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Nelumal A, the active principle from *Ligularia nelumbifolia*, is a novel farnesoid X receptor agonist

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ABSTRACT

A series of 29 oxyprenylated and azoprenylated phenylpropanoids were chemically synthesized and tested in transfected cultured HepG2 cells by means of the dual-luciferase assay as farnesoid X receptor (FXR) agonists, using the endogenous ligand chenodeoxycholic acid (CDCA) as reference drug. Among the tested molecules, three compounds, namely auraptene, nelumol A, and nelumal A showed a potency comparable to the endogenous ligand, with the latter natural product having a level of activity slightly superior to CDCA. Nelumal A is thus of interest as a valuable potential novel lead compound in the search for FXR agonists.

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The nuclear receptor family of transcription factors is intimately involved in the regulation of a wide variety of metabolic processes, including synthesis of endogenous compounds as well as metabolism of both endogenous and exogenous substances. In humans, 49 members of this family have been isolated and identified.[1] Nuclear receptors are modular in nature, being made up of a set of distinct domain types, though the structure of these domains varies extensively across the family. The N-terminus comprises a variable domain containing the AF-1 motif, which is highly conserved between nuclear receptor family members and is implicated in ligand independent transactivation. This N-terminal domain is followed by the DNA binding domain (DBD), consisting of two Zn fingers specific for recognizing the specific DNA response elements in the promoter regions of target genes. Connected to the DBD via a highly variable hinge region is the ligand binding domain (LBD), which contains the active ligand binding pocket, shaped to bind to the distinct class of ligands recognized by the nuclear receptor. At the C-terminal end of the ligand binding domain is the AF-2 motif, which is thought to be involved in ligand dependent transactivation.[1] The C-terminal end of the protein is also involved in protein–protein binding, for a number of nuclear receptors forms homo- or heterodimers upon activation by ligands.[2]

The farnesoid X receptor (FXR; NR1I3) is a member of the Class II subset of nuclear receptors, and as such forms a heterodimer with retinoid X receptor (RXR) upon ligand activation.[3] Upon activation, FXR targets response elements in the promoter regions of target genes; these are involved in various pathways, primarily bile acid homeostasis, lipoprotein metabolism, and glucose metabolism.[4] Though initially isolated as a receptor capable of being transactivated by farnesol, the most potent agonists of FXR are bile acids and their metabolic derivatives,[5] indicating its role as a major step in the moderation of bile acid homeostasis. Due to its involvement in specific metabolic pathways, FXR is predominantly expressed in the liver, intestine, kidneys, and adrenal glands, though is poorly expressed in tissues such as brain, heart, and lung.[6]

The ligand profile of FXR has been shown in recent years to extend beyond bile acids to both endogenous compounds as well as exogenous compounds, both natural and synthetic. Wang et al. have shown that FXR could be transactivated by androsterone, an androgenic metabolite of testosterone, suggesting that the metabolic pathways induced by FXR may be linked to sex steroid levels.[7] As well, it has been shown that the cholesterol derivative oxysterol 22-(*R*)-hydroxycholesterol induced FXR transactivation.[8] Several natural products have been found to be agonists of FXR, including bile acid derivatives, marchatins, and ginkgolic acids,[9] as well as synthetic compounds, such as the potent activator GW4064 and its derivatives.[10,11] These compounds were tested to determine their potential as analytical tools or therapeutic agents. GW4064 and its derivatives have been powerful analytical tools, though their therapeutic efficacy has been limited due to bioavailability issues with the crystalline forms, an issue being addressed using self-emulsifying drug delivery systems.[12]

Dysfunction in the activity of FXR leads to several disease states and for this reason FXR represents an important pharmacological target. The loss of FXR in mice ($FXR^{-/-}$ mice) has been shown to

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result in type 2 diabetes, hypertriglyceridemia, cholestasis, and cholesterol gallstone disease.[13] These effects are due to the disruption in the homeostasis of bile acids, triglycerides, and glucose that is modulated by the transactivation of FXR. As well, $FXR^{-/-}$ mice were found to spontaneously develop hepatic cancers, likely due to the disruption of homeostatic balances within the liver, specifically in bile acid homeostasis, as well as dysfunction in hepatic regeneration, which FXR has been shown to be intimately involved in.[4] As such, FXR is an important therapeutic target for dealing with a number of hepatic metabolic diseases, as well as hepatic cancers, and therefore isolation of compounds that would act as potent agonists for FXR could be an important step in dealing with these diseases.

Oxyprenylated natural products, such as isopentenyloxy-(C_5), geranyloxy-(C_{10}), and farnesyloxy-(C_{15}) related compounds, represent a family of secondary metabolites that were considered for years to be merely biosynthetic intermediates of the more widespread C-prenylated derivatives. These secondary metabolites have been recognized in the last two decades as interesting and valuable biologically active phytochemicals. Approximately 300 compounds have been isolated and structurally characterized from plants, primarily from the families Rutaceae, Compositae, Guttiferae, and Leguminosae, comprising several edible vegetables and fruits. The phytochemistry and pharmacology of prenyloxyphenylpropanoids was recently reviewed.[13]

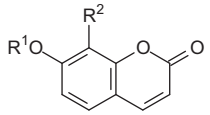
As a continuation of our ongoing studies aimed to better characterize the phytochemical and pharmacological properties of natural and semisynthetic oxyprenylated and azoprenylated phenylpropanoids, in this work we synthesized and investigated the effect of 29 selected compounds belonging to this group on FXR using a whole cell reporter assay system.

The chemical structures of the compounds that we studied are illustrated in Figure 1.

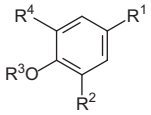
The main natural sources of 7-isopentenyloxycoumarin (**1**), auraptene (**2**), 8-hydroxy-7-isopentenyloxy-coumarin (**3**), lacinartin (**4**), collinin (**5**), boropinic acid (**6**), 4′-geranyloxyferulic acid (**7**), geranyloxy-*p*-coumaric acid (**9**), valencic acid (**10**), boropinal (**14**), (2*E*)-3-(4-((*E*)-3,7-dimethylocta-2,6-dienyloxy)-3-methoxyphenyl)acrylaldehyde (**15**), nelumal A (**16**), (2*E*)-3-(4-((*E*)-3,7-dimethylocta-2,6-dienyloxy)-3-methoxyphenyl)prop-2-en-1ol (**22**), boropinol C (**23**), nelumol A (**24**), 4-hydroxycordoin (**25**), 4′-geranyloxyisoliquiritigenin (**26**), and *N*-isopentenylanthranilic acid (**27**) have been described previously.[13–15]

Geranyloxy-*p*-benzoic acid (**11**) has been previously extracted from enzymatic extracts of *Piper crassinervium* Kunth (Piperaceae),[16] *p*-isopentenyloxybenzaldehyde (**12**) has been isolated from the leaf oil of *Clausena anisata* Hook f. (Rutaceae),[17] geranyloxyvanillin (**13**) has been obtained from the apolar extracts of *Crithmum maritimum* L. (Apiaceae),[18] 3,5-dimethoxy-4-isopentenyloxybenzyl alcohol (**17**) has been extracted in form of angelate ester from the roots of *Erechtites hieracifolia* (L.) Raf ex DC.(Asteraceae),[19] etrogol (**18**) and its acetate (**19**) has been isolated from *Citrus* spp. (Rutaceae),[20] 3-(4-geranyloxyphenyl)-1-ethanol (**20**) is a juvenile hormone of several insect species,[21] 3-(4-isopentenyloxyphenyl)-1-propanol (**21**) has been obtained from the roots of *Fagara zanthoxyloides* Lam.[22] and *Zanthoxylum wutaiense* Chen (Rutaceae),[23] *N*-acetyl-*O*-isopentenyl-L-tyrosine (**28**) has been extracted from the fungus *Pithomyces ellis*,[24] and finally lawsone 2-isopentenyl ether (**29**) has been isolated from the fungus *Streptocarpus dunnii*.[25]

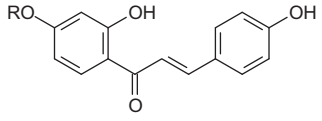
The synthesis of compounds (**1–10**), (**14–16**), and (**22–26**) was accomplished according to the procedures described previously.[14] Geranyloxy-*p*-benzoic acid (**11**), *p*-isopentenyloxybenzaldehyde (**12**), geranyloxyvanillin (**13**), etrogol (**18**), 3-(4-geranyloxyphenyl)-1-ethanol (**20**) 3-(4-isopentenyloxyphenyl)-1-propanol (**21**), *N*-acetyl-*O*-isopentenyl-L-tyrosine (**28**), and lawsone 2-isopentenyl ether (**29**) were synthesized starting from the corresponding commercially available phenolic derivatives by alkylation with either geranyl or 3,3-dimethylallyl bromide using K_2CO_3 as the base in refluxing acetone (Scheme 1).



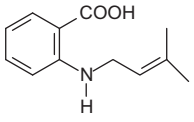
1 R^1= isopentenyl, R^2 = H
2 R^1= geranyl, R^2 = H
3 R^1= isopentenyl, R^2 = OH
4 R^1= isopentenyl, R^2 = OCH_3
5 R^1= geranyl, R^2 = OCH_3



6 R^1= *trans* CH=CH-COOH, R^2 = OCH_3, R^3 = isopentenyl, R^4 = H
7 R^1= *trans* CH=CH-COOH, R^2 = OCH_3, R^3 = geranyl, R^4 = H
8 R^1= *trans* CH=CH-COOH, R^2 = H, R^3 = isopentenyl, R^4 = H
9 R^1= *trans* CH=CH-COOH, R^2 = H, R^3 = geranyl, R^4 = H
10 R^1= COOH, R^2 = H, R^3 = isopentenyl, R^4 = H
11 R^1= COOH, R^2 = H, R^3 = geranyl, R^4 = H
12 R^1= CHO, R^2 = H, R^3 = isopentenyl, R^4 = H
13 R^1= CHO, R^2 = OCH_3, R^3 = geranyl, R^4 = H
14 R^1= *trans* CH=CHCHO, R^2 = OCH_3, R^3 = isopentenyl, R^4 = H
15 R^1= *trans* CH=CHCHO, R^2 = OCH_3, R^3 = geranyl, R^4 = H
16 R^1= *trans* CH=CHCHO, R^2 = OCH_3, R^3 = geranyl, R^4 = OCH_3
17 R^1= CH_2OH, R^2 = OCH_3, R^3 = isopentenyl, R^4 = OCH_3
18 R^1= CH_2CH_2OH, R^2 = H, R^3 = isopentenyl, R^4 = H
19 R^1= CH_2CH_2OAc, R^2 = H, R^3 = isopentenyl, R^4 = H
20 R^1= CH_2CH_2OH, R^2 = H, R^3 = geranyl, R^4 = H
21 R^1= $CH_2CH_2CH_2OH$, R^2 = H, R^3 = isopentenyl, R^4 = H
22 R^1= *trans* CH=$CHCH_2OH$, R^2 = OCH_3, R^3 = geranyl, R^4 = H
23 R^1= *trans* CH=$CHCH_2OH$, R^2 = OCH_3, R^3 = isopentenyl, R^4 = OCH_3
24 R^1= *trans* CH=$CHCH_2OH$, R^2 = OCH_3, R^3 = geranyl, R^4 = OCH_3



25 R = isopentenyl
26 R= geranyl



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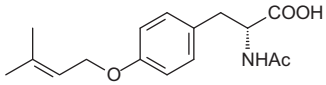

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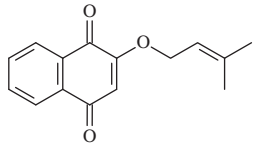

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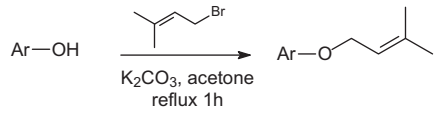
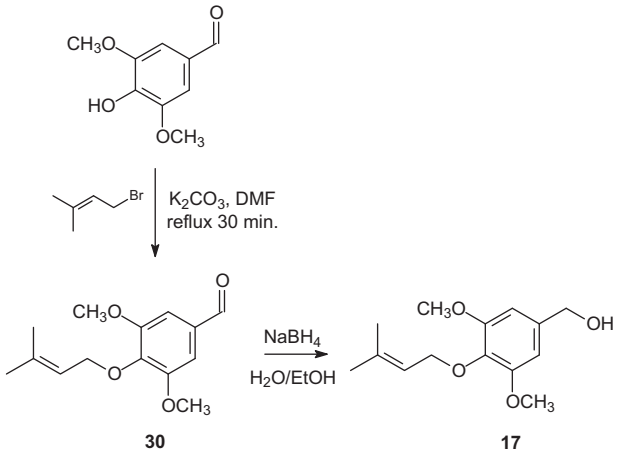
Figure 1. Illustration of the chemical structures studied. The 29 compounds that we studied belong to six chemical groups: coumarins (**1–5**), cinnamic and benzoic acids (**6–11**, **27**), benzaldehydes and cinnamaldehydes (**12–16**), cinnamyl alcohols (**17–24**), chalcones (**25** and **26**) amino acid derivatives (**28**), and quinones (**29**).



Scheme 1. Isopentenylation of aryl ethers.



Scheme 2. Synthesis of compounds **30** and **17**.

This simple but very efficient and 'clean' reaction provided the desired products after crystallization (*n*-hexane) with the following yields: 50% (compound **11**), 80% (compound **12**), 96% (compound **13**), 93% (compound **18**), 85% (compound **20**), 84% (compound **21**), 80% (compound **28**), and 69% (compound **29**).

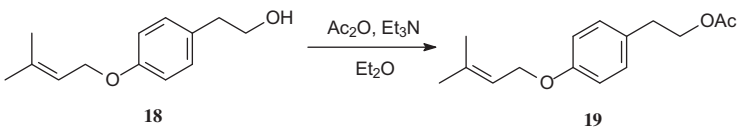
3,5-Dimethoxy-4-isopentenyloxybenzyl alcohol (**17**) was obtained by a two-step synthesis (Scheme 2) from commercially available syringaldehyde, that was first alkylated in position 4 employing similar reaction conditions as depicted above with the only modification of substituting acetone with DMF. The use of an aprotic polar solvent was needed due to the steric hindrance exerted by the two $-OCH_3$ groups in position 3 and 5 to the alkylation of the phenol group. Intermediate (**30**) was obtained in 91% yield and was then reduced to the benzyl alcohol derivative (**17**) in 99% yield with $NaBH_4$ using a $EtOH/H_2O$ mixture as the solvent at room temperature.

Etrogol acetate (**19**) was synthesized in 93% yield from etrogol (**18**) using Ac_2O and Et_3N as the base in Et_2O at room temperature (Scheme 3).

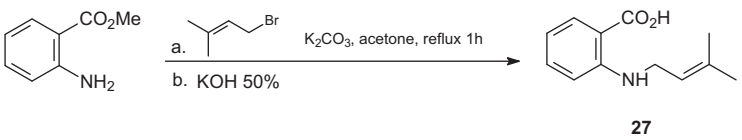
Finally *N*-isopentenylanthranilic acid (**27**) was obtained in 91% yield from commercially available methyl anthranilate that was first N-alkylated with 3,3-dimethylallyl bromide, using the same experimental procedure as above, and then submitted to alkaline hydrolysis with 50% KOH at 80 °C to provide the desired product (Scheme 4).[26]

All compounds were then assessed for their effect in a FXR reporter assay in transfected HepG2 cells. Chemicals were tested at 7 concentration levels, namely 0.1, 1.0, 5.0, 10, 25, 50, and 100 μM, DMSO was used as a negative control at 0.05% v/v, and CDCA was used as a positive control. Table 1 reports the level of activation of FXR by the synthesized compounds on FXR as measured by means of the dual-luciferase assay. Non-active samples are shaded light grey, while strong agonists are shaded dark grey. Weak and medium agonists are unshaded. Figure 2 illustrates the dose response curves for the strong agonists auraptene, nelumal A and nelumol A compared to CDCA.

From the results obtained and reported in Table 1 it is evident that only three compounds, namely auraptene (**2**), nelumal A (**16**), and nelumol A (**24**) can be regarded as FXR agonists exerting an appreciable activity. All three of these compounds were recently found to exert appreciable pharmacological activities: auraptene (**2**) is nowadays well recognized as an anti-cancer, anti-bacterial, anti-protozoal, anti-fungal, anti-inflammatory, and anti-oxidant agent[27] in in vitro, in whole cells, and/or in in vivo systems, and recently an effective remedy against metabolic disorders involving liver functionality[28,29]; nelumol A (**24**), and nelumal A (**16**) were shown to exert mild to good activity against several different human cancer cell lines.[30–33] The effective interaction disclosed by our study between auraptene and FXR, which is expressed strongly in liver and is involved in its functionality as stated in the Introduction, can shed light on the mechanism of action of this geranyloxycoumarin as a hepatoprotective agent.[28,29] On the other hand, it is well known that FXR is also a key component of cancer pathogenicity. The central role that FXR plays in bile acid homeostasis implicates it as a potentially important factor in preventing tumorigenesis in the liver. It has been found that hepatic tumors can be initiated through hepatic injury due to elevated bile acid levels, including through dietary supplementation.[15] A similar generation of hepatic tumors, due to disruption of bile acid homeostasis, has been seen in FXR knockout mice, indicating FXR plays a role as a metabolic suppressor of tumorigenesis.[34,35] FXR also appears to play a pivotal role as a tumorigenesis repressor in the intestinal tract; however it appears that its modulation of bile acid levels is not primary in this preventative role.[36] In the intestine, FXR is down-regulated in tumor progenitor cells, resulting in the disruption of several cellular pathways that are involved in mucosal barrier maintenance and induction of apoptosis in aberrant cells. As such, induction of FXR expression in these aberrant cells, and its increased transactivation, could result in prevention of intestinal tumorigenesis. In this context the observed interaction between auraptene and FXR could partly account for the reported properties



Scheme 3. Synthesis of etrogol acetate.



Scheme 4. Synthesis of compound **27**.

Table 1
Effect of the 29 compounds under study on the activity of FXR relative to DMSO control

Compound	Dose (μM)						
	0.1	1	5	10	25	50	100
1[#]	1.198 ± 0.145	1.044 ± 0.075	1.194 ± 0.072	1.368 ± 0.133	1.536 ± 0.128[*]	1.626 ± 0.130[*]	2.119 ± 0.139[*]
2[#]	0.895 ± 0.186	1.005 ± 0.105	1.017 ± 0.061	1.260 ± 0.165	2.084 ± 0.294	5.897 ± 0.780[*]	11.668 ± 1.424[*]
3[#]	0.758 ± 0.055	0.961 ± 0.060	1.158 ± 0.072	1.477 ± 0.074[*]	2.172 ± 0.121[*]	3.265 ± 0.237[*]	5.544 ± 0.277[*]
4[#]	0.893 ± 0.088	1.177 ± 0.128	1.621 ± 0.089	2.485 ± 0.137[*]	3.485 ± 0.237[*]	5.878 ± 0.465[*]	9.431 ± 0.368[*]
5[#]	0.860 ± 0.088	1.141 ± 0.161	1.591 ± 0.256	2.134 ± 0.247[*]	3.051 ± 0.286[*]	4.322 ± 0.420[*]	6.491 ± 0.392[*]
6[#]	0.725 ± 0.065	0.796 ± 0.103	1.095 ± 0.123	1.253 ± 0.152	1.222 ± 0.108	1.661 ± 0.112[*]	2.203 ± 0.218[*]
7[#]	1.111 ± 0.084	1.152 ± 0.072	1.320 ± 0.109	1.490 ± 0.111[*]	1.877 ± 0.168[*]	2.180 ± 0.114[*]	4.541 ± 0.243[*]
8[#]	0.723 ± 0.072	0.854 ± 0.069	0.936 ± 0.100	1.114 ± 0.114	1.312 ± 0.147	1.632 ± 0.128[*]	2.366 ± 0.166[*]
9[#]	0.894 ± 0.059	0.969 ± 0.062	0.990 ± 0.047	1.177 ± 0.080	1.408 ± 0.048[*]	1.794 ± 0.105[*]	2.673 ± 0.192[*]
10[#]	1.107 ± 0.037	1.063 ± 0.074	1.196 ± 0.098	1.677 ± 0.231[*]	2.315 ± 0.220[*]	3.023 ± 0.207[*]	4.180 ± 0.293[*]
11[#]	0.902 ± 0.035	0.899 ± 0.053	0.993 ± 0.095	1.054 ± 0.065	1.348 ± 0.039[*]	1.854 ± 0.026[*]	2.820 ± 0.111[*]
12[#]	1.015 ± 0.111	1.113 ± 0.128	1.185 ± 0.088	1.162 ± 0.087	1.285 ± 0.091[*]	1.718 ± 0.117[*]	2.654 ± 0.132[*]
13[#]	0.914 ± 0.091	1.105 ± 0.032	1.430 ± 0.066[*]	1.707 ± 0.062[*]	2.335 ± 0.115[*]	3.715 ± 0.144[*]	5.742 ± 0.126[*]
14	0.932 ± 0.049	0.923 ± 0.066	0.965 ± 0.101	0.922 ± 0.077	0.884 ± 0.064	0.985 ± 0.090	1.079 ± 0.103
15	0.929 ± 0.098	1.020 ± 0.136	1.170 ± 0.075	1.033 ± 0.087	1.033 ± 0.120	1.130 ± 0.124	1.003 ± 0.071
16[#]	0.833 ± 0.073	0.966 ± 0.059	1.347 ± 0.077	1.885 ± 0.152	3.519 ± 0.193[*]	6.949 ± 0.482[*]	15.322 ± 0.539[*]
17	0.875 ± 0.173	0.970 ± 0.144	1.014 ± 0.125	1.178 ± 0.170	1.241 ± 0.242	1.122 ± 0.154	1.176 ± 0.198
18[#]	1.178 ± 0.132	1.182 ± 0.081	1.220 ± 0.087	1.353 ± 0.051	1.664 ± 0.052[*]	2.970 ± 0.136[*]	3.804 ± 0.087[*]
19	0.857 ± 0.061	0.899 ± 0.079	0.786 ± 0.064	1.022 ± 0.152	1.079 ± 0.167	1.021 ± 0.078	1.304 ± 0.074
20[#]	0.865 ± 0.049	1.315 ± 0.088	1.428 ± 0.034	1.735 ± 0.074[*]	2.181 ± 0.091[*]	4.432 ± 0.361[*]	7.619 ± 0.209[*]
21[#]	1.091 ± 0.183	0.690 ± 0.140	0.798 ± 0.022	0.939 ± 0.124	1.293 ± 0.232	1.398 ± 0.197	1.531 ± 0.140[*]
22[#]	0.932 ± 0.095	1.054 ± 0.065	1.272 ± 0.188	1.272 ± 0.052	2.071 ± 0.101[*]	3.019 ± 0.112[*]	4.488 ± 0.255[*]
23	0.731 ± 0.071	0.972 ± 0.114	1.123 ± 0.119	1.117 ± 0.138	1.282 ± 0.194	1.202 ± 0.170	1.279 ± 0.183
24[#]	1.050 ± 0.088	1.044 ± 0.057	1.223 ± 0.111	1.577 ± 0.070[*]	1.860 ± 0.049[*]	4.072 ± 0.240[*]	10.855 ± 0.253[*]
25[#]	0.712 ± 0.044	0.757 ± 0.071	0.822 ± 0.115	0.877 ± 0.074	1.269 ± 0.119	1.929 ± 0.143[*]	4.827 ± 0.181[*]
26[#]	0.911 ± 0.056	0.896 ± 0.080	1.128 ± 0.177	1.200 ± 0.121	1.168 ± 0.107	1.389 ± 0.078	2.669 ± 0.368[*]
27[#]	0.821 ± 0.081	0.885 ± 0.094	0.816 ± 0.096	1.248 ± 0.206	1.635 ± 0.289[*]	2.225 ± 0.214[*]	3.410 ± 0.285[*]
28	1.002 ± 0.142	1.194 ± 0.189	1.237 ± 0.158	1.190 ± 0.166	1.125 ± 0.228	1.248 ± 0.125	1.123 ± 0.120
29[#]	0.896 ± 0.090	1.198 ± 0.041	1.764 ± 0.130[*]	2.011 ± 0.125[*]	2.660 ± 0.138[*]	3.986 ± 0.203[*]	7.128 ± 0.260[*]
DMSO	1.000 ± 0.099	—	—	—	—	—	—
CDCA	0.744 ± 0.067	1.038 ± 0.073	1.393 ± 0.075	2.210 ± 0.326[*]	3.662 ± 0.163[*]	6.920 ± 0.456[*]	13.375 ± 0.258[*]

Activity levels are expressed as mean fold changes relative to the control values obtained from the DMSO treated cells with standard errors. Experiments were conducted in duplicate, repeated three times.
[*]Significant fold change (p <0.05) compared to DMSO controls.
[#]Compounds with a dose dependent effect (p <0.001), as determined by linear regression.

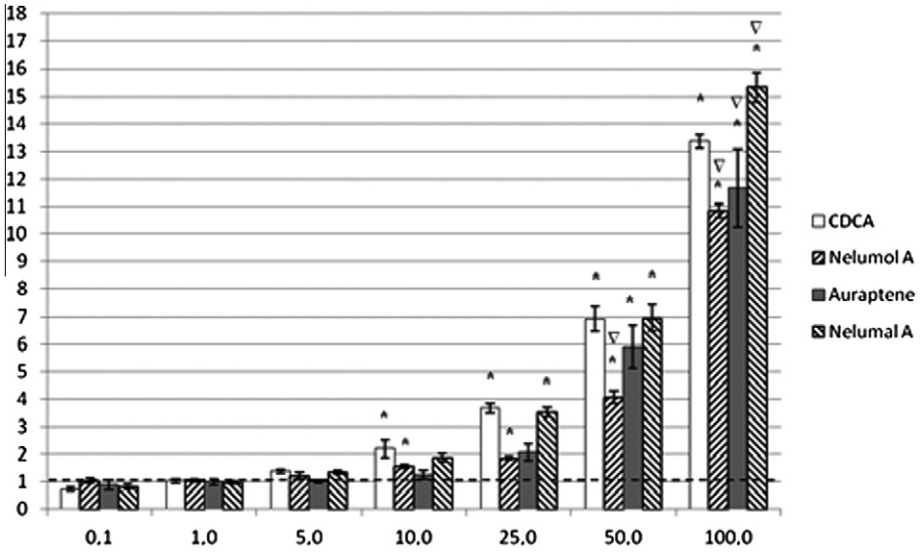


Figure 2. Response of FXR to CDCA and strong agonists in fold response of the DMSO control (dashed line). Significant fold change (p <0.05) compared to the control is denoted by (∗), while significant fold changes (p <0.05) to CDCA treatment at the same concentration are denoted by (). Data from duplicates repeated three times (mean ± SEM).

of this geranyloxycoumarin as a dietary chemoprotective agent against colon adenoma and adenocarcinoma.[37,38] Although some reported naturally occurring FXR ligands, like gingkolic acid and its derivatives, seem to act as membrane disrupters thus leading to the observed cytotoxicity,[39] this is not the case of compounds tested herein. Although some have shown cytotoxic effects on cancer cell lines, like recently reported, including auraptene (**2**)[40] and nelumal A (**16**)[15] the integrity of the cell structure after in vitro incubation with a prenyloxy molecule was assessed by quantitative videomicroscopy.

In terms of structure–activity relationships, the geranyl substituted compounds were found to be in general more active than those having an isopentenyloxy side chain. The higher activity recorded for nelumal A (**16**) compared to the respective cinnamyl alcohol nelumol A (**24**) led us to hypothesize that the presence of an α,β-unsaturated conjugated aldehyde as the C3 portion of the phenylpropanoid core of these natural product, is a crucial structural requirement for the biological activity, whereas its reduction to an allylic alcohol or its oxidation to carboxylic acid tended to decrease or abolish the effect. Nevertheless, another important structural feature, namely the 3,5-dimethoxy substituted aromatic ring, could be revealed from results reported in Table 1. In fact, comparing the activity of nelumal A (**16**) and its analogue (2*E*)-3-(4-((*E*)-3,7-dimethylocta-2,6-dienyloxy)-3-methoxyphenyl)acrylaldehyde (**15**), it was found that the first was about 15-fold more potent than the latter as a FXR agonist, highlighting the importance of substitution of the hydrogen in position 5 of the aromatic ring with a methoxy group. Also the presence of a coumarin nucleus, that remains chemically stable once inside the cell[41] and so cannot be related to cinnamic acid derivatives (**6**)–(**9**) in terms of SAR considerations, seemed to provide a certain level of effective interaction as a FXR agonist, auraptene (**2**) being the most potent compound compared to the other three coumarins, lacinartin (**3**), 8-hydroxy-7-isopentenyloxycoumarin (**4**), and collinin (**5**). In this case, however, only the *O*-geranyl side chain, together with the α-benzopyrone ring, seemed to be a crucial structural requirement for the observed activity. Taken together, these considerations address to the fact that the pattern of substitution in the aromatic ring is crucial to structurally define a novel lead compound as FXR agonist. Moreover the configuration of the conjugated double bond seems to have less importance to this aim, being *trans* (like in nelumal A (**16**)) or *cis*, like in auraptene (**2**), substituted compound active to a comparable extent. A more pivotal role on the contrary seems to be played by the terminal moiety of the C3 skeleton in the phenylpropanoid core of these natural products, for which polar groups, like carboxylic acids or alcohols as found in compounds (**6**)–(**9**) and (**22**)–(**24**) tended to decrease the activity as FXR agonists, while moieties featured by low to medium polarity like a lactone ring or an aldehyde, as found in nelumal A (**16**) and auraptene (**2**), seemed to enhance this kind of effect.

In this manuscript we described for the first time the interaction of some natural prenyloxyphenylpropanoids with FXR. We discovered that three of these compounds could be claimed to good to very good agonists of this class of receptor. Nelumal A was seen to be the most potent one and on this basis it could be useful to identify a novel class of FXR ligand using (**16**) as the lead compound. The naturally widespread geranyloxycoumarin auraptene (**2**) also displayed an appreciable amount of activity. As reported previously,[28] this compound is also found in several edible fruits and vegetables. This consideration, together with the statement that FXR plays a pivotal role in liver homeostasis and hepatic syndromes, could be useful in exploring the effect of dietary feeding with vegetable-containing auraptene, like agrumes, on the chemoprevention of liver diseases. In the same way, we recently provided evidence for the chemoprevention of colon cancer by the same natural product.[37,38,40]

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.03.057.

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26. Analytical data of all adducts obtained by chemical synthesis described herein were in full agreement with those already reported in the literature for the same compounds (see Ref. 15).
*7-Isopentenyloxycoumarin (**1**):* Anal. Calcd for $C_{14}H_{14}O_3$: C, 73.03; H, 6.13; O, 20.84. Found: C, 73.01; H, 6.16; O, 20.81.
*Auraptene (**2**):* Anal. Calcd for $C_{19}H_{22}O_3$: C, 76.48; H, 7.43; O, 16.09. Found: C, 76.52; H, 7.46; O, 16.05.
*8-Hydroxy-7-isopentenyloxy-coumarin (**3**):* Anal. Calcd for $C_{14}H_{14}O_4$: C, 68.28; H, 5.73; O, 25.99. Found: C, 68.24; H, 5.76; O, 25.96.
*Lacinartin (**4**):* Anal. Calcd for $C_{15}H_{16}O_4$: C, 69.22; H, 6.20; O, 24.59. Found: C, 69.19; H, 6.16; O, 24.55.
*Collinin (**5**):* Anal. Calcd for $C_{20}H_{24}O_4$: C, 73.15; H, 7.37; O, 19.49. Found: C, 73.12; H, 7.34; O, 19.47.
*Boropinic acid (**6**):* Anal. Calcd for $C_{15}H_{18}O_4$: C, 68.69; H, 6.92; O, 24.40. Found: C, 68.72; H, 6.94; O, 24.36.
*4′-Geranyloxyferulic acid (**7**):* Anal. Calcd for $C_{20}H_{26}O_4$: C, 72.70; H, 7.93; O, 19.37. Found: C, 72.69; H, 7.94; O, 19.34.
*Isopentenyloxy-p-coumaric acid (**8**):* Anal. Calcd for $C_{14}H_{16}O_3$: C, 72.39; H, 6.94; O, 20.66. Found: C, 72.36; H, 6.99; O, 20.64.
*Geranyloxy-p-coumaric acid (**9**):* Anal. Calcd for $C_{19}H_{24}O_3$: C, 75.97; H, 8.05; O, 15.98. Found: C, 75.93; H, 8.01; O, 15.96.
*Valencic acid (**10**):* Anal. Calcd for $C_{12}H_{14}O_3$: C, 69.89; H, 6.84; O, 23.27. Found: C, 69.92; H, 6.80; O, 23.24.
*Geranyloxy-p-benzoic acid (**11**):* Anal. Calcd for $C_{17}H_{22}O_3$: C, 74.42; H, 8.08; O, 17.49. Found: C, 74.38; H, 8.03; O, 17.45.
*p-Isopentenyloxybenzaldehyde (**12**):* Anal. Calcd for $C_{12}H_{14}O_2$: C, 75.76; H, 7.42; O, 16.82. Found: C, 75.78; H, 7.38; O, 16.82.
*Geranyloxyvanillin (**13**):* Anal. Calcd for $C_{18}H_{24}O_3$: C, 74.97; H, 8.39; O, 16.64. Found: C, 74.99; H, 8.33; O, 16.65.
*Boropinal (**14**):* Anal. Calcd for $C_{15}H_{18}O_3$: C, 73.15; H, 7.37; O, 19.49. Found: C, 73.18; H, 7.33; O, 19.45.
*(2E)-3-(4-((E)-3,7-Dimethylocta-2,6-dienyloxy)-3-methoxyphenyl)acrylaldehyde (**15**):* Anal. Calcd for $C_{20}H_{26}O_3$: C, 76.40; H, 8.33; O, 15.27. Found: C, 76.45; H, 8.31; O, 15.25.
*Nelumal A (**16**):* Anal. Calcd for $C_{21}H_{28}O_4$: C, 73.23; H, 8.19; O, 18.58. Found: C, 73.20; H, 8.22; O, 18.55.
*3,5-Dimethoxy-4-isopentenyloxybenzyl alcohol (**17**):* Anal. Calcd for $C_{14}H_{20}O_4$: C, 66.65; H, 7.99; O, 25.36. Found: C, 66.60; H, 7.94; O, 25.35.

*Etrogol (**18**):* Anal. Calcd for $C_{13}H_{18}O_2$: C, 75.69; H, 8.80; O, 15.51. Found: C, 75.66; H, 8.82; O, 15.54.
*Etrogol acetate (**19**):* Anal. Calcd for $C_{15}H_{20}O_3$: C, 72.55; H, 8.12; O, 19.33. Found: C, 72.50; H, 8.13; O, 19.37.
*3-(4-Geranyloxyphenyl)-1-ethanol (**20**):* Anal. Calcd for $C_{18}H_{26}O_2$: C, 78.79; H, 9.55; O, 11.66. Found: C, 78.83; H, 9.53; O, 11.67.
*3-(4-Isopentenyloxyphenyl)-1-propanol (**21**):* Anal. Calcd for $C_{14}H_{20}O_2$: C, 76.33; H, 9.15; O, 14.52. Found: C, 76.31; H, 9.11; O, 14.57.
*(2E)-3-(4-((E)-3,7-Dimethylocta-2,6-dienyloxy)-3-methoxyphenyl)prop-2-en-1ol (**22**):* Anal. Calcd for $C_{20}H_{28}O_3$: C, 75.91; H, 8.92; O, 15.17. Found: C, 75.87; H, 8.86; O, 15.20.
*Boropinol C (**23**):* Anal. Calcd for $C_{16}H_{22}O_4$: C, 69.04; H, 7.97; O, 22.99. Found: C, 69.00; H, 7.92; O, 22.94.
*Nelumol A (**24**):* Anal. Calcd for $C_{21}H_{30}O_4$: C, 72.80; H, 8.73; O, 18.47. Found: C, 72.77; H, 8.70; O, 18.44.
*4-Hydroxycordoin (**25**):* Anal. Calcd for $C_{20}H_{20}O_4$: C, 74.06; H, 6.21; O, 19.73. Found: C, 74.02; H, 6.18; O, 19.77.
*4′-Geranyloxyisoliquiritigenin (**26**):* Anal. Calcd for $C_{25}H_{28}O_4$: C, 76.50; H, 7.19; O, 16.31. Found: C, 76.46; H, 7.18; O, 16.27.
*N-Isopentenylanthranilic acid (**27**):* Anal. Calcd for $C_{12}H_{15}NO_2$: C, 70.22; H, 7.37; N, 6.82; O, 15.59. Found: C, 70.23; H, 7.38; N 6.78; O, 15.56.
*N-Acetyl-O-isopentenyl-L-tyrosine (**28**):* Anal. Calcd for $C_{16}H_{21}NO_4$: C, 65.96; H, 7.27; N, 4.81; O, 21.97. Found: C, 65.93; H, 7.23; N 4.78; O, 21.96.
*Lawsone 2-isopentenyl ether (**29**):* Anal. Calcd for $C_{15}H_{14}O_3$: C, 74.36; H, 5.82; O, 19.81. Found: C, 74.31; H, 5.76; O, 19.84.
*Isopentenyloxysyringaldehyde (**30**):* Anal. Calcd for $C_{14}H_{18}O_4$: C, 67.18; H, 7.25; O, 25.57. Found: C, 67.17; H, 7.22; O, 25.54.

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