Synthesis of 2-Amino-4*H*-thiazolo[5,4-*b*]indole and Characterization of its Colored Conversion Products with Protein Tyrosine Phosphatase Inhibitory Activity

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In DMSO-solution 2-amino-4*H*-thiazolo[5,4-*b*]indole is converted into a complex mixture of colored products. The three major conversion end-products, of which two are inhibitors of protein tyrosine phosphatases (PTPs), were isolated by chromatographic methods and their structures characterized by spectroscopic analysis, including NMR and MS combined with computer assisted structure elucidation, and, finally, confirmed by independent chemical synthesis. Synthesis of 2-amino-4*H*-thiazolo[5,4-*b*]indole as well as its *N*-acetyl derivatives prepared from either oxindole or 2-bromo-1-(2-nitro-phenyl)ethanone is described.

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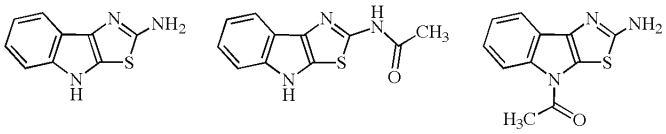
Introduction.

Protein-tyrosine phosphatases (PTPs) are critically involved in the regulation of intracellular signaling pathways that control cellular metabolism, differentiation and mitogenicity (reviewed in [1]). Inhibition of PTPs that negatively control protein tyrosine kinases (PTKs) would potentially lead to enhanced (duration and perhaps amplitude) signaling [2]. Therefore, PTPs are considered attractive therapeutic targets in different disease states where improved PTK signaling is warranted, including type 2 diabetes [3]. Although it has been known for more than a decade that certain PTPs are negatively regulating the insulin receptor signaling pathway, the exact identity of these enzymes remained elusive. Based on *in vitro* studies, several PTPs have been proposed as negative regulators of insulin signaling, including PTPα [4-8], PTP-LAR [9], and PTP1B [10-12]. Importantly, recent studies on PTP1B knockout mice provided significant support for the view that PTP1B is a key regulator of insulin signaling. The lack of PTP1B gave rise to both increased insulin sensitivity and resistance to diet-induced obesity, thus pointing to this phosphatase as a relevant therapeutic target in type 2 diabetes and obesity [13].

We therefore initiated high throughput screening (HTS) of the Novo Nordisk compound library using PTP1B and a ^{33}P-phosphorylated peptide as substrate. Several inhibitors were identified and one of these seemed to be 2-amino-4*H*-thiazolo[5,4-*b*]indole, **1** since a sample containing this compound relatively potently inhibited PTP1B. However, NMR and MS analysis of the original sample revealed the presence of two components, namely the expected compound, **1** and an acetyl-derivative thereof, represented by either formula **2** or **3** (Scheme 1). For unambiguous structure determination of the acetate and for separate biological testing of single compounds, **1**, **2**, and **3** were synthesized. Surprisingly, when testing for inhibitory activity against PTP1B neither of the compounds **1**, **2**, and **3** displayed any activity. In contrast, colored products formed during storage of **1** in DMSO-solution inhibited PTP1B efficiently.

Scheme 1



1 **2** **3**

We here report the isolation, structural characterization and biological activity of the major conversion products derived from compound **1**. Furthermore, we describe the independent synthesis of the major conversion product to confirm the spectroscopy derived structure, and, finally we describe the synthesis of compounds **1**, **2** and **3**.

Results.

Preparation and Purification of Conversion Products.

Automated HTS revealed that a solution of the aminothiazole **1**, contaminated with minor amounts of its *N*-acetyl derivative **3**, the synthesis of which is described below, exhibited relatively potent PTP1B inhibitory activity. The surprising observation that freshly prepared solutions of **1** and **3** were unable to inhibit PTP1B prompted us to further investigate the chemical stability of **1** in solution. Immediately after preparation, solutions of **1** in DMSO appeared colorless and inactive against PTP1B, but the

progress of a chemical reaction at room temperature was evident from the appearance of a red color with a concomitant increase in the PTP inhibitory activity. Increasing the storage temperature accelerated this development and a solution with K_{ic} = 100 μ*M* improved to 6 μ*M* after 3 days at 37° (or 1.4 μ*M* by heating at 80° for one hour). The reaction mixture obtained by heating a DMSO-solution of **1** at 80° for sixteen hours was dominated by three major, colored components (Figure 1), which were separated and isolated by reversed phase HPLC as described in the experimental section.

TFA salt. Liberation of the corresponding free base resulted in a colour change from red to blue (see UV-VIS data in experimental section). For reasons of solubility and spectral quality the NMR analysis were performed on a solution of the TFA-salt in DMSO-d_6 added approximately one additional equivalent of TFA. ^{1}H-NMR, including ^{1}H,^{1}H-COSY, established the presence of eight aromatic protons in two separated four-proton spin systems. Additional ^{1}H-resonances at δ 12.68 (1H, sharp) and δ 9.30 (2H, broad) were attributed to isolated NH and NH_2^+ groups, respectively. The ^{13}C-NMR spectrum exhibited

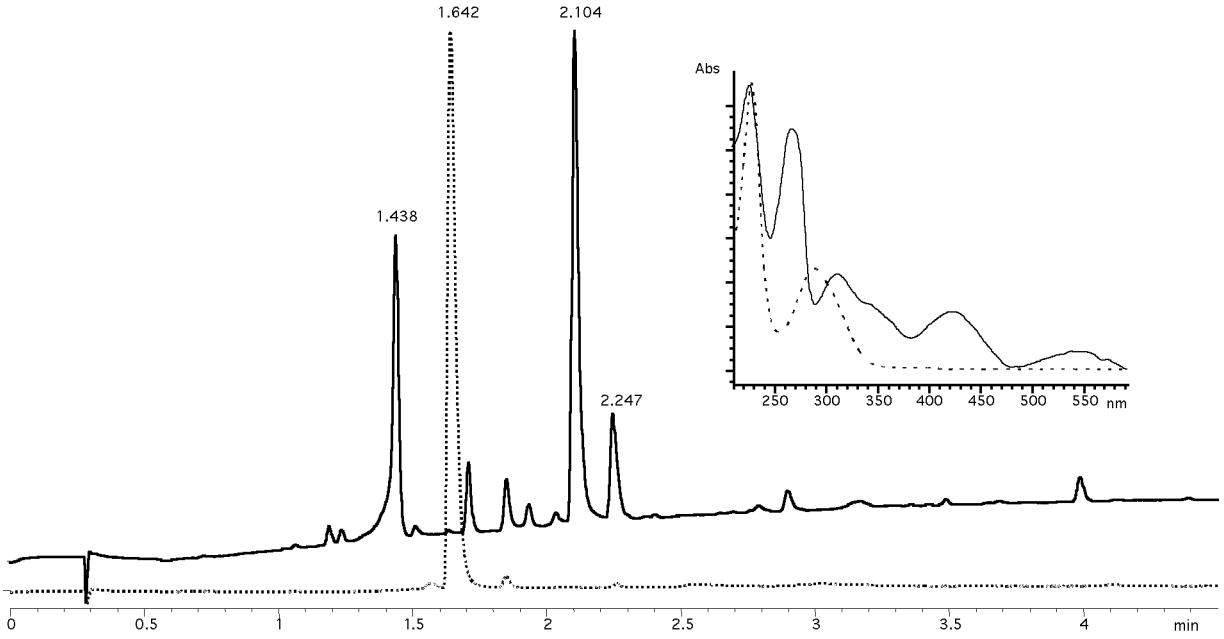


Figure 1. HPLC trace detected at 300 nm of a fresh DMSO-solution of **1** (dotted line) and the reaction mixture obtained after heating at 80 ° for 16 hours (solid line). The retention times for **1**, **4**, **5** and **6** are 1.64, 1.44, 2.10, and 2.25 minutes, respectively. The insert shows the UV-spectra of **1** (dotted line) and **5** (solid line). Chromatographic details are given in Experimental.

Structural Characterization.

Structure and Biochemical Activity of Yellow Conversion Product.

NMR analysis including single- and multiple-bond ^{1}H,^{13}C-correlation experiments (HSQC and HMBC) established the structure of the yellow product as indole-2,3-dione, **4** (isatin). The identity was supported by EI-MS, exhibiting a molecular ion peak at *m*/*z* 147 and, finally, confirmed by spectral and chromatographic comparison with an authentic sample of isatin. Isatin was found to be unable to inhibit PTPs.

Structure and Biochemical Activity of the Red Conversion Product.

The HPLC-purification scheme (as outlined in the experimental section) using an acidic mobile phase afforded the major conversion product as a red, amorphous

seventeen individual signals corresponding to aromatic or carbonyl carbon atoms. ^{1}H,^{13}C-HSQC data served to assign carbon atoms directly coupled to protons and the ^{1}H,^{13}C-HMBC experiment to extend the carbon framework by observation of long-range ^{1}H,^{13}C-couplings to quaternary carbon atoms. High-resolution FAB-MS (*m*/*z* 287.0945 $[M+H]^+$) in conjunction with the NMR established contents of protons and carbon atoms, implied the molecular composition of the protonated species to be $C_{17}H_{11}N_4O$. In conjunction with ^{1}H,^{15}N-connectivities and ^{15}N-chemical shifts obtained by the ^{15}N-versions of the heteronuclear shift correlation experiments (^{1}H,^{15}N-HSQC and -HMBC) the substructures and fragments presented in Figure 2 were established. Key, diagnostic ^{1}H,^{13}C- and ^{1}H,^{15}N-HMBC correlations are depicted by arrows, and nitrogen atoms are labelled with their recorded ^{15}N-chemical shifts, except for one (N^d) which exhibited no detectable direct or long-range couplings.

Computer-generation of all structures conforming to the established molecular formula, substructures and fragments by the structure generation program NMRSAMS (Version 2.0, Spectrum Research, LLC, Madison, Wisconsin, USA) resulted in hundreds of possible structures if no further constraints on the constitution were included. However, the number of candidate structures could be reduced significantly by taking into account a few additional spectroscopy-derived restrictions. First, the low field chemical shift for the carbonyl carbon atom (δ_C 181.8) excluded an amide. Second, the chemical shift of N^a (δ_N 146.2) excluded a double bond heteroaromatic nitrogen. Third, lack of absorptions in the IR spectrum in the 2100-2400 cm^{-1} range excluded the presence of a nitrile, and, finally, a strong NOE observed between the protons (δ 9.30) on the basic nitrogen (N^c) and the aromatic proton (δ 8.36) attached to substructure **A** (Figure 2) demonstrated their close proximity in space. By including these restrictions structures **5**, **5a**, **5b** and **5c** (Scheme 2) were found to be the only plausible structural candidates. Of note independent chemical synthesis (see below) confirmed the structure **5** for the major conversion product.

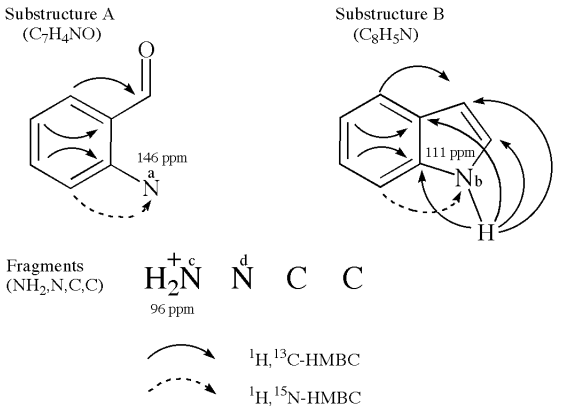
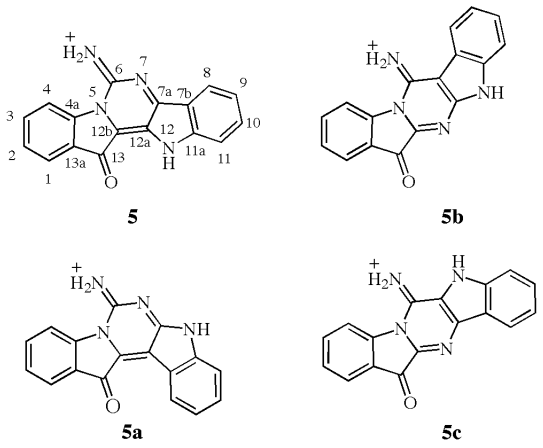


Figure 2. Fragments and NMR-derived substructures **A** and **B** of major conversion product with key, diagnostic long-range $^1H,^{13}C$- and $^1H,^{15}N$-HMBC correlations depicted by arrows. Nitrogen atoms (except for N^d) are labeled with their ^{15}N-chemical shifts.

The presence of a basic nitrogen with double bond character is in accordance with the relatively low-field chemical shift for the basic nitrogen, N^c (96.2 ppm), and the ^{13}C-resonance at 148.8 ppm attributable to the carbon atom attached to N^c. In comparison, the guanidinium carbon atom in diphenyl guanidine was in DMSO-d_6 observed to resonate between δ_C 147 (free base) and 155 (excess TFA), and the ^{15}N-chemical shift for the imino-nitrogen recorded in a $^1H,^{15}N$-HSQC experiment under acidic conditions (excess TFA to obtain sharp NH-resonances) was δ_N 82.8.

The low-field chemical shift for C-7a (δ_C 162.8) is explained by delocalization of the positive charge at N^c involving a resonance structure carrying the positive charge on C-7a.

Scheme 2



The purified conversion product **5** exhibited K_{ic} values against PTP1B and PTPα of 2.6 and 1.0 μ*M*, respectively, and appears to account for the major part of the increase in enzyme inhibition observed by heat treatment of compound **1** in DMSO-solution. Compound **5** was also found to inhibit PTPβ, PTPε, CD45, LAR and SHP1 in the 1-5 μ*M* range, indicating that **5** is a general inhibitor of PTPs.

Structure and Biochemical Activity of the Blue Conversion Product.

The minor conversion product, obtained as a blue solid, exhibited NMR characteristics very similar to those recorded for **5**, except for the lack of signals corresponding to protons attached to a basic nitrogen and the occurrence of an additional six-proton singlet signal at δ_H 3.18 corresponding to two equivalent methyl groups. $^1H,^{13}C$-HSQC and $^1H,^{13}C$-HMBC experiments furnished evidence for substructures **A** and **B** (Figure 2) identical to those of **5**. The chemical shift for the carbon atoms corresponding to the two equivalent methyl groups (δ_C 31.8) suggested their geminal attachment to a sulphur atom. Additionally, a long-range correlation observed in the $^1H,^{15}N$-HMBC spectrum from the methyl groups to a nitrogen atom resonating at 145 ppm demonstrated that the sulphur atom is attached to a nitrogen atom, all in accordance with the presence of a *S*,*S*-dimethylsulfilimine group.

The HRFAB-MS spectrum exhibited a molecular ion peak at *m/z* 347.0979 M^+ in correspondence with the molecular composition $C_{19}H_{15}N_4OS$, leading to **6** as the proposed structure of the blue compound (Scheme 3). Expected NOE-interaction was observed between the *S*-methyl groups and an aromatic proton (δ 8.81) attached to substructure **A**. The presence of a DMSO-derived *S*,*S*-dimethylsulfilimine group was further substantiated by using DMSO-d_6 as the solvent during the conversion of **1** to **6**. In this case the isolated blue product **6** exhibited in its FAB mass spectrum a molecular ion peak at *m/z* 353 corresponding to $C_{19}H_9D_6N_4OS$. Compound **6** inhibited PTP1B with K_{ic} = 2.6 μ*M*.

Reaction Mechanism and Independent Synthesis.

To rationalize the formation of **5** and **6** from **1** the time course of the reaction was investigated further. Thus, a solution of **1** in DMSO was heated at 80° and samples were analyzed by LC-MS over a period of 26 hours. In parallel an identical experiment, except for using DMSO-d_6 as the solvent, was conducted allowing identification of intermediates and products carrying DMSO-derived methyl groups. In addition to the starting material **1** (*m/z* 190, $[C_9H_7N_3S+H]^+$) and end-products isatin, **4** (*m/z* 148, $[C_8H_5NO_2+H]^+$), **5** (*m/z* 287, $[C_{17}H_{10}N_4O+H]^+$) and **6** (*m/z* 347 $[C_{19}H_{15}N_4OS]^+$ and 353 $[C_{19}H_9D_6N_4OS]^+$), four major intermediate components were detected. Three exhibited *m/z* 311 and one *m/z* 371 and 377 for the non-deuterated and deuterated species, respectively. The time dependent concentration profiles of individual species were established by extracting selective ion traces corresponding to the masses of (pseudo) molecular ions of each component as shown in Figure 3. Initially a rapid disappearance of **1** and a concomitant build-up of the *m/z* 311 and *m/z* 371/377 intermediates are observed. The intermediates are then slowly disappearing in concert with formation of the products **4**, **5** and **6**. Based on these findings we suggest that **4**, **5** and **6** are formed from **1** according to Scheme 3. In brief, the aminothiazole moiety in **1** is believed to ring open which results in a thiourea-substituted indole **7**. The indole is either dimerized in an oxidative manner to compound **8** liberating one equivalent of water or forms a thiourea-substituted oxindole **9**, which is hydrolysed directly to **4** (isatin) perhaps *via* a cyanoimine. Formation of the bis(cyanoimino) intermediate *m/z* 311 ($[M+H]^+$, $C_{18}H_{11}N_6$) **10** proceeds *via* elimination of hydrogen sulfide perhaps in a way similar to that described for the formation of aryl aminocyans [14]. Cyclisation of **10** yields compound **11**, containing the pentacyclic ring system pyrimido[1,6-*a*: 5,4-*b*']diindole [15], which is hydrolyzed to **5** or reacts with DMSO under the formation of water and a *S,S*-dimethylsulfilimine species **12** which is finally hydrolysed to **6**. Heating a HPLC purified sample of **5** at 80° in DMSO does not convert it to **6,** demonstrating that **6** is not derived from **5** but from an earlier intermediate. Additional evidence for the proposed routes to **5** and **6** outlined in Scheme 3 was obtained by synthesis of the postulated intermediate **10** from indole and cyanogen azide as described by Gompper *et al*. [16]. Heating a solution of **10** in DMSO at 80° transformed the compound into a mixture of which **5** and **6** are major and minor components, respectively. Isatin (**4**) was not detected in this mixture, which supports its formation *via* a separate route not involving compound **10**.

Synthesis of **1**, **2** and **3**.

To unequivocally identify the acetate derivative of **1** present in the HTS-sample routes offering the possibility for a regioselective introduction of the *N*-acetyl group in *N*-(4*H*-thiazolo[5,4-*b*]indole-2-yl)acetamide, **2** and 1-(2-aminothiazolo-[5,4-*b*]indol-4-yl)ethanone, **3** were explored as outlined in Scheme 4. The routes should provide the possibility of having

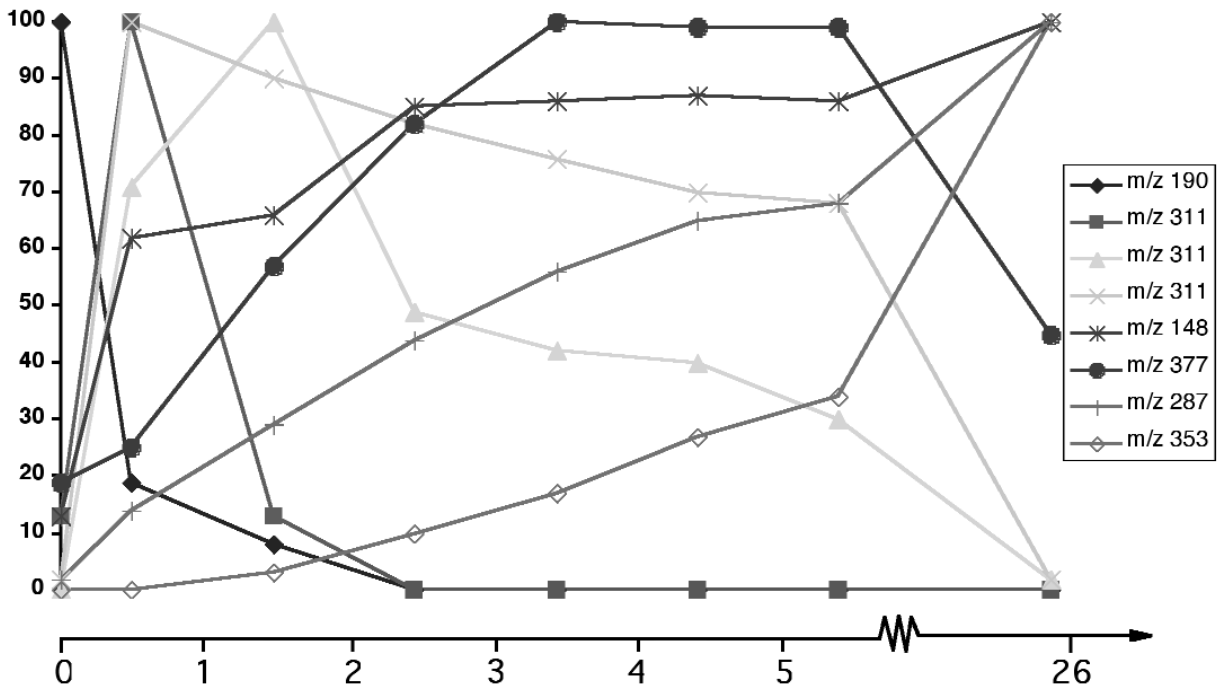
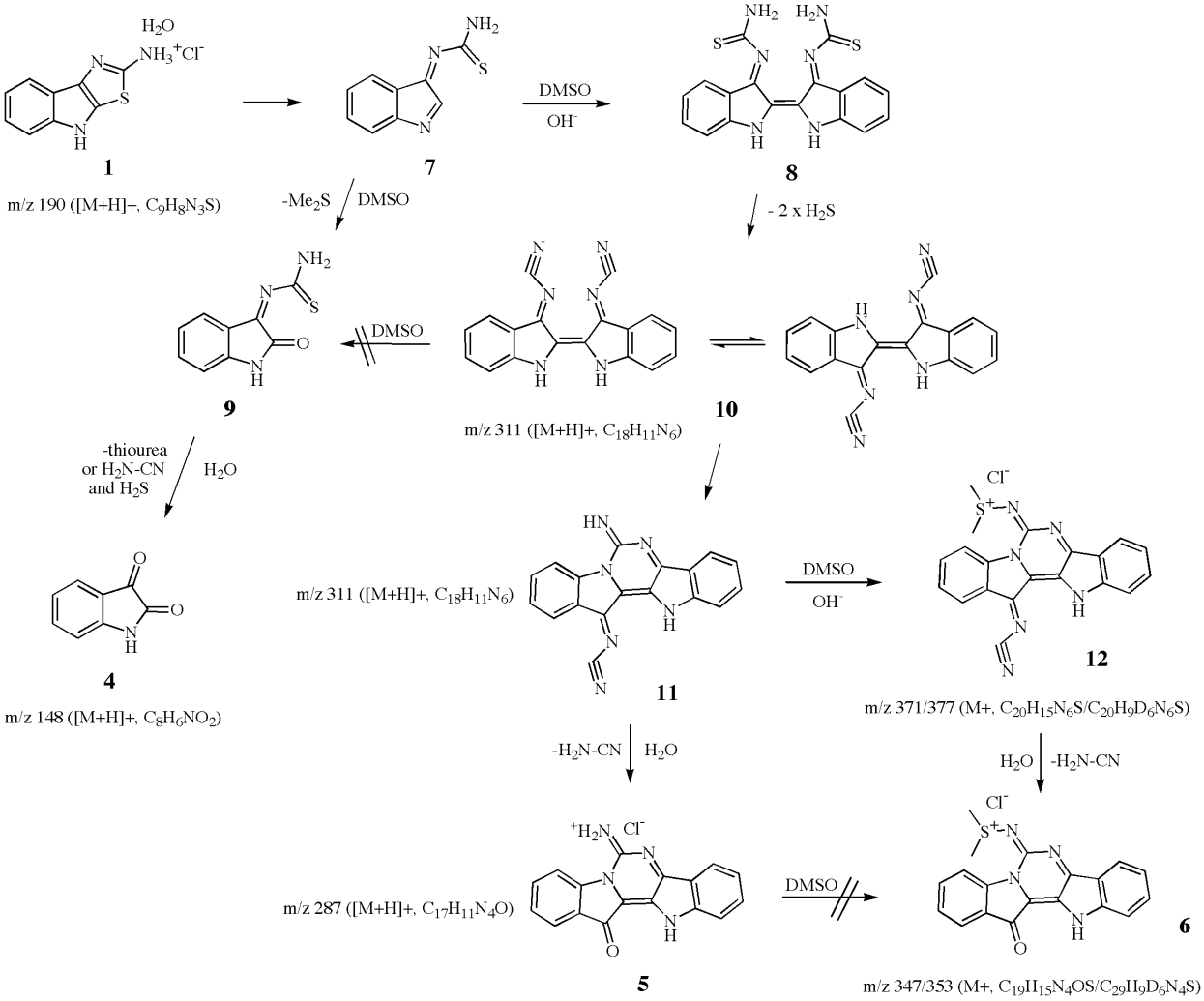


Figure 3. Relative concentration profiles of starting material **1** (*m/z* 190, ◆), products **4** (*m/z* 148, ✳), **5** (*m/z* 287, +), and **6** (*m/z* 353, ◇) and intermediates (*m/z* 311, ×, ■, ▲ and 377, ◆) observed by LC-MS during heating of **1** in DMSO-d_6 at 80° over a period of 26 hours.

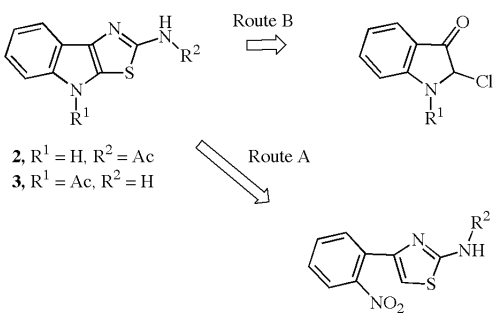
Scheme 3



either the 2-aminothiazole ring (route **A**) or the indole ring system (route **B**) formed prior to the final ring closure affording the thiazolo[5,4-*b*]indole ring system, which would allow for a regioselective introduction of the acetyl group.

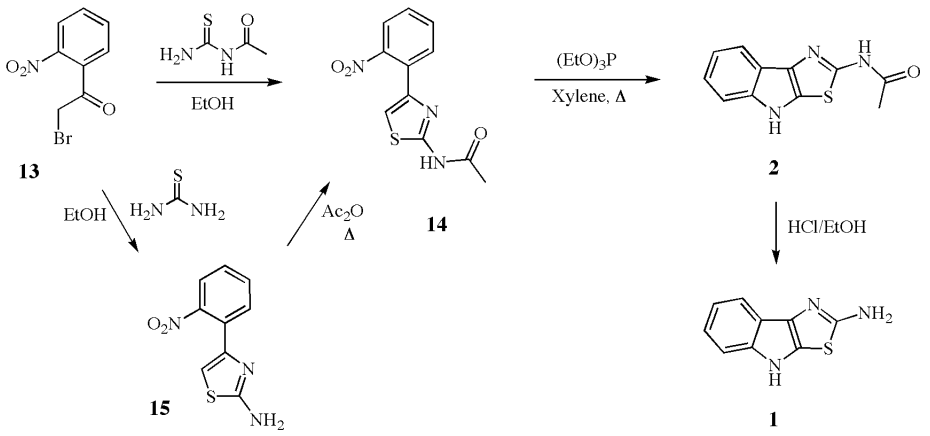
2-Amino-4*H*-thiazolo[5,4-*b*]indoles are to our knowledge unprecedented within the chemical literature. Potts and Marshall [17] have described the synthesis of 2-phenyl-4*H*-thiazolo[5,4-*b*]indole wherein the thiazole ring was formed prior to the final ring closure. Using a slightly modified procedure, compound **1** and **2** were synthesized. In brief, treatment of 2-bromo-1-(2-nitrophenyl)ethanone, **13**, as shown in Scheme 5, with *N*-acetyl thiourea afforded the thiazole compound **14**, which was also obtained *via* treatment of **13** with thiourea followed by acetylation with acetic acid anhydride of the intermediate 2-amino-4-(2-nitrophenyl)thiazole, **15**. Deoxygenation of aromatic nitro compounds with triethyl phosphite [18] affords products expected from the corresponding nitrenes. In the present case, electrophilic attack of the nitrene intermediate on the unsubstituted 5-thiazole position afforded the desired *N*-(4*H*-thiazolo[5,4-*b*]indol-2-yl)acetamide, **2**. Deacetylation using hydrochloric acid in ethanol afforded 2-amino-4*H*-thiazolo[5,4-*b*]indole, **1**, which proved identical with the major component in the HTS-sample based on NMR, HPLC and MS data, whereas compound **2** exhibited NMR and HPLC properties different from those recorded for the minor component in the HTS-sample.

Scheme 4

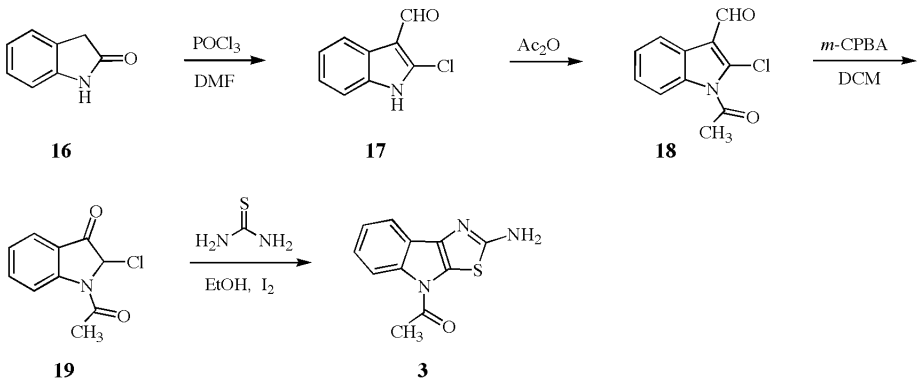


1-(2-Aminothiazolo[5,4-*b*]indol-4-yl)ethanone, **3** was synthesized using route **B** (Scheme 4) from oxindole, **16**

Scheme 5



Scheme 6



which offers the possibility of introducing the acetyl group at the indole nitrogen atom prior to the thiazole formation as shown in Scheme 6. Fused 2-aminothiazoles are most conveniently synthesized by condensing an α-haloketone with thiourea [19] which suggests 1-acetyl-2-chloro-1,2-dihydroindol-3-one [20], **19** as a key intermediate synthesized in three steps as outlined in Scheme 6. Oxindole, **16** was chloroformylated using phosphorus oxychloride in DMF followed by acetylation of the indole nitrogen atom with acetic acid anhydride which afforded 1-acetyl-2-chloro-1*H*-indol-3-carbaldehyde, **18**. Baeyer-Villiger oxidation of the formyl group using *m*-chloroperbenzoic acid (*m*-CPBA) yielded the acetylindole, **19** which underwent cyclisation when treated with thiourea in ethanol affording the desired 1-(2-aminothiazolo[5,4-*b*]indol-4-yl)ethanone, **3** exhibiting NMR and HPLC characteristics identical with those of the minor component in the HTS-sample.

EXPERIMENTAL

Melting points were determined in open capillary tubes with a BÜCHI 535 melting point apparatus and are uncorrected. The ^{1}H -, ^{13}C - and ^{1}H,^{13}C-heteronuclear nmr spectra (HSQC and HMBC) were obtained in DMSO-d_6 or chloroform-*d* at 300 K on Bruker DRX400, DRX300 or DRX200 instruments equipped with 5 mm selective inverse *z*-gradient probes. ^{1}H,^{15}N-HSQC (optimized for $^{1}J_{NH}$ = 101 Hz) and HMBC (two individual experiments optimized for $^{n}J_{NH}$ = 2.5 and 7 Hz, respectively) experiments were performed on a Bruker AMX2-400 instrument equipped with a 5 mm broadband inverse *z*-gradient probe. ^{1}H and ^{13}C chemical shifts (δ-values) are reported in ppm downfield from tetramethylsilane. ^{15}N chemical shifts are reported relative to nitromethane at 379.5 ppm. LC-MS analysis was performed on a HP1100 MSD equipped with an electro spray interface. EI mass spectra, at 70 eV ionisation potential, and high-resolution fast atom bombardment (HR-FAB) mass spectra were recorded on a JEOL AX505W instrument. Microanalyses were carried out on a Perkin-Elmer Model 240 elemental analyzer. Merck Kieselgel 60 F_{254} on glass - or aluminium plates were used for tlc monitoring, detection by UV (254 nm).

Synthesis of *N*-(4*H*-thiazolo[5,4-*b*]indol-2-yl)acetamide **2**.

N-[4-(2-Nitrophenyl)-thiazol-2-yl]acetamide (**14**).

A mixture of 1-acetyl-2-thiourea (9.7 g, 0.082 mole) and 2-bromo-1-(2-nitrophenyl)ethanone (10.0 g, 0.041 mole) in ethanol (150 mL) was heated at reflux temperature for 20 minutes. After cooling to room temperature the precipitate was filtered off, washed with ethanol (2 × 50 mL) and dried *in vacuo* at 50°, which afforded **14** in 62 % yield (6.7 g), mp 216-218°; ^{1}H

Table 1

^{1}H and ^{13}C NMR data (δ, ppm) for Compounds **5** and **6** in DMSO-d_6

Position [a]	Red compound, **5** ^{1}H	^{13}C/^{15}N	Blue compound, **6** ^{1}H	^{13}C/^{15}N [b]
1	8.10 (d, *J* = 7.8 Hz)	125.8	8.00 (d, *J* = 7.5 Hz)	125.1
2	7.69 (t, *J* = 7.8 and 7.6 Hz)	129.1	7.59 (t, *J* = 7.5 and 7.5 Hz)	128.3
3	8.00 (t, *J* = 7.6 and 8.3 Hz)	137.2	7.92 (t, *J* = 7.5 and 8.2 Hz)	137.1
4	8.36 (d, *J* = 8.3 Hz)	117.2	8.81 (d, *J* = 8.2 Hz)	119.4
4a		145.6		147.1
5		146		n.o. [c]
6		148.8		n.o. [c]
7		n.o. [c]		n.o. [c]
7a		162.8		162.3
7b		117.2		117.5
8	8.12 (d, *J* = 8.0 Hz)	124.2	8.17 (d, *J* = 7.9 Hz)	124.3
9	7.38 (t, *J* = 8.0 and 7.7 Hz)	122.7	7.33 (t, *J* = 7.9 and 7.5 Hz)	122.3
10	7.88 (t, *J* = 7.7 and 8.2 Hz)	137.4	7.83 (t, *J* = 7.5 and 8.2 Hz)	137.0
11	7.58 (d, *J* = 8.2 Hz)	113.9	7.52 (d, *J* = 8.2 Hz)	113.7
11a		151.9		151.8
12	12.68 (s)	111	12.40 (s)	110
12a		121.7		120.7
12b		122.2		n.o. [c]
13		181.8		183.2
13a		123.9		123.7
NH_2	9.30 (bs)	96		145
$S(Me)_2$			3.18 (s)	31.8

[a] Atom numbering as indicated in Scheme 2; [b] ^{13}C-Chemical shifts extracted from HSQC and HMBC spectra; [c] Not observed. Carbon or nitrogen atoms for which no direct- or long range correlations were observed.

Table 2

K_{ic} values (μ*M*) of HTS-hit and Compounds **1**-**3** and Conversion Products **4**-**6** as Inhibitors of two Different Protein-Tyrosine Phosphatases, PTP1B and PTPα

Compound	PTP1B	PTPα
HTS HIT	50	30
1	75-300	100-160
2	>1000	>1000
3	>400	>400
4	> 1000	> 1000
5	2.6	1
6	2.6-16	1.7-6.5

nmr (DMSO-d_6): δ 2.15 (s, 3H), 7.50 (s, 1H), 7.59 (dt, 1H), 7.72 (dt, 1H), 7.80 (dd, 1H), 7.89 (dd, 1H), 12.2 (bs, 1H).

Anal. Calcd. for $C_{11}H_9N_3O_3S$: C, 50.18; H, 3.45; N, 15.96. Found: C, 50.28; H, 3.28; N, 15.53.

2-Amino-4-(2-nitrophenyl)thiazole (**15**).

A mixture of thiourea (12.8 g, 0.17 mole) and 2-bromo-1-(2-nitrophenyl)ethanone (20.5 g, 0.084 mole) in ethanol (150 mL) was heated at reflux temperature for 30 minutes. After cooling to room temperature the volatiles were evaporated *in vacuo*. The residue was dissolved in water (150 mL), the pH was adjusted to 7 by addition of 8 *N* sodium hydroxide and extracted with ethyl acetate (2 × 200 mL). The combined extracts were washed with brine (2 × 100 mL), dried ($MgSO_4$), filtered and the solvent evaporated *in vacuo*, which afforded **15** in 88 % yield (16.3 g), mp 105-107°; ^{1}H nmr (chloroform-*d*): δ 5.05 (bs, 2H), 6.67 (s, 1H), 7.44 (dt, 1H), 7.58 (dt, 1H), 7.67 (dd, 1H), 7.73 (dd, 1H).

Anal. Calcd. for $C_9H_7N_3O_2S$: C, 48.86; H, 3.19; N, 18.99. Found: C, 49.26; H, 3.22; N, 18.69.

N-[4-(2-Nitrophenyl)thiazol-2-yl]acetamide (**14**).

A mixture of **15** (15.0 g, 0.07 mole) and sodium acetate (12.8 g, 0.16 mole) in acetic acid anhydride (200 mL) was stirred at room temperature for 18 hours. The precipitate was isolated by filtration, washed with water (3 × 80 mL), heptane (2 × 100 mL) and dried *in vacuo* at 50° for 18 hours, which afforded **14** in 73 % yield (13.0 g), mp 217-218°; ^{1}H nmr (DMSO-d_6): δ 2.16 (s, 3H), 7.51 (s, 1H), 7.60 (dt, 1H), 7.73 (dt, 1H), 7.78 (dd, 1H), 7.89 (dd, 1H), 12.17 (s, 1H).

Anal. Calcd. for $C_{11}H_9N_3O_3S$: C, 50.18; H, 3.45; N, 15.96. Found: C, 50.16; H, 3.43; N, 15.77.

N-(4*H*-Thiazolo[5,4-*b*]indol-2-yl)acetamide (**2**).

A mixture of compound **14** (9.3 g, 0.035 mole), triethyl phosphite (20 mL) and xylene (70 mL) was heated at reflux temperature for 18 hours. After cooling the precipitate was isolated by filtration, washed with diethyl ether (3 × 20 m) and dried *in vacuo* at 50°, which afforded **2** in 61 % yield (5.0 g), mp > 250°; ^{1}H nmr (DMSO-d_6): δ 2.16 (s, 3H), 7.11 (t, 1H), 7.28 (t, 1H), 7.48 (d, 1H), 7.74 (d, 1H), 11,41 (s, 1H), 12.15 (bs, 1H, N*H*COMe).

Anal. Calcd. for $C_{11}H_9N_3OS$: C, 57.13; H, 3.92; N, 18.17. Found: C, 57.50; H, 3.93; N, 18.13.

Synthesis of 2-Amino-4*H*-thiazolo[5,4-*b*]indole **1**.

2-Amino-4*H*-thiazolo[5,4-*b*]indole Hydrochloride (**1**).

To a mixture of compound **2** (3.5 g, 0.015 mole) in ethanol (50 mL) was added concentrated hydrochloric acid (17.5 mL). The reaction mixture was refluxed for 6 hours and stirred at room temperature over night. The precipitate was isolated by filtration, washed with ethanol (2 × 20 mL) and dried *in vacuo* at 50°,

which afforded **1** in 59 % yield (2.0 g), mp 248-250°; ^{1}H nmr (DMSO-d_6): δ 7.14 (t, 1H), 7.21 (t, 1H), 7.59 (d, 1H), 7.81 (d, 1H), 9.48 (bs, 2H, *NH*$_2$), 11.45 (s, 1H, *NH*).

Anal. Calcd. for $C_9H_7N_3S$, 1×HCl, 1×H_2O: C, 44.36; H, 4.14; N, 17.24. Found: C, 44.60; H, 4.25; N, 17.07.

Synthesis of 1-(2-Aminothiazolo[5,4-*b*]indol-4-yl)ethanone **3**.

1-(2-Aminothiazolo[5,4-*b*]indol-4-yl)ethanone (**3**).

A mixture of thiourea (2.8 g, 36.3 mmoles), compound **19** [19] (3.8 g, 18 mmoles) and iodine (4.6 g, 18 mmoles) in absolute ethanol (100 mL) was heated at reflux temperature for 1 hour. After cooling to room temperature the volatiles were evaporated *in vacuo*. The residue was suspended in a mixture of water (100 mL) and ethyl acetate (100 mL) and stirred for 30 minutes. The solid was filtered off, suspended in ethyl acetate (25 mL) and stirred at reflux temperature for 5 minutes. This was repeated with ethanol (40 mL) instead of ethyl acetate. The solid was filtered off, suspended in a mixture of water (20 mL) and diethyl ether (20 mL) and stirred at room temperature for 15 minutes, filtered off and dried *in vacuo* at 50° for 18 hours, which afforded **3** in 8 % yield (0.35 g), mp > 250° Decomp; MS (EI) m/z 231 (M$^+$, 50), 189 (100), 162 (75), 144 (18), 129 (30), 102 (32); ^{1}H nmr (DMSO-d_6): δ 2.77 (bs, 3H), 7.18 (bs, 2H, *NH*$_2$), 7.33 (m, 2H), 7.65 (m, 1H), 8.03 (bs, 1H, Ar-*H*5).

Anal. Calcd. for $C_{11}H_9N_3OS$: C, 57.13; H, 3.92; N, 18.17. Found: C, 56.70; H, 3.90; N, 18.00.

Preparation and Isolation of Isatin (**4**), 6-Imino-12*H*-pyrimido[1,6-*a*:5,4-*b*']diindole-13-one (**5**) and S,S-Dimethyl-13-oxo-12*H*-pyrimido[1,6-*a*:5,4-*b*']diindole-6-imino Sulfonium (**6**).

2-Amino-4*H*-thiazolo[5,4-*b*]indole, **1** (100 mg) dissolved in DMSO (3.0 mL) was heated in a sealed vial at 80° for 16 hours. The components of the resulting dark solution were (in five portions) separated by preparative reversed phase HPLC (Column: YMC RP18 (15 µm), 250 × 20 mm; Gradient: 10 → 50 % CH_3CN (0.05 % TFA) (50 min), 50 → 100 % CH_3CN (0.05 % TFA) (15 minutes), flow rate 15 mL/minute; Detection: UV 210/550 nm). Fractions were analyzed by reversed phase HPLC using a Nucleosil 100 (3 µm, 60 × 4 mm) column eluted with a linear gradient from H_2O (0.1 % H_3PO_4) to CH_3CN over a period of 6 minutes at a flow rate of 2.7 mL/minute, and employing diode array detection at 200-600 nm. Appropriate pooling of fractions yielded isatin, **4** (25 mg), the red compound **5** (25 mg), and the blue compound **6** (4 mg), all obtained as amorphous solids. **5**: ^{1}H- ,^{13}C - and ^{15}N nmr: see table 1. HRFAB-MS: Calcd. for $C_{17}H_{10}N_4O$ 287.0942; Found 287.0945 ([M+H]$^+$). EI-MS: 286 (100, M$^+$), 258 (28, [M-CO]$^+$), 182 (5), 156 (6), 143 (7), 129 (6), 103 (10), 76 (5). ir (potassium bromide): 3398, 3126, 1681, 1640, 1614, 1572, 1203 and 1129 cm^{-1}. uv (λ max nm (log ε)) (MeOH + TFA): 265 (4.20), 313 (3.87), 415 (3.65), and 539 (3.36); (MeOH + NaOH): 281 (4.26), 333 (3.85), 450 (2.98), 605 (3.51), and 640 (3.47). **6:** ^{1}H -, ^{13}C- and ^{15}N nmr: see table 1; HRFAB-MS: Calcd. for $C_{19}H_{15}N_4OS$ 347.0966: Found 347.0979 (M$^+$); ir (potassium bromide): 3436, 1691, 1605, 1200, and 1123 cm^{-1}; uv (λ max nm (log ε)) (MeOH + TFA): 277 (4.33), 328 (3.79), 444 (3.22), and 576 (3.40); (MeOH + NaOH): 249 (4.23), 282 (4.27), 352 (3.85), 458 (3.53) and 750 (3.01).

Enzyme Kinetics.

Determination of Inhibitor Constants, K_{ic}.

The enzyme reactions were carried out using standard conditions essentially as described by Burke *et al*. [21]. The assay conditions were as follows. Appropriately diluted inhibitors (4 different concentrations: diluted 1, 3, 9 and 27 fold in DMSO) were added to microtiter plate wells containing different concentrations of the substrate, *p*-nitrophenyl phosphate (usual range: 0.31 to 20 m*M* - final assay concentration). The buffer used was 100 m*M* sodium acetate pH 5.5, 50 m*M* sodium chloride, 0.1 % (w/v) bovine serum albumin, 5 m*M* DTT (dithiothreitol) and 10 % DMSO from the inhibitor solution (total volume 100 µL). The reactions were started by addition of the enzyme to the wells and were carried out at 25° for 1 hour. The reactions were stopped by addition of NaOH. The enzyme activity was determined by measurement of the absorbance at 405 nm with appropriate corrections for absorbance of the compounds and *p*-nitrophenyl phosphate. The data were analyzed using nonlinear regression hyperbolic fit to classical Michaelis Menten enzyme kinetic models. Inhibition is expressed as K_{ic} values in µ*M*, assuming mixed type inhibition.

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