CHAPTER 4

CONCLUSION

4.1) CE with immobilized cells for drugs screening

There are several distinct advantages for using whole cells as the stationary phase of affinity capillary electrophoresis: (i) isolation of (unstable) receptors is not required, (ii) the stability of whole cells is improved by immobilization, and (iii) the whole cells are easily coated on a fused-silica capillary column via the guidance of poly-L-lysine template. We have demonstrated this concept of whole-cell immobilized affinity capillary electrophoresis by a protocol using the capillary column coated with the Chinese hamster ovary cells containing overexpressing endothelin receptor A. As shown in Fig. 4.1 and Table 1, the capillary column prepared as such exhibits excellent affinity for separation and identification of endothelin receptor A antagonists. There is a good correlation between the relative inhibition of the ET-1 induced $[Ca^{2+}]_i$ and the retention time of the examined compound on the capillary column coated with ET_A-overexpression CHO cells. This affinity capillary electrophoresis method only requires a very small quantity of sample, and offers a reliable assessment of a library of compounds in a relatively short period. The affinity capillary electrophoresis with immobilized whole cells would have great promise to be developed as a high-throughput screening method based on the specific receptor-ligand interactions.

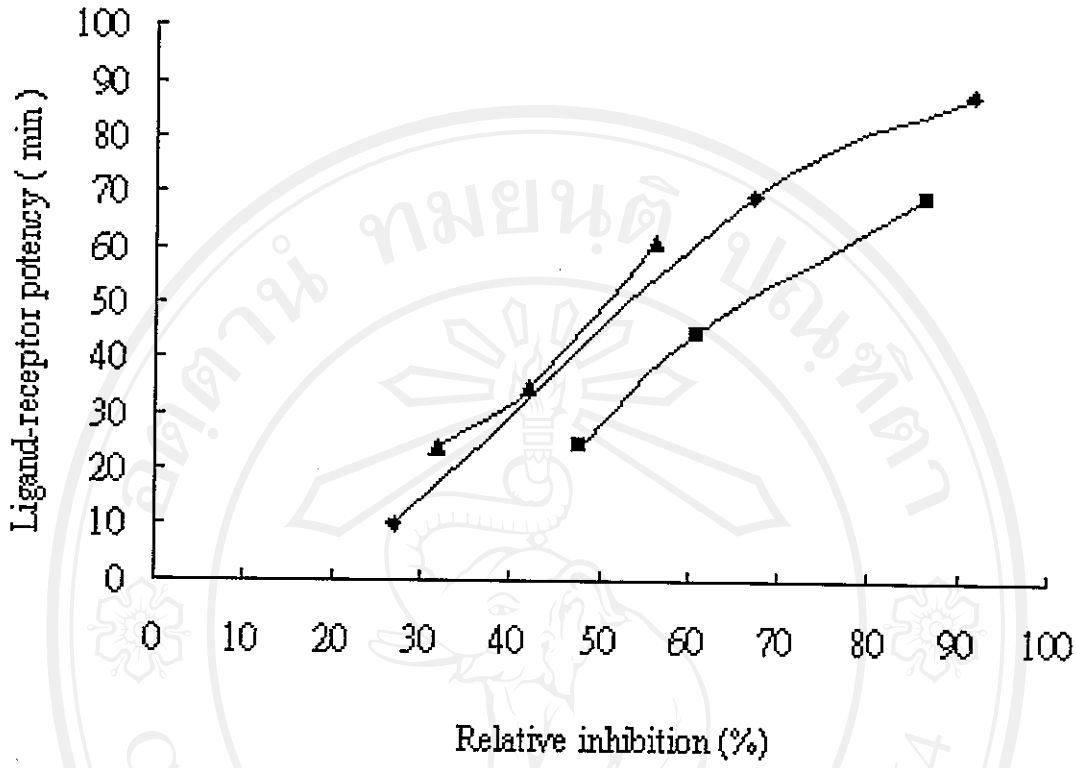

Figure 4.1 Correlation between the retention time of the examined compound on the capillary column coated with ETA-overexpression CHO cells and the relative inhibition of the ET-1 induced increase of intracellular calcium ion concentration. In each line, the stronger affinity of a compound toward ETA shows a longer retention time and more potent inhibitory effect.

■ line: from top to bottom are JKC302, BQ123 and ET-1(16-21).

◆ line: from top to bottom are SB209670, JMF310 and YHK891.

▲ line: from top to bottom are Magnolol, Geniposide and Honokiol

Table 4.1 The percentage of relative inhibition of induced increasing $[Ca^{2+}]_i$ concentration and the retention time of ACE method (a capillary colum with the stationary phase of immobilized ETA overexpression CHO cells) for the evaluated compounds.

Evaluated compound	Relative inhibition (%)	Retention time (min)
JKC302	86.3	69.7
BQ123	60.9	45.0
ET-1 (16-21)	47.8	24.2
SB209670	91.4	87.8
JMF310	67.0	69.7
YHK891	27.0	10.0
Magnolol	56.0	61.4
Geniposide	42.0	34.7
Honokiol	32.0	24.1

4.2) ET_A antagonists nonpeptides(I): carbazolothiophene-2-carboxylic acid derivative

The SmI_2-promoted three-component coupling reaction of thiophene-2-carboxylate, indole-2-carbaldehyde and acetophenone provides an expedient route to a series of tetracyclic carbazolothiophene compounds bearing the indole and thiophene rings. Among these samples, 9-benzyl-4-methyl-4-(4-hydroxyphenyl)-10-oxo-4,10-dihydrocarbazolo[2,3-*b*]thiophene-2-carboxylic acid shows the most potent inhibition against the endothelin-1 induced increase of

intracellular calcium ion concentration. In summary, a series of tetracyclic compounds 7-22 bearing the indole and thiophene rings were prepared in an expedient fashion. The functional assay indicated that one of these samples (compound 18) can serve as a lead compound for future exploration of potent endothelin receptor antagonists (Table 4.2).

Table 4.2 The percentage of relative inhibition of induced increasing $[Ca^{2+}]_i$ concentration for the 7-22 of carbazolothiophene-2-carboxylic acid compounds.

Evaluated compound	Relative inhibition (%)
7	51
9	55
10	79
11	69
12	86
13	79
14	0
15	33
16	15
17	0
18	95
19	75
20	0
21	0
22	0

4.3) ET_A antagonists nonpeptides(II): 1,4-benzodiazepine-2,5-diones derivatives

The 1,4-benzodiazepine-2,5-diones with the anisole or phenol moiety, 26, 43 and 45, show the significant inhibition due to the oxygen atom of the anisole or phenol moiety, and the ester may chelate zinc ion in physiological conditions, even the absence of carboxylate.

We demonstrated that a whole cell stationary phase consisting of ET_A-over-expressing CHO cells provides a successful ACE protocol for the screening of the ET_A-specific ligands. The peptide and non-peptide ET_A antagonists were satisfactorily resolved on ACE, in accordance to the order of their affinity and antagonist potency toward ET_A. A series of nonpeptide compounds bearing the indole and thiophene rings were prepared in an expedient fashion. The functional assay indicated that one of these samples can serve as a lead compound for future exploration of potent endothelin receptor antagonists. The structure-activity relationship also awaits further investigation.

Table 4.3 The percentage of relative inhibition of induced increasing $[Ca^{2+}]_i$ concentration for the 23-61 of 1,4-benzodiazepine-2,5-dione compounds.

evaluated compound	relative inhibition (%)
23	61
24	80
25	69
26	92
27	71
28	92
29	69
30	92
31	85
32	77
33	65
34	27
35	53
36	64
37	58
38	75
39	63
40	75
41	81
42	76
43	100
44	36
45	100
46	43
47	81
48	55
49	0
50	80
51	85
52	72
53	73
54	72
55	58
56	75
57	50
58	60
59	90
60	82
61	81