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Structure-Based Design and Synthesis of Novel Hybrid Molecules Derived from Anthranilic Acid as Drug Candidates †

Miglena Milusheva [1,2,*], Vera Gledacheva [3], Mihaela Stoyanova [2], Mina Todorova [2], Iliyana Stefanova [3] and Stoyanka Atanasova Nikolova [2]

[1] Department of Bioorganic Chemistry, Faculty of Pharmacy, Medical University of Plovdiv, 4002 Plovdiv, Bulgaria
[2] Department of Organic Chemistry, Faculty of Chemistry, University of Plovdiv, 4000 Plovdiv, Bulgaria; miglena.milusheva@uni-plovdiv.bg (M.S.); minatodorova@uni-plovdiv.bg (M.T.); stoyanova@uni-plovdiv.bg (S.A.N.); tanya@uni-plovdiv.bg
[3] Department of Medical Physics and Biophysics, Faculty of Pharmacy, Medical University of Plovdiv, 4002 Plovdiv, Bulgaria; vera.gledacheva@mu-plovdiv.bg (V.G.); iliyana.stefanova@mu-plovdiv.bg (I.S.)
* Correspondence: miglena.milusheva@mu-plovdiv.bg
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Abstract

Hybrid molecules, integrating multiple pharmacophores within a single scaffold, represent a modern strategy in drug discovery, offering improved selectivity and safety. Anthranilic acid is a versatile building block with diverse biological activities. Here, we designed and synthesized novel anthranilic acid-based hybrids with enhanced pharmacokinetic potential. Cheminformatics tools guided library design, followed by amide bond formation between anthranilic acid derivatives and substituted 2-phenylethylamines. Purification and structural characterization were achieved via NMR, IR, and HRMS. The compounds exhibited favorable predicted ADME/Tox profiles and synthetic accessibility. These results provide a foundation for further biological evaluation toward therapies for smooth muscle dysfunction and inflammation.

Keywords: anthranilic acid derivatives; hybrid molecule synthesis; amide bond formation; structure-based design; 2-phenylethylamines; computational chemistry

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1. Introduction

Modern drug discovery increasingly relies on molecular hybridization, a strategy in which two or more pharmacophoric elements are combined into a single scaffold (Figure 1). This approach enables modulation of multiple biological targets simultaneously, potentially yielding compounds with improved selectivity, synergistic efficacy, and reduced side effects, compared to single-target agents. Recent reviews have highlighted that hybrid molecules are especially promising in tackling complex, multifactorial diseases such as inflammation, cancer, metabolic syndrome, and neurodegeneration [1,2].

Anthranilic acid is an advantaged scaffold in medicinal chemistry, on account of its dual reactive groups (–NH_2 and –COOH) which allow for diverse derivatization. Various recent studies report anthranilic acid derivatives with potent biological activities, including anti-inflammatory, antimicrobial, anticonvulsant, enzyme inhibition, and receptor modulatory effects [3,4]. For example, novel anthranilic acid hydrazones have

shown potent inhibition of cholinesterases and α-glycosidase with favourable ADMET profiles [5]. A very recent work describes hybridization of quinoline with anthranilic acid to produce compounds with strong in vitro and in vivo anti-inflammatory effects and good drug-likeness [6].

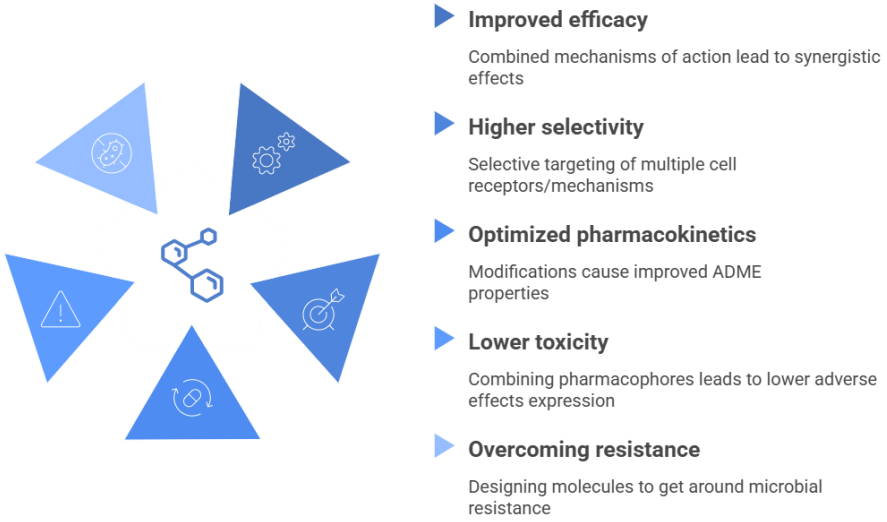


Figure 1. Advantages of hybrid molecules in novel drug design (visualized with Napkin AI).

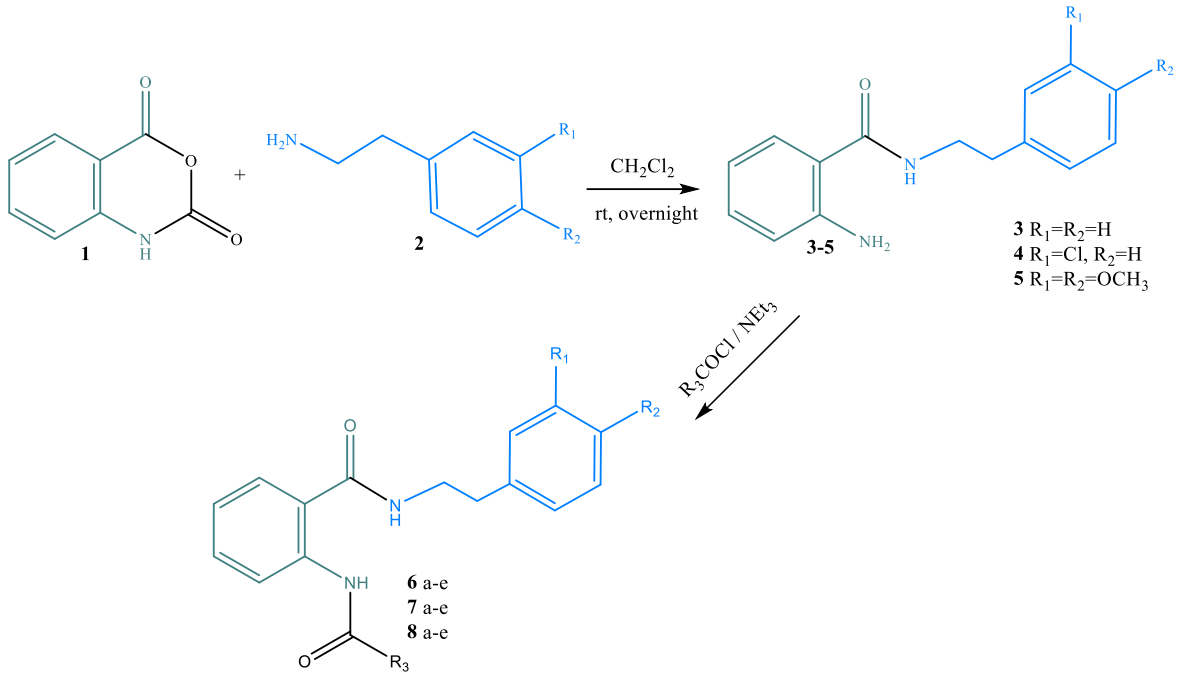
In this context, our study aims to design and synthesize novel anthranilic acid-based hybrid molecules that not only retain the broad spectrum of biological activities associated with anthranilic analogues, but also exhibit improved pharmacokinetic and pharmacodynamic properties via rational hybrid design and in silico filtering.

2. Materials and Methods

A combination of cheminformatics tools was used to guide the design of a focused library of target compounds. Publicly available software products (SwissADME [7], PASS Online [8], ProTox 3.0 [9]) were used for the in silico screening to identify the most favourable candidates for synthesis. The main criteria in this process included properties such as synthetic availability, Lipinski's rule of 5, gastrointestinal absorption, blood-brain barrier (BBB) permeability, antispasmodic activity, and toxicity.

The synthetic procedure (Scheme 1) relied on an efficient ring-opening reaction of isatoic anhydride with substituted 2-phenylethylamines at room temperature [10–14]. Obtaining the desired hybrids (**3–5**) was monitored chromatographically (TLC). The hybrids were acylated with various alkyl- and aryl-substituted acyl chlorides ($R_3 = CH_3$, C_6H_5, CH_2-C_6H_5, 2-Cl-C_6H_4, $CH(Cl)C_6H_5$), yielding the desired diamides **6–8 a–e** with high purity.

All compounds were purified and characterized using standard spectroscopic techniques, including NMR, IR, and HRMS, and melting point temperature determination.



Scheme 1. Synthetic procedure for obtaining the desired hybrid diamides **6–8 a–e**.

3. Results and Discussion

The applied in silico approaches, including cheminformatics and ADME/Tox predictions, generated a comprehensive dataset on the physicochemical, pharmacokinetic, and toxicological profiles of the tested structures. Based on the analysis of key parameters, three phenylethylanthranilamide compounds and their diamide derivatives were identified as the most promising candidates.

Lipophilicity, a major determinant of oral bioavailability, was found to be below the critical threshold of logP = 5. This finding is consistent with Lipinski's Rule of Five, which states that orally active drugs are more likely to succeed when not violating more than one of the following criteria: molecular weight ≤ 500 Da, logP ≤ 5, ≤ 5 hydrogen bond donors, and ≤ 10 hydrogen bond acceptors [15]. Compliance with these guidelines is crucial, since a significant proportion of drug candidates fail at early stages of development due to poor ADME properties [16].

Further ADME predictions revealed high gastrointestinal absorption and blood–brain barrier (BBB) permeability. While BBB penetration is advantageous in the context of central nervous system drug discovery, particularly for neurodegenerative disorders, it also raises safety concerns due to the risk of neurotoxicity [17].

Preliminary toxicity assessment placed the compounds in toxicity class 4, with calculated LD_{50} values ranging from 1000 to 2025 mg/kg. The SwissADME tool (Figure 2) confirmed overall organ safety, yet predicted approximately 70% probability of respiratory and neurotoxicity. In this study, such findings are interpreted as potentially favorable, since BBB penetration is a desired feature for the conceptualized therapeutic application. Comparable or even higher toxicity levels have been reported for clinically used drugs in this therapeutic area, suggesting that the investigated structures remain within an acceptable safety range for further pharmacological exploration [18,19].

Application of the synthetic methodology allowed for the efficient synthesis of three hybrid molecules and fifteen diamides in total, with practical yields ranging between 78–83% [12–14].

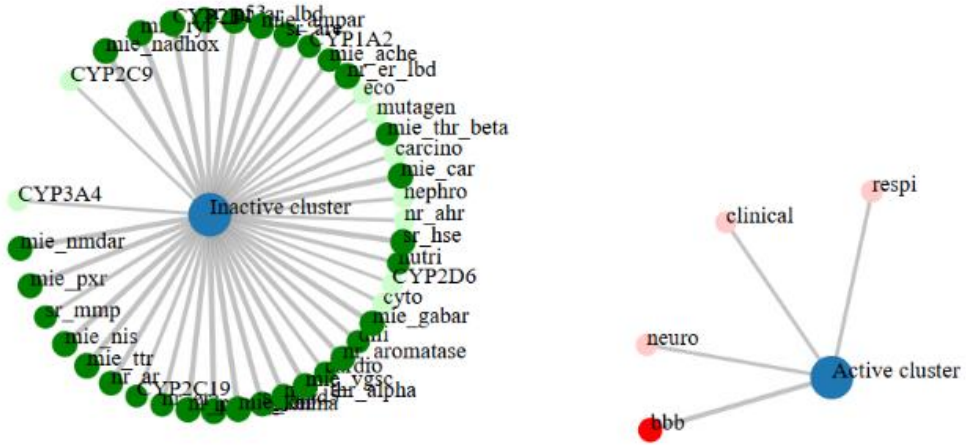


Figure 2. Toxicity calculation results for compound **6b**, obtained with SwissADME.

4. Conclusions

The resulting hybrid structures integrate multiple pharmacophores and demonstrate favorable predicted ADME properties and toxicity. The synthetic procedures were efficient and reproducible, yielding structurally confirmed compounds ready for further biological exploration.

The generated hybrid structures show promising in silico drug-likeness and synthetic accessibility. This work provides a strong foundation for developing new small molecules aimed at treating disorders involving smooth muscle dysfunction and inflammation. The biological effects of all hybrids are to be thoroughly studied as a next step of the novel drug candidates design and development project.

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Abbreviations

The following abbreviations are used in this manuscript:

ADME	Absorption, distribution, metabolism, excretion
Tox	Toxicity
BBB	Blood-brain barrier
IR	Infrared spectroscopy
NMR	Nuclear magnetic resonance spectroscopy

HRMS	High resolution mass spectrometry
TLC	Thin-layer chromatography

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