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Synthesis, anticancer activity and pharmacokinetic analysis of 1-[(substituted 2-alkoxyquinoxalin-3-yl)aminocarbonyl]-4-(hetero)arylpiperazine derivatives

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ABSTRACT

Based on the anticancer activity of novel quinoxalinyl-piperazine compounds, 1-[(5 or 6-substituted alkoxyquinoxalinyl)aminocarbonyl]-4-(hetero)arylpiperazine derivatives published in *Bioorg. Med. Chem.* **2010**, *18*, 7966, we further explored the synthesis of 7 or 8-substituted quinoxalinyl piperazine derivatives. From in vitro studies of the newly synthesized compounds using human cancer cell lines, we identified some of the 8-substituted compounds, for example **6p**, **6q** and **6r**, which inhibited the proliferation of various human cancer cells at nanomolar concentrations. Compound **6r**, in particular, showed the lowest IC_{50} values, ranging from 6.1 to 17 nM, in inhibition of the growth of cancer cells, which is better than compound **6k** (compound **25** in the reference cited above). In order to select and develop a leading compound among the quinoxaline compounds with substitutions on positions 5, 6, 7 or 8, the compounds comparable to compound **6k** in in vitro cancer cell growth inhibition were chosen and their pharmacokinetic properties were evaluated in rats. In these studies, compound **6k** showed the highest oral bioavailability of 83.4%, and compounds **6j** and **6q** followed, with 77.8% and 57.6%, respectively. From the results of in vitro growth inhibitory activities and the pharmacokinetic study, compound **6k** is suggested for further development as an orally deliverable anticancer drug.

1. Introduction

Since AG1295, which has a relatively simple quinoxaline structure, has been shown to selectively block tyrosine kinases such as PDGFR,[1–3] the quinoxaline pharmacophore has become an attractive scaffold for the development of active chemotherapeutic agents. As a result of such efforts, the pharmacological data of several anticancer drugs containing a quinoxaline ring has been reported in many journals.[4–9] For example, the quinoxaline anticancer drugs XK469 (2-(4-((7-chloro-2-quinoxalinyl)oxy)phenoxy)propionic acid) and CQS (chloroquinoxaline sulfonamide) were found to have activity against solid tumors[10–12] and have been studied in clinical trials.[13–15]

There is also a report suggesting unsubstituted quinoxaline compounds containing piperazine as candidates for anticancer agents; one of the unsubstituted quinoxalinyl-piperazine compounds was shown to exhibit microtubule-inhibiting activity.[16,17]

Recently we reported the anticancer activities of 5 or 6-substituted quinoxaline compounds containing piperazine.[18] Compound **6k**, 1-(3,5-dimethoxyphenyl)-4-[(6-fluoro-2-methoxyquinoxalin-3-yl)aminocarbonyl] piperazine, especially showed strong inhibition of the growth of human cancer cells (IC_{50}, 11–21 nM), and also induced cell cycle arrest at G2/M and apoptosis in cancer cells by the downregulation of Bcl-2 protein level.[18] This compound also significantly inhibited the growth of drug-resistant cancer cells, and has shown potential use in combination therapy with known anticancer drugs such as palcitaxel, doxorubicin, gemcitabine, 5-FU and cisplatin.[18]

The strong anticancer activity shown in our previous study by compound **6k**, which has 6-fluoro on the quinoxaline ring and 3,5-dimethoxyphenyl piperazine, inspired us to investigate other substituted quinoxaline compounds containing the piperazine group to find an even stronger compound. In view of the previous rationale that was more focused on the quinoxaline derivatives substituted on the 5 or 6 position, and containing a piperazine group, and in continuation of an ongoing program aiming to find a new lead with potential anticancer activity, a series of novel 7- or 8-substituted compounds (substituted at R_3 or R_4 of compound **6** in Scheme 1) at the quinoxaline ring of 1-[(2-alkoxyquinoxalin-3-yl)aminocarbonyl-4-(hetero)arylpiperazine derivatives (shown

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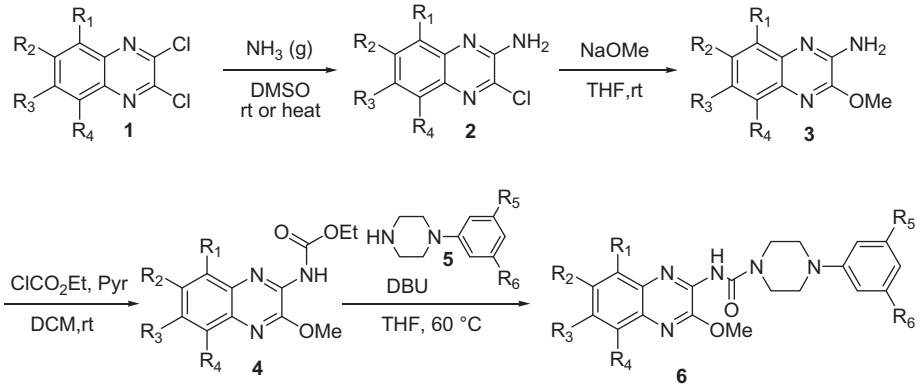
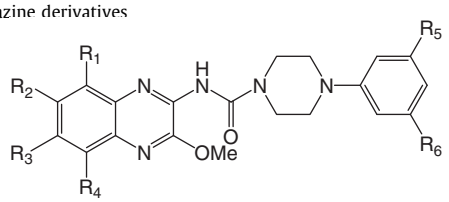


Scheme 1.

Table 1
Structures of 1-[(substituted 2-alkoxyquinoxalin-3-yl)aminocarbonyl]-4-(hetero)arylpiperazine derivatives



Compound	R_1	R_2	R_3	R_4	R_5 and R_6
6a[*]	Me	H	H	H	Me
6b[*]	Me	H	H	H	OMe
6c[*]	OMe	H	H	H	Me
6d[*]	OMe	H	H	H	OMe
6e[*]	F	H	H	H	Me
6f[*]	F	H	H	H	OMe
6g[*]	H	Me	H	H	Me
6h[*]	H	Me	H	H	OMe
6i[*]	H	OMe	H	H	Me
6j[*]	H	F	H	H	Me
6k[*]	H	F	H	H	OMe
6l	H	H	Me	H	Me
6m	H	H	OMe	H	Me
6n	H	H	F	H	Me
6o	H	H	H	Me	Me
6p	H	H	H	Me	OMe
6q	H	H	H	OMe	Me
6r	H	H	H	OMe	OMe

[*] From Ref. 18.

in Table 1) were synthesized and tested against various types of human cancer cell lines, as a second optimization process. We selected several active compounds such as **6p**, **6q** and **6r**, and conducted a pharmacokinetic study in rats, together with representative 5 or 6-substituted compounds including compound **6k,** to develop a promising and orally deliverable anticancer drug.

2. Chemistry

1-[(7 or 8-Substituted alkoxyquinoxalinyl)aminocarbonyl]-4-(hetero)arylpiperazine derivatives were regiospecifically synthesized from the corresponding 2,3-dichloroquinoxaline starting materials as described in Scheme 1 by application of the same reaction sequence as that for the synthesis of 6-substituted alkoxyquinoxalinyl)aminocarbonyl]-4-(hetero)arylpiperazine derivatives described earlier.[18]

Sequential substitution reactions by treatment of 2,3-dichloroquinoxaline starting materials with ammonia followed by methoxide afforded 3-amino-2-methoxy derivatives through the formation of 3-amino-2-chloroquinoxaline derivatives. The final quinoxalinyl-piperazine compounds were prepared from the reactions of carbamate derivatives of 3-amino-2-methoxy intermediates with arylpiperazines.

3. Results

3.1. In vitro cell growth inhibition

The in vitro cytotoxicity experiments were performed with the synthetic 1-[(7 or 8-substituted alkoxyquinoxalinyl)aminocarbonyl]-4-(hetero)arylpiperazine derivatives (Table 1) in human cancer cell lines using a colorimetric sulphorhodamine B (SRB) assay.[19] The IC_{50} values (Table 2) varied significantly and were dependent on the substitution on the quinoxaline ring but also on substituents on the phenyl ring. The data from our previous paper indicated that compounds containing methyl or methoxy group at the C-5 position of the quinoxaline ring possessed stronger anticancer activities with 3,5-dimethoxyphenyl-piperazine group than 3,5-dimethylphenyl-piperazine group. Compounds with the 3,5-dimethoxyphenyl-piperazine group together with 6-methyl or 6-F substitution on the quinoxaline ring showed more potent anticancer activity than those with the 3,5-dimethylphenyl-piperazine group. This stronger activity of compounds with 3,5-dimethoxyphenyl-piperazine group also applied to the compounds containing methyl or methoxy at the C-8 position of the quinoxaline ring (see Table 2). In the case of the compounds with substitutions at the C-7 position, the compound **6l**, which contains a methyl group at the C-7 position of the quinoxaline ring, displayed stronger activity than the equivalent C-7 methoxy compound (**6m**). However, their IC_{50} values are higher than those of the 6- or 8-substituted compounds. Among the compounds tested, compound **6r**, which has both a methoxy group at the C-8 position of the quinoxaline ring and the 3,5-dimethoxyphenyl-piperazine group, was found to be the most active compound (IC_{50} from 6.1 to 17 nM in the cell lines tested), and followed by compound **6k**.

3.2. The prediction of physicochemical properties

We also checked the physicochemical properties of the compounds listed in Table 1 using, to save time and cost, an in silico prediction method for pharmacokinetics, as shown in Table 3. As discussed by Lipinski, molecular properties are closely related to the oral bioavailability of a drug.[20] In particular, membrane permeation is considered as a common requirement for oral bioavailability. It is well known that membrane permeability shows a good correlation with *C*Log*P*.[21] Hann et al. suggested lead-likeness as

Table 2
Inhibition of cell growth (IC_{50}, μM) by quinoxaline-piperazine compounds against human cancer cell lines

	6b*	6g*	6j*	6k*	6l	6m	6n	6o	6p	6q	6r
Hs578T	0.055 ± 0.025	0.15 ± 0.018	0.041 ± 0.0050	0.024 ± 0.0028	0.32 ± 0.095	0.49 ± 0.067	0.50 ± 0.085	0.16 ± 0.019	0.020 ± 0.0062	0.048 ± 0.0038	0.015 ± 0.0032
UMRC-2	0.030 ± 0.0031	0.050 ± 0.030	0.022 ± 0.0081	0.013 ± 0.0021	0.057 ± 0.0063	0.27 ± 0.020	0.32 ± 0.022	0.092 ± 0.024	0.014 ± 0.0023	0.045 ± 0.0031	0.0061 ± 0.0013
PANC-1	0.054 ± 0.0067	0.28 ± 0.14	0.024 ± 0.0042	0.021 ± 0.0044	0.28 ± 0.018	0.47 ± 0.054	0.56 ± 0.034	0.18 ± 0.049	0.028 ± 0.0079	0.063 ± 0.0031	0.017 ± 0.0023
A549	0.056 ± 0.0083	0.097 ± 0.0099	0.023 ± 0.0042	0.021 ± 0.0038	0.21 ± 0.027	0.35 ± 0.068	0.42 ± 0.073	N/A	0.028 ± 0.0035	0.068 ± 0.0092	0.017 ± 0.0023
MKN-45	0.057 ± 0.0059	0.080 ± 0.0080	0.027 ± 0.0015	0.020 ± 0.0017	0.18 ± 0.0080	0.27 ± 0.018	0.25 ± 0.017	N/A	0.024 ± 0.0013	0.064 ± 0.0035	0.015 ± 0.0008
HepG2	0.063 ± 0.0096	0.081 ± 0.0093	0.021 ± 0.0034	0.019 ± 0.0035	0.19 ± 0.013	0.56 ± 0.072	0.48 ± 0.077	N/A	0.033 ± 0.0053	0.062 ± 0.0084	0.017 ± 0.0022
HCT116	0.050 ± 0.0078	0.10 ± 0.0014	0.025 ± 0.0022	0.020 ± 0.0022	0.20 ± 0.018	0.38 ± 0.033	0.40 ± 0.021	N/A	0.026 ± 0.0031	0.068 ± 0.0071	0.015 ± 0.0006
PC-3	0.057 ± 0.011	0.11 ± 0.0017	0.022 ± 0.0035	0.021 ± 0.0053	0.25 ± 0.0053	0.58 ± 0.048	0.70 ± 0.12	N/A	0.023 ± 0.0024	0.059 ± 0.0071	0.015 ± 0.0008
HeLa	0.062 ± 0.0059	0.10 ± 0.0058	0.024 ± 0.0017	0.021 ± 0.0013	0.22 ± 0.015	0.38 ± 0.026	0.37 ± 0.0088	N/A	0.025 ± 0.0013	0.065 ± 0.0038	0.017 ± 0.0008
SK-MEL-28	0.064 ± 0.0019	0.13 ± 0.019	0.022 ± 0.0054	0.020 ± 0.0054	0.25 ± 0.063	0.50 ± 0.13	0.55 ± 0.15	N/A	0.033 ± 0.0074	0.069 ± 0.0063	0.014 ± 0.0015
OVCAR3	0.039 ± 0.0069	0.065 ± 0.0065	0.025 ± 0.0080	0.012 ± 0.0013	0.14 ± 0.0071	0.29 ± 0.016	0.29 ± 0.021	N/A	0.018 ± 0.0025	0.053 ± 0.0057	0.012 ± 0.0011

* From *Ref.* 18.

Table 3
The physicochemical properties of quinoxaline-piperazine compounds

Compound	*C*Log*P*[a]	Solubility (*S*)[b]	PSA[c]	BBB[d]	hERG[e]
6a	4.75	−5.699	59.338	0.219	Ok
6b	3.78	−4.732	77.198	−0.375	Ok
6c	4.65	−5.19	68.268	−0.078	Weak
6d	3.68	−4.207	86.128	−0.671	Ok
6e	4.43	−5.409	59.338	0.132	Ok
6f	3.46	−4.442	77.198	−0.461	Ok
6g	4.75	−5.684	59.338	0.219	Ok
6h	3.78	−4.716	77.198	−0.375	Ok
6i	4.65	−5.179	68.268	−0.078	Ok
6j	4.43	−5.447	59.338	0.132	Ok
6k	3.46	−4.479	77.198	−0.461	Ok
6l	4.75	−5.676	59.338	0.219	Ok
6m	4.65	−5.162	68.268	−0.078	Ok
6n	4.43	−5.439	59.338	0.132	Ok
6o	4.75	−5.691	59.338	0.219	Ok
6p	3.78	−4.723	77.198	−0.375	Ok
6q	4.65	−5.173	68.268	−0.078	Weak
6r	3.68	−4.188	86.128	−0.671	Ok

[a] These values are calculated using Tripos Sybyl-X1.3.[23]
[b] Aqueous solubility; $S < -8.0$, extremely low; $-8.0 < S < -6.0$, very low, possible; $-6.0 < S < -4.0$, low; $-4.0 < S < -2.0$, good; $-2.0 < S < 0.0$, optimal; $S > 0.0$, too soluble, using Accelrys ADMET module.[24]
[c] Polar surface area for each molecule using a 2D structure.
[d] The predicted blood–brain barrier using Accelrys ADMET module; BBB > 0.0, high penetrate; −0.52 < BBB < 0, medium penetrate; BBB < −0.52, low penetrate.
[e] The hERG inhibition predicted from Ref. 25.

*C*Log*P* values less than 4.2, and −5 < solubility < 0.5.[22] In addition, we calculated BBB (blood–brain barrier) and hERG (human ether-a-go-go related gene) inhibition. All compounds showed stable values for BBB and hERG inhibition although **6c** and **6q** gave weak inhibition—about 30–40% at 10 μM.

3.3. Pharmacokinetic analysis

Shown in Figure 1 are the serum concentration versus time curves of the selected compounds in Sprague–Dawley male rats following a single dose given iv or po. The compounds were measured in the plasma for 24 h post-dosing. Table 4 also shows that there were marked differences among the tested compounds in some pharmacokinetic parameters, including the maximal serum concentrations (C_{max}), the half-lives ($T_{1/2}$), the systemic clearance (CL), the area under the curves (AUC) and the oral bioavailability (*F*). Interestingly, among the six compounds, compound **6r**, which has the strongest anticancer activity in inhibition of cell growth, showed a relatively low level of oral bioavailability (32.4% *F*). Compounds **6b** and **6p** also showed a low level of oral bioavailability. On the other hand, compounds **6j** and **6k** displayed a high level of oral bioavailability, 77.8% and 83.4%, respectively. Compound **6k** showed the second best anti-cancer activities against the tested cancer cell lines but showed the highest oral bioavailability (*F*), indicating satisfactory plasma levels after oral administration. The systemic clearance (CL) of compound **6k** was 0.61 l/hr/kg in male rats, which corresponds to only about 18.9% of the hepatic blood flow of the rat. The apparent volume of distribution at a steady state (V_{ss}) in male rats was estimated to be 4.5 l/kg, indicating that compound **6k** is likely to be readily distributed out of the vascular space. The terminal half-life ($T_{1/2}$) in male rats was 6.5 and 7.9 h after iv or po administration, respectively.

4. Discussion

We reported previously that compound **6k** was characterized as a G2/M-specific cell cycle inhibitor, with strong inhibition of

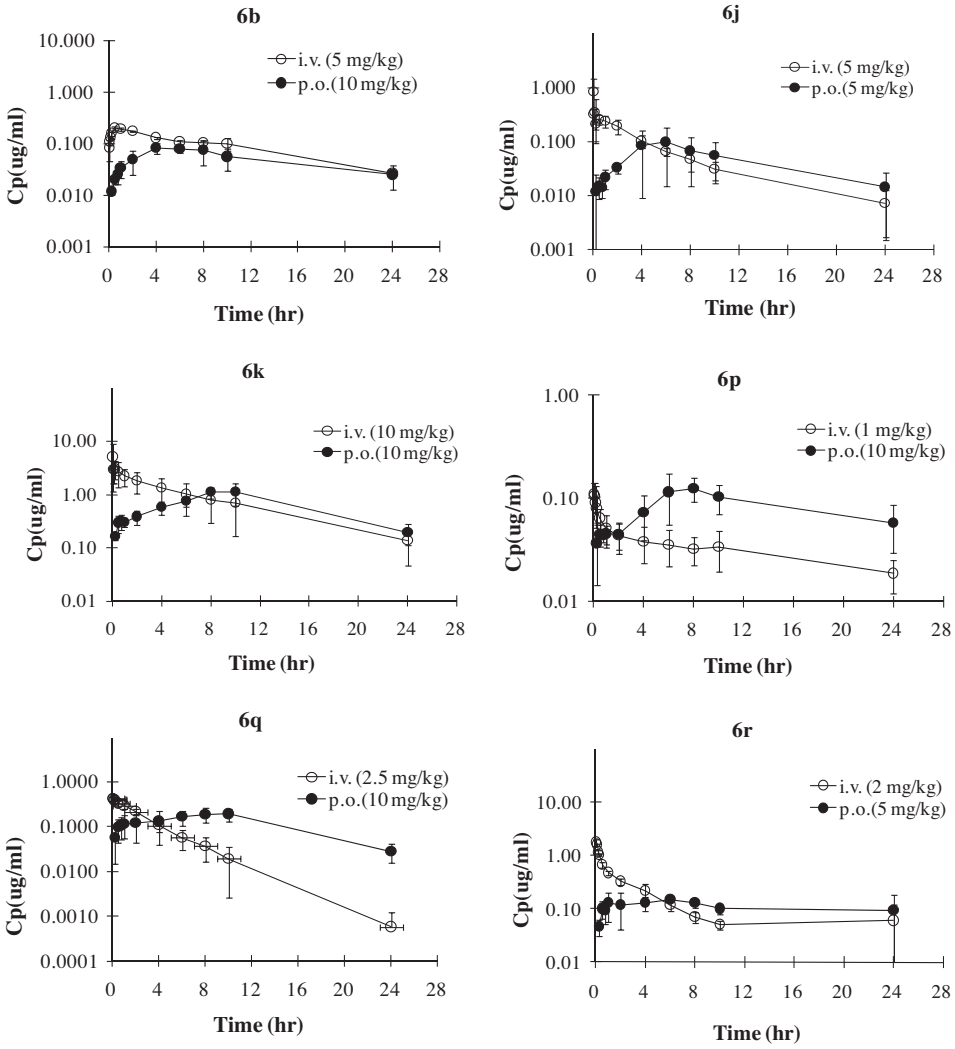


Figure 1. Plasma concentration versus time plots for compounds **6b**, **6j**, **6k**, **6p**, **6q** and **6r** after a single iv (○) or oral (●) administration to male Sprague–Dawley rats.

Table 4
Pharmacokinetic parameters of compounds **6b**, **6j**, **6k**, **6p**, **6q** and **6r** after an iv and oral administration to SD male rats ($n = 3$).

Parameters	**6b**	**6j**	**6k**	**6p**	**6q**	**6r**
iv						
C_{max} (μg/mL)	—	—	—	—	—	—
T_{max} (h)	—	—	—	—	—	—
$T_{1/2}$ (h)	8.1 ± 0.93	5.6 ± 1.37	6.5 ± 2.37	17.8 ± 4.39	2.7 ± 0.99	7.4 ± 1.47
AUC_{0-24h} (ug h/mL)	2.2 ± 0.29	1.4 ± 0.23	19.2 ± 10.98	0.78 ± 0.285	1.31 ± 0.335	3.22 ± 0.173
$AUC_{0-\infty}$ (μg h/mL)	2.5 ± 0.29	1.5 ± 0.29	20.6 ± 11.48	1.24 ± 0.391	1.36 ± 0.304	3.76 ± 0.689
Cl (L/kg h)	2.0 ± 0.25	3.4 ± 0.61	0.61 ± 0.365	0.86 ± 0.262	1.89 ± 0.395	0.54 ± 0.090
V_{ss} (L/Kg)	22.8 ± 3.08	18.8 ± 2.53	4.5 ± 1.88	22.2 ± 11.24	6.23 ± 1.759	4.9 ± 1.71
MRT (h)	7.8 ± 0.31	4.7 ± 0.97	6.0 ± 0.99	9.4 ± 0.13	2.9 ± 1.01	6.2 ± 2.83
po						
C_{max} (μg/mL)	0.09 ± 0.024	0.09 ± 0.098	1.2 ± 0.37	0.14 ± 0.037	0.21 ± 0.064	0.17 ± 0.034
T_{max} (h)	6.7 ± 1.16	6.0 ± 2.0	8.7 ± 1.16	7.3 ± 2.31	9.3 ± 1.16	4.7 ± 2.31
$T_{1/2}$ (h)	10.7 ± 0.84	6.7 ± 1.37	7.9 ± 1.72 ($n = 2$)	24.1 ± 9.63 ($n = 2$)	6.5 ($n = 1$)	10.9 ± 2.85 ($n = 2$)
AUC_{0-24h} (μg h/mL)	1.2 ± 0.45	1.12 ± 0.872	15.9 ± 4.15	1.9 ± 0.45	3.01 ± 1.028	2.61 ± 1.008
$AUC_{0-\infty}$ (μg h/mL)	1.6 ± 0.64	1.27 ± 1.044	17.5 ± 4.85 ($n = 2$)	4.5 ± 0.001 ($n = 2$)	4.07 ($n = 1$)	2.72 ± 0.106 ($n = 2$)
Cl (L/kg h)	—	—	—	—	—	—
V_{ss} (L/kg)	—	—	—	—	—	—
MRT (h)	9.6 ± 0.50	8.6 ± 0.75	9.6 ± 0.18	11.2 ± 0.95	8.9 ± 0.47	10.8 ± 1.92
F (%)	27.3	77.8	83.4	25.6	57.6	32.4

C_{max}: maximum serum concentration, T_{max}: time to maximum concentration, $T_{1/2}$: half-life, AUC: area under the curve, CL: systemic clearance, V_{ss}: volume of distribution at a steady state, MRT: mean residence time, F: bioavailability.

the growth of human cancer cells and also induction of apoptosis by the downregulation of Bcl-2 protein level.[18] Although compound **6k** was the strongest inhibitor in the previous study, it belongs to a small group of 5 or 6-substituted quinoxaline com-

pounds and thus it led us to explore other substituted quinoxaline compounds containing the piperazine group. In the current study, we designed and synthesized several compounds substituted at the 7- or 8-position of the quinoxaline ring (shown in Table 1), and tested them against human cancer cells. In an in vitro cell study (Table 2), we found that compounds with a 3,5-dimethoxyphenyl-piperazine group as well as 8-methyl (**6p**) or 8-methoxy (**6r**) substitution on the quinoxaline ring displayed similar or better anti-cancer activity as compound **6k**. Compound **6r** especially showed the possibility of being a new leading compound, as it had the lowest IC_{50} range. In order to select the most reliable candidate compounds in terms of drug development, we are required to select some representative compounds from each of the substitution groups, that is, C-5 or C-6 or C-8 substituted compounds. Compound **6b** with C-5 substitution, compounds **6j** and **6k** with C-6 substitution and compounds **6p**, **6q** and **6r** with C-8 substitution were selected based on their both in vitro activities in inhibition of cell growth and the predicted physicochemical properties and the pharmacokinetic analyses performed in rats. Compounds substituted at the C-7 position were excluded from the pharmacokinetic study because they had relatively less activity in cell growth inhibition. From the PK data (Table 4), it is obvious that substitution on the phenyl-piperazine group as well as at the quinoxaline ring has a direct impact on $T_{1/2}$, F and AUC of the compounds. Among the six compounds for which there is pharmacokinetic data, the lead compound **6k** exhibited good properties suitable for a drug, such as oral bioavailability (*F*; 83%), half-life ($T_{1/2}$; 7.9 h in oral), and plasma levels. *C*Log*P* of compound **6k** is about 3.46 and water solubility is low (Table 3), which will require a formulation study to achieve efficient oral delivery of the compound. Oral administration of lipophilic drugs has been challenged during the past 20 years and the development of orally available anticancer drugs has made great progress using several platform technologies such as nanosize formulation,[26–28] lipid based delivery systems[29,30] or polymeric micelles.[31] More than forty five oral agents have been approved for the treatment of cancer in US[32] and it is expected that more novel oral anticancer drugs will be in the market in the near future. So from the viewpoints of a potent anticancer activity and good pharmacokinetic properties, compound **6k** could be a promising orally available anticancer drug and guarantees further ADME and toxicological studies.

5. Conclusion

In conclusion, we synthesized a series of 5-, 6-, 7- or 8-substituted quinoxalinyl-piperazine compounds in the previous and present studies and tested them against human cancer cell lines for the selection of a final lead compound(s). Actually, we found that compounds **6p** or **6r** displayed similar or better anticancer activity compared to compound **6k** in inhibition of cell growth in vitro. However these second tier compounds (**6p** and **6r)** do not have better pharmacokinetic properties compared to the primary compound **6k**. Finally, among representative compounds that were substituted at the C-5-, C-6-, C-7-, or C-8 position on the quinoxaline phenyl ring, our in vitro cell line and pharmacokinetic studies suggest that compound **6k** has a strong anti-cancer efficacy and desirable pharmacokinetic properties. These data support further development of compound **6k** to achieve an orally available anticancer drug.

6. Experimental

6.1. Chemistry

6.1.1. General

All chemicals were reagent grade and used as purchased. Reactions were monitored by TLC analysis using Merck Silica Gel 60 F-254 thin layer plates. Flash column chromatography was carried out on Merck Silica Gel 60 (230–400 mesh). The crude products were purified by parallel chromatography using Quad3™. ^{1}H NMR spectra were recorded in δ units relative to deuterated solvent as an internal reference using a Bruker 300 or 500 MHz NMR instrument. LC–MS analysis was performed on an ESI mass spectrometer with PDA detection. LC–MS area % purities of all products were determined by LC peak area analysis (XTerraMS C_{18} column, 4.6 mm × 100 mm; PDA detector at 200–400 nm; gradient, 5–95% CH_3CN/H_2O).

6.1.2. Compound synthesis

The analytical data of compounds **6a**–**6k** and all of the synthetic procedure of the intermediate compounds **1**–**4** can be found in our previous paper.[18] Compounds **6m**–**6r** were synthesized by the same way with the preparation of the compound **6l**.

6.1.2.1. Representative synthetic procedure of 1-(3,5-dimethylphenyl)-4-[(3-methoxy-6-methylquinoxalin-2-yl)amino carbonyl] piperazine (6l). To a stirred solution of ethyl-(3-methoxy-6methylquinoxalin-2-yl)carbamate (**4l**) (9.7 mg, 0.037 mmol) and 1-(3,5-dimethoxyphenyl)piperazine (12 mg, 0.054 mmol) in tetrahydrofuran (2 ml) at room temperature was added DBU (8 mg, 0.05 mmol). The resulting mixture was stirred at 60 °C for 27 h, concentrated in vacuo to remove the solvent and purified by SiO_2 column chromatography (*n*-hexane/ethyl acetate = 2:1) to yield the desired compound **6l** (14.2 mg, 95%): mp 132 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.29 (s, 6H), 2.50 (s, 3H), 3.18–3.29 (m, 4H), 3.73–3.80 (m, 4H), 4.12 (s, 3H), 6.58 (s, 3H), 7.31 (d, J = 8.4 Hz, 1H), 7.43 (s, 1H), 7.55 (s, 1H), 7.72 (d, J = 8.4 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$); δ 153.6, 151.6, 151.3, 148.8, 139.0, 137.4, 137.0, 135.9, 128.9, 127.0, 126.1, 122.6, 114.8, 55.4, 49.6, 45.3, 21.8, 21.6; LC-MS (ESI) *m/z* 406.20 ($[M+H]^+$); MS–MS (EI) *m/z* M^+ for $C_{23}H_{27}N_5O_2$ calcd 405.220, found 406.059 (M+1).

6.1.2.2. 1-[(3,6-Dimethoxyquinoxalin-2-yl)aminocarbonyl]-4-(3,5-dimethylphenyl)piperazine (6m). In 90% yield; mp 142–145 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.29 (s, 6H), 3.26 (s, 4H), 3.75 (s, 4H), 3.90 (s, 3H), 4.14 (s, 3H), 6.59 (s, 3H), 6.99–7.20 (m, 3H), 7.73–7.75 (m, 1H); LC-MS (ESI) *m/z* 422.20 ($[M+H]^+$).

6.1.2.3. 1-[(6-Fluoro-3-methoxyquinoxalin-2-yl)aminocarbonyl]-4-(3,5-dimethylphenyl)piperazine (6n). In 61% yield; mp 148 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.29 (s, 6H), 3.24 (m, 4H), 3.74 (t, J = 8 Hz, 4H), 4.15 (s, 3H), 6.56(d, J = 19 Hz, 3H), 7.24 (m, 1H), 7.40 (dd, J = 4.5, 11 Hz, 1H), 7.80 (dd, J = 9.6, 5.5 Hz, 1H), 14,47 (br s, 1H); LC–MS (ESI) *m/z* 410.20 ($[M+H]^+$).

6.1.2.4. 1-(3,5-Dimethylphenyl)-4-[(3-methoxy-5-methylquinoxalin-2-yl)aminocarbonyl] piperazine (6o). In 81% yield; mp 201 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.29 (s, 6H), 2.56 (s, 3H), 3.21–3.23 (m, 4H), 3.74–4.05 (m, 3H), 4.15 (s, 4H), 6.57–6.59 (m, 3H), 7.25–7.30 (m, 3H), 7.54 (br s, 1H); LC–MS (ESI) *m/z* 406.00 ($[M+H]^+$).

6.1.2.5. 1-(3,5-Dimethoxyphenyl)-4-[(3-methoxy-5-methylquinoxalin-2-yl)aminocarbonyl] piperazine (6p). In 61% yield; mp 172 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.56 (s, 3H), 3.23–3.25 (m, 4H), 3.79 (s, 6H), 3.92–3.93 (m, 4H), 4.14 (s, 3H), 6.05–6.06 (m, 1H), 6.12 (d, J = 1.8 Hz, 2H), 7.26–7.28 (m, 3H), 7.52–7.54 (m, 1H); LC–MS (ESI) *m/z* 437.97 ($[M+H]^+$).

6.1.2.6. 1-[(3,5-Dimethoxyquinoxalin-2-yl)aminocarbonyl]-4-(3,5-dimethylphenyl)piperazine (6q). In 66% yield; mp 130 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.26–2.29 (m, 6H), 3.18–3.30

(m, 4H), 3.74–3.80 (m, 3H), 4.01–4.04 (m, 3H), 4.10–4.22 (m, 7H), 6.59 (s, 2H), 6.78–6.84 (m, 1H), 6.93–6.96 (m, 1H), 7.26–7.45 (m, 3H); LC–MS (ESI) *m/z* 421.99 ($[M+H]^+$).

6.1.2.7. 1-(3,5-Dimethoxyphenyl)-4-[(3,5-dimethoxyquinoxalin-2-yl)aminocarbonyl]piperazine (6r). In 81% yield; mp 102 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.20–3.32 (m, 4H), 3.74–3.81 (m, 9H), 4.01–4.04 (m, 3H), 4.12 (br s, 1H), 4.19–4.22 (m, 3H), 6.06–6.07 (m, 1H), 6.11–6.12 (m, 2H), 6.79–6.84 (m, 1H), 6.93–6.96 (m, 1H), 7.26–7.29 (m, 1H), 7.41–7.44 (m, 1H); LC–MS (ESI) *m/z* 453.96 ($[M+H]^+$).

6.2. Biology

6.2.1. Growth of cancer cells

Human cancer cell lines were obtained from the following sources: OVCAR-3, Hs578T, HeLa, PC3, HepG2, A549, PANC-1, SK-MEL-28 and HCT116 from the American Type Culture Collection (Manassas, VA); MKN-45 from DSMZ (German Collection of Microorganisms and Cell Cultures), Braunschweig, Germany; UMRC2 (kidney) from the US National Cancer Institute (Bethesda, MD, USA). All cell lines, except Hs578T, HCT116, UMRC-2 and PANC-1, were grown in RPMI1640 medium (Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum (FBS), 1 mM sodium pyruvate, 10 mM HEPES, and 100 units/mL penicillin–streptomycin (P/S). Hs578T, HCT116, UMRC-2 and PANC-1 cells were maintained in Dulbecco's modified Eagle's medium (DMEM) (Invitrogen, Carlsbad, CA) supplemented with 10% FBS, 10 mM HEPES, 100 units/mL P/S, and 2 mM L-glutamine. All cells were incubated at 37 °C under 5% CO_2 and humidified air.

6.2.2. Cell growth inhibition assay

Growth inhibition of human cancer cells by the synthetic quinoxalinyl-piperazine compounds was assessed by the sulphorhodamine B (SRB) assay,[19] along with DMSO as a control. Different human cancer cell lines were treated with compounds at various concentrations. After a 96 h incubation, surviving cells were fixed with trichloroacetic acid, washed and stained with sulphorhodamine B. Absorbance was measured at 530 nm using Benchmark Plus Microplate reader (Bio-Rad Laboratories, Hercules, CA). The drug concentration that inhibited the cell growth by 50% (IC_{50}) was calculated using the KaleidaGraph software program (Synergy software, Reading, PA).

6.3. Pharmacokinetic studies and analysis

Sprague–Dawley rats (adult males, 250–300 g) were fasted overnight, and the femoral vein (for iv administration of the compound) and jugular vein (for blood sampling) of each rat was cannulated with polyethylene tubing. The selected compounds dissolved in 30% polyethyleneglycol (PEG) in saline were administered in a single dose to rats either intravenously or orally. A round-head needle with a syringe was used for oral dosing. At various time points after administration, blood samples (0.5 ml) were collected from the jugular vein, transferred to heparin-coated tubes and centrifuged to separate off the plasma. The plasma was stored at -80 °C until assayed. Compound concentrations in plasma were determined by LC/MS/MS analysis (Q TRAP mass spectrometer, Applied Biosystem, USA). Pharmacokinetic parameters were analyzed by using the Winolin software program. The area under the curve (AUC) was calculated using the trapezoidal rule extrapolated to infinity. The terminal elimination half-life, systemic clearance and volume of distribution at steady state were determined. The extent of absolute oral bioavailability (F) was estimated by comparing the AUC values after intravenous and oral administration of the compounds.

6.4. Statistical analysis

Analytical data were processed using Analyst software Version 1.4.1. (Applied Biosystems, Concord, Canada). Pharmacokinetic parameters were obtained by standard non-compartmental analysis of the plasma concentration-time profiles using PK Solutions 2.0 (Summit Research Services, Montrose, CO, USA).

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2011.12.026.

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