

High-throughput screening for human collagenase 1 inhibitors¹

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KEY WORDS high-throughput screening; drug screening; matrix metalloproteinase

ABSTRACT

AIM: To establish a high-throughput method for inhibitor screening using a recombinant collagenase catalytic domain. **METHODS:** Human collagenase 1 catalytic domain protein was expressed in *E coli* and used for screening a set of 2720 compounds in a high-throughput fashion. **RESULTS:** The screening was accomplished within 2 h and 10 min with consumption of each compound at 4 μg . Sixty-six compounds were identified with > 60 % inhibitory activity at 20 mg/L, among which 44 compounds were confirmed by subsequent testing at multiple concentrations. The most potent compound showed an IC_{50} at 4.3 $\mu\text{mol/L}$, and there were total 15 compounds with IC_{50} less than 20 $\mu\text{mol/L}$. **CONCLUSION:** The high-throughput method using the recombinant collagenase is fast, effective and practical in identifying inhibitors.

INTRODUCTION

Drug discovery is largely still an empirical process and relies heavily on accidental findings and random screening of samples from various sources. Recent advances in combinatorial chemistry and natural product chemistry could offer a large number of pure compounds or mixtures for such screening, and high-throughput screening (HTS) technologies have been widely applied to drug discovery process. The combination of sample

preparation from combinatorial synthesis and natural product isolation with enzyme/receptor-based and cell-based HTS methods will certainly accelerate the drug lead generation for further development as useful therapeutic agents.

Collagenase 1 is a member in the matrix metalloproteinase (MMP) family that participates in normal connective tissue remodeling by degrading protein components in connective tissues^[1]. MMP have also implicated in several diseases involved in connective tissue degradation such as cancer metastasis, arthritis, multiple sclerosis, and periodontal diseases^[2]. Extensive researches have been carried out both academically and by pharmaceutical industry to find effective and selective inhibitors for MMPs as potential drugs for such diseases^[3].

The protein sequence of human collagenase 1 is typical among MMPs with distinctive region for the catalytic domain. Evidence has been shown that the MMP catalytic domains usually have similar catalytic efficiency as the full length MMPs and can be used as a tool for screening MMP inhibitors^[4-7]. We report here the development of an HTS assay using a recombinant human collagenase 1 catalytic domain (C1CD) protein, and the screening results from 2720 pure synthetic compounds.

MATERIALS AND METHODS

Materials and instruments The EST clone (Genbank number: AA082736, EST clone number: 549904) containing the gene for human collagenase 1 catalytic domain were purchased from Genome Systems (St Louis, MO, USA). Thiopeptolide Ac-Pro-Leu-Gly-thioester-Leu-Leu-Gly-OEt was purchased from Bachem Bioscience (King of Prussia, PA, USA). Other reagents and solvents used in the experiments are analytic grade or reagent grade as appropriate.

The plasmid pGEMEX-1 and *E coli* strain JM109/DE3 were purchased from Promega (Madison, WI, USA). Restriction enzymes and other enzymes used in DNA cloning were purchased locally.

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Samples used in the HTS assays are all small organic compounds with purity higher than 90 %, either purchased or collected from different sources with wide structural diversity.

Biomek 2000 liquid handling workstation from Beckman (Fullerton, CA, USA) was used for liquid handling, and SPECTRAMax 340 96-well plate reader from Molecular Devices (Sunnyvale, CA, USA) was used for kinetic monitoring and end point readings.

Sample preparation Individual compounds (1 mg each) were dissolved in 200 μ L of Me₂SO as 5 g/L solutions. The solution was diluted 5 fold by adding 20 μ L of the solution to 80 μ L of Me₂SO in the sample wells (A2-H11) of 96-well polypropylene plates as mother plates with the layout format shown in Fig 1, and the concentration was 1 g/L for each compound. All mother plates were duplicated to polystyrene plates as daughter plates for screening by transferring 2 μ L of the solution using the Biomek 2000 workstation.

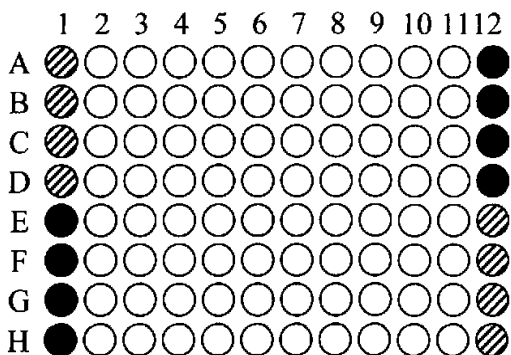


Fig 1. Layout of samples (open circles), blanks (shaded circles), and controls (filled circles) on the 96-well plate. Rows A to H and columns 1 to 12 are shown.

Cloning, expression, and purification of C1CD protein Several MMP catalytic domains have been cloned and expressed in *E coli*^(4-6, 8,9). The gene of the C1CD protein was amplified from the EST clone with the primers CCGATC*TCTAGA*TTT-GTCCTCACT-GAGGGGAA and CGCAGT*GAA*TTCTTATCCATATA-TGGCTTGGATGC (the coding sequences are underlined and the restriction sites are in italics) with introduction of restriction sites for *Nhe*I and *Eco*R I. The DNA fragment was cloned into the plasmid pGEMEX-1 for expression in *E coli* under the control of a T7 promoter. The recombinant plasmid pGEMEX-C1CD containing the C1CD gene was transformed into an *E coli* strain JM109/

DE3. The transformed cells were cultured in 2XTY medium in a 2-L fermentor, and the expression of C1CD protein was induced by addition of isopropyl- β -D-thiogalactopyranoside (IPTG).

The C1CD protein was obtained as insoluble protein. The cells were lysed by sonication, and the pellet after centrifugation was washed with 50 mmol/L Tris (pH 7.6) 3 times before dissolving in urea 6 mol/L. The C1CD protein in urea was added dropwise to a refolding buffer (50 mmol/L Tris, pH 7.6, 10 mmol/L CaCl₂, and 10 μ mol/L ZnCl₂) stirred at 4 $^{\circ}$ C. The mixture was centrifuged, and the supernatant containing the refolded C1CD protein was loaded to a Q-Sepharose column equilibrated previously with 50 mmol/L Tris (pH 7.6) and 10 mmol/L CaCl₂. The C1CD protein was eluted with a linear gradient from 0 to 1 mol/L NaCl in the Tris/CaCl₂ buffer, and fractions containing C1CD protein were collected and concentrated. The C1CD protein was active and showed as a single band by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis.

C1CD activity assay The C1CD activity was evaluated using the thiopeptolide Ac-Pro-Leu-Gly-thioester-Leu-Leu-Gly-OEt as the substrate⁽¹⁰⁾. Cleavage of the thiopeptolide generates a free thiol group, which reacts immediately with 5,5-dithiobis(2-nitrobenzoic acid (DTNB) to produce a substance with UV absorption ($\epsilon_{412\text{nm}} = 13\ 600\ \text{M}^{-1}\cdot\text{cm}^{-1}$)⁽¹¹⁾. The hydrolysis process can be monitored kinetically on 96-well plate.

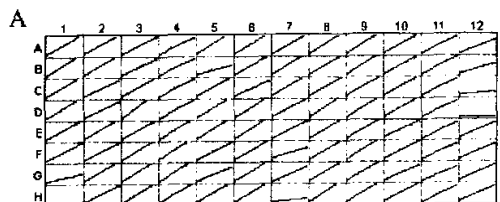
In a total 100 μ L assay volume, the final concentrations were 50 mmol/L Tris (pH 7.0), 10 mmol/L CaCl₂, 1 mmol/L DTNB, 100 μ mol/L thiopeptolide, and C1CD protein and inhibitors at different concentrations. The hydrolysis was monitored at 412 nm on SPECTRAMax 340 plate reader for 2-5 min at room temperature.

C1CD HTS activity assay On the daughter plates, Me₂SO solvent (2 μ L) was distributed in eight blank wells (A1-D1, E12-H12), and controls are in another eight wells (E1-H1, A12-D12) using known MMP inhibitor Galardin solution in Me₂SO (2 μ L; 3 μ mol/L, 1 μ mol/L, 333 nmol/L, and 111 nmol/L) (Fig 1).

An assay mix (88 μ L), which contains 10 μ L of 500 mmol/L Tris (pH 7.0), 1 μ L of 1 mol/L CaCl₂, 10 μ L of 1 mmol/L thiopeptolide, 1 μ L of 100 mmol/L DTNB (in ethanol), and 66 μ L of water, was added to each well, followed by addition of 10 μ L of C1CD protein (200 nmol/L in 50 mmol/L Tris, pH 7.6, and 10

mmol/L CaCl₂) in 12 successions with 8 channel pipette on Biomek 2000 to all wells. The plate was transferred to the plate reader for immediate reading at 412 nm. UV absorption change was monitored for 3 min, and slope of the absorption change was recorded (Fig 2).

The average of the slopes for the 8 blanks was used as 100 % activity. The slope from control wells or compound wells was divided by the average, giving percent activity in the presence of the inhibitor Galardin or the screening compound (Fig 2).



B

35.7	34.8	32.0	29.8	42.6	37.9	35.7	39.9	39.1	37.7	37.3	33.3
40.5	37.9	29.1	28.3	16.7	34.7	38.9	38.0	39.1	36.7	38.6	25.4
38.2	29.0	36.2	31.3	34.4	26.6	37.6	36.0	35.7	37.6	37.5	8.6
38.4	29.4	46.4	32.3	38.3	38.6	34.2	41.5	37.5	37.4	35.4	0.4
34.8	38.3	34.7	37.9	38.9	39.1	31.5	37.8	35.9	32.6	34.8	37.4
30.6	32.5	39.3	39.8	31.6	36.2	19.9	38.2	39.5	35.4	35.7	37.3
13.9	38.1	38.9	40.8	27.2	37.1	29.7	38.1	36.3	29.7	31.7	37.9
0.4	30.7	38.8	36.1	38.3	37.8	7.0	43.0	37.6	36.7	35.9	38.2

C

94	92	84	79	112	100	94	105	103	99	98	88
107	100	77	75	44	92	102	100	103	97	102	67
101	76	95	82	91	70	99	95	94	99	99	23
101	78	122	85	101	102	90	109	99	99	93	1
92	101	92	100	103	103	83	100	95	86	92	99
81	86	104	105	83	95	52	101	104	93	94	98
37	100	103	107	72	98	78	100	96	78	84	100
1	81	102	95	101	100	19	113	99	97	95	101

Fig 2. Typical data conversion from kinetic reading to percent activity. **A.** Raw data showing the absorption change at 412 nm over a period of 3 min in each of the 96-wells (layout of samples, blanks, and controls as shown in Fig 1). **B.** Slopes for the absorption change in each well. **C.** Converted percent activity in each well.

RESULTS

Characterization of C1CD activity The recombinant C1CD protein, like other MMPs, cleaves several synthetic chromogenic and fluorogenic substrates. We used the thiopeptolide Ac-Pro-Leu-Gly-thioester-Leu-Leu-Gly-OEt as substrate and monitored its hydrolysis by UV absorption at 412 nm. The purified C1CD protein showed *K_m* of (750 ± 150) μmol/L and *k_{cat}* of (24.6 ± 2.4) s⁻¹ in hydrolyzing the thiopeptolide, similar to literature values⁽¹²⁾.

Since Me₂SO was used in dissolving compounds for the HTS assays, we investigated the effects of Me₂SO on C1CD activity. As increase of Me₂SO concentration, the activity of C1CD decreased accordingly. The C1CD protein showed (91.6 % ± 6.4%) activity at 1% Me₂SO, (82.9 % ± 1.9 %) activity at 2 % Me₂SO, (75.3 % ± 4.8 %) activity at 5 % Me₂SO, and (65.0 % ± 6.8 %) activity at 10 % Me₂SO, compared with no Me₂SO as 100 % activity. The data suggested that presence of 2 % Me₂SO in the HTS assay did not significantly affect the C1CD enzymatic activity. In the presence of 2 % Me₂SO, the rate of the thiopeptolide hydrolysis showed good linear increase up to 400 nmol/L C1CD we tested. For a reasonable signal to noise ratio, we chose 20 nmol/L of C1CD protein for the HTS assays.

High throughput screening of 2720 compounds

After initial experiments mentioned above, we defined the final condition for the HTS assay as 20 nmol/L C1CD protein, 100 μmol/L thiopeptolide, 1 mmol/L DTNB, 50 mmol/L Tris (pH 7.0), 10 mmol/L CaCl₂, 2% Me₂SO, and 20 mg/L screening compounds, in a total volume of 100 μL. Me₂SO solvent was used as blanks, and Galardin in Me₂SO at 60, 20, 6.7, and 2.2 nmol/L final concentrations was used as controls.

The total 2720 pure compounds were accommodated on 34 96-well plates. With 2 μL each of blanks (Me₂SO solvent), controls (Galardin in Me₂SO), and compounds (in Me₂SO) distributed on the 96-well plates, assay mix (Tris, CaCl₂, DTNB, and the thiopeptolide) and the C1CD protein were added separately and sequentially, before kinetically monitoring the UV absorption at 412 nm for 3 min. The addition of assay mix and C1CD protein took less than 3 min for each plate and was carried out simultaneously as the kinetically monitoring, so that the total assay time for each plate was limited by the plate reading. It took a little over 3 min for each plate, and we finished the HTS assay in 2 h 10 min for 34 plates with total 2720 compounds.

Compounds with no effect on C1CD activity showed the slope of UV absorption change similar to the blanks, while those compounds with C1CD inhibition changed the slope to a reduced number by different degrees, reflecting their inhibitory potency on C1CD (Fig 2). A fraction of compounds (55 out of the total 2720 compounds) showed irregular slopes due to precipitation during the reading, and their data were not useable. However, deep color or slight cloudy appearance affected endpoint readings but

did not seem to affect kinetic reading.

As expected, most compounds showed no or weak inhibition. There were 227 compounds showed 20 % - 40 % inhibition, 70 compounds showed 40 % - 60 % inhibition, 40 compounds showed 60 % - 80 % inhibition, 26 compounds showed larger than 80 % inhibition, and the rest showed no inhibition or less than 20 % inhibition at the assay concentration of 20 mg/L. We took the 66 compounds with higher than 60 % inhibition as hits for further characterization, and the hit rate was 2.4 % of the total 2720 compounds.

Quality controls in the HTS assay In order to handle a large number of compounds in an HTS assay and manage huge amount of information generated, it is the common practice to assay compounds only at a single empirical concentration with hope to identify hits for further investigation. To differentiate fluctuations due to liquid handling and instrument reading from real inhibition, we tested the precision in pipetting and instrument reading in situations we used for our HTS assays.

We first tested the precision of pipetting by Biomek 2000 liquid handling workstation using the dye Bromophenyl Blue (BPB) as the indicator, measuring absorption at 592 nm on 96-well plates. When 2 μ L of BPB in Me₂SO was added to dry plates, simulating compound distribution to daughter plates, the CV was 7 % across the entire plate (Fig 3), and when 10 μ L of BPB in 50 mmol/L Tris (pH 7.0) was added to 90 μ L of 50 mmol/L Tris (pH 7.0), simulating our enzyme addition, the CV was within 6 % (Fig 3).

We also investigated the fluctuations in thiopeptidase hydrolysis by C1CD kinetically with Me₂SO solvent in places of the 80 compounds in Me₂SO, and we found that the readings for the last three columns showed gradual decrease with the right most column being the lowest (Fig 4). The abnormal reading could not be due to pipetting error, since we did not see such trend in BPB tests mentioned above. The likely reason is that the right side of the plate is close to the drawer opening, and the lower temperature there may contribute to the slower hydrolysis kinetically. We applied correction factors to each column for kinetic data, and we obtained a uniform data across the entire plate with the CV at 4% (Fig 5). We observed the same trend in the data from the 34 sample plates, so that we also applied the same correction factors to each column for those data.

We used the known MMP inhibitor Galardin as the control at four final concentrations (60, 20, 6.7, and 2.2 nmol/L). Consistently, Galardin showed C1CD

A

0.72	0.73	0.73	0.69	0.72	0.82	0.69	0.69	0.68	0.70	0.69	0.68	0.71	<i>0.05</i>
0.72	0.69	0.68	0.69	0.69	0.68	0.67	0.70	0.70	0.69	0.70	0.68	0.69	<i>0.02</i>
0.71	0.70	0.68	0.69	0.67	0.80	0.67	0.67	0.67	0.67	0.67	0.68	0.68	<i>0.05</i>
0.71	0.69	0.68	0.67	0.67	0.66	0.66	0.66	0.67	0.67	0.69	0.68	0.68	<i>0.02</i>
0.69	0.67	0.67	0.66	0.67	0.70	0.66	0.66	0.67	0.67	0.67	0.67	0.67	<i>0.02</i>
0.69	0.67	0.66	0.67	0.66	0.65	0.64	0.66	0.66	0.67	0.76	0.78	0.68	<i>0.06</i>
0.68	0.68	0.67	0.64	0.64	0.65	0.74	0.65	0.77	0.73	0.78	0.64	0.69	<i>0.07</i>
0.70	0.72	0.70	0.70	0.71	0.67	0.67	0.67	0.70	0.85	0.91	0.86	0.74	<i>0.11</i>
0.70	0.69	0.68	0.68	0.68	0.70	0.67	0.67	0.69	0.71	0.73	0.71	0.71	<i>0.07</i>
0.02	0.03	0.03	0.03	0.04	0.09	0.04	0.02	0.05	0.09	0.11	0.10		

B

0.52	0.59	0.59	0.58	0.60	0.56	0.57	0.51	0.56	0.58	0.56	0.55	0.56	<i>0.05</i>
0.49	0.56	0.58	0.59	0.57	0.58	0.53	0.63	0.56	0.59	0.62	0.53	0.57	<i>0.07</i>
0.47	0.51	0.58	0.59	0.64	0.53	0.60	0.59	0.59	0.63	0.52	0.58	0.57	<i>0.09</i>
0.53	0.59	0.59	0.58	0.65	0.52	0.65	0.57	0.59	0.55	0.58	0.56	0.58	<i>0.07</i>
0.54	0.56	0.61	0.59	0.57	0.58	0.58	0.58	0.59	0.52	0.64	0.53	0.57	<i>0.06</i>
0.52	0.54	0.56	0.59	0.62	0.57	0.51	0.61	0.58	0.59	0.57	0.58	0.57	<i>0.06</i>
0.51	0.60	0.63	0.59	0.58	0.58	0.59	0.57	0.59	0.58	0.59	0.57	0.58	<i>0.05</i>
0.52	0.59	0.57	0.61	0.62	0.59	0.58	0.54	0.64	0.53	0.60	0.55	0.58	<i>0.07</i>
0.51	0.57	0.59	0.59	0.61	0.56	0.58	0.57	0.59	0.57	0.58	0.55		
0.04	0.05	0.04	0.02	0.05	0.05	0.07	0.06	0.04	0.06	0.06	0.03		0.06

Fig 3. Precision of liquid addition by Biomek 2000 under our assay condition. A. BPB (2 μ L, Me₂SO solution) was added to a dry 96-well plate; B. BPB(10 μ L, aqueous solution) was added to 90 μ L of aqueous solution. The absorbance was measured at 592 nm. Averages for each column and row are shown as bold face, and CV are shown as italic.

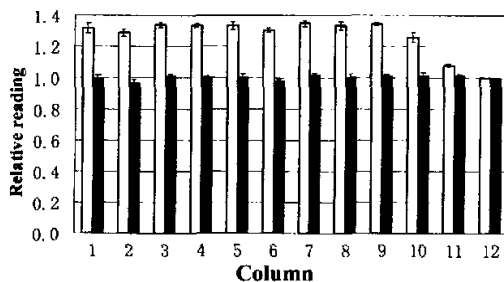


Fig 4. Uniformity on the 96-well plates during the hydrolysis of thiopeptidase by monitoring the hydrolysis kinetically. Open bars are raw data, and filled bars are the data after applying the correction factors.

inhibition with the expected potency with IC₅₀ at 11.8 nmol/L using the pooled data from the 34 plates (Fig 6). Galardin inhibited collagenase 1 at K_i of 0.4 nmol/L^[13], and it is likely that Galardin was titrating C1CD protein under our HTS assay condition since our C1CD protein final concentration was 20 nmol/L.

With these tests on precision of pipetting and instrument reading, as well as data from the control compound Galardin, we understand the degree of fluctuation due to pipetting and reading, and we can comfortably conclude that we would most likely see an inhibition if the compounds to be screened has a significant inhibitory activity

41.4	35.7	42.3	42.4	41.1	39.2	41.1	42.0	42.2	41.6	43.4	36.4	41.1	0.05
38.0	40.8	40.3	39.8	39.7	38.4	41.3	41.9	42.0	41.8	39.7	34.7	40.3	0.03
39.6	38.3	40.2	42.0	38.9	38.8	39.7	38.9	39.9	41.4	40.2	19.8	39.8	0.03
39.6	37.6	40.5	39.4	39.9	42.5	42.0	42.9	41.5	41.4	40.4	2.9	40.7	0.04
38.7	38.0	38.8	38.4	39.0	38.8	39.9	39.6	39.4	41.4	40.4	41.3	39.5	0.03
34.8	38.0	38.4	38.6	39.0	38.5	41.1	39.6	38.9	40.7	40.3	39.1	39.3	0.03
23.2	42.1	39.1	39.6	39.0	38.7	39.9	39.2	39.8	40.9	41.0	40.1	39.9	0.03
1.4	37.6	42.1	39.1	41.8	39.1	41.5	39.2	41.3	42.0	40.3	38.1	40.2	0.04
39.7	38.5	40.2	39.9	39.8	39.3	40.8	40.4	40.6	41.4	40.7	39.6		
0.04	0.05	0.04	0.04	0.03	0.03	0.02	0.04	0.03	0.01	0.03	0.03		0.04

Fig 5. Fluctuation of kinetic reading on SPECTRAMax 340 plate reader. Me₂SO solvent (2 μL) was in compound wells and blank wells, and Galardin in Me₂SO (2 μL) was in control wells with concentration of 60, 20, 6.7, and 2.2 nmol/L. The absorbance changes were recorded at 412 nm for 3 min, and the readings were used for calculating average and CV. Averages for each column and row (values for control wells were excluded) are shown as bold face, and CV are shown as italic.

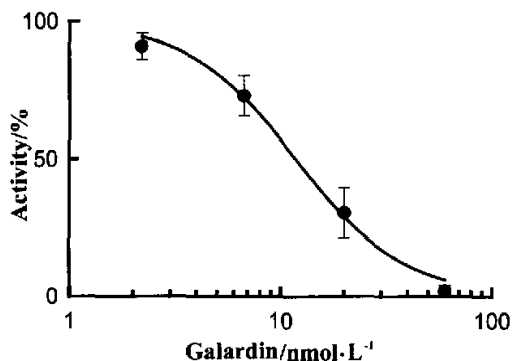


Fig 6. Inhibition of CICD activity by Galardin from pooled data from 34 96-well plates.

at the concentration of 20 mg/L under our HTS condition.

Characterization of the hit compounds The 66 compounds with inhibition higher than 60 % were further investigated at 8 concentrations (33 mg/L, 11 mg/L, 3.7 mg/L, 1.2 mg/L, 407 μg/L, 136 μg/L, 45 μg/L, and 15 μg/L). Among those tested, 44 compounds showed IC₅₀ lower than 20 mg/L, generating a confirming rate at 67 %. Other 22 compounds showed either IC₅₀ higher than 20 mg/L or showed precipitation and were considered false positives.

After taking their molecular weights into account for each active compounds, the best compound obtained from this screening showed an IC₅₀ at 4.2 μmol/L, followed by those with IC₅₀ at 7.1, 8.4, 8.7 μmol/L. There were total 15 compounds with IC₅₀ lower than 20 μmol/L, and 20 compounds with IC₅₀ between 20 and 40

μmol/L, demonstrating the power of the HTS assay in identifying leads for MMP inhibitors. These compounds are under further structure-activity analysis and structural modification for developing potent and selective MMP inhibitors.

DISCUSSION

Random screening has gone through a throughput improvement in recent years, thanks for the advances in sample preparation, assay development, and screening technologies. What took 1 year to screen 10 000 compounds 10 years ago, it takes only days or less to screening even 100 000 compounds^[14]. Usually, the capability of screening 10 000-100 000 compounds per day is considered as high-throughput. We carried out the screening of 2720 compounds during 2 h 10 min, and with sufficient supply of compounds, reagents, and disposables, we could screen close to 10 000 compounds in 8 h and close to 30 000 compounds over a 24-h period, qualifying our screening as a high-throughput screening.

However, we should point out that although the actual screening time was short, it took much longer for weighing a large number of compounds, making mother plates and daughter plates before the actual screening, in addition to the time spent on assay development and recombinant protein production. With the assay throughput reaches a certain level, the bottleneck for improving further the overall throughput will be the plate preparation before the screening and hit confirmation and characterization after the screening.

We achieved this throughput with only limited automation equipments. The two main instruments used are Beckman's Biomek 2000 for liquid handling, and Molecular Devices' SPECTRAMax 340 with a total capital investment of around USD \$ 80 000. The main cost for the screening was on reagents and plastics, which was under RMB ¥ 10 or USD \$ 1 for each wells.

Another advantage in our HTS assay is saving compounds. At 20 mg/L final compound concentration, only 4-μg compound was used in each well. This low demand on compounds is especially helpful for compounds hard obtained from natural sources. Once a Me₂SO solution was made from 1 mg compound, it can be used for several assays, increasing the chance of finding its biological activity.

One unique feature in our HTS assay is monitoring of the hydrolysis kinetically, in contrast to endpoint determination used by most people in pharmaceutical indus-

try. The kinetic monitoring measures the change of UV absorption over a period of time, and thus, it tolerates the interferences from color or other fixed absorption and loosens the requirement for accurate timing of reading. It does require transferring plate once the addition of reagents was finished. Without mechanical devices for automated plate transfer, plate transfer manually could be labor-intensive. Therefore, with proper arrangement of personnel and automation for labor-intensive steps, the kinetic methods could be very useful techniques for certain types of drug screening.

With the collagenase I inhibitor HTS screening, we have demonstrated that HTS assays can be carried out with limited instruments and budgets. With the small-scale screening, we have found C1CD inhibitors with low micromolar inhibitory potency, offering the clues for further drug discovery and development.

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人一型胶原酶抑制剂的高通量筛选¹

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关键词 高通量筛选; 药物筛选; 基质金属蛋白酶

目的: 以重组胶原酶催化区建立高通量药物筛选模型, 筛选胶原酶抑制剂。 **方法:** 在大肠杆菌中表达了人的一型胶原酶的催化区, 并以此建立了高通量药物筛选模型。对一批共 2720 个纯化合物进行了高通量筛选。 **结果:** 胶原酶催化区的高通量筛选中, 完成 2720 个纯化合物的筛选只用了 2 小时 10 分, 每个化合物的用量是 4 μg 。高通量筛选发现 66 个化合物在 20 mg/L 的浓度下具有大于 60% 的抑制活性, 其中有 44 个化合物的抑制活性被随后的多浓度测试所证实。筛选发现的最好化合物的 IC_{50} 为 4.3 $\mu\text{mol/L}$, 共有 IC_{50} 小于 20 $\mu\text{mol/L}$ 的化合物 15 个。 **结论:** 重组胶原酶催化区的高通量药物筛选模型具有快速、微量、准确的特点。

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