St John's wort, a herbal antidepressant, activates the steroid X receptor

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Abstract

St John's wort (SJW), an extract of the medicinal plant *Hypericum perforatum*, is widely used as a herbal antidepressant. Recently, this agent has been found to adversely affect the metabolism of various coadministered drugs. Steroid X receptor (SXR), an orphan nuclear receptor, induces hepatic cytochrome P450 gene expression in response to diverse endogenous steroids, xenobiotics and drugs. Here, we report that, when coexpressed with SXR, a reporter construct derived from the cytochrome P450 3A promoter is activated by St John's wort. A GAL4-SXR ligand binding domain (LBD) fusion mediates concentration-dependent transactivation by SJW, whereas a mutant GAL4-SXR fusion,

containing substitutions in key residues in a transactivation domain, is inactive. SJW recruits steroid receptor coactivator-1 to SXR in a two-hybrid assay and competes with radiolabelled ligand in binding studies, suggesting it interacts directly with the receptor LBD. Of two constituents of SJW, we find that hyperforin, but not hypericin, mediates both transactivation and coactivator recruitment by SXR. Our observations suggest that SXR activation by St John's wort mediates its adverse interaction with drugs metabolised via the CYP 3A pathway. Future development of SJW derivatives lacking SXR activation, may enable its antidepressant and drug-metabolising properties to be dissociated.

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Introduction

St John's wort, an extract of a goatweed herb (*Hypericum perforatum*) belonging to the Hypericaceae family, has been used for centuries as a natural remedy for the treatment of a number of conditions. When applied topically, it may have wound healing properties. Taken orally, it is used in the treatment of anxiety states and insomnia. In addition, it has been phenomenally successful as a herbal antidepressant. Hypericum extracts used either as self-medication or prescribed anti-depressant accounted for 111 million daily doses sold in Germany in 1997 and total European sales figures in 1998 amounted to US\$ 6 billion. In the USA, St John's wort is marketed as a food supplement and sales increased by 2800% in one year (Ernst E. 1999). A recent meta-analysis of several placebo-controlled trials indicated that it is at least as effective as conventional antidepressants with fewer side-effects (Linde K *et al.* 1996), suggesting that its popularity is not misplaced.

The nuclear receptor superfamily includes protein targets of steroid and thyroid hormones, retinoids and vitamin D, as well as a growing number of orphan receptors whose cognate ligands and function remain to be elucidated. The human steroid X receptor (SXR), was shown to be activated by a wide range of endogenous and synthetic steroids (Blumberg B *et al.* 1998) and its murine counterpart PXR, is particularly responsive to pregnanes such as pregnenolone 16 α -carbonitrile (Kliwer SA *et al.* 1998). However, it is now recognised that SXR is also activated by drugs which are

potent inducers of hepatic cytochrome P450 3A enzyme activity such as rifampicin and clotrimazole (Blumberg B *et al.* 1998), (Lehmann JM *et al.* 1998), (Bertilsson G *et al.* 1998). Consonant with this, SXR is most highly expressed in liver and intestine and the receptor forms heterodimers with RXR which bind to specific response elements in human and murine CYP 3A promoters which mediate gene induction in response to these drugs (Blumberg B *et al.* 1998), (Lehmann JM *et al.* 1998), (Bertilsson G *et al.* 1998). These observations are unified by the concept that SXR regulates both the hepatic catabolism of endogenous steroids and metabolism of drugs via CYP 3A, by inducing enzyme activity in response to these compounds. Furthermore, this concept provides a mechanism for the well-recognised observation that rifampicin enhances the metabolism of glucocorticoids (Edwards OM *et al.* 1974) and sex steroids and interacts adversely with drugs that are metabolised via the CYP 3A pathway (Michalets EL. 1998).

Recently, a number of case reports have documented important interactions between St John's wort and various prescribed drugs. In women on oral contraceptives, breakthrough bleeding with potential loss of contraceptive efficacy, presumably due to decreased circulating sex steroid levels, has been reported (Ernst E. 1999). Co-medication with St John's wort also reduces blood levels of an immunosuppressant (cyclosporin), anticonvulsants (e.g. carbamazepine, phenobarbitone) and HIV protease inhibitors (e.g. indinavir) that are known to be metabolised by the CYP 3A pathway, and a bulletin highlighting these

adverse effects has been issued by the Committee on Safety of Medicines/Medicines Control Agency in the U.K (Anonymous MCA, 2000).

We therefore hypothesised that St John's wort might exert these effects via activation of SXR. Here, we report that SJW enhances the transcriptional activity of SXR comparably to rifampicin. This activation is dose-dependent and mediated by the receptor ligand binding domain. SJW also promotes recruitment of the coactivator SRC-1 to SXR and displaces radiolabelled ligand bound to SXR, suggesting a direct interaction with the receptor. Of the two putative active constituents of SJW, we show that hyperforin but not hypericin induces transcriptional activation and SRC-1 recruitment by SXR. Our data suggests that SXR activation by SJW mediates some of its adverse interactions with other drugs.

Materials and methods

Plasmid constructs

Flag-epitope tagged full length human SXR expression vector was constructed by inserting a PCR amplified cDNA fragment encompassing residues 1-434 (Blumberg B *et al.* 1998) downstream of the CMV promoter and Flag epitope in pCMV2Flag (Sigma, Poole, UK). Gal4SXR contains DNA sequences encompassing residues 107 to 434 of the SXR ligand binding domains (LBD) linked to the Gal4 DNA binding domain (DBD) in pSG424 (Tone Y *et al.* 1994). The L424A /E427A SXR double mutants were generated by PCR amplifying fragments containing either full length SXR or its LBD from the wild type receptor cDNA using a reverse primer, incorporating single nucleotide substitutions (underlined) corresponding to the codon changes (5'-CTCGAGGTCGACTCAGCTACCTGTGATGCCGAACAA CGCCTGCATGGCGGGCGTAGC), and cloning into pCMV2Flag or pSG424 respectively. All constructs were fully verified by sequencing. Wild type and mutant SXR LBD fragments were cloned downstream of the transactivation domain of VP16 in AASV (Tone Y *et al.* 1994) to yield VP16-SXR fusions. Gal4SRC contains the central nuclear receptor interaction domain ($\alpha\alpha$ 570 to 780) of human SRC1 fused in frame to the Gal4 DBD in pSG424 (Kalkhoven E *et al.* 1998).

Reporter constructs containing two copies of SXR response elements from either the murine (DR3TKLUC) (Lehmann JM *et al.* 1998) or human (ER6TKLUC) (Bertilsson G *et al.* 1998) cytochrome p450 3A gene promoters, were generated by inserting annealed oligonucleotide pairs (murine forward 5'-TCGAAGACAGTTCATGAAGTTCATCTAGATC GATCAGACAGTTCATGAAGTTCATCTAGATC;

Reverse 5'-GATCGATCTAGATGAAGTTCATGAAGTCTCTGATCGATCTAGATGAAGTTCATGAAGTGTCT,

human forward 5'-TCGAGAATATGAAGTCAAAGGAG GTCAGTGAGTAGAATATGAAGTCAAAGGAGGTCAGTG AGTG;

reverse 5'-TCGACACTCACTGACCTCCTTTGATGTAC

TATTCTACTCACTGACCTCCTTTGAGTTCATAGTC) into pA3TKLUC (Tone Y *et al.* 1994), upstream of the thymidine kinase promoter and luciferase. UASTKLUC contains two copies of a Gal4 binding site in pA3TKLUC as described previously (Tone Y *et al.* 1994). The reference plasmid Bos- β Gal contains the promoter of the human elongation factor 1 α linked to β -galactosidase (Tone Y *et al.* 1994).

Reagents and chemicals

Rifampicin and hypericin were obtained from Sigma (Poole, Dorset, UK). A dried ethanolic extract of St John's Wort, containing at least 0.3% hypericin, 3% hyperforin, was obtained from Essential Nutrition (Brough, United Kingdom). Hyperforin was obtained from HWI Analytik (Rheinzabern, Germany). All compounds were dissolved in DMSO.

Cell culture and transfection assays

JEG-3 human choriocarcinoma cells were cultured in Optimem (GIBCO, UK) supplemented with 2% (v:v) foetal bovine serum (FBS) and 1% penicillin/streptomycin/fungizone and transferred to phenol red-free DMEM with 4% resin-stripped FBS and 1% penicillin/streptomycin prior to transfection. 24 well plates of cells were transfected by a 4h exposure to calcium phosphate with each well containing 50ng of receptor expression vector, 500ng of reporter construct and 100ng of the internal control plasmid Bos β -gal. In two hybrid assays 50ng of Gal4-SRC1 and VP16-SXRLBD fusions were used together with reporter and internal control plasmids as above. Following a 36h incubation with vehicle or compound, cells were lysed, luciferase activity measured and normalised using β -galactosidase activity as described previously (Tone Y *et al.* 1994).

Ligand binding assays

The ligand binding domains of SXR and its heterodimeric partner RXR were coexpressed by *in vitro* transcription/translation (Promega, UK). The expressed protein complex was saturated with 3 H-corticosterone (Amersham Pharmacia Biotech, UK), an SXR ligand. Then, either vehicle (4% v/v DMSO) or rifampicin (400 μ M) or St John's wort (4% v/v of solution), was added to aliquots of ligand-bound protein and incubated for 30 min at 4°C. Bound and free radioligand were separated by application to 0.3% polyethylenimine-treated glass-fibre filters, followed by extensive washing and then counts bound were quantified by liquid scintillation.

Results

JEG-3 cells were cotransfected with a full length human SXR expression vector together with a reporter construct (DR3TKLUC), containing two copies of the SXRE from the murine CYP 3A gene linked to the thymidine kinase promoter and luciferase. Exposure to rifampicin (20 μ M), enhanced transcriptional activity by 7.5 fold and incubation with St

Figure 1 St John's wort activates transcription via SXR A) JEG-3 cells were transfected with DR3TKLUC and full length SXR (left panels) or UASTKLUC and either wild type (Gal-SXR) or mutant (Gal-SXRm) Gal4-receptor fusions (centre, right panels) and incubated with vehicle, 20μM rifampicin or 10μg/ml St John's wort. Luciferase reporter activity was normalised for β-galactosidase and expressed as fold activation compared with values in vehicle-treated cells. B) Cells, transfected with UASTKLUC and wild type Gal-SXR, were incubated with increasing concentrations of rifampicin (0.1 to 20μM) or St John's wort (0.1 to 10μg/ml). The activation by rifampicin (solid line) or SJW (dotted line) are expressed as a percentage of the maximal transcriptional response with each agent.

John's wort (10μg/ml) elicited a comparable five fold response (Fig 1a, left panels), whereas the reporter construct alone was not activated by either compound (data not shown). To determine whether this activation was mediated by the receptor ligand binding domain, a Gal4 DBD-SXR LBD fusion was coexpressed with its cognate reporter (UASTKLUC), containing two 17-mer Gal4 binding sites. Rifampicin and SJW were comparably effective, inducing reporter activity by

~6 or 5 fold respectively (Fig 1a, middle panels). In other nuclear receptors, an amphipathic α-helix at the C-terminus of the LBD mediates ligand-dependent transactivation (AF-2) function (Danielian PS *et al.* 1992), (Tone Y *et al.* 1994). Accordingly, a Gal4-SXR mutant in which highly conserved hydrophobic (Leu 424) and charged (Glu427) residues in this domain are changed to alanine, was generated and tested with rifampicin and SJW and found to be transcriptionally inactive (Fig 1a, right panels), suggesting that drug-induced transcriptional activation is dependent on receptor AF-2 function. The relative potencies of rifampicin versus St John's wort were compared by exposure of cells transfected with Gal4-SXR and UASTKLUC to increasing concentrations of each compound. Both agents augmented transcriptional activity in a dose-dependent manner (Rifampicin 0-20 μM; St John's wort 0-10 μg/ml), reaching a comparable maximal response (Fig 1b).

The putative active ingredients in St John's wort mediating its antidepressant effect include naphodianthrones (hypericin) and phloroglucinols (hyperforin). We therefore tested these compounds for receptor activation in our assay system. Hypericin failed to activate UASTKLUC via Gal4-SXR even at a concentration of 1000ng/ml which greatly exceeds the blood levels of this compound achieved following St John's wort (Weiser D. 1991). In contrast, hyperforin activated UASTKLUC in a dose-dependent manner achieving a 7.5 fold maximal response at 1000ng/ml (Fig 2). Furthermore, hyperforin also activated full length SXR, enhancing the activity of reporter constructs containing receptor response elements from either the human (ER6) or murine (DR3) CYP 3A gene promoters (Fig 3).

Figure 2 Hyperforin but not hypericin activates Gal4-SXR. JEG-3 cells, transfected with UASTKLUC and Gal4-SXR, were incubated with vehicle (open bars) or increasing concentrations of hypericin (checked bars, 10, 100, 1000 ng/ml) or hyperforin (dotted bars, 50, 100, 500, 1000ng/ml). Results were calculated and expressed as in Fig1a.

Figure 3 Hyperforin activates human and murine CYP 3A response elements via SXR. JEG-3 cells, transfected with ER6TKLUC (left panels) or DR3TKLUC (right panels) and full length SXR, were incubated with vehicle (open bars), 100ng/ml (shaded bars) or 500ng/ml (black bars) hyperforin. Results are expressed as in Fig 1a.

A hallmark of nuclear receptors is their ability to recruit transcriptional coactivators in a ligand-dependent manner. Accordingly, we used a mammalian two hybrid assay to test whether a VP16-SXRLBD chimera could be recruited to a fusion (Gal4-SRC1), containing the DBD of Gal4 linked to the central nuclear receptor interaction domain of steroid receptor coactivator 1, in the presence of rifampicin, St John's wort or its active ingredients. Rifampicin and St John's wort augmented receptor-coactivator association comparably by 7 to 8 fold. In contrast, hypericin failed to promote any association, whereas hyperforin mediated significant SXR-SRC1 interaction equivalent to rifampicin or SJW (Fig 4a, left panels). We then tested the mutant VP16-SXRLBD chimera, containing substitutions which abolish its transcriptional activity (Fig 1a). In keeping with this, drug-mediated receptor-coactivator association with rifampicin, St John's wort or hyperforin was also lost (Fig 4a, right panels).

To determine whether SJW interacts directly with SXR, we tested it in radiolabelled ligand binding assays. When incubated with an *in vitro*-synthesised SXR-RXR complex, St John's wort displaced ^3H -corticosterone binding with comparable efficacy to rifampicin (Fig 4b).

Discussion

We have shown that St John's wort activates a reporter gene containing xenobiotic response elements from the CYP 3A gene promoter via coexpressed SXR. The induction is dose-dependent and of comparable magnitude to the effects of rifampicin, a known CYP 3A activator, in the same system. The response to SJW is mediated by the SXR LBD which is known to contain a powerful ligand-dependent transactivation

Figure 4 A) Drug-dependent recruitment of SRC1 by SXR. JEG-3 cells, transfected with UASTKLUC, Gal4-SRC1 and either wild type (left panels) or mutant (right panels) VP16-SXRLBD fusions, were incubated with vehicle, 20 μM rifampicin, 10 $\mu\text{g}/\text{ml}$ St John's wort, 1000ng/ml hypericin or 500ng/ml hyperforin as indicated. Results were calculated as in Fig 1a. b) St John's wort interacts directly with SXR. An *in vitro* translated SXR-RXR complex was incubated with ^3H -corticosterone ligand plus either vehicle (4% v/v DMSO), or rifampicin (400 μM) or St John's wort (4%v/v). The results are expressed as a percentage of the maximal ligand bound with vehicle alone.

(AF-2) function (Blumberg B *et al.* 1998). In keeping with this, mutation of hydrophobic and charged residues that are conserved in other nuclear receptors and critical for transactivation (Tone Y *et al.* 1994), (Danielian PS *et al.* 1992), abolishes the response. We also find that St John's wort recruits SRC1, a nuclear receptor coactivator that mediates AF-2 activity, to the SXR LBD. In addition, SJW competes with

radiolabelled ligand for SXR binding, suggesting that it interacts directly with the receptor LBD.

There is compelling evidence to suggest that such SXR activation by SJW is relevant *in vivo*. The urinary excretion of 6 β -hydroxycortisol, a specific marker of steroid metabolism via the CYP 3A pathway (Ged C *et al.* 1989), is enhanced following treatment with known enzyme inducers such as rifampicin (Yeung JHK *et al.* 1997). When used as an antidepressant, typically 900mg of SJW is taken orally in daily divided doses (Philipp M *et al.* 1999). When administered to normal volunteers at this dosage for 14 days, a recent study showed that St John's wort significantly enhanced urinary 6 β -hydroxycortisol excretion (Kerb R *et al.* 1997). Although St John's wort is a mixture of naphodianthrones, phloroglucinols and flavonoids, there is evidence to indicate that its beneficial central nervous system effects are mediated by hypericin or hyperforin, and most commercial preparations contain at least 0.3% and 3% of each compound respectively. Following the administration of 300 to 1200mg of SJW, it is known that plasma hypericin levels ranged from 1.5 to 5ng/ml (Weiser D. 1991). Hypericin is a naphodianthrone and bears some superficial structural similarity to rifampicin. However, despite this, we found that hypericin did not activate SXR, even at concentrations 200 fold greater than achieved *in vivo*. Pharmacokinetic studies show that blood levels of hyperforin range from 100 to 250ng/ml, following a 900mg dose of St John's wort (Biber A *et al.* 1998). In our transfection assays, hyperforin induced reporter activity via both Gal4-SXR (Fig 2) and full length receptor (Fig 3), 3 to 8 fold over a similar 50 to 500ng/ml concentration range. Our data strongly suggest that hyperforin is the active constituent of St John's wort which mediates SXR activation by the latter. Very recently, in studies conducted in parallel with our work, another group has reported that St John's wort induces CYP3A4 expression in human hepatocytes and activates SXR and that hyperforin is the likely receptor ligand (Moore LB *et al.* 2000a). Hyperforin is a phloroglucinol which is structurally different from rifampicin. This is consistent with previous observations that SXR interacts with a large number of structurally dissimilar steroids and xenobiotics (Blumberg B *et al.* 1998), (Lehmann JM *et al.* 1998), (Bertilsson G *et al.* 1998), suggesting that this receptor has an unusually promiscuous ligand binding pocket.

St John's wort has been reported to interact adversely with drugs metabolised via other pathways. For example, it decreases serum levels of digoxin which is metabolised via the P-glycoprotein drug transporter (John A *et al.* 1999) and reduces levels of theophylline and warfarin which are metabolised via CYP1A2 and CYP2C9 pathways respectively (Nebel A *et al.* 1999), (Kaminsky LS *et al.* 1997). Although nuclear receptor response elements have not been described hitherto in these gene promoters, it is possible that St John's wort might also induce these enzymes via SXR or perhaps the constitutive androstane receptor (CAR)-another nuclear receptor that is activated by xenobiotics (Moore LB *et al.* 2000b).

SXR is also activated by endocrine-disruptor chemicals (nonylphenol, phthalic acid) (Masuyama H *et al.* 2000), suggesting that this receptor not only regulates the metabolism of endogenous steroids but also catabolism of environmental agents. The activation of SXR by St John's wort is consistent with this notion and it is tempting to speculate that this receptor has evolved to protect the host from potentially harmful plant toxins ingested as the herbaceous component of dietary intake.

Given the widespread use of St John's wort, relatively few cases of adverse drug interactions have been reported. One explanation is that such effects have been missed; alternatively, there may be inter-individual variation in susceptibility to its adverse effects, perhaps mediated by genetic variation in SXR or the CYP 3A gene.

Finally, hyperforin has been shown to inhibit neurotransmitter uptake in synaptosomal preparations (Chatterjee SS *et al.* 1998), suggesting that it is the constituent which mediates the antidepressant activity of St John's wort. This raises the future possibility that modified derivatives lacking SXR activation but with preserved neuropharmacological properties can be developed.

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References

- Anonymous MCA 2000 Reminder: St John's wort (Hypericum perforatum) interactions. *Current Problems in Pharmacovigilance* **26** 6-7.
- Bertilsson G, Heidrich J, Svensson K, Asman M, Jendeborg L, Sydow-Backman M, Ohlsson R, Postlind H, Blomquist P & Berkenstam A 1998 Identification of a human nuclear receptor defines a new signaling pathway for CYP3A induction. *Proceedings of the National Academy of Sciences of the United States of America* **95** 12208-12213.
- Biber A, Fischer H, Romer A & Chatterjee SS 1998 Oral bioavailability of hyperforin from hypericum extracts in rats and human volunteers. *Pharmacopsychiatry* **31** (Suppl 1) 36-43.
- Blumberg B, Sabbagh W, Jr., Juguilon H, Bolado J, Jr., van Meter CM, Ong ES & Evans RM 1998 SXR, a novel steroid and xenobiotic-sensing nuclear receptor. *Genes and Development* **12** 3195-3205.
- Chatterjee SS, Bhattacharya SK, Wonnemann M, Singer A & Muller WE 1998 Hyperforin as a possible antidepressant component of hypericum extracts. *Life Sciences* **63** 499-510.
- Danielian PS, White R, Lees JA & Parker MG 1992 Identification of a conserved region required for hormone dependent transcriptional activation by steroid hormone receptors. *EMBO Journal* **11** 1025-1033.
- Edwards OM, Courtenay-Evans RJ, Galley JM, Hunter J & Tait AD 1974 Changes in cortisol metabolism following rifampicin therapy. *Lancet* **2** 549-551.
- Ernst E 1999 Second thoughts about safety of St John's wort. *Lancet* **354** 2014-2016.
- Ged C, Rouillon JM, Pichard L, Combalbert J, Bressot N, Bories P, Michel H, Beaune P & Maurel P 1989 The increase in urinary

- excretion of 6 beta-hydroxycortisol as a marker of human hepatic cytochrome P450III_A induction. *British Journal of Clinical Pharmacology* **28** 373-387.
- Johne A, Brockmoller J, Bauer S, Maurer A, Langheinrich M & Roots I 1999 Pharmacokinetic interaction of digoxin with an herbal extract from St John's wort (*Hypericum perforatum*). *Clinical Pharmacology Therapeutics* **66** 338-345.
- Kalkhoven E, Valentine JE, Heery DM & Parker MG 1998 Isoforms of steroid receptor co-activator 1 differ in their ability to potentiate transcription by the oestrogen receptor. *EMBO Journal* **17** 232-243.
- Kaminsky LS & Zhang ZY 1997 Human P450 metabolism of warfarin. *Pharmacology Therapeutics* **73** 67-74.
- Kerb R, Bauer S, Brockmoller J & Roots I 1997 Urinary 6- β -hydroxycortisol excretion rate is affected by treatment with hypericum extract. *European Journal of Clinical Pharmacology* **52** A186 (abstr).
- Kliewer SA, Moore JT, Wade L, Staudinger JL, Watson MA, Jones SA, McKee DD, Oliver BB, Willson TM, Zetterstrom RH, Perlmann T & Lehmann JM 1998 An orphan nuclear receptor activated by pregnanes defines a novel steroid signaling pathway. *Cell* **92** 73-82.
- Lehmann JM, McKee DD, Watson MA, Willson TM, Moore JT & Kliewer SA 1998 The human orphan nuclear receptor PXR is activated by compounds that regulate CYP3A4 gene expression and cause drug interactions. *Journal of Clinical Investigation* **102** 1016-1023.
- Linde K, Ramirez G, Mulrow CD, Pauls A, Weidenhammer W & Melchart D 1996 St John's wort for depression - an overview and meta-analysis of randomised clinical trials. *British Medical Journal* **313** 253-258.
- Masuyama H, Hiramatsu Y, Kunitomi M, Kudo T & MacDonald PN 2000 Endocrine disrupting chemicals, phthalic acid and nonylphenol, activate Pregnane X receptor-mediated transcription. *Molecular Endocrinology* **14** 421-428.
- Michalets EL 1998 Update: clinically significant cytochrome P-450 drug interactions. *Pharmacotherapy* **18** 84-112.
- Moore LB, Goodwin B, Jones SA, Wisely GB, Serabjit-Singh CJ, Willson TM, Collins JL & Kliewer SA 2000a St. John's wort induces hepatic drug metabolism through activation of the pregnane X receptor [In Process Citation]. *Proceedings of the National Academy of Sciences of the United States of America* **97** 7500-7502.
- Moore LB, Parks DJ, Jones SA, Bledsoe RK, Consler TG, Stimmel JB, Goodwin B, Liddle C, Blanchard SG, Willson TM, Collins JL & Kliewer SA 2000b Orphan nuclear receptors constitutive androstane receptor and pregnane X receptor share xenobiotic and steroid ligands. *Journal of Biological Chemistry* **275** 15122-15127.
- Nebel A, Schneider BJ, Baker RK & Kroll DJ 1999 Potential metabolic interaction between St. John's wort and theophylline. *Annals of Pharmacotherapy* **33** 502.
- Philipp M, Kohnen R & Hiller KO 1999 Hypericum extract versus imipramine or placebo in patients with moderate depression: randomised multicentre study of treatment for eight weeks [published erratum appears in BMJ 2000 Feb 5;320(7231):361]. *British Medical Journal* **319** 1534-1538.
- Tone Y, Collingwood TN, Adams M & Chatterjee VKK 1994 Functional analysis of a transactivation domain in the thyroid hormone β receptor. *Journal of Biological Chemistry* **269** 31157-31161.
- Weiser D 1991 Pharmakokinetik von Hypericin nach oraler Einnahme des Johanniskraut-Extraktes LI 160. *Nervenheilkunde* **10** 318.
- Yeung JHK, Wong JKL & Park BK 1997 Development of a monoclonal antibody to 6 β -hydroxycortisol and its application in an enzyme-linked immunosorbent assay (ELISA) for 6 β -hydroxycortisol in urine. *Journal of Pharmacological and Toxicological Methods* **38** 71-79.