Non-Steroidal LXR Agonists; An Emerging Therapeutic Strategy for the Treatment of Atherosclerosis

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Abstract: The Liver X Receptor (LXR)   and   isoforms are members of the type II nuclear receptor family which function as obligate heterodimers with the Retinoid X Receptor (RXR). Upon agonist binding, the DNA Binding Domain (DBD) of LXR interacts with LXR response elements on target genes to initiate transcription. A number of genes have been shown to be modulated by LXR function, including the ATP-binding cassette transporter A1 (ABCA1). ABCA1 is involved in the process of reverse cholesterol transport (RCT) from macrophages in atherosclerotic plaques to high-density lipoproteins (HDL) in the plasma. Both homozygous and heterozygous mutations in ABCA1 result in conditions characterised by decreased levels of HDL and an earlier onset of atherosclerosis. A number of other genes are upregulated by LXR activation which would be expected to have either pro- or anti-atherogenic effects. One such target gene is sterol regulatory element binding protein-1c (SREBP-1c), which is involved in the process of lipogenesis leading to increased levels of triglycerides which are pro-atherogenic. The complexity of LXR responses, however, makes it difficult to extrapolate the 'positive' or 'negative' effects of each target gene in isolation to a conclusion as to the outcome in humans when all target genes are being modulated in concert. This review will cover the structural features and associated biological data of non-steroidal LXR modulators claimed for the treatment of cardiovascular disease, as well as highlighting preferred compounds where this information can be discerned. In addition to this patent information a précis of literature data relevant to the utility of specific compounds in the treatment of cardiovascular disease will be given where available.

Keywords: Liver X receptor (LXR), alpha ( ) and beta ( ) isoforms, ATP-binding cassette transporter A1 (ABCA1), reverse cholesterol transport (RCT), atherosclerosis.

1. INTRODUCTION

Atherosclerotic coronary artery disease (CAD) is the leading cause of mortality in industrialised countries and over the next two decades is predicted to become the leading cause of death worldwide [1, 2]. There are many biological mechanisms involved in the initiation, progression and activation of atherosclerotic lesions with one of these being the uptake of oxidised low-density lipoprotein (LDL) by macrophages present in the fatty streaks/plaques in the arterial wall [3, 4]. This results in the accumulation of cholesterol esters, leading to the formation of foam cells. Many biological processes are subsequently triggered, including the production of inflammatory mediators, recruitment of inflammatory cells [5], smooth muscle cell migration and apoptosis. Subsequent activation of atherosclerotic plaques can result in rupture of the fibrous cap, exposing the thrombogenic contents of the plaque core, leading to thrombotic clot formation, myocardial infarction or cerebral ischemia.

Reverse cholesterol transport (RCT) is the process by which cholesterol is transported from peripheral tissues and becomes incorporated in high-density lipoproteins (HDL) which are transported to the liver for metabolism [6]. Epidemiological studies and clinical trials have identified that decreased levels of HDL (and elevated levels of LDL) are pro-atherogenic. The ATP-binding cassette transporter A1 (ABCA1) is a crucial component in the process of RCT and has been shown to be modulated by the Liver X Receptors [7-11]. Patients with Tangiers disease, and the milder heterozygous form, familial hypoalphalipoproteinemia (FHA), have been shown to possess mutations in the ABCA1 gene. In Tangiers disease this homozygous mutation results in a virtual absence of circulating HDL cholesterol, and a premature onset of atherosclerosis [12-20]. Therefore, increasing RCT through modulation of the ABCA1/LXR pathway represents an attractive potential therapeutic mechanism for the treatment of atherosclerosis [8-11, 21, 22]. A number of other genes are upregulated by LXR activation which may have 'positive' or 'negative' effects on atherosclerosis [8-11, 21, 22]. One such target gene is sterol regulatory element binding protein-1c (SREBP-1c), which is involved in the process of lipogenesis leading to increased levels of triglycerides which are pro-atherogenic [8-11, 21, 22].

The liver X receptor (LXR)   and   isoforms [23] are members of the type II nuclear receptor family that includes peroxisome proliferator activated receptors (PPAR), farnesoid X receptors (FXR) and the pregnane X receptor (PXR) [7-9, 24-26]. The LXR isoforms share 77% sequence homology, with   having highest expression in liver, intestine, adipose tissue, and macrophages and   being more widely expressed [27]. LXR has a conserved nuclear receptor structure, which contains both a DNA binding domain (DBD) and a ligand binding domain (LBD) and functions as an obligate heterodimer with the retinoid X

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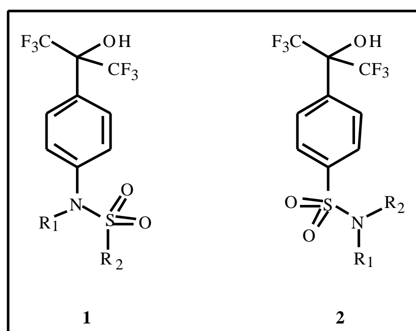
receptor (RXR). Oxidised derivatives of cholesterol have been shown to act as activators of LXRs at physiologically relevant concentrations e.g. 24(*S*),25-epoxycholesterol [28-33]. Upon agonist binding, the LXR/RXR heterodimer recruits co-activators containing an LXXLL sequence, binds to specific LXR response elements on target genes and recruits the basal transcription machinery which causes an increase in transcription. The LXR/RXR heterodimer can be activated by an LXR agonist or an RXR agonist or synergistically by both.

This article will focus on reviewing patent applications for non-steroidal LXR modulators, claimed for the treatment of cardiovascular disease, published until May 2005. This review will cover the structural features and associated biological data of the compounds claimed as well as highlighting preferred compounds where this information can be discerned. In addition to this patent information a précis of literature data relevant to the utility of these agents in the treatment of cardiovascular disease will be given where available.

2. LIVER X RECEPTOR MODULATORS

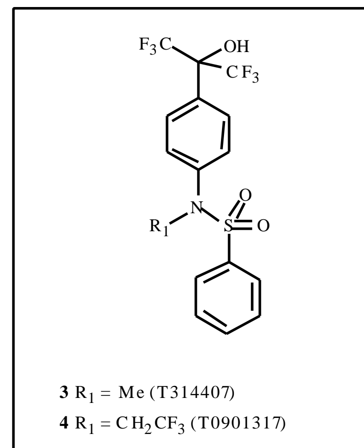
2.1. Tularik Inc.

Tularik Inc. was the first company to file a patent application specifically describing a series of non-steroidal LXR modulators, which were covered by wide generic structural claims [34]. The compounds are claimed for use in the treatment of disease states associated with cholesterol metabolism, particularly atherosclerosis and hypercholesterolemia [34]. Analysis of both exemplified and claimed compounds suggests that the hexafluoroisopropanol containing generic structures (**1**) and (**2**) are preferred. For (**1**) and (**2**) the preferred substitution for R_1 is CH_2CF_3 and R_2 is a halo-substituted phenyl ring or chloro-substituted thiophene.



Biological activity for the exemplified compounds was assessed in both a chimeric luciferase reporter gene assay (GAL4-LXR) in HEK293 cells and a peptide sensing assay. An EC_{50} of less than 2μM is observed in each assay for structure (**1**) where R_1 is hydrogen and R_2 is phenyl.

A further patent application has been filed based on the same generic structures (**1** and **2**) and methods as the initial filing [34] for raising HDL cholesterol in mammals [35]. Specific examples include (**3**) (R_1 = Me, T314407) and (**4**) (R_1 = CH_2CF_3, T0901317). T314407 (**3**) was shown to bind to the LBD of LXR   with an EC_{50} of 0.2μM using a LXR -GST fusion protein binding assay. T314407 (**3**) was also shown to compete with the oxysterol 24(*S*),25-epoxycholesterol for binding to LXR   LBD. In a two week study, oral administration of T0901317 (**4**) (5 mg/kg and 50 mg/kg) to male and female C57BL/6 mice caused an increase in total plasma cholesterol (TC), HDL cholesterol and plasma triglyceride levels. As the prototypical non-steroidal LXR agonist T0901317 (**4**) has been utilised in a number of additional studies relevant to cardiovascular pharmacology which will be described later in more detail.



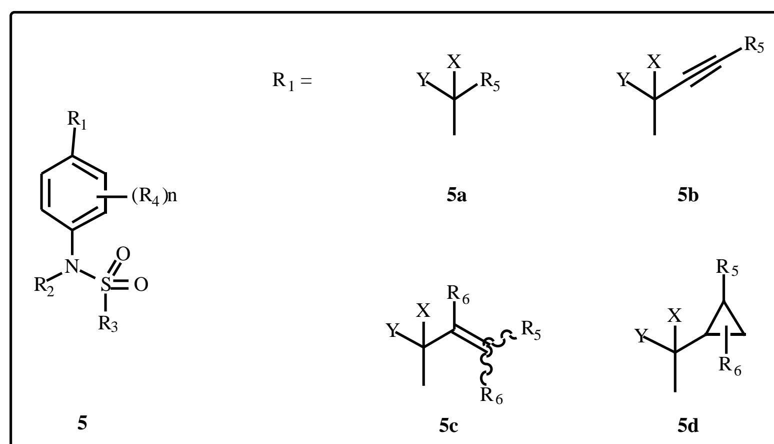
3 R_1 = Me (T314407)
4 R_1 = CH_2CF_3 (T0901317)

Tularik Inc. has also claimed a further selection of compounds capable of specifically regulating LXR and treating LXR-responsive diseases, particularly hypercholesterolemia, atherosclerosis and hyperlipoproteinemia [36]. Again cell based reporter gene and peptide sensor assays are used for determining LXR activity but no specific biological data is reported. The claimed compounds are outlined by the general formula (**5**) where R_1 is represented by structures (**5a-5d**). A large number of substituents are within the scope of the patent application, but the 94 synthetic examples favour structures (**5a-5c**).

Preferred embodiments of the invention covered by generic structure (**5**) are where R_2 is alkyl, branched alkyl, cycloalkyl, benzyl and substituted benzyl, R_3 is substituted aryl or heteroaryl and R_4 is simple substitution e.g. methyl. Within (**5a-5c**), X is preferred as alcohol, alkyl ether or hydrogen and the Y substituent is most preferred as CF_3 although alkyl and H are also exemplified. Within (**5a**) R_5 is preferred as phenyl, substituted phenyl, benzyl, pyridyl, pyrimidinyl, pyrazolyl, aminoalkyl, branched alkyl, cycloalkyl, sulfonamide, amide, acid or ester substituents. Within (**5b**) R_5 is preferred as imidazolyl, phenyl, benzimidazoyl, thiazolyl or pyrrolyl substituents. Tularik Inc. has filed a second patent application based around the generic structure (**5a**). The substitution pattern covered in this application is where X is alcohol or hydrogen, Y is 1-methoxyethyl-pyrrol-2-yl or 1-ethoxyethyl-pyrrol-2-yl and R_5 is hydrogen, methyl, ethyl, n-propyl or CF_3 [37].

2.1.1. Pharmacological Activity of T0901317 (4)

T0901317 (**4**) has been utilised in a number of biological studies to elucidate the role and potential for LXR modulation in various therapeutic indications. For the



purpose of this review data generated relevant to the potential utility of T0901317 (**4**) in cardiovascular pharmacotherapy will be briefly discussed. For a more in depth review and discussion the reader is directed to the primary references and those references cited therein.

In Vitro LXR Activity

T0901317 (**4**) has been shown to be an efficacious LXR agonist *in vitro* in both cell free and cell based assay systems. In a cell free scintillation proximity binding assay (SpA) T0901317 (**4**) was found to possess an IC_{50} of 20nM with identical EC_{50} values being measured in both a cell free peptide recruitment assay and a cell based reporter gene assay [38]. The level of potency seen was in all cases considerably higher than that possessed by the endogenous ligand 24(*S*),25-epoxycholesterol. Data has also been reported which shows that T0901317 (**4**) has both PXR and FXR agonist activity *in vitro* [38, 39]. Upregulation of CYP3A in primary rat and mouse hepatocytes, mediated by PXR receptor function, has also been shown upon treatment with T0901317 (**4**) [40]. Whilst not prohibitive these effects must provide a cautionary note on the use of T0901317 (**4**) as a pharmacological tool to investigate LXR function.

LXR Target Gene Regulation T0901317 (4)

A number of studies have now been published on the effects of T0901317 (**4**) on the regulation of LXR target genes involved in reverse cholesterol transport (RCT), lipogenesis and inflammatory responses and a number of these are summarised in the following section.

ATP-Binding Cassette Transporters

The membrane bound ATP binding cassette transporters (ABC) A1, G1, G5 and G8 are LXR target genes [8-11, 41], which have received considerable attention as targets for anti-atherosclerotic therapy due to their involvement in RCT from various tissues and cell types [3, 6, 8-11]. Mutations in the ABCA1 gene can result in Tangiers disease whereas mutations in G5 and G8 genes results in the autosomal recessive disorder Sitosterolaemia (or Phytosterolaemia); both conditions are associated with premature atherosclerotic disease [12-20, 42-46]. Treatment of $LDLr^{-/-}$ mice with T0901317 (**4**) for 8 weeks (3 and 10 mg/kg/day p.o.) has been shown to result in increased expression of ABCA1 protein in aortic root sections [47]. The expression of ABCA1 was co-localised with macrophages as detected by immunostaining [47].

In a more recent study dosing C57BL/6 mice with T0901317 (**4**) (50mg/kg/day p.o. for 6 days) resulted in a significant increase in levels of ABCA1 in duodenal (10 fold) and liver tissue (2 fold), with a similar 2 fold induction also being observed for ABCG1 [48]. In addition to these *in vivo* observations, induction of ABCA1 mRNA was also seen upon treatment of HepG2, differentiated THP-1, J7774 cells as well as in cultured murine peritoneal macrophages [48].

In another study, male mixed strain (A129/C57BL6) mice fed a cholesterol rich diet (0.2%) and dosed with T0901317 (**4**) (50mg/kg) showed increased mRNA levels of ABCG5 and G8 in the liver and intestine after acute (12h) or repeat dosing (10 days) [49].

Sterol-Regulatory Element Binding Protein-1c (SREBP-1c), Acetyl-CoA Carboxylase (ACC) and Fatty Acid Synthase (FAS)

SREBP-1c, ACC and FAS are involved in lipogenesis, a process which ultimately leads to increased levels of 'pro-atherogenic' plasma triglycerides. To examine the *in vivo* effects of T0901317 (**4**) on these genes, studies were performed in C57BL/6 mice:

1. Male mice fed a chow diet containing 6.2% fat and 0.01% cholesterol were dosed for 4 days with T0901317 (**4**) (10mg/kg/day) or vehicle by oral gavage [50]. Expression of SREBP-1c, ACC and FAS were found to be increased between 2 and 5 fold in the livers of T0901317 (**4**) treated animals as compared to controls.

2. In a further study, significant induction of the expression of SREBP-1c (12 fold), FAS (9 fold) and ACC (3 fold) was seen in the liver tissue of mice dosed with T0901317 (**4**) (50mg/kg/day, p.o.) for 6 days [48].

In vitro SREBP-1c, ACC and FAS gene regulation data has also been published and is summarised below:

1. Treatment of THP-1 cells with T0901317 (**4**) has also been shown to result in an increase in FAS [51]. This effect occurs directly from the binding of the LXR/RXR

heterodimer to a DR-4 LXR response element on the FAS gene as well as indirectly through upregulation of SREBP-1c.

2. Treatment of rat hepatoma cells (McA-RH7777) with T0901317 (**4**) (10μM) resulted in a selective upregulation of SREBP-1c mRNA over 1a and/or 2, which was not observed after treatment with either 24(S),25 epoxycholesterol or 24(*S*)-hydroxycholesterol [52].

3. Treatment of HepG2, differentiated THP-1 and J7774 cells with T0901317 (**4**) (10μM) also resulted in an upregulation of mRNA for SREBP-1c [48].

Lipoprotein Lipase

Lipoprotein lipase (LPL) is involved in the hydrolysis of the constituent triglycerides in lipoproteins [6], and a DR-4 LXR response element has been identified on the LPL gene. Treatment of male A129 mice with T0901317 (**4**) (50 mg/kg/day contained in food) for 7 days resulted in an upregulation of LPL mRNA in liver as compared to controls. No similar upregulation was found in muscle, kidney, adrenal, intestine or cardiac tissue [53].

Phospholipid Transfer Protein

Phospholipid transfer protein (PLTP) is a modulator of HDL size and composition through its role in transferring phospholipids and cholesterol from very low density lipoprotein (VLDL) and chylomicrons to HDL during lipolysis by LPL [6]. Treatment over 7 days of C57/BL6 mice with T0901317 (**4**) (1, 10, 50 and 100 mg/kg/day) resulted in a dose dependent increase in plasma PLTP activity with a 201% increase at 100 mg/kg. Hepatic PLTP mRNA levels were also found to be increased in a dose dependent fashion with a six fold increase at 100mg/kg [54].

Apolipoprotein E (ApoE)

ApoE is present in triglyceride rich VLDL particles which are synthesised in the liver and function to transport fatty acids to adipose and muscle tissue [3]. An LXR response element is present on the ApoE gene and ApoE expression was found to be upregulated in murine peritoneal macrophages and adipose tissue but not in hepatic tissue [55].

Angiopoietin-Like Protein 3

Angiopoietin-like protein 3 (Angptl3) is a vascular endothelial growth factor, which is involved in the regulation of lipid metabolism [56]. Treatment of C57BL/6 mice with 10mg/kg/day T0901317 (**4**) for 7 days resulted in an increase in Angptl3 mRNA in liver tissue [57]. A concentration dependent increase in Angptl3 mRNA was also observed in HepG2 cells.

Matrix Metalloproteinase-9

Matrix metalloproteinases (MMPs) are zinc endopeptidases which have been shown to be present in atherosclerotic plaques. MMPs are involved in the degradation of substrates, including collagen in the fibrous cap of plaques, weakening the fibrous cap and rendering the plaque at an increased risk of rupture [3, 58-60]. Treatment of murine peritoneal macrophages with T0901317 (**4**) (2μM) was found to reduce MMP-9 mRNA expression and attenuate its induction by pro-inflammatory stimuli including lipopolysaccharide (LPS), IL-1 and TNF- [58]. The involvement of LXR receptor function in this effect was further strengthened by the observation that MMP-9 levels were unchanged after treatment of macrophages obtained from LXR / double knockout mice.

Tissue Factor

Exposure of blood to tissue factor (TF) triggers the extrinsic pathway of the coagulation cascade. TF has been shown to be abundantly expressed in macrophages and the lipid rich core in atherosclerotic plaques and plays a pivotal role in thrombus formation associated with plaque rupture. The expression of TF within macrophages is upregulated by pro-inflammatory stimuli including TNF , IL-1 and LPS. Preincubation of peritoneal macrophages from male C57BL/6 mice with T0901317 (**4**) prior to stimulation with LPS (100ng/mL) resulted in a dose-dependent decrease in TF mRNA concentrations as determined by quantitative real time chain reaction (QRT-PCR) assays over the 24 hours timecourse of the experiment [61]. The LXR receptor dependency of this effect was shown by the fact that treatment of peritoneal macrophages with agonists (tested at 1μM) for PPAR (Wy14643), PPAR (rosiglitazone), PPAR (GW501516) and FXR (GW4064) had minimal effects on TF expression. Pretreatment with T0901317 (**4**) (1μM) also reduced the induction of TF mRNA by stimulation with IL-1 (20ng/mL) or TNF (20ng/mL). Dose-dependent reductions in LPS induced TNF mRNA and protein secretion were also shown after pretreatment of peritoneal macrophages with T0901317 (**4**). No effect was seen on LPS induced IL-1 or IL-6 protein secretion upon similar treatment. In follow up experiments pretreatment of human monocytes for 18 hours with T0901317 (**4**) led to a significant dose-dependent reduction in LPS induced TF activity and mRNA levels as well as a reduction in TF protein expression. The question of the *in vivo* relevance of these findings was then tackled by dosing C57BL/6 mice with T0901317 (**4**) (3mg/kg/day) for 7 days prior to the i.p. administration of LPS (4mg/kg). Pretreatment with T0901317 (**4**) led to a significant reduction of TF mRNA levels in both kidney and lung tissue whilst having no effect on baseline expression. TF mRNA levels in atherosclerotic lesions of LDLr$^{-/-}$ mice were also shown to be reduced in a dose-dependent manner upon treatment with T0901317 (**4**) (3 and 10mg/kg/day) for 8 weeks. This reduction in TF mRNA in atherosclerotic lesions was accompanied by the expected increase in ABCA1 mRNA.

Osteopontin

Osteopontin (OPN) is a proinflammatory cytokine expressed in atherosclerotic lesions [62]. Secretion of OPN by macrophages, vascular smooth muscle cells (VSMCs) and endothelial cells (ECs) can promote monocyte adhesion, migration, activation as well as cytokine and MMP expression [63, 64]. *In vivo* data has been published linking OPN, and in particular macrophage derived OPN, to the promotion of atherosclerotic development. Pretreatment of murine macrophages (RAW 264.7) with T0901317 (**4**) (5μM) for 24 hours prior to stimulation with TNF , IL-1 , INF- or LPS resulted in a reduction of OPN protein and

mRNA levels as compared to controls [65]. This effect was shown to be dose dependent for TNF (20ng/ml) induction of OPN expression upon preincubation with T0901317 (**4**) (0.1 to 5μM). Similar reductions in TNF induced OPN mRNA and protein expression were observed in human peripheral blood monocytes and THP-1 monocytes upon pretreatment with T0901317 (**4**) (5μM). As with previous studies the *in vivo* relevance of these findings was investigated by administration of T0901317 (**4**) to C57BL/6 mice for 7 days prior to isolation of peritoneal macrophages. Sufficient exposure to T0901317 (**4**) was indicated by quantitation of a ~4 fold induction of ABCA1 in peritoneal macrophages from the treatment group. OPN protein and mRNA levels were decreased in macrophages from T0901317 (**4**) treated mice compared to controls with 76% and 64% decrease in OPN mRNA and protein levels respectively [65].

In vivo Atherosclerosis Studies

T0901317 (4) has been used to study *in vivo* anti-atherosclerotic effects in mice. However, a number of differences exist between rodents and humans with respect to LXR receptor target genes which can make it difficult to confidently translate effects in mouse models to that expected in humans [66-71]. In addition, there are a number of differences with respect to lipoprotein profiles and cholesterol trafficking between rodents and humans which can also complicate extrapolation of effects seen in rodents to those expected in man [66-71]. A summary of these are highlighted below:

1. Mice and rat LXR upregulates Cytochrome P450 7a (Cyp7a), the rate determining enzyme in the metabolism of cholesterol, an effect that does not occur with human LXR (the human Cyp7a gene does not contain an LXR response element). Treatment of rat hepatocytes with T0901317 (**4**) (1 μM) gave a robust induction of Cyp7a (~9 fold) mRNA levels whilst having no effect on short heterodimer partner (SHP) expression [66]. The same experiments were performed in human hepatocytes, however in this case treatment with T0901317 (**4**) resulted in an induction of SHP expression which was paralleled by a repression in Cyp7a expression.
2. Cholesterol ester transfer protein (CETP) is present in human cells but not mice and is an LXR target gene [67, 68]. CETP equilibrates cholesterol ester across various lipoprotein particles with its net effect being the transfer of sterol ester from HDL to triglyceride-rich lipoproteins such as VLDL and LDL.
3. LXR is upregulated by LXR agonists in human cells but not mice. T0901317 (**4**) caused a dose dependant increase in expression of hLXR but not hLXR in hTHP-1 cells [69-71]. No LXR autoinduction was observed in primary human adipocytes or hepatocytes, in a human intestinal cell line, in cultured mouse cells or *in vivo* in mice. Three LXR response elements were identified in the hLXR promoter region. One has strong affinity for both LXR /RXR and LXR /RXR while the other two show affinity for LXR /RXR only.

The *in vivo* atherosclerosis studies using T0901317 (**4**) are described below:

1. Oral treatment of C57BL/6 mice with T0901317 (**4**) (5 and 50 mg/kg) resulted in a 2-3 fold increase in plasma triglycerides (TG) [38]. Increases in plasma very low density lipoprotein (VLDL) TG occurred with comparable increases in plasma total cholesterol (mainly HDLc) and HDL-phospholipid. In contrast to humans, which use LDL for the transport of most plasma cholesterol, mice carry a predominant portion of their cholesterol on HDL. T0901317 (**4**) was also tested in hamsters, which have lipoprotein cholesterol distributions more closely resembling humans [38]. Similar increases in plasma triglycerides were quantified in the hamster.
2. In male C57BL/6 mice, treatment with T0901317 (**4**) (20μmol/kg/day ~ 10mg/kg/day, o.d. by gavage for 4 days) caused a significant increase in plasma cholesterol levels as well as phospholipids and HDL (59% increase) [72]. Hepatic total cholesterol was decreased in treated mice, phospholipid concentrations were unchanged and a marked increase in hepatic triglyceride content (>8 fold) was evident. Biliary cholesterol excretion was found to be increased 2.7 fold upon treatment with T0901317 (**4**), although bile flow, bile salt and phospholipid output were not affected. Fecal bile salt loss was increased upon treatment of C57BL/6 mice with T0901317 (**4**) with a concomitant increase in fecal neutral sterol output of 187%. These effects were shown to be independent of ABCA1 using wild type DBA/1 and ABCA1$^{-/-}$ mice [72].
3. Male C57BL/6 mice fed a chow diet containing 6.2% fat and 0.01% cholesterol were dosed T0901317 (**4**) (10 mg/kg/day, o.d. by gavage) or vehicle for 4 days [50]. The livers from the treated mice were significantly heavier than those of control animals and showed a 10 fold increase in triglyceride content as well as a slight decrease in cholesterol ester content. Treatment with T0901317 (**4**) was also found to increase the rate of VLDL-TG production by 2.6 fold and an increase in mean VLDL particle diameter of 48%. The treated groups also had increased plasma concentrations of free cholesterol, cholesterol esters, phospholipids and free fatty acids.
4. Treatment of LDLr$^{-/-}$ mice fed an atherogenic diet (1.25% cholesterol, 7.5% cocoa butter and 0.5% sodium cholate) with T0901317 (**4**) (3 and 10 mg/kg/day) for 8 weeks was found to dramatically reduce aortic atherosclerotic lesion area [-57% (3mg/kg), -71% (10mg/kg)], without affecting plasma total cholesterol levels [47]. This anti-atherogenic effect was correlated with the plasma concentration of T0901317 (**4**), but not with the dose dependent increase in HDLc which was seen in the treatment group. Triglyceride elevation was observed at week 1, but gradually declined during the administration period and completely recovered to the same level as control by week 8, as did the significant increase in chylomicron cholesterol levels seen at weeks 1 and 5. Analysis of the atherosclerotic lesions for ABCA1 protein showed a significant expression of ABCA1 which was co-localised with macrophages.

5. In another study, wild type (129S6SvEv x C57BL/6) and ABCG5/G8 double knockouts were treated with T0901317 (**4**) for 7 days (diets containing 0.025% of T0901317 (**4**) were made by mixing powdered chow diet with pure T0901317 (**4**)) [73]. In wild type mice treatment resulted in a 3 fold increase in biliary cholesterol concentrations, a 25% reduction in fractional cholesterol absorption, and a 4 fold elevation in fecal neutral sterol excretion. In contrast, treatment with T0901317 (**4**) did not significantly affect any of these parameters in ABCG5/G8 double knockout mice, indicating that ABCG5 and ABCG8 are required for changes in dietary and biliary sterol trafficking as a result of LXR agonism.

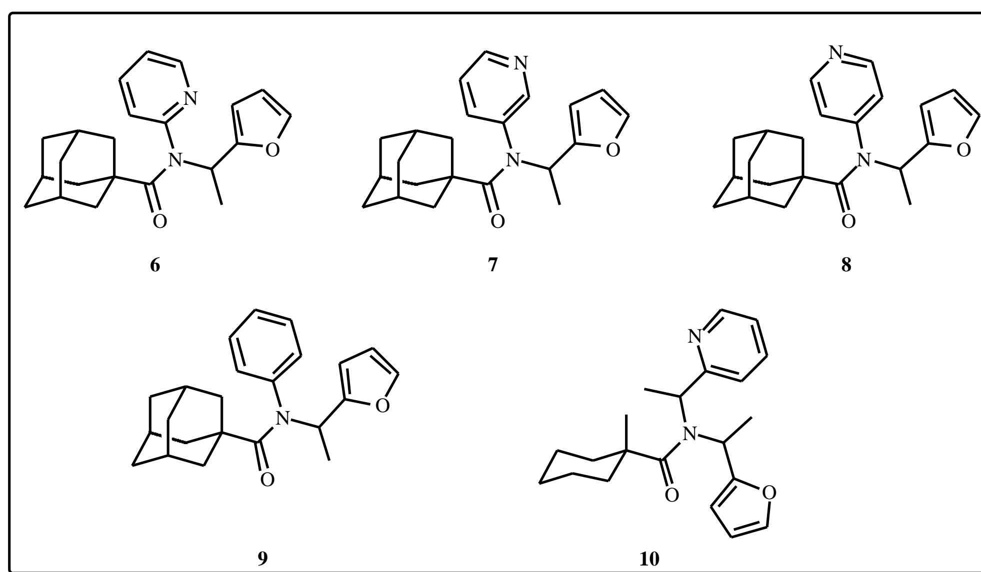
6. Treatment of LDLr$^{-/-}$ mice fed a Western diet (0.15% cholesterol wt/wt, 21% fat wt/wt) with T0901317 (**4**) (10mg/kg/day by gavage) for 6 weeks resulted in a 70% reduction in lesion area in comparison to controls as measured by en face analysis [74]. This effect was demonstrative of a 'preventive' effect on lesion development upon commencement of LXR agonist treatment. Also of note in this study was the finding that T0901317 (**4**) treatment also resulted in a 62% en face reduction in lesion area when compared to mice assessed at 'baseline'. This demonstrated for the first time a LXR agonist induced regression of established atherosclerotic lesions as measured by en face analysis. Utilisation of a macrophage specific antibody also showed that treatment with T0901317 (**4**) resulted in a 48% reduction in macrophage positive areas in aortic root lesions. Analysis of aortic lesions from T0901317 (**4**) treated mice for mRNA expression levels of the macrophage marker CD68 showed a significant decrease of 45% coupled to a significant increase in ABCA1 mRNA expression of 67%. Utilising a bone marrow transplantation protocol to selectively ablate LXR from macrophages the authors of the study were able to show a 60% reduction in atherosclerosis in mice with macrophages that express LXR upon treatment with T0901317 (**4**). This was in stark contrast to the finding that treatment with T0901317 (**4**) had no inhibitory effect on atherosclerosis in mice with macrophages devoid of LXR receptor function. Taken together these results suggest a pivotal role for macrophage LXR receptor function in the anti-atherosclerotic action of LXR agonists.

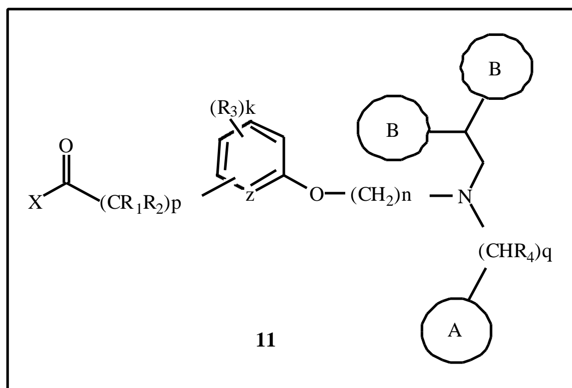
Tularik Inc. has filed a patent application on a series of amide LXR modulators structurally distinct from T0901317 (**4**), with biological activity being established by employing a GAL4-LXR chimeric luciferase reporter gene assay in HEK293 cells [75]. Ten examples are given with preferred activity data being given for the five examples (**6-10**) shown below. Compounds of this nature have also been reported to be prepared via a synthetic route employing solid phase methodology [76].

2.2. GSK

GlaxoSmithKline's initial filing in the field of LXR agonists was published in 2002 claiming a series of alkylaminoalkoxy arylalkanoate derivatives having formula (**11**) and the use of these derivatives for the treatment of cardiovascular and atherosclerotic disease [77]. The utility of these derivatives for increasing reverse cholesterol transport, inhibiting cholesterol absorption, increasing HDL cholesterol and decreasing LDL cholesterol was also claimed.

Whilst no detailed biological data is given, a peptide recruitment assay using a fragment of the NR recognition sequence of steroid receptor co-activator-1 (SRC1) applicable for assaying LXR and agonist activity was described. Preferred compounds are claimed to achieve 100% activation of LXR relative to 24(*S*),25-epoxycholesterol in this assay whilst possessing 'selectivity' for LXR . This isoform selectivity is described as compounds possessing an EC_{50} value for LXR of >10 fold lower than that for LXR . Preferred compounds dosed at <10μM, are also claimed to upregulate the expression of

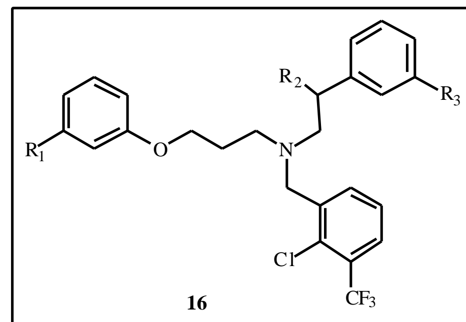




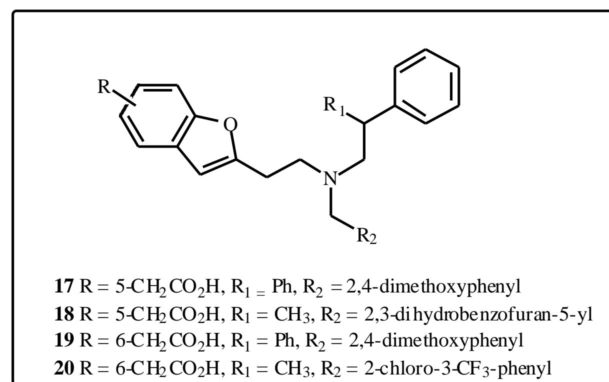
ABCA1 mRNA in a murine macrophage cell line (RAW 264.7), as measured by quantitative polymerase chain reaction (Q-PCR). Compounds (**12-15**) are particularly preferred. Compound (**12**, GW3965) has been utilised in a number of additional biological studies relevant to cardiovascular pharmacology which will be described later in more detail.

The initial application [77] was followed in 2003-2005 by the publication of seven further applications expanding the intellectual property protection around the alkylaminoalkoxy arylalkanoate chemotype [78-84]. The first three of these applications [78-80] contain similar therapeutic indication claims and *in vitro* activity criteria to those described in the initial filing [77]. The remaining four patent applications contain broadly similar claims on the treatment of LXR mediated diseases to those discussed previously [77], although no specific claims were made on the use of the compounds to either increase HDL or lower LDL concentrations [81-84]. In addition to this difference the *in vitro* activity criteria was also modified from the earlier applications to classify compounds as active if they attained 20% activation of LXR relative to 24(*S*),25-epoxycholesterol in an identical assay format (previous = 100%) [81-84].

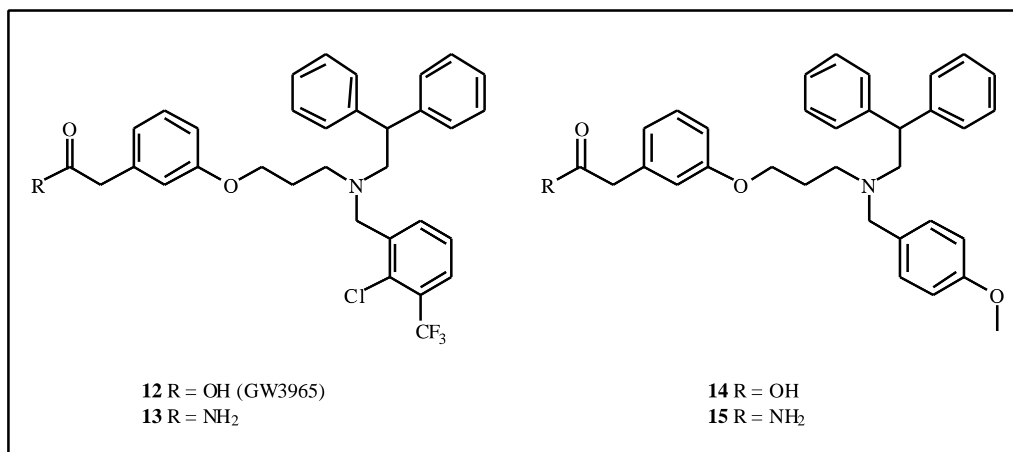
The preferred compounds from the first of these applications covered by generic structure (**16**) where R_2 is methyl or $OC(O)CH_3$ and R_3 is hydrogen or chlorine [78]. In contrast to this relatively narrow exemplification in these areas the preferred compounds contain a wide range of R_1 substituents with $(CH_2)_2OH$, 1,2,4-triazol-3-yl-CH_3, 1,2,3,4-tetrazol-5-yl-CH_3, 1,2,3,4-tetrazolyl-CH_3,CH_2CO_2H, $CH_2CO_2CH_3$, 1-cyclobutane-CO_2H, $NHCH_2CH_3$, $(CH_2)_2NHCH_2$-(1H-imidazol-2-yl-CH_3), $NHSO_2CH_3$, $(CH_2)_2NH CH_2CO_2CH_3$, 1-pyrrolidine-2-CO_2H, NHCO-2-furan, 2-morpholin-4-yl-CH_2CH_3, piperazine-1-yl-CH_2CO_2H, C $(CH_3)_2$ CO_2H, 4-methylpiperazine-1-yl or piperidine-4-CO_2H all being claimed.



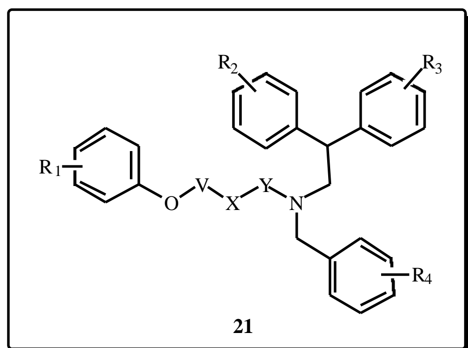
The second application claims benzofuran-5-acetic acid derivatives and preferred compounds were identified (**17-20**) [79].



The third of the applications published in 2003 covering the alkylaminoalkoxy arylalkanoate chemotype provides



exemplification for substitution of the propyl linker which until this point had received relatively little attention [80].



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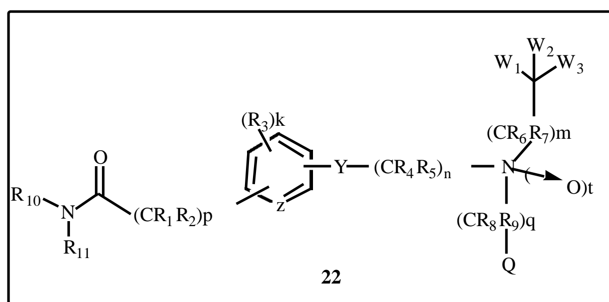
The preferred compounds from this application are covered by generic structure (**21**) where R_2 and R_3 are H, 3-F or 2-Cl; R_1 is CH_2CO_2H, $CH_2CO_2CH_3$, $(CH_2)_2CO_2H$, $CH(CH_3)CH_2CO_2H$, 2-Cl-4-$CH_2CO_2CH_3$ or 2-CH_3-5-CO_2H; R_4 is 2-chloro-3-trifluoromethyl, 2,4-dimethoxy, 2-fluoro-4-methoxy or 4-isopropyl and the groups V, X and Y are either all CH_2 or one of the groups V, X or Y is $CHCH_3$.

In the fourth application, compounds having the generic structure (**22**) are claimed [81].



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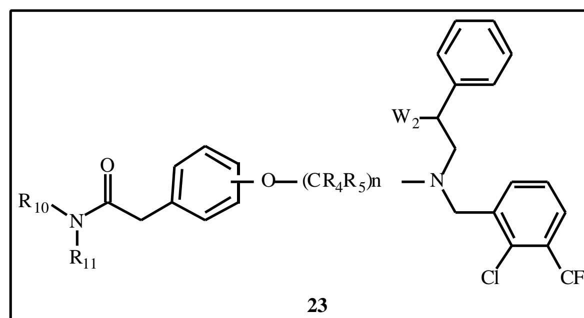
More specific embodiments of this invention comprise compounds depicted by formula (**23**) where R_4 and R_5 are each independently selected from H and methyl; R_{10} and R_{11} are each independently selected from H, methyl, ethyl, imidazol-2-ylmethyl-, 5-bromo-thiophenyl-2-ylmethyl-, thiophen-2-ylmethyl-, 2-methoxyethyl-, 2-dimethylaminoethyl-, 2 morpholino-4-ylethyl-, 2-methoxy-1-methylethyl-, 2-methoxyethyl-, furan-2-ylmethyl-, 3-methylisoxazol-5-ylmethyl-, 2-thiomorpholin-4-ylethyl-, 2-pyrrolidin-1-ylethyl-, pyridin-3-ylmethyl-, 2-pyridinylmethyl-, 3-phenoxyethyl-, 3-isopropoxypropyl-, 3-methoxypropyl-, 5-methyl[1,3,4]oxadiazol-2-ylmethyl-, 4-methylthiazol-2-ylmethyl-, 1-thiophen-2-ylethyl, thiophen-3-ylmethyl-, 5-methyl-4H-[1,2,4]triazol-3-ylmethyl-, pyridin-2-ylmethyl, tetrahydrofuran-2-ylmethyl, 1-ethylpyrrolidin-2-ylmethyl, octyl, decyl, 2-(2-hydroxyethyl)ethyl-, 1-carboxythiophen-2-ylmethyl, phenyl, methylsulfonyl, phenylsulfonyl or R_{10} and R_{11} are cyclised to form azetidinyl, pyrrolidinyl, piperidinyl, azepinyl, 4-methylpiperazin-1-yl, or morpholinyl-4-yl substitution; n is 3; and W_2 is unsubstituted phenyl or methyl.

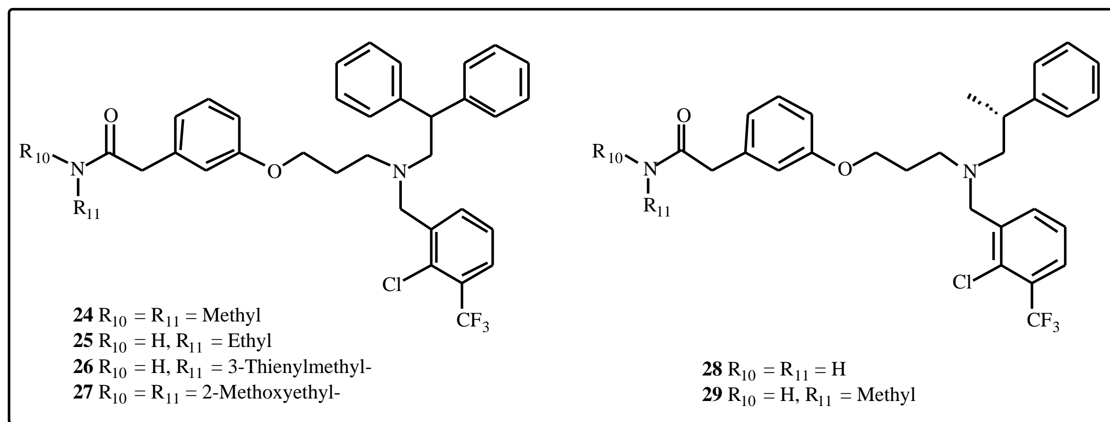


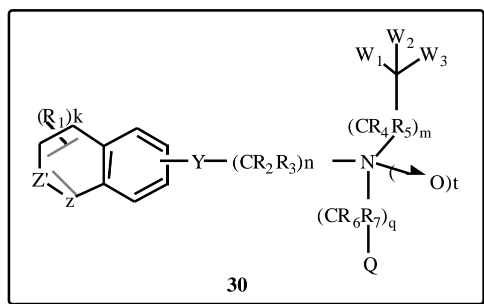
23

Preferred compounds (**24**-**29**) were identified, with the focus appearing to be on expanding protection of the phenylmethylcarboxy moiety in conjunction with the well established 2,2-diphenylethyl and (2-chloro-3-trifluoromethyl)phenylmethyl groups.

The fifth application claims compounds with the generic formula (**30**) [82]. The focus of the exemplification and preferred examples centre around the fused bicyclic moiety in conjunction with the established 2,2-diphenylethyl and (2-chloro-3-trifluoromethyl)phenylmethyl groups.

More specific embodiments of this invention comprise compounds depicted by formulae (**31** - **33**) where Rx is $-NH_2$, $-NHCH_3$, $-NHCH_2CH_3$, $-N(CH_3)_2$, $-N(CH_2CH_3)_2$,-





$NHCO_2C(CH_3)_3$, $-CO_2H$ or $–NHSO_2CH_3$ and Q is a phenyl group substituted by two or three groups selected from fluoro, chloro, trifluoromethyl and methoxy.

Nine preferred compounds (**34**-**42**) were identified, with seven (**34**-**40**) of these belonging to the aminonapthyl series and single examples from the 1,2,3,4-tetrahydronapthalene (**41**) and 1,2,3,4-tetrahydroquinoline (**42**) series.

The sixth application claims 5-membered aromatic heterocycle-containing compounds of the generic formula (**43**) [83].

More specific embodiments of this invention comprise compounds depicted by formula (**44**) where R_1 is selected from $-CH_3$, $-CO_2CH_3$, $-CH_2CO_2H$, $-CH_2CON(CH(CH_3)_2)_2$, $-CH_2CONHCH_2$-furan-2-yl, $-CH_2CO$-morpholin-4-yl, $-CH_2CO$-thiomorpholin-4-yl, $-CH_2CO$-pyrrolidin-1-yl, $-CH_2CH_2N(CH(CH_3)_2)_2$ and $-CH_2CH_2NHCH_2$-furan-2-yl; X is O or $NH(R_1)$.

Eleven preferred compounds (**45**-**55**) are described in claim 19, with all but two of these possessing a substituted isoxazole moiety in conjunction with the established 2,2-diphenylethyl and (2-chloro-3-trifluoromethyl)phenylmethyl groups.

Lastly within the ‘alkylaminoalkoxy arylalkanoate chemotype’ a patent application claiming indole-containing compounds with the generic structure (**56**) was published in 2005 [84].

More preferred embodiments of this invention comprise compounds of generic formula (**57**) where n is 3; each R_2 and R_3 are H or one R_2 or R_3 is methyl and each of the other R_2 and R_3 is H; X is H, $-CH_3$, $-CH_2CH_3$, $-CH_2CH{=}CH_2$, $-CH_2CO_2H$, $-C(CH_3)_2CO_2H$, $-CH_2CONHCH_3$, $-CH_2CONHCH_2CH_3$, $-CH_2CONHCH_2$-furan-2-yl, $-CH_2CONH$-benzyl, $-CH_2CO$-morpholin-4-yl, $-CH_2CO$-thiomorpholin-4-yl, $-CH_2CO$-pyrrolidin-1-yl, $-CH_2CH_2NHCH_3$, $-CH_2CH_2NHCH_2CH_3$, $-CH_2CH_2NHCH_2$-furan-2-yl, $-CH_2CH_2$-morpholin-4-yl, $-CH_2CH_2$-thiomorpholin-4-yl, $-CH_2CH_2$-pyrrolidin-1-yl, $-SO_2CH_3$, -SO2CH2CH3, $-SO_2CH_2CH_2CH_3$, or $-SO_2CH(CH_3)_2$, Q is a phenyl group substituted by two or three groups selected from fluoro, chloro, trifluoromethyl and methoxy and W_2 is unsubstituted phenyl or methyl.

There are 49 exemplified compounds contained within the application which cover structural modification in groups X, (CR2R3)n, W_2 and Q from generic structure (**57**). This is in contrast to the previous applications described where exemplified compounds have largely focussed on exemplification of changes to the moiety distal from the basic nitrogen whilst retaining (2-chloro-3-trifluoromethyl) phenylmethyl as Q and unsubstituted phenyl as W_2. A selection of the exemplified compounds from this application is shown below (**58**-**83**). Whether this reflects an increased level of interest in this indole sub-series within GSK remains to be seen from subsequent patent applications and/or publications relating to biological activity.

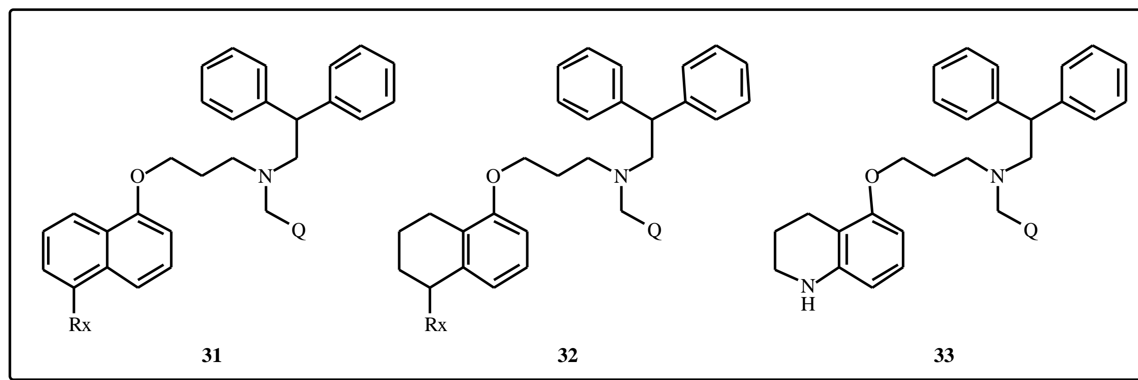
2.2.1. Pharmacological Activity of GW3965 (12)

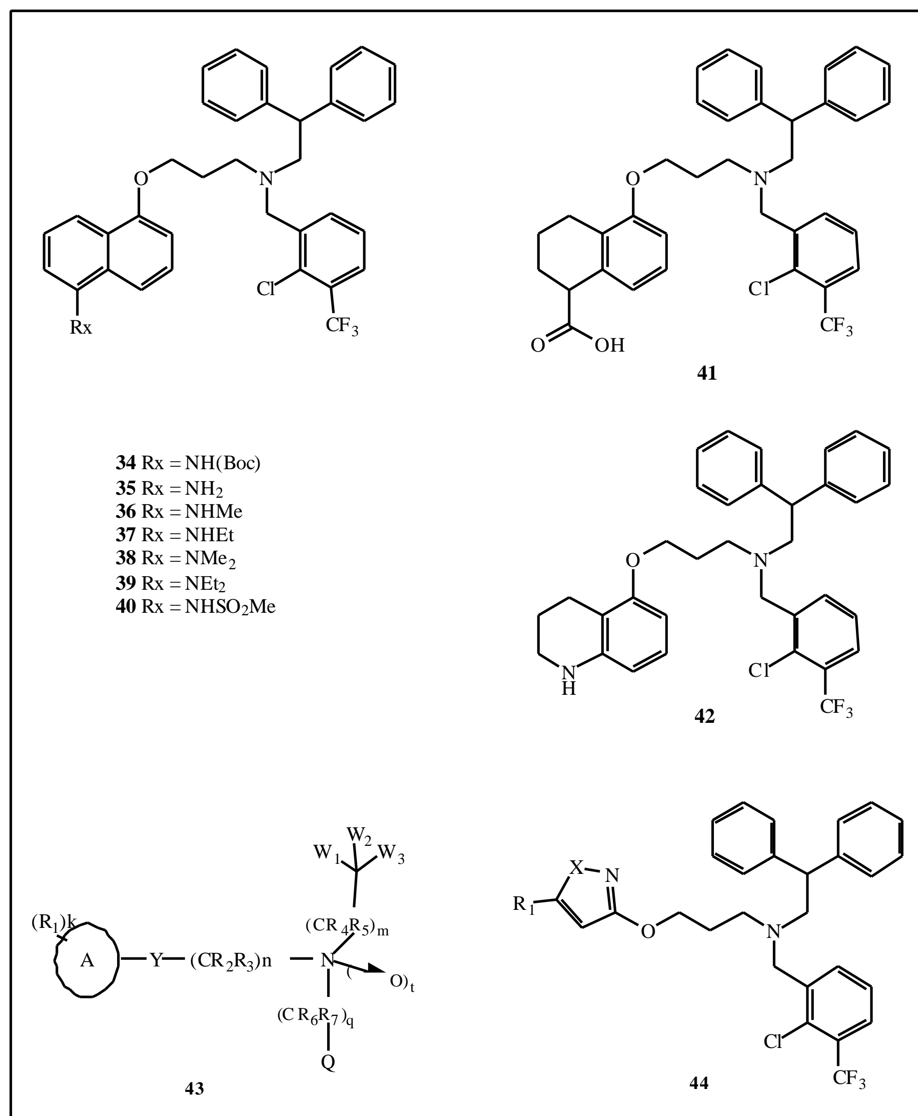
In Vitro LXR Activity of GW3965 (12)

GW3965 (**12**) has an *in vitro* activity at hLXR in two assays: an EC_{50} of 125nM for recruitment of a 24 amino acid fragment of the steroid receptor coactivator 1 (SRC1) as measured in the cell-free ligand sensing assay (LiSA) and an EC_{50} of 190nM in a cell based LXR -GAL4 chimeric reporter gene assay [85]. The *in vitro* activity of analogues (**13**-**15**) related to GW3965 (**12**) have also been published [85].

LXR Target Gene Regulation: GW3965 (12)

As with T0901317 (**4**) discussed previously a number of studies have now been published on the effects of GW3965 (**12**) on the regulation of LXR target genes involved in reverse cholesterol transport (RCT), lipogenesis and inflammatory responses and a number of these are summarised in the following section.

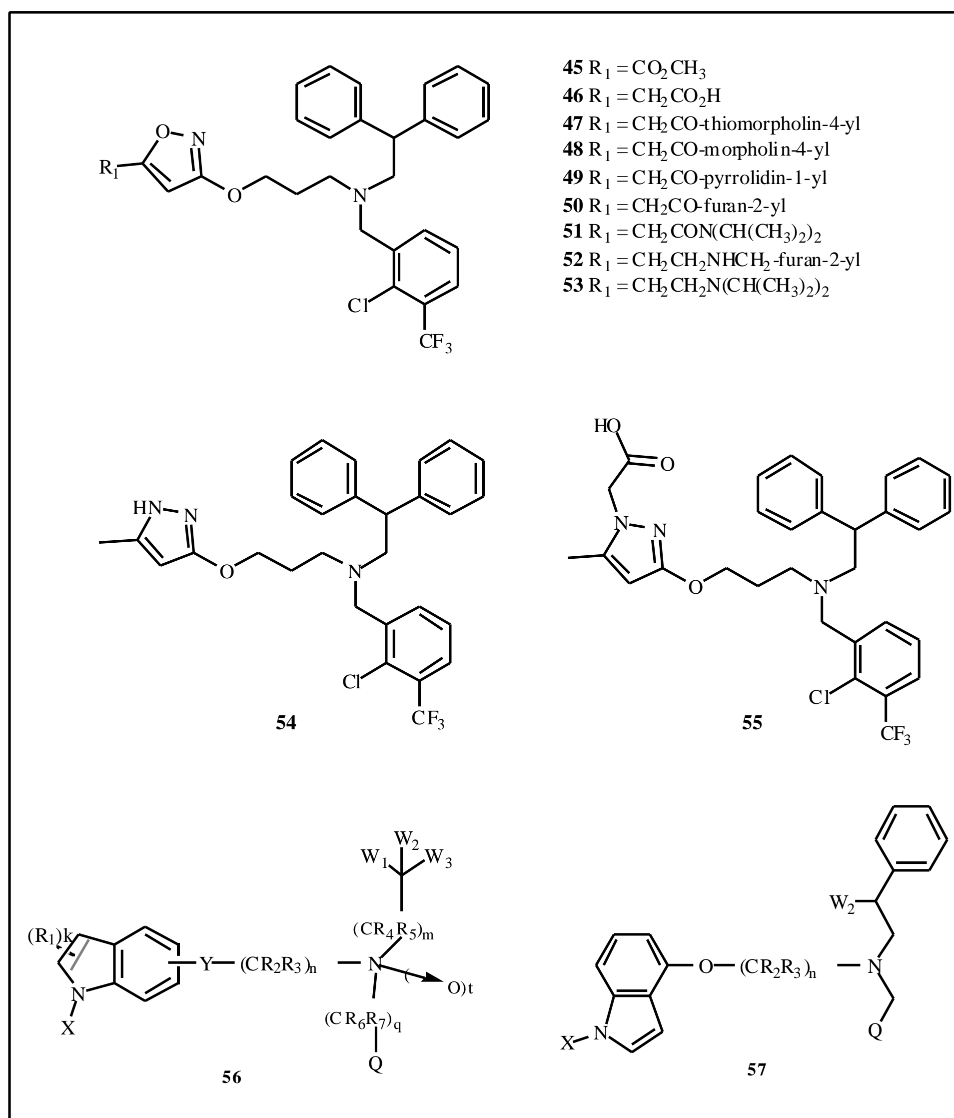




ATP-Binding Cassette (ABC) Transporter - ABCA1

The *in vivo* effects of GW3965 (**12**) on ABCA1 levels has been assessed, with data from a number of these studies being summarised in the following section:

1. ApoE$^{-/-}$ mice were treated with GW3965 (**12**) (10 mg/kg/day, contained in diet) to examine its effects on ABCA1 mRNA expression in the liver and small intestine. mRNA was measured by quantitative real-time PCR, at three time points (2 weeks and 4 days, 4 weeks and 12 weeks) [86]. A strong induction of ABCA1 (~55 fold) was observed in the intestine at 2 weeks and 4 days which had declined to 3 fold by 12 weeks [86]. All three time points revealed minimal effects on ABCA1 expression in the liver.
2. In a second experiment in ApoE$^{-/-}$ mice, treatment for 4 days with GW3965 (**12**) (10 mg/kg/day, contained in diet) caused a 2 fold increase in ABCA1 expression in the aorta [86].
3. An increase in ABCA1 expression was observed in the small intestine (8 fold) and in peripheral macrophages (7 fold) of C57BL/6 mice treated with GW3965 (**12**) (10 mg/kg, b.i.d.) for 3 days [71]. Mice were treated for 14 days in this study but no data was given for other time points [71].
4. Female FVB mice treated with GW3965 (**12**) (35 mg/kg/day, contained in food) for 10 days also showed a significant, 9 fold upregulation of ABCA1 in the small intestine as measured by RT-PCR [87].
5. C57BL/6 mice treated with GW3965 (**12**) (10 mg/kg/day or 50 mg/kg/day) for 6 days showed a significant upregulation of ABCA1 mRNA in the liver (1.6 fold at 10 mg/kg and 2.2 fold at 50 mg/kg). In the duodenum ABCA1 mRNA was upregulated by 4-5 fold at both doses [48].



ATP-Binding Cassette (ABC) Transporters - ABCG1, ABCG2, ABCG5 and ABCG8

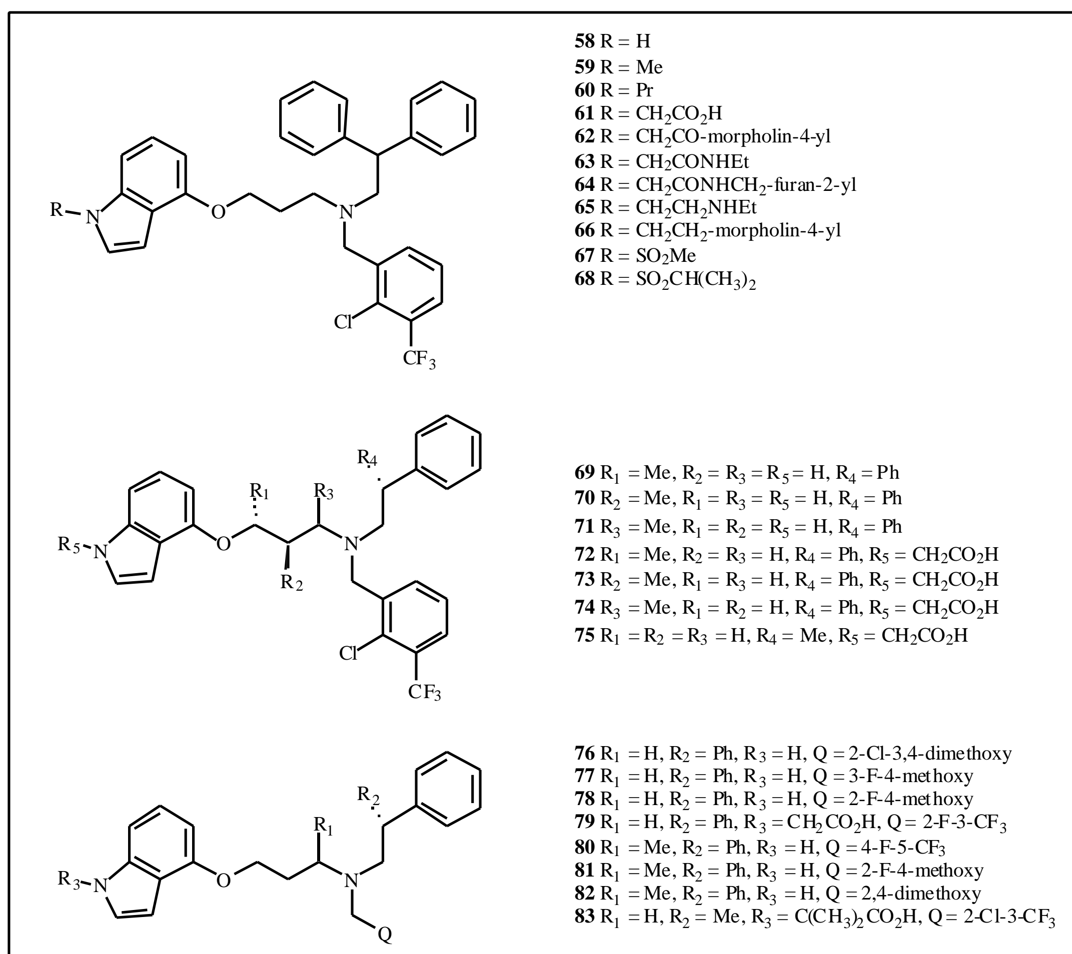
Female FVB mice were treated with GW3965 (**12**) (35 mg/kg/day, contained in food) for 10 days to examine its effects on the ABCG transporters in the small intestine. A 13 fold increase in ABCG1 mRNA (RT-PCR) was observed which was correlated with an increase in protein expression (Western Blotting). ABCG5 (~3 fold) and ABCG8 (~3 fold) mRNA were upregulated but ABCG2 was not. Protein expression of ABCG5 was shown to be upregulated at the brush border membranes of the small intestine [87]. C57BL/6 mice treated with GW3965 (**12**) (10 mg/kg/day or 50 mg/kg/day) for 6 days showed a significant upregulation of ABCG1 mRNA in the liver (1.9 fold at 10 mg/kg and 2.5 fold at 50 mg/kg) [48].

Scavenger Receptor B1 (SR-B1)

SR-B1 is a cell surface membrane protein with a high affinity for HDL [6]. SR-B1 is responsible for the internalisation of the HDL-derived cholesterol esters and the recycling of HDL apolipoproteins, such as ApoA1 [6]. Female FVB mice treated with GW3965 (12) (35 mg/kg/day, contained in food) for 10 days had a 2 fold upregulation of SR-B1 mRNA in the small intestine with no significant effect on Niemann-Pick C1 Like 1 (NPC1L1), acyl-coenyzme A:cholesterol acyltransferase 2 (ACAT2) or 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR) mRNA [87].

Sterol-Regulatory Element Binding Protein-1c (SREBP-1c), Acetyl-Coa Carboxylase (ACC) And Fatty Acid Synthase (FAS)

Treatment of ApoE$^{-/-}$ mice with GW3965 (**12**) (10 mg/kg/day, contained in diet) for 12 weeks showed an increase in the expression of SREBP-1 in the liver (1.5 fold) and small intestine (2 fold) [86]. C57BL/6 mice treated with GW3965 (**12**) (10 mg/kg/day or 50 mg/kg/day) for 6 days showed a significant upregulation of SREBP-1c mRNA in the liver (3.4 fold at 10 mg/kg and 5.6 fold at 50 mg/kg). In the duodenum SREBP-1c mRNA was upregulated to similar



levels at both doses [48]. FAS (2.3 fold) and ACC (1.5 fold) mRNA were also significantly upregulated in the liver at 50 mg/kg but not 10 mg/kg.

Cytochrome P450 7a (Cyp 7a)

ApoE$^{-/-}$ mice treated with GW3965 (12) (10 mg/kg/day, contained in diet) for 12 weeks showed an increased expression of Cyp7a in the liver at 2 weeks and four days (2.5 fold) and at 4 weeks (4 fold) which had decreased by week 12 [86].

Matrix Metalloproteinase-9 (MMP-9)

ApoE$^{-/-}$ mice treated for 3 days with GW3965 (**12**) showed a down-regulation of MMP-9 in the aorta [88]. This down-regulation also occurred *in vitro* in mouse macrophages with no effects being observed for MMP-12 and -13 expression [58]. This effect does not appear to be through direct interaction of the LXR DNA binding domain with the MMP-9 gene as sequence analysis has not identified any potential LXR response elements in the promoter region of the MMP-9 gene [58].

Phospholipid Transfer Protein (PLTP)

GW3965 (**12**) induces expression of PLTP in mice, differentiated THP-1 cells, HepG2 cells, and in primary human hepatocytes. C57BL/6 mice treated with GW3965 (**12**) (30 mg/kg/day by gavage) for 5 days showed an increase in PLTP mRNA in liver, intestine and adipose tissues [89]. Treatment of ApoE$^{-/-}$ mice with GW3965 (**12**) (10 mg/kg/day by gavage) for 5 days caused an upregulation of PLTP mRNA in the aorta. The promoter region of the human PLTP gene does contain an LXR response element.

Anti-Inflammatory Effects

Inflammation is recognised to play a key role in atherosclerosis [5] and GW3965 (**12**) treatment has been shown to illicit anti-inflammatory effects in a number of studies which are summarised below:

1. GW3965 (**12**) has been shown to inhibit the expression of inflammatory mediators, such as iNOS, COX-2, and IL-6, in macrophages stimulated by LPS or bacterial infection [88]. A down-regulation of iNOS and IL-6 mRNA was observed in stimulated murine peritoneal macrophages treated for 18 hours with GW3965 (**12**). No effect on the expression of iNOS and MCP-1 mRNA was observed in the aortas of apoE$^{-/-}$ mice after 3 days treatment with GW3965 (**12**) [88].
2. Treatment of macrophages (RAW 264.7) with TNF- , IL-1 INF- or LPS induces the expression of osteopontin mRNA and protein. Pre-treatment of the macrophages with GW3965 (**12**) (3μM) for 24 hours

before they were stimulated with TNF- , IL-1 INF- or LPS was shown to block this induction [65].

3. Tissue factor expression can be induced in mouse peritoneal macrophages by inflammatory stimuli such as LPS. Pre-treatment of macrophages with 1, 0.1 and 0.01µM of GW3965 (**12**) for 18 hours before stimulation with LPS for 6 hours caused a significant reduction in the expression of tissue factor mRNA. The same protocol was used in human monocytes which showed a significant reduction in tissue factor mRNA and activity at 1µM, 0.1µM but not 0.01µM [61].

4. Anti-inflammatory effects have also been observed in a mouse model of contact dermatitis where a reduction in ear weight and ear thickness was measured [88, 90].

In vivo Atherosclerosis and Lipid Modulation Studies

1. Treatment of male LDLR$^{-/-}$ mice on a high fat diet containing GW3965 (**12**) (10mg/kg/day, contained in diet) for 12 weeks led to a significant decrease in total cholesterol and unesterified cholesterol and no significant changes in triglyceride or HDL levels [86]. A statistically significant 50% reduction in average aortic lesion area (en face analysis) and a statistically significant 35% reduction in lesion area in aortic root sections (Oil Red O staining) was observed in this study [86].

2. Treatment of male ApoE$^{-/-}$ mice on a normal chow diet containing GW3965 (**12**) (10mg/kg/day, contained in diet) for 12 weeks gave no significant changes in total cholesterol and HDL cholesterol, a significant decrease in very-low-density lipoprotein and increase in triglycerides [86]. A statistically significant 47% reduction in the lesion area of aortic root sections (Oil Red O staining) was observed in this study [86].

3. Oral dosing of GW3965 (**12**) (10mg/kg, b.i.d.) for 14 days to C57BL/6 mice showed an increase in plasma HDLc levels measured at days three, seven and fourteen [85].

4. Female FVB mice treated with GW3965 (**12**) (35 mg/kg/day, contained in food) for 10 days showed a 27% increase in plasma cholesterol which was shown by fast protein liquid chromatography to be mainly HDL-size fractions, while liver triglycerides had increased by 106% relative to vehicle [87]. In a separate fecal-dual isotope study cholesterol absorption was reduced by 50% after treatment with GW3965 (**12**). This study used GW3965 (**12**) (35 mg/kg/day, contained in food) for 10 days and after day 6 the mice received a 150µL medium chain tryglyceride oil containing [^{14}C]cholesterol and [^{3}H]sitostanol [87].

5. C57BL/6 mice were treated with GW3965 (**12**) (20mg/kg/day, 60 mg/kg/day or 100 mg/kg/day) for 3 days. Plasma HDL cholesterol levels were significantly increased at the 60 mg/kg (18%) and 100 mg/kg (20%) doses. Unlike T0901317 (**4**) (50 mg/kg/day), GW3965 (**12**) had no effect (at any dose) on liver triglycerides, size or colour [91]. In the liver, GW3965 (**12**) was shown to be a weaker inducer of SREBP1-c and FAS (2-4 fold) than T0901317 (**4**). Using a co-transfection assay with GALR4-LXR , it was shown that the EC_{50} for LXR in HEPG2 cells was low (1.2µM) compared with the HEK-293 cell line (0.29µM). The GW3965 (**12**) activity in HepG2 cells was 10 times lower than that of T0901317 (**4**) (EC_{50} = 0.11µM). A similar difference was observed for LXR activity in HepG2 cells (T0901317 (**4**) EC_{50} = 0.2µM, GW3965 (**12**) EC_{50} = 4µM). The authors used a peptide recruitment assay to show that one difference between the two compounds was in recruitment of the CBP protein (T0901317 (**4**) EC_{50} = 0.03µM, GW3965 (**12**) EC_{50} = 0.36µM). Other peptides, such as SRC-1 and DRIP, were recruited to similar extents by both compounds.

6. C57BL/6 mice treated with GW3965 (**12**) (10 mg/kg/day or 50 mg/kg/day) for 6 days showed an increase in total cholesterol, triglycerides and VLDL-cholesterol at both doses. A significant increase in HDL-cholesterol was only observed at the 50 mg/kg dose [91].

Pharmacokinetic Profile of GW3965 (12)

The pharmacokinetic data obtained in mice GW3965 (**12**) (after a 10mg/kg dose) gave an oral bioavailability of 70%, a C_{max} of 12.7µg/mL and a $t_{1/2}$ of 2 hours [85].

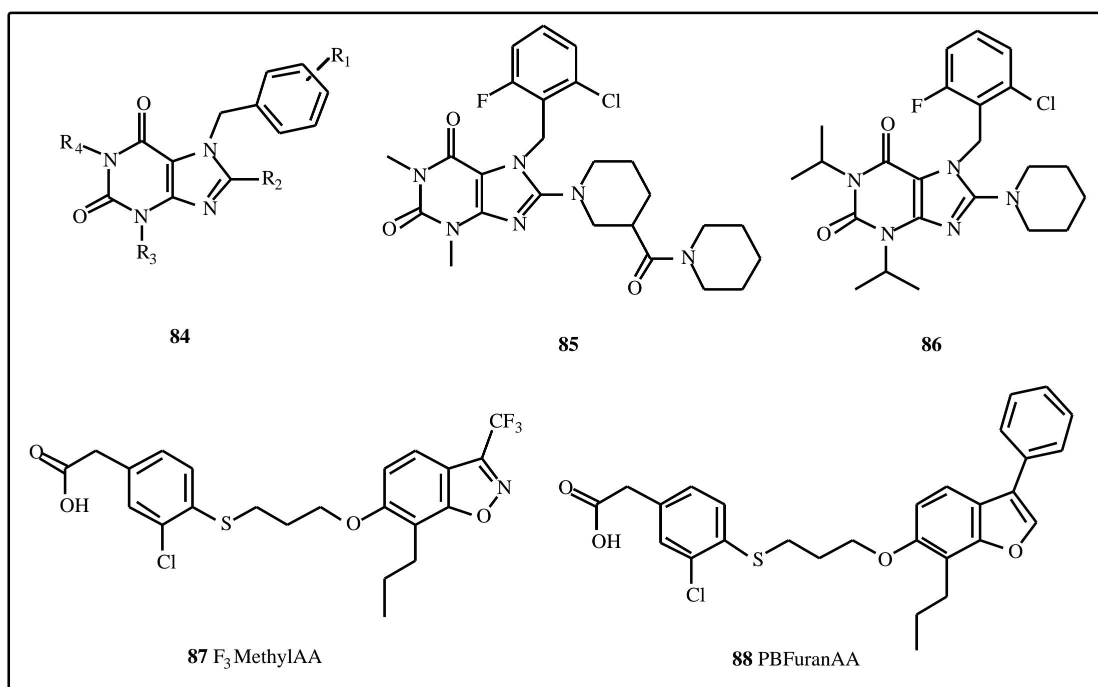
A patent application from GSK has also been filed claiming a series of xanthine based compounds of general structure (**84**) for the treatment or prevention of a LXR mediated disease or condition, including cardiovascular disease and atherosclerosis [92]. R_1 may be selected from halo, alkyl or nitro with the 2-chloro-6-fluoro substitution being most preferred in the majority of examples. R_2 may be a substituted or unsubstituted aryl, heteroaryl, halo, alkyl, cycloalkyl or piperidine. R_3 and R_4 are generally alkyl groups. Compounds (**85**) and (**86**) constitute preferred examples. An LXR FRET assay was employed to determine the activity of synthesised compounds using 24(*S*),25-epoxycholesterol as the control.

Compound (**85**) was reported to have a pEC_{50} = 6.6 for LXR (74% control) and pEC_{50} = 7.3 for LXR (69% control). Compound **86** was reported to have a pEC_{50} = 6.8 for LXR (49% control) and pEC_{50} = 7.7 for LXR (44% control).

2.3. Merck and Co.

F_3MethylAA (**87**), is a benzisoxazole-containing LXR agonist (covered in the scope of a PPAR patent from Merck and Co. [93]) with a Ki of 13nM at LXR and a Ki of 8nM at LXR (as measured by a scintillation proximity binding assay) [94]. The closely related structure PBFuranAA (**88**) was shown to have a low affinity in the binding assay. Compounds (**87**) and (**88**) also possess agonist activity at one or more of the PPAR isoforms, whilst showing no activity at the glucocorticoid, estrogen, thyroid, farnesoid X, pregnane X or retinoid X receptors at 10µM [95].

Compound (**87**) (5µM) was shown to upregulate ABCA1 mRNA in human primary hepatocytes, and macrophages and ABCG1 and SREBP1 mRNA in human macrophages (as measured by RT-PCR). Cyp7a was shown to be upregulated in rat primary hepatocytes but not in human primary hepatocytes, again highlighting the species differences. Five



84 **85** **86**

87 F_3MethylAA **88** PBFuranAA

days oral dosing of Sprague Dawley rats with compound (**87**) (30 mg/kg/day by gavage) caused an upregulation of liver Cyp7a mRNA and enzyme levels and compound (**88**) showed no effects on liver Cyp7a mRNA after the same dosing regime.

Merck and Co. have subsequently filed four patent applications which all contain the benzisoxazole unit found in F_3MethylAA (**87**) [96-99]. The first patent application has benzisoxazole derivatives of type (**89**) which are claimed as novel LXR ligands for the possible treatment of dyslipidemic conditions, particularly depressed levels of HDL cholesterol [96]. Analysis of the preferred claims and experimental examples suggests that the CF_3 group is the most preferred group at R_1 (with R_1 = CH_2-tert-butyl being the only other synthesised example) and the propyl group at R_2 is fixed for all synthetic examples. A methyl group appears to be favoured for the X group with other synthetic examples including X = H. The Y group incorporates pyridyls (e.g. **90**), alkyls unsubstituted or substituted with an acid group (e.g. **91**) and also incorporates benzyl derivatives unsubstituted or substituted with an acid group.

Synthetic examples in which the X and Y groups are combined to form groups such as morpholine are also reported. In all synthetic examples, the linker chain has three carbon atoms (n = 3). Scintillation proximity binding assay results are claimed to give IC_{50} values of 900nM for one isoform and 5000nM for the other.

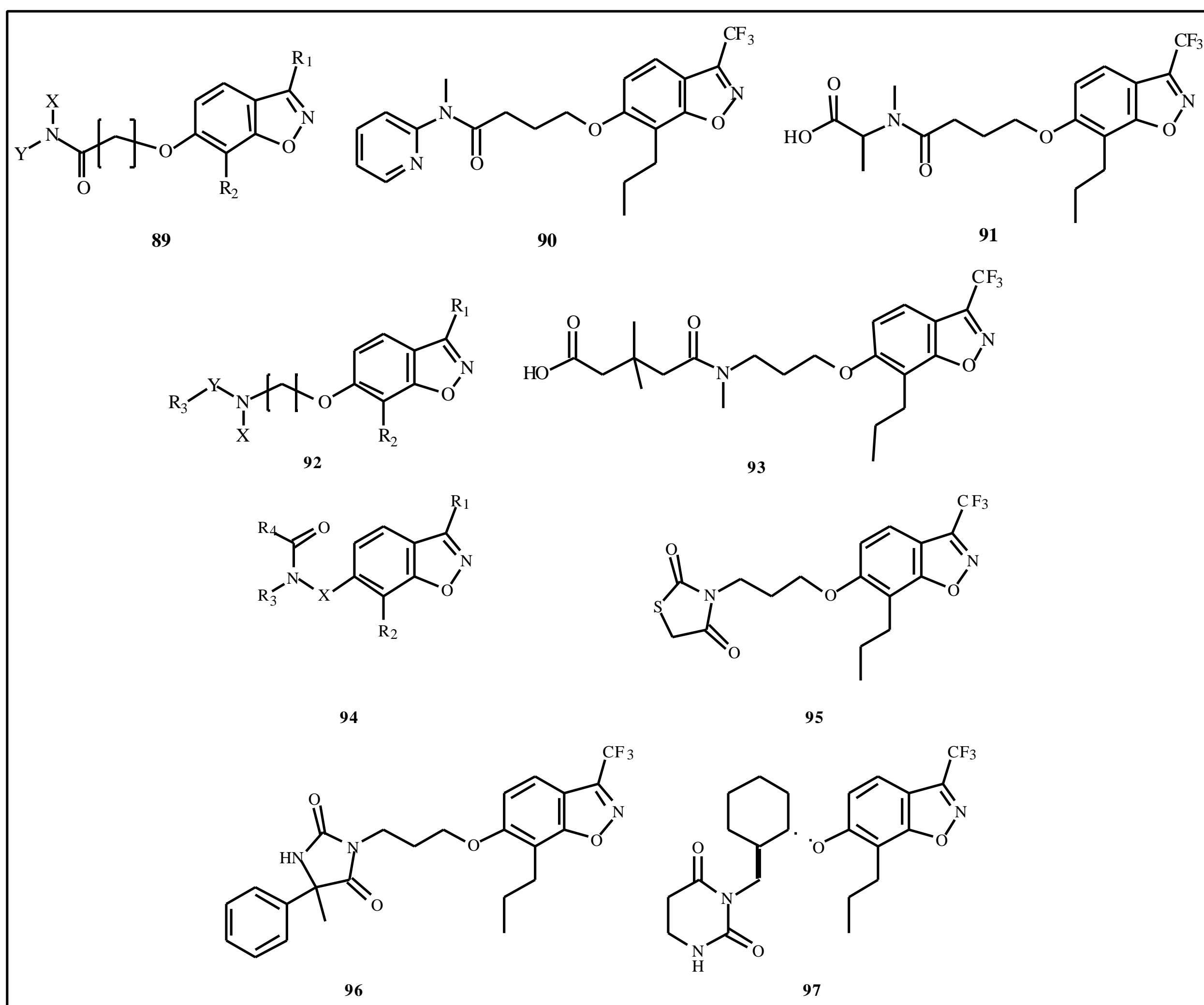
The second patent application claims benzisoxazole analogues of generic formula (**92**) as novel LXR ligands [97]. Analysis of the patent highlights similar preferences for the substitution of the benzisoxazole as the previous filing [96], in that all synthetic examples have R_1 = CF_3 and R_2 = propyl. Furthermore, in the majority of examples X = CH_3 with the exception of X = H for two examples. All synthetic examples include Y = CO and R_3 generally contains alkyls or aromatic groups unsubstituted or substituted with an acid group (e.g. **93**). In all synthetic examples, the linker chain has three carbon atoms (n=3). Scintillation proximity binding assay results are claimed to give IC_{50} values of 2000nM for LXR and from 20nM to >50000nM for LXR .

Compound (**93**) caused a 3.3 fold induction in ABCA1 mRNA levels, in human primary macrophages, at 1μM and 13.6 fold at 10μM.

The third patent application based around the benzisoxazole core claims compounds of general structure (**94**) [98]. Analysis of the patent suggests that the CF_3 group is the most preferred group at R_1 (with R_1 = CH_2-tert-butyl or phenyl being the only other synthesised examples) and the propyl group at R_2 is fixed for all synthetic examples. The X group can be a C4 alkyl, propyloxy, cycloalkyl or cycloalkyloxy. R_3 and R_4 are cyclised to form 5, 6 or 7-membered heterocyclic rings. Compounds (**95**-**97**) are illustrative synthetic examples of preferred compounds.

Scintillation proximity binding assay results for preferred compounds are claimed to give IC_{50} values of 300nM for at least one of the LXR or LXR receptors. Compounds (**95**) and (**96**) have been shown to increase cholesterol efflux from human CaCo-2 cells.

The most recent patent application claims urea based compounds of generic structure (**98**) [99]. The preferred compounds are highlighted by structure (**99**) with R_3 being methyl or ethyl and R_4 being hydrogen or methyl. There are a greater number of R_5 groups claimed including methyl, ethyl, propyl, isopropyl, carboxyethyl, carboxypropyl, pyridyl (1, 2 and 3), 4-methyl-2-pyridyl, 5-chloro-2-pyridyl, 5-carboxy-2-pyridyl, 1-oxo-2-pyridyl, pyrimidinyl (2 and 4), 2-pyrazinyl, 5-terazolyl, 5-methyl-3-oxazolyl, phenyl, 4-carboxyphenyl.



Merck and Co. has reported a structurally distinct series of LXR agonists from previous benzisoxazole filings [96-99] which are claimed to stimulate the expression of the ABC1 gene, to raise serum HDL levels and to treat atherosclerosis and related conditions [100]. The acetyl-podocarpic dimer (APD) (**100**) is the only synthetic example given in the application and has an IC_{50} = 80nM for LXR , and an IC_{50} = 40nM for LXR in a scintillation proximity binding assay. Treatment of human primary macrophages with APD (**100**) (0.1μM) caused a 6.9 fold increase in ABCA1 mRNA levels.

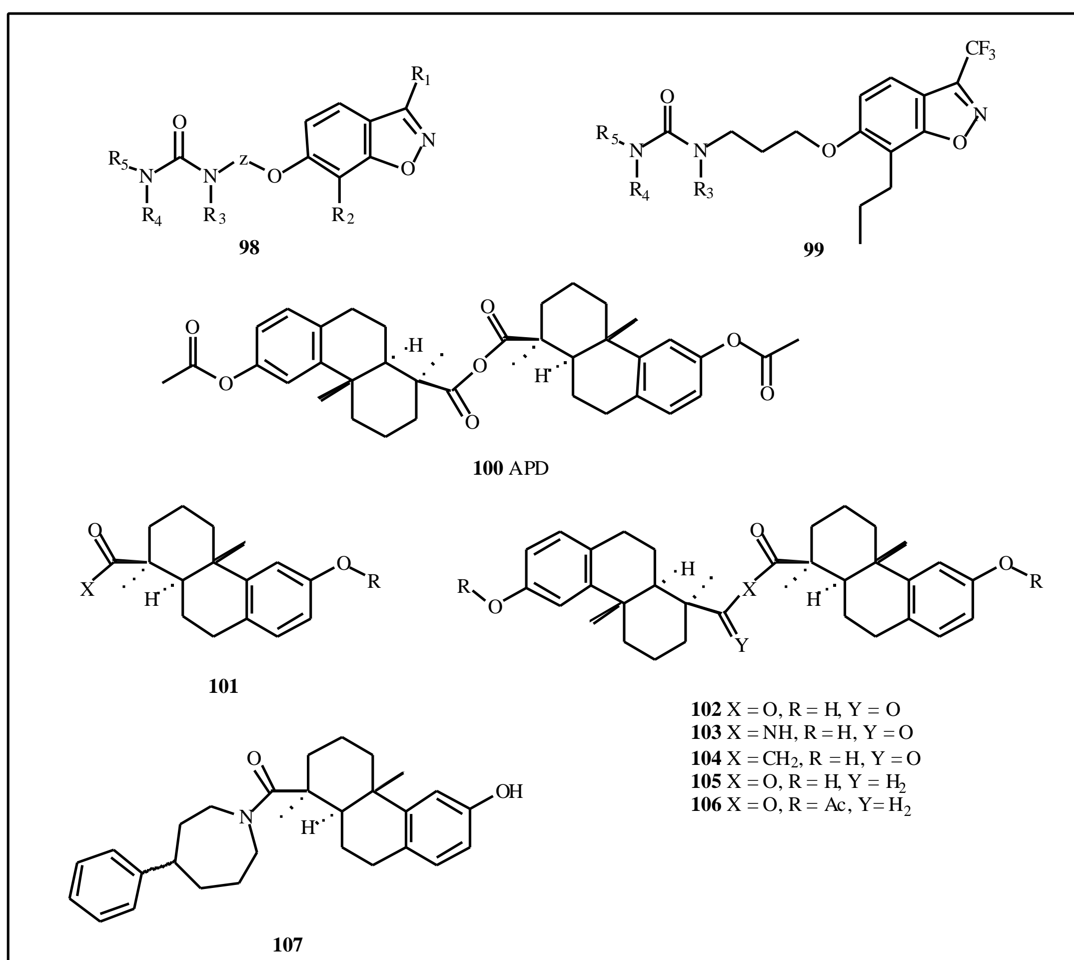
In a related publication, APD (**100**) was also shown to induce ABC mRNA levels and increase efflux of both cholesterol and phospholipids, from THP-1 macrophages, primary human fibroblasts, CaCo-2 and human primary monocyte-macrophage cell types [101].

A follow up patent application based on APD (**100**) claims compounds of formula (**101**) as novel LXR agonists useful in the treatment of dyslipidemic conditions, particularly depressed levels of HDL cholesterol [102]. From analysis of the claims and experimental examples R_1 is generally a hydrogen atom, however, two examples of R_1 being acetyl are also given. Bicyclic examples (**102-106**) appear to be preferred, as well as the monocyclic 4-phenyl-homopiperidin-1-ylamide derivative (**107**).

Scintillation proximity binding assay results are claimed to give IC_{50} values of 500nM for LXR and 300nM for LXR . Experiments in human primary macrophages with compound (**103**, 1μM) have shown a 10 fold induction of ABCA1 mRNA levels. To examine the effects of compound (**103**) on lipid profiles it was dosed (10 mg/kg, b.i.d.) to hamsters and male C57BL/6 mice for 8 days. In hamsters, there was an increase in total plasma cholesterol (28%), HDL-cholesterol (38%) and in triglyceride levels (51%). A similar result was observed in mice with increases of 26% for total plasma cholesterol, 19% for HDL-cholesterol and 26% for triglyceride levels [103].

2.4. AstraZeneca

AstraZeneca has filed a patent application claiming a series of pyrrole-2,3-dione derivatives of the general formula (**108**) as LXR and/or modulators for the treatment and/or prevention of conditions including atherosclerosis, lipid disorders and metabolic syndrome [104].



There are 27 synthetic examples given in the patent application, which contain a selection of groups for R_1 e.g. 2-methoxyethyl (6 examples), pyridine-3-ylmethyl (5 examples), (6-aminopyridine-3-yl)methyl (3 examples) and trifluoroethyl (2 examples), with single examples of 4-dimethylaminobenzyl, pyridine-2-ylmethyl, pyridine-4-ylmethyl and (5-methylisoxazole-3-yl)methyl. R_2 is mostly 4-substituted phenyl rings e.g. 4-methoxyphenyl (14 examples), 4-difluoromethoxyphenyl (3 examples), 4-trifluoromethoxyphenyl (2 examples) and morpholino-4-ylphenyl (3 examples), with single examples of hydrogen and 2-acetyl-5-benzofuranyl.

LXR modulator activity of claimed compounds can be demonstrated in either peptide recruitment or chimeric transactivation assay format. The peptide recruitment assay for LXR and utilises a fragment of steroid receptor co-activator-1, with 22(*R*)-hydroxycholesterol at 50μM used as the 100% control. The transactivation assay utilises a chimeric protein consisting of LXR or ligand binding domain with GAL4 transcription factor DNA binding domain in U2/OS osteosarcoma cells, with in this case T0901317 (**4**) at 1μM being used as the 100% control. The compounds in the patent are claimed to have an EC_{50} <50μM in the LXR and/or peptide recruitment and/or GAL4-LXR transactivation assay.

Peptide recruitment data is given for 2 of the 27 examples with compound (**109**) and (**110**) possessing EC_{50} values of 150 and 110nM respectively.

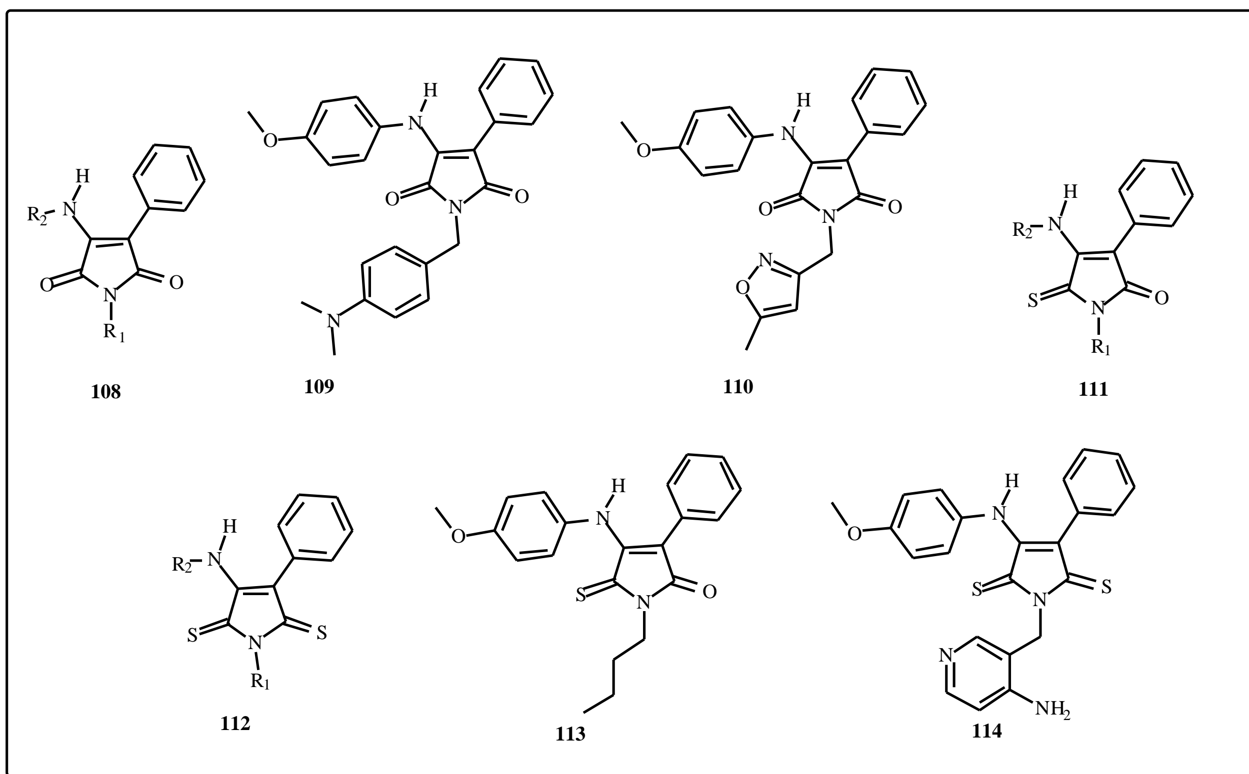
AstraZeneca has filed a further patent application claiming a series of 5-thioxo-1,5-dihydro-2H-pyrrol-2-ones (**111**) and 1H-pyrrole-2,5-dithione (**112**) derivatives as LXR and/or modulators for the treatment and/or prevention of conditions including atherosclerosis, lipid disorders and metabolic syndrome [105]. The same assays and activity criteria are used as in the previous filing [104].

There are 20 synthetic examples given in the patent application, in which R_1 is (6-aminopyridine-3-yl)methyl (4 examples) and there are 2 examples of each of the following groups: 2-methoxyethyl, pyridine-3-ylmethyl, pyridine-4-ylmethyl, butyl, 2,2,2-trifluoroethyl, benzyl, methyl and ethyl. R_2 is 4-methoxyphenyl (16 examples), 4-difluoromethoxyphenyl (2 examples) and morpholino-4-ylphenyl (2 examples).

Peptide recruitment data is given for 2 of the 27 examples with compound (**113**) and (**114**) possessing EC_{50} values of 90 and 140nM respectively.

2.5. X-Ceptor/Sankyo

X-Ceptor Therapeutics has filed a patent application based on pyridone derivatives of general structure (**115**) for



modulating the activity of LXR [106]. The 140 claims are broad but synthetic examples favour R_1 = substituted aryl but can be 1-substituted piperidin-4-yl, heteroaryl (e.g. thiophen-2-yl), benzyl, naphthyl, R_2 = H, R_3 = CF_3, R_4 = CN but can be CH_2NHCOalkyl and R_5 = benzyl or substituted benzyl (e.g. 2,4-dimethylbenzyl) but can be piperidin-1-yl, phenethyl, CH_2heteroaryl (e.g. CH_2pyridyl), alkyloxyalkyl. Several assays are reported, including a scintillation proximity binding assay and a transactivation assay. Representative data was given in these assays for selected compounds e.g. (**116**, LXR Ki = 0.69μM, LXR Ki = 0.45μM, LXR EC_{50} = 3.4μM), (**117**, LXR Ki = 0.51μM, LXR Ki = 0.12μM, LXR EC_{50} = 1.2μM) and (**118**, LXR Ki = 0.36μM, LXR Ki = 0.23μM, LXR EC_{50} = 0.58μM).

X-Ceptor/Sankyo have filed a second patent application claiming fused pyrimidine-4(3H)-one derivatives (**119**) which exhibit anti-arteriosclerotic and anti-inflammatory activity through their ability to regulate LXR [107].

Examination of the patent reveals that preferred compounds of generic structure (**120**) contain the hexafluoroisopropanol unit found in the Tularik compound, T0901317 (**4**).

For the preferred compounds R_1 is benzyl or substituted benzyl (e.g. 4-Cl, 4-OMe, 4-Me, 4-CF_3, 3-CF_3, 3-F, 2-F, 2,3-Di-F) with one example of CF_3 and cyclobutyl.

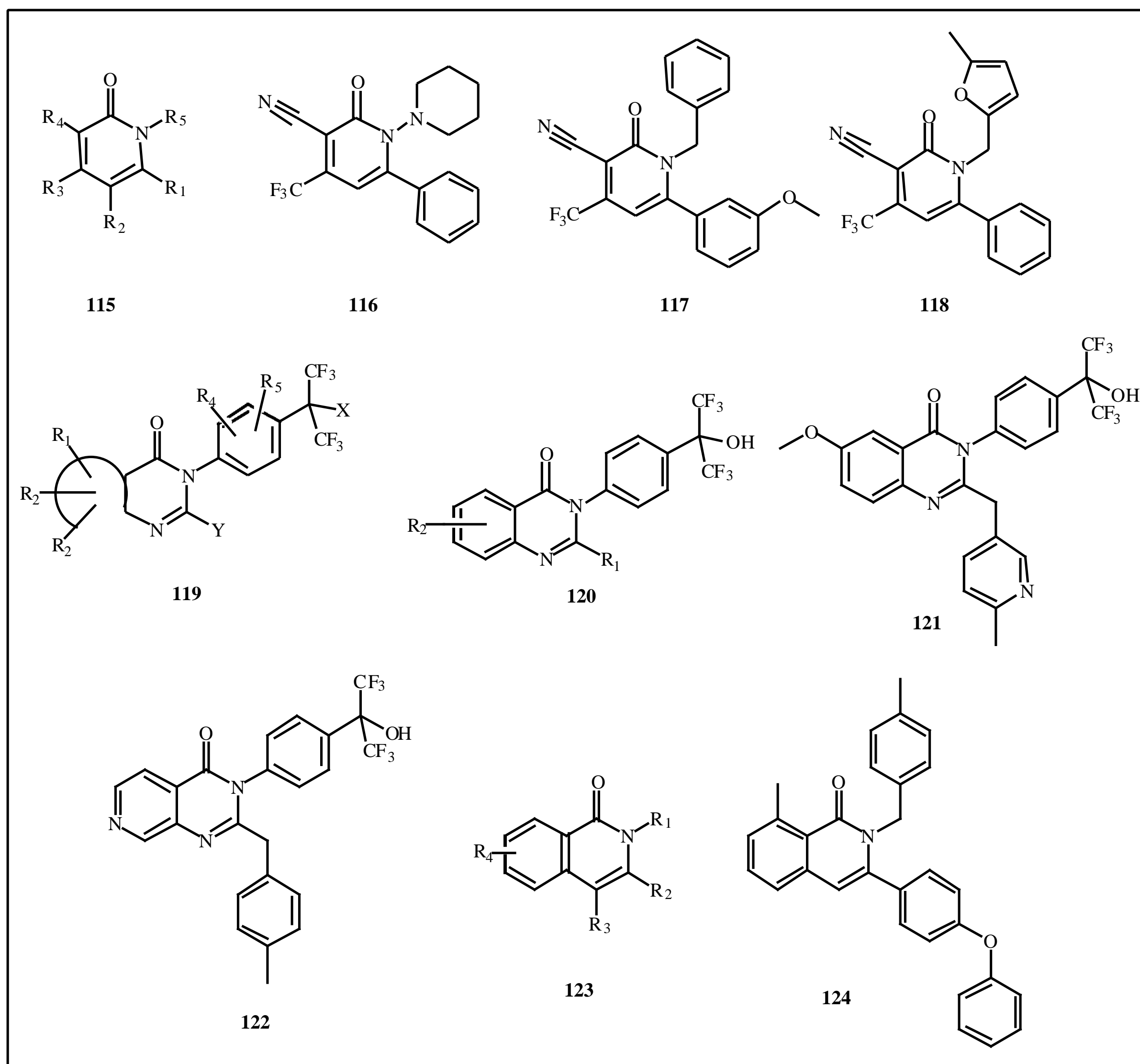
R_2 has many examples of simple substitution in positions 5, 6 and 7 e.g. H, 5-Cl, 5-Me, 5-OH 6-OH, 6-Br, 6-Cl, 7-CF_3, 7-OH, 7-OH but only one 8 substituent (8-Cl). There are also examples of disubstitution e.g. 6,7-dimethoxy, 5,6-dimethyl, 6-Cl-7-Me. Most of the mono and disubstituted R_2 compounds have R_1 = benzyl.

The synthetic examples in which the phenyl ring of the quinazolinone is replaced with thiophene and pyridine are not included in the preferred list in claim 48. A number of assays are used including a scintillation proximity assay (SpA) for LXR and using ^{3}H-24(*S*),25-epoxycholesterol. There is data presented for 91 compounds, of which the most potent are compounds (**121**, LXR pKi = 32nM, LXR pKi = 12nM) and (**122**, LXR pKi = 28nM, LXR pKi = 7nM).

A follow up patent from X-Ceptor/Sanko [108] is based around the same chemical series as general structure (**119**).

A further patent application from X-Ceptor/Sankyo claims a series of isoquinoline derivatives (**123**) as LXR modulators for the treatment or prevention of diseases associated with altered cholesterol transport, reverse cholesterol efflux, fatty acid metabolism, cholesterol absorption, cholesterol excretion or cholesterol metabolism [109].

The scope of the preferred compounds is broad and includes R_1 as benzyl, substituted benzyl e.g. 2,4-dimethylbenzyl, biphenyl-4-ylmethyl, substituted heteroaryl-methyl e.g. pyridine-3ylmethyl, 5-methylfuran-2-ylmethyl, thiophen-2-ylmethyl or alkyl e.g. cyclohexylmethyl and 2,2-dimethylpropyl. R_2 is aromatic e.g. phenyl, furanyl, thiophenyl, benzothiophenyl, benzofuranyl, substituted aromatics e.g. 4-phenoxyphenyl, tolyl, 5-bromothiophen-2-yl, (3,5-bis-trifluoromethylphenyl)thiophen-2-yl, R_3 is H and R_4 is H, 5-Cl, 5-F, 6-Me, 7-Me, 7-F, 8-Me, 8-Cl, 8-OMe, 5,6-diCl and 6,7-diCl are also claimed.



Several assays are described and the SpA binding activity of 14 compounds is given as a range: Ki >500nM or Ki is between 500nM and 150nM or Ki is between 10nM and 150nM (e.g. **124**).

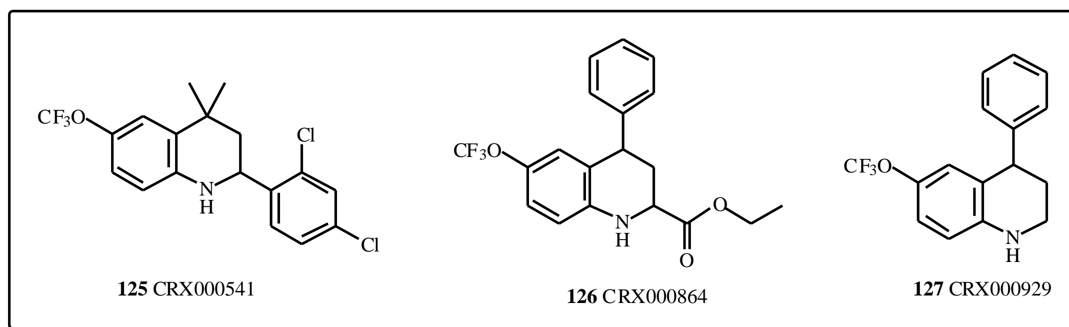
2.6. CareX

CareX have recently filed three patent applications around a tetrahydroquinoline framework with broad structural claims [110-112]. Due to the breadth of the claims the following review will focus on the exemplified and specifically claimed compounds paying particular attention to those with associated biological data.

There are seventeen compounds exemplified by synthesis in the first application [110], with CRX000541 (**125**), CRX000864 (**126**) and CRX000929 (**127**) being specifically claimed. Stereochemistry is not defined for (**125**-**127**) with all possible isomers and mixtures being claimed.

No detailed biological activity is mentioned for the exemplified compounds although ranges of activity are outlined. Binding to the LXR ligand binding domain is preferred to be between 10nM and 500nM. Compounds are also claimed to modulate LXR function, the magnitude of the function being measured by a transactivation assay and western-blot analysis of the up-regulation of target genes involved in cholesterol trafficking. Mention is also made of the partial agonist activity of the compounds within this patent and an indication that compounds may differentially regulate LXR target gene expression though no data is supplied to support these claims. Accordingly, compositions of the invention are claimed to possess the ability to increase the expression of cholesterol trafficking gene ABCA1 but have limited ability to increase the expression of the 'lipogenic genes' FAS, SREBP-1c and Angptl3. Preferred compound activity is described as <20% induction of the lipogenic genes as compared to T0901317 (**4**).

In the second CareX patent application [111], sixteen compounds are specifically claimed, all of which are exemplified by synthesis, with limited biological data included for selected analogues. Data is available for two compounds in a scintillation proximity binding assay using



125 CRX000541 **126** CRX000864 **127** CRX000929

radiolabelled T0901317 (**4**) as the competitor standard. CRX000823 (**128**) and CRX000987 (**129**) both have a binding Ki of between 250-1000nM at the human LXR ligand binding domain. The binding Ki at human LXR is between 1000-3000nM.

Resolution of CRX000987 (**129**) into its constituent enantiomerically pure *syn* isomers (CRX001093 and CRX001094) was acheived by chiral HPLC or by using a chiral auxiliary strategy. CRX000987 (**129**), CRX001093 and CRX001094 all show functional activity in a transactivation assay with EC_{50} values at LXR in the range of 250-1000nM. Compounds of the invention are also reported to promote upregulation of genes implicated in cholesterol efflux, with CRX000987 (**129**), CRX001093 and CRX001094 giving 80-100% Vmax (maximal response with reference to T0901317 (**4**)) for ABCA1 expression. Genes involved with lipogenesis such as FAS and SREBP-1c were also investigated together with Angptl3, although, no information on the test concentration(s) used in these assays is given. Compounds of the invention are also claimed to promote cholesterol efflux from THP-1 cells *in vitro* though no biological data is presented.

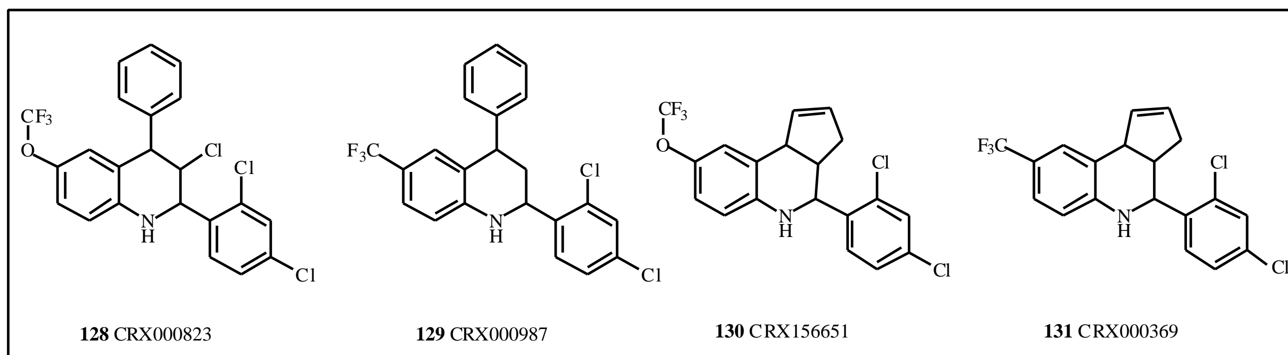
In the final CareX patent application [112], tricyclic compounds related to those previously disclosed are claimed as LXR modulators. Specific claims are made on 162 compounds with 189 compounds exemplified by synthesis. Of the specified compounds two compounds are more preferred, CRX156651 (**130**) or the pure enantiomers CRX000909 and particularly CRX000908, and CRX000369 (**131**) or the pure enantiomers CRX001045 and CRX001046.

CRX156651 (**130**) was also screened using a luciferase based transactivation assay, giving an EC_{50} of 305nM. It is also suggested that the compound is a partial agonist relative to T0901317 (**4**). CRX156651 (**130**) activates ABCA1 gene expression in a dose dependant manner giving an ED_{50} of 2.9μM, with this efficacy being 63% of GW3965 (**12**). CRX155651 (**130**) also up-regulates the FAS and SREBP1c genes with ED_{50} values of 593nM and 377nM respectively. CRX155651 (**130**) also activates the Angptl3 gene in a dose dependant manner, the efficacy being 44% of GW3965 (**12**) with an ED_{50} of 7.5μM. Finally, CRX155651 (**130**) is shown to promote cholesterol efflux from THP-1 cells *in vitro*. The efficacy of this compound in this assay is claimed to be ~70% of GW3965 (**12**).

Further profiling of this compound *in vivo* suggests that the compound can have a beneficial effect on HDL-cholesterol levels. C57BL/6 mice were dosed with 10mg/kg/day for 7 days prior to assaying for HDL. Data is also presented to suggest that CRX 156651 (**130**) does not increase either plasma or liver triglyceride levels.

2.7. Chugai Seiyaku Kabushiki Kaisha

Chugai Seiyaku Kabushiki Kaisha has filed two patent applications covering structures containing the hexafluoroisopropanol moiety present in T0901317 (**4**) [113, 114]. The first series of compounds has the hexafluoroisopropanol fragment attached to a 5 or 6 membered ring [113]. Preferred aromatic 5-membered rings contain at least two heteroatoms, with thiazole (**132**) being the most preferred. The most preferred 6-membered aromatic ring containing structures represented by (**133**) are the 1, 2 or 3-pyridyl moiety with optional substitution (R_1). The most preferred 5-membered non aromatic heterocyclic ring is the isoxazoline (**134**). The ability of 3 analogues to induce ABCA1 expression in THP-1 cells was disclosed, with the



128 CRX000823 **129** CRX000987 **130** CRX156651 **131** CRX000369

most potent compound (**135**) giving a 2.5 fold increase at 10μM.

In the second patent application compounds have the general structure (**136**) where R_1 is selected from a 3-7 membered heterocyclic ring containing 0-3 heteroatoms, a urea or thiourea [114]. The ability of 2 analogues to induce ABCA1 expression in THP-1 cells was disclosed, with the most potent compound (**137**) giving a 3.7 fold increase at 10μM.

Chugai Seiyaku Kabushiki Kaisha has claimed a further series of 1,3-thiazoles as LXR modulators for the treatment of cardiovascular diseases [115]. The compounds claimed in this application are covered by generic structure (**138**) with R_1 and R_2 being cyclised to form pyrimidines, pyridines, pyridazines and benzenes.

The rings formed upon cyclisation of R_1 and R_2 are further substituted with a variety of urea, carbamates and amides which often contain aromatic rings. Compound (**139**) showed a 2.9 fold increase in ABCA1 expression in THP-1 cells at 10μM.

2.8. Pharmacia Corporation

Pharmacia Corporation has filed two patent applications containing the hexafluoroisopropanol fragment present in T0901317 (**4**) [116, 117]. The first patent application describes aniline derivatives of general structure (**140**) where R_1 is preferred as H, alkyl or substituted alkyl [116], R_2 is often an ester substituted alkyl chain and may be joined to R_1, or alternatively R_1 is fused to the aromatic ring, R_3 is generally H, alkyl or aryl and can be joined with R_4 to form a cycloalkyl ring. Exemplified compounds are claimed to have an EC_{50} <50μM in a LXR reporter gene transactivation assay.

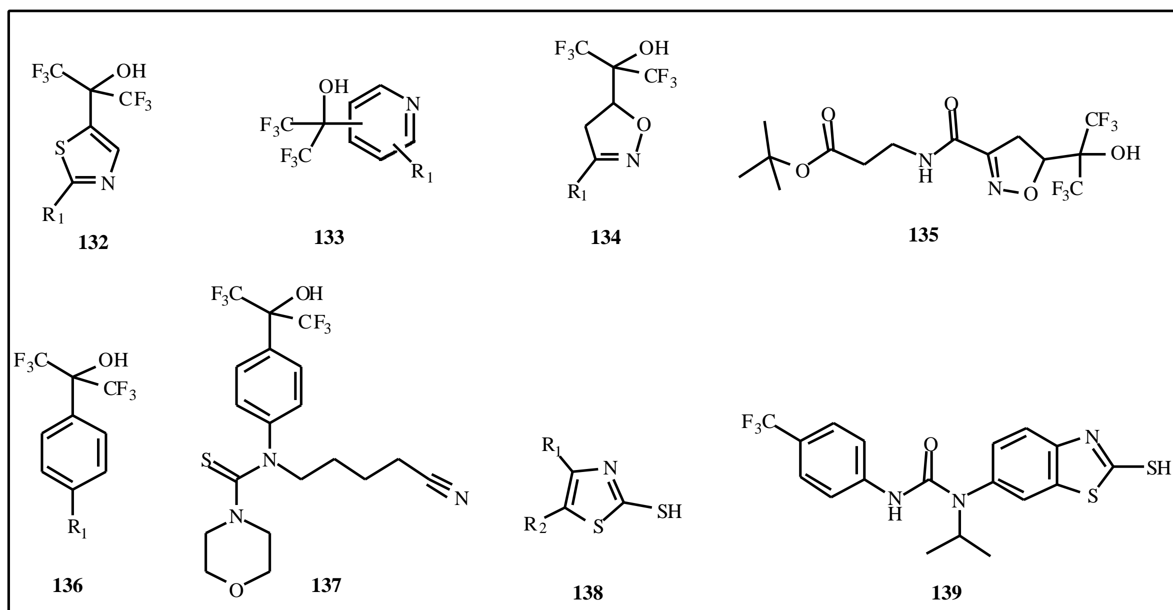
The claims of the second patent application cover sulfone derivatives containing the hexafluoroisopropanol group [117], covered by generic structure (**141**) where R_1 is generally a substituted or un-substituted phenyl, R_2 is H, alkyl or substituted benzoyl and in one example R_2 and R_3 are both methyl. As with the earlier application [135] exemplified compounds are claimed to have EC_{50} <50μM, as determined by an LXR reporter gene transactvation assay.

Moving away from the hexafluoroisopropanol group Pharmacia Corporation recently filed a patent application claiming aromatic thioether derivatives and their use in the treatment and/or prevention of LXR-modulated conditions including atherosclerosis, dyslipidemia and diabetes [118]. Again exemplified compounds covered by generic structure (**142**) are claimed to have an EC_{50} value of <50μM in an LXR reporter gene transactivation assay. The general substitution has R_1 as 2-Cl-4,5-OCH_2O, 2-OMe-5-NO_2, 2-OMe, 3-NO_2-4-OMe, 2,5-diOMe, 3-CF_3, -3,4-OCH_2O-, -2,3-OCH_2OCH_2-, 2-pyridyl, 2-OMe-5-(substituted)benzyl amino, 2-OMe-5-acylamino, R_2 as tert-butyl, thiophene, alkyl, haloalkyl, aryl, haloaryl, cycloalkyl, substituted alkyl and R_3 as H, Me, Cl and F.

2.9. PheneX Pharmaceuticals AG

PheneX have filed a patent application claiming compounds having the general formula (**143**) which bind to LXR or acting as 'gene selective agonists' [119]. The utility of these compounds in the treatment of LXR mediated diseases is also claimed, in particular the treatment of hypercholesterimia, obesity or other diseases associated with elevated LDL levels.

Embodiments of the invention are described by structures (**144** & **145**). Within generic structure (**145**), R_1 and R_3 are H, R_2 and R_4 are H, iodo, bromo, chloro, fluoro, hydroxy-methyl, trifluoromethyl, methoxy, amino, alkylated amino or nitro; R_5 is H, alkyl, preferably isopropyl, vinyl, 2-isobutyl, substituted alkyl, cycloalkyl and substituted cycloalkyl, aryl and substituted aryl, alkylphenyl or substituted phenylalkyl;



R_8 is alkyl or substituted alkyl, substituted alkyl, cycloalkyl or substituted cycloalkyl, alkylphenyl or substituted phenylalkyl, preferably benzyl or (furan-2-yl)-methyl or (thiophen-2-yl)-methyl and R_9 is alkyl or substituted alkyl, cycloalkyl or substituted cycloalkyl, alkylphenyl or substituted phenylalkyl, preferably (ethyl)-propion-1-yl.

Compounds of this invention were screened using a mammalian one hybrid (M1H) assay, allowing the measurement of dose dependant activation of a luciferase reporter gene mediated through GAL4-LXR -LBD or GAL4-LXR -LBD constructs in HEK293 cells. Six preferred compounds (**146**-**151**) are claimed due to their ability to act as either full or partial agonists of LXR with EC_{50} values ranging from 0.12μM to 5μM for compounds (**146-148** & **150,151**).

It is also stated in example 3 of the patent that LN7033 (**151**) activates LXR LBD mediated luciferase activity to a greater extent than with LXR , although no comparative data is given and as such the veracity of this claim is hard to establish.

Comparative data on the ability of LN7033 (**151**) and T0901317 (**4**) to increase the abundance of mRNA of LXR target genes (ABCA1 & ABCG1 in THP-1 cells; FAS and SREBP-1c in HepG2 cells) is described in the application. This data indicates robust and similar levels of mRNA upregulation for LXR , ABCA1 and ABCG1 in THP-1 cells for both LN7033 (**151**) and T0901317 (**4**) when tested at 10μM. In contrast LN7033 (**151**) (10μM) does not appear to upregulate FAS and SREBP-1c in HepG2 cells whereas treatment with T0901317 (**4**) (10μM) leads to a robust upregulation of these LXR target genes in line with the results seen in THP-1 cells. Further evidence is provided for a gene selective effect through demonstration of increased cholesterol efflux from differentiated THP-1 cells coupled to no effect on triglyceride mass in HepG2 cells upon treatment with LN7033 (**151**) (10μM) as compared to treatment with T0901317 (**4**) (10μM).

2.10. Eli Lilly and Company

Eli Lilly and Company has filed a patent application claiming tricyclic compounds and their use in the modulation of LXR activity for the treatment and/or prevention of LXR-mediated diseases including dyslipidemia and/or atherosclerosis [120].

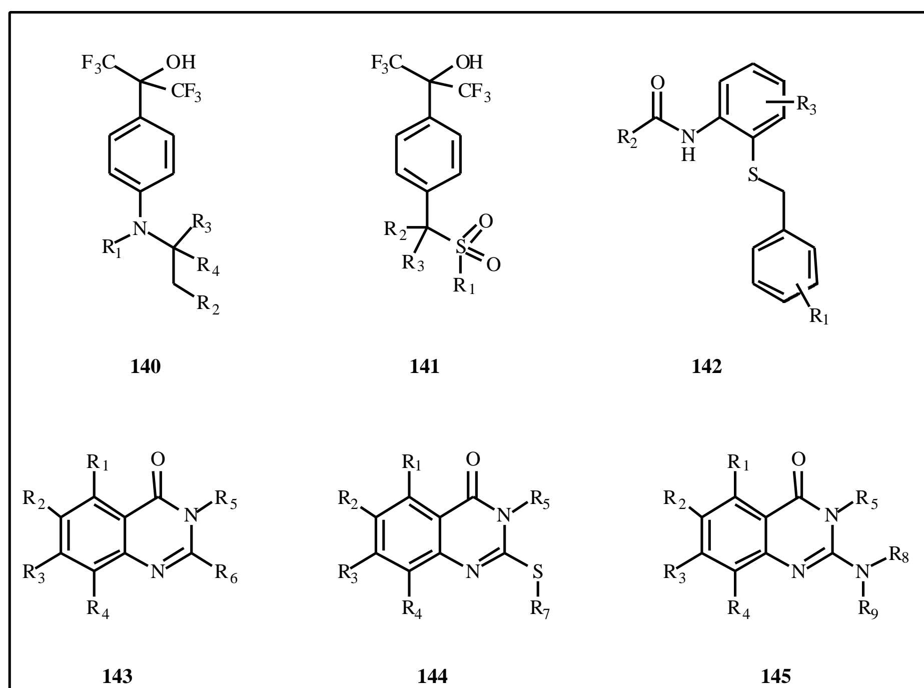
Four synthetic compounds are exemplified with substitution as shown (**152**-**155**). Compound (**152**) caused an induction of ABCA1 mRNA expression with an EC_{50} of 3.9μM.

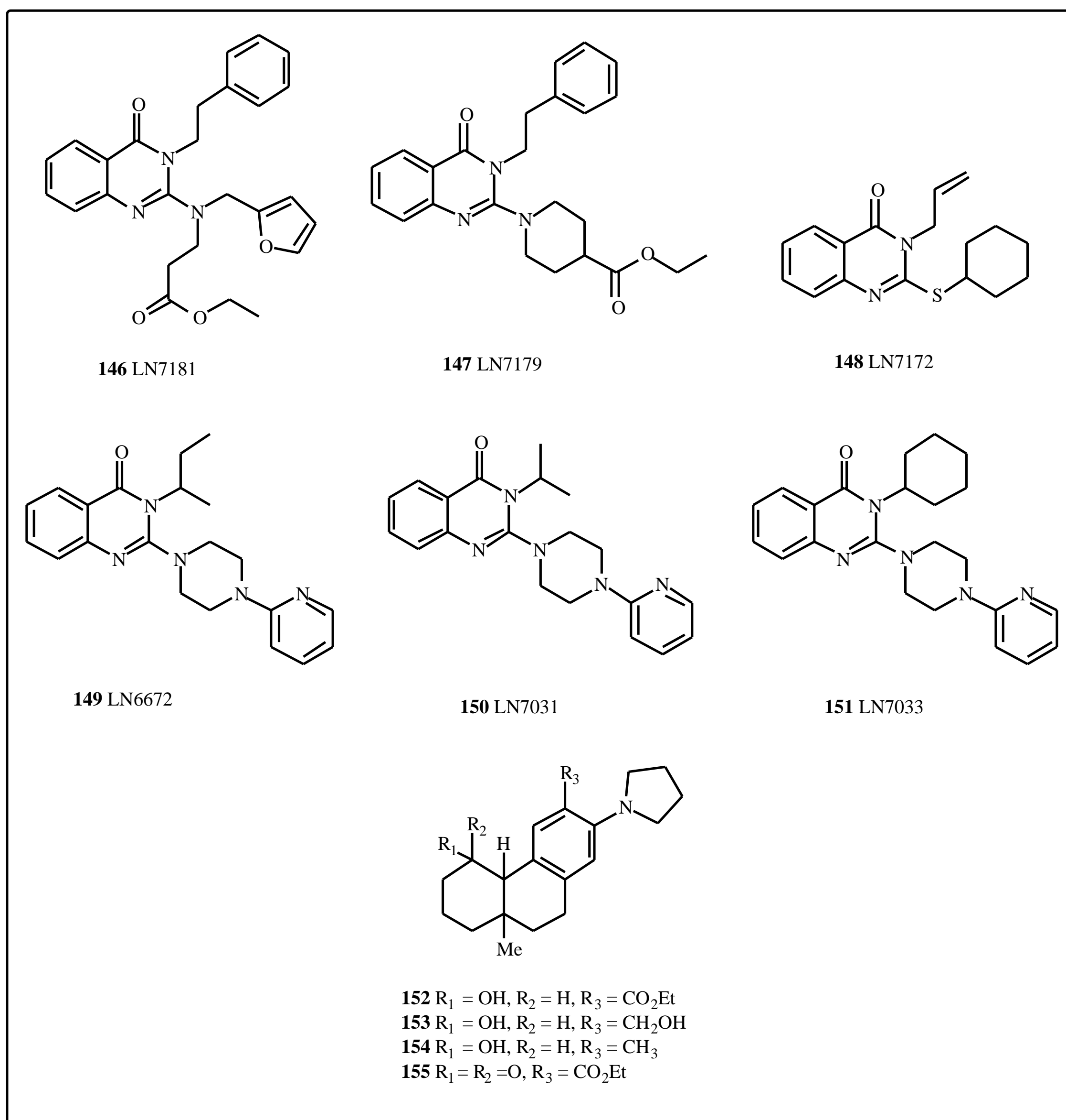
2.11. Amgen

Amgen have filed a patent application [121] claiming a series of compounds bearing a strong resemblance to the later Tularik patents, covered by general structures (**156** & **157**).

Substitution at R_{11} is broad but a review of the embodiments exemplified by synthesis show that substituted phenyl, pyrazolyl and thienyl are most favoured. R_2 is preferably alkyl, fluoroalkyl e.g. $CH_2CH_2CF_3$ (**158**) or cycloalkyl e.g. cyclopropylmethyl (**159**) substitution. R_3 is preferred as phenyl or substituted phenyl. Generally X is OH although occasionally this is masked as the methyl ether. In 85 out of 86 synthetic examples Y is CF_3, with one example of Y being CH_2CF_3. No specific biological data is given and only a brief mention is made of the ability of these compounds to modulate LXR function and/or upregulate LXR expression.

Function of the compounds in the invention can be demonstrated in a binding assay or a cell-based assay e.g. a





transient transfection assay. A broad claim that compounds disclosed in the application can lower cholesterol levels in hamsters fed a high cholesterol diet is made although no data is presented to lend credence to this claim.

2.12. Galderma Research and Development

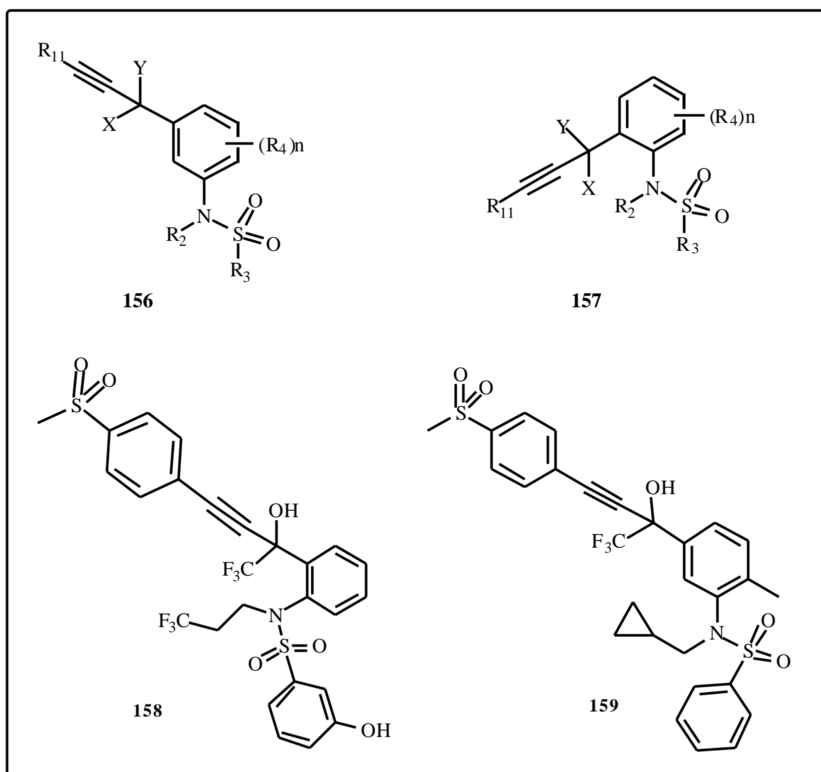
Galderma R&D recently filed a patent application [122] claiming 4-substituted piperidine amide compounds of general structure (**160**) and their use in human and veterinary medicine.

There are a 89 specifically claimed compounds with a number of these being particularly preferred (**160**). Preferably Ar is a phenyl group, X and Y are oxygen atoms, R_2 is a methyl or propyl group and R_1 contains the broadest substitution pattern. Only two compounds are exemplified by synthesis (**161** & **162**) and three further compounds are exemplified by reference to their LXR activity (**163**-**165**). A transactivation assay is outlined employing a luciferase reporter gene format to record the activity of these three compounds with T0901317 (**4**) being used as a reference.

Compounds of the invention are primarily claimed for the treatment of skin disorders, however complaints of the cardiovascular system such as arteriosclerosis and lipid metabolism complaints are specifically claimed.

3. CURRENT AND FUTURE DEVELOPMENTS

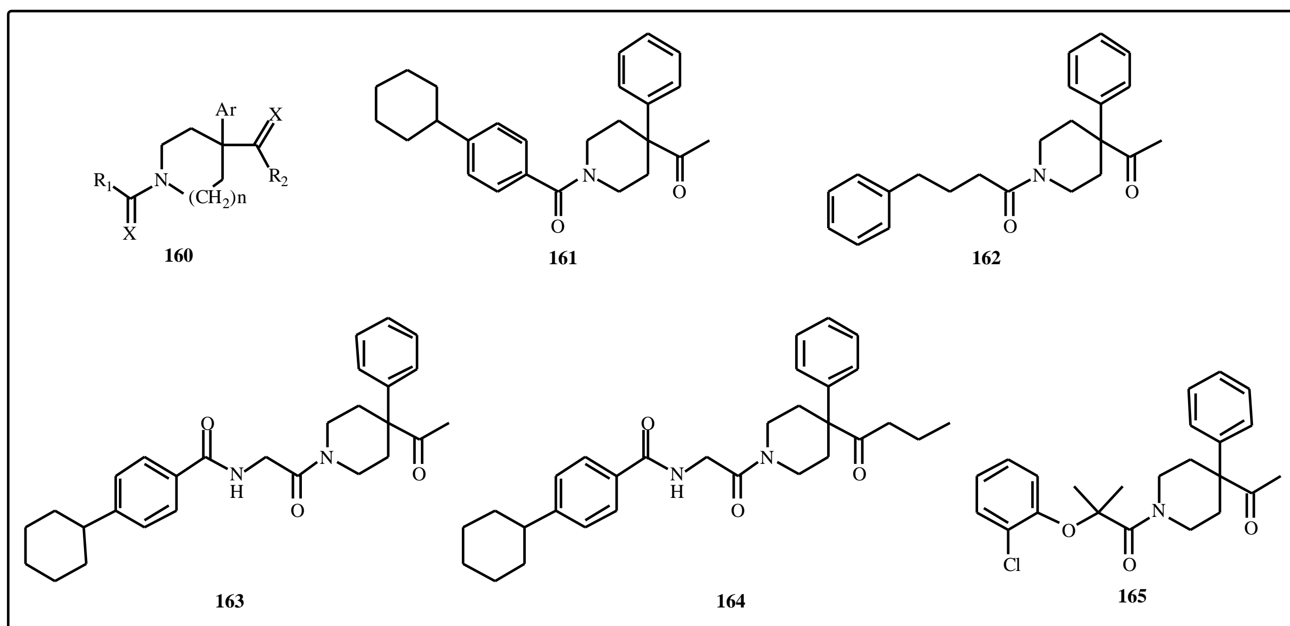
The knowledge of LXR pharmacology has increased rapidly over the last few years. In particular the ability of LXR agonists to promote RCT from macrophages and to attenuate atherosclerosis development in murine models has stimulated interest in the development of new ligands as potential agents for the treatment of atherosclerosis. This increase in interest from the pharmaceutical industry is reflected in the number of different chemotypes being claimed in patent applications as LXR modulators. Design of new ligands has been facilitated by the availability of X-ray



crystallography data which has highlighted key interactions between the ligand and the LXR ligand binding domain which are crucial for agonist activity [123-126]. These studies have also highlighted the large size and predominantly hydrophobic nature of the ligand binding cavity, which at least in part explains the 'relatively' high molecular weight and lipophilicity of the ligands disclosed to date. However, the availability of crystal structure data for LXR and related NRs, e.g. PXR, should aid the design of selective LXR / agonists with improved physicochemical properties.

LXR agonists from various chemotypes have been shown to modulate the expression of a number of genes involved in cholesterol transport, lipid metabolism and inflammation, many of which are inter-related. Due to this complexity it is difficult to extrapolate the pro- or anti-atherogenic effects of each target gene in isolation to a conclusion as to the outcome when all target genes are being modulated in concert. The most profiled LXR agonists, T0901317 (**4**) and GW3965 (**12**), activate both and isoforms and have been shown to upregulate LXR target genes e.g. ABCA1 and SREBP-1 *in vitro* and in rodents after oral administration. Both T0901317 (**4**) and GW3965 (**12**) are currently in pre-clinical development and at present the full selectivity, pharmacokinetic, pharmacodynamic and toxicological profiles of these compounds have yet to be reported. Whether non-selective LXR / agonism will result in an overall positive or negative effect on atherosclerosis will only be elucidated when further *in vivo* testing is reported in species with a higher predictive capacity for humans e.g. with respect to lipoprotein profiles and cholesterol metabolism, and ultimately from clinical evaluation in patients.

Future chemical focus will be influenced by pharmacological or clinical data suggesting that isoform, gene or tissue selective LXR modulators are required. For instance, one can hypothesise that selective LXR agonists may provide a fruitful strategy for maximising RCT whilst having a lesser effect on hepatic lipogenesis. This hypothesis is supported by the observations that LXR expression is higher in the liver than LXR suggesting that LXR activity is predominant in modulation of lipogenesis. From the available biostructural information it would appear that the design of isoform selective agonists will prove challenging due to the high level of similarity in the residues surrounding co-crystallised ligands in LXR and [123-126]. Analogous to the tissue selective estrogen receptor modulators (SERMs), a macrophage or ABCA1 selective LXR agonist would also be expected to provide an improved pharmacodynamic profile [127]. The ability for LXR to modulate gene expression is dependant on the availability of its heterodimerisation partner, RXR, as well as specific co-activators and/or co-repressors. Over the coming years, it is likely that we will see an advance in the understanding of which co-activators and co-repressors are involved in target gene modulation in different tissues relevant to LXR function. Armed with this knowledge it may then be possible to exploit these specific interactions to aid in the design and profiling of LXR modulators which possess the desired pharmacodynamic effects on RCT whilst having little or no effects on lipogenesis.



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