PART A

POLYPHARMACOLOGY: A SAFETY CONCERN IN DRUG DISCOVERY
The concern of the public, regulators, and litigators for rare but occasionally fatal or disabling effects of drugs was crystallized in the 1990s by two cases for which a precise, biochemical understanding of the basis of the effects ultimately became well understood, those of fen-phen (fenfluramine, dexfenfluramine) and terfenadine (Seldane). While generally receiving more attention in the press, idiosyncratic hepatotoxicity is thought to result from uncertain and perhaps highly complex or multifactorial immunologic mechanisms coupled to biotransformation-related events. There are potentially multiple molecular targets involved, rendering predictive strategies difficult. In contrast, the cardiac valvular effects of serotonergic agents due to 5HT2B agonism, and the fatal ventricular arrhythmias of the commonly used antihistamine, terfenadine, resulting from hERG K⁺ channel inhibition, are biochemically discrete. Facile counterscreens to these unwanted off-target pharmacologies permit the creation of drugs specifically devoid of these liabilities. The relevance of the discrete polypharmacology of these two cases has been extended for 5HT2B agonism to other appetite suppressants in the demonstration of a class effect. Selective agents, such as lorcaserin (Figure 1.1), lack 5HT2B agonism and do not cause valvulopathies. Following the identification of hERG as a relatively common off target, many compounds have been discontinued or have received “blackbox” labeling. Safe antihistamines no longer carry this liability. These two examples represent retrospective discoveries of major off-target safety issues. Recognizing the importance of off-target effects prior to selecting a lead for clinical advancement, a selectivity strategy was devised for the dipeptidyl peptidase IV (DPP4) class of drugs for type 2 diabetes. Proactive identification of inhibitors highly selective over DPP2, DPP8, and DPP9 at projected clinically relevant concentrations led to the development of sitagliptin (Figure 1.1), which has proved safe in a broad patient population. The relevance and precise understanding of off-target polypharmacology
FIGURE 1.1 Structures of the appetite suppressants fenfluramine, dexfenfluramine, and lorcaserin; the antihistamine terfenadine and its metabolite, fexofenadine; the fungicide ketoconazole; and the antidiabetics, sitagliptin and vildagliptin.
in these two cases has driven the desire, as exemplified by sitagliptin, to reduce or eliminate interactions with hERG, 5HT2B, and all other off targets and thereby make safer drugs. Specifically, countless patients who would have been susceptible to the consequences of off-target polypharmacology now lead healthy lives.

What is understood of the role and function of safety pharmacology groups varies widely in the pharmaceutical industry. Different resourcing and scientific models exist, ranging from the checkbox description of the minimum datasets required by the ICH S7A and S7B guidelines, through to a more resource-intensive, broader evaluation of specific cardiovascular, respiratory, and neurologic endpoints in vivo, and broader determination of biochemical selectivity in both cell-based and biochemical assays [1,2]. In this latter model, findings observed in initial biochemical profiling screens or in more formal drug safety evaluation in nonclinical species are used to optimize subsequent leads to reduce unwanted off-target activities. In general, safety pharmacology engages the drug discovery research phase as a largely predictive science, merging with drug safety evaluation, which is both predictive (to humans) and reactive in the determination of compound liabilities in agnostic whole-animal screens. The concept of polypharmacology or molecular promiscuity [3] and its consequences are generally incorporated into the workings of safety pharmacology groups. An understanding of the impact of selectivity versus promiscuity is emerging and guiding the evolving strategies for how safety pharmacology groups work to reduce compound attrition and improve drug safety.

The high prevalence of obesity in the developed world, with an understanding of how obesity predisposes to type 2 diabetes, cardiovascular disease, a variety of cancers, and other diseases, and quite simply human vanity, has driven the discovery, development, and subsequent abuse of appetite suppressant drugs. Multiple neuronal mechanisms contribute to the complex phenomenon of appetite and are reflected in the complexity of appetite suppressive pharmacologic approaches, and possibly the relatively low efficacy of engaging single mechanisms of appetite suppression [4]. Fenfluramine and its more potent stereoisomer, dexfenfluramine (Figure 1.1), were developed as moderately efficacious anorectic agents stimulating 5HT (5-hydroxytriptamine or serotonin) transmission by the CNS by increasing levels of 5HT. This was shown to occur for these drugs and their major metabolites, norfenfluramines, by acting as substrates for serotonin transporter on serotonergic neurons (SERT) and not dopamine or noradrenaline transporters [4]. These drugs were withdrawn from the market because of a high incidence of cardiac valve disease (CVD), especially in those patients either prescribed or self-administered substantially higher than recommended dosages [5].

Although at the time it was known that 5HT is mitogenic in certain systems, the relatively weak association (odds ratio of 2) between drug administration and CVD was not understood. Broad screening of receptors (receptorome) led, in part, to a detailed evaluation of the 5HT2 family of 5HT receptors [6]. A series of experiments spanning several years identified the high expression of the receptor for 5HT2B on cardiac valvular endothelium, and revealed that elevated plasma 5HT concentrations can drive mitogenesis through agonism of the 5HT2B receptor, that agonism of the 5HT2B receptor by fenfluramines and their metabolites likely underpins cardiac valvular pathology, and that an interactions of 5HT with 5HT2C drives appetite control [5].
Identification of the desired molecular target for appetite control (5HT2C) versus the putative cause of valvulopathy (5HT2B) spawned drug discovery programs driving selectivity between these two proteins. Through these and other research programs, the author and industry colleagues have recognized the frequency with which aminergic receptors, transporters, and degradative enzymes (monamine oxidase A and B) are off-target hits for a large variety of clinical indications, but especially those drugs that are CNS-active. While 5HT2B agonism is rare and serious, 5HT2B antagonism does not appear to be associated with clinical consequences despite the cardiac phenotype of the knockout mouse of the same receptor. Lorcaserin was recently submitted for registration with the FDA as a 5HT2C-selective appetite suppressant. While this selectivity appeared to successfully avoid the predisposition to CVD [7], an unusual constellation of preclinical rodent carcinogenicity findings more recently led to the initial rejection of the drug application. Possibly related to the difficulty in building selectivity into drugs specifically targeting 5HT receptors, identifying 5HT2C agonists without a variety of unexpected preclinical off-target effects has prove extremely difficult for all companies working in this field.

Since the discovery of the role of the myocardial K⁺ channel, hERG, in drug-induced QT interval prolongation, and secondarily an association with the rare ventricular arrhythmia, torsades de pointes (TdP), drug leads have been carefully evaluated and profiled in vitro and in vivo for this liability. Although an imperfect association exists between hERG inhibition, QT prolongation, and TdP, studiously avoiding these effects preclinically, and rigorous early clinical screening has markedly reduced the incidence of this liability. TdP has plagued repolarization-delaying antiarrhythmics, various antihistamines, antipsychotics, antimicrobials, and other drugs [8,9]. Before development of the molecule-level hypotheses that led to chemists developing a structure–activity relationship (SAR) for selectivity of desirable pharmacology over hERG, several market withdrawals of commonly used agents occurred. Terfenadine (Seldane) was a commonly used and highly effective antihistamine in the late 1980s and early 1990s. In 1990, Monahan et al. [10] described the first association (exclusive of drug overdose) of symptomatic TdP occurring with the use of terfenadine in a patient who was taking the recommended prescribed dose of this drug in addition to cefaclor, ketoconazole, and medroxyprogesterone. Measured serum concentrations of terfenadine and its main metabolite showed that excessive levels of parent terfenadine and proportionately reduced concentrations of metabolite, suggesting inhibition of terfenadine metabolism by ketoconazole-mediated inhibition of drug metabolism through cytochrome 3A4 [10,11]. This was subsequently proved. In fact, ketoconazole both increased parent terfenadine (Figure 1.1) while reducing concentrations of the active acid metabolite fexofenadine [11]. This is consistent with the generally poor cell penetration of acids, the knowledge that hERG inhibitors act on the intracellular side of the K⁺ channel, and that the SAR for this interaction frequently involves drug basicity. The interactions of fexofenadine with hERG are profoundly reduced compared to terfenadine, rendering this drug and subsequently improved antihistamines much safer with respect to cardiovascular risk.

Following many years in which potentially avoidable off-target effects of drugs were first identified in clinical trials, strategies were developed to attempt to identify and
eliminate such effects prior to lead selection. The eventual development of highly selective DPP4 inhibitors with optimal safety profiles in humans derived from this strategy.

The desired pharmacology of DPP4 inhibitors in type 2 diabetes is the inhibition of cleavage of the incretinlike hormones, GLP1 (glucagonlike peptide-1) and GIP (gastrointestinal polypeptide) in plasma. Normally, GLP1 is rapidly hydrolyzed (half-life \( t < 1 \) min) by DPP4. GLP1 acts to reduce plasma glucose by stimulating pancreatic islet \( \beta \)-cell insulin secretion. Through inhibition of its cleavage, the action of GLP1 may be prolonged, thereby lowering blood glucose and improving glucose homeostasis [12]. Using selective tool molecules for homologous proteinases (an inhibitor of DPP8/9), and of quiescent cell proline dipeptidases (QPP), effects relating to their inhibition, including alopecia, thrombocytopenia, reticulocytopenia, enlarged spleen, multiorgan histopathologic changes, and gastrointestinal toxicity, were shown \textit{in vivo}. In addition, study of the DPP8/9 inhibitor \textit{in vitro} suggested attenuation of human T-cell responses. In parallel studies, selective inhibitors of DPP4 did not demonstrate toxicity in either rats or dogs [13]. In addition to these homologous proteinases, efforts to maintain selectivity of DPP4 over DPP2 have also been sought [14]. Clinical results with sitagliptin suggest that an optimal safety profile for this new class of antihyperglycemic may have resulted from the proactive medicinal chemistry strategy to obtain high selectivity of DPP4 over homologous proteinases. However, some less selective DPP4 inhibitors are also used safely in patients, suggesting that these drugs are either selective within the exposure range employed in patients, or that the selectivity itself is not important [14]. The delayed full registration in the United States of the less selective DPP4 inhibitor, vildagliptin (Figure 1.1), may relate in part to preclinical safety issues, possibly secondary to DPP nonselectivity.

Two examples of clearly avoidable but retrospectively discovered off targets, in part, drove the pharmaceutical industry to seek drugs with greater selectivity. Complementing this approach is an example in which proactively building biochemical selectivity into a drug may have contributed to an overall safer medicine. The definition of selectivity has been broadened from specific off targets to broader classes of relatively higher-frequency off-target hits. When drugs are then classified by high nonselectivity (\textit{molecular promiscuity}) versus highly selectivity, a compelling correlation between clinical safety and biochemical selectivity emerges [3]. The science of these concepts is aptly described by the term \textit{polypharmacology}.

REFERENCES


