Synthesis and biological evaluation of novel quinoline analogs of ketoprofen as multidrug resistance protein 2 (MRP2) inhibitors

Fatemeh Mosaffa 1, Farzin Hadizadeh 1, 2, Faezeh Fathi 1, Zahra Eslami Nasab 1, Tahereh Pourzahed 1, Sayyed Mohammad Aboutorabzade 2, Razieh Ghodsi 1, 2*

1 Biotechnology Research Center, Institute of Pharmaceutical Technology, Mashhad University of Medical Sciences, Mashhad, Iran
2 Department of Medicinal Chemistry, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran

ABSTRACT

Objective(s): A new series of quinoline analogs of ketoprofen was designed and synthesized as multidrug resistance protein 2 (MRP2) inhibitors using ketoprofen as the lead compound.

Materials and Methods: The cytotoxic activity of the compounds was evaluated against two cancer cell lines including A2780/RCIS (MRP2-overexpressing ovarian carcinoma), A2780, drug-sensitive ovarian carcinoma using MTT assay. Compounds showing low toxicity in MTT test were selected to investigate their MRP inhibition activity. MRP2 inhibitory potency was evaluated by determination of the uptake amount of fluorescent 5-carboxy fluorescein diacetate (5-CFDA) substrate, by A2780/RCIS in the presence of the selected compounds. Mode of interaction between synthesized ligands and homology modeled MRP2 was investigated by MOE software.

Results: Compound 6d, a 4-carboxy quinoline possessing dimethoxy phenyl in position 2 of quinoline ring, showed the most MRP2 inhibition activity among all the quinolines and more than the reference drug ketoprofen. MRP2 inhibition activity of compound 7d was less in comparison to that of compound 6d, indicating that carboxyl group in position 4 of quinoline may interact with MRP2. Docking studies showed that compound 7d methyl ester of 6d, interacted less compared to its parent 6d, which is consistent with biological results.

Conclusion: This study indicates that 6- or 8-benzoyl-2-arylquinoline is a suitable scaffold to design MRP2 inhibitors. The position of benzoyl in quinoline ring is important in inhibition of MRP2. Generally, MRP2 inhibition activity of compound 7d was less in comparison to that of 6d, indicating that carboxyl group in position 4 of quinoline may interact with MRP2.

Introduction

Cancer is the reason of 25% of all deaths in developed countries (1). Although chemotherapy is the collective way for treatment of different cancers, it fails to treat most cancer patients with advanced disease due to the occurrence of drug resistance (2, 3). One of the most essential mechanisms underlying MDR (multidrug resistance) is the overexpression of adenosine triphosphate (ATP)-binding cassette (ABC) super-family of transporters, which efflux both cytotoxic agents and targeted anticancer drugs using ATP driven energy (4). One important class of the ABC family is the human multidrug resistance-associated protein (MRP) family which comprises seven members. Numerous members of the MRP family especially MRP1 and MRP2 are implicated in the detoxification and defense of the host against xenobiotic materials. They are also expected to cause drug resistance by their ability in moving a wide range of anticancer drugs out of the cells and their occurrence in many different types of cancers (5).

NSAIDs (Non-steroidal anti-inflammatory drugs) have been administrated as analgesic, antipyretic and anti-inflammatory agents for several years (6, 7). NSAIDs also have been widely considered for their anti-tumorigenic and chemosensitive properties (8, 9).

Please cite this article as:
Mosaffa F, Hadizadeh F, Fathi F, Eslami Nasab Z, Pourzahed T, Aboutorabzade SM, Ghodsi R. Synthesis and biological evaluation of novel quinoline analogs of ketoprofen as multidrug resistance protein 2 (MRP2) inhibitors. Iran J Basic Med Sci 2021; 24:815-825. doi: 10.22038/ijbms.2021.54554.12265
A wide variety of NSAIDs like indomethacin and ketoprofen inhibited MRP2 and MRP4 facilitated methotrexate transport at concentrations to which the transporters may be unprotected under therapeutic conditions. As ketoprofen is a well-known MRP2 inhibitor (14) and some quinoline derivatives such as quinine (27-31) reported as MRP modulators, we designed novel 2-(aryl)quinolines possessing ketoprofen scaffold as MRP2 inhibitors. The rational for the design of these compounds is represented in Figure 2. The cytotoxic activity of the synthesized compounds was evaluated against two human cancer cell lines including A2780/RCIS, cisplatin resistant human ovarian carcinoma (MRP2-overexpressing ovarian carcinoma); A2780, drug-sensitive ovarian carcinoma. Compounds showed low to moderate toxicity in MTT test were selected to investigate their MRP 2 inhibition activity. Moreover, trying to explain the results of biological experiments, docking studies of the selected compounds into the homology-modeled human MRP2, were carried out.

**Materials and Methods**

**Chemistry**

All reagents, chemicals and solvents used in this research were bought from Merck AG and Aldrich Chemical. Melting points were assessed using a Thomas–Hoover capillary apparatus. Infrared spectra were attained by a Perkin Elmer (Model 1420) spectrometer. To acquire 1H NMR spectra Bruker FT-500 and 300 MHz instruments (Bruker Biosciences, USA) was used and A Bruker FT-300 MHz instrument was used to obtain 13C NMR spectra. Chloroform-D and DMSO-D6 were used as solvents. Coupling constant (J) values are measured in hertz (Hz) and spin multiples are given as s (singlet), d (double), t (triplet), q (quartet), m (multiplet). The mass spectra were assessed using a 3200 QTRAP LCMS triple quadrupole mass spectrometer (LCMS) with an electrospray ionization (ESI) interface.

**General procedure for preparation of 6- or 8-benzoyl-2-aryquinoline-4-carboxylic acid (Doebner reaction)**

A solution of appropriate benzaldehyde (9.45 mmol) and pyruvic acid (1.25g, 14.3 mmol) in acetic acid (10 ml) was heated for 40 min then 2- or 4-aminobenzophenone (10 mmol) was added to the solution and refluxed overnight. After cooling, the formed precipitate was filtered and washed with hexane and recrystallized in ethanol.

**8-Benzoyl-2-phenylquinoline-4-carboxylic acid (4a)**

Yield: 25%; mp=247-249 °C; 1H NMR (300MHz-DMSO-d6): δ (ppm)7.34-7.45 (m, 3H, phenyl H 3&H4&H5), 7.48-7.51 (t, 2H, benzoyl H &H7, J=9Hz), 7.58-7.65 (t, 1H, benzoyl H 4, J=9 Hz), 7.68-7.70 (m,4H, benzoyl H1&H2& phenyl H 2&H6), 7.83-7.86 (t, 1H, quinoline H 7, J=9 Hz), 7.97-8.00 (dd, 1H, quinoline H 5, J=9Hz, J=2.5Hz), 8.5 (s,1H, quinoline H 1), 8.84-8.87 (dd, 1H, quinoline H 5, J=9Hz, J=2.5Hz), 13.05 (s, 1H, COOH); 13C NMR (DMSO-d6, 75 MHz): δ 128.39, 130.08, 131.26, 130.39, 137.74, 138.33, 138.95, 139.56, 155.83, 167.87, 198.01; LC-MS(ESI):352.0 (M-1).

**Figure 1.** Chemical structures of ketoprofen, quinine and our designed quinoline derivatives possessing ketoprofen scaffold as MRP2 inhibitors

**Figure 2.** The uptake amount of the fluorescent 5-carboxy fluorescein diacetate (5-CFDA) substrate, by A2780/RCIS in the presence of compounds 6d, 7b, 7d and ketoprofen
8-Benzyl-2-(p-tolyl)quinoline-4-carboxylic acid (4c)

Yield: 33%; mp=252-254 °C; IR (KBr): ν (cm⁻¹) 2965.68 (OH) 1710.50, 1659.41 (C=O); ¹H NMR (300MHz-DMSO-d₆): δ 2.30 (s, 3H, methyl), 7.15-7.17 (d, 2H, 4-methyl phenylH₃ &H₄; δ 6.71 (t, 1H, quinolineH₃; δ 7.69-7.71 (dd, 2H, phenyl H₂ &H₆, J=9Hz); 7.40 (s, 1H, quinoline H₅); 8.15-8.18 (d, 2H, quinoline H₆ &H₇, J=9Hz); 14.17 (s, 1H, COOH); ¹³C NMR (DMSO-d₆, 75 MHz): δ 21.27, 130.62, 131.04, 133.38, 134.44, 135.01, 138.16, 139.05, 139.45, 140.55, 146.40, 153.78, 167.87, 198.09; LC-MS(ESI): 366.0 (M-1).

8-Benzyl-2-(3,4-dimethoxyphenyl)quinoline-4-carboxylic acid (4d)

Yield: 22%; mp=266-268 °C; IR (KBr): ν (cm⁻¹) 2960.9 (OH) 1700.7, 1677.8 (C=O); ¹H NMR (300MHz-DMSO-d₆): δ 3.87 (s, 3H, OCH₃), 7.15-7.17 (d, 2H, 4-methyl phenylH₃ &H₄; δ 7.68-7.70 (m, 5H, phenyl), 7.74-7.77 (t, 2H, quinolineH₂ &H₃, J=9Hz); 8.25 (s, 1H, quinolineH₈); 8.54 (s, 1H, quinolineH₃); 9.08-9.12 (s, 1H, quinoline H₅), 14.04 (s, 1H, COOH); ¹³C NMR (DMSO-d₆, 75 MHz): δ 120.81, 123.12, 127.49, 129.09, 129.52, 130.28, 130.50, 130.68, 130.99, 133.36, 135.68, 137.33, 137.92, 138.81, 150.31, 158.45, 167.49, 195.70; LC-MS(ESI): 412.0 (M-1).

General procedure for preparation of methyl 6-methoxy-2-arylquinoline-4-carboxylic acid

2-arylquinoline-4-carboxylic acid (4 or 6) (2 mmol) and potassium carbonate (10 mmol) were mixed. Methyl iodide (10 mmol) and acetone (10 ml) were added. The reaction mixture was refluxed. After 5 hours, the solvent was evaporated in vacuo and the residue was added to the residual mixture. The product was collected by filtration and dried to obtain pure product.

Methyl 8-benzyl-2-phenylquinoline-4-carboxylate (5a)

Yield: 25%; mp=248-250 °C; IR (KBr): ν (cm⁻¹) 3055(ΩH) 1700.7, 1663.3 (C=O); ¹H NMR (300MHz-DMSO-d₆): δ (ppm) 7.57-7.64 (m, 5H, phenyl), 7.74-7.77 (t, 1H, benzoyl H₇; δ 7.65-7.73 (2H, benzoyl H₂ &H₃, δ 7.46-7.49 (m, 3H, quinoline H₁ &H₂, J=9Hz); 7.84-7.87 (dt, 2H, benzoyl H₂ &H₃, J=9Hz, J=2.5Hz); 8.14-8.17 (d, 1H, quinolineH₇; J=9Hz, J=2.5Hz); 8.26-8.29 (d, 1H, quinoline H₈, J=9Hz); 8.32-8.35 (dd, 2H, benzoyl H₂ &H₃, J=9Hz); 8.57 (s, 1H, quinoline H₈); 8.14 (s, 1H, COOH); ¹³C NMR (DMSO-d₆, 75 MHz): δ 120.81, 123.12, 127.49, 129.09, 129.52, 130.28, 130.50, 130.68, 130.99, 133.36, 135.68, 137.33, 137.92, 138.81, 150.31, 158.45, 167.49, 195.70; LC-MS(ESI): 352.0 (M-1).
Methyl 8-benzoyl-2-(4-fluorophenyl)quinoline-4-carboxylate (5b)

Yield: 72%; mp=183-185 °C; IR (KBr): ν (cm⁻¹) 3449, 3073, 2941, 1724.2, 1658.4 (C=O); 1H NMR (300MHz-CDCl₃): δ (ppm) 2.37 (s, 3H, 4-methylphenyl), 3.81 (s, 3H, methoxy), 7.27-7.29 (d, 2H, phenyl H3 &H5, J=9Hz), 7.49 (t, 2H, 4-methylphenyl H3 &H5, J=9Hz), 8.19-8.22 (d, 1H, quinoline H7, J=9Hz), 8.4 (s, 1H, quinoline H8), 8.8 (s, 1H, quinoline H5); 13C NMR (CDCl₃, 75 MHz): δ 21.44, 52.90, 121.03, 121.73, 127.12, 127.15, 127.85, 128.20, 129.43, 129.53, 129.75, 132.61, 133.04, 133.43, 133.95, 135.90, 140.25, 146.89, 155.87, 166.66, 198.37; LC-MS(ESI): 382.2(M+1), 404.2(M+23).

Methyl 6-benzoyl-2-(p-tolyl)quinoline-4-carboxylate (5c)

Yield: 78%; mp=178-180 °C; IR (KBr): ν (cm⁻¹) 3449, 3073, 2941, 1729.61, 1656.83 (C=O); 1H NMR (300MHz-CDCl₃): δ (ppm) 2.25 (s, 3H, CH₃), 4.03 (s, 3H, OCH₃), 7.02-7.04 (d, 2H, 4-methylphenyl H&H₂, J=6Hz), 7.30-7.33 (dd, 2H, phenyl H₂, J=19Hz, J=2.5Hz), 7.43-7.46 (t, 1H, phenyl H₆, J=19Hz), 7.50-7.53 (dd, 2H, phenyl H₂, J=19Hz, J=7.5Hz), 7.59-7.62 (t, 1H, quinoline H₇, J=9Hz), 7.70-7.73 (dd, 2H, 4-methylphenyl H₂&H₆, J=6Hz), 7.81-7.83 (dd, 1H, quinoline H₈, J=6.25Hz), 8.31 (s, 1H, quinoline H₅), 8.79-8.82 (dd, 1H, quinoline H₆, J=9Hz, J=2.5Hz); 13C NMR (CDCl₃, 75 MHz): δ 21.32, 52.88, 119.98, 123.67, 127.12, 127.15, 127.85, 128.20, 129.43, 129.53, 129.75, 132.61, 133.04, 133.43, 133.95, 135.90, 140.25, 146.89, 155.87, 166.66, 198.37; LC-MS(ESI): 382.2(M+1), 404.2(M+23).

Methyl 8-benzoyl-2-(3,4-dimethoxyphenyl)quinoline-4-carboxylate (5d)

Yield: 22%; mp=266-268 °C; IR (KBr): ν (cm⁻¹) 3449, 3073, 2941, 1724.2, 1658.4 (C=O); 1H NMR (300MHz-CDCl₃): δ (ppm) 3.84 (s, 3H, methoxy), 3.81 (s, 3H, methoxy), 4.02 (s, 3H, methoxy), 6.74-6.77 (d,1H,3,4-dimethoxyphenyl H₅, J=9Hz), 7.18-7.21 (d, 1H, quinoline H₇, J=9Hz), 7.28-7.47 (m, 3H, phenyl H&H₂&H₃, J=3,4,4-dimethoxyphenyl H₇, J=9Hz), 7.61-7.64 (t, 1H, quinoline H₉, J=9Hz), 7.74-7.81 (m, 3H, phenyl H₂&H₆&H₇, J=3,4,4-dimethoxyphenyl H₉), 8.31 (s, 1H, quinoline H₈), 8.78-8.81 (dd, 1H, quinoline H₅, J=19Hz, J=2.5Hz); 13C NMR (CDCl₃, 75 MHz): δ 29.72, 55.69, 55.95, 109.78, 110.56, 119.78, 119.87, 123.41, 127.09, 127.71, 128.25, 129.44, 129.92, 130.66, 132.77, 135.42, 138.76, 139.50, 149.30, 150.91, 155.27, 166.68, 198.19; LC-MS(ESI): 428.2(M+1), 451.2(M+23).

Methyl 6-benzoyl-2-phenylquinoline-4-carboxylate (7a)

Yield: 52%; mp=145-147 °C; IR (KBr): ν (cm⁻¹) 1729, 1649 (C=O); 1H NMR (300MHz-CDCl₃): δ (ppm) 3.93 (s, 3H, methoxy), 7.41-7.58 (m, 6H, benzoyl H&H₂&H₃&phenyl H₂&H₃&H₄&H₅&H₆, 1H), 7.82-7.84 (d, 2H, benzoyl H₂&H₆, J=9Hz), 8.11-8.24 (m, 4H, phenyl H₂&H₆&quinoline H₁, H₂, H₃, 1H), 8.4 (s, 1H, quinoline H₅), 9.12 (s, 1H, quinolinine=H); 13C NMR (CDCl₃, 75 MHz): 52.92, 56.05, 56.13, 110.27, 111.06, 120.74, 128.02, 128.67, 129.84, 129.93, 130.35, 130.41, 130.72, 131.72, 136.53, 137.39, 149.59, 150.68, 151.29, 158.15, 166.41, 169.16; LC-MS(ESI): 428.2(M+1), 451.2(M+23).

Biological assays

Cytotoxicity assay

The MTT assay was done by seeding 5.0×10³ human cancer cells per well in 96-well plates (32-41). Following overnight incubation of the cells in 5% CO₂ at
37°C, culture medium of each well was exchanged with medium having reference anticancer drug, cisplatin (0-100 µM) or different concentrations of newly synthesized quinolines (0-100 µM) or ketoprofen. Then cells were incubated for 72 hr. MTT solution (25 µl, 4 mg ml⁻¹) was added to each well and the cells were incubated at 37 °C for 3 hr. Finally, formazan crystals were dissolved in DMSO (100 µl) and absorbance was read in a plate reader (Synergy H4, USA) at 540 nm.

**MDR reversal studies**

The MTT based assay was done by seeding 5000 cancer cells per 180 µl RPMI complete culture medium in each well of 96-well culture. Cisplatin was applied at concentrations of 12.5, 25, 50 and 100 µM in both A2780 and A2780/RCIS cancer cells in absence or presence of highest non-toxic concentrations of synthesized compounds. Cells were then incubated (37 °C in 5% CO₂ incubator) for 48 hr. Then 25 µl of MTT solution (4 mg ml⁻¹) were added to each well and then incubated at 37 °C (3 hr). At the end of incubation, formazan crystals were dissolved in DMSO (100 µl) and plates were read in a plate reader (Synergy H4, USA) at 540 nm. This experiment was done in triplicate determination each time.

**Flow cytometric efflux assay**

Microplates containing 1×10⁶ resistant cells in each well were incubated with 10 µM of 5-CFDA for 60 min. After washing, synthesized compounds were added and the cells were further incubated (60 min). Cells were washed with ice-cold PBS (two times) and harvested. After centrifugation, supernatants were removed and cells suspended in ice-cold PBS. Samples were analyzed by a BD FACS Calibur Flow Cytometer (BD Biosciences, San Jose, USA). Fluorescence intensity of substrate accumulated in the cells was measured with FlowJo 7.6.1 data analysis software (Oregon, USA). Cells treated with ketoprofen were used as controls.

**Molecular modeling**

Mode of interaction between synthesized ligands and homology modeled ABCG2 (MRP2) was investigated by docking. 2D structure of chemicals was organized in Chem Draw Ultra 12.0 software and 3D structures were arranged by Chem Draw Ultra 12.0 software using molecular mechanic force filed pre-optimization monitored by MM2 calculation. Further modification such as polar hydrogen addition was achieved by MOE software. Synthesized chemicals were docked into the binding site of MRP2 by MOE software. The docking simulations were done using triangle matcher placement algorithm with London dG scoring function and force field as refinement method. For each compound, the top-score docking poses were selected for final ligand-target interaction analysis using LigX module in MOE Software.

**Results**

**Synthesis**

A one-step Doebner reaction was used to make 2-arylquinoline-4-carboxylic acid derivatives. As shown in scheme 1, 2- or 4-aminobenzophenone (1), substituted benzaldehyde (2) and pyruvic acid (3) were refluxed in acetic acid to obtain 4-carboxy quinolines (4 and 6) (43) and then esterification of carboxylic acid group was performed using methyl iodide in acetone (43) to afford the novel quinoline-4-methyl esters (5 and 7). The compounds were characterized by nuclear magnetic resonance, infrared spectroscopy and mass spectroscopy.

**Biological evaluation**

**In vitro cytotoxic effects**

Mahdizadeh et al. (44) examined the basic level of the mRNA expression of MRP1 and MRP2 in A2780/RCIS cells and sensitive parental A2780 cell line. They

**Scheme 1.** Reagents and conditions: (a) acetic acid, reflux (b) K₂CO₃, CH₃I, Acetone, reflux
reported that the MRP1 mRNA level in the resistant cell line (A2780/RCIS) was 1.29 times more than its expression level in sensitive cells (A2780 cells). Also, their results displayed that the expression level of MRP2 mRNA in the A2780/RCIS (resistant cell line) was much more (13 times) than the MRP2 mRNA level in parental A2780 cells. To identify ideal MRP inhibitors reversing MDR at non-toxic concentrations, cytotoxicity of the quinoline compounds against parental sensitive A2780 cells and their resistant sublines A2780/RCIS cells which overexpress MRP2 was evaluated by MTT assay. Cisplatin and ketoprofen were selected as controls. Most of our compounds exhibited negligible or much lower cytotoxic effect in both cancer cells. As depicted in Table 1, four quinoline derivatives 5a, 6b, 6c and 7b showed moderate cytotoxic activity with IC\textsubscript{50} in the range of 31.95-84.41 μM. However, the other quinolines did not display cytotoxic activity at concentrations below 100 μM.

**Reversal of MRP -mediated MDR by quinoline derivatives**

The reversal of multidrug resistance by the new quinoline derivatives was evaluated in drug-resistant cancer cell line with overexpression of MRP2 (A2780/RCIS). The multidrug resistant cancer cell lines are remarkably resistant to the corresponding substrate anticancer drugs. We determined the cytotoxicity of cisplatin, in A2780/RCIS, multidrug resistant ovarian carcinoma cells (MRP2-overexpressing ovarian carcinoma cell line) and A2780, drug-sensitive ovarian carcinoma cells. The resulting IC\textsubscript{50} values are shown in Table 2. Our compounds are two groups, the first group is 8-benzoyl quinoline derivatives and the second group which is the isomers of the first group is 6-benzoyl quinoline derivatives. Compounds 4c, 5a, 5b and 5c from the first group and 6d, 7a, 7b and 7d from the second group at 30 μM concentration (almost the highest common non-toxic concentration between all synthetized compounds) exerted MDR reversal, and increased the anticancer activity of cisplatin in the human MRP2 overexpressing cell line A2780/RCIS. Compound 7d from the second group possessing dimethoxy phenyl in position 2 of quinoline exerted the most MDR reversal activity, and enhanced the cytotoxicity of cisplatin more than the other quinolines.

**Biological evaluation of the MRP2 inhibition**

Compounds exerted MDR reversal, and enhanced the cytotoxicity of cisplatin in the human MRP2 overexpressing cell line A2780/RCIS, including 4c, 5a, 5b, 5c (from the first group), 6d, 7a, 7b and 7d (from the second group) were selected to investigate their MRP2 inhibition activity. MRP2 inhibition was evaluated by the determination of the uptake amount of the fluorescent 5-carboxy fluorescein diacetate (5-CFDA) substrate, by A2780/RCIS ovarian carcinoma cells overexpressing MRP2 in the presence of the selected compounds. Compounds from the first group 4c, 5a, 5b, 5c did not show significant MRP2 inhibitory activity at the concentration below 200 μM. Compound 4c showed the most potent MRP2 inhibitory activity in the first group in concentration of 500 μM in a dose-dependent manner (data not shown).

When compounds from the second group tested at the concentration of 30 μM, none of the compounds

---

Table 1. The in vitro antiproliferative activities of quinolines, ketoprofen and cisplatin against A2780 (drug-sensitive ovarian carcinoma cells) and A2780/RCIS (multidrug resistant ovarian carcinoma cells)

| Compound | X | R\textsubscript{1} | R\textsubscript{2} | R\textsubscript{3} | R\textsubscript{4} | A2780 IC\textsubscript{50} (μM) | A2780/RCIS IC\textsubscript{50} (μM) |
|----------|---|-----------------|-----------------|-----------------|-----------------|------------------|------------------|
| 4a       | OH | H               | H               | H               | CO\textsubscript{2}H | >100              | >100              |
| 4b       | OH | H               | F               | H               | CO\textsubscript{2}H | >100              | >100              |
| 4c       | OH | H               | CH\textsubscript{3} | H               | CO\textsubscript{2}H | >100              | >100              |
| 4d       | OH | OCH\textsubscript{3} | OCH\textsubscript{3} | H               | CO\textsubscript{2}H | >100              | >100              |
| 5a       | OCH\textsubscript{2}H | H               | H               | H               | CO\textsubscript{2}H | 67.38±17          | >100              |
| 5b       | OCH\textsubscript{2}H | H               | F               | H               | CO\textsubscript{2}H | >100              | >100              |
| 5c       | OCH\textsubscript{2}H | H               | CH\textsubscript{3} | H               | CO\textsubscript{2}H | >100              | >100              |
| 5d       | OCH\textsubscript{2}H | OCH\textsubscript{3} | OCH\textsubscript{3} | H               | CO\textsubscript{2}H | >100              | >100              |
| 6a       | OH | H               | H               | CO\textsubscript{2}H | H               | >100              | 84.41±2.4         |
| 6b       | OH | H               | F               | CO\textsubscript{2}H | H               | >100              | >100              |
| 6c       | OH | H               | CH\textsubscript{3} | CO\textsubscript{2}H | H               | 75.35±2.9         | >100              |
| 7a       | OCH\textsubscript{2}H | H               | H               | CO\textsubscript{2}H | H               | >100              | >100              |
| 7b       | OCH\textsubscript{2}H | H               | F               | CO\textsubscript{2}H | H               | 31.95±1.07        | >100              |
| 7c       | OCH\textsubscript{2}H | H               | CH\textsubscript{3} | CO\textsubscript{2}H | H               | >100              | >100              |
| 7d       | OCH\textsubscript{2}H | OCH\textsubscript{3} | OCH\textsubscript{3} | CO\textsubscript{2}H | H               | >100              | >100              |
| Ketoprofen |     |                 |                 |                 |                 | >100              | >100              |
| Cisplatin |     |                 |                 |                 |                 | 4.6±0.6           | 57.67±4.6         |

*aCompound concentration required to inhibit tumor cell proliferation by 50%. Data are presented as the mean ± SD from the dose−response curves of three independent experiments*
except 6d and 7d were found to inhibit the efflux of 5-carboxyfluresin diacetate in A2780/RClS cells (data not shown) compound 6d, a 4-carboxy quinoline possessing dimethoxy phenyl in position 2 of quinoline ring, showed the most potent MRP2 inhibition among all the tested quinolines in a dose-dependent manner and more than the reference drug ketoprofen. Surprisingly, compound 7d which exerted the most MDR reversal, and enhanced the cytotoxicity of cisplatin more than the other quinolines did not show the most MRP2 inhibition activity.

Docking studies

As X-ray crystallization of the MRP2 protein is not accessible, the lone structural information existing to date is a bacterial ABC transporter. A previous structure of a bacterial MDR-ABC transporter MsbA has been remoted due to incorrect topological assignments resulted from low resolution of the X-ray diffraction data (45). However, using the obsolete PDB entry a homology model of ABCC2/MRP2 has been built to predict the binding of our quinolines.

Homology modeling of ABCC2 (MRP2)

The 1545-amino acid human ABCC2 (MRP2) contains two nucleotide binding domains (46) and up to 17 transmembrane helices distributed within three transmembrane domains (TMD), 1, 2, and 3. It has been shown that the amino terminal TMD1 of ABCC1 is not essential for substrate transport. So experiments have focused on TMD2 and 3. Sequence for ABCC2 had swiss port entry Q92887. As it was described previously (47) we used lipid flippase MsbA (chain A and B) with Data Bank entry: 1pf4 as a model for TMD-2 and 3. We used MOE2019 for homology modeling with its default settings. We used residues Lys329, Met 440, Ser 444, Gln 447, Ile 476, Ile 479, Gln 543, Cys 544, Val 546, Phe 550, Thr 553, Val 557, Ser 558, Phe 562, Asn 587, Ile 588, Leu 589, Arg 591, Met 595, Met 598, Met 599 as binding site. MOE2019 with its default setting was employed for docking studies (Figure 3).

To explain the results of biological experiments docking studies of ketoprofen, compounds 7d and 6d into the homology-modeled human MRP-2, were carried out (Figure 3). As mentioned above, compound 6d, a 4-carboxy quinoline possessing dimethoxy phenyl in position 2 of quinoline ring, showed the most potent MRP2 inhibition among all the tested quinolines in a dose-dependent manner and more than the reference drug ketoprofen. Studying ligand interaction mode of 6d by LigX module of MOE software revealed that O and

Table 2. The cytotoxicity of cisplatin, in A2780/RClS, multidrug-resistant ovarian carcinoma cells (MRP2-overexpressing ovarian carcinoma cell line) alone or in the presence of compounds

| Compound | A2780/RClS IC<sub>50</sub> (μM) | Compound | A2780/RClS IC<sub>50</sub> (μM) |
|----------|-----------------------------|----------|-----------------------------|
| Cis +4a  | 53.62±3.45                  | Cis +6a  | 64.55±1.7                   |
| Cis +4b  | 63.21±2.96                  | Cis +6b  | 62.84±2.1                   |
| Cis +4c  | 37.34±3.87                  | Cis +6c  | 56.27±3.2                   |
| Cis +4d  | 52.67±4.36                  | Cis +6d  | 35.54±0.9                   |
| Cis +5a  | 45.23±4.12                  | Cis +7a  | 48.06±0.51                  |
| Cis +5b  | 48.34±3.35                  | Cis +7b  | 25.17±1.6                   |
| Cis +5c  | 28.56±5.34                  | Cis +7c  | ND                          |
| Cis +5d  | 54.34±3.95                  | Cis +7d  | 14.88±1.1                   |
| Cisplatin| 57.67±4.61                  |          |                             |

Figure 3. TMD2 (blue) and TMD3 (yellow) model for ABCC2
H atoms of carboxyl group of 6d, could form hydrogen bonds with MET 595 and MET 598 (Figure 4). The O atom of benzoyl group made hydrogen bond with ARG 393. Methoxy groups of 6d can made contact with the backbone of several amino acid residues, like Phe 591 and Phe 550. Compound 7d methyl ester of 6d, interacted less compared to its parent 6d. As shown in Figure 4, the O atom of benzoyl group of 7d made hydrogen bond with ARG 393, the same as that of 6d, but esterification of 6d led to eliminate the hydrogen bonds with MET 595 and MET 598. Ketoprofen also interacted less than its derivatives 6d and 7d. As shown in Figure 4, the O atom of benzoyl group of ketoprofen made hydrogen bond with ARG 393, the same as that of 6d and 7d. O atom of hydroxyl group of ketoprofen, could form hydrogen bonds with MET 595, the same as 6d. Although ketoprofen possess carboxyl group which forms hydrogen bond with target, but its binding energy is more than its derivatives 6d and 7d (Table 3), indicating that the quinoline ring causes the carboxyl group to be placed in a direction that can interact more with the target, and also dimethoxy phenyl ring provided additional interactions with the target.

### Discussion

This study indicates that 6- or 8-benzoyl-2-aryquinoline is a suitable scaffold (template) to design MRP2 inhibitors. The position of benzoyl in quinoline ring is important in inhibition of MRP2. Generally, 8-benzoyl-2-aryquinolines showed more activity compared to their isomers (6-benzoyl-2-aryquinolines). Compound 6d, a 4-carboxy quinoline possessing dimethoxy phenyl in position 2 of quinoline ring, showed the most potent MRP inhibition among all the tested quinolines in a dose-dependent manner and more than the reference drug ketoprofen. MRP2 inhibition activity of compound 7d was less in comparison to that of 6d, indicating that carboxyl group in position 4 of quinoline may interact with MRP2. These hydrophobic interactions and hydrogen bonds formation of compounds with homology modeled MRP2 can describe inhibitory effect of these compounds. Docking studies showed that compound 7d methyl ester of 6d, interacted less compared to its parent 6d, which is consistent with biological results.

### Conclusion

Benzoyl-2-aryquinoline is a suitable template to design MRP2 inhibitors. The position of benzoyl in quinoline ring is important in inhibition of MRP2. Carboxyl group in position 4 of quinoline may interact with MRP2. Docking studies described the biological results and is consistent with biological results.

### Acknowledgment

We are grateful to Research Deputy of Mashhad University of Medical Sciences, Mashhad (Iran) for financial support of this study as part of thesis of Faezeh Fathi.

### Conflicts of Interest

The authors declare that there is no conflict of interests.

### References

1. Siegel RL, Miller KD, Jemal A. CA Cancer J Clin 2015;65:5-29.
2. Cozzi P. The discovery of a new potential anticancer drug: a case history. Farmaco 2003;58:213-220.
3. Liang Xj, Chen C, Zhao Y, Wang PC. Circumventing tumor resistance to chemotherapy by nanotechnology. Methods Mol Biol 2010;596:467-488.
4. Beretta GL, Cassinelli G, Pennati M, Zucolo V, Gatti L. Overcoming ABC transporter-mediated multidrug resistance: The dual role of tyrosine kinase inhibitors as multitargeting agents. Eur J Med Chem 2017;142:271-289.
5. Staud F, Pavek P. Breast cancer resistance protein (BCRP/ABCG2). Int J Biochem Cell Biol 2005;37:720-725.
6. Hosseinzadeh H, Mazaheri F, Ghodsi R. Pharmacological effects of a synthetic quinoline, a hybrid of tomoxiprole and naproxen, against acute pain and inflammation in mice: a behavioral and docking study. Iran J Basic Med Sci 2017;20:446-450.
7. Zarghi A, Afraei S. Selective COX-2 Inhibitors: A Review of Their Structure-Activity Relationships. Iran J Pharm Res 2011;10:655-683.
8. Thun MJ, Henley SJ, Patrono C. Nonsteroidal anti-inflammatory drugs as anticancer agents: mechanistic, pharmacologic, and clinical issues. J Natl Cancer Inst 2002;94:252-266.
9. Perkovic I, Butula I, Kralj M, Martin-Kleiner I, Balzarini J, Hadjipavlou-Litina D, et al. Novel NSAID 1-acyl-4-cycloalkyl/arylsemicarbazides and 1-acyl-5-benzoxyl/hydroxy carbamoylcarbazides as potential anticancer agents and antioxidant. Eur J Med Chem 2012;51:227-238.
10. Amin R, Kamitani H, Sultana H, Taniura S, Islam A, Sho A, et al. Aspirin and indomethacin exhibit antiproliferative effects and induce apoptosis in T98G human glioblastoma cells. Neuril Res 2003;25:370-376.
11. Carrett-Dias M, Votto AP, Figueira Dde M, Almeida DV, Vallochi AL, D’Oca MG, et al. Anti-MDR and antitumoral action of acetylsalicylic acid on leukemic cells. Biosci Rep 2011;31:391-398.
12. Draper MP, Martell RL, Levy SB. Indomethacin-mediated reversal of resistance and drug efflux in human and murine cell lines overexpressing MRP, but not P-glycoprotein. Br J Cancer 1997;75:810-815.
13. Duffy CP, Elliott CJ, O'Connor RA, Heenan MM, Coyle S, Cleary IM, et al. Enhancement of chemotherapeutic drug toxicity to human tumour cells in vitro by a subset of non-stereoidal anti-inflammatory drugs (NSAIDs). Eur J Cancer 1998;34:1250-1259.

14. El-Sheikh AA, van den Heuvel JJ, Koenderink JB, Russel FG. Interaction of nonsteroidal anti-inflammatory drugs with multidrug resistance protein (MRP) 2/ABCC2 and MRP4/ABCC4-mediated methotrexate transport. J Pharmaco Exp Ther 2007;320:229-235.

15. Gruber BM, Bubko L, Krzyzton-Russjan J, Anuszewska EL. Synergistic action of doxorubicin and sulindac in human cervix carcinoma cells - studies on possible mechanisms. Med Sci Mon Int Med J Exp Clin Res 2010;16: 45-51.

16. Maguire AR, Plunkett SJ, Papot S, Clynes M, O'Connor R, Touhey S. Synthesis of indomethacin analogues for evaluation as modulators of MRP activity. Bioorg Med Chem 2001;9:745-462.

17. O'Connor R, Heenan M, Connolly L, Larkin A, Clynes M. Increased anti-tumour efficacy of doxorubicin when combined with sulindac in a xenograft model of an MRP-1-positive human lung cancer. Anticancer Res 2004;24:457-466.

18. O'Connor R, O'Leary M, Ballot J, Collins CD, Kinsella P, Mager DE, et al. Phase I clinical and pharmacokinetic study of the multidrug resistance protein-1 (MRP-1) inhibitor sulindac, in combination with epirubicin in patients with advanced cancer. Cancer Chemother Pharmacol 2007;59:79-87.

19. Roller A, Bahr OR, Streifer J, Winter S, Heneka M, Deininger M, et al. Selective potentiation of drug cytotoxicity by NSAID in human glioma cells: the role of COX-1 and MRP. Biochem Bioph Res Comm 1999;259:600-605.

20. Rosenbaum C, Rohrs S, Muller O, Waldmann H. Modulation of MRP-1-mediated multidrug resistance by indomethacin analogues. J Med Chem 2005;48:1179-1187.

21. Touhey S, O'Connor R, Plunkett S, Maguire A, Clynes M. Structure-activity relationship of indomethacin analogues for MRP-1, COX-1 and COX-2 inhibition. Identification of novel chemotherapeutic drug resistance modulators. Eur J Cancer 2002;38:1661-1670.

22. Zhang L, Liu L, Zheng C, Wang Y, Nie X, Shi D, et al. Synthesis and biological evaluation of novel podothylloptoxin-NSAIDs conjugates as multifunctional anti-MDR agents against resistant human hepatocellular carcinoma Bel-7402/5-FU cells. Eur J Med Chem 2017;131:81-91.

23. Anuchapreeda S, Thanaranattakorn P, Sittipreechacharn S, Timo S, Chanarat P, Limtrakul P. Inhibitory effect of curcumin on MDR1 gene expression in patient leukemic cells. Arch Pharm Res 2006;29:866-873.

24. Nakamichi N, Ishimoto T, Yamachi Y, Masuo Y, Kato Y. Screening to identify multidrug resistance-associated protein inhibitors with neuroblastoma-selective cytotoxicity. Biol Pharm Bull 2016;39:1638-1645.

25. O’CONNOR R. The pharmacology of cancer resistance. Anticancer Res 2007;27:1267-1272.

26. Elsheikh AA, van den Heuvel JJ, Koenderink JB, Russel FG. Interaction of nonsteroidal anti-inflammatory drugs with multidrug resistance protein (MRP) 2/ABCC2 and MRP4/ABCC4-mediated methotrexate transport. J Pharmaco Exp Ther 2007;320:229-235.

27. Rijpma SR, van den Heuvel JJ, van der Velden M, Sauerwein RW, Russel FG, Koenderink JB. Atovaquone and quinone antimalarials inhibit ATP binding cassette transporter activity. Malar J 2014;13:359-367.

28. Nakamura T, Oka M, Aizawa K, Soda H, Fukuda M, Terashi K, et al. Direct interaction between a quinoline derivative, MS-209, and multidrug resistance protein (MRP) in human gastric cancer cells. Biochem Bioph Res Comm 1999;255:618-624.

29. Wu CP, Klokouzas A, Hladky SB, Ambudkar SV, Barrand MA. Interactions of melfoxime with ABC proteins, MRP1 (ABCC1) and MRP4 (ABCC4) that are present in human red cell membranes. Biochem Pharmacol 2005;70:500-510.

30. Gekeler V, Ise W, Sanders KH, Ulrich WR, Beck J. The leukotriene LTD4 receptor antagonist MK571 specifically modulates MRP associated multidrug resistance. Biochem Biophys Res Comm 1995;208:345-352.

31. Karthikeyan C, Malla R, Ashby CR, Jr., Amawi H, Abbott KL, Moore J, et al. Pyrimidol[1' ,2' :2,3']pyrazolo[3,4-b]quinolines: Novel compounds that reverse ABCG2-mediated resistance in cancer cells. Cancer Lett 2016;376:118-126.

32. Behbahanis F, Tabeshpour J, Mirzaei S, Golmakanyooin S, Tayarani-Najaran Z, Ghasemi A, et al. Arch Pharm 2019;352:1800307–1800318.

33. Ghosdi R, Azizi E, Ferlin MG, Pezzi V, Zarghi A, Design, synthesis and biological evaluation of 4-(imidazolylmethyl)-2-arylquinoline derivatives as aromatase inhibitors and anti-breast cancer agents. Lett Drug Des Discov 2016;13:89-97.

34. Ghosdi R, Azizi E, Zarghi A. Design, synthesis and biological evaluation of 4-(imidazolylmethyl)-2-(4-methylsulfonylphenyl)-quinoline derivatives as selective COX-2 inhibitors and in-vitro anti-breast cancer agents. Iran J Basic Med Sci 2016;19:15-16.

35. Golmakanyooin S, Askari VR, Abnous K, Zarghi A, Ghosdi R. Synthesis, characterization and in-vitro evaluation of novel naphthoquinone derivatives and related imines: Identification of new anti cancer leads. Iran J Pharma Res 2019;18:16-29.

36. Jafari F, Baghaya H, Lavaeep E, Hadizadeh F, Soltani F, Moallemzadeh H, et al. Design, synthesis and biological evaluation of novel benzo- and tetrahydrobenzo-[h]quinoline derivatives as potential DNA-intercalating anti-tumor agents. Eur J Med Chem 2019;146:292-303.

37. Karimikia E, Behravan J, Zarghi A, Ghandadi M, Malayeri SO, Ghosdi R. Colchicine-like β-acetamidoketones as inhibitors of microtubule polymerization: Design, synthesis and biological evaluation of in vitro anti-cancer activity. Iran J Basic Med Sci 2019;22:1138-1146.

38. Malayeri SO, Abnous K, Arab A, Akaberi M, Mehris S, Zarghi A, et al. Design, synthesis and biological evaluation of 7-(aryl)-2,3-dihydro-[1,4]dioxino[2,3-g]-quinoline derivatives as potential Hsp90 inhibitors and anti-cancer agents. Bioorg Med Chem 2017;25:1294-1302.

39. Malayeri SO, Tayarani-Najaran Z, Behbahani FS, Rashidi R, Delpazir S, Ghosdi R. Synthesis and biological evaluation of benzox[furo][3,4-e][1,4]diazepin-1-one derivatives as potential anticancer agents. Bioorg Chem 2018;80:631-638.

40. Mirzaei S, Eisvand F, Hadizadeh F, Mosaffa F, Ghosodi A, Ghosdi R. Design, synthesis and biological evaluation of novel 5,6,7-trimethoxy-N-aryl-2-styrylquinolin-4-amines as potential anticancer agents and tubulin polymerization inhibitors. Bioorg Chem 2020;98:103711.

41. Mirzaei S, Hadizadeh F, Eisvand F, Mosaffa F, Ghosdi R. Synthesis, structure-activity relationship and molecular docking studies of novel quinoline-chalcone hybrids as potential anticancer agents and tubulin inhibitors. J Mol Struct 2020;1202: 127310.

42. Zarghi A, Ghosdi R. Design, synthesis, and biological evaluation of ketoprofen analogs as potent cytochrome-p450-2 inhibitors. Bioorg Med Chem 2010;18:5585-5592.

43. Aboutorabzadeh SM, Mosaffa F, Ghasemi A, Ghosdi R. Design, synthesis, and biological evaluation of 6-methoxy-2-arylquinolines as potential P-glycoprotein inhibitors. Iran J Basic Med Sci 2018;21:9-18.

44. Mahdizadeh S, Karimi G, Behravan J, Arabzadeh S, Lage H, Kalalinia F. Crocin suppresses multidrug resistance in MRP...
overexpressing ovarian cancer cell line. Daru 2016;24:17-24.
45. Xing L, Hu Y, Lai Y. Advancement of structure-activity relationship of multidrug resistance-associated protein 2 interactions. AAPSJ 2009;11:406-413.
46. Nies AT, König J, Cui Y, Brom M, Spring H, Keppler D. Structural requirements for the apical sorting of human multidrug resistance protein 2 (ABCC2). Eur J Biochem 2002;269:1866-1876.
47. Williamson G, Aeberli I, Miguet L, Zhang Z, Sanchez MB, Crespy V, et al. Interaction of positional isomers of quercetin glucuronides with the transporter ABCC2 (cMOAT, MRP2). Drug Metab Dispos 2007;35:1262-1268.