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Identification and synthesis of major metabolites of Vasopressin V_2-receptor agonist WAY-151932, and antagonist, Lixivaptan® ☆,☆☆

Albert J. Molinari,[a,*] Eugene J. Trybulski,[a] Jehan Bagli,[b] Susan Croce,[b] John Considine,[c] Jian Qi,[d] Kadum Ali,[d] William DeMaio,[e] Lynne Lihotz[e] and David Cochran[b]

[a]*Chemical and Screening Sciences, Wyeth Research, 500 Arcola Road, Collegeville, PA 19426, USA*
[b]*Chemical and Screening Sciences, Wyeth Research, Princeton, NJ 08543-8000, USA*
[c]*Chemical Development, Wyeth Research, Pearl River, NY 10965, USA*
[d]*Chemical and Screening Sciences, Wyeth Research, Pearl River, NY 10965, USA*
[e]*Biotransformation, Wyeth Research, 500 Arcola Road, Collegeville, PA 19426, USA*

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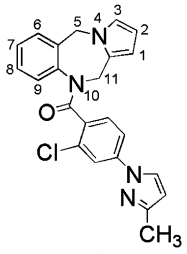
Abstract—Small molecule agonists and antagonists of the V_2-vasopressin receptor have been discovered and have undergone clinical trials. In conjunction with these discovery programs, the synthesis and biological testing of various metabolites associated with these clinical targets were actively pursued. We now report the results of our synthetic efforts and the corresponding biological data generated for several of the metabolites of WAY-151932 and CL-347985 (Lixivaptan®).

Vasopressin is a nonapeptide posterior pituitary regulatory hormone also known as anti-diuretic hormone.[1,2] It directly and indirectly influences water balance, osmotic salt balance, and blood pressure through a series of vasopressin receptors.[3] The V_2-receptors, located in the distal collecting tubule of the kidney, are G-protein mediated receptors which balance the re-absorption and elimination of the body's free-water content. Discovery programs were initiated to identify small molecule agonists and antagonists of this receptor subtype to treat medical conditions, such as nocturnal enuresis for an agonist, and congestive heart failure and liver cirrhosis for an antagonist.

The programs produced two small molecules, a V_2-receptor agonist[4,5] **1**, and a V_2-receptor antagonist[6–8] **2**, Lixivaptan®. Both compounds entered clinical trials. Over the course of the program it was necessary to synthesize and test the major metabolites associated with each compound.



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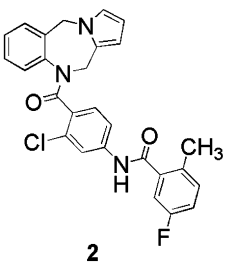

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The metabolites were in general first detected in rat and dog blood samples by LC/MS. Possible metabolite structures were proposed based on general principles of human metabolism. The proposed metabolites were subsequently synthesized, structures determined, and

Keywords: Vasopressin; V_2-receptor agonist; V_2-receptor antagonist; Lixivaptan®.

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* Corresponding author. Tel.: +1 484 865 4408; fax: +1 484 865 9399; e-mail: Molinaa@wyeth.com

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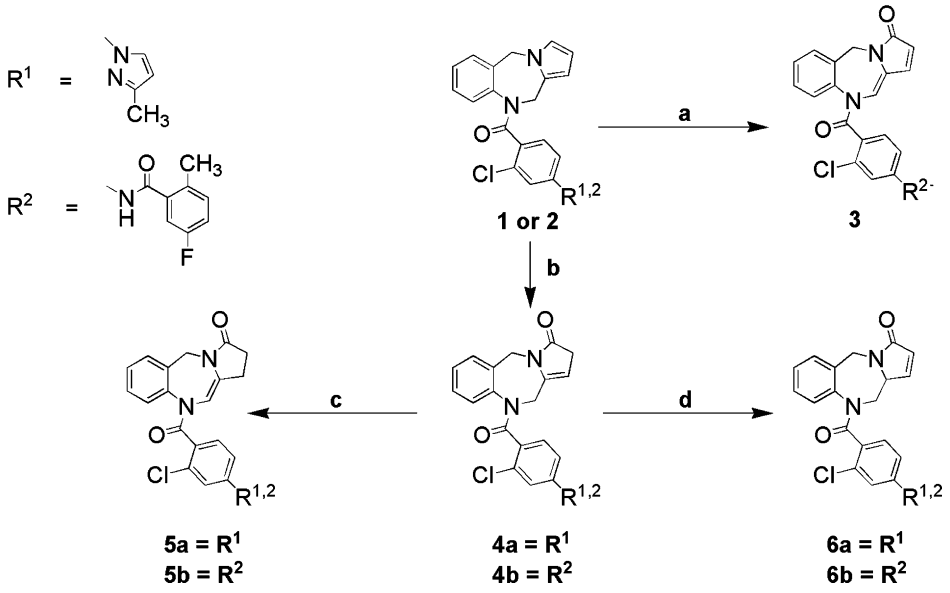


Scheme 1. Reagents and conditions: (a) 3,3-dimethyldioxirane in acetone; (b) m-CPBA (1 equiv), −40 °C > −10 °C, DCM; (c) silica gel or acid at room temperature in MeOH, or 230 °C (neat) 3 min; (d) 2.5 grade alumina, EtOAc–DCM, 25 °C.

their presence confirmed in rat, dog, and/or human blood samples collected from patients participating in the clinical trials.[9–12] The synthesized compounds were later tested for biological activity.

Chemistry: preparation of proposed pyrrole metabolites. The pyrrole ring of the tricyclic pyrrolo[2,1-*c*][1,4]benzodiazepine template was the most sensitive region of the molecule toward oxidative metabolism. In Scheme 1, preferential chemical oxidation in the pyrrole ring of vasopressin agonist **1** and antagonist **2** occurred readily with purified 3-chloroperoxybenzoic acid (>90%) in dichloromethane at −40 °C to afford kinetic oxidation products **4a** and **4b**. Both compounds were sufficiently stable to enable isolation, characterization, and cold storage as solids; however, they independently isomerized under a variety of conditions. Under acidic conditions, silica gel at room temperature, or heated neat at 230 °C, the kinetic oxidation products underwent a 1,3-sigmatropic shift of the double bond from the five-membered ring to the seven-membered ring to afford the thermodynamic compounds **5a** and **5b** exclusively. However; under basic conditions, such as potassium carbonate or basic alumina, an alternative migration from the 1,11a-β–γ to the 1,2-α–β double bond afforded compounds **6a** and **6b** exclusively. Secondary oxidation products associated with the pyrrole ring were also identified with compound **2**. Thus, oxidation of **2** with 3,3-dimethyldioxirane afforded compound **3**, which was not further tested biologically.

The position of the double bond in compound **5b** was deduced from homonuclear proton decoupling experiments as shown in Figure 1b and c. Thus, irradiation of **5b** at 6.32 ppm simplified the 1-α and 1-β proton coupling patterns from a (dddd) into a (ddd). In addition, an attached proton (APT) carbon–hydrogen experiment, shown in Figure 1d, clearly showed three upfield carbon chemical shifts (carbons 1, 2, and 5), each attached to two protons. With the structure of compound **5b** determined, the structural assignments of compounds **4b** and **6b** were deduced from analysis of their respective NMR spectra. Compound **4b** had a broad singlet at 3.00 ppm representing the 2-CH_2 protons, and compound **6b** had olefinic protons at 4.72 and 5.19 ppm with allylic coupling to proton **11a**. In analogous fashion the assignments of compounds **4a**, **5a**, and **6a** were made by comparison with the spectra previously obtained for compounds **4b**, **5b**, and **6b**.

In Scheme 2, further oxidation of **4b** with a second equivalent of m-CPBA afforded the 1-position alcohol via the proposed oxirane intermediate **7**, followed by ring opening with the 3-chlorobenzoic acid formed in situ to provide **8**, and subsequent β-elimination to generate the allylic alcohol **9**.

As shown in Scheme 3, the α–β conjugated enone of **6b** is susceptible to 1,4 conjugate addition and subsequent attack by biological nucleophiles, such as reduced glutathione (GSH). Treatment of **6b** with GSH afforded the glutathione adduct **10**, which was proposed as a biliary metabolite by our biotransformation group. While prepared, co-injection of **10** with drug treated bile was not conducted to confirm the proposal.

Preparation of proposed diazepine metabolites. In Scheme 4, a three-step derivatization of methyl anthranilate readily affords compounds **12a** and **12b**. In Schemes 5 and 6, treatment of pyrrole or a pyrrole ester with 2-nitrobenzyl bromide, followed by catalytic reduction of the nitro group, acylation of the corresponding aniline, and either Vilsmeier reaction conditions or hydrolysis afforded the compounds **14a** and **14b** or **15a** and **15b**, respectively.

Preparation of proposed pyrazole metabolites. It was discovered that the 3-methyl group in the pyrazole moiety

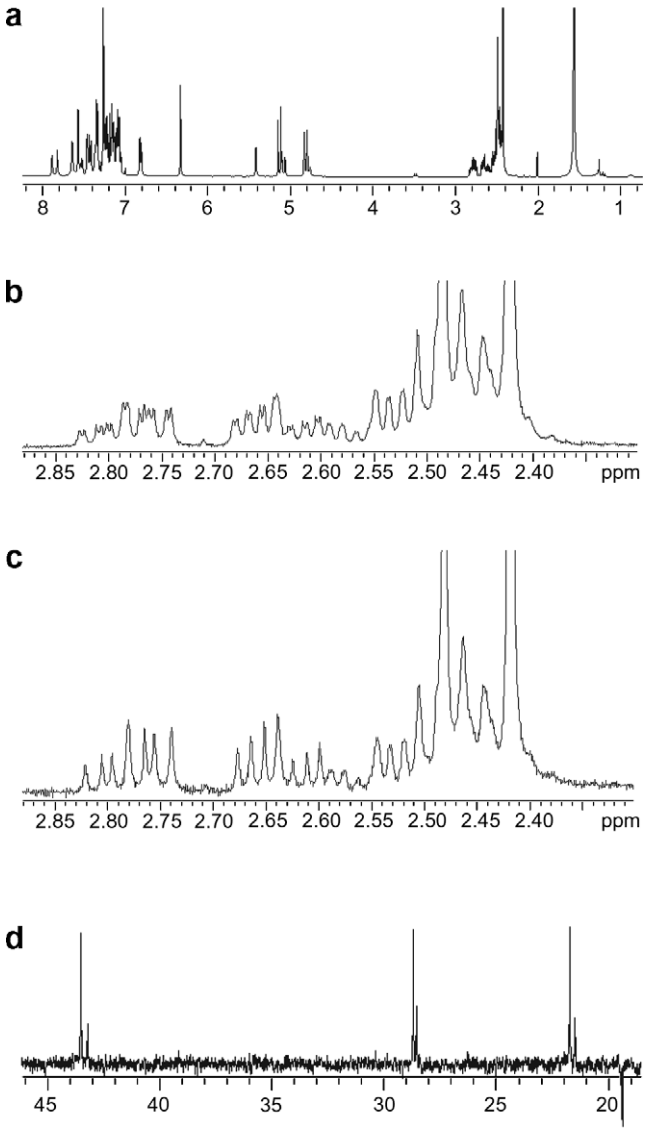
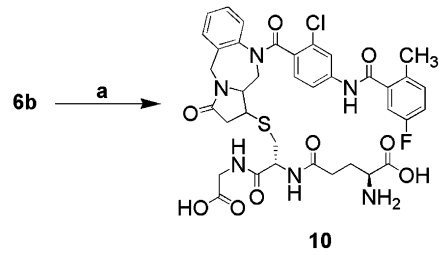
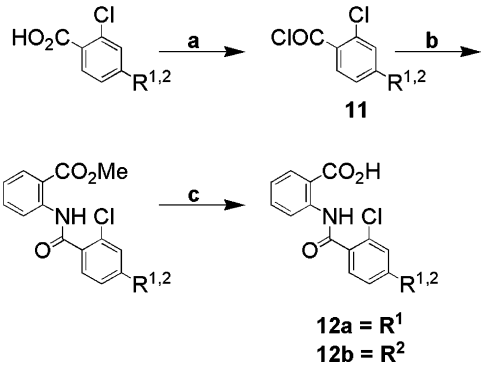


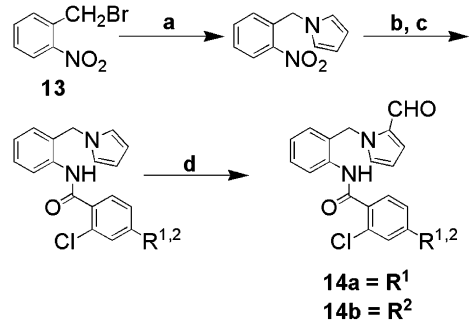
Figure 1. (a) ^{1}H NMR spectrum of **5b** in $CDCl_3$, 400 MHz. (b) Upfield region of the coupled ^{1}H spectrum in $CDCl_3$, 400 MHz. (c) Decoupled spectrum, same upfield region, $CDCl_3$, 400 MHz, irradiated at 6.32 ppm. (d) Expanded upfield view of APT spectrum.



Scheme 3. Reagents and conditions: (a) Reduced glutathione (GSH), TEA, MeCN–H_2O, 25 °C, 12 h.

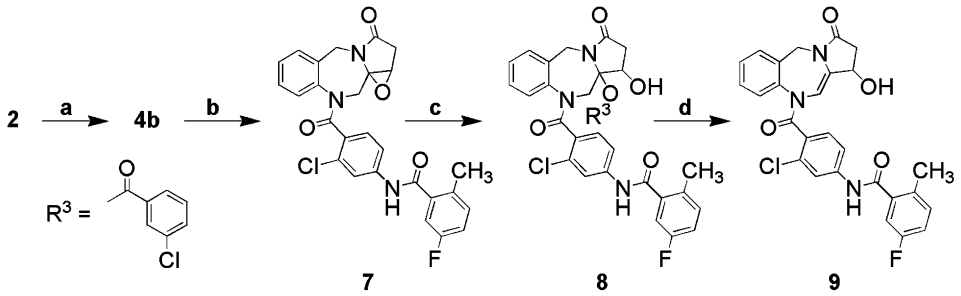


Scheme 4. Reagents and conditions: (a) $(COCl)_2$, DMF (cat), DCM; (b) methyl anthranilate, TEA, DCM; (c) 1 N NaOH, H_2O/THF.

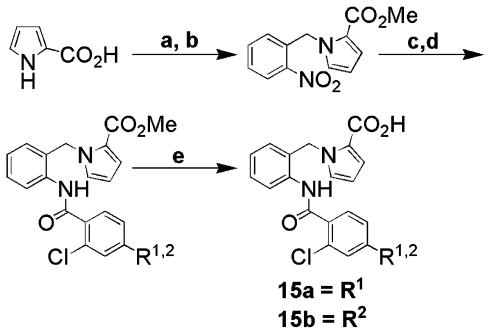


Scheme 5. Reagents and conditions: (a) Pyrrole, NaOH, Bu_4NBr; (b) Pd/C, H_2; (c) **11**, TEA, DCM; (d) DMF–$POCl_3$.

of **1** was also sensitive to biotransformation. Our successful synthetic approach involved the early oxidation of the 3-methylpyrazole group. Thus, preparation and conversion of 2-chloro-4-(3-methyl-1*H*-pyrazol-1-yl)-benzoic acid to the diacid chloride **16** was achieved as shown in Scheme 7. Reaction with 10,11 dihydro-5*H*-benzo[*e*]pyrrolo[1,2-*a*][1,4]diazepine[13] led to amide formation, followed by quenching with methanol to afford



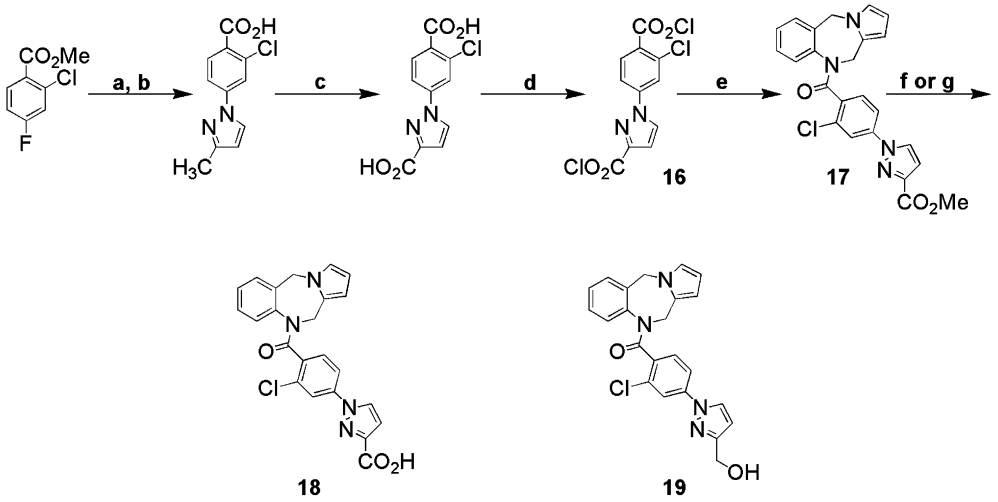
Scheme 2. Reagents and conditions: (a) m-CPBA (1st equiv); (b) m-CPBA (2nd equiv), −10 °C, DCM; (c) 3-chlorobenzoic acid (in situ); (d) spontaneous β-elimination.



Scheme 6. Reagents and conditions: (a) MeI, K_2CO_3, DMF; (b) **13**, NaH, THF; (c) 10% Pd/C, EtOAc; (d) **11**, TEA, DCM; (e) 1 N NaOH, THF–H_2O.

the key intermediate, **17**. Since both regioisomers were formed in this reaction, chromatographic purification afforded **17** as the major isomer. Finally, either hydrolysis with sodium hydroxide in methanol or reduction with lithium borohydride in THF afforded compounds **18** and **19**, respectively.

Biological results. Compound **2**, Lixivaptan®, was extensively metabolized in rats, dogs, and humans, and the major metabolic pathways were oxidation of the pyrrole and diazepine rings. In vitro metabolism by liver microsomes of rats, dogs, and humans generated metabolites **4b**, **5b**, **6b**, **12b**, and **15b**. Metabolites **6b**, **12b**, and **15b** were observed in rats. Metabolites **5b**, **6b**, **12b**, and **15b** were observed in dogs and humans. Metabolites were identified by LC/MS analysis of plasma, urine, and feces of animals and humans. Confirmation of the proposed structures was obtained by comparison of UV and mass spectral data of the isolated metabolites with the corre-



Scheme 7. Reagents and conditions: (a) 3-methylpyrazole, KH, DMF; (b) LiOH, THF; (c) 1 N KOH (1 equiv), $KMnO_4$ (5.25 equiv), 60–65 °C, 4 h; (d) oxalyl chloride, DMF (cat), DCM; (e) 10,11-dihydro-5*H*-benzo[*e*]pyrrolo[1,2-*a*][1,4]diazepine, DIEA, DCM, excess MeOH, chromatographic purification of **17**; (f) 1 N NaOH, MeOH, 40 °C, 30 min; (g) 2 M $LiBH_4$–THF (excess), 65 °C, 1.5 h.

Table 1. Biological assays for selected metabolites of **1**

Metabolite	Human-V_2 binding[a or b]			Human-V_{1a} binding[c]			Human oxytocin binding[d]			Rat urine volume[e,f] at 10 mpk po (% Inc/Dec urine volume[g])
	Concn (nM)	% Inh	IC_{50} (nM)	Concn (nM)	% Inh	IC_{50} (nM)	Concn (nM)	% Inh	IC_{50} (nM)	
1			80.3[a]			778			353	−82%
4a	1000	40		1000	−3		1000	+9		0%
5a			1214[a]	1000	+4		na[h]		na	0%
6a	1000	16		1000	−8		1000	−4		+16%
12a	1000	3		1000	−7		1000	−12		
15a	1000	1		1000	−11		1000	−10		
18	na		na	na		na	na		na	0%
19	na		na	na		na	na		na	−82%

Assays include human-V_2, V_{1a}, and oxytocin in vitro receptor binding and rat urine volume in vivo results.
[a] Expressed in human-V_2 subtype transfected in murine fibroblast LV-2 cell lines.
[b] Expressed in human-V_2 subtype transfected Chinese Hamster Ovary (CHO) cells.
[c] Expressed in human-V_{1a} subtype transfected CHO cells.
[d] Expressed in human oxytocin subtype transfected CHO cells.
[e] Pre water loaded (30 mL) rats.
[f] Negative control vehicle.
[g] Urine volume ratio expressed as a percentage calculated from (Drug urine volume) − (Control urine volume)/(Control urine volume) × 100.
[h] na, not available.

Table 2. Biological assays for selected metabolites of **2**

Metabolite	Human-V_2 binding[a or b]			Human-V_{1a} binding[c]			Human oxytocin binding[d]			Rat urine volume[e,f] at 10 mpk po (% Inc/Dec urine volume[g])
	Concn (nM)	% Inh	IC_{50} (nM)	Concn (nM)	% Inh	IC_{50} (nM)	Concn (nM)	% Inh	IC_{50} (nM)	
2			1.2[a]			124			519	+740
4b	na[h]		na	na		na	na		na	+131%
5b			11			1000			1000	+133%
6b			34			1000			1000	−26%
9	na		na	na		na	na		na	+102%
12b[i]			1000			1000			1000	−6%
14b	100	3		100	−6		100	12		
15b[i]			1000			1000			1000	+6.9%

Assays include human-V_2, V_{1a}, and oxytocin in vitro receptor binding and rat urine volume in vivo results.
[a] Expressed in human-V_2 subtype transfected in murine fibroblast LV-2 cell lines.
[b] Expressed in human-V_2 subtype transfected Chinese Hamster Ovary (CHO) cells.
[c] Expressed in human-V_{1a} subtype transfected CHO cells.
[d] Expressed in human oxytocin subtype transfected CHO cells.
[e] Rats water restricted during test.
[f] Negative control vehicle.
[g] Urine volume ratio expressed as a percentage calculated from (Drug urine volume) − (Control urine volume)/(Control urine volume) × 100.
[h] na, not available.
[i] Administered intravenously.

sponding synthetic standards. In addition metabolites **5b**, **6b**, **12b**, and **15b** were confirmed by co-injection chromatography. In vitro metabolism of individual compounds was conducted to better understand the metabolic pathways of Lixivaptan®. Metabolites **5b** and **6b** were formed, possibly through common intermediate **4b**, early in the metabolic pathway. Oxidation of the diazepine ring at carbons 5 and 11 with subsequent ring opening afforded metabolites **12b** and **15b**, respectively, and were formed late in the metabolic pathway.

In a similar fashion in vitro metabolism of compound **1** in liver microsomes of rats, dogs, or humans generated metabolites **4a**, **5a**, and **6a**. Metabolites **4a**, **12a**, **15a**, **18**, and **19** were also observed in either rat or dog plasma and identified by LC/MS. Metabolites of **1** in human plasma were not investigated.

Biological testing included in vitro human-V_2, V_{1a}, and oxytocin receptor binding assays,[14] and in vivo rat urine volume assays.[15] All metabolites underwent testing except compounds **3**, **10**, and **14a**, and the results are presented in Tables 1 and 2. For the vasopressin agonist **1** only metabolite **19**[15] was active and equipotent with **1** in the rat urine volume assay. For the vasopressin antagonist **2** all metabolites had either significantly reduced activity (**4b**, **5b**, and **9**) or no activity at all (**6b**, **12b**, and **15b**). In general, the integrity of the pyrrolobenzodiazepine ring was important to maintain significant activity.

Summary. In summary, a highly efficient synthesis of 17 proposed metabolites over two clinical programs was developed and executed, and further testing confirmed the majority of those metabolites. One metabolite, **19**, was found to be biologically equipotent with its clinical lead, **1**.

Acknowledgments

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References and notes

1. Trybulski, E. J. *Ann. Rep. Med. Chem* **2001**, 36.
2. Jard, S. *Adv. in Experimental Med. & Biol* **1998**, *449*, 1.
3. Breyer, M. D.; Ando, Y. *Ann. Rev. Physiol* **1994**, *56*, 711.
4. Caggiano, T. J. *Drugs Future* **2002**, *27*, 248.
5. Dusza, J. P.; Chan, P. S.; Albright, J. D.; Bagli, J. F.; Failli, A. A.; Ashwell, M. A.; Molinari, A. J.; Caggiano, T. J.; Trybulski, E. J. U.S. Patent 6,511,974, 2003.
6. Albright, J. D.; Reich, M. F.; Delos Santos, E. G.; Dusza, J. P.; Sum, F. W.; Venkatesan, A. M.; Coupet, J.; Chan, P. S.; Ru, X.; Mazandarani, H.; Bailey, T. J. *Med. Chem.* **1998**, *41*, 2442.
7. Chan, P. S.; Coupet, J.; Park, H.; Lai, f.; Hartupee, D.; Cervoni, P.; Dusza, J.; Albright, J.; Ru, X.; Mazandarani, H.; Tanikella, T.; Shepherd, C.; Ochalski, L.; Bailey, T.; Lock, T.; Yeung, W.; Ning, X.; Taylor, J.; Spinelli, W. *Adv. Exp. Med. Biol.* **1998**, *449*, 439.
8. Albright, J. D.; Reich, M. F.; Sum, F. W.; Delos Santos, E. G. U.S. Patent 5,516,774, 1996.
9. Wong, F., et al. *Hepatology* **2003**, *37*, 182.
10. Guyader, D., et al. *Hepatology* **2002**, *36*, 1197.
11. Decaux, G. *J. Lab. Clin. Med.* **2001**, *138*, 18.
12. Martinez-Castelao, A. *Curr. Opin. Invest. Drugs* **2001**, *2*, 525.
13. Albright, J. D.; Venkatesan, A. M.; Aranapakam, M.; Dusza, J. P.; Sum, F. W. U.S. Patent 5,753,648, 1998.
14. Failli, A. A.; Sanders, W. J.; Trybulski, E. J.; U.S. Patent 7,022,699, 2006.
15. Molinari, A. J.; Trybulski, E. J.; U.S. Patent 7,138,393, 2006.