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Efficient and mild deamination procedure for 1-aminoanthraquinones yielding a diverse library of novel derivatives with potential biological activity

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ABSTRACT

A convenient in situ method is described for reductive removal of the amino group in position 1 of the anthraquinone (AQ) moiety. The reaction proceeds smoothly within a few minutes yielding novel AQ derivatives in excellent yields. Diazonium salt formation is followed by reduction with zinc in ethanol. The method has been applied to a variety of 1-amino-AQ derivatives. It allows access to a large library of new AQ derivatives which possess potential as pharmacological tools for studying purinergic signaling, and as potential drugs, for example, for the treatment of cancer and cardiovascular diseases.

1-Aminoanthraquinone (1-amino-AQ) derivatives (**1–5**, Fig. 1) are an important class of dyestuffs used for the coloring of textiles, including natural and synthetic fibers, and there is a continuous interest in optimizing this class of compounds.[1–4] A wide range of blue colors can be obtained depending on the substitution pattern of the 1-amino-AQ scaffold. 1-Amino-4-bromo-2-sulfoanthraquinone (**6**, bromaminic acid) is a key intermediate for the preparation of 1-amino-AQ derivatives. The presence of an amino group in the 1-position of the anthraquinone moiety along with a *p*-substituted amino function appears to be essential for obtaining the blue color. Syntheses of derivatives of the blue dyes which are lacking the amino group in the 1-position of the anthraquinone moiety have not been investigated yet.

In recent years, the AQ dye Reactive Blue 2 (**1**, RB-2 Fig. 1) as well as other related AQ derivatives, including Acid Blue 25 (**4**) and Acid Blue 129 (**5**) have been found to interact with nucleotide-binding proteins.[5,6] Reactive Blue 2 (**1**) is the classical non-selective purine P2 receptor antagonist.[5,6] Furthermore, RB-2 inhibits ectoenzymes, for example, ecto-5′-nucleotidase (eN, CD73),[7] ecto-nucleoside triphosphate diphosphohydrolase (E-NTPDase1,2,3)[8] and nucleotide pyrophosphatases (NPP1 and NPP3).[9] These ectoenzymes are hydrolyzing extracellular nucleotides (P2 receptor ligands) eventually forming the respective nucleosides, particularly the P1 receptor ligand adenosine.[10–12] Recently we have developed new synthetic methodologies to easily access 1-amino-substituted 4-alkyl-, 4-aryl-, and 4-aralkyl-AQ derivatives.[13–15] Within this class of compounds, we identified and successfully optimized potent and selective P2 receptor antagonists,[16–19] and ecto-nucleotidase inhibitors.[7,8] We found that a negatively charged group, for example, sulfonate or carboxylate, in the 2-position of the anthraquinone moiety is essential for the activity of the compounds, while replacing it by a neutral group such as methyl abolished the activity toward all nucleotide-binding targets that have been tested so far.[7,8,16–19] The potency and target-selectivity of the synthesized compounds were found to be highly dependent on the substituent present in the 4-position of the AQ core.[5–8,13,14,16–19]

So far, synthetic efforts in the field have been mainly directed toward 4-aryl- or 4-(ar)alkyl-amino-substituted 1-amino-AQ derivatives. While the 1-amino group appears to be required for obtaining blue colored compounds, its role for interaction with potential drug targets is yet unknown. 1-Amino-AQ derivatives are easily accessible in large amounts and high yields by microwave-assisted Ullmann coupling reaction of the commercially available 1-amino-AQ derivative bromaminic acid (BAA, **6**, Fig. 1), or its bioisosteric analog **7**, respectively, with the appropriate amines.[13,14] In the present study we developed a method for obtaining a library of AQ derivatives lacking the 1-amino function in order to allow for investigating the role of the amino group with regard to the biological activity of the AQ derivatives.

In the course of our investigations on the structure–activity relationships of AQ derivatives as P2 receptor antagonists and ecto-nucleotidase inhibitors we observed that the significance of the amino group in the 1-position on their biological activity was unknown. The amino group is present in all commercially available

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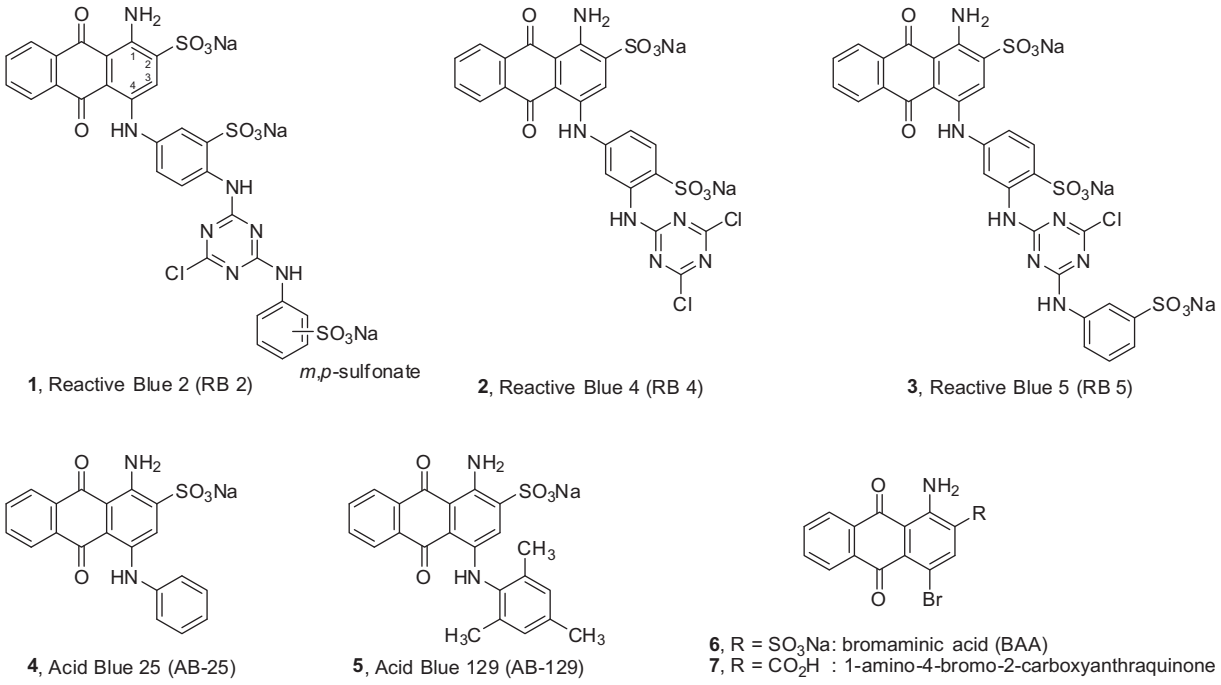


Figure 1. Structures of some commercially available anthraquinone dyes (**1–5**) and 4-bromoanthraquinone derivatives (**6–7**) used for Ullmann coupling reactions.

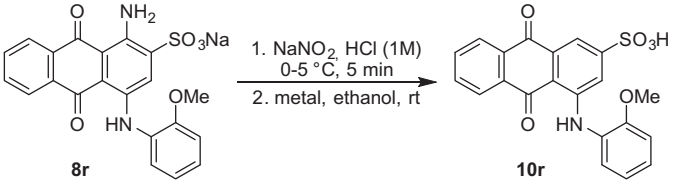
AQs that have been discovered to antagonize P2 receptors and/or inhibit ectonucleotidases (for examples see Fig. 1). It might be required as a hydrogen bond donor for interacting with the target proteins.

Bromaminic acid (**6**) and 1-amino-4-bromo-2-carboxyanthraquinone (**7**) are key intermediates in the preparation of important AQ derivatives, and patents have been filed describing their preparation even on a large scale. However, the syntheses of BAA analogs or RB-2 analogs that are lacking the amino group have so far not been described in the literature. This may be due to difficulties in the synthesis including regioselective bromination and/or sulfonation of the anthraquinone core. We tried to synthesize a derivative of compound **7** which lacks the amino group. Carboxylate **7** was selected as a synthetic target rather than sulfonate **6** since in medicinal chemistry a carboxylate group is a bioisosteric replacement for a sulfonate group. Both functional groups share similar physicochemical properties resulting in potentially similar biological activities, but a carboxylate function confers more drug-like properties, for example, improved bioavailability, to the compound due to its higher pK_a value as compared to the sulfonate group.[20,21]

We started our synthesis from commercially available 2-carboxyanthraquinone and tried to selectively brominate the 4-position by applying several bromination reagents, but all of our attempts failed, mainly due to solubility problems. We therefore synthesized the ethyl ester of 2-carboxyanthraquinone, thereby solving the solubility problem. However, bromination in position 4 of the anthraquinone moiety could not be achieved in a regioselective manner (for more details see Supplementary data). It is much easier to brominate 1-amino-2-sulfo- (or 2-carboxy) anthraquinone to obtain **6** (or **7**) in excellent yields because the presence of the amino group in position 1 of the anthraquinone core will direct the bromination toward the 4-position, while sulfonate or carboxylate functions deactivate the aromatic system because of their electron-withdrawing properties.

We subsequently aimed at deaminating compound **6** (BAA, Fig. 1) to obtain the desired 4-bromo-2-sulfoanthraquinone. First we diazotized the amino function using concentrated sulfuric acid and sodium nitrite in the presence of water, followed by the addition of zinc, ice, and ethanol, and the mixture was left stirring for 2 h at 110 °C.[22] However, only ca. 1% of the desired product was detected by HPLC-MS. The main product was 2-sulfoanthraquinone (80%). Two isomers of hydroxy-2-sulfoanthraquinone were also detected, probably the 1-hydroxy- and the 4-hydroxy-2-sulfoanthraquinone (for more details see Supplementary data). After unsuccessful attempts to synthesize de-amino-BAA (**6**) and the de-amino derivatives of its bioisosteric analog (**7**) we decided to deaminate the final products, the 4-(ar)alkylamino- and anilino-substituted anthraquinone derivatives. At first we explored the effects of some non-noble metals, namely nickel (Ni), copper (Cu), and zinc (Zn), on the reductive deamination of 1-amino-4-(2-methoxyphenyl)-2-sulfoanthraquinone (**8r**, PSB-0716, Table 1), a potent $P2Y_2$ receptor antagonist.[16] Ten equivalents of the applied

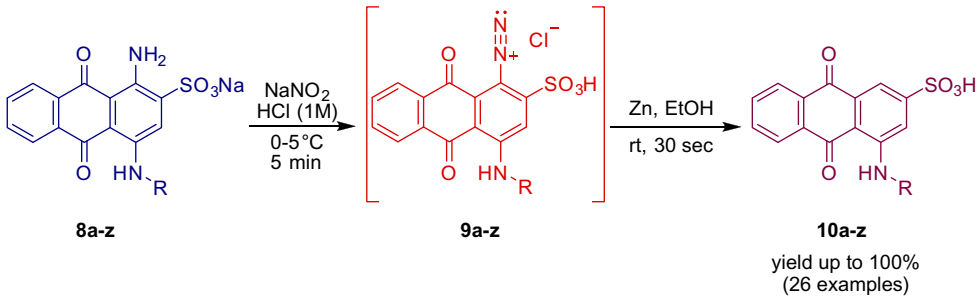
Table 1
Exploring the impact of non-noble metals on the reductive deamination of PSB-0716 (**8r**)[a]



Entry	Metal	Metal equiv	Reaction time (step 2)	Yield[b]
1	Ni	10	30 min	75
2	Cu	10	15 min	77
3	Zn	10	30 s	86
4	Zn	5	3 min	81
5	—	—	7 days	0

[a] Reaction conditions: PSB-716 (45 mg, 0.1 mmol) was dissolved in 5 mL of 1 M HCl then cooled down to 0-5 °C in an ice bath. Then portion-wise addition of $NaNO_2$ (14 mg, 0.2 mmol) dissolved in water (0.5 mL) was added and the mixture was stirred for 5 min. It was then warmed up to rt followed by the addition of the metal and ethanol (5 mL) and left stirring at rt.

[b] Isolated yield.



Scheme 1. Reductive deamination of 1-aminoanthraquinone derivatives.

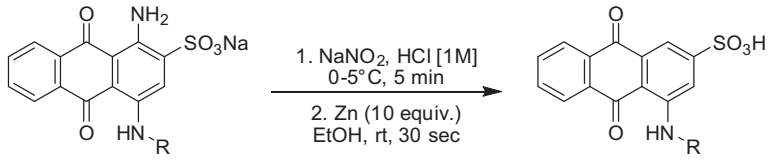
metal were required to reduce the diazonium salt indicated by the evolution of N_2 gas from the intermediate product, and color change of the solution from red to purple. The required reaction time increased in the following sequence: Zn < Cu < Ni (Table 1, entries 1–3).

Thus, Zn was superior to the other metals, and the reaction was completed within less than one minute (ca. 30 s), while Cu and Ni required 15, or 30 min, respectively, for complete conversion. Entry 4 in Table 1 shows 5 equiv of Zn to be sufficient for converting the intermediate into compound **10r** within only 3 min. No reaction was observed in the absence of any metal even after 7 days (Table 1, entry 5). Interestingly the intermediate compound of **8r** (the diazonium salt, **9r**, Scheme 1) appeared to be stable for such a long period of time in the presence of ethanol. After 7 days we added Zn (10 equiv) to the mixture and within less than 1 min the reaction was completed with very high isolated yield (80%).

We found that at least 5 equiv of Zn are essential for completion of the reaction, since 1–4 equiv were not sufficient. Even when the reaction was performed for longer times, stirring the reaction mixture at room temperature for up to 10 h, the intermediate diazonium salt (**9r**), detected by a red spot on the RP-TLC plate, was still observed.

The newly developed method (10 equiv Zn/ethanol system) was then applied to a diverse range of 1-amino-AQ derivatives bearing

Table 2
Synthesis of products **10a-z** via a new reductive deamination protocol using a zinc/ethanol system[a]



Entry	R	Products [**10a–z**]	Yield[b] (%)	Purity[c] (%)	Color[d]
1	Propyl	**10a**	96	98.8	Dark red
2	Isobutyl	**10b**	73	98.9	Brown
3	Cyclohexyl	**10c**	100	98.6	dark brown
4	*p*-Chlorophenethyl	**10d**	79	97.8	Red
5	2′-Hydroxyphenethyl	**10e**	88	99.6	Dark red
6, Acid Blue 25	Phenyl	**10f**	79	100	Purple
7	*p*-Fluorophenyl	**10g**	93	99.1	Dark purple
8	*o*-Chlorophenyl	**10h**	85	100	Dark red
9	*m*-Chlorophenyl	**10i**	81	96.7	Brown
10	*p*-Chlorophenyl	**10j**	85	96.9	Purple
11	*p*-Bromophenyl	**10k**	77	96.5	Purple
12	*m*-Methylphenyl	**10l**	84	95.8	Dark brown
13	*p*-Methylphenyl	**10m**	100	100	Purple
14, Acid Blue 129	2,4,6-Trimethylphenyl	**10n**	60	99.4	Dark red
15	*o*-Ethylphenyl	**10o**	87	99.7	Purple
16	*o*-Hydroxyphenyl	**10p**	76	97.5	Dark purple
17	*p*-Hydroxyphenyl	**10q**	89	98.5	Dark red
18, PSB-0716[16]	*o*-Methoxyphenyl	**10r**	86	95.3	Purple
19	*p*-Acetaminophenyl	**10s**	75	95.0	Purple
20	4′-Chloro-2′-methylphenyl	**10t**	100	99.7	Dark Purple
21, PSB-0952[19]	2′-Carboxy-4′-fluorophenyl	**10u**	75	97.7	Purple
22	2′-Carboxy-4′-hydroxyphenyl	**10v**	57	96.1	Dark purple
23	1-Naphthyl	**10w**	89	96.6	Dark purple
24	2-Naphthyl	**10x**	89	97.4	Purple
25	4″-Fluoro-4′-phenoxyphenyl	**10y**	74	98.7	Purple
26, PSB-0739[17]	4′-Anilino-3′-sulfophenyl	**10z**	87	97.8	Dark purple

[a] Reaction conditions: [26]starting material (**8a–z**, 0.1 mmol) was dissolved in 5 mL of 1 M HCl then cooled down to 0–5 °C in an ice bath. Subsequently $NaNO_2$ (14 mg, 0.2 mmole) dissolved in water (0.5 mL) was added portion-wise, and the mixture was stirred for 5 min. It was then warmed up to rt followed by the addition of ethanol (5 mL) and zinc (65 mg, 1 mmol, 10 equiv) and left stirring at rt for <1 min.
[b] Isolated yield.
[c] Purity measured by HPLC-MS-UV using a diode array detector (DAD) as previously described.[14]
[d] Color of the solid isolated compound.

different substituents in the 4-position of the anthraquinone core including (ar)alkylamino residues (Table 2, entries 1–5) and anilino substituents (Table 2, entry 6–26) with mono-, di-, or tri-substituted phenyl groups (Table 2, entries 6–22), α- or β-naphthyl residues (Table 2,entries 23, 24,) and derivatives with large 4-substituent containing an additional aromatic ring (Table 2, entries 25, 26). Good to excellent isolated yields were obtained (57–100%, Table 2). The reaction can easily be followed by visual detection. All three stages of the reaction have different colors (Scheme 1): the starting materials (**8a–z**) are blue, the intermediate diazonium salts (**9a–z**) are red, while the products show a purple color (**10a–z**).

In conclusion we have developed a novel, fast, mild, and convenient one-pot two-step protocol for the reductive removal of the aromatically bound amino-groups present in the 1-position of 2,4-substituted AQ derivatives using a zinc/ethanol system. Twenty-three new AQ derivatives (Table 2, entries 1, 2, 4, 5, 7–11, 13–26,) were synthesized. Two compounds (Table 2, entries 3 and 12) have been described in one patent reference with neither synthetic procedure nor spectral data provided,[23,24] while one compound of the present series (Table 2, entry 6) had been isolated as a by-product (yield of 1%) in the synthesis of Acid Blue 25 (**8f**, Table 2).[25] The new compounds were obtained in excellent isolated yields (up to 100%). The intermediate diazonium salt (**9r**) was found to be stable for at least several days in ethanol at rt in the absence of metal. The newly developed method has been applied to a series of 1-amino-AQ (26 examples) including pharmacologically active derivatives, such as the potent ecto-5′-NT inhibitor PSB-0952 (**8u**, Table 2), and the $P2Y_{12}$ antagonist PSB-0739 (**8z**, Table 2).[17,18] The new AQ derivatives are now available for biological testing and will be investigated in due course.

Acknowledgments

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Supplementary data

Supplementary (experimental procedures and NMR spectra for all compounds (**10a**–**z**)) data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2012.09.011.

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26. *General deamination procedure for the synthesis of the products **10a–z***
To a 50 mL round-bottomed flask equipped with a magnetic stirring bar was added 1-amino-anthraquinone (1-amino-AQ) derivative (**8a–z**, 0.1 mmol) dissolved in 1 M HCl (5.0 mL) then cooled to 0–5 °C in an ice bath, followed by the drop wise addition of $NaNO_2$ (0.2 mmol, 2 equiv) dissolved in 0.5 mL distilled water while the temperature was kept between 0–5 °C for 5 min. The mixture was then allowed to warm up to rt, and zinc powder (10 equiv) was added together with 5 mL of ethanol, and the reaction was stirred at rt for ca. 30 s. The reaction can easily be followed due to the color change that is observed during the course of the reaction: the starting blue material turns red after diazotization while the final product has a purple color. The reaction was also followed by RP-TLC using acetone: water (2:3) as the eluent. Then the product was purified by flash column chromatography using reversed phase silica gel (RP-18) and water. The polarity of the eluent was then gradually decreased by the addition of acetone in the following steps: 5%, 20%, 40%, and 60%. Fractions containing purple product were collected. For some compounds the last step of purification (RP-18 flash chromatography) had to be repeated twice to obtain pure product (⩾95% purity as determined by LC–MS/UV and elemental analysis, to an accuracy of within ±0.4%). The pooled fractions containing a purple color were collected and evaporated under vacuum until all acetone and most of the water phase were removed. The remaining water was then removed by a freeze dryer to yield products **10a**–**z**.