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Synthesis, Characterisation and Biological Evaluation of Some 5-Trifluoromethylisoxazole Derivatives

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Abstract:

A series of 4-(aryldiazenyl)-5-trifluoromethyl substituted isoxazole derivatives (**3a-n** and **3aa-an**) was synthesized to explore their biological potential. All compounds were characterized by analyzing their FT-IR, ¹H/¹³C/¹⁹F NMR spectroscopic, and mass spectrometric data. The DFT calculations further supported the structural assignment of the hydrazono form (**3**), among the possible tautomeric structures. The results of *in vitro* antimicrobial evaluation revealed that among all, seven candidates have shown good potential out of which compound **3ak** emerged as the most potent antimicrobial agent, exhibiting remarkable antibacterial activity against *Staphylococcus aureus* (*S. aureus*) and *Bacillus subtilis* (*B. subtilis*) with MIC value of 80 µg/mL, comparable to Ciprofloxacin (MIC = 62.5 µg/mL); and significantly higher antifungal potential against *Candida albicans* (*C. albicans*) with MIC value of 320 µg/mL than Amphotericin B (MIC = 1280 µg/mL). Antioxidant screening revealed that **3j**, **3l**, **3aa**, **3ae**, **3ah-ak** and **3al-an** displayed strong antioxidant potential with EC₅₀ values of 62.5–125 µg/mL, in comparison to ascorbic acid (EC₅₀ =

132.7 $\mu\text{g/mL}$). In addition, **3g**, **3n**, **3f**, **3m**, **3af**, **3ag**, **3am**, and **3an** displayed excellent and **3b**, **3e**, **3aa**, **3ae**, and **3h** demonstrated significant DNA protective effects against UV-induced damage at 60 $\mu\text{g/mL}$ concentration. Hence, the results of the present study demonstrated that the synthesized 5-trifluoromethyl substituted isoxazoles have significant antioxidant potential along with good DNA protective potential.

Keywords: Antimicrobial, Antioxidant, Density functional theory, DNA protection, Trifluoromethylisoxazoles

1. Introduction

The rapid emergence of multidrug-resistant (MDR) microbial pathogens has become one of the most serious global healthcare challenges, substantially limiting the therapeutic effectiveness of currently available antimicrobial drugs [1]. The continuous rise in bacterial resistance to conventional antibiotics, particularly among Gram-positive and Gram-negative strains, has stimulated extensive efforts toward the discovery of structurally novel antimicrobial agents with improved efficacy and reduced resistance potential [2]. In parallel, fungal infections caused by opportunistic pathogens such as *C. albicans* have also increased significantly, especially in immunocompromised patients. Resistance to commonly used antifungal agents has further complicated treatment strategies [3].

Simultaneously, oxidative stress generated by excessive production of reactive oxygen species (ROS) further aggravates the deterioration of health conditions [4] and has been recognized as a major factor responsible for cellular damage and the progression of several pathological disorders, including inflammation, carcinogenesis, aging, cardiovascular diseases, and neurodegenerative disorders [5,6]. ROS-induced oxidative damage to proteins, lipids, and nucleic acids may also result in DNA strand cleavage and mutagenesis [7]. Therefore, compounds capable of exhibiting antioxidant and DNA protective properties, along with antibacterial and antifungal potential, are highly desirable and remain an important objective in medicinal chemistry for the development of a new class of multifunctional therapeutic agents.

Among the various heterocyclic scaffolds, isoxazole (**I**) derivatives have attracted considerable attention because of their broad spectrum of biological and pharmacological activities [8–10]. Isoxazoles are known to exhibit antimicrobial, antifungal, antioxidant, anticancer, anti-inflammatory, antiviral, antitubercular, and neuroprotective properties [11–14]. Moreover, several

clinically important drugs, including Valdecoxib (II) [15], Leflunomide (III) [16], Sulfamethoxazole (IV) [17], and Zonisamide (V) [18], possess an isoxazole nucleus, highlighting the significance of this privileged pharmacophore in modern drug discovery (Fig.1).

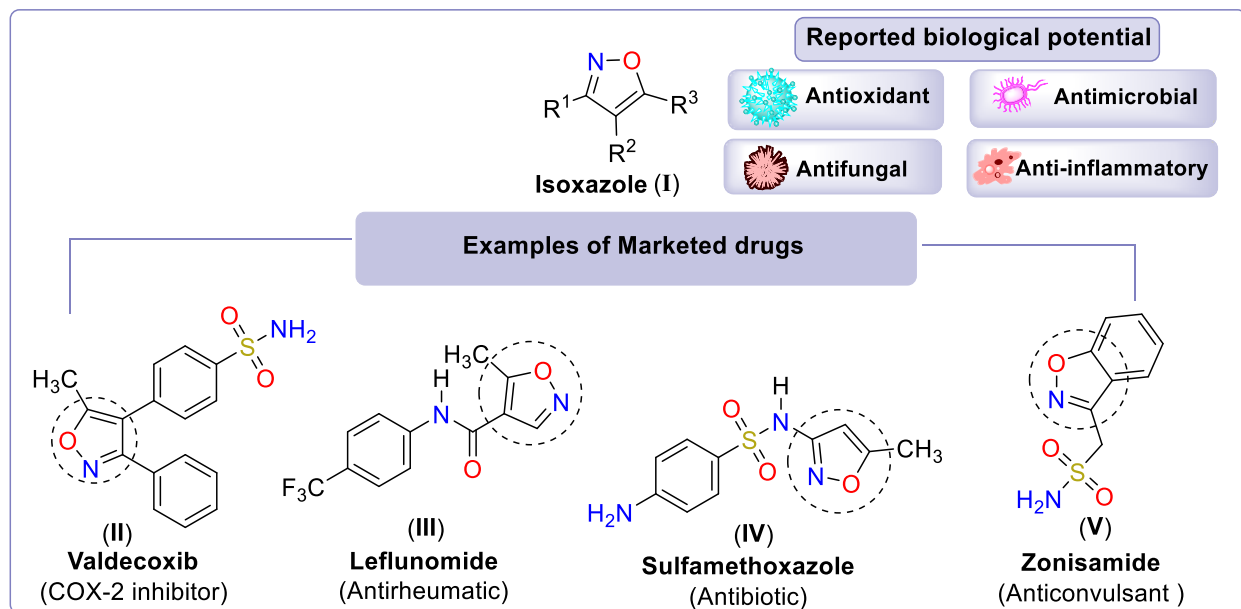


Figure 1: Some important drugs containing the isoxazole nucleus.

Recent investigations have demonstrated that aryl-substituted and fused isoxazole derivatives exhibit promising antimicrobial and antioxidant properties owing to enhanced π -conjugation and favorable electronic interactions with biological targets. The 3-halo-4,5-dihydroisoxazole scaffold has emerged as a promising pharmacophore in medicinal chemistry, with its chemical reactivity and biological activity being strongly modulated by the nature of the halogen substituent[19].

In recent years, fluorinated heterocyclic compounds have attracted substantial attention because the incorporation of fluorine-containing substituents can profoundly influence the physicochemical, electronic, and pharmacokinetic properties of bioactive molecules [20–23]. Among fluorinated functionalities, the trifluoromethyl (CF_3) group is considered one of the most important pharmacophoric units in medicinal chemistry because of its strong electron-withdrawing nature, high electronegativity, lipophilicity, and metabolic stability[24,25]. Chemically, the CF_3 group alters the electron density distribution within the molecular framework through a strong $-I$ inductive effect, thereby modulating the molecular polarity, acidity/basicity, dipole moment, and intermolecular interactions [26,27]. The incorporation of the CF_3 group also increases membrane permeability and receptor-binding affinity while reducing metabolic degradation, leading to improved pharmacological performance [24,28]. As shown in **Figure 2**, 5-trifluoromethyl-2-

isoxazoline derivatives (**VI**) [29] and (*R, S*)-2-amino-3-(3-hydroxy-5-trifluoromethylisoxazol-4-yl)propanoic acid (trifluoro-AMPA) (**VII**) [30] have been developed as ectoparasiticides and highly potent AMPA receptor agonists, respectively. Previous studies demonstrated that trifluoromethyl-containing isoxazole derivatives often display superior biological activities compared to non-fluorinated analogs [31]. In particular, Pattanayak et al. reported that the introduction of the CF₃ functionality into isoxazole-based molecules (**VIII**) resulted in nearly eight-fold enhancement in anticancer activity relative to the corresponding non-fluorinated analogs [32]. Similarly, Bonacorso et al. demonstrated that fluorinated isoxazole systems exhibit enhanced biological performance owing to favorable electronic distribution and increased molecular stability [33].

Another important class of biologically active molecules includes azo-linked aromatic compounds containing the aryldiazenyl (–N=N–) functionality [34]. Azo derivatives are known to possess significant antibacterial, antifungal, antioxidant, anticancer, and DNA protective activities [35,36]. The azo linkage enhances π -electron delocalization and molecular conjugation, thereby improving charge-transfer capability and intermolecular interactions with biological targets [37]. In addition, the presence of electron-donating and electron-withdrawing substituents on the azo-linked aromatic ring substantially influences the molecular electronic distribution and redox behavior [38]. Incorporating aryldiazenyl functionality into heterocyclic systems improves radical-scavenging activity and DNA protective properties because of enhanced electron delocalization and stabilization of free radical intermediates [39,40]. Moreover, azo-linked isoxazoles, such as, 5-amino-4-[(4-methoxyphenyl)diazenyl]-3-methylisoxazole (**IX**) [41] and 3-(2-thien-2-yl)-4-arylazo-5-hydroxy-5-trifluoromethyl- Δ^2 -isoxazoline derivatives (**X**) [42,43], exhibited improved antitumor and antioxidant activities, respectively, owing to increased hydrophobic interactions and stronger binding with microbial biomolecules [44].

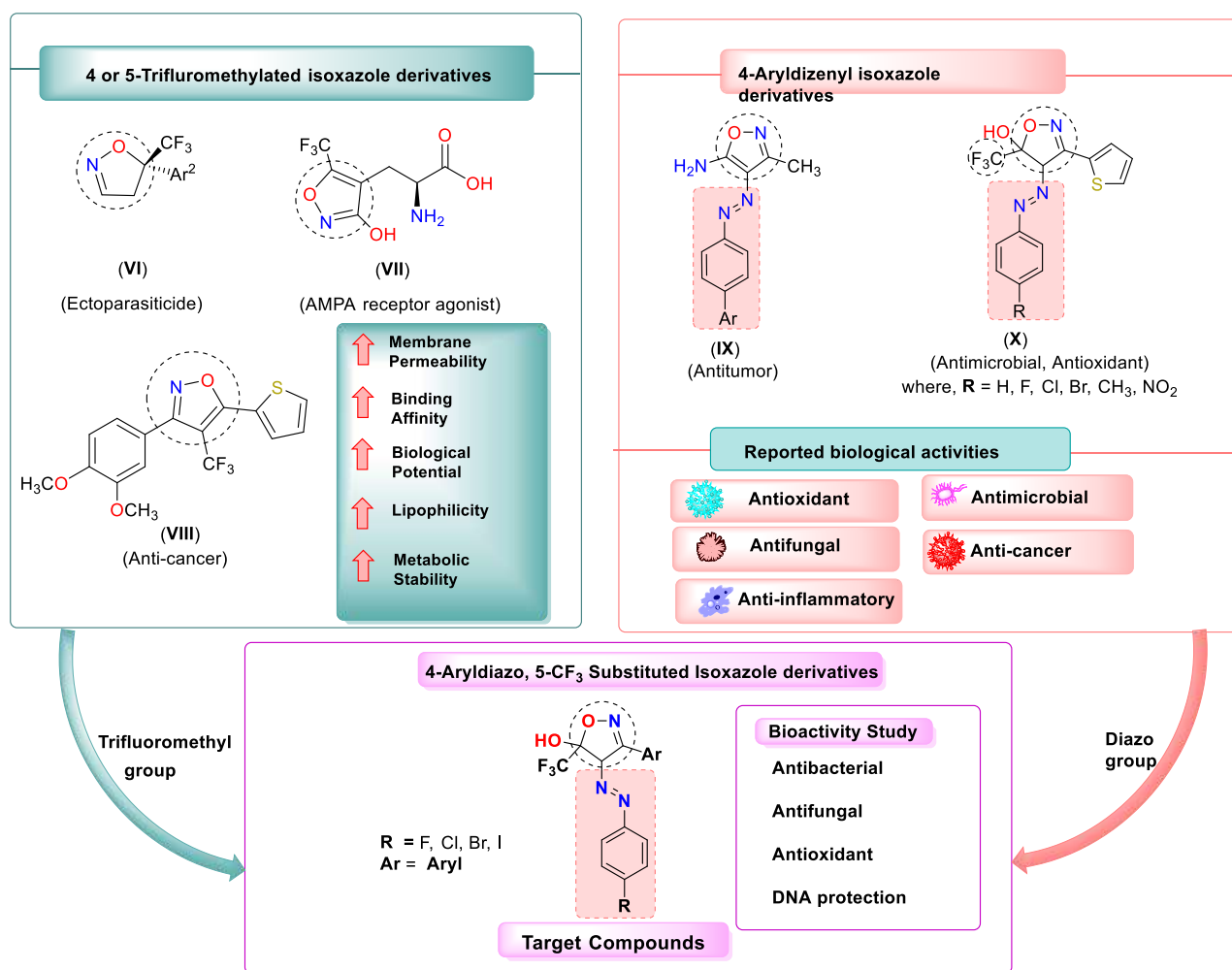


Figure 2: Some biologically potent isoxazoles (VI-X), and design of the target compounds.

Thus, substitution modulates the electronic distribution and biological responsiveness of the resulting molecules [45]. It has been reported that arylazo functionality not only contributes to extended conjugation and color intensity but also enhances radical scavenging and potential DNA-protective properties [46,47]. Further, halo-substitution on the isoxazoline moiety has led to the development of potent anticancer agents [12,42,48].

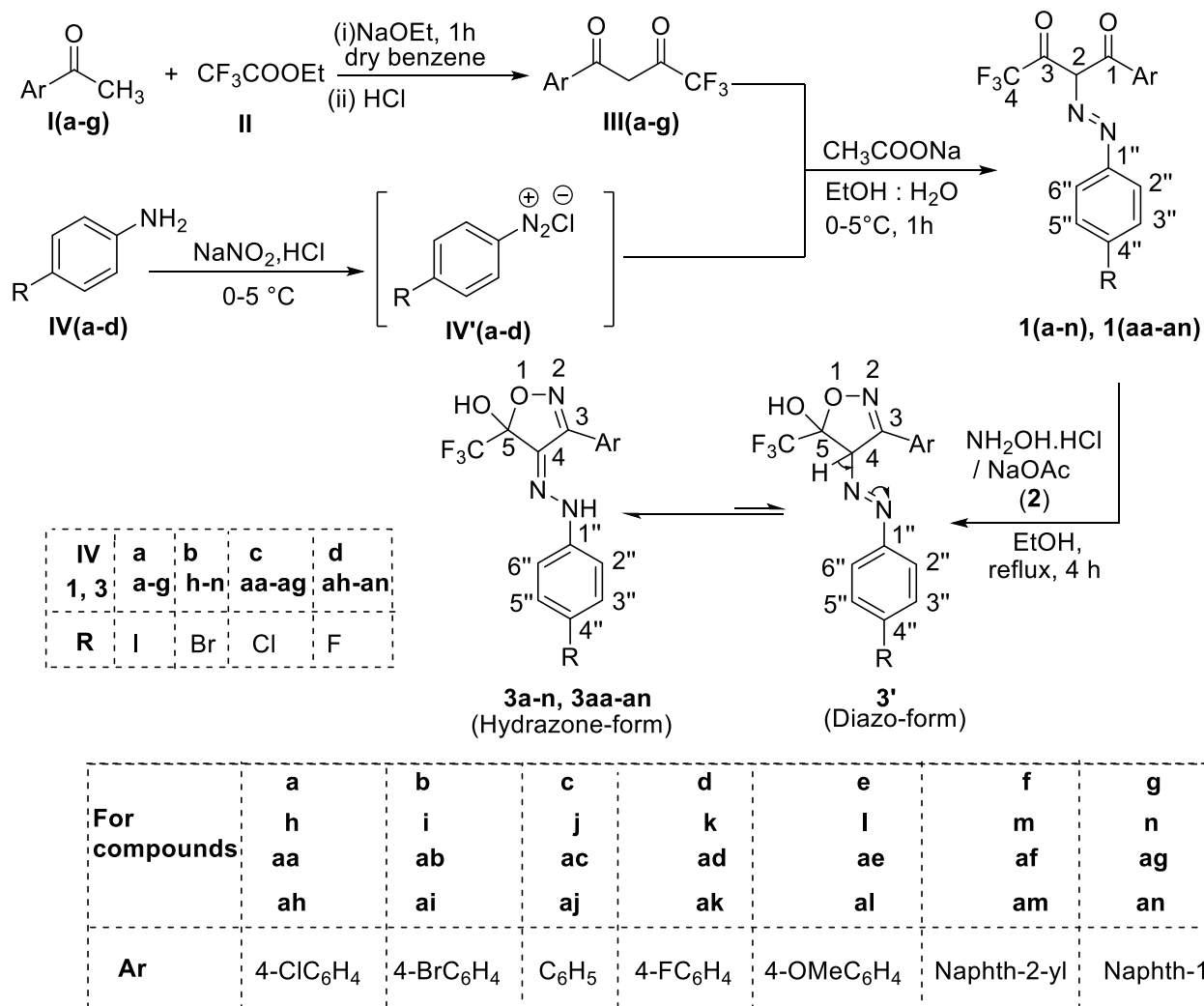
Molecular hybridization has emerged as a powerful strategy in medicinal chemistry for the design of multifunctional molecules through the integration of two or more biologically active pharmacophores into a single molecular framework [47]. Literature reports indicate that Hybrid molecules containing isoxazole and fluorinated functionalities often exhibit superior biological activities owing to the synergistic enhancement of their electronic and physicochemical properties [50]. However, studies involving the simultaneous incorporation of isoxazole, trifluoromethyl, and aryldiazene functionalities within a single scaffold remain relatively less explored.

Therefore, in continuation of our ongoing efforts toward the synthesis of biologically active fluorinated heterocycles [43], herein we report the synthesis of a new series of twenty-eight (*E*)-4-(aryldiazenyl)-3-aryl-5-hydroxy-5-trifluoromethyl-4,5-dihydroisoxazole derivatives with the anticipation that integration of these three pharmacologically important moieties could generate structurally enriched molecules with multifunctional biological potential. The study also provides insights into the influence of trifluoromethyl, aryl, and aryldiazenyl groups attached to positions-5, 3, and 4 of the isoxazoline ring on the biological potential of the synthesized molecular hybrids.

2. Results and Discussion

2.1 Chemistry

A series of (*E*)-3-aryl-4-(aryldiazenyl)-5-hydroxy-5-trifluoromethyl-4,5-dihydroisoxazoles (**3'**) were prepared by adopting a multistep procedure according to **Scheme 1**. To achieve the final product, the starting precursors, 1-aryl-4,4,4-trifluoromethyl- β -diketones (**IIIa-g**), were prepared in our laboratory by the Claisen condensation of aryl methyl ketones (**Ia-g**) with ethyl trifluoroacetate (**II**) in the presence of sodium ethoxide, according to a previously reported procedure [51].



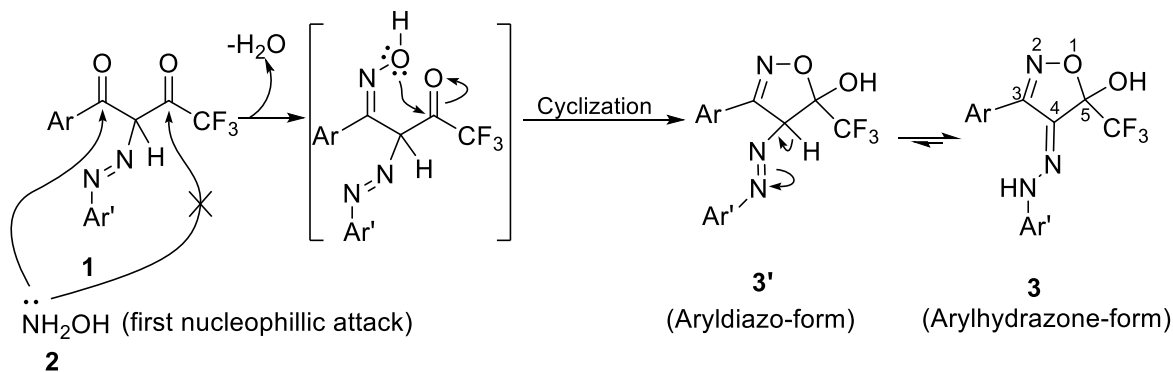
Scheme 1: Multistep synthesis of (*E*)-3-aryl-4-(aryldiazenyl)-5-hydroxy-5-trifluoromethyl-4,5-dihydroisoxazoles (**3'**) or (*E*)-3-aryl-4-(2-(aryldiazenylidene)-5-hydroxy-5-trifluoromethyl-4,5-dihydroisoxazoles (**3**)

The reaction of different *p*-halo substituted aromatic primary amines (**IVa-d**) with sodium nitrite in an acidic medium at 0–5 °C yielded the corresponding aryldiazonium salts (**IV'a-d**), which, on subsequent treatment with various 1-aryl-4,4,4-trifluoromethylbutan-1,3-diones (**III**) in presence of sodium acetate, gave the key intermediates, (*E*)-1-(4-aryl)-4,4,4-trifluoro-2-(4-aryldiazenyl)butane-1,3-diones (**1**) in good yields [52]. The reaction of different 1-(4-aryl)-4,4,4-trifluoro-2-(4-aryldiazenyl) butane-1,3-diones (**1**) with hydroxylamine hydrochloride (**2**) in the presence of sodium acetate under reflux conditions in ethanol afforded the final products (**3**) in good yields.

The structures of the obtained products were assigned based on a combination of FT-IR, NMR (^1H , ^{13}C , and ^{19}F) spectroscopy, mass spectrometry, and DFT studies. The appearance of a characteristic peak corresponding to the C=O stretching vibration at 1698 cm^{-1} and an N=N stretching peak in the range of $1510\text{--}1560\text{ cm}^{-1}$ in the FT-IR spectra of **1** confirms the formation of diazenyl-substituted trifluoromethyl- β -diketones (**1**). In contrast, the disappearance of a peak due to the carbonyl stretching vibration in the FT-IR spectra of the final products (**3**) confirmed cyclization and the formation of the isoxazole ring. Furthermore, the appearance of the NH stretching peak around $3290\text{--}3300\text{ cm}^{-1}$ indicates that the final product exists mainly in the arylhydrazone form (**3**) rather than the diazo (**3'**) form [53–55].

The ^1H NMR spectrum of a representative intermediate, **1a**, revealed that eight aromatic protons resonated at δ 7.28–7.80 ppm, and one additional singlet signal (OH/NH) appeared at δ 10.25 ppm, confirming the formation of **1**. In the NMR spectrum of the final product **3a** (a representative compound), two broad singlet signals (D_2O exchangeable) were observed at δ 9.86 and 10.45 ppm, corresponding to the OH and NH groups, respectively. The eight aromatic protons were attributed to the range of δ 7.26–7.98 ppm. In the ^{13}C NMR spectrum of compound **1a**, the carbonyl carbon signals appeared due to C-1 and C-3 at δ 189.68 and 175.32 ppm ($^2J_{\text{C-F}} = 33.20\text{ Hz}$), respectively. The disappearance of these two signals in ^{13}C NMR spectrum of **3a** further supports the cyclization and formation of the desired product. Carbon-5 of the isoxazole ring resonated at δ 98 ppm ($^2J_{\text{CF}_3} = 35.21\text{ Hz}$), while C-4 and C-3 gave singlets at δ 131 and 154 ppm, respectively[56]. Moreover, a sharp singlet was observed at δ -78 ppm in the ^{19}F NMR spectrum, confirming the presence of the trifluoromethyl group at position-5 of the isoxazole ring, which is in close agreement with the reported values[57]. Further structural confirmation of **3a** was provided by the mass spectral data, which showed a molecular ion peak (M^+) at $m/z = 495.9$, in accordance with the molecular formula $\text{C}_{16}\text{H}_{10}\text{ClF}_3\text{IN}_3\text{O}_2$. In addition, DFT studies indicated that the hydrazone form is more stable than the azo-form owing to its lower HOMO–LUMO energy gap, which reflects greater electronic delocalization and molecular stability; detailed results are provided in Section 2.4.

The probable mechanism involved in the formation of 5-trifluoromethyl-5-hydroxyisoxazoles (**3**) is shown in **Scheme 2**. According to literature reports, nucleophilic attack by the NH_2 of hydroxylamine occurs at the more reactive carbonyl carbon (i.e., COAr) of the diketone, which then cyclizes, followed by deprotonation to form an isoxazole ring [58].



Scheme 2: The probable mechanism involved in the formation of **3** and **3'**.

2.2 Biological activity

2.2.1 Antimicrobial activity

All synthesized 5-trifluoro-5-hydroxyisoxazole derivatives (**3**) were screened to explore their *in vitro* antibacterial and antifungal potential against five well-known human pathogenic strains using the agar-well diffusion method [59]. The selected four bacterial strains, namely *S. aureus* and *B. subtilis* (Gram-positive), *Escherichia coli* (*E. coli*), and *Pseudomonas aeruginosa* (*P. aeruginosa*) (Gram-negative), along with one yeast strain, *C. albicans*, are associated with a wide range of infectious diseases and are routinely employed for the biological evaluation of newly synthesized antimicrobial agents to assess their broad-spectrum therapeutic potential[60]. Preliminary results were recorded by measuring the inhibition zones (IZ) of bacterial or fungal growth around the wells for each tested compound at 4000 µg/mL. The results revealed that most of the synthesized derivatives displayed moderate to significant antimicrobial activity against both bacterial and fungal strains (**Figs. 3** and **4**). Minimum inhibitory concentration (MIC) values were determined using the two-fold serial dilution method for the selected compounds, which showed inhibition zones greater than 16 mm compared to ciprofloxacin (22–26.6 mm) and Amphotericin-B (17.6 mm) as positive controls.

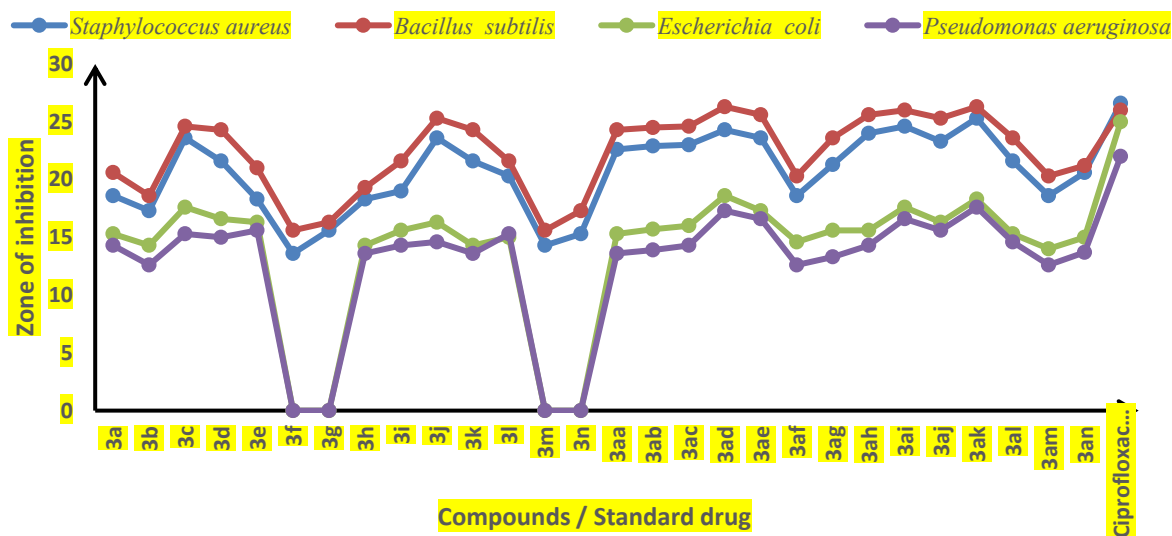


Figure 3: Zones of inhibition (in mm) of **3a-an** in comparison to Ciprofloxacin for antibacterial study at 4000 µg/mL

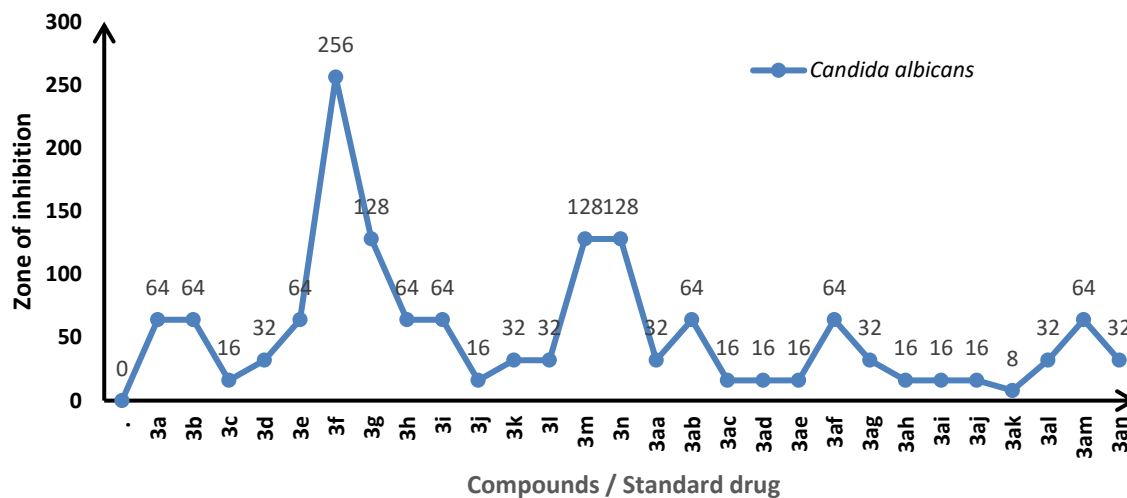


Figure 4: Zones of inhibition (in mm) of **3a-an** in comparison to Amphotericin B for antifungal study at 4000 µg/mL

The results revealed that among all the synthesized compounds, **3ak** emerged as the most potent broad-spectrum antimicrobial agent, exhibiting the lowest MIC value of 80 µg/mL against both *S. aureus* and *B. subtilis* and 320 µg/mL against *C. albicans*. The compound demonstrated excellent antibacterial potential, with IZ of 25.3 and 26.3 mm in comparison with IZ of Ciprofloxacin 26.6

and 26.0 mm against *S. aureus* and *B. subtilis*, respectively. Furthermore, against *C. albicans*, **3ak** displayed an inhibition zone of 16.3 mm, relative to Amphotericin-B (17.6 mm) (Table 1).

Table :1 Inhibitory potential of the most active compounds against each microbial strain.

Microbial strains	Most active compound(s)	MIC (µg/mL)	Inhibitory potential* (w.r.t. standard)	Zone of inhibition (mm)	Standard drug (MIC)
<i>S. aureus</i>	3ak	80	~78% of standard	25.3	Ciprofloxacin (62.5)
<i>B. subtilis</i>	3ad, 3ak	80	~78% of standard	26.3, 26.3	Ciprofloxacin (62.5)
<i>E. coli</i>	3ad, 3ak	640	Lower than standard	18.6, 18.3	Ciprofloxacin (62.5)
<i>P. aeruginosa</i>	3ak	640	Lower than standard	17.6	Ciprofloxacin (62.5)
<i>C. albicans</i>	3ak	320	Significantly higher than standard	16.3	Amphotericin-B (1280)

In case of Gram-positive bacteria, seven (7) compounds, **3j**, **3ad**, **3ae**, **3ah**, **3ai**, **3aj**, and **3ak** exhibited excellent antibacterial activity against *B. subtilis* with MIC values of 80 µg/mL, relative to Ciprofloxacin (MIC = 62.5 µg/mL) and exhibited 25.3–26.3 mm inhibitory potential relative to Ciprofloxacin (26.00 mm). Additionally, eight (8) compounds, **3c**, **3d**, **3k**, **3aa**, **3ac**, **3ag**, **3al**, and **3an**, displayed moderate potential with MIC values of 160 µg/mL and IZ of 23.6 -24.6 mm.

In contrast, the synthesized derivatives showed relatively moderate potential against Gram-negative bacterial strains (*E. coli* and *P. aeruginosa*), which may be attributed to the presence of a highly impermeable lipopolysaccharide-rich outer membrane that restricts the penetration of antimicrobial agents [61]. Among the tested compounds, **3ad** and **3ak** exhibited activity against *E. coli* with MIC values of 640 µg/mL, relative to Ciprofloxacin (250µg/mL). In addition, compound **3ak** demonstrated the highest potential against *P. aeruginosa*, exhibiting an inhibition zone of 17.6 mm, compared to ciprofloxacin (22.0 mm).

Antifungal screening against *C. albicans* revealed moderate to excellent activity of several compounds. Compound **3ak** exhibited the highest antifungal potency with an MIC of 320 µg/mL and an inhibition zone of 16.3 mm, in comparison to Amphotericin-B (1280µg/mL and 17.6 mm, respectively). Compounds **3ad** and **3ae** also demonstrated significant antifungal activity, with MIC values of 640 µg/mL and inhibition zones of 16.3 mm (92.6%) and 15.3 mm (86.9%), respectively,

compared to Amphotericin-B. Furthermore, compounds **3d**, **3ah**, and **3ai** exhibited inhibition zones of 14.3 mm (81.3%), 14.6 mm (83.0%), and 15.0 mm (85.2%), respectively, indicating antifungal activity comparable to Amphotericin-B. In contrast, compounds **3b**, **3i**, **3j**, **3ab**, **3al**, **3am**, and **3an** showed only minimal antifungal activity, with MIC values of 2560 µg/mL and inhibition zones ranging from 12.9–14.2 mm, corresponding to approximately 73.3–80.7% inhibitory activity relative to Amphotericin-B (Fig.5).

Overall, the synthesized compounds exhibited comparatively higher activity against gram-positive bacteria than gram-negative strains, as evidenced by larger inhibition zones and lower MIC values. Among the tested microorganisms, *B. subtilis* was the most susceptible. Among all the candidates, sixteen (16) compounds showed good antibacterial activity against *B. subtilis*, while nine (9) exhibited notable activity against *S. aureus*. Similarly, seven (7) compounds displayed promising antifungal potential against *C. albicans*.

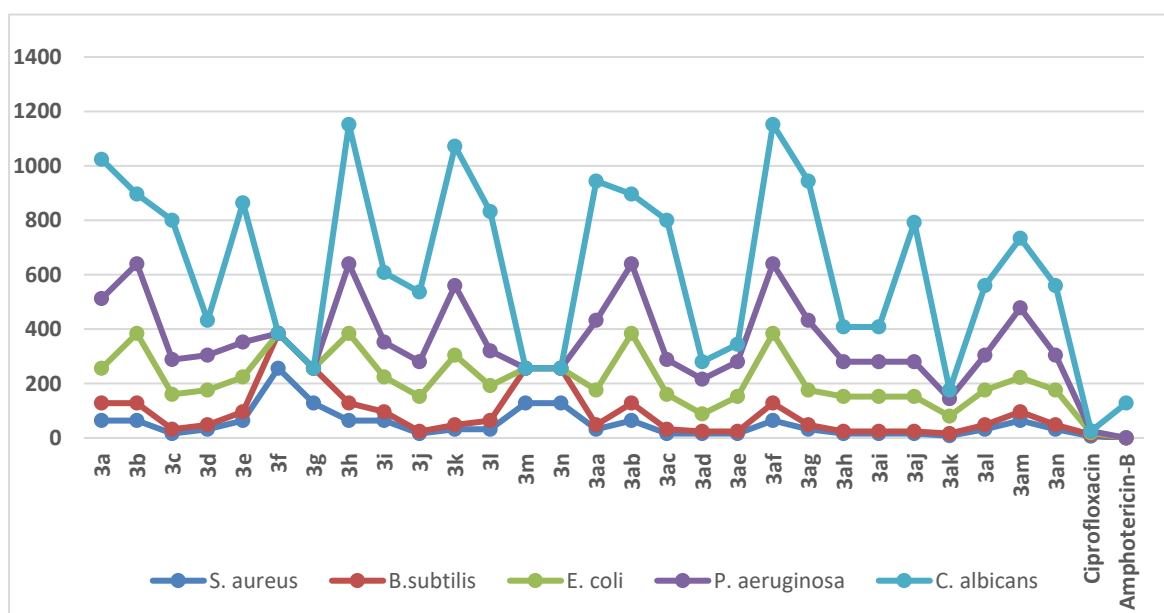


Figure 5: Antimicrobial activity of compounds **3a-an** expressed in MIC (µg/mL)

The antimicrobial activity of the synthesized derivatives was strongly influenced by the nature of both the halogen substituent (R) and aryl moiety (Ar). A clear trend emerged, with fluorinated and chlorinated analogs generally exhibiting superior antimicrobial activity compared to their corresponding brominated and iodinated derivatives. This observation suggests that the smaller atomic size and favorable electronic characteristics of fluorine and chlorine provide a more suitable

balance of lipophilicity and target interaction, leading to enhanced microbial growth inhibition[62].

In the antibacterial study, the fluorinated series showed the most promising activity, particularly against *B. subtilis* and *S. aureus*. Among all the compounds, derivative **3ak** (R = F, Ar = *p*-F-C₆H₄) displayed the highest antibacterial potency, indicating that the simultaneous presence of fluorine on both the isoxazole framework and aryl ring is highly beneficial for activity. Other fluorinated derivatives, including **3aj** (Ar = C₆H₅) and **3al** (Ar = *p*-OMe-C₆H₄), exhibited notable antibacterial effects, further highlighting the positive contribution of fluorine substitution.

The influence of the aryl group was also evident. In general, compounds bearing phenyl or *para*-substituted phenyl rings demonstrated better antibacterial activity than the corresponding 1-naphthyl and 2-naphthyl analogs. The lower activity of the naphthyl-containing derivatives may be attributed to the increased steric bulkiness of these substituents, which might reduce the ability of the molecules to efficiently interact with biological targets[63]. Interestingly, this unfavorable steric effect was less pronounced in the fluorinated series, where compounds **3am** and **3an** retained appreciable antibacterial activity despite the presence of bulky naphthyl groups, suggesting that fluorine substitution can partly compensate for steric limitations.

A comparison within the chloro-, bromo-, and iodo-substituted series further revealed that derivatives containing phenyl, *p*-fluorophenyl, and *p*-methoxyphenyl groups generally exhibited greater antibacterial activity than those with *p*-bromophenyl, *p*-chlorophenyl, 1-naphthyl, or 2-naphthyl substituents. These findings indicate that both electronic and steric factors play important roles in displaying antibacterial efficacy.

The antifungal results followed a similar trend. Compounds containing fluorine and chlorine exhibited markedly higher activity against *C. albicans* than brominated and iodinated compounds. Once again, derivative **3ak** (R = F, Ar = *p*-F-C₆H₄) emerged as the most active member of the series, emphasizing the favorable contribution of fluorine substitution toward antifungal activity. Collectively, these observations demonstrate that the antimicrobial activity within this series is governed by a delicate interplay of electronic effects, lipophilicity, and steric factors, with fluorinated derivatives consistently emerging as the most potent antimicrobial candidates.

2.2.2 Antioxidant activity

All synthesized compounds were also investigated for their free radical scavenging effects on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals to explore their antioxidant potential. The DPPH assay measures the ability of a compound to scavenge free radicals by donating an electron and/or hydrogen atom to the stable DPPH radical (DPPH•). Upon reduction, the characteristic violet color of DPPH gradually faded, accompanied by a decrease in absorbance at 515 nm. The antioxidant activity observed for the synthesized compounds may therefore arise from their capacity to interact with and neutralize the radical species. The extent of radical scavenging is influenced by the electronic characteristics of the molecules, particularly the nature of the substituents and the degree of conjugation, which can facilitate electron donation and stabilize the resulting radical intermediates. Accordingly, compounds with more favorable electronic environments exhibited enhanced DPPH scavenging activity.

All compounds were studied in the concentration range of 100-6.25 µg/mL to evaluate DPPH radical scavenging ability and determine their EC₅₀ values. Ascorbic acid, used as the reference standard, exhibited excellent antioxidant activity with an EC₅₀ value of 13.27 ± 1.10 µg/mL and 87.83% radical scavenging at 100 µg/mL.

Among the synthesized derivatives, six (6) compounds (**3ah**, **3ai**, **3aj**, **3al**, **3am** and **3an**) demonstrated excellent antioxidant activity with EC₅₀ values in the range of 6.25–12.5 µg/mL, comparable to the reference standard (13.27 ± 1.10 µg/mL). Furthermore, five (5) compounds (**3j**, **3l**, **3aa**, **3ae**, and **3ak**) displayed promising antioxidant potential, with EC₅₀ values ranging from 12.5–25 µg/mL (**Fig.6** and **Fig.7**).

Moderate antioxidant activity was observed for ten (10) compounds, **3b**, **3d**, **3f**, **3g**, **3m**, **3ab**, **3ac**, **3ad**, **3af** and **3ag**.

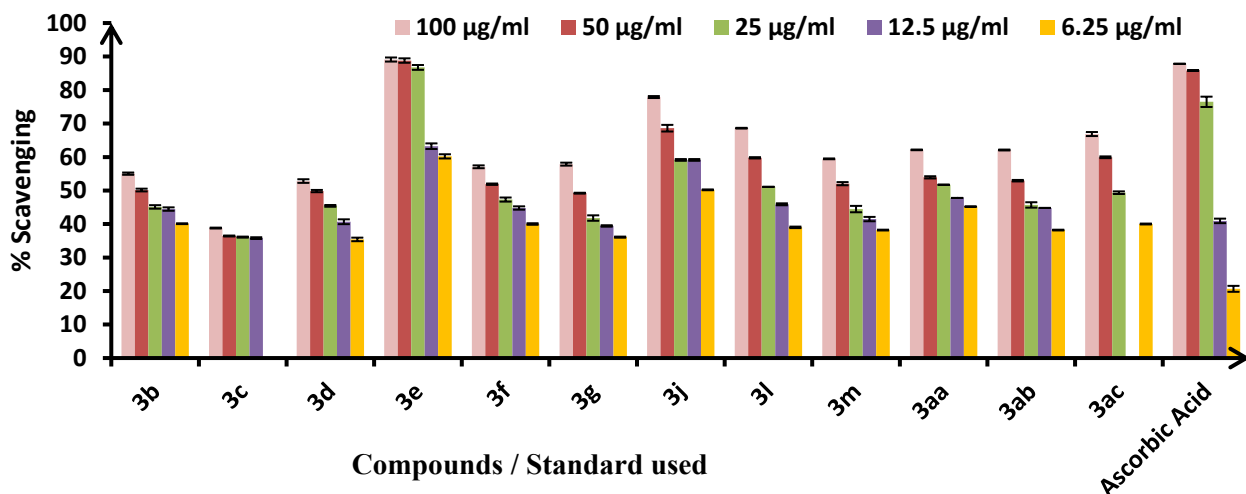


Figure 6: Scavenging activity of 3b-ac in reference to ascorbic acid

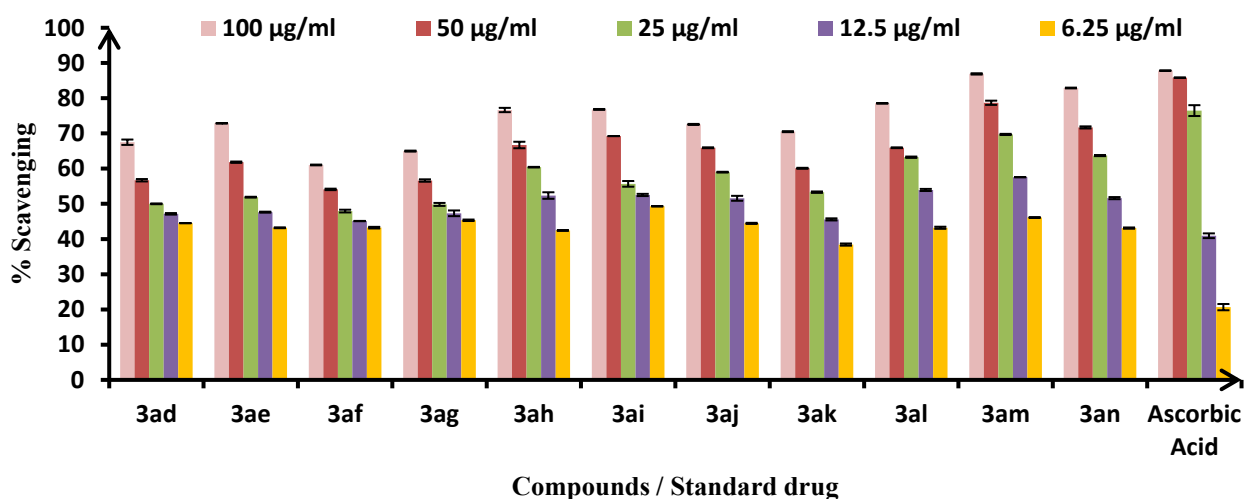


Figure 7: Comparison of (%) scavenging activity of 3ad-an in reference to ascorbic acid

Structure–activity relationship (SAR) analysis of the antioxidant data revealed a significant influence of both the halogen substituent (R) and aryl group (Ar) on radical-scavenging activity. Among the investigated derivatives, compounds bearing R = F consistently exhibited superior antioxidant activity compared to the corresponding Cl-, Br-, and I-substituted analogs. The enhanced activity of the fluorinated derivatives may be attributed to the favorable electronic

properties of fluorine, which facilitate the stabilization of the resulting radical species following hydrogen atom or electron transfer.

The nature of the aryl substituent plays a crucial role in determining the antioxidant potency. Irrespective of the halogen present, derivatives containing a *p*-methoxyphenyl (4-OMe-C₆H₄) group generally displayed enhanced radical-scavenging activity. The electron-donating methoxy group is expected to increase electron density within the π -conjugated framework via resonance (+R) or (+M) effect. This enhanced electron density strengthens the overall radical-scavenging capacity and thermodynamic favorability for hydrogen atom transfer (HAT) to stabilize the generated radical intermediate[64]. This effect is reflected in the excellent antioxidant activity observed for compounds such as **3ae** and related analogs.

A notable influence of the ring size and aromatic substitution pattern was also observed. Compounds bearing a 2-naphthyl substituent exhibited higher antioxidant activity than their corresponding 1-naphthyl analogs, regardless of the nature of R. This behavior may arise from more effective resonance delocalization of the unpaired electron in the radical form, resulting in greater stabilization and enhanced free radical scavenging ability.

Overall, the antioxidant activity of the synthesized trifluoromethylated isoxazole derivatives appears to be governed primarily by electronic effects and radical stabilization energy. Fluorine substitution at R, presence of electron-donating methoxy groups, and incorporation of a 2-naphthyl moiety were identified as the most favorable structural features for antioxidant activity, as evidenced by the low EC₅₀ values of compounds **3ah**, **3ai**, **3aj**, **3al**, **3am**, and **3an**.

2.2.3 UV-mediated DNA damage protection activity

Agarose gel electrophoresis was used to explore the DNA damage protection potential of the synthesized isoxazoles (**3**). The protective potential of the tested compounds was assessed by comparing the appearance of bands and their intensities in the control (C) and test compounds under UV irradiation. It has been reported in the literature that on single-strand breaking, the supercoiled (SC) form changes into the more relaxed open circular (OC) form; however, double-strand breaking leads to the transformation of the SC form into the linear circular (LC) form[65]. The experimental results of the present investigation revealed that in the absence of UV irradiation, plasmid DNA existed in the SC form (**lane 1 of Fig. 8**); however, on exposure to UV light, most of it was transformed into the OC form, along with a very low-intensity LC form, as shown in **lane 2 of Figure 8**.

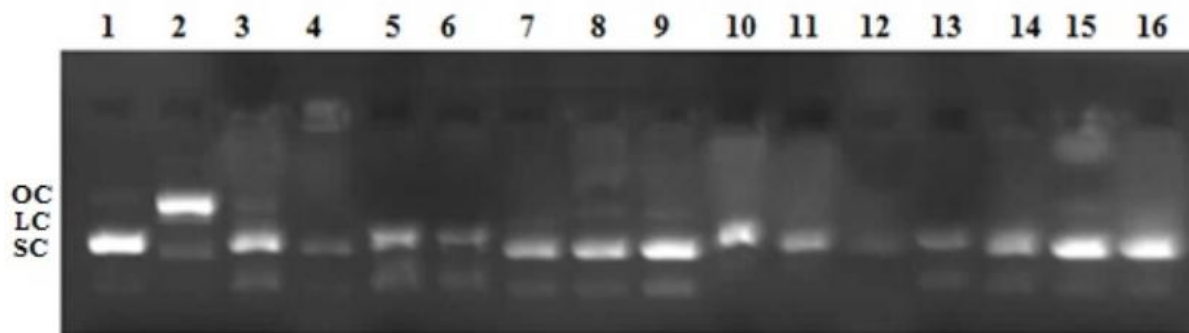


Figure 8: Effect of compounds on plasmid DNA under UV-irradiation (**3a-3n**).

(extracted from **Figure 29S**)

Lane-1: DNA + DMSO without UV, Lane-2: DNA + DMSO + UV, Lane-3: DNA + **3a** + UV, Lane-4: DNA + **3b** + UV, Lane-5: DNA + **3c** + UV, Lane-6: DNA + **3d** + UV, Lane-7: DNA + **3e** + UV, Lane-8: DNA + **3f** + UV, Lane-9: DNA + **3g** + UV, Lane-10: DNA + **3h** + UV, Lane-11: DNA + **3i** + UV, Lane-12: DNA + **3j** + UV, Lane-13: DNA + **3k** + UV, Lane-14: DNA + **3l** + UV, Lane-15: DNA + **3m** + UV, Lane-16: DNA + **3n** + UV

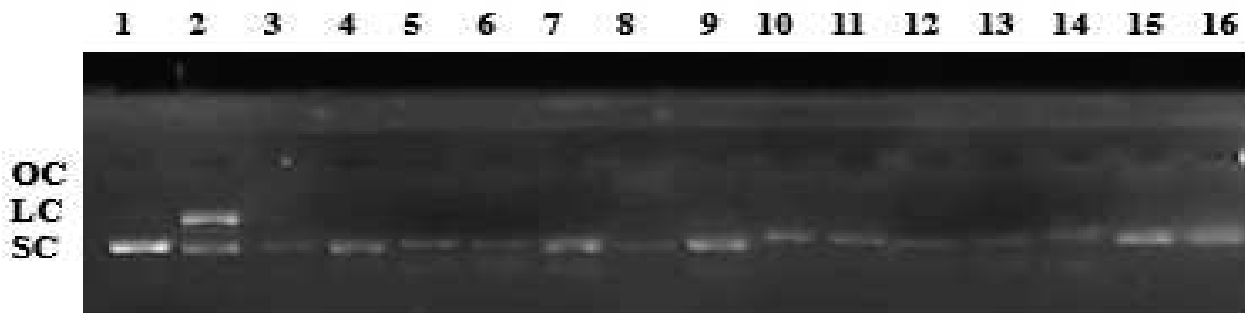


Figure 9: Effect of compounds on plasmid DNA under UV-irradiation (**3aa-3an**). (extracted from **Figure 30S**)

Lane-1: DNA + DMSO without UV, Lane-2: DNA + DMSO + UV, Lane-3: DNA + **3aa** + UV, Lane-4: DNA + **3ab** + UV, Lane-5: DNA + **3ac** + UV, Lane-6: DNA + **3ad** + UV, Lane-7: DNA + **3ae** + UV, Lane-8: DNA + **3af** + UV, Lane-9: DNA + **3ag** + UV, Lane-10: DNA + **3ah** + UV, Lane-11: DNA + **3ai** + UV, Lane-12: DNA + **3aj** + UV, Lane-13: DNA + **3ak** + UV, Lane-14: DNA + **3al** + UV, Lane-15: DNA + **3am** + UV, Lane-16: DNA + **3an** + UV

It has been observed that **3g**, **3n**, **3f**, **3m**, **3af**, **3ag**, **3 am**, and **3an** displayed excellent and **3b**, **3e**, **3aa**, **3ae**, and **3h** demonstrated moderate DNA damage-protecting effects at 60 µg/mL concentration under UV radiation. Whereas **3j** and **3aj** degrade the DNA completely. Therefore, out of twenty compounds, thirteen are most potent against DNA protection.

Overall, the synthesized trifluoromethylated isoxazole derivatives demonstrated promising antimicrobial activity, DNA damage protection potential, and antioxidant potential, highlighting their importance as multifunctional bioactive scaffolds.

2.3 Substitution pattern versus bioactivity

All the synthesized compounds (**3**) were evaluated for their antimicrobial, antioxidant, and DNA-damage protective activities. The relationship between substitution patterns and bioactivity is summarized in **Fig. 10**. When R = F or Cl, the compounds exhibited a good antibacterial profile against *B. subtilis*, whereas 1-naphthyl and 2-naphthyl substitutions resulted in diminished activity, which could be due to steric hindrance from the large moiety. Based on antifungal screening, 4-fluorophenyl substitution at the 3-position of the isoxazole ring resulted in excellent activity against *C. albicans*. Fluorine and chlorine substitutions resulted in better antimicrobial activity than bromine and iodine. In terms of antioxidant activity, fluorine substitution exhibited excellent scavenging potential, irrespective of Ar substitution at position-3 of the isoxazole ring, compared to other halogen substituents. The other compounds exhibited moderate antioxidant activity. Furthermore, DNA protection study revealed that the nature of the aryl substituent significantly influenced the activity of the synthesized isoxazoles. Compounds bearing a naphthyl moiety exhibited the most pronounced protection against UV-induced *pBR322* plasmid DNA damage at 60 µg/mL, whereas the phenyl-substituted derivatives showed poor protective activity, resulting in significant DNA degradation. Overall, the isoxazoles with fluorine substitution displayed enhanced activity.

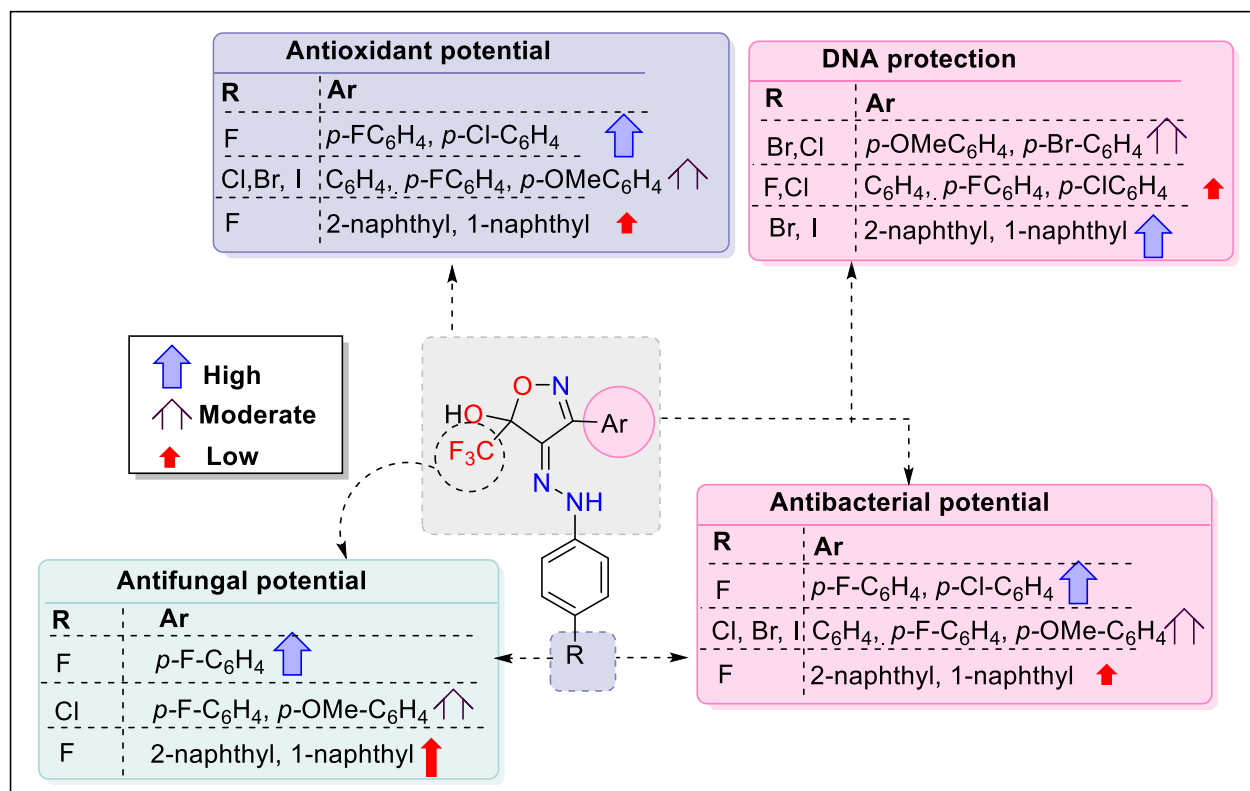


Figure 10: Substitution patterns vs bioactivity

2.4 Computational study

2.4.1 Molecular electrostatic potential

Azo compounds exist in equilibrium with their corresponding hydrazone tautomers, and the relative stability of these forms is often governed by electronic and structural factors[66]. Since tautomerism can significantly influence the physicochemical, spectroscopic, and biological properties of a molecule, it is important to identify the preferentially stabilized form[67]. Therefore, to gain deeper insight into the structural characteristics of the synthesized isoxazole derivatives, density functional theory (DFT) calculations were performed on representative compound **3aj** in both its azo (**3'aj**) and hydrazone (**3aj**) forms using the B3LYP functional with the 6-311G(d, p) basis set. The molecular electrostatic potential (MEP) maps the electrostatic potential onto a constant electron density surface and is routinely employed to locate reactive sites and assess electrophilic and nucleophilic reactivity[68]. The MEP surface is represented by a color scale, where red and green regions correspond to low electrostatic potential associated with high electron density, whereas blue regions indicate high electrostatic potential arising from electron-deficient areas and proton repulsion [69]. The MEP surfaces for both forms **3'aj** and **3aj** were

generated using density functional theory (DFT) based on the optimized geometry, as shown in **Figure 11**. The maximum positive region, represented by the blue color, is the favored site for nucleophilic attack, and the negative region, represented by the red color, is the favored site for electrophilic attack[70]. The pronounced red regions indicate strong electronegativity and susceptibility to nucleophilic attack, whereas the blue regions indicate potential electrophilic sites. The minimum total electronic energies calculated for the studied structures **3'aj** and **3aj** were 1330.62 Hartree and -1330.63 Hartree, respectively, while the calculated dipole moments were 3.49 and 3.68 Debye, respectively. The slightly lower energy of the hydrazone form (**3aj**) indicates that it is thermodynamically more stable than the corresponding azo tautomer (**3'aj**). This observation is further supported by the higher dipole moment (3.68 Debye) compared to that of the azo form (3.49 Debye), reflecting a greater degree of charge separation and electronic stabilization. Therefore, the DFT results only support the preferential existence of the hydrazone tautomer in the studied isoxazole system.

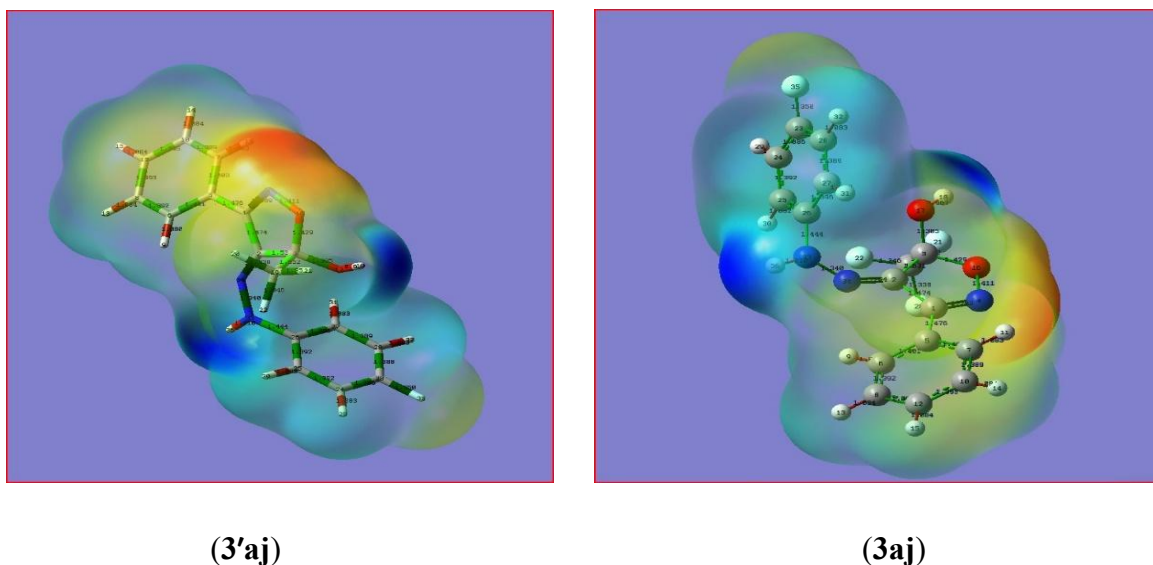


Figure 11: Optimised geometry of structures of **3'aj** & **3aj** measured using DFT calculations at B3LYP /6-311G level

2.4.2 Frontier Molecular Orbital (FMO)

To further investigate the relative stabilities of the azo (**3'aj**) and hydrazone (**3aj**) forms, frontier molecular orbital (FMO) analysis was performed at the B3LYP/6-311G(d, p) level of theory. FMO analysis is widely employed to explain the optical and electronic properties of organic compounds [71]. Understanding the HOMO and LUMO, and their properties, such as energy difference, is

useful for gauging the chemical reactivity of molecules. During molecular interactions, the LUMO accepts electrons, and its energy corresponds to the electron affinity (EA), whereas the HOMO represents an electron donor, and its energy is associated with the ionization potential (IP) [72]. The HOMO-LUMO energy gap explains the concluding charge-transfer interaction within the molecule and is useful for determining the molecular electrical transport properties. In this study, large energy difference was observed between HOMO-LUMO of **3aj** as compared to **3aj'**. The small gap suggests a soft, highly polarizable system with enhanced charge-transfer ability and increased chemical reactivity, whereas larger gaps are typically associated with greater thermodynamic stability and reduced reactivity[73,74].

Within the conceptual DFT framework, global reactivity descriptors, such as electronegativity (χ), chemical potential (μ), global hardness (η), softness (S), and electrophilicity index (ω), provide quantitative insights into the stability and reactivity of organic molecules. The chemical hardness (η), derived from the ionization potential (IP) and electron affinity (EA), reflects the resistance to charge transfer, whereas the softness ($S=1/\eta$) represents the ease of electronic redistribution within the system [75–77]. Electronegativity (χ) describes the electron-attracting ability, and the chemical potential ($\mu = -\chi$) indicates the tendency of electrons to escape; more negative μ values are associated with enhanced thermodynamic stability [78]. The electrophilicity index, defined as [$\omega = \mu^2/2\eta$], is a robust measure of electron-accepting capability, with higher values signifying a stronger electrophilic character [79]. The calculated global reactivity descriptors for the structures **3'aj** and **3aj** are listed in **Table 2**. The electrophilicity index values of $\omega = 0.126025$ and 0.125833 , respectively, indicate the pronounced electrophilic nature of these compounds. The relatively low global hardness ($\eta = 0.08291$ eV), together with a negative chemical potential ($\mu = -0.14456$ eV), confirms the soft character, high polarizability, and enhanced charge-transfer ability of the studied compounds. The dipole moment (μ) is associated with molecular stability in polar environments [52]. The calculated dipole moments are listed in **Table 2**. These parameters provide a deeper insight into the stability of the investigated tautomers.

Table 2: The optimized geometries of HOMO and LUMO, Energy calculated at the B3LYP/6-311G(d,p) level and values of different parameters in Hartree

Compounds	HOMO	LUMO	$\Delta E = \text{LUMO} - \text{HOMO}$	Ionization Potential IP	Electron Affinity (E_A)	Electron negativity (χ)	Chemical potential (μ)	Global Hardness (η)	Softness (S)	Electrophilicity index (ω)	Dipole Moment (D)
3'aj	 $E_{\text{HOMO}} = -0.2385$	 $E_{\text{LUMO}} = -0.0894$	0.1491	0.2385	0.0894	0.16400	-0.1640	0.0746	13.14	0.1804	3.49
3aj	 $E_{\text{HOMO}} = -0.2329$	 $E_{\text{LUMO}} = -0.0732$	0.1597	0.2329	0.0732	0.1531	-0.1531	0.0798	12.52	0.1467	3.68

3 Materials and methods

3.1 Chemistry

The melting points were determined using the open capillary method and are not corrected. FT-IR spectra of the compounds were recorded on an FT-IR spectrometer (Model RZX (Perkin Elmer) using KBr pellets. The ^1H , ^{13}C , and ^{19}F NMR spectra were recorded on a Bruker Advance II 400 NMR Spectrometer at 400, 100, and 375 MHz, respectively; the chemical shifts are expressed on the δ -scale downfield from TMS as an internal standard. Mass spectra were recorded on a Waters Micromass Q-ToF Micro Mass spectrometer equipped with electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) sources with mass ranges of 4000 and 20000 amu in quadrupole and ToF, respectively. The absorbance for antioxidant activity was recorded using a Spectro 250 Analytikena UV spectrophotometer. Compounds **1** and **3** were prepared as previously reported [57,80].

3.1.1 Synthesis of (*E*)-1-(4-aryl)-4,4,4-trifluoro-2-(4-aryldiazenyl)butane-1,3-diones (**1**)

General Procedure: An appropriate *p*-substituted aniline (0.02 mol) was dissolved in a mixture of conc. HCl and water (20 mL, 1:1). It was cooled to 0 °C and a cold aq. solution of sodium nitrite (0.02 mol in 10 mL water) was added to it slowly by maintaining the temperature at 5°C. The cold diazotized solution was added dropwise to a pre-cooled mixture of an appropriate 1-aryl-4,4,4-trifluorobutane-1,3-dione (0.02 mol) and sodium acetate (0.06 mol) in 20 mL of 50% ethanol. The

reaction mixture was stirred for 1 h, and the separated crystals were filtered, washed with water, dried, and recrystallized from ethanol to obtain the final product.

3.1.1.1 (*E*)-1-(4'-Chlorophenyl)-4,4,4-trifluoro-2-((4''-iodophenyl)diazenyl)butane-1,3-dione (**1a**):

Color: pale yellow; Yield: 8.63 g, 85%; Observed m.p. 121 °C; IR ($\nu_{\max}$, cm^{-1}): 1698 (CO str.), 3208 (OH str.), 3378 (NH str.); ^1H NMR (400 MHz; CDCl_3 + $\text{DMSO}-d_6$, δ_{H}): 7.28 (d, 2H, 2'', 6''-H, $^3J_{\text{H-H}} = 8.82$ Hz), 7.60 (d, 2H, 3', 5'-H, $^2J_{\text{H-H}} = 8.56$ Hz), 7.74 (d, 2H, 3'', 5''-H, $^3J_{\text{H-H}} = 8.72$ Hz), 7.80 (d, 2H, 2', 6'-H, $^3J_{\text{H-H}} = 8.84$ Hz), 12.50 (s, 1H, NH or OH, D_2O exchangeable); ^{13}C NMR (100 MHz; CDCl_3 + $\text{DMSO}-d_6$, δ_{C}): 89.59 (C-4''), 116.93 (q, 4-CF₃, $^1J_{\text{C-F}} = 292.77$ Hz), 118.25 (C-2'', 6''), 129.06 (C-3', 5'), 130.50 (C-2', 6'), 131.13 (C-2), 134.30 (C-1'), 138.08 (C-3'', 5''), 139.45 (C-4'), 141.72 (C-1''), 175.32 (C-3, $^2J_{\text{C-F}} = 33.20$ Hz), 189.68 (C-1); ^{19}F NMR ($\text{DMSO}-d_6$, δ_{F}): -69.19 (s, 3F, CF₃).

Other derivatives (**1b-n**, **1aa-an**) were synthesized using a similar procedure.

All experimental details, FT-IR, NMR (^1H and ^{13}C), and data of all synthesized compounds are provided in the Supporting Information.

3.1.2 Synthesis of (*E*)-4-(aryldiazenyl)-3-aryl-5-hydroxy-5-(trifluoromethyl)-4,5-dihydroisoxazoles (**3**)

General procedure: An ethanolic (25 mL) mixture of hydroxylamine hydrochloride (5 mmol) and sodium acetate (5 mmol) was refluxed in a water bath for 15 min (pH = 6.7). Aryl-1, 1, 1-trifluorobutane-2, 4-dione (5 mmol) was subsequently added to the reaction mixture, which was then refluxed for 4 h. The reaction was monitored using TLC. The solvent was evaporated, and the residue was extracted with chloroform. The organic phase was dried over anhydrous sodium sulfate and chloroform was distilled off to obtain the final product, **3**.

3.1.2.1 (*E*)-3-(4'-Chlorophenyl)-4-((4''-iodophenyl)diazenyl)-5-hydroxy-5-trifluoromethyl-4,5-dihydroisoxazole (**3a**): Color: Yellow ; Yield: 2.14 g, 87%; Observed m.p. 200-201 °C; IR ($\nu_{\max}$, cm^{-1}): 3005 (OH str.), 3292 (NH str.); ^1H NMR (400 MHz; $\text{DMSO}-d_6$, δ_{H}): 7.26 (d, 2H, 2'', 6''-H, $^1J_{\text{H-H}} = 8.80$ Hz), 7.63-7.66 (m, 4H, 3', 5', 3'', 5''-H), 7.98 (d, 2H, 2', 6'-H, $^1J_{\text{H-H}} = 8.56$ Hz), 9.86 (bs, 1H, OH, D_2O exchangeable), 10.45 (bs, 1H, NH, D_2O exchangeable); ^{13}C NMR (100 MHz; $\text{DMSO}-d_6$, δ_{C}): 85.72 (C-4''), 98.52 (q, C-5, $^2J_{\text{C-F}} = 35.21$ Hz), 117.00 (C-2'', 6''), 122.05 (q, 5-CF₃, $^1J_{\text{C-F}} = 288.75$ Hz), 125.59 (C-1'), 129.03 (C-3', 5'), 129.40 (C-2', 6'), 131.95 (C-4), 135.56 (C-4'), 137.74 (C-3'', 5''), 143.12 (C-1''), 154.60 (C-3); ^{19}F NMR ($\text{DMSO}-d_6$, δ_{F}): -78.87 (s, 3F, CF₃); MS:

496 (m/z, M^+) Calcd for $C_{16}H_{10}ClF_3IN_3O_2$: 494.9458, Found: 493.9265 (M^+-1), and (m/z, M^++2)
 497 calcd. 496.9429, Found: 495.9168 (M^+-2) in a ratio showing a typical chlorine isotope profile (3:1).
 498 3.1.2.2 (*E*)-3-(4'-Bromophenyl)-4-((4''-iodophenyl)diazenyl)-5-hydroxy-5-trifluoromethyl-4,5-
 499 dihydroisoxazole (**3b**): Color: Bright yellow; Yield: 2.37 g, 88%; Observed m.p. 202 °C; IR
 500 ($\nu_{\max}$, cm^{-1}): 3009 (OH str.), 3292 (NH str.); ^1H NMR (400 MHz; $CDCl_3$ + DMSO- d_6 , δ_H): 7.14
 501 (d, 2H, 2'', 6''-H, $^1J_{H-H}$ = 8.76 Hz), 7.54 (d, 2H, 3'', 5''-H, $^1J_{H-H}$ = 8.72 Hz), 7.64 (d, 2H, 3', 5'-H,
 502 $^1J_{H-H}$ = 8.56 Hz), 7.86 (d, 2H, 2', 6'-H, $^1J_{H-H}$ = 8.52 Hz), 9.64 (bs, 1H, OH, D_2O exchangeable),
 503 10.16 (bs, 1H, NH, D_2O exchangeable); ^{13}C NMR (100 MHz; $CDCl_3$ + DMSO- d_6 , δ_C): 90.32 (C-
 504 4''), 103.40 (q, C-5, $^2J_{C-F}$ = 35.21 Hz), 121.88 (C-2'', 6''), 126.93 (q, 5-CF₃, $^1J_{C-F}$ = 288.75 Hz),
 505 129.61 (C-4'), 131.19 (C-1'), 131.61 (C-4), 134.53 (C-2', 6'), 136.86 (C-3', 5'), 142.81 (C-3'', 5''),
 506 148.15 (C-1''), 159.66 (C-3); ^{19}F NMR (DMSO- d_6 , δ_F) δ : -78.85 (s, 3F, CF₃); MS: (m/z, M^+) Calcd
 507 for $C_{16}H_{10}BrF_3IN_3O_2$: 538.8953, Found: 537.8217 (M^+-1), and (m/z, M^++2) calcd. 540.89433,
 508 Found: 539.8227 (M^+-2) in the ratio showing a typical bromine isotope profile (1:1)
 509 3.1.2.3 (*E*)-4-((4''-Iodophenyl)diazenyl)-3-phenyl-5-hydroxy-5-trifluoromethyl-4,5-
 510 dihydroisoxazole (**3c**): Color: Dark yellow; Yield: 1.91 g, 83%; Observed m.p. 187 °C; IR ($\nu_{\max}$,
 511 cm^{-1}): 3009 (OH str.), 3297 (NH str.); ^1H NMR (400 MHz; DMSO- d_6 , δ_H): 7.13 (d, 2H, 2'', 6''-H,
 512 $^1J_{H-H}$ = 8.76 Hz), 7.45-7.47 (m, 3H, 3', 4', 5'-H), 7.52 (d, 2H, 3'', 5''-H, $^1J_{H-H}$ = 8.76 Hz), 7.90-7.92
 513 (m, 2H, 2', 6'-H), 9.62 (bs, 1H, OH, D_2O exchangeable), 10.11 (bs, 1H, NH, D_2O exchangeable);
 514 ^{13}C NMR (100 MHz; DMSO- d_6 , δ_C): 84.90 (C-4''), 98.39 (q, C-5, $^2J_{C-F}$ = 35.21 Hz), 116.48 (C-2'',
 515 6''), 121.93 (q, 5-CF₃, $^1J_{C-F}$ = 287.74 Hz), 126.23 (C-1'), 127.54 (C-3', 5'), 128.45 (C-2', 6'), 132.77
 516 (C-4), 130.44 (C-4'), 137.54 (C-3'', 5''), 142.97 (C-1''), 155.21 (C-3); ^{19}F NMR (DMSO- d_6 , δ_F): -
 517 78.89 (s, 3F, CF₃); HRMS: (m/z, M^++1) Calcd for $C_{16}H_{11}F_3IN_3O_2$: 461.9882, Found: 461.9922
 518 (M^++1).
 519 3.1.2.4 (*E*)-3-(4'-Fluorophenyl)-4-((4''-iodophenyl)diazenyl)-5-hydroxy-5-trifluoromethyl-4,5-
 520 dihydroisoxazole (**3d**): Color: Dusky yellow; Yield: 1.96 g, 83%; Observed m.p. 190-191 °C; IR
 521 ($\nu_{\max}$, cm^{-1}): 3008 (OH str.), 3292 (NH str.); ^1H NMR (400 MHz; DMSO- d_6 , δ_H): 7.25 (d, 2H, 2'',
 522 6''-H, $^1J_{H-H}$ = 8.84 Hz), 7.38-7.44 (m, 2H, 3', 5'-H), 7.64 (d, 2H, 3'', 5''-H, $^1J_{H-H}$ = 8.84 Hz), 8.00-
 523 8.05 (m, 2', 6'-H), 9.84 (bs, 1H, OH, D_2O exchangeable), 10.42 (bs, 1H, NH, D_2O exchangeable);
 524 ^{13}C NMR (100 MHz; DMSO- d_6 , δ_C): 85.65 (C-4''), 98.40 (q, C-5, $^2J_{C-F}$ = 35.21 Hz), 116.01 (d, C-
 525 3', 5', $^2J_{C-F}$ = 21.13 Hz), 116.95 (C-2'', 6''), 122.05 (q, 5-CF₃, $^1J_{C-F}$ = 288.75 Hz), 123.25 (d, C-1',
 526 $^4J_{C-F}$ = 3.02 Hz), 130.08 (d, C-2', 6', $^3J_{C-F}$ = 9.05 Hz), 132.24 (C-4), 137.23 (C-3'', 5''), 143.14 (C-

1"), 154.60 (C-3), 163.45 (d, C-4', $^1J_{C-F} = 248.51$ Hz); ^{19}F NMR (DMSO- d_6 , δ_F): - 78.89 (s, 3F, CF₃), 109.33 (s, 1F, 4"-F); HRMS: (m/z, $M^+ + 1$) Calcd for C₁₆H₁₀F₄IN₃O₂: 491.9987 (M^+), Found: 492.0029 ($M^+ + 1$).

3.1.2.5 (*E*)-4-((4"-Iodophenyl)diazenyl)-3-(4'-methoxyphenyl)-5-hydroxy-5-trifluoromethyl-4,5-dihydroisoxazol3 (**3e**): Color: Creamish white; Yield: 1.98, 81%; Observed m.p. 182 °C; IR (ν_{max} , cm⁻¹): 3002 (OH str.), 3297 (NH str.); ^1H NMR (400 MHz; CDCl₃ + DMSO- d_6 , δ_H): 3.79 (s, 3H, 4'-OMe), 7.02 (d, 2H, 3', 5'-H, $^1J_{H-H} = 8.76$ Hz), 7.17 (d, 2H, 2", 6"-H, $^1J_{H-H} = 8.64$ Hz), 7.55 (d, 2H, 3", 5"-H, $^1J_{H-H} = 8.64$ Hz), 7.87 (d, 2H, 2', 6'-H, $^1J_{H-H} = 8.76$ Hz), 9.64 (bs, 1H, OH, D₂O exchangeable), 10.17 (bs, 1H, NH, D₂O exchangeable); ^{13}C NMR (100 MHz; CDCl₃ + DMSO- d_6 , δ_C): 55.21 (4'-OMe), 85.08 (C-4"), 98.40 (q, C-5, $^2J_{C-F} = 35.21$ Hz), 114.13 (C-3', 5'), 116.69 (C-2", 6"), 119.00 (C-1'), 122.05 (q, 5-CF₃, $^1J_{C-F} = 288.75$ Hz), 129.09 (C-2', 6'), 133.12 (C-4), 137.63 (C-3", 5"), 143.17 (C-1"), 154.67 (C-3), 161.09 (C-4'); ^{19}F NMR (DMSO- d_6 , δ_F): - 78.89 (s, 3F, CF₃); MS: (m/z, $M^+ + 1$) Calcd for C₁₆H₁₃F₄IN₃O₃: 491.9987, Found: 492.0029 ($M^+ + 1$)

3.1.2.6 (*E*)-4-((4"-Iodophenyl)diazenyl)-3-(naphthalen-2'-yl)-5-hydroxy-5-trifluoromethyl-4,5-dihydroisoxazole (**3f**): Color: Sandy yellow; Yield: 2 g, 78%; Observed m.p. 201 °C; IR (ν_{max} , cm⁻¹): 3011 (OH str.), 3299 (NH str.); ^1H NMR (400 MHz; CDCl₃ + DMSO- d_6 , δ_H): 7.22 (d, 2H, 2", 6"-H, $^1J_{H-H} = 8.60$ Hz), 7.53-8.00 (m, 8H, 3", 5", 3', 4', 5', 6', 7', 8'-H), 8.62 (s, 1'-H), 9.73 (bs, 1H, OH, D₂O exchangeable), 10.27 (bs, 1H, NH, D₂O exchangeable); ^{13}C NMR (400 MHz; CDCl₃ + DMSO- d_6 , δ_H): 85.08 (C-4"), 98.40 (q, C-5, $^2J_{C-F} = 35.21$ Hz), 116.69 (C-2", 6"), 122.05 (q, 5-CF₃, $^1J_{C-F} = 288.75$ Hz), 124.18, 124.60, 126.16, 126.63, 127.69, 128.87, 129.60, 129.77, 131.95 (C-4), 132.06, 135.84, 137.63 (C-3", 5"), 143.17 (C-1"), 154.67 (C-5); ^{19}F NMR (DMSO- d_6 , δ_F): - 78.82 (s, 3F, CF₃); HRMS: (m/z, $M^+ + 1$) Calcd for C₂₀H₁₃F₃IN₃O₂: 512.0038, Found: 512.0080 ($M^+ + 1$).

3.1.2.7 (*E*)-4-((4"-Iodophenyl)diazenyl)-3-(naphthalen-1'-yl)-5-hydroxy-5-trifluoromethyl-4,5-dihydroisoxazole (**3g**): Color: Geryish-green ; Yield: 2g, 78%; Observed m.p. 170 °C; IR (ν_{max} , cm⁻¹): 3007 (OH str.), 3295 (NH str.); ^1H NMR (400 MHz; CDCl₃ + DMSO- d_6 , δ_H): 6.28 (d, 2H, 2", 6"-H, $^1J_{H-H} = 8.80$ Hz), 7.34 (d, 2H, 3", 5"-H, $^1J_{H-H} = 8.76$ Hz), 7.46-8.08 (m, 7H, 2', 3', 4', 5', 6', 7', 8'-H), 9.65 (bs, 1H, OH, D₂O exchangeable), 10.19 (bs, 1H, NH, D₂O exchangeable); ^{13}C NMR (100 MHz; CDCl₃ + DMSO- d_6 , δ_C): 85.09 (C-4"), 98.42 (q, C-5, $^2J_{C-F} = 35.21$ Hz), 116.70 (C-2", 6"), 122.07 (q, 5-CF₃, $^1J_{C-F} = 288.75$ Hz), 123.18, 123.60, 124.16, 126.63, 127.70, 128.89, 129.62, 129.79, 131.95 (C-4), 132.08, 135.86, 137.69 (C-3", 5"), 143.17 (C-1"), 154.67 (C-5); ^{19}F

558 NMR (DMSO-*d*₆, δ_F): - 78.83 (s, 3F, CF₃); HRMS: (m/z, M⁺+1) Calcd for C₂₀H₁₃F₃IN₃O₂:
559 512.0038, Found: 512.0081 (M⁺+1).

560 3.1.2.8 (*E*)-4-((4''-Bromophenyl)diazenyl)-3-(4'-chlorophenyl)-5-hydroxy-5-trifluoromethyl-4,5-
561 dihydroisoxazole (**3h**): Color: Lemon yellow; Yield: 1.95 g, 87%; Observed m.p. 178 °C; IR ($\nu_{\max}$,
562 cm⁻¹): 3005 (OH str.), 3293 (NH str.); ¹H NMR (400 MHz; CDCl₃ + DMSO-*d*₆, δ_H): 7.34 (d, 2H,
563 2'', 6''-H, ¹J_{H-H} = 8.96 Hz), 7.43 (d, 2H, 3'', 5''-H, ¹J_{H-H} = 8.92 Hz), 7.57 (d, 2H, 3', 5'-H, ¹J_{H-H} =
564 8.56 Hz), 8.00 (d, 2H, 2', 6'-H, ¹J_{H-H} = 8.60 Hz), 9.72 (bs, 1H, OH, D₂O exchangeable), 10.27 (bs,
565 1H, NH, D₂O exchangeable); ¹³C NMR (100 MHz; CDCl₃ + DMSO-*d*₆, δ_C): 98.52 (q, C-5, ²J_{C-F} =
566 36.21 Hz), 114.11 (C-4''), 116.23 (C-2'', 6''), 121.89 (q, 5-CF₃, ¹J_{C-F} = 288.75 Hz), 125.54 (C-1'),
567 128.70 (C-3', 5'), 129.09 (C-2', 6'), 131.72 (C-3'', 5''), 132.22 (C-4), 135.72 (C-4'), 142.36 (C-1''),
568 154.36 (C-3); ¹⁹F NMR (DMSO-*d*₆, δ_F): - 78.84 (s, 3F, CF₃); MS (m/z, M⁺) Calcd for
569 C₁₆H₁₀BrClF₃N₃O₂: 446.9597 (M⁺) 448.9577 (M⁺+2), Found: 445.9171 (M⁺-1), 447.9198 (M⁺+2-
570 1).

571 3.1.2.9 (*E*)-3-(4'-Bromophenyl)-4-((4''-bromophenyl)diazenyl)-5-hydroxy-5-trifluoromethyl-4,5-
572 dihydroisoxazole (**3i**): Color: Creamish yellow; Yield: 2.19 g, 89%; Observed m.p. 174 °C; IR
573 ($\nu_{\max}$, cm⁻¹): 3006 (OH str.), 3296 (NH str.); ¹H NMR (400 MHz; CDCl₃ + DMSO-*d*₆, δ_H): 7.36 (d,
574 2H, 2'', 6''-H, ¹J_{H-H} = 8.92 Hz), 7.44 (d, 2H, 3'', 5''-H, ¹J_{H-H} = 8.92 Hz), 7.73 (d, 2H, 3', 5'-H, ¹J_{H-H}
575 = 8.56 Hz), 7.92 (d, 2H, 2', 6'-H, ¹J_{H-H} = 8.56 Hz), 9.77 (bs, 1H, OH, D₂O exchangeable), 10.35
576 (bs, 1H, NH, D₂O exchangeable); ¹³C NMR (100 MHz; CDCl₃ + DMSO-*d*₆, δ_C): 98.54 (q, C-5, ²J<sub>C-
577 F</sub> = 36.21 Hz), 114.10 (C-4''), 116.38 (C-2'', 6''), 121.93 (q, 5-CF₃, ¹J_{C-F} = 288.75 Hz), 124.35 (C-
578 4'), 125.97 (C-1'), 129.39 (C-2', 6'), 131.72 (C-3', 5'), 131.76 (C-3'', 5''), 132.06 (C-4), 142.46 (C-
579 1''), 154.53 (C-3); ¹⁹F NMR (DMSO-*d*₆, δ_F): - 78.83 (s, 3F, CF₃); MS (m/z, M⁺) Calcd for
580 C₁₆H₁₀Br₂F₃N₃O₂: 490.9092 (M⁺), 492.9071 (M⁺+2), 494.9051 (M⁺+4), Found: 489.917 (M⁺)
581 491.817 (M⁺+2) 493.916 (M⁺+4).

582 3.1.2.10 (*E*)-4-((4'-Bromophenyl)diazenyl)-3-phenyl-5-hydroxy-5-trifluoromethyl-4,5-
583 dihydroisoxazole (**3j**): Color: Pale yellow; Yield: 1.78 g, 86%; Observed m.p. 172 °C; IR ($\nu_{\max}$,
584 cm⁻¹): 3002 (OH str.), 3294 (NH str.); ¹H NMR (400 MHz; CDCl₃ + DMSO-*d*₆, δ_H): 7.35 (d, 2H,
585 2'', 6''-H, ¹J_{H-H} = 8.88 Hz), 7.44 (d, 2H, 3'', 5''-H, ¹J_{H-H} = 8.88 Hz), 7.52-7.56 (m, 3H, 3', 4', 5'-H),
586 7.96-7.99 (m, 2H, 2', 6'-H), 9.73 (bs, 1H, OH, D₂O exchangeable), 10.30 (bs, 1H, NH, D₂O
587 exchangeable); ¹³C NMR (100 MHz; CDCl₃ + DMSO-*d*₆, δ_C): 98.38 (q, C-5, ²J_{C-F} = 36.22 Hz),
588 113.98 (C-4''), 116.22 (C-2'', 6''), 121.99 (q, 5-CF₃, ¹J_{C-F} = 287.74 Hz), 126.84 (C-1'), 127.59 (C-

3', 5'), 128.56 (C-2', 6'), 130.52 (C-4'), 131.75 (C-3'', 5''), 132.59 (C-4), 142.54 (C-1''), 155.33 (C-3); ¹⁹F NMR (DMSO-*d*₆, δ_F): - 78.89 (s, 3F, CF₃); HRMS: (m/z, M⁺+1) Calcd for C₁₆H₁₁BrF₃N₃O₂: 414.0020, Found: 414.0066 (M⁺+1).

3.1.2.11 (*E*)-3-(4'-Fluorophenyl)-4-((4''-bromophenyl)diazenyl)-5-hydroxy-5-trifluoromethyl-4,5-dihydroisoxazole (**3k**): Color: Light yellow; Yield: 1.83 g, 85%; Reported m.p. 150–154 °C; Observed m.p. 176 °C; IR (ν_{max}, cm⁻¹): 3007 (OH str.), 3295 (NH str.); ¹H NMR (400 MHz; CDCl₃ + DMSO-*d*₆, δ_H): 7.31-7.36 (m, 4H, 3', 5', 2'', 6''-H, ¹J_{H-H} = 8.80 Hz), 7.44 (d, 3'', 5''-H, ¹J_{H-H} = 8.84 Hz), 8.02-8.05 (m, 2', 6'-H), 9.73 (bs, 1H, OH, D₂O exchangeable), 10.31 (bs, 1H, NH, D₂O exchangeable); ¹³C NMR (100 MHz; CDCl₃ + DMSO-*d*₆, δ_C): 98.40 (q, C-5, ²J_{C-F} = 36.22 Hz), 114.04 (C-4''), 115.70 (d, C-3', 5', ²J_{C-F} = 22.13 Hz), 116.28 (C-2'', 6''), 121.94 (q, 5-CF₃, ¹J_{C-F} = 288.75 Hz), 123.21 (d, C-1', ⁴J_{C-F} = 4.02 Hz), 129.87 (d, C-2', 6', ³J_{C-F} = 9.05 Hz), 131.73 (C-3'', 5''), 132.40 (C-4), 142.46 (C-1''), 154.40 (C-3), 163.43 (d, C-4', ¹J_{C-F} = 249.51 Hz); ¹⁹F NMR (DMSO-*d*₆, δ_F) δ : - 78.89 (s, 3F, CF₃), 109.38 (s, 1F, 4''-F); MS (m/z) Calcd for C₁₆H₁₀BrF₄N₃O₂: 430.9893 (M⁺), 432.9872 (M⁺+2),

3.1.2.12 (*E*)-4-((4''-Bromophenyl)diazenyl)-3-(4'-methoxyphenyl)-5-hydroxy-5-trifluoromethyl-4,5-dihydroisoxazole (**3l**): Color: Brownish yellow; Yield: 1.86 g, 84%; Observed m.p. 172-173 °C; IR (ν_{max}, cm⁻¹): 3009 (OH str.), 3295 (NH str.); ¹H NMR (400 MHz; CDCl₃ + DMSO-*d*₆, δ_H): 3.87 (s, 3H, 4'-OMe), 7.07 (d, 2H, 3', 5'-H, ¹J_{H-H} = 8.88 Hz), 7.32 (d, 2H, 2'', 6''-H, ¹J_{H-H} = 8.88 Hz), 7.43 (d, 2H, 3'', 5''-H, ¹J_{H-H} = 8.88 Hz), 7.95 (d, 2H, 2', 6'-H, ¹J_{H-H} = 8.88 Hz), 9.64 (bs, 1H, OH, D₂O exchangeable), 10.15 (bs, 1H, NH, D₂O exchangeable); ¹³C NMR (100 MHz; CDCl₃ + DMSO-*d*₆, δ_C): 55.07 (4'-OMe), 98.13 (q, C-5, ²J_{C-F} = 35.22 Hz), 113.87 (C-4''), 113.92 (C-3', 5'), 116.01 (C-2'', 6''), 118.98 (C-1'), 121.94 (q, 5-CF₃, ¹J_{C-F} = 287.74 Hz), 129.00 (C-2', 6'), 131.70 (C-3'', 5''), 133.18 (C-4), 142.44 (C-1''), 154.57 (C-3), 161.03 (C-4'); ¹⁹F NMR (DMSO-*d*₆, δ_F): - 78.89 (s, 3F, CF₃); HRMS: (m/z, M⁺+1) Calcd for C₁₇H₁₃BrF₃N₃O₃: 444.0126, Found: 444.0170 (M⁺+1) .

3.1.2.13 (*E*)-4-((4''-Bromophenyl)diazenyl)-3-(naphthalen-2'-yl)-5-hydroxy-5-trifluoromethyl-4,5-dihydroisoxazole (**3m**): Color: Brown; Yield: 1.82 g, 79%; Observed m.p. 179 °C; IR (ν_{max}, cm⁻¹): 3009 (OH str.), 3299 (NH str.); ¹H NMR (400 MHz; CDCl₃ + DMSO-*d*₆, δ_H): 7.41 (d, 2H, 2'', 6''-H, ¹J_{H-H} = 8.92 Hz), 7.48 (d, 2H, 3'', 5''-H, ¹J_{H-H} = 8.88 Hz), 7.58-8.21 (m, 6H, 3', 4', 5', 6', 7', 8'-H), 8.68 (s, 1H, 1'-H), 9.78 (bs, 1H, OH, D₂O exchangeable), 10.39 (bs, 1H, NH, D₂O exchangeable); ¹³C NMR (100 MHz; CDCl₃ + DMSO-*d*₆, δ_C): 98.16 (q, C-5, ²J_{C-F} = 35.21 Hz),

114.00 (C-4"), 116.27 (C-2", 6"), 121.98 (q, 5-CF₃, ¹J_{C-F} = 287.74 Hz), 124.09, 124.24, 126.70, 127.40, 127.53, 128.03, 128.18, 128.68, 131.79 (C-3", 5"), 132.39, 133.65 (C-4), 142.63 (C-1"), 155.03 (C-3); ¹⁹F NMR (DMSO-*d*₆, δ_F): - 78.89 (s, 3F, CF₃); HRMS: (m/z, M⁺+1) Calcd for C₂₀H₁₃BrF₃IN₃O₂: 464.0177, Found: 464.0226 (M⁺+1).

3.1.2.14 (*E*)-4-((4"-Bromophenyl)diazenyl)-3-(naphthalen-1'-yl)-5-hydroxy-5-trifluoromethyl-4,5-dihydroisoxazole (**3n**): Color: Dark brown ;Yield: 1.68 g, 73%; Observed m.p. 170 °C; IR (ν_{max}, cm⁻¹): 3009 (OH str.), 3296 (NH str.); ¹H NMR (400 MHz; CDCl₃ + DMSO-*d*₆, δ_H): 7.04 (d, 2", 6"-H, ¹J_{H-H} = 8.92 Hz), 7.27 (d, 3", 5"-H, ¹J_{H-H} = 8.88 Hz), 7.57-8.12 (m, 7H, 2', 3', 4', 5', 6', 7', 8'-H), 9.76 (bs, 1H, OH, D₂O exchangeable), 10.38 (bs, 1H, NH, D₂O exchangeable) ¹³C NMR (100 MHz; CDCl₃ + DMSO-*d*₆, δ_C): 98.18 (q, C-5, ²J_{C-F} = 35.21 Hz), 113.91 (C-4"), 116.09 (C-2", 6"), 121.99 (q, 5-CF₃, ¹J_{C-F} = 287.74 Hz), 123.20, 123.62, 124.18, 126.69, 127.70, 128.89, 129.62, 129.79, 131.72 (C-3", 5"), 132.08, 133.19 (C-4), 135.86, 142.44 (C-1"), 154.60 (C-3); ¹⁹F NMR (DMSO-*d*₆, δ_F): - 78.83 (s, 3F, CF₃); HRMS: (m/z, M⁺+1) Calcd for C₂₀H₁₃BrF₃IN₃O₂: 464.0177, Found: 464.0222 (M⁺+1).

3.1.2.15 (*E*)-3-(4'-Chlorophenyl)-4-((4"-chlorophenyl)diazenyl)-5-hydroxy-5-trifluoromethyl-4,5-dihydroisoxazole (**3aa**): Color: Yellow; Yield: 1.80 g, 89 %; Observed m.p. 169 °C; IR (ν_{max}, cm⁻¹): 2995 (OH str.), 3293 (NH str.); ¹H NMR (400 MHz; CDCl₃ + DMSO-*d*₆, δ_H): 7.33 (d, 2H, 2", 6"-H, ¹J_{H-H} = 8.92 Hz), 7.43 (d, 2H, 3", 5"-H, ¹J_{H-H} = 8.88 Hz), 7.61 (d, 2H, 3', 5'-H, ¹J_{H-H} = 8.52 Hz), 8.00 (d, 2H, 2', 6'-H, ¹J_{H-H} = 8.56 Hz), 9.80 (bs, 1H, OH, D₂O exchangeable), 10.41 (bs, 1H, NH, D₂O exchangeable); ¹³C NMR (100 MHz; CDCl₃ + DMSO-*d*₆, δ_C): 98.52 (q, C-5, ²J_{C-F} = 35.22 Hz), 116.00 (C-2", 6"), 121.99 (q, 5-CF₃, ¹J_{C-F} = 288.75 Hz), 125.60 (C-1'), 126.28 (C-4), 128.85 (C-3', 5'), 128.92 (C-3", 5"), 129.25 (C-2', 6'), 131.91 (C-4"), 135.62 (C-4'), 142.10 (C-1"), 154.50 (C-3); ¹⁹F NMR (DMSO-*d*₆, δ_F): -78.89 (s, 3F, CF₃); MS: (m/z, M⁺) Calcd for C₁₆H₁₀Cl₂F₃N₃O₂: 403.0102, Found: 402.0289 (M⁺-1), (m/z, M⁺+2) calcd. 405.0073, Found: 404.0250 (M⁺+2-1), and (m/z, M⁺+4) calcd. 407.0043, Found: 406.0495 (M⁺+4-1).

3.1.2.16 (*E*)-3-(4'-Bromophenyl)-4-((4"-chlorophenyl)diazenyl)-5-hydroxy-5-trifluoromethyl-4,5-dihydroisoxazole (**3ab**): Color: Pale Yellow ; Yield: 2 g, 90%; Observed m.p. 170 °C; IR (ν_{max}, cm⁻¹): 2997 (OH str.), 3295 (NH str.); ¹H NMR (400 MHz; CDCl₃ + DMSO-*d*₆, δ_H): 7.32 (d, 2H, 2", 6"-H, ¹J_{H-H} = 8.92 Hz), 7.42 (d, 2H, 3", 5"-H, ¹J_{H-H} = 8.92 Hz), 7.75 (d, 2H, 3', 5'-H, ¹J_{H-H} = 8.56 Hz), 7.92 (d, 2H, 2', 6'-H, ¹J_{H-H} = 8.52 Hz), 9.79 (bs, 1H, OH, D₂O exchangeable), 10.40 (bs, 1H, NH, D₂O exchangeable); ¹³C NMR (100 MHz; CDCl₃ + DMSO-*d*₆, δ_C): 98.34 (q, C-5,

651 $^2J_{C-F}$ = 36.21 Hz), 116.00 (C-2", 6"), 121.93 (q, 5-CF₃, $^1J_{C-F}$ = 288.75), 124.34 (C-4'), 125.98 (C-
652 1'), 126.27 (C-4), 128.92 (C-3", 5"), 129.44 (C-2', 6'), 131.77 (C-3', 5'), 131.83 (C-4"), 142.08 (C-
653 1"), 154.58 (C-3); ^{19}F NMR (DMSO-*d*₆, δ_F): - 78.81 (s, 3F, CF₃); MS: (m/z, M⁺) Calcd for
654 C₁₆H₁₀BrClF₃N₃O₂: 446.9597 (M⁺), 448.9568 (M⁺+2) Found: 445.9092 (M⁺-1), 447.9240 (M⁺+2-
655 1).

656 3.1.2.17 (*E*)-4-((4"-Chlorophenyl)diazenyl)-3-phenyl-5-hydroxy-5-trifluoromethyl-4,5-dihydro-
657 isoxazole (**3ac**): Color: Citron Yellow ;Yield: 1.47 g, 80%; Observed m.p. 165°C; IR (ν_{max} , cm⁻¹):
658 2998 (OH str.), 3293 (NH str.); ^1H NMR (400 MHz; CDCl₃ + DMSO-*d*₆, δ_H): 7.30 (d, 2H, 2", 6"-
659 H, $^1J_{H-H}$ = 8.56 Hz), 7.39 (d, 2H, 3", 5"-H, $^1J_{H-H}$ = 8.56 Hz), 7.55 (appeared as singlet, 3H, 3', 4',
660 5'-H), 7.98-7.99 (m, 2H, 2', 6'-H), 9.74 (bs, 1H, OH, D₂O exchangeable), 10.27 (bs, 1H, NH, D₂O
661 exchangeable); ^{13}C NMR (100 MHz; CDCl₃ + DMSO-*d*₆, δ_C): 98.38 (q, C-5, $^2J_{C-F}$ = 36.22 Hz),
662 115.72 (C-2", 6"), 121.93 (q, CF₃, J = 288.75), 126.23 (C-4), 126.85 (C-1'), 127.57 (C-3', 5'),
663 128.52 (C-2', 6'), 128.85 (C-3", 5"), 130.48 (C-4'), 132.51 (C-4"), 142.05 (C-1"), 155.30 (C-3); ^{19}F
664 NMR (DMSO-*d*₆, δ_F): - 78.84 (s, 3F, CF₃); HRMS: (m/z, M⁺+1) Calcd for C₁₆H₁₁ClF₃N₃O₂:
665 370.0525, Found: 370.0567 (M⁺+1).

666 3.1.2.18 (*E*)-4-((4"-Chlorophenyl)diazenyl)-3-(4'-fluorophenyl)-5-hydroxy-5-trifluoromethyl-4,5-
667 dihydroisoxazole (**3ad**): Color: Amber yellow; Yield: 1.56 g, 81%; Observed m.p. 162 °C; IR
668 (ν_{max} , cm⁻¹): 2998 (OH str.), 3297 (NH str.); ^1H NMR (400 MHz; CDCl₃ + DMSO-*d*₆, δ_H): 7.30-
669 7.37 (m, 4H, 3', 5', 2", 6"-H), 7.41 (d, 2H, 3", 5"-H, $^1J_{H-H}$ = 8.76 Hz), 8.02-8.05 (m, 2H, 2', 6'-H),
670 9.77 (bs, 1H, OH, D₂O exchangeable), 10.34 (bs, 1H, NH, D₂O exchangeable); ^{13}C NMR (100
671 MHz; CDCl₃ + DMSO-*d*₆, δ_C): 98.40 (q, C-5, $^2J_{C-F}$ = 35.21 Hz), 115.75 (d, C-3', 5', $^3J_{C-F}$ = 22.13
672 Hz), 115.89 (C-2", 6"), 121.99 (q, 5-CF₃, $^1J_{C-F}$ = 288.75 Hz), 123.24 (d, C-1', $^4J_{C-F}$ = 3.02 Hz),
673 126.24 (C-4), 128.88 (C-3", 5"), 129.90 (d, C-2', 6', $^3J_{C-F}$ = 8.05 Hz), 132.23 (C-4"), 142.08 (C-1"),
674 154.44 (C-3), 163.43 (d, C-4', $^1J_{C-F}$ = 249.51 Hz); ^{19}F NMR (DMSO-*d*₆, δ_F): - 78.81 (s, 3F, CF₃),
675 109.39 (s, 1F, 4"-F);

676 3.1.2.19 (*E*)-4-((4"-Chlorophenyl)diazenyl)-3-(4'-methoxyphenyl)-5-hydroxy-5-
677 (trifluoromethyl)-4,5-dihydroisoxazole (**3ae**): Color: Brown; Yield: 1.65 g, 83%; Reported m.p.
678 108-112°C; Observed m.p. 165 °C; IR (ν_{max} , cm⁻¹): 2995 (OH str.), 3299 (NH str.); ^1H NMR (400
679 MHz; CDCl₃ + DMSO-*d*₆, δ_H): 3.86 (s, 3H, 4'-OMe), 7.09 (d, 2H, 3', 5'-H, $^1J_{H-H}$ = 8.76 Hz), 7.32
680 (d, 2H, 2", 6"-H, $^1J_{H-H}$ = 8.80 Hz), 7.41 (d, 2H, 3", 5"-H, $^1J_{H-H}$ = 8.84 Hz), 7.95 (d, 2H, 2', 6'-H,
681 $^1J_{H-H}$ = 8.76 Hz), 9.71 (bs, 1H, OH, D₂O exchangeable), 10.27 (bs, 1H, NH, D₂O exchangeable);

¹³C NMR (100 MHz; CDCl₃ + DMSO-*d*₆, δ_C): 55.15 (4'-OMe), 98.13 (q, C-5, ²J_{C-F} = 35.21 Hz), 114.06 (C-3', 5'), 115.75 (C-2'', 6''), 119.01 (C-1'), 122.06 (q, 5-CF₃, ¹J_{C-F} = 287.74 Hz), 126.07 (C-4), 128.89 (C-3'', 5''), 129.06 (C-2', 6'), 132.92 (C-4''), 142.17 (C-1''), 154.66 (C-3), 161.05 (C-4'); ¹⁹F NMR (DMSO-*d*₆, δ_F): -78.87 (s, 3F, CF₃); HRMS: (m/z, M⁺+1) Calcd for C₁₇H₁₃ClF₃N₃O₃: 400.0631, Found: 400.0635 (M⁺+1).

3.1.2.20 (*E*)-4-((4''-Chlorophenyl)diazenyl)-3-(naphthalen-2'-yl)-5-hydroxy-5-trifluoromethyl-4,5-dihydroisoxazole (**3af**): Color: Turmeric yellow ; Yield: 71.59 g, 76%; Observed m.p. 162 °C; IR (ν_{max}, cm⁻¹): 2999 (OH str.), 3299 (NH str.); ¹H NMR (400 MHz; CDCl₃ + DMSO-*d*₆, δ_H): 7.35 (d, 2H, 2'', 6''-H, ¹J_{H-H} = 8.84 Hz), 7.47 (d, 2H, 2'', 6''-H, ¹J_{H-H} = 8.92 Hz), 7.59-8.22 (m, 6H, 3', 4', 5', 6', 7', 8'-H), 8.69 (s, 1H, 1'-H), 9.79 (bs, 1H, NH, D₂O exchangeable), 10.40 (bs, 1H, NH, D₂O exchangeable); ¹³C NMR (100 MHz; CDCl₃ + DMSO-*d*₆, δ_C): 98.50 (q, C-5, ²J_{C-F} = 35.21 Hz), 115.86 (C-2'', 6''), 122.09 (q, 4-CF₃, ¹J_{C-F} = 287.74 Hz), 124.12, 124.26, 126.24 (C-4), 126.71, 127.41, 127.54, 128.05, 128.20, 128.68, 128.94 (C-3'', 5''), 132.40, 132.44 (C-4''), 133.66, 142.21 (C-1''), 155.07 (C-3); ¹⁹F NMR (DMSO-*d*₆, δ_F): -78.91 (s, 3F, CF₃); HRMS: (m/z, M⁺+1) Calcd for C₂₀H₁₀ClF₄N₃O₂: 420.0682, Found: 420.0723 (M⁺+1).

3.1.2.21 (*E*)-4-((4''-Chlorophenyl)diazenyl)-3-(naphthalen-1'-yl)-5-hydroxy-5-trifluoromethyl-4,5-dihydroisoxazole (**3ag**): Color: Greenish yellow Yield: 1.52 g, 73%; Observed m.p. 160 °C; IR (ν_{max}, cm⁻¹): 2994 (OH str.), 3298 (NH str.); ¹H NMR (400 MHz; CDCl₃ + DMSO-*d*₆, δ_H): 7.00-8.18 (m, 11H, 2', 3', 4', 5', 6', 7', 8', 3'', 5'', 2'', 6''-H), 9.66 (bs, 1H, OH, D₂O exchangeable), 10.24 (bs, 1H, NH or OH, D₂O exchangeable); ¹³C NMR (400 MHz; CDCl₃ + DMSO-*d*₆, δ_H): 98.16 (q, C-5, ²J_{C-F} = 35.21 Hz), 115.77 (C-2'', 6''), 122.09 (q, 4-CF₃, ¹J_{C-F} = 287.74 Hz), 123.20, 123.62, 124.18, 126.09 (C-4), 126.69, 127.70, 128.89 (C-3'', 5''), 128.91, 129.62, 129.79, 132.08, 132.92 (C-4''), 135.86, 142.17 (C-1''), 154.67 (C-3); ¹⁹F NMR (DMSO-*d*₆, δ_F): -78.90 (s, 3F, CF₃); HRMS: (m/z, M⁺+1) Calcd for C₂₀H₁₀ClF₄N₃O₂: 420.0682, Found: 420.0725 (M⁺+1).

3.1.2.22 (*E*)-3-(4''-Chlorophenyl)-4-((4'-fluorophenyl)diazenyl)-5-hydroxy-5-trifluoromethyl-4,5-dihydroisoxazole (**3ah**): Color: Greyish yellow ;Yield: 1.53 g, 73%; Observed m.p. 160 °C; IR (ν_{max}, cm⁻¹): 2994 (OH str.), 3298 (NH str.); ¹H NMR (400 MHz; CDCl₃ + DMSO-*d*₆, δ_H): 7.00-8.18 (m, 11H, 2', 3', 4', 5', 6', 7', 8', 3'', 5'', 2'', 6''-H), 9.66 (bs, 1H, OH, D₂O exchangeable), 10.24 (bs, 1H, NH or OH, D₂O exchangeable); ¹³C NMR (400 MHz; CDCl₃ + DMSO-*d*₆, δ_H): 98.16 (q, C-5, ²J_{C-F} = 35.21 Hz), 115.77 (C-2'', 6''), 122.09 (q, 4-CF₃, ¹J_{C-F} = 287.74 Hz), 123.20, 123.62, 124.18, 126.09 (C-4), 126.69, 127.70, 128.89 (C-3'', 5''), 128.91, 129.62, 129.79, 132.08, 132.92

(C-4"), 135.86, 142.17 (C-1"), 154.67 (C-3); ¹⁹F NMR (DMSO-*d*₆, δ_F): -78.90 (s, 3F, CF₃); HRMS: (m/z, M⁺+1) Calcd for C₁₆H₁₀ClF₄N₃O₂: 387.04, Found: 386.03794 (M⁺+1).

3.1.2.23 (*E*)-3-(4"-Bromophenyl)-4-((4'-fluorophenyl)diazenyl)-5-hydroxy-5-trifluoromethyl-4,5-dihydroisoxazole (**3ai**): Color: Sunny yellow ; Yield: 1.92 g, 89%; Observed m.p. 165 °C; Lit m.p. 166 °C [40]; IR (ν_{max}, cm⁻¹): 3129 (OH str.), 3320 (NH str.); ¹H NMR (300 MHz; CDCl₃, δ_H): 3.78 (bs, 1H, OH, D₂O exchangeable), 7.03-7.10 (m, 4H, 2", 3", 5" and 6"-H), 7.56 (d, 2H, 3', 5'-H, ¹J_{H-H} = 8.1 Hz), 7.80 (d, 2H, 2', 6'-H, ¹J_{H-H} = 8.4 Hz), 9.21 (bs, 1H, NH, D₂O exchangeable); ¹³C NMR (100 MHz; CDCl₃, δ_C): 98.75 (q, C-5, ²J_{C-F} = 38.25 Hz), 115.27 (d, C-2", 6", ³J_{C-F} = 8.25 Hz), 116.43 (d, C-3", 5", ²J_{C-F} = 23.25 Hz), 121.75 (q, CF₃, ¹J_{C-F} = 285.75), 125.12 (C-4'), 125.77 (C-1'), 129.54 (C-2', 6'), 131.32 (C-4), 131.93 (C-3', 5'), 138.18 (d, C-1", ⁴J_{C-F} = 3 Hz), 155.13 (C-3), 159.18 (d, C-4", ¹J_{C-F} = 240.75 Hz); ¹⁹F NMR (DMSO-*d*₆, δ_F): - 78.89 (s, 3F, CF₃), 109.37 (s, 1F, 4"-F). -HRMS: (m/z, M⁺+1) Calcd for C₁₆H₁₀BrF₄N₃O₂: 432.17, Found: 431.921012 (M⁺+1).

3.1.2.24 (*E*)-4-((4"-Fluorophenyl)diazenyl)-3-phenyl-5-hydroxy-5-trifluoromethyl-4,5-dihydroisoxazole (**3aj**): Color: Pale yellow ;Yield: 1.50 g, 80%; Observed m.p. 164 °C; Lit m.p. 166 °C [40]; IR (ν_{max}, cm⁻¹): 3122 (OH str.), 3319 (NH str.); ¹H NMR (300 MHz; CDCl₃, δ_H): 3.80 (bs, 1H, OH, D₂O exchangeable), 6.97-6.99 (m, 4H, 2', 3', 5', 6', 2", 3", 5", 6"-H), 7.41-7.44 (m, 3H, 3', 4', 5'-H), 7.91 (d, 2H, 2', 6'-H, ¹J_{H-H} = 7.6 Hz), 9.27 (bs, 1H, NH, D₂O exchangeable); ¹³C NMR (300 MHz; CDCl₃, δ_H): 98.40 (q, C-5, ²J_{C-F} = 38.25 Hz), 115.16 (d, C-2", 6"-H), 116.20 (d, C-3", 5", ²J_{C-F} = 23.25 Hz), 121.70 (q, CF₃, ¹J_{C-F} = 285.75), 126.36 (d, C-1', ⁴J_{C-F} = 285.75), 128.58 (d, C-2', 6', ³J_{C-F} = 8.25 Hz), 128.16 (d, 3', 5'-H, ²J_{C-F} = 21.75 Hz), 130.98 (d, C-4', ¹J_{C-F} = 249.75 Hz), 132.11 (C-4), 138.45 (d, C-1", ⁴J_{C-F} = 3.00 Hz), 156.02 (C-3), 158.96 (C-4"); ¹⁹F NMR (DMSO-*d*₆, δ_F): - 78.81 (s, 3F, CF₃), 109.36 (s, 1F, 4"-F); HRMS: (m/z, M⁺+1) Calcd for C₁₆H₁₁F₄N₃O₂: 354.0821, Found: 354.0863 (M⁺+1).

3.1.2.25 (*E*)-3-(4'-Fluorophenyl)-4-((4'-fluorophenyl)diazenyl)-5-hydroxy-5-trifluoromethyl-4,5-dihydroisoxazole (**3ak**): Color: Sandy yellow ;Yield: 1.54 g, 78%; Observed m.p. 169-170 °C; Lit m.p. 170 °C [40]; IR (ν_{max}, cm⁻¹): 3130 (OH str.), 3328 (NH str.); ¹H NMR (300 MHz; CDCl₃, δ_H): 3.82 (bs, 1H, OH, D₂O exchangeable) 7.06-7.18 (m, 6H, 3', 5', 2", 3", 5", 6"-H), 7.97 (dd, 2H, 2', 6'-H, ¹J_{H-H} = 7.6 Hz, ⁴J_{H-F} = 3.3 Hz), 9.23 (bs, 1H, NH, D₂O exchangeable); ¹³C NMR (100 MHz; CDCl₃, δ_C): 98.97 (q, C-5, ²J_{C-F} = 36.00 Hz), 114.93 (d, C-2", 6", ³J_{C-F} = 7.50 Hz), 115.65 (C-3', 5'), 116.06 (d, C-3", 5", ²J_{C-F} = 23.25 Hz), 122.13 (q, CF₃, ¹J_{C-F} = 287.25 Hz), 123.23 (C-1'),

129.91 (C-2', 6'), 133.27 (C-4), 138.79 (d, C-1", $^4J_{C-F}$ = 3.0 Hz), 154.52 (C-3), 158.59 (d, C-4", $^1J_{C-F}$ = 240.75 Hz), 164.02 (C-4'); ^{19}F NMR (DMSO- d_6 , δ_{F}): - 78.89 (s, 3F, CF_3), 109.36 (s, 1F, 4"-F).

3.1.2.26 (*E*)-4-((4"-Fluorophenyl)diazenyl)-3-(4'-methoxyphenyl)-5-hydroxy-5-trifluoromethyl-4,5-dihydroisoxazole (**3al**): Color: brownish yellow ; Yield: 1.63 g, 80 %; Observed m.p. 143 °C; Lit m.p. 145-146 °C [40]; IR (ν_{max} , cm^{-1}): 3123 (OH str.), 3320 (NH str.); ^1H NMR (300 MHz; CDCl_3 , δ_{H}): 3.81 (bs, 1H, OH, D_2O exchangeable), 3.83 (s, 3H, OCH_3), 6.92 (d, 2H, 3', 5'-H, $^1J_{\text{H-H}}$ = 8.4 Hz), 7.02-7.05 (m, 4H, 2", 3", 5" and 6"-H), 7.90 (d, 2H, 2', 6'-H, $^1J_{\text{H-H}}$ = 8.4 Hz), 9.22 (bs, 1H, NH, D_2O exchangeable); ^{13}C NMR (100 MHz; CDCl_3 , δ_{C}): 55.32 (4"-OMe), 98.86 (q, C-5, $^2J_{C-F}$ = 36 Hz), 113.99 (C-3', 5'), 115.11 (d, C-2", 6", $^3J_{C-F}$ = 7.5 Hz), 116.20 (d, C-3", 5", $^2J_{C-F}$ = 22.25 Hz), 118.71 (C-1'), 122.08 (q, CF_3 , $^1J_{C-F}$ = 285.75), 129.60 (C-2', 6'), 132.44 (C-4), 138.53 (d, C-1", $^4J_{C-F}$ = 3 Hz), 155.15 (C-3), 158.77 (d, C-4", $^1J_{C-F}$ = 240.75 Hz), 161.61 (C-4'); ^{19}F NMR (DMSO- d_6 , δ_{F}): - 78.85 (s, 3F, CF_3), 109.37 (s, 1F, 4"-F); HRMS: (m/z, $\text{M}^+ + 1$) Calcd for $\text{C}_{17}\text{H}_{13}\text{F}_4\text{N}_3\text{O}_3$: 384.0927, Found: 384.0966 ($\text{M}^+ + 1$).

3.1.2.27 (*E*)-4-((4"-Fluorophenyl)diazenyl)-3-(naphthalen-2'-yl)-5-hydroxy-5-trifluoromethyl-4,5-dihydroisoxazole (**3am**): Color: Dark yellow Yield: 1.68 g, 78%; Observed m.p. 159 °C; IR (ν_{max} , cm^{-1}): 3129 (OH str.), 3323 (NH str.); ^1H NMR (300 MHz; CDCl_3 , δ_{H}): 3.81 (bs, 1H, OH, D_2O exchangeable), 7.02-7.05 (m, 4H, 2", 3", 5" and 6"-H), 7.53-8.00 (m, 7H, 2', 3', 4', 5', 6', 7', 8'-H), 8.62 (s, 1'-H), 9.27 (bs, 1H, NH, D_2O exchangeable); ^{13}C NMR (100 MHz; CDCl_3 , δ_{C}): 98.86 (q, C-5, $^2J_{C-F}$ = 36 Hz), 115.11 (d, C-2", 6", $^3J_{C-F}$ = 7.5 Hz), 116.20 (d, C-3", 5", $^2J_{C-F}$ = 22.25 Hz), 122.08 (q, 5- CF_3 , $^1J_{C-F}$ = 285.75), 124.17, 124.62, 126.18, 126.69, 127.71, 128.87, 129.60, 129.77, 132.06, 132.44 (C-4), 138.53 (d, C-1", $^4J_{C-F}$ = 3 Hz), 135.84, 155.15 (C-3), 158.77 (d, C-4", $^1J_{C-F}$ = 240.75 Hz); ^{19}F NMR (DMSO- d_6 , δ_{F}): -78.90 (s, 3F, CF_3), 109.37 (s, 1F, 4"-F); HRMS: (m/z, $\text{M}^+ + 1$) Calcd for $\text{C}_{20}\text{H}_{13}\text{F}_4\text{N}_3\text{O}_2$: 404.0977, Found: 404.1018 ($\text{M}^+ + 1$).

3.1.2.28 (*E*)-4-((4"-Fluorophenyl)diazenyl)-3-(naphthalen-2'-yl)-5-hydroxy-5-trifluoromethyl-4,5-dihydroisoxazole (**3an**): Color: Greenish yellow Yield: 1.55 g, 72%; Observed m.p. 149 °C; IR (ν_{max} , cm^{-1}): 3128 (OH str.), 3320 (NH str.); ^1H NMR (300 MHz; CDCl_3 , δ_{H}): 3.86 (bs, 1H, OH, D_2O exchangeable), 7.02-7.07 (m, 4H, 2", 3", 5" and 6"-H), 7.53-8.00 (m, 7H, 2', 3', 4', 5', 6', 7', 8'-H), 9.29 (bs, 1H, NH, D_2O exchangeable); ^{13}C NMR (100 MHz; CDCl_3 , δ_{C}): 98.86 (q, C-5, $^2J_{C-F}$ = 36 Hz), 115.11 (d, C-2", 6", $^3J_{C-F}$ = 7.5 Hz), 116.20 (d, C-3", 5", $^2J_{C-F}$ = 22.25 Hz), 122.08 (q, 5- CF_3 , $^1J_{C-F}$ = 285.75), 123.20, 123.62, 124.18, 126.69, 127.70, 128.89, 129.62, 129.79, 132.08, 132.44 (C-4), 135.86, 138.53 (d, C-1", $^4J_{C-F}$ = 3 Hz), 155.15 (C-3), 158.77 (d, C-4", $^1J_{C-F}$ = 240.75

Hz); ^{19}F NMR ($\text{DMSO}-d_6$, δ_{F}): - 78.92 (s, 3F, CF_3), 109.38 (s, 1F, 4"-F); HRMS: (m/z , $\text{M}^+ + 1$) Calcd for $\text{C}_{20}\text{H}_{13}\text{F}_4\text{N}_3\text{O}_2$: 404.0977, Found: 404.107 ($\text{M}^+ + 1$).

3.2 Biological activity

3.2.1 Antimicrobial activity

The antimicrobial activity of the synthesized compounds was evaluated against clinically important microbial strains relevant to human infections. The selected test organisms included five microbial strains. These strains include two Gram-positive bacteria (*S. aureus* MTCC 96 and *B. subtilis* MTCC 121), two Gram-negative bacteria (*E. coli* MTCC 1652 and *P. aeruginosa* MTCC 741), and one yeast strain (*C. albicans* MTCC). These microorganisms were selected to assess the broad-spectrum antimicrobial potential of the synthesized derivatives. All microbial cultures were purchased from the Microbial Type Culture Collection (MTCC) at IMTECH, Chandigarh. Bacterial strains were subcultured on nutrient agar (NA), and yeast was cultured on malt extract agar (MEA) plates.

Determination of zone of inhibition

The zones of inhibition for all the synthesized compounds were evaluated using the agar well diffusion assay[81]. Inoculum suspensions of the test microorganisms were prepared using 16-h-old cultures adjusted to 10^8 cfu/mL, referring to the 0.5 McFarland standard. A total of 20 mL of agar medium (NA for antibacterial and MEA for antifungal) was poured into each Petri plate, and the plates were swabbed with 100 μL of inoculum of the test microorganisms and kept for 15 min for adsorption. Wells were bored into the seeded agar plates using a sterile cork borer of diameter 8 mm, and these were loaded with a 100 μL volume with a concentration of 4.0 mg/mL of each compound reconstituted in dimethylsulphoxide (DMSO). All plates were incubated at 37 $^{\circ}\text{C}$ for 24 h. The antimicrobial activity of each compound against the test organisms was evaluated by measuring the zone of inhibition using a zone reader (Hi Antibiotic zone scale). Ciprofloxacin and amphotericin B were used as positive controls for bacterial and yeast strains, respectively. This procedure was performed in triplicates for each organism.

Determination of minimum inhibitory concentration (MIC)

The MIC of the various compounds against bacterial and yeast strains was tested using a modified agar well diffusion method [82,83]. The agar dilution method was used for MIC determination owing to its robustness, reproducibility, and ability to provide clear and reliable endpoints for evaluating the antibacterial activities of synthetic compounds. In this protocol, a two-fold serial

dilution of each compound was prepared by first reconstituting the compound in dimethyl sulfoxide (DMSO), followed by dilution in DMSO to achieve a decreasing concentration range of 4–0.0625 mg/mL. A 100 µL volume of each dilution was introduced into wells (in triplicate) in the agar plates seeded with 100 µL of a standardized inoculum (10^8 cfu/mL) of the test microbial strain. All test plates were incubated aerobically at 37 °C for 24 h and observed for inhibition zones. The MIC, defined as the lowest concentration of the chemical compound that inhibited microbial growth, was indicated by a clear zone of inhibition and recorded for each test organism. Ciprofloxacin and amphotericin B were used as positive controls, and DMSO was used as a negative control.

3.2.2 Free radical-scavenging of the compounds using DPPH analysis

DPPH radical scavenging activity is based on the fact that the methanolic solution of DPPH, which imparts a vivid purple color and gives a strong absorption band at 515 nm, is reduced in the presence of an antioxidant compound [56]. Different concentrations of the compounds under evaluation (50–400 µg/mL) were added to 4mL of a DPPH solution (120 µM) in methanol and incubated at 37 °C temperature for 30 min in the dark. The absorbance was determined at 515 nm, and the percentage of free radical scavenging (%) was calculated using the following equation:

$$\text{Scavenging \%} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$$

where A_{control} is the absorbance of the control reaction (containing all reagents except the test compounds), and A_{sample} is the absorbance of the test compound. Ascorbic acid was used as a positive control, and the tests were conducted in triplicate.

3.2.3 UV-mediated plasmid DNA damage-protecting activity

Treatment of plasmid DNA with the samples

The stock solutions of all the tested compounds were prepared by dissolving 0.005 g of the compound in 0.5 mL of DMSO. All synthesized compounds **3a-u** (50 µg) in DMSO were added separately to a volume of 2 µL containing plasmid DNA in *Tris* 10 mM, EDTA 0.01 mM, pH 8.0 (TE) buffer solution. The same volume of DMSO used to prepare the solution of the tested compounds was added to **A** and control **C**. The reaction volumes, except **A** were held in polyethylene microcentrifuge tube caps, which were directly irradiated on the surface of a trans-illuminator (8000 mW/cm) at 360 nm for 1 h at room temperature. Thereafter, **A**, control (**C**), and test samples were incubated at 37 °C for 0.5 h.

Agarose gel electrophoresis

After the treatment, electrophoresis was performed according to the method described below [57]: To a 2 mL 50X Tris-acetate-EDTA (TAE) buffer (40 mM Tris, 20 mM acetic acid, 1 mM EDTA, pH 8.0), 98 mL of autoclaved water was added to obtain a 1X TAE buffer solution. Agarose (0.8 g) was dissolved by boiling the mixture. When the gel reached a temperature of 55 °C, 10 mg/mL of ethidium bromide (EtBr) was added, and the mixture was poured into a gel cassette fitted with a comb. The gel was then allowed to solidify. The comb was carefully removed, and the gel was placed in an electrophoresis chamber flooded with Tris-acetate-EDTA buffer (40 mM Tris, 20 mM acetic acid, 1 mM EDTA, pH 8.0). The treated DNA sample mixed with 6 loading dye (0.25% bromophenol blue and 30% glycerol) was carefully loaded into the wells along with the control (C) and A, and electrophoresis was carried out at 5V/cm for 2.0 h, after the bands were observed under a UV trans illuminator.

4. Conclusion

In the present study, a series of twenty-eight 4-(aryldiazenyl)-3-aryl-5-hydroxy-5-trifluoromethyl-4,5-dihydroisoxazole derivatives were successfully synthesized using a multistep approach and comprehensively characterized using FT-IR, ¹H, ¹³C, and ¹⁹F NMR spectroscopy, along with mass spectrometry. The data revealed that the compounds exist in the arylhydrazone form (**3**) instead of the aryl diazo form (**3'**), which was further supported by DFT calculations. The synthesized compounds were evaluated for exploring *in vitro* antibacterial, antifungal, antioxidant, and DNA damage-protective activities. Biological screening demonstrated that **3j**, **3ad**, **3ae**, **3ah**, **3ai**, **3aj**, and **3ak** exhibited significant antibacterial activity against *B. subtilis*, while **3ak** showed strong inhibition against *S. aureus* with nearly 78% efficacy compared to Ciprofloxacin. Antifungal evaluation revealed that **3k** displayed significantly higher activity against *C. albicans* than Amphotericin B, whereas **3ad** and **3ae** showed comparatively lower enhanced activity. Antioxidant studies indicated that compounds **3e**, **3j**, **3ah-aj**, and **3al-an** possessed remarkable radical-scavenging potential, outperforming Ascorbic Acid. Furthermore, **3g**, **3n**, **3f**, **3m**, **3af**, **3ag**, **3am**, **3an**, **3b**, **3e**, **3aa**, **3ae**, and **3h** demonstrated significant DNA-protective ability against UV-induced damage at 60 µg/mL. Overall, the present work provides important insights into the structural features and biological potential of 5-trifluoromethylated isoxazoline derivatives, highlighting their significance as promising multifunctional bioactive candidates for future medicinal and pharmaceutical applications.

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Authors' contribution

Kamalneet Kaur: Writing-Original draft preparation, Investigation, Validation. **Aman Kumar:** Writing, Investigation, Validation. **Nisha:** Writing, Investigation, Validation. **Ekta:** Investigation, Validation. **Chetan Sharma:** Investigation, Validation. **Vishal Sharma:** Investigation, Validation. **Sundeep Jaglan:** Investigation, Validation. **Rakesh Kumar Bhardwaj:** Investigation, Software. **Ramesh Kataria:** Data Curation, Formal analysis. **Vikas Beniwal:** Investigation, Formal analysis. **Vinod Kumar:** Writing-Review & Editing, Resources, Conceptualization, Supervision

Declarations

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Competing interests

No potential competing interest was reported by the author(s).

Clinical trial registration details

No clinical trials were performed; hence, it is not applicable.

Data declaration:

All data supporting the results of this study are original and available within the research paper and its Supplementary Information.

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900 References

- 901 [1] Md.A. Salam, Md.Y. Al-Amin, M.T. Salam, J.S. Pawar, N. Akhter, A.A. Rabaan, M.A.A.
902 Alqumber, Antimicrobial Resistance: A Growing Serious Threat for Global Public Health,
903 Healthcare (Basel) 11 (2023) 1946. <https://doi.org/10.3390/healthcare11131946>.
- 904 [2] G. Muteeb, M.T. Rehman, M. Shahwan, M. Aatif, Origin of Antibiotics and Antibiotic
905 Resistance, and Their Impacts on Drug Development: A Narrative Review, Pharmaceuticals
906 (Basel) 16 (2023) 1615. <https://doi.org/10.3390/ph16111615>.
- 907 [3] S. Tahlan, S. Singh, H. Dey, M. Kaira, K.C. Pandey, Recent research frontiers of heterocycles
908 as antifungal Agents: Insights from the past five years, European Journal of Medicinal
909 Chemistry 295 (2025) 117801. <https://doi.org/10.1016/j.ejmech.2025.117801>.
- 910 [4] Z. Liu, Z. Ren, J. Zhang, C.-C. Chuang, E. Kandaswamy, T. Zhou, L. Zuo, Role of ROS and
911 Nutritional Antioxidants in Human Diseases, Front Physiol 9 (2018) 477.
912 <https://doi.org/10.3389/fphys.2018.00477>.
- 913 [5] V.P. Reddy, Oxidative Stress in Health and Disease, Biomedicines 11 (2023) 2925.
914 <https://doi.org/10.3390/biomedicines11112925>.
- 915 [6] Y. Qin, C. Qian, W. Li, Q. Wang, Q. Sheng, Z. Chen, W. Zhang, W. Li, G. Ge, Z. Yan, D.
916 Geng, Oxidative Stress: Molecular Mechanisms, Diseases, and Therapeutic Targets,
917 MedComm (2020) 7 (2026) e70600. <https://doi.org/10.1002/mco2.70600>.
- 918 [7] G. Pizzino, N. Irrera, M. Cucinotta, G. Pallio, F. Mannino, V. Arcoraci, F. Squadrito, D.
919 Altavilla, A. Bitto, Oxidative Stress: Harms and Benefits for Human Health, Oxid Med Cell
920 Longev 2017 (2017) 8416763. <https://doi.org/10.1155/2017/8416763>.
- 921 [8] N. Agrawal, P. Mishra, The synthetic and therapeutic expedition of isoxazole and its analogs,
922 Med Chem Res 27 (2018) 1309–1344. <https://doi.org/10.1007/s00044-018-2152-6>.
- 923 [9] Y. Shinde, B. Khairnar, S. Bangale, Exploring the Diverse Biological Frontiers of Isoxazole:
924 A Comprehensive Review of its Pharmacological Significance, ChemistrySelect 9 (2024)
925 e202401423. <https://doi.org/10.1002/slct.202401423>.
- 926 [10] G. Kumar, R. Shankar, 2-Isoxazolines: A Synthetic and Medicinal Overview,
927 ChemMedChem 16 (2021) 430–447. <https://doi.org/10.1002/cmdc.202000575>.
- 928 [11] A. Arzine, S.A. Assou, L.E. Bouamri, M. Chalkha, A. Nakkabi, S. Chtita, R.M. Aljowaie,
929 M.A.M. Aboul-Soud, M.E. Hassouni, J.P. Giesy, M.E. Yazidi, New isoxazole-based
930 heterocyclic hybrids with dual antimicrobial and antioxidant bioactivity: integrated synthesis,
931 in vitro assessment, and computational exploration, RSC Advances 16 (2026) 13515–13528.
932 <https://doi.org/10.1039/D6RA01005A>.
- 933 [12] G.J. Martis, S.L. Gaonkar, Advances in isoxazole chemistry and their role in drug discovery,
934 RSC Adv 15 (n.d.) 8213–8243. <https://doi.org/10.1039/d4ra08339c>.

- 935 [13] A. Shaik, R.R. Bhandare, K. Palleepati, S. Nissankararao, V. Kancharlapalli, S. Shaik,
936 Antimicrobial, Antioxidant, and Anticancer Activities of Some Novel Isoxazole Ring
937 Containing Chalcone and Dihydropyrazole Derivatives, *Molecules* 25 (2020) 1047.
938 <https://doi.org/10.3390/molecules25051047>.
- 939 [14] K. Vashisht, P. Sethi, A. Bansal, P. Bansal, Antimicrobial activity of isoxazole derivatives:
940 A brief overview, *Vietnam Journal of Chemistry* 63 (2025) 195–213.
941 <https://doi.org/10.1002/vjch.202400029>.
- 942 [15] A.R. Reddy, G. Goverdhan, A. Sampath, K. Mukkanti, P.P. Reddy, R. Bandichhor,
943 Application of [3 + 2]-Cycloaddition in the Synthesis of Valdecixib, *Synthetic*
944 *Communications* 42 (2012) 639–649. <https://doi.org/10.1080/00397911.2010.528572>.
- 945 [16] R.B. Mrid, N. Bouchmaa, H. Ainani, R. El Fatimy, G. Malka, L. Mazini, Anti-rheumatoid
946 drugs advancements: New insights into the molecular treatment of rheumatoid arthritis,
947 *Biomedicine & Pharmacotherapy* 151 (2022) 113126.
948 <https://doi.org/10.1016/j.biopha.2022.113126>.
- 949 [17] R. Preyra, L.E. Eddin, F. Ahmadi, A. Jafari, F.T. Muanda, Safety of sulfamethoxazole–
950 trimethoprim for the treatment of bacterial infection in outpatient settings: A systematic
951 review and meta-analysis with active comparator disproportionality analysis, *Br. J. Clin.*
952 *Pharmacol.* 91 (2025) 1632–1648. <https://doi.org/10.1002/bcp.70051>.
- 953 [18] B.E. Gidal, T. Resnick, M.C. Smith, J.W. Wheless, Zonisamide, *Neurol Clin Pract* 14 (2024)
954 e200210. <https://doi.org/10.1212/CPJ.000000000000200210>.
- 955 [19] A. Zana, A. Galbiati, Synthesis and Reactivity of 3-Halo-4,5-dihydroisoxazoles: An
956 Overview, *ChemistrySelect* 6 (2021) 8249–8261. <https://doi.org/10.1002/slct.202101779>.
- 957 [20] C. Rizzo, S. Amata, I. Pibiri, A. Pace, S. Buscemi, A. Palumbo Piccionello, FDA-Approved
958 Fluorinated Heterocyclic Drugs from 2016 to 2022, *Int J Mol Sci* 24 (2023) 7728.
959 <https://doi.org/10.3390/ijms24097728>.
- 960 [21] S. Mukherjee, U. Rani, G. Chandra, S. Pal, Recent advances in the synthesis of fluorinated
961 heterocycles and a review of recent FDA-approved fluorinated drugs, *Org. Biomol. Chem.*
962 23 (2025) 7821–7851. <https://doi.org/10.1039/D5OB00997A>.
- 963 [22] K.P. Melnykov, O. Tavlui, A. Skreminskiy, Y.O. Kuchkovska, O.O. Grygorenko, Impact of
964 Fluoroalkyl Substituents on the Physicochemical Properties of Saturated Heterocyclic
965 Amines, *Chemistry* 28 (2022) e202201601. <https://doi.org/10.1002/chem.202201601>.
- 966 [23] A. Abbas, T. Farghaly, K. Dawood, Recent progress in therapeutic applications of fluorinated
967 five-membered heterocycles and their benzo-fused systems, *RSC Advances* 14 (2024)
968 33864–33905. <https://doi.org/10.1039/d4ra05697c>.
- 969 [24] M. Novás, M.J. Matos, The Role of Trifluoromethyl and Trifluoromethoxy Groups in
970 Medicinal Chemistry: Implications for Drug Design, *Molecules* 30 (2025) 3009.
971 <https://doi.org/10.3390/molecules30143009>.

- 972 [25] A.S. Nair, A.K. Singh, A. Kumar, S. Kumar, S. Sukumaran, V.P. Koyiparambath, L.K.
 973 Pappachen, T.M. Rangarajan, H. Kim, B. Mathew, FDA-Approved Trifluoromethyl Group-
 974 Containing Drugs: A Review of 20 Years, Processes 10 (2022) 2054.
 975 <https://doi.org/10.3390/pr10102054>.
- 976 [26] Y. Zafrani, G. Parvari, D. Amir, L. Ghindes-Azaria, S. Elias, A. Pevzner, G. Fridkin, A.
 977 Berliner, E. Gershonov, Y. Eichen, S. Saphier, S. Katalan, Modulation of the H-Bond
 978 Basicity of Functional Groups by α -Fluorine-Containing Functions and its Implications for
 979 Lipophilicity and Bioisosterism, J. Med. Chem. 64 (2021) 4516–4531.
 980 <https://doi.org/10.1021/acs.jmedchem.0c01868>.
- 981 [27] S. Schiesser, H. Chepliaka, J. Kollback, T. Quennesson, W. Czechtizky, R.J. Cox, N-
 982 Trifluoromethyl Amines and Azoles: An Underexplored Functional Group in the Medicinal
 983 Chemist's Toolbox, J. Med. Chem. 63 (2020) 13076–13089.
 984 <https://doi.org/10.1021/acs.jmedchem.0c01457>.
- 985 [28] P. Bhattarai, T.A. Trombley, R.A. Altman, On the Metabolic Stability of Fluorinated Small
 986 Molecules: A Physical Organic Chemistry Perspective, J Med Chem 69 (2026) 1916–1941.
 987 <https://doi.org/10.1021/acs.jmedchem.5c02359>.
- 988 [29] H. Kawai, S. Okusu, E. Tokunaga, N. Shibata, Enantioselective Synthesis of 5-
 989 Trifluoromethyl-2-isoxazolines and Their N-Oxides by [Hydroxy(tosyloxy)iodo]benzene-
 990 Mediated Oxidative N–O Coupling, European Journal of Organic Chemistry 2013 (2013)
 991 6506–6509. <https://doi.org/10.1002/ejoc.201301096>.
- 992 [30] U. Madsen, B. Ebert, P. Krogsgaard-Larsen, E. Wong, Synthesis and pharmacology of (*RS*)-
 993 2-amino-3-(3-hydroxy-5-trifluoromethyl-4-isoxazolyl)propionic acid, a potent AMPA
 994 receptor agonist, European Journal of Medicinal Chemistry 27 (1992) 479–484.
 995 [https://doi.org/10.1016/0223-5234\(92\)90181-Y](https://doi.org/10.1016/0223-5234(92)90181-Y).
- 996 [31] P.K. Mykhailiuk, Fluorinated Pyrazoles: From Synthesis to Applications, Chem. Rev. 121
 997 (2021) 1670–1715. <https://doi.org/10.1021/acs.chemrev.0c01015>.
- 998 [32] P. Pattanayak, S. Nikhitha, D. Halder, B. Ghosh, T. Chatterjee, Exploring the impact of
 999 trifluoromethyl (–CF₃) functional group on the anti-cancer activity of isoxazole-based
 1000 molecules: design, synthesis, biological evaluation and molecular docking analysis, RSC Adv
 1001 14 (n.d.) 18856–18870. <https://doi.org/10.1039/d4ra02856b>.
- 1002 [33] H.G. Bonaccorso, E.P. Pittaluga, L.M.F. Porte, F.M. Libero, A.F. Junges, N. Zanatta, M.A.P.
 1003 Martins, Unexpected Metal-Free Fluorination and Oxidation at the C-4 Position of Pyrazoles
 1004 Promoted by Selectfluor, Synlett 26 (2015) 2009–2013. <https://doi.org/10.1055/s-0034-1381050>.
- 1006 [34] N.A. Razali, Z. Jamain, Synthesis, chemical identification and biological application of Azo-
 1007 based molecules containing different terminal group: A review, Journal of Molecular
 1008 Structure 1284 (2023) 135329. <https://doi.org/10.1016/j.molstruc.2023.135329>.

- 1009 [35] T. Tahir, M. Ashfaq, H. Asghar, M.I. Shahzad, R. Tabassum, A. Ashfaq, Medicinal
1010 Importance of Azo and Hippuric Acid Derivatives, [Http://Www.Eurekaselect.Com](http://Www.Eurekaselect.Com) (n.d.).
1011 <https://www.eurekaselect.com/article/91999> (accessed May 21, 2026).
- 1012 [36] D. Prasad Mishra, P. Kumar Sahu, B. Acharya, S. Prasad Mishra, S. Bhati, A review of the
1013 synthesis and application of azo dyes and metal complexes for emerging antimicrobial
1014 therapies, *Results in Chemistry* 10 (2024) 101712.
1015 <https://doi.org/10.1016/j.rechem.2024.101712>.
- 1016 [37] M. Azzouzi, S.E. El Hadad, N. Ikken, S. Fait, M.M. Ndjogo, M. Chaoui Roqai, M. Timinouni,
1017 R. Daoud, A. Oussaid, Synthesis, Characterization, and Biological Evaluation of Novel Azo-
1018 Based Imidazo[1,2-a]pyridine Derivatives: In Vitro and In Silico Investigations, *ACS Omega*
1019 10 (n.d.) 55842–55867. <https://doi.org/10.1021/acsomega.5c07351>.
- 1020 [38] A. Ghosh, S. Guria, A. Adhikary, Unveiling the diverse roles of azo groups in the
1021 performance of small molecule probes: A review, *Coordination Chemistry Research* 2 (2025)
1022 100009. <https://doi.org/10.1016/j.cocr.2025.100009>.
- 1023 [39] M.S. Ayoup, I. Shawki, H. Abdel-Hamid, D.A. Ghareeb, S. Ashraf, M. El-Atawy, N.S.
1024 Alharbi, J.Y. Al Nawah, M.M.F. Ismail, Antioxidant evaluation of novel 3-Aryl-5-
1025 phenoxy-1,2,4-oxadiazoles as radical scavengers: Synthesis, characterization, and *ADME*
1026 studies, *Results in Chemistry* 16 (2025) 102508.
1027 <https://doi.org/10.1016/j.rechem.2025.102508>.
- 1028 [40] M.M. El-Samoly, E.M. El-Fakharany, Y.A. El-Maradny, A.E. Abdallah, F.A. Taher, N.T.A.
1029 Dawoud, D.R. Lotfy, Novel diazenyl chalcones with a 2-arylidene hydrazineylidene thiazole
1030 moiety: synthesis, anticancer potential, spectroscopic characterisation, and molecular
1031 docking studies, *RSC Adv* 16 (n.d.) 3894–3908. <https://doi.org/10.1039/d5ra07769a>.
- 1032 [41] W.S. Hamama, M.E. Ibrahim, H.H. Zoorob, Synthesis and Biological Evaluation of Some
1033 Novel Isoxazole Derivatives, *Journal of Heterocyclic Chemistry* (2016).
1034 <https://doi.org/10.1002/jhet.2589>.
- 1035 [42] R. Aggarwal, A. Bansal, A. Mittal, Synthesis and antimicrobial activity of 3-(2-thienyl)-4-
1036 arylazo-5-hydroxy-5-trifluoromethyl- Δ^2 -isoxazolines and 3-(2-thienyl)-4-arylazo-5-
1037 trifluoromethylisoxazoles, *Journal of Fluorine Chemistry* 145 (2013) 95–101.
1038 <https://doi.org/10.1016/j.jfluchem.2012.10.005>.
- 1039 [43] V. Kumar, K. Kaur, Fluorinated isoxazolines and isoxazoles: A synthetic perspective, *Journal*
1040 *of Fluorine Chemistry* 180 (2015) 55–97. <https://doi.org/10.1016/j.jfluchem.2015.09.004>.
- 1041 [44] K. Vashisht, P. Sethi, A. Bansal, T. Singh, R. Kumar, H.S. Tuli, S. Saini, Synthesis,
1042 characterization of isoxazole derivatives and evaluation of their antibacterial, antioxidant and
1043 anticancer activity, *Eur J Clin Exp Med* 22 (2024) 376–387.
1044 <https://doi.org/10.15584/ejcem.2024.2.25>.
- 1045 [45] A. Altharawi, B. Kumar Chagaleti, M. A. Alamri, Y. Riadi, T. Aldakhil, A.I.M. Youssef, A.
1046 Oubella, R. A. Haggam, Synthesis, molecular modelling, and evaluation of new mono- and

- 1047 bis-isoxazolines as antibacterial agents: in vitro and in silico analysis, RSC Advances 16
1048 (2026) 26399–26420. <https://doi.org/10.1039/D5RA09902A>.
- 1049 [46] A. Kumar, P. Jhila, M. Brahma, M. Kumar, P. Kumari, J. Singh, D. Kumar, M. Maruthi, A.
1050 Ansari, S. Singh, R. Kataria, V. Kumar, Design, synthesis, and characterization of new *bis*-
1051 Pyrazoles as potential bioactive agents: Integrated *In-vitro* and *In-silico* studies, Journal of
1052 Molecular Structure 1367 (2026) 146153. <https://doi.org/10.1016/j.molstruc.2026.146153>.
- 1053 [47] K. Kaur, V. Kumar, V. Beniwal, V. Kumar, K.R. Aneja, V. Sharma, S. Jaglan, Novel (E)-1-
1054 aryl-2-(3,5-dimethyl-4-(aryldiazenyl)-1H-pyrazol-1-yl)ethanones: solvent-free synthesis and
1055 antimicrobial, antioxidant and UV-mediated DNA damage protective activity studies, Med
1056 Chem Res 24 (2015) 4023–4036. <https://doi.org/10.1007/s00044-015-1452-3>.
- 1057 [48] K. Kaur, V. Kumar, A.K. Sharma, G.K. Gupta, Isoxazoline containing natural products as
1058 anticancer agents: A review, European Journal of Medicinal Chemistry 77 (2014) 121–133.
1059 <https://doi.org/10.1016/j.ejmech.2014.02.063>.
- 1060 [49] P. Pinheiro, L. Franco, T. Montagnoli, C. Fraga, Molecular hybridization: a powerful tool for
1061 multitarget drug discovery, Expert Opinion on Drug Discovery 19 (2024) 1–20.
1062 <https://doi.org/10.1080/17460441.2024.2322990>.
- 1063 [50] H.M. Sampath Kumar, L. Herrmann, S.B. Tsogoeva, Structural hybridization as a facile
1064 approach to new drug candidates, Bioorganic & Medicinal Chemistry Letters 30 (2020)
1065 127514. <https://doi.org/10.1016/j.bmcl.2020.127514>.
- 1066 [51] J.C. Reid, M. Calvin, Some New β -Diketones Containing the Trifluoromethyl Group^{1a}, J.
1067 Am. Chem. Soc. 72 (1950) 2948–2952. <https://doi.org/10.1021/ja01163a038>.
- 1068 [52] V. Kumar, R. Aggarwal, S. Singh, The Reaction of Hydroxylamine with Aryl
1069 Trifluoromethyl- β -diketones: Synthesis of 5-Hydroxy-5-trifluoromethyl- Δ^2 -isoxazolines and
1070 Their Dehydration to 5-Trifluoromethylisoxazoles, Cheminform 37 (2006).
1071 <https://doi.org/10.1002/chin.200644133>.
- 1072 [53] M. Al-Sheikh, H.Y. Medrasi, K. Usef Sadek, R.A. Mekheimer, Synthesis and Spectroscopic
1073 Properties of New Azo Dyes Derived from 3-Ethylthio-5-cyanomethyl-4-phenyl-1,2,4-
1074 triazole, Molecules 19 (2014) 2993–3003. <https://doi.org/10.3390/molecules19032993>.
- 1075 [54] A. Sethukumar, B. Arul Prakasam, Spectral and cyclic voltammetric studies on some
1076 intramolecularly hydrogen bonded arylhydrazones: Crystal and molecular structure of 2-(2-
1077 (3-nitrophenyl)hydrazono)-5,5-dimethylcyclohexane-1,3-dione, Journal of Molecular
1078 Structure 963 (2010) 250–257. <https://doi.org/10.1016/j.molstruc.2009.11.001>.
- 1079 [55] Z. Ma, A.M. Maharromov, I.A. Aliyev, I.N. Aliyeva, M.N. Kopylovich, G.I. Amanullayeva,
1080 K.T. Mahmudov, A.J.L. Pombeiro, New arylhydrazones of β -diketones and their optical and
1081 thermal properties, Journal of Molecular Structure 1019 (2012) 16–20.
1082 <https://doi.org/10.1016/j.molstruc.2012.03.054>.

- 1083 [56] V.M. Muzalevskiy, Z.A. Sizova, M.S. Nechaev, V.G. Nenajdenko, Acid-Switchable
1084 Synthesis of Trifluoromethylated Triazoles and Isoxazoles via Reaction of CF₃-Ynones with
1085 NaN₃: DFT Study of the Reaction Mechanism, *International Journal of Molecular Sciences*
1086 23 (2022) 14522. <https://doi.org/10.3390/ijms232314522>.
- 1087 [57] V. Kumar, R. Aggarwal, S.P. Singh, The reaction of hydroxylamine with aryl
1088 trifluoromethyl- β -diketones: Synthesis of 5-hydroxy-5-trifluoromethyl- Δ^2 -isoxazolines and
1089 their dehydration to 5-trifluoromethylisoxazoles, *Journal of Fluorine Chemistry* 127 (2006)
1090 880–888. <https://doi.org/10.1016/j.jfluchem.2006.03.009>.
- 1091 [58] P. Pattanayak, T. Chatterjee, Synthesis of (4-Trifluoromethyl)isoxazoles through a Tandem
1092 Trifluoromethyloximation/Cyclization/Elimination Reaction of α,β -Unsaturated Carbonyls,
1093 *J. Org. Chem.* 88 (2023) 5420–5430. <https://doi.org/10.1021/acs.joc.2c03053>.
- 1094 [59] R. Hulankova, Methods for Determination of Antimicrobial Activity of Essential Oils In
1095 Vitro—A Review, *Plants* 13 (2024) 2784. <https://doi.org/10.3390/plants13192784>.
- 1096 [60] M. Ratajczak, M.M. Kubicka, D. Kamińska, P. Sawicka, J. Długaszewska, Microbiological
1097 quality of non-sterile pharmaceutical products, *Saudi Pharmaceutical Journal* 23 (2015) 303–
1098 307. <https://doi.org/10.1016/j.jsps.2014.11.015>.
- 1099 [61] H. Ma, F.J. Irudayanathan, W. Jiang, S. Nangia, Simulating Gram-Negative Bacterial Outer
1100 Membrane: A Coarse Grain Model, *J. Phys. Chem. B* 119 (2015) 14668–14682.
1101 <https://doi.org/10.1021/acs.jpcb.5b07122>.
- 1102 [62] E. Henary, S. Casa, T.L. Dost, J.C. Sloop, M. Henary, The Role of Small Molecules
1103 Containing Fluorine Atoms in Medicine and Imaging Applications, *Pharmaceuticals* 17
1104 (2024) 281. <https://doi.org/10.3390/ph17030281>.
- 1105 [63] B.X. Li, K. Yamanaka, X. Xiao, Structure–activity relationship studies of naphthol AS-E and
1106 its derivatives as anticancer agents by inhibiting CREB-mediated gene transcription,
1107 *Bioorganic & Medicinal Chemistry* 20 (2012) 6811–6820.
1108 <https://doi.org/10.1016/j.bmc.2012.09.056>.
- 1109 [64] J. Chen, J. Yang, L. Ma, J. Li, N. Shahzad, C.K. Kim, Structure-antioxidant activity
1110 relationship of methoxy, phenolic hydroxyl, and carboxylic acid groups of phenolic acids,
1111 *Sci Rep* 10 (2020) 2611. <https://doi.org/10.1038/s41598-020-59451-z>.
- 1112 [65] M.B. Hahn, Rapid quantitative analysis of double-stranded plasmid DNA with capillary gel
1113 electrophoresis for applications in quality control and radiation research, *Sci Rep* 15 (2025)
1114 1068. <https://doi.org/10.1038/s41598-025-85132-w>.
- 1115 [66] A. Ünal, B. Eren, E. Eren, Investigation of the azo-hydrazone tautomeric equilibrium in an
1116 azo dye involving the naphthalene moiety by UV–vis spectroscopy and quantum chemistry,
1117 *Journal of Molecular Structure* 1049 (2013) 303–309.
1118 <https://doi.org/10.1016/j.molstruc.2013.06.037>.

- 1119 [67] P.V. Bharatam, O.R. Valanju, A.A. Wani, D.K. Dhaked, Importance of tautomerism in drugs,
1120 Drug Discovery Today 28 (2023) 103494. <https://doi.org/10.1016/j.drudis.2023.103494>.
- 1121 [68] A.M. Köster, M. Leboeuf, D.R. Salahub, Molecular Electrostatic Potentials from Density
1122 Functional Theory, Theoretical and Computational Chemistry (1996).
1123 [https://doi.org/10.1016/S1380-7323\(96\)80042-2](https://doi.org/10.1016/S1380-7323(96)80042-2).
- 1124 [69] G. Demirtaş, N. Dege, H. İçbudak, Ö. Yurdakul, O. Büyükgüngör, Experimental and DFT
1125 Studies on Poly[di- μ 3-acesulfamato-O,O':O';O':O,O-di- μ -acesulfamato-O,O; N-di- μ -aqua-
1126 dicalcium(II)] Complex, J Inorg Organomet Polym 22 (2012) 671–679.
1127 <https://doi.org/10.1007/s10904-012-9679-7>.
- 1128 [70] Fazilath Basha A, F. Liakath Ali Khan, S. Muthu, M. Raja, Computational evaluation on
1129 molecular structure (Monomer, Dimer), RDG, ELF, electronic (HOMO-LUMO, MEP)
1130 properties, and spectroscopic profiling of 8-Quinolinesulfonamide with molecular docking
1131 studies, Computational and Theoretical Chemistry 1198 (2021) 113169.
1132 <https://doi.org/10.1016/j.comptc.2021.113169>.
- 1133 [71] L. Padmaja, C. Ravikumar, D. Sajan, I. Hubert Joe, V.S. Jayakumar, G.R. Pettit, O. Faurskov
1134 Nielsen, Density functional study on the structural conformations and intramolecular charge
1135 transfer from the vibrational spectra of the anticancer drug combretastatin-A2, Journal of
1136 Raman Spectroscopy 40 (2009) 419–428. <https://doi.org/10.1002/jrs.2145>.
- 1137 [72] K. Fukui, T. Yonezawa, H. Shingu, A Molecular Orbital Theory of Reactivity in Aromatic
1138 Hydrocarbons, J. Chem. Phys. 20 (1952) 722–725. <https://doi.org/10.1063/1.1700523>.
- 1139 [73] E.-B.R. M, E.-S. E, K.T. I, Thermal and temperature dependence of electrical conductivity
1140 studies on Zn, Cd and Hg hydrazone complexes., Journal of Thermal Analysis 39 (1993) 65–
1141 74.
- 1142 [74] V. Choudhary, A. Bhatt, D. Dash, N. Sharma, DFT calculations on molecular structures,
1143 HOMO–LUMO study, reactivity descriptors and spectral analyses of newly synthesized
1144 diorganotin(IV) 2-chloridophenylacetohydroxamate complexes, Journal of Computational
1145 Chemistry 40 (2019) 2354–2363. <https://doi.org/10.1002/jcc.26012>.
- 1146 [75] C. de Llano, Ground states of conjugated molecules. XI. Improved treatment of
1147 hydrocarbons, Journal of the American Chemical Society (1969).
1148 <https://doi.org/10.1021/JA01032A001>.
- 1149 [76] N.L. Allinger, J.J. Maul, Sister Mary J. Hickey, Conformational analysis. LXXIV. Phenol
1150 and anisole derivatives, J. Org. Chem. 36 (1971) 2747–2752.
1151 <https://doi.org/10.1021/jo00818a005>.
- 1152 [77] V. Arjunan, S. Thillai Govindaraja, A. Jayapraksh, S. Mohan, Structural, vibrational and
1153 nuclear magnetic resonance investigations of 4-bromoisoquinoline by experimental and
1154 theoretical DFT methods, Spectrochimica Acta Part A: Molecular and Biomolecular
1155 Spectroscopy 107 (2013) 62–71. <https://doi.org/10.1016/j.saa.2013.01.037>.

- 1156 [78] N.S. Babu, D. Jayaprakash, Global and Reactivity Descriptors Studies of Cyanuric Acid
 1157 Tautomers in Different Solvents by using of Density Functional Theory (DFT), International
 1158 Journal of Science and Research (IJSR) 4 (2015) 615–620.
 1159 <https://doi.org/10.21275/19051501>.
- 1160 [79] G. Banuppriya, R. Sribalan, V. Padmini, Synthesis and characterization of curcumin-
 1161 sulfonamide hybrids: Biological evaluation and molecular docking studies, Journal of
 1162 Molecular Structure 1155 (2018) 90–100. <https://doi.org/10.1016/j.molstruc.2017.10.097>.
- 1163 [80] J.C. Reid, M. Calvin, Some New β -Diketones Containing the Trifluoromethyl Group^{1a},
 1164 Journal of the American Chemical Society 72 (1950). <https://doi.org/10.1021/ja01163a038>.
- 1165 [81] A.S. Shijila Rani, S. Babu, A. Anbukumaran, S. Veeramani, V. Ambikapathy, S. Gomathi,
 1166 G. Senthilkumar, Prospective Approaches of *Pseudonocardia alaniniphila* Hydrobionts for
 1167 *Litopenaeus vannamei*, in: D. Dhanasekaran, A. Sankaranarayanan (Eds.), Advances in
 1168 Probiotics, Academic Press, 2021: pp. 327–348. [https://doi.org/10.1016/B978-0-12-822909-](https://doi.org/10.1016/B978-0-12-822909-5.00021-6)
 1169 [5.00021-6](https://doi.org/10.1016/B978-0-12-822909-5.00021-6).
- 1170 [82] J.M. Andrews, Determination of minimum inhibitory concentrations, J Antimicrob
 1171 Chemother 48 (2001) 5–16. https://doi.org/10.1093/jac/48.suppl_1.5.
- 1172 [83] M. Balouiri, M. Sadiki, S.K. Ibnsouda, Methods for *in vitro* evaluating antimicrobial activity:
 1173 A review, Journal of Pharmaceutical Analysis 6 (2016) 71–79.
 1174 <https://doi.org/10.1016/j.jpha.2015.11.005>.
- 1175 [84] V. Bondet, W. Brand-Williams, C. Berset, Kinetics and Mechanisms of Antioxidant Activity
 1176 using the DPPH.Free Radical Method, LWT - Food Science and Technology 30 (1997) 609–
 1177 615. <https://doi.org/10.1006/fstl.1997.0240>.
- 1178 [85] T. Taj, R.R. Kamble, T.M. Gireesh, R.K. Hunnur, S.B. Margankop, One-pot synthesis of
 1179 pyrazoline derivatised carbazoles as antitubercular, anticancer agents, their DNA cleavage
 1180 and antioxidant activities, Eur J Med Chem 46 (2011) 4366–4373.
 1181 <https://doi.org/10.1016/j.ejmech.2011.07.007>.

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