Medicinal Chemistry Research (2019) 28:1182–1196
https://doi.org/10.1007/s00044-019-02363-z

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and in vitro evaluation of substituted tetrahydroquinoline-isoxazole hybrids as anticancer agents

Fausto M. Güiza[1] · Yenny Bueno Duarte[1,2] · Stelia C. Mendez-Sanchez[1,2] · Arnold R. Romero Bohórquez[1]

Received: 3 March 2019 / Accepted: 20 May 2019 / Published online: 1 June 2019

Abstract

A series of isoxazole linked to 4-(2-oxopyrrolinidyl-1)-tetrahydroquinoline derivatives was efficiently synthesized. The synthetic route started with the formation of the corresponding *N*-propargyl tetrahydroquinoline derivatives via cationic Povarov reaction. Tetrahydroquinoline-isoxazole hybrid systems (**3a**–**p**) were obtained with good yields (42–88%) through a 1,3-dipolar cycloaddition reaction with a click chemistry approach. These compounds have been tested for their in vitro cytotoxic activity against four different human cancer cell lines, lung (A549), liver (HepG2), and melanoma murine (B16F10) using the conventional MTT assay. Among all tetrahydroquinoline-isoxazole hybrids synthesized, compounds **3a**, **3e**, **3j**, and **3m** showed promising in vitro activity against HepG2 cancer cell line with considerable selectivity. Compounds **3a** ($IC_{50} = 6.80$ µM, SI = 14.7) and **3j** ($IC_{50} = 5.20$ µM, SI > 16.1) exhibited the highest cytotoxic effect. The death pathway related to cytotoxicity of the compound **3j** showed necrotic characteristics selectively on the tumor cell line, also showed an improved in vitro activity against the tested reference drug (*oxaliplatin*), without significant affectation on the viability of hepatocytes. In general, results suggested that these type of hybrid compounds might have therapeutic potential in future investigations on hepatocellular carcinoma.

Keywords Tetrahydroquinoline · Isoxazole · Cationic Povarov reaction · 1,3-dipolar cycloaddition · Hybrids

Introduction

Cancer is a multifactorial disease to arise when cells are dividing at an uncontrollable rate. This disease has been characterized by the presence of mutations, independent cell proliferation of mitogen, high genetic instability and invasion of other tissues (Cooper and Husman 2007; Abdel Rahman et al. 2013; Hensley et al. 2016). The most common types of cancer treatment are surgery, chemotherapy, radiation therapy, targeted therapy, and immunotherapy, though almost all patient receive chemotherapy. Several chemotherapeutic drugs have been developed to treat cancer that includes DNA-alkylating agents as *oxaliplatin*, antimetabolites as *gemcitabine*, (Ishiguro and Toi 2012) antitumor antibiotics as *doxorubicin*, antimitotic agents as *paclitaxel* (Reyes-Habito and Roh 2014). *Oxaliplatin* is commonly used as reference drug due that it is a platinum-based chemotherapeutic agent with in vitro and in vivo efficacy against many tumor cell lines, including some that are resistant to *cisplatin* and *carboplatin* (Raymond et al. 1998). However, the effectiveness of chemotherapy has suffered diminution by many factors including systemic toxicity due to lack of specificity, rapid drug metabolism, high treatment cost and both intrinsic and acquired drug resistance (Alfarouk et al. 2015; Tartarone et al. 2013; Zhu et al. 2013). The difficulties encountered in the treatment available demonstrate the importance of design and development of novel therapeutic molecules.

Hybrid compounds are new chemical entities that combined two different heterocyclic moieties conjugating them

These authors contributed equally: Yenny Bueno Duarte, Fausto M. Güiza

Supplementary Information The online version of this article (https://doi.org/10.1007/s00044-019-02363-z) contains supplementary material, which is available to authorised users.

✉ Arnold R. Romero Bohórquez
arafrom@uis.edu.co

[1] Grupo de Investigación en Compuestos Orgánicos de Interés Medicinal (CODEIM), Parque Tecnológico Guatiguará, Universidad Industrial de Santander, Piedecuesta, Bucaramanga A. A. 678, Colombia

[2] Grupo de investigación en Bioquímica y Microbiología (GIBIM), Parque Tecnológico Guatiguará, Universidad Industrial de Santander, Piedecuesta, Bucaramanga A.A. 678, Colombia

into a single skeleton. Their design is perhaps one of the most rational and relevant approaches to develop or improve the drug efficiency in the last decade (Muregi and Ishih 2010). Such compounds may show synergy in the pharmacological effect of some of its heterocyclic fragments, besides delaying the potential development of drug-resistance and consequently to reduce of costs for the design-oriented to specific therapeutic targets of novel drugs. There is an important number of these hybrid compounds which have presented important biological properties including anticancer (Kerru et al. 2017), antifungal (Fang et al. 2017), antimalarial (Agarwal et al. 2017), and antileishmanial activity (Sangshetti et al. 2015).

On the other hand, tetrahydroquinoline compounds play an important role in bioorganic and medicinal chemistry (Sridharan et al. 2011; Kumar et al. 2009). This structural scaffold is present in many agents of pharmaceutical interest and exhibit a wide range of biological activities, such as anti-inflammatory (Liu et al. 2009), neurotropic (Goli et al. 2017), antibiotic (Asolkar et al. 2004), antipsychotic (Singer et al. 2005), antitubercular (Kumar et al. 2011), estrogenic receptors (Chen et al. 2007), and anti-oxidants (Rudenko et al. 2014), among others. Besides, several studies have reported cytotoxic activity of derivatives tetrahydroquinolines on lymphomas, liver, breast, and lung cancer cells (Hou et al. 2013, Alqasoumi et al. 2010; Lam et al. 2013; Faidallah and Rostom 2013). In the same vein, isoxazole moiety is present in many bioactive compounds (Sysak and Obmińska-Mrukowicz 2017; Zhang et al. 2018). Some isoxazole derivatives have shown biological potential as antimicrobial agents (Darwish et al. 2014; Chauhan et al. 2012), anticonvulsant (Eddington et al. 2002), anti-inflammatory (Palusa et al. 2011), antituberculosis (Palanisamy et al. 2013), and anti-depressant (Liu et al. 2011). Moreover, for this kind of compounds the cytotoxicity and antiproliferative activity versus to cervix, colon, lung, melanoma, ovary, breast, prostate, kidney, and leukemia tumor cell lines, have been established (Veeraswamy et al. 2012; Kumbhare et al. 2012; Palanisamy et al. 2013; Cirrincione et al. 2010). It is worth mentioning that, for some isoxazole derivatives the arrest cell cycle and the inhibitory effect on membrane receptors have been determined (Al-Sanea et al. 2013). Additionally, some compounds proved out to be important in the processes of cell differentiation and proliferation, as well as, in the regulation of cell cycle and apoptosis in cancer cells (Kamal et al. 2011; Palanisamy et al. 2013; Cirrincione et al. 2010).

In continuation of the efforts for synthesize novel bioactive and selective heterocycles (Fonseca-Berzal et al. 2013; Romero Bohórquez et al. 2012), specially tetrahydroquinoline-isoxazole hybrids (Bueno et al. 2018; Álvarez Santos et al. 2019), in this work sixteen (16) tetrahydroquinoline-isoxazole hybrid compounds (THQ-isoxazole) were synthesized. In vitro cytotoxicity activity against different cancer cell lines, including human lung (A549), human liver (HepG2), melanoma murine (B16F10), cervical (HeLa), and one normal cell line (kidney VERO) were evaluated. In addition, for the compound with the highest cytotoxic effect was possible to establish the more probable mechanism of cell death.

Materials and methods

Chemistry

All reagents were purchased from Merck, J.T. Baker, and Sigma Aldrich Chemical Co. and used without further purification. Reaction progress was monitored using thin layer chromatography on PF254 TLC aluminum sheets from Merck. Column chromatography was performed using Silica gel (60–120 mesh) and solvents employed were of analytical grade. Melting points (uncorrected) were determined using a Fisher-Johns melting point apparatus. IR spectra were recorded on an FT-IR Bruker Tensor 27 spectrophotometer coupled to Bruker platinum ATR cell. Mass spectrometry ESI-MS analyses were conducted on an ESI-IT Amazon X (Bruker Daltonics) with direct injection, operating in Full Scan at 300 °C and 4500 V in the capillary, using nitrogen as nebulizer gas with reflux of 8 L min^{-1} and 30 psi. The elemental analysis of the different compounds was performed in ThermoScientific CHNS-O analyzer equipment (Model Flash 2000).

Nuclear magnetic resonance (NMR) spectra (^{1}H and ^{13}C) were measured on a Bruker Ultrashield-400 spectrometer (400 MHz ^{1}H NMR and 100 MHz ^{13}C NMR), using $CDCl_3$ as solvent and TMS as reference. *J* values are reported in Hz; chemical shifts are reported in ppm (δ) relative to the solvent peak (residual $CHCl_3$ in $CDCl_3$ at 7.26 ppm for protons). Signals were designated as follows: s, singlet; d, doublet; dd, doublet of doublets; ddd, doublet of doublets of doublets; t, triplet; td, triplet of doublets; q, quartet; m, multiplet, and br, broad.

General procedure for the synthesis of molecular hybrid Tetrahydroquinoline/Isoxazole 3a–p

The first synthetic step for obtaining the tetrahydroquinoline/isoxazole hybrids of interest consisted in the preparation of the corresponding N-propargyl tetrahydroquinolines **1a–d** via the three-component cationic Povarov reaction. Hybrids were synthesized following the previous method described by Rodríguez et al. (2016). Generated stable and easily isolable compounds, a solution of the corresponding N-propargyl-4-(2′-oxopirrolidin-1′-il)-1,2,3,4-tetrahydroquinolines (1 mmol) and the respective aldoximes **2a**–

d (3 mmol) in dichloromethane (10 mL), were poured into a 50 mL round-bottom flask and vigorously stirred at room temperature for 10 min. Then, triethylamine (TEA) (0.1 mmol) was added and the reaction was placed in an ice bath until the temperature dropped to 0 °C, subsequently, 8 mL of solution 10% w/v of NaOCl (8.6 mmol) was added drop by drop to the mixture. The reaction was monitored by TLC and after 4–6 h, the mixture was diluted with water (30 mL) and extracted with ethyl acetate (3 × 20 mL). The organic phase was separated and dried over Na_2SO_4, and concentrated in vacuo. Resulting products were purified using column chromatography (silica gel, petroleum ether-ethyl acetate).

5-((4′-(2″-oxopyrrolidin-1″-yl)-3′,4′-dihydroquinolin-1′(2′H)-yl)methyl)-3-phenylisoxazole (3a) Orange oil; IR (ATR) ν_{max}: 3465.2, 2931.2, 1670.0, 1497.2, 1438.9, 1167.6, 1017.5, 665.2 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 2.00 (2H, m, H-4″), 2.14 (2H, m, H-3′), 2.49 (2H, m, H-3″), 3.19 (2H, m, H-5″), 3.50 (2H, m, H-2′), 4.56 (1H, d, J = 17.5 Hz, Ha-11′), 4.62 (1H, d, J = 17.5 Hz, Hb-11′), 5.41 (1H, dd, J = 9.2, 5.4 Hz, H-4′), 6.37 (1H, s, H-4), 6.66 (1H, d, J = 8.6 Hz, H-8′), 6.70 (1H, dd, J = 7.4, 1.0 Hz, H-5′), 6.89 (1H, td, J = 7.4, 1.1 Hz, H-6′), 7.10 (1H, tdd, J = 7.8, 1.6, 0.7 Hz, H-7′), 7.42 (3H, m, H_{Ar}-3, H_{Ar}-4, H_{Ar}-5), 7.74 (2H, m, H_{Ar}-2, H_{Ar}-6); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 18.4 (C-4″), 26.7 (C-3′), 31.5 (C-3″), 43.8 (C-5″), 47.7 (C-11′), 48.0 (C-4′), 48.2 (C-2′), 100.4 (C-4), 112.0 (C-8′), 117.1 (C-6′), 120.1 (C-4a′), 126.9 (C_{Ar}-2, C_{Ar}-6), 127.9 (C-5′), 128.8 (C-7′), 128.8 (C_{Ar}-1), 129.0 (C_{Ar}-3, C_{Ar}-5), 130.2 (C_{Ar}-4), 145.0 (C-8a′), 162.5 (C-3), 170.0 (C-5), 175.7 (C-2″); MS (ESI-IT) *m/z*: 289.1 $[M\text{-}C_4H_8NO]^+$, 374.1 $[M+H]^+$, 396.1 $[M+Na]^+$, 769.1 $[2M+Na]^+$; Anal. calcd for $C_{23}H_{23}N_3O_2$: C, 73.97; H, 6.21; N, 11.25%. Found: C, 73.49; H, 6.34; N, 11.25%.

3-(4-methoxyphenyl)-5-((4′-(2″-oxopyrrolidin-1″-yl)-3′,4′-dihydroquinolin-1′(2′H)-yl)methyl)isoxazole (3b) Reddish oil; IR (ATR) ν_{max}: 3442.9, 2961.2, 1672.5, 1422.3, 1250.9, 1017.8, 834.9, 531.9 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 2.04 (2H, m, H-4″), 2.12 (2H, m, H-3′), 2.48 (2H, td, J = 7.7, 1.0 Hz, H-3″), 3.18 (2H, m, H-5″), 3.48 (2H, m, H-2′), 3.81 (3H, s, 4-OCH_3), 4.53 (1H, d, J = 17.7 Hz, Ha-11′), 4.59 (1H, d, J = 17.7 Hz, Hb-11′), 5.40 (1H, dd, J = 9.4, 5.6 Hz, H-4′), 6.32 (1H, s, H-4), 6.64 (1H, d, J = 8.5 Hz, H-8′), 6.68 (1H, dd, J = 7.4, 0.9 Hz, H-5′), 6.88 (1H, td, J = 7.7, 1.4 Hz, H-6′), 6.92 (2H, d, J = 8.9 Hz, H_{Ar}-3, H_{Ar}-5), 7.09 (2H, td, J = 7.4, 1.2 Hz, H-7′), 7.67 (2H, d, J = 8.9 Hz, H_{Ar}-2, H_{Ar}-6); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 18.3 (C-4″), 26.6 (C-3′), 31.5 (C-3″), 43.8 (C-5″), 47.6 (C-11′), 47.9 (C-4′), 48.1 (C-2′), 55.4 (4-O$\underline{C}H_3$), 100.1 (C-4), 111.9 (C-8′), 114.2 (C_{Ar}-2, C_{Ar}-6), 117.6 (C-6′), 120.0 (C-4a′), 121.3 (C_{Ar}-1), 127.8 (C-5′), 128.2 (C_{Ar}-3, C_{Ar}-5), 128.7 (C-7′), 145.0 (C-8a′), 161.0 (C_{Ar}-4), 162.0 (C-3), 169.7 (C-5), 175.7 (C-2″); MS (ESI-IT) *m/z*: 426.1 $[M+Na]^+$, 829.1 $[2M+Na]^+$; Anal. calcd for $C_{24}H_{25}N_3O_3$: C, 71.44; H, 6.25; N, 10.41%. Found: C, 71,03; H, 6.11; N, 10.12%.

5-((4′-(2″-oxopyrrolidin-1″-yl)-3′,4′-dihydroquinolin-1′(2′H)-yl)methyl)-3-(3,4,5-trimethoxyphenyl)isoxazole (3c) Dark brown oil; IR (ATR) ν_{max}: 3437.0, 2939.4, 1668, 1421.5, 1236.3, 11245.6, 1001.0. 842.9 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 2.07 (4H, m, H-4″, H-3′), 2.50 (2H, m, H-3″), 3.24 (2H, m, H-5″), 3.53 (2H, m, H-2′), 3.87 (3H, s, 4-OCH_3), 3.91 (6H, s, 3-OCH_3, 5-OCH_3), 4.51 (1H, d, J = 16.7 Hz, Ha-11′), 4.64 (1H, d, J = 16.7 Hz, Hb-11′), 5.41 (1H, m, H-4′), 6.32 (1H, s, H-4), 6.56 (1H, d, J = 7.1 Hz, H-5′), 6.64 (1H, d, J = 10.1 Hz, H-8′), 6.83 (1H, m, H-7′), 6.99 (2H, m, H_{Ar}-2, H_{Ar}-6), 7.29 (1H, m, H-6′); ^{13}C NMR (100 MHz, $CDCl_3$) (ppm): 18.3 (C-4″), 26.4 (C-3′), 31.2 (C-3″), 43.5 (C-5″), 47.8 (C-11′), 48.4 (C-4′), 50.0 (C-2′), 56.4 (3-O$\underline{C}H_3$, 5-O$\underline{C}H_3$), 61.0 (4-O$\underline{C}H_3$), 100.5 (C-4), 104.0 (C_{Ar}-2, C_{Ar}-6), 113.2 (C-8′), 122.0 (C-6′), 122.5 (C_{Ar}-1), 124.1 (C-4a′), 127.1 (C-5′), 128.6 (C-7′), 139.6 (C_{Ar}-4), 143.5 (C-8a′), 153.7 (C_{Ar}-3, C_{Ar}-3), 162.4 (C-3), 169.5 (C-5), 175.0 (C-2″); MS (ESI-IT) *m/z*: 486.0 $[M+Na]^+$, 520.1 $[M+Cl+Na]^+$; Anal. calcd for $C_{26}H_{29}N_3O_5$: C, 67.37; H, 6.31; N, 9.07%. Found: C, 67.78; H, 6.17; N, 8.81%.

5-((6′-methyl-4′-(2″-oxopyrrolidine-1″-yl)-3′,4′-dihydroquinolin-1′(2′H)-yl)methyl)-3-phenylisoxazole (3d) Reddish oil, IR (ATR) ν_{max}: 3374.9, 2952.5, 1667.7, 1421.3, 1285.8, 1093.9, 905.4. 694.7 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 2.03 (2H, m, H-4″), 2.20 (2H, m, H-3′), 2.19 (3H, s, 6′-CH_3), 2.50 (2H, m, H-3″), 3.19 (2H, m, H-5″), 3.45 (2H, m, H-2′), 4.54 (1H, d, J = 17.4 Hz, Ha-11′), 4.59 (1H, d, J = 17.4 Hz, Hb-11′), 5.38 (1H, dd, J = 9.0, 5.4 Hz, H-4′), 6.36 (1H, s, H-4), 6.58 (1H, d, J = 8.4 Hz, H-8′), 6.71 (1H, d, J = 1.8 Hz, H-5′), 6.91 (1H, dd, J = 8.4, 2.1 Hz, H-7′), 7.42 (3H, m, H_{Ar}-3, H_{Ar}-5, H_{Ar}-4), 7.74 (2H, m, H_{Ar}-2, H_{Ar}-6); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 18.4 (C-4″), 20.5 (6′-$\underline{C}H_3$), 27.0 (C-3′), 31.6 (C-3″), 43.9 (C-5″), 47.87 (C-11′), 47.8 (C-4′), 48.2 (C-2′), 100.4 (C-4), 112.2 (C-8′), 120.2 (C-6′), 126.9 (C_{Ar}-2, C_{Ar}-6), 127.0 (C-4a′), 128.5 (C-5′), 128.9 (C_{Ar}-1), 129.0 (C_{Ar}-3, C_{Ar}-5), 129.4 (C-7′), 130.1 (C_{Ar}-4), 142.8 (C-8a′), 162.5 (C-3), 170.2 (C-5), 175.7 (C-2″); MS (ESI-IT) *m/z*: 410.1 $[M+Na]^+$, 797.2 $[2M+Na]^+$; Anal. calcd for $C_{24}H_{25}N_3O_2$: C, 74.39; H, 6.50; N, 10.84%. Found: C, 74.78; H, 6.37; N, 10.61%.

3-(4-methoxyphenyl)-5-((6′-methyl-4′-(2″-oxopyrrolidin-1″-yl)-3′,4′-dihydroquinolin-1′(2′H)-yl)methyl)isoxazole (3e) Reddish oil; IR (ATR) ν_{max}: 3367.6, 2957.8, 1664.5, 1425.3, 1251.8, 1176.5, 839.0 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.92 (4H, m, H-3′, H-4″), 2.21 (2H, s, 6′-

CH_3), 2.48 (2H, m, H-3″), 3.08 (2H, m, H-5″), 3.31 (2H, m, H-2′), 3.84 (3H, s, 4-OCH_3), 4.31 (1H, d, $J = 16.5$ Hz, Ha-11′), 4.38 (1H, d, $J = 16.5$ Hz, Hb-11′), 5.36 (1H, dd, $J =$ 9.2, 7.2 Hz, H-4′), 6.59 (1H, s, H-4), 6.71 (1H, d, $J =$ 1.8 Hz, H-5′), 6.87 (1H, m, H-8′), 6.94 (2H, dd, $J = 6.1$, 1.9 Hz, H_{Ar}-3, H_{Ar}-5), 7.10 (1H, d, $J = 1.9$ Hz, H-7′), 7.72 (2H, dd, $J = 6.1$, 2.1 Hz, H_{Ar}-2, H_{Ar}-6); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 18.2 (C-4″), 20.6 (6′-$\underline{C}H_3$), 26.3 (C-3′), 31.2 (C-3″), 43.0 (C-5″), 47.0 (C-11′), 47.7 (C-4′), 48.7 (C-2′), 55.4 (4-O$\underline{C}H_3$), 100.7 (C-4), 114.3 (C_{Ar}-2, C_{Ar}-6), 121.5 (C_{Ar}-1), 126.9 (C-8′), 127.7 (C-4a′), 128.2 (C_{Ar}-3, C_{Ar}-5), 129.1 (C-7′), 130.7 (C-5′), 133.4 (C-6′), 142.2 (C-8a′), 162.0 (C_{Ar}-4), 162.2 (C-3), 171.1 (C-5), 175.8 (C-2″); MS (ESI-IT) *m/z*: 474.1 $[M+Cl+Na]^+$, 925.1 $[2M+2Cl+Na]^+$; Anal. calcd for $C_{25}H_{27}N_3O_3$: C, 71.92; H, 6.52; N, 10.06%. Found: C, 73.72; H, 6.68; N, 9.86%.

5-((6′-methyl-4′-(2″-oxopyrrolidin-1″-yl)-3′,4′-dihydroquinolin-1′(2′H)-yl)methyl)-3-(3,4,5-trimethoxyphenyl)isoxazole (3f) Reddish oil; IR (ATR) ν_{max}: 3392.7, 2935.6, 1668.4, 1421.5, 1124.5, 1001.0, 524.9 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 2.00 (2H, m, H-4″), 2.15 (2H, m, H-3′), 2.19 (3H, s, 6′-CH_3), 2.51 (2H, m, H-3″), 3.20 (2H, m, H-5″), 3.48 (2H, m, H-2′), 3.86 (3H, s, 4-OCH_3), 3.90 (6H, s, 3-OCH_3, 5-OCH_3), 4.51 (1H, d, $J = 17.6$ Hz, Ha-11′), 4.62 (1H, d, $J = 17.6$ Hz, Hb-11′), 5.4 (1H, dd, J = 9.2, 5.6 Hz, H-4′), 6.32 (1H, s, H-4), 6.56 (1H, d, $J =$ 8.4 Hz, H-8′), 6.7 (1H, d, $J = 2.0$ Hz, H-5′), 6.91 (1H, dd, J = 8.4, 2.0 Hz, H-7′), 6.96 (2H, s, H_{Ar}-2, H_{Ar}-6); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 18.4 (C-4″), 20.5 (6′-$\underline{C}H_3$), 26.9 (C-3′), 31.6 (C-3″), 43.8 (C-5″), 48.0 (C-4′), 48.0 (C-11′), 48.4 (C-2′), 56.4 (3-O$\underline{C}H_3$, 5-O$\underline{C}H_3$), 61.1 (4-O$\underline{C}H_3$), 100.3 (C-4), 104.1 (C_{Ar}-2, C_{Ar}-6), 112.2 (C-8′), 120.2 (C_{Ar}-1), 124.6 (C-4a′), 128.3 (C-5′), 129.4 (C-7′), 133.6 (C-6′), 139.6 (C_{Ar}-4), 142.8 (C-8a′), 153.6 (C_{Ar}-3, C_{Ar}-5), 162.4 (C-3), 170.3 (C-5), 175.7 (C-2″); MS (ESI-IT) *m/z*: 391.1 $[M-C_4H_7NO]^+$, 478.2 $[M+H]^+$, 500.2 $[M+Na]^+$, 539.1 $[M+C_2H_8CONa]^+$, 977.3 $[2M+Na]^+$; Anal. calcd for $C_{27}H_{31}N_3O_5$: C, 67.91; H, 6.54; N, 8.80%. Found: C, 68.33; H, 6.69; N, 9.04%.

5-((6′-methoxy-4′-(2″-oxopyrrolidin-1″-yl)-3′,4′-dihydroquinolin-1′(2′H)-yl)methyl)-3-phenylisoxazole (3g) Dark brown oil; IR (ATR) ν_{max}: 3393.5, 2949.2, 1661.4, 1502.8, 1421.3, 1285.3, 1039.9, 769.9 cm^{-1}; **^{1}H** NMR (400 MHz, $CDCl_3$) δ (ppm): 2.00 (2H, m, H-4″), 2.13 (2H, m, H-3′), 2.48 (2H, m, H-3″), 3.18 (2H, m, H-5″), 3.42 (2H, m, H-2′), 3.68 (3H, s, 6′-OCH_3), 4.49 (1H, d, $J = 17.4$ Hz, Ha-11′), 4.57 (1H, d, $J = 17.4$ Hz, Hb-11′), 5.39 (1H, dd, $J = 9.3$, 5.6 Hz, H-4′), 6.35 (1H, s, H-4), 6.49 (1H, dd, $J = 2.9$, 0.7 Hz, H-5′), 6.61 (1H, d, $J = 9.0$ Hz, H-8′), 6.69 (1H, dd, $J = 8.9$, 2.9 Hz, H-7′), 7.42 (3H, m, H_{Ar}-3, H_{Ar}-4, H_{Ar}-5), 7.73 (2H, m, H_{Ar}-2, H_{Ar}-6); **^{13}C** NMR (100 MHz, $CDCl_3$) δ (ppm): 18.4 (C-4″), 26.8 (C-3′), 31.4 (C-3″), 43.7 (C-5″), 48.7 (C-4′), 48.2 (C-11′), 48.4 (C-2′), 55.8 (6′-O$\underline{C}H_3$), 100.4 (C-4), 113.5 (C-8′), 113.7 (C-5′), 114.1 (C-7′), 121.8 (C_{Ar}-1), 126.8 (C_{Ar}-2, C_{Ar}-6), 128.8 (C-4a′), 128.9 (C_{Ar}-3, C_{Ar}-5), 130.1 (C_{Ar}-4), 139.4 (C-8a′), 152.1 (C-6′), 162.5 (C-3), 170.2 (C-5), 175.7 (C-2″); MS (ESI-IT) *m/z*: 426.1 $[M+Na]^+$, 829.1 $[2M+Na]^+$; Anal. calcd for $C_{24}H_{25}N_3O_3$: C, 71.44; H, 6.25; N, 10.41%. Found: C, 71.08; H, 6.39; N, 10.13%.

3-(4-methoxyphenyl)-5-((6′-methoxy-4′-(2″-oxopyrrolidin-1″-yl)-3′,4′-dihydroquinolin-1′(2′H)-yl)methyl)isoxazole (3h) Dark red oil; IR (ATR) ν_{max}: 3398.5, 2937.5, 1662.6, 1427.3, 1249.8, 1174.6, 1026.1, 837.1 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 2.00 (2H, m, H-4″), 2.12 (2H, m, H-3′), 2.48 (2H, m, H-3″), 3.09 (2H, m, H-5″), 3.42 (2H, m, H-2′), 3.69 (3H, s, 6′-OCH_3), 3.82 (3H, s, 4-OCH_3), 4.48 (1H, d, $J = 17.6$ Hz, Ha-11′), 4.56 (1H, d, $J = 17.6$ Hz, Hb-11′), 5.36 (1H, dd, $J = 8.9$, 6.5 Hz, H-4′), 6.29 (1H, s, H-4), 6.49 (1H, d, $J = 0.9$ Hz, H-5′), 6.61 (1H, d, $J = 8.9$ Hz, H-8′), 6.70 (1H, dd, $J = 8.9$, 2.7, Hz, H-7′) 6.92 (2H, dd, $J =$ 6.8, 2.1 Hz, H_{Ar}-3, H_{Ar}-5), 7.67 (2H, dd, $J = 6.8$, 2.1 Hz, H_{Ar}-2, H_{Ar}-6); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 18.4 (C-4″), 264.9 (C-3′), 31.2 (C-3″), 44.0 (C-5″), 47.0 (C-11′), 48.0 (C-4′), 48.2 (C-2′), 55.4 (4-O$\underline{C}H_3$), 55.8 (6′-O$\underline{C}H_3$), 100.2 (C-4), 111.7 (C-8′), 113.5 (C-5′), 114.1 (C-7′), 114.3 (C_{Ar}-2, C_{Ar}-6), 121.2 (C_{Ar}-1), 128.2 (C_{Ar}-3, C_{Ar}-5), 129.0 (C-4a′), 130.4 (C_{Ar}-4), 138.3 (C-8a′), 152.1 (C-6′), 161.6 (C-3), 169.8 (C-5), 175.7 (C-2″); MS (ESI-IT) *m/z*: 456.1 $[M+Na]^+$, 889.2 $[2M+Na]^+$; Anal. calcd for $C_{25}H_{27}N_3O_4$: C, 69.27; H, 6.28; N, 9.69%. Found: C, 69.64; H, 6.42; N, 9.92%.

5-((6′-methoxy-4′-(2″-oxopyrrolidin-1″-yl)-3′,4′-dihydroquinolin-1′(2′H)-yl)methyl)-3-(3,4,5-trimethoxyphenyl)isoxazole (3i) Red oil; IR (ATR) ν_{max}: 3373.4, 2937.5, 1664.5, 1421.5, 1238.3, 1124.5, 824.9 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 2.04 (2H, m, H-4″), 2.15 (2H, m, H-3′), 2.51 (2H, m, H-3″), 3.22 (2H, m, H-5″), 3.50 (2H, m, H-2′), 3.70 (3H, s, 6′-OCH_3), 3.87 (3H, s, 4-OCH_3), 3.9 (6H, s, 3-OCH_3, 5-OCH_3), 4.48 (1H, d, $J = 17.4$ Hz, Ha-11′), 4.62 (1H, d, $J = 17.4$ Hz, Hb-11′), 5.43 (1H, dd, $J = 9.6$, 5.7 Hz, H-4′), 6.31 (1H, s, H-4), 6.50 (1H, dd, $J = 2.9$, 0,8 Hz, H-5′), 6.61 (1H, d, $J = 9.0$ Hz, H-8′), 6.71 (1H, dd, $J = 9.0$, 2.9 Hz, H-7′), 6.97 (2H, s, H_{Ar}-2, H_{Ar}-6); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 18.4 (C-4″), 26.9 (C-3′), 31.5 (C-3″), 43.6 (C-5″), 48.2 (C-4′), 48.4 (C-11′), 48.6 (C-2′), 55.9 (6′-O$\underline{C}H_3$), 56.4 (3-O$\underline{C}H_3$, 5-O$\underline{C}H_3$), 61.1 (4-O$\underline{C}H_3$), 100.4 (C-4), 104.1 (C_{Ar}-2, C_{Ar}-6), 113.4 (C-5′), 113.6 (C-8′), 114.1 (C-7′), 121.9 (C_{Ar}-1), 124.4 (C-4a′), 139.4 (C_{Ar}-4), 139.7 (C-8a′), 152.2 (C-6′), 153.7 (C_{Ar}-3, C_{Ar}-5), 162.4 (C-3), 170.3 (C-5), 175.8 (C-2″); MS (ESI-IT) *m/z*: 516.1 $[M+Na]^+$, 1009.2 $[2M+Na]^+$; Anal. calcd for $C_{27}H_{31}N_3O_6$:

C, 65.71; H, 6.33; N, 8.51%. Found: C, 65.49; H, 6.21; N, 8.30%.

5-((6′-chloro-4′-(2″-oxopyrrolidin-1″-yl)-3′,4′-dihydroquinolin-1′(2′H)-yl)methyl)-3-(phenyl)isoxazole (3j) Dark red oil; IR (ATR) ν_{max}: 3427.5, 2930.4, 1657.9, 1496.6, 1127.6, 806.6, 771.4 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 2.05 (2H, m, H-4″), 2.12 (2H, m, H-3′), 2.50 (2H, m, H-3″), 3.20 (2H, m, H-5″), 3.50 (2H, m, H-2′), 4.52 (1H, d, J = 17.5 Hz, Ha-11′), 4.60 (1H, d, J = 17.5 Hz, Hb-11′), 5.37 (1H, dd, J = 9.6, 5.9 Hz, H-4′), 6.37 (1H, s, H-4), 6.57 (1H, d, J = 8.9 Hz, H-8′), 6.83 (1H, dd, J = 2.5, 1.0 Hz, H-5′), 7.04 (1H, dd, J = 8.9, 2.5 Hz, H-7′), 7.43 (3H, m, H_{Ar}-3, H_{Ar}-4, H_{Ar}-5), 7.75 (2H, m, H_{Ar}-2, H_{Ar}-6); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 18.3 (C-4″), 26.4 (C-3′), 31.2 (C-3″), 43.5 (C-5″), 47.7 (C-11′), 47.8 (C-4′), 48.2 (C-2′), 100.5 (C-4), 113.2 (C-8′), 121.9 (C-4a′), 122.6 (C-6′), 126.9 (C_{Ar}-2, C_{Ar}-6), 127.3 (C-5′), 128.6 (C-7′), 128.7 (C_{Ar}-1), 129.0 (C_{Ar}-3, C_{Ar}-5), 130.3 (C_{Ar}-4), 143.6 (C-8a′), 162.6 (C-3), 169.4 (C-5), 175.8 (C-2″); MS (ESI-IT) *m/z*: 430.0 $[M+Na]^+$, 837.1 $[2M+Na]^+$; Anal. calcd for $C_{23}H_{22}ClN_3O_2$: C, 67.73; H, 5.44; N, 10.30%. Found: C, 67.36; H, 5.58; N, 10.09%.

5′-((6′-chloro-4′-(2″-oxopyrrolidin-1″-yl)-3′,4′-dihydroquinolin-1′(2′H)-yl)methyl)-3-(4-methoxyphenyl)isoxazole (3k) Orange oil; IR (ATR) ν_{max}: 3415.8, 2976.1, 1678.0, 1431.2, 1253.7, 1178.5, 1028.0, 837.1 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 2.05 (2H, m, H-4″), 2.13 (2H, m, H-3′), 2.51 (2H, m, H-3″), 3.20 (2H, m, H-5″), 3.50 (2H, m, H-2′), 3.82 (3H, s, 4-OCH_3), 4.50 (1H, d, J = 17.6 Hz, Ha-11′), 4.58 (1H, d, J = 17.6 Hz, Hb-11′), 5.37 (1H, dd, J = 9.6, 5.4 Hz, H-4′), 6.31 (1H, s, H-4), 6.57 (1H, d, J = 8.9 Hz, H-8′), 6.83 (1H, dd, J = 2.6, 1.0 Hz, H-5′), 6.93 (2H, dd, J = 6.1, 2.2 Hz, H_{Ar}-3, H_{Ar}-5), 7.03 (1H, ddd, J = 8.9, 2.6, 0.8 Hz, H-7′), 7.67 (2H, dd, J = 6.1, 2.2 Hz, H_{Ar}-2, H_{Ar}-6); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 18.4 (C-4″), 26.4 (C-3′), 31.4 (C-3″), 43.7 (C-5″), 47.7 (C-11′), 47.8 (C-4′), 48.2 (C-2′), 55.4 (4-O<u>C</u>H_3), 100.2 (C-4), 113.3 (C-8′), 114.4 (C_{Ar}-2, C_{Ar}-6), 121.2 (C_{Ar}-1), 121.8 (C-4a′), 122.5 (C-6′), 127.3 (C-5′), 128.3 (C_{Ar}-3, C_{Ar}-5), 128.6 (C-7′), 143.6 (C-8a′), 161.1 (C_{Ar}-4), 162.2 (C-3), 169.1 (C-5), 175.9 (C-2″); MS (ESI-IT) *m/z*: 460.1 $[M+Na]^+$, 897.1 $[2M+Na]^+$; Anal. calcd for $C_{24}H_{24}N_3O_3$: C, 65.83; H, 5.52; N, 9.60%. Found: C, 67.47; H, 5.66; N, 9.48%.

5-((6′-chloro-4′-(2″-oxopyrrolidin-1′-yl)-3′,4′-dihydroquinolin-1′(2′H)-yl)methyl)-3-(3,4,5-trimethoxiphenyl)isoxazole (3l) Brown oil; IR (ATR) ν_{max}: 3431.3, 2936.2, 1667.7, 1582.3, 1420.3, 1122.9, 999.4, 842.3, 732.8 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 2.07 (2H, m, H-4″), 2.14 (2H, m,H-3′), 2.52 (2H, m, H-3″), 3.22 (2H, m, H-5″), 3.52 (2H, m, H-2′), 3.87 (3H, s, 4-OCH_3), 3.9 (6H, s, 3-OCH_3, 5-OCH_3), 4.51 (1H, d, J = 17.6 Hz, Ha-11′), 4.64 (1H, d, J = 17.6 Hz, Hb-11′), 5.40 (1H, dd, J = 9.9, 5.4 Hz, H-4′), 6.31 (1H, s, H-4), 6.57 (1H, d, J = 8.8 Hz, H-8′), 6.84 (1H, dd, J = 2.5, 0.9 Hz, H-5′), 6.96 (2H, s, H_{Ar}-2, 6-H_{Ar}-6), 7.05 (1H, dd, J = 8.8, 2.6 Hz, H-7′); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 18.4 (C-4″), 26.4 (C-3′), 31.4 (C-3″), 43.5 (C-5″), 47.8 (C-11′), 47.9 (C-4′), 48.4 (C-2′), 56.5 (3-O<u>C</u>H_3, 5-O<u>C</u>H_3), 61.1 (4-O<u>C</u>H_3), 100.4 (C-4), 104.1 (C_{Ar}-2, C_{Ar}-6), 113.2 (C-8′), 122.0 (C-6′), 122.7 (C_{Ar}-1), 124.2 (C-4a′), 127.2 (C-5′), 128.7 (C-7′), 139.8 (C_{Ar}-4), 143.6 (C-8a′), 153.7 (C_{Ar}-3, C_{Ar}-5), 160.5 (C-3), 169.6 (C-5), 175.8 (C-2″); MS (ESI-IT) *m/z*: 520.1 $[M+Na]^+$, 1017.2 $[2M+Na]^+$, 1513.0 $[3M+Na]^+$; Anal. calcd for $C_{26}H_{28}ClN_3O_5$: C, 62.71; H, 5.67; N, 8.44. Found: C, 63.12; H, 5.53; N, 8.68%.

5-((6′-chloro-4′-(2″-oxopyrrolidin-1″-yl)-3′,4′-dihydroquinolin-1′(2′H)-yl)methyl)-3-(3,4-dimethoxiphenyl)isoxazole (3m) Viscous yellow oil; IR (ATR) ν_{max}: 3397.1, 2935.2, 1665.7, 1420.3, 1257.9, 1022.1, 853.8, 764.7 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 2.04 (2H, m, H-3′), 2.16 (2H, m, H-4″), 2.50 (2H, m, H-3″), 3.24 (2H, m, H-5″), 3.48 (2H, m, H-2′), 3.89 (3H, s, 4-OCH_3), 3.91 (3H, s, 3-OCH_3), 4.49 (1H, d, J = 17.6 Hz, Ha-11′), 4.59 (1H, d, J = 17.6 Hz, Hb-11′), 5.36 (1H, dd, J = 9.7, 5.3 Hz, H-4′), 6.31 (1H, s, H-4), 6.56 (1H, d, J = 8.8 Hz, H-8′), 6.82 (1H, dd, J = 2.5, 1.0 Hz, H-5′), 6.87 (1H, d, J = 8.3 Hz, H_{Ar}-5), 7.02 (1H, dd, J = 8.8, 2.5 Hz, H-7′), 7.22 (1H, dd, J = 8.3, 2.0 Hz, H_{Ar}-6), 7.34 (1H, d, J = 2.0 Hz, H_{Ar}-2); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 18.3 (C-4″), 26.4 (C-3′), 31.3 (C-3″), 43.6 (C-5″), 47.7 (C-2′), 47.8 (C-4′), 48.3 (C-11′), 56.0 (3-O<u>C</u>H_3), 56.1 (4-O<u>C</u>H_3), 100.2 (C-4), 109.2 (C_{Ar}-2), 111.0 (C_{Ar}-5), 113.2 (C-8′), 120.1 (C_{Ar}-6), 121.4 (C_{Ar}-1), 121.9 (C-4a′), 122.5 (C-6′), 127.2 (C-5′), 128.6 (C-7′), 143.5 (C-8a′), 149.3 (C_{Ar}-3), 150.7 (C_{Ar}-4), 162.2 (C-3), 169.2 (C-5), 175.8 (C-2″); MS (ESI-IT) *m/z*: 456.1 $[[M-Cl]+H]^+$, 490.1 $[M+Na]^+$, 597.1 $[2M+Na]^+$; Anal. calcd for $C_{25}H_{26}ClN_3O_4$: C, 64.17; H, 5.60; N, 8.98%. Found: C, 63.82; H, 5.45; N, 8.76%.

5-((8′-chloro-6′-methyl-4′-(2″-oxopyrrolidin-1″-yl)-3′,4′-dihydroquinolin-1′(2′H)-yl)methyl)-3-(3,4-dimethoxyphenyl)isoxazole (3n) Beige solid; M.p. 123–125 °C; IR (ATR) ν_{max}: 3477.8, 2966.4, 1656.8, 1429.2, 1255.6, 1143.8, 1016.4, 856.4 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.89 (2H, m, H-3′), 2.00 (2H, m, H-4″), 2.23 (3H, s, 6′-CH_3), 2.48 (2H, m, H-3″), 3.12 (2H, m, H-5″), 3.26 (2H, m, H-2′), 3.91 (3H, s, 4-OCH_3), 3.94 (3H, s, 3-OCH_3), 4.33 (1H, d, J = 16.3 Hz, Ha-11′), 4.38 (1H, d, J = 16.3 Hz, Hb-11′), 5.38 (1H, dd, J = 8.8, 7.1 Hz, H-4′), 6.63 (1H, s, H-4), 6.73 (1H, t, J = 1.0 Hz, H-5′), 6.91 (1H, d, J = 8.3 Hz, H_{Ar}-5), 7.11 (1H, d, J = 2.1 Hz, H-7′), 7.30 (1H, dd, J = 8.3, 2.0 Hz, H_{Ar}-6), 7,40 (1H, d, J = 2.0 Hz, H_{Ar}-2); ^{13}C NMR

(100 MHz, $CDCl_3$) δ (ppm): 18.2 (C-4″), 20.7 (6′-$\underline{C}H_3$), 21.4 (C-3′), 31.3 (C-3″), 43.0 (C-5″), 47.0 (C-2′), 47.7 (C-4′), 49.8 (C-11′), 56.0 (4-O$\underline{C}H_3$), 56.1 (3-O$\underline{C}H_3$), 100.8 (C-4), 109.2 (C_{Ar}-2), 111.0 (C_{Ar}-5), 120.0 (C_{Ar}-6), 121.8 (C_{Ar}-1), 127.0 (C-5′), 127.8 (C-8′), 129.2 (C-6′), 130.7 (C-7′), 133.5 (C-4a′), 142.3 (C-8a′), 149.3 (C_{Ar}-3), 150.6 (C_{Ar}-4), 162.4 (C-3), 171.3 (C-5), 175.8 (C-2″); MS (ESI-IT) *m/z*: 482.0 $[M+H]^+$, 504.1 $[M+Na]^+$, 985.1 $[2M+Na]^+$; Anal. calcd for $C_{26}H_{28}ClN_3O_4$: C, 64.79; H, 5.86; N, 8.72%. Found: C, 65.23; H, 5.69; N, 8.94%.

5-((8′-chloro-6′-methoxy-4′-(2″-oxopyrrolidin-1″-yl)-3′,4′-dihydroquinolin-1′(2′H)-yl)methyl)-3-(3,4-dimethoxyphenyl)isoxazole (3o) Brown solid; M.p. 128–129 °C; IR (ATR) ν_{max}: 3363.8, 2943.3, 2839.1, 1670.3, 1429.2, 1261.4, 1136.0, 1020.3, 854.4 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.90 (2H, m, H-3′), 2.06 (2H, m, H-4″), 2.47 (2H, m, H-3″), 3.14 (2H, m, H-5″), 3.30 (2H, m, H-2′), 3.72 (3H, s, 6′-OCH_3), 3.91 (3H, s, 4-OCH_3), 3.93 (3H, s, 3-OCH_3), 4.28 (1H, d, $J = 16.3$ Hz, Ha-11′), 4.34 (1H, d, $J = 16.3$ Hz, Hb-11′), 5.39 (1H, t, $J = 8.2$ Hz, H-4′), 6.50 (1H, d, $J = 2.9$, 0.9 Hz, H-5′), 6.62 (1H, s, H-4), 6.90 (1H, dd, $J = 2.8$, 0.8 Hz, H-7′), 6.91 (1H, d, $J = 8.5$ Hz, H_{Ar}-5), 7.29 (1H, dd, $J = 8.5$, 2.0 Hz, H_{Ar}-6), 7,40 (1H, d, $J = 2.0$ Hz, H_{Ar}-2); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 18.3 (C-4″), 21.6 (C-3′), 31.2 (C-3″), 43.0 (C-5″), 46.9 (C-2′), 48.0 (C-4′), 49.9 (C-11′), 55.8 (6′-O$\underline{C}H_3$), 56.0 (4-O$\underline{C}H_3$), 56.0 (5-O$\underline{C}H_3$), 100.8 (C-4), 109.2 (C_{Ar}-2), 111.1 (C_{Ar}-5), 111.7 (C-5′), 115.9 (C-7′), 120.0 (C_{Ar}-6), 121.7 (C_{Ar}-1), 129.0 (C-8′), 130.4 (C-4a′), 138.3 (C-8a′), 149.3 (C_{Ar}-3), 150.6 (C_{Ar}-4), 155.5 (C-6′), 162.4 (C-3), 171.3 (C-5), 175.7 (C-2″); MS (ESI-IT) *m/z*: 486.2 $[[M-Cl]+Na]^+$, 520.1 $[M+Na]^+$, 1017.2 $[2M+Na]^+$; Anal. calcd for $C_{26}H_{28}ClN_3O_5$ (497.98 g/mol): C, 62.71; H, 5.67; N, 8.44%. Found: C, 62.23; H, 5.83; N, 8.67%.

5-((6′,8′-chloro-4′-(2″-oxopyrrolidin-1″-yl)-3′,4′-dihydroquinolin-1′(2′H)-yl)methyl)-3-(3,4-dimethoxyphenyl)isoxazole (3p) Orange solid; M.p. 157–158 °C; IR (ATR) ν_{max}: 3118.8, 2954.9, 1662.6, 1427.3, 1249.8, 1026.1, 854.4 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.91 (2H, m, H-3′), 2.02 (2H, m, H-4″), 2.48 (2H, m, H-3″), 3.13 (2H, m, H-5″), 3.28 (2H, m, H-2′), 3.92 (3H, s, $C_{Ar\text{-}4}$-OCH_3), 3.94 (3H, s, $C_{Ar\text{-}3}$-OCH_3), 4.36 (1H, d, $J = 16.4$ Hz, Ha-11′), 4.43 (1H, d, $J = 16.4$ Hz, Hb-11′), 5.39 (1H, t, $J = 8.3$ Hz, H-4′), 6.61 (1H, s, H-4), 6.92 (1H, d, $J = 8.4$, H_{Ar}-5), 6.92 (1H, d, $J = 0.8$ Hz, H-7′), 7.29 (1H, dd, $J = 8.3$, 2.0 Hz, H_{Ar}-6), 7.30 (1H, d, $J = 0.8$ Hz, H-5′), 7.40 (1H, d, $J = 2.0$ Hz, H_{Ar}-2); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 18.2 (C-4″), 21.4 (C-3′), 31.1 (C-3″), 43.0 (C-5″), 47.3 (C-2′), 47.8 (C-4′), 49.4 (C-11′), 56.1 (4-O$\underline{C}H_3$), 56.1 (3-O$\underline{C}H_3$), 100.9 (C-4), 109.2 (C_{Ar}-2), 111.1 (C_{Ar}-5), 120.1 (C_{Ar}-6), 121.6 (C_{Ar}-1), 126.3 (C-5′), 128.3 (C-8′), 128.6 (C-6′), 129.9 (C-7′), 130.4 (C-4a′), 143.6 (C-8a′), 149.4 (C_{Ar}-3), 150.7 (C_{Ar}-4), 162.5 (C-3), 170.8 (C-5), 175.8 (C-2″); MS (ESI-IT) *m/z*: 490.1 $[[M-Cl]+Na]^+$, 524.0 $[M+Na]^+$, 1027.0 $[2M+Na]^+$; Anal. calcd for $C_{25}H_{25}Cl_2N_3O_4$: C, 59.77; H, 5.02; N, 8.36%. Found C, 62.31; H, 5.16; N, 8.55%.

In vitro anticancer evaluation

MTT assay

The in vitro cytotoxicity of all compounds synthesized were evaluated on cancer cell lines, including, human lung (A549), human liver (HepG2) and melanoma murine (B16F10), besides, of normal (non-human) cells of kidney (VERO). The cell line melanoma murine was grown in RPMI and the cell lines A549, HepG2, and VERO were grown in EMEM medium containing 10% fetal bovine serum and gentamicin (50 µg/mL) at 37 °C in a 5% CO_2 humidified incubator. Cells were plated (7.5×10^3 cells/well) in 96-well dishes. After overnight incubation, cells were treated with varying concentrations of compounds (5, 25, 50, and 100 µM) for 48 h. Oxaliplatin (1, 5, 25, 50, and 100 µM) was used as a positive control. After incubation, cell viability was performed using MTT method (Mosmann 1983), the absorbance was measured at 540 nm using a microplate reader *multiskan go*. Results were expressed as the half-maximal inhibitory concentration (IC_{50}) of the cell viability. Additionally, the selectivity index (SI): IC_{50} normal cell (VERO)/IC_{50} tumor cell was reported. For the compound with the best IC_{50}, the result was expressed as a percentage of viable cells in comparison to the control.

Hepatoxicity viability

Isolation and primary culture of adult rat hepatocytes, according to the Shen et al. (2013) protocol, were performed. Isolated hepatocytes (viability > 60%) were cultured in 6 cm glass Petri dishes (1×10^6 cells/box) in M199 culture medium and incubated at 37 °C with CO_2 atmosphere (5%) for 3 h for total adherence. Subsequently, hepatocytes were treated with different concentrations of hybrid compound **3j** (5, 25, 50, and 100 µM) dissolved in Hepatozyme-SFM culture medium (supplemented with 100 µg/mL streptomycin) and 0.1% DMSO. As a negative control, cells were used without the addition of the drug, grown in Hepatozyme-SFM medium. After 24 h of treatment, the cells were released using trypsin-EDTA solution (pH 7.3) and resuspended in M199 culture medium with 10% FBS to inactivate trypsin. The viability of the hepatocytes was determined by staining with 0.4% (w/v) trypan blue counts in Neubauer's chamber (90 µL of cell suspension plus 10 µL of trypan blue).

Measurement of intracellular reactive oxygen species

Intracellular reactive oxygen species (ROS) were estimated using 2′,7′-dichlorofluorescein-diacetate (H_2DCFDA). HepG2 cells were plated (2.0×10^4 cells/well) in 96-well dishes. After incubation for 24 h, cells were incubated with H_2DCFD (50 μM) for 30 min. Afterward, cells were washed with phosphate buffered saline (PBS) and treated with the compound **3j** (25 μM). H_2O_2 (400 μM) was used as a positive control. Immediately, the fluorescence was measured for 240 min by Varioskan Flash Spectral Scan plate reader (Thermo Fisher Scientific, Waltham, MA, USA) with excitation/emission wavelengths set to 485/525 (LeBel et al. 1992).

Mitochondrial transmembrane potential ($\Delta\psi_m$)

Mitochondrial depolarization was evaluated using the method of incorporation of Rho-123. HepG2 cells were plated (1×10^5 cells/well) in 6-well dishes. After incubation for 24 h, cells were incubated with the hybrid **3j** (25 μM) for 24 h. Doxorubicin (1.0 μM) was used as a positive control. Then, the cells were released with trypsin and were washed twice with PBS and incubated with Rhodamine 123 (1.0 μg/mL) for 15 min at 37 °C in the dark. Afterward, cells were washed and resuspended with PBS ant 30 °C. Fluorescence of rhodamine 123 was determined using FACSCanto II (Becton Dickinson, Heidelberg, Germany) (Cury-Boaventura et al. 2004).

Induction of apoptosis-Annexin V/sytox

HepG2 cells were plated (1×10^5 cells/well) in 6-well dishes. After incubation for 24 h, cells were incubated with the hybrid **3j** (25 μM) for 24 h. Doxorubicin (1.0 μM) was used as a positive control. Then, to detect cell population in viable, early and late apoptosis stage, the APC Annexin V/Dead Cell Apoptosis Kit (Invitrogen) was used following the manufacturer′s instructions. Afterward, cells were analyzed by FACSCanto II flow cytometer (Becton Dickinson, Heidelberg, Germany) (Vermes et al. 1995).

Statistical analysis

All assays were performed in triplicate in three independent assays, and the obtained values were analyzed and expressed as mean ± standard error of the mean (SEM). Statistical analysis was carried out by analysis of variance (one-way ANOVA) followed by a Tukey test.

In silico ADME screening

Absorption, distribution, metabolism, and excretion (ADME) properties for tetrahydroquinoline-isoxazole hybrids **3a**–**p** were calculated based on virtual screening analysis, in order to predict some physical and pharmaceutical properties. Physicochemical parameters, including the Lipinski′s rule of five (Lipinski et al. 2012), were calculated using the Molinspiration virtual platform service (http://www.molinspiration.com/services/). This software allows drawing chemical structures and calculating numerous drug-relevant descriptors that could indicate their potential use as drugs.

Results and discussion

Chemistry

In order to access easily and efficiently to the tetrahydroquinoline-isoxazole hybrids of interest, in this work, a route that involves a two-step synthesis was designed. First, the corresponding N-propargyl tetrahydroquinolines derivatives (**1a**–**d**) were obtained via cationic Povarov reaction, which results from a nucleophilic addition kind Mannich, followed by an intramolecular Friedel-Craft electrophilic substitution reaction. These N-propargyl THQs were synthesized and then properly characterized, according to the previous work described by Rodriguez et al. (2016). Once the tetrahydroquinoline derivatives with propargyl fragment were prepared, the hybrid systems tetrahydroquinoline-isoxazole **3a**–**p** were obtained under mild conditions reaction and good yields (42–88%). The reaction for obtaining the corresponding isoxazole core occurred simple and effectively, through 1,3-dipolar cycloaddition reaction with a click chemistry approach. In this opportunity, the nitrile oxides (dipole) was obtained in situ using aqueous NaOCl commercial solution as an oxidizing agent, from the aldoximes **2a**–**d**, previously prepared and without purifying. These dipoles, reacted with the corresponding N-propargyl THQs (dipolarophile) with high regio-specificity, allowing the obtaining of 3,5-regioisomers as unique products (Table 1; Scheme 1).

The structure of all hybrid tetrahydroquinoline-isoxazole compounds synthesized was determined by IR and MS and confirmed by ^{1}H NMR and ^{13}C NMR spectroscopy. The analysis of IR spectra of the molecular hybrids showed the presence of all absorption bands associated with the principal functional groups present in the structure, besides the absence of the absorption band around 3220–3230 cm^{-1}, corresponding to the vibration of tension associated with the C_{sp}-H bond of the terminal alkyne fragment in the N-propargyl THQs precursors, was observed. Similarly, in the spectra of ^{1}H NMR signals for each proton of the molecule were shown, as well as the absence of a triplet signal around 2.10–2.50 ppm corresponding to C_{sp}-H proton of the N-propargyl THQs. Therefore, the appearance of singlet signal

associated with the proton H-4 of the isoxazole ring (6.29–6.63 ppm), which unequivocally corroborated the isoxazole ring formation and therefore, confirming the structure for tetrahydroquinoline-isoxazole hybrids synthesized.

It is worth noting that during the synthesis of the isoxazole core, when aldoximes **2d** were using, chlorinated products were obtained. It is presumed that the formation of this product is due to hypochlorous acid (HOCl) that coexists in the NaOCl aqueous equilibrium, which facilitated the corresponding aromatic electrophilic substitution reaction (Acelas et al. 2018; Nwaukwa and Keehn 1989).

Table 1 Physico-chemical parameters of novel tetrahydroquinoline/isoxazole hybrids **3a–p**

Comp.[a]	R_1	R_2	R_3	R_4	Yield (%)[b]	M.p. (°C)[c]
3a	H	H	H	H	55	Orange oil
3b	H	H	OCH_3	H	65	Reddish oil
3c	H	OCH_3	OCH_3	OCH_3	75	Brown oil
3d	CH_3	H	H	H	72	Reddish oil
3e	CH_3	H	OCH_3	H	64	Reddish oil
3f	CH_3	OCH_3	OCH_3	OCH_3	63	Reddish oil
3g	OCH_3	H	H	H	42	Brown oil
3h	OCH_3	H	OCH_3	H	74	Red oil
3i	OCH_3	OCH_3	OCH_3	OCH_3	74	Red oil
3j	Cl	H	H	H	55	Red oil
3k	Cl	H	OCH_3	H	51	Orange oil
3l	Cl	OCH_3	OCH_3	OCH_3	85	Brown oil
3m	Cl	H	OCH_3	OCH_3	68	Yellow oil
3n	CH_3	H	OCH_3	OCH_3	58	123–125
3o	OCH_3	H	OCH_3	OCH_3	86	128–129
3p	Cl	H	OCH_3	OCH_3	88	157–158

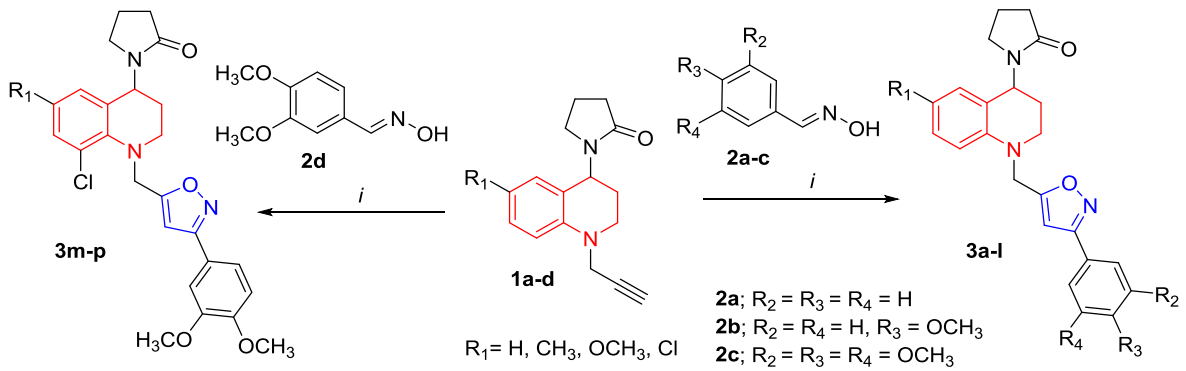
[a]Compounds **3m–p** were isolated as the major product trough to unexpected halogenation of the hybrids desired

[b]Yields after column chromatography

[c]Uncorrected

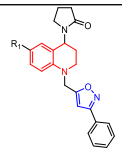
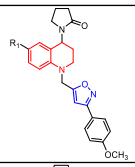
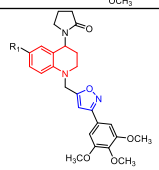
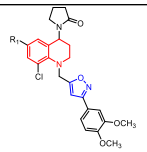
In vitro anticancer evaluation

Hybrid compounds **3a–p** synthesized were screened for their in vitro cytotoxicity, using the colorimetric method MTT against A549 (lung), HepG2 (hepatocarcinoma), B16F10 (melanoma murine) cancer cell lines and VERO (non-human mammalian kidney cells). Cytotoxic activity was tested to 48 h, using four (4) concentrations (5, 25, 50, and 100 μM). These results, including the values for the reference compound (oxaliplatin), were expressed as IC_{50} (μM) and summarized in the Table 2, which for greater comprehension was divided in four (4) different series of compounds that maintain in common the aryl group attached to the C-3 position of heterocyclic fragment of isoxazole. As shown in the table, several of the tetrahydroquinoline-isoxazole hybrids tested present promising in vitro activity against the cancer cell lines evaluated and low toxicity towards VERO normal cells. In general, hybrid **3j** (series 1) was the most cytotoxic compound against A549 ($IC_{50} = 16.9 \pm 6.4$ μM), while hybrid **3c** (series 4) showed the highest cytotoxicity on B16F10 ($IC_{50} = 23.6 \pm 1.2$ μM). In the case of HepG2 cancer cell line, moderate to good cytotoxicity were observed for seven (7) evaluated hybrids. Stand out compounds **3a** and **3j** (series 1, $IC_{50} = 6.8 \pm 0.71$ and 5.2 ± 1.9 μM, respectively), **3e** (series 2, $IC_{50} = 10.9 \pm 2.5$ μM), and **3m** (series 3, $IC_{50} = 10.2 \pm 2.6$ μM), which showed an IC_{50} value significantly less than the observed for standard drug *oxaliplatin* ($IC_{50} = 22.4 \pm 1.1$ μM). Hybrids **3a** (SI > 14.7) and **3j** (SI = 16.1) also showed the best selectivity towards HepG2 cancer cells in comparison to non-tumorigenic VERO cells, even much greater than for the reference drug oxaliplatin (SI = 2.3). In fact, compound **3j** against HepG2 cancer line was four (4) times more cytotoxic and seven (7) times more selective, compared to the results obtained with oxaliplatin (Fig. 1). The cytotoxicity and selectivity shown by some tetrahydroquinoline-isoxazole hybrids tested suggests that these compounds can be considered as promising chemotherapeutic agents against hepatocellular carcinoma



Scheme 1 Synthesis of tetrahydroquinoline/isoxazole hybrids **3a–p** derivatives. Reagents and conditions: (i) NaOCl (10% w/v), CH_2Cl_2, r.t

Table 2 In vitro cytotoxic activity using MTT assay of new tetrahydroquinoline/isoxazole hybrids **3a–p**

Comp.	Series	Cytotoxicity, IC_{50} [μM] [a]				Ratio IC_{50} [b]		
		A549	HepG2	B16F10	VERO	A549/VERO	HepG2/VERO	B16F10/VERO
3a		35.3 ± 8.8	**6.8 ± 0.71**	87.9 ± 1.5	>100	>2.8	**>14.7**	>1.1
3d		45.2 ± 3.5	26.2 ± 4.2	84.1 ± 2.3	>100	>2.2	>3.8	>1.2
3j		**16.9 ± 3.4**	**5.2 ± 1.9**	87.3 ± 13.0	82.2 ± 19.6	**4.9**	**16.1**	0.94
3g		72.7 ± 12.9	43.9 ± 6.9	50.4 ± 2.5	94.0 ± 3.9	1.1	1.9	1.6
3b		52.0 ±17.8	19.5 ± 17.3	>100	>100	>1.9	>5.1	>1.0
3e		nd	**10.9 ± 2.5**	>100	>100	–	**>8.2**	>1.0
3k		>100	27.6 ± 1.6	>100	>100	>1.0	>3.6	>1.0
3h		62.9 ± 3.9	nd	87.6 ± 8.3	>100	>1.6	–	>1.1
3c		31.0 ± 0.7	78.6 ± 2.5	**23.6 ± 1.2**	34.7 ± 5.3	1.1	0.44	1.5
3f		58.5 ± 2.2	74.9 ± 0.04	>100	91.56 ± 1.6	1.6	1.2	0.83
3l		34.4 ± 7.2	>100	63.2 ± 3.4	68.6 ± 2.1	1.9	>0.7	1.1
3i		88.2 ± 10.2	>100	>100	91.9 ± 12.6	1.0	>0.9	>0.9
3m		39.9 ± 7.2	**10.2 ± 2.6**	88.2 ± 20.5	>100	>2.5	**>9.8**	1.1
3n		>100	>100	>100	>100	>1.0	>1.0	>1.0
3o		22.7 ± 6.4	>100	>100	>100	4.4	>1.0	>1.0
3p		nd	nd	>100	>100	–	–	1.0
L-OHP [c]		31.2 ±1.7	22.4 ± 1.1	4.9 ±1.2	51.5 ± 2.2	1.6	2.3	10.5

nd not determinate

Bold values indicates the more significative results

[a] IC_{50} value corresponding to the compound concentration required to inhibit tumor cell proliferation by 50%

[b] Ratio of the IC_{50} values corresponding to the selectivity index (SI): IC_{50} normal cell (VERO)/IC_{50} tumor cell

[c] Oxaliplatin (L-OHP) was used as positive controls

(HCC), which is the third type of cancer with the highest prevalent worldwide (Germano et al. 2013; Pesi et al. 2016) and perhaps the most difficult to treat, due to it is frequently associated with cirrhosis (Zhao et al. 2015).

Structure–activity relationship (SAR) analysis base on cytotoxicity data of this series of hybrids showed that is not possible to establish a coherent relationship between these structures and its toxicity effect. However, compounds **3a**, **3d**, **3g**, and **3j** (series 1), that conserve a phenyl group linked to the C-3 position of isoxazole moiety, showed the best and more remarked cytotoxic effect against A549 and HepG2 cells. In general, the cytotoxicity effect tends to decrease when substituents different to proton on the phenyl group are introduced, with the unique exception of hybrid **3e** where the 4-methoxyphenyl group are attached to the C-3 position of the isoxazole core (series 2), which turned out to be moderately cytotoxic against the HepG2 cell line (Table 2). Besides, the cytotoxic activities of some hybrids tetrahydroquinoline-isoxazole with trimethoxyphenyl and dimethoxyphenyl substituents on isoxazole ring (**3c**, **3l**, and **3m**) were lightly more cytotoxic than against B16F10 than HepG2 and A549 cancer cells. The foregoing contrasts with the fact that trimethoxyaryl and dimethoxyaryl structural moiety, in several cases, have been used as pharmacophoric fragments in the design of analogs of the potent anticancer agent combretastatin A4 (Duan et al. 2016). These results can be explained under the premise that more active hybrid compounds would have a different mode of action those combretastatin A4 derivatives, which are directed towards the inhibition of tubulin (Kumar et al. 2018; Sharma et al. 2017).

Compound 3j induces necrosis on HepG2 cells and low cytotoxicity on normal cells

Hepatocellular carcinoma (HCC) is the most prevalent type of cancer and has the highest mortality rate in the world.

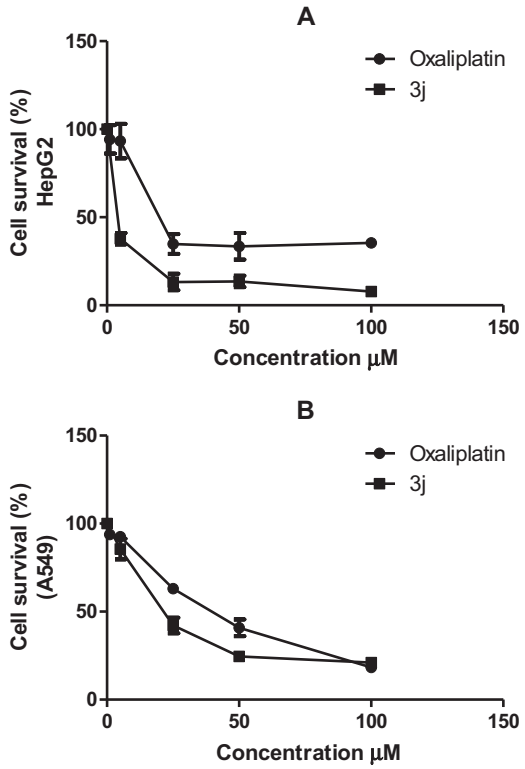


Fig. 1 Effects of **3j** and oxaliplatin on cell viability. **a** The viability of the HepG2 cell line after **3j** and oxaliplatin treatment for 48 h. Values represent the mean ± SD of percentage of viable cells in comparison to the control (data represent two independent experiments, each in triplicate). Significant differences ($p < 0.05$) were found in the percentage of cell death at all doses tested. **b** The viability of the A549 cell line after **3j** and oxaliplatin treatment for 48 h. Values represent the mean ± SD of the percentage of viable cells in comparison to the control (data represent two independent experiments, each in triplicate). Significant differences ($p < 0.05$) were found in the percentage of cell death at all doses tested

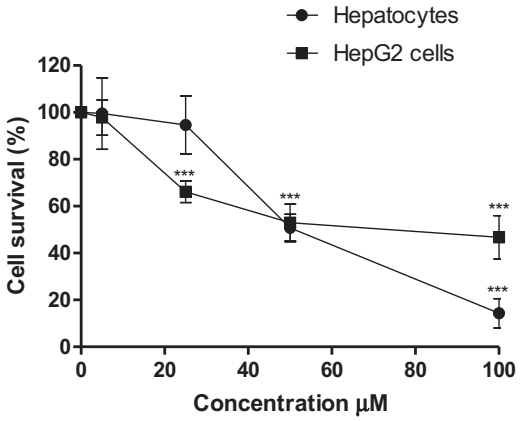


Fig. 2 Cytotoxic effect of **3j** hybrid on HepG2 cells and hepatocytes treatment for 24 h. Values represent the mean ± SD of the percentage of viable cells in comparison to the control (data represent two independent experiments, each in triplicate). ** and *** denotes values significantly different from the control or between the different treatments at $p < 0.05$ and $p < 0.001$

Various surgical, chemotherapeutic and molecular therapy procedures are part of the treatment. However, there are several difficulties related to side effects, surgical complications and resistance to chemotherapeutic and molecular therapy (Manzione et al. 2008; Shen et al. 2013). In this sense, THQ-isoxazole hybrid **3j** exhibited cytotoxic activity in hepatocarcinoma cells (HepG2) and low cytotoxicity on normal cells.

The selectivity of the emerging antitumor compounds is one of the most important characteristics of potential drugs. Figure 2 shows the cytotoxic activity of the hybrid compound **3j** in hepatocarcinoma cells and in rat hepatocytes. As observed in Fig. 2, compound **3j** reduced HepG2 cells viability by about 40% at 25 µM, and no cytotoxicity was observed in an assay for concentrations less than 50 µM in rat hepatocytes. These results suggest that the compound **3j** is selectively cytotoxic to HepG2 cells.

HepG2 cells were treated with the hybrid **3j** (25 and 50 µM) for 24 h and were evaluated by flow cytometry with Annexin V and sytox. Figure 3 shows the diagrams for cells without treatment (Fig. 3a), drug doxorubicin as a positive control (Fig. 3b) and treatments (25 and 50 µM) (Fig. 3c, d). Treatment with the 25 and 50 µM showed a significant increase with respect to the control in cells labeled with Annexin V (−)/Sytox (+) of 21.5 and 40.4%, respectively. These results indicate that HepG2 cells treated with the **3j** hybrid at concentrations 25 and 50 µM have a necrotic cell death mechanism.

Necrosis was originally considered an accidental and unregulated cell death. Evidence has shown that necrosis can be induced and proceed in a regulated manner like apoptosis (Su et al. 2016). Necroptosis is a major form of regulated necrosis, but regulated necrosis may also include other forms, such as oxytosis, ferroptosis, NETosis, pyronecrosis, and pyroptosis (Berghe et al. 2014). This mechanism of regulated cell death (RCD) is gaining interest; it could become an alternative to increasing cases of resistance to chemotherapeutic treatment that induces apoptosis (Wu et al. 2012).

In order to elucidate the factors that trigger necrosis by the **3j** hybrid, the generation of ROS by fluorometric analysis using the DFCH-DA method was determined, H_2O_2 (400 µM) was used as a positive control of the assay. Compound **3j** and positive control showed an increase in ROS as a function of time up to ~60 min, after, which a tendency to stabilize up was observed (Fig. 4). Hybrid **3j** generated an increase in ERO of ~150% and H_2O_2 exceeded 250% when was compared to no threated cells.

Likewise, the effect of **3j** on the mitochondrial membrane potential of HepG2 cells using rhodamine (Rho-123) was determined by flow cytometry and the results obtained are shown in Fig. 5. Hybrid **3j** generates mitochondrial

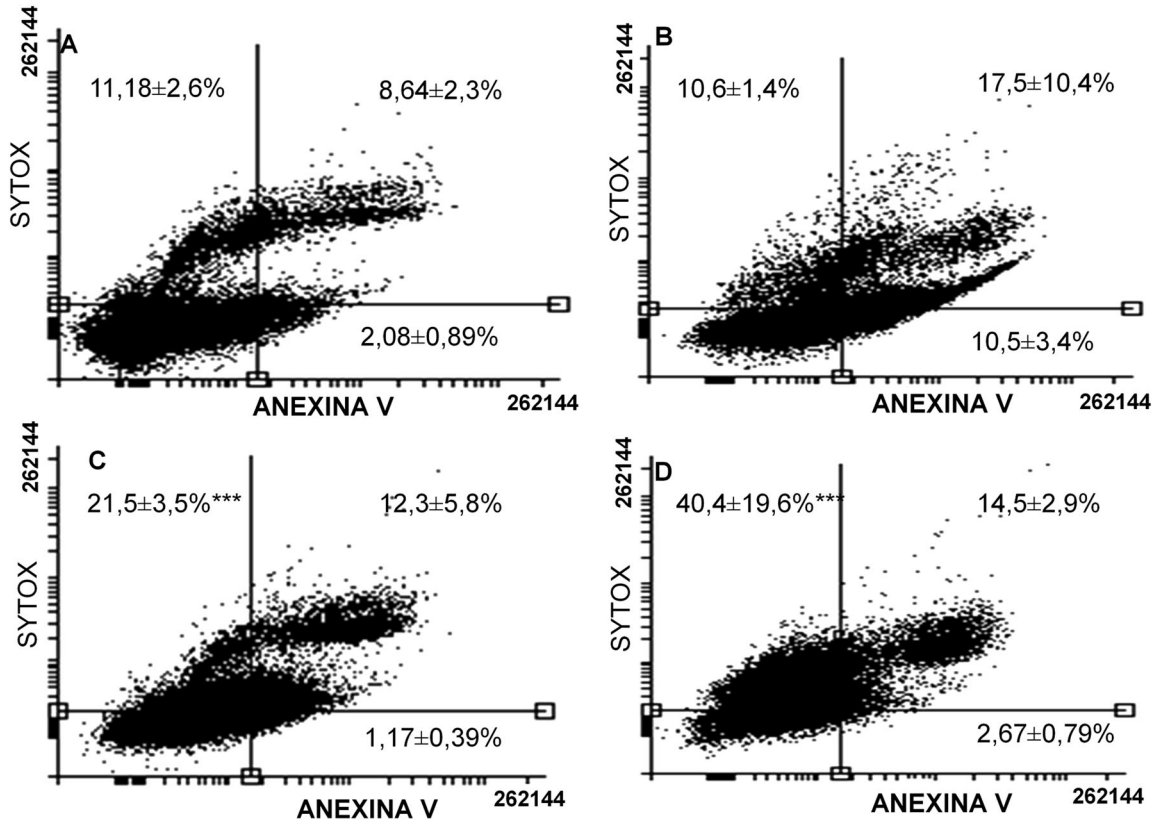


Fig. 3 Annexin V-Sytox staining of HepG2 treated with **3j** compound. The cells were seeded with or without hybrid **3j** at 25 and 50 µM for 24 h. Then the cells were collected with trypsin and 30,000 events were analyzed by flow cytometry. **a** Control, **b** Doxorubicin 1.0 µM, **c** **3j** 25 µM, **d** **3j** 50 µM. The figures show representative dot—plot with the different cell populations: left bottom = labeled cells; left top = Sytox labeled; right top = doubly labeled; right bottom = annexin V labeled. The results were expressed as mean ± SD of three independents experiments. *** denotes values significantly different from the control or between the different treatments at $p < 0.001$

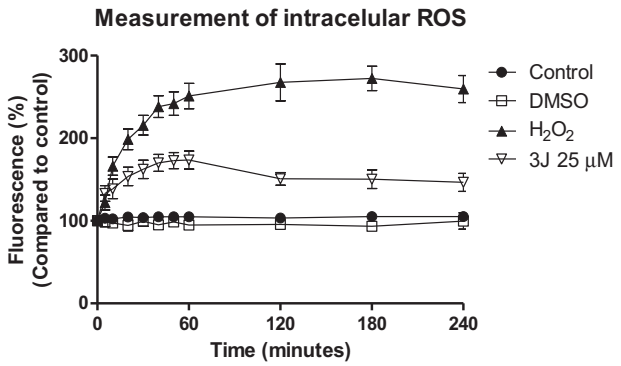


Fig. 4 Reactive oxygen species (ROS) after treatment with hybrid **3j**. HepG2 cells were pretreated with DCFH-DA dissolved in PBS, treated with the hybrid compound **3j** (25 µM) and reading the fluorescence was performed for 240 min. The experiments represent the mean ± SD of the triplicate of three independent experiments. Compound **3j** (25 µm) and H_2O_2 (400 µm) as positive control, presented significant differences with respect to the control $p < 0.05$

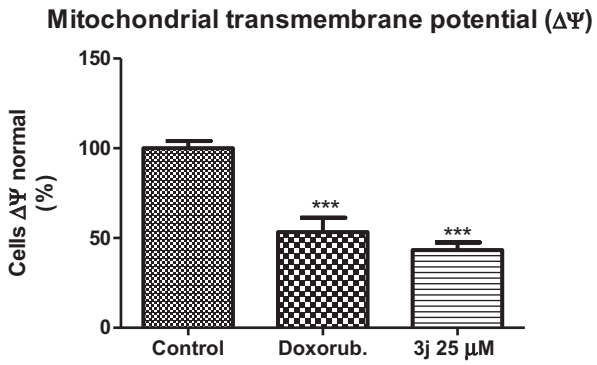


Fig. 5 Effect of **3j** compound on mitochondrial transmembrane potential. The results were determined by flow cytometric analysis after 24 h of incubation. Doxorubicin 1.0 µM was used as a positive control. A total of 30,000 events were analyzed in each experiment as a control. The results were expressed as mean ± SD of three independents experiments. *** denotes values significantly different from the control or between the different treatments at $p < 0.001$

depolarization in HepG2 cells, in the concentration of 25 µM during 24 h of treatment. Likewise, the drug doxorubicin, taken as a positive control, affected the membrane potential of these cells.

Hybrid **3j** generates ERO (Fig. 4), probably because of the inhibition on the transport of electron on mitochondrial respiratory chain, specifically from the complex I to III (Álvarez Santos et al. 2019), which ultimately affects the

mitochondrial membrane potential (Fig. 5) and probably the synthesis of ATP (Divakaruni et al. 2014). Mitochondria exert control over the mechanism of RCD by many factors; one of them is the generation of ROS (Marshall and Baines 2014). Additionally, the major generation of ROS in mitochondria is by inhibitions of the complex I (Fato et al. 2009), so these results clearly suggest that the cell death induced by **3j** hybrid is regulated by the mitochondria. In the same way, the HepG2 cells have reduced complex I activity and ROS overproduction, associated with mtDNA mutation generated by tumorigenesis (Gao et al. 2011). The HepG2 cells may be more vulnerable to cell death induced by oxidative stress than normal cells, due to their high levels of ROS (Anderson et al. 2014; Gorrini et al. 2013; Reczek and Chandel 2017), these observations could explain the selectivity of compound **3j** for HepG2 cells, without affecting hepatocytes in comparable concentrations.

Necroptosis mechanism uses components that are different from the apoptotic pathway. Cancer cells that are resistant to apoptosis agents may be sensitive to necroptosis inducers. In other words, apoptosis needs the activation of caspases, but necroptosis can terminate cancer cells when caspases are inhibited or defective (Su et al. 2016). It has been shown that only necroptosis is induced if there is pharmacological inhibition or genetic ablation of the apoptotic pathway, suggesting that necroptosis is an alternative to ensure cell death when apoptosis fails (Philipp et al. 2016). Other compounds such as tanshinone IIA have reported the simultaneous induction of apoptosis and necrosis in A549 and HepG2 cells (Lin et al. 2016). In addition, the compounds obatoclax and icaritin induce necrosis in thyroid and colorectal cancer cell (Champa et al. 2016; Zhou et al. 2016).

In silico ADME screening

The principal theoretical molecular descriptors, including the number of hydrogen donors (nNHOH), number of hydrogen acceptors (nNO), number of rotatable bonds (NRB), molecular weight (MW), and topological polar surface area (TPSA) for membrane permeation, prerequisite for the bioavailability, were calculated (Table 3). Here, it was found that 14 of the tetrahydroquinoline-isoxazole hybrids tested do not present any violation, two compounds **3k** (Log $p = 5.01$) and **3n** (Log $p = 5.01$) showed one (1) violation and the hybrid **3p** (MW = 501.40 g, Log $p = 5.24$) present two (2) the Lipinski′ rule violations. The obtained calculations demonstrate that most analyzed tetrahydroquinoline-isoxazole hybrid compounds, including the promising hybrid **3j**, showed high bioavailability properties, similar to oxaliplatin (Table 3).

Table 3 Main descriptors calculated for new tetrahydroquinoline/isoxazole hybrids **3a**–**p** using Molinspiration software

Comp.	Mol. formula[a]	MW (g/mol)	Parameters					
			Log *p*	TPSA, Å	nON[b]	nOHNH[c]	RBN[d]	Violations
3a	$C_{24}H_{24}N_2O_2$	372.47	4.34	46.34	4	0	4	0
3b	$C_{25}H_{26}N_2O_3$	402.49	4.39	55.58	5	0	5	0
3c	$C_{27}H_{30}N_2O_5$	462.55	3.97	74.04	7	0	7	0
3d	$C_{25}H_{26}N_2O_2$	386.49	4.76	46.34	4	0	4	0
3e	$C_{26}H_{28}N_2O_3$	416.52	4.82	55.58	5	0	5	0
3f	$C_{28}H_{32}N_2O_5$	476.57	4.39	74.04	7	0	7	0
3g	$C_{25}H_{26}N_2O_3$	402.49	4.37	55.58	5	0	5	0
3h	$C_{26}H_{28}N_2O_4$	432.52	4.42	64.81	6	0	6	0
3i	$C_{28}H_{32}N_2O_6$	492.57	4.00	83.28	8	0	8	0
3j	$C_{24}H_{23}ClN_2O_2$	406.91	4.99	46.34	4	0	4	0
3k	$C_{25}H_{25}ClN_2O_3$	436.94	5.05	55.56	5	0	5	1
3l	$C_{27}H_{29}ClN_2O_5$	496.99	4.62	74.04	7	0	7	0
3m	$C_{26}H_{27}ClN_2O_4$	466.96	4.64	64.81	6	0	6	0
3n	$C_{27}H_{29}ClN_2O_4$	480.99	5.01	64.81	6	0	6	1
3o	$C_{27}H_{29}ClN_2O_5$	496.99	4.62	74.04	7	0	7	0
3p	$C_{26}H_{26}Cl_2N_2O_4$	501.40	5.24	64.81	6	0	6	2
L-OHP	$C_8H_{14}N_2O_4Pt$	397.29	−4.96	85.82	6	4	0	0

[a]Confirmed by elemental analysis with ±0.5% of calculated values

[b]Number of hydrogen bond acceptors

[c]Number of hydrogen bond donors

[d]Number of rotatable bonds, Reference drug: Oxaliplatin (L-OHP)

Prediction results by TPSA parameters for the compounds **3a**–**p** (Table 3) showed TPSA values between 46.34 and 74.04 Å^2. For the particular case of **3j** (TPSA = 46.34 Å^2) value was less than 60 Å^2, indicating that this compound has a good membrane permeability, as well a good penetration of the blood-brain barrier.

Conclusions

Many types of cancer show resistance towards apoptosis (Holohan et al. 2016; Mohammad et al. 2015), demonstrating a clinical need for alternative treatment strategies. This paper showed that hybrid compounds of THQ-isoxazoles have a cytotoxic effect on at least one of the tumor cell lines analyzed. Hybrid **3j** showed the best cytotoxic effect on the HepG2 and A549 tumor lines and selective cytotoxicity to HepG2 cells, by promoting cell death with necrotic characteristics, while it did not affect the viability of hepatocytes. This compound might be considered as promising candidates to hepatocellular carcinoma treatment.

Acknowledgements This research was supported by "Patrimonio Autónomo, Fondo Nacional de Financiamiento para la Ciencia Francisco José de Caldas" (Colciencias) contract code 110265842934. FMG thanks COLCIENCIAS for his fellowship.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Abdel Rahman AM, Ryczko M, Pawling J, Dennis JW (2013) Probing the hexosamine biosynthetic pathway in human tumor cells by multitargeted Tandem Mass Spectrometry. ACS Chem Biol 8:2053–2062

Acelas M, Kouznetsov VV, Romero Bohórquez AR (2018) Facile and highly diastereo and regioselective synthesis of novel octahydroacridine-isoxazole and octahydroacridine-1,2,3-triazole molecular hybrids from citronella essential oil. Mol Divers 23:189–193

Agarwal D, Gupta RD, Awasthi SK (2017) Are antimalarial hybrid molecules a close reality or a distant dream? Antimicrob Agents Chemother 61:1–12

Alfarouk KO, Stock C-M, Taylor S, Walsh M, Muddathir AK, Verduzco D, Bashir AHH, Mohammed OY, Elhassan GO, Harguindey S, Reshkin SJ, Ibrahim ME, Rauch C (2015) Resistance to cancer chemotherapy: failure in drug response from ADME to P-gp. Cancer Cell Int 15:71.

Anderson A, Bowman A, Boulton SJ, Manning P, Birch-Machin MA (2014) A role for human mitochondrial complex II in the production of reactive oxygen species in human skin. Redox Biol 2:1016–1022

Alqasoumi SI, Al-Taweel AM, Alafeefy AM, Ghorab MM, Noaman E (2010) Discovering some novel tetrahydroquinoline derivatives bearing the biologically active sulfonamide moiety as a new class of antitumor agents. Eur J Med Chem 45:1849–1853

Al-Sanea MM, El-Deeb IM, Lee SH (2013) Design, synthesis and in-vitro screening of new 1h-pyrazole and 1,2-isoxazole derivatives as potential inhibitors for ROS and mapk14 kinases. Bull Korean Chem Soc 34:437–442

Álvarez Santos MR, Duarte YB, Güiza FM, Romero Bohórquez AR, Mendez-Sanchez SC (2019) Effects of new tetrahydroquinoline-isoxazole hybrids on bioenergetics of hepatocarcinoma Hep-G2 cells and rat liver mitochondria. Chem Biol Interact. https://doi.org/10.1016/j.cbi.2019.02.002

Asolkar RN, Schroeder D, Heckmann R, Lang S, WagnerDoebler I, Laatsch H (2004) Marine bacteria XXVII. Helquinoline, a new tetrahydroquinoline antibiotic from Janibacter limosus Hel 1. J Antibiot 57:17–23

Berghe TV, Linkermann A, Jouan-Lanhouet S, Walczak H, Vandenabeele P (2014) Regulated necrosis: the expanding network of non-apoptotic cell death pathways. Nat Rev Mol Cell Biol 15:135–147

Bueno Y, Ramírez JV, Güiza FM, Méndez SC, Romero-Bohórquez AR (2018) Synthesis and in vitro evaluation of cytotoxic effect on cervical cancer cells (HELA) of tetrahydroquinoline-isoxazole hybrid derivatives. Res J Pharm Biol Chem Sci 9:420–432

Champa D, Orlacchio A, Patel B, Ranieri M, Shemetov AA, Verkhusha VV, Cuervo AM, Di Cristofano A (2016) Obatoclax kills anaplastic thyroid cancer cells by inducing lysosome neutralization and necrosis. Oncotarget 7:34453–34471

Chauhan R, Siddiqi AA, Dwivedi J (2012) An approach to regioselective synthesis of pyrazole and isoxazole derivatives and study of their antimicrobial effect. Pharm Chem J 46:316–320

Chen W, Lin Z, Ning M, Yang C, Yan X, Xie Y et al. (2007) Aza analogues of equol: novel ligands for estrogen receptor beta. Bioorg Med Chem 15:5828–5836

Cirrincione G, Barraja P, Diana P, Carbone A, Kelter G, Fiebig H-H (2010) Synthesis and antitumor activity of 2,5-bis(3'-indolyl)-furans and 3,5-bis(3'-indoliy)-isoxazoles, nortopsentin analogues. Bioorg Med Chem 18:4524–4529

Cooper G, Husman R (2007) La célula. Marbán Libros, Madrid

Cury-Boaventura MF, Pompéia C, Curi R (2004) Comparative toxicity of oleic acid and linoleic acid on Jurkat cells. Clin Nutr 23:721–732

Darwish ES, Atia KA, Farag AM (2014) Synthesis and antimicrobial evaluation of some isoxazole based heterocycles. Heterocycles 89:1393–1411

Divakaruni AS, Paradyse A, Ferrick DA, Murphy AN, Jastroch M (2014) Analysis and interpretation of microplate-based oxygen consumption and pH data. Methods Enzymol 547:275–307

Duan Y-T, Man R-J, Tang D-J, Yao Y-F, Tao X-X, Yu C, Liang X-Y, Makawana JA, Zou MJ, Wang Z-C, Zhu H-L (2016) Design, synthesis and antitumor activity of novel link-bridge and B-ring modified combretastatin A-4 (CA-4) analogues as potent antitubulin agents. Sci Rep 6:25387

Eddington ND, Cox DS, Roberts RR, Butcher RJ, Edafiogho IO, Stables JP, Cooke N, Goodwin AM, Smith CA, Scott KR (2002) Synthesis and anticonvulsant activity of enaminones: 4. Investigations on isoxazole derivatives. Eur J Med Chem 37:635–648

Faidallah HM, Rostom S (2013) Synthesis, in vitro antitumor evaluation and DNA-binding study of novel tetrahydroquinolines and some derived tricyclic and tetracyclic ring systems. Eur J Med Chem 63:133–143

Fang X-F, Li D, Reddy VK, Gopala L, Gao W-W, Zhou C-H (2017) Novel potentially antifungal hybrids of 5-flucytosine and fluconazole: design, synthesis and bioactive evaluation. Bioorg Med Chem Lett 27:4964–4969

Fato R, Bergamini C, Bortolus M, Maniero AL, Leoni S, Ohnishi T, Lenaz G (2009) Differential effects of mitochondrial Complex I inhibitors on production of reactive oxygen species. Biochim Biophys Acta 1787:384–392

Fonseca-Berzal C, Merchán DR, Romero Bohórquez AR, Escario JA, Kouznetsov VV, Gómez-Barrio A (2013) Selective activity of 2,4-diaryl-1,2,3,4-tetrahydroquinolines on Trypanosoma cruzi epimastigotes and amastigotes expressing β-galactosidase. Bioorg Med Chem Lett 23:4851–4856

Gao W, Xu K, Li P, Tang B (2011) Funtional roles of superoxide and hydrogen peroxide generated by mitochondrial DNA mutation in regulating tumorigenicity of HepG2 cells. Cell Biochem Funct 29:400–407

Germano D, Tinessa V, Barletta E, Cannella L, Daniele B (2013) Targeted therapy for advanced hepatocellular cancer in the elderly: focus on sorafenib. Drugs Aging 30:887–892

Goli N, Mainkar PS, Kotapalli SS, Tejaswini K, Ummanni R, Chandrasekhar S (2017) Expanding the tetrahydroquinoline pharmacophore. Bioorg Med Chem Lett 27:1714–1720

Gorrini C, Harris IS, Mak T (2013) Modulation of oxidative stress as an anticancer strategy. Nat Rev Drug Discov 12:931–947

Hensley CT, Wasti AT, DeBerardinis RJ (2016) Glutamine and cancer: cell biology, physiology, and clinical opportunities. J Clin Invest 123:3678–3684

Holohan C, Van Schaeybroeck S, Longley DB, Johnston PG (2016) Cancer drug resistance: an evolving paradigm. Nat Rev Cancer 13:714–726

Hou X, Luo H, Zhong H, Wu F, Zhou M, Zhang W, Han X, Yan G, Zhang M, Lu L, Ding Z, He G, Li R (2013) Analysis of furo[3,2-c]tetrahydroquinoline and pyrano[3,2-c]tetrahydroquinoline derivatives as antitumor agents and their metabolites by liquid chromatography/electrospray ionization tandem mass spectrometry. Rapid Commun Mass Spectrom 27:1222–1230

Ishiguro H, Toi M (2012) How to dose cytotoxic chemotherapeutic drugs. Curr Top Pharmacool 16:67–81

Kamal A, Bharathi EV, Reddy JS, Ramaiah MJ, Dastagiri D, Reddy MK, Viswanath A, Reddy TL, Shaik TB, Pushpavalli SN, Bhadra MP (2011) Synthesis and biological evaluation of 3,5-diaryl isoxazoline/isoxazole linked 2,3-dihydroquinazolinone hybrids as anticancer agents. Eur J Med Chem 46:691–703

Kerru N, Singh P, Koorbanally N, Raj R, Kumar V (2017) Recent advances (2015–2016) in anticancer hybrids. Eur J Med Chem 142:179–212

Kumar S, Bawa S, Gupta H (2009) Biological activities of quinoline derivatives. Min Rev Med Chem 9:1648–1654

Kumar A, Srivastava S, Gupta G, Chaturvedi V, Sinha S, Srivastava R (2011) Natural product inspired diversity oriented synthesis of tetrahydroquinoline scaffolds as antitubercular agent. ACS Comb Sci 13:65–71

Kumar B, Sharma P, Gupta VP, Khullar M, Singh S, Dogra N, Kumar V (2018) Synthesis and biological evaluation of pyrimidine bridged combretastatin derivatives as potential anticancer agents and mechanistic studies. Bioorg Chem 78:130–140

Kumbhare RM, Kosurkar UB, Janaki Ramaiah M, Dadmal TL, Pushpavalli SN, Pal-Bhadra M (2012) Synthesis and biological evaluation of novel triazoles and isoxazoles linked 2-phenyl benzothiazole as potential anticancer agents. Bioorg Med Chem Lett 22:5424–5427

Lam KH, Lee KK, Gambari R, Wong RS, Cheng GY, Tong SW, Chan KW, Lau FY, Lai PB, Wong WY, Chan AS, Kok SH, Tang JC, Chui CH (2013) Preparation of galipea officinalis hancock type tetrahydroquinoline alkaloid analogues as anti-tumour agents. Phytomedicine 20:166–171

LeBel CP, Ischiropoulos H, Bondy SC (1992) Evaluation of the probe 2′,7′-dichlorofluorescin as an indicator of reactive oxygen species formation and oxidative stress. Chem Res Toxicol 5:227–231

Lin CY, Chang TW, Hsieh WH, Hung MC, Lin IH, Lai SC, Tzeng YJ (2016) Simultaneous induction of apoptosis and necroptosis by Tanshinone IIA in human hepatocellular carcinoma HepG2 cells. Cell Death Discov 2:16065.

Lipinski CA, Lombardo F, Dominy BW, Feeney PJ (2012) Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv Drug Deliv Rev 64:4–17

Liu AL, Shu SH, Qin HL, Lee SM, Wang YT, Du GH (2009) In vitro anti-influenza viral activities of constituents from Caesalpinia sappan. Planta Med 75:337–339

Liu J, Yu LF, Eaton JB, Caldarone B, Cavino K, Ruiz C, Terry M, Fedolak A, Wang D, Ghavami A, Lowe DA, Brunner D, Lukas RJ, Kozikowski AP (2011) Discovery of isoxazole analogues of sazetidine-A as selective α4β2-nicotinic acetylcholine receptor partial agonists for the treatment of depression. J Med Chem 54:7280–7288

Manzione L, Grimaldi AM, Romano R, Ferrara D, Dinota A (2008) Hepatocarcinoma: from pathogenic mechanisms to target therapy. Oncol Rev 2:214–222

Mohammad RM, Muqbil I, Lowe L, Yedjou C, Hsu HY, Lin LT, Siegelin MD, Fimognari C, Kumar NB, Dou QP, Yang H (2015) Broad targeting of resistance to apoptosis in cancer. Semin Cancer Biol 35:S78–S103

Marshall KD, Baines CP (2014) Necroptosis: is there a role for mitochondria? Front Physiol. 5:323.

Mosmann T (1983) Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. J Immunol Methods 65:55–63

Muregi FW, Ishih A (2010) Next-generation antimalarial drugs: hybrid molecules as a new strategy in drug design. Drug Dev Res 71:20–32

Nwaukwa SO, Keehn PM (1989) Ring chlorination of benzenoid compounds using calcium hypochlorite [$Ca(OCl)_2$]. Synth Commun 19:799–804

Palanisamy P, Jenniefer SJ, Muthiah PT, Kumaresan S (2013) Synthesis, characterization, antimicrobial, anticancer, and antituberculosis activity of some new pyrazole, isoxazole, pyrimidine and benzodiazepine derivatives containing thiochromeno and benzothiepino moieties. RSC Adv 3:19300–19310

Palusa SKG, Udupi RH, Himabindu V (2011) Synthesis, antimicrobial and anti-inflammatory studies of isoxazole analogues of rosuvastatin. Der Pharma Chem 3:39–50

Pesi B, Giudici F, Moraldi L, Montesi G, Romagnoli S, Pinelli F, Batignani G (2016) Hepatocellular carcinoma on cirrhosis complicated with tumoral thrombi extended to the right atrium: results in three cases treated with major hepatectomy and thrombectomy under hypothermic cardiocirculatory arrest and literature review. World J Surg Oncol 14:83.

Philipp S, Sosna J, Adam D (2016) Cancer and necroptosis: friend or foe? Cell Mol Life Sci 73:2183–2193

Raymond E, Faivre SJ, Woynarowski JM, Chaney SG (1998) Oxaliplatin: mechanism of action and antineoplastic activity. Semin Oncol 25 2(suppl 5):4–12

Reczek CR, Chandel NS (2017) The two faces of reactive oxygen species in cancer. Annu Rev Cancer Biol 1:4.1–4.20

Reyes-Habito CM, Roh EK (2014) Cutaneous reactions to chemotherapeutic drugs and targeted therapy for cancer: part II. Targeted therapy. J Am Acad Dermatol 71:217.e1–217.e11

Rodríguez YA, Gutiérrez M, Ramírez D, Alzate-Morales J, Bernal CC, Güiza FM, Romero Bohórquez AR (2016) Novel N-allyl/propargyl tetrahydroquinolines: synthesis via three-component cationic imino Diels–Alder reaction, binding prediction, and evaluation as cholinesterase inhibitors. Chem Biol Drug Des 88:498–510

Romero Bohórquez AR, Escobar P, Leal SM, Kouznetsov VV (2012) In vitro activity against Trypanosoma cruzi and Leishmania

chagasi parasites of 2,4-diaryl 1,2,3,4-tetrahydroquinoline derivatives. Lett Drug Des Discov 9:802–808
Rudenko DA, Shavrina TV, Shurov SN, Zykova SS (2014) Synthesis and antioxidant activity of tricyclic compounds containing a 5,6,7,8-tetrahydroquinoline moiety. Pharm Chem J 48:100–103
Sangshetti JN, Khan K, Firoz AK, Abhishek AA, Rohidas P, Rajendra H (2015) Antileishmanial drug discovery: comprehensive review of the last 10 years. RSC Adv 5:32376–32415
Sharma S, Gupta MK, Saxena AK, Singh Bedi PM (2017) Thiazolidinone constraint combretastatin analogs as novel antitubulin agents: design, synthesis, biological evaluation and docking studies. Anticancer Agents Med Chem 17:230–240
Shen YC, Ou DL, Hsu C, Lin KL, Chang CY, Lin CY, Liu SH, Cheng AL (2013) Activating oxidative phosphorylation by a pyruvate dehydrogenase kinase inhibitor overcomes sorafenib resistance of hepatocellular carcinoma. Br J Cancer 108:72–81
Singer JM, Barr BM, Coughenour LL, Walters MA (2005) 8-Substituted 3,4-dihydroquinolinones as a novel scaffold for atypical antipsychotic activity. Bioorg Med Chem Lett 15:4560–4563
Sridharan V, Suryavanshi PA, Menéndez JC (2011) Advances in the chemistry of tetrahydroquinolines. Chem Rev 111:7157–7259
Su Z, Yang Z, Xie L, Dewitt JP, Chen Y (2016) Cancer therapy in the necroptosis era. Cell Death Differ 23:748–756
Sysak A, Obmińska-Mrukowicz B (2017) Isoxazole ring as a useful scaffold in a search for new therapeutic agents. Eur J Med Chem 137:292–309
Tartarone A, Lazzari C, Lerose R, Conteduca V, Improta G, Zupa A, Bulotta A, Aieta M, Gregorc V (2013) Mechanisms of resistance to EGFR tyrosine kinase inhibitors gefitinib/erlotinib and to ALK inhibitor crizotinib. Lung Cancer 81:328–36
Veeraswamy B, Kurumurthy C, Kumar GS, Rao PS, Thelakkat K, Kotamraju S, Narsaiah B (2012) Synthesis of novel 5-substituted isoxazole-3-carboxamide derivatives and cytotoxicity studies on lung cancer cell line. Indian J Chem Sect B 51:1369–1375
Vermes I, Haanen C, Steffens-Nakken H, Reutellingsperger C (1995) A novel assay for apoptosis flow cytometric detection of phosphatidylserine expression on early apoptotic cells using fluorescein labelled Annexin V. J Immunol Methods 184:39–51
Wu W, Liu P, Li J (2012) Necroptosis: an emerging form of programmed cell death. Crit Rev Oncol Hematol 82:249–258
Zhang HZ, Zhao ZL, Zhou CH (2018) Recent advance in oxazole-based medicinal chemistry. Eur J Med Chem 144:444–492
Zhao J-J, Yan T, Zhao H, Zhou J-G, Huang Z, Zhang Y-F, Cai J-Q (2015) Evaluation of eight different clinical staging systems associated with overall survival of Chinese patients with hepatocellular carcinoma. Chin Med J 128:316–321
Zhou C, Chen Z, Lu X, Wu H, Yang Q, Xu D (2016) Icaritin activates JNK-dependent mPTP necrosis pathway in colorectal cancer cells. Tumor Biology 37:3135–3144
Zhu Y, Cheng Y, Li A (2013) Mechanisms of drug resistance to sorafenib in hepatocellular carcinoma. Chin Pharmacol Bull 6:752–755