Publisher: African Networks on Ethnomedicines
Web page: /http://journals.sfu.ca/africanem/index.php/ajtcam/index
http://dx.doi.org/10.4314/ajtcam.v9i3.20

DISCOVERY OF POTENT, ORALLY ACTIVE COMPOUNDS OF TYROSINE KINASE AND SERINE/THREONINE-PROTEIN KINASE INHIBITOR WITH ANTI-TUMOR ACTIVITY IN PRECLINICAL ASSAYS

Yun-Qing Qiu[1], Jue Zhou[2], Xin-Shan Kang[3], Jian-Zhong Shen-Tu[1], Lie-Ming Ding[3], Fen-Lai Tan[3], Jing Guo[1], Lan-Juan Li[1*]

[1]State Key Laboratory for Diagnosis and Treatment of Infectious Disease, the First Affiliated Hospital, School of Medicine, Zhejiang University, 310003. Hangzhou, China. [2]College of Food Science and Biotechnology, Zhejiang Gongshang University, 310012. Hangzhou, China. [3]Zhejiang Beta Pharma Co., Ltd. 311100. Hangzhou, China.

***Email:** ljli@zju.edu.cn

Abstract

Traditional medicines have become the most productive source of leads for drugs development, particularly as anti-cancer agents. Various screening approaches are being applied. Sorafenib, a multikinase inhibitor, is used to treat primary kidney cancer (advanced renal cell carcinoma) and advanced primary liver cancer. A small library of compounds analogous to sorafenib were designed and screened for the treatment of liver cancer. Multiple members of the family in an assay panel of tyrosine kinase family and serine/threonine-protein kinase family, including VEGFR, Abl, Aurora A, p 38, Lck, Src, PDGFR, Flt3, c-RAF, c-KIT, MEK(MAPKK) were selected to test these compounds. Analysis of the selectivity patterns for these compounds shows specificity for many kinase families. IC_{50} were measured for the selected compounds. Multiple compounds have very similar kinase inhibition profiles of VEGFR, Flt3, FGFR to that of sorafenib. The IC_{50} of c-RAF of BB1 is lower than sorafenib. The IC_{50} of c-RAF of BB3-12 is higher than that of sorafenib. For Flt3, IC_{50} of BB1-4 is less than sorafenib. The IC_{50} value of KDR of BB1-10 is less than sorafenib. especially against c-RAF, PDGFR, c-KIT, KDR compared to sorafenib. These compounds are potent Raf1 and Flt4 kinase inhibitors.

Key words: Tyrosine kinase inhibitors; Serine/threonine-protein kinase inhibitors; Sorafenib; Clinical hepatotoxicity.

Introduction

Traditional medicines have become the most productive source of leads for drugs development, particularly as anti-cancer agents. Combinatorial chemistry approaches are being based on the scaffolds of traditional and alternative medicines to create screening libraries that closely resemble drug-like compounds. Various screening approaches are being developed and data mining and virtual screening techniques are being applied. At the present time, sorafenib approved by the FDA in 2005, is a multikinase inhibitor, targeting several serine/threonine and receptor tyrosine kinases (RAF kinase, VEGFR-2,VEGFR-3, PDGFR-beta, KIT, and FLT-3)(Hu et al., 2011). Sorafenib, is used to treat primary kidney cancer (advanced renal cell carcinoma) and advanced primary liver cancer (hepatocellular carcinoma). At the onset of the medicinal chemistry programme that led to sorafenib, there were no marketed kinase inhibitors in the field of oncology. However, before the approval of sorafenib, two first-generation targeted anticancer agents —Gleevec (Novartis) and Tarceva (Genentech), which target Bcr–Abl and EGFR, respectively — had begun to show clinical benefit (Capdeville et al., 2002), (Dowell et al., 2005) In multiple clinical trials, sorafenib is as both combination and single-agent therapies.

Analysis of newly introduced drugs states that sorafenib has reached the market from an origin in

Publisher: African Networks on Ethnomedicines
Web page: /http://journals.sfu.ca/africanem/index.php/ajtcam/index
http://dx.doi.org/10.4314/ajtcam.v9i3.20

high-throughput screening of combinatorial chemistry libraries. Tyrosine kinase inhibitor was discontinued from clinical development due to unexpected hepatotoxicity in cancer patients (Feng et al., 2009). Some drug has clinical toxicity and exerted its hepatotoxicity via both hepatocellular injury and hepatobiliary cholestatic mechanisms. The direct cytotoxic effect, hepatobiliary disposition and inhibition of active canalicular transport of bile constituents have been evaluated in established human hepatocyte models and *in vitro* transporter systems. The major human hepatic uptake transporter, multidrug resistance protein 1 (MDR1) and breast cancer resistance protein, was involved in hepatobiliary clearance. Evidence suggests three major mechanisms of medicine used clinically for the treatment of cholestatic liver diseases: (1) protection of cholangiocytes against cytotoxicity of hydrophobic bile acids, resulting from modulation of the composition of mixed phospholipid-rich micelles, reduction of bile acid cytotoxicity of bile and, possibly, decrease of the concentration of hydrophobic bile acids in the cholangiocytes; (2) stimulation of hepatobiliary secretion, putatively via $Ca^{(2+)}$ and protein kinase C-alpha-dependent mechanisms and/or activation of *p38*(MAPK) and extracellular signal-regulated kinases (Erk) resulting in insertion of transporter molecules (e.g., bile salt export pump, BSEP, and conjugate export pump, MRP2) into the canalicular membrane of the hepatocyte and, possibly, activation of inserted carriers; (3) protection of hepatocytes against bile acid-induced apoptosis, involving inhibition of mitochondrial membrane permeability transition (MMPT), and possibly, stimulation of a survival pathway. But effects on disease progression have not been further evaluated at the molecular level (Paumgartner and Beuers, 2002).

Tyrosine kinase is an enzyme that can transfer a phosphate group from ATP to a protein in a cell (Stepanek et al., 2011). It functions as an "on" or "off" switch in many cellular functions. Receptor tyrosine kinases (RTK) are the high-affinity cell surface receptors for many polypeptide growth factors, cytokines, and hormones. Of the 90 unique tyrosine kinase genes identified in the human genome, 58 encode receptor tyrosine kinase proteins.(Robinson et al., 2000) This gene encodes a member of the Ser/Thr protein kinase family. This kinase is regulated through direct phosphorylation by *p38* MAP kinase. In conjunction with *p38* MAP kinase, this kinase is known to be involved in many cellular processes including stress and inflammatory responses. The ABL1 proto-oncogene encodes a cytoplasmic and nuclear protein tyrosine kinase. Serine/threonine-protein kinases select specific residues to phosphorylate on the basis of residues that flank the phosphoacceptor site, which together comprise the *consensus sequence*. Aurora A kinase known as serine/threonine-protein kinase 6 is an enzyme that in humans is encoded by the AURKA gene.

Methods

We have recently disclosed a novel series of compounds as inhibitors of tyrosine kinases serine/threonine-protein kinases based on the source of traditional medicines. A minimized binding mode of the ATP pocket of the ligand-binding Pocket model is shown in Figure 1. A limited set of small hydrophobic groups may be substituted on this phenyl ring at very specific positions since the pocket possesses a limited tolerance for larger groups (Figure 1). The sketch of the components of the library and synthesis scheme is shown in Figure 2.

Based on the scaffold from nature products, we synthesized 50 compounds with the R1, R2 resion sit for the pocket. A small library of compounds analogous to sorafenib were designed and screened against multiple members of the tyrosine kinase and serine/threonine-protein kinase. To determine the kinase profiler and IC_{50} profiler, the reference inhibitor control compounds have been introduced in the kinase profiler and IC_{50} profiler express operational procedure. The structure of the tested compounds is shown in Table 1. Some tyrosine kinase and serine/threonine-protein kinase were selected to test the kinase profiler and IC_{50} (Table 2).

Publisher: African Networks on Ethnomedicines
Web page: /http://journals.sfu.ca/africanem/index.php/ajtcam/index
http://dx.doi.org/10.4314/ajtcam.v9i3.20

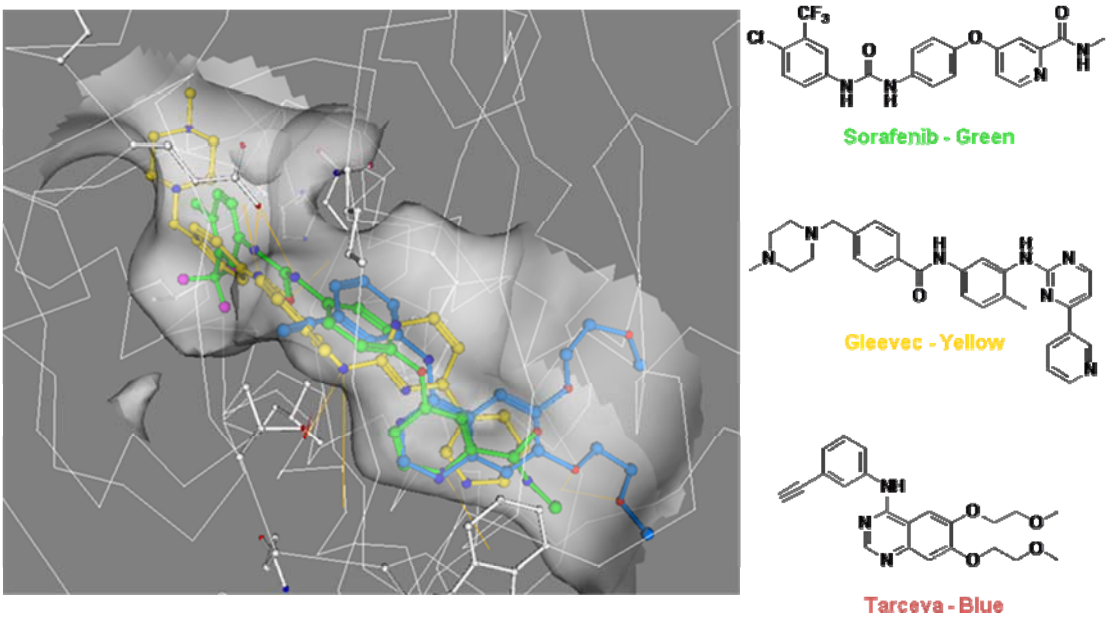


Figure 1: Ligand-binding Pocket model

Note: Green refers to sorafenib; Yellow refers to gleevec; Blue refers to tarceva.

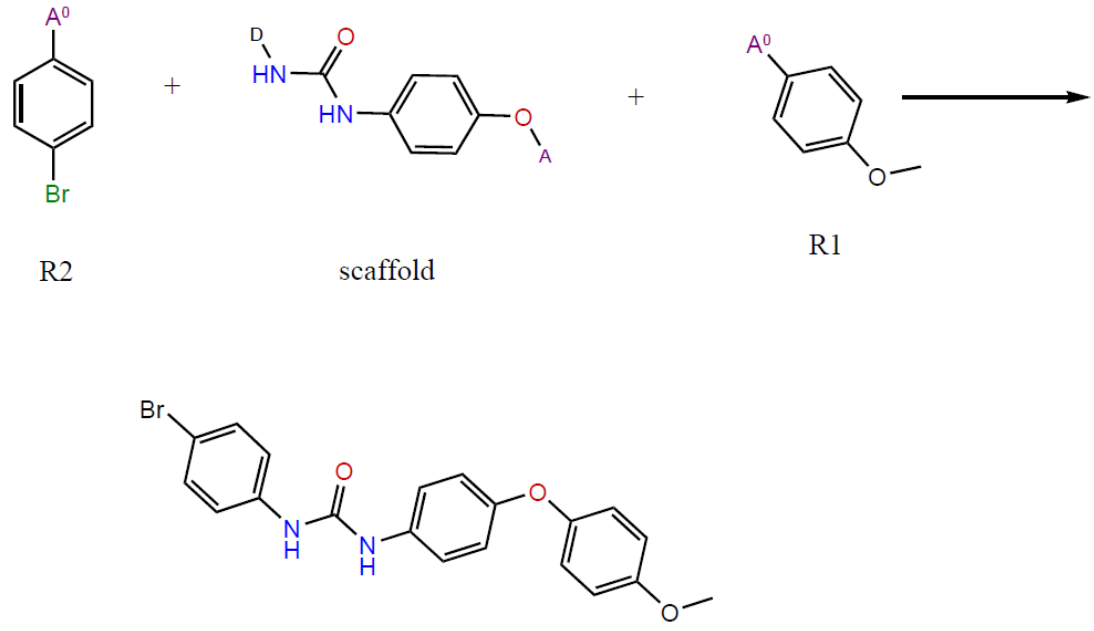
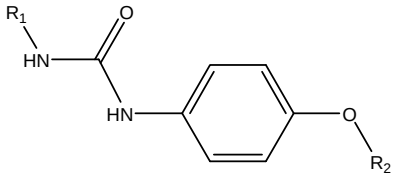


Figure 2: Sketch of the components of the library and the end products

Publisher: African Networks on Ethnomedicines
Web page: /http://journals.sfu.ca/africanem/index.php/ajtcam/index
http://dx.doi.org/10.4314/ajtcam.v9i3.20

Table 1: The structure of the tested compounds



Compounds	R_1	R_2
BB1	1-Br-4-MePh	1H-imidazole
BB2	1-Cl-4-MePh	Morpholin-4-amine
BB3	1-F-4-MePh	Morpholinomethyl 4-methylbenzenesul
BB4	5-methylisoxazole	2-morpholinoethyl 4-methylbenzenesulfonate
BB5	2-methylnaphthalene	4-methoxybenzenamine
BB6	p-xylene	4-chloropyridine
BB7	3-methylisoxazol-5-amine	3-chloropyridine
BB8	3-methylisoxazole	N,4-dimethylpicolinamide
BB9	toluene	2-Cl-5-nitropyridine
BB10	4-chloropyridine	N-(3-aminopyridin-2-yl)acetamide
BB11	1-methoxy-3-methylbenzene	Pyrimidine-4,5-diamine
BB12	1-fluoro-2-methyl-4-(trifluoromethyl)benzene	4,6-dichloropyrimidin-5-amine

Table 2: Selected kinases

Family	Kinase	Full name
Tyrosine kinase	c-Kit (CD117)	tyrosine-protein kinase Kit or
	Flt3 (CD 135)	fms-like tyrosine kinase receptor-3
	RTKs (VEGFR)	Receptor tyrosine kinases (vascular endothelial growth factor receptors)
	RTKs (FGFR1)	fibroblast growth factor receptor 1
	RTKs (EGFR)	epidermal growth factor receptor
	RTKs(PDGFRα, PDGFRβ)	Platelet-derived growth factor receptor
	Abl	V-abl Abelson murine leukemia viral oncogene homolog
	Lck	lymphocyte-specific protein tyrosine kinase
	Src	proto-oncogenic tyrosine kinase
	KDR	type III receptor tyrosine kinase (vascular endothelial growth factor receptor 2)

Publisher: African Networks on Ethnomedicines
Web page: /http://journals.sfu.ca/africanem/index.php/ajtcam/index
http://dx.doi.org/10.4314/ajtcam.v9i3.20

Serine/threonine-protein kinase	*p38* (SAPK2a)	mitogen-activated protein (MAP) kinase-activated protein kinase 2
	MEK(MAPKK)	MAP kinase cascade
	c-RAF	RAF proto-oncogene serine/threonine-protein kinase
	Aurora-A	serine/threonine-protein kinase 6
	CDK2/cyclinA	Cyclin-dependent kinase 2

Kinase Profiler and IC_{50} Profiler

A family of validated kinase targets was identified with specific structural features. The composition of the dilution buffer for each kinase is shown in Table 3. All kinases are pre-diluted to a 10x working concentration prior to addition into the assay. The Kinase Profiler of ATP concentration is shown in Table 4.

Table 3. Buffer composition of kinase dilution

Buffer composition	Kinase (s)
50 mM Tris pH 7.5, 0.1 mM EGTA, 0.1 mM Na3VO4,	c-Kit
0.1% β-mercaptoethanol,	c-RAF
1 mg/ml BSA	Flt3
50 mM Tris pH 7.5, 0.1 mM EGTA,	PDGFRα
0.1% β-mercaptoethanol, 1 mg/ml BSA	SAPK2a
50 mM Tris pH 7.5, 0.05% β-mercaptoethanol,	RTKs (VEGFR)
1 mg/ml BSA	EGFR
25 mM Tris pH 7.5, 0.1 mM EGTA,	FGFR1
0.1% β-mercaptoethanol, 1 mg/ml BSA	MEK1
20 mM MOPS pH 7.0, 1 mM EDTA,	Abl
0.1% β-mercaptoethanol, 0.01% Brij-35,	Aurora-A
5% glycerol, 1 mg/ml BSA	CDK2/cyclinA
20 mM HEPES pH 7.6, 0.15 M NaCl,	Lck
0.1 mM EGTA, 5 mM DTT, 0.1% Triton X-100,	*p38*
50% glycerol	MAPKAP-K3
180 mM HEPES pH 7.4, 3.6 mM DTT,	PDGFRα
0.07% Brij-35	PDGFRβ
40 mM HEPES pH 7.4, 1 mg/ml BSA	
20 mM HEPES pH 7.4, 0.03% Triton X-100	
50 mM Na β-glycerophosphate pH 7.0, 0.1% β-mercaptoethanol, 0.1 mM EGTA, 1 mg/ml BSA	

Publisher: African Networks on Ethnomedicines
Web page: /http://journals.sfu.ca/africanem/index.php/ajtcam/index
http://dx.doi.org/10.4314/ajtcam.v9i3.20

Table 4: Kinase Profiler of ATP concentration

Kinase	Km Assay Conc
Abl	45μM
Abl(T315I)	10μM
Aurora-A	15μM
c-Kit	200μM
c-RAF	45μM
CDK2/cyclinA	45μM
EGFR	10μM

IC_{50} was measured for the selected compounds with the best activities below 50 nM in Upstate BioPredict Inc. Each protein kinase has been paired with an ATP-competitive inhibitor that is included on every assay plate as a reference blank.

Abl

In a final reaction volume of 25 μl, Abl (m) (5-10 mU) is incubated with 8 mM MOPS pH 7.0, 0.2 mM EDTA, 50 μM EAIYAAPFAKKK, 10 mM MgAcetate and [γ-33P-ATP] (specific activity approx. 500 cpm/pmol, concentration as required).The reaction is initiated by the addition of the MgATP mix. After incubation for 40 minutes at room temperature, the reaction is stopped by the addition of 5 μl of a 3% phosphoric acid solution. 10 μl of the reaction is then spotted onto a P30 filtermat and washed three times for 5 minutes in 75 mM phosphoric acid and once in meth\anol prior to drying and scintillation counting.

c-KIT

In a final reaction volume of 25 μl, c-KIT (5-10 mU) is incubated with 8 mM MOPS pH 7.0, 0.2 mM EDTA, 10 mM MnCl2, 0.1 mg/ml poly(Glu, Tyr) 4:1, 10 mM MgAcetate and [γ-33P-ATP] (specific activity approx. 500 cpm/pmol, concentration as required). The reaction is initiated by the addition of the MgATP mix. After incubation for 40 minutes at room temperature, the reaction is stopped by the addition of 5 μl of a 3% phosphoric acid solution. 10 μl of the reaction is then spotted onto a Filtermat A and washed three times for 5 minutes in 75 mM phosphoric acid and once in methanol prior to drying and scintillation counting.

Lck

In a final reaction volume of 25 μl, Lck (5-10 mU) is incubated with 50 mM Tris pH 7.5, 0.1 mM EGTA, 0.1 mM Na3VO4, 250 μM KVEKIGEGTYGVVYK (Cdc2 peptide), 10 mM MgAcetate and [γ-33P-ATP] (specific activity approx. 500 cpm/pmol, concentration as required). The reaction is initiated by the addition of the MgATP mix. After incubation for 40 minutes at room temperature, the reaction is stopped by the addition of 5 μl of a 3% phosphoric acid solution. 10 μl of the reaction is then spotted onto a P30 filtermat and washed three times for 5 minutes in 75 mM phosphoric acid and once in methanol prior to drying and scintillation counting.

MEK1

In a final reaction volume of 25 μl, MEK1 (1-5 mU) is incubated with 50 mM Tris pH 7.5, 0.2 mM EGTA, 0.1% β-mercaptoethanol, 0.01% Brij-35, 1 μM inactive MAPK2 (m), 10 mM MgAcetate and cold ATP (concentration as required). The reaction is initiated by the addition of the MgATP. After incubation for 40 minutes at room temperature, 5 μl of this incubation mix is used to initiate a MAPK2 (m) assay, which is described on page 12 of this book.

PDGFR

In a final reaction volume of 25 μl, PDGFRα (5-10 mU) is incubated with 8 mM MOPS pH 7.0, 0.2 mM EDTA, 0.1 mg/ml poly (Glu, Tyr) 4:1, 10 mM MnCl2, 10 mM MgAcetate and [γ-33P-ATP] (specific activity approx. 500 cpm/pmol,

Publisher: African Networks on Ethnomedicines
Web page: /http://journals.sfu.ca/africanem/index.php/ajtcam/index
http://dx.doi.org/10.4314/ajtcam.v9i3.20

concentration as required). The reaction is initiated by the addition of the MgATP mix. After incubation for 40 minutes at room temperature, the reaction is stopped by the addition of 5 µl of a 3% phosphoric acid solution. 10 µl of the reaction is then spotted onto a filtermat A and washed three times for 5 minutes in 75 mM phosphoric acid and once in methanol prior to drying and scintillation counting.

SAPK2a

In a final reaction volume of 25 µl, SAPK2a (5-10 mU) is incubated with 25 mM Tris pH 7.5, 0.02 mM EGTA, 0.33 mg/ml myelin basic protein, 10 mM MgAcetate and [γ-33P-ATP] (specific activity approx. 500 cpm/pmol, concentration as required). The reaction is initiated by the addition of the MgATP mix. After incubation for 40 minutes at room temperature, the reaction is stopped by the addition of 5 µl of a 3% phosphoric acid solution. 10 µl of the reaction is then spotted onto a P30 filtermat and washed three times for 5 minutes in 75 mM phosphoric acid and once in methanol prior to drying and scintillation counting.

Results

A small library of compounds analogous to sorafenib were designed and screened against multiple members of the family at Millipore-Upstate BioPredict Inc in an assay panel. Analysis of the selectivity patterns for these compounds shows specificity for many kinase families. IC_{50} were measured for the selected compounds. The potentially comprise an alternative group of compounds that are highly interesting, as for Flt3 and KDR, IC_{50} of BB1, BB2, BB3, BB4 is less than sorafenib. To assess the potential of these compounds to inhibit kinases, we screened the compounds at Upstate BioPredict Inc. The tested compounds were also shown to potently inhibit the wild-type kinases including KDR, FGFR1 and other RTKs involved in tumorigenesis (c-Kit, Flt-3 and RET) *in vitro*. Figure 2 shows the comparison between the IC_{50} values determined by functional assays for different kinases. In these kinases, the IC_{50} values of VEGFR, Flt3, FGFR is similar to sorafenib. Some compounds show difference, especially against c-RAF, PDGFR, c-KIT, KDR compared to sorafenib. The IC_{50} of c-RAF of BB1(2µM) is lower than that of sorafenib (6µM). The IC_{50} of c-RAF of BB3(85µM),BB4(91µM), BB5(97µM), BB6(105µM), BB7(91µM), BB8(101µM), BB9(50µM), BB10(79µM), BB11(85µM) and BB12(94µM) is higher than that of sorafenib(6µM). The IC_{50} value of Flt3 of BB1 (1µM), BB2(4µM), BB3(3µM) and BB4(5µM) is less than sorafenib (58µM). The IC_{50} value of KDR of BB1(4µM), BB2(4µM),BB3(4µM), BB4(3µM), BB5(25µM), BB6(8µM), BB7(22µM), BB8(11µM), BB9(69µM) and BB10(7µM) is less than sorafenib (90µM). These results of the multiple compounds have very similar kinase inhibition profile to that of sorafenib. *In vitro* assays confirmed that these compounds (compound BB71A and compound 28) are potent *in vitro* inhibitor of Raf1 kinase and Flt4 (IC_{50} of 2 nM and 1 nM) (Figure 2). However, similar to sorafenib, these compounds had no significant inhibitory effect on PKCγ, PKA, PKCα, MEK1, Met, IGF-1R.

Discussion

Some anti-tumor agent contained in traditional Chinese medicine, has been extensively used, such as quercetin, baicalein, baicalin, salvianolic acid B and emodin. In the present study, we investigated the selectivity patterns for the compounds which showed specificity for tyrosine kinase and serine/threonine-protein kinase families. IC_{50} were measured for the selected compounds (Gao et al., 2011). Clinical cases reported that fatal acute liver failure occurred when paracetamol (acetaminophen) was co-administrated with some tyrosine kinase inhibitors. The direct inhibition of UDP-glucuronosyltransferase activities has been identified as a mechanism of potentiation of paracetamol hepatotoxicity (Liu et al., 2011). Some tyrosine kinase inhibitors have increased propensity to cause hepatotoxicity, which may be fatal in

Publisher: African Networks on Ethnomedicines
Web page: /http://journals.sfu.ca/africanem/index.php/ajtcam/index
http://dx.doi.org/10.4314/ajtcam.v9i3.20

	Sorafenib IC50s	BB1 @ 0.5 µM	BB2 @ 0.5 µM	BB3 @ 0.5 µM	BB4 @ 0.5 µM	BB5 @ 0.5 µM	BB6 @ 0.5 µM	BB7 @ 0.5 µM	BB8 @ 0.5 µM	BB9 @ 0.5 µM	BB10 @ 0.5 µM	BB11 @ 0.5 µM	BB12 @ 0.5 µM
c-RAF(h)	6	2	11	86	91	97	105	91	101	50	79	85	94
Flt4(h) - VEGFR3	20	2	1	6	4	34	22	23	12	39	11	96	80
Flt3(h)	58	1	4	3	5	61	92	53	88	38	78	104	110
PDGFRβ(h)	57	90	68	97	73	110	72	105	72	110	89	118	115
cKit(h)	68	84	73	58	9	68	10	70	13	90	23	100	46
KDR(h)	90	4	4	4	3	25	8	22	11	69	7	105	101
FGFR1(h)	580	9	98	70	89	108	112	108	105	105	108	108	112
Flt1(h) - VEGFR1		10	13	8	9	14	15	13	13	19	15	49	63
SAPK2a(h)		28	69	106	113	111	115	110	120	107	115	112	114
TrkA(h)		32	75	89	85	95	108	91	93	97	101	101	105
PDGFRα(h)		43	32	32	12	53	17	45	14	54	26	80	55
Lck(h)		56	95	93	93	92	94	95	100	97	103	92	100
EphB4(h)		57	106	109	61	113	101	113	101	111	90	119	107
Abl(h)		66	109	114	116	111	101	111	100	114	114	112	115
FGFR3(h)		82	118	116	116	98	96	103	101	111	109	120	109
Aurora-A(h)		83	87	102	107	103	104	101	99	101	107	111	102
PKCγ(h)	10000	93	93	96	100	105	106	105	101	106	100	116	95
PKA(h)	10000	102	106	70	71	71	109	69	72	68	69	72	68
PKCα(h)	10000	103	69	108	106	103	106	105	105	108	107	108	108
MEK1(h)	10000	104	112	113	114	103	114	112	115	107	114	107	112
Met(h)	10000	105	94	110	116	103	112	107	108	108	112	112	109
IGF-1R(h)	10000	107	112	107	112	111	114	106	111	106	108	111	107
CDK2/cyclinA(h)		108	110	113	115	99	110	109	104	105	110	111	112
PKBα(h)	10000	110	110	111	107	106	116	110	106	109	115	107	114
CDK1/cyclinB(h)	10000	113	120	120	118	118	115	107	110	119	118	117	121
cSRC(h)		114	121	113	111	103	116	106	103	106	111	102	119
MLK1(h)		122	114	117	129	112	111	121	118	115	105	129	127
Pim-1(h)	10000	124	127	128	124	104	114	109	119	122	118	117	121
EGFR(h)	10000	125	119	100	104	100	109	109	116	117	124	117	110
JAK3(h)		125	121	137	136	118	125	123	127	121	128	103	116

Figure 3: Kinase analysis profile of some compounds

Note: Each cell represents a compound (the structure of compound is shown in Table 1), with numbers as the residual activities of each kinase in the presence of 5 µM individual compound. Green color means great inhibition; Black color means 50% inhibition; Red color means worse inhibition.

rare cases (Keisner and Shah, 2011). The Raf serine/threonine kinase isoforms (A-Raf, B-Raf and Raf) are the first kinases in the MAPK cascade and are pivotal regulators of cellular proliferation and survival (Pruitt et al., 2002). McDonald et al. (McDonald et al., 1999) had developed an approach for the high-throughput screening (HTS) and identification of selective Raf/MEK/ERK enzyme inhibitors using tumour cell lines that contained oncogenic *k-ras* and/or *b-raf* mutations demonstrated upregulated signalling through the Raf–MEK–ERK pathway. We designed and analysed multiple compounds that have very similar kinase inhibition profiles of VEGFR, Flt3, FGFR to that of sorafenib. Some compounds, especially against c-RAF, PDGFR, c-KIT, KDR compared to sorafenib. The IC_{50} of c-RAF of BB1(2µM) is lower than that of sorafenib (6µM). The IC_{50} of c-RAF of BB3(85µM),BB4(91µM), BB5(97µM), BB6(105µM), BB7(91µM), BB8(101µM), BB9(50µM), BB10(79µM), BB11(85µM) and BB12(94µM) is higher than that of sorafenib(6µM). The IC_{50} value of Flt3 of BB1 (1µM), BB2(4µM), BB3(3µM) and BB4(5µM) is less than sorafenib (58µM). The IC_{50} value of KDR of BB1(4µM), BB2(4µM),BB3(4µM), BB4(3µM), BB5(25µM), BB6(8µM), BB7(22µM), BB8(11µM), BB9(69µM) and BB10(7µM) is less than sorafenib (90µM).

Acknowledgement

This study was supported by an International Cooperation Grant from Science Technology Department of Zhejiang Province (Agreement Number: 2009C14030)

References

Publisher: African Networks on Ethnomedicines
Web page: /http://journals.sfu.ca/africanem/index.php/ajtcam/index
http://dx.doi.org/10.4314/ajtcam.v9i3.20
Capdeville, R., E. Buchdunger, J. Zimmermann, and A. Matter. (2002). Glivec (ST1571, Imatinib), a rationally developed, targeted anticancer drug. Nature Reviews Drug Discovery **1:**493-502.

Dowell, J., J.D. Minna, and P. Kirkpatrick. (2005). Erlotinib hydrochloride. Nature Reviews Drug Discovery **4:**13-14.

Feng, B., J.J. Xu, Y.A. Bi, R. Mireles, R. Davidson, D.B. Duignan, S. Campbell, V.E. Kostrubsky, M.C. Dunn, A.R. Smith, and H.F. Wang. (2009). Role of hepatic transporters in the disposition and hepatotoxicity of a HER2 tyrosine kinase inhibitor CP-724,714. Toxicol Sci **108:**492-500.

Gao, N., S. Cheng, A. Budhraja, Z. Gao, J. Chen, E.H. Liu, C. Huang, D. Chen, Z. Yang, Q. Liu, P. Li, Z. Zhang, and X. Shi. (2011). Ursolic acid induces apoptosis in human leukemia cells and exhibits antileukemic activity in nude mice through Akt pathway. Br J Pharmacol

Hu, S., H. Niu, H. Inaba, S. Orwick, C. Rose, J.C. Panetta, S. Yang, S. Pounds, Y. Fan, C. Calabrese, J.E. Rehg, D. Campana, J.E. Rubnitz, and S.D. Baker. (2011). Activity of the Multikinase Inhibitor Sorafenib in Combination With Cytarabine in Acute Myeloid Leukemia. J Natl Cancer Inst

Keisner, S.V., and S.R. Shah. (2011). Pazopanib: the newest tyrosine kinase inhibitor for the treatment of advanced or metastatic renal cell carcinoma. Drugs **71:**443-454.

Liu, Y., J. Ramirez, and M.J. Ratain. (2011). Inhibition of paracetamol glucuronidation by tyrosine kinase inhibitors. Br J Clin Pharmacol **71:**917-920.

McDonald, O.B., W.J. Chen, B. Ellis, C. Hoffman, L. Overton, M. Rink, A. Smith, C.J. Marshall, and E.R. Wood. (1999). A scintillation proximity-assay for the Raf/MEK/ERK Kinase cascade: High-throughput screening and identification of selective enzyme inhibitors. Analytical Biochemistry **268:**318-329.

Paumgartner, G., and U. Beuers. (2002). Ursodeoxycholic acid in cholestatic liver disease: mechanisms of action and therapeutic use revisited. Hepatology **36:**525-531.

Pruitt, K., W.M. Pruitt, G.K. Bilter, J.K. Westwick, and C.J. Der. (2002). Raf-independent deregulation of p38 and JNK mitogen-activated protein kinases are critical for Ras transformation. Journal of Biological Chemistry **277:**31808-31817.

Robinson, D.R., Y.M. Wu, and S.F. Lin. (2000). The protein tyrosine kinase family of the human genome. Oncogene **19:**5548-5557.

Stepanek, O., T. Kalina, P. Draber, T. Skopcova, K. Svojgr, P. Angelisova, V. Horejsi, A. Weiss, and T. Brdicka. (2011). Regulation of SRC-family kinases involved in T-cell receptor signaling by protein tyrosine phosphatase CD148. J Biol Chem