Regioselective One-Pot Synthesis, Biological Activity and Molecular Docking Studies of Novel Conjugates N-(p-Aryltriazolyl)-1,5-benzodiazepin-2-ones as Potent Antibacterial and Antifungal Agents

Asma Nsira 1, Hasan Mtiraoui 1, Sami Chniti 1,*, Hanan Al-Ghulikah 2,*, Rafik Gharbi 3 and Moncef Msaddek 1

1 Laboratory of Heterocyclic Chemistry Natural Products and Reactivity/CHPNR, Department of Chemistry, Faculty of Science of Monastir, University of Monastir, Monastir 5000, Tunisia; asma_nsira@yahoo.fr (A.N.); mtiraoui1hasan@gmail.com (H.M.); samichniti@yahoo.fr (S.C.); moncefmsadek@gmail.com (M.M.)
2 Department of Chemistry, College of Sciences, Princess Nourah bint Abdulrahman University, P.O. Box 84428, Riyadh 11671, Saudi Arabia
3 Laboratory of Applied Chemistry and Environment, Department of Chemistry, Faculty of Science of Monastir, University of Monastir, Monastir 5000, Tunisia; raf.gharbi@yahoo.fr

* Correspondence: haalghulikah@pnu.edu.sa; Tel.: +966-11823-6011

Abstract: Novel 1,2,3-triazolo-linked-1,5-benzodiazepinones were designed and synthesized via a Cu(I)-catalyzed 1,3-dipolar alkyne-azide coupling reaction (CuAAC). The chemical structures of these compounds were confirmed by 1H NMR, 13C NMR, HMBC, HRMS, and elemental analysis. The compounds were screened for their in vitro antibacterial and antifungal activities. Several compounds exhibited good to moderate activities compared to those of established standard drugs. Furthermore, the binding interactions of these active analogs were confirmed through molecular docking.

Keywords: 1,5-benzodiazepin-2-ones; azides; click chemistry; CuAAC; N-triazolo-benzodiazepinones; antibacterial activity; antifungal activity; docking

1. Introduction

The development of new therapeutic agents is one of the major goals in medicinal chemistry research [1]. Generally, evidence that agents are modulating more than one target may develop a wider field of therapeutic applications compared to single-target drugs [2,3]. Hence, the actual increase in interest in agent discovery is already addressing multiple biological targets for many therapeutic treatments [4,5].

One of the privileged structures that have been recently updated by Patchett et al. [6,7] is the 1,5-benzodiazepine (BZD) derivatives that have been repeatedly reported to display tranquilizing, muscular relaxant, anticonvulsant, hypnotic, and sedative effects [8–10]. Actually, the use of this class of scaffolds is not only limited to anxiety and stress conditions but also seemingly minor changes in their structures that can produce a host of different biological activities [11]. Accordingly, polycyclic BZD derivatives A, B, and C have proven their bioactivity against peptides hormone (A), interleukin converting enzymes (B), and potassium blockers (C) [12–14] (Figure 1).

Moreover, in previous work, our research group has reported the production and subsequent determination of photoluminescence properties of an understudied family of 1,5-benzodiazepin-2-one derivatives. Furthermore, the recent work published by Chiraz Ismail et al. reports on the synthesis of some fluorescent N-triazolo-1,5-benzodiazepine-2-ones [15,16].
Figure 1. Polycyclic BZD derivatives A, B, and C.

Because the $N$-functionalization of benzodiazepines is highly desired for the development of novel powerful molecular targets [17], it appears that the $N$-1,2,3-triazolo-1,5-benzodiazepine scaffold has great importance due to the remarkable biological relevance of such combination [18,19]. Triazoles belong to an important class of heterocycles. They display an ample spectrum of biological activities and are widely employed as pharmaceuticals and agrochemicals [20–22]. More particularly, the 1,2,3-triazole derivatives that exhibit favorable physicochemical properties interact with different biological targets through hydrogen bonding and dipole interactions, improving both the potency and specificity of the resulting analogs [23,24].

Thus, and as a continuation of our ongoing research to synthesize novel 1,5-benzodiazepine derivatives bearing a triazole moiety [25], we turn our attention to designing novel hybrid conjugates of 1,2,3-triazoles tethered to 1,5-benzodiazepines namely the $N$-triazolo-1,5-benzodiazepin-2-ones. On the other hand, the click chemistry methodology is one of the most used strategies for simple access to these compounds, particularly the Cu(I)-catalyzed 1,3-dipolar alkyne-azide coupling reaction (CuAAC) [26]. In addition, derivatives $4a−i$ and $6a−c$ were evaluated for their antibacterial and antifungal potentials, and further molecular docking of synthesized compound 6 was also performed.

2. Results and Discussion

Our synthetic strategy for building the $N$-triazolo-1,5-benzodiazepine scaffolds is based on the CuAAC reaction and involves the preparation of $N$-alkynic benzodiazepine $2a−c$ reacted with aromatic azides $3a−d$. Thus, the key intermediate BZD 2 was primarily prepared following the method of E. Latteman et al. [27,28]. We treated compound $1a−c$ with propargyl bromide in the presence of sodium hydride as a base in THF. Eventually, DMF was found to be especially effective in this reaction for weakening the bromine-carbon bond [29]. Under these experimental conditions, the reaction monitored by TLC showed the formation of a single product that was identified, based on its spectral data, as the $N$-prop-2-yn-1,5-benzodiazepin-2-one $2a−c$. Note here that compound $2a$ has already been prepared by our research team [13]. In addition, H. Ahabchane and co-workers have prepared BZD derivatives but are limited in substitution patterns [30].

The $^1$H NMR spectrum of compound $2a−c$ recorded at 300 MHz in CDCl$_3$ exhibited characteristic signals from which chemical shifts and multiplicities we were able to assign the propargyl group. Thus, for compound $2b$, taken as an example, the spectrum showed a doublet at 4.25 ppm ($J = 4.80$ Hz) corresponding to the methylene group at C-1″ coupled with the acetylenic proton H-3″ which appears as a triplet at 2.30 ppm ($J = 4.80$ Hz).

Taking notes that the propargylation of the benzodiazepine could obviously occur either on the hydroxyl or on the amide function [31], the presence of the deshielded phenolic hydrogen singlet observed at $\sim$13.95 ppm excluded from the beginning the formation of the $O$-prop-2-yn-1,5-benzodiazepin-2-one (Scheme 1). Particularly in $2b$, the non-equivalence of the methylene protons H-3 (a pair of two doublets at 3.00 ppm ($J = 12.6$ Hz) and 4.70 ppm($J = 16.8$ Hz)) is undoubtedly consistent with partial non-planarity of the hep-
tatomic ring. Similarly, this result is also cited in our previously described N-isopropylated-1,5-benzodiazepine-2-one [32,33].

Scheme 1. Synthesis of alkynes 2a–c.

The reluctance of the hydroxyl group to react was rationalized in terms of a steric hindrance due to a strong intermolecular hydrogen bonding between the hydroxyl group (13.95 ppm) and the nitrogen of the imine C=N functionality at the 5-position of the diazepine ring [34].

The analysis of the $^{13}$C NMR spectra recorded at 75.47 MHz came comforting the obtention of the N-propargyl-1,5-benzodiazepinone 2b that showed a peak at 37.1 ppm (C-1″), 72.0 ppm (C-3″) as well as 78.1 ppm (C-2″) of the propargyl group.

The corresponding azides 3a–c were prepared according to the reported method via a devastation reaction of p-substituted aniline using NaN$_3$ and a diluted solution of HCl in ethanol at 0 °C followed by treatment with NaN$_3$ [35].

The coupling of azides 3a–c and the N-propargyl-1,5-benzodiazepin-2-ones 2a–c was carried out in DCM at room temperature using CuI as catalyst and triethylamine as an additive base. Very interesting pentacyclic compounds 4a–I were then isolated in suitable yields (Scheme 2). The reaction parameters were optimized using the N-propargyl-1,5-benzodiazepines 2a, the azides 3c, and the CuI as catalysts. The reaction did occur whatever the solvent used. Replacing acetonitrile with toluene increased the yields owing to a better solubility of the starting materials (entries 2 and 4). The reaction resulted in comparable yields when performed at room temperature or under gentle heating. On the other hand, an increase in the amount of the catalyst (from 5 to 10 mol%) did not modify the yield (entries 6 and 7).

Scheme 2. Copper-catalyzed click reactions of azide 3a–c with N-propargylbenzodiazepine 2a–c.

However, the excess of CuI probably caused a decrease in the performance due to the deposition of copper species on the dipole and the low solubility of cuprous iodide in triethylamine (entries 8). DCM proved to be by far the most suitable solvent at room temperature (entries 6) (Table 1)
Table 1. Optimization of Cu(I)-catalyzed 1,3-dipolar cyclization for the synthesis of 1,2,3-triazoles 4a–i.

| Entry | CuI(equiv) | Solvent | Temp (°C) | Time (h) | Additive | Yield (%) |
|-------|------------|---------|-----------|----------|----------|-----------|
| 1     | CuI(5)     | Acetonitrile | rt        | 6 h      | TEA      | 18        |
| 2     | CuI(5)     | Acetonitrile | 60        | 6 h      | TEA      | 49        |
| 3     | CuI(5)     | Toluene   | rt        | 6 h      | TEA      | 30        |
| 4     | CuI(5)     | Toluene   | 60        | 6 h      | TEA      | 68        |
| 5     | CuI(5)     | DCM       | rt        | 4 h      | -        | 40        |
| 6     | CuI(5)     | DCM       | rt        | 4 h      | TEA      | 89        |
| 7     | CuI(10)    | DCM       | rt        | 4 h      | TEA      | 92        |
| 8     | CuI(20)    | DCM       | rt        | 4 h      | TEA      | 83        |

Bold in entry highlights the optimal reaction conditions: a Alkyne 2a (1 mmol) and two equivalents of phenylazide 3e in the indicated solvent. b Referred to the starting alkyne 2a. c 2 eq were used. d Isolated yield after column chromatography based on the starting dipolarophile 2a.

Thus, one can state that the use of 1 m mole N-propargyl-1,5-benzodiazepine 2a–c, aromatic azide 3a–c (2 eq) at rt for 4 h in DCM as a solvent with CuI (5 mol%) as catalyst and triethylamine (2.5 eq) as an additive [36] are the best experimental conditions to generate the series of new N-triazolyl-1,5-benzodiazepin-2-one 4a–i in suitable yield (Scheme 2 (Table 2)).

Table 2. One-pot synthesis of 1,4-disubstituted 1,2,3-triazole 4a–i.

| Entry | Compound | R_1 | R_2 | Time (h) | Yield of 6 (%) |
|-------|----------|-----|-----|----------|----------------|
| 1     | 4a       | H   | H   | 4 h      | 87             |
| 2     | 4b       | H   | OCH_3 | 4 h     | 89             |
| 3     | 4c       | H   | NO_2 | 4 h      | 78             |
| 4     | 4d       | CH_3 | H   | 4 h      | 82             |
| 5     | 4e       | CH_3 | OCH_3 | 4 h     | 83             |
| 6     | 4f       | CH_3 | NO_2 | 4 h      | 79             |
| 7     | 4g       | Cl   | H   | 4 h      | 78             |
| 8     | 4h       | Cl   | OCH_3 | 4 h     | 81             |
| 9     | 4i       | Cl   | NO_2 | 4 h      | 77             |

In particular we have observed that the solubility of the 1,2,3-triazole-BZD conjugates 4a–i is enhanced in most of the organic solvents. This may be attributed to the new functionalities present in these novel conjugates.

Unambiguous proofs for the obtained products 4a–i were obtained from their ^1H/^13C NMR and 2D NMR spectra, which were consolidated by HRMS and elemental analysis (see Supplementary Materials).

As mentioned in the introduction, the effects of such benzodiazepines on the nervous system are abundantly described in the literature [37]. Moreover, interesting biological activities are observed with some analog derivatives, but their very low hydrosolubility can restrict their applications. Generally, when glycopyranosyl is attached to the nitrogen of the heptatonic ring systems, it can increase the water solubility and confer amphiphilic properties.

Obviously, there is no single function for oligosaccharides. Perhaps their most important function is to serve as recognition markers. Additionally, oligosaccharides have the ability to alter the intrinsic properties of the molecules to which they are attached [38].

Accordingly and encouraged by the above interesting result, we have extended this method to the synthesis of novel N-galactopyranosyl-N-triazolo-1,5-benzodiazepines.
Therefore, we screened an azido galactopyranosyl [39], a choice that was not fortuitous insofar as our research team has used it for the synthesis of some optically active pyrazolines [40–43].

As exemplified in (Scheme 3), the reaction proceeded smoothly to completion, and the corresponding N-galactopyranosyl-N-triazolo-1,5-benzodiazepinones products 6a–c were obtained after 8 h with excellent yields and with high purity (Table 3).

![Scheme 3. Copper-catalyzed reactions of galactopyranose azide 5 with N-propargyl benzodiazepine 2a–c.](image)

Table 3. One-pot synthesis of 1,4-disubstituted 1,2,3-triazoles 6a–c.

| Entry | Compound | R   | Time (h) | Yield of 6 (%) |
|-------|----------|-----|----------|----------------|
| 1     | 6a       | H   | 8 h      | 85             |
| 2     | 6b       | CH₃ | 8 h      | 84             |
| 3     | 6c       | Cl  | 8 h      | 80             |

To find the optimal experimental conditions for the reaction, the cycloaddition reaction was firstly carried out in different solvents: DCM, acetonitrile, toluene, and a mixture of DMF/H₂O at room temperature and under reflux. Finally, it was found that DMF/H₂O(8:2) was the most suitable solvent (Table 4).

Table 4. Optimization of Cu(I)-catalyzed 1,3-dipolar cyclization for the synthesis of 1,2,3-triazoles 6a a.

| Entry | CuI(equiv) b | Solvent        | Temp (°C) | Time (h) | Additive c | Yield (%) d |
|-------|-------------|----------------|-----------|----------|------------|-------------|
| 1     | CuI(5)      | Acetonitrile   | rt        | 12 h     | TEA        | 18          |
| 2     | CuI(5)      | Acetonitrile   | 60        | 12 h     | TEA        | 49          |
| 3     | CuI(5)      | DCM            | rt        | 8 h      | TEA        | 56          |
| 4     | CuI(5)      | DMF            | 60        | 12 h     | TEA        | 68          |
| 5     | CuI(5)      | DMF            | rt        | 12 h     | TEA        | 45          |
| 6     | CuI(5)      | DMF/H₂O (8:2)  | rt        | 8 h      | TEA        | 89          |
| 7     | CuI(5)      | Toluene        | rt        | 8 h      | TEA        | 17          |

Bold in entry highlights the optimal reaction conditions: a Alkyne 2a (1 mmol) and two equivalents of galactopyranose azide 5. b Referred to the starting alkyne 2a. c 2 eq were used. d Based on the initial dipolarophile, isolated yield after column chromatography 2a. e 2 eq were used.

The use of both dimensional and bidimensional NMR spectroscopy techniques allowed one to deduce unambiguously the exclusive formation of the regioisomeric species, namely the 1,4-triazoles (as exemplified for 6b).
Four singlets integrating three hydrogens each and corresponding to the methyl of the galactopyranose part appeared at 1.34, 1.36, 1.47, and 1.50. A peak integrating one proton was also observed at 7.69 ppm and assigned as the characteristic H-5"triazolic hydrogen.

A minor influence of the triazole group was observed on the proton H-1" in front of the triazole ring at C-1, which has a chemical shift of 4.25 ppm in the precursor 2b and a two doublet at 4.75 ppm and 5.09 ppm in 6b. Furthermore, a modest downfield shift was observed for the galactose H-6′′′ signal due to the influence of the triazole ring at C-4, changing from 3.55 ppm in azide5 to approximately 4.52 ppm in the triazole products for 6 series.

The resulting 1,4-regioisomers were evidenced by the presence in the NOESY spectrum of an NOE between the triazolic proton H-5" and the H-5′′′ proton of the galactopyranosyl moiety, in addition to another NOE between the proton H-5′′′ and H-6′′′. Such regiospecificity agrees with that cited in the literature [44].

To our knowledge, the obtention of these N-galactopyranosyl-N-triazolo-1,5-benzodiazepinones conjugates 6a-c is very demanded given the interesting pharmacological properties of some analogs so far reported by I. Carvalho et al. As a matter of fact, they proved to be moderate to weak TcTS (Trypanosoma cruzi and its cell surface trans-sialidase) inhibitors in vitro [45].

Most prepared 1,5-benzodiazepin-2-ones were evaluated for antibacterial and antifungal activity in order to survey the possible biological activities of this class of compounds [46,47].

2.1. Biological Activity

2.1.1. Antibacterial Activity

Were tested in vitro for antibacterial activity against an array of eight bacteria using streptomycin as a control, with the findings expressed as MIC in g/mL. (Table 5). The obtained data revealed that all the tested compounds 4a–i showed suitable inhibition against all strains. Particularly compounds 4d (R1 = Me, R2 = H) and 4e (R1 = Me, R2 = OMe) in series 1 might be the major active compounds, and they all showed a similar activity potential, especially against S. epidermidis (MIC = 32 µg/mL) showing values better than the reference antibiotic. Most of the tested compounds displayed poor activity against E. coli and S. typhimurium. Further, toward B. cereus, derivatives 4e seems to contribute better (MIC = 32 µg/mL) than the other analogs followed by 4d (MIC = 64 µg/mL) whereas, against S. aureus, compound 4f (R1 = Me, R2 = NO2) displayed the highest activity (MIC= 32 µg/mL). Toward M. luteus, derivative 4e was found to be the most active compound, followed by 4d and 4g. Moreover, compounds 4d then 4e due to hydrogen atom and methoxy group in the phenyl para-position, respectively, showed the best values for the antibacterial activity compared to other analogs against E. fæcalis. Furthermore, toward L. monocytogenes, also 4d and 4e displayed noticeable antibacterial activity. On the other hand, as depicted in Table 5, the obtained data demonstrate that all the tested compounds 6a–c showed better values of the antibacterial potential compared to compounds 4a–i. Finally, these findings clearly showed the importance of the added fragments to the 1,5-benzodiazepine 1 via the methylene linker to confer activity, essentially the nature of the aromatic system and the galactopyranosyl attached to the triazole ring in the activity.

| Compounds | S. epidermidis(+) | B. cereus(+) | S. aureus(+) | M. luteus(+) | E. coli(−) | P. aeruginosa(−) | E. fæcalis(+) | S. typhimurium(−) | L. monocytogenes(+) |
|-----------|------------------|-------------|-------------|--------------|------------|------------------|--------------|------------------|-------------------|
| 4a        | 125              | 250         | 250         | 250          | 250        | 250              | 250          | 250              | 125               |
| 4b        | 64               | 125         | 250         | 250          | 125        | 125              | 125          | 250              | 550               |
| 4c        | 125              | 64          | 125         | 125          | 125        | 125              | 125          | 250              | 125               |
| 4d        | 32               | 64          | 64          | 64           | 64         | 64               | 64           | 125              | 64                |
| 4e        | 32               | 32          | 32          | 125          | 125        | 125              | 64           | 125              | 32                |
| 4f        | 64               | 125         | 32          | 125          | 125        | 500              | 125          | 125              | 250               |

Table 5. Antibacterial activities of 4a–i and 6a–b: minimum inhibitory concentration (MIC).
Table 5. Cont.

| Compounds | S. epidermidis(+) | B. cereus(+) | S. aureus(+) | M. luteus(+) | E. coli(−) | P. aeruginosa(−) | E. fecalis(+) | S. typhimurium(−) | L. monocytophages(+) |
|-----------|-------------------|--------------|--------------|--------------|------------|-----------------|--------------|-------------------|---------------------|
| 4g        | 64                | 250          | 125          | 64           | 250        | 250             | 250          | 125               | 125                 |
| 4h        | 64                | 125          | 125          | 125          | 250        | 250             | 125          | 550               | 255                 |
| 4i        | 125               | 125          | 250          | 500          | 250        | 250             | 125          | 550               | 125                 |
| 6a        | 32                | 32           | 64           | 64           | 125        | 64              | 32           | 125               | 32                  |
| 6b        | 64                | 32           | 32           | 32           | 125        | 125             | 32           | 125               | 32                  |
| 6c        | 32                | 32           | 32           | 64           | 125        | 64              | 32           | 125               | 32                  |
| Streptomycin | 64             | 78           | 50           | 78           | 256        | 100             | 62.5         | 256               | 50                  |

MICs are given in µg/mL. (+): Gram-positive bacteria, (−): Gram-negative bacteria.

2.1.2. Antifungal Activity

The target compounds 4a–i and 6a–c were assayed for inhibitory activity against clinically important pathogenic fungi such as the Candida albicans and the Aspergillus flavus. Ketoconazole was used as the reference drug (Table 6). All the titled compounds showed good to moderate inhibition against the tested fungal pathogens. Particularly, compound 4d (R1 = Me, R2 = H) revealed excellent activity against both the Candida albicans and the Aspergillus flavus.

Table 6. Antifungal activities of 2a–c, 4a–i, and 6a–b: minimum inhibitory concentration (MIC).

| Compounds | Aspergillus niger | Candida albicans |
|-----------|------------------|------------------|
| 4a        | 500              | 250              |
| 4b        | 125              | 250              |
| 4c        | 125              | 125              |
| 4d        | 64               | 32               |
| 4e        | 125              | 64               |
| 4f        | 250              | 125              |
| 4g        | 500              | 500              |
| 4h        | 125              | 250              |
| 4i        | 125              | 125              |
| 6a        | 64               | 64               |
| 6b        | 64               | 32               |
| 6c        | 125              | 64               |
| Ketoconazole | 500            | H2O              |

MICs are given in µg/mL.

Furthermore, the tested compounds 6a (R = H) and 6b (R = OMe) showed suitable activity against Aspergillus flavus with MIC = 64 µg/mL, which was significantly more potent than Ketoconazole. Toward Candida albicans, 6b (MIC = 32 µg/mL) seems to be the most active, followed by its analogs 6a and 6c (R = Cl). These obtained results suggest that the galactopyranosyl part on the C-4 triazole ring of compound 6b is favorable for enhancing antifungal activity.

2.2. Molecular Docking Studies

A molecular docking study of the newly synthesized compound of series 1 (4a–i) and series 2 (6a–c) was conducted to gain insights into its probable mechanism of action. Indeed, the crystallized structure of Staphylococcus epidermidis TcaR in complex with streptomycin (PDB code: 4EJW) was taken as the target receptor, and the binding pocket was validated by performing redocking of the ligand (Streptomycin). The binding pocket and the interaction of the ligand in complex with the target receptor are shown in Figure 2. Molecular docking calculations of all the test compounds were carried out with Auto Dock vina software. The docked ligand with the lowest binding free energy was used for analysis in Table 7.
Figure 2. (A) is the 3D docking picture of reference ligand «Streptomycin» (the cyan one), (A’) is the 2D docking picture of reference ligand «Streptomycin», (B) is the 3D docking picture of the most active compound in series 1 (the cyan one), (B’) is the 2D docking picture of the most active compound in series 1, (C) is the 3D docking picture of the most active compound in series 2 (the cyan one), (C’) is the 2D docking picture of the most active compound in series 2.
Table 7. Docking binding energies (kcal mol\(^{-1}\)) of promising antibacterial agents.

| Compound | Free Binding Energy |
|----------|---------------------|
| 4a       | −9.4                |
| 4b       | −9.8                |
| 4c       | −9.6                |
| 4d       | −9.6                |
| 4e       | −9.9                |
| 4f       | −9.0                |
| 4g       | −9.7                |
| 4h       | −9.8                |
| 4i       | −9.6                |
| 6a       | −11.1               |
| 6b       | −11.1               |
| 6c       | −11.4               |
| Streptomycin | −9.2         |

As can be seen from the results, the molecular docking for the representative compounds: the most active derivative in series 1 is BZD 4e, the most active derivative in series 2 is compound 6c, and the redocked «streptomycin» showed that the ligands were well oriented toward the active site gorge. Thus, 4e formed a conventional hydrogen bond with GLN-B-61 through its hydroxyl group besides a Pi-Donor hydrogen bond with GLN-A-31. In addition, the ligand 4e was oriented to a hydrophobic pocket composed of ALA-A-24 and ALA-A-38 with Pi-Alkyl interactions. The methylbenzodiazepine ring contributed to shaping interaction with HIS-A-42 and Alkyl interaction with VAL-A-63. The methoxytriazole moiety formed Pi-Alkyl interactions with ALA-B-24 and ALA-B-38 besides a stacking interaction with HIS-B-42 (Figure 2B,B’).

On the other hand, ligand 6c set up H-bonds with ASN-B-20 and HIS-A-42 through its N-galactopyranosyl and BZD pharmacophores, respectively. This finding demonstrates the crucial role of the N-galactopyranosyl in series 2 (compounds 6a–c) linked to the triazole ring, which took the place of the aryl group in series 1 (compounds 4a–i). Furthermore, 6c formed some interesting Alkyl interactions with residues: VAL-A-63, ALA-B-24, LEU-B-27, ALA-B-38, and HIS-B-42 via its N-galactopyranosyl fragment, which displayed a Pi-Sigma interaction with HIS-B-42. In addition, derivative 6c showed Amide-Pi stacked with SER-A-41 and hydrophobic Pi-Alkyl and Alkyl interactions with VAL-B-63. (Figure 2C,C’).

From these results, it can be inferred that docked compound, especially derivative 6c, probably showed its antibacterial activity in a similar way as that of the Streptomycin antibiotic (Figure 2A,A’) by interfering with the functioning of epidermidis TcaR in complex with streptomycin receptor.

3. Materials and Methods

3.1. Instruments and Methods

Toluene and methylene chloride (DCM) were obtained from MBRAUN’s MB SPS-800 apparatus and dried according to conventional protocols. Unless otherwise specified, cyclohexane, ethyl acetate (EtOAc), acetonitrile (CH\(_3\)CN), and diethyl ether (OEt\(_2\)) were acquired in ACS-grade quality and utilized without additional purification. Unless otherwise noted, commercially available reagents were utilized without further purification.

\(^1\)H and \(^13\)C NMR spectra were recorded with an AC-300 Bruker spectrometer with tetramethylsilane as an internal reference. Chemical shifts are reported in parts per million. Two-dimensional NMR experiments were performed with an Avance-300 Bruker spectrometer. Multiplicities are described as s (singlet), d (doublet), dd, dd, etc. (doublet of doublets), t (triplet), and m (multiplet). High-resolution mass spectra of compounds 4b, 4e, and 4g were performed within a Hewlett-Packard 5890/5970 GC mass spectrometer. Elemental analysis was recorded on a PERKIN–ELMER 240B microanalyzer.

All the reactions were followed by TLC using aluminum sheets of Merck silica gel 60 F254, 0.2 mm. The spots were visualized through illumination with a UV lamp (\(\lambda = 254\) nm).
and/or staining with KMnO₄. Column chromatography purifications were performed on silica gel (40–63 µm) carried out on Merck DC Kiesel gel 60 F-254 aluminum sheets. The starting material 1a–c was prepared according to the literature [8]. Melting points of benzodiazepines 2a–c, 4a–l, and 6a–c were determined on a Buchi 510 capillary melting point apparatus.

3.2. Synthesis of N-Propargyl-1,5-benzodiazepinones (2a–c)

NaH (60% in mineral oil, 0.88 g, 2.4 mmol, 1.2 equiv.) was added to a solution of 4-(2′-hydroxyphenyl)-1,5-benzodiazepin-2-one 1a–c (2 mmol) in DMF (15 mL) at 0 °C under nitrogen. Before the mixture was stirred for 10 to 15 min and propargyl bromide, 1.2 equiv was added. The reaction mixture was maintained at room temperature for 6 h. The reaction mixture was kept at room temperature. The raw ingredient was poured into distilled water, and dichloromethane was used to extract it. The organic layers were mixed together and dried over anhydrous MgSO₄, then filtered and concentrated under reduced pressure. The crude substance was purified using silica gel column chromatography (80:20 hexane/EtOAc).

3.2.1. 1-prop-2-ynyl-4-(2-Hydroxyphenyl)-3H-1,5-benzodiazepin-2-one (2a)

This compound was prepared according to the literature method [13].

| Compound | Yield | Color | Melting point (°C) | NMR Data | Mass Spectrometry |
|----------|-------|-------|-------------------|----------|------------------|
| 2a       | 377 mg | Yellow | 136–138          | δH 2.30 (t, 1H, H-3′′), 3.00 (d, 1H, H-3b, J = 12.3 Hz), 4.25 (d, 2H, CH₂-1′′, J = 2.4 Hz), 4.70 (d, 1H, H-3a, J = 17.1 Hz), 6.93 (t, 1H, H-3′), 7.01 (d, 1H, H-3′, J = 7.5 Hz), 7.15 (dd, 1H, H-7), 7.23 (d, 1H, H-6, J = 3.9 Hz), 7.40 (t, 1H, H-4′), 7.51 (s, 1H, H-9), 7.83 (d, 1H, H-6′, J = 6.6 Hz), 13.95 (s, 1H, OH). |
| 2b       | 377 mg | Yellow | 136–138          | δH 2.30 (t, 1H, H-3′′), 3.00 (d, 1H, H-3b, J = 12.3 Hz), 4.25 (d, 2H, CH₂-1′′, J = 2.4 Hz), 4.70 (d, 1H, H-3a, J = 17.1 Hz), 6.93 (t, 1H, H-3′), 7.01 (d, 1H, H-3′, J = 7.5 Hz), 7.15 (dd, 1H, H-7), 7.23 (d, 1H, H-6, J = 3.9 Hz), 7.40 (t, 1H, H-4′), 7.51 (s, 1H, H-9), 7.83 (d, 1H, H-6′, J = 6.6 Hz), 13.95 (s, 1H, OH). |

3.2.2. 1-prop-2-ynyl-4-(2-Hydroxyphenyl)-8-chloro-3H-1,5-benzodiazepin-2-one (2c)

Yield 452 mg (60%). Yellow solid, m.p 176–178 °C. 1H NMR (300 MHz, CDCl₃) δH 2.37 (t, 1H, H-3′′), 3.03 (d, 1H, H-3a, J = 12.3 Hz), 4.27 (d, 2H, CH₂-1′′, J = 2.4 Hz), 4.74 (d, 1H, H-3b, J = 17.1 Hz), 6.98 (t, 1H, H-5′), 7.04 (d, 1H, H-3′, J = 8.4 Hz), 7.34 (d, 1H, H-7, J = 8.7 Hz), 7.44 (t, 1H, H-4′), 7.46 (s, 1H, H-9), 7.69 (d, 1H, H-6, J = 8.7 Hz), 7.89 (d, 1H, H-6′, J = 7.8 Hz), 13.74 (s, 1H, OH). 13C NMR (75.47 MHz, CDCl₃) δC 20.7 (C-8a), 37.0 (C-3), 38.1 (C-2′′), 72.0 (C-3′′), 78.4 (C-2′′), 117.7 (C-3′), 118.6 (C-1′), 121.0 (C-5′), 121.7 (C-9), 126.7 (C-4′), 127.7 (C-6), 133.4 (C-9a), 135.5 (C-5a), 137.7 (C-8), 161.6 (C-2′), 163.5 (C-2′), 164.5 (C-4). Anal. Calcd for C₁₈H₁₆ClN₂O₂: C, 66.57; H, 4.03; N, 8.63; found: C, 66.01; H, 4.12; N, 8.69.

3.3. General Procedure for the Synthesis of Compounds (4a–i)

Cul (5.0 mg, 0.025 mmol, 5 mol percent) and the corresponding phenyl azide 3a–e derivative were added to a mixture of compounds 2a–c (0.5 mmol, 1 eq) and Et₃N (2.0 eq, 134 l, 1 mmol) in DCM (20 mL) (1.0 mmol, 2.0 eq). At room temperature, the reaction mixture was stirred for 4 h. The filtrate was concentrated under reduced pressure after the crude reaction was filtered using Celite®. Flash column chromatography on silica gel (Cyclohexane/EtOAc from 100:0 to 90:10) was used to purify the crude substance, yielding pure 4a–i in 77–89% yields.
3.3.1. 4-(2-Hydroxyphenyl)-1-((1-phenyl)-1H-1,2,3-triazol-4-yl)methyl)-1,5-benzodiazepin-3H-2-one (4a)

Yield 178 mg (87%). Yellow solid, m.p. 201–203 °C. 1H NMR (300 MHz, CDCl3) δH 3.00 (d, 1H, H-3a, J = 12.00 Hz), 4.25 (d, 1H, H-3b, J = 2.4 Hz), 4.90 (d, 1H, H-1a′′, J = 14.7 Hz), 5.25 (d, 1H, H-1b′′, J = 15 Hz), 6.96 (t, 1H, H-7), 7.05 (d, 1H, H-6, J = 8.4 Hz), 7.32 (d, 1H, H-9), J = 7.5 Hz), 7.41–7.45 (m, 4H, H-4′, H-5′, H-4″, H-8), 7.48 (t, 2H, H-3′, H-5′), 7.70 (d, 2H, H-2′′, H-6′′), J = 7.5 Hz), 7.86 (d, 1H, H-3, J = 8.1 Hz), 8.11 (s, 1H, H-5″), 8.13 (d, 1H, H-6′, J = 8.1 Hz), 13C NMR (75.47 MHz, CDCl3) δC 38.2 (C-3), 44.8 (C-1″), 117.9 (C-1′), 118.3 (C-6), 119.1 (C-2″′, C-6″″), 120.4 (C-6″), 122.5 (C-5″), 126.1; 126.7; 134.2 (C-4″, C-5″, C-8, C-4″″), 128.8 (C-3′), 129.3 (C-3″′, C-5″″), 129.7 (C-9a), 135.2 (C-5a), 136.9 (C-1″′′), 138.2 (C-4″″), 162.1 (C-2′), 164.9 (C-4), 165.1 (C-2). Anal. Calcd for C24H19N2O2 (409.45): C, 70.40; H, 4.68; N, 17.10; found: C, 70.22; H, 4.37; N, 17.16.

3.3.2. 4-(2-Hydroxyphenyl)-1-((1-(4-metoxyphenyl))-1H-1,2,3-triazol-4-yl)methyl)-1,5-benzodiazepin-3H-2-one (4b)

Yield 189 mg (86%). Yellow solid, m.p. 174–176 °C. 1H NMR (300 MHz, CDCl3) δH 3.02 (d, 1H, H-3a, J = 12.00 Hz), 3.88 (s, 3H, OCH3), 4.27 (d, 1H, H-3b, J = 12.00 Hz), 4.96 (d, 1H, H-1a′, J = 15.00 Hz), 5.27 (d, 1H, H-1b″, J = 15.30 Hz), 6.98–7.09 (m, 4H, H-arom), 7.34–7.49 (m, 4H, H-arom), 7.62 (d, 2H, H-3″, H-5″′, J = 7.80 Hz), 7.88 (d, 1H, H-arom, J = 8.1 Hz), 8.05 (s, 1H, H-5″), 8.16 (d, 1H, H-6′, J = 8.10 Hz), 13C NMR (75.47 MHz, CDCl3) δC21.08 (CH), 8.16 (d, 1H, H-arom, J = 8.10 Hz), 144.2 (C-4), 159.8 (C-4″), 162.2 (C-4″″), 164.9 (C-4), 165.0 (C-2′). Anal. Calcd for C25H21N3O2 (391.43): C, 76.33; H, 4.82; N, 15.94; found: C, 76.82; H, 4.89; N, 16.06. HRMS (ESI+): calcld. for C25H21N3NaO2[M+Na]+: 460.1749; found: 460.1763.

3.3.3. 4-(2-Hydroxyphenyl)-1-((1-(4-nitrophenyl))-1H-1,2,3-triazol-4-yl)methyl)-1,5-benzodiazepin-3H-2-one (4c)

Yield 165 mg (78%). Yellow solid, m.p. 225–227 °C. 1H NMR (300 MHz, CDCl3) δH 3.04 (d, 1H, H-3a, J = 12 Hz), 4.28 (d, 1H, H-3b, J = 12 Hz), 5.05 (d, 1H, H-1a′, J = 15 Hz), 5.26 (d, 1H, H-1b″, J = 15.3 Hz), 6.99 (t, 1H, H-arom), 7.08 (d, 1H, H-arom, J = 8.10 Hz), 7.34–7.50 (m, 4H, H-arom), 7.88 (d, 1H, H-arom, J = 7.80 Hz), 7.95 (d, 2H, H-3″′, H-5″″, J = 9.00 Hz), 8.07 (d, 1H, H-6′, J = 8.10 Hz), 8.19 (s, 1H, H-5″), 8.41 (d, 2H, H-2″′, H-6″″, J = 9.00 Hz). 13C NMR (75.47 MHz, CDCl3) δC = 38.2 (C-3), 44.6 (C-1″), 117.9 (C-1′), 118.3 (C-arom), 119.2 (C-arom), 120.4 (C-arom), 122.3 (C-arom), 123.1 (C-5″), 125.5 (C-arom), 126.3 (C-arom), 127.0 (C-arom), 127.6 (C-arom), 129.3 (C-arom), 134.3 (C-9a), 134.9 (C-1″′′), 138.4 (C-5a), 141.0 (C-4″), 147.3 (C-4″″) 162.2 (C2′), 164.1 (C-4), 165.2 (C-2′). Anal. Calcd for C23H18N2O4 (454.14): C, 63.43; H, 3.99; N, 18.49; found: C, 63.15; H, 4.09; N, 18.23.

3.3.4. 4-(2-Hydroxyphenyl)-8-methyl-1-((1-phenyl)-1H-1,2,3-triazol-4-yl)methyl)-1,5-benzodiazepin-3H-2-one (4d)

Yield 173 mg (82%). Yellow solid, m.p. 202–204 °C. 1H NMR (300 MHz, CDCl3) δH 2.40 (s, 3H, CH3-8a), 2.99 (d, 1H, H-3a, J = 12.00 Hz), 4.23 (d, 1H, H-3b, J = 12.30 Hz), 4.91 (d, 1H, H-1a″, J = 15.30 Hz), 5.22 (d, 1H, H-1b″, J = 15.30 Hz), 6.94 (t, 1H, H-arom), 7.03 (d, 1H, H-arom, J = 8.4 Hz), 7.22 (s, 1H, H-9), 7.39 (t, 2H, H-arom), 7.48 (t, 2H, H-arom), 7.69 (d, 2H, H-arom, J = 8.4 Hz), 7.84 (dd, 1H, H-arom, J = 7.8 Hz), 7.96 (d, 1H, H-arom, J = 8.4 Hz), 8.07 (s, 1H, H-5″). 13C NMR (75.47 MHz, CDCl3) δC 20.7 (CH3-8a), 38.2 (C-3), 44.7 (C-1″), 118.2 (C-1′), 119.1 (C-arom), 118.1 (C-arom), 120.4 (C-arom), 122.4 (C-5″), 123.0 (C-arom), 126.9 (C-arom), 126.8 (C-arom), 128.7 (C-arom), 129.3 (C-arom), 129.7 (C-arom), 132.8 (C-9a),
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134.0 (C-arom), 135.1 (C-5a), 136.1 (C-8), 138.0 (C-1‴′), 144.5 (C-4″), 162.2 (C-2″′), 163.8 (C-4), 164.9 (C-2). Anal. Calcd for C_{25}H_{21}N_{5}O_{2} (423.17): C, 70.91; H, 5.00; N, 16.54; found: C, 70.51; H, 5.09; N, 16.24.

3.3.5. 4-(2-Hydroxyphényl)-8-méthyl-1-((1-méthoxyphényl)-1H-1,2,3-triazol-4-yl)méthyl)-1,5-benzodiazipépin-3H-2-one (4e)

Yield 187 mg (83%). Yellow solid, m.p. 184–186 °C. ^1H NMR (300 MHz, CDCl_{3}) δH 2.40 (s, 6H, CH_{3}-8a), 2.98 (d, 1H, H-3a, J = 12.00 Hz), 3.85 (s, 3H, OCH_{3}-4‴′′′), 4.22 (d, 1H, H-3b, J = 12.30 Hz), 4.92 (d, 1H, H-1a‴′, J = 15.00 Hz), 5.21 (d, 1H, H-1b‴′, J = 15.30 Hz), 6.94–7.05 (m, 4H, H-arom), 7.18 (m, 1H, H-arom), 7.21 (s, 1H, H-9), 7.39 (t, 1H, H-arom), 7.58 (d, 2H, H-2‴″′, H-6‴″′, J = 9.00 Hz), 7.84 (d, 1H, H-6″′, J = 8.10 Hz), 7.97 (s, 1H, H-5‴″′), 13.95 (1s, 1H, OH). ^13C NMR (75.47 MHz, CDCl_{3}) δC 19.7 (CH_{3}-8a), 20.0 (CH_{3}-4‴′′′), 38.3 (C-3), 44.6 (C-1‴′), 117.0 (C-1′), 117.2 (C-arom), 118.0 (C-arom), 121.5 (C-arom), 122.3 (C-3‴′′′), 125.9 (C-arom), 126.9 (C-arom), 128.2 (C-arom), 129.4 (C-arom), 131.8 (C-arom), 133.0 (C-arom) 135.1 (C-9a), 137.0 (C-5a), 143.3 (C-4‴′′′), 158.8 (C-1‴″′), 161.2 (C-4‴″′), 162.3 (C-2″′′), 162.8 (C-4), 163.9 (C-2). Anal. Calcd for C_{26}H_{23}N_{3}O_{3} (453.18): C, 68.86; H, 5.11; N, 15.44; found: C, 69.12; H, 5.29; N, 15.46; HRMS (ESI+): calcd. for C_{26}H_{24}N_{3}O_{3}[M+H]+: 454.1879; found: 454.1879.

3.3.6. 4-(2-Hydroxyphényl)-8-méthyl-1-((1-(4-nitrophényl)-1H-1,2,3-triazol-4-yl)méthyl)-1,5-benzodiazipépin-3H-2-one (4f)

Yield 185 mg (79%). Yellow solid, m.p. 235–237 °C. ^1H NMR (300 MHz, CDCl_{3}) δH 2.41 (s, 6H, CH_{3}-8a), 3.01 (d, 1H, H-3a, J = 12.00 Hz), 4.23 (d, 1H, H-3b, J = 12.00 Hz), 5.03 (d, 1H, H-1a‴′, J = 15.00 Hz), 5.24 (d, 1H, H-1b‴′, J = 15.00 Hz), 6.95 (t, 1H, H-arom), 7.00 (d, 1H, H-arom, J = 8.10 Hz), 7.16 (m, 1H, H-arom), 7.22 (s, 1H, H-9), 7.41 (t, 1H, H-arom), 7.80 (m, 2H, H-arom), 7.90 (d, 2H, H-2‴″′, H-6‴″′, J = 9.50 Hz), 8.12 (s, 1H, H-5‴″′), 8.37 (d, 2H, H-3‴″′, H-5‴″′, J = 9.00 Hz). ^13C NMR (75.47 MHz, CDCl_{3}) δC 19.7 (CH_{3}-8a), 37.2 (C-3), 43.5 (C-1‴′′), 117.3 (C-1′), 118.1 (C-arom), 119.4 (C-arom), 121.1 (C-arom), 121.8 (C-5‴″′), 124.4 (C-arom), 125.9 (C-arom), 127.6 (C-arom), 128.2 (C-arom), 131.5 (C-arom), 133.1 (C-9a), 135.4 (C-5a), 137.2 (C-8), 140.0 (C-1‴″′), 144.4 (C-4‴′′′), 146.2 (C-4‴″′), 161.2 (C-2″′), 162.9 (C-4), 164.0 (C-2). Anal. Calcd for C_{26}H_{29}N_{4}O_{4} (468.15): C, 64.10; H, 4.30; N, 17.94; found: C, 63.85; H, 4.19; N, 17.64.

3.3.7. 4-(2-Hydroxyphényl)-8-chloro-1-((1-phényl)-1H-1,2,3-triazol-4-yl)méthyl)-1,5-benzodiazipépin-3H-2-one (4g)

Yield 173 mg (78%). Yellow solid, m.p. 246–248 °C. ^1H NMR (300 MHz, CDCl_{3}) δH 2.99 (d, 1H, H-3a, J = 12.30 Hz), 4.29 (d, 1H, H-3b, J = 12.30 Hz), 4.85 (d, 1H, H-1a‴′, J = 13.50 Hz), 5.27 (d, 1H, H-1b‴′, J = 14.10 Hz), 6.99 (t, 1H, H-arom), 7.07 (d, 1H, H-arom, J = 8.40 Hz), 7.41–7.50 (m, 4H, H-arom), 7.53 (t, 1H, H-arom), 7.74 (d, 2H, H-2‴″′, H-6‴″′, J = 9.00 Hz), 7.88 (d, 1H, H-arom, J = 7.20 Hz), 8.19 (s, 1H, H-5‴″′), 13.64 (1s, 1H, OH). ^13C NMR (75.47 MHz, CDCl_{3}) δC 38.4 (C-3), 44.7 (C-1‴″′), 117.8 (C-1′), 118.4 (C-arom), 119.3 (C-arom), 120.5 (C-arom), 124.6 (C-arom), 126.4 (C-arom), 128.9 (C-arom), 129.4 (C-arom), 129.8 (C-arom), 131.3 (C-8), 133.9 (C-4‴″′), 134.5 (C-arom), 139.2 (C-4‴″′), 162.2 (C-2″′), 164.6 (C-4), 164.9 (C-2). Anal. Calcd for C_{24}H_{18}ClN_{2}O (443.11): C, 64.94; H, 4.09; N, 15.78; found: C, 65.24; H, 3.89; N, 16.08.HRMS (ESI+): calcd. for C_{24}H_{18}BrClN_{2}O_{2}[M+Br]^+: 522.1324; found: 522.1324.

3.3.8. 4-(2-Hydroxyphényl)-8-chloro-1-((1-méthoxyphényl)-1H-1,2,3-triazol-4-yl)méthyl)-1,5-benzodiazipépin-3H-2-one (4h)

Yield 191 mg (81%). Yellow solid, m.p. 236–238 °C. ^1H NMR (300 MHz, CDCl_{3}) δH 2.99 (d, 1H, H-3a, J = 12.30 Hz), 3.89 (s, 3H, OCH_{3}-4‴′′′), 4.28 (d, 1H, H-3b, J = 12.30 Hz), 4.86 (d, 1H, H-1a‴′, J = 15.00 Hz), 5.25 (d, 1H, H-1b‴′, J = 15.00 Hz), 6.98–7.09 (t, 4H, H-arom),
7.28 (dd, 1H, H-arom, J = 8.7 Hz), 7.37 (s, 1H, H-9), 7.44 (t, 1H, H-arom), 7.63 (d, 2H, H-2′′′′, H-6′′′′, J = 9.00 Hz), 7.86 (d, 1H, H-arom, J = 8.10 Hz), 8.05 (s, 1H, H-5′′′′), 8.28 (d, 1H, H-arom, J = 7.8 Hz), 13.80 (1s, 1H, OH). 13C NMR (75.47 MHz, CDCl3) δC 37.2 (C-3′), 43.8 (C-1′′′′), 54.6 (OCH3-4′′′′), 113.7 (C-7), 116.8 (C-1′), 117.3 (C-arom), 118.2 (C-arom), 121.0 (C-arom), 122.2 (C-5′), 125.4 (C-arom), 127.0 (C-arom), 128.3 (C-arom), 129.3 (C-arom), 131.8 (C-8′), 133.3 (C-9a), 134.9 (C-1′″′), 135.8 (C-4′′′′′), 158.8 (C-4′′′′′), 161.1 (C-2′), 163.1 (C-4), 163.6 (C-2). Anal. Calcld for C23H20ClN3O2 (473.13): C, 63.36; H, 4.25; N, 15.29; found: C, 65.07; H, 4.46; N, 14.78.

3.3.9. 4-(2-Hydroxyphényl)-8-chloro-1-((1-phenyl)-1H,1,2,3-triazol-4-yl)méthyl)-1,5-benzodiazépín-3H-2-one (4i)

Yield 178 mg (77%). Yellow solid, m.p. > 250 °C. 1H NMR (300 MHz, CDCl3) δH 3.01 (d, 1H, H-3a, J = 12.00 Hz), 4.29 (d, 1H, H-3b, J = 12.30 Hz), 4.91 (d, 1H, H-1a″″, J = 15.30 Hz), 5.25 (d, 1H, H-1b″″, J = 15.00 Hz), 7.00 (t, 1H, H-arom), 7.07 (d, 1H, H-arom, J = 8.10 Hz), 7.38 (dd, 1H, H-arom, J = 9.00 Hz), 7.46 (m, 2H, H-arom), 7.86 (dd, 1H, H-arom, J = 8.10 Hz), 7.97 (d, 2H, H-3′′′′, H-5′′′′′, J = 9.00 Hz), 8.09 (d, 1H, H-arom, J = 8.70 Hz), 8.24 (s, 1H, H-5′′′′′), 8.42 (d, 2H, H-2′′′′′, H-6′′′′′, J = 9.00 Hz), 13.64 (1s, 1H, OH). 13C NMR (75.47 MHz, CDCl3) δC 38.3 (C-3′), 44.6 (C-1′″), 117.6 (C-1′), 118.4 (C-arom), 119.3 (C-arom), 120.5 (C-arom), 122.6 (C-arom), 124.4 (C-arom), 125.4 (C-arom), 126.5 (C-arom), 127.6 (C-arom), 129.4 (C-arom), 131.5 (C-8′), 133.6 (C-9a), 134.7 (C-1′″′), 140.9 (C-4′′′′′), 147.3 (C-4′′′′′), 162.2 (C-2′′), 164.8 (C-4), 164.9 (C-2). Anal. Calcld for C24H17ClN3O4 (488.10): C, 58.96; H, 3.51; N, 17.19; found: C, 59.26; H, 3.41; N, 17.00.

3.4. General Procedure for the Synthesis of Compounds (6a–c)

Cul (5.0 mg, 0.025 mmol, 5 mol percent) and the suitable galactopyranosyl azide 5 (1 mmol, 2 eq) were added to a combination of compounds 2a–c (0.5 mmol, 1 eq) and Et3N (2 eq, 134 µL, 1 mmol) in DMF/H2O (8/2). For 8 h, the reaction mixture was stirred at room temperature. The raw material was put into distilled water and extracted with dichloromethane after being filtered through Celite®. Flash column chromatography on silica gel (Cyclohexane/EtOAc from 100:1 to 90:10) was used to purify the crude substance, yielding pure 6a–c in 80–85% yields.

3.4.1. 4-(2-Hydroxyphényl)-1-(1-(3aR, 5R, 5aS, 8aS, 8bR)-2,2,7,7-tetraméthyltrèthralydhydro-3aH-bis([1,3]dioxolo)[4,5-b:4′,5′-d]pyran-5-yl)méthyl)-1H-1,2,3-triazol-4-yl)méthyl)-1H-1,5-benzodiazépin-2-one (6a)

Yield 202 mg (85%). Yellow solid, m.p. 156–158 °C. 1H NMR (300 MHz, CDCl3) δH 1.29; 1.37; 1.42; 1.50 (s, 3H, CH3), 2.97 (d, 1H, H-3a, J = 12.00 Hz), 4.12 (m, 3H, H-3b, H-3′′′′′, H-5′′′′′), 4.29 (m, 1H, H-2′′′′′), 4.45 (m, 1H, H-4′′′′′), 4.56 (m, 2H, CH2-6′′′′′), 4.86 (d, 1H, H-1a″″, J = 15.00 Hz), 5.21 (d, 1H, H-1b″″, J = 15.30 Hz), 5.51 (s, 1H, H-1′″″′′, 6.96–8.19 (m, 8H, H-arom), 8.06 (s, 1H, H-5′′′′′), 13.95 (1s, 1H, OH). 13C NMR (75.47 MHz, CDCl3) δC 23.9; 24.3; 25.4; 31.0 (CH2-Sucre), 37.7 (C-3′), 44.0 (C-1′″′), 50.3 (C-6′′′′′), 61.5 (C-2′′′′′), 67.1 (C-3′′′′′), 70.3 (C-4′′′′′), 70.7 (C-5′′′′′), 96.2 (C-1′″″′′), 108.5 (C-isop), 109.4 (C-isop), 117.5 (C-1′), 117.7 (C-3′), 118.2 (C-arom), 119.0 (C-arom), 123.2 (C-arom), 125.8 (C-arom), 126.9 (C-arom), 127.5 (C-arom), 128.8 (C-quat), 129.3 (C-arom), 133.5 (C-quat), 134.0 (C-arom), 134.8 (C-8) 137.9 (C-4′′′′′), 161.7 (C-2′′′′′), 163.8 (C-4′′′′′), 164.2 (C-2). Anal. Calcld for C30H33N3O5 (575.24): C, 62.60; H, 5.78; N, 12.17; found: C, 63.75; H, 6.36; N, 11.68.

3.4.2. 4-(2-Hydroxyphényl)-8-méthyl-1-(1-(3aR, 5R, 5aS, 8aS, 8bR)-2,2,7,7-tetraméthyltrèthralydhydro-3aH-bis([1,3]dioxolo)[4,5-b:4′,5′-d]pyran-5-yl)méthyl)-1H-1,2,3-triazol-4-yl)méthyl)-1H-1,5-benzodiazépin-2-one (6b)

Yellow solid, yield 247 mg (84%), m.p. 140–142 °C. 1H NMR (300 MHz, CDCl3) δH 1.34; 1.36; 1.47; 1.50 (s, 3H, CH3), 2.41 (s, 3H, CH3-8a), 2.95 (d, 1H, H-3a, J = 12.00 Hz),
4.12 (m, 3H, H-3b, H-3‴′), H-5‴′), 4.25 (m, 1H, H-2‴′), 4.36 (m, 1H, H-4‴′), 4.52 (m, 2H, CH₂-6‴′), 4.75 (d, 1H, H-1a″, J = 15.00 Hz), 5.09 (d, 1H, H-1b″, J = 15.30 Hz), 5.40 (s, 1H, H-1‴′), 6.87–7.95 (m, 7H, H-arom), 7.69 (s, 1H, H-5‴′), 13.95 (1s, 1H, OH). 13C NMR (75.47 MHz, CDCl₃) δ 13.31; 1.39; 1.45; 1.52 (s, 3H, CH₃), 2.97 (d, 1H, H-3a, J = 12.00 Hz), 4.17 (m, 3H, H-3b, H-3‴′, H-5‴′), 4.32 (m, 1H, H-2‴′), 4.49 (m, 1H, H-4‴′), 4.59 (m, 1H, CH₂-6‴′), 4.86 (d, 1H, H-1a″, J = 15.00 Hz), 5.21 (d, 1H, H-1b″, J = 15.30 Hz), 5.54 (s, 1H, H-1‴′), 6.98–8.21 (m, 8H, H-arom), 8.08 (s, 1H, H-5‴′), 13.95 (1s, 1H, OH). 13C NMR (75.47 MHz, CDCl₃) δ 13.31; 1.39; 1.45; 1.52 (s, 3H, CH₃), 2.97 (d, 1H, H-3a, J = 12.00 Hz), 4.17 (m, 3H, H-3b, H-3‴′, H-5‴′), 4.32 (m, 1H, H-2‴′), 4.49 (m, 1H, H-4‴′), 4.59 (m, 1H, CH₂-6‴′), 4.86 (d, 1H, H-1a″, J = 15.00 Hz), 5.21 (d, 1H, H-1b″, J = 15.30 Hz), 5.54 (s, 1H, H-1‴′), 6.98–8.21 (m, 8H, H-arom), 8.08 (s, 1H, H-5‴′), 13.95 (1s, 1H, OH). 13C NMR (75.47 MHz, CDCl₃) δ 13.31; 1.39; 1.45; 1.52 (s, 3H, CH₃), 2.97 (d, 1H, H-3a, J = 12.00 Hz), 4.17 (m, 3H, H-3b, H-3‴′, H-5‴′), 4.32 (m, 1H, H-2‴′), 4.49 (m, 1H, H-4‴′), 4.59 (m, 1H, CH₂-6‴′), 4.86 (d, 1H, H-1a″, J = 15.00 Hz), 5.21 (d, 1H, H-1b″, J = 15.30 Hz), 5.54 (s, 1H, H-1‴′), 6.98–8.21 (m, 8H, H-arom), 8.08 (s, 1H, H-5‴′), 13.95 (1s, 1H, OH).

3.5.1. Antibacterial Tests

Microbial Inhibitory Concentration

Microdilution assay The MICs of the compounds were determined by microdilution [48] using standard inocula of 2 × 10⁶ CFU/mL. Serial dilutions of the test compounds were prepared in DMSO. A bacterial fluid (1 mL of 0.5 McFarland standard) was added to each tube. The MIC was visually determined after incubation for 18 h at 37 °C.

3.5.2. Antifungal Activity

The antifungal activity of compounds 4a–I and 6a–c was tested against two fungal species, namely: Aspergillus flavus and Candida albicans. These fungi were obtained from the (Department of Clinical biology, Laboratory of Analysis, Treatment and valorization of Pollutants of the Environment and Products, Faculty of Pharmacy of Monastir). They were cultured at 25 °C on potato dextrose agar (PDA) medium one week before use.

3.5.3. Molecular Docking Procedure

Molecular docking simulations were performed by Auto Dock 4.2 program package [49]. The optimization of all the geometries of compounds was carried out using ACD (3D viewer) software (http://www.filefacts.com/acad3d-viewer-freeware-info, accessed on 25 March 2022). The three-dimensional structure of PDB (PDB: 4EJW) was obtained from the RSCB protein data bank [50]. First, the water molecules were eliminated, and the missing hydrogens and Gasteiger charges were added to the system during the preparation of the receptor input file. Then, AutoDock Tools were used for the preparation of the corresponding ligand and protein files (PDB QT). Subsequently, pre-calculation of the
grid maps was performed using Auto Grid to save much time during docking. Next, the docking calculation was carried out using a grid per map with $40 \times 40 \times 40 \text{Å}^3$ points of (PDB: 4EJW) in addition to a grid-point spacing of 0.375 Å, which was centered on the receptor in order to determine the active site. The visualization and analysis of interactions were performed using Discovery Studio 2017R2 (https://www.3dsbiovia.com/products/collaborative-science/biovia-discovery-studio/, accessed on 25 March 2022).

4. Conclusions

In our study, novel conjugates $N$-triazolo-1,5-benzodiazepinones 4a–i and 6a–c were designed and synthesized. In fact, we have incorporated 1,2,3-triazole at the first position of the heptatomic ring with either linkage employing the Cu(I)-catalyzed 1,3-dipolar alkyneneazide coupling reaction (CuAAC). Compounds synthesized by this method are of high quality, allowing for simple purification and screening in a high throughput manner. Some of them were screened for their antimicrobial activity and have shown good to moderate antibacterial and antifungal activities. Even though the inhibition levels are only at µM levels, we believe these novel classes of $N$-triazolo-1,5-benzodiazepin-2-ones could find applications in biology. Our strategy, therefore, lays the foundations for the future exploration of more potent and selective $N$-functionalized-1,5-benzodiazepinones. To understand the mechanism of antibacterial activity and binding mode of these novel derivatives inside the binding pocket of the crystallized structure of Staphylococcus epidermidis TcaR in complex with streptomycin and to confirm the experimental results, molecular docking studies were performed.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/molecules27134015/s1, Figure S1: NMR spectra 1H (300 MHz, CDCl3) of compound 4b; Figure S2: NMR spectra 13C (75,47 MHz, CDCl3) of compound 4b; Figure S3: DEPT 135 of compound 4b; Figure S4: CHcorr spectra of compound 4b; Figure S5: NMR spectra 1H (300 MHz, CDCl3) of compound 6b; Figure S6: NMR spectra 1H (300 MHz, CDCl3) of compound 6b; Figure S7: NOESY Spectra of compound 6b; Figure S8: COSY 1H-1H spectra of compound 6b; Figure S9: COSY 1H-1H spectra of compound 6b; Figure S10: NMR spectra 1H (300 MHz, CDCl3) of compound 6b; Figure S11: DEPT 135 (75,47 MHz, CDCl3) of compound 6b; Figure S12: CHcorr Spectra of compound 6b; Figure S13: COSY Spectra of compound 6b; Figure S14: NOESY Spectra of compound 6b; Figure S15: HRMS Spectra of compound 4b; Figure S16: HRMS Spectra of compound 4g; Figure S17: NMR spectra 1H (300 MHz, CDCl3) of compound 2c; Figure S18: DEPT 135 of compound 2c; Figure S19: NMR spectra 1H (300 MHz, CDCl3) of compound 4d; Figure S20: NMR spectra 13C (75,47 MHz, CDCl3) of compound 4d; Figure S21: NMR spectra 1H (300 MHz, CDCl3) of compound 4e; Figure S22: NMR spectra 13C (75,47 MHz, CDCl3) of compound 4e; Figure S23: NMR spectra 1H (300 MHz, CDCl3) of compound 4f; Figure S24: NMR spectra 13C (75,47 MHz, CDCl3) of compound 4f; Figure S25: NMR spectra 1H (300 MHz, CDCl3) of compound 6b; Figure S26: NMR spectra 13C (75,47 MHz, CDCl3) of compound 4b; Figure S27: NMR spectra 1H (300 MHz, CDCl3) of compound 4i; Figure S28: NMR spectra 13C (75,47 MHz, CDCl3) of compound 4i.

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