Supporting Information for

Evaluation of kasugamycin as a chiral selector in capillary electrophoresis

Table S1 Physical and chemical properties of KAS.

Item	Description
Synonyms	Kasumin; Kasurabcide
Classification	Aminoglycoside antibiotic; Agricultural antibiotic
Appearance	White to light yellow powder
Chemical formula	$C_{14}H_{25}N_3O_9$
Relative molecular mass	379.363
Melting point	236 ℃
LogP	-2.06
Solubility	Insoluble in several organic solvents (methanol, ethanol, benzene, etc); Easily soluble in water (about 1g/8mL at room temperature)
pKa	3.23
Number of chiral centers	10

Table S2 Comparable properties of some typical antibiotics chiral selectors.

Kind	Representative	Consumption	Water solubility	Ultraviolet absorption	Price	Stereoselectivity
Glycopeptides	Vancomycin	Small	Moderate	Strong	Expensive	Strong
Ansamycins	Rifampicin	Moderate	Poor	Strong	Moderate	Moderate
Macrolides	Clarithromycin	Large	Poor	Weak	Moderate	Moderate
β-lactams	Penicillin G	Small	Moderate	Strong	Cheap	Moderate
Tetracyclines	Doxycycline	Moderate	Moderate	Strong	Expensive	Moderate
Lincosamides	Clindamycin phosphate	Moderate	Good	Weak	Expensive	Strong
Aminoglycosides	Kanamycin	Moderate	Moderate	Weak	Cheap	Weak
	KAS (this work)	Moderate	Good	Weak	Moderate	Moderate

Table S3 Effect of KAS concentration on chiral separations.

Rs	KAS concentration (mM)				
Analytes	40	50	60	70	80
EPH(PSE)	5.40	6.57	8.64	8.32	7.75
QIN(QID)	0.55	1.21	1.52	1.50	1.44
CIN(CID)	0.55	1.18	1.51	1.47	1.43

Conditions: capillary temperature, 25 ℃; separation voltage, 8 kV; BGE, 40 mM borax buffer containing 20% methanol (v/v) for QIN(QID), CIN(CID) or none of organic modifiers for EPH(PSE) and 40-80 mM KAS; buffer pH, 8.0 for QIN(QID) and CIN(CID) or 8.2 for EPH(PSE).

Table S4 Effect of separation voltage on chiral separations.

Rs	Separation voltage (kV)			
Analytes	6	8	10	12
EPH(PSE)	8.06	8.64	7.11	5.62
QIN(QID)	1.45	1.52	1.16	0.80
CIN(CID)	1.43	1.51	1.10	0.79

Conditions: capillary temperature, 25 ℃; separation voltage, 6-12 kV; BGE, 40 mM borax buffer containing 20% methanol (v/v) for QIN(QID), CIN(CID) or none of organic modifiers for EPH(PSE) and 60 mM KAS; buffer pH, 8.0 for QIN(QID) and CIN(CID) or 8.2 for EPH(PSE).

Table S5 Chiral separation results with KAS as a chiral selector.

	Migration time		Number of theoretical plates		Resolution	Selectivity factor
Analytes	t_1 (min)	t_2 (min)	N_1	N_2	Rs	α
EPH (PSE)	7.238	8.680	41925	32163	8.64	1.200
QIN (QID)	17.745	18.172	69746	57718	1.52	1.024
CIN (CID)	17.468	17.872	74348	51224	1.51	1.023
PRO	9.521	9.869	11793	22909	1.04	1.037
AML	11.823	/	/	/	<0.5	/
OFL	25.275	/	/	/	<0.5	/

Conditions: capillary temperature, 25 °C; separation voltage, +8 kV; BGE, 40 mM borax buffer containing 20% methanol (v/v, 0% for PRO, AML, EPH and PSE) and 60 mM KAS; buffer pH, 8.0 (8.2 for EPH and PSE).

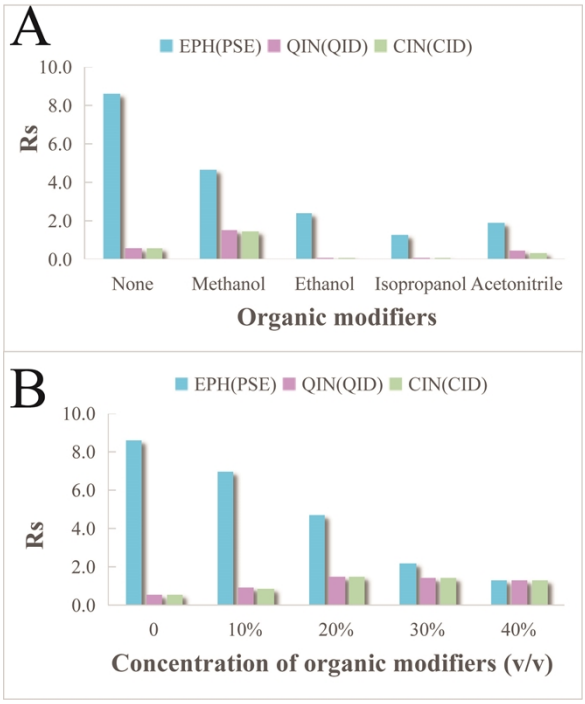

Fig.S1 (A) Chiral separations with different organic solvents as additives. Conditions: capillary temperature, 25 °C; voltage, 8 kV; BGE, 40 mM borax buffer containing 20% organic modifiers (v/v) and 60 mM KAS; buffer pH, 8.0 for QIN(QID) and CIN(CID) or 8.2 for EPH(PSE). (B) Effect of methanol concentration on enantioseparation. Conditions: capillary temperature, 25 °C; voltage, 8 kV; BGE, 40 mM borax buffer containing 0-40% methanol (v/v) and 60 mM KAS; buffer pH, 8.0 for QIN(QID) and CIN(CID) or 8.2 for EPH(PSE).

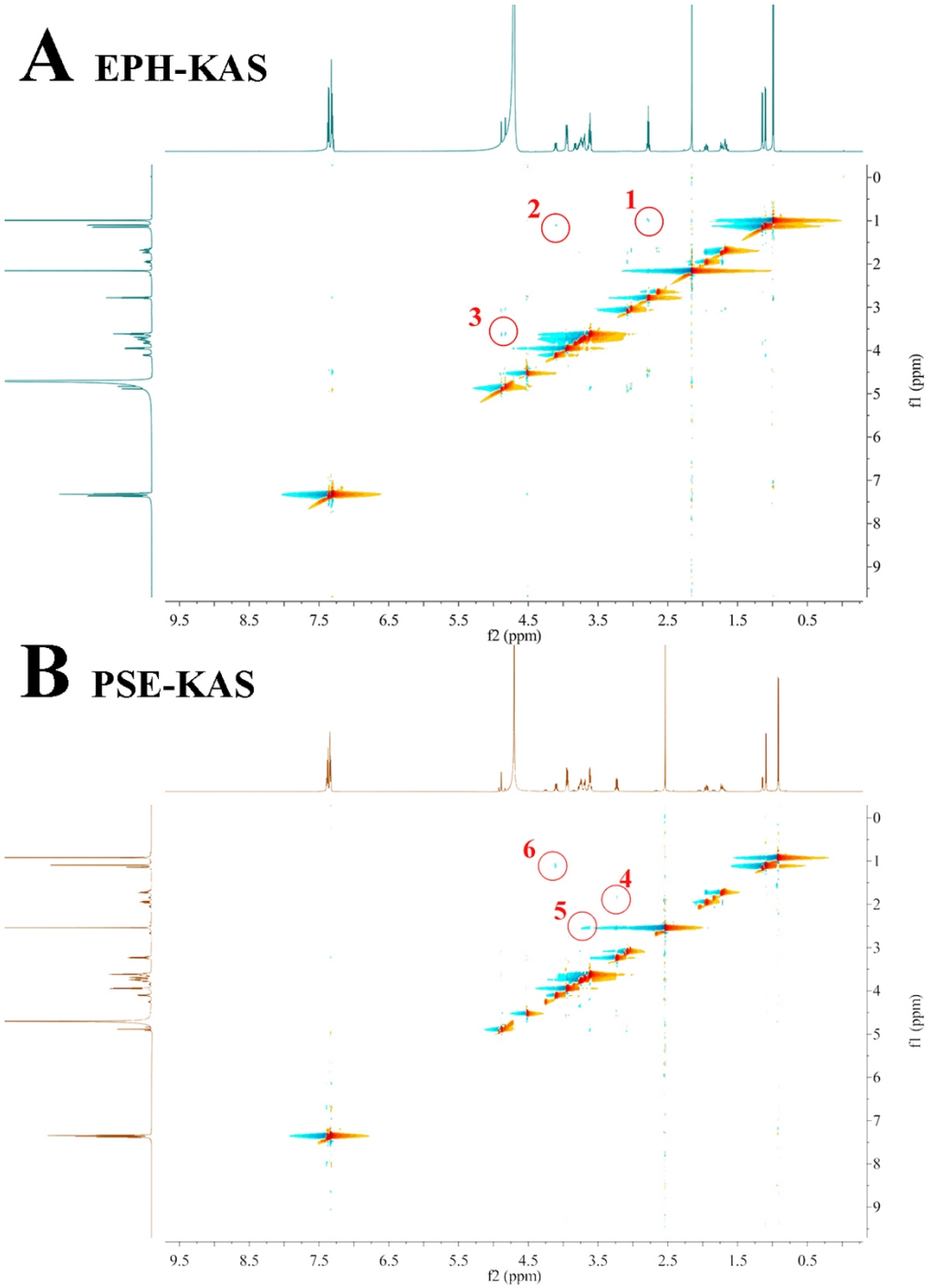

Fig.S2 2D ROESY NMR spectra of (A) EPH and KAS (1:1); (B) PSE and KAS (1:1) in D_2O at pH* 8.2.

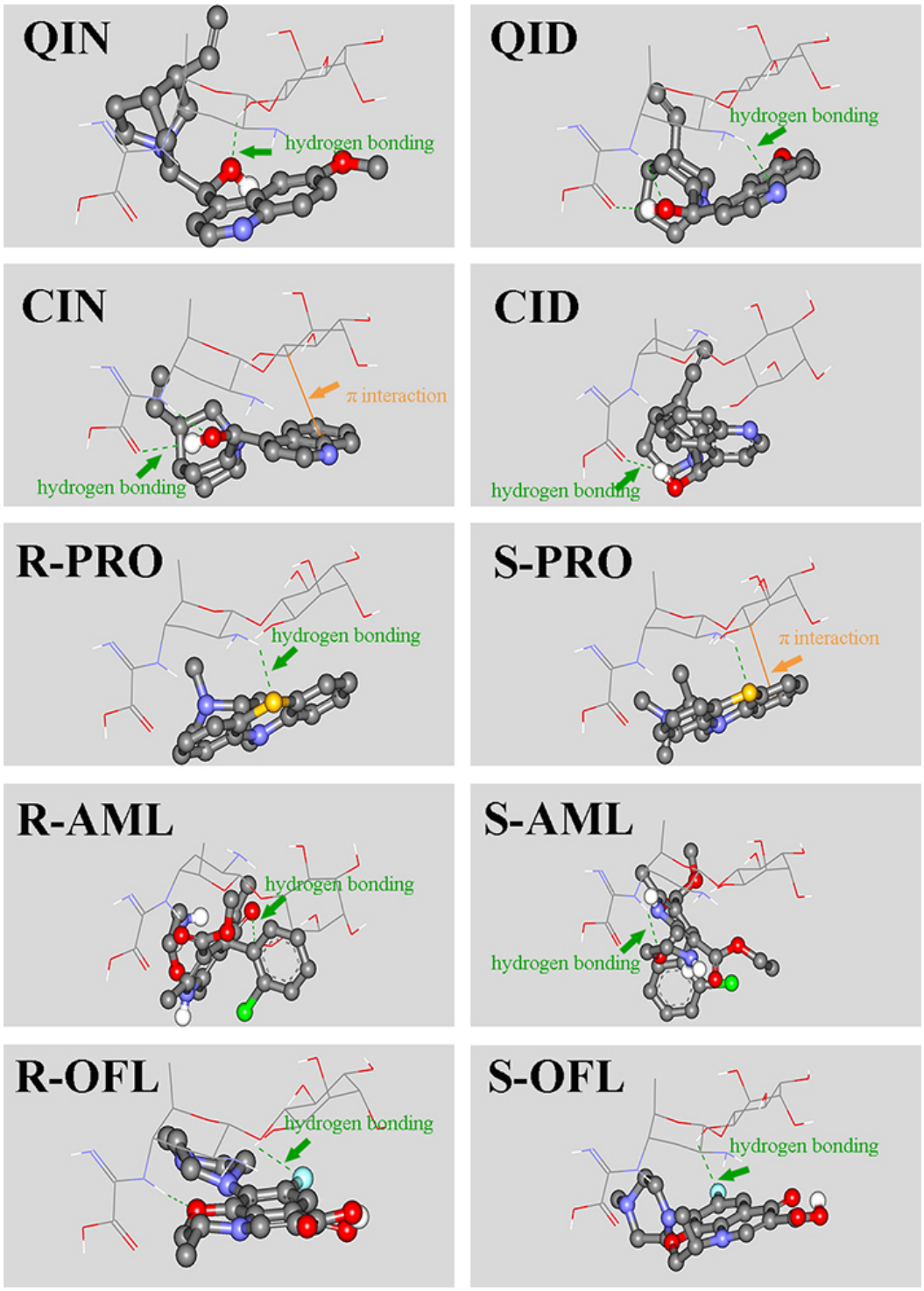

Fig.S3 Molecular modeling conformations of other model drugs in KAS separation system. The hydrogen bonding is indicated by green dotted line, the π interaction is indicated by orange solid line. C grey, H white, O red, N blue, S yellow, Cl green, F light blue.