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Design, Synthesis, Anti-Microbial Evaluation, and Docking Studies of New *N*-Benzyl-3-Indole Derivatives

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Abstract

New 3-hydrazinecarbothioamide-*N*-benzylindol derivatives **3a**,**b** have been synthesized by the reaction of *N*-benzyl-3-acetylindol **1** with hydrazinecarbothioamide **2a/b**. Then compounds **3a**,**b** were cyclized with different α-halocarbonyl derivatives to produce thiazolidinone and thiazole compounds in moderate to excellent yields. The anti-microbial activities were screened for all the newly synthesized compounds. The experimental results indicated that only hydrazinecarbothioamides **3a**,**b** showed good anti-microbial activities toward all the studied microbes compared with the used references. Despite that, compound **12e** had anti-bacterial efficacy versus *E. coli*, compound **12d** had anti-microbial activity against *C. albicans*, and compounds **12c** and **12d** had anti-fungal action against *A. niger*. Also, compounds **3a** and **3b** exhibited higher MIC values than ciprofloxacin. A molecular docking study was conducted for the most potent compounds **3a**,**b** to assess theoretically the anti-microbial effect on the crystal structures of *S. aureus* and *E. coli* DNA gyrase B enzymes. The results of molecular docking proved that hydrazinecarbothioamides **3a,b** had good binding energy and good binding mode actions towards the DNA gyrase B enzymes through their action with the main amino acids of the aimed enzymes in a similar way to the native inhibitor, consequently preventing cell division that leads to the death of bacteria.

***Keywords*:** Hydrazinecarbothioamide; Thiazolidinone; Thiazole; Anti-microbial activities; DNA gyrase B.

1. Introduction

The synthesis of nitrogen-containing heterocyclic compounds is of great interest to researchers as they can be used as essential structural components and useful functional materials to develop several bioactive compounds. Many of these compounds have been successfully used with high bioavailability, low toxicity, and curative effects in the prevention and treatment of numerous types of diseases and disorders. They represent a wide range of potential uses in drug discovery. Among different kinds of *N*-heteroaromatics, the indole derivatives, thiazolidinone, and thiazole compounds represent key building blocks to developing several bioactive compounds [1-6].

Indole is a five-membered pyrrole ring fused to a six-membered benzene ring naturally formed by bacteria. Indole derivatives are a core structure in many synthetically and naturally bioactive compounds. Their diverse biological characteristics and potential properties in synthetic chemistry were well established in litterateurs [7-17]. The pharmacological properties of indole derivatives including analgesic [7], anti-malarial [8], anti-microbial [9], anti-viral [10], anticonvulsant [11], anti-hypertensive [12], anti-HIV activities [13], anti-asthmatic [14], anti-Alzheimer [15], anti-diabetic [16], and anti-depressant [17] were documented.

Furthermore, compounds bearing hydrazinecarbothioamide (thiosemicarbazide) moiety have a wide range of established biological potencies, including anti-bacterial [18], anti-fungal [19], anti-oxidant [20], anti-tubercular [21], anti-cancer [22], and anti-convulsant [23]. In addition,

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hydrazinecarbothioamide are significant core structure units of many bioactive compounds, including 4-thiazolidinones [24], 1,3,4-thiadiazoles [25], 1,3,4-oxadiazoles [25], and 1,2,4-triazole-3-thiones [26, 27].

On the other hand, many thiazolidinone and thiazole compounds are important classes in pharmacology due to their biological activities and therapeutic potential, making them noteworthy in drug discovery development and some are applied as drugs [28, 29]. Over the past four decades, much research work has been carried out to explore the diverse and potentially useful applications of thiazolidinone and thiazole compounds [30]. Thiazolidinones were classically discovered as hypoglycemic agents (blood sugar-lowering) [29]. Pioglitazone [31] which is one of the thiazolidinedione (TZD) family has been approved as a drug that is primarily used to treat type 2 diabetes mellitus [32]. The recent years have witnessed a wide diversity of their activities, starting from anti-inflammatory [33,34], anti-hypertensive [35], anti-proliferative [36] to anti-tumor [34]. Furthermore, the thiazole ring and its derivatives have established an identity as anti-infective [37], anti-inflammatory [38], and anti-cancer agents [39]. Furthermore, thiazolidinone and thiazole compounds have exhibited significant anti-microbial characteristics against an assortment of pathogens, such as viruses, bacteria, and fungi [40-43]. However, even though the arising of anti-biotic-resistant bacteria causes a great health issue, they were explored as potential agents in combating infectious diseases [44-45].

Considering the attributes mentioned above and in continuation of our trials to control this issue or reduce its effects [46-48], we herein present easy and efficient strategies for the synthesis of novel hydrazinecarbothioamide, thiazolidinone, and thiazole compounds bearing the *N*- benzyl indole moiety. The idea behind designing the new compounds is to combine two potential pharmacophore structures into one molecule. It was imagined that this molecular hybrid containing these moieties might be produced as lead anti-microbial agents [49-53].

The potencies of the synthesized products as anti-microbial agents were evaluated. Additionally, the study used a molecular docking strategy to evaluate the interactions between the prospective compounds and the DNA gyrase B enzymes responsible for regulating bacterial reproduction.

2. Experimental

General

Melting points of synthesized compounds were measured using an open capillary tube on a digital Gallen-Kamp MFB-595 instrument; melting points were not corrected. FTIR spectra were recorded using KBr disc on a JASCO FT-IR 6100 (JASCO, Japan). Mass spectra were carried out at 70 eV on an MS-50 Kratos (A.E.I.) spectrometer given with a data system. Using DMSO-d6 and TMS as an internal standard,13C NMR (75 MHz) and 1H NMR (300 MHz) spectra were recorded on a Bruker model (300 MHz) Ultra Shield NMR spectrometer. Units of measurement for chemical shifts are δ ppm. The elemental analysis of the new compounds was accomplished at Cairo University's Microanalysis Lab in Giza, Egypt, by using the basic elementar Analysensysteme GmbH - vario EL III Element Analyzer, Germany. Microchemical analysis (C/H/N) was used to measure the purity of all new products, and the observed values were agreed well (±0.2%) with the calculated values. Materials and reagents were purchased from Aldrich and standard techniques were used to dry the solvents. Thin-layer chromatography (TLC, silica gel F254 plates (Merck)) was used to monitor the progress of reactions and the homogeneity of the products.

Synthesis

General procedure for preparation of benzyl-1H-indol-3-yl)ethylidene)-N-(aryl)hydrazine-1-carbothioamides **3a**,**b**

10 mmol of aryl hydrazinecarbothioamide **2a/b** (1.8 g for **2a**, 2.0 g for **2b**) was added to a solution of 1-(1-benzyl-1*H*-indol-3-yl)ethenone (**1**) (2.5 g, l0 mmol) in ethanol (30 mL) containing a few drops of acetic acid. The mixture was heated under reflux temperature for ~2 h (TLC). The solid material was collected after cooling and then crystallized from ethanol to afford the target carbothioamides **3a**,**b**.

2-(1-(1-Benzyl-1H-indol-3-yl)ethylidene)-N-(p-tolyl)hydrazine-1-carbothioamide (**3a**). Bright yellow needles; 82%; mp 179-181°C; IR (KBr): ν = 3440, 3458 (2NH), 1615 (C=N), 1267 (C=S) cm^{-1}; ^{1}H NMR (300 MHz, DMSO): δ = 2.30, 2.44 (2s, 6H, 2Me), 5.47 (s, 2H, CH_2), 7.16–7.35 (m, 10H, Ar–H), 7.49-7.51 (d, 1H, J_{HH} = 6 Hz, Ar–H), 7.59-7.61 (d, 2H, J_{HH} = 6 Hz, Ar-H), 8.21(s, 1H, pyrrole), 9.61, 10.51 (2br, 2H, 2*H*N, D_2O exch) ppm; ^{13}C-NMR (75 MHz, DMSO): δ = 178.5, 137.6, 136.9, 135.6, 134.1, 133.3, 130.4, 129.6, 128.6, 127.5, 126.4, 125.4, 123.2, 121.6, 120.8, 119.9, 113.0, 110.1, 49.7, 22.7, 13.8 ppm; EI-MS (70 eV): m/z (%) = 412 (M^+, 30). Anal. Calcd for $C_{25}H_{24}N_4S$ (412.56): C, 72.78; H, 5.86; N, 13.58. Found: C, 72.99; H, 5.61; N, 13.80%.

2-(1-(1-Benzyl-1H-indol-3-yl)ethylidene)-N-(4-chlorophenyl)hydrazine-1-carbothioamide (**3b**). Bright yellow needles; 80%; mp 195-197°C; IR (KBr): ν = 3442, 3455 (2NH), 1618 (C=N), 1270 (C=S) cm^{-1}; ^{1}H NMR (300 MHz, DMSO): δ = 2.45 (s, 3H, CH_3), 5.48 (s, 2H, CH_2), 7.18-7.19 (d, 1H, J_{HH} = 3 Hz, Ar–H), 7.24–7.35 (m, 7H, Ar–H), 7.40-7.43 (d, 2H, J_{HH} = 9 Hz, Ar–H), 7.49-7.51 (d, 1H, J_{HH} = 6 Hz, Ar-H), 7.76-7.79 (d, 2H, J_{HH} = 9 Hz, Ar-H), 8.22(s, 1H, pyrrole), 9.75, 10.66 (2br, 2H, 2NH, D_2O exch) ppm; ^{13}C-NMR (75 MHz, DMSO): δ = 179.3, 138.7, 136.9, 135.8, 134.1, 133.3, 132.5, 129.7, 128.6, 128.1, 127.5, 127.1, 123.3, 122.5, 121.6, 119.7, 112.8, 110.6, 50.2, 14.5 ppm; EI-MS (70 eV): m/z (%) = 434 (M^+ + 2, 28), 432 (M^+, 32). Anal. Calcd for $C_{24}H_{21}ClN_4S$ (432.97): C, 66.58; H, 4.89; N, 12.94. Found: C, 66.80; H, 4.64; N, 12.72%.

General procedure for the synthesis of thiazolidine derivatives **6a**,**b**

Equimolar amounts of carbothioamides **3a/b** (1 mmol) (0.4 g for **3a**, 0.4 g for **3b**), ethyl 2-bromoacetate (**4**) (0.17 g, 1 mmol), and fused sodium acetate (0.25 g, 3 mmol) in absolute ethanol (15 mL) were treated under reflux for 6–7 h (TLC). After cooling, The formed solid was filtered, washed with EtOH, dried, and crystallized from MeOH to give the corresponding products **6a**,**b**.

2-((1-(1-Benzyl-1H-indol-3-yl)ethylidene)hydrazineylidene)-3-(p-tolyl)thiazolidin-4-one (**6a**). White solid; 81%; mp 80-82 °C; IR (KBr): ν = 1655 (C=N), 1638 (C=O) cm^{-1}; ^{1}H NMR (300 MHz, DMSO): δ = 2.21, 2.38 (2s, 6H, 2*Me*), 4.09 (s, 2H, CH_2/thiazolidine), 5.45 (s, 2H, CH_2N), 7.16–7.32 (m, 11H, Ar–H), 7.47-7.49 (d, 1H, J_{HH} = 6 Hz, Ar–H) 8.10 (s, 1H, pyrrole), 8.47-8.50 (dd, 1H, J_{HH} = 6.3 Hz, Ar–H) ppm; ^{13}C-NMR (75 MHz, DMSO): δ = 169.8, 163.1, 158.9, 137.7, 136.9, 135.9, 135.7, 129.7, 129.3, 128.6, 127.1, 126.7, 126.3, 125.4, 121.6, 120.5, 119.1,110.6, 108.4, 48.7, 40.3, 22.1, 16.1 ppm; EI-MS (70 eV): m/z (%) = 452 (M^+, 30). Anal. Calcd for $C_{27}H_{24}N_4OS$ (452.58): C, 71.66; H, 5.35; N,12.38. Found: C, 71.91; H, 5.18; N, 12.16%.

2-((1-(1-Benzyl-1H-indol-3-yl)ethylidene)hydrazineylidene)-3-(4-chlorophenyl)thiazolidin-4-one (**6b**). White solid; 85%; mp > 250 °C; IR (KBr): ν = 1660 (C=N), 1635 (C=O) cm^{-1}; ^{1}H NMR (300 MHz, DMSO): δ = 2.22 (s, 3H, Me), 4.10 (s, 2H, CH_2/thiazolidine), 5.45 (s, 2H, CH_2N), 7.17–7.31 (m, 7H, Ar–H), 7.49-7.52 (d, 3H, J_{HH} = 9 Hz, Ar–H) 7.59-7.62 (d, 2H, J_{HH} = 9 Hz, Ar–H) 8.11 (s, 1H, pyrrole), 8.50-8.52 (d, 1H, J_{HH} = 6 Hz, Ar–H) ppm; ^{13}C-NMR (75 MHz, DMSO): δ = 171.8, 160.0, 159.3, 137.6, 137.0, 134.2, 133.0, 132.8, 130.0, 128.9, 128.6, 127.5, 127.1, 123.3, 122.5, 121.0, 119.3, 112.9, 110.6, 49.4, 40.4, 15.4 ppm; EI-MS (70 eV): m/z (%) = 474 (M^+ + 2, 22), 472 (M^+, 28). Anal. Calcd for $C_{26}H_{21}ClN_4OS$ (472.99): C, 66.02; H, 4.48; N, 11.85. Found: C, 66.23; H, 4.25; N, 11.63%.

General procedure for the synthesis of dihydrothiazole derivatives **9a**,**b**

Mixing equimolar amounts of hydrazine-1-carbothioamides **3a**,**b** (1 mmol) (0.4 g for **3a**, 0.4 g for **3b**) and chloroacetone (**7**) (0.1 g, 1 mmol) in EtOH (10 mL) containing TEA (~0.07 mL) was treated under reflux for ~9 h (TLC). After cooling; the solid was collected, filtered, washed with EtOH, dried, and then crystallized from MeOH afforded the corresponding dihydrothiazole derivatives **9a**,**b**.

2-((1-(1-Benzyl-1H-indol-3-yl)ethylidene)hydrazineylidene)-4-methyl-3-(p-tolyl)-2,3-dihydrothiazole (**9a**). Pale gray solid; 85%; mp 195-197 °C; IR (KBr): ν = 1645 (C=N) cm^{-1}; ^{1}H NMR (300 MHz, DMSO): δ = 1.88, 2.14, 2.16 (3s, 9H, 3Me), 5.43 (s, 2H, CH_2N), 6.18 (s, 1H, H-thiazole) 7.13–7.23 (m, 2H, Ar–H), 7.25-7.29 (m, 7H, Ar–H), 7.48-7.51 (dd, 2H, J_{HH} = 9.3 Hz, Ar–H), 7.57, 7.60 (2d, 2H, J_{HH} = 3.3, J_{HH} = 2.1 Hz, Ar–H), 7.90 (s, 1H, pyrrole), 8.50-8.53 (dd, 1H, J_{HH} = 6, 3 Hz, Ar–H) ppm; ^{13}C-NMR (75 MHz, DMSO): δ = 164.1, 157.7, 139.5, 137.7, 136.9, 136.2, 135.7, 133.4, 130.8, 129.5, 128.6, 127.5, 127.1, 126.3, 121.6, 120.5, 119.5, 110.6, 108.4, 95.1, 50.0, 21.7, 15.1, 13.6 ppm; EI-MS (70 eV): m/z (%) = 450 (M^+, 27). Anal. Calcd for $C_{28}H_{26}N_4S$ (450.60): C, 74.63; H, 5.82; N, 12.43. Found: C, 74.85; H, 5.60; N, 12.21%.

2-((1-(1-Benzyl-1H-indol-3-yl)ethylidene)hydrazineylidene)-3-(4-chlorophenyl)-4-methyl-2,3-dihydrothiazole (**9b**). Bright green substance; 88%; mp 183-185 °C; IR (KBr): ν = 1640 (C=N) cm^{-1}; ^{1}H NMR (300 MHz, DMSO): δ = 1.99, 2.57 (s, 2H, Me), 5.58 (s, 2H, CH_2N), 6.12 (s, 1H, H-thiazole), 7.16–7.34 (m, 8H, Ar–H), 7.49-7.53 (m, 2H, Ar–H), 7.77-7.82 (q, 2H, J_{HH} = 6 Hz, Ar–H), 8.24 (s, 1H, pyrrole), 8.68-8.69 (d, 1H, J_{HH} = 3 Hz, Ar–H) ppm; ^{13}C-NMR (75 MHz, DMSO): δ = 162.9, 158.1, 139.1, 138.3, 137.0, 136.0, 135.1, 133.1, 131.3, 130.6, 128.7, 128.2, 127.1, 125.6, 122.4, 120.9, 119.2, 110.3, 108.1, 95.3, 49.5, 15.8, 12.7 ppm; EI-MS (70 eV): m/z (%) = 473 (M^+ + 2, 20), 471 (M^+, 26). Anal. Calcd for $C_{27}H_{23}ClN_4S$ (471.02): C, 68.85; H, 4.92; N, 11.90. Found: C, 68.62; H, 4.69; N, 11.67%.

General procedure for the synthesis of diazenyl-2,3-dihydrothiazole derivatives **12a-f**

Mixing equimolar amounts of hydrazine-1-carbothioamides **3a**,**b** (1 mmol) (0.4 g for **3a**, 0.4 g for **3b**) and hydrazonoyl chloride derivatives **10a-c** (1 mmol); 2-oxo-*N*-phenylpropanehydrazonoyl chloride (**10a**, 0.2 g), 2-oxo-*N*-(*p*-tolyl)propanehydrazonoyl chloride (**10b**, 0.21 g), or *N*-(4-chlorophenyl)-2-oxopropanehydrazonoyl chloride (**10c**, 0.23 g) in EtOH (20 mL) containing TEA (~0.07 mL) was treated under reflux for 10–12 h (TLC). After cooling the reaction medium, the precipitate was collected, filtered, washed with ethanol, dried, and then crystallized from methanol to give the corresponding diazenyl derivatives **12a-f**.

2-((1-(1-Benzyl-1H-indol-3-yl)ethylidene)hydrazineylidene)-4-methyl-5-(phenyldiazenyl)-3-(p-tolyl)-2,3-dihydrothiazole (**12a**). Red solid; 82%; mp 98-100 °C; IR (KBr): ν = 1629 (C=N), 1482 (N=N) cm^{-1}; ^{1}H NMR (300 MHz, DMSO): δ = 2.20, 2.39, 2.42 (3s, 9H, 3Me), 5.45 (s, 2H, CH_2N), 7.26–7.41 (m, 11H, Ar–H), 7.49-7.51 (d, 2H, J_{HH} = 6 Hz, Ar–H), 7.74-7.87 (m, 4H, Ar–H), 8.05 (s, 1H, pyrrole), 8.55-8.58 (d, 1H, J_{HH} = 9 Hz, Ar–H) ppm; ^{13}C-NMR (75 MHz, DMSO): δ = 157.7, 152.3, 147.7, 137.7, 136.9, 135.7, 133.4, 132.5, 130.0, 129.7, 129.3, 128.6, 128.1, 127.5, 127.1, 125.4, 123.3, 122.5, 121.6, 121.0, 120.5, 117.5, 114.7, 110.6, 49.4, 20.9, 15.5, 13.1 ppm; EI-MS (70 eV): m/z (%) = 554 (M^+, 29). Anal. Calcd for $C_{34}H_{30}N_6S$ (554.72): C, 73.62; H, 5.45; N, 15.15. Found: C, 73.84; H, 5.22; N, 14.97%.

2-((1-(1-Benzyl-1H-indol-3-yl)ethylidene)hydrazineylidene)-4-methyl-3-(p-tolyl)-5-(p-tolyldiazenyl)-2,3-dihydrothiazole (**12b**). Red powder; 84%; mp 130-132 °C; IR (KBr): ν = 1622 (C=N), 1408 (N=N) cm^{-1}; ^{1}H NMR (300 MHz, DMSO): δ = 2.19, 2.36, 2.40, 2.42 (4s, 12H, $4CH_3$), 5.45 (s, 2H, CH_2N), 7.18–7.33 (m, 10H, Ar–H), 7.39-7.41 (d, 2H, J_{HH} = 6 Hz, Ar–H), 7.48-7.51 (d, 2H, J_{HH} = 9 Hz, Ar–H), 7.65-7.67 (d, 2H, J_{HH} = 6 Hz, Ar–H), 8.05 (s, 1H, pyrrole), 8.54-8.56 (d, 1H, J_{HH} = 6 Hz, Ar–H) ppm; ^{13}C-NMR (75 MHz, DMSO): δ = 161.5, 160.2, 154.7, 144.2, 139.5, 138.2, 137.4, 136.6, 135.0, 131.2, 130.7, 130.0, 128.7, 128.5, 127.3, 126.8, 126.1, 123.3, 121.9, 121.1, 119.9, 115.1, 109.9, 92.7, 52.7, 21.5, 21.2, 15.9, 11.7 ppm; EI-MS (70 eV): m/z (%) = 568 (M^+, 24). Anal. Calcd for $C_{35}H_{32}N_6S$ (568.74): C, 73.91; H, 5.67; N, 14.78. Found: C, 73.69; H, 5.44; N, 14.54%.

2-((1-(1-Benzyl-1H-indol-3-yl)ethylidene)hydrazineylidene)-5-((4-chlorophenyl)diazenyl)-4-methyl-3-(p-tolyl)-2,3-dihydrothiazole (**12c**). Yellowish brown solid; 75%; mp > 250 °C; IR (KBr): ν = 1625 (C=N), 1465 (N=N) cm^{-1}; ^{1}H NMR (300 MHz, DMSO): δ = 2.30, 2.38, 2.44 (3s, 9H, $3CH_3$), 5.47 (s, 2H, CH_2N), 7.16-7.19 (d, 2H, J_{HH} = 9 Hz, Ar–H), 7.25–7.31 (m, 11H, Ar–H), 7.49-7.50 (d, 1H, J_{HH} = 6 Hz, Ar–H), 7.58-7.61 (d, 2H, J_{HH} = 9 Hz, Ar–H), 8.21 (s, 1H, pyrrole), 8.51-8.53 (d, 1H, J_{HH} = 6 Hz, Ar–H) ppm; ^{13}C-NMR (75 MHz, DMSO): δ = 160.5, 158.9, 151.0, 145.4, 139.1, 138.2, 137.3, 136.6, 135.0, 133.8, 131.6, 130.1, 129.1, 128.6, 127.5, 126.8, 125.8, 123.4, 121.7, 121.6, 119.8, 113.0, 109.7, 91.8, 52.5, 21.4, 21.3, 10.2 ppm; EI-MS (70 eV): m/z (%) = 591 (M^+ + 2, 19), 589 (M^+, 23). Anal. Calcd for $C_{34}H_{29}ClN_6S$ (589.16): C, 69.31; H, 4.96; N, 14.26. Found: C, 69.19; H, 4.74; N, 14.02%.

2-((1-(1-Benzyl-1H-indol-3-yl)ethylidene)hydrazineylidene)-3-(4-chlorophenyl)-4-methyl-5-(phenyldiazenyl)-2,3-dihydrothiazole (**12d**). Brownish red solid; 87%; mp 172-174 °C; IR (KBr): ν = 1620 (C=N), 1485 (N=N) cm^{-1}; ^{1}H NMR (300 MHz, DMSO): δ = 2.20, 2.45 (2s, 6H, $2CH_3$), 5.46 (s, 2H, CH_2N), 7.25–7.52 (m, 12H, Ar–H), 7.66–7.77 (m, 5H, Ar–H), 8.08 (s, 1H, pyrrole), 8.52-8.55 (d, 1H, J_{HH} = 9 Hz, Ar–H) ppm; ^{13}C-NMR (75 MHz, DMSO): δ = 163.2, 160.4, 159.3, 151.3, 149.8, 140.3, 137.4, 136.5, 136.0, 131.5, 130.7, 129.2, 128.9, 128.6, 127.7, 127.5, 125.6, 122.7, 121.9, 120.5, 119.1, 112.8, 109.5, 95.3, 51.7, 19.1, 11.0 ppm; EI-MS (70 eV): m/z (%) = 577 (M^+ + 2, 15), 575 (M^+, 24). Anal. Calcd for $C_{33}H_{27}ClN_6S$ (575.13): C, 68.92; H, 4.73; N, 14.61. Found: C, 69.11; H, 4.54; N, 14.40%.

2-((1-(1-Benzyl-1H-indol-3-yl)ethylidene)hydrazineylidene)-3-(4-chlorophenyl)-4-methyl-5-(p-tolyldiazenyl)-2,3-dihydrothiazole (**12e**). Pale green substances; 86%; mp 194-196 °C; IR (KBr): ν = 1617 (C=N), 1410 (N=N) cm^{-1}; ^{1}H NMR (300 MHz, DMSO): δ = 2.23, 2.44, 2.73 (3s, 9H, $3CH_3$), 5.47 (s, 2H, CH_2N), 7.16-7.19 (t, 2H, J_{HH} = 6 Hz, Ar–H), 7.24–7.34 (m, 8H, Ar–H), 7.40, 7.51 (2d, 4H, J_{HH} = 8.7, J_{HH} = 6.9 Hz, Ar–H), 7.76-7.79 (d, 2H, J_{HH} = 9 Hz, Ar–H), 8.22 (s, 1H, pyrrole), 8.66-8.67 (d, 1H, J_{HH} = 3 Hz, Ar–H) ppm; ^{13}C-NMR (75 MHz, DMSO): δ = 160.6, 159.8, 150.0, 146.3, 140.0, 137.9, 136.9, 136.4, 132.9, 130.0, 129.0, 128.8, 128.4, 127.6, 127.4 126.8, 124.7, 123.9, 120.8, 119.5, 117.6, 110.7, 109.0, 92.1, 51.9, 20.2, 19.8, 10.1 ppm; EI-MS (70 eV): m/z (%) = 591 (M^+ + 2, 13), 589 (M^+, 20). Anal. Calcd for $C_{34}H_{29}ClN_6S$

(589.16): C, 69.31; H, 4.96; N, 14.26. Found: C, 69.10; H, 4.74; N, 14.01%.

2-((1-(1-benzyl-1H-indol-3-yl)ethylidene)hydrazineylidene)-3-(4-chlorophenyl)-5-((4-chlorophenyl)diazenyl)-4-methyl-2,3-dihydrothiazole (**12f**). Gray solid; 83%; mp 206-208 °C; IR (KBr): ν = 1623 (C=N), 1468 (N=N) cm^{-1}; ^1H NMR (300 MHz, DMSO): δ = 2.45, 2.84 (2s, 6H, $2CH_3$), 5.48 (s, 2H, CH_2N), 7.17-7.18 (t, 2H, J_{HH} = 3 Hz, Ar–H), 7.24–7.35 (m, 9H, Ar–H), 7.41-7.44 (d, 2H, J_{HH} = 9 Hz, Ar–H), 7.49-7.51 (d, 1H, J_{HH} = 6 Hz, Ar–H), 7.76-7.79 (d, 2H, J_{HH} = 9 Hz, Ar–H), 8.22 (s, 1H, pyrrole), 8.62-8.64 (d, 1H, J_{HH} = 6 Hz, Ar–H) ppm; ^{13}C-NMR (75 MHz, DMSO): δ = 164.7, 162.0, 158.3, 154.2, 142.5, 141.2, 139.4, 137.6, 135.0, 134.2, 133.0, 130.0, 129.0, 128.6, 127.5, 127.1, 126.7, 126.2, 125.5, 122.1, 120.8, 119.5, 109.9, 108.2, 50.3, 14.6, 11.7 ppm; EI-MS (70 eV): m/z (%) = 611 (M^+ + 2, 10), 609 (M^+, 21). Anal. Calcd for $C_{33}H_{26}Cl_2N_6S$ (609.57): C, 65.02; H, 4.30; N, 13.79. Found: C, 64.89; H, 4.09; N, 13.55%.

Alternate synthesis of compound **12a** *from thiazole derivative* **9a** [54-57]

2 mmol of sodium acetate trihydrate (0.27 g) was added to a solution of 2 mmol of compound **9a** (0.9 g) in ethanol (20 mL). After 15 minutes of stirring, the mixture was cooled to a temperature between 0 to 5 ºC. A newly prepared cold solution of diazonium chloride was added to the recently prepared cold liquid [the diazonium chloride solution was prepared by treatment of 2 mmol of aniline **13** (0.19 g) with 1.5 mL of 6 *M* HCl and 2 mmol sodium nitrite ($NaNO_2$) (0.14 g) dissolved in 4 mL of H_2O]. After adding the *in situ* prepared diazonium salt, the mixture was stirred for 30 minutes in the ice bath (0–5 °C) and then left for 8 h in a fridge (4 °C). The crude solid was filtered, washed with H_2O, dried, and crystallized from ethanol to produce the corresponding product **12a**. The product **12a** was similar to that gained from the reaction of **3a** with **10a** in all its aspects (mp, mixed mp, and IR spectra), but with a yield of 85%.

Anti-microbial activity

Anti-microbial behaviors of all newly synthesized compounds **3a,b**, **6a,b**, **9a,b**, and **12a-f** were tested in vitro by the cup diffusion agar method [58] versus a panel of bacteria, yeast, and fungi pathogens, such as Gram-positive- Staphylococcus aureus ATCC 6538-P; Gram-negative- Escherichia coli ATCC 25933; yeast- Candida albicans ATCC 10231 and fungus-Aspergillus niger NRRL-A326. Regarding yeast and bacteria, agar plates of nutrients were inoculated heavily and regularly with 0.1mL of 105-106 cells/mL. The anti-fungal properties were screened using Potato dextrose agar plates seeded by 0.1mL (106 cells/mL) of the fungal inoculum. In inoculated plates, 100 µl of tested compounds dissolved in DMSO (10 mg in 2 mL DMSO) were put into the initial holes. After that, plates remained for 2-4 hours at a temperature of 4°C to promote maximal diffusion. After that, in the case of bacteria, the plates were incubated for 24 hours at 37 °C while in the case of fungi, the plates were incubated for 48 hours at 30 °C in a vertical position to ensure maximum growth of the organisms. The anti-microbial properties of the tested samples were determined by measuring the diameter of the inhibition zone expressed in millimeters (mm). The test was repeated more than once, and the mean reading was listed. Cycloheximide and Neomycin were applied as anti-fungal and anti-bacterial standards, respectively.

Minimal inhibitory concentration measurement

The anti-microbial behavior of the two active compounds **3a**, **3b**, and the reference drug Ciprofloxacin was then screened following the method described by Sarker et al., (2007) [59]. In some details three various microbes were used namely: *S. aureus* ATCC 6538 (G-ve bacterium), *E. coli* ATCC 25933 (G-ve bacterium), and *C. albicans* ATCC 10231 (yeast). Resazurin dye was prepared by dissolving 6.75mg in 10 mL distilled water. 96-well plates were used in which 100 µl of nutrient broth were distributed and 100 µl of each compound (in duplicates) were placed in the first cells and serial dilutions were done leaving the last cell in each raw as reference. In each well 10 µl of the microbial stock culture were added followed by 10 µl of resazurin. All steps had been performed under aseptic conditions. Any color change of the dye was considered a positive result.

Molecular docking method

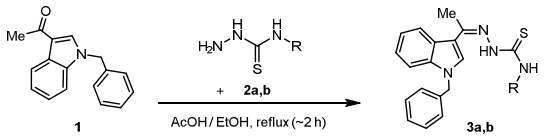
The molecular docking screening of the two compounds under study with the X-ray crystallographic construction of *S. aureus* DNA gyrase B (PDB: 3U2D) and *E. coli* DNA gyrase B (PDB: 1S14) was accomplished by employing PyRx tools Autodock Vina (version 1.1.2) [60]. Original ligands alongside H_2O molecules were extracted from the proteins using the VEGA ZZ 2.3.2 tool, then Kollman charges and polar hydrogen were added, followed by transformation to PDBQT format by Autodock Vina tools. The compounds under study are saved as a mol file, protonated, minimized, and then changed into a pdb file using Open Babel software. The formed pdb file was converted to Autodock Vina tools to establish some torsions and

pdbqt file creation. Auto Grid with a grid box was used to create the grid map. Taking the binding energy into account, many docked poses were established for each molecule. The lowest binding energy with 0 Å RMSD (root mean square deviation) was considered appropriate and most complex with the receptor for study. Engaging BIOVIA Discovery Studio 2021, the top poses' binding modes and binding interactions were visually studied.

3. Results and Discussion

Chemistry

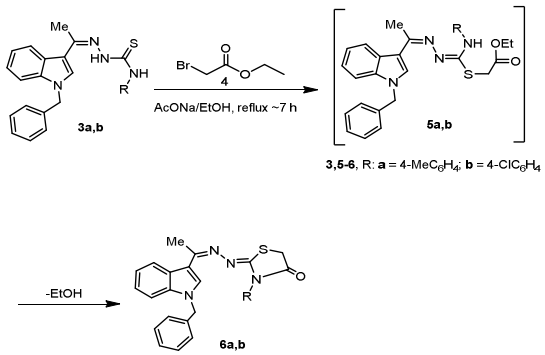
The new key compounds, 2-(1-(1-benzyl-1*H*-indol-3-yl)ethylidene)-*N*-(aryl)hydrazine-1-carbothio-amides **3a** and **3b** could be gained from the condensation reaction of *N*-benzyl-3-acetylindol **1** with the appropriate aryl hydrazinecarbothioamide **2a/b** in an ethanol (EtOH) solution with few drops of acetic acid (AcOH) (Scheme 1). The reactions were completed under reflux giving the carbothioamides **3a/b** in ~81% yields and characterized according to standard methods. The IR spectrum of **3a** exhibited absorption bands at 3440, 3458, 1615, and 1267 cm^{-1} owing to 2NH, C=N, and C=S, respectively. ^{1}H NMR spectrum disclosed two singlet signals at δ 2.30, and 2.44 ppm owing to 2Me groups, one singlet signal at δ 5.47 ppm due to the methylene moiety (CH_2), and two exchangeable signals at δ 9.61 and 10.51 ppm due to 2NH.



2-3, R: **a** = 4-MeC_6H_4; **b** = 4-ClC_6H_4

Scheme 1. preparation of hydrazine-1-carbothioamides **3a**,**b**

The carbothioamide derivatives **3a** and **3b** were subjected to cyclization with different halogenated compounds **4**, **7**, and **10a-c** in the hope of obtaining the biologically active derivatives of the thiazolidinone and the thiazole moieties. Thus, when equimolar amounts of carbothioamide **3a/b** and ethyl 2-bromoacetate (**4**) were mixed in absolute ethanol (EtOH) in the presence of fused sodium acetate (3 mmol) at reflux temperature for the proper time (TLC) the corresponding substituted thiazolidine derivatives **6a**,**b** obtained in good yields (~83%). The formation of **6a**,**b** involved an initial nucleophilic substitution with loss of HBr followed by cyclization through the extrusion of ethanol molecules from the resulting intermediates **5a**,**b** to give the thiazolidinone **6a**,**b** (Scheme 2). This mechanism was previously reported in similar cases [54, 55, 61].

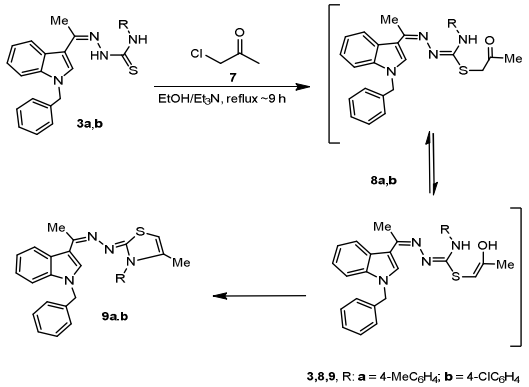


3,5-6, R: **a** = 4-MeC_6H_4; **b** = 4-ClC_6H_4

Scheme 2. synthesis of thiazolidine derivatives **6a,b**

The structures of thiazolidinones **6** were delineated from their spectroscopic properties. As a representative example, the IR spectrum of compound **6a** exhibited the disappearance of 2NH and C=S peaks at 3440, 3458, and 1267 Cm^{-1} respectively, and the appearance of a new peak at 1638 cm^{-1} according to C=O moiety. In its ^{1}H NMR, the two methyl group protons were displayed at 2.21 and 2.38 ppm, while the CH_2 protons of the thiazolidine appeared at 4.09 ppm and the methylene protons of the CH_2N signal were exhibited at 5.45 ppm. Moreover, the ^{13}C-NMR spectrum showed the lack of a C=S signal at 178.5 ppm and the appearance of a new carbon signal at 169.8 ppm belongs to C=O moiety.

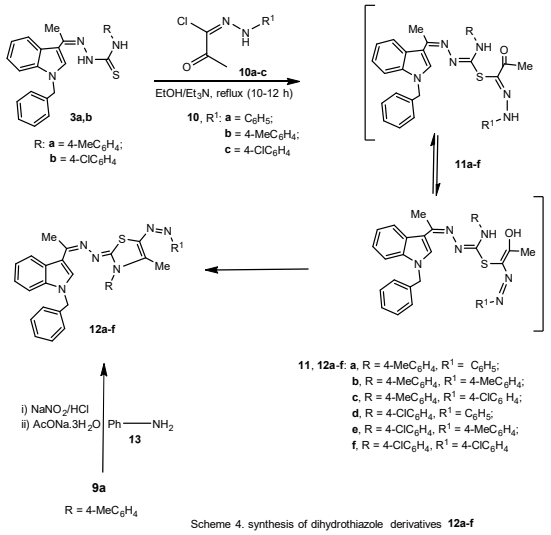
Furthermore, treating compounds **3a**,**b** with chloroacetone (**7**) in an ethanol solution has a catalytic quantity of triethylamine (TEA) at reflux temperature afforded the corresponding substituted thiazole derivatives **9a**,**b**, in high yields ~87%. As described in Scheme 3. Structures **9a** and **9b** were identified from their spectroscopic data. The ^{1}H NMR spectrum of **9a** showed the three methyl protons at 1.88, 2.14, and 2.16 ppm while the methylene protons (CH_2N) appeared at 5.43 ppm.



3,8,9, R: **a** = 4-MeC_6H_4; **b** = 4-ClC_6H_4

Scheme 3. synthesis of dihydrothiazole derivatives **9a,b**

Finally, treating the carbothioamides **3a**,**b** with hydrazonyl chlorides **10a**-**c** in ethanol/containing TEA (~3 drops) under thermal conditions afforded the dihydrothiazole derivatives **12a**-**f** in high yields (75-87%) as displayed in Scheme 4. ^{1}H- and ^{13}C-NMR spectroscopy, mass spectroscopy (EI), and elemental analysis confirmed the purity and structure of compounds **12a**-**f**. The IR spectrum of structure **12a** revealed bands at 1629 and 1482 cm^{-1} due to the (C=N), (N=N) groups, and the disappearance of NH and C=S peaks. Moreover, its ^{1}H NMR spectrum displayed signals at δ = 2.20, 2.39, and 2.42 ppm because of the 9H of 3 methyl groups and signal at 5.45 ppm due to 2H of CH_2 protons (CH_2N).



Scheme 4. synthesis of dihydrothiazole derivatives **12a-f**

On the other hand, 2-((1-(1-benzyl-1*H*-indol-3-yl)ethylidene)hydrazineylidene)-4-methyl-5-(phenyldiazenyl)-3-(*p*-tolyl)-2,3-dihydrothiazole (**12a**) could be synthesized by a different route from the reaction of compound **9a** in ethanolic sodium acetate trihydrate ($AcONa.3H_2O$) with diazonium chloride was prepared *in situ* from aniline **13** and sodium nitrite ($NaNO_2$)/HCl (Scheme 4) [54, 55]. The formed compound was found to be similar in all of its characters (spectra, mp, and mixed mp) with the dihydrothiazole derivative **12a** produced from the reaction of carbothioamide **3a** with hydrazonyl chloride **10a** but the yield is higher.

Anti-microbial evaluation

A preparatory evaluation of all new compounds **3a,b**, **6a,b**, **9a,b**, and **12a-f** in addition to the two reference drugs Neomycin and cycloheximide took place in vitro versus a panel of Gram-negative and Gram-positive bacteria, yeast, and fungi pathogens applying the method of cup diffusion agar [50, 58], and the results are presented in Table 1.

As listed in Table 1, the anti-microbial efficiency results of the evaluated compounds, a small number of compounds exhibited positive effects on the studied organisms. while the majority of the examined compounds offered no reaction on most of the tested organisms. The resulting data exposed that only compounds **3a** and **3b** showed anti-bacterial properties against the Gram-positive (G+ve) test strain (*S. aureus*) with inhibition values of 22 and 28 mm, respectively. Compounds **3a**, **3b**, and **12e** had anti-bacterial efficacy versus the Gram-negative (G-ve) test strain (*E. coli*) with inhibition values of 23, 26, and 14 mm, respectively. The inhibition values of compounds **3a**, **3b,** and **12d** as anti-microbial agents against the yeast test strain (*C. albicans*) are 24, 29, and 15 mm, respectively. Compounds **12c** and **12d** had anti-fungal action against *A. niger* with values of inhibition are 39 and 16 mm, respectively.

Table 1. Zone of growth inhibition (mm) of the new **3a,b**; **6a,b**; **9a,b**; and **12a-f** against some microbial pathogens.

compound	Clear zone (mm)			
	Strain			
	Gram-positive	Gram-negative	yeast	fungus
	S. aureus	*E. coli*	*C. albicans*	*A. niger*
3a	22	23	24	NA
3b	28	26	29	NA
6a	NA	NA	NA	NA
6b	NA	NA	NA	NA
9a	NA	NA	NA	NA
9b	NA	NA	NA	NA
12a	NA	NA	NA	NA
12b	NA	NA	NA	NA
12c	NA	NA	NA	39
12d	NA	NA	15	16
12e	NA	14	NA	NA
12f	NA	NA	NA	NA

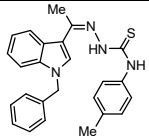
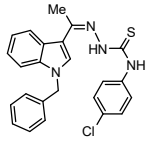
S. aureus, Staphylococcus aureus ATCC 6538-P; *E. coli, Escherichia coli* ATCC 25933; *C. albicans, Candida albicans* ATCC 10231 and *A. niger, Aspergillus niger* NRRL-A326; NA, not active.

It is possible to recognize definite aspects of the structure-activity relationships for the samples under study. Relating the anti-microbial activity of hydrazinecarbothioamide derivatives **3a**,**b**, just the two of them displayed good results toward the tested pathogens. On the contrary, the majority of the compounds studied like thiazolidinone and thiazole derivatives exhibited no action on the tested organisms. The main factor of these results is owing to the existence of the CH_2-functional group and (NH-CS-NH) bridge of the *N*-benzyl-1*H*-indole hydrazinecarbothioamide scaffold. Cyclization of hydrazinecarbothioamides **3a** and **3b** with halogenated derivatives, **4**, **7**, and **10a-c** afforded the cyclic structure like thiazolidinone and thiazole derivatives containing the indole moiety. The results listed above mention that the cyclization of

hydrazine-carbothioamides does not enhance the efficiency of anti-microbial properties. Cyclization of hydrazinecarbothioamides causes a bad impact on anti-microbial efficacy. Despite that, the cyclized structure, dihydrothiazole **12c** showed good anti-microbial activity only against the tested *Aspergillus niger* organisms. In the case of compound **12d** resulted in moderate activities against *Candida albicans* and *Aspergillus niger*. While compound **12e** gave reasonable efficacy against the examined *Escherichia coli* organisms only. These data reflect the importance of the presence of the *N*-benzyl-1*H*-indole hydrazine-carbothioamide moiety in the molecule, which probably enhances the effectiveness.

Subsequently, the minimum concentrations demanded to limit the growth [(MIC), minimum inhibitory concentration (µg/mL)] were studied for the lead hydrazinecarbothioamides **3a** and **3b** as well as for the reference drug ciprofloxacin applying the process mentioned by Sarker *et al*., (2007) [59]. The MIC values of the examined samples (**3a** and **3b**) are represented in Table 2. Results indicated compound **3a** had MIC values of 625, 312.5, and 625 µg/mL versus *S. aureus*, *E. coli,* and *C. albicans*, respectively. Compound **3b** exhibited MIC values of 312.5, 312.5, and 312.5 µg/mL versus *S. aureus*, *E. coli,* and *C. albicans*, respectively. Both compounds **3a** and **3b** exhibited higher MIC values as compared to the reference drug ciprofloxacin.

Table 2. Minimum inhibitory concentrations (MIC) (µg/mL) of **3a**, **3b**, and **Ciprofloxacin** against different pathological strains.

Sample	Strain		
	Gram-positive	Gram-negative	yeast
	S. aureus	*E. coli*	*C. albicans*
 **3a**	625	312.5	625
 **3b**	312.5	312.5	312.5
Cipro	19.53	39.06	78.13

S. aureus, Staphylococcus aureus ATCC 6538-P; *E. coli, Escherichia coli* ATCC 25933; *C. albicans, Candida albicans* ATCC 10231 and Cipro, ciprofloxacin.

Molecular docking

Supported by the anti-microbial influence outcomes, compounds **3a** and **3b** were selected for the molecular docking study towards DNA gyrase B (a primary site of action for both Gram-positive and Gram-negative bacteria)[62] DNA gyrase plays an essential role in bacterial reproduction and compaction [63]. DNA gyrase inhibition prevents the relaxation of supercoiled DNA that is required for reproduction and so prevents cell division consequently causing the death of bacteria [64, 65].

The X-ray crystallographic constructions of *S. aureus* DNA gyrase B (PDB: 3U2D) and *E. coli* DNA gyrase B (PDB: 1S14) were downloaded from the website https://www.rcsb.org (20-05-2024). The original ligands (08B) of *S. aureus* DNA gyrase B (PDB: 3U2D) and (NOV) of *E. coli* DNA gyrase B (PDB: 1S14) were re-docked in the enzyme's active cave of the original proteins to validate the authenticity of molecular docking analyze. 08B of S. aureus DNA gyrase B (PDB: 3U2D) showed a docking value of −8.0 kcal/mol with the formation of hydrogen bonds with the essential amino acids ARG144, THR173, and ASP81 as before reported [66]. Regarding E. coli DNA gyrase B (PDB: 1S14), NOV exhibited a docking value of −8.3 kcal/mol and re-generated the connections with the main amino acids THR1163, ASP1069, Asp1077, ASN1042, and ARG1072 via hydrogen bonds as reported [67].

The molecular docking assessment of the studied compounds 3a and 3b towards S. aureus DNA gyrase B, PDB: 3U2D

Figure 1 displays the docking results of compounds **3a** and **3b** towards the DNA gyrase B of *S. aureus* (PDB: 3U2D). Compound **3a** presented a binding energy score of -7.9 kcal/mol. Additionally, it demonstrated good binding interactions with the active pocket of DNA gyrase B of *S. aureus* (PDB: 3U2D) *via* three conventional hydrogen bonds with the residue amino acids ASP81, ASP57, and ASN54, in addition to π-π interactions with the residue amino acids ILE102, ILE86 (π-alkyl), THR173 (π-sigma), ASP57, GLU58 (π-anion), and ASN54 (amid-π-stacked) (Figures 1a and 1c). On the other hand, compound **3b** revealed a binding energy score of -7.7 kcal/mol and established good binding interactions with the active pocket of DNA gyrase B of *S. aureus* (PDB: 3U2D). **3b** showed four hydrophilic interactions with the residue amino acids ASP57, ASP81, PRO87, and ASN54. In addition, compound **3b** showed many π-π interactions with the residue amino acids THR173 (π-sigma), ILE86, ILE102 (π-alkyl), and GLU58 (π-anion) (Figures 1b and 1d).

In sum, both **3a** and **3b** showed good superimposition toward the active pocket of DNA-gyrase of *S. aureus via* the interaction with the key amino acids as the original ligand ASP81 and THR173 (Figure 1a-d). These results highlight the importance of the *N*-benzyl-1*H*-indole moiety, where the CH_2-functional group of the benzyl core

interacted with the main amino acid (ASP81) besides the interaction of the phenyl moiety of the benzyl core with the main amino acid (THR173).

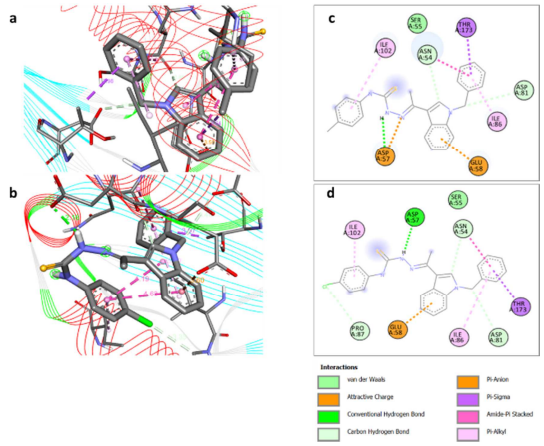


Figure 1. (**a** & **b**): The 3D configuration of compounds **3a** and **3b** inside the active pocket of DNA gyrase B of *S. aureus* (PDB: 3U2D); (**c** & **d**): The 2D representation of compounds **3a** and **3b** inside the active pocket of DNA gyrase B of *S. aureus* (PDB: 3U2D).

*The molecular docking assessment of the studied compounds **3a** and **3b** towards E. coli DNA gyrase B, PDB: 1S14*

Figure 2 displays the docking results of compounds **3a** and **3b** towards the DNA gyrase B of *E. coli* (PDB: 1S14). **3a** showed a binding energy score of -7.5 kcal/mol. Its binding mode inside the active pocket of the DNA gyrase B of *E. coli* (PDB: 1S14) demonstrated three hydrophilic interactions with the residue amino acids ASP1069, ASN1042, and THR1163 through two NH functional groups and the CS functional group. Also, it showed many π-π interactions with the residue amino acids VAL1165 (π-sigma), ARG1072, GLU1046 (π-anion), VAL1039, MET1074, PRO1075, and ALA1086 (π-alkyl) (Figure 2c). On the other hand, compound **3b** showed a binding energy score equal to -7.5 kcal/mol. The binding mode of **3a** recreated the same interaction of compound **3a** inside the active pocket of the DNA gyrase B of *E. coli* (PDB: 1S14) (Figure 2d).

Finally, both **3a** and **3b** showed the same behavior towards the active pocket of the DNA gyrase B of *E. coli* (PDB: 1S14) and confirmed the potency of both compounds via their interaction with the main amino acids ARG1072, ASN1042, THR1163, and ASP1069 as the original ligand. These results shads the importance of the (NH-CS-NH) bridge beside the indole moiety, where the NH-CS-NH interacted with three main amino acids (ASP1069, ASN1042, and THR1163) besides the interaction of the indole moiety with the main amino acid (ARG1072).

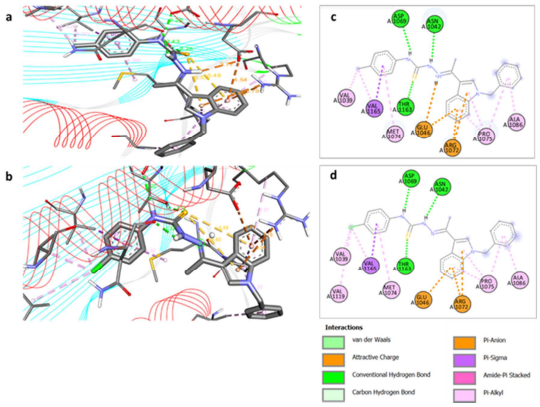


Figure 2. (**a** & **b**): The 3D configuration of compounds **3a** and **3b** inside the active pocket of DNA gyrase B of *E. coli* (PDB: 1S14); (**c** & **d**): The 2D representation of compounds **3a** and **3b** inside the active pocket of DNA gyrase B of *E. coli* (PDB: 1S14).

4. Conclusion

In conclusion, the current study provided a convenient approach for the synthesis of a series of new hydrazinecarbothioamide, thiazolidinone, and thiazole derivatives bearing an *N*- benzyl indole-based moiety. The new compounds have been designed and synthesized in good to excellent yields. In this investigation, we have also tried to establish connections between the bioactive indole moiety and other moieties that may have pharmacological activity to evaluate them as anti-microbial compounds. The biological assay results elucidated that both hydrazinecarbothioamides **3a** and **3b** are the most active anti-microbial biotics and only as they exhibited significant inhibition potency towards all the studied pathogens with high MIC values compared with the reference drug ciprofloxacin. The potencies of both compounds **3a** and **3b** could be attributed to the presence of the (NH-CS-NH) bridge, which could explain the dramatic lowering or even loss of activities when **3a/3b** were cyclized. The experimental results showed that the dihydrothiazoles **12c** and **12d** had moderate anti-fungal inhibition against *A. niger*. While compounds **12d** and **12e** showed mild potencies against the yeast test strain (*C. albicans*) and the Gram-negative test strain (*E. coli*), respectively. Except for compounds **3a, 3b, 12c, 12d,** and **12e**, all the other synthesized compounds had no anti-microbial activity on the microbial test strains.

Molecular docking studies were conducted and the results confirmed that both **3a** and **3b** showed good binding energy and binding mode interactions with two aim DNA gyrase B enzymes, and recreating reactions with the key amino acids similar to the

native inhibitor. These results are interesting and highlight the potential of compounds **3a** and **3b** for further exploration as anti-microbial agents.

5. Conflicts of interest

There are no conflicts to declare.

6. Formatting of funding sources

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7. Acknowledgments

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8.References

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