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Discovery of C-shaped aurone human neutrophil elastase inhibitors

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S. D. Lucas,*†[a] M. P. Carrasco,†[a] L. M. Gonçalves,[a] R. Moreira[a] and R. C. Guedes*[a]

An activity screening of a library of aurones led to the identification of sub-micromolar HNE inhibitors. The activity is rationalized by a C-shape conformation that allows tight binding to human neutrophil elastase S1 and S2 pockets.

Human Neutrophil Elastase (HNE, EC 3.4.21.37) is a serine protease of the chymotrypsin superfamily that is stored in the primary azurophilic granules of polymorphonuclear neutrophils. HNE is naturally involved in the degradation of diverse structural proteins, such as elastin, or functional proteins toward the activation of other proteases, such as matrix metalloproteinases, but also plays an important role on pathogen killing by cleaving structural proteins of the outer cell wall of gram-negative bacteria. On the other hand, the imbalance between HNE and its endogenous inhibitors leads to severe tissue injuries associated to the onset and progression of several diseases such as chronic obstructive pulmonary disease (COPD, reported by the World Health Organization as the third leading cause of death worldwide), rheumatoid arthritis, pulmonary emphysema and psoriasis.[1] Moreover, it has been postulated that HNE can contribute to non-small cell lung cancer progression.[2]

A wide range of chemical scaffolds with different mechanisms of action against HNE were reported over the last decades, nevertheless only Sivelestat (ONO-5046), an acylating agent from Ono Pharmaceuticals, has been launched at the Japanese market for acute lung conditions, however failed FDA approval. Hence, there is an urgent need for new scaffolds toward HNE inhibition. One of the main pitfalls of the drug discovery process toward HNE-related diseases is the target flexibility due to induced-fit events upon the binding of different ligands.[3, 4] For example a potent non-covalent dihydropyrimidone inhibitor was co-crystallised with HNE revealing a unique orientation addressing HNE S1 and S2 subsites, in which the formation of a deep and well-defined cavity at S2 was first described (Fig. 1).[4] The shape complementarity between the induced S2 cavity and the P2 moiety of the inhibitor opens the way to a new potential "C-shaped" HNE inhibitor discovery (Fig. 1). Moreover, Bayer HealthCare recently disclosed BAY 85-8501, a picomolar dihydropyrimidone that also presents a frozen bioactive conformation toward tight S1 and S2 pocket interactions. BAY 85-8501 is currently in Phase 2 clinical trials for safety and efficacy evaluation in patients with non-cystic fibrosis bronchiectasis.[5]

Our group has been engaged in the development of HNE inhibitors with new architectures such as the potent oxo-β-lactam class,[6] or kojic acid derived inhibitors identified by a computer-aided campaign.[7, 8] The exploitation of the reactivity of covalent inhibitors also allowed us to design a selective fluorescent activity-based probe, as a tool for molecular functional analysis for HNE-related disease proteomes.[9] Following our journey pursuing new lead structures for HNE inhibition we envisaged the aurone derivatives as potential C-shape candidates toward adequate conformation for HNE non-covalent inhibition (Fig. 1). An in-house aurone library with 25 derivatives was then screened against HNE in order to evaluate the conformation-activity relationship within a diverse collection of compounds with a wide range of substituents around the aurone scaffold.

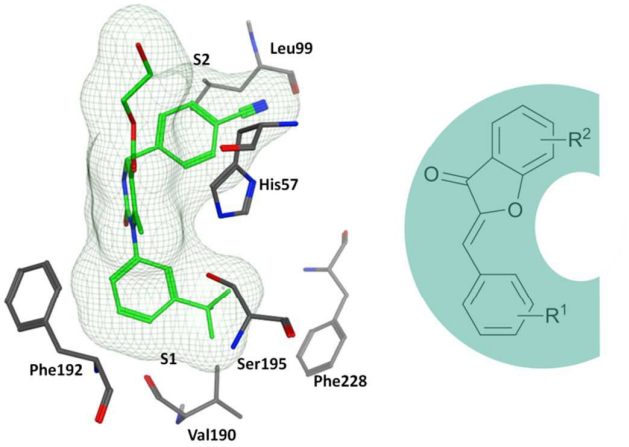

Fig. 1 Structure of HNE in complex with a dihydropyrimidone inhibitor with "C-shape" conformation (PDB 3Q77)[4] and envisaged aurone scaffold.

[a] Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa. Av. Prof. Gama Pinto, 1649-003 Lisboa, Portugal. sdlucas@ff.ulisboa.pt; rguedes@ff.ulisboa.pt

† These authors contributed equally to the experimental part of this work. Electronic Supplementary Information (ESI) available: Chemistry, molecular modeling and *in vitro* assays. See DOI: 10.1039/x0xx00000x

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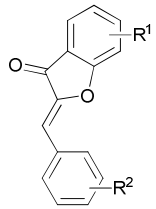
The therapeutic potential of aurones has been highlighted with recent studies disclosing their anti-cancer,[10, 11] antimicrobial,[12] antiparasitic,[13] anti-viral,[14] and anti-inflammatory[15] activities. In addition, aurones can also act as modulators of ABC drug transporters[16] and present inhibitory activity against acetylcholinesterase[17] and MAO-B[18]. However, no report on the conformation-activity relationship as major responsible for biological outcome has been described.

A library of aurones was previously developed by our group in order to probe its activity against *Plasmodium falciparum*.[13] These were synthesized by aldol condensation of benzofuranones with the appropriately substituted benzaldehydes, leading to the desired aurones with Z configuration, the thermodynamically more stable isomer.[13] Microwave assisted synthesis using palladium catalyzed protocols was also used for aurone scaffold extension. Envisaged as potential C-shape HNE inhibitors, aurone activity was screened at 10 μM in order to identify aurone hits toward HNE inhibition (Table 1).

Table 1. Aurone library assayed against HNE.



Compd	R^1	R^2	HNE act [I]10μM %[a]	IC_{50} (HNE)/ μM±SD	LE
1	H	2-Br	100	ND	ND
2	H	3-Br	100	ND	ND
3	H	4-Br	100	ND	ND
4	H	4-Ph	110	ND	ND
5	H	4-(C_6H_4-4'-F)	87	ND	ND
6	H	4-(C_6H_4-4'-Cl)	67	ND	ND
7	H	4-(C_6H_4-4'-CHO)	105	ND	ND
8	H	4-(3'-Quinoline)	88	ND	ND
9	H	4-[5'-(Pyridin-2'-NH_2)]	82	ND	ND
10	H	4-NMe_2	10	2.9±0.8	0.39
11	H	4-(NHPh)	22	1.4±0.8	0.34
12	H	4-(NHBn)	19	0.9±0.3	0.34
13	H	4-(OC_6H_4-4'-Me)	100	ND	ND
14	H	4-(OC_6H_4-4'-Cl)	36	0.5±0.3	0.35
15	H	4-Bn	58	4.4±2.6	0.31
16	H	3-Ph	69	ND	ND
17	H	3-(C_6H_4-4'-Cl)	41	0.5±0.3	0.37
18	H	3-(C_6H_4-4'-CHO)	49	1.1±0.3	0.33
19	H	3-(3'-Quinoline)	87	ND	ND
20	H	3-Bn	44	2.9±1.4	0.32
21	7-OMe	H	87	ND	ND
22	6-OH	H	76	ND	ND
23	6-OH	4-NMe_2	19	5.1±1.6	0.35
24	6-OH	4-(OC_6H_4-4'-Me)	49	ND	ND
25	6-OH	4-(OC_6H_4-4'-Cl)	41	1.5±0.4	0.31

[a]Remaining HNE activity in the presence of [I]=10 μM. ND- non determined.

Inspection of Table 1 reveals that aurones with small R^2 substituents at ring B are inactive (e.g. **1**-**3**). Similarly, larger and rigid R^2 groups at C-4 lead to poorly active compounds (e.g. **4**-**9**). The exception were aurones with an amine (**10** and **12**), aniline (**11**) or 4-(4'-chloro)phenoxy (**14**) groups, with IC_{50} values ranging from 0.51 to 2.9 μM. These results may reflect a lack of flexibility imparted by rigid C-4 substituents that does not favor the C-shape conformation in the active site, which is recovered in the presence of a more flexible linker. In contrast, aurones with aryl groups at C-3 were well-tolerated indicating that 3-substitution pattern may favor the C-shape conformation in the active site of HNE. For example, moving the 4-chlorophenyl substituent from C-4 (**6**) to C-3 (**17**) led to a dramatic increase of potency.

Kinetic studies were performed for aurone **12**. The inhibitor and substrate were added to the enzyme and product formation was monitored over 120 min, at different inhibitor concentrations (Fig. 2). Progress curve analysis show concentration but no time-dependent inhibition, in line with a competitive mechanism.[7, 19, 20]

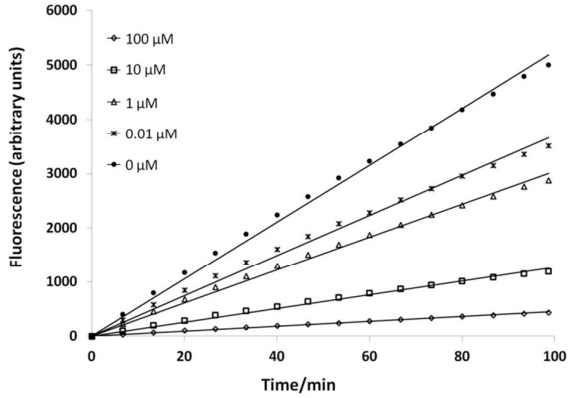


Fig. 2 Plots of progress curves for HNE inhibition by aurone **12** (0-100 μM). No time-dependent inhibition was observed and lines indicate linear best fits.

In order to have a molecular insight for aurone HNE inhibitors mode of action, molecular docking studies were performed using GOLD version 5.2.0 software and HNE 3D coordinates from PDB ID: 3Q77, where HNE forms a complex that revealed the ability of a non-covalent dihydropyrimidone inhibitor to adopt a induced-fit C-shape inactivation of the enzyme.[4, 21]

The docked poses for the active aurones clearly show the ability of these derivatives (**10**, **12**, **14**, **17**, **20** and **25**, Fig. 3) to form a well-defined C-shape that promotes the induced-fit toward HNE inhibition. We can observe for all derivatives that the aurone ring is well accommodated in the S2 pocket establishing π-π stacking interactions with His57 and should be responsible for the induced-fit event leading to the movement of Leu99 toward a solvent accessible area resulting in a deeper S2 pocket. Moreover, the active compounds present appropriate size and conformation for a correct pose that well-fits both S1 and S2 pockets (Fig. 3). In addition, the presence of a hydroxyl group at C-6 is well tolerated at the P2 moiety of the aurone but does not lead to extra favorable interactions (**25**, Fig. 3). Weaker inhibitors as **10** and **20** show a suitable C-shape with the aurone occupying the S2 pocket, nevertheless the inhibitor length does not allow a fulfilling of the

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deep S1 pocket. When we observe the docked poses for the inactive compounds **2** and **13** (Fig. 3), we can foresee that compound **2** is too small to promote the induced-fit as the aurone does not completely fit toward the S2 pocket end while the same happens on the opposite side of the molecule where the phenylbromide moiety is not well accommodated in S1 deep subsite, disrupting the possibility for tight binding to HNE. On the other hand compound **13** has extended size with a methyl group at the opposite end of the aurone and, in agreement with its inactivity, we were unable to find docked poses that suggest the occupancy of both S1 and S2 subsites.

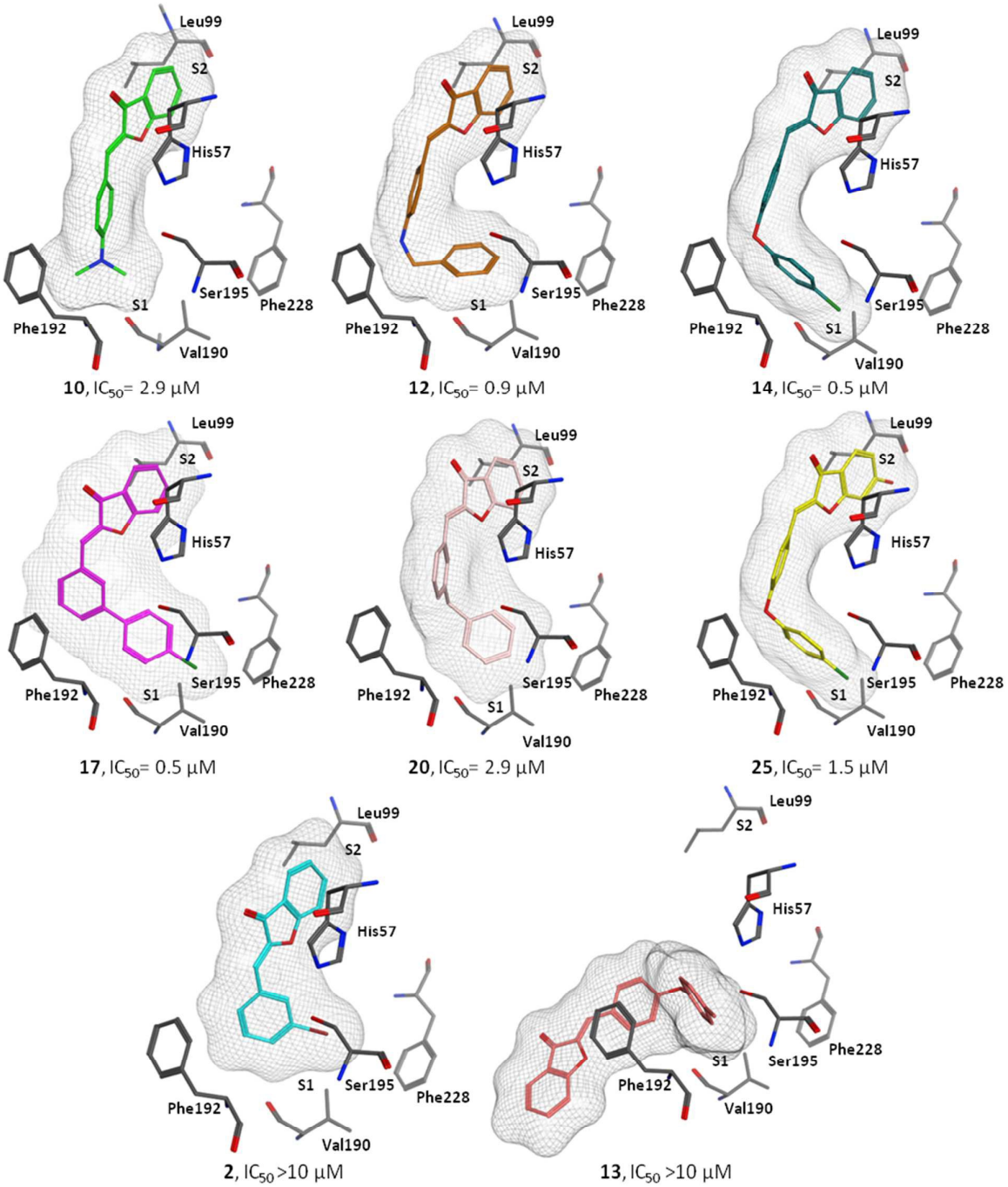

Fig. 3 Docked poses for aurone human neutrophil inhibitors using GOLD5.2 software. The ability for a well-defined C-shape conformation leads to increased potency due to S1 and S2 complementarity. Pictures performed using MOE package.[22]

Ligand efficiency (LE) is a useful metric assessing the druggability of leads and targets.[23] Regarding the acceptable value for LE, the aurones now disclosed as HNE inhibitors are suitable as lead compounds for further development (Table 1). Moreover, the

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assayed aurones were shown to be non-cytotoxic against cultured human cells (HEK293T) presenting EC_{50} values, in general, higher than 100 μM, leading to selectivity indices (SI = EC_{50}(HEK293T)/IC_{50}(HNE)) higher than 20.[13] Hence, as anticipated the aurone scaffold acts as a C-shape non-covalent inhibitor of HNE depending on the scaffold decoration that might lead to further development toward more potent HNE inhibitors with adequate drug like properties.

Conclusions

A new class of human neutrophil elastase based on the aurone scaffold was discovered, presenting sub-micromolar activities. The ability of aurones to form a C-shape conformation depending on the substitution pattern may role induced-fit events that allow tight binding to HNE S1 and S2 pockets. The suitable ligand efficiency and the lack of cytotoxicity opens the way for further development of aurone HNE inhibitors and computer-aided techniques will be valuable tools for the design of more potent molecules with adequate C-shape complementarity to HNE S1 and S2 subsites.

Acknowledgments

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