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Synthesis and structure determination of SCR7, a DNA ligase inhibitor

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ABSTRACT

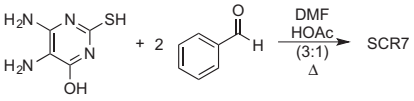
In contrast to a published report, reaction of 4,5-diamino-6-hydroxy-2-mercaptopyrimidine with 2 equiv of aromatic aldehydes produces a mixture of 6,7-diaryl-2-thioxopteridine-4-one and 6,7-diaryl-2-thioxo-7,8-dihydropteridine-4-one rather than a diimine. These compounds represent the correct structure for SCR7, a substance reported to be an inhibitor of nonhomologous end-joining, a DNA repair pathway. The dihydropteridine can be isolated as a minor product, and it can be oxidized to the pteridine.

Introduction

During the course of our work with human DNA ligase inhibitors,[1,2] we encountered a report describing SCR7, reported to be a selective inhibitor of DNA Ligase IV.[3] The proposed structure for SCR7 is a diimine with structure A (Fig. 1).[3] Based on our analysis of ^{1}H and ^{13}C NMR spectra, structure A is not isolated when following the published procedure for the synthesis of SCR7. Herein, we provide evidence that the major compound obtained from the published procedure is pteridine **1a**. Furthermore, based on our analysis of a sample originating in the lab of the authors, the compound used for the reported biological studies is 7,8-dihydropteridine **2a**, a minor product of the reaction that forms SCR7. Full details of our biological studies with these compounds were reported elsewhere,[4] but in our hands, both **1a** and **2a** are inhibitors of all three human DNA ligases. Pteridines **1a** and **1b** are known compounds with biological activity. Both compounds have been previously shown to be active as nematocides,[5,6] and **1a** has recently been shown to inhibit replication of HIV-1, although it is also toxic to host cells.[7]

Synthesis and structure determination of pteridines

The published procedure[3] for the synthesis of SCR7 consists of heating 4,5-diamino-6-hydroxy-2-mercaptopyrimidine with 2 equivalents of benzaldehyde for 3 h under reflux (155 degrees) in a 3:1 mixture of DMF and acetic acid (Eq. 1).



(1)

Following this procedure, we obtained a yellow solid upon addition of water to the cooled reaction mixture. Based on NMR analysis, we conclude that this crude material is approximately a 2:1 mixture of compound **1a** and the previously published ligase inhibitor L189, (which Raghavan refers to as SCR6). Consistent with the published report, we obtained L189 as the sole product when we reacted 4,5-diamino-6-hydroxy-2-mercaptopyrimidine with 1 equiv of benzaldehyde overnight at room temperature. The published purification of SCR7 consists of recrystallization from DMF–ethanol.[3] In our hands, an orange solid can be crystallized in very low yields from DMF-ethanol, but it is still not pure. NMR analysis showed it to be a 1:1.5 mixture of compound **1a** and L189 (enriched in L189 relative to the crude precipitate). Compound **1a** is best purified by column chromatography eluting with 2:1 CH_2Cl_2/ethyl acetate. A typical yield of compound **1a** by this route is 39%. The synthesis of pteridines from the reaction of 4,5-diaminopyrimidines with aromatic aldehydes has been previously described,[8] and Ochoa used the same method to synthesize compound **1a**.[5] SCR7 is now commercially available from XCessBio (with the published structure in their online catalog), however, the material they sold to us was actually compound **1a**.

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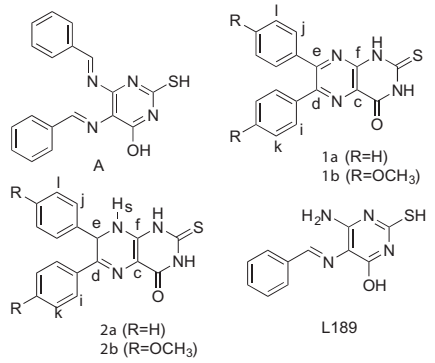
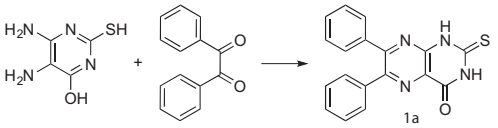


Figure 1. Structures of compounds produced in reaction of 4,5-diamino-6-hydroxy-2-mercaptopyrimidine with benzaldehyde or *p*-anisaldehyde. A = structure proposed by Raghavan for product obtained when the pyrimidine is heated with 2 equivalents of benzaldehyde,[3] **1a** = structure of major isolated product under these conditions; **2a** = structure of minor isolated product; L189 = previously reported product[1] obtained through reaction of the pyrimidine with 1 equiv of benzaldehyde at room temperature.

Structure A is not consistent with either the published NMR data[3] or the NMR for the compound that we isolated. Specifically, the two imine substituents in a hypothetical structure A would be inequivalent due to the asymmetry of the pyrimidine ring, so the spectrum for structure A should contain two singlets (not a multiplet) for the two inequivalent imine hydrogens around 9.6 ppm. In addition, the spectrum for structure A should contain two inequivalent doublets between 7.8 and 7.9 ppm integrating to 4 hydrogens (*ortho* hydrogens on the phenyl rings), a series of peaks between 7.3 and 7.4 ppm integrating to 6 hydrogens (*meta* and *para* hydrogens on phenyl rings), and no peaks around 8.1 ppm. The published NMR data were most likely obtained from a mixture of compound **1** and L189, which is the result of following the published procedure exactly. The reported peak at δ 12.80 and part of the reported multiplet between δ 7.53 and δ 7.36 are consistent with our spectrum for compound **1a**. The reported peaks at δ 11.97, 9.64, 7.88–7.86, and 7.53–7.36 are present in L189. The only peak that does not belong to either compound is the peak from 8.11 to 8.08, so it must be due to another impurity.

The more established route to pteridines involves the reaction of α-diketones with 4,5-diaminopyrimidines. For example, reaction of the 4,5-diamino-2-mercapto-4-hydroxypyrimidine with benzil is reported to produce compound **1a** in 40% yield (Eq. 2).[9]



(2)

When we carried out the synthesis of compound **1a** starting from these reagents but using a more current procedure,[10] the material we obtained was physically and spectroscopically identical to the sample that we purified from the benzaldehyde reaction as described above. The mass spectrum of compound **1a** indicates that the molecular mass of the compound is 332, 2 mass units less than that of structure A suggesting loss of 2 hydrogen atoms. Furthermore, the high resolution mass spectrum of compound **1a** confirms a molecular formula of $C_{18}H_{12}N_4OS$.

The ^{1}H NMR spectrum of compound **1a** is difficult to interpret because all of the aromatic peaks overlap, and the only other hydrogens in the molecule are the downfield amide hydrogens. There is a single recent report containing ^{1}H NMR data for compound **1a** in $CDCl_3$, however that spectrum is also different from our spectrum, and the literature report suggests their route produces a different tautomer. As a result, we decided to synthesize a related compound (**1b**) using the same method as our synthesis of **1a**. In the ^{1}H NMR spectrum of **1b**, the signals for hydrogens i, j, k, and l (Fig. 1) on the two inequivalent aromatic rings are all well-resolved doublets, and our spectrum matches the data reported in the literature.

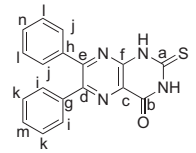
Given the overlap of signals in the ^{1}H NMR, and the fact that all of the carbons in the pteridine core are quaternary, ^{13}C NMR is a better tool to support our contention that **1a** is the structure of the major product formed under the conditions reported for the synthesis of SCR7. Raghavan et al. do not report any ^{13}C NMR data in their Article,[3] but ^{13}C data for compounds **1a**[5] and **1b**[6] have been previously reported. Our ^{13}C data for compound **1b** matches the data reported in the literature exactly. There are slight discrepancies between our data and the literature data for compound **1a**, but through the use of DEPT, HMQC, and HMBC spectra, we have unambiguously assigned all of the carbons in the pteridine core of compounds **1a** and **1b** (Table 1).

Synthesis and structure determination of 7,8-dihydropteridines

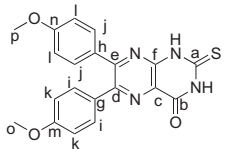
We analyzed a sample of SCR7 that originated in the authors's lab by ^{1}H, ^{13}C, DEPT, and COSY NMR spectroscopy, expecting it to be identical to the material that we isolated from the reaction (compound **1a**). However, the NMR spectra are not consistent with structure A, compound **1a**, or L189. Based on a detailed analysis of the spectra, we conclude that this compound is actually a novel 7,8-dihydropteridine with structure **2a**. Specifically, the peak for H_i at δ 7.83 is separated from the rest of the aromatic hydrogens, suggesting that these hydrogens are ortho to a 'typical' C=N double bond rather than a pteridine ring system. The doublet at δ 7.52 disappears upon shaking the NMR sample with D_2O, suggesting that it is an N—H (H_q). The H—H COSY spectrum contains cross peaks between the resonances at δ 7.52 and δ 6.02, indicating that the peak at δ 6.02 is H_e. The absence of this distinctive peak in the NMR data reported for SCR7 suggests that compound **2a** was not a significant component of the NMR sample analyzed for the original Article. The ^{13}C NMR spectrum contains a peak at δ 52.3 which indicates an aliphatic carbon, and according to the DEPT spectra, this carbon bears a single hydrogen (H_e). The peak assigned to carbon c is further upfield in **2a**, as compared to **1a**, which is consistent with an ordinary pyrimidine ring system rather than a pteridine ring system. TLC analysis indicates that compound **2a** is much more polar than compound **1a**, also consistent with the N—H bond in the proposed structure.

We were unable to isolate any of compound **2a** using the published purification procedure, but we were able to observe it as a minor product in the NMR spectra of the crude product. In order

Table 1
^{13}C NMR assignments of selected carbons in **1a** and **1b**

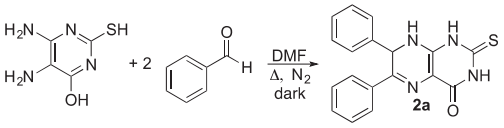


1a carbons	δ
a	175.7
b	158.5
c	127.2
d	147.0
e	155.9
f	149.1
g	137.7
h	137.1



1b carbons	δ
a	175.5
b	158.6
c	126.4
d	146.6
e	155.2
f	148.7
m/n	160.6, 159.7
o/p	55.35, 55.25

to increase the yield of compound **2a** relative to compound **1a**, we modified the reaction conditions by omitting acetic acid, and carrying out the reaction under nitrogen. Furthermore, we developed a different workup procedure to take advantage of the polarity difference. Even under these improved conditions, pure compound **2a** can only be isolated in 22% yield (Eq. 3).



(3)

The ^{1}H, ^{13}C, DEPT, and COSY spectra provided evidence that compound **2a** is a dihydropteridine, but the question remained of whether the correct structure is a 5,6-dihydropteridine or a 7,8-dihydropteridine (Fig. 2). Both structures have been reported

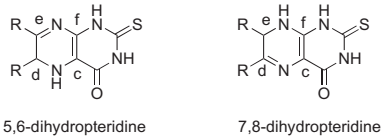
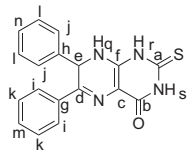
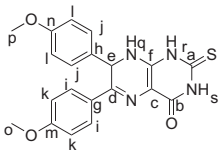


Figure 2. 5,6-Dihydropteridine versus 7,8-dihydropteridine.

Table 2
^{1}H and ^{13}C NMR assignments for **2a** and **2b**



2a	^{1}H	^{13}C
a	—	173.1
b	—	157.6
c	—	104.3
d	—	146.7
e	6.02	52.3
f	—	143.2
g	—	136.1
h	—	140.1
i	7.83	126.4
j	7.27	128.56
k	Unassigned	Unassigned
l	Unassigned	Unassigned
m	Unassigned	Unassigned
n	Unassigned	Unassigned
q	7.52	—
r or s	12.07	—
r or s	12.00	—

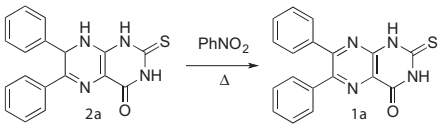


2b	^{1}H	^{13}C
a	—	172.8
b	—	157.6
c	—	104.2
d	—	146.8
e	5.91	51.8
f	—	143.0
g	—	128.8
h	—	132.4
i	7.76	128.1
j	7.19	128.3
k	6.92	114.0
l	6.88	114.5
m	—	160.6
n	—	159.3
o	3.75	55.3
p	3.68	55.2
q	7.33	—
r or s	11.95	—
r or s	11.88	—

for similar compounds with an oxygen at the 2-position.[11] With the ability to synthesize and purify compound **2a**, we were able to record HMQC and HMBC spectra for the compound. Resonances that we were able to assign based on these 2D spectra can be found in Table 2. The presence of an HMBC cross peak between H_e (6.02 ppm) and C_f (143.2 ppm) confirms the 7,8-dihydropteridine structure. If the 5,6-dihydropteridine structure were correct, and the hydrogen were on carbon d, there would be a cross peak to carbon c instead.

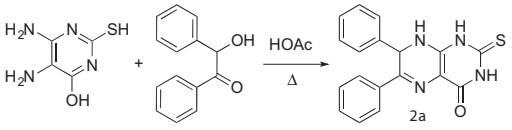
Due to the overlap of most aromatic resonances in the ^{1}H NMR spectrum of **2a**, we were unable to assign all of the protons and carbons even using 2D techniques. To confirm the assignments, we synthesized compound **2b** in 8% isolated yield by the same method. All of the protons and carbons can be unambiguously assigned for compound **2b** (Table 2). The specific HMBC cross peaks that confirm the 7,8-dihydropteridine structure are between H_e (5.91 ppm), and C_f (143.0 ppm), between H_i (7.76 ppm) and C_d (146.8 ppm), and between H_j (7.19 ppm) and C_e (51.8 ppm).

To provide further evidence in support of our proposed structure for compounds **2a** and **2b**, we carried out an oxidation of each compound in refluxing nitrobenzene, conditions which are known to promote dehydrogenation to produce aromatic systems[12] (Eq. 4). Compound **1a** was isolated in 44% yield, and compound **1b** was isolated in 49% yield with no evidence of any other by-products.



(4)

The synthesis of similar dihydropteridines from α-hydroxyketones has been reported.[11,13] In analogous fashion, reaction of 4,5-diamino-6-hydroxy-2-mercaptopyrimidine with benzoin in acetic acid affords compound **2a** in 42% yield (Eq. 5).



(5)

Figure 3 outlines the most likely mechanism of the reaction between 4,5-diamino-6-hydroxy-2-mercaptopyrimidine and aromatic aldehydes. Under mild conditions, only one amino group reacts with benzaldehyde to form an imine and give L189. Under more forcing conditions, the second amino group presumably reacts the same way as the first to give the bis-imine (structure A). Not only did we not isolate a compound with structure A, there was no evidence of such a structure in crude reaction mixtures. It is not surprising that structure A cannot be isolated, since in the literature, analogous compounds are only postulated as unstable intermediates. For example, reaction of *o*-phenylenediamine with benzaldehyde produces a substituted benzimidazole.[14] Structure

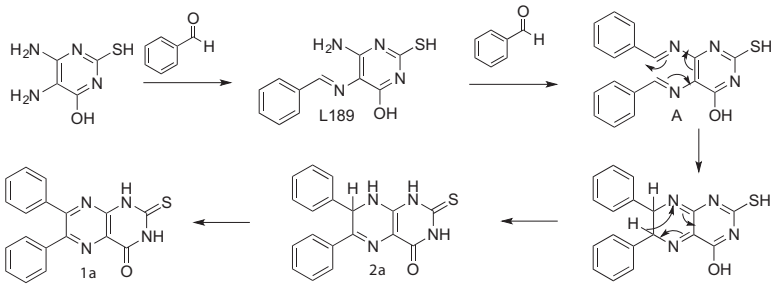


Figure 3. Possible mechanism of formation of compounds **2a** and **1a** through L189 and structure A.

A then undergoes a 6π electrocyclic ring closure to produce another intermediate which has not been identified or isolated. This intermediate undergoes a [1,5] sigmatropic rearrangement to give dihydropteridines **2a** or **2b**. The dihydropteridine is oxidized under the reaction conditions to give the fully aromatic pteridine **1a** or **1b**.

We have demonstrated that the reaction conditions reported to produce SCR7 actually produce a mixture of **1a** and **2a**, with **2a** being a novel compound. The scope of this dihydropteridine synthesis and the optical spectroscopic properties of the dihydropteridines are currently under investigation.

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Supplementary data

Supplementary data (experimental procedures (synthetic procedures, and compound characterization)) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2016.06.037. These data include MOL files and InChiKeys of the most important compounds described in this article.

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