Drug Evolution Concept in Drug Design: 1. Hybridization Method†

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A novel concept, "drug evolution", is proposed to develop chemical libraries that have a high probability of finding drugs or drug candidates. It converts biological evolution into chemical evolution. In this paper, we present "hybridization" drug evolution, which is the equivalent of sexual recombination of parental genomes in biological evolution. The hybridization essentially shuffles the building blocks of the parent drugs and ought to drug(s); no drug evolution can otherwise occur. We hybridized two drugs, benzocaine and metoclopramide and generated 16 molecules that include the parent drugs, four known drugs, and two molecules whose therapeutic activities are reported. The unusually high number of drugs and drug candidates in the library encourages high expectations of finding new drug(s) or drug candidate(s) within the remaining eight compounds. Interestingly, the therapeutic applications of the eight drugs or drug candidates in the library are fairly diverse as 38 therapeutic applications and 25 molecular targets are counted. Therefore, the library fits as a general chemical library for unspecified therapeutic activities. The hybridization of other two drugs, aspirin and cresotamide, is also described to demonstrate the generality of the method.

Introduction

The completion of the human genome sequence has enabled the understanding of the genetic and molecular bases of diseases and the identification of new molecular targets, followed by their validations for drug development. In response to the new molecular targets and ever-increasing global competition, new drugs must be developed in a short period of time. Furthermore, the era of continuous growth and profitability of the pharmaceutical industry is coming to an end due to the high costs of drug development (~$800 million/drug). Thus, methods for rapid and cost-effective drug development are in urgent need.

If the new target is homologous to a known target(s), then the accumulated information, such as structure of the target protein, ligand−protein interactions at the binding site, or quantitative structure−activity relationship (QSAR) of the lead compounds, is incorporated to build a knowledge-based chemical library. This type of library has a range of hundreds to thousands of compounds and is referred to as a focused chemical library. Due to the accumulated knowledge on the homologous target(s), drug development tends to be rapid and efficient. For example, the success of HIV protease inhibitors in preventing progression to AIDS was based on the transition state analogues of the homologous protease renin.[1]

However, if the new target has no or poor homology with known targets, then the lead compounds may be discovered from screening a general chemical library that is designed for unspecified targets. In response to the rapidly growing diversity of new molecular targets, the general chemical library emphasizes a diversity of chemical structures. With the recent advances in combinatorial chemistry, the library may contain up to millions of compounds that can be evaluated by high throughput screenings (HTS). However, the high cost of the general chemical library and HTS can be a problem. Even if the cost of each chemical and the cost of assay per chemical is low, screening 50 000−200 000 compounds, which is the typical size of HTS in the pharmaceutical industry, may cost up to millions of dollars.[2]

The quality of the chemicals and the number of compounds in libraries were improved by excluding nondruglike molecules, which do not satisfy druglike constraints such as the size, the hydrophobicity, the hydrogen bond formation capability, the characteristic structures of known drug pharmacophores, etc. As most organic compounds do not satisfy such criteria of druglikeness, excluding the nondruglike molecules substantially reduced the cost of synthesis. Furthermore, many druglike molecules are commercially available at relatively low cost per compound. Such general chemical libraries were extensively used, resulting in the discovery of a large number of lead compounds. Nevertheless, their outcome in the drug development has been poor. Absorption, distribution, metabolism, excretion, and toxicity (ADMET) are the recent indexes of druglikeness, and they contribute to the prediction of the behavior of a compound in vivo. Although ADMET assays are still time-consuming and are associated with high costs, there has been some progress in this field. The synergistic use of experimental and computational ADMET models makes the application of ADMET models more reliable in the virtual screening of small molecules.[3]

A general method, which does not depend on whether the new target is homologous to the known targets or not, was proposed by Holland. He converted biological evolution to chemical evolution and used optimization techniques called genetic algorithms (GAs), which are evolutionary search and optimization strategies based

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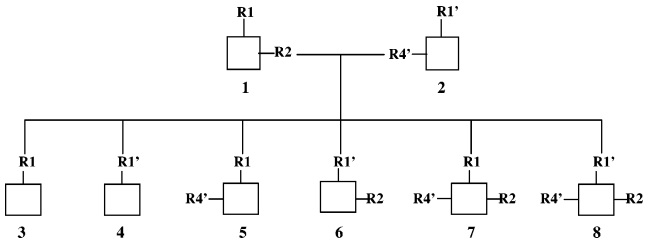


Figure 1. Proposed schematic for shuffling the building blocks in the hybridization method.

on the Darwinian model of natural selection and evolution.[4] GAs allow a dynamically evolving population of molecules to gradually improve by competing for the best performance.[5] Combination of the building blocks is the primary source of the GA's search power to produce meaningful solutions.[6]

In this article, we propose an even more general approach that works for unspecified targets. Analogously to Holland's method, we transform biological evolution to chemical evolution. The biological evolution methods could be sexual recombination of parental genes, mutation, chimera, horizontal gene transfer, etc. In the article, we describe the conversion of the sexual recombination of parental genes into chemical evolution. Other drug evolution methods derived from biological evolution will be described elsewhere.

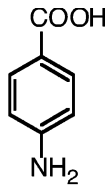
Methods

Hybridization Method. There are different ways to incorporate the advantages of sexual recombination of parental genomes into chemical evolution. Here, we mimic the fertilization step by merging two parent drug molecules. The parent molecules are selected from the small organic drug molecules that are accepted by the human body. These molecules are justified by assuming that the scaffolds and building blocks of drugs are easily accepted by the human body, therefore, being suitable for further drug development. As an initial trial of the drug evolution, we selected parent molecules that had the same scaffold. The same scaffold may be regarded as the restriction of the sexual recombination between male and female of one species.

In an analogous manner to the way gene recombination occurs between the corresponding chromosomes, the building blocks are shuffled at the same site on the scaffold. Figure 1 illustrates a proposed schematic for the shuffling of the building blocks.

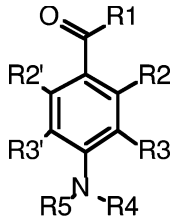
Molecule 1 is substituted with building blocks R1 and R2 at the first and second substitution sites, respectively and molecule 2 with building blocks R1′ and R4′ at the first and fourth substitution sites, respectively. The building blocks R1 and R1′ in the parent molecules are shuffled at the same first substitution site in such a way that the second-generation molecules have a building block of either R1 or R1′. The parent molecule 2 has no building blocks at the second substitution site. Thus, the second-generation molecules have either R2 or no building blocks at the second substitution site. The parent molecule 1 has no building blocks at the fourth substitution site. Thus, the second-generation molecules have either R4′ or no building blocks at the fourth substitution site. Accordingly, six second-generation molecules result from the combination of the building blocks of the two parent molecules 1 and 2.

Selection of Scaffolds. In the previous article, we introduced *p*-aminobenzoic acid (PABA) as a scaffold to generate general chemical libraries, because it has the following suitable properties:

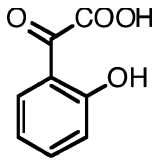


PABA

(1) the PABA scaffold shows up frequently in the structure of drugs, as 184 drugs among 12 111 drugs in the Negwer's database[7] retain the structure of PABA, (2) the PABA-containing drugs show diverse therapeutic activities as 84 therapeutic applications are listed in Negwer's database, (3) the scaffold fits to easy combinatorial synthesis as PABA contains two reactive functional groups (carboxyl and amino groups), and (4) the possibilities of designing novel PABA-containing compounds are very high. For example, just by shuffling the building blocks used in 184 PABA-containing drugs at the corresponding substitution sites, ∼4 million compounds, most of them novel, can be generated. The general chemical structure of PABA containing compounds is shown below.



The generality of the method was demonstrated with another scaffold, salicylic acid (SA), because it also fulfills the four properties outlined above for PABA: (1) the SA scaffold



is frequently used in drugs structure, as 381 drugs from among the 12 111 drugs in the Negwer's database contain SA, (2) the SA-containing drugs show diverse therapeutic activities as 137 therapeutic applications are listed in the 381 SA-containing drugs, (3) the SA scaffold fits to easy combinatorial synthesis as SA contains two reactive functional groups (carboxyl and hydroxyl groups), and (4) the possibilities of designing novel SA-containing compounds are very high. For example, just by shuffling the building blocks used in 381 SA-containing drugs at the same substitution sites, ∼30 billion compounds, most of which are novel, can be generated.

PABA Hybridization Library. We selected benzocaine and metoclopramide (compounds **1** and **16**, respectively, in Figure 2) as parent drugs that contain

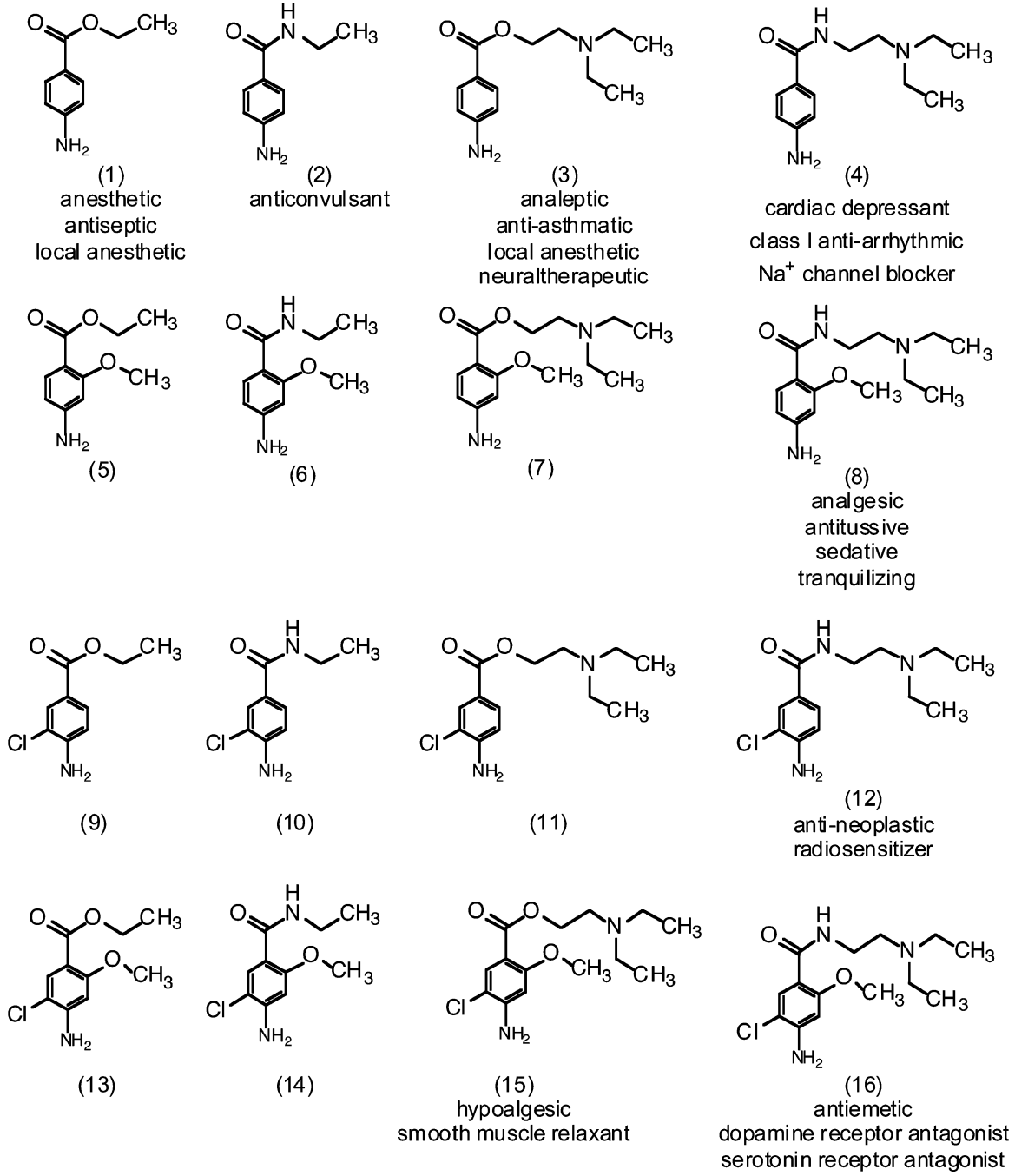
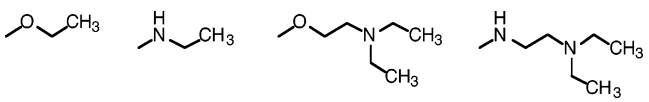
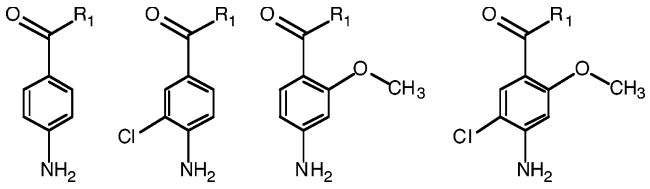


Figure 2. Structures of the 16 compounds in the PABA hybridization library.

PABA as a scaffold. Benzocaine is an anesthetic, local anesthetic, and antiseptic drug, whereas metoclopramide is an antiemetic and stomachic drug. It is noteworthy that these two drugs have different activities, therefore the hybridization may be compared to the diploid combinations of which new alleles might express new functions. The R1 building blocks of benzocaine and metoclopramide are ethyl ester and *N*-(2-diethylaminoethyl)-amide, respectively. Including the mixing of ester and amide bonds, the hybridization of the two building blocks generates four R1 building blocks as shown below:

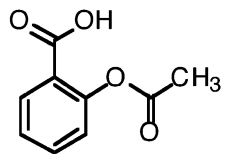


Similarly, the substituents of the aromatic ring at the second and fifth positions in metoclopramide generate four aromatic substitutions: The combination of the

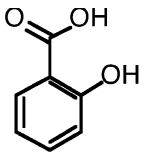


above building blocks generates 16 compounds (Figure 2) including the parent molecules benzocaine and metoclopramide.[8]

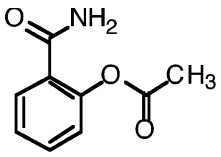
SA Hybridization Library. To generate a new chemical library using the SA scaffold, we used aspirin



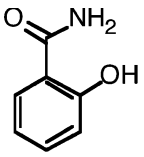
(17)
analgesic
anti-arthritic
antineoplastic
antineuralgic
antipyretic
antirheumatic
immunostimulant
platelet aggregation
inhibitor



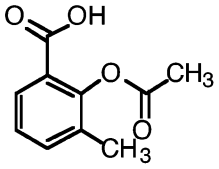
(18)
analgesic
anti-arthritic
antidiarrheal
anti-inflammatory
antineuralgic
antipyretic
antirheumatic
anti-thrombotic
bismuch therapeutic
dermatic
keratolytic
sclerosing agent
topical antiseptic



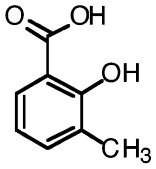
(19)
antipyretic



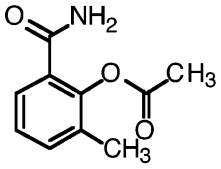
(20)
analgesic
antipyretic



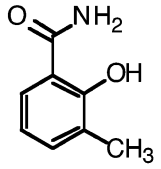
(21)
antineuralgic
antipyretic
antirheumatic



(22)
analgesic
antiseptic
metabolic



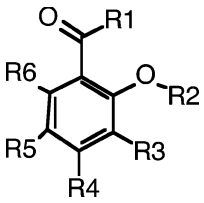
(23)



(24)
analgesic
antipyretic
muscle relaxant

Figure 3. Structures of the Eight compounds in the salicylic acid hybridization library.

and cresotamide (compounds **17** and **24**, respectively, in Figure 3) as parent drugs. Aspirin has a large spectrum of therapeutic applications: analgesic, anti-arthritic, antineoplastic, antineuralgic, antipyretic, anti-rheumatic, immunostimulant, and platelet aggregation inhibition, whereas cresotamide is known as an analgesic, antipyretic, and muscle relaxant drug. It is noteworthy that these two drugs share two activities, analgesic and antipyretic, therefore the hybridization may be compared to the diploid combinations of which new alleles might express synergistic evolution of the parental beneficial genes. The general chemical structure of salicylic acid containing compounds is shown below:



The R1 building block of aspirin and cresotamide is hydroxyl and amino, respectively. The hybridization at the first position generates carboxyl and amide groups. Similarly, the substituents of the aromatic ring at the second position in aspirin and cresotamide are acetyl group and hydrogen, respectively, generating two substitutions of acetyl group and hydrogen. The substituents of the aromatic ring at the third position in aspirin and cresotamide are hydrogen and methyl group, respectively, generating two substitutions of hydrogen and methyl group. The combination of these building blocks generates eight molecules that include the parent aspirin and cresotamide, as shown in Figure 3.

Validation of the Hybridization Library. The hybridization of drugs can be carried out for any pair of drugs or group of drugs. The hybridization using over 10 000 drugs generates practically an infinite number of hybridized molecules of which only a limited number of the molecules can be developed as drugs. Thus, because most drug hybridization will not generate drug(s), the validation of the hybridized molecules is required. A drug-evolution library is successful if it generates at least one drug. Because we cannot predict if the library contains novel drugs or drug candidates in advance, we can only examine if the library contains known drugs or drug candidates. The superiority of the second-generation drug(s) compared to the parental drugs is hardly established without thorough evaluation. Consequently, we validate the drug-evolution library if it contains at least one drug in the second-generation molecules. Because the hybridization generates drug(s) in the validated drug-evolution library, there is a reasonably high probability to find more drug(s) or drug candidate(s) in the remaining molecules

of the library. If the library contains unusually high number of known drugs or drug candidates, then the library is enriched with drugs or drug candidates, and therefore there is an even higher probability to discover novel drug(s) or drug candidate(s) in the remaining molecules of the library. We highlight such a library as a "hot spot" library. Moreover, if the therapeutic applications of the known drugs or drug candidates in the "hot spot" library are diverse, then the library is expected to have a wide range of therapeutic activities and can serve as a general chemical library for drug discovery. If the therapeutic activities focus on certain applications, then the library will serve as a focused library of certain areas of therapeutic applications. A further advantage of a "hot spot" library is the accumulated ADMET information on the known drugs, which may give some good insight into the ADMET of the remaining compounds in the library due to the structural similarity among the compounds.

Results and Discussion

The Hybridized Library of Benzocaine and Metoclopramide. The hybridization of two drugs, benzocaine and metoclopramide, generates a library of 16 compounds including the parent drugs (Figure 2). Some of the therapeutic activities are briefly listed in Figure 2. Literature and patent searches for the 16 compounds were further carried out. Some of the therapeutic or biological activities and targets located during this search are described below.

Compound 1, Ethyl 4-Aminobenzoate (Benzocaine). Benzocaine is a commonly used local anesthetic drug,[9] which inhibits the propagation of action potentials by blocking voltage-gated Na^+ channels.[10] Benzocaine shows antiinfective activity against *Campylobacter pylori*[11] and antifungal activity by inhibiting mycelial growth, especially against *Aspergillus*. It also inhibits the biosynthesis of aflatoxin.[12] Benzocaine is approved for treating obesity by altering taste.[13] In animals and plants, benzocaine affects the fertilization potential of *Xenopus* eggs[14] and inhibits photosynthetic activity at the water-splitting site of photosystem II in pea chloroplasts.[15] At the molecular level, benzocaine inhibits mitochondrial ATPase,[16] liver cholesterol esterase,[17] acyl-CoA−cholesterol acyltransferase,[18] and lecithin−cholesterol acyltransferase.[19]

Compound 2, 4-Amino-*N*-ethyl-benzamide. Compound **2** is an anticonvulsant.[20] However, it is not listed as a drug in Negwer's database.

Compound 3, 2-Diethylaminoethyl 4-Aminobenzoate (Procaine). Procaine is a local anesthetic agent that blocks Na^+ and K^+ ion channels.[21] Procaine is also used in the treatment of severe forms of bronchial asthma.[22] Furthermore, it protects neurons against ischemic damage by blocking Ca^{2+} channels and inhibiting the release of Ca^{2+} from intracellular Ca^{2+} stores.[23] Procaine has cytostatic activity against human cancer cells through the inhibition of DNA methyltransferases.[24] It has immunological effects and inhibits adhesion, phagocytosis, and production of superoxide anion and hydrogen peroxide in neutrophils,[25] possibly through the inhibition of phospholipase D activity.[26] Procaine reduces the thermal stability of transmembrane domain of Ca^{2+} ATPase and sensitizes cells to hyperthermia.[27] Procaine stimulates the growth of plants and microorganisms such as bacteria, yeast, algae, and protozoa.[28] Besides being a drug by itself, procaine is also used to formulate antibiotic penicillin G.[29] At the molecular level, procaine binds to various receptors or membrane proteins such as opioid receptors,[30] 5-HT_3 receptor,[31] nicotinic acetylcholine receptor,[32] and Na, K ATPase.[33] It also binds to a dopamine transporter and inhibits dopaminergic neurotransmission.[34,35]

Compound 4, 4-Amino-*N*-(2-diethylaminoethyl)-benzamide (Procainamide). Procainamide, a class Ia antiarrhythmic drug, was widely used to prevent or treat ventricular tachycardia.[36,37] It modulates inward Na^+ current, delayed rectifier outward K^+ current, and ATP sensitive K^+ current.[38] At the molecular level, it inhibits NADPH-dependent lipid peroxidation and scavenges hydroxyl radicals.[39] It binds to cardiac muscarinic receptors and inhibits the uptake of choline.[40]

Compound 5, Ethyl 4-Amino-2-methoxybenzoate. Compound **5** was used as a reagent in the synthesis of dopaminergic D3 antagonists and of hyperlipidemia and atherosclerosis agents.[41,42] However, no biological activities have been reported for it.

Compound 6, 4-Amino-*N*-ethyl-2-methoxybenzamide. Compound **6** is an intermediate in the synthesis of its antibacterial sulfonamide derivative.[43] No biological activity of this compound has been reported though.

Compound 7, 2-Diethylaminoethyl 4-Amino-2-methoxybenzoate. Compound **7** is registered with CAS number 100862-14-4; however, we could not find any information in the literature.

Compound 8, *N*-(2-Diethylaminoethyl) 4-Amino-2-methoxybenzamide. Compound **8** is an analgesic and sedative as well as an efficient tranquilizing agent,[44,45] but it is not listed as a drug in Negwer's database.

Compound 9, Ethyl 4-Amino-3-chlorobenzoate. Compound **9** is an intermediate in the synthesis of antidiabetic agents[46] and antiinflammatory agents.[47] It is also used as a reagent in the synthesis of herbicides and of insecticides.[48] However, no biological activity of compound **9** has been reported.

Compound 10, Ethyl 4-Amino-3-chloro-*N*-ethylbenzamide, and Compound 11, 2-Diethylaminoethyl 4-Amino-3-chlorobenzoate. Compounds **10** and **11** are novel compounds.

Compound 12, 4-Amino-*N*-(2-diethylaminoethyl)-3-chlorobenzamide (Declopramide). Declopramide is an antiarrhythmic drug and part of a new class of chemosensitizers and radiosensitizers through inhibition of DNA repair.[49] At the molecular level, it induces apoptosis by activation of caspases or by inhibition of $I\kappa B\alpha$ breakdown and the NF-κB rescue pathway.[47,50,51] It also binds to 5-HT_3 receptors, which are important in controlling vomiting.[49]

Compound 13, Ethyl 4-Amino-5-chloro-2-methoxybenzoate. Compound **13** is an intermediate in the synthesis of metoclopramide.[52] However, no biological activity has been reported.

Compound 14, 4-Amino-5-chloro-*N*-ethyl-2-methoxybenzamide. Compound **14** is a novel compound.

Compound 15, 2-Diethylaminoethyl 4-Amino-5-chloro-2-methoxybenzoate (SDZ 205,557). SDZ 205,-

557 mediates muscle contraction or relaxation within the gut and stimulates heart muscle contractions by antagonizing 5-HT_4 receptors.[53,54] It also has a hypoalgesic effect on cutaneous pain by antagonizing 5-HT_4 receptors.[55] Furthermore, SDZ 205,557 was used as a research reagent on the cognitive functions of 5-HT_4 receptors.[56,57]

Compound 16, 4-Amino-5-chloro-*N*-(2-diethylaminoethyl)-2-methoxybenzamide (Metoclopramide). Metoclopramide is a widely used antiemetic agent through its triple action of antagonizing central dopaminergic receptors, both central and peripheral 5-HT_3 receptors, and peripheral 5-HT_4 receptors.[58] Due to its antiemetic effects, it is used as an adjunctive in diagnostic procedures (intestinal intubation, contrast radiography of the gastrointestinal tract, diagnosis of gastroesophageal reflux disease).[59,60] Metoclopramide is prescribed for the treatment of anorexia nervosa.[61] It is also immunostimulatory, because its dopamine antagonism upregulates prolactin.[62] Metoclopramide could potentially be used in the treatment of neurogenic bladder, orthostatic hypotension, tumor-associated gastroparesis, nonprolactinemic amenorrhea, failure to thrive, Tourette's syndrome, hiccups, and migraine therapy.[63]

The Validation of the Hybridized Library of Benzocaine and Metoclopramide. The therapeutic and biological activities and the target of the above eight drugs or drug candidates are listed in Tables 1 and 2, respectively.

It is noteworthy that most of the 38 therapeutic applications and 25 molecular targets listed in Tables 1 and 2 are not shared among the eight drugs or drug candidates. Consequently, the therapeutic applications or biological activities of the remaining eight compounds cannot be predicted in advance, and a wide range of assays may have to be carried out before their therapeutic activities are identified. However, once such therapeutic activities are discovered in one or more of the remaining eight compounds, their probability to become drugs or at least drug candidates with no or minimum modification is high due to the enriched content in the known drugs and drug candidates (50%) of the library. It should be also emphasized that in such a small drug-enriched library three compounds are novel.

The Hybridized Library of Aspirin and Cresotamide. The library derived from aspirin and cresotamide is also surprisingly enriched with drugs; seven compounds **17**, **18**, **19**, **20**, **21**, **22**, and **24** out of the eight compounds are classified as drugs in Negwer's database (shown in Figure 3). Surprisingly, the remaining compound **23** is novel to our best knowledge. In such a chemical library, the probability of finding therapeutic activities for compound **23** as a drug or drug candidate is very high, validating the SA hybridization′ library represented in Figure 3. Moreover, the analgesic, antipyretic, antineuralgic, and antirheumatic activities are commonly reported in some of the seven drugs. This implies that some of the activities of compound **23** may be the same or in the vicinity of the activities of the seven drugs.

The beauty of this method is that there are no requirements of any previous knowledge for target diseases or molecules or for assumptions regarding the biological activities of the resulted compounds. It simply

Table 1. Therapeutic Applications Corresponding to the 16 Compounds in the PABA-Containing Hybrid Library

therapeutic applications and biological activities	compound no.
affecting fertilization potential	**1**
analgesic	**8**
antiemetic	**16**
antifungal	**1**
anticonvulsant	**2**
antineoplastic	**3**, **12**
class Ia antiarrhythmic	**4**
controlling vomiting	**12**
contraction or relaxation of muscle	**15**
growth promotion of plants and microorganisms	**3**
hypoalgesic effect on cutaneous pain	**15**
immunostimulatory	**16**
inhibition of adhesion	**3**
inhibition of choline uptake	**4**
inhibition of dopaminergic neurotransmission	**3**
inhibition of hydrogen peroxide production	**3**
inhibition of phagocytosis	**3**
inhibition of photosynthetic activity	**1**
inhibition of superoxide anion production	**3**
local anesthetic	**1**, **3**
management of functional gut disorder	**16**
neuroprotective against ischemic damage	**3**
radiosensitizer	**12**
sedative	**8**
sensitization of cells to hyperthermia	**3**
tranquilizing	**8**
treatment of anorexia nervosa	**16**
treatment of bronchial asthma	**3**
treatment of failure to thrive	**16**
treatments of neurogenic bladder	**16**
treatment of obesity	**1**
treatment of hiccups	**16**
treatment of migraine	**16**
treatment of nonprolactinemic amenorrhea	**16**
treatment of orthostatic hypotension	**16**
treatment of tachycardia	**4**
treatment of tumor-associated gastroparesis	**16**
treatment of Tourette's syndrome	**16**

Table 2. Interacting Targets Related to the 16 Compounds in the PABA-Containing Hybrid Library

targets	compound no.
activation of caspases	**12**
agonist of 5-HT_4 receptors	**16**
antagonist of dopaminergic receptors	**16**
antagonist of 5-HT_4 receptors	**15**
binding to cardiac muscarinic receptors	**4**
binding to dopamine transporter	**3**
binding to 5-HT_3 receptors	**3**, **12**, **16**
binding to Na, K-ATPase	**3**
binding to nicotinic acetylcholine receptor	**3**
binding to opioid receptors	**3**
Ca^{2+} channel blocker	**3**
hydroxyl radical scavenger	**4**
inhibition of acyl-CoA−cholesterol acyltransferase	**1**
inhibition of chloesterol esterase	**1**
inhibition of DNA methyltransferases	**3**
inhibition of IκBα breakdown and the NF-κB rescue pathway	**12**
inhibition of lecithin−chlolesterol acyltransferase	**1**
inhibition of mitochondrial ATPase	**1**
inhibition of NADPH-dependent lipid peroxidation	**4**
inhibition of phospholipase D	**3**
K^+ channel blocker	**3**
modulation of Na^+ and K^+ currents	**4**
Na^+ channel blocker	**1**, **3**
reduction of thermal stability of Ca^{2+} ATPase	**3**
water-splitting site of PSII	**1**

uses the knowledge of existing drugs or drug candidates. It should be noted that the exemplified method of hybridization is not limited to just two drugs but can be applied to a larger number.

Sunscreen Activity. PABA has a strong UV absorbance around 260 nm ($\epsilon = 13\,000$) and, therefore, was used as a sunscreen agent against UV-B (280−320 nm) for many years.[56] We found that all of the 16 compounds in Figure 2 have higher absorbance at UV-A (320−400 nm) and UV-B regions than that of PABA; especially, **13** has absorbance at 275 nm ($\epsilon = 15\,000$) and 310 nm ($\epsilon = 13\,000$).

Other biological activities for the 16 PABA derivatives and the 8 salicylic acid derivatives will be reported elsewhere after extensive screenings against various activities and targets. Incidentally, some PABA analogues in our drug-evolution libraries showed potent neurogeneration activities that will be reported elsewhere. The generality of the hybridization method is further demonstrated by applying it to more complex scaffolds of nucleotide base and steroid, resulting in half of the hybridized second-generation molecules are known drugs.

Conclusions

A new concept, drug evolution is introduced. It incorporates the methods of biological evolution into drug development. This concept consists of evolving the existing drugs in order to develop new ones, in particular drugs and drug candidates corresponding to a wide range of diseases or medicinal targets, and to design general libraries of such compounds, drug evolution libraries. The sexual recombination of parental genomes in biological evolution was successfully used to build two sets of drug evolution libraries that use PABA and salicylic acid as scaffolds. The libraries were validated as the hybridization of the parental drugs generated drug(s). Indeed, 50% of the 16 compounds in the PABA hybridization library are drugs or drug candidates, and 87% of the 8 compounds in the salicylic acid hybridization library are drugs. The probability of finding drug(s) or drug candidate(s) within the remaining molecules of the libraries is high in such drug-enriched libraries; yet no previous knowledge of targeted diseases or target molecules was used, and no assumptions regarding the biological activities of the resulting compounds were made.

Experimental Section

Purification and Identification. All of the synthetic products were purified using a preparative HPLC (C-8 or C-18 reverse-phase column, in acetonitrile gradients in water, 0.1% trifluoroacetic acid (TFA)) at ≥98% purity. The purity was established using an analytical HPLC system (Waters Symmetry 3.5 μm; 50 mm × 2.1 mm C-18 reverse-phase column, 10 min gradient 0−80% acetonitrile in water, 0.1% TFA; flow rate 0.8 mL/min (system 1) or Vydac 250 mm × 4.6 mm C-18 reverse-phase column, 60 min gradient 10−80% acetonitrile in water, 0.1% TFA; flow rate 1.0 mL/min (system 2)). The purified compounds were identified using a Sciex API III mass spectrometer (Sciex, Ontario, Canada) and ^{1}H and ^{13}C NMR using a Bruker AMX2-500.

UV Absorbance Measurements. The UV absorption spectra of the PABA-containing 16 compounds were measured in water (compounds **1**, **2**, **3**, **4**, **7**, **8**, **11**, **12**, **15**, and **16**) or in an aqueous solution containing 0.1% methanol (compounds **5**, **6**, **9**, **10**, **13**, and **14**) at 20 μM concentration. The instrument operated was a CARY 3E UV−vis spectrophotometer (Varian, Palo Alto, CA) with a 10-mm quartz cell. The experiments were done at room temperature, and the sample solutions were pH 6. The scanning speed was 100 nm/min, and the baseline was corrected by the absorbance of the solvent.

Materials. All reagents, chemicals, and solvents purchased from commercial sources were used without further purification unless otherwise noted. Benzocaine (**1**) was purchased from TCI (Portland, OR). Procaine hydrochloride (**3**) was purchased from Aldrich (Milwaukee, WI). Procainamide hydrochloride (**4**) was purchased from RBI (Natick, MA). Metoclopramide hydrochloride (**16**) was purchased from ICN (Costa Mesa, CA).

Compound 2, 4-Amino-*N*-ethyl-benzamide. 4-Boc-amino-*N*-ethyl-benzamide. To a solution of ethylamine hydrochloride (4 g, 50 mmol) in water (10 mL), dichloromethane (DCM, 10 mL) was added. The aqueous phase was brought to pH 12 by adding concentrated NaOH solution. The free amine was extracted with DCM (3 × 10 mL), and the combined organic layers were washed with brine and dried over sodium sulfate. 4-Boc-aminobenzoic acid (400 mg, 2 mmol), *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (TBTU, 650 mg), and *N*,*N*-diisopropylethylamine (DIEA, 350 μL) were added to the DCM solution of ethylamine and left overnight at room temperature. After evaporation of the solvent, the residue was dissolved in ethyl acetate, washed with 10% sodium carbonate, water, and brine, and dried over sodium sulfate. The removal of the solvent afforded the desired compound, which was further used without purification. t_R = 29.4 min (system 2). MS [M + 1]: 265.2.

***N*-Ethyl 4-Aminobenzamide.** 4-Boc-amino-*N*-ethyl-benzamide (300 mg, 1.1 mmol) was dissolved in 20 mL of TFA solution containing water (0.5 mL) and triisopropylsilane (0.5 mL) and left for 3 h at room temperature. After the solvents were evaporated, the residue was dissolved in 1 N HCl and washed with ethyl acetate. The aqueous layer was adjusted to pH 12 by adding sodium carbonate, and the product was extracted with ethyl acetate. The organic layer was washed with 10% sodium carbonate, water, and brine and dried over sodium sulfate. After evaporation, the residue was purified using preparative HPLC and transformed into acetate. t_R = 9.15 min (system 2). MS [M + 1]: 165.1. ^{1}H NMR (500 MHz, $(CD_3)_2CO$): δ (ppm) 1.13 (t, J = 6.8 Hz, 3H), 3.33 (q, J = 6.8 Hz, 2H), 6.63 (d, J = 9.0 Hz, 2H), 7.62 (d, J = 7.9 Hz, 2H).

Compound 5, Ethyl 4-Amino-2-methoxybenzoate Acetate. Ethyl 2-Methoxy-4-nitrobenzoate. 2-Methoxy-4-nitrobenzoic acid was esterified according to a standard procedure (i.e., the acid (1.0 g, 5 mmol) was dissolved in ethanol (50 mL) in the presence of sulfuric acid (0.5 mL) and gently refluxed for 3 h). The solvent was removed in vacuo, and the residue was poured into 10% sodium carbonate and extracted with ethyl acetate. The organic layer was washed with 10% sodium carbonate, water, and brine and dried over sodium sulfate. The solution was evaporated to dryness, and the resulting yellow residue was further used without purification. t_R = 34.14 min (system 2). MS [M + 1]: 226.3.

Ethyl 4-Amino-2-methoxybenzoate. Ethyl 2-methoxy-4-nitrobenzoate (200 mg, 0.9 mmol) was dissolved in methanol (30 mL) and hydrogenated at room temperature under atmospheric pressure over 10% palladium on carbon. The reaction was completed after 3 h. The catalyst was filtered off, and the resulting residue after evaporation was purified by preparative HPLC and transformed into acetate. t_R = 19.48 min (system 2). MS [M + 1]: 196.2. ^{1}H NMR (500 MHz, $(CD_3)_2CO$): δ (ppm) 1.25 (t, J = 6.8 Hz, 3H), 3.75 (s, 3H), 4.15 (q, J = 6.8 Hz, 2H), 6.23 (d, J = 7.9 Hz, 1H), 6.29 (s, 1H), 7.58 (d, J = 9 Hz, 1H).

Compound 6, 4-Amino-*N*-ethyl-2-methoxybenzamide Acetate. *N*-Ethyl-2-methoxy-4-nitrobenzamide. To a solution of ethylamine hydrochloride (4 g, 50 mmol) in water (10 mL), DCM (10 mL) was added. The aqueous phase was brought to pH 12 by adding concentrated NaOH solution. The free amine was extracted with DCM (3 × 10 mL), and the combined organic layers were washed with brine and dried over sodium sulfate. 2-Methoxy-4-nitrobenzoic acid (400 mg,

2 mmol), TBTU (650 mg), and DIEA (350 μL) were added to the DCM solution of ehtylamine and left overnight at room temperature. After evaporation of the solvent, the residue was dissolved in ethyl acetate, washed with 10% sodium carbonate, water, and brine, and dried over sodium sulfate. The removal of the solvent afforded the desired compound, which was further used without purification. t_R = 24.8 min (system 2). MS [M + 1]: 225.3.

4-Amino-*N*-ethyl-2-methoxybenzamide. *N*-Ethyl-2-methoxy-4-nitrobenzamide (400 mg, 1.8 mmol) was dissolved in methanol (50 mL) and hydrogenated at room temperature under atmospheric pressure over 10% palladium on carbon. The reaction was completed in 5 h. The catalyst was filtered off, and after evaporation the residue was purified by preparative HPLC and transformed into acetate. t_R = 13.56 min (system 2). MS [M + 1]: 195.4. ^{1}H NMR (500 MHz, $(CD_3)_2CO$): δ (ppm) 1.13 (t, J = 6.8 Hz, 3H), 3.34 (q, J = 6.8 Hz, 2H), 3.88 (s, 3H), 6.3 (d, J = 7.9 Hz, 1H), 6.32 (s, 1H), 7.8 (d, J = 9 Hz, 1H).

Compound 7, 2-Diethylaminoethyl 4-Amino-2-methoxybenzoate Acetate. 2-Diethylaminoethyl 2-Methoxy-4-nitrobenzoate. 2-Methoxy-4-nitrobenzoic acid (600 mg, 3.0 mmol) was dissolved in 20 mL of toluene containing *N*,*N*-diethylaminoethanol (400 μL, 30 mmol) and sulfuric acid (3 mL). The mixture was gently heated on a water bath for 1 h and left overnight at room temperature. The reaction solution was then poured onto 10% sodium carbonate, the organic layer was separated, and the product in the aqueous layer was further extracted with ethyl acetate. The combined organic layers were washed with 10% sodium carbonate, water, and brine and dried over sodium sulfate. The removal of the solvent afforded the desired compound, which was further used without purification. t_R = 25.1 min (system 2). MS [M + 1]: 297.3.

2-Diethylaminoethyl 4-Amino-2-methoxybenzoate. 2-Diethylaminoethyl 2-methoxy-4-nitrobenzoate (200 mg; 0.67 mmol) was dissolved in methanol (30 mL) and hydrogenated at room temperature under atmospheric pressure over 10% palladium on carbon. The reaction was completed in 4 h. The catalyst was filtered off, and the residue resulted after evaporation was purified by preparative HPLC and transformed into acetate. t_R = 13.76 min (system 2). MS [M + 1]: 267.2. ^{1}H NMR (500 MHz, $(CD_3)_2CO$): δ (ppm) 1.36 (t, J = 6.8 Hz, 6H), 3.39 (q, J = 6.8 Hz, 4H), 3.55 (t, J = 4.5 Hz, 2H), 3.78 (s, 3H), 4.6 (t, J = 4.5 Hz, 2H), 6.26 (d, J = 7.9 Hz, 1H), 6.35 (s, 1H), 7.68 (d, J = 9 Hz, 1H).

Compound 8, 4-Amino-*N*-(2-diethylaminoethyl)-2-methoxybenzamide Acetate. *N*-(2-Diethylaminoethyl)-2-methoxy-4-nitrobenzamide. 2-Methoxy-4-nitrobenzoic acid (500 mg, 2.5 mmol) was dissolved in DMF (10 mL). 2-Diethylaminoethylamine (360 μL, 2.5 mmol), TBTU (800 mg), and DIEA (550 μL) were added, and the mixture was left at room temperature for 2 h. The mixture was then poured onto 10% sodium carbonate and extracted with ethyl acetate. The organic layer was washed with 10% sodium carbonate, water, and brine and dried over sodium sulfate. After evaporation, the product was further used without purfication. t_R = 23.3 min (system 2), MS [M + 1]: 296.5.

4-Amino-*N*-(2-diethylaminoethyl)-2-methoxybenzamide. *N*-(2-Diethylaminoethyl)-2-methoxy-4-nitrobenzamide (200 mg, 0.67 mmol) was dissolved in methanol (30 mL) and hydrogenated at room temperature under atmospheric pressure over 10% palladium on carbon. The reaction was completed in 4 h. The catalyst was filtered off, and the resulting residue after evaporation was purified by preparative HPLC and transformed into acetate. t_R = 12.50 min (system 2). MS [M + 1]: 266.2. ^{1}H NMR (500 MHz, $(CD_3)_2CO$): δ (ppm) 1.33 (t, J = 6.8 Hz, 6H), 3.28 (m, 6H), 3.77 (t, J = 4.5 Hz, 2H), 3.88 (s, 3H), 6.31 (d, J = 7.9 Hz, 1H), 6.38 (s, 1H), 7.82 (d, J = 9 Hz, 1H).

Compound 9, Ethyl 4-Amino-3-chlorobenzoate Acetate. To a solution of ethyl 4-aminobenzoate (compound **1**) (8.25 g; 50 mmol) in acetonitrile (100 mL), heated to boiling point, *N*-chlorosuccinimide (7.0 g; 53 mmol) was gradually added. The mixture was refluxed for 5 h and left overnight at room temperature. Acetonitrile was evaporated in vacuo, and the solid residue was dissolved in DCM, washed with 5% NaOH, water, and brine, and dried over sodium sulfate. The product was purified using preparative HPLC and transformed into acetate. t_R = 33.58 min (system 2). MS [M + 1]: 200.1. ^{1}H NMR (500 MHz, $(CD_3)_2CO$): δ (ppm) 1.31 (t, J = 6.8 Hz, 3H), 4.25 (q, J = 6.8 Hz, 2H), 5.66 (bs, 2H), 6.89 (d, J = 7.9 Hz, 1H), 7.68 (d, J = 9 Hz, 1H), 7.83 (d, J = 1.8 Hz, 1H).

Compound 10, 4-Amino-3-chloro-*N*-ethyl-benzamide Acetate. 4-Amino-3-chlorobenzoic Acid. Ethyl 4-amino-3-chlorobenzoate acetate (compound **9**) (1.0 g, 5.0 mmol) was refluxed for 3 h in a mixture of methanol (50 mL) and water (100 mL) containing NaOH (2 g). The mixture was concentrated, acidified, and extracted with ethyl acetate. The organic phase was washed with water and brine and dried over sodium sulfate. The product was further used without purification. t_R = 4.75 min (system 1). MS [M + 1]: 172.2.

4-Amino-3-chloro-*N*-ethyl-benzamide. To a solution of ethylamine hydrochloride (4.0 g, 50 mmol) in water (10 mL), DCM (10 mL) was added. The aqueous phase was brought to pH 12 by adding concentrated NaOH solution. The free amine was extracted with DCM (3 × 10 mL), and the combined organic layers were washed with brine and dried over sodium sulfate. 4-Amino-3-chlorobenzoic acid (350 mg, 2.0 mmol), TBTU (650 mg), and DIEA (350 μL) were added to the DCM solution and left overnight at room temperature. After evaporation of the solvent, the residue was dissolved in ethyl acetate, washed with 10% sodium carbonate, water, and brine and dried over sodium sulfate. After evaporation, the residue was purified by preparative HPLC and transformed into acetate. t_R = 18.06 min (system 2). MS [M + 1]: 198.9. ^{1}H NMR (500 MHz, $(CD_3)_2CO$): δ (ppm) 1.13 (t, J = 6.8 Hz, 3H), 3.34 (q, J = 6.8 Hz, 2H), 5.45 (bs, 2H), 6.85 (d, J = 7.9 Hz, 1H), 7.46 (bs, 1H), 7.60 (d, J = 9 Hz, 1H), 7.78 (d, J = 1.8 Hz, 1H).

Compound 11, 2-Diethylaminoethyl 4-Amino-3-chlorobenzoate Acetate. To a solution of 2-diethylaminoethyl 4-aminobenzoate (compound **3**) (500 mg, 1.8 mmol) in acetonitrile (40 mL), heated to boiling point, *N*-chlorosuccinimide (256 mg, 1.9 mmol) was gradually added. The mixture was refluxed for 5 h and left overnight at room temperature. Acetonitrile was evaporated in vacuo, and the solid residue was dissolved in DCM, washed with 5% NaOH, water, and brine, and dried over sodium sulfate. The product was purified using preparative HPLC and transformed into acetate. t_R = 19.36 min (system 2). MS [M + 1]: 270.9. ^{1}H NMR (500 MHz, $(CD_3)_2CO$): δ (ppm) 1.36 (t, J = 6.8 Hz, 6H), 3.36 (q, J = 6.8 Hz, 4H), 3.61 (t, J = 4.1 Hz, 2H), 4.66 (t, J = 4.5 Hz, 2H), 5.75 (bs, 2H), 6.9 (d, J = 7.9 Hz, 1H), 7.78 (d, J = 9 Hz, 1H), 7.88 (d, J = 1.8 Hz, 1H).

Compound 12, 4-Amino-3-chloro-*N*-(2-diethylaminoethyl)-benzamide Acetate. To a solution of 4-amino-*N*-(2-diethylaminoethyl)-benzamide (compound **4**) (500 mg, 1.8 mmol) in acetonitrile (40 mL) heated to the boiling point, *N*-chlorosuccinimide (256 mg, 1.9 mmol) was gradually added. The mixture was refluxed for 5 h and left overnight at room temperature. Acetonitrile was evaporated in vacuo, and the solid residue was dissolved in DCM, washed with 5% NaOH, water, and brine, and dried over sodium sulfate. The product was purified using preparative HPLC and transformed into acetate. t_R = 16.1 min (system 2). MS [M + 1]: 269.9. ^{1}H NMR (500 MHz, $(CD_3)_2CO$): δ (ppm) 1.32 (t, J = 6.8 Hz, 6H), 3.31 (q, J = 6.8 Hz, 4H), 3.41 (t, J = 4.5 Hz, 2H), 3.76 (q, J = 4.5 Hz, 2H), 5.44 (bs, 2H), 6.85 (d, J = 7.9 Hz, 1H), 7.68 (d, J = 9 Hz, 1H), 7.85 (s, 1H), 8.8 (bs, 1H).

Compound 13, Ethyl 4-Amino-5-chloro-2-methoxybenzoate Acetate. A solution of 4-amino-5-chloro-2-methoxybenzoic acid (0.40 g, 2.0 mmol) in ethanol (50 mL) was treated with sulfuric acid (0.5 mL) and gently refluxed at the boiling point temperature overnight. The solvent was removed in vacuo, and the residue was poured into 10% sodium carbonate and extracted with ethyl acetate. The organic layer was washed with 10% sodium carbonate, water, and brine and dried over sodium sulfate. After removal of the solvent, the

resulting residue was purified using preparative HPLC and transformed into acetate. t_R = 30.97 min (system 2) MS [M + 1]: 230.0. ^{1}H NMR (500 MHz, $(CD_3)_2CO$): δ (ppm) 1.28 (t, J = 6.8 Hz, 3H), 3.77 (s, 3H), 4.19 (q, J = 6.8 Hz, 2H), 5.55 (bs, 2H), 6.55 (s, 1H), 7.69 (s, 1H).

Compound 14, 4-Amino-5-chloro-*N*-ethyl-2-methoxybenzamide Acetate. To a solution of ethylamine hydrochloride (4.0 g, 50 mmol) in water (10 mL), DCM (10 mL) was added. The aqueous phase was brought to pH 12 by adding concentrated NaOH solution. The free amine was extracted with DCM (3 × 10 mL), and the combined organic layers were washed with brine and dried over sodium sulfate. 4-Amino-5-chloro-2-methoxybenzoic acid (400 mg, 2 mmol), TBTU (650 mg), and DIEA (350 μL) were added to the DCM solution of ethylamine, and the mixture was left overnight at room temperature. After evaporation of the solvent, the residue was dissolved in ethyl acetate, washed with 10% sodium carbonate, water, and brine, and dried over sodium sulfate. After evaporation, the residue was purified by preparative HPLC and transformed into acetate. t_R = 22.73 min (system 2). MS [M + 1]: 229.0. ^{1}H NMR (500 MHz, $(CD_3)_2CO$): δ (ppm) 1.12 (t, J = 6.8 Hz, 3H), 3.32 (m, 2H), 3.9 (s, 3H), 5.39 (d, J = 9.3 Hz, 2H), 6.59 (s, 1H), 7.84 (d, J = 2.4 Hz, 1H), 7.95 (s, 1H).

Compound 15, 2-Diethylaminoethyl 4-Amino-5-chloro-2-methoxybenzoate Acetate. 4-Amino-5-chloro-2-methoxybenzoic acid (400 mg, 2.0 mmol) was dissolved in toluene (20 mL) containing *N*,*N*-diethylaminoethanol (400 μL, 30 mmol) and sulfuric acid (3 mL). The mixture was gently heated with stirring on a water bath for 1 h and then left overnight at room temperature. After the mixture was poured onto 10% sodium carbonate, the organic layer was separated, and the aqeous layer was further extracted with ethyl acetate. The combined organic phases were washed with 10% sodium carbonate, water, and brine and dried over sodium sulfate. After evaporation, the product was purified by preparative HPLC and transformed into acetate. t_R = 18.9 min (system 2). MS [M + 1]: 301.3. ^{1}H NMR (500 MHz, $(CD_3)_2CO$): δ (ppm) 1.35 (t, J = 6.8 Hz, 6H), 3.31 (m, 6H), 3.8 (s, 3H), 4.62 (t, J = 4.5 Hz, 2H), 5.85 (bs, 2H), 6.57 (s, 1H), 7.75 (s, 1H).

Compound 23, 2-Acetoxy-3-methylbenzamide. 2-Acetoxy-3-methylbenzoic Acid. Acetic anhydride (10 mL) was added to 3-methylsalicylic acid (4.0 g, 26 mmol), the mixture was stirred at room temperature for 30 min, and then sulfuric acid (0.5 mL) was added dropwise. The mixture was stirred at room temperature until the solid dissolved completely and was then heated on a boiling water bath for 10 min. Water (50 mL) was added, the mixture was stirred until the disappearance of the oily layer, and then it was heated for another 10 min and left to cool at room temperature. The homogeneous mixture was treated with saturated sodium bicarbonate solution (30−50 mL) and then acidified with 3 N HCl. After the mixture was cooled in an ice bath, a white, fluffy precipitate formed and was filtered off to give the desired product (4.0 g), which was used in the next step without further purification. t_R = 5.98 min (system 1). MS [M + 1]: 195.0.

2-Acetoxy-3-methylbenzamide. To a solution of 2-acetoxy-3-methylbenzoic acid (1.0 g, 5.2 mmol) in DMF (20 mL), diisopropylcarbodiimide (0.65 g, 5.2 mmol) was added and the mixture was stirred at room temperature for 30 min and then treated with 3 mL 2 M NH_3/ethanol (0.087 g, 5.2 mmol). The reaction mixture was further stirred at room temperature for 3 h, treated afterward with saturated sodium carbonate solution (30−50 mL), and extracted with ethyl acetate. The organic phase was washed with water and brine and dried over sodium sulfate to afford, after evaporation, the desired product, which was purified by preparative HPLC. t_R = 9.155 min (system 1). MS [M + 1]: 194.0. ^{1}H NMR (500 MHz, $(CD_3)_2CO$): δ (ppm) 2.36 (s, 3H), 5.16 (q, J = 6.65 Hz, 3H), 7.27 (t, J = 7.46, 1H), 7.61 (d, J = 7.47 Hz, 1H), 7.83 (d, J = 7.6 Hz, 1H), 8.00 (bs, $-NH_2$).

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