Journal of Medicinal Chemistry

Article

pubs.acs.org/jmc

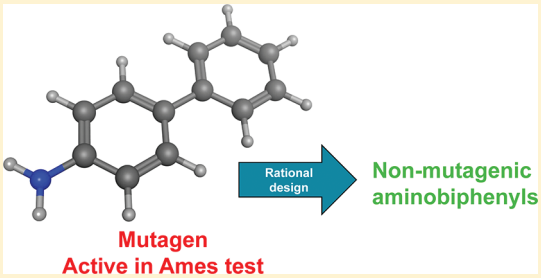
Rationally Designing Safer Anilines: The Challenging Case of 4-Aminobiphenyls

Alan M. Birch, Sam Groombridge, Robert Law, Andrew G. Leach,* Christine D. Mee, and Carolin Schramm

AstraZeneca Pharmaceuticals, Mereside, Alderley Park, Macclesfield, SK10 4TG, U.K.

Supporting Information

ABSTRACT: We describe how we have been able to design 4-aminobiphenyls that are nonmutagenic (inactive in the Ames test). No such 4-aminobiphenyls were known to us, but insights provided by quantum mechanical calculations have permitted us to design and synthesize some examples. Importantly, the quantum mechanical calculations could be combined with predictions of other properties of the compounds that contained the 4-aminobiphenyls so that these remained druglike. Having found compounds that are not active, the calculations can provide insight into which factors (electronic and conformational in this case) are important. The calculations provided SAR-like information that was able guide the design of further examples of 4-aminobiphenyls that are not active in the Ames test.



INTRODUCTION

During the course of a recent drug discovery program aimed at finding potential treatments for diabetes, a chemical series containing an embedded 4-aminobiphenyl was found to have a number of attractive properties. However, 4-aminobiphenyl itself is a known human carcinogen and many related compounds are found to be active in a bacterial mutagenicity (Ames) test. A compound containing an Ames active aromatic amine was not considered acceptable for this indication, and so 4-aminobiphenyls were sought that were not active. In recent studies it had been found that the electrophilicity (computed with quantum mechanics) of metabolites derived from aromatic amines correlates with the likelihood of the aromatic amines being active in the Ames test.[1] The insight provided by these calculations has permitted us to design, make, and assess the first 4-aminobiphenyls that we are aware of that are not active in the Ames test. More importantly, this has been done in a rational fashion and has incorporated a view of the predicted properties of the compound that would contain the 4-aminobiphenyl. Hence, the physical and pharmaceutical properties of target molecules were not sacrificed in order to remove potential mutagenic metabolites. We believe that this provides a new paradigm for designing safer compounds while remaining within druglike chemical space.

BACKGROUND

Many marketed and candidate drug molecules contain amino-substituted aromatic substructures, occasionally with a free amino group but most commonly where the amine has been incorporated into amide or sulfonamide groups or into a heterocyclic ring. Amino aromatics may give rise to safety concerns, as many have been shown to be mutagenic in the Ames test and therefore may be carcinogenic in animals.[2] In the situation where an Ames positive amino aromatic is embedded in a more complex structure, concern persists that the free amine could be liberated through common metabolic processes such as amidolysis and N-dealkylation. It is therefore preferable to ensure that only safe aromatic amines are embedded in potential drugs.

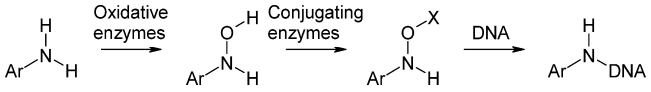
The Ames test is part of the standard test battery of genetic toxicity tests in the International Conference on Harmonisation set of technical requirements for registration of pharmaceuticals for human use and, therefore, is required by regulators worldwide to support clinical trials and registration.[3] In this test,[4−6] compounds are applied to strains of *S. typhimurium* or *E. coli* that have been mutated so that they are unable to grow in the absence of a particular exogenous amino acid. In the two-strain test reported here, the TA98 and TA100 strains of *S. typhimurium* require histidine to grow. Compounds that cause particular mutations of these bacteria are able to cause reversion to the state that is able to produce histidine. Hence, exposure of these bacteria to mutagens is manifest as a colony that is able to grow in a histidine free environment. In particular, TA98 detects frameshift mutations while TA100 detects base-pair substitution mutations.

Many compounds, including most aromatic amines, are not inherently mutagenic, but metabolism generates species that are inherently mutagenic. This is achieved in the Ames test by incorporating S9 fraction from the livers of rats in which metabolic activity has been induced; in this study, this employed Aroclor 1254.[6,7] Aromatic amines are generally thought to yield mutagens after N-hydroxylation often with subsequent O-conjugation to create an electrophilic species

Received: January 30, 2012
Published: April 4, 2012

(such as an ester, sulfate, or phosphate) that is able to react with DNA (Scheme 1).[8−15]

Scheme 1. Cascade of Reactions That Is Postulated to Link Aromatic Amines with DNA Damage and Mutation (X = COR, SO_2R, $PO(R)_2$, etc.)



4-Amino-1,1′-biphenyl compounds give rise to particular concern, as the parent molecule **1a** (Figure 1) is an established

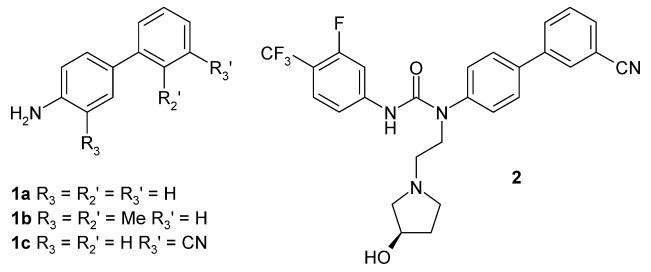


Figure 1. Some 4-aminobiphenyls that have been described as causing mutagenesis and related genetic toxicity and an MCH-1 compound that was abandoned.

human carcinogen[2] and 4-amino-2′,3-dimethyl-1,1′-biphenyl **1b** has been used extensively to induce a variety of cancers in rodent models.[16] Workers at Schering-Plough reported a series of biarylurea MCH-1 antagonists in which one promising compound **2** contained embedded the 4-amino-3′-cyano derivative **1c**.[17] The free aniline **1c** was found to be highly mutagenic in the Ames test, so compound **2** was abandoned even though in vivo drug metabolism studies showed no evidence for liberation of **1c**. It was decided that any risk of aminobiphenyl exposure, regardless of extent, was unacceptable in the context of antiobesity therapy. A number of other aminobiphenyls were described in the Organon data set disclosed by Bentzien et al. and illustrated in Figure 2.[18] All

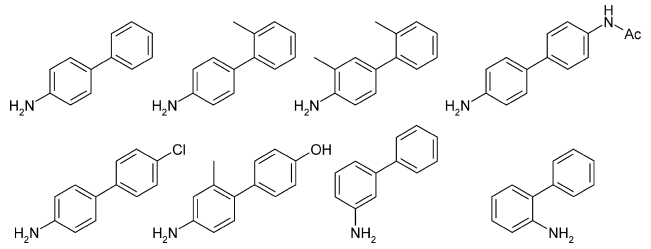


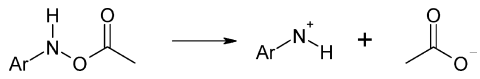
Figure 2. Aminobiphenyls reported by Bentzien et al. to be active in an Ames test.[18]

of these compounds were described as positive in the Ames test, suggesting that within this particular chemical class it is particularly challenging to avoid mutagenicity; indeed no aminobiphenyls were known to us that were not positive in an Ames test.

In previous diabetes programs at AstraZeneca in which compounds with embedded aromatic amines have caused concern, the approach taken to remove the risk of mutagenicity has been to exchange the aromatic amine for others that are known to not cause a response in the Ames test, either employing examples known from the literature or by exhaustive profiling of putative aromatic amine metabolites.[19] As a consequence, the properties of the resulting compounds in which the aromatic amines are embedded have often been dictated by the tolerated aromatic amines. It would be preferential to be able to design compounds whose risk of embedding Ames positive aromatic amines has already been mitigated. In this way, a more holistic view of the properties of any designed compound can be taken. Researchers at Bristol-Myers Squibb have disclosed how predictions from a QSAR based model could be used to guide design, although it is difficult to have such an approach suggest what compound to make next (the inverse QSAR problem).[20]

In 2009, Leach et al. described how the probability of an aromatic amine being active in the Ames test could be correlated with the dissociation energy for the process shown in Scheme 2.[1] Similar correlations have been found by

Scheme 2. Dissociation Process Studied by Quantum Mechanical Calculations To Model Mutagenicity in the Ames Test



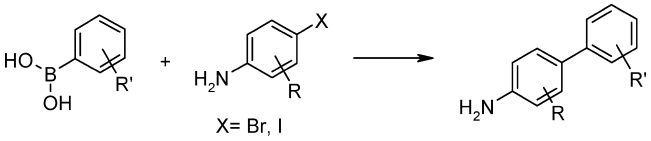
others.[13,14,18,21,22] It is possible that Scheme 2 represents the relevant rate-limiting step in the biochemical process that leads to mutation in the assay conditions. It is equally possible that this reaction energy simply provides a general view of the electrophilicity of the assortment of metabolites that might be formed from any given aromatic amine and that might react through a range of mechanisms. In particular, the acetate ester had been selected for computational convenience as a surrogate for O-conjugates that are good leaving groups such as esters in general, sulfates, and phosphates. Other calculations performed by Leach et al. sought to model the liability toward cytochrome P450 mediated oxidation and the reactivity of the nitroso compound that results from further oxidation of the hydroxylamine. Neither of these was found to be as reliable a guide to activity in the Ames test as the reaction in Scheme 2. From the perspective of compound design it is important to note that this property has been found to correlate with activity in the Ames test more significantly than any other individual descriptor and that it is chemically understandable such that compounds can be proposed in a rational way to modulate it. In this report we disclose how this approach was applied to design nonmutagenic (Ames negative) aminobiphenyls.

■ COMPOUND SYNTHESIS

Compounds that were evaluated in the Ames test (Table 2) were prepared by Suzuki−Miyaura coupling (Schemes 3−5) using microwave irradiation. The boronic acids described by the generic structure in Scheme 3 were in several cases unavailable or gave poor results in the protocol described. In these cases they were substituted by commercial trifluoroborate salts (Scheme 5) or boron pinacol esters synthesized from the corresponding aryl halides (Scheme 4). These reagents have been shown to slowly release the free boronic acid into the reaction mixture, thereby minimizing its degradation.[23] Both aniline and nitrobenzene starting materials were utilized with, in the latter case, hydrogenation of the crude nitrobiphenyl, yielding the aminobiphenyl product.

dx.doi.org/10.1021/jm3001295 | J. Med. Chem. 2012, 55, 3923−3933

Scheme 3. Synthesis of Compounds Using Methods A and B[a]



[a]Reagents and conditions: Method A: $PdCl_2(dppf)-CH_2Cl_2$, K_3PO_4, DME, EtOH, H_2O. Method B: premixed $PdCl_2(dtbpf)/K_3PO_4$, MeCN, H_2O.

RESULTS AND DISCUSSION

During the course of a drug discovery project aimed at the production of an antidiabetic compound, optimization was performed on a series that contained an embedded 4-aminobiphenyl. Naturally, this prompted concerns about the potential mutagenicity of such compounds should they be liberated. As genotoxic compounds are subject to very tight control, arguments about the possibility or otherwise of these embedded anilines being released are unlikely to be relevant in a program aimed at chronic medication for diabetics.[3,17,19] The approach taken was therefore to identify 4-aminobiphenyls that are Ames negative.

However, the potency and pharmaceutical properties of the elaborated compounds containing those anilines were also of concern. During earlier phases of the project, a significant number of substitution patterns had been proposed as alternatives to the simple biphenyl group and all of these had been tracked in an in-house database as part of our Web-based collaborative design tools.[24] The various structures that had been proposed (for reasons unrelated to mutagenicity) which ought to include many with relatively attractive properties were extracted from this database and transformed into a virtual database of 327 aromatic amines.

As described by Leach et al., the higher the computed energy change is for the reaction in Scheme 2, the lower is the proportion of aromatic amines that are active in the Ames test.[1] As shown in Figure 3a for 308 of the 312 aromatic amines described by Leach et al., the dissociation energy changes for the reaction in Scheme 2 tend to be lower for compounds that are found to be active in an Ames test (shown as red bars) compared to those found to be inactive (shown as green bars).[25] In Figure 3b these individual bars are plotted as ratios of active to inactive compounds in bins that are 10 kcal/mol wide (the point at X kcal/mol is the ratio of active to inactive for all aromatic amines with a computed energy change in the range $X - 5$ kcal/mol to $X + 5$ kcal/mol). The statistical software package JMP is able to fit a continuous function linking the energy change to this ratio, and this is given as eq 1 and plotted in Figure 3b.[26]

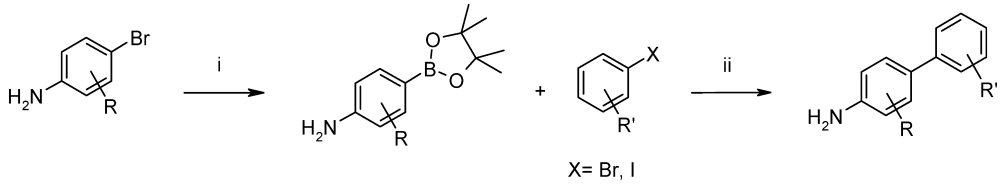
$$P(\text{active}) = \frac{1}{1 + e^{(0.056E_{\text{thermal}} - 8.012)}} \quad (1)$$

In eq 1, P(active) is the proportion of compounds that are active and is equated here to the probability of a new compound being active and E_{thermal} is the energy change including thermal corrections (for 298 K) computed for the reaction given in Scheme 2 by B3LYP/6-31G*.[27−30] The quantity P(active) has two roles: when used retrospectively to describe compounds whose activity is known, it indicates the proportion of compounds with equivalent E_{thermal} values that would be active and hence can indicate whether the compound's activity is surprising or not; when used prospectively, it is equated to the probability that a compound that has not yet been made and tested will be found to be active in the Ames test.

The key observation is that aromatic amines with a higher value of E_{thermal} will have lower probability of being active. At no point does that probability drop to 0% or reach 100%. Indeed, as the overlapping distributions in the bar chart in Figure 3a show, there are active and inactive aromatic amines represented across most of the E_{thermal} range. In addition to any inaccuracy in the calculations or issues with how relevant gas phase calculations might be, there remain many other factors that determine whether an aromatic amine is active in the Ames test. Aromatic amines can be inactive in the test even when the computed electrophilicty is high for several reasons including the following: they may not be soluble; they may not be metabolized or may be metabolized in a fashion that does not generate mutagens; the activated metabolite may not be stable or permeable enough when activated to reach the DNA. Similarly, aromatic amines that are computed to not be electrophilic enough to be active may nonetheless be active in the Ames test by using a different mechanism such as reacting via a different metabolite or by intercalating with DNA. Such factors confound, in part at least, analysis of how well the relevant electrophilic reactivity is computed. Despite these complexities, it is worth recalling the notion enunciated by Box that "all models are wrong, but some are useful".[31] It is hoped to highlight the utility of this model in the remainder of the text.

A set of 142 4-aminobiaryls that were found in the design database were subject to QM calculations. The details are provided in the section Computational Methods. The range of probabilities of being active that were obtained highlighted the challenge that would be faced (Figure 4). Whereas in general it is observed that approximately 35% of all aromatic amines that have been tested are active in the AstraZeneca Ames test, the mean probability of being active in the Ames test computed for these anilines is 52%.

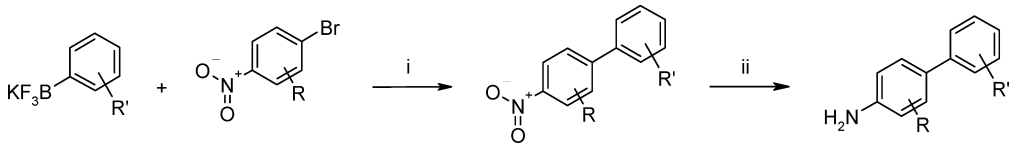
Scheme 4. Synthesis of Compounds Using Method C[a]



[a]Reagents and conditions: (i) $PdCl_2(dppf)-CH_2Cl_2$, CH_3COOK, bis(pinacolato)diboron, dioxane; (ii) $PdCl_2(dppf)-CH_2Cl_2$, K_3PO_4, DME, EtOH, H_2O.

dx.doi.org/10.1021/jm3001295 | J. Med. Chem. 2012, 55, 3923−3933

Scheme 5. Synthesis of Compounds Using Method D[a]



[a]Reagents and conditions: (i) $PdCl_2(dppf)-CH_2Cl_2$, K_3PO_4, DME, EtOH, H_2O; (ii) 10% Pd on C, H_2, EtOAc or EtOH.

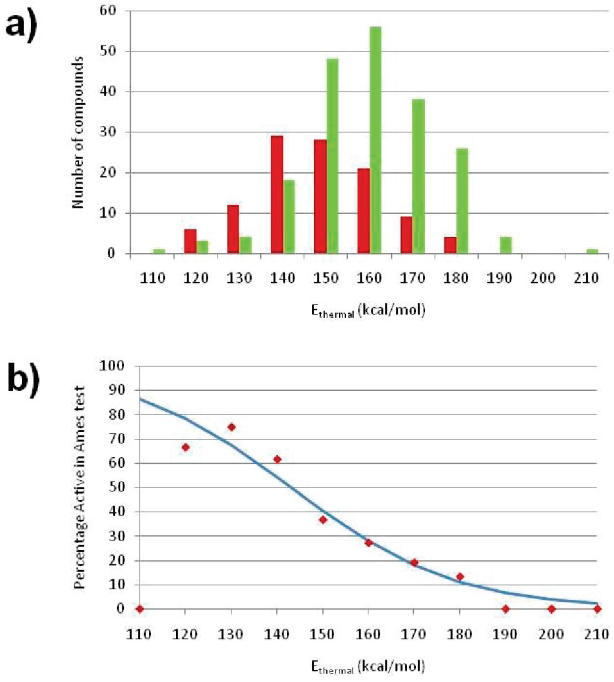


Figure 3. (a) Distribution of dissociation energies including thermal corrections for the process in Scheme 2 for 308 compounds found to be active (red bars) or inactive (green bars) in the AstraZeneca Ames test. (b) Proportion of the same 308 compounds in bins 10 kcal/mol wide that are active in the Ames test at different values of $E_{thermal}$ are shown as red points, and the curve fitted through the unbinned data set is shown in blue.

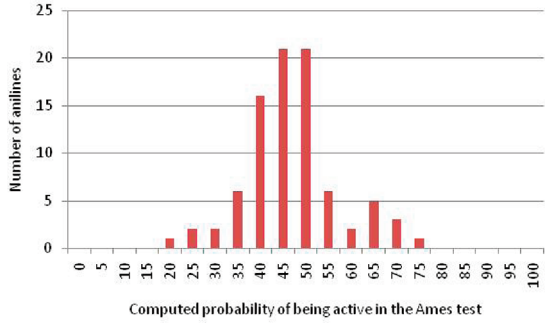


Figure 4. Distribution of probability of being active, *P*(active), found for a set of aminobiphenyls of interest to an antidiabetes drug discovery project.

In terms of compound design, the power of being able to compute this probability is that it can now be considered alongside other properties of the final elaborated molecule. In Figure 5, the molecular weight and clogP of each compound with an embedded aniline are plotted against the probability of being active in the Ames test for the aniline. In blue are the compounds that have a molecular weight less than 500 and clogP less than 3, thought to be reasonable upper limits for these properties for this particular project. Selecting those anilines with a probability of less than 50% of being active in the Ames test and satisfying these two property filters suggest that 26 of the 142 potential compounds are most likely to be of interest.

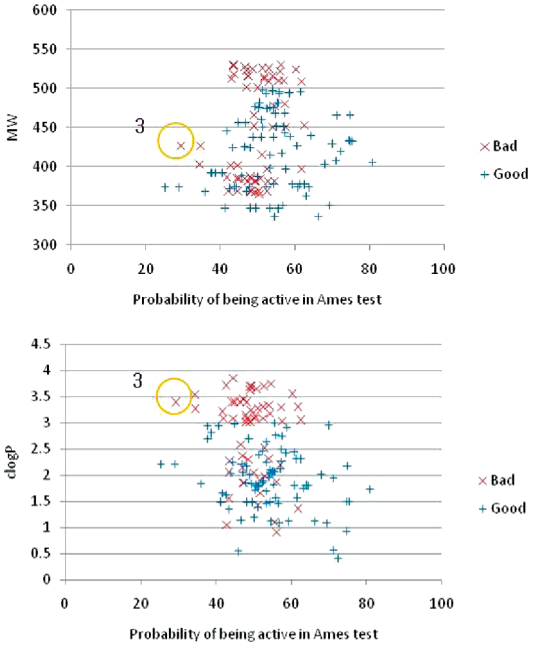


Figure 5. Computed properties (molecular weight at the top and clogP at the bottom) for a range of compounds with embedded anilines are plotted against the *P*(active) for the embedded aniline.

The initial output as summarized in Figures 4 and 5 provided more than just suggestions of useful compounds. It suggested some SAR that was used to develop other candidates for synthesis. For instance, the point corresponding to compound **3** is circled in the plots in Figure 5. This aniline (Figure 6) has an attractively low probability of being active in the Ames test, but the elaborated compound resulting from it was computed to have a lipophilicity that is higher than desired. Therefore, the chlorine atom that was not believed to be critical for primary potency was deleted; compound **3** was not selected for

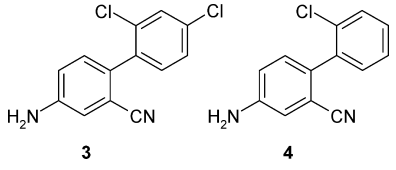


Figure 6. An aminobiphenyl (**3**) highlighted by a circle in Figure 5. Compound **4** was selected as being a less lipophilic example that retains a low probability of being active in the Ames test.

dx.doi.org/10.1021/jm3001295 | *J. Med. Chem.* 2012, 55, 3923−3933

synthesis but was replaced by compound **4** which was computed to have a probability of being active of 32%.

Examining the data set in more detail highlighted some interesting SAR among the various difluoro substituted anilines that were included (Figure 7). While the physical properties of this set of compounds are very similar, the probability of being active in the Ames test varies significantly and shows a systematic advantage of having fluorine meta to the aniline group. Comparing **8** with **5**–**7** shows that a fluorine at the meta position on the same ring as the aniline group is more beneficial than a fluorine at any of the positions on the distal ring. Among the positions on that distal ring, the meta position also is the best place to put fluorine as shown by **7** compared to **5** and **6**. Meanwhile, having a second fluorine in a meta position on the same ring as the aniline, as in **9**, enhances the effect even further.

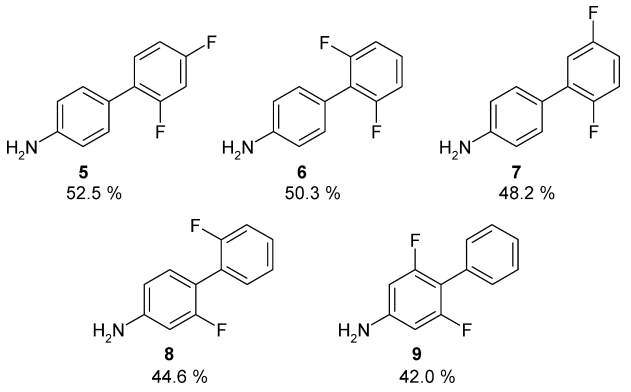


Figure 7. Examples of difluoro substituted 4-aminobiphenyls that were included in the initial enumeration and that suggested some key SARs. Beneath each structure is their computed probability of being active.

In light of this information, it was decided to modify the aniline **10**, which was at that point the one embedded in one of the preferred compounds for primary potency, by the addition of a fluorine to give aniline **11** (Figure 8). Aniline **10** had been computed to have an $E_{thermal}$ of 143.6 kcal/mol corresponding to 49% probability of being active (and was subsequently found to be active in the Ames test), and the SAR outlined above suggested strongly that **11** would have a decreased chance of being active in the Ames test. Two further anilines that were included in compounds of interest to the project, **12** and **13**, were also modified in this way to give **14** and **15**. The compounds containing **12** and **13** were accessed by a route that did not go through the aromatic amine, and so neither of them were made or tested in the Ames test. The selected compounds, **11**, **14**, and **15**, were computed to have probabilities of being active of 41%, 44%, and 30%, respectively (lower than their unmodified relatives **10**, **12**, and **13**).

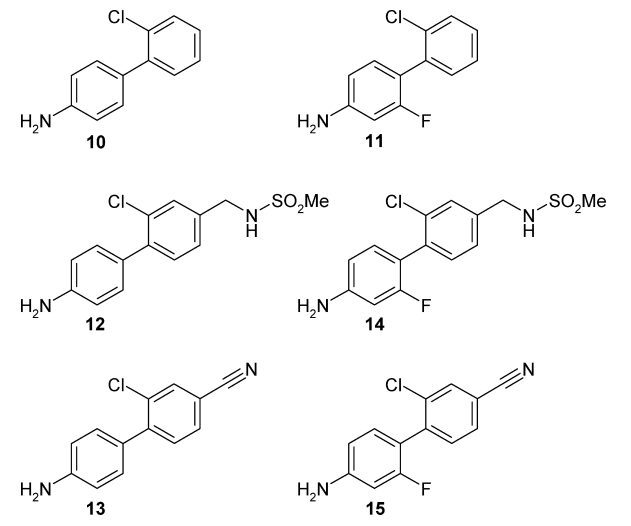
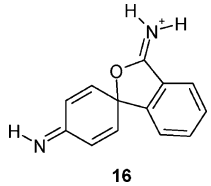


Figure 8. Anilines **10**, **12**, and **13** were of particular interest to a drug discovery project and were modified in light of computed SAR to **11**, **14**, and **15** which are less likely to be active in the Ames test.

Having selected compounds **4**, **11**, **14**, and **15** for synthesis, these were prepared and tested in a two-strain Ames test. Pleasingly, three of these four anilines were found to be inactive in the Ames test. Compound **14** was the only one that was active. Considering the precedent for Ames activity in aminobiphenyl compounds, we considered this a significant achievement.

Further exploration of the SAR for these compounds probed the effect of a range of small substituents (F, Cl, Me, CN, CONHMe, NHAc, OMe, and SO_2Me) at all of the positions around the biphenyl core either alone or in combination with the meta fluorine highlighted already. In Table 1, the value of the change in $E_{thermal}$ caused by addition of these substituents at each position is given compared to the parent 4-aminobiphenyl, which has $E_{thermal}$ of 138.8 kcal/mol. These changes are color coded, with those that increase this quantity shaded green and those that decrease this property shaded red. The change caused by the combination of each group with the meta fluorine is also provided and shows that this group generally increases $E_{thermal}$ by about 4.9 kcal/mol no matter which other groups it is combined with. An inspection of the curve in Figure 3b shows that, particularly in the region of the inflection point at 143 kcal/mol, a change of this size could cause a radical decrease in the likelihood of a compound being active in the Ames test.

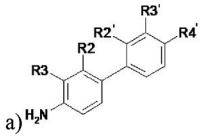
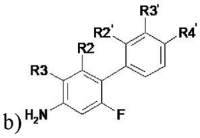
The various contributions given in Table 1 generally correlate well with the σ values provided by Hansch et al., and plots of these correlations are provided in the Supporting Information. It is of note that the correlation is better with σ_p than with σ_m for groups at R2 but not for R3′, which suggests that R3′ behaves more like a "normal" meta position than R2 where substituents will also have an effect on the conformation of the biphenyl bond. Studies in the wider set of anilines have found that σ values have a value within specific series but do not provide the generality that is achieved with the quantum mechanical calculations. One significant outlier in the correlations with σ_p is CONHMe at the R2′ position. The geometry optimization of the nitrenium species for this yielded the spirocyclic cation **16**. It remains untested whether this effect is of experimental relevance.



To help to ground this SAR in experiment, it was decided to make and test the set of compounds in which both F and Cl are introduced at each position around the biphenyl framework. As both substituents are computed to be beneficial at all points, it was hoped that a number of simple 4-aminobiphenyls might be

dx.doi.org/10.1021/jm3001295 | J. Med. Chem. 2012, 55, 3923–3933

Table 1. Changes in $E_{thermal}$ in kcal/mol Compared to Parent 4-Aminobiphenyl Caused by Addition of (a) Substituents at Various Positions (Columns 2−6) and (b) the Same Comparisons for the Dual Effect of Adding the Substituent along with a Fluorine at the Meta Position Shown[a]

	a) 					b) 				
Group	R3	R2	R2'	R3'	R4'	R3	R2	R2'	R3'	R4'
F	+0.3	+4.9	+1.9	+3.9	+0.3	+4.9	+10.0	+8.2	+8.9	+5.1
Cl	+1.5	+7.6	+4.8	+4.8	+1.9	+6.4	+12.5	+10.8	+9.7	+6.5
Me	-5.4	+0.6	+0.3	-2.0	-4.2	-0.7	+5.6	+6.2	+2.8	+0.5
CN	+9.9	+12.6	+9.5	+10.0	+9.8	+15.2	+17.0	+15.2	+15.1	+14.7
CONHMe	-0.7	+1.0	-14.9	+0.1	+1.4	+3.8	+5.5	-12.0	+5.3	+6.2
NHAc	-15.6	+1.7	+0.5	-1.3	-10.3	-12.8	+7.2	+5.2	+3.4	-6.2
OMe	-12.4	-3.3	-7.0	-2.3	-10.3	-8.1	+2.0	-1.1	+2.6	-6.0
SO_2Me	+9.6	+12.0	+7.1	+7.1	+9.0	+14.9	+16.9	+12.0	+12.2	+14.0

[a]Cells shaded green are ones where $E_{thermal}$ increases, and those shaded red are ones where $E_{thermal}$ decreases.

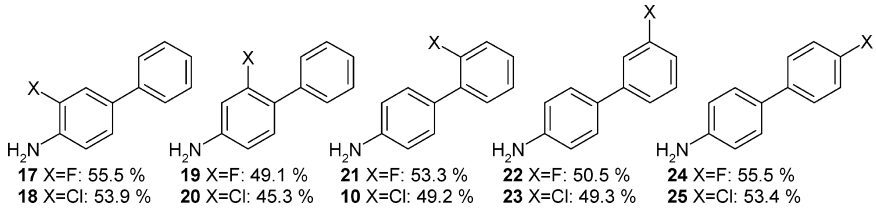


Figure 9. 4-Aminobiphenyl compounds in which F and Cl are systematically placed at each position that were made and tested. Beneath each structure is their computed probability of being active.

identified that are not active in the Ames test. These compounds and their computed probabilities of being active in the Ames test are shown in Figure 9. Most have a computed probability of about 50% of being active in the Ames test. The most likely to be active are the fluoro substituted examples, particularly **17** and **24**. The least likely to be active is the chloro substituted **20**. When these compounds were tested experimentally, it was surprising that all of them except for **20** were active in the Ames test. This suggests that the computational method does a good job of ranking potential candidates but also hints that the biphenyl architecture of these compounds may make them even more likely to be active than an average aromatic amine with equivalent $E_{thermal}$.

This SAR suggested that all of the singly substituted aminobiphenyls that had been of interest to the drug discovery project are Ames positive. However, the beneficial effect of the meta-fluoro had been established experimentally by comparing **11** and **10**. A range of other anilines were therefore selected for synthesis, all including this feature and other substitution patterns computed to be beneficial. This set included compound **8** shown in Figure 7. These were selected both opportunistically based on availability of reagents and on the grounds of SAR relating to the primary target. The structures of this set of compounds are shown in Figure 10, along with their computed probability of being active, all of which are low for

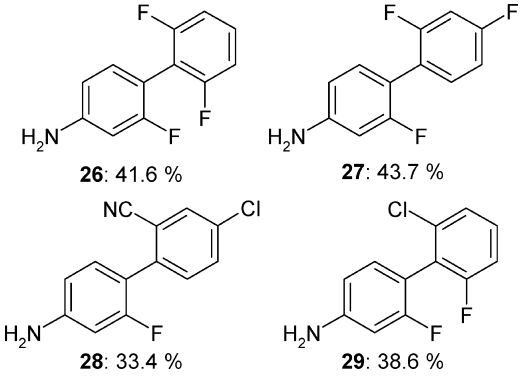


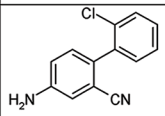
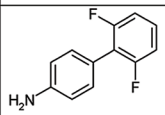
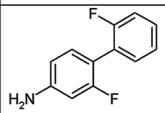
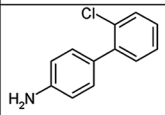
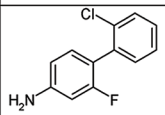
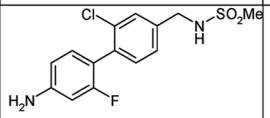
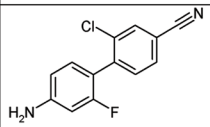
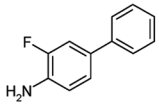
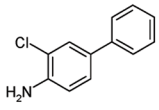
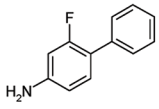
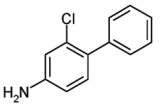
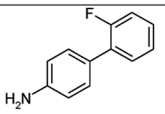
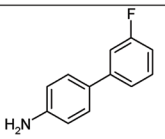
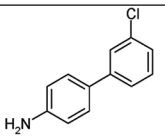
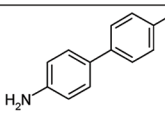
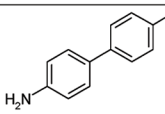
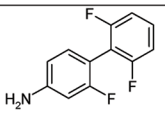
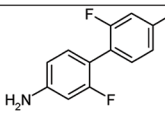
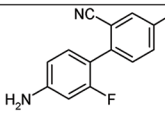
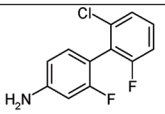
Figure 10. A set of 4-aminobiphenyls that were selected for synthesis. Beneath each structure is their computed probability of being active.

this class. These were also all made and tested along with compound **6**, which provides a comparator for compound **26** to probe the effect of the meta-fluoro group. All of the compounds **26**−**29** were found *not* to be active in the Ames test, while by contrast compound **6**, which is the same as **26** but lacking the meta-fluoro, was found to be active in the Ames test, further emphasizing the beneficial effect of this fluorine substituent.

During the course of these investigations, 20 anilines had been made, purity checked, and tested in a two-strain Ames

dx.doi.org/10.1021/jm3001295 | J. Med. Chem. 2012, 55, 3923−3933

Table 2. Summary Results of the Two-Strain Ames Test for the Aminobiphenyl Compounds Designed, Made, and Tested As Described

Compound Number	Structure	$E_{thermal}$ in kcal/mol	P(active)	Activity in the absence of S9	Activity in the presence of S9[a]
4		156.9	32 %	*TA98* Negative *TA100* Negative	*TA98* Negative *TA100* Negative
6		142.9	50 %	*TA98* Negative *TA100* Negative	*TA98* x 7 at 500 *TA100* x 3 at 160
8		147.0	45 %	*TA98* Negative *TA100* Negative	*TA98* Negative *TA100* Negative
10		143.6	49 %	*TA98* Negative *TA100* Negative	*TA98* x 11 at 160 *TA100* x 5 at 160
11		149.6	41 %	*TA98* Negative *TA100* Negative	*TA98* Negative *TA100* Negative
14		147.4	44 %	*TA98* Negative *TA100* Negative	*TA98* x 3 at 1600 *TA100* Negative
15		158.2	30 %	*TA98* Negative *TA100* Negative	*TA98* Negative *TA100* Negative
17		139.1	56 %	*TA98* Negative *TA100* Negative	*TA98* x 54 at 50 *TA100* x 27 at 16
18		140.3	54 %	*TA98* Negative *TA100* Negative	*TA98* x 54 at 50 *TA100* x 37 at 50
19		143.7	49 %	*TA98* Negative *TA100* Negative	*TA98* x 2 at 160 *TA100* x 3 at 160
20		146.4	45 %	*TA98* Negative *TA100* Negative	*TA98* Negative *TA100* Negative
21		140.7	53 %	*TA98* Negative *TA100* Negative	*TA98* x 26 at 160 *TA100* x 10 at 160
22		142.7	50 %	*TA98* Negative *TA100* Negative	*TA98* x 38 at 160 *TA100* x 13 at 160
23		143.6	49 %	*TA98* Negative *TA100* Negative	*TA98* x 47 at 160 *TA100* x 12 at 50
24		139.1	56 %	*TA98* Negative *TA100* Negative	*TA98* x 43 at 160 *TA100* x 21 at 160
25		140.6	53 %	*TA98* Negative *TA100* Negative	*TA98* x 54 at 160 *TA100* x 14 at 500
26		149.1	42 %	*TA98* Negative *TA100* Negative	*TA98* Negative *TA100* Negative
27		147.6	44 %	*TA98* Negative *TA100* Negative	*TA98* Negative *TA100* Negative
28		155.4	33 %	*TA98* Negative *TA100* Negative	*TA98* Negative *TA100* Negative
29		151.3	39 %	*TA98* Negative *TA100* Negative	*TA98* Negative *TA100* Negative

[a]The maximum increase over control is given along with the corresponding compound quantity in μg/plate.

test, the results of which are summarized in Table 2.[32] The details of each experiment are provided as Supporting Information. These data reveal that in all cases, anilines in this set are inactive in the absence of the S9 fraction, indicating that all of them require metabolic activation to exert their mutagenic effect. All examples, apart from compound **14**, have the same result (either active or inactive) in both the TA98 and TA100 strains. Compound **14** shows a weak effect in the TA98 strain and is negative in the TA100 strain. In general this set of anilines have a larger numerical effect in the TA98 strain than in TA100.

One question that arises concerns the origin of both the elevated risk with aminobiphenyls and the reduced risk caused by the meta-fluorine atom that are reflected in changes in

dx.doi.org/10.1021/jm3001295 | J. Med. Chem. 2012, 55, 3923−3933

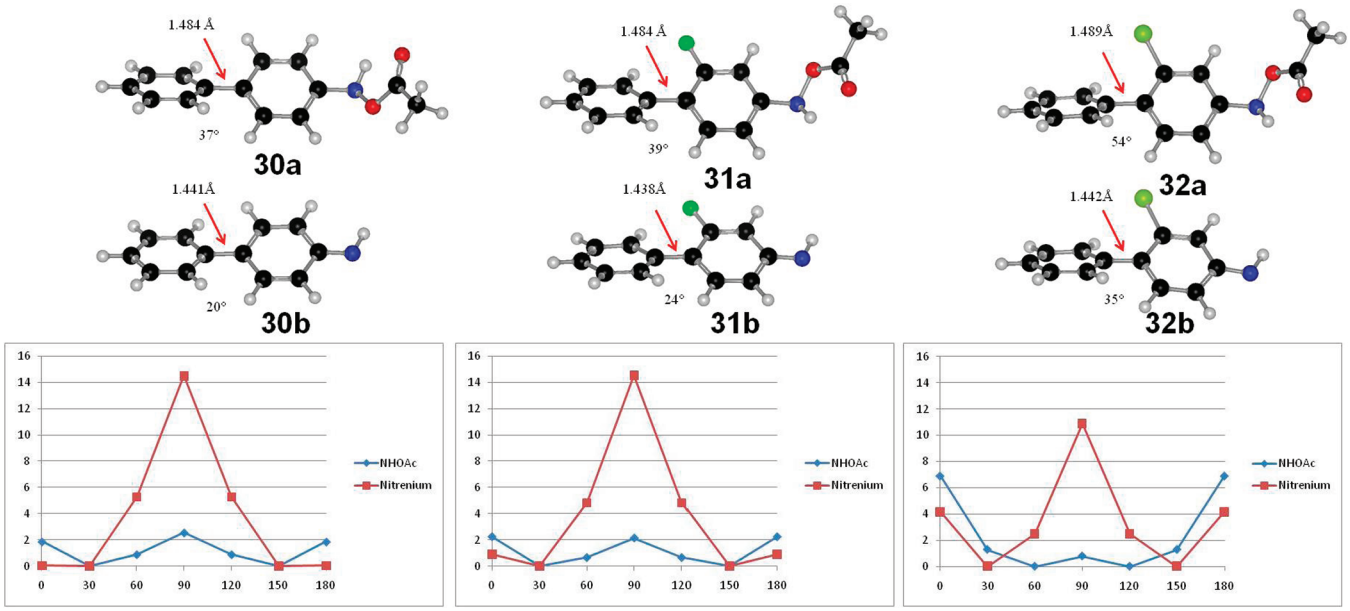


Figure 11. Structures optimized with B3LYP/6-31G* for the ArNHOAc and $ArNH^+$ species involved in Scheme 2. **30a,b** correspond to the derivatives of 4-aminobiphenyl. **31a,b** correspond to 2-fluoro-4-aminobiphenyl, and **32a,b** correspond to 2-chloro-4-aminobiphenyl. Below the structures are shown the profiles for rotation about the biphenyl bond in each structure with blue curves for ArNHOAc and red curves for the nitrenium ion. The *x*-axis is the inter-ring dihedral, and the *y*-axis is energy in kcal/mol relative to the lowest energy point.

$E_{thermal}$. There are a number of factors that may be important. The σ_p of phenyl is −0.01 and the σ_m of fluoro is 0.34, suggesting that phenyl at the para position is weakly electron donating which would stabilize a nitrenium or partially positive charged equivalent while fluorine at the meta position is electron withdrawing and would have the opposite effect.[33] It is worth noting that the effect of fluorine at the para position would be much diminished (it has a σ_p of 0.06), suggesting that the innate electronegativity of fluorine, which will tend to withdraw electrons in the plane of the aromatic ring, is counteracted by back-donation into the π-system at the para (and presumably ortho) position.[33]

One property of the aminobiphenyls that is slightly different from the general set of simple aromatic amines is that they have a higher lipophilicity. All of the 4-aminobiphenyls listed in Table 2 have clogP between 3 and 4 except for **14**. One effect of this is that these anilines and many of their metabolites will have a combination of molecular weight and lipophilicity that permits them to readily permeate the range of membranes that would otherwise prevent them from getting from the site at which metabolism takes place to the site at which the damage to DNA takes place.[34,34,35] The higher lipophilicity of these compounds will also tend to make them more readily metabolized. A final property that is slightly different for this set compared to aromatic amines in general is that substitution around the biphenyl bond can have a conformational effect that might change a substituent's ability to modulate the electrophilicity. Quantum mechanical calculations can provide insight into such phenomena. Two sets of calculations have been performed, one in which the various biphenyl structures are constrained to be flat and the second in which the energy profile for rotation about the biphenyl bond is examined.[36]

When both the ArNHOAc species and the nitrenium ions are constrained to have a dihedral angle of 0° between the two phenyl rings, the dissociation energy corresponding to Scheme 2 goes down by 1.7 kcal/mol (from 142.7 to 141.0 kcal/mol) for the species with no substituents, **30a** dissociating to **30b**, suggesting that the flat conformation benefits more from the electron donating effect of the phenyl ring than the twisted one. The structures of the lowest energy conformations of the two key species **30a** and **30b** are shown in Figure 11 and reveal that there tends to be a flattening of the dihedral in the nitrenium ion. The ability to delocalize the cation more effectively throughout the framework in a flatter structure is in direct opposition to the steric clashing around the bond linking the two phenyl rings.

For the corresponding species with a meta-fluoro substituent (**31a** and **31b**), the dissociation energy goes down by only 1.4 kcal/mol (from 147.6 to 146.2 kcal/mol) when the inter-ring dihedral is constrained to be 0°, suggesting that the fluorine actually *reduces* the effect of the twist that is present in even the unsubstituted case. This effect also manifests itself in the geometry of the nitrenium species: for the unsubstituted example, **30b**, the bond linking the two phenyl rings has a length of 1.441 Å but is 0.003 Å shorter when a fluorine is added to give **31b**, although this shorter distance is offset slightly by an increased dihedral angle between the rings. By contrast, with a meta-chloro substituent, the dissociation energy goes down by 2.7 kcal/mol (from 150.4 to 147.7 kcal/mol) when all species are constrained to be flat, suggesting that the extra twist induced by the chlorine and manifest in significantly larger dihedral angles in **32a** and **32b** makes a significant contribution to the decreased probability of being active for this particular substitution pattern in the aminobiphenyls.

Changes in lipophilicity and the degree of twisting in the aniline could also contribute to changes in the liability of the compounds toward metabolism, and so it is interesting to note that neither property is able to provide any further distinction among active and inactive compounds. The degree of twisting in either the reactant ArNHOAc or the product nitrenium correlates reasonably well with $E_{thermal}$, but neither is able to rationalize the differences between active and inactive compounds in the Ames test as effectively as $E_{thermal}$. In particular, compounds **10** and **14** are both computed to have

dx.doi.org/10.1021/jm3001295 | J. Med. Chem. 2012, 55, 3923−3933

significant twisting in both species but are active in the Ames test. It is also of interest that adding a fluorine meta to an aromatic amine is not sufficient to make it inactive either in 4-aminobiphenyls (for example, compound **19** is active in the Ames test) or more generally.

Within the compound set described here, all 4-aminobiphenyls with $E_{thermal}$ above 145 kcal/mol are inactive in the Ames test while those with $E_{thermal}$ lower than this cutoff are active with one exception. This is compound **14**, which has a molecular weight of 329. It was noted by McCarren et al. that the specificity of this kind of prediction is diminished for aromatic amines that have a molecular weight greater than 250;[22] compound **14** is the only one in this set with molecular weight in this range. Although $E_{thermal}$ discriminates very well between active and inactive aminobiphenyls, this does not preclude other electrophilic metabolites such as the arylnitroso deriving from further oxidation from being relevant. Calculations in which methylamine was used as a model nucleophile adding to ArNO to give either ArN(OH)NHMe or ArN=NMe were performed to examine if they were also capable of achieving this discrimination and were found not to be able to do so (computed energy differences are in the Supporting Information).

CONCLUSIONS

A computed probability of being active in the Ames test can help design safer aromatic amines, even in challenging chemical series such as 4-aminobiphenyls. These calculations can be combined with property calculations for the compounds that contain the embedded aromatic amines to make an improved design process. These calculations can be used to obtain SAR that can suggest safer aromatic amines to make. In particular, adding fluorine or chlorine meta to the aromatic amine has been found to reduce the risk of activity in the Ames test. In the aminobiphenyls, reduced Ames risk as reflected in higher $E_{thermal}$ values can be attributed to the blend of electron donating and withdrawing properties of substituents and their conformational effect. In particular, the meta-fluorine substituent flanking a para-phenyl group has an electron withdrawing effect that is reduced by conformational effects whereas for an equivalent chlorine substituent the two effects complement one another. By providing an idea of the likelihood of aromatic amines being active in the Ames test, this approach allows a potential liability to be avoided in a rational way. As a consequence, this property can be considered alongside other computed properties when designing molecules to enable an improved holistic approach to drug design.

EXPERIMENTAL METHODS

All solvents and chemicals used were reagent grade. Anhydrous solvents were purchased from Sigma Aldrich. Flash column chromatography was carried out using Redisep or Crawford prepacked silica cartridges (4−330 g) and elution was with an Isco Companion system. Following isolation, compounds were purified to >95% purity (UV and NMR) by silica gel chromatography and reverse phase preparative high performance liquid chromatography (HPLC) purification carried out using a Waters XBridge Prep C18 OBD column, 5 μm silica, 50 mm diameter, 150 mm length.

Mass spectrometry data were recorded by liquid chromatography−mass spectrometry (LCMS) on a Waters 2790 separations module with a Phenomenex 5 μm C18 50 mm × 2 mm column, a Waters 996 photodiode array detector, and Waters Micromass ZQ mass spectrometer, with detection by UV at 254 nm. Purity was >95% for all test compounds as determined by this HPLC/MS method. ^{1}H NMR spectra were recorded on a Bruker Ultrashield 400 Plus at 400 MHz in the indicated deuterated solvent. Chemical shifts are reported in ppm relative to tetramethylsilane (TMS) (0.00 ppm) or solvent peaks as the internal reference, and coupling constant (J) values are reported in hertz (Hz). Merck precoated thin layer chromatography (TLC) plates were used for TLC analysis. For workup, solutions were dried over anhydrous magnesium sulfate and the solvent was removed by rotary evaporation under reduced pressure. Microwave reactions were carried out in a Biotage initiator microwave reactor. General methods by which all compounds were prepared are described for exemplar compounds. Full experimental details for all other compounds are provided in the Supporting Information.

General Method A: 2,2′-Difluorobiphenyl-4-amine (8). A mixture of 2-fluorophenylboronic acid (2.06 g, 14.74 mmol), 4-bromo-3-fluoroaniline (2.00 g, 10.53 mmol), $PdCl_2$(dppf)−CH_2Cl_2 adduct (0.430 g, 0.53 mmol), and tripotassium phosphate (2.68 g, 12.63 mmol) in DME (10 mL), ethanol (5 mL), and water (2.5 mL) was heated to 110 °C for 60 min by microwave irradiation. The suspension was cooled, filtered through Celite, and evaporated to dryness. The residue was diluted with 2-methyltetrahydrofuran (60 mL), washed sequentially with water (30 mL) and saturated brine (30 mL), dried over $MgSO_4$, and concentrated to afford crude product. This was purified by chromatography (EtOAc/isohexane = 5−20%) and then by preparative basic HPLC, eluting with 5−95% water−acetonitrile (+0.5% NH_3) to provide the title compound (0.178 g, 8%) as a white solid. ^{1}H NMR ($CDCl_3$) 3.83 (s, 2H), 6.50 (ddd, J = 2.3, 10.0, 14.0 Hz, 2H), 7.09−7.2 (m, 3H), 7.26−7.38 (m, 2H). MS m/z: $(M + H)^+$ = 206.08. HPLC purity = 100%.

General Method B: 4′-Fluorobiphenyl-4-amine (24). A mixture of 4-iodoaniline (0.750 g, 3.42 mmol), 4-fluorophenylboronic acid (0.623 g, 4.45 mmol), and premixed 1,1-bis(di-*tert*-butylphosphino)ferrocenepalladium dichloride/tripotassium phosphate (5 mol % catalyst and 200 mol % base) (1.57 g) in acetonitrile (12 mL) and water (4 mL) was heated to 100 °C for 3 h by microwave irradiation. The suspension was cooled, filtered through Celite, and evaporated to dryness. The residue was diluted with EtOAc (100 mL) and washed sequentially with water (100 mL) and saturated brine (100 mL), dried over $MgSO_4$, and concentrated to afford crude product. The crude product was purified by chromatography (EtOAc/isohexane = 10−70%) and then by preparative basic HPLC, eluting with 5−95% water−acetonitrile (+0.5% NH_3) to provide the title compound (0.435 g, 67%) as a white solid. ^{1}H NMR (DMSO-d_6) 5.18 (s, 2H), 6.58−6.66 (m, 2H), 7.12−7.21 (m, 2H), 7.25−7.34 (m, 2H), 7.48−7.58 (m, 2H). MS m/z: $(MH)^+$ = 188.28. HPLC purity = 100%.

General Method C(i): 3-Fluoro-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline. A mixture of 4-bromo-3-fluoroaniline (5.00 g, 26.31 mmol), potassium acetate (7.75 g, 78.94 mmol), $PdCl_2$(dppf)−CH_2Cl_2 adduct (1.50 g, 1.84 mmol), and bis(pinacolato)diboron (8.02 g, 31.58 mmol) in dioxane (100 mL), ethanol (5 mL), and water (2.5 mL) was heated at 80 °C for 24 h. This was then evaporated to dryness, dissolved in EtOAC (400 mL) and water (400 mL), and filtered through Celite. The organic layer was separated, dried over $MgSO_4$, and concentrated to afford crude product which was purified by chromatography (CH_2Cl_2) to provide the title compound (2.80 g, 45%) as a yellow solid. ^{1}H NMR ($CDCl_3$) 1.33 (s, 12H), 3.93 (s, 2H), 6.30 (dd, J = 2.1, 11.3 Hz, 1H), 6.41 (dd, J = 2.1, 8.1 Hz, 1H), 7.51 (dd, J = 7.0, 8.0 Hz, 1H). MS m/z: $(M + H)^+$ = 238.45.

Method C(ii): 4′-Amino-4-chloro-2′-fluorobiphenyl-2-carbonitrile (28). General method A was followed with 3-fluoro-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline (0.750 g, 3.16 mmol) in place of a boronic acid and 2-bromo-5-chlorobenzonitrile (0.685 g, 3.16 mmol). The crude product was purified by chromatography (EtOAc/isohexane = 5−20%) to provide the title compound (0.118 g, 15%) as a white solid. ^{1}H NMR ($CDCl_3$) 3.95 (s, 2H), 6.51 (ddd, J = 2.2, 10.1, 14.2 Hz, 2H), 7.17 (t, J = 8.4, 8.4 Hz, 1H), 7.41 (dd, J = 1.3, 8.5 Hz, 1H), 7.57 (dd, J = 2.3, 8.5 Hz, 1H), 7.70 (d, J = 2.2 Hz, 1H). MS m/z: $(M + H)^+$ = 247.21. HPLC purity = 100%.

dx.doi.org/10.1021/jm3001295 | J. Med. Chem. 2012, 55, 3923−3933

General Method D(i): 2,2′,6′-Trifluorobiphenyl-4-amine (26). 1-Bromo-2-fluoro-4-nitrobenzene (1.00 g, 4.55 mmol), potassium (2,6-difluorophenyl)trifluoroborate (1.50 g, 6.82 mmol), $PdCl_2$(dppf)−CH_2Cl_2 adduct (0.186 g, 0.23 mmol), and tripotassium phosphate (1.16 g, 5.45 mmol) were suspended in DME (16 mL), ethanol (8 mL), and water (4 mL) and degassed under vacuum, and the atmosphere was replaced with nitrogen. The mixture was heated to 110 °C for 2.5 h in the microwave reactor and cooled to room temperature. The reaction mixture was evaporated to dryness, redissolved in EtOAc (100 mL), and washed with water (100 mL) and saturated brine (100 mL). The organic layer was filtered through Celite, dried over $MgSO_4$, filtered, and evaporated. The crude product was purified by chromatography (0−15% EtOAc in isohexane) to afford 2,2′,6′-trifluoro-4-nitrobiphenyl (0.600 g, 52.1%) as a beige solid.

General Method D(ii). Palladium (10%) on carbon (37.8 mg, 0.04 mmol) was added to 2,2′,6′-trifluoro-4-nitrobiphenyl (0.600 g, 2.37 mmol) in ethanol (100 mL) under nitrogen. The reaction flask was evacuated and purged 3 times, and the suspension was stirred under an atmosphere of hydrogen at room temperature for 20 h and then filtered through Celite and evaporated. It was then purified by chromatography (5−30% EtOAc in isohexane) and then HPLC, eluting with 5−95% water−acetonitrile (+0.5% NH_3) to afford 2,2′,6′-trifluorobiphenyl-4-amine (361 mg, 68.2%) as a white solid. ^{1}H NMR (DMSO-d_6) 5.64 (s, 2H), 6.45 (ddd, J = 2.2, 10.5, 14.8 Hz, 2H), 7.02 (t, J = 8.4, 8.4 Hz, 1H), 7.15 (t, J = 8.1, 8.1 Hz, 2H), 7.42 (tt, J = 6.6, 6.6, 8.2, 8.2 Hz, 1H). MS m/z $(M - H)^-$ = 222.36. HPLC purity = 100%.

Ames Test. Standard plate incorporation assays were performed using *Salmonella typhimurium* strains TA98 and TA100 according to published methods.[6,37] Tests were performed with amounts up to a maximum of 5000 μg/plate in the absence and presence of S9 from the livers of rats pretreated with Aroclor 1254 (purchased from Moltox Inc., Boone, NC, U.S.). All test compounds were dissolved in dimethylsulfoxide (DMSO). In all tests, there were three plates for the solvent control, for each level of test compound, and for positive control groups. The S9-independent positive controls were the following: for TA100, sodium azide, 0.5 μg/plate; for TA98, 2-nitrofluorene. The positive control in tests with S9 was 2-aminoanthracene at 2 μg/plate for both strains. All the compounds were tested at AstraZeneca, Alderley Park, U.K.

Computational Methods. All calculations were performed in Gaussian 09 and employed the B3LYP functional and 6-31G* basis set.[27−30] Starting geometries were generated from SMILES strings by CORINA.[38] Two conformations about the Ar−N bond were generated for each species. All optimized geometries were subjected to frequency calculations to verify them as minima. Full coordinates and energies for each species are provided in the Supporting Information.

■ ASSOCIATED CONTENT

Supporting Information

Full experimental details for Ames tests summarized in Table 2, dependence upon Hammett parameters of $E_{thermal}$, experimental details for other compounds, geometries and energies from QM calculations, and dihedral angles and nitroso energy changes. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Phone: +44 1625 231853. E-mail: andrew.leach@astrazeneca.com.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors are grateful to Mike O'Donovan for careful reading of and improvements to the manuscript.

■ ABBREVIATIONS USED

SAR, structure−activity relationship; DME, 1,2-dimethoxyethane; DMSO, dimethylsulfoxide; dppf, 1,1′-bis(diphenylphosphino)ferrocene; dtbpf, 1,1′-bis(di-*tert*-butylphosphino)ferrocene; EtOAc, ethyl acetate; DCM, dichloromethane; rt, room temperature

■ REFERENCES

(1) Leach, A. G.; Cann, R.; Tomasi, S. Reaction energies computed with density functional theory correspond with a whole organism effect; modelling the Ames test for mutagenicity. *Chem. Commun. (Cambridge, U. K.)* **2009**, 1094−1096.

(2) *Some Aromatic Amines, Organic Dyes, and Related Exposures*; IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. *99*; International Agency for Research on Cancer: Lyon, France, 2010; pp 1−658.

(3) ICH Expert Working Group. Guidance on Genotoxicity Testing and Data Interpretation for Pharmaceuticals Intended for Human Use. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, ICH S2(R1); ICH: Geneva, 2011.

(4) Ames, B. N.; Whitfield, H. J., Jr. Frameshift mutagenesis in Salmonella. *Cold Spring Harbor Symp. Quant. Biol.* **1966**, *31*, 221−225.

(5) Ames, B. N.; Lee, F. D.; Durston, W. E. Improved bacterial test system for the detection and classification of mutagens and carcinogens. *Proc. Nat. Acad. Sci. U.S.A.* **1973**, *70*, 782−786.

(6) Mortelmans, K.; Zeiger, E. The Ames Salmonella/microsome mutagenicity assay. *Mutat. Res.* **2000**, *455*, 29−60.

(7) Easterbrook, J.; Fackett, D.; Li, A. P. A comparison of Aroclor 1254-induced and uninduced rat liver microsomes to human liver microsomes in phenytoin O-deethylation, coumarin 7-hydroxylation, tolbutamide 4-hydroxylation, S-mephenytoin 4′-hydroxylation, chloroxazone 6-hydroxylation and testosterone 6β-hydroxylation. *Chem.-Biol. Interact* **2001**, *134*, 243−249.

(8) Beland, F. A.; Kadlubar, F. F. Formation and persistence of arylamine DNA adducts in vivo. *Environ. Health Perspect.* **1985**, *62*, 19−30.

(9) Kadlubar, F. F.; Beland, F. A. Chemical properties of ultimate carcinogenic metabolites of arylamines and arylamides. *ACS Symp. Ser.* **1985**, *283*, 341−370.

(10) Wild, D. A novel pathway to the ultimate mutagens of aromatic amino and nitro compounds. *Environ. Health Perspect.* **1990**, *88*, 27−31.

(11) Turesky, R. J.; Guengerich, F. P.; Guillouzo, A.; Langouet, S. Metabolism of heterocyclic aromatic amines by human hepatocytes and cytochrome P4501A2. *Mutat. Res., Fundam. Mol. Mech. Mutagen.* **2002**, *506−507*, 187−195.

(12) Novak, M.; Rajagopal, S. Correlations of nitrenium ion selectivities with quantitative mutagenicity and carcinogenicity of the corresponding amines. *Chem. Res. Toxicol.* **2002**, *15*, 1495−1503.

(13) Borosky, G. L. Ultimate carcinogenic metabolites from aromatic and heterocyclic aromatic amines: a computational study in relation to their mutagenic potency. *Chem. Res. Toxicol.* **2007**, *20*, 171−180.

(14) Borosky, G. L. Carcinogenic carbocyclic and heterocyclic aromatic amines: a DFT study concerning their mutagenic potency. *J. Mol. Graphics Modell.* **2008**, *27*, 459−465.

(15) Himmelstein, M. W.; Boogaard, P. J.; Cadet, J.; Farmer, P. B.; Kim, J. H.; Martin, E. A.; Persaud, R.; Shuker, D. E. G. Creating context for the use of DNA adduct data in cancer risk assessment: II. Overview of methods of identification and quantitation of DNA damage. *Crit. Rev. Toxicol.* **2009**, *39*, 679−694.

(16) Siraki, A. G.; Chan, T. S.; Galati, G.; Teng, S.; O'Brien, P. J. N-Oxidation of aromatic amines by intracellular oxidases. *Drug Metab. Rev.* **2002**, *34*, 549−564.
(17) McBriar, M. D.; Guzik, H.; Shapiro, S.; Paruchova, J.; Xu, R.; Palani, A.; Clader, J. W.; Cox, K.; Greenlee, W. J.; Hawes, B. E.; Kowalski, T. J.; O'Neill, K.; Spar, B. D.; Weig, B.; Weston, D. J.; Farley, C.; Cook, J. Discovery of orally efficacious melanin-concentrating hormone receptor-1 antagonists as antiobesity agents. Synthesis, SAR, and biological evaluation of bicyclo[3.1.0]hexyl ureas. *J. Med. Chem.* **2006**, *49*, 2294−2310.
(18) Bentzien, J.; Hickey, E. R.; Kemper, R. A.; Brewer, M. L.; Dyekjaer, J. D.; East, S. P.; Whittaker, M. An in silico method for predicting ames activities of primary aromatic amines by calculating the stabilities of nitrenium ions. *J. Chem. Inf. Model.* **2010**, *50*, 274−297.
(19) Block, M. H.; Boyer, S.; Brailsford, W.; Brittain, D. R.; Carroll, D.; Chapman, S.; Clarke, D. S.; Donald, C. S.; Foote, K. M.; Godfrey, L.; Ladner, A.; Marsham, P. R.; Masters, D. J.; Mee, C. D.; O'Donovan, M. R.; Pease, J. E.; Pickup, A. G.; Rayner, J. W.; Roberts, A.; Schofield, P.; Suleman, A.; Turnbull, A. V. Discovery and optimization of a series of carbazole ureas as NPY5 antagonists for the treatment of obesity. *J. Med. Chem.* **2002**, *45*, 3509−3523.
(20) Hamann, L. G.; Manfredi, M. C.; Sun, C.; Krystek, S. R.; Huang, Y.; Bi, Y.; Augeri, D. J.; Wang, T.; Zou, Y.; Betebenner, D. A.; Fura, A.; Seethala, R.; Golla, R.; Kuhns, J. E.; Lupisella, J. A.; Darienzo, C. J.; Custer, L. L.; Price, J. L.; Johnson, J. M.; Biller, S. A.; Zahler, R.; Ostrowski, J. Tandem optimization of target activity and elimination of mutagenic potential in a potent series of *N*-aryl bicyclic hydantoin-based selective androgen receptor modulators. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 1860−1864.
(21) Borosky, G. L. Quantum chemical studies on ultimate carcinogenic metabolites from polycyclic aromatic hydrocarbons. *Curr. Med. Chem.* **2008**, *15*, 2901−2920.
(22) McCarren, P.; Bebernitz, G. R.; Gedeck, P.; Glowienke, S.; Grondine, M. S.; Kirman, L. C.; Klickstein, J.; Schuster, H. F.; Whitehead, L. Avoidance of the Ames test liability for aryl-amines via computation. *Bioorg. Med. Chem.* **2011**, *19*, 3173−3182.
(23) Lennox, A. J. J.; Lloyd-Jones, G. C. The slow-release strategy in Suzuki−Miyaura coupling. *Isr. J. Chem.* **2010**, *50*, 664−674.
(24) Plowright, A. T.; Johnstone, C.; Kihlberg, J.; Pettersson, J.; Robb, G.; Thompson, R. A. Hypothesis driven drug design: improving quality and effectiveness of the design-make-test-analyse cycle. *Drug Discovery Today* **2012**, *17*, 56−62.
(25) Four of the 312 anilines originally studied had been tested more than once and found to have different classifications as active or inactive. Rather than perform a detailed analysis of each case, these four examples have been excluded from this calibration curve.
(26) *JMP*, version 8; SAS Institute Inc.: Cary, NC.
(27) Becke, A. D. Density-functional thermochemistry. III. The role of exact exchange. *J. Chem. Phys.* **1993**, *98*, 5648−5652.
(28) Lee, C.; Yang, W.; Parr, R. G. Development of the Colle−Salvetti correlation-energy formula into a functional of the electron density. *Phys. Rev. B: Condens. Matter* **1988**, *37*, 785−789.
(29) Hariharan, P. C.; Pople, J. A. Influence of polarization functions on MO hydrogenation energies. *Theor. Chim. Acta* **1973**, *28*, 213−222.
(30) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Mennucci, B.; Petersson, G. A.; Nakatsuji, H.; Caricato, M.; Li, X.; Hratchian, H. P.; Izmaylov, A. F.; Bloino, J.; Zheng, G.; Sonnenberg, J. L.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Vreven, T.; Montgomery, J. A., Jr.; Peralta, J. E.; Ogliaro, F.; Bearpark, M.; Heyd, J. J.; Brothers, E.; Kudin, K. N.; Staroverov, V. N.; Kobayashi, R.; Normand, J.; Raghavachari, K.; Rendell, A.; Burant, J. C.; Iyengar, S. S.; Tomasi, J.; Cossi, M.; Rega, N.; Millam, N. J.; Klene, M.; Knox, J. E.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Martin, R. L.; Morokuma, K.; Zakrzewski, V. G.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Dapprich, S.; Daniels, A. D.; Farkas, Ö.; Foresman, J. B.; Ortiz, J. V.; Cioslowski, J.; Fox, D. J. *Gaussian 09*, revision A.1; Gaussian, Inc.: Wallingford, CT, 2009.
(31) Box, G. E. P. *Empirical Model-Building and Response Surfaces*; Wiley: New York, 1987.
(32) O'Donovan, M. R.; Mee, C. D.; Fenner, S.; Teasdale, A.; Phillips, D. H. Boronic acids: a novel class of bacterial mutagen. *Mutat. Res., Genet. Toxicol. Environ. Mutagen.* **2011**, *724*, 1−6.
(33) Hansch, C.; Leo, A.; Unger, S. H.; Kim, K. H.; Nikaitani, D.; Lien, E. J. Aromatic substituent constants for structure−activity correlations. *J. Med. Chem.* **1973**, *16*, 1207−1216.
(34) Waring, M. J. Defining optimum lipophilicity and molecular weight ranges for drug candidates: molecular weight dependent lower log D limits based on permeability. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 2844−2851.
(35) Camenisch, G.; Alsenz, J.; van de Waterbeemd, H.; Folkers, G. Estimation of permeability by passive diffusion through Caco-2 cell monolayers using the drugs' lipophilicity and molecular weight. *Eur. J. Pharm. Sci* **1998**, *6*, 313−319.
(36) Calculations in which constraints are imposed cannot incorporate vibrational corrections, as the simple harmonic potential approximation breaks down. So the energies reported in this section are uncorrected electronic energies and should not be compared directly with energy changes reported elsewhere in this paper.
(37) Maron, D. M.; Ames, B. N. Revised methods for the Salmonella mutagenicity test. *Mutat. Res., Environ. Mutagen. Relat. Subj.* **1983**, *113*, 173−215.
(38) *CORINA, Molecular Networks*; GmbH Computer Chemie Langemarckplatz 1: Erlangen, Germany.