Drug Evolution Concept in Drug Design: 2. Chimera Method[†]

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Abstract: The drug evolution method represents a novel approach towards efficient rational drug design by implementing the drug evolution concept to the creation and development of general chemical libraries with the purpose of allowing the identification of drug candidates with improved odds and lesser costs than the traditional drug design strategies. As another example of successful translation of the biological evolution into chemical evolution, the chimera method comprises the grafting of selected building blocks, identified through a basic search within a drug library, onto the same substitution sites on a rationally chosen scaffold. The method allows the creation of a library containing both drugs and prospective drug candidates without any priorly required knowledge on the pursued disease or molecular target. Two libraries having scaffolds derived from *para*-aminobenzoic acid and salicylic acid have exemplified the application of the chimera method. The validation of the method has been achieved through the high number of recognized drugs within the library, which exhibit in the same time a wide variety of therapeutic activities and interact with a broad spectrum of molecular targets. The drug-enriched chimera libraries are expected to provide a highly efficient access to novel drug candidates whose unspecified therapeutic effects should be further revealed through high-throughput screening.

Key Words: Drug library, drug evolution, chimera method, scaffold, building blocks, *para*-aminobenzoic acid.

INTRODUCTION

The combinatorial boom, the virtually infinite number of compounds that are synthetically manageable, has fascinated and challenged chemists ever since the introduction of the concept. Independent of the design of the library, the same question is omnipresent: which compounds should be produced from the huge pool of possibilities once the chemistry is confirmed and the relevant building blocks are known? The days in which compounds were generated just to fill up the company's inventories, without considering any design or filtering criteria, are long gone. In fact, most of the early combinatorial chemistry libraries have now been largely eliminated from the standard screening sets due to the disappointing results obtained after biological testing [1]. Moreover, there is a huge gap in productivity within the biopharmaceutical industry, as the output does not keep the pace with the increase in research and development spending. Although high hopes have been held for combinatorial chemistry, high throughput screening and genomics, the huge investments in these technologies have yielded so far only few products [2]. Hence, there is now a clear trend to move away from huge and diverse randomly generated combinatorial libraries towards more focused smaller drug-like sub-libraries. There are several approaches to design and generate targeted compound collections by using the mechanism of action of a biological target, the ligand motif-based library design or, often, virtual screening tools to search through chemical space for topologically similar entities using known compounds as references. Another answer to the continuous demand for lead compounds' generation is to develop novel strategies and more realistic methods that can be used to overcome some of the problems of the random chemical libraries.

The previous article in this series [3] described drug evolution as a new concept for designing and generating high quality, general chemical libraries of biologically active compounds. The drug evolution concept stems from the observation that nature develops new species through evolutionary processes starting from the already existing species. Alike nature, it would be more efficient to develop new drugs by evolving the already existing ones rather than designing them from scratch. Similar to Holland's genetic algorithm [4], the concepts of biological evolution have been translated into chemical evolution. The previous article presented the conversion of the genetic method of sexual recombination into a method in chemical evolution called hybridization. The present article illustrates the adaptation of chimera from biology to a similar chemical evolution notion, the chemical chimera. The mythological chimera, a fire-breathing three-headed monster with the body of a lion, the tail of a serpent, and a goat's head sprouting out of the back, is a composite creature that can be regarded as a mixture of two or more species in one body. More specifically, the biological chimera is considered an organism comprised of cell lines from a variety of sources, having more than one genetically distinct population of cells that originated from more than one zygote, and would present recognizable features acquired from both cell lines.

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† NRC Publication No. 47486.

1573-4064/06 $50.00+.00

METHODS

Chimera Method

Although they are quite rare, probably due to disruptive internal conflicts that compromise the organism's normal function, natural chimeric organisms occur, as proven by a small number of examples [5]. The fate of the transient coexistence of two different cell lines in one organism is determined by those benefits that outbalance costs. If the benefits deriving from integration of two cell lines in one organism are greater than the incumbent costs, the chimera will be viable and will express some macroscopic characteristics imprinted in the genes in both cell lines. A few general net benefits of chimerism, such as increased genetic variability, synergism, or size-specific attributes enhancing fitness [6] have been identified in the case of cellular chimeras. Nevertheless, the most outstanding example of biological chimerism is undoubtedly the eukariotic cell itself. The theory of endosymbiosis [7], explaining the formation of eukaryotic cells with organelles through the engulfment by protists of aerobes and cyanobacterias which turned over a long time into mitochondria and chloroplasts, respectively, advocates for considering the eukaryotic cell a genetic chimera descended from the association of different organisms [8]. An extrapolation of the chimera concept in biological systems can be exemplified at the molecular level by the cell-surface receptors. Tyrosine kinase receptors, while exhibiting well conserved juxtamembrane and catalytic domains, have incorporated different sequences of amino acids next to the phosphorylated tyrosine in the C-terminal tail to be distinctively recognized by the SH2 domain of the signaling proteins in order to achieve specificity, and show various combination of structural motifs in the extracellular domain that enables them to bind with high affinity to an unique growth factor [9]. Every individual association of the standard juxtamembrane and catalytic domains with the structurally variable extracellular domain and C-terminal tail which allows each of the members of tyrosine kinase receptors family to accomplish its particular role must have been the result of an evolutionary process driven by the necessity to regulate various signaling pathways [10]. Chimeric tyrosine kinase receptors having altered extracellular ligand-binding domains are useful in studies of these receptors' biological and biochemical properties [11,12], but receptor tyrosine kinases can also become potent chimeric oncoproteins that cause cellular transformation when mutated or altered structurally [13], thus emphasizing the importance of the nature of the extracellular domain to the receptor's activity. In a manner similar to the association of different extracellular domains to the invariable juxtamembrane and catalytic domains which creates various chimeric tyrosine kinase receptors, one could produce drug libraries by using a choice of building blocks to be attached to the same scaffold. Moreover, as cells generate the chimeric receptors by mixing and matching the already existing modules, the building blocks and scaffolds required in the generation of a chimeric drug library should be enlisted from such available chemical entities identified in drugs.

The practical approach in applying the notion of chemical chimerism in drug evolution is formally conceived as grafting preferred building blocks onto a chosen scaffold. The preference for particular building blocks is the key aspect of chemical chimerism in the same way the preference for certain parent compounds is central in hybridization method in drug evolution. The same premise of selecting candidates for supplying building blocks among small organic drug molecules that are easily accepted and well tolerated by the human body applies in the case of chemical chimerism as well. The examples in this paper used to illustrate the chimera method in drug evolution comprise the same scaffold, a limitation that could be perceived as a translation of biological chimerism confined to the same species, but not meant to exclude the application of chemical chimerism using different scaffolds as a version of interspecies biological chimerism. However, the odds of finding useful chemical chimeras among those generated from different scaffolds is probably lower than in the case of chemical chimeras derived from the same scaffold, very much in the same manner that interspecies chimeras are less viable than biological chimeras produced within the same species.

The building blocks identified as most suitable for being grafted on the scaffold are always placed in chimera molecules at the same site where they were spotted as being meaningful. Assuming that three building blocks have been recognized as relevant for a primary substitution site on the scaffold and that other three have been considered significant for a secondary substitution site, nine chimera molecules will be generated through the grafting of these building blocks on the scaffold, as shown in Fig. (**1**).

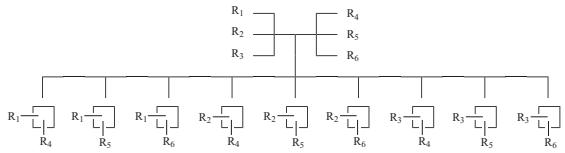


Fig. (1). Proposed schematic for grafting building blocks onto a scaffold according to the chimera.

Selection of Scaffolds

The previous article in the series described our criteria for the choice of scaffolds and emphasized our preference for *p*-aminobenzoic acid (PABA) and salicylic acid (SA) depicted in Fig. (**2**). The choice of the same scaffolds to generate general chemical libraries using the chimera method is justifiable for the reasons outlined there.

Selection of the Building Blocks

A detailed analysis of the substitution pattern in the currently in use drugs containing the chosen scaffold is required in order to establish the preferred building blocks to be grafted on the scaffold when applying the chimera method. Two criteria are to be observed in the selection of the building blocks. First, a convenient building block is one found in multiple drugs. Second, it is preferably for the identified building blocks to be encountered in drugs exhibiting various activities, because it is the functional diversity rather than the structural diversity that would give

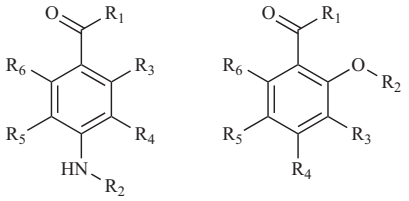


Fig. (2). *para*-Aminobenzoic acid (PABA) and salicylic acid (SA) scaffolds.

rise to a good general library that is not designed for a specified target or disease. The selection of the building blocks for the two chosen scaffolds has been performed using the Negwer database [14].

PABA Chimera Library

para-Aminobenzoic acid scaffold, defined according to Fig. (**2**) as a benzene ring *para*-disubstituted with a carbonyl group whose carbon atom has the ability to form a bond with substituent R_1 and an amino group that can be further substituted by R_2, offers multiple opportunities to be modified by incorporating frequently used building blocks [15]. Five such building blocks have been identified in PABA-containing drugs as frequently used substituents R_1 of the carbonyl group in PABA scaffold. The structure of these building blocks are shown in Fig. (**3**), together with the numbers of PABA-containing drugs sharing these building blocks and the number of therapeutic uses or activities of these drugs.

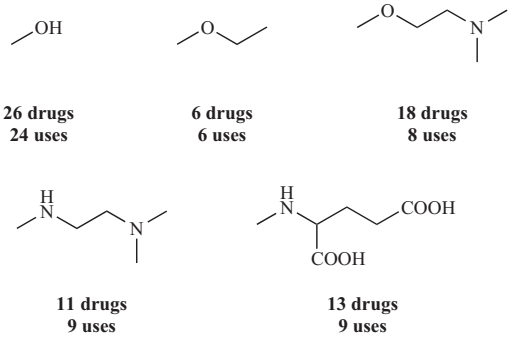


Fig. (3). Most common building blocks identified as R_1 substituents of PABA scaffold in PABA-containing drugs and the count of these drugs' therapeutic applications.

Similarly, three building blocks have been identified as frequently used substituents of the amino group in PABA-containing drugs. Fig. (**4**) presents the structure of these building blocks, the numbers of PABA-containing drugs sharing these building blocks, and the numbers of therapeutic uses or activities of these drugs.

Three aromatic substitution patterns have been identified as frequently used building blocks of the aromatic ring in PABA-containing drugs. These building blocks are shown in Fig. (**5**), together with the numbers of PABA-containing drugs sharing these building blocks and the numbers of therapeutic uses or activities of these drugs.

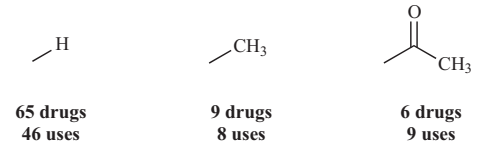


Fig. (4). Most common building blocks identified as R_2 substituents of PABA scaffold in PABA-containing drugs and the count of these drugs' therapeutic applications.

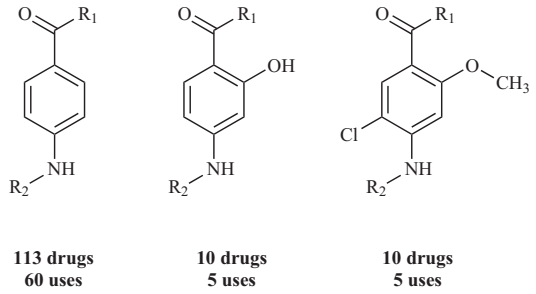


Fig. (5). Most common building blocks identified as ring substituents of PABA scaffold in PABA-containing drugs and the count of these drugs' therapeutic applications.

The combination of these three groups of building blocks generates 45 compounds, out of which a number of 10 are drugs presenting 24 therapeutic uses or activities. As this 22% drug density in the library is high enough, and the number of therapeutic uses or activities is satisfactorily high, the library containing these 45 PABA derivatives represents a "hot spot".

SA Chimera Library

Salicylic acid makes another excellent example as a scaffold that can be further modified by incorporating building blocks frequently identified in SA-containing drugs or biologically active compounds. This scaffold's benzene ring is *ortho*-disubstituted with a carbonyl group whose carbon atom can be further substituted with R_1, and a hydroxyl group that can adopt a substituent R_2. A thorough search using Negwer's database led to ten frequently used building blocks. A first group comprises three of these building blocks as substituent R_1 of the carbonyl group in SA scaffold, a second group consists of two substituents R_2 of the hydroxyl group, a third group including three building blocks is formed by the substituent R_3 *ortho* to the hydroxyl moiety, and the fourth group counting two building blocks refers to the substituent R_5 *para* to the hydroxyl moiety, as illustrated in Fig. (**6**).

The combination of these building blocks generates a library of 36 ($3 \times 2 \times 3 \times 2$) compounds, out of which a number of 15 compounds, highlighted in Fig. (**7**), are known drugs (42% of drug density) having 24 therapeutic uses or activities. Again, the therapeutic uses or biological activities

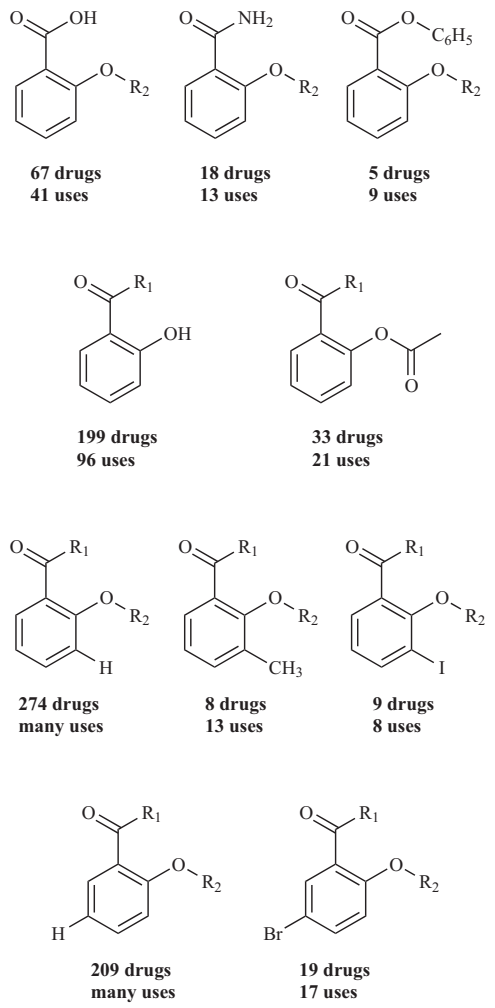


Fig. (6). Frequent building blocks in SA-containing drugs and the number of these drugs' therapeutic uses.

that may be identified among the remaining 21 compounds of this group are not predictable.

Validation of the Chimera Library

The design of chemical chimeras using the described algorithm can be carried out for any group of drugs. An attempt to generate chemical chimeras using over 10,000 drugs would result in a practically infinite number of molecules, but only a limited number of them can be developed into drugs. Similar to a library created by employing the hybridization method, a chimera-containing library would be a successful one if includes drug(s) or drug candidate(s), irrespective of the fact that they are known or novel. Because an estimation regarding the drug density in a newly generated chimera library cannot be obtained a priori, the library's content should be searched for known drugs or drug candidates after its design had been achieved. If the library contains a high number of known drugs or drug candidates, one can consider this library as enriched with drugs or drug candidates, it can be validated as a "hot spot" library. Furthermore, a "hot spot" library bears a high probability to harbor more drug candidate(s) within the remaining molecules. Moreover, if the therapeutic applications or biological activities of the known drugs or drug candidates in the chimera "hot spot" library are diverse, it is expected that the remnant molecules in the library also exhibit a wide range of therapeutic applications or biological activities, and the "hot spot" chimera library can serve as a general chemical library for drug discovery. If the therapeutic applications only focus on particular applications, the "hot spot" chimera library can serve as a library focused on certain areas of therapeutic applications.

RESULTS AND DISCUSSIONS

PABA Chimera Library

Application of the chimera concept to generate a library having *para*-aminobenzoic acid as a scaffold and using eleven building blocks as structural elements to be grafted onto the scaffold afforded a 45-membered library given in Fig. (**8**). The results of the literature search conducted with the view to reveal the therapeutic or biological activities of these compounds are presented below for each compound.

4-Aminobenzoic acid (PABA) (1) is an essential growth factor for many species of bacteria, which use it as a precursor in the biosynthesis of folic acid. Plants [16], fungi, and parasites such as *Plasmodium falciparum* [17] also use PABA as an intermediate in the same biosynthetic pathway, which makes PABA a perfect target for the development of antibacterial and antifungal agents [18]. PABA was the most commonly used sunscreen agent after WWII [19], but its use declined due to dermatological side effects such as contact or photocontact dermatitis [20,21], inhibition of enzymatic formation of melanin precursors and increase of melanin formation by increasing non-enzymatic oxidation of DOPA [22], or DNA damage after UV irradiation [23]. These side effects could be diminished if the contact of skin with PABA-containing formulations is minimized by immobilizing the sunscreen agent on a neutral support such as cyclodextrins [24,25] or mica [26]. PABA has also been long known as an antirickettsial agent potentially useful for the treatment of several types of typhus [27-29]. Tromboxane B_2 production is inhibited by PABA, apparently due to this compound's interference with the mobilization and/or utilization of intracellular Ca^{2+} [30]. Several hitherto unknown or less investigated properties of PABA, such as capacity of inducing endogenous interferon, antioxidant, anticoagulant, fibrinolytic, or immunomodulating activities, have been recently summarized in a review [31].

Ethyl 4-aminobenzoate (benzocaine) (2) is a local anesthetic that blocks neuronal impulses propagation or generation by inhibiting Na^+ current [32,33] through binding to individual voltage-gated Na^+ channels [34] and either occluding ion flow or promoting channel inactivation [35]. The location of the binding site for local anestetics [36] allowed the development of benzocaine into a target-specific drug which dinamically interacts with a distinct molecular site on the Na^+ channel protein [37]. Benzocaine's mechanism

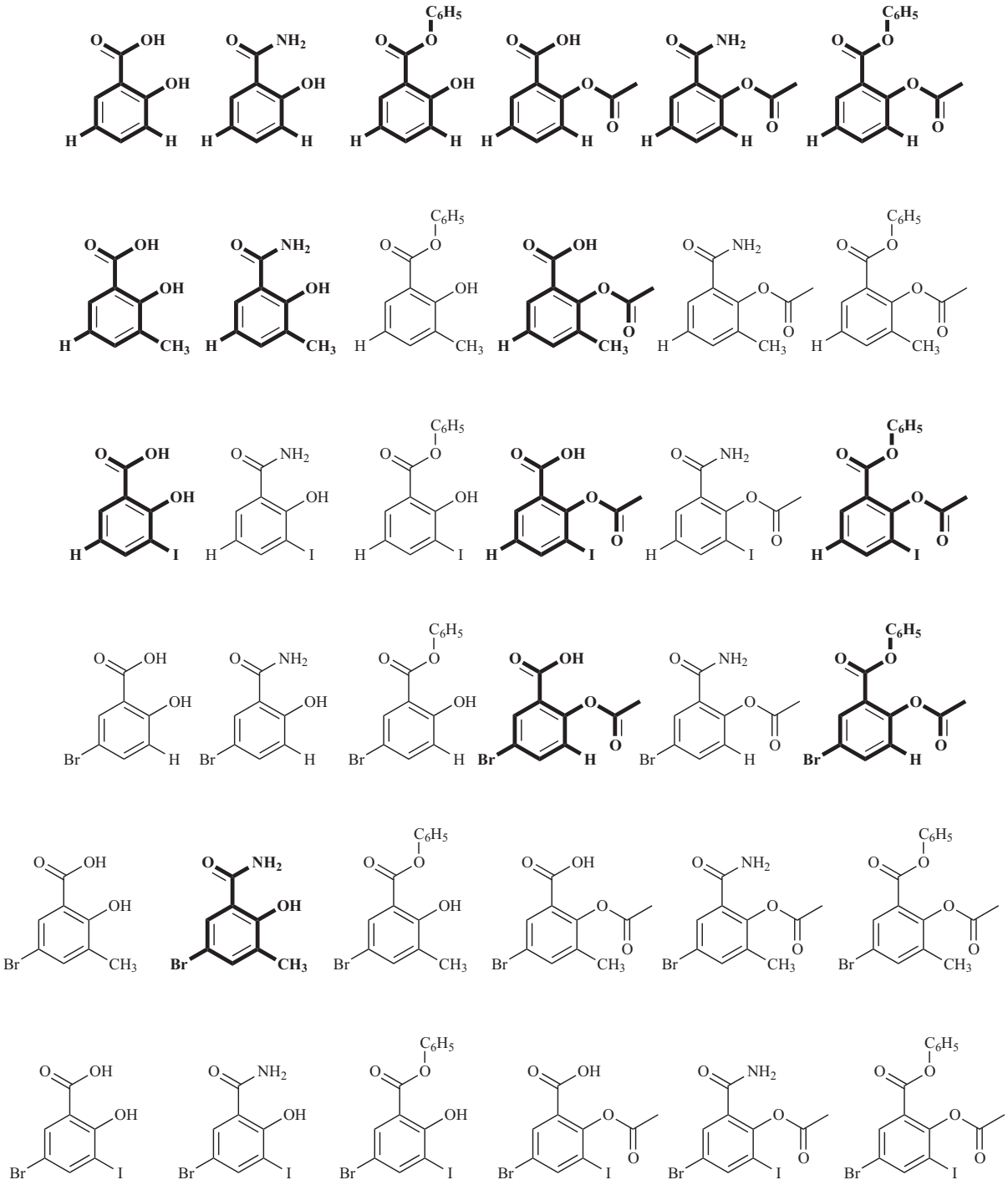
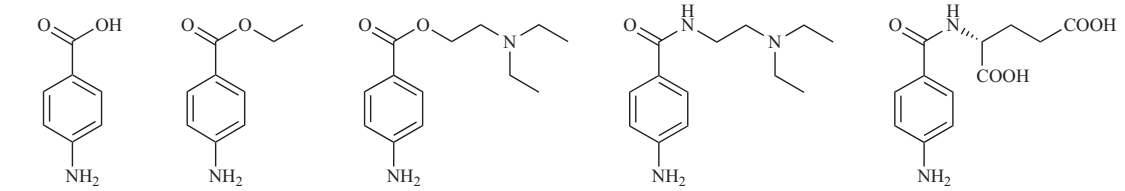


Fig. (7). SA chimera drug library.

of action may extend beyond the classical blockade of Na^+ channels as it was also shown to bind to NK1 (substance P) tachykinin receptor [38], an important transmiter/modulator in nociception. The electron-donating properties of benzocaine and other local anesthetics [39] correlate well with their Na^+ channel blocking activities, substantiating a



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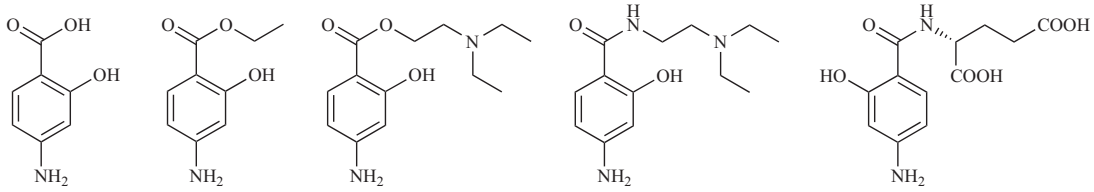

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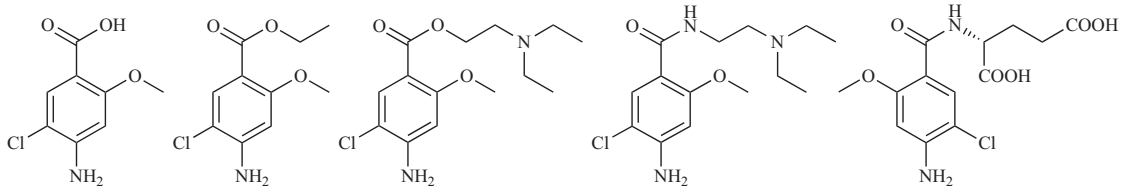

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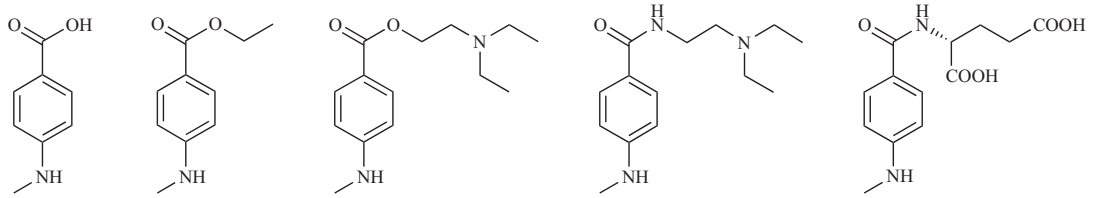

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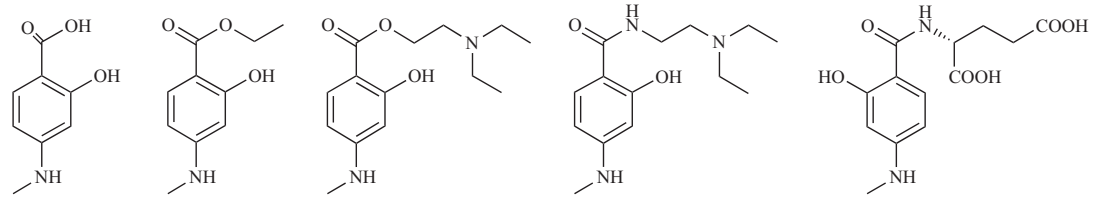

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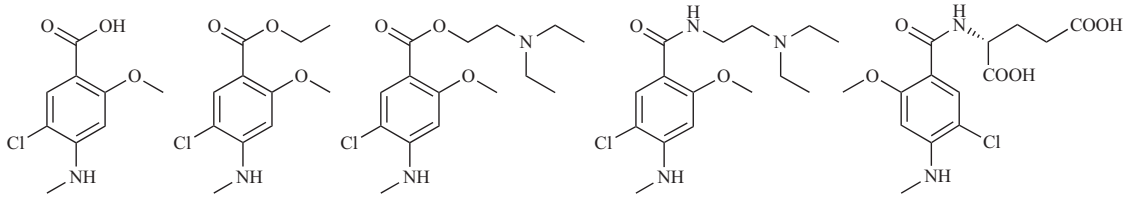

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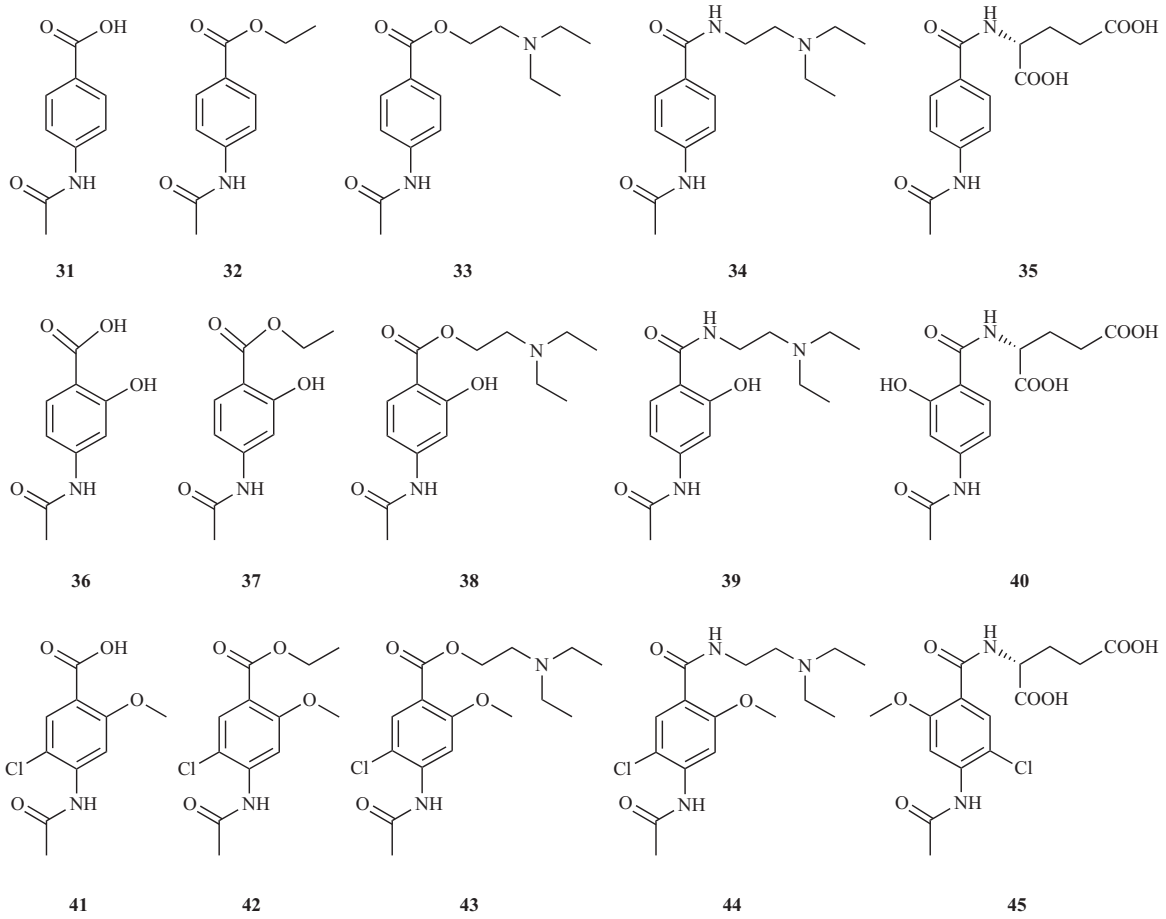

Fig. (8). PABA chimera drug library.

proposed redox model of ion channels [40]. Furthermore, benzocaine inhibits several G-protein–coupled receptors, such as thromboxane A_2 receptor functioning [41], muscarinic m1 [42] and m3 receptors [43] acetylcholine signaling, or lysophosphatidic acid signaling [44]. Several other ion channels are also blocked by benzocaine, such as K^+ channels [45] or Ca^{2+} channels [46]. Benzocaine-containing formulations were bactericidal or fungicidal towards six species of microorganisms commonly found within the oral cavity [46], and inhibited the growth of *Campylobacter pylori* [48]. By acting upon nerve endings of the papillae of the tongue, benzocaine reduces the ability to detect different degrees of sweetness [49], and has been used for treating obesity by altering taste [50]. Benzocaine was shown to inhibit platelet aggregation induced by adenosine phosphate – an effect that increases after its incapsulation into liposomes [51]. At molecular level, benzocaine affects ion-pumping enzymes Na,K-ATPase [52] and Ca^{2+}-ATPase [53], inhibits platelet-aggregation primed polymorphonuclear neutrophil [54], liver cholesterol esterase [55], acyl-CoA-cholesterol acyltransferase [56], and lecithin-cholesterol acyltransferase [57].

2-Diethylaminoethyl 4-aminobenzoate (procaine) (3) also belongs to local anesthetics which act as potent blocker of Na^+ currents [58], exhibiting less effect on voltage-dependent K^+ currents in peripheral nerve [59]. Its inhibitory action towards voltage-dependent Ca^{2+} channels in smooth muscle cells [60] and rat sensory neurons [61] has also been proven. Procaine restores membrane potential after the ischemic insult [62], and protects neurons from ischemia through suppression of the direct-current potential shift and inhibition of both the release of Ca^{2+} from the Ca^{2+} intra-cellular stores and the influx of Ca^{2+} from the extracellular space [63], while being less neurotoxic than other local anesthetics [64]. Regarded as a less toxic compound useful in cancer therapy addressing DNA methylation [65], procaine causes global DNA hypomethylation, demethylation and re-expression of a CpG-island-associated gene (*RARβ2*), and growth inhibition in breast cancer cells [66]. Its complex with cisplatin is an antitumour agent able to induce growth inhibition and trigger apoptosis in tumors [67], having in the same time a considerably lower toxicity than cisplatin itself [68,69]. Procaine inhibits platelet activating factor-induced platelet aggregation [70], possibly through changes in

erythrocyte shape [71]. Its long known antimicrobial property [72-74] has been exploited for the development of an injectable form of procaine penicillin useful for the treatment of sexually transmitted diseases gonorrhea and syphilis [75,76]. Retrosternal administration of procaine in severe forms of bronchial asthma was found to improve the respiration parameters, hemodynamics, and blood gas composition [77]. Procaine affects adhesion, phagocytosis, and the production of superoxide anion and hydrogen peroxide in neutrophils [78] through inhibition of phospholipase D activation [79]. The observed sensitization to hyperthermia and induction of heat shock proteins in cells induced by procaine can be explained in part by the increased destabilization of transmembrane domain of the band III protein of the red blood cell and the transmembrane domain of the Ca^{2+}-ATPase [80]. Moreover, procaine also affects the activity of Na,K-ATPase [81], an effect that can account for its antisickling properties [82]. Reports of procaine binding to dopamine [83], serotonin [84], muscarinic [85], nicotinic acetylcholine [86], opioid [87], and *N*-methyl-*D*-aspartate [88] receptors are also available.

4-Amino-*N*-(2-(diethylamino)ethyl)benzamide (procainamide) (4) is a class Ia antiarrhythmic drug used for short-term treatment of ventricular tachycardia and a variety of supraventricular tachycardias, primarily atrial flutter and atrial fibrillation [89,90]. It acts as a sodium channel blocker that modulates inward Na^+ current [91,92], also showing an influence on the delayed rectifier outward and ATP-sensitive K^+ currents [93], HERG K^+ channels [94], and Ca^{2+} channels [95]. Procainamide has been proposed as a non-nucleoside inhibitor of DNA methylation that causes global DNA hypomethylation and restores expression of the detoxifier gene GSTP1 in prostate cancer cells in which it has been silenced by hypermethylation [96], induces demethylation and re-expression of the *ER, RARβ,* and *p16* genes [97], and decreases the tamoxifen resistance by inducing over-expression of the estrogen receptor beta in breast cancer patients [98]. Moreover, it is chemoprotective against cisplatin and diminishes the weight loss subsequent to treatment [99], reduces cisplatin hepatotoxicity [100] and nephrotoxicity [101] by forming a complex, and modulates cisplatin antitumor activity [102]. At molecular level, procainamide binds to muscarinic receptors [103,104] leading to inhibiting the uptake of choline [105], and inhibits cardiac mitochondrial lactate dehydrogenase [106] and myocardial Na(+)-K(+)-ATPase [107]. Furthermore, procainamide inhibits sympathetic nerve activity [108], platelet aggregation and thromboxane B2 production [109], and inhibits NADPH-dependent lipid peroxidation while scavenging hydroxyl radicals [110].

(*S*)-2-(4-Aminobenzoylamino)pentanedioic acid (5) is a folate catabolite [111] arising from the cleavage of the C^9-N^{10} bond [112] and excreted as its *N*-acetylated derivative (35), which results from the action of arylamine *N*-acetyltransferases (NAT) on compound (5) as substrate [113]. 4-Aminobenzoyl-L-glutamic acid is therefore used for the preparation of folic acid [114] and other antifolates [115]. Compound (5) is a high affinity inhibitor of cell-free dihydropteroate synthesizing system of *Escherichia coli* [116], and affects the sodium-dependent L-[3H]glutamate transport activity in brain [117], but it is not listed as a drug in Negwer database.

4-Amino-2-hydroxybenzoic acid (*para*-aminosalicylic acid, PAS) (6) is the earliest known bacteriostatic anti-tuberculosis drug, but its usage slowly diminished as more easily tolerated antibiotics became available [118]. The lack of resistance of even highly drug-resistant strains of *Mycobacterium* to PAS [119] have prompted its reemergence in therapy as one of the most important second line antimycobacterial agent for the treatment of multi-drug resistant tuberculosis [120]. The mechanism of action of PAS as antimycobacterial is supposed to rely either on its ability to act as a folate antagonist [121], or on the inhibition of mycobactin synthesis [122]. PAS potentiates the activity of isoniazid and streptomycin [123], and is usually recommended to be used in combination chemotherapy [124,125]. Its efficiency can be enhanced through receptor-mediated delivery as a conjugate with maleylated bovine serum albumin [126], direct lung delivery by aerosol particles [127], or by using a twice-daily regimen of Paser, a PAS granule formulation [128]. PAS proved as efficient as its isomer, 5-aminosalicylic acid, in the treatment of inflammatory bowel diseases [129], lacking in the same time the adverse side effects of the latter [130]. Different types of colitis can be remedied both by oral [131] and rectal [132] administration of PAS, and even a colonic delivery of the drug [133] has been developed for patients who do not tolerate enemas. The ability of PAS to scavenge superoxide radicals [134] is likely to play a role in its efficiency in treating inflammatory bowel diseases [135]. Moreover, PAS is able to trap HOCl produced by activated neutrophils [136].

Ethyl 4-amino-2-hydroxybenzoate (7) was claimed as being effective in improving the condition of patients with psoriasis [137] or in sunscreen compositions for protection against UV light [138], and was investigated with respect of its antituberculostatic activity [139], but it is not listed as a drug in Negwer database.

2-Diethylaminoethyl 4-amino-2-hydroxybenzoate (hydroxyprocaine) (8) is a local anesthetic [140] exhibiting antibacterial properties [141] which have recommended its use in combination with penicillin in the therapy of tuberculosis [142] and leprosy [143]. Pascaine, a 1 : 1 combination of hydroxyprocaine with PAS, shows anti-arrhythmic effects [144].

4-Amino-*N*-(2-(diethylamino)ethyl)-2-hydroxybenzamide (9) has been presented in a patent describing a series of benzamides [145], but no biological activity has been reported for it.

(*S*)-2-(4-Amino-2-hydroxybenzoylamino)pentanedioic acid (10) is novel.

4-Amino-5-chloro-2-methoxybenzoic acid (11) is a starting material for the preparation of stomachic metoclopramide (14), peristaltic stimulant cisapride, antiemetic clebopride, gastrokinetic renzapride, and smooth muscle relaxant SDZ 205-557 (13), but it is not listed as a drug in Negwer database.

Ethyl 4-amino-5-chloro-2-methoxybenzoate (12) is mentioned in several patents as intermediate in the synthesis of pharmacologically relevant amides derived from 4-amino-

5-chloro-2-methoxybenzoic acid, but no biological activity has been reported for it.

2-Diethylaminoethyl 4-amino-5-chloro-2-methoxybenzoate (SDZ 205-557) (13) is presented as a potent and selective 5-HT_4 serotonin receptor antagonist in isolated guinea pig ileum [146], which makes compound (13) a practical tool for the characterization of this receptor [147] or its involvement in biological events such as regulation of cognitive processes [148]. However, it should be mentioned that antagonism of 5-HT_3 receptor has also been reported for this compound [149]. SDZ 205-557 antagonizes cocaine-induced hyperactivity [150] and modifies the disinhibitory profile of diazepam [151], the latter effect supporting its use in the treatment and/or prophylaxis of anxiety [152]. Visceral and cutaneous nociception was mediated by SDZ 205-557's action on 5-HT_4 receptors [153]. Furthermore, SDZ 205-557 was claimed useful in the treatment of urinary incontinence [154] and gastrointestinal disorders associated with upper gut motility and/or emesis [155], and deemed to be able to overcome gastrointestinal effects of serotonin reuptake inhibitors [156].

4-Amino-5-chloro-*N*-(2-(diethylamino)ethyl)-2-methoxybenzamide (metoclopramide) (14) is a stimulant of the motility of the upper gastrointestinal tract and blocks emesis, which makes it useful in the control of nausea and vomiting [157], the prevention of radiotherapy-induced emesis [158], and especially delayed high-dose cisplatin-induced emesis [159]. Due to metoclopramide's prokinetic actions in esophagus, it is also employed for the management of gastroesophageal reflux disease [160]. The pharmacological effects of metoclopramide are believed to be due to a combination of a relatively weak serotonin 5-HT_3 and dopamine D_2 receptors antagonism and serotonin 5-HT_4 agonism [161]. Metoclopramide is the preferable therapy for migraines in emergency departments [162]; given alone [163] or in combination with aspirin [164] during a migraine attack, metoclopramide improves the adsorption of orally administered antimigraine drugs, thereby increasing speed of headache relief, and reduces nausea and vomiting triggered by pain directly [165]. A pain-relieving effect of metoclopramide was recorded even in the treatment of ureteral colic [166]. As an inducer of DNA strand break and inhibitor of DNA repair [167], metoclopramide is a radiosensitizer which potentiates radiation-induced cytotoxicity [168] in its acidic formulation Primperan® or better as a novel neutral formulation Neu-sensamide [169]. Moreover, metoclopramide also acts a chemosensitizer that modulates the antitumor effects of cisplatin [170] presumably by increased formation of cisplatin-DNA adducts [171]. The mechanisms underlying the cytotoxic effects of meclopramide also include the induction of apoptosis [172] *via* the inhibition of NFκB activation in murine 70Z/3 cells [173], or through activation of the caspase cascade *via* the mitochondrial pathway and arrest of cells in the G_2/M phase [174]. The inhibition of NFκB activation [175], which in turn inhibits TNFα, is also the foundation for the newly discovered anti-inflammatory properties of metoclopramide [176].

(*S*)-2-(4-Amino-5-chloro-2-methoxybenzoylamino)pentanedioic acid (15) has not been previously reported in the literature.

4-(Methylamino)benzoic acid (16) is a starting material in the preparation of *p*-(methylamino)benzoyl-L-glutamic acid (20) [177] and served for the synthesis of potentially cytotoxic aminomethylpteridines [178]. *para*-(Methylamino)-benzoic acid provided 100% protection against α-amanitin poisoning [179], and protected against convulsions and mortality and against pulmonary lesions caused by hyperbaric oxygen in mice [180]. Recently, compound (16) has been involved in the synthesis of a γ-glutamyl tripeptide containing an internally quenched fluorophore as a substrate for recombinant rat γ-glutamyl hydrolase [181], in the large scale production of the κ-opioid receptor agonist CJ-15,161 [182], in the preparation of antitussive 4-hydroxypiperidine derivatives [183], or the synthesis of a NO-releasing prodrug as an antitumor agent [184].

Ethyl 4-(methylamino)benzoate (17) was also involved in the synthesis of antitumor and antifolate analogues of methotrexate [185,186], but no biological activity has been reported for it.

2-Diethylaminoethyl 4-(methylamino)benzoate (18) is a *N*-alkylated procaine analogue having a 3.5-fold local anesthetic potency compared to the parent compound [187]. Its effect on the glucose transport in human erythrocite [188] and eel electroplax and cobra venom acetylcholinesterase [189] have been investigated, but it is not listed as a drug in Negwer database.

***N*-(2-(Diethylamino)ethyl)-4-(methylamino)benzamide** (19) was not found in the up-to-date literature.

(*S*)-2-(4-(Methylamino)benzoylamino)pentanedioic acid (20) is a starting material for the preparation of methotrexate [190] or its analogs [191,192], or an intermediate in the synthesis of a tripeptidic substrate for γ-glutamyl hydrolase [181], but no biological activity has been reported for it.

2-Hydroxy-4-(methylamino)benzoic acid (21) seems to have no reported biological activity.

Ethyl 2-hydroxy-4-(methylamino)benzoate (22), **2-diethylaminoethyl 2-hydroxy-4-(methylamino)benzoate** (23), ***N*-(2-(diethylamino)ethyl)-2-hydroxy-4-(methylamino) benzamide** (24), and **(*S*)-2-(2-hydroxy-4-(methylamino) benzoylamino)pentanedioic acid** (25) are all novel compounds.

5-Chloro-2-methoxy-4-(methylamino)benzoic acid (26) is a starting material for the synthesis of a series of carboxamides having a high affinity for 5-HT_3 and D_2 receptors [193], dopamine receptors [194], 5-HT_4 receptor agonists [195], or exhibiting gastric prokinetic [196], anxiolytic [197] and anti-emetic activity [198], but no biological activity has been reported for it.

Ethyl 5-chloro-2-methoxy-4-(methylamino)benzoate (27) and **2-diethylaminoethyl 5-chloro-2-methoxy-4-(methylamino)benzoate** (28) have not been previously reported.

5-Chloro-*N*-(2-(diethylamino)ethyl)-2-methoxy-4-(methylamino)benzamide (29) is mentioned in two papers investigating the dopamine receptor system in canine caudate nucleus [199,200].

(*S*)-2-(5-Chloro-2-methoxy-4-(methylamino)benzoylamino)pentanedioic acid (30) is a novel compound.

4-Acetylaminobenzoic acid (31), also known as Acedoben, is to be found as a complex containing the salt of 4-acetylaminobenzoic acid with *N*,*N*-dimethylaminopropan-2-ol and inosine in a 3 : 1 ratio in Inoprinosine (or Inosiplex, or Inosine pranobex) [201], an antiviral drug useful for retarding neurological deterioration and prolonging life in patients with slowly progressive subacute sclerosing panencephalitis [202], and for the treatment of HIV infections [203], or as an immunomodulatory agent in the therapy of other infections [204]. Isoprinosine's possible mechanism of action in AIDS patients by preventing *Pneumocystis carinii* pneumonia relies on the ability of 4-acetylaminobenzoic acid to inhibit dihydropteroate synthetase [205]. 4-Acetylaminobenzoic acid represents one major metabolic products of *para*-aminobenzoic acid [206] through acetylation by *N*-acetyltransferases [207]. Compound (31**)** was also found to inhibit platelet aggregation [208] and enhance mitogenesis of human lymphocytes [209].

Ethyl 4-acetylaminobenzoate (32) is an intermediate in the synthesis of some influenza neuraminidase inhibitors [210] and monoamine oxidase inhibitors [211], but no biological activity has been reported for it.

2-Diethylaminoethyl 4-acetylaminobenzoate (33) is a novel compound.

4-Acetylamino-*N*-(2-(diethylamino)ethyl)benzamide (acecainide) (34) is a class III antiarrhythmic agent [212] mainly through the inhibition of K^+ currents due to cardiac K^+ channels blockage [213]. On the other hand, acecainide produces relaxation of tracheal smooth muscle due to the activation of K^+ channels and ameliorates bronchoconstriction [214]. Because acecainide is formed *in vivo* as a major hepatic procainamide metabolite having a 2-3-fold plasma concentration than that of the parent compound, its accumulation may cause adverse effects [215]. Acecainide exhibits a cardiac vagolytic action related to its ability to bind to cardiac muscarinic receptors [103], and decreased the biosynthesis of both phosphatidylcholine and phosphatidylethanolamine [216].

(*S*)-2-(4-Acetylaminobenzoylamino)pentanedioic acid (35) is an urinary folate catabolite [111] together with *para*-aminobenzoylglutamic acid (5) from which is generated *in vivo* [113], but it is not listed as a drug in Negwer database.

4-Acetylamino-2-hydroxybenzoic acid (36) is the major metabolite of PAS [217] able to impair interferon-γ induced HLA-DR expression [218] through the inhibition of this modulator binding to its receptor [219]. Although 4-acetylamino-2-hydroxybenzoic acid exhibits analgesic properties similar to aspirin [220] and is able to inhibit neoplasm [221] and xanthine oxidase [222], it is not a drug.

Ethyl 4-acetylamino-2-hydroxybenzoate (37) is an intermediate in the synthesis of histamine release inhibitors [223] or antiarhythmics [224], but no biological activity has been reported for it.

2-Diethylaminoethyl 4-acetylamino-2-hydroxybenzoate (38) is a novel compound.

4-Acetylamino-*N*-(2-(diethylamino)ethyl)-2-hydroxybenzamide (39) has only been mentioned as a one of the liver metabolites of procainamide [225], but no biological activity has been reported for it.

(*S*)-2-(4-Acetylamino-2-hydroxybenzoylamino)pentanedioic acid (40) has not been reported in the literature.

4-Acetylamino-5-chloro-2-methoxybenzoic acid (41) is a reactant in the preparation of peptide inhibitors of caspase [226], K^+ channel modulators [227], gastric prokinetic agents [228], anticonvulsants [229], 5-HT_4 agonists [230], 5-HT_3 antagonists [231], or selective D_3 and/or D_4 antagonists [232], but no biological activity has been recorded for it.

Ethyl 4-acetylamino-5-chloro-2-methoxybenzoate (42), and **2-diethylaminoethyl 4-acetylamino-5-chloro-2-methoxybenzoate** (43) have not been previously reported in the literature.

4-Acetylamino-5-chloro-*N*-(2-(diethylamino)ethyl)-2-methoxybenzamide (44) is one of the *N*-substituted benzamides discovered as potent inhibitors of NFκB activation [173], but it is not listed as a drug in Negwer database.

(*S*)-2-(4-Acetylamino-5-chloro-2-methoxybenzoylamino) pentanedioic acid (45) is a novel compound.

Validation of the PABA Chimera Library

The biological activities and the reported molecular targets of the ten known drugs in the PABA chimera library are summarized in Table **1** and Table **2**, respectively.

Table 1. Therapeutic Applications and Biological Activities of the 45 Compounds in the PABA Chimera Library

therapeutic applications and biological activities	compound
analgesic	(36)
antagonizer of cocaine-induced activity	(13)
antiemetic	(14)
antiinflammatory	(14)
antimicrobial	(2, 3, 8)
antioxidant	(1)
antirickettsial	(1)
antisickling	(3)
antitumour	(3, 36)
antiviral	(31)
chemoprotection against cisplatin	(4)
cisplatin activity modulator	(4, 14)
class Ia antiarrhythmic	(4)
class III antiarrhythmic	(34)
DNA demethylation	(3, 4)

(Table 1. Contd….)

therapeutic applications and biological activities	compound
DNA hypomethylation	(3, 4)
enhancer of lymphocytes mitogenesis	(31)
fibrinolytic	(1)
gastrointestinal motility stimulant	(14)
growth promoting factor	(1)
HOCl scavenger	(6)
immunomodulator	(1)
induction of apoptosis	(14)
induction of DNA strand break	(14)
inhibition of DNA repair	(14)
inhibition of interferon production	(1)
inhibition of lipid peroxidation	(4)
inhibition of neutrophil adhesion and phagocytosis	(3)
inhibition of platelet aggregation	(1, 2, 3, 4, 31)
inhibition of superoxide and hydrogen peroxide production in neutrophils	(3)
inhibition of sympathetic nerve activity	(4)
local anesthetic	(2, 3, 8, 18)
management of gastroesophageal reflux disease	(14)
neuroprotective	(3)
nociceptive	(13)
protective against α-amanitin poisoning	(16)
protective against mortality caused by hyperbaric oxygen	(16)
radiosenzitizer	(14)
regulation of cognitive processes	(13)
sensitization of cells to hyperthermia	(3)
smooth muscle relaxant	(4, 34)
sunscreen agent	(1, 7)
superoxide scavenger	(6)
therapy of migraine	(14)
treatment of asthma	(3)
treatment of anxiety	(13)
treatment of gastrointestinal disorders	(13)
treatment of HIV infections	(31)
treatment of inflammatory bowel diseases	(6)
treatment of psoriazis	(7)
treatment of obesity	(2)
treatment of sexually transmitted diseases	(3)
treatment of subacute sclerosing panencephalitis	(31)
treatment of tachycardia	(4)
treatment of urinary incontinence	(13)
tuberculostatic	(6)
vagolytic action	(34)

Table 2. Interacting Targets Related to the 45 Compounds in the PABA Chimera Library

Targets	compound
activation of caspase	(14)
activation of phospholipase D	(3)
agonism of 5-HT_4 receptors	(14)
antagonism of D_2 receptors	(14)
antagonist of 5-HT_3 receptors	(13, 14)
antagonist of 5-HT_4 receptors	(13)
binding to Ca-ATPase	(2, 3)
binding to dopamine receptors	(3, 29)
binding to muscarinic receptors	(2, 3, 4, 34)
binding to Na,K-ATPase	(2, 3, 4)
binding to nicotinic acetylcholine receptors	(3)
binding to NK1 tachykinin receptor	(2)
binding to *N*-methyl-*D*-aspartate receptors	(3)
binding to opioid receptors	(3)
binding to serotonin receptors	(3)
Ca^{2+} channel blocker	(2, 3, 4)
inhibition of acetylcholine signaling	(2)
inhibition of acyl-CoA-cholesterol acyltransferase	(2)
inhibition of cholesterol esterase	(2)
inhibition of choline uptake	(4)
inhibition of dihydropteroate synthetase	(5, 31)
inhibition of interferon-γ binding to receptor	(36)
inhibition of lactate dehydrogenase	(4)
inhibition of lecithin-cholesterol acyltransferase	(2)
inhibition of lysophosphatidic acid signaling	(2)
inhibition of neutrophils	(2)
inhibition of NFκB activation	(14, 44)

(Table 2. Contd….)

targets	compound
inhibition of phosphatidylcholine and phosphatidylethanolamine biosynthesis	(34
inhibition of thromboxane A_2 receptor	(2)
inhibition of xanthine oxidase	(36)
K^+ channel blocker	(2, 3, 4, 34)
modulator of L-[3H]glutamate transport activity	(5)
Na^+ channel blocker	(2, 3, 4)

A good deal of the therapeutic applications, biological activities and targets listed in Tables **1** and **2** are not common to the ten acknowledged drugs, leading to the conclusion that the biological activities potentially exhibited by the remaining 35 compounds have a fair chance of being different from those recorded for the known drugs, without discounting the possibility of some presenting a few of the same pharmacological properties as these recognized ten drugs. Because no expected therapeutic applications for the 35 non-drugs can be *a priori* assumed, the only way of establishing these compounds biological activities relies on their high-throughput screening using a broad variety of assays. Regardless the inherent costs of such extensive screening, the odds of finding among these compounds a drug candidate showing an interesting novel pharmacological action or simply being an improved, more efficient and/or side effects lacking alternative of a structurally related known drug are significantly high and definitely within a range that makes the screening worth of a try. Even in the worst-case scenario, the costs associated with the screening offer a better risk-versus-reward trade-off compared to other drug discovery strategies. The front-runner drug once discovered within these 35 aspirants, its likelihood of actually becoming a drug or at least a drug candidate to be further developed through minor chemical modifications also runs high, the rich content in drugs of this particular chimera library standing witness to this assertion. Furthermore, this approach offers a technological solution to the declining productivity of the biopharmaceutical industry and a valid alternative to *de novo* drug discovery, mainly because the development time required for a novel drug discovered among these candidates is shorter due to the reduction in the time of the lead optimization and clinical stages. The identified drug candidate already possessing the essential structural features for the expression of a good pharmacological activity, the drug design required for its optimization should be minimal, and the drug candidate's structural resemblance with another well-established drug having the same scaffold (and possibly part of the drug library under investigation) may straightforwardly point towards the beneficial chemical modification. The same structural likeliness of the drug candidate with a drug presenting the same pharmacological profile as the candidate could hint that the analogy is also reproduced in the possible side effects of the drug candidate, allowing a swift identification of some of the existing safety issues even before clinical testing and, if these issues prove to be insurmountable through further drug design, preventing a commitment in time and money difficult to recover.

The chimera approach in drug discovery does not require any previous elaborate information referring to the identification and validation of a target, nor it entails specific knowledge on a targeted disease or an innovative educated hypothesis for drug design developed over the years. The available data on the currently in use drugs and a statistical study based on a virtual screening of an existing database such as Negwer are sufficient enough to produce in an inexpensive manner a general drug library prone to afford upon an efficiently conducted high-throughput assay a certain number of lead compounds to be later developed into drugs.

CONCLUSIONS

As a part of our continuing efforts to develop novel approaches for drug discovery, a second method belonging to the larger context of the drug evolution concept has been described and used to create two small-sized drug-enriched general libraries based on *para*-aminobenzoic and salicylic acid derivatives. The chimera method deeply relies on the successful identification and selection of the relevant scaffold and the corresponding appropriate building blocks to be subsequently grafted on the scaffold at the very same substitution sites they have been originally spotted in a manner formally recalling the conceiving of the ancient chimera. The choice of a good scaffold and relevant building blocks from a drug database will ensure a solid foundation for a library exhibiting a high content of drug-like molecules, as validated by the recognition of an unusually elevated number of established drugs. Due to the multiple pharmacological actions and targets retrieved from the up to date literature for the large number of drugs present in this library, one can speculate over the usefulness of the chimera method in promoting drug discovery, not through a costly *de novo* strategy, but merely by using an efficiently set-up high-throughput screening to identify the prospective candidates and the existing knowledge on the drugs in the newly created drug library.

ACKNOWLEDGMENTS

The authors are grateful to Drs. Piotr Stefanowicz and Pierre de Macedo for useful discussions. The technical assistance of Concordia co-op students David Bryant, Jonah Prevost Kirkwood, Melissa McKinney, and Benedicte Patureau is greatly acknowledged. The authors are thankful to Natural Sciences and Engineering Research Council of Canada for the financial support of this work.

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Received: 19 September, 2005 Revised: 25 October 2005 Accepted: 26 October, 2005