Bioorganic & Medicinal Chemistry Letters 18 (2008) 3887–3890

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Hepatitis C NS5B polymerase inhibitors: 4,4-Dialkyl-1-hydroxy-3-oxo-3, 4-dihydronaphthalene-3-yl benzothiadiazine derivatives

Douglas K. Hutchinson [a,*], Teresa Rosenberg [a], Larry L. Klein [a], Todd D. Bosse [a], Daniel P. Larson [a], Wenping He [a], Wen W. Jiang [a], Warren M. Kati [a], William E. Kohlbrenner [a], Yaya Liu [a], Sherie V. Masse [a], Tim Middleton [a], Akhteruzzaman Molla [a], Debra A. Montgomery [a], David W. A. Beno [b], Kent D. Stewart [c], Vincent S. Stoll [c], Dale J. Kempf [a]

[a] Department of Antiviral Research, Global Pharmaceutical Research and Development, Abbott Laboratories, Abbott Park, IL 60064, USA
[b] Department of Drug Metabolism and Pharmacokinetics, Global Pharmaceutical Research and Development, Abbott Laboratories, Abbott Park, IL 60064, USA
[c] Department of Structural Biology, Global Pharmaceutical Research and Development, Abbott Laboratories, Abbott Park, IL 60064, USA

ARTICLE INFO

Article history:
Received 31 March 2008
Revised 10 June 2008
Accepted 12 June 2008
Available online 18 June 2008

Keywords:
Hepatitis C
HCV
HCV NS5B polymerase
Antiviral

ABSTRACT

4,4-Dialkyl-1-hydroxy-3-oxo-3.4-dihydronaphthalene-3-yl benzothiadiazine derivatives were synthesized and evaluated as inhibitors of genotypes 1a and 1b HCV NS5B polymerase. A number of these compounds exhibited potent activity against genotypes 1a and 1b HCV polymerase in both enzymatic and cell culture activities. A representative compound also showed favorable pharmacokinetics in the rat.

Hepatitis C is a positive-stranded RNA virus of the *Flaviviridae* family which was first identified in 1989.[1] Since that time, the hepatitis C virus (HCV) has been found to be a major cause of cirrhosis, hepatocellular carcinoma, as well as liver failure, and is the most common cause in developed countries of the need for liver transplantation.[2] HCV is most commonly transmitted by blood transfusions, hemodialysis, and intravenous drug use, and is quite insidious in that clinical manifestations can be mild or non-extant for years or even decades after initial infection. It is estimated by the Centers for Disease Control (CDC) that 2.7 million Americans and a total of 170 million individuals worldwide are chronically infected with HCV, this number being approximately 3% of the world population.[3] In addition, the CDC estimates that 8–10 thousand deaths each year in the United States alone are related to HCV, and this number is expected to triple over the next two decades.

It can be seen that clinical manifestations of HCV infection represent a major impending healthcare problem. Unfortunately, the current treatment regimens utilizing pegylated interferon-α-2a (PEG-IFN-α, PEG-Intron®) in combination with ribavarin (Rebetrol® and Copegus®) are extremely expensive and are accompanied by serious systemic side effects.[4,5] This is complicated by the existence of six major genotypes of HCV (genotypes 1–6), of which genotypes 1 and 2 are the most common in the United States, Europe, and Japan.[6] Sustained virologic response (SVR) in patients infected with HCV genotypes 2 and 3 approaches 80% after a 24-week treatment regimen of PEG-IFN-α/ribavarin. In contrast, SVR in patients infected with genotype 1 is less than 50% even after extended treatment for 48 weeks.[4,5] Consequently there is a great medical need for new HCV chemotherapeutic agents that would give SVR against genotypes other than 2 or 3.

The cloning of HCV in 1989[1] eventually led to identification of several targets for small molecule intervention in the virus life cycle.[7,8] Of these, inhibitors of the NS3/NS4A protease/helicase and the NS5B RNA-dependent RNA polymerase (RdRp) have been the most actively investigated.[8] Drug discovery efforts aimed at inhibiting the HCV NS5B RdRp have disclosed both nucleoside and non-nucleoside inhibitors.[8] One series of non-nucleoside inhibitors that show promise in both enzymatic and cell-based replication assays using the subgenomic HCV replicon are the benzothiadiazines, of which structure **1** is representative.[9] This series of compounds, originally prepared as diuretics,[10] was found to be potent inhibitors of HCV NS5B polymerase in a manner noncompetitive with respect to GDP.[10] Mechanistically these compounds were found to inhibit initiation of viral RNA synthesis by preventing the binding of the RNA primer to the enzyme[11] (Fig. 1).

As part of ongoing drug discovery efforts at Abbott, hydrazine derivatives, such as **2**, were found to be potent inhibitors of HCV

* Corresponding author. Tel.: +1 847 938 8441; fax: +1 847 938 2756.
E-mail address: douglas.hutchinson@abbott.com (D.K. Hutchinson).

0960-894X/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2008.06.043

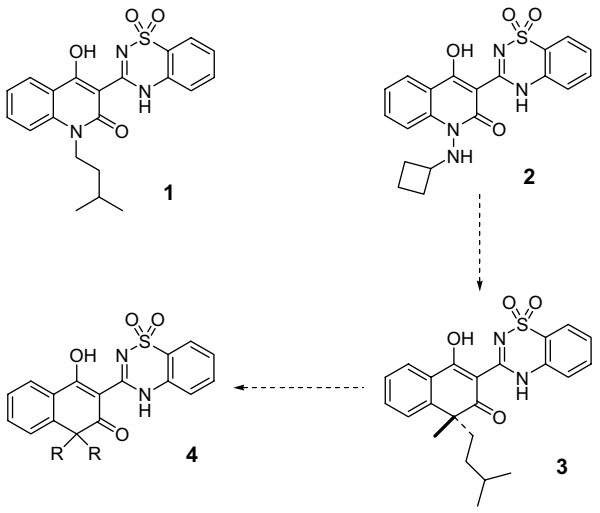


Figure 1. Genesis of dialkyl benzothiadiazines.

polymerase both in enzymatic and cell-based replication assays.[12] While some analogs of **2** exhibited good pharmacological properties, others showed extremely poor solubility and were difficult to formulate for in vivo studies. Postulating that replacement of the nitrogen atom of the dihydroquinolinedione ring system of **1** with a carbon atom, as in structure **3**, would interfere with the compound's π-stacking ability, it would follow that such compound would be less crystalline, and therefore more soluble. A similar approach was used by Rosen to improve the solubility of a series of fluoroquinolone antibacterial agents.[13] Preparation of **3** would, however, necessitate an asymmetric synthesis or a resolution, the difficulty of which would not be justified for a speculative series of analogs. Consequently, the preparation of a symmetrically disubstituted analog series, illustrated by structure **4**, was undertaken for proof of concept.

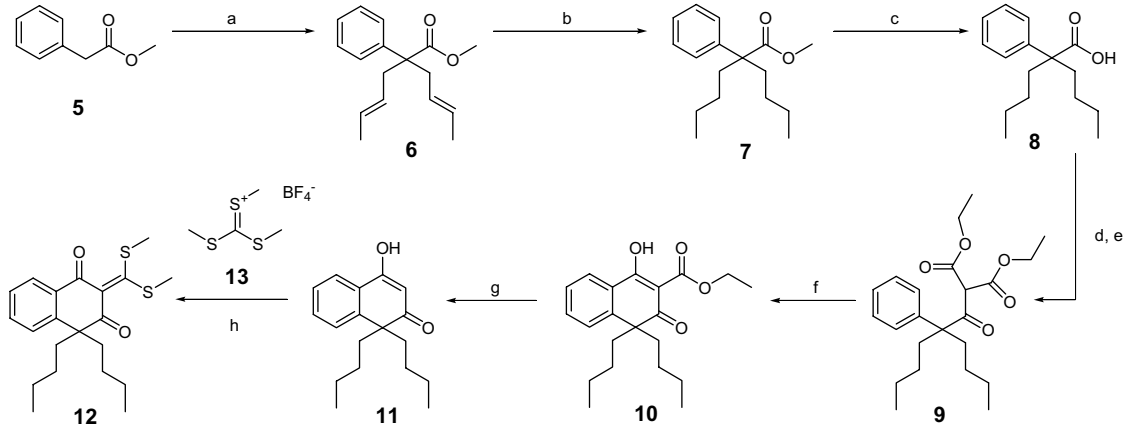
The synthesis of the 4,4-dialkyl-1-hydroxy-3-oxo-3.4-dihydronaphthalene ring system was easily accomplished by a modification of the method of Buckle.[14] The synthesis of the *gem*-dibutyl series was typical of that used for all analog series investigated, and is summarized in Scheme 1. As shown, alkylation of methyl phenylacetate (**5**) with crotyl bromide afforded the di-(2-butenyl) compound **6** in high yield. After hydrogenation of the double bonds and hydrolysis of the ester,[12,15] the acid **8** was treated with an excess of oxalyl chloride in the presence one equivalent of DMF[13,16] to cleanly afford the acid chloride. Subsequent treatment with diethyl malonate under the conditions of Rathke[17] afforded ketodiester **9** in good overall yield. Cyclization of **9** was readily accomplished by stirring in methanesulfonic acid overnight, affording diketo ester **10** in good yield. After hydrolysis/decarboxylation, resultant diketone **11** was treated with salt **13** to afford dithioketene acetal **12** in moderate to good yields.[18]

Precursors to the benzothiadiazine ring system were synthesized by methods summarized below in Scheme 2. As shown, amination of benzenesulfonamide **14** afforded **15** in 90% yield.[19] Reduction of the nitro group and treatment with methanesulfonyl chloride gave the key methanesulfonamide **16**. For other substitituents on the benzothiadiazine ring system, synthetic intermediates were prepared by modifications of methods developed by Topliss.[20] As shown, benzylation of nitrophenol **17** followed by nucleophilic aromatic displacement with benzyl mercaptan, afforded thioether **20** in excellent overall yield. Oxidation of **20** with chlorine gas and then quenching the intermediate sulfonyl chloride with aqueous ammonia, afforded the nitro benzenesulfonamide **21** in 89% overall yield. Reduction of the nitro group with iron in the presence of ammonium chloride afforded aniline **22** in good yield.

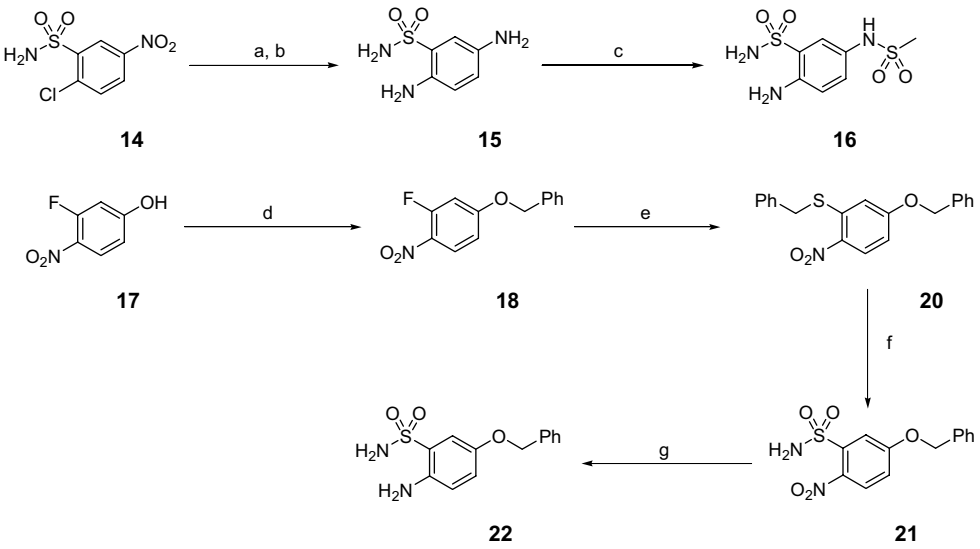
Final assembly and functional group interconversions of analogs for biological evaluation are summarized in Scheme 3. As shown, treatment of ketenedithioacetal **12** with amino sulfonamide **22** in refluxing dioxane afforded benzothiadiazine **23** in good yield. Compound **23** was then transformed into the oxyacetamide analog **25** in excellent overall yield using routine chemical transformations. In a similar manner, treatment of **12** with **16** afforded methanesulfonamide **26** in good yield.

A number of structurally diverse compounds synthesized in an analogous manner 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. These data are summarized in Table 1 below (see Supplementary material for biological methods).

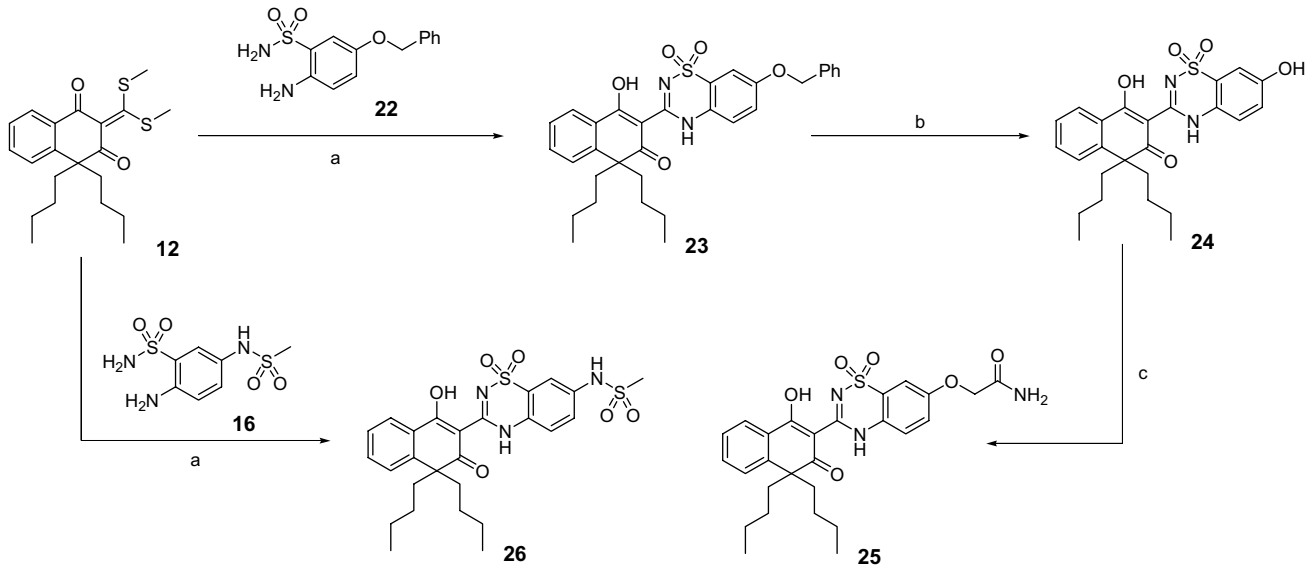
As shown, a wide range of activities were observed for the *gem*-dialkyl benzothiadiazines. Based on published crystal structure data of structurally related inhibitors in the benzothiadiazine series, it can be postulated that the *gem*-dimethyl analogs (**27** and **28**) would show, at best, modest levels of activities, since they lack a longer hydrocarbon chain to project into the hydrophobic pocket below the plane of the ring systems.[21] Consistent with this theory, increasing the chain length improved the potency, with *gem*-dipropyl methanesulfonamide **31** showing a nearly 10-fold increase in activity over its *gem*-dimethyl counterpart **28**. In most cases exam-



Scheme 1. Reagents and conditions: (a) crotyl bromide, NaH, NaI, THF, Δ, 95%; (b) H_2 (45 psi), 10% Pd/C, MeOH, 95%; (c) $KOSiMe_3$ (5 equiv), THF, Δ, 90%; (d) oxalyl chloride (3 equiv), DMF (1 equiv), hexanes; (e) diethyl malonate, $MgCl_2$, TEA, CH_3CN, 95% overall; (f) methanesulfonic acid, RT, 60%; (g) 1:1 1 N HCl–THF, Δ, 70%; (h) **13**, pyridine, dioxane, Δ, 70%.



Scheme 2. Reagents and conditions: (a) $(NH_4)_2CO_3$, $CuSO_4$, NH_4OH, 120 °C, sealed tube, 90%; (b) $Na_2S_2O_4$, NaOH, 70%; (c) MsCl, pyridine, CH_2Cl_2, 60%; (d) benzyl bromide, Cs_2CO_3, TBAI, DMF, 100%; (e) benzyl mercaptan, Na_2CO_3, EtOH, Δ, 90%; (f) i—Cl_2, HOAc; 0 °C; ii—NH_4OH, CH_2Cl_2, 89% overall; (g) Fe, NH_4Cl, MeOH–H_2O, Δ, 72%.



Scheme 3. Reagents and conditions: (a) dioxane, 100 °C, 75%; (b) H_2, 10% Pd/C, EtOAc, 90%, (c) ICH_2CONH_2, TBAI, Cs_2CO_3, DMF, 90%.

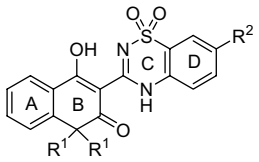
ined, the methanesulfonamide substituent on the D ring gave 2- to 4-fold better activities than those obtained using the oxyacetamide substituent. The *gem*-dibutyl series showed the best overall in vitro activity in this series, with *gem*-dibutyl oxyacetamide derivative **25** and methanesulfonamide derivative **26** showing submicromolar IC_{50} values of 10 and 73 nM against HCV strains 1a and 1b, respectively. In addition, *gem*-dibutyl methanesulfonamide **26** also showed submicromolar levels of activity in the replicon assays, against both the 1a and 1b strains. While the *iso*-amyl side chain had afforded potent analogs in previous series, in this case the *gem*-*iso*-amyl compounds **34** and **35** showed less activity than their *n*-butyl counterparts, possibly because of the increased energy cost of displacing more water molecules from the space above the plane of the rings at the binding site. Consequently, while the *iso*-amyl group may be ideal for binding in the hydrophobic pocket in other analog series,[21] the *n*-butyl group may represent a compromise between binding affinity and the energy cost of displacing waters from the binding site.

The pharmacokinetic behavior of *gem*-dibutyl methanesulfonamide **26** in the rat was examined using both intravenous and oral doses of 5 mg/kg. As shown in Table 2, the compound shows low clearance with a moderate volume of distribution. With oral dosing, the compound shows a half-life of 4.3 h, with a maximum plasma concentration of 0.58 μg/mL occurring at 3 h. With exposure levels obtained from oral dosing being similar to those obtained from intravenous dosing, these data correspond to a bioavailability of 94%.

The pharmacokinetic behavior of **26** was even more interesting when liver concentrations were determined. As shown in Table 3, much higher levels of **26** were found in the liver than in plasma, up to 12 h after dosing. Maximum liver concentrations of 21 μg/g were achieved 6 h after dosing, whereas a 0.41-μg/mL concentration was achieved in the plasma, this corresponding to a 48:1 liver to plasma ratio.

In conclusion, it can be stated that while most of the compounds in this study had only modest activity, the *gem*-dibutyl

Table 1
Biochemical and cell culture activity of *gem*-dialkyl benzothiadiazines



Compound	R^1	R^2	Polymerase 1a IC_{50} (μM)[a]	Polymerase 1b IC_{50} (μM)[a]	Replicon 1a EC_{50} (μM)[a,b]	Replicon 1b EC_{50} (μM)[a,b]
27	CH_3	H	2.2	—	—	—
28	CH_3	$NHSO_2CH_3$	0.93	6.26	—	—
29	*n*-Propyl	H	2.20	—	—	—
30	*n*-Propyl	OCH_2CONH_2	0.24	—	>10.0	0.81
31	*n*-Propyl	$NHSO_2CH_3$	0.099	0.13	0.60	0.022
32	*n*-Butyl	H	0.99	2.27	—	—
25	*n*-Butyl	OCH_2CONH_2	0.26	0.33	9.64	0.29
26	*n*-Butyl	$NHSO_2CH_3$	0.010	0.073	0.28	0.048
33	*iso*-Amyl	H	0.82	—	—	—
34	*iso*-Amyl	OCH_2CONH_2	0.32	—	10.10	>3.0
35	*iso*-Amyl	$NHSO_2CH_3$	0.068	0.39	0.42	—
1			0.54	0.42	—	0.25
2			0.28	0.48	—	0.10

[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 material.
[b] Assay run with 5 % fetal calf serum.

Table 2
Mean pharmacokinetic parameters for **26** in rat at 5 mg/kg[a]

IV dose (5 mg/kg)					Oral dose (5 mg/kg)				
$t_{1/2}$[b]	Vss	Vβ	AUC	CLp	$t_{1/2}$[b]	C_{max}	T_{max}	AUC	F
4.3	1.87	6.11	7.3	0.70	4.3	0.58	3.00	6.4	94.3

$t_{1/2}$ (h); Vss (L/kg); Vβ (L/kg); $AUC^{0-\infty}$ (μg h/mL); CLp (L/h kg).
[a] All parameters are means of values obtained from each of three rats.
[b] Harmonic mean.

Table 3
Mean liver/plasma levels of **26** after 5 mg/kg oral dose in rat[a]

	Concentration (μg/g or μg/mL)			
Time (h)	1	6	12	24
Liver concentration	14.2	20.8	4.6	0.15
Plasma concentration	0.45	0.41	0.25	0.0
Liver/plasma ratio	34.3	48.2	17.3	—

[a] All parameters are means of values obtained for **26** from each of three rats.

methanesulfonamide **26** showed surprisingly good activity both in enzymatic and cell culture assays. Most notably, **26** showed good pharmacokinetic behavior in the rat and showed outstanding selective penetration into the liver. These data taken together suggested that further work on analogs of **26** with unsymmetrical alkyl substitution on the B ring would be justified, and these studies have been ongoing in our laboratories.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.06.043.

References and notes

1. Choo, Q. L.; Kuo, G.; Weiner, A. J.; Overby, L. R.; Bradley, D. W.; Houghton, M. *Science* **1989**, *244*, 359.
2. Poordad, F. F.; Tran, T.; Martin, P. *Expert Opin. Emerging Drugs* **2003**, *8*, 9.
3. Alter, M. J.; Kruszon-Moran, D.; Nainan, O. V.; McQuillan, G. M.; Gao, F.; Moyer, L. A.; Kaslow, R. A.; Margolis, H. S. *N. Engl. J. Med.* **1999**, *341*, 556.
4. Manns, M. P.; McHutchison, J. G.; Gordon, S. C.; Rustgi, V. K.; Shiffman, M.; Reindollar, R.; Goodman, Z. D.; Koury, K.; Ling, M.-H.; Albrecht, J. K. *Lancet* **2002**, *347*, 975.
5. Fried, M. W.; Shiffman, M. L.; Reddy, K. R.; Smith, C.; Marinos, G.; Goncales, F. L., Jr.; Haussinger, D.; Diego, M.; Carosi, G.; Dhumeaux, D.; Craxi, A.; Lin, A.; Hoffman, J.; Yu, J. *N. Engl. J. Med* **2002**, *347*, 975.
6. Simmonds, P. J. *Hepatology* **1999**, *31*, 54.
7. Moradpour, D.; Brass, V.; Gosert, R.; Woelk, B.; Blum, H. E. *Trends Mol. Med.* **2002**, *8*, 476.
8. Gordon, C. P.; Keller, P. A. *J. Med. Chem.* **2005**, *48*, 1.
9. 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.
10. (a) Ukrainets, I. V.; Taran, E. A.; Gorokhova, O. V.; Jaradat, N. A.; Voronina, L. N.; Porokhnyak, I. V. *Chem. Heterocycl. Compd. (Engl. Trans.)* **2000**, *36*, 346; (b) Kovalenko, S. N.; Chernykh, V. P.; Shkarlat, A. I.; Ukrainets, I. V.; Gridasov, V. I.; Rudnev, S. A. *Chem. Heterocycl. Compd. (Engl. Trans.)* **1998**, *34*, 791.
11. Gu, B.; Johnston, V. K.; Gutshall, L. L.; Nguyen, T. T.; Gontarek, R. R.; Darcy, M. G.; Tedesco, R.; Dhanak, D.; Duffy, K. J.; Kao, C. C.; Sarisky, R. T. *J. Biol. Chem.* **2003**, *278*, 16602.
12. Pratt, J. K.; Donner, P.; McDaniel, K. F.; Maring, C. J.; Kati, W. M.; Mo, H.; Mittleton, 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.
13. Rosen, T.; Chu, D. T. W.; Lico, I. M.; Fernandes, P. B.; Marsh, K.; Shen, L.; Cepa, V. G.; Pernet, A. G. *J. Med. Chem.* **1988**, *31*, 1598.
14. Buckle, D. R.; Cantello, B. C. C.; Smith, H.; Smith, R. J.; Spicer, B. A. *J. Med. Chem.* **1977**, *20*, 1059. Also see: Hutchinson, D.K. et al. U.S. Patent Application US 2005/0107364, May 19, 2005.
15. Laganis, E. D.; Chenard, B. L. *Tetrahedron Lett.* **1984**, *25*, 5831.
16. Ward, D. E.; Rhee, C. K. *Tetrahedron Lett.* **1991**, *32*, 7165.
17. Rathke, M. W.; Cowan, P. J. *J. Org. Chem.* **1985**, *50*, 2622.
18. (a) Barbero, M.; Cadamuro, S.; Digani, I.; Fochi, R.; Gatti, A.; Regondi, V. *Synthesis* **1988**, 22; (b) Gompper, R.; Kutter, E. *Chem. Ber.* **1965**, *98*, 1365.
19. Walter, L. A. *J. Am. Chem. Soc.* **1943**, *65*, 738.
20. Topliss, J. G.; Sherlock, M. H.; Reimann, H.; Konzelman, L. M.; Shapiro, E. P.; Pettersen, B. W.; Schneider, H.; Sperber, N. *J. Med. Chem.* **1963**, *6*, 122.
21. 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.