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Design, synthesis and antimicrobial evaluation of novel 1-phenyl/hetaryl-4-arylazo-3,5-bistrifluoromethyl-1*H*-pyrazole derivatives

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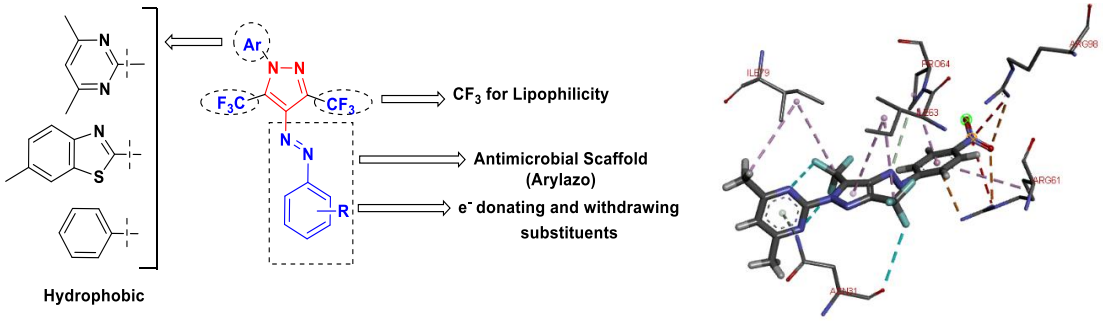
Abstract

3,5-Bistrifluoromethyl-4-arylazo-1H-pyrazoles and 3,5-bistrifluoromethyl-4-arylazo-4,5-dihydro-1H-pyrazoles were designed as potential antimicrobial agents. The synthesis of these pyrazole derivatives were achieved by the reaction of aryl/heteroaryl hydrazines with 1,1,1,5,5,5-hexafluoro-3-arylazopentane-2,4-diones in ethanol at reflux temperature. The synthesized derivatives were evaluated for their antimicrobial potential against one fungal and seven clinical bacterial strains. The results of the antimicrobial screening revealed that nearly all of the bistrifluoromethylpyrazoles have comparable or better antimicrobial potential than the standard drugs amoxicillin and fluconazole. Acute toxicity studies of these compounds have also been carried out on normal mammalian and plant cell lines which revealed that these compounds are safe to normal cells. Molecular docking studies of these compounds with the active site of DNA gyraseB (*S. aureus,* PDB id: 3G75) were also performed which revealed efficient binding interactions with good docking scores.



Keywords: Antimicrobial, pyrazoles, trifluoromethyl, docking studies

Introduction

From the last few decades it has been observed that marketed antibiotic drugs are becoming less and less effective day by day probably due to the overuse of antibiotics and increased antimicrobial drug resistance.[1,2] According to WHO priority list 2018 on antibiotic-resistant bacteria, the bacteria common in the community like *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi* and *Shigella spp.* should be included in studies for long term plans to develop new antibacterial drugs because of their increased antibiotic resistance.[3] Moreover, the fungal pathogens such as *Candida sp.*, one of the leading causes of nosocomial infections, are becoming resistant to available antifungal drugs.[4] Thus, due to these reasons once easily treatable diseases are becoming difficult to cure which in turn is increasing the burden of healthcare. This ultimately is affecting the society in terms of economy and individual's life.[5] Therefore, the development of new strategies are required to address these global challenges due largely to antibiotic resistance in bacteria.[6,7]

The pyrazole moiety is reported to possess various therapeutic applications[8,9,10] including antimicrobial properties. 3,5-Dimethyl-4-arylazopyrazole derivatives **1**, **2** and **3** (Figure 1) are reported as antimicrobial,[11,12,13] antioxidant and antitumor agents.[14] Recently, 3,5-diamino-4-(2'-nitrophenylazo)-1-aryl/heteroarylpyrazole derivatives **4** were studied for their antimicrobial potential by our research group which revealed promising results.[15] The slight structural modifications for achieving new molecular properties in heterocyclic moieties can lead to novel and better therapeutic agents which can be achieved through regulation of physicochemical properties and addition of new functionalities to the core scaffold.[16] Fluorine-containing functionalities when incorporated to heterocyclic scaffold, like pyrazoles, make notable differences from their non-fluorinated counterparts.[17,18]

Trifluoromethyl group, one of the most commonly used fluorine-containing functionality has improved several important properties including lipophilicity, metabolic stability and permeability of the parent compound.[19] Due to these properties trifluoromethyl group is present in many heterocyclic scaffolds of great pharmaceutical and agrochemical utility.[20] For instance 1-pyrimidinyl-3-trifluoromethylpyrazole derivative (**5**) was found to be good anti-inflammatory agent (COX-2 inhibitor)[21] (Figure 1). Trifluoromethylpyrazoles are present as core scaffold in widely used drugs such as Resaxaban (anticoagulant) (**6**).[22]

However, it was observed that examples of bistrifluoromethylpyrazole derivatives are very rare. The compound **7**, a bistrifluoromethylpyrazole, was considered to be tissue-selective androgen receptor modulator (Cytokine production inhibitor)[23] (Figure 1). Bistrifluoromethylpyrazoles are rarely studied for their antimicrobial potential to the best of our knowledge. Moreover, 4-arylazopyrazoles/isooxazoles have been synthesized and explored for their different biological activities in our lab and were found to possess good therapeutic potential.[15,24,25] From the literature studies it was found that trifluoromethyl-4-arylazopyrazoles are important heterocyclic motifs exhibiting high therapeutic potential. Encouraged by the literature reports, and our studies based on trifluoromethyl and arylazopyrazoles, it was envisaged to synthesize novel trifluoromethyl-4-arylazopyrazoles for their antimicrobial studies.

Antibacterial activity of the synthesized derivatives against various antibiotic resistant bacterial strains was also evaluated for assessing their therapeutic potential. Additionally, antifungal activity of these compounds was also evaluated against *Candida albicans* strain. Moreover, an acute toxicity study of the target compounds on normal cells were evaluated by using one mammalian cell line i.e. mouse fibroblast cell line and one plant cell line i.e. plant seed germination cell line.

Design of 3,5-bistrifluoromethyl-4-arylazo-1-aryl/hetarylpyrazoles

While designing novel therapeutic agent, the molecular hybridization works as one of the finest techniques which includes expanding a number of biolabile moieties. It usually comprises the union of different kind of pharmacophoric moieties of comparable effect in a molecule, which brings considerable alteration in the therapeutic potential of the core compound. Keeping in mind these hybridization techniques and taking an inspiration from the ongoing research on hybrid pyrazole molecules having trifluoromethyl and arylazo groups and in continuation to our previous work on fluorinated pyrazoles [21,26] and antimicrobial activity studies,[15,27,28] we have designed new hybrid pyrazole derivatives in search of new antimicrobial agents (Figure 2).

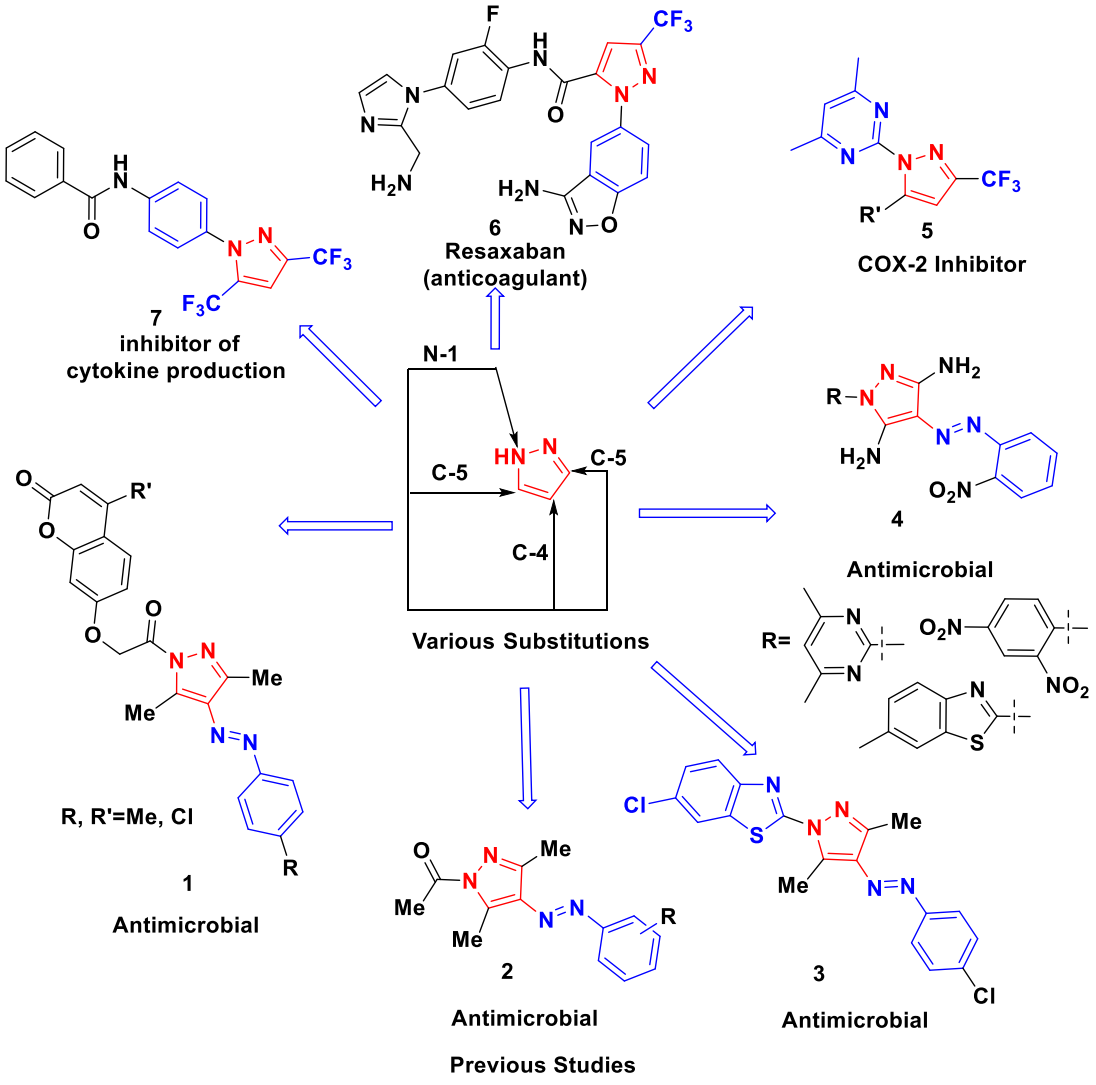


Figure 1. Reported modifications on pyrazole moiety for biological activities.

The structural remodelling involved i) The pyrazole ring as core moiety was retained, ii) trifluoromethyl groups were introduced at C-3 and C-5 position of pyrazole ring with an aim to increase the lipophilicity, efficacy and bioavailablity of the molecules, iii) arylazo group was introduced at position-4 of pyrazole ring for anti-microbial activity, and iv) Phenyl/benzothiazolyl/4,6-dimethylpyrimidinyl rings were introduced at position-1 of the pyrazole nucleus to make hydrophobic interactions in the biological system. We have also explored the effect of electron releasing and electron withdrawing groups on 4-arylazo moiety. The design of

these molecules was also inspired from our recent publication with 3,5-diamino-4-(2'-nitrophenylazo)-1-aryl/heteroarylpyrazoles (**5**) (Figure 1) as antimicrobial agents.[15] So, we have designed these compounds to get the desired molecules having 1-aryl/heteroayl and pyrazole groups along with bioactive moieties into a single scaffold that would efficiently act as promising antimicrobial agents.

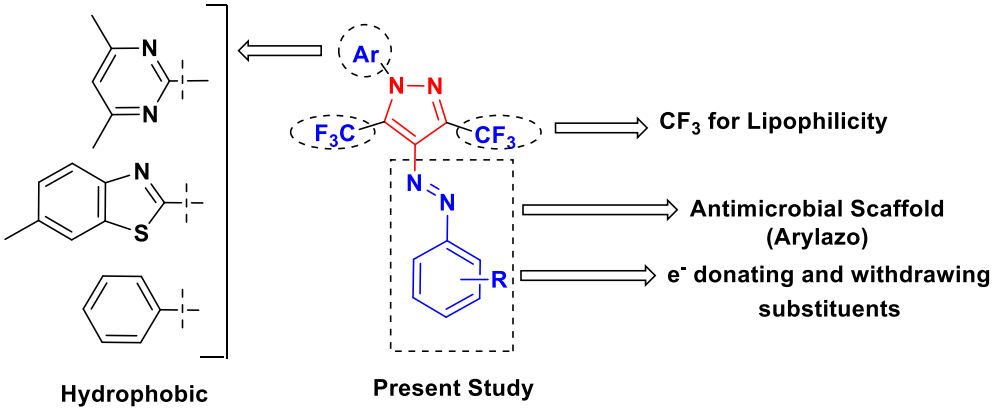
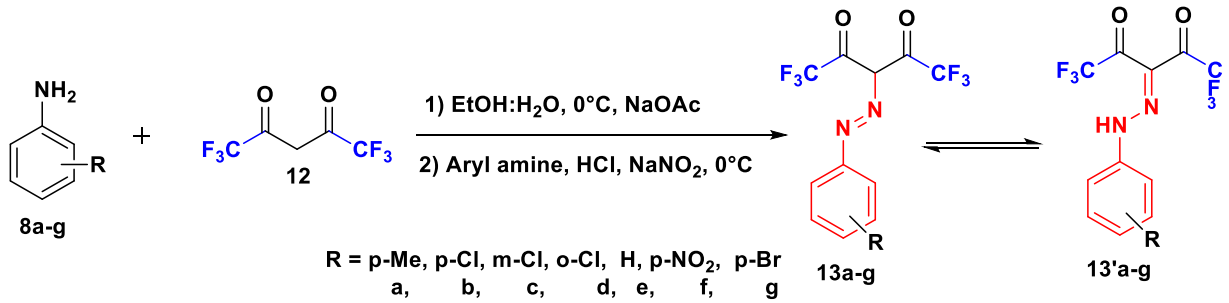


Figure 2. Design of novel pyrazole derivatives.

Results and Discussion

Chemistry

The starting compound 2-hydrazino-4,6-dimethylpyrimidine (**14a**) and 2-hydrazino-6-methylbenzothiazole (**14b**) were prepared following the reported synthetic procedure procedure.[29] Hexafluorinated-1,3-dione **12** and phenyl hydrazine (**14c**) are commercially available. The key intermediates 1,1,1,5,5,5-hexafluoro-3-arylazopentane-2,4-diones **13** were synthesized by condensing aryl diazonium salts (which were in turn generated *in situ* by diazotization of corresponding arylamines **8a-g**) with hexafluorinated-1,3-dione **12** using reported literature methods[25,30] (Scheme 1).

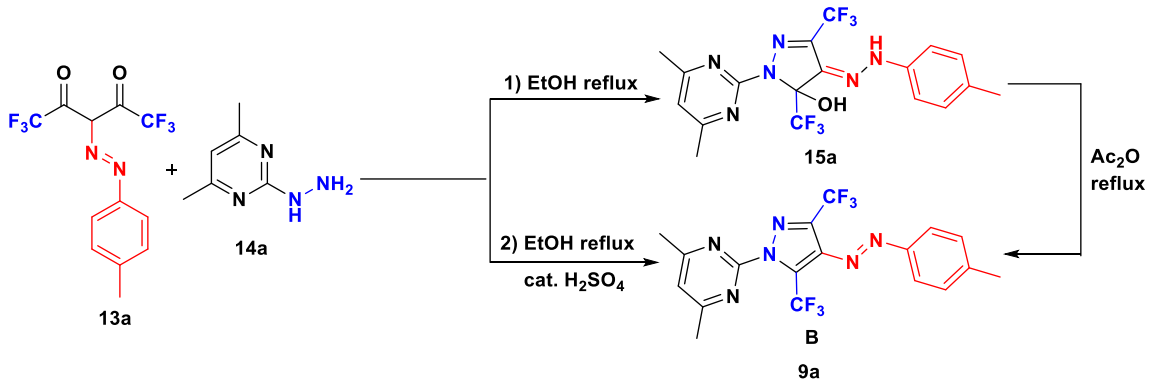


Scheme 1. Synthesis of 1,1,1,5,5,5-hexafluoro-3-arylazopentane-2,4-dione **(13).**

Subsequently, a model substrate reaction of 1,1,1,5,5,5-hexafluoro-3-(4-methylphenylazo)pentane-2,4-dione (**13a**) with 2-hydrazinyl-4,6-dimethylpyrimidine (**14a**) in ethanol at reflux temperature (Scheme 2) was carried out in order to get the 1-(4,6-dimethylpyrimidin-2-yl)-4-(4-methylphenylazo)-3,5-bistrifluoromethylpyrazole (**9a**). The reaction was monitored by checking TLC (ethyl acetate: petroleum ether,

40:60, *v/v* as eluent) at regular intervals. After 3 hours of reflux, it was observed that there is complete consumption of reactants along with formation of a new spot on TLC indicating the completion of reaction.

The solvent was removed in vacuo which resulted in precipitation of the final product formed as a solid. The solid product thus obtained was filtered under suction, dried and recrystallized with ethanol which was later characterized by spectroscopic studies involving IR, ^{1}H NMR, ^{13}C NMR and high resolution mass spectrometry.



Scheme 2. Synthesis of 4,5-dihydro-1*H*-pyrazole **15a** and pyrazole **9a**.

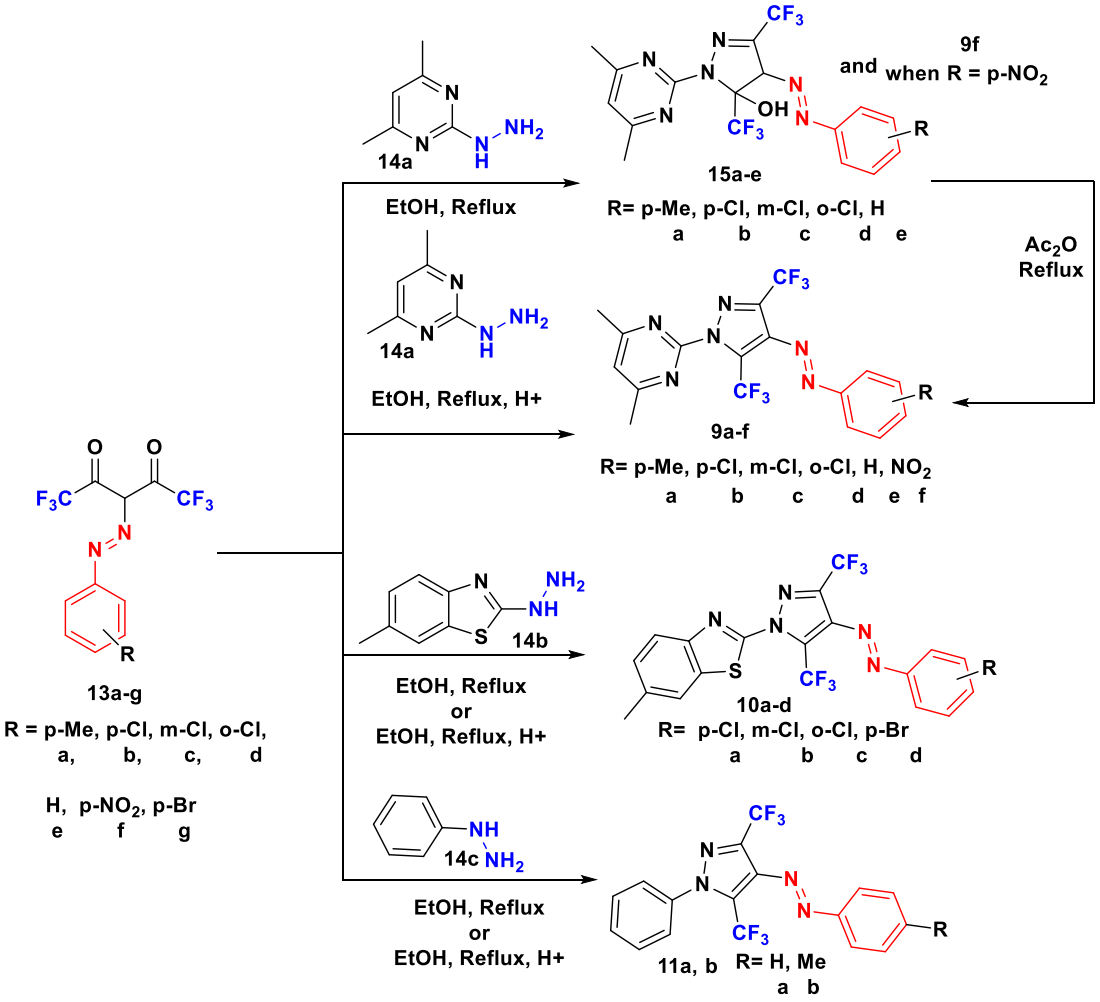
The IR spectrum of the solid product displayed two characterstic broad absorption peaks at 3313 cm^{-1} and 3540 cm^{-1} along with multiple peaks from 1560 cm^{-1} to 1480 cm^{-1} (for C=N & C=C) and a band at 1450 cm^{-1} (for N=N). ^{1}H NMR spectrum of this compound, showed two peaks in the aliphatic region at δ 2.31 and 2.47 ppm having three and six proton intensity, respectively and a singlet of one proton intensity at δ 6.73 ppm corresponding to the 5-H of pyrimidine ring in the aromatic region. Additionally, a regular para substitution pattern of phenyl protons was also observed in aromatic region which confirmed the successful condensation of reactants to give the product. It was interesting to note that two broad singlets at δ 2.61 and 9.31 ppm were also present, corresponding to the protons of OH and NH groups, indicating the product as 1-(4,6-dimethylpyrimidin-2-yl)-4-(4-methylphenylazo)-3,5-bistrifluoromethyl-5-hydroxy-4,5-dihydro-1H-pyrazole (pyrazoline **15a)** not 1-(4,6-dimethylpyrimidin-2-yl)-4-(4-methylphenylazo)-3,5-bistrifluoromethylpyrazole (pyrazole **9a)**. The mass spectra of the product also supported the formation of 5-hydroxy-4,5-dihydro-1H-pyrazole **15a** with a peak at *m/z* = 447.1344, for the molecular ion rather than the peak at *m/z* = 429.1241 for pyrazole **9a**.

The substrate scope of the reaction was explored by performing the reactions with various 1,1,1,5,5,5-hexafluoro-3-arylazopentane-2,4-diones (**13b-e**) by changing electron withdrawing and electron releasing substituents at position-4 of arylazo group under similar reaction conditions to get 5-hydroxy-4,5-dihydro-1H-pyrazoles (**15 b-e**). From the reactions and our earlier studies on the reactions of trifluoromethylpentane-2,4-diones and hydrazines[21,25], it was concluded that under neutral conditions, the presence of CF_3 being a strong EWG, at 3,5-positions of 4,5-dihydro-1H-pyrazole **15a** made it unsusceptible to undergo dehydration.

Taking a lead from the above and our previous observations,[21,25] we have envisaged to explore these reactions under acidic conditions by conducting the reactions in presence of 2 drops of Conc. H_2SO_4. The reaction resulted in exclusive formation of a desired pyrazole **9a** (Scheme 2) instead of 4,5-dihydro-1H-pyrazole **15a** as indicated by the TLC, which was confirmed by various spectroscopic techniques (^{1}H NMR, ^{13}C, ^{19}F NMR and IR spectroscopy and Mass Spectrometery). Moreover, it was observed that dehydration of 4,5-

dihydro-1H-pyrazole **15a** also resulted in formation of corresponding pyrazole **9a** on treatment with acetic anhydride (Scheme 2).

The ^{1}H NMR spectra of compound **9a** displayed a doublet of two proton intensity at δ 7.84-7.86 ppm and a doublet of two proton intensity at δ 7.32-7.34 ppm for phenyl protons. Compound **9a** also exhibited a singlet of one proton intensity at δ 7.20 ppm for pyrimidinyl proton along with two singlets of three and six proton intensity for methyl protons in aliphatic region at δ 2.45 and 2.62 ppm which was consistent to our previous studies.[21] The disappearance of signals for the protons of NH and OH groups confirmed it as dehydrated and fully aromatized pyrazole **9a**. The HRMS spectrum of product showed molecular ion peak at 429.1241 which also supported the product as pyrazole **9a**. Moreover, ^{19}F spectra of this compound displayed two peaks one at δ -61.92 and another at -55.6 ppm for 3-trifluoromethyl and 5-trifluoromethyl groups respectively which is in accordance to the reported literature values of 3 and 5-trifluoromethylpyrazoles.[31]



Scheme 3. Synthesis of title compounds (**9a-f, 10a-d, 11a-b** and **15a-e**).

The reaction of 2-hydrazinyl-4,6-dimethylpyrimidine (**14a**) with corresponding 1,1,1,5,5,5-hexafluoro-3-arylazopentane-2,4-dione (**13a-e)** provided respective 1-(4,6-dimethylpyrimidin-2-yl)-4-arylazo-3,5-bistrifluoromethyl-5-hydroxy-4,5-dihydro-1H-pyrazole (**15 a-e**) under neutral conditions within 3-4 hours. The -4,5-dihydro-1H-pyrazole derivatives (**15 a-e**) thus formed were converted into respective 1-(4,6-dimethylpyrimidin-2-yl)-4-(arylazo)-3,5-bistrifluoromethylpyrazole **9 a-e** derivatives under acidic condition or

by treatment with acetic anhydride (Scheme 3). However, the reaction of 2-hydrazinyl-4,6-dimethylpyrimidine (**14a)** with 1,1,1,5,5,5-hexafluoro-3-(4-nitrophenylazo)pentane-2,4-dione (**13f**) resulted in the formation of corresponding pyrazole derivative (**9f**) but the reaction took 7 hours for complete consumption of reactants as indicated by TLC (Table 1).

Table 1. Optimization of reaction conditions

S. No.	1,1,1,5,5,5-hexafluoro-3-arylazo-pentane-2,4-dione **13**	Hydrazine **14**	Reaction conditions	Time taken (hours)	Product
1	**13 a-e**	**a**	EtOH, reflux	3-4	**15 a-e**
2	**13 f**	**a**	EtOH, reflux	7	**15f**
3	**13 f**	**a**	EtOH,reflux, H^+	3	**15f**
4	**13a-e**	**a**	EtOH, reflux, H^+	2-3	**9a-e**
5	**13 b-d, 13g**	**b**	EtOH, reflux,	8-9	**10 a-d**
6	**13 b-d, 13g**	**b**	EtOH, reflux, H^+	2-3	**10 a-d**
7	**13 b-d, 13g**	**c**	EtOH,reflux	8-9	**11 a-b**
8	**13 a, 13e**	**c**	EtOH,reflux, H^+	3-4	**11 a-b**

Scope and efficiency of the protocol was also explored by condensing 1,1,1,5,5,5-hexafluoro-3-arylazopentane-2,4-dione (**13**) with appropriate 6'-methylbenzothiazolyl or phenyl hydrazine (**14 b** or **14c**). In case of benzothiazolyl and phenyl hydrazine (**14b** and **14c**) reaction also resulted in the formation of corresponding pyrazole derivatives (**10a-d** and **11a-b**) (Scheme 3). However, it was observed that under neutral conditions reaction took longer time for completion (8-9 hours) as compared to acid catalyzed conditions (3-4 hours).

Molecular docking study

DNA gyrase, is an enzyme present in bacteria. It is structurally similar to topoisomerase II, essential for DNA replication and protein synthesis. It acts on the twists and writhes of a covalently closed DNA which makes it a potential target to for antibacterial agents. It contains two subunits of each GyrA and GyrB. Antibacterial agents usually produce their antibacterial effect by inhibition of DNA gyrase. Most of the antibacterial compounds bind with either GyrA subunit or block the ATP-binding site of GyrB subunit. The compounds which can inhibit ATP-ase activity of the DNA gyraseB can be potential therapeutic agents as antibacterials. We have evaluated the docking score and type of interactions between the compounds **9a-f, 10a-d, 11a,b** and **15a-e** and *S. aureus* DNA gyraseB (PDB code 3G75). The results obtained revealed that hexafluoropyrazole derivatives have promising binding potential with active DNA gyrase enzyme (Table S2, and see Supplementary Figure S1-S17). The binding free energy and types of possible interactions of our docked compounds as well as the interacting amino acid residues are represented in Table S2. In molecular modelling studies, 5-hydroxyhexafluoro-4,5-dihydro-1H-pyrazole derivatives core of **15a-e** demonstrated Hydrogen bonding, π-alkyl, π-carbon, π-cation, halogen and unfavourable attractive charge interactions with corresponding amino acid residues like Asn35, Ile67, Arg61, Arg65, Glu39, Glu35, Gly62, Ile78 and Pro68 of DNA gyrase (Figure S13-S17). CF_3-group at position-3 and 5 of hexafluoropyrazole scaffold in most of the compounds showed hydrogen bond interaction with one of the Asn31, Ile63, Glu35 and Thr127. However, compounds **9e**, **9f**, **10a** and **10c** showed no H-bond interactions. Further, aryl/heteroaryl rings at position-1

and 4 of most of the compounds **9a-f, 10a-d** and **11a,b** showed alkyl/π-alkyl interactions with Pro64, Ile63, Ile79, Ile28, Ile129, and Val56. Compound **9f** showed unfavourable ++ interactions and attractive charge interactions by NO_2 group with Arg61 and Arg98 (Figure S6 and S7). Compound **9c** showed amide π-stacked interactions from π electrons of pyrimidine ring with Asn31. Compounds **9e, 10a, 10b** and **10c** displayed best docking score with -8.3, -8.3, -8.1 and -8.3 kcal/mol binding energies, respectively, marginally better than for other compounds and significantly higher than amoxicillin.

Pyrazole derivative **9e** have made pi-cation/pi-anion interactions with Arg61 and Arg98 through N/O of 4-nitrophenyl group, which might played significant role in its better dock score. Benzothiazolyl derivatives have made interactions through 6'-methyl and benzothiazol ring for its increased dock score. It can be inferred that all the benzothiazolyl derivatives have better dock score than phenyl and pyrimidinyl derivatives. It is also interesting to note here that almost all the pyrazole derivatives have higher dock score than the average dock score of -4,5-dihydro-1H-pyrazole derivatives (-6.84 kcal/mol) and hence can be better antimicrobial agents.

ADMET properties

Total polar surface area (TPSA) and Lipinski's rule of five

The *in silico* studies of TPSA, Log S and Lipinski's rule of five can help in predicting the potential of drug molecule. Most of the well-known drugs generally have a log S value of greater than -4. The compounds which have low solubility are not well absorbed by the body and therefore cannot act as good drugs.

The data from *in silico* studies helps to assess the pharmacokinetic properties such as solubility, lipophilicity, and TPSA which are critical for drug development. Table S3 summarizes the data of solubility, total polar surface area (TPSA), and calculated Lipinski's rule of five for the synthesized compounds *viz.* **9a-f**, **10a-d**, **11a-b** and **15a-e**. The Log S value (solubility) ranges from -7.92 for compound 10d to -5.36 for compound 15e, indicating that the compounds with more negative values have lower solubility. A high value of TPSA suggests high polarity of the compound. The calculated TPSA values of compounds varies from 42.54 for compounds 11a and 11b to 114.14 for compound 9e. The molecular weights of these compounds ranges from 384 for compound 11a to 534 for compound 10d. Further, the calculated lipophilicity (cLog P) is found to be highest for compound 10d (cLog P =6.96) and lowest for compound 15a (cLog P 3.94), suggesting the solubility of compounds in fats versus water. Number of hydrogen bond acceptors (nHBA) in these compounds ranges from 9 to 13, while the number of hydrogen bond donors (nHBD) is 0 to 2 which influences the ability of these compounds to engage in hydrogen bonding. The value of rotatable bonds (RB) for these compounds is in between 5 to 6, which affects the flexibility of these compounds. The number of violations (nVio) to Lipinski's rule of five for these compounds is consistently 0 which indicates that all these compounds comply with this rule. Overall toxicity of a compounds depends upon its structural fragments.

s, and bioavailability score of the compounds **9a-f**, **10a-d**, **11a-b** and **15a-e**. It is also evident from the data that all these compounds have no risk related to mutagenicity, tumorigenicity, irritancy, or reproductive effects. Further, all the compounds have druglikeness which suggest that these compounds have potential to be drug. The bioavailability score data of the compounds **9a-f**, **10a-d**, **11a-b** and **15a-e** have revealed that all these compounds has a consistent bioavailability score of 0.55, which indicate that these have a uniform potential for absorption and effectiveness in the body. It can be concluded that all these compounds have equal potential for bioavailability and can be considered safe and viable for further drug developments.

Biological Activity

Antimicrobial evaluation

The antimicrobial activity of compounds (**9a-f, 10a-d, 11a,b** and **15a-e**) was evaluated *in vitro* according to Clinical and Laboratory Standard Institute (CLSI) guidelines using seven clinical bacterial strains: *Pseudomonas argunosa (ATCC 15442), Bacillus cereus (ATCC 11770), Escherichia coli (MTCC 143)*, *Listeria monocytogenes (MTCC 657)*, *Salmonella typhi (MTCC 733)*, *Staphylococcus aureus (ATCC 6538P)*, and *Shigella flexneri (ATCC 9199)* and one fungal strain *Candida albicans (MTCC 183)*. The minimum inhibitory concentration of synthesized compounds (**9a-f, 10a-d, 11a,b** and **15a-e**) corresponding to antimicrobial activity ranged from 31.25 µg/ml to 3.125 µg/ml and results have been presented in Table S5. Antimicrobial potential of the compounds was compared with amoxycillin, a routinely used standard antibiotic, and fluconazole, a well-established antifungal drug.

Interestingly, most of the pyrimidinyl derivatives **9a-f** demonstrated good to excellent antibacterial activity (indicated by green entries), but anifungal results were not found so much significant. All these derivatives **9a-f**, were found to be good antibacterial agents equipotent to standard drug Amoxicillin against *E. coli, L. monocytogenes* and *S. flexneri* with low MIC values of 3.12 µg/ml. Out of all the tested derivatives, only compound **9b** have shown equipotent activity against *P. aeruginosa* as that of standard drug. Compounds **9d**, **9e** demonstrated commendable activity against *S. aureus* and *S. typhi*. Except **9e** all compounds were found to exhibit good potency against *B. cereus.*

Among benzothiazolylpyrazole derivative **10a-d**, the compounds **10b** and **10d** were found to be the less active against tested bacterial strains with higher MIC values than the standard drug but compound **10d** compounds displayed equi potent antifungal activity with MIC value equal to the standard drug fluconazole.

Compound **10a** showed excellent activity against *L. monocytogenes* and *B. cereus* while compound **10c** were found to show potent activity against *P. argunosa* and *S. typhi* bacterial species.

Among phenyl derivatives **11a,b** compound **11a** showed poor antibacterial activity but it was found to be best antifungal agent, while Compound **11b** was found to be active against *S. typhi, L. monocytogenes, B. cereus* and *S. flexneri* bacterial strains and inactive against fungal strain *C. albicans*.

3,5-Bistrifluoromethyl-4-arylazo-4,5-dihydro-1H-pyrazoles (**15a-e**) were found less active against all the tested bacterial strains and fungal strain *C. albicans* than standard drug. All the -4,5-dihydro-1H-pyrazole derivatives (**15a-e**) showed notable antibacterial activity against *B. cereus* and *S. aureus* with MIC value of 6.25 µg/ml. The derivative **15b** showed against *E.coli*, **15c** showed against *L. monocytogenes, S. flexneri* and **15d** showed against *S.typhi* good antibacterial activity.

Structure activity relationship

Structure-activity relationship analysis revealed that the incorporation of a 2,4-dimethylpyrimidine ring enhanced antibacterial activity, whereas the inclusion of a 6-methylbenzothiazole ring and a phenyl ring at position-1 of the pyrazole ring reduced antibacterial potency. Conversely, the introduction of 2,4-dimethylpyrimidine ring reduced the antifungal activity while presence of 6'-methylbenzothiazole ring and phenyl ring enhanced this activity. In the case of 1-benzothiazolyl-3,5-bistrifluoromethylpyrazoles **10a-d**, the addition of a 4-bromophenylazo group at position-4 of the pyrazole ring improved antifungal efficacy. Similarly, for 1-phenyl-3,5-bistrifluoromethylpyrazoles **(11a-b)**, the incorporation of a 4-methylphenylazo group at the same position was favourable for antifungal activity. Furthermore, it was observed that all the -4,5-dihydro-1H-pyrazole derivatives **15a-e** exhibit lower potency compared to the corresponding pyrazole derivatives and the standard reference drugs.

Acute Toxicity studies:

Acute toxicity study is a type of preclinical investigation that evaluates the immediate toxicological effects of a substance following a single or short term exposure typically 24 hours.[32] It helps in drug development,

chemical safety assessment to determine the potential toxicity of a substance when first exposed to an organism.

Compounds **9a-f, 10a-d, 11a,b** and **15a-e** at very high concentration of 1mg/ml were screened for their acute toxicity on normal cell line including one mammalian cell line (mouse fibroblast cell line) and one plant cell line (plant seed germination cell line) using MTT assay. The results of the acute toxicity studies are given in Table S6. From the acute toxicity results it was observed that all the compounds showed 90-100% cell viability which indicated that compounds have minimal or no cytotoxic effect on normal cells. These results are highly favourable for their safety profile, especially if the compounds are intended for pharmaceutical or therapeutic applications.

Conclusions

The present study deals with design, synthesis, characterization and biological evaluation of bistrifluoromethyl-4-arylazopyrazole derivatives **9a-f, 10a-d, 11a,b** and bistrifluoromethyl-4-arylazo-4,5-dihydro-1*H*-pyrazole **15a-e** as antimicrobial agents. Docking studies revealed that the pyrazole derivative with benzothiazole (**10a-c**) ring at position-1 are promising interactions to be drug molecules. ADMET studies revealed that all the compounds can be considered safe and capable for future drug development. Almost all the bistrifluoromethyl-4-arylazopyrazole/-4,5-dihydro-1*H*-pyrazole derivatives have displayed promising antimicrobial activity results with comparable or better MIC values than the standard drugs amoxicillin and fluconazole used for respective activities. The compound **9d** was identified as broad-spectrum antibacterial agents. Compound **11a** was found to be selective antifungal agent. None of the compounds was found toxic to normal mammalian (Mouse Fibfroblast cell) and plant cell (Plant seed germination) lines.

Experimental Section

General. Melting point of the compounds were determined in open capillaries with an digital melting point apparatus (MEPA) and are uncorrected, the IR spectra of the compounds were recorded on Buck Scientific IR M-500 spectrophotometer using KBr pellets (ν_{max} in cm^{-1}), ^{1}H and ^{13}C NMR spectra on a Bruker instrument at 400 and 100 MHz, respectively, using deuteriochloroform as a solvent. Chemical shifts are expressed in δ-scale downfield from TMS as an internal standard. ^{19}F NMR spectra were run on DPX 400 at 376 MHz, using deuteriochloroform as a solvent. The internal standard for ^{19}F spectra was fluorotrichloromethane, setting the $CFCl_3$ signal at δ 0.0. Mass analyses were performed at Sophisticated Analytical Instrument Facility, Panjab University, Chandigarh, India.

General synthetic procedure for 1-(4,6-dimethylpyrimidin-2-yl)-4-(4-methylphenylazo)-3,5-bistrifluoromethyl-5-hydroxy-4,5-dihydro-1*H*-pyrazole 15a

An ethanolic mixture of 1,1,1,5,5,5-hexafluoro-3-(4-methylphenylazo)pentane-2,4-dione **13a** (0.001 mol) 2-hydrazino-4,6-dimethylpyrimidine **14a** (0.001 mol) was refluxed on water bath. TLC of the reaction revealed complete consumption of reactants on 6-7 hr heating the reaction mixture at *reflux* temperature. The solvent was removed in vacuo and solid thus obtained was filtered, dried and recrystallized with ethanol to get final product bistrifluoromethyl-4,5-dihydro-1H-pyrazole **15a**.

Compounds **15b-e** were also synthesized using the same procedure with respective starting materials as reactants.

General synthetic procedure for 1-Phenyl/benzothiazolyl/4,6-dimethylpyrimidin-3,5-bistriflurormethyl-4-arylazo-1*H*-pyrazoles 9a-f, 10a-d and 11a-b.

An ethanolic mixture of 1,1,1,5,5,5-hexafluoro-3-arylazopentane-2,4-dione **13** (0.001 mol) and corresponding aryl/hetarylhydrazines (**14a-c**) (0.001 mol) in presence of catalytic amount of sulphuric acid was refluxed on water bath for 3-4 hr. Solvent was removed in vacuo and the solid thus obtained was filtered, dried and recrystallized with ethanol to get the corresponding bistrifluoromethylpyrazoles (**9**/**10/11).**

1-(4,6-dimethylpyrimidin-2-yl)-4-(4-methylphenylazo)-3,5-bistrifluoromethyl-5-hydroxy-4,5-dihydro-1*H*-pyrazole (15a). Mp 118-120 °C, Yield 78%, IR (KBr, cm^{-1}): 3570, 3223, 2152, 1670, 1648, 1610; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.33 (s, 1H, N-H); 7.13-7.16 (d, 2H, *J* = 8.8Hz, 2'',6''-H); 7.06-7.09 (d, 2H, *J* = 8.8Hz, 3'',5''-H); 6.75 (s, 1H, 5'-H); 2.63 (s, 1H, O-H); 2.49 (s, 6H, 4', 6'-CH_3); 2.33 (s, 3H, 4''-CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): 157.81, 139.74, 133.25, 130.04126.79, 115.19, 114.22, 24.18, 20.89; MS (EI) *m/z*: calcd. for $C_{18}H_{16}F_6N_6O$: 447.1290 $[M+1]^+$; found: 447.1344; $[M+1]^+$; Elemental analysis: Calcd. for $C_{18}H_{16}F_6N_6O$: C, 48.44; H, 3.61; N, 18.83% Found: C, 48.37; H, 3.56; N, 18.80%.

4-(4-Chlorophenylazo)-1-(4,6-dimethylpyrimidin-2-yl)-5-hydroxy-3,5-bistrifluoromethyl-4,5-dihydro-1*H*-pyrazole (15b). Mp 120-124 °C, Yield 80%, IR (KBr, cm^{-1}): 3563, 3213, 2151, 1668, 1642, 1603;^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.335 (s, 1H, N-H); 7.304 (d, 2H, *J* = 8.4Hz, 2'',6''-H); 7.111 (d, 2H, *J* = 8.4Hz, 3'',5''-H); 6.770 (s, 1H, 5'-H); 2.486 (s, 6H, 4', 6'-CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): 168.45, 157.73, 140.69, 129.57, 128.50,128.37, 124.64, 120.74, 118.04, 115.39, 115.36, 24.03; MS (EI) *m/z*: calcd. for $C_{17}H_{13}ClF_6N_6O$: 467.0477 $[M+1]^+$; found: 467.0829; $[M+1]^+$; 469.0804; $[M+1+2]^+$; Elemental analysis: Calcd. for $C_{17}H_{13}ClF_6N_6O$: C, 43.74; H, 2.81; N, 18.00% Found: C, 43.63; H, 2.70; N, 17.92%.

4-(3-Chlorophenylazo)-1-(4,6-dimethylpyrimidin-2-yl)-5-hydroxy-3,5-bistrifluoromethyl-4,5-dihydro-1*H*-pyrazole (15c). Mp 123-126 °C, Yield 80%, IR (KBr, cm^{-1}): 3558, 3218, 2159, 1663, 1640, 1605;^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.906 (s, 1H, N-H); 7.546 (d, 1H, *J* = 8.4Hz, 6''-H); 7.319 (d, 1H, *J* = 8.0Hz, 4''-H); 7.288 (d, 1H, *J* = 8.0Hz, 2''-H); 6.966 (t, 1H, *J* = 7.6Hz, 5''-H) 6.76 (s, 1H, 5'-H); 2.614 (s, 1H, O-H); 2.479 (s, 6H, 4', 6'-CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): 170.039, 168.485, 157.723, 138.350, 130.037, 129.428, 128.241, 123.568, 119.544, , 115.425, 114.942, 24.096; MS (EI) *m/z*: calcd. for $C_{17}H_{13}ClF_6N_6O$: 467.0477 $[M+1]^+$; found: 467.0837; $[M+1]^+$; 469.0812; $[M+1+2]^+$; Elemental analysis: Calcd. for $C_{17}H_{13}ClF_6N_6O$: C, 43.74; H, 2.81; N, 18.00% Found: C, 43.61; H, 2.68; N, 17.93%.

4-(2-Chlorophenylazo)-1-(4,6-dimethylpyrimidin-2-yl)-5-hydroxy-3,5-bistrifluoromethyl-4,5-dihydro-1*H*-pyrazole (15d). Mp 144-146 °C, Yield 77%, IR (KBr, cm^{-1}): 3553, 3212, 2154, 1658, 1646, 1609; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.33 (s, 1H, N-H); 7.281 (m, 1H, 4''-H); 7.185 (t, 1H, *J* = 2.0Hz, 4''-H); 7.01-7.05 (m, 2H, *J* = 8.0Hz, 3'', 5'' -H); 6.77 (s, 1H, 5'-H); 2.49 (s, 6H, 4', 6'-CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): 157.70, 143.20, 135.48, 130.59, 128.92, 120.697, 117.999, 115.42, 114.36, 112.42, 24.11; MS (EI) *m/z*: calcd. for $C_{17}H_{13}ClF_6N_6O$: 467.0477 $[M+1]^+$; found: 467.0831; $[M+1]^+$; 469.0806; $[M+1+2]^+$; Elemental analysis: Calcd. for $C_{17}H_{13}ClF_6N_6O$: C, 43.74; H, 2.81; N, 18.00% Found: C, 43.63; H, 2.70; N, 17.92%.

1-(4,6-Dimethylpyrimidin-2-yl)-4-(phenylazo)-5-hydroxy-3,5-bistrifluoromethyl-4,5-dihydro-1*H*-pyrazole (15e). Mp 104-106 °C, Yield 85%, IR (KBr, cm^{-1}): 3555, 3216, 2157, 1660, 1641, 1611;^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.36 (s, 1H, N-H); 7.37-7.32 (m, 2H, 2'',6''-H); 7.19-7.16 (m, 2H, 3'',5''-H); 7.08-7.04 (m, 1H, 4''H); 6.76 (s, 1H, 5'-H); 2.48 (s, 6H, 4', 6'-CH_3); 1.61 (s, 1H, O-H); ^{13}C NMR (100 MHz, $CDCl_3$): 157.77, 141.95, 129.49, 127.49, 123.50, 115.16, 114.18, 24.03; MS (EI) *m/z*: calcd. for $C_{17}H_{14}F_6N_6O$: 451.1133 $[M+H_3O]^+$; found: 451.0754; $[M+H_3O]^+$; Elemental analysis: Calcd. for $C_{17}H_{14}F_6N_6O$: C, 47.23; H, 3.26; N, 19.44% Found: C, 47.18; H, 3.12; N, 19.38%.

1-(4,6-Dimethylpyrimidinyl)-4-(4-methylphenylazo)-3,5-bistrifluoromethyl-1*H*-pyrazole (9a). Mp 125-127 °C, Yield 85%, IR (KBr, cm^{-1}): 2155, 1664, 1638, 1613; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.84-7.86 (d, 2H, *J* = 8Hz, 2'',6''-H); 7.53-7.55 (d, 2H, *J* = 8Hz, 3'',5''-H); 7.20 (s, 1H, 5'-H); 2.62 (s, 6H, 4', 6'-CH_3); 2.45 (s, 3H, 4''-CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): 169.96, 143.55, 129.93, 123.45, 120.88, 23.92, 21.76; ^{19}F NMR (376 MHz, $CDCl_3$): -61.92, -55.60; MS (EI) *m/z*: calcd. for $C_{18}H_{14}F_6N_6$: 429.1184 $[M+1]^+$; found: 429.1241 $[M+1]^+$; Elemental analysis: Calcd. for $C_{18}H_{14}F_6N_6$: C, 50.47; H, 3.29; N, 19.62% Found: C, 50.32; H, 3.16; N, 19.45%.

1-(4,6-Dimethylpyrimidinyl)-4-(4-chlorophenylazo)-3,5-bistrifluoromethyl-1*H*-pyrazole (9b). Mp 153-155 °C, Yield 87%, IR (KBr, cm^{-1}): 2152, 1667, 1637, 1618;; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.88-7.90 (d, 2H, *J* = 8Hz, 2'',6''-H); 7.50-7.52 (d, 2H, *J* = 8Hz, 3'',5''-H); 7.21 (s, 1H, 5'-H); 2.63 (s, 6H, 4', 6'-CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): 177.87, 173.47, 170.03, 129.61, 124.63, 121.01, 23.92; ^{19}F NMR (376 MHz, $CDCl_3$): -62.09, -55.61; MS (EI) *m/z*: calcd. for $C_{17}H_{11}ClF_6N_6$: 449.0638 $[M+1]^+$; found: 449.0723 $[M+1]^+$, 451.0696 $[M+1+2]^+$; Elemental analysis: Calcd. for $C_{17}H_{11}ClF_6N_6$: C, 45.50; H, 2.47; N, 18.73% Found: C, 45.34; H, 2.31; N, 18.56%.

1-(4,6-Dimethylpyrimidinyl)-4-(3-chlorophenylazo)-3,5-bistrifluoromethyl-1*H*-pyrazole (9c). Mp 145-149 °C, Yield 84%, IR (KBr, cm^{-1}): 2159, 1658, 1642, 1619; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.90-7.91 (s, 1H, 4''-H); 7.84-7.87 (s, 1H, 5''-H); 7.46-7.53 (m, 2H, 2'',6''-H); 7.22 (s, 1H, 5'-H); 2.63 (s, 6H, 4', 6'-CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): 170.05, 153.27, 135.41, 132.49, 130.36, 122.61, 122.46, 121.05, 23.91; ^{19}F NMR (376 MHz, $CDCl_3$): -62.14, -55.60 MS (EI) *m/z*: calcd. for $C_{17}H_{11}ClF_6N_6$: 449.0638 $[M+1]^+$; found: 449.0731 $[M+1]^+$, 451.0708 $[M+1+2]^+$; Elemental analysis: Calcd. for $C_{17}H_{11}ClF_6N_6$: C, 45.50; H, 2.47; N, 18.73% Found: C, 45.31; H, 2.32; N, 18.61%.

1-(4,6-Dimethylpyrimidinyl)-4-(2-chlorophenylazo)-3,5-bistrifluoromethyl-1*H*-pyrazole (9d). Mp 147-151 °C, Yield 82%, IR (KBr, cm^{-1}): 2156, 1654, 1639, 1609; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.72-7.74 (dd, 1H, J1 = 8 Hz, J2 = 1.6 Hz, 6''-H); 7.58-7.60 (dd, 1H, J_1 = 8 Hz, J_2 = 1.2 Hz, 3''-H); 7.43-7.47 (td, 1H, J_1 = 8 Hz, J_2 = 1.6 Hz, 4''-H); 7.33-7.37 (td, 1H, J_1 = 8 Hz, J_2 = 1.2 Hz, 5''-H); 7.21 (s, 1H, 5'-H); 2.63 (s, 6H, 4', 6'-CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): 170.03, 155.73, 148.95, 137.12, 133.34, 131.19, 127.28, 121.03, 117.39, 23.90; ^{19}F NMR (376 MHz, $CDCl_3$): -62.33, -55.70; MS (EI) *m/z*: calcd. for $C_{17}H_{11}ClF_6N_6$: 449.0638 $[M+1]^+$; found: 449.0615 $[M+1]^+$, 451.0626 $[M+1+2]^+$; Elemental analysis: Calcd. for $C_{17}H_{11}ClF_6N_6$: C, 45.50; H, 2.47; N, 18.73% Found: C, 45.30; H, 2.29; N, 18.62%.

1-(4,6-Dimethylpyrimidinyl)-4-(4-nitrophenylazo)-3,5-bistrifluoromethyl-1*H*-pyrazole (9e). Mp 166-170 °C, Yield 87%, IR (KBr, cm^{-1}): 2159, 1658, 1556, 1642, 1619, 1352; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.40-8.42 (d, 2H, *J* = 8Hz, 2'',6''-H); 8.06-8.08 (d, 2H, *J* = 8Hz, 3'',5''-H); 7.24 (s, 1H, 5'-H); 2.64 (s, 6H, 4', 6'-CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): 170.15, 155.31, 152.86, 149.65, 124.90, 123.98, 121.22, 23.92; ^{19}F NMR (376 MHz, $CDCl_3$): -62.65, -55.87; MS (EI) *m/z*: calcd. for $C_{17}H_{11}F_6N_7O_2$: 460.3124 $[M+1]^+$; found: 460.981 $[M+1]^+$; Elemental analysis: Calcd. for $C_{17}H_{11}F_6N_7O_2$: C, 44.45; H, 2.41; N, 21.35% Found: C, 44.34; H, 2.28; N, 21.16%.

1-(4,6-Dimethylpyrimidinyl)-4-phenylazo-3,5-bistrifluoromethyl-1*H*-pyrazole (9f). Mp 108-112 °C, Yield 90%, IR (KBr, cm^{-1}): 2157, 1655, 1628, 1600;^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.94-7.97 (m, 2H, 2'',6''-H); 7.53-7.55 (m, 3H, 3'',4'',5''-H); 7.21 (s, 1H, 5'-H); 2.63 (s, 6H, 4', 6'-CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): 169.92, 152.48, 132.55, 129.21,123.34, 120.89, 23.85; ^{19}F NMR (376 MHz, $CDCl_3$): -62.01, -55.60; MS (EI) *m/z*: calcd. for $C_{17}H_{12}F_6N_6$: 415.1028 $[M+1]^+$; found: 415.1019 $[M+1]^+$; Elemental analysis: Calcd. for $C_{17}H_{12}F_6N_6$: C, 49.28; H, 2.92; N, 20.28% Found: C, 49.15; H, 2.81; N, 20.04%.

1-(6-Methylbenzothiazol-2-yl)-4-(4-chlorophenylazo)-3,5-bis(trifluoromethyl)-1*H*-pyrazole (**10a**). Mp 208-210 °C, Yield 77%, IR (KBr, cm^{-1}): 1656, 1573, 1446, 1233, 1135; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.94–7.96 (d, 1H, *J* = 8.0 Hz, 4'-H); 7.89-7.91 (d, 2H, *J* = 8.0 Hz, 3'',5''-H); 7.70 (s, 1H, 7'-H); 7.51-7.53 (d, 2H, *J*=8.0 Hz, 2'',6''-H); 7.37-7.39 (d, 1H, *J*=8.0 Hz, 5'-H); 2.53 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): 150.75, 148.2, 139.0, 137.1, 134.3, 129.6, 128.7, 124.6, 123.7, 121.2, 21.7; MS (EI) *m/z*: : calcd for $C_{19}H_{10}ClF_6N_5S$: 490.0250 $[M+1]^+$; found:

490.0239, $[M+1]^+$, 492.0234, $[M+1+2]^+$ (3:1); Elemental analysis: Calcd. for $C_{19}H_{10}ClF_6N_5S$: C, 46.59; H, 2.06; N, 14.30% Found: C, 46.12; H, 1.65; N, 13.73%.

1-(6'-Methylbenzothiazol-2'-yl-4-(3''-chlorophenylazo)-3,5-bistrifluoromethyl-1*H*-pyrazole (10b). Mp 152-154 °C, Yield 75%, IR (KBr, cm^{-1}): 1653, 1570, 1443, 1235, 1134; ^1H NMR (400 MHz, $CDCl_3$) δ: 7.94–7.96 (d, 1H, *J* = 8.0 Hz, 4'-H); 7.90-7.91 (m, 1H, 6''-H); 7.85-7.88 (m, 1H, 3''-H); 7.70-7.71 (t, 1H, *J* = 0.8 Hz, 7'-H); 7.48-7.55 (m, 2H, 2'',4''-H); 7.37-7.40 (m, 1H, 5'-H); 2.53 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): 153.1, 148.3, 137.1, 135.4, 134.4, 132.5, 130.5, 128.7, 123.8, 122.6, 122.5, 121.3, 21.7; MS (EI) *m/z*: calcd for $C_{19}H_{10}ClF_6N_5S$: 490.0250 $[M+1]^+$; found: 490.0239, $[M+1]^+$, 492.0234, $[M+1+2]^+$ (3:1); Elemental analysis: Calcd. for $C_{19}H_{10}ClF_6N_5S$: C, 46.59; H, 2.06; N, 14.30% Found: C, 46.21; H, 1.71; N, 13.90%.

1-(6'-Methylbenzothiazol-2'-yl-4-(2''-chlorophenylazo)-3,5-bistrifluoromethyl-1*H*-pyrazole (10c). Mp 159-161 °C, Yield 73%, IR (KBr, cm^{-1}): 1655, 1580, 1456, 1250, 1134; ^1H NMR (400 MHz, $CDCl_3$) δ: 7.59–7.61 (d, 1H, *J*=8.0 Hz, 4'-H); 7.54-7.56 (m, 2H, 4'',5''-H); 7.33-7.36 (m, 1H, 7'-H); 7.28-7.32 (m, 1H, 6''-H); 7.22-7.25 (m, 1H, 3''-H); 6.96-7.03 (m, 1H, 5'-H); 2.45 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): 161.7, 147.4, 137.9, 134.4, 130.4, 129.4, 129.0, 128.2, 128.0, 124.2, 123.9, 121.3, 120.5, 119.7, 115.0, 21.4; MS (EI) *m/z*: calcd for $C_{19}H_{10}ClF_6N_5S$: 489.0250; found: 491.0239 $[M+1]^+$, $[M+1]^+$, 492.0234, $[M+1+2]^+$ (3:1); Elemental analysis: Calcd. for $C_{19}H_{10}ClF_6N_5S$: C, 46.59; H, 2.06; N, 14.30% Found: C, 46.23; H, 1.77; N, 13.84%.

1-(6'-Methylbenzothiazol-2'-yl)-4-(4''-bromophenylazo)-3,5-bistrifluoromethy-1*H*-pyrazole (10d). Mp 224-226 °C, Yield 77%, IR (KBr, cm^{-1}): 1659, 1575, 1445, 1236, 1134; ^1H NMR (400 MHz, $CDCl_3$) δ: 7.94–7.96 (d, 1H, *J*=8.0 Hz, 4'-H); 7.81-7.83 (d, 2H, *J*=8.0 Hz, 3'',5''-H); 7.69-7.70 (m, 3H, *J*=8.0 Hz, 7',2'',6''-H); 7.37-7.39 (d, 1H, *J*=8.0 Hz, 5'-H); 2.53 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): 151.1, 148.2, 137.1, 134.3, 132.6, 128.7, 127.7, 124.8, 123.7, 121.2, 21.7; MS (EI) *m/z*: calcd for $C_{19}H_{10}BrF_6N_5S$: 535.9724 $[M+1]^+$; Found: 535.9708 [M+1]; 537.9699, $[M+1+2]^+$ (1:1); Elemental analysis: Calcd. for $C_{19}H_{10}BrF_6N_5S$: C, 42.71; H, 1.89; N, 13.11% Found: C, 42.32; H, 1.51; N, 12.77%.

1-Phenyl-4-phenylazo-3,5-bistrifluoromethyl-1*H*-pyrazole (**11a)**. Mp 107-109 °C, Yield 81%, IR (KBr, cm^{-1}): 1650, 1565, 1438, 1220; ^1H NMR (400 MHz, $CDCl_3$) δ: 7.59-7.62 (m, 3H, 2',6',4'-H), 7.34–7.37 (m, 2H, 2'',6''-H), 7.19-7.24 (m, 4H, 3',5',3'',5''-H), 7.00–7.03 (m, 1H, 4''-H); ^{13}C NMR (100 MHz, $CDCl_3$): 152.8, 142.8, 138.9, 130.6, 130.1, 129.7, 129.5, 129.2, 129.1, 127.1, 126.5, 125.9, 123.1; MS (EI) *m/z*: calcd for $C_{17}H_{10}F_6N_4$: 385.0810 $[M+1]^+$; Found: 385.0770 $[M+1]^+$; Elemental analysis: Calcd. for $C_{17}H_{10}F_6N_4$: C, 53.13; H, 2.62; N, 14.585% Found: C, 52.81; H, 2.29; N, 13.87%.

1-Phenyl-4-(4-methylphenylazo)-3,5-bistrifluoromethyl-1*H*-pyrazole (**11b)**. Mp 124-126 °C, Yield 75%, IR (KBr, cm^{-1}): 1696, 1589, 1389, 1227, 1134; ^1H NMR (400 MHz, $CDCl_3$) δ: 7.82-7.84 (d, 2H, *J* = 8.0 Hz, 3'',5''-H); 7.51-7.56 (m, 5H, 2',3',4',5',6'-H); 7.31-7.33 (d, 2H, *J* = 8.0 Hz, 2'',6''-H); 2.44 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): 150.82, 143.43, 138.65, 134.25, 130.05, 129.95, 129.48, 129.45, 129.43, 126.0, 123.36, 123.33, 123.30, 21.7; MS (EI) *m/z*: calcd for $C_{18}H_{12}F_6N_4$: 400.0966 $[M+1]^+$; found: 399.0954 $[M+1]^+$; Elemental analysis: Calcd. for $C_{18}H_{12}F_6N_4$: C, 54.28; H, 3.04; F, N, 14.07% Found: C, 53.91; H, 2.89; N, 13.69%.

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Supplementary Material

All the spectral data is available in the supplementary material file of this manuscript.

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