4 (5.73/-)
				Renal Cancer	*ACHN* (5.70/4.00)
					TK-10 (5.85/5.49)
					UO-31 (5.71/-)
				Breast Cancer	*HS 578T* (5.58/-)
30* **748183**	5.66 (0.96, 2.62)	4.62 (1.13, 1.75)	4.12 (1.13, 1.25)	Leukemia	*SR* (5.56/-)
				NSC Lung Cancer	*HOP-92* (5.89/5.55)
				CNS Cancer	*SF-539* (5.76/5.47)
					SNB-75 (5.80/5.51)
					U251 (5.70/5.45)
				Melanoma	*MALME-3M* (5.71/-)
				Ovarian Cancer	*OVCAR-3* (5.74/-)
					OVCAR-4 (5.73/-)
				Renal Cancer	*ACHN* (5.69/5.45)
					CAKI-1 (6.62/5.75)
					TK-10 (5.68/5.42)
					UO-31 (5.77/5.51
				Breast Cancer	*HS 578T* (5.61/5.36)
31 **729564**	4.10 (2.37, 2.47)	4.00 (0.0, 0.0)	4.00 (0.0, 0.0)	Leukemia	*SR* (6.47/4.00)
				CNS Cancer	*SF-539* (4.52/4.00)
36 **740735**	4.00 (0.0, 0.0)	4.00 (0.0, 0.0)	4.00 (0.0, 0.0)	-	-
37 **735619**	4.16 (1.40, 1.56)	4.02 (1.07, 1.09)	4.00 (0.0, 0.0)	NSC Lung Cancer	*HOP-92* (5.56/5.09)
				Melanoma	*MALME-3M* (4.46/4.00)
39 **735618**	4.08 (3.92, 4.00)	4.00 (0.02, 0.02)	4.00 (0.00, 0.00)	-	-

* Data of double assay

Table 3.

Compounds	*Tbb* inhibition, %		Compounds	*Tbb* inhibition, %	
	10 μg/mL	1 μg/mL		10 μg/mL	1 μg/mL
1	16,78	0,05	**23**	20,79	41,25
5	35,82	-3,91	**25**	5,77	5,99
9	10,80	0,18	**26**	6,86	2,91
17	59,75	31,34	**29**	44,18	7,36
18	5,78	-7,12	**30**	28,59	11,27
19	-9,98	5,46	**32**	12,16	3,93
21	-0,53	20,93	**35**	8,13	8,15
22	60,78	2,91			

Highlights for the manuscript **"Isothiocoumarin-3-carboxylic acid derivatives: synthesis, anticancer and antitrypanosomal activity evaluation"**

by **Danylo Kaminskyy, Anna Kryshchyshyn, Ihor Nektegayev, Olexandr Vasylenko, Philippe Grellier & Roman Lesyk**

- Isothiocoumarin-3-carboxylic acid derivatives synthesis based on 5-ylidenerhodanines
- 4-Phenylthiazol-2-yl-amide (**30**) showed prominent anticancer activity ($GI_{50} \approx 1.5$ µM)
- Synthesized compounds didn't exhibit significant antitrypanosomal (*Tbb*) effects

Supplementary material

Isothiocoumarin-3-carboxylic acid derivatives: synthesis, anticancer and antitrypanosomal activity evaluation

Danylo Kaminskyy[a], Anna Kryshchyshyn[a], Ihor Nektegayev[a], Olexandr Vasylenko[b], Philippe Grellier[c] & Roman Lesyk[a]

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National Academy of Science of Ukraine,
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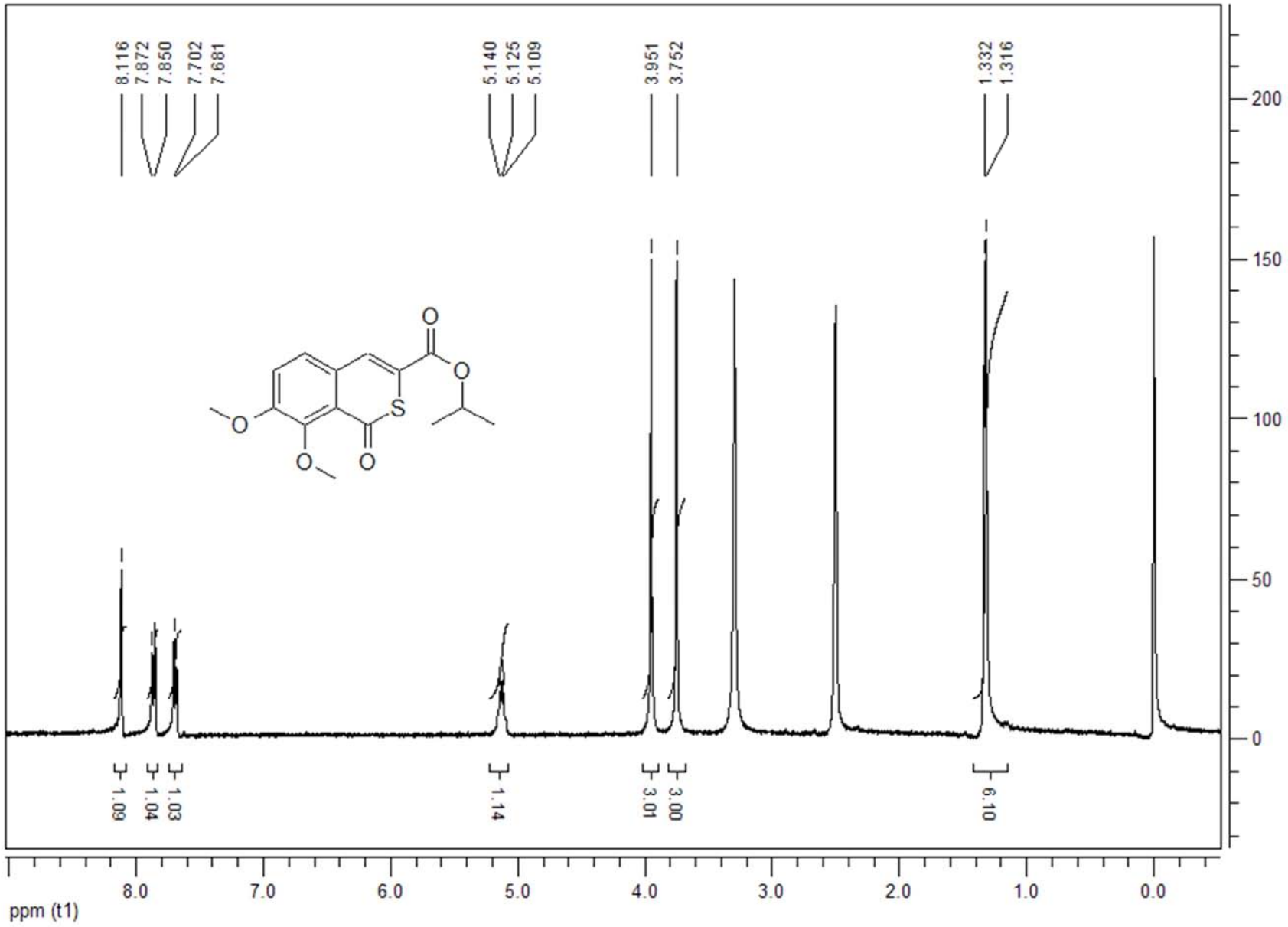
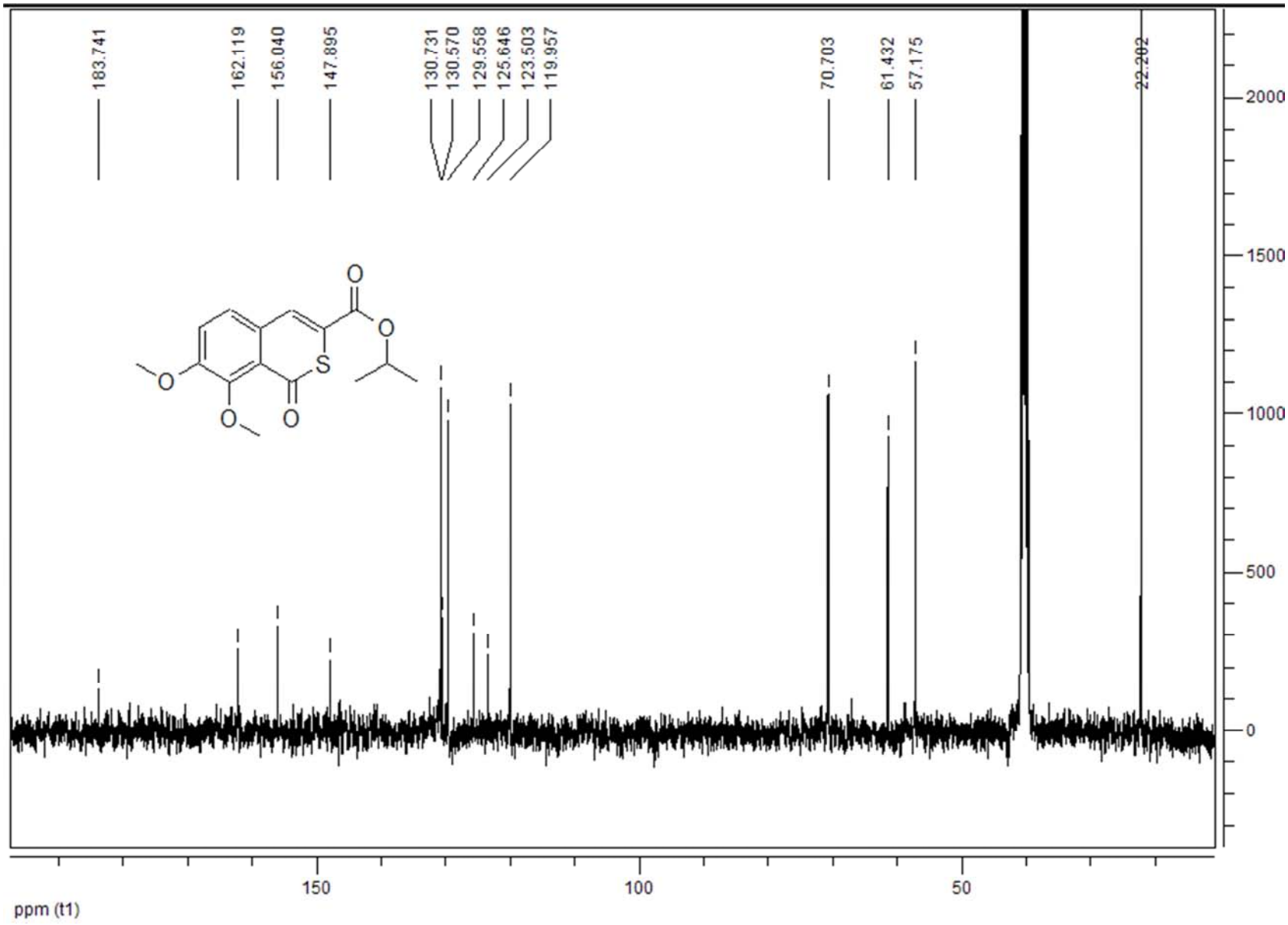
[c] National Museum of Natural History,
UMR 7245 CNRS-MNHN, team APE, CP 52,
57 Rue Cuvier, 75005 Paris, France

Contents:

7,8-Dimethoxy-1-oxo-1H-isothiochromene-3-carboxylic acid isopropyl ester (8).

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57.175
22.202
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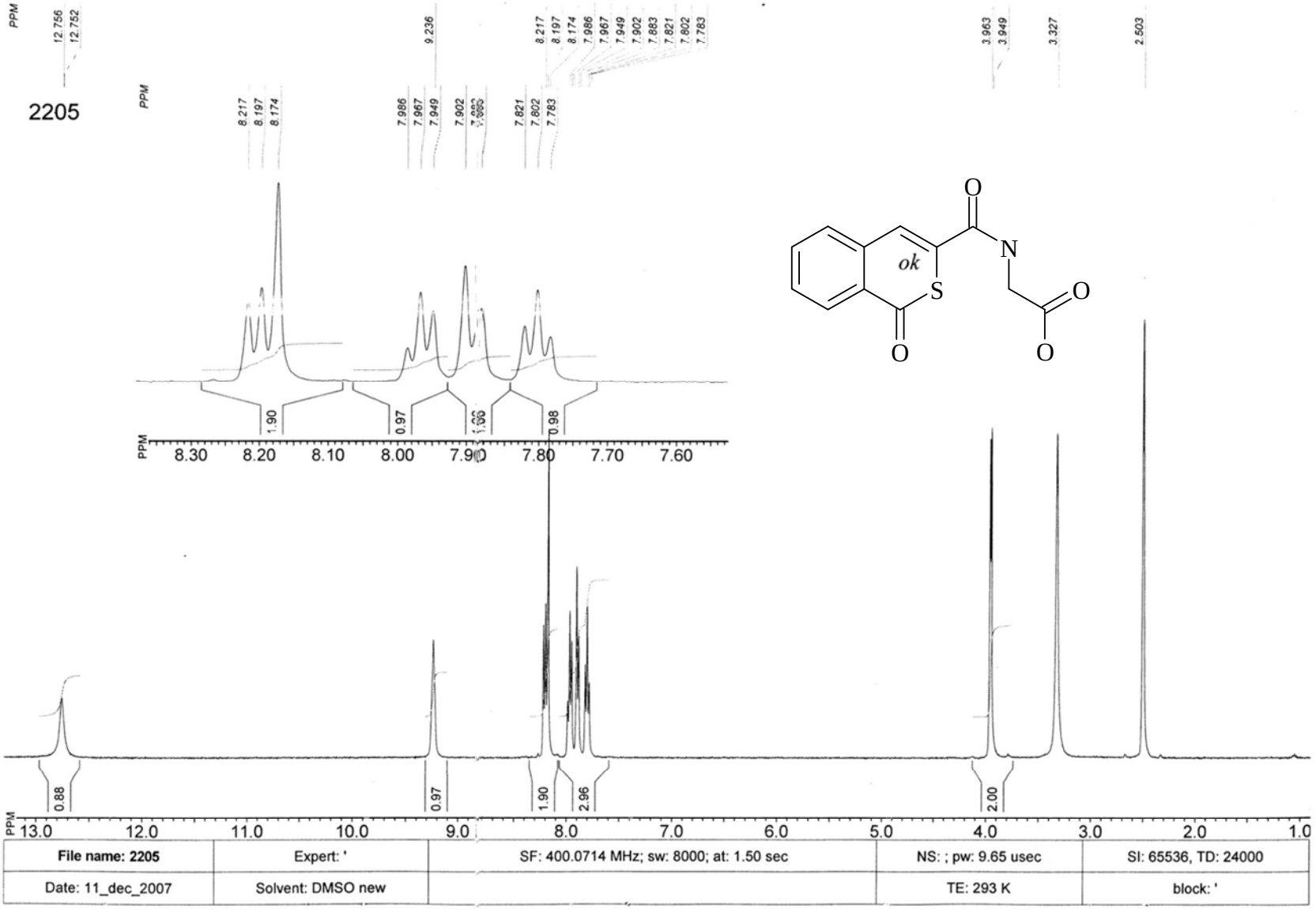
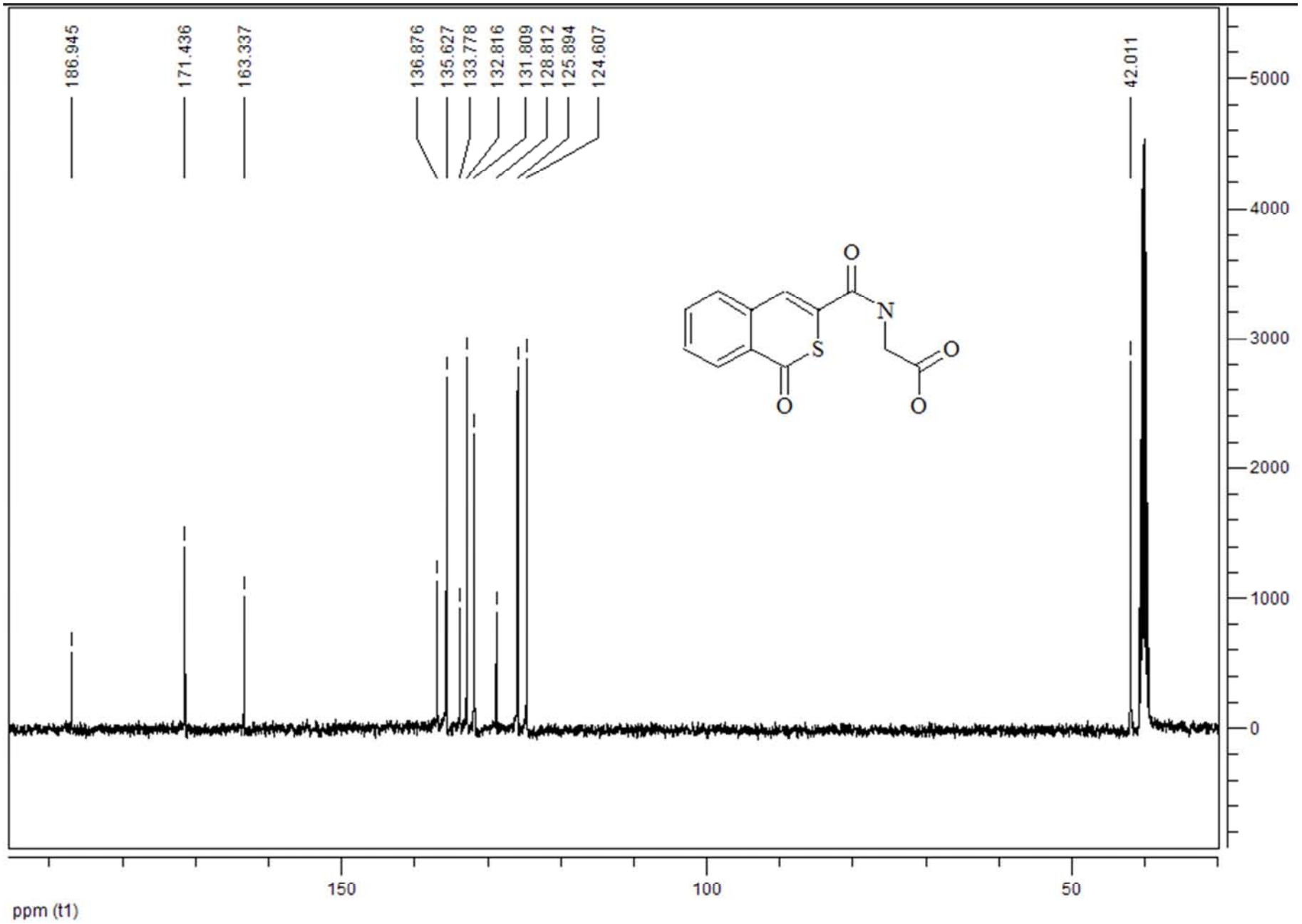
1000
500
0
150
100
50
ppm (t1)

[(1-Oxo-1H-isothiochromene-3-carbonyl)-amino]-acetic acid (9).

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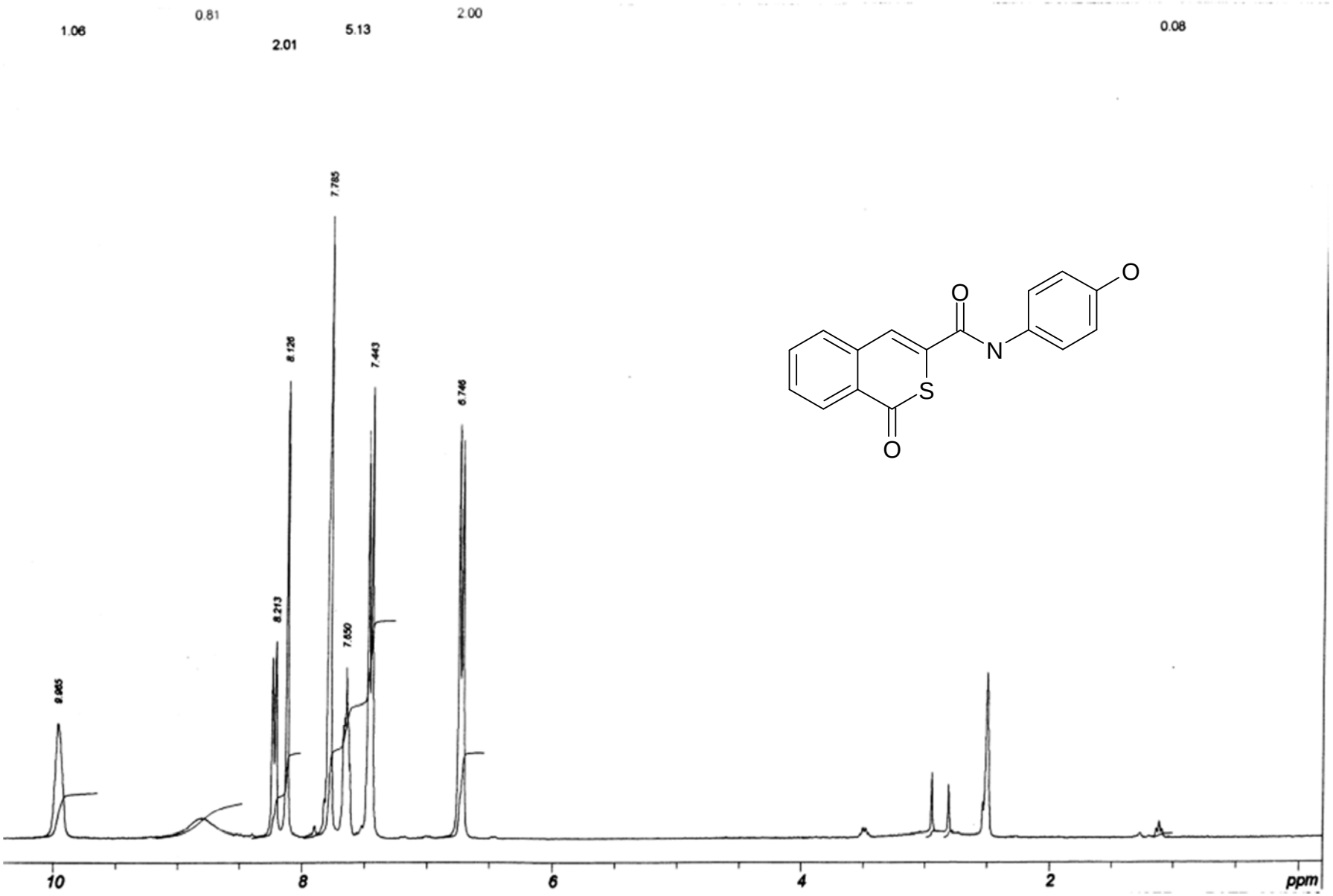
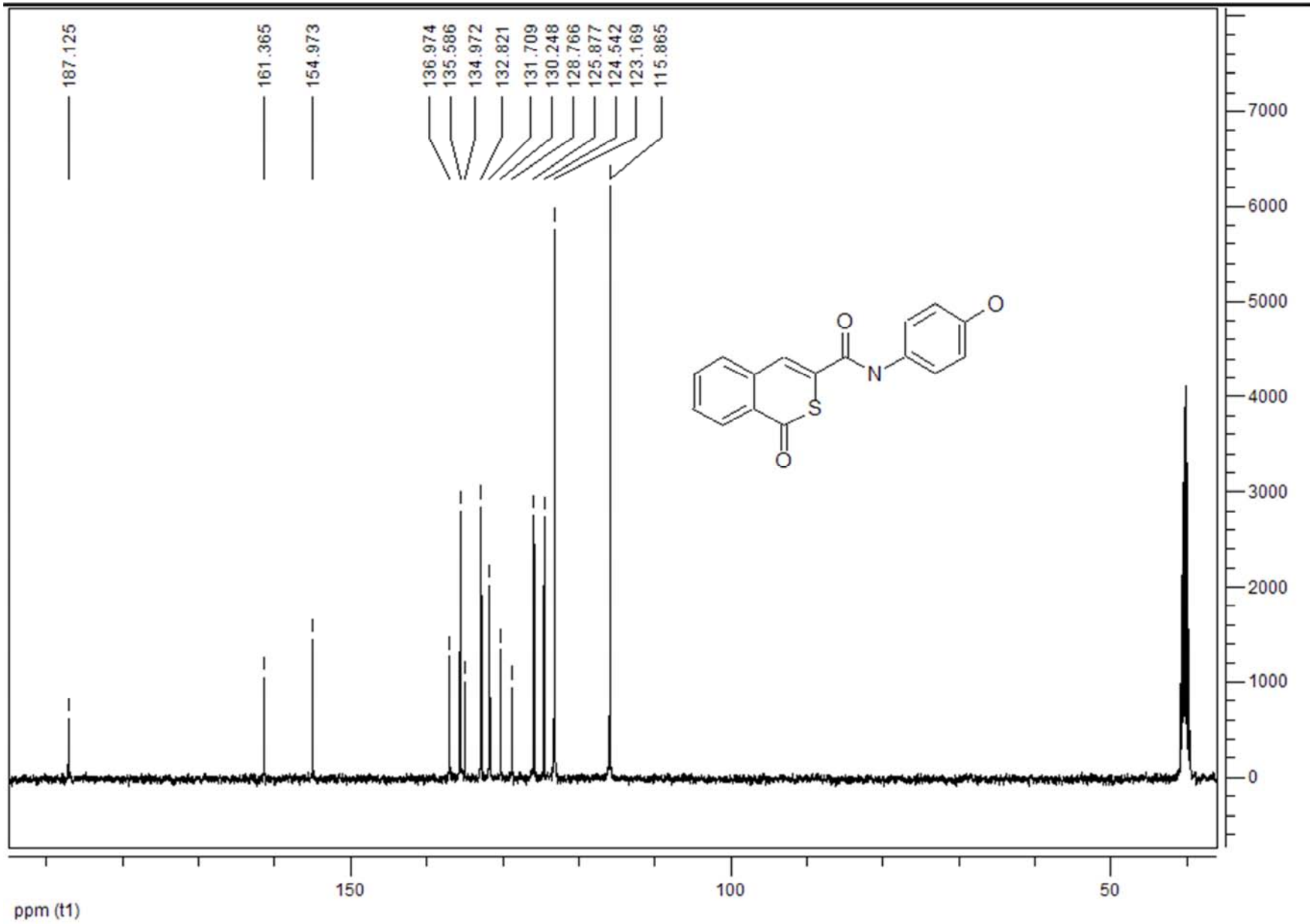
171.436
163.337
136.876
135.627
133.778
132.816
131.809
128.812
125.894
124.607
42.011
O
N
S
O
O
O
5000
4000
3000
2000
1000
0
150
100
50
ppm (t1)

1-Oxo-1H-isothiochromene-3-carboxylic acid (4-hydroxyphenyl)-amide (14)

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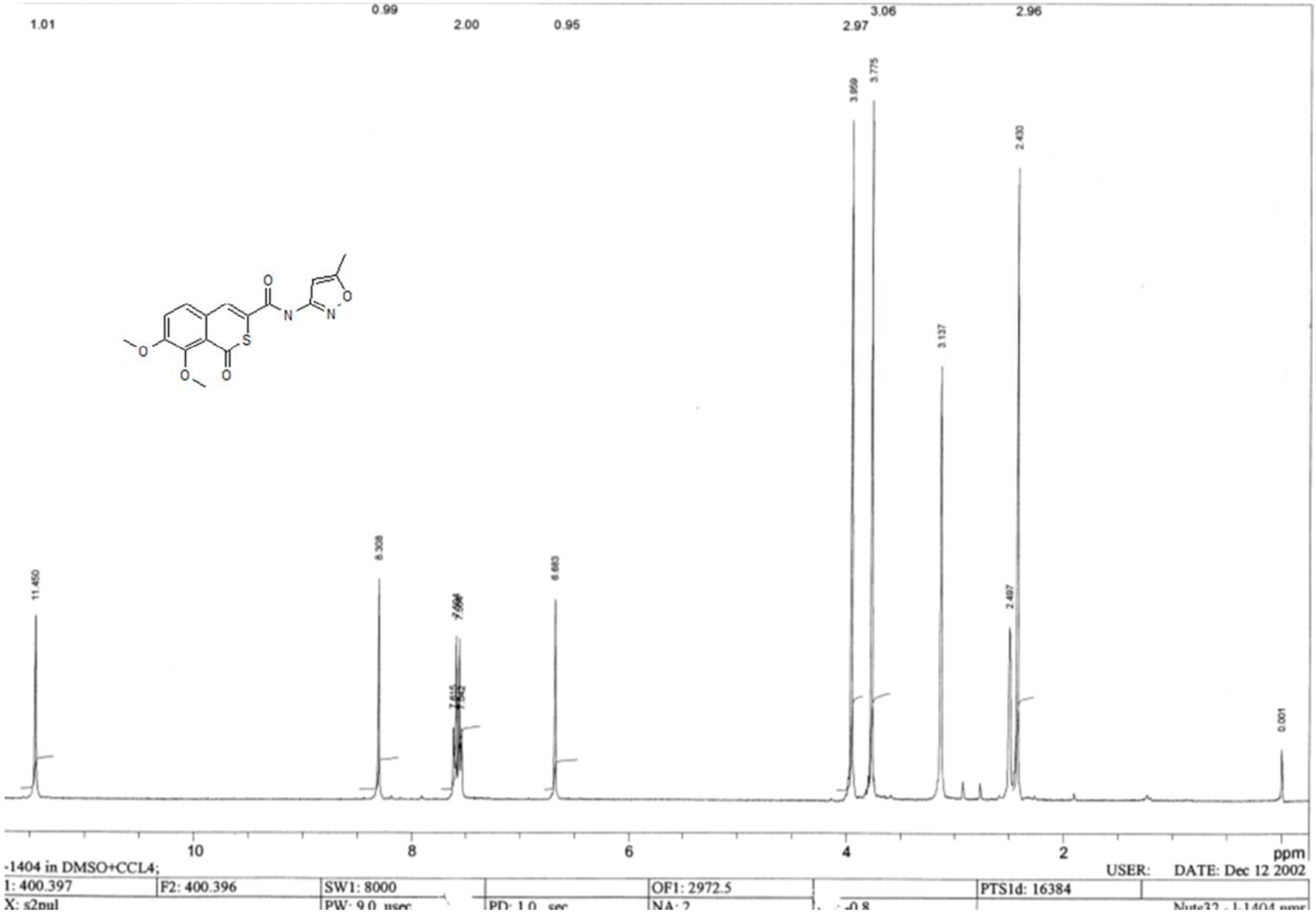
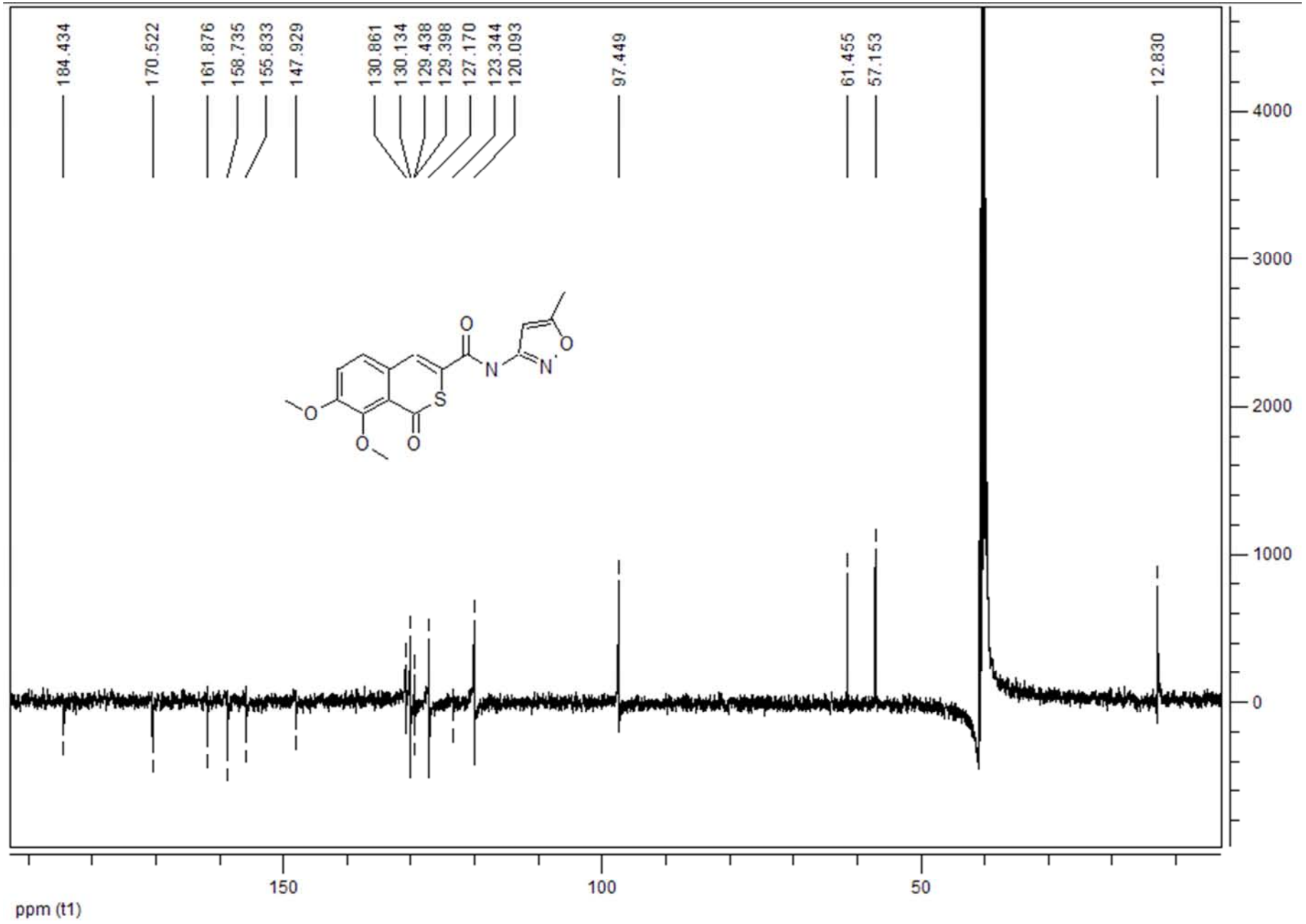
161.365
154.973
136.974
135.586
134.972
132.821
131.709
130.248
128.766
125.877
124.542
123.169
115.865
O
N
S
O
O
7000
6000
5000
4000
3000
2000
1000
0
150
100
50
ppm (t1)

7,8-Dimethoxy-1-oxo-1H-isothiochromene-3-carboxylic acid (5-methylisoxazol-3-yl)-amide (32)

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155.833
147.929
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130.134
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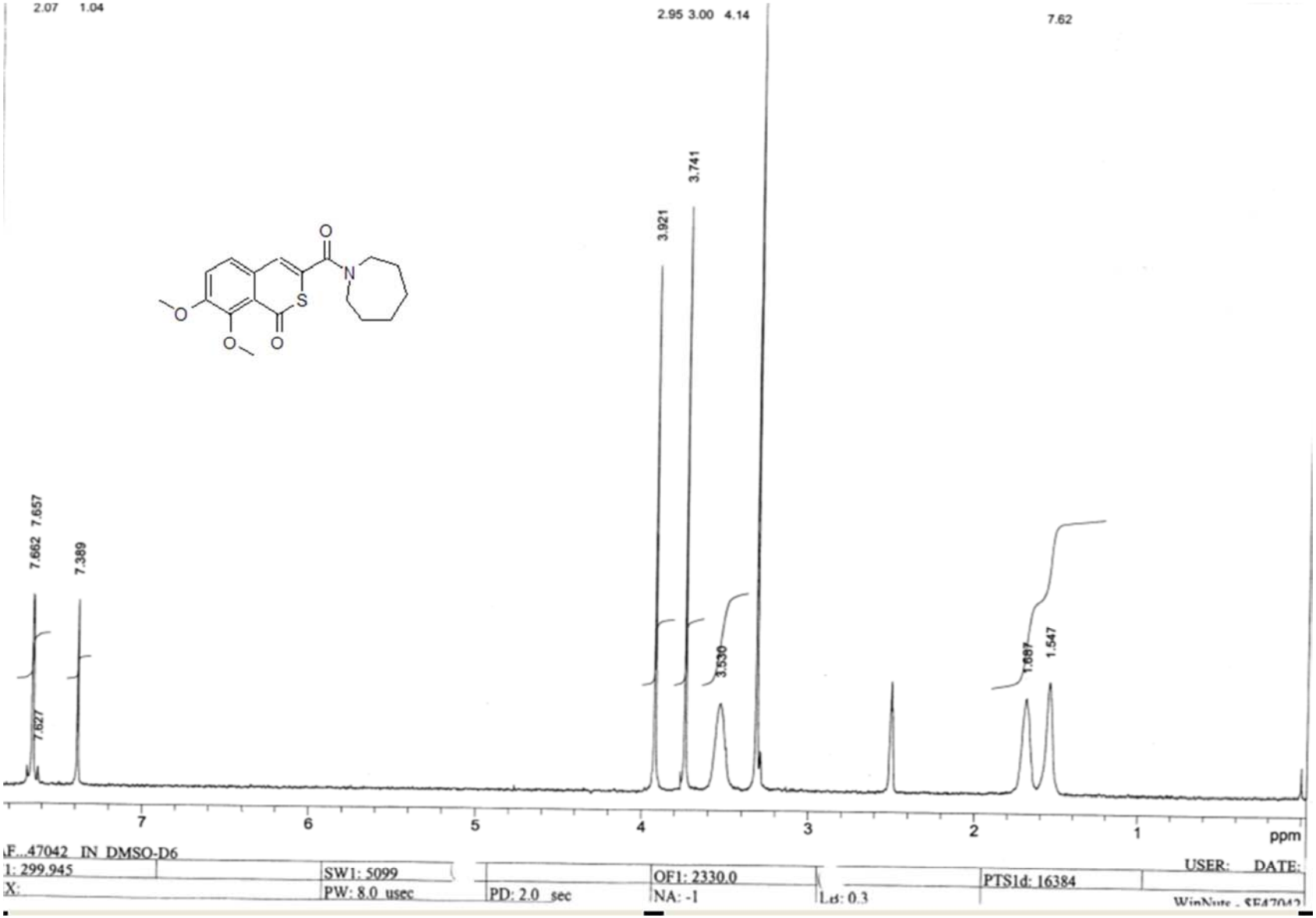
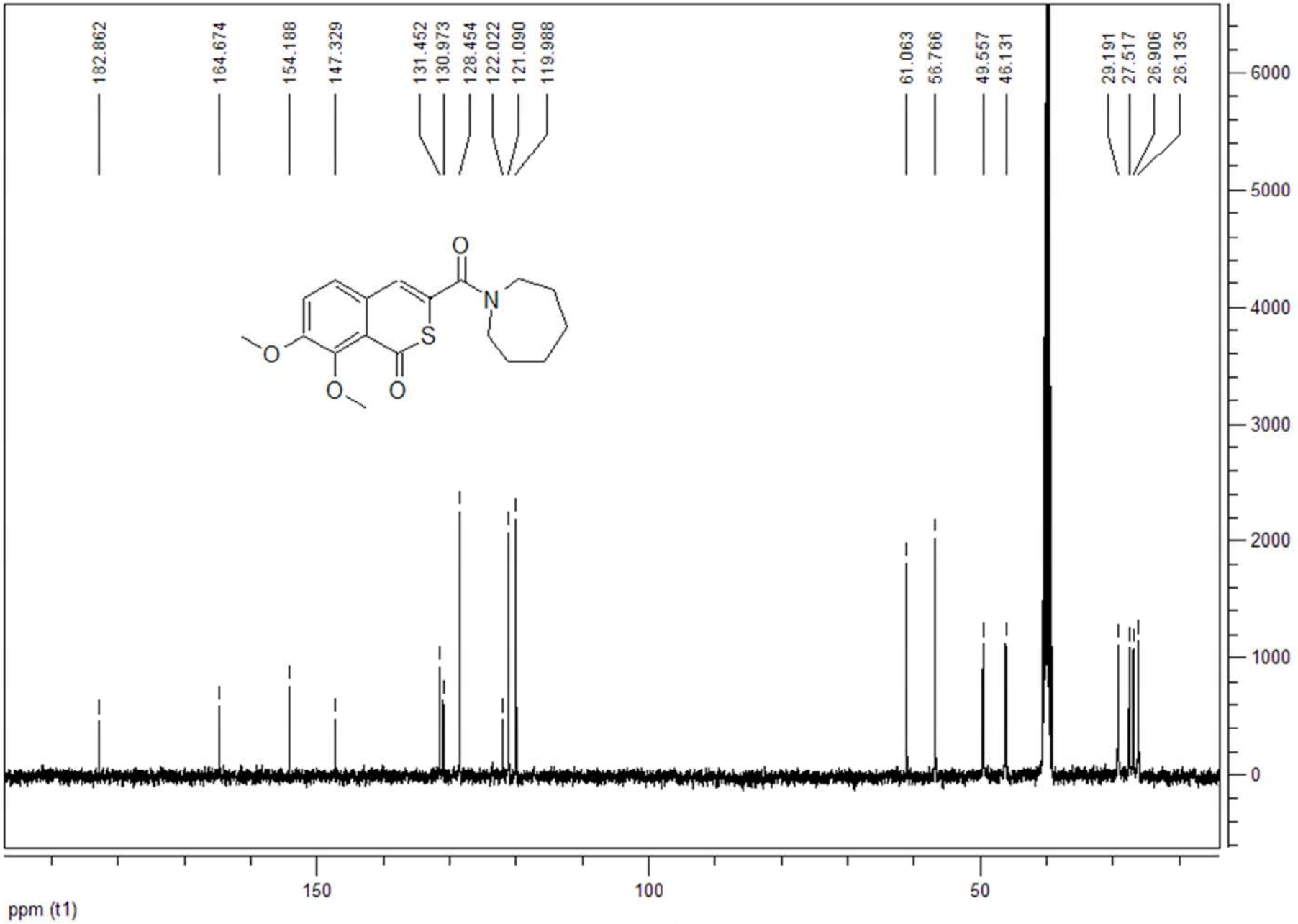
129.398
127.170
123.344
120.093
97.449
61.455
57.153
12.830
4000
3000
2000
1000
0
150
100
50
ppm (t1)

3-(Azepane-1-carbonyl)-7,8-dimethoxy-isothiochromen-1-one (37)

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154.188
147.329
131.452
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128.454
122.022
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119.988
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46.131
29.191
27.517
26.906
26.135
O
N
S
O
O
O
6000
5000
4000
3000
2000
1000
0
150
100
50
ppm (t1)