Saudi Pharmaceutical Journal 26 (2018) 1069–1072

Contents lists available at ScienceDirect

Saudi Pharmaceutical Journal

journal homepage: www.sciencedirect.com

Original article

Novel plant inducers of PXR-dependent cytochrome P450 3A4 expression in HepG2 cells

Mohammed S. Al-Dosari *, Mohammad K. Parvez *

Department of Pharmacognosy, College of Pharmacy, King Saud University, Riyadh 11451, Saudi Arabia

ARTICLE INFO

Article history:
Received 19 February 2018
Accepted 22 May 2018
Available online 01 June 2018

Keywords:
Pregnane X receptor
Cytochrome P450
CYP3A4
Plant extracts
Luciferase

ABSTRACT

The cytochrome P450 3A4 (CYP3A4) is the most abundant CYP450 enzyme involved in the metabolism of endogenous products and xenobiotics, including prescription drugs and herbals. Modulation of hepatic CYP3A4 gene expression via nuclear receptors, like pregnane X receptor (PXR), is a major cause of adverse effects like drug-unresponsiveness and toxicity. In the present study, ethanol extracts of 58 medicinal plants, belonging to 27 families, were evaluated for potential activities in CYP3A4 induction in HepG2 cells by reporter gene assay. For PXR-mediated CYP3A4 induction, a 50 μg/ml concentration was used for all non-cytotoxic plants extracts. Rifampicin (10 μM) and DMSO (0.1%) were used as standard inducer and untreated (negative) control, respectively. The comparative fold-induction of CYP34A by the plant extracts in relation to the untreated control was determined. As a result, *Dodonaea angustifolia* (2.62 fold; $P < 0.0001$) was found to be the most promising inducer of CYP3A4, followed by *Euphorbia tirucalli* (1.95 fold; $P = 0.0004$), *Alternanthera pungens* (1.74 fold, $P = 0.0035$), and *Ficus palmata* (1.65 fold; $P = 0.0097$). Further phytochemical characterizations of the active plants are therefore, warranted.

1. Introduction

Cytochrome P450 (CYP) is a superfamily of drug-metabolizing enzymes that is involved in the metabolism of endogenous compounds, xenobiotics and pharmaceuticals (Anzenbacher and Anzenbacherová, 2001). The major CYP involved in the hepatic metabolism of most of the drugs include CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4 (Anzenbacher and Anzenbacherová, 2001; Al-Dosari and Parvez, 2016). Since CYP is involved in phase-1 metabolism of >70% of prescription drugs, modulation of its expressions is a major cause of adverse drug-drug interactions, including decreased drug efficacy (Tyagi et al., 2010; Zhang et al., 2010). Expression of CYP3A4 is markedly induced both *in vivo* and in cultured human hepatocytes *in vitro* in response to a variety of xenobiotics (eg., dexamethasone and rifampicin) as well as medicinal herbs like, St. John's wort (*Hypericum perforatum*) (Kolars et al., 1992; Schuetz and Guzelian, 1984; Zhou and Lai, 2008; Harmsen et al., 2008; Quatrochi and Guzelian, 2001; Moore et al., 2000).

Further, gene expression of CYP is regulated by a set of nuclear receptors in response to a wide spectrum of xenobiotics (Pelkonen et al., 1998; Quatrochi and Guzelian, 2001; Al-Dosari and Parvez, 2016). Of these, the pregnane X receptor (PXR), also known as xenobiotic or pregnane-activated receptor regulates CYP3A4 gene induction (Matic et al., 2007; Hustert et al., 2001). The most common clinical implication for the PXR activation is the drug-drug interactions, mediated by the upregulation of CYP3A4. Notably, PXR itself is activated by rifampicin and other xenobiotics (Lehmann et al., 1998) as well as plant secondary metabolites like, hyperforin from *H. perforatum* (Zhou and Lai, 2008, Moore et al., 2000). In addition to this, PXR and its target genes also play an important role in maintaining normal physiological function and homeostasis. For example, artemisinin (*Artemisia annua*), piperine (*Piper nigrum*) and notoginsenoside (*Panax notoginseng*), the known activators of PXR, have been shown to prevent severity of colonic inflammatory bowel disease by inducing CYP3A4 expression (Hu et al., 2015, 2014; Zhang et al., 2015).

Hepatic PXR has broad substrate specificity and thus may be activated by a large number of chemically-diverse secondary metabolites found in dietary supplements and therapeutic herbs. Since such natural products are often orally consumed, the high concentration of their phytoconstituents in gut and liver may

* Corresponding authors.
E-mail addresses: mdosari@ksu.edu (M.S. Al-Dosari), mohkhalid@ksu.edu.sa (M.K. Parvez).
Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.jsps.2018.05.016
1319-0164/

potentially affect the CYP activity. In this report, we therefore, intended to screen the novel PXR-dependent CYP3A4 activation potential of 58 medicinal plants of 27 families using cultured hepatocytes and reporter gene assay.

2. Experimental methods

2.1. Plant materials and extraction

The studied plant extracts included pre-identified, non-cytotoxic medicinal plants (Table 1) with their traditionally known or published therapeutic values (Arbab et al., 2017). The dried plant parts were ground to a coarse powder using mortar-pestle and extracted with 80% ethanol (Merck, Germany) for three days with periodic shaking and filtered using Whatman No. 1 paper (Sigma, Germany). After removal of the solvent under reduced pressure using rotary evaporator (4 °C) and complete drying, their yield percentage were calculated. Stock of each extract (100 mg/ml) was prepared by dissolving in dimethyl sulfoxide (DMSO, Sigma, USA), and stored at -20 °C.

2.2. Cell culture and reagents

Human hepatoblastoma cell line, HepG2 (López-Terrada et al., 2009) was maintained in T75 culture flask (Corning, USA) in RPMI-1640 medium (Gibco, Thermo Fisher Scientific, Inc., MA,

Table 1
List of medicinal plants (n = 58) screened for PXR-mediated CYP3A4 induction activity.

No.	Specimen no.	Plants name	Family	Plant part used
1	16011	*Achyranthe aspera*	Amaranthaceae	Shoots
2	16391	*Alternanthera pungens*		Shoots
3	16189	*Amaranthus alba*		Shoots
4	16196	*Avera Javanica*		Shoots
5	16198	*Flaveria trineriva*	Asteraceae	Shoots
6	16083	*Pulicaria crispa*		Shoots
7	16075	*Pergularia tomentosa*	Asclepiadaceae	Shoots
8	16318	*Eruca sativa*	Brassicaceae	Leaves, Stems
9	15841	*Capparis decidua*	Capparaceae	Stems
10	16195	*Atriplex suberecta*	Chenopodiaceae	Shoots
11	15496	*Combretum molle*	Combretaceae	Bark
12	798	*Guiera senegalensis*		Leaves
13	16075	*Ipomoea cairica*	Convolvulaceae	Shoots
14	16179	*Juniperus phonicea*	Cupressaceae	Leaves, Stems
15	16194	*Juniperus procera*		Leaves, Stems
16	15830	*Cleome droserifolia*	Crassulaceae	Shoots
17	16275	*Coccinia grandis*	Cucurbitaceae	Leaves, Stems
18	16393	*Corallocarpus epigeus*		Leaves
19	16395	*Momordica balsamina*		Leaves
20	16181	*Chenopodium ambrosioides*		Arial parts
21	16197	*Chenopodium glaucum*		Leaves, Stem
22	16172	*Euphorbia tirucalli*	Euphorbiaceae	Stems
23	16084	*Euphorbia hirta*		Shoots
24	15189	*Jatropha curcas*		Seeds
25	14005	*Ricinus communis*		Leaves
26	16281	*Acacia mellifera*	Fabaceae	Leaves
27	16221	*Acacia hamulosa*		Leaves, Stems
28	16387	*Acacia asak*		Leaves
29	16385	*Acacia ehrenbergiana*		Stems
30	16390	*Acacia laeta*		Stems
31	16389	*Acacia oerfota*		Stems
32	15007	*Acacia salicina*		Leaves
33	14977	*Acacia tortilis*		Stems
34	16182	*Albizia procera*		Leaves
35	16035	*Delonix elata*		Leaves
36	16183	*Delonix regia*		Leaves
37	16392	*Indigofera coerulea*		Shoots
38	16390	*Indigofera tinctoria*		Shoots
39	160322	*Senna obtusifolia*		Fruits
40	155009	*Senna occidentalis*		Fruits
41	16245	*Senna alexandrina*		Leaves
42	16301	*Fumaria parviflora*	Fumariaceae	Leaves, Stems
43	16043	*Marrubium vulgare*	Labiatae	Shoots
44	15716	*Cassytha filiformis*	Lauraceae	Stems
45	16082	*Abutilon figarianum*	Malvaceae	Leaves
46	16080	*Ficus benghalensis*	Moraceae	Leaves, Stems
47	15448	*Ficus palmata*		Leaves
48	16085	*Psidium guajava*	Myrtaceae	Leaves
49	16184	*Boerhavia diffusa*	Nyctaginaceae	Leaves
50	16177	*Bougainvillea spectabilis*		Leaves
51	16185	*Argemone ochroleuca*	Papaveraceae	Shoots
52	16186	*Rumex dentatus*	Polygonaceae	Shoots
53	16173	*Citrus maxima*	Rutaceae	Leaves
54	15787	*Dodonea angustifolia*	Sapindaceae	Leaves
55	15604	*Daturai noxia*	Solanaceae	Leaves
56	16386	*Solanum surrattense*		Leaves
57	12788	*Clerodendrum inerme*	Verbenaceae	Leaves, Stems
58	560	*Balanites aegyptiaca*	Zygophyllaceae	Bark

USA), supplemented with 10% heat-inactivated bovine serum (Gibco, MA, USA), 1× penicillin-streptomycin (Gibco, MA, USA) and 1× sodium pyruvate (GE Healthcare Life Sci., UT, USA) in an incubator at 37 °C with 5% CO_2 supply. Dimethyl sulphoxide (DMSO; Sigma, Germany) was used as carrier to prepare stocks of plants extracts or compounds as well as negative control. Rifampicin (Sigma, Germany) was used as standard PXR-mediated CYP3A4 inducer or positive control.

2.3. Plasmid DNA preparations

The nuclear receptor expression vector *pCDG-hPXR* and CYP3A4 firefly-luciferase reporter construct *pGL3-CYP3A4-XREM* were kind gifts from Dr. Ron Evans (The Salk Institute for Biological Studies, La Jolla, USA) and Dr. Richard Kim (Department of Physiology and Pharmacology, University of Western Ontario, London, Canada), respectively. The renilla-luciferase expression plasmid (*pRL-TK*; Promega, USA) served as internal control. All plasmid DNA were transformed into DH5α XL competent cells (Invitrogen, USA) by the heat-shock method and plated on ampicillin (50 μg/ml) containing agar plates. Following an overnight incubation at 37 °C, bacterial colonies were picked and plasmids (Qiagen Plasmid Mini-prep Kit, Germany) were screened by restriction digestion. Further, DNA stocks were prepared (Qiagen Plasmid Maxi-prep Kit, Germany), quantified (Nanodrop 3300) and stored at −20 °C.

2.4. Transient transfection

HepG2 cells were seeded in 24-well culture plates (Corning, USA) and incubated overnight to reach up to 60–70% confluency. Next day, cells were co-transfected with *pGL3-CYP3A4-XREM* (CYP, 400 ng), *pCDG-hPXR* (PXR, 400 ng) and *pRL-TK* (200 ng), using transfection reagent FuGENE6 (Promega, USA) per well. A mock transfection control (negative, without plasmid) was also included. For a 24-well plate (200 μl media/well), the amount of FuGENE6 to DNA per well (3:1) was optimized as per the FuGENE6 manual. After 24 h, the medium was removed and 200 μl/well of fresh medium containing DMSO (0.1%) or rifampicin (10 μM) or plant extracts (50 μg/ml) was added. The treated cells were further incubated for 24 h at 37 °C. The cells were transfected in triplicate for all samples, including controls.

2.5. Luciferase reporter gene assay

After 24 h of treatment (48 h post-transfection), the reporter activities of firefly-luciferase and renilla-luciferase were measured with the Dual-Luciferase Reporter Assay System (Promega, USA) according to the manufacturer's manual. Briefly, reagents were brought to room temperature (RT), and reconstituted. Meanwhile, media were discarded and cells were carefully washed with 1× PBS (200 μl/well). The passive lysis buffer (1× PLB; 60 μl/well) was added and cells were allowed to lyse for 15 min at RT by gentle rocking (Heidolph DuoMax 1030, Heidolph instruments, GmBH, Germany). Total cell lysates were properly mixed and carefully harvested into pre-labeled 1.5 ml Eppendorf tubes. Lysates were quickly cleared at 10000 rpm for 30 sec (Eppendorf 5415D, USA) and placed in ice bath. The assay was instantly performed in round bottom high-clarity polypropylene tubes (5 ml; Falcon, USA) with 100 μl Luciferase Assay Reagent II or Stop & Glo Reagent and 10 μl lysate, using an illuminometer (Berthhold Lumat LB9507, Berthhold Technologies, USA). The assay was performed in triplicate for all samples and repeated. The firefly-luciferase signal was normalized to renilla-luciferase signal for each sample. To calculate fold-inductions, the ratios of all tested extracts were compared with normalized signals of the control. To determine the potency of the active extracts, concentrations <50 μg/ml were also tested. All tests were performed in triplicate and repeated twice. Data was analyzed and represented as bar graph (Excel 2010; Microsoft, OK, USA).

2.6. Statistical analysis

All experiments were performed in triplicate and data were presented as the mean ± standard error, and were analyzed by One Way ANOVA using GraphPad Prism 7.04. The statistical differences between the control (CYP + PXR only) and treatment groups were carried out using Dunnett's Test (P value <0.05).

3. Results

3.1. PXR-dependent CYP3A4 induction by plant extracts

For PXR-dependent CYP3A4 induction, a 50 μg/ml concentration was used for all non-cytotoxic plants ethanol extracts. Of the 58 plants extracts screened, four showed induction of

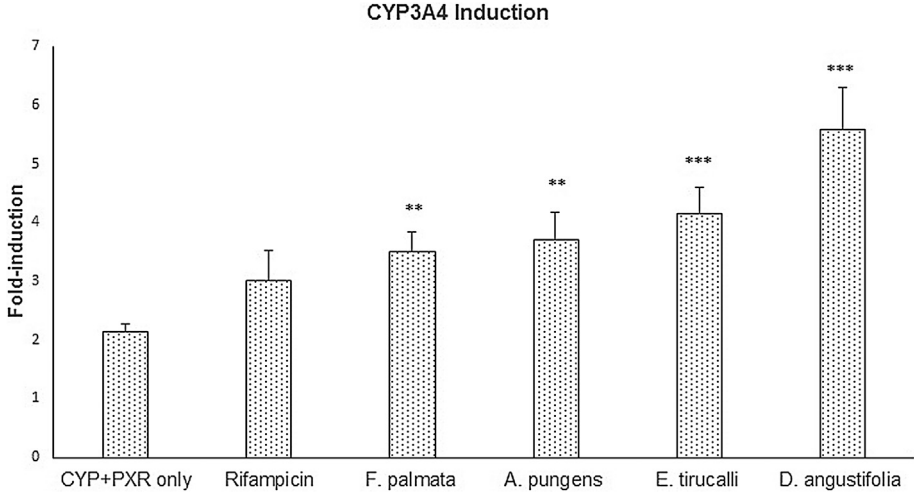

Fig. 1. Reporter gene assay, showing fold-activation of PXR-mediated CYP3A4 expression in HepG2 cells upon treatment with rifampicin (10 μM) and different plant extracts (50 μg/ml). Data are presented as the mean ± standard error (n = 3). $^{**}P < 0.01$, $^{***}P < 0.001$ vs. CYP + PXR only (DMSO control) group. PXR: pCDG-hPXR; CYP: pGL3-CYP3A4-XREM; F. palmate: *Ficus palmate*; A. pungens: *Alternanthera pungens*; E. tirucalli: *Euphorbia tirucalli*; D. angustifolia: *Dodonaea angustifolia*.

PXR-mediated CYP3A4 expression. The comparative fold-induction of CYP34A by the plant extracts in relation to the untreated (DMSO) control was determined (Fig. 1). The induction activities of the tested plants were in the order, *Dodonaea angustifolia* (2.62 fold, $P < 0.0001$), *Euphorbia tirucalli* (1.95 fold, $P = 0.0004$), *Alternanthera pungens* (1.74 fold, $P = 0.0035$), *Ficus palmata* (1.65 fold, $P = 0.0097$); and the rifampicin mediated fold of induction was 1.42 ($P = 0.1209$).

4. Discussion

The PXR-mediated CYP3A4 expression is markedly induced in cultured human hepatocytes in response to a variety of xenobiotics and drugs, including some bioactive plant products. In this study, we have therefore, screened ethanol extracts of 58 medicinal plants using HepG2 cell culture and dual-luciferase assay for their PXR-mediated CYP3A4 activation potential. All the non-toxic extracts were tested at the safe concentration 50 μg/ml as compared to a similar study where extracts at 100 μg/ml doses were used (Mooiman et al., 2013).

Dodonaea angustifolia (sand olive) occurs naturally in Arabia and southern Africa, including Australia and New Zealand. An important traditional medicine of Africa, its leaves decoction is used for fever, colds, flu, stomachache, measles, tuberculosis and skin rashes (van Heerden et al., 2000). However, very limited studies have been published on *Dodonaea angustifolia* and none on its chemical constituents compared to its other species. A single study has reported presence of flavonoids, reducing sugars, alkaloids, saponins and tannin in its leaves (Amabeoku et al., 2001). Here, we have for the first time, demonstrated its very promising activation potential of PXR-mediated CYP3A4 expression in HepG2 cells.

Euphorbia tirucalli (firestick or milk bush) has a wide distribution in Arabia and Africa, including many other tropical regions. It is a hydrocarbon plant that produces a poisonous latex, possibly convertible to biofuel (Hastilestari et al., 2013). It is used in traditional medicine for cancer, excrescence, tumors, warts, asthma, cough, earache, neuralgia and rheumatism (Duke, 1983). In this study, we report a novel PXR-mediated CYP3A4 activation property of *Euphorbia tirucalli* in HepG2 cells.

Alternanthera pungens (Kunth) is a ruderal plant of roadsides, path verges and waste places. Though a native of South America, it is also reported from other tropical countries including India (Jakhar and Dahiya, 2017). Compared to its other species, *Alternanthera pungens* is poorly studied. In a very recent study, its crude extract is shown to have a wide spectrum antibacterial activity as well as good antioxidant potential (Jakhar and Dahiya, 2017). Here, we have demonstrated the novel PXR-mediated CYP3A4 activation potential *Alternanthera pungens* in HepG2 cells.

Ficus palmata (Fegra or Wild Himalayan Fig) occurs in North West India, Afghanistan, Iran, Arabia and Africa (Joshi et al., 2014). It is used as hypoglycemic, anti-tumour, anti-ulcer, anti-diabetic, lipid lowering and antifungal remedy, including nephro-hepatoprotective effect (Joshi et al., 2014). In this report, we have shown a novel PXR-mediated CYP3A4 inducing activity of *Ficus palmata* in HepG2 cells.

5. Conclusion

Our screening of ethanol extracts of 58 medicinal plants using HepG2 cells and reporter gene assay, has demonstrated the novel PXR-mediated CYP3A4 gene induction potential of four plants. Of these, *Dodonaea angustifolia* was found to be the most promising CYP3A4 activator, followed by *Euphorbia tirucalli*, *Alternanthera pungens*, and *Ficus palmata*. Further phytochemical characterizations, including isolation of active principles are therefore, warranted.

References

Amabeoku, G.J., Eagles, P., Scott, G., Mayeng, I., Springfield, E., 2001. Analgesic and antipyretic effects of *Dodonaea angustifolia* and *Salvia africana-lutea*. J. Ethnopharmacol. 75, 117–124.

Anzenbacher, P., Anzenbacherová, E., 2001. Cytochromes P450 and metabolism of xenobiotics. Cell. Mol. Life Sci. 58, 737–747.

Arbab, A.H., Parvez, M.K., Al-Dosari, M.S., Al-Rehaily, A.J., 2017. *In vitro* evaluation of novel antiviral activities of 60 medicinal plants extract against hepatitis B virus. Exp. Ther. Med. 14, 626–634.

Al-Dosari, M.S., Parvez, M.K., 2016. Genetic polymorphisms of drug eliminating enzymes and transporters. Biomed. Genet. Genom. 1, 44–50.

Duke, J., 1983. Euphorbia tirucalli L., Handbook of Energy Crops. Purdue University Centre for New Crops and Plant Products. Available at: <www.hort.purdue.edu>.

Hastilestari, B.R., Mudersbach, M., Tomala, F., Vogt, H., Biskupek-Korell, B., Van Damme, P., Guretzki, S., Papenbrock, J., 2013. *Euphorbia tirucalli* L.-comprehensive characterization of a drought tolerant plant with a potential as biofuel source. PLoS One 8, e63501.

Harmsen, S., Koster, A.S., Beijnen, J.H., Schellens, J.H., Meijerman, I., 2008. Comparison of two immortalized human cell lines to study nuclear receptor-mediated CYP3A4 induction. Drug Metab. Dispos. 36, 1166–1171.

Hustert, E., Zibat, A., Presecan-Siedel, E., Eiselt, R., Mueller, R., Fuss, C., Brehm, I., Brinkmann, U., Eichelbaum, M., Wojnowski, L., Burk, O., 2001. Natural protein variants of pregnane X receptor with altered transactivation activity toward CYP3A4. Drug Metab. Dispos. 29, 1454–1459.

Hu, D., Wang, Y., Chen, Z., Ma, Z., You, Q., Zhang, X., Liang, Q., Tan, H., Xiao, C., Tang, X., Gao, Y., 2015. The protective effect of piperine on dextran sulfate sodium induced inflammatory bowel disease and its relation with pregnane X receptor activation. J. Ethnopharmacol. 169, 109–123.

Hu, D., Wang, Y., Chen, Z., Ma, Z., You, Q., Zhang, X., Zhou, T., Xiao, Y., Liang, Q., Tan, H., Xiao, C., Tang, X., Zhang, B., Gao, Y., 2014. Artemisinin protects against dextran sulfate-sodium-induced inflammatory bowel disease, which is associated with activation of the pregnane X receptor. Eur. J. Pharmacol. 738, 273–284.

Jakhar, S., Dahiya, P., 2017. Antimicrobial, antioxidant and phytochemical potential of Alternanthera pungens HB&K. J. Pharm. Sci. Res. 9, 1305–1311.

Joshi, Y., Joshi, A.K., Prasad, N., Juyal, D., 2014. A review on *Ficus palmata* (Wild Himalayan Fig). J. Phytopharmacol. 3, 374–377.

Kolars, J.C., Schmiedlin-Ren, P., Schuetz, J.D., Fang, C., Watkins, P.B., 1992. Identification of rifampin-inducible P450IIIA4 (CYP3A4) in human small bowel enterocytes. J. Clin. Invest. 90, 1871–1878.

Lehmann, J.M., McKee, D.D., Watson, M.A., Willson, T.M., Moore, J.T., Kliewer, S.A., 1998. The human orphan nuclear receptor PXR is activated by compounds that regulate CYP3A4 gene expression and cause drug interactions. Proc. Natl. Acad. Sci. USA 95, 12208–12213.

Matic, M., Mahns, A., Tsoli, M., Corradin, A., Polly, P., Robertson, G.R., 2007. Pregnane X receptor: promiscuous regulator of detoxification pathways. Int. J. Biochem. Cell Biol. 39, 478–483.

Moore, L.B., Goodwin, B., Jones, S.A., Wisely, G.B., Serabjit-Singh, C.J., Willson, T.M., Collins, J.L., Kliewer, S.A., 2000. St. John's wort induces hepatic drug metabolism through activation of the pregnane X receptor. Proc. Natl. Acad. Sci. USA 97, 7500–7502.

Mooiman, K.D., Maas-Bakker, R.F., Moret, E.E., Beijnen, J.H., Schellens, J.H., Meijerman, I., 2013.. Milk thistle's active components silybin and isosilybin: novel inhibitors of PXR-mediated CYP3A4 induction. Drug Metab. Dispos. 41, 1494–1504.

López-Terrada, D., Cheung, S.W., Finegold, M.J., Knowles, B.B., 2009. HepG2 is a hepatoblastoma-derived cell line. Hum. Pathol. 40, 1512–1515.

Pelkonen, O., Mäenpää, J., Taavitsainen, P., Rautio, A., Raunio, H., 1998. Inhibition and induction of human cytochrome P450 (CYP) enzymes. Xenobiotica 28, 1203–1253.

Quatrochi, L.C., Guzelian, P.S., 2001. CYP3A regulation: from pharmacology to nuclear receptors. Drug Met. Disposit. 29, 615–622.

Schuetz, E.G., Guzelian, P.S., 1984. Induction of cytochrome P450 by glucocorticoids in rat liver. II. Evidence that glucocorticoids regulate induction of cytochrome P450 by a nonclassical receptor mechanism. J. Biol. Chem. 259, 2007–2012.

Tyagi, L.K., Singh, M., Singh, V., Singh, N., Tyagi, S.N., Kori, M.L., 2010. Herb-drug interactions: emerging threat and their management. Bot. Res. Int. 3, 1–13.

van Heerden, F.R., Viljoen, A.M., van Wyk, B.E., 2000. The major flavonoid of *Dodonaea angustifolia*. Fitoterapia 71, 602–604.

Zhang, L., Reynolds, K.S., Zhao, P., Huang, S.M., 2010. Drug interactions evaluation: an integrated part of risk assessment of therapeutics. Toxicol. Appl. Pharmacol. 243, 134–145.

Zhang, J., Ding, L., Wang, B., Ren, G., Sun, A., Deng, C., Wei, X., Mani, S., Wang, Z., Dou, W., 2015. Notoginsenoside R1 attenuates experimental inflammatory bowel disease via pregnane X receptor activation. J. Pharmacol. Exp. Ther. 352, 315–324.

Zhou, S.F., Lai, X., 2008. An update on clinical drug interactions with the herbal antidepressant St. John's wort. Curr. Drug Metab. 9, 394–409.