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# Hepatitis C NS5B polymerase inhibitors: Functional equivalents for the benzothiadiazine moiety

Douglas K. Hutchinson *, Charles A. Flentge, Pamela L. Donner, Rolf Wagner, Clarence J. Maring, Warren M. Kati, Yaya Liu, Sherie V. Masse, Tim Middleton, Hongmei Mo, Debra Montgomery, Wen W. Jiang, Gennadiy Koev, David W. A. Beno, Kent D. Stewart, Vincent S. Stoll, Akhteruzzaman Molla, Dale J. Kempf

*Department of Antiviral Research, Department of Drug Metabolism and Pharmacokinetics, Department of Structural Biology, Global Pharmaceutical Research and Development, Abbott Laboratories, Abbott Park, IL 60064, USA*

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ABSTRACT

A series of quinoline derivatives was synthesized as potential bioisosteric replacements for the benzothiadiazine moiety of earlier Hepatitis C NS5B polymerase inhibitors. Several of these compounds exhibited potent activity in enzymatic and replicon assays.



Benzothiadiazine derivatives, such as **1**[1] and **2**,[2] shown in Figure 1, are of great interest since they have been found to be potent inhibitors of the RNA-dependent RNA polymerase (RdRp) of the hepatitis C virus (HCV).

A number of SAR studies have shown that modification of the *N*-alkyl-4-hydroxy-3,4-dihydroquinoline-2-one moiety of **1**,[1] such as that of the *gem*-dialkyl derivative **2**,[2] can have profound effects on both the antiviral activity of these compounds, and on their pharmacological properties.

While these and other studies[3] have focused primarily on changes to the AB ring system of **1**, much less has appeared concerning changes to the benzothiadiazine CD ring system. Notably, disclosures by Roche[4] and more recently by Anadys[5] have described compounds such as **3** and **4** (see Fig. 1), which exhibit potent antiviral activity in both enzymatic and cell culture assays, despite the replacement of one of the nitrogen atoms of the benzothiadiazine moiety with a carbon atom, as in **3**, or with the saccharin moiety, as in **4**. In view of the great portent of these findings, we wish to describe our own efforts in this area.

As part of our efforts to understand the importance of each of the structural features of benzothiadiazine **2**, we sought to understand qualitatively how much binding energy was gained by having the sulfonyl moiety in the CD ring system. A number of groups have published crystal structures suggesting that the C-ring sulfonyl group participates in a water-mediated hydrogen bond with the hydroxyl group of serine-288 in the palm site of the HCV NS5B polymerase.[1b,5b,c] To probe the importance of this interaction to the in vitro activity, we attempted to prepare quinazoline derivative **10**. As shown in Scheme 1, borane reduction[6] of nitro

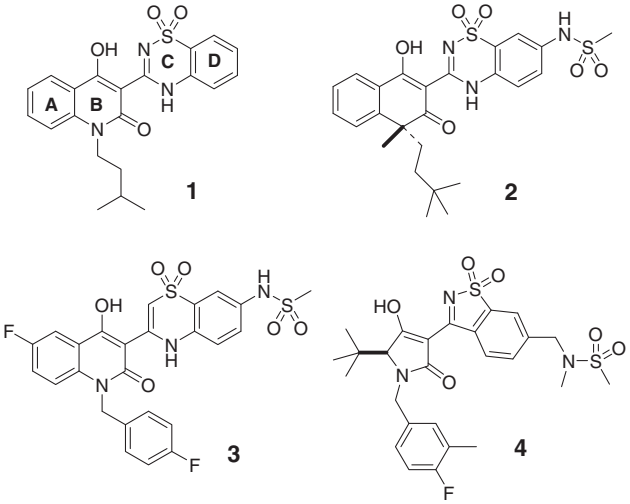


**Figure 1.** Benzothiadiazine analog series and CD ring replacements.

* Corresponding author. Tel.: +1 847 838 5899.
*E-mail address:* hutsmi@sbcglobal.net (D.K. Hutchinson).

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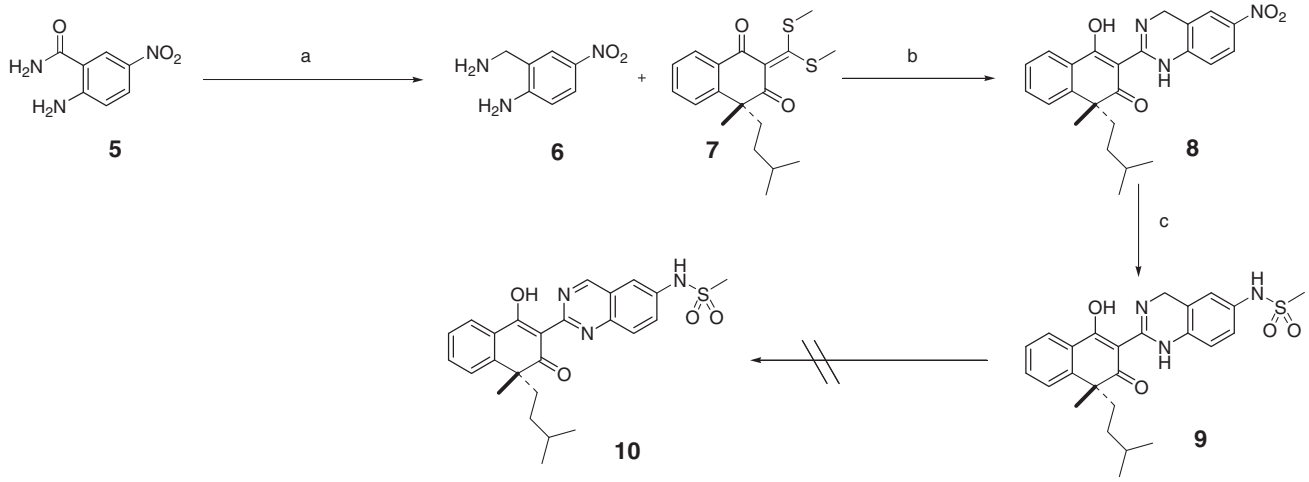


**Scheme 1.** Reagents and conditions: (a) (i) $BH_3$–$Me_2S$, THF; (ii) HCl, MeOH, 30% overall; (b) dioxane, $Et_3N$, 100 °C, 38%; (c) (i) $H_2$, Pt/C, EtOAc; (ii) MsCl, pyridine, 55% overall.

amide **5**[7] afforded diamine **6** in low yield as its bis-hydrochloride. Treatment of this material with ketenedithioacetal **7**[2b,d] afforded the corresponding dihydroquinazoline **8** in moderate yield.

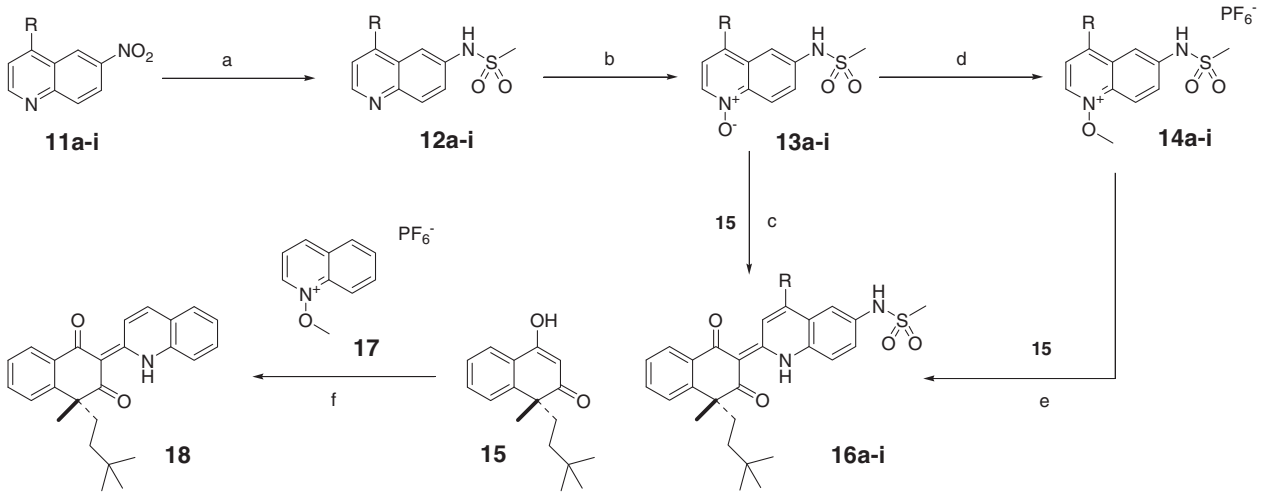
After reduction and methanesulfonamide formation, dihydroquinazoline **9** was obtained in 55% overall yield. Unfortunately, using a variety of oxidation conditions (DDQ, etc.), we were unable to oxidize **9** to the desired quinazoline **10**. Testing of **9**, however, against HCV polymerases from genotypes **1a** and **1b** showed $IC_{50}$ values of 0.83 and 4.2 μM, respectively.

Surmising that the modest activity of **9** was due in part to lowered propensity of the four rings to assume coplanarity, we decided to prepare the corresponding quinoline derivatives. The general synthetic route is summarized below in Scheme 2. As shown, commercially available nitro quinoline derivatives **11** were reduced to corresponding amine and treated with methanesulfonyl chloride to afford methanesulfonamides **12** in good overall yield. Oxidation with MCPBA readily afforded amine oxides **13**. While amine oxides **13** could be treated with diketone **15**[2b] in the presence of warm acetic anhydride, to afford quinoline derivatives **16** directly,[8] such a process in our hands seldom afforded the desired products in yields higher than 20%, and purification was complicated by the presence of many unidentified side products.

Consequently, we utilized relatively unknown chemistry published by Schnekenburger,[9] whereby an N-methoxy quinoline salt is allowed to react with a 1,3-dicarbonyl system in the presence of an amine base. Thus, amine oxides **13** were treated with methyl triflate, and after a salt exchange with hexafluorophosphoric acid,[10] the N-methoxy quinoline salts **14** could be isolated in good overall yield. Treatment of a cold DMF solution of diketone **15** and Hünig's base with a solution of salts **14** cleanly afforded quinoline derivatives **16** in 48–78% yields (see Supplementary data for a detailed preparation of **16a**). Unsubstituted compound **18** was prepared in an analogous manner from *N*-methoxyquinoline hexafluorophosphate (**17**). All of the compounds **16** and **18** were bright yellow in color, due to the favored hydrogen bond-stabilized enaminedione tautomer shown, which predominates in both solution and the solid state.[11]

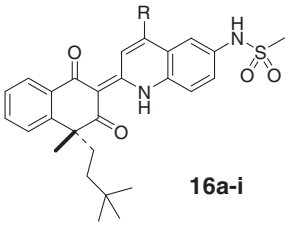
Compounds **16** were evaluated in biochemical assays against HCV NS5B polymerases from genotypes **1a** and **1b**. In addition, the activities of these compounds in cell culture against the HCV subgenomic replicons from genotypes **1a** and **1b** were also determined. Results are summarized above in Table 1 (see Supplementary data for biological methods).

Prototypical compound **16a** (R = H) showed surprisingly good activity in both enzymatic and cell culture assays, being

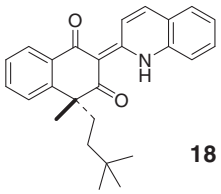


**Scheme 2.** Reagents and conditions. (a) (i) $H_2$, Pd/C, EtOAc; (ii) MsCl, pyridine, 70% overall; (b) MCPBA, $CH_2Cl_2$, 92%; (c) **15**, $Ac_2O$, Δ, <25%; (d) (i) MeOTf, $CH_2Cl_2$; (ii) $HPF_6$, EtOH–$H_2O$, 56%; (e) **15**, DIEA, DMF, 0 °C, 48–78%; (f) DIEA, DMF, 0 °C, 69%.

**Table 1**
Enzymatic and cell culture activities of quinoline derivatives




**16a-i**




**18**

| Compound | R | Polymerase **1a** $IC_{50}$[a] (nM) | Polymerase **1b** $IC_{50}$[a] (nM) | Replicon **1a** $EC_{50}$[a,b] (nM) | Replicon **1b** $EC_{50}$[a,b] (nM) |
|---|---|---|---|---|---|
| **2** | — | 4 | 7 | 4 | 2 |
| **16a** | H | 17 | 29 | 82 | 22 |
| **18** | H | 1680 | 5550 | — | — |
| **16b** | $CH_3$ | 32 | 52 | 135 | 20 |
| **16c** | *iso*-Amyl | 4300 | 1020 | — | — |
| **16d** | $CONMe_2$ | 72 | 238 | 932 | 99 |
| **16e** | CONHMe | 133 | 333 | 1040 | 245 |
| **16f** | $CO_2Et$ | 315 | 739 | 3080 | 660 |
| **16g** | $CH_2OH$ | 12 | 56 | 781 | 50 |
| **16h** | CHO | 15 | 25 | 1200 | 168 |
| **16i** | $CO_2H$ | 3 | 5 | 3320 | 221 |

[a] Both $IC_{50}$ and $EC_{50}$ values are means of at least two independent determinations, standard deviation ±10%. Detailed protocols can be found in Supplementary data.
[b] Assay run with 5% fetal calf serum.

approximately 4-fold less active in enzymatic assays than highly active comparator compound **2**. In general, activity in the enzymatic assays decreased with increasing size of the C-ring 4-position substituent, where activity fell off by a factor of two with methyl substitution as with **16b**, and then precipitously with the bulky isoamyl substituent as with **16c**. A similar trend with increasing steric bulk was observed in the series of *N*,*N*-dimethyl amide **16d** and *N*-methylamide **16e**, where all of these compounds showed much lower activity. An interesting trend was observed in going from ethyl ester **16f**, which only showed modest activity, to carbinol **16g** and aldehyde **16h**, which showed nearly identical activity to **16a** in both **1a** and **1b** enzymatic assays. Surprisingly, 4-position carboxylate **16i** showed similar enzymatic activity to comparator compound **2**.

Unfortunately, only prototypical compound **16a** showed useful levels of activity in cell culture assays. Preliminary experiments to determine intracellular concentration[12] of highly active analog **16i** suggest that this compound (and by inference, the others) did not readily penetrate the cell membrane of the hepatocytes used in these assays.

Assuming that these compounds bind to the palm site of the polymerase, their enzymatic activities can be rationalized by comparison with polymerase inhibitors whose binding modes have been determined by X-ray crystallography studies published in the RCSB Protein Data Bank.[4c,5c] The major expected binding interactions are summarized below in Figure 2 for compound **16i**. It can be seen that as long as the C-4 position of the C-ring has a small substituent (such as H with **16a**) the binding will be stabilized by, inter alia, the hydrogen bond interactions with the B-ring carbonyl and the NH of Tyr 448, the methanesulfonamide group with Asn 291 and Asp 318, as well as the neohexyl side chain with the hydrophobic pocket. Thus, without the C-ring sulfonyl group, compound **16a** shows less potent polymerase **1a** and **1b** enzymatic activities by only a factor of 3–4-fold as compared to compound **2**. Even a methyl group at C-4, as in compound **16b**, can be accommodated by the enzyme, albeit with activity half that of **16a**. When the C-4 substituent becomes sterically larger, as with an isoamyl group (**16c**), an ethoxycarbonyl group (**16f**), or a carboxamide (**16d** and **16e**), the substituent in the normal binding orientation would force the group into making severe steric interactions with the depression on the enzyme that interacts with the sulfonyl group, thus forcing the molecule to bind in a less favorable orientation, this being reflected in much higher $IC_{50}$ values.

More subtle factors seem to come into play in the case of compounds **16g–i**. Not surprisingly considering the activity of carboxamides **16d** and **16e**, carboethoxy analog **16f** shows only modest activity, but both hydroxymethyl derivative **16g** and aldehyde analog **16h** have similar activity to unsubstituted analog **16a**. More striking was the activity of carboxylic acid **16i**, which was essentially equipotent to comparator compound **2**. Molecular mechanics programs such as Chem3D® predict that the carboxyl group of **16i** and the carboxaldehyde of **16h** would be co-planar with the quinoline ring in the absence of other interactions. In the binding site of the enzyme, however, it is conceivable that these groups could rotate so that they were oriented orthogonally to the plane of the quinoline ring. If this were the case, they would, as shown in Figure 2, be able to project into the site on the enzyme that normally interacts with the sulfonyl group and participate in the water-mediated hydrogen bond with Serine 288. Similar stabilizing interactions could also be envisioned for hydroxymethyl analog **16g**, and carboxaldehyde **16h**. Other interactions with the

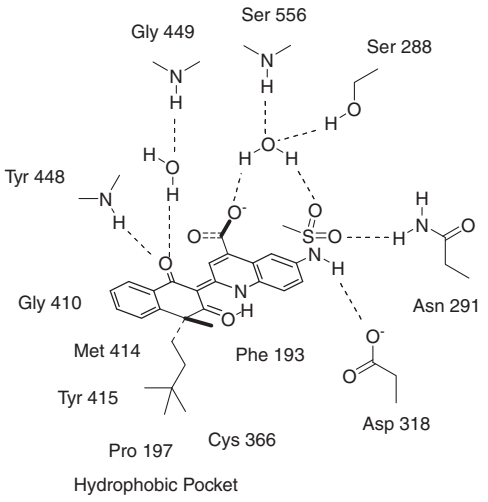



**Figure 2.** Graphical description of binding interactions of compound **16i** with the NS5B polymerase palm site.[4c,5c]

methanesulfonyl group on the D-ring would be similar to those noted in the published crystal structure of benzothiazine **3**,[4c] nicely rationalizing the potent enzymatic activity of carboxylic acid **16i**. To the extent that this rationale is valid, the quinoline-4-carboxylic acid moiety of compound **16i** could be viewed as a bioisosteric replacement for the benzothiadiazine moiety.

While compound **16i** may mimic the binding site interactions of comparator benzothiadiazine **2**, it must be noted that unsubstituted compound **16a** is only 5–6-fold less active than **16i**. This relatively modest difference considering the major loss of activity upon removal of the methanesulfonamide moiety as with compound **18**, suggests that these interactions are more important to the compound's binding to the enzyme than those of the sulfonyl group on the C-ring.

It can therefore be postulated that given efforts to optimize the other structural features of this or a similar series of molecules, good activity could be obtained without the need for the sulfonyl group of the C-ring. Such efforts are being vigorously pursued in our laboratories.

In conclusion, we have developed a series of compounds closely related to our earlier *gem*-dialkyl benzothiadiazines that exhibited, in some cases, excellent activity in enzymatic assays against genotypes **1a** and **1b** HCV NS5b polymerases, despite radical modification of the C-ring. In one case a 4-quinoline carboxylic acid **16i** gave essentially equivalent enzymatic activity as highly potent benzothiadiazine derivative **2**. The surprising level of activity of unsubstituted compound **16a** suggests that the contribution of the sulfonyl group of earlier benzothiadiazine analogs is far less important than previously appreciated.

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## Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.12.067.

## References and notes

1. (a) Dhanak, D.; Duffy, K. J.; Johnston, V. K.; Lin-Goerke, J.; Darcy, A. N.; Gu, B.; Silverman, C.; Gates, A. T.; Nonnemacher, M. R.; Earnshaw, D. L.; Casper, D. J.; Kaura, A.; Baker, A.; Greenwood, C.; Gutshall, L. L.; Maley, D.; DelVecchio, A.; Macarron, R.; Hofmann, G. A.; Alnoah, Z.; Cheng, H.-Y.; Chan, G.; Khandekar, S.; Keenan, R. M.; Sarisky, R. T. *J. Biol. Chem.* **2002**, *277*, 38322; (b) Tedesco, R.; Shaw, A. N.; Bambal, R.; Chai, D.; Concha, N. O.; Darcy, M. G.; Dhanak, D.; Fitch, D. M.; Gates, A.; Gerhardt, W. G.; Halegoua, D. L.; Han, C.; Hofmann, G. A.; Johnston, V. K.; Kaura, A. C.; Liu, N.; Keenan, R. M.; Lin-Goerke, J.; Sarisky, R. T.; Wiggall, K. J.; Zimmerman, M. N.; Duffy, K. J. *J. Med. Chem.* **2006**, *49*, 971.
2. (a) Krueger, A. C.; Madigan, D. L.; Green, B.; Hutchinson, D. K.; Jiang, W. W.; Kati, W. M.; Liu, Y.; Maring, C. J.; Masse, S. V.; McDaniel, K. F.; Middleton, T. R.; Mo, H.; Molla, A.; Montgomery, D. A.; Ng, T. I.; Kempf, D. J. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 2289; (b) Bosse, T. D.; Larson, D. P.; Wagner, R.; Hutchinson, D. K.; Rockway, T. W.; Kati, W. J.; Liu, Y.; Masse, S.; Middleton, T.; Mo, H.; Montgomery, D.; Jiang, W.; Koev, G.; Kempf, D. J.; Molla, A. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 586; (c) Hutchinson, D. K.; Rosenberg, T.; Klein, L. L.; Bosse, T. D.; Larson, D. P.; He, W.; Jiang, W. W.; Kati, W. M.; Kohlbrenner, W. E.; Liu, Y.; Masse, S. V.; Middleton, T.; Molla, A.; Montgomery, D. A.; Beno, D. W. A.; Stewart, K. D.; Stoll, V. S.; Kempf, D. J. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 3887; (d) Wagner, R.; Larson, D. P.; Beno, D. W. A.; Bosse, T. D.; Darbyshire, J. F.; Gao, Y.; Gates, B.; He, W.; Henry, R. F.; Hernandez, L. E.; Hutchinson, D. K.; Jiang, W. W.; Kati, W. M.; Klein, L. L.; Koev, G.; Kohlbrenner, W.; Krueger, A. C.; Liu, J.; Liu, Y.; Long, M. A.; Maring, C. J.; Masse, S. V.; Middleton, T.; Montgomery, D. A.; Pratt, J. K.; Stuart, P.; Molla, A.; Kempf, D. J. *J. Med. Chem.* **2009**, *52*, 1659.
3. (a) Pratt, J. K.; Donner, P.; McDaniel, K. F.; Maring, C. J.; Kati, W. M.; Mo, H.; Middleton, T.; Liu, Y.; Ng, T.; Xie, Q.; Zhang, R.; Montgomery, D.; Molla, A.; Kempf, D. J.; Kohlbrenner, W. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1577; (b) Donner, P. L.; Xie, Q.; Pratt, J. K.; Maring, C. J.; Kati, W.; Jiang, W.; Liu, Y.; Koev, G.; Masse, S.; Montgomery, D.; Molla, A.; Kempf, D. J. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 2735.
4. (a) Hendricks, R. T.; Spencer, S. R.; Blake, J. B.; Fell, J. B.; Fischer, J. P.; Stengel, P. J.; Leveque, V. J. P.; LePogam, S.; Rajyaguru, S.; Najera, I.; Josey, J. A.; Swallow, S. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 410; (b) Blake, J. F.; Fell, J. B.; Fischer, J. P.; Hendricks, R. T.; J. Robinson, J. E.; Spencer, S. R.; Stengel, P. J. U.S. Patent Application US 2006/0040927, February 23, 2006.; (c) Vicente, J. V.; Hendricks, R. T.; Smith, D. B.; Fell, J. B.; Fischer, J.; Spencer, S. R.; Stengel, P. J.; Mohr, P.; Robinson, J. E.; Blake, J. F.; Hilgenkamp, R. K.; Yee, C.; Adjabeng, G.; Elworthy, T. R.; Tracy, J.; Chin, E.; Li, J.; Wang, B.; Bamberg, J. T.; Stephanson, R.; Oshiro, C.; Harris, S. F.; Ghate, M.; Leveque, V.; Najera, I.; Le Pogam, S.; Rajyaguru, S.; Ao-leong, G.; Alexandrova, L.; Larrabee, S.; Brandl, M.; Briggs, A.; Sukhtankar, S.; Farrell, R.; Xu, B. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 3642.
5. (a) Ellis, D. A.; Blazel, J. K.; Webber, S. E.; Tran, C. V.; Dragovich, P. S.; Sun, Z.; Ruebesam, F.; McGuire, H. M.; Xiang, A. X.; Zhao, J.; Li, L.-S.; Zhou, Y.; Han, Q.; Kissinger, C. R.; Showalter, R. E.; Lardy, M.; Shah, A. M.; Tsan, M.; Patel, R.; LeBrun, L. A.; Kamran, R.; Bartkowski, D. M.; Nolan, T. G.; Norris, D. A.; Sergeeva, M. V.; Kirkovsky, L. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 4628; (b) Dragovich, P. S.; Blazel, J. K.; Ellis, D. A.; Han, Q.; Kamran, R.; Kissinger, C. R.; LeBrun, L. A.; Li, L.-S.; Murphy, D. E.; Noble, M.; Patel, R. A.; Ruebesam, F.; Sergeeva, M. V.; Shah, A. M.; Showalter, R. E.; Tran, C. V.; Tsan, M.; Webber, S. E.; Kirkovsky, L.; Zhou, Y. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 5635; (c) Rubesam, F.; Murphy, D. E.; Tran, C. V.; Li, L.-S.; Zhou, J.; Dragovich, P. S.; McGuire, H. M.; Ziang, A. X.; Sun, Z.; Ayida, B. K.; Blazel, J. K.; Kim, S. H.; Zhou, Y.; Han, Q.; Kissinger, C. R.; Webber, S. E.; Showalter, R. E.; Shah, A. M.; Tsan, M.; Patel, R. A.; Thompson, P. A.; LeBrun, L. A.; Hou, H. J.; Kamran, R.; Sergeeva, M. V.; Bartkowski, D. M.; Nolan, T. G.; Norris, D. A.; Khandurina, J.; Brooks, J.; Okamoto, E.; Kirkovsky, L. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 6404; the correct entry code is **3H2L** at <http://www.rcsb.org>
6. Brown, H. C.; Narasimhan, S.; Choi, Y. M. *Synthesis* **1981**, 441.
7. Yale, H. L.; Kalkstein, M. *J. Med. Chem.* **1967**, *10*, 334.
8. Yousif, M. M.; Saeki, S.; Hamana, M. *Chem. Pharm. Bull.* **1982**, *30*, 2326.
9. Mehnert, J.; Schnekenburger, J. *Arch. Pharm. (Weinheim)* **1988**, *321*, 897.
10. Most literature examples of N-methoxy pyridine or quinoline salts (see for example: Katritzky, A. R.; Lunt, E. *Tetrahedron* **1969**, *25*, 4291) describe isolation as the corresponding perchlorates. Due to the explosive nature of perchlorates in general, we sought to avoid such an unnecessary hazard, and as a result found that the hexafluorophosphate counterion afforded nicely crystalline non-hygroscopic salts which could be easily purified by trituration with ether. Several of these salts, notably **14a** and **17**, remain free flowing after storage at ambient temperature for >2 years following completion of this project.
11. Dobosz, R.; Kolehmainen, E.; Valkonen, A.; Ośmiałowski, B.; Gawinecki, R. *Tetrahedron* **2007**, *63*, 9172.
12. Colletti, L. M.; Liu, Y.; Koev, G.; Richardson, P. L.; Chen, C.-M.; Kati, W. *Anal. Biochem.* **2008**, *383*, 186.