RESEARCH ARTICLE

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Pharmacological Evaluation of Theophylline Containing Variant Acetylene Derivatives for Antimicrobial, Antioxidant Activities and Molecular Docking Studies

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ABSTRACT

Theophylline is structurally and pharmacologically similarity to theobromine and caffeine, and is readily found in nature, being present in tea and cocoa. In our present work, we studied in vitro antimicrobial and anti-oxidant activity of ten compounds of theophylline containing acetylene by standard methods. Anti-bacterial activity was studied by agar plate method and minimum inhibitory concentration (MIC) method, anti-fungal studied by MIC method. Anti-oxidants by 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) and nitric oxide (NO) Scavenging activity, further docking was performed using Auto Dock software.

Anti-bacterial activity was performed on ten compounds, compound 2, 4, 5, 15, and 19 showed the MIC of 9.375 µg/mL equal to standard oxytetracycline. Among the anti-fungal activity of the ten compounds, compound 2, 4, 5, 15, and 19 shows the good antifungal activity with MIC of 9.375 µg/mL. DPPH scavenging activity was studied in ten compounds with 5 concentrations from 5-25 µg/mL with ascorbic acid as a standard. Highest concentration of 25 µg/mL of ascorbic acid showed the % of inhibition 95.13 and compound 19 showed the better % of inhibition of 84.97 followed by compound 5 and compound 15. NO was estimated using Griess Illosvosy reaction were compound 19 showed the better % of inhibition of 86.57 followed by compound 2 and 5. In ABTS, compound 4 showed the better % of inhibition of 84.36 followed by compound 15 and 19. The docking studies demonstrated that the compounds 2, 4, 5, 15, and 19 exhibited good binding affinities towards target protein. Based on the present in-vitro studies we conclude that theophylline containing acetylenes compounds are the promising agents for anti-microbial and anti-oxidant. Further in-vivo studies are needed to use these compounds in any of the applications.

Keywords: Acetylene derivatives, Antibacterial, Antifungal, Antioxidant, Molecular docking, Theophylline.

1. INTRODUCTION

In medicinal chemistry and organic synthesis, acetylenes are the most valuable and critical elegance of compounds. The metabolites of acetylenes belong to a class of molecules having the triple bonds and they are determined in fungi, flora, marine invertebrates, and microorganisms.[1] Unfortunately, most natural products themselves are not suitable for administration as drugs. But natural products served as an effective source of drug throughout the history.[2] Theophylline was selected as lead which belongs to the class called xanthine family, mainly found in the beans of cocoa and it is highly present in criollo cocoa beans to an extent of 3.7 mg/kg and is also found in brewed tea. It is reported to have a wide range of various biological activities such as treatment of respiratory diseases like asthma, chronic obstructive pulmonary disease (COPD), and other airway diseases for more than 75 years. Theophylline containing acetylene derivatives have been reported as dipeptidyl peptidase

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4 (DPP-4) inhibitor with G protein-coupled receptor (GPR) 119 agonist particularly in the treatment of type 2 diabetes mellitus. Other biological activities possessed by theophylline includes anticancer, anti-microbial, and antidepressant activity.[3]

So, in the present work few of the theophylline containing acetylenes were studied for their antioxidant, antibacterial, and antifungal activity.

2. MATERIAL AND METHODS

Theophylline containing acetylene derivatives were obtained as a gift sample, which were prepared according to the procedure described by Ruddarraju *et al* (Table 1).[4]

The bacterial strains of *Staphylococcus aureus* (MTCC96), *Bacillus Subtilis* (MTCC220), *E. coli* (MTCC119), *Pseudomonas aeruginosa* (MTCC647) were obtained from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh.

Oxytetracycline and amphotericin B standard drugs for antibacterial and antifungal activity were obtained from Leo Bio Care Pvt Ltd, Kukatpally, Hyderabad, India.

The fungal strains of *Aspergillus Brasilienses* (ATCC10145) and *Candida albicans* (MTCC10145) were obtained from Horizon Biolabs Pvt Ltd, Balanagar, Hydereabad, India.

2.1. Media Preparation for Anti-bacterial Activity

2.1.1. Nutrient Agar Medium

Nutrient agar medium is most commonly used medium and the components are peptone (5.0 g), beef extract (3.0 g), agar (15.0 g), sodium chloride (5.0 g), distilled water (1000 mL), pH was adjusted to 7.0 (Table 2). After mixing the ingredients in to the distilled water it was melted in the water bath and sterilized by autoclaving at 15 lbs pressure of 121°C for 15 minutes.

Table 1: Structure of different compounds of theophylline containing acetylene derivatives

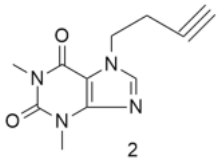
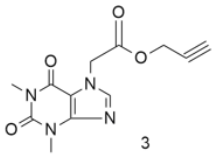
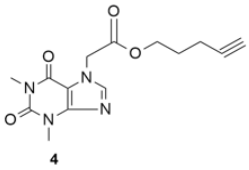
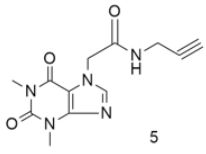
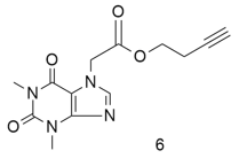
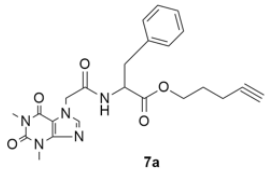
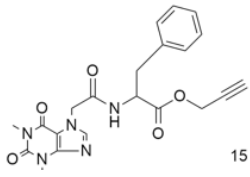
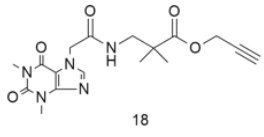
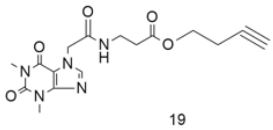
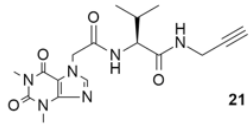
Structure with compound code	*IUPAC name*
 2	7-(but-3-yn-1-yl)-1-methyl-1H-purine 2,6(3H.7H)-dione
 3	prop-2-yn-1-yl 2-(1,3-dimethyl-2,6-dioxo-2.3-dihydro-1H-purin 7(6H)-yl)acetate
 4	but-3-yn-1-yl 2-(1-methyl-2,6-dioxo-2,3-dihydro-1H purin-7(6H)-yl)acetate
 5	2-(1,3-dimethyl-2,6-dioxo-2,3-dihydro-1H-purin-7(6H) yl)-N-(prop-2-yn-1-yl) acetamide
 6	but-3-yn-1-yl 2-(1,3-dimethyl-2,6-dioxo-2,3 dihydro-1H-purin-7(6H)-yl)acetate
 7a	pent-4-yn-1-yl 2-(2-(1,3-dimethyl-2,6-dioxo-2,3-dihydro-1H-purin-7(6H)-yl)acetamido)-3 phenylpropanoate
 15	prop-2-yn-1-yl 2-(2-(1,3-dimethyl-2,6-dioxo-2,3 dihydro-1H-purin-7(6H)-yl)acetamido)-3 phenylpropanoate
 18	7-(5,5-dimethyl-2,6-dioxodec-9-yn-1-yl)-1,3 dimethyl-1H-purine-2,6(3H 7H)-dione
 19	but-3-yn-1-yl 3-(2-(1,3-dimethyl-2,6-dioxo-2,3 dihydro-1H-purin-7(6H)-yl)acetamido) propanoate
 21	2-(1,3-dimethyl-2,6-dioxo-2,3-dihydro-1H purin-7(6H)-yl)acetic acid

Table 2: Media Composition for Nutrient Agar

Nutrient Agar	
Peptic digest of animal tissue	5.000
Sodium chloride	5.000
Beef extract	1.500
Yeast extract	1.500
Agar	15.000
Final pH (at 25°C)	7.4 ± 0.2

2.1.2. Preparation of Stock Solution

The stock culture of each organism was prepared by taking two nutrient agar slants and sub-culturing each confirmed test organism aseptically. One set slant was kept as stock culture and another as working set. The cultures of bacteria in their appropriate agar slants were stored at 4°C and used as stock cultures. One counter glycerol stock was also maintained at 20°C.

2.1.3. Inoculum Preparation

The selected bacterial pathogens were inoculated into nutrient broth (liquid medium) and incubated at 37°C for 24 hours and the suspensions were checked to provide approximately 10–5 CFU/mL.

2.2. Antibacterial Activity

2.2.1. Agar Well Diffusion Method

Anti-bacterial activity of compounds was studied by Agar well-diffusion method with four concentrations (12.5, 25, 50, and 100 µg), tested against different bacterial pathogens such as *S. aureus, B. Subtilis, E. coli, P. aeruginosa.* The plates were incubated at 37°C for 18–24 hours and at the end of the experiment the diameter of the inhibition zone (mm) was measured and the activity index was calculated. The readings were taken in three different fixed directions and the average values were recorded.

2.2.2. Minimum Inhibitory Concentration (MIC)

To determine the MIC, Serial dilution procedure was followed with five concentrations of test compounds of 6.25, 12, 25, 50, and 100 µg. For this, sterile tubes were labeled with concentration and dispensed with the 1 mL sterile media using sterile micropipette. After the addition of compound 100 µg/mL to tube 1, serial dilutions were made till tube 5 (6.5 µg/mL). Later we added the inoculum of 100 µL to each test tube. Same procedure was followed for all testing compounds. Test tubes were incubated at 37°C for 24 hours. All the results obtained were recorded in terms of MIC.

2.3. Antifungal Activity

The anti-fungal activity of compounds was studied by serial tube dilution method and calculated the MIC values using the RPMI1640 medium. Fungal Strain *A. Brasilienses* and *C. albicans* were used for antifungal studies. The compounds of 6.25, 10, 25, 50, and 100 µg were tested against fungal pathogens and MIC values were recorded.

2.4. DPPH Free Radical Scavenging Activity

The free radical scavenging activity of the compounds was analysed by the 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) method according to ebrahimzadeh, and bahramian.[5] In this assay, varying concentrations (5, 10, 15, 20 and 25 mg/mL) of compound in 1 mL of methanol solution of DPPH (0.2 mM) was used. There is a need to prepare fresh DPPH 0.2 mM and set optical density (OD) value to 0.8. If the OD is less than 0.8 add DPPH or more than 0.8 add methanol. The mixture was thoroughly mixed and incubated for 30 min. The optical density of the solution was then measured at 517 nm using Hitachi 2010 spectrophotometer. Percent inhibition of antioxidant activity was calculated by using the following formula and readings of test sample are compared with that of ascorbic acid (Vitamin C) (Positive control).

Percentage of inhibition of DPPH = ((Control OD – Test OD) / Control OD) X 100.

2.5. Nitric Oxide (NO) Activity

Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide (NO), which interacts with oxygen to produce nitrite ions, which can be estimated using Griess Illosvosy reaction.[6,7] Scavengers of NO compete with oxygen, leading to reduced production of NO and a pink colored chromophore is formed. The absorbance of these solutions was measured at 540 nm against the corresponding blank solutions. Percentage inhibition was calculated as

NO scavenging activity (%) = (A0 –A1) /A0 ×100

Where A0 is the absorbance of the control, and A1 is the absorbance of the sample.

2.6. ABTS Radical-Scavenging Activity

ABTS radical-scavenging activity of the compounds were determined according to Re *et al.*[8] The ABTS.+cation radical was produced by the reaction between 5 mL of 14 mM ABTS solution and 5 mL of 4.9 mM potassium persulfate ($K_2S_2O_8$) solution, stored in the dark at room temperature for 16 hours. Before the use, this solution was diluted with ethanol to get an absorbance of 0.700 ± 0.020 at 734 nm. The compounds at various concentrations with 1mL of ABTS solution was mixed and its absorbance was recorded at 734 nm. Ethanol blanks were run in each assay, and all measurements were done after at least 6 min. Similarly, the reaction mixture of standard group was obtained by mixing 950 µL of ABTS.+ solution and 50 µL of butylated hydroxytoluene (BHT). As for the antiradical activity, ABTS scavenging ability was expressed as IC_{50} (µg/mL).

The inhibition percentage of ABTS radical was calculated using the following formula.

ABTS scavenging activity (%) = (A0 –A1) /A0 ×100

2.7. Molecular Docking

The docking was performed for antimicrobial activity by using Auto Dock Tools 4.2 by default setting in the

software.[9] The grid is generated by using the co-ordinates of co-crystal ligand. The 3D crystal structure of target protein was downloaded from RCSB Protein Data Bank and used as a docking model. Water molecules and co-crystalized hetero molecules were removed from the target protein. The ligand structures were constructed using ChemDraw ultra 19.0 software and then converted to 3D structures and saved in .pdb format. The ligand energies were minimized using MOPAC (semiempirical quantum mechanics) with AM1 MOZYME geometry acceleration with 100 iterations. A total of ten different confirmations were generated for each docked ligand. The co-crystal ligand SM7 In redocked with the target protein to validate the results.

3. RESULTS

3.1. Antibacterial Activity

Antibacterial activity of ten compounds 2, 3, 4, 5, 6, 7A, 15, 18, 19, and 21 was carried out using the *S. aureus* and *Bacillus* as gram positive; *E. coli* and *Pseudomonas aeruginosa* as gram negative culture by agar plate method and serial tube dilution methods. Oxytetracycline was used as standard and it showed the MIC of 9.375 *µg*/mL. Along with standard the antibacterial activity of ten compounds was studied with five concentrations 6.25, 12.50, 25, 50, and 100 µg/mL. Compound 2, 4, 5 ,15, and19 showed the MIC of 9.375 µg/mL equal to standard oxytetracycline (Table 3). Other five compounds 3, 6, 7A, 18, and 21 showed the anti-bacterial activity at higher concentration of 100 µg/mL (Data not shown).

3.2. Antifungal Activity

The antifungal activity of the compounds was studied by MIC method using the fungal cultures *A. brasilinesis* and *C. albicans* with five concentrations 6.25, 12.5, 25, 50, and 100 µg/mL.[10]

Among the ten compounds, compound 2, 4, 5, 15, and 19 showed good antifungal activity with MIC of 9.375 µg/mL (Table 4). Other compounds showed antifungal activity with *A. brasilinesis* and *C. albicans* at higher concentration of 100 µg/mL.

3.3. Ani-oxidant Activities

3.3.1. DPPH Activity

Many researchers and previous studies have suggested that antioxidants reduce the risk for chronic diseases like cancer, diabetes, and heart disease etc,.

The antioxidant activity of compounds was studied through free radical DPPH scavenging activity described by Brand-Williams *et al.*[11] In this method, the DPPH absorbance was measured by spectrophotometric method at 517 nm, ascorbic acid used as standard.

Table 3: Antibacterial Activity of Various Theophylline Containing Acetylene Derivatives

Tube No.		*1*	*2*	*3*	*4*	*5*
Concentration (µg/ml)		*100*	*50*	*25*	*12.5*	*6.25*
Oxytetracycline						
Turbidity	B.subtilis	-	-	-	-	+
	P. aeruginosa	-	-	-	+	+
	E.coli	-	-	-	-	+
	S.aureus	-	-	-	-	+
Compound 2						
Turbidity	*B.subtilis*	-	-	-	+	+
	P. aeruginosa	-	-	-	+	+
	E.coli	-	-	-	+	+
	S.aureus	-	-	-	+	+
Compound 4						
Turbidity	*B.subtilis*	-	-	-	+	+
	P. aeruginosa	-	-	-	+	+
	E.coli	-	-	-	+	+
	S.aureus	-	-	-	+	+
Compound 5						
Turbidity	*B.subtilis*	-	-	-	+	+
	P. aeruginosa	-	-	-	+	+
	E.coli	-	-	-	+	+
	S.aureus	-	-	-	+	+
Compound 15						
Turbidity	*B.subtilis*	-	-	-	+	+
	P. aeruginosa	-	-	-	+	+
	E.coli	-	-	-	+	+
	S.aureus	-	-	-	+	+
Compound 19						
Turbidity	*B.subtilis*	-	-	-	+	+
	P. aeruginosa	-	-	-	+	+
	E.coli	-	-	-	+	+
	S.aureus	-	-	-	+	+

Table 4: Antifungal Activity of Various Theophylline Containing Acetylene Derivatives

Tube No.		*1*	*2*	*3*	*4*	*5*
Concentration (µg/ml)		*100*	*50*	*25*	*12.5*	*6.25*
Amphotericin B						
Turbidity	C. albicans	-	-	-	-	-
	A. brasiliensis	-	-	-	-	+
Compound 2						
Turbidity	*C. albicans*	-	-	-	+	+
	A. brasiliensis	-	-	-	+	+
Compound 4						
Turbidity	*C. albicans*	-	-	-	+	+
	A. brasiliensis	-	-	-	+	+
Compound 5						
Turbidity	*C. albicans*	-	-	-	+	+
	A. brasiliensis	-	-	-	+	+
Compound 15						
Turbidity	*C. albicans*	-	-	-	+	+
	A. brasiliensis	-	-	-	+	+
Compound 19						
Turbidity	*C. albicans*	-	-	-	+	+
	A. brasiliensis	-	-	-	+	+

The DPPH scavenging activity were studied with 5 concentrations from 5–25 µg/mL. At highest concentration of 25 µg/mL, ascorbic acid showed the % of inhibition of 95.13 and compound 19 showed the better % of inhibition of 84.97 followed by compound 5 showed the % of inhibition of 81.47, and compound 15 showed the % of inhibition of 79.06. Compound 7A showed the least % of inhibition with 22.63 at 25 µg/mL concentration (Table 5).

3.4. Nitric Oxide (NO) Activity

NO plays an important role in human life and measurement of NO is essential anti-oxidant parameter. NO was estimated using Griess Illosvosy reaction and absorbance was measured at 540 nm and NO % inhibition was calculated.[6,7] At the highest concentration of 25 µg/mL, Ascorbic acid showed the % of inhibition of 89.24 and compound 19 showed the better % of inhibition of 86.57 followed by compound 2 which showed the % of inhibition of 85.18 and compound 5 showed the % of inhibition of 80.96. Compound 6 showed the lowest % of inhibition with 32.67 at 25 µg/mL concentration (Table 6).

3.5. ABTS Radical-scavenging Activity

The anti-oxidant property of ten compounds was studied against 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS).[12] ABTS is a chemical compound used to observe the reaction kinetics of specific enzymes. ABTS assay measures the relative ability of antioxidants to scavenge the ABTS generated which has a dark blue color, is reduced by an antioxidant into colorless ABTS, which can be measured spectrophotometrically.[5] At highest concentration of 25 µg/mL, ascorbic acid showed the % of inhibition 91.14 and compound 4 showed the better % of inhibition of 84.36 followed by compound 15 which showed the % of inhibition of 82.13 and compound 19 showed the % of inhibition of 71.44. Compound 6 showed the lowest % of inhibition with 27.49 at 25 µg/mL concentration (Table 7).

3.6. Molecular Docking for Antimicrobial Activity

The structural relation of theophylline derivatives with binding of DNA Gyrase was identified using molecular docking. The compounds 2, 4, 5, 15, and 19 exhibited good binding affinities towards target protein. Hydrogen and hydrophobic interactions were played vital role in the binding and were influenced the docking results. Almost ten different conformations per each ligand was generated and the best pose was depicted in the Figure. Further, the compounds 2, 4, 5, 15, and 19 and co-crystalized ligand were occupied the same binding site (Figure 1). The binding energies of 2, 4, 5, 15, and 19 were -5.472, -5.909, -8.362, -6.517, and -5.862 Kcal/mol, respectively (Table 8). The co-crystal ligand exhibited -8.47 Kcal/mol binding energy. Amino acid residues in the active site of the target protein interacted with the docked ligands were represented in the Table 8.

Table 5: DPPH Scavenging Activity of Compounds

S. No	Test Compound	Concentration (µg/mL)	% Inhibition
1	Ascorbic acid	5	54.62 ± 0.167
		10	68.16 ± 0.298
		15	82.18 ± 0.234
		20	91.41 ± 0.651
		25	95.13 ± 0.524
2	Compound 2	5	12.36 ± 0.180
		10	22.49 ± 0.213
		15	36.52 ± 0.208
		20	49.14 ± 0.752
		25	65.18 ± 0.242
3	Compound 3	5	5.69 ± 0.108
		10	9.68 ± 0.116
		15	12.81 ± 0.257
		20	19.72 ± 0.233
		25	24.13 ± 0.437
4	Compound 4	5	14.97 ± 0.125
		10	26.54 ± 0.146
		15	41.62 ± 0.320
		20	54.17 ± 0.517
		25	72.64 ± 0.822
5	Compound 5	5	18.19 ± 0.362
		10	32.64 ± 0.434
		15	47.56 ± 0.342
		20	63.83 ± 0.581
		25	81.47 ± 0.625
6	Compound 6	5	9.15 ± 0.113
		10	14.61 ± 0.152
		15	19.72 ± 0.129
		20	27.34 ± 0.352
		25	40.23 ± 0.294
7	Compound 7A	5	3.88 ± 0.117
		10	7.12 ± 0.186
		15	9.67 ± 0.153
		20	15.85 ± 0.101
		25	22.63 ± 0.142
8	Compound 15	5	15.60 ± 0.135
		10	32.37 ± 0.822
		15	46.52 ± 0.374
		20	67.61 ± 0.253
		25	79.06 ± 0.625
9	Compound 18	5	7.22 ± 0.067
		10	15.68 ± 0.153
		15	20.93 ± 0.260
		20	25.64 ± 0.518
		25	32.69 ± 0.564
10	Compound 19	5	19.64 ± 0.251
		10	36.53 ± 0.217
		15	47.82 ± 0.346
		20	69.64 ± 0.483
		25	84.97 ± 0.721
11	Compound 21	5	5.96 ± 0.238
		10	12.35 ± 0.143
		15	16.57 ± 0.214
		20	23.61 ± 0.203
		25	31.02 ± 0.546

Table 6: NO Activity of Compounds

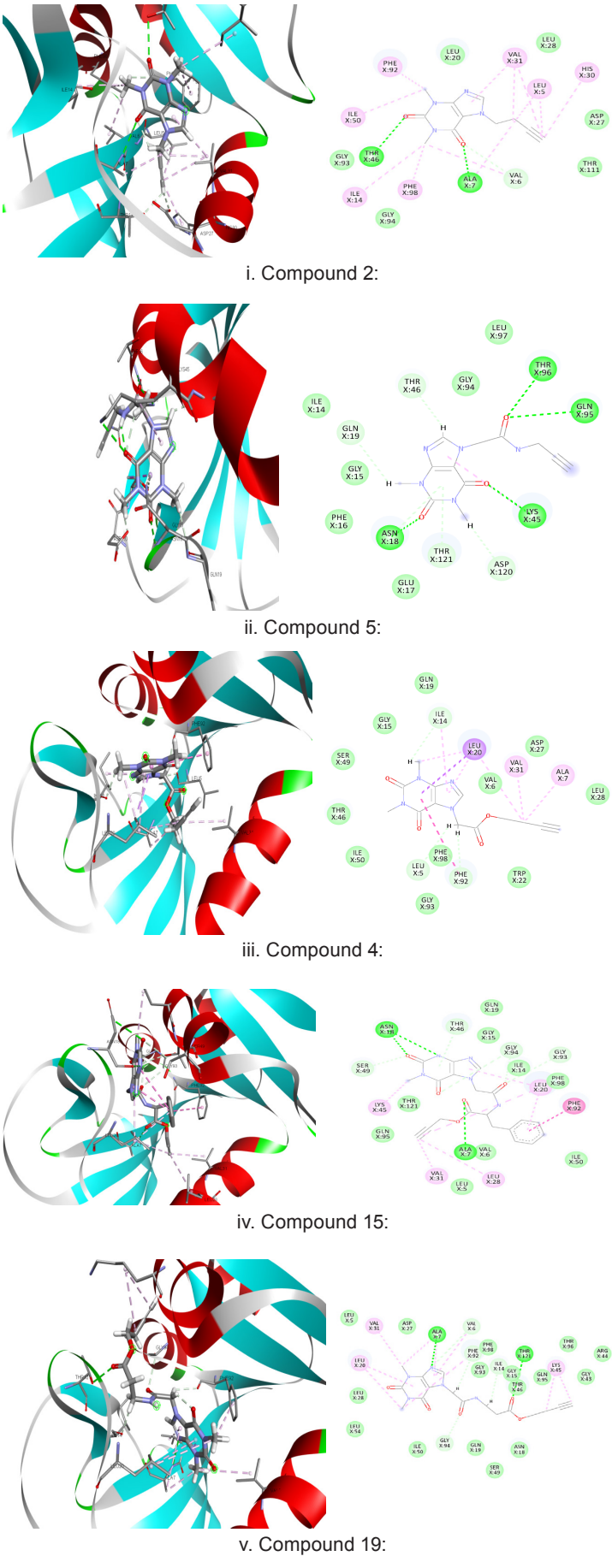
S. No	Test Compound	Concentration (µg/mL)	% Inhibition
1	Ascorbic acid	5	19.63 ± 0.224
		10	34.51 ± 0.357
		15	56.97 ± 0.388
		20	73.17 ± 0.512
		25	89.24 ± 0.751
2	Compound 2	5	15.64 ± 0.212
		10	31.69 ± 0.256
		15	49.87 ± 0.427
		20	71.52 ± 0.462
		25	85.18 ± 0.616
3	Compound 3	5	6.17 ± 0.213
		10	10.36 ± 0.262
		15	16.82 ± 0.218
		20	21.08 ± 0.297
		25	38.15 ± 0.255
4	Compound 4	5	12.36 ± 0.144
		10	26.87 ± 0.132
		15	36.51 ± 0.118
		20	55.64 ± 0.143
		25	67.32 ± 0.164
5	Compound 5	5	18.27 ± 0.367
		10	39.11 ± 0.452
		15	57.34 ± 0.618
		20	71.26 ± 0.723
		25	80.96 ± 0.815
6	Compound 6	5	5.72 ± 0.108
		10	9.63 ± 0.163
		15	16.42 ± 0.257
		20	22.19 ± 0.228
		25	32.67 ± 0.318
7	Compound 7A	5	15.18 ± 0.351
		10	24.57 ± 0.226
		15	41.16 ± 0.653
		20	52.19 ± 0.459
		25	67.54 ± 0.612
8	Compound 15	5	13.51 ± 0.245
		10	28.67 ± 0.367
		15	42.19 ± 0.552
		20	57.94 ± 0.591
		25	77.82 ± 0.738
9	Compound 18	5	7.12 ± 0.326
		10	12.62 ± 0.344
		15	21.53 ± 0.401
		20	31.09 ± 0.543
		25	46.58 ± 0.528
10	Compound 19	5	23.14 ± 0.315
		10	41.43 ± 0.427
		15	60.22 ± 0.551
		20	73.18 ± 0.631
		25	86.57 ± 0.642
11	Compound 21	5	10.27 ± 0.248
		10	17.26 ± 0.253
		15	26.89 ± 0.361
		20	38.42 ± 0.342
		25	55.21 ± 0.384

Table 7: ABTS Scavenging Activity of Compounds

S. No	Test Compound	Concentration (µg/mL)	% Inhibition
1	Ascorbic acid	5	24.57 ± 0.261
		10	56.12 ± 0.418
		15	74.06 ± 0.342
		20	82.92 ± 0.657
		25	91.14 ± 0.816
2	Compound 2	5	13.24 ± 0.152
		10	23.19 ± 0.188
		15	37.10 ± 0.163
		20	52.14 ± 0.158
		25	68.17 ± 0.254
3	Compound 3	5	8.35 ± 0.127
		10	14.68 ± 0.143
		15	23.41 ± 0.362
		20	31.34 ± 0.428
		25	40.62 ± 0.636
4	Compound 4	5	21.18 ± 0.152
		10	39.16 ± 0.417
		15	61.14 ± 0.364
		20	75.92 ± 0.322
		25	84.36 ± 0.918
5	Compound 5	5	14.28 ± 0.225
		10	25.61 ± 0.367
		15	37.04 ± 0.414
		20	55.07 ± 0.462
		25	69.03 ± 0.514
6	Compound 6	5	3.73 ± 0.112
		10	7.45 ± 0.193
		15	13.66 ± 0.140
		20	18.38 ± 0.281
		25	27.49 ± 0.324
7	Compound 7A	5	13.18 ± 0.106
		10	22.69 ± 0.164
		15	36.54 ± 0.367
		20	43.27 ± 0.322
		25	51.52 ± 0.351
8	Compound 15	5	21.43 ± 0.425
		10	33.17 ± 0.214
		15	50.26 ± 0.562
		20	67.48 ± 0.473
		25	82.13 ± 0.468
9	Compound 18	5	6.32 ± 0.234
		10	9.56 ± 0.228
		15	13.48 ± 0.462
		20	20.73 ± 0.417
		25	28.15 ± 0.483
10	Compound 19	5	16.39 ± 0.325
		10	29.72 ± 0.267
		15	42.14 ± 0.381
		20	56.35 ± 0.314
		25	71.44 ± 0.362
11	Compound 21	5	12.35 ± 0.118
		10	19.67 ± 0.144
		15	28.93 ± 0.352
		20	42.35 ± 0.419
		25	56.97 ± 0.403

4. DISCUSSION

Present work studied the biological activities of theophylline containing acetylene compounds for anti-oxidant and antimicrobial activities by standard methods. Ruddarraju *et al.* studied the theophylline containing acetylene compounds α- amylase enzyme



i. Compound 2:

ii. Compound 5:

iii. Compound 4:

iv. Compound 15:

v. Compound 19:

Figure 1: Molecular Docking Studies of Theophylline Derivatives for Antimicrobial Activity

Table 8: Molecular Docking Studies of Theophylline Derivatives for Antimicrobial Activity

Antimicrobial activity			
Compound	*DOCK SCORE (Kcal/Mol)*	*H-Bond*	*Hydrophobic*
2	-5.472	ALA7(3.05),THR46(3.39),VAL6(3.17),PHE92(2.84),MOL159(2.52),ASP27(2.76),THR111(2.64)	ALA7(4.16),VAL31(4.36),VAL6(5.21),ILE14(4.37),LEU5(4.37),4.37(4.37)
5	-5.909	ASN18(2.65),LYS45(3.04),GLN95(3.06),THR96(2.94),MOL159(2.14),THR46(2.29),ASP120(2.14),MOL159(MOL159),GLN19(2.93),ASN18(3.11),THR121(3.50)	THR121(3.43),LYS45(5.03)
19	-8.362	ALA7(3.10),THR121(2.87),VAL6(3.26),GLY94(3.57),PHE92(2.53),MOL159(2.21),ILE14(2.39),	ALA7(3.59),LYS45(4.34),LEU20(5.41),VAL31(4.75),LYS45(4.03),PHE92(4.59)
15	-6.517	ASN18(3.04),ALA7(2.93),ASN18(2.93),SER49(3.50),GLY93(3.58),GLY93(3.58),GLY94(3.29),GLY94(3.54),THR46(3.74)	MOL159 (5.49),MOL159(4.17),PHE92(5.34
4	-5.862	LYS721(3.37),THR830(3.336)	THR766(3.69), THR830(3.95) LYS721(4.979)

inhibitiory activity and further in-vivo anti-diabetic activity by intake of excessive Fatty Diet- Streptozotocin (HFD-STZ) model in rat and results showed that the animal treated with the Theophylline containing various acetylene derivatives) reversed and controlled the progression of the disease compared to the standard.[4] S.N. Mangasuli *et al.* studied the in vitro anti-tubercular activity and anti-microbial activity and reported for the coumarin-theophylline hybrids compounds 3a showed the anti-tubercular activity and compound 3a and 3f showed significant anti-microbial activity.[13] Ruddarraju *et al* studied the antibacterial activity of theophylline containing acetylene derivatives and theophylline containing 1,2,3-triazole derivatives with variant nucleoside derivatives and reported the Compounds 11, 21 and 26 showed the antibacterial activity against Escherichia coli, Staphylococcus aureus, Bacillus cereus and Pseudomonas aeruginosa organisms with significant minimum inhibitory concentrations (MIC).[4] In the present work the antibacterial activity of ten compounds (2, 3, 4, 5, 6, 7A, 15, 18, 19, and 21) was studied. Out of these ten compounds, compound 4 showed the good anti-bacterial activity with MIC of 9.375 µg/mL and zone of inhibition of 26.8 mm at concentration of 100 µg/mL followed by compound 19 a showed the MIC of 9.375 µg/mL and zone of inhibition of 26.5 mm at concentration of 100 µg/ml. Compound 15, 2 and 4 showed the MIC of 9.375 µg/mL and zone of inhibition of 26.4, 26.2 mm at concentration of 100 µg/mL. Along with antibacterial activity the compounds were also screened for antifungal activity were 2, 4, 5,15, and 19 showed the MIC of 9.375 µg/mL at 100 µg/mL against the *C. albicans* and *A. brasiliensis,* these results were almost similar to antibacterial results. Many studies reported that number of antioxidants can reduce the risk of tumours and heart diseases and they are helpful in scavenging Reactive Oxygen Species (ROS) which involved in the onsets of those diseases.[14-24] Devarakonda Srinivas *et al* studied the in vitro antioxidant evaluation of novel theophylline derivatives and reported the C-17 showed DPPH and c-16 and c-17 showed the nitrogen scavenging activity.[25] R. Ranjani, A. T. Sathiya Vinotha studied the randomised controlled study of theophyllines on oxidative stress and observed the significant increase of oxidative enzymes.[26] In present work studied the theophylline containing acetylene ten compounds anti-oxidant properties of DPPH, ABTS and NO Scavenging activity by standard methods. The DPPH scavenging activity were studied with concentrations from 5–25 µg/mL and the concentration of 25 µg/mL, Compound 19 showed the 84.97% of higher inhibition and Compound 7A showed the 22.63% of least inhibition. NO estimated using Griess Illosvosy reaction with concentrations of 5- 25 µg/mL, Compound 19 showed the 86.57 % of higher inhibition and Compound 6 shows the 32.67 % of lowest inhibition in studied ten compounds. Along with DPPH and NO, studied the ABTS scavenging activity of these ten compounds with concentration of 5-25 µg/mL and Ascorbic acid used as standard. The structural relation of theophylline derivatives with binding of EGFR Target & DNA Gyrase Target was identified using molecular docking by using Auto DockTools 4.2. The compounds 2, 4, 5, 15, and 19 exhibited very good binding affinities towards target protein. Hydrogen and hydrophobic interactions were played vital role in the binding and were influenced the docking results.[27,28]

5. CONCLUSION

In the present study the anti-microbial and anti-oxidant activities of the ten compounds (2, 3 4, 5, 6, 7A, 15, 18, 19, and 21 was studied. Compounds 2, 4, 5, 15, and 19 showed good antimicrobial and anti-oxidant activity. Based on the present in-vitro studies we conclude that theophylline containing acetylene compounds are the promising usage as antimicrobial and anti-oxidants. The compounds 2, 4, 5, 15, and 19 exhibited very good binding affinities towards target protein. Hydrogen and hydrophobic interactions play vital role in the binding and the same was observed in the docking results. Further in-vivo studies need to be studied for the use of these compounds in any of these applications.

REFERENCES

1. Siddiq A, Dembitsky V. Acetylenic anticancer agents. *Anticancer Agents Med Chem*. 2008;8(2):132-170. doi:10.2174/187152008783497073
2. Dias DA, Urban S, Roessner U. A historical overview of natural products in drug discovery. *Metabolites*. 2012;2(2):303-336. doi:10.3390/metabo2020303
3. Profire LE, Sunel V, Lupascu D, Baican MC, Bibire N, Vasile C. New theophylline derivatives with potential pharmacological activity. *Farmacia*. 2010;58(2):170-6.
4. Ruddarraju RR, Kiran G, Murugulla AC, *et al*. Design, synthesis and biological evaluation of theophylline containing variant acetylene derivatives as α-amylase inhibitors. *Bioorg Chem*. 2019;92:103120. doi:10.1016/j.bioorg.2019.103120
5. Ebrahimzadeh MA, Bahramian F. Antioxidant Activity of Crataegus pentaegyna Subsp. elburensis Fruits Extracts. *Pak J Biol Sci*. 2009;12(5):413-9.
6. Ivanov VM. The 125th anniversary of the Griess reagent. *Journal of Analytical chemistry*. 2004;59(10):1002-1005.
7. Cos P, Ying L, Calomme M, Hu JP, Cimanga K, Poel BV, Pieters L, Vlietinck AJ, Berghe DV. Structure-activity relationship and classification of falvonoids as inhibition of xanthine oxidase and superoxide scavengers. *J Nat Prod*. 1998;61:71-76.
8. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved

ABTS radical cation decolorization assay. *Free Radic Biol*. 1999;26:1231-7.

9. Morris GM, Huey R, Lindstrom W, Sanner MF, Belew RK, Goodsell DS, Olson AJ. AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. *Journal of computational chemistry*. 2009;30(16):2785-91.
10. Tretyakova EV, Salimova EV, Parfenova LV. Synthesis and Antimicrobial and Antifungal Activity of Resin Acid Acetylene Derivatives. *Russian Journal of Bioorganic Chemistry*. 2019;45(6):545-51.
11. Brand-Williams W, Cuvelier ME, Berset CL. Use of a free radical method to evaluate antioxidant activity. *LWT-Food science and Technology*. 1995;28(1):25-30.
12. Cano A, Arnao MB. ABTS/TEAC (2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)/Trolox®-Equivalent Antioxidant Capacity) radical scavenging mixed-mode assay. Measurement of Antioxidant Activity & Capacity: Recent Trends and Applications. *Chichester: Wiley*. 2018:117-39.
13. Mangasuli SN, Hosamani KM, Devarajegowda HC, Kurjogi MM, Joshi SD, Synthesis of coumarin-theophylline hybrids as a new class of anti-tubercular and anti-microbial agents, *Eur. J. Med. Chem*. 2018;146:747–756.
14. Rao AV, Agarwal S. Role of antioxidant lycopene in cancer and heart disease. *Journal of the American College of Nutrition*. 2000;19(5):563-9.
15. Hertog MG, Sweetnam PM, Fehily AM, Elwood PC, Kromhout D. Antioxidant flavonols and ischemic heart disease in a Welsh population of men: the Caerphilly Study. *The American journal of clinical nutrition*. 1997;65(5):1489-94.
16. Diaz MN, Frei B, Vita JA, Keaney Jr JF. Antioxidants and atherosclerotic heart disease. New England Journal of Medicine. 1997 Aug 7;337(6):408-16.
17. Hollman PC, Feskens EJ, Katan MB. Tea flavonols in cardiovascular disease and cancer epidemiology. *Proceedings of the Society for Experimental Biology and Medicine*. 1999;220(4):198-202.
18. Duthie GG, Duthie SJ, Kyle JA. Plant polyphenols in cancer and heart disease: implications as nutritional antioxidants. *Nutrition research reviews*. 2000;13(1):79-106.
19. Battin EE, Brumaghim JL. Antioxidant activity of sulfur and selenium: a review of reactive oxygen species scavenging, glutathione peroxidase, and metal-binding antioxidant mechanisms. *Cell biochemistry and biophysics*. 2009;55(1):1-23.
20. Mandal S, Hazra B, Sarkar R, Biswas S, Mandal N. Assessment of the antioxidant and reactive oxygen species scavenging activity of methanolic extract of Caesalpinia crista leaf. *Evidence-Based Complementary and Alternative Medicine*. 2011;2011.
21. Suzuki YJ, Tsuchiya M, Packer L. Thioctic acid and dihydrolipoic acid are novel antioxidants which interact with reactive oxygen species. *Free radical research communications*. 1991;15(5):255-63.
22. Siriwardhana N, Lee KW, Jeon YJ, Kim SH, Haw JW. Antioxidant activity of Hizikia fusiformis on reactive oxygen species scavenging and lipid peroxidation inhibition. *Food Science and Technology International*. 2003;9(5):339-46.
23. Sueishi Y, Hori M, Ishikawa M, Matsu-Ura K, Kamogawa E, Honda Y, Kita M, Ohara K. Scavenging rate constants of hydrophilic antioxidants against multiple reactive oxygen species. *Journal of clinical biochemistry and nutrition*. 2014:13-53.
24. Tong L, Chuang CC, Wu S, Zuo L. Reactive oxygen species in redox cancer therapy. *Cancer letters*. 2015;367(1):18-25.
25. Srinivas D, Venkatesh D, Prasad DK, Bakshi V, Kiran G. In vitro evaluation of antioxidant activity of novel theophylline derivatives. *Methods*. 2018;6(7): 24-29
26. Ranjani R, Vinotha ATS. A prospective randomized controlled study: Theophylline on oxidative stress and steroid sensitivity in chronic obstructive pulmonary disease patients. *Int J Pharm Investig*. 2017;7(3):119-124
27. Patil R, Das S, Stanley A, Yadav L, Sudhakar A, Varma AK. Optimized hydrophobic interactions and hydrogen bonding at the target-ligand interface leads the pathways of drug-designing. *PloS one*. 2010;5(8):e12029.
28. Fikrika H, Ambarsari L, Sumaryada T. Molecular docking studies of catechin and its derivatives as anti-bacterial inhibitor for glucosamine-6-phosphate synthase. InIOP Conference Series: *Earth and Environmental Science*. 2016;31(1):012009).

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