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Synthesis and nematocide activity of *S*-glycopyranosyl-6,7-diarylthiolumazines

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Dedicated to the memory of Dr. Manfred Stud

Abstract—6,7-Diaryl derivatives of mono and di-*S*-glycopyranosylthiolumazine derivatives **5**–**8** were prepared to test their nematocide activity. In vitro tests against *Caenorhabditis elegans* were performed and it was found that monosubstituted derivatives **5**–**7** showed higher activity than the corresponding unsubstituted 2-thiolumazines **1**–**3**, whilst 2-*S*,4-*S*-di-glycopyranosylpteridine derivative **8** was inactive in contrast to unsubstituted derivative **4**. In order to check whether the lack of activity of **8** was due to the two bulky substituents of the pteridine nucleus, 2-*S*,4-*S*-dimethyl derivative **9** was synthesized and assayed showing also lack of activity. A theoretical study on the stability of the different possible tautomers of compound **4** was carried out in an attempt to explain some, in appearance, anomalous ^{13}C NMR data of this compound.

1. Introduction

It is estimated that about 1.3–2.0 billion people in the world suffer from helminth infections. Although direct mortality is low, nematode parasites cause serious morbidity mainly in the 'Third World'. The infections in humans and livestock are controlled by anthelmintic drugs, but the chemotherapy of many helminth infections is complicated by the widespread development of drug resistance to most of the broad-spectrum drugs in many parasite species of livestock animals.[1,2] Furthermore, there is an increased awareness of the potential problem of anthelmintic resistance in the treatment and control of human helminths.[3] Despite the availability of highly effective, broad-spectrum agents, there is always an urgent need for safer, more convenient and more environmentally friendly products that will overcome the ever present threat of resistance development.[4] A prioritized list of research objectives in veterinary parasitology includes the need of new drugs.[5] In spite of this recommendation, not many articles on this topic have appeared recently.

In previous papers, we have described the synthesis and nematocide activity of several 6,7-diarylpteridines bearing different substituents, none of them being a glycosidic moiety.[6–8] Many of these compounds showed interesting nematocide properties, and structure–activity relationship studies, using the Comparative Molecular Field Analysis (CoMFA)[9] and neural networks technique,[7] were carried out. It was observed that *N*-methylation decreased the nematocide activity whilst *S*-methylation increased it.[6,7] In order to investigate the effect of a carbohydrate substituent on the nematocide

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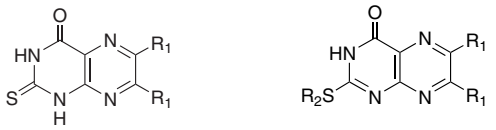


1, R_1 = phenyl
2, R_1 = 2-thienyl
3, R_1 = *p*-methoxyphenyl

5 R_1 = phenyl,
6 R_1 = 2-thienyl
7 R_1 = *p*-methoxyphenyl
5, 6, 7, R_2 = 6-(1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranosyl)

Scheme 1. Structure of pteridine derivatives **1**–**3** and **5**–**7** previously synthesized.

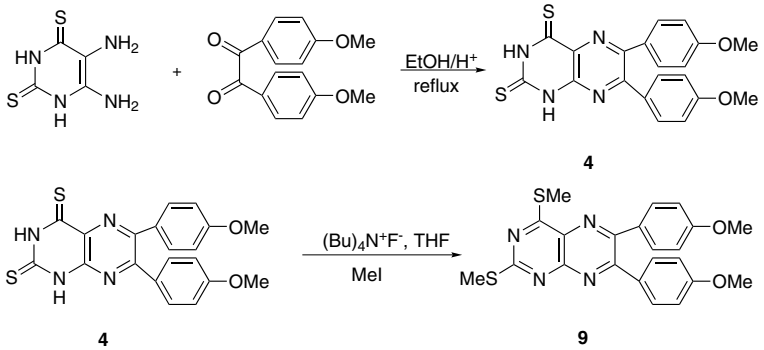
activity of pteridines, monosubstituted 2-*S*-(6-deoxy-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranos-6-yl) derivatives (**5**–**7**) of 6,7-diaryl-2-thiolumazines (**1**–**3**) (Scheme 1) had previously been synthesized.[10] To check the effect of the introduction of a second glycosidic substituent on the nematocide activity, the synthesis of the unknown 6,7-bis(*p*-methoxyphenyl)-2,4-pteridinedithione **4** and its corresponding 2-*S*,4-*S*-bis(6-deoxy-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranos-6-yl) derivative **8** has now been performed. *S*,*S*′-Dimethyl substituted derivative **9** has also been synthesized in order to check if the potential nematocide property of **8** could be mediated by bulky substituents such as carbohydrate moieties. In this work, the nematocide activity of pteridines bearing carbohydrate moieties **5**–**8**, as well as, pteridines **4** and **9** has been evaluated using *Caenorhabditis elegans* as model. *C. elegans* has become a popular model system for searching nematocide activity, since it is easy to maintain and has a very fast life-cycle.[11]

2. Results and discussion

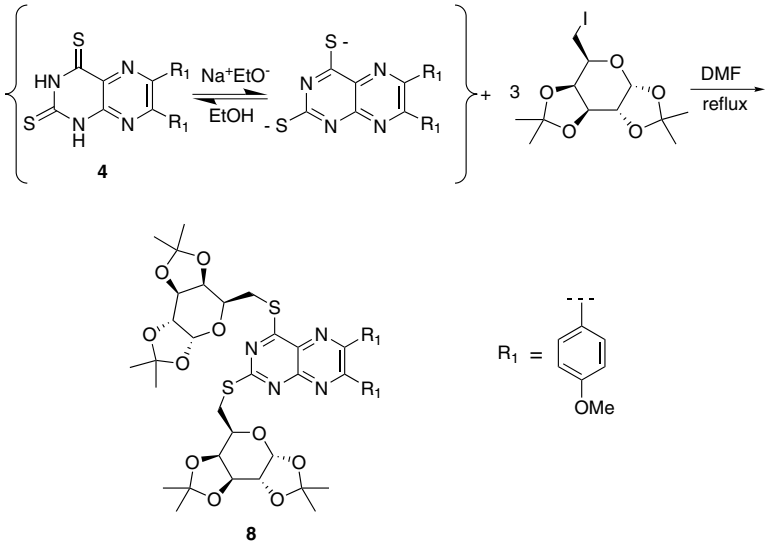
2.1. Chemistry

6,7-Diaryl-2(1*H*)-thioxo-4(3*H*)-pteridinones **1** and **2**[6] and 6,7-bis(*p*-methoxyphenyl)-2(1*H*)-thioxo-4(3*H*)-pteridinone **3**[7] had previously been synthesized by us following two different methods.[12,13] 6,7-Bis-(*p*-methoxyphenyl)-2,4(1*H*,3*H*)-pteridinedithione **4** has now been synthesized, in moderate yield (45%), by condensation between 4,5-diamino-2,6-dimercaptopyrimidine and *p*-dimethoxybenzil following the usual method to obtain pteridines.[12] *S*-Methylation of **4** using tetrabutylammonium fluoride (TBAF), as base, and an excess of methyl iodide, as alkylating agent, afforded dimethyl derivative **9** (see Scheme 2). Attempts to obtain the corresponding *S*-monomethyl derivatives were unsuccessful due to the similar reactivity against alkylating agents of both thioxo groups. So, when equimolar amounts of alkylating agent and pteridine **4** were used, only a complex and inseparable mixture, probably of 2-*S*- and 4-*S*-monomethylated derivatives, *S*,*S*′-dimelthylated derivative and starting material was obtained. An excess of alkylating agent afforded *S*,*S*′-dimethyl derivative.

A previous report on the synthesis of *S*-, *N*- and *O*-glycosides derived of compound **1** had appeared in the literature.[14] 6,7-Diaryl-2-[(6′-deoxy-1′,2′:3′,4′-di-*O*-isopropylidene-α-D-galactopyranos-6′-yl)thio]pteridin-4-one derivatives **5**–**7** were previously prepared by us from 6,7-diaryl-2-thiolumazines **1**–**3**, respectively.[10] In agreement with previous results[15] in the synthesis of 2-[(6′-deoxy-1′,2′:3′,4′-di-*O*-isopropylidene-α-D-galactopyran-6′-yl)thio]-4-hydroxypyrimidine from 4-hydroxy-2-mercaptopyrimidine, these kind of heterocycles can generate an *S*-anion by aromatization of the pyrimidine ring, and acting as a nucleophile replacing a good leaving group. Thus, *S*-glycosylation of **1**–**3** using 6-*O*-tosyl-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose as glycosylating agent and sodium ethoxide as strong base afforded derivatives **5**–**7**. To obtain the *S*,*S*′-disubstituted derivative **8**, from compound **4**, identical reaction conditions were attempted. Results, under these conditions, were unsuccessful, since, only decomposition of starting base was observed. Glycosylation reaction of **4** was finally achieved by using an excess of 6-iodo-6-deoxy-1,2:3,4-di-*O*-isopropyliden-α-D-galactopyranose instead the corresponding tosyl derivative (see Scheme 3). *S*,*S*′-Diglycopyranosyl derivative **8** was obtained in similar yield as compounds **5**–**7**. The ^{1}H NMR spectrum of derivative **8** is very simple for a disubstituted derivative. However, ^{13}C NMR data (Table 2) of C-2 and C-4 are in agreement with a double substitution and are comparable to those of dimethyl derivative **9**. On the other hand, analysis of compound **8** using FAB-MS shows a peak (892) corresponding to the molecular weight.



Scheme 2. Synthetic pathway of compounds **4** and **9**.



Scheme 3. Synthetic pathway of compound **8**.

Table 1. ^{13}C NMR chemical shifts[a] of pteridine derivatives **1**–**9**

Compound	C-2	C-4	C-4a	C-6	C-7	C-8a	Carbon of substituents other than pteridine nucleus
1[b,6]	179.2	162.6	121.0	146.0	157.3	148.3	137.8, 137.6, 130.2, 130.0, 129.1, 128.3
2[b,6]	175.7	157.9	126.4	141.5	149.0	146.6	139.5, 139.2, 132.5, 130.7, 129.3, 128.9, 127.7
3[b,7]	175.8	159.2	126.6	147.0	156.1	149.6	160.1, 161.1, 132.0, 131.4, 130.7, 129.8, 114.5, 114.3, 55.8
4[b]	172.8	187.3	129.6	142.8	156.0	149.9	161.1, 160.1, 131.7, 131.3, 130.8, 130.6, 114.2, 55.6
5[c,10]	161.1*	160.5*	128.9	151.9	158.3	152.8	137.6, 129.9, 129.8, 129.6, 129.3, 128.1, 109.6, 108.9, 96.4, 71.8, 70.8, 70.5, 66.6, 31.7, 26.0–24.3
6[c,10]	161.5*	159.8*	129.2	143.3	152.2	150.1	140.3, 139.9, 131.8, 130.4, 129.7, 128.3, 127.9, 109.7, 109.1, 95.6, 71.8, 70.9, 70.6, 66.8, 31.8, 26.1–24.4
7[c,10]	160.8*	160.2*	128.7	151.3	159.9	152.5	159.9, 131.5, 131.2, 131.3, 130.1, 113.6, 109.5, 108.8, 96.3, 71.7, 70.8, 70.5, 66.5, 55.1, 31.5, 25.9–24.3
8[c]	169.1*	173.3*	128.3	149.3	158.7	150.4	160.1, 159.6, 130.7, 130.4, 112.8, 112.7, 109.5, 108.8, 95.6, 70.9, 70.6, 69.9, 69.5, 65.5, 65.1, 54.3, 30.4, 28.6, 25.9, 25.0, 24.8
9[c]	172.0*	175.1*	129.8	150.6	160.1	151.8	161.5, 161.0, 132.1, 131.8, 130.8, 114.2, 114.1, 55.7, 15.3, 13.0
9[b]	170.3*	174.3*	128.7	149.7	159.4	151.1	160.6, 160.1, 131.5, 131.1, 130.1, 113.8, 113.7, 55.3, 14.3, 12.2

[a] δ in ppm.
[b] DMSO-d_6 as solvent.
[c] $CDCl_3$ as solvent.
* Assignments could be interchanged.

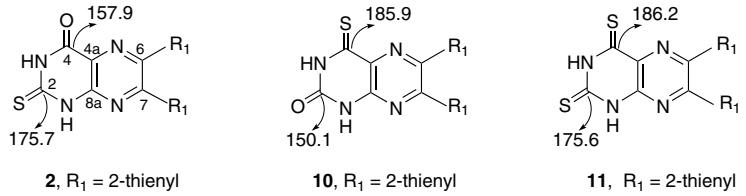
Table 2. Calculated energies (E, hartrees), relative energies (ΔE, kcal mol^{-1}) in gas phase and in the COSMO model, and dipole moments (μ, Debye) of tautomers **12A**–**12B**

	Method	**12A**			**12B**		
		E	ΔE	μ	E	ΔE	μ
Vacuum	B3LYP/6-31G*	−1246.450073	0.00	4.50	−1246.42598	15.12	2.77
Vacuum	DMol	−1240.132822	0.00	3.58	−1240.108598	15.20	2.28
$CHCl_3$	COSMO/DMol	−1240.146679	0.00	—	−1240.124451	13.95	—
H_2O	COSMO/DMol	−1240.153048	0.00	—	−1240.131829	13.31	—

In this case preparation of *S*-monosubstituted derivatives was also unsuccessful and only inseparable complex mixtures were obtained when glycosylating agent and starting base were used in equimolar amount. Galactopyranose iodide was obtained by treatment of the protected galactose with I_2/Ph_3P.[16]

2.2. ^{13}C NMR study

In order to compare ^{13}C NMR data, ^{13}C chemical shifts of new compounds **4**, **8** and **9** together with those previously reported for compounds **1**–**3**, **5**–**7** are shown in Table 1. The NMR study of compound **4** was carried



Scheme 4. C-2 and C-4 Chemical shifts of oxo and thioxo pteridines **2**, **10** and **11**.

out in DMSO-d_6 solution because this compound is very insoluble in $CDCl_3$. ^{13}C chemical shifts of compound **4** were assigned by comparing the chemical shifts of the corresponding 2-thioxo,4-oxo-derivatives **1**–**3**. On the other hand, the correct assignment for C-2 and C-4 was checked by comparison with the corresponding chemical shifts of related derivatives **2**, **10** and **11** previously reported[6] (see Scheme 4).

As can be seen in Table 1 and Scheme 4, the chemical shift of C-4 is shifted downfield about 30 ppm by changing an oxo group (compounds **2** and **3**) by a thioxo group (compounds **4** and **11** about 187 ppm), whilst C-2 chemical shift's changes from 150 ppm to about 175 ppm when an oxo is transformed into a thioxo group (Scheme 4).

NMR spectra of soluble compounds in $CDCl_3$ (**5**–**9**) were recorded in this solvent. In order to compare ^{13}C chemical shifts of compounds **4** and **9**, the ^{13}C NMR spectrum of **9** was also recorded in DMSO-d_6 showing both spectra only slight chemical shift differences. Comparing ^{13}C NMR spectra of unsubstituted derivative **4** and *S*,*S*′-dimethyl derivative **9** (recorded both in DMSO-d_6), it is interesting to point out whilst the C-4 signal appears shifted 13 ppm to upfield in **9**, as expected for the change of an N–C=S to N=C–S-group (compare C-2 chemical shifts of **1**–**3** to those **5**–**7**), the signal corresponding to C-2 only changes slightly (172.8–170.3 ppm). One possible explanation, of this anomalous fact, could be the existence of compound **4** mainly in the form of tautomer **B**, in DMSO solution (see Scheme 5).

2.3. Theoretical study on tautomerism

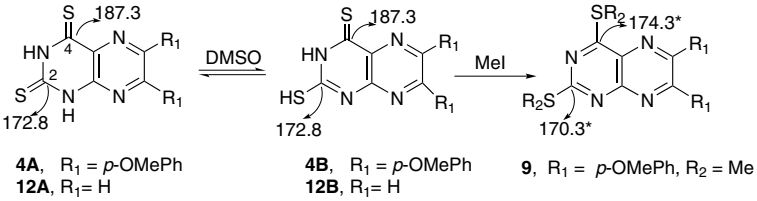
In view of these results, we decided to study the tautomerism in a more simple pteridine, **12**, in gas phase and in solution by theoretical molecular orbital calculations. In principle, 2,4-(1*H*,3*H*)-pteridinedithione **12** can exist as six possible tautomers, however, only tautomers **A** and **B** were calculated, form **A** as the probably more stable tautomer and form **B** as the tautomer compatible with ^{13}C NMR data. Thus, the study in gas phase was performed using two density functional methods, a B3LYP/6-31G*[17,18] and a local density functional method (LDF), which include electron correlation[19–21] with DMol program.[22] Results obtained indicate that in the gas phase tautomer **A** is more stable (15 kcal mol^{-1}) than tautomer **B** (Table 2) and therefore, the form present in the gas phase.

To study the relative stability of the two tautomers in solution, the solvation effect has been considered via the COSMO method[23,24] implemented in DMol. The Conductor-like Screening Model (COSMO) is a continuum solvation model, where the solute molecule forms a cavity within the dielectric continuum of permittivity ε that represents the solvent.

The results obtained with COSMO model in water or in chloroform, collected in Table 2, indicate that tautomer **A** is more stable than tautomer **B**, 13 or 14 kcal mol^{-1}, in water or chloroform, respectively. The theoretical results in solution from COSMO model provide a stabilization of tautomer **B**, about 2 kcal mol^{-1} in relation to gas phase; however, the large stability difference of tautomer **A** in relation to **B** indicates that only form **A** should be present in chloroform or water. On the other hand, the value of dipole moments in vacuum indicates that tautomer **A** would be more favoured than tautomer **B** in polar solvents.

2.4. Biological study

The in vitro nematocide activity of compounds **4**–**9** was tested against larvae of *C. elegans*. The use of *C. elegans*, a free-living nematode, for the in vitro test is a safe and fast method to evaluate the nematocide activity of compounds.[25] In order to compare with the data of pteridines previously evaluated (**1**–**3**),[6,7] the same method and conditions were used. All compounds were tested at concentration of 100 μg/mL, subsequently, com-



Scheme 5. Tautomers **A** and **B** of **4** and **12** and C-2, C-4 data of **4** and **9** in DMSO solution.

Table 3. Nematocide in vitro activity of compounds **1**–**9** against *C. elegans*

Compd	IC_{50} (μg/mL)	IC_{50} (mM)
1[6]	50	1.72×10^{-1}
2[6]	>100	$>2.91\times10^{-1}$
3[7]	25	6.38×10^{-2}
4	25	6.13×10^{-2}
5	24	4.18×10^{-2}
6	10	1.99×10^{-2}
7	15	2.37×10^{-2}
8	>100	$>1.12\times10^{-1}$
9	>100	$>2.29\times10^{-1}$
Mb	1–0.5	2.67×10^{-3}

pounds showing activity higher than 50% were tested at lower concentration (50, 25, 10, 2 and 0.5 μg/mL) to determine the inhibition concentration required to obtain up to 50% (IC_{50}) in the reduction of population growth of nematode.

Compounds **1**, **3**–**7** showed more than 90% of reduction in the nematode population growth at the higher concentration tested (100 μg/mL); thus, these compounds present remarkable nematocide activity at this concentration. However, compound **8** exhibited only 40% of reduction and compounds **2** and **9** no reduction at this concentration. The nematocide activity of compounds **1**, **3**–**7** were assayed at lower concentrations and their IC_{50} in μg/mL and mM concentrations are gathered in Table 3 together with those corresponding to mebendazole (**Mb**) used as standard. The most active pteridines, now tested, are the *S*-mono-glycosyl derivatives **6** and **7**. *S*-Mono-glycosyl derivatives present higher nematocide activity than the corresponding unsubstituted derivatives (comparing IC_{50} of **1** and **5**, **2** and **6** and **3** and **7** in Table 3) mainly in the case of 6,7-dithienyl derivative **2**. Substitution of 4-oxo by 4-thioxo group did not modify the activity (comparing **3** and **4**). When a second substituent, *S*-glycosyl or *S*-methyl, is introduced in the 4 position of pteridine the activity is completely lost (comparing **7** to **8** and **9**).

3. Conclusions

Results of theoretical calculations showed that the most stable tautomer in the gas phase is form **A**. Although, results in solution indicated a slight stabilization of tautomer **B**, the most stable tautomer is still tautomer **A**.

Except for derivatives **2**, **8** and **9**, the pteridines studied exhibited nematocide activity, although none of them reached the activity of mebendazole used as standard. Substitution at 2-*S*-position enhances nematocide activity, whilst disubstitution at 2-*S*,4-*S*-positions eliminates this activity. We have not found the reason to explain the lack of activity of the *S*,*S*′-unsubstituted-6,7-dithienyl derivative **2** in contrast to the activity of compounds **1**, **3** and **6**.

The inactivity of compounds **8** and **9** could be due to the absence of acid protons in the molecule; however, 1-*N*-methyl, 2-*S*-methyl-substituded derivatives of some 6,7-diarylthiolumazines, without acid protons, showed nematocide activity.[6,7] On the other hand, the inactivity of *S*,*S*′-diglycosyl derivative **8** could not be due to its excessive bulkiness because the *S*,*S*′-dimethyl derivative **9** is also inactive, unless, one admits that the steric hindrance of *S*-methyl group at C-4 position should be enough to diminish nematocide activity.

4. Experimental section

4.1. Chemistry

Melting points were determined with a Kofler hot-stage apparatus and are uncorrected. Flash column chromatography was performed on Merck silica gel (230–400 mesh). Analytical TLC was performed on aluminium sheets coated with 0.2 mm layer of silica gel 60 F254. ^1H and ^{13}C NMR were recorded on a Varian Gemini-200 at 200 and 50 MHz, respectively, for compounds **4** and **9**, and on a Bruker-500 at 500 and 175 MHz, respectively, for compound **8**, using tetramethylsilane (TMS) as internal standard. MS of compound **8** was registered by fast atomic bombardment (FAB) technique on a ZAB-SEQ4F spectrometer, using glycerol matrix.

4.2. 6,7-Bis(*p*-methoxyphenyl)-2,4(1*H*,3*H*)-pteridinedithione 4

To a solution of 4,5-diamino-2,6-dimercaptopyrimidine (0.87 g, 5 mmol) in ethanol (50 mL), *p*-dimethoxybenzyl (1.35 g, 5 mmol) and some drops of hydrochloric acid were added under reflux. The solutions was kept on refluxing for 6 h. Then, the reaction mixture was concentrated and the solid obtained suspended in hexane (100 mL). The suspension was heated under reflux over 30 min in order to remove the excess of α-dicarbonylic compound. The remaining solid was filtered and purified by column chromatography using CH_2Cl_2/CH_3OH (30/1 v/v) as eluent. Compound **4** (0.94 g) was obtained in 45% yield: mp = 283–285 °C (CH_3OH/H_2O). ^1H NMR (DMSO-d_6) δ (ppm): 13.84 (br s, ^1H), 13.75 (br s, 1H), 7.44 (d, $^3J = 8.8$ Hz, 2H), 7.36 (d, $^3J = 8.8$ Hz, 2H), 6.95 (d, $^3J = 8.8$ Hz, 2H), 6.93 (d, $^3J = 8.8$ Hz, 2H) 3.78 (s, 6H). Anal. Calcd for $C_{20}H_{16}N_4O_2S_2$: C, 58.82; H, 3.92; N, 13.72; S, 15.68. Found: C, 59.10; H, 4.07; N, 14.02; S, 16.00.

4.3. 2,4-Bis[(6′-deoxy-1′,2′:3′,4′-di-*O*-isopropylidene-α-D-galactopyranos-6′-yl)thio]-6,7-bis(*p*-methoxypheyl)pteridine 8

6,7-Di-(*p*-methoxyphenyl)-2,4-pteridinedithione (230 mg, 0.56 mmol) were placed into a 25 mL round flask, and 1.7 mL of a solution of sodium ethoxide were added (the later solution was prepared by dissolution of 190 mg on metallic sodium in 20 mL of ethanol). The suspension was stirred in a warm bath (45 °C), then evaporated to dryness and to this solid, 557 mg

(1.5 mmol) of 6-deoxy-6-iodo-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (synthesized as it was described in literature[16]) and 10 mL of DMF, were added. The mixture was heated to reflux until no changes were detected by TLC (approximately 4 h). The mixture was concentrated under reduced pressure, purified by flash chromatography using cyclohexane/acetone (9:1 v/v) as eluent and the title compound was obtained as a syrup (208 mg, 40.8% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 7.58 (d, $^3J = 8.7$ Hz, 2H), 7.54 (d, $^3J = 8.7$ Hz, 2H), 6.85 (d, $^3J = 8.7$ Hz, 4H), 5.57 (d, $^3J = 4.7$ Hz, 2H), 4.31 (dd, $^3J = 4.7$ Hz, $^3J = 2.6$ Hz, 2H), 4.65 (dd, $^3J = 7.5$ Hz, $^3J = 2.6$ Hz, 1H), 4.63 (dd, $^3J = 7.5$ Hz, $^3J = 2.6$ Hz, 1H), 4.45 (m, 2H), 4.23 (m, 1H), 4.16 (m, 1H), 3.84 (s, 6H), 3.63 (dd, $^2J = 13.9$ Hz, $^3J = 6.2$ Hz), 3.52 (dd, $^2J = 13.9$ Hz, $^3J = 7.7$ Hz), 1.48–1.29 (12H). MS *m/z*: 892. (M). Anal. Calcd for $C_{44}H_{52}N_4O_{12}S_2$: C, 59.19; H, 5.38; N, 6.28; S, 7.77. Found: C, 59.33; H, 5.69; N, 6.15; S, 7.60.

4.4. 6,7-Bis(*p*-methoxyphenyl)-2,4-bis(methylthio)-pteridine 9

To a solution of 2,4-pteridinedithione **4** (0.82 g, 2 mmol), in tetrahydrofurane (14 mL) containing tetrabutylammonium fluoride (THF/TBAF 1/5), methyl iodide (1.15 g, 8 mmol) was added dropwise. The reaction mixture was stirred at 40 °C for 3 h. The resulting mixture was dried under vacuum and the residue purified by column chromatography using CH_2Cl_2/CH_3OH (30/1 v/v) as eluent. Dimethyl derivative **9** (0.26 g, 30% yield) was obtained as a yellow solid. Mp 203–204 °C (DMF/H_2O). ^{1}H NMR ($CDCl_3$) δ (ppm): 7.58 (d, $^3J = 8.8$ Hz, 2H), 7.54 (d, $^3J = 8.8$ Hz, 2H), 6.87 (d, $^3J = 8.8$ Hz, 2H), 6.84 (d, $^3J = 8.8$ Hz, 2H), 3.83 (s, 6H), 2.77 (s, 3H), 2.65 (s, 3H). ^{13}C NMR ($CDCl_3$) δ (ppm): 175.1, 172.0, 161.5, 161.0, 160.1, 151.8, 150.6, 132.1, 131.8, 130.8, 129.8, 114.2, 114.1, 55.7, 15.3, 13.0. Anal. Calcd for $C_{22}H_{20}N_4O_2S_2$: C, 60.55; H, 4.59; N, 12.84; S, 14.68. Found: C, 60.14; H, 4.65; N, 12.55; S, 14.25.

4.5. Theoretical calculations

The studied compounds were built with standard bond lengths and angles by using the molecular modelling package Insight.[27] All the structures were fully optimized without any symmetry restrictions in the gas phase and the simulated solvent environment.

The DFT method was performed using the Gaussian 98 package.[28] The standard 6-31G* basis set with the density functional calculation (DFT) B3LYP functional[17,18] were used.

The LDF calculations were carried out using the DMol program[22] distributed by Accelrys Inc. A double zeta numerical basis set with polarization functions in all the atoms and the Perdew–Wang local correlation[29] were used. The geometry of the molecules was optimized until the gradient was smaller than 0.001 au. The solvation effect has been studied using the COSMO method[23,24] implemented in DMol.

4.6. Nematocide activity test

The compounds were tested for nematocide activity against the free-living nematode *C. elegans* following the method of Simpkin and Coles[25] with slight modifications.[26] Tests were carried out in 24-well plates (Costar®) and four wells were used for each experimental group. To each well, 1 mL of culture medium was added followed by 5 μL of the appropriate compound solution. The products were dissolved in dimethylsulfoxide and the same concentration of solvent (0.5%) was incorporated to control wells. Finally, 0.5 mL of culture medium containing 10–15 *C. elegans* larvae (L2 or L3 obtained of synchronous cultures) was added to each well.

The effect of compounds on the development and reproductive capacity of *C. elegans* was determined by comparing the populations levels attained in the control and test wells after an incubation period of 7 days at 20 ± 1 °C. Inhibition concentration (IC_{50}) was calculated from the reduction percentage of nematode population growth at different doses of test compounds in relation with controls.

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