2-oxo-1,2-dihydroquinoline-4-carboxylic acid derivatives as potent modulators of ABCB1-related drug resistance of mouse T-lymphoma cells

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PII: S2405-8300(20)30207-X
DOI: https://doi.org/10.1016/j.cdc.2020.100501
Reference: CDC 100501

To appear in: *Chemical Data Collections*

Received date: 24 July 2020
Accepted date: 28 July 2020

Please cite this article as: Yassir Filali Baba , Houria Misbahi , Youssef Kandri Rodi , Younes Ouzidan , El Mokhtar Essassi , Klaudia Vincze , Márta Nové , Márió Gajdács , Joseph Molnár , Gabriella Spengler , Ahmed Mazzah , 2-oxo-1,2-dihydroquinoline-4-carboxylic acid derivatives as potent modulators of ABCB1-related drug resistance of mouse T-lymphoma cells, *Chemical Data Collections* (2020), doi: https://doi.org/10.1016/j.cdc.2020.100501

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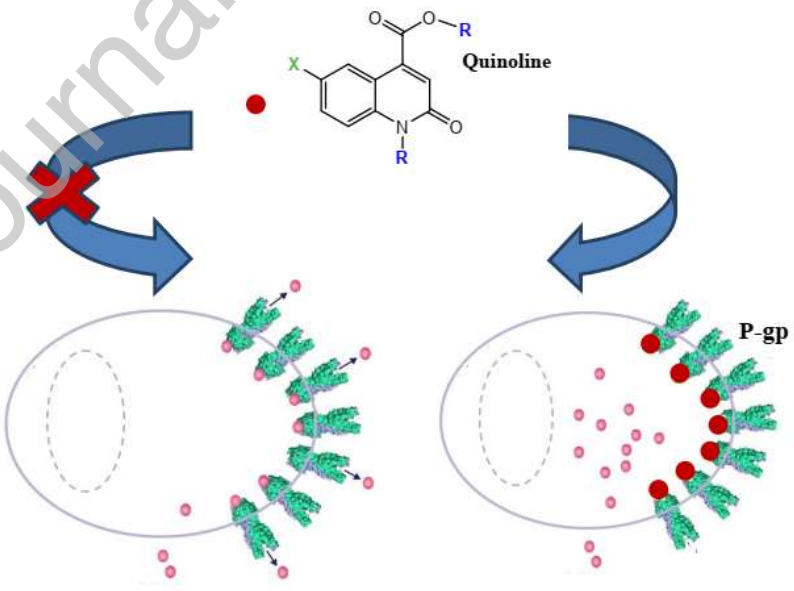
Authors: Yassir Filali Baba[a], Houria Misbahi[a], Youssef Kandri Rodi[a],Younes Ouzidan[a,b], El Mokhtar Essassi[c], Klaudia Vincze[d], Márta Nové[d], Mário Gajdács[d], Joseph Molnár[d], Gabriella Spengler[d], Ahmed Mazzah[e]

Affiliation: *[a]Laboratory of Applied Organic Chemistry, Faculty of Science and Technology, Sidi Mohamed Ben Abdellah University, BP 2202 Fez, Morocco. [b]Laboratory of Physical Chemistry and Bioorganic Chemistry, Faculty of Science and Technology, Hassan II University, BP 146 Mohammedia 28800, Morocco. [c]Laboratory of Heterocyclic Organic Chemistry URAC21, Faculty of Sciences, University of Mohamed V, Rabat, Morocco. [d]Department of Medical Microbiology and Immunobiology, Faculty of Medicine, University of Szeged; Dóm tér 10, 6720 Szeged, Hungary. [e]USR 3290 Miniaturisation pour l'analyse, la synthèse et la protéomique, 59655 Villeneuve d'Ascq Cedex, Université Lille1, France*

Contact email: younes.ouzidan@usmba.ac.ma /younes.ouzidan@fstm.ac.ma

Abstract: Multidrug resistance (MDR) of cancer cells is a major cause of therapeutic failure. One of the mechanisms of MDR is the overexpression of efflux pump such as ABCB1. The use of ABCB1 inhibitors constitute an important strategy for reversing MDR. Thus, this study aimed to synthesize a novel 2-oxo-1,2-dihydroquinoline-4-carboxylic acid derivatives and evaluate their biological activities *in vitro* using parental (PAR) and multidrug resistant (MDR; ABCB1-overexpressing) mouse T-lymphoma cells. The cytotoxic activity and selectivity of the tested compounds were assessed by MTT method. The ABCB1 modulating activity was measured by rhodamine 123 accumulation assay using flow cytometry. The results showed that the compounds **2b**, **2c** and **3b** exerted cytotoxic activity with $IC50_{MDR}$ value of 9.09 µM, 71.14 µM and 19.09 µM, respectively. The most active compound **8c** should be considered as a lead compound for further derivatization and additional biological assays.

Graphical abstract



Keywords: Quinoline, multidrug resistance, P-glycoprotein, ABCB1, efflux pump.

Specifications Table

Subject area	*Organic Chemistry, Biochemistry, Spectroscopy*
Compounds	*Quinolone derivatives*
Data category	*Spectral data, synthesized*
Data acquisition format	*Process and analysis data*
Data type	*analyzed*
Procedure	*The compound was synthesized, characterized, and investigated regarding their cytotoxicity and their ABCB1-modulating properties against parental and ABCB1-overexpressing MDR mouse T-lymphoma cells.*
Data accessibility	*Data is with this article or in public repository.*

1. Rationale

Cancer is the second leading cause of death worldwide [1]. This disease is a consequence of a disruption of physiological cell functions. Cell resistance to multiple chemotherapy drugs is considered as the main difficulty to develop the efficient therapy. The multidrug resistance (MDR) can be developed by multiple mechanisms [2].

The main mechanisms of resistance are those that lead to the removal of chemotherapy drugs. The ATP-binding cassette (ABC) transporters have been reported to play a critical role in this process [3]. ABC transporters are a superfamily of membrane proteins consisting of 48 members. ABCB1, also called P-glycoprotein is the first ABC transporter discovered and has been extensively studied in recent years [4]. As other ABC transporters, ABCB1 is recognized to produce resistance by lowering intracellular concentration of chemotherapy drug in ATP-depending manner [4]. It is known to transport various drugs including the vinca alkaloids, anthracyclines, etoposide, taxanes, bisantrene, mitoxantrone, and the histone deacetylase inhibitor depsipeptide [4,5].

Targeting ABCB1 has led to develop ABCB1 inhibitors that are able to block transport of substrates and consequently increase intracellular concentration of drugs [6]. In clinical trials, many inhibitors have been studied, but definitive proof that inhibition of drug efflux can improve clinical outcome has not been forthcoming [7]. The first generation of ABCB1 inhibitors, such as verapamil, are substrates of these pumps and competitively inhibit the efflux of other compounds, often at concentrations that would not be achievable in vivo without considerable toxic side effects. In contrast, the second generation, such as valspodar, and the third generation, of ABCB1 inhibitors are the products of high-throughput screening (HTS), and they exert their activities without causing severe side effects, as their activity is specific and selective for these MDR transporters [8]. Therefore, the development of ABCB1-transporter inhibitors appears to be an interesting concept to explore, as their use as adjuvants could improve the therapy of many tumor-related pathologies in humans.

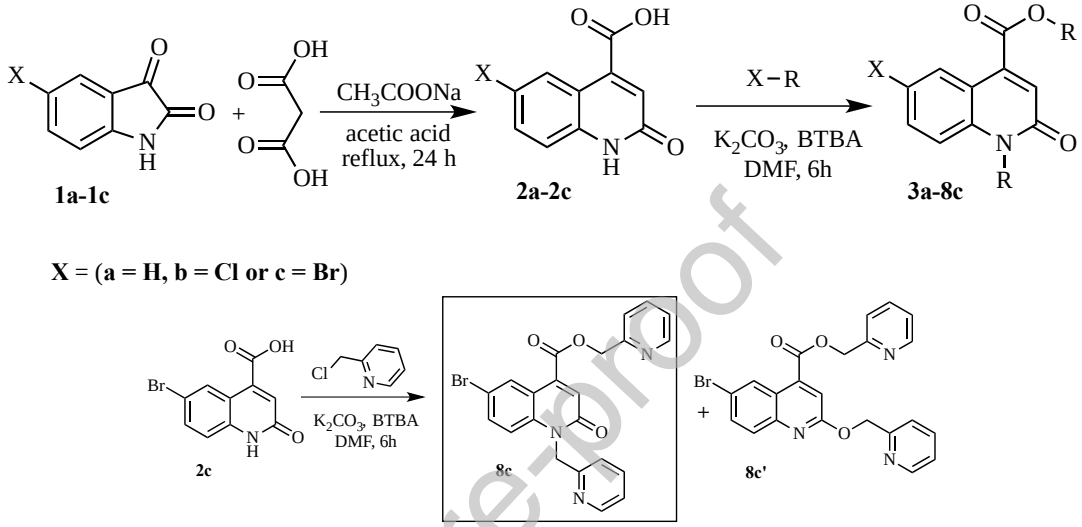
Despite the great structural diversity observed in efflux pump inhibitors, some common characters have been identified. They are generally nitrogenous heterocycles, with a low basic character and with the presence of an aromatic nucleus [9-18]. Certain derivatives of quinoline have shown various interesting biological properties and a favorable pharmacological profile. It was reported that the compounds with antitumor activity containing a quinoline moiety may act as cytostatic agents, [19-21] or as inhibitors of the topoisomerase-II enzyme, interfering with DNA replication [22]. A novel 1,2-dihydroquinoline derivative anticancer agent and its delivery to tumor cells using cationic liposomes has also been described [23].

Thus, in this study, a series of 2-oxo-1,2-dihydroquinoline-4-carboxylic acid derivatives (18 compounds) were prepared and evaluated for their cytotoxicity effect and their ABCB1-modulating properties against parental and ABCB1-overexpressing MDR mouse T-lymphoma cells.

2. Procedure

2.1. Synthesis.

The structures of the compounds studied are summarized in Scheme 1 and Table 1. The products were obtained in two steps procedure as described previously for some products [24-29]. First, 2-oxo-1,2-dihydroquinoline-4-carboxylic acid derivatives **2a**, **2b** and **2c** were prepared from condensation of various isatin (1H-indole-2,3-dione) and malonic acid. The mixture was refluxed in acetic acid in the presence of sodium acetate for 24 hours. Then, the compounds (**2a**-**2c**) were alkylated using different alkyl reagents in the presence of K_2CO_3 in DMF for 6 hours. The tertiobutylammonium bromide (TBAB) was used as catalyst. In these conditions, N-alkyl derivatives were obtained in good yields. However, for the alkylation of **2c** with picolyl chloride hydrochloride, we have been able to isolate, in addition to the expected product **8c**, the compound **8c'** resulting from O-alkylation.



Scheme 1. Structures of 2-oxo-1,2-dihydroquinoline-4-carboxylic acid derivatives (2a-8c, 8c')

Table 1. Substituents of 2-oxo-1,2-dihydroquinoline-4-carboxylic acid derivatives (2a-8c, 8c').

Compounds	X	R
2a	H	H
2b	Cl	H
2c	Br	H
3a	H	CH_3
3b	Cl	CH_3
4a	H	CH_2CH_3
4b	Cl	CH_2CH_3
4c	Br	CH_2CH_3
5b	Cl	$CH_2CH{=}CH_2$
5c	Br	$CH_2CH{=}CH_2$
6a	H	$CH_2C{\equiv}CH$
6c	Br	$CH_2C{\equiv}CH$
7a	H	$CH_2C_6H_5$
7c	Br	$CH_2C_6H_5$
8a	H	$CH_2C_5H_5N$
8b	Cl	$CH_2C_5H_5N$
8c	Br	$CH_2C_5H_5N$

8c'	Br	$CH_2C_5H_5N$

2.1.1. General procedure synthesis of the compounds 2a-2c:

To a solution of 10 mmol of isatin and 10 mmol of malonic acid in 30 mL of acetic acid was added 1 mmol of sodium acetate. The mixture was refluxed for 24 h. After cooling, 100 mL of ice water are added. The precipitate obtained was washed several times with ethanol.

2.1.2. General procedure synthesis of compounds 3a-8c'

To a solution of 10^{-3}mol of compound 2a-2c in 10 mL of DMF were added 2.510^{-3}mol of alkylating agent 4.10^{-3}mol of K_2CO_3 and 0.0110^{-3} mol of tetran-butylammonium bromide (TBAB). The reaction mixture was stirred at room temperature in DMF for 6 h. After removal of the salts by filtration, the solvent was evaporated under reduced pressure and the residue obtained is dissolved in dichloromethane. The organic phase is dried over Na_2SO_4 then concentrated under vacuum. The compound obtained was purified by chromatography on a column of silica gel (eluent: ethyl acetate/hexane (1/3)).

2.2. Materials and methods

An FT-IR spectrum was recorded directly without dilution in KBr pellets using a JASCO FTIR-4160 spectrometer in the range of 4000 – 400 cm^{-1} and at a resolution of ± 2 cm^{-1}.

The spectroscopic characterization of the synthesized compounds is achieved by recording NMR spectra (Bruker Avance DPX300). TLC and column chromatography were performed using silica plates and silica gel, respectively.

2.3. Biological activities

Afterwards, working solutions were prepared by dilution in water, the concentration of DMSO was below 1 % in all the experiments.

Other chemicals used in the study as reagents were: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; Sigma-Aldrich, St Louis, MO, USA), sodium dodecyl sulphate (SDS; Sigma-Aldrich, St Louis, MO, USA), rhodamine 123 (R123; Sigma, St. Louis, MO, USA), verapamil (EGIS Hungarian Pharmaceutical Company, Budapest, Hungary) and dimethyl sulfoxide (DMSO; Sigma-Aldrich, St Louis, MO, USA). Stock solutions of R123 were prepared in phosphate buffered saline and verapamil was dissolved in water. All solutions were prepared on the day of the assay.

2.3.1. Cell lines.

L5178Y mouse T-cell lymphoma cells (PAR) (ECACC Cat. No. 87111908, obtained from FDA, Silver Spring, MD, USA) were transfected with pHa MDR1/A retrovirus, as previously described by Cornwell et al [30]. The ABCB1-expressing cell line L5178Y (MDR) was selected by culturing the infected cells with colchicine. The L5178Y human ABCB1-transfected subline was cultured in McCoy's 5A medium (Sigma-Aldrich, St Louis, MO, USA) supplemented with 10% heat-inactivated horse serum (Sigma-Aldrich, St Louis, MO, USA), 200 mM L-glutamine (Sigma-Aldrich, St Louis, MO, USA) and a penicillin-streptomycin (Sigma-Aldrich, St Louis, MO, USA) mixture in concentrations of 100 U/L and 10 mg/L, respectively. The cell lines were incubated at 37˚C, in a 5% CO_2, 95% air atmosphere.

2.3.2. Assay for cytotoxic effect.

The effects of increasing concentrations of the tested 2-oxo-1,2-dihydroquinoline-4-carboxylic acid derivatives on cell growth were tested in 96-well microtiter plates. The parental (PAR) and multidrug resistant (MDR) mouse T-lymphoma cells were cultured using McCoy's 5A medium supplemented with 10% heat-inactivated horse serum. The density of the cells was adjusted to $1x10^4$ cells per well (in 100 µL of medium per well) and then added to the 96-well flat-bottomed microtiter plates containing the dilutions of the tested compounds. The culture plates were incubated at 37°C, in a 5% CO_2, 95% air atmosphere.

The culture plates were incubated at 37°C for 24 h; at the end of the incubation period, 20 µL of MTT (Sigma) solution (from a stock solution of 5 mg/mL) were added to each well. After incubation at 37˚C for 4 h, 100 µL of

sodium dodecyl sulfate (SDS) (Sigma) solution (10% in 0.01 M HCl) were added to each well and the plates were further incubated at 37˚C overnight. Cell growth was determined by measuring the optical density (OD) at 540/630 nm with Multiscan EX ELISA reader (Thermo Labsystems, Cheshire, WA, USA) [31]. Inhibition of the cell growth was determined according to the formula below:

$$IC_{50} = 100 - \left[\frac{OD\,sample - OD\,medium\,control}{OD\,cell\,control - OD\,medium\,control} \right] \times 100$$

Rhodamine 123 accumulation assay. This method, previously described elsewhere, is a fluorescence-based detection system which uses verapamil as reference inhibitor of the ABCB1 efflux pump [32]. The parental and multidrug resistant (MDR) subline of mouse T-lymphoma cells were adjusted to a density of 2 x 10^6 cells/mL and re-suspended in serum-free McCoy's 5A medium and distributed in 500 µL aliquots. The tested compounds (1 and 10 µL from a stock solution of 1 mM, respectively) were added at different concentrations (final concentrations of 2 µM and 20 µM, respectively). Verapamil was used as positive control at 20 µM (from a 5 mg/mL stock solution) and DMSO was used as solvent control (at 2 V/V%). The samples were incubated for 10 min at room temperature, then 10 µL (5.2 µM final concentration) of rhodamine 123 were added to the samples and the cells were incubated for 20 minutes at 37˚C, washed twice with phosphate buffered saline (PBS) and re-suspended in 1 mL PBS for analysis. The fluorescence intensity of the gated cell population was measured with a Partec CyFlow flow cytometer (Partec, Munster, Germany). The mean fluorescence intensity was calculated for the treated MDR and parental mouse T-lymphoma cells lines as compared to the untreated cells [33,34]. The fluorescence activity ratio (FAR) was calculated based on the following equation which relates the measured fluorescence values:

$$FAR = \frac{MDR_{treated} / MDR_{control}}{parental_{treated} / parental_{control}};$$

$$Quotient = 100\ x\ (FAR_{compound}/FAR_{verapamil})$$

3. Data, value and validation

3.1. Chemistry (Characteristic and spectroscopic data)

1,2-dihydro-2-oxoquinoline-4-carboxylic acid: 2a

Yield: 90%; Physical state: gray solid; mp>350°C; NMR ^{1}H (300 MHz, DMSO): 6.86 (s, 1H, CH), 7.2-8.16 (m, 4H, CH_{arom}), 12.17 (s, 1H, NH); 13.9 (s, 1H, OH). NMR ^{13}C (75 MHz, DMSO): 167.2 (COOH), 163.0 (C=O), 141.7(Cq), 139.8 (Cq), 131.3 (CH_{arom}), 126.5 (CH_{arom}), 123.8 (CH_{arom}), 122.6 (CH_{arom}), 116.2 (CH_{arom}). MS (DIC / NH_3): m/z = 189 $[MH]^+$.

6-chloro-1,2-dihydro-2-oxoquinoline-4-carboxylic acid: 2b

Yield: 83%; Physical state: gray solid; mp: >350 °C; NMR ^{1}H (300 MHz, DMSO): 6.91 (s, 1H, CH), 7.32 (d, 1H, $^3J_{H-H}$ = 9Hz, CH_{arom}), 7.72 (d, 1H, $^3J_{H-H}$ = 9 Hz, CH_{arom}), 8.22 (d, 1H, $^4J_{H-H}$ = 1.8Hz, CH_{arom}), 12.22 (s, 1H, NH), 14.05 (s, 1 H, OH). NMR ^{13}C (75 MHz, DMSO): 166.69 (COOH), 161.24 (C=O), 139.64(Cq), 138.7 (Cq), 131.14 (CH_{arom}), 126.7 (Cq), 126.07 (CH_{arom}), 125.77 (CH_{arom}), 118.08 (Cq), 117.42 (CH_{arom}). MS (DIC / NH_3): m/z = 223.5 $[MH]^+$.

6-bromo-1,2-dihydro-2-oxoquinoline-4-carboxylic acid: 2c

Yield: 85%; Physical state: gray solid; mp: >350 °C; NMR ^{1}H (300 MHz, DMSO): 6.98 (s, 1H, CH), 7.30 (d, 1H, $^3J_{H-H}$ = 9Hz, CH_{arom}), 7.7 (dd, 1H, $^3J_{H-H}$ = 9 Hz, $^4J_{H-H}$ = 1.8 Hz, CH_{arom}), 8.42 (d, 1H, $^4J_{H-H}$ = 1.8Hz, CH_{arom}), 12.22 (s, 1H, NH), 14.05 (s, 1H, OH).

NMR ^{13}C (75 MHz, DMSO): 166.7 (COOH), 161.2 (C=O), 139.6(Cq), 139 (Cq), 133.8 (CH_{arom}), 128.7 (CH_{arom}), 125.9 (CH), 118.3 (CH_{arom}), 117.9 (Cq), 114.5 (CH_{arom}). MS(DIC / NH_3): m/z = 268 $[MH]^+$.

Methyl 1,2-dihydro-1-methyl-2-oxoquinoline-4-carboxylate: 3a

Yield: 75%; Physical state: mauve solid; mp: 134 °C; NMR ^{1}H (300 MHz, $CDCl_3$): 3.74 (s, 3H, CH_3), 4.0 (s, 3H, CH_3), 7.28 (s, 1H, CH), 7.35 (d,1H, $^{3}J_{H-H}$ = 9.3 Hz, CH_{arom}), 7.57(dd, 1H, $^{3}J_{H-H}$ = 11.1 Hz, $^{4}J_{H-H}$ = 2.4Hz, CH_{arom}), 8.4 (d, 1H, $^{4}J_{H-H}$ = 2.4Hz, CH_{arom}).

NMR ^{13}C (75 MHz, $CDCl_3$): 165.22 (C=O), 160.92 (C=O), 138.97-137.09 (Cq, Cq), 131.18 (C CH_{arom}), 128.60 (Cq), 126.70 (CH_{arom}) 125.9 (CH), 118.50 (Cq), 115.78 (CH_{arom}), 52.95 (CH_3), 29.97 (CH_3). HRMS (ESI): m/z calculated for $C_{12}H_{11}NO_3$ $[M+H]^+$: 218.08117, found 218.08117.

Methyl 6-chloro-1,2-dihydro-1-methyl-2-oxoquinoline-4-carboxylate: 3b

Yield: 75%; Physical state: gray solid; mp: 134 °C; NMR ^{1}H (300 MHz, $CDCl_3$): 3.74 (s, 3H, CH_3), 4.0 (s, 3H, CH_3), 7.28 (s, 1H, CH), 7.35 (d,1H, $^{3}J_{H-H}$ = 9.3 Hz, CH_{arom}), 7.57(dd, 1H, $^{3}J_{H-H}$ = 11.1 Hz, $^{4}J_{H-H}$ = 2.4Hz, CH_{arom}), 8.4 (d, 1H, $^{4}J_{H-H}$ = 2.4Hz, CH_{arom}).

NMR ^{13}C (75 MHz, $CDCl_3$): 165.22 (C=O), 160.92 (C=O), 138.97(Cq), 137.09 (Cq), 131.18 (CH_{arom}), 128.60 (Cq), 126.70 (CH_{arom}) 125.9 (CH), 118.50 (Cq), 115.78 (CH_{arom}), 52.95 (CH_3), 29.97 (CH_3). HRMS (ESI): m/z calculated for $C_{12}H_{10}{}^{35}ClNO_3$ $[M+H]^+$ 252.04220, found 252.04219.

Ethyl 1-ethyl-1,2-dihydro-2-oxoquinoline-4-carboxylate: 4a

Yield: 86%; Physical state: Violet crystals; mp: 93 °C; NMR ^{1}H (300 MHz, $CDCl_3$): 1.39 (t, 3H, $^{3}J_{H-H}$ = 7.2Hz, CH_3), 1.42 (t, 3H, $^{3}J_{H-H}$ = 6.9 Hz, CH_3), 4.40 (q, 2H, $^{3}J_{H-H}$ = 6.9 Hz, CH_2), 4.46 (q, 2H, $^{3}J_{H-H}$ = 7.2 Hz, CH_2), 7.20 (s, 1H, CH), 7.30 (m, 1H, CH_{arom}), 7.45 (d, 1H, $^{3}J_{H-H}$ = 8.1 Hz, CH_{arom}); 7.62 (m, 1H, CH_{arom}); 8.35 (d, 1H, $^{4}J_{H-H}$ = 1.5 Hz, CH_{arom}).

NMR ^{13}C (75 MHz, $CDCl_3$): 165.46 (C=O), 160.95 (C=O), 139.36(Cq), 138.90 (Cq), 131.07 (CH_{arom}), 127.37 (CH_{arom}), 124.20 (CH_{arom}) 122.45 (CH), 117.80 (Cq), 114.35 (CH_{arom}), 61.99 (CH_2), 37.64 (CH_2), 14.38 (CH_3), 12.63 (CH_3). HRMS (ESI): m/z calculated for $C_{14}H_{15}NO_3$ $[M+H]^+$ 246.11247, found 246.11246.

Ethyl 6-chloro-1-ethyl-1,2-dihydro-2-oxoquinoline-4-carboxylate: 4b

Yield: 88%; Physical state: Marron crystals; mp: 122 °C; NMR ^{1}H (300 MHz, $CDCl_3$): 1.35 (t, 3H, $^{3}J_{H-H}$ = 7.2Hz, CH_3), 1.42 (t, 3H, $^{3}J_{H-H}$ = 7.2Hz, CH_3), 4.34 (q, 2H, $^{3}J_{H-H}$ = 7.2Hz, CH_2), 4.45 (q, 2H, $^{3}J_{H-H}$ = 7.2 Hz, CH_2), 7.24 (s, 1H, CH), 7.34 (d, 1H, $^{3}J_{H-H}$ = 9.3 Hz, CH_{arom}), 7.53 (dd, 1H, $^{3}J_{H-H}$ = 9.3 Hz, $^{4}J_{H-H}$ = 2.4 Hz, CH_{arom}), 8.44 (d, 1H, $^{4}J_{H-H}$ = 2.7 Hz, CH_{arom}).

NMR ^{13}C (75 MHz, $CDCl_3$):164.80 (C=O), 160.47 (C=O), 137.91(Cq), 137.35 (Cq), 131.06 (CH_{arom}), 128.22 (Cq), 126.83 (CH_{arom}), 125.71 (CH_{arom}), 118.82 (Cq), 115.64 (CH_{arom}), 62.16(CH_2), 37.84(CH_2), 14.10(CH_3), 12.57(CH_3).

Ethyl 6-bromo-1-ethyl-1,2-dihydro-2-oxoquinoline-4-carboxylate: 4c

Yield: 79%; Physical state: Green solid; mp: 114 °C; NMR ^{1}H(300 MHz, $CDCl_3$): 1.35 (t, 3H, $^{3}J_{H-H}$ = 7.14Hz, CH_3), 1.45 (t, 3H, $^{3}J_{H-H}$ = 7.13Hz, CH_3), 4.35 (q, 2H, $^{3}J_{H-H}$ = 7.14 Hz, CH_2), 4.45 (q, 2H, $^{3}J_{H-H}$ = 7.14 Hz, CH_2), 7.43 (s, 1H, CH), 7.5 (d, 1H, $^{3}J_{H-H}$ = 8.8 Hz, CH_{arom}), 7.65 (dd, 1H, $^{3}J_{H-H}$= 2.32 Hz, CH_{arom}), 8.52 (s, 1H, $^{4}J_{H-H}$ = 2.3Hz, CH_{arom}).

NMR ^{13}C(75 MHz, $CDCl_3$): 164.79 (C=O), 160.47 (C=O), 138.25(Cq), 137.33 (Cq), 133.84 (CH_{arom}), 129.86 (CH_{arom}), 125.65 (CH), 119.26 (Cq), 115.92 (CH_{arom}), 115.67 (Cq), 62.18 (CH_2), 37.82 (CH_2) 14.11 (CH_3), 12.56 (CH_3). HRMS (ESI): m/z calculated for $C_{14}H_{14}{}^{79}BrNO_3$ $[M+H]^+$ 324.02298, found 324.02298, m/z calculated for $C_{14}H_{14}{}^{81}BrNO_3$ $[M+H]^+$ 326.02093, found 326.02093

Allyl 1-allyl-6-chloro-1,2-dihydro-2-oxoquinoline-4-carboxylate: 5b

Yield: 68%; Physical state: White solid; mp: 94 °C; NMR ^{1}H(300 MHz, $CDCl_3$): 4.9 (m, 4H, $2CH_2$=CH), 5.0-5.4 (m, 4H, $2CH_2CH$), 5.9 (m, 2H, $2CH_2$=CH), 7.3(s,1H, CH), 7.33 (d, 1H, $^{3}J_{H-H}$ = 5.1Hz,CH $_{arom}$), 7.5 (dd, 1H, $^{3}J_{H-H}$ = 11.4Hz, $^{4}J_{H-H}$ = 2.4Hz, CH $_{arom}$), 8.4 (d, 1H, $^{3}J_{H-H}$ = 2.4Hz, CH_{arom}).

NMR ^{13}C(75 MHz, $CDCl_3$): 164.42 (C=O), 160.59 (C=O), 138.28(Cq), 137.50 (Cq), 131.12 (CH_{arom}), 131.08 (CH_2=CH), 130.97 (CH_2=CH), 128.59 (Cq), 125.66 (CH_{arom}), 125.73 (CH), 119.61 (Cq), 118.66 (CH_2CH), 117.61 (CH_2CH), 116.55 (CH_{arom}), 66.65 (CH_2=CH), 45.03 (CH_2=CH). HRMS (ESI): m/z calculated for $C_{16}H_{14}{}^{35}ClNO_3$ $[M+H]^+$ 304.07350, found 304.07350.

Allyl 1-allyl-6-bromo-1,2-dihydro-2-oxoquinoline-4-carboxylate: 5c

Yield: 71%; Physical state: Yellow solid; mp: 97 °C; NMR ^{1}H(300 MHz, $CDCl_3$): 4.9 (m, 4H, 2 CH_2=CH), 5.1-5.5 (m, 4H, 2 CH_2CH), 6.0 (m, 2H, 2 CH_2=CH), 7.23-7.32(m, 3H, 2 $CH_{arom,}$ CH), 7.65 (dd, 1H, $^{3}J_{H-H}$ = 10.8Hz, $^{4}J_{H-H}$ = 1.8 Hz CH $_{arom}$), 8.6 (d, 1H, $^{3}J_{H-H}$ = 8.1Hz CH $_{arom}$). NMR ^{13}C(75 MHz, $CDCl_3$): 164.39 (C=O), 160.55 (C=O), 138.69(Cq), 137.44 (Cq), 133.87 (CH_{arom}), 131.07 (CH_2=CH), 130.94 (CH_2=CH), 129.66 (CH_{arom}), 125.68 (CH), 119.60 (Cq), 119.06 (Cq), 117.61 (CH_2CH), 116.81 (CH_{arom}), 116.0 (CH_2CH), 66.65 (CH_2=CH), 44.98(CH_2=CH). HRMS (ESI): m/z calculated for $C_{16}H_{14}{}^{79}BrNO_3$ $[M+H]^+$ 348.02298, found 348.02299, m/z calculated for $C_{16}H_{14}{}^{81}BrNO_3$ $[M+H]^+$ 350.02093, found 350.02092

Prop-2-ynyl 1,2-dihydro-2-oxo-1-(prop-2-ynyl)quinoline-4-carboxylate: 6a

Yield: 94%; Physical state: Yellow crystal; mp: 162 °C; NMR ^{1}H(300 MHz, $CDCl_3$): 2.88 (t, 1H, $^{3}J_{H-H}$ = 5.1 Hz, $^{4}J_{H-H}$ = 2.4 Hz, C≡CH), 2.59 (t, 1H, $^{3}J_{H-H}$ = 5.1 Hz, $^{4}J_{H-H}$ = 2.4 Hz, C≡CH), 5.0 (d, 2H, $^{4}J_{H-H}$ = 2.4 Hz CH_2), 5.1(d, 2H, $^{4}J_{H-H}$ = 2.4 Hz CH_2), 7.32 (s, 1H,CH), 7.34 (m, 1H,CH_{arom}), 7.6 (d, 1H, $^{3}J_{H-H}$ = 8.4 Hz, CH_{arom}), 7.68 (m, CH_{arom}), 8.38 (dd, 1H, $^{3}J_{H-H}$ = 8.1Hz, $^{4}J_{H-H}$ = 1.2Hz CH_{arom}).

NMR ^{13}C(75 MHz, $CDCl_3$): 164.32 (C=O), 160.25 (C=O), 139.04-138.5 (Cq-Cq), 131.41 (CH_{arom}), 127.26 (CH_{arom}), 124.49 (CH_{arom}) 123.18 (CH), 117.55 (Cq), 115.78 (CH_{arom}),75.89 (C≡CH), 72.82 (C≡CH), 53.31(CH_2), 31.97(CH_2).

Prop-2-ynyl 6-bromo-1,2-dihydro-2-oxo-1-(prop-2-ynyl)quinoline-4-carboxylate: 6c

Yield: 80%; Physical state: Orange solid; mp: 176 °C; NMR ^{1}H(300 MHz, $CDCl_3$): 2.3 (t, 1H, $^{3}J_{H-H}$ = 5.1 Hz, $^{4}J_{H-H}$ = 2.7 Hz, C≡CH), 2.61 (t, 1H, $^{3}J_{H-H}$ = 5.1 Hz, $^{4}J_{H-H}$ = 2.4 Hz, C≡CH), 5.0 (d, 2H, $^{4}J_{H-H}$ = 2.4 Hz CH_2), 5.1(d, 2H, $^{4}J_{H-H}$ = 2.4 Hz CH_2), 7.33 (s, 1H,CH), 7.45 (d, 1H, $^{3}J_{H-H}$ = 9.3 Hz, CH_{arom}), 7.76 (dd, 1H, $^{3}J_{H-H}$ = 11.4Hz, $^{4}J_{H-H}$ = 2.1Hz CH $_{arom}$), 8.6 (d, 1H, $^{4}J_{H-H}$ = 2.1Hz CH_{arom}).

NMR ^{13}C(75 MHz, $CDCl_3$): 163.75 (COOH), 159.83 (C=O), 137.96(Cq), 136.96 (Cq), 131.41 (CH_{arom}), 127.26 (CH_{arom}), 125.96 (CH), 118.99 (Cq), 116.57 (CH_{arom}), 116.49 (Cq), 76.12 (C≡CH), 73.27 (C≡CH), 53.52 (CH_2), 32.10 (CH_2).

Benzyl 1-benzyl-1,2-dihydro-2-oxoquinoline-4-carboxylate: 7a

Yield: 76%; Physical state: Orange solid; NMR ^{1}H(300 MHz, $CDCl_3$): 5.46 (s, 2H, CH_2), 5.59 (s, 2H, CH_2), 7.20-7.49 (m, 14H, CH_{arom}), 8.39 (d, 1H, ^{3}J = 8.1 Hz, CH_{arom}). NMR ^{13}C(75 MHz, $CDCl_3$): 165.10 (C=O), 161.50 (C=O), 139.86-139.10 (Cq, Cq), 135.83 (Cq), 135.05 (Cq), 131.16, 128.87, 128.87, 128.78, 128.7, 127.42, 127.20, 126.53, 124.29, 122.78, 115.43 (CH_{arom}), 117.74 (Cq), 67.73 (CH_2), 46.25 (CH_2). HRMS (ESI): m/z calculated for $C_{24}H_{19}NO_3$ $[M+H]^+$ 370.14377, found 370.14377.

Benzyl 1-benzyl-6-bromo-1,2-dihydro-2-oxoquinoline-4-carboxylate: 7c

Yield: 65%; Physical state: Yellow solid; mp: 134 °C, NMR ^{1}H(300 MHz, $CDCl_3$): 4.05 (m, 4H, 2CH_2), 6.90 (d, 1H, $^{3}J_{H-H}$ = 9 Hz, CH_{arom}), 7.10-7.41 (m, 12H, CH_{arom}), 8.07 (d, 1H, ^{4}J = 2.4 Hz, CH_{arom}).

NMR ^{13}C(75 MHz, $CDCl_3$): 164.90 (C=O), 161.45 (C=O), 156.08 (2Cq), 154.86 (Cq),149.70 (Cq), 149.39 (Cq), 139.92(Cq), 139.06 (Cq), 137.11 (CH_{arom}), 136.98 (CH_{arom}), 131.40 (CH_{arom}), 127.20 (CH_{arom}), 124.33 (CH_{arom}), 123.24 (CH_{arom}), 122.62 (CH_{arom}), 121.87 (2CH_{arom}), 121.58 (CH_{arom}), 117.65 (Cq), 115.72 (CH_{arom}), 68.02 (CH_2), 48.37 (CH_2).

(pyridin-2-yl)methyl 1,2-dihydro-2-oxo-1-((pyridin-2-yl)methyl)quinoline-4-carboxylate: 8a

Yield: 92%; Physical state: Yellow crystals; mp: 143 °C, NMR ^{1}H(300 MHz, $CDCl_3$): 5.57 (s, 2H, CH_2), 5.71 (s, 2H, CH_2), 7.13-7.32 (m, 4H, CH_{arom}), 7.41 (s, 1H, CH_{arom}), 7.46-7.51 (m, 3H, CH_{arom}), 7.59 (td, 1H, ^{3}J = 7.7 Hz, ^{4}J = 1.8 Hz CH_{arom}), 7.77 (td, 1H, ^{3}J = 7.7 Hz, ^{4}J = 1.8 Hz CH_{arom}), 8.41 (d, 1H, ^{3}J = 8.1 Hz, CH_{arom}), 8.6 (m, 1H, CH_{arom}), 8.66 (m, 1H, CH_{arom}). NMR ^{13}C(75 MHz, $CDCl_3$): 165.02 (C=O), 161.43 (C=O),156.16(Cq), 154.93 (Cq), 149.70 (CH_{arom}), 149.37 (CH_{arom}), 139.99 (Cq),137.02 (CH_{arom}), 136.89 (CH_{arom}), 131.33, 127.20, 128.87, 128.78, 128.7, 127.42, 127.20, 126.53, 124.29, 122.78, 115.43 (CH_{arom}), 117.74(Cq), 67.73(CH_2), 46.25(CH_2). HRMS (ESI): m/z calculated for $C_{22}H_{17}N_3O_3$ $[M+H]^+$ 372.13427, found 372.13429.

(pyridin-2-yl)methyl 6-chloro-1,2-dihydro-2-oxo-1-((pyridin-2-yl)methyl)quinoline-4-carboxylate: 8b

Yield: 83%; Physical state: Yellow solid; mp: 152 °C, NMR ^{1}H(300 MHz, $CDCl_3$): 5.55 (s, 2H, CH_2), 5.68 (s, 2H, CH_2), 7.05-7.50 (m, 9H, CH_{arom}), 8.34 (m, 1H, CH_{arom}), 8.7 (m, 1H, CH_{arom}). NMR ^{13}C(75 MHz, $CDCl_3$): 164.79 (C=O), 161.32 (C=O),155.75 (Cq), 154.67 (Cq), 149.88 (CH_{arom}), 149.72 (CH_{arom}), 138.96 (Cq), 137.57 (Cq), 137.24 (CH_{arom}), 137.01,

134.19, 129.22, 125.46, 123.51, 122.93, 122.67, 122.01 (CH_{arom}), 119.35 (Cq), 117.12 (CH_{arom}), 116.53 (Cq), 68.81(CH_2), 48.32(CH_2).

(pyridin-2-yl)methyl 6-bromo-1,2-dihydro-2-oxo-1-((pyridin-2-yl)methyl)quinoline-4-carboxylate: 8c

Yield: 48%; Physical state: Yellow solid; mp: 156 °C, NMR ^{1}H(300 MHz, $CDCl_3$): 5.56 (s, 2H, CH_2), 5.66 (s, 2H, CH_2), 7.15-7.80 (m, 9H, CH_{arom}), 8.56 (m, 1H, CH_{arom}), 8.67 (m, 1H, CH_{arom}). NMR ^{13}C(75 MHz, $CDCl_3$): 164.43 (C=O), 161.02 (C=O),155.67 (Cq), 154.64 (Cq), 149.79 (CH_{arom}), 149.42 (CH_{arom}), 138.91 (Cq), 137.72 (Cq), 137.18 (CH_{arom}), 136.99, 134.16, 129.70, 125.66, 123.31, 122.80, 121.93, 121.83 (CH_{arom}), 119.09 (Cq), 117.43 (CH_{arom}), 116.35 (Cq), 68.13(CH_2), 48.42(CH_2). HRMS (ESI): m/z calculated for $C_{22}H_{16}{}^{79}BrN_3O_3$ $[M + H]^+$ 450.04478, found 450.04478, m/z calculated for $C_{22}H_{16}{}^{81}BrN_3O_3$ $[M + H]^+$ 452.04273, found 452.04273.

(pyridin-2-yl)methyl 2-((pyridin-2-yl)methoxy)-6-bromo-1,2-dihydroquinoline-4-carboxylate: 8c'

Yield: 18%; Physical state: White solid; mp: 118 °C, NMR ^{1}H(300 MHz, CDCl3): 5.58 (s, 2H, CH_2), 5.69 (s, 2H, CH_2), 7.23-7.33 (m, 2H, CH_{arom}), 7.42-7.56 (m, 2H, CH_{arom}), 7.69-7.80 (m, 2H, CH_{arom}), 8.66 (t, 2H, 3J = 6 Hz, CH_{arom}), 8.96 (s, 1H, CH_{arom}). NMR ^{13}C(75 MHz, CDCl3):164.80 (C=O), 160.95 (C=O),156.64 (Cq), 154.95 (Cq), 149.75 (CH_{arom}), 149.48 (CH_{arom}), 146.15 (Cq), 136.99 (CH_{arom}), 136.37, 136.42 (Cq), 133.38 (CH_{arom}), 129.0, 128.66, 123.39, 123.25, 122.74, 122.04, 121.92 (CH_{arom}), 119.50 (Cq), 116.0 (CH_{arom}), 68.66(CH_2), 68.00 (CH_2). HRMS (ESI): m/z calculated for $C_{22}H_{16}{}^{79}BrN_3O_3$ $[M+ H]^+$ 450.04478, found 450.04478, m/z calculated for $C_{22}H_{16}{}^{81}BrN_3O_3$ $[M+ H]^+$ 452.04273, found 452.04273.

3.2. Biology

3.2.1. Results

It can be observed, that apart from compounds **2b**, **2c**, and **3b** (with IC_{50MDR} values of 9.09 µM, 71.14 µM and 19.09 µM, respectively), none of the tested compounds exerted cytotoxic activity against the parental and MDR subline of mouse T-lymphoma cells (Table II.). The abovementioned three compounds presented with slight selectivity towards the MDR subline (SI values between 1.19–2.56).

Among the nineteen tested compounds, 7 compounds (**5c**, **5b**, **6c**, **7a**, **7c**, **8c** and **8c'**) presented with potent ABCB1-modulating activity at similar concentration as the positive control verapamil (20 µM), with FAR values ranging between 12.86 and 250.13 vs. $FAR_{verapamil}$=8.34. As denoted by the FAR quotients in Table III., after treatment with the previously mentioned compounds in the rhodamine 123 accumulation assay, the fluorescence was enhanced by 154.24–2999.39%, compared to verapamil. There were cases when the efflux pump modulatory effect of some compounds (**7a**, **7c**, **8c'** and **8c**, with $FAR_{2\ \mu M}$ values of 11.34, 28.70, 8.22 and 224.70) concurred or exceeded the inhibitory activity of verapamil at 2 µM In contrast, compounds **2a**, **2b**, **2c**, **3a**, **3b**, **4a**, **4b**, **4c**, **6a**, **8a** and **8b** did not exert significant efflux pump modulatory activities in either concentrations tested (with FAR quotients ranging between 9.19 and 46.39%).

Table 2. Cytotoxicity of tested compounds against parental (PAR) and multidrug resistant (MDR) mouse lymphoma cells and selectivity indices (SI).

	IC_{50} (µM)		
Compounds	PAR (A)	MDR (B)	SI (B/A)
2a	>100	>100	-
2b	23.26 ± 5.48	9.09 ± 3.16	2.56
2c	>100	71.14 ± 3.21	>1.41
3a	>100	>100	-
3b	22.66 ± 1.77	19.09 ± 1.53	1.19
4a	>100	>100	-
4b	>100	>100	-

4c	>100	>100	-
5b	>100	>100	-
5c	>100	>100	-
6a	>100	>100	-
6c	>100	>100	-
7a	>100	>100	-
7c	>100	>100	-
8a	>100	>100	-
8b	>100	>100	-
8c	>100	>100	-
8c'	>100	>100	-
DMSO	>2 V/V%	>2 V/V%	-

DMSO: dimethyl-sulfoxide; SD: standard deviation; SI: Selectivity Index; SI<3 values denote slight selectivity, 3<SI<6 values indicate moderate selectivity, whereas SI<6 indicates strong selectivity [35].

Table 3. Rhodamine 123 accumulation assay in multidrug resistant (MDR) mouse T-lymphoma cells overexpressing the ATP-binding cassette transporter protein B1.

Compounds	Concentrations (μM)	Fluorescence activity ratio (FAR)	FAR quotient (%)
Verapamil	20	8.34	-
2a	2	1.29	15.42
	20	1.91	22.93
2b	2	1.43	17.20
	20	0.98	11.73
2c	2	1.53	18.29
	20	1.49	17.88
3a	2	1.23	14.74
	20	1.16	13.92
3b	2	0.98	11.74
	20	3.03	36.31
4a	2	0.77	9.19
	20	2.12	25.46
4b	2	1.61	19.32
	20	3.87	46.39
4c	2	0.80	9.61
	20	1.02	12.28
5c	2	1.82	21.84
	20	**56.23**	674.29
5b	2	3.64	43.68
	20	**57.94**	694.77

6a	2	0.84	10.02
	20	0.94	11.32
6c	2	1.24	14.88
	20	**12.86**	154.24
7a	2	**11.34**	135.92
	20	**65.23**	782.14
7c	2	**28.70**	344.19
	20	**49.77**	596.79
8a	2	0.85	10.16
	20	0.84	10.03
8b	2	0.87	10.38
	20	0.76	9.10
8c'	2	**8.22**	98.62
	20	**65.39**	784.16
8c	2	**224.70**	2694.37
	20	**250.13**	2999.39
DMSO	2 V/V%	0.78	9.32

3.2.1. Discusion

In the present work, we have tested 18 novel 2-oxo-1,2-dihydroquinoline-4-carboxylic acid derivatives synthesized in our laboratory for their cytotoxic activities and their potency as MDR reversing agents associated with ABCB1 transporter-related chemotherapeutic resistance. Seven of the compounds tested were found to be especially potent modulators of the ABCB1 efflux pump (their activity was 1.54-29.99-fold higher than verapamil's) in a concentration-dependent manner, while the most active compound 8c was 26.94-fold more potent, than verapamil in concentrations that are ten times smaller. Barring a few exceptions, none of the tested derivatives showed cytotoxicity on the tested cell lines, which may well be the advantage of these compounds, as this property would allow for their administrations as adjuvants *in vivo* in case of efflux pump-related drug resistance, without the fear of collateral toxicity caused by these agents. This is underlined by our data, since all the potent efflux pump inhibitor compounds had an IC_{50} values higher than 100 µM, while they exerted their activity on the ABCB1 transporter in much lower concentrations. Our results suggest that derivatives with a bromine group at position 6, together with derivatives that are dialkylated with benzyl or 2-(methyl)pyridine, allyl- or propargyl groups have presented the best modulatory effects on P-glycoprotein. Derivative 8c which carries a bromine group at the position 6, has O-alkylation and N-alkylation with 2-(methyl)pyridine showed the most promising inhibition of ABCB1-related multidrug resistance. Thus, it should be considered as a lead compound for further derivatization and additional biological assays.

By comparing the results (**table 3** and **fig. 1**), we note that the two derivatives having in position 6: a chlorine **5b** (FAR = 57.93) and a bromine **5c** (FAR = 56.23), have almost the same effect on MDR reversion activity at a concentration of 20 µM.

Similarly the comparison between **6a** (H) (FAR = 0.94) and **6c** (Br) (FAR = 12.86) at 20 µM shows that the bromination of the studied pattern gives it a strong ability to inhibit Pgp.

Comparing the two derivatives **5c** and **6c**, we see that the disubstitution with allyl was more favorable than that with propargyl.

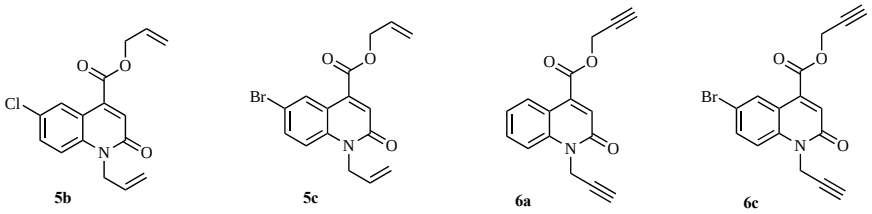


Fig 1: *Product with a good anti-MDR activity*

By comparing anti-MDR activity profile in both compounds **7a** and **7c**, we can notice that bromine presence is favorable in anti-MDR activity at a concentration of 2 µM. Besides that, at 20 µM the two products **7a** and **7c** have almost the same activity. It is possible to compare the two isomers of positions **8c** and **8c'**, and we find that N-alkylated product **8c** is the best for the reversion activity than the O-alkylated product **8c'**. Otherwise, we can conclude that the derivative disubstituted by pyridin-2-ylmethyl **8c** has an excellent inhibitory power compared to the derivative disubstituted by benzyl **7c** (**fig. 2**).

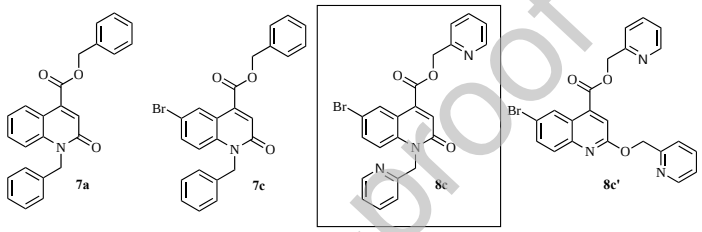


Fig 2: *Product with a good anti-MDR activity*

4. Conclusion

The activity of 18 derivates with various side-chains was compared with the aim of determining the effect of the nature of this chain on the MDR reversion. The best activity was obtained with **8c** which bear a picolyl side chain. In addition, the N-alkylation products seems be more interesting than O-alkylated ones. This is clearly demonstrated by comparing **8c** and **8c'** which are isomers of positions (N-alkylated and O-alkylated products respectively). The effect of halogen on the activity studied. Comparing products with Br, Cl and H, demonstrated that Br seemed to be the best substituent in position 6.

ACKNOWLEDGEMENTS

This study was supported by the European Union and the State of Hungary, co-financed by the European Social Fund in the framework of TÁMOP 4.2.4. A/2-11-1-2012-0001 'National Excellence Program'. Gabriella Spengler was supported by the János Bolyai Research Scholarship of the Hungarian Academy of Sciences. Mário Gajdács and Gabriella Spengler received funding from the Márton Áron Research Programme (2017/18) financed by the Hungarian Ministry of Foreign Affairs and Trade. Mário Gajdács was supported by the ÚNKP-17-3 New National Excellence Program of the Ministry of Human Capacities. The study was supported by the Szeged Foundation for Cancer Research.

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