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

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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.

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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

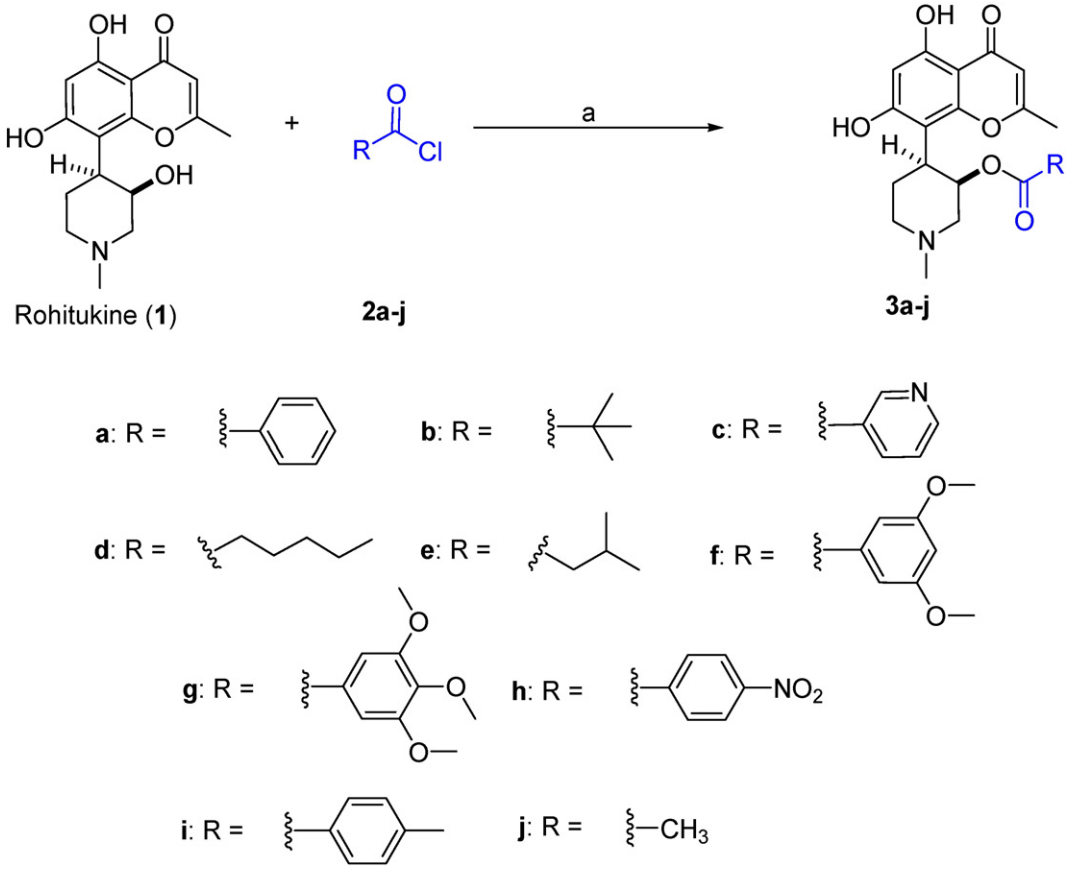

Fig. 1. Synthesis of ester prodrugs **3a–j** of rohitukine (**1**). Reagents and conditions: (a) DMF, TEA, 0–5 °C, 50–70%. Acid chlorides *viz.* **2d**, **2f**, **2g** and **2h** were synthesized using oxalyl chloride with hexanoic acid, 2,5-dimethoxybenzoic acid, 2,3,5-trimethoxybenzoic acid and 4-nitrobenzoic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