ACADEMIA ROMÂNĂ
Revue Roumaine de Chimie
http://web.icf.ro/rrch/

Rev. Roum. Chim.,
2018, *63*(12), 1123-1134

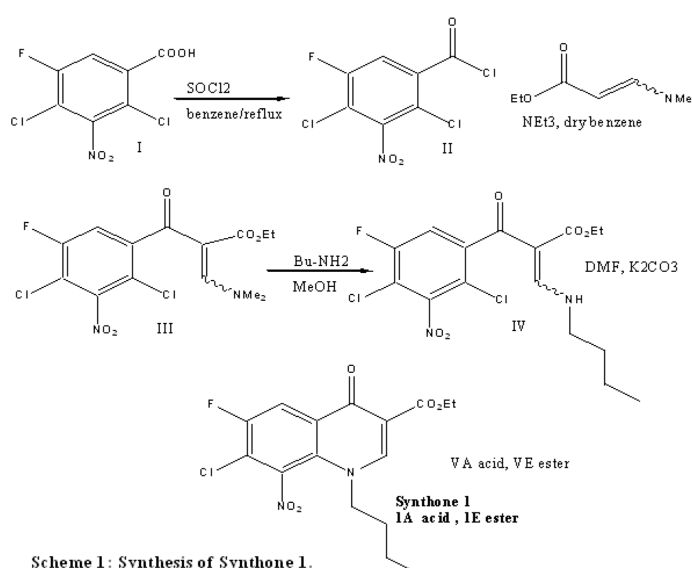
IN VITRO MODULATION OF PANCREATIC LIPASE AND PROLIFERATION OF OBESITY RELATED COLORECTAL CANCER CELL LINE PANEL BY NOVEL SYNTHETIC FLUOROQUINOLONES[**]

Yousef ALABSI,[a] Yusuf AL-HIARI,[a] Violet KASABRI,[*a] Shereen ARABIYAT,[b] Rabab BASHITI,[a] Sundos ALALAWI[a] and Rula AL-SHAHRABI[a]

[a]The University of Jordan, School of Pharmacy, Queen Rania Street, Amman 11942, Jordan
[b] Salt College, Al-Balqa Applied University, Salt 19117, Jordan

Received December 21, 2017

Ten novel fluoroquinolones (FQs) were investigated *in vitro* for pancreatic lipase (PL) inhibitory propensities in comparison to orlistat, the robust reference agent for potency and efficacy determinations. In comparison to the antineoplastic agent cisplatin; the assessment of antiproliferative properties of these novel FQs in a panel of colorectal carcinoma (CRC) cell lines (HT29, HCT116, SW620, CACO2, SW480) and normal periodontal ligament fibroblasts for safety examination was performed. Antioxidant properties were proposed as the mechanism of antiproliferative activity, as seen by the DPPH test used to analyze radical scavenging potency of FQs vs. ascorbic acid was undertaken. Compounds **3b** and **4b** manifested moderate activity in PL inhibition (IC_{50} value µM: 2.3 and 2.8 respectively) vs. orlistat (0.2 µM) antilipolytic properties. The amino group of compounds revealed higher potency compared to nitro and triazolo groups, also among each group, the *meta* substituted compounds were more potent compared to the *ortho* and *para*. Moreover, compounds **1A, 4a** and **4c** revealed high antiproliferative potency (IC_{50} values (µM): 1.1 (significantly more potent) on cell line HT29, 12.4 on cell line HT29 and 7.1 on cell line SW620, respectively) with appreciable higher safety compared to cisplatin (6.9 µM on cell line HT29). As for antioxidative radical scavenging potentials, novel FQs compounds **4a**, **5a**, **3b**, and **4c** exerted antioxidant powers ((IC_{50} values (µM) 35.9, 67.1, 49.7, and 38.6, respectively)) comparable to (no significant difference in **4a** and **4c**) natural antioxidative ascorbic acid (38.8 µM), same compounds demonstrated appreciable CRC antiproliferative effects.



Scheme 1: Synthesis of Synthone 1.

INTRODUCTION

Obesity is increasing at alarming rates causing a substantial burden on health care systems. [1] There are many links between obesity and other debilitating diseases like asthma, depression and musculoskeletal abnormalities; [2,3] as for cancer, it is estimated that obesity is causing 30% of cancer

[*] Corresponding author: hotice162@gmail.com
[**]Supplementary information on http://web.icf.ro/rrch/ or http://revroum.lew.ro/

cases. [4,5] One of the most commonly prescribed obesity treatment medications is orlistat. It is an inhibitor of enzyme pancreatic lipase (PL) responsible for triglycerides breakdown, and inhibition of such enzyme hinders the transformation of dietary fats to an absorbable form.[6,7] FQs and related triazolo-fluoroquinoles were reported as stronger PL inhibitors and antiproliferative compounds.[8,9] Previous studies discovered natural PL inhibitors, [10,11] other research developed synthetic PL inhibitors,[12,13a,b] Antioxidant properties of chemopreventive compounds are the suspected mechanism in many conducted studies.[14,15a,b,c] The aim in this study is to test novel FQ compounds that can be used for antiobesity and anticancer management with fewer side effects. Their anticipated chemopreventive-antioxidative action mechanism may aid combating obesity-related CRC.

RESULTS AND DISCUSSION

In vitro testing of FQ compounds for inhibitory PL activity

In this study, The IC_{50} value of the standard compound orlistat was 0.2 µM, which was comparable to the values cited in the literature.[17] A set of 10 novel FQ compounds were developed and tested for PL, and anticolorectal adenocarcinoma proliferative properties. Most compounds showed good inhibitory PL activity with IC_{50} values below 30 µM. In fact, 2 compounds revealed some excellent inhibitory PL activity. PL-IC_{50}values of compounds **3b** and **4b** (2.3±0.2 and 2.8±0.4, µM, respectively) demonstrate their extraordinary PL inhibitory efficacy. Although both exhibited weaker activity compared to the reference drug orlistat with a statistically significant deference ($P < 0.001$), however they are the only available novel synthetic compounds to achieve PL inhibitory potency below 5 µM along with those prepared by our group.[16] The IC_{50} value of the standard compound orlistat was 0.2 µM, which was comparable to the values cited in the literature.[17] In this present study, the tested compounds were recognized for their dose-dependent PL inhibitory activity. Figure 1 below demonstrates comparison between FQs chemical groups and substitution positions in relation to their PL modulation. It can be noticed that among each chemical group, the *meta* substituted compound revealed higher potency contrary to *ortho* and *para* substituted compounds, also the amino group of compounds revealed higher potency compared to the nitro and triazolo compounds.

Letters (**a**, **b** and **c**) represent test compounds names within the related group number as follows Nitro group (**3a**, **3b** and **3c**), Reduced group (**4a**, **4b** and **4c**), Triazole group (**5a**, **5b** and **5c**). While **a** indicate *ortho* position of the methoxy group at the C7 methoxy-aniline substitution, **b** represent *meta* position of the methoxy group and **c** represent *para* position.

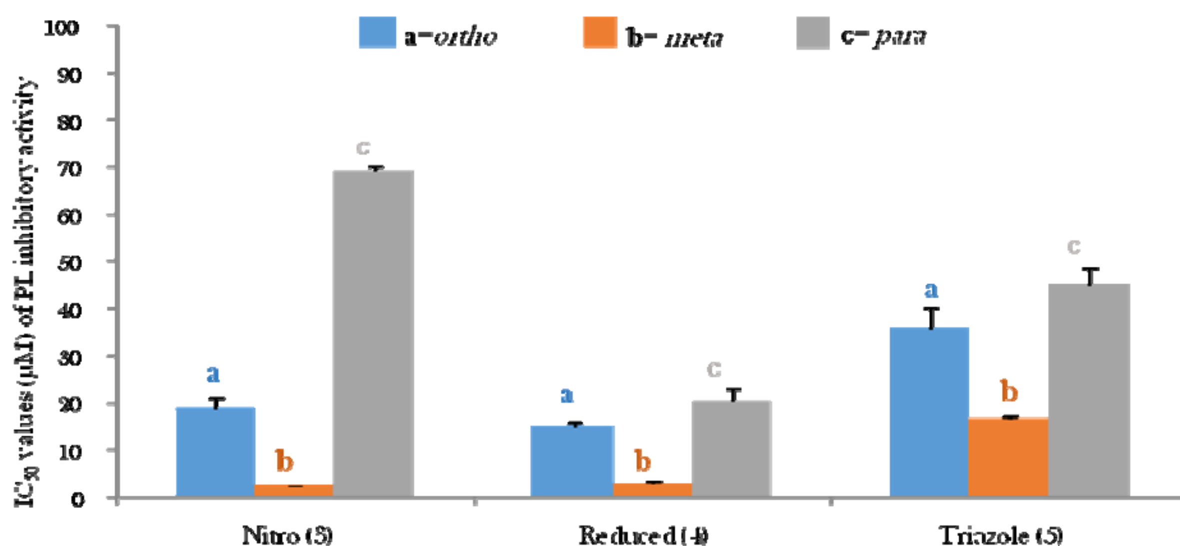


Fig. 1 – IC_{50} values (µM) of PL inhibitory activity of FQ test compounds.

Table 1

PL inhibitory IC_{50} values μM (μg/mL) of orlistat and test FQ compounds

Treatment	PL-IC_{50}value μM (μg/mL)	C Log P	P value
1A	257.6±15.3 (95.5±5.7)	3.312	<0.0001
3a	18.8±2.1 (8.1±0.9)	4.93	<0.0001
4a	14.9±0.9 (6±0.4)	3.62	<0.0001
5a	35.9±4.4(14.4±1.7)	4.39	<0.0001
3b	**2.3±0.2** (1±0.1)	4.93	0.0001
4b	**2.8±0.4** (1.1±0.2)	3.62	0.0001
5b	16.5±0.6 (6.8±0.3)	4.39	<0.0001
3c	69.1±1.2 (29.7±0.5)	4.93	<0.0001
4c	20.2±2.7 (8.1±1.1)	3.62	0.0002
5c	24.3±3.5 (10.4±1.1)	4.39	0.0003
Orlistat	0.2±0 (0.1±0)	8.56	-

Results are mean ± SD (n = 3-4 independent replicates). ClogP was calculated using ChemDrawUltraV 11, 2008. *P* value is calculated by unpaired t-test between test compound IC_{50} values μM and orlistat IC_{50} value μM using GraphPad Prism software version 5.01. * when $P < 0.05$ and ** when $P < 0.01$ or 0.001.

Table 2

In vitro antiproliferative activities IC_{50} values (μM) (mean ± SD)
and selective cytotoxicity index of cisplatin and selected Test FQ compounds on CRC cell lines

Treatment	HT29	HCT116	SW620	SW480
1A	**1.1±0.1**(**307**)	**30.3±4 (11)**	25±3.9**(13)**	12.4±1.7 (**26.8**)
4a	**12.2±0.8**(4)	111.9±14.4 (0.4)	240±40.6 (0.2)	54.2±9.8 (0.9)
5a	25.4±2 (**26.6**)	232.2±25.5 (2.8)	422±36.9 (1.6)	81.31±10.7 (8)
3b	35.4±5.1 (7.2)	388.7±19.8 (0.6)	85.7±16.9 (3)	168.8±21.2 (7)
4c	25.1±2.7 (1.7)	33.8±4.9 (1.4)	**7.1±0.3 (6.8)**	52±7.6 (0.9)
5c	288.6±28 (0.5)	78.2±6 (1.5)	56±0.3 (2.2)	100±17.2 (1.2)
Cisplatin	6.9±0.5 (5.2)	38±0.1 (0.2)	5.7±0.9 (1.2)	5.3±0.7 (1.3)

Antiproliferative activity assay of studied compounds in obesity related CRC cell lines

Antiproliferative effectiveness of tested FQ derivatives against a panel of CRC cell lines and PDL fibroblasts was demonstrated with respective IC_{50} values (Table 2). Each cell line manifested a distinctive response profile to the set of 10 test compounds. Cisplatin antiproliferative efficacies in all colorectal carcinomas were further illustrated, last column indicates selective cytotoxicity index (the same Table). It can be noticed from table 4 that; compound **1A** exerted significantly ($P<0.001$) more potent cytotoxic activity than cisplatin in cell line HT29. In addition, compound **4c** demonstrated equal cytotoxic potency compared cisplatin on both cell lines HT116 and SW620 with no statistical deference ($P>0.05$) in comparable IC_{50}values. Table 3 below summarises promising FQ compounds with their selective cytotoxicity index.

To unambiguously portray and elaborate extracted data from IC_{50} values, a ratio indicator called selective cytotoxicity index is used to express ratio of antiproliferative IC_{50} exerted on normal cell line divided by IC_{50} value of investigated neoplastic cell line. Selective cytotoxicity index can predict safety of studied compounds by indicating its selective cytotoxicity for neoplastic cells compared to normal cells. [18] For example; in cell line HT29, cisplatin control drug exhibit index of 1, in other words, same concentration exerts same effect on both normal and neoplastic cell lines, this indicates poor selective cytotoxicity and safety, however; test compound **1A** revealed an outstanding index of 307 In other words, to achieve equal activity on normal cell lines 307 times the concentration is need, which characterise extremely high selective cytotoxicity for neoplastic cells and accordingly high safety. Compared to reference antineoplastic cisplatin, this study has revealed that 3 FQs of the tested compounds demonstrated a high antiproliferative properties on 5 CRC cell lines. In fact, some of these newly prepared FQs demonstrated much higher potency than the reference antineoplastic cisplatin. Furthermore, these

3 compounds unveiled an outstanding selective cytotoxicity to CRC cell lines over normal one; with superior selective cytotoxicity index exhibited by 2 compounds. Such advantage provides an excellent solution to a laborious and tedious challenge often faced in developing anticancer medication (Tables 2 and 3).[19] Most of anticancer drugs cannot differentiate between normal and neoplastic cells, rendering them very toxic and producing very hazardous side effects. In fact, some of the reported side effects is new neoplastic tissues developed, because of high activity of indiscriminate killing of cells that will induce a rebound uncontrolled growth (American Cancer Society, 2017), which is cancer per se. Developing a more selective compound with a high selective cytotoxicity index[18] can achieve a safety profile that seldom found in anticancer medications. Each cell line manifested a distinctive response profile to the set of 10 test compounds. In cell line HT29; Test compound **1A** exerted quite potent (1.1μM IC_{50}) antiproliferative activity and significantly more potent (P<0.001) than antineoplastic cisplatin (6.9μM IC_{50}). Moreover, compound **1A** exhibited an outstanding selective cytotoxicity index of 307, in other terms, to a certain amount of this compound, for each 307CRC cells infarct by compound, only one normal cell infarct will occur by the same amount of the compound. This indicates significant safety profile, unlike the antineopalstic cisplatin that only posed a selective cytotoxicity index of 1, presenting indiscrimination and lack of targeting. Moreover, compound **1A** exerted outstanding activity on all tested cell lines, offering more potent effect than reference drug against three cell lines. Compound **4a** and **5a** (*ortho* substituted aniline) also demonstrated relatively less competitive potency compared to cisplatin (12.2, 25.4 and 6.9 μM IC_{50}, respectively).Same compounds exhibited superior selective cytotoxicity index of 4 and 26 respectively; with clear safety profile manifested on PDL fibroblasts. Test compounds **3b** and **4c** also showed advantage over antineoplastic cisplatin in comparison of safety with diminished activity on normal cell line. A promising finding exhibited in test compound **3c** revealed promising predictable safety with complete lack of cytotoxicity on normal cell line (Fibroblasts) within tested concentration range of 0.1-200 μg/mL; however, posing less potency compared to antineoplastic cisplatin on cell line HT29.

In cell line HCT116, comparing results in this line can be deceptive from first sight, since cisplatin drug and test compound **1A** in this cell line displayed equality in IC_{50} values, however, if we take into consideration the factor of selective cytotoxicity, test compound **1A** exhibited an appreciable selective cytotoxicity index of 11, implying that compound **1A** had 11 times more antiproliferative potency on neoplastic cell line HCT116 than normal cell line. On the other hand, cisplatin posed an IC_{50} value to all cell lines exceeding that of normal fibroblasts cell line. In other terms, very poor selective cytotoxicity index of 0.2 indicating that cisplatin is more selective to normal cell line than HCT116 cell line. A brief digression, this implies that the use of cisplatin in patients with tissue morphology resembling cell line HCT116 could produce disastrous consequences since this drug is more selective to normal tissue than CRC cells in this case. More about this cell line, fortunately test compound **4c** manifested comparable (statistically no significant deference (P>0.05)) potency to cisplatin (33 and 38 μM, respectively) with superior selective cytotoxicity index of 1.4 characterizing a safety of 7 times compared to cisplatin. Although test compound **5c** was less potent than cisplatin, it revealed a selective cytotoxicity index of 4, also providing more safety (Tables 2 and 3).

Cell line SW620, test compound **1A** exhibited potency of 25μM compared to 5.7 μM in cisplatin; nonetheless, it unveiled a promising selective cytotoxicity index of 13, implying superior selective cytotoxicity and hence safety in test compound **1A**. Compound **4c** demonstrated an excellent potency manifested by IC_{50} value 7.1μM that is very competitive (statistically no significant deference (P > 0.05)) to that of cisplatin, with a high selective cytotoxicity index of 6.8.This provides an excellent candidate for research and development. Cell line SW480, competitive antiproliferative activity of compound **1A** manifested by IC_{50} potency of 12.4 μM, with a considerable selective cytotoxicity index of 27, featuring high safety. From chemistry point of view few observations can be addressed. Compound **1A** with no phenyl at C7 has revealed the best and comparable activity. It is observed that, adding a phenyl substitution at C-7 has reduced antiproliferative activity against all cell lines (Figure 2). On the other hand, the activities of our compounds were mainly exerted against HT29 cell line by *ortho* anisidine derivatives **3a**, **4a** and **5a** (Figure 2). Figure 2 below demonstrates comparison between FQs chemical groups and substitution positions in relation to cytotoxic activity on HT29 colorectal cell line.

Table 3

IC_{50} values of in vitro antiproliferative activities of cisplatin and test FQ compounds on CRC cell lines

	Cytotoxicity (as of % Control) IC_{50} value (µg/mL (µM))						selective cytotoxicity index♦
Treatment	HT29	HCT116	SW620	CACO2	SW480	PDL Fibroblasts	Value (related cell line)
1A	**0.4±0(1.1**±0.1)**	**11.2±1.5 (30.3*±4)**	**9.3±1.5 (25**±3.9)**	24.1±4.0 (65**±10.8)	4.6±0.5 (12.4**±1.4)	123.1±17.6 (331.9**±47.4)	**307.5** (HT29)
3a	22±0 (51**±0.1)	657.6±103.3 (1531.4**±240.7)	NI	309.7±29.2 (721.1**±68.1)	441.7±50.9 (1029**±118)	13.0±0.5 (30.3**±1.2)	0.6 (HT29)
4a	4.9±0.3 **(12.2**±0.8)**	44.7±5.8 (111.9**±14.4)	95.8±16.2 (240**±40.6)	50.3±5.4 (126**±13.4)	21.6±3.9 (54.2**±9.8)	19.6±2.6 (49.2**±9.4)	4.0 (HT29)
5a	10.4±0.8 **(25.4**±2.0)**	95.3±10.5 (232.2**±25.5)	173.2±15.1 (422**±36.9)	39.5±4.1 (96.3**±10.1)	33.4±4.3 (81.3**±10.47)	273.6±30.6 (666.8**±74.5)	**27.3** (HT29)
3b	15.2±2.2 **(35.4**±5.1)**	166.9±8.5 (388.7**±19.8)	36.8±7.1 (85.7**±16.5)	34.4±5.9 (80.1**±13.7)	72.5±9.1 (168.8**±21.2)	108.8±15.7 (253.4**±36.6)	7.3 (HT29)
4b	43.4±6.4 (108.6**±16.2)	38.2±1.9 (95.6**±4.7)	27.3±2.4 (68.3**±6.0)	50.9±6.1 (127.4**±1.3)	32.3±4.9 (80.9**±12.3)	49.1±5.6 (123**±14.3)	1.8 (SW620)
5b	150.8±16.9 (367.4**±41.2)	237.5±4 (578.8**±9.8)	47.7±2.3 (116.2**±5.6)	381±11.4 (928.4**±27.9)	109.1±15.8 (265.8**±38.5)	292.4±54 (712.5**±131.5)	6.2 (HT29)
3c	29.6±6.3 (68.9**±14.7)	NI	48.7±9.2 (113.3**±21.5)	176.0±20.7 (409.8**±48.3)	902.9±25.4 (2102.8**±59)	NI	NI
4c	10±1.1 **(25.1**±2.7)**	13.5±1.9 **(33.8 NS ±4.9)**	2.8±0.1 **(7.1 NS ±0.3)**	23.3±3.9 (58.3**±9.8)	21.0±3 (52.6**±7.6)	17.1±2.8 (48.6**±6.10)	**7** (SW620)
5c	93.8±11.5 (228.6**±28.1)	32.1±2.5 (78.2**±6)	23±1.9 (56**±4.5)	60.6±6.1 (135.5**±14.8)	41.0±7 (100**±17.2)	50.6±1.4 (123.3**±3.4)	2.2 (SW620)
Cisplatin	2.1±0.2 (6.9±0.5)	11.4±0 (38±0.1)	1.7±0.3 (5.7±0.9)	0.4±0 (1.3±0)	1.6±0.2 (5.3±0.7)	2.1±0.2 (7±0.7)	5.2 (CACO2)

♦Only highest selective cytotoxicity index is shown that calculated in least IC_{50} value among CRC cell line stated in same column.

Results are mean ± SD (n = 3-4 independent replicates). IC_{50} values (concentration at which 50% inhibition of cell proliferation took place in comparison to non-induced basal 72 h incubations) were calculated within 0.1-200 µg/mL range. NI is lack of cytotoxicity within the tested 0.1-200 µg/mL concentration range. P-value calculated by unpaired t-test between test compound IC_{50} values and cisplatin's (µM) using GraphPad Prism software version 5.0.1.* when $P < 0.05$ and ** when $P < 0.01$ or 0.001, NS: not significantly different from reference agent.

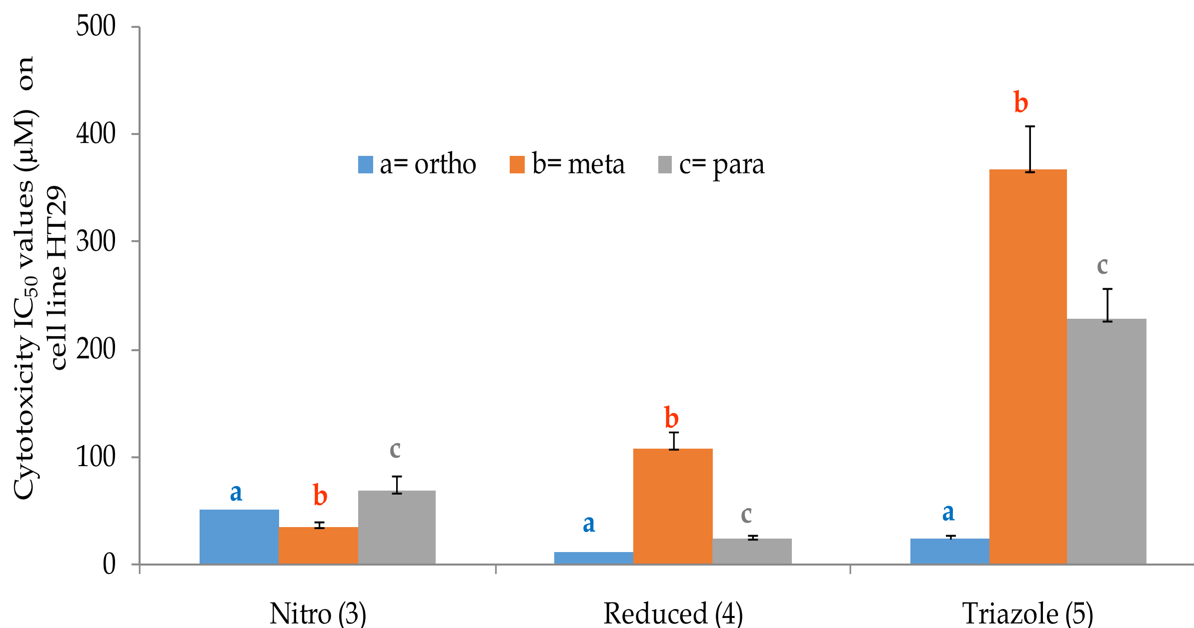


Fig. 2 – **Cytotoxicity IC_{50} values (µM) of tested FQs on cell line HT29** Numbers (**3**,**4**,**5**) represent test compounds related group as follows Nitro group (**3**), Reduced group (**4**), Triazole group (**5**). Other part is letters **a, b** and **c**, litter **a** indicate *ortho* position of the methoxy group at the C7 methoxy-aniline substitution, **b** represent *meta* position of the methoxy group and **c** represent *para* position.

Antioxidant properties of tested compounds

In comparison to ascorbic acid, the robust and classical radical scavenging and antioxidative reference agent; compounds **4a** and **4c** were equally potent radical scavenging treatments (statistically no significant deference (P > 0.05)) (Table 4). Other compounds also exerted comparable antioxidant activity. Specifically, compounds **5a** and **4b** were ascribed moderate radical scavenging efficacy in the tested range of 0.2-100 g/mL; the same range was used for ascorbic acid as well (the same table). In correspondence with the hypothesis that radical scavenging properties could illuminate antiproliferative activity, several findings in current study on our set of test compounds shed some light to that theory.

In Table 5 below, a direct insight is observed about correlation between antioxidant and antiproliferative activity. Apart from compound 1A, it was noticed that the most active antiproliferative FQs **4a**, **5a**, **4b**, **4c** are the only FQs which showed radical scavenging effects (Table 5). In fact, the highest cytotoxic activity IC_{50} values were at **4a** and **4c**, have also displayed the highest DPPH radical scavenging activity similar (statistically no significant deference (P > 0.05)) to ascorbic acid (Table 3). Furthermore, compounds **5a** and **4b** showed appreciable radical scavenging IC_{50} potency.

Table 4

IC_{50} values µM (µg/mL) of DPPH radical scavenging activity of ascorbic acid and FQ compounds

Treatment	IC_{50} value µM (µg/mL)	P value
1A	**NI**	
3a	**NI**	
4a	**35.9 ^{NS} ±1.5 (14.3±0.6)**	**0.3606**
5a	**67.1**±3.1 (27.6±1.3)**	**0.0003**
3b	**NI**	
4b	**49.7**±1 (19.9±0.4)**	**0.0028**
5b	**NI**	
3c	**NI**	
4c	**38.6^{NS}±2.6 (15.4±1.1)**	**0.9306**
5c	1020.8**±67.5 (418.9±27.7)	<0.0001
Ascorbic acid	38.8±2.7 (6.8±1)	-

Results are mean ± SD (n = 3-4 independent replicates). IC_{50} value is concentration at which 50% reduction of DPPH took place in comparison to non-induced basal incubations. NI: Non-Inhibitory in the tested 0.2-100 µg/mL range of concentration. P value is calculated by unpaired t-test between test compound IC_{50} values µM and ascorbic acid IC_{50} value µM using GraphPad Prism software version 5.01.* when P < 0.05 and ** when P < 0.01 or 0.001, NS: not significantly different from reference agent.

Table 5
Relation of DPPH radical scavenging activity of promising FQ compounds with their colorectal cytotoxicity

Treatment	DPPH radical scavenging activity IC_{50} value (µM)	Cytotoxicity IC_{50} values (µM) (related cell line)	Hoffman's *et al.*, (2011)[41] selective cytotoxicity index
4a	35.9±1.5	12.2 ±0.8 (HT29)	4
5a	67.1±3.1	25.4 ±2.0 (HT29)	26.2
4b	49.7±1	68.3±6.0 (SW620)	1.8
4c	38.6±2.6	7.1±0.3 (SW620)	6.8
Ascorbic acid	38.8±2.7		

Within our novel FQs, noticeable antioxidant activity of potent antiproliferative compounds, supports the hypothesis of antioxidant mechanism in contributing to chemo preventive and antiproliferative activity. Such phenomenon has a precedent in the literature, which also augments to this hypothesis. [20]

EXPERIMENTAL

Synthetic preparation

Materials and instruments

All reagents and chemicals of analytical grade were procured from Sigma (Dorset, UK); unless otherwise stated. All chemicals, reagents and solvents were used directly without further purification. Starting materials involved in the preparation are: 2,4-Dichloro-5-fluoro-3-nitrobenzoic acid was purchased from Sigma-Aldrich (St. Luis, MO, USA). Ethyl 3-(*N*,*N*-dimethyl-amino) acrylate and butyl amine were from Acros, Belgium. Primary aromatic amines; *o*-anisidine, *m*-anisidine, and *p*-anisidine are from Merck, (Darmstadt, Germany. Reducing agent, anhydrous stannous chloride crystals, was purchased from Fluka, (Switzerland). Sodium nitrate was purchased from Sigma Aldrich (St. Luis, MO, USA). Orlistat (Sigma, St. Luis, MO, USA), Crude porcine PL type II, (Sigma, St. Luis, MO, USA, EC 3.1.1.3), Tris-HCl buffer (2.5 mM, pH 7.4, Promega Corp. WI, USA) *para*-nitrophenyl butyrate (*p*-NPB, Sigma, St. Luis, MO, USA), cisplatin (Sigma, St. Luis, MO, USA), SpectroScan 80D UV-VIS spectrophotometer (Sedico Ltd., Nicosia, Cyprus). CRC cell lines HT29, HCT116, SW620, SW480 and CACO2 were cultured in DMEM containing 10% FBS (Bio Whittaker, Verviers, Belgium). Fetal bovine serum (FBS), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) Buffer, L-glutamine, gentamicin, penicillin, and streptomycin sulfate (Sigma, St. Luis, MO, USA). Sulforhodamine B (SRB; Santa Cruz Biotechnology, Inc. Texas, USA). Melting points (mp) were determined in open capillaries on a Stuart scientific electro-thermal melting point apparatus (Stuart, Staffordshire, UK) and are uncorrected. Thin layer chromatography (TLC) was performed on 10 x 10 cm^2 aluminum plates pre-coated with fluorescent silica gel GF254 (ALBET, Germany) and was visualized using UV lamp (at 254nm wave length/ short wave length/ long wavelength). Mobile phase mixtures were: 95:5 chloroform-methanol ($CHCl_3$-MeOH) (system 1) and 50:50 (*n*-hexane - Ethyl actate) (system 2), in some cases were studied compound did not mobilize, alternative system with mobile phase mixtures of system (**3**): Chloroform: methanol: formic acid ($CHCl_3$: MeOH: FA) (98: 2: 1) was used. TLC plates were visualized under UV lamp, Spectroline cabinet, Model CX-20, (USA). Nuclear Magnetic Resonance Spectra (NMR) were recorded on Bruker, Avance DPX-300 spectrometer, Bruker Avance-400 (400 MHz) Ultrashield spectrometer, and on Bruker 500 MHz-Avance III (500 MHz) at Hamdi Mango Center at University of Jordan. Chemical shifts are reported in ppm related to tetramethylsilane (TMS) as the internal standard. Deuteriated dimethylsulfoxide (DMSO-d_6) and deuteriated chloroform ($CDCl_3$) were used as solvents in sample preparation, otherwise is indicated. The chemical shifts were reported in ppm relative to tetramethylsilane (TMS), which was used as an internal reference standard. ^{1}H NMR data are reported (see appendix) in the following way; chemical shift (ppm), (multiplicity, coupling constant (Hz), number of protons, the corresponding proton(s). High resolution mass spectra (HRMS) were measured in positive ion mode using electrospray ionization (ESI) technique by collision-induced dissociation on a Bruker APEX-4 (7 Tesla) instrument. The samples were dissolved in acetonitrile, diluted in spray solution (methanol/water 1:1 v/v + 0.1 formic acid) and infused using a syringe pump with a flow rate of 2µL/min. External calibration was conducted using Arginine cluster in a mass range m/z 175-871. Synthesizing 1-Butyl-7-chloro-6-fluoro-8-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**1A**), introduction of (*ortho*-methoxy-aniline) substitutions at C-7 (intermediate **2**), hydrolysis (intermediate **3**), reduction (intermediate **4**) and formation of triazol-derivatives (intermediate **5**) was carried out according to the following schemes (Schemes 1 and 2) procedure.[21] Synthesis of synthone **1A** was carried out successfully for the first time producing novel intermediates **I-V** and final synthones **1A** and **1E** (Scheme 1) in large amounts and high yields (≈ 90 %).

***Ethyl (2Z)-2-[(2,4-dichloro-5-fluoro-3-nitrophenyl)carbonyl]-3-(dimethylamino)prop-2-enoate* (III) or *Ethyl-3-(N,N-dimethylamino)-2-(2,4-dichloro-5-fluoro-3-nitrobenzoyl) acrylate* (III) (Scheme 1)**

This compound was prepared by refluxing a mixture of 2,4-dichloro-5-fluoro-3-nitrobenzoic acid (**I**), (10.2 g, 40 mmol), and thionyl chloride ($SOCl_2$) (19.0 g, 160 mmol), dissolved in dry benzene (120 ml) at 75-80 °C for 3–4 h under anhydrous conditions. The mixture was then distilled off under reduced pressure to remove solvent and excess thionyl chloride. Dry benzene was then added twice (2 x 20 ml) into the reaction vessel and the mixture was re-distilled so as to remove traces of thionyl chloride. The resulting 2,4-dichloro-5-fluoro-3-nitrobenzoyl chloride (**II**), formed as thick oil, was used as such for the next step without further purification. To a stirred and cooled (5-10 °C) solution of ethyl 3-(*N*,*N*-dimethyl-amino)acrylate (6.3 g, 44 mmol) and triethylamine (4 ml, 8.1 g, 80 mmol) in dry benzene (50 ml), a solution of the crude acid chloride (prepared above) in dry benzene (25 ml)

was added drop by drop. The resulting mixture was stirred continuously for 2 h at room temperature under anhydrous conditions. Then, the solution was refluxed at 90 °C for 90 minutes. This crude product was evaporated to dryness, re-dissolved in chloroform; the chloroform was extracted with water (30 ml) and dried (with anhydrous $MgSO_4$). The solvent, chloroform, was then evaporated to dryness under reduced pressure. The residual product (about 20 ml) was soaked in methanol (10 ml) whereby the title compound **III** was obtained as a yellowish powder that was collected by suction filtration and dried, mp = 140-141 °C (decomposition), yield 13.8 g (91 %), *Rf* value in system (1) = 0.89 and in system (2) = 0.850. **^{1}H NMR** (500 MHz, $CDCl_3$): δ 0.95 (t, J = 7.1 Hz, 3H, OCH_2CH_3), 2.97 (s, 3H) and 3.37 (s, 3H) [$N(CH_3)_2$], 3.94 (q, J = 7.1 Hz, 2H, OCH_2Me), 7.27 (d, $^3J_{H-F}$ = 8.2 Hz, 1H, H-6′), 7.91 (br s, 1H, *N*-C(3)-H). **^{13}C NMR** (75 MHz, $CDCl_3$): δ 13.8 (CH_3CH_2), 43.3, 48.4 [$N(CH_3)_2$], 60.2 (CH_2Me), 100.9 (C-2), 114.5 (d,$^2J_{C-F}$ = 23.3 Hz, C-4′), 116.9 (d, $^2J_{C-F}$ = 23.1 Hz, C-6′), 118.2 (d, $^3J_{C-F}$ = 4.5 Hz, C-1′), 144.2 (d, $^3J_{C-F}$ = 6 Hz, C-3′), 148.8 (br d, $^4J_{C-F}$ = 1.3 Hz, C-2′), 156.6 (d, $^1J_{C-F}$ = 254 Hz, C-5′), 160.5 (*N*-C-3), 166.5 (CO_2Et), 185.1 (C = O). HRMS ((+ve)-ESI): *m/z* calculated for $C_{14}H_{14}Cl_2FN_2O_5$ $[M+H]^+$: 379.02638, found: 379.02591. Calculated for $C_{14}H_{13}Cl_2FN_2O_5$ (379.17): C, 44.35; H, 3.46; N, 7.39. Found: C, 44.30; H, 3.38; N, 7.62.

Ethyl (2Z)-2-[(2,4-dichloro-5-fluoro-3-nitrophenyl)carbonyl]-3-(Butylamino)prop-2-enoate (IV) or *Ethyl 2-(2, 4-dichloro-5-fluoro-3-nitrobenzoyl)-3-butylamino-acrylate* (IV)

This compound was prepared by stirring a solution of ethyl-3-(*N,N*-dimethylamino)-2-(2,4-dichloro-5-fluoro-3-nitrobenzoyl) acrylate **III** (13.5 g, 36 mmol)[22] in 20 ml methanol followed by drop-wise addition of butyl amine (5.6 g, 71mmol, 2M excess) at -5° C for 30 minutes. A dark greenish precipitate started to form in the first 10 minutes of the reaction, at the end of the reaction, the precipitate was filtered, washed with few milliliters of diethyl ether and kept for the next step. It was obtained as a faint white solid, mp = 155-157 °C (decomposition), yield ≈ 13.5 g (93 %), *Rf* value in system (**2** (ethAc:Hex, 1:1)) = 0.57. **^{1}H-NMR**(500 MHz, DMSO-d_6): δ 0.78, 0.84 (Z/E, 2t, J = 7.25, 7.05 Hz, 3H, NH--CH_{3}.4''), 0.87, 0.91 (*Z*/*E*, 2t, J = 7.25, 7.05 Hz, 3H, OCH_2CH_3), 1.26 (q, J = 7.25 Hz, 2H, NH—CH_2.3''), 1.54(q, J = 7.25Hz, 2H, NH—CH_2.2''), 3.47 (Z/E, m, J = 6. 1Hz, 2H, NH—CH_2.1''), 3.85 (*Z*/*E*, m, 2H, OCH_2), 7.77 (d, $^3J_{H-F}$ = 8.5 Hz, 1H, H-6′), 8.22, 8.26 (*Z*/*E*, 2d, J = 14.70, 14.81 Hz, 1H, *N*-C(3)-H), 9.76, 10.87 (*Z*/*E*, 2 br d, J = 16.o, 16.5 Hz, 1H, exchangeable *N*-H). **^{13}C NMR** (125 MHz, DMSO-d_6):δ 13.63, 13.92 (Z/E, NH--CH_3.4''), 13.94, 14.11 (Z/E, OCH_2CH_3), 19.52 (NH—CH_2.3''),32.41 (NH—CH_2.2''),50.00, 50.10 (Z/E, NH—CH_2.1''),59.41, 59.63 (Z/E, OCH_2CH_3), 98.17, 98.69 (Z/E, C-2), 112.66, 112.85 (Z/E, d,$^2J_{C-F}$ = 23.74 Hz, C-4′), 116.95 (d, $^2J_{C-F}$ = 15.18, 22.88 Hz, C-6′), 117.03 (d, $^3J_{C-F}$ = 6.1 Hz, C-1′), 145.38, 145.44 (Z/E, d, $^3J_{C-F}$ = 6.8 Hz, C-3′), 147.99 (br d, C-2′), 156.83 (d, $^1J_{C-F}$ = 251.22 Hz, C-5′), 160.70, 161.39 (Z/E, *N*-C-3), 165.95, 166.98 (Z/E, CO_2Et), 186.62, 186.81 (Z/E, C = O). HRMS (ESI, +ve): calculated for $C_{16}H_{17}Cl_2FN_2NaO_5$ $[M+Na]^+$ (429.03962); found (429.05679).

Ethyl 7-chloro-1-butyl-6-fluoro-8-nitro-4-oxo-1, 4-dihydroquinoline-3-carboxylate (synthone 1E)

This compound was prepared by the cyclization process of the resulted **IV**. Compound **IV** (12 g, 29.5mmol) was treated with potassium carbonate (8.15 g, 59mmol) in dimethylformamide (DMF, 50 ml) and the mixture was heated at 85 °C with continuous stirring for 1 hour. The reaction mixture was then poured onto crushed ice (500 g) with vigorous stirring for 10-15 min. A gummy brown layer was formed and filtered by suction filtration and left to dry in dark. The compound was obtained as a faint yellow solid, mp = 172-175 °C (decomposition), yield ≈ 10.55 g (97 %), *Rf* value in system (**1**) = 0.62.

^{1}H-NMR (500MHz, DMSO-d_6): δ 0.80 (t, J=7.3 Hz, 3H, CH_3-4’), 1.16 (m,2H, CH_2-3’), 1.24 (t, J=7.1, 3H, OCH_2 $C\underline{H_3}$), 1.55 (m, 2H, CH_2-2’), 3.97 (t, J=7.6 Hz, 2H, N-CH_2-1’), 4.21 (q, J=7.1, 2H, OCH_2), 8.29 (d, $^3J_{H-F}$=8.6 Hz, 1H, H-5), 8.66 (S, 1H, H-2). **^{13}C –NMR** (125 MHZ, DMSO-d6): δ13.45 (CH_3-4’), 14.39 (O-$CH_2\underline{C}H_3$), 19.09 (CH_2-3’), 32.27 (CH_2-2’), 54.56 (N-CH_2-1’), 60.59 ($O\underline{C}H_2$), 111.81 (C-3), 115.5 (d, $^2J_{C-F}$=22.5 Hz, C-5), 120.61 (d, $^2J_{C-F}$=23.7Hz, C-7), 128.7 (C-8a), 130.9 (d, $^3J_{C-F}$= 5.7 Hz, C-4a), 140.45 (C-8), 152.78 (C-2), 154.01 (d, $^1J_{C-F}$=248.33 Hz, C-6), 163.96 (CO_2Et), 170.18 (C-4). HRMS (ESI, +ve): calculated for $C_{16}H_{16}ClFN_2NaO_5$ $[M+Na]^+$ (393.06294) Found (393.06951); LRMS (ESI, +ve), m/z: 393 (M+Na,100%), 389 (13%), 302 (15%), 274 (48%), 230 (19%).

7-Chloro-1-butyl-6-fluoro-8-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (Synthone 1A)

This compound was prepared by vigorously stirring of cloudy suspension of Ethyl 7-chloro-1-butyl-6-fluoro-8-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylate synthone **1E** (10.0 g, 26.97mmol) in 150 ml mixture of (36 N H_2SO4, H_2O and 96% Ethanol (1:1:1)). The reaction was heated at 80-85 °C under reflux conditions for 48 h. Thereafter, the reaction mixture was cooled, poured onto crushed ice (250 g) and the resulting heavy precipitate was collected, washed with cold water (2 x 20 ml), dried and recrystallized from a mixture of chloroform and Methanol. It was obtained as an off-white solid, mp = 222-224 °C, yield ≈ 8.5g (92 %), *R f* value in system (**1**) = 0.38. **H-NMR** (500MHz, DMSO-d6): 0.81 (t, J=7.3 Hz, 3H, CH_3-4’), 1.17 (m,2H, CH_2-3’), 1.61 (m, 2H, CH_2-2’), 4.15 (t, J=7.5 Hz, 2H, N-CH2-1’), 8.48 (d,$^3J_{H-F}$ =8.4 Hz, 1H, H-6), 8.99 (s, 1H, H-2), 13.85 (br s, 1H, COOH).

^{13}C –NMR (125 MHz, DMSO-d6): δ13.45 (CH_3-4’), 19.06 (CH_2-3’), 32.45 (CH_2-2’), 55.47 (N-CH_2-1’), 109.79 (C-3), 115.21 (d, $^2J_{C-F}$=22.8 Hz, C-5), 122.28 (d, $^2J_{C-F}$ =23.6, Hz, C-7), 128.90 (C-8a), 128.92 (C-4a), 140.8 (C-8), 153.48 (C-2), 154.01 (D, $^1J_{C-F}$=250.4 Hz, C-6), 164.94 (COOH), 175.63 (C-4). HRMS (ESI, -ve): calculated for C14H11ClFN2O5 $[M^+$-H]$^+$(341.03405 ^{35}Cl, 343.03109 ^{37}Cl) Found (341.03460 ^{35}Cl, 343.03129 ^{37}Cl). LRMS (ESI, -ve): [M^+ -H, m/z%] $^+$ (341, 75%), (343, 25%).

Synthesis of synthone 5a-c (5a) (Scheme 2)

Note: For this work, compounds **2a**, **3a**,**4a**, **5a** were prepared and reported. Compounds **2-5 b-c** were prepared by other study team.[23]

Synthesis of ethyl -1-butyl-6-fluoro-7-(2-methoxy-phenylamino)-8-nitro-4-oxo-1,4-dihydro-quinoline-3-carboxylate (2a)

Three molar equivalents of *o*-anisidine (2.99g, 24.3mmol) were added into a solution (**1E**), 3.0g, 8.1mmol) and 15 ml of dimethylsulfoxide (DMSO) as a solvent and few drops of pyridine then was refluxed at 65-70°C under anhydrous conditions for (2-3) days. The reaction mixture was monitored until no starting material remained then was left to crystallize

at room temperature. The product was filtered and washed, left to dry in dark place to give bright orange crystals; yield ≈ 86% (3.2g); mp = 199-200°C; *Rf* value in system 1 = 0.65.

^{1}H-NMR (500MHz, DMSO-d_6): δ 0.83 (t, J=7.25 Hz, 3H, CH_3-4'), 1.13 (q, J=7.3 Hz, 2H, CH_2-3'), 1.29 (t, J=7.05 Hz, 3H, OCH_2CH_3), 1.59 (q, J=7.1Hz, 2H, CH_2-2'), 3.71 (s, 3H, $ArOCH_3$), 4.00 (t, J=7.35 Hz, 2H, N-CH_2-1'), 4.26 (q, J=7.05 Hz, 2H, OCH_2), 6.86 (dd, J=7.25, 7.45 Hz, 1H, ArCH-5''), 6.96 (d, J=7.65, Hz, 1H, ArCH-3''), 6.99-7.08 (m, 2H, ArCH-4'', ArCH-6''), 8.04 (d,$^3J_{H-F}$=11.8 Hz, 1H, H-5), 8.11 (s, 1H, Ar-NH), 8.65 (s, 1H, H-2). **^{13}C –NMR** (125 MHZ, DMSO-d6); δ13.43 (CH_3-4'), 14.44 (OCH_2CH_3), 19.15 (CH_2-3'), 31.85 (CH_2-2') ,54.84 (NCH_2-1'), 55.83 ($ArOCH_3$), 60.40 (OCH_2), 111.92 (C-3), 111.97 (C-5''), 114.77 (d, $^2J_{C-F}$=21.3 Hz, C-5), 120.69 (C-3''), 120.92 (C-4''), 124.37 (C-8a), 124.45 (C-6''), 130.57 (C-4a), 130.80 (C-8), 132.42 (d, $^3J_{C-F}$=16 Hz, C-7), 134.30 (C-1''), 151.09 (C-2''), 151.95 (C-2), 152.85 (d, $^1J_{C-F}$=250.48 Hz, C-6), 164.33 (COOEt), 170.55 (C-4). HRMS (ESI, -ve): *m/z* calculated for $C_{23}H_{23}FN_3O_6$ [M-H]$^+$: 456.15709, found: 456.15776.

***Synthesis of 1-butyl-6-fluoro-7-(2-methoxy-phenylamino)-8-nitro-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid* (3a)**

A vigorously stirred suspension of (**2a**), 2.0g, 4.37 mmol) in 12N HCl (30mL) and ethanol (15 mL) was heated at 80-85 °C under reflux conditions. Progress of the ester hydrolysis was monitored by TLC and was completed within 36h. Thereafter, the reaction mixture was cooled, poured onto crushed ice (250 g) and the resulting pale orange precipitate was collected, washed with cold water (2 x 25 mL) and left to dry. Yield ≈ 80% (1.5 g). Mp = 206-208 °C; *Rf* value in system 1 = 0.26. **^{1}H-NMR** (500MHz, DMSO-d_6): δ0.77 (t, J=7.3 Hz, 3H, CH_3-4'), 1.09 (q, J=7.2 Hz, 2H, CH_2-3'), 1.57 (q, J=6.9 Hz, 2H, CH_2-2'), 3.63 (s, 3H, OCH_3), 4.14 (q, J=7.15 Hz,2H, N-CH_2-1'), 6.85 (dd, J=7.40 Hz 7.40 Hz, 1H, ArH-5''), 6.97 (d, J=7.98 Hz, 1H, ArH-3''), 7.06 (m, 2H, ArH-4'' and ArH-6''), 8.08 (d,$^3J_{H-F}$=11.8 Hz, 1H, H-5), 8.49 (s, 1H, NH), 8.92 (s, 1H, H-2), 14.61(br s, 1H, COOH).

^{13}C –NMR (125 MHZ, DMSO-d6): δ13.42 (CH_3-4'), 19.13 (CH_2-3'), 32.04 (CH_2-2'), 55.85 (N-CH_2-1'), 55.91 (OCH_3), 109.41 (C-3), 111.77 (C-5''), 113.52 (d, $^2J_{C-F}$=21.6 Hz, C-5), 120.52 (d, $^3J_{C-F}$=7.5 Hz, C-4a), 120.61 (C-3''), 122.59 (C-6''), 125.53 (C-4''), 129.68 (C-8), 131.42 (C-1''), 132.48 (C-8a), 134.71 (d, $^2J_{C-F}$=15.13 Hz, C-7), 152.21 (C-2), 152.23 (C-2''),152.57 (d, $^1J_{C-F}$=253.18 Hz, C-6), 165.48 (COOH), 175.63 (C-4). HRMS (ESI, -ve): calculated for $C_{21}H_{19}FN_3O_6$ [M^+-1]$^+$ (428.12579) Found (428.13675); LRMS (ES, -ve) m/z calc. for $C_{21}H_{19}FN_3O_6$ (429): Found 429.13 (M+, 16%), 428.137 (14%), 393.287 (27%), 365.255 (26%), 299.136; (13%), 283.270 (40%), 281.256 (23%), 281.256 (23%), 255.238 (100%), 253.223 (32%).

***Synthesis of 8-amino-1-butyl-6-fluoro-7-(2-methoxy-phenylamino)-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid* (4a)**

A mixture of (**3a**, 1.0g, 2.3mmol) in 6.5mL of 12N HCl was left stirring in ice bath (0-5°C) for 15 minutes. After that, the ice bath was removed and (1.77g, 9.3mmol, 4Mexcess) stannous chloride ($SnCl_2$) was added portion wise and the reaction mixture left stirring overnight and was monitored by TLC until completion. Then, the reaction mixture was poured on crushed ice to precipitate a brown-orange product that is collected by filtration and left to dry. Yield =0.62g (≈ 66%). mp = 201-203°C (decomposition); *Rf* value in system 1 = 0.14. **^{1}H NMR**(500 MHz, DMSO-d6):δ0.88 (t, J=7.4 Hz, 3H, CH_3-4'), 1.06 (m, J=7.3 Hz, 2H, CH_2-3'), 1.53 (m, J=7.2 Hz, 2H, CH_2-2'), 3.88 (s, 3H, OCH_3), 4.82 (t, J=7.0 Hz, 2H, N-CH_2-1'), 5.56 (br s, 2H, NH_2), 6.20 (d, J=7.65 Hz, 1H, ArH-3''), 6.70 (dd, J=7.60 Hz 7.90 Hz, 1H, ArH-5''), 6.77 (dd, J=7.55 Hz, 7.75 Hz, 1H, ArH-4''), 6.98 (d, J=7.9 Hz, 1H, ArH-6''), 7.16 (br s, 1H, NH, exchangeable), 7.47 (d,$^3J_{H-F}$=9.6 Hz, 1H, H-5), 8.94 (s, 1H, H-2), 14.65 (br s, 1H, COOH, exchangeable). **^{13}C –NMR** (125 MHZ, DMSO-d6): δ13.66 (CH_3-4'), 19.08 (CH_2-3'), 32.27 (CH_2-2'), 55.82 (OCH_3), 56.46 (N-CH_2-1'), 99.81 (d, $^2J_{C-F}$=23.31 Hz, C-5), 107.02 (C-3), 111.23 (C-5''), 112.39 (C-3''), 119.44 (C-4''), 120.77 (C-6''), 122.72 (d, $^2J_{C-F}$=15.40 Hz, C-7), 126.98 (d, $^3J_{C-F}$=9.34 Hz, C-4a), 127.25 (C-8a), 134.35 (C-8), 139.86 (C-1''), 148.20 (C-2''), 151.47 (C-2), 157.23 (d, $^1J_{C-F}$=244.88 Hz, C-6), 166.55 (COOH), 177.38 (C-4). HRMS ((+ve)-ESI): *m/z* calculated for $C_{21}H_{22}FN_3NaO_4$ [M+Na]$^+$: 422.14920, found: 422.14866.

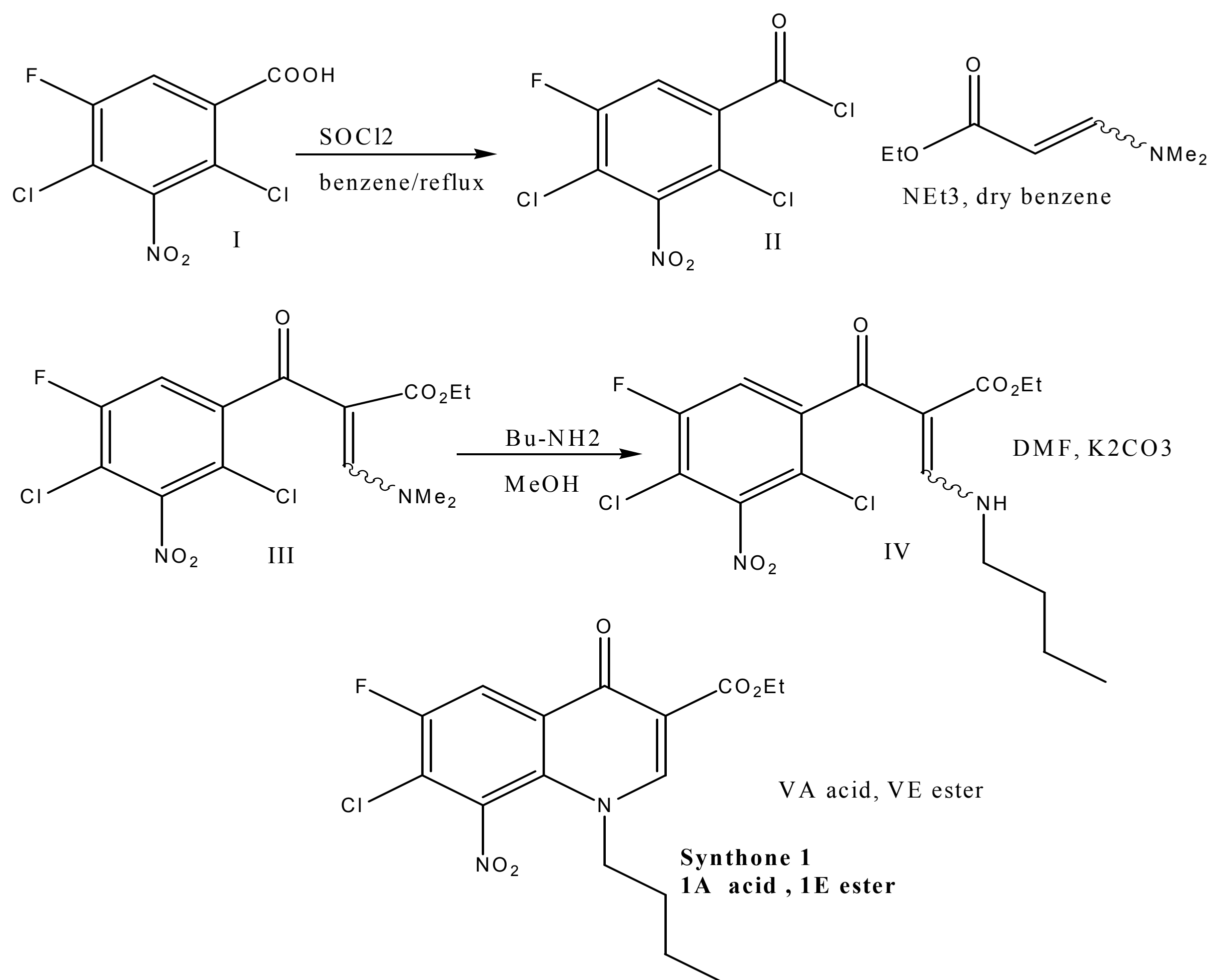
***Synthesis of 9-butyl-4-fluoro-3-(2-methoxy-phenyl)-6-oxo-6,9-dihydro-3H-[1,2,3]triazolo[4,5-h]quinoline-7-carboxylic acid* (5a)**

Compound **5a** was synthesized through cyclization/ diazotization of preceding reduced acid **4a**. Compound **4a** (0.50g, 1.25mmol) was placed in RBF, then 20mL aqueous HCl was added and the mixture left stirring in ice bath (0-5°C) for 15 minutes. $NaNO_2$ (0.086g, 1mmol) dissolved in 10mL H_2O is added drop wise. The reaction mixture was left stirring overnight. Progress of cyclization reaction was monitored by TLC and was completed within 24 h. Thereafter, the reaction mixture was cooled, poured onto crushed ice (250g) and the resulting brown precipitate was collected, washed with cold water (2 x 20mL) and left to dry. Yield= 0.21g (≈ 42 %). mp = 186-189°C; *Rf* value in system 1= 0.38. **^{1}H NMR** (500 MHz, DMSO-d6): δ0.97 (t, J=7.6 Hz, 3H, CH_3-4'), 1.47 (m, 2H, CH_2-3'), 1.94 (m, 2H, CH_2-2'), 3.78 (s, 3H, OCH_3), 5.26 (brs, 2H, N-CH_2-1'), 7.26 (dd, J = 7.42 Hz, J = 7.45 Hz, 1H, CH-5"), 7.41 (d, J= 8.41 Hz, 1H, CH-3"), 7.71 (m, 1H, CH-4", CH-6"), 7.77 (d, J=7.05 Hz, 1H, CH-6"), 8.13 (d, $^3J_{H-F}$ = 9.70 Hz, 1H, H-5), 9.17 (s, 1H, H-8), 15.17 (brs, 1H, COOH).

^{13}C NMR (75 MHz, DMSO-d6): 13.76 (CH_3-4'), 19.21 (CH_2-3'), 31.95 (CH_2-2'), 56.31 (OCH_3), 57.34 (N-CH_2-1'), 109.01 (d, $^2J_{C-F}$ = 20.25 Hz, C-5), 109.26 (C-7), 112.94 (C-5"), 121.02 (C-3"), 123.48(C-9a), 124.46 (C-5a), 128.43 (C-4"), 128.88 (C-3a), 130.76 (C-9b), 132.89 (C-6"), 139.26 (C-1"), 147.25 (d, $^1J_{C-F}$ = 251.23 Hz, C-4), 149.55 (C-8), 154.35 (C-2"), 165.98 (COOH), 176.42 (C-6). HRMS ((+ve)-ESI): *m/z* calculated for $C_{21}H_{20}FN_4O_4$[M+H]$^+$:411.14686, found:411.14631.

Synthone **2a-c** derivatives using the acid path way blended by the reaction of primary amine (aliphatic or aromatic) with synthone **1**, Scheme **2**. This procedure involves the reaction of the ester 1E and the primary amine under reflux and anhydrous conditions, dimethylsulphoxide (DMSO) as a solvent and drops of pyridine. This method provides pure product with high yield for most of compounds without purification or chromatographic separation that were needed in acid pathway (Scheme 2). The hydrolysis of esters 2 into acid derivatives 3 was carried out successfully using ethanolic acidic condition by refluxing at 80-90°C for 24-48 h (Scheme 2). The reaction was followed up by TLC upon completion. Pouring the reaction mixture into ice led to precipitation of pure products (**3a-c**).

***In vitro* enzymatic inhibition assays of each derivative produced by substitution at C-7, the hydrolysis, reduction and triazolo- corresponding compounds 3-5.**

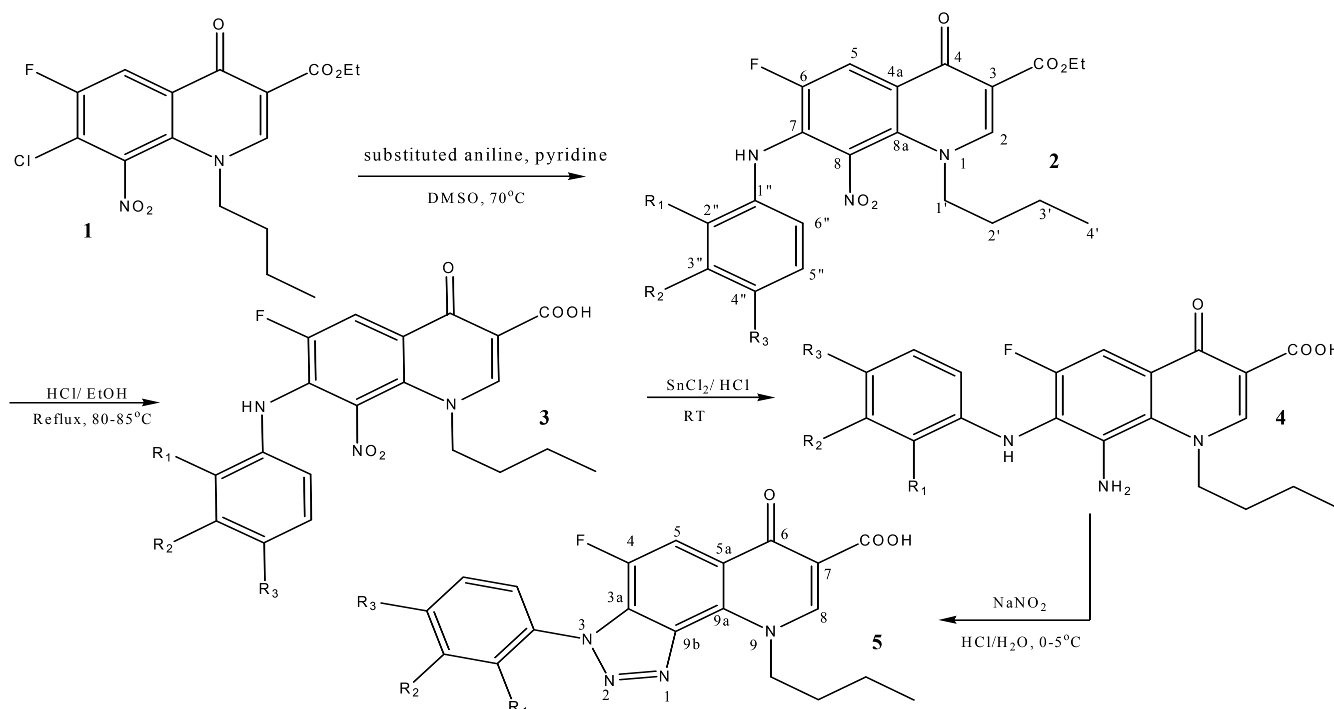


Scheme 1 – Synthesis of Synthone1.

Preparation of the test compounds and orlistat for the in vitro PL activity assay and quantification of PL activity by spectrophotometric assay.

Orlistat (the robust and reliable antilipolytic standard; 10 mg), was dissolved in DMSO (10 mL) to give a stock solution (1 mg/mL), which was used to make six different working solutions with concentrations in the range of 0.625 – 20 µg/mL. Thereafter, 20 µL aliquots of each working solution was used in the reaction mixture to give final concentrations in the range of 0.0125–0.4 µg/mL. Compounds I-V(**1**), **4a, 5a** acids and **4aE, 5aE** esters were prepared in this work and all spectral data are reported herein, whereas compounds **4b, 5b** acids and **4bE, 5bE** esters /**4c, 5c** acids and **4cE, 5cE** esters were previously prepared by our team in an ongoing new antimicrobial study[23] and used in this work for comparison. Furthermore, the test compounds were initially dissolved in DMSO to give three stock solutions, which were subsequently diluted to give five different stock solutions (0.01–100 mg/mL). Thereafter, 20 µL aliquots of each stock solution were used in the reaction mixture to give the final concentration range (0.2 – 2000 µg/mL). Crude porcine PL type II (0.5 mg/mL) was suspended in Tris-HCl buffer (pH 7.4) to a final concentration of 200 units/mL. A 100 µM solution of *para*-nitrophenyl butyrate in DMSO was used as the PL substrate. Aliquots (0.1 mL) of the PL solution were added to the reaction mixtures and the volumes were made up to 1 mL with Tris-HCl buffer. The PL was preincubated with different concentrations of the test material for at least 1 min prior to the addition of the substrate. The reactions were maintained at 37 °C and initiated by the addition of 5 µL of the *p*-NPB substrate solution. The *p*-nitrophenol released during the reaction was measured at 410 nm using a spectrophotometer over a minimum of five time points (1–5 min), against a blank of the same mixture containing the denatured enzyme. The catalytic activity of PL was determined colorimetrically by measuring its activity towards the hydrolysis of *p*-NPB to *p*-nitrophenol.[24a-d, 25]

The activity of PL in this reaction was quantified by measuring the increase in the rate of the release of *p*-nitrophenol from the slope of the linear segment of the absorbance *versus* time profiles.[8, 24] The percentage of residual PL activity was determined for all of the test compounds relative to the control compounds, to calculate the concentration required to inhibit the activity of PL by 50% (i.e., the IC_{50}). All of the assays were performed in triplicate and the calculated activities reported as the mean values ± SD (n=3). The PL inhibition values (%) were calculated according to the following formula: Inhibition (%) = 100 − [(B/A) × 100], where A is the PL activity in the absence of an inhibitor or test compound and B is the PL activity in the presence of an inhibitor or test compound.



Scheme 2 – Synthesis of FQs 1-5.

Scheme code	Toludine derivative	R_1	R_2	R_3
a	2-methoxy aniline	OMe	H	H
b	3- methoxy aniline	H	OMe	H
c	4- methoxy aniline	H	H	OMe

In vitro antiproliferative assay

Obesity-related colorectal cell lines HT29, [26] HCT116, [26] SW620, [26] CACO2[27] and SW480[28] were cultured in high glucose DMEM containing 10% FBS, HEPES Buffer (10 mM), L-glutamine (2 mM), gentamicin (50 µg/mL), penicillin (100 U/mL), and streptomycin sulfate (100 mg/mL). The cytotoxicity measurements were determined using SRB by a colorimetric assay for cytotoxicity screening at wavelength 570 nm, [29, 30] it is considered one of the most reliable and consistent method for cell viability assay. [31, 32] Human periodontal ligament fibroblasts (PDL) [33] are a primary cell culture for verification of selective cytotoxicity with the least antiproliferative IC_{50} value obtained. As a robust and classical antineoplastic reference agent, cisplatin (1-100 µg/mL) was recruited for comparison purpose. [34] All of the assays were performed in triplicate and the calculated antiproliferative activities were reported as IC_{50} mean values ± SD (n=3).To describe the safety of the test compounds; Selective cytotoxicity index is calculated by dividing antiproliferative IC_{50} value of tested compound on normal cell line by least antiproliferative IC_{50} value of the same compound on specific pathological cell line. [18]

Antioxidant capacity

Utilising 0.2 mM DPPH solution diluted with MeOH, then mixing each test compound as well as ascorbic acid with DPPH solution, in a concentration ratio of 1:1 using 96-well plate, treated solution is incubated one hour isolated from light. Finally, change in absorbance at 517nm was measured, [35a,b, 36] and compared to control solution of ascorbic acid, which is the most frequently used standard antioxidant solution. [37, 38] Calculation of radical scavenging activity by DPPH or inhibition (in) of DPPH was determined by following equation where A represents photometric absorbance: (in %) = ((A control – A sample)/ A control) x 100%. [39, 40]

Statistical analysis

The values are presented as mean ± SD (standard deviation) of 3-4 independent experiments. Statistical differences between reference agent and different treatment compounds were determined using GraphPad Prism software unpaired t-test (version 5.01 for windows; GraphPad software, San Diego, CA, USA). Values were considered significantly different if $P < 0.05$ and highly significantly different if $P < 0.01$ and $P < 0.001$.

CONCLUSIONS

In this study, a set of 10 novel FQ compounds were developed and tested for PL, and colorectal adenocarcinoma antiproliferative properties.

Compounds (**3b** and **4b**) revealed appreciable inhibitory PL activity. This finding can open the avenue for more investigation and development of drug candidates and design of pharmaceutical lead compounds.

Comparable to reference antineoplastic cisplatin, 4 of the tested compounds (**1A, 4a**, **5a** and **4c**) demonstrated high antiproliferative properties on 5 CRC cell lines.

On the other hand, same compounds have revealed satisfactory antioxidant activity, which can be implicit as potential mechanism for their antiproliferative activity.

Furthermore, these compounds unveiled an outstanding selective cytotoxicity to CRC cell lines over normal one. High selective cytotoxicity provides an appreciable safety feature.

These findings can provide cornerstone for developing cancer treatments with more safe and effective CRC cell targeting.

Action mechanism of promising FQs' enzyme inhibition and apoptogenic antiproliferative efficacies maybe delineated in future studies.

For future work; these active synthetic compounds propensities are warranted further in vivo models' testing and clinical trials for effective utilization as antiobesity therapeutic agents.

*Acknowledgements***:** The authors would like to thank the Deanship of Academic Research & Quality Assurance at the University of Jordan for funding this work [1630 and 1745] and continuous support. We are grateful for the Scientific Research Fund-The Ministry of Higher Education [MPH/ 1/05/2014].

REFERENCES

1. World Health Organization, WHO, "Obesity and overweight fact shee, **2016.**
2. J. Krishnan, C. Danzer, T. Simka, J. Ukropec, K. M. Walter, S. Kumpf, P. Mirtschink, B. Ukropcova, D. Gasperikova and T. Pedrazzini, *Genes and Development.,* **2012**, *26*, 259-270.
3. T. Gurevich-Panigrahi, S. Panigrahi, E. Wiechec and M. Los, *Curr. Med. Chem.,* **2009**, *16*, 506-521.
4. Y. Ma, Y. Yang and F. Wang, *PLoS One.,* **2013**, *8*, 53916.
5. American Cancer Society, *Colorectal Cancer Facts and Figures 2017-2019.,* **2017**, 861717.
6. M. Baretic, *Digestive Diseases.,* **2012**, *30*, 168-172.
7. A.Ballinger and S. R. Peikin, *Euro. J. Pharmaco.,* **2002**, *440*, 109-117.
8. S. Arabiyat, "Synthesis And Evaluation Of Novel Triazolo-Quinolones As Potential Pancreatic Lipase Inhibitors", *PhD dissertation*, The University of Jordan, **2016.**
9. S. Arabiyat, Y. AL-Hiari, K. Y. Bustanji, H. Zalloum and V. Kasabri, *Rev. Roum. Chim.,* **2016**, *61*, 871-879.
10. T. Buchholz and M. F. Melzig, *Planta Medica.,* **2015**, *81*, 771-783
11. K. Weibel, P. Hadvary, E. Hochuli, E. Kupfer and H. Lengsfeld, *J. Antibiotics (Tokyo).,* **1987**, *40*, 1081-1085.
12. Y. M. Al-Hiari, A. K. Shakya, M. H. Alzweiri and T. Aburjaiand R. Abu-Dahab, *Farmacia.,* **2014**, *62*, 570-588.
13. (a) S. Arabiyat, V. Kasabri, Y. Al-Hiari, Y. Bustanji, R. Albashiti, I. Almasri and D. Sabbah, *Chem. Biol. Drug Design.,* **2017**, 10.1111, 13049. (b) N. A. Lunagariya, N. K. Patel, S. C. Jagtapand and K. K. Bhutani, *EXCLI Journal,* **2014**, *13*, 897
14. H. Kurt, A. Celik and M. Kelleci, *Oncology Letters.,* **2015**, *10*, 3177-3182.
15. A. Y. Wang, H. Su, Y. Gu, X. Song and J. Zhao, *OncoTargets and Therapy.,* **2017**, *10*, 4065–4079. (b) M. Amararathna, M. R. Johnston and H. V. Rupasinghe, *Int. J. Molec. Sci.,* **2016**, *17*, 1352. (c) J. Neuwirthová, B. Gál, P. Smilek and P. Urbánková, *Clinical Oncology,* **2017**, *30*, 106-114.
16. S. Arabiyat, Y. AL-Hiari, K. Y. Bustanji, H. Zalloum and V. Kasabri, *Rev. Roum. Chim.,* **2016**, *61*, 871-879.
17. D. Bentley, A.-M. Young, L. Rowell, G. Gross, J. Tardio and D. Carlile, *J. Cardio. Pharmaco.,* **2012**, *60*, 390-396.
18. H. Hoffmann, A. Kunz, V. Simona, P. Palesea and M., Shaw, *Proceed. Nat. Acad. Sci.,* **2011**, *108*, 5777–5782.
19. FF. Hefti, *BioMedCentral Neurosci.,* **2008**, *9*, 7-16.
20. M. Amararathna, M. R. Johnston and H. V. Rupasinghe, *Int. J. Molecul. Sci.,* **2016**, *17*, 1352.
21. Y. M. Al-Hiari, V. N. Kasabri, A. K. Shakya, M. H. Alzweiri, F. U. Afifi, Y. K. Bustanji and I. M. Al-Masri, *Med. Chem. Research.,* **2014**, *23*, 1-11.
22. Y. Al-Hiari, M. Al-Mazari, I. S. Shakya, A. K. Darwish and R. M. Abu-Dahab, *Molecules.,* **2007a**, *12*, 1240-1258.
23. S. Hasan, "Antimicrobial effect of new fluoroquinolones on clinically important microorganisms", *MSc Thesis*, The University of Jordan, **2018**.
24. (a)A. Dechakhamphu and N. Wongchum, *Asian Pacific J. Tropical Biomed.,* **2015**, *5*, 1042-1045.(b) Y. Bustanji, I. M. Al-masri, M. Mohammad, M. Hudaib, K. Tawaha, H. Tarazi and H. S. Alkhatib, *J. Enz. Inhib. Med. Chem.,* **2011**, *26*, 453-459. (c) Y. Bustanji, A. Issa, M. Mohammad, M. Hudaib, K. Tawah, H. Alkhatib, I. Almasri and B. Al-Khalidi, *J. Med. Plants Research,* **2010**, *4*, 2235-2242. (d) Y. Bustanji, M. Mohammad, M. Hudaib, K. Tawaha, I. M. Al-Masri, H. S. Alkhatib, A. Issa and F. Q. Alali, *Jordan J. Pharma. Sci.,* **2011**, *4*, 81-88.
25. R. B. Birari and K. K. Bhutani, *Drug Discovery Toda.,* **2007**, *12*, 879-889.
26. G. Kaur and M. Dufour, *Spermatogenesis,* **2012**, *2*, 1–5.
27. *American Type Tissue Culture*, **2016**, [Caco2] (ATCC® HTB-37™)
28. *American Type Tissue Culture*, **2014**, [SW-480] (ATCC® CCL228™)
29. L. Riss, R. A Moravec, L. Niles, S. Duellman, H. A. Benink, J. Worzella and L. Minor, "Assay Guidance Manual", 2015, Bookshelf ID: NBK144065.
30. V. Vichai and K. Kirtikara, *Nature Protocols.,* **2006**, *1*, 1112 – 1116.
31. P. Keepers, E. Pizao, G. Peters, J. V. Ark-Otte, B. Winograd and M. Pinedo, *Eur. J. Cancer.,* **1991**, *27*, 897 900.
32. V. Tonde, M. Joubert and D. Cromarty, *Biomed. Central Research Notes*, **2015**, *8*,47.
33. *American Type Tissue Culture*, **2014**, (PDL) (ATCC® CRL-2522™)
34. V. Kasabri, F. U. Afifi, R. Abu-Dahab, N. Mhaidat, Y. K. Bustanji, I. F. Abaza and S. Mashallah, *Rev. Roum. Chim.,* **2014**, *59*, 693-705.
35. (a)N. Liang and D. Kitts, *Molecules.*, **2014**, *19*, 180-19208. (b) G. Marinova and V. Batchvarov, *Bulgarian J. Agric. Sci.,* **2011**, *17*, 11-24
36. Q. Shen, B. Zhang, R. Xu, Y. Wang, X., Ding and P. Li, *Anaerobe*, **2010**, *16*, 380-386.
37. M. A. Hidayat, A. Fitri and B. Kuswandi, *Acta Pharma. Sinica B*., **2017**, *7*, 395–400.
38. P. Sharma and K. Bhat, *Food Chem.*, **2009**, *113*, 1202–1205.
39. E. Shalaby and S. Shanab, *Indian J. Geo-marine Sci.*, **2013**, *42*, 556- 564.
40. K. Paulpriya, M. Lincy, P. Tresina and V. Mohan, *J. BioInnovation*, **2015**, *4*, 257-268.