NUCLEAR RECEPTORS

Blocking without obstruction

Research in the May issue of *Nature Structural Biology* has shown that a small molecule known as THC antagonizes oestrogen receptor-β (ER-β) by means of a novel mechanism. THC blocks transcription without the usual physical obstruction of the positioning of helix 12 in the ER-β protein, which is necessary for receptor activation. Interestingly, whereas THC acts as an antagonist of ER-β, it is an agonist of ER-α. This study solved the crystal structures of THC bound to the ligand-binding domains of both ER-α and ER-β, in order to investigate the mechanisms of THC agonism and antagonism.

Both ER-α and ER-β mediate the physiological effects of both endogenous and synthetic oestrogens. These receptors are members of the nuclear-receptor superfamily of ligand-regulated transcription factors. The ligand-binding domains of ER-α and ER-β each have a transcriptional activation function, which is responsive to agonists, including oestrogen 17β-oestradiol (E2) and diethylstilbestrol. Antagonists, such as 4-hydroxytamoxifen and raloxifene, block the activation-function activity. Agonists stabilize a conformation of the receptor in which helix 12 lies across the opening of the binding pocket, thereby allowing the receptor to interact with the transcriptional co-activators that mediate ligand-dependent transcription of the receptor. Most ER antagonists have bulky side chains that cannot fit within the binding pocket, and so ER-β helix 12 cannot adopt the agonist-bound conformation, which occludes binding to transcriptional co-activators. But because THC lacks the bulky side chain that is typical of other ER antagonists, it must antagonize ER-β through a different mechanism.

Comparing the crystal structures of THC bound to either ER-β or ER-α shows that although THC binds to both chains in a similar manner overall, it fails to stabilize several of the binding-pocket interactions with ER-β in comparison to those with ER-α. The slight differences in sequence between the two ERs means that crucial residues in ER-β adopt a random-coil conformation instead of a helical one. This does not favour the agonist-bound conformation of helix 12, and actually stabilizes an

HIGH-THROUGHPUT SCREENING

Birds of a feather…

Many hits from high-throughput and virtual screening programmes are subsequently found to have peculiar, undesirable characteristics — they act non-competitively, show little relationship between structure and activity, and have poor selectivity. Such hits can waste much time and effort, but despite their common occurrence, the underlying reasons for their behaviour have remained unknown. Now, writing in the *Journal of Medicinal Chemistry*, McGovern *et al.* provide evidence that these compounds, although structurally unrelated, share the ability to aggregate, which results in them being falsely detected as hits in screening assays.

Initially, the authors investigated 15 diverse compounds described as inhibitors of one or more protein or nucleic-acid targets, and found that the compounds were also micromolar inhibitors of several unrelated enzymes, including β-lactamase, chymotrypsin, dihydrofolate reductase and β-galactosidase. Furthermore, inhibition of various targets was decreased in the presence of bovine serum albumin, a common sign of nonspecific binding. But most intriguingly, the apparent inhibition constants (IC_{50} values) of all the compounds worsened considerably when the concentration of one of the test targets, β-lactamase, was increased tenfold, in contrast to the behaviour of an established competitive inhibitor.

To account for the extreme sensitivity of the screening hits to the molar ratio of inhibitor to enzyme, the authors considered the hypothesis that the active inhibitor might be an aggregate of many molecules. Dynamic light-scattering experiments (DLS) of aqueous solutions of the hits indicated the presence of particles with apparent diameters ranging from 95 to 400 nm — much larger than the target enzymes, which are 20 nm at most in their longest dimension. The presence of aggregates was confirmed by transmission electron microscopy, and also backed up by further experiments on enzyme kinetics.

So, are aggregate-forming nonspecific inhibitors commonplace in corporate screening collections? To investigate this possibility, the authors tested 30 compounds from the Pharmacia Corporation library, biasing the selection of these compounds towards those that register as hits in multiple screens against different targets. Of these 30, 20 inhibited β-lactamase and chymotrypsin at micromolar concentrations. As with the previous compounds, inhibition worsened as enzyme concentration was increased, and DLS indicated the presence of large particles.

It thus seems that compounds that inhibit enzymes by forming aggregates could often be artificially raising the hit rates in high-throughput screens for new drug leads. Several important questions remain to be answered, such as how the molecules are arranged in the aggregates, and how the aggregates inhibit the enzymes. Increased understanding should lead to computational or screening methods to rapidly eliminate these phoney hits from future screening programmes.

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References and links

ORIGINAL RESEARCH PAPER McGovern, S. L. *et al.* A common mechanism underlying promiscuous inhibitors from virtual and high-throughput screening. *J. Med. Chem.* **45**, 1712–1722 (2002)