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Synthesis and Biological Activity of Ethyl 2-(substituted benzylthio)-4-(3′-(ethoxycarbonyl)biphenyl-4-yl)-6-methyl-1,4-dihydropyrimidine-5-carboxylate Derivatives

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In the present study, a new series of ethyl 2-(substituted benzylthio)-4-(3′-(ethoxycarbonyl)-biphenyl-4-yl)-6-methyl-1,4-dihydropyrimidine-5-carboxylate derivatives was synthesized. The newly synthesized compounds were characterized by ^{1}H-NMR, mass and C, H, N analyses. All newly synthesized compounds were screened for their antibacterial (*Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus pyogenes* and *Klebsiella pneumoniae*) and antifungal (*Aspergillus flavus*, *Aspergillus fumigatus*, *Candida albicans*, *Penicillium marneffei* and *Trichophyton mentagrophytes*) activity. The results revealed that all synthesized compounds have a significant biological activity against the tested microorganisms. Compounds **8a**, **8b**, **8c**, **8e**, **8f**, **8i**, and **8j** exhibited good antimicrobial activity.

Keywords: Antibacterial activities / Antifungal activities / Biphenyl pyrimidine

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Introduction

Heterocycles are ubiquitous among pharmaceutical compounds [1]. Pyrimidine moiety is an important class of *N*-containing heterocyclics, which are widely used as key building blocks for pharmaceutical agents. Pyrimidine moiety exhibits a wide spectrum of pharmacophores as it acts as bactericidal, fungicidal [2], analgesic [3], antihypertensive [4] and antitumor agent [5]. Among the pyrimidine containing heterocyclics, thiouracils are used as for antiinflammatory and virucidal agents [6]. Also, preclinical data from literature survey indicate continuing research in poly substituted pyrimidines as potential antitumor agents [7]. The biological and synthetic significance places this scaffold at a prestigious position in medicinal chemistry research. The key role pyrimidines play in cellular processes has made them valuable leads for drug discovery. Another important class of pyrimidine is 2-thiopyrimidine (2-TP) and its derivatives, which are also well known as 2-mercaptopyrimidine compounds [8]. In 2-TP ring sulfur atom serves as an interesting replacement for the existing oxygen atom bonded to C-2 in uridine base [9]. Due this reason, 2-TPs have attracted substantial interest of synthetic-biochemists [10]. A European patent [11] revealed the scope of 2-TP derivatives in preparation of cardiotonic drugs. Pathak et al. have evaluated and reported the existence of primary activity of 2-TP derivatives against *Mycobacterium tuberculosis* (Mtb) [12].

Biginelli, in 1893, reported one-step synthesis of 3,4-dihydropyrimidin-2(1*H*)-one by three-component condensation of aldehydes, ethyl acetoacetate and urea in alcohol using strong mineral acid [13]. These Biginelli compounds possess several pharmaceutical properties like antibacterial, antiviral, antiinflammatory, antihypertensive and antitumor agents [14]. In continued quest of new antimicrobial agents, we designed and synthesized novel biphenylpyrimidines having substituted benzylthio groups. Structures of the products were characterized by ^{1}H-NMR, LCMS mass spectrometry and elemental analysis. Results of biological activities indicate that some compounds possess potential antimicrobial activity.

Results and discussion

Chemistry

The synthesis of ethyl 2-(substituted benzylthio)-4-(3′-(ethoxycarbonyl)biphenyl-4-yl)-6-methyl-1,4-dihydropyrimidine-5-

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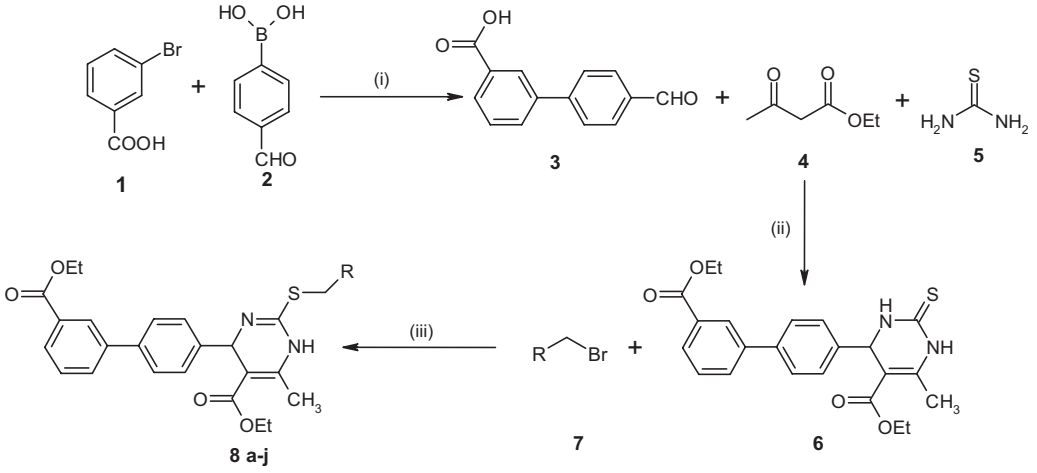
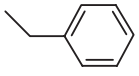
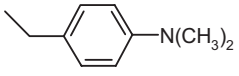
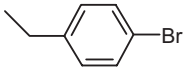
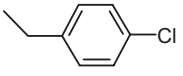
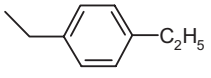
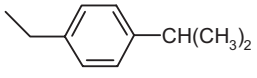
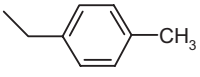
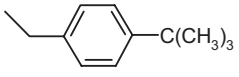
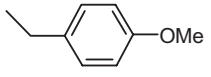
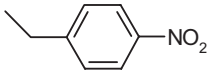
carboxylate derivatives **8a–j** was achieved through the versatile and efficient synthetic route outlined in Scheme 1. The desired compounds were synthesized as follows. Initially, when 3-bromobenzoic acid (**1**) was treated with 4-formylphenylboronic (**2**) acid in the presence of cesium carbonate and bis(triphenylphosphine)palladium(II) chloride, it afforded the corresponding 4′-formyl-biphenyl-3-carboxylic acid (**3**). Compound **3** reacted with ethyl acetoacetate (**4**), thiourea (**5**) in ethanol and a few drops of HCl to obtain 4-(3′-ethoxycarbonyl-biphenyl-4-yl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylic acid ethyl ester (**6**) [15]. Finally, ethyl 2-(substituted benzylthio)-4-(3′-(ethoxycarbonyl)biphenyl-4-yl)-6-methyl-1,4-dihydropyrimidine-5-carboxylate derivatives (**8a–j**) were synthesized by the reaction of (**6**), substituted benzyl halides (**7**) and potassium carbonate in DMF solution.

Its structural assignment was proved by spectroscopic analyses. Its ^{1}H-NMR spectrum revealed singlet signals at δ 10.20 and 13.10 ppm due to CHO and COOH proton, respectively, beside an aromatic multiplet in the region of δ 7.48–8.50 ppm. Moreover, the LCMS mass spectrum showed *m*/*z* at 227 (M+H). The structure of compound **4** was proven by its analytical and spectral analyses. The LCMS mass spectrum showed at *m*/*z* 425 (M+H). ^{1}H-NMR (DMSO-d_6) showed the protons at δ 8.95–9.15 ppm due to 2 NH protons and at δ 5.20 ppm due to CH protons. The target molecules (**8a–j**) were also proved by their analytical and spectral analyses. ^{1}H-NMR



Compound	R	Compound	R
8a		8f	
8b		8g	
8c		8h	
8d		8i	
8e		8j	

Reagents and conditions: (i) $Pd(PPh_3)_2Cl_2$, Cs_2CO_3, dioxane, reflux, 10 h; (ii) HCl, reflux, 16 h; (iii) DMF, K_2CO_3, 1 h.

Scheme 1. Synthetic pathway for compounds **8 a–j**.

(DMSO-d_6) showed proton regions at δ 3.35 to 3.45, δ 5.10 to 5.25 ppm due to NH, SCH_2 protons and at δ 4.10 to 4.21 ppm due to CH_2 protons.

Compounds (**8a–j**) were screened for their antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Streptococcus pyogenes* by comparing them with standard ciprofloxacin, and antifungal activity against *Aspergillus fumigatus*, *Aspergillus flavus*, *Trichophyton mentagrophytes*, *Penicillium marneffei* and *Candida albicans* was compared with the standard fungicide amphotericin. It is interesting to observe that the maximum compounds had very good antifungal and antibacterial activity.

Pharmacological assay

Antibacterial assay

A standard inoculum (1–2 × 10^7 colony-forming unit (c.f.u)/cm^3 0.5 McFarland standards) was introduced onto the surface of sterile agar plates, and a sterile glass spreader was used for even distribution of the inoculum. The discs measuring 6.25 mm in diameter were prepared from Whatmann no.1 filter paper and sterilized by dry heat at 140°C for 1 h. The sterile disc previously soaked in a known concentration of the test compounds were placed in nutrient agar medium. Solvent and growth controls were kept. The plates were inverted and incubated for 24 h at 37°C. The inhibition zones were measured and compared with the controls. Minimum inhibitory concentration (MIC) was determined by broth dilution technique. The nutrient broth, which contained logarithmic serially two-fold diluted amount of test compound and controls was inoculated with approximately 5 × 10^5 c.f.u of actively dividing bacteria cells. The cultures were incubated for 24 h at 37°C and the growth was monitored visually and spectrophotometrically. The lowest concentration (highest dilution) required to arrest the growth of bacteria was regarded as minimum inhibitory concentrations (MIC). Ciprofloxacin was used as a standard drug. The diameter of the zone of inhibition and minimum inhibitory concentration values are given in Table 1.

The newly synthesized compounds were screened for their antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus pyogenes* and *Klebsiella pneumoniae* (recultured) bacterial strains by disc diffusion method [16, 17]. The investigation of antibacterial screening data revealed that all the tested compounds showed moderate to good bacterial inhibition. The compounds **8a**, **8b**, **8e**, **8f**, **8i** and **8j** showed very good activity against all the bacterial strains.

Antifungal assay

Sabourauds agar media was prepared by dissolving 1 g peptone, 4 g D-glucose, and 2 g agar in 100 cm^3 distilled water, and adjusting pH to 5.7 using buffer. Normal saline was used to make a suspension of spore of fungal strain for lawning. A loop full of particular fungal strain was transferred to 3 cm^3 saline to get a suspension of corresponding species. 20 cm^3 of agar media was poured into each Petri dish. Excess of suspension was decanted and the plates were dried by placing in a incubator at 37°C for 1 h. Using an agar punch, wells were made and each well was labeled. A control was also prepared in triplicate and maintained at 37°C for 3–4 d. The inhibition zones in diameter were measured and compared with the controls. The nutrient broth, which contained logarithmic serially two-fold diluted amount of test compound and controls was inoculated with approximately 1.6 × 10^4-6 × 10^4 c.f.u cm^{-3}. The cultures were incubated for 48 h at 35°C and the growth was monitored. The lowest

Table 1. Antibacterial activity of pyrimidine-5-carboxylate derivatives, **8a–j** (zone of inhibition).

Compound no.	*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Klebsiella pneumoniae*	*Streptococcus pyogenes*
8a	20 (6.25)	25 (6.25)	29 (6.25)	18 (6.25)	23 (6.25)
8b	23 (6.25)	28 (6.25)	30 (6.25)	18 (6.25)	21 (6.25)
8c	10 (12.5)	–	–	17 (6.25)	11 (6.25)
8d	8 (25)	23 (6.25)	9 (25)	–	8 (12.5)
8e	22 (6.25)	27 (6.25)	32 (6.25)	20 (6.25)	24 (6.25)
8f	21 (6.25)	29 (6.25)	32 (6.25)	20 (6.25)	23 (6.25)
8g	10 (12.5)	15 (25)	–	–	17 (12.5)
8h	12 (12.5)	–	21 (6.25)	–	8 (25)
8i	21 (6.25)	24 (6.25)	29 (6.25)	19 (6.25)	23 (6.25)
8j	21 (6.25)	26 (6.25)	32 (6.25)	21 (6.25)	24 (6.25)
Standard[a]	24 (6.25)	30 (6.25)	33 (6.25)	23 (6.25)	25 (6.25)

[a]Ciprofloxacin was used as standard. – Indicates bacteria are resistant to the compounds at >100 μg/mL, MIC values are given in brackets. MIC (μg/mL) = minimum inhibitory concentration, i.e. lowest concentration to completely inhibit bacterial growth. In parenthesis - Zone of inhibition in mm.

Table 2. Antifungal activity of pyrimidine-5-carboxylate derivatives, **8a–j** (zone of inhibition).

Compound no	*Aspergillus fumigatus*	*Aspergillus flavus*	*Trichophyton mentagrophytes*	*Penicillium marneffei*	*Candida albicans*
8a	22 (6.25)	22 (6.25)	25 (6.25)	22 (6.25)	20 (6.25)
8b	8 (25)	–	12 (12.5)	–	17 (6.25)
8c	22 (6.25)	20 (6.25)	22 (6.25)	25 (6.25)	17 (6.25)
8d	15 (6.25)	–	7 (25)	21 (6.25)	18 (6.25)
8e	5 (25)	18 (6.25)	–	12 (12.5)	17 (6.25)
8f	24 (6.25)	21 (6.25)	21 (6.25)	23 (6.25)	18 (6.25)
8g	11 (12.5)	12 (25)	–	–	14 (12.5)
8h	9 (25)	–	12 (12.5)	9 (25)	10 (12.5)
8i	22 (6.25)	19 (6.25)	20 (6.25)	23 (6.25)	19 (6.25)
8j	21 (6.25)	26 (6.25)	32 (6.25)	21 (6.25)	24 (6.25)
Standard	25 (6.25)	21 (6.25)	23 (6.25)	25 (6.25)	19 (6.25)

– Indicates fungus is resistant to the compounds at >100 mg/mL, MIC values are given in brackets. MIC (mg/mL) = minimum inhibitory concentration, ie. lowest concentration to completely inhibit fungal growth. Zone of inhibition in mm. Amphotericin was used as standard.

concentration (highest dilution) required to arrest the growth of fungus was regarded as minimum inhibitory concentrations (MIC). Amphotericin B was used as the standard drug. The diameter of zone of inhibition and minimum inhibitory concentration values are given in Table 2.

Newly prepared compounds were screened for their antifungal activity against *Aspergillus flavus*, *Aspergillus fumigatus*, *Candida albicans*, *Penicillium marneffei* and *Trichophyton mentagrophytes* (recultured) in DMSO by serial plate dilution method [18, 19]. The antifungal screening data showed moderate to good activity. Compounds **8a**, **8c**, **8f**, **8i** and **8j** emerged as very active against all the fungal strains.

Conclusion

In the present study, novel ethyl 2-(substituted benzylthio)-4-(3′-(ethoxycarbonyl)-biphenyl-4-yl)-6-methyl-1,4-dihydropyrimidine-5-carboxylate derivatives were synthesized and evaluated for their antibacterial and antifungal activity. The investigation of antibacterial screening data reveals that among the 10 compounds screened, five compounds showed good antibacterial activity and six compounds showed fungal inhibition almost equivalent to that of standard.

Experimental

Chemistry

All reagents and solvents were purchased and used without further purification. Melting points were determined on a Fisher–Johns melting point apparatus and were uncorrected. Crude products were purified by column chromatography on silica gel of 60–120 mesh. NMR spectra were recorded on a Varian 300 MHz spectrometer for ^{1}H-NMR. 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 preparationof 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, 8H, Ar-H), 10.20 (s, 1H), 13.10 (s, 1H, COOH); LCMS (*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), were added biaryl aldehyde (1.1 mmol) and thiourea (2 mmol) in ethanol. 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°C; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 1.15–1.35 (t, 6H), 2.35 (s, 3H), 4.10–4.40 (q, 4H), 5.20 (s, 1H), 7.10–8.60 (m, 8H, Ar-H), 8.95–9.15 (s, 2H); LCMS (*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-(substituted benzylthio)-4-(3′-(ethoxycarbonyl)biphenyl-4-yl)-6-methyl-1,4-dihydropyrimidine-5-carboxylate ***8a–j***

An ice cold solution of the cyclic compound 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-L round bottomed flask equipped with magnetic stirrer and stirred for 1 h. The residual portion was poured on to crushed ice, neutralized with dilute acid and the obtained product ethyl 2-(substituted benzylthio)-4-(3′-(ethoxycarbonyl)biphenyl-4-yl)-6-methyl-1,4-dihydropyrimidine-5-carboxylate derivatives (**8a–j**) was collected by filtration.

Ethyl 2-(benzylthio)-4-(3′-(ethoxycarbonyl)biphenyl-4-yl)-6-methyl-1,4-dihydropyrimidine-5-carboxylate ***8a***

Pale yellow solid, 69% yield; m.p. 196–197°C; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 1.19–1.35 (t, 6H), 2.10 (s, 1H), 2.35 (s, 3H), 3.45 (s, 1H), 4.05–4.33 (q, 4H), 5.10 (s, 2H), 7.20–8.55 (m, 13H). LCMS (*m*/*z*): 515 (M+H, 100%); anal. calcd. for $C_{30}H_{30}N_2O_4S$: C, 70.05; H, 5.90; N, 5.45. Found: C, 69.84; H, 5.95; N, 5.70.

Ethyl 2-(4-bromobenzylthio)-4-(3′-(ethoxycarbonyl)-biphenyl-4-yl)-6-methyl-1,4-dihydropyrimidine-5-carboxylate ***8b***

Yellow solid, 64% yield; m.p. 214–216°C; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 1.20–1.40 (t, 6H), 2.15 (s, 1H), 2.32 (s, 3H), 3.45 (s, 1H), 4.10–4.35 (q, 4H), 5.25 (s, 2H), 7.10–8.60 (m, 12H). LCMS (*m*/*z*): 595 (M+2H, 100%); anal. calcd. for $C_{30}H_{29}BrN_2O_4S$: C, 60.71; H, 4.92; N, 4.72. Found: C, 60.52; H, 4.71; N, 4.90.

Ethyl 4-(3′-(ethoxycarbonyl)biphenyl-4-yl)-2-(4-ethylbenzylthio)-6-methyl-1,4-dihydropyrimidine-5-carboxylate ***8c***

Yellow solid, 75% yield; m.p. 175–177°C; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 1.15–1.30 (t, 9H), 2.10 (s, 1H), 2.30 (s, 3H), 2.40–2.55 (q, 2H), 3.40 (s, 1H), 4.10–4.35 (q, 4H), 5.15 (s, 2H), 6.75–8.55 (m, 12H). LCMS (*m*/*z*): 543 (M+H, 100%); anal. calcd. for $C_{32}H_{34}BrN_2O_4S$: C, 70.82; H, 6.31; N, 5.16. Found: C, 70.91; H, 6.18; N, 5.32.

Ethyl 4-(3′-(ethoxycarbonyl)biphenyl-4-yl)-2-(4-methylbenzylthio)-6-methyl-1,4-dihydropyrimidine-5-carboxylate ***8d***

Pale yellow solid, 65% yield; m.p. 224–225°C; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 1.15–1.30 (t, 6H), 2.10 (s, 1H), 2.30–2.45 (s, 6H), 3.45 (s, 1H), 4.10–4.35 (q, 4H), 5.10 (s, 2H), 6.90–8.55 (m, 12H). LCMS (*m*/*z*): 529 (M+H, 100%); anal. calcd. for $C_{31}H_{32}N_2O_4S$: C, 70.43; H, 6.10; N, 5.30. Found: C, 70.03; H, 5.90; N, 5.45.

Ethyl 4-(3′-(ethoxycarbonyl)biphenyl-4-yl)-2-(4-methoxybenzylthio)-6-methyl-1,4-dihydropyrimidine-5-carboxylate ***8e***

Yellow solid, 76% yield; m.p. 171–173°C; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 1.10–1.35 (t, 6H), 2.15 (s, 1H), 2.35 (s, 3H), 3.45 (s, 1H), 4.15–4.40 (q, 4H), 5.20 (s, 2H), 6.60–8.75 (m, 12H). LCMS (*m*/*z*): 545 (M+H, 100%); anal. calcd. for $C_{31}H_{32}N_2O_5S$: C, 68.36; H, 5.92; N, 5.14. Found: C, 68.17; H, 5.69; N, 5.02.

Ethyl 2-(4-(dimethylamino)benzylthio)-4-(3′-(ethoxycarbonyl)biphenyl-4-yl)-6-methyl-1,4-dihydropyrimidine-5-carboxylate ***8f***

Pale yellow solid, 82% yield; m.p. 210–212°C; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 1.10–1.35 (t, 6H), 2.15 (s, 1H), 2.30 (s, 3H), 2.50–2.85 (s, 6H), 3.45 (s, 1H), 4.10–4.45 (q, 4H), 5.25 (s, 2H), 6.65–8.65 (m, 12H). LCMS (*m*/*z*): 558 (M+H, 100%); anal. calcd. for $C_{32}H_{35}N_3O_4S$: C, 68.92; H, 6.33; N, 7.53. Found: C, 68.23; H, 6.09; N, 7.65.

Ethyl 2-(chlorobenzylthio)-4-(3′-(ethoxycarbonyl)biphenyl-4-yl)-6-methyl-1,4-dihydropyrimidine-5-carboxylate ***8g***

Yellow solid, 80% yield; m.p. 252–254°C; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 1.15–1.35 (t, 6H), 2.10 (s, 1H), 2.35 (s, 3H), 3.45 (s, 1H), 4.15–4.45 (q, 4H), 5.22 (s, 2H), 7.10–8.60 (m, 12H). LCMS (*m*/*z*): 549 (M+H, 100%); anal. calcd. for $C_{30}H_{29}ClN_2O_4S$: C, 65.62; H, 5.32; N, 5.10. Found: C, 65.43; H, 5.14; N, 4.95.

Ethyl 4-(3′-(ethoxycarbonyl)biphenyl-4-yl)-2-(4-isopropylbenzylthio)-6-methyl-1,4-dihydropyrimidine-5-carboxylate ***8h***

Pale yellow solid, 78% yield; m.p. 185–187°C; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 0.95–1.35 (m, 12H), 2.10 (s, 1H), 2.28 (s, 3H), 2.60 (m, 1H), 3.35 (s, 1H), 4.10–4.40 (q, 4H), 5.10 (s, 2H), 7.15–8.65 (m, 12H). LCMS (*m*/*z*): 557 (M+H, 100%); anal. calcd. for $C_{33}H_{36}N_2O_4S$: C, 71.20; H, 6.52; N, 5.03. Found: C, 70.85; H, 6.27; N, 5.25.

Ethyl 2-(4-tert-butylbenzylthio)-4-(3′-(ethoxycarbonyl)-biphenyl-4-yl)-6-methyl-1,4-dihydropyrimidine-5-carboxylate ***8i***

Pale yellow solid, 71% yield; m.p. 202–204°C; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 1.10–1.48 (m, 15H), 2.10 (s, 1H), 2.30 (s, 3H), 3.40 (s, 1H), 4.10–4.35 (q, 4H), 5.10 (s, 2H), 7.10–8.55 (m,12H). LCMS (*m*/*z*): 571 (M+H, 100%); anal. calcd. for $C_{34}H_{38}N_2O_4S$: C, 71.55; H, 6.71; N, 4.91. Found: C, 71.19; H, 6.54; N, 4.68.

Ethyl 4-(3′-(ethoxycarbonyl)biphenyl-4-yl)-6-methyl-2-(4-nitrobenzylthio)-1,4-dihydropyrimidine-5-carboxylate ***8j***

Yellow solid, 67% yield; m.p. 221–223°C; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 1.18–1.40 (t, 6H), 2.15 (s, 1H), 2.30 (s, 3H), 3.45 (s, 1H), 4.10–4.35 (q, 4H), 5.25 (s, 2H), 7.10–8.65 (m, 12H). LCMS (*m*/*z*): 560 (M+H, 100%); anal. calcd. for $C_{30}H_{29}N_3O_6S$: C, 64.39; H, 5.19; N, 7.53. Found: C, 64.13; H, 5.28; N, 7.82.

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