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RESEARCH ARTICLE

# Reversal of multidrug resistance in cancer cells by novel asymmetrical 1,4-dihydropyridines

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**Abstract** Multidrug resistance (MDR) is an important obstacle that limits the efficacy of chemotherapy in many types of cancer. In this study, 14 novel asymmetrical DHPs possessing pyridyl alkyl carboxylate substitutions at $C_3$ and alkyl carboxylate groups at $C_5$ in addition to a nitroimidazole or nitrophenyl moiety at $C_4$ position were synthesized. Calcium channel blocking (CCB) activity was measured in guinea pig ileal longitudinal smooth muscle. Cytotoxicity was tested on 4 human cancer cell lines, while MDR reversal capacity was examined on P-glycoprotein overexpressing doxorubicin resistant MES-SA-DX5 and compared with non-resistant MES-SA cells. Compounds showed different CCB ($IC_{50}$: 29.3 nM–4.75 μM) and cytotoxic activities ($IC_{50}$: 6.4 to more than 100 μM). Several compounds having nitrophenyl moiety at $C_4$, could significantly reverse resistance to doxorubicin at 0.5 and 1 μM. The most active ones were **7e** and **7g** containing ethyl carboxylate and isopropyl carboxylate at $C_5$, respectively. CCB activity, which is considered an undesirable effect for these agents, of **7e** and **7g** were 33 and 20 times lower than nifedipine, respectively. In conclusion, the newly synthesized asymmetrical DHP compounds showed promising MDR reversal and antitumoral activities with low CCB effects and could be of therapeutic value in drug resistant cancer.

**Keywords** Cancer · Multidrug resistance · Dihydropyridines · Cytotoxicity · Calcium channel blocking

**Abbreviations**
DHPs Dihydropyridines
MDR Multidrug resistance

## Introduction

Cancer is one of the most important causes of death all over the world among various racial and ethnic groups (King and Robins 2006). Different therapeutic modalities such as surgery, radiotherapy and chemotherapy are used for treatment of cancer, among which chemotherapy is one of the most common methods (Kasper et al. 2005). Successful treatment of cancer by chemotherapy is to a large extent dependent on the effectiveness of cytotoxic anticancer drugs, which are used either alone or in combination with other methods. Unfortunately, in the recent years, many types of cancer have become either intrinsically resistant to all initial chemotherapeutic treatments or acquire resistance to a broad spectrum of these agents over time, a phenomenon called multidrug resistance (MDR) (Ejendal and Hrycyna 2002; Szakacs et al. 2006; Borst et al. 2007).

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MDR appears to have diverse and complex mechanisms. One of the most accepted classifications is the division to classical or ATP-binding cassette (ABC)-transporters-mediated MDR on the one hand and atypical MDR that includes other mechanisms on the other hand (Teodori et al. 2002; Zarrin et al. 2010). P-glycoprotein (P-gp) also known as MDR protein 1 or ABC sub-family B member 1 is one of the main causes of typical MDR that limits the effectiveness of chemotherapy (Stavrovskaya and Stromskaya 2008).

Because of the importance of MDR in failure of chemotherapy in many instances, a number of studies have focused on the development of MDR reversal agents (Miri and Mehdipour 2008; Eid et al. 2012). 1,4-Dihydropyridines (DHPs) have been introduced as a class of calcium channel blockers (Edraki et al. 2009). In the beginning of the 1980s, it was discovered that calcium channel blockers are able to inhibit the process of MDR (Tsuruo et al. 1981; Tsuruo et al. 1982). Several studies have been performed since then in order to find new DHP derivatives for reversal of MDR. For instance, some derivatives of niguldipine and their pyridine counterparts (Zhou et al. 2005a, b) as well as compounds bearing pyridyl groups on 3 and 5 positions of DHP nucleus have been synthesized and tested (Tasaka et al. 2001; Mehdipour et al. 2007; Foroughinia et al. 2008). Furthermore, Zhou et al. have introduced some DHP derivatives, which are effective on typical MDR including MDR-associated protein 1 and Breast cancer related protein-mediated MDR (Zhou et al. 2005a, b).

We have recently synthesized some novel DHP analogues that contain pyridyl group at $C_3$ and $C_5$ in addition to a nitroimidazole moiety at $C_4$ that show good MDR reversal activity (Mehdipour et al. 2007; Foroughinia et al. 2008). All DHP derivatives tested in our laboratory so far have had symmetrical structures, but in the present study we decided to examine the effect of asymmetry at $C_3$ and $C_5$ positions on MDR reversal activity of these compounds. Besides, the cytotoxic effect of the newly synthesized DHPs were measured on 4 different human cancer cell lines, and their calcium channel antagonistic activity, was also evaluated.

## Materials and methods

### Physical measurements

The melting points were measured on a hot stage apparatus (Electrothermal, Essex, UK) and were uncorrected. Elemental analyses (C, H, N) were undertaken on **7a**–**7n** by the Microanalytical Department, Central Laboratories for Research, Shiraz University of Medical Sciences and were within 0.4 % of the calculated value. The $^1$H-NMR spectra were performed on a Burker-AdvanceDPX-500 MHz in $d_1$-chloroform. Tetramethylsilane was used as the internal standard. The mass spectra were obtained with a Hewlett-Packard spectrometer [Hewlett-Packard (HP) 6890, Böblingen, Germany] with a direct inlet system at 70 eV, The IR spectra were measured with a Perkin-Elmer spectrometer (KBr disk) (Perkin-Elmer, Waltham, MA). All spectra confirmed the structure of the synthesized compounds.

### Synthesis of aryl acetoacetate (**3a**–**3c**)

A mixture of 16.67 mM of corresponding alcohol **1a**–**1c** (Sigma-Aldrich Chemie GmbH, Deisenhofon, Germany) and 16.67 mmol 2,2,6-trimethyl-4H-1,3-dioxin-4-one (Sigma-Aldrich Chemie GmbH, Deisenhofon, Germany) were refluxed in 10 ml xylene and stirred vigorously at a temperature of 150 °C for 45 min. The formation of final product was confirmed using thin layer chromatography (TLC) (>90 %). Afterwards, the reaction mixture was cooled and the xylene removed. The product was purified by TLC on silica gel with chloroform–methanol (90/10) as the mobile phase to give pure compounds **3a**–**3c**.

*Pyridine-2-yl-propyl-3-oxobutanoate (**3a**) yield: 91 %*

IR (KBr): υ1743 (C=O, ester), 1716 (C=O, ketone), 2958 $cm^{-1}$ (C–H aromatics).

*Pyridine-3-yl-propyl-3-oxobutanoate (**3b**) yield: 93 %*

IR (KBr): υ 1748 (C=O, ester), 1717 (C=O, ketone), 2958 $cm^{-1}$ (C–H aromatics).

*Pyridine-4-yl-propyl-3-oxobutanoate (**3c**) yield: 95 %*

IR (KBr): υ1742 (C=O, ester), 1716 (C=O, ketone), 2963 $cm^{-1}$ (C–H aromatics).

### Synthesis of alkyl 3-aminocrotonate (**5a**–**5c**)

A solution of alkyl acetoacetic esters **4a**–**4c** (2 mmol) (Merck, Darmstadt, Germany) and ammonium acetate (3 mmol) in 5 ml ethanol were refluxed in 10 ml ethanol and stirred vigorously at a temperature of 90 °C for 24 h. Then, the reaction mixture was cooled and ethanol removed. IR spectra of the compounds were recorded to confirm the structure of **5a**–**5c**. Then, the compounds were immediately used in subsequent reactions.

*Methyl 3-aminocrotonate (**5a**)*

IR (KBr): υ 1716 (C=O, ester), 3511, 3333 $cm^{-1}$ ($NH_2$).

*Ethyl 3-aminocrotonate (**5b**)*

IR (KBr): υ 1716 (C=O, ester), 3511, 3332 $cm^{-1}$ ($NH_2$).

*Isopropyl 3-aminocrotonate (**5c**)*

IR (KBr): υ 1716 (C=O, ester), 3509, 3332 $cm^{-1}$ ($NH_2$).

General procedure for the synthesis of asymmetrical derivatives of 1,4-DHP (**7a**–**7n**)

A solution of compounds **5a**–**5c**, **3a**–**3c** and aryl or heteryl aldehydes were protected from light and refluxed in ethanol (30 ml) for 24 h. after cooling, the solution was concentrated under reduced pressure and purified by TLC on silica gel with chloroform–ethanol (95–5 %). The product was recrystallized from diethyl ether–petroleum ether to give pure compounds **7a**–**7n** as prism crystals.

*3-Methyl-5-(3-(pyridine-4-yl)propyl)-2,6-dimethyl-4-(4-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate (**7a**)*

$^1$H-NMR ($CDCl_3$, 500 Hz): δ 1.90–1.94 (m, 2H, $COOCH_2\underline{CH_2}CH_2$), 2.34–2.37 (2s, 6H, $C_2$–$CH_3$ and $C_6$–$CH_3$), 2.55 (t, 2H, $COOCH_2CH_2\underline{CH_2}$, j = 7.2 Hz), 3.65 (s,3H,$COO\underline{CH_3}$), 4.03–4.09 (m, 2H, $COO\underline{CH_2}CH_2CH_2$), 5.10 (s, 1H, $C_4$–H), 5.91 (brs, 1H, NH–DHP), 7.02 (d, 2H, $C_{2,6}$H-pyridyl, j = 5.8 Hz), 7.44 (d, 2H, $C_{2,6}$H-phenyl, j = 8.7 Hz), 8.07 (d, 2H, $C_{3,5}$H-phenyl, j = 8.7 Hz), 8.47 (d, 2H, $C_{3,5}$H-pyridyl, j = 6.0 Hz).

Found C, 63.91; H, 5.56; N, 9.30 %. Anal. ($C_{24}H_{25}N_3O_6$) requires C, 63.85; H, 5.58; N, 9.31 %.

MS: (*m/z*) 434 (5), 420 (4), 343 (11), 329 (100), 297 (6), 196 (3), 136 (8), 120 (22), 106 (13), 92 (14), 77 (4), 51 (2).

IR (KBr): υ 3278 (NH), 3077 (CH-aromatic), 2947 (CH-aliphatic), 1699 (CO), 1342, 1508 $cm^{-1}$ ($NO_2$).

*3-Methyl-5-(3-(pyridine-3-yl)propyl)-2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate (**7b**)*

$^1$H-NMR ($CDCl_3$, 500 Hz): δ 1.89–1.95 (m, 2H, $COOCH_2\underline{CH_2}CH_2$), 2.36–2.38 (2s, 6H, $C_2$–$CH_3$ and $C_6$–$CH_3$), 2.57 (t, 2H, $COOCH_2CH_2\underline{CH_2}$, j = 7.7 Hz), 3.65 (s, 3H, $COO\underline{CH_3}$), 4.01–4.12 (m, 2H, $COO\underline{CH_2}CH_2CH_2$), 5.10 (s, 1H, $C_4$–H), 5.87 (brs, 1H, NH–DHP), 7.18–7.20 (dd, 1H, $C_5$H-pyridyl), 7.37 (t, 1H, $C_5$H-phenyl, j = 7.9 Hz), 7.41 (d, 1H, $C_6$H-phenyl, j = 7.8 Hz), 7.64 (d, 1H, $C_6$H-pyridyl, j = 7.6 Hz), 7.99 (d, 1H, $C_4$H-phenyl, j = 8.2 Hz), 8.11 (s, 1H, $C_2$H-phenyl), 8.37 (s, 1H, $C_2$H-pyridyl), 8.43 (d, 1H, $C_4$H-pyridyl, j = 3.5 Hz).

Found C, 63.78; H, 5.56; N, 9.29 %. Anal. ($C_{24}H_{25}N_3O_6$) requires C, 63.85; H, 5.58; N, 9.31 %.MS: (*m/z*) 452 (7), 434 (4), 420 (6), 329 (33), 297 (72), 120 (22), 106 (13), 92 (50), 78 (7), 51 (5).

IR (KBr): υ 3188 (NH), 3057 (CH-aromatic), 2925 (CH-aliphatic), 1682 (CO), 1344, 1526 $cm^{-1}$ ($NO_2$).

*3-Ethyl-5-(3-(pyridine-4-yl)propyl)-2,6-dimethyl-4-(4-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate (**7c**)*

$^1$H-NMR ($CDCl_3$, 500 Hz): δ 1.23 (t, 3H, $COOCH_2\underline{CH_3}$, j = 7.1 Hz), 1.90–1.93 (m, 2H, $COOCH_2\underline{CH_2}CH_2$), 2.36 (2s, 6H, $C_2$–$CH_3$ and $C_6$–$CH_3$), 2.56 (t, 2H, $COOCH_2CH_2\underline{CH_2}$, j = 7.7 Hz), 4.04–4.12 (m, 4H, $COO\underline{CH_2}CH_2CH_2$ and $COO\underline{CH_2}CH_3$), 5.11 (s, 1H, $C_4$–H), 5.87 (brs, 1H, NH–DHP), 7.02 (d, 2H, $C_{2,6}$H-pyridyl, j = 5.9 Hz), 7.45 (d, 2H, $C_{2,6}$H-phenyl, j = 8.7 Hz), 8.08 (d, 2H, $C_{3,5}$H-phenyl, j = 8.7 Hz), 8.47 (d, 2H, $C_{3,5}$H-pyridyl, j = 6.0 Hz).

Found C, 64.41 H, 5.83; N, 9.06 %. Anal. ($C_{25}H_{27}N_3O_6$) requires C, 64.50; H, 5.85; N, 9.03 %.

MS: (*m/z*) 466 (1), 448 (40), 343 (100), 329 (4), 297 (44), 271 (4), 196 (21), 136 (11), 120 (58), 106 (13), 93 (20), 77 (6).

IR (KBr): υ 3194 (NH), 3075 (CH-aromatic), 2951 (CH-aliphatic), 1688, 1703 (CO), 1343, 1513 $cm^{-1}$ ($NO_2$).

*3-Methyl-5-(3-(pyridine-3-yl)propyl)-2,6-dimethyl-4-(2-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate (**7d**)*

$^1$H-NMR ($CDCl_3$, 500 Hz): δ 1.86–1.92 (m, 2H, $COOCH_2\underline{CH_2}CH_2$), 2.34 (2s, 6H, $C_2$–$CH_3$ and $C_6$–$CH_3$), 2.47 (t, 2H, $COOCH_2CH_2\underline{CH_2}$, j = 7.8 Hz), 3.58 (s, 3H, $COO\underline{CH_3}$), 3.99–4.12 (m, 2H, $COO\underline{CH_2}CH_2CH_2$), 5.78 (s, 1H, $C_4$–H), 5.89 (brs, 1H, NH–DHP), 7.16–7.19 (dd, 1H, $C_5$H-pyridyl), 7.23 (t, 1H, $C_4$H-phenyl, j = 8.6 Hz), 7.43 (d, 1H, $C_6$H-phenyl, j = 8.4), 7.47 (t, 1H, $C_5$H-phenyl, j = 8.5 Hz), 7.53 (d, 1H, $C_6$H-pyridyl, j = 1.3 Hz), 7.68 (d, 1H, $C_3$H-phenyl, j = 7.0 Hz), 8.30 (s, 1H, $C_2$H-pyridyl), 8.40 (d, 1H, $C_4$H-pyridyl, j = 4.8 Hz).

Found C, 63.79; H, 5.57; N, 9.34 %. Anal. ($C_{24}H_{25}N_3O_6$) requires C, 63.85; H, 5.58; N, 9.31 %.

MS: (*m/z*) 452 (1), 434 (36), 420 (2), 343 (1), 329 (16), 297 (31), 270 (55), 196 (13), 136 (8), 120 (87), 106 (23), 92 (100), 77 (13), 51 (8).

IR (KBr): υ 3188 (NH), 3067 (CH-aromatic), 2944 (CH-aliphatic), 1699, 1688 (CO), 1342, 1508 $cm^{-1}$ ($NO_2$).

*3-Ethyl-5-(3-(pyridine-4-yl)propyl)-2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate (**7e**)*

$^1$H-NMR ($CDCl_3$, 500 Hz): δ 1.23 (t, 3H, $COOCH_2\underline{CH_3}$, j = 7.11 Hz), 1.90–1.95 (m, 2H, $COOCH_2\underline{CH_2}CH_2$), 2.37 (2s, 6H, $C_2$–$CH_3$ and $C_6$–$CH_3$), 2.56 (t, 2H, $COOCH_2CH_2\underline{CH_2}$, j = 7.7 Hz), 4.06–4.16 (m, 4H, $COO\underline{CH_2}CH_2CH_2$ and $COO\underline{CH_2}CH3$), 5.11 (s, 1H, $C_4$–H), 5.94 (brs, 1H, NH–DHP), 7.03 (d, 2H, $C_{2,6}$H-pyridyl,

j = 5.9 Hz), 7.37 (t, 1H, $C_5$H-phenyl, j = 7.9 Hz), 7.64 (d, 1H, $C_6$H-phenyl, j = 7.7 Hz), 7.99 (d, 1H, $C_4$H-phenyl, j = 7.8 Hz), 8.14 (s, 1H, $C_2$H-phenyl), 8.47 (d, 2H, $C_{3,5}$H-pyridyl, j = 5.9 Hz).

Found C, 64.41; H, 5.83; N, 9.01 %. Anal. ($C_{25}H_{27}N_3O_6$) requires C, 64.50; H, 5.85; N, 9.03 %.

MS: (*m/z*) 448 (35), 343 (100), 329 (4), 297 (50), 196 (16), 136 (9), 120 (18), 106 (13), 92 (12), 77 (2), 51 (1).

IR (KBr): υ 3272 (NH), 3068 (CH-aromatic), 2957 (CH-aliphatic), 1697 (CO), 1348, 1524 $cm^{-1}$ ($NO_2$).

*3-Isopropyl-5-(3-(pyridine-2-yl)propyl)-2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate (**7f**)*

$^1$H-NMR ($CDCl_3$, 500 Hz): δ 1.10–1.26 (2d, 6H, CO-OCH($\underline{CH_3}$)$_2$, j = 6.2, 6.2), 2.02–2.07 (m, 2H, CO-OCH$_2\underline{CH_2}$CH$_2$), 2.34–2.37 (2s, 6H, $C_2$–$CH_3$ and $C_6$–$CH_3$), 2.75 (t, 2H, COOCH$_2$CH$_2\underline{CH_2}$, j = 7.6 Hz), 4.04–4.12 (m, 2H, COO$\underline{CH_2}$CH$_2$CH$_2$), 4.93–4.98 (m, 1H, COO$\underline{CH}$(CH$_3$)$_2$), 5.09 (s, 1H, $C_4$–H), 5.72 (brs, 1H, NH–DHP), 7.04 (d, 1H, $C_6$H-pyridyl, j = 7.7 Hz), 7.09 (t, 1H, $C_4$H-pyridyl, j = 8.4 Hz), 7.35 (t, 1H, $C_5$H-phenyl, j = 8.8 Hz), 7.56 (t, 1H, $C_5$H-pyridyl, j = 7.0 Hz), 7.65 (d, 1H, $C_6$H-phenyl, j = 7.7 Hz), 7.98 (d, 1H, $C_4$H-phenyl, j = 8.2 Hz), 8.14 (s, 1H, $C_2$H-phenyl), 8.50 (d, 1H, $C_3$H-pyridyl, j = 4.8 Hz).

Found C, 64.99; H, 6.13; N, 8.73 %. Anal. ($C_{26}H_{29}N_3O_6$) requires C, 65.12; H, 6.10; N, 8.76 %.

MS: (*m/z*) 462 (3), 420 (3), 343 (3), 196 (5), 136 (5), 120 (100), 106 (13), 93 (10), 78 (2).

IR (KBr): υ 3207 (NH), 3089 (CH-aromatic), 2976 (CH-aliphatic), 1695, 1670(CO), 1346, 1525 $cm^{-1}$ ($NO_2$).

*3-Isopropyl-5-(3-(pyridine-3-yl)propyl)-2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate (**7g**)*

$^1$H-NMR ($CDCl_3$, 500 Hz): δ 1.11–1.26 (2d, 6H, CO-OCH($\underline{CH_3}$)$_2$, j = 6.2, 6.2 Hz), 1.88–1.94 (m, 2H, CO-OCH$_2\underline{CH_2}$CH$_2$), 2.35–2.38 (2s, 6H, $C_2$–$CH_3$ and $C_6$–$CH_3$), 2.58 (t, 2H, COOCH$_2$CH$_2\underline{CH_2}$, j = 7.7 Hz), 4.01–4.11 (m, 2H, COO$\underline{CH_2}$CH$_2$CH$_2$), 4.94–4.99 (m, 1H, COO$\underline{CH}$(CH$_3$)$_2$), 5.10 (s, 1H, $C_4$–H), 5.80 (brs, 1H, NH–DHP), 7.18–7.21 (dd, 1H, $C_5$H-pyridyl), 7.37 (t, 1H, $C_5$H-phenyl, j = 8.8 Hz), 7.41 (d, 1H, $C_6$H-phenyl, j = 7.7 Hz), 7.64 (d, 1H, $C_6$H-pyridyl, j = 7.7 Hz), 7.99 (d, 1H, $C_4$H-phenyl, j = 8.3 Hz), 8.14 (s, 1H, $C_2$H-phenyl), 8.37 (s, 1H, $C_2$H-pyridyl), 8.43 (d, 1H, $C_4$H-pyridyl, j = 4.7 Hz).

Found C, 65.07; H, 6.12; N, 8.77 %. Anal. ($C_{26}H_{29}N_3O_6$) requires C, 65.12; H, 6.10; N, 8.76 %.

MS: (*m/z*) 480 (2), 461 (46), 447 (3), 343 (10), 329 (1), 297 (100), 196 (25), 136 (15), 120 (40), 106 (26), 92 (77), 77 (10), 51 (6).

IR (KBr): υ 3208 (NH), 3087 (CH-aromatic), 2979 (CH-aliphatic), 1695 (CO), 1347, 1522 $cm^{-1}$ ($NO_2$).

*3-Isopropyl-5-(3-(pyridine-4-yl)propyl)-2,6-dimethyl-4-(4-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate (**7h**)*

$^1$H-NMR ($CDCl_3$, 500 Hz): δ 1.12–1.24 (2d, 6H, CO-OCH($\underline{CH_3}$)$_2$, j = 6.2, 6.2 Hz), 1.88–1.94 (m, 2H, CO-OCH$_2\underline{CH_2}$CH$_2$), 2.34–2.36 (2s, 6H, $C_2$–$CH_3$ and $C_6$–$CH_3$), 2.56 (t, 2H, COOCH$_2$CH$_2\underline{CH_2}$, j = 7.7 Hz), 4.03–4.09 (m, 2H, COO$\underline{CH_2}$CH$_2$CH$_2$), 4.95–4.98 (m, 1H, COO$\underline{CH}$(CH$_3$)$_2$), 5.10 (s, 1H, $C_4$–H), 5.86 (brs, 1H, NH–DHP), 7.02 (d, 2H, $C_{2,6}$H-pyridyl, j = 5.8 Hz), 7.45 (d, 2H, $C_{2,6}$H-phenyl, j = 8.7 Hz), 8.07 (d, 2H, $C_{3,5}$H-phenyl, j = 8.7 Hz), 8.47 (d, 2H, $C_{3,5}$H-pyridyl, j = 5.9 Hz).

Found C, 64.98; H, 6.11; N, 8.79 %. Anal. ($C_{26}H_{29}N_3O_6$) requires C, 65.12; H, 6.10; N, 8.76 %.

MS: (*m/z*) 480 (2), 462 (39), 448 (2), 420 (10), 434 (4), 392 (8), 375 (2), 297 (46), 271 (18), 196 (19), 136 (8), 120 (30), 106 (8), 92 (24), 77 (8), 51 (5).

IR (KBr): υ 3196 (NH), 3076 (CH-aromatic), 2971 (CH-aliphatic), 1701, 1674 (CO), 1346, 1511 $cm^{-1}$ ($NO_2$).

*3-Ethyl-5-(3-(pyridine-3-yl)propyl)-2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate (**7i**)*

$^1$H-NMR ($CDCl_3$, 500 Hz): δ 1.23 (t, 3H, COOCH$_2\underline{CH_3}$, j = 7.5 Hz), 1.90–1.93 (m, 2H, COOCH$_2\underline{CH_2}$CH$_2$), 2.34–2.38 (2s, 6H, $C_2$–$CH_3$ and $C_6$–$CH_3$), 2.57(t, 2H, COOCH$_2$CH$_2\underline{CH_2}$, j = 7.7 Hz), 4.04–4.11 (m, 4H, COO$\underline{CH_2}$CH$_2$CH$_2$ and COO$\underline{CH_2}$CH$_3$), 5.11 (s, 1H, $C_4$–H), 5.91 (brs, 1H, NH–DHP), 7.20 (t, 1H, $C_5$H-pyridyl), 7.37 (t, 1H, $C_5$H-phenyl, j = 7.9 Hz), 7.41 (d, 1H, $C_6$H-phenyl, j = 7.7 Hz), 7.64 (d, 1H, $C_6$H-pyridyl, j = 7.5 Hz), 7.99 (d, 1H, $C_4$H-phenyl, j = 7.8 Hz), 8.13 (s, 1H, $C_2$H-phenyl), 8.37 (s, 1H, $C_2$H-pyridyl), 8.44 (d, 1H, $C_4$H-pyridyl).

Found C, 64.71; H, 5.90; N, 9.09 %. Anal. ($C_{25}H_{27}N_3O_6$) requires C, 64.50; H, 5.85; N, 9.03 %.

MS: (*m/z*) 466 (2), 447 (45), 420 (3), 343 (44), 329 (6), 297 (91), 196 (29), 136 (13), 120 (25), 106 (21), 92 (56), 78 (8), 51 (5).

IR (KBr): υ 3206 (NH), 3081 (CH-aromatic), 2979 (CH-aliphatic), 1692 (CO), 1342, 1522 $cm^{-1}$ ($NO_2$).

*3-Methyl-5-(3-(pyridine-3-yl)propyl)-2,6-dimethyl-4-(1-methyl-5-nitro-1-imidazole-2-yl)-1,4-dihydropyridine-3,5-dicarboxylate (**7j**)*

$^1$H-NMR ($CDCl_3$, 500 Hz): δ 1.92–1.98 (m, 2H, CO-OCH$_2\underline{CH_2}$CH$_2$), 2.25–2.26 (2s, 6H, $C_2$–$CH_3$ and $C_6$–$CH_3$), 2.63 (t, 2H, COOCH$_2$CH$_2\underline{CH_2}$, j = 7.7 Hz), 3.68 (s, 3H, N–$CH_3$), 4.08–4.16 (m, 2H, COO$\underline{CH_2}$CH$_2$CH$_2$), 4.21 (s, 3H, COO$\underline{CH_3}$), 5.14 (s, 1H, $C_4$–H), 7.19–7.21 (dd, 1H, $C_5$H-pyridyl), 7.45 (d, 1H, $C_6$H-pyridyl, j = 7.7 Hz), 7.95 (s, 1H, H–Imidazole), 8.41 (s, 1H, $C_2$H-pyridyl), 8.45 (d, 1H, $C_4$H-pyridyl, j = 3.3 Hz), 8.45 (brs, 1H, NH–DHP).

Found C, 58.22; H, 5.55; N, 15.41 %. Anal. ($C_{22}H_{25}N_5O_6$) requires C, 58.01; H, 5.53; N, 15.38 %.

MS: (*m/z*) 438 (33), 329 (21), 297 (41), 272 (7), 120 (29), 106 (25), 92 (50), 51 (4).

IR (KBr): υ 3423(NH), 3081(CH-aromatic), 2979(CH-aliphatic), 1700 (CO), 1375, 1501 $cm^{-1}$ ($NO_2$).

*3-Methyl-5-(3-(pyridine-2-yl)propyl)-2,6-dimethyl-4-(1-methyl-5-nitro-1-imidazole-2-yl)-1,4-dihydropyridine-3,5-dicarboxylate (**7k**)*

$^1$H-NMR ($CDCl_3$, 500 Hz): δ 2.07–2.10 (m, 2H, CO-$OCH_2\underline{CH_2}CH_2$), 2.25–2.27 (2s, 6H, $C_2$–$CH_3$ and $C_6$–$CH_3$), 2.77 (t, 2H, $COOCH_2CH_2\underline{CH_2}$, j = 7.1 Hz), 3.68 (s, 3H, N–$CH_3$), 4.11–4.18 (m, 2H, $COO\underline{CH_2}CH_2CH_2$), 4.20 (s, 3H, $COO\underline{CH_3}$), 5.13 (s, 1H, $C_4$–H), 7.07 (d, 1H, $C_4$H-pyridyl, j = 7.8 Hz), 7.10 (d, 1H, $C_6$H-pyridyl, j = 4.9), 7.57 (t, 1H, $C_5$H-pyridyl, j = 7.15 Hz), 7.94 (s, 1H, H-imidazole), 8.22 (brs, 1H, NH–DHP), 8.50 (d, 1H, $C_3$H-pyridyl, j = 4.0 Hz).

Found C, 58.10; H, 5.51; N, 15.36 %. Anal. ($C_{22}H_{25}N_5O_6$) requires C, 58.01; H, 5.53; N, 15.38 %.

MS: (*m/z*) 438 (6), 329 (21), 120 (100), 93 (10), 78 (5), 51 (2).

IR (KBr): υ 3423 (NH), 3185 (CH-aromatic), 2947 (CH-aliphatic), 1703, 1667 (CO), 1374, 1508 $cm^{-1}$ ($NO_2$).

*3-Ethyl-5-(3-(pyridine-3-yl)propyl)-2,6-dimethyl-4-(1-methyl-5-nitro-1-imidazole-2-yl)-1,4-dihydropyridine-3,5-dicarboxylate (**7l**)*

$^1$H-NMR ($CDCl_3$, 500 Hz): δ 1.24 (t, 3H, $COOCH_2\underline{CH_3}$, j = 7.1 Hz), 1.93–1.98 (m, 2H, $COOCH_2\underline{CH_2}CH_2$), 2.27 (s, 6H, $C_2$–$CH_3$ and $C_6$–$CH_3$), 2.63 (t, 2H, $COOCH_2CH_2\underline{CH_2}$, j = 7.7 Hz), 4.10–4.16 (m, 4H, $COO\underline{CH_2}CH_2CH_2$ and $COO\underline{CH_2}CH3$), 4.22 (s, 3H, N–$CH_3$), 5.14 (s, 1H, $C_4$–H), 7.19–7.21 (dd, 1H, $C_5$H-pyridyl), 7.44 (d, 1H, $C_6$H-pyridyl, j = 7.8 Hz), 7.95 (s, 1H, H–imidazole), 8.41 (s, 1H, $C_3$H-pyridyl), 8.44 (d, 1H, $C_4$H-pyridyl, j = 3.4 Hz).

Found C, 58.79; H, 5.82; N, 14.89 %. Anal. ($C_{23}H_{27}N_5O_6$) requires C, 58.84; H, 5.80; N, 14.92 %.

MS: (*m/z*) 452 (46), 329 (6), 297 (33), 273 (2), 196 (11), 120 (15), 106 (13), 92 (22), 51 (2).

IR (KBr): υ 3438 (NH), 3081 (CH-aromatic), 2933 (CH-aliphatic), 1698, 1674 (CO), 1373, 1500 $cm^{-1}$ ($NO_2$).

*3-Methyl-5-(3-(pyridine-4-yl)propyl)-2,6-dimethyl-4-(1-methyl-5-nitro-1-imidazole-2-yl)-1,4-dihydropyridine-3,5-dicarboxylate (**7m**)*

$^1$H-NMR ($CDCl_3$, 500 Hz): δ 1.93–1.99 (m, 2H, CO-$OCH_2\underline{CH_2}CH_2$), 2.26 (s, 6H, $C_2$–$CH_3$ and $C_6$–$CH_3$), 2.61 (t, 2H, $COOCH_2CH_2\underline{CH_2}$, j = 7.7 Hz), 3.68 (s, 3H, N–$CH_3$), 4.07–4.17 (m, 2H, $COO\underline{CH_2}CH_2CH_2$), 4.21 (s, 3H, $COO\underline{CH_3}$), 5.14 (s, 1H, $C_4$–H), 7.06 (d, 2H, $C_{2,6}$H-pyridyl, j = 6.0 Hz), 7.95 (s, 1H, H–imidazole), 8.25 (brs, 1H, NH–DHP), 8.49 (d, 2H, $C_{3,5}$H-pyridyl, j = 6.0 Hz).

Found C, 58.09; H, 5.56; N, 15.41 %. Anal. ($C_{22}H_{25}N_5O_6$) requires C, 58.01; H, 5.53; N, 15.38 %.

MS: (*m/z*) 455 (2), 438 (100), 329 (37), 297 (28), 120 (26), 106 (11), 93 (11), 51 (2).

IR (KBr): υ 3423 (NH), 3081 (CH-aromatic), 2924 (CH-aliphatic), 1701 (CO), 1376, 1501 $cm^{-1}$ ($NO_2$).

*3-Isopropyl-5-(3-(pyridine-2-yl)propyl)-2,6-dimethyl-4-(1-methyl-5-nitro-1-imidazole-2-yl)-1,4-dihydropyridine-3,5-dicarboxylate (**7n**)*

$^1$H-NMR ($CDCl_3$, 500 Hz): δ 1.17–1.24 (2d, 6H, CO-$OCH(\underline{CH_3})_2$, j = 6.2, 6.3 Hz), 2.07–2.10 (m, 2H, CO-$OCH_2\underline{CH_2}CH_2$), 2.27–2.28 (2s, 6H, $C_2$–$CH_3$ and $C_6$–$CH_3$), 2.78 (t, 2H, $COOCH_2CH_2\underline{CH_2}$, j = 7.3 Hz), 4.14–4.17 (m, 2H, $COO\underline{CH_2}CH_2CH_2$), 4.22 (s, 3H, N–$CH_3$), 5.01–5.03 (m, 1H, $COO\underline{CH}(CH_3)_2$), 5.10 (s, 1H, $C_4$–H), 7.08 (d, 1H, $C_4$H-pyridyl, j = 8.2 Hz), 7.10 (d, 1H, $C_6$H-pyridyl, j = 8.7 Hz), 7.57 (t, 1H, $C_5$H-pyridyl, j = 8.5 Hz), 7.95 (s, 1H, H–imidazole), 8.50 (d, 1H, $C_3$H-pyridyl, j = 4.1 Hz).

Found C, 59.58; H, 6.06; N, 14.49 %. Anal. ($C_{24}H_{29}N_5O_6$) requires C, 59.62; H, 6.05; N, 14.48 %.

MS: (*m/z*) 466 (12), 196 (2), 136 (3), 120 (100), 106 (4), 92 (6), 78 (2).

IR (KBr): υ 3428 (NH), 3081 (CH-aromatic), 2978 (CH-aliphatic), 1700, 1671 (CO), 1373, 1508 $cm^{-1}$ ($NO_2$).

## Pharmacology

Male albino guinea pigs (300–450 g) were purchased from Shiraz University Animal House Department. They had free access to standard rodent chow and tap water ad libitum and were housed at 23 ± 2 °C temperatures, 55 ± 10 % humidity, and on a 12 h dark/light cycle. The feeding was discontinued 1 day before starting the in vitro tests. The animals were sacrificed and their intestines were removed above the ileocecal valve. Smooth muscle segments of about 1 cm length were mounted under a resting tension of 500 mg and were maintained at 37 °C in a 20 ml Jacketed organ bath containing oxygenated (95 % $O_2$ and 5 % $CO_2$) physiological saline solution of the following compositions: NaCl 137 mM, $CaCl_2$ 1.8 mM, KCl 2.7 mM, $MgSO_4$ 1.1 mM, $NaHPO_4$ 0.4 mM, $NaHCO_3$ 12 mM and glucose 5 mM. The muscle was equilibrated for 1 h with a solution changing every 15 min. The contractions were recorded with a forced displacement transducer (Hugo Sachs, March-Hugstetten and Germany) on a physiograph (Hugo Sachs). All compounds were dissolved in DMSO and the same volume of solvent was used as the negative control, while nifedipine was used as the positive control. The contraction was elicited

with 80 mM KCl. The contractile response was taken as the 100 % value for the tonic (slow) component of the response. Test compounds were added in cumulative doses after the maximum response caused by KCl addition. Test compound-induced relaxation of contraction was expressed as the percent of the control and $IC_{50}$ values were determined from the contraction-response curves (Mehdipour et al. 2007; Foroughinia et al. 2008).

### Cell lines and cell culture

HeLa (human cervical adenocarcinoma), LS180 (human colon adenocarcinoma), MCF-7 (human breast adenocarcinoma) and Raji (human B lymphoma) cells were obtained from the National Cell Bank of Iran, Pasteur Institute, Tehran, Iran. All cell lines were maintained in RPMI 1640 supplemented with 10 % FBS, and 100 U/ml penicillin-G and 100 µg/ml streptomycin. MES-SA and MES-SA-DX5 cells were first grown in Opti-MEM media and then gradually adapted to RPMI in the course of 4 weeks. Cells were grown in monolayer cultures, except for Raji cells, which were grown in suspension, at 37 °C in humidified air containing 5 % $CO_2$.

### Cytotoxicity assay

Cell viability following exposure to synthetic compounds was estimated by using the MTT reduction assay (Mosmann 1983; Miri et al. 2011). MCF-7 and Raji cells were plated in 96-well microplates at a density of $5 \times 10^4$ cells/ml (100 µl per well). LS180 and HeLa cells were plated at densities of $1 \times 10^5$ and $2.5 \times 10^4$ cells/ml, respectively. Control wells contained no drugs and blank wells contained only growth medium for background correction. After overnight incubation at 37 °C, half of the growth medium was removed and 50 µl of medium supplemented with different concentrations of synthetic compounds dissolved in DMSO were added in triplicate. Plates with Raji cells were centrifuged before this procedure. DMSO concentration in wells did not exceed 0.5 %. Cells were further incubated for 72 h, except for HeLa cells, which were incubated for 96 h. At the end of the incubation time, the medium was removed and MTT was added to each well at a final concentration of 0.5 mg/ml and plates were incubated for another 4 h at 37 °C. Then formazan crystals were solubilized in 200 µl DMSO. The optical density was measured at 570 nm with background correction at 655 nm using a Bio-Rad microplate reader (Model 680). The percentage of inhibition of viability compared to control wells was calculated for each concentration of the compound and $IC_{50}$ values were calculated with the software CurveExpert version 1.34 for Windows. Each experiment was repeated four times. Data are presented as mean ± SD

### MDR reversal assay

MES-SA-DX5 cells that over-express P-gp were used as a model of typical MDR, which are developed from their parental drug sensitive MES-SA (uterine sarcoma) cells. Cells were seeded in 96 well plates with a density of $3 \times 10^4$ cells/ml. Various concentrations of test compounds were added in triplicate followed by different concentrations of doxorubicin after 1 h. Cells were then further incubated for 72 h and the viability was measured with the MTT assay as described above.

## Results and Discussion

Fourteen novel asymmetrical dihydropyridines (DHPs) were synthesized and their calcium channel blocking (CCB), cytotoxic and MDR reversal properties were examined.

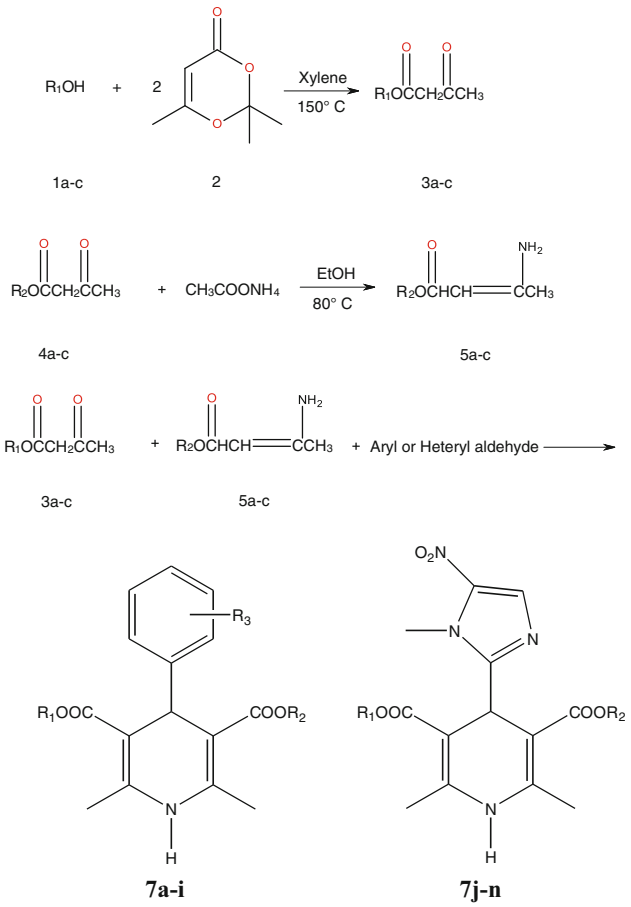
### Chemistry

The synthesis of the 1,4-DHP derivatives **7a–7n** was achieved following the steps outlined in Scheme 1 based on modified method of Dagnino et al. (1986). Reaction of alcohol **1a–1c** with 2,2,6-trimethyl-4-H-1,3-dioxin-4-one 2 afforded the corresponding acetoacetic esters **3a–3c** (>90 % yield) (Hyatt et al. 1984). Reaction of acetoacetic esters **4a–4c** with ammonium acetate produced the corresponding alkyl 3-aminocrotonate **5a–5c**. The asymmetrical analogues, **7a**–7**n**, were synthesized by a modified Hantzch reaction (~5–30 % yield). These compounds were purified by preparative thin-layer chromatography and recrystallized, and then characterized by mass spectroscopy, IR and $^1$H NMR. The yield and melting point of final compounds are summarized in Table 1.

### Pharmacology

In vitro CCB activities of compounds were determined in guinea pig ileal longitudinal smooth muscle (GPILSM) and the molar concentration of the test compound required to produce 50 % inhibition of GPILSM ($IC_{50}$) was determined (Table 1). This model has been used by several investigators for measurement of CCB activity (Iman et al. 2011).

Synthesized compounds **7a–7n** exhibited weak to moderate CCB activity ($IC_{50}$ range: 134.0 nM–4.8 µM) relative to the reference drug nifedipine ($IC_{50}$: 68.9 nM), except for compound **7b**, which was about twofolds stronger than nifedipine ($IC_{50}$: 29.3 nM). Considering that CCB activity is an undesired effect as long as MDR reversal drug development is concerned, it is supposed that **7b** is not a good choice for further development since its strong CCB activity would




**Scheme 1** Synthetic pathway of target compounds **7a**–**7n**

certainly limit the dosing for MDR reversal. On the other hand, from this point of view, compounds with CCB $IC_{50}$ values in the micromolar range (**7a**, **7d**, **7e**, **7g**, **7i**, **7k**) seem to be good candidates for further development. Comparison of these asymmetric compounds with previously synthesized symmetric compounds (Mehdipour et al. 2007; Foroughinia et al. 2008) shows that both symmetrical and asymmetrical compounds have same range of CCB activity ($IC_{50}$ values $\cong 10^{-7}$ M) and they are at least ten folds weaker than nifedipine. Therefore, it seems that the presence of at least one pyridyl group at $C_3$ or $C_5$ is sufficient for reduction of CCB activity as a side effect in this context. No evident difference could be observed between the CCB activity of compounds bearing nitrophenyl moiety (**7a**–**7i** compounds) or imidazole group (**7j**–**7n** compounds) at $C_4$ position, suggesting that substitutions at this position do not considerably alter the CCB activity.

## Cytotoxicity

The cytotoxic activities of synthesized compounds were evaluated on four human cancer cell lines and $IC_{50}$ values were calculated (Table 2).

Most of the DHP compounds demonstrated cytotoxicity in all 4 different cancer cells, including solid tumor (HeLa, LS180, MCF-7) and hematopoietic malignancy cells (Raji). The lowest observed $IC_{50}$ belonged to the compound **7c** in Raji (human B lymphoma) cells ($6.4 \pm 2.1$ μM). Compound **7c** had also the lowest $IC_{50}$ against MCF-7 cell line ($16.0 \pm 3.4$ μM). Comparison of the effect of DHP compounds on the 4 cell lines used in this study, showed that they generally had the highest $IC_{50}$ values on LS180 (human colon adenocarcinoma) cells (except for compound **7e**).

As for the structure–cytotoxic activity relationships of studied compounds, **7a**–**7i** that contain nitrophenyl moiety at $C_4$ position had $IC_{50}$ values in the range between 6.4 and 64.4 μM against cancer cells. These were much more active than compounds **7j**–**7n** bearing imidazole ring at $C_4$, which mostly had $IC_{50}$ values of higher than 100 μm against cancer cells. Therefore, it is clear that the substituent at the $C_4$ position has a great impact on the cytotoxic effect of these compounds.

Other substitutions at $C_3$ and $C_5$ positions had also some effects on the cytotoxic activity; however, these effects were smaller compared to the effect of $C_4$ substitution. Compounds **7c** and **7h** have low $IC_{50}$ values against most of the cell lines. Both of these compounds have 3-(pyridine-4-yl)propyl at their $C_3$ position and a 4-nitrophenyl group at their $C_4$ position. Therefore, it seems that the combination of these 2 moieties confers high cytotoxic activity to these compounds. On the other hand, comparison of the $IC_{50}$ values of **7g** and **7b** compounds against cancer cells shows that the former compound is more active than the later in all cell lines. Since **7g** differs from **7b** in having an isopropyl ester instead of a methyl ester at $C_5$ position, the presence of a longer alkyl ester at $C_5$ seems to improve the activity. Indeed, all compounds possessing this moiety at $C_5$ position (**7f**, **7g** and **7h**) have low $IC_{50}$ values against all cancer cell lines.

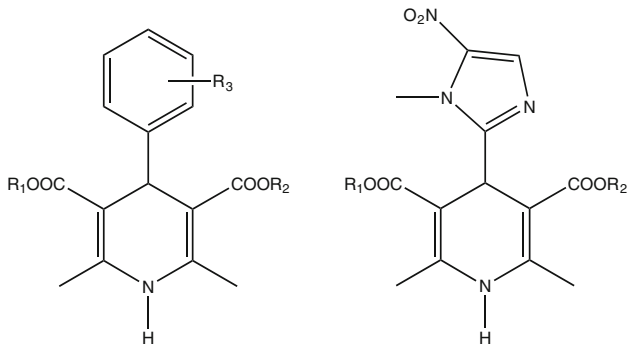
## MDR reversal

MDR reversal activity of DHP derivatives was assessed on MES-SA-DX5 cells that over-express P-gp and are resistant to doxorubicin compared to their non-resistant parental MES-SA cells (Figs. 1, 2).

Over-expression of P-gp, which pumps the cytotoxic agents out of the cell, is a well-known cause of MDR in cancer cells (Chen and Sikic 2012). MES-SA-DX5 cells over-express P-gp and hence are an established cell model for typical MDR (Koo et al. 2008; Angelini et al. 2012).

The efficiency of the test compounds in increasing the cytotoxicity of doxorubicin in MES-SA-DX5 cells was therefore determined as an index of their MDR reversal capacity. Most of DHP compounds bearing nitrophenyl

**Table 1** Physical properties and calcium channel antagonist activities of synthetic dihydropyridines




7a-i 7j-n

| Compound | $R_1$ | $R_2$ | $R_3$ | Mp (°C) | Yield (%) | $IC_{50} \pm SEM$ (M)[a] | *n* |
|---|---|---|---|---|---|---|---|
| **7a** | 3-(Pyridine-4-yl)propyl | Methyl | 4-Nitro | 152–154 | 14.4 | $(4.23 \pm 1.72) \times 10^{-6}$ | 3 |
| **7b** | 3-(Pyridine-3-yl)propyl | Methyl | 3-Nitro | 138–140 | 5.3 | $(2.93 \pm 2.28) \times 10^{-8}$ | 4 |
| **7c** | 3-(Pyridine-4-yl)propyl | Ethyl | 4-Nitro | 114–118 | 29.4 | $(4.57 \pm 1.76) \times 10^{-7}$ | 4 |
| **7d** | 3-(Pyridine-3-yl)propyl | Methyl | 2-Nitro | 178–180 | 24.543 | $(1.08 \pm 0.68) \times 10^{-6}$ | 3 |
| **7e** | 3-(Pyridine-4-yl)propyl | Ethyl | 3-Nitro | 167–170 | 24.7 | $(2.26 \pm 0.89) \times 10^{-6}$ | 4 |
| **7f** | 3-(Pyridine-2-yl)propyl | Isopropyl | 3-Nitro | 120–122 | 19.7 | $(2.32 \pm 1.18) \times 10^{-7}$ | 4 |
| **7g** | 3-(Pyridine-3-yl)propyl | Isopropyl | 3-Nitro | 114–116 | 13.2 | $(1.39 \pm 0.52) \times 10^{-6}$ | 4 |
| **7h** | 3-(Pyridine-4-yl)propyl | Isopropyl | 4-Nitro | 180–182 | 29.4 | $(1.34 \pm 0.32) \times 10^{-7}$ | 4 |
| **7i** | 3-(Pyridine-3-yl)propyl | Ethyl | 3-Nitro | 116–118 | 26.5 | $(1.30 \pm 0.73) \times 10^{-6}$ | 4 |
| **7j** | 3-(Pyridine-3-yl)propyl | Methyl | – | 158–162 | 15.0 | $(2.74 \pm 1.77) \times 10^{-7}$ | 3 |
| **7k** | 3-(Pyridine-2-yl)propyl | Methyl | – | 150–152 | 9.7 | $(4.75 \pm 1.62) \times 10^{-6}$ | 4 |
| **7l** | 3-(pyridine-3-yl)propyl | Ethyl | – | 166–168 | 7.8 | $(1.61 \pm 0.36) \times 10^{-7}$ | 4 |
| **7m** | 3-(Pyridine-4-yl)propyl | Methyl | – | 174–178 | 18.7 | $(2.79 \pm 1.03) \times 10^{-7}$ | 4 |
| **7n** | 3-(Pyridine-2-yl)propyl | Isopropyl | – | 138–140 | 9.7 | $(3.75 \pm 0.24) \times 10^{-7}$ | 3 |
| Nifedipine | Methyl | Methyl | 2-Nitro | | | $(6.89 \pm 2.00) \times 10^{-8}$ | 3 |

[a] Calcium channel antagonist activity was measured in guinea pig ileal longitudinal smooth muscle

**Table 2** Cytotoxic activity of synthetic dihydropyridines on human cancer cell lines

| Compound | IC50 (μM) | | | |
|---|---|---|---|---|
| | Hela | LS180 | MCF-7 | Raji |
| **7a** | 13.2 ± 2.6 | 26.9 ± 10.0 | 24.3 ± 2.9 | 24.7 ± 8.7 |
| **7b** | 24.8 ± 6.4 | 40.9 ± 7.0 | 37.5 ± 3.4 | 19.5 ± 11.5 |
| **7c** | 27.2 ± 2.4 | 31.8 ± 5.9 | 16.0 ± 3.4 | 6.4 ± 2.1 |
| **7d** | 26.5 ± 8.5 | 41.9 ± 5.4 | 30.9 ± 5.8 | 18.4 ± 13.8 |
| **7e** | 33.5 ± 9.5 | 63.2 ± 14.1 | 37.6 ± 10.2 | 64.4 ± 34.4 |
| **7f** | 14.8 ± 6.6 | 39.1 ± 11.8 | 24.3 ± 7.1 | 19.3 ± 10.0 |
| **7g** | 20.0 ± 3.5 | 26.0 ± 8.2 | 20.3 ± 5.5 | 18.5 ± 8.3 |
| **7h** | 12.6 ± 1.1 | 28.4 ± 9.2 | 18.7 ± 3.0 | 21.7 ± 6.4 |
| **7i** | 22.4 ± 5.2 | 39.1 ± 11.5 | 18.1 ± 5.0 | 17.8 ± 10.9 |
| **7j** | >100 | >100 | >100 | >100 |
| **7k** | >100 | >100 | >100 | >100 |
| **7l** | >100 | >100 | >100 | >100 |
| **7m** | 60.4 ± 21.2 | >100 | >100 | >100 |
| **7n** | >100 | >100 | 88.9 ± 55.3 | >100 |
| Doxorubicin | 0.511 ± 0.112 | 0.061 ± 0.011 | 0.237 ± 0.182 | 0.184 ± 0.187 |

Values are presented as mean ± SD of 3–4 experiments

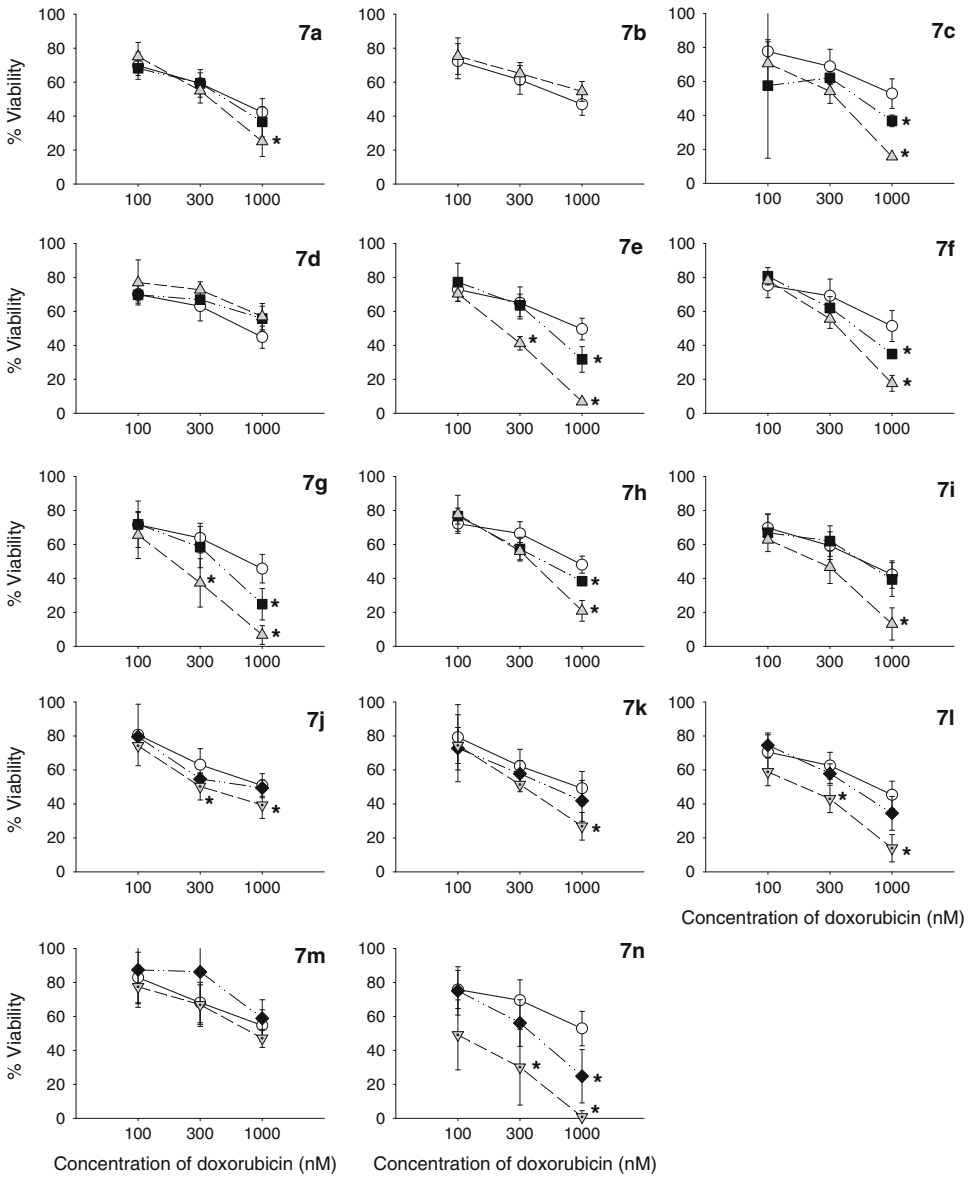



**Fig. 1** Effect of newly synthesized dihydropyridine derivatives (**7a–7n**) on resistance to doxorubicin in MES-SA-DX5 cells. Doxorubicin-resistant cells were preincubated alone (*open circle*) or with DHP compounds [**7a–7i** at 0.5 (*filled square*) and 1 μM (*filled triangle*); **7j–7n** at 5 (*filled diamond*) and 10 μM (*filled inverted triangle*)] for 1 h and then exposed to different concentrations of doxorubicin for 72 h. Cell viability was measured with the MTT assay and expressed as percentage compared to untreated control cells. Significant versus doxorubicin alone (in the absence of test compound) at $^*p < 0.05$

moiety at $C_4$ position (including **7a**, **7c**, **7e**, **7g**, **7h** and **7i**) were able to reverse MDR in resistant cells at the concentrations of 0.5 and/or 1 μM, while the same concentrations were ineffective on non-resistant cells. The lack of effect in non-resistant cells suggests that the mechanism of action of these compounds is probably through interference with P-gp.

The most effective compounds were **7e** and **7g**, which at the concentration of 1 μM significantly increased the effect of 300 nM doxorubicin and also at the concentrations of 0.5 and 1 μM augmented the effect of 1 μM doxorubicin. In the order of efficiency, these derivatives were followed by compounds **7c**, **7f** and **7h**, which significantly reversed the resistance to 1 μM doxorubicin at the concentrations of 0.5 and 1 μM. Compounds **7i** and **7a** reversed the resistance to 1 μM doxorubicin only at the higher concentration of 1 μM, while compounds **7b** and **7d** were not effective either on resistant or non-resistant cells. Concentrations higher than 1 μM of compounds **7a–7i** induced direct cytotoxicity on cell lines (data not shown), therefore these concentrations were not tested for MDR reversal, as the direct cytotoxic effect of the test compound would have been confounded with the MDR reversal effect.

On the other hand, compounds possessing nitroimidazole at $C_4$ position (**7j–7n**) were not effective on resistant cells at 0.5 and 1 μM concentrations, however some of them were selectively effective on MES-SA-DX5 cells at 5 and 10 μM. Compound **7n** appeared to be the most effective DHP in this subgroup, since at the concentration of 5 μM it was able to significantly reverse the effect of 1 μM doxorubicin. Compounds **7j** and **7l** at the concentration of 10 μM were able to significantly reverse the effect of

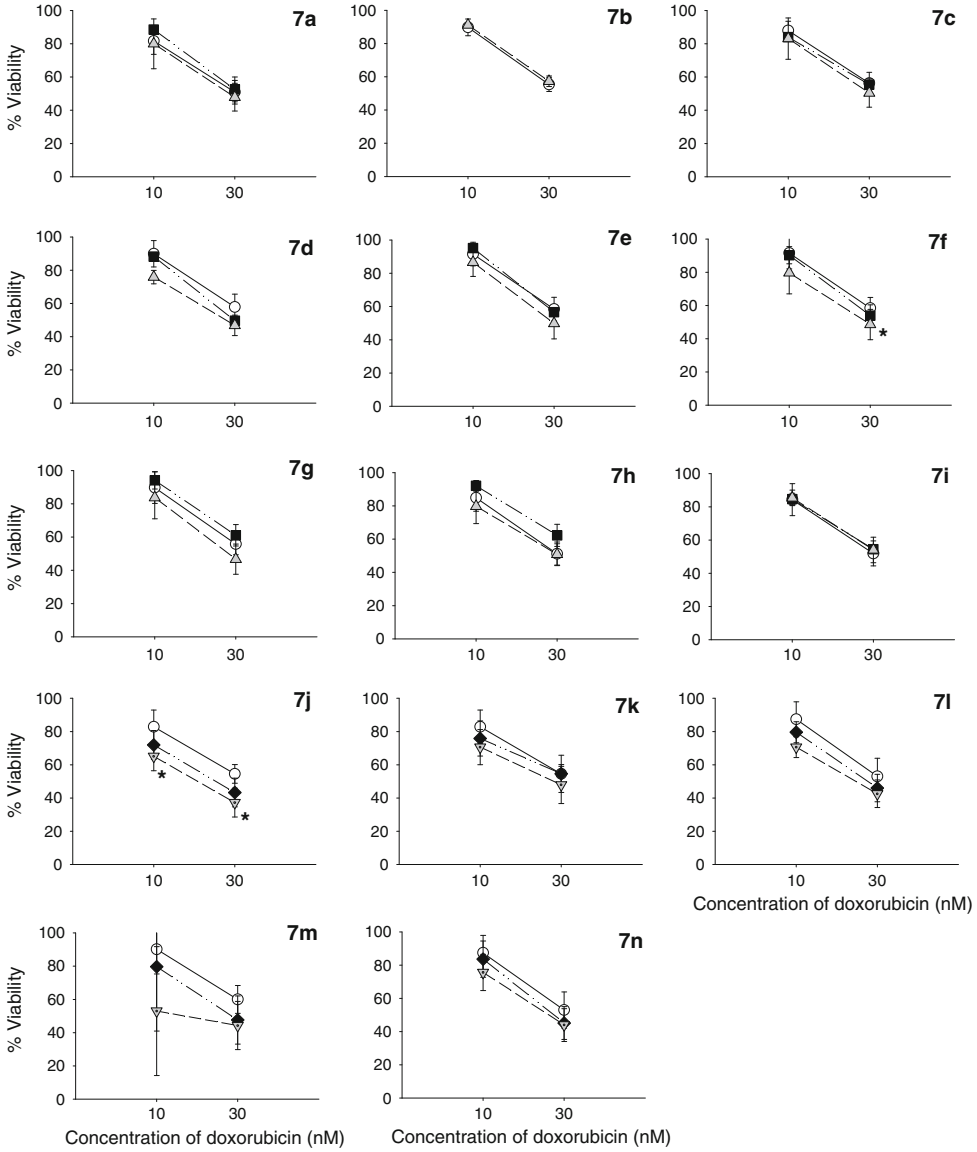



**Fig. 2** Effect of newly synthesized dihydropyridine derivatives (**7a–7n**) on resistance to doxorubicin in MES-SA cells. Nonresistant cells were incubated alone (*open circle*) or with DHP compounds [**7a–7i** at 0.5 (*filled square*) and 1 μM (*filled triangle*); **7j–7n** at 5 (*filled diamond*) and 10 μM (*filled inverted triangle*) for 1 h and then exposed to different concentrations of doxorubicin for 72 h. Cell viability was measured with the MTT assay and expressed as percentage compared to untreated control cells. Significant versus doxorubicin alone (in the absence of test compound) at $^*p < 0.05$

300 nM and 1 μM doxorubicin. Compound **7k** was also able to selectively reverse resistance to 1 μM doxorubicin at the concentration of 10 μM. None of these compounds had any direct cytotoxic effect on cell lines at the tested concentrations (data not shown).

In conclusion, MDR is one of the main mechanisms that limits the efficacy of chemotherapeutic agents in many types of cancer, therefore MDR reversal agents could be of great help in management of drug resistant cancer. 1,4-DHPs have been in clinical use as CCB agents for a long time, but they are also able to reverse MDR in cancer cells. In this project, in continuation of our previous studies, we synthesized novel asymmetrical derivatives of DHP and measured their cytotoxic, MDR reversing and also CCB activity, with the latest being considered a side effect in this case. Based on the obtained results, it appears that compounds **7e** and **7g** possess high MDR reversal and cytotoxic activities and at the same time low CCB properties. These characteristics make these compounds very good candidates for MDR reversal and antitumoral drug development.

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**Conflict of interest** Authors declare that they have no conflict of interest.

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