Synthesis and Biological Evaluation of Novel Conjugated Coumarin-Thiazole Systems

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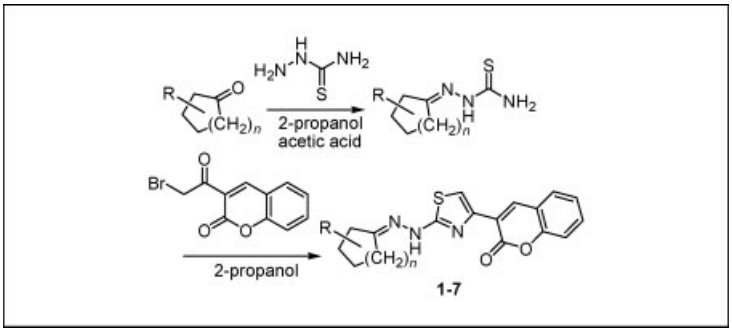
Received August 2, 2008
DOI 10.1002/jhet.110
Published online 5 May 2009 in Wiley InterScience (www.interscience.wiley.com).



Seven new 2,4-disubstituted thiazoles have been synthesized by Hantzsch condensation and assayed for several biological activities for a preliminary screening. They have been fully characterized by elemental analysis, UV–Vis spectroscopy, TG-DTA, IR, and ^{1}H NMR as well. These structures display different pharmacological (antimicrobic activity, citotoxicity, and hMAO inhibition) and industrial properties (complete transparency in the region between 465–800 nm and high thermal stability) varying on their substituents and could be considered as good lead compounds for further developments.

J. Heterocyclic Chem., **46**, 575 (2009).

INTRODUCTION

In the last few years, researchers have renewed interest in 3-(thiazol-4-yl)coumarins [1–6] for their industrial applications as fluorescent probes and laser dyes [7], for their biological activity as pharmaceutical agents [8–10] and because they could be useful for the construction of the diazepinone ring [11]. In addition, π-conjugated systems are known to show interesting electronic, optical, and electric properties [12]. On the basis of our experience in the synthesis of thiazole and coumarin [13,14] derivatives, we were pursued, therefore, to synthesize a series of novel heterocyclic systems characterized by the association of a coumarin nucleus with a thiazole ring linked to a cycloaliphatic group, whose presence and influence on this kind of molecules have not been described so far (Table 1).

RESULTS AND DISCUSSION

The coumarin-thiazole derivatives (**1–7**) were prepared by Hantzsch condensation between the appropriate thiosemicarbazone and 3-α-bromo-acetyl coumarin in 2-propanol at room temperature according to a protocol used in our laboratory [15]. In these conditions, the isolation of pure products in 86–99% yield was made simply by filtration under vacuum. All the structures were fully supported by elemental analysis and spectral data as reported in the experimental section and in Table 2. In particular, we obtained compound **3** and **4** as racemates, because of the presence of a chiral center in the structure. Knowing that the stereochemistry could affect deeply different biological activities and being commercially available the pure (*R*)-(+) enantiomer of 3-methylcyclohexanone, we planned a stereocontrolled synthesis in order to obtain derivative **5** with correlated (*R*)-(+) absolute configuration. The stereochemistry has been preserved by the stereoconservative synthetic pattern which did not interest the methyl on the cycloaliphatic moiety. The enantiomeric excess has been determined by HPLC on the Chiralpak AS-H chiral stationary phase (CSP) using the mixture *n*-hexane-2-propanol 70:30 (v/v) as a mobile phase: ee $>$ 99%; $[\alpha]_D^{20} = -20$ (0.1, EtOH).

The compounds, correctly analyzed for their molecular formula, showed in the IR spectrum strong bands at 1710 and 1600 cm^{-1} due to the presence of a δ-lactone C=O and C=N group, respectively.

The UV–visible spectral analysis was carried out in methanol as solvent and the data are collected in Table

Table 1

Structure and CLogP (ChemDraw Ultra 8.0) of compounds **1–7**.

Compound	*n*	R	CLogP
1	1	H	3.28
2	2	H	3.84
3	2	2-CH_3	4.36
4	2	3-CH_3	4.36
5	2	(*R*)-(+)-3-CH_3	4.36
6	2	4-CH_3	4.36
7	3	H	4.40

Table 3

MIC values (μg/mL) of some derivatives against eight strains of *Candida spp* and **C** = Clotrimazole.

Compound	*C. albicans* (2 strains)	*C. sakè* (strain)	*C. parapsilopis* (strains)	*C. krusei* (strains)
1	4	32	>128	>128
2	8	16	16	8–16
7	8–16	16	8	8–>128
C	8	8	8	8

2. The absorption spectra of all derivatives are generally characterized by sharp absorption bands in the region of 230–250 nm and 290–300 nm with the λ_{max} independent upon the substituent and size of the cycloaliphatic moiety. Accordingly, the coumarin local π-π excitation appears to be the main type of electronic transition and complete transparency was observed in the region between 465–800 nm.

Further we have also studied the thermal stability of **1–7** using TG-DTA method (heat rate: 10°C/min, Ar flow: 100 cc/min). All the compounds exhibited a decomposition temperature (T_d) in the range 290–315°C, a feature which could be significant for NLO applications because it is superior to that reported (T_d = 290°C) for the benchmark 4-dimethylamino-4′-nitrostilbene (DANS) [16].

From a biological point of view, evaluating the influence of the bioactive coumarin nucleus on the C4 position of the thiazole as a modulator of the biological activity and correlating the results with those obtained in our previous papers [15,17,18], some of the synthesized compounds (which all show calculated log *P* values < 5) have been widely assayed for a preliminary antimicrobic screening against eight strains of pathogenic fungi (Table 3), several types of routine clinical Gram-positive and negative isolates (MIC values > 128 μg/mL, data not shown), and 20 clinical strains of *Helycobacter pylori* (Table 4). Clotrimazole and Metronidazole were used as standard antifungal and antibacterial drug, respectively.

The results showed that all compounds possess an antibacterial activity targeting selectively towards Metronidazole resistant strains of *Helicobacter pylori* and some *Candida albicans* strain isolates.

In addition, we evaluated the low citotoxic effect of the most representative compound (**7**) against EAhy, a human cell line obtained from a hybridoma between HUVEC and epithelial cells from a lung carcinoma. The viability of cells exposed to test compounds was estimated by the Trypan Blue dye exclusion assay after 24 h of incubation at 37°C. Cells incubated with culture medium alone represented the control and the cell viability was always greater than 97%. Data represent the arithmetic mean ± SD of at least three independent experiments (Table 5).

In the end, all the newly synthesized compounds were investigated for their ability to inhibit both human MAO isoforms by measuring their effects on the production of H_2O_2 from *p*-tyramine, using the Amplex Red MAO assay kit. Microsomial MAO isoforms prepared from insect cells (BTI-TN-5B1-4) infected with recombinant baculovirus containing cDNA inserts for hMAO-A and hMAO–B. Data were calculated as pIC_{50} for both isoforms and pSI referred to log selectivity index = $pIC_{50\ (MAO\text{-}A)} - pIC_{50\ (MAO\text{-}B)}$. The data represent mean values of at least five separate experiments and Clorgyline and *R*-(−)-deprenyl were used as standard drugs (Table 6).

Despite their inhibitory activity in the micromolar range, in examining the influence of the structural variation, some of them (in particular those which did not present substituents on the cycloaliphatic moiety) possessed a moderate A-selectivity. From a qualitative point

Table 2

UV–visible absorption of derivatives **1–7** (MeOH).

Compound	λ_{ab} (nm)	$\varepsilon \times 10^4$
1	293.74	1.60
2	293.57	1.85
3	291.61	1.29
4	292.03	0.88
5	295.37	0.66
6	298.52	1.23
7	292.30	1.24

Table 4

MIC values (μg/mL) of some derivatives and **M** = Metronidazole against 20 *H. pylori* strains.

Compound	Metronidazole sensitive strains (15 strains)	Metronidazole resistant strains (strains)
1	16–32	16–32
2	8–32	8–32
7	8–32	8–32
M	0.5–2	64–>128

Table 5

Citotoxicity of derivative **7** expressed as cell survival fraction ± SD (%).

	Concentration (μg/mL)			
Compound	50	5	0.5	0.05
7	52.94 ± 3.8	81.82 ± 5.6	93.75 ± 4.7	97.50 ± 1.8

of view, it must be highlighted the different selective hMAO inhibition of compound **5** (pure (*R*)-(+) enantiomer) in relation to **4** (racemate) bearing the same substituent in the same position.

CONCLUSION

We reported the synthesis, the spectral characterization, and a preliminary biological evaluation of seven novel heterocyclic systems. According to the biological results, these compounds exhibited a promising antimicrobic activity expecially oriented towards Metronidazole resistant strains of *Helycobacter pylori* and an interesting anti-*Candida* spectrum comparable to that of Clotrimazole. It has been also found a low cytotoxic effect against EAhy, a human immortalized cell line, which could justify a safe and therapeutic use of these derivatives. They further displayed a moderate selective hMAO-A inhibition which is probably affected by the steric hindrance of bulky coumarin nucleus inside the active site. All these results could be useful to evaluate new substitutions on the coumarin nucleus in order to enhance biological activities.

EXPERIMENTAL

The chemicals, solvents for synthesis, and spectral grade solvents were purchased from Aldrich (Italy) without further purification. Melting points (uncorrected) were determined automatically on a FP62 apparatus (Mettler-Toledo). ^{1}H NMR spectra were recorded at 400 MHz on a Bruker spectrometer. Chemical shifts are expressed as δ units (parts per millions) relative to the solvent peak. Coupling constants *J* are valued in Hertz (Hz). IR spectra were registered on a Perkin-Elmer FTIR Spectrometer Spectrum 1000 in potassium bromide. Elemental analysis for C, H, and N were recorded on a Perkin-Elmer 240 B microanalyzer and the analytical results were within ±0.4% of the theoretical values for all compounds. All reactions were monitored by TLC on 0.2 mm thick silica gel plates (60 F254 Merck). UV–vis spectra were recorded on a Perkin-Elmer UV–vis Spectrometer Lambda 10. Thermal analysis was performed under an inert gas flux using TGA/DTA (Pyris Diamond Perkin-Elmer, Waltham, MA).

Typical procedure for the thiosemicarbazones synthesis. The appropriate cycloaliphatic ketone (50 mmol) was dissolved in 100 mL of 2-propanol and stirred at room temperature with an equimolar amounts of thiosemicarbazide for 24 h with acetic acid as catalyst. The desired thiosemicarbazone precipitated from reaction mixture was filtered and crystallized from suitable solvent and dried.

Typical procedure for the Hantzsch protocol for the preparation of derivatives 1–7. Equimolar amounts of the prepared thiosemicarbazones (50 mmol) and 3-α-bromo-acetyl coumarin [19] (50 mmol), both dissolved in 2-propanol, were reacted at room temperature under magnetic stirring for 2 h. The precipitate was filtered, washed with petroleum ether and diethyl ether, and dried to give compounds **1–7** in 86–99% yields. All the yields are on isolated basis. The characterization data are given in Tables 1 and 2.

3-(2-(2-Cyclopentylidenehydrazynyl)thiazol-4-yl)-2*H*-chromen-2-one (1). Yellow crystals, 98% yield, mp 207–209°C; ^{1}H NMR ($CDCl_3$): δ 1.87–1.89 (m, 2H, cyclopentyl), 1.95–1.97 (m, 2H, cyclopentyl), 2.55 (s, 2H, cyclopentyl), 2.64 (s, 2H, cyclopentyl), 7.37–7.39 (m, 1H, C_5H-thiaz.), 7.49–7.69 (m, 2H, Ar), 7.81–7.83 (m, 2H, Ar), 8.59 (s, 1H, CH=), 12.20 (br s, 1H, NH, D_2O exch.); Anal. Calcd. for $C_{17}H_{15}N_3O_2S$: C, 62.75; H, 4.65; N, 12.91. Found: C, 62.77; H, 4.67; N, 12.92.

3-(2-(2-Cyclohexylidenehydrazynyl)thiazol-4-yl)-2*H*-chromen-2-one (2). Dark yellow crystals, 98% yield, mp 180–182°C; ^{1}H NMR ($CDCl_3$): δ 1.64–1.71 (m, 4H, cyclohexyl), 1.86 (s, 2H, cyclohexyl), 2.58 (s, 2H, cyclohexyl), 2.69 (s, 2H, cyclohexyl), 7.36 (s, 1H, C_5H-thiaz.), 7.61–7.63 (m, 2H, Ar), 7.80–7.85 (m, 2H, Ar), 8.62 (s, 1H, CH=), 12.39 (s, 1H, NH, D_2O exch.); Anal. Calcd. for $C_{18}H_{17}N_3O_2S$: C, 63.70; H, 5.05; N, 12.38. Found: C, 63.68; H, 5.04; N, 12.39.

3-(2-(2-(2-Methylcyclohexylidenehydrazynyl)thiazol-4-yl)-2*H*-chromen-2-one (3). Off white crystals, 86% yield, mp 171–173°C; ^{1}H NMR ($CDCl_3$): δ 1.16–1.18 (d, J = 6.3 Hz, 3H, CH_3), 1.21–1.23 (m, 2H, cyclohexyl), 1.27–2.04 (m, 5H, cyclohexyl), 2.50 (s, 1H, cyclohexyl), 3.01 (s, 1H, cyclohexyl), 7.38 (s, 1H, C_5H-thiaz.), 7.62–7.64 (m, 2H, Ar), 7.82–7.84 (m, 2H, Ar), 8.60 (s, 1H, CH=), 12.17 (br s, 1H, NH, D_2O exch.); Anal. Calcd. for $C_{19}H_{19}N_3O_2S$: C, 64.57; H, 5.42; N, 11.89. Found: C, 64.59; H, 5.40; N, 11.88.

3-(2-(2-(3-Methylcyclohexylidenehydrazynyl)thiazol-4-yl)-2*H*-chromen-2-one (4). Yellow crystals, 99% yield, mp 188–190°C; ^{1}H NMR ($CDCl_3$): δ 1.03–1.05 (d, J = 6.3 Hz, 3H, CH_3), 1.21–1.23 (m, 2H, cyclohexyl), 1.26–2.08 (m, 5H, cyclohexyl), 2.50 (s, 1H, cyclohexyl), 3.01 (s, 1H, cyclohexyl), 7.38 (s, 1H, C_5H-thiaz.), 7.80 (s, 2H, Ar), 7.91 (s, 2H, Ar), 12.20 (br s, 1H, NH, D_2O exch.); Anal. Calcd. for $C_{19}H_{19}N_3O_2S$: C, 64.57; H, 5.42; N, 11.89. Found: C, 64.56; H, 5.43; N, 11.90.

Table 6

hMAO inhibition and hMAO-A selectivity of derivatives **1–7**.

Compound	pIC_{50} hMAO-A	pIC_{50} hMAO-B	pSI
1	4.39	<4.00	>0.39
2	5.28	<4.00	>1.28
3	5.04	5.12	−0.08
4	4.96	4.97	−0.01
5	5.11	5.02	0.09
6	5.15	4.87	0.28
7	5.19	<4.00	>1.19
Clorgyline	8.29	4.20	−4.09
R-(−)-deprenyl	4.17	7.77	3.60

(*R*)-(+)-3-(2-(2-(Methylcyclohexylidenehydrazynyl)thiazol-4-yl)-2*H*-chromen-2-one (5). Yellow crystals, 99% yield, mp 200–201°C; ^{1}H NMR ($CDCl_3$): δ 1.02–1.04 (d, J = 6.3 Hz, 3H, CH_3), 1.26–1.27 (m, 2H, cyclohexyl), 1.79–1.96 (m, 5H, cyclohexyl), 2.95–2.99 (m, 2H, cyclohexyl), 7.38 (s, 1H, C_5H-thiaz.), 7.62–7.66 (m, 2H, Ar), 7.80–7.84 (m, 2H, Ar), 8.62 (s, 1H, CH=), 12.18 (br s, 1H, NH, D_2O exch.); Anal. Calcd. for $C_{19}H_{19}N_3O_2S$: C, 64.57; H, 5.42; N, 11.89. Found: C, 64.59; H, 5.44; N, 11.90.

3-(2-(2-(4-Methylcyclohexylidenehydrazynyl)thiazol-4-yl)-2*H*-chromen-2-one (6). Dark orange crystals, 86% yield, mp 175–177°C; ^{1}H NMR ($CDCl_3$): δ 0.99 (s, 3H, CH_3), 1.75 (s, 1H, cyclohexyl), 1.99–2.09 (m, 3H, cyclohexyl), 2.19–2.31 (m, 2H, cyclohexyl), 2.55–2.56 (m, 1H, cyclohexyl), 3.06 (s, 1H, cyclohexyl), 7.32 (s, 1H, C_5H-thiaz.), 7.64 (s, 2H, Ar), 7.84 (s, 2H, Ar), 8.59 (s, 1H, CH=), 12.17 (s, 1H, NH, D_2O exch.); Anal. Calcd. for $C_{19}H_{19}N_3O_2S$: C, 64.57; H, 5.42; N, 11.89. Found: C, 64.59; H, 5.42; N, 11.90.

3-(2-(2-(Cycloheptylidenehydrazynyl)thiazol-4-yl)-2*H*-chromen-2-one (7). Yellow crystals, 98% yield, mp 184–186°C; ^{1}H NMR ($CDCl_3$): δ 1.64–1.68 (m, 6H, cycloheptyl), 1.86–1.88 (m, 2H, cycloheptyl), 2.57–2.59 (m, 2H, cycloheptyl), 2.68–2.71 (m, 2H, cycloheptyl), 7.27 (s, 1H, C_5H-thiaz.), 7.38–7.40 (m, 2H, Ar), 7.62–7.64 (t, 1H, Ar), 7.81–7.82 (t, 1H, Ar), 8.62 (s, 1H, CH=), 11.95 (s, 1H, NH, D_2O exch.); Anal. Calcd. for $C_{19}H_{19}N_3O_2S$: C, 64.57; H, 5.42; N, 11.89. Found: C, 64.58; H, 5.41; N, 11.89.

HPLC enantioseparation. HPLC enantioseparation was performed using stainless-steel Chiralpak AS-H (250 mm × 4.6 mm I.D.) columns (Chiral Technologies Europe, Illkirch, France). HPLC-grade solvents were supplied by Carlo Erba (Milan, Italy). The HPLC apparatus consisted of a Perkin-Elmer (Norwalk, CT) 200 LC pump equipped with a Rheodyne (Cotati, CA) injector, a 50-μL sample loop, a HPLC Dionex TCC-100 oven (Sunnyvale, CA), and a Perkin-Elmer UV detector model 290.

Specific rotations of enantiomers of compound **4**, dissolved in ethanol, were measured at 589 nm by a Perkin-Elmer polarimeter model 241 equipped with a Na lamp. The volume of the cell was 1 mL and the optical path 10 cm. The system was set at a temperature of 20°C using a Neslab RTE 740 cryostat.

Acknowledgments. This work was supported by grants from MURST (Italy). We also are very thankful to Prof. Francisco Orallo of the Department of Pharmacology, Faculty of Pharmacy, University of Santiago de Compostela for performing hMAO inhibition assays.

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