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Synthesis of 1,2,3-triazole incorporated monocarbonyl curcumin analogues as potent antitubercular, antifungal and antioxidant agents

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Abstract: Curcumin is an active component of turmeric with potent therapeutic properties, but it is limited due to its poor solubility, stability and bioavailability. To enhance its efficacy, we designed a series of twelve dimeric 1,2,3-triazoles incorporated monocarbonyl curcumin analogue (**7a-l**) and evaluated for their *in vitro* antitubercular, antifungal and antioxidant activities against their respective strains. Most of the compounds shows good antitubercular as well as antioxidant activities. Among the newly synthesized series, compound **7h** was found as most potent antitubercular as well as antioxidant agent. The compound **7b**, **7i**, **7j** and **7l** were shows good antitubercular activity against *Mtb* H37Rv whereas, compound **7b**, **7i** and **7l** also shows good antioxidant activity. In support to activities, *in silico* ADME properties prediction have been also carried out in this study.

Keywords: Dimeric 1,2,3-triazoles, Monocarbonyl curcumin analogues, Antitubercular activity, Antioxidant activity, Antifungal activity.

Introduction

A “Golden Drug” from every kitchen of India is curcumin (*Curcuma longa*), commonly known as termeric. Curcumin and its analogues have been widely shows various therapeutic activities like wound healing capacity, anticancer, antibacterial, antifungal, antiviral, anti-inflammatory, antioxidant, antidiabetic and treats skin diseases [1]. Owing to the potential importance of curcumin derivatives as key moieties in drug discovery, the worldwide efforts have made in the last few decades by many researchers in the synthesis and bioevaluation

of various curcumin analogues. Chemically, curcumin is a bis-α, β-unsaturated β-diketones, that is generally limited due to its poor solubility, stability and bioavailability. To overcome these limitations, the diketo core moiety of curcumin has been modified in to its monocarbonyl analogues [2-5]. Literature survey also reveals that monocarbonyl curcumin analogues shows better activity as well as solubility as compared to curcumin analogues.

Similarly, the 1,2,3-triazoles conjugation also helps to increases the solubility, stability and infuse capability to readily interact with biological targets through hydrogen bonding and dipole interactions [6]. Among the various triazoles, the 1,4-disubstituted-1,2,3-triazole derivatives have been found to be promising drug core moiety [7]. It is also acts as bioisostere of amide bond due to which it can easily bind with high affinity to many biological targets [8]. It is also very easy to synthesize by Cu (I)-catalyzed 1,3-dipolar cycloaddition of azide and alkyne by using Sharpless and Meldal's greener approach of click chemistry [9]. Literature survey reveals that the 1,2,3-triazole scaffolds have shown various biological activities [10]. Because of these excellent biological as well as stable physical properties of 1,2,3-triazoles, many scientist looking forward to synthesize the therapeutic lead bearing 1,2,3-triazoles.

Tuberculosis (TB) is an ancient infectious lung disease caused by pathogenic bacterium *Mycobacterium tuberculosis* (*Mtb*) [11]. According to WHO, near about one third of world's population is infected by *Mtb* [12]. *Mtb* has a unique ability to resist against regularly used anti-TB drugs. Therefore, the available existing anti-TB drugs are unable to cure the TB. Hence, there is an urgent need to develop newer chemical entities to cure TB. Sometimes there is a co-infection of *Mtb* with opportunistic pulmonary fungal pathogen was observed at lungs [13]. In such cases there were no any clinical sign of existence observed by many physicians as it is hindered by TB [14]. Therefore, sometimes patient may lead to death even after TB was cured completely. Hence, there is a need for such promising anti-TB drugs that kills fungal pathogens too. During the treatment of tuberculosis due to intake of high doses of anti-TB drugs over a long period of time in body causes oxidative stress in TB patients. It is occurred due to the free radicals burst from activated macrophages and various anti-TB drugs [15]. The antioxidants are normally used to prevent the formation of free radicals or scavenge the generation of reactive oxygen species (ROS) [16]. Therefore, if the antitubercular drugs exhibits the antioxidant activity too then, it is possible to avoid the oxidative stress and ultimately pulmonary tissue inflammation in TB patients.

By considering the therapeutic significance of the above here, we have reported the synthesis of dimeric 1,2,3-triazoles incorporated monocarbonyl curcumin analogues by using various piperidin-4-ones as a source of monocrbonyl moiety and their antitubercular, antifungal and antioxidant activity evaluations. In addition to this, we have also performed *in silico* ADME properties prediction for newly synthesized compound.

Results and discussion

Chemistry

Molecular hybridization is a common strategy used for developing new therapeutic agents by uniting two or more active pharmacophores into a single molecular framework. Therefore, the designed scaffolds used for the synthesis of desired titled compounds were originated from molecular hybridization of pharmacophoric fragments of 1,2,3-triazole [17] and monocarbonyl analogues bearing 1,2,3-triazoles [18] as shown in **Figure 2**.

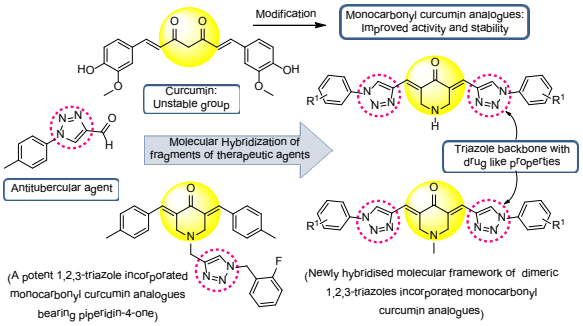
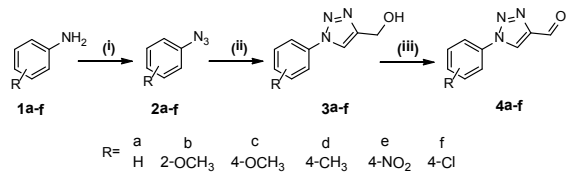


Figure 2. Molecular hybridization designing strategy for the synthesis of dimeric 1,2,3-triazoles incorporated monocarbonyl curcumin analogues.

The newly designed scaffold was broadly divided into three segments that is dimeric triazoles, monocarbonyl linker and substitutional variant unit. The dimeric 1,2,3-triazoles acts as a main backbone of the newly designed compounds. It shows drug like properties with increase in metabolic stability, water solubility and infuse capability to interact with biological targets through hydrogen bonding and dipole interactions. The monocarbonyl linker of piperidin-4-ones from newly designed compound shows modified structure of curcumin analogues that improves the properties of activity as well as stability of compounds as compared to curcumin. The substitutinal variant units helps to control lipophilicity and shows pharmacophoric properties.

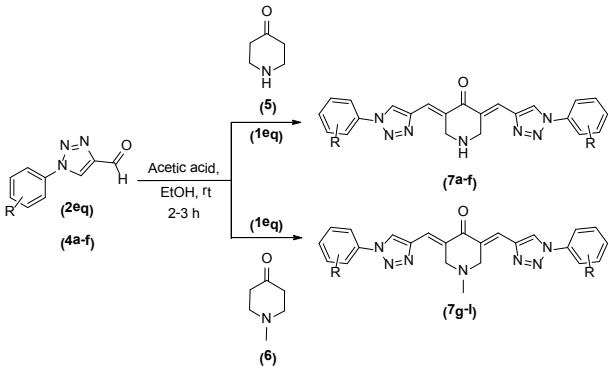
As per the designed strategy, we have planned and synthesized the titled compounds (**7a-l**) *via* multistep synthetic route. Before the synthesis of final titled compounds, the starting material, 1-phenyl-1H-1,2,3-triazole-4-carbaldehydes (**4a-f**) were synthesized by using reported procedure [19] as depicted in **Scheme 1**.



Reagents and conditions: (i) $NaNO_2$, HCl, 0 °C; NaN_3, 2-4 h, rt; (ii) propargyl alcohol, $CuSO_4$, sodium ascorbate, PEG-400:H_2O (1:1), 25 h, rt; (iii) CrO_3:2Py, CH_2Cl_2, 3 h, rt.

Scheme 1. Synthetic route for 1-phenyl-1H-1,2,3-triazole-4-carbaldehydes.

The freshly synthesized 1-phenyl-1H-1,2,3-triazole-4-carbaldehydes (**4a-f**) were crystallised and then the pure form of compounds as a starting material was allowed to react with piperidin-4-ones (**5, 6**) in ethanol and acetic acid at room temperature to form yellow solids with good yields (**Scheme 2**). The compounds with turmeric yellow colour is one of the characteristics that confirms the formation of titled curcumin analogues (**7a-l**).



Scheme 2. Synthesis of dimeric 1,2,3-triazoles incorporated monocarbonyl curcumin analogues using various piperidin-4-ones (**7a-l**)

All the compounds have been characterized using ^{1}H NMR, ^{13}C NMR, IR and HRMS spectral analysis for the more confirmation of the synthesized compounds. One of the representative ^{1}H NMR spectrum of compound (**7i**), displays peaks, at δ 2.25, 3.86 and 9.04 ppm as a singlet, due to the N-CH_3, O-CH_3 and triazoly-H, respectively. The presence of three characteristic carbon signals are observed at δ 25.6, 53.3 and 194.4 ppm in ^{13}C NMR spectrum of (**7i**) owing to the signals of N-CH_3, O-CH_3 and carbonyl carbon, respectively. The HRMS

spectrum further strengthen the structure assigned to (**7i**) as it displays $[M+H]^+$ ion peak at m/z 484.2120 for the molecular formula $C_{26}H_{26}N_7O_3$ and equivalent to the calculated $[M+H]^+$ ion peak at 484.2019.

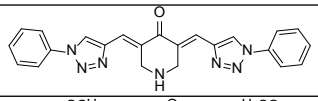
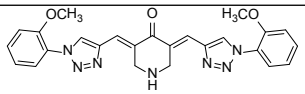
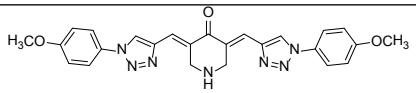
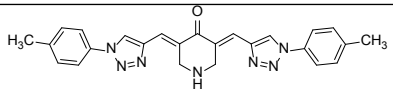
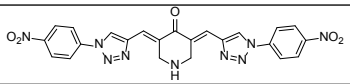
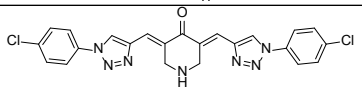
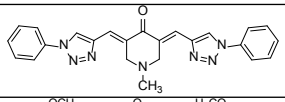
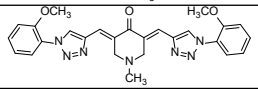
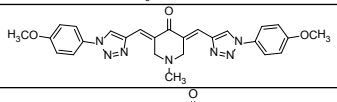
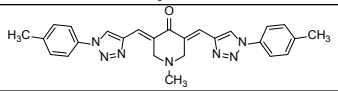
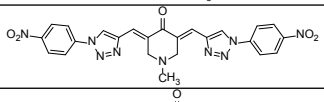
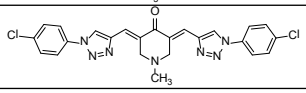
Biological assay

All the newly synthesized dimeric 1,2,3-triazoles incorporated monocarbonyl curcumin analogues (**7a-l**) were evaluated for their *in vitro* antitubercular, antifungal and antioxidant activity against their respective strains. The results of activities were compared with respective standards used for its comparative studies.

In vitro antitubercular activity

All the titled compounds were evaluated for

Table 1. *In vitro* antitubercular activity of compounds (**7a-l**)

Entry	Structures	MIC (µg/mL)
7a		>25
7b		**12.5**
7c		>25
7d		>25
7e		>25
7f		>25
7g		**25**
7h		**6.25**
7i		**12.5**
7j		**12.5**
7k		>25
7l		**12.5**
Isoniazid		**0.1**
Rifampicin		**0.2**
Ethambutol		**1.56**
Ciprofloxacin		**1.56**

their *in vitro* antitubercular activity against *Mtb* H37Rv by using Microplate Almar Blue Assay (MABA) [20]. The obtained results were compared with the standard drugs Isoniazid, Rifampicin, Ethambutol and Ciprofloxacin as shown in **Table 1**. It was observed that, compound **7h** was the most potent antitubercular agent among the newly synthesized series with MIC 6.25 µg/mL.

Other than compound **7h** from the newly synthesized series, the compounds **7b**, **7i**, **7j** and **7l** were also displayed promising antitubercular activity with MIC 12.5 µg/mL. Compound **7g** was also found as potent antitubercular agent with MIC 25 µg/mL. From the obtained results, it can be found as the curcumin analogues bearing 1-methylpiperidin-4-one shows better antitubercular activity than piperidin-4-one bearing curcumin analogues. It was also observed that there is effect of substitutional variant group on the activity results. The compounds having 2-OCH_3 group found as most potent antitubercular agents synthesized from both types of curcumin analogues that is either piperidin-4-one or 1-methylpiperidin-4-one. The compounds having 4-OCH_3, 4-CH_3 and 4-Cl variant groups synthesized from 1-methylpiperidin-4-one were also found to be potent antitubercular agent.

From the above results, most of the compounds shows potency and therefore, it offers an attractive lead series for the discovery of new therapeutic antitubercular agents in future by generating more similar analogues of synthesized compounds.

In vitro antifungal and antioxidant activity

All the synthesized compounds were screened for their *i*n vitro antifungal activity against four different human pathogenic fungal strains by using standard agar dilution method [21] and the obtained results were compared with standard antifungal drug, Miconazole. All the compounds have been also evaluated for antioxidant activity by using DPPH radical scavenging method [16] and the results were compared with BHT. All the antifungal as well as antioxidant activity results were well summarised in **Table 2**.

Table 2. In vitro antifungal and antioxidant activity of synthesized compounds (**7a-l**)

Compounds	MIC (µg/mL) against fungal strains				IC_{50} (µg/mL) DPPH Scavenging antioxidant activity
	CA	***FO***	***AF***	***AN***	
7a	228.1	249.4	*	*	20.40± 0.21
7b	179.2	164.4	172.9	131.7	**11.37± 0.34**
7c	166.2	139.4	187.3	159.3	19.61± 0.49
7d	138.4	203.0	169.1	175.1	18.20± 0.32
7e	244.1	*	*	209.7	26.36± 0.51
7f	*	*	*	*	30.18± 0.21
7g	*	238.2	*	**8**	34.23± 0.01
7h	*	*	*	*	**09.03± 0.14**
7i	131.1	117.4	216.3	206.4	**13.11± 0.00**
7j	171.3	166.2	177.3	192.5	19.62± 0.34
7k	137.2	160.1	133.3	169.2	22.39± 0.21
7l	144.1	139.1	201.5	163.8	**15.46± 0.07**
Miconazole	**25**	**25**	**12.5**	**25**	NT
BHT	NT	NT	NT	NT	**16.47± 0.18**

CA: *Candida albicans* (NCIM 3471); FO: *Fusarium oxysporum* (NCIM 1332); AF: *Aspergillus flavus* (NCIM 539); AN: *Aspergillus niger* (NCIM 1196); NT: Not Tested; Note*- No activity up to 250 mg/mL; BHT: Butylated Hydroxy Toluene.

Among all the synthesized compounds none compound shows promising antifungal activity except compound **7g**, which shows potent antifungal activity against only one of the fungal strain *Aspergillus niger* with MIC 8 µg/mL. Therefore, the activity results reveals that compounds of the series didn't active as antifungal agents but most of the compounds from the series shows good antioxidant activity.

From **Table 2**, it was observed that compounds

7b, **7h**, **7i** and **7l** were shows good to better antioxidant activities as compared to BHT standard used for the comparative study, which having IC_{50} values ranging from 09.03-15.46 µg/mL. Among the potent antioxidant agents compound **7h** observed as most potent antioxidant agent with IC_{50} value 09.03 µg/mL. The next potent antioxidant agent from series was compound **7b** with IC_{50} value 11.37 µg/mL. Both the compounds **7h** and **7b** contains 2-OCH_3 variant group, synthesized from 1-methylpiperidin-4-one and piperidin-4-one, respectively. Similarly compounds **7i** (IC_{50} value 13.11 µg/mL) and **7l** (IC_{50} value 15.46 µg/mL) bearing 4-OCH_3 and 4-Cl variant groups, respectively synthesized from 1-methylpiperidin-4-one were shows good antioxidant activity. The obtained results reveals that most of the newly synthesized compounds can act as potent antioxidant agents.

In silico ADME prediction

The newly synthesized curcumin analogues have been also predicted for their in *silico* ADME properties. In this study, we have calculated molecular volume (MV), molecular weight (MW), logarithm of partition coefficient (mi LogP), number of hydrogen bond acceptors (n-ON), number of hydrogen bonds donors (n-OHNH), topological polar surface area (TPSA), number of rotatable bonds (n-ROTB) and Lipinski's rule of five [22] of all the newly synthesized compounds using Molinspiration online property calculation toolkit [23]. Absorption (% ABS) of all the derivatives of the series was also calculated by using following formula.

$$\% \text{ ABS} = 109-(0.345 \times \text{TPSA}) \text{ [24]}$$

Drug-likeness model score (a collective property of physico-chemical properties, pharmacokinetics and pharmacodynamics of a compound is represented by a numerical value) of each and every compound was computed by MolSoft software [25]. A molecule is considered to develop an orally active drug if the drug violates only one of the Lipinski's rule of five having following four valid criteria's: miLog P (octanol-water partition coefficient) ≤5, molecular weight ≤500, number of hydrogen bond acceptors ≤10 and number of hydrogen bond donors ≤5. The results of ADME predictions of all the newly synthesized curcumin analogues (**7a-l**) were summarised in **Table 3**.

[a]Percentage Absorption; [b]Topographical polar

Table 3. In silico ADME properties prediction of the compounds (**7a-l**)

Entry	% ABS[a]	TPSA[b] (A^2)	n-ROTB[c]	MV[d]	MW[e]	miLog[f]	n-ON[g]	n-OH NH[h]	Lipinski violations[i]	Drug likeness model score
Rule	-	-	-	-	<500	≤ 5	<10	<5	≤ 1	-
7a	77.77	90.53	4	361.36	409.45	1.85	8	1	0	-0.45
7b	71.40	109.00	6	412.45	469.50	2.29	10	1	0	-1.00
7c	71.40	109.00	6	412.45	469.50	1.97	10	1	0	-0.94
7d	77.77	90.53	4	394.48	437.51	2.75	8	1	0	-0.81
7e	46.15	182.18	6	408.03	499.45	1.77	14	1	1	-0.85
7f	77.77	90.53	4	388.43	478.34	3.21	8	1	0	-0.59
7g	46.15	81.75	4	378.30	423.48	2.45	8	0	0	0.44
7h	74.43	100.21	6	429.39	483.53	2.89	10	0	0	-0.43
7i	74.43	100.21	6	429.39	483.53	2.56	10	0	0	-0.30
7j	46.15	81.75	4	411.42	451.53	3.35	8	0	0	-0.16
7k	49.18	173.39	6	424.97	513.47	2.37	14	0	2	-0.11
7l	46.15	81.75	4	405.37	492.37	3.81	8	0	0	0.25

surface area; [c]Number of rotatable bonds; [d]Molecular volume; [e]Molecular Weight; [f]Lipophilicity; [g]No. of hydrogen bond acceptors; [h]No. of hydrogen bond acceptors; [i]Number of violations.

From the **Table 3**, it was observed that most of the property predictions were found in an acceptable range and possess average to good potential to develop orally active drug molecules. A molecule is considered to develop an orally active drug if the drug violates only one of the Lipinski's rule of five. All the compound of the series follows Lipinski's rule of five except compound **7k**. It was also observed that all the synthesized compounds exhibited moderate to good % ABS ranging from 46.15 to 77.77 %. Therefore, most of the compounds from series able to be an orally active drug in future.

Experimental

Materials and methods

All the chemicals were of laboratory grade and purchased from commercial suppliers Spectrochem, Rankem, Alfa Aesar, Sigma Aldrich were used without further purification. Purity and completion of reaction time of all synthesized compounds were monitored by thin layer chromatography (TLC) using silica gel 60-F_{254} precoated on aluminum sheets as an adsorbent, Merck, Germany and visualization was accomplished by iodine/ultraviolet light. Melting points of all the synthesized compounds were determined in open capillary tubes and are uncorrected. The IR spectra (In KBr pellets) were recorded on Brukar FT-IR spectrometer. ^{1}H NMR spectra were recorded on a Brukar DRX-300 and 300 MHz NMR spectrometer using tetramethylsilane (TMS) as an internal standard and chemical shifts are in δ (ppm). The splitting pattern abbreviations are designed as singlet (s); doublet (d); double doublet (dd); triplet (t); quartet (q) and multiplet (m). ^{13}C NMR spectra were recorded on a Brukar DRX-75 and 125 MHz NMR in $CDCl_3$/DMSO-d_6. High-resolution mass spectra (HRMS) were obtained using Agilent 6520 (Q-TOF) ESI-HRMS instrument.

General Procedure for synthesis of titled compounds (7a-l)

A mixture of 0.01 mol of piperidin-4-one **(5, 6)** and 0.02 mol of freshly prepared substituted 1,2,3-triazolyl aldehydes **(4a-f)** were stirred for 2-3 hours at room temperature in catalytic amount of acetic acid and ethanol. The progress of reaction was monitored by TLC. After completion of reaction, the reaction mass was poured on ice and obtained solid was filtered and dried well. The final products **(*7a-l*)** were purified by crystallization. The crystallization was carried out in EtOH:DMF (3:1) solvent system. All the 1,2,3-triazole incorporated curcumin analogues were obtained as yellowish crystals.

(*3E,5E*)-3,5-bis((1-phenyl-*1H*-1,2,3-triazol-4-yl)methylene)piperidin-4-one (7a): The compound (**7a**) was obtained by acid catalyzed reaction between freshly synthesized starting material (**4a**) and piperidin-4-one (**5**) as creamish yellow solid; Yield: 78 %; MP: 235-237 °C; IR ν max/cm^{-1} 3446, 3159, 1646 (C=O); ^{1}H NMR (400 MHz, DMSO-d6, δ_H ppm) 2.01 (s, 1H, N-H), 3.78 (s, 4H, piperidin-4-one-CH_2), 7.40 (t, 2H, Ar-H), 7.55 (t, 4H, Ar-H), 7.62 (s, 2H, CH), 7.68 (d, 4H, Ar-H), 8.89 (s, 2H, triazolyl-H); ^{13}C NMR (100 MHz, $CDCl_3$, δ_C ppm) 56.4, 117.9, 119.5, 120.8, 121.9, 132.7, 133.6, 135.8, 160.2, 188.5; HRMS (ESI) calcd. For $C_{23}H_{20}N_7O$ $[M+H]^+$: 410.1651; found: 410.1687.

(*3E,5E*)-3,5-bis((1-(2-methoxyphenyl)-*1H*-1,2,3-triazol-4-yl)methylene)piperidin-4-one (7b): The compound (**7b**) was obtained by acid catalyzed reaction between freshly synthesized

starting material (**4b**) and piperidin-4-one (**5**) as pale yellow solid; Yield: 81 %; MP: 205-207 °C; IR ν max/cm^{-1} 3454, 3123, 1655 (C=O); ^{1}H NMR (400 MHz, DMSO-d6, δ_H ppm) 2.12 (s, 1H, N-H), 3.93 (s, 4H, piperidin-4-one-CH_2), 4.73 (s, 6H, O-CH_3), 7.19-7.88 (m, 8H, Ar-H), 7.56 (s, 2H, CH), 8.97 (s, 2H, triazolyl-H); ^{13}C NMR (100 MHz, $CDCl_3$, δ_C ppm) 52.4, 61.3, 118.6, 120.6, 121.2, 123.4, 130.3, 133.6, 135.5, 156.6, 160.1, 162.6, 191.2; HRMS (ESI) calcd. For $C_{25}H_{24}N_7O_3$ $[M+H]^+$: 470.1862; found: 470.1870.

(*3E,5E*)-3,5-bis((1-(4-methoxyphenyl)-*1H*-1,2,3-triazol-4-yl)methylene)piperidin-4-one (7c): The compound (**7c**) was obtained by acid catalyzed reaction between freshly synthesized starting material (**4c**) and piperidin-4-one (**5**) as bright yellow solid; Yield: 83 %; MP: 244-234 °C; IR ν max/cm^{-1} 3446, 3159, 1646 (C=O); ^{1}H NMR (400 MHz, DMSO-d6, δ_H ppm) 2.13 (s, 1H, N-H), 3.95 (s, 4H, piperidin-4-one-CH_2), 4.81 (s, 6H, O-CH_3), 7.17 (d, 4H, Ar-H), 7.53 (s, 2H, CH), 7.77 (d, 4H, Ar-H), 8.96 (s, 2H, triazolyl-H); ^{13}C NMR (100 MHz, $CDCl_3$, δ_C ppm) 52.4, 61.2, 118.8, 120.4, 121.5, 131.3, 133.7, 135.4, 158.2, 160.1, 192.1; HRMS (ESI) calcd. For $C_{25}H_{24}N_7O_3$ $[M+H]^+$: 470.1862; found: 470.1874.

(*3E,5E*)-3,5-bis((1-(p-tolyl)-*1H*-1,2,3-triazol-4-yl)methylene)piperidin-4-one (7d): The compound (**7d**) was obtained by acid catalyzed reaction between freshly synthesized starting material (**4d**) and piperidin-4-one (**5**) as dark yellow solid; Yield: 80 %; MP: 207-209 °C; IR ν max/cm^{-1} 3446, 3159, 1646 (C=O); ^{1}H NMR (400 MHz, DMSO-d6, δ_H ppm) 2.09 (s, 1H, N-H), 3.46 (s, 6H, CH_3), 3.92 (s, 4H, piperidin-4-one-CH_2), 7.18 (d, 4H, Ar-H), 7.57 (s, 2H, CH), 7.86 (d, 4H, Ar-H), 9.01 (s, 2H, triazolyl-H); ^{13}C NMR (100 MHz, $CDCl_3$, δ_C ppm) 38.1, 52.4, 60.3, 119.1, 120.7, 121.2, 131.3, 133.8, 135.7, 160.1, 192.4; HRMS (ESI) calcd. For $C_{25}H_{24}N_7O$ $[M+H]^+$: 438.1964; found: 438.1972.

(*3E,5E*)-3,5-bis((1-(4-nitrophenyl)-*1H*-1,2,3-triazol-4-yl)methylene)piperidin-4-one (7e): The compound (**7e**) was obtained by acid catalyzed reaction between freshly synthesized starting material (**4e**) and piperidin-4-one (**5**) as dark yellow solid; Yield: 80%; MP: >270 °C; IR ν max/cm^{-1} 3442, 3167, 1658 (C=O); ^{1}H NMR (400 MHz, DMSO-d6, δ_H ppm) 2.11 (s, 1H, N-H), 3.96 (s, 4H, piperidin-4-one-CH_2), 7.23 (d, 4H, Ar-H), 7.61 (s, 2H, CH), 7.89 (d, 4H, Ar-H), 9.13 (s, 2H, triazolyl-H); ^{13}C NMR (100 MHz, $CDCl_3$, δ_C ppm) 53.8, 60.6, 120.5, 120.9, 130.7, 132.9, 135.1, 157.1, 162.8, 194.4; HRMS (ESI) calcd. For $C_{23}H_{18}N_9O_5$ $[M+H]^+$: 500.1353; found: 500.1361.

(*3E,5E*)-3,5-bis((1-(4-chlorophenyl)-*1H*-1,2,3-triazol-4-yl)methylene)piperidin-4-one (7f): The compound (**7f**) was obtained by acid catalyzed reaction between freshly synthesized starting material (**4f**) and piperidin-4-one (**5**) as dark yellow solid; Yield: 85 %; MP: >270 °C; IR ν max/cm^{-1} 3449, 3161, 1650 (C=O); ^{1}H NMR (400 MHz, DMSO-d6, δ_H ppm) 2.06 (s, 1H, N-H), 4.16 (s, 4H, piperidin-4-one-CH_2), 7.33 (d, 4H, Ar-H), 7.60 (s, 2H, CH), 7.86 (d, 4H, Ar-H), 9.12 (s, 2H, triazolyl-H); ^{13}C NMR (100 MHz, $CDCl_3$, δ_C ppm) 55.8, 61.2, 120.8, 121.0, 130.2, 132.8, 135.7, 158.1, 163.0, 190.9; HRMS (ESI) calcd. For $C_{23}H_{18}Cl_2N_7O$ $[M+H]^+$: 478.0872; found: 478.0883.

(*3E,5E*)-1-methyl-3,5-bis((1-phenyl-*1H*-1,2,3-triazol-4-yl)methylene)piperidin-4-one (7g): The compound (**7g**) was obtained by acid catalyzed reaction between freshly synthesized starting material (**4a**) and 1-methylpiperidin-4-one (**6**) as light yellow solid; Yield: 87 %; MP: 217-221 °C; IR ν max/cm^{-1} 3150, 1656 (C=O); ^{1}H NMR (400 MHz, DMSO-d6, δ_H ppm) 2.22 (s, 3H, N-CH_3), 3.98 (s, 4H, piperidin-4-one-CH_2), 7.43 (t, 2H, Ar-H), 7.57 (t, 4H, Ar-H), 7.63 (s, 2H, CH), 7.69 (d, 4H, Ar-H), 8.99 (s,

2H, triazolyl-H); ^{13}C NMR (100 MHz, $CDCl_3$, δ_C ppm) 26.7, 58.4, 119.7, 121.5, 121.8, 123.1, 133.2, 133.9, 136.3, 162.2, 190.4; HRMS (ESI) calcd. For $C_{24}H_{22}N_7O$ $[M+H]^+$: 424.1808; found: 424.1811.

(*3E,5E*)-3,5-bis((1-(2-methoxyphenyl)-*1H*-1,2,3-triazol-4-yl)methylene)-1-methylpiperidin-4-one (7h): The compound (**7h**) was obtained by acid catalyzed reaction between freshly synthesized starting material (**4b**) and 1-methylpiperidin-4-one (**6**) as light yellow solid; Yield: 80 %; MP: 167-170 °C; IR ν max/cm^{-1} 3132, 1647 (C=O); ^{1}H NMR (400 MHz, DMSO-d6, δ_H ppm) 2.25 (s, 3H, N-CH_3), 4.01 (s, 4H, piperidin-4-one-CH_2), 4.77 (s, 6H, O-CH_3), 7.21-7.85 (m, 8H, Ar-H), 7.53 (s, 2H, CH), 8.99 (s, 2H, triazolyl-H); ^{13}C NMR (100 MHz, $CDCl_3$, δ_C ppm) 27.1, 52.8, 61.7, 118.8, 120.3, 121.5, 123.2, 130.7, 133.5, 135.8, 156.7, 160.1, 162.4, 190.0; HRMS (ESI) calcd. For $C_{26}H_{26}N_7O_3$ $[M+H]^+$: 484.2019; found: 484.2123.

(*3E,5E*)-3,5-bis((1-(4-methoxyphenyl)-*1H*-1,2,3-triazol-4-yl)methylene)-1-methylpiperidin-4-one (7i): The compound (**7i**) was obtained by acid catalyzed reaction between freshly synthesized starting material (**4c**) and 1-methylpiperidin-4-one (**6**) as bright yellow solid; Yield: 73 %; MP: 235-257 °C; IR ν max/cm^{-1} 3150, 1658 (C=O); ^{1}H NMR (400 MHz, DMSO-d6, δ_H ppm) 2.25 (s, 3H, N-CH_3), 3.97 (s, 4H, piperidin-4-one-CH_2), 4.86 (s, 6H, O-CH_3), 7.19 (d, 4H, Ar-H), 7.59 (s, 2H, CH), 7.87 (d, 4H, Ar-H), 9.04 (s, 2H, triazolyl-H); ^{13}C NMR (100 MHz, $CDCl_3$, δ_C ppm) 25.6, 53.4, 60.9, 118.5, 120.2, 121.6, 130.3, 133.6, 135.1, 157.5, 162.8, 194.4; HRMS (ESI) calcd. For $C_{26}H_{26}N_7O_3$ $[M+H]^+$: 484.2019; found: 484.2120.

(*3E,5E*)-1-methyl-3,5-bis((1-(p-tolyl)-*1H*-1,2,3-triazol-4-yl)methylene)piperidin-4-one (7j): The compound (**7j**) was obtained by acid catalyzed reaction between freshly synthesized starting material (**4d**) and 1-methylpiperidin-4-one (**6**) as bright yellow solid; Yield: 77 %; MP: 256-258 °C; IR ν max/cm^{-1} 3149, 1651 (C=O); ^{1}H NMR (400 MHz, DMSO-d6, δ_H ppm) 2.32 (s, 3H, N-CH_3), 3.49 (t, 6H, CH_3), 3.90 (s, 4H, piperidin-4-one-CH_2), 7.18 (d, 4H, Ar-H), 7.47 (s, 2H, CH), 7.89 (d, 4H, Ar-H), 9.03 (s, 2H, triazolyl-H); ^{13}C NMR (100 MHz, $CDCl_3$, δ_C ppm) 27.5, 37.8, 53.4, 61.7, 119.3, 120.7, 121.4, 131.5, 133.8, 136.1, 160.5, 191.2; HRMS (ESI) calcd. For $C_{26}H_{26}N_7O$ $[M+H]^+$: 452.2121; found: 452.2127.

(*3E,5E*)-1-methyl-3,5-bis((1-(4-nitrophenyl)-*1H*-1,2,3-triazol-4-yl)methylene) piperidin-4-one (7k): The compound (**7k**) was obtained by acid catalyzed reaction between freshly synthesized starting material (**4e**) and 1-methylpiperidin-4-one (**6**) as dark yellow solid; Yield: 81 %; MP: 260-263 °C; IR ν max/cm^{-1} 3170, 1653 (C=O); ^{1}H NMR (400 MHz, DMSO-d6, δ_H ppm) 2.14 (s, 3H, N-CH_3), 3.93 (s, 4H, piperidin-4-one-CH_2), 7.21 (d, 4H, Ar-H), 7.63 (s, 2H, CH), 7.88 (d, 4H, Ar-H), 9.10 (s, 2H, triazolyl-H); ^{13}C NMR (100 MHz, $CDCl_3$, δ_C ppm) 29.1, 52.9, 61.3, 121.5, 121.9, 131.1, 132.9, 136.1, 158.2, 162.4, 190.1; HRMS (ESI) calcd. For $C_{24}H_{20}N_9O_5$ $[M+H]^+$: 514.1509; found: 514.1513.

(*3E,5E*)-3,5-bis((1-(4-chlorophenyl)-*1H*-1,2,3-triazol-4-yl)methylene)-1-methylpiperidin-4-one (7l): The compound (**7l**) was obtained by acid catalyzed reaction between freshly synthesized starting material (**4f**) and 1-methylpiperidin-4-one (**6**) as bright yellow solid; Yield: 88 %; MP: 247-250 °C; IR ν max/cm^{-1} 3173, 1660 (C=O); ^{1}H NMR (400 MHz, DMSO-d6, δ_H ppm) 2.13 (s, 3H, N-CH_3), 3.96 (s, 4H, piperidin-4-one-CH_2), 7.20 (d, 4H, Ar-H), 7.59 (s, 2H, CH), 7.83 (d, 4H, Ar-H), 9.11 (s, 2H, triazolyl-H); ^{13}C NMR (100 MHz, $CDCl_3$, δ_C ppm) 28.3, 51.9, 60.8, 121.8, 122.3, 131.8, 132.3, 136.2, 158.7, 160.4, 192.9;

HRMS (ESI) calcd. For $C_{24}H_{20}Cl_2N_7O$ $[M+H]^+$: 492.1028; found: 492.1033.

Experimental protocol for biological activity

***In vitro* MTB MABA assay**

The antitubercular activity of newly synthesized Compounds **(3a-j)** have been screened for their *in vitro* effects against *Mtb* H37Rv (ATCC 27294) by using microplate Almar Blue assay (MABA) [20] for determination of MIC in triplicates. The MIC (in µg/mL) was recorded as the lowest concentration/highest dilution of the compounds/control drugs that completely inhibited the growth of MTB cultures. The MIC values of compounds **(3a-r)** have been compared with standard drugs (Rifampicin, Isoniazid, Ethambutol and Ciprofoxacin). The experimental method for antitubercular activity is briefly described as follows-

Initially, the inoculum was prepared from fresh LJ medium re-suspended in 7H9-S medium (7H9 broth, 0.1% casitone, 0.5% glycerol, supplemented oleic acid, albumin, dextrose, and catalase [OADC]), adjusted to a McFarland tube No. 1, and diluted 1:20; 100 µl was used as inoculum. Each drug stock solution was thawed and diluted in 7H9-S at four-fold the final highest concentration tested. Serial two-fold dilutions of each drug were prepared directly in a sterile 96-well microtiter plate using 100µl 7H9-S. A growth control containing no antibiotic and a sterile control were also prepared on each plate. Sterile water was added to all perimeter wells to avoid evaporation during the incubation. The plate was covered, sealed in plastic bags and incubated at 37°C in normal atmosphere. After 7 days incubation, 30 µl of alamar blue solution was added to each well, and the plate was re-incubated overnight. A change in colour from blue (oxidised state) to pink (reduced) indicated the growth of bacteria, and the MIC was defined as the lowest concentration of drug that prevented this change in colour.

***In vitro* Antifungal activity**

Antifungal activity was determined by standard agar dilution method as per CLSI (formerly, NCCLS) guidelines [21]. The synthesized compounds and standard miconazole were dissolved in DMSO solvent. The medium yeast nitrogen base was dissolved in phosphate buffer pH 7 and it was autoclaved at 110 °C for 10 min. With each set, a growth control without the antifungal agent and solvent control DMSO were included. The fungal strains were freshly subcultured onto Sabouraud dextrose agar (SDA) and incubated at 25 °C for 72 h. The fungal cells were suspended in sterile distilled water and diluted to get 105 cells/mL. Ten microliters of standardized suspension was inoculated onto the control plates and the media incorporated with the antifungal agents. The inoculated plates were incubated at 25 °C for 48 h. The readings were taken at the end of 48 and 72 h. The MIC was the lowest concentration of drug preventing growth of macroscopically visible colonies on drug containing plates when there was visible growth on the drug free control plates.

General procedure for (evaluation of DPPH radical scavenging) antioxidant activity

The antioxidant activity of all the synthesized compounds have been assessed *in vitro* by the 1,1-diphenyl-2- picrylhydrazyl (DPPH) radical scavenging assay [15] and the results were compared with standard synthetic antioxidant BHT (Butylated Hydroxy Toluene). The hydrogen atom or electron donation ability of the compounds were measured from the bleaching of the purple coloured methanol solution of 1,1-diphenyl-1-picrylhydrazyl (DPPH). The spectrophotometric assay uses the stable radical DPPH as a reagent. 1 mL of various concentrations of the test compounds (5, 10, 25, 50 and 100 mg/mL) in methanol was added

to 4mL of 0.004% (w/v) methanol solution of DPPH. The reaction mixture was incubated at 37 °C. The scavenging activity on DPPH was determined by measuring the absorbance at 517 nm after 30 min. All tests were performed in triplicate and the mean values were entered. The percent of inhibition (I %) of free radical production from DPPH was calculated by the following equation

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

Where, $A_{control}$ is the absorbance of the control (DPPH radical without test sample)

A_{sample} is the absorbance of the test sample (DPPH radical with test sample). The control contains all reagents except the test samples.

Conclusion

In conclusion, we reports synthesis and biological evaluation of new dimeric 1,2,3-triazoles incorporated monocarbonyl curcumin analogues using various piperidin-4-ones (**7a-l**). This series of hybrid compounds exhibited potent antitubercular and antioxidant agents. Among the newly synthesized series, compound **7h** was found as a most potent antitubercular as well as antioxidant agent with MIC value 6.25 µg/mL and IC_{50} value 09.03 µg/mL, respectively. The compounds **7b**, **7i**, **7j** and **7l** were shows good antitubercular activity with MIC value 12.5 µg/mL. Also the compounds **7b**, **7i** and **7l** were found as better antioxidant agents than BHT. Compound **7g** was observed as the only potent antifungal agent against one of the fungal strain *Aspergillus niger* with 12.5 µg/mL MIC. From the above activity results, it can be conclude that the library of newly synthesized titled compounds (**7a-l**) were offers an attractive lead series for the discovery of potent antitubercular as well as antioxidant agents.

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