Development and characterization of novel and selective inhibitors of cytochrome P450 CYP26A1, the human liver retinoic acid hydroxylase

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Abstract

Cytochrome P450 CYP26 enzymes are responsible for all-*trans*-retinoic acid (a*t*RA) clearance. Inhibition of CYP26 enzymes will increase endogenous a*t*RA concentrations and is an attractive therapeutic target. However, the selectivity and potency of the existing a*t*RA metabolism inhibitors towards CYP26A1 and CYP26B1 is unknown, and no selective CYP26A1 or CYP26B1 inhibitors have been developed. Here the synthesis and potent inhibitory activity of the first CYP26A1 selective inhibitors is reported. A series of non-azole CYP26A1 selective inhibitors was identified with low nM potency. The lead compound 3-{4-[2-(5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphthalen-2-yl)-1,3-dioxolan-2-yl] phenyl}4 propanoic acid (**24**) had 43-fold selectivity towards CYP26A1 with an IC_{50} of 340 nM. Compound **24** and its two structural analogs also inhibited a*t*RA metabolism in HepG2 cells resulting in increased potency of a*t*RA towards RAR activation. The identified compounds have potential to become novel treatments aiming to elevate endogenous a*t*RA concentrations and may be useful as cotreatment with a*t*RA to combat therapy resistance.

Graphical Abstract

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Supporting Information. Additional characterization data, ^{1}H and ^{13}C-NMR spectra, compound purity.

All-*trans*-retinoic acid → CYP26A1 (IC$_{50}$: 340 nM) → Inactive Metabolites
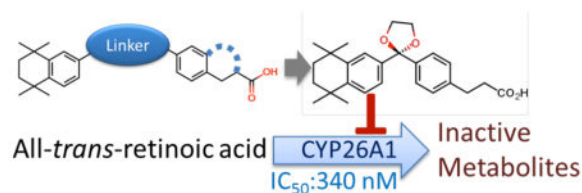
Keywords

Cytochrome P450; retinoic acid; CYP26; retinoic acid receptor

INTRODUCTION

Retinoic acid (RA) is the endogenous active metabolite of vitamin A (retinol). It is an essential regulator of cell growth, cell cycle and differentiation.[1,2] RA exists in several isomeric forms, all-*trans* retinoic acid (a*t*RA), 13-*cis*-retinoic acid (13-*cis*RA), 9,13-di*cis*-retinoic acid (9,13-di*cis*RA), and 9-*cis*-retinoic acid (9-*cis*RA).[3] Of these isomers, a*t*RA is believed to be the main biologically active form exhibiting its activity through binding predominantly to nuclear retinoic acid receptors (RARs). Through its binding to nuclear receptors and regulating gene transcription, a*t*RA plays a critical role in many biological processes including reproduction, maintenance of skin and epithelia, regulation of the immune system and T- and B-cell function, and in fetal development.[4–6] In addition, imbalance in vitamin A and RA homeostasis has been implied in development and progression of several human diseases such as acne, psoriasis and ichthyosis, type II diabetes, neurodegenerative diseases and some cancers.[1] The potential of RA in the treatment of human diseases has been manifested in the success of 13-*cis*RA (Accutane) in treatment of acne and neuroblastoma, and the effectiveness of a*t*RA in the treatment of acute promyelocytic leukemia (APL). However, the use and long term efficacy of a*t*RA has been limited in all of the therapeutic areas by its high clearance that limits oral administration of this agent, and the resistance that develops towards a*t*RA due to autoinduction of its metabolism.[7] Additionally 13*cis*-RA via isomerization to a*t*RA, and a*t*RA both cause broad activation of RARs that is believed to be responsible for some of the untoward side effects of these agents. Due to these shortcomings, the full clinical and therapeutic potential of retinoids has not been reached.

The clearance of a*t*RA is predominantly mediated by cytochrome P450 family 26 enzymes (CYP26).[8,9] The CYP26 family has three isoforms: CYP26A1, CYP26B1 and CYP26C1 which are expressed in a tissue specific manner. In the human liver CYP26A1 is the predominant CYP26 enzyme responsible for majority of the hepatic clearance of *at*RA.[10] CYP26B1 protein is not detected in human liver and the expression of this isoform appears to be restricted to extrahepatic sites.[11] The biological role and expression pattern of CYP26C1 is not well characterized but this enzyme appears to be responsible for 9-*cis*RA metabolism and be less critical in regulating RA concentrations than CYP26A1 and CYP26B1.

As a*t*RA is predominantly cleared by the CYP26 enzymes, it has been proposed that inhibition of the CYP26 enzymes will increase a*t*RA concentrations in target tissues, and inhibition of CYP26 enzymes during a*t*RA or 13-*cis*RA therapy could be used to combat disease resistance.[12] However, inhibitors of CYP26 that have been tested in humans (Figure 1) commonly suffer from broad spectrum CYP inhibition resulting in untoward side effects. Examples of this include the inhibition of steroid metabolism by liarozole (compound **1**) and the drug-drug interactions seen with ketoconazole (compound **2**).[7] The lack of specificity of these inhibitors is likely due to the imidazole and triazole moieties in the compounds that are known to coordinate with the heme iron providing additional potency towards CYP inhibition. Although more selective triazole and imidazole containing CYP26 inhibitors have been developed,[7] none of these compounds has yet reached human trials and therefore their drug-drug interaction potential in vivo is unknown. In addition, none of the existing inhibitors of CYP26s have been evaluated for specificity between the CYP26 enzymes and therefore the benefits and shortcomings of selective or broad CYP26 inhibition have not been established.

CYP26 enzymes appear to have cell type and tissue specific expression,[11] and therefore it is likely that specific CYP26A1 or CYP26B1 inhibitors would be beneficial for increasing RA concentrations in selected target tissues without causing broad side effects. Specifically, since CYP26A1 appears to be the predominant CYP26 enzyme in the liver, selective inhibition of CYP26A1 is expected to decrease systemic clearance of a*t*RA and therefore be useful for combatting therapy resistance in individuals being treated with a*t*RA or 13*cis*RA. In addition, it is likely that use of CYP26A1 specific inhibitor will selectively increase RA concentrations in the liver. Notably, based on conditional knock-out mouse studies, CYP26B1 inhibition could be a liability for male reproduction as testes cell type specific CYP26B1 knock-out mice show adverse effects on the testes.[14] Similarly, T-cell specific CYP26B1 knockouts have shown that CYP26B1 regulates iTreg and Th17 cell polarization,[15] an effect that may be a liability for a broad CYP26 inhibitor. A CYP26A1 selective inhibitor would avoid both of these effects and likely be beneficial when systemic a*t*RA concentrations are targeted.

The aim of this study was to identify novel non-azole inhibitors of CYP26 enzymes that would be selective for CYP26A1 and devoid of CYP26B1 inhibition. A series of novel CYP26A1 selective inhibitors was identified with low nM potency for CYP26A1 inhibition. The lead compound from the series had 43-fold selectivity towards CYP26A1 in comparison to CYP26B1 and other P450 enzymes and inhibited CYP26A1 with an IC_{50} of 340 nM. The lead compound and two of its structural analogs also inhibited a*t*RA metabolism in HepG2 cells resulting in increased potency of a*t*RA towards RAR activation in these cells. The identified compounds are the first CYP26A1 selective inhibitors discovered and have potential to become novel treatments aiming to elevate endogenous RA concentrations in circulation and in the liver. These compounds would also be useful as cotreatment with a*t*RA to combat therapy resistance likely caused by increased systemic clearance of a*t*RA and autoinduciton of CYP26A1, particularly in the liver.

RESULTS

Chemistry

Initial coupling of 2-bromo-1-(5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphthalen-2-yl)ethan-1-one with methyl-4-hydroxybenzoate in the presence of K_2CO_3 in methyl ethyl ketone under microwave conditions, gave ester **4** in good yield (Scheme 1). It was further hydrolyzed under basic conditions to afford carboxylic acid **5** in good yield. Reduction of the ketone moiety of compound **4** to alcohol by treatment with $NaBH_4$, followed by basic hydrolysis at the ester position, gave carboxylic acid **6**. Reaction of **4** with hydroxylamine hydrochloride in MeOH under reflux formed oxime isomers E **(7)** and Z (**8**), in moderate and good yield, respectively. Z-isomer **8** was further hydrolyzed in aqueous K_2CO_3 to provide carboxylic acid **9**.

Friedel-Crafts acylation[16,17] of 1,1,4,4-tetramethyl-1,2,3,4-tetrahydronaphthalene (TTN) with methyl 4-(chlorocarbonyl)benzoate and methyl 6-(chlorocarbonyl)naphthalene-2-carboxylate in CH_2Cl_2 in the presence of $AlCl_3$ yielded the expected ketones **10a** and **10b** (Scheme 2). The ester moieties of **10a** and **10b** were then hydrolyzed in basic conditions to respectively afford carboxylic acids **11a** and **11b** in excellent yields. Further, treatment of the ketone **10a** and **10b** with ethylene glycol[17] resulted in the formation of ketals **12a** and **12b** which after basic hydrolysis, afforded the expected carboxylic acids **13a** and **13b**. Likewise thioketalization of **10a** and **10b** was achieved by reaction with ethanedithiol in the presence of boron trifluoride diethyl etherate ($BF_3•Et_2O$), giving the thioketals **14a** and **14b** in excellent yields which after basic hydrolysis, gave carboxylic acids **15a** and **15b**.

Friedel-Crafts acylation[16] of TTN by 4-iodobenzoyl chloride according to the previously described procedure, afforded ketone **16** in good yield (Scheme 3). Heck[18,19] coupling reaction of **16** with methyl acrylate under palladium catalysis proceeded smoothly to give alkene **17**. Compound **17** was then hydrolyzed under basic conditions, to give carboxylic acid **18**. Hydrogenation of **17** under Pd/C catalysis not only reduced the alkene moiety, but also the carbonyl moiety resulting in the formation of benzylbenzene **19** which upon subsequent basic hydrolysis provided the corresponding carboxylic acid **20**. Ketalization of **17** was carried out according to the previously described procedure, to afford ketal **21** in moderate yield which after basic hydrolysis gave carboxylic acid **22**. Hydrogenation of **21** in presence of Pd/C followed by hydrolysis gave carboxylic acid **24**. The ketal moiety of **23** was cleaved with iodine in acetone under reflux according to a modified procedure of Sun et al,[20] to afford ketone **25** which after saponification yielded to the carboxylic acid **26**. Thioketalization of **17** followed by hydrolysis provided carboxylic acid **28**. The alkene moiety of **28** was found to be resistant to hydrogenation using Pd-C or Wilkinson's catalyst. Therefore, ketal **29** was obtained directly from the thioketalization of ketone **25** which after basic hydrolysis gave carboxylic acid **30**.

Sulfone **34** was prepared in 4 steps starting from TTN. TTN was first converted to disulfide **31** (Scheme 4) by treatment with chlorosulfonic acid followed by heating in presence of zinc in hydrochloric acid.[21] Disulfide **31** was then reduced and coupled with ethyl 3-(4-bromophenyl)propanoate in presence of $PdCl_2(dppf)$ and zinc,[22] to give diaryl sulfide **32**.

Oxidation of **32** with oxone produced the expected sulfone **33** which after hydrolysis under basic condition afforded carboxylic acid **34** in good yield.

To explore the role of the atoms of oxygen (or sulfur) from the dioxolane (dithiolane) moiety, we investigated the activity of a novel series of compounds with a sulfide moiety or a sulfoxide moiety as a linker in between the two aromatic rings. Synthesis started with preparation of iodo TTN derivative **35** from TTN (Scheme 5).[23] Diaryl sulfides **36a** and **36b** were then prepared by nickel catalyzed coupling with the iodo derivative **32** in the presence of polymer-supported borohydride in butanol, to respectively provide the 4- and 3-mercaptophenylacetic acid methyl esters.[24] Methyl esters **36a** and **36b** were obtained as mixtures with their butyl ester analogues (25% butyl ester). Biaryl sulfides **36a** and **36b** were then oxidized with oxone in methanol to afford the expected biaryl sulfones **37a** and **37b**. Compounds **36a**, **36b**, **37a** and **37b** were then hydrolyzed under basic conditions to afford the desired carboxylic acids **38a**, **38b**, **39a** and **39b** respectively in good to excellent yields.

Enzyme Inhibition and structure-activity relationship

A method was previously developed using recombinant CYP26A1 microsomes and 9-*cis*-RA as a substrate to test for inhibition of CYP26A1.[25] Previously, 9-*cis*-RA was chosen as the screening substrate instead of a*t*RA due to the lack of substrate and metabolite depletion in the screening assays with 9-*cis*-RA, and the lower affinity of 9-*cis*-RA to CYP26A1 when compared to a*t*RA allowing screening of inhibitors at substrate concentration at or below the K_m value.[25] Similar to CYP26A1, a*t*RA is a high affinity substrate of CYP26B1 and the metabolites formed by CYP26B1 are also substrates of CYP26B1[11] confounding kinetic inhibition measurements. To allow characterization of inhibition of CYP26B1, 9-*cis*-RA turnover by recombinant CYP26B1 was also characterized. 9-*cis*-RA was found to be a substrate of CYP26B1 and the formation of the metabolite that coeluted with 9-*cis*-4-OH-RA was NADPH dependent. The K_m and V_{max} for 9-*cis*-4-OH-RA formation by CYP26B1 were of 555 nM and 3.6 pmol/min/pmol P450, respectively. Based on the K_m value, all compounds were evaluated for CYP26B1 inhibition at a substrate (9-*cis*-RA) concentration of 100 nM (concentration $\ll K_m$ to increase sensitivity and decrease the dependence of IC_{50} values on inhibition mechanism).

In an effort to identify lead CYP26A1 specific inhibitors, we decided to focus our screening on synthetic analogues of RA. The compounds were selected on the basis of structural similarity with a*t*RA, the endogenous substrate of CYP26A1, and a small number of analogues were prepared to obtain an initial characterization of the structure-activity relationships (SAR) of CYP26A1 binding and selectivity. This approach was based on prior work showing that some RAR agonists bind and inhibit CYP26A1.[25] The classic RAMBAs (compounds **1** and **3**) and three commercially available synthetic retinoids **40** (bexarotene) (Table 1), **13a** (SR11237[26]) (Table 2), and **15b** (MM11253[27]) (Table 1), and two close analogues **13b** and **15a**, were tested for their CYP26A1 and CYP26B1 inhibition potency. This screening showed that both the RAMBAs and synthetic retinoids inhibited CYP26A1 and CYP26B1 (Table 1 and 2). The azole containing compound **3** inhibited both CYP26A1 and CYP26B1 potently whereas compound **1** was a more potent inhibitor of CYP26B1 than

CYP26A1. Neither one of these compounds provided sufficient specificity and selectivity to inhibit a*t*RA metabolism specifically by CYP26A1.

Compounds **40, 13a** and **15a** had IC_{50} values in the micromolar range and did not demonstrate significant selectivity for CYP26A1, whereas the RARγ selective antagonist **15b**[28] had a 0.061 μM IC_{50} for CYP26A1 and 1.03 μM IC_{50} for CYP26B1 (Table 1) demonstrating a 17-fold higher IC_{50} towards CYP26B1 than CYP26A1. Compound **13b**, the ketal homologue of **15b** displayed the same CYP26B1 inhibitory activity but exhibited a reduced CYP26A1 activity compared to the thioketal analogues. Replacement of the original dithiolane linker of **15b** by a carbonyl in compounds **11b** and **18**, and synthesis of their shorter homologues **11a** and **15a** resulted in a reduction of CYP26A1 inhibitory activities and a modest enhancement of CYP26B1 inhibition for compound **11b**. All these data together indicate that the geometry of the thioketal ring and the distance between the carboxylic acid and the thioketal moiety are crucial for the CYP26A1 inhibitory activity. Our subsequent research efforts to complete SAR of synthetic retinoids led to the synthesis of compounds **5**, **6** and **9.** We decided to incorporate polar groups in the spacer between the two aromatic moieties to explore potential hydrogen bonding interaction with CYP26A1. The distance between the carboxylic acid and the tetramethyltetrahydronaphthalene moiety was kept in the same range as for compound **15b.** The naphthyl moiety was replaced by a methyleneoxyphenyl moiety to minimize potential steric hindrance that would prevent hydrogen bonding. However, such a chemical modification did not produce significant improvement in affinity towards CYP26A1. We also explored the effect of potential steric hindrance on compound **11b** by replacing the naphthyl moiety by a styryl moiety (**18**) This change led to a dramatic loss of CYP26B1 inhibition and a 2 to 3-fold reduction in CYP26A1 activity. A similar trend was observed for the CYP26B1 activity of the styryl derivative **22** whereas CYP26A1 activity was comparable to the naphthyl analogue **13b.** Surprisingly compound **28**, the styryl analogue of compound **15b** was not active towards CYP26A1. While **15b** is a potent inhibitor of CYP26A1 it is not an ideal inhibitor to be developed further due to its high RAR affinity.[28] Therefore, **15b** was chosen as a lead compound for the design and synthesis of novel inhibitors that would be free of RAR binding (agonist or antagonist), and inhibition of other P450s except CYP26A1. We decided to explore the effect of the replacement of the naphthyl group bearing the carboxylic acid by a phenethyl group on CYP26 and RAR activities. Phenethyl derivative **24** displayed a lower CYP26B1 activity compared to the naphthyl analogue **13b** while CYP26A1 activity was only reduced by 2 to 3-fold. CYP26A1 inhibitory activity for the thioketal analogue **30** was in the same range of compound **24** whereas CYP26B1 activity was increased. We also explored whether it is possible to improve CYP26A1 inhibitory activity and selectivity by modifying the substituents on the one-carbon between the two ring systems of compound **24**. These will result in the modification of the spatial orientation of the two aromatic moieties. Such a chemical modification did not produce significant improvement in CYP26 inhibition for analogues **20**, **26** and **34**. In the course of our initial exploratory work on the SARs for this novel series of CYP26 inhibitors, we also explored whether it is possible to impact the spatial positioning of the carboxylic moiety by shortening the phenethyl moiety. The thioethers (**38a, 38b**) and the sulfones (**39a, 39b**) lacked inhibitory activity towards CYP26A1 and CYP26B1.

In summary, in contrast to the relatively robust nanomolar inhibition of CYP26A1 by the dithiolane and dioxolane compounds in this series, and the micromolar inhibition of CYP26A1 by majority of the remaining compounds, the inhibitory potency of the compounds (**5–39**) towards CYP26B1 was variable with only compounds **11b, 30** and **5** showing submicromolar potency towards CYP26B1. Interestingly, compounds **5, 6, 9, 11b, 34** were more potent inhibitors of CYP26B1 than CYP26A1 suggesting that interactions between these inhibitors and the CYP26 enzymes within the active site are different allowing development of CYP26 isoform selective inhibitors. Compounds **15b** and **13b** were approximately 10–20 fold more potent inhibitors of CYP26A1 than CYP26B1. The replacement of the naphthyl group in the dioxolane compound **13b** with a saturated or an unsaturated two carbon chain (**22** and **24**) led to over 10-fold decrease in the CYP26B1 inhibition with a lower effect on CYP26A1 inhibition. Therefore, these changes increased the CYP26A1 selectivity to >40-fold between CYP26B1 and CYP26A1. In contrast, with the dithiolane compound **15b**, replacement of the naphthyl with an unsaturated or a saturated two carbon chain led to either considerable decrease in CYP26A1 inhibition (**28**) or loss of CYP26A1 selectivity (**30**).

RAR activation

The initial lead compound in the series, **15b**, is a known RAR antagonist[28] and therefore it is possible that also the novel compounds identified will possess off-target effects via RAR binding. Hence, the two most promising selective inhibitors, compounds **22** and **24,** as well as compound **30** which was a potent non-selective CYP26A1/B1 inhibitor, were assessed for RAR activation. We first used a luciferase-based reporter assay to screen compounds **22**, **24** and **30** for RARα and RARγ activation at a concentration of 1 μM. Screen was accomplished using human RARα and RARγ reporter assay kits provided by Indigo Biosciences as reported previously. No measurable RARα and RARγ activation was detected for the three compounds (Data not shown). We then used the HepG2 cells, which have robust a*t*RA metabolism activity and RAR mediated effects on gene transcription[29] to test the effects of compounds **22**, **24** and **30** on RAR activation in a cell system. The induction of the mRNA of the classic RAR target genes CYP26A1 and RARβ was evaluated after treatment with each of the novel compounds in comparison to vehicle and a*t*RA (Figure 2). As shown, none of the three compounds resulted in activation of the pool of RARs in the HepG2 cells at a test concentration of 1 μM, a concentration at least 5 times greater than the IC_{50} value of these compounds towards CYP26A1.

Inhibition of RA metabolism and Enhancement of RA potency

To further determine the biological activity of the lead compounds, their efficacy to increase the potency of a*t*RA was tested in the HepG2 cells. It has been previously shown that a*t*RA is efficiently metabolized in HepG2 cells and that the metabolism of *at*RA in HepG2 cells is predominantly mediated by CYP26A1.[29] Therefore, RA metabolism in HepG2 cells and the activation of RARs (CYP26A1 and RARβ induction) by a*t*RA in these cells provide a reliable test system for cellular activity of the compounds of interest. When HepG2 cells were treated with 100 nM a*t*RA in combination with 1 μM of compounds **22**, **24** and **30**, the magnitude of induction of RARβ and CYP26A1 was significantly increased when compared

to *at*RA treatment alone (Figure 2), demonstrating that all three compounds potentiate the effect of a*t*RA in cells presumably via inhibiting a*t*RA metabolism and increasing the cellular concentrations of a*t*RA. Importantly the induction of RARβ and CYP26A1 following treatment with 100 nM a*t*RA together with the inhibitors was significantly greater than the induction observed following treatment with 1 μM a*t*RA alone demonstrating a considerable increase in the potency of a*t*RA by the inhibitors. Furthermore these data demonstrate that these compounds do not exhibit an RAR antagonistic activity.

To test whether the increased induction of RAR target genes in the presence of the inhibitors and *at*RA was due to inhibition of a*t*RA depletion in the cells, the concentrations of a*t*RA in the media at the end of the 24-hour treatment were measured. Table 3 summarizes the effects of the lead compounds on a*t*RA depletion in HepG2 cells. All three CYP26 inhibitors significantly increased a*t*RA concentrations in the cell media with compound **30** having the greatest effect, approximately 9-fold increase in a*t*RA concentrations in the media. Compound **22** increased a*t*RA concentration 3.5-fold and compound **24** increased a*t*RA concentration in the media by 2-fold. This increase in a*t*RA concentrations correlated well with the observed magnitude of effect on potentiating a*t*RA mediated RAR activation in cells with compound **30** also having the greatest effect on increasing a*t*RA mediated induction of RARβ and CYP26A1.

Selectivity for CYP26A1

To determine the selectivity of the lead compounds towards other P450 enzymes the inhibition of CYP2D6, CYP3A4, CYP2C19 and CYP2B6 by compounds **22**, **24** and **30** was evaluated. The inhibition data is summarized in Table 4. When screened at 10 μM inhibitor concentration, compounds **22** and **24** were free of any CYP3A4 inhibition while compound **30** inhibited CYP3A4 by 25%. The IC_{50} values for CYP3A4 inhibition for compounds **22** and **30** were 30 μM and 7.5 μM respectively. For compound **24** no concentration dependent inhibition of CYP3A4 was observed up to 100 μM with maximum 40% inhibition observed, demonstrating that compound **24** is unlikely to result in CYP3A4 inhibition at concentrations required to inhibit CYP26A1. Similarly, compound **24** was free of any significant inhibition of CYP2D6, CYP2C19 and CYP2B6 suggesting that this compound may become a novel CYP26A1 selective inhibitor. In contrast compound **22** inhibited CYP2D6 by 50% at 10 μM concentration and compound **30** inhibited CYP2B6 by 70% at 10 μM.

DISCUSSION

Inhibition of *at*RA metabolism has been a potential drug target for some decades now and several promising inhibitors of overall *at*RA metabolism have emerged from variety of drug development programs in industry and academia.[7] Yet, in the development of the inhibitors of a*t*RA metabolism or CYP26, the role of inhibition of the different CYP26 isoforms has never been addressed. In fact, the screening data in this study shows that talarozole is a potent inhibitor of both CYP26A1 and CYP26B1 while liarozole is a preferential inhibitor of CYP26B1. This difference in their inhibition profile likely contributes to some differences in their *in vivo* pharmacology and side effect profile. As CYP26A1 and CYP26B1 are

expressed in a tissue specific manner,[11] selective inhibition of either CYP26 isoform holds great promise in organ specific targeting of the retinoid system. This study is the first to test and identify CYP26 isoform specific inhibitors with the goal of identifying a CYP26A1 selective inhibitor that can be used to inhibit a*t*RA metabolism in the liver. Compound **24** has 43-fold specificity towards CYP26A1 compared to CYP26B1 and it emerged as the lead inhibitor in this novel structural series. The selectivity of compound **24** together with compounds **15b**, **13b** and **22** is striking as up to now only minor differences in the biochemical structure function between CYP26A1 and CYP26B1 have been observed. Both enzymes use a*t*RA as a substrate, bind a*t*RA with high affinity and form very similar metabolites from a*t*RA although at slightly different product ratios.[11] This is despite the fact that the two CYP26 enzymes only share about 40% sequence similarity and hence would be expected to be structurally and functionally different. As the CYP26 enzymes have proven exceedingly difficult to express and purify in recombinant systems, no X-ray crystal structure or low-resolution structural information is available for these enzymes at present. Therefore the discovery of these novel selective inhibitors of CYP26A1 provide critical insight to the differences in the active site features and structure-activity relationships of these two enzymes and provide a lead for future development of selective inhibitors of the two enzymes.

The novel compounds in this series were developed using known RAR ligands as lead compounds and by exploring synthetically feasible modifications that could reduce RAR binding activity without compromising CYP26A1 inhibition. Replacement of the naphthyl group of the dioxolane derivative **13b** by a phenethyl moiety in compound **24** appears to have a minor impact on CYP26A1 inhibitory activity while reducing CYP26B1 activity. The results in HepG2 cells show that indeed the lead compounds **22**, **24** and **30** were devoid of RAR modulation while maintaining the CYP26 inhibition potency. In addition, the studies in HepG2 cells showed that compound **24** and the two other potent inhibitors in this series, compounds **22** and **30,** were effective at inhibiting a*t*RA metabolism in cell culture systems and they potentiated the activity of a*t*RA in the cell system. To mimic the in vivo endogenous a*t*RA concentrations in cells, the HepG2 cells were treated with 100 nM a*t*RA and under these conditions, the lead inhibitors increased the activity of a*t*RA by 5–100 fold and decreased a*t*RA metabolism significantly. The HepG2 cells have been previously shown to be devoid of CYP26B1 expression although CYP26B1 expression is somewhat inducible in these cells.[30] Similarly HepG2 cells do not express significant levels of the common drug metabolizing enzymes such as CYP3A4 and CYP2C enzymes. Therefore it is likely that the effect of the inhibitors on a*t*RA activity in these cells is solely due to CYP26A1 inhibition.

In this investigation, we present a broad range of experimental data on a novel series of retinoid-based CYP26A1 inhibitors. Within this series, a SAR could be established that showed that lipophilic bulk induced by a dioxolane or a dithiolane on the spacer appears to be required for enhanced CYP26A1 inhibitory activity. Dioxolane analogues **22** and **24** were identified as selective CYP26A1 inhibitors. Classic CYP26 inhibitors have all included a triazole or imidazole functional group to increase their affinity to CYP26s *via* coordination to the heme iron.[31,32] However, a*t*RA itself binds to CYP26A1 with 10 nM affinity suggesting that high affinity inhibitors devoid of the imidazole or triazole group prone to

broad spectrum CYP inhibition can be developed. Indeed, this study shows that nM inhibitors of both CYP26A1 and CYP26B1 free of imidazole or triazole functionality can be synthesized. These inhibitors were also free of inhibition of off-target CYPs such as CYP3A4, CYP2C19 and CYP2D6 at concentrations relevant for CYP26A1 inhibition. However, these compounds do include a ketal that may be metabolically labile and compound **22** is an unsaturated acid. These functional groups are potential in vivo liabilities of these compounds and require further study. Nevertheless, these compounds provide the first structural leads for selective CYP26A1 inhibition, and have the potential to become the first selective CYP26A1 inhibitors for testing in preclinical models. Eventually these compounds may become useful for treatment of diseases that would require increasing systemic or liver a*t*RA concentrations or in inhibiting a*t*RA metabolism when a*t*RA is administered clinically to combat therapy resistance that develops due to CYP26A1 induction by a*t*RA.

EXPERIMENTAL SECTION

General Experimental methods

Moisture sensitive reactions were performed in an inert, dry atmosphere of argon in flame dried glassware. Air sensitive liquids were transferred via syringe or cannula through rubber septa. Reagent grade solvents were used for extraction and flash chromatography. THF was distilled from Na/benzophenone under argon; dichloromethane (CH_2Cl_2) and chloroform ($CHCl_3$) were distilled from CaH_2 under argon. All other reagents including 2-bromo-1-(5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphthalen-2-yl)ethan-1-one and 1,1,4,4-tetramethyl-1,2,3,4-tetrahydronaphthalene and solvents which were purchased from commercial sources, were used directly without further purification. The progress of reactions was checked by analytical thin-layer chromatography (Sorbent Technologies, Silica G TLC plates w/UV 254). Flash column chromatography was performed using prepacked Biotage SNAP/ZIP cartridges on a Biotage Isolera One instrument. Microwave reactions were performed using a Biotage Initiator instrument. The solvent compositions reported for all chromatographic separations are on a volume/volume (v/v) basis. ^{1}HNMR spectra were recorded at 400 or 500 MHz and are reported in parts per million (ppm) on the δ scale relative to tetramethylsilane as an internal standard. ^{13}CNMR spectra were recorded at 100 or 125 MHz and are reported in parts per million (ppm) on the δ scale relative to $CDCl_3$. Melting points were determined on a Stuart melting point apparatus from Bibby Scientific Limited and are uncorrected. LC-UV and high resolution mass spectrometry (HRMS) analyses were conducted on a Waters ACQUITY UPLC-series liquid chromatography system equipped with a diode array detector and LCT PREMIER XE[TM] time of flight (TOF) mass spectrometer operated on the electrospray ionization mode. Compounds were separated using a Phenomenex (Torrance, CA) NX-C18 column (50×4.60 mm) with 3 μm particle size and gradient elution at mobile phase flow rate of 0.4 mL/min with solvents A: water with 0.1% formic acid and B: acetonitrile. The gradient was from 90% A 10% B to 2% A 98% B over 6.6 min then held isocratic till 13 min followed by return to initial conditions by 15 min. Compound purity was assigned on the basis of UV detection data at 254-nM detection by comparing relative peak areas of the signals. All final compounds were more than 95% pure.

General procedure for ester hydrolysis using K₂CO₃ (method A)—To a solution of ester (0.25 mmol) in MeOH (4 mL) was added aqueous K_2CO_3 (0.4 mL, 2.0 M) dropwise. The reaction mixture was stirred at 80°C for 1 hour. Completion of reaction was assessed by TLC. The reaction was then cooled (0 °C), acidified to pH 2 with 1.0 N HCl, and extracted with EtOAc (3×10 mL). The combined organic phases were dried ($MgSO_4$) and concentrated under reduced pressure.

General procedure for ester hydrolysis using LiOH (method B)—To a solution of the ester (0.11 mmol) in anhydrous THF (0.5 mL) was added aqueous LiOH (0.25 mL, 1.0 N) dropwise. The reaction mixture was refluxed for 2 hours or stirred overnight at room temperature. The reaction was then cooled (0 °C), acidified to pH 2 with 1.0 N HCl, and extracted with EtOAc (3×10 mL). The combined organic phases were dried ($MgSO_4$) and concentrated.

General procedure for Friedel-Crafts acylation (method C)—Acyl chloride (11.7 mmol) and 1,1,4,4-tetramethyl-1,2,3,4-tetrahydronaphthalene material (2.0 g, 10.6 mmol) were weighed into a round-bottomed flask equipped with a stir bar and 24 mL dichloromethane added. Stirring commenced and $AlCl_3$ (3.10 g, 23.3 mmol) was added slowly. After completion of the addition, stirring was continued at room temperature overnight. The reaction was then poured into an ice solution (40 mL), acidified with a 20% HCl solution (10 mL) and the organic layer removed. Then extracted further with 2×40 mL EtOAc. The organic layers were combined and washed with water (40 mL) and brine respectively, dried over $MgSO_4$, filtered and concentrated under reduced pressure.

General procedure for formation of dioxolane (method D)—Diarylketone (2.0 mmol) was treated with ethylene glycol (2 mL) and a catalytic amount of *p*TsOH (20 mg) in toluene (10 mL) at 145°C overnight using a Dean Stark trap. After cooling to room temperature, the mixture was washed with saturated $NaHCO_3$(aq.), brine, dried over $MgSO_4$ and concentrated under reduced pressure.

General procedure for formation of dithiolane (method E)—To a solution of diarylketone (1.14 mmol) in CH_2Cl_2 (2 mL) at 0°C under argon, was added a solution of $(CH_2SH)_2$ (144 μL, 1.71 mmol) in CH_2Cl_2 (0.5 mL) followed by $BF_3 \cdot Et_2O$ (217 μL, 1.71 mmol). The resulting mixture was stirred at 0°C for 1 h and then warmed to room temperature overnight. The reaction was quenched by pouring the mixture into saturated $NaHCO_3$, and the mixture was extracted with 20 mL CH_2Cl_2. The combined organic layers were dried ($MgSO_4$) and concentrated.

General procedure for hydrogenation or hydrogenolysis (method F)—Alkene (515 mg, 1.36 mmol) was placed in a hydrogenation apparatus equipped with a magnetic stir bar and EtOH/EtOAc (30 mL) added followed by Pd/C (240 mg). H_2 gas was introduced at 20 psi pressure and reacted at room temperature for 7 hrs. The black solution was filtered using a celite pad and concentrated under reduced pressure.

General procedure for coupling thioaryl to aryl halides (method G)—To a mixture of thioaryl (0.71 mmol, 1 eq.) and aryl halides (0.71 mmol, 1 eq.) in dioxane (5 mL)

and butanol (5 mL), were added the borohydride, polymer supported (650 mg, 2.5 – 5 mmol/g, 3 eq.) and (bpy)$_2$NiBr$_2$ (70 mg, 0.2 eq.). The mixture was heated to 145°C under an atmosphere of nitrogen. The polymer beads were removed by filtration, and the mixture was concentrated to dryness before purification by flash chromatography using the following gradient system, (cyclohexane/dichloromethane): (20/80) to (0/100).

General procedure for oxidation of sulfide to sulfone (method H)—To a solution sulfide (0.26 mmol) in THF (7 mL), was added a solution of oxone (475 mg, 0.775 mmol, 3 eq.) in water (4 mL). The reaction was stirred overnight at room temperature. Water (10 mL) was added and the mixture was extracted with dichloromethane (3×50 mL).

Methyl-4-(2-oxo-2-(5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphthalen-2-yl)ethoxy)benzoate (4): A mixture 500 mg (1.62 mmol) of the commercially available 2-bromo-1-(5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphthalen-2-yl)ethan-1-one, 271 mg of the commercially available methyl 4-hydroxybenzoate (1.78 mmol) and K$_2$CO$_3$ (246 mg, 1.78 mmol) in 10 ml of methyl ethyl ketone was heated 2 times at 100°C for 20 minutes in the microwave and then filtered and concentrated on a rotary evaporator under vacuum. The product was purified by crystallization with a (heptane/ethyl acetate: 70/30) solution to give the expected compound (450 mg; 73%) as a white solid. mp = 112–114°C. ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.98 (d, J = 8.9 Hz, 2H), 7.97 (d, J = 1.9 Hz, 1H), 7.72 (dd, J = 8.3, 1.9 Hz, 1H), 7.43 (d, J = 8.3 Hz, 1H), 6.95 (d, J = 8.9 Hz, 2H), 5.32 (s, 2H), 3.88 (s, 3H), 1.71 (s, 4H), 1.32 (s, 6H), 1.31 (s, 6H). ^{13}C NMR (126 MHz, CDCl$_3$) δ 193.34, 166.63, 161.72, 152.00, 145.85, 131.74, 131.58, 127.14, 126.68, 125.02, 123.32, 114.32, 70.48, 51.87, 34.80, 34.70, 34.58, 34.42, 31.73, 31.53. MS (TOF ESI+): m/z calcd for C$_{24}$H$_{29}$O$_4^+$ (M+H)$^+$ calcd. 381.21, found 381.20.

4-(2-Oxo-2-(5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphthalen-2-yl)ethoxy)benzoic acid (5): Compound **4** (200 mg, 0.53 mmol) was stirred at 80°C with sodium hydroxide (200 mg) in a mixture of EtOH, THF and water (10 mL, 10 mL and 1.5 mL) for 12 hours. The reaction was then cooled at r.t., acidified to pH 2 with 1.0 N HCl, and extracted with EtOAc (3 × 20 mL). The combined organic phases were dried (MgSO$_4$) and concentrated under reduced pressure. The residue was purified by crystallization in a mixture of heptane and EtOAc (70/30) to provide 149 mg (77%) of a white solid. mp = 162–163°C. ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.06 (d, J = 8.9 Hz, 2H), 7.98 (d, J = 1.7 Hz, 1H), 7.72 (dd, J = 8.3, 1.8 Hz, 1H), 7.43 (d, J = 8.3 Hz, 1H), 6.97 (d, J = 8.9 Hz, 2H), 5.34 (s, 2H), 1.72 (s, 4H), 1.32 (s, 6H), 1.31 (s, 6H). ^{13}C NMR (101 MHz, CDCl$_3$) δ 193.30, 170.88, 162.50, 152.11, 145.95, 132.40, 131.80, 127.21, 126.77, 125.07, 122.38, 114.51, 70.56, 34.86, 34.77, 34.66, 34.47, 31.77, 31.57. HPLC (t= 8.12 min, 96%). MS (TOF ESI+) for C$_{23}$H$_{27}$O$_4^+$ (M+H)$^+$ calcd. 367.20, found 367.19.

4-(2-Hydroxy-2-(5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphthalen-2-yl)ethoxy)benzoic acid (6): To a solution of compound **4** 95 mg (0,25 mmole) dissolved in 3 mL of THF under nitrogen, was carefully added 10 mg of sodium borohydride(0,25 mmole). The mixture was stirred 3 h at room temperature. The residue was taken up in 10 mL water, and the aqueous layer was extracted with 3×10 mL EtOAc. The combined organic extract was washed with

30 mL water and 30 mL brine respectively. The organic solution was dried ($MgSO_4$), filtered, and concentrated to give 75 mg (79%) of a colorless oil. The resulting ester (70 mg, 0.18 mmol) was stirred at 80°C with sodium hydroxide (70 mg) in a mixture of EtOH, THF and water (3mL, 3mL and 0.5 mL) for 12 hours. The reaction was then cooled at r.t., acidified to pH 2 with 1.0 N HCl, and extracted with EtOAc (3 × 10 mL). The combined organic phases were dried ($MgSO_4$) and concentrated under reduced pressure. The residue was purified by crystallization in a mixture of heptane and EtOAc (70/30) to provide 40 mg (60%) of a white solid. mp = 156–158°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06 (d, J = 8.8 Hz, 2H), 7.38 (d, J = 1.5 Hz, 1H), 7.34 (d, J = 8.1 Hz, 1H), 7.21 (dd, J = 8.1, 1.6 Hz, 1H), 6.97 (d, J = 8.9 Hz, 2H), 5.11 (dd, J = 8.3, 3.4 Hz, 1H), 4.23 – 4.04 (m, 2H), 1.69 (s, 4H), 1.35 – 1.22 (m, 12H).^{13}C NMR (101 MHz, $CDCl_3$) δ 171.39, 162.97, 145.28, 145.17, 136.35, 132.39, 126.92, 124.46, 123.41, 122.09, 114.37, 73.45, 72.63, 35.07, 34.99, 34.35, 34.19, 31.87, 31.85, 31.83. HPLC (t= 8.34 min, 97%). MS (TOF ESI+) for $C_{23}H_{29}O_4{}^+$ (M+H)$^+$ calcd. 369.21, found 369.21.

(E)-Methyl-4-(2-(hydroxyimino)-2-(5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphthalen-2-yl)ethoxy-)benzoate (7):

The E-isomer was recrystallized from EtOAc/heptane (3/7) to give 69 mg (24%) of a white solid. mp = 146–149°C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.21 (s, 1H), 7.98 (d, J = 9.0 Hz, 2H), 7.59 (d, J = 1.8 Hz, 1H), 7.40 (dd, J = 8.3, 1.9 Hz, 1H), 7.35 (d, J = 8.3 Hz, 1H), 6.98 (d, J = 9.0 Hz, 2H), 4.95 (s, 2H), 3.88 (s, 3H), 1.69 (s, 4H), 1.28 (s, 6H), 1.25 (s, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.79, 161.93, 153.08, 146.73, 144.85, 131.56, 127.49, 126.93, 126.48, 125.51, 123.11, 114.55, 69.32, 51.91, 34.88, 34.83, 34.34, 34.29, 31.78, 31.65. MS (TOF ESI+) for $C_{24}H_{30}NO_4{}^+$ (M+H)$^+$ calcd. 396.22, found 396.21.

(Z)-Methyl-4-(2-(hydroxyimino)-2-(5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphthalen-2-yl)ethoxy-)benzoate (8):

A solution of compound **5** (275 mg, 0.72 mmol) in MeOH (7 mL) was treated with hydroxylamine hydrochloride (100 mg, 1.45 mmol) and pyridine (235 μL, 2.9 mmol), and the mixture was heated at reflux for 6 h. The mixture was cooled to room temperature, and the MeOH was removed in vacuo. The residue was taken up in 20 mL water, and the aqueous layer was extracted with 3×30 mL EtOAc. The combined organic extract was washed with 20 mL water and 20 mL brine respectively. The organic solution was dried ($MgSO_4$), filtered, and concentrated. The residue was purified by flash column chromatography (heptane-ethyl acetate 100:0 v/v increasing to 70:30 v/v)to yield the 2 isomers E and Z. The Z-isomer was recrystallized from EtOAc/ heptane to give 165 mg (71%) of a white solid. mp = 123–125°C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.79 (s, 1H), 7.97 (d, J = 8.8 Hz, 2H), 7.60 (d, J = 1.9 Hz, 1H), 7.39 (dd, J = 8.3, 1.9 Hz, 1H), 7.29 (d, J = 8.3 Hz, 1H), 6.98 (d, J = 8.8 Hz, 2H), 5.32 (s, 2H), 3.88 (s, 3H), 1.67 (s, 4H), 1.27 (s, 6H), 1.26 (s, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.81, 161.81, 155.20, 146.73, 144.91, 131.59, 130.12, 126.69, 125.35, 124.01, 123.09, 114.25, 59.86, 51.91, 34.93, 34.85, 34.30, 34.29, 31.78, 31.65. MS (TOF ESI+) for $C_{24}H_{30}NO_4{}^+$ (M+H)$^+$ calcd. 396.22, found 396.21.

(Z)-4-(2-(Hydroxyimino)-2-(5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphthalen-2-yl)ethoxy)benzoic acid (9):

Compound **8** (100 mg, 0.25 mmol) was hydrolysed according

to method A. The residue was purified by flash column chromatography (DCM-MeOH 100:0 v/v increasing to 95:5 v/v) to provide a white solid (25 mg, 27%). mp = 208–211°C. ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.96 (d, J = 8.9 Hz, 2H), 7.58 (d, J = 1.9 Hz, 1H), 7.48 (s, 1H), 7.39 (dd, J = 8.3, 1.9 Hz, 1H), 7.28 (d, J = 8.3 Hz, 1H), 6.99 (d, J = 8.9 Hz, 2H), 5.33 (s, 2H), 1.68 (s, 4H), 1.26 (s, 6H), 1.25 (s, 6H). HPLC (t= 8.14 min, 95%). HRMS (TOF ESI+) for C$_{23}$H$_{28}$NO$_4$$^+$ (M+H)$^+$ calcd. 382.2018, found 382.2012.

Methyl-4-(5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphthalene-2-carbonyl)benzoate (10a): Compound **10a** was prepared according to method C starting from 2.0 g of the commercially available 1,1,4,4-tetramethyl-1,2,3,4-tetrahydronaphthalene (10.6 mmol). The crude was crystallized from hot EtOAc by addition of 20 mL MeOH, resulting in white crystals after cooling. Yield 2.15 g (58%). mp = 140–142°C. ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.15 (d, J = 8.4 Hz, 2H), 7.83 (d, J = 8.4 Hz, 2H), 7.79 (d, J = 1.8 Hz, 1H), 7.54 (dd, J = 8.2, 1.9 Hz, 1H), 7.41 (d, J = 8.2 Hz, 1H), 3.97 (s, 3H), 1.72 (s, 4H), 1.32 (s, 6H), 1.29 (s, 6H). ^{13}C NMR (101 MHz, CDCl$_3$) δ 195.88, 166.40, 150.78, 145.30, 141.88, 134.15, 132.91, 129.67, 129.39, 128.89, 127.35, 126.70, 52.40, 34.81, 34.74, 34.41, 31.74, 31.60. MS (TOF ESI+) for C$_{23}$H$_{27}$O$_3$$^+$ (M+H)$^+$ calcd. 351.20, found 351.20.

Methyl-6-(5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphthalene-2-carbonyl)-2-naphthoate (10b): Compound **10b** was synthesized according to method C starting from 875 mg of 1,1,4,4-tetramethyl-1,2,3,4-tetrahydronaphthalene (4.65 mmol). The compound **10b** crystallized from hot EtOAc by addition of 20 mL MeOH, resulting in white crystals after cooling (1.94 g, 69%). mp = 134–136°C. ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.67 (s, 1H), 8.30 (s, 1H), 8.13 (dd, J = 8.6, 1.6 Hz, 1H), 8.06 (d, J = 8.5 Hz, 1H), 7.98 (dd, J = 8.5, 1.6 Hz, 2H), 7.97 (d, J = 8.6 Hz, 1H), 7.87 (d, J = 1.8 Hz, 1H), 7.60 (dd, J = 8.2, 1.8 Hz, 1H), 7.44 (d, J = 8.2 Hz, 1H), 4.01 (s, 3H), 1.74 (s, 4H), 1.34 (s, 6H), 1.31 (s, 6H). ^{13}C NMR (101 MHz, CDCl$_3$) δ 196.21, 166.86, 150.47, 145.26, 137.33, 134.59, 134.45, 134.13, 130.91, 130.68, 129.49, 129.33, 128.94, 127.44, 126.69, 126.66, 126.08, 52.40, 34.85, 34.78, 34.73, 34.43, 31.76, 31.63. MS (TOF ESI+) for C$_{27}$H$_{29}$O$_3$$^+$ (M+H)$^+$ calcd. 401.21, found 401.21.

4-(5,5,8,8-Tetramethyl-5,6,7,8-tetrahydronaphthalene-2-carbonyl)benzoic acid (11a): Compound **10a** (60 mg, 0.17 mmol) was hydrolyzed according to method A. The resultant oil was triturated with heptanes to yield compound **11** as a white solid (52 mg, 91%). mp = 194–195°C. ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.24 (d, J = 8.3 Hz, 2H), 7.87 (d, J = 8.3 Hz, 2H), 7.81 (d, J = 1.8 Hz, 1H), 7.55 (dd, J = 8.2, 1.8 Hz, 1H), 7.42 (d, J = 8.2 Hz, 1H), 1.73 (s, 4H), 1.33 (s, 6H), 1.30 (s, 6H). ^{13}C NMR (101 MHz, CDCl$_3$) δ 195.84, 170.91, 150.97, 145.41, 142.74, 134.03, 131.89, 130.05, 129.75, 128.91, 127.42, 126.76, 34.81, 34.77, 34.74, 34.43, 31.76, 31.61, 26.91. HPLC (t= 8.82 min, 100%). HRMS (TOF ESI+) for C$_{22}$H$_{25}$O$_3$$^+$ (M+H)$^+$ calcd. 337.1804, found 337.1811.

6-(5,5,8,8-Tetramethyl-5,6,7,8-tetrahydronaphthalene-2-carbonyl)-2-naphthoic acid (11b): Compound **10b** (220 mg, 0.55 mmol) was hydrolyzed according to method A. The crude was crystalized from cyclohexane to provide compound **11b** as a white solid (90 mg, 95%). mp = 224–225°C. ^{1}H NMR (400 MHz, MeOD) δ 8.70 (s, 1H), 8.30 (s, 1H), 8.15 (d, J = 8.5 Hz, 1H), 8.08 (d, J = 8.5 Hz, 1H), 7.98 (t, J = 8.0 Hz, 2H), 7.86 (s, 1H), 7.61 (d, J = 8.2

Hz, 1H), 7.46 (d, J = 8.2 Hz, 1H), 1.75 (s, 4H), 1.35 (s, 6H), 1.32 (s, 6H). ^{13}C NMR (101 MHz, MeOD) δ 196.84, 168.40, 150.59, 145.17, 136.98, 134.34, 134.32, 134.12, 130.94, 130.80, 129.70, 129.45, 129.30, 128.88, 127.30, 126.59, 126.33, 126.27, 34.65, 34.58, 34.26, 31.53, 31.40. HPLC (t= 9.40 min, 95%). HRMS (TOF ESI+) for $C_{26}H_{27}O_3^+$ (M+H)$^+$ calcd. 387.1960, found 387.1998.

Methyl-4-(2-(5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphthalen-2-yl)-1,3-dioxolan-2-yl)benzoate (12a): Compound **10a** (705 mg, 2.01 mmol) was treated treated according to method D. After work-up, the residue was purified by flash column chromatography (cyclohexane-EtOAc 100:0 v/v increasing to 60:40 v/v) to yield a white solid, 641 mg (81%). mp= 148–148°C. ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.00 (d, J = 8.3 Hz, 2H), 7.61 (d, J = 8.3 Hz, 2H), 7.42 (d, J = 1.8 Hz, 1H), 7.23 (d, J = 8.3 Hz, 1H), 7.17 (dd, J = 8.2, 1.8 Hz, 1H), 4.14 – 4.07 (m, 2H), 4.07 – 3.97 (m, 2H), 3.90 (s, 3H), 1.65 (s, 4H), 1.23 (s, 12H). ^{13}C NMR (101 MHz, CDCl$_3$) δ 166.88, 147.43, 144.86, 144.65, 138.21, 129.61, 129.47, 126.45, 126.17, 123.82, 123.25, 109.22, 64.90, 52.07, 35.04, 34.99, 34.31, 34.09, 31.83, 31.76. MS (TOF ESI+) for $C_{25}H_{31}O_4^+$ (M+H)$^+$ calcd. 395.22, found 395.23.

Methyl-6-(2-(5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphthalen-2-yl)-1,3-dioxolan-2-yl)-2-naphthoate (12b): Compound **11b** (570 mg, 1.42 mmol) was treated according to method D. The residue was purified by flash column chromatography (cyclohexane-EtOAc 100:0 v/v increasing to 60:40 v/v) to yield a white solid (557 mg, 88%). mp= 182–184°C. ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.57 (s, 1H), 8.07 (s, 1H), 8.05 (dd, J = 8.6, 1.5 Hz, 1H), 7.89 (dd, J = 8.5, 5.1 Hz, 2H), 7.67 (dd, J = 8.6, 1.6 Hz, 1H), 7.52 (s, 1H), 7.24 (d, J = 9.1 Hz, 2H), 4.19 – 4.11 (m, 2H), 4.11 – 4.02 (m, 2H), 3.97 (s, 3H), 1.65 (s, 4H), 1.25 (s, 6H), 1.23 (s, 6H). ^{13}C NMR (101 MHz, CDCl$_3$) δ 167.22, 144.81, 144.63, 142.46, 138.37, 135.12, 132.07, 130.67, 129.38, 128.63, 127.59, 126.45, 125.43, 125.35, 124.55, 123.85, 123.47, 109.51, 64.98, 52.20, 35.06, 35.00, 34.32, 34.10, 31.85, 31.77. MS (TOF ESI+) for $C_{29}H_{33}O_4^+$ (MH+) calcd. 445.24, found 445.23.

4-(2-(5,5,8,8-Tetramethyl-5,6,7,8-tetrahydronaphthalen-2-yl)-1,3-dioxolan-2-yl)benzoic acid (13a): Compound **12a** (236 mg, 0.59 mmol) was hydrolyzed according to method A. The crude was crystalized from cyclohexane and washed to provide compound **13a** as a white solid (161 mg, 72%). mp = 242–243°C. ^{1}H NMR (400 MHz, CDCl$_3$) δ 12.05 (bs, 1H), 8.07 (d, J = 8.5 Hz, 2H), 7.65 (d, J = 8.5 Hz, 2H), 7.44 (d, J = 1.8 Hz, 1H), 7.24 (d, J = 8.3 Hz, 1H), 7.18 (dd, J = 8.3, 1.9 Hz, 1H), 4.15 – 4.08 (m, 2H), 4.08 – 3.99 (m, 2H), 1.65 (s, 4H), 1.24 (s, 6H), 1.23 (s, 6H). ^{13}C NMR (101 MHz, CDCl$_3$) δ 171.55, 148.46, 144.93, 144.72, 138.13, 130.14, 128.67, 126.50, 126.30, 123.79, 123.24, 109.19, 64.94, 35.05, 35.00, 34.33, 34.11, 31.84, 31.77. HPLC (t= 8.85 min, 98%). HRMS (TOF MS ES+) for $C_{24}H_{29}O_4^+$ (MH+) calcd. 381.2066, found 381.2038.

6-(2-(5,5,8,8-Tetramethyl-5,6,7,8-tetrahydronaphthalen-2-yl)-1,3-dioxolan-2-yl)-2-naphthoic acid (13b): Compound **12b** was hydrolyzed according to method B. The crude oil was purified by flash column chromatography (cyclohexane-EtOAc-HOAc 100:0:0.1 v/v increasing to 80:20:0.1 v/v) to provide a white solid (30 mg, 63%). mp = 222–223°C. ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.68 (s, 1H), 8.12 (d, J = 8.6 Hz, 2H), 7.94 (dd, J = 8.5, 4.6 Hz,

2H), 7.70 (dd, J = 8.6, 1.3 Hz, 1H), 7.52 (s, 1H), 7.24 (d, J = 6.1 Hz, 2H), 4.17 (m,, 2H), 4.13 – 4.03 (m, 2H), 1.65 (s, 4H), 1.25 (s, 6H), 1.23 (s, 6H). ^{13}C NMR (101 MHz, CDCl$_3$) δ 172.09, 144.86, 144.67, 142.95, 138.32, 135.60, 132.02, 131.77, 129.58, 128.82, 126.68, 126.48, 125.60, 125.52, 124.60, 123.87, 123.46, 109.50, 65.01, 35.06, 35.00, 34.33, 34.11, 31.85, 31.77. HPLC (t= 7.74 min, 95%). HRMS (TOF ESI+) for C$_{28}$H$_{31}$O$_4$ $^+$ (M+H)$^+$ calcd. 431.2222, found 431.2222.

Methyl-4-(2-(5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphthalen-2-yl)-1,3-dithiolan-2-yl)benzoate (14a): Compound **10a** (400 mg, 1.14 mmol) was treated according to method E. The crude residue was purified by flash column chromatography (cyclohexane-DCM 100:0 v/v increasing to 60:40 v/v) to yield **14a** as a white solid (482 mg, 99%). mp= 103–105°C. ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.95 (d, J = 8.7 Hz, 2H), 7.72 (d, J = 8.6 Hz, 2H), 7.45 (d, J = 2.1 Hz, 1H), 7.22 (dd, J = 8.4, 2.1 Hz, 1H), 7.17 (d, J = 8.3 Hz, 1H), 3.90 (s, 3H), 3.52 – 3.41 (m, 2H), 3.41 – 3.30 (m, 2H), 1.65 (s, 4H), 1.24 (s, 6H), 1.20 (s, 6H). ^{13}C NMR (101 MHz, CDCl$_3$) δ 166.79, 150.39, 144.37, 144.03, 140.08, 129.12, 128.77, 128.37, 126.24, 125.34, 52.07, 40.23, 35.02, 34.98, 34.33, 34.02, 31.77, 31.71. MS (TOF ESI+) for C$_{25}$H$_{31}$O$_2$S$_2$$^+$ (M+H)$^+$ calcd. 427.18, found 427.18.

Methyl-6-(2-(5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphthalen-2-yl)-1,3-dithiolan-2-yl)-2-naphthoate (14b): Compound **10b** (0.83 g, 2.07 mmol) was treated according to method E. The crude was purified by flash column chromatography (cyclohexane-EtOAc 100:0 v/v increasing to 95:5 v/v) to yield **14b** as a white solid(851 mg, 86%). mp= 153–155°C. ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.55 (s, 1H), 8.24 (d, J = 1.1 Hz, 1H), 8.06 (dd, J = 8.6, 1.5 Hz, 1H), 7.87 (d, J = 8.6 Hz, 1H), 7.84 (d, J = 8.8 Hz, 1H), 7.67 (dd, J = 8.7, 1.9 Hz, 1H), 7.53 (d, J = 2.1 Hz, 1H), 7.24 (dd, J = 8.6, 1.5 Hz, 1H), 7.18 (d, J = 8.4 Hz, 1H), 3.97 (s, 3H), 3.57 – 3.45 (m, 2H), 3.44 – 3.33 (m, 2H), 1.65 (s, 4H), 1.25 (s, 6H), 1.19 (s, 6H). ^{13}C NMR (101 MHz, CDCl$_3$) δ 167.23, 145.05, 144.41, 144.06, 139.86, 134.81, 131.49, 130.38, 128.91, 128.60, 128.24, 127.64, 126.36, 126.26, 125.92, 125.63, 125.53, 52.20, 40.18, 35.05, 35.01, 34.35, 34.04, 31.77, 31.73. MS (TOF ESI+) for C$_{29}$H$_{33}$O$_2$S$_2$$^+$ (M+H)$^+$ calcd. 477.20, found 477.19.

4-(2-(5,5,8,8-Tetramethyl-5,6,7,8-tetrahydronaphthalen-2-yl)-1,3-dithiolan-2-yl)benzoic acid (15a): Compound **14a** (100 mg, 0.23 mmol) in MeOH (5 mL) was hydrolyzed according to method A. The crude was crystalized from cyclohexane to provide compound **15a** as a white solid (90 mg, 95%). mp = 211–212°C. ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.03 (d, J = 8.6 Hz, 2H), 7.76 (d, J = 8.6 Hz, 2H), 7.46 (d, J = 2.0 Hz, 1H), 7.22 (dd, J = 8.4, 2.1 Hz, 1H), 7.18 (d, J = 8.3 Hz, 1H), 3.53 – 3.43 (m, 2H), 3.42 – 3.32 (m, 2H), 1.65 (s, 4H), 1.25 (s, 6H), 1.20 (s, 6H). ^{13}C NMR (101 MHz, CDCl$_3$) δ 171.58, 151.50, 144.44, 144.10, 139.98, 129.80, 128.49, 127.83, 126.30, 126.21, 125.33, 40.28, 35.02, 34.99, 34.35, 34.03, 31.78, 31.72. HPLC (t= 9.35 min, 95%). HRMS (TOF MS ES+) for C$_{24}$H$_{29}$O$_2$S$_2$ $^+$ (MH+) calcd. 413.1609, found 413.1648.

6-(2-(5,5,8,8-Tetramethyl-5,6,7,8-tetrahydronaphthalen-2-yl)-1,3-dithiolan-2-yl)-2-naphthoic acid (15b): To a suspension of the ester **14b** (680 mg, 1.43 mmol) in 75% aqueous MeOH (21 mL) was added KOH (0.77 g), and the mixture was stirred at 80°C for 3

h during which time the compound dissolved. The solution was cooled to room temperature, acidified with 1 N HCl, and a white solid precipitated out. The solid was filtered, washed with water and allowed to dry to yield a white solid, 621 mg (94%). mp= 270–272°C. ^{1}H NMR (400 MHz, DMSO) δ 13.08 (bs, 1H), 8.54 (s, 1H), 8.29 (s, 1H), 8.04 (d, J = 8.6 Hz, 1H), 8.00 (d, J = 8.7 Hz, 2H), 8.00 – 7.96 (m, 1H), 7.53 (dd, J = 8.7, 1.7 Hz, 1H), 7.47 (d, J = 1.9 Hz, 1H), 7.23 (d, J = 8.4 Hz, 1H), 7.14 (dd, J = 8.4, 1.8 Hz, 1H), 3.59 – 3.47 (m, 2H), 3.44 – 3.27 (m, 2H), 1.60 (s, 4H), 1.20 (s, 6H), 1.13 (s, 6H). ^{13}C NMR (101 MHz, DMSO) δ 167.35, 144.88, 143.82, 143.49, 140.23, 134.15, 131.02, 129.84, 129.02, 128.63, 128.45, 127.76, 126.25, 125.76, 125.41, 125.28, 124.99, 76.31, 34.41, 34.36, 33.90, 33.67, 31.55, 31.43. HPLC (t= 9.97 min, 95%). HRMS (TOF ESI+) for $C_{28}H_{31}O_2S_2{}^+$ (M+H)$^+$ calcd. 463.1765, found 463.1769.

(4-Iodophenyl)(5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphthalen-2-yl)methanone (16):
Compound **16** was prepared according to method C starting from 1.90 g of the commercially available 1,1,4,4- tetramethyl-1,2,3,4-tetrahydronaphthalene (10 mmol). After work-up, the crude product was purified by flash column chromatography to yield compound **16** as a yellow oil, 3.57 g (85%). ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.84 (d, J = 8.4 Hz, 2H), 7.76 (d, J = 1.8 Hz, 1H), 7.51 (dd, J = 8.2, 2.0 Hz, 1H), 7.52 (d, J = 8.4 Hz, 2H), 7.40 (d, J = 8.2 Hz, 1H), 1.72 (s, 4H), 1.31 (s, 6H), 1.29 (s, 6H). ^{13}C NMR (101 MHz, CDCl$_3$) δ 195.71, 150.44, 145.20, 137.43, 137.31, 134.27, 131.42, 128.72, 127.21, 126.63, 99.67, 34.81, 34.73, 34.69, 34.39, 31.74, 31.60. MS (TOF ESI+) for $C_{21}H_{24}IO^+$ (M+H)$^+$ calcd. 419.09, found 419.09.

(E)-Methyl 3-(4-(5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphthalene-2-carbonyl)phenyl)acrylate (17): Prepared according to the modified procedure reported by Heck *et al.*[9, 10] To a 50-mL round bottomed flask equipped with a magnetic stir bar was added 1.65 g (3.94 mmol) of iodide **16** and methyl acrylate (1.0 mL, 9.86 mmol). Pd(OAc)$_2$ (9 mg, 0.04 mmol), (2-Tol)$_3$P (48 mg, 0.16 mmol) and Et$_3$N (2.2 mL, 15.78 mmol) were then simultaneously added and a cooled condenser attached. The solution was heated at 100°C for 6 hours. The reaction mixture was allowed to cool to room temperature and 20 mL EtOAc was added After filtration, the solution was concentrated under reduced pressure and purified by flash column chromatography (cyclohexane-EtOAc 100:0 v/v increasing to 95:5 v/v) to yield **17** as a yellow solid (1.22 g, 82%). mp= 138–140°C. ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.82 (d, J = 8.3 Hz, 2H), 7.79 (d, J = 1.8 Hz, 1H), 7.75 (d, J = 16.1 Hz, 1H), 7.63 (d, J = 8.3 Hz, 2H), 7.54 (dd, J = 8.2, 1.9 Hz, 1H), 7.41 (d, J = 8.2 Hz, 1H), 6.55 (d, J = 16.0 Hz, 1H), 3.83 (s, 3H), 1.72 (s, 4H), 1.32 (s, 6H), 1.30 (s, 6H). ^{13}C NMR (101 MHz, CDCl$_3$) δ 195.88, 167.16, 150.58, 145.36, 143.72, 139.42, 137.90, 134.59, 130.65, 128.90, 127.90, 127.44, 126.76, 120.09, 52.01, 34.98, 34.90, 34.85, 34.55, 31.90, 31.77. MS (TOF MS ES+) for $C_{25}H_{29}O_3$ (MH+) calcd. 377.21, found 377.21.

(E)-3-(4-(5,5,8,8-Tetramethyl-5,6,7,8-tetrahydronaphthalene-2-carbonyl)phenyl)acrylic acid (18): Compound **17** (0.82 mmol) was hydrolyzed according to method B. The resultant oil was purified by flash column chromatography (cyclohexane-EtOAc-HOAc 100:0:0.1 v/v increasing to 80:20:0.1 v/v) to provide compound **18** as a white solid (278 mg, 93%). mp = 220–221°C. ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.85 (d, J = 16.8 Hz, 1H), 7.84 (d, J = 8.0 Hz, 1H), 7.80 (d, J = 1.8 Hz, 1H), 7.66 (d, J = 8.2 Hz, 2H), 7.54 (dd, J = 8.2, 1.8 Hz, 1H), 7.41

(d, J = 8.2 Hz, 1H), 6.57 (d, J = 16.0 Hz, 1H), 1.73 (s, 4H), 1.32 (s, 6H), 1.30 (s, 6H). ^{13}C NMR (101 MHz, CDCl$_3$) δ 195.94, 171.95, 150.72, 145.91, 145.41, 139.81, 137.49, 134.51, 130.70, 128.95, 128.21, 128.03, 127.48, 126.80, 119.58, 114.86, 34.98, 34.90, 34.88, 34.57, 31.91, 31.77. HPLC (t= 10.06 min, 80%, (E)-isomer). HRMS (TOF ESI−) for C$_{24}$H$_{25}$O$_3^-$ (M−H)$^-$ calcd. 361.1804 found 361.1817.

Methyl-3-(4-((5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphthalen-2-yl)methyl)phenyl)propanoate (19):

Compound **17** (515 mg, 1.36 mmol) was treated according to method F. The crude was purified on by column chromatography (cyclohexane-ethyl acetate: 100:0 v/v increasing to 90:10 v/v) to provide a colorless oil (479 mg, 93%). ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.19 (d, J = 8.1 Hz, 1H), 7.15 – 7.08 (Aromatic, 5H), 6.90 (dd, J = 8.1, 1.9 Hz, 1H), 3.89 (s, 2H), 3.65 (s, 3H), 2.91 (t, J = 7.9 Hz, 2H), 2.61 (t, J = 7.9 Hz, 2H), 1.66 (s, 4H), 1.25 (s, 12H). ^{13}C NMR (101 MHz, CDCl$_3$) δ 173.52, 144.87, 142.56, 139.40, 138.15, 138.03, 129.17, 128.40, 126.97, 126.69, 126.21, 51.70, 41.41, 35.87, 35.30, 35.25, 34.30, 34.06, 32.02, 32.00, 30.67.

3-{4-[(5,5,8,8-Tetramethyl-5,6,7,8-tetrahydronaphthalen-2-yl)methyl]phenyl}9 propanoic acid (20):

Compound **19** (422 mg, 1.16 mmol) was hydrolyzed according to method B. The crude was purified by flash column chromatography (DCM-MeOH 100:0 v/v increasing to 95:5 v/v) to provide compound **20** as a white solid (301 mg, 77%). ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.20 (d, J = 8.1 Hz, 1H), 7.12 (5H), 6.90 (dd, J = 8.1, 1.8 Hz, 1H), 3.89 (s, 2H), 2.92 (t, J = 7.8 Hz, 2H), 2.66 (t, J = 7.8 Hz, 2H), 1.66 (s, 4H), 1.25 (s, 12H). mp= 142–143°C. HPLC (t= 9.83 min, 95%). HRMS (TOF ESI+) for C$_{24}$H$_{31}$O$_2^+$ (M+H)$^+$ calcd. 351.2324, found 351.2321.

(E)-Methyl-3-(4-(2-(5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphthalen-2-yl)-1,3-dioxolan-2-yl)- phenyl)acrylate (21):

Compound **17** (650 mg, 1.73 mmol) was treated according to method D. The crude was puridied by flash column chromatography (cyclohexane-EtOAc 100:0 v/v increasing to 80:20 v/v). Compound **21** was obtained as a white solid, 385 mg (53%). mp= 155–157°C. ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.67 (d, J = 16.0 Hz, 1H), 7.55 (d, J = 8.4 Hz, 2H), 7.48 (d, J = 8.3 Hz, 2H), 7.45 (d, J = 1.9 Hz, 1H), 7.23 (d, J = 8.2 Hz, 1H), 7.17 (dd, J = 8.2, 1.9 Hz, 1H), 6.42 (d, J = 16.0 Hz, 1H), 4.12 – 4.05 (m, 2H), 4.05 – 3.99 (m, 2H), 1.65 (s, 4H), 1.24 (s, 6H), 1.23 (s, 6H). ^{13}C NMR (101 MHz, CDCl$_3$) δ 167.56, 144.94, 144.92, 144.77, 144.62, 138.51, 134.09, 128.06, 126.86, 126.60, 123.91, 123.47, 118.03, 109.42, 77.48, 77.16, 76.84, 65.02, 51.84, 35.22, 35.16, 34.47, 34.25, 32.00, 31.93. MS (TOF ESI+) for C$_{27}$H$_{33}$O$_4^+$ (M+H)$^+$ calcd. 421.24, found 421.24.

(E)-3-(4-(2-(5,5,8,8-Tetramethyl-5,6,7,8-tetrahydronaphthalen-2-yl)-1,3-dioxolan-2-yl)phenyl)- acrylic acid (22):

Compound **21** was hydrolyzed according to method B. T The crude was purified by flash column chromatography (DCM-MeOH 100:0 v/v increasing to 95:5 v/v) to provide compound **22** as a white solid, 50 mg (56%). mp = 241–243°C. ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.77 (d, J = 15.9 Hz, 1H), 7.57 (d, J = 8.3 Hz, 2H), 7.51 (d, J = 8.3 Hz, 2H), 7.45 (d, J = 1.6 Hz, 1H), 7.23 (d, J = 8.3 Hz, 1H), 7.17 (dd, J = 8.2, 1.7 Hz, 1H), 6.43 (d, J = 16.0 Hz, 1H), 4.21 – 3.93 (m, 4H), 1.65 (s, 4H), 1.25 (s, 6H), 1.23 (s,

6H). ^{13}C NMR (101 MHz, CDCl$_3$) δ 172.04, 146.81, 145.43, 144.97, 144.81, 138.45, 133.74, 128.37, 126.94, 126.62, 123.92, 123.46, 117.42, 109.40, 65.04, 35.22, 35.16, 34.48, 34.26, 32.01, 31.93. HPLC (t= 9.60 min, 100%). HRMS (TOF ESI+) for C$_{26}$H$_{31}$O$_4^+$ (MH$^+$) calcd. 407.2222, found 407.2228.

Methyl 3-{4-[2-(5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphthalen-2-yl)-1,3-dioxolan-2-yl]phenyl}propano ate (23): Compound **21** (750 mg, 1.78 mmol) was treated according to method F. Further purification by flash column chromatography (cyclohexane-dichloromethane 80:20 v/v increasing to 0:100 v/v) yielded compound **23** as a white solid, 520 mg (70%). mp= 120–122°C. ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.38 – 7.49 (m, 3H), 7.19 – 7.24 (m, 3H), 7.10 – 7.19 (m, 3H), 3.93 – 4.13 (m, 4H), 3.66 (s, 3H), 2.93 (t, J = 7.84 Hz, 2H), 2.61 (t, J = 7.84 Hz, 2H), 1.65 (s, 4H), 1.24 (2s, 12H). ^{13}C NMR (101 MHz, CDCl3) δ 173.3, 144.5, 144.4, 140.5, 140.2, 138.8, 128.0, 126.4, 126.3, 123.9, 123.5, 109.6, 64.8, 51.6, 35.6, 35.2, 35.1, 34.3, 34.1, 31.9, 31.8, 30.6. MS (ESI+) [M+H]$^+$ 423.24, calc. 423.25.

3-{4-[2-(5,5,8,8-Tetramethyl-5,6,7,8-tetrahydronaphthalen-2-yl)-1,3-dioxolan-2-yl] phenyl}4 propanoic acid (24): Compound **23** (250 mg, 0.60 mmol) was hydrolyzed according to method B. No further purification is needed after work-up. Compound **24** was obtained as a white solid (220 mg, 91%). mp= 219°–220°C. ^{1}H NMR (400 MHz, CDCl3) δ ppm 7.44 (m, 3 H), 7.19 – 7.24 (d, J=8.28 Hz 1 H), 7.17 (m, 3 H), 3.97 – 4.09 (m, 4 H), 2.94 (t, J=7.84 Hz, 2 H), 2.66 (t, J=7.78 Hz, 2 H), 1.65 (s, 4 H), 1.24 (s, 6 H),1.23 (s, 6H). ^{13}C NMR (101 MHz, CDCl$_3$) δ ppm 178.53, 144.53, 144.46, 140.58, 139.83, 138.78, 128.00, 126.46, 126.36, 123.88, 123.50, 109.56, 64.80, 35.40, 30.26, 35.14, 35.09, 34.33, 34.12, 31.89, 31.83. HPLC (t= 9.13 min, 99%). HRMS (TOF ESI−) for C$_{26}$H$_{31}$O$_4^-$ (M−H)$^-$ calcd. 407.2222 found 407.2216.

Methyl-3-[4-(5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphthalene-2-carbonyl) phenyl] propanoate (25): A mixture of compound **23** (265 mg, 0.63 mmol, 1 eq.), iodine (160 mg, 0.63 mmol, 1 eq.) and 4Å molecular sieves (250 mg) in acetone (6 mL) was stirred for 8 hours under reflux according to a modified method of Sun et al.[20] After work-up and purification by flash column chromatography (cyclohexane-DCM 80:20 v/v increasing to 20:80 v/v, compound **25** was obtained as a colorless oil (150 mg, 63%). ^{1}H NMR (400 MHz, CDCl3) δ ppm 7.71 – 7.80 (m, 3 H), 7.53 (d, J=8.16 Hz, 1 H), 7.39 (d, J=8.16 Hz, 1 H), 7.31 (d, J=7.91 Hz, 2 H), 3.69 (s, 3 H), 3.04 (t, J=7.78 Hz, 2 H), 2.69 (t, J=7.72 Hz, 2 H), 1.72 (s, 4 H), 1.31 (s, 6 H), 1.30 (s, 6H). ^{13}C NMR (101 MHz, CDCl3) δ ppm 196.25, 173.01, 149.95, 145.11, 145.01, 136.17, 134.89, 130.42, 128.71, 128.12, 127.29, 126.45, 51.72, 35.21, 34.89, 34.81, 34.66, 34.40, 31.76, 31.64, 30.86. MS (TOF ESI+) for C$_{25}$H$_{31}$O$_3^+$ (M +H)$^+$ calcd. 379.23, found 379.22.

3-[4-(5,5,8,8-Tetramethyl-5,6,7,8-tetrahydronaphthalene-2-carbonyl)phenyl] propanoic acid (26): Compound **25** (50 mg, 0.132 mmol) was hydrolyzed according to method B. No further purification was needed after work-up. Compound **25** was obtained as a white solid (45 mg, 94%). mp= 153°–155°C. ^{1}H NMR (400 MHz, CDCl3) δ 7.70 – 7.83 (m, 3H), 7.53 (dd, J = 1.82, 8.25 Hz, 1H), 7.39 (d, J = 8.25 Hz, 1H), 7.32 (d, J = 8.16 Hz, 2H), 3.05 (t, J = 7.72 Hz, 2H), 2.75 (t, J = 7.72 Hz, 2H), 1.72 (s, 6H), 1.31 (2s, 12H). ^{13}C NMR (101 MHz,

CDCl3) δ ppm 196.31, 150.01, 145.03, 144.76, 136.24, 134.84, 130.46, 128.73, 128.12, 127.30, 126.47, 35.01, 34.88, 34.80, 34.66, 34.40, 31.76, 31.64, 30.51. HPLC (t= 8.96 min, 99%). HRMS (TOF ESI−) (M−H)− calcd. 363.1960, found 363.1975.

(E)-Methyl-3-(4-(2-(5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphthalen-2-yl)-1,3-dithiolan-2-yl)- phenyl)acrylate (27): Compound **17** (347 mg, 0.92 mmol) was treated according to method E. The crude was purified by flash column chromatography (cyclohexane-EtOAc 100:0 v/v increasing to 90:10 v/v) to provide **27** a colorless oil (380 mg, 91%). ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.67 (d, J = 16.2 Hz, 1H), 7.66 (d, J = 8.4 Hz, 2H), 7.50 (d, J = 2.0 Hz, 1H), 7.44 (d, J = 8.4 Hz, 2H), 7.22 (dd, J = 8.4, 2.1 Hz, 1H), 7.18 (d, J = 8.3 Hz, 1H), 6.42 (d, J = 16.0 Hz, 1H), 3.80 (s, 3H), 3.50 – 3.40 (m, 2H), 3.42 – 3.32 (m, 2H), 1.66 (s, 4H), 1.25 (s, 6H), 1.21 (s, 6H). ^{13}C NMR (101 MHz, CDCl$_3$) δ 167.59, 147.72, 144.48, 144.42, 144.11, 140.44, 133.28, 129.03, 127.73, 126.37, 125.58, 117.95, 51.85, 40.37, 35.20, 35.16, 34.51, 34.18, 31.94, 31.88. MS (TOF ESI+) for $C_{27}H_{33}O_2S_2^+$ (M+H)$^+$ calcd. 453.1922, found 453.1916.

(E)-3-(4-(2-(5,5,8,8-Tetramethyl-5,6,7,8-tetrahydronaphthalen-2-yl)-1,3-dithiolan-2-yl)phenyl)- acrylic acid (28): Compound **27** (86 mg, 0.19 mmol) was hydrolyzed according to method B. Without any further purification, compound **28** is obtained as a white solid, 81 mg (97%). mp = 234–236°C. ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.77 (d, J = 15.9 Hz, 1H), 7.68 (d, J = 8.4 Hz, 2H), 7.50 (d, J = 2.0 Hz, 1H), 7.47 (d, J = 8.4 Hz, 2H), 7.22 (dd, J = 8.4, 2.1 Hz, 1H), 7.18 (d, J = 8.3 Hz, 1H), 6.43 (d, J = 16.0 Hz, 1H), 3.46 (ddd, J = 12.3, 9.4, 7.4 Hz, 2H), 3.42 – 3.34 (m, 2H), 1.66 (s, 4H), 1.25 (s, 6H), 1.21 (s, 6H). ^{13}C NMR (101 MHz, CDCl$_3$) δ 171.89, 148.29, 146.63, 144.52, 144.16, 140.37, 132.92, 129.11, 128.04, 126.40, 126.38, 125.57, 117.27, 40.40, 35.20, 35.16, 34.52, 34.19, 31.95, 31.89. HPLC (t= 10.02 min, 100%). HRMS (TOF ESI+) for $C_{26}H_{31}O_2S_2^+$ (M+H)$^+$ calcd. 439.1765, found 439.1765.

Methyl-3-{4-[2-(5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphthalen-2-yl)-1,3-dithiolan-2-yl]phenyl}propanoate (29): Compound **25** (142 mg, 0.375 mmol) was treated according to method E. The crude was purified by flash column chromatography (cyclohexane/EtOAc 95:5 v/v increasing to 75:25 v/v) to provide a colorless oil (116 mg, 68%). ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.54 (d, J = 8.4 Hz, 2H), 7.52 (d, J = 2.1 Hz, 1H), 7.22 (dd, J = 8.4, 2.2 Hz, 1H), 7.16 (d, J = 8.4 Hz, 1H), 7.11 (d, J = 8.3 Hz, 2H), 3.67 (s, 3H), 3.48 – 3.29 (m, 4H), 2.93 (t, J = 7.9 Hz, 2H), 2.62 (t, J = 7.9 Hz, 2H), 1.65 (s, 4H), 1.24 (s, 6H), 1.21 (s, 6H). ^{13}C NMR (101 MHz, CDCl3) δ ppm 173.32, 144.05, 143.64, 142.86, 140.91, 139.30, 128.50, 127.66, 126.28, 126.05, 125.54, 51.61, 40.04, 35.49, 35.10, 35.07, 34.33, 33.99, 31.78, 31.74, 30.41. MS (TOF ESI+) for $C_{27}H_{35}O_2S_2O_2^+$ (M+H)$^+$ calcd. 455.21, found 455.21.

3-{4-[2-(5,5,8,8-Tetramethyl-5,6,7,8-tetrahydronaphthalen-2-yl)-1,3-dithiolan-2-yl]phenyl}propanoic acid (30): Compound **29** (102 mg, 0.224 mmol) was hydrolyzed according to method B. Without any further purification, compound **30** was obtained as a white solid (80 mg, 82%). mp= 165–167°C. ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.55 (d, J = 8.3 Hz, 2H), 7.52 (d, J = 2.1 Hz, 1H), 7.22 (dd, J = 8.4, 2.2 Hz, 1H), 7.16 (d, J = 8.4 Hz, 1H), 7.12 (d, J = 7.4 Hz, 2H), 3.49 – 3.32 (m, 4H), 2.94 (t, J = 7.8 Hz, 2H), 2.67 (t, J = 7.8 Hz,

2H), 1.65 (s, 4H), 1.25 (d, $J = 1.7$ Hz, 6H), 1.21 (s, 6H). ^{13}C NMR (101 MHz, CDCl3) δ ppm 177.50, 144.10, 143.68, 143.04, 140.91, 139.00, 128.58, 127.67, 126.32, 126.08, 125.57, 40.06, 35.15, 35.11, 34.35, 34.02, 31.80, 31.76, 30.11. HPLC (t= 9.67 min, 96%). HRMS (TOF ESI–) for $C_{26}H_{31}O_2S_2{}^-$ (M−H)$^-$ calcd. 439.1765, found 439.1743.

5,5,8,8-Tetramethyl-5, 6, 7,8-tetrahydro-naphthalene-2-disulfide (31): 5,5,8,8-Tetramethyl-5, 6, 7,8-tetrahydro-naphthalene-2-disulfide was prepared according to a procedure described by Boiteau et al.[21] mp= 83–85°C. ^{1}H NMR (400 MHz, CDCl3) δ ppm 7.41 (d, J=1.88 Hz, 2 H), 7.28 (dd, J=8.28, 1.88 Hz, 2 H), 7.23 (d, J=8.28 Hz, 2 H), 1.65 (s, 8 H), 1.21 (s, 12 H,) 1.24 (s, 12 H). ^{13}C NMR (101 MHz, CDCl3) δ ppm 145.79, 144.46, 134.05, 127.45, 126.89, 125.93, 34.97, 34.95, 34.45, 34.15, 31.75. MS (TOF ESI+) for $C_{28}H_{39}S_2{}^+$ (M+H)$^+$ calcd. 439.24, found 439.25.

Ethyl-3-{4-[(5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphthalen-2-yl)sulfanyl] phenyl} propanoate (32): Compound **32** was prepared according to the method described by Fukuzawa et al.[22] 5, 5,8,8-Tetramethyl-5, 6, 7,8-tetrahydro-naphthalene-2-disulfide (100 mg, 0.228 mmol, 0.5eq.), PdCl$_2$(dppf) (17 mg, 0.023 mmol, 0.05 eq.), and zinc (36 mg, 0.547 mmol, 1.2 eq.) were placed in a flask and then a solution of ethyl 3-(4-bromophenyl) propanoate (115 mg, 0.456 mmol, 1 eq.) in THF (3 mL) was added. The mixture was refluxed for 24 h and diluted with Et$_2$O (30 mL) after cooling. The precipitate was removed by filtration and the filtrate was washed with brine and dried over CaSO$_4$. After concentration of the organic layer, the crude was concentrated and grossly purified by column chromatography (cyclohexane-DCM 95:5 v/v increasing to 55:45 v/v). A mixture of **32** and ethyl 3-(4-bromophenyl) propanoate (30%) was obtained (125 mg). It was used in the next step without any further purification.

Ethyl 3-[4-(5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphthalene-2-sulfonyl)phenyl] propanoate (33): Compound **32** (125 mg, 0.32 mmol) was treated according to method H. After drying over CaSO$_4$ and evaporation, the crude was purified by flash column chromatography (cyclohexane-DCM 80:20 v/v increasing to 0:100 v/v). Compound **33** was obtained as a white solid (82 mg, 87%). mp= 105–106°C. ^{1}H NMR (400 MHz, CDCl$_3$) δ ppm 7.88 – 7.92 (m, 1 H), 7.85 (m, J=8.16 Hz, 2 H), 7.58 (dd, J=8.41, 1.51 Hz, 1 H), 7.39 (d, J=8.41 Hz, 1 H), 7.33 (m, J=8.16 Hz, 2 H), 4.10 (q, J=7.11 Hz, 2 H), 2.99 (t, J=7.53 Hz, 2 H), 2.62 (t, J=7.53 Hz, 2 H), 1.68 (s, 4 H), 1.28 (s, 6 H), 1.25 (s, 6 H),1.19 (t, J=7.11 Hz, 3 H). ^{13}C NMR (101 MHz, CDCl$_3$) δ ppm 172.28, 150.80, 146.45, 146.21, 140.05, 138.55, 129.18, 127.81, 127.77, 125.89, 124.49, 60.63, 35.14, 34.69, 34.66, 34.61, 34.55, 31.70, 31.57, 30.68, 14.15. MS (TOF ESI+) for $C_{50}H_{65}S_2O_4{}^+$ (2M+H)$^+$ calcd. 857.40, found 857.40.

3-[4-(5,5,8,8-Tetramethyl-5,6,7,8-tetrahydronaphthalene-2-sulfonyl)phenyl] propanoic acid (34): 3-Compound **33** (66 mg, 0.014 mmol) was hydrolyzed according to method B. With no further purification, compound **34** is obtained as a white solid (53 mg, 85%). mp= 211°C. ^{1}H NMR (400 MHz, METHANOL-d_4) δ ppm 7.90 (d, J=2.01 Hz, 1 H), 7.87 (m, J=8.41 Hz, 2 H), 7.65 (dd, J=8.41, 2.01 Hz, 1 H), 7.56 (d, J=8.41 Hz, 1 H), 7.48 (m, J=8.41 Hz, 2 H), 3.01 (t, J=7.59 Hz, 2 H), 2.65 (t, J=7.59 Hz, 2 H), 1.75 (s, 4 H), 1.32 (s, 6H), 1.30

(s, 6H). ^{13}C NMR (101 MHz, METHANOL-d_4) δ ppm 176.28, 152.55, 148.81,31. 147.99,87, 141.29, 140.25, 130.73, 129.42, 128.83, 127.00, 125.71, 36.03, 35.87, 35.78, 32.18, 32.02. HPLC (t= 8.44 min, 99%). HRMS (TOF ESI−) for $C_{23}H_{27}O_4S^-$ (M−H)$^-$ calcd. 399.1630, found 399.1658.

6-Iodo-1,1,4,4-tetramethyl-1,2,3,4-tetrahydronaphthalene (35): 6-iodo-1,1,4,4-tetramethyl-1,2,3,4-tetrahydronaphthalene was prepared according to Christie et al.[23]

Methyl-2-{4-[(5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphthalen-2-yl)sulfanyl] phenyl}acetate (36a): Compound **35** (305 mg, 0.97 mmol) was treated according to method G. A colorless oil was obtained (225 mg, 60%) as a mixture of compound **36a** and its butyl ester derivative (25%). ^{1}H NMR (400 MHz, CDCl$_3$) δ ppm 7.35 (d, J=1.88 Hz, 1 H), 7.22 – 7.25 (m, 3 H), 7.16 – 7.21 (m, 2 H), 7.09 (dd, J=8.28, 1.51 Hz, 1 H), 3.69 (s, 3 H), 3.59 (s, 2 H), 1.67 (s, 4 H), 1.24 (s, 6 H), 1.26 (s, 6 H). ^{13}C NMR (101 MHz, CDCl$_3$) δ ppm 171.82, 146.10, 144.52, 135.78, 132.16, 130.95, 130.25, 129.97, 129.91, 129.10, 127.63, 52.09, 40.70, 34.97, 34.93, 34.37, 34.15, 31.75. MS (TOF ESI+) for $C_{46}H_{57}O_4S_2Na^+$ (2M+Na)$^+$ calcd. 759.36, found 759.35.

Methyl-2-{3-[(5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphthalen-2-yl)sulfanyl] phenyl} acetate (36b): Compound **35** (305 mg, 0.97 mmol) was treated according to method G. A colorless oil (210 mg, 55%) was obtained as a mixture of compound **36b** and its butyl ester derivative (25%). ^{1}H NMR (400 MHz, CDCl$_3$) δ ppm 7.34 (d, J=1.88 Hz, 1 H), 7.23 (m, 3 H), 7.14 – 7.18 (m, 1 H), 7.07 – 7.14 (m, 2 H), 3.67 (s, 3 H), 3.57 (s, 2 H), 1.68 (s, 4 H), 1.24 (s, 6 H), 1.27 (s, 6 H). ^{13}C NMR (101 MHz, CDCl3) δ ppm 171.62, 146.11, 144.52, 137.28, 134.83, 130.80, 130.43, 130.18, 129.16, 129.02, 128.42, 127.62, 127.31, 52.05, 40.97, 34.96, 34.93, 34.37, 34.15, 31.75, 31.71. MS (TOF ESI+) for $C_{46}H_{57}O_4S_2Na^+$ (2M +Na)$^+$ calcd. 759.36, found 759.35.

Methyl-2-[4-(5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphthalene-2-sulfonyl) phenyl]acetate (37a): Compound **36a** (105 mg, 0.27 mmol) was treated according to method H. After drying over CaSO$_4$ and evaporation, the crude was purified by flash column chromatography (cyclohexane/dichloromethane): (80/20) to (0/100). An off-white solid was obtained (61 mg, 54%) as a mixture of compound **37a** and its butyl ester derivative (5%). ^{1}H NMR (400 MHz, CDCl3) δ ppm 7.86 – 7.93 (m, 3 H), 7.58 (dd, J=8.41, 2.01 Hz, 1 H), 7.40 (m, 3 H), 3.69 (s, 3 H), 3.67 (s, 2H), 1.68 (s, 4 H), 1.29 (s, 6 H), 1.26 (s, 6 H). ^{13}C NMR (101 MHz, CDCl3) δ ppm 170.99, 150.99, 146.54, 141.05, 139.24, 138.36, 130.29, 130.19, 127.88, 126.00, 124.61, 52.32, 40.84, 34.74, 34.69, 34.63, 34.58, 31.72, 31.59. MS (TOF ESI+) for $C_{46}H_{57}O_8S_2^+$ (2M+H)$^+$ calcd. 801.34, found 801.34.

Methyl-2-[3-(5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphthalene-2-sulfonyl) phenyl] acetate (37b): Compound **36b** (90 mg, 0.23 mmol) was treated according to method H. After drying over CaSO$_4$ and evaporation, the crude was purified by flash column chromatography (cyclohexane/dichloromethane): (80/20) to (0/100). Compound **37b** was obtained as a thick oil (50 mg, 51%). ^{1}H NMR (400 MHz, CDCl3) δ ppm 7.90 (d, J=2.01 Hz, 1 H), 7.88 (s, 1 H), 7.83 (dt, J=7.00, 1.65 Hz, 1 H), 7.59 (dd, J=8.41, 1.88 Hz, 1 H), 7.43 – 7.52 (m, 2 H), 7.40 (d, J=8.41 Hz, 1 H), 3.69 (s, 5 H), 1.68 (s, 4 H), 1.29 (s, 6 H), 1.26 (s,

6 H). ^{13}C NMR (101 MHz, CDCl$_3$) δ ppm 170.98, 150.99, 146.54, 142.39, 138.30, 135.38, 133.94, 129.43, 128.34, 127.84, 126.44, 126.01, 124.55, 52.25, 40.70, 34.72, 34.67, 34.61, 34.55, 31.70, 31.57. MS (TOF ESI+) for C$_{46}$H$_{57}$O$_8$S$_2^+$ (2M+H)$^+$ calcd. 801.34, found 801.34.

2-{4-[(5,5,8,8-Tetramethyl-5,6,7,8-tetrahydronaphthalen-2-yl)sulfanyl] phenyl}acetic acid (38a): Compound **36a** (50 mg, 0.136 mmol) was hydrolyzed according to method B. With no further purification, compound **38a** was obtained as a white solid (43 mg, 99%). mp = 87°C. ^{1}H NMR (400 MHz, CDCl$_3$) δ ppm 7.33 – 7.37 (m, 1 H), 7.15 – 7.25 (m, 5 H), 7.07 – 7.12 (m, 1 H), 3.60 (s, 2 H), 1.67 (s, 4 H), 1.27 (s, 6 H), 1.24(s, 6 H). ^{13}C NMR (101 MHz, CDCl$_3$) δ ppm 177.38, 146.17, 144.67, 136.36, 131.30, 130.71, 130.50, 130.03, 129.97, 129.80, 129.34, 127.70, 40.52, 34.99, 34.96, 34.41, 34.19, 31.79. HPLC (t= 9.50 min, 95%). HRMS (TOF ESI−) for [(C$_{22}$H$_{26}$O$_2$S)$_2$−1]$^-$ (2M−H)$^-$ calcd. 707.3229, found 707.3204.

2-{3-[(5,5,8,8-Tetramethyl-5,6,7,8-tetrahydronaphthalen-2-yl)sulfanyl]phenyl} acetic acid (38b): Compound **36b** (50 mg, 0.136 mmol) was hydrolyzed according to method B. With no further purification, compound **38b** was obtained as a colorless oil (43 mg, 99%). ^{1}H NMR (400 MHz, CDCl$_3$) δ ppm 7.34 (d, J=1.88 Hz, 1 H), 7.19 – 7.24 (m, 3 H), 7.14 – 7.19 (m, 1 H), 7.07 – 7.13 (m, 2 H), 3.58 (s, 2 H), 1.67 (s, 4 H), 1.27 (s, 6 H), 1.22 (s, 6H). ^{13}C NMR (101 MHz, CDCl$_3$) δ ppm 177.31, 146.20, 144.63, 137.55, 134.15, 130.68, 130.50, 130.30, 129.25, 129.14, 128.58, 127.68, 127.41, 34.99, 34.98, 34.40, 34.19, 31.79, 31.75. HPLC (t= 9.45 min, 95%). HRMS (TOF ESI−) for C$_{22}$H$_{25}$O$_2$S$^-$ (2M−H)$^-$ calcd. 707.3229, found 707.3286.

2-[4-(5,5,8,8-Tetramethyl-5,6,7,8-tetrahydronaphthalene-2-sulfonyl)phenyl]acetic acid (39a): Compound **37a** (45 mg, 0.112 mmol) was hydrolyzed according to method B. With no further purification, compound **39a** was obtained as white solid (31 mg, 72%). mp = 214°C. ^{1}H NMR (400 MHz, CDCl$_3$) δ ppm 7.87 – 7.93 (m, 3 H), 7.58 (dd, J=8.41, 2.01 Hz, 1 H), 7.40 (m, 3 H), 3.70 (s, 2 H), 1.68 (s, 4 H), 1.29 (s, 6 H), 1.25 (s, 6 H). ^{13}C NMR (101 MHz, CDCl$_3$) δ ppm 175.32, 151.05, 146.56, 141.30, 138.42, 138.23, 130.25, 127.92, 127.88, 126.01, 124.60, 34.74, 34.68, 34.62, 34.55, 31.71, 31.59. HPLC (t= 8.32 min, 99%). HRMS (TOF ESI−) for [(C$_{22}$H$_{26}$O$_4$S)$_2$−1]− (M−H)− calcd. 771.3025, found 771.3063.

2-[3-(5,5,8,8-Tetramethyl-5,6,7,8-tetrahydronaphthalene-2-sulfonyl)phenyl]acetic acid (39b): Compound **38b** (45 mg, 0.112 mmol) was hydrolyzed according to method B. With no further purification, compound **39b** was obtained as white solid (41 mg, 95%). mp = 75°C. ^{1}H NMR (400 MHz, CDCl$_3$) δ ppm 7.89 (s, 2 H), 7.84 (d, J=6.27 Hz, 1 H), 7.56 – 7.63 (m, 1 H), 7.43 – 7.52 (m, 2 H), 7.39 (d, J=8.41 Hz, 1 H), 3.72 (s, 2 H), 1.67 (s, 4 H), 1.27 (s, 6 H), 1.25 (s, 6 H). ^{13}C NMR (101 MHz, CDCl$_3$) δ ppm 175.74, 151.05, 146.59, 142.48, 138.22, 134.75, 134.01, 129.49, 128.46, 127.86, 126.62, 126.04, 124.54, 34.73, 34.66, 34.61, 34.56, 31.68, 31.57. HPLC (t= 8.27 min, 99%). HRMS (TOF ESI−) for C$_{22}$H$_{25}$O$_4$S$^-$ (M−H)− calcd. 385.1474, found 385.1494.

Crystallography

Compounds **7** and **8** yielded crystals of suitable quality for X-ray diffraction by slow evaporation of ethyl acetate/heptane solutions. These crystals were used to confirm the structures using X-ray data collected at 90 K, with Cu Kα radiation (λ=1.54178 Å) on a Bruker Kappa Apex-II diffractometer. Crystals of **8** are triclinic, space group P-1 with Z=2, R=0.036, CCDC 896010. Crystals of **7** are monoclinic, space group P2$_1$/c with Z=4, R=0.065, CCDC 896011. There is a conformational disorder of the six-membered ring carrying the four methyl groups. The CIFs have been deposited at the Cambridge Crystallographic Data Centre and can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif.

CYP26A1 and CYP26B1 inhibition assays

CYP26A1 and CYP26B1 were expressed in Sf9 cells and used as microsomal fractions supplemented with rat P450 reductase expressed in *Escherichia coli* as described previously.[11,33] Incubations were performed with 5 pmol of P450 (CYP26A1 or CYP26B1) and 10 pmol of P450 reductase. The purified rat reductase was added to CYP26A1 or CYP26B1 microsomes, and allowed to incorporate into the membrane for 10 min at room temperature. The final volume of each incubation sample was then brought to 1 ml by adding 100 mM potassium phosphate (KPi) buffer, pH 7.4, 9-*cis*-RA, and, when appropriate, inhibitor or solvent. Compounds were dissolved in methanol or dimethyl sulfoxide, and final solvent amounts in the incubations were kept at 1%. The samples were preincubated for 5 min at 37°C before the reaction was initiated with NADPH (final concentration 1 mM). Incubation times were 1 minute and 5 minutes for CYP26A1 and CYP26B1 incubations respectively.

To determine whether the RA isomer 9-*cis*-RA is a substrate of CYP26B1, 9-*cis*-RA was incubated with CYP26B1. The formation of 9-*cis*-4-OH-RA by CYP26B1 was measured by HPLC as described previously for CYP26A1.[25] Product formation was linear from 1 minute to 8 minutes. The K$_m$ and k$_{cat}$ of 9-*cis*-RA hydroxylation by CYP26B1 was determined by incubating 8 different concentrations of 9-*cis*-RA between 50 nM and 1000 nM with CYP26B1. Five minutes after reactions were initiated with NADPH the reactions were quenched with 5 ml of ethyl acetate, acitretin was added as an internal standard, samples extracted, evaporated to dryness, reconstituted in methanol and analyzed by HPLC as described previously.[25] A standard curve of 9-*cis*-4-OH-RA was used to quantify product formation by analysis of the peak area of the primary metabolite on an HPLC. The Michaelis-Menten equation was fit to the data using GraphPad Prism (GraphPad Software Inc., San Diego, CA), and the K$_m$ and V$_{max}$ values were obtained from this fit. 9-*cis*-RA was then used as the substrate for subsequent assays of inhibitor potency at 100 nM concentration.

Twenty novel compounds, **1**, **3** and two RAR ligands **15b** and **13a** were tested as inhibitors of CYP26A1 and CYP26B1. The formation of 9-*cis*-4-OH-RA metabolite was monitored and the percent activity remaining in the presence of the inhibitor in comparison to the solvent only control was quantified. For IC$_{50}$ determination, 6–8 concentrations of the inhibitor spanning below and above the predicted IC$_{50}$ were tested, and each concentration

was analyzed in triplicate. The IC_{50} values were determined by nonlinear regression using GraphPad Prism, according to eq. 1:

$$100\% \cdot \frac{V_i}{V} = \left(\frac{V_i}{V}\right)_{min} \cdot 100\% + \frac{((V_i/V)_{max} - (V_i/V)_{min} \cdot 100\%)}{(1 + 10^{(I - logIC_{50})})} \quad (1)$$

in which 100% *(V_i/V) is the percentage of activity remaining at a given inhibitor (I) concentration, $(V_i/V)_{max}$*100% is the fitted maximum percentage activity remaining, and $(V_i/V)_{min}$*100% is the minimum percentage activity remaining. For compounds with IC_{50} values less than 100 nM, all fits were corrected for inhibitor depletion, and the K_d was determined using the Morrison equation as described previously [15] according to eq. 2:

$$[EI] = \frac{[E] + [I] + K_d - \sqrt{([E] + [I] + K_d)^2 - 4[E][I]}}{2} \quad (2)$$

in which K_d is the affinity constant of the inhibitor, [I] is the concentration of inhibitor, [E] is the concentration of enzyme, and [EI] is the concentration of the enzyme-inhibitor complex.

RAR activation and induction of RARγ and CYP26A1 in HepG2 cells

The HepG2 cells were obtained and cultured in a humidified incubator at 37 °C under a 5% atmosphere of carbon dioxide using DMEM (Dulbecco's Modified Eagle Medium) containing 4.5g/L D-glucose, L-glutamine, and 110mg/L Sodium Pyruvate supplemented with 10% FBS and 1% penicillin. (Life Technologies, Grand Island, NY) For all treatments, cells were plated into six well plates in 2 mL media and allowed to adhere for 24 hours prior to treatments.

To test whether any of the candidate compounds activate RAR, HepG2 cells were treated for 24 hours in 2 mL media with 0.1% DMSO (vehicle control), and the test compounds at a concentration of 1 μM in 0.1% DMSO. To determine whether the test compounds inhibited a*t*RA metabolism in the HepG2 cells and hence potentiated the RAR activation (CYP26A1 and RARγ induction) cells were treated with either vehicle control (0.1% DMSO+0.01% ethanol), 10, 100 or 1000nM a*t*RA or 1 μM of each test compound together with 100 nM a*t*RA. Each treatment was performed as triplicate. At the completion of each treatment, the media was collected for measurement of a*t*RA concentration and cells were harvested for mRNA extraction. To each well, 300 μL of TRI reagent (Invitrogen, Grand Island, NY) was added and mRNA extracted according to manufacturers recommendations. mRNA concentration was quantified using the Nanodrop 2000c Spectrophotometer. (Thermofisher Sci., Waltham, MA) and cDNA generated from 1 μg mRNA using Taqman® Gene expression reagents (Applied Biosystems, Carlsbad, CA). RT-PCR was used to quantify CYP26A1 mRNA (StepOnePlus™, Applied Biosystems, Carlsbad, CA) as previously described.[30] TaqMan real-time gene expression master mix and PCR primers and fluorescent probes were obtained from Applied Biosystems (Foster City, CA). Probes were labeled with the 5′-reporter dye 5-carboxyfluorescein and a nonfluorescent black hole quencher on the 3′-end. Primer and probe pairs used included: CYP26A1 (Hs00175627_m1, FAM), GAPDH (Hs99999905_m1, VIC). GAPDH was used as the housekeeping gene and all assays were done as multiplexes. All triplicate samples were analyzed in singlet. Changes in target

mRNA were measured using relative quantification (fold-difference) and the C_T method.[30] The effect of the inhibitors on *at*RA metabolism in HepG2 cells was determined using LC-MS/MS as previously described,[29] using an AB Sciex QTRAP 4500 mass spectrometer coupled with an Agilent 1290 Infinity UHPLC and Agilent Zorbax C18 column (3.5 μm, 2.1 mm × 100 mm) using negative ion electrospray detection

Inhibition of CYP3A4, CYP2C19, CYP2D6 and CYP2B6

The inhibition of drug metabolizing P450 enzymes by the lead compounds was tested using midazolam (CYP3A4), (S)-mephenytoin (CYP2C19), dextromethorphan (CYP2D6) and bupropion (CYP2B6) as selective substrates as previously described.[34,35] Briefly, human liver microsomes were incubated with the substrate and inhibitor in 100mM potassium phosphate buffer. All incubations were initiated with NADPH and quenched with ice cold acetonitrile at the end of the incubation period. Product formation was measured by LC-MS/MS using an AB Sciex 4500 mass spectrometer (AB Sciex, Foster City, CA) coupled to a Shimadzu UFLC XR DGU-20A5 (Shimadzu Scientific Instruments, Columbia, MD).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

ABBREVIATIONS

CYP	Cytochrome P450
HRMS	High resolution mass spectrometry
RA	retinoic acid
RAR	retinoic acid receptor
TTN	1,1,4,4-tetramethyl-1,2,3,4-tetrahydronaphthalene

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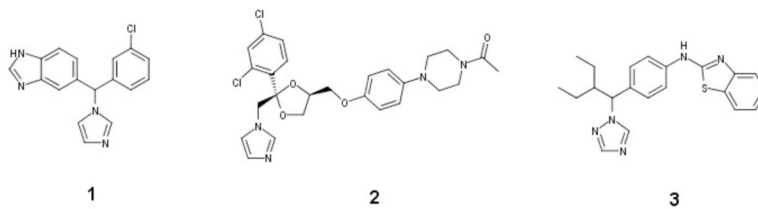
Figure 1.
Chemical structures of previously identified CYP26 inhibitors, **1** (liarozole), **2**
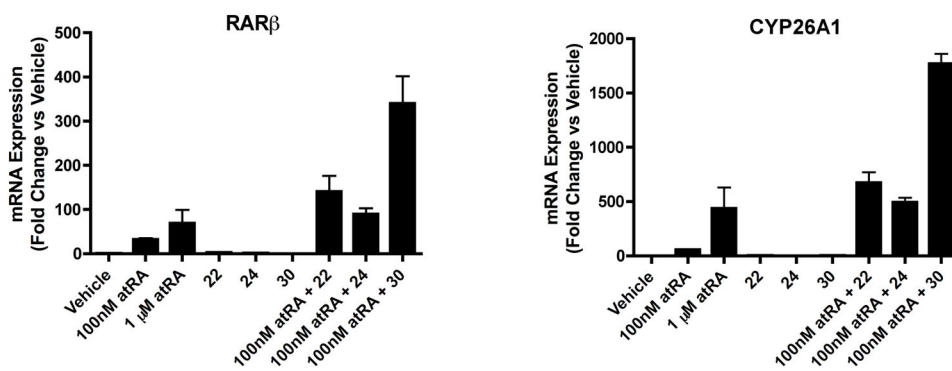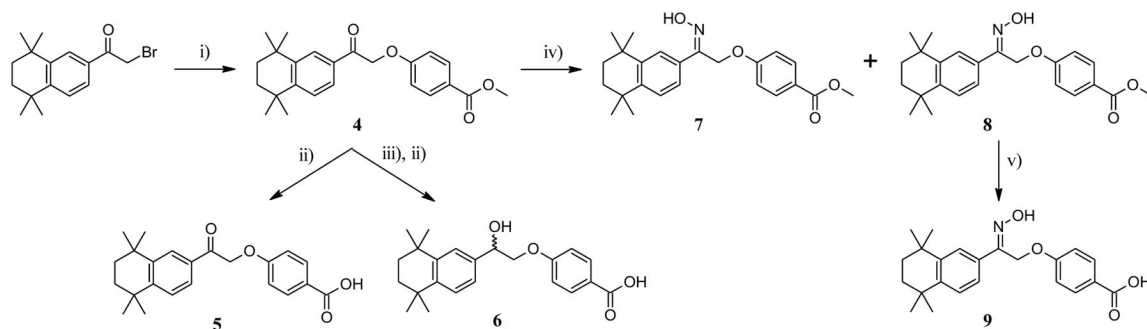(ketoconazole), and **3** (talarozole also called R115866[13]).

Figure 2.
Induction of RARβ and CYP26A1 mRNA in HepG2 cells as measured by rt-PCR. HepG2 cells were treated with 100 nM or 1 μM a*t*RA or with 1 μM compound **22**, **24** or **30** alone or in combination with 100 nM a*t*RA.
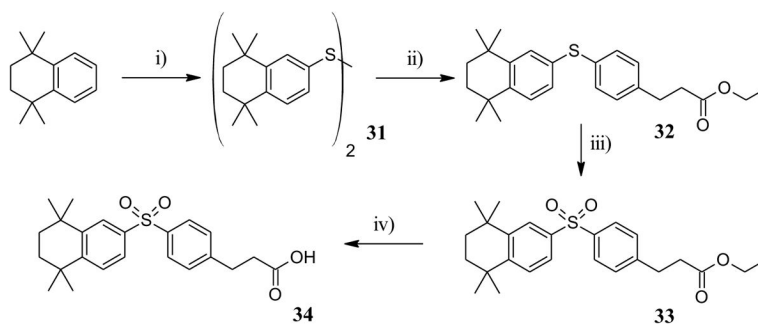
Scheme 1a.
aReagents and conditions: (i) Methyl-4-hydroxybenzoate, K_2CO_3/MEK, μW, 100°C, 2×20 min; (ii) NaOH, THF. EtOH, H_2O, 80°C, 12 h; (iii) NaBH4, THF, r.t., 1 h; (iv) $H_2NOH.HCl$/ MeOH, pyridine, reflux, 6 h; (v) K_2CO_3 (2M)/MeOH, reflux, 80°C.
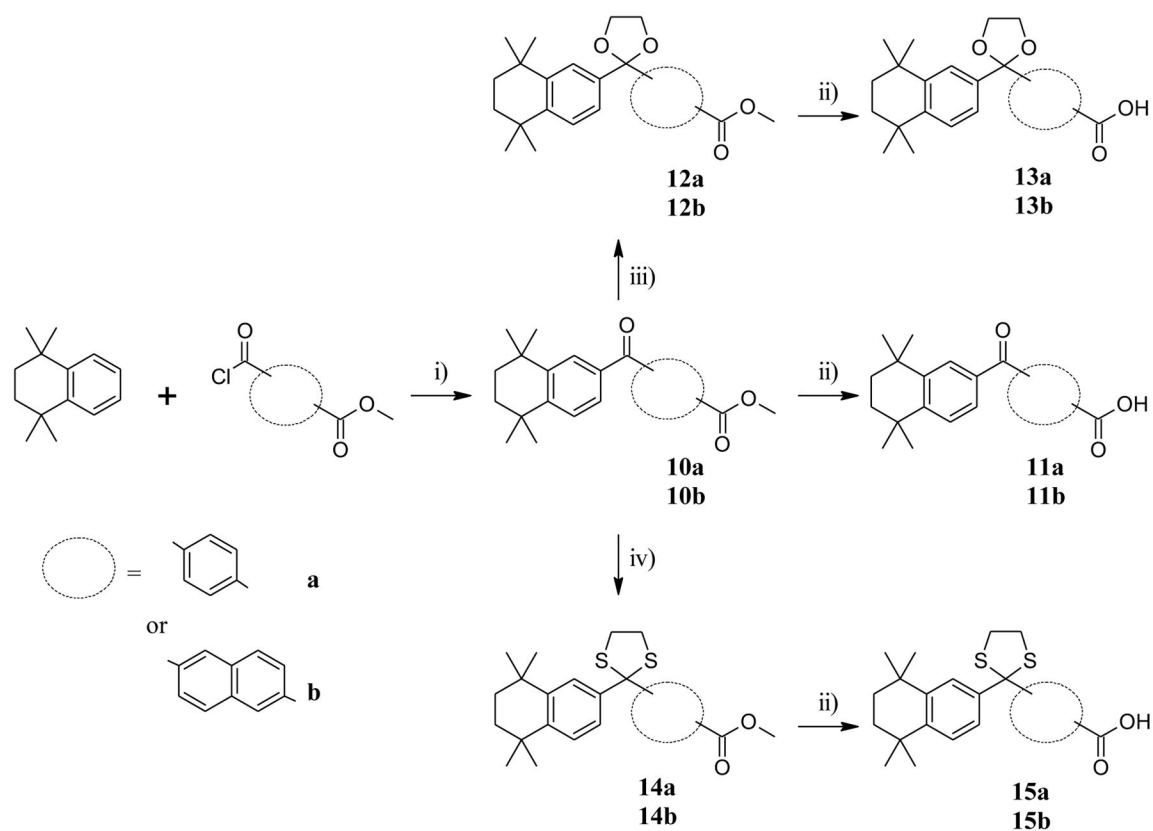
Scheme 2[a]

[a]Reagents and conditions: (i) AlCl₃, CH₂Cl₂, r.t., overnight; (ii) K₂CO₃ (2M)/MeOH, reflux, 80°C,; (iii) (CH₂OH)₂, pTsOH, toluene, reflux, overnight; (iv) (CH₂SH)₂, BF₃.Et₂O, CH₂Cl₂, r.t., 0.5–1h.
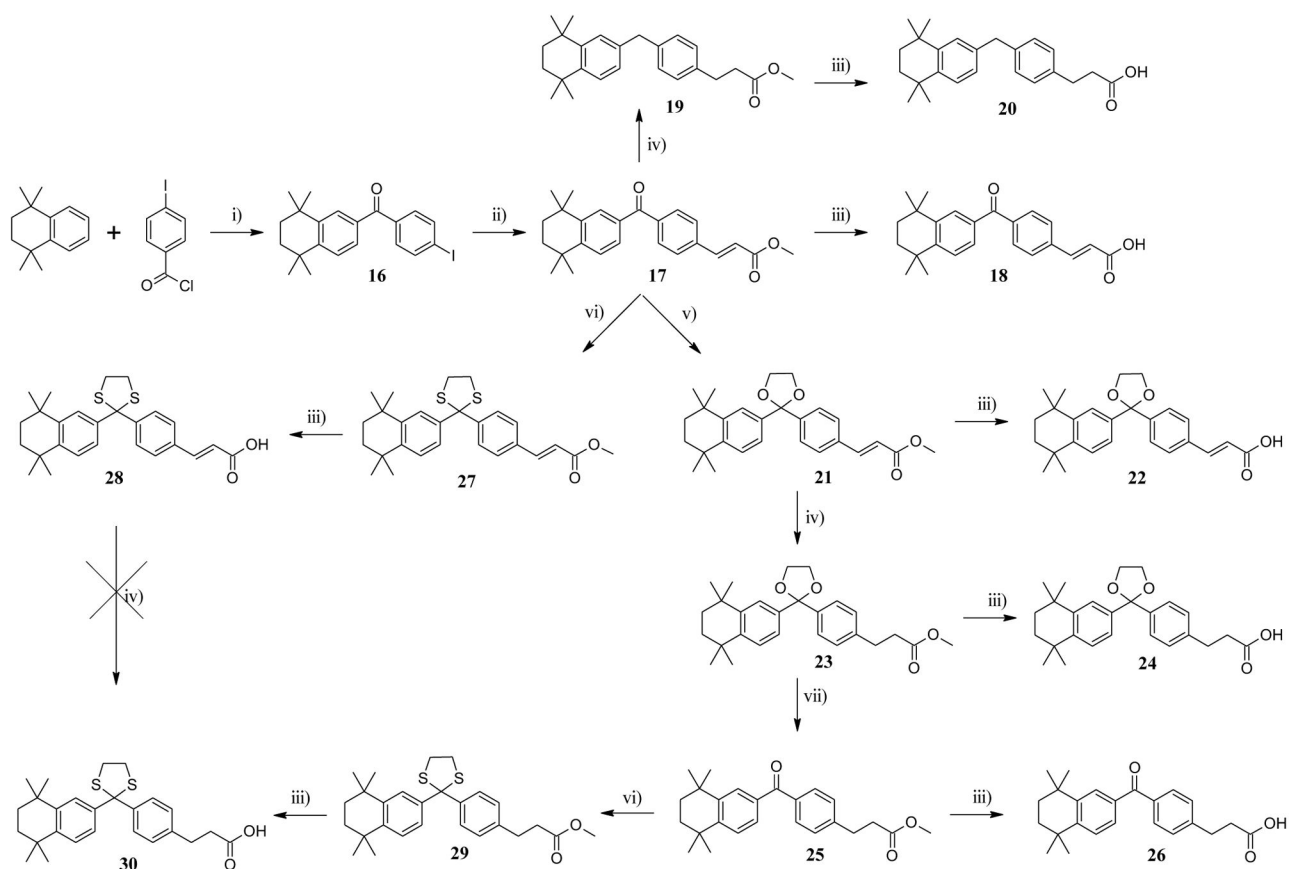
Scheme 3[a]

[a]Reagents and conditions: (i) AlCl₃, CH₂Cl₂, r.t., overnight; (ii) methyl acrylate, Pd(OAc)₂, NEt₃, DMF, 100°C, 6 h; (iii) LiOH aq. 1N, THF, reflux, 3 h or r.t., overnight; (iv) H₂, Pd/C, EtOH/EtOAc 20 psi, 7–12 h; (v) (CH₂OH)₂, pTsOH, toluene, reflux, overnight; (vi) (CH₂SH)₂, BF₃.Et₂O, CH₂Cl₂, r.t., 0.5–1 h; (vii) I₂, acetone, 4Å MS, reflux, 16 h.
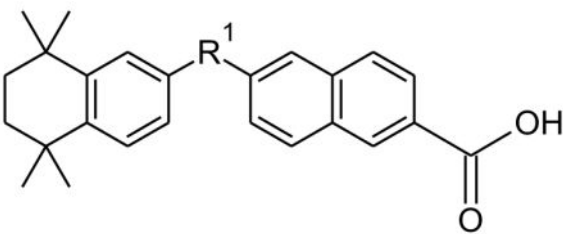
Scheme 4[a]

[a]Reagents and conditions: i) 1) Chlorosulfonic acid, 0°C to r.t., 3h, 2) Zn, EtOH, conc. HCl, reflux 45 min, r.t. overnight; ii) ethyl 3-(4-bromophenyl)propanoate, PdCl$_2$(dppf), Zn, THF, reflux, 24h; iii) oxone, H$_2$O, MeOH, 0°C to r.t., 12h; iv) LiOH 1N, THF, H$_2$O, r.t. 12h.
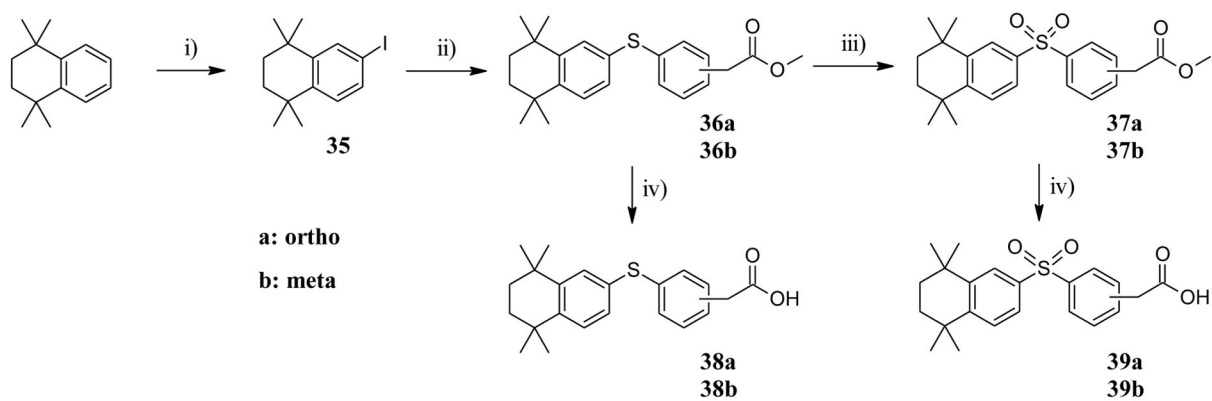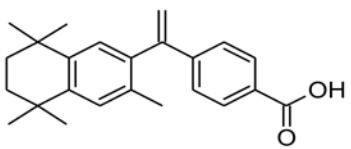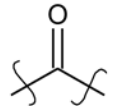
Scheme 5[a]

[a]Reagents and conditions: i) I_2, H_2SO_4, HIO_4, AcOH, H_2O, 70°C ii) 3- or 4-mercaptophenylacetic acid methyl ester, borohydride, polymer supported, $(bpy)_2NiBr_2$, dioxane, ButOH, 130–145°C, 3 h to 5 h; iii) oxone, H_2O, MeOH, 0°C to r.t., 12 h; iv) LiOH 1N, THF, H_2O, r.t. 12h.
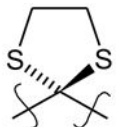
Table 1

IC$_{50}$ values and 95% confidence intervals of the IC$_{50}$ for the RAMBAs and synthetic retinoids against CYP26A1 and CYP26B1 for compounds **1**, **3**, **40**, and the naphthyl analogues **11b**, **13b** and **15b**.
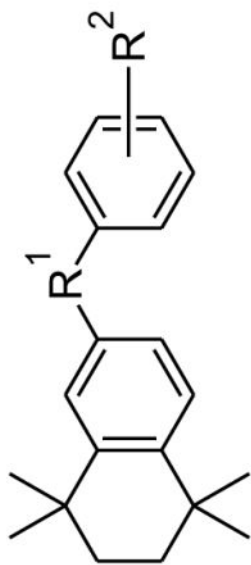

Compound	R1	Activity (μM)	
		CYP26A1	CYP26B1
1	–	1.9 (1.5–2.3)	0.018 (0.013–0.027)
3	–	0.0051[a] (0.0034–0.0068)	0.00046[a] (0.000069–0.00085)
40	–	13.5 (8.6–21.3)	5.9 (2.6–13.3)
11b		1.63 (0.95–2.9)	0.52 (0.4–0.7)
13b		0.11 (0.05–0.24)	1.03 (0.62–1.7)
15b		0.06 (0.04–0.10)	1.03 (0.69–1.5)

[a]Inhibition constant determined based on tight binding quadratic equation.

Table 2

IC$_{50}$ values and 95% confidence intervals of the IC$_{50}$ for the novel CYP26 inhibitors against CYP26A1 and CYP26B1.

Cpd	R1	R2	Activity (μM)	
			CYP26A1	**CYP26B1**
5		*para*	4.0 (2.5–6.3)	0.93 (0.61–1.4)
6		*para*	2.8 (1.7–4.8)	1.4 (0.77–2.6)
9		*para*	3.5 (2.2–5.6)	1.4 (0.4–4.8)
11a		*para*	>25	>25

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Cpd	R1	R2		Activity (µM)	
				CYP26A1	CYP26B1
13a			para	7.8 (2.8–21.4)	12.6[b]
15a			para	1.3 (0.64–2.54)	1.1 (0.1–11.7)
18			para	4.7	>5
20			para	1.66	1.7 (0.8–3.5)
22			para	0.24 (0.10–0.59)	>10
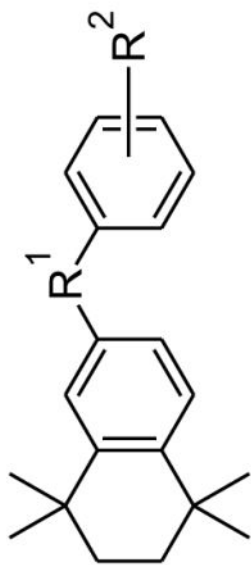
Cpd	R1	R2	Activity (μM)	
			CYP26A1	CYP26B1
24		*para*	0.34 (0.06–2.0)	14.5 (3.5–59.7)
26		*para*	>5	>5
28		*para*	>10	4.4 (1.8–10.6)
30		*para*	0.27 (0.15–0.47)	0.68 (0.2–2.4)
34		*para*	>5	6.2
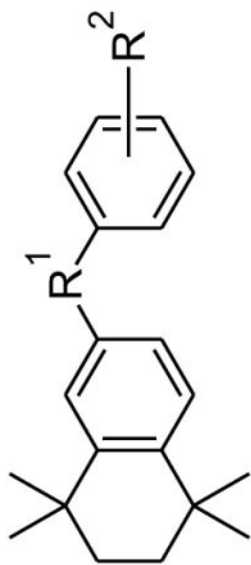
Cpd	R1	R2		Activity (µM)	
				CYP26A1	CYP26B1
38a		*para*		10.6 (5–23)	>5
38b		*para*		>5	>5
39a		*para*		>5	>5
39b		*meta*		>5	>5

[b]Maximum inhibition 59% at 20µM

Table 3

a*t*RA concentration in cell media after 24 hour treatment

Treatments	*at*RA in cell media (nM)
Vehicle	0.49 ± 0.33
100nM *at*RA	13.2 ± 0.14
1μM *at*RA	214 ± 13.6
1μM **22**	1.09 ± 0.52
1μM **24**	0.27 ± 0.09
100nM a*t*RA + 1μM **22**	45.5 ± 2.8 [*]
100nM a*t*RA + 1μM **24**	25.6 ± 3.4 [*]
100nM a*t*RA + 1μM **30**	113 ± 9 [*]

[*]significantly different from the 100 nM RA treatment

Table 4

Percent inhibition for each of the tested CYP enzymes when screened with 10 μM of CYP26 inhibitors. NI indicates no inhibition observed. *shows that activity in the presence of the inhibitor was significantly different (p<0.01) from that in control.

Compound	CYP3A4	CYP2D6	CYP2C19	CYP2B6
22	NI	49 ± 7*	NI	NI
24	NI	NI	NI	NI
30	75 ± 8*	NI	NI	30 ± 1*

*activity in the presence of the test inhibitor (22 or 30) was significantly different from control