Discovery, Synthesis and Activity Evaluation of Novel Compounds Bearing 1,2,4-triazolo[3,4-b][1,3,4]thiadiazine Moiety: A Review

1,2,4-triazolo[3,4-b][1,3,4]thiadiazin Artığı İçeren Yeni Bileşiklerin Keşfi, Sentezi ve Biyolojik Aktiviteleri: Bir Derleme

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ABSTRACT

Lately, much interest has been focused on the chemistry and the biological activity of fused heterocyclic compounds carrying nitrogen atoms because of their utility in various applications, and over the years N-bridged heterocyclic systems derived from 1,2,4-triazoles attracted the interest of researchers owing to the hopeful promise of their pharmacological activities such as antimicrobial, antifungal, molluscicidal, nematicidal, analgesic, anti-inflammatory, anticancer, phosphodiesterase 4 inhibitor, acetylcholinesterase, butyrylcholinesterases and alkaline phosphatase inhibitor. The fused ring of triazole and thiadiazines, named as triazolothiadiazines, represents a specific and important class of N-bridged heterocycles with their remarkable and wide range of biological activity and there are not enough studies that include the current developments associated with the new synthesis techniques and novel biological evaluation results of 1,2,4-triazolo[3,4-b][1,3,4] thiadiazine derivatives. In an attempt to overcome this deficiency in the literature, we deeply researched the literature and formed a review study about the discovery, synthesis and activity evaluation of new compounds bearing 1,2,4-triazolo[3,4-b][1,3,4]thiadiazine moiety within the years of 1996-2019. We aimed to provide scientists with a wide data resource about 1,2,4-triazolo[3,4-b][1,3,4]thiadiazine derivatives, thus helping them perform a more organized and fertile drug discovery operation during their experimental studies.

Keywords: 1,2,4-triazolo[3,4-b][1,3,4]thiadiazine, synthesis, biological activity

ÖZ

Son zamanlarda, geniş ölçekte çeşitlilik gösteren uygulamalardaki kullanımları nedeniyle azot atomu taşıyan kaynaşmış heterosiklik bileşiklerin kimyası ve biyolojik aktivitesi üzerine olan ilgi artmış ve yoğunlaşmıştır. Yıllar boyunca 1,2,4-triazollerden türetilen azot köprüli heterosiklik sistemler antimikrobiyal, antifungal, molluscidal, nematisidal, analjezik, anti-inflamatuar, antikanser, fosfodiesteraz-4 inhibitörü, etsetkolinesteraz, butyrylcolinesteraz ve alcalen fosfataz inhibitörü gibi farklı biyolojik yönerde, umut vadeden farmakolojik aktiviteler göstermiş ve buna bağlı olarak araştırmacılarnın ilgisini çekmiştir. Triazo ve tiyadiazin halkalarının kaynaşmasıyla oluşan triazolotiyadiazinler azot köprüli heterosiklik bileşikler arasında önemli ve dikkat çekici bir alt sınıf oluşturmuştur, buna karşın 1,2,4-triazolo[3,4-b][1,3,4]tiyadiazin türevlerinin aç gönül sentez teknikleri ve yeni biyolojik aktivite bulgularını içeren çalışmalar literatürde yeterli oranda bulunmamaktadır. Bu noktadan hareketle 1996-2019 tarih aralığına referans alarak 1,2,4-triazolo[3,4-b][1,3,4]tiyadiazin yapısı taşıyan yeni bileşiklerin keşfi, sentezi ve aktivite değerlendirilmesi ile ilişkilidir bir derleme çalışması yürütülmüştür. Araştırmacılarnın daha organize ve verimli bir ilaç keşfi prosesi gerçekleştirmelerine yardımcı olmak amacıyla.

Anahtar Kelimeler: 1,2,4-triazolo[3,4-b][1,3,4]tiyadiazin, sentez, biyolojik aktivite
INTRODUCTION

1,2,4-triazolo[3,4-b][1,3,4]thiadiazines are 9-membered heterocyclic compounds containing 4 carbon atoms, 4 nitrogen atoms and 1 sulfur atom, and the main structure of the compound is formed by fused triazole and thiaizaizine rings. The structure is capable of acting as both a hydrogen bond acceptor and a hydrogen bond donor. This qualification gives the group the characteristic of being a specific pharmacophore group capable of making significant interactions with the active site of various target receptors. In addition, due to the polar nature of the structure, the triazole moiety can increase the solubility of the ligand and thereby the pharmacokinetic profile of the drug is positively affected. 1,2,4-triazolo[3,4-b][1,3,4]thiadiazine derivatives have been reported in the literature by their antimicrobial, antifungal, molluscicidal and nematocidal, analgesic, anti-inflammatory, anticancer, phosphodiesterase-4 inhibitor, acetylcholinesterase, butyrylcholinesterase and alkaline phosphatase inhibitor activity. Due to the wide pharmacological effect of 1,2,4-triazolo[3,4-b][1,3,4]thiadiazine derivatives, researchers are interested in the synthesis of novel compounds bearing the 1,2,4-triazolo[3,4-b][1,3,4]thiadiazine moiety.

| Compound | Ar     | R     | R'    | Compound | Ar     | R     | R''   |
|----------|--------|-------|-------|----------|--------|-------|-------|
| 3a       | C₆H₅   | C₂H₅  | Et    | 4a       | C₆H₅   | C₂H₅  | Me    |
| 3b       | 4-MeC₆H₄| C₂H₅  | Et    | 4b       | 4-CIC₆H₄| C₂H₅  | Me    |
| 3c       | 4-ClC₆H₄| C₂H₅  | Et    | 4c       | C₆H₅   | H     | Me    |
| 3d       | 4-BrC₆H₄| C₂H₅  | Et    | 4d       | C₆H₅   | n-C₃H₇| Me    |
| 3e       | 4-FC₆H₄ | C₂H₅  | Et    | 4e       | C₆H₅   | C₂H₅  | n-Pr  |
| 3f       | 4-NO₂C₆H₄| C₂H₅  | Et    | 4f       | 4-CIC₆H₄| C₂H₅  | n-Pr  |
| 3g       | C₆H₅   | H     | Et    | 4g       | C₆H₅   | H     | n-Pr  |
| 3h       | C₆H₅   | n-C₃H₇| Et    | 4h       | C₆H₅   | n-C₃H₇| n-Pr  |
| 3i       | C₆H₅   | C₂H₅  | i-Pr  | 4i       | C₆H₅   | C₆H₅  | Me    |
| 3j       | 4-ClC₆H₄| C₆H₅  | i-Pr  | 4j       | C₆H₅   | C₆H₅  | n-Pr  |
| 3k       | C₆H₅   | H     | i-Pr  | 5a       | C₆H₅   | C₆H₅  | Me/n-Pr|
| 3l       | C₆H₅   | n-C₃H₇| i-Pr  | 5b       | 4-CIC₆H₄| C₆H₅  | Me/n-Pr|
| 3m       | C₆H₅   | C₂H₅  | Et    | 5c       | C₆H₅   | H     | Me/n-Pr|
| 3n       | C₆H₅   | C₂H₅  | i-Pr  | 5d       | C₆H₅   | n-C₃H₇| Me/n-Pr|
|          |        |       |       | 5e       | C₆H₅   | Et    | C₆H₅  |

Scheme 1. The synthesis pathway of the novel 1,2,4-triazolo[3,4-b][1,3,4]thiadiazine derivatives.
Biological Activity of 1,2,4-triazolo[3,4-b][1,3,4]thiadiazine Derivatives

Compounds bearing the 1,2,4-triazolo[3,4-b][1,3,4]thiadiazine ring structure are disclosed in the literature as antimicrobial, antifungal, molluscicidal, analgesic, anti-inflammatory, anticancer, phosphodiesterase-4 inhibitor, acetylcholinesterase, butyrylcholinesterase and alkaline phosphatase inhibitor agents.

Antimicrobial and antifungal activity

A series of novel 7H-7-alkoxy-3-alkyl/phenyl-6-aryl-s-triazolo[3,4-b][1,3,4]thiadiazines were synthesized by Pundeer et al. and the antimicrobial and antifungal activity of the compounds were evaluated. The biological activities of the compounds were compared with the antibacterial ciprofloxacin and antifungal amphotericin-B. The activity results showed that the novel compounds possess significant activity against the gram-positive bacteria, Staphylococcus aureus, and Bacillus subtilis and the yeasts, Saccharomyces cerevisiae and Candida albicans.

In another study, a series of novel 4-(alkylidene/arylidene)-amino-5-(2-furanyl)-2,4-dihydro-3H-1,2,4-triazole-3-thiones and 6-aryl-3-(2-furanyl)-7H-1,2,4-triazolo[3,4-b][1,3,4]thiadiazines were synthesized by Ergenç et al. and the antimicrobial and antifungal activity of the compounds were evaluated. Of the novel compounds tested, 2b, 2g and 4f were found as active against Staphylococcus aureus and/or Staphylococcus epidermidis, whereas all exhibited different degrees of antifungal activity.

Table 1. In vitro antimicrobial activity of the tested compounds through agar well diffusion method

| Compounds | Diameter of growth of inhibition zone (mm)* |
|-----------|---------------------------------------------|
|           | S. aureus | B. subtilis | S. cerevisiae | C. albicans |
| 3a        | 13.6      | 18.3       | -            | -           |
| 3b        | 12.3      | 17         | 10.6         | -           |
| 3c        | -         | -          | 18.3         | 15.6        |
| 3d        | 12.3      | 13.6       | -            | -           |
| 3e        | -         | -          | 13.6         | 13          |
| 3f        | -         | -          | 15.6         | 13.3        |
| 3g        | 16.3      | 20.3       | 13.6         | 13.6        |
| 3h        | 13.6      | 17.6       | -            | -           |
| 3i        | 13.6      | 12.3       | -            | -           |
| 3j        | 11.3      | 12.3       | 11.3         | -           |
| 3k        | 14.6      | 13.6       | 13.3         | -           |
| 3l        | 12.3      | 14.6       | -            | -           |
| 3m        | 24.3      | 17.6       | 17.6         | 15.6        |
| 3n        | 18.3      | 15.6       | 13.3         | -           |
| 4a        | 22.3      | 21.6       | 15.3         | 16.3        |
| 4b        | 19.3      | 16.3       | 12.6         | 13.0        |
| 4c        | 19.6      | 18.6       | 13.6         | 13.3        |
| 4d        | 16.0      | 18.3       | 12.6         | -           |
| 4e        | 14.0      | 13.6       | -            | -           |
| 4f        | 12.6      | 16.3       | 10.3         | -           |
| 4g        | 15.6      | 18.3       | -            | -           |
| 4h        | 13.6      | 16.3       | 15.3         | -           |
| 4i        | 25.6      | 18.0       | 18.6         | -           |
| 4j        | 20.6      | 17.3       | 17.3         | -           |
| Ciprofloxacin | 26.6      | 24.0       | Nt           | Nt          |
| Amphotericin-B | Nt        | Nt         | 13.6         | 14.3        |

(* Values, including diameter of the well (8mm), are means of three replicates
(-) no activity, Nt not tested

Table 1. In vitro antimicrobial activity of the tested compounds through agar well diffusion method

| Compounds | Diameter of growth of inhibition zone (mm)* |
|-----------|---------------------------------------------|
|           | S. aureus | B. subtilis | S. cerevisiae | C. albicans |
| 3a        | 13.6      | 18.3       | -            | -           |
| 3b        | 12.3      | 17         | 10.6         | -           |
| 3c        | -         | -          | 18.3         | 15.6        |
| 3d        | 12.3      | 13.6       | -            | -           |
| 3e        | -         | -          | 13.6         | 13          |
| 3f        | -         | -          | 15.6         | 13.3        |
| 3g        | 16.3      | 20.3       | 13.6         | 13.6        |
| 3h        | 13.6      | 17.6       | -            | -           |
| 3i        | 13.6      | 12.3       | -            | -           |
| 3j        | 11.3      | 12.3       | 11.3         | -           |
| 3k        | 14.6      | 13.6       | 13.3         | -           |
| 3l        | 12.3      | 14.6       | -            | -           |
| 3m        | 24.3      | 17.6       | 17.6         | 15.6        |
| 3n        | 18.3      | 15.6       | 13.3         | -           |
| 4a        | 22.3      | 21.6       | 15.3         | 16.3        |
| 4b        | 19.3      | 16.3       | 12.6         | 13.0        |
| 4c        | 19.6      | 18.6       | 13.6         | 13.3        |
| 4d        | 16.0      | 18.3       | 12.6         | -           |
| 4e        | 14.0      | 13.6       | -            | -           |
| 4f        | 12.6      | 16.3       | 10.3         | -           |
| 4g        | 15.6      | 18.3       | -            | -           |
| 4h        | 13.6      | 16.3       | 15.3         | -           |
| 4i        | 25.6      | 18.0       | 18.6         | -           |
| 4j        | 20.6      | 17.3       | 17.3         | -           |
| Ciprofloxacin | 26.6      | 24.0       | Nt           | Nt          |
| Amphotericin-B | Nt        | Nt         | 13.6         | 14.3        |

(* Values, including diameter of the well (8mm), are means of three replicates
(-) no activity, Nt not tested
Molluscicidal and Nematicidal activity

A series of pyrazolyl-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-6-yl)-2H-pyran-2-one derivatives were synthesized by Penta et al. and the novel compounds were evaluated for their in vitro antimicrobial activity against gram-positive bacteria (Staphylococcus aureus and Bacillus subtilis), gram-negative bacteria (Escherichia coli and Klebsiella pneumoniae), anti-fungal activity against Candida albicans, and nematicidal activity against Meloidogyne incognit14. It was found that, among the newly synthesized compounds, there were compounds having excellent antimicrobial and nematicidal activity against tested bacteria, fungi and nematodes.

In another study, a series of 3-(2,4-dichlorophenoxy)methyl)-1,2,4-triazolo(thiadiazoles and thia diazines) were synthesized by El Shehry et al. and the novel compounds were evaluated for their molluscicidal activity6. The compounds 3, 4b, 8 and 10 exhibited significant molluscicidal activities.

Analgesic and anti-inflammatory activity

A series of 3,6-disubstituted 7H-1,2,4-triazolo[3,4-b][1,3,4]thiadiazines were synthesized by Aytaç et al. and the novel compounds were evaluated for their analgesic/anti-inflammatory activity3. Among the newly synthesized compounds, the compounds 4, 1c, 2b and 4c showed the highest anti-inflammatory activity. Also compounds 2, 3, 4b, 3a and 4b showed higher or similar analgesic activity to that of aspirin at the 100 mg/kg dose level. The activity results showed that some of the novel

| Compounds | S. aureus | B. subtilis | S. cerevisiae | C. albicans |
|-----------|-----------|-------------|---------------|-------------|
| 3a        | 256       | 64          | -             | -           |
| 3b        | >256      | 128         | >256          | -           |
| 3c        | -         | -           | 32            | 64          |
| 3d        | >256      | 256         | -             | -           |
| 3e        | -         | -           | 128           | 128         |
| 3f        | -         | -           | 64            | 128         |
| 3g        | 128       | 64          | 128           | -           |
| 3h        | 256       | 128         | -             | -           |
| 3i        | 256       | >256        | -             | -           |
| 3j        | >256      | >256        | >256          | -           |
| 3k        | 256       | 256         | 128           | -           |
| 3l        | >256      | 256         | -             | -           |
| 3m        | 16        | 128         | 32            | 64          |
| 3n        | 128       | 256         | 128           | -           |
| 4a        | 32        | 32          | 64            | 64          |
| 4b        | 64        | 128         | 128           | 128         |
| 4c        | 64        | 64          | 128           | 128         |
| 4d        | 128       | 64          | 256           | -           |
| 4e        | 256       | 256         | -             | -           |
| 4f        | >256      | 128         | >256          | -           |
| 4g        | 128       | 64          | -             | -           |
| 4h        | 256       | 128         | 64            | -           |
| 4i        | 16        | 64          | 32            | -           |
| 4j        | 64        | 128         | 64            | -           |
| Ciprofloxacin | 128     | 64          | 128           | 128         |
| Amphotericin-B  | Nt   | Nt          | 100           | 100         |

(-) no activity, Nt not tested
Values, including diameter of the well (8mm), are means of three replicates
Scheme 2. The synthesis of the novel compounds

| Compound | R        | Mp [°C] | Yield [%] | Formula (molecular mass)         |
|----------|----------|---------|-----------|----------------------------------|
| 2b       | 4-BrC₆H₄ | 219-220 | 67        | C₁₃H₉BrN₄O₅S (349.21)           |
| 2g       | 2-(5-nitro-2-furyl)-ethenyl | 219-220 | 87        | C₁₃H₉N₅O₄S (333.31)            |
| 4f       | 4-NO₂C₆H₄ | >300    | 91        | C₁₄H₉N₅O₃S (327.32)           |

Scheme 3. The reagents and the synthesis pathway
compounds possess significant activity and have potential for being a new analgesic/anti-inflammatory agent.

**Anticancer activity**

A series of 1,2,4-triazolo[3,4-b][1,3,4]thiadiazine derivatives were synthesized by Ahmad et al. and the novel compounds were evaluated for their anticancer activity. In this study, compounds having a triazolothiadiazine nucleus were found as potentially active anticancer molecules.

**Phosphodiesterase-4 Inhibitor Activity**

A series of 1,2,4-triazolo[3,4-b][1,3,4]thiadiazine derivatives were synthesized by Baeeri et al. and the new compounds were evaluated for their phosphodiesterase-4 (PDE-4) inhibitor activity. The novel compounds were tested on cultured NIH-3T3 cells to analyze their safety and activity in NIH-3T3 mouse fibroblastic cells in comparison with rolipram, which is a selective PDE-4 inhibitor. Extracellular cyclic guanosine monophosphate (cGMP) and cyclic adenosine monophosphate (cAMP) concentrations were evaluated to understand the PDE inhibition rate. The results showed that all tested compounds caused a marked increase in the concentration of cAMP, whereas the concentration of cGMP stayed approximately unchanged.

**Acetylcholinesterase, butyrylcholinesterases and alkaline phosphatase inhibitor activity**

In another study, a series of 1,2,4-triazolo[3,4-b][1,3,4]thiadiazole and 1,2,4-triazolo[3,4-b][1,3,4]thiadiazine derivatives were synthesized by Khan et al. and the new compounds were evaluated for their acetylcholinesterase, butyrylcholinesterases and alkaline phosphatase inhibitor activity. According to activity results, the novel compounds showed significant biological activity.

**RESULTS**

As a result of our study, it has been detected that there are many different 1,2,4-triazolo[3,4-b][1,3,4]thiadiazine derivatives that exhibit serious pharmacological activity and possess the potential to be a leading compound. However, in the literature, there are not enough studies supported by computer-aided drug design techniques associated with 1,2,4-triazolo[3,4-b][1,3,4]thiadiazine derivatives. Computer-aided drug design tools potentially minimize time and cost in drug discovery processes.

Table 3. Nematicidal activity of the tested compounds

| Compounds | 24h | | | 48h | | |
|------------|-----|-----|-----|-----|-----|-----|
|            | 250 (µg/ml) | 150 (µg/ml) | 50 (µg/ml) | 250 (µg/ml) | 150 (µg/ml) | 50 (µg/ml) |
| 5a         | 5    | 3    | 2    | 8    | 5    | 4    |
| 5b         | 5    | 3    | 2    | 9    | 6    | 4    |
| 6a         | 42   | 28   | 15   | 55   | 33   | 26   |
| 6b         | 8    | 5    | 2    | 11   | 6    | 3    |
| 6c         | 35   | 20   | 10   | 51   | 28   | 19   |
| 6d         | 18   | 10   | 6    | 28   | 16   | 10   |
| 6e         | 40   | 23   | 19   | 44   | 28   | 20   |
| 6f         | 67   | 43   | 32   | 85   | 63   | 45   |
| 6g         | 52   | 35   | 20   | 73   | 55   | 28   |
| 6h         | 5    | 3    | 1    | 8    | 5    | 3    |
| 6i         | 5    | 3    | 2    | 9    | 5    | 3    |
| 6j         | 5    | 3    | 2    | 8    | 6    | 3    |
| 6k         | 5    | 3    | 2    | 12   | 5    | 3    |
| 6l         | 3    | 0    | 0    | 5    | 2    | 0    |
| 6m         | 2    | 0    | 0    | 3    | 2    | 0    |
| DMSO       | 0    | 0    | 0    | 0    | 0    | 0    |
taking advantage of computer-aided drug design technology, researchers may be able to design potentially active and original molecules. Researchers may also carry out in silico simulations using the software to determine binding modes of the compounds with the related target and calculate potential drug-likeness and other properties that are related to absorption, distribution, metabolism,
excretion, and the toxicity of the compounds. The overall results obtained from molecular modeling studies and the pharmacological responses of the synthesized molecules can provide insight into the synthesis of more efficient target-specific agents, which might also have higher selectivity and activity. Hence, researchers definitely should continue their drug discovery investigations and researches should be supported by computer-aided drug design techniques.
Table 4. Physico-chemical properties of the novel 1,2,4-triazolo[3,4-b][1,3,4]thiadiazine derivatives

| Compounds | R | Molecular formula | Physical state | Melting Point (°C) | % Yield | Molecular Weight |
|-----------|---|-------------------|----------------|-------------------|---------|------------------|
| 4a        |   | C_{20}H_{25}N_{4}S | Brown sticky liquid | - | 65 | 354.470 |
| 4b        |   | C_{27}H_{40}N_{4}S | Brown sticky liquid | - | 62 | 452.631 |
| 4c        |   | C_{27}H_{40}N_{4}O_{S} | Brown sticky liquid | - | 60 | 468.630 |
| 4d        |   | C_{27}H_{40}N_{4}O_{S} | Brown sticky liquid | - | 60 | 468.630 |

Scheme 7. Rolipram (1), alkoxy-substituted 3,6-diphenyl-7H-1,2,4-triazolo[3,4-b][1,3,4]thiadiazines (2), and some new 6-(3-(cyclopentyloxy)-4-methoxyphenyl)-3-aryl-7H-1,2,4-triazolo[3,4-b][1,3,4]thiadiazines (3)
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