Strong anti-steatotic and anti-fibrotic effects of novel FXR agonists in a murine NASH model that resembles human NASH

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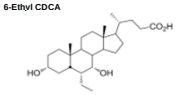
Abstract

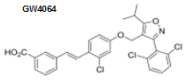
Non-Alcoholic Steatohepatitis (NASH) is a liver disease with signs of steatosis, liver inflammation and fibrosis manifested histologically. Metabolic Syndrome, Obesity and hepatic insulin resistance, in particular, are considered as driving factors in the etiology of NASH, therefore any animal model that tries to resemble the human NASH situation has to take these factors into account. Currently, there is no dedicated treatment approved for NASH and due to the growing incidence of this disease in developed countries, there is also an increasing demand for a pharmacotherapy that addresses the aforementioned factors. Stelic Institute & Co, has developed a novel murine model for NASH that closely mimicks the human NASH situation in the sense that the animals display major hallmarks of human NASH including a moderate increases in transaminases and plasma lipids. The animals develop the entire cascade of metabolically induced liver disease from fatty liver / steatosis over NASH, to fibrosis, cirrhosis and ultimately hepatocellular carcinoma (HCC). Phenex AG has developed novel potent non-steroidal FXR agonists with a good pharmacokinetic profile that were tested in the Stelic mouse NASH model. We used three different administration regimen from five (5) to sixteen (16) weeks of administration to test for preventive as well as for curative effects. As an outcome, our new FXR agonists Px20350 and Px20606 show superior effects at doses of 10 and 30 mpk/d in reducing the liver inflammation, steatosis and fibrosis compared to the positive control and also compared to 6-Ethyl CDCA (10 mpk/d) and Pioglitazone (10mpk/d) which were used as benchmark cpds. Thus, we believe that our new FXR agonists provide an excellent option as a new pharmacotherapy for the treatment of NASH

FXR agonists and other compounds used in this study

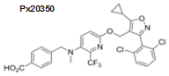
6-Ethyl CDCA



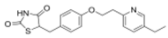
GW4064



Px20350



Pioglitazone



	FRET		GAL4 reporter	
	EC_{50} [nM]	Efficacy [%]	EC_{50} [nM]	Efficacy [%]
6-Ethyl CDCA	90	98	150	95
GW4064	20	100	35	100
Px20350	12	109	30	81

Stelic Animal model / Study Design:

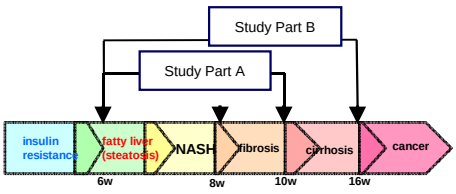
C57/bl6 are injected with a single dose of Streptotoxin (STZ), three days after birth to destroy appr. 80% of all pancreatic β-cells. At the age of 4 weeks animals are put on a high fat diet. This combined treatment results in the development of steatosis, fibrosis, cirrhosis and finally hepatocellular carcinoma (HCC) along with hyperglycemia and moderate hyperlipidemia thus closely resembling human NASH.

The study was subdivided into parts A and B:

age 5 weeks to -Study Part A (intervention- preventive mode): N = 8 animals per group were treated from the 10 weeks with the indicated doses of compound.

5 weeks to -Study Part B (intervention- curative mode): N = 8 animals per group were treated from the age 16 weeks with the indicated doses of compound.

Group	Mouse / Compound	Dose
1	Normal C57 mice	n/a
2	NASH pos. control	n/a
3	NASH / Px20350	30mpk/d
4	NASH / Px20350	10mpk/d
5	NASH / Px20606	30mpk/d
6	NASH / Px20606	10mpk/d
7	NASH / Pioglitazone	10mpk/d
8	NASH / 6-ECDCA	10mpk/d

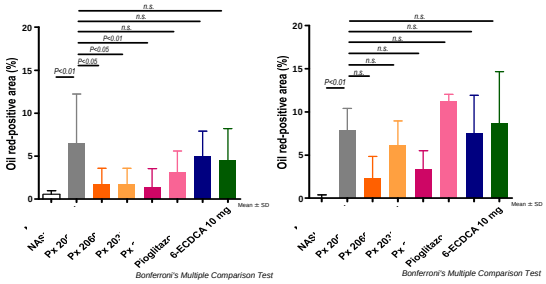


Results:

1) Quantitative imaging analysis of oil red O stained liver sections

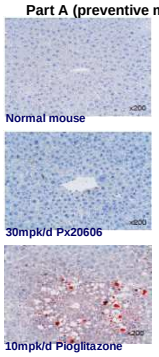
Part A (preventive mode)

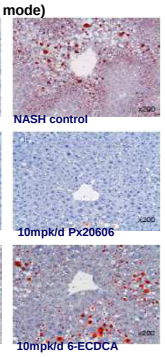
Part B (curative mode)



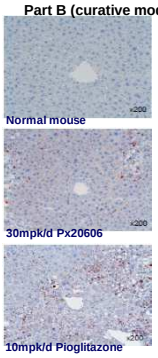
Oil red o stain for lipid droplets

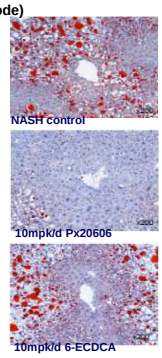
Part A (preventive mode)





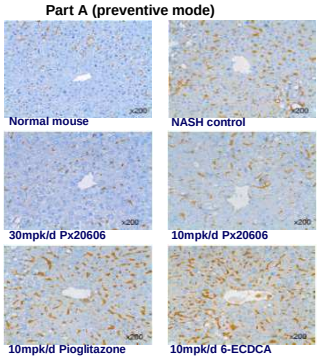
Part B (curative mode)



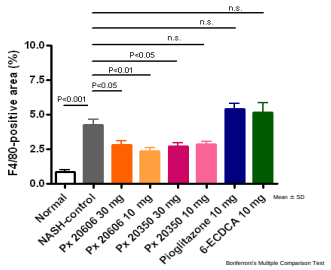


2) F4/80 stain for activated macrophages

Part A (preventive mode)

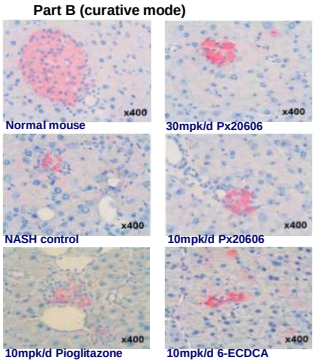


Quantitative imaging analysis of F4/80 stained liver sections



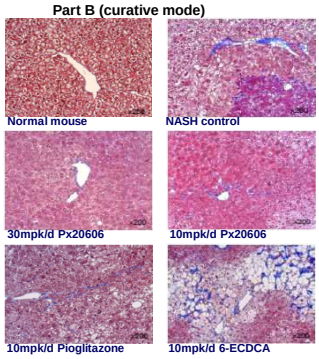
3) Insulin stain in pancreas

Part B (curative mode)



4) Masson´s Trichrome for fibrosis (blue)

Part B (curative mode)



5) NASH scoring (Part A- preventive mode)

	Steatosis	Lobular inflammation	Hepatocyte ballooning	total NASH SCORE
normal C57 mice	0,00	0,00	0,00	**0,00**
NASH pos. control	2,00	1,25	2,00	**5,25**
NASH / Px20606 (30mpk/d)	0,63	1,13	1,25	**3,00**
NASH / Px20606 (10mpk/d)	1,25	0,75	1,63	**3,63**
NASH / Px20350 (30mpk/d)	0,75	1,13	1,38	**3,25**
NASH / Px20350 (10mpk/d)	1,00	1,25	1,63	**3,88**
NASH / Pioglitazone (10mpk/d)	1,43	1,57	1,71	**4,71**
NASH / 6-ECDCA(10mpk/d)	1,25	1,63	1,50	**4,38**

We draw two major conclusions from our studies:

1.) The Stelic mouse model seems to be very appropriate to test drugs for their anti-NASH effects because one can monitor steatosis, fibrotisation and inflammation in a murine NASH model which closely resembles the human histopathology.

2.) Our potent synthetic non-steroidal FXR agonists show superior anti-steatotic, anti-inflammatory and general hepatoprotective properties compared to Pioglitazone and 6-Ethyl CDCA as a PPARγ and a steroidal FXR agonist, respectively.