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Non-nucleoside inhibitors of HCV NS5B polymerase. Part 1: Synthetic and computational exploration of the binding modes of benzothiadiazine and 1,4-benzothiazine HCV NS5b polymerase inhibitors

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ABSTRACT

The importance of internal hydrogen bonding in a series of benzothiadiazine and 1,4-benzothiazine NS5b inhibitors has been explored. Computational analysis has been used to compare the protonated vs. anionic forms of each series and we demonstrate that activity against HCV NS5b polymerase is best explained using the anionic forms. The syntheses and structure–activity relationships for a variety of new analogs are also discussed.

Approximately 200 million people worldwide are infected with Hepatitis C virus (HCV), the leading cause of cirrhosis of the liver.[1] Infection with HCV can lead to hepatocellular carcinoma, and ultimately, liver failure requiring transplantation surgery.[2] The current best treatment option consists of a combination of pegylated interferon-α-2a and ribavirin, a broad-spectrum anti-viral agent.[3] The limited efficacy and potential for severe side-effects of this approach continues to drive the search for more efficacious and targeted therapies.

In 2002 GSK reported that compound **1** inhibited the HCV NS5b polymerase with an IC_{50} of 0.03 μM (Fig. 1).[4a] Compound **2** was later revealed as a more potent analog with an enzyme IC_{50} of 0.01 μM and a subgenomic HCV replicon IC_{50} of 0.04 μM. A key feature of these compounds is their ability to form two internal hydrogen bonds, as shown in Figure 1. As described, these hydrogen bonds are thought to stabilize the bound conformation, thus contributing to the potency observed for this class of NS5b inhibitors.[4b]

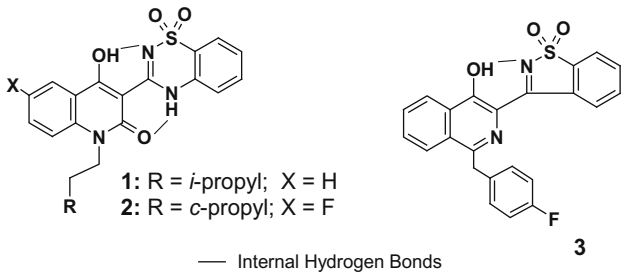

Figure 1. Internal hydrogen bond networks for 4-hydroxyquinolone-benzothiadiazines **1** and **2** and isoquinoline **3**.

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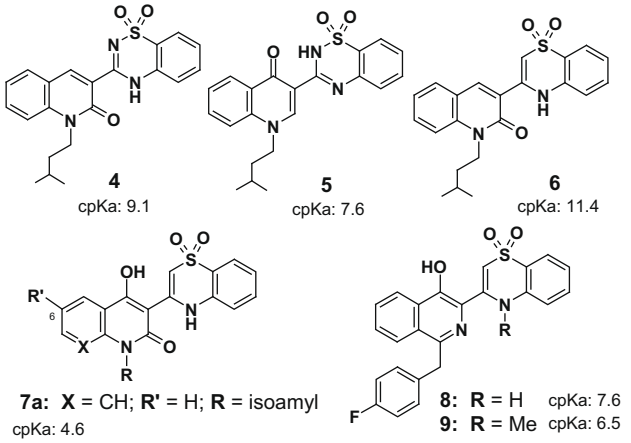
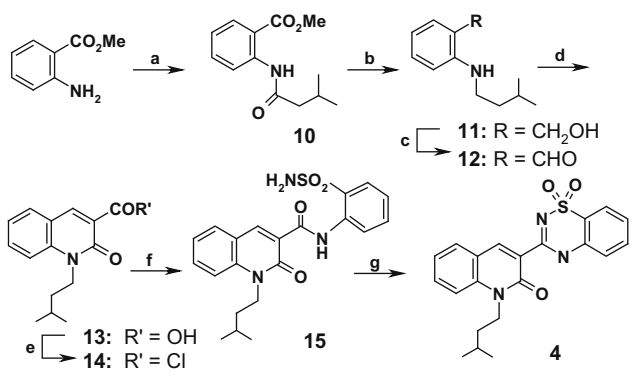


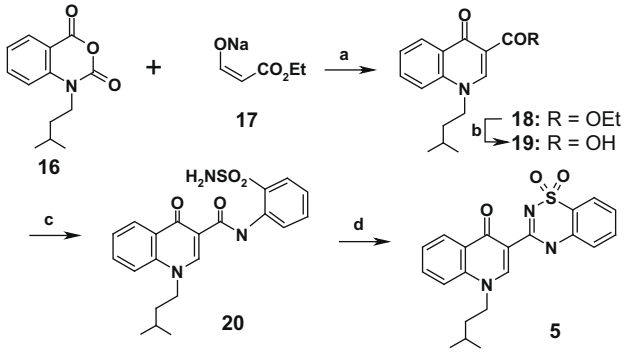
Figure 2. Set of analogs with the capacity to form only a single internal hydrogen bond.

We recently described a new series of 3-hydroxyisoquinoline derived NS5b inhibitors (Fig. 1, compound **3**), which demonstrated reasonable NS5b inhibitory potency (IC_{50}: 2.2 μM).[5] While **3** can also exist in an internal hydrogen-bond stabilized conformation similar to that of **2**, we hypothesized that a delocalized enolic anion (pK_a of **3** = 7.3) is the active form that interacts with the NS5B polymerase enzyme. To test this hypothesis further, we designed a set of compounds related to **1** and **3** (Fig. 2) that would probe both the importance of this key hydrogen bond and the anionic binding model. Compounds **4** and **5**[4c] can only form one internal hydrogen bond to the thiadiazine ring system, and neither compound can form a delocalized anion in the quinolone ring. The combined 2-quinolone and 1,4-thiazine ring system in **6** lacks both hydrogen bond elements along the upper portion of the ring system. Compounds **7**, **8** and **9** also possess the 1,4-thiazine system, but retain a hydrogen bond donating OH group on the left-hand ring system and have the ability to form delocalized enolic anions. Both internal hydrogen bonds have been blocked entirely in compound **9**. In general, measured pK_a's for compounds across these series have tracked very closely with calculated pK_a (cpK_a) values.[6]

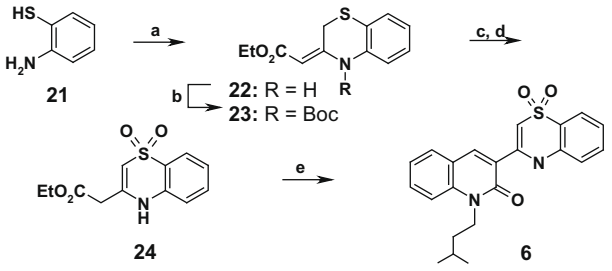
Our synthesis of compound **4** is shown in Scheme 1. 2-Aminomethylbenzoate was acylated using 3-methylbutanoyl chloride to give **10**, which was reduced with lithium aluminum hydride to give the anilino alcohol **11**. Oxidation to the aldehyde **12** was accomplished using MnO_2. Treatment with Meldrum's acid followed by chlorination gave quinolone acyl chloride **14** in modest yield. Reaction with 2-amino-benzenesulfonamide and subsequent ring closure of **15** using Cs_2CO_3 in ethanol at reflux provided **4**.



Scheme 1. Reagents: (a) 3-methylbutanoyl chloride, $NaHCO_3$, THF; (b) LAH, THF; (c) MnO_2, DCM; (d) Meldrum's acid, ethylenediamine, MeOH, AcOH; (e) $SOCl_2$; (f) 2-aminobenzenesulfonamide, toluene; (g) Cs_2CO_3, EtOH.



Scheme 2. Reagents and conditions: (a) DMF, 110 °C; (b) THF, NaOH; (c) (1) oxalyl chloride, toluene; (2) 2-aminobenzenesulfonamide, TEA, toluene; (d) Cs_2CO_3, EtOH, reflux.



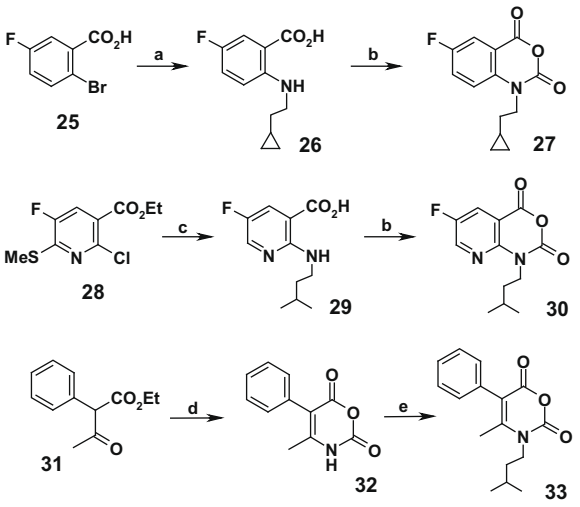
Scheme 3. Reagents and conditions: (a) ethyl 4-chloro-3-oxobutanoate, MeOH; (b) BOC_2O, THF, DMAP; (c) MCPBA, THF; (d) TFA, CH_2Cl_2; (e) compound **12**, THF, 130 °C; then aq $NaHCO_3$ workup.

The synthesis of the 1*H*-quinolin-4-one **5** was carried out by reaction of the isatoic anhydride **16**[7] and the sodium enolate **17**[8] to give the quinolone ester **18** (Scheme 2). Saponification to the acid was achieved in quantitative yield using NaOH in THF. Activation with oxalyl chloride and reaction with 2-aminobenzene-sulfonamide gave intermediate **20**. Using conditions similar to that found in Scheme 1, the product was cyclized with $CsCO_3$ in ethanol.

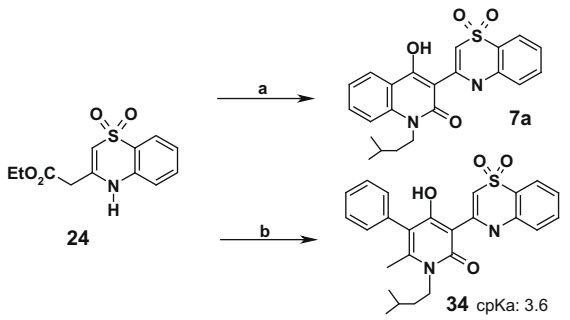
The 1,4-thiazine **6** was synthesized using the route shown in Scheme 3. Alkylation of 2-aminothiophenol **21** with ethyl 4-chloro-3-oxobutanoate resulted in a spontaneous ring closure to give 1,4-benzothiazine **22**. Direct oxidation of **22** proved problematic. However, following acylation with Boc_2O, intermediate **23** underwent efficient oxidation and spontaneous olefin migration using MCPBA. Condensation of the deprotected 1,4-thiazine dioxide **24** (as the TFA salt) with aldehyde **12** in a sealed tube at 130 °C gave the desired cyclized product **6** in low (15%) yield.

The syntheses of a variety of key isatoic anhydride intermediates are outlined in Scheme 4. An S_nAr reaction on 2-bromo-5-fluoro-benzoic acid using 2-cyclopropylethylamine gave aniline **26**. Treatment with triphosgene in the presence of K_2CO_3 provided isatoic anhydride **27**.[4d] Desulfurization of **28**[9] followed by amine displacement and saponification gave the key aza intermediate **29**, which was converted to anhydride **30** as previously described. Additional variously substituted anhydrides were prepared in a similar fashion using readily available materials. Keto-ester **31** was first converted to cyclic anhydride **32** using urethane and $POCl_3$. Alkylation of the sodium salt of **32** with isoamylbromide gave the monocyclic precursor **33** in good (73%) yield.

Following Scheme 5, the 1,4-thiazines **7a** and **34** were synthesized using the key 1,4-thiazine dioxide intermediate **24** from Scheme 3. Reaction of **24** with the appropriately substituted isatoic anhydrides gives the desired products in modest yield. In the case of **34**, use of DBU gave poor conversion, however, switching to the sodium salt of **24** led to a modest (41%) yield of the desired 1,4-thiazine.



Scheme 4. Reagents and conditions: (a) 2-cyclopropylethylamine HCl, K_2CO_3, $CuBr_2$, THF; (b) triphosgene, K_2CO_3, EtOAc; (c) (1) Ra-Ni, EtOH; (2) isoamylamine, DIEA, EtOH, 150 °C (sealed tube); (3) aq NaOH, dioxane (d) urethane, $POCl_3$, 90 °C; (e) isoamylbromide, NaH, DMA, 80 °C (sealed tube).



Scheme 5. Reagents and conditions: (a) compound **16**, DBU, THF/EtOAc, THF; (b) compound **33**, NaH, THF, reflux.

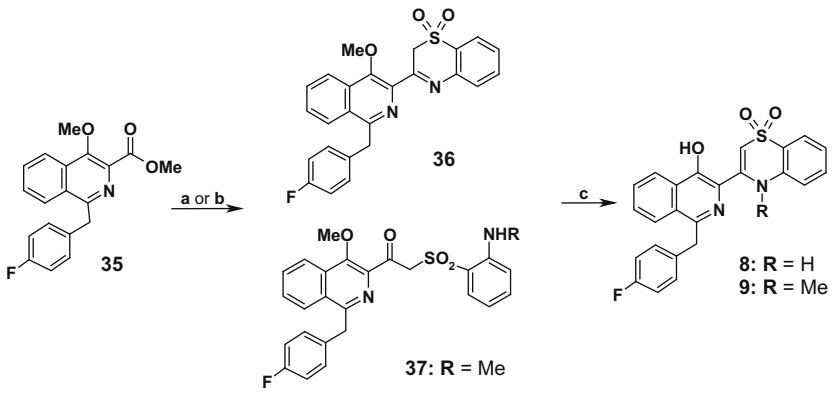
The preparation of isoquinoline-1,4-thiazines **8** and **9** is outlined in Scheme 6. Acylation of the dianion of 2-methanesulfonyl-phenylamine with **35**[5] led to the spontaneously cyclized intermediate imine **36**. Deprotection of this imine with BBr_3 gave the tautomeric 1,4-benzothiazine dioxide **8** as a single product. Direct methylation of **8** with MeI/K_2CO_3 proved unsuccessful, giving primarily the C-alkylated imine product. Switching to the *N*-methyl aniline precursor in the acylation step gave **37** as the sole product. Treatment with BBr_3 led directly to the desired *N*-methyl 1,4-thiazine **9**.

Table 1
NS5b and replicon inhibitory potency of compounds **1**–**9** and **34**

Compound #	NS5b Enzyme IC_{50} (μM)	GT-1b Replicon EC_{50} (μM)
1	1.1	0.87
2	0.1	0.26
3	2.2	0.44
4	682	nd
5	>1000	nd
6	868	nd
7a	2.3	2.5
8	6.9	7.6
9	19.5	nd
34	87	nd

Heterocycles **1–9** and **34** were tested against the isolated HCV NS5b polymerase (GT-1b) and in a replicon assay system.[10] Table 1 summarizes the potency data for this set of compounds. Analogs **4** and **5**, which retain the thiadiazine moiety found in compounds **1** and **2** but lack an ionizable 4-hydroxy group, showed greater than two orders of magnitude loss in potency against the enzyme. Compound **6**, which differs from **4** by a single N-for-C exchange, exhibited a similar ca. 800-fold drop in activity compared to **1**. The 1,4-thiazine **7a**, now with an ionizable 4-hydroxy quinolone group, inhibited the NS5b enzyme with an IC_{50} of 2.3 μM and showed promising replicon activity (2.5 μM). This finding suggested the conformational stabilization provided by two internal hydrogen bonds was not an absolute requirement for NS5B activity within this general class of inhibitors. The expanded 6-membered 1,4-thiazine ring of **8** is less well tolerated in the isoquinoline series (6.9 μM vs 2.2 μM for **3**), and as expected, methylation of the nitrogen (i.e., **9**) further reduced activity. The mono-cyclic 1,4-thiazine analog **34** is notably less active against the NS5b enzyme, regardless of its greater ionization potential.

Publication of the X-ray co-crystal structure of **1** complexed with the NS5b polymerase demonstrated that this class of inhibitors adopts a shallow U-shaped conformation in the active site, with the internal H-bonding partners aligned as depicted in Figure 1.[4b,11] Given the general similarity of compounds **1** and **7**, it is reasonable to assume that they may inhibit the NS5b polymerase in a similar fashion, while maintaining key contacts with the polymerase. However, it would seem unlikely that 1,4-thiazines **7** can adopt a similar shallow U-shaped conformation in the NS5b active site, without a significant energetic cost, due to the lack of complimentary H-bond partners. To investigate this more fully, we



Scheme 6. Reagents and conditions: (a) 2-methanesulfonyl-phenylamine; 2.2 equiv *n*-BuLi, THF, −78 °C; (b) (2-methanesulfonylphenyl)methylamine, 2.2 equiv *n*-BuLi, THF, −78 °C; (c) BBr_3, DCM.

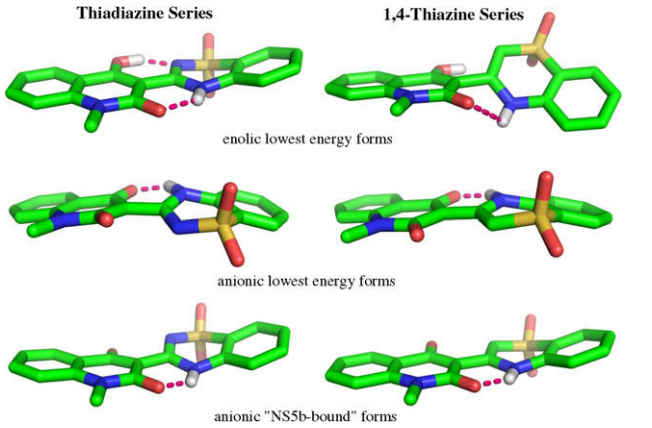
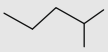
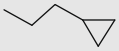
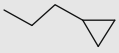
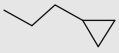
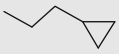
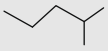
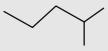
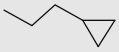
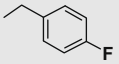
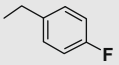
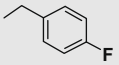
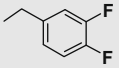
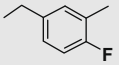
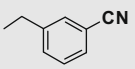


Figure 3. Lowest energy conformations of the enolic (top row) and anionic (middle row) thiadiazine and 1,4-thiazine series prototypes. Bottom row: computed NS5B-bound conformations for each anionic form (i.e., ca. 180° shifted about the central C–C bond).

Table 2
NS5b and replicon inhibitory potency of compounds **7a–o**

Compound #	X	R′	R	NS5b IC_{50} (μM)	Replicon EC_{50} (μM)
7a	CH	H		2.3	2.5
7b	CH	F		1.1	1.2
7c	CH	Cl		2.4	1.6
7d	CH	Me		1.3	0.64
7e	CH	MeO		3.8	7.1
7f	N	H		1.4	nd
7g	N	F		0.83	8.6
7h	N	H		2.7	nd
7i	CH	H		17.5	46
7j	CH	H		0.72	0.79
7k	CH	F		0.7	0.7
7l	CH	Me		1.5	1.5
7m	CH	F		1.2	1.5
7n	CH	F		0.4	0.8
7o	CH	F		37	nd

computed the energy of the 'unbound' (i.e., lowest energy) structures of *N*-methyl enolic prototypes of **1** and **7** via ab initio molecular orbital calculations at the 6-311++G(d,p)//6-311++G(d,p) level of theory with the GAMESS program.[12] We then compared these minimum energy conformations with the published thiadiazine structure.

For the thiadiazine enolic prototype, we found the lowest energy conformation corresponds to a doubly hydrogen bonded structure, illustrated in Figure 3 (top row). Interestingly, our computed lowest energy structure is not fully co-planar, but rather adopts a U-shaped conformation quite similar to that in the published thiadiazine/NS5b X-ray co-crystal structure. The 'bend' in our computed structure is caused by the boat-like geometry of the thiadiazine ring.[13]

The minimum energy structure of the 1,4-thiazine enolic prototype was found to possess a substantially different orientation of the quinolone and 1,4-thiazine rings. The computed torsion for the C(OH)=C–C=C(SO_2) angle is 51.5°, compared to 2.6° for the equivalent C(OH) =C–C=N(SO_2) angle in the quinolone thiadiazine prototype (Fig. 3; top row). Based on the published X-ray co-crystal of **1**, it is unlikely that this lowest energy structure of the 1,4-thiazine prototype corresponds to an 'active' conformation. Constraining the torsion angle connecting the 1,4-thiazine and quinolone rings to match that of the thiadiazine series gave a structure ca. 12 kcal/mol higher in energy. Given the relative inhibitory potencies of the 1,4-thiazine (2.3 μM) and thiadiazine (1.1 μM) series of compounds, it seems unlikely that a 12 kcal/mol conformational penalty must be overcome in order for the 1,4-thiazines to bind to the NS5b polymerase.

We sought to develop an alternate binding model for these compounds through consideration of the acidic nature of the hydroxy quinolone ring system; we hypothesized that the anionic species, resulting from loss of the 2-quinolone hydroxyl proton, may be the active form of the inhibitors.[5,6] Upon computing the lowest energy and 'NS5b-bound' conformations for the anionic form of each of the prototypes, we found a shift in the conformational preference for both series. Interestingly, both anionic forms now prefer 'inverted' conformations corresponding to a ca. 180° shift in the relative orientation of the quinolone and either of the thiadiazine or 1,4-thiazine rings (Fig. 3; middle row). The lowest energy conformation for the anionic thiadiazine prototype is ca. 1.5 kcal/mol lower in energy than the bound conformation, while the lowest energy conformation of the 1,4-thiazine is preferred by only ca. 1.1 kcal/mol (Fig. 3; middle vs bottom row). Both anionic prototypes display the characteristic shallow U-shaped conformation due to a boat-like thiadiazine/1,4-thiazine ring. Since the anionic prototypes for both series require only a modest amount of energy to adopt the predicted NS5b binding mode, these computational results are in excellent accord with the data from the biochemical assays in Table 1, and further support our anionic binding hypothesis.[14]

A more detailed analysis of the structure–activity relationships within the 1,4-thiazine series is outlined in Table 2. Based on our own determination of structure–activity limitations within the original thiadiazine series, we chose to focus only on small substitutions at C-6, combined with a limited set of lipophilic N-substituents. Compounds **7b–o** were prepared as outlined in Scheme 5, using the appropriately substituted isotoic anhydrides (Scheme 4). Small C-6 substituents were tolerated when combined with either the cyclopropylethyl (**7b–e**) or the 4-fluorobenzyl (**7j–l**) lipophilic sidechain. The 1*H*-naphthyridone analogs (**7f–h**) also showed similar NS5b potencies, but were significantly less active compared with a similar thiadiazine series[15] and **7g** showed a further 10-fold loss in the replicon assay (8.6 μM). In general, the NS5b and replicon potencies across this series show only minor variations. Two key exceptions showing notable losses in potency are the 2-fluorobenzyl (**7i**) and 3-cyanobenzyl (**7o**) analogs. Com-

pared with **7o**, the smaller, less-polar 4-fluoro-3-methylbenzyl analog **7n** is two orders of magnitude more potent (0.4 μM vs 37 μM). Somewhat surprisingly, replicon potency generally tracked closely with NS5b potency. Cellular permeabilities based on a 7-day caco cell assay are quite reasonable (**7b**, **7d**, **7j**; P_{app} = 6.4, 9.4, 4.4×10^{-6} cm/s, respectively), however, a 21-day caco cell assay suggests significant efflux occurs across the series (**7b**, **7k**, **7n**; P_{app} AB/BA = 0.6/7.1, 0.6/4.9, $0.5/5.3 \times 10^{-6}$ cm/s, respectively).

A novel class of hydroxy quinolone thiadiazine inhibitors of HCV NS5b polymerase was recently described.[4a,4b] A key feature of this class of inhibitors is that they can form two internal hydrogen bonds which help to stabilize an NS5b-bound-like conformation, thus contributing their inhibitory potency. We have further tested this hypothesis via synthesis of a series of closely related molecules that contain varying degrees of hydrogen bonding functionality. Our results suggest an alternate key feature of these hydroxy quinolone based inhibitors is the acidic nature of this functionality, leading to their interaction with the enzyme as anions. From our effort, a novel series of hydroxy quinolone 1,4-thiazine derived inhibitors has emerged with sub-micromolar potencies against the HCV NS5b polymerase enzyme.

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References and notes

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11. PDB code: 2fvc.
12. GAMESS Version 27 June 2005 (R2): Schmidt, M. W.; Baldridge, K. K.; Boatz, J. A.; Elbert, S. T.; Gordon, M. S.; Jensen, J. J.; Koseki, S.; Matsunaga, N.; Nguyen, K. A.; Su, S.; Windus, T. L.; Dupuis, M.; Montgomery, J. A. *J. Comput. Chem.* **1993**, *14*, 1347. All stationary points were verified as true minima via computation of the corresponding analytical frequencies.
13. For comparison, the fully co-planar benzothiadiazine structure was found to be ca. 0.80 kcal/mol higher in energy than the boat form, and shown to be a transition state via computation of the analytical frequencies. A search of the CSD for thiadiazine containing molecules yields a number of relevant examples. In the majority of small molecule X-ray structures the thiadiazine fragment is largely planar, though subtle puckering is noted in a few examples. In light of the ab initio results, the planar conformation of the thiadiazine group is likely due to crystal packing effects. CSD, Version 5.13, April 1997 release. Entries CACTAZ0001, DIAZOX0001, JIFYUS0001, JIFZAZ0001, SEFMEV0001, SEFMIZ0001.
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