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# Assessment of mutagenic potential in a series of compounds structurally related to 2-amino-3-methylimidazo [4,5-*f*]quinoline (IQ)

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**Summary** — The bacterial mutagenicity of a series of compounds related to the mutagen 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ) was determined in the Ames test using *Salmonella* strain TA-1538. Thiazolo[4,5-*f*]quinoline-2-amine **3** and its 4,5-dihydro derivative **4**, where the *N*(3)-Me moiety of IQ has been replaced by a sulfur atom, were both potent mutagens. A structure–mutagenicity study was performed in a group of aminothiazole compounds related to **3**. In addition to the aromatic $NH_2$ group, a fully aromatic tricyclic structure is required for mutagenicity. Enzymatic oxidation can provide the required aromaticity to partially reduced analogues, but more extensively reduced compounds, or compounds where the central ring cannot be oxidized, are not mutagenic.

**mutagenicity / Ames / IQ / aminothiazole**

## Introduction

The carcinogenicity of numerous aromatic amines is well established [1]. The ubiquitous presence of this type of compounds [2] and their potential impact on human health [3] justify the study of their biological activity. Especially important is the ability to rationalize and even predict the relationship between the structure of aromatic amines and their mutagenicity/carcinogenicity [4, 5].

Of particular interest among the aromatic amines is a group of amino-imidazo-azaarenes, represented by 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ, **1**) and 2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline (MeIQ, **2**), produced by the pyrolysis of proteins during the cooking of fish and meat [6, 7]. These compounds are among the most potent frameshift mutagens in the *Salmonella* mutagenicity test [8, 9] and have been shown to be genotoxic and tumorigenic in animals [10, 11]. The structure–activity relationships of IQ and related compounds have been extensively studied [12, 13]. Particular interest has been paid to the role of the primary amine, the *N*(3)-methyl group, the *N*(6) nitrogen atom, and the effect of additional methyl substitution throughout the polyaromatic system [14–17]. However, little is known about compounds structurally related to IQ but lacking full aromaticity.

Tricyclic aminothiazoles **3** and **4**, which are very closely related to IQ, have been identified as potent mutagens in *Salmonella typhimurium* strain TA-1538 in the presence of enzymatic activation [18]. These compounds were synthesized in connection with the preclinical development of the antipsychotic compound **5** [19]. Although **5** is not mutagenic in this test, the structural similarity between IQ and some of the synthetic intermediates in the preparation of **5**, including **3** and **4**, led us to investigate the structure–mutagenicity relationship for this type of compound. In addition to exploring whether the same structural requirements for mutagenicity in the amino imidazo azaarenes series (*ie*, IQ) exist in the aminothiazole series, we were particularly interested in the activity of non-aromatic analogues such as **5**. Since, as mentioned above, the mutagenicity of non-aromatic analogues of IQ has not been studied in detail, it was felt that the information gained from this study might be applicable to such compounds as well.

This paper presents our results with a group of aminothiazole compounds (**3–8**, **11** and **13**) in a mutagenicity screen against *Salmonella* strain TA-1538,

the more sensitive strain in our earlier work with **3** and **4** [18]. The key structural features associated with mutagenicity in this bacterial strain are then discussed in the light of these results.

## Chemistry

4,5-Dihydrothiazolo[4,5-*f*]quinolin-2-amine **4** [20], 6-methyl-4,5,5*a*,6,7,8-hexahydrothiazolo[4,5-*f*]quinolin-2-amine **5** [19], 4-(3-pyridinyl)-2-thiazolamine **6** [21], and *trans*-4,5,5*a*,6,7,8,9,9*a*-octahydrothiazolo[4,5-*f*] quinolin-2-amine **7** [20] were synthesized according to published procedures. The syntheses of compounds **3**, **8**, **11**, and **13** are outlined in schemes 1–3. Oxidation of **4** with DDQ in dichloromethane provided the fully aromatic analogue **3** in 94% yield. Reaction of **4** with sodium nitrite in hydrochloric acid, followed by reaction with hypophosphorous acid, produced the deaminated analogue **8** in 33% yield (scheme 1). For the synthesis of **11**, reaction of **9** with acrolein in methanol provided a cyclic adduct that was treated with DBU to produce a mixture of dihydropyridine compounds that was subsequently oxidized with DDQ to give **10** in 61% overall yield. Reaction of **10** with thiourea and iodine at 100°C produced the target compound **11** in 73% yield (scheme 2). Compounds **5** and **12** were prepared by previously described methodology [20], namely reaction of **4** with an alkyl

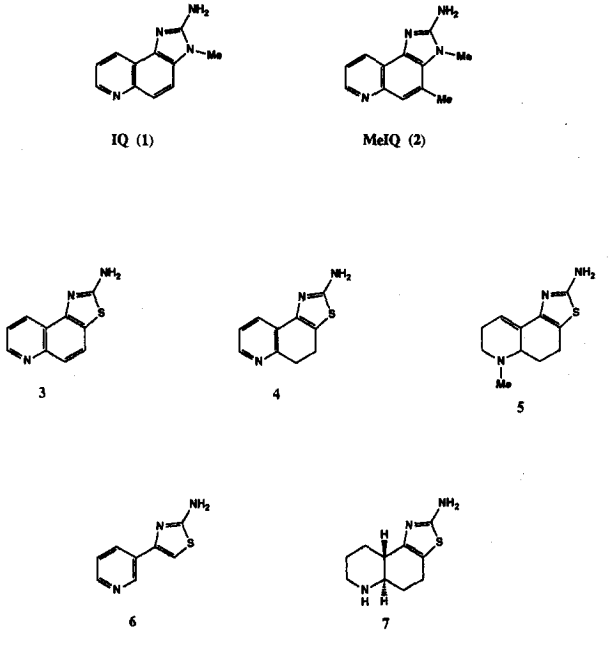
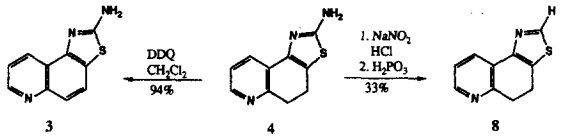
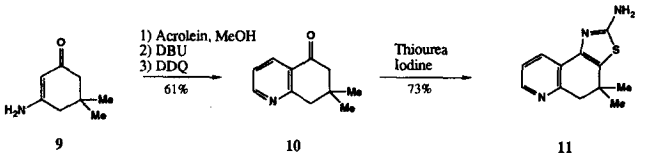


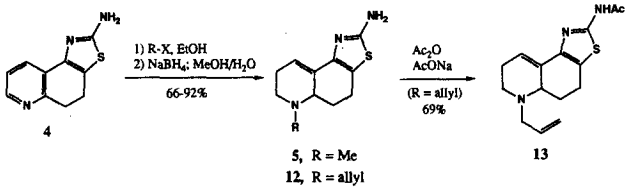

**Fig 1.** Structures of aminothiazole and thiazole compounds.




**Scheme 1.** Synthesis of compounds **3** and **8**.




**Scheme 2.** Synthesis of compound **11**.




**Scheme 3.** Synthesis of compound **13**.

halide, followed by $NaBH_4$ reduction of the corresponding quaternary salt in methanol/water (1:1). Reaction of **12** with acetic anhydride in the presence of sodium acetate provided amide **13** in 69% yield.

## Results and discussion

Table I shows the results obtained with the present series of thiazole compounds. As previously reported, compounds **3** and **4** are mutagenic in TA-1538 (82-fold background at 10 μg/plate, and 22-fold background at 100 μg/plate, respectively) [18]. Fully aromatic aminothiazole **3**, the direct analogue of IQ, is more potent than **4**, which is partially reduced. Thiazole **8**, which lacks the primary amino group, was found to be inactive up to 3000 μg/plate. Compound **11**, an analogue of **4** with geminal dimethyl substitution in the central ring, was found to be weakly mutagenic, with a maximal response 3-fold over background at 316 μg/plate. Aminothiazole **6**, which is structurally related to **3** and **4** but lacks the central ring, was inactive up to 10 000 μg/plate. Compound **5**, a tetrahydropyridine analogue of **3** and **4** is non-mutagenic. Compound **13**, a tetrahydropyridine compound related to **5** but without a free $NH_2$ group, was inactive at all concentrations tested. Finally, **7** which has the pyridine and central rings completely saturated, was also found to be inactive. Thus, out of the 8 compounds tested, only **3** and **4** were found to be

**Table I.** Mutagenicity of test compounds in *Salmonella* strain TA-1538.

| Compd | Conc. (μg/plate) | Revertant Colony Counts | Relative Activity | Compd | Conc. (μg/plate) | Revertant Colony Counts | Relative Activity |
|---|---|---|---|---|---|---|---|
| 3 | 0.0 | 16.7 ± 2.6 | 1.0 | 7 | 0.0 | 30.7 ± 2.6 | 1.0 |
| | 1.0 | 225.0 ± 12.0 | 13.5 | | 1.0 | 25.0 ± 4.6 | 0.8 |
| | 3.2 | 814.0 ± 68.7 | 48.7 | | 3.2 | 24.3 ± 7.3 | 0.8 |
| | 10.0 | 1339.7 ± 148.6 | 80.2 | | 10.0 | 20.0 ± 4.3 | 0.6 |
| | 31.6 | 1351.0 ± 78.9 | 80.9 | | 31.6 | 26.0 ± 2.2 | 0.8 |
| | 100 | 1373.3 ± 160.9 | 82.2 | | 100 | 25.7 ± 9.5 | 0.8 |
| | 316 | 918.7 ± 104.2 | 55.0 | | 316 | 26.7 ± 0.9 | 0.9 |
| | 1000 | 845.7 ± 166.3 | 50.6 | | 1000 | 29.0 ± 6.5 | 0.9 |
| | 3160 | 0.0 ± 0.0 | -- | | 3160 | 34.0 ± 9.9 | 1.1 |
| | 10000 | 0.0 ± 0.0 | -- | | 10000 | 41.7 ± 8.2 | 1.4 |
| 4 | 0.0 | 27.3 ± 2.0 | 1.0 | 8 | 0.0 | 18.3 ± 4.7 | 1.0 |
| | 1.0 | 71.3 ± 13.3 | 2.6 | | 1.0 | 19.3 ± 2.6 | 1.0 |
| | 3.2 | 181.3 ± 12.4 | 6.6 | | 3.2 | 17.3 ± 4.5 | 0.9 |
| | 10.0 | 310.7 ± 7.0 | 11.4 | | 10.0 | 15.0 ± 4.6 | 0.8 |
| | 31.6 | 495.3 ± 29.4 | 18.1 | | 31.6 | 15.0 ± 2.9 | 0.8 |
| | 100 | 605.7 ± 91.7 | 22.2 | | 100 | 24.0 ± 4.6 | 1.3 |
| | 316 | 441.3 ± 57.1 | 16.2 | | 316 | 21.3 ± 2.0 | 1.2 |
| | 1000 | 128.7 ± 47.5 | 4.7 | | 1000 | 29.7 ± 5.7 | 1.6 |
| | | | | | 3160 | 21.3 ± 1.7 | 1.1 |
| | | | | | 10000 | 0.3 ± 0.5 | -- |
| 5 | 0.0 | 19.3 ± 3.7 | 1.0 | 11 | 0.0 | 18.3 ± 4.7 | 1.0 |
| | 625 | 19.3 ± 4.1 | 1.0 | | 1.0 | 24.0 ± 3.3 | 1.3 |
| | 1250 | 27.7 ± 1.7 | 1.4 | | 3.2 | 19.0 ± 2.2 | 1.0 |
| | 2500 | 21.0 ± 2.4 | 1.1 | | 10.0 | 21.3 ± 0.9 | 1.2 |
| | 5000 | 18.3 ± 2.0 | 0.9 | | 31.6 | 21.7 ± 3.8 | 1.2 |
| | 10000 | 23.0 ± 5.0 | 1.2 | | 100 | 41.0 ± 2.2 | 2.2 |
| | | | | | 316 | 62.3 ± 10.9 | 3.4 |
| | | | | | 1000 | 14.7 ± 0.5 | 0.8 |
| | | | | | 3160 | 0.7 ± 0.9 | -- |
| | | | | | 10000 | 0.0 ± 0.0 | -- |
| 6 | 0.0 | 18.3 ± 4.7 | 1.0 | 13 | 0.0 | 19.0 ± 4.1 | 1.0 |
| | 1.0 | 16.7 ± 2.5 | 0.9 | | 0.7 | 22.7 ± 6.0 | 1.2 |
| | 3.2 | 19.7 ± 5.0 | 1.1 | | 2.1 | 22.0 ± 1.4 | 1.2 |
| | 10.0 | 18.0 ± 5.7 | 1.0 | | 6.7 | 22.0 ± 6.4 | 1.2 |
| | 31.6 | 16.0 ± 3.7 | 0.9 | | 21.1 | 24.0 ± 0.0 | 1.3 |
| | 100 | 20.0 ± 3.7 | 1.1 | | 66.7 | 32.0 ± 13.5 | 1.7 |
| | 316 | 20.0 ± 5.0 | 1.1 | | 211 | 35.0 ± 7.8 | 1.8 |
| | 1000 | 20.0 ± 4.6 | 1.1 | | 667 | 40.7 ± 3.1 | 2.1 |
| | 3160 | 15.3 ± 0.9 | 0.8 | | 2110 | 35.3 ± 4.7 | 1.8 |
| | 10000 | 10.7 ± 7.6 | 0.6 | | 6670 | 32.3 ± 0.5 | 1.7 |

potent mutagens in TA-1538, and one compound, **11**, was identified as a very weak mutagen. The relative potency of some of these compounds is better represented in figure 2, which describes the activity of **3**, **4** and **5** in TA-1538. It should be pointed out that even though TA-1538 was the most sensitive strain to the effects of **3** and **4** [18], the rest of the compounds described in this report were not tested in any other strain. Thus, our results do not exclude that compounds found to be inactive in TA-1538 might be active in other strains.

In order to interpret the results presented in this paper, it may be useful to briefly outline the generally accepted mechanisms of aromatic amine mutagenicity. As indicated above, many arylamines (and arylamides) are known mutagens and carcinogens in animals, including man [1]. However, most are not mutagenic prior to metabolic activation. In general, *N*-hydroxylation by microsomal mixed function monooxygenases occurs in various tissues and is widely accepted as a common pathway for the metabolic activation of these compounds to the ultimate carcinogens [25] (see fig 3). The *N*-hydroxy derivatives of aromatic amines such as IQ and MeIQ have been shown to be directly mutagenic without need for further metabolic activation [26–28] and to bind co-valently to DNA *in vitro* [29, 30]. However, there exists ample evidence that the acetylated and/or sulfated forms of

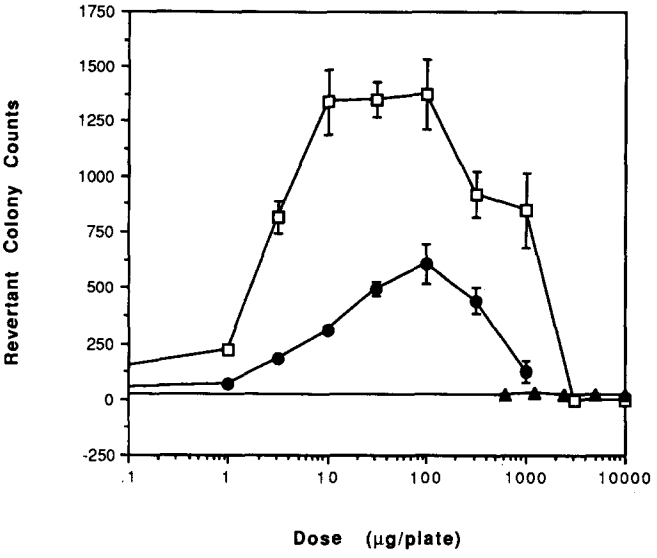



**Fig 2.** *In vitro* mutagenicity of **3–5**; —□—, compound **3**; —●—, compound **4**; —▲— compound **5**.

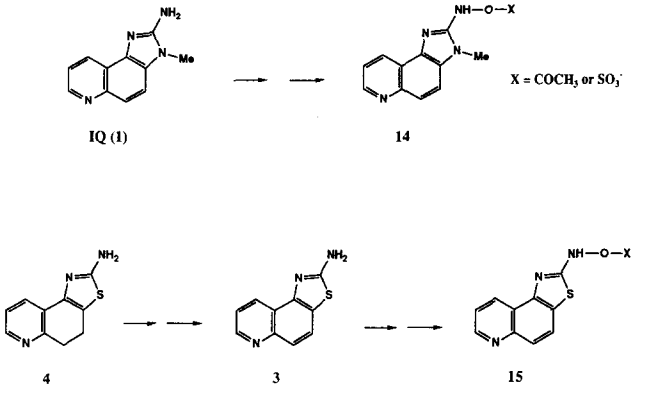
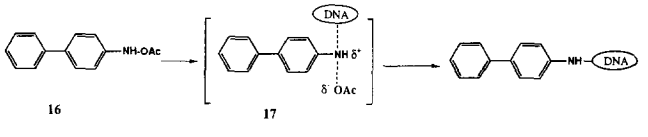



**Fig 3.** Proposed pathway for the enzymatic activation of aromatic amines.

these hydroxylamines (*ie*, **14**) may be the ultimate mutagens in the metabolic activation of aryl amines [31–33]. *N*-Acetoxy arylamines are extremely reactive electrophiles and have been shown to react with nucleosides *in vitro* [34], and to alkylate DNA both *in vivo* and in *vitro* [29, 35]. At the onset of our work it was speculated that the aminothiazole analogues of IQ might be metabolically activated in a manner similar to IQ, and that structural features that affect the formation and electrophilic reactivity of the postulated *N*-acetoxy derivatives would have a major effect on their mutagenicity.

The mutagenic activity of **3** after enzymatic activation can be understood if we consider its structural similarity with IQ. The only difference is the replacement of N-Me with a sulfur atom. *A priori*, this relatively minor change might have been expected to affect the mutagenicity of the compound, since demethylated IQ (replacement of N-Me with N-H) dramatically reduces its mutagenic potency [12, 13]. Based on the accepted mechanism of enzymatic activation of IQ, we postulate the *N*-acetoxy species **15** as the likely ultimate mutagen from **3** (fig 3). The mutagenicity of **4**, on the other hand, could be due to its *N*-acetoxy derivative or to the same intermediate **15** postulated for **3**. This would require enzymatic oxidation of the central ring in **4**. We favor the notion of the fully aromatic intermediate, since **11**, the *gem*-dimethyl analogue of **4**, is only weakly mutagenic. In this case, since metabolic oxidation of the central ring is not feasible without major structural rearrangement, we may assume that the weak mutagenicity expressed by **11** probably represents the effect that would be observed for **4** if it were not metabolically oxidized to **3**. The importance of the oxidized central ring will be discussed later in this paper. The non-mutagenicity of **8** is consistent with the lack of activity of desamino-IQ [14, 15]. Since these compounds are no longer arylamines, they cannot form the activated *N*-acetoxy type of intermediate.

Bicyclic analogue **6**, lacking a central ring, is also non-mutagenic. Subtle structural changes to the central ring of these aminothiazoles should not affect enzymatic *N*-hydroxylation and subsequent acetyl (or sulfonyl) group transfer. The basic ring frameworks of **3**, **4**, **6** and **11** are quite similar. The pyridine and thiazole rings of **3**, **4** and **11** are locked in a fairly rigid coplanar (or near coplanar) arrangement. Based on our own molecular modeling with compounds related to **6**, a near-coplanar conformation should also be energetically favored for this compound [21]. Thus, we may assume that the drastic differences in mutagenicity observed between **3** and **4**, on the one hand, and **6** and **11** on the other, are probably not due to major differences in the ability to form the corresponding *N*-acetoxy or *N*-sulfonyloxy derivatives, but rather due to differences in the electrophilic reactivity of these postulated intermediates.




**Scheme 4.** DNA alkylation.

While 4-aminobiphenyl is extremely mutagenic following enzymatic activation, 3-aminobiphenyl is non-mutagenic [36]. This appears to be due not to differences in the extent of oxidative activation of the amine group of these compounds, but rather to a decreased electrophilic reactivity of the activated *N*-oxidized form of 3-aminobiphenyl [36]. This suggests that the process of DNA alkylation by aromatic *N*-acetoxy species is very sensitive to structural features that stabilize a transient partial positive charge on the nitrogen atom. In the biphenyl examples, this appears to be the main determining factor for the electrophilic reactivity of these species. These observations suggest that DNA alkylation by aromatic *N*-acetoxy compounds may possess some $S_N1$ character. Thus, as shown in scheme **4**, a partial positive charge on the aniline nitrogen atom can be better delocalized, and hence stabilized, by a *para* phenyl than a *meta* phenyl substituent. This increased stabilization may account for the higher mutagenicity of **16** over its *meta* isomer. It may be possible to discuss the structure/mutagenicity relationship of other aromatic amines in a similar manner. That is, structural elements that facilitate the delocalization of the developing positive charge on nitrogen during the $N^{(+)}\text{---}^{(-)}OAc$ bond cleavage in metabolically derived *N*-acetoxy species, *ie* **17** should contribute to the reactivity of such compounds. This greater reactivity will translate into a greater level of DNA alkylation and, ultimately, increased mutagenicity and/or carcinogenicity.

As shown in figure 4, the partial positive charge that may develop on the nitrogen atom during alkylation of *N*-acetoxy-IQ is greatly delocalized over all 3 rings. The extensive charge delocalization in $[IQ]^{\delta+}$ may account for the high reactivity of IQ. In a similar manner, the corresponding ion derived from **3** may be stabilized by charge delocalization over all 3 rings (fig 4), and this may account for its high mutagenicity. When the pyridine and central rings of the tricyclic aminothiazole compounds are not aromatic (**5**, **7** and **11**) or, as in the case of **6**, the central ring is missing,

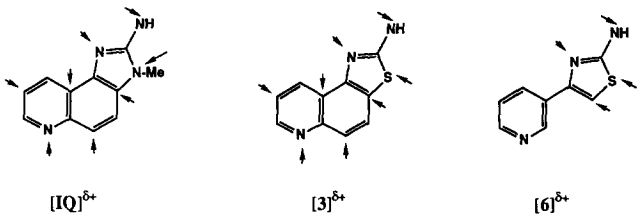



**Fig 4.** Charge delocalization in some postulated transition states. (The arrows indicate the atoms that will contribute to the delocalization of a partial positive charge generated at the amine nitrogen.)

the positive charge would appear to be delocalized only into the thiazole ring, a situation similar to 3-aminobiphenyl, and this would account for the complete lack of mutagenicity observed with these compounds. Our results also suggest that the tetrahydropyridine or piperidine rings of compounds such as **5**, **7** and **13** are not readily oxidized, under the assay conditions, to the corresponding pyridine ring. The lack of activity of **13**, an acetylated compound that might be expected to become deacetylated during S9 activation, may also result from the lack of metabolic oxidation of the tetrahydropyridine ring.

## Conclusion

In spite of the large number of mutagenic aromatic amines described in the literature, our results indicate that there exist very tight structural requirements for mutagenicity within the thiazole analogues of IQ described here. Compounds **3** and **4** were found to be potent mutagens, the latter presumably by oxidation to the former. With the exception of **11**, which was weakly active, all other compounds tested were found to be nonmutagenic. The structure–mutagenicity relationship established for **3** and other tricyclic aminothiazoles suggests that structural features that facilitate charge delocalization during reaction of the presumed ultimate mutagen with DNA are an important determinant of mutagenicity. These structural requirements for mutagenicity include an amine group and a fully aromatic tricyclic structure, or the ability to aromatize under enzymatic activation. Our results confirm that while some aromatic amines are extremely potent mutagens, very closely related analogues may be inactive.

## Experimental procedures

Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Proton NMR spectra were recorded on an IBM WP100SY NMR spectrometer (100 MHz) or a Varian XL200 NMR spectrometer (200 MHz) and were consistent with the proposed structures. The peaks are described in ppm downfield from TMS (internal standard). The mass spectra were obtained on a Finnigan 4500 mass spectrometer or a VG analytical 7070E/HF mass spectrometer; the spectra are described by the molecular peak (M) and its relative intensity as well as the molecular weight of the base peak (100%). Elemental analyses were performed by the Analytical Research Section at Parke-Davis (Ann Arbor, MI); analyses indicated by the symbols of the elements were within ± 0.4% of the theoretical values. Medium pressure liquid chromatography (MPLC) was performed on silica gel, E Merck, grade 60, 230–400 mesh, 60 A, with a RB-SY pump (FMI).
*Warning:* As indicated above, compounds **3** and **4** are strong mutagens in the Ames assay and should be handled with extreme caution.

*Thiazolo[4,5-f]quinolin-2-amine **3***
A mixture of **4** [20](5.0 g; 24.6 mmol) and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (5.7 g; 24.6 mmol) in 500 ml dichloromethane was stirred at room temperature under a nitrogen atmosphere for 2 h. The mixture was made basic with $NH_4OH$ and the solid formed was filtered. From the filtrate, the organic layer was dried ($MgSO_4$) and evaporated. The residue was pooled with the filtered solid to give 4.63 g (94%) of crude **3**. A sample of this crude material was recrystallized from ethanol/ethyl acetate to give **3**, mp: 250–253°C (dec). $^1$H-NMR (DMSO–$d_6$): 7.55 (dd, $J$ = 8.6 and 4.2 Hz, [$H_8$]); 7.67 (d, $J$ = 9.0 Hz, [$H_5$]); 7.80 (s, [2 x $NH_2$]); 8.06 (d, $J$ = 9.0 Hz, [$H_4$]); 8.68 (dd, $J$ = 8.6 and 1.7 Hz, [$H_9$]); 8.86 (dd, $J$ = 4.2 and 1.7 Hz, [$H_7$]). MS: 201 (M, 100). Anal($C_{10}H_7N_3S$•O.1$H_2O$).

*4,5-Dihydrothiazolo[4,5-f]quinoline **8***
An ice-cold solution of 1 N sodium nitrite (74 ml; 74 mmol) was added dropwise to a solution of **4** [20](10.0 g; 49.2 mmol) in 250 ml concentrated HCl at –10°C. The mixture was stirred at –10°C for 1 h, and an ice-cold solution of 30% hypophosphoric acid (13.5 ml; 61.4 mmol) was added dropwise. The mixture was allowed to warm up to room temperature overnight. The reaction mixture was cooled in an ice bath and carefully basified with $NH_4OH$. The product was extracted into chloroform (3 x 500 ml). The combined organic extracts were dried ($MgSO_4$), filtered and concentrated. The crude product was purified by medium pressure chromatography (2% methanol in chloroform) to give 3.14 g (33%) of **8**, mp: 77–79°C. $^1$H-NMR ($CDCl_3$): 3.07–3.12 (m, [2 x $H_5$]); 3.17–3.22 (m, [2 x $H_4$]); 7.14 (dd, $J$ = 7.7 and 4.9 Hz, [$H_8$]); 8.07 (dd, $J$ = 7.7 and 1.6 Hz, [$H_9$]); 8.31 (dd, $J$ = 4.9 and 1.6 Hz, [$H_7$]); 8.62 (s, [$H_2$]). MS: 188 (M, 100). Anal($C_{10}H_8N_2S$).

*7,8-Dihydro-7,7-dimethyl-5(6H)-quinolinone **10** [22]*
Acrolein (32 ml; 0.479 mol) was added dropwise to an ice-cold solution of 3-amino-5,5-dimethylcyclohexen-2-one **9**, obtained from Aldrich Chemical Co (59.9 g; 0.430 mol) and 2 ml glacial acetic acid in 600 ml methanol. The mixture was stirred at room temperature overnight. To the reaction mixture was added 1,8-diazobicyclo[5.4.0]undec-7-ene (65 ml; 0.435 mol), and the reaction flask was equipped with a distillation head. The methanol was slowly distilled off and gradually replaced with 4-methyl-2-pentanone. Heating was continued until the temperature of the distillate reached 110°C. To the cooled solution was added 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (98.7 g; 0.435 mol), the mixture was stirred at room temperature for 1 h, and filtered through a pad of Celite. The filtrate was concentrated and dissolved in 500 ml 10% HCl. After washing with ethyl acetate (2 x 500 ml), the aqueous solution was cooled, made basic with $NH_4OH$, and extracted with ethyl acetate. The organic extract was washed with brine, dried ($MgSO_4$), and evaporated. The residue was purified by medium pressure chromatography (5% methanol in chloroform) to give 46.2 g of **10** (61% yield) as a light brown oil. $^1$H-NMR ($CDCl_3$): 1.03 (s, [6 x $CH_3$]); 2.47 (s, [2 x $H_6$]); 2.96 (s, [2 x $H_8$]); 7.21 (dd, $J$ = 7.7 and 4.7 Hz, [$H_3$]); 8.17 (dd, $J$ = 7.7 and 1.8 Hz, [$H_4$]); 8.62 (dd, $J$ = 4.7 and 1.8 Hz, [$H_2$]). MS: 175 (M, 69); 119 (100). Anal($C_{11}H_{13}NO$).

*4,5-Dihydro-4,4-dimethylthiazolo[4,5-f]quinolin-2-amine **11***
A mixture of **10** (8.65 g; 49.4 mmol), thiourea (11.27 g; 148.1 mmol) and iodine (18.79 g; 74.0 mmol) was heated in a steam bath, under a nitrogen atmosphere for 4 h. The mixture was suspended in 250 ml hot 10% HCl, and filtered through Celite. The filtrate was made basic with $NH_4OH$ and extracted

into chloroform. The organic extract was dried ($MgSO_4$) and concentrated *in vacuo* to give 8.30 g (73%) of **11**. The crude product was recrystallized from acetonitrile, mp: 223–226°C. $^1$H-NMR (DMSO–$d_6$): 1.20 (s, [6 x $CH_3$]); 2.97 (s, [2 x $H_5$]); 7.10 (s, [2 x $NH_2$]); 7.23 (dd, $J$ = 7.7 and 4.9 Hz, [$H_8$]); 7.77 (dd, $J$ = 7.7 and 1.7 Hz, [$H_9$]); 8.27 (dd, $J$ = 4.9 and 1.7 Hz, [$H_7$]). MS:231 (M,16); 216 (100). Anal($C_{12}H_{13}N_3S$).

*6-(2-Propenyl)-4,5,5a,6,7,8-hexahydrothiazolo[4,5-f]quinolin-2-amine* ***12***

This compound was prepared in 66% yield from **4** by the literature procedure [20]. Mp: 174–183°C. $^1$H–NMR (DMSO–$d_6$): 1.26–1.43 (m, [1 x $H_5$]); 1.98–2.19 (m, [1 x $H_5$]); 2.23–2.35 (m, [2 x $H_8$ and 1 x $H_4$]); 2.56–2.65 (m, [1 x $H_4$ and 1 x $H_7$]); 2.84–3.00 (m, [2 x N-$CH_2$-CH = $CH_2$ and 1 x $H_7$]); 3.53 (dd, $J$ = 14.3 and 4.8 Hz, [$H_{5a}$]); 5.15 (d, $J$ = 10.4 Hz, [1 x $CH_2$-CH = $CH$); 5.22 (d, $J$ = 17.1 Hz, [1 x $CH_2$-CH = $CH$); 5.81–5.97 (m, [$CH$ = $CH_2$]); 6.08 (d, $J$ = 5.3 Hz, [$H_9$]); 6.78 (broad s, [2 x $NH_2$]). MS: 247 (M, 50); 178 (100). Anal($C_{13}H_{17}N_3S$).

*N-[4,5,5a,6,7,8-Hexahydro-6-(2-propenyl)thiazolo[4,5-f]quinolin-2-yl)acetamide* ***13***

A suspension of **12** (10.0 g; 40.4 mmol) and anhydrous sodium acetate (6.6 g; 80.4 mmol) in 200 ml acetic anhydride was refluxed under nitrogen for 3 h (upon heating, all solids went into solution). The mixture was concentrated *in vacuo*. The solid residue was taken up into chloroform, washed with 5% $NH_4OH$, dried ($MgSO_4$), filtered and concentrated. The residue was purified by medium pressure chromatography (silica; 2% methanol in chloroform) to give 8.1 g (69%) of **13** as a tan solid, mp: 226–228°C (dec). $^1$H-NMR ($CDCl_3$): 1 .53–1.74 (m, [1 x $H_5$]); 2.10 (s, [3 x $CH_3$]); 2.13–2.89 (m, [1 x $H_5$, 1 x $H_4$, and 2 x $H_8$]); 2.93–3.13 (m, [1 x $H_4$, 2 x $H_7$, and 2 x N-$CH_2$-CH = $CH_2$]); 3.63 (dd, $J$ = 14.1, 4.8 Hz, [$H_{5a}$]); 5.19–5.29 (m, [2 x $CH_2$-CH = $CH_2$]); 5.87–6.07 (m, [$CH$ = $CH_2$]); 6.33–6.35 (broad s, [$H_9$]); 11.02 (s, [NH]). MS: 289 (M, 46); 220 (100). Anal($C_{15}H_{19}N_3OS$).

In order to rule out the possibility of mutagenic impurities in the test samples, the purity of compounds found active in the Ames test was routinely assayed by HPLC (Supelco LC18DB–5 μ (4.6 x 250 mm) column; mobile phase: 0.05 M $(NH_4)H_2PO_4$/methanol (58:42), adjusted to pH 3.5 with $H_3PO_4$).

*Biological assays*

Ames tests were conducted with metabolic activation in strain TA-1538, the most sensitive bacterial strain to this class of compounds, as determined in earlier studies [18]. This strain, which detects frameshift mutations, was obtained from B Ames (Berkeley, CA) and stored at –80°C. The master culture was regrown from a frozen stock and checked for retention of strain characteristics for each test. The tests were conducted according to standard methods [23, 24]. Metabolic activation was provided by plate incorporation of an Aroclor 1254 induced rat liver microsomal fraction (S9) purchased from Organon Technika Corp and was included at a concentration of 10 μl/plate. Drugs, bacteria, S9 fraction, and top agar were plated on top of Ames minimal agar (Vogel–Bonner E broth containing 2% dextrose and 1.5% Noble agar). The resulting colonies were counted by image analysis after incubation at 37°C for 72 h. A compound was considered mutagenic if the response exceeded 3-fold background in a concentration-dependent manner. All compounds were stored at room temperature in amber bottles. Drugs were dissolved in dimethyl sulfoxide (DMSO) and tested at concentrations up to 10 mg/plate or the limits of solubility.

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## References

1 Radomski JL (1979) *Annu Rev Pharmacol Toxicol* 19, 129–157
2 Weisburger EK, Russfield AB, Homburger F, Weisburger JH, Boger E, Van Dongen CG, Chu KC (1978) *J Environ Pathol Toxicol* 2, 325–356
3 Kriek E (1979) *Environmental Carcinogenesis* (Emmelot P, Kriek E, eds) Elsevier/North-Holland Press, Amsterdam, 143-164
4 Yuta K, Jurs PC (1981) *J Med Chem* 24, 241–251
5 Klopman G, Frierson MR, Rosenkranz HS (1985) *Environ Mutagen* 7, 625–644
6 Sugimura T (1982) *Cancer* 49, 1970–1984
7 Sugimura T, Sato S (1983) *Cancer Res* 43(suppl), 2415s–2421s
8 Kasai H, Yamaizumi Z, Wakabayashi K, Nagao M, Sugimura T, Yokoyama S, Miyazawa T, Spingarn N, Weisburger JH, Nishimura S (1980) *Proc Jpn Natl Acad B* 56, 278-283
9 Kasai H, Yamaizumi Z, Shiomi T, Yokoyama S, Miyazawa T, Wakabayashi K, Nagao M, Sugimura T, Nishimura S (1981) *Chem Lett* 485–488
10 Ohgaki H, Kusama K, Matsukura N, Morino K, Hasegawa H, Sato S, Takayama S, Sugimura T (1984) *Carcinogenesis* 5, 921–924
11 Wild D, Gocke E, Harnasch D, Kaiser G, King MT (1985) *Mutat Res* 156, 93-102.
12 Wild D, Kaiser G, King MT, Harnasch D (1986) *Genetic Toxicology of the Diet.* Alan R Liss, NY, 145–154
13 Kaiser G, Harnasch D, King M-T, Wild D (1986) *Chem Biol Interactions* 57, 97–106
14 Barnes WS, Lovelette CA, Tong C, Williams GM, Weisburger JH (1985) *Carcinogenesis* 6, 441–444
15 Grivas S, Jagerstad M (1984) *Mutat Res* 137, 29–32
16 Nagao M, Wakabayashi K, Kasai H, Nishimura S, Sugimura T (1981) *Carcinogenesis* 2, 1147–1149
17 Jagerstad M, Grivas S (1985) *Mutat Res* 144, 131–136
18 Kropko ML, Jaen JC, Theiss JC, Wold S, Caprathe BW, Wise LD (1992) *Mutat Res Lett* 281, 233–238
19 Jaen JC, Caprathe BW, Wise LD, Pugsley TA, Meltzer LT, Heffner TG (1991) *Bio Med Chem Lett* 1, 539–543
20 Caprathe BW, Jaen JC, Wise LD, Heffner TG, Pugsley TA, Meltzer LT, Parvez M (1991) *J Med Chem* 34, 2736–2746
21 Jaen JC, Wise LD, Caprathe BW, Tecle H, Bergmeier S, Humblet C, Heffner TG, Meltzer LT, Pugsley TA (1990) *J Med Chem* 33, 311–317
22 Curran ACW (1976) *J Chem Soc Perkin Trans 1*, 975–977
23 Maron DM, Ames BN (1983) *Mutat Res* 113, 173–215
24 Belser WL, Shaffer SD, Bliss RD, Hynds PM, Yamamoto L, Pitts JN, Winer JA (1981) *Environ Mutagen* 3, 123-139
25 Kato R, Kamataki T, Yamazoe Y (1983) *Environ Health Perspect* 49, 21–25
26 Okamoto T, Shudo K, Hashimoto Y, Kosuge T, Sugimura T, Nishimura S (1981) *Chem Pharm Bull* 29, 590–593
27 Yamazoe Y, Shimada M, Kamataki T, Kato R (1983) *Cancer Res* 43, 5768–5774

28 Niwa T, Yamazoe Y, Kato R (1982) *Mutat Res* 95, 159–170
29 Snyderwine EG, Wirth PJ, Roller PP, Adamson RH, Sato S, Thorgeirsson SS (1988) *Carcinogenesis* 9, 411–418
30 Okamoto T, Shudo K, Hashimoto Y, Kosuge T, Sugimura T, Nishimura S (1981) *Chem Pharm Bull* 29, 590–593
31 Nagao M, Fujita Y, Wakabayashi K, Sugimura T (1983) *Biochem Biophys Res Commun* 114, 626–631
32 Shinohara A, Saito K, Yamazoe Y, Kamataki T, Kato R (1985) *Carcinogenesis* 6, 305–307
33 Lai C-C, Miller EC, Miller JA, Liem A (1988) *Carcinogenesis* 9, 1295–1302
34 Famulok M, Bosold F, Boche G (1989) *Tetrahedron Lett* 321–325
35 Hashimoto Y, Shudo K, Okamoto T (1980) *Biochem Biophys Res Commun* 96, 355–362
36 Ioannides C, Lewis DFV, Trinick J, Neville S, Sertkaya NN, Kajbaf M, Gorrod JW (1989) *Carcinogenesis* 10, 1403–1407