

Study on the Synthesis and Cytotoxicity of New Quinophenoxazine Derivatives

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We have synthesized several new quinophenoxazine analogues and tested their cytotoxicity activities. The results showed that the compounds, 4a and 4b, possessing phenyl ring in the structure have almost same pharmacological capacity with A-62176. This finding suggests that the phenyl ring portion is important to this series of compounds for the activity expression.

Key words fluoroquinolone; quinophenoxazine; cytotoxicity

The fluoroquinolone class analogues represented by Norfloxacin (**1**)¹⁾ and Ciprofloxacin (**2**)²⁾ have been clinically important and widely used as antibacterial agents. These class drugs are known to show pharmacological activity by inhibiting DNA gyrase and topoisomerase IV.^{3,4)} Because of the excellent antibacterial efficacy, a number of fluoroquinolone analogues have been prepared and tested for the development of new antimicrobial agents with improved pharmacological efficacy.^{3,4)} The similar mechanistic aspects between bacterial and eukaryotic DNA topoisomerase II attracted researchers to screen the possibilities of usage for antitumor action of fluoroquinobenzoxazine analogues.^{5–7)} In the series of the compounds synthesized previously, A-62176 (**3**) prepared by Abbott researchers⁵⁾ showed good pharmacological activity against several human and murine cancer cell lines. This compound possesses tetracyclic ring system in which another phenyl moiety is fused into the benzoxazine core of **1** and **2** (Fig. 1). Recently, University of Arizona researchers have prepared a few A-62176 analogues, which have extended aromatic ring system. The *in vitro* cytotoxicity test results showed some of these compounds exhibited better antitumor activity than A-62176 (**3**).^{8–10)} These information suggested that modification of the phenyl ring moiety of **3** can modulate the biological capacity of this compound. In order to prepare new quinophenoxazine analogues of **3** possessing enhanced pharmacological profile, we have synthesized six analogues (Fig. 2), one known and five new ones, and tested their cytotoxicity activities *in vitro* against several human cancer cell lines.

Results and Discussion

According to the previous model study of the complex, **3** and DNA, the intercalation of tetracyclic ring of **3** into the DNA base pairs is important. In addition, the chelation of Mg²⁺ and β -keto acid and the amino group of the side chain on **3** provides more stabilization.⁸⁾ Based on this information we have designed and synthesized six quinophenoxazine ana-

logues. Basically the designed compounds contained diverse aromatic rings including pyridine, quinoline and anthraquinone. Synthesis was conducted with some modifications of the published method.^{5,8)} Chart 1 describes a representative synthetic pathway for the compound **4**. All series of compounds were prepared with the same synthesis procedure with **4a**. The key *O*-aminoaromatic alcohol compounds (**5a–f**) were purchased or synthesized. Especially, **5b**¹¹⁾ and **5f**¹²⁾ were prepared by the methods in the references. The *in situ* coupling of compound **5a** and product of ethyl 2,3,4,5-tetrafluorobenzoyl acetate (**6**) and triethyl orthoformate in acetic anhydride provided **7a**. Double ring cyclization under NaHCO₃ basic condition in DMF generated **8a**. Regioselective nucleophilic substitution reaction at C-2 position was accomplished with 3(*S*)-(t-butoxycarbonylamino)pyrrolidine and **8a** in pyridine solvent. Final hydrolysis of ethyl carboxylate and removal of protection part from amino group produced **4a**. With this procedure, six analogues including one known **4a**⁶⁾ and five new quinophenoxazines **4b–f** were prepared.

In order to see the anticancer effect, we tested all the compounds against several human cancer cell lines. The data are summarized in Table 1. In these results we could observe the structure–activity relationship. Apparently, no compounds showed better biological efficacy compared to A-62176 (**3**) used as reference (values shown in the parenthesis in Table 1). The compounds, **4a** and **b**, possessing phenyl ring moiety like A-62176 exhibited almost same antitumor activity against cell lines tested regardless of the substituents on the

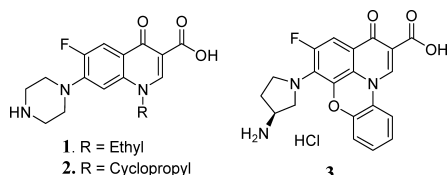


Fig. 1. Structures of Norfloxacin (**1**), Ciprofloxacin (**2**), and A-62176 (**3**)

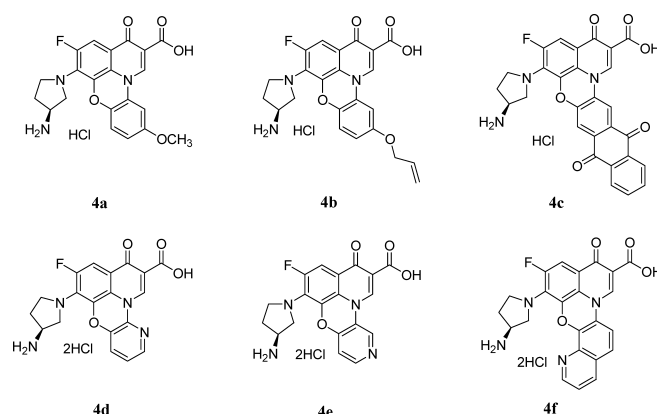
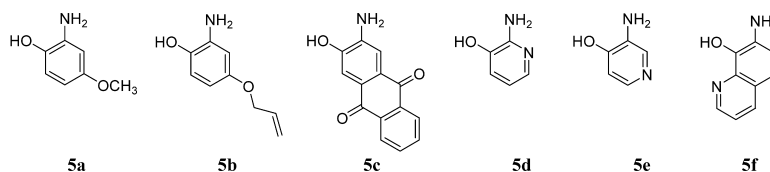
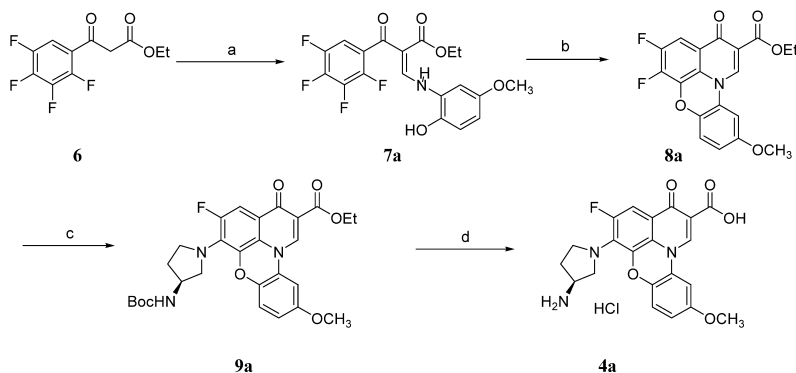


Fig. 2. Structures of Target Compounds **4a–f**

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A) *O*-Aminoaromatic alcohols

B) General synthetic method



(a) (i) $\text{CH}(\text{OEt})_3$, Ac_2O , 130°C , 4 h (ii) **5a**, Pyr/ CH_2Cl_2 , o/n; (b) NaHCO_3 , DMF, 110°C , 2 h; (c) 3-(*S*)-(t-Butoxycarbonylamino)pyrrolidine, Pyr, 110°C , 40 h; (d) (i) 1N KOH, EtOH, 80°C , 30 min, (ii) 2N HCl, EtOH, 80°C , 3 h

Chart 1. Preparation of Target Compounds **4a–f**Table 1. Cytotoxicity (IC_{50} , μM) of Compounds **4a–f** against Cancer Cells

Compound/Cell line	HT29-colon	Du145-prostate	MiaPaCa-pancreatic
4a	0.03 (0.03 ^a)	0.02 (0.02)	0.01 (0.02)
4b	0.05 (0.03)	0.04 (0.02)	0.02 (0.02)
4c	40.0 (0.29)	12.8 (0.84)	>100 (0.64)
4d	2.20 (0.84)	2.49 (0.58)	2.31 (0.85)
4e	ND ^b)	ND	ND
4f	ND	ND	ND

a) The values in the parenthesis indicate one corresponding to A-62176. b) ND represents not determined.

phenyl ring. But replacement of phenyl ring of A-62176 with other components, anthraquinone, pyridine, and quinoline, significantly reduced or abolished antitumor capacity. It is interesting to find the reduction on activity of **4d**. One possible explanation of this phenomenon could be that the introduction of nitrogen atom in the phenyl ring might alter the π - π stacking interaction between DNA and **4d**.⁸⁾ The result obtained from **4e** also suggested that the location of nitrogen atom would be important factor for the activity expression. When nitrogen is moved to one more carbon away from quinobenzoxazine core, the biological capacity completely disappeared. We also expected that introduction of intercalation property of anthraquinone group could increase the biological efficacy. In contrast to our expectation, anthraquinone moiety did not give positive substitution effect.

With the preliminary results of the cytotoxicity test on small numbers of quinophenoxazine analogues, it is likely the phenyl ring moiety of A-62176 is important component for the biological efficacy. More biological and structural

studies should be investigated for better understanding and preparing new potential compounds as new drug candidates.

Experimental

General Method for Chemistry ^1H -NMR spectra were taken on Bruker AMX 500 at University of Arizona Cancer Center. Chemical shifts (δ) are in parts per million (ppm) relative to tetramethylsilane as an internal standard and coupling constants (J values) are in hertz (Hz). Low-resolution and high-resolution mass spectral data were recorded at mass spectroscopy center in the University of Arizona. The solvents and reagents used for synthesis were of the best commercial grade available and were used without further purification unless noticed.

General Procedure for the Preparation of Compounds **7a–f** A solution of ethyl 2,3,4,5-tetrafluorobenzoyl acetate (**6**) (1 eq) in triethyl orthoformate (3 eq) and acetic anhydride (8.4 eq) was stirred at 130°C (4 h). The solvent was removed *in vacuo*. The oil residue was dissolved in CH_2Cl_2 and corresponding *O*-aminophenol compounds (**5a–f**) (1.0–1.2 eq) in CH_2Cl_2 /pyridine was added. After stirring at room temperature (17 h), solvent was removed under reduced pressure. The residue was purified by silica gel column chromatography to give products as isomeric mixture; the values in the parenthesis indicate another set of isomer.

Ethyl 3-(2-Hydroxy-5-methoxyphenylamino)-2-(2,3,4,5-tetrafluorobenzoyl)acrylate (**7a**): Orange solid (81%); *R*_f: 0.67 (40% EtOAc/hexane); ^1H -NMR (CDCl_3 , 500 MHz) δ : 1.16 (1.05) (3H, t, $J=6.4$ Hz), 3.82 (3H, s), 4.15 (4.40) (2H, q, $J=6.4$ Hz), 6.64 (6.84) (1H, m), 6.91 (1H, m), 7.09 (7.06) (1H, m), 8.62 (8.56) (1H, d, $J=13.5$ Hz), 12.68 (1H, d, $J=16.3$ Hz).

Ethyl 3-(2-Hydroxy-5-allyloxyphenylamino)-2-(2,3,4,5-tetrafluorobenzoyl)acrylate (**7b**): Yellow solid (84%); *R*_f: 0.53 (30% EtOAc/hexane); ^1H -NMR (CDCl_3 , 500 MHz) δ : 1.17 (1.04) (3H, t, $J=7.2$ Hz), 4.16 (2H, q, $J=7.2$ Hz), 4.53 (4.52) (2H, d, $J=5.4$ Hz), 5.32 (5.30) (1H, d, $J=10.1$ Hz), 5.43 (1H, d, $J=17.3$ Hz), 6.07 (1H, m), 6.66 (6.63) (1H, dd, $J=8.6$, 2.1 Hz), 6.79 (1H, d, $J=8.6$ Hz), 6.93 (1H, d, $J=2.1$ Hz), 7.10 (7.20) (1H, dd, $J=12.9$, 8.0 Hz), 8.62 (8.56) (1H, d, $J=13.9$ Hz).

Ethyl 3-(3-Hydroxy-9,10-dioxo-9,10-dihydroanthracen-2-ylamino)-2-(2,3,4,5-tetrafluorobenzoyl)acrylate (**7c**): Orange solid (70%); *R*_f: 0.36 (10%/10% MeOH/ CHCl_3 /hexane); ^1H -NMR (CDCl_3 , 500 MHz) δ : 1.24 (1.04) (3H, t, $J=7.2$ Hz), 4.24 (4.17) (2H, q, $J=7.2$ Hz), 7.14 (7.26) (1H, m), 7.85 (2H, m), 8.35 (2H, m), 8.36 (8.23) (1H, s), 8.31 (8.20) (1H, s), 8.77

(1H, d, $J=13.0$ Hz), 12.75 (11.67) (1H, d, $J=12.9$ Hz).

Ethyl 3-(3-Hydroxypyridin-2-yl-amino)-2-(2,3,4,5-tetrafluorobenzoyl)-acrylate (**7d**): Yellow solid (96%); $^1\text{H-NMR}$ (CDCl_3 , 500 MHz) δ : 1.24 (1.09) (3H, t, $J=7.0$ Hz), 4.20 (2H, q, $J=7.0$ Hz), 7.04 (6.99) (1H, dd, $J=7.5$, 4.4 Hz), 7.17 (7.12) (1H, d, $J=7.5$ Hz), 7.99 (7.92) (1H, d, $J=4.4$ Hz), 9.33 (9.16) (1H, d, $J=13.3$ Hz), 12.71 (11.48) (1H, d, $J=13.3$ Hz).

Ethyl 3-(8-Hydroxyquinolin-7-yl-amino)-2-(2,3,4,5-tetrafluorobenzoyl)-acrylate (**7f**): Red solid (64%); $^1\text{H-NMR}$ (CDCl_3 , 500 MHz) δ : 1.09 (1.19) (3H, t, $J=7.2$ Hz), 4.19 (2H, q, $J=7.2$ Hz), 7.23 (7.14) (1H, dd, $J=7.6$, 1.8 Hz), 7.46 (7.61) (1H, dd, $J=8.0$, 4.3 Hz), 7.48 (7.62) (1H, dd, $J=8.8$, 1.8 Hz), 8.20 (1H, dd, $J=7.1$, 1.2 Hz), 8.77 (8.86) (1H, d, $J=14.2$ Hz), 8.84 (1H, dd, $J=11.2$, 1.2 Hz), 11.68 (12.98) (1H, d, $J=14.0$ Hz).

General Procedure for the Preparation of Compounds 8a–f Compounds **7a–f** (1 eq) and NaHCO_3 (5 eq) were placed in DMF under nitrogen and the mixture was refluxed at 110°C (2 h). Solvent was removed under reduced pressure and the residue was dissolved with CHCl_3 and water. Undissolved material was filtered and the organic layer was separated. After removing the solvent, the residue was purified by silica gel column chromatography to give products: Compound **8b** was directly used for next reaction without spectroscopic identification.

Ethyl 1,2-Difluoro-8-methoxy-4-oxo-4H-pyrido[3,2,1-*kl*]phenoxazine-5-carboxylate (**8a**): Orange solid (90%); $^1\text{H-NMR}$ (CDCl_3 , 500 MHz) δ : 1.44 (3H, t, $J=6.4$ Hz), 3.88 (3H, s), 4.46 (2H, q, $J=7.1$ Hz), 6.81 (1H, dd, $J=8.9$, 2.4 Hz), 7.01 (1H, d, $J=2.4$ Hz), 7.16 (1H, d, $J=8.9$ Hz), 7.79 (1H, dd, $J=10.0$, 7.8 Hz), 8.92 (1H, s).

Ethyl 1,2-Difluoro-4-oxo-4H-pyrido[3,2,1-*kl*](1,4-dioxonaphtho)[2,3-*c*]phenoxazine-5-carboxylate (**8c**): Orange solid (recrystallized from CHCl_3) (73%); R_f : 0.70 (5% MeOH/ CHCl_3); $^1\text{H-NMR}$ (CDCl_3 , 500 MHz) δ : 1.48 (3H, t, $J=7.1$ Hz), 4.50 (2H, q, $J=7.1$ Hz), 7.84 (1H, dd, $J=9.9$, 8.8 Hz), 7.88 (1H, d, $J=13.4$ Hz), 7.87 (1H, m), 8.05 (1H, s), 8.34 (2H, m), 8.38 (1H, s), 9.11 (1H, s).

Ethyl 1,2-Difluoro-4-oxo-4H-pyrido[3,2,1-*kl*]-7-azaphenoxazine-5-carboxylate (**8d**): Light yellow solid (78%); R_f : 0.34 (50% EtOAc/hexane); $^1\text{H-NMR}$ (CDCl_3 , 500 MHz) δ : 1.45 (3H, t, $J=7.0$ Hz), 4.43 (2H, q, $J=7.0$ Hz), 7.27 (1H, dd, $J=8.0$, 4.3 Hz), 7.49 (1H, d, $J=8.0$ Hz), 7.81 (1H, dd, $J=9.7$, 8.0 Hz), 8.19 (1H, d, $J=4.3$ Hz), 9.71 (1H, s).

Ethyl 1,2-Difluoro-4-oxo-4H-pyrido[3,2,1-*kl*]-8-azaphenoxazine-5-carboxylate (**8e**): Light yellow solid (18%); R_f : 0.58 (6% MeOH/ CHCl_3); $^1\text{H-NMR}$ ($\text{CDCl}_3+\text{CD}_3\text{OD}$, 500 MHz) δ : 1.27 (3H, t, $J=7.1$ Hz), 4.24 (2H, q, $J=7.1$ Hz), 7.05 (1H, d, $J=5.4$ Hz), 7.62 (1H, dd, $J=9.9$, 9.9 Hz), 8.32 (1H, d, $J=5.4$ Hz), 8.74 (1H, s), 8.94 (1H, s).

Ethyl 1,2-Difluoro-4-oxo-4H-pyrido[3,2,1-*kl*]pyridino[3,2-*c*]phenoxazine-5-carboxylate (**8f**): Gray solid (95%); R_f : 0.35 (1% MeOH/ CHCl_3); $^1\text{H-NMR}$ (CDCl_3 , 500 MHz) δ : 1.47 (3H, t, $J=7.1$ Hz), 4.48 (2H, q, $J=7.1$ Hz), 7.54 (1H, dd, $J=8.2$, 4.2 Hz), 7.72 (1H, d, $J=9.2$ Hz), 7.75 (1H, d, $J=9.2$ Hz), 7.82 (1H, dd, $J=10.0$, 7.6 Hz), 8.19 (1H, dd, $J=8.2$, 1.2 Hz), 9.07 (1H, s), 9.10 (1H, dd, $J=4.2$, 1.2 Hz).

General Procedure for the Preparation of Compounds 9a–f Compounds **8a–f** (1 eq) and 3-(*S*)-(tert-butoxycarbonylamino)pyrrolidine (3 eq) were dissolved in anhydrous pyridine and the mixture was stirred under nitrogen at 110°C (24–40 h). The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography to give products.

Ethyl 1-(3-(*S*)-(tert-butoxycarbonylamino)pyrrolidin-1-yl)-2-fluoro-8-methoxy-4-oxo-4H-pyrido[3,2,1-*kl*]phenoxazine-5-carboxylate (**9a**): Orange solid (88%); R_f : 0.43 (20% MeOH/ CHCl_3); $^1\text{H-NMR}$ (CDCl_3 , 500 MHz) δ : 1.43 (3H, t, $J=6.9$ Hz), 1.48 (9H, s), 1.96 (1H, m), 2.23 (1H, m), 3.53 (1H, m), 3.61 (1H, m), 3.84 (3H, s), 3.88 (2H, m), 4.35 (1H, m), 4.41 (2H, q, $J=6.9$ Hz), 6.70 (1H, dd, $J=8.8$, 1.7 Hz), 6.85 (1H, d, $J=1.7$ Hz), 6.98 (1H, d, $J=8.8$ Hz), 7.56 (1H, d, $J=13.5$ Hz), 8.70 (1H, s).

Ethyl 1-(3-(*S*)-(tert-butoxycarbonylamino)pyrrolidin-1-yl)-2-fluoro-8-allyloxy-4-oxo-4H-pyrido[3,2,1-*kl*]phenoxazine-5-carboxylate (**9b**): Yellow solid (77%); $^1\text{H-NMR}$ (CDCl_3 , 500 MHz) δ : 1.43 (3H, t, $J=7.1$ Hz), 1.46 (9H, s), 1.95 (1H, m), 2.23 (1H, m), 3.52 (1H, m), 3.61 (1H, m), 3.83 (1H, m), 3.88 (1H, m), 4.34 (1H, m), 4.42 (2H, q, $J=7.1$ Hz), 4.55 (2H, d, $J=5.2$ Hz), 5.34 (1H, d, $J=10.6$ Hz), 5.45 (1H, d, $J=17.2$ Hz), 6.04 (1H, m), 6.70 (1H, dd, $J=9.0$, 2.4 Hz), 6.90 (1H, d, $J=2.4$ Hz), 6.96 (1H, d, $J=9.0$ Hz), 7.55 (1H, d, $J=13.5$ Hz), 8.69 (1H, s).

Ethyl 1-(3-(*S*)-(tert-butoxycarbonylamino)pyrrolidin-1-yl)-2-fluoro-4-oxo-4H-pyrido[3,2,1-*kl*](1,4-dioxonaphtho)[2,3-*c*]phenoxazine-5-carboxylate (**9c**): Red solid (80%); R_f : 0.37 (2% MeOH/ CHCl_3); $^1\text{H-NMR}$ (CDCl_3 , 500 MHz) δ : 1.47 (3H, t, $J=7.1$ Hz), 1.50 (9H, s), 1.98 (1H, m), 2.27 (1H, m), 3.56 (1H, m), 3.74 (1H, m), 3.92 (1H, m), 4.01 (1H, m), 4.37 (1H, m),

4.48 (2H, q, $J=7.1$ Hz), 7.63 (1H, d, $J=13.6$ Hz), 7.83 (1H, s), 7.85 (2H, m), 8.26 (1H, s), 8.34 (2H, m), 8.94 (1H, s).

Ethyl 1-(3-(*S*)-(tert-butoxycarbonylamino)pyrrolidin-1-yl)-2-fluoro-4-oxo-4H-pyrido[3,2,1-*kl*]-7-azaphenoxazine-5-carboxylate (**9d**): Yellow solid (97%); R_f : 0.73 (12.5% MeOH/EtOAc); $^1\text{H-NMR}$ (CDCl_3 , 500 MHz) δ : 1.45 (3H, t, $J=7.1$ Hz), 1.48 (9H, s), 1.94 (1H, m), 2.24 (1H, m), 3.51 (1H, m), 3.63 (1H, m), 3.84 (1H, m), 3.92 (1H, m), 4.3 (1H, m), 4.41 (2H, q, $J=7.1$ Hz), 7.17 (1H, dd, $J=7.6$, 4.1 Hz), 7.34 (1H, d, $J=7.6$ Hz), 7.62 (1H, d, $J=13.6$ Hz), 8.09 (1H, d, $J=4.1$ Hz), 9.58 (1H, s).

Ethyl 1-(3-(*S*)-(tert-butoxycarbonylamino)pyrrolidin-1-yl)-2-fluoro-4-oxo-4H-pyrido[3,2,1-*kl*]-8-azaphenoxazine-5-carboxylate (**9e**): Yellow solid (80%); R_f : 0.35 (6% MeOH/ CHCl_3); $^1\text{H-NMR}$ (CDCl_3 , 500 MHz) δ : 1.39 (3H, t, $J=7.1$ Hz), 1.45 (9H, s), 1.95 (1H, m), 2.18 (1H, m), 3.46 (1H, m), 3.63 (1H, m), 3.81 (1H, m), 3.90 (1H, m), 4.29 (1H, m), 4.36 (2H, q, $J=7.1$ Hz), 6.93 (1H, d, $J=5.4$ Hz), 7.49 (1H, d, $J=13.7$ Hz), 8.36 (1H, d, $J=5.4$ Hz), 8.63 (1H, s), 8.73 (1H, s).

Ethyl 1-(3-(*S*)-(tert-butoxycarbonylamino)pyrrolidin-1-yl)-2-fluoro-4-oxo-4H-pyrido[3,2,1-*kl*]pyridino[3,2-*c*]phenoxazine-5-carboxylate (**9f**): Yellow solid (99%); R_f : 0.21 (2% MeOH/ CHCl_3); $^1\text{H-NMR}$ (CDCl_3 , 500 MHz) δ : 1.46 (3H, t, $J=7.1$ Hz), 1.50 (9H, s), 2.17 (1H, m), 2.38 (1H, m), 3.64 (1H, m), 3.77 (2H, m), 4.07 (1H, m), 4.32 (1H, m), 4.46 (2H, q, $J=7.1$ Hz), 7.51 (1H, dd, $J=7.7$, 4.1 Hz), 7.64 (1H, d, $J=12.8$ Hz), 7.67 (2H, s), 8.17 (1H, d, $J=7.7$ Hz), 8.95 (1H, s), 9.10 (1H, br s).

General Procedure for the Preparation of Compounds 4a–f Compounds **9a–f** (1 eq) was mixed with aqueous 1 N KOH in ethanol (1 : 2) and refluxed at 80°C (30 min). And then aqueous 2 N HCl (0.5 ml) in ethanol (3 : 2) was added into the reaction mixture. After 3 h more refluxing, the reaction mixture was cooled and solid precipitated was filtered. Ethanol was added to the solid and the mixture was heated. The solid collected after filtration was dried *in vacuo* to give products.

1-(3-(*S*)-Pyrrolidin-1-yl)-2-fluoro-8-methoxy-4-oxo-4H-pyrido[3,2,1-*kl*]phenoxazine-5-carboxylic Acid HCl (**4a**): Orange solid (97%). $^1\text{H-NMR}$ ($\text{CD}_3\text{OD}+\text{DMSO}-d_6$, 500 MHz) δ : 2.10 (1H, m), 2.37 (1H, m), 3.78 (2H, m), 3.89 (3H, s), 3.94 (2H, m), 3.98 (1H, m), 6.97 (1H, dd, $J=8.8$, 2.1 Hz), 7.23 (1H, d, $J=8.8$ Hz), 7.36 (1H, d, $J=2.1$ Hz), 7.53 (1H, d, $J=13.5$ Hz), 9.12 (1H, s). FAB-MS m/z : 412.1305 (Calcd for $\text{C}_{21}\text{H}_{19}\text{N}_3\text{O}_5\text{F}$: 412.1309). MS m/z : 412 [$\text{M}+\text{H}$] $^+$.

1-(3-(*S*)-Pyrrolidin-1-yl)-2-fluoro-8-allyloxy-4-oxo-4H-pyrido[3,2,1-*kl*]phenoxazine-5-carboxylic Acid HCl (**4b**): Yellow solid (68%). $^1\text{H-NMR}$ ($\text{DMSO}-d_6$, 500 MHz) δ : 2.00 (1H, m), 2.26 (1H, m), 3.74 (2H, m), 3.87 (2H, m), 3.93 (1H, m), 4.69 (2H, d, $J=5.0$ Hz), 5.29 (1H, dd, $J=10.5$, 1.0 Hz), 5.44 (1H, dd, $J=17.2$, 1.0 Hz), 6.05 (1H, m), 7.00 (1H, dd, $J=9.0$, 2.3 Hz), 7.27 (1H, d, $J=9.0$ Hz), 7.51 (1H, d, $J=13.5$ Hz), 7.56 (1H, d, $J=2.3$ Hz), 9.18 (1H, s). FAB-MS m/z : 438.1469 (Calcd for $\text{C}_{23}\text{H}_{21}\text{N}_3\text{O}_5\text{F}$: 438.1465). MS m/z : 438 [$\text{M}+\text{H}$] $^+$.

1-(3-(*S*)-Pyrrolidin-1-yl)-2-fluoro-4-oxo-4H-pyrido[3,2,1-*kl*](1,4-dioxonaphtho)[2,3-*c*]phenoxazine-5-carboxylic Acid HCl (**4c**): Brown solid (87%). $^1\text{H-NMR}$ ($\text{CD}_3\text{OD}+\text{DMSO}-d_6$, 500 MHz) δ : 2.25 (1H, m), 2.50 (1H, m), 3.91 (1H, m), 3.97 (1H, m), 4.09 (2H, m), 4.15 (1H, m), 7.51 (1H, d, $J=13.5$ Hz), 7.91 (1H, s), 7.93 (2H, m), 8.23 (1H, d, $J=7.2$ Hz), 8.26 (1H, d, $J=7.2$ Hz), 8.45 (1H, s), 8.94 (1H, s). MS m/z : 512 [$\text{M}+\text{H}$] $^+$.

1-(3-(*S*)-Pyrrolidin-1-yl)-2-fluoro-4-oxo-4H-pyrido[3,2,1-*kl*]-7-azaphenoxazine-5-carboxylic Acid 2HCl (**4d**): Yellow solid (69%). $^1\text{H-NMR}$ ($\text{DMSO}-d_6$, 500 MHz) δ : 2.03 (1H, m), 2.35 (1H, m), 3.74 (3H, m), 3.88 (1H, m), 3.95 (1H, m), 7.49 (1H, dd, $J=7.7$, 4.2 Hz), 7.52 (1H, d, $J=13.6$ Hz), 7.76 (1H, d, $J=7.7$ Hz), 8.24 (1H, d, $J=4.2$ Hz), 9.54 (1H, s). FAB-MS m/z : 383.1151 (Calcd for $\text{C}_{19}\text{H}_{16}\text{N}_4\text{O}_4\text{F}$: 383.1156). MS m/z : 383 [$\text{M}+\text{H}$] $^+$.

1-(3-(*S*)-Pyrrolidin-1-yl)-2-fluoro-4-oxo-4H-pyrido[3,2,1-*kl*]-8-azaphenoxazine-5-carboxylic Acid 2HCl (**4e**): Yellow solid (70%). $^1\text{H-NMR}$ ($\text{CD}_3\text{OD}+\text{DMSO}-d_6$, 500 MHz) δ : 2.03 (1H, m), 2.49 (1H, m), 3.14 (1H, m), 3.21 (1H, m), 3.38 (1H, m), 3.63 (1H, m), 3.98 (1H, m), 6.63 (1H, dd, $J=6.8$, 1.8 Hz), 7.70 (1H, d, $J=12.1$ Hz), 7.96 (1H, d, $J=6.8$ Hz), 8.32 (1H, s), 8.56 (1H, d, $J=5.4$ Hz). FAB-MS m/z : 383.1143 (Calcd for $\text{C}_{19}\text{H}_{16}\text{N}_4\text{O}_4\text{F}$: 383.1156). MS m/z : 383 [$\text{M}+\text{H}$] $^+$.

1-(3-(*S*)-Pyrrolidin-1-yl)-2-fluoro-4-oxo-4H-pyrido[3,2,1-*kl*]pyridino[3,2-*c*]phenoxazine-5-carboxylic Acid 2HCl (**4f**): Yellow solid (73%). $^1\text{H-NMR}$ (CD_3OD , 500 MHz) δ : 2.25 (1H, m), 2.54 (1H, m), 3.82 (1H, m), 3.87 (1H, m), 4.01 (1H, m), 4.15 (1H, m), 4.33 (1H, m), 7.56 (1H, d, $J=12.7$ Hz), 7.70 (1H, dd, $J=8.3$, 4.2 Hz), 7.85 (1H, d, $J=8.3$ Hz), 8.43 (1H, d, $J=8.3$ Hz), 9.04 (1H, d, $J=4.2$ Hz), 9.22 (1H, s). FAB-MS m/z : 433.1307 (Calcd for $\text{C}_{23}\text{H}_{18}\text{N}_4\text{O}_4\text{F}$: 433.1312). MS m/z : 433 [$\text{M}+\text{H}$] $^+$.

Cytotoxicity Test¹³⁾ Cancer cells were purchased from the American Tissue Culture Collection (Rockville, MD, U.S.A.) and cultured by the supplier's instructions. Exponentially growing cells ((1–2) $\times 10^3$ cells) in 0.1 ml

of medium were seeded on day 0 in a 96-well microtiter plate. On day 1, 0.1 ml aliquots of medium containing graded concentrations of compound were added to the cell plates. On day 4, the cell cultures were incubated with 50 μ l of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (1 mg/ml in Dulbecco's phosphate buffered saline) for 4 h at 37 °C. The resulting formazan precipitate was dissolved with 200 μ l of 0.04 M HCl in isopropyl alcohol. For determination of the IC₅₀ values, the absorbance readings at 570 nm were fitted to the four-parameters logistic equation.

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- 11) Synthesis of 4-allyloxy-2-aminophenol (**5b**)

The reaction scheme shows the synthesis of 4-allyloxy-2-aminophenol (**5b**) from catechol. Step (a) involves the reaction of catechol with allyl iodide and K₂CO₃ in acetone to form 4-allyloxyphenol. Step (b) involves the nitration of 4-allyloxyphenol with HNO₃ in AcOH to form 4-allyloxy-2-nitrophenol. Step (c) involves the reduction of the nitro group to an amino group using SnCl₂ in EtOH to yield the final product, 4-allyloxy-2-aminophenol (**5b**).
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