European Journal of Pharmaceutical Sciences 92 (2016) 203–211

Contents lists available at ScienceDirect

European Journal of Pharmaceutical Sciences

journal homepage: www.elsevier.com/locate/ejps

Modulating lipophilicity of rohitukine via prodrug approach: Preparation, characterization, and *in vitro* enzymatic hydrolysis in biorelevant media☆

Vikas Kumar [a,b], Sonali S. Bharate [a,*], Ram A. Vishwakarma [b,c,**]

[a] Preformulation Laboratory, CSIR-Indian Institute of Integrative Medicine, Canal Road, Jammu 180001, India
[b] Academy of Scientific and Innovative Research (AcSIR), CSIR-Indian Institute of Integrative Medicine, Canal Road, Jammu 180001, India
[c] Medicinal Chemistry Division, CSIR-Indian Institute of Integrative Medicine, Canal Road, Jammu 180001, India

ARTICLE INFO

Article history:
Received 26 May 2016
Received in revised form 27 June 2016
Accepted 11 July 2016
Available online 12 July 2016

Keywords:
Prodrugs
Drug discovery
Rohitukine
Solubility
Lipophilicity
Enzymatic hydrolysis

ABSTRACT

Rohitukine is a medicinally important natural product which has inspired the discovery of two anticancer clinical candidates. Rohitukine is highly hydrophilic in nature which hampers its oral bioavailability. Thus, herein our objective was to improve the drug-like properties of rohitukine via prodrug-strategy. Various ester prodrugs were synthesized and studied for solubility, lipophilicity, chemical stability and enzymatic hydrolysis in plasma/esterase. All prodrugs displayed lower aqueous solubility and improved lipophilicity compared with rohitukine, which was in accordance with the criteria of compounds in drug-discovery. The stability of synthesized prodrugs was evaluated in buffers at different pH, SGF, SIF, rat plasma and in esterase enzyme. The rate of hydrolysis in all incubation media was dependent primarily on the acyl promoieties. Hexanoyl ester prodrug of rohitukine, **3d**, was stable under chemical conditions; however it was completely hydrolyzed to rohitukine, in plasma and in esterase in 4 h. Hexanoate ester **3d** appeared to be the most promising prodrug as it remained intact at gastric/intestinal pH and was completely transformed to the parent compound in plasma as desired for an ideal prodrug. The data presented herein, will help in designing prodrugs with desired physicochemical properties in future in structurally similar chemotypes.

1. Introduction

Prodrugs are chemically oriented drug delivery tools wherein intrinsic physicochemical properties of parent drug/s are modulated through a temporary chemical change by the covalent attachment of a chemical moiety (promoiety). The prodrug being a completely new chemical, it possesses a different physicochemical profile which might allow easier drug delivery. These are bioreversible derivatives of drug/s that undergo an enzymatic and/or chemical transformation *in vivo* to release the parent drug, which can exert the desired pharmacological effect (Guarino, 2011).

There are many literature reports wherein prodrugs have been utilized for increasing aqueous solubility in general and/or modulating the pH dependent aqueous solubility. Fosphenytoin is a highly water-soluble prodrug of anticonvulsant drug phenytoin that is administered intramuscularly (IM) and intravenously (IV). Fospropofol is administered through IV bolus injection and is a phosphoryloxymethyl prodrug of anesthetic propofol (Rautio et al., 2008). The others include sulindac (non-steroidal anti-inflammatory) (Davies and Watson, 1997; Duggan et al., 1977), miproxifene phosphate, TAT–59 (anticancer) (Masuda et al., 1998; Nomura et al., 1998), fosamprenavir (antiviral) (Chapman et al., 2004; Furfine et al., 2004; Wire et al., 2006), estramustine phosphate (anticancer) (Bergenheim and Henriksson, 1998; Perry and McTavish, 1995), prednisolone phosphate (glucocorticoid) (Heimbach et al., 2007; Sousa, 1991), fludarabine phosphate (antiviral) (Boogaerts et al., 2001; Heimbach et al., 2007; Heimbach et al., 2003) etc. There are examples of prodrugs that are synthesized with an objective to modulate solubility in negative direction. To improve lipophilicity of a drug molecule, prodrugs are prepared to mask polar and ionizable groups within the compound so as to improve its oral delivery. Increasing lipophilicity of compound/drug promotes membrane permeation and thus oral absorption. These examples include enalapril (angiotensin converting enzyme inhibitor) (Beaumont et al., 2003; Todd and Heel,

Abbreviations: log D, distribution coefficient; log P, partition coefficient; NCEs, new chemical entities; PBS, phosphate buffer saline; SGF, Simulated gastric fluid; SIF, Simulated intestinal fluid; RP-HPLC, reverse-phase high-performance liquid chromatography; t_R, retention time; UV, ultraviolet; HMBC, Heteronuclear Multiple Bond Correlation; GI_{50}, Concentration for 50% growth inhibition.

☆ IIIM communication number: IIIM/1917/2016.

* Correspondence to: Sonali S. Bharate, Preformulation Laboratory, CSIR-Indian Institute of Integrative Medicine, Canal Road, Jammu 180001, India.
** Corresponding author.
E-mail addresses: sonalibharate@gmail.com (S.S. Bharate), ram@iiim.ac.in (R.A. Vishwakarma).

http://dx.doi.org/10.1016/j.ejps.2016.07.010
0928-0987/

1986), pivampicillin (β-lactam antibiotic) (Ehrnebo et al., 1979; Jusko and Lewis, 1973), oseltamivir (anti-influenza) (Bardsley-Elliot and Noble, 1999; Doucette and Aoki, 2001; McClellan and Perry, 2001), adefovir dipivoxil (antiviral) (Dando and Plosker, 2003; Noble and Goa, 1999), tenofovir disoproxil (antiviral) (Chapman et al., 2003; Gallant and Deresinski, 2003; Shaw et al., 1997), famciclovir (antiviral) (Gudmundsson and Antman, 2007; Hodge et al., 1989; Simpson and Lyseng-Williamson, 2006), ximelagatran (anticoagulant) (Eriksson et al., 2003; Gustafsson et al., 2001), MGS0210 (glutamate receptor antagonist) (Nakamura et al., 2006; Yasuhara et al., 2006) etc. This illustrates how sometimes the dramatic lowering of solubility can be an advantage for enhancing drug delivery and thus oral bioavailability.

Compounds having too many hydroxyl groups often impart polar properties (e.g. rohitukine, propranolol, timolol, penciclovir) and may lead to phase II metabolism. Besides this, due to hydroxyl groups, the properties of parent compound can be manipulated via prodrug approach. Acylation, alkylation, or reduction may lead to a less polar prodrug however phosphorylation can lead to a more soluble prodrug (Dhareshwar and Stella, 2007). Many drugs are efficiently absorbed from the gut however they often demonstrate limited systemic bioavailability due to first-pass metabolism or are inactivated before reaching the systemic circulation. This first-pass metabolism is well reported in drugs bearing the phenolic hydroxyl group, resulting in low bioavailability after oral administration and, thus, limiting their usefulness. The inactivation of these drugs in the gut and/or liver is due to sulfation, glucuronidation, or methylation of the hydroxyl group (George, 1981; Longcope et al., 1985). The approach to circumvent first-pass metabolism of alcohols or phenols is to administer drug orally as a prodrug that may minimize the metabolism in the gut. It is reported that hydrophilic groups *viz.* hydroxyl, thiol, carboxyl, phosphate, or an amine group on the parent drug can be transformed to more lipophilic alkyl or aryl esters. These prodrugs get readily converted to their active species by esterases, which are present throughout the body (Huttunen et al., 2011; Liederer and Borchardt, 2006). The attractiveness of this prodrug approach is that the alkyl chain length can be modified to obtain accurately the preferred lipophilicity.

Rohitukine (**1**, Fig. 1) is a naturally occurring chromone alkaloid, isolated for the first time from *Amoora rohituka* (Roxb.) (Harmon and Weiss, 1979) and later from *Dysoxylum binectariferum* Hook. (Meliaceae) (Naik et al., 1988). It is reported to possess anti-inflammatory as well as anticancer activity (Jain et al., 2012; Mohanakumara et al., 2010; Safia et al., 2015). Recently, we reported (Kumar et al., 2016) *in vitro* cytotoxicity of rohitukine in a panel of 20 cancer cell lines comprising of leukemia, pancreatic, prostate, breast and CNS cancer cell lines. It showed promising cytotoxicity in leukemia cells HL-60 and Molt-4 with GI_{50} values of 10 and 12 μM, respectively. It also showed good cytotoxicity in breast cancer cell lines MDAMB-231 and MDAMB-468 with GI_{50} values of 13 and 17 μM, respectively. The toxicity of rohitukine was also assessed in normal cell lines (fR2, and HEK-293) in order to demonstrate its selectivity toward tumor cells. Rohitukine was found to be non-toxic to normal cells (GI_{50} > 50 μM), indicating its excellent therapeutic window. It is also a very potent inhibitor of cyclin-dependent kinases Cdk-2 and Cdk-9 showing IC_{50} values of 7.3 and 0.3 μM. Rohitukine has also been reported to possess several other pharmacological activities including antidyslipidemic (Mishra et al., 2014), antiadipogenic (Varshney et al., 2014), gastroprotective (Singh et al., 2011), antifertility (Keshri et al., 2007) and antileishmanial activities (Lakshmi et al., 2007).

Rohitukine led to the discovery of two clinical candidates *viz.* flavopiridol and P-276-00. This success clearly indicates that rohitukine

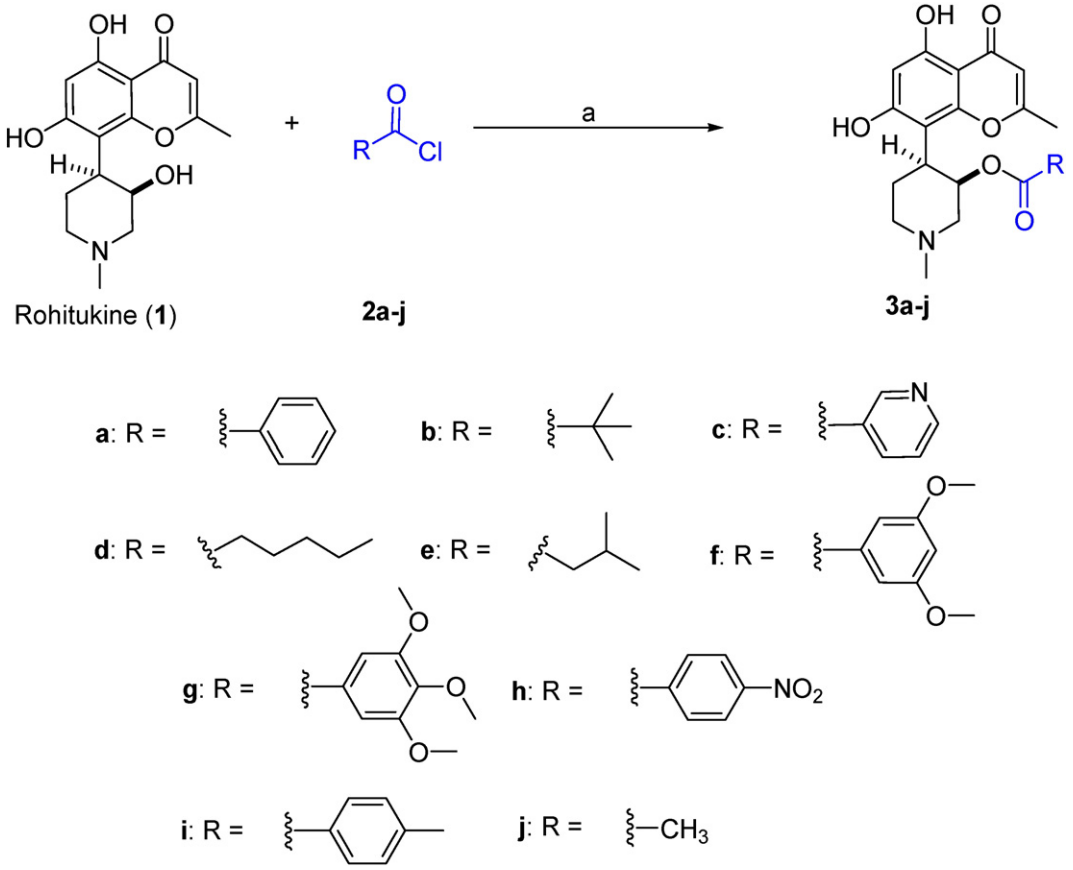

Fig. 1. Synthesis of ester prodrugs **3a–j** of rohitukine (**1**). Reagents and conditions: (a) DMF, TEA, 0–5 °C, 50–70%. Acid chlorides *viz.* **2d**, **2f**, **2g** and **2h** were synthesized using oxalyl chloride with hexanoic acid, 2,5-dimethoxybenzoic acid, 2,3,5-trimethoxybenzoic acid and 4-nitrobenzoic acid respectively. Reagent **2i** was synthesized using 4-methyl benzoic acid and 4-methylbenzoyl chloride. Reaction conditions were dry THF at 0–5 °C.

is a precious natural product and it has further potential to produce more lead candidates. We have determined thermodynamic equilibrium solubility of rohitukine in bio-relevant media namely phosphate buffer saline pH 7.4 (PBS), simulated gastric fluid pH 1.2 (SGF) and simulated intestinal fluid pH 6.8 (SIF). The thermodynamic equilibrium solubility of rohitukine in water, PBS (pH 7.4), SGF (pH 1.2) and SIF (pH 6.8) was found to be 10.246 ± 0.24, 16.936 ± 0.25, 33.366 ± 0.99 and 18.905 ± 0.25 mg/mL, respectively. Both, log P and log D was determined experimentally and was −0.55. This demonstrated hydrophilic nature of rohitukine. It is reported that oral bioavailability of rohitukine in male Sprague Dawley rats is ~30% (Chhonker et al., 2014; Varshney et al., 2014). Attempts have never been made to modulate physicochemical properties namely lipophilicity and solubility of rohitukine. Here, we report the design and synthesis of ester prodrugs of rohitukine with modulated/improved drug-like properties (Fig. 1). We have synthesized ester prodrugs of rohitukine using different promoieties to identify an appropriate promoieties which will give prodrug/s with balanced lipophilicity and aqueous solubility. The thermodynamic equilibrium solubility of synthesized prodrugs was determined in water, PBS (pH 6.8), SGF (pH 1.2) and SIF (pH 6.8). The experimental log P (water/octanol) and log D (PBS pH 7.4/octanol) were determined by miniaturized shake flask method. The chemical stability of synthesized ester prodrugs were evaluated in buffers at pH 1.2, 4.0, 6.8 and 7.4. The enzymatic stability of synthesized prodrugs was also evaluated in SGF, SIF, plasma and esterase from porcine liver.

2. Materials and methods

2.1. Materials

Sodium dihydrogen phosphate, potassium phosphate monobasic, sodium acetate, boric acid, disodium hydrogen phosphate (Alfa Aesar), sodium chloride (Loba Chemie Pvt. Ltd.), pepsin from porcine stomach (Sigma-Aldrich), hydrochloric acid (SD Fine Chemicals), monobasic potassium phosphate (Alfa Aesar), pancreatin from porcine pancreas (Sigma-Aldrich), potassium chloride (Ranbaxy laboratories Ltd.), and sodium hydroxide (Qualigens Fine Chemicals) were used for preparation of buffers and biorelevant media for the study. HPLC grade methanol and acetonitrile (SD Fine Chemicals, Mumbai) was used throughout the study. *n*-Octanol (Sigma Aldrich) was used for partition and distribution coefficient experiments.

HPLC (Shimadzu, LC-6AD), reversed-phase C18 column (NeoSphere, 5 μm, 250 mm × 4.6 mm), reversed-phase C8 column (Discovery HS, 5 μm, 250 mm × 4.6 mm), Spectramax Plus 384 plate reader (Molecular devices), vortex (IKA vortex Genius 3), microplate shaker (Grant-bio PMS-1000 Microplate Shaker, Digital), pH meter (Thermo electron cooperation orion 20A+), microcentrifuge 5430R (Eppendorf), sonicator and micropipettes were used for the study.

^{1}H, ^{13}C and DEPT NMR spectra were recorded on Brucker-Avance DPX FT-NMR 500 and 400 MHz instruments. ^{13}C NMR spectra were recorded at 125 MHz or 100 MHz. ESI-MS spectra were recorded on Agilent 1100 LC-Q-TOF machine. IR spectra were recorded on Perkin-Elmer IR spectrophotometer. Melting points were recorded on digital melting point apparatus.

2.2. Synthesis and purification of ester prodrugs **3a–j** of rohitukine

Rohitukine (500 mg, 1.64 mM, 1 equiv.) and triethylamine (TEA, 2 equiv.) was dissolved in dry DMF (*N*,*N*-dimethylformamide). The resulting mixture was stirred for 10 min under nitrogen atmosphere in an ice bath. To this solution, the respective acid chloride (1 equiv.) was added drop-wise under stirring. The resulting reaction mixture was stirred at room temperature for 1 h and the reaction was quenched by addition of chloroform. DMF and chloroform was evaporated under reduced pressure. The residue was dissolved in 25 mL of chloroform and washed with 10 mL of water (saturated with potassium carbonate), brine and water respectively. The organic layer was dried over sodium sulfate and further concentrated under reduced pressure. The synthesized esters (Fig. 1, **3a–j**) of rohitukine were purified using silica gel column chromatography by elution with methanol-chloroform.

HPLC purity of compounds was carried out using RP-C18 column (NeoSphere, 250 mm × 4.6 mm, 5 μm). Mobile phase consisted of methanol: water (75:25 v/v) at flow rate of 1.0 mL/min (pump, Shimadzu LC-6AD). The column oven (CTO-10ASVP) temperature was 25 °C and the injection volume (SIL-20A HT Prominence autosampler) was 20 μL. The detector was diode array detector (SPD-M20A, Prominence, Shimadzu) and the detection wavelength was 254 nm.

2.2.1. 5,7-Dihydroxy-8-(3-hydroxy-1-methylpiperidin-4-yl)-2-methyl-4H-chromen-4-one (**1**, rohitukine)

Creamish-white solid; HPLC purity: 99.46% (t_R = 1.9 min); m.p. 126–128 °C; ^{1}H NMR (400 MHz, CD_3OD): δ 6.15 (s, 1H), 5.97 (s, 1H), 4.14 (brs, 1H), 3.62–3.59 (m, 1H), 3.49 (d, *J* = 8.4 Hz, 1H), 3.38 (t, *J* = 4.4 Hz, 1H), 3.35 (d, *J* = 4 Hz, 1H), 3.22–3.20 (m, 1H), 3.15 (d, *J* = 12 Hz, 1H), 2.82 (s, 3H), 2.32 (s, 3H), 1.75 (d, *J* = 8 Hz, 1H); ^{13}C NMR (100 MHz, CD_3OD): δ 183.0, 167.7, 163.0, 160.7, 156.5, 107.4, 106.4, 104.0, 99.4, 66.7, 60.4, 55.4, 42.9, 35.7, 21.9, 18.9; IR ($CHCl_3$) ν_{max} 3584, 3312, 2956, 2920, 2870, 1741, 1653, 1460, 1378, 1247, 1082, 1020, 771 cm^{-1}; ESI-MS: *m/z* 306.01 $[M + H]^+$; HRMS: *m/z* 306.1367 calcd for $C_{16}H_{20}NO_5 + H^+$ (306.1341).

2.2.2. 4-(5,7-Dihydroxy-2-methyl-4-oxo-4H-chromen-8-yl)-1-methylpiperidin-3-yl benzoate (**3a**)

Cream white powder; HPLC purity: 97.09% (t_R = 5.1 min); m.p. 235–237 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 12.81 (s, 1H), 7.83 (d, *J* = 12 Hz, 2H), 7.51 (t, *J* = 8, 12 Hz, 1H), 7.34 (t, *J* = 8, 16 Hz, 2H), 6.27 (s, 1H), 5.83 (s, 1H), 5.55 (brs, 1H), 3.99 (s, 1H), 3.20–3.11 (m, 2H), 2.86–2.68 (m, 2H), 2.51 (s, 1H), 2.45 (s, 3H), 2.09 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 182.61, 166.08, 165.92, 163.72, 160.81, 156.77, 133.251, 129.75, 129.429, 128.37, 108.18, 104.40, 101.13, 70.01, 55.96, 52.25, 44.58, 24.43, 20.04; IR ($CHCl_3$) ν_{max} 3585, 2926, 2852, 2684, 1715, 1660, 1587, 1422, 1389, 1269, 1185, 1115, 967, 845, 754 cm^{-1}; ESI-MS: *m/z* 410.00 $[M + H]^+$; HRMS: *m/z* 410.1589 calcd for $C_{23}H_{24}NO_6 + H^+$ (410.1604).

2.2.3. 4-(5,7-Dihydroxy-2-methyl-4-oxo-4H-chromen-8-yl)-1-methylpiperidin-3-yl pivalate (**3b**)

Light yellow solid; HPLC purity: 97.29% (t_R = 9.8 min); m.p. 178–179 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 12.88 (s, 1H), 6.31 (s, 1H), 6.02 (s, 1H), 5.23–5.20 (m, 1H), 3.79 (s, 1H), 3.15–3.10 (m, 2H), 2.90 (s, 1H), 2.67 (d, *J* = 8 Hz, 1H), 2.46 (s, 3H), 2.37 (s, 3H), 2.05–1.95 (m, 1H), 1.45–1.36 (m, 1H), 1.02 (s, 9H); ^{13}C NMR (125.76 MHz, $CDCl_3$): δ 182.74, 178.28, 166.08, 163.31, 160.73, 156.63, 108.46, 104.43, 104.29, 100.49, 69.82, 59.94, 56.95, 46.03, 44.86, 38.82, 26.88, 24.53, 20.51; IR ($CHCl_3$) ν_{max} 3584, 3389, 2957, 2923, 1724, 1660, 1590, 1462, 1389, 1277, 1187, 1140, 846, 762 cm^{-1}; ESI-MS: *m/z* 390.00 $[M + H]^+$; HRMS: *m/z* 390.1899 calcd for $C_{21}H_{28}NO_6 + H^+$ (390.1917).

2.2.4. 4-(5,7-Dihydroxy-2-methyl-4-oxo-4H-chromen-8-yl)-1-methylpiperidin-3-yl nicotinate (**3c**)

Light yellow powder; HPLC purity: 97.42% (t_R = 20.0 min); m.p. 208–210 °C; ^{1}H NMR [400 MHz, $(CD_3)_2CO$]: δ = 12.92 (s, 1H), 9.01 (s, 1H), 8.62 (dd, *J* = 1.6, 4.8 Hz, 1H), 8.14 (s, 1H), 7.37–7.34 (m, 1H), 6.15 (s, 1H), 5.86 (s, 1H), 5.25 (brs, 1H), 3.45 (d, *J* = 16 Hz, 1H), 3.27 (d, *J* = 12 Hz, 1H), 3.13 (d, *J* = 16 Hz, 1H), 2.97 (s, 1H), 2.31 (s, 3H), 2.89 (s, 3H), 1.72 (d, *J* = 8 Hz, 1H); ^{13}C NMR [125.76 MHz, $(CD_3)_2CO$]: δ 182.54, 167.07, 164.47, 162.44, 160.83, 156.80, 153.36, 150.54, 136.77, 126.32, 123.43, 107.66, 105.46, 104.11, 98.77, 72.27, 58.60, 56.51, 45.43, 37.12, 25.29, 19.48; IR ($CHCl_3$) ν_{max} 3584, 2924, 2853, 2790, 1722, 1660, 1591, 1420, 1389, 1278, 1187, 1116, 1025, 967, 846, 742 cm^{-1}; ESI-MS: *m/z* 411.00 $[M + H]^+$; HRMS: *m/z* 411.1547 calcd for $C_{22}H_{23}N_2O_6 + H^+$ (411.1556).

*2.2.5. 4-(5,7-Dihydroxy-2-methyl-4-oxo-4H-chromen-8-yl)-1-methylpiperidin-3-yl hexanoate (**3d**)*

White powder; HPLC purity: 97.14% (t_R = 3.3 min); m.p. 203–205 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 12.80 (s, 1H), 6.10 (s, 1H), 6.03 (s, 1H), 5.16 (d, *J* = 4 Hz, 1H), 3.72–3.69 (m, 1H), 3.15–3.05 (m, 1H), 2.67 (s, 1H), 2.45–2.42 (m, 1H), 2.34 (s, 6H), 2.62–2.20 (m, 3H), 1.78 (s, 1H), 1.54–1.47 (m, 3H), 1.28–1.18 (m, 4H), 0.86 (t, *J* = 8, 16 Hz, 3H); ^{13}C NMR (125.76 MHz, $CDCl_3$): δ 182.77, 172.9, 166.02, 162.44, 160.95, 108.64, 105.04, 104.9, 101.10, 71.77, 57.91, 54.76, 51.61, 45.66, 34.29, 33.43, 31.13, 24.87, 24.34, 22.28, 20.51, 13.89; IR ($CHCl_3$) ν_{max} 3357, 2927, 2855, 2351, 1731, 1659, 1589,1420, 1389, 1259, 1185, 1117, 1007, 845, 755 cm^{-1}; ESI-MS: *m/z* 404.00 $[M + H]^+$; HRMS: *m/z* 404.2102 calcd for $C_{22}H_{30}NO_6 + H^+$ (404.2073).

*2.2.6. 4-(5,7-Dihydroxy-2-methyl-4-oxo-4H-chromen-8-yl)-1-methylpiperidin-3-yl 3-methylbutanoate (**3e**)*

Cream white powder; HPLC purity: 94.5% (t_R = 4.7 min); m.p. 187–188 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 12.81 (s, 1H), 6.19 (s, 1H), 5.94 (s, 1H), 5.12 (brs, 1H), 3.50–3.45 (m, 1H), 3.07 (d, *J* = 12 Hz, 2H), 2.93–2.89 (m, 1H), 2.37–2.33 (m, 1H), 2.29 (s, 3H), 2.27 (s, 3H), 2.17–2.2 (m, 1H), 2.05–1.92 (m, 2H), 1.82–1.70 (m, 2H), 0.67 (d, *J* = 8 Hz, 6H); ^{13}C NMR (125.76 MHz, CD_3Cl): δ 182.79, 172.50, 166.19, 162.48, 160.85, 156.29, 108.45, 105.07, 104.77, 100.51, 71.27, 58.23, 55.42, 45.73, 43.36, 34.62, 25.50, 24.82, 22.13, 20.50; IR ($CHCl_3$) ν_{max} 3333, 2957, 2925, 2851, 2443, 1730, 1660, 1589, 1421, 1389, 1292, 1186, 1081, 754 cm^{-1}, ESI-MS: *m/z* 390.00 $[M + H]^+$; HRMS: *m/z* 390.1882 calcd for $C_{21}H_{28}NO_6 + H^+$ (390.1917).

*2.2.7. 4-(5,7-Dihydroxy-2-methyl-4-oxo-4H-chromen-8-yl)-1-methylpiperidin-3-yl 3,5-dimethoxybenzoate (**3f**)*

White powder; HPLC purity: 98.94% (t_R = 4.542 min); m.p. 182–183 °C; ^{1}H NMR (400 MHz, CD_3OD): δ 7.14 (s, 2H), 6.64 (s, 1H), 6.23 (s, 1H), 5.88 (s, 1H), 5.46 (brs, 1H), 3.79 (s, 6H), 3.61–3.58 (m, 1H), 3.40–3.36 (m, 1H), 3.28–3.21 (m, 2H), 2.69 (d, *J* = 16 Hz, 1H), 2.44 (s, 3H), 2.41–2.38 (m, 1H), 2.34 (s, 3H), 1.90 (d, *J* = 12 Hz, 1H); ^{13}C NMR (100 MHz, CD_3Cl): δ 182.63, 166.16, 165.98, 163.52, 160.82, 160.56, 160.14, 156.70, 131.60, 108.24, 107.26, 106.87, 105.35, 104.38, 104.35, 100.94, 70.39, 55.99, 55.47, 55.30, 52.62, 44.52, 32.11, 24.29, 20.17; IR ($CHCl_3$) ν_{max} 3584, 3332, 2923, 2849, 1715, 1660, 1594, 1426, 1389, 1233, 1205, 1156, 1050, 845, 763 cm^{-1}; ESI-MS: *m/z* 470.00 $[M + H]^+$; HRMS: *m/z* 470.1822 calcd for $C_{25}H_{28}NO_8 + H^+$ (470.1815).

*2.2.8. 4-(5,7-Dihydroxy-2-methyl-4-oxo-4H-chromen-8-yl)-1-methylpiperidin-3-yl 3,4,5-trimethoxybenzoate (**3g**)*

White powder; HPLC purity: 98.25% (t_R = 9.67 min); m.p. 228–230 °C; ^{1}H NMR (400 MHz, $CDCl_3$ + CD_3OD) δ: 7.23 (s, 2H), 6.10 (s, 1H), 5.74 (s, 1H), 5.44 (brs, 1H), 3.76 (s, 6H), 3.72 (s, 3H), 3.52–3.48 (m, 1H), 3.30–3.23 (m, 1H), 3.19–3.15 (m, 2H), 2.61 (d, *J* = 12 Hz, 1H), 2.38 (s, 3H), 2.33–2.28 (m, 1H), 2.15 (s, 3H), 1.77 (d, *J* = 12 Hz, 1H); ^{13}C NMR (100 MHz, CD_3Cl + CD_3OD) δ: 184.22, 168.74, 167.51, 164.41, 161.83, 158.12, 154.28, 143.79, 126.82, 108.99, 108.45, 106.48, 105.20, 99.98, 72.47, 61.24, 60.10, 57.91, 57.14, 46.43, 38.49, 30.74, 26.18, 20.366; IR ($CHCl_3$) ν_{max} 3381.97, 2978.35, 2946.27, 2882.52, 2739.21, 2605.55, 2498.58, 2321.53, 1661.27, 1590.66, 1476.06, 1397.68, 1173.34, 1126.68, 1037.01, 851.08, 807.56 cm^{-1}; ESI-MS: *m/z* 500.00 $[M + H]^+$; HRMS: *m/z* 500.1929 calcd for $C_{26}H_{30}NO_9 + H^+$ (500.1921).

*2.2.9. 4-(5,7-Dihydroxy-2-methyl-4-oxo-4H-chromen-8-yl)-1-methylpiperidin-3-yl 4-nitrobenzoate (**3h**)*

Yellow powder; HPLC purity: 97.23% (t_R = 8.4 min); m.p. = 118–120 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 12.79 (s, 1H), 8.19 (d, *J* = 8 Hz, 2H), 8.04 (d, *J* = 8 Hz, 2H), 6.23 (s, 1H), 5.90 (s, 1H), 5.55–5.49 (m, 1H), 4.02–3.93 (m, 1H), 3.25–3.05 (m, 2H), 2.98–2.81 (m, 2H), 2.73–2.67 (m, 1H), 2.44 (s, 3H), 2.20 (s, 3H), 2.13–2.02 (m, 1H); ^{13}C NMR (125 MHz, CD_3Cl) δ: 182.39, 166.12, 164.43, 162.89, 160.62, 156.64, 150.15, 135.43, 130.72, 130.43, 123.55, 122.97, 108.17, 104.75, 104.15, 99.80, 72.69, 58.0, 55.97, 45.75, 36.20, 25.06, 20.36; IR ($CHCl_3$) ν_{max} 3385, 2923, 2852, 2280, 1724, 1660, 1526, 1418, 1387, 1351, 1271, 1186, 1115, 844, 757 cm^{-1}; ESI-MS: *m/z* 454.80 $[M + H]^+$; HRMS: *m/z* 455.1461 calcd for $C_{23}H_{23}N_2O_8 + H^+$ (455.1454).

*2.2.10. 4-(5,7-Dihydroxy-2-methyl-4-oxo-4H-chromen-8-yl)-1-methylpiperidin-3-yl 4-methylbenzoate (**3i**)*

Off white powder; HPLC purity: 96.27% (t_R = 4.6 min); m.p. 236–238 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 12.68 (s, 1H), 7.61 (d, *J* = 8 Hz, 2H), 6.95 (d, *J* = 8 Hz, 2H), 6.07 (s, 1H), 5.79 (brs, 1H), 5.29 (s, 1H), 3.96 (s, 1H), 3.19–3.06 (m, 3H), 2.45 (d, *J* = 12 Hz, 1H), 2.27–2.17 (m, 10H), 1.87–1.77 (m, 1H); ^{13}C NMR (100 MHz, CD_3Cl): δ 182.62, 166.45, 166.06, 163.27, 160.60, 156.88, 143.66, 129.40, 128.81, 127.18, 108. 03, 105.02, 104.21, 100.25, 70.93, 57.54, 54.75, 45.45, 34.63, 24.90, 21.59, 20.29; IR ($CHCl_3$) ν_{max} 3584, 2923, 2852, 2786, 2326, 1711, 1660, 1612, 1588, 1389, 1271, 1180, 1080, 980, 753 cm^{-1}; ESI-MS: *m/z* 424.10 $[M + H]^+$; HRMS: *m/z* 424.1765calcd for $C_{24}H_{26}NO_6 + H^+$ (424.1760).

*2.2.11. 4-(5,7-Dihydroxy-2-methyl-4-oxo-4H-chromen-8-yl)-1-methylpiperidin-3-yl acetate (**3j**)*

Cream white powder; HPLC purity: 99.0% (t_R = 17.7 min); m.p. 145–147 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 12.81 (s, 1H), 6.30 (s, 1H), 6.03 (s, 1H), 5.14 (brs, 1H), 3.71–3.66 (m, 1H), 3.20–3.08 (m, 2H), 2.82 (brs, 1H), 2.50–2.44 (m, 1H), 2.42 (s, 3H), 2.37 (s, 3H), 2.35 (brs, 1H), 2.03 (s, 3H), 1.83 (d, *J* = 16 Hz, 1H); ^{13}C NMR (100 MHz, CD_3OD): δ 182.40, 170.22, 166.97, 162.38, 159.95, 156.34, 106.73, 104.49, 103.20, 97.89, 69.98, 57.76, 55.64, 44.07, 35.60, 23.79, 18.94, 18.39; IR ($CHCl_3$) ν_{max} 3584, 2924, 2852, 2792, 1737, 1660, 1588, 1389, 1258, 1116, 1081, 846, 754 cm^{-1}; ESI-MS: *m/z* 348.00 $[M + H]^+$; HRMS: *m/z* 348.1455 calcd for $C18H_{22}NO_6 + H^+$ (348.1447).

2.3. Thermodynamic equilibrium solubility studies

Solubility of synthesized prodrugs was determined by miniaturized shake-flask method (Bharate and Vishwakarma, 2015). Briefly, an excess of the compound was added into 1.5 mL Eppendorf tube containing 200 μL of dissolution medium (water/PBS/SGF/SIF). Eppendorf tubes were shaken at 300 rpm (IKA® MS 3 digital shaker) for 24 h at room temperature in order to achieve equilibrium. After equilibration, the tubes were centrifuged at 16 RCF (G force) for 10 min using Eppendorf centrifuge 5430R. Supernatants were analyzed, to find out amount of drug dissolved in respective dissolution medium. The concentration of compound in dissolution media *viz*. water, PBS (pH 7.4), SGF (pH 1.2) and SIF (pH 6.8) was determined as follow. Calibration curve of compound in water, PBS, SGF and SIF was plotted in the concentration range of 0-160 μg/mL (0, 5, 10, 20, 40, 80 and 160 μg/mL). The optical absorbance of standard solutions and tests were measured at 254 nm, using microplate reader (SpectraMax Plus384), against a blank. The concentration of compound in the dissolution medium was calculated using calibration equation. The analysis was performed in triplicate for each compound.

2.4. Determination of log P and log D

Log P and log D was determined as per our recently published and validated protocol (Bharate et al., 2016). Briefly, in 1.5 mL Eppendorf tube, 200 μL of stock solution (1000 μg/mL prepared in *n*-octanol) was added and volume was made up to 1 mL with 300 μL of presaturated *n*-octanol and 500 μL of presaturated aqueous phase (water/PBS pH 7.4). The Eppendorf tubes were shaken overnight at 500 rpm and centrifuged at 16,000 RCF (G force) for 20 min so as to separate aqueous and organic layer. The supernatants were removed using glass syringe to avoid contamination of aqueous and organic aliquots with each other. For the experiment, presaturated solutions were used. For log P and log D experiments, organic phase consisted of *n*-octanol saturated

with water and PBS (pH 7.4), respectively. The aqueous phase consisted of water and PBS (pH 7.4) saturated with *n*-octanol. The concentration of compound in organic and aqueous phase was determined by HPLC method. The HPLC analysis employed reversed-phase C18 column (NeoSphere, 5 μm, 250 mm × 4.6 mm, Hexon Laboratories Pvt. Ltd., India) using photodiode detector (SPD-M20A, Prominence, Shimadzu). Isocratic mobile phase consisting of 3:1 (v/v) methanol and 0.1% v/v acetic acid was used for the analysis. Injection volume was 20.0 μL (SIL-20A HT Prominence auto-sampler) with flow rate of 1.0 mL/min (pump, LC-6AD Shimadzu liquid chromatography). The column oven temperature was kept at 25 °C (column oven, CTO-10ASVP). Each determination was performed in triplicate.

2.5. In-vitro stability studies

2.5.1. Chemical (non-enzymatic) hydrolysis in different buffers at pH 1.2, 4.0, 6.8 and 7.4

To test stability of compound, different buffers were prepared as per the protocol given in Indian Pharmacopoeia 2007 (Anonymous, 2007). The buffers consisted of hydrochloric acid buffer pH 1.2, phosphate buffer pH 4.0 and phosphate buffered saline (pH 6.8 and pH 7.4) (Mattarei et al., 2013; Yan et al., 2010; Zhang et al., 2013). A known concentration of compound dissolved in DMSO was incubated in buffers of pH 1.2, 4.0, 6.8 and 7.4 (120 μg/mL) at 37 °C for 24 h. From the incubated solutions, aliquot of 50 μL was removed at two time points i.e. at zero time and after 24 h. These aliquots were quenched by adding 200 μL of ice-cold methanol. The resulting solution was centrifuged at 17,000*g* for 10 min at 4 °C. The supernatant was analyzed, using HPLC conditions as mentioned in Table 1, to determine AUC (area under the curve) of the corresponding prodrug ester and its metabolite (parent compound i.e. rohitukine), if any. The experiment was performed in triplicate for each compound.

2.5.2. Stability in biorelevant media, SGF (pH 1.2) and SIF (pH 6.8)

Biorelevant media *viz*. SGF (pH 1.2) and SIF (pH 6.8) were prepared as per the protocol given in USP 2000 (Anonymous, 2000). The stability of compound in biorelevant media *viz*. SGF and SIF was determined as per the protocol mentioned in Section 2.5.1. The solution of compound was incubated in SGF and SIF at 37 °C and aliquot was removed at 0, 4 and 8 h. The HPLC analysis was performed to find out degradation/hydrolysis of ester prodrug to rohitukine, if any, using HPLC method as described in Table 1. Each analysis was performed in triplicate.

2.5.3. Stability in rat plasma

The blood was collected from the jugular vein of rat. It was centrifuged at 17,000*g* for 10 min to get plasma. The stock solution of compound in DMSO was added to preheated plasma at 37 °C to get concentration of 40 μg/mL. One volume of compound stock solution (10 mg/mL) was mixed with 249 volumes of plasma. Aliquot of 50 μL was withdrawn at 0, 5, 15, 30, 60, 120 and 240 min. Extraction and analysis methods were similar as mentioned in Section 2.5.1 and Table 1. Experiments were performed in triplicate.

2.5.4. Stability in esterase from porcine liver (PLE)

A solution of PLE (150 units/mL) was prepared in PBS (pH 7.4) (Legigan et al., 2013). The stock solution of compound was added to preheated PLE solution at 37 °C to get compound concentration of 40 μg/mL and 120 μg/mL. Aliquot of 50 μL was withdrawn at 0, 5, 15, 30, 60, 120 and 240 min. Extraction and analysis methods (Table 1) were similar as mentioned in Section 2.5.1. Each analysis was performed in triplicate. The hydrolysis half-life was calculated using non-linear exponential decay model by graph-pad prism 6.01.

3. Results and discussion

3.1. Synthesis of ester prodrugs of rohitukine

Designing ester prodrugs is one approach for modifying/altering physicochemical properties such as solubility and/or lipophilicity of a compound having hydroxyl groups. It has been reported that ester prodrugs of a compound can be an effective way to optimize the drug delivery properties. The abundance of esterase present in body fluids is responsible for a reversible conversion of prodrug to the parent form (Borkar et al., 2015).

It is reported that branched acyl groups such as pivalic acid chloride, **2b**, as promoieties have been used into prodrugs specifically for their higher lipophilicity and slower chemical and enzymatic hydrolysis in comparison to the corresponding linear analogs e.g. epinephrine, dipivefrine (Hussain and Truelove, 1976; Wei et al., 1978). Higher alkyl chain fatty acids like hexanoic acid chloride, **2d**, have been utilized to generate lipophilic prodrugs such as haloperidol decanoate, a prodrug of haloperidol (Beresford and Ward, 1987). It is also reported that increase in degree of branching or length of the acyl side chain, **2b** and **2e**, will result in lipophilic prodrugs. This also slows down chemical and enzymatic hydrolysis in comparison to corresponding linear analogs (Irwin and Belaid, 1988).

The ester prodrugs were synthesized as depicted in Fig. 1. Rohitukine on treatment with various acyl chlorides resulted in formation of rohitukine-O-esters. The ^{1}H NMR spectrum of all prepared esters showed downfield chemical shift of OH-linked —*CH* hydrogen of piperidine ring. The signal for OH-linked —*CH* hydrogen appears at δ 4.14 ppm whereas this was shifted to δ 5.12–5.55 ppm. This gave us the indication that esterification has taken place at hydroxyl of piperidine ring. In order to have final confirmation, one of the representative

Table 1
HPLC methods and retention times of compounds **3a**–**j**.

Compound	Details of method[a]	Retention time (min)	
		Compound	Rohitukine
3a	Isocratic, mobile phase 70:30–0.1% v/v CH_3COOH:MeOH, RP-C8 column (Discovery HS, 5 μm, 250 mm × 4.6 mm), run time - 30 min	7.1	2.4
3b	Isocratic, mobile phase 70:30–0.1% v/v CH_3COOH:MeOH, RP-C8 column (Discovery HS, 5 μm, 250 mm × 4.6 mm), run time - 20 min	6.1	2.3
3c	Gradient, RP-C18 column (NeoSphere, 5 μm, 250 mm × 4.6 mm), run time - 35 min, mobile phase A is 0.1% v/v CH_3COOH and B is CH_3CN, gradient - B is 5% for 0–2 min, 15% from 2 to 10 min, 35% from 12 to 20 min, 70% from 22 to 23 min, 25% from 30 to 30.01, 5% from 30.01 to 35 min	19.8	16.9
3d	Isocratic, mobile phase 70:30–0.1% v/v CH_3COOH:MeOH, RP-C8 column (Discovery HS, 5 μm, 250 mm × 4.6 mm), run time - 30 min	12.1	2.5
3e	Isocratic, mobile phase 70:30–0.1% v/v CH_3COOH:MeOH, RP-C8 column (Discovery HS, 5 μm, 250 mm × 4.6 mm), run time - 30 min	6.2	2.5
3f	Isocratic, mobile phase 70:30–0.1% v/v CH_3COOH:MeOH, RP-C8 column (Discovery HS, 5 μm, 250 mm × 4.6 mm), run time - 20 min	12.9	2.49
3g	Isocratic, mobile phase 70:30–0.1% v/v CH_3COOH:MeOH, RP-C8 column (Discovery HS, 5 μm, 250 mm × 4.6 mm), run time - 30 min	7.6	2.5
3h	Isocratic, mobile phase 70:30–0.1% v/v CH_3COOH:MeOH, RP-C8 column (Discovery HS, 5 μm, 250 mm × 4.6 mm), run time - 30 min	8.5	2.5
3i	Isocratic, mobile phase 70:30–0.1% v/v CH_3COOH:MeOH, RP-C8 column (Discovery HS, 5 μm, 250 mm × 4.6 mm), run time - 30 min	12.6	2.5
3j	Gradient, RP-C18 column (NeoSphere, 5 μm, 250 mm × 4.6 mm), run time - 32 min, mobile phase A is phosphate buffer pH 7.4 and B is CH_3CN, gradient - B is 5% for 0–1 min, 15% from 1 to 5 min, 20% from 6 to 9 min, 25% from 10 to 14 min, 35% from 14.5 to 21 min, 70% from 21 to 22 min, 20% from 28 to 29 min, 5% from 29 to 30 min	16.0	12.3

[a] Flow rate - 1.0 mL/min; column oven temperature - 37 °C; detection wavelength - 254 nm; injection volume - 10 μL.

Table 2
Experimental aqueous solubility, log P and log D of rohitukine and its ester prodrugs.[a]

Compound	Thermodynamic equilibrium solubility (mg/mL)				Partition coefficient at 25 °C	Distribution coefficient at 25 °C
	Water	PBS (pH 7.4)	SGF (pH 1.2)	SIF (pH 6.8)	Log P (water/*n*-octanol)	Log D (PBS pH 7.4/*n*-octanol)
1	10.246	16.936	33.366	18.905	-0.55 ± 0.063	-0.55 ± 0.098
3a	1.979	0.971	75.043	14.142	2.328 ± 0.087	2.325 ± 0.060
3b	7.382	12.036	26.375	21.300	0.786 ± 0.119	1.659 ± 0.040
3c	>3.000	>3.000	>3.000	>3.000	0.303 ± 0.025	0.381 ± 0.068
3d	0.253	0.306	63.431	8.990	2.206 ± 0.002	2.434 ± 0.005
3e	11.005	7.082	17.573	10.939	−0.183 ± 0.015	2.262 ± 0.221
3f	1.150	0.469	36.100	5.392	0.419 ± 0.120	2.833 ± 0.194
3g	58.650	17.175	155.150	17.400	0.239 ± 0.078	1.804 ± 0.019
3h	9.394	3.842	24.477	1.488	2.091 ± 0.119	1.309 ± 0.053
3i	1.280	0.167	50.810	1.555	0.728 ± 0.036	3.037 ± 0.144
3j	>68.58	>62.84	>80.83	>35.36	0.413 ± 0.020	0.588 ± 0.016

[a] All values are average of three determinations and are represented as mean ± SD.

ester (**3j**) was studied for HMBC (Heteronuclear Multiple Bond Correlation) correlations. The carbonyl of acetyl moiety showed *3J*-correlation with the OH-linked —*CH* hydrogen, which confirmed the position of esterification.

3.2. Determination of aqueous solubility, partition coefficient (log P) and distribution coefficient (log D)

Thermodynamic equilibrium solubility of synthesized ester prodrug (**3a–j**) was determined in water, PBS (pH 7.4), SGF (pH 1.2) and SIF (pH 6.8). Determination of log P and log D is a way of assessing lipophilicity of a compound. Lipophilicity is associated with physicochemical and physiological properties of the compound such as permeability through a membrane. It is described in most cases as partition between two phases (hydrophilic and hydrophobic) (Wang et al., 2007). We determined the effect of different substituent (-R groups, aliphatic and aromatic) on aqueous solubility, partition coefficient and distribution coefficient of rohitukine ester prodrugs (Table 2).

Compounds **3a**, **3b**, **3d**, **3f**, **3h** and **3i** possesses low aqueous solubility in comparison to rohitukine and they were categorized as highly soluble as per the criteria and solubility classification (compounds with solubility > 100 μg/mL are considered to be highly soluble) for discovery projects and in medicinal chemistry (Bharate and Vishwakarma, 2015; Kerns and Di, 2008b).

The log P and log D values had opposite trend wherein synthesized prodrug esters **3a–j** were hydrophobic (log P and log D in the range of −0.1 to 2.4) as compared to rohitukine (both log P and log D, −0.55). These synthesized compounds possess remarkable solubility in water, PBS (pH 7.4), SGF (pH 1.2) and SIF (pH 6.8). At the same time, log P and log D was in the optimum range for oral administration and absorption (0–3). Compounds with lower log P (<0) are more polar and have poor lipid bilayer permeability however the compounds with higher log P (>3) are more non-polar and have poor aqueous solubility (Kerns and Di, 2008a). Based on the aqueous solubility, log P and log D values of **3a–j**, ester prodrugs namely **3a**, **3d**, **3f** and **3h** possess optimal lead-like physicochemical properties *viz.* solubility (>100 μg/mL) and lipophilicity (0–3).

3.3. In-vitro stability studies

The stability of synthesized ester prodrugs of rohitukine (**3a–j**) toward hydrolysis was evaluated in different media: 1) Buffer solutions

Table 3
Stability of ester prodrugs **3a–j** of rohitukine: chemical (non-enzymatic) and enzymatic hydrolysis.

	Chemical (non-enzymatic) hydrolysis				Enzymatic hydrolysis				
	% hydrolyzed after 24 h				% hydrolyzed after 8 h		% hydrolyzed after 4 h		
	HCl buffer pH 1.2[a]	Phosphate buffer pH 4.0[a]	PBS pH 6.8[a]	PBS pH 7.4[a]	SGF (pH 1.2)[a]	SIF (pH 6.8)[a]	Plasma[b]	Esterase from porcine liver[b]	Esterase from porcine liver[a]
1	0	0	0	0	0	2.4 ± 0.3	0.1 ± 0.2	–	–
3a	0.4 ± 0.1	0.9 ± 0.5	1.5 ± 0.1	1.9 ± 0.1	0.03 ± 0.01	6.3 ± 0.5	5.5 ± 5.2	23.9 ± 3.5	27.4 ± 0.5
3b	0	0.1 ± 0. 2	0.3 ± 0.5	0.7 ± 0.6	0.01 ± 0.1	11.1 ± 1.2	0	2.6 ± 2.2	0
3c	0.5 ± 0.3	9.9 ± 1.2	6.8 ± 2.0	16.2 ± 2.8	0.5 ± 0.0	0	36.5 ± 1.9	0	ND
3d	**2.2 ± 1.9**	**3.5 ± 1.2**	**0**	**2.5 ± 1.5**	**0.04 ± 0.05**	**6.6 ± 1.6**	**96.8 ± 1.3**	**100 ± 0.1**	**100 ± 0.1**
3e	1.5 ± 0.1	0.7 ± 0.1	0.8 ± 0.1	1.4 ± 0.06	0	8.9 ± 0.4	14.3 ± 1.9	29.3 ± 2.7	23.5 ± 0.7
3f	0.07 ± 0.03	1.3 ± 0.4	9.8 ± 7.6	1.3 ± 0.9	0	11.3 ± 0.8	18.7 ± 2.1	17.7 ± 0.2	20.2 ± 1.1
3g	0.09 ± 0.03	0.5 ± 0.03	1.4 ± 0.3	1.4 ± 0.4	0.3 ± 0.5	16.9 ± 0.7	0	0	0
3h	0.3 ± 0.1	13.8 ± 1.7	27.8 ± 1.6	32.8 ± 0.6	0	23.8 ± 2.1	32.4 ± 0.8	40.8 ± 5.0	41.9 ± 2.6
3i	0	0	0	0	1.0 ± 0.5	0	11.7 ± 1.1	34.8 ± 0.8	20.9 ± 0.4
3j	4.3 ± 1.2	0	2.0 ± 1.3	0	11.2 ± 0.5	0	80.4 ± 1.5	0	ND

Results are expressed as mean ± SD, n = 3; ND, not determined.
Bold entry indicates prodrug with ideal properties.
[a] Compound concentration is 120 μg/mL.
[b] Compound concentration is 40 μg/mL.

of different pH *viz*. 1.2, 4.0, 6.8 and 7.4 to examine their chemical hydrolysis, 2) SGF (pH 1.2) and SIF (pH 6.8) to evaluate their hydrolysis in biorelevant media, and 3) rat plasma and esterase from porcine liver to understand enzymatic hydrolysis of synthesized prodrugs. The hydrolysis pattern of compounds in biorelevant media, plasma and esterase from porcine liver seems likely the same as under conditions similar to those they may encounter following oral administration.

3.3.1. Chemical (non-enzymatic) hydrolysis in different buffers at pH 1.2, 4.0, 6.8 and 7.4

The % hydrolyzed after incubation of compounds in respective buffer media for 24 h was determined and the values are shown in Table 3. The obtained results indicated that the hydrolysis rate of ester prodrugs primarily depend on the ester promoieties. Ideally, prodrugs must be stable to chemical hydrolysis across a pH range, however they should undergo rapid and quantitative breakdown to yield the parent compound post absorption in plasma and in presence of esterase enzyme (Beaumont et al., 2003; Testa and Mayer, 2003; Zawilska et al., 2013). All ester prodrugs **3a**–**j** were stable to hydrolysis in above mentioned buffers however compounds **3c** and **3h** were hydrolyzed to the extent of ~32% in phosphate buffer solutions at pH 6.8 and 7.4.

3.3.2. Stability in biorelevant media, SGF (pH 1.2) and SIF (pH 6.8)

In vitro enzymatic hydrolysis pattern of ester prodrugs was studied in SGF (pH 1.2) and SIF (pH 6.8) at 37 °C. To mimic *in vivo* gastric and intestinal digestion, simulated gastric and intestinal fluids, containing the most relevant esterase activities in the stomach and/or intestine were used for *in vitro* studies. The % hydrolysis of the compounds is depicted in Table 3. The hydrolysis rates of **3i** and **3j** in SGF (pH 1.2) was ~1–3 times faster than the corresponding rates in HCl buffer (pH 1.2). It was also observed that prodrugs **3a**, **3b**, **3e**, **3f**, **3g** and **3h** were hydrolyzed at faster rate ~ 3–15 times higher in comparison to the hydrolysis rate in PBS (pH 6.8 and 7.4). However, the hydrolysis pattern of **3h** in PBS (pH 6.8 and 7.4) did not change in SIF (pH 6.8). This may be due to the fact that chemical hydrolysis is already so fast that the contribution of enzyme activity becomes negligible.

3.3.3. Stability in rat plasma and esterase from porcine liver (PLE)

In vitro enzymatic hydrolysis of ester prodrugs was studied in rat plasma and in esterase from porcine liver at 37 °C. The hydrolysis rate of **3c**, **3d**, **3e**, **3f** and **3j** in plasma and esterase are faster than the corresponding rate in PBS (pH 7.4), SGF (pH 1.2) and SIF (pH 6.8). This indicated that the hydrolysis of ester prodrugs is facilitated by enzymes. However, the hydrolysis pattern of **3a**, **3b**, **3g** and **3i** in PBS (pH 7.4), SGF (pH 1.2) and SIF (pH 6.8) did not show much change in plasma and in esterase from porcine liver. The data is given in Table 3. The hydrolytic half-life of **3d** in rat plasma and esterase from porcine liver was calculated using graph-pad prism 6.01 software and was found to be 56.85 and 36.7 min, respectively. The hydrolytic half-life of **3c**, **3e**, **3f** and **3j** was >4 h in plasma and in esterase from porcine liver.

Briefly, the compound **3d** was found to have ideal properties that are required for any prodrug. The compound was very stable to chemical

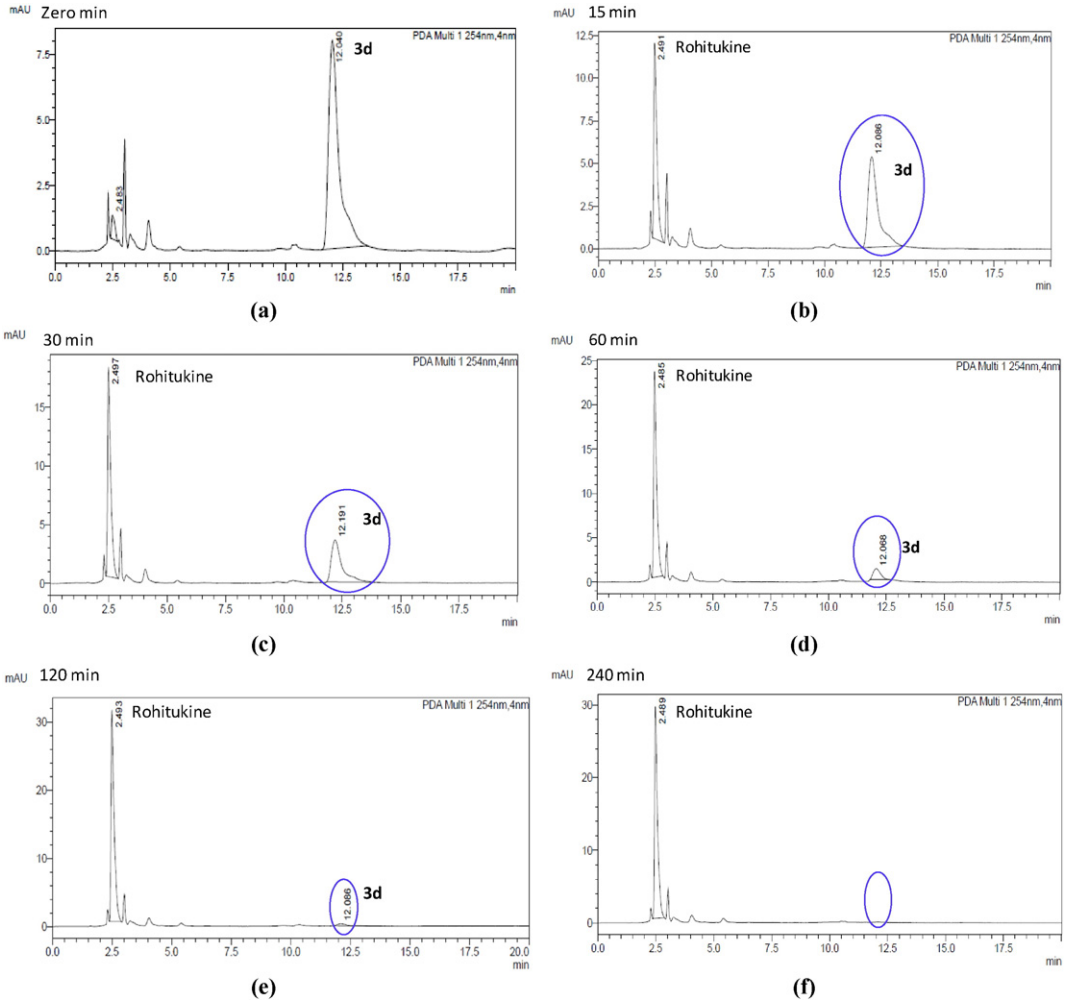

Fig. 2. HPLC chromatograms of compound **3d** recorded at various time points showing hydrolysis of **3d** to rohitukine in esterase from porcine liver. (a) Zero min; (b) 15 min; (c) 30 min; (d) 60 min; (e) 120 min and (f) 240 min.

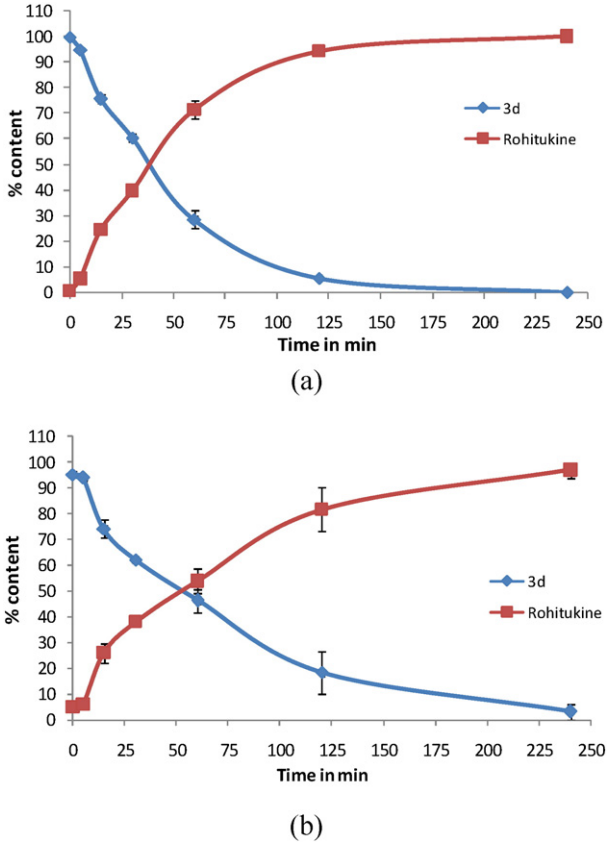

Fig. 3. Time versus concentration profile of 3d and rohitukine (a) in esterase enzyme and (b) in rat plasma.

hydrolysis in buffer solutions of different pH and in SGF (pH 1.2) and SIF (pH 6.8). However, it was completely converted to parent compound i.e. rohitukine in rat plasma and in presence of esterase from porcine liver in ~4 h as depicted in Fig. 2. The % remaining of **3d** at zero, 5, 15, 30, 60, 120 and 240 min was 99.616 ± 0.109, 94.672 ± 0.250, 75.531 ± 1.711, 60.328 ± 11.757, 28.534 ± 3.442, 5.634 ± 0.178 and 0.00 ± 0.00, respectively. Similarly, upon incubation of **3d** with esterase enzyme, it was observed that **3d** was converted to rohitukine as depicted in Fig. 2a–f. The % rohitukine at zero, 5, 15, 30, 60, 120 and 240 min was 0.383 ± 0.109, 5.327 ± 0.250, 24.468 ± 1.711, 39.671 ± 11.757, 71.465 ± 3.442, 94.365 ± 0.178 and 100.00 ± 0.00, respectively. After 240 min of incubation (Fig. 2e), a complete conversion of **3d** to rohitukine was observed. The time versus concentration profile of prodrug **3d** and rohitukine in esterase enzyme and plasma is presented in Fig. 3a and b, respectively. The bioconversion of **3d** to rohitukine was negligible or very slow in PBS (pH 7.4), SGF and SIF. The plausible reason for slow transformation of 3d in SGF and SIF compared with esterase enzyme (and rat plasma) could be that the rohitukine hexanoate (ester prodrug) is a substrate for plasma esterase with $t_{1/2}$ of 57 min. However, it is not a substrate for pepsin and or pancreatic enzymes (amylase, lipase, and protease) which are present gastrointestinal tract.

4. Conclusion

The ester prodrugs of rohitukine were synthesized and investigated for the desirable properties *viz*. solubility and lipophilicity. The data obtained in this study demonstrated that ester prodrugs of rohitukine possess better physicochemical properties, namely solubility and lipophilicity, in comparison to rohitukine which was in accordance with the criteria that is required in drug discovery. All ester prodrugs were highly soluble (>100 μg/mL) in biorelevant media namely PBS, SGF and SIF. Log P and log D of all ester prodrugs was found to be in the range of 0–3 which is as per the criteria required for the compounds in drug discovery. The best identified prodrug **3d** was substrates for the enzymes in plasma and other esterase leading to its conversion to the parent compound, rohitukine. The selection of appropriate ester moiety was essential to control the conversion of ester to free rohitukine in the presence of esterase enzymes. Preventing hydrolysis of ester prodrug in SGF (pH 1.2) and SIF (pH 6.8) was necessary to retain the molecule sufficiently lipophilic to achieve intestinal absorption. Thus, lipophilic prodrugs of compounds containing hydroxyl group may have potential to improve the transport of the prodrugs. The ester prodrugs of rohitukine with improved physicochemical properties were designed, synthesized and evaluated for the first time.

Acknowledgements

V. K. is thankful to UGC for the award of research fellowship. S.S.B. is a research associate (Reference No. 45/33/2013/NAN-BMS) receiving financial support from ICMR. The work was supported by CSIR 12th FYP grant # BSC-0205.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.ejps.2016.07.010.

References

Anonymous, 2000. Solutions. The United States Pharmacopoeia, pp. 2724–2728.
Anonymous, 2007. Reagents and solutions. Indian Pharmacopoeia 1, 239–317.
Bardsley-Elliot, A., Noble, S., 1999. Oseltamivir. Drugs 58, 851–860.
Beaumont, K., Webster, R., Gardner, I., Dack, K., 2003. Design of ester prodrugs to enhance oral absorption of poorly permeable compounds: challenges to the discovery scientist. Curr. Drug Metab. 4, 461–485.
Beresford, R., Ward, A., 1987. Haloperidol decanoate. A preliminary review of its pharmacodynamic and pharmacokinetic properties and therapeutic use in psychosis. Drugs 33, 31–49.
Bergenheim, A.T., Henriksson, R., 1998. Pharmacokinetics and pharmacodynamics of estramustine phosphate. Clin. Pharmacokinet. 34, 163–172.
Bharate, S.S., Vishwakarma, R.A., 2015. Thermodynamic equilibrium solubility measurements in simulated fluids by 96-well plate method in early drug discovery. Bioorg. Med. Chem. Lett. 25, 1561–1567.
Bharate, S., Kumar, V., Vishwakarma, R., 2016. Determining partition coefficient (Log P), distribution coefficient (Log D) and ionization constant (pKa) in early drug discovery. Comb. Chem. High Throughput Screen. 19, 461–469.
Boogaerts, M.A., Van Hoof, A., Catovsky, D., Kovacs, M., Montillo, M., Zinzani, P.L., Binet, J.L., Feremans, W., Marcus, R., Bosch, F., Verhoef, G., Klein, M., 2001. Activity of oral fludarabine phosphate in previously treated chronic lymphocytic leukemia. J. Clin. Oncol. 19, 4252–4258.
Borkar, N., Li, B., Holm, R., Hakansson, A.E., Müllertz, A., Yang, M., Mu, H., 2015. Lipophilic prodrugs of apomorphine I: preparation, characterisation, and *in vitro* enzymatic hydrolysis in biorelevant media. Eur. J. Pharm. Biopharm. 89, 216–223.
Chapman, T., McGavin, J., Noble, S., 2003. Tenofovir disoproxil fumarate. Drugs 63, 1597–1608.
Chapman, T.M., Plosker, G.L., Perry, C.M., 2004. Fosamprenavir: a review of its use in the management of antiretroviral therapy-naive patients with HIV infection. Drugs 64, 2101–2124.
Chhonker, Y.S., Chandasana, H., Kumar, D., Mishra, S.K., Srivastava, S., Balaramnavar, V.M., Gaikwad, A.N., Kanojiya, S., Saxena, A.K., Bhatta, R.S., 2014. Pharmacokinetic and metabolism studies of rohitukine in rats by high performance liquid-chromatography with tandem mass spectrometry. Fitoterapia 97, 34–42.
Dando, T., Plosker, G., 2003. Adefovir dipivoxil: a review of its use in chronic hepatitis B. Drugs 63, 2215–2234.
Davies, N.M., Watson, M.S., 1997. Clinical pharmacokinetics of sulindac. A dynamic old drug. Clin. Pharmacokinet. 32, 437–459.
Dhareshwar, S.S., Stella, V.J., 2007. Prodrugs of alcohols and phenols. In: Stella, V.J., Borchardt, R.T., Hageman, M.J., Oliyai, R., Maag, H., Tilley, J.W. (Eds.), Prodrugs. Springer New York, pp. 731–799.
Doucette, K.E., Aoki, F.Y., 2001. Oseltamivir: a clinical and pharmacological perspective. Expert. Opin. Pharmacother. 2, 1671–1683.
Duggan, D.E., Hare, L.E., Ditzler, C.A., Lei, B.W., Kwan, K.C., 1977. The disposition of sulindac. Clin. Pharmacol. Ther. 21, 326–335.
Ehrnebo, M., Nilsson, S.O., Boreus, L.O., 1979. Pharmacokinetics of ampicillin and its prodrugs bacampicillin and pivampicillin in man. J. Pharmacokinet. Pharmacodyn. 7, 429–451.
Eriksson, U., Bredberg, U., Hoffmann, K., Thuresson, A., Gabrielsson, M., Ericsson, H., Ahnoff, M., Gislén, K., Fager, G., Gustafsson, D., 2003. Absorption, distribution, metabolism, and excretion of ximelagatran, an oral direct thrombin inhibitor, in rats, dogs, and humans. Drug Metab. Dispos. 31, 294–305.

Furfine, E.S., Baker, C.T., Hale, M.R., Reynolds, D.J., Salisbury, J.A., Searle, A.D., Studenberg, S.D., Todd, D., Tung, R.D., Spaltenstein, A., 2004. Preclinical pharmacology and pharmacokinetics of GW433908, a water-soluble prodrug of the human immunodeficiency virus protease inhibitor amprenavir. Antimicrob. Agents Chemother. 48, 791–798.

Gallant, J.E., Deresinski, S., 2003. Tenofovir disoproxil fumarate. Clin. Infect. Dis. 37, 944–950.

George, C., 1981. Drug metabolism by the gastrointestinal mucosa. Clin. Pharmacokinet. 6, 259–274.

Guarino, V.R., 2011. Modulating solubility through prodrugs for oral and IV drug delivery. In: Rautio, J. (Ed.), Prodrugs and Targeted Delivery: Towards Better ADME Properties. WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim, pp. 111–131.

Gudmundsson, O.S., Antman, M., 2007. Famciclovir: a prodrug of penciclovir. In: Stella, V., Borchardt, R., Hageman, M., Oliyai, R., Maag, H., Tilley, J. (Eds.), Prodrugs: Challenges and Rewards. AAPS Press/Springer, New York, pp. 531–539.

Gustafsson, D., Nyström, J., Carlsson, S., Bredberg, U., Eriksson, U., Gyzander, E., Elg, M., Antonsson, T., Hoffmann, K., Ungell, A., Sörensen, H., Någård, S., Abrahamsson, A., Bylund, R., 2001. The direct thrombin inhibitor melagatran and its oral prodrug H 376/95: intestinal absorption properties, biochemical and pharmacodynamic effects. Thromb. Res. 101, 171–181.

Harmon, A.D., Weiss, U., 1979. The structure of rohiturine, the main alkaloid of *Amoora rohituka* (syn. *Aphanafhxis polystachya*) (Meliaceae). Tetrahedron Lett. 85, 721–724.

Heimbach, T., Oh, D., Li, L., Rodríguez-Hornedo, N., Garcia, G., Fleisher, D., 2003. Enzyme-mediated precipitation of parent drugs from their phosphate prodrugs. Int. J. Pharm. 261, 81–92.

Heimbach, T., Fleisher, D., Kaddoumi, A., 2007. Overcoming poor aqueous solubility of drugs for oral delivery. In: Stella, V., Borchardt, R.T., Hageman, M., Oliyai, R., Maag, H., Tilley, H. (Eds.), Prodrugs: Challenges and Rewards. AAPS Press/Springer, New York, pp. 155–212.

Hodge, R.A.V., Sutton, D., Boyd, M.R., Harnden, M.R., Jarvest, R.L., 1989. Selection of an oral prodrug (BRL 42810; famciclovir) for the antiherpesvirus agent BRL 39123 [9-(4-hydroxy-3-hydroxymethylbut-1-yl)guanine; penciclovir]. Antimicrob. Agents Chemother. 33, 1765–1773.

Hussain, A., Truelove, J., 1976. Prodrug approaches to enhancement of physicochemical properties of drugs iv: novel epinephrine prodrug. J. Pharm. Sci. 65, 1510–1512.

Huttunen, K.M., Raunio, H., Rautio, J., 2011. Prodrugs—from serendipity to rational design. Pharmacol. Rev. 63, 750–771.

Irwin, W., Belaid, K., 1988. Drug-delivery by ion-exchange. Stability of ester prodrugs of propranolol in surfactant and enzymatic systems. Int. J. Pharm. 48, 159–166.

Jain, S.K., Bharate, S.B., Vishwakarma, R.A., 2012. Cyclin-dependent kinase inhibition by flavoalkaloids. Mini-Rev. Med. Chem. 12, 632–649.

Jusko, W.J., Lewis, G.P., 1973. Comparison of ampicillin and hetacillin pharmacokinetics in man. J. Pharm. Sci. 62, 69–76.

Kerns, E., Di, L., 2008a. Lipophilicity. In: Kerns, E., Di, L. (Eds.), Drug-like Properties: Concepts, Structure Design and Methods: From ADME to Toxicity Optimization. Elsevier's Science & Technology Rights, Oxford, UK, pp. 43–47.

Kerns, E., Di, L., 2008b. Solubility. In: Kerns, E., Di, L. (Eds.), Drug-like Properties: Concepts, Structure Design and Methods: From ADME to Toxicity Optimization. Elsevier's Science & Technology Rights, Oxford, UK, pp. 56–85.

Keshri, G., Oberoi, R.M., Lakshmi, V., Pandey, K., Singh, M.M., 2007. Contraceptive and hormonal properties of the stem bark of *Dysoxylum binectariferum* in rat and docking analysis of rohitukine, the alkaloid isolated from active chloroform soluble fraction. Contraception 76, 400–407.

Kumar, V., Guru, S.K., Jain, S.K., Joshi, P., Gandhi, S.G., Bharate, S.B., Bhushan, S., Bharate, S.S., Vishwakarma, R.A., 2016. A chromatography-free isolation of rohitukine from leaves of *Dysoxylum binectariferum*: evaluation for *in vitro* cytotoxicity, Cdk inhibition and physicochemical properties. Bioorg. Med. Chem. Lett. http://dx.doi.org/10.1016/j.bmcl.2016.1006.1046.

Lakshmi, V., Pandey, K., Kapil, A., Singh, N., Samant, M., Dube, A., 2007. *In vitro* and *in vivo* leishmanicidal activity of *Dysoxylum binectariferum* and its fractions against Leishmania donovani. Phytomedicine 14, 36–42.

Legigan, T., Clarhaut, J., Renoux, B., Tranoy-Opalinski, I., Monvoisin, A., Jayle, C., Alsarraf, J., Thomas, M., Papot, S., 2013. Synthesis and biological evaluations of a monomethylauristatin E glucuronide prodrug for selective cancer chemotherapy. Eur. J. Med. Chem. 67, 75–80.

Liederer, B., Borchardt, R., 2006. Enzymes involved in the bioconversion of ester-based prodrugs. J. Pharm. Sci. 95, 1177–1195.

Longcope, C., Gorbach, S., Goldin, B., Woods, M., Dwyer, J., Warram, J., 1985. The metabolism of estradiol; oral compared to intravenous administration. J. Steroid Biochem. 23, 1065–1070.

Masuda, H., Ikeda, K., Nagamachi, N., Nagayama, S., Kawaguchi, Y., 1998. Metabolic fate of TAT-59 (4th report): species difference, dose response and protein binding. Yakuri to Chiryo 26, 809–828.

Mattarei, A., Azzolini, M., Carraro, M., Sassi, N., Zoratti, M., Paradisi, C., Biasutto, L., 2013. Acetal derivatives as prodrugs of resveratrol. Mol. Pharm. 10, 2781–2792.

McClellan, K., Perry, C.M., 2001. Oseltamivir: a review of its use in influenza. Drugs 61, 263–283.

Mishra, S., Tiwari, S., Shrivastava, S., Sonkar, R., Mishra, V., Nigam, S., Saxena, A., Bhatia, G., Mir, S., 2014. Pharmacological evaluation of the efficacy of *Dysoxylum binectariferum* stem bark and its active constituent rohitukine in regulation of dyslipidemia in rats. J. Nat. Med. http://dx.doi.org/10.1007/s11418-11014-10830-11413.

Mohanakumara, P., Sreejayan, N., Priti, V., Ramesha, B.T., Ravikanth, G., Ganeshaiah, K.N., Vasudeva, R., Mohan, J., Santhoshkumar, T.R., Mishra, P.D., Ram, V., Shaanker, R.U., 2010. *Dysoxylum binectariferum* Hook. f (Meliaceae), a rich source of rohitukine. Fitoterapia 81, 145–148.

Naik, R., Kattige, S., Bhat, S., Alreja, B., Souza, N.D., Rupp, R., 1988. An antiinflammatory cum immunomodulatory piperidinylbenzopyranone from dysoxylum binectariferum: isolation, structure and total synthesis. Tetrahedron 44, 2081–2086.

Nakamura, M., Kawakita, Y., Yasuhara, A., Fukasawa, Y., Yoshida, K., Sakagami, K., Nakazato, A., 2006. *In vitro* and *in vivo* evaluation of the metabolism and bioavailability of ester prodrugs of MGS0039 (3-(3,4-dichlorobenzyloxy)-2-amino-6-fluorobicyclo[3.1.0]hexane-2,6-dicarb oxylic acid), a potent metabotropic glutamate receptor antagonist. Drug Metab. Dispos. 34, 369–374.

Noble, S., Goa, K.L., 1999. Adefovir dipivoxil. Drugs 58, 479–487.

Nomura, Y., Abe, O., Enomoto, K., Fujiwara, K., Tominaga, T., Hayashi, K., Uchino, J., Takahashi, M., Hayasaka, A., Asaishi, K., Okazaki, M., Abe, R., Kimishima, I., Kajiwara, T., Haga, S., Shimizu, T., Miyazaki, I., Noguchi, M., Yoshida, M., Miura, S., Taguchi, T., Oota, J., Sakai, K., Kinoshita, H., Tashiro, H., 1998. Phase I study of TAT-59 (a new antiestrogen) in breast cancer. Gan To Kagaku Ryoho 25, 553–561.

Perry, C.M., McTavish, D., 1995. Estramustine phosphate sodium. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic efficacy in prostate cancer. Drugs Aging 7, 49–74.

Rautio, J., Kumpulainen, H., Heimbach, T., Oliyai, R., Oh, D., Järvinen, T., Savolainen, J., 2008. Prodrugs: design and clinical applications. Nat. Rev. Drug Discov. 7, 255–270.

Safia, Kamil, M., Jadiya, P., Sheikh, S., Haque, E., Nazir, A., Lakshmi, V., Mir, S., 2015. The chromone alkaloid, rohitukine, affords anti-cancer activity via modulating apoptosis pathways in A549 cell line and yeast Mitogen Activated Protein Kinase (MAPK) pathway. PLoS One 10. http://dx.doi.org/10.1371/journal.pone.0137991.

Shaw, J., Sueoko, C., Oliyai, R., Lee, W., Arimilli, M., Kim, C., Cundy, K., 1997. Metabolism and pharmacokinetics of novel oral prodrugs of 9-[(R)-2-(phosphonomethoxy)propyl]adenine (PMPA) in dogs. Pharm. Res. 14, 1824–1829.

Simpson, D., Lyseng-Williamson, K.A., 2006. Famciclovir: a review of its use in herpes zoster and genital and orolabial herpes. Drugs 66, 2397–2416.

Singh, N., Singh, P., Shrivastva, S., Mishra, S.K., Lakshmi, V., Sharma, R., Palit, G., 2011. Gastroprotective effect of anti-cancer compound rohitukine: possible role of gastrin antagonism and H(+) K (+)-ATPase inhibition. Naunyn Schmiedeberg's Arch. Pharmacol. 385, 277–286.

Sousa, F., 1991. The bioavailability and therapeutic effectiveness of prednisolone acetate vs. prednisolone sodium phosphate: a 20-year review. CLAO J. 17, 282–284.

Testa, B., Mayer, J.M., 2003. The hydrolysis of carboxylic acid ester prodrugs. In: Testa, B., Mayer, J.M. (Eds.), Hydrolysis in Drug and Prodrug Metabolism. Chemistry, Biochemistry, and Enzymology. Verlag Helvetica Chimica Acta, Postfach, CH8042 Zürich, Switzerlaand, pp. 420–535.

Todd, P.A., Heel, R.C., 1986. Enalapril. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic use in hypertension and congestive heart failure. Drugs 31, 198–248.

Varshney, S., Shankar, K., Beg, M., Balaramnavar, V.M., Mishra, S.K., Jagdale, P., Srivastava, S., Chhonker, Y.S., Lakshmi, V., Chaudhari, B.P., Bhatta, R.S., Saxena, A.K., Gaikwad, A.N., 2014. Rohitukine inhibits *in vitro* adipogenesis arresting mitotic clonal expansion and improves dyslipidemia *in vivo*. J. Lipid Res. 55, 1019–1032.

Wang, J.J., Sung, K.C., Huang, J.F., Yeh, C.H., Fang, J.Y., 2007. Ester prodrugs of morphine improve transdermal drug delivery: a mechanistic study. J. Pharm. Pharmacol. 59, 917–925.

Wei, C., Anderson, J., Leopold, I., 1978. Ocular absorption and metabolism of topically applied epinephrine and a dipivalyl ester of epinephrine. Invest. Ophthalmol. Vis. Sci. 17, 315–321.

Wire, M.B., Shelton, M.J., Studenberg, S., 2006. Fosamprenavir: clinical pharmacokinetics and drug interactions of the amprenavir prodrug. Clin. Pharmacokinet. 45, 137–168.

Yan, Y.-D., Kim, H.-K., Seo, K.-H., Lee, W.S., Lee, G.-S., Woo, J.-S., Yong, C.-S., Choi, H.-G., 2010. The physicochemical properties, in vitro metabolism and pharmacokinetics of a novel ester prodrug of EXP3174. Mol. Pharm. 7, 2132–2140.

Yasuhara, A., Nakamura, M., Sakagami, K., Shimazaki, T., Yoshikawa, R., Chaki, S., Ohta, H., Nakazato, A., 2006. Prodrugs of 3-(3,4-dichlorobenzyloxy)-2-amino-6-fluorobicyclo[3.1.0]hexane-2,6-dicarboxylic acid (MGS0039): a potent and orally active group II mGluR antagonist with antidepressant like potential. Bioorg. Med. Chem. 14, 4193–4207.

Zawilska, J.B., Wojcieszak, J., Olejniczak, A.B., 2013. Prodrugs: a challenge for the drug development. Pharmacol. Rep. 65, 1–14.

Zhang, Y., Sun, J., Gao, Y., Jin, L., Xu, Y., Lian, H., Sun, Y., Sun, Y., Liu, J., Fan, R., Zhang, T., He, Z., 2013. A carrier-mediated prodrug approach to improve the oral absorption of antileukemic drug decitabine. Mol. Pharm. 10, 3195–3202.