

Supporting information

Material and methods

Forchlorfenuron, N-(2-chloro-4-pyridinyl)-N'-(3-hydroxyphenyl) (CAS: 76947-71-2), 1,3 -Diphenylurea, Diuron, Carbamazepine, Ketanserin, Pemigatinib (purity \geq 99 %) were purchased from J&K Scientific Co., Ltd. (Beijing, China). Ovalbumin (OVA), Bovine albumin (BSA), and goat anti-mouse IgG were acquired through Sigma-Aldrich (St. Louis, MO, USA). Ethoxylated oleyl alcohol (On-870), polyethylene glycol (PEG, MW: 4000), and Tween 20 solution were supplied by Thermo Fisher Scientific Co., Ltd. (Shanghai, China). Sample pad, absorbent pad, conjugate pad and polyvinyl-chloride (PVC) backing card were provided by Kinbio Tech. Co., Ltd. (Shanghai, China). Nitrocellulose (NC) membranes were provided by Shenzhen BSK Industrial Co., Ltd. The portable colloidal gold immunoassay analyzer was obtained from Suzhou Helmen Precision Instruments Co., Ltd. (Jiangsu, China). The XYZ Platform Dispenser was acquired from Shanghai Kinbio Tech. Co., Ltd. (Shanghai, China). All animals were purchased from Home-SPF Biotechnology Co., Ltd. (Beijing, China). All experiments were approved by the Jiangnan University Animal Welfare and Ethics Committee (JN.NO20251027B090181 [140]) and conducted in strict compliance with the EU Directive 2010/63 on the protection of animals used for scientific purposes.

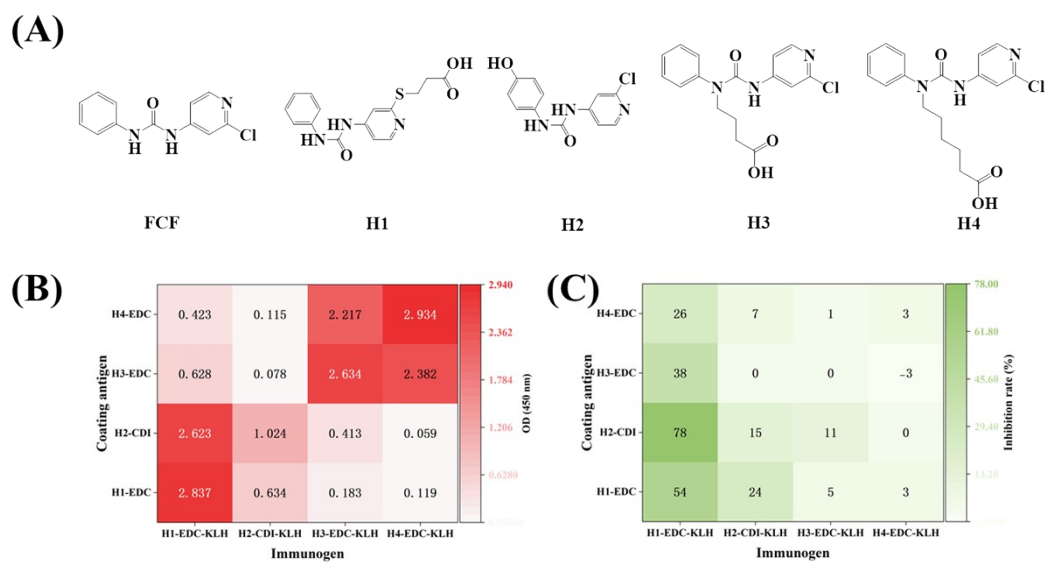


Fig. S1. (A) Structure of FCF and five designed haptens. The titers (B) and inhibition rates (C) of different immunogens under different coating antigens were determined.

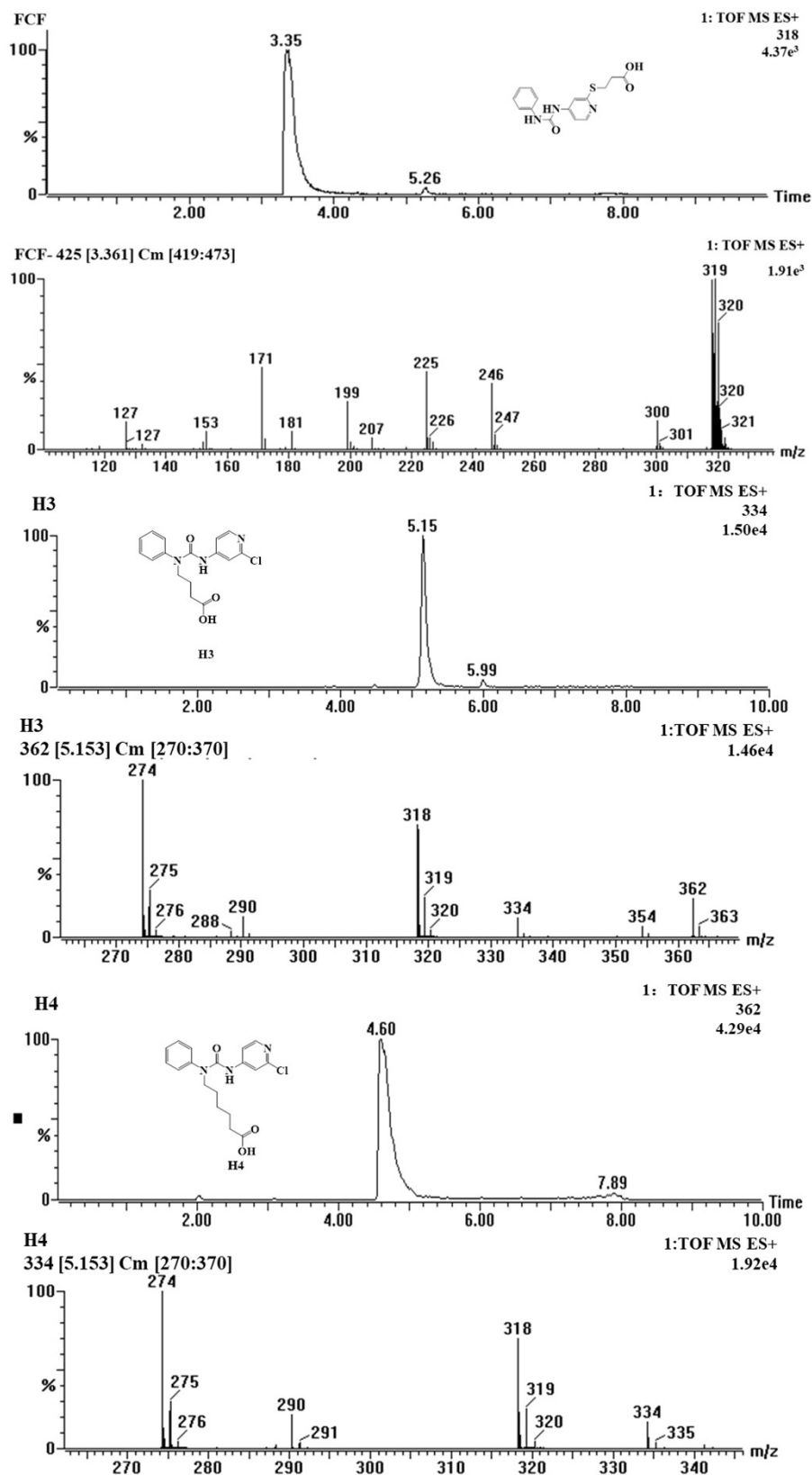


Fig. S2. Verify the structure of the H1, H3 and H4 by LC-MS/MS.

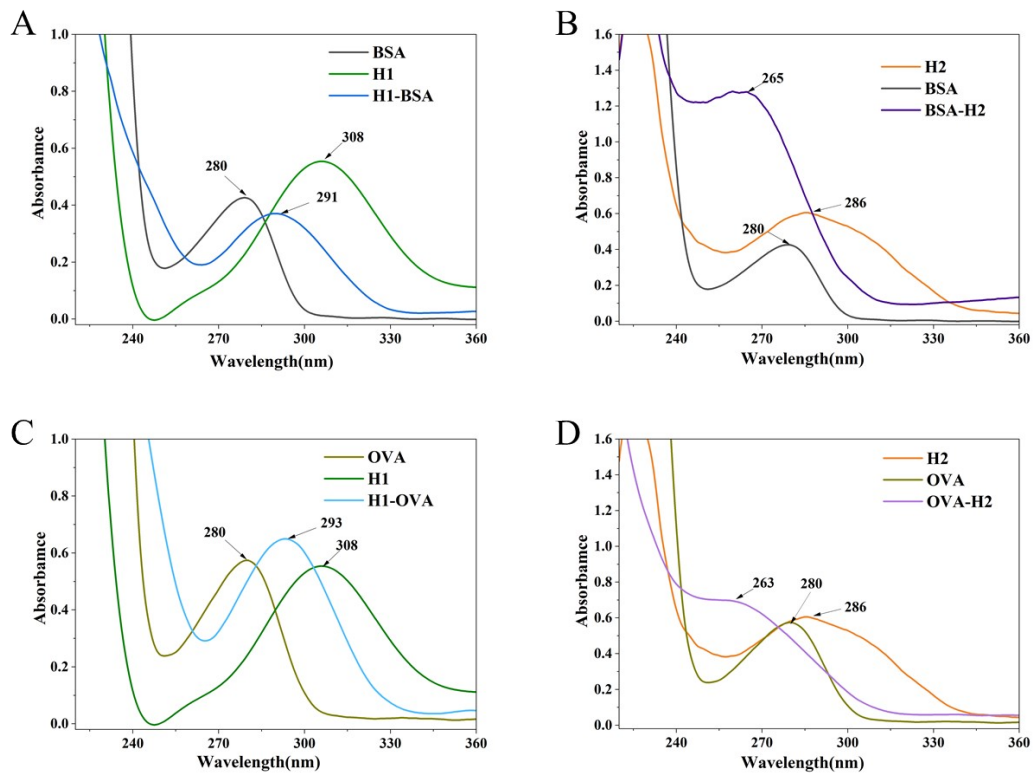


Fig. S3. The result of UV-Vis spectroscopy characterization of FCF antigens.

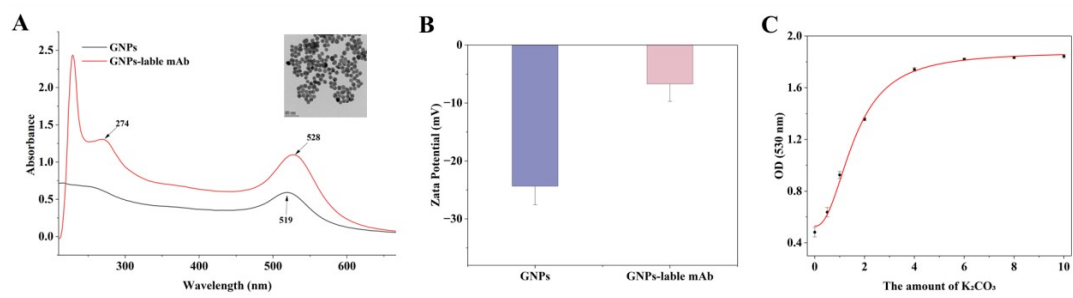


Fig. S4. Characterization data of AuNPs and AuNPs-mAb.

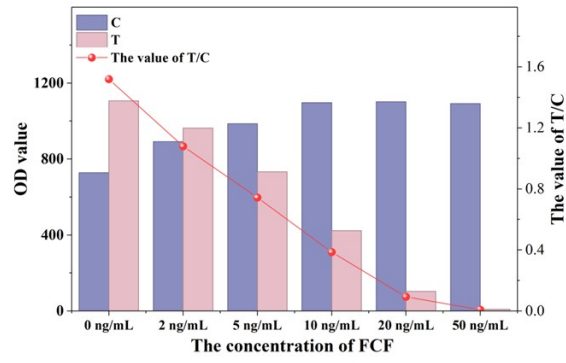


Fig. S5. Performance of single T-line ICA.

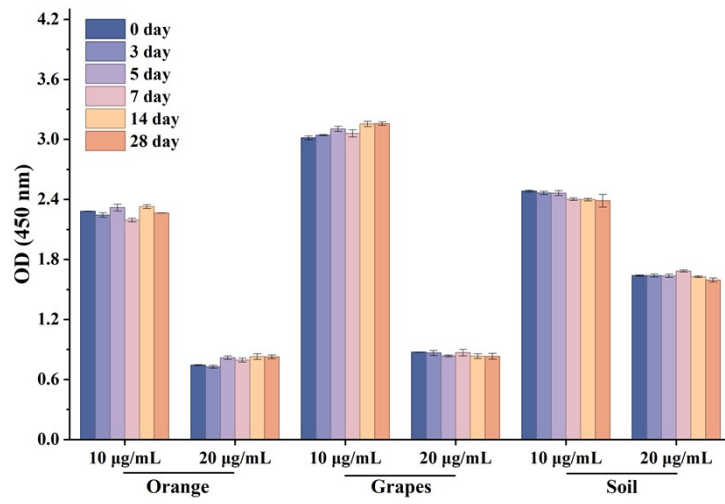


Fig. S6. Effects of storage time on the output data of ICA.

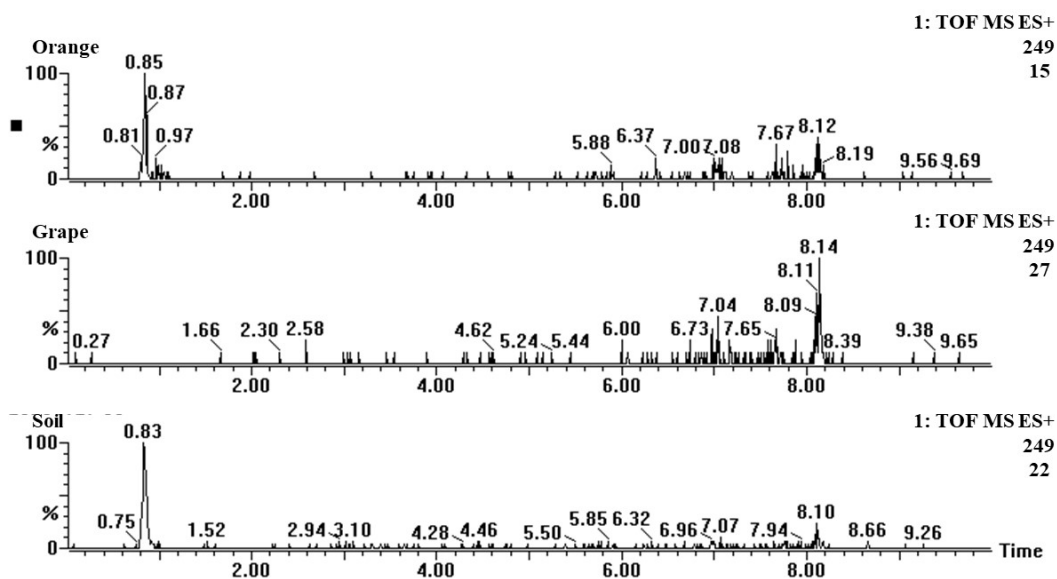


Fig. S7. Analysis negative samples by LC-MS.

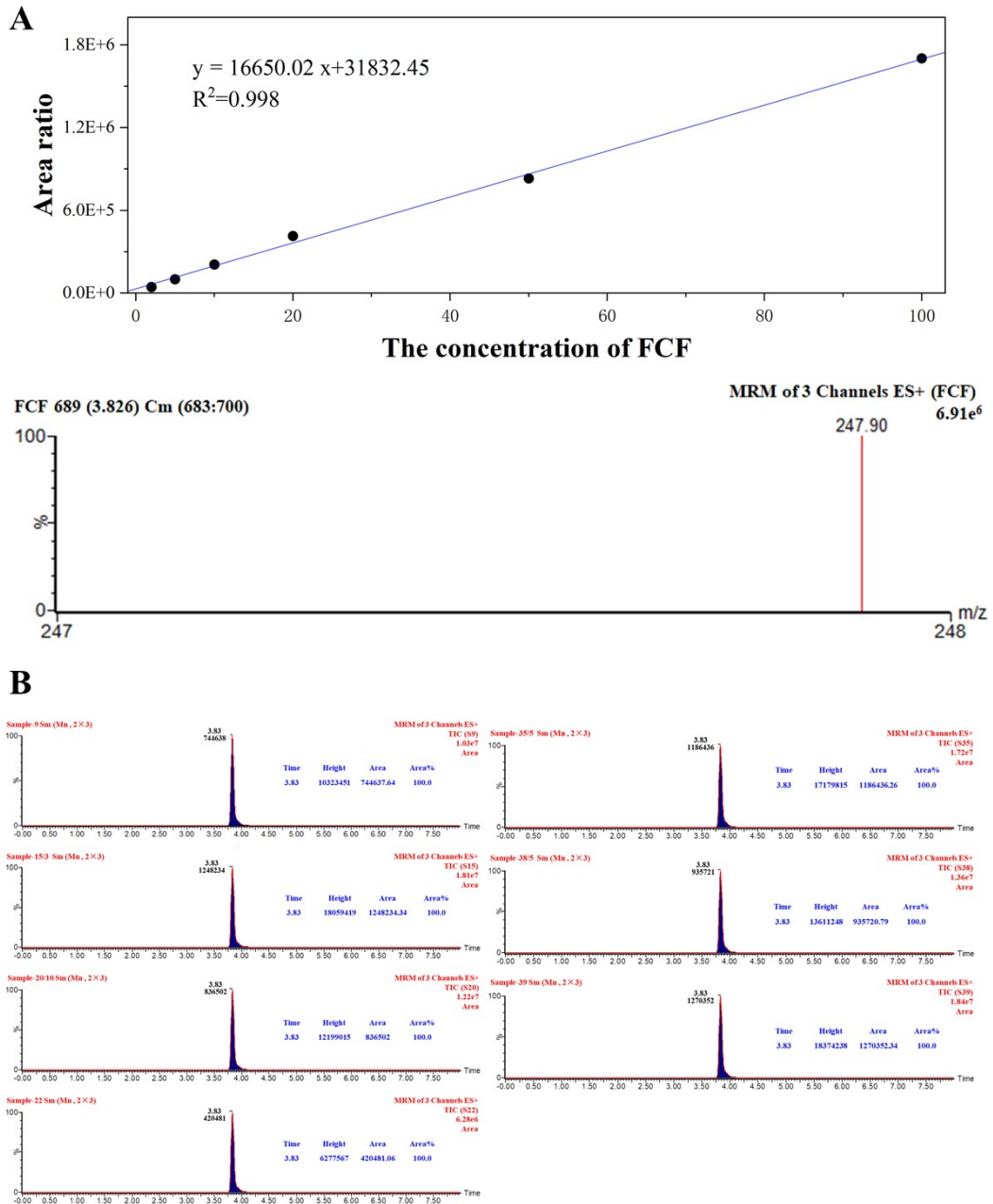
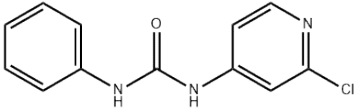
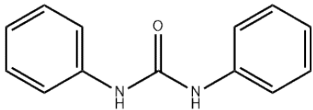
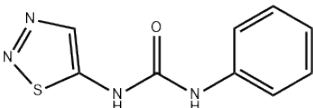
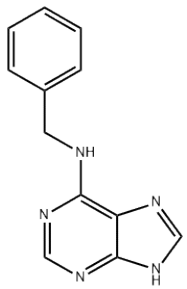
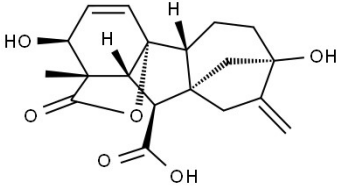
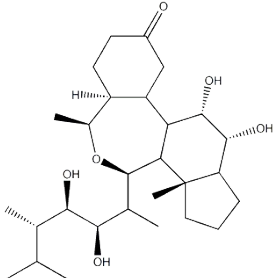
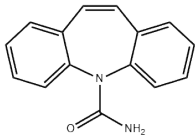
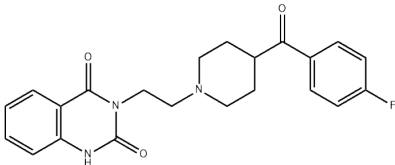
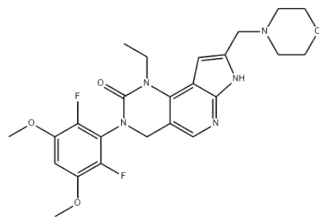


Fig. S8. Analysis positive samples by LC-MS.

Table. S1. Cross-reactivity of structural analogues.

Analogs	Structure	IC50 (ng/mL)	CR (%)
Forchlorfenuron		0.10086	100
1,3 -Diphenylurea		10.854	0.93
Thidiazuron		64.292	0.16
6-benzylaminopurine		>100	<0.1
Gibberellic acid		>100	<0.1
Brassinolide		>100	<0.1
Carbamazepine		>100	<0.1
Ketanserin		>100	<0.1

Pemigatinib



>100

<0.1

Table. S2. Recovery of FCF in different samples by the Immunochromatographic assay strip and LC-MS/MS (n = 3).

Samples	Spiked value of target ($\mu\text{g}/\text{kg}$)	Immunochromatographic assay strip			LC-MS/MS		
		Detection value ($\mu\text{g}/\text{kg}$)	Recovery	CV	Detection value ($\mu\text{g}/\text{kg}$)	Recovery	CV
		Mean	(%)	(%)	Mean	(%)	(%)
Orange	0	ND	NC	NC	ND	NC	NC
	5	4.928 ± 0.075	98.57	1.53	4.950 ± 0.082	98.99	1.65
	20	22.015 ± 1.078	110.08	4.90	21.801 ± 2.332	109.00	9.14
	200	192.340 ± 2.162	96.17	1.12	206.543 ± 3.833	103.27	1.86
FCF Grapes	0	ND	NC	NC	ND	NC	NC
	5	5.059 ± 0.056	101.19	1.10	4.945 ± 0.06	98.90	1.20
	20	20.363 ± 1.063	101.81	5.22	20.513 ± 0.634	102.57	3.09
	200	202.421 ± 3.834	101.21	1.89	193.704 ± 4.581	96.85	2.36
Soil	0	ND	NC	NC	ND	NC	NC
	5	5.245 ± 0.111	104.89	2.12	4.978 ± 0.144	99.55	2.89
	20	18.450 ± 0.531	92.25	2.88	22.576 ± 1.912	112.88	8.47
	200	205.333 ± 6.825	102.67	3.32	207.224 ± 1.965	103.61	0.95

^a ND, not detectable.^b NC, not calculated.

Table. S3. Types of real samples (n = 5).

Serial Number	Types of Samples
No. 1 ~ No. 11	Orange Samples
No. 12 ~ No. 27	Grapes samples
No. 28 ~ No. 40	Soil Samples