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Synthesis, structure analysis, and antibacterial activity of some novel 10-substituted 2-(4-piperidyl/phenyl)-5,5-dioxo[1,2,4]triazolo[1,5-*b*][1,2,4]benzothiadiazine derivatives

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Abstract—A new series of 10-substituted 5,5-dioxo-5,10-dihydro[1,2,4]triazolo[1,5-*b*]-[1,2,4]benzothiadiazine arylsulfonamide derivatives (**10a**–**j** and **13a**–**f**) was synthesized. The structures of these compounds were confirmed on the basis of spectral data, elemental analysis, X-ray analysis, and quantum chemical calculations. These compounds were evaluated for their efficacy as antibacterial agents against various Gram-positive and Gram-negative strains of bacteria. Amongst these compounds **10f** and **10i** were the most active compounds against *Escherichia coli* and **13e** against *E. coli* as well as *Bacillus subtilis*. Moreover, other compounds also showed potent inhibitory activity in comparison to the standard drugs.

The demand for novel chemotherapeutic antibacterial agents remains attractive in the field of medicinal chemistry. After many years of extensive studies on the structural modification of known antibacterial scaffolds, it is increasingly becoming difficult to deliver new leads. The focus of such antibacterials' research has, therefore, moved to the identification of novel chemical classes of bacterial targets. In fact, in the past 40 years only two new chemical classes of antibiotics, oxazolidinone (linezolid)[1] and the lipopeptide (daptomycin),[2] have been introduced to the market and existing antibiotics are directed at a small number of targets, mainly cell wall, DNA, and protein biosynthesis.

The sulfonamide group is considered as a pharmacophore which is present in a number of biologically active molecules, particularly in antimicrobial agents.[3–5] In addition, numerous sulfonamide derivatives have been reported as carbonic anhydrase inhibitors,[6] anticancerous,[7] and anti-inflammatory agents.[8] The discovery of sulfonamides as antibacterial agents in the early 1930s was the beginning of one of the most fascinating areas of chemotherapeutic agents and once used successfully in the treatment of a variety of bacterial infections. However, the rapid emergence of sulfonamide resistance organisms and the development of more potent drugs have limited their clinical use. Some organisms are resistant to all approved antibiotics and can only be treated with experimental and potentially toxic drugs. For these reasons, there is an overwhelming need to develop more effective antibacterial agents to treat infections caused by antibiotic resistant bacterial pathogens.

Sulfonamides exert their effect by targeting on dihydropteroate synthase (DHPS) enzyme, which catalyzes folic acid pathway in bacteria and some eukaryotic cells,[9] but it is not present in human cells.[10] This is the basis for the selective effect of sulfonamides on bacteria and for their broad spectrum of antibacterial activity. 4*H*-1,2,4-Benzothiadiazine 1,1-dioxides can be considered as cyclic sulfonamide class of molecules and have been extensively studied as potassium channel openers, for example, diazoxide.[11] Moreover, these compounds have exhibited marked inhibitory activity[12] against Gram-positive bacteria and recently we have investigated this structural core for its anti-*Mycobacterium* activity.[13]

Keywords: Triazolobenzothiadiazines; Aryl sulfonamides; Antibacterial activity; X-ray crystallography; Quantum chemical calculations.
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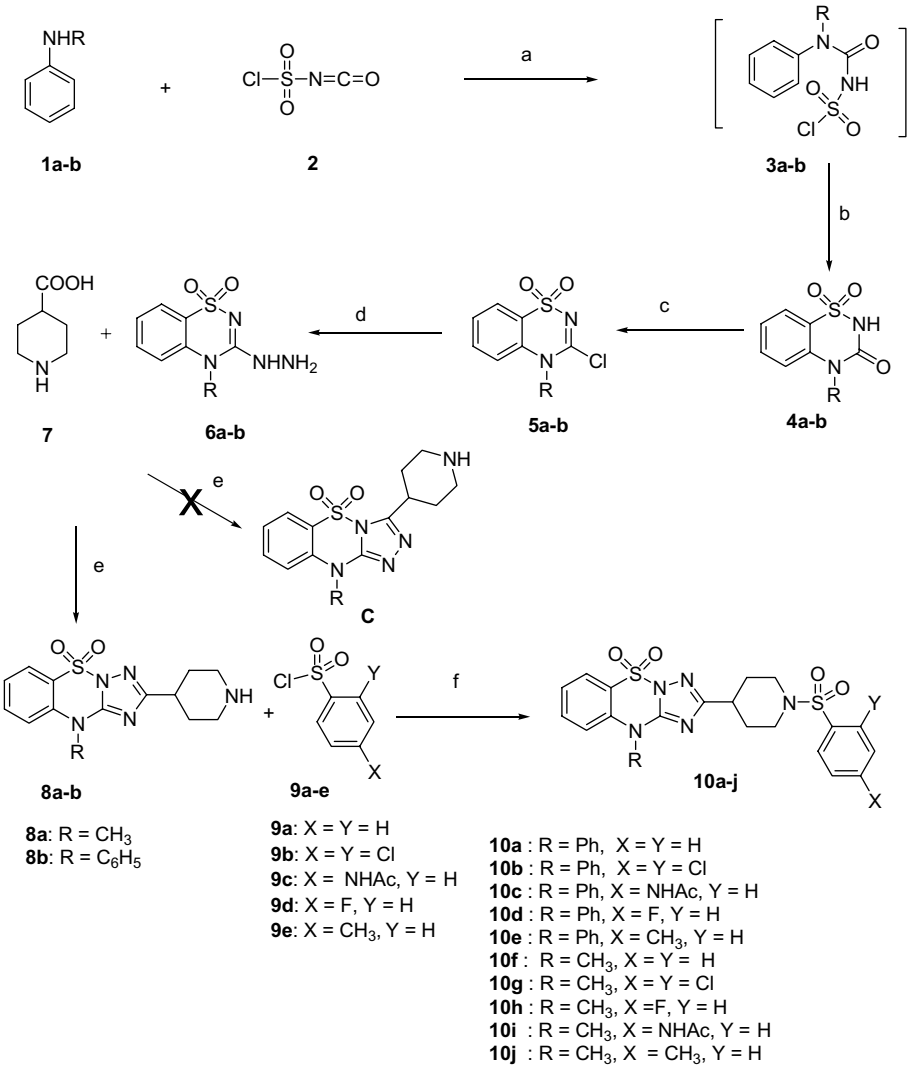
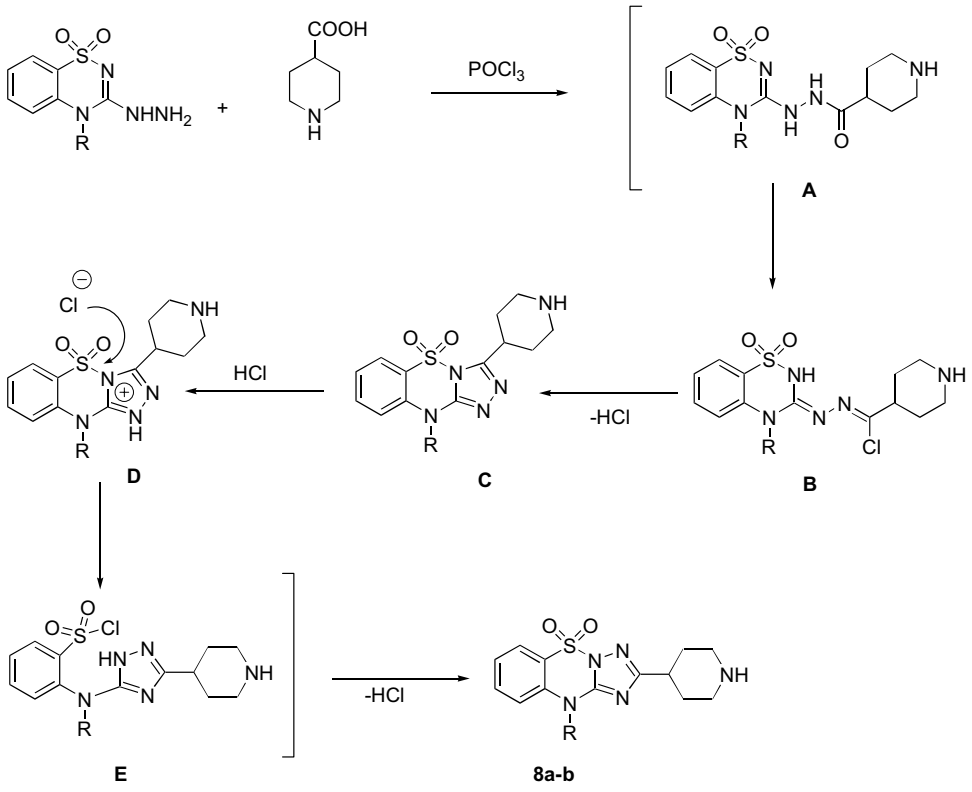
On the basis of these findings and in continuation of our search on 1,2,4-benzothiadiazine derivatives with potential biological activity, we have synthesized[14] new molecules based on 1,2,4-benzothiadiazine 1,1-dioxide ring system by incorporating arylsulfonamide functionality. The arylsulfonamide structural motif is well known for exhibiting antibacterial activity.[15–20] This article describes the synthesis and antibacterial activity of some novel triazolobenzothiadiazine linked arylsulfonamides (**10a**–**j** and **13a**–**f**). The structure of these molecules has been confirmed by X-crystallography and the rearrangement of the ring is explained on the basis of relative energies and proton affinities. Almost all compounds showed promising antibacterial activity against Gram-positive and Gram-negative bacterial strains.

The preparation of the starting materials, 3-hydrazino-4-methyl/phenyl-4*H*-1,2,4-benzothiadiazine 1,1-dioxides (**6a** and **b**), was accomplished by the synthetic sequences as previously reported[21] (Scheme 1). The synthesis of triazolo fused benzothiadiazines (**8a** and **b**) was carried out by refluxing 3-hydrazino-4-methyl/phenyl-4*H*-1,2,4-benzothiadiazine 1,1-dioxides (**6a** and **b**) and isonipecotic acid (**7**) in phosphorus oxychloride.[22] The target sulfonamide linked triazolobenzothiadiazines **10a**–**j** were obtained by the condensation of **8a** and **b** with substituted arylsulfonyl chlorides (**9a**–**e**) in pyridine[23] (Scheme 1).

A probable mechanism[22] of the reaction course is shown in Scheme 2. Treatment of 3-hydrazino-4-methyl-4*H*-1,2,4-benzothiadiazine 1,1-dioxide and isonipecotic acid with $POCl_3$ gives 3-(*N*′-piperidinoyl)benzothiadiazine hydrazide of type **A**, which undergoes chlorination to give chloro derivative of type **B**. This undergoes intramolecular ring closure with evolution of HCl to give triazolo[4,3-*b*][1,2,4]benzothiadiazine (**C**). Protonation of triazole ring **D** in acidic condition to form a sulfonyl chloride **E** by SO_2–N bond cleavage takes place which in turn cyclizes to more stable triazolo[1,5-*b*][1,2,4]benzothiadiazines (**8a** and **b**) with minimization of potential energies. Structures of the compounds **8a** and **b**, and final products **10a**–**j** (Scheme 1) were confirmed by the elemental analysis, IR, ^1H, ^{13}C NMR, and mass



Scheme 1. Reagents and conditions: (a) CH_3NO_2, 0 °C, rt, 30 min; (b) $AlCl_3$, 120 °C, 30 min; (c) PCl_5, 190 °C, 30 min; (d) $N_2H_4{\cdot}H_2O$, $CHCl_3$, 10 °C; (e) $POCl_3$, 110 °C, 3–4 h; (f) pyridine, rt, 4 h.



Scheme 2. Proposed mechanism of the formation of 10-substituted 2-piperidyl[1,2,4]triazolo[1,5-*b*][1,2,4]benzothiadiazines **8a** and **b**.

spectrometry. However, the spectroscopic data did not allow straightforward discrimination between expected benzo[*e*][1,2,4]triazolo[4,3-*b*][1,2,4]thiadiazines' **C** structure and the alternative benzo[*e*][1,2,4]triazolo[1,5-*b*][1,2,4]thiadiazines **8a** and **b** (Scheme 1). Therefore, X-ray crystallography[24] was undertaken on representative compound **10b** to investigate further discrete structural aspects of this compound. The compound **10b** was subjected to single-crystal X-ray analysis to prove the alternative benzo[*e*][1,2,4]triazolo[1,5-*b*][1,2,4]thiadiazines **8a** and **b** (Fig. 1 and Scheme 1). Crystal of compound **10b** was grown by slow evaporation from a mixture of solvents, that is, methanol, chloroform, and diisopropylether (2:3:1).

Similarly, 3-(*N*′-arylsulfonyl)triazolobenzothiadiazine derivatives (**13a**–**f**) were obtained by the reaction of

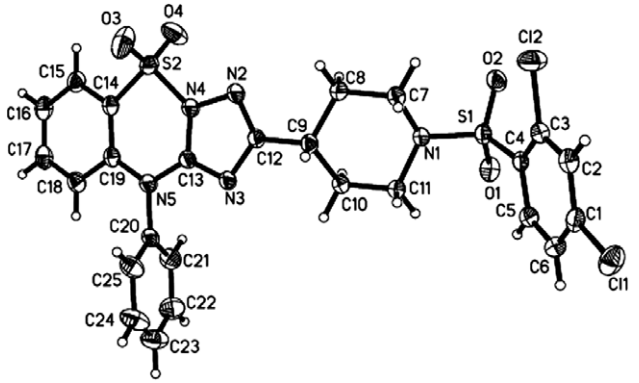


Figure 1. ORTEP view showing the atom-labeling scheme with thermal ellipsoids drawn at 30% probability for compound **10b**.

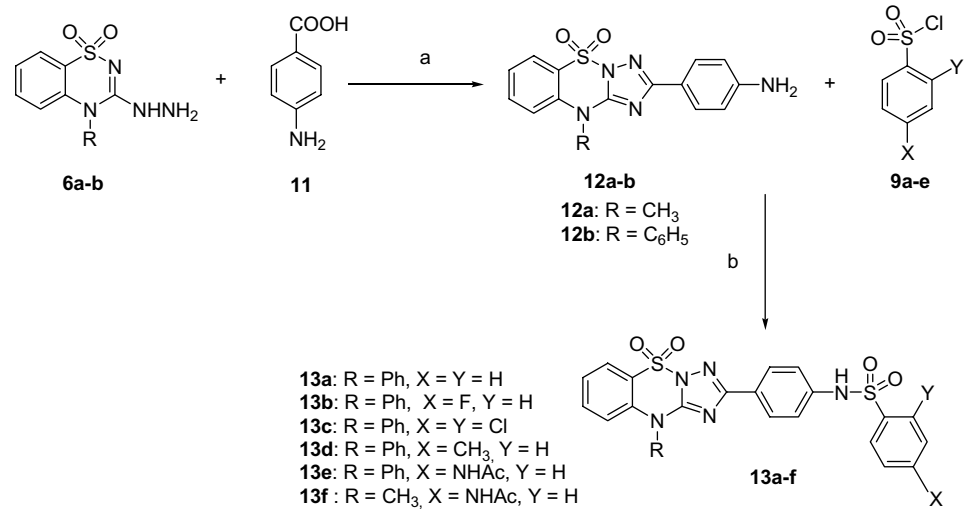
the compounds **6a** and **b** and *p*-amino benzoic acid (**11**) by refluxing in phosphorus oxychloride followed by the condensation of **12a** and **b** with arylsulfonyl chlorides (**9a**–**e**) in pyridine (Scheme 3).

Quantum chemical calculations were employed to corroborate the observed experimental results. A two-model system was studied to explain the preferential formation of the structure **8a** over **C** (Fig. 2). All the calculations were carried out by using the Gaussian 03 program package.[25] Geometry optimization and frequency calculations were performed on all the structures considered at B3LYP/6-31G* level. Further, the proton affinities were also calculated at the same level of theory.

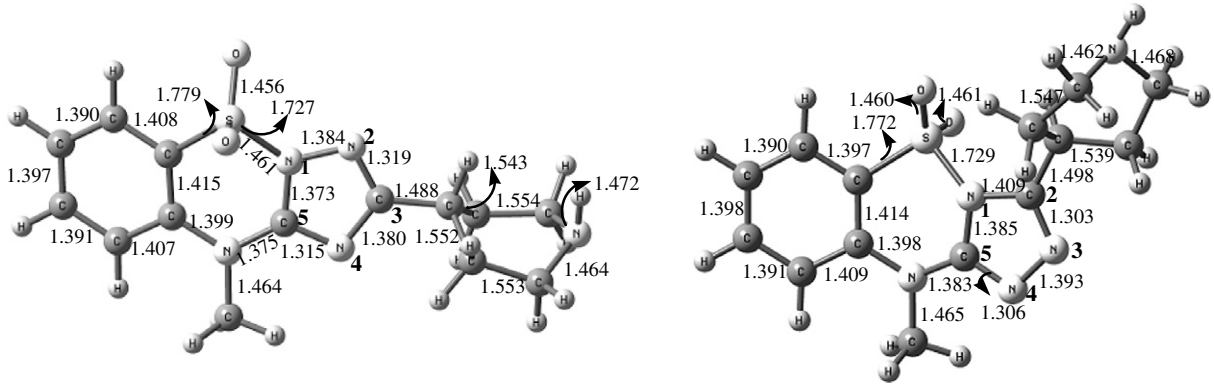
Both these structures (**8a** and **C**) were found to be minima on the potential energy surface. The calculated relative energies are given in parentheses. Based on the calculated relative energies it was observed that **8a** is more stable than **C** and these energy values are shown in Figure 2. Highest occupied molecular orbital and lowest unoccupied molecular orbital gap for **8a** is 4.44 eV and **C** is 4.35 eV. This is evident as the HOMO–LUMO difference is higher, stability of the molecule is considered as higher. Furthermore, in order to explain the reactivity of these molecules a gas-phase proton affinity (PA) has been calculated for the following reaction:

$$B + H^+ \rightleftharpoons BH^+$$

where B is the base form and BH^+ is the protonated form. The PAs were calculated by using the equation given below.



Scheme 3. Reagents and conditions: (a) $POCl_3$, 110 °C, 3–4 h; (b) pyridine, rt, 4 h.



8a (0.0 kcal/mol) **C** (6.8 kcal/mol)

Figure 2. Optimized geometries (Å) and relative energies of model systems **8a** and **C** at B3LYP/6-31G* level.

$$PA = -\Delta H = \Delta E_{tot} + \Delta ZPE + 5/2\ RT$$

where ΔH is the enthalpy change of the protonation reaction, E_{tot} is the electron energy at 0 K, ZPE is the zero point correction energy, R is the molar gas constant, and T is the absolute temperature in Kelvin.

In the case of **8a**, proton additions at the N2 and N4 positions gave proton affinities 240.6 and 238.0 kcal/mol, respectively, whereas in case of **C**, proton addition at the N4 and N3 positions gave proton affinities 248.1 and 252.1 kcal/mol, respectively. These proton affinity results depict that the proton addition takes place preferably in **C**. These results also indicate that the ring rearrangement takes place in structure **C**. Moreover, overall results of quantum chemical calculations are in accordance with the experimental observations and confirm that the combined relative energies and proton affinities provide rationale for the observed rearrangement that takes place in this reaction.

The antibacterial activity of the synthesized compounds (**10a**–**j** and **13a**–**f**) was tested by disk diffusion method[26,27] against various Gram-positive and Gram-negative bacterial strains of *Escherichia coli* MTCC 448, *Pseudomonas aeruginosa* MTCC 424, *Klebsiella pneumoniae* MTCC 618, *Staphylococcus epidermidis* MTCC 435, *Bacillus subtilis* MTCC 441, and *Vibrio species*. The antibacterial activity was compared with those of some standard antibacterial agents like sulfanilamide and sulfadiazine. From the results in Table 1, most of the compounds showed broad spectrum of inhibitory activity against bacterial strains. Compounds **10f**, **10i**, and **13e** were found to be the most active against *E. coli* (MIC 8 μg/disk); however, these compounds showed comparable activity with sulfadiazine against other organisms. Similarly, compounds **13a**, **13d**, and **13e** were very effective against *B. subtilis*. All these compounds displayed more potent inhibitory activity than the standard drugs against *K. Pneumoniae*. Overall, good to improved antibacterial activity was observed for most of the compounds against all the bacterial strains used in the study and their MIC ranged from 16 to 64 μg/disk in comparison to the controls.

In summary, the synthesis and screening of antibacterial activity for a novel series of 10-substituted 5,5-dioxo[1,24,]triazolo[1,5-*b*][1,2,4]benzothiadiazine

Table 1. Antibacterial activity of compounds of [1,2,4]triazolo[1,5-*b*]-benzothiadiazine arylsulfonamide derivatives (**10a–j** and **13a–f**)

Compound	MIC (μg/disk)					
	E. coli[a]	*P. aeruginosa*[a]	*K. pneumoniae*[a]	*S. epidermidis*[b]	*B. subtilis*[b]	*Vibrio sps*[a]
10a	16	16	32	32	16	32
10b	16	32	32	32	16	16
10c	16	32	32	32	16	32
10d	16	32	32	32	16	16
10e	16	32	16	32	16	32
10f	8	64	32	32	16	32
10g	16	64	16	16	16	32
10h	16	32	16	32	32	32
10i	8	32	32	32	32	16
10j	16	64	64	32	16	32
13a	16	32	32	32	8	32
13b	16	32	64	16	16	32
13c	16	16	32	32	16	32
13d	16	32	32	32	8	32
13e	8	32	16	32	8	32
13f	16	32	32	32	16	32
SA	>128	>512	>64	>512	>128	>512
SZ	16	32	>64	32	16	16

[a] Gram-negative.
[b] Gram-positive; SA, sulfanilamide; SZ, sulfadiazine.

arylsulfonamide derivatives was investigated. Moreover, rearrangement of the fused triazolobenzothiadiazine was studied by using X-ray crystallography and quantum chemical calculations. Compounds **10f**, **10i**, and **13e** were found to be the most active against *E. coli* and **13a**, **13d**, and **13e** against *B. subtilis*. Almost all the compounds have shown significant antibacterial activity in comparison to the controls. Therefore, such compounds would represent a fruitful matrix for the development of a new class of antibacterial agents using triazolobenzothiadiazine arylsulfonamides as a promising scaffold for further investigations.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.07.043.

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14. General procedures for the synthesis of compounds **10a–j**. 3-Hydrazino-4-methyl/phenyl-4*H*-1,2,4-benzothiadiazine 1,1-dioxides (**6a** and **b**, 6.63 mmol) and piperidine 4-carboxylic acid (**7**, 7.79 mmol) were taken in phosphorus oxychloride (5 mL) and refluxed for 4–5 h in oil bath under nitrogen atmosphere. The reaction mixture was then cooled to room temperature, poured onto crushed ice, and neutralized with sodium bicarbonate. The aqueous layer was extracted with ethyl acetate (4× 50 mL) dried over anhydrous Na_2SO_4, and concentrated under vacuum to get **8a** and **b**. Compounds **8a** and **b** (1 mmol) and substituted benzenesulfonyl chlorides (**9a–e**, 1.2 mmol) were taken in dry pyridine (5 mL) and stirred at room temperature until starting materials were consumed as monitored by TLC. The reaction mixture was diluted with 1 M HCl, then the obtained precipitate was filtered, dried, and further purified by column chromatography (hexane/ethyl acetate) to afford the final products (**10a–j**). Spectral data for compound **10b**: ^{1}H NMR (300 MHz, $CDCl_3$): 8.12 (d, J = 8.30, 1H), 7.98 (d, J = 8.30 Hz, 1H), 7.60–7.70 (m, 3H), 7.52 (m, 2H), 7.32–7.44 (m, 4H), 6.74 (d, J = 8.30 Hz, 1H), 3.79 (td, J = 12,84, 3.77 Hz, 2H), 2.91 (dt, J = 10.57, 3.77 Hz, 2H), 2.74–2.86 (m, 1H), 2.05 (dd,

J = 10.57, 3.77 Hz, 2H), 1.85 (dq, J = 10.57, 3.77 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 167.8, 153.3, 139.2, 137.7, 136.2, 135.4, 134.4, 133.2, 132.8, 131.9, 130.8, 130.2, 128.9, 127.1, 124.5, 123.8, 121.7, 117.2, 45.1, 35.1, 29.5; ESI-MS m/z = 590 $(M+1)^+$, 592 $(M+3)^+$.

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24. Crystal data of **10b**. $C_{25}H_{21}Cl_2N_5O_4S_2$; Data were collected on a Bruker SMART Apex CCD detector with graphite monochromated Mo Kα radiation (λ = 0.71073 Å). Data collection was performed and unit cell was initially refined using SMART. The crystals were triclinic, $P\bar{1}$, with a = 10.5944(5) Å, b = 11.0090(5) Å, c = 11.5280(5) Å, α = 88.034(1)°, β = 84.082(1)°, γ = 80.500(1)°, V = 1318.84(10) Å^3, Z = 2, ρ_{calc} = 1.487 Mg/m^3, μ = 0.447 mm^{-1}, and $F(000)$ = 608. Data reduction was performed using SAINT.[28] The structure was solved by direct methods using SHELXS97 and refinement was carried out by full-matrix least-squares technique using SHELXL97.[29] Anisotropic displacement parameters were included for all non-hydrogen atoms. All H atoms were included using a riding model. The final R value ($R1$) was 0.0326 for 4231 observed [$I > 2\sigma(I)$] reflections and 0.0357 for all 4639 reflections using 343 parameters, and goodness-of-fit was 1.041. Full crystallographic details of **10b** have been deposited at the Cambridge Crystallographic Data Centre and allocated the deposition number CCDC 641916.
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27. *Antibacterial assay:* All the test compounds were dissolved in DMF of 2 mg/mL. Empty sterilized disks of 6 mm were impregnated with compounds in the range from 8 to 64 μg/disk and placed in triplicate in the medium inoculated with fresh bacteria ($1–5 \times 10^4$ cfu mL^{-1}) on the agar surface of the culture plates. The plates were incubated at 35 °C for 24 h for zone of inhibition, if any, around the disks. Lowest concentration of the test substance exhibiting no visible growth of bacteria on the plate was considered to be minimum inhibitory concentrations. Sulfanilamide and sulfadiazine were used as positive controls whereas, the equivalent amount of solvent (DMF) did not exhibit any activity in the assay.
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