ARTICLE

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Large-scale prediction and testing of drug activity on side-effect targets

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Discovering the unintended 'off-targets' that predict adverse drug reactions is daunting by empirical methods alone. Drugs can act on several protein targets, some of which can be unrelated by conventional molecular metrics, and hundreds of proteins have been implicated in side effects. Here we use a computational strategy to predict the activity of 656 marketed drugs on 73 unintended 'side-effect' targets. Approximately half of the predictions were confirmed, either from proprietary databases unknown to the method or by new experimental assays. Affinities for these new off-targets ranged from 1 nM to 30 μM. To explore relevance, we developed an association metric to prioritize those new off-targets that explained side effects better than any known target of a given drug, creating a drug–target–adverse drug reaction network. Among these new associations was the prediction that the abdominal pain side effect of the synthetic oestrogen chlorotrianisene was mediated through its newly discovered inhibition of the enzyme cyclooxygenase-1. The clinical relevance of this inhibition was borne out in whole human blood platelet aggregation assays. This approach may have wide application to de-risking toxicological liabilities in drug discovery.

Adverse drug reactions (ADRs) can limit the use of otherwise effective drugs. Next to lack of efficacy, they are the leading cause for attrition in clinical trials of new drugs[1–3] and are more prominent still in the failure of molecules to advance from pre-clinical research into human trials[4]. Some ADRs are caused by modulation of the primary target of a drug[5], others result from non-specific interactions of reactive metabolites[6]. In many cases, however, ADRs are caused by unintended activity at off-targets. Notorious examples of off-target toxicity include that of the appetite suppressant fenfluramine–phentermine (fen–phen), which was withdrawn from the market after numerous patient deaths. These owed to the activation of the 5-hydroxytryptamine-2B (5-HT_{2B}) receptor by one of its metabolites, norfenfluramine, leading to proliferative valvular heart disease[7]. Similarly, well-known drugs, such as the antihistamine terfenadine, have been withdrawn because they caused arrhythmias and death, which have been attributed to their off-target inhibition of the human *ether-à-go-go*-related gene potassium channel (hERG, also known as KCNH2)[8,9]. Prediction of unknown off-target drug interactions might prevent such disastrous drug toxicities, which are often detected only after fatalities in the clinic, and might allow safer molecules to be prioritized for pre-clinical development. Methods to systematically predict off-targets, and associate these with side effects, have thus attracted intense interest[10–16], frequently in the form of either chemical genomics[17,18] or informatics[19–26] approaches.

Whereas the informatics methods have never been tested systematically on a large scale, in principle they can be deployed against thousands of targets. Here we present a large-scale, prospective evaluation of safety target prediction using one such method, the similarity ensemble approach (SEA)[25–27]. SEA calculates whether a molecule will bind to a target based on the chemical features it shares with those of known ligands, using a statistical model to control for random similarity. Because SEA relies only on chemical similarity, it can be applied systematically and, for those targets that have known ligands, comprehensively. For 656 drugs approved for human use (Supplementary Table 1), targets were predicted from among 73 proteins (Supplementary Table 2 and Methods) with established association of ADRs[22,28], for which assays were available at Novartis. Encouragingly, many of the predictions were confirmed, often at pharmacologically relevant concentrations. This motivated us to develop a guilt-by-association metric that linked the new targets to the ADRs of those drugs for which they are the primary or well-known off-targets, creating a drug–target–ADR network. The applicability and the limitations of this approach will be considered.

Testing the predictions

The 656 drugs were computationally screened for their likelihood to bind to 73 targets (Supplementary Table 2) using SEA[25–27]. The targets belong to the Novartis *in vitro* safety panels based on their association with ADRs[22,28]. Here we insisted that they also be described in the ChEMBL database[29], enabling correspondence with SEA predictions (Supplementary Table 2). ChEMBL annotates more than 285,000 ligands modulating more than 1,500 different human targets with affinities better than 30 μM. SEA calculated the similarity of each drug versus each set of ligands for the 73 targets, comparing the overall set similarity to a model of such expected at random. For instance, the sodium channel blocker aprindine loosely resembled the set of histamine H_1 ligands; although no single H_1 ligand was strongly similar to the drug (Table 1), the overall similarity of the set was much greater than expected at random, leading to a highly significant SEA expectation value (E value) of 5×10^{-26} between aprinidine and H_1 receptor ligands. Only 1,644 of the more than 47,000 possible drug–target pairs had significant E values. Of these, 403 were already known in ChEMBL and so were trivially confirmed; we do not consider these further. Of the remaining 1,241 predictions, 348 (28%) were unknown to ChEMBL, but could be found in proprietary ligand–target databases that were unavailable to SEA (see Methods). The remaining

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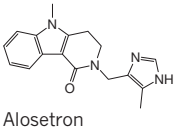
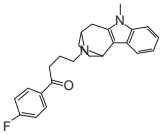
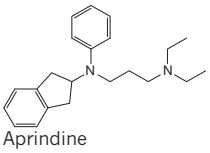
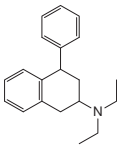
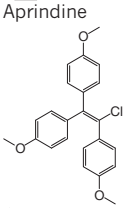
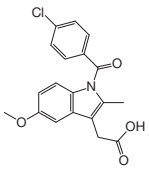
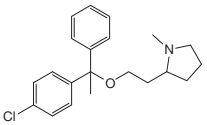
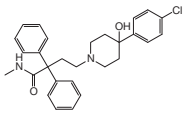
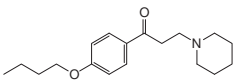
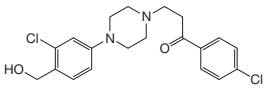
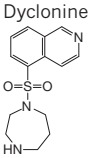
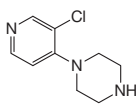
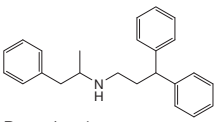
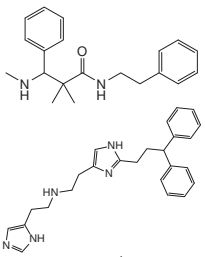
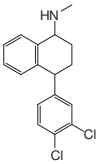
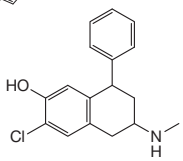
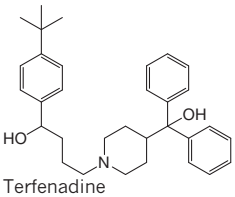
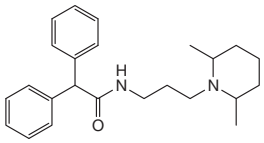
Table 1 | New drug-off-target predictions confirmed by *in vitro* experiment

Drug	Closest chEMBL molecule	Tc value	Target	SEA *E* value	IC_{50} (μM)	Closest known target	BLAST *E* value
 Alosetron		0.25	HTR2B	10.6×10^{-17}	0.02	KCNH7	3.6×10^{2}
 Aprindine		0.38	HRH1	5.0×10^{-26}	0.78	SCN5A	3.3×10^{-1}
 Chlorotrianisene		0.31	COX-1	1.9×10^{-17}	0.16	ESR1	9.0×10^{2}
 Clemastine		0.31	SLC6A4	1.1×10^{-14}	0.42	KCNH2	6.1×10^{1}
 Dyclonine		0.36	DRD4	1.5×10^{-17}	4.1	SLC6A3	2.3×10^{2}
 Fasudil		0.37	ADRA2A	1.1×10^{-7}	4.0	CCR2	1.5×10^{-9}
 Prenylamine		0.31	OPRM1	1.1×10^{-8}	1.8	CACNA1G	3.5×10^{0}
		0.30	HRH1	3.2×10^{-66}	7.9	SCN5A	3.3×10^{-1}
 Sertraline		0.33	HRH2	5.1×10^{-45}	1.4	HTR4	6.8×10^{-51}
 Terfenadine		0.24	SCN5A	8.9×10^{-8}	7.1	CYP2D6	5.9×10^{1}

Representative, confirmed predictions are shown. Tc values determined using ECFP_4-based molecular similarity to the closest ChEMBL reference molecule in the target set. The closest known target is a known target of the drug that has highest sequence similarity to the predicted target. The BLAST *E* value is based on the sequence identity of the predicted target to the closest known target; values greater than 10^{-5} represent unrelated proteins.

893 predictions represented previously unexplored drug–target associations.

Of these predictions, 694 were tested at Novartis. For 478, activity was less than 25% at 30 μM; these were considered disproved. For another 65 predictions, activity was between 25 and 50% at 30 μM; these were considered ambiguous. Finally, for 151 of the new drug–target predictions, half-maximum inhibitory concentration (IC_{50}) values of less (better) than 30 μM were measured in concentration–response curves (Fig. 1a and Supplementary Fig. 1). In 125 cases, the drugs had an IC_{50} value better than 10 μM, and in 48 cases activities were sub-micromolar (Table 1, Supplementary Table 3 and Supplementary Fig. 1). In summary, of the 1,042 predictions that were tested (694 by assay,

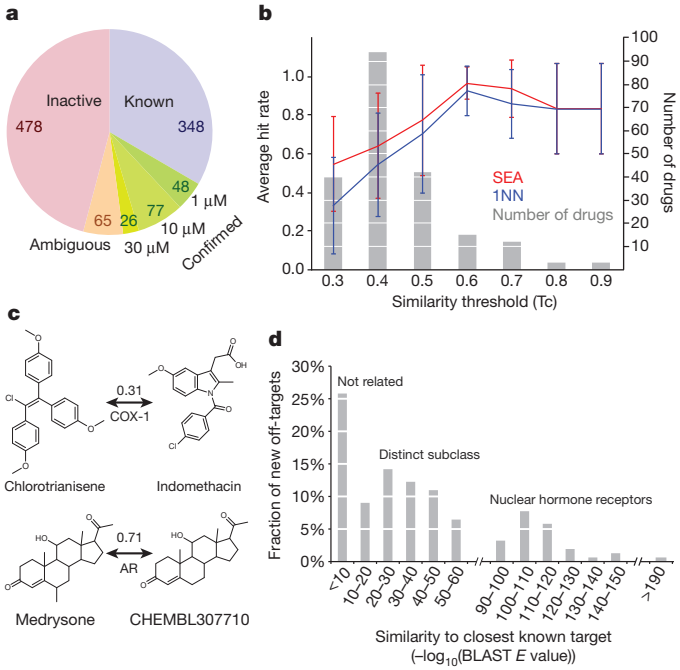


Figure 1 | Predicting off-targets, and their novelty. **a**, Success of SEA predictions. Known: predicted off-targets confirmed by proprietary databases. Confirmed: predictions tested *in vitro* achieving $IC_{50} < 30\ \mu M$. Ambiguous: predictions with 25–50% activity at 30 μM. Inactive: <25% activity. **b**, SEA enriched for non-trivial similarity. Drugs were binned (grey) by lowest Tc values yielding valid SEA predictions. Hit rates of SEA (red) and 1NN (blue) are shown. Error bars denote s.d. **c**, Non-intuitive (chlorotrianisene) and straightforward (medrysone) SEA predictions, with Tc values to closest references shown. Chlorotrianisene is only marginally similar to indomethacin, but is (correctly) predicted for COX-1. AR, androgen receptor. **d**, Sequence similarities of each confirmed drug off-target to the closest known target of the drug.

348 by databases), 48% were confirmed either in proprietary databases, unknown to the method and to those undertaking the SEA calculation, or in Novartis assays in full concentration responses, and just under 46% were disproved (Fig. 1a).

In assessing these results, one would like to compare the true predictions with the false-positive and the false-negative predictions. Whereas this work offers guidance on false-positive predictions, we can only address false negatives for a few compounds (Supplementary Results). Among these was astemizole, which had affinities ranging from 0.1 to 9 μM on the 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} serotonin receptors and the histamine H_2 and dopamine D_2 receptors, as measured in other projects at Novartis. These targets were missed owing to a charge post-filter, separate from SEA itself, which excluded compounds with net charge dissimilar from the reference ligands[30]. Astemizole was improperly assigned[31] a charge of +2, wrongly differentiating it from the known ligands; the SEA *E* values linking astemizole to these targets were themselves between 10^{-25} to 10^{-27}. Other failures could be attributed to SEA itself. For instance, promazine bound to the histamine H_1 and H_2 receptors with low to mid-nanomolar affinities, but the SEA *E* values at 10^{-4} to 10^{-3} were below our significance cut-off. This work was undertaken with ChEMBL_2 as a source of ligand–target association; had we used the more recent ChEMBL_10, the H_1 receptor would have been predicted with an *E* value of 10^{-9} (see http://sea.bkslab.org), and had we used ChEMBL_12 and a newer version of SEA, both targets would have been predicted. Clearly, with its reliance on topology and on inference from known ligand–target associations, SEA will have false negatives.

A key question is whether the new predictions were in any way surprising. One way to evaluate this is to compare the similarity of drugs predicted for new targets with the closest previously known ligand for that target. We used Tanimoto coefficients (Tc), which compare the groups in common between two molecules, here represented by ECFP_4 fingerprints. Tc values between nearest molecules were small, often less than 0.4 (ref. 32); visual inspection of these pairs confirms the dissimilarity suggested by the low Tc values (Table 1). More systematically, SEA may be compared with a method that predicts targets based only on the one-nearest neighbour (1NN) model (Fig. 1b). For close analogues (Tc values > 0.7; Fig. 1c), the fraction of true positives was comparable between 1NN and SEA approaches (Fig. 1b). But across most similarity thresholds, SEA substantially outperformed 1NN, and by nearly twofold in the low similarity range. Thus, for the Rho kinase inhibitor fasudil, SEA predicted only the adrenergic α_{2A} receptor, with an *E* value of 1.1×10^{-7}, which was experimentally confirmed ($IC_{50} = 4\ \mu M$). This occurred despite the low similarity of the closest known α_2 ligand, which had a Tc value of 0.37 to fasudil. Conversely, at this similarity threshold the 1NN model predicted nine targets, only three of which were confirmed (Supplementary Table 4). For chlorotrianisene, two of the three targets predicted by SEA were confirmed; conversely, at its 0.31 Tc for cyclooxygenase-1 (COX-1, also known as PTGS1) the 1NN model predicted ten targets, only two of which were confirmed.

We also investigated how often the new off-target would have been obvious based on sequence similarity of the targets[25,26,33]. We calculated the BLAST sequence similarity of predicted targets to any known target of a drug (Table 1 and Supplementary Table 3). Of the 151 new off-target predictions, 39 (26%) had BLAST *E* values greater (worse) than 10^{-5}, suggesting the previously known targets shared no sequence similarity with the new off-targets (Table 1, Supplementary Table 3 and Fig. 1d). For example, the anaesthetic dyclonine was shown to bind the histamine H_2 receptor (HRH2), whereas the closest known target was the $Na_v1.8$ channel (SCN10A), which has no significant sequence similarity (BLAST *E* value > 1) and is functionally unrelated to the H_2 receptor. Similarly, the anti-nausea drug alosetron antagonized the 5-HT_{2B} receptor with an IC_{50} of 18 nM, although 5-HT_{2B} has no sequence similarity to the ion channel targets of this drug (Table 1). Chlorotrianisene potently inhibits the enzyme COX-1, which is unrelated by sequence to the primary nuclear hormone receptor of this drug, the oestrogen receptor (Table 1).

Associating *in vitro* targets with ADRs

To assess the potential clinical relevance of the discovered targets systematically, we developed a quantitative score that associated *in vitro* activity with patient ADRs. We enumerated all possible target–ADR pairs for 2,760 drugs with available adverse event annotations, expressing as an enrichment score the co-occurrence of pairs that were more common than expected by chance (Supplementary Table 5). For example, 'abdominal pain upper' has been reported for 45 drugs that interact with COX-1. The ADR abdominal pain upper was linked with 6,046 drug–target pairs, whereas COX-1 was linked with 2,188 drug–ADR pairs; there were a total of 681,797 target–ADR pairs overall. Thus the pair abdominal pain upper–COX-1 was enriched 2.3-fold above random (Methods), with a χ^2 *P* value of 9.9×10^{-9}. A total of 3,257 significant target–ADR associations were identified (Supplementary Table 5).

Having identified new off-targets for the drugs, and linked these with observed ADRs, we sought drug–target–ADR connections that illuminate the clinical relevance of the predictions. Of the 151 confirmed new drug–target associations tested at Novartis, 82 were significantly associated with one or more ADR, resulting in a total of 247 drug–target–ADR links. In 116 cases, the enrichment factor (EF) of the new drug–target–ADR link was stronger than that for any previously known target (Table 2 and Supplementary Table 6). For example, prenylamine was found to bind the histamine H_1 receptor (HRH1), which we associate with a sedation ADR (EF = 4.9). By contrast, none of the known targets of prenylamine was associated

Table 2 | Characteristic new, confirmed targets associated with ADRs of the drugs

Drug name	Target	Activity (μM) (median)	AUC (μM h)	C_{max} (μM)	Adverse event	EF ratio	Alternative target	Comparable drug
Chlorotrianisene	COX-1	0.16	NA	NA	Abdominal pain upper	2.32	None	None
					Rash	1.79	None	None
Clemastine	SLC6A4	0.42	NA	NA	Sleep disorder	2.15	None	None
Cyclobenzaprine	HRH1	0.02	0.16–4.10 (0.69)	0.01–0.13 (0.06)	Ataxia	1.73	None	**Desipramine**
					Somnolence	1.49	None	**Aripiprazole**
Diphenhydramine	SLC6A3	4.33	2.57–3.42 (3.00)	0.26–0.26 (0.26)	Tremor	2.02/1.90	SCN10A	**Citalopram**
Loxapine	CHRM2	1.12	0.03–0.43 (0.21)	0.02–0.41 (0.14)	Tachycardia	2.08/1.97	CHRM1	Sibutramine
Methylprednisolone	PGR	1.30	0.09–10.76 (1.28)	0.06–2.11 (0.31)	Depression	3.87/2.49	NR3C1	Flutamide
Prenylamine	HRH1	7.87	0.12–0.12 (0.12)	1.20–1.20 (1.20)	Sedation	4.94	None	None
Ranitidine	CHRM2	5.56	5.66–121.90 (9.67)	1.14–9.11 (2.12)	Constipation	1.63	None	Haloperidol
Ritodrine	OPRM1	9.18	0.03–0.32 (0.11)	0.01–0.15 (0.04)	Hyperhidrosis	3.21	None	**Oxycodone**

ADRs are listed that are more strongly associated with the predicted target than any known target, together with pharmacokinetic data (AUC and C_{max}), where available. Numbers in parentheses denote the median value, and the range denotes the minimal and maximal reported value. Where pharmacokinetics (PK) and pharmacodynamic activity (PD) were available, drugs have been identified that behave comparably to the predicted drug and also cause the adverse event. The EF ratio is the ADR–target enrichment for the predicted/best alternative known target ratio. Comparable drugs are those that are known to bind the predicted target (bold denotes predicted target is the primary target), share the ADR and behave similarly in terms of PK and PD (see Methods). NA, not available.

with this side effect. For other cases, known targets represented an alternative explanation for an ADR. For instance, we found that diphenhydramine binds to the dopamine transporter (SLC6A3; Table 2), which is associated with tremor[34]. Although tremor was also associated with one of the known targets of diphenhydramine, sodium channel SCN10A (EF = 1.9)[35], its association with the dopamine transporter was higher (EF = 2.02), indicating a possible mechanistic link with the new off-target. Conversely, the 'dry mouth' side effect of diphenhydramine was better explained by its known antagonism of the M_3 muscarinic receptor (CHRM3; EF = 2.45, Supplementary Table 5).

We asked whether the affinity of a drug for its predicted ADR–target was relevant given its pharmacology, comparing the predictions against other drugs with similar pharmacodynamics and pharmacokinetics (Table 2). This was possible for 36 drug–target–ADR links (Supplementary Table 6). For instance, cyclobenzaprine was shown to bind to the histamine H_1 receptor at 21 nM, whereas its median maximal plasma concentration (C_{max}) was 61 nM; nine other drugs binding the H_1 receptor in the nanomolar range with comparable C_{max} values were found (Table 2). Although some of the measured drug–target affinities were moderate, the pharmacokinetic data often confirmed that they were nevertheless relevant. For instance, the affinity of ranitidine (Zantac) for the M_2 muscarinic receptor, which we associate with its constipation ADR, is only 5.6 μM. Nevertheless, with an area under the curve (AUC) value of 5.7 to 122 μM h (minimal and maximal reported values) in plasma, this association seems plausible. Similarly, diphenhydramine has an IC_{50} value of only 4.3 μM against the dopamine transporter, a target that we associate with the tremor side effect of the drug. Nevertheless, the AUC value of diphenhydramine of 2.6 to 3.4 μM h supports the relevance of its modest IC_{50} value.

Drug–target–ADR networks

Network graphs help to visualize the new and known drug–target links, and the adverse events with which they are associated (Fig. 2a–c). For example, the oestrogen receptor (ESR1) modulator chlorotrianisene was found to inhibit COX-1, with an affinity substantially better than its affinity for ESR1. Drugs that modulate the two proteins can share two of the adverse reactions of chlorotrianisene ('erythema multiforme' and 'oedema'), but 'rash' and 'abdominal pain upper' link only to drugs inhibiting COX-1, and these are both associated with chlorotrianisene almost uniquely among the oestrogen receptor modulators (Fig. 2c and Supplementary Table 5). For prenylamine, a new G-protein-coupled receptor (GPCR) cluster (HRH1, OPRM1 and ADRB2) emerges that is unrelated to the primary ion channel activity of the drug, but uniquely link to its sedative and myocardial infarction ADRs (Fig. 2b). For domperidone, its known activity at dopamine receptors is associated with a Parkinsonism-like phenotype ('hyperprolactinaemia' and 'extrapyramidal disorder'), whereas 'somnolence' associates only with the newly discovered opioid activity (Fig. 2a).

Several of the drug–target–ADR associations that emerged were surprising. Among them were the association of the muscle relaxant cyclobenzaprine with somnolence, the H_2 antagonist ranitidine with constipation, and chlorotrianisene with upper abdominal pain (Table 2). Cyclobenzaprine caught our attention because even its mechanism of action target for muscle relaxation has not been characterized, and its association with the off-target discovered here, the H_1 receptor (IC_{50} = 20 nM), precedes the identification of its primary target. The central nervous system H_1 receptor is strongly associated with somnolence, consistent with the ADR of the drug, and supported by its pharmacokinetics. Similarly, the constipation effect of ranitidine is consistent with its activity on the M_2 muscarinic receptor. Although its affinity for M_2 is moderate at 5.5 μM, the pharmacokinetics make this affinity relevant to this ADR.

Perhaps the most compelling demonstration of a drug–target–ADR association is one *in vivo*, or in an accepted *in vivo* biomarker. The observation that chlorotrianisene was a potent COX-1 inhibitor seemed a reasonable explanation for the upper abdominal pain (epigastralgia) side effect provoked by the drug, and one that lent itself to direct testing in an accepted biomarker. Epigastralgia is a well-known ADR of non-steroidal anti-inflammatory drugs (NSAIDs), which inhibit the cyclooxygenase enzymes COX-1 and COX-2. COX-1 has housekeeping effects in the gastric mucosa[36], and its inhibition can lead to mucosal thinning and gastroduodenal ulceration, leading to upper gastric pain and the thousands of annual hospitalizations that are associated with NSAID use[37]. NSAIDs also inhibit platelet aggregation by direct inhibition of their endogenous COX-1 enzyme[38]. Intriguingly, this effect is unreported for other synthetic oestrogens, which, to the contrary, are more likely to promote platelet aggregation[39,40]. A widely accepted model for platelet aggregation may be run *ex vivo*, in whole blood, allowing one to test for target engagement of COX-1 in this effect.

Accordingly, collagen-induced platelet aggregation was measured in freshly drawn human blood from six healthy volunteer donors. Acetylsalicylic acid, the active ingredient in aspirin, inhibited platelet aggregation by 42–48% at 250 μM. The more potent NSAID indomethacin inhibited platelet aggregation by 50% at 50 μM. Chlorotrianisene inhibited platelet aggregation in whole blood with a potency almost indistinguishable from that of indomethacin, and more potently than acetylsalicylic acid (Fig. 2d). These results are consistent with an *in vivo* inhibitory activity of chlorotrianisene on COX-1, and with the epigastralgia that is among its common side effects.

Drug and target promiscuity

To investigate overall patterns of drug and target promiscuity, we integrated the experimental results from this and other Novartis studies. The most promiscuous target was the voltage-gated sodium channel

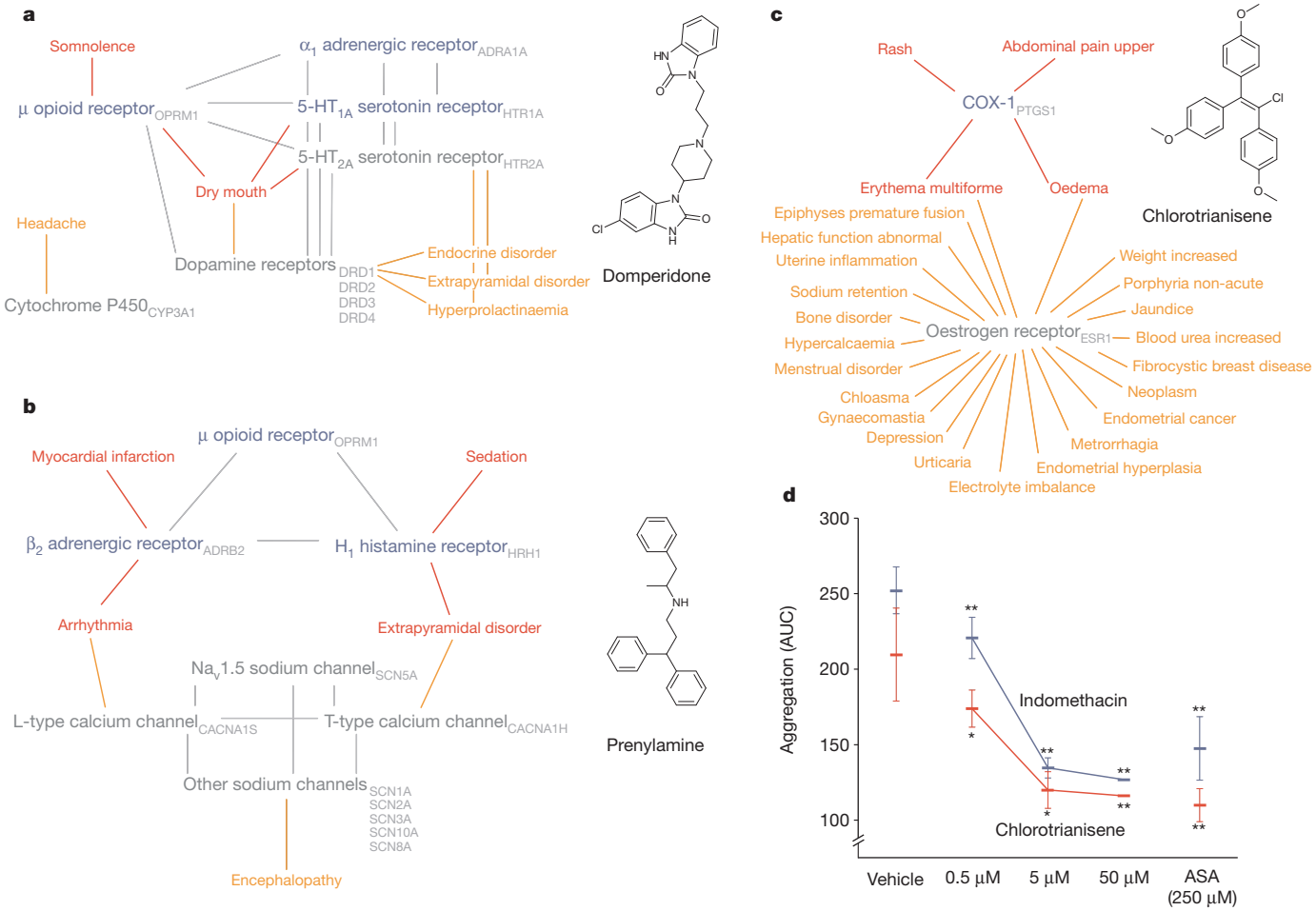


Figure 2 | Off-target networks. **a**–**c**, Off-target networks for three drugs. Known targets of the drugs are grey, whereas newly predicted targets are blue; the adverse events associated with each are orange and red, respectively. Red adverse events are significantly (EF > 1, q value < 0.05) associated with the new off-targets. Targets related by sequence are connected by grey edges. **d**, Chlorotrianisene inhibits platelet aggregation. Two independent experiments (red and blue) are shown for chlorotrianisene and indomethacin, respectively. ASA, acetylsalicylic acid (positive control); vehicle denotes negative control. $*P < 0.05$, $**P < 0.01$ (paired Student's t-test differences compared with vehicle control). Error bars denote s.d.

(SCN5A), to which 70 out of 126 (56%) tested drugs bound (Fig. 3a). From a target family standpoint, however, this was an exception, as most other promiscuous targets were small molecule-recognizing GPCRs; of non-GPCRs, only SCN5A and the ion channel hERG were targeted more than average (>13% of drugs tested). Transporters had mid-range promiscuity; enzymes, nuclear receptors and ligand-gated ion channels were less promiscuous, whereas peptide-recognizing receptors were hit least of all (Supplementary Tables 2 and 7).

Inverting this analysis, the most promiscuous drug, chlorhexidine, hit 34 out of 54 (64%) targets against which it was tested, and another nine drugs were active on more than 50% of their tested targets (Fig. 3b and Supplementary Table 8). Twenty-five drugs bound to proteins from among all major target classes. Highly promiscuous drugs were often lipophilic and cationic at physiological pH (Fig. 3b)[41,42].

Predicting off-targets and adverse events

This study begins to quantify drug polypharmacology at scale: the 656 drugs considered here each modulated an average of seven safety targets, sometimes across several classes, and more than 10% of the drugs acted on nearly half (45%) of the 73 targets (Fig. 3b). It is sobering that this promiscuity is observed for approved human drugs, which have typically already been optimized to minimize toxicity. For lead molecules that are progressing towards the clinic, this level of off-target promiscuity might be higher still[28]. Anticipating these off-targets is difficult, as they can be unrelated in sequence and structure to the primary targets of a drug, and even known target–ADR associations are not always straightforward. Two results of this study begin to address these challenges. First, of the 1,042 predicted drug–target associations that were tested, 48% were confirmed (Fig. 1a). With 46% of the predictions disproved, the method remains imperfect, but this rate may nevertheless be high enough to prioritize compound classes and targets for testing. Second, a guilt-by-association metric can link off-targets with ADRs. A three-way association between drugs, molecular targets and ADRs may be systematically calculated and interpreted (Fig. 2a–c).

Surprisingly, drugs often modulated off-targets unrelated to their primary target. Of the 151 off-targets that were confirmed by new experiment, 39 were unrelated by sequence to any of the known drug targets (Fig. 1d). For example, the antitussive clemastine and the antihistamine diphenhydramine (an active ingredient in products such as Tylenol PM), both of which act on the histamine H_1 GPCR, also modulate the serotonin transporter (5-HTT, also known as SLC6A4), to which the primary target is unrelated by sequence or structure. Conversely, the serotonin transporter inhibitor sertraline acts on the histamine H_2 GPCR. The activity of drugs on targets that are unrelated by sequence or structure to their primary targets can seem capricious and certainly makes prioritization of likely targets more difficult. A ligand-based approach offers an orthogonal view of target relationships and so can illuminate similarities that are opaque from a molecular biology perspective. The converse is also true, and the two views will often be complementary.

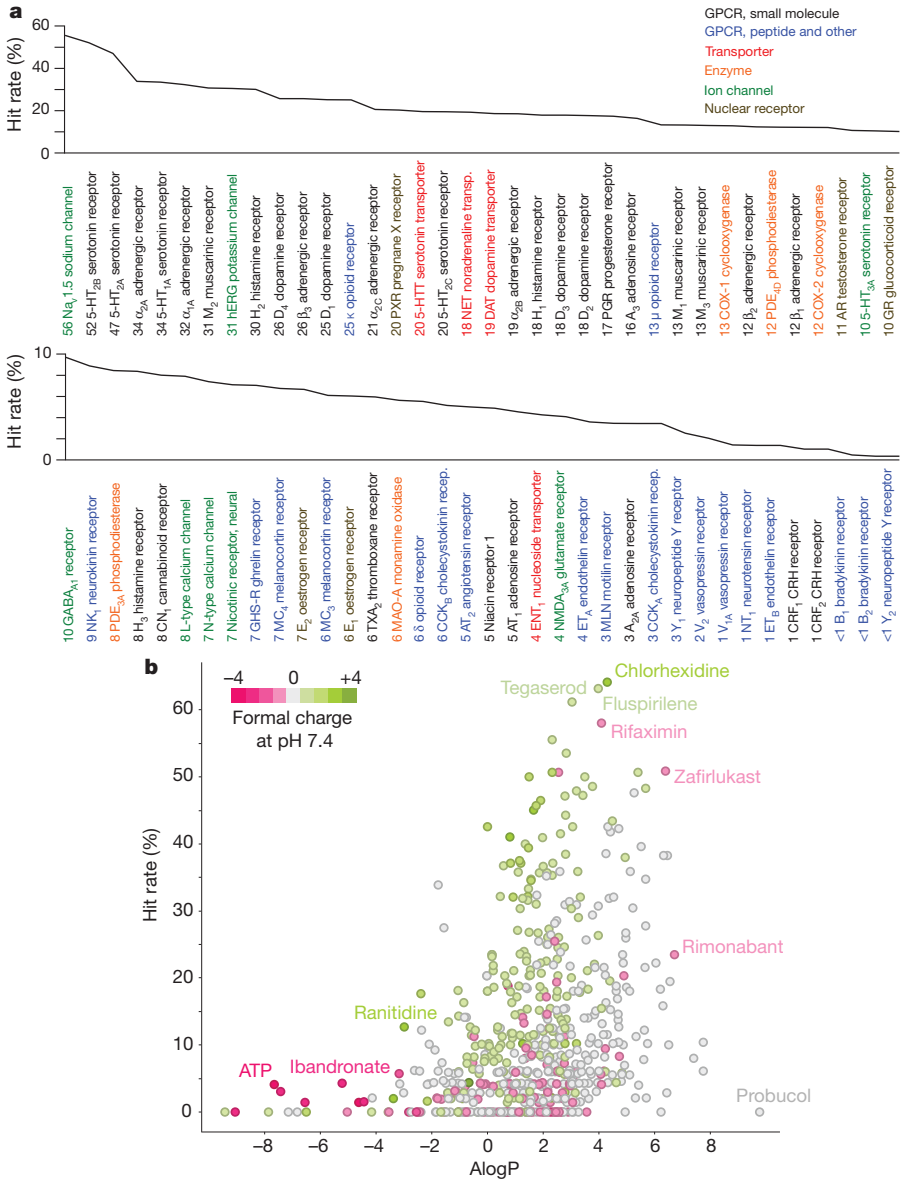


Figure 3 | Target and drug promiscuity. **a**, Target promiscuity. Targets are sorted based on the percentage of drugs hitting the target below 30 μM (indicated by numbers next to target names). Colours code for seven distinct target classes. **b**, Promiscuous drugs are often hydrophobic and cationic. Each point represents one drug. Ionization at pH 7.4 and AlogP (calculated octanol–water partitioning coefficient) values were calculated from drug structures. Hit rate denotes the percentage of targets the drug binds below 30 μM.

The association between chlorotrianisene, COX-1 and epigastralgia illustrates the potential of the approach. The therapeutic target of chlorotrianisene, the oestrogen nuclear hormone receptor, bears no sequence or structural similarity to the COX-1 enzyme, but the likelihood of cross-activity between the two targets is articulated by ligand similarity (Table 1). Correspondingly, the linking of abdominal pain and COX-1 only emerges when one quantitatively compares the ADRs of known COX-1 inhibitors to what one would expect at random. The potent inhibition of platelet aggregation by chlorotrianisene in whole blood (Fig. 2d) is consistent with the systemic, *in vivo* activity of this drug at relevant concentrations on COX-1.

Certain shortcomings of the method should not escape the reader's attention. Almost 46% of the predicted drug–target associations were disproved, and the method, which is inference-based, undoubtedly has important false negatives. As such, SEA cannot replace compound-target testing. What it can do is identify compounds early in development for possible liabilities that would ordinarily be identified only much later in drug progression. Similarly, the guilt-by-association method is inference-based and mechanism-naive, and so will miss some target–ADR associations and make others that are invalid. Also, only some side effects fall into the remit of this approach, which assumes an off-target mechanism. Side effects, like other pharmacological events, have a strong exposure component, and can result from complex interactions with regulatory networks[43]. Thus, topical drugs such as econazole or chlorhexidine, although promiscuous *in vitro*, have fewer ADRs than expected because they never achieve sufficient systemic exposure *in vivo*. Conversely, a drug such as tacrolimus might be relatively selective *in vitro*, but is associated with several side effects owing to its broad immunosuppressant effects.

These caveats should not obscure the potential of this approach to predict and understand drug side effects. The method was used automatically at scale, without human intervention. Whereas its predictions were sometimes disproved, they were just as often confirmed. If some of the targets it suggested required no imaginative leap—as when a steroid was predicted for a new nuclear hormone receptor—a quarter of the confirmed targets were unrelated by sequence or structure to any of the known targets of the drugs (Fig. 1c, d, Table 1 and Supplementary Table 2). Pragmatically, the ability to calculate drug–target–ADR networks provides a tool to anticipate liabilities among candidate drugs being advanced towards the clinic, or yet earlier, for prioritization of chemotypes in preclinical series. If such networks cannot replace direct experimentation, they can usefully prioritize off-targets for consideration. As we struggle to develop new therapeutics, this and related approaches[11,19,21–24,44,45] can identify molecules with liabilities early in their development, and so focus effort on those candidates that are least subject to them.

METHODS SUMMARY

A collection of on-hand 656 US Food and Drug Administration-approved drugs was computationally screened against a panel of 339 molecular targets representing the species-specific expansion of 73 target assays used in Novartis safety panels. Each of the 339 target proteins was represented by its set of known ligands, as extracted from the ChEMBL_2 database. The two-dimensional structural similarity of a drug to the ligand set of a target was quantified as an *E* value using the SEA, then subjected to a molecular charge filter. Predictions were tested retrospectively using proprietary databases including GeneGo Metabase, Thompson Reuters Integrity, Drugbank and GVKBio; new predictions were tested prospectively in Novartis *in vitro* assays. Binding assays, and, when available, functional assays were performed including scintillation proximity, fluorometric imaging, filtration, fluorescence polarization, patch clamp, time-resolved fluorescence resonance energy transfer, and homogenous time-resolved fluorescence assays. Concentration-response curves were calculated using XLfit (v.2 or v.4, IDBS) or corresponding in-house software. All curves were redrawn using GraphPad PRISM v.5. Adverse drug reaction data were extracted from the World Drug Index and encoded using the medicinal dictionary for regulatory affairs (MedDRA). Using target annotations from GeneGo Metabase, Integrity, Drugbank, ChEMBL and GVKBio, target–ADR pairs for all drugs were enumerated. Disproportionality analysis in conjunction with a chi-squared test for association was carried out for all drug–target pairs. The false discovery rate was controlled using the Benjamini–Hochberg correction for multiple hypothesis testing. Pharmacokinetic data were extracted from Integrity. For target and drug promiscuity analysis, combined external and internal target annotations were used. The computational workflow apart from SEA was implemented in Pipeline Pilot version 8 and statistical analyses were performed in R. Platelet aggregometry was performed in human blood for chlorotrianisene and indomethacin, with acetylsalicylic acid as a positive control.

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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Author Contributions SEA calculations were undertaken by M.J.K. Target–ADR associations, networks and promiscuity analysis were by E.L. *In vitro* assays were directed by S.W., J.H. and L.U. PK and PD experiments were conducted by E.W. and P.L. Platelet aggregation study was designed and carried out by L.U. and S.C. Chlorotrianisene solubility and aggregation were conducted by A.K.D. The project was conceived and planned by B.K.S., J.J., D.M. and L.U. Overall analysis and writing was largely by E.L., M.J.K., B.K.S. and L.U. All authors contributed to the manuscript.

Author Information Reprints and permissions information is available at www.nature.com/reprints. The authors declare competing financial interests: details accompany the full-text HTML version of the paper at www.nature.com/nature. Readers are welcome to comment on the online version of this article at www.nature.com/nature. Correspondence and requests for materials should be addressed to J.J. (jeremy.jenkins@novartis.com), B.K.S. (shoichet@cgl.ucsf.edu) or L.U. (laszlo.urban@novartis.com).

METHODS

Virtual target profiling of drugs. We assembled a set of 656 drugs (Supplementary Table 1) available for internal prospective testing together with 73 assay targets for which Novartis safety panel assays[28] were available. To compare activity annotations across databases, each target was mapped to human genes using Entrez gene and ChEMBL target identifiers (Supplementary Table 2). For target prediction, the 73 targets were represented by 339 orthologous proteins from human, rat, mouse, bovine and sheep, using the ChEMBL_2 database (released 25 March 2010); ligands for these targets with affinities $\leq 1\ \mu M$ were grouped into sets for the SEA calculation.

We computationally screened the 656 drugs against the 339-target panel, using 1024-bit folded ECFP_4 (ref. 46) and 2048-bit Daylight[47] fingerprints independently, with the Tc value as the similarity metric. Tc values lie between 0 and 1, in which 1 corresponds to perfect overlap of two fingerprints. Where both fingerprints yielded the same SEA prediction, we took the prediction with the lower (that is, stronger) E value, unless otherwise noted. The maximum pair-wise Tc value was used in the 1NN model.

Predictions with $E < 10^{-4}$ were retained. As a final step, we subjected the SEA predictions to a pass/no-pass charge filter, to de-prioritize those predictions in which the total charge of the drug did not match the charges calculated for at least 5% of the known ligands of the predicted target[30,31]. This resulted in 4,195 drug–ChEMBL target pairs that were subsequently mapped to the 73 target panel, resulting in 1,644 unique predictions (the difference reflects the orthologous redundancies).

Testing predictions. Many SEA predictions could be confirmed by interrogation of proprietary databases, available at Novartis but unavailable in San Francisco where the calculations were performed. These included the Thompson Reuters Integrity (http://thomsonreuters.com/products_services/science/science_products/a-z/integrity, accessed January 2011), GeneGo Metabase (version 6.2, http://www.genego.com, accessed January 2011), and GVKBio (http://www.gvkbio.com/, accessed January 2011). In addition, we also compared predictions with the ChEMBL_11 (ref. 29) and Drugbank 3.0 (ref. 48) databases. For comparison across data sources, compounds were represented using the non-stereo-specific part of InChiKeys[49].

For prospective evaluation of the remaining predictions we used binding and functional assay data from internal Novartis profiling efforts, carried out in parallel to the SEA study. For some targets, functional assays were also available. Full concentration–responses curves were plotted for any compound with at least 50% inhibition or activity at the maximal tested concentration (30 μM; Supplementary Fig. 1). For detailed assay descriptions, see Supplementary Methods and Supplementary Table 2.

Comparison to a 1NN model. We evaluated two 1NN models, using either ECFP_4 or Daylight fingerprints. Each drug was compared with all reference ligands of a target. The highest Tc value resulting from that comparison was assigned to the drug–target pair. For each drug, we identified the lowest Tc value that yielded valid SEA predictions using the respective fingerprint, and collected all drug–target pairs with Tc scores above that threshold, irrespective of the SEA E value. We counted the predictions confirmed in the proprietary databases or by experiment at Novartis. We calculated an adjusted hit rate:

$$\text{Adjusted hit rate} = (\text{number of true positives} + 1)/(\text{number of total predictions} + 1).$$

The additional count for both numerator and denominator distinguishes cases in which no predictions were confirmed, but one method or the other predicted fewer targets. For example, SEA predicted four targets for bezafibrate, none of which were confirmed (Supplementary Table 4). However, at the corresponding Tc threshold of 0.37, the ECFP_4 1NN model identified 12 potential targets, none of which was confirmed. The adjusted fraction for SEA is 0.2 ((0+1)/(4+1)), whereas the adjusted fraction for the 1NN model is 0.077 (1/13). We monitored the average adjusted hit rate for ten similarity threshold bins ranging from 0 to 1.

BLAST target comparison. To investigate how closely the predicted targets were related to already known primary or off-targets, we calculated a target similarity matrix for all known and predicted targets found in our study. Amino acid sequences of all targets were assembled from UniProt[50]. Sequences were compared in a pair-wise manner using BLASTp as implemented in Pipeline Pilot (version 8, http://www.accelrys.com)[51]. Target sequence similarity was quantified using BLAST E values. Target pairs with values smaller than 10^{-5} were considered related by sequence.

Target and drug promiscuity. Targets were classified using the ChEMBL target taxonomy, which consists of eight levels. The first three levels were used here to distinguish between small molecule and peptide GPCRs, as well as voltage- and ligand-gated ion channels (Supplementary Table 5). In-house and literature drug–target annotations were combined, and annotations with $IC_{50} < 30\ \mu M$ were counted as hits. The lipophilicity of drugs was assessed by calculating AlogP values in Pipeline Pilot. Negative values correspond to hydrophilic compounds, and positive values to lipophilic compounds.

Associations between targets and ADRs. ADRs were extracted from the World Drug Index (WDI, http://thomsonreuters.com/products_services/science/science_products/a-z/world_drug_index/, accessed March 2011) and mapped to preferred terms from the medicinal dictionary for regulatory affairs (MedDRA)[52]. MedDRA organizes adverse reaction terms in a hierarchy reaching from low-level terms to system organ classes at the highest level. Original WDI terms were first mapped to low-level terms in the MedDRA hierarchy using text mining components in Pipeline Pilot (version 8). Low-level terms serve as synonyms for preferred terms in MedDRA. These preferred terms were used to identify each adverse event uniquely. For example, the low-level terms 'dry mouth' and 'xerostomia' both map to the preferred term dry mouth. This resulted in 1,685 unique ADR terms, 2,760 unique drug structures with ADR annotations, and a total of 51,101 drug–ADR pairs. Using drug–target associations from databases used for testing predictions, we enumerated all target–ADR pairs (681,797 total). The assessment was done separately for binding, antagonist and agonist annotations. Assuming that each ADR could potentially occur owing to any of the targets hit by the drug, we enumerated all possible target–ADR pairs for each drug. Target–ADR pairs occurring more than ten times were retained. The number of observations for each unique pair was then compared with the expected number of observations given the overall distribution of activity and adverse effect annotations. An enrichment score was calculated for each target–ADR pair:

$$\text{EF} = p/(A \times T/P)$$

in which p is the co-occurrence of target X and ADR Y, A is the number of times ADR Y was linked to any drug–target pair, T is the number of times target X was linked with any drug–ADR pair, and P is the total number of target–ADR pairs.

To assess the statistical significance of found associations, we applied the chi-squared test for association based on contingency tables calculated for each unique target–ADR pair with an EF score greater than one. The false discovery rate was controlled using Benjamini–Hochberg correction in R (version 2.12, http://www.r-project.org)[53]. P values and q values (that is, P values corrected for multiple hypothesis testing), as well as the χ^2 statistic were calculated using the R statistical package. A total of 3,257 associations with a q value of <0.05 were retained (Supplementary Table 5).

Adverse reactions associated with predicted targets. Enrichment factors of predicted target–ADR pairs were compared with the association of ADRs with any known targets of each drug. We prioritized adverse reactions that were stronger associated with the predicted than with any known target (that is, had a higher EF score). To prioritize further adverse reactions that were probably due to the newly predicted target we extracted pharmacokinetic data from Thompson Reuters Integrity. Maximal plasma concentration (C_{max}) and cumulative concentration (AUC) values measured in humans were assembled. Activity data were assembled from quantitative sources (ChEMBL_11 and GVKBio) for drugs that were not part of the predictions, but shared ADRs with the prediction drugs. Drugs were identified for each prediction and associated ADR that satisfied the following three criteria: (1) they shared the ADR with the prediction drug; (2) they were not more than ten times more active at the predicted target; and (3) their C_{max} value and/or AUC value was not more than ten times higher than for the prediction drug.

Platelet aggregation inhibition. Human blood samples from six healthy volunteer male donors were used to perform platelet aggregometry with a multiplate impedance aggregometer (Dynabyte Medical) as follows: chlorotrianisene or indomethacin was added to whole blood at final concentrations of 0.5, 5 and 50 μM, and incubated at room temperature for 10 min; platelet aggregation was induced with 1 $\mu g\ ml^{-1}$ collagen and measured at 37 °C for 15 min; control aggregations were measured with vehicle only, and with 250 μM acetylsalicylic acid. Statistical analysis was performed using two-tailed Student's t-tests and $P \leq 0.05$ was considered significant. A detailed description can be found in the Supplementary Methods.

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