



Short communication

Development of MIF/IL-1 β biosensors for discovery of critical quality attributes and potential allergic rhinitis targets from clinical real-world data by intelligent algorithm coupled with in vitro and vivo mechanism validation

Lijuan Ma^{a,1}, Yanfei Zheng^{b,1}, Ji Wang^{b,1}, Qianqian Li^a, Jingqi Zeng^a, Zijian Wang^a, Tingjun Hou^c, Yang Zhang^d, Mingshuang Li^a, Rongmin Shen^b, Xuemei Chen^b, Jingbo Qin^b, Leting Lei^a, Qing Xia^e, Qi Wang^{b,f,**}, Yanjiang Qiao^{a,f,***}, Zhisheng Wu^{a,f,*}

^a Beijing University of Chinese Medicine, School of Chinese Materia Medica, Beijing, 102488, China

^b Beijing University of Chinese Medicine, School of Traditional Chinese Medicine, Beijing, 102488, China

^c Zhejiang University, College of Pharmaceutical Sciences, Zhejiang, 310058, China

^d Chinese Academy of Sciences, Institute of Semiconductors, Beijing, 100083, China

^e Biology Institute of Shandong Academy of Sciences, Shandong, 250014, China

^f Pharmaceutical Engineering and New Drug Development of TCM of Ministry of Education, Beijing, 102488, China

ARTICLE INFO

Keywords:

High electron mobility transistor biosensor
Clinical real-world data
Intelligent algorithm
Allergic rhinitis
Macrophage migration inhibitory factor
Interleukin-1 β

ABSTRACT

There are still huge challenges from clinical real-world data to accurate targets and critical quality attributes (CQAs) for effective treatment of allergic rhinitis (AR). Here, we present a novel integrated strategy that biosensors and intelligent algorithms were used to angle AR targets and CQAs from clinical real world. Firstly, bagging and boosting partial least squares discrimination analysis (PLS-DA) and Monte-Carlo sampling were proposed to screen accurate AR targets. Macrophage migration inhibitory factor (MIF) and Interleukin-1 β (IL-1 β) potential targets were obtained based on large-scale analysis of one thousand proteins and in-depth precise screening of seventy proteins. Furthermore, high electron mobility transistor (HEMT) biosensors were fabricated and successfully modified by MIF and IL-1 β potential targets with a low detection concentration as 1 pM and quantitative range from 1 pM to 10 nM. Surprisingly, through MIF/IL-1 β biosensors, we angled 5-O-methylvisammoside, amygdalin, and cnicifugoside three CQAs. The strong interaction was discovered among three CQAs and MIF/IL-1 β biosensors with almost all K_D up to 10^{-11} M. Finally, interaction among three CQAs and MIF/IL-1 β biosensors were evaluated by in vitro and vivo experiments. In this paper, two critical potential targets and three effective CQAs for AR treatment were discovered and validated by biosensor and advanced algorithms. It provides a superior integrated idea for angling critical targets and CQAs from clinical real-world data by biosensors and informatics.

1. Introduction

Allergic rhinitis (AR) is a common chronic hypersensitivity reaction (Kurganskiy et al., 2021; Lancet, 2008), of which the incidence ranks first among all allergic diseases (Wheatley and Togias, 2015). With the growing side-effect shocking caused by large-scale use of antibiotics and

antihistamines (Karaki et al., 2013), clinical real-world data have become increasingly essential to accurate targets and effective critical quality attributes (CQAs) for AR (Aljabban et al., 2019; Kurtz et al., 2019). How to achieve the goal from clinical data to critical targets and CQAs? Several commercial techniques have been extensively explored, such as fluorescence, enzyme linked immunosorbent assay, and surface

* Corresponding author. Beijing University of Chinese Medicine, School of Chinese Materia Medica, Beijing, 102488, China.

** Corresponding author. Beijing University of Chinese Medicine, School of Traditional Chinese Medicine, Beijing, 102488, China.

*** Corresponding author. Beijing University of Chinese Medicine, School of Chinese Materia Medica, Beijing, 102488, China.

E-mail addresses: wangqi710@126.com (Q. Wang), yjqiao@bucm.edu.cn (Y. Qiao), wzs@bucm.edu.cn (Z. Wu).

¹ The authors contributed equally to this work.

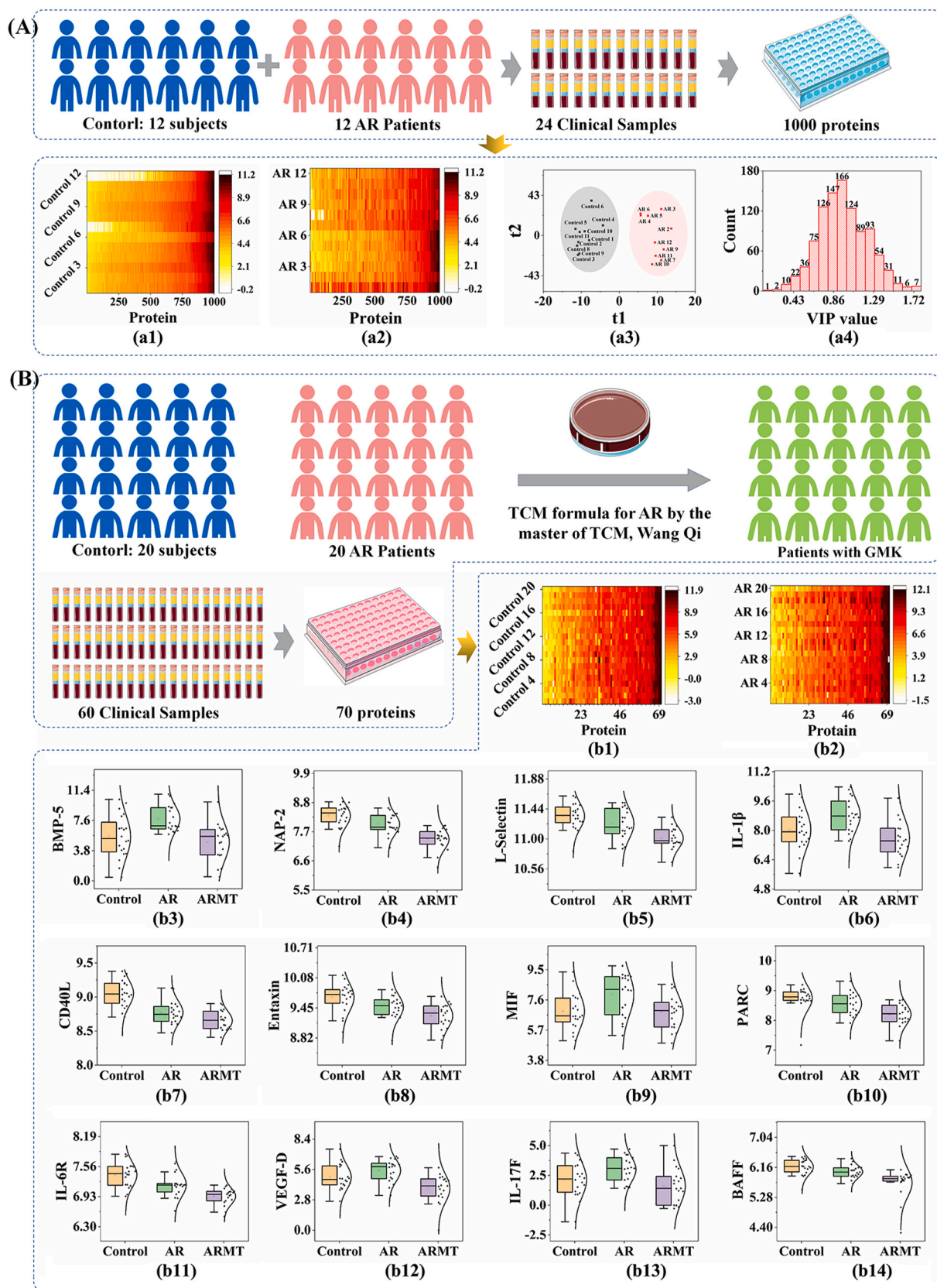


Fig. 1. (A) Large-scale screening from one thousand proteins. (a1) - (a2) The visualized heat map of one thousand proteins content in control group and AR group; (a3) Classification results of control group and AR group; (a4) The VIP distribution of one thousand proteins. (B) In-depth screening from seventy important proteins. (b1) - (b2) The visualized heat map of seventy proteins content in control group and AR group; (b3) - (b14) The box charts of 12 differential proteins in these three groups.

plasmon resonance. While there are still challenges of low-sensitivity and high-cost in the system of biomacromolecules and CQAs.

For this, biosensor is a powerful and promising tool (Park et al., 2020; Wang et al., 2013; Miao et al., 2017; Zamfir et al., 2013). The global biosensor market was up to \$8.2 billion in 2009, and expected to grow by 6.3% annually (Scognamiglio et al., 2010). A centerpiece in successful clinical application of the biosensors is sensitivity and accuracy. At present, the detection limit (DL) of commercial biosensors can be measured as low as nM between biomacromolecules. Nevertheless, how to reduce the DL of the interaction between biomacromolecule and small molecule is still an urgent problem.

High electron mobility transistor (HEMT) biosensor (Ding et al., 2014; Pulikkathodi et al., 2018) is used widely in molecular recognition because of its low DL (Lee et al., 2016; Tomioka et al., 2012). Its DL is 0.1 pg/mL in detecting prostate-specific antigens (Li et al., 2014). It also exhibits high sensitivity and dynamic range, offering extremely low DL in clinical real word (Saragadharan et al., 2018). Previously, our team successfully developed a HEMT biosensor, which realized the application on high-throughput screening for bitter substances (Wang et al., 2018).

However, given representativeness of small samples and reliability of the biosensor, there are still challenges for the integration of clinical real-word data and biosensors. A centerpiece in successful combination of these is intelligence algorithms (Li et al., 2020). On the one hand, it is useful to reduce errors in the rare clinical real-world data. Partial least squares (PLS) regression is a classical multivariable algorithm (Wu et al., 2013). Moreover, systematic modeling was developed for small-sample analysis, such as bagging and boosting PLS (Galvao et al., 2006; Pan et al., 2015; Zhao et al., 2018). On the other hand, intelligence algorithms are also essential for the biosensor application (Baronas et al., 2010). It has been introduced to analytical characteristics of actual biosensors since 1970s. This opens the idea of the biosensor application in clinical real word.

Here, the rare clinical real-world samples and ourselves bagging/boosting PLS algorithm were integrated to screen critical AR targets. Furthermore, HEMT biosensors modified by the critical potential targets were developed to angle CQAs for AR treatment. Finally, the performance and applicability of biosensors were investigated by in vivo and vitro experiments. These provide a perfect strategy to angle critical targets and CQAs for AR treatment.

2. Materials and methods

2.1. Materials and instruments

The interaction was realized by Chi660E electrochemical workstation (Chenhua Instrument, China)). The elements on the HEMT chip were measured by X-ray photoelectron spectroscopy (XPS) (ThermoFisher, USA). Other materials and instruments were shown in Supplementary Material.

2.2. Clinical real-word sample

12 healthy people (Control 1, Control 2, ...Control 12) and 12 AR patients (AR 1, AR 2, ...AR 12) were selected for the large-scale screening of one thousand proteins in clinical trials. Furthermore, for in-depth screening of seventy critical proteins, 40 subjects were selected and divided into two groups, 20 healthy (Control 1, Control 2, ...Control 20) and 20 AR patients (AR 1, AR 2, ...AR 20). In addition, 20 AR patients treated by Guominkang (GMK) were the third group (ARMT 1, ARMT 2, ...ARMT 20). Notably, GMK is one decoction proposed by Academician Wang Qi. Its excellent efficacy on AR patients and CQAs were showed in Supplementary Material Fig. S1 and Table S2.

The informed written consent from all participants was obtained prior to the research. With the funding of the National Natural Science Foundation of China (81473554), the ethical review approval was

proved by the ethics board/committee of Beijing University of Chinese Medicine, and numbered as BJZYDX-LL-2014011.

2.3. Large-scale screening of critical AR targets from clinical real-word data by advanced algorithm

Bagging and boosting PLS discrimination analysis (PLS-DA) combined with Monte-Carlo sampling was used for large-scale screening of one thousand proteins from above clinical real-word samples. Furthermore, seventy critical proteins were discovered and used for in-depth screening of AR targets. The specific protocol was provided in Supplementary Material.

2.4. Fabrication and evaluation of biosensors by critical AR targets

Furthermore, the potential targets screened from clinical real-word data were immobilized on the HEMT chip. XPS was introduced to verify the immobilization of the targets with a monochromatic Al Ka source and a hemispherical electron energy analyzer. The DL, quantitation limit (QL), detection range, and specificity of biosensors were investigated by Chi660E electrochemical workstation and our previous algorithms. The drain-source current ($I_{DS-V_{DS}}$) was measured with the voltage as 2.0 V and sampling rate as 10 ns.

2.5. Angling of CQAs for AR by MIF and IL-1 β biosensor

Eleven concentrations of CQAs gradients ranging from 0.1 pM to 1.0 mM were measured by the biosensor. 0.1 M phosphate buffered saline (PBS) solution was used as the control. Each test was repeated three times. Dissociation constant (K_D) was calculated to angle CQAs (Saragadharan et al., 2018).

2.6. Validation of biosensor application by in vitro and vivo investigation

In vitro validation of biosensor applicability was investigated by network pharmacology and molecular docking. Furthermore, in vivo validation was implemented by the inhibition bioactivity of CQAs on migration of zebrafish macrophages. Here, zebrafish were divided into control, model, positive drug, and CQAs groups (62.5, 125, 250, 500, 1000 μ M). Each group was paralleled six times. Specific protocol was provided in Supplementary Material.

2.7. Statistical analysis

Statistical analyses of clinical real-word data and biosensor results were performed on SPSS13.0 (International Business Machines Corporation, USA). Bagging and boosting PLS-DA algorithm were written by ourself and completed on MATLAB R2016a (MathWorks Inc, USA).

3. Results and discussion

3.1. Discovery of MIF and IL-1 β potential targets by large-scale and in-depth screening from clinical real-word data coupled with intelligence algorithm

Fig. 1 showed the critical potential targets from clinical real-word data. Firstly, by large-scale analysis of one thousand proteins, seventy proteins were screened by orthogonal-PLS-DA (OPLS-DA) coupled with variable importance projection (VIP) (Fig. 1(A)). Furthermore, these seventy proteins were used for in-depth screening. We found twelve proteins had remarkable differences among the three groups, which were showed in Fig. 1(B) and Supplementary Material Table S3. Grati-fyingly, bagging and boosting PLS-DA models overcome the challenge of poor reliability of small-sample analysis. Validation results showed in Supplementary Material Table S4, indicating these twelve proteins were critical to recognize AR patients. In which, MIF and IL-1 β changed

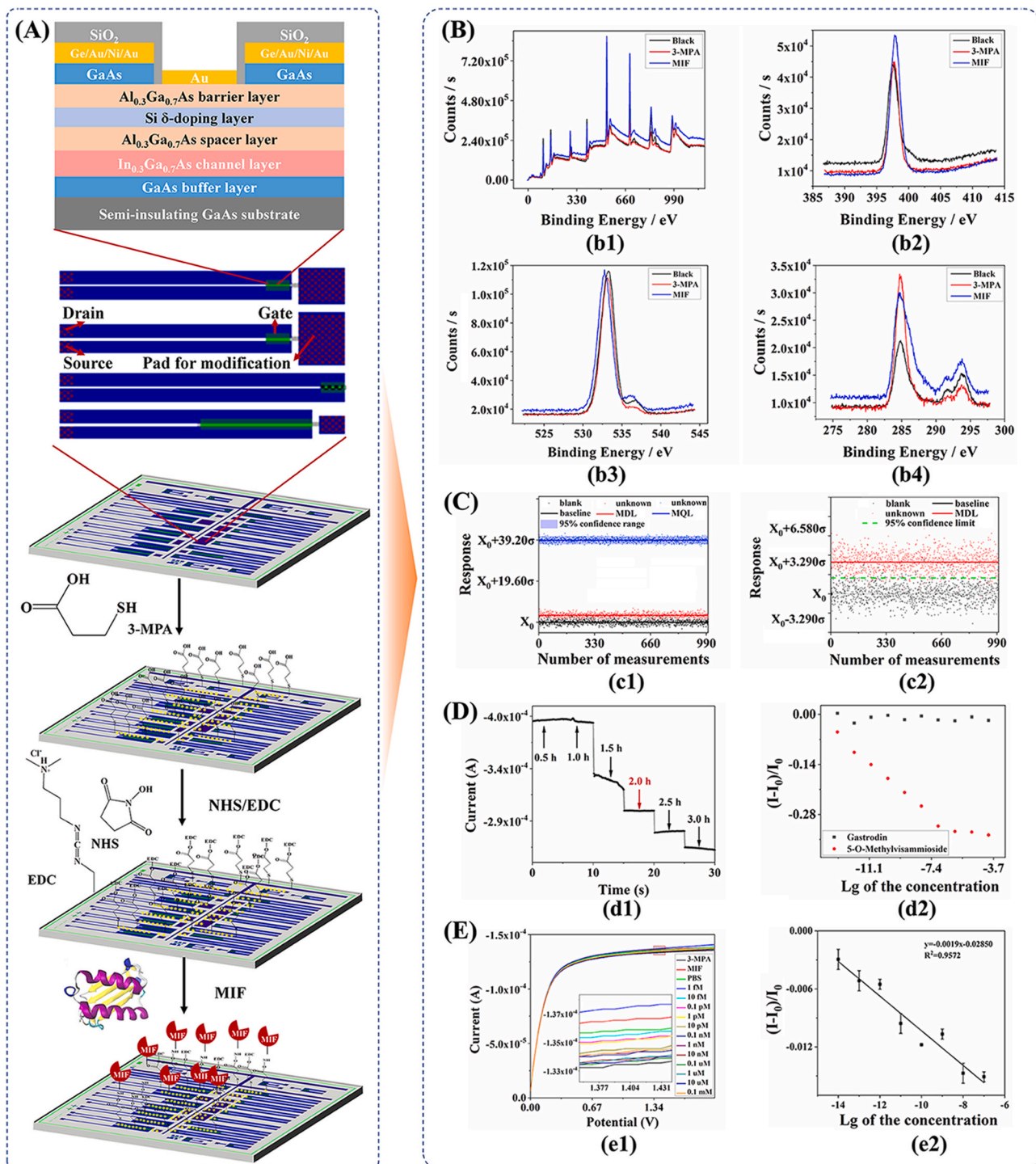


Fig. 2. (A) The procedure of fabrication of MIF-HEMT biosensor. (B) XPS results of the protein modified biosensor. (b1) full scan peak, (b2) 1s orbital of C, (b3) 1s orbital of N, (b4) 1s orbital of O. (C) The schematic diagram of DL and QL error of one thousand simulation experiments. (c1) DL, (c2) QL. (D) Investigation of the time of protein modification (d1), the sensitivity and quantitative concentration range (d2). (E) (e1) $I_{DS}-V_{DS}$ of MIF-HEMT biosensor at different concentrations of ISO-1, (e2) The linear model with Lg of the concentration as the abscissa and $\Delta I/I_0$ as the ordinate.

significantly, are two critical potential targets of AR.

3.2. Fabrication and evaluation of biosensors of critical potential targets by MIF and IL-1 β

Fig. 2(A) showed the fabrication protocol of HEMT biosensor. As the results of XPS (Fig. 2(B)), the peak height and half peak width of N1s increased significantly after target immobilization, and the peak centers

were at 397.6 (NH₂) and 397.7 EV(NH₃⁺). Markedly, a new carbonyl peak appeared at 292.0 EV. Simultaneously, the carbonyl group was activated by carboxyl activation and finally formed a peptide bond with the amino group (294.0 EV), indicating the target was successfully modified on the biosensor.

Furthermore, the DL and QL of HEMT biosensor was evaluated in Fig. 2(C). Fig. 2(d1). It showed markedly that 2 h is the best time for protein modification. Interestingly, biosensor had high specificity of

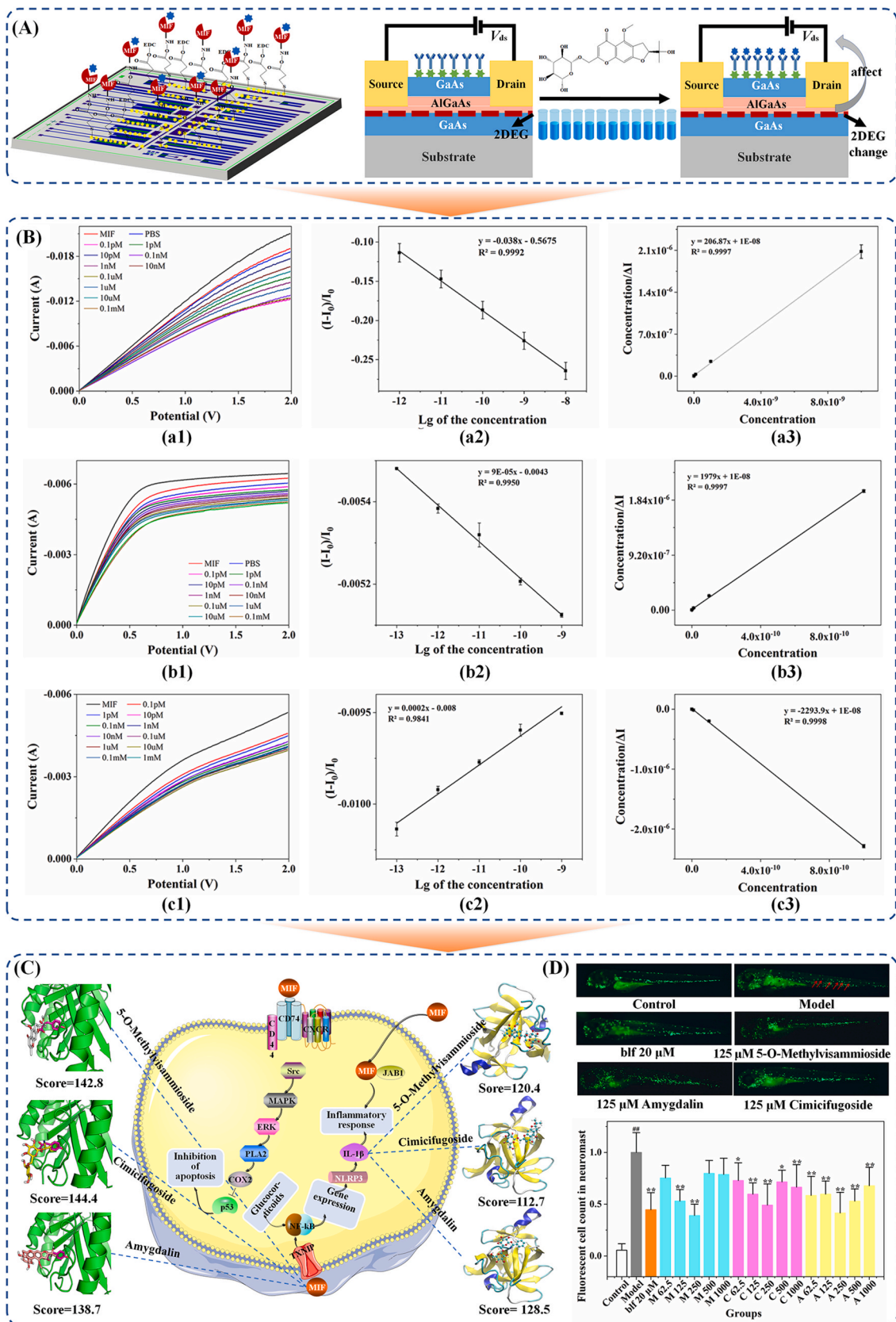


Fig. 3. (A) The schematic diagram of the measure process. (B) The interaction results of 5-O-methylvisammoside, amygdalin, and cimiticifugoside. (a1) I_{DS} - V_{DS} regularity decreases with increasing concentration of 5-O-methylvisammoside in the range from 1.0 pM to 10.0 nM, (b1) cimiticifugoside, (c1) amygdalin. (a2) The linear fitting between $\lg$ of 5-O-Methylvisammoside concentration and $\Delta I/I_0$, (b2) cimiticifugoside, (c2) amygdalin. (a3) $[Ag]/\Delta I$ versus $[Ag]$ of 5-O-methylvisammoside, (b3) cimiticifugoside, (c3) amygdalin. (C) The potential action mechanism of 3 candidates from GMK on AR. (D) The inhibition of three CQAs with different concentration on the migration of zebrafish macrophages.

specific reorganization of 5-O-methylvisamminoside, as shown in Fig. 2(d2). Besides, Fig. 2(E) showed the interaction result of positive control between MIF and ISO-1. There was a perfect linear fitting ranged from 1.0 fM to 0.1 μ M, which was an appropriate quantitative detection range.

3.3. A successive application and validation of MIF/IL-1 β biosensors on precise angling of CQAs

3.3.1. Successive application of MIF/IL-1 β biosensors on precise angling of CQAs for AR

According to the protocol of Fig. 3(A), Fig. 3(B) showed the interaction results that the K_D of 5-O-methylvisamminoside, amygdalin, and cimicifugoside were 48.34, 4.360, and 5.503 pM, respectively. Notably, three CQAs all have perfect affinity with MIF. Simultaneously, gastrodin cannot bound to MIF in negative control.

Similarly, we found the strong interaction between IL-1 β and amygdalin, cimicifugoside and 5-O-methylvisamminoside that the K_D were 6.121 pM, 44.86 nM, and 12.23 pM, respectively. The interaction detail and relative standard deviation (RSD) were shown in detail in Supplementary Material Fig. S2 and Table S5. It exhibits high sensitivity and dynamic range of MIF/IL-1 β biosensors. These indicated MIF and IL-1 β were indeed critical potential targets, and these candidates were indeed CQAs for AR.

3.3.2. Validation of MIF/IL-1 β biosensor application by in vitro and vivo investigation

Fig. 3(C) showed in vitro validation results of biosensor application. The LibDockScore of molecular docking revealed strong interaction between CQAs and MIF/IL-1 β . Nuclear factor kappa-B (NF- κ B) was one key pathway of these CQAs. Notably, MIF and IL-1 β were two essential potential targets in NF- κ B pathway, suggesting NF- κ B was indeed a vital pathway of these CQAs for AR treatment.

Furthermore, in vivo validation by inhibition bioactivity on zebrafish macrophage migration shown in Fig. 3(D). The result showed 250 μ M of 5-O-methylvisamminoside, cimicifugoside, and amygdalin had better antiallergic effect on inhibiting macrophage migration. There was stronger inhibition bioactivity in these CQAs than that in positive group. Most importantly, it showed these CQAs could inhibit the migration of macrophages through MIF, resulting in a decrease of IL-1 β and playing the role of AR treatment. The above evidences verified the strong applicability of the MIF/IL-1 β biosensors and informatics.

4. Conclusion

Integrating of clinical real-world data and bagging/boosting-PLS-DA, MIF and IL-1 β HEMT biosensors have been successfully developed with a low DL and QL. Its detection concentration was low as 1.0 pM and quantitative range from 1.0 pM to 0.1 μ M. According to the above biosensors and intelligence algorithms, we triumphantly angled three CQAs for AR, almost all K_D of which come up to 10^{-11} M. Furthermore, the strong applicability of biosensors were verified by in vitro and vivo mechanism investigation. Finally, a superior idea was proposed for the integrated innovation of clinical real-world data, biosensors, intelligence algorithms, and methodological verification. Taken together, the present study provides a novel guideline for the angling of critical potential targets and CQAs by biosensor and informatics. Future works will be focused on developing multi-taste-receptor-modified biosensor array to angle CQAs with efficacy for Chinese materia medica.

CRedit authorship contribution statement

Lijuan Ma: Conceptualization, Data curation, Formal analysis,

Software, Writing – original draft, Writing – review & editing, Visualization, Validation. **Yanfei Zheng:** Collection of clinical real-world samples. **Ji Wang:** Collection of clinical real-world samples. **Qianqian Li:** Investigation. **Jingqi Zeng:** Algorithm. **Zijian Wang:** Experiment. **Tingjun Hou:** Experiment. **Yang Zhang:** Fabrication of HEMT. **Mingshuang Li:** Visualization. **Rongmin Shen:** Collection of clinical real-world samples. **Xuemei Chen:** Experiment. **Jingbo Qin:** Investigation. **Leting Lei:** Investigation. **Qing Xia:** Experiment. **Qi Wang:** Supervision. **Yanjiang Qiao:** Supervision. **Zhisheng Wu:** Conceptualization, Funding acquisition, Project administration, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work was co-supported by National Science Fund for Excellent Young Scholars (82022073), Natural Science Foundation of China (81773914), National Key Research and Development Program of China (2018YFC1706900, 2019YFC1711200).

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.bios.2021.113608>.

References

- Aljabban, J., McDermott, S., Wanner, R.A., Salhi, H., Aljabban, N., Mukhtar, M., Mohammed, Z., Aljabban, H., Panahiazar, M., Hadley, D., 2019. J. Allergy Clin. Immunol. 143 (2), Ab185-Ab185.
- Baronas, R., Ivanauskas, F., Kulys, J., 2010. Springer. Dordrecht, New York.
- Ding, K., Wang, C.Y., Zhang, B.T., Zhang, Y., Guan, M., Cui, L.J., Zhang, Y.W., Zeng, Y.P., Lin, Z., Huang, F., 2014. IEEE Electron. Device Lett. 35 (3), 333–335.
- Galvao, R., Arajio, M., Martins, M., José, G., Pontes, M., Silva, E.C., Saldanha, T., 2006. Chemometr. Intell. Lab. Syst. 81 (1), 60–67.
- Karaki, M., Akiyama, K., Mori, N., 2013. Auris Nasus Larynx 40 (3), 277–281.
- Kurganskiy, A., Creer, S., Vere, N.D., Griffith, G.W., Skjth, C.A., 2021. Sci. Adv. 7.
- Kurtz, D.M., Esfahani, M.S., Scherer, F., Soo, J., Alizadeh, A.A., 2019. Cell 178 (3), 1.
- Lancet, T., 2008. Lancet 371 (9630), 1.
- Lee, H.H., Bae, M., Jo, S.H., Shin, J.K., Son, D.H., Won, C.H., Lee, J.H., 2016. Sensor. Actuator. B Chem. 234, 316–323.
- Li, J.D., Cheng, J.J., Miao, B., Wei, X.W., Xie, J., Zhang, J.C., Zhang, Z.Q., Wu, D.M., 2014. J. Micromech. Microeng. 24 (7).
- Li, Q.Q., Zeng, J.Q., Lin, L., Zhang, J., Zhu, J.Y., Yao, L., Wang, S.Y., Yao, Z.Q., Wu, Z., 2020. Food Chem. 332, 127356.
- Miao, X.S., Cui, Q.Y., Wu, H.H., Qiao, Y.J., Zheng, Y.F., Wu, Z.S., 2017. Acta Pharm. Sin. B 3 (7), 137–145.
- Pan, X.N., Li, Y., Wu, Z.S., Zhang, Q., Zhou, Z., Shi, X.Y., Qiao, Y.J., 2015. Sensors 15 (4), Basel, Switzerland.
- Park, S.J., Kim, J., Kang, S., Cha, H.J., Min, D.H., 2020. Sci. Adv. 6 (22), eaaz8201.
- Pulikkathodi, A.K., Sarangadharan, I., Hsu, C.P., Chen, Y.H., Hung, L.Y., Lee, G.Y., Chyi, J.I., Lee, G.B., Wang, Y.L., 2018. Sensor. Actuator. B Chem. 257, 96–104.
- Sarangadharan, I., Regmi, A., Chen, Y.W., Hsu, C.P., Chen, P.C., Chang, W.H., Lee, G.Y., Chyi, J.I., Shiesh, S.C., Lee, G.B., Wang, Y.L., 2018. Biosens. Bioelectron. 100, 282–289.
- Scognamiglio, V., Pezzotti, G., Pezzotti, I., Cano, J., Buonasera, K., Giannini, D., Giardi, M.T., 2010. Microchim. Acta. 170 (3–4), 215–225.
- Tomoka, K., Yoshimura, M., Fukui, T., 2012. Nature 488 (7410), 189.
- Wang, T., Hu, N., Cao, J., Wu, J., Su, K., Wang, P., 2013. Biosens. Bioelectron. 49 (Complete), 9–13.
- Wang, Z.X., Zhang, Y.X., Zeng, Y.L., Li, X., Chen, Z., Luo, J.M., Zhang, Y., Zhang, Y.L., Qiao, Y.J., 2018. J. Agric. Food Chem. 66 (44), 11663–11671.
- Wheatley, L.M., Togias, A., 2015. N. Engl. J. Med. 372 (5), 456–463.
- Wu, Z.S., Peng, Y.F., Chen, W., Xu, B., Ma, Q., Shi, X.Y., Qiao, Y.J., 2013. Biosens. Bioelectron. 137, 394–399.
- Zamfir, L.G., Rotariu, L., Bala, C., 2013. Biosens. Bioelectron. 46, 61–67.
- Zhao, N., Ma, L.J., Huang, X.G., Liu, X.N., Qiao, Y.J., Wu, Z.S., 2018. Front. Chem. 6, 1–7.

Supplementary Materials

Development of MIF/IL-1 β Biosensors for Discovery of Critical Quality Attributes and Potential Allergic Rhinitis Targets from Clinical Real-world data by Intelligent Algorithm coupled with in Vitro and Vivo Mechanism Validation

Lijuan Ma^{a, 1}, Yanfei Zheng^{b, 1}, Ji Wang^{b, 1}, Qianqian Li^a, Jingqi Zeng^a, Zijian Wang^a, Tingjun Hou^c, Yang Zhang^d, Mingshuang Li^a, Rongmin Shen^b, Xuemei Chen^b, Jingbo Qin^b, Leting Lei^a, Qing Xia^e, Qi Wang^{b, f*}, Yanjiang Qiao^{a, f*}, Zhisheng Wu^{a, f*}

^a Beijing University of Chinese Medicine, School of Chinese Materia Medica, Beijing, China, 102488.

^b Beijing University of Chinese Medicine, School of Traditional Chinese Medicine, Beijing, China, 102488.

^c Zhejiang University, College of Pharmaceutical Sciences, Zhejiang, China, 310058.

^d Chinese Academy of Sciences, Institute of Semiconductors, Beijing, China, 100083.

^e Biology Institute of Shandong Academy of Sciences, Shandong, China, 250014.

^f Pharmaceutical Engineering and New Drug Development of TCM of Ministry of Education, Beijing, China, 102488.

*Corresponding author: Zhisheng Wu, Yanjiang Qiao, Qi Wang.

Email: wzs@bucm.edu.cn, yjqiao@bucm.edu.cn, wangqi710@126.com.

¹The authors contributed equally to this work.

1 Materials and Methods

1.1 Materials and instruments

Standards of Cimicifugoside (CAS: 84272-85-5; Lot: 3438), Amygdalin (CAS: 29883-15-6; Lot: 5930), and 5-O-Methylvisammioside (CAS: 80681-45-4; Lot: 2075) was supplied by Shanghai Nature-standard Technical Service Co., Ltd (Shanghai). The Chinese herb medicine Fangfeng (20150724), Wumei (20151210), Chantui (20150805), Lingzhi (20150705), Shouwuteng (20150915), and Tianma (20150812) was purchased from Ben Cao Fang Yuan Medicine Co. LTD. (Beijing, China). Macrophage Migration Inhibitory Factor (MIF), Interleukin 1 beta (IL-1 β), 10 mM polarization beam splitter (PBS) solution, 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC), and N-hydroxysuccinimide (NHS) was purchased from Zhongnong Boxin (Beijing) Technology Co., Ltd. Rat interleukin 4, and rat immunoglobulin E were supplied by Cusabio Biotech Co., Ltd. QAH-CUST kit was purchased from RayBiotech, USA. Fire-fighting pertussis toxin and 10% chloral hydrate were donated by China-Japan Friendship Hospital. Methanol and acetonitrile were purchased from Fisher Company (USA).

Analysis of serum samples was performed on an Ultra-performance liquid chromatography-quadrupole-electrostatic field/orbitrap high-resolution mass spectrometry (Thermo-Fisher, USA). Fluorescence detection was implemented on a fluorescence scanner InnoScan 300 Microarray Scanner (Innopsys, France).

1.2 The critical quality attributes (CQAs) identification of Guominkang (GMK)

1.2.1 The AR animal model for CQAs identification of GMK

The CQA is a physical, chemical, biological, or microbiological property or characteristic that should be within an appropriate limit, range, or distribution to ensure the desired product quality. CQAs, proposed by Food and Drug Administration, are generally associated with the drug substance, excipients, intermediates (in-process materials), and drug product. CQAs was identified as follow:

Firstly, 60 SPF male rats were randomly divided into two groups. Control group includes 10 rats, and model group includes 50 rats. According to the laboratory animal management standards, the living temperature of animals was 18-22 °C, the relative humidity was 38~40%. The 21-day experimental period was initiated after 3-day acclimatization.

On the first day of basic sensitization stage, a mixture of saline (1 mL), egg white protein (1.5 mg), aluminum hydroxide sol (3 mg), and inactivated pertussis bacteria was injected

subcutaneously into the four paws of the rat. Specifically, the forefoot was injected into 0.2 mL mixture and the hind paw was 0.3 mL. On the 5th day, another mixture of saline (1 mL), egg white protein (1.0 mg), aluminum hydroxide sol (2 mg) was injected into the five subcutaneous sites on the back with 0.2 mL. In the second week, five rats have been placed in a transparent box with an atomizing device. The transparent box was atomized with 0.5% egg albumin (OVA) physiological saline for about 15 min. In the last week, rats were instilled with 5% egg protein solution once a day in the nose. Each nostril was instilled with the mixture of 30 μ L. The control group was injected with saline.

Based on the successful AR modeling, rats of model group were divided to model group, positive drug group, low-dose GMK group, medium-dose GMK group, and high-dose GMK group. After intragastric administration for 7 days, rats were bled from the orbits at 15 min, 30 min, 60 min, 90 min, 120 min, 150 min, 240 min, 480 min, and 720 min, respectively. Serum samples were obtained by 10-min centrifugation with 3000 rpm in 4 $^{\circ}$ C, and stored at -80 $^{\circ}$ C.

The above serum samples were vortexed for 3 min, and centrifuged for 20 min (4 $^{\circ}$ C, 10000rpm) with 1.5 mL methanol. The supernatant was blown dry with nitrogen at 37 $^{\circ}$ C, and then reconstituted with 400 μ L of methanol. The supernatant after 10-min centrifuging was used as the analytical sample. Each of the above serum was taken 500 μ L and dissolved in 1.5 ml of methanol. The samples were vortexed for 3 min and then centrifuged for 20 min (4 $^{\circ}$ C, 10000rpm). Next, each sample was blown dry with nitrogen at 37 $^{\circ}$ C and reconstituted with 400 μ L of methanol. Finally, each sample was centrifuged for 10 min (4 $^{\circ}$ C, 10000rpm). The supernatants were collected as the animal serum samples for the detection of CQAs.

1.2.2 The animal serum for CQAs identification of GMK by UPLC-MS

The animal serum samples for the detection of CQAs were analyzed by Waters ACQUITY UPLC HSS T3 Column (1.7 μ m, 2.1 $\times$ 100 mm). The column temperature was set as 40 $^{\circ}$ C, detection wavelength as 327 nm, and the volume as 10 μ L. Mobile phase A was an aqueous solution containing 0.1% formic acid and 4 mM ammonium formate, and B was an acetonitrile solution containing 0.1% formic acid and 4 mM ammonium formate. The flow rate was set as 300 μ L/min. The schedule of gradient elution was shown in **Table S1**.

Table S1 The schedule of gradient elution for the identification of CQAs.

Time (min)	A	B
0	90%	10%

1	90%	10%
10	0%	100%
12	0%	100%

Analysis of the constituents was implemented by HESI positive ion acquisition mode with 30 arb sheath gas and 10 arb auxiliary gas. The spray voltage was set as 3.5 kV, the capillary temperature as 325 °C, and the heater temperature as 350 °C. The scan was carried out with Full Scan/ddMS2 mode in the range of 100- 800 m/z. The resolution is MS Full Scan 70000 FWHM and MS/MS 17500 FWHM. NCE is 35 eV.

1.3 Large-scale screening of critical potential AR targets from clinical real-word data by advanced algorithm

1.3.1 The inclusion and exclusion criteria of the clinical real-word samples

Inclusion criteria: (1) Clinical subjects are judged as patients with typical allergic rhinitis (AR) and healthy subjects, respectively. (2) Clinical subjects had moderate to severe persistent allergic rhinitis, according to the international diagnostic criteria for allergic rhinitis established by the World Health Organization (WHO). (3) Clinical subjects of AR patients are judged by the positive allergen skin prick test or the positive specific immunoglobulin E (Ig E) antibody. (4) Clinical subjects of healthy sample are judged by the normal physical indicators. (5) Clinical subjects are aged 18-65 years old. (6) All clinical subjects include half male and half female.

Exclusion criteria: (1) Clinical subjects have diseases other than allergic rhinitis. (2) Clinical subjects have allergic urticaria, eczema, purpura, and other allergic diseases. (3) Clinical subjects have other respiratory infections within 14 days, such as asthma, cough variant asthma, etc. (4) Clinical subjects have chronic sinusitis or other non-allergic rhinitis, such as vasomotor rhinitis, infectious rhinitis, taste rhinitis, non-allergic rhinitis with eosinophilia syndrome, drug-induced rhinitis, etc. (5) Clinical subjects have organic disease in the nasal cavity or undergo nasal cavity surgery. (6) Clinical subjects have undergone specific immunotherapy or systemic hormone therapy in the past year. (7) Clinical subjects have mental illness (8) Clinical subjects are taking other chronic disease drugs. (9) Clinical subjects are pregnant and lactating women. (10) Clinical subjects are poorly compliant considered by clinical investigators.

Before blood collection, clinical real-word samples need to avoid drinking and tiredness one day, while women need to avoid menstruation. In the morning, 5 mL of venous blood was extracted by full-time medical staff of the hospital. Each blood sample was anticoagulated with heparin.

Then, serum was separated from the above blood within two hours. Finally, the serum samples were stored at - 80 °C and used as the clinical real-world samples.

1.3.2 Large-scale screening coupled with in-depth screening for critical potential AR targets

Large-scale screening from one thousand proteins and in-depth screening from seventy important proteins for critical AR targets were carried out by the protein chips provided by Guangzhou Raybiotech Biotechnology Co. Ltd. The specific testing protocol was in accordance with the instructions of this protein chip kit. Axon genepix 4000 B (American molecular instrument company) was used to scan antibody sandwich chip kit. The PMT was set as 650, wavelength was 532 nm, and resolution was 10um. AAH-BLG-1000 software was used for data pre-analysis.

1.4 Fabrication and evaluation of biosensors by critical potential AR targets

A 500 nm GaAs buffer layer was used as the foundation. Subsequently, a 15 nm $\text{In}_{0.3}\text{Ga}_{0.7}\text{As}$ channel layer, a 4 nm $\text{Al}_{0.3}\text{Ga}_{0.7}\text{As}$ spacer layer, a Si δ -doping layer, a 25 nm $\text{Al}_{0.3}\text{Ga}_{0.7}\text{As}$ barrier layer and a 30 nm GaAs cap layers was put on layer by layer.

Firstly, 3-mercaptopropionic acid (3-MPA) with 10 mM was used to form a layer of Au-S bond on the HEMT chip for 24 h. Next, washing the chip 3 times by water, a mixture of 30 μL EDC (20 mM) and 30 μL NHS (50 mM) were used for carboxyl activation. After the stable amine adducts were generated, 100 $\mu\text{g}/\text{mL}$ MIF dissolved in 0.1 M PBS was incubated on the HEMT chip at 4 °C.

1.5 Validation of biosensor application by in vitro and vivo investigation

1.5.1 In vivo validation of MIF/IL-1 β biosensor application by molecular docking

The protein crystal structure files of MIF and IL-1 β were downloaded from Protein Data Bank (PDB). The crystal structure with high resolution and corresponding bioactive ligands is preferred. The PDB ID of MIF is human protein 6b1k, and the PDB ID of IL-1 β is 5r8q. The clean protein tool in Discovery Studio 2.5 (DS 2.5, Accelrys Inc., San Diego,CA, USA) was used to pretreatment the protein. It included deleting the ligand and water molecules, completing incomplete residues, deleting redundant protein conformation, hydrogenating, and allocating related charges. Libdock program was used for molecular docking, in which 'formation method' was determined as 'BEST', and the other options were set as default settings.

After the original ligand is taken out, it is redocked into the active pocket. If the root mean square deviation (RMSD) is less than or equal to 2, it shows that the related parameters can simulate the binding mode of the ligand and the protein, which could be used as the active pocket

of the protein. cimirubin, 5-O-methylvisamycin, amygdalin and gastrodin were docked with MIF and IL-1 β protein, respectively. Discovery Studio 2.5 software operation analysis tool was used to process docking results. Libdockscore was calculated to evaluate the best pose.

1.5.2 In vivo validation of MIF/IL-1 β biosensor application by zebrafish

Zebrafish embryo acquisition: the female and male zebrafish were fed separately and regularly with artificial granular diet and newly hatched nauplii of *Artemia*. Healthy sexually mature zebrafish were put into the mating tank according to the ratio of female/male as 1/1. The fertilized eggs were obtained at 9-10 o'clock in the next day. Then, at 28 °C, they were transferred to zebrafish embryo culture water which was a mixture of NaCl (5.0 mM), KCl (0.17 mM), CaCl₂ (0.4 mM), MgSO₄ (0.16 mM).

Healthy zebrafish embryos were selected and randomly inhaled into 24 well plates with 10 embryos per well. All above embryos were divided to four groups, the control group, model group, positive drug group, and CQAs group. 20 μ M ibuprofen solution was set as positive drug. The CQAs group was given 5, 125, 250, 500 and 1000 μ M 5-O-methylvisammioside, cimiruboside, and amygdalin, respectively. While the control group and model group were given zebrafish culture water. Next, 20 μ M 5H₂O·CuSO₄ was added to all groups after 24 hours of administration. Then, 6 zebrafish were randomly selected from each group and anesthetized with 0.22% Tricaine for 1 min. Finally, the zebrafish were photographed under the Stereofluorescence Microscope (Zeiss AXIO Zoom V16, Zeiss, Oberkochen, Germany). The migration number of macrophages in each group was recorded by Image-Pro Plus software.

2. Results and Discussion

2.1 The CQAs identification of GMK and Discovery of MIF and IL-1 β potential targets from clinical real-word data coupled with intelligence algorithm

2.1.1 The CQAs identification of GMK by UPLC-MS of animal serum

A total ion currency (TIC) profile of the mass spectrum in serum was shown in **Figure S1(a-b)**. According to the [M+H]⁺ and fragment peaks of the active pharmacological ingredients, there were 3 constituents of GMK migrating to blood, cimiruboside, 5-O-methylvisamminol, and 5-O-methylvisammioside. The results were shown in **Figure S1(c-d)**, in which, there was no these 3 constituents in the control group, while these 3 constituents could be detected at 15min, 30min, 60min, 90min, 120min, 150min, 240min, and 480min in ARMT group.

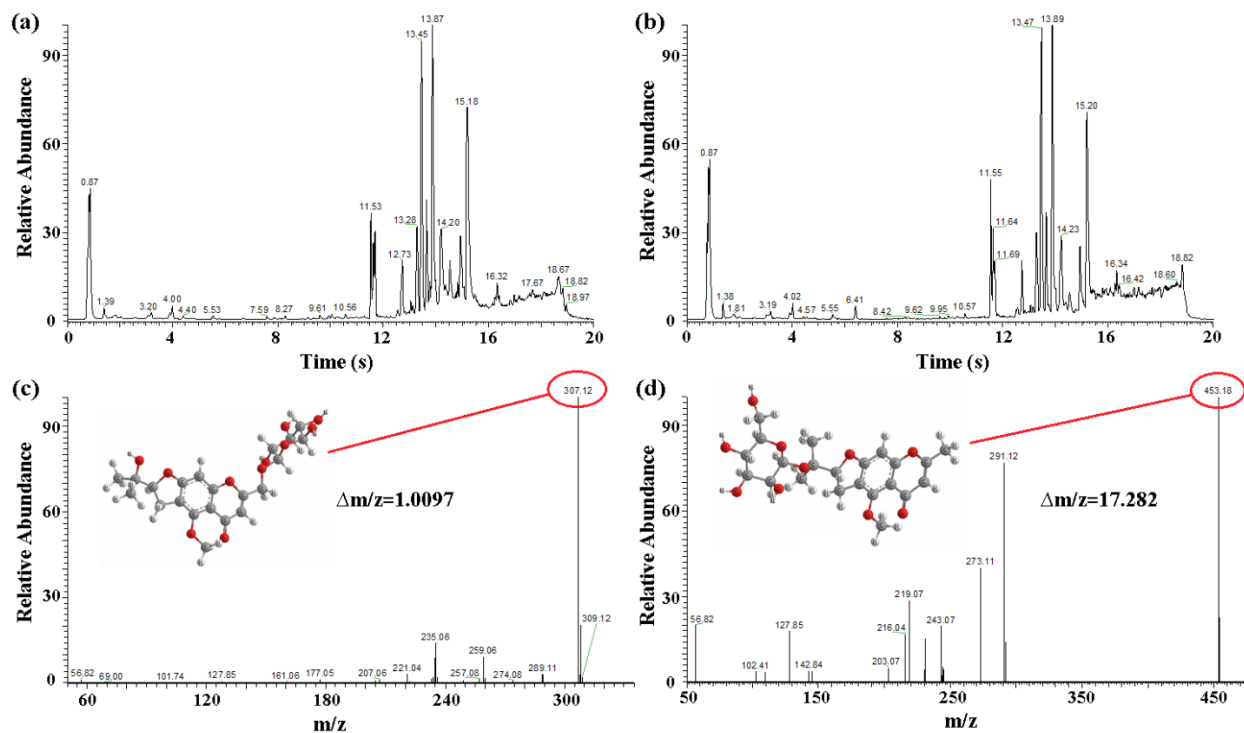


Figure S1 A total ion current (TIC) profile of the mass spectrum in rat serum, (a) the control group, (b) AR group treated with GMK, (c) the secondary mass spectrum of cimicifugoside, (d) the secondary mass spectrum of 5-O-methylvisammioside.

2.1.2 The reliability of clinical real-word data by the efficacy of GMK on ARMT group

Table S2 shows the Nasal Symptom Score (NSS), concomitant Simultaneous Nasal Symptom Score (SNSS) and quality of life Score (QLS) of ARMT group. Notably, TNSS was significantly improved, and the improvement was most obvious after eight weeks, after 4 weeks of continuous administration of allergy. TNNSS did not improve at the 4th week of treatment ($P > 0.05$), and the score decreased significantly ($P < 0.01$) at eighth week and decreased at twelfth week ($P < 0.05$). In addition, RQLQ showed the greatest impact (7.00 ± 0.53 points). The symptoms decreased significantly with the extension of treatment time. These above indicated that our clinical real-word data was reliable enough.

Table S2 The reliability of clinical real-word data by the efficacy of GMK on ARMT group.

Evaluation Indicators	Groups	Before treatment	In treatment (4 weeks)	In treatment (8 weeks)	In treatment (12 weeks)
TNSS	Group AR	27.86±1.48	23.36±1.34	12.41±1.39	10.32±2.02
	P value	/	0.001	0.001	0.001
	rhinobyon	6.41±0.71	5.82±0.67	3.56±0.53	2.59±0.56
	P value	/	0.001	0.001	0.073
	rhinorrhoea	7.14±0.47	4.36±0.38	1.96±0.41	2.5±0.55
	P value	/	0.001	0.001	0.283
	rhinocnesmus	7.00±0.46	6.68±0.42	3.59±0.39	2.41±0.54
	P value	/	0.05	0.001	0.034
	sneezing	7.32±0.39	6.50±0.36	3.27±0.44	2.82±0.61
	P value	/	0.001	0.001	0.279
TNNSS	Group AR	36.27±2.47	34.27±2.65	17.41±2.39	9.60±2.64
	P value	/	0.177	0.001	0.011
	runny nasopharynx	4.14±0.63	3.41±0.62	1.59±0.48	1.46±0.46
	P value	/	0.001	0.024	0.808
	lacrimation	3.18±0.65	2.90±0.58	3.72±0.48	0.86±0.41
	P value	/	0.162	0.208	0.001
	itching in eye	6.46±0.61	6.18±0.60	2.00±0.51	1.46±0.51
	P value	/	0.137	0.001	0.47
	Conjunctival congestion	3.64±0.69	3.46±0.67	1.50±0.44	0.55±0.35
	P value	/	0.296	0.002	0.047
NNSS	pain in the nose and palate	2.91±0.70	2.55±0.59	1.18±0.33	0.59±0.32
	P value	/	0.042	0.031	0.254
	headache	2.73±0.62	2.55±0.56	1.14±0.35	0.64±0.28
	P value	/	0.042	0.009	0.126
	itching in ear	3.96±0.60	3.91±0.57	2.59±0.48	1.05±0.35
	P value	/	0.665	0.082	0.005
	pharynx urticant cough	4.55±0.60	4.23±0.59	2.32±0.54	1.82±0.47
	P value	/	0.005	0.012	0.444

	hyposmia	4.18±0.69	4.09±0.69	0.64±0.36	1.09±0.50
	P value	/	0.162	0.001	0.357
	asthma	1.23±0.42	1.14±0.38	0.31±0.20	0.09±0.09
	P value	/	0.329	0.003	0.329
TQLS	Group AR	42.90±2.74	34.18±2.69	19.23±2.83	10.82±2.61
	P value	/	0.001	0.001	0.001
	daily life	7.00±0.53	5.73±0.53	3.36±0.61	2.14±0.59
	P value	/	0.001	0.001	0.008
	sleep	4.23±0.59	4.77±0.65	2.77±0.61	1.77±0.67
	P value	/	0.396	0.001	0.032
	no nasal inflammation	4.23±0.59	3.27±0.57	1.63±0.46	1.00±0.32
	P value	/	0.001	0.001	0.224
QLS	behavior	5.77±0.63	4.55±0.55	2.32±0.42	1.27±0.39
	P value	/	0.001	0.001	0.036
	emotion	4.96±0.69	3.91±0.66	2.41±0.56	1.36±0.43
	P value	/	0.001	0.009	0.031
	pars nasalis	7.96±0.43	6.59±0.45	2.81±0.27	2.18±0.44
	P value	/	0.001	0.001	0.12
	eye	6.59±0.63	6.09±0.51	2.91±0.48	1.09±0.39
	P value	/	0.102	0.001	0.001

2.1.3 Discovery of MIF and IL-1 β potential targets by large-scale and in-depth screening from clinical real-word data coupled with intelligence algorithm

An independent sample T test was used to analyze the different protein between groups AR and control after log-transformation. Paired T-test was used to analyze protein content differences between groups AR and ARMT in after log-transformation. **Table S3** showed the twelve common proteins with remarkable differences among the three groups, control groups, AR group, and ARMT group.

197 **Table S3** The information of differenced proteins among control, AR, and ARMT groups.

Uniprot ID	Name	Mean of AR	SD of AR	Mean of Control	SD of Control	Mean of ARMT	SD of ARMT	AR/Control	ARMT/AR
P29965	CD40L	8.7722	0.1618	8.6579	0.1338	9.0707	0.1782	1.0132	1.0340
P08887	IL6RA	7.1790	0.2441	6.9379	0.1662	7.4113	0.2372	1.0348	1.0323
P14151	LYAM1	11.2229	0.2108	11.0017	0.1491	11.3626	0.1438	1.0201	1.0125
P02775	CXCL7	7.9864	0.3741	7.4057	0.3796	8.3642	0.3222	1.0784	1.0473
P55774	CCL18	8.5577	0.3551	8.1525	0.3998	8.8260	0.1656	1.0497	1.0314
Q9Y275	TN13B	6.0327	0.1788	5.6988	0.4044	6.2089	0.1835	1.0586	1.0292
P51671	CCL11	9.4693	0.2361	9.2777	0.2386	9.6972	0.2719	1.0207	1.0241
P22003	BMP5	7.8152	1.5729	4.5401	2.5858	5.7778	2.5300	1.7214	0.7393
Q16552	IL17	2.9981	1.0338	1.5967	1.3971	2.1885	1.4213	1.8777	0.7300
P14174	MIF	7.9528	1.2831	6.8379	0.9850	6.8745	1.1157	1.1630	0.8644
O43915	VEGFD	5.5073	0.9930	3.8519	1.2281	4.9412	1.1220	1.4298	0.8972
P01584	IL1 β	8.8315	0.8622	7.5677	0.9879	8.0902	1.0420	1.1670	0.9161

198 * AR refers to the AR group; Mean of AR refers to the mean of the protein contents in AR group; SD of AR refers to the standard deviation of the protein
199 contents in AR group; Control refers to the control group; Mean of Control refers to the mean of the protein contents in Control group; SD of AR refers to
200 the standard deviation of the protein contents in Control group; ARMT refers to the ARMT group; Mean of ARMT refers to the mean of the protein contents
201 in ARMT group; SD of ARMT refers to the standard deviation of the protein contents in ARMT group. 'AR/Control' refers to Mean of AR / Mean of
202 Control, 'ARMT/AR' refers to Mean of ARMT / Mean of AR.

Subjects in control group and AR group were divided into calibration set (Cal) and validation set (Val) using K-S method. The percentage of Cal/Val was 30/9, 25/14, and 20/19, respectively. PLS-DA, bagging PLS-DA and boosting PLS-DA models were established respectively. The prediction results of validation set are shown in **Table S4**. With the decrease of the sample number of the calibration set, the prediction performance of the verification set gradually worse. When Cal/Val was 25/14, the prediction performance of the model is better among others ratios. Comparing the different modeling methods on the prediction ability, bagging PLS-DA and boosting PLS-DA can effectively improve the prediction performance of the model of small sample analysis.

When all seventy proteins were considered, the accuracy and durability of the discrimination model were low due to the interference of irrelevant proteins. When forty-two differential proteins of control group and AR group were used, the prediction performance showed that the model was likely to misjudge control group. When eighteen differential proteins of AR group and ARMT group was taken, the prediction performance of the model was better. Furthermore, with twelve common differential proteins of these three groups as variables, the model gives better prediction performance. And fewer variables are conducive to improve the durability of the model. These results demonstrated that chemometric discriminant model can accurately identify the allergic and mild subjects in clinical real-word by these twelve proteins.

221 **Table S4** The T-tests of differenced proteins among control, AR and ARMT groups.

Variable	Model	Cal: Val = 30: 9		Cal: Val = 25: 14		Cal: Val = 20: 19	
		AR	Control	AR	Control	AR	Control
		(n=6)	(n=3)	(n=10)	(n=4)	(n=13)	(n=6)
All proteins (n=70)	PLS-DA	6	3	9	4	10	6
	Bagging-PLS-DA	6	3	10	4	10	6
	Boosting-PLS-DA	6	3	10	4	10	6
Differential proteins between AR and control (n=42)	PLS-DA	6	2	9	3	10	6
	Bagging-PLS-DA	6	2	9	3	11	6
	Boosting-PLS-DA	6	2	10	3	11	6
Differential proteins between AR and ARMT (n=18)	PLS-DA	6	3	10	4	10	6
	Bagging-PLS-DA	6	3	10	4	11	6
	Boosting-PLS-DA	6	3	10	4	10	6
Differential proteins among AR, control and ARMT (n=12)	PLS-DA	6	3	10	4	10	6
	Bagging-PLS-DA	6	3	10	4	10	6
	Boosting-PLS-DA	6	3	10	4	7	6

222 * AR refers to the AR group; Control refers to the control group; ARMT refers to the ARMT group; n means the
223 number of the proteins; Cal refers to calibration set; Val refers to validation set.

2.2 A successive application and validation of IL-1 β biosensor on precise angling of CQAs

The interaction between IL-1 β and CQAs were showed in **Figure S2**. The K_D of 5-O-methylvisammioside, amygdalin, cimicifugoside were 6.121 pM, 44.85 nM, and 12.23 pM, respectively. **Table S5** showed the interaction detail among CQAs and MIF/IL-1 β , in which, almost all RSD of K_D were less than 5. The result indicated that our biosensors had high applicability and reliability.

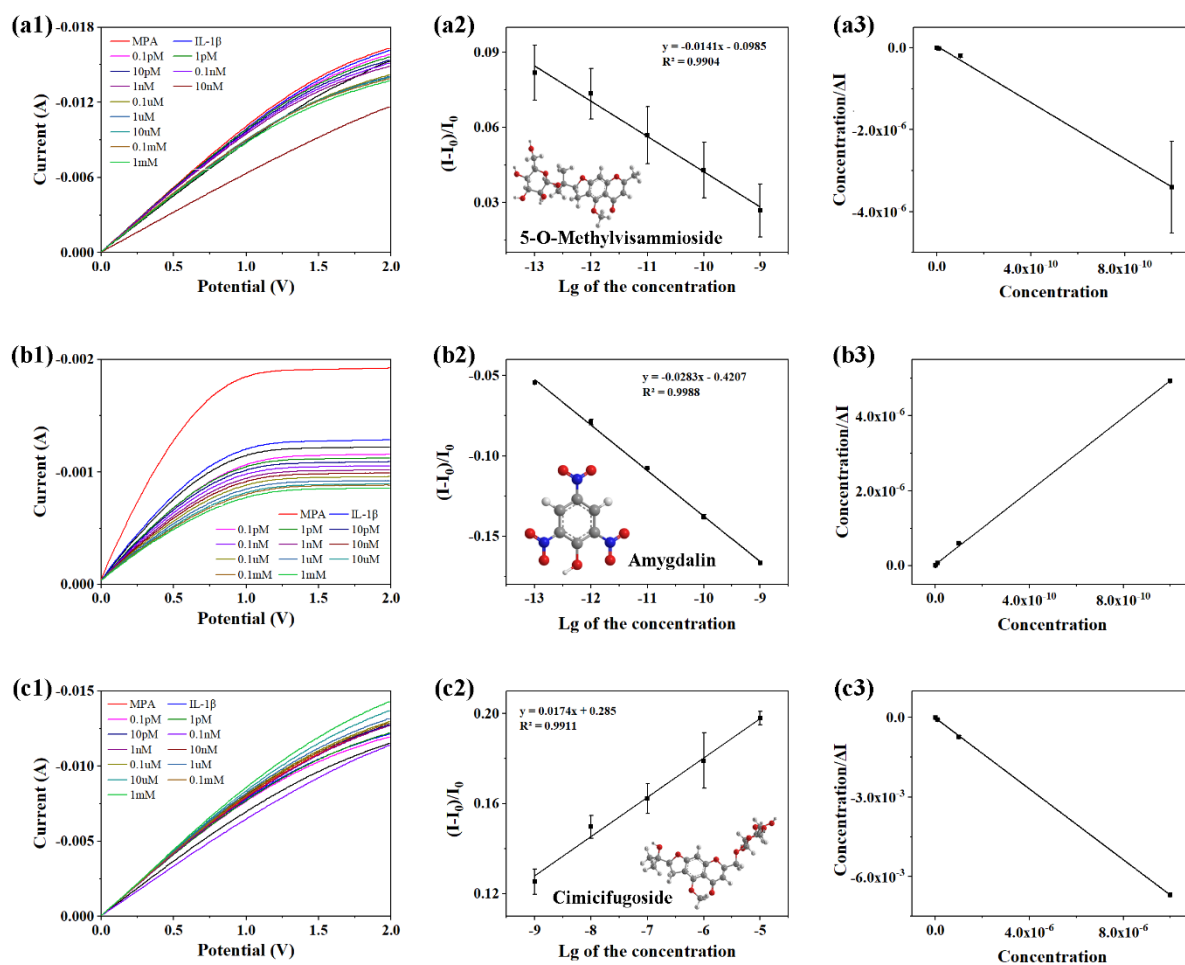


Figure S2 The interaction results of 5-O-methylvisammioside, amygdalin, and cimicifugoside. (a1) I_{DS} - V_{DS} regularity decreases with increasing concentration of 5-O-methylvisammioside, (b1) amygdalin, (c1) cimicifugoside. (a2) The linear fitting between the Lg of 5-O-methylvisammioside concentration and $\Delta I/I_0$, (b2) amygdalin, (c2) cimicifugoside; (a3) $[Ag]/\Delta I$ versus $[Ag]$ of 5-O-methylvisammioside, (b3) amygdalin, (c3) cimicifugoside.

237 **Table S5** The interaction detail among CQAs and critical AR targets, MIF and IL-1β.

Targets	Candidates	Kd (M)			Mean	Standard Deviation	Relative Standard Deviation (RSD)
MIF	5-O-Methylvisammioside	4.66×10 ⁻¹¹	5.15×10 ⁻¹¹	4.72×10 ⁻¹¹	4.84×10 ⁻¹¹	2.67×10 ⁻¹²	5.518
	Cimicifugoside	5.00×10 ⁻¹²	5.10×10 ⁻¹²	5.06×10 ⁻¹²	5.05×10 ⁻¹²	5.26×10 ⁻¹⁴	1.040
	Amygdalin	4.36×10 ⁻¹²	4.30×10 ⁻¹²	4.42×10 ⁻¹²	4.36×10 ⁻¹²	5.52×10 ⁻¹⁴	1.267
IL-1β	5-O-Methylvisammioside	1.23×10 ⁻¹¹	1.07×10 ⁻¹¹	1.37×10 ⁻¹¹	1.22×10 ⁻¹¹	1.48×10 ⁻¹²	12.11
	Cimicifugoside	4.44×10 ⁻⁸	4.53×10 ⁻⁸	4.49×10 ⁻⁸	4.49×10 ⁻⁸	4.79×10 ⁻¹⁰	1.067
	Amygdalin	6.09×10 ⁻¹²	6.12×10 ⁻¹²	6.15×10 ⁻¹²	6.12×10 ⁻¹²	2.95×10 ⁻¹⁴	0.4826

2.3 Validation of MIF/IL-1 β biosensor application by in vitro and vivo investigation

2.3.1 In vivo validation of MIF/IL-1 β biosensor application by molecular docking

To verify the strong applicability of the MIF/IL-1 β biosensors, the mechanism of CQAs was investigated by network pharmacology and molecular docking. The essential pathways with high correlation with AR were shown in **Figure S3(a)**, mainly including tumor necrosis factor (TNF), nuclear factor kappa-B (NF- κ B), mitogen-activated protein kinase (MAPK) and other inflammation related pathways. **Figure S3(b)** showed the vital biochemical process of AR intervention by GMK. As a result, GMK plays a critical role in AR are mainly through the inflammatory reaction process, immune stress process, NF- κ B transcription factor activity, RNA polymerase II promoter transcription, and so on.

The LibDockScore among three CQAs and the critical AR targets, MIF/IL-1 β were shown in the **Table S6**. Among them, cimicifugoside has the strongest affinity to MIF, with a score of 144.387. Its interaction with MIF is manifested by the formation of hydrogen bonds with the LYS32 amino acid residues; and the formation of hydrophobic forces with PRO1, TYR36, PRO33, PHE113, ILE64 and TRP108 amino acid residues.

Simultaneously, amygdalin has the strongest affinity to IL-1 β . The score of which was 128.454. It binds to IL-1 β through the formation of hydrogen bonds with LEU80, LEU134 and GLN81 amino acid residues; and the formation of hydrophobic forces with LEU26, VAL132 and LEU82 amino acid residues.

The LibDockScore of molecular docking revealed strong interaction between CQAs and MIF/IL-1 β . NF- κ B was one key pathway of these CQAs. Notably, MIF and IL-1 β were two essential targets in NF- κ B pathway, suggesting NF- κ B was indeed a vital pathway of these CQAs for AR treatment. In vitro experiments have successfully verified that the angling results of our biosensors were reliable.

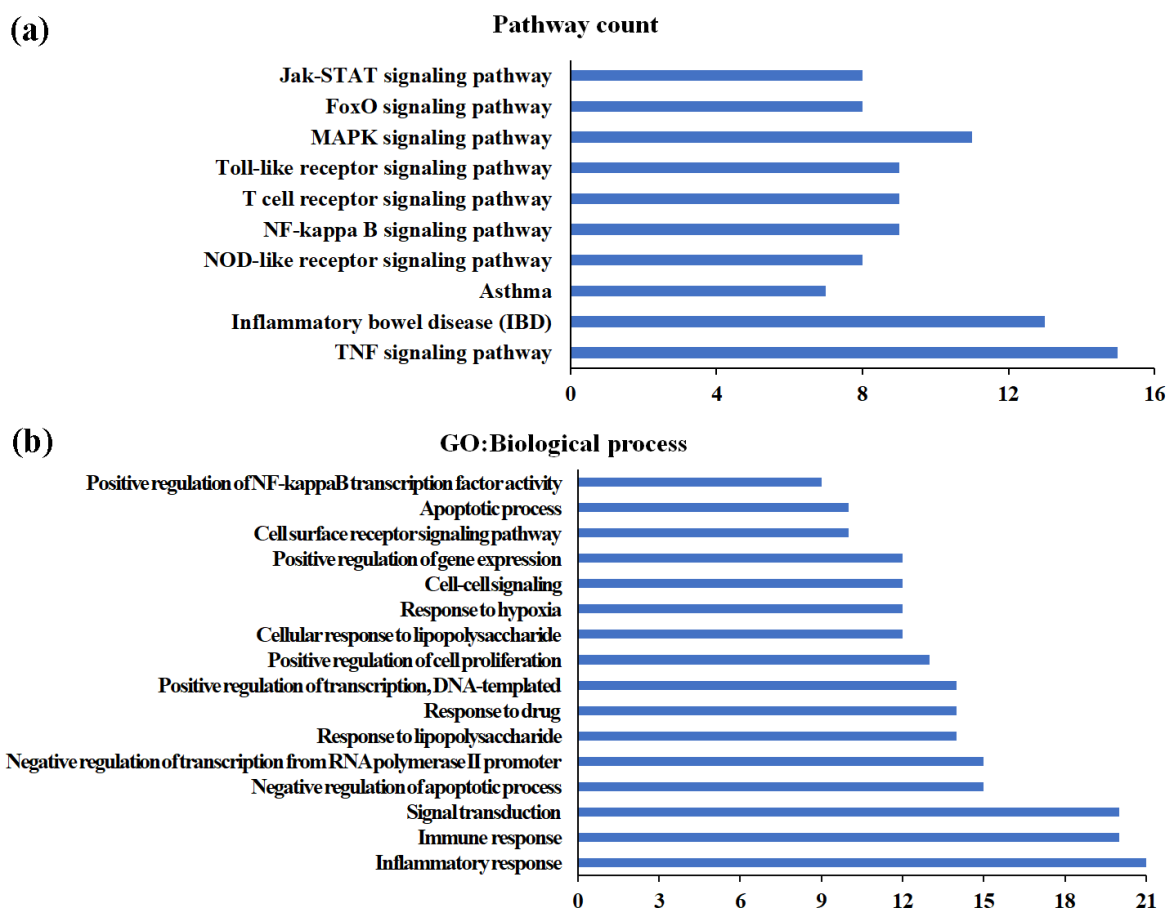


Figure S3 (a) The essential pathways with high correlation with AR. (b) The essential biochemical process of AR intervention by GMK.

Table S6 The interaction prediction results of candidates from GMK with MIF and IL-1 β by molecular docking.

Potential candidates	LibDockScore	
	MIF	IL-1 β
Cimicifugoside (CID_441913)	144.387	112.746
5-O-Methylvisammioside (CID_21670038)	142.781	120.44
Amygdalin (CID_656516)	138.678	128.454
Gastrodin (CID_115067)	122.097	100.245

2.3.2 In vivo validation of MIF/IL-1 β biosensor application by zebrafish

Zebrafish macrophage migration was normalized as a percentage of the model group. As a result, 5-O-methylvisamminoside has inhibitory effect at 125 μ M and 250 μ M, and the inhibitory effect was the strongest at 250 μ M, even stronger than that of the positive control group. Cimicifugoside and amygdalin all had inhibitory effects at five concentrations (62.5, 125, 250, 500 and 1000 μ M), and the inhibitory effect was the strongest at 250 μ M. It is stronger than that of the positive control group. These indicated that the 5-O-methylvisammioside, cimicifugoside and amygdalin with 250 μ M concentration had better antiallergic effect on

276 inhibiting macrophage migration. The above evidence verified the strong applicability of the
277 MIF/IL-1 β biosensors and informatics.