How were new medicines discovered?

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Abstract | Preclinical strategies that are used to identify potential drug candidates include target-based screening, phenotypic screening, modification of natural substances and biologic-based approaches. To investigate whether some strategies have been more successful than others in the discovery of new drugs, we analysed the discovery strategies and the molecular mechanism of action (MMOA) for new molecular entities and new biologics that were approved by the US Food and Drug Administration between 1999 and 2008. Out of the 259 agents that were approved, 75 were first-in-class drugs with new MMOAs, and out of these, 50 (67%) were small molecules and 25 (33%) were biologics. The results also show that the contribution of phenotypic screening to the discovery of first-in-class small-molecule drugs exceeded that of target-based approaches — with 28 and 17 of these drugs coming from the two approaches, respectively — in an era in which the major focus was on target-based approaches. We postulate that a target-centric approach for first-in-class drugs, without consideration of an optimal MMOA, may contribute to the current high attrition rates and low productivity in pharmaceutical research and development.

Investment in drug research and development (R&D) has increased substantially in recent decades, but the annual number of truly innovative new medicines approved by the US Food and Drug Administration (FDA) has not increased accordingly, and attrition rates are very high[1]. Indeed, in a recent analysis[2] it was noted that without a dramatic improvement in R&D productivity, the pharmaceutical industry cannot sustain sufficient innovation to replace the loss of revenues due to patent expirations for successful products.

The authors of this analysis[2] also considered R&D productivity in two dimensions: efficiency and effectiveness. R&D efficiency represents the ability to translate inputs (such as ideas, investment and effort) into defined outputs (such as milestones that represent resolved uncertainties), whereas R&D effectiveness can be considered as the ability to produce outputs with certain intended and desired qualities. A key efficiency variable for increased productivity is the probability of technical success. If the probability of technical success could be increased (by reducing attrition) for any given drug candidate or, ideally, for a portfolio of drug candidates, then productivity would increase accordingly. The authors also suggested that target selection may be one of the most important determinants of attrition and overall R&D productivity[2].

Since the dawn of the genomics era in the 1990s, the main focus of drug discovery has been on drug targets, which are typically proteins that appear to have a key role in disease pathogenesis[3–5]. Modification of target activity provides a rational basis for the discovery of new medicines; a target-centric approach provides a specific biological hypothesis to be tested and a starting point for the identification of molecules to do this with. Tremendous advances have been made in the development of new tools to identify targets (for example, RNA interference) and compounds that interact with these targets (for example, high-throughput target-based screening assays that are applicable to key protein families such as G protein-coupled receptors and kinases). Structure-based tools that can be used to aid lead identification and optimization for some targets have also been developed, including X-ray crystallography and computational modelling and screening (virtual screening).

However, despite the power of these tools to identify potential drug candidates, R&D productivity remains a crucial challenge for the pharmaceutical industry, which raises questions about the possible limitations of a target-centric approach to drug discovery. Indeed, before the introduction of target-based approaches, drug discovery was driven primarily by phenotypic assays, often with limited knowledge of the molecular mechanisms of disease. Nevertheless, the pharmaceutical industry was successful in the discovery and development of new

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innovative medicines; it has therefore been suggested that the more limited use of phenotypic screening in recent years has contributed to the current lack of success in drug R&D[6,7].

These two different approaches to drug discovery — target-based screening and phenotypic screening — each have advantages and disadvantages. The strengths of the target-based approach include the ability to apply molecular and chemical knowledge to investigate specific molecular hypotheses, and the ability to apply both small-molecule screening strategies (which can often be achieved using high-throughput formats) and biologic-based approaches, such as identifying monoclonal antibodies. A disadvantage of the target-based approach is that the solution to the specific molecular hypotheses may not be relevant to the disease pathogenesis or provide a sufficient therapeutic index.

A strength of the phenotypic approach is that the assays do not require prior understanding of the molecular mechanism of action (MMOA), and activity in such assays might be translated into therapeutic impact in a given disease state more effectively than in target-based assays, which are often more artificial. A disadvantage of phenotypic screening approaches is the challenge of optimizing the molecular properties of candidate drugs without the design parameters provided by prior knowledge of the MMOA. An additional challenge is to effectively incorporate new screening technologies into phenotypic screening approaches, which is important for addressing the traditional limitation of some of these assays: a considerably lower throughput than target-based assays.

In order to gain a better understanding of the factors that could contribute to the high attrition rates, and to provide insights that might help to reduce attrition and increase R&D productivity, we decided to investigate the approaches that were used in the discovery of recently introduced medicines. To achieve this, we analysed the characteristics of the new molecular entities (NMEs) and new therapeutic biologics that were approved by the FDA during the 10-year period between 1999 and 2008 by examining the discovery approach, the MMOA and whether the drug was first in its class.

Data and analysis

Numbers of NMEs. In the 10-year period between 1999 and 2008, the FDA approved 183 small-molecule drugs, 20 imaging agents and 56 new therapeutic biologics (259 agents overall). Out of these, 75 drugs were identified as first-in-class or with novel MMOAs based on the information provided in the product labels on the FDA website (see the Drugs@FDA website), and primary research and review publications (TABLE 1; Supplementary information S1 (table)). The specific sources for each drug are referenced in TABLE 1 and in Supplementary information S2 (box).

Discovery approaches. We divided the list of 259 agents into three general categories: first-in-class drugs (75 drugs), follower drugs (164 drugs) and imaging agents (20 agents; these were not further analysed). A list of all the drugs and their classification is provided in Supplementary information S1 (table) and a brief description of the discovery history of first-in-class drugs is provided in Supplementary information S2 (box). We categorized the method of discovery of each new drug as target-based, phenotypic-based, modification of a natural substance, biologic-based or other (see Supplementary information S1 (table)). Overall, 100 NMEs were discovered using target-based approaches, 58 NMEs were discovered using phenotypic-based approaches, 18 NMEs were based on modifications of natural substances and 56 of the agents were biologics. All of the biologics can be considered to have been discovered using a target-based approach, and the main focus of our analysis is on the methods of discovery for small-molecule first-in-class NMEs and follower NMEs; that is, small molecules that are in the same class as a previously approved NME.

MMOA. The MMOA of the NMEs was analysed because the limitations of a target-based approach with respect to the MMOA have been highlighted[8–10], and because the MMOA is a characteristic of drugs that has received less attention with regard to its connection to attrition. For the purpose of this article, MMOA is defined as the biochemical mechanism through which the structural interactions between the drug and its target(s) result in a functional response[10–12], which is important in both drug efficacy and safety (BOX 1). The MMOA can affect how efficiently a binding interaction is coupled to the functional response, which can be assessed by considering biochemical efficiency (BOX 2).

For instance, resistance to the ATP-competitive kinase inhibitors gefitinib and erlotinib — which target the epidermal growth factor receptor (EGFR) kinase — has been shown to be due to mutations that alter the ATP binding site in such a way that they increase the affinity of the EGFR kinase domain for ATP. The functional consequence of these resistance mutations is therefore to enable ATP to compete more effectively with gefitinib and erlotinib[12,13]. This provides an explanation for the mechanism of resistance to these rapidly reversible ATP-competitive inhibitors, and also provides an explanation as to why irreversible covalent binding inhibitors overcome this resistance[13].

An example of how the therapeutic utility of drugs that function through interaction with a receptor is influenced by their MMOA is provided by the tissue-selective functional effects of the selective oestrogen receptor modulators (SERMs), which are mediated by SERM-induced structural changes in the oestrogen receptor[14]. Binding to the receptor initiates a series of molecular events, which culminate in the activation or repression of specific genes. The SERMs tamoxifen and raloxifene bind at the same site within the core of the ligand-binding domain, but with different binding modes that are translated into distinct conformations of the transactivation domain of the receptor. Transcriptional regulation of the oestrogen receptor is a complex process that involves the participation of co-activators and co-repressors, and the

New molecular entities (NME). A medication containing an active ingredient that has not been previously approved for marketing in any form in the United States.

Table 1 | **First-in-class small-molecule new molecular entities approved by the FDA: 1999–2008**

Drug (trade name; company)	Therapeutic area	Target type	Molecular mechanism of action	Refs
Discovered through phenotypic screening				
Aripiprazole (Abilify; Bristol-Myers Squibb/Otsuka Pharmaceutical)	CNS	Receptor	Conformational/partial agonist	74,75, 80–84
Azacitidine (Vidaza; Celgene/Pfizer)	Cancer	Enyzme	Irreversible inhibition	69,85
Caspofungin (Cancidas; Merck)	Infectious disease	Enzyme	Noncompetitive inhibition	71,86
Cilostazol (Pletal; Otsuka)	Cardiovascular	Enzyme	Inhibition	87
Cinacalcet (Sensipar; Amgen)	Metabolic	Receptor	Allosteric activator	29
Daptomycin (Cubicin; Cubist)	Infectious disease	NA (disrupts bacterial membrane)	Unknown	88
Docosanol (Abreva; Avanir Pharmaceuticals)	Infectious disease	Unknown	Unknown	89–92
Ezetimibe (Zetia; Merck)	Cardiovascular	Transporter	Slow binding kinetics	30
Fulvestrant (Faslodex; AstraZeneca)	Cancer	Receptor	Antagonist-induced degradation	47,93,94
Levetiracetam (Keppra; UCB Pharma)	CNS	Unknown	Unknown	95
Linezolid (Zyvox; Pfizer)	Infectious disease	Enzyme	Conformational	28,96,97
Lubiprostone (Amitiza; Sucampo Pharmaceuticals)	Gastrointestinal	Unknown	Unknown	98–100
Memantine (Namenda; Forest)	CNS	Receptor	Uncompetitive and fast binding kinetics	101–103
Miglustat (Zavesca; Actelion)	Rare diseases	Enzyme	Reversible inhibition	104,105
Nateglinide (Fastic; Novartis/Astellas)	Metabolic	Unknown	Fast binding kinetics	106–108
Nelarabine (Arranon; GlaxoSmithKline)	Cancer	DNA (nucleoside analogue)	Nucleotide chain termination	109–113
Nitazoxanide (Alinia; Roche)	Infectious disease	Enzyme	Irreversible and redox	78,79
Nitisinone (Orfadin; Syngenta)	Rare diseases	Enzyme	Irreversible	114–116
Pemirolast (Alamast; Senten)	Immune modulation	Unknown	Unknown	117
Ranolazine (Ranexa; Gilead)	Cardiovascular	Unknown	Unknown	118–121
Retapamulin (Altabax; GlaxoSmithKline)	Infectious disease	Enzyme	Allosteric inhibitor	122
Rufinamide (Inovelon; Novartis)	CNS	Unknown	Unknown	123,124
Sinecatechins (Veregen; Medigene)	Infectious disease	Unknown	Unknown	125
Sirolimus (Rapamune; Pfizer)	Immune modulation	Enzyme	Conformational/inhibition	70,126
Varenicline (Chantix; Pfizer)	CNS	Ion channel	Conformational/partial agonist	76
Vorinostat (Zolinza; Merck)	Cancer	Enzyme	Equilibrium kinetics	127,128
Ziconotide (Prialt; Elan Pharmaceuticals)	Pain and/or CNS	Ion channel	Equilibrium kinetics	31
Zonisamide (Excegran; Dainippon Pharmaceuticals)	CNS	Unknown	Unknown	129
Discovered through target-based screening				
Aliskiren (Tekturna; Novartis)	Cardiovascular	Enzyme	Equilibrium binding	38,130
Aprepitant (Emend; Merck)	Gastrointestinal	Receptor	Slow binding kinetics	46
Bortezomib (Velcade; Millenium Pharmaceuticals)	Cancer	Enzyme	Equilibrium binding	131,132
Bosentan (Tracleer; Actelion)	Cardiovascular	Receptor	Equilibrium binding	37
Conivaptan (Vaprisol; Astellas Pharma)	Metabolic	Receptor	Equilibrium binding	133
Eltrombopag (Promacta; GlaxoSmithKline)	Immune	Receptor	Noncompetitive agonist	36
Gefitinib (Iressa; AstraZeneca)	Cancer	Enzyme	Stabilize inactive conformation	41,42

Table 1 cont. | **First-in-class small-molecule new molecular entities approved by the FDA: 1999–2008**

Drug (trade name; company)	Therapeutic area	Target type	Molecular mechanism of action	Refs
Imatinib (Gleevec; Novartis)	Cancer	Enzyme	Stabilizes inactive conformation	49
Maraviroc (Celsentri; Pfizer)	Infectious disease	Receptor	Conformational and/or allosteric	134–137
Mifepristone (Mifeprex; Aventis Pharma)	Reproductive	Receptor	Conformational antagonist	138–141
Orlistat (Xenical; Roche)	Metabolic	Enzyme	Irreversible	35,142
Raltegravir (Isentress; Merck)	Infectious disease	Enzyme	Traps conformational state	39,40,143, 144
Ramelteon (Rozerem; Takeda Pharmaceuticals)	CNS	Receptor	Equilibrium binding	72,145
Sitagliptin (Januvia; Merck)	Metabolic	Enzyme	Equilibrium binding	33,146
Sorafenib (Nexavar; Bayer)	Cancer	Enzyme	Conformation state-specific inhibition	44
Sunitinib (Sutent; Pfizer)	Cancer	Enzyme	Conformation state-specific inhibition	147–150
Zanamivir (Relenza; GlaxoSmithKline)	Infectious disease	Enzyme	Equilibrium binding	34,151
Discovered based on natural substrate or natural substance				
Acamprosate (Campral; Merck)	CNS	Ion channel	Conformational channel modulator	152
Aminolevulinic acid (Levulan; Berlex)	Dermatology	NA (photosensitizer)	Redox	153,154
Fondaparinux (Arixtra; Sanofi)	Cardiovascular	Enzyme	Irreversible	155–157
Sapropterin (Kuvan; BioMarin)	Rare diseases	Enzyme	Cofactor	158–161
Verteporfin (Visudyne; QLT)	Ocular	NA (photoreaction)	Redox	77,162

CNS, central nervous system; FDA, US Food and Drug Administration; NA, not applicable.

different conformations presumably change the affinity of the receptor for the interacting co-activators and co-repressors. The change in co-repressor affinity alters the composition of the distinct cellular co-regulatory complexes that modulate the functional transcriptional activity[14].

The MMOA can also differentiate similar drugs with respect to their therapeutic indications. At the structural level, aspirin is an irreversible inhibitor of cyclooxygenases, whereas ibuprofen and naproxen are reversible inhibitors. All three molecules bind to cyclooxygenase enzymes at the same substrate binding site. However, the irreversible MMOA of aspirin differentiates its functional use as an antiplatelet drug from the reversible inhibitors, because this MMOA translates into a long-lasting action of aspirin in platelets, as platelets do not have the capacity to resynthesize new enzymes[15,16].

There are many different biochemical features of an MMOA through which molecular interactions can contribute to a specific functional response. These include residence time[10,17–19], irreversible binding[20], transient binding[21,22], and uncompetitive[22,23] and non-competitive[10] inhibition mechanisms (BOX 1). It has been proposed that drugs should be activated by the pathological state that they are intended to inhibit[22,23]. Allosteric inhibition and activation are important for the pharmacological modulation of many receptors and channels[24,25]. Voltage- or frequency-dependent channel blockade can also influence a selective pharmacological response[26,27]. Given the importance of the MMOA to the therapeutic effects of NMEs, we consider it further in the following sections.

Discovery of first-in-class medicines

NMEs that were discovered through phenotypic screening. The 28 first-in-class small-molecule NMEs that were discovered in phenotypic screens either came from intentional targeting of a specific phenotype (25 NMEs) or through serendipity (3 NMEs) (FIG. 1). The intentional approaches were based on assays that measured a specific physiological phenomenon, with little understanding of the MMOA. In many cases, the newly discovered molecules were subsequently used to identify MMOAs for the physiological phenomena. For example, the oxazolidinone antibiotics (such as linezolid) were initially discovered as inhibitors of Gram-positive bacteria but were subsequently shown to be protein synthesis inhibitors that target an early step in the binding of *N*-formylmethionyl-tRNA to the ribosome[28]. This is also illustrated by the calcium receptor allosteric activator cinacalcet[29], the sterol transporter inhibitor ezetimibe[30] and the N-type calcium channel blocker ziconotide[31]; these drugs were initially discovered using phenotypic assays.

The majority of discoveries focused on using specific chemical classes in which prior knowledge contributed to matching the chemical class with the phenotype — for example, screening nucleoside analogues as potential anticancer and antiviral agents. Random library screening was also successful for ezetimibe, linezolid, pemirolast, retapamulin, rufinamide and sirolimus. An additional approach was to use phenotypic screening to identify new MMOAs for established targets, which led to the discovery of the partial agonists aripiprazole and varenicline, and the full antagonist fulvestrant (see Supplementary information S2 (box) for details). It is

Box 1 | **Molecular mechanism of action**

The molecular mechanism of action (MMOA) is defined here as the interaction between a drug and its target (or targets) that creates a specific response. These specific molecular interactions link structure to function in such a manner as to provide a therapeutically effective and safe response. In this context, the MMOA is differentiated from mechanism of action (MOA), which describes the mechanism in the context of the physiological response — such as antihistamines, anti-inflammatory, and so on.

There are many facets of this interaction that ultimately result in the desired therapeutic outcome. For example, the site of interaction (allosteric or orthosteric), molecular descriptors of the binding interaction (such as affinity and binding kinetics), the functional impact (for example, receptor agonism, modulation or antagonism) and the specificity of the functional outcome (for example, activation of specific signalling pathways) all contribute to the MMOA and affect the ultimate pharmacological response.

Possible MMOAs at a target are listed below, together with selected examples of drugs that act through these MMOAs.

Kinetic mechanisms

For kinetic mechanisms, the pharmacological response to the drug is primarily driven by binding kinetics and residence time at the target[12,17–19].

Equilibrium binding. The response to the drug is represented by the equilibrium dissociation constant (K_i) to the target. The binding has sufficiently fast on and off rates (k_{on} and k_{off}) to allow equilibrium to be reached and is thereby sensitive to competition with physiological substrates and/or ligands (for example, bosentan, an endothelin receptor antagonist; and aliskiren, a renin inhibitor)[37,38,68].

Slow kinetics. Non-equilibrium and irreversible mechanisms involve slow association and/or dissociation rates (k_{on} and k_{off}) that do not allow equilibrium to be reached and are less sensitive to competition with physiological substrates and/or ligands (for example, orlistat binds irreversibly to the active site serine of pancreatic lipase, azacitidine irreversibly binds to DNA methyltransferases and candesartan has a slow dissociation rate from the angiotensin II receptor)[17–20,35,63,69].

Conformational mechanisms

For conformational mechanisms, binding of the drug to the target involves a conformational change in the target that couples drug binding to a response (for example, sirolimus binds to the peptidylprolyl isomerase FKBP12, which stabilizes a conformation that subsequently inhibits the kinase activity of mammalian target of rapamycin; and fulvestrant induces a conformation of the oestrogen receptor that is subsequently degraded)[8–11,47,70].

Noncompetitive inhibition and/or antagonism. This is a form of MMOA in which the drug binds to a site on the target that is distinct from the physiological substrate- and/or ligand-binding site that results in an inhibition of the response (for example, caspofungin is a noncompetitive inhibitor of 1,3-β-D-glucan synthase owing to the observation that its IC_{50} (half-maximal inhibitory concentration) is not influenced by substrate concentrations)[68,71].

Uncompetitive inhibition and/or antagonism. An uncompetitive MMOA is contingent on prior activation of the target by the physiological effector (the substrate or the ligand). This means that the same amount of drug blocks higher concentrations of the physiological effector to a greater degree than lower concentrations. For example, memantine is an uncompetitive antagonist that binds only to the activated form of the NMDA receptor. The potency of the inhibition of the NMDA receptor by memantine increases at higher concentrations of glutamate (the physiological ligand) [22,23,68].

Full agonism. Maximal efficacy is produced following drug binding to the receptor and subsequent receptor activation (for example, ramelteon mimics the activity of melatonin for the melatonin receptor through binding at the orthosteric site with efficient coupling to activate specific signalling pathways) [72,73].

Partial agonism. This is a form of MMOA in which only partial efficacy is produced following drug binding to the orthosteric site on the receptor (for example, aripiprazole is a partial agonist of the dopamine D2 receptor and varenciline is a partial agonist of the nicotinic acetylcholine receptors)[73,74–76].

Allosteric modulator. This mechanism involves regulation of the biological activity of the target by binding of a drug at a site other than the binding site for the endogenous substrate and/or ligand (allosteric site) (for example, cinacalcet is an allosteric modulator of the calcium receptor by binding to the allosteric site) [29,73].

Redox mechanisms

Redox is short for reduction–oxidation reactions in which the pharmacological response to the drug is a consequence of electron transfer between the drug and a physiological target. For example, generation of hydroxyl radicals by verteporfin is thought to contribute to its ability to damage cells, and the antiprotozoal activity of nitazoxanide is believed to be due in part to interference with the pyruvate–ferredoxin oxidoreductase enzyme-dependent electron transfer reaction, which is essential to anaerobic energy metabolism[77–79].

worth noting that several of these NMEs (for example, nelarabine, azacitidine and nitazoxanide) were initially described decades before their approval and before the development of new molecular screening approaches. Many of these NMEs were also derived from natural substances, including the nucleoside analogues nelarabine and azacitidine, the PGE1 derivative lubiprostone and the fatty acid docosanol. Ziconotide, sirolimus and retapamulin were derived from natural products.

NMEs that were developed as synthetic and/or modified versions of natural substances, or discovered by screening such substances. A small fraction of the first-in-class NMEs (5 out of 75) were developed as synthetic versions of natural substances (that were sometimes slightly modified), including the modified heparin fondaparinux, the porphyrin verteporfin, the biopterin cofactor sapropterin, the porphyrin precursor aminolevulinic acid and the acetylated homotaurine

acamprosate (FIG. 1c). Additionally, in some cases, natural substances provided starting points for small-molecule phenotypic screening (10 NMEs (FIG. 1a)) and target-based discovery (3 NMEs (FIG. 1b)). In total, 18 out of the 50 (36%) first-in-class small-molecule NMEs originated from natural substances. These numbers are consistent with those reported by Newman and Cragg[32] for the percentage of all medicines derived from natural products, and the supposition that libraries that are derived from natural substances provide good chemical starting points for optimization. For example, two NMEs that were discovered using a target-specific strategy — ramelteon, which targets melatonin receptors, and mifepristone, which is a progesterone receptor modulator — were derived from the modification of natural ligands.

Target-based approaches. Target-based approaches led to the discovery of 17 of the 50 first-in-class small-molecule NMEs. Various approaches contributed to these discoveries, and they are illustrated by the following examples. Sitagliptin, an inhibitor of the protease dipeptidyl peptidase 4 (DPP4), was discovered in an iterative discovery approach that was aimed at optimizing metabolic properties while retaining efficacy[33]. A computer-assisted drug design strategy that was based on the crystal structure of the influenza viral neuraminidase led to the identification of zanamivir[34]. A target-directed screening of microbial broths from soil organisms resulted in the discovery of a very potent, selective and irreversible inhibitor of pancreatic lipases, which was named lipstatin (orlistat)[35]. Eltrombopag was identified by screening small-molecule libraries for the ability to activate a reporter molecule in thrombopoietin (TPO)-dependent cell lines. Lead compounds were initially identified and then optimized for their biological effect and pharmaceutical properties[36]. In a programme that was aimed at discovering non-peptide endothelin receptor antagonists, a class of substituted arylsulphonamidopyrimidines was identified in a chemical compound library, which led to the discovery of bosentan[37].

However, knowledge of the targets did not necessarily lead to an easy path to discovery. For example, although renin had been a clear target for the treatment of hypertension for decades, the development of orally active renin inhibitors, which culminated in the discovery of the NME aliskiren[38], was a major challenge.

The development of six of the NMEs that were discovered by target-based approaches involved subsequent identification of their effective MMOA at the target that was selected for the initial screening strategies. The kinase inhibitors gefitinib, imatinib, sorafenib and sunitinib block kinase activation; the HIV integrase inhibitor raltegravir traps an intermediate complex between the enzyme and nucleic acid; and maraviroc is an allosteric antagonist of the the CC chemokine receptor type 5. These inhibitors represent successes of the target-based strategy, but they also highlight that the optimal MMOA at the target may not be apparent at the time of initiating the discovery strategy. For example, the HIV1 integrase inhibitor raltegravir was only discovered after several MMOAs had been investigated using different assay formats[39,40]. The diketo acids that led to the discovery of raltegravir were eventually found to block the strand transfer reaction, and this MMOA provided good *in vivo* efficacy. The importance of the assay format in the identification of compounds with effective MMOAs at a chosen target is also illustrated by the discovery of gefitinib, which is thought to act by sequestering the EGFR and its ligand into inactive receptor–ligand complexes[41]. Screening for activity in A431 vulval squamous carcinoma cells was the assay format that led to the identification of gefitinib and its MMOA[42].

The neurokinin-1 receptor antagonist aprepitant and the proteasome inhibitor bortezomib were originally discovered with a view to targeting different indications to those that they were first approved for (Supplementary information S2 (box)). Repositioning was also involved for three of the NMEs that were discovered through phenotypic assays: miglustat, azacitidine and nitisinone (Supplementary information S2 (box)).

Biologics. Biologics that were approved under biologics license applications and large peptide molecules that were approved as NMEs (for example, enfuvirtide and pegvisomant) accounted for 25 (33%) out of the 75 first-in-class medicines (FIG. 1d). The biologics were further categorized according to their pharmacological action as described by Leader, Baca and Golan[43]. The pharmacological actions of these biologics included enzyme replacement (agalsidase-β, alglucosidase alfa, galsulfase, idursulfase and laronidase), augmenting existing pathways (drotrecogin-α, exenatide, palifermin, pramlintide and romiplostim), providing a novel function (rasburicase), interfering with a molecular activity (alemtuzumab, abatacept, anakinra, alefacept, bevacizumab, cetuximab, eculizumab, efalizumab, enfuvirtide,

Box 2 | **Biochemical efficiency**

The dose of a drug required to achieve the desired physiological response depends on its biochemical efficiency[10,11]. This is defined as 'binding affinity/functional response', which is equivalent to K_i/EC_{50} (effector concentration for half-maximal response). Good biochemical efficiency enables efficacy at lower drug concentrations and increases the therapeutic index. It is a property of many approved medicines[10,11].

There are many factors that can influence the shift in dose–response curves between binding and functional assays, including:

- Pharmacokinetics and ADME (absorption, distribution, metabolism and excretion) properties
- Assay relevance (is the functional assay appropriate for the target? Are the assays technically accurate?)
- The involvement of the target in the functional readout and biology
- The molecular mechanism of action (MMOA)

Although all of these factors can and do contribute to the relationship between binding affinity and the functional response, the role of the MMOA is not always considered. The concept of biochemical efficiency was introduced to quantify this possibility[10,11]. When biochemical efficiency is used as a measure of an optimal MMOA, it is important that the other mitigating factors are eliminated. For example, when evaluating biochemical efficiency, the assays must be run in the absence of serum (or plasma) to eliminate the shift in IC_{50} (half-maximal inhibitory concentration) owing to serum protein binding.

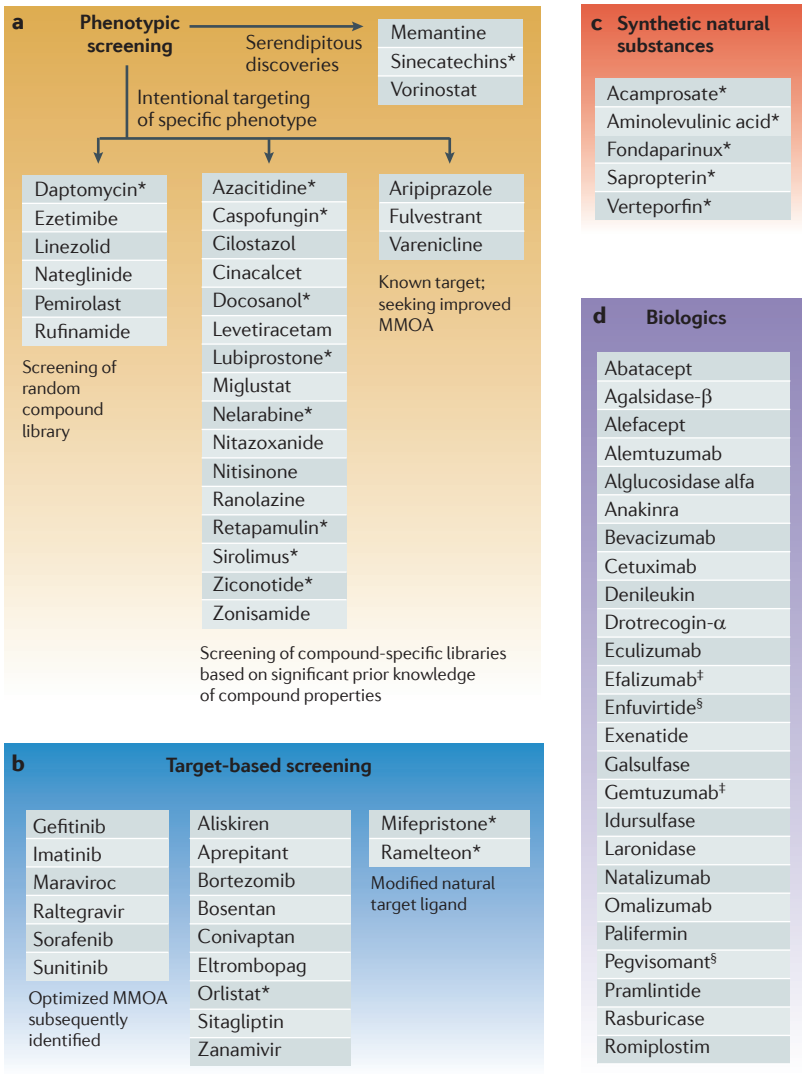

Figure 1 | **Discovery strategies used to identify first-in-class medicines.** The strategies that were used were categorized as being based on phenotypic screening (**a**), target-based strategies (**b**), synthetic versions of natural substances or very close derivatives (**c**) and biologics (**d**). Phenotypic strategies were further subdivided into intentional screening with random compound libraries or compound-specific libraries, optimization for molecular mechanism of action (MMOA) and serendipitous discoveries. Drugs that were identified through target-based screening that involved optimization of a natural ligand or identification of the optimal MMOA are highlighted. *Drugs that are derived from natural substances. ‡These medicines have been withdrawn from the market. §Although enfuvirtide and pegvisomant were approved as new molecular entities, for the purpose of this analysis they have been treated as biologics, given that they are both much larger than typical small-molecule drugs (see Supplementary information S2 (box)).

omalizumab, pegvisomant and natalizumab); and delivering other compounds or proteins (denileukin diftitox and gemtuzumab). Thus, the majority of these biologics function by interfering with a molecular activity and, as mentioned above, all of these biologics can be considered to have been discovered using a target-based approach.

Both first-in-class small molecule NMEs and biologics were approved for two targets: EGFR kinase (the small-molecule EGFR kinase inhibitor gefitinib and the EGFR-specific monoclonal antibody cetuximab) and TPO (the small-molecule TPO receptor agonist eltrombopag and the 'peptibody' TPO receptor agonist romiplostim). Three first-in-class medicines also act by inhibiting vascular endothelial growth factor (VEGF) signalling: the VEGF-specific monoclonal antibody bevacizumab, and the small-molecule VEGF receptor kinase inhibitors sunitinib, which also inhibits KIT, and sorafenib, which was originally discovered on the basis of its inhibition of RAF kinase[44].

Strategies according to disease area. Evaluation of the discovery strategy by disease area showed that a phenotypic approach was the most successful for central nervous system disorders and infectious diseases, whereas target-based approaches were most successful in cancer, infectious diseases and metabolic diseases (TABLE 2). Biologics accounted for most of the new medicines that act by modulating the immune system and 50% of the new medicines for cancer.

Discovery of follower drugs

There were 164 follower drugs, out of which 83 (51%) were discovered via target-based approaches, 30 (18%) via phenotypic assays and 31 (19%) were biologics (FIG. 2) (Supplementary information S1 (table)). Seven (4%) of the follower drugs were prodrugs or combinations of previously approved medicines. Considering NMEs alone, target-based approaches accounted for 62% (83 out of 133) of the small-molecule NMEs. The ratio of NMEs from target-based approaches to those from phenotypic screening increased during the final 4 years of the analysis (FIG. 3b).

Molecular mechanism of action

The majority of small-molecule first-in-class NMEs had MMOAs that involved inhibiting the activity of enzymes or modulating receptors (FIG. 4). This trend is consistent with the findings of Imming and colleagues[4] in their analysis of the nature and number of all drug targets. The pharmacological responses were often achieved by binding to the target protein to elicit a positive or negative response.

For the first-in-class NMEs and biologics, many different biochemical mechanisms mediated the drug response at the target (BOX 1). These included reversible, irreversible and slow binding kinetics; competitive, uncompetitive and noncompetitive interactions between physiological substrates/ligands and drugs; as well as inhibition, activation, agonism, partial agonism, allosteric activation and induced degradation.

Illustrative examples in which stimulation of a biological response was achieved included: exenatide, which mimics a natural peptide (glucagon-like peptide 1 (GLP1)) but is resistant to degradation by the protease DPP4 (REF. 45); sitagliptin, which prevents degradation of endogenous GLP1 by inhibiting DPP4 (REF. 33); and cinacalcet, which is an allosteric activator of the calcium-sensing receptor[29].

Illustrative examples in which inhibition or antagonism of a biological response was achieved included: aprepitant, which is a competitive antagonist of the neurokinin-1 receptor[46]; orlistat, which is an irreversible inhibitor of lipase enzymes[35]; fulvestrant, which induces

Table 2 | **Discovery of first-in-class NMEs by therapeutic area**

Disease area	Target-based screening	Phenotypic screening	Biologics
Infectious diseases	3	7	1
Immune	1	0	6
Cancer	5	3	8
Central nervous system	1	7	1
Metabolic	3	2	2
Cardiovascular	2	3	0
Gastrointestinal	1	1	1
Others	1	3	1
Rare diseases	0	2	5

NME, new molecular entity.

degradation of the oestrogen receptor[47]; bevacizumab, which binds to VEGF, thereby preventing its interaction with its cell surface receptors[48]; and imatinib, which inhibits the BCR–ABL kinase by stabilizing its inactive conformation[49] (see Supplementary information S2 (box)) for further details on these and other MMOAs).

Importantly, simple equilibrium binding at the target was rarely sufficient for the translation of drug binding to the target into a therapeutically useful response — a subtle aspect of drug action that is underappreciated. These results are consistent with the previous conclusion[10] that "two components are important to the MMOA. The first component is the initial mass action-dependent interaction. The second component requires a coupled biochemical event to create a transition away from mass-action equilibrium". It is also consistent with the opinions expressed by Imming and colleagues[4] in their analysis of drug targets, in which they emphasized the need to consider the dynamics of the drug–target interactions, because "in situations in which the dynamic actions of the drug substance stimulate, or inhibit, a biological process, it is necessary to move away from the descriptions of single proteins, receptors and so on and to view the entire signal chain as the target".

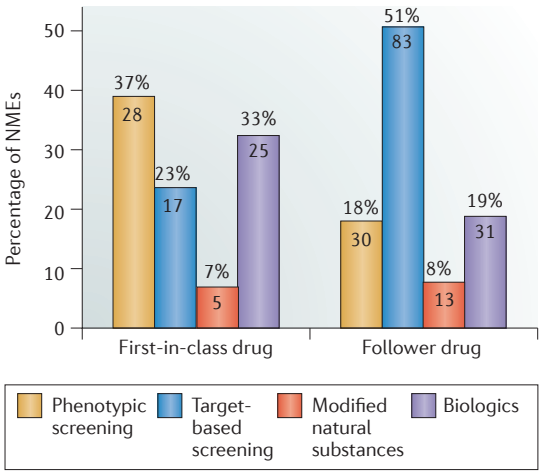

Figure 2 | **The distribution of new drugs discovered between 1999 and 2008, according to the discovery strategy.** The graph illustrates the number of new molecular entities (NMEs) in each category. Phenotypic screening was the most successful approach for first-in-class drugs, whereas target-based screening was the most successful for follower drugs during the period of this analysis. The total number of medicines that were discovered via phenotypic assays was similar for first-in-class and follower drugs — 28 and 30, respectively — whereas the total number of medicines that were discovered via target-based screening was nearly five times higher for follower drugs versus first-in-class drugs (83 to 17, respectively).

The diversity of the MMOAs of the new drugs analysed in this article is not surprising. Physiological and drug mechanisms provide numerous examples of how diversity and complexity in the MMOA can provide robust, selective and timely functional responses. For example, nuclear receptor ligands can induce ligand-specific structural conformations that can be uniquely coupled to the physiological system to provide functionally selective responses[14]. Such conformational changes might not be detectable by X-ray crystallography studies; indeed, this was recently demonstrated for the β_2-adrenergic receptor — there was no discernable difference in the conformation of the receptor when it was bound to an inverse agonist or an antagonist[50]. The functions of many enzymes are also regulated by specific structural changes. For example, receptor tyrosine kinase activation requires conformational changes that are facilitated by ligand binding[51], and many proteases have inhibitory domains that must be proteolytically cleaved for enzyme activation[52]. Both kinetics and conformation contribute to the specificity of high-fidelity nucleotide incorporation by DNA polymerases. Kinetic analysis has shown that the nucleotide substrate-induced structural change has a key role in discriminating between correct and incorrect base pairs, by governing whether a nucleotide will be retained and incorporated or rapidly released[53].

Discussion

A principal observation from this analysis is that the majority of small-molecule first-in-class NMEs that were discovered between 1999 and 2008 were first discovered using phenotypic assays (FIG. 2): 28 of the first-in-class NMEs came from phenotypic screening approaches, compared with 17 from target-based approaches. This is despite the current focus of small-molecule drug discovery on target-based approaches. A possible contributing factor to this trend could have been a lag time between the introduction of new technologies and strategies, and their impact in terms of the number of approved first-in-class NMEs derived from these approaches. However, such a lag is not strongly apparent in a comparison of the cumulative number of NMEs from the two approaches during the period analysed (FIG. 3a).

This observation, along with further analysis of the MMOA of the first-in-class NMEs, leads us to propose that a focus on target-based drug discovery, without accounting sufficiently for the MMOA of small-molecule first-in-class medicines, could be a technical reason contributing to high attrition rates. Our reasoning for

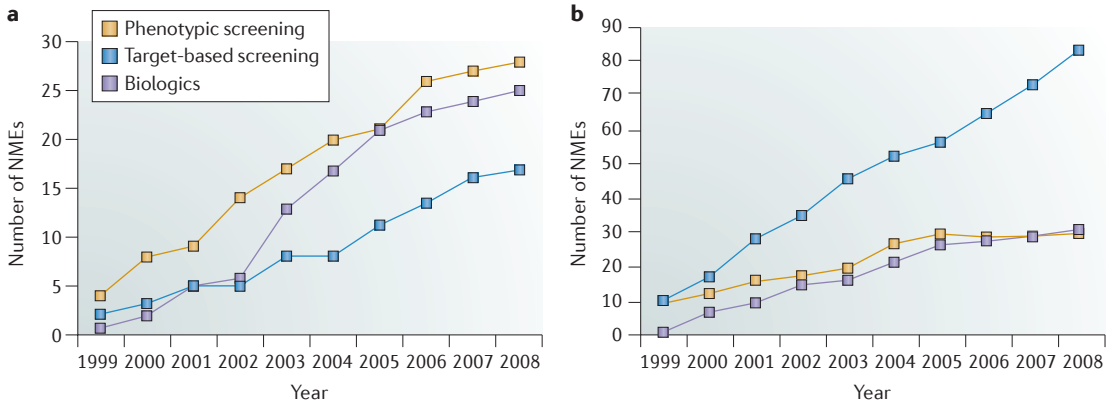

Figure 3 | **Cumulative distribution of new drugs by discovery strategy. a** | First-in-class drugs. A lag is not strongly apparent in a comparison of the cumulative number of small-molecule new molecular entities (NMEs) that were discovered from the different approaches during the period analysed. **b** | Follower drugs. For follower drugs, the ratio of small-molecule NMEs discovered through target-based screening to those discovered through phenotypic screening appears to increase in the second half of the time period.

this proposal is that the MMOA is a key factor for the success of all approaches, but is addressed in different ways and at different points in the various approaches.

In the more common target-based approach, drug discovery is generally hypothesis-driven, and there are at least three hypotheses that must be correct to result in a new drug. The first hypothesis, which also applies to other discovery approaches, is that activity in the preclinical screens that are used to select a drug candidate will translate effectively into clinically meaningful activity in patients. The other two hypotheses are that the target that is selected is important in human disease and that the MMOA of drug candidates at the target in question is one that is capable of achieving the desired biological response. Successful target-based discovery of first-in-class drugs with tolerable safety profiles requires the time and resources to investigate all three hypotheses. In particular, the importance of hypothesis testing to identify an appropriate MMOA may be an underappreciated challenge that — if neglected — could contribute to increased attrition rates for such approaches. In other words, it is clearly difficult to rationally identify the specific molecular interactions from all of the potential dynamic molecular interactions that will contribute to an optimal MMOA. Thus, the key biochemical nuances that are important for the translation of the molecular interaction (between a drug and the target) to an optimal pharmacological response could be missed with target-based approaches.

By contrast, in the case of phenotypic-based screening approaches, assuming that a screening assay that translates effectively to human disease is available or can be identified, a potential key advantage of this approach over target-based approaches is that there is no preconceived idea of the MMOA and target hypothesis. This could considerably aid the identification of molecules with appropriate targets (and possibly multiple targets) and MMOAs, which might be less likely to emerge rapidly, if at all, from pursuing a focused target-based hypothesis. However, two limitations of phenotypic-based screening approaches should also be noted. First, it will often be necessary to characterize the MMOA of active molecules that are identified in phenotypic screens to aid the optimization of a drug candidate, but substantial progress has been made in approaches to achieve this — for example, approaches based on RNA interference[54,55]. Second, phenotypic assays are often lower in throughput than standard target-based assays, although considerable progress has also been made in recent years to automate such assays and increase their throughput[56–58].

Finally, as has often been noted in reviews of the role of natural products in drug discovery[32,59], discovery strategies that are based on natural substances have an inherent advantage: the biology, target and MMOA are often likely to be have been optimized already through evolution, and so modifying such substances can be a fruitful approach. Similarly, some of the biologics that have been approved are harnessing endogenous mechanisms in a rational way — for example, by providing a natural protein that is reduced in a given disease state, as is the case for enzyme replacement therapies for lysosomal storage disorders. In other cases though, it is apparent that the precise MMOA of biologics might also be important in their biological effects, as illustrated by the differences in the properties of two monoclonal antibodies that target CD20 on B cells[60] — rituximab and ofatumumab — although neither of these were approved in the 10-year period we studied. Telling *et al.*[60] conclude that the recognition of a novel epitope cooperates with a slow off-rate in determining the activity of CD20 monoclonal antibodies in the activation of complement and the induction of tumour cell lysis.

The importance of the MMOA is further supported by the evolution of the MMOA within drug classes, from the first-in-class molecule to the best-in-class molecule, which is not widely appreciated. For example, in some cases in which there is no mechanism-based toxicity, the evolution of drugs in a given class towards the best-in-class has been associated with slower dissociation rates at the target. This has been observed with antihistamines

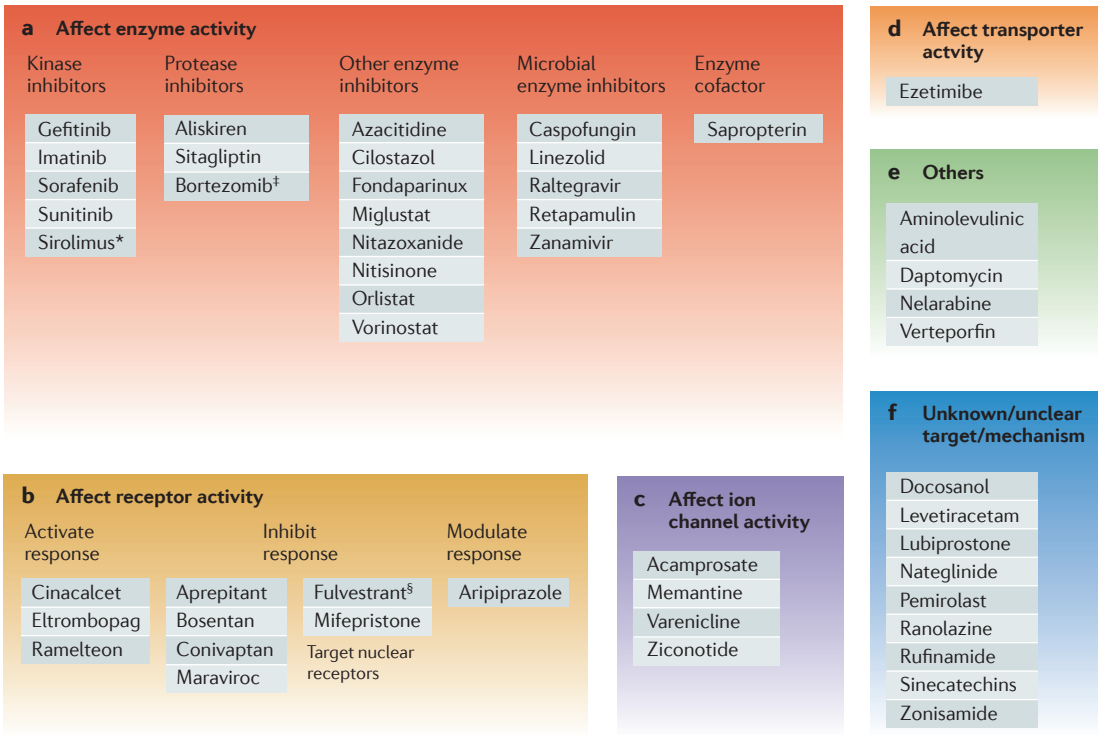

Figure 4 | **Activities of first-in-class small-molecule new molecular entities.** Nearly half (22 out of 50) of the first-in-class small-molecule drugs that were approved between 1999 and 2008 affected enzyme activity (**a**). The molecular mechanisms of action (MMOAs) of these drugs included reversible, irreversible, competitive and noncompetitive inhibition, blocking activation and stabilizing the substrate. The next largest group of targets (10 drugs) were receptors (**b**), most of which were G protein-coupled receptors. Their MMOAs included agonism, partial agonism, antagonism and allosteric modulation. Two drugs — fulvestrant and mifepristone — targeted nuclear receptors. Four of the drugs targeted ion channel activity (**c**); their MMOAs included uncompetitive antagonism and partial agonism. One drug, ezetimibe, targeted the activity of a transporter (**d**). The remaining drugs had other activities (**e**), or unclear targets or MMOAs (**f**). Of the NMEs with other activities, two had a unique MMOA: verteporfin, a porphyrin that catalyses the generation of reactive oxygen species and is used for photodynamic therapy; and daptomycin, which has an MMOA that involves disruption of bacterial membranes. For details of the discovery and activities of each drug, see Supplementary information S2 (box). *Sirolimus binds to the protein FKBP12 and the sirolimus–FKBP12 complex inhibits the kinase activity of mammalian target of rapamycin, whereas the other four kinase inhibitors target receptor tyrosine kinases. ‡Bortezomib inhibits the 26S proteasome — a multiprotein complex — by inhibiting the chymotryptic-like activity of the proteasome. §Fulvestrant acts by promoting receptor degradation.

(desloratadine)[61], antimuscarinics (tiotropium)[62] and angiotensin receptor blockers (candesartan)[63,64]. Conversely, in drug classes with mechanism-based toxicity, MMOAs that increase the therapeutic index have been identified, as illustrated by SERMs such as raloxifene[14,65]. A decrease in the number of iterations required to identify an optimal MMOA for first-in-class drugs could accelerate lead discovery and reduce late-stage attrition, thereby increasing R&D productivity.

With regard to the discovery of follower drugs, the opposite trend was seen compared to first-in-class drugs, with target-based approaches accounting for 83 (51%) of these NMEs and phenotypic-based approaches accounting for 30 (18%) NMEs. The reversal of the trend is presumably the result of drug developers taking advantage of knowledge of a previously identified MMOA to effectively use target-based tools. The timing of the use of these tools may also be important. A recent report by DiMasi and Faden[66] on follower drugs shows that research on a large percentage of follower drugs was initiated before first-in-class approval. The authors[66] concluded that "drug development can often be characterized as a race in which several firms pursue investigational drugs with similar chemical structures or with the same mechanism of action before any drug in the class obtains regulatory marketing approval". That is, it appears that once a mechanism of action or a chemical class with the potential to be developed into a drug is discovered, multiple organizations within the pharmaceutical industry may pursue it vigorously. In drug discovery, this race may contribute to the escalating costs, as there is only room for a few drugs in a class. Additionally, the analysis by DiMasi and Faden[66] only captures the drug classes that have been approved; if the costs for organizations involved in a race around a hypothesis that was later proven to be incorrect are also considered, the total costs could be substantially higher.

The increased reliance on hypothesis-driven target-based approaches in drug discovery has coincided with the sequencing of the human genome and an apparent

belief by some that every target can provide the basis for a drug. As such, research across the pharmaceutical industry as well as academic institutions has increasingly focused on targets, arguably at the expense of the development of preclinical assays that translate more effectively into clinical effects in patients with a specific disease. In our analysis, we found that there are numerous diverse MMOAs for approved new first-in-class drugs, but drug discovery at present appears to be dominated by a 'one size fits all' approach, in which drugs are optimized for binding affinity with less consideration for binding kinetics and conformation. For optimal application of target-based approaches, it is important to consider how efficiently binding is coupled to the response (BOX 2). However, the molecular descriptors for the coupling factors may not be accurately captured by only considering binding affinity. Furthermore, an excessive focus on affinity at a given target could lead to compromises being made in pharmacokinetic properties that are critical for the success of drugs, which has also been postulated to be an underlying factor for current attrition rates[67].

Reducing the impact of technical uncertainty on the later, more costly stages of drug development through a 'quick win/fast fail' strategy has been proposed as a solution to the current problems with R&D productivity[2]. However, this strategy does not address the key issues that contribute to the greater technical uncertainty and associated risk of failure. Our analysis leads us to conclude that the identification of an optimal MMOA has been a key factor contributing to the success of phenotypic screening in the discovery of the first-in-class NMEs in the 10-year period we studied. Thus, we consider that technical risk and, consequently, overall attrition in drug development could be decreased for first-in-class drugs through the development and greater use of translational phenotypic assays, and by considering diverse MMOAs when using a target-based, hypothesis-driven strategy.

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