Exploration of Nitroaromatic Antibiotics via Sanger’s Reagent: Synthesis, In Silico, and Antimicrobial Evaluation

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ABSTRACT: Facile synthesis of molecular hybrids containing a 2,4-dinitrophenyl moiety was achieved via nucleophilic aromatic substitution of the fluoride anion of Sanger’s reagent (2,4-dinitrofluorobenzene) with various N, S, and O nucleophiles, considered as bioactive moieties. Antimicrobial evaluation of the new hybrids was carried out using amoxicillin and nystatin as antibacterial and antifungal reference standards, respectively. MIC test results identified the compounds 3, 4, and 7 as the most active hybrids against standard strains and multidrug-resistant strains (MDR) of *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. Most of the hybrids displayed two times the antibacterial activity of AMOX against MDR *Pseudomonas aeruginosa*, *E. coli*, and a standard strain of *P. aeruginosa* (ATCC 29853), while demonstrating a weak antifungal profile against *Candida albicans*. Selectivity profiles of the promising compounds 3, 4, 6, 7, 8, and 11 on WI-38 human cells were characterized, which indicated that compound 3 is the safest one (CC₅₀ 343.72 µM). The preferential anti-Gram-negative activity of our compounds led us to do docking studies on DNA gyrase B. Docking revealed that the potential antimicrobial compounds fit well into the active site of DNA gyrase B. Furthermore, in silico absorption, distribution, metabolism, and excretion (ADME) predictions revealed that most of the new compounds have high gastrointestinal absorption and a good oral bioavailability with no BBB permeability.

1. INTRODUCTION

Contagious diseases generally occur in tropical and subtropical communities. The virulence of such diseases is competent to multiply in human hosts, justifying their continuous survival. Microbes are progressively modifying and provoking resistant strains to contemporary clinical medicines. The developing antibiotic resistance crisis, due to drug-resistant Gram-positive and Gram-negative bacteria, constitutes urgency for the evolution of improved medications to cure these infections.¹

Nitroaromatic compounds having one or more nitro functionalities directly bonded to an aromatic group exhibit biological assets, permitting their clinical usage as antibiotics, for instance, metronidazole I, niclosamide II, chloramphenicol III, and tinidazole IV, as shown in Figure 1.²⁻⁵

Nitroaromatic antibiotics necessitate reductive bioactivation via metabolism, converting the NO₂ group to nitric oxide (NO) or reactive nitrogen species (RNS) for antimicrobial activity. This is carried out using nitroreductases (NTRs), which are found almost exclusively in bacteria and certain eukaryotes but not found in humans.⁶ Therefore, nitroreductases could be used to catalyze the release of antibiotic sites specifically in humans, concentrating the active drug in the place of an infection. The compound would only deliver the medicine in large amounts if an infection grows, averting the overuse of antibiotics, addressing the issue of bacteria developing antibiotic resistance, and potentially reducing or eliminating side effects from antibiotics.⁷

In spite of the antibacterial and antiparasitic properties of nitroaromatic antibiotics, drugs having nitro groups frequently evince mutagenicity and intolerable toxicity profiles, which have hampered further progress of this drug group. The potent therapeutic action of these compounds drives the search for nonmutagenic and selectively toxic nitroaromatic antibiotics that kill infectious organisms without causing harm to the host cells.³⁻⁸

Antimicrobial resistance turned out to be a remarkable threat to worldwide public health, hence bringing about fundamental demands for new drugs with better therapeutic effectiveness. In this matter, molecular hybridization is based on the combination of pharmacophoric moieties of different bioactive substances to produce a new hybrid compound with improved affinity and efficacy. In this study, we report a library of molecular hybrids composed of 2,4-dinitrobenzene attached directly to various

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privileged scaffolds that confer desirable therapeutic potentials, e.g., imidazole,10 piperazine,11 methyl thiouracil,12 substituted quinoxalinone,13 or attached via an O/S linker to phenol,14 8-hydroxyquinoline,15−17 or benzo[d]thiazole.18 Minimum inhibition concentrations (MICs, μg/mL) of the nitroaromatic series were assessed against both multidrug resistant strains (MDR) and standard strains of Gram-positive (Staphylococcus aureus ATCC 25923) and Gram-negative (Escherichia coli ATCC 25922) and (Pseudomonas aeruginosa ATCC 29853) bacterial strains and a clinical strain of Candida albicans.

Furthermore, molecular docking was performed to understand the binding and interactions of the promising hybrids inside the active site of the target enzyme. Sanger’s reagent (1-fluoro-2,4-dinitrobenzene) was used to predict the sequence of the N-terminal of peptides by Sanger in 1945.19 Sanger’s reagent is a good reagent to synthesize smooth hybrids of organic molecules containing dinitrophenyl moieties, via nucleophilic aromatic substitution for the fluoride anion under mild conditions.20

2. RESULTS AND DISCUSSION

2.1. Chemistry. The target hybrids 3−11 were synthesized in good yields via nucleophilic aromatic substitution of fluoride11−23 of the starting material 2,4-dinitrofluorobenzene with various N, S, and O nucleophiles 1a−i, namely, (3-(3-oxo-3,4-dihydroquinolin-2-yl)-1-phenyl-1H-pyrazol-5-yl)-methylacetate (1a), 3-methyquinolin-2(1H)-one (1b), 3-benzylquinolin-2(1H)-one (1c), benzo[d]thiazole-2-thiol (1d), 1-methyl-2-thiouracil (1e), 8-hydroxyquinoline (1f), 2,6-di-tert-butylphenol (1g), piperazine (1h), and imidazole (1i), under reflux in acetone as an aprotic solvent in the presence of anhydrous K2CO3 to enhance nucleophilic aromatic substitutions, as shown in Scheme 1. The structures of 2,4-dinitrophenyl derivatives 3−11 were established based on their spectral data.1 H NMR spectra of 3−11 showed aromatic proton signals in the following range of δH: 8.99−6.83 ppm. The aliphatic protons of 3−5 of Ar-CH2/CH3 appeared at a δH of

Figure 1. Structures of FDA-approved antibiotic drugs containing NO2 groups.

Scheme 1. Synthesis of Dinitroaromatic Derivatives 3−11 via Sanger’s Reagent

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Table 1. Antimicrobial Activities of New Compounds (MIC μg/mL)

| compds No. | S. aureus (ATCC 25923) | S. aureus clinical | E. coli ATCC 25922 | E. coli MDR | P. aeruginosa (ATCC 29853) | P. aeruginosa MDR | fungi MIC |
|------------|-------------------------|-------------------|-------------------|-------------|---------------------------|-----------------|------------|
| 3          | >500                    | 500               | >500              | 64.5        | 125                       | 125             | >500       |
| 4          | 32.25                   | 16.125            | 125               | 125         | 125                       | 125             | 125        |
| 5          | >500                    | 500               | >500              | >500        | 125                       | 125             | >500       |
| 6          | >500                    | 500               | 500               | >500        | >500                      | 125             | 125        |
| 7          | 64.5                    | 32.25             | 64.5              | 125         | 125                       | 125             | 125        |
| 8          | 125                     | 125               | 125               | >500        | >500                      | >500            | >500       |
| 9          | >500                    | 500               | >500              | >500        | >500                      | 125             | >500       |
| 10         | >500                    | >500              | >500              | >500        | >500                      | >500            | >500       |
| 11         | 125                     | 125               | 125               | 125         | 125                       | 125             | 125        |
| AMOX       | ≤7.813                  | ≤7.813            | 62.5              | 250         | >500                      | >500            | <2         |

Table 2. Cytotoxicity Data (IC50 μM) of Promising Hits

| compd no. | 3 | 4 | 7 | 8 | 11 |
|-----------|---|---|---|---|----|
| CC50 (μM) | 343.72 ± 12.76 | 61.22 ± 1.98 | 120.69 ± 4.05 | 243.05 ± 8.43 | 213.28 ± 6.74 |

5.24, 2.02, and 4.47 ppm respectively. 1H NMR of 9 and 10 showed symmetry where the di-tert-butyl protons in 9 appeared as a singlet signal equivalent for 18 protons at a δ of 1.34 ppm and also a singlet signal at a δ of 3.30 ppm equivalent four symmetrical CH2 groups of the piparazine moiety of compound 10 equivalent for 8 protons. 13C NMR spectra of compounds 3–5 showed the carbonyl of quinolinate at δC values ranging at 135.4, 154.4, and 153.9 ppm, respectively. The di-tert-butyl carbons of 9 were detected at a δC of 34.6 and 30.1 ppm corresponding to quaternary carbon and methyl carbons, respectively.

2.2. Biological Evaluation. 2.2.1. MIC Determination against Various Bacteria. The antimicrobial activity of the newly synthesized compounds was performed by microbroth dilution assay used for the determination of minimum inhibitory concentrations (MICs) according to the CLSI reference standards. In this study, the MIC test was applied to assess the antibacterial properties of the target compounds 3–11 toward standard strains of the Gram-negative bacteria E. coli (ATCC 25922) and P. aeruginosa (ATCC 29853) and Gram-positive bacteria S. aureus (ATCC 25923), in addition to clinical strains of Gram-negative bacteria including E. coli and P. aeruginosa and Gram-positive bacteria S. aureus; also, antifungal activity against a clinical isolate of C. albicans using amoxicillin (AMOX) and nystatin (NYS) as antibacterial and antifungal reference standards, respectively, is evaluated as shown in Table 1.

2.2.2. SAR Study. Exceptionally, 3 exhibited a promising antibacterial result (MIC 64.5 μg/mL) against the multidrug resistant strain of E. coli, which represents four times the activity of AMOX (MIC 250 μg/mL). In addition, the antimicrobial study revealed that the hybrid of 1-(2,4-dinitrophenyl)-3-methylquinoxaline-2(1H)-one (4) showed at least fourfold (MIC 125 μg/mL) the antimicrobial activity of AMOX (MIC > 500 μg/mL) against both standard and clinical strains of P. aeruginosa. Moreover, it showed twofold (MIC 125 μg/mL) the antimicrobial activity of AMOX (MIC 250 μg/mL) against MDR E. coli. Also, it demonstrated less than or equal to half potency (MIC 16.125 μg/mL) of AMOX (MIC ≤ 7.8125 μg/mL) against the resistant Gram-positive strain of S. aureus and half potency (MIC 125 μg/mL) of AMOX (MIC 62.5 μg/mL) against the standard strain of E. coli. Noticeably, the other quinoline hybrids 3 and 5 showed similar enhanced MIC to 4 toward standard and clinical strains of P. aeruginosa.

Concerning the hybrid with 2-((2,4-dinitrophenyl)thio)-1-methylpyrimidin-4(1H)-one (7), it elicited approximately (MIC 64.5 μg/mL) the same MIC of AMOX (MIC 62.5 μg/mL) toward the standard strain of E. coli; what is more is that it elicited double the activity of AMOX against MDR E. coli and at least fourfold the activity of AMOX against both standard and MDR strains of P. aeruginosa. This hybrid was active against the clinical strain of Candida; it showed a moderate activity (MIC 16.125 μg/mL) compared to that of the standard antifungal drug NYS.

The hybrids 2,4-dinitrophenyl-imidazole (11) and 8-(2,4-dinitrophenoxy)quinoline (8) are equipotent toward all tested microorganisms (MIC 125 μg/mL); they displayed twofold the antibacterial activity of AMOX (MIC 250 μg/mL) against MDR E. coli and showed at least fourfold the potency of AMOX (MIC > 500 μg/mL) against both MDR and a standard strain of P. aeruginosa (ATCC 29853). A similar activity profile toward MDR E. coli and both MDR and a standard strain (ATCC 29853) of P. aeruginosa was noticed for 9. Among all screened compounds, 2-[(2,4-dinitrophenyl)thio]benzo[d]thiazole (6) and bis(2,4-dinitrophenoxy)piperazine (10) exhibited low antibacterial activities, as shown in Table 1.

2.2.3. In Vitro Cytotoxicity Screening Against the Human Cell Line WI-38. In order to evaluate the selective toxicity of the most active compounds 3, 4, 7, 8, and 11, they were tested against the human diploid lung fibroblast cell line WI-38 using MTT assay, as shown in Table 2 and Figure 2. Interestingly, compound 3 possessed a remarkable nontoxic effect on these normal WI-38 cells (CC50 343.72 μM). Hence, this compound is further evaluated by in vitro assays as a DNA gyrase inhibitor.

2.3. Enzyme Assessment of DNA Gyrase. Compound 3 is selected, being the safest compound, for further evaluation against the bacterial DNA gyrase enzyme.

2.3.1. DNA Gyrase Supercolling Assay. Compound 3 was screened for its ability to inhibit DNA gyrase supercoiling (using E. coli DNA gyrase), using TopoGEN DNA gyrase assay kit protocol TG1003. Ciprofloxacin and novobiocin are used as

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reference standards. The results revealed that it inhibited DNA gyrase supercoiling at the micromolar level, \( \text{IC}_{50} 0.869 \ \mu\text{M} \), which is more than that of novobiocin (\( \text{IC}_{50} 1.23 \ \mu\text{M} \)); however, it displayed lower inhibitory activity than that of ciprofloxacin (\( \text{IC}_{50} 0.282 \ \mu\text{M} \)), as shown in Table 3.

2.3.2. DNA Gyrase ATPase Assay. Compound 3 was further tested in DNA gyrase ATPase assay using a commercially available \( E. \text{coli} \) DNA Gyrase ATPase assay Kit (inspiralis).28 It

![Figure 2](image.png)

Figure 2. Cytotoxic effect of compounds 3 (A), 4 (B), 7 (C), 8 (D), and 11 (E) on the human cell line WI-38.

| compound  | \( E. \text{coli} \) DNA gyrase \( \text{IC}_{50} \) (\( \mu\text{M} \)) |
|-----------|---------------------------------|
| 3         | 0.16 ± 0.007 0.869 ± 0.046      |
| ciprofloxacin | 0.282 ± 0.015            |
| novobiocin  | 0.09 ± 0.004 1.23 ± 0.04      |

Table 3. DNA Gyrase Assays for Compound 3
Figure 3. continued
Figure 3. Proposed three-dimensional (3D) and two-dimensional (2D) binding modes of compounds 3 (A), 4 (B), 7 (C), 8 (D), 11 (E), and ligand (F).
inhibited the ATPase activity of DNA gyrase (IC\textsubscript{50} 0.16 µM) approximately in the same range of the novobiocin reference standard (IC\textsubscript{50} 0.09 µM), as shown in Table 3.

2.4. Molecular Docking Study. In recent decades, the DNA gyrase enzyme has been identified as one of the most verified and researched targets for the generation of novel antibacterial medicines.\textsuperscript{29,30} The critical importance of DNA gyrase in bacterial survival and absence in higher eukaryotes make such an enzyme an appropriate target for developing new therapeutics in terms of selective toxicity.\textsuperscript{31}

In this study, the preferential activity of our compounds toward Gram-negative bacteria routes us to further explore of their binding interactions by docking into the active site of DNA gyrase B.

A docking study was conducted using MOE 2014.09 software to show the possible interactions between our novel promising compounds and bacterial DNA gyrase B. Here, our target compounds 3, 4, 7, 8, and 11 were docked to the ATP binding site of \textit{E. coli} DNA gyrase B (PDB code: 4DUH), as shown in Figure 3A–E, respectively. The outcome of our docking study is illustrated in Table 4 and Figures 3 and 4. The studied compounds were found to interact with the ATP active site, with binding energies from −5.378 to −6.803 kcal/mol. Compound 4 revealed the best docking score of −6.803 kcal/mol, while the ligand exhibited a docking score of −6.712 kcal/mol. Redocking of the cocrystallized ligand revealed an RMSD value of 0.7068 Å, indicating the validity of the used docking protocol. It showed H-bond interactions with the important amino acids Arg76, Gly101, and Arg136, as shown in Figure 3F.

Interestingly, in all the docked compounds 3, 4, 7, 8, and 11, the 2,4-dinitrophenyl motif was buried in a hydrophobic cavity lined with Arg76, Ile78, and Pro79 residues. Also, one of the nitro groups of these derivatives developed hydrogen bond interactions with the crucial residue Arg136, which is known to be a critical amino acid for all ATPase inhibitors binding.\textsuperscript{35} Concerning the 2,4-dinitrophenyl-3-methylquinoxalin-2-one (3) and 4, another hydrogen bond interaction was observed between the quinoxaline-2-one oxygen atom and Arg76. Additionally, Gly102 was involved in the Pi-H interaction with the quinoxaline moiety of compound 4. In the case of the pyrimidine derivative 7, the dinitrophenyl moiety reacted with the active site through H-bonds with Arg136 and Pi-H interactions with Lys103. The pyrimidine ring of compound 7 was also incorporated in the binding to the active site through forming Pi-H interactions with Gly102. In the imidazole derivative 11, the 2,4-dinitrophenyl moiety displayed the same binding pattern in which one of the nitro groups formed H-bonds with the essential Arg136 residue, while the second nitro group acted as a hydrogen bond acceptor for Lys103. Furthermore, the phenyl ring was involved in the Pi-H interaction with Lys103. On the other hand, the binding to Arg136 was the only observed H-bond interaction in the case of compound 8.

2.5. In Silico Physicochemical and Pharmacokinetic Property Prediction. Swiss ADME was used to assess the physicochemical and pharmacokinetic characteristics of the promising compounds 3, 4, 7, 8, and 11.\textsuperscript{36,37} As shown in Table 5, compounds 4, 7, 8, and 11 displayed no violations for Lipinski’s rule (Log P ≤ 5, number of hydrogen bond donors ≤ 5, M\textsubscript{W} ≤ 500, and number of hydrogen bond acceptors ≤ 10), while compound 3 revealed only one violation (M\textsubscript{W} > 500). High-topological polar surface areas were observed for all compounds (TPSA = 109.46–170.65 Å\textsuperscript{2}). All of the compounds have 3–8 rotatable bonds, suggesting structural flexibility to their biological target.

Concerning the pharmacokinetic properties of the screened compounds as shown in Table 6, it was noticed that all compounds have no BBB permeation, and consequently, they are not predictable to show CNS side effects. In addition, low gastrointestinal absorption was observed in the case of compounds 3 and 7, while compounds 4, 8, and 11 displayed high GI absorption ability.

The most important element determining absorption is bioavailability that is a measure of the amount of chemical in the plasma. All of the tested compounds have high scores of bioavailability of 0.55 except compound 3 that showed a bioavailability score of 0.17. Swiss ADME also performed pan-assay interference compound screening and found no alerts for any of the compounds.

3. CONCLUSIONS

A series of hybrids containing 2,4-dinitrophenyl moieties were prepared via nucleophilic aromatic substitution of the fluoride anion of Sanger’s reagent by various N, S, and O nucleophiles.
and the ecytotoxicity test proved the safety of this promising hit. has acceptable pharmacokinetics and drug likeness properties, assays proved its inhibitory activity toward DNA gyrase. Compounds (fl)

Melting points were recorded using a Thermo Scientific perfromed using a Flash 2000 organic elemental analyzer. no: 1002D, 220

band proton decoupling at 125 MHz. All chemical shifts (δ) were recorded using the UDEFT pulse sequence and broad IR (KBr) Spectra were used as an internal deuterium lock. 13C NMR spectra were recorded using a Perkin Elmer; νmax (cm−1) data were recorded using a Perkin Elmer; νmax (cm−1)

Overall, these results present compound 3 as a promising scaffold on which other molecules can be modeled to deliver new antimicrobial agents with improved potency safety and pharmacokinetic properties. The docking analysis unveiled the capability of the promising hybrid to well-accommodate the ATP binding site of E. coli DNA gyrase B. Moreover, in vitro assays proved its inhibitory activity toward DNA gyrase enzymes. ADME calculations indicated that this compound has acceptable pharmacokinetics and drug likeness properties, and the cytotoxicity test proved the safety of this promising hit.

4. EXPERIMENTAL SECTION

All reactions were carried out in dried glassware. NMR spectra were measured using a JEOLJNM ECA 500. The deuterated solvent was used as an internal deuterium lock. 13C NMR spectra were recorded using the UDEFT pulse sequence and broad band proton decoupling at 125 MHz. All chemical shifts (δ) are stated in units of parts per million (ppm) and presented using TMS as the standard reference point. CHN analyses were performed using a Flash 2000 organic elemental analyzer. Melting points were recorded using a Thermo Scientific, model no: 1002D, 220−240 °C; 200 W; 50/60 °C and are uncorrected. IR (KBr) νmax (cm−1) data were recorded using a Perkin Elmer; Fourier transform infrared (FT-IR) Spectrum BX; and Bruker tensor 37 FT-IR. Reaction time was monitored by TLC on Merck silica gel aluminum cards (0.2 mm thickness) with a fluorescent indicator at 254 nm. Visualization of the TLC during monitoring of the reaction was done using a UV VILBER LOURMAT 4 w-365 or 254 nm tube. 4.1. Chemistry. 4.1.1. General Method for the Synthesis of Compounds (3−11). To a solution of aromatic nucleophiles Ar−i (1.0 mmol) in dry acetone (20 mL), anhydrous potassium carbonate (2.0 mmol) was added; then, the reaction mixture was stirred under reflux for 20 min. Sanger’s reagent (1.2 mmol) was added,38 and the mixture was heated under reflux for the reported time; the reaction progress was monitored with TLC. After reaction completion, the mixture was cooled and then poured onto cold water (100 mL); the formed precipitate of the desired product was filtered, washed with water, dried, and recrystallized from ethanol to give the desired products 3−11. 4.1.2. 1-N-(2,4-Dinitro phenyl)-3-[5-(acetoxymethyl)-1-Phenyl pyrazol-3-yl]quinolin-2-one (3). Yellowish-white powder (0.43 g, 82%); Rf = 0.75 (EToAc/n-hexane 1:1); m.p = 210−212 °C; IR(KBr) νmax (cm−1): 3163, 3108, 3057, 1738, 1682, 1604, 1536, 1491, 1445; 1H NMR (500 MHz, dimethyl sulfoxide (DMSO-d6)) δH: 8.99 (d, J = 3.5 Hz, 1H, Ar-H); 8.73 (dd, J = 11.5 Hz, J = 3.5 Hz, 1H, Ar-H); 8.18−8.22 (m, 1H, Ar-H); 8.07 (d, J = 11.5 Hz, 1H, Ar-H); 7.78−7.84 (m, 2H, Ar-H); 7.49−7.73 (m, 7H, Ar-H); 5.24 (s, 2H, −CH2); 2.02 (s, 3H, CO−CH3) Figure S1; 13C NMR (125 MHz, DMSO-d6) δC: 170.1, 153.4, 150.4, 146.9, 144.8, 142.1, 139.9, 139.7, 139.3, 138.5, 131.6, 130.3, 129.9, 129.6, 129.2, 128.9, 127.3, 126.9, 125.4, 125.1, 121.1, 119.6, 56.2, 20.6, Figure S2; Anal. calcd for C18H13N3O2: C, 59.32; H, 3.45; N, 15.96; Found C, 59.4; H, 3.58; N, 15.86.

4.1.3. 1-(2,4-Dinitrophenyl)-3-methylquinolin-2(1H)-one (5). Colorless crystals (0.28 g, 85%); Rf = 0.675 (EToAc/n-hexane 1:1); m.p = 128−130 °C; IR(KBr) νmax (cm−1): 3102, 3032, 1607, 1533, 1504, 1479; 1H NMR (500 MHz, DMSO-d6) δH: 8.92 (d, J = 3.5 Hz, 1H, Ar-H); 8.70 (dd, J = 9.0 Hz, J = 2.5 Hz, 1H, Ar-H); 8.0 (d, J = 9.0 Hz, 2H, Ar-H); 7.64−7.70 (m, 2H, Ar-H); 7.59 (dd, J = 8.0 Hz, J = 1.5 Hz, 1H, Ar-H); 2.74 (s, 3H, −CH3) Figure S3; 13C NMR (125 MHz, DMSO-d6) δC: 154.4, 149.7, 147.3, 144.5, 141.8, 139.5, 137.9, 130.1, 129.9, 128.5, 128.0, 127.0, 126.8, 121.8, 20.2, Figure S4; Anal. calcd for C12H12N2O: C, 55.22; H, 3.09; N, 17.17; Found C, 55.43; H, 3.15; N, 17.25.

4.1.4. 3-Benzyl-1-(2,4-dinitrophenyl)quinoxalin-2(1H)-one (11). Pale-yellow powder (0.26 g, 84%)).

Table 5. In Silico Prediction of Physicochemical Properties of the Compounds 3, 4, 7, 8, and 11

| comp no. | GIT absorption | BBB permeability | bioavailability score | PAINS alert | Lipinski’s violation | TPSA | no. of rotatable bonds |
|----------|----------------|------------------|-----------------------|------------|---------------------|------|----------------------|
| 3        | low            | no               | 0.17                  | 0          | 1                   | 170.65 | 8                    |
| 4        | high           | no               | 0.55                  | 0          | 0                   | 126.53 | 3                    |
| 7        | low            | no               | 0.55                  | 0          | 0                   | 151.83 | 4                    |
| 8        | high           | no               | 0.55                  | 0          | 1                   | 113.76 | 4                    |
| 11       | high           | no               | 0.55                  | 0          | 1                   | 109.46 | 3                    |

Table 6. In Silico Prediction of Pharmacokinetic Properties of the Compounds 3, 4, 7, 8, and 11

| comp no. | GIT absorption | BBB permeability | bioavailability score | PAINS alert | bioavailability | TPSA | Permeability | score | alert |
|----------|----------------|------------------|-----------------------|------------|-----------------|------|--------------|-------|-------|
| 3        | low            | no               | 0.17                  | 0          | 126.53          | 3    | 0.75         | 0.75  | 3     |
| 4        | high           | no               | 0.55                  | 0          | 151.83          | 4    | 0.75         | 0.75  | 4     |
| 7        | low            | no               | 0.55                  | 0          | 113.76          | 4    | 0.75         | 0.75  | 4     |
| 8        | high           | no               | 0.55                  | 0          | 109.46          | 3    | 0.75         | 0.75  | 3     |
| 11       | high           | no               | 0.55                  | 0          | 109.46          | 3    | 0.75         | 0.75  | 3     |
8.64 (m, 2H, Ar-H); 7.88 (d, J = 8.5 Hz, 1H, Ar-H); 7.07 (d, J = 6 Hz, 1H, Ar-H); 2.24 (s, 3H, –CH3), Figure S9; 13C NMR (125 MHz, DMSO-d6) δ C: 171.5, 166.6, 160.4, 148.5, 144.8, 141.8, 129.8, 126.8, 121.6, 103.8, 13.4, Figure S10; Anal. calcd for C11H8N4O5S: C, 42.86; H, 2.68; N, 13.77.

8.61 (d, J = 2.5 Hz, 1H, Ar-H); 8.43 (dd, J = 2.5 Hz, 1H, Ar-H); 7.82 (dd, J = 7 Hz, 1H, Ar-H); 7.74 (t, J = 7.5 Hz, 1H, Ar-H); 7.62 (q, J = 13 Hz, 1H, Ar-H); 6.83 (d, J = 10 Hz, 1H, Ar-H), Figure S11; 13C NMR (125 MHz, DMSO-d6) δ C: 156.2, 151.1, 148.1, 140.9, 139.7, 138.1, 136.7, 129.9, 129.5, 127.1, 127.0, 122.6, 121.9, 121.6, 118.4, Figure S12; Anal. calcd for C15H14N6O6: C, 45.94; H, 3.37; N, 20.09; Found C, 46.11; H, 2.68; N, 18.02.

8.64 (m, 2H, Ar-H); 7.88 (d, J = 8 Hz, 1H, Ar-H); 7.49 (s, 1H, Ar-H); 7.11 (s, 1H, Ar-H); 7.08 (s, 1H, Ar-H); 7.03 (t, J = 2.25 Hz, 1H, Ar-H); 7.02 (d, J = 2 Hz, 1H, Ar-H); 6.87 (d, J = 2.25 Hz, 1H, Ar-H); 6.85 (d, J = 8 Hz, 1H, Ar-H); 7.82 (d, J = 7 Hz, 1H, Ar-H); 7.74 (t, J = 7.5 Hz, 1H, Ar-H); 7.62 (q, J = 13 Hz, 1H, Ar-H); 6.83 (d, J = 10 Hz, 1H, Ar-H), Figure S11; 13C NMR (125 MHz, DMSO-d6) δ C: 155.5, 148.8, 145.9, 141.0, 139.7, 132.9, 126.6, 125.6, 124.4, 119.4, 34.6, 30.1, Figure S14; Anal. calcd for C20H24N2O5: C, 64.50; H, 6.50; N, 7.52; Found C, 64.71; H, 6.68; N, 7.80.

8.15 (d, J = 2.5 Hz, 1H, Ar-H); 8.12 (d, J = 8 Hz, 1H, Ar-H); 7.43 (s, 1H, Ar-H); 7.11 (s, 1H, Ar-H); 1.34 (s, 18H, t-Bu-H), Figure S13; 13C NMR (125 MHz, DMSO-d6) δ C: 155.5, 148.8, 145.9, 141.0, 139.7, 132.9, 126.6, 125.6, 124.4, 119.4, 34.6, 30.1, Figure S14; Anal. calcd for C20H24N2O5: C, 64.50; H, 6.50; N, 7.52; Found C, 64.71; H, 6.68; N, 7.80.

8.15 (d, J = 2.5 Hz, 1H, Ar-H); 8.12 (d, J = 8 Hz, 1H, Ar-H); 7.43 (s, 1H, Ar-H); 7.11 (s, 1H, Ar-H); 1.34 (s, 18H, t-Bu-H), Figure S13; 13C NMR (125 MHz, DMSO-d6) δ C: 155.5, 148.8, 145.9, 141.0, 139.7, 132.9, 126.6, 125.6, 124.4, 119.4, 34.6, 30.1, Figure S14; Anal. calcd for C20H24N2O5: C, 64.50; H, 6.50; N, 7.52; Found C, 64.71; H, 6.68; N, 7.80.

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Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.1c06383.

Copy of 1H NMR and 13C NMR of all synthesized compounds (PDF)

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Notes

The authors declare no competing financial interest.

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