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The influence of halogen substituents on the biological properties of sulfur-containing flavonoids

Lucian Gabriel Bahrin [a,b], Laura Gabriela Sarbu [a], Henning Hopf [b], Peter G. Jones [c], Cornelia Babii [d], Marius Stefan [d,*], Mihail Lucian Birsa [a,b,*]

[a] Department of Chemistry, Al.I. Cuza University of Iasi, 11 Carol I, 700506 Iasi, Romania
[b] Institute of Organic Chemistry, Technical University of Braunschweig, Hagenring 30, D-38106 Braunschweig, Germany
[c] Institute of Inorganic and Analytical Chemistry, Technical University of Braunschweig, Hagenring 30, D-38106 Braunschweig, Germany
[d] Department of Biology, Al.I. Cuza University of Iasi, 11 Carol I, 700506 Iasi, Romania

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ABSTRACT

A series of halogen-substituted tricyclic flavonoids containing a 1,3-dithiol-2-ylium moiety has been synthesized from the corresponding 3-dithiocarbamic flavanones. The influence of halogen substituents on the antibacterial properties of the tricyclic flavonoids has been investigated against *Staphylococcus aureus* and *Escherichia coli*. On going from fluorine to iodine, these compounds exhibit good to excellent inhibitory properties against both Gram-positive and Gram-negative pathogens. These results suggest that size is the main factor for the change in potency rather than polarity/electronics.

1. Introduction

Drug-resistant microorganisms are a major concern to modern-day society.[1] The extensive, sometimes negligent use of antibiotics over the past few decades has inevitably led to the appearance of drug-resistant pathogens.[2] Certain strains of *Escherichia coli*, *Klebsiella pneumoniae* and *Staphylococcus aureus*, to name but a few, have developed the ability to produce extended-spectrum β-lactamases (ESBLs) that render β-lactam antibiotics ineffective.[1,3,4] Other antibiotic groups, such as quinolones[5] and macrolides,[6] also prove ineffective in some cases. In this context, new antibiotics, preferably based on new molecular scaffolds, are required.[7]

Flavonoids are a diverse group of polyphenolic plant secondary metabolites. As a consequence of the various patterns on the C_6–C_3–C_6 backbone, more than 9000 flavonoids are known today.[8] Their wide range of biological activities has attracted a great deal of attention in the past few decades. Flavonoids have been found to display anticancer,[9,10] anti-inflammatory,[11] cardio-protective,[12,13] neuro-protective,[14] antiviral,[15] antifungal[16] and antibacterial[17,18] properties. In a previous paper,[19] we reported the antibacterial activity of a series of flavonoids containing a 1,3-dithiolium moiety. In one particular case, structure **1** (Fig. 1), minimum inhibitory concentrations (MIC) of 0.48 μg/mL for *Staphylococcus aureus* and 3.9 μg/mL for *Escherichia coli* were recorded. With MIC values less than 10 μg/mL being of interest,[20] we decided to extend our previous research and investigate the influence of the two halogen substituents bound to structure **1** on the antimicrobial activity of this class of tricyclic flavonoids.

It should be underlined that 1,3-dithiolium salts as a general class of compounds are not known to exhibit antibacterial properties. A literature survey on the non-ionic similar flavonoids reveals that a similar tricyclic condensed structure, carbon skeleton only, represents a class of selective estrogen receptor β agonists (SERBAs);[21] no related tricyclic structures with antibacterial activities were reported so far.

2. Results and discussion

2.1. Chemistry

The synthesis of tricyclic flavonoids **5a–o** has been accomplished by the hetero cyclocondensation of dithiocarbamic esters **4a–o** (Scheme 1). The later flavanones have been synthesized from phenacyl dithiocarbamates **2a–c**, obtained following an already established procedure,[22,23] and aminals **3a–d**, which were

* Corresponding authors.
E-mail addresses: stefanm@uaic.ro (M. Stefan), lbirsa@uaic.ro (M.L. Birsa).

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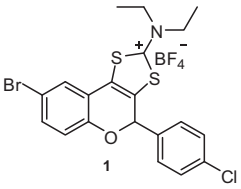

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Figure 1. Molecular structure of tricyclic flavonoid **1**.

synthesized using morpholine and the corresponding halogenated benzaldehydes. Usually the closure of the benzopyran ring is accomplished by the reaction of a 2-hydroxyacetophenone with an aldehyde under basic conditions. However, due to the side-reactions provided by a free aldehyde in a basic environment, it is of interest to use a protected but reactive aldehyde. One of these methods is to convert the aldehyde to an aminal by reacting this with two equivalents of a secondary amine. The aminal is a versatile reagent for this type of ring closure since in an intermediate step it release one equivalent of secondary amine that acts subsequently as a mild catalyst.

After 2 h in refluxing ethanol, the desired products were isolated as non-separable mixtures of *syn*- and *anti*-isomers, as presented in Figure 2. The ^{1}H NMR spectra indicated the presence of a pair of signals for each H-2 and H-3 hydrogen atom. Based on the calculated coupling constants, we were able to distinguish between the isomers where both hydrogen atoms are on the same side of the benzopyran ring (*syn*, $^3J \sim 3.8$–4.2 Hz), or on opposite sides of it (*anti*, $^3J \sim 8.9$–9.9 Hz). In each case, the *anti*-isomers predominate, probably because they are less hindered sterically. Thus, for twelve of the fifteen flavanones, the *anti* to *syn* ratio was approximately 70/30 (Fig. 2); for the other three, this ratio was even larger, up to 96/4 for compound **4g**.

The structures of dithiocarbamic flavanones **4** are supported by spectral and analytical data. Thus, the ^{1}H NMR spectra indicate the disappearance of the phenolic hydrogen atom (~12 ppm) and of the singlet corresponding to the methylene group (~4.8 ppm) from phenacyl dithiocarbamates **2**. At the same time, new signals corresponding to the aromatic ring provided by the aminal were recorded; corresponding signals are also observed in the ^{13}C NMR spectra. The MS spectra confirm the molecular weight calculated for flavanones **4**. Moreover, the structures of dithiocarbamic flavanones **4g**, **4h**, **4m** and **4o** have been unambiguously established by X-ray analysis (Figs. 3 and 4). We have able to grow single crystals by the slow cooling of the mixture of isomers in ethanol. All measured structures revealed the *anti*-isomer. Structural data for compounds **4g**, **4h**, **4m** and **4o** are presented in Table 1.

Tricyclic flavonoids **5** have been obtained via acid catalyzed cyclization of flavanones **4**. The reactions were performed using a mixture of sulfuric and acetic acid (1:3, v/v);[26,27] after 30 min at 80 °C, an aqueous solution of sodium tetrafluoroborate was added to the resulting solution, which contains the 1,3-dithiolium cation, in order to isolate compounds **5** as white solids.

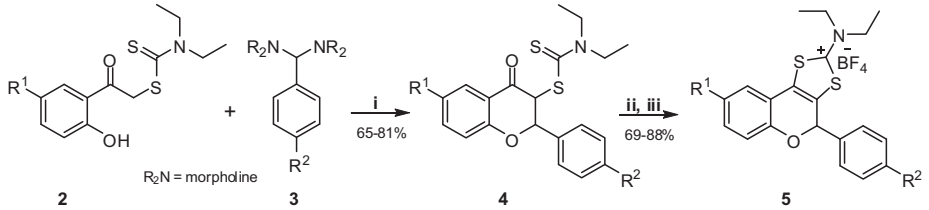
The cyclization of dithiocarbamates **4** to tricyclic flavonoids **5** is accompanied by important spectral changes. Thus, IR spectroscopy shows the absence of the carbonyl absorption bands (~1685 cm^{-1}) and the presence of new strong and broad absorption bands (~1050 cm^{-1}) from the tetrafluoroborate anion. In the ^{1}H NMR spectra, the doublets corresponding to the H-3 hydrogen atoms are no longer present; at the same time, the signals of the H-2 hydrogen atoms are shifted to ca. 6.80 ppm and become singlets. The ^{13}C NMR spectra confirm the absence of the carbonyl and thiocarbonyl atoms (187 and 191 ppm) and show a new signal at ca. 184 ppm corresponding to the positive 1,3-dithiol-2-ylium carbon atom. The ESI-MS spectra also supports the cyclization of flavanones **4** to **5**, showing the molecular ion of the positively charged tricyclic moiety.

As mentioned before, the cyclization of dithiocarbamic flavanones **4** in a mixture of sulfuric and acetic acid resulted in a homogeneous solution that contains the 1,3-dithiolium cation. Various salts of the 1,3-dithiolium derivatives can be obtained by adding the corresponding counter-ion into the obtained solutions. However, the isolation of the 1,3-dithiolium salts is limited to their solubility in water. For this reason, no chlorides were isolated so far using this method; hydrogen sulfates are also often water soluble. Perchlorates are easily isolated by adding 70% $HClO_4$ and water. Several perchlorate containing compounds have been previously reported to exhibit antimicrobial activity.[28] For this reasons we decided to isolate the tricyclic 1,3-dithiolium salts as tetrafluoroborates; the ion-dissociation or the formation of a tight ion pair has been reported to affect the antimicrobial activities with the following order chloride > tetrafluoroborate > perchlorate > hexafluorophosphate.[29]

2.2. Biological study

2.2.1. In vitro antibacterial activity

In our previous study,[19] we established that dithiocarbamic flavanones of type **4** do not display any antibacterial activity. For this reason, we decided to test only tricyclic flavonoids of type **5** and to investigate the influence of the halogen substituents on their antibacterial properties. Furthermore, we also noticed a correlation between antibacterial activity and the nature of the substituents of tricyclic flavonoids. Surprisingly, among the investigated flavonoids, the compound with $R^2 = OCH_3$ displayed a lower antibacterial



2 3 4 5

		a	b	c*	d	e	f	g	h	i	j	k	l	m	n	o
2	R^1	H	F	Cl	-	-	-	-	-	-	-	-	-	-	-	-
3	R^2	H	F	Cl	Br	I	-	-	-	-	-	-	-	-	-	-
4, 5	R^1	H	H	H	H	H	F	F	F	F	F	Cl	Cl	Cl	Cl	Cl
	R^2	H	F	Cl	Br	I	H	F	Cl	Br	I	H	F	Cl	Br	I

* Compounds **4c and 5c** were previously reported.[19]

Scheme 1. Reagents and conditions: (i) EtOH, reflux 2 h; (ii) $AcOH/H_2SO_4$ = 1:3 (v/v), 80 °C, 30 min; (iii) $NaBF_4$ (aq).