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Full Paper

An Efficient and Convenient Approach for the Synthesis of Novel Pyrazolo[1,2-*a*]triazole-triones and Evaluation of their Antimicrobial Activities

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A new protocol for the efficient synthesis of novel pyrazolo[1,2-*a*]triazole-1,3,5-triones has been developed. The synthesis was achieved by reaction of aromatic aldehydes, Meldrum's acid, and 4-phenylurazole in the presence of a catalytic amount of glacial AcOH at 80°C. The structural assignment has been unambiguously confirmed by X-ray analysis. The present finding provides an environmentally benign, efficient, and promising synthetic strategy for the synthesis of libraries with diversity. These compounds were tested in vitro for their antibacterial activity against four strains, namely *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Pseudomonas aeruginosa* and antifungal activity against two fungal strains, namely *Aspergillus niger* and *Aspergillus flavus*.

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Introduction

Green chemistry is recognised as a pioneering concept, which widely reports intrinsic atom economy, energy savings, waste reduction, easy work ups, and the avoidance of hazardous chemicals.[1] The development of a simple, eco-friendly reaction protocol for the synthesis of highly functionalized compound libraries of medicinal motifs is an attractive area of research in both academia and the pharmaceutical industry.[2] In this context, multicomponent reactions (MCRs)[3] have emerged as a powerful tool to achieve synthetic efficiency, as they have unique advantages such as convergence, operational simplicity, facile automation, one pot reactions, atom and step economy (PASE),[4] in addition to diminished waste generation.

Heterocyclic compounds occur very widely in nature and are essential to life. Among a large variety of nitrogen containing heterocyclic compounds, heterocycles containing a urazole (1,2,4-triazolidine-3,5-dione) moiety are of interest because they constitute an important class of natural and non-natural products, many of which have a myriad of biological functions such as anticonvulsant,[5] antifungal,[6] herbicidal,[7] hypolipidemic,[8] and insecticidal activities.[9] They are also utilised in the production of anti-tumour drugs[10] and in polymeric materials.[11] Urazoles have been used as laboratory reagents for the preparation of novel heterocyclic compounds.[12]

The reported methods for the preparation of pyrazolo [1,2-*a*]triazole-1,3,5-trione ring systems involve: (i) reaction of pyrazolonecarboxylates with isocyanate in the presence of an equimolar amount of a tertiary amine[13] and (ii) an ene-type reaction of 4-phenyl-1,2,4-triazoline-3,5-dione and 5-methylfuran-2(3*H*)-one.[14] As far as we know, there is no report concerning the isocyanate-free synthesis of pyrazolo [1,2-*a*]triazole-1,3,5-triones by an MCR methodology. This encouraged us to develop an environmentally benign synthetic methodology for pyrazolo[1,2-*a*]triazole-trione derivatives. We envisaged that a one-pot three-component synthesis of pyrazolo[1,2-*a*]triazole-triones from aromatic aldehydes, 4-phenyl urazole, and a component containing a $–CH_2CO–$ moiety could be handy in the present case. In order to maintain the pyrazole ring of pyrazolo[1,2-*a*]triazole-trione, the Meldrum's acid appeared as an appropriate third component that would eventually allow the construction of the pyrazolo [1,2-*a*]triazole-trione framework.

Results and Discussion

Chemistry

We report herein a new, convenient, diversity oriented, and highly efficient green protocol for the synthesis of novel pyrazolo[1,2-*a*]triazole-1,3,5-triones by the three component condensation of aldehydes **1**, Meldrum's acid, and 4-phenylurazole in the presence of a catalytic amount of glacial AcOH at 80°C under solvent free conditions. The reaction conditions were optimized using 4-fluorobenzaldehyde, Meldrum's acid, and 4-phenylurazole in equimolar amounts as a model system. Initially a control experiment confirmed that the reaction did not proceed in the absence of a catalyst (entries 1 and 2, Table 1).

The model reaction was then performed in the presence of different catalysts, such as *p*-toluene sulfonic acid (*p*-TSA), H_2SO_4, ceric ammonium nitrate (CAN), Et_3N, L-proline, La $(OTf)_3$, and glacial AcOH (10 mol-% each) in refluxing ethanol.

The reactions performed using 10 mol-% of *p*-TSA, H_2SO_4, and CAN as catalysts were found to be incomplete and gave complex reaction mixtures (entries 3–5, Table 1). Reactions using Et_3N and L-proline led to the formation of 5-(4-fluorobenzylidene)-2,2-dimethyl-1,3-dioxane-4,6-dione by Knoevenagel condensation of an aldehyde and Meldrum's acid (entries 6 and 7, Table 1). The reaction using $La(OTf)_3$ as catalyst furnished the desired product 7-(4-fluorophenyl)-2-phenyldihydropyrazolo[1,2-*a*][1,2,4]triazole-1,3,5(2*H*)-trione (**2a**) albeit in low yield (entry 8, Table 1). To our delight, glacial AcOH gave the desired **2a** in 66 % yield in 5 h (entry 9, Table 1). When the reaction was attempted under solvent free conditions, using glacial AcOH (10 mol-%) as catalyst at 80°C, the reaction was complete in 4 h and yielded 75 % of desired **2a** after a simple work up (entry 10, Table 1). When the reaction was conducted using 20 mol-% of the glacial AcOH, it required a shorter time for completion (3.5 h) and yielded 92 % of **2a** while upon increasing the amount of the catalyst to 30 mol-%, no significant improvement in the time or yield of the product **2a** was observed (entries 11 and 12, Table 1).

The optimized reaction conditions of employing 20 mol-% of glacial AcOH at 80°C was utilised for a variety of substrates to explore the synthetic scope and generality of this cascade reaction and to generate a small library of functionalized pyrazolo[1,2-*a*]triazole-trione derivatives. Notably, a wide range of aromatic aldehydes bearing electron-withdrawing as well as electron-donating groups underwent the reactions smoothly under the optimized reaction conditions to give the desired pyrazolo[1,2-*a*]triazole-triones **2a**–**q** in high yields (Scheme 1).

Table 1. Optimization of reaction conditions for the synthesis of 7-(4-fluorophenyl)-2- phenyldihydropyrazolo[1,2-*a*][1,2,4]triazole-1,3,5 (2*H*)-trione (2a)[A]

Entry	Catalyst	Catalyst [mol-%]	Solvent	Time [h]	Temp. [°C]	Yield **2a** [%]
1	None	–	H_2O	24	100	–[B]
2	None	–	EtOH	24	80	–[B]
3	*p*-TSA	10	EtOH	8	80	–[C]
4	H_2SO_4	10	EtOH	8	80	–[C]
5	CAN	10	EtOH	8	80	–[C]
6	Et_3N	10	EtOH	24	80	–[D]
7	L-Proline	10	EtOH	24	80	–[D]
8	$La(OTf)_3$	10	EtOH	5	80	43
9	Gl. AcOH	10	EtOH	5	80	66
10	Gl. AcOH	10	–	4	80	75
11	Gl. AcOH	20	–	3.5	80	92
12	Gl. AcOH	30	–	3.5	80	93

[A]Reaction of 4-fluorobenzaldehyde (**2a**, 1.0 mmol), Meldrum's acid (1.0 mmol), and 4-phenylurazole (1.0 mmol).
[B]No reaction.
[C]Incomplete reaction with a number of spots observed by TLC including arylidene Meldrum's acid.
[D]The product formed was identified as arylidene Meldrum's acid.

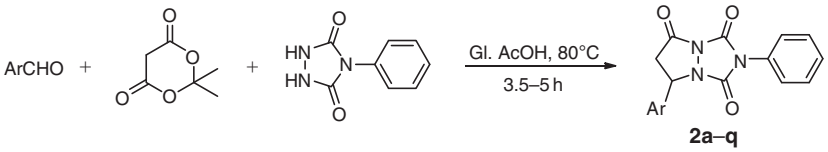
Reactions employing aromatic aldehydes with electron-withdrawing groups were faster and gave higher yields of the products compared with reactions of aldehydes with electron-donating groups as the presence of electron-withdrawing groups on the aryl ring make the arylidene intermediate **A** more electrophilic, thereby allowing a facile attack of the 4-phenylurazole on **A** (Table 2, entries 1–17). Interestingly, when the catalytic system was applied to terephthaldehyde (1.0 mmol), Meldrum's acid (2.0 mmol), and 4-phenylurazole (2.0 mmol), it led to the synthesis of **2r** (Scheme 2) (Table 2, entry 18). All the products were identified on the basis of IR, ^{1}H NMR, ^{13}C NMR, and mass spectra.

In addition, the structures of the synthesized novel pyrazolo [1,2-*a*]triazole-trione derivatives **2** have been confirmed by the single crystal X-ray diffraction analysis of compound **2m** (Fig. 1). A single crystal of **2m** suitable for X-ray diffraction was obtained by evaporation of $CHCl_3$/hexane solutions at room temperature. The calculated structure provides accurate molecular shapes, especially in terms of the pyramidal geometry at N1 and N3 and planar geometry at N2. The angle between the two planes containing the triazole ring and phenyl ring is 83.75° whereas that between the planes of pyrazole and phenyl rings is 61.17°. The two five-membered rings deviate very slightly from planarity as evidenced by the angle between the planes containing them, i.e. 32.47°. All bond lengths and angles are normal and in agreement with similar compounds.[15]

A mechanistic explanation for the formation of pyrazolo[1,2-*a*]triazole-trione derivatives is provided in Scheme 3. The formation of pyrazolo[1,2-*a*]triazole-trione derivatives **2** can be rationalized by initial formation of arylidene Meldrum's acid **A** by the Knoevenagel condensation between an aldehyde **1** and

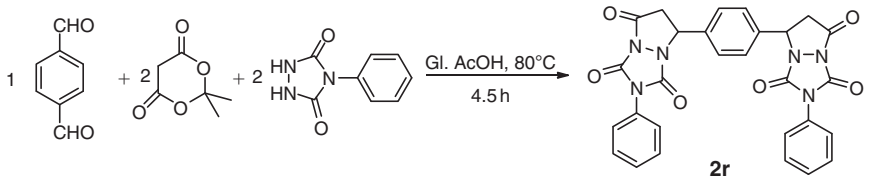
Table 2. Synthesis of pyrazolo[1,2-*a*]triazole-triones 2a–r using glacial AcOH at 80°C

Entry	Product	Ar [ArCHO]	Time [h]	Yield [%]
1	**2a**	4-FC_6H_4	3.5	92
2	**2b**	4-ClC_6H_4	4	85
3	**2c**	3,4,5-$(CH_3O)_3C_6H_2$	4	80
4	**2d**	4-$(CN)C_6H_4$	4.5	87
5	**2e**	3-ClC_6H_4	4	83
6	**2f**	2,4-$Cl_2C_6H_3$	4	79
7	**2g**	3-$(NO_2)C_6H_4$	5	76
8	**2h**	C_6H_5	4	81
9	**2i**	4-$CH_3C_6H_4$	5	78
10	**2j**	3-FC_6H_4	3.5	90
11	**2k**	2-FC_6H_4	4	86
12	**2l**	2-$(F_3C)C_6H_4$	4	81
13	**2m**	2-ClC_6H_4	4	82
14	**2n**	3-$(F_3C)C_6H_4$	3.5	85
15	**2o**	4-$(F_3C)C_6H_4$	3.5	79
16	**2p**	4-$(Me_2CH)C_6H_4$	4.5	77
17	**2q**	2-(Br),4-(F)C_6H_3	3.5	79
18	**2r**	4-$(OHC)C_6H_4$	4.5	84



2a–q

Scheme 1.



Scheme 2.

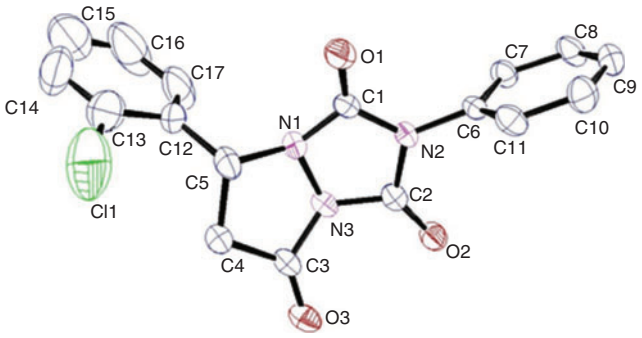


Fig. 1. X-ray crystallographic structure of **2m**.

Meldrum's acid. Subsequently 4-phenylurazole undergoes a Michael-type addition to arylidene Meldrum's acid **A** to give intermediate **B** which undergoes cyclization with concomitant loss of acetone and carbon dioxide to afford the desired compound **2**. The proposed reaction pathway was confirmed by preparing 4-chlorobenzylidene Meldrum's acid independently and then carrying out its reaction with 4-phenylurazole in the presence of glacial acetic acid which led to the formation of the desired product **2b**, thus supporting the pathway.

Pharmacology

A total of 18 novel pyrazolo[1,2-a]triazole-triones **2a**–**r** were screened for their antibacterial and antifungal activity. The tested chemical compounds possessed variable antibacterial activity against Gram-positive (*Staphylococcus aureus, Bacillus subtilis*) bacteria and antifungal activity against *Aspergillus niger* and *A. flavus.* However, the tested chemical compounds did not exhibit any activity against Gram-negative bacteria. Positive controls produced significantly sized inhibition zones against the tested bacteria and fungi. However, the negative control produced no observable inhibitory effect against any of the test organisms.

All the tested compounds showed a zone of inhibition ranging between 14 and 20 mm against the bacteria. On the basis of the zone of inhibition produced against the test bacterium, compound **2f** was found to be most effective against *B.subtilis* and *S. aureus*, with a zone of inhibition of 20.3 and 18.3 mm, respectively. However other tested compounds showed moderate antibacterial activity (Table 3).

The minimal inhibitory concentration (MIC) of all the compounds **2a**–**r** ranged between 64 and 256 $\mu g\,mL^{-1}$ against the tested bacteria. Compound **2f**, having 2,4-dichloro substitution, was found to be best as it exhibited the lowest MIC of 64 $\mu g\,mL^{-1}$ against *S. aureus*. Six compounds, namely **2a**, **2f**, **2h**, **2k**, **2o**, and **2q** showed the lowest MICs of 64 $\mu g\,mL^{-1}$ against *B.subtilis* (Table 4). A further perusal of activity data revealed that compounds with halogen substitution at the 2 or 4 positions and an unsubstituted phenyl ring were more active among all the pyrazolo[1,2-*a*]triazole-triones, e.g. 2,4-dichloro, 4-F, 2-F, 4-CF_3, 2-Br, 4-F, and 4-H.

Of the 18 chemical compounds screened for their antifungal activity, two compounds, namely **2f** and **2h** showed more than 50 % inhibition of mycelial growth against *A. niger* and four compounds, **2f**, **2h**, **2o**, and **2q** showed more than 50 % inhibition of mycelial growth against *A. flavus* (Table 5). The compound with an unsubstituted phenyl ring, **2h**, was found to be most effective with a maximum growth inhibition index, although compounds with halogen substitution at the 2 or 4 position also exhibited higher activity, e.g. 2,4-dichloro, 4-F_3C, 2-Br, and 4-F.

Among all the tested chemical compounds, compound **2f** showed good antibacterial and antifungal activity. Therefore, this compound can be further explored for its toxicity.

Conclusion

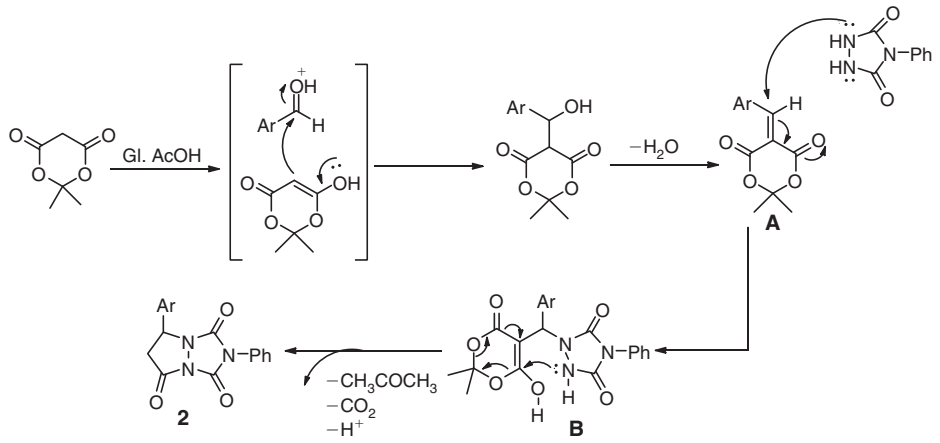
In conclusion, we have reported a new multicomponent reaction for the synthesis of novel pyrazolo[1,2-*a*]triazole-triones, by reaction of aldehydes, Meldrum's acid, and 4-phenylurazole. Mild reaction conditions and high isolated yields are the remarkable advantages of this protocol. These derivatives were evaluated for their antimicrobial activities, of which, some have shown good antibacterial and antifungal activities.

Experimental

All the chemicals were purchased from Sigma–Aldrich or Merck and were used as received. Thin-layer chromatography (GF_{254}) was used to monitor reaction progress. Melting points were determined on a Tropical labequip apparatus and were uncorrected. IR (KBr) spectra were recorded on a Perkin–Elmer FTIR spectrophotometer and the values are expressed as ν_{max} in cm^{-1}. Mass spectra were recorded on a Waters LCT Micromass spectrometer. The ^{1}H, ^{19}F, and ^{13}C NMR spectra were recorded on a Jeol JNM ECX-400P spectrometer at 400, 376, and 100 MHz, respectively, using TMS as an internal standard. The chemical shift values are recorded on a δ scale and the coupling constants (J) are in Hz. X-Ray intensity data were collected on an Oxford Diffraction Xcalibur CCD diffractometer with graphite monochromated $Mo_{K\alpha}$ radiation (λ 0.71073 Å).

Data Collection and Refinement

The intensity data for compound **2m** was collected on an Oxford Xcalibur CCD diffractometer equipped with graphite monochromatic $Mo_{K\alpha}$ radiation (λ 0.71073 Å) at 298(2) K. A multiscan absorption correction was applied. The structure was solved by direct methods and refined by full-matrix least-squares refinement techniques on F^2 using *SHELXL-97*.[16] The coordinates of non-hydrogen atoms were refined anisotropically using *SHELXL-97*. The positions of hydrogen atoms were obtained from difference Fourier maps and were included in the final cycles of refinement. All calculations were done using the *Wingx* software package.[17] Complete crystallographic data (excluding factors) of **2m** have been deposited at



Scheme 3. Proposed reaction pathway for the synthesis of **2**.

Table 3. Antibacterial activity of compounds 2a–r through agar well diffusion method

Compound	Diameter of growth of inhibition zone[A] [mm]	
	Staphylococcus aureus	*Bacillus subtilis*
2a	16.3	17.6
2b	15.0	15.6
2c	16.3	17.0
2d	15.3	16.3
2e	16.3	17.6
2f	18.3	20.3
2g	15.3	17.3
2h	17.6	18.6
2i	14.6	15.6
2j	14.3	15.3
2k	14.6	15.6
2l	14.6	15.3
2m	14.3	15.6
2n	15.6	16.6
2o	17.6	18.6
2p	15.3	16.3
2q	16.6	18.6
2r	15.3	17.3
Ciprofloxacin	26.6	24.0

[A]Values, including diameter of the well (8 mm), are means of three replicates.

Table 4. Minimum inhibitory concentration of compounds 2a–r by using a modified agar well diffusion method

Compound	Minimum inhibitory concentration [μg mL^{-1}]	
	Staphylococcus aureus	*Bacillus subtilis*
2a	128	64
2b	256	128
2c	128	128
2d	128	128
2e	128	128
2f	64	64
2g	128	128
2h	128	64
2i	256	128
2j	256	128
2k	256	64
2l	256	128
2m	256	128
2n	128	128
2o	64	64
2p	128	128
2q	128	64
2r	128	128
Ciprofloxacin	6.25	6.25

the Cambridge Crystallographic Data Centre under number CCDC 936196.

Crystal Data

$C_{17}H_{12}ClN_3O_3$, M 341.75, orthorhombic, space group P 21 21 21, a 6.0132(4), b 11.8864(7), c 23.1834(17), V 1657.04 (19) Å^3, Z 4, F(000) 704, D_C 1.370 mg m^{-3}, λ 0.71073 Å, absorption coefficient 0.250 mm^{-1}. Selected bond angles: C(1)–N(1)–N(3), 107.8(4); C(1)–N(1)–C(5), 124.2(4); N(3)–N(1)–C(5), 110.3(4); C(2)–N(2)–C(1), 112.3(4); C(2)–N(2)–C(6), 123.1(4); C(1)–N(2)–C(6), 122.9(4); C(2)–N(3)–C(3), 126.5(4); C(2)–N(3)–N(1), 108.1(3); and C(3)–N(3)–N(1), 110.4(4).

*General Procedure for the Synthesis of Pyrazolo[1,2-a] triazole-trione Derivatives **2a–q***

A mixture of aryl aldehyde **1** (1.0 mmol), Meldrum's acid (1.0 mmol), 4-phenylurazole (1.0 mmol), and glacial AcOH (20 mol-%) was placed in a 25 mL round-bottomed flask and the contents were stirred magnetically in an oil-bath maintained at 80°C for an appropriate time as indicated in Table 2. The progress of the reaction was monitored by TLC (eluent: methanol–chloroform, 10 : 90 v/v). After completion of the reaction, the reaction mixture was allowed to cool at room temperature and diluted with cold H_2O. A solid eventually separated out. The solid was filtered under vacuum, and washed with water to remove residual acetic acid. The crude product so obtained was recrystallized from $CHCl_3$/hexane (80 : 20, v/v) to afford the pure product **2**. The products were characterized by IR, ^{1}H NMR, ^{13}C NMR, ^{19}F NMR, and mass spectra and an X-ray crystallographic study.

Reaction of terephthaldehyde (**1r**, 1.0 equiv.) required 2.0 equiv. of Meldrum's acid and 2.0 equiv. of 4-phenylurazole for completion to give the bis-condensation product, 7,7′-(1,4-phenylene)bis(2-phenyldihydropyrazolo[1,2-*a*][1,2,4]triazole-1,3,5(2*H*)-trione) (**2r**).

Table 5. Antifungal activity of compounds 2a–r through poisoned food method

Compound	Mycelial growth inhibition [%]	
	Aspergillus niger	*Aspergillus flavus*
2a	47.7	48.8
2b	35.5	37.7
2c	44.4	48.8
2d	41.1	43.3
2e	43.3	45.5
2f	51.1	53.3
2g	41.1	43.3
2h	53.3	57.7
2i	43.3	45.5
2j	38.8	41.1
2k	35.5	37.7
2l	37.7	42.2
2m	34.4	37.7
2n	35.5	38.8
2o	46.6	51.1
2p	38.8	42.2
2q	48.8	53.3
2r	42.2	46.6
Fluconazole	81.1	77.7

*Spectroscopic Data of Compounds **2a–r***

*7-(4-Fluorophenyl)-2-phenyldihydropyrazolo[1,2-a][1,2,4]triazole-1,3,5(2H)-trione (**2a**)*

White solid. Yield: 92 %. Mp 171–173°C. ν_{max} (KBr)/cm^{-1} 2929, 1751, 1719. δ_H (400 MHz, $CDCl_3$) 7.46–7.40 (m, 7H, ArH), 7.14 (t, *J* 8.8, 2H, ArH), 5.45 (t, *J* 8.04, 1H, CH), 3.58 (dd, *J* 9.52, 18.32, 1H, CH*H*CO), 3.17 (dd, *J* 7.32, 17.56, 1H, CH*H*CO). δ_F (376 MHz, DMSO) −114.13 (s, 1F, ArF). δ_C (100 MHz, $CDCl_3$) 163.05, 151.09, 143.66, 132.56, 130.23, 129.35, 128.95, 128.13 (d, $^3J_{C-F}$ 7.63), 125.56, 116.46 (d, $^2J_{C-F}$ 21.93), 58.01, 43.08. *m*/*z* (ESI) 348.50 ($[M + Na]^+$). Anal. Calc. for $C_{17}H_{12}FN_3O_3$ 325.09.

*7-(4-Chlorophenyl)-2-phenyldihydropyrazolo[1,2-a][1,2,4]triazole-1,3,5(2H)-trione (**2b**)*

White solid. Yield: 85 %. Mp 151–153°C. ν_{max} (KBr)/cm^{-1} 2929, 1752, 1685. δ_H (400 MHz, $CDCl_3$) 8.01 (d, *J* 8.04, 1H, ArH), 7.45–7.38 (m, 8H, ArH), 5.44 (t, *J* 8.8, 1H, CH), 3.60 (dd, *J* 9.52, 18.32, 1H, CH*H*CO), 3.18 (dd, *J* 8.08, 18.32, 1H, CH*H*CO). δ_C (100 MHz, $CDCl_3$ + DMSO) 171.03, 152.47, 135.34, 133.35, 130.93, 128.54, 128.23, 128.03, 127.17, 125.14, 124.90, 55.61, 35.89. *m*/*z* (ESI) 364.12 ($[M + Na]^+$), 366.12 ($[M + Na + 2]^+$). Anal. Calc. for $C_{17}H_{12}ClN_3O_3$ 341.06.

*2-Phenyl-7-(3,4,5-trimethoxyphenyl)dihydropyrazolo[1,2-a][1,2,4]triazole-1,3,5(2H)-trione (**2c**)*

White solid. Yield: 80 %. Mp 230–232°C. ν_{max} (KBr)/cm^{-1} 2937, 1746, 1720. δ_H (400 MHz, $CDCl_3$) 7.41 (s, 5H, ArH), 6.60 (s, 2H, ArH), 5.39 (t, *J* 8.04, 1H, CH), 3.85 (s, 6H, OCH_3), 3.82 (s, 3H, OCH_3), 3.55 (dd, *J* 9.52, 18.32, 1H, CH*H*CO), 3.15 (dd, *J* 7.32, 17.56, 1H, CH*H*CO). δ_C (100 MHz, $CDCl_3$ + DMSO) 166.00, 153.21, 153.05, 145.58, 137.10, 134.78, 130.95, 129.00, 128.50, 126.42, 103.95, 59.91, 58.39, 55.95, 42.73. *m*/*z* (ESI) 420.05 ($[M + Na]^+$). Anal. Calc. for $C_{20}H_{19}N_3O_6$ 397.13.

*4-(1,3,7-Trioxo-2-phenylhexahydropyrazolo[1,2-a][1,2,4]triazol-5-yl)benzonitrile (**2d**)*

White solid. Yield: 87 %. Mp 165–166°C. ν_{max} (KBr)/cm^{-1} 2925, 1752, 1720. δ_H (400 MHz, $CDCl_3$) 7.73 (d, *J* 8.08, 2H, ArH), 7.56 (d, *J* 7.36, 2H, ArH), 7.45–7.42 (m, 5H, ArH), 5.51 (t, *J* 8.80, 1H, CH), 3.61 (dd, *J* 9.52, 18.32, 1H, CH*H*CO), 3.14 (dd, *J* 8.04, 18.32, 1H, CH*H*CO). δ_C (100 MHz, $CDCl_3$) 162.74, 150.93, 144.03, 141.95, 133.19, 129.43, 129.12, 126.97, 125.54, 118.04, 113.27, 57.96, 42.71. *m*/*z* (ESI) 355.14 ($[M + Na]^+$). Anal. Calc. for $C_{18}H_{12}N_4O_3$ 332.09.

*7-(3-Chlorophenyl)-2-phenyldihydropyrazolo[1,2-a][1,2,4]triazole-1,3,5(2H)-trione (**2e**)*

White solid. Yield: 83 %. Mp 115–116°C. ν_{max} (KBr)/cm^{-1} 2929, 1749, 1718. δ_H (400 MHz, $CDCl_3$) 7.43 (s, 5H, ArH), 7.36 (s, 3H, ArH), 7.30 (s, 1H, ArH), 5.42 (t, *J* 8.40, 1H, CH), 3.57 (dd, *J* 9.52, 17.60, 1H, CH*H*CO), 3.15 (dd, *J* 6.60, 17.60, 1H, CH*H*CO). δ_C (100 MHz, $CDCl_3$) 163.36, 150.80, 143.99, 134.72, 131.82, 130.35, 130.27, 129.33, 128.91, 127.77, 127.23, 125.60, 55.98, 41.91. *m*/*z* (ESI) 364.12 ($[M + Na]^+$), 366.11 ($[M + Na + 2]^+$). Anal. Calc. for $C_{17}H_{12}ClN_3O_3$ 341.06.

*7-(2,4-Dichlorophenyl)-2-phenyldihydropyrazolo[1,2-a][1,2,4]triazole-1,3,5(2H)-trione (**2f**)*

White solid. Yield: 79 %. Mp 128–130°C. ν_{max} (KBr)/cm^{-1} 2942, 1736, 1717. δ_H (400 MHz, $CDCl_3$) 7.53 (d, *J* 8.08, 1H, ArH), 7.47–7.44 (m, 5H, ArH), 7.42–7.40 (m, 1H, ArH), 7.37–7.34 (m, 1H, ArH), 5.73 (t, *J* 8.04, 1H, CH), 3.76 (dd, *J* 9.52, 18.32, 1H, CH*H*CO), 3.06 (dd, *J* 8.04, 18.32, 1H, CH*H*CO). δ_C (100 MHz, $CDCl_3$) 162.91, 150.98, 143.83, 135.71, 133.33, 132.58, 130.25, 130.17, 129.40, 129.04, 128.21, 128.14, 125.57, 55.71, 41.72. *m*/*z* (ESI) 398.00 ($[M + Na]^+$), 400.00 ($[M + Na + 2]^+$). Anal. Calc. for $C_{17}H_{11}Cl_2N_3O_3$ 375.02.

*7-(3-Nitrophenyl)-2-phenyldihydropyrazolo[1,2-a][1,2,4]triazole-1,3,5(2H)-trione (**2g**)*

White solid. Yield: 76 %. Mp 188–190°C. ν_{max} (KBr)/cm^{-1} 2927, 1760, 1723, 1417. δ_H (400 MHz, $CDCl_3$) 8.28 (s, 1H, ArH), 8.22 (d, *J* 8.24, 1H, ArH), 7.76 (d, *J* 7.8, 1H, ArH), 7.63 (t, *J* 8.24, 1H, ArH), 7.41 (m, 5H, ArH), 5.55 (t, *J* 8.92, 1H, CH), 3.57 (dd, *J* 9.64, 17.88, 1H, CH*H*CO), 3.11 (dd, *J* 8.68, 18.32, 1H, CH*H*CO). δ_C (100 MHz, $CDCl_3$) 163.39, 152.06, 148.67, 144.30, 139.37, 132.21, 130.51, 130.10, 129.39, 129.08, 125.63, 124.00, 121.44, 57.79, 42.87. *m*/*z* (ESI) 375.09 ($[M + Na]^+$). Anal. Calc. for $C_{17}H_{12}N_4O_5$ 352.08.

*2,7-Diphenyldihydropyrazolo[1,2-a][1,2,4]triazole-1,3,5(2H)-trione (**2h**)*

White solid. Yield: 81 %. Mp 166–168°C. ν_{max} (KBr)/cm^{-1} 2940, 1739, 1696. δ_H (400 MHz, $CDCl_3$) 7.39 (s, 10H, ArH), 5.40 (s, 1H, CH), 3.56 (s, 1H, CH*H*CO), 3.16 (s, 1H, CH*H*CO). δ_C (100 MHz, $CDCl_3$ + DMSO) 164.42, 152.47, 144.52, 137.61, 129.74, 127.77, 127.68, 127.52, 127.26, 127.12, 126.24, 125.02, 124.73, 124.37, 57.05, 41.91. *m*/*z* (ESI) 330.23 ($[M + Na]^+$). Anal. Calc. for $C_{17}H_{13}N_3O_3$ 307.10.

*2-Phenyl-7-(4-methylphenyl)dihydropyrazolo[1,2-a][1,2,4]triazole-1,3,5(2H)-trione (**2i**)*

White solid. Yield: 78 %. Mp 200–202°C. ν_{max} (KBr)/cm^{-1} 2925, 1752, 1720, 759. δ_H (400 MHz, $CDCl_3$) 7.45 (s, 4H, ArH), 7.40–7.31 (m, 1H, ArH), 7.25 (s, 4H, ArH), 5.44 (s, 1H, CH), 3.57 (br s, 1H, CH*H*CO), 3.2 (br s, 1H, CH*H*CO) 2.34 (s, 3H,

CH_3). δ_C (100 MHz, $CDCl_3$ + DMSO) 163.85, 152.92, 151.24, 138.98, 134.08, 129.87, 129.30, 129.13, 128.84, 128.64, 127.40, 126.00, 125.51, 58.27, 43.07, 21.06. *m/z* (ESI) 344.37 ([M + Na]$^+$). Anal. Calc. for $C_{18}H_{15}N_3O_3$ 321.11.

*7-(3-Fluorophenyl)-2-phenyldihydropyrazolo[1,2-a][1,2,4]triazole-1,3,5(2H)-trione (**2j**)*

White solid. Yield: 90 %. Mp 153–155°C. ν_{max} (KBr)/cm^{-1} 2929, 1758, 1718. δ_H (400 MHz, $CDCl_3$) 7.43–7.32 (m, 6H, ArH), 7.16–7.09 (m, 2H, ArH), 7.06–7.02 (m, 1H, ArH), 5.41 (t, *J* 8.08, 1H, CH), 3.56 (dd, *J* 9.52, 18.32, 1H, CH*H*CO), 3.13 (dd, *J* 8.08, 18.32, 1H, CH*H*CO). δ_C (100 MHz, $CDCl_3$) 162.90, 151.13, 143.69, 139.30 (d, $^3J_{C-F}$ 6.68), 131.18 (d, $^3J_{C-F}$ 8.59), 130.21, 129.37, 128.99, 125.58, 121.67, 116.32 (d, $^2J_{C-F}$ 20.98), 113.38 (d, $^2J_{C-F}$ 22.89), 57.84, 42.99. *m/z* (ESI) 348.17 ([M + Na]$^+$). Anal. Calc. for $C_{17}H_{12}FN_3O_3$ 325.09.

*7-(2-Fluorophenyl)-2-phenyldihydropyrazolo[1,2-a][1,2,4]triazole-1,3,5(2H)-trione (**2k**)*

White solid. Yield: 86 %. Mp 178–180°C. ν_{max} (KBr)/cm^{-1} 2925, 1752, 1720. δ_H (400 MHz, DMSO) 7.70 (t, *J* 7.32, 1H, ArH), 7.51 (d, *J* 6.88, 2H, ArH), 7.46–7.44 (m, 4H, ArH), 7.29 (t, *J* 8.24, 2H, ArH), 5.62 (t, *J* 8.72, 1H, CH), 3.62 (dd, *J* 10.08, 18.32, 1H, CH*H*CO), 3.24 (dd, *J* 8.24, 17.88, 1H, CH*H*CO). δ_C (100 MHz, DMSO) 166.05, 158.41, 153.28, 145.63, 130.84, 130.50 (d, $^3J_{C-F}$ 7.62), 129.11, 128.81 (d, $^2J_{C-F}$ 20.97), 126.57, 126.07 (d, $^3J_{C-F}$ 11.45), 124.89, 115.75 (d, $^2J_{C-F}$ 20.02) 52.96, 41.25. *m/z* (ESI) 348.17 ([M + Na]$^+$). Anal. Calc. for $C_{17}H_{12}FN_3O_3$ 325.09.

*2-Phenyl-7-(2-(trifluoromethyl)phenyl)dihydropyrazolo[1,2-a][1,2,4]triazole-1,3,5(2H)-trione (**2l**)*

White solid. Yield: 81 %. Mp 232–234°C. ν_{max} (KBr)/cm^{-1} 2928, 1752, 1728. δ_H (400 MHz, $CDCl_3$) 7.84–7.82 (m, 1H, ArH), 7.72–7.69 (m, 2H, ArH), 7.57–7.47 (m, 6H, ArH), 5.86 (t, *J* 8.04, 1H, CH), 3.65 (dd, *J* 9.52, 18.32, 1H, CH*H*CO), 3.07 (dd, *J* 7.32, 18.32, 1H, CH*H*CO). δ_C (100 MHz, $CDCl_3$) 162.70, 151.01, 143.75, 142.21, 136.14, 133.32, 132.15, 130.20, 129.37, 129.14, 129.00, 128.02, 126.61, 125.53, 54.84, 44.04. *m/z* (ESI) 398.21 ([M + Na]$^+$). Anal. Calc. for $C_{18}H_{12}F_3N_3O_3$ 375.08.

*7-(2-Chlorophenyl)-2-phenyldihydropyrazolo[1,2-a][1,2,4]triazole-1,3,5(2H)-trione (**2m**)*

White solid. Yield: 82 %. Mp 170–172°C. ν_{max} (KBr)/cm^{-1} 2927, 1752, 1719. δ_H (400 MHz, $CDCl_3$) 7.56 (d, *J* 7.32, 1H, ArH), 7.46–7.31 (m, 8H, ArH), 5.78 (t, *J* 8.24, 1H, CH), 3.78 (dd, *J* 10.08, 18.76, 1H, CH*H*CO), 3.09 (dd, *J* 7.80, 18.80, 1H, CH*H*CO). δ_C (100 MHz, $CDCl_3$) 163.22, 150.69, 143.88, 134.66, 131.84, 130.39, 130.30, 129.35, 128.94, 127.80, 127.23, 125.60, 55.98, 41.91. *m/z* (ESI) 364.18 ([M + Na]$^+$), 366.18 ([M + Na + 2]$^+$). Anal. Calc. for $C_{17}H_{12}ClN_3O_3$ 341.06.

*2-Phenyl-7-(3-(trifluoromethyl)phenyl)dihydropyrazolo[1,2-a][1,2,4]triazole-1,3,5(2H)-trione (**2n**)*

White solid. Yield: 85 %. Mp 154–156°C. ν_{max} (KBr)/cm^{-1} 2927, 1762. δ_H (400 MHz, $CDCl_3$) 7.67–7.54 (m, 4H, ArH), 7.43–7.38 (m, 5H, ArH), 5.51 (t, *J* 8.92, 1H, CH), 3.59 (dd, *J* 9.64, 18.36, 1H, CH*H*CO), 3.14 (dd, *J* 8.24, 18.32, 1H, CH*H*CO). δ_C (100 MHz, $CDCl_3$) 163.13, 151.63, 143.96, 138.17, 130.17, 130.02, 129.49, 129.38, 129.01, 126.07, 125.59, 123.11, 58.04, 43.01. *m/z* (ESI) 398.09 ([M + Na]$^+$). Anal. Calc. for $C_{18}H_{12}F_3N_3O_3$ 375.08.

*2-Phenyl-7-(4-(trifluoromethyl)phenyl)dihydropyrazolo[1,2-a][1,2,4]triazole-1,3,5(2H)-trione (**2o**)*

White solid. Yield: 79 %. Mp 138–140°C. ν_{max} (KBr)/cm^{-1} 2925, 1669, 772. δ_H (400 MHz, $CDCl_3$) 7.68 (s, 2H, ArH), 7.56 (s, 2H, ArH), 7.42 (s, 5H, ArH), 5.59 (s, 1H, CH), 3.65 (s, 1H, CH*H*CO), 3.11 (s, 1H, CH*H*CO). δ_F (376 MHz, DMSO) −62.66 (s, 3F, $ArCF_3$). δ_C (100 MHz, $CDCl_3$) 163.39, 151.53, 144.07, 141.05, 130.18, 129.39, 129.00, 126.78, 126.42, 125.85, 58.40, 43.57. *m/z* (ESI) 398.46 ([M + Na]$^+$). Anal. Calc. for $C_{18}H_{12}F_3N_3O_3$ 375.08.

*7-(4-Isopropylphenyl)-2-phenyldihydropyrazolo[1,2-a][1,2,4]triazole-1,3,5(2H)-trione (**2p**)*

White solid. Yield: 77 %. Mp 175–177°C. ν_{max} (KBr)/cm^{-1} 2961, 1706, 1677. δ_H (400 MHz, $CDCl_3$) 7.46–7.43 (m, 4H, ArH), 7.37–7.32 (m, 2H, ArH), 7.20 (s, 3H, ArH), 5.74 (t, *J* 5.16, 1H, CH), 3.32 (dd, *J* 6.60, 17.56, 1H, CH*H*CO), 3.12 (dd, *J* 5.12, 17.60, 1H, CH*H*CO), 2.92–2.85 (m, 1H, C*H*(CH_3)$_2$), 1.23 (d, *J* 7.32, 6H, CH_3). δ_C (100 MHz, $CDCl_3$) 175.57, 153.74, 152.13, 149.41, 132.68, 130.91, 129.20, 128.49, 127.31, 127.07, 125.72, 55.38, 36.84, 33.75, 23.84. *m/z* (ESI) 350.39 ([M + H]$^+$). Anal. Calc. for $C_{20}H_{19}N_3O_3$ 349.14.

*7-(2-Bromo-4-fluorophenyl)-2-phenyldihydropyrazolo[1,2-a][1,2,4]triazole-1,3,5(2H)-trione (**2q**)*

White solid. Yield: 79 %. Mp 224–226°C. ν_{max} (KBr)/cm^{-1} 2926, 1764, 1720. δ_H (400 MHz, $CDCl_3$) 7.58 (s, 1H, ArH), 7.48 (s, 4H, ArH), 7.42 (s, 1H, ArH), 7.36–7.34 (m, 1H, ArH), 7.70 (s, 1H, ArH), 5.71 (t, *J* 8.08, 1H, CH), 3.82 (dd, *J* 9.52, 18.32, 1H, CH*H*CO), 3.04 (dd, *J* 8.08, 18.32, 1H, CH*H*CO). δ_C (100 MHz, $CDCl_3$) 162.76, 161.10, 151.17, 143.87, 135.01 (d, $^3J_{C-F}$ 8.59), 130.14, 129.43, 129.10, 125.61, 117.75 (d, $^2J_{C-F}$ 22.88), 114.85, 114.54, 57.66, 41.81. *m/z* (ESI) 425.96 ([M + Na]$^+$). Anal. Calc. for $C_{17}H_{11}BrFN_3O_3$ 403.00.

*7,7′-(1,4-Phenylene)bis(2-phenyldihydropyrazolo[1,2-a][1,2,4]triazole-1,3,5(2H)-trione) (**2r**)*

White solid. Yield: 84 %. Mp 154–156°C. ν_{max} (KBr)/cm^{-1} 2929, 1752, 1720. δ_H (400 MHz, $CDCl_3$) 7.53 (s, 2H, ArH), 7.48–7.38 (m, 12H, ArH), 5.51 (t, *J* 9.52, 2H, CH), 3.61–3.54 (m, 2H, CH*H*CO), 3.21–3.14 (m, 2H, CH*H*CO). δ_C (100 MHz, $CDCl_3$ + DMSO) 171.02, 152.25, 136.95, 130.96, 129.17, 128.11, 127.13, 128.84, 55.64, 35.80. *m/z* (ESI) 559.87 ([M + Na]$^+$). Anal. Calc. for $C_{28}H_{20}N_6O_6$ 536.14.

Antimicrobial Assay

Test Microorganisms

A total of six microbial strains were selected on the basis of their clinical importance in causing diseases in humans. Two Gram-positive bacteria (*Staphylococcus aureus* MTCC 96 and *Bacillus subtilis* MTCC 121), two Gram-negative bacteria (*Escherichia coli* MTCC 1652 and *Pseudomonas aeruginosa* MTCC 741), and two fungi, *Aspergillus niger* and *A. flavus*, the ear pathogens isolated from the patients of Kurukshetra,[18] were used in the present study for evaluation of antimicrobial activity of the chemical compounds. All the bacterial cultures were procured from Microbial Type Culture Collection (MTCC), IMTECH, Chandigarh. The bacteria were subcultured on Nutrient agar whereas fungi were grown on Sabouraud's dextrose agar.

Antibacterial Activity

The antibacterial activity of 18 chemical compounds was evaluated by the agar well diffusion method. All the microbial cultures were adjusted to 0.5 McFarland standard, which is visually comparable to a microbial suspension of $\sim 1.5 \times 10^6$ cfu mL^{-1}. Mueller Hinton agar medium (20 mL) was poured into each Petri plate and plates were swabbed with a 100 µL inocula of the test microorganisms and kept for 15 min for adsorption. Using a sterile cork borer of 8 mm diameter, wells were bored into the seeded agar plates and these were loaded with 100 µL of 2.0 mg mL^{-1} of each compound reconstituted in dimethyl sulfoxide (DMSO). All the plates were incubated at 37°C for 24 h. The antibacterial activity of each compound was evaluated by measuring the zone of growth inhibition against the test organisms with a zone reader (HiAntibiotic zone scale). DMSO was used as a negative control whereas Ciprofloxacin was used as a positive control. This procedure was performed in three replicate plates for each organism and the mean values of the diameter of inhibition zones ± standard deviations were calculated.[19]

Determination of the MIC of Chemical Compounds

The MIC is the lowest concentration of an antimicrobial compound that will inhibit the visible growth of a microorganism after overnight incubation. The MIC of the various compounds against bacterial and yeast strains was tested through a macrodilution tube method.[20] In this method, a 2-fold serial dilution of each chemically synthesized compound was prepared by first reconstituting the compound in DMSO followed by dilution in Nutrient broth (bacteria) and Malt yeast broth (yeast) to achieve a decreasing concentration range of 512 to 0.5 µg mL^{-1} in the sterile tubes. Each dilution was seeded with 100 µL of the standardized microbial inoculum (1.5×10^6 cfu mL^{-1}). The inoculated culture tubes were incubated at 37°C for 24 h. A set of tubes containing only broth was kept as control. Afterwards, incubation tubes were examined for changes in turbidity as an indicator of growth. Ciprofloxacin and amphotericin B were used as a positive control while DMSO was a negative control.

Antifungal Activity

The antifungal activity of 18 chemical compounds was evaluated by a poisoned food technique.[21] The moulds were grown on Sabouraud dextrose agar (SDA) at 25°C for 7 days and used as inocula. The 15 mL of molten SDA (45°C) was poisoned by the addition of 100 µL of each compound having a concentration of 2.0 mg mL^{-1} reconstituted in DMSO, poured into a sterile Petri plate, and allowed to solidify at room temperature. The solidified poisoned agar plates were inoculated at the centre with fungal plugs (8 mm diameter) obtained from the colony margins and incubated at 25°C for 7 days. DMSO was used as the negative control whereas Fluconazole was used as the positive control. The experiments were performed in triplicate. The diameter of fungal colonies was measured and expressed as percent myelial inhibition.

$$\text{Percent inhibition of myelial growth} = [(dc - dt)/dc] \times 100$$

where *dc* is the average diameter of the fungal colony in the negative control sets and *dt* is the average diameter of the fungal colony in the experimental sets.

Supplementary Material

Copies of ^{1}H, ^{19}F, and ^{13}C NMR spectra are available on the Journal's website.

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