A Platinum Open Access Journal for Organic Chemistry

Paper

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Pyrazole-tethered isoxazoles: hypervalent iodine-mediated, metal-free synthesis and biological evaluation

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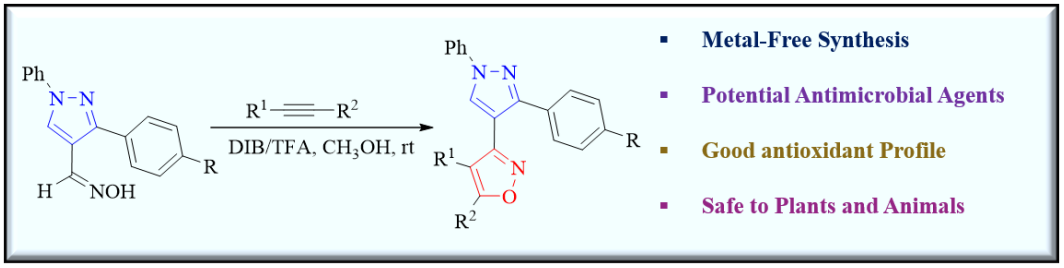
Dedicated to Alan R. Katritzky and Charles W. Rees

Received mm-dd-yyyy **Accepted** mm-dd-yyyy **Published on line** mm-dd-yyyy

to be inserted by editorial office

Abstract

In the present work, a hypervalent iodine-mediated, metal-free route for the synthesis of a series of di- and tri-substituted pyrazole-tethered isoxazole derivatives has been utilized *via* [3+2]-cycloaddition reaction of nitrile oxides with alkynes. All the synthesized isoxazole derivatives were characterized by FTIR, ^{1}H NMR, ^{13}C NMR, and HRMS data. The structure of one of the products, ethyl-3-(1,3-diphenyl-1*H*-pyrazol-4-yl)-5-phenylisoxazole-4-carboxylate, was confirmed by X-ray analysis. All synthesized compounds were screened for antimicrobial activity and compared to the standard drug, Amoxicillin. Some of the compounds exhibited antibacterial activity comparable to or higher than Amoxicillin. Moreover, the synthesized compounds exhibited moderate to excellent antioxidant activity. The cytotoxicity of all products was investigated against the mouse fibroblast (animal) and plant seed germination cell line (*Vigna radiata*).



Keywords: Pyrazole-tethered isoxazoles, metal-free, hypervalent iodine, single crystal x-ray, biological activity

Introduction

Heterocyclic compounds are indispensable scaffolds in organic and pharmaceutical chemistry because of their wide pharmaceutical and synthetic applications.[1,2] Among them, pyrazoles and isoxazoles have been identified as important pharmacophores of many bioactive molecules such as cloxacillin, leflunomide, ibotenic acid, lonazolac, etc (Figure 1).[3-8] Pyrazoles, isoxazoles and their derivatives exhibited numerous metabolic and biological activities such as antimalarial, antimicrobial, antiviral, anti-inflammatory, antioxidant, anticancer and HIV inhibitory activities etc.[9-20] The presence of two electronegative heteroatoms significantly enhances biological activities because heteroatoms can make hydrogen donor–acceptor (D-A) interactions with various enzymes and receptors.[21] Moreover, the isoxazole derivatives can be transformed into numerous significant synthetic units, namely: β-hydroxy ketones, β-hydroxy nitriles, α,β-unsaturated oximes, and β-dicarbonyl compounds, etc.[22] During past years, various methodologies such as cycloaddition, cycloisomerization, condensation and functionalizations have been explored for the synthesis of isoxazoles using homogeneous and heterogeneous catalysts.[23-25] Among them, [3+2] cycloaddition of the alkynes with nitrile oxides is one of the most widely used methods.[26-28] Generally, nitrile oxides are produced by the dehydration of nitro compounds, dehydrohalogenation of hydroxyiminoyl chloride and oxidative dehydrogenation of aldoximes.[28-32] Disubstituted isoxazoles have also been synthesized using Ru(II) and Cu(I) as catalyst.[28] However, due to the toxic nature of metal complexes, a metal-free approach is beneficial in this field. In this regard, hypervalent iodine reagents were found as efficient reagents for the generation of nitrile oxides under metal-free conditions.[33]

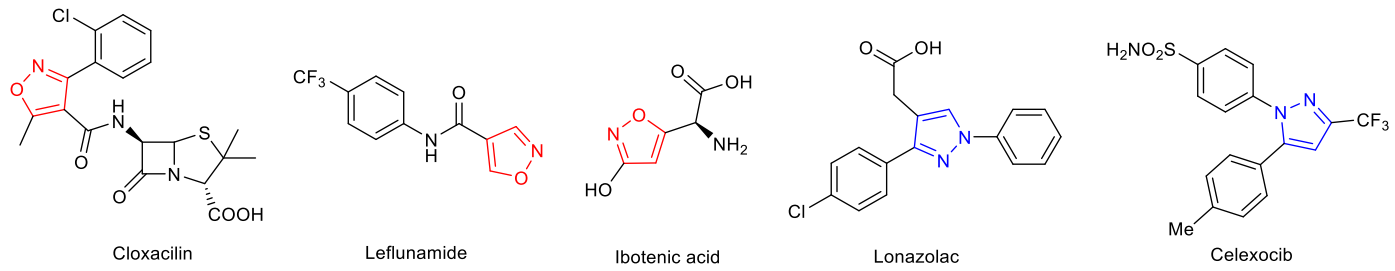


Figure 1. Structures of some useful drugs containing isoxazole/pyrazole ring

Hypervalent iodine (HVI) reagents are readily available, eco-friendly and mild oxidizing reagents. Owing to these characteristic properties and similar reactivity patterns with heavy metal oxidants such as, Pb(IV), Hg(II) and Tl(III), HVI reagents are superior alternatives to the heavy metal oxidants.[34,35] HVI reagents have been extensively explored in synthetic chemistry for various organic transformations.[36,37] The literature reports illustrate that isoxazoles can be efficiently synthesized by the reaction of oxime with alkyne using various HVIs such as hydroxy(tosyloxy)iodobenzene (HTIB), bis(trifluoroacetoxy)iodobenzene (BTI), diacetoxyiodobenzene (DIB) and dichloroiodobenzene, as stoichiometric reagents.[38-43] Besides, the hypervalent iodine-mediated synthesis of heterocyclic moieties, isoxazoles have also been reported by using a catalytic amount of HVI reagents in the presence of terminal oxidant.[40-43] The reaction of HVI reagents with aldoxime generates nitrile oxide which undergoes [3+2] cycloaddition reaction with alkyne to form the corresponding product. In a strategy developed by Peddinti *et al*, di- and tri-substituted isoxazole derivatives were synthesized from aldoxime and alkyne by employing diacetoxyiodobenzene.[44] In another report by Mukhtar *et al*, some coumarin- and flavone-based isoxazole derivatives can be accessed *via* a one-pot reaction of aryl benzaldehyde, hydroxylamine hydrochloride and *o*-propargyl coumarin/flavones. The coumarin- and flavone-based isoxazole derivative were

found as efficient antimicrobial agents.[45] Moreover, pyrrolo-isoxazoles have been synthesized using 2-iodobenzoic acid in the presence of *m*-CPBA and triflic acid.[46] In addition to hypervalent iodine reagents, the synthesis of isoxazoles has also been achieved by using *tert*-butyl hypoiodite and sodium iodide.[47] Motivated by biological activities associated with pyrazole and isoxazole moieties coupled with our ongoing interest in the field of HVI reagents, a series of pyrazole-tethered isoxazole derivatives **1a-g** and **2a-f** have been synthesized from easily available starting materials by the use of diacetoxyiodobenzene under metal-free conditions.

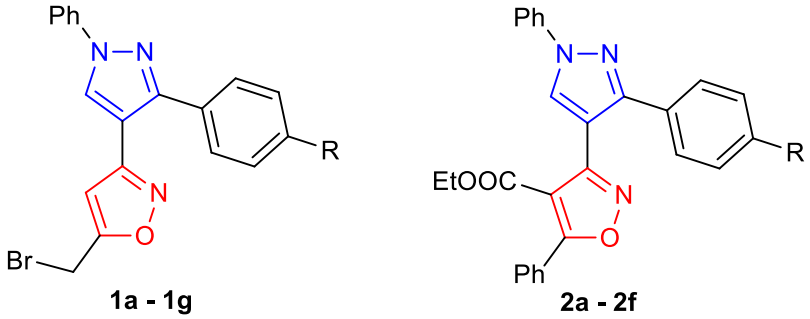
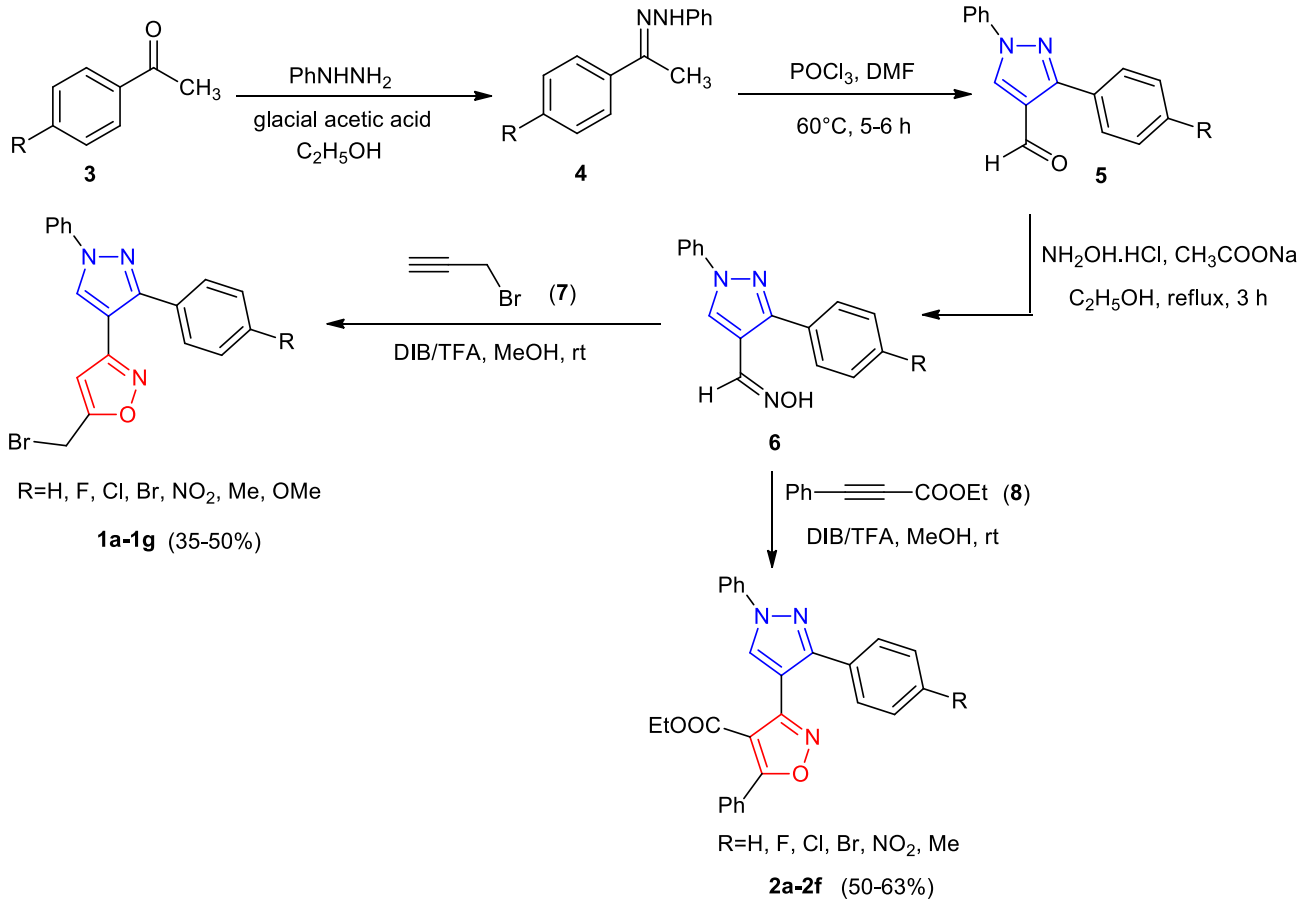


Figure 2. Structures of newly synthesized pyrazole-tethered isoxazoles **1a-g** and **2a-f**

Results and Discussion

Chemistry

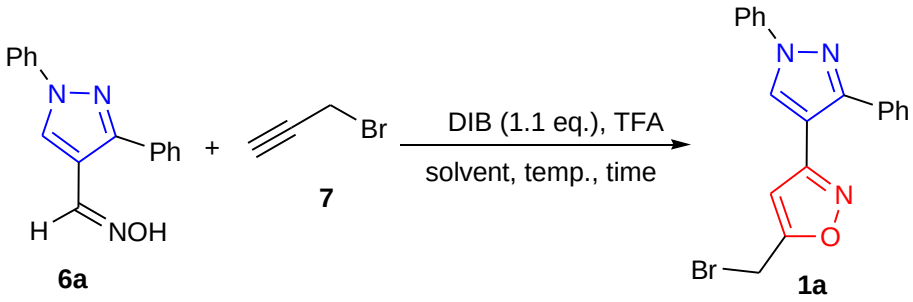
The synthesis of the title compounds **1a-g** and **2a-f** is outlined in Scheme 1. Structures of these compounds were interpreted based on their spectral data viz.; FTIR, ^{1}H NMR, ^{13}C NMR and HRMS. 1-Phenyl-3-aryl-4-formylpyrazoles **5** and their corresponding oximes **6** needed for the synthesis of compounds **1a-g** and **2a-f** were synthesized by the reported procedure.[48,49] Acetophenone phenylhydrazone **4** was synthesized by the reaction of phenylhydrazine with appropriate acetophenone **3** using glacial acetic acid as a catalyst. The resulting hydrazone **4** on Vilsmeier-Haack reaction afforded corresponding 1-phenyl-3-aryl-4-formylpyrazoles **5** in 70-80% yield. The thus obtained formylpyrazole **5** was further refluxed with hydroxylamine hydrochloride ($NH_2OH{\cdot}HCl$) and sodium acetate (CH_3COONa) in ethanol for 3 h that afforded the oximes in 60-75% yield. To optimize the reaction conditions for synthesizing pyrazole-tethered isoxazoles, 1,3-diphenyl-pyrazolyl aldoxime **6a** and propargyl bromide **7** were selected as model substrates.



Scheme 1. Synthesis of pyrazole-tethered isoxazoles **1** and **2**

The reaction of DIB/TFA with oxime results in the *in situ* generation of nitrile oxide which slowly dimerized to form oxadiazole-*N*-oxide.[50] Therefore, the solution of DIB/TFA was added portionwise, and resulting nitrile oxide was captured by propargyl bromide to form the resulting isoxazole. In a preliminary attempt, a solution of oxime **6a** and propargyl bromide (**7**) in acetonitrile/water (2:1) was treated with diacetoxyiodobenzene (DIB) which resulted in a complex mixture and reaction didn't complete even after 12 h of the reaction (Table 1, entry 1 and 2). The addition of a catalytic amount of trifluoroacetic acid (TFA) gave the corresponding pyrazole-tethered isoxazole **1a** in 22% yield (Table 1, entry 3). The addition of TFA to the reaction mixture may lead to the generation of mixed iodonium carboxylates such as PhI(OAc)(OCOCF$_3$).[51] The increase in the concentration of propargyl bromide to 2.5 equivalents gave the desired product in 25% yield (Table 1, entry 4). A range of solvents were screened to improve the yield of desired products, and the best results were obtained with methanol (CH_3OH) affording isoxazole **1a** in 42% yield in 4.5 h (Table 1, entry 6). Further, the effect of temperature was also studied, and any change in the temperature did not improve the yield of the reaction (Table 1, entries 2 and 5). All the optimization experiments are summarized in Table 1. The synthesis of isoxazole was confirmed by ^{1}H NMR, ^{13}C NMR and HRMS data. ^{1}H NMR data of **1a** showed a singlet at δ_H 6.14 due to C-H of isoxazole ring while singlet at δ_H 4.43 confirmed the presence of -CH_2 group. In ^{13}C NMR, peak at δ_C 18.27 appeared due to the Carbon of -CH_2Br. The HRMS data showed *m/z* peaks at 380.0434 and 382.0417 in 1:1 ratio corresponding to $[M+H]^+$ and $[M+H+2]^+$.

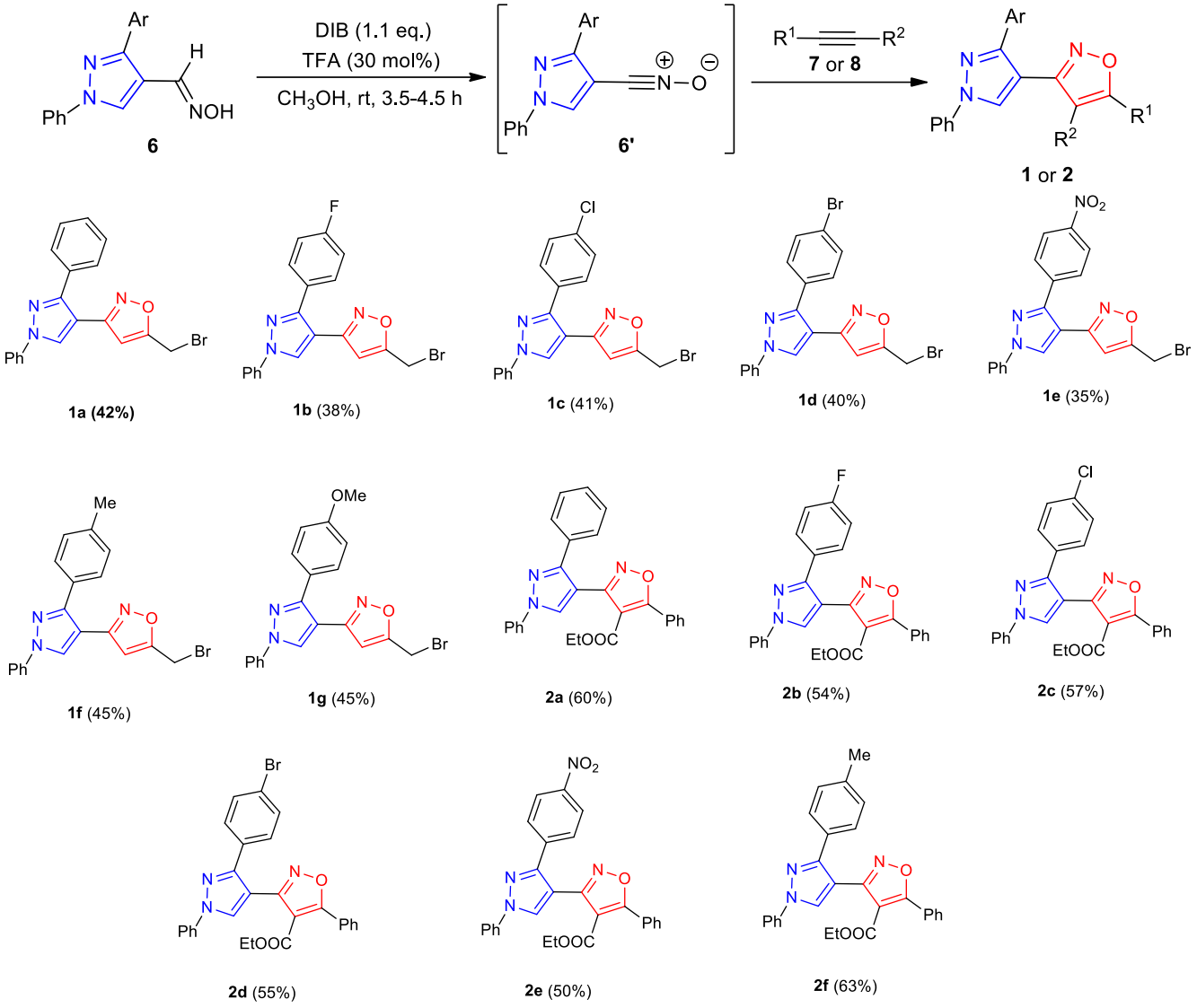
Table 1. Optimization for the synthesis of **1a**



Entry	7 (eq.)	Solvent	TFA (mol%)	Time (h)	Temp. (°C)	Isolated Yield (%)
1	1.5	CH_3CN/H_2O (2:1)	-	12	rt	Complex mixture
2	1.5	CH_3CN/H_2O (2:1)	-	10	60	Mixture
3	2.0	CH_3CN/H_2O (2:1)	30	8	rt	22
4	2.5	CH_3CN/H_2O (2:1)	30	7.5	rt	25
5	2.5	CH_3CN/H_2O (2:1)	30	9	0	Mixture
6	2.5	MeOH	30	4.5	rt	42
7	2.5	EtOH	30	5	rt	35
8	2.5	$MeOH/H_2O$ (5:1)	30	5.5	rt	32

After optimization, the scope of above synthetic approach was extended towards a range of oximes **6** with dipolarophiles **7** and **8** (Table 2). The reaction of propargyl bromide (**7**) was performed with acetophenones **6a-g** having electron-deficient or electron-rich groups, which afforded the corresponding pyrazole-tethered isoxazole derivatives **1a-g** in 35-45% yield. Ethyl phenylpropiolate (**8**) gave the corresponding pyrazole-tethered isoxazole derivatives **2a-f** in 45-63% yield.

Table 2. Substrate scope for [3+2] cycloaddition of nitrile oxide with dipolarophile **7** or **8**



Further, the structure of ethyl-3-(1,3-diphenyl-1*H*-pyrazol-4-yl)-5-phenylisoxazole-4-carboxylate (**2a**) was also confirmed by single-crystal XRD data, and the ORTEP view of compound **2a** is given in Figure 3. Details of XRD data for **2a** are provided in supplementary information as Figure S1.

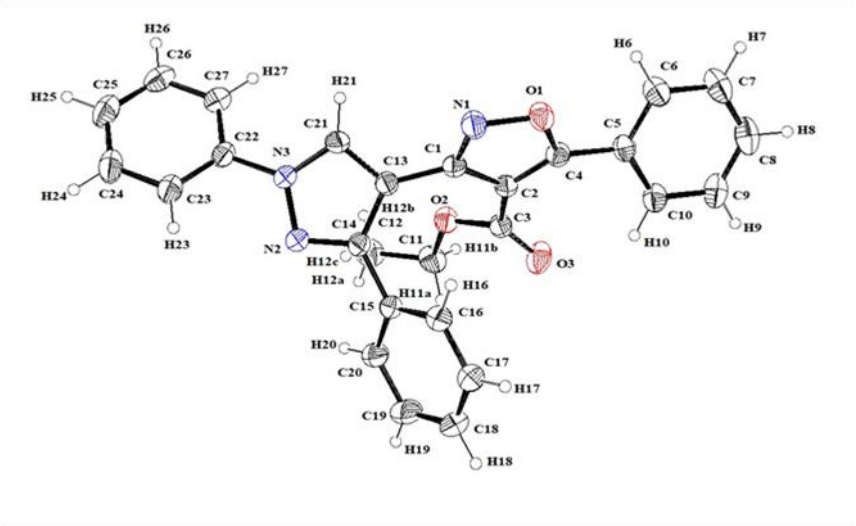


Figure 3. ORTEP view of ethyl-3-(1,3-diphenyl-1*H*-pyrazol-4-yl)-5-phenylisoxazole-4-carboxylate (**2a**) (CCDC No. 2343634)

Biology/Pharmacology

Antimicrobial Activity

The antimicrobial activity of compounds **1a-g** and **2a-f** was screened against different bacterial and fungal strains (Table 3). It was observed that compounds **1a-g** and **2a-f** have excellent antibacterial activities. The target compounds **1a**-**c** showed either equivalent (8.2 □M) or better (7.5-7.8 □M) anti-microbial activity in comparison with the commercial drug, Amoxicillin. It was observed that the compounds with *p*-fluoro and *p*-chloro substituents **1b-c** and **2b-c** in the aryl ring at C-3 position of pyrazole moiety possessed higher antibacterial potential. The synthesized compounds **1** and **2** were better inhibitors against *Candida albicans MTCC 183.* Compounds **1d**, **2b** and **2c** showed approximately three-fold better inhibition (MIC value 6.6-6.9 □M) against *Candida albicans* (fungal strain) (Table 3).

Table 3. MIC values of compounds **1a–g** and **2a–f** against tested microbes

Compounds	Minimum Inhibitory Concentration (μM)[a]							
	Gram-negative bacterial strain				Gram-positive bacterial strain			Fungal strain
	P. aeruginosa	*E. coli*	*S. typhi*	*S. flexneri*	*S. aureus*	*L. monocytogenes*	*B. cereus*	*C. albicans*
1a	8.2	8.2	8.2	8.2	8.2	8.2	8.2	8.2
1b	7.8	7.8	7.8	7.8	7.8	7.8	7.8	7.8
1c	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5
1d	13.6	13.6	13.6	13.6	13.6	13.6	13.6	6.8
1e	14.7	14.7	14.7	14.7	14.7	14.7	14.7	14.7
1f	15.8	15.8	15.8	15.8	7.9	7.9	7.9	7.9
1g	7.6	7.6	7.6	7.6	7.6	15.2	15.2	7.6
2a	7.2	14.3	14.3	7.2	14.3	14.3	14.3	7.2
2b	6.9	13.8	6.9	6.9	6.9	13.8	13.8	6.9
2c	6.6	13.3	13.3	6.6	6.6	6.6	6.6	6.6
2d	12.1	12.1	12.1	12.1	12.1	12.1	12.1	12.1
2e	6.5	13.0	13.0	6.5	13.0	13.0	6.5	13.0
2f	6.9	13.9	13.9	6.9	13.9	13.9	6.9	13.9
Amoxicillin	8.5	8.5	8.5	8.5	8.5	8.5	8.5	0
Fluconazole	-	-	-	-	-	-	-	20.4

[a] Mean of three replicates

Antioxidant activity

Antioxidants play an indispensable role in quenching free radicals as they can donate electrons and prevent the damage caused by free radicals.[52] The antioxidant activity of **1a-g** and **2a-f** is presented in Table 4. These results illustrated that the synthesized compounds **1** and **2** exhibited moderate to excellent antioxidant activity.

Table 4. Antioxidant profile of compounds **1a-g** and **2a-f**

Compounds	% RSA	Compounds	% RSA
1a	83.2	**2a**	88.23
1b	76.92	**2b**	85.2
1c	89.12	**2c**	89.34
1d	89.32	**2d**	88.19
1e	65.67	**2e**	76.11
1f	88.65	**2f**	83.35
1g	81.34	**Ascorbic acid control**	96.78

RSA: Radical Scavenging activity

Cytotoxic Study

The synthesized compounds **1** and **2** were tested against plant (*Vigna radiata*) and animal (mouse fibroblast) cell lines using DMSO as control (Table 5). Analysis of the results suggested that these compounds **1a-g** and **2a-f** are safer towards the examined cell lines.

Table 5. Cytotoxic study of compounds **1a-g** and **2a-f**

Compounds	Mouse Fibroblast cell % Cell Viability	Plant seed germination	Compounds	Mouse Fibroblast cell % Cell Viability	Plant seed germination
1a	93.24	100	**2a**	92.32	100
1b	91.56	100	**2b**	93.24	100
1c	94.11	100	**2c**	89.31	100
1d	94.54	100	**2d**	95.27	100
1e	94.33	100	**2e**	94.1	100
1f	93.98	100	**2f**	93.34	100
1g	96.21	100	**DMSO Control**	23.12	70

Conclusions

In this study, a metal-free route is demonstrated to access a series of di- and tri-substituted pyrazole-tethered isoxazoles **1a-g** and **2a-f** *via* [3+2] cycloaddition of *in-situ* generated 1,3-dipolar species (nitrile oxide) and a dipolarophile (alkyne). The targeted compounds were successfully synthesized in 35-63% yield. Further, all synthesized compounds were screened for their biological activity towards gram-positive and gram-negative bacterial strains. The biological investigation of compounds **1** and **2** against various microbial strains indicated that these compounds can be quite valuable from the pharmacological point of view. Among these compounds, products **1a**-**c** possessed excellent activity against the tested gram-positive and gram-negative bacterial strains. Moreover, isoxazoles **1a-g** and **2a-f** exhibited higher antifungal activity as compared to the standard drug, Fluconazole. Further, screening of the antioxidant profile of synthesized compounds **1** and **2** revealed that these compounds exhibited good to excellent antioxidant activity with % RSA up to 89.34%.

Experimental Section

General: All commercial chemicals were used as received without any further purification. Phenylhydrazine, phosphorus oxychloride ($POCl_3$), trifluoroacetic acid, hydroxylamine hydrochloride, sodium acetate, acetophenone and its derivatives were obtained from Central Drug House (CDH). Propargyl bromide, ethyl phenylpropiolate, diacetoxyiodobenzene (DIB), ascorbic acid and DPPH were purchased from Sigma Aldrich. The purity was checked by TLC using iodine and UV chamber as visualizing agents. The preparative TLC plates were purchased from Sigma Aldrich (Merck). The IR spectra were recorded on Perkin Elmer FT-IR Spectrometer (UTAR Two) at CIL, J. C. Bose UST YMCA, Faridabad. ^{1}H and ^{13}C NMR spectral data were recorded either on Bruker Advance Neo 300 or 500 MHz NMR spectrometers from THSTI, Faridabad or SAIF, Panjab University (P.U.).

Chandigarh. Tetramethylsilane (TMS) was used as an internal standard for NMR data. The high-resolution mass spectral data (HRMS) was recorded on Maldi-TOF Synapt XS HD Mass Spectrometer at SAIF, P.U. Chandigarh. Chemical shifts (δ) are expressed in δ (ppm). Abbreviations 's' for singlet, 'd' for doublet, 'dd' for doublet of doublet, 't' for triplet and 'm' for multiplet are used for NMR assignments. Oxford-Rigaku Analytical X-ray Supernova system coupled with a four-axis KAPA goniometer module with a HyPix3000 detector with a micro-focus Molybdenum (Mo) source was used for recording single crystal X-ray data under nitrogen atmosphere at the Department of Chemistry, P. U. Chandigarh. The biological assays for the antimicrobial, anti-oxidant activity as well as for the cytotoxic study are provided in supplementary information as S2.

General procedure for the synthesis of pyrazole-tethered isoxazoles 1 and 2

Synthesis of acetophenone phenylhydrazones 4: Acetophenone phenylhydrazones were synthesized by the literature-reported procedure.[53] The melting points of acetophenone phenylhydrazones **4** matched with reported values.

1-Phenyl-3-aryl-4-formylpyrazoles 5.[48] 1-Phenyl-3-aryl-4-formylpyrazoles were synthesized by the reported procedure. The melting point of all 4-formylpyrazoles was matched with the literature values.

1-Phenyl-3-aryl-pyrazolyl aldoximes 6.[49] 1-Phenyl-3-aryl-pyrazolyl aldoximes **6** were synthesized by the reported procedure. The IR and melting point of these compounds agreed with those reported in the literature.

General procedure for the synthesis of 1-phenyl-3-aryl-pyrazolyl isoxazole 1 and 2. To a solution 1-phenyl-3-aryl-pyrazolyl aldoxime (**6**, 1 mmol) and alkyne **7**/**8** (2.5 mmol) in 2 mL methanol, a solution of DIB (1.1 mmol) in methanol (1.5 mL) along with 2 drops of TFA were added portion-wise over 40 min. The resulting reaction mixture was stirred at rt (room temperature) till the completion of the reaction (4-5 h) as checked by TLC. After the completion of the reaction, the methanol was evaporated and the residue thus obtained was purified by chromatographic separation to get pure pyrazole-tethered isoxazoles **1** and **2**.

5-(Bromomethyl)-3-(1,3-diphenyl-1*H*-pyrazol-4-yl)isoxazole (1a). Colorless solid; 42% yield; mp 130-132 °C; IR (υ, cm^{-1}): 3022, 2843, 1599, 1571, 1464; ^{1}H NMR ($CDCl_3$, 500 MHz) δ (ppm): 8.40 (s, 1H, pyrazole ring H), 7.79 (d, 2H, *J* 7.8 Hz, ArH), 7.65 (dd, 2H, *J* 7.6, 2.2 Hz, ArH), 7.51-7.44 (m, 5H, ArH), 7.35 (t, *J* 7.6 Hz, 1H, ArH), 6.14 (s, 1H, isoxazole ring H), 4.43 (s, 2H, -CH_2Br); ^{13}C NMR ($CDCl_3$, 126 MHz) δ (ppm): 167.3, 156.6, 151.8, 139.5, 132.3, 129.6, 129.4, 128.8, 128.5, 127.6, 127.2, 119.3, 110.3, 103.5, 18.5; HRMS (ESI) *m/z* calculated for $C_{19}H_{14}BrN_3O$ $[M+H]^+/[M+2+H]^+$ 380.0398/382.0378, found 380.0434/382.0417.

5-(Bromomethyl)-3-[3-(4-fluorophenyl)-1-phenyl-1*H*-pyrazol-4-yl]isoxazole (1b). Colorless solid; 38% yield; mp 150-152 °C; IR (υ, cm^{-1}): 2923, 2865, 1607, 1575, 1478; ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 8.38 (s, 1H, pyrazole ring H), 7.78 (d, *J* 7.9 Hz, 2H, ArH), 7.65 (dd, *J* 8.4, 5.5 Hz, 2H, ArH), 7.50 (t, *J* 7.8 Hz, 2H, ArH), 7.35 (t, *J* 7.4 Hz, 1H, ArH), 7.14 (t, *J* 8.5 Hz, 2H, ArH), 6.15 (s, 1H, isoxazole ring H), 4.44 (s, 2H, -CH_2Br); ^{13}C NMR (126 MHz, $CDCl_3$) δ (ppm): 167.5, 163.1 (d, $^1J_{C\text{-}F}$ 248.5 Hz), 156.4, 150.8, 139.4, 130.6 (d, $^3J_{C\text{-}F}$ 8 Hz), 129.6, 128.4 (d, $^4J_{C\text{-}F}$ 3.4 Hz), 127.7, 127.3, 119.3, 115.5 (d, $^2J_{C\text{-}F}$ 21.6 Hz), 110.2, 103.4, 18.4; HRMS (ESI) *m/z* calculated for $C_{19}H_{13}BrFN_3O$ $[M+H]^+/[M+2+H]^+$ 398.0304/400.0284, found 398.0286/400.0275.

5-(Bromomethyl)-3-[3-(4-chlorophenyl)-1-phenyl-1*H*-pyrazol-4-yl]isoxazole (1c). Colorless solid; 41% yield; mp 94-96 °C; IR (υ, cm^{-1}): 2923, 2844, 1599, 1463; ^{1}H NMR ($CDCl_3$, 500 MHz) δ (ppm): 8.37 (s, 1H, pyrazole ring H), 7.79-7.76 (m, 2H, ArH), 7.64-7.61 (m, 2H, ArH), 7.52-7.48 (m, 2H, ArH), 7.44-7.40 (m, 2H, ArH), 7.38-7.34 (m, 1H, ArH), 6.18 (s, 1H, isoxazole ring H), 4.45 (s, 2H, -CH_2Br); ^{13}C NMR ($CDCl_3$, 126 MHz) δ (ppm): 167.5, 156.4, 150.5, 139.4, 134.8, 130.8, 130.1, 129.6, 128.8, 127.9, 127.4, 119.4, 110.3, 103.5, 18.4; HRMS (ESI) *m/z* calculated for $C_{19}H_{13}BrClN_3O$ $[M+H]^+/[M+2+H]^+/[M+4+H]^+$ 414.0009/415.9998/417.9886, found 414.0006/415.9986/417.9971.

5-(Bromomethyl)-3-[3-(4-bromophenyl)-1-phenyl-1*H*-pyrazol-4-yl]isoxazole (1d). Colorless solid; 40% yield; mp 120-122 °C; IR (υ, cm^{-1}): 2923, 2852, 1609, 1582, 1463; ^{1}H NMR (500 MHz, $CDCl_3$) *δ* (ppm): 8.36 (s, 1H, pyrazole ring H), 7.78 (dd, 2H, *J* 8.8, 1.3 Hz, ArH), 7.54-7.58 (m, 4H, ArH), 7.51-7.48 (m, 2H, ArH), 7.35 (t, 1H, *J* 7.4 Hz, ArH), 6.18 (s, 1H, isoxazole ring H), 4.45 (s, 2H, -CH_2Br); ^{13}C NMR (126 MHz, $CDCl_3$) *δ* (ppm): 167.6, 156.4, 150.5, 139.4, 131.7, 131.3, 130.3, 129.6, 127.9, 127.4, 123.1, 119.3, 110.2, 103.4, 18.4; HRMS (ESI) *m/z* calculated for $C_{19}H_{13}Br_2N_3O$ $[M+H]^+/[M+2+H]^+/[M+4+H]^+$ 457.9503/459.9483/461.9463 found 457.9535/459.9513/461.9485.

5-(Bromomethyl)-3-[3-(4-nitrophenyl)-1-phenyl-1*H*-pyrazol-4-yl]isoxazole (1e). Pale yellow solid; 35% yield; mp 156-158 °C; IR (υ, cm^{-1}): 2923, 2845, 1614, 1575, 1456; ^{1}H NMR (500 MHz, $CDCl_3$) *δ* (ppm): 8.36 (s, 1H, pyrazole ring H), 8.30 (dd, *J* 8.8, 3.0 Hz, 2H, ArH), 7.94 (d, *J* 8.6 Hz, 2H, ArH), 7.79 (t, *J* 7.0 Hz, 2H, ArH), 7.53 (t, *J* 7.8 Hz, 2H, ArH), 7.40 (q, *J* 6.9 Hz, 1H, ArH), 6.26 (s, 1H, isoxazole ring H), 4.48 (s, 2H, -CH_2Br); ^{13}C NMR (126 MHz, $CDCl_3$) *δ* (ppm): 168.1, 157.0, 149.1, 138.8, 130.2, 129.7, 129.4, 128.6, 127.8, 123.8, 123.2, 119.5, 110.9, 103.5, 18.3; HRMS (ESI) *m/z* calculated for $C_{19}H_{13}BrN_4O_3$ $[M+H]^+/[M+2+H]^+$ 425.0249/427.0229 found 425.0267/427.0244.

5-(Bromomethyl)-3-[3-(*p*-tolyl)-1-phenyl-1*H*-pyrazol-4-yl]isoxazole (1f). Colorless solid; 45% yield; mp 118-120 °C; IR (υ, cm^{-1}): 2919, 2845, 1609, 1583, 1463; ^{1}H NMR (500 MHz, $CDCl_3$) *δ* (ppm): 8.39 (s, 1H, pyrazole ring H), 7.79 (d, *J* 7.9 Hz, 2H, ArH), 7.58-7.45 (m, 4H, ArH), 7.34 (t, *J* 7.4 Hz, 1H, ArH), 7.24 (d, 2H, Ar), 6.16 (s, 1H, isoxazole ring H), 4.43 (s, 2H, -CH_2Br), 2.42 (s, 3H, -CH_3); ^{13}C NMR (126 MHz, $CDCl_3$) *δ* (ppm): 167.2, 156.7, 151.9, 139.6, 138.7, 129.6, 129.4, 129.2, 128.7, 127.5, 127.1, 119.5, 110.1, 103.5, 21.4, 18.5; HRMS (ESI) *m/z* calculated for $C_{20}H_{16}BrN_3O$ $[M+H]^+/[M+2+H]^+$ 394.0555/396.0534 found 394.0556/396.0536.

5-(Bromomethyl)-3-[3-(4-methoxyphenyl)-1-phenyl-1*H*-pyrazol-4-yl]isoxazole (1g). Colorless solid; 45% yield; mp 126-128 °C; IR (υ, cm^{-1}): 2925, 2851, 1612, 1526, 1460; ^{1}H NMR (500 MHz, $CDCl_3$) *δ* (ppm): 8.38 (s, 1H, pyrazole ring H), 7.81-7.74 (m, 2H, ArH), 7.61-7.55 (m, 2H, ArH), 7.52-7.45 (m, 2H, ArH), 7.37-7.30 (m, 1H, ArH), 7.01-6.94 (m, 2H, ArH), 6.16 (s, 1H, isoxazole ring H), 4.44 (s, 2H, -CH_2Br), 3.87 (s, 3H, -OCH_3); ^{13}C NMR ($CDCl_3$, 126 MHz) *δ* (ppm): 167.1, 156.7, 151.3, 139.7, 130.2, 129.7, 129.2, 127.6, 127.2, 124.8, 119.4, 114.1, 110.1, 103.6, 55.4, 18.6; HRMS (ESI) *m/z* calculated for $C_{20}H_{16}BrN_3O_2$ $[M+H]^+/[M+2+H]^+$ 410.0504/412.0483, found 410.0523/412.0503.

Ethyl 3-(1,3-diphenyl-1*H*-pyrazol-4-yl)-5-phenylisoxazole-4-carboxylate (2a). Colorless solid; 60% yield; mp 144-146 °C; IR (υ, cm^{-1}): 2921, 1718, 1606, 1582, 1571, 1473, 1377; ^{1}H NMR (500 MHz, $CDCl_3$) *δ* (ppm): 8.31 (s, 1H, pyrazole ring H), 7.99-7.96 (m, 2H, ArH), 7.84-7.81 (m, 2H, ArH), 7.69-7.66 (m, 2H, ArH), 7.55-7.48 (m, 5H, ArH), 7.40-7.28 (m, 4H, ArH), 3.80 (q, *J* 7.1 Hz, 2H, -$COOCH_2$), 0.96 (t, *J* 7.1 Hz, 3H, -CH_3); ^{13}C NMR ($CDCl_3$, 126 MHz) *δ* (ppm): 172.6, 161.6, 156.8, 151.9, 139.7, 132.7, 131.4, 129.6, 129.0, 128.9, 128.6, 128.5, 128.3, 127.5, 127.0, 126.7, 119.2, 109.0, 109.0, 61.1, 13.5; HRMS (ESI) *m/z* calculated for $C_{27}H_{21}N_3O_3$ $[M+H]^+$ 436.1661, found 436.1685.

Ethyl 3-[3-(4-fluorophenyl)-1-phenyl-1*H*-pyrazol-4-yl]-5-phenylisoxazole-4-carboxylate (2b). Colorless solid; 54% yield; mp 140-142 °C; IR (υ, cm^{-1}): 2925, 1725, 1596, 1582, 1481, 1385; ^{1}H NMR (300 MHz, $CDCl_3$) *δ* (ppm): 8.29 (s, 1H, pyrazole ring H), 7.94 (dd, *J* 7.7, 1.9 Hz, 2H, ArH), 7.79 (d, *J* 8.1 Hz, 2H, ArH), 7.69-7.59 (m, 2H, ArH), 7.50 (q, *J* 6.9 Hz, 5H, ArH), 7.33 (t, *J* 7.4 Hz, 1H, ArH), 7.04 (t, *J* 8.7 Hz, 2H, ArH), 3.85 (q, *J* 7.1 Hz, 2H, -$COOCH_2$), 0.97 (t, *J* 7.1 Hz, 3H, -CH_3); ^{13}C NMR ($CDCl_3$, 126 MHz) *δ* (ppm): 172.7, 161.6, 156.7, 151.0, 139.6, 132.4, 131.5, 129.63, 129.59, 129.4, 129.3, 129.1, 128.9, 128.5, 127.0, 126.6, 119.2, 115.6, 109.1, 61.1, 13.6; HRMS (ESI) *m/z* calculated for $C_{27}H_{20}FN_3O_3$ $[M+H]^+$ 454.1567, found 454.1554.

Ethyl 3-[3-(4-chlorophenyl)-1-phenyl-1*H*-pyrazol-4-yl]-5-phenylisoxazole-4-carboxylate (2c). Colorless solid; 57% yield; mp 80-82 °C; IR (υ, cm^{-1}): 2923, 1722, 1599, 1583, 1476, 1382; ^{1}H NMR (500 MHz, $CDCl_3$) *δ* (ppm): 8.31 (s, 1H, pyrazole ring H), 7.95 (dt, 2H, *J* 8.5, 1.6 Hz, ArH), 7.81 (dd, 2H, *J* 7.4, 1.4 Hz, ArH), 7.63 (dt, 2H, *J* 8.5,

2.7 Hz, ArH), 7.56-7.49 (m, 5H, ArH,), 7.37-7.33 (m, 3H ArH), 3.88 (q, *J* 7.2 Hz, -$COOCH_2$), 0.99 (t, 3H, *J* 7.2 Hz, -CH_3), ^{13}C NMR (126 MHz, $CDCl_3$) *δ* (ppm): 172.8, 161.6, 156.6, 150.7, 139.6, 134.3, 131.5, 131.3, 129.6, 129.2, 128.9, 128.8, 128.7, 128.5, 127.1, 126.6, 119.3, 109.2, 108.9, 61.2, 13.6; HRMS (ESI) *m/z* calculated for $C_{27}H_{20}Cl_2N_3O_3$ $[M+H]^+$/$[M+2+H]^+$ 470.1271/472.1244, found 470.1280/472.1261.

Ethyl 3-[3-(4-bromophenyl)-1-phenyl-1*H*-pyrazol-4-yl]-5-phenylisoxazole-4-carboxylate (2d). Colorless solid; 45% yield; mp 110-112 °C; IR (υ, cm^{-1}) 2924, 1721, 1597, 1581, 1474, 1383; ^{1}H NMR (500 MHz, $CDCl_3$) *δ* (ppm): 8.30 (s, 1H, pyrazole ring H), 8.00-7.92 (m, 2H, ArH), 7.84-7.78 (m, 2H, ArH), 7.58-7.55 (m, 3H), 7.54-7.52 (m, 2H), 7.52-7.48 (m, 5H), 7.39-7.32 (m, 1H), 3.88 (q, *J* 7.1 Hz, 2H, -$COOCH_2$), 0.99 (t, *J* 7.1 Hz, 3H, -CH_3).^{13}C NMR (126 MHz, $CDCl_3$) *δ* (ppm): 172.9, 161.7, 156.7, 150.9, 139.7, 131.9, 131.8, 131.7, 129.8, 129.4, 129.3, 129.1, 128.7, 127.3, 126.7, 122.7, 119.4, 109.4, 108.9, 61.3, 13.7; HRMS (ESI) *m/z* calculated for $C_{27}H_{20}BrN_3O_3$ $[M+H]^+$/$[M+2+H]^+$ 514.0766/516.0746 found 514.0764/516.0751.

Ethyl 3-[3-(4-nitrophenyl)-1-phenyl-1*H*-pyrazol-4-yl]-5-phenylisoxazole-4-carboxylate (2e). Yellow solid; 50% yield; mp 150-151 °C; IR (υ, cm^{-1}) 2923, 1718, 1598, 1573, 1465, 1383; ^{1}H NMR (500 MHz, $CDCl_3$) *δ* (ppm): 8.36 (s, 1H, pyrazole ring H), 8.26-8.21 (m, 2H, ArH), 7.97-7.94 (m, 2H, ArH), 7.91-7.88 (m, 2H, ArH), 7.84-7.81 (m, 2H, ArH), 7.58-7.50 (m, 5H, ArH), 7.42-7.37 (m, 1H, ArH), 3.93 (q, *J* 7.1 Hz, 2H, -$COOCH_2$), 0.99 (t, *J* 7.1 Hz, 3H, -CH_3); ^{13}C NMR (126 MHz, $CDCl_3$) *δ* (ppm): 173.3, 161.4, 156.3, 149.5, 147.5, 139.4, 139.3, 131.7, 129.9, 129.7, 129.1, 128.6, 128.2, 127.6, 126.5, 123.8, 119.4, 109.9, 108.8, 61.2, 13.6; HRMS (ESI) *m/z* calculated for $C_{27}H_{20}N_4O_5$ $[M+H]^+$ 481.1512 found 481.1490.

Ethyl 3-[3-(*p*-tolyl)-1-phenyl-1*H*-pyrazol-4-yl]-5-phenylisoxazole-4-carboxylate (2f). Colorless solid; 63% yield; mp 130-131 °C; IR (υ, cm^{-1}) 2921, 1718, 1586, 1570, 1464, 1376; ^{1}H NMR (500 MHz, $CDCl_3$) *δ* (ppm): 8.29 (s, 1H, pyrazole ring H), 7.97 (dt, 2H, *J* 6.5, 1.4 Hz, ArH), 7.82 (dd, 2H, *J* 7.3, 1.3 Hz, ArH), 7.57-7.55 (m, 2H, ArH), 7.48-7.54 (m, 5H, ArH), 7.34 (t, 1H, *J* 7.4 Hz, ArH),7.17 (d, 2H, *J* 7.9 Hz, ArH), 3.81 (q, *J* 7.1 Hz, 2H, -$COOCH_2$), 2.35 (s, 3H), 0.96 (t, *J* 7.1 Hz, 3H, -CH_3); ^{13}C NMR (126 MHz, $CDCl_3$) *δ* (ppm): 172.5, 161.7, 156.9, 151.9, 139.8, 138.1, 131.4, 129.9, 129.5, 129.3, 128.90, 128.87, 128.5, 127.4, 126.9, 126.7, 119.2, 109.2, 109.1, 61.1, 21.3, 13.5; HRMS (ESI) *m/z* calculated for $C_{28}H_{23}N_3O_3$ $[M+H]^+$ 450.1817 found 450.1812.

Acknowledgements

RK acknowledges the Department of Science and Technology for PURSE grant {SR/PURSE/2022/126}and J. C. Bose UST, YMCA, Faridabad for seed grant {R&D/SG/2020- 21/166}. RK and BU are grateful to DST, Haryana {HSCSIT/R&D/2022/164} for financial support. BU acknowledges DST, for SERB-POWER Grant, {SPG/2021/004027}. VVZ is thankful to the National Science Foundation (CHE-2243793) for support of his research on hypervalent iodine chemistry. Rimi is grateful to the J. C. Bose UST, YMCA, Faridabad for the award of the University Research Scholarship (URS). The authors acknowledge the central Instrumentation Laboratory (CIL) of J. C. Bose UST for providing the instrumentation facilities.

Supplementary Material

Single crystal XRD structure, ^{1}H NMR, ^{13}C NMR and HRMS data are provided in the Supplementary Information. Moreover, the biological assays used for the antimicrobial, antioxidant activity and cytotoxicity are also provided in the SI.

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