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Synthesis and Biological Studies of Novel Biphenyl-3,5-dihydro-2*H*-thiazolopyrimidines Derivatives

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ABSTRACT. A new series of ethyl 2-(4-substitutedbenzylidene)-5-(3'-(ethoxycarbonyl)biphenyl-4-yl)-7-methyl-3-oxo-3,5-dihydro-2*H*-thiazolo[3,2-a]pyrimidine-6-carboxylate derivatives (**8a-j**) were synthesized. The newly synthesized compounds were characterized by **IR**, **^{1}H NMR**, **^{13}C NMR**, **LCMS mass** and **C**, **H**, **N** analyses. All newly synthesized compounds were screened for their *In vitro* antioxidant activity (S*cavenging of hydrogen peroxide, Scavenging of nitric oxide radical, and Lipid peroxidation inhibitory activity*), antibacterial (*Escheria coli, Pseudonmonas aeruginosa* (gram-negative bacteria), *Bacillus subtillis, Staphylococcus aureus* (gram-positive bacteria)) and antifungal (*Candida albicans Aspergillus niger*) studies.

Key words: Biphenyl-3,5-dihydro-2*H*-thiazolopyrimidines derivatives, Antioxidant activity, Antibacterial, Antifungal activities

INTRODUCTION

Heterocycles are ubiquitous to among pharmaceutical compounds.[1-3] Pyrimidine moiety is an important class of *N*-containing heterocycles widely used as key building blocks for pharmaceutical agents. It exhibits a wide spectrum of pharmacophore activities, as it can act as bactericidal, fungicidal[4,5] analgesic,[6] antihypertensive[7] and anti-tumor agents.[8] Among the substituted pyrimidines, thiouracils are well known for anti-inflammatory and virucidal agents.[9] Also, preclinical data from literature survey indicate continuing research in polysubstituted pyrimidine as potential anti-tumor agents.[10-12] The biological and synthetic significance places this scaffold at a prestigious position in medicinal chemistry research.

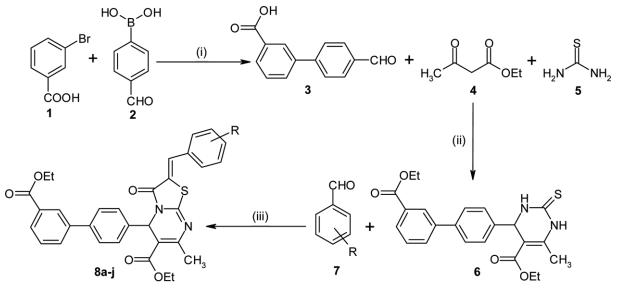
The 3,4-dihydropyrimidin-2(1*H*)-ones have recently emerged as important target molecules due to their therapeutic and pharmacological properties[13] such as antiviral,[14] antimitotic,[15] anticarcinogenic,[16] antihypertensive[17,18] and noteworthy, as calcium channel modulators.[19,20] Additionally, their particular structure has been found in natural marine alkaloid batzelladine A and B which are the first low molecular weight natural products reported in the literature to inhibit the binding of HIV gp-120 to CD4 cells, so disclosing a new field towards the development of AIDS therapy.[21] Thiazoles and their derivatives are also found to be associated with various biological activities such as antibacterial, antifungal and anti-inflammatory.[22-25] In recent past we have reported thiosubstituted pyrimidines which exhibited good biological activity.[5] Prompted by the chemotherapeutic importance of pyrimidine derivatives and in a view to synthesize bioactive molecules,[26] it was contemplated to synthesize a series of novel fused pyrimidine derivatives possessing 4-biphenyl moiety and study their biological properties.

In continued quest of new anti oxidant activity and antimicrobial agents, we designed and synthesized novel biphenyl-3,5-dihydro-2*H*-thiazolopyrimidines derivatives. Structures of the products were characterized by IR, ^{1}H-NMR, ^{13}C NMR, LC-MS mass spectrometry and elemental analysis. Results of biological activities indicate that some compounds possess potential antioxidant and antimicrobial activity.

RESULTS AND DISCUSSION

Chemistry

The synthesis of ethyl 2-(4-substitutedbenzylidene)-5-(3'-(ethoxycarbonyl)biphenyl-4-yl)-7-methyl-3-oxo-3,5-dihydro-2*H*-thiazolo[3,2-a]pyrimidine-6-carboxylate derivatives (**8a-j**) was achieved through the versatile and efficient synthetic route outlined in *Scheme* 1. The desired compounds were synthesized as follows. Initially, when 3-bromobenzoic acid (**1**) was treated with 4-formylphenylboronic (**2**) acid in the presence of cesium carbonate and bis(triphenylphosphine)palladium(II) chloride, it afforded the corresponding 4'-Formyl-biphenyl-3-carboxylic acid (**3**). The compound (**3**) was reacted with ethyl acetoace-



Compound	R	Compound	R
8a	H	**8f**	4-$N(CH_3)_2$
8b	4-Br	**8g**	4-Cl
8c	4-C_2H_5	**8h**	4-$CH(CH_3)_2$
8d	4-CH_3	**8i**	4-$C(CH_3)_3$
8e	4-OCH_3	**8j**	4-NO_2

Reagents and conditions: (i) $Pd(PPh_3)_2Cl_2$, Cs_2CO_3, Dioxane, reflux, 10h; (ii) HCl, reflux, 16h; (iii) $ClCH_2COOH$, AcONa, Ac_2O/AcOH, reflux, 2h.

***Scheme* 1.** Synthetic pathway for compound **8 a-j**.

tate (**4**), thiourea (**5**) in ethanol and a few drops of HCl to obtained 4-(3'-ethoxycarbonyl-biphenyl-4-yl)-6-methyl-2-thioxo-1,2,3,4-tetrahydro-pyrimidine-5-carboxylic acid ethyl ester (**6**).[27] Finally, ethyl 2-(4-substitutedbenzylidene)-5-(3'-(ethoxycarbonyl)biphenyl-4-yl)-7-methyl-3-oxo-3,5-dihydro-2*H*-thiazolo[3,2-a]pyrimidine-6-carboxylate derivatives (**8a-j**) were synthesized by the reaction of (**6**), substituted benzaldehydes (**7**) and chloro acetic acid in the presence of sodium acetate.

All the synthesized compounds were obtained in good to high yields. Products were purified and characterized by various spectroscopic techniques. The IR spectra of compounds (**6a-j**) showed characteristic absorption bands at 2981-2969 cm^{-1}, 1721-1706 cm^{-1}, 1632-1606, and 1568-1525 cm^{-1} corresponding to the C-H_{str}, C=O_{str}, C=N_{str}, and C=C_{str} functions in the structures. Similarly the ^{1}H NMR spectra showed peaks due to in the range of δ 1.10-1.48 for OCH_2-($\underline{CH_3}$)$_2$, δ 2.30-2.39 for Ar-$\underline{CH_3}$, δ 4.05-4.45 for -O$\underline{CH_2}CH_3$, δ 6.18-6.24 for -$\underline{CH}$ and δ 6.60-8.75 for =CH & Ar-H. The mass spectrum of all the compounds showed molecular ion peak at M+H, at M+2H corresponding to its molecular formula, which confirmed its chemical structure. The IR, ^{1}H NMR, ^{13}C NMR, LCMS mass spectra and elemental analysis showed the structure of various novel ethyl 2-(4-substitutedbenzylidene)-5-(3'-(ethoxycarbonyl)biphenyl-4-yl)-7-methyl-3-oxo-3,5-dihydro-2*H*-thiazolo[3,2-a]pyrimidine-6-carboxylate derivatives (**8a-j**).

***Table* 1.** Antioxidant activity (IC_{50} values) of compounds (**8a-j**)

Compounds	IC_{50} (Mean ± S.D) µg/mL		
	Scavenging of NO radical	Scavenging of H_2O_2	Lipid peroxidation inhibitory activity
8a	56 ± 0.052	32 ± 0.318	39 ± 0.066
8b	60 ± 0.066	49 ± 0.121	43 ± 0.318
8c	65 ± 0.453	58 ± 0.318	78 ± 0.045
8d	63 ± 0.183	53 ± 0.066	68 ± 0.087
8e	69 ± 0.318	66 ± 0.162	63 ± 0.162
8f	59 ± 0.453	41 ± 0.087	46 ± 0.453
8g	62 ± 0.045	46 ± 0.024	57 ± 0.279
8h	54 ± 0.024	36 ± 0.121	37 ± 0.318
8i	46 ± 0.333	30 ± 0.183	34 ± 0.087
8j	44 ± 0.279	29 ± 0.087	31 ± 0.121
Standard	47 ± 0.087	33 ± 0.121	26 ± 0.333

S.D.=standard deviation (Average of three determination); Standard=Ascorbic acid.

PHARMACOLOGICAL ASSAY

Antioxidant Activity

All the synthesized compounds **8a-j** were screened for their *in vitro* antioxidant activity by various methods such as *scavenging of hydrogen peroxide*, *scavenging of nitric oxide radical*, and *lipid peroxidation inhibitory activity*. *In vitro* antioxidant activity of synthesized compound is summarized in *Table* 1.

The investigation of antioxidant screening revealed that some of the tested compounds showed moderate to good antioxidant activity. The novel biphenyl-3,5-dihydro-2*H*-thiazolopyrimidines derivatives (**8a-j**) showed more promising antioxidant activity as compared to that of standard, ascorbic acid. This could be due the availability of free thiazolopyrimidine group. In scavenging of nitric oxide radical techniques, compounds **8j** and **8i** showed low IC_{50} value than the standard. While, **8j**, **8i** and **8a** have shown more potent activity in scavenging of hydrogen peroxide. This may be due to additional aliphatic group present on benzene ring in the structure. All the compounds showed higher IC_{50} value than the standard by lipid peroxidation inhibitory activity. Derivatives with aliphatic group on benzene ring having good antioxidant activity compared with the other compounds in their series. **8h**, **8a**, **8f**, **8b**, **8g** and **8d** exhibited moderate to good antioxidant activity.

Antimicrobial Activity

The antimicrobial activities of the compounds (**8a-j**) were tested against *Escheria coli, Pseudonmonas aeruginosa* (gram-negative bacteria), *Bacillus subtillis* and *Sta-*

***Table* 2.** Antifungal activities of compounds[a] (**8a-j**)

Compounds	Candida albicans			Aspergillus niger		
	25 μg mL^{-1} ± S.D.	50 μg mL^{-1} ± S.D.	100 μg mL^{-1} ± S.D.	25 μg mL^{-1} ± S.D.	50 μg mL^{-1} ± S.D.	100 μg mL^{-1} ± S.D.
8a	9.62 ± 0.58	12.58 ± 0.58	19.76 ± 1.53	11.56 ± 0.58	13.00 ± 0.00	20.66 ± 0.58
8b	12.04 ± 0.58	15.20 ± 0.58	21.16 ± 1.16	11.90 ± 1.73	14.12 ± 0.61	21.16 ± 0.61
8c	10.86 ± 0.58	12.30 ± 0.47	18.00 ± 2.00	10.14 ± 0.47	13.10 ± 0.58	20.36 ± 1.53
8d	9.10 ± 0.58	13.83 ± 0.61	21.46 ± 1.53	12.10 ± 0.58	15.80 ± 0.61	22.36 ± 0.58
8e	10.38 ± 0.58	13.72 ± 1.16	17.72 ± 1.16	10.24 ± 2.08	12.24 ± 1.16	18.26 ± 2.08
8f	13.44 ± 0.58	17.88 ± 0.61	21.76 ± 0.61	11.23 ± 0.58	14.00 ± 0.00	23.78 ± 1.53
8g	11.48 ± 0.58	14.86 ± 1.16	20.16 ± 1.53	12.16 ± 0.58	15.06 ± 0.63	22.40 ± 0.58
8h	9.41 ± 0.58	13.48 ± 1.63	17.00 ± 1.53	10.80 ± 0.58	12.12 ± 0.27	18.94 ± 0.47
8i	10.58 ± 0.61	14.46 ± 0.47	15.46 ± 1.53	9.21 ± 0.58	12.62 ± 0.27	18.12 ± 0.58
8j	10.43 ± 0.58	13.30 ± 0.27	17.72 ± 0.63	9.48 ± 0.58	11.74 ± 0.61	17.16 ± 1.16
Standard	12.89 ± 0.58	15.26 ± 1.73	23.10 ± 1.53	14.89 ± 0.47	17.13 ± 0.58	26.14 ± 0.61

S.D.=standard deviation; Standard=Amphotericin-B; [a]Zone of inhibition.

phylococcus aureus (gram-positive bacteria) and two fungi *Candida albicans* and *Aspergillus niger* and the results were reported as zone of inhibition. The results of preliminary antifungal testing of the compounds **8a-j** are shown in *Table* 2. All the compounds exhibited moderate to good antifungal activity. Compound **8f** exhibited potent activity against *C. albicans* and compounds **8f** and **8g** exhibited good activity than the standard. The compounds **8f**, **8g**, **8d**, **8a**, and **8b** exhibited moderate to good activity against *A. niger*.

The results of preliminary antibacterial testing of compounds (**8a-j**) are shown in *Table* 3. The results revealed that, all the novel biphenyl-3,5-dihydro-2*H*-thiazolopyrimidines derivatives (**8a-j**) were showing good to potent

***Table* 3.** Antibacterial activity of the compounds[a] (**8a-j**)

Compounds	*Escheria coli*			*Pseudomonas aeruginosa*		
	25 μg mL^{-1} ± S.D.	50 μg mL^{-1} ± S.D.	100 μg mL^{-1} ± S.D.	25 μg mL^{-1} ± S.D.	50 μg mL^{-1} ± S.D.	100 μg mL^{-1} ± S.D.
8a	10.48 ± 0.47	14.72 ± 0.58	17.39 ± 1.16	8.21 ± 2.09	10.23 ± 0.58	16.12 ± 1.73
8b	15.10 ± 2.09	22.28 ± 1.16	27.49 ± 0.47	12.67 ± 0.26	16.06 ± 0.45	26.25 ± 0.61
8c	12.96 ± 0.17	14.18 ± 0.45	18.04 ± 1.16	9.19 ± 1.16	12.12 ± 2.09	19.85 ± 0.26
8d	12.54 ± 1.71	13.12 ± 0.58	16.11 ± 1.16	9.67 ± 1.53	12.72 ± 0.58	18.82 ± 1.73
8e	11.10 ± 0.58	10.18 ± 0.47	14.03 ± 0.17	8.29 ± 1.16	11.92 ± 0.26	16.73 ± 0.58
8f	14.72 ± 0.45	19.48 ± 1.71	25.02 ± 1.16	11.79 ± 0.61	17.02 ± 2.09	25.67 ± 0.26
8g	13.16 ± 0.61	20.48 ± 0.58	26.90 ± 1.16	12.15 ± 1.16	16.31 ± 0.58	23.88 ± 1.73
8h	10.26 ± 0.26	12.36 ± 0.47	16.18 ± 0.61	9.90 ± 1.16	12.26 ± 0.58	17.42 ± 0.58
8i	13.48 ± 0.58	17.27 ± 0.58	24.42 ± 1.16	10.16 ± 0.17	15.00 ± 2.09	21.12 ± 0.58
8j	13.12 ± 0.47	16.00 ± 0.58	22.90 ± 1.16	10.28 ± 1.16	12.82 ± 0.58	20.33 ± 1.73
Standard	15.78 ± 0.17	21.10 ± 1.16	28.71 ± 0.58	13.36 ± 1.53	18.70 ± 0.45	30.33 ± 0.61
Compounds	*Bacillus subtilis*			*Staphylococcus aureus*		
	25 μg mL^{-1} ± S.D.	50 μg mL^{-1} ± S.D.	100 μg mL^{-1} ± S.D.	25 μg mL^{-1} ± S.D.	50 μg mL^{-1} ± S.D.	100 μg mL^{-1} ± S.D.
8a	10.19 ± 1.16	14.04 ± 0.58	18.14 ± 1.16	10.12 ± 0.37	15.05 ± 0.26	18.65 ± 1.53
8b	14.78 ± 1.53	19.26 ± 0.58	28.00 ± 1.16	15.76 ± 2.09	20.84 ± 0.45	26.67 ± 1.16
8c	12.92 ± 1.16	14.11 ± 1.16	18.30 ± 1.53	10.92 ± 0.45	14.10 ± 1.16	17.71 ± 1.53
8d	12.88 ± 1.16	15.05 ± 0.58	18.67 ± 0.61	11.82 ± 0.17	12.18 ± 1.71	16.49 ± 0.61
8e	11.21 ± 1.53	14.82 ± 0.47	20.90 ± 0.47	10.12 ± 0.58	13.48 ± 0.58	16.52 ± 0.58
8f	14.18 ± 2.09	16.71 ± 1.16	25.39 ± 1.53	13.07 ± 0.63	17.06 ± 1.16	22.00 ± 0.47
8g	13.42 ± 1.16	17.16 ± 0.58	26.18 ± 2.00	14.60 ± 2.09	18.26 ± 0.61	24.32 ± 0.58
8h	11.62 ± 1.53	15.10 ± 0.45	19.25 ± 1.61	10.33 ± 0.58	14.10 ± 0.58	18.67 ± 0.17
8i	13.92 ± 1.16	14.23 ± 1.16	23.06 ± 1.53	12.33 ± 0.61	17.38 ± 1.16	22.82 ± 0.47
8j	14.42 ± 1.16	15.10 ± 2.09	21.80 ± 0.61	12.67 ± 0.71	16.12 ± 0.45	20.30 ± 0.17
Standard	15.14 ± 0.78	20.23 ± 0.47	31.82 ± 1.53	15.39 ± 0.58	22.41 ± 2.09	29.48 ± 0.17

S.D.=standard deviation; Standard=Streptomycin; [a]Zone of inhibition

antibacterial activity against all the tested strains of bacteria. While the entire derivatives showed moderate to potent activity against *Bacillus subtilis*. Amongst all the derivatives in series i.e. (**8a-j**), the halogenated and amino derivatives exhibited potent antibacterial activity. As compare to standard streptomycin, compounds **8b**, **8f** and **8g** exhibited good to potent activity against all the tested strains. While compounds **8i**, **8j**, **8c**, **8d**, **8e** and **8h** were moderately active. The other compounds were weakly active against the tested organism.

EXPERIMENTAL

All reagents and solvents were purchased and used without further purification. Melting points were determined on a Fisher-Johns melting point apparatus were uncorrected. Crude products were purified by column chromatography on silica gel of 60-120 mesh. IR spectra were obtained on a Perkin Elmer BX serried FT-IR 5000 spectrometer using KBr pellet. NMR spectra were recorded on a varian 300 MHz spectrometer for ^{1}H NMR. The ^{13}C NMR spectra were recorded on JEOL. The chemical shifts were reported as ppm down field using TMS as an internal standard. LCMS Mass spectra were recorded on a MASPEC low resolution mass spectrometer operating at 70 eV.

General procedure for the preparation of 4'-Formyl-biphenyl-3-carboxylic acid (3)

To a stirred solution of 3-bromobenzoic acid (1 mmol) in dioxane : water (20 mL, 4:1) was added cesium carbonate (1.5 mmol) followed by addition of 4-formylphenylboronic acid (1.1 mmol) and the resulting solution was stirred and degassed under nitrogen for 30 min. Bis(triphenylphosphine) palladium(II) chloride (1.5 mmol) was added and the reaction mixture was stirred at reflux temperature for 8 h. After completion (monitored by TLC), solvent was removed under reduced pressure and diluted with water. The aqueous phase was acidified by dilute HCl and then extracted with ethyl acetate (100 mL). The combined organic layers were dried (Na_2SO_4) and concentrated under reduced pressure. The crude compound was purified by column chromatography to afford the 4'-Formyl-biphenyl-3-carboxylic acid (**3**) as pale yellow solid, 65% yield. ^{1}H NMR (300 MHz, DMSO-d_6) 7.48-8.50 (m, Ar-H, 8H), 10.20 (s, 1H), 13.10 (s, 1H, COOH); Mass (m/z): 227 (M+H, 100%).

General procedure for the preparation of 4-(3'-Ethoxycarbonyl-biphenyl-4-yl)-6-methyl-2-thioxo-1,2,3,4-tetrahydro-pyrimidine-5-carboxylic acid ethyl ester (6)

To a stirred solution of ethyl acetoacetate (4) (1 mmol), biaryl aldehyde (1.1 mmol) and thiourea (2 mmol) in ethanol were added in the given order. It was followed by addition of catalytic amount of HCl. The resulting solution was stirred at reflux for 12 h. After completion, solvent was removed under reduced pressure and the residue obtained was extracted with ethyl acetate. The combined organic layers were dried (Na_2SO_4) and concentrated under reduced pressure. The crude compound was purified by column chromatography to afford the 4-(3'-Ethoxycarbonyl-biphenyl-4-yl)-6-methyl-2-thioxo-1,2,3,4-tetrahydro-pyrimidine-5-carboxylic acid ethyl ester (**6**) as solid.

Yellow solid, 50% yield; M.p. 141-142 ºC; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.15 (t, 6H), 2.35 (s, 3H), 4.10 (q, 4H), 5.20 (s, 1H), 7.10-8.60 (m, Ar-H, 8H), 8.95 (s, 2H); Mass (m/z): 425 (M+H, 100%); Anal. Calcd. for $C_{23}H_{24}N_2O_4S$: C, 65.07; H, 5.70; N, 6.60. Found: C, 64.84; H, 5.67; N, 6.66.

General procedure for the preparation of ethyl 2-(4-substitutedbenzylidene)-5-(3'-(ethoxycarbonyl)biphenyl-4-yl)-7-methyl-3-oxo-3,5-dihydro-2*H*-thiazolo[3,2-a]pyrimidine-6-carboxylate (8a-j)

An ice cold solution of cyclic compound of 4-(3'-Ethoxycarbonyl-biphenyl-4-yl)-6-methyl-2-thioxo-1,2,3,4-tetrahydro-pyrimidine-5-carboxylic acid ethyl ester (**6**) (1 mmol) in DMF (4 vol), potassium carbonate (1.5 mmol) and substituted benzyl halides (1.3 mmol) was taken in a 1 liter round bottomed flask equipped with magnetic stirrer and stirred for 1 hour. The residual portion was poured on to crushed ice, neutralized with dilute acid and the product obtained ethyl 2-(substitutedbenzylthio)-4-(3'-(ethoxycarbonyl)biphenyl-4-yl)-6-methyl-1,4-dihydropyrimidine-5-carboxylate derivatives **(8a-j)** was collected by filtration.

Ethyl 2-benzylidene-5-(3'-(ethoxycarbonyl)biphenyl-4-yl)-7-methyl-3-oxo-3,5-dihydro-2*H*-thiazolo[3,2-a]pyrimidine-6-carboxylate (8a)

Yield: 78%; mp 258-259 ºC; IR (KBr) υ 2976 (C-H), 1710 (C=O), 1606 (C=N), 1541 (C=C), cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.19 (t, 6H, $OCH_2\underline{CH_3}$), 2.35 (s, 3H, $Ar\underline{CH_3}$), 4.05 (q, 4H, $O\underline{CH_2}CH_3$), 6.18 (s, 1H, -CH), 7.20-8.55 (m, 14H, =CH & Ar-H); ^{13}C NMR (DMSO-d_6, δ, ppm) 13.6, 13.9, 19.8, 54.8, 62.5, 63.2, 114.5, 121.3, 124.7, 125.2, 126.1, 126.5, 127.0, 127.6, 127.9, 128.2, 129.3, 130.9, 133.6, 137.4, 139.2, 141.1, 141.9, 154.8, 162.3, 166.6, 167.4, 171.2; LCMS: (*m/z*) 553 [M+H]. *Anal*. Calcd. for $C_{32}H_{28}N_2O_5S$: C, 69.55; H, 5.11; N, 5.08. Found: C, 69.59; H, 5.17; N, 5.01.

Ethyl 2-(4-bromobenzylidene)-5-(3'-(ethoxycarbonyl) biphenyl-4-yl)-7-methyl-3-oxo-3,5-dihydro-2*H*-thiazolo [3,2-a]pyrimidine-6-carboxylate (8b)

Yield: 88%; mp 203-205 ºC; IR (KBr) υ 2974 (C-H),

1708 (C=O), 1613 (C=N), 1528 (C=C), 754 (C-Br) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.20 (t, 6H, $OCH_2\underline{CH_3}$), 2.39 (s, 3H, $Ar\underline{CH_3}$), 4.10 (q, 4H, $O\underline{CH_2}CH_3$), 6.23 (s, 1H, -CH), 7.10-8.60 (m, 13H, =CH & Ar-H); LCMS: (*m*/*z*) 632 [M+2H]. *Anal*. Calcd. for $C_{32}H_{27}BrN_2O_5S$: C, 80.85; H, 4.32; N, 4.44. Found: C, 80.76; H, 4.37; N, 4.39.

Ethyl 2-(4-ethylbenzylidene)-5-(3'-(ethoxycarbonyl) biphenyl-4-yl)-7-methyl-3-oxo-3,5-dihydro-2*H*-thiazolo[3,2-a]pyrimidine-6-carboxylate (8c)

Yield: 56%; mp 198-200 °C; IR (KBr) υ 2969 (C-H), 1711 (C=O), 1609 (C=N), 1546 (C=C) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.15 (t, 9H, $OCH_2\underline{CH_3}$ & -$ArCH_2\underline{CH_3}$), 2.34 (s, 3H, $Ar\underline{CH_3}$), 2.40 (q, 2H, -$Ar\underline{CH_2}CH_3$), 4.10 (q, 4H, $O\underline{CH_2}CH_3$), 6.20 (s, 1H, -CH), 6.75-8.55 (m, 13H, =CH & Ar-H); LCMS: (*m*/*z*) 581 [M+H]. *Anal*. Calcd. for $C_{34}H_{32}N_2O_5S$: C, 70.32; H, 5.55; N, 4.82. Found: C, 70.36; H, 5.48; N, 4.85.

Ethyl 2-(4-methylbenzylidene)-5-(3'-(ethoxycarbonyl) biphenyl-4-yl)-7-methyl-3-oxo-3,5-dihydro-2*H*-thiazolo[3,2-a]pyrimidine-6-carboxylate (8d)

Yield: 87%; mp 235 °C; IR (KBr) υ 2976 (C-H), 1710 (C=O), 1617 (C=N), 1544 (C=C) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.15 (t, 6H, $OCH_2\underline{CH_3}$), 2.30 (s, 6H, $Ar\underline{CH_3}$), 4.10 (q, 4H, $O\underline{CH_2}CH_3$), 6.18 (s, 1H, -CH), 6.90-8.55 (m, 13H, =CH & Ar-H); ^{13}C NMR (DMSO-d_6, δ, ppm) 13.7, 13.9, 19.9, 24.3, 55.1, 61.8, 63.2, 113.2, 121.3, 124.8, 125.6, 126.2, 126.4, 127.1, 127.7, 128.3, 129.1, 130.6, 131.3, 136.8, 137.2, 139.5, 141.6, 141.8, 154.9, 162.3, 166.5, 167.4, 171.2; LCMS: (*m*/*z*) 567 [M+H]. *Anal*. Calcd. for $C_{33}H_{30}N_2O_5S$: C, 69.94; H, 5.34; N, 4.94. Found: C, 70.01; H, 5.39; N, 4.87.

Ethyl 2-(4-methoxybenzylidene)-5-(3'-(ethoxycarbonyl)biphenyl-4-yl)-7-methyl-3-oxo-3,5-dihydro-2*H*-thiazolo[3,2-a]pyrimidine-6-carboxylate (8e)

Yield: 82%; mp 265-266 °C; IR (KBr) υ 2980 (C-H), 1706 (C=O), 1606 (C=N), 1568 (C=C) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.10 (t, 6H, $OCH_2\underline{CH_3}$), 2.38 (s, 3H, $Ar\underline{CH_3}$), 3.78 (s, 3H, OCH_3), 4.15 (q, 4H, $O\underline{CH_2}CH_3$), 6.28 (s, 1H, -CH), 6.60-8.75 (m, 13H, =CH & Ar-H); ^{13}C NMR (DMSO-d_6, δ, ppm) 13.8, 14.2, 21.2, 54.8, 55.3, 62.7, 63.5, 115.2, 123.1, 124.4, 125.8, 126.0, 126.3, 127.8, 128.6, 129.6, 130.0, 131.1, 131.8, 137.9, 141.0, 141.9, 142.6, 154.6, 160.2, 162.6, 166.5, 167.6, 171.3; LCMS: (*m*/*z*) 583 [M+H]. *Anal*. Calcd. for $C_{33}H_{30}N_2O_6S$: C, 68.02; H, 5.19; N, 4.82. Found: C, 67.95; H, 5.24; N, 4.79.

Ethyl 2-(4-(dimethylamino)benzylidene)-5-(3'-(ethoxycarbonyl)biphenyl-4-yl)-7-methyl-3-oxo-3,5-dihydro-2*H*-thiazolo[3,2-a]pyrimidine-6-carboxylate (8f)

Yield: 92%; mp 210-212 °C; IR (KBr) υ 2973 (C-H), 1718 (C=O), 1610 (C=N), 1538 (C=C) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.10 (t, 6H, $OCH_2\underline{CH_3}$), 2.39 (s, 3H, $Ar\underline{CH_3}$), 2.84 (s, 6H, -$N(\underline{CH_3})_2$), 4.10 (q, 4H, $O\underline{CH_2}CH_3$), 6.24 (s, 1H, -CH), 6.65-8.65 (m, 13H, =CH & Ar-H); LCMS: (*m*/*z*) 596 [M+H]. *Anal*. Calcd. for $C_{34}H_{33}N_3O_5S$: C, 68.55; H, 5.58; N, 7.05. Found: C, 68.61; H, 5.60; N, 6.98.

Ethyl 2-(4-chlorobenzylidene)-5-(3'-(ethoxycarbonyl) biphenyl-4-yl)-7-methyl-3-oxo-3,5-dihydro-2*H*-thiazolo[3,2-a]pyrimidine-6-carboxylate (8g)

Yield: 77%; mp 244-246 °C; IR (KBr) υ 2981 (C-H), 1721 (C=O), 1628 (C=N), 1552 (C=C), 823 (C-Cl) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.15 (t, 6H, $OCH_2\underline{CH_3}$), 2.38 (s, 3H, $Ar\underline{CH_3}$), 4.15 (q, 4H, $O\underline{CH_2}CH_3$), 6.24 (s, 1H, -CH), 7.10-8.60 (m, 13H, =CH & Ar-H); LCMS: (*m*/*z*) 587 [M+H]. *Anal*. Calcd. for $C_{32}H_{27}ClN_2O_5S$: C, 65.48; H, 4.65; N, 4.78. Found: C, 65.55; H, 4.59; N, 4.82.

Ethyl 2-(4-isopropylbenzylidene)-5-(3'-(ethoxycarbonyl)biphenyl-4-yl)-7-methyl-3-oxo-3,5-dihydro-2*H*-thiazolo[3,2-a]pyrimidine-6-carboxylate (8h)

Yield: 88%; mp 187-189 °C; IR (KBr) υ 2972 (C-H), 1714 (C=O), 1618 (C=N), 1528 (C=C), cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.95-1.35 (m, 12H, $OCH_2\underline{CH_3}$), 2.33 (s, 3H, $Ar\underline{CH_3}$), 2.60 (m, 1H, -$Ar\underline{CH}(CH_3)_2$), 4.10 (q, 4H, $O\underline{CH_2}CH_3$), 6.20 (s, 1H, -CH), 7.15-8.65 (m, 13H, =CH & Ar-H); LCMS: (*m*/*z*) 595 [M+H]. *Anal*. Calcd. for $C_{35}H_{34}N_2O_5S$: C, 70.68; H, 5.76; N, 4.73. Found: C, 70.73; H, 5.68; N, 4.69.

Ethyl 2-(4-tert-butylbenzylidene)-5-(3'-(ethoxycarbonyl)biphenyl-4-yl)-7-methyl-3-oxo-3,5-dihydro-2*H*-thiazolo[3,2-a]pyrimidine-6-carboxylate (8i)

Yield: 74%; mp 211-212 °C; IR (KBr) υ 2974 (C-H), 1713 (C=O), 1616 (C=N), 1538 (C=C) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.10-1.48 (m, 15H, $OCH_2\underline{CH_3}$ & -$ArC(\underline{CH_3})_3$), 2.35 (s, 3H, $Ar\underline{CH_3}$), 4.10 (q, 4H, $O\underline{CH_2}CH_3$), 6.22 (s, 1H, -CH), 7.10-8.55 (m, 13H, =CH & Ar-H); LCMS: (*m*/*z*) 609 [M+H]. *Anal*. Calcd. for $C_{36}H_{36}N_2O_5S$: C, 71.04; H, 5.97; N, 4.60. Found: C, 70.97; H, 6.02; N, 4.57.

Ethyl 2-(4-nitrobenzylidene)-5-(3'-(ethoxycarbonyl) biphenyl-4-yl)-7-methyl-3-oxo-3,5-dihydro-2*H*-thiazolo [3,2-a]pyrimidine-6-carboxylate (8j)

Yield: 69%; mp 219-220 °C; IR (KBr) υ 2980 (C-H), 1720 (C=O), 1632 (C=N), 1555 (C=C) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.18 (t, 6H, $OCH_2\underline{CH_3}$), 2.39 (s, 3H, $Ar\underline{CH_3}$), 4.10 (q, 4H, $O\underline{CH_2}CH_3$), 6.28 (s, 1H, -CH), 7.10-8.65 (m, 13H, CH & Ar-H); ^{13}C NMR (DMSO-d_6, δ, ppm) 13.6, 14.1, 21.3, 54.8, 63.1, 63.9, 115.2, 121.4, 123.8, 124.8, 125.4, 126.1, 127.4, 128.3, 128.9, 130.9, 131.7, 138.2, 140.8, 141.2, 141.9, 142.0, 148.5, 154.7, 162.4, 166.6, 167.7, 171.4; LCMS: (*m*/*z*) 598 [M+H]. *Anal*. Calcd. for $C_{32}H_{27}N_3O_7S$: C, 64.31; H, 4.55; N, 7.02. Found: C, 64.42; H, 4.61; N, 6.95.

PHARMACOLOGICAL SCREENING

Antioxidant Screening (in vitro)

Hydrogen Peroxide Scavenging Activity:

A solution of hydrogen peroxide (20 mM) was prepared in phosphate buffer saline (pH 7.4). Various concentrations (12.5, 25, 50, 100 μg/mL) of 1 mL of the test samples or standard, ascorbic acid[28] in methanol were added to 2 mL of hydrogen peroxide solution in phosphate buffer saline. The absorbance was measured at 230 nm after 10 min.[29]

Nitric Oxide Scavenging Activity:

The reaction mixture (6 mL) containing sodium nitroprusside (10 μM, 4 mL), phosphate buffer saline (pH 7.4, 1 mL) and test samples or standard, ascorbic acid solution in dimethyl sulphoxide (1 mL) at various concentrations (12.5, 25, 50, 100 μg/mL) was incubated at 25 ºC for 150 min. After incubation, 0.5 mL of reaction mixture containing nitrite ion was removed, 1 mL of sulphanillic acid reagent was added to this, mixed well and allowed to stand for 5 min for completion of diazotization. Then, 1 mL of naphthyl ethylene diamine dihydrochloride was added, mixed and allowed to stand for 30 min in diffused light. A pink colored chromophore was formed. The absorbance was measured at 640 nm.[30]

Lipid Peroxidation Inhibitory Activity:

Egg lecithin (3 μg/mL phosphate buffer, pH 7.4) was sonicated in an ultrasonic sonicator for 10 min to ensure proper liposome formation. Test samples or standard, ascorbic acid (100 mL) of different concentrations (12.5, 25, 50, 100 μg/mL) were added to liposome mixture (1 mL); the control was without test sample. Lipid peroxidation was induced by adding ferric chloride (10 mL, 400 mM) and L-ascorbic acid (10 mL, 200 μM). After incubation for 1 h at 37 ºC the reaction was stopped by adding hydrochloric acid (2 mL, 0.25 N) containing trichloroacetic acid (150 μg/mL), thiobarbituric acid (3.75 mg/mL) and butylated hydroxy anisole (0.50 μg/mL). The reaction mixture was subsequently boiled for 15 min, cooled, centrifuged at 1000 rpm for 15 min and the absorbance of the supernatant was measured at 532 nm.[31]

For all the above antioxidant methods, experiments were done in triplicate and average is taken, the % inhibition at different concentration was calculated by the following formula

$$\% \text{ Inhibition} = [1-(V_t/V_c)] \times 100$$

Where, V_t mean absorption of test compound, V_c mean absorption of control.

The IC_{50} value was derived from the % inhibition at different concentration.

Antimicrobial Activity:

Applying the agar plate diffusion technique all of the newly synthesized compounds were screened in vitro for antibacterial activity against *E. coli, P. aeruginosa* (Gram-negative), *S. aureus, B. subtilis* (Gram-positive) at 25, 50 and 100 μg/mL concentrations, respectively. Streptomycin (binds to the 16SrRNA of the bacterial ribosome, interfering with the binding of formylmethionyl-tRNA to the 30S subunit therefore prevents initiation of protein synthesis and leads to death of microbial cell) was chosen as a standard drug.[32] Streptomycin is an antibiotic that inhibits both gram-positive and gram-negative bacteria, and is therefore a useful broad spectrum antibiotic. Similarly, the antifungal screening of the compounds was carried out in vitro by paper disc method against two fungi *A. niger* and *C. albicans* by using Amphotericin-B (binds to plasma membrane sterols like ergosterol. The fungi cells have large amount of ergosterol in plasma membrane. The ergosterol facilitates the attachment of amphotericin B, which act as ionophores and cause leakage of cations like K^+) as a standard.[33,34]

CONCLUSION

In conclusion, we have described simple and efficient protocol for the synthesis of novel biphenyl-3,5-dihydro-2*H*-thiazolopyrimidines derivatives (**8a-j**) with good yields. All the synthesized compounds have been investigated for their anti-oxidant, antibacterial and antifungal activities. With our newly synthesized compounds, it is evident that **8j** and **8i** have highest antioxidant activity; **8b**, **8f** and **8g** have antibacterial activity; and **8f** has antifungal activity. Accordingly, these novel class of biphenyl-3,5-dihydro-2*H*-thiazolopyrimidines derivatives reported from our laboratory emerge as a valuable lead series with great potential to be used as anti-oxidant activity, antibacterial and antifungal agents, and as promising candidates for further efficacy evaluation.

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REFERENCES

1. Eicher, T.; Hauptmann, S. *The Chemistry of Heterocycles*, 2nd ed.; Wiley-VCH: Weinheim, 2003.
2. Suresh, M.; Jonnalagadda, S. B.; Rao, C. V. *Oriental. J. Chem.* **2011**, *27*, 127.
3. Suresh, M.; Lavanya, P.; Rao, C. V. *Arabian. J. Chem.* 2011. DOI: 10.1016/j.arabjc.2011.02.004.
4. El-Gazzar, A. R. B. A.; Hafez, H. N. *Acta Chim. Slov.* **2008**, *55*, 359.
5. Suresh, M.; Jonnalagadda, S. B. *Arch. Pharm. Chem. Life Sci*. 2011. (In Press)
6. Regnier, G.; Canevar, L.; Le, R. J.; Douarec, J. C.; Halstop, S.; Daussy, J. *J. Med. Chem*. **1972**, *15*, 295.
7. Winter, C. A.; Fisley, E. A. R.; Nuss, G. W. *Proc. Soc. Exp. Biol. Med*. **1962**, *111*, 544.
8. Suguira, K.; Schmid, A. F.; Schmid, M. M.; Brown, F. G. *Cancer Chemother. Rep. Part.* **1973**, *23*(1), 231.
9. Mojtahedi, M. M.; Saidi, M. R.; Shirzi, J. S.; Bolourtchian, M. *Syn. Commun*. **2002**, *32*, 851.
10. Maquoi, E.; Sounni, N. E.; Devy, L.; Olivier, F.; Frankenne, F.; Krell, H-W.; Grams, F.; Foidart, J-M.; Noel, A. *Clin. Cancer Res*. **2004**, *10*, 4038.
11. Huang, M.; Wang, Y.; Collins, M.; Mitchell, B. S.; Graves, L. M. *Mol. Pharmacol*. **2002**, *62*, 463.
12. Von Bubnoff, N.; Darren, R.; Veach, W.; Miller, T.; Li, W.; Sanger, J.; Peschel, C.; Bornmann, W. G.; Clarkson, B.; Duyster, J. *Cancer Res*. **2003**, *63*, 6395.
13. Kappe, C. O. *Eur. J. Med. Chem*. **2000**, *35*, 1043.
14. Hurst, E. W.; Hull, R. *J. Med. Pharm. Chem*. **1961**, *3*, 215.
15. Mayer, T. U.; Kapoor, T. M.; Haggarty, S. J.; King, R. W.; Schreiber, S. I.; Mitchison, T. J. *Science*. **1999**, *286*, 971.
16. Kato, T. *Jpn. Kokai Tokkyo Koho* **1984**, *974*, 59190.
17. Atwal, K. S.; Swanson, B. N.; Unger, S. E.; Floyd, D. M.; Moreland, S.; Hedberg, A.; OReilly, B. C. *J. Med. Chem*. **1991**, *34*, 806.
18. Rovnyak, G. C.; Atwal, K. S.; Hedberg, A.; Kimball, S. D.; Moreland, S.; Gougoutas, J. Z.; OReilly, B. C.; Schwartz, J.; Malley, M. F. *J. Med. Chem*. **1992**, *35*, 3254.
19. Kappe, C. O. *Molecules* **1998**, *3*, 1.
20. Jauk, B.; Pernat, T.; Kappe, C. O. *Molecules* **2000**, *5*, 227.
21. Patil, A. D.; *et al. J. Org. Chem*. **1995**, *60*, 1182.
22. Hans, N. *Swiss Patent* 592103, 1977; *Chem. Abstr.* **1978**, *88*, 22886.
23. Wilson, K. N.; Utig, C. R.; Subhasinghe, N.; Hoffmann, J. B.; Rudolph, N. J.; Soll, R.; Molloy, C. J.; Bone, R.; Green, D. *Bioorg. Med. Chem. Lett*. **2001**, *11*, 915.
24. Berlin, K. D.; Herd, M. D. *Proc. Okla. Acad. Sci.* **1991**, *71*, 29.
25. Holla, B. S.; Malini, K. V.; Rao, B. S.; Sarojini, B. K.; Kumari, N. S. *Eur. J. Med. Chem.* **2003**, *38*, 313.
26. Holla, B. S.; Mahalinga, M.; Ashok, M.; Karegoudar, P. *Phosphorus, Sulfur Silicon Relat. Elem*. **2006**, *181*, 1427.
27. Rahul, R. N.; Shinde, D. B. *J. Heterocyclic Chem*. **2010**, 47, 33.
28. Ismaili, L.; Nadaradjane, A.; Nicod, L.; Guyon, C.; Xicluna, A.; Robert, J.; Refouvelet, B. *Eur. J. Med. Chem*. **2008**, *43*, 1270.
29. Jayaprakasha, G. K.; Jaganmohan, R. L.; Sakariah, K. K. *Bioorg. Med. Chem*. **2004**, *12*, 5141.
30. Marcocci, L.; Packer, L.; Droy-lefaix, M. T.; Sekaki, A.; Gondes-albert, M. *Methods Enzymol*. **1994**, *234*, 462.
31. Duh, P. D.; Yen, G. H. *Food. Chem*. **1997**, *60*, 639.
32. Bakavoli, M.; Bagherzadeh, G.; Vaseghifar, M.; Shiri, A.; Pordel, M.; Mashreghi, M.; Pordeli, P.; Araghi, M. *Eur. J. Med. Chem*. **2010**, *45*, 647.
33. Rang, H. P.; Dale, M. M.; Ritter, J. M.; Moore, P. K. *Pharmacology*, 3rd ed.; Churchill Livingstone: New York, 2003.
34. Yakaiah, T.; Lingaiah, B. V. P.; Narsaiah, B.; Pranay Kumar, K.; Murthy, U. S. N. *Eur. J. Med. Chem*. **2008**, *43*, 341.