Syntheses for 2-Amino and 2-Mercapto-2*H*-1,4-benzoxazin-3(4*H*)-one and 2*H*-1,4-Benzothiazin-3(4*H*)-one as Aza and Thio Analogues of the Natural Product *Blepharigenin*

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2-Amino-2*H*-1,4-benzoxazin-3(4*H*)-one **3**, 2-amino-2*H*-1,4-benzothiazin-3(4*H*)-one **4**, 2-mercapto-2*H*-1,4-benzoxazin-3(4*H*)-one **7**, and 2-mercapto-2*H*-1,4-benzothiazin-3(4*H*)-one **8** representing aza and thio analogues of the natural product's aglucone *Blepharigenin* (2-hydroxy-2*H*-1,4-benzoxazin-3(4*H*)-one) from *Gramineae* and *Acanthaceae* species have been synthesized for the first time from their 2-bromo precursors **1** and **2**. Attempts to similarly prepare the 4-hydroxy derivatives of **7** and **8**, which would represent new thio analogues of the naturally occurring cyclic hydroxamic acid, 2,4-dihydroxy-2*H*-1,4-benzoxazin-3(4*H*)-one, have failed.

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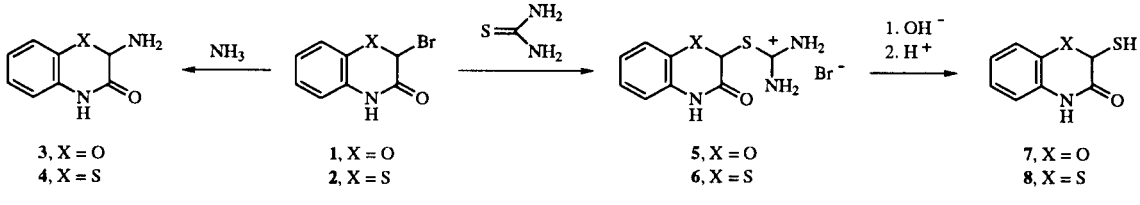
Hemiacetals with the 2*H*-1,4-benzoxazin-3(4*H*)-one skeleton have been found to occur in the form of 2-β-D-glucosides as allelo chemicals in different plant species. Thus, both the lactam (2*R*)-2-β-D-glucopyranosyloxy-4-hydroxy-2*H*-1,4-benzoxazin-3(4*H*)-one (*Blepharin,*) from *Blepharis edulis* (*Acanthaceae*) [1], *Secale cereale L.* (*Gramineae*) [2] and *Zea mays L.* (*Gramineae*) [3] and the cyclic hydroxamic acid (2*R*)-2-β-D-glucopyranosyloxy-2,4-dihydroxy-2*H*-1,4-benzoxazin-3(4*H*)-one from *e. g. Secale cereale L.* [4] have been isolated. Their aglucones 2-hydroxy-2*H*-1,4-benzoxazin-3(4*H*)-one (*Blepharigenin*) and 2,4-dihydroxy-2*H*-1,4-benzoxazin-3(4*H*)-one, respectively, are liberated by β-glucosidase on an injury of the plant by a pest attack. Benzoxazinoid hemiacetals of these leads are under current investigation for their role in the plant resistance against microbial diseases and insects [5,6] and in allelopathic interactions [7]. Recently, we have described syntheses leading both to 2-hydroxy-7-methoxy-2*H*-1,4-benzoxazin-3(4*H*)-one and 2,4-dihydro-7-methoxy-2*H*-1,4-benzoxazin-3(4*H*)-one, the 7-methoxy derivatives of *Blepharigenin* and 2,4-dihydro-2*H*-1,4-benzoxazin-3(4*H*)-one [8,9], respectively, and to 2,4,7-trihydroxy-2*H*-1,4-benzoxazin-3(4*H*)-one, 2,4-dihydro-2*H*-1,4-benzoxazin-3(4*H*)-one [10], as well as syntheses which gave rise to 2-hydroxy-2*H*-1,4-benzothiazin-3(4*H*)-one and 2,4-dihydroxy-2*H*-1,4-benzothiazin-3(4*H*)-one [11], representing the first thio analogues of *Blepharigenin* and 2,4-dihydro-2*H*-1,4-benzoxazin-3(4*H*)-one. Furthermore, we have reported on conditions for the diastereoselective glucosidation of two benzoxazinoid hemiacetals to form acetal glucosides [12].

Based on this work, we are interested in the syntheses of further compounds which are heteroanalogues of the hemiacetals with the 1,4-benzoxazinone and 1,4-benzothiazinone skeleton, respectively, and can be regarded as possible precursors for aza and thio analogous acetal glucosides.

Results and Discussion.

Hitherto, both the 2-amino and 2-mercapto derivatives of the 2*H*-1,4-benzoxazin-3(4*H*)-one and 2*H*-1,4-benzothiazin-3(4*H*)-one skeletons have not yet been described.

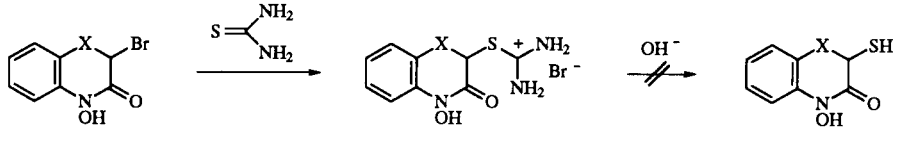


3, X = O
4, X = S

1, X = O
2, X = S

5, X = O
6, X = S

7, X = O
8, X = S



9, X = O
10, X = S

11, X = O
12, X = S

Furthermore, to the best of our knowledge, in general cyclic OR/NH_2-acetals and SR/NH_2-acetals, *i.e.* representatives of 2-amino-1-oxacyclanes and 2-amino-1-thiacyclanes, respectively, have not been cited at all [13]. We decided to prepare them from the starting 2-bromo-2*H*-1,4-benzoxazin-3(4*H*)-one **1** and 2-bromo-2*H*-1,4-benzothiazin-3(4*H*)-one **2**.

Therefore, **1** and **2**, respectively, were treated with a solution of gaseous ammonia in tetrahydrofuran to afford 2-amino-2*H*-1,4-benzoxazin-3(4*H*)-one **3** and 2-amino-2*H*-1,4-benzothiazin-3(4*H*)-one **4**, respectively, in good yield. This method proved to be superior to the treatment of the bromo precursors with concentrated aqueous ammonia, which always resulted in a mixture of the desired 2-amino compound accompanied by the 2-hydroxy derivative as by-product.

The 2-mercapto group has been introduced following a two step method [14] which includes formation and alkaline cleavage of the corresponding isothiouronium bromides. Hence, both *S*-[2*H*-1,4-benzoxazin-3(4*H*)-on-2-yl]isothiouronium bromide (**5**) and *S*-[2*H*-1,4-benzothiazin-3(4*H*)-on-2-yl]isothiouronium bromide (**6**), have been obtained in good yield by reaction of their bromo precursors **1** and **2**, respectively, with thiourea in acetone. Similarly, nucleophilic substitution by thiourea of the 2-bromo compounds **9** and **10**, which are the 4-hydroxy derivatives of compounds **1** and **2**, gave rise to *S*-[4-hydroxy-2*H*-1,4-benzoxazin-3(4*H*)-on-2-yl]isothiouronium bromide **11** and *S*-[4-hydroxy-2*H*-1,4-benzothiazin-3(4*H*)-on-2-yl]isothiouronium bromide **12**, respectively. The four isothiouronium bromides obtained, showed different behavior when subjected to aqueous sodium hydroxide for cleavage of the isothiouronium unit, depending on whether there is a lactam or a cyclic hydroxamic acid function present in the heterocyclic moiety of the molecule. Whereas the lactam salts **5** and **6** reacted to form the target 2-mercapto-2*H*-1,4-benzoxazin-3(4*H*)-one **7** and 2-mercapto-2*H*-1,4-benzothiazin-3(4*H*)-one **8**, respectively, their 4-hydroxy analogues **11** and **12** underwent a decomposition that could not be prevented by changing the experimental conditions. Hence, the hitherto unknown 2-SH and 1-S/2-SH analogues of 2,4-dihydro-2*H*-1,4-benzoxazin-3(4*H*)-one intended to gain by these reactions could not be obtained. Therefore, attempts to protect the cyclic hydroxamic acid unit before developing the 2-mercapto function are in progress. The 2-mercapto lactam **7** is a new representative of the rare cyclic monothio hemiacetals with exocyclic sulfur atom, hitherto *e.g.* 2-tetrahydropyranthiol has been described [15]. Compound **7** is the structural isomer of 2-hydroxy-2*H*-1,4-benzothiazin-3(4*H*)-one, the cyclic monothio hemiacetal with endocyclic sulfur atom, we have recently described [11]. Furthermore, the cyclic dithio hemiacetal **8** is one of the rare examples for 2-mercapto-1-thiacyclanes [14]. Finally, a noteworthy observation has been made on the sulfur containing heterocycles **4** and **8**. It is a feature of their ^1H nmr spectra that, in contrast to **3** and **7**, no coupling is to be observed between the proton(s) of the 2-substituent and 2-H. This behavior is not fully understood yet, but obviously caused by the sulfur atom in position 1.

In summary, the lactams **3**, **4**, **7**, and **8** represent aza and thio analogues of *Blepharigenin* and are interesting subjects for investigations of their bioactivity and for the synthesis of aza and thio analogous acetal glucosides of *Blepharin*.

EXPERIMENTAL

Melting points were determined on a Boetius micro hot-stage apparatus and are corrected. Elemental analyses were performed on a Heraeus CHN-O-Rapid analyzer. The nmr spectra were recorded on a Varian Gemini 200 spectrometer at 199.975 MHz for ^1H and at 50.289 MHz for ^{13}C with hexamethyldisiloxane as the internal standard. The ir spectra were obtained on a Carl Zeiss Jena Specord M 80 spectrometer in potassium bromide. Mass spectra were recorded on a Finnigan MAT 212 spectrometer (70 eV EI ionisation, source temperature 200°). The following educts were prepared according to the literature: 2-bromo-2*H*-1,4-benzoxazin-3(4*H*)-one (**1**) [16], 2-bromo-2*H*-1,4-benzothiazin-3(4*H*)-one (**2**) [11], 2-bromo-4-hydroxy-2*H*-1,4-benzoxazin-3(4*H*)-one (**9**) [17], and 2-bromo-4-hydroxy-2*H*-1,4-benzothiazin-3(4*H*)-one (**10**) [11].

General Procedure for the Synthesis of the 2-Amino Compounds **3** and **4**.

The appropriate bromo precursor (5 mmoles) was added to a saturated solution of gaseous ammonia in tetrahydrofuran (100 ml) and stirred for 10 minutes until tlc monitoring showed completeness of the reaction. The ammonium bromide which precipitated was filtered off, the filtrate concentrated *in vacuo* to a volume of 5 ml and diluted with ethyl acetate (50 ml). The resulting solution was washed with water (3 x 20 ml), dried (magnesium sulfate) and evaporated to dryness to give an off-white solid which was recrystallized from chloroform.

2-Amino-2*H*-1,4-benzoxazin-3(4*H*)-one (**3**).

Precursor **1** was reacted to give 740 mg (90%) **3** as colorless crystals, mp 185-187° dec (chloroform); ir: ν 1670, 1600, 1480, 1400 cm^{-1}; ^1H nmr (DMSO-d_6): δ 3.14 (d, 2H, NH_2, J = 8.4 Hz), 5.11 (t, 1H, 2-H, J = 8.4 Hz), 6.88-6.99 (m, 4H, aromatics), 10.54 (s, 1H, NH); ^{13}C nmr (DMSO-d_6): δ 82.6 (C-2), 115.9 (C-8a), 117.7 (C-5), 122.2 (C-8), 123.3 (C-7), 127.8 (C-6), 142.1 (C-4a), 164.3 (C-3); ms: m/z 164 (M^+, 19), 163 (68), 109 (100), 80 (45).

Anal. Calcd. for $C_8H_8N_2O_2$: C, 58.53; H, 4.91; N, 17.06. Found: C, 58.20; H, 5.11; N, 17.35.

2-Amino-2*H*-1,4-benzothiazin-3(4*H*)-one (**4**).

Precursor **2** was reacted to give 760 mg (84%) **4** as pale beige crystals, mp 151-153° dec (chloroform); ir: ν 1675, 1580, 1475, 1390 cm^{-1}; ^1H nmr (DMSO-d_6): δ 3.32 (s, 2H, NH_2), 4.63 (s, 1H, 2-H), 6.95-7.29 (m, 4H, aromatics), 10.51 (s, 1H, NH); ^{13}C nmr

(DMSO-d_6): δ 54.3 (C-2), 117.1 (C-8a), 118.7 (C-5), 123.0 (C-8), 126.7 (C-7), 128.3 (C-6), 136.5 (C-4a), 165.4 (C-3); ms: m/z 180 (M^+, 38), 165 (41), 151 (74), 136 (100), 125 (36).

Anal. Calcd. for $C_8H_8N_2OS$: C, 53.32; H, 4.47; N, 15.54; S, 17.79. Found: C, 53.09; H, 4.68; N, 15.23; S, 17.97.

General Procedure for the Synthesis of the Isothiouronium Bromides **5**, **6**, **11**, and **12**.

To a solution of the corresponding bromo precursor (1.0 mmole) in dry acetone (5 ml) was added a solution of thiourea (84 mg, 1.1 mmoles) in dry acetone (2 ml) at 0° with stirring. After 2 additional hours of stirring the crystals precipitated were filtered off and dried *in vacuo*. Analyses proved the products thus obtained to be pure without recrystallization. Due to their rapid decomposition at mp temperature we abstained from recording ms data under standard conditions.

S-[2*H*-1,4-Benzoxazin-3(4*H*)-on-2-yl]isothiouronium Bromide (**5**).

Precursor **1** was reacted to give 277 mg (91%) **5** as pale yellow powder, mp 176-177° dec; ir: ν 2950, 1720, 1645, 1505 cm^{-1}; ^1H nmr (DMSO-d_6): δ 5.47 (s, 1H, 2H), 6.93-7.11 (m, 4H, aromatics), 9.66 (s, 4H, 2 x NH_2), 10.77 (s, 1H, NH); ^{13}C nmr (DMSO-d_6): δ 90.6 (C-2), 115.8 (C-5), 117.6 (C-8), 122.6 (C-7), 123.2 (C-6), 127.0 (C-4a), 140.9 (C-8a), 162.8 (-$C(NH_2)_2$), 166.9 (C-3).

Anal. Calcd. for $C_9H_{10}BrN_3O_2S$: C, 35.54; H, 3.31; N, 13.82. Found: C, 35.57; H, 3.58; N, 13.48.

S-[2*H*-1,4-Benzothiazin-3(4*H*)-on-2-yl]isothiouronium Bromide (**6**).

Precursor **2** was reacted to give 266 mg (83%) **6** as colorless powder, mp 171-173° dec; ir: ν 1670, 1640, 1580, 1480 cm^{-1}; ^1H nmr (DMSO-d_6): δ 6.04 (s, 1H, 2-H), 7.09-7.48 (m, 4H, aromatics), 9.39 (s, 4H, 2 x NH_2), 11.38 (s, 1H, NH); ^{13}C nmr (DMSO-d_6): δ 44.4 (C-2), 114.6 (C-5), 118.0 (C-8), 124.5 (C-7), 128.6 (C-6), 128.7 (C-4a), 136.1 (C-8a), 160.5 (-$C(NH_2)_2$), 166.5 (C-3).

Anal. Calcd. for $C_9H_{10}BrN_3OS_2$: C, 33.76; H, 3.15; N, 13.12. Found: C, 33.39; H, 3.44; N, 13.07.

S-[4-Hydroxy-2*H*-1,4-benzoxazin-3(4*H*)-on-2-yl]isothiouronium Bromide (**11**).

Precursor **9** was reacted to give 263 mg (82%) **11** as pale beige powder, mp 160-162° dec; ir: ν 2950, 1680, 1645, 1500 cm^{-1}; ^1H nmr (DMSO-d_6): δ 5.69 (s, 1H, 2-H), 7.05-7.27 (m, 4H, aromatics), 8.41 (s, 4H, 2 x NH_2), 9,75 (s, 1H, N-OH); ^{13}C nmr (DMSO-d_6): δ 92.3 (C-2), 113.3 (C-5), 117.4 (C-8), 122.8 (C-7), 124.0 (C-6), 129.1 (C-4a), 140.9 (C-8a), 151.4 (-$C(NH_2)_2$), 157.9 (C-3).

Anal. Calcd. for $C_9H_{10}BrN_3O_3S$: C, 33.76; H, 3.15; N, 13.13. Found: C, 33.94; H, 3.33; N, 13.02.

S-[4-Hydroxy-2*H*-1,4-benzothiazin-3(4*H*)-on-2-yl]isothiouronium Bromide (**12**).

Precursor **10** was reacted to give 300 mg (89%) **12** as pale yellow powder, mp 185-186° dec; ir: ν 1675, 1645, 1610, 1355 cm^{-1}; ^1H nmr (DMSO-d_6): δ 6.27 (s, 1H, 2-H), 7.21-7.55 (m, 4H, aromatics), 9.41 (s, 4H, 2 x NH_2), 11.19 (s, 1H, N-OH); ^{13}C nmr (DMSO-d_6): δ 45.9 (C-2), 115.6 (C-5), 116.2 (C-8), 125.1 (C-7), 128.8 (C-6), 128.9 (C-4a), 138.2 (C-8a), 156.9 (-$C(NH_2)_2$), 166.1 (C-3).

Anal. Calcd. for $C_9H_{10}BrN_3O_2S_2$: C, 32.15; H, 3.00; N, 12.50. Found: C, 32.54; H, 3.16; N, 12.32.

General Procedure for the Synthesis of the 2-Mercapto Compounds **7** and **8**.

To a solution of the appropriate isothiouronium bromide precursor (0.5 mmole) in water (10 ml) was dropwise added a solution of sodium hydroxide (60 mg, 1.5 mmoles) in water (2 ml) under an argon atmosphere at 0° with stirring. After 2 additional hours of stirring the solution was acidified to *p*H 3 with 25% sulfuric acid. The crystals precipitated were filtered off and recrystallized from chloroform.

2-Mercapto-2*H*-1,4-benzoxazin-3(4*H*)-one (**7**).

Isothiouronium bromide **5** was reacted to give 73 mg (80%) **7** as colorless crystals, mp 165-167° (chloroform); ir: ν 2495, 1660, 1600, 1490 cm^{-1}; ^1H nmr (DMSO-d_6): δ 4.19 (d, 1H, SH, J = 5.5 Hz), 6.07 (d, 1H, 2-H, J = 5.5 Hz), 6.95-7.06 (m, 4H, aromatics), 10.90 (s, 1H, NH); ^{13}C nmr (DMSO-d_6): δ 75.4 (C-2), 116.1 (C-8a), 118.2 (C-5), 123.7 (C-8), 123.8 (C-7), 127.6 (C-6), 139.8 (C-4a), 162.8 (C-3); ms: m/z 181 (M^+, 53), 148 (27), 136 (100), 121 (58), 108 (35).

Anal. Calcd. for $C_8H_7NO_2S$: C, 53.03; H, 3.89; N, 7.73; S, 17.69. Found: C, 52.90; H, 3.75; N, 7.62; S, 17.50.

2-Mercapto-2*H*-1,4-benzothiazin-3(4*H*)-one (**8**).

Isothiouronium bromide **6** was reacted to give 65 mg (66%) **8** as pale yellow crystals, mp 194-196° dec (chloroform); ir: ν 1671, 1586, 1481, 1370 cm^{-1}; ^1H nmr (DMSO-d_6): δ 3.82 (s, 1H, SH), 4.99 (s, 1H, 2-H), 7.02-7.39 (m, 4H, aromatics), 10.77 (s, 1H, NH); ^{13}C nmr (DMSO-d_6): δ 39.0 (C-2), 116.0 (C-8a), 117.3 (C-5), 123.7 (C-8), 127.9 (C-7), 128.6 (C-6), 136.9 (C-4a), 163.7 (C-3); ms: m/z 197 (M^+, 28), 164 (63), 136 (100), 109 (24).

Anal. Calcd. for $C_8H_7NOS_2$: C, 48.71; H, 3.58; N, 7.10; S, 32.50. Found: C, 48.41; H, 3.88; N, 7.34; S, 32.20.

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