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Synthesis of selective SRPK-1 inhibitors: Novel tricyclic quinoxaline derivatives

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Abstract—SR protein-specific kinase-1 (SRPK-1) has been identified as a validated target for hepatitis B virus (HBV). A series of novel tricyclic quinoxaline derivatives was designed and synthesised as potential kinase inhibitory antiviral agents and was found to be active and selective for SRPK-1 kinase. Most of these novel compounds have drug-like properties according to experimentally determined Log*P* and Log*S* values.

1. Introduction

SR protein kinases (SRPKs) and their substrates, the SR family of serine/arginine-rich pre-mRNA splicing factors, appear to be key regulators of alternative splicing. Their splicing activity is regulated by reversible serine phosphorylation of their carboxyl-terminal serine/threonine motifs by a number of interesting kinases, including the serine/threonine protein kinases SRPK-1 and SRPK-2, and the scleroderma autoantigen topoisomerase I. Both SRPK-1 and SRPK-2 are most likely the cellular protein kinases mediating hepatitis B virus (HBV) core protein phosphorylation during viral infection and therefore represent important host cell targets for therapeutic intervention in HBV infection.[1]

The quinoxaline scaffold can be found in a number of biologically active compounds especially antibacterial,[2] anti-inflammatory[3] and antitumour[4–6] drug candidates. Their kinase inhibitory activity might be responsible for their therapeutic effects. Gazit et al. prepared several quinoxaline derivatives, which act as inhibitors of platelet derived growth factor receptor kinase (PDGF). The most active compound was a tricyclic benzo[*g*]quinoxaline (**1**, IC_{50} = 0.9 μM);[7] therefore we synthesised a series of its derivatives. The compounds were tested on different targets and it was found that 2,3-di-thiophen-2′-yl-benzo[*g*]quinoxaline **2** had significant and selective inhibitory activity against SRPK-1 kinase (Fig. 1).

Even though compound **2** was very active in the enzyme assay, it suffered from high lipophilicity and poor water solubility. These properties make it difficult to develop this compound into a pharmaceutical agent. Therefore, we modified the structure with hydrophilic moieties[8] and tested the resulting compounds on SRPK-1 kinase assay (Table 3) and on a selectivity panel of 19 kinase assays (Table 4).

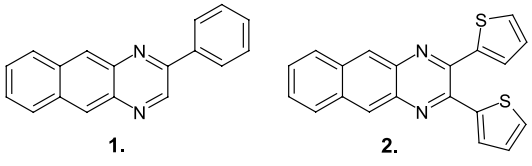

Figure 1. Tricyclic benzo[*g*]quinoxaline leads.

Keywords: SRPK-1; Quinoxaline; Kinase inhibitor.
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2. Physicochemical properties

About one-half of the potential drug candidates fail because of poor ADME properties, which may be partially related to the Log *P* and water solubility (*S*, [mol/l]) values of the compound. According to generally accepted criteria of drug-likeliness, the Log *P* value of a drug candidate must be below 5. The concentration range where the compounds are active requires that Log *S* be at least 6. We have experimentally determined the Log *S* and Log *P* values of the compounds by a dedicated HPLC method.[9] We found that all modified derivatives of **2** had lower lipophilicity than the parent compound. The Log *P* value of **2** is 5.98, much higher than those of compounds **6a**–**c**, **15** and **16**, which had Log *P* values of <4.5. Replacement of the first aromatic ring with alkylene-dioxo derivatives (**17**–**20**) should also improve the physicochemical properties of this class of compounds. Fifteen out of eighteen newly synthesised compounds had higher water solubility than Log *S* = −2 and ten compounds had lower lipophilicity than Log *P* = 5. Vichem Chemie Research Ltd has developed an automatic QS(P)AR program called 3DNET4W.[10–12] By using 3DNET4W software we have also calculated the Log *P* values of selected quinoxalines and benzo[*g*]quinoxalines to compare the experimental and predicted values. We found that our software had good Log *P* predicting capability (Table 1).[13,14]

3. Chemistry

Since the carboxyl function is very suitable for enhancing the water solubility of the molecules, they were inserted into parent compound **2**, obtaining **6a**–**d**, **7** and **13**. A general synthetic route for the preparation of benzo[*g*]quinoxalines disubstituted with thenil-alkyl-carboxylic acids was developed as outlined in Scheme 1 (Table 2). The symmetric key intermediate compounds **4a**–**d** were obtained via commercially available 2- or 3-thiophene-alkyl-carboxylic acids **3a**–**d** which were reacted with thionyl chloride and esterified with methanol, then acylated with oxalyl chloride in the presence of aluminium chloride in Friedel–Crafts reaction. These disubstituted thenil-esters **4a**–**d** were reacted with naphthalene-2,3-diamine in ethanol, yielding **5a**–**d**. Esters were hydrolysed with aq NaOH–THF, producing the appropriate alkyl-carboxylic acids **6a**–**d** (Scheme 1). The dicarboxylic acid derivative without alkyl linker **7** was prepared from lithiated **2** and carbon dioxide (Scheme 2).

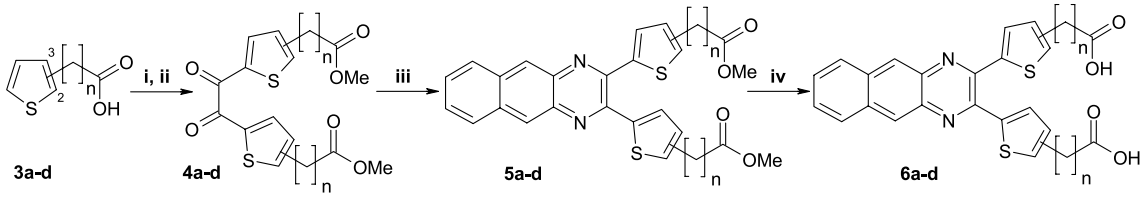
Carboxyl group was also inserted onto the position 7 of **2**, producing 2,3-di-thiophen-2′-yl-benzo[*g*]quinoxaline-7-carboxylic acid **13**. This synthesis started from commercially available 2,3-dimethoxy-naphthalene **8** as shown in Scheme 3. Its Friedel–Crafts acylation with acetic anhydride resulted in **9**, which was then converted to 6,7-dimethoxy-naphthalene-2-carboxylic acid **10** with aq NaOCl at 65 °C. The cleavage of methoxy groups with 48% aq HI afforded **11**, which was aminated in cc NH_4OH on 120 bar (**12**). This compound was condensed with 2,2′-thenil in EtOH, yielding the desired compound **13**.

We modified the first aromatic ring of **2** with alkylene-dioxo derivatives (**17**–**20**) and substituted the middle phenyl ring with pyrazine, obtaining **23**. Compounds **17**–**20** and **23** were prepared from 4,5-dimethoxy-benzene-1,2-diamine **14** (Scheme 4). Condensation of **14** with 2,2′-thenil in ethanol followed by methoxy cleavage with HBr in glacial acetic acid at 100 °C provided 2,3-di-thiophen-2-yl-quinoxaline-6,7-diol **16**. Compounds **17**–**19** were afforded by cyclization of **16** with corresponding alkyl-dihalogenides. The carboxyl-derivative **20** was obtained by treatment of **16** with

Table 1. Physicochemical properties of selected quinoxaline derivatives

Compound	Log *P*	*C* Log *P*[a]	Log *S*	Compound	Log *P*	*C* Log *P*[a]	Log *S*
2	5.98	6.33	−3.05	**7**	5.03	4.78	−0.11
5a	4.95	5.02	−1.02	**13**	5.40	5.64	−0.60
5b	5.00	5.04	−1.07	**15**	4.00	4.92	−1.08
5c	5.00	5.15	−2.29	**16**	4.20	4.14	−1.10
5d	5.50	5.90	−2.12	**17**	4.80	5.06	−1.98
6a	3.30	4.00	−0.13	**18**	5.00	4.94	−1.08
6b	4.11	3.99	−0.24	**19**	5.00	4.98	−1.19
6c	4.28	4.16	−0.19	**20**	4.70	4.01	−0.07
6d	4.77	4.89	−0.10	**23**	4.79	5.08	−0.78

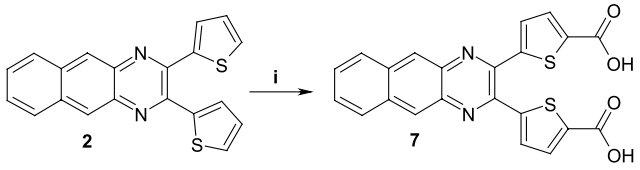
[a] 3DNET4W software; *C* Log *P* = calculated Log *P*.



Scheme 1. Reagents and conditions: (i) thionyl chloride/MeOH, rt, 3 h; (ii) oxalyl chloride/$AlCl_3$, abs DCM, rt, 3 h; (iii) naphthalene-2,3-diamine, EtOH, 79 °C, 2 h; (iv) aq NaOH/THF, rt, 3 h, then 1 N HCl.

Table 2. Side-chain modification on thiophene rings

Compounds	Position of modification	*n*
3–6 a	2	1
3–6 b	3	1
3–6 c	2	2
3–6 d	2	3

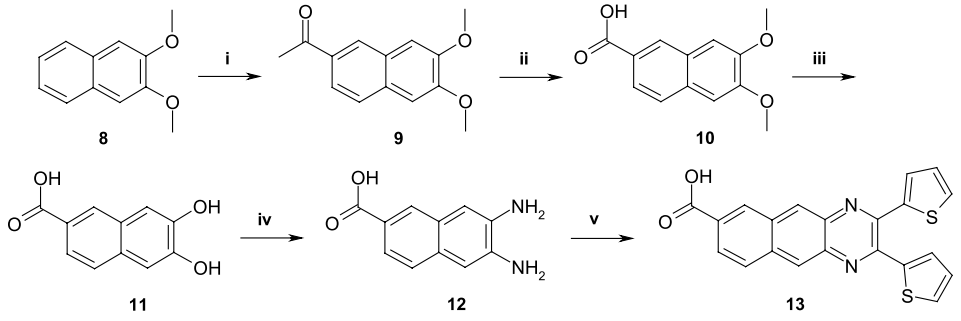


Scheme 2. Reagents and conditions: (i) LDA/THF, −78 °C, 2 h, then CO_2, rt, 3 h.

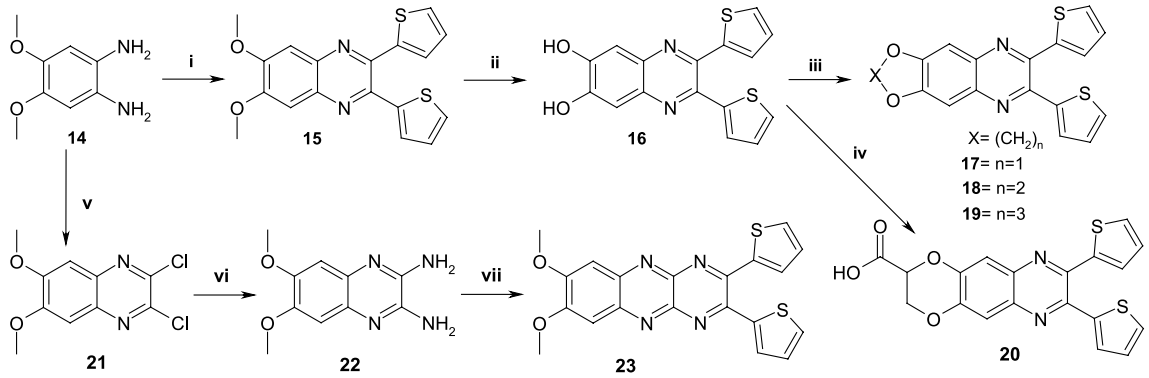
2,3-dibromo-propionic acid ethyl ester, and triethylamine in EtOH, followed by removing the ester function with aq NaOH. Compound **23** was also prepared in three steps from **14**. The first one was cyclization of **14** with oxalic acid, and then quinoxaline-2,3-dione was converted to dichloro-quinoxaline **21** with phosphorus oxychloride. Compound **21** was substituted with ammonia to give diamino-quinoxaline **22**, which was reacted with 2,2′-thenil in ethanol to produce the desired diaza derivative **23**.

4. Biological results

The compounds were tested on SRPK-1 assay and 10 compounds were found to inhibit SRPK-1 activity to lower than 10 μM IC_{50} (Table 3).[15] The most active compound on the SRPK-1 kinase assay was the compound **13** (IC_{50} = 0.04 μM). The dose–activity curve of **13** is presented in Figure 2. For statistical studies, we calculated the Hill parameter and linear regression of the dose–activity curve. We found good correlation between observed and predicted values of doses and activity (r^2 = 0.9734), and the shape of the dose–activity curve was ideal as well (Hill = 0.62). Inhibition specificity of selected quinoxalines and benzo[*g*]quinoxalines was investigated on a selectivity panel of 19 kinases (Table 4).[15] All compounds were measured at 10 μM concentration in triplicate. It seems squarely that carboxylic acid derivatives **6a–d**, **13**, **20** and the diaza derivative **23** exhibited high inhibitory activity against SRPK-1 kinase. Alkyl-carboxylic acid derivatives **6a–d** showed higher inhibition of SRPK-1 kinase than ester analogues **5b–d**. The compounds **6a** and **b**, which have an acetic acid group at 2 or 3 position on the thiophene moiety, exhibited medium inhibitory activity towards EGFR tyrosine kinase and MAPK-ERK1 kinase even at 10 μM concentration. When carboxyl group was inserted into the position 7 of **2** (compound **13**), we found that besides the excellent SRPK-1 inhibitory activity, c-Src inhibitory activity was detected. Replacement of the first phenyl ring with alkylene-dioxo derivatives (**17–19**)



Scheme 3. Reagents and conditions: (i) Ac_2O, $AlCl_3$/nitrobenzene, 0 °C, 2 h; (ii) aq NaOCl, 65 °C, 2 h; (iii) 48% HI, reflux, 5 h; (iv) cc NH_4OH, 120 atm, 180 °C, 48 h; (v) 2,2′-thenil/EtOH, reflux, 2 h.



Scheme 4. Reagents and conditions: (i) 2,2′-thenil/EtOH, reflux, 2 h; (ii) HBr/glacial acetic acid, 100 °C, 3 h; (iii) Br–X–Br/10% aq NaOH/dichloromethane, 20–100 °C; (iv) (1) 2,3-dibromo-propionic acid ethyl ester, TEA/EtOH, rt, 3 h, then (2) aq NaOH; (v) (1) oxalic acid/HCl/water, reflux, 2 h, (2) $POCl_3$/reflux, 2 h; (vi) cc NH_4OH, 120 atm, 180 °C, 48 h; (vii) 2,2′-thenil/EtOH, reflux, 2 h.

Table 3. Inhibitory activity of selected quinoxalines on SRPK-1 kinase assay

Compound	SRPK-1 IC_{50}, μM[a]
2	1.36 (±0.05)
5b	11.75 (±0.09)
5c	6.13 (±1.67)
5d	na
6a	1.28 (±0.05)
6b	0.64 (±0.03)
6c	0.87 (±0.01)
6d	0.82 (±0.10)
7	10 (±0.03)
13	0.04 (±0.02)
15	16.31 (±0.25)
16	9.61 (±0.28)
17	28.49 (±0.72)
18	23.36 (±0.80)
19	na
20	0.95 (±0.04)
23	1.14 (±0.01)

[a] Values are means of three experiments; standard deviation is given in parentheses (na = not active).

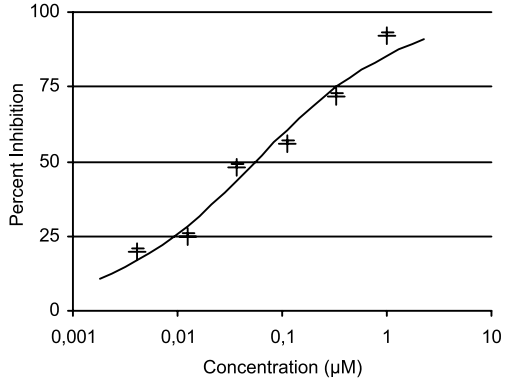


Figure 2. Dose–activity curve of compound **13** on SRPK-1 kinase assay.

decreased SRPK-1 activity and resulted in high c-Src inhibitory potency. Compounds **17** and **18** exhibited medium activity against PDGFR-beta tyrosine kinase. The dimethoxy-quinoxaline **15**, which is the open form of **18,** did not show inhibitory activity against any kinase. Very interestingly, the addition of a carboxyl group at the position 2 of **18** (compound **20**) resulted in a drastic change of activity. Compound **20** did not show the inhibition of c-Src kinase, but specific and high inhibitory activity was observed in the case of SRPK-1 kinase. The diaza derivative **23** did not have significantly modified activity, but it showed higher selectivity and lower lipophilicity than compound **2**.

According to the selectivity data, the most potent and selective compounds against SRPK-1 kinase were the carboxyl derivatives **6a–d**, **20** and the diaza derivative **23**. These compounds had higher selectivity than the parent compound **2** and besides the kinase inhibitory activity, they had better physicochemical properties as well. The most potent compound on SRPK-1 kinase assay was the compound **13** (IC_{50} = 0.04 μM) but it showed high activity on c-Src kinase assay as well.

5. Conclusion

In summary, we have synthesised a series of novel tricyclic quinoxaline analogues as SRPK-1 inhibitors with advantageous physicochemical properties. We found that carboxylic acid derivatives **6a–d, 20** and the diaza compound **23** had high and more selective inhibitory activity against SRPK-1 kinase than the parent compound **2**. The most active compound against SRPK-1 was **13** but it showed lower selectivity. Correlation was found between the structure and the SRPK-1 inhibitory activity. According to the structure–activity relationships, we found that position of carboxyl group and replacement of the first phenyl ring of **2** to alkylene-dioxo derivatives can cause a drastic change

Table 4. Inhibitory activity of selected quinoxalines in a selectivity panel of 19 kinase assays (%)

Compounds[a]	**2**	**5b**	**5c**	**5d**	**6a**	**6b**	**6c**	**6d**	**7**	**13**	**15**	**16**	**17**	**18**	**19**	**20**	**23**
c-Src	20	8	19	−12	14	29	70	35	20	**83** (±3)	10	−3	**71** (±3)	**84** (±1)	**75** (±6)	19	9
AKT1/PKBa	12	1	14	−6	15	17	0	−13	12	−10	−6	−7	−6	−12	−13	4	−4
Abl	4	−10	−8	15	10	−7	3	−11	−13	11	−2	1	14	11	10	−6	−1
CDK2/CycA(h)	3	−4	12	25	8	−5	−4	7	3	−11	−5	7	−4	1	−7	10	27
CK1-alpha	7	3	14	30	−2	18	40	24	7	10	−1	9	9	8	−5	15	2
EGFR	6	11	32	8	52	54	40	32	49	40	10	25	35	34	26	39	20
GSK-3beta	6	2	7	24	47	22	23	13	51	4	−13	5	−1	−15	−12	33	4
IKK-beta	6	14	31	16	25	37	54	43	29	42	10	3	28	38	22	14	10
InsR	55	−16	−12	−11	5	−14	−5	−11	−19	42	−7	−4	29	48	34	−12	−7
Jnk1a1	6	−10	−10	9	−3	−23	−5	−12	−25	20	−13	−3	18	24	15	−15	−15
Kit	8	1	12	5	15	17	52	33	12	56	−6	−7	38	46	22	4	−4
MAPK-ERK1	11	11	13	8	52	54	11	6	49	18	10	25	14	31	21	39	20
PDGFR-beta	15	9	13	47	10	22	49	37	23	68	7	−11	54	61	24	5	5
ROCK2	19	4	5	5	5	18	18	0	19	15	2	−18	31	41	32	−1	−1
RSK1	6	13	31	38	23	27	30	20	31	3	15	6	1	19	17	14	48
c-Raf	16	17	18	42	39	40	18	12	16	6	11	3	21	4	−5	12	8
cMet	43	−2	39	12	27	21	32	7	−4	49	−3	0	34	42	27	−2	12
P56Lck	−19	4	5	2	−9	8	−3	9	−19	47	9	1	41	53	39	−2	−7
P70S6K	8	−5	11	−7	17	10	29	18	14	5	−9	−8	6	−11	2	−1	−2

[a] Values were determined at 10 μM concentration in triplicate; standard deviation is given in parentheses.

in activity. These new compounds can be starting points for the development of novel antiviral agents.

Acknowledgments

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13. Determination of solubility: Compounds were submitted for the solubility assay as 10 mM solutions in DMSO in a mother (96-well NUNC plate) plate. From this plate two daughter plates were derived: a STANDARD plate containing compounds in DMSO solution and a sample plate of the compounds in PBS (Fluka Phosphate-Buffered Saline; pH 7.4). Five microlitres of each sample with 95 μL PBS was dispensed in the SAMPLE plate (96-well NUNC plate). After 1 h of equilibration time, the SAMPLE plate was filtered onto the FILTER plate (Millipore hydrophilic PTFE filter plate) by centrifugation (10 min at 1500 rpm). And then 10 μL of each well of STANDARD and FILTER plates (including the blanks) was injected into the HPLC device. The same procedure was carried out with our internal standards using compounds of the chromatography hydrophobicity index test mixture according to Valkó et al. (1997). Stock solutions of internal standards such as benzimidazole, 5-phenyl-1*H*-tetrazole, theophyllin, 8-phenylteophylin, indole, acetofenone, propiofenone, butirofenone, valerofenone (all from Sigma) were prepared with DMSO (5 mg/ml solution) and 5 μL of each standard was mixed in 95 μL PBS and then subjected to chromatography measurements. Solubility of the compounds was calculated from the gradient retention times corrected with the slope and intercept values obtained from the test curve.

$$\text{Solubility(mg/ml)} = \frac{\text{peak area 'sample'}}{\text{peak area 'std'}/10 \ * \ \text{sample inj}(\mu\text{L})/\text{std conc}(\mu\text{g/ml})/1000}$$

Log *S* [M] = Log_{10} (concentration (mg/ml)/molecular weight).
14. Measurement of Log *P* and Log *S* by HPLC: During high performance liquid chromatography measurements, the following system was applied. RP-C18 column (LiChrospher), 10 cm, 4.6 I.D. with a particle size of 10 μm.

Eluents:
A eluent: 50 mM ammonium acetate, pH 7.4
B eluent: 100% acetonitrile
Linear gradient system:

Time (min)	B (%)
0–1	0
1–8	100
8–11	100
11–12	0
12–16	0

The flow rate was 1 mL/min.
Temperature: 25 °C
Detection: λ = 230 and 254 nm.
For Log *P* calibration, the following test compounds were used.

No.	Name	Log *P*
1	Theophylline	−0.05
2	Colchicine	0.92
3	5-Phenyl-1*H*-tetrazole	1.42
4	Benzimidazole	1.55
5	Acetophenone	1.66
6	8-Phenyl-theophylline	2.05
7	Indole	2.14
8	Propiophenone	2.2
9	Butyrophenone	2.73
10	Valerophenone	3.26

15. General description of the kinase assays: the kinase activity was assayed in 96-well microtitre plates at a final compound concentration of 10 μM in a total volume of

50 μl. Compounds were dissolved in 100% DMSO to prepare a 10 mM stock solution and then diluted with buffer to reach a 10 μM (or the required) final concentration. The kinase concentration was used to yield 10% ATP turnover. The ATP concentration was used at the $K_{m\,ATP}$ and 12.5 μCi/ml adenosine 5′-[γ-^{33}P]triphosphate. The substrate concentration was used at 5-fold excess over the K_m for the substrate. The reaction was incubated for 1 h at room temperature and stopped by addition of 10 μl of 50 mM EDTA. The assay was transferred to a 96-well MAPH filter plate (Millipore). The radioactivity was counted on a Microbeta microplate counter.