Synthesis, characterization and in vitro biological screening of 4-hydroxy naphthalen-1-yl, naphtho[1,2-b]furan, benzo[h]chromene and 5,6-dihydropyridazine derivatives containing sulfonamide moiety

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Abstract: In this study, a series of 4-((4-hydroxynaphthalen-1-yl)diazenyl)benzenesulfonamides have been prepared by subsequent diazotization of sulfonamide derivatives and coupling with 1-naphthol in alkaline medium. Cyclization of 4-((4-hydroxynaphthalen-1-yl)diazenyl)benzenesulfonamides with cinnamic acid in the presence of a basic catalyst afforded the novel naphtho[1,2-b]furans. Also, 4-((4-hydroxynaphthalen-1-yl)diazenyl)benzenesulfonamides can be cyclized with α-cyanocinnamionitriles to afford 2-amino-3-cyano-4-phenyl-4H-benzo[h]chromenes. 4-(4-amino-3,5-dicyano-6-iminopyridazin-1(6H)-yl)benzenesulfonamides were obtained at room temperature by treatment of 2-amino-1,1,3-tricyanopropene with a diazonium salt of sulfonamide derivatives. The structures of newly synthesized compounds were confirmed by analytical data and spectroscopic techniques. The antimicrobial activity of the obtained compounds was assessed in vitro by qualitative and quantitative (minimum inhibitory concentration) (MIC) assays.

Keywords: sulfonamide; azobenzene; naphtho[1,2-b]furan; benzo[h]chromene; pyridazine.

Introduction

Substituted azobenzene have attracted considerable attention based on their various physical and chemical properties, such as bright colors, good stability, low flammability, and rapid, reversible photo-isomerization 1-3. Aromatic azo compounds are widely used in the chemical industry as dyes, pigments 4,6, food additives 7, indicators 8, radical reaction initiators 9 and therapeutic agents 9,10. Also, azobenzenes have shown promising applications in photo-optical media 11, photo-switches 12, photo-mechanical systems 13, micro patterning 14, nonlinear optical media 15, molecular shuttles 16, nanotubes 17, and in the manufacture of protective eye glasses and filters 18.

Naphthofuran derivatives have also been reported to possess diverse biological activities, including antityrosinase, antioxidant and antibacterial 19. Naphtho[1,2-b]furans are very important structural units found in diverse natural and synthetic products 20. They possess a broad spectrum of biological activities and have been used as precursors for the synthesis of bioactive materials. Naphtho[1,2-b]furan-4,5-dione (NFD, Fig. 1), a 1,2-furanonaphtho quinone, was originally isolated from avicennia marina belonging to the family Avicenniaceae and can be synthesized by a chemical process. NFD was found to show potent cytotoxicity against human cancer cell lines, including KB (human epidermoid carcinoma, IC50 = 3.05 ± 0.195 µM), HeLa (human cervical carcinoma,IC50 = 2.85 ± 0.210 µM) and HepG2 (human hepatocellular carcinoma, IC50 = 3.00 ± 0.040 µM) cell lines 21.
Recently, Chen et al. 22 discover, N-(naphtho[1,2-b]furan-5-yl) benzene sulfonamides (Fig. 1), as novel selective inhibitors of triple-negative breast cancer (TNBC).

Chromene derivatives are very an important class of heterocyclic compounds, widely distributed in natural products. Chromene and its derivatives have also been recognized as one type of ‘privileged medicinal scaffolds’ due to their unique pharmacological and biological activities 23. Dong et al. 24,25 designed and prepared a series of 4-amino-2H-benzo[h]-chromen-2-one and 4-amino-7,8,9,10-tetrahydro-2H-benzo[h]chromen-2-one derivatives based on the potent anticancer agents neo-tanshinlactone and its 4-ethyl analogue 26.

Sulfonamides are another important compounds family for the medicinal industry, and they are now extensively used drugs for the treatment or conservation of different illnesses 27. In clinical medicine, they have been used as anticancer 28, antimicrobial 29, antiobesity 30, carbonic anhydrase 31 and acetylcholinesterase inhibitor agents for Alzheimer’s disease 32.

In view of the above-mentioned benefits and in continuation of our interest in biologically active compounds 33-37, we report herein the synthesis of some novel 4-((4-hydroxynaphthalen-1-yl)diazenyl)-benzene sulfonamides3a-e, naphtho[1,2-b]-furans 6a-c,benzo[h]chromenes8a,b and 4-(4-amino-3,5-dicyano-6-imino-5,6-dihydropyridazin-1(4H)-yl)-benzenesulfonamides 13a-e containing a sulfonamido moiety to evaluate their antimicrobial biological activity.

![Naphtho[1,2-b]furan-4,5-dione (NFD) and N-(naphtho[1,2-b]furan-5-yl) benzenesulfonamides](image)

Figure 1.

**Results and Discussion**

**Syntheses and characterizations of the compounds**

A series of 4-((4-hydroxynaphthalen-1-yl)diazenyl)benzenesulfonamides 3a-e were synthesized by coupling of diazonium salt of sulfonamide derivatives 1a-e with 1-naphthol 2 in presence of 10% sodium hydroxide (Scheme 1). Diazotization was carried out in the presence of nitrosyl chloride at 0-5 °C. The structure of compounds 3a-e was determined by their elemental analysis and spectral data. Elementary analysis indicated that sulfur was present. The infrared spectra of all isolated compounds were consistent with the assumed structures. The infrared spectra of compounds 3a-e showed the presence of absorption band at 3357-3448 cm⁻¹ which is characteristic of the hydroxyl group beside two absorptions bands for azo and sulfone groups. The representative ¹HNMR spectrum of compound 3a (DMSO-d₆) shown 7.1, 7.75, 8.35, 8.80 (4d, 4H, naphtho-H), 7.47, 7.58 (2m, 2H, naphtho-H), 7.94, 8.24 (2d, 4H, AB-system), 8.10 (s, 2H, NH₂ exchangeable with D₂O), 12.36 (br, 1H, OH exchangeable with D₂O). The molecular ion peak of compound 3e was observed at m/z 410 (42.83%) corresponding to the molecular formula Cₗ₉H₇N₂O₆S₂, and the base peak was found in the spectrum at m/z 65. The enolic–OH groups of all the compounds were chemically detected by the treatment with a FeCl₃ solution, which gives characteristic color.
Scheme 1. Synthesis of 4-((4-hydroxynaphthalen-1-yl)diazenyl)benzenesulfonamides 3a-e

The reactivity of 4-((4-hydroxynaphthalen-1-yl)diazenyl)benzenesulfonamides 3 towards some carbon electrophiles was investigated. Thus, it has been found that the reaction of 4-((4-hydroxynaphthalen-1-yl)diazenyl)benzenesulfonamides 3a-c with cinnamic acid 4 in refluxing $N,N$-dimethylformamide (DMF) containing catalytic amounts of piperidine gave naphtho[1,2-b]furans 6a-c rather than the expected naphthopyran 5 (Scheme 2).

Scheme 2. Synthesis of naphtho[1,2-b]furans 6a-c
The structures of compounds 6a-c were established by spectroscopic tools as well as elemental analyses data. The infrared spectra of compounds 6a-c indicated the absence of the hydroxyl and carbonyl absorption bands. The $^1$H NMR spectrum of compound 6a (DMSO-$d_6$) 7.25-7.62 (m, 7H, Ph-H and naphtho-H), 8.20 (s, 1H, furan-H), 8.32, 8.39(2d, 2H, naphtho-H), 8.45, 8.50(2s, 3H, naphtho-H and NH$_2$, exchangeable with D$_2$O), 7.98, 8.55 (2d, 4H, AB-system). The mass spectrum of compound 6b showed a molecular ion peak at m/z 469 (12.15%) compatible with molecular formula C$_{25}$H$_{19}$N$_5$O$_3$S. The base peak was found in the spectrum at m/z 55. Also, the mass spectrum of compound 6c showed a molecular ion peak at m/z 510 (7.64%) corresponding to the molecular formula C$_{27}$H$_{18}$N$_4$O$_3$S$_2$. The molecular ion of compound 6c underwent fragmentation to produce a peak of m/z 57, corresponding to the base peak (Scheme 3).

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{scheme3.png}
\caption{Scheme 3. Fragmentation pattern of naphtho[1,2-b]furan 6c}
\end{figure}

The formation of 6 from the reaction of 3 with 4 is assumed to proceed via initial addition naphtholate anion (C-2) in 3 to the activated double bond in 4 to yield the non-isolable intermediate Michael adduct (A) followed by intramolecular cyclization and subsequent decarboxylation to afford the naphthofurans 6 (Scheme 4).

The reaction of compound 3a with $\alpha$-cyanocinnaminitriles was investigated. Thus, the reaction of compound 3a with $\alpha$-cyanocinnaminitriles 7 in refluxing DMF in the presence of piperidine afforded 2-amino-3-cyano-4-phenyl-4H-benzo[h]chromenes 8a,b. The structure of 8 was supported by elemental analysis and spectral data. The infrared spectra of compounds 8a, b displayed absorption bands for NH$_2$, C≡N, N=N and SO$_2$ functions. The mass spectrum of compound 8a showed a molecular ion peak at m/z 481 (1.64%) corresponding to the molecular formula C$_{26}$H$_{19}$N$_5$O$_3$S. The formation of 8 from the reaction of 3a with 7 is assumed to proceed via initial addition of naphtholate anion (C-2) in 3a to the activated double bond in 7 to yield the non-isolable intermediate Michael adduct (B) followed by intramolecular cyclization through nucleophilic addition of the hydroxyl group to the cyano group and tautomerization $^{38}$ to afford benzochromene 8 (Scheme 5).
Scheme 4. Formation of naphtho[1,2-b]furans 6

Scheme 5. 2-amino-3-cyano-4-phenyl-4H-benzo[h]chromenes 8a-b
Pyridazine and its derivatives have been extensively investigated because of their important role especially in medicinal chemistry, a large variety of biological activities being described: antibacterial, antifungus, antituberculosis, antiviral, anti-inflammatory, anticancer, cardiovascular disorders. Thus, treatment of 2-amino-1,1,3-tricyanopropene with a diazonium salt of sulfonamide derivatives at room temperature gave 4-(4-amino-3,5-dicyano-6-iminopyridazin-1(6H)-yl)benzenesulfonamide derivatives via intramolecular cyclization of through nucleophilic addition of the nitrogen atom to the cyano group and tautomerization (Scheme 6).

Scheme 6. 4-(4-amino-3,5-dicyano-6-imino-5,6-dihydropyridazin-1(4H)-yl)benzenesulfonamides

**Antimicrobial activity and minimal inhibition concentration**

The newly synthesized compounds were evaluated for their in-vitro antibacterial activity against *Staphylococcus aureus, Bacillus subtilis* as examples of Gram-positive bacteria, *Proteus vulgaris* and *Escherichia coli* as examples of Gram-negative bacteria, using two standard antibiotics, Ampicillin, and Gentamycin as reference drugs and antifungal potential against a representative panel of fungal strains i.e. *Aspergillus fumigatus* (filamentous fungi), and *Candida albicans* (yeast), using one standard antibiotic, Amphotericin B as reference drug. The compounds were tested for their activity at a concentration of 10 mg/mL using inhibition zone diameter in mm as a criterion for the antimicrobial activity, and the results are shown in (Table 1). Based on the results, the newly synthesized compounds tested displayed variable in-vitro antimicrobial activities under these screening conditions. Interestingly, the tested compounds exhibited significant antifungal activities against the filamentous fungus (*Aspergillus fumigatus*) and unicellular yeast (*Candida albicans*). The highest antifungal activity was detected for compound 3b followed by 3a, 3e, 3d, 3c, respectively. However, compound 3e exhibited the highest activity against Gram-positive bacteria, *Staphylococcus aureus* as compared with the standard antibiotic, Ampicillin,
followed by 3b, 3a, 8a and 3d, respectively. On the other hand, compound 3b exhibited the highest activity against Gram-positive bacteria, *Bacillus subtilis* followed by 3a, 3e, 3d and 6a, respectively. Moreover, the tested Gram-negative bacteria; *Proteus vulgaris* was highly susceptible to compound 3e followed by, 3d, 8a, 3b, 3a, 6a and 6b as compared with the standard antibiotic, Gentamycin. The order of activity against *Escherichia coli* was 3e > 3b > 3a > 3d > 6a > 8a > 3c > 6c > 6b > 8b.

**Table 1.** *In-vitro* antimicrobial activities of the synthesized compounds tested at 10 mg/mL by well diffusion agar assay and expressed as inhibition zone diameter (mm) in the form of mean ± standard deviation.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Fungi</th>
<th>Gram positive bacteria</th>
<th>Gram negative bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>C. albicans</em> ATCC 10231</td>
<td><em>A. fumigatus</em> RCMB 002568</td>
<td><em>S. Aureus</em> RCMB 010012</td>
</tr>
<tr>
<td>3a</td>
<td>33.5±1.6</td>
<td>29±0.7</td>
<td>20.3±0.5</td>
</tr>
<tr>
<td>3b</td>
<td>34.1±1.7</td>
<td>30.7±1.5</td>
<td>21.6±1.3</td>
</tr>
<tr>
<td>3c</td>
<td>13±0.7</td>
<td>20.3±1.1</td>
<td>15.8±0.5</td>
</tr>
<tr>
<td>3d</td>
<td>14.2±0.6</td>
<td>26±1.2</td>
<td>17.1±0.8</td>
</tr>
<tr>
<td>3e</td>
<td>26.1±1.2</td>
<td>27.4±0.8</td>
<td>22.3±1.4</td>
</tr>
<tr>
<td>6a</td>
<td>17.1±0.7</td>
<td>15.7±0.9</td>
<td>16.3±1.1</td>
</tr>
<tr>
<td>6b</td>
<td>0</td>
<td>0</td>
<td>13.4±0.9</td>
</tr>
<tr>
<td>6c</td>
<td>11.4±0.9</td>
<td>0</td>
<td>13.3±0.8</td>
</tr>
<tr>
<td>8a</td>
<td>14.5±1.1</td>
<td>16±0.8</td>
<td>18.2±0.9</td>
</tr>
<tr>
<td>8b</td>
<td>14.6±0.8</td>
<td>12.9±0.3</td>
<td>10.2±0.4</td>
</tr>
<tr>
<td>11a</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>11b</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>11c</td>
<td>0</td>
<td>0</td>
<td>9.8±0.6</td>
</tr>
<tr>
<td>11d</td>
<td>0</td>
<td>0</td>
<td>8.7±0.5</td>
</tr>
<tr>
<td>11e</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Amphotericin B</td>
<td>25.7±1.3</td>
<td>24.8±1.4</td>
<td>-</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>-</td>
<td>-</td>
<td>27.8±0.6</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Amphotericin B, ampicillin and gentamycin were used as standard drugs against the tested fungi, Gram-positive and Gram-negative bacteria, respectively.

The antimicrobial activities of the synthesized compounds were also tested to determine the minimum inhibitory concentration as shown in Table 2. Moreover, compound 3b showed the highest activity (MIC values ranged from 4.9 to 312.5 µg/ml), followed by 3a (MIC 4.9-625 µg/ml), 3e (MIC 9.8-625 µg/ml), and 3d (MIC 9.8-625 µg/ml).
Table 2. The antibacterial activities of the synthesized compounds expressed as minimum inhibitory concentration (µg/ml).

<table>
<thead>
<tr>
<th>Compound</th>
<th>C. albicans ATCC 10231</th>
<th>A. fumigatus RCMB 002568</th>
<th>S. aureus RCMB 010012</th>
<th>B. subtilis NRRL B-543</th>
<th>Proteus vulgaris ATCC 13315</th>
<th>E. coli ATCC 25955</th>
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<tbody>
<tr>
<td>3a</td>
<td>4.9</td>
<td>4.9</td>
<td>39</td>
<td>156</td>
<td>625</td>
<td>39</td>
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<tr>
<td>3b</td>
<td>4.9</td>
<td>4.9</td>
<td>156</td>
<td>156</td>
<td>312.5</td>
<td>39</td>
</tr>
<tr>
<td>3c</td>
<td>1250</td>
<td>156</td>
<td>625</td>
<td>5000</td>
<td>1250</td>
<td>625</td>
</tr>
<tr>
<td>3d</td>
<td>625</td>
<td>9.8</td>
<td>312.5</td>
<td>625</td>
<td>312.5</td>
<td>156</td>
</tr>
<tr>
<td>3e</td>
<td>9.8</td>
<td>9.8</td>
<td>39</td>
<td>625</td>
<td>156</td>
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<td>6b</td>
<td>NA*</td>
<td>NA</td>
<td>625</td>
<td>5000</td>
<td>625</td>
<td>2500</td>
</tr>
<tr>
<td>6c</td>
<td>1250</td>
<td>NA</td>
<td>625</td>
<td>2500</td>
<td>625</td>
<td>2500</td>
</tr>
<tr>
<td>8a</td>
<td>625</td>
<td>625</td>
<td>312.5</td>
<td>625</td>
<td>625</td>
<td>625</td>
</tr>
<tr>
<td>8b</td>
<td>625</td>
<td>1250</td>
<td>2500</td>
<td>1250</td>
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<td>2500</td>
</tr>
<tr>
<td>Amphotericin B</td>
<td>9.8</td>
<td>2.44</td>
<td>-</td>
<td>-</td>
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<td>-</td>
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<tr>
<td>Ampicillin</td>
<td>-</td>
<td>-</td>
<td>1.22</td>
<td>0.6</td>
<td>9.76</td>
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<tr>
<td>Gentamycin</td>
<td>-</td>
<td>-</td>
<td>9.76</td>
<td>4.88</td>
<td>0.6</td>
<td>1.22</td>
</tr>
</tbody>
</table>

* NA: No activity

Conclusion
A series of novel 4-((4-hydroxynaphthalen-1-yl)diazenyl) benzenesulfonamides, naphtho[1,2-b]furans, benzo[h]chromenes and 4-(4-amino-3,5-dicyano-6-imino-5,6-dihydropyridazin-1(4H)-yl)benzenesulfonamides were synthesized to evaluate their antimicrobial biological activity with the hope of discovering new structure leads serving as antimicrobial agents.

Experimental
All analyses were done at the Microanalytical Center, Cairo University, Cairo (Egypt). Melting points (uncorrected) were determined in open capillaries on a Gallenkamp melting point apparatus (Sanyo Gallenkamp, Southborough, UK). IR spectra (KBr discs) were recorded using a Shimadzu FT-IR 8400S spectrophotometer (Shimadzu, Kyoto, Japan). Infrared (IR) Spectra were recorded as KBr disks. NMR Spectra were recorded on a Bruker spectrophotometer (Bruker, Karlsruhe, Germany). 1H spectrum was run at 400 MHz in deuterated dimethylsulfoxide (DMSO-d6). Chemical shifts are expresses in values (ppm) relative to TMS as an internal standard. Mass spectral data were given by a GCMS-QP1000 EX-spectrometer (Shimadzu, Kyoto, Japan) at 70 eV. All reagents used were of the Analytical grade. Compounds α-cyanocinnamnitrites 7 41 and 2-amino-1,1,3-tricyanopropene 9 42 have been synthesized as previously reported.

General Procedure for Synthesis of 4-((4-hydroxynaphthalen-1-yl)diazenyl)benzenesulfonamides 3a-e:
Sulfonamide (0.01 mole) was suspended in water (50 ml). Hydrochloric acid (10 ml, 36%) was added dropwise to this well stirred. The mixture was gradually heated up to 70 °C till clear solution obtained. The solution was cooled to 0-5 °C in an ice bath. A solution of NaNO2 (0.5 mg) in water (5ml) previously cooled to 0 °C, was then added over a period 5 minutes with
stirring. 1-naphthal (0.01 mole) was dissolved in 10% NaOH (10 ml) and then put ice to cool to 5 °C. Then, diazonium salt solution was added occasionally stirring very slowly to the 1-naphthal solution. The reaction mixture was left to complete for 15 min and occasional stirring; then the formed precipitate was filtered and dried in air and then recrystallized from proper solvent to give 3.

4-((4-hydroxynaphthalen-1-yl)diazeyln)-benzenesulfonamide 3a.
Brown crystals (ethanol), Yield: 82%, m.p. 214-219 °C; IR(KBr, cm⁻¹): 3445 (OH), 3274 (NH), 1627 (C=C), 1594 (N=N), 1355, 1155 (S=O);¹H NMR (DMSO-_d₆, ppm): 7.24, 7.72, 7.98, 8.16(4d, 4H), 7.55, 7.62(2m, 2H), 7.88, 8.26 (2d, 4H), 7.36 (s, 2H, NH₂ exchangeable with D₂O), 9.45 (s, 1H, OH, exchangeable with D₂O); Anal. Calcd. for C₁₃H₁₁N₂O₂S: C, 56.80; H, 3.89; N, 12.58; S, 9.65.

N-carbamimido-4-((4-hydroxynaphthalen-1-yl)diazeyln)-benzenesulfonamide 3b.
Brown crystals (ethanol), Yield: 83%, m.p. 246-248 °C; IR(KBr, cm⁻¹): 3441 (OH), 3239 (NH₂), 3227, 3223 (2NH), 1633 (C=C), 1596 (N=N), 1355, 1165 (S=O);¹H NMR (DMSO-_d₆, ppm): 7.28, 7.72, 8.14 (3d, 3H), 7.49-7.57(2m, 2H), 7.82, 788 (2d, 4H), 6.75 (s, 2H, NH₂ exchangeable with D₂O), 7.61, 7.80 (2s, 2H, 2NH exchangeable with D₂O), 10.03 (s, 1H, OH exchangeable with D₂O); Anal. Calcd. for C₁₀H₁₀N₂O₃S: C, 55.27; H, 4.09; N, 18.96; S, 8.68. Found: C, 55.12; H, 3.95; N, 18.76; S, 8.42.

4-((4-hydroxynaphthalen-1-yl)diazeyln)-N-(thiazol-2-yl)benzenesulfonamide 3c.
Brown crystals (ethanol), Yield: 86%, m.p. 302-304 °C; IR(KBr, cm⁻¹): 3415 (OH), 3274 (NH), 1627 (C=C), 1594 (N=N), 1517, 1138 (S=O);¹H NMR (DMSO-_d₆, ppm): 7.30, 7.75, 8.13, 8.15(4d, 4H), 7.58, 7.71 (2m, 2H), 7.86, 7.92(2d, 4H), 6.73, 7.26 (2d, 2H, H-thiazole), 12.74 (br, H, OH exchangeable with D₂O), 10.05 (s, 1H, OH, exchangeable with D₂O); MS: 410 (M⁺, 42.83 %), 393 (3.17), 382 (28.92 %), 346 (62.87 %), 247 (17.45 %), 219 (4.18 %), 92 (93.25 %), 76 (19.77 %), 65 (100 %); Anal. Calcd. for C₁₀H₁₀N₂O₃S₇: C, 55.60; H, 3.44; N, 13.65; S, 15.62. Found: C, 55.46; H, 3.28; N, 13.47; S, 15.42.

4-((4-hydroxynaphthalen-1-yl)diazeyln)-N-(5-methylisoxoazol-3-yl)benzenesulfonamide 3d.
Brown crystals (ethanol), Yield: 80%, m.p. 242-244 °C; IR(KBr, cm⁻¹): 3448 (OH), 3107 (NH), 2924 (CH-aliph.), 1632 (C=C), 1595 (N=N), 1327, 1139 (S=O);¹H NMR (DMSO-_d₆, ppm): 2.36 (s, 3H, CH₃), 6.25 (s, 1H, H-oxazole), 7.25, 7.68, 8.04, 8.12(4d, 4H), 7.51, 7.63 (2m, 2H), 7.88, 7.97(2d, 4H), 11.26 (s, 1H, NH exchangeable with D₂O), 10.02 (s, 1H, OH exchangeable with D₂O); Anal. Calcd. for C₁₃H₁₁N₂O₃S: C, 58.81; H, 3.95; N, 13.72; S, 7.85. Found: C, 58.64; H, 3.79; N, 13.54; S, 7.68.

4-((4-hydroxynaphthalen-1-yl)diazeyln)-N-(pyrimidin-2-yl)benzenesulfonamide 3e.
Dark brown crystals (ethanol), Yield 78%, m.p. 132-134 °C; IR(KBr, cm⁻¹): 3377 (OH), 3225 (NH), 1625 (C=C), 1581 (N=N), 1316, 1155 (S=O);¹H NMR (DMSO-_d₆, ppm): 6.99, 8.36 (m, d,3H, H-pyrimidine), 7.23, 7.69, 8.06, 8.15 (4d, 4H), 7.52, 7.60 (2m, 2H), 7.91, 7.98 (2d, 4H), 11.30 (s, H, NH exchangeable with D₂O), 9.98 (s, 1H, OH, exchangeable with D₂O); Anal. Calcd. for C₁₀H₁₀N₂O₃S: C, 59.25; H, 3.73; N, 17.27; S, 7.91. Found: C, 58.94; H, 3.64; N, 17.13; S, 7.64.

**General Procedure for Synthesis of naphtho[1,2-b]furans 6a-c.**

To a mixture of compound 3 (0.01 mole) and cinnamic acid 4 (0.01 mole) in DMF (10 ml), a few drops piperidine was added. The reaction mixture was refluxed for 2 h. After cooling, the precipitate was filtered and recrystallized from proper solvent to give 6.

4-((3-phenyl)naphtho[1,2-b]furan-5-yl)diazeyln)-benzenesulfonamide 6a.
Brown crystals (ethanol), Yield: 79%, m.p. 180-182 °C; IR(KBr, cm⁻¹): 3266 (NH₂), 1624 (C=C), 1594 (N=N), 1316, 1155 (S=O);¹H NMR (DMSO-_d₆, ppm): 7.28-7.55 (m, d, 5H, Ph-H), 8.18 (s, 1H, furan-H), 8.32(s, 1H), 8.20, 8.52(2d, 2H), 7.42, 7.56 (2m, 2H), 7.90, 8.06(2d, 4H), 7.30(s, 2H, NH₂ exchangeable with D₂O); Anal. Calcd. for C₁₀H₁₀N₂O₃S: C, 67.43; H, 4.01; N, 11.23; S, 7.50. Found: C, 67.28; H, 3.87; N, 11.14; S, 7.38.

N-carbamimido-4-((3-phenyl)naphtho[1,2-b]furan-5-yl)diazeyln)-benzenesulfonamide 6b.
Dark brown crystals (ethanol), Yield: 82%, m.p. 205-207 °C; IR(KBr, cm⁻¹): 3440 (NH₂), 3328, 3271 (2NH), 1632 (C=C), 1595 (N=N), 1307, 1131 (S=O);¹H NMR (DMSO-_d₆, ppm): 7.32-7.58 (m, d, 5H, Ph-H), 8.17(s, 1H, furan-H), 8.32(s, 1H), 8.17, 8.43 (2d, 2H), 7.60, 7.67 (2m, 2H), 7.89, 7.96(2d, 4H), 6.74(s, 2H,
NH₂, exchangeable with D₂O), 7.75, 8.08(2s, 2H, 2NH, exchangeable with D₂O).

MS: 469 (M⁺; 12.15 %), 453 (8.95 %), 426 (10.10 %), 398 (6.67 %), 392(6.67%), 362 (14.58 %), 198 (11.76 %), 135 (8.31 %), 78 (81.71 %), 57 (100 %). Anal. Calcd. for C₂₅H₂₈N₂O₅S: C, 63.95; H, 4.08; N, 10.22; S, 6.83. Found: C, 63.78; H, 3.97; N, 10.10; S, 6.65.

4-((3-phenyl-naphtho[1,2-b]furan-5-yl)diazeyl)-N-(thiazol-2-yl)benzenesulfonamide 6c.

Brown crystals (ethanol), Yield: 84%, m.p.193-195 °C; IR(KBr, cm⁻¹): 3475 (NH, exchangeable with D₂O), 3431, 2928 (2NH, 2H, NH exchangeable with D₂O), 1602 (C=O), 1589 (C=C), 1483 (NH, exchangeable with D₂O);

1H NMR (DMSO-d₆, ppm): 7.57, 7.92, 8.02 (2d, 4H), 7.71, 7.97 (2H), 7.93, 8.02 (2d, 4H), 12.64(s, NH, exchangeable with D₂O);

MS: 510 (M⁺; 7.64 %), 494 (4.64 %), 482 (6.39 %), 446 (4.76 %), 433 (4.14 %), 319 (3.38 %), 267 (3.45 %), 241 (7.58 %), 239 (3.29 %), 226 (4.26 %), 209 (4.07 %), 177 (4.57 %), 149 (4.76 %), 100 (100 %), 56 (32.02 %), 52 (7.89 %). Anal. Calcd. for C₂₅H₂₈N₂O₅S: C, 63.51; H, 3.55; N, 10.97; S, 12.56. Found: C, 63.38; H, 3.39; N, 10.75; S, 12.32.


To a mixture of compound 3 (0.01 mole) and α-cyanocinnamonic acid (0.01 mole) in DMF (10 ml), a few drops triethylamine was added. The reaction mixture was refluxed for 1 h. After cooling, the precipitate was filtered and recrystallized from proper solvent to give 8.

4-((2-amino-3-cyano-4-phenyl-4H-benzo[h]chromen-6-yl)diazeyl)benzenesulfonamide 8a.

Brown crystals (ethanol), Yield: 84%, m.p.90-92 °C; IR(KBr, cm⁻¹): 3355, 3270 (2NH₂), 3054 (CH- arom.), 2190 (CN), 1631 (C=O), 1593 (N=N), 1357, 1156 (S=O); ¹H NMR(DMSO-d₆, ppm): 4.90 (s, 1H, 4H-pyran), 7.23-7.32 (m, d, 5H, Ph-H), 7.88(s, 1H), 8.12, 8.27(2d, 2H), 7.49, 7.56(2m, 2H), 7.90, 8.25(2d, 4H), 6.88, 7.21 (2s, 4H, 2NH₂, exchangeable with D₂O); MS: 481 (M⁺; 1.64 %), 465 (1.53 %), 455 (1.55 %), 452 (25.81 %), 401 (2.42 %), 404 (4.62 %), 373 (12 %), 327 (8.13 %), 297 (100 %), 269 (39.18 %), 219 (11.1 %), 65 (26.78 %).

Anal. Calcd. for C₂₅H₂₆N₂O₅S: C, 64.85; H, 3.98; N, 14.54; S, 6.66. Found: C, 64.63; H, 3.74; N, 14.24; S, 6.39.

4-((2-amino-3-cyano-4-(p-tolyl)-4H-benzo[h]chromen-6-yl)diazeyl)benzenesulfonamide 8b.

Brown crystals (ethanol), Yield: 87%, m.p.135-137 °C; IR(KBr, cm⁻¹): 3424, 3385 (2NH₂), 3034 (CH-arom), 2870 (CH- aliph), 2186 (CN), 1631 (C=C), 1594 (N=N), 1358, 1153 (S=O); ¹H NMR (DMSO-d₆, ppm): 2.35 (s, 3H, CH₃), 4.82 (s, 1H, 4H-pyran), 7.13, 7.21 (2d, 4H, Ph-CH₃), 7.87(s, 1H), 8.13, 8.38(2d, 2H), 7.51, 7.54(2m, 2H), 8.15, 7.81(2d, 4H), 6.89, 7.23 (2s, 4H, 2NH₂, exchangeable with D₂O);

Anal. Calcd. for C₂₇H₂₆N₂O₅S: C, 65.44; H, 4.27; N, 14.13; S, 6.47. Found: C, 65.38; H, 4.09; N, 13.95; S, 6.28.

General Procedure for Synthesis of 4-(4-amino-3,5-dicyano-6-iminopyrazidin-1(6H)-yl)benzenesulfonamides 11a-c.

Sulfonamide (0.01 mole) was suspended in water (50 ml). Hydrochloric acid (10 ml, 36%) was added drop wise to this well stirred. The mixture was gradually heated up to 70 °C till clear solution obtained. The solution was cooled to 0-5 °C in an ice bath. A solution of NaNO₂ (0.5 gm) in water (5ml) previously cooled to 0 °C, was then added over a period 5 minutes with stirring. 2-Amino-1,1,3-tricyanopropene 9 (0.01 mole) was dissolved in ethanol (10 ml) in the presence of sodium acetate (1 gram) and then put ice to cool to 5 °C. Then, diazonium salt solution was added occasionally stirring very slowly to the 2-Amino-1,1,3-tricyanopropene solution. The reaction mixture was left to complete for 3h and occasional stirring; the formed precipitate was filtered and dried in air and then recrystallized from proper solvent to give 11.

4-(4-amino-3,5-dicyano-6-iminopyrazidin-1(6H)-yl)benzenesulfonamide 11a.

Yellow crystals (ethanol), Yield: 88%, m.p.158- 160 °C; IR(KBr, cm⁻¹): 3378, 3312 (2NH₂), 3211 (NH), 2219, 2199 (2C=N), 1620 (C=N), 1326, 1159 (S=O); ¹H NMR (DMSO-d₆, ppm): 6.65, 7.20 (2br., 4H, 2NH₂, exchangeable with D₂O), 7.80, 8.21(2d, 4H, AB-system), 9.85(br., 1H, NH, exchangeable with D₂O). Anal. Calcd. for C₁₂H₁₃N₂O₃S: C, 45.71; H, 2.88; N, 31.10; S, 10.17. Found: C, 45.56; H, 2.64; N, 30.94; S, 10.04.

4-(4-amino-3,5-dicyano-6-iminopyrazidin-1(6H)-yl)-N-carbamimidoylbenzenesulfonamide 11b.

Yellow crystals (ethanol), Yield: 82%, m.p.>300 °C; IR(KBr, cm⁻¹): 3450-3211 (NH+sNH₂), 2220, 2202 (2C=N), 1620 (C=N), 1352, 1138 (S=O); ¹H NMR (DMSO-d₆, ppm): 6.85, 7.10(2br., 4H, 2NH₂, exchangeable with D₂O), 7.63, 7.95 (2d, 4H, AB-
system), 7.55, 8.52, 10.10(br., 3H, 3NH, exchangeable with D2O).
Anal. Calcd. for C13H11N2O3S:C: 43.69; H: 3.10; N: 35.28; S: 8.97. Found: C: 43.46; H: 2.92; N: 35.13; S: 8.87.

4-(4-amino-3,5-dicyano-6-iminopyrazidin-1(6H)-yl)-N-(thiazol-2-yl)benzenesulfonylamide 11c
Yellow crystals (ethanol), Yield: 85%, m.p.>300 °C; IR(KBr, cm-1): 3430 (NH2), 3320, 3214 (2NH), 2216, 2201 (2C=O), 1622 (C=N), 1330, 1147 (S=O); 1HNMR (DMSO-d6, ppm): 6.84, 7.77 (2d, 2H, thiazole), 7.70, 7.95 (2d, 4H, AB-system), 6.70 (s, 2H, NH2, exchangeable with D2O), 9.85, 12.16 (2br., 2H, 2NH, exchangeable with D2O).

4-(4-amino-3,5-dicyano-6-iminopyrazidin-1(6H)-yl)-N-(5-methylisoxazol-2-yl)benzenesulfonylamide 11d
Yellow crystals (ethanol), Yield: 80%, m.p.>300 °C; IR(KBr, cm-1): 3311 (NH2), 3225, 3192 (2NH), 2217, 2199 (2C=O), 1618 (C=N), 1330, 1159 (S=O); 1HNMR (DMSO-d6, ppm): 2.35 (s, 3H, CH3), 6.15 (s, 1H, oxazole-H), 7.72, 8.12 (2d, 4H, AB-system), 7.52 (s, 2H, NH2, exchangeable with D2O), 9.76, 11.18 (2s, 2H, 2NH, exchangeable with D2O); Anal. Calcd. for C18H12N2O3S: C: 70.48; H: 4.30; N: 11.49; S: 7.59. Found: C: 70.36; H: 4.35; N: 11.51; S: 7.65.

4-(4-amino-3,5-dicyano-6-iminopyrazidin-1(6H)-yl)-N-(pyrimidin-2-yl)benzenesulfonylamide 11e
Yellow crystals (ethanol), Yield: 88%, m.p.>300 °C; IR(KBr, cm-1): 3434 (NH2), 3228, 3123 (2NH), 2216, 2201 (2C=O), 1621 (C=N), 1339, 1155 (S=O); 1HNMR (DMSO-d6, ppm): 6.98, 8.42 (m, d, 3H, H-pyrimidine), 7.68, 8.15 (2d, 4H, AB-system), 7.90 (s, 2H, NH2, exchangeable with D2O), 9.65, 11.26 (2s, 2H, 2NH, exchangeable with D2O); Anal. Calcd. for C18H11N2O3S: C: 71.00; H: 4.09; N: 11.73; S: 7.90. Found: C: 71.04; H: 4.09; N: 11.75; S: 7.90.

4-(4-amino-3,5-dicyano-6-iminopyrazidin-1(6H)-yl)-N-(pyrimidin-2-yl)benzenesulfonylamide 11f
Yellow crystals (ethanol), Yield: 88%, m.p.>300 °C; IR(KBr, cm-1): 3434 (NH2), 3228, 3123 (2NH), 2216, 2201 (2C=O), 1621 (C=N), 1339, 1155 (S=O); 1HNMR (DMSO-d6, ppm): 6.98, 8.42 (m, d, 3H, H-pyrimidine), 7.68, 8.15 (2d, 4H, AB-system), 7.90 (s, 2H, NH2, exchangeable with D2O), 9.65, 11.26 (2s, 2H, 2NH, exchangeable with D2O); Anal. Calcd. for C18H11N2O3S: C: 71.00; H: 4.09; N: 11.73; S: 7.90. Found: C: 71.04; H: 4.09; N: 11.75; S: 7.90.

Biological evaluation
All microbial strains were provided from the culture collection of the Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University, Cairo, Egypt. The antimicrobial activity was investigated on a dozen of newly synthesized compounds in order to increase the selectivity of these derivatives towards test microorganisms using the agar diffusion method using Mueller-Hinton agar medium for bacteria and Sabouraud’s agar medium for fungi. Briefly, 100 μl of the test bacteria/fungi were grown in 10 mL of fresh media until they reached a count of approximately 10⁸ cells/mL for bacteria or 10⁶ cells/mL for fungi. All the newly synthesized compounds were weighed and dissolved in dimethyl sulfoxide to prepare extract stock solution.

One hundred μL of each sample at 5 mg/mL was added to each well (10 mm diameter holes cut in the agar gel). The plates were incubated for 24-48 h at 37 °C (for bacteria and yeast) and 48 h at 28 °C (for filamentous fungi). After incubation, the microorganism’s growth was observed. Ampicillin and Gentamycin were used as standard antibacterial drugs while amphotericin B was used as a standard antifungal drug. The resulting inhibition zone diameters were measured in millimeters and used as a criterion for the antimicrobial activity. If an organism is placed on the agar, it will not grow in the area around the well if it is susceptible to the chemical. This area of no growth around the disc is known as a Zone of inhibition. The size of the clear zone is proportional to the inhibitory action of the compound under investigation. Solvent controls (DMSO) were included in every experiment as negative controls. DMSO was used for dissolving the tested compounds and showed no inhibition zones, confirming that it does not influence the growth of the tested microorganisms. The active compounds were further investigated to determine their antimicrobial activity expressed regarding minimum inhibitory concentration (MIC) using the modified agar well diffusion method that mentioned above. Different concentrations of each active compound were tested and compared with standard drugs. The MIC was then determined as the lowest concentration inhibiting the growth of the organism after 24-48 h.

Conflict of interest
The authors declare that they have no conflict of interest.

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