Bioorganic & Medicinal Chemistry Letters 20 (2010) 4355–4358

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

LuxR-dependent quorum sensing: Computer aided discovery of new inhibitors structurally unrelated to *N*-acylhomoserine lactones

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ARTICLE INFO

Article history:
Received 11 May 2010
Revised 11 June 2010
Accepted 12 June 2010
Available online 17 June 2010

Keywords:
Virtual screening
Molecular docking
Quorum sensing
Antagonist
LuxR
AHL

ABSTRACT

A virtual screening, involving flexible docking sequences within the LuxR, TraR and LasR binding sites, was used as a structural binding sites similarity filter to specifically target conserved residues in the proteins of the LuxR family (namely Tyr62, Trp66, Tyr70, Asp79, Trp94 for LuxR). This docking-based screening, employing a genetic algorithm, was performed on a 2344 chemical compounds library, together with empirical binding free energy (ΔG_{bind}) calculations. Docking results were analysed, and the compounds detected with reproducible low ΔG_{bind} values or identified as being in the top 120 for most of the docking sequences, were selected as hits candidates which interact with conserved residues. Biological evaluation with LuxR-dependent quorum sensing led to the discovery of some new inhibitors, namely tamoxifen, sertraline, pimethixene, terfenadine, fendiline and calmidazolium. Notably, calmidazolium was identified as one of the most potent AHL-structurally unrelated inhibitors of LuxR-dependent quorum sensing, with an IC_{50} value of 7.0 ± 0.2 μM.

Bacteria are able to communicate with each other using a specific system called 'Quorum Sensing' (QS). This cell to cell communication system is based on the correlation between population size and the concentration of small diffusible chemical messengers called autoinducers. When a critical concentration is reached, target genes encoding for phenotypes such as bioluminescence, biofilm formation or virulence are expressed allowing bacteria to adapt their behaviour to their environment.[1–6] Consequently, QS is now targeted for the design of potential antibacterial drugs.[7–10] In Gram negative bacteria, quorum sensing involves *N*-acyl homoserine lactones (AHLs) as autoinducers, associated with transcriptional regulators belonging to proteins of the LuxR family. Most studies describing the discovery of LuxR-protein antagonists are based on the rational design of AHL analogues or on biological screenings of compound libraries.[11] To date, in silico screening of ligand databases, which has become a key methodology in drug discovery,[12–15] has been only moderately employed to discover new QS inhibitors,[16–18] especially using LuxR-type proteins. In 2008, Zeng et al. reported the virtual screening of active compounds from Traditional Chinese Medicines with antibacterial activity using docking simulations and the TraR protein.[19] More recently, Yang et al. described an interesting study involving docking-based virtual screening, within the binding site of LasR, using structural alignment with the natural ligand, 3-oxo-C_{12}-HSL which led to the discovery of new antagonists, namely salicylic acid, nifuroxazide, and chlorzoxazone.[20]

As a part of our research programme, aimed at discovering potential modulators of the AHLs-dependent transcriptional regulators,[21–23] we describe here a virtual screening using a genetic algorithm and involving flexible docking sequences within the LuxR, TraR and LasR binding sites. This protocol was used as a structural binding sites similarity filter to specifically target conserved residues in the LuxR-proteins. Indeed, as shown in other studies,[24,25] agonistic or antagonistic activities are related to the interactions of ligands with the conserved residues (namely Tyr62, Trp66, Tyr70, Asp79, Trp94 for LuxR). Thus, four docking sequences for each protein were performed then analysed in order to select compounds that target specifically these residues due to structural homology.

The docking-based virtual screening, employing a genetic algorithm (GADock)[26–28] which randomly generates conformations within a binding site, was performed with 2344 biologically relevant compounds from the Chembank subset chemical library of Ligand.Info Meta-Database.[14,29,30] Chembank was, in fact, created for the discovery of small molecules that modulate specific biological pathways using a chemical genetics approach.[31,32] Among the 2344 structures, 2289 compounds[33] were docked within a binding site and ranked, according to the binding free energy calculations (ΔG_{bind}),[28] resulting in one docking sequence. Subsequently, with reference to four docking sequences within each binding site of LuxR, TraR and LasR (12 sequences, 27468 docking experiments),

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doi:10.1016/j.bmcl.2010.06.081

all the docking results were analysed as follows: (i) compounds detected with reproducible low ΔG_{bind} values and high ranks were selected; (ii) like manoalide, known to be a QS inhibitor[34] and identified six times in the top 120, compounds detected at least seven times in the top 120 (according to the docking sequences) were selected and identified as potential hit candidates targeting conserved residues (Fig. 1).

Some of the hit candidates can be classified according to their biological activity, with lipids arising from the arachidonic acid cascade (leukotriene or prostaglandin derived compounds), vitamin A derived compounds, steroid hormones (β-estradiol or progesterone derived compounds), and several compounds known as calcium channel modulators (vitamin D3 derived compounds, flunarizine, cinnarazine, fendiline, calmidazolium and torasemide). Finally, clofoctol, tamoxifen, mitotane, sertraline, pimethixene, fenvalerate, terfenadine and manoalide were also identified. It was interesting to note that some potential QS modulators are related to hormones or to calcium channel modulators, suggesting a possible bacteria-host cross-talk as already proposed in the literature.[35,36] Interestingly, manoalide has also been reported as being a calcium channel inhibitor.[37] In addition, AHLs have been shown to modulate host cell responses through calcium signalling.[38] Natural AHL ligands (3-oxo-C_6-, 3-oxo-C_8-, 3-oxo-C_{12}-HSL) were not selected by this screening protocol, although a consistent influence of the size of the hydrophobic acyl chain on the ΔG_{bind} values was observed, with better affinity when increasing the chain length,

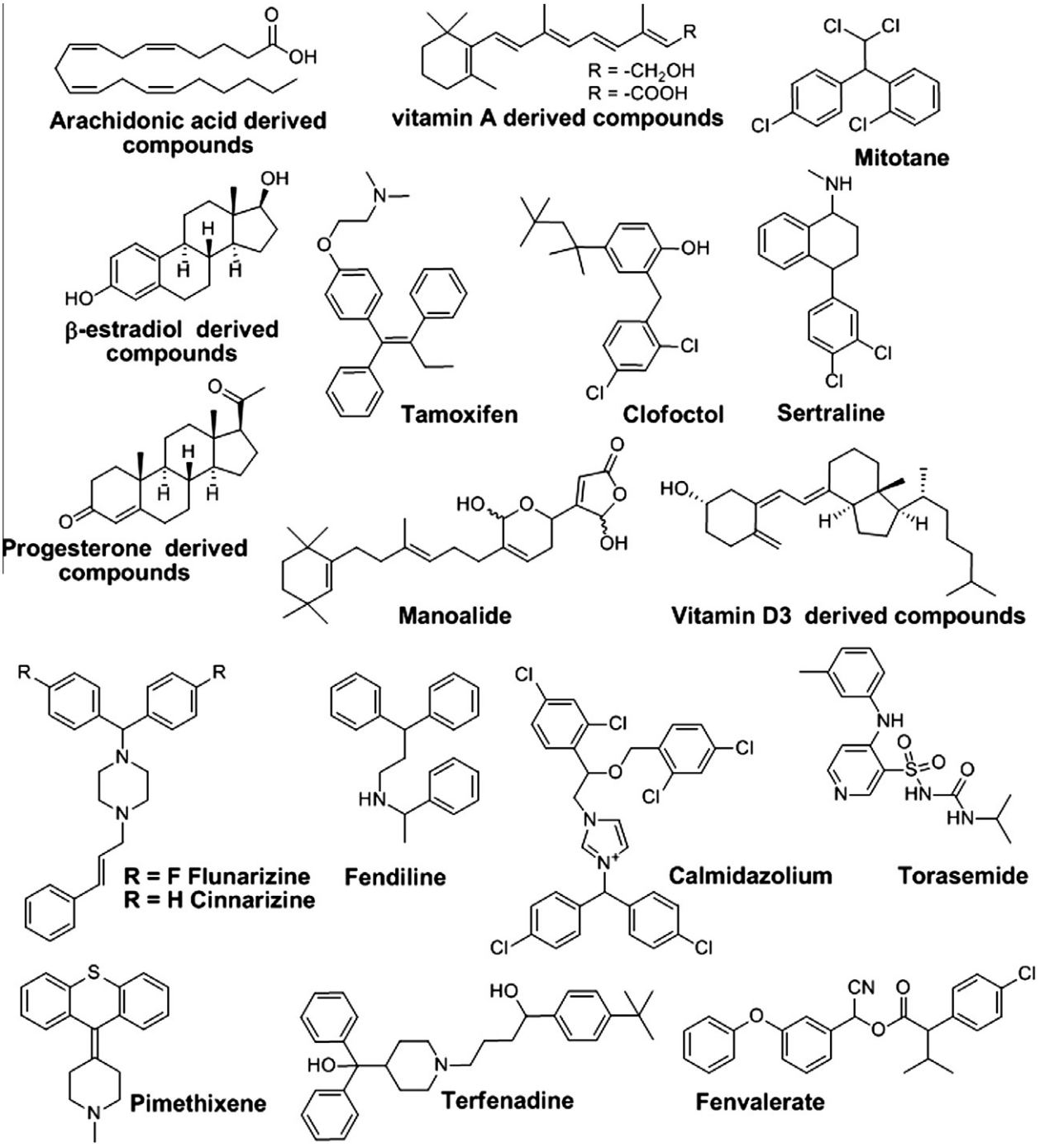

Figure 1. Structures of compounds identified as hit candidates.

Table 1
Inhibition of bioluminescence obtained with bioactive compounds

Compounds	IC_{50}[a,b] (μM)
Tamoxifen	≈40 μM
Sertraline	27 (±2)
Pimethixene	56 (±3)
Terfenadine	92 (±2)
Fendiline	91 (±2)
Calmidazolium	7.0 (±0.2)

[a] Concentration (μM) required to reduce to 50% intensity (IC_{50}) the bioluminescence induced by 200 nM of 3-oxo-C_6-HSL.
[b] Values are the means of three experiments; standard deviation is given in brackets.

suggesting that the protocol is mainly sensitive to hydrophobic interactions.

With the exception of manoalide, already identified as a QS inhibitor, arachidonic acid, vitamin D3, retinol and retinoic acid, β-estradiol and progesterone, as representative compounds of related derivatives, and all the other compounds were evaluated for their ability to modulate bioluminescence in the *Vibrio fischeri* QS system by agonising or antagonising the LuxR receptor.[21] Whereas none of them were found to induce bioluminescence at concentrations up to 200 μM, tamoxifen, sertraline, pimethixene, terfenadine, fendiline and calmidazolium interfered with bioluminescence in the *V. fischeri* QS system induced by 3-oxo-hexanoyl-l-homoserine lactone (3-oxo-C_6-HSL). In the case of tamoxifen, the dose–response relationship upturned slightly at high concentrations, a phenomenon which has been observed in previous studies.[39] However, this compound showed inhibition of bioluminescence, with a decrease to 73.6 ± 0.5% at 20 μM and to 53 ± 3% at 40 μM. Sertraline, pimethixene terfenadine, and fendiline also inhibited 3-oxo-C_6-HSL-induced bioluminescence and exhibited dose–response activity, with an IC_{50} ranging from 27 to 92 μM. Interestingly, calmidazolium was identified as a potent LuxR-dependent AHL-structurally unrelated QS inhibitor, with an IC_{50} value of 7.0 ± 0.2 μM (Table 1).

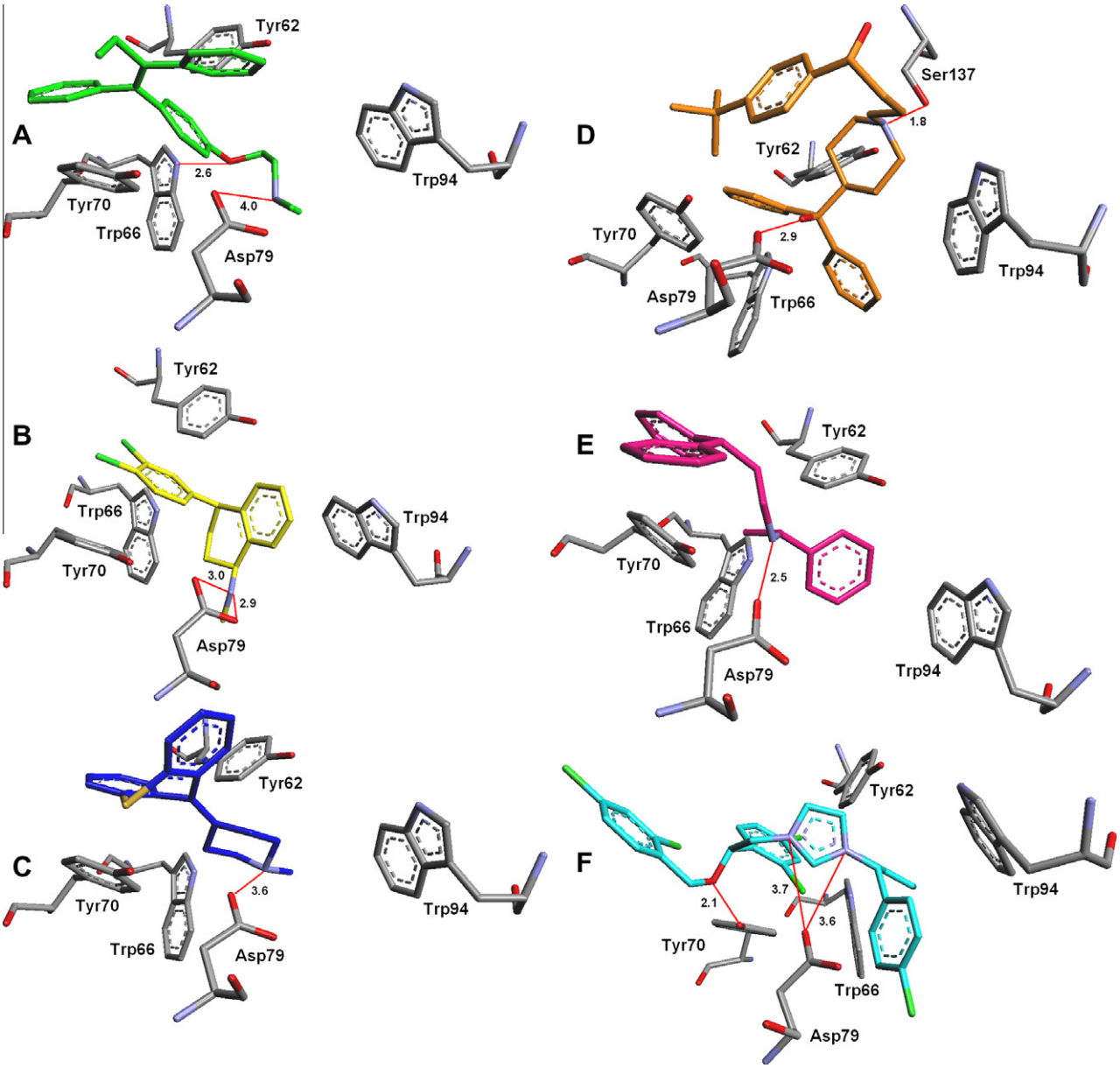

Figure 2. Magnified views of proposed binding modes within the LuxR binding site displaying interactions between conserved residues in the LuxR family and the newly identified inhibitors tamoxifen (A), sertraline (B), pimethixene (C), terfenadine (D), fendiline (E) and calmidazolium (F).

A molecular modelling study was carried out to investigate further the structure–activity relationship. Docking experiments were individually conducted on each antagonist within the ligand binding site of LuxR. The docking results led to the binding modes, presented in Figure 2, with conserved residues in the LuxR family (see Supplementary data for an overall representation). Careful analysis of the complexes revealed several attractive interactions between these compounds and the conserved aromatic residues. Indeed, the aromatic moiety of these compounds interacts tightly with Tyr62, Tyr70 and Trp66. In addition, Trp94 is involved in attractive interactions with the aromatic or benzhydryl groups of sertraline, terfenadine, fendiline and calmidazolium (Fig. 2B, D–F). Polar groups of these compounds also interact through hydrogen bonds, with Trp66 (Fig. 2A) or with Ser137 (Fig. 2D), a conservatively replaceable residue in the LuxR family. Importantly, the residue Asp79, an essential residue conserved in the LuxR family,[22] is involved in hydrogen bonds or in electrostatic interactions with amine groups (Fig. 2A–E) or with the positively charged imidazolium ring of calmidazolium (Fig. 2F). This compound interacts tightly with the LuxR binding site, not only through the interactions already cited but also via a hydrogen bond between the oxygen atom of the ether function and Tyr70, as well as several halogen bonds.[40] All these interactions could explain the potent activity of calmidazolium.

To summarise, we have described here an efficient docking-based virtual screening of a 2344 chemical compounds library targeting the binding sites of LuxR-type proteins. The screening protocol included four sequences of docking simulations, performed with LuxR, TraR, and LasR, in order to specifically target the conserved residues. Interestingly, some compounds identified as potential QS modulators belong to hormones or calcium channel modulators, thus providing indications of a possible cross-talk between bacteria and the host. A biological evaluation of the LuxR-dependent QS system led to the discovery of six new QS inhibitors: tamoxifen, sertraline, pimethixene, terfenadine, fendiline and calmidazolium. In addition, calmidazolium was identified as one of the most potent LuxR-dependent QS inhibitors structurally unrelated to AHL.

Acknowledgements

Financial support from MESR, CNRS, and the 'Cluster de recherche Chimie/infectiologie de la Région Rhône-Alpes' is gratefully acknowledged. M.S. is the recipient of a scholarship from the cluster. We warmly thank Dr. Sylvie Reverchon for helpful discussions, Julien Wawrzyniak for assistance during biological evaluation and V. James for improving the English of the manuscript.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.06.081.

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