RESEARCH ARTICLE

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Antiproliferative Activities of Lipophililic Fluoroquinolones-Based Scaffold Against a Panel of Solid and Liquid Cancer Cell Lines

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Abstract

Objectives: In this work, 9 lipophilic-acid chelating FQs (fluoroquinolones) comprising chelating groups have been prepared, characterized and screened for in vitro cytotoxicity, radical scavenging and antiinflammation propensities. **Methods:** Using sulforhodamine B colorimetric bioassay vs. cisplatin; FQs-inflicted reductions' of viability against breast T47D and MCF7, Pancreatic PANC-1, colorectal HT29, HCT116, SW620, CACO2, SW480 and Leukaemia K562 cancer cell lines were examined in quadruplicates/dose/cell line. Parameters including potency, toxicity, and selectivity (potency/toxicity) have been reported along with DPPH- and NO- radicals' scavenging capacities -as their molecular action mechanism- in comparison to ascorbic acid and indomethacin respectively. Using Griess assay in Lipopolysaccharide (LPS) prompted RAW264.7 macrophages; mitigation of inflammation was investigated. **Results:** nitroFQ 3b, unlike the rest of FQs in PANC1 and MCF7 cells, exhibited remarkably superior NO-radical scavenging/antiinflammation capacity to indomethacin with respective antiproliferative IC_{50} values (<50μM) 49 vs. cisplatin's 122 and 6 vs. cisplatin's 28 ($p<0.01$-0.001; n=4). Reduced FQ 4b of significantly dual DPPH-NO scavenging propensities exerted exceptionally substantial micromolar antiproliferation in colorectal cancer cells with respective antiproliferative IC_{50} values (<50μM) of HCT116 0.84< HT29 1.6<PANC1 5.7<SW620 9.2 vs. cisplatins', ($p<0.01$-0.001; n=4). FQ 5a of superb NO radical reduction effect had antiproliferative IC_{50} value (<50μM) of 37.6 in PANC1 cells. In breast cancer T47D the ascending order of pronounced nano-micromolar antiproliferative IC_{50} values (<50μM) was 4d<3d<4a<4b<3b (0.009<0.59<10<15<41 vs. cisplatins', $p<0.01$-0.001; n=4). Both 4d and 4b displayed both DPPH-NO radicals reduction –related cytotoxicities. NO radical scavengers 3d and 3b as well as DPPH radical scavenger 4a exerted highly appreciably relevant antineoplastic affinities. **Conclusion:** Acidic groups and C8-C7 ethylene diamine Chelation Bridge along with bulky dual halogenations can be substantially associated with molecular action mechanisms of FQs cytotoxicities, antioxidative and antiinflammation effects, collectively.

Keywords: Quinolones-Fluoroquinolones-NO-radical scavenging-Sulphorodhamine B- Cisplatin

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Introduction

Malignancy is a congregation of diseases labeled by the uncontrolled development and spread of irregular cells. Drug resistance represents the main barrier to malignance management and patient survival. In some cases, the adverse effects of chemotherapy are severe as the disease itself, this is due to the nonspecific effect of antineoplastic drugs on the malignancy cells and normal cells alike and as well effected on organ functions. Consequently, the discovery of new compounds that have less resistance with fewer side effects is the main goal. Anti-tumor medications are classified either as cell killing medications (cytotoxic) or as antiproliferative medications (cytostatic), both of which prevent the growth of malignant cells (Sharma et al., 2020). To reduce major barriers to the effectiveness of chemotherapy, such as drug resistance and severe adverse effects, many FQs derivatives have been demonstrated to have anticancer activity (Yadav and Talwar, 2019). FQs have been found to have a cytotoxic activity towards tumor cells due to mechanical resemblances with sequence homologies of affecting topoisomerase enzymes. Anticancer properties of some antibacterial agents such as gemifloxacin, ciprofloxacin, moxifloxacin, and levofloxacin are clinically established. Gemifloxacin has antineoplastic action in the colon malignancy cell lines (Mamdooh et al., 2019). More recently, our group researched gemofloxacin as excellent antiproliferative

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agent against 8 cancer cell lines including liquid and solid cancer cell lines (AlKhalil et al., 2020). Vosaroxin is the first quinolone derivative in a novel class of antineoplastic agents; it makes replication-dependent DNA destruction by intercalating DNA and stopping topoisomerase II, which prompts tumour cell apoptosis (Hawtin et al., 2010a,b). Numerous reports show that the anti-tumour effectiveness of FQs can be improved by increasing the lipophilicity of new FQs compounds (Korolyov et al., 2010).Also, Several halogenated FQs C-7 anilines showed outstanding activities against cancer cell lines (Arabiyat et al., 2016a; b; 2017).

Materials and Methods

Study aims

Reportedly 7-anilino FQs exhibited appreciable antiproliferative capacities in vitro. Presently in this study the newly synthesized lipophilic FQ compounds have been investigated for possible selective antiproliferative propensities on malignant cell lines, due to their lipophilic and chelator nature. Accordingly, the objectives of this work are to synthesize 9 novel compounds of halogenated 7-anilino FQs and to evaluate their selective antineoplastic activity on breast cancer T47D, MCF7, Pancreatic PANC1, leukemia K562, 'colorectal cancer (CRC) HT29, HCT116, SW620, $CACO_2$, and SW480 as well as fibroblast cells. Subsequent determinations of their potential of DPPH- and NO- radicals scavenging properties as anticipated molecular cytotoxicity action mechanisms are implemented.

Results

DPPH radical scavenging effects and antiinflammatory properties in LPS-prompted RAW264.7 macrophages of tested of FQs/TFQs vs. respective reference agents (Table 1)

Apart from bioactive reduced FQs of series 4 (4d<4a<4b of respective IC_{50} values (μM) of 33<90<100 vs. vitamin C's 124; p<0.05-0.001; n=4); The rest of the both series 3 and 5 FQs/TFQs were significantly ineffective DPPH- radical scavengers (IC_{50} values (μM) ≥ 1000) as compared to ascorbic acid (p<0.001; n=4).

The inhibitory bioactivities of the compounds against LPS-induced NO production in RAW 264.7 macrophages were tested by the Griess assay. Evidently unlike the rest of FQs/TFQs derivatives; reduced 4b exhibited remarkably superior NO-radical scavenging vs. indomethacin (IC_{50} values (<50 μM) of 5 vs. indomethacin's 119.5; p<0.001; n=4) in LPS-induced inflammation in RAW264.7 macrophages. The tested compounds 3b<3d<3a<5b<5a<4d (respective IC_{50} values (<100 μM) of 69.3<71.2<72.3<78<84<85vs. indomethacin's 119.5; p<0.05-0.001; n=4) had reasonably appreciable antiinflammatory effects. Most notably, none of the tested compounds of the 3 new FQs series had cytotoxity in RAW264.7 macrophages over 72h incubations as checked by SRB bioassay.

Antiproliferative activity (as of %Control) of new tested compounds of 3 FQs series 3 and 4 and TFQs 5 vs. respective reference agent in cancer cell lines (Tables 2a,b)

Using the SRB assay; HTS for antiproliferative activity of tested new FQs/TFQs against cancer K562, PANC1, T47D and MCF7 cell lines as well as colorectal HT29, HCT116, SW620, CACO2, SW480 cancer cell lines was demonstrated with respective IC_{50} values. Each cell line showed a different response profile to each of the set of tested FQs/TFQs. Cisplatin antiproliferative efficacies in all cancer cell lines were further illustrated. Except for reduced 4b of comparable affinity to cisplatin'sundifferential cytotoxicity, the rest of proven antiproliferative FQs/TFQs had satisfactory selectivity indices further indicative of their safety profiles in normal cells' monolayers.

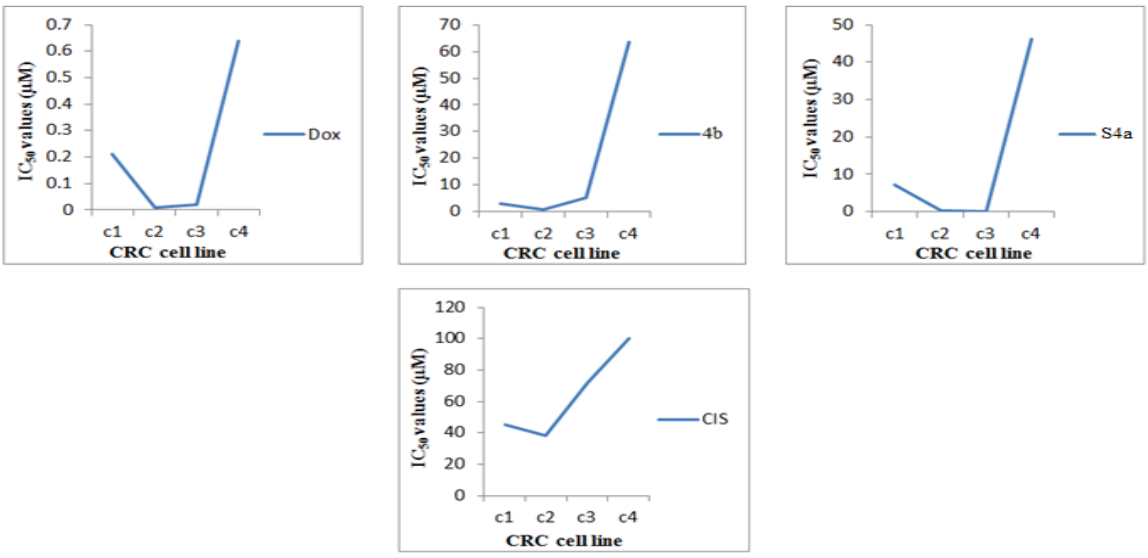


Figure 1.Similarity in Antiproliferation Pattern and IC_{50} Comparison between Doxorubicin (Dox), S4a and 4b against CRC cell lines (HCT29 (c1), HCT116 (c2), SW620 (c3), CACO2 (c4).

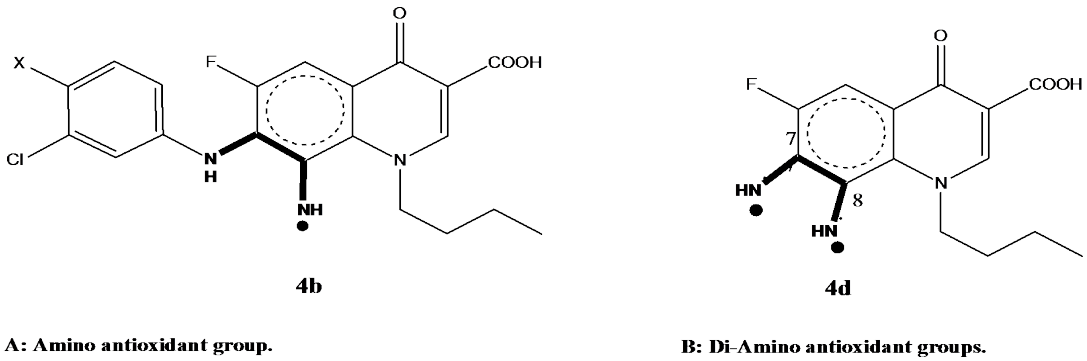


Figure 2. Acid Scavenging Amino Group N both 4b and 4d

NitroFQs (Tables 2a,b)

Despite their lack of cytotoxicity in leukemia K562 wells; 3b in ascending order of growth suppression in MCF>T47D>PANC1 cancer cells (IC_{50} values (μM); 6<41<49 vs. respective cisplatins' 28<57<122; p<0.05-0.001; n=4). Exquisitely 3d exhibited nanomolar affinities for antineoplastic potencies in T47D breast carcinoma 72h incubations (IC_{50} values (μM) 0.6 vs. 57 of cisplatin's; p<0.01; n=4). In GI system; All NitroFQs were substantially ineffective against all colorectal cancer 72h growth suppression wells when compared to cisplatins' (p<0.05-0.001; n=4).

Table 1. IC_{50} Values (μM; μg/mL) of *in vitro* DPPH-Radical Scavenging Properties and Antiinflammatory Activities of the Tested FQs/TFQs vs. respective reference agents

Code	DPPH radical scavenging IC_{50} value μM (μg/mL)+	NOS- IC_{50} value μM (μg/mL)++
NitroFQs		
3a	NI	72.33±12.24**
		(31.38±5.31)
3b	NI	69.31±1.16****
		(30.07±0.5)
3d	9708.5±1783.48***	71.16±4.81***
	(4546.1±835.1)	(33.32±2.25)
Reduced FQs		
4a	89.77±0.53***	139.78±8.81*
	(36.25±0.21)	(56.45±3.56)
4b	110.03±4.31*	5.39±0.74****
	(44.43±1.74)	(2.17±0.3)
4d	33.02±0.69***	85.00±2.8***
	(14.47±0.3)	(37.25±1.23)
TFQs		
5a	10449.20±1244.01***	84.22±4.95***
	(4334.43±516.03)	(34.93±2.06)
5b	NI	77.73±11.92**
		(32.24±4.94)
5d	1714.1±166.33***	2157.10±394.68***
	(770.08±74.73)	(969.10±177.32)
Reference Drug	Ascorbic acid	Indomethacin
	123.94±6.22	119.49±3.02
	(21.83±1.10)	(42.75±1.08)

Results are mean ± SD (n = 4 independent replicates);+ IC_{50} values (concentration at which 50% inhibition of DPPH in comparison to non-induced basal 30 minutes incubations or cell proliferation in comparison to non-induced basal incubations) were calculated within testing dose range; ++ The IC_{50} value is the concentration at which 50% inhibition of Nitric oxide synthase took place in comparison to non-induced basal 24h incubations; P-value is calculated by unpaired t-test between test compound IC_{50} values μM and ascorbic acid IC_{50} values μM (DPPH) or Indomethacin IC_{50} values μM (NOS) using GraphPad Prism software version 5.0.1.* When P<0.05 and ** when P<0.01 or 0.001, *** when P< 0.001or 0.0001 , **** when P<0.0001, NS, not significantly different from reference agent.

Reduced FQs (Tables 2a,b)

In GI system; reduced 4b exhibited significantly superior potencies to those of antineoplastic cisplatin (with IC_{50} value (μM) 1.6 <45 in HT29; 5.7<122 in PANC1; 9.2<72 in SW620; 46<100 in CACO2; 70.3<96 in SW480; p<0.01-0.001; n=4). Unequivocally 4b exhibited nanomolar affinities for antineoplastic potencies in HCT116 carcinoma 72h incubations (IC_{50} values (μM) 0.84 vs. 38.3 of cisplatin's; p<0.001; n=4). Superbly 4a exhibited the unmatched and marked micromolar cytotoxicity in both HT29 (IC_{50} values (μM); 55<cisplatin's 72; p<0.05; n=4), and PANC1 (74<cisplatin's 122; p<0.01; n=4).

Unlike TFQs in K562 leukemia and breast T47D cancer cells 72h incubations; reduced FQs proved an ascending order 4d<4a<4b of growth inhibition (IC_{50} values (μM);73<74<88 vs. cisplatin's 180; p<0.01; and 0.009 (9nM)<10<15 vs. cisplatin's 57; p<0.01-0.001; n=4 respectively); In MCF7 4b<cisplatin's<4a (IC_{50} values (μM) 22<cisplatin's 28<58; p<0.01; n=4) order of pronounced antineoplastic capacity posed 4b equipotent to cisplatin but 4a of inferior potency.

TFQs(Tables 2a,b)

TFQs in ascending order of 4d<4a<4b TFQs 5 (a, b and d) lacked antineoplastic efficacies in leukemia and breast MCF7 cancer cell lines when compared to cisplatin's (p<0.01-0.001; n=4). In GI system; While 5d in pancreatic PANC1 and 5b in breast T47D cancer cells were equipotent to cisplatin's; 5a<5b in pancreatic PANC1 and 5d in breast T47D cancer cells had greater potency vs. cisplatin's (IC_{50} values (μM); p<0.05-0.001; n=4). In GI system; All TFQs were substantially ineffective against all colorectal cancer 72h growth inhibition wells; Unequivocally in colorectal SW620 cancer monolayers 5d displayed marked antiproliferation of comparable

Table 2a. IC_{50} Values (μM (μg/mL) of *In vitro* 'Antiproliferative Activities of Cisplatin and New FQs Compounds

	Cytotoxicity (as of %Control) IC_{50} value μM (μg/mL)					
	HT29	HCT116	SW620	CACO2	SW480	PANC1
NitroFQs						
3a	190.91±6.83****	97.11±4.12***	271.77±51.08**	309.92±51.66**	283.57±42.49**	114.1 ±9.01NS
	(82.82±2.96)	(42.13±1.79)	(117.9±22.16)	(134.45±22.41)	(123.02±18.43)	(49.5±3.91)
3b	58.78±1.25*	77.91±4.66***	253.45±10.95****	269.24±14.89****	602±43.65****	49.1±6.06***
	(25.5±.54)	(33.8±2.02)	(109.95±4.75)	(116.8±6.46)	(261.21±19.07)	(21.3±2.63)
3d	432.49±19.84****	303±15.22****	193.03±11.56****	467.65±58.99***	670.14±89.59***	359.47±47.94**
	(189.55±8.70)	(132.8±6.67)	(84.6±5.07)	(204.96±25.85)	(293.71±39.26)	(157.55±21.01)
Reduced FQs						
4a	95.44±1.75***	105.99±3.12 ***	54.96±6.66*	264.47±14.22****	77.38±9.2*	74.29±12.06**
	(38.54±.71)	(42.8±1.26)	(22.8±2.76)	(106.8±5.74)	(32.1±3.81)	(30±4.87)
4b	1.610±0. 223 ***	0.84±0. 1 ****	9.19±.67 ****	63.64±6.54**	70.33±4.89**	5.7±.17****
	(.65±.09)	(0.34±.04)	(3.71±0.27)	(25.7±2.64)	(28.4±1.98)	(2.3±.07)
4d	171.91±4.67****	172.34±3.57****	90.76±8.34 *	283.03±31.06***	154.62±18.84**	111.05±4.98NS
	(80.5±2.19)	(80.7±1.67)	(42.5±3.91)	(132.53±14.54)	(72.4±8.82)	(52±2.33)
TFQs						
5a	222.03±13.82****	127.94±10.19***	119.85±1.09***	217.449±6.85****	688.04±28.79****	37.61±3.4****
	(92.1±5.52)	(53.07±4.23)	(48.4±.44)	(90.2±2.84)	(277.85±11.62)	(15.6±1.41)
5b	143.68±6.01 ****	84.09±6.25***	110.17±2.95***	222.87±17.05***	151.76±18.02**	55.69±8.56***
	(59.6±2.49)	(34.88±2.59)	(45.7±1.22)	(92.45±7.07)	(62.95±7.47)	(23.1±3.55)
5d	268.02±31.33****	166.38±2.67****	73.68±4.64 NS	441.48±98.15 **	164.83±14.82**	135±16.58NS
	(120.41±14.08)	(74.75±1.2)	(33.1±2.08)	(198.34±44.09)	(74.05±6.66)	(60.65±7.45)
Cisplatin	45.13±7.9	38.3±6	71.8±5.38	100.4±5.82	95.68±5.45	122.25±8.43
	(13.54±2)	(11.5±1.9)	(21.54±1.61)	(30.21±1.75)	(28.71±1.63)	(36.68±2.53)

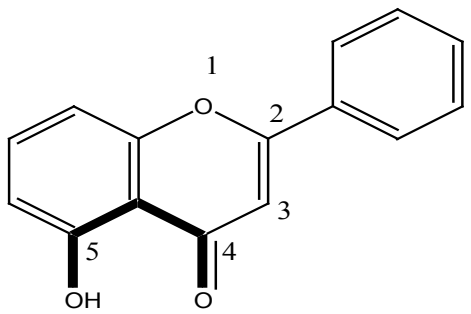
Results are mean ± SD (n = 4 independent replicates). IC_{50} values (concentration at which 50% inhibition of cell proliferation took place in comparison to non-induced basal 72 h incubations) were calculated within 0.1-200 μg/mL range. NI is lack of cytotoxicity within the tested 0.1-200 μg/mL concentration 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, *** when P< 0.001or 0.0001 , **** when P<0.0001, , NS, not significantly different from reference agent

effectiveness to cisplatin's (with IC_{50} value (μM) 74 vs. cisplatin's 72; p>0.05; n=4).

Discussion

Chemistry

Synthesis of synthon 2(a-c) was conducted following the ester pathway. The reaction proceeded smoothly between the halogenated aniline with synthon 1E.The procedure affords high yields of the products 2(a-c) mainly upon simple crystallization / precipitation process. It was assumed that all products were correct since one spot appeared on TLC. No further characterization was carried out at this stage. All crude products were directly



flavone
5-Hydroxy-4-one

Flavanoids

ethylene diamine

4b

Figure 3. Chelator Groups in Flavonoids and FQ-4b

Table 2b. IC_{50} Values (μM (μg/mL) of *In vitro* Antiproliferative Activities of Cisplatin and Tested FQs/TFQs Compounds with their Respective Selectivity Indices in PDL Fibroblasts

	Cytotoxicity (as of %Control) IC_{50} value μM (μg/mL)				
	K562	T47D	MCF7	PDL Fibroblasts	Selectivity Index* Value (related cell line)
NitroFQs					
3a	225.34±14.11*	45.81±1.46NS	63.62±5.72**	41.05±3.16 ***	0.9
	(91±5.7)	(18.5±0.59)	(27.6±2.48)	(17.81±1.37)	(T47D)
3b	350.38±63.81*	40.57±3.29*	5.99±1.23**(2.6±.53)	16.83±1.89***	2.81
	(152±27.68)	(17.6±1.43)		(7.3±.82)	(MCF7)
3d	NI	0.59±.046***	390.16±36.11****	124.58±6.08****	211.15
		(0.26±0.02)	(171±15.82)	(54.6±2.66)	(T47D)
Reduced FQs					
4a	76.35±12.91**	10.14±2.08***	57.95±7.78**	47.05±8.13***	4.64
	(33.12±5.6)	(4.4±0.9)	(23.4±3.14)	(19±3.28)	(T47D)
4b	88.65±13.45**	14.91±0.47**	21.62±1.87NS	0.14±0.014****	0.17
	(35.8±5.43)	(6.02±0.19)	(8.73±.75)	(0.055±0.01)	(HCT116)
4d	72.56±11.35**	0.009±0.011***	159.31±25.7***	74.1±3.45****	8233.3
	(32.6±5.1)	(.004±0.005)	(74.6±12.04)	(34.7±1.62)	(T47D)
TFQs					
5a	1086.039±76.71****	172.61±15.92***	74.32±10.98**	39.78±0.64****	1.06
	(450.5±31.80)	(71.6±6.6)	(30.83±4.56)	(16.5±0.27)	(Panc1)
5b	342.18±57.09**	70.88±8.86 NS	54.24±3.71**	41.05±6.15***	0.76
	(141.94±57.09)	(29.4±3.68)	(22.5±1.54)	(17.03±2.55)	(MCF7)
5d	NI	41.18±3.65*	171.39±26.29***	50.08±5.15***	1.22
		(18.5±1.64)	(77±11.81)	(22.5±2.32)	(T47D)
Cisplatin	180.20±21	57±8.7	28.13±4.4	5.07±0.3	0.18
	(54.07±6.30)	(17.1±2.7)	(8.44±1.32)	(1.52±0.09)	(MCF7)

Results are mean ± SD (n = 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, *** when P< 0.001or 0.0001, **** when P<0.0001, NS, not significantly different from reference agent

hydrolyzed to the acid 3(a-c). The hydrolysis step to obtain 3 was carried out using ethanolic HCl at high temperature. Although TLC has displayed one spot for all derivatives 3 a-c, NMR analysis revealed that 3b was highly pure, whereas 3a was major. Detailed analysis of 3c has furnished the side product 3d indicating clearly that the target 3c did not form or it was broken upon formation due to tough conditions used. This reaction and product 3d was deeply investigated for optimization. The reaction was repeated following the acid pathway to prepare 3c. The acid 1A was coupled directly using reflux conditions with 2,4-dichloroanile.Although the reaction took less time than the ester pathway, it yielded again the side product 3d directly. Upon proceeding with reduction to 4c and diazotization to 5c, it was found that the reduced 7 amino (4d) and the triazolodiamino (5d) were obtained. In depth investigation of 3c reaction has shown that time was the most critical in the formation of the target 3c or side product 3d. The reaction shifted toward target 3c when the time the experiment was reduced to 2-3 days. Reducing the time interval less than 2 days or the temperature hindered the product formation and the starting 1A did not react. Although 3c was obtained, it was not possible to separate this product from either the starting 1A or 3d since all have close Rf values. Neither chromatography nor recrystallization gave 3c. We can state that such toned balance was also apparent within the ester path way as well, especially for 2a. The reaction proceeded by formation of the product 2c in ester pathway, or 3c in acid pathway parallel with breaking of the 7-haloaniline part to form 3d. In this work we suggest a potential explanation for such rare mechanism depending on acid and heat catalysis. Protonation of the N atom of C-7 aniline has led to electron withdrawal effect from the benzene ring. Breaking this weak polarizable bond to neutralize the ammonium charge led to the formation benzene carbocation and the 7-amino FQ 3d. The carbocation was stabilized by a hydroxyl group from the aqueous media leading to formation of phenolic side product.

In vitro antiproliferative activity

Our previous work has established the FQ scaffold (substituted 7-haloaniline-6-fluoro-8-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid)with good antiproliferative activity. Therefore, this work aims at synthesizing novel lipophilic FQs by substituting the site of chloro aniline at C-7 and baring N-Butyl in N1, as seen in Scheme 1 (Supplementary). Halogenated FQs

3-5 (a-b) have revealed again excellent antiproliferative activity against all cell lines tested including five CRC cell lines, MCF7, T47D, and PANC1 cell lines with IC_{50} values less than 100μM for most of them. On the other hand, the 7-amino derivatives 3-5(d) showed much lower activity against same cell lines tested five CRC cell lines, MCF7, T47D, and PANC1. This for sure concludes that the 7-halogenated anilines in both a(2-chloro aniline) and b(3-chloro aniline) derivatives is essential for activity compared to absence of aniline substituent. This clearly indicates the importance lipophilic FQs toward anticancer activity provided by the lipophilic aniline side chain at C-7. Worth to mention that the activity of these derivatives against MCF7, T47D, and PANC1 were much stronger than colorectal cell lines with exception to reduced 4b The reduced series 4a and 4b were the strongest compounds with superior or equipotent activity to the reference cisplatin against all cell lines. In fact compound 4b was more potent than the reference drug cisplatin against all CRC cell lines HT29, HCT116, SW620, $CACO_2$, and SW480 with IC_{50} 1.61±0. 223, 0.84±0.1, 9.19±0.67, 63.64±6.54 and 70.33±4.89 respectively.The reduced series (4a-b) have exhibited the uppermost antiproliferative activity with the five cell lines followed by nitro series (3a-b) in the case of HT29 or triazolo series (5a-b) in the case of HCT116. The reduced 3-chloro 4b has demonstrated the topmost antiproliferative CRC activity against the three cell lines (SW620, HCT116, and HT29) in all series with nanomolar IC_{50} value mainly against HCT116 (840 nM). Moreover, 4b displayed IC_{50} value below 10 μM against the other cell lines SW620 and HT29. The order of activity among the most active series 4 is (b, 3-Cl) >(a, 2-Cl)>>>(d, 7-amino). All compounds including synthons 3, 4 and 5 have shown weak activity against $CACO_2$ and SW480 with IC_{50} value above 100μM.Similar to the colorectal activity pattern, Tables 2a, b reveal that the reduced series 4a and 4b exhibited the strongest activity against MCF7 and PANC1 cell lines followed by triazolo and nitro derivatives. The order for T47D cell lines was reduced (4) > nitro (3) >triazolo (5). The 3-chloro aniline derivate 4b was the most potent against the 3 cell lines with remarkable activity against T47D cell line detailing IC_{50} values of 14.91 μM. The order of activity of the reduced series was T47D >PANC1> MCF7 cell lines. Strange enough, the reduced and nitro derivatives of 3d and 4d have expressed strong activity against T47D with IC_{50} values in nM range. Against the norm, this abnormal outstanding activity can be explained by the fact that these derivatives work by a different mechanism compared to the highly lipophilic 3b and 4b. Although the activity against K562 was weak or non-inhibitory, only the reduced series 4(a, b, and d) have displayed satisfactory activity against K562 cancer cell lines with IC_{50} values less than 100 μM. Finally it is worth mentioning that all compounds are safe when compared to fibroblasts with have high IC_{50} values compared to reference drug (cisplatin) in PDL cells. Furthermore, they have selectivity index higher than cisplatin indicating high safety and selectivity profile for these compounds. Only 4b has a close value to cisplatin which means it is still selective and safe.

Mechanistic studies/SAR studies

The research started by comparing 3 clinically used antibacterial FQs for their antiproliferative properties including gemofloxacin, levofloxacin and ciprofloxacin (AlKhalil et al, 2020). The work revealed gemofloxacin as strong antiproliferative FQ against many cancer cell lines tested and topped the reference cisplatin activity against K562 cell line. This finding spotted the light on C-8 N atom shared between both gemofloxacin and vosaroxin since only FQs with C-8 N displayed anticancer properties In fact this has drawn our attention that the Hydrogen acceptor atom in both drugs at C-8 may be the key player in anticancer FQ hits.4d has revealed much weaker activity compared to 4a and 4b. This difference in activity rises from the C-7 aniline in a and b compared to d, signifying the importance of lipophilicity. Lipophilic substitution imposed from aniline seems to be essential for activity. Furthermore, lipophilic nature allows cancer cell membrane penetration through phospholipid bilayer which is different from active efflux pump of antibacterial FQ drugs.Our results display clearly that the reduced series 4 were more active than 3 and 5. This activity is due only to C-amino group at C-8 in all series, 'Scheme 1(Supplementary), Tables 2a, b. The amino group has increased polarity and total number of HB acceptor/donor (H-B: A/D ratio). This clearly indicated that increasing number of both hydrogen bond donor and acceptor is a major requirement for anticancer FQ from our scaffold. It is known that increasing total number of H-B does increase possibility of H-B with more receptors. It was also important that increased values of donor are more significant. This means that 8-amino is essential for activity as the case in all reduced and provides good vicinity for target interaction with FQ through the freely accessible interfacing H-B. Supported by weak activity of the 4a (2-chloro) and 4d (which has 7-amino), it seems that the 7,8-ethylene diamine bridge created with reduced series offers an excellent chelator group between C-7 and C-8. Moreover, the aromatic amine of C-8 is similar to hydroxyl phenol in which both provide good radical scavenging antioxidant groups. The amino group provides stability of free ionizable electron radical (formed on the nitrogen atom) through resonance which makes such group as antioxidant group, Figure 2. The data of this work have showed that 3-chloro derivative 4b was more active than 2-chloro 4a against most cell lines. This chloro benzene driven activity is limited to positions 3' and 4' but not 2'. This indirectly enlightened the importance of free vicinity or space in front of C-7 and C-8, indicating the importance of C-8 amino group and the 7-8-ethylene diamine bridge in anticancer activity. It possible that chloro atom at position 2' come in close proximity to C- 7 and 8 cosmos and sterically hindered the receptor interaction through occupying the space in front of the 7-8 ethylene diamine system, mainly in reduced 4 series.

The flexibility at C-3'' allows the choro atom to contribute freely in the tridentate metal complex in 4b, since it points out forward toward the C-8 amine creating a short distance and come in close proximity to contribute in Metal-complex. However, the steric repulsion between the N-1 butyl and C-2'' chloro atom in 4a pushes the

chloro atom far away hindering its contribution in the chelation complex. This fore sure can lessen the activity of 4a. More support to this assumption comes from the 3D of the most stable conformer of chloro at C-2'' (4a). The 2'' chloro atom points out backward and away from the vicinity of the 2 N atoms, making the chloro atom far from the vicinity of the complex together with the phenyl ring which is also pushed away with its chloro atom.

This ratify with no doubt that the free COOH-4-oxo-quinoline scaffold is essential requirement for anticancer FQs. Not only it provides ionic interaction, this acidic group provides also hydrogen bond acceptor through carbonyl oxygen and hydrogen bond donor through OH. Chelation is another mechanism since carboxylate is another reported mechanism for all antibacterial FQs.

We can propose initial SAR for anticancer FQ to include:

1- Acidic compounds: through 3-COOH group

2- Chelator: through 4-oxo-3-COOH group and C8, 7-ethylene diamine

3- Lipophilicity N1-Butyl and C7-halo aniline

4- Comprising nitro and amine group at C-8: very essential for activity

5- Increasing number of hydrogen bond donor and acceptor is the major important requirement.

6- Encompassing one or more chloro substitution at the aniline (4' or 3'-chloro, far from vicinity of the diamine bridge).

It was anticipated that our compounds share the same mechanism of action as vosaroxin since both have the same structural scaffold and close functionalities. Similar to vosaroxin, compounds 4(a-c) are proposed to uncover eukaryotic topoisomerase II inhibition potency explaining the anticancer potentialities revealed within this research. This assumption is illuminated indirectly by the fact that active compounds FQs have different and lower IC_{50} values against many cells compared to the reference cisplatin with no conclusive pattern. In fact, 4b was more active than reference agent against all cell lines (with nanomolar values in some) that was unparalleled by the reference. This may be an indication of a different mechanism of action from the alkylating mechanism of the reference. These outcomes clearly and unambiguously that our FQs do work by a different mechanism from DNA interfering agent cisplatin. Having the eukaryotic topoisomerase II inhibitor doxorubicin in our labs and availability of successful assay, we have decided to compare the activity of our active FQ 4b, doxorubicin and Khaleel et al., (2021) active compound S4a. The 2 compounds were investigated against CRC cell lines HT29, HCT116 and SW620 and $CACO_2$ cell lines respectively (Figure 1). Surprisingly, Figure 2 illustrates that both 4b and S4a do synchronize with doxorubicin pattern rather than cisplatin. This pattern and mode similarity suggests that our FQ 4b has closer or similar mechanism of action to the topoisomerase II inhibitor doxorubicin. All tested compounds 4b and S4a and doxorubicin showed strong IC_{50} values against HT29, HCT116 and SW620 respectively but weaker values on most resistant $CACO_2$. Since vosaroxin works as eukaryotic topoisomerase II inhibitor (Abbas and Stuart, 2012), these outcomes fortifying the assumption that our FQs potentially act as topoisomerase II inhibitors as one potential target of their antiproliferative mechanism.We do believe that this activity comes mainly from the extra number of H-B A/D provided by C-8 amino group in 4b. Accordingly, the huge number of H-B A/D groups can explain the tremendous activity of doxorubicin over FQ 4b. It is worth saying also that extra chelation groups in doxorubicin might increment such activity. We do have confidence that the most important structural feature for selective eukaryotic topoisomerase II inhibitor shared by all active FQs, doxorubicin and even vosaroxin is the extra number of H-B. These groups are provided by C-8 amino group in 4N, C-8 N atom in vosaroxin and beta hydroxyl-1-one group in doxorubicin. We would like here to enforce the role of such groups in providing also a chelation mechanism that is also reported in anticancer therapy. Increasing the number of chelator groups is more likely to enhance activity as the case of doxorubicin. We rationalize the role of C-8 amino to provide a chelator group also to further optimize a more active FQs in the future. We do conclude by introducing a universal chelator group in cancer therapy. The advantage of chelation is to provide an inhibition against many enzymes/targets intra and extracellular increasing the potency of any agent and the broadness of activity (more than one cell line). The fact that 4b was active against most cell lines tested with extraordinary IC_{50} values can be explained by a universal mechanism only. This for sure support our assumption that 4b works by chelation as a universal mechanism. Moreover, we would like to introduce the term (universal drug) based on these results. We do expect that such chelator based FQs could offer efficient treatment against many types of cancer from one side, and against metastatic types of cancers. Moreover, we do anticipate that such chelators work on different enzymes/targets and exhibit many biological activities. This proposition can further be validated and rationalized by testing the antioxidant and antiinflammatory activities as seen in next parts of this work. Finally this research has shed some light on potential anticancer chelator FQs with safe profile. Further mechanistic studies are worth following before taking them to clinical testing especially to explore their target receptors.

Antioxidative capacities

Based on the evidence that was highlighted about the positive relationship between the antioxidant and antiproliferative properties of several compounds, the DPPH assay was used in this research to assess antioxidant characteristics of our compounds and to understand their antiproliferative properties. Furthermore, this new biological activity was carried out to validate the universality of chelation mechanism in biology. The most active antiproliferative series 4 (reduced) were the only group having an antioxidant activity (Table 1). Also, compound d has shown the strongest antioxidant (4d>4a>4b). Based on these results, we conclude that the radical scavenging activity may also be a potential antiproliferation mechanism, which is due to the presence of the free radical scavenging amino group (NH_2).From a chemistry point of view, free radical

scavenging groups can be involved in both activities; anticancer and antiproliferation. Such ionizable groups include phenolic OH and aniline NH_2 group. Since FQs 4a and b contain ionizable NH_2 at C-8, they can offer both potencies. The extra antioxidative activities of 4d can be explained by 7 amino and 8 amino groups and for sure explain their different mechanism of actions from 4b. Furthermore, antioxidativechelators are well known to exhibit antiproliferative activities. In fact, both 4b and 4d showed excellent potencies as antioxidant and anticancer compounds, possibly due to again chelator ethylene diamine Groups, (Figure 2). The difference in lipophilicity does explain antiproliferative properties of 4b over 4d although both have the ethylene diamine; this indicates clearly that our lipophilic FQs work intracellular via topoisomerase interaction since they are lipophilic whereas 4d worked extracellular only as in DPPH assay.

Antiinflammatory activity

Chronic inflammation is one of the most significant risk factors for cancer formation and development, and thus antiinflammatory drugs can reduce the risk of developing different types of cancer. Therefore, we investigated the effectiveness of the new nine FQs compounds as antiinflammatory drugs. We assayed our novel FQs against LPS-induced nitric oxide (NO) production in RAW 264.7 cell line to verify the antiinflammatory activity of these drugs. Table 1 displays the IC_{50} values of anti-inflammatory activity of FQs alongside indomethacin as a reference. The compounds 3b, 3d, 4b, 4d, and 5a exerted an exceedingly remarkable and superior antiinflammatory effects ($p<0.0001$ vs. indomethacin). Compounds 3a and 5b were moderately more efficacious than indomethacin. Table 1 clearly showed that the strongest antiproliferative 4b revealed the strongest antiinflammatory activity with IC_{50} value of 5.39±0.74. 4b showed 24 fold stronger than the reference indomethacin with IC_{50} value of 119.49±3.02. Again, this incriminates that strongest antiproliferative compounds with ethylene diamine chelating bridge as 4b showed the superlative antiinflammatory potency. Chemistry wise, it seems that the C-7 and C-8 ethylene diaminechelator group was a universal group that served different activities through metal chelation. Principally flavonoids with these 3 activities were never connected to this universal chelator group as 5-hydroxy-4-one within flavonoids, Figure 3 (Matsjeh et al., 2017). In fact, this is the first time that we introduce chelation as universal ethylene diamine group in FQs and 5-hydroxy-4-one within flavonoids explaining the 3 activities. Surprisingly, the well documented chelator-4-oxo-3-COOH group in FQs has minimal role in any of these activities, suggesting the amine chelators as universal groups only.

Conclusive remarks and future directives

Explicitly marked spectrum of bioeffects of heterocyclic lipophilic FQs correlated to their acidic groups and C8-C7 ethylene diamine Chelation Bridge along with bulky dual halogenations. It was anticipated that chloro aniline contributed the tridentate complex. In effect these derivatives exhibited their multi-bioactivity due to universal chelation mechanism. Collectively, this work reveals lipophilic-acidic chelator FQs with dual antiinflammation/antioxidative free radical scavenging and antiproliferation propensities as authentic agents for the anticancer chemotherapy/prevention. The only incurred limitation was the untested spectrum of anticipated antimicrobial effectiveness for these 9 novel FQs. Future work includes investigating potential topoisomerase and HDAC mitigation capacities of FQs. Further preparation of new FQs similar to 4b by substituting hexyl on N1 position rather than butyl to increase the lipophilicity of compound can be planned; Optimizing chloroaniline positions and number on new FQs; Testing our compounds against other cell lines such as cervical cancer, melanoma, and lung cell lines; Investigation of the mechanism and pharmacokinetic properties of active hits such as 4b; Investigating toxicity and safety of active hit 4b toward clinical testing; Evaluating the new FQs compounds for possible antibacterial activity and Investigation of the active hit 4d for in vivo radical scavenging activity .

Author Contribution Statement

All authors contributed equally towards rationale conceptualization, experimental design, data collection and analyses, manuscript write up and proofreading.

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Conflicts of interests

Authors declare no conflict of interest.

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