Synthesis and Anti-bacterial Activity of New Series of 4-(Substituted biphenyl-4-yl)-6-methyl-2-oxo/thioxo-1,2,3, 4-tetrahydro-pyrimidine-5-carboxylic Acid Ethyl Ester

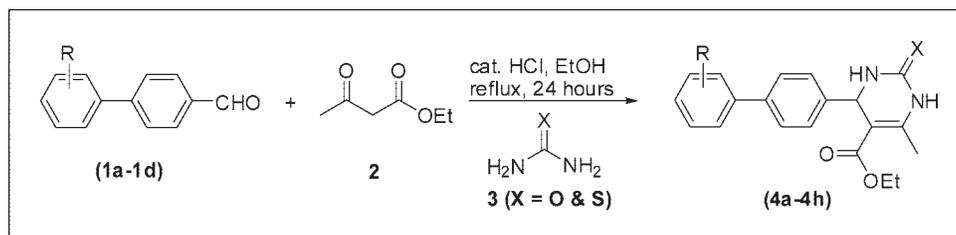Rahul R. Nagawade and Devanand B. Shinde*

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New series of 4-(substituted biphenyl-4-yl)-6-methyl-2-oxo-1,2,3,4-tetrahydro-pyrimidine-5-carboxylic acid ethyl ester and 4-(substituted biphenyl-4-yl)-6-methyl-2-thioxo-1,2,3,4-tetrahydro-pyrimidine-5-carboxylic acid ethyl ester has been synthesized and the structures of the new compounds were established on the basis of ^{1}H NMR, Mass (ES/MS), elemental analysis, and melting point. *In-vitro* antibacterial activity (MIC activity) was evaluated and compared with standard drugs ciprofloxacin, sparfloxacin, and trovafloxacin. Most of the compounds in this new series have shown moderate antibacterial activity against both Gram-positive and Gram-negative organisms.

INTRODUCTION

Numerous antibiotics have been prescribed and found to be effective on various infectious disorders. However, the appearance of multidrug-resistant Gram-positive bacteria, in particular, methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant Enterococci (VRE) is causing a serious menace. Moreover, the emergence of vancomycin-resistant MRSA can be anticipated in foreseeable future. For the treatment of these intractable infections, a new anti-infectious agent is needed. The synthetic antibiotics include the sulphonamide drugs, nitrofuran derivative, pyridine- carboxylic acid analogues, fluoroquinolones, and oxazolidinones. The semi-synthetic antibiotics include the penicillins, cephalosporins, tetracyclines, and macrolides. Among them, the sulphonamide drugs, nitrofuran derivatives, penicillins, and tetracyclines are scarcely used in clinical therapy. Quinolone antibiotics are widely prescribed drugs because of their safety, good tolerance, broad antibacterial spectrum, and less resistance [1–4]. Macrolide antibiotics, including Erythromycin and related compounds, continue to be an important therapeutic class against Gram-positive organisms, with second generation macrolides such as Clarithromycin and Azithromycin being widely prescribed due to their efficacy, safety, and lack of serious side effects [5]. Oxazolidinone antibacterial agents [6] are newer class of synthetic antibac-

terial agents with activity against Gram-positive bacteria. Linezolid [7] is well known as first promising candidate of oxazolidinone and works effectively against numerous serious Gram-positive human pathogens caused by MRSA and VRE. Quinolines and naphthyridines are also emerging as a new class of synthetic antibacterial agents.

- Dihydropyrimidones and their thione analogues have been reported to exhibit wide range of biological activities such as antiviral, antitumor agents, anti-carcinogenic, anti-inflammatory, analgesic, and most important anti-hypertensive activity. Dihydropyrimidinones (DHPMS) have now been recognized as vital drugs in the antihypertensive treatment as well as calcium channel blockers, α-1α-antagonists, and neuropeptide Y (NPY) antagonists [8]. Some of them are batzeladine alkaloids, which have been found to be potent HIV gp 120-CD4 inhibitors [9]. DHPMS and their sulfur analogues are pharmacologically important because of their antibacterial, antitumor, and anti-inflammatory properties [10]. The biological activity of some recently isolated alkaloids has also been attributed to the presence of dihydropyrimidinone moiety in the corresponding molecule [11]. Since dihydropyrimidones and thiones have been reported to exhibit wide range of biological activity and so far none of

Scheme 1
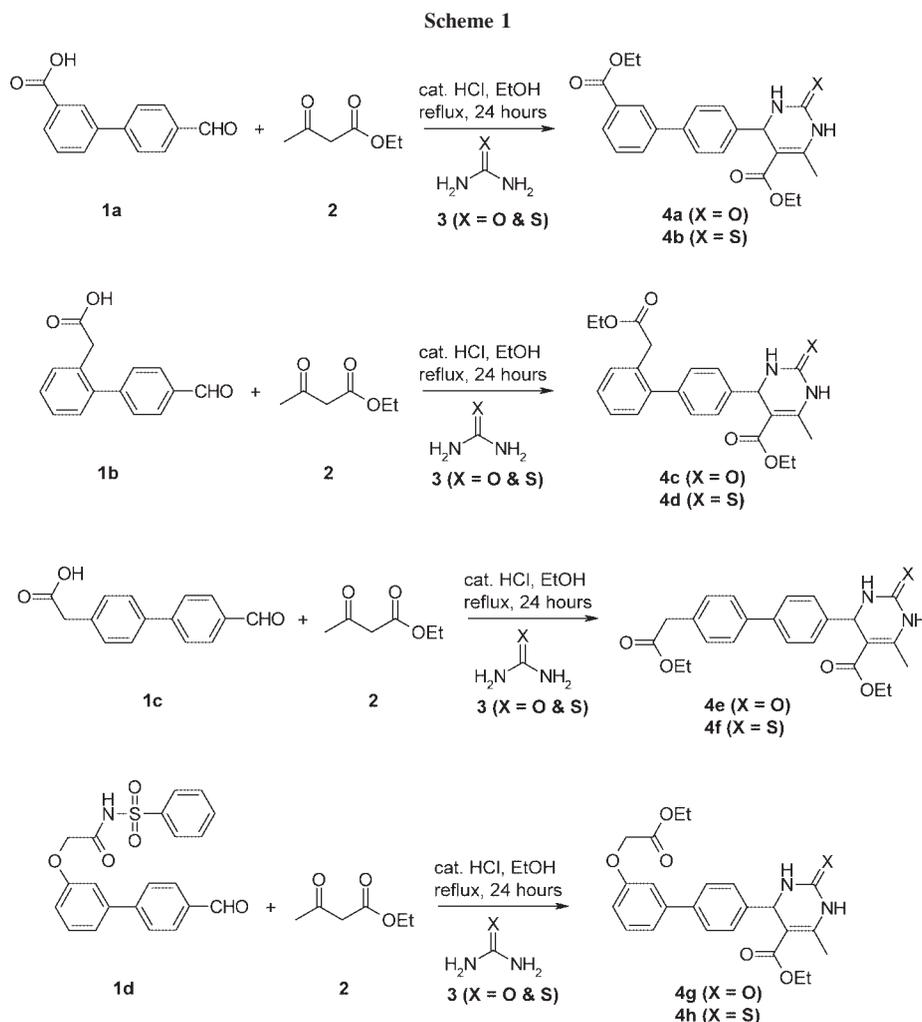
them have been evaluated for anti-bacterial activity, we decided to synthesise new series of dihydropyrimidones and thiones and evaluate them for antibacterial activity.

• In 1893, Biginelli [12] reported the first synthesis of DHPMS by a simple one-pot condensation reaction of ethyl acetoacetate, benzaldehyde, and urea. In the following decades, the original cyclocondensation reaction had been extended widely to include variation in all three components, allowing access to a large number of multifunctionalized DHPMS derivatives. However, it suffers from low yields of the product particularly in the case of substituted aromatic and aliphatic aldehydes. Recently, several methods have been reported for preparing dihydropyrimidines using different lewis acid such as $BF_3.OEt_2$, $LaCl_3$, $La(OTf)_3$, $Yb(OTf)_3$ $ZnCl_2$, $ZnBr_2$, $ZrCl_4$, $BiCl_3$, $Bi(OTf)_3$, $LiBr$, $LiClO_4$, $Mn(OAc)_3$, CAN, $FeCl_3.6H_2O$, $NiCl_2.6H_2O$, etc.[13] as well as protic acids such as H_2SO_4, HOAc, conc. HCl [14] as promoters.

• Oxazolidinones, a new class of antibacterial agent has many promising candidates with biaryl moiety [15]. The excellent in vitro antibacterial activity (MIC activity) has been attributed because of biaryl part and accordingly new series of biaryl dihydropyrimidones and thiones has been synthesized.

Herein, we describe the synthesis, characterization, and biological evaluation of new series of 4-(substituted biphenyl-4-yl)-6-methyl-2-oxo-1,2,3,4-tetrahydro-pyrimidine-5-carboxylic acid ethyl ester and 4-(substituted biphenyl-4-yl)-6-methyl-2-thioxo-1,2,3,4-tetrahydro-pyrimidine-5-carboxylic acid ethyl ester as potential antibacterials along with their in vitro biological activity (MIC activity). The synthesis of [4-(substituted biphenyl-4-yl)-6-methyl-2-oxo-1,2,3,4-tetrahydro-pyrimidine-5-carboxylic acid ethyl ester and 4-(substituted biphenyl-4-yl)-6-methyl-2-thioxo-1,2,3,4-tetrahydro-pyrimidine-5-carboxylic acid ethyl ester is outlined in Scheme 1.

Scheme 2

Scheme 4
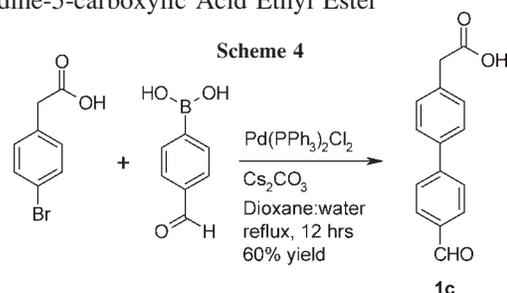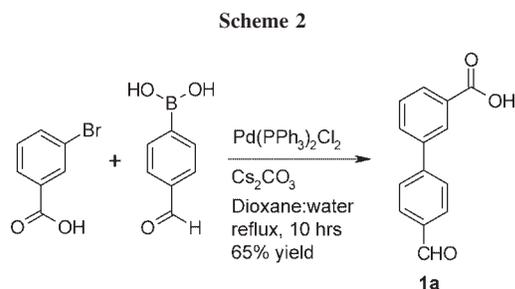
The synthesis is achieved in two parts: first part involves the synthesis of differentially substituted biaryl aldehydes and the second part involves Biginelli condensation of these biaryl aldehydes with ethylacetoacetate and urea or thiourea to afford the desired compounds. The biaryl aldehydes **1a** (4′-formyl-biphenyl-3-carboxylic acid), **1b** (4′-formyl-biphenyl-2-yl-acetic acid), and **1c** (4′-formyl-biphenyl-4-yl-acetic acid) have been synthesized by Suzuki coupling of 4-formylphenyl-boronic acid with respective aryl bromide using bis(triphenylphosphine) palladium(II) chloride as a catalyst and cesium carbonate as a base in dioxane: water as solvent at reflux temperature (Schemes 2–4).

N-[2-(4′-Formyl-biphenyl-2-yloxy)-acetyl]-benzene-sulfonamide (1d). The synthesis starts with alkylation of 3-bromo phenol (**i**) with chloroacetic acid in water using sodium hydroxide as base to afford (2-bromo-phenoxy)-acetic acid (**ii,** CAS#1798-99-8). Second step involves reaction of compound **ii** (acid chloride) with benzene sulphonamide in DCM using triethylamine as a base to afford N-[2-(2-bromo-phenoxy)-acetyl]-benzene-sulfonamide (**iii**). Third step involves Suzuki coupling of compound **iii** with 4-formylphenylboronic acid using bis(triphenyl-phosphine)palladium(II) chloride as a catalyst and cesium carbonate as a base in dioxane: water at reflux temperature to afford the desired biaryl aldehyde **1d** (Scheme 5).

Second part of synthesis involves Biginelli condensation of these biaryl aldehydes (**1a, 1b, 1c,** and **1d**) with ethylacetoacetate (**2**) and urea/thiourea (**3**) in ethanol using catalytic HCl to afford the desired dihydropyrimidones and thiones (**4a–4h,** Scheme 1). Since the reaction is carried out with catalytic HCl in ethanol, all the final
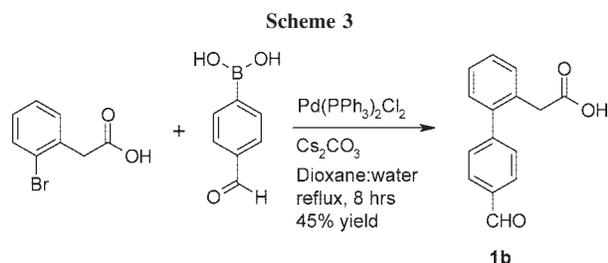
compounds are isolated as respective ethyl esters (**4a–4h,** Scheme 1). The benzenesulphonamido side chain was incorporated in biaryl aldehyde since sulphonamides have been reported to exhibit good antibacterial activity. However, during the reaction, the benzenesulphonamido portion of biaryl aldehyde was hydrolysed to acid and finally isolated as ethyl ester (**4g, 4h,** Scheme 1). The Biginelli condensation of biaryl aldehyde, ethylacetoacetate, and urea is clean and high yielding (60–70% yield) when compared with thiourea, where the yields are between 45 and 60%.

The new 4-(substituted-biphenyl-4-yl)-6-methyl-2-oxo/thioxo-1,2,3,4-tetrahydro-pyrimidine-5-carboxylic acid ethyl esters prepared above were tested *in vitro versus* a panel of Gram-positive and Gram-negative clinical isolates. Minimum inhibitory concentration (MIC) was determined by standard agar dilution method as per NCCLS guidelines and the values are shown in Table 1. The data of ciprofloxacin, sparfloxacin, and trovafloxacin were used as reference standards.

Results of biological evaluation. Most of the compounds in the series have shown moderate antibacterial activity against both Gram-positive and Gram-negative strains, except for *Pseudomonas aeruginosa* and *E. faecium*, where all the tested compounds were inactive (MIC > 32 μg/ml) in comparison with ciprofloxacin, sparfloxacin, and trovafloxacin as reference standards. In all the cases, the thioxo analogues have shown one-fold better activity with respect to their oxo counterparts.

In summary, the synthesis of new 4-(substituted-biphenyl-4-yl)-6-methyl-2-oxo/thioxo-1,2,3,4-tetrahydro-pyrimidine-5-carboxylic acid ethyl esters has been described. Most of the compounds in the series have shown moderate antibacterial activity against *S. aureus*, *S. epidermis*, *E. coli*, and *Klebsiella species* as compared to ciprofloxacin, sparfloxacin, and trovafloxacin. Since the preliminary series of novel dihydropyrimidones and thiones have shown moderate antibacterial activity, the possibility remains that more structural variation might change/ improve the activity profile. In connection with this notion, we point out for the first time dihydropyrimidones and their thione analogues as potential antibacterial agents and suggest that the further study is warranted.

Scheme 3

Scheme 5
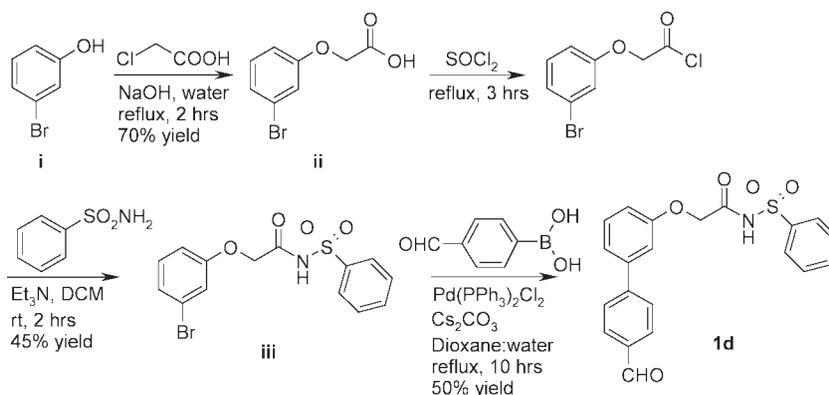
EXPERIMENTAL

Melting points were determined on Quality Precise apparatus and are uncorrected. ^{1}H NMR spectra were recorded on Bruker 400-MHz spectrometer. Chemical shifts are reported in δ units (ppm) relative to TMS as internal standard. Electron spray ionization mass spectra (ES-MS) were recorded on Water-Micromass Quattro-II spectrometer. All the solvents and reagents used were of AR grade and were used without further purification.

4′-Formyl-biphenyl-3-carboxylic acid (1a). To a stirred solution of 3-bromobenzoic acid (1 g, 4.98 mmol) in dioxane: water (20 mL, 4:1) was added cesium carbonate (4.85 g, 14.92 mmol) followed by addition of 4-formylphenylboronic acid (0.90 g, 5.96 mmol) and the resulting solution was stirred and degassed under nitrogen for 15 min. Bis(triphenylphosphine) palladium(II) chloride (0.17 g, 0.24 mmol) was added and the reaction mixture was stirred at reflux temperature for 10 h. After completion (monitored by TLC, 1:9 MeOH: CHCl3 as

Table 1

Minimum inhibitory concentration (µg/mL) of 4-(substituted-biphenyl-4-yl)-6-methyl-2-oxo/thioxo-1,2,3,4-tetrahydro-pyrimidine-5-carboxylic acid ethyl ester.

Comp.	P. aeruginosa (n = 26)			S. aureus (n = 80)			E. coli (n = 54)			S. epidermidis (n = 33)		
	MIC 50	MIC 90	Range	MIC	MIC	Range	MIC 50	MIC 90	Range	MIC50	MIC 90	Range
4a		Inactive (>32)		16	>32	8–32	8	>16	4–>32	16	>32	8–32
4b		Inactive (>32)		8	>16	4–>32	8	>16	2–>32	8	>16	4–>32
4c		Inactive (>32)		16	>32	8–32	8	>16	4–>32	16	>32	8–32
4d		Inactive (>32)		8	>16	4–>32	8	>16	2–>32	8	>16	4–>32
4e		Inactive (>32)		16	>32	8–32	8	>16	4–>32	16	>32	8–32
4f		Inactive (>32)		8	>16	4–>32	8	>16	2–>32	8	>16	4–>32
4g		Inactive (>32)		16	>32	8–32	8	>16	4–>32	16	>32	8–32
4h		Inactive (>32)		8	>16	4–>32	8	>16	2–>32	8	>16	4–>32
Cipro.	0.25	1	0.12–4	1	4	0.5–8	0.007	0.015	0.003–0.03	1	4	0.5–8
Spar.	1.0	2	0.25–4	1	2	0.5–4	0.007	0.03	0.003–0.03	1	2	0.5–4
Trova.	0.25	1	0.06–4	0.5	1	0.25–4	0.002	0.015	0.003–0.03	0.5	1	0.25–4

	Klebsiella sp. (n = 24)			E. faecium (n = 16)			E. faecalis (n = 24)		
	MIC 50	MIC 90	Range	MIC 50	MIC 90	Range	MIC 50	MIC 90	Range
4a	8	>32	2–>32		Inactive (>32)		8	>32	2–>32
4b	8	>16	2–>32		Inactive (>32)		8	>16	2–>32
4c	8	>32	2–>32		Inactive (>32)		8	>32	2–>32
4d	8	>16	2–>32		Inactive (>32)		8	>16	2–>32
4e	8	>32	2–>32		Inactive (>32)		8	>32	2–>32
4f	8	>16	2–>32		Inactive (>32)		8	>16	2–>32
4g	8	>32	2–>32		Inactive (>32)		8	>32	2–>32
4h	8	>16	2–>32		Inactive (>32)		8	>16	2–>32
Cipro	0.01	0.02	0.01–0.5	>16	>16	NA	2	8	0.5–16
Spar.	0.01	0.02	0.01–0.25	>16	>16	NA	1	4	0.5–8
Trova.	0.025	0.1	0.06–0.4	16	16	NA	1	2	0.5–4

n = number of strains tested.

mobile phase), solvent was removed under reduced pressure and diluted with water. The aqueous phase was acidified upto pH 4 by dilute HCl and then extracted with ethyl acetate (3 × 25 mL). The combined organic layers were dried (Na$_2$SO$_4$) and concentrated under reduced pressure. The crude compound was purified by column chromatography (60–120 mesh silica gel) eluting with methanol: chloroform (2:98) to afford the title compound **1a** as light yellow solid (0.70 g, 65% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.65 (t, 1H), 7.95–7.97 (d, J = 8.08 Hz, 2H), 8.00–8.03 (m, 4H), 8.26 (s, 1H), 10.07 (s, 1H), 13.20 (s, 1H, COOH); Mass (m/z): 228 (M + H, 100%).

(4′-Formyl-biphenyl-2-yl)-acetic acid (1b). To a stirred solution of 2-bromophenylacetic acid (1 g, 4.65 mmol) in dioxane: water (20 mL, 4:1) was added cesium carbonate (4.53 g, 13.95 mmol) followed by addition of 4-formylphenylboronic acid (0.84 g, 5.58 mmol) and the resulting solution was stirred and degassed under nitrogen for 15 min. Bis(triphenyl-phosphine)palladium(II) chloride (0.16 g, 0.23 mmol) was added and the reaction mixture was stirred at reflux temperature for 8 h. After completion (monitored by TLC, 1:9 MeOH: CHCl3 as mobile phase), solvent was removed under reduced pressure and diluted with water. The aqueous phase was acidified upto pH 4 by dilute HCl and then extracted with ethyl acetate (3 × 25 mL). The combined organic layers were dried (Na$_2$SO$_4$) and concentrated under reduced pressure. The crude compound was purified by column chromatography (60–120 mesh silica gel) eluting with methanol: chloroform (2:98) to afford the title compound 1b as yellow solid (0.49 g, 45% yield). M.p. 171–173°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.54 (s, 2H), 7.35–7.40 (m, 2H), 7.53 (d, J = 8.16 Hz, 2H), 7.89–7.92 (m, 2H), 7.96–8.02 (m, 2H), 10.07 (s, 1H), 12.31 (bs, 1H, COOH); Mass (m/z): 241 (M + H, 100%).

(4′-Formyl-biphenyl-4-yl)-acetic acid (1c). To a stirred solution of 4-bromophenylacetic acid (1 g, 4.65 mmol) in dioxane: water (20 mL, 4:1) was added cesium carbonate (4.53 g, 13.95 mmol) followed by addition of 4-formylphenylboronic acid (0.84 g, 5.58 mmol) and the resulting solution was stirred and degassed under nitrogen for 15 min. Bis(triphenyl-phosphine)palladium(II) chloride (0.16 g, 0.23 mmol) was added and the reaction mixture was stirred at reflux temperature for 12 h. After completion (monitored by TLC, 1:9 MeOH: CHCl3 as mobile phase), solvent was removed under reduced pressure and diluted with water. The aqueous phase was acidified upto pH 4 by dilute HCl and then extracted with ethyl acetate (3 × 25 mL). The combined organic layers were dried (Na$_2$SO$_4$) and concentrated under reduced pressure. The crude compound was purified by column chromatography (60–120 mesh silica gel) eluting with methanol: chloroform (2:98) to afford the title compound 1c as yellow solid (0.65 g, 60% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.64 (s, 2H), 7.40 (d, J = 8 Hz, 2H), 7.72 (d, J = 7.96 Hz, 2H), 7.91 (d, J = 8.08 Hz, 2H), 7.99 (d, J = 7.96 Hz, 2H), 10.05 (s, 1H), 12.41 (s, 1H, COOH); Mass (m/z): 241 (M + H, 100%).

(3-Bromo-phenoxy)-acetic acid (ii). To a stirred solution of 3-bromo phenol (i) (5 g, 28 mmol) in water (50 mL) was added NaOH (1.34 g, 33.6 mmol) followed by addition of chloroacetic acid (2.89 g, 30.8 mmol) and the resulting solution was refluxed for 2 h. After completion, the reaction mixture was acidified upto pH 4 by 6N HCl and the resulting mixture was extracted ethyl acetate (3 × 50 mL). The combined

organic layers were dried (Na$_2$SO$_4$) and concentrated under reduced pressure to afford the title compound ii as yellow solid (4.67 g, 70% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.72 (s, 2H), 6.92–6.95 (m, 1H), 7.12–7.15 (m, 2H), 7.24 (t, J = 7.84, 1.96 Hz, 1H), 13.09 (s, 1H, COOH); Mass (m/z): 232 (M + H, 100%).

N-**[2-(3-Bromo-phenoxy)-acetyl]-benzenesulfonamide (iii).** A stirred solution of compound ii (2.5 g, 10.82 mmol) in SOCl$_2$ (15 mL) was refluxed under nitrogen atmosphere for 3 h. After completion, solvent was removed completely under reduced pressure to afford the acid chloride. To this acid chloride solution dissolved in DCM (25 mL) was added triethylamine (3 mL, 21.64 mmol) followed by addition of benzenesulphonamide (2.03 g, 12.98 mmol) under cooling. After addition, the reaction mixture was stirred at room temperature for additional 2 h. After completion, the reaction mixture was quenched with water (25 mL) and the organic phase was separated, dried (Na$_2$SO$_4$), and concentrated under reduced pressure. The crude compound was purified by column chromatography (60–120 mesh silica gel) eluting with 1:1 ethyl acetate: hexane to afford the title compound iii (1.80 g, 45% yield). M.p. 207–210°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.70 (s, 2H), 6.81–6.84 (dd, J = 2.32, 1.84 Hz, 1H), 7.02 (t, J = 2.2, 1.96 Hz, 1H), 7.12 (d, J = 8.08 Hz, 1H), 7.19 (m, 1H), 7.63 (m, 2H), 7.72 (m, 1H), 7.92 (m, 2H), 12.49 (bs, 1H, COOH); Mass (m/z): 371 (M + H, 100%).

N-**[2-(4′-Formyl-biphenyl-3-yloxy)-acetyl]-benzenesulfonamide (1d).** To a stirred solution of *N*-[2-(3-bromo-phenoxy)-acetyl]-benzenesulfonamide (iii) (1 g, 2.70 mmol) in dioxane: water (20 mL, 4:1) was added cesium carbonate (2.63 g, 8.10 mmol) followed by addition of 4-formylphenylboronic acid (0.49 g, 3.24 mmol) and the resulting solution was stirred and degassed under nitrogen for 15 min. Bis(triphenylphosphine)palladium(II) chloride (0.094 g, 0.13 mmol) was added and the reaction mixture was stirred at reflux temperature for 10 h. After completion (monitored by TLC, 1:9 MeOH: CHCl3 as mobile phase), solvent was removed under reduced pressure and diluted with water. The aqueous phase was acidified upto pH 4 by dilute HCl and then extracted with ethyl acetate (3 × 25 mL). The combined organic layers were dried (Na$_2$SO$_4$) and concentrated under reduced pressure. The crude compound was purified by column chromatography (60–120 mesh silica gel) eluting with methanol: chloroform (1:99) to afford the title compound **1d** as yellow solid (0.53 g, 50% yield). M.p. 143–145°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.77 (s, 2H), 6.88 (d, J = 7.84 Hz, 1H), 7.13 (s, 1H), 7.32–7.39 (m, 2H), 7.55–7.59 (m, 2H), 7.61–7.65 (m, 1H), 7.81 (d, J = 8.08 Hz, 2H), 7.92 (d, J = 7.80 Hz, 2H), 7.99 (d, J = 8.12 Hz, 1H), 10.06 (s, 1H), 12.52 (bs, 1H, COOH); Mass (m/z): 396 (M + H, 100%).

General procedure for synthesis of compounds 4a to 4h. To a stirred solution of ethyl acetoacetate (**2**) (2 mmol) in ethanol was added biaryl aldehyde (**1a-1d**) (2 mmol), urea/thiourea (**3**) (3 mmol) followed by addition of 0.5 mL of 6N HCl. The resulting solution was stirred at reflux for 16 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$_2$SO$_4$) and concentrated under reduced pressure. The crude compound was purified by column chromatography (60–120 mesh silica gel) eluting with ethyl acetate: hexane (1:1) to afford the title compounds **4a–4h** as solid.

4-(3′-Ethoxycarbonyl-biphenyl-4-yl)-6-methyl-2-oxo-1,2,3,4-tetrahydro-pyrimidine-5-carboxylic acid ethyl ester (4a). Light yellow solid; 65% yield; M.p. 128–130°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.12 (t, $J = 7.08, 7.08$ Hz, 3H), 1.33 (t, $J = 7.12, 7.08$ Hz, 3H), 2.26 (s, 3H), 3.98–4.03 (q, $J = 7.08, 7.16$ Hz, 2H), 4.31–4.37 (q, $J = 7.04, 7.16$ Hz, 2H), 5.20 (d, $J = 2.96$ Hz, 1H), 7.35 (d, $J = 8.24$ Hz, 2H), 7.59–7.63 (m, 2H), 7.66 (d, $J = 8.28$ Hz, 2H), 7.91–7.95 (m, 2H), 8.15 (bs, 1H, NH), 9.25 (s, 1H, NH); Mass (m/z): 409 (M + H, 100%); Anal. Calcd. for $C_{23}H_{24}N_2O_5$: C, 67.63; H, 5.92; N, 6.86. Found: C, 67.88; H, 5.97; N, 6.92.

4-(3′-Ethoxycarbonyl-biphenyl-4-yl)-6-methyl-2-thioxo-1,2,3,4-tetrahydro-pyrimidine-5-carboxylic acid ethyl ester (4b). Yellow solid, 50% yield; M.p. 141–142°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.15 (t, $J = 7.12, 7.08$ Hz, 3H), 1.33 (t, $J = 7.12, 7.04$ Hz, 3H), 2.40 (s, 3H), 4.02–4.06 (q, $J = 7.12, 7.12$ Hz, 2H), 4.31–4.36 (q, $J = 7.08, 7.08$ Hz, 2H), 5.36 (s, 1H), 7.27 (d, $J = 8.16$ Hz, 2H), 7.55–7.64 (m, 5H), 7.90–7.94 (m, 2H), 8.14 (s, 1H); 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.

4-(2′-Ethoxycarbonylmethyl-biphenyl-4-yl)-6-methyl-2-oxo-1,2,3,4-tetrahydro-pyrimidine-5-carboxylic acid ethyl ester (4c). Off-white solid; 60% yield; M.P. 94–96°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.05 (t, $J = 6.76, 6.68$ Hz, 3H), 1.15 (t, $J = 6.48, 5.72$ Hz, 3H), 2.26 (s, 3H), 3.58 (s, 2H), 3.90–3.94 (q, $J = 6.78, 6.92$ Hz, 2H), 3.99–4.03 (q, $J = 7.02, 7.02$ Hz, 2H), 5.19 (s, 1H), 7.21–7.33 (m, 7H), 7.62 (m, 1H), 7.79 (s, 1H), 9.23 (s, 1H); Mass (m/z): 423 (M + H, 100%); Anal. Calcd. for $C_{24}H_{26}N_2O_5$: C, 68.23; H, 6.20; N, 6.63. Found: C, 68.57; H, 6.25; N, 6.68.

4-(2′-Ethoxycarbonylmethyl-biphenyl-4-yl)-6-methyl-2-thioxo-1,2,3,4-tetrahydro-pyrimidine-5-carboxylic acid ethyl ester (4d). Light yellow solid; 50% yield; M.p. 99–101°C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.04 (t, $J = 7.16, 7.08$ Hz, 3H), 1.12 (t, $J = 7.08, 7.04$ Hz, 3H), 2.30 (s, 3H), 3.58 (s, 2H), 3.89–3.95 (q, $J = J = 7.00, 7.04$ Hz, 2H), 4.02–4.05 (q, $J = 7.08, 7.12$ Hz, 2H), 5.21 (d, $J = 3.60$ Hz, 1H), 7.20–7.26 (m, 5H), 7.31–7.34 (m, 3H), 9.71 (s, 1H, NH), 10.39 (s, 1H, NH); Mass (m/z): 439 (M + H, 100%); Anal. Calcd. for $C_{24}H_{26}N_2O_4S$: C, 65.73; H, 5.98; N, 6.39. Found: C, 65.41; H, 5.93; N, 6.35.

4-(4′-Ethoxycarbonylmethyl-biphenyl-4-yl)-6-methyl-2-oxo-1,2,3,4-tetrahydro-pyrimidine-5-carboxylic acid ethyl ester (4e). Yellow solid; 65% yield; M.p. 146–148°C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.12 (t, $J = 7.08, 7.08$ Hz, 3H), 1.19 (t, $J = 7.08, 7.08$ Hz, 3H), 2.26 (s, 3H), 3.69 (s, 2H), 3.97–4.03 (q, $J = 7.08, 7.04$ Hz, 2H), 4.06–4.11 (q, $J = 7.16, 7.08$ Hz, 2H), 5.17 (d, $J = 3.08$ Hz, 1H), 7.30–7.34 (m, 4H), 7.60 (m, 4H), 7.78 (s, 1H, NH), 9.24 (s, 1H, NH); Mass (m/z): 423 (M + H, 100%); Anal. Calcd. for $C_{24}H_{26}N_2O_5$: C, 68.23; H, 6.20; N, 6.63. Found: C, 67.95; H, 6.16; N, 6.59.

4-(4′-Ethoxycarbonylmethyl-biphenyl-4-yl)-6-methyl-2-thioxo-1,2,3,4-tetrahydro-pyrimidine-5-carboxylic acid ethyl ester (4f). Yellow solid; 55% yield; M.p. 134–136°C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.06-1.20 (m, 6H), 2.28 (s, 3H), 3.67 (s, 2H), 3.99–4.07 (m, 4H), 5.18 (s, 1H), 7.25–7.32 (m, 4H), 7.56–7.62 (m, 4H), 9.68 (s, 1H, NH), 10.36 (s, 1H, NH); Mass (m/z): 439 (M + H, 100%); Anal. Calcd. for $C_{24}H_{26}N_2O_4S$: C, 65.73; H, 5.98; N, 6.39. Found: C, 65.38; H, 6.03; N, 6.44.

4-(3′-Ethoxycarbonylmethoxy-biphenyl-4-yl)-6-methyl-2-oxo-1,2,3,4-tetrahydro-pyrimidine-5-carboxylic acid ethyl ester (4g). Yellow solid; 50% yield; M.p. 151–152°C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.12 (t, $J = 7.12, 7.04$ Hz, 3H), 1.20 (t, $J = 7.20, 7.12$ Hz, 3H), 2.26 (s, 3H), 3.97–4.03 (q, $J = 6.96, 7.08$ Hz, 2H), 4.14–4.20 (q, $J = 7.12, 7.12$ Hz, 2H), 4.85 (s, 2H), 5.17 (d, $J = 2.96$ Hz, 1H), 6.89–6.92 (dd, $J = 1.96, 1.92$ Hz 1H), 7.16 (bs, 1H), 7.23 (d, $J = 7.92$ Hz, 1H), 7.30 (d, $J = 8.20$ Hz, 2H), 7.36 (t, $J = 7.88, 8.00$ Hz, 1H), 7.62 (d, $J = 8.24$ Hz, 2H), 7.79 (s, 1H, NH), 9.25 (s, 1H, NH); Mass (m/z): 439 (M + H, 100%); Anal. Calcd. for $C_{24}H_{26}N_2O_6$: C, 65.74; H, 5.98; N, 6.39. Found: C, 66.02; H, 5.93; N, 6.43.

4-(3′-Ethoxycarbonylmethoxy-biphenyl-4-yl)-6-methyl-2-thioxo-1,2,3,4-tetrahydro-pyrimidine-5-carboxylic acid ethyl ester (4h). Yellow solid; 40% yield; M.p. 163–165°C, ^{1}H NMR (400 MHz, CDCl$_3$) δ 1.19 (t, $J = 7.12, 7.12$ Hz, 3H), 1.29 (t, $J = 7.12, 7.12$ Hz, 3H), 2.37 (s, 3H), 4.10–4.12 (q, $J = 7.08, 7.12$ Hz, 2H), 4.25–4.30 (q, $J = 7.12, 7.16$ Hz, 2H), 4.66 (s, 2H), 5.44 (d, $J = 2.40$ Hz, 1H), 6.87 (d, $J = 8.12$ Hz, 1H), 6.99 (d, $J = 7.36$ Hz, 1H), 7.10 (s, 1H), 7.17 (d, $J = 7.92$ Hz, 1H), 7.32–7.35 (m, 4H); Mass (m/z): 455 (M + H, 100%); Anal. Calcd. for $C_{24}H_{26}N_2O_5S$: C, 63.42; H, 5.77; N, 6.16. Found: C, 63.11; H, 5.83; N, 6.23.

MIC determination. *Bacterial isolates.* The strains were collected from major hospitals of India during the period 2003–2006 and were identified by standard laboratory procedures.

MIC was determined by standard agar dilution method as per CLSI (formerly, NCCLS) guidelines (Approved Standard M7-A6, vol. 23, 2003) on Mueller-Hinton agar containing serial two-fold dilutions of the compounds. Strains were grown in Tryptic Soya Broth (TSB, HiMedia, India) for 18–24 h. The overnight grown cultures were diluted appropriately so that the final density is approximately 10^7 CFU/mL. A portion of this diluted broth was transferred to seed block of a multipoint inoculator (Applied Quality Services, United Kingdom). The inoculating pins were standardized to inoculate 1–2 μL of this broth, so that the final CFU was 1×10^4–5×10^4 CFU/ spot. The inoculated plates were allowed to stand until the moisture in the inoculum spot has been absorbed in the media.

The inoculated plates were inverted and incubated for 18–24 h at 35°C in an ambient air incubator (Newtronic, India). After the completion of incubation period, the plates were read visually.

MIC was defined as the lowest concentration that inhibited the growth of strain completely. Drug free plates were used to ensure the growth of strains. *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as quality control strains for each run of MIC determination.

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