IJPSR (2017), Vol. 8, Issue 4 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 15 September, 2016; received in revised form, 14 November, 2016; accepted, 25 November, 2016; published 01 April, 2017

NEW GALACTOSYLATED NSAIDs PRODRUGS IN A GREEN CONTEXT: SYNTHESIS AND STABILITY

Salvatore Magliocca [*1], Federica Sodano [2], Maria Nieddu [3], Lucia Burrai [3], Gianpiero Boatto [3] and Maria Grazia Rimoli [1]

Department of Pharmacy [1], "Federico II" University of Naples, 80131 Naples, Italy.
Department of Drug Science and Technology [2], University of Turin, 10125 Turin, Italy.
Department of Chemistry and Pharmacy [3], University of Sassari, 07100 Sassari, Italy.

Keywords:

Prodrugs, Green Chemistry, Ionic Liquids, Stability, Galactose, NSAIDs

Correspondence to Author:
Salvatore Magliocca

Department of Pharmacy, "Federico II" University of Naples, via D. Montesano 49, 80131 Naples, Italy.

E-mail: magliocca.s@gmail.com

ABSTRACT: Prodrug approach is a new frontier that reinforces the concept of green chemistry in the pharmaceutical chemistry field. The combination of a suitable synthesis and the strategy of the prodrug is of huge advantage for pharmaceutical industries. In this paper, we compare the conventional synthesis of new galactosylated NSAIDs prodrugs with green strategies and demonstrate how we can move away from the use of harmful substances using a suitable method for the synthesis of new compounds. So, we decided to investigate the use of potentially cleaner solvents and reagents such as room temperature ionic liquids, hydrochloric acid and glacial acetic acid. Furthermore, the chemical and enzymatic stabilities of new NSAIDs prodrugs were evaluated in order to determine both their stability in an aqueous solution at pH=1 and pH=7.4 and their feasibility in undergoing enzymatic cleavage by plasma to regenerate the original drug. Results concerning green synthetic approach clearly showed a reduction of time and waste. Moreover, these prodrugs are susceptible to degradation in rat plasma but stable to chemical hydrolysis.

INTRODUCTION: The number of new drugs approved annually continues to decrease, due to the fact that new compounds with pharmacological activity tend to encounter increasingly more difficulty in satisfying all requirements such as solubility, bioavailability, stability, etc. Thus, the pharmaceutical industry is undoubtedly facing a series of challenges.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.8(4).1575-81

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.8 (4).1575-81

This is brought about by the fact that, in order to synthesize new molecules, classic molecular synthesis methods are used and this involves a large amount of materials during the initial stage and finally, at the end of the whole drug producing process, a great many by-products are produced [1]. One of the most important methods used for the synthesis of new compounds is esterification. The conventional method often involves the use of chlorinated solvents causing the organic contamination of final product and are therefore referred to as 'residual solvents' or 'organic volatile impurities' [2]. These reasons are urging pharmaceutical industries to find new advantageous strategies to obtain new drugs. One successful solution is the "green chemistry".

The concept of green chemistry [3] has become a tool for promoting sustainable development in laboratories and industry. The twelve principles of green chemistry [4] are the guidelines addressed to those who want to follow this philosophy. These principles emphasize either the need for using safer and less toxic solvents, or the elimination of solvents and reduction of the use of reagents and auxiliaries, including lowering energy consumption through the use of milder reaction conditions [5], avoiding derivatization as well as preferring renewable source-based substrates [6].

In other words, they provide a framework for actions that can be taken to make chemical products and processes more environmentally benign. These actions are developed representing different areas of chemistry, for example, organic synthesis, chemical engineering, or analytical chemistry.

In this paper we describe and compare the synthesis with conventional and green methods of six galactosylated NSAIDs prodrugs: ketoprofen (a), flurbiprofen (b), ketorolac (c), ibuprofen (d), indomethacin (e) and mefenamic acid (f). On these prodrugs, except for the galactosyl prodrug of ketorolac, were made the chemical and enzymatic stability in order to evaluate the release of the parent drugs themselves. The choice to synthesize these prodrugs is due to the encouraging results achieved with Ketogal, a galactosylated prodrug of ketorolac patented by our research group. This prodrug showed to be a potential candidate for a slower and sustained release form of ketorolac, and above all, compared with its parent drug is much less ulcerogenic while preserving its high pharmacological efficacy [7, 8].

However, the synthesis of this compound follows the classic methods of organic chemistry, while with the desire to avoid the use of unsuitable reagents in chemistry as well as dichloromethane (DCM), trifluoroacetic acid (TFA), etc., so we decided to investigate the use of potentially cleaner solvents and reagents such as ionic liquids, hydrochloric acid and glacial acetic acid. Ionic liquids are generally defined as salts that melt either at or below 100 °C. When the ionic liquids are free-flowing liquids at room temperature, they named room temperature ionic liquids (RTILs). Furthermore they display undetectable vapor pressure throughout the entire temperature range, good electrical conductivity, an interesting mixing behavior with other liquids, including selective solubility of gases and they are recyclable. Thanks to real advantages in terms of handling practicalities, the list of ionic liquids increases daily. The cations are generally bulky, asymmetric ammonium or phosphonium salts, or hetero aromatics, with low symmetry, weak intermolecular interactions and low charge densities [9-10].

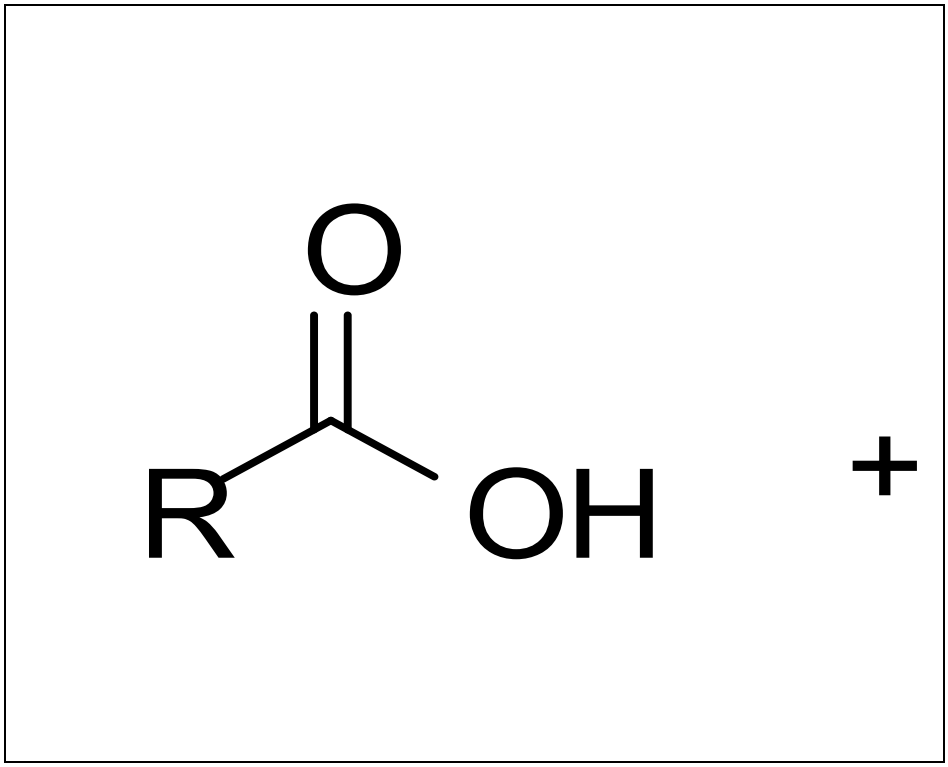
Above all, imidazolium salts, are currently receiving a lot of attention in chemistry and have already proved to be useful solvents in organic synthesis. Some of the reactions already successfully carried out in imidazolium salts include Diels–Alder [11], Wittig [12], the Suzuki cross-coupling [13], Heck [14], oxidations [15], reductions [16], and hydrogenations [17]. In this paper we used 1-butyl-3-methylimidazolium hexafluorophosphate [bmim][PF_6], because it is one of the most widely studied RTILs to date.

MATERIALS AND METHODS:

Chemicals: All the NSAIDs (a-f), [bmim][PF_6], 1,2,3,4-di-O-isopropylidene-D-α - galactopyranose, N-ethyl-N′-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC•HCl), TFA, 4-(dimethyl amino)-pyridine (DMAP) and all the solvents used for the synthesis and stability test were purchased from Sigma-Aldrich and VWR. The course of reactions and purity of products were controlled by TLC (silica gel 60F254s; Merck) and spots were detected by exposure to iodine. Flash chromatography was performed on Merck silica gel (0.040-0.063 mm). ESI mass spectra were recorded with an Applied Biosystems API 2000.

^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on a Varian Mercury 400 spectrometer operating at 400 MHz. Chemical shift values are reported in δ units (ppm) relative to TMS used as the internal standard.

Synthesis of NSAIDs prodrugs: The synthesis, as depicted in **Scheme 1**, was carried out through two steps: esterification of parent drug with DIPG and then cleavage of ketals.



SCHEME 1 : SYNTHESIS OF GALACTOSYLATED NSAIDS PRODRUGS. REAGENTS AND CONDITIONS IN CLASSIC METHOD: (i) EDC•HCl, DMAP, DCM, r.t., 12h; (ii) TFA, DCM. REAGENTS AND CONDITIONS IN GREEN METHOD: (i) EDC•HCl, DMAP, [bmim][PF_6], 35°C; (ii) HCl 1N, GLACIAL ACETIC ACID (GAA), ACETONITRILE (ACN), 50°C, REFLUX.

Classic procedure of synthesis:

Synthesis of compounds 1(a-f),intermediate products : Each NSAIDs (a-f) (1 mol) was reacted with 1,2,3,4-di-O-isopropylidene - D-α-galacto pyranose (1 mol) and EDC•HCl (1 mol) in presence of the DMAP (0.1 mol). The reagents were dissolved in a suitable amount of anhydrous DCM and the reaction mixture was stirred at room temperature for 12 hours. The organic phase for each reaction was extracted several times with water, dried over anhydrous sodium sulphate, filtered, and concentrated in vacuo. The crude reaction product was purified on a chromatography column with silica gel and eluted with dichloromethane.

Synthesis of compounds 2(a-f), final products: Every compound 1(a-f) (1 mol) was dissolved in a suitable amount anhydrous DCM and was added TFA (10 mol). The reaction was then stirred at room temperature for 48 hours. Evaporation of the solvents gives a residue which was purified on a chromatography column with silica gel by using chloroform as eluent in a gradient of methanol to obtain the final prodrugs: **2(a, b, c, d, e, f)**

Green procedure of synthesis

Synthesis of compounds 1(a-f), intermediate products: Each NSAIDs (a-f) (2 mmol) with 1,2,3,4-di-O-isopropylidene-D - α- galactopyranose (1 mmol) and EDC•HCl (2.4 mmol) were dissolved in [bmim][PF_6] (3.4 mL) in presence of DMAP (0.1 mmol). Each reaction mixtures were stirred at 35 °C for 6 hours. The products extracted from ionic liquids with diethyl ether and the crude were purified on a chromatography column with silica gel and eluted by hexane/ether giving the intermediate products.

Synthesis of compounds 2(a-f), final products: The de-protection step was carried out dissolving every intermediate compound (1 mmol) in enough ACN and then hydrochloric acid 1N (1.4 mL) and GAA (2.8 mL) was added. The mixtures were stirred at reflux for 40 min. Evaporation of the solvent gave a residue which was purified on a chromatography column with silica gel by using ethyl acetate as eluent to obtain the final prodrugs: **2(a-f).**

^{1}H, ^{13}C NMR and MS data :

Diacetone 6' - O - Ketoprofen -D-galacto pyranoside (1a) : ^{1}H-NMR ($CDCl_3$): δ 1.30, 1.31, 1.40, 1.41 (4s, 12H, ketals); 1.50 (d, 3H, -CH_3); 3.70 (m, 1H, -CH); 4.05 (m, 1H, 4'-H); 4.15 (m, 1H, 5'-H); 4.20 (m, 2H, 6'-H); 4.40 (m, 1H, 2'-H); 4.50 (m, 1H, 3'-H); 5.35 (m, 1H, 1'-H); 7.31 (m, 2H, 4''-H and 5''-H); 7.36 (m, 2H, 3'''-H and 5'''-H); 7.45 (m, 1H, 4'''-H); 7.52 (d, 1H, 6''-H); 7.56 (s, 1-H, 2''-H); 7.7 (m, 2H, 2'''-H and 6'''-H). ^{13}C-NMR ($CDCl_3$): δ 17 (CH_3); 20 e 22 (4CH_3-ketals); 40 (CH); 65 (C-6'); 66.7 (C-4'); 71 (C-5'); 71.5 (C-2'); 72 (C-3'); 96 (C-1'); 109 e 111(C-ketals); 128 (C-3''' and C-5'''); 129 (C-5'' and C-6''); 130 (C-2''' and C-6'''); 131 (C-2''); 132 (C-4'''); 133 (C-4''); 135 (C-3''); 139 (C-1'''); 140 (C-1''); 174 (CO-ester); 196 (CO-ketone). m/z: 497 $(M + H)^+$. Anal. ($C_{28}H_{32}O_8$) C, H, O.

Ketoprofen-D-galactos-6'-yl ester (2a) : ^{1}H-NMR (CD_3OD): δ 1.50 (d, 3H, -CH_3); 3.45 (m, 1H, 4'-H); 3.70 (m, 1H, -CH); 3.80 (m, 1H, 5'-H); 4.20 (m, 2H, 6'-H); 4.30 (m, 1H, 2'-H); 4.40 (m, 1H, 3'-H); 5.15 (m,1H, 1'-H); 7.31 (m, 2H, 4''-H and 5''-H); 7.36 (m, 2H, 3'''-H and 5'''-H); 7.45 (m, 1H, 4'''-H); 7.52 (d, 1H, 6''-H); 7.56 (s, 1-H, 2''-H); 7.7 (m, 2H, 2'''-H and 6'''-H). ^{13}C-NMR (CD_3OD): δ 17 (CH_3); 40 (CH); 64 (C-6'); 68 (C-4'); 70 (C-5'); 73 (C-2'); 74 (C-3'); 93 and 97 (C-1'); 128 (C-3''' and C-5'''); 129 (C-5'' and C-6''); 130 (C-2''' and C-6'''); 131 (C-2''); 132 (C-4'''); 133 (C-4''); 135 (C-3''); 139 (C-1'''); 140 (C-1''); 174 (CO-ester); 196 (CO-ketone). m/z: 417 $(M + H)^+$. Anal. ($C_{22}H_{24}O_8$) C, H, O. mp: 123-124 °C.

Diacetone 6' - O- Flurbiprofen – D - galacto pyranoside (1b): ^{1}H-NMR ($CDCl_3$): δ 1.30, 1.31, 1.40, 1.41 (4s, 12H, ketals); 1.5 (d, 3H, -CH_3); 3.70 (m, 1H, -CH); 4.05 (m, 1H, 4'-H); 4.15 (m, 1H, 5'-H); 4.20 (m, 2H, 6'-H); 4.40 (m, 1H, 2'-H); 4.50 (m, 1H, 3'-H); 5.35 (m, 1H, 1'-H); 7.17 (m, 2H, 6- and 5-biph); 7.36 (s, 1H, 3-biph); 7.43 (m, 3-H, 9-, 10- and 11-biph); 7.52 (m, 2-H, 8- and 12-biph). ^{13}C-NMR ($CDCl_3$): δ 18 (CH_3); 20 e 22 (4CH_3-ketals); 45 (CH); 65 (C-6'); 66.7 (C-4'); 71 (C-5'); 71.5 (C-2'); 72 (C-3'); 96 (C-1'); 109 and 111 (C-ketals); 115 (3-biph); 124 (5-biph); 127 (10-biph); 128 (8- and 12-biph); 129 (9- and 11-biph); 131 (6-biph); 135 (4-biph); 142 (7-biph); 159 (1-biph); 160 (2-biph); 174 (CO ester). m/z: 487 $(M + H)^+$. Anal. ($C_{27}H_{31}FO_7$) C, H, O.

Flurbiprofen-D-galactos-6'-yl ester (2b): ^{1}H-NMR (CD_3OD): δ 1.50 (d, 3H, -CH_3); 3.45 (m, 1H, 4'-H); 3.70 (m, 1H, -CH); 3.80 (m, 1H, 5'-H); 4.20 (m, 2H, 6'-H); 4.30 (m, 1H, 2'-H); 4.40 (m, 1H, 3'-H); 5.15 (m, 1H, 1'-H); 7.17 (m, 2H, 6- and 5-biph); 7.36 (s, 1H, 3-biph); 7.43 (m, 3-H, 9-, 10- and 11-biph); 7.52 (m, 2-H, 8- and 12-biph). ^{13}C-NMR (CD_3OD): δ 18 (CH_3); 45 (CH); 64 (C-6'); 68 (C-4'); 70 (C-5'); 73 (C-2'); 74 (C-3'); 91 e 96 (C-1'); 115 (3-biph); 124 (5-biph); 127 (10-biph); 128 (8- and 12-biph); 129 (9- and 11-biph); 131 (6-biph); 135 (4-biph); 142 (7-biph); 159 (1-biph); 160 (2-biph); 174 (CO ester). m/z: 407 $(M + H)^+$. Anal. ($C_{21}H_{23}FO_7$) C, H, O. mp: 126-127 °C.

Diacetone 6'-O-Ketorolac-D-galactopyranoside (1c) and Ketorolac-D-galactos-6'-yl ester (2c): The ^{1}H and ^{13}C NMR data and MS data were already reported in our previous paper [7].

Diacetone 6'-O-Ibuprofen-D-galactopyranoside (1d): ^{1}H-NMR ($CDCl_3$): δ 1.01 (2s, 6H, -CH_3 ibu); 1.30, 1.31, 1.40, 1.41 (4s, 12H, ketals); 1.50 (d, 3H, -CH_3); 2.22 (m, 1H, -CH ibu); 2.51 (d, 2H, -CH_2 ibu); 3.78 (m, 1H, -CH); 4.05 (m, 1H, 4'-H); 4.15 (m, 1H, 5'-H); 4.20 (m, 2H, 6'-H); 4.40 (m, 1H, 2'-H); 4.50 (m, 1H, 3'-H); 5.35 (m, 1H, 1'-H); 7.1 (2d, 4-H, ph). ^{13}C-NMR ($CDCl_3$): δ 13 (CH_3); 20 e 22 (4CH_3-ketals); 23 (2CH_3 ibu); 29 (CH ibu), 40 (CH); 44.5 (CH_2 ibu); 65 (C-6'); 66.7 (C-4'); 71 (C-5'); 71.5 (C-2'); 72 (C-3'); 96 (C-1'); 109 e 111 (C-ketals); 128 (C-2 ph and C-6 ph); 129 (C-3 ph and C-5 ph); 132 (C-1 ph); 140 (C-4 ph); 174 (CO-ester). m/z: 449 $(M + H)^+$. Anal. ($C_{25}H_{36}O_7$) C, H, O.

Ibuprofen-D-galactos-6'-yl ester (2d): ^{1}H-NMR ($CDCl_3$): δ 1.01 (2s, 6H, -CH_3 ibu); 1.5 (d, 3H, -CH_3); 2.0 (m, 1H, -CH ibu); 2.51 (d, 2H, -CH_2 ibu); 3.78 (m, 1H, -CH); 3.45 (m, 1H, 4'-H); 3.5 (m, 1H, 5'-H); 3.8 (m, 2H, 6'-H); 4.20 (m, 1H, 2'-H); 4.40 (m, 1H, 3'-H); 5.15 (m,1H, 1'-H); 7.1 (2d, 4-H, ph). ^{13}C-NMR ($CDCl_3$): δ 13 (CH_3); 23 (2CH_3 ibu); 29 (CH ibu), 40 (CH); 44.5 (CH_2 ibu); 64 (C-6'); 68 (C-4'); 70 (C-5'); 73 (C-2'); 74 (C-3'); 93 and 97 (C-1'); 128 (C-2 ph and C-6 ph); 129 (C-3 ph and C-5 ph); 132 (C-1 ph); 140 (C-4 ph); 174 (CO-ester). m/z: 369 $(M + H)^+$. Anal. ($C_{19}H_{28}O_7$) C, H, O. mp: 137-138 °C.

Diacetone 6' - O – Indomethacin – D - galacto pyranoside (1e): ^{1}H-NMR ($CDCl_3$): δ 1.30, 1.31, 1.40, 1.41 (4s, 12H, ketals); 2.30 (s, 3H, -CH_3 indo); 3.39 (s, 2H, -CH_2 indo); 3.73 (s, 3H, -OCH_3 indo); 4.05 (m, 1H, 4'-H); 4.15 (m, 1H, 5'-H); 4.20 (m, 2H, 6'-H); 4.40 (m, 1H, 2'-H); 4.50 (m, 1H, 3'-H); 5.35 (m, 1H, 1'-H); 6.22 (s, 1-H, 5-indo); 6.70 (2d, 2H, 7- and 8-indo); 7.40 (2d, 2H, 3- e 5-ph); 7.70 (2d, 2H, 2- and 6-ph). ^{13}C-NMR ($CDCl_3$): δ 6 (CH_3 indo); 20 and 22 (4CH_3-ketals); 33 (CH_2 indo); 56 (OCH_3 indo); 65 (C-6'); 66.7 (C-4'); 71 (C-5'); 71.5 (C-2'); 72 (C-3'); 96 (C-1'); 102 (C-5 indo); 106 (C-3 indo); 108.5 (C-7 indo); 109 and 111 (C-ketals); 112 (C-8 indo); 128 (C-4 indo and C-9 indo); 128.5 (C-1 ph); 129 (C-3 ph e C-5 ph); 132 (C-2 ph and C-6 ph); 135 (C-2 indo); 140 (C-4 ph); 155 (C-6 indo); 168 (CO indo); 174 (CO-ester). m/z: 600 $(M + H)^+$. Anal. ($C_{31}H_{34}ClNO_9$) C, H, Cl, N, O.

Indomethacin-D-galactos-6'-yl ester (2e): ^{1}H-NMR (CD_3OD): δ 2.3 (s, 3H, -CH_3 indo); 3.39 (s, 2H, -CH_2 indo); 3.45 (m, 1H, 4'-H); 3.50 (m, 1H, 5'-H); 3.70 (s, 3H, -OCH_3 indo); 3.80 (m, 2H, 6'-H); 4.20 (m, 1H, 2'-H); 4.40 (m, 1H, 3'-H); 5.15 (m,1H, 1'-H); 6.22 (s, 1-H, 5-indo); 6.70 (2d, 2H, 6- and 7-indo); 7.40 (2d, 2H, 3- and 5-ph); 7.70 (2d, 2H, 2- and 6-ph). ^{13}C-NMR (CD_3OD): δ 6 (CH_3 indo); 33 (CH_2 indo); 56 (OCH_3 indo); 64 (C-6'); 68 (C-4'); 70 (C-5'); 73 (C-2'); 74 (C-3'); 93 and 97 (C-1'); 102 (C-5 indo); 106 (C-3 indo); 108.5 (C-7 indo); 112 (C-8 indo); 128 (C-4 indo and C-9 indo); 128.5 (C-1 ph); 129 (C-3 ph and C-5 ph); 132 (C-2 ph and C-6 ph); 135 (C-2 indo); 140 (C-4 ph); 155 (C-6 indo); 168 (CO indo); 174 (CO-ester). m/z:520 $(M+H)^+$. Anal. ($C_{25}H_{26}ClNO_9$) C, H, Cl, N, O. mp: 204-205 °C.

Diacetone 6'-O-Mefenamic-D-galactopyranoside (1f): ^{1}H-NMR ($CDCl_3$): δ 1.34-1.36(d, 6H, ketals); 1.49, 1.52 (d, 6H, ketals); 2.16 (s, 3H,3''' -CH3); 2.32 (s, 3H, 2'''-CH_3); 4.23 (m, 1H, 4'-H); 4.36 (m, 1H, 5'-H); 4.45 (m, 2H, 6'-H); 4.50 (m, 1H, 2'-H); 4.60-4.70 (d, 1H, 3'-H); 5.57-5.58 (d, 1H, 1'-H); 6.66 (t, 6'''-H, ph);6.71-6.73 (d, 4'''H,ph);7.01-7.03 (d, 6''-H, ph); 7.10 (t, 5'''-H, ph); 7.13 (t, 4''-H, ph) 7.227.23 (d, 5''-H, ph); 7.97-8.00(d, 3''-H, ph). ^{13}C-NMR ($CDCl_3$): δ 13.96 (3'''-CH_3 ,ph); 20.6 (2'''-CH_3, ph); 26 (CH_3, ketals); 63 (C-6'); 66 (C-2'); 70.6 (C-3'); 70.7 (C-4') 71(C-5'); 96 (C-1'); 108.8 e 109.7 (C-ketals); 111 (C-2'' ph); 113.6 (C-6''' ph);116 (C4'' ph); 123 (C-4''' ph); 125.9 (C-6'' ph); 126.8 (C-2''' ph); 131.7 (C-5''' ph); 132.6 (C-3'' ph); 134.2 (C-5'' ph); 138.2 (C-3''' ph); 138.7 (C-1''' ph); 149.5 (C-1'' ph); 168.4 (CO-ester) m/z: 484 $(M + H)^+$. Anal. ($C_{27}H_{33}NO_7$) C, H, N, O.

Mefenamic-D-galactos-6'-yl ester (2f): ^{1}H-NMR (CD_3OD): δ 2.14 (s, 3H,3''' -CH3); 2.31 (s, 3H, 2'''-CH3); 3.49 (m, 1H, 4'-H); 3.5 (m, 1H, 3'-H); 3.9 (m, 2H, 2'-H); 4.1 (m, 1H, 5'-H); 4.50(m, 1H, 6'-H); 5.18-5.20 (d, 1H, 1'-H); 6.6 (t, 4'''-H e 6'''-H, ph); 7.02-7.03 (d, 6''-H, ph); 7.7 (t, 4''-H e 5'''-H, ph); 7.24 (t, 5''-H, ph) 7.957.97 (d, 3''-H, ph); ^{13}C-NMR (CD_3OD): δ 12.7 (3'''-CH3 ,ph); 19 (2'''-CH3, ph); 63.7 (C-6'); 69.1(C-4'); 69.8 (C-3'); 72.7 (C-5') 73.4(C-2'); 93 and 97 (C-1'); 110.4 (C-2'' ph); 113.1 (C-6''' ph);115.8 (C-4'' ph); 123 (C-4''' ph); 125.7 (C-6'' ph); 126.6 (C-2''' ph); 131.4 (C-5''' ph); 132 (C-3'' ph); 134 (C-5'' ph); 137.9 (C-3''' ph); 138.4 (C-1''' ph); 149.5 (C-1'' ph); 168.3 (CO-ester) m/z: 404 $(M + H)^+$. Anal. ($C_{21}H_{27}NO_7$) C, H,N, O. mp: 263-267 °C.

Stability Test of Prodrugs: Solutions were prepared by dissolving an aliquot of compounds 2(a, b, d, e, f) in pH 7.4 phosphate buffer, in hydrochloric acid 0.1 N (pH 1) or in rat plasma. The solution was maintained at 37°C and aliquots were withdrawn every 1 h for chromatographic analysis, until 8 h of incubation. A prefixed time, an aliquot of plasma was extracted with acetonitrile (1:2) and the solutions were vortex mixed and centrifuged at 3000 RPM (1000 g) for 10 minutes. The surnatant was transferred into appropriate vial and analyzed. The hydrolysis of compounds was followed by HPLC- diode array detection described after. Pseudo-first-order half-times (t½) for the chemical and enzymatic hydrolysis were calculated from the linear slopes of plots of the logarithm of remaining ester against time.

HPLC System: Chromatographic separations were performed using a 1090L Liquid Chromatograph (Hewlett-Packard, Palo Alto, USA) equipped with a DAD HP1040A. All separations were accomplished on a Phenomenex Luna C18 (250 x 4.6 mm, particle size 5 µm). The mobile phase consisted of acetonitrile and aqueous phosphoric acid 1 mM pH 3 [68:32], only for the compound 2f the mobile phase consisted of methanol and

aqueous phosphoric acid 2 mM pH 3 [90:10]. The flow-rate was 1 mL/min with an injection volume of 20 µL. All reagents and solvents were of analytical grade. Deionised and distilled water was purified through a Milli Q system (Millipore). Retention times and selected wavelengths were: ketoprofen (7 min; 250 nm), 2a (9 min, 250 nm); flurbiprofen (11 min; 250 nm), 2b (6 min, 250 nm); ibuprofen (7 min, 220 nm), 2d (16 min, 220 nm); indomethacin (6.2 min; 313 nm), 2e (4.6 min, 313 nm); mefenamic acid (3.2 min; 280 nm); 2f (2.6 min; 280 nm).

RESULTS AND DISCUSSION:

Evaluation of classic and green synthesis of NSAIDs prodrugs: As you can see, by comparing the yield obtained by both approaches, the green method leads to substantial reduction of chlorinated solvents in both steps. In fact, esterification was made by RTIL which is recyclable by extraction with ether and purged with nitrogen and ready for another reaction producing the ester product in a good yield; moreover, the excess of NSAIDs was recovered by means of a simple extraction with water and $NaHCO_3$ and the unreacted DIPG was recovered by using chromatography (**Table 1**). In the second step, after several attempts, the best procedure to obtain the finals products proved to be the treatment of intermediate compounds with ACN, HCl 1N and GAA solution at reflux for 40 min. Moreover the adopted cleavage procedure gave rise to a substantial reduction of the intermediate monoketal product that appears during the cleavage process with TFA, causing problems during purification (**Table 2**).

TABLE 1: % YIELD OF COMPOUNDS 1(a, b, c, d, e, f)

Compounds	[bmim][PF_6]	DCM
1a	72	51
1b	70	61
1c	65	75
1d	58	52
1e	56	74
1f	63	60

TABLE 2: % YIELD OF COMPOUNDS 2(a, b, c, d, e, f).

Compounds	HCl, ACN, GAA	DCM, TFA
2a	53	55
2b	72	53
2c	51	65
2d	56	55
2e	53	55
2f	45	40

Stability test of new NSAIDs prodrugs: These prodrugs appeared quite stable at pH 7.4 (t1/2 > 8 h) and at pH 1 (t1/2>8 h). No chemical degradation products have been generated from its hydrolysis. So, from this first analysis we can assume than the prodrugs should not cause any gastrolesive effects during the transit in the stomach, since the parent drug was not recovered at pH 1. They showed a great handling in physiological solution, so they could be use for extemporaneous preparations without fear to have the ester bond hydrolysis. Futhermore, the optimal release of free NSAIDs following enzymatic hydrolysis allows us to suppose that the parent drugs will keep their pharmacological activity *in vivo* experiments (**Table 3**).

TABLE 3: CHEMICAL AND ENZYMATIC STABILITY OF PRODRUGS

Prodrugs	pH 7.4 t½ (h)	pH 1 t½ (h)	Plasma t½ (h)
2a	>8	>8	4
2b	>8	>8	1
2d	>8	>8	2
2e	>8	>8	2
2f	>8	>8	6

CONCLUSION: The synthesis of six different galactosylated NSAIDs prodrugs obtained by the classic and green methods, clearly demonstrated that the latter is more efficient both in respect to the synthesis yield as well as in respect to the reduction of toxic substances which could remain trapped in the product even after purification. Furthermore, the possibility to reuse RTILs avoids the use of a fresh solvent each time and reduces the amount of solvent to be consumed and waste to be disposed; the prodrug strategy itself could be inserted into the green chemistry concept. The reason resides on a marketed parent drug and a non-toxic carrier, linked by an easily hydrolysable bond. The encouraging outcomes found by stability tests where the prodrugs have a satisfactory stability at pH 1 and pH 7.4 and the capacity to regenerate parent drugs makes us suppose that the new synthetized prodrugs can achieve the same pharmacological results of NSAIDs themselves but without drawbacks. For all these reasons, the prodrugs strategy combined with a green synthesis is innovative way to reduce the waste of both time and resources therefore providing a product with greater purity.

ACKNOWLEDGEMENTS: We are grateful to Thérèse Nicola Marshall for proofreading the manuscript.

CONFLICT OF INTEREST STATEMENT: The authors declare that there are no conflicts of interest.

REFERENCES:

1. Marrucho IM, Branco LC, Rebelo LPN. Ionic liquids in pharmaceutical applications. Annu Rev Chem Biomol Eng 2014; 5: 527-546.
2. Siódmiak T, Marsza MP, Proszowska A .Ionic liquids: a new strategy in pharmaceutical synthesis. Mini Rev Org Chem 2012; 9(2): 203-208.
3. Anastas PT: Green Chemistry Theory and Practice, Oxford University Press: Oxford, UK, 1998.
4. Poliakoff M, Fitzpatrick JM, Farren TR, Anastas PT. Green chemistry: science and politics of change. Science 2002; 297(5582): 807–810.
5. Kirchoff MM. Promoting green engineering through green chemistry. Environ Sci Technol 2003; 37: 5349–5353.
6. Horvath IT, Anastas PT. Innovations and green chemistry. Chem Rev 2007; 107: 2169–2173.
7. Curcio A, Sasso O, Melisi D, Nieddu M, La Rana G, Russo R, Gavini E, Boatto G, Abignente E, Calignano A, Rimoli MG. Galactosyl prodrug of ketorolac: synthesis, stability, and pharmacological and pharmacokinetic evaluations. J Med Chem 2009; 52(12): 3794-3800.
8. Stewart Italia S.R.L.,Milano, Rimoli MG, Calignano A, Cuomo R, Boatto G: A galactosylated pro-drugs of non-steroidal anti-inflammatories with improved pharmacokinetic characteristics and reduced toxicity of the starting drug. U.S. Patent US8551958, 08 October, 2013 and Europe Patent EP2320951, 16 January, 2016.
9. Handy ST: Applications of ionic liquids in science and technology. InTech first edition 2011.
10. Ratti R. Ionic liquids: synthesis and applications in catalysis. Adv Chem 2014; 2014: 1-16.
11. Beniwal V, Manna A, Kumar A. Spectacular rate enhancement of the Diels–Alder reaction at the ionic liquid/n-hexane interface. Chem Phys Chem 2016; 17: 1969 – 1972.
12. Kavetsou E, Detsi A. Ionic liquids as solvents and catalysts for the green synthesis of coumarins. J Crit Rev 2016; 3(3): 50-55.
13. Leadbeater NE. Opening an Aladdin's cave: the Suzuki coupling in a room-temperature ionic liquid. Chem Commun 2014; 50: 1515-1518.
14. Gyton MR, Cole ML, Harper JB. Ionic liquid effects on Mizoroki–Heck reactions: more than just carbine complex formation. Chem Commun 2011; 47: 9200–9202.
15. Mohammad A, Inamuddin: Green solvents II: properties and applications of ionic liquids. Springer 2012.
16. Zhao SF, Horne M, Bond AM, Zhang J. Electrochemical reduction of aromatic ketones in 1-butyl-3-methylimidazolium-based ionic liquids in the presence of carbon dioxide: the influence of the ketone substituent and the ionic liquid anion on bulk electrolysis product distribution. Phys Chem Chem Phys 2015; 17(29):19247-19254.
17. Uchimoto H, Tsuji T, Kawasaki I, Arimitsu K, Yasui H, Yamashita M, Ohta S, Nishide K. Preparation of chiral ligands connected with quaternary ammonium group for recyclable catalytic asymmetric transfer hydrogenation in ionic liquid. Chem Pharm Bull 2015; 63(3): 200–209.

How to cite this article:
Magliocca S, Sodano F, Nieddu M, Burrai L, Boatto G and Rimoli MG: New galactosylated NSAIDs prodrugs in a green context: synthesis and stability. Int J Pharm Sci Res 2017; 8(4): 1575-81.doi: 10.13040/IJPSR.0975-8232.8(4).1575-81.