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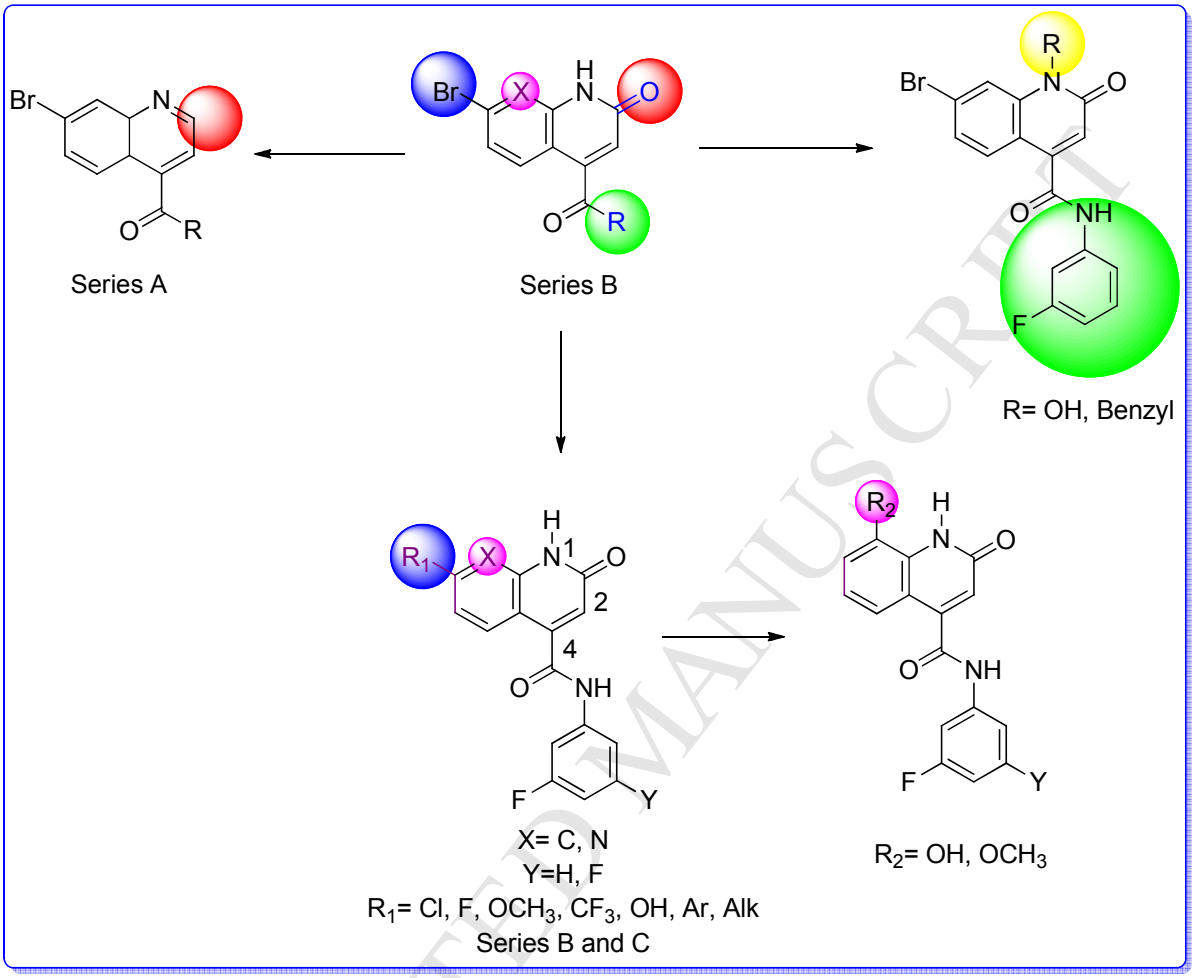

Synthesis and biological evaluation of novel 2-oxo-1,2-dihydroquinoline-4-carboxamide derivatives for the treatment of esophageal squamous cell carcinoma

Mai I. Shahin,[a,b] Joyeeta Roy,[a] Maha Hanafi, [a,c] Dongyao Wang,[a,d] Urarika Luesakul,[a,e] Yifeng Chai,[d] Nongnuj Muangsin,[e] Deena S. Lasheen,[b] Dalal A. Abou El Ella,[b,f] Khaled A. Abouzid,[b] and Nouri Neamati*[a]

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Abstract

No new and effective treatments have been approved for the treatment of esophageal squamous cell carcinoma (ESCC) in the past decade. Cisplatin and 5-fluoruracil are the most commonly used drugs for this disease. In order to develop a new class of drugs effective in our ESCC phenotypic screens, we began a systematic approach to generate novel compounds based on the 2-oxo-1,2-dihydroquinoline-4-carboxamide fragment. Herein, we report on the synthesis and initial assessment of 55 new analogues in two ESCC cell lines. Some of the active analogues with IC_{50} values around 10 µM were tested in three additional cell lines. Our structure-activity relationships revealed remarkable alterations in the anti proliferative activities upon modest chemical modifications and autophagy modulation is a suggested mechanism of action.

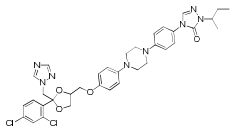
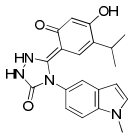
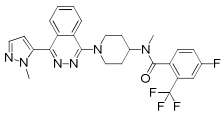
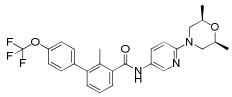
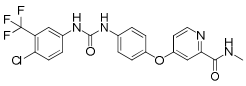
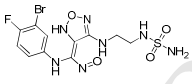
Keywords

Quinoline-4-carboxamide; esophageal squamous cell cancer; autophagy; structure-activity relationships; chloroquine.

1. Introduction

The two predominant histological types of esophageal cancers are esophageal squamous cell cancer (ESCC) and adenocarcinoma (AC) [1-3]. These cancers are different in terms of epidemiology, pathogenesis, tumor biology, and prognosis [4-9]. Recent studies have demonstrated striking similarities between ESCC and head and neck cancers [10]. From >400,000 deaths from esophageal cancer in the world each year, 80% are due to ESCC [11-13]. ESCC is an aggressive disease with poor prognosis and is typically diagnosed in late stage of the disease. ESCC is the fourth leading cause of cancer death in the United States with a 5-year survival rate of 19%. The standard of care treatment consists of the classical modalities including surgery, radiotherapy, and the combination of two old drugs, cisplatin and 5-FU [2],[14-16]. Unfortunately, only 20–40% of patients show significant response. Therefore, novel drugs targeting key genes or pathways implicated in ESCC are needed to effectively treat this disease. In recent years, neoadjuvant chemotherapy followed by surgery have been adopted as a first-line treatment for advanced ESCC tumor [17]. Selected small molecule drugs with different biological targets that are currently under clinical development are listed in **Table 1**. Despite all these substantial efforts, no significant improvements have been observed in the overall survival rates of ESCC patients. Therefore, there is an unmet need for developing new effective therapeutic agents for ESCC.

Table1. Selected examples of small molecule drugs under clinical trials for ESCC treatment

Agent	Structure	Mode of action	Trial stage	Tumor type	Trial number	References
Itraconazole		Hedgehog pathway inhibition	Early phase I	Esophageal cancer	NCT02749513	[18]
Ganetespib (STA-9090)		HSP90 inhibition	Phase I	Esophageal cancer	NCT02389751	[19]
Taladegib (LY2940680)		Hedgehog pathway inhibition	Phase I/II	Esophageal cancer	NCT02530437	[20]
Sonidegib (LDE225)		Hedgehog pathway inhibition	Phase I	Esophageal cancer	NCT0213892	[20]
Sorafenib		VEGFR inhibition	Phase II	Esophageal cancer Gastroesophageal Junction Cancer Gastric Adenocarcinoma Gastroesophageal Junction Adenocarcinoma Recurrent Esophageal Carcinoma Recurrent Gastric Carcinoma Unresectable Esophageal Carcinoma	NCT00917462	[21]
Epacadostat (INCB024360)		IDO1 inhibition	Phase II		NCT03196232	[22]

Autophagy, an evolutionarily conserved cellular response, mitigates stresses through catabolic degradation of intracellular components to maintain hemostasis. The impact of autophagy is investigated in many diseases especially cancer [23, 24]. Its dual role in cancer suggests that autophagy modulation through inhibition or activation can be an effective strategy for treating cancer [25]. Despite its complex role in cancer, many clinical interventions are thought to be

through autophagy inhibition [26]. The solely clinically available drugs suppressing autophagy are chloroquine and hydroxychloroquine. Chloroquine in combination with various drugs has been explored as an effective strategy to treat different types of cancers including ESCC [27]. The mechanistic studies revealed that chloroquine exerts its anti-tumor activity mainly through autophagy inhibition [28, 29]. A recent study reported that autophagy inhibition sensitizes esophageal cancer cells to radiation [30].

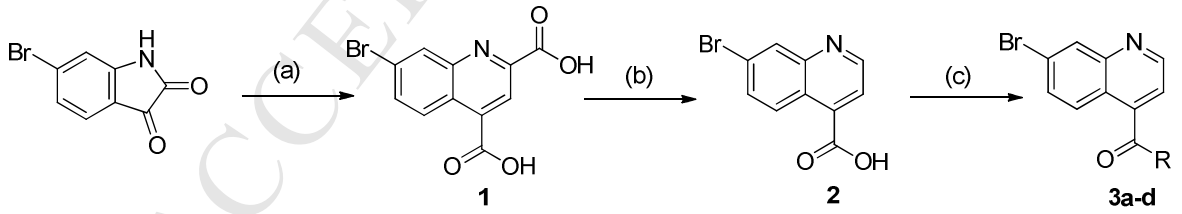
In this context, 2-oxo-1,2-dihydroquinoline-4-carboxamide fragment was used as a core scaffold to synthesize three series of compounds consisting of 55 derivatives. They were evaluated for their inhibition of cell proliferation in two representative ESCC cell lines, KYSE410 and KYSE70. We used the 2-oxo-1,2-dihydroquinoline-4-carboxamide fragment for synthetic elaboration to design novel compounds for the following reasons: 1. This fragment is drug like (MW = 264, tPSA = 58, cLogP = 1.3, H-bond donor= 2, H-bond acceptor = 2). 2. This fragment is novel because it is not present in any clinically used drug. 3. 2-Oxo-1,2-dihydroquinoline-4-carboxamide can be readily synthesized from commercially available isatin derivatives and malonic acid. 4. This fragment is amenable to facile modifications producing a large library of novel analogues with desirable pharmacokinetics and pharmaceutics properties. The stepwise optimization strategy presented in this study yielded compounds with novel mechanisms to treat ESCC. Therefore, the optimized compounds are suitable for further developments paving the way for designing second-generation compounds for *in vivo* studies.

2. Chemistry

Series A, B, and C were synthesized according to **Schemes 1** and **2a,b**. 7-Bromoquinoline-2,4-dicarboxylic acid (**1**) and 7-substituted-2-oxo-1,2-dihydroquinoline-4-carboxylic acid (**5a**, **5c, 5d**

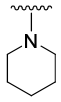
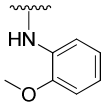
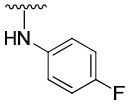
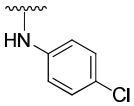
and **5e**) were synthesized by reacting the commercially available 6-substituted isatin with pyruvic acid sodium salt and malonic acid respectively as reported [31, 32]. Similar synthetic method was applied to prepare compound **14** from the commercially available 7-methoxyindoline-2,3-dione. 6-Methoxy-1H-pyrrolo[2,3-b]pyridine-2,3-dione (**4**) was synthesized by Pyridinium chlorochromate mediated oxidation of 6-methoxy-1H-pyrrolo[2,3-b]pyridine [33]. Decarboxylation at position 2 of compound **1** was achieved by refluxing in nitrobenzene as reported [34]. The commercially available 7-fluoro-2-oxo-1,2-dihydroquinoline-4-carboxylic acid **(5b)** and the synthesized carboxylic acids **2**, **5a, 5c, 5d, 5e and 5f** were reacted with the corresponding amines to give the targeted compounds (**Schemes 1** and **2a, b**; Series A and B). BBr_3-mediated demethylation of **6x** and **15** afforded the corresponding hydroxy analogs **6y** and **16** [35]. To replace the bromo atom with the acetylene moiety, bis(triphenylphosphine)palladium chloride was used as a catalyst to couple of trimethylsilylacetylene **6i**. Compound **8** was further deprotected to yield the free acetylenic derivative (**9**) [36, 37]. Compound **6i** was benzylated using benzyl chloride and potassium carbonate in DMF to yield **10** [38].

Scheme 1: Synthetic route to series A analogs



Reagents and conditions: (a) Pyruvic acid sodium salt, NaOH, reflux 2h; (b) Nitrobenzene, reflux, overnight; (c) R^1NH_2, dichloromethane, HATU, TEA, rt, overnight.

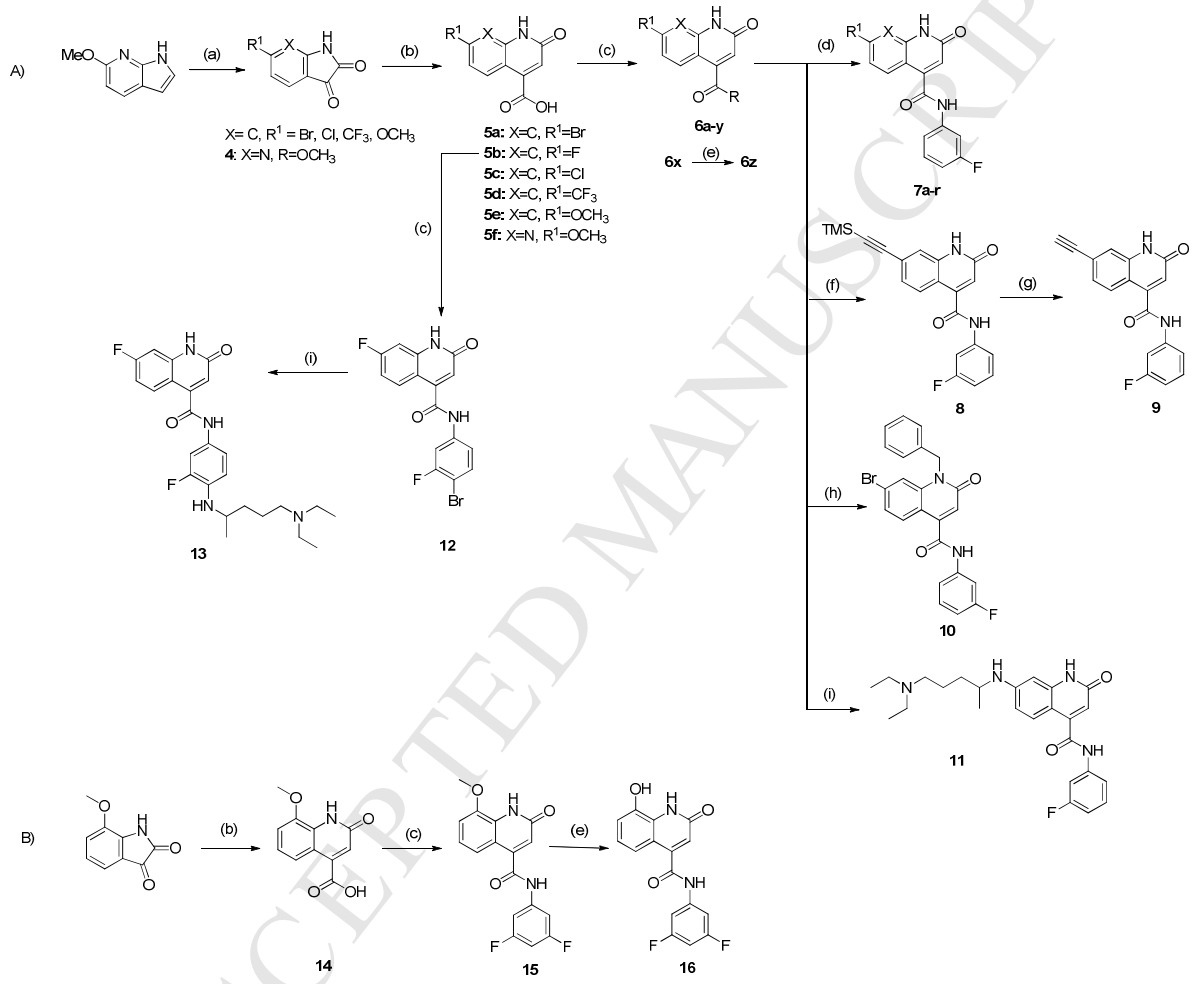
Table2. Cytotoxicity ± SD of compounds **3a-d** and cisplatin in two ESCC cell lines

Entry	R	IC_{50} (μM)	
		KYSE410	KYSE70
Cisplatin	—	9.1 ± 0.7	10.0 ± 1.9
3a		>20	>20
3b		>20	>20
3c		>20	>20
3d		>20	>20

Coupling the bromo-derivatives with various boronic acid under Suzuki–Miyaura cross-coupling conditions afforded series C analogues. This was achieved by refluxing the bromo-derivative and the boronic acid in dioxane: water (4:1) using cesium carbonate as base and [1,1'-bis(diphenylphosphino)ferrocene]palladium(II) dichloride (Pd(dppf)Cl_2) as the palladium source [39]. The *N*,*N*-diethylpentane-1,4-diamine moiety was appended to the corresponding bromo derivatives **6i** and **12** under conditions of Buchwald Hartwig amination. This coupling afforded derivatives **11** and **13** using Xphos: (2-Dicyclohexylphosphino-2′,4′,6′-triisopropylbiphenyl) and $Pd_2(dba)_3$: (Tris(dibenzylideneacetone)dipalladium(0)) as catalysts in the presence of sodium tert-butoxide as a base [40]. Compound **18** was synthesized according to the pathway outlined in **Scheme 3**. Chlorination of *N*-(3-fluorophenyl)-2-oxo-1,2-dihydroquinoline-4-carboxamide using $POCl_3$ resulted in **17** that was further oxidized using *m*CPBA (meta-chloroperoxybenzoic acid)

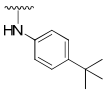
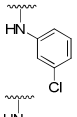
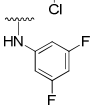
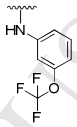
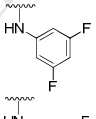
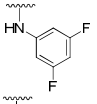
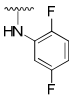
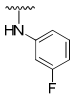
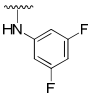
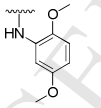
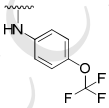
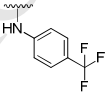
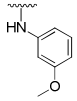
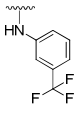
to yield the *N*-oxide intermediate. This intermediate was directly converted to the targeted compound **18** using 10% aq. HCl under reflux condition [41].

Scheme2a,b: Synthetic route of series B and C analogs

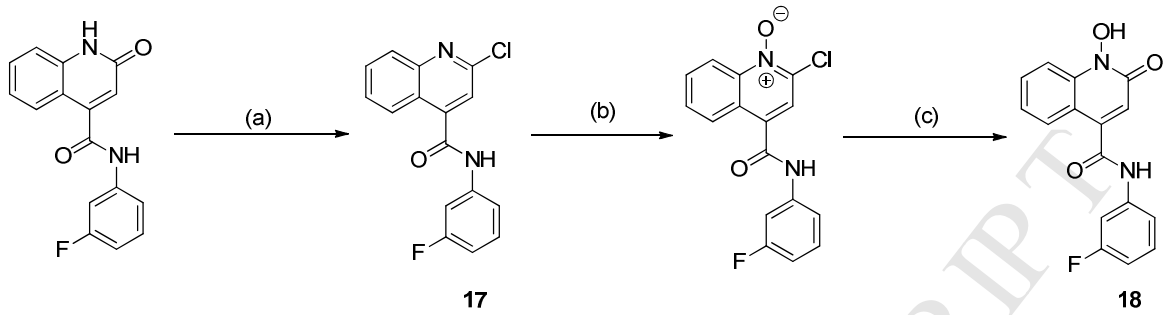


Reagents and conditions: (a) Pyridinium chlorochromate, SiO_2, $AlCl_3$, dichloroethane, reflux, overnight; (b)Malonic acid, AcOH, reflux, overnight; (c) R^2NH_2, dichloromethane, HATU, TEA, rt, overnight; (d) $R^3B(OH)_2$, Cs_2CO_3, $Pd(dppf)Cl_2$, rt, overnight; (e) BBr_3, dichloromethane, 0° C, 4h; (f) Trimethylsilylacetylene, $PdCl_2(PPh_3)_2$, TEA, 80° C, 8h; (g) Methanol, K_2CO_3, rt, 3h; (h) Benzyl chloride, DMF, K_2CO_3, rt, overnight; (i) Novoldiamine, $Pd_2(dba)_3$, Xphos, NaOtBu, dioxane, 90 °C, 16 h

Table 3. Cytotoxicity ± SD of compounds **6a-z, 13, 15** and **16** in two ESCC cell lines

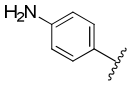
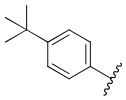
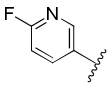
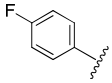
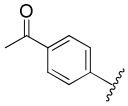
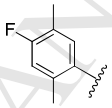
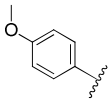
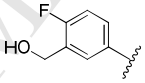
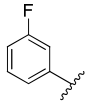
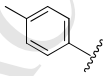
Entry	R^1	R	X	IC_{50} (μM) KYSE410	IC_{50} (μM) KYSE70	Entry	R^1	R	X	IC_{50} (μM) KYSE410	IC_{50} (μM) KYSE70
Cisplatin	--	--	--	9.1 ± 0.7	10.0 ± 1.9	6o	Br		C	>20	>20
6a	Br		C	>20	>20	6p	Br		C	>20	>20
6b	Br		C	>20	>20	6q	Br		C	>20	>20
6c	Br		C	>20	>20	6r	Br		C	11.2 ± 2.2	10.7 ±0.0
6d	Br		C	>20	>20	6s	Br		C	14.0 ± 0.7	>20
6e	Br		C	>20	>20	6t	F		C	>20	>20
6f	Br		C	9.8 ±3.5	11.0 ± 0.3	6u	F		C	>20	>20
6g	Br		C	>20	>20	6v	Cl		C	11.0 ± 0.7	9.0 ± 0.2
6h	Br		C	>20	>20	6w	CF_3		C	17.9 ± 3.1	14.2 ± 0.8
6i	Br		C	7.3 ± 1.1	8.9 ± 0.1	6x	OCH_3		C	>20	>20
6j	Br		C	>20	>20	6y	OCH_3		N	>20	>20
6k	Br		C	>20	>20	6z	OH		C	>20	>20
6l	Br		C	>20	>20	13	--	—	—	>20	>20
6m	Br		C	>20	>20	15	--	--	—	>20	>20
6n	Br		C	>20	>20	16	—	--	—	>20	>20

Scheme 3:



Reagents and conditions: (a) $POCl_3$, 3 h, reflux; (b) mCPBA, dichloromethane, rt, 4h; (c) 10% aq HCl, reflux, overnight

Table 4. Cytotoxicity ± SD of compounds **7a-r**, **9**, **10, 11**and **18** in two ESCC cell lines

Entry	R^1	IC_{50} (μM)		Entry	R^1	IC_{50} (μM)	
		KYSE410	KYSE70			KYSE410	KYSE70
Cisplatin	—	9.1 ± 0.7	10.0 ± 1.9	**7l**		>20	>20
7a		>20	>20	**7m**		12.1 ± 2.2	10.5 ± 0.6
7b		>20	>20	**7n**		>20	>20
7c		10.3 ± 1.1	3.3 ± 0.1	**7o**		11.6 ± 6.4	>20
7d		>20	>20	**7p**		>20	>20
7e		18.0 ± 1.8	>20	**7q**		>20	>20
7f		4.3 ±2.2	5.1 ± 2.8	**7r**		13.6 ± 4.3	9.7 ± 0.3
7g		10.9 ± 1.1	12.0 ± 0.7	**9**		9.7 ± 1.0	9.5 ± 0.1
7h		>20	>20	**10**	—	>20	>20
7i		>20	>20	**11**	—	>20	>20
7j		>20	>20	**18**	—	>20	>20
7k		>20	>20				

3. Results and discussion

3.1. Lead optimization and SAR analysis

To design compounds containing the 2-oxo-1,2-dihydroquinoline-4-carboxamide fragment, various analogues were synthesized through stepwise structure optimization process. MTT assay was performed to assess the antiproliferative potency of all compounds in KYSE410 and KYSE70 cell lines (**Tables 2, 3** and **4**). Cisplatin, a clinically used drug was used as control. As shown in **Fig. 1**, four positions were explored by applying different modifications. First, series A and B were synthesized in parallel with different aliphatic and aromatic fragments at position 4. Compounds **6i** was the most active with IC_{50} values 7.3 ± 1.1 and 8.9 ± 0.1 µM, respectively in KYSE410 and KYSE70 cell lines. Having another fluorine atom in the other *meta* position in compound **6r** maintained similar IC_{50} values around 10 µM in both cell lines. This illustrates the importance of having a fluorine atom at a *meta* position of the phenyl ring. Other derivatives bearing different substituents on this phenyl ring on different positions did not produce potent compounds (e.g. compound **6o** which has a tertiary butyl group on the *para* position). The dose response curve and the colony formation assay of the active compound **6r** and the inactive compound **6o** in comparison to cisplatin is shown in **Fig. 2**.

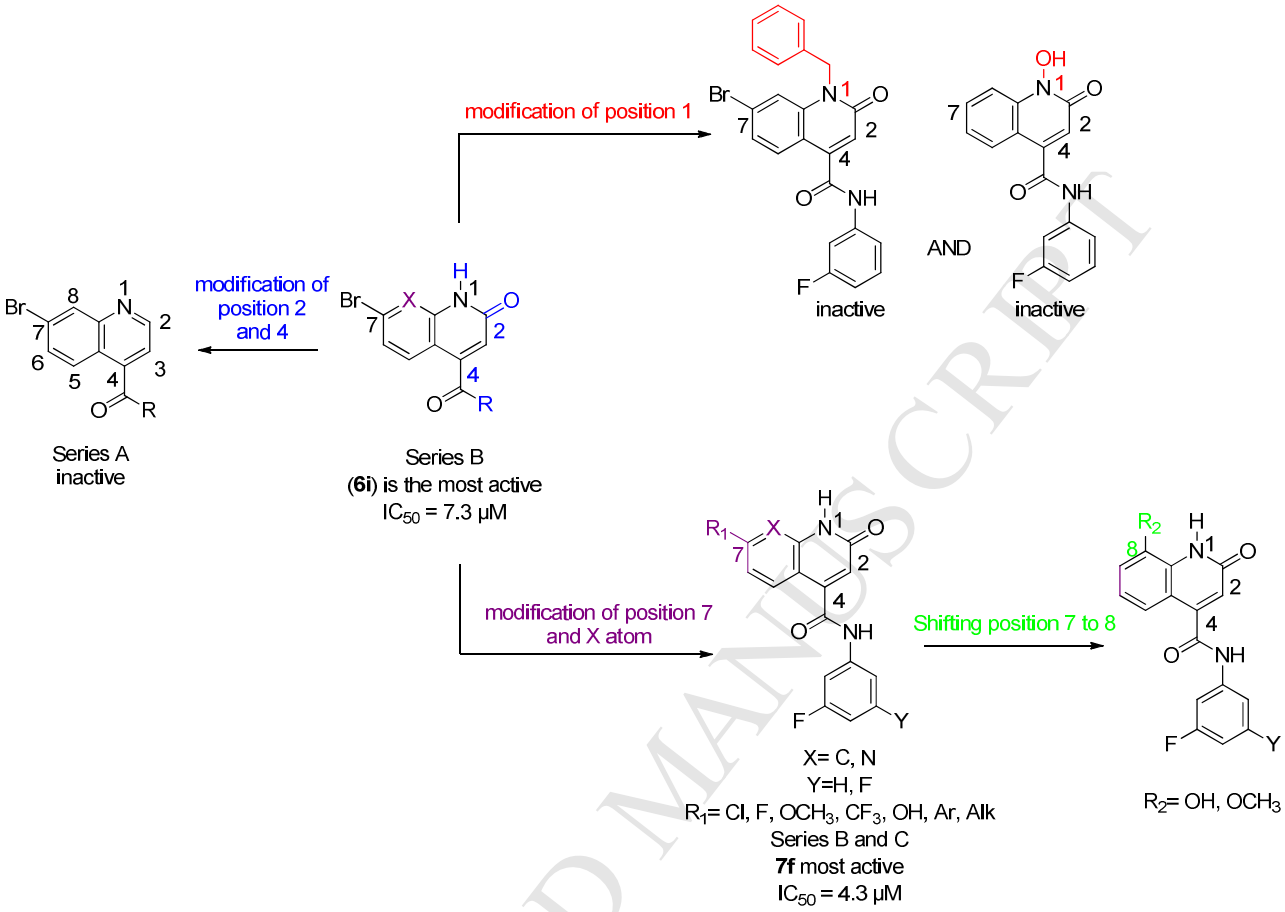


Fig. 1. Lead optimization paradigm

The replacement of the bromo atom at position 7 with the fluoro, chloro, trifloromethyl, methoxy, hydroxyl, vinyl and acetylene groups negatively affects the activity. Also, neither the bioisosteric replacement of quinolone with naphthyridine nor shifting the substitution to be at position 8 instead of position 7 showed improvement in the activities. Since **6i** and **6r** are acceptable starting point for optimization, further modifications were carried out as follow: **Step 1**: Position 1 modifications: We synthesized compounds **10** and **18** to test the effect of hydrophilic and hydrophobic moieties at Position 1. Compound **10**, with a hydrophobic group showed diminished activity. Compound **18** bearing a hydrophilic hydroxyl group at position 1

did not improve activity. This suggests the importance of having 2-oxo-1,2-dihydroquinoline skeleton without any modifications at position 1. **Step 2**: Position 7 optimization: Starting from compound **6i**, further optimization was carried out on position 7. Series C (compounds **7a-r**) were synthesized using Suzuki-Miyaura cross coupling reaction where the bromo atom was replaced with different aromatic and aliphatic rings. As illustrated in **Table 4**, the *para* fluoro substituted phenyl ring (compound **7c**) showed moderate potency where the activity decreased by moving this fluoro atom to m*eta* then to *ortho* positions. Similar cytotoxicity profile was observed by introducing a pyran and a 2-fluoropyridine fragments on compounds **7m** and **7o,** respectively. Compound **7f** having a thiophene ring showed IC_{50} values of 4.3 and 5.1 µM in KYSE410 and KYSE70, respectively.

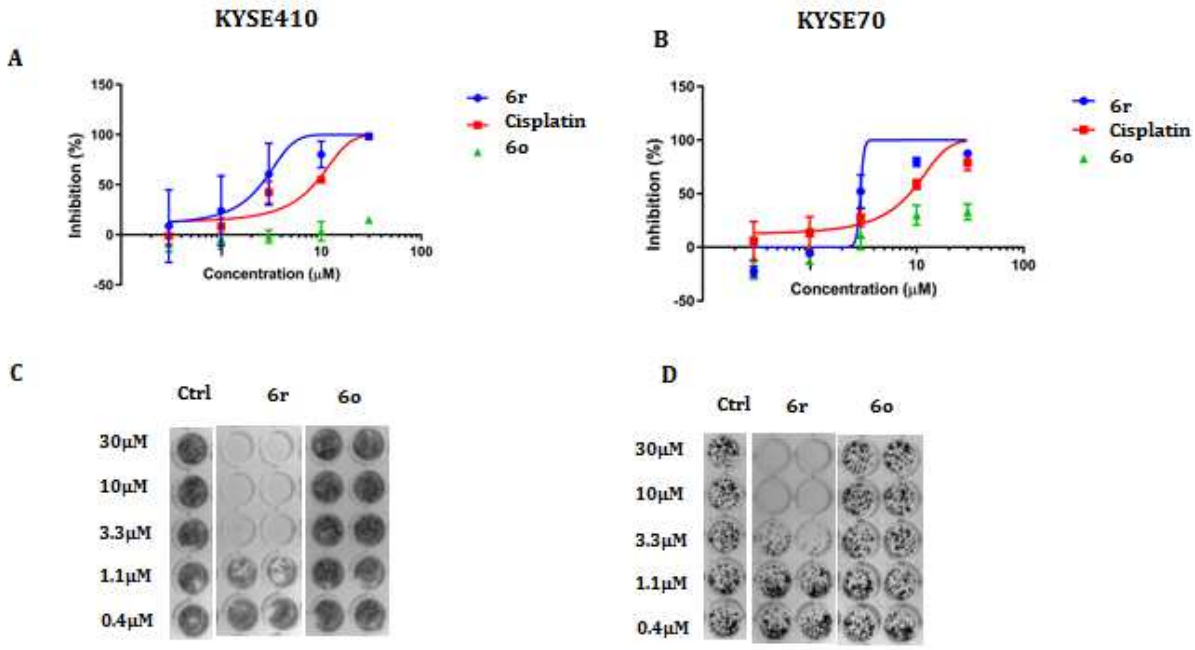


Fig. 2. Compounds with a *meta* fluoro substitutions are active. (A) and (B) Compound **6r** but not **6o** is more potent than cisplatin in KYSE410 and KYSE70 cells, respectively. (C) and (D) Compound **6r** completely block formation of colonies at doses above 3 µM in KYSE410 and KYSE70 cells.

Step 3: Adding the *N*,*N*-diethylpentane-1,4-diamine moiety: It was important to synthesize examples of compounds having this feature to test the effect on both cytotoxicity and autophagy process. That's why we synthesized compounds **11** and **13** having this group in two variant positions. Despite the drop in the cytotoxicity, they behaved like chloroquine in its inhibitory activity of autophagy pathway as will be discussed in the western blot results.

From all the synthesized compounds, the active compounds (**6f**, **6i**, **6r**, **7c**, **7f** and **7m**) were further tested in pancreatic cancer MIA PaCa-2 and two ovarian cancer OVCAR8 and SKOV3 cell lines. Compound **7f** showed best cytotoxic activity over the other compounds that only showed moderate cytotoxicity against them. (**Table 5**).

Table 5. IC_{50} ± SD (μM) values of compounds **6f, 6i, 6r, 7c, 7f** and **7m** in KYSE410, KYSE70, MIA PaCa-2, OVCAR8 and SKOV3 cell lines using MTT assay.

Compound	KYSE410	KYSE70	MIA PaCa-2	OVCAR8	SKOV3
6f	9.8 ±3.5	11.0 ± 0.3	21.3 ± 6.0	>20	>20
6i	7.3 ± 1.1	8.9 ± 0.1	7.5 ± 3.6	5.8 ± 1.1	10.6 ± 3.8
6r	11.2 ± 2.2	10.7 ± 0.1	7.8 ± 1.2	12.1 ± 4.6	10.8 ± 1.7
7c	10.3 ± 1.1	3.3 ± 0.1	19.3 ± 2.5	22.3 ± 2.6	16.6 ± 2.2
7f	4.3 ±2.2	5.1 ± 2.8	4.5 ± 1.6	13.4 ± 4.4	6.7 ± 2.0
7m	12.1 ± 2.2	10.5 ± 0.6	8.8 ± 1.6	21.8 ± 0.8	13.3 ± 4.6

3.2. Lead compounds decrease bafilomycin-induced LC3B levels

To further investigate the mechanism of action of the lead compounds, the levels of LC3B, a marker of autophagy, were measured upon treatment with compounds **6i** and **6r**. The experiment was carried out in the presence and absence of bafilomycin-A1, a lysosome inhibitor. Bafilomycin-A1 inhibits autophagy at late stages and results in increased levels of LC3B. The

autophagy inhibitor chloroquine was used as a control. In contrast to chloroquine, compounds **6i** and **6r** cause a decrease in the bafilomycin-induced LC3B levels after 24h treatment (**Fig. 3A and 3B**). To confirm these results, we repeated the experiment using different concentrations of compound **6r**. The Western blot analysis showed a concentration-dependent decrease in the bafilomycin-induced LC3B levels (**Fig. 3C**). Furthermore, we tested whether proteasome inhibition will block the effect of **6r**. We pre-treated the cells with the proteasome inhibitor MG132 for 1h prior to a 24h treatment with **6r**. The results showed no decrease in LC3B levels in the presence of MG132 suggesting that the effect of **6r** on LC3B levels are mediated by the proteasome. In conclusion, the results show that the lead compounds are effective in decreasing LC3B levels in the presence of bafilomycin.

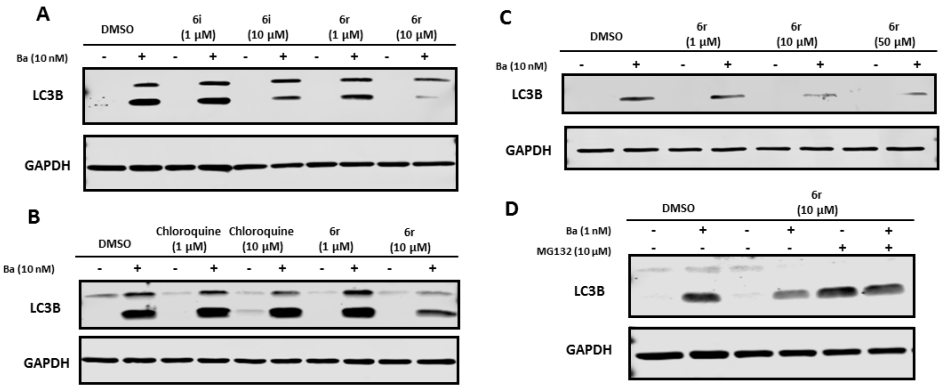


Fig. 3. Lead compounds decrease bafilomycin-induced LC3B levels. (A) MIA PaCa-2 cells treated with compounds **6i** and **6r** (1 µM and 10 µM each) show a decrease in the bafilomycin-induced LC3B levels after 24h. (B) KYSE410 treated withchloroquine (1 and 10 µM) show increase in the LC3B level compared to compound **6r** (1 µM and 10 µM) that shows a decrease in the LC3B level. (C) KYSE410 cells treated with compound **6r** (1 µM, 10 µM and 50 µM) show a concentration-dependent decrease in the bafilomycin-induced LC3B levels after 24h. (D) KYSE410 cells pre-treated with 10 µM MG132 for 1h shows no significant decrease in the LC3B levels upon treatment with compound **6r** (10 µM, 24 h).

We extended our studies to test the effect of the structural modifications on the autophagy-related mechanism. We synthesized analogs **11** and **13** that contain the *N*,*N*-diethylpentane-1,4-

diamine fragment, which is an important moiety in chloroquine-mediated inhibition of autophagy. As expected, we observed an increase in LC3B levels compared to DMSO treatment.

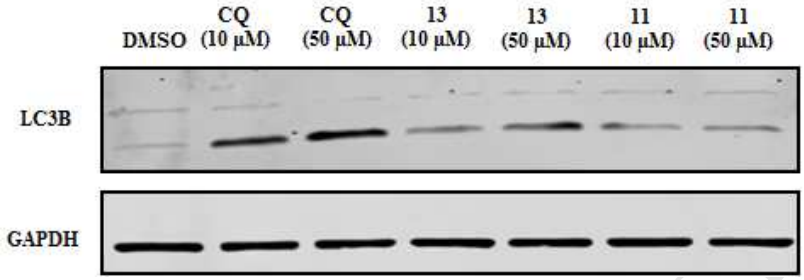


Fig. 4. Compounds 11 and 13 increase LC3B levels. KYSE410 cell were treated with chloroquine, compounds **11** and **13** for 24h (10 µM and 50 µM). All three compounds induce an increased expression of LC3B compared to the DMSO control.

*3.3. Compound **7f** arrests cells in G2/M phase*

To better elucidate the mechanism of action of the leads, representative compounds were tested for their effect on altering cell cycle. Although compounds **6i**, **6f**, and **6r** and chloroquine showed no significant effect on cell cycle, compound **7f** arrested cells in G2/M phase (**Fig. 4**).

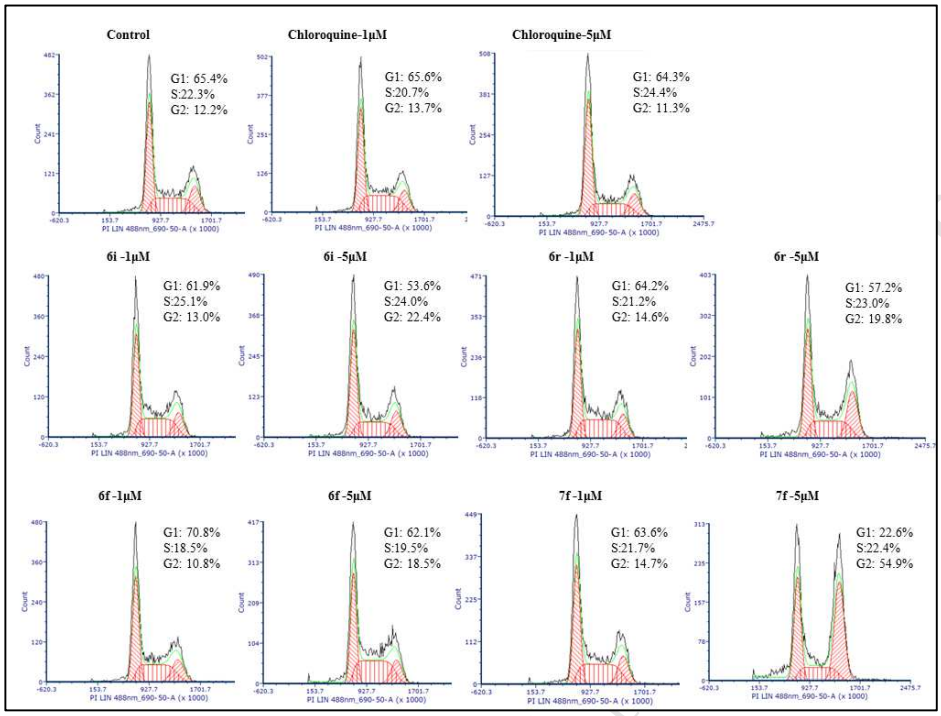


Fig. 4. Compound **7f** arrests cells in G2/M phase. Cells were treated with increasing concentrations of lead compounds, stained with propidium iodide and analyzed by flow cytometry.

4. Conclusions

We synthesized 55 new compounds containing a drug-like 2-oxo-1,2-dihydroquinoline-4-carboxamide fragment to select novel lead compounds to treat ESCC. All compounds were initially tested in two ESCC cell lines using MTT and colony formation assays and active compounds were tested in three additional cell lines. Overall, several compounds showed potency comparable to cisplatin. Mechanistic studies of the lead compounds confirmed inhibition of autophagy as the mechanism of action. From the cell cycle analysis, **7f** was found to be the only derivative that can cause cell cycle arrest at the G2/M phase. Our study suggests that development of new anti-cancer drugs based on 2-oxo-1,2-dihydroquinoline-4-carboxamide warrants further investigations due to their unique mechanism of action.

5. EXPERIMENTAL METHODS

5.3. Chemistry

All commercial reagents and anhydrous solvents were purchased and used without further purification. Column chromatography was performed using normal phase silica gel on a Biotage Isolera™ flash chromatography system. Proton nuclear magnetic resonance (^{1}H NMR) spectroscopy was recorded on a Bruker Avance 300 NMR spectrometers. LC-MS were performed on a Shimadzu LC-MS-2020 system. The purity of the final products was confirmed to be > 95% as determined by HPLC/MS and ^{1}H NMR. Acetonitrile/water solvent mixture was used as an eluent for the HPLC.

5.3.1. *7-Bromoquinoline-2,4-dicarboxylic acid (**1**)* was prepared as previously reported [31].

5.3.2. *7-Bromoquinoline-4-carboxylic acid (**2**).* Decarboxylation was done by refluxing compound **1** (1 g) in 10 mL of nitrobenzene overnight. The reaction mixture was evaporated under reduced pressure and the resulting solid was crystallized from hexane to give brown solid in 67% yield (570 mg).

5.3.3. *General synthetic method for compounds (**3a-d**).* The carboxylic acid (0.8 mmol), HATU (hexafluorophosphate azabenzotriazole tetramethyl uranium) (455 mg, 1.2 mmol) and triethylamine (324 mg, 3.2 mmol) were stirred for 30 minutes in 20 mL dichloromethane. The corresponding amine (1.19 mmol) was added to the reaction mixture and stirred overnight. The resulting solid was filtered, washed with dichloromethane and crystallized from methanol unless otherwise noted. Purification was carried out over silica gel chromatography till purity reached > 95%.

5.3.3.1. *7-Bromoquinolin-4-yl)(piperidin-1-yl)methanone (**3a**).* Purification was carried out using silica gel chromatography (Dichloromethane:MeOH 9.8:0.2). Brown solid (23 mg, 9% yield). LC-MS m/z 319 [M + H] +; ^{1}H NMR (300 MHz, Methanol-d4) δ 8.97 (d, J = 4.4 Hz, 1H), 8.36 – 8.28 (m, 1H), 7.89 – 7.75 (m, 2H), 7.53 (d, J = 4.4 Hz, 1H), 3.88 (t, J = 5.2 Hz, 2H), 3.19 (q, J = 5.7, 4.0 Hz, 2H), 1.77 (d, J = 4.4 Hz, 4H), 1.49 (dt, J = 11.3, 5.2 Hz, 2H).

5.3.3.2. *7-Bromo-N-(2-methoxyphenyl)quinoline-4-carboxamide (**3b**).* Buff solid (20 mg, 7% yield). LC-MS m/z 357 [M + H]+; ^{1}H NMR (300 MHz, DMSO-d6) δ 10.06 (s, 1H), 9.06 (d, J = 4.3 Hz, 1H), 8.35 (s, 1H), 8.19 (d, J = 9.1 Hz, 1H), 7.92 – 7.84 (m, 2H), 7.75 (d, J = 4.4 Hz, 1H), 7.24 (d, J = 7.6 Hz, 1H), 7.13 (d, J = 8.5 Hz, 1H), 7.02 (dd, J = 8.1, 6.5 Hz, 1H), 3.85 (s, 3H).

5.3.3.3. *7-Bromo-N-(4-fluorophenyl)quinoline-4-carboxamide (**3c**).* Yellow solid (41 mg, 15% yield). LC-MS m/z 345 [M + H] +; ^{1}H NMR (300 MHz, Methanol-d4) δ 9.06 – 8.98 (m, 1H), 8.33 (d, J = 2.2 Hz, 1H), 8.19 (dd, J = 9.0, 1.5 Hz, 1H), 7.88 – 7.71 (m, 4H), 7.22 – 7.10 (m, 2H).

5.3.3.4. *7-Bromo-N-(4-chlorophenyl)quinoline-4-carboxamide (**3d**).* White solid (54 mg, 19% yield). LC-MS m/z 361 [M + H] +; ^{1}H NMR (300 MHz, Methanol-d4) δ 9.05 – 8.95 (m, 1H), 8.30 (d, J = 2.1 Hz, 1H), 8.17 (dd, J = 9.2, 1.5 Hz, 1H), 7.82 (s, 1H), 7.80 – 7.73 (m, 3H), 7.46 – 7.35 (m, 2H).

5.3.4. *6-Methoxy-1H-pyrrolo[2,3-b]pyridine-2,3-dione (**4**).* Pyridinium chlorochromate (1.6 g, 7.5 mmol) was mixed with silica gel (1.6 g, 70-230 mesh) and transferred to a round-bottom flask containing dichloroethane (20 mL). To the resulting orange suspension was added a solution of azaindole (450 mg, 3 mmol) in dichloroethane (10 mL) while stirring at room temperature. To this, $AlCl_3$ (5 mg, 0.039 mmol) was added, and the reaction mixture was stirred at 80 °C. Completion of the reaction was monitored by TLC. After completion of the reaction,

the solid was filtered under suction through a sintered funnel layered with silica gel. The filtrate was evaporated. Crude product was subjected to the next reaction.

5.3.5. *General synthetic method for compounds (**5a**,**5c-f** and **14**).* Synthesis was achieved according to the reported procedure. [32]

*5.1.6. General synthetic method for compounds (**6a-y**, **12** and **15**).* Same procedure as for compounds **3a-d**.

*5.1.6.1. 7-Bromo-4-(piperidine-1-carbonyl)quinolin-2(1H)-one (**6a**).* Brown solid (48 mg, 18% yield). LC-MS m/z 335 [M + H] +; ^{1}H NMR (300 MHz, DMSO-d6) δ 11.98 (s, 1H), 7.54 (d, J = 1.8 Hz, 1H), 7.46 – 7.24 (m, 2H), 6.46 (s, 1H), 3.17 (d, J = 4.8 Hz, 4H), 1.60 (d, J = 4.7 Hz, 4H), 1.44 (s, 2H).

*5.1.6.2. 7-Bromo-N-(2-methoxyphenyl)-2-oxo-1,2-dihydroquinoline-4-carboxamide (**6b**).* White solid (150 mg, 51% yield). LC-MS m/z 373 [M + H] +; ^{1}H NMR (300 MHz, DMSO-d6) δ 12.04 (s, 1H), 10.00 (s, 1H), 7.87 – 7.77 (m, 1H), 7.72 (d, J = 8.6 Hz, 1H), 7.56 (s, 1H), 7.47 – 7.37 (m, 1H), 7.29 – 7.17 (m, 1H), 7.11 (d, J = 8.2 Hz, 1H), 7.00 (t, J = 7.7 Hz, 1H), 6.69 (s, 1H), 3.84 (s, 3H).

*5.1.6.3. 7-Bromo-N-(4-methoxyphenyl)-2-oxo-1,2-dihydroquinoline-4-carboxamide (**6c**).* White solid (30% yield). LC-MS m/z 373 [M + H] +; ^{1}H NMR (300 MHz, DMSO-d6) δ 12.07 (s, 1H), 10.61 (s, 1H), 7.68 (dd, J = 13.5, 8.6 Hz, 3H), 7.56 (s, 1H), 7.39 (d, J = 8.7 Hz, 1H), 6.95 (d, J = 8.5 Hz, 2H), 6.74 (s, 1H), 3.75 (s, 3H).

*5.1.6.4. 7-Bromo-N-(4-chlorophenyl)-2-oxo-1,2-dihydroquinoline-4-carboxamide (**6d**).* Buff solid (88 mg, 33% yield). LC-MS m/z 377 [M + H] +; ^{1}H NMR (300 MHz, DMSO-d6) δ 12.10 (s, 1H), 10.88 (s, 1H), 7.78 (d, J = 8.5 Hz, 2H), 7.69 (d, J = 8.8 Hz, 1H), 7.59 – 7.55 (m, 1H), 7.45 (d, J = 8.2 Hz, 2H), 7.39 (d, J = 9.3 Hz, 1H), 6.79 (s, 1H).

5.1.6.5. 7-Bromo-N-(2,4-difluorophenyl)-2-oxo-1,2-dihydroquinoline-4-carboxamide ***(6e)***. Yellow solid (66 mg, 22% yield). LC-MS m/z 379 [M + H] +; ^{1}H NMR (300 MHz, DMSO-d6) δ 7.86 – 7.66 (m, 2H), 7.57 (d, J = 1.9 Hz, 1H), 7.40 (ddd, J = 11.7, 8.9, 2.3 Hz, 2H), 7.17 (dd, J = 9.8, 7.8 Hz, 1H), 6.78 (s, 1H).

5.1.6.6. 7-Bromo-2-oxo-N-(pyridin-2-yl)-1,2-dihydroquinoline-4-carboxamide ***(6f)***. Yellow solid (49 mg, 18% yield). LC-MS m/z 344 [M + H] $^{+}$; ^{1}H NMR (300 MHz, DMSO-d6) δ 12.05 (s, 1H), 11.27 (s, 1H), 8.39 (d, J = 4.5 Hz, 1H), 8.22 (d, J = 7.9 Hz, 1H), 7.89 (t, J = 8.1 Hz, 1H), 7.69 – 7.48 (m, 2H), 7.43 – 7.33 (m, 1H), 7.22 (t, J = 6.1 Hz, 1H), 6.73 (s, 1H).

5.1.6.7. 7-Bromo-N-(4-fluorophenyl)-2-oxo-1,2-dihydroquinoline-4-carboxamide ***(6g)***. White solid (86 mg, 30% yield). LC-MS m/z 361 [M + H] +; ^{1}H NMR (300 MHz, DMSO-d6) δ 12.08 (s, 1H), 10.81 (s, 1H), 7.86 – 7.64 (m, 3H), 7.57 (d, J = 2.0 Hz, 1H), 7.39 (dd, J = 8.8, 2.0 Hz, 1H), 7.23 (t, J = 8.8 Hz, 2H), 6.78 (s, 1H).

5.1.6.8. 7-Bromo-N-(2,5-difluorophenyl)-2-oxo-1,2-dihydroquinoline-4-carboxamide ***(6h)***. Yellow solid (42 mg, 14% yield). LC-MS m/z 379 [M + H] $^{+}$; ^{1}H NMR (300 MHz, DMSO-d6) δ 10.76 (s, 1H), 7.84 (s, 1H), 7.70 (d, J = 8.7 Hz, 1H), 7.57 (d, J = 1.8 Hz, 1H), 7.41 (d, J = 8.9 Hz, 2H), 7.15 (s, 1H), 6.78 (s, 1H); ^{13}C NMR (101 MHz, DMSO) δ 164.91, 161.46, 158.06 (d, J =240.4 Hz, 2.0 Hz), 151.25 (d, J = 245.4 Hz, 2.0 Hz), 144.99, 140.77, 128.09, 126.65 (dd, J = 15.1 Hz, 12.1 Hz), 125.61, 124.54, 121.59, 118.44, 117.26 (dd, J = 23.2 Hz, 9.1 Hz), 115.59, 113.25 (dd, J = 24.2 Hz, 8.1 Hz), 112.48 (d, J = 27.3 Hz).

5.1.6.9. 7-Bromo-N-(3-fluorophenyl)-2-oxo-1,2-dihydroquinoline-4-carboxamide ***(6i)***. White solid (111 mg, 39% yield). LC-MS m/z 361 [M + H] +; ^{1}H NMR (300 MHz, DMSO-d6) δ 12.15 – 12.07 (m, 1H), 10.95 (s, 1H), 7.71 (t, J = 10.2 Hz, 2H), 7.61 – 7.35 (m, 4H), 7.00 (t, J = 8.2 Hz, 1H), 6.80 (s, 1H); ^{13}C NMR (101 MHz, DMSO) δ 164.55, 162.52 (d, J= 242.2 Hz), 161.51,

145.35, 140.82, 140.60 (d, J = 11.1 Hz), 130.96 (d, J = 9.1 Hz), 128.21, 125.65, 124.59, 121.36, 118.45, 116.28 (d, J= 2.0 Hz), 115.46, 111.28 (d, J = 21.2 Hz), 107.33 (d, J = 26.3 Hz).

5.1.6.10. 7-Bromo-N-(2,5-dimethoxyphenyl)-2-oxo-1,2-dihydroquinoline-4-carboxamide ***(6j)***. White solid (80 mg, 25% yield). LC-MS m/z 403 [M + H] +; ^{1}H NMR (400 MHz, DMSO-d6) δ 12.03 (s, 1H), 9.96 (s, 1H), 7.71 (d, J = 8.6 Hz, 1H), 7.57 (dd, J = 6.8, 2.5 Hz, 2H), 7.41 (dd, J = 8.7, 2.0 Hz, 1H), 7.03 (d, J = 9.0 Hz, 1H), 6.79 (dd, J = 8.9, 3.1 Hz, 1H), 6.69 (s, 1H), 3.76 (s, 6H).

5.1.6.11. 7-Bromo-2-oxo-N-(4-(trifluoromethoxy)phenyl)-1,2-dihydroquinoline-4-carboxamide ***(6k)***. White solid (68 mg, 20% yield). LC-MS m/z 427 [M + H] +; ^{1}H NMR (300 MHz, DMSO-d6) δ 12.12 (s, 1H), 10.96 (s, 1H), 7.92 – 7.82 (m, 2H), 7.70 (d, J = 8.6 Hz, 1H), 7.57 (d, J = 2.0 Hz, 1H), 7.40 (td, *J* = 6.3, 3.2 Hz, 3H), 6.81 (s, 1H).

5.1.6.12. 7-Bromo-2-oxo-N-(4-(trifluoromethyl)phenyl)-1,2-dihydroquinoline-4-carboxamide ***(6l)***. White solid (98 mg, 30% yield). LC-MS m/z 411 [M + H] +; ^{1}H NMR (300 MHz, DMSO-d6) δ 12.10 (s, 1H), 11.11 (s, 1H), 7.97 (d, J = 8.4 Hz, 2H), 7.77 (d, J = 8.4 Hz, 2H), 7.69 (d, J = 8.6 Hz, 1H), 7.57 (s, 1H), 7.40 (dd, J = 8.5, 1.9 Hz, 1H), 6.84 (s, 1H).

5.1.6.13. 7-Bromo-N-(3-methoxyphenyl)-2-oxo-1,2-dihydroquinoline-4-carboxamide ***(6m)***. White solid (77 mg, 26% yield). LC-MS m/z 373 [M + H] +; ^{1}H NMR (300 MHz, DMSO-d6) δ 12.08 (s, 1H), 10.72 (s, 1H), 7.69 (d, J = 8.6 Hz, 1H), 7.57 (s, 1H), 7.47 – 7.35 (m, 2H), 7.29 (d, J = 5.8 Hz, 2H), 6.75 (d, J = 7.8 Hz, 2H), 3.76 (s, 3H); ^{13}C NMR (101 MHz, DMSO) δ 164.32, 161.56, 159.99, 145.69, 140.82, 140.10, 130.10, 128.23, 125.63, 124.52, 121.14, 118.43, 115.59, 112.74, 110.27, 106.30, 55.55

5.1.6.14. 7-Bromo-2-oxo-N-(3-(trifluoromethyl)phenyl)-1,2-dihydroquinoline-4-carboxamide ***(6n)***. White solid (59 mg, 18% yield).LC-MS m/z 411 [M + H] +; ^{1}H NMR (300 MHz, DMSO-

d6) δ 12.13 (s, 1H), 11.08 (s, 1H), 8.26 (s, 1H), 7.94 (d, J = 8.1 Hz, 1H), 7.73 (d, J = 8.7 Hz, 1H), 7.68 – 7.60 (m, 1H), 7.60 – 7.48 (m, 2H), 7.39 (dd, J = 8.6, 2.0 Hz, 1H), 6.86 (s, 1H).

5.1.6.15. 7-Bromo-N-(4-(tert-butyl)phenyl)-2-oxo-1,2-dihydroquinoline-4-carboxamide ***(6o)***. White solid (85 mg, 27% yield). LC-MS m/z 399 [M + H] +; ^{1}H NMR (300 MHz, DMSO-d6) δ 12.09 (s, 1H), 10.69 (s, 1H), 7.67 (t, J = 8.5 Hz, 3H), 7.56 (d, J = 2.0 Hz, 1H), 7.40 (d, J = 8.5 Hz, 3H), 6.74 (s, 1H), 1.28 (s, 9H).

5.1.6.16. 7-Bromo-2-oxo-N-(3,4,5-trifluorophenyl)-1,2-dihydroquinoline-4-carboxamide ***(6p)***. White solid (170 mg, 54% yield). LC-MS m/z 397 [M + H] $^{+}$; ^{1}H NMR (300 MHz, DMSO-d6) δ 12.15 (s, 1H), 11.11 (s, 1H), 7.77 – 7.60 (m, 3H), 7.57 (d, J = 1.8 Hz, 1H), 7.48 – 7.31 (m, 1H), 6.83 (s, 1H).

5.1.6.17 7-Bromo-N-(3-chlorophenyl)-2-oxo-1,2-dihydroquinoline-4-carboxamide ***(6q)***. White solid (159 mg, 53% yield).LC-MS m/z 377 [M + H] +; ^{1}H NMR (300 MHz, DMSO-d6) δ 12.12 (s, 1H), 10.93 (d, J = 6.1 Hz, 1H), 7.96 (s, 1H), 7.78 – 7.52 (m, 3H), 7.40 (dd, J = 8.1, 4.2 Hz, 2H), 7.23 (d, J = 7.6 Hz, 1H), 6.81 (d, J = 4.0 Hz, 1H).

5.1.6.18. 7-Bromo-N-(3,5-difluorophenyl)-2-oxo-1,2-dihydroquinoline-4-carboxamide ***(6r)***. White solid (168 mg, 56% yield). LC-MS m/z 379 [M + H] +; ^{1}H NMR (300 MHz, DMSO-d6) δ 12.15 (s, 1H), 11.12 (s, 1H), 7.70 (d, J = 8.6 Hz, 1H), 7.57 (d, J = 2.0 Hz, 1H), 7.52 – 7.44 (m, 2H), 7.40 (dd, J = 8.7, 2.0 Hz, 1H), 7.06 (tt, J = 9.5, 2.6 Hz, 1H), 6.83 (d, J = 1.5 Hz, 1H).

5.1.6.19. 7-Bromo-2-oxo-N-(3-(trifluoromethoxy)phenyl)-1,2-dihydroquinoline-4-carboxamide ***(6s)***. White solid (59 mg, 26% yield). LC-MS m/z 427 [M + H] $^{+}$; ^{1}H NMR (300 MHz, DMSO-d6) δ 12.14 (s, 1H), 11.05 (s, 1H), 7.93 (s, 1H), 7.83 – 6.98 (m, 6H), 6.84 (s, 1H).

5.1.6.20. 7-Fluoro-N-(3-fluorophenyl)-2-oxo-1,2-dihydroquinoline-4-carboxamide ***(6t)***. White solid (121 mg, 51% yield). LC-MS m/z 301 [M+H]+; ^{1}H NMR (300 MHz, DMSO-d6) δ 12.15

(s, 1H), 10.97 (s, 1H), 7.89 – 7.61 (m, 2H), 7.55 – 7.31 (m, 2H), 7.13 (ddt, J = 13.1, 9.2, 4.5 Hz, 2H), 7.00 (t, J = 8.3 Hz, 1H), 6.72 (s, 1H).

5.1.6.21. N-(3,5-Difluorophenyl)-7-fluoro-2-oxo-1,2-dihydroquinoline-4-carboxamide ***(6u)****.* White solid (133 mg, 53% yield). LC-MS m/z 319 [M+H]+; ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.17 (s, 1H), 11.12 (s, 1H), 7.81 (dd, J = 8.9, 6.0 Hz, 1H), 7.48 (dd, J = 9.3, 2.3 Hz, 2H), 7.21 – 6.95 (m, 3H), 6.75 (s, 1H).

5.1.6.22. 7-Chloro-N-(3,5-difluorophenyl)-2-oxo-1,2-dihydroquinoline-4-carboxamide ***(6v)****.* Brown solid (122 mg, 46% yield). LC-MS m/z 335 [M+H]+, 376 [M+H+ MeCN]+; ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.16 (s, 1H), 11.12 (s, 1H), 7.78 (d, *J* = 8.7 Hz, 1H), 7.48 (d, J = 8.6 Hz, 2H), 7.42 (d, J = 2.1 Hz, 1H), 7.28 (dd, J = 8.7, 2.1 Hz, 1H), 7.06 (t, J = 9.3 Hz, 1H), 6.82 (s, 1H).

5.1.6.23. N-(3,5-Difluorophenyl)-2-oxo-7-(trifluoromethyl)-1,2-dihydroquinoline-4-carboxamide ***(6w)****.* White solid (128 mg, 44% yield). LC-MS m/z 369 [M+H]+, 410 [M+H+ MeCN]+; ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.34 (s, 1H), 11.17 (s, 1H), 7.99 (d, J = 8.4 Hz, 1H), 7.71 (s, 1H), 7.52 (dd, J = 15.1, 8.6 Hz, 2H), 7.07 (t, J = 9.3 Hz, 1H), 6.99 (s, 1H), 6.56 (s, 1H).

5.1.6.24. N-(3,5-Difluorophenyl)-7-methoxy-2-oxo-1,2-dihydroquinoline-4-carboxamide ***(6x)****.* Brown solid (126 mg, 48% yield). LC-MS m/z 372 [M + H+ MeCN] +; ^{1}H NMR (300 MHz, DMSO-d6) δ 11.93 (s, 1H), 11.06 (s, 1H), 7.65 (d, J = 8.8 Hz, 1H), 7.49 (d, J = 8.8 Hz, 2H), 7.03 (d, J = 9.3 Hz, 1H), 6.86 (dd, J = 12.1, 3.2 Hz, 2H), 6.57 (s, 1H), 3.83 (s, 3H).

5.1.6.25. N-(3,5-Difluorophenyl)-7-methoxy-2-oxo-1,2-dihydro-1,8-naphthyridine-4-carboxamide ***(6y)****.* White solid (113 mg, 43% yield). LC-MS m/z 373 [M + H+ MeCN] +; ^{1}H NMR (300 MHz, DMSO-d6) δ 12.39 (s, 1H), 11.07 (s, 1H), 8.09 (d, J = 8.8 Hz, 1H), 7.49 (d, J = 9.0 Hz, 2H), 7.05 (s, 1H), 6.83 – 6.60 (m, 2H), 3.95 (s, 3H).

*5.1.6.26. N-(3,5-Difluorophenyl)-8-methoxy-2-oxo-1,2-dihydroquinoline-4-carboxamide (**15**).* White solid (102 mg, 39% yield). LC-MS m/z 372 [M + H+ MeCN] +; 1H NMR (300 MHz, DMSO-d6) δ 11.21 (s, 1H), 11.13 (s, 1H), 7.49 (d, J = 8.8 Hz, 2H), 7.36 – 7.11 (m, 3H), 7.05 (t, J = 9.2 Hz, 1H), 6.78 (s, 1H), 3.93 (s, 3H).

*5.1.7. N-(3,5-Difluorophenyl)-7-hydroxy-2-oxo-1,2-dihydroquinoline-4-carboxamide (**6z**).* To a solution of **6x** (10 mg, 0.03 mmol) in dichloromethane, BBr_3 (1M in dichloromethane) (3 equiv) was added at 0 ºC and stirred at that temperature for 4h. After completion of the reaction as monitored by TLC analysis, work up was done using dichloromethane and water. The crude product was purified by column chromatography. White solid (5 mg, 52% yield). LC-MS m/z 358 [M + H+ MeCN] +; ^{1}H NMR (300 MHz, DMSO-d6) δ 11.83 (s, 1H), 11.03 (s, 1H), 10.34 (s, 1H), 7.51 (dd, J = 16.0, 8.8 Hz, 3H), 7.04 (t, J = 9.4 Hz, 1H), 6.77 (s, 1H), 6.69 (d, J = 8.0 Hz, 1H), 6.47 (s, 1H).

*5.1.8. N-(3,5-Difluorophenyl)-8-hydroxy-2-oxo-1,2-dihydroquinoline-4-carboxamide (**16**).* Same procedure as compound **6z**. White solid (4 mg, 41% yield). LC-MS m/z 358 [M + H+ MeCN] +; 1H NMR (300 MHz, DMSO-d6) δ 11.09 (s, 1H), 10.89 (s, 1H), 10.50 (s, 1H), 7.48 (d, J = 8.7 Hz, 2H), 7.15 (d, J = 6.4 Hz, 1H), 7.10 – 6.96 (m, 3H), 6.73 (s, 1H).

*5.1.9. General synthetic method for compounds (**7a-r**)*: To a suspension of compound **6i** (0.76 mmol) in 3 mL dioxane:water (4:1), the boronic acid reagent (0.76 mmol) and Cs_2CO_3 (270 mg, 0.83 mmol) was added. The mixture was purged using nitrogen gas then $PdCl_2$(dppf) (10 mg, 0.014 mmol) was added under inert condition. The mixture was heated to 100 °C for 24 h under argon and then cooled to room temperature. Filtration using celite, then water (30 mL) was added and the resulting mixture was extracted with EtOAc. The organic phase was dried over

$MgSO_4$, filtered, and dried under vacuum. The crude product was purified by flash chromatography (Dichloromethane:MeOH 9.8:0.2) to afford the compounds described below.

*5.1.9.1. N-(3-Fluorophenyl)-2-oxo-7-phenyl-1,2-dihydroquinoline-4-carboxamide (**7a**).* Brown solid (77 mg, 28% yield). LC-MS m/z 359 [M + H]+ ; ^{1}H NMR (300 MHz, DMSO-d6) δ 7.88 – 7.75 (m, 2H), 7.70 (d, J = 8.4 Hz, 3H), 7.64 (s, 1H), 7.60 – 7.38 (m, 5H), 7.02 (s, 1H), 6.76 (s, 1H).

*5.1.9.2. 7-(4-(Tert-butyl)phenyl)-N-(3-fluorophenyl)-2-oxo-1,2-dihydroquinoline-4-carboxamide (**7b**).* Brown solid (50 mg, 16 % yield). LC-MS m/z 415 [M + H]+; ^{1}H NMR (300 MHz, DMSO-d6) δ 12.11 (s, 1H), 10.98 (s, 1H), 7.96 (s, 1H), 7.81 (d, J = 9.3 Hz, 1H), 7.72 (d, J = 7.9 Hz, 2H), 7.62 (d, J = 5.5 Hz, 1H), 7.54 (d, J = 7.7 Hz, 3H), 7.35 (d, J = 7.7 Hz, 2H), 7.05 – 6.96 (m, 1H), 6.75 (s, 1H), 1.27 (s, 9H).

*5.1.9.3. N-(3-Fluorophenyl)-7-(4-fluorophenyl)-2-oxo-1,2-dihydroquinoline-4-carboxamide (**7c**).* Brown solid (132 mg, 46% yield). LC-MS m/z 377 [M + H] +; ^{1}H NMR (300 MHz, DMSO-d6) δ 12.11 (s, 1H), 10.98 (s, 1H), 7.89 – 7.68 (m, 4H), 7.62 – 7.30 (m, 6H), 7.10 – 6.91 (m, 1H), 6.76 (d, J = 1.6 Hz, 1H).

*5.1.9.4. 7-(4-Acetylphenyl)-N-(3-fluorophenyl)-2-oxo-1,2-dihydroquinoline-4-carboxamide (**7d**).* Brown solid (159 mg, 52% yield). LC-MS m/z 401 [M + H] +; ^{1}H NMR (300 MHz, DMSO-d6) δ 11.00 (s, 1H), 8.11 (d, J = 8.7 Hz, 2H), 7.95 – 7.26 (m, 8H), 7.02 (s, 1H), 6.80 (s, 1H), 2.64 (s, 3H).

*5.1.9.5. N-(3-Fuorophenyl)-7-(4-methoxyphenyl)-2-oxo-1,2-dihydroquinoline-4-carboxamide (**7e**).* Brown solid (98 mg, 33% yield). LC-MS m/z 389 [M + H] +; ^{1}H NMR (300 MHz, DMSO-d6) δ 12.06 (s, 1H), 10.97 (s, 1H), 7.95 – 7.26 (m, 8H), 7.20 – 6.87 (m, 3H), 6.72 (s, 1H), 3.82 (s, 3H); ^{13}C NMR (101 MHz, DMSO) δ 165.02, 163.76 (d, J = 242.2 Hz), 161.84, 160.13, 145.75,

142.75, 140.75 (d, J = 11.1 Hz), 140.38, 131.63, 131.03, 130.94, 128.51 (2C), 126.78, 121.17, 120.18, 116.29 (d, J= 2.0 Hz), 115.12 (2C), 112.98, 111.21 (d, J = 21.2 Hz), 107.31 (d, J = 26.3 Hz), 55.75.

*5.1.9.6. N-(3-Fluorophenyl)-2-oxo-7-(thiophen-3-yl)-1,2-dihydroquinoline-4-carboxamide (**7f**).* Brown solid (45 mg, 16% yield). LC-MS m/z 365 [M + H] +; ^{1}H NMR (300 MHz, DMSO-d6) δ 12.04 (s, 1H), 10.97 (s, 1H), 7.97 (t, J = 1.9 Hz, 1H), 7.78 (s, 1H), 7.73 (dd, J = 6.0, 3.7 Hz, 2H), 7.65 (s, 1H), 7.60 (dd, J = 8.6, 1.8 Hz, 1H), 7.55 – 7.39 (m, 3H), 7.09 – 6.92 (m, 1H), 6.81 – 6.61 (m, 1H); ^{13}C NMR (101 MHz, DMSO) δ 164.97, 162.55 (d, J = 242.4 Hz), 161.79, 145.69, 140.71, 140.70 (d, J = 11.1 Hz), 140.40, 137.90, 130.99 (d, J = 10.0 Hz), 128.31, 126.81, 126.42, 123.37, 121.15, 120.31, 116.28 (d, J = 2.0 Hz), 115.36, 112.89, 111.22 (d, J = 21.2 Hz), 107.32 (d, J = 26.3 Hz).

*5.1.9.7. N,7-bis(3-fluorophenyl)-2-oxo-1,2-dihydroquinoline-4-carboxamide (**7g**).* Brown solid (35 mg, 12% yield). LC-MS m/z 377 [M + H] +; ^{1}H NMR (300 MHz, DMSO-d6) δ 11.01 (s, 1H), 7.80 (dd, J = 21.5, 9.9 Hz, 2H), 7.66 – 7.35 (m, 7H), 7.29 (dd, J = 9.4, 6.7 Hz, 1H), 7.01 (t, J = 8.3 Hz, 1H), 6.78 (s, 1H).

*5.1.9.8. 7-(2-Fluorophenyl)-N-(3-fluorophenyl)-2-oxo-1,2-dihydroquinoline-4-carboxamide (**7h**).* White solid (60 mg, 21%yield). LC-MS m/z 377 [M + H] +; ^{1}H NMR (300 MHz, DMSO-d6) δ 12.14 (s, 1H), 10.99 (s, 1H), 7.88 – 7.71 (m, 2H), 7.64 – 7.30 (m, 8H), 7.01 (ddd, J = 10.4, 8.3, 2.1 Hz, 1H), 6.80 (d, J = 1.5 Hz, 1H).

*5.1.9.9. N-(3-Fluorophenyl)-2-oxo-7-(p-tolyl)-1,2-dihydroquinoline-4-carboxamide (**7i**).* White solid (59 mg, 21%yield). LC-MS m/z 373 [M + H]+; ^{1}H NMR (300 MHz, DMSO-d6) δ 12.07 (s, 1H), 10.97 (s, 1H), 7.87 – 7.69 (m, 2H), 7.66 – 7.24 (m, 8H), 7.00 (t, J = 8.4 Hz, 1H), 6.74 (s, 1H), 2.37 (s, 3H).

*5.1.9.10. 7-Cyclopropyl-N-(3-fluorophenyl)-2-oxo-1,2-dihydroquinoline-4-carboxamide (**7j**).* White solid (50 mg, 20% yield), LC-MS m/z 324 [M+H]+; ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.05 (s, 1H), 10.95 (s, 1H), 7.79 – 7.69 (m, 2H), 7.58 (t, J = 7.9 Hz, 1H), 7.48 (t, J = 7.1 Hz, 1H), 7.41 (t, J = 8.8 Hz, 1H), 7.23 (t, J = 7.6 Hz, 1H), 7.07 – 6.92 (m, 1H), 6.74 (s, 1H), 4.13 (q, J = 5.2 Hz, 1H), 3.17 (d, J = 5.2 Hz, 4H).

*5.1.9.11. N-(3-Fluorophenyl)-2-oxo-7-(pyridin-4-yl)-1,2-dihydroquinoline-4-carboxamide (**7k**).* Pale yellow solid (137 mg, 50% yield). LC-MS m/z 360 [M+H]+; 401 [M+H+CH_3CN]+. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.18 (s, 1H), 11.00 (s, 1H), 8.72 (s, 2H), 7.89 (d, J = 8.4 Hz, 1H), 7.81 – 7.59 (m, 5H), 7.54 – 7.32 (m, 2H), 7.01 (t, J = 7.9 Hz, 1H), 6.83 (s, 1H).

*5.1.9.12. 7-(4-Aminophenyl)-N-(3-fluorophenyl)-2-oxo-1,2-dihydroquinoline-4-carboxamide (**7l**).* Yellow solid (96 mg, 34% yield). LC-MS m/z 374 [M+H]+. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.95 (s, 1H), 10.94 (s, 1H), 7.73 (dd, J = 15.3, 10.0 Hz, 2H), 7.57 – 7.34 (m, 6H), 7.00 (t, J = 8.3 Hz, 1H), 6.73 – 6.58 (m, 3H), 5.45 (s, 2H).

*5.1.9.13. 7-(3,6-Dihydro-2H-pyran-4-yl)-N-(3-fluorophenyl)-2-oxo-1,2-dihydroquinoline-4-carboxamide (**7m**).* Yellow solid (45 mg, 16% yield). LC-MS m/z 365 [M+H]+; 406 [M+H+CH_3CN]+. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.01 (s, 1H), 10.95 (s, 1H), 7.72 (dd, J = 14.2, 10.4 Hz, 2H), 7.55 – 7.36 (m, 3H), 7.07 – 6.94 (m, 1H), 6.70 (s, 1H), 6.57 (s, 1H), 6.42 (s, 1H), 4.27 (d, J = 2.9 Hz, 2H), 3.85 (t, J = 5.4 Hz, 2H), 2.47 (m, 2H).

*5.1.9.14. N-(3-Fluorophenyl)-7-(6-fluoropyridin-3-yl)-2-oxo-1,2-dihydroquinoline-4-carboxamide (**7n**).* White solid (160 mg, 56% yield). LC-MS m/z 378 [M+H]+, 419 [M+H+CH_3CN]+; ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.15 (s, 1H), 10.99 (s, 1H), 8.58 (d, J = 2.5 Hz, 1H), 8.31 (td, J = 8.0, 2.6 Hz, 1H), 7.86 (d, J = 8.4 Hz, 1H), 7.76 (d, J = 11.3 Hz, 1H),

7.65 – 7.55 (m, 2H), 7.52 (d, J = 8.3 Hz, 1H), 7.49 – 7.41 (m, 1H), 7.37 (dd, J = 8.6, 2.8 Hz, 1H), 7.07 – 6.93 (m, 1H), 6.80 (s, 1H).

*5.1.9.15. N-(3-Fluorophenyl)-7-(2-fluoropyridin-3-yl)-2-oxo-1,2-dihydroquinoline-4-carboxamide (**7o**)*. White solid (134 mg, 47% yield). LC-MS m/z 378 [M+H]+, 419 [M+H+CH_3CN]+. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.17 (s, 1H), 11.00 (s, 1H), 8.32 (d, J = 4.9 Hz, 1H), 8.19 (ddd, J = 9.8, 7.5, 1.9 Hz, 1H), 7.87 (d, J = 8.5 Hz, 1H), 7.82 – 7.71 (m, 1H), 7.63 (s, 1H), 7.59 – 7.37 (m, 4H), 7.08 – 6.94 (m, 1H), 6.82 (s, 1H).

*5.1.9.16. 7-(4-Fluoro-2,5-dimethylphenyl)-N-(3-fluorophenyl)-2-oxo-1,2-dihydroquinoline-4-carboxamide (**7p**)*. Light yellow solid (160 mg, 52% yield). LC-MS m/z 405 [M+H]+, 446 [M+H+CH_3CN]+. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.07 (s, 1H), 10.98 (s, 1H), 7.76 (dd, J = 9.7, 4.7 Hz, 2H), 7.46 (dt, J = 22.8, 8.0 Hz, 2H), 7.30 (s, 1H), 7.24 – 7.07 (m, 3H), 7.00 (t, J = 8.3 Hz, 1H), 6.77 (s, 1H), 2.23 (d, J = 12.0 Hz, 6H).

*5.1.9.17. 7-(4-Fluoro-3-(hydroxymethyl)phenyl)-N-(3-fluorophenyl)-2-oxo-1,2-dihydroquinoline-4-carboxamide (**7q**)*. White solid (127 mg, 41% yield). LC-MS m/z 407 [M+H]+, 448 [M+H+CH_3CN]+. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.09 (s, 1H), 10.98 (s, 1H), 7.86 – 7.71 (m, 3H), 7.63 (s, 2H), 7.52 (d, J = 8.8 Hz, 2H), 7.44 (q, J = 7.7 Hz, 1H), 7.32 (t, J = 9.2 Hz, 1H), 7.01 (t, J = 8.4 Hz, 1H), 6.76 (s, 1H), 5.45 (t, J = 5.6 Hz, 1H), 4.64 (d, J = 5.4 Hz, 2H).

*5.1.9.18. N-(3-Fluorophenyl)-2-oxo-7-vinyl-1,2-dihydroquinoline-4-carboxamide (**7r**)*. White solid (112 mg, 48% yield). LC-MS m/z 309 [M + H] +; ^{1}H NMR (300 MHz, DMSO-d6) δ 12.04 (s, 1H), 10.95 (s, 1H), 7.80 – 7.64 (m, 1H), 7.53 – 7.32 (m, 3H), 7.00 (t, J = 8.6 Hz, 1H), 6.82 (dd, J = 17.5, 10.9 Hz, 1H), 6.71 (d, J = 1.7 Hz, 1H), 6.57 (s, 2H), 5.94 (d, J = 17.5 Hz, 1H), 5.44 (d, J = 10.9 Hz, 1H).

*5.1.10. N-(3-Fluorophenyl)-2-oxo-7-((trimethylsilyl)ethynyl)-1,2-dihydroquinoline-4-carboxamide (**8**).* To a solution of the **6i** (0.1 mmol), $PdCl_2(PPh_3)_2$ (0.1 mmol), CuI (0.02 mmol) and triethylamine (1.5 mL) was added trimethylsilane acetylene (1.2 mmol). The resulting mixture was stirred at 80 °C under nitrogen atmosphere for 8 hours. After the complete consumption of starting material, the reaction mixture was filtered through celite and purified by HPLC.

*5.1.11. 7-Ethynyl-N-(3-fluorophenyl)-2-oxo-1,2-dihydroquinoline-4-carboxamide (**9**).* TMS-ethynyl substrate (0.05 mmol) were dissolved in 1.5 mL MeOH and anhydrous K_2CO_3 (0.16 mmol) was added under nitrogen atmosphere. The reaction mixture was stirred at room temperature for 3 h. Upon completion, the reaction was filtered through celite and the filtrate was evaporated under reduced pressure and purified by HPLC. White solid (12 mg, 48% yield). LC-MS m/z 309 [M + H] +; ^{1}H NMR (300 MHz, DMSO-d6) δ 12.04 (s, 1H), 10.95 (s, 1H), 7.80 – 7.64 (m, 1H), 7.53 – 7.32 (m, 3H), 7.00 (t, J = 8.6 Hz, 1H), 6.82 (dd, J = 17.5, 10.9 Hz, 1H), 6.71 (d, J = 1.7 Hz, 1H), 6.57 (s, 2H), 5.94 (d, J = 17.5 Hz, 1H), 5.44 (d, J = 10.9 Hz, 1H).

*5.1.12. 1-Benzyl-7-bromo-N-(3-fluorophenyl)-2-oxo-1,2-dihydroquinoline-4-carboxamide (**10**).* Benzyl chloride (35 mg, 0.27 mmol) was added to a solution of **6i** (50 mg, 0.13 mmol) in 2 mL DMF and K_2CO_3 and stirred overnight. The reaction mixture was poured onto water and extracted using EtOAc. The organic phase was dried over $MgSO_4$, filtered, and dried under vacuum. The residue was purified using flash chromatography (Dichloromethane:MeOH 9.5:0.5) resulting in white solid of **10** (13 mg, 21% yield). LC-MS m/z 451 [M + H] +; ^{1}H NMR (300 MHz, Methanol-d4) δ 7.84 (d, *J* = 8.6 Hz, 1H), 7.76 – 7.66 (m, 3H), 7.56 – 7.41 (m, 2H), 7.35 (dt, *J* = 13.8, 7.2 Hz, 4H), 7.26 (d, *J* = 7.3 Hz, 3H), 7.01 (s, 1H), 5.66 (s, 2H).

*5.1.13. General synthetic method for compounds (**11** and **13**)*: To a suspension of the bromo derivative (1 equiv.) in 2 mL dioxane were added N^1,N^1-diethylpentane-1,4-diamine (1.3 equiv.), NaO^{t}Bu (1.5 equiv.) and XPhos (0.05 equiv.). The mixture was purged with argon gas then $Pd_2(dba)_3$ (0.015 equiv.) was added. The mixture was heated at 90 °C for 16 h under argon and then cooled to room temperature. Filtration using celite as filter aid was followed by purification by flash chromatography (9.8 Dichloromethane: 0.2 MeOH) and HPLC to afford the targeted compounds.

*5.1.13.1. 7-((5-(Diethylamino)pentan-2-yl)amino)-N-(3-fluorophenyl)-2-oxo-1,2-dihydroquinoline-4-carboxamide (**11**)*: White solid (1.1 mg, 9% yield). LC-MS m/z 439 [M + H] +; ^{1}H NMR (300 MHz, DMSO-d6) δ 11.55 (s, 1H), 10.80 (s, 1H), 7.77 – 7.68 (m, 1H), 7.51 – 7.45 (m, 1H), 7.44 – 7.33 (m, 2H), 7.01 – 6.88 (m, 1H), 6.60 – 6.49 (m, 2H), 6.42 – 6.36 (m, 1H), 6.23 (s, 1H), 3.18 – 2.94 (m, 6H), 2.07 – 2.04 (m, 1H), 1.76 – 1.44 (m, 4H), 1.29 – 1.05 (m, 9H).

*5.1.13.2. N-(4-((5-(Diethylamino)pentan-2-yl)amino)-3-fluorophenyl)-7-fluoro-2-oxo-1,2-dihydroquinoline-4-carboxamide (**13**)*: Yellow solid (2 mg, 15% yield). LC-MS m/z 499 [M + H+ MeCN] +; ^{1}H NMR (300 MHz, DMSO-d6) δ 12.11 (s, 1H), 10.57 (s, 1H), 9.07 (s, 1H), 7.81 (dd, J = 8.7, 6.1 Hz, 1H), 7.58 (d, J = 13.9 Hz, 1H), 7.29 (d, J = 8.6 Hz, 1H), 7.11 (t, J = 9.9 Hz, 2H), 6.79 (t, J = 9.5 Hz, 1H), 6.65 (s, 1H), 3.53 (s, 1H), 3.27 – 2.82 (m, 6H), 1.57 (d, J = 52.6 Hz, 4H), 1.38 – 0.80 (m, 9H).

*5.1.14. 2-Chloro-N-(3-fluorophenyl)quinoline-4-carboxamide (**17**).* *N*-(3-Fluorophenyl)-2-oxo-1,2-dihydroquinoline-4-carboxamide (500 mg, 1.67 mmol) was treated with $POCl_3$ (2 mL) and refluxed for 3 h. After completion of the reaction, the mixture was poured into dry ice and extracted with dichloromethane. The crude compound was concentrated and a white solid was

obtained as product (300 mg, 60%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.05 (s, 1H), 10.95 (s, 1H), 7.80 – 7.69 (m, 2H), 7.58 (t, J = 7.5 Hz, 1H), 7.49 (t, J = 7.5 Hz, 1H), 7.41 (dd, J = 10.1, 7.8 Hz, 1H), 7.23 (t, J = 7.6 Hz, 1H), 7.07 – 6.92 (m, 1H), 6.74 (d, J = 1.6 Hz, 1H).

*5.1.15. N-(3-Fluorophenyl)-1-hydroxy-2-oxo-1,2-dihydroquinoline-4-carboxamide (**18**).* To a solution of compound **17** (150 mg, 0.5 mmol) in dichloromethane was added *m*-CPBA (172 mg, 1 mmol) and stirred at rt for 4h. After the complete consumption of the starting material, the reaction mixture was extracted with Dichloromethane (30 mL) and the extracts were washed few times with water (2 × 15 mL) followed by brine. The organic phase was concentrated under reduced pressure to yield the crude product that was not purified and subjected to the next reaction. The crude (30 mg, 0.09 mmol) was dissolved in 10% aqueous HCl and refluxed overnight. The crude product was freeze dried and purified by HPLC to obtain compound **18** (8 mg, 28%) as white solid. LC-MS m/z 299 [M+H]+, 340 [M+H+CH_3CN]+. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.97 (s, 1H), 7.77 (dt, J = 25.5, 7.7 Hz, 4H), 7.60 – 7.27 (m, 4H), 7.00 (d, J = 7.6 Hz, 1H), 6.56 (s, 1H).

5.2. Biological evaluation

5.2.6. Cell culture

KYSE70 and KYSE410 cells (Sigma-Aldrich Company) and OVCAR-8 , SKOV3-3, MIA PaCa-2 (ATCC and the National Cancer Institute, Developmental Therapeutics Program, Bethesda, MD) were maintained in RPMI-1640 medium supplemented with 10% heat-inactivated fetal bovine serum, FBS (Invitrogen). Cells were grown as monolayers at 37 ºC in a humidified atmosphere of 5 % CO_2. To remove adherent cells from the flask for subculture and counting, cells were washed with PBS, incubated with a small volume of 0.25% trypsin-EDTA solution (Mediatech Company, USA), resuspended with cell culture medium, and centrifuged. All

experiments were carried out using cells in the exponential growth phase. Cells were routinely checked for *mycoplasma* contamination by using PlasmoTest (InvivoGen).

5.2.7. Colony formation assay

Colony formation assays were conducted as described [42]. Briefly, KYSE410 cells (250 cells/well) and KYSE70 cells (250 cells/well) were seeded on 96-well plates and allowed to attach. After 24 hours, serial dilutions of the corresponding compounds were added to the culture medium and incubated at 37 ºC. Cells were cultured until colonies were formed (7 days for KYSE410 cells and KYSE70 cells), then subsequently washed, stained with crystal violet solution (2%) for 1 hour, and thoroughly washed with water.

5.2.8. Growth inhibition assay

Growth inhibition was assessed using a (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (MTT) assay as described [43] Cells were seeded in 96-well microtiter plates, allowed to attach for 24 hours followed by addition of corresponding compounds to the culture medium. After 72 hours, cells were incubated with 0.3 mg/mL MTT (Amresco) for an additional 3 hours at 37°C. After removal of the supernatant, DMSO was added to the wells and the absorbance was read at 570 nm. All assays were conducted in triplicate. Percentages of cell growth inhibition are expressed as: (1− A/C) × 100% (A and C are the absorbance values from experimental and control cells, respectively). Inhibitory concentration 50% (IC_{50}) values were determined for each drug from a plot of log (drug concentration) versus percentage of cell growth inhibition. Standard deviations were calculated using the IC_{50} values obtained from at least 3 independent experiments.

5.2.9. Western blotting analysis

MIA PaCa-2 and KYSE410 cells were treated with compounds at the indicated concentrations for 24h. Protein samples were prepared by lysing cells in RIPA buffer containing protease inhibitor cocktail (Roche) and diluted in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) protein sample buffer. Samples were heated for 5 min at 100 °C. Protein concentrations were measured using the BCA assay (Thermo fisher Co., USA) according to the manufacturer's instructions. Equal amount of proteins were loaded on 15% SDS-PAGE gel. After electrophoresis, gels were transferred to a polyvinylidene difluoride (PVDF) membrane (Millipore) and incubated with primary antibodies overnight at 4 °C. The membranes were then washed with tris buffered saline with tween (TBST) and incubated with anti-rabbit antibody. The images were analyzed by Image Studio Lite software (LiCor Biosciences). Anti-LC3B was obtained from Invitrogen Life Technologies and anti-GAPDH was obtained from Cell Signaling Technology.

5.2.10. Cell cycle analysis

Cell cycle distribution was determined by staining DNA with propidium iodide (PI). Briefly, cells (1x E6) were incubated with or without compounds for 24 and 48 h. Cells were washed with cold phosphate-buffered saline (PBS) and fixed in 70% ethanol at -20C for 12 h. After washing with PBS, cells were stained with cold PI solution (40 mg/mL PI and 100 mg/mL RNase in PBS) for 30 min at room temperature in the dark. The percentage of cells in different phases of the cell cycle was determined by a flow cytometer (BD Bioscience) and analyzed using FlowJo software.

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Highlights:

- Synthesized quinoline derivatives are effective in killing esophageal cancer cells
- Several compounds showed cytotoxicity comparable to cisplatin
- Lead compounds modulate autophagy mechanism
- Lead compounds arrest cell cycle