Bioorganic & Medicinal Chemistry 17 (2009) 3580–3587

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

6-Amino-4-oxo-1,3-diphenyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbonyl derivatives as a new class of potent inhibitors of Interleukin-8-induced neutrophil chemotaxis

Sara Cesarini [a,*], Andrea Spallarossa [a], Angelo Ranise [a], Olga Bruno [a], Nicoletta Arduino [b], Maria Bertolotto [b], Franco Dallegri [b], Massimiliano Tognolini [c], Thomas Gobbetti [c], Elisabetta Barocelli [c]

[a] Dipartimento di Scienze Farmaceutiche, Universitá di Genova, Viale Benedetto XV 3, I-16132 Genova, Italy
[b] Dipartimento di Medicina Interna, Universitá di Genova, Viale Benedetto XV, 6;16132 Genova, Italy
[c] Dipartimento di Scienze Farmacologiche, Biologiche e Chimiche Applicate, Universitá di Parma, Viale G.P. Usberti 27/A, 43100 Parma, Italy

ARTICLE INFO

Article history:
Received 29 December 2008
Revised 2 April 2009
Accepted 6 April 2009
Available online 11 April 2009

Keywords:
Neutrophil
Chemotaxis
Interleukin-8
Inhibitors
Peritonitis

ABSTRACT

A series of 6-amino-4-oxo-1,3-diphenyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbonyl derivatives was synthesized. The compounds demonstrated to be novel, potent and selective inhibitors of Interleukin-8-induced human neutrophil chemotaxis. A SAR study was performed by varying the carbonyl function at position 5 and the chain linked to the amino group at position 6 of the scaffold. All the compounds of the series displayed inhibitory activity at nano- or picomolar concentrations against Interleukin-8-driven migration and no activity against fMLP- and C5a-induced chemotaxis. The binding tests of selected compounds on CXCR1 and CXCR2 receptors were negative. The most potent derivative showed in vivo efficacy in a mouse model of Zymosan-induced peritonitis.

1. Introduction

Neutrophils are key cellular elements of innate immune system, providing protection from invading microorganisms, and play a critical role in inflammatory response.[1–3] Chemotaxis (i.e., directed migration in a gradient of chemotactic stimuli, in contrast to random cell migration, which is called chemokinesis) enables neutrophils to rapidly reach the site of infection and destroy the invading pathogens.[4,5] Chemotaxis is induced by chemoattractants that include chemokines such as Interleukin-8 (IL-8), bacterial products such as the peptide N-formyl-L-methionyl-L-leucyl-L-phenylalanine (fMLP), the product of the complement cascade C5a anaphylotoxin, platelet activating factor (PAF), and products of arachidonic acid metabolism such as leukotriene B4 (LTB4). The binding of the chemoattractants to specific receptors located on the neutrophil membrane (e.g., CXCR1 and CXCR2 receptors for IL-8; FPR and FPRL1 receptors[6] for fMLP; C5aR and C5L2 receptors[7] for C5a; PAFR for PAF; BLT1 and BLT2 for LTB4)[8] causes a series of cytoplasmic events resulting in the actin cytoskeleton re-organisation that drives neutrophil motility.[9] In particular, when chemotactic factors bind to these seven-transmembrane-domain receptors, heterotrimeric Gi proteins are activated and, downstream from them, a complex interrelated signalling network is activated: stimulation of phospholipase Cβ results in hydrolysis of phosphatidylinositol 4,5-bisphosphate, generating diacylglycerol which activates protein kinase C isoforms, and inositol 1,4,5-triphosphate which releases calcium from intracellular stores; activation of phosphoinositide 3-kinase (PI3K) results in increased production of phosphatidylinositol 3,4,5-trisphosphate and phosphatidylinositol 3,4-bisphosphate; activation of the small GTP-binding proteins of the Rho family and of the mitogen-activated protein kinase (MAPK) cascade, as well as activation of protein phosphatases and tyrosine kinases.[10–12]

Compounds able to inhibit neutrophil chemotaxis are potentially useful for the treatment of acute and chronic disorders associated with excessive inflammatory responses and neutrophil-mediated tissue damage, such as rheumatoid arthritis, psoriasis, inflammatory bowel disease, asthma, chronic obstructive pulmonary disease, acute respiratory distress syndrome, ulcerative colitis, post-ischemia reperfusion injury and transplant rejection.[13–19] Figure 1 illustrates examples of IL-8 induced neutrophil chemotaxis inhibitors under clinical trials: SB656933 and SCH527123, CXCR2-selective and CXCR1/CXCR2 competitive antagonists, respectively,[20–22] and the CXCR1 non-competitive antagonist Reparixin, which binds to an allosteric pocket in the transmembrane

* Corresponding author. Tel.: +39 010 353 8361; fax: +39 010 353 8358.
E-mail address: sara.cesarini@unige.it (S. Cesarini).

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doi:10.1016/j.bmc.2009.04.006

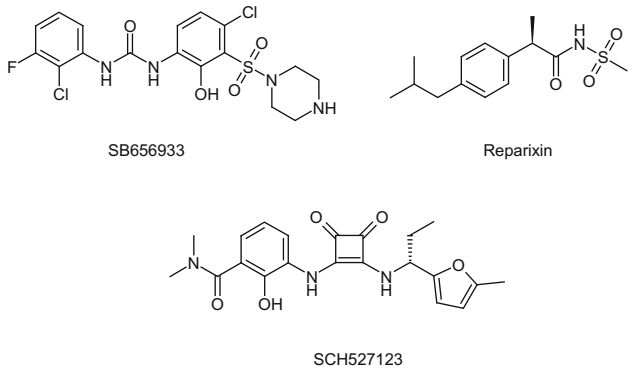


Figure 1. Chemical structures of SB656933, Reparixin and SCH527123.

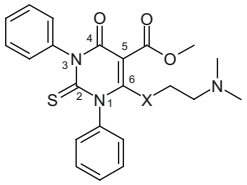
region of CXCR1 and blocks the agonist-induced receptor signalling in the intracellular compartment, not directly affecting the IL-8 binding affinity.[22,23]

During investigations on the pharmacological potential of 1,3-diphenyl-2-thioxo-2,3-dihydropyrimidin-4(1H)-one derivatives, compound **1** (Fig. 2) emerged as a potent inhibitor of human neutrophil chemotaxis induced by IL-8 (IC_{50} = 3.32 ± 0.11 nM), not endowed with cytotoxic activity like its isoster sulfur analogue **I**.[24] The isosteric replacement of the sulfur atom with the NH group is one of the strategies often successfully applied in medicinal chemistry and that has led to the discovery of new drugs.[25,26] In order to perform a structure–activity relationship (SAR) study, a series of analogues of **1** was synthesized (**14–29**, Table 1). Initially, the (dimethylamino)ethylamino chain at position 6 was maintained constant and group R_1 was modified by substituting the methyl with bulkier alkyl groups (ethyl **15**, isopropyl **16**) or by replacing the ester function with a methylketone (**14**). Successively, the effects of the variations on the chain linked to the amino group at position 6 on the inhibitory activity were investigated. Thus, (i) the dimethylamino substructure was replaced by the diethylamino moiety (**17** and **18**), by cycloaliphatic amines (piperidine **20**, morpholine **21**, N-methylpyrrolidine **22**) and by aromatic moieties bearing basic functions (2-pyridyl **23**, aminophenyl **24**) or acidic functionalities (4-aminosulfonylphenyl **25** and 3-indolyl **26**); (ii) the ethyl spacer was lengthened to propyl (**19**); (iii) the (dimethylamino)ethyl chain was replaced by the methoxy- (**27**) or dimethoxy- (**28**) -ethyl moiety; (iv) the (dimethylamino)ethylamino substructure was incorporated in the 4-methylpiperazinyl ring (**29**).

2. Chemistry

The synthesis of **6–9** (Scheme 1) was accomplished by a one-pot procedure previously described by some of us for the synthesis of **6** and **8**.[24,27] The synthetic method requires the reaction of methyl acetoacetate (**2**) or the suitable dialkyl malonate (**3–5**) with 3 equiv (equiv) of phenylisothiocyanate in dry DMF in the presence of 2 equiv of sodium hydride. Compounds **6–9** were obtained in high yields (83–89%). The successive methylation of **6–9**, performed with iodomethane in DMF at 65 °C in the presence of sodium bicarbonate as a base, afforded the corresponding thiomethyl-derivatives **10–13** in very high yields (93–97%) and allowed to obtain a good leaving group (–SMe) at the position 6 of the scaffold for the following step of nucleophilic aromatic substitution. The methylation occurred at the sulfur atom at position 6, instead of at the oxygen atom at position 4, because, although the enol–thione tautomer is conformationally stabilized by a strong intramolecular hydrogen bond[24,27] (as depicted in Scheme 1), the soft electrophile iodomethane prefers to react at the soft nucleophilic site, that is, sulfur atom (cheto–thiol tautomer), as confirmed by ^{1}H NMR (singlet at δ 2.30–2.31) and ^{13}C NMR spectral data (thiomethyl signal at δ 18.14–18.57 and signal of the amidic carbonyl at position 4 at δ 156.45–156.54).

The displacement of the thiomethyl group with dialkylaminoalkyl-, cycloaminoethyl-, phenylaminoethyl-, (di) methoxyethyl- and (hetero)arylethyl-amines and 4-methylpiperazine led to compounds **1** and **14–29** (Table 1). Different reaction times and temperatures and number of equivalents of amine have been used depending on the different reactivity of the amines employed. The work-up simply required filtrations or extractions and the final products were purified by crystallization. The yields ranged from 47% to 95%. The structure of **1** and **14–29** was confirmed by IR, ^{1}H NMR and, for compounds **1**, **16–18**, **20**, **22**, **25**, **26** and **29**, by ^{13}C NMR spectral data. When tautomerism was possible (i.e., for **1** and **14–28**, Fig. 3), only one tautomer was detected. Both the enaminone form T_1 and the enol–imine form T_2 can be stabilized by an intramolecular hydrogen bond (Fig. 3). However, the presence of a sharp band at 3140–3365 cm^{-1} in the IR spectra (N–H stretching) and of a signal at δ 157.67–158.98 in the ^{13}C NMR spectra (amidic carbonyl at position 4) suggests that T_1 is favoured. This would be supported also by the presence of a broad singlet, exchangeable with deuterium oxide, at δ 5.94–8.00 in the ^{1}H NMR spectra recorded in DMSO-d_6 or $CDCl_3$. In this context, the higher δ value of this signal in the ^{1}H NMR spectrum of **14** (δ 11.92) might be due to the stronger N–H···O hydrogen bond that the ketone carbonyl is able to form (the ketone carbonyl is a better hydrogen bond acceptor than the ester carbonyl).



1: X = NH
I: X = S

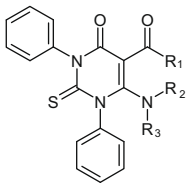
Figure 2. Chemical structure of compounds **1** and **I**.

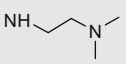
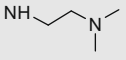
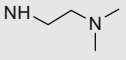
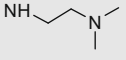
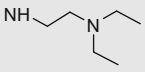
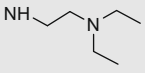
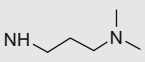
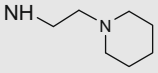
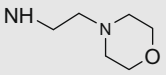
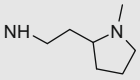
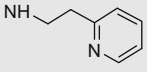
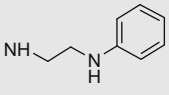
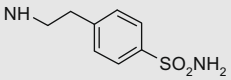
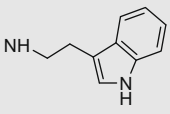
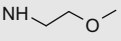
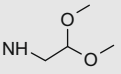
3. Biological results and discussion

Compounds **1** and **14–29** were tested to evaluate the inhibition of IL-8- and fMLP-induced human neutrophil migration (the results are expressed as IC_{50} values, Table 1) and the cytotoxicity against neutrophils. The most potent IL-8-driven chemotaxis inhibitors were assayed also for their ability to block the human neutrophil migration induced by C5a anaphylotoxin. To shed some light on the mechanism of action, selected compounds were submitted to a binding assay on CXCR1 and CXCR2. The compound emerged from the in vitro assays as the most potent chemotaxis inhibitor was tested in vivo in a mouse model of peritonitis, using Dexamethasone as reference drug (Table 2).

No compounds showed cytotoxic activity against neutrophils, the percentage of viable cells never being lower than 94%. All the tested molecules blocked the IL-8-induced chemotaxis, while they were inactive against fMLP-driven migration. All compounds displayed IC_{50} values (Table 1) in the nanomolar or even picomolar concentration range (derivatives **15–18**, **23** and **24**). The most potent analogues resulted to be the ethyl and isopropyl esters **15–18** incorporating the dimethyl- or diethyl-aminoethyl chain and they showed no inhibitory activity against C5a-induced chemotaxis.

Table 1
Chemical structures and chemotaxis inhibition values of compounds **1** and **14–29**[a]



Compound	R_1	NR_2R_3	Inhibition of IL-8-induced neutrophil migration IC_{50} (nM)
1	OCH_3		3.32 ± 0.11
14	CH_3		6.86 ± 0.76
15	OCH_2CH_3		0.10 ± 0.05
16	$OCH(CH_3)_2$		0.18 ± 0.07
17	OCH_2CH_3		0.02 ± 0.01
18	$OCH(CH_3)_2$		0.21 ± 0.10
19	OCH_2CH_3		3.89 ± 0.69
20	OCH_2CH_3		85.44 ± 8.20
21	OCH_2CH_3		171.63 ± 8.71
22	OCH_2CH_3		1.61 ± 0.12
23	OCH_2CH_3		0.49 ± 0.14
24	OCH_2CH_3		0.61 ± 0.13
25	OCH_3		10.79 ± 4.21
26	OCH_3		29.64 ± 8.14
27	OCH_2CH_3		1.36 ± 0.47
28	OCH_3		31.22 ± 1.24
29	CH_3		14.8 ± 3.25

[a] Neutrophil chemotaxis was evaluated in Boyden Chamber Migration assay, after stimulation with 10^{-9} M IL-8 or 10^{-8} M fMLP or 10^{-9} M C5a (C5a only for **15–18**), in the absence and in the presence of tested compounds. The net migration was determined by subtracting spontaneous migration, that is, the distance travelled by neutrophils in the absence of the stimulus, from the distance travelled by neutrophils towards the stimulus. The concentration of each compound giving 50% inhibition (IC_{50}) of net migration was obtained from nonlinear regression analysis of concentration–response curves, and the results reported are the mean of at least three experiments. No inhibitory activity was observed in fMLP- or C5a-induced chemotaxis.

Regarding the substituent R_1 of the carbonyl function at position 5, the ethoxy group demonstrated to be more beneficial than propoxy (**15** vs **16**; **17** vs **18**) and much more favourable than methoxy and methyl (**15** vs **14** and **1**).

Among the compounds bearing the aminoethylamino linker at position 6, the diethylamino derivatives **17** and **18** showed very high potency, similar to the corresponding dimethylamino analogues **15** and **16**, and resulted to be much more effective than the cycloamino congeners (compare **20** and **21** with **17** and **15**). The lengthening of the ethyl spacer to propyl produced an approximately 39-fold decrease in potency (compare **19** with **15**).

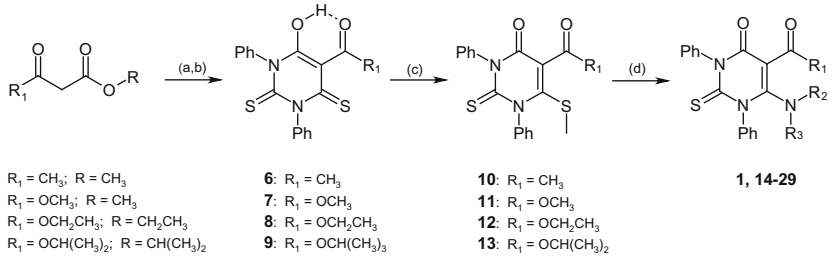
In compound **20**, the isosteric substitution of the methylene at piperidine position 4 with an oxygen atom (**21**) caused the potency halving, while the replacement of the six-membered ring with N-methylpyrrolidine (five-membered ring with the basic and possible H-bond acceptor amino group shifted) afforded an approximately 53-fold increase in inhibitory activity (**22** vs **20**). Moreover, the exchange of the cycloaliphatic amines with basic but aromatic moieties, such as the 2-pyridyl ring (**23**) and the aniline substructure (**24**), led to picomolar inhibitors from five to six-fold less potent than the corresponding dimethylamino analogue **15** (R_1 = OEt).

Also the substitution of the dimethylamino group of compound **1** (R_1 = OMe) with aromatic moieties bearing acidic functions (4-aminosulfonylphenyl **25** and 3-indolyl **26**) caused a drop in potency, in particular in the case of the bulky indole ring (**25** and **26** vs **1**).

The replacement of the basic dimethylamino function with the neutral and smaller methoxy group led to an approximately 14-fold decrease in inhibitory activity (compare **27** with **15**). The introduction of another methoxy group was not beneficial (compare **28** with **27** and **1**).

The rigidification of the (dimethylamino)ethylamino chain of compound **14** in a 4-methylpiperazinyl ring led to about the halving of the potency (**29** vs **14**). According to this result, the presence of the H-bond donor function (NH) at position 6 does not seem to be essential for inhibitory activity.

The selectivity of the series in inhibiting the human neutrophil chemotaxis induced by IL-8 prompted us to investigate the possible ability of these molecules to interfere with the binding of IL-8 to CXCR1 and CXCR2 receptors with a mechanism of competitive



Scheme 1. Reagents and conditions: (a) NaH (2 equiv), Ph–N=C=S (3 equiv), dry DMF, 0 °C, 1 h, then rt, 20 h. (b) H_3O^+; (c) $NaHCO_3$ (1.1 equiv), CH_3I (1 equiv), DMF, 65 °C, 1 h; (d) NHR_2R_3 (1–2 equiv), rt or 80 °C, 1.5–48 h. The structures of **1** and **14–29** are listed in Table 1.

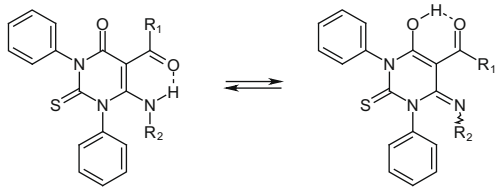


Figure 3. General structure of compounds **1** and **14–28** in the enaminone and enol–imine forms.

Table 2
Effect of compound **17** on cell and granulocyte recruitment as well as on protein concentration enhancement induced by Zymosan in peritoneal cavity of mice[a]

Compound	No. peritoneal cells (million/cavity)	No. peritoneal granulocytes (million/cavity)	Peritoneal protein concentration (μg/mL)
Saline	6.2 ± 1.2	1.9 ± 0.4	551 ± 203
Saline + Zymosan	31.5 ± 3.4	27.1 ± 3.5	1961 ± 160
17 + Zymosan	23.8 ± 2.7	17.7 ± 1.6**	1521 ± 170
Dexamethasone + Zymosan	22.0 ± 1.8	18.5 ± 1.6**	1404 ± 144

** $P < 0.01$ by Anova one-way followed by Bonferroni's post-test compared to saline + Zymosan. Data are expressed as mean ± SEM of **8–12** independent data.

[a] Animals received compound **17** (50 mg/kg os) or Dexamethasone (3 mg/kg os) 1 h before intraperitoneal injection of Zymosan (1 mg/mouse). Peritoneal fluid was collected and examined microscopically 4 h later.

antagonism. However, the binding tests of **17**, **22**, **23**, **24** and **27** on CXCR1 and CXCR2 receptors turned out to be negative (data not shown), thus suggesting that these new chemotaxis inhibitors might affect intracellular signal transduction activated by IL-8.

Interestingly, compound **17** demonstrated in vivo activity in a mouse model of peritonitis (induced by the intraperitoneal administration of Zymosan). Table 2 shows the effects of **17** on cell and granulocyte recruitment (neutrophils constitute the majority of granulocytes, which include also eosinophils and basophils in small percentage) as well as on protein concentration enhancement induced by Zymosan in peritoneal cavity of mice. The treatment with compound **17** caused a significant decrease of granulocytes present in the peritoneal cavity compared to negative control. **17** displayed similar activity to Dexamethason used at a 17-fold lower dose.

4. Conclusions

A new class of IL-8-induced neutrophil chemotaxis inhibitors was identified and a SAR study was performed by varying the carbonyl function at position 5 and the chain linked to the amino group at position 6 of the scaffold. All the compounds of the series displayed inhibitory activity at nano- or picomolar concentrations, with selectivity against fMLP- and C5a-induced chemotaxis. Derivative **17** emerged as the most potent compound in vitro and showed in vivo efficacy in a mouse model of peritonitis.

The investigation of the mechanism of action of the title compounds is in progress. The understanding of the molecular target will provide useful information to modify the structure of this new class of inhibitors in order to improve their pharmacological properties.

5. Experimental protocols

5.1. Chemistry

5.1.1. General

All chemicals were purchased by Sigma–Aldrich Chemical Co., Alfa Aesar and Lancaster and used without further purification. Solvents were reagent grade. DMF was dried on molecular sieves (5 Å 1/16″ inch pellets). Organic solutions were dried over anhydrous sodium sulfate. Thin layer chromatography (TLC) system for routine monitoring the course of reactions and confirming the purity of analytical samples employed aluminium-backed silica gel plates (Merck DC-Alufolien Kieselgel 60 F_{254}): petroleum ether/ethyl acetate or CH_2Cl_2/methanol were used as developing solvents and detection of spots was made by UV light and/or by iodine vapours. The organic solutions were evaporated using a rotatory evaporator operating at reduced pressure of about 10–20 Torr. Yields were not optimized. Melting points were determined on a Fisher-Johns apparatus and are uncorrected. IR spectra were recorded on a Perkin Elmer 398 spectrometer as KBr discs. ^1H and ^{13}C NMR spectra were recorded on a Varian Gemini 200 instrument at 200 and 50.30 MHz, respectively, employing solutions in DMSO-d_6 or $CDCl_3$. Chemical shifts were reported in ppm units relative to the internal reference tetramethylsilane, and the splitting patterns were described as follows: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet) and br s (broad singlet). The first order values reported for coupling constants *J* were given in hertz. Elemental analyses were performed by an EA1110 Elemental Analyser (Fison-Instruments, Milan) and were within ± 0.4% of the theoretical values. The synthesis of compounds **5** and **7** was accomplished according to the published procedure.[24,27]

5.1.2. Synthesis of alkyl 6-hydroxy-1,3-diphenyl-2,4-dithioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate 7 and 9

A 60% sodium hydride dispersion in mineral oil (12 g, 0.30 mol) was added in portions to a stirred and ice-cooled solution of dimethylmalonate (34.29 mL, 0.30 mol) (for **7**) or diisopropylmalonate (56.98 mL, 0.30 mol) (for **9**) in dry DMF (300 mL). When hydrogen evolution ceased, phenylisothiocyanate (107.48 mL, 0.90 mol) was added, followed after 15 min by another amount of sodium hydride dispersion (12 g, 0.30 mol). After stirring at 0 °C for 1 h, and then at rt for 20 h, 1.0 L of an aqueous 0.6 M ammonium chloride solution was added to the reaction mixture. After extraction with

diethyl ether/petroleum ether (1:1) (5 × 400 mL), the aqueous layer was cooled and acidified with a 6 M HCl solution (300 mL). The precipitate was filtered, washed with water, dried under vacuum and purified by crystallization from CH_2Cl_2/acetone.

5.1.2.1. Methyl 6-hydroxy-1,3-diphenyl-2,4-dithioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate 7. Mp 275 °C (dec); yield: 84%. IR (KBr) cm^{-1}: 1739, 1674, 1563. ^1H NMR ($CDCl_3$) δ: 3.92 (s, 3H, CH_3), 7.21–7.55 (m, 10H, arom. H), 9.05 (br s, 1H, OH). Anal. Calcd for $C_{18}H_{14}N_2O_3S_2$: C, 58.36; H, 3.81; N, 7.56; S, 17.31. Found: C, 58.57; H, 3.89; N, 7.74; S, 17.04.

5.1.2.2. Isopropyl 6-hydroxy-1,3-diphenyl-2,4-dithioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate 9. Mp > 300 °C; yield: 83%. IR (KBr) cm^{-1}: 2980, 1696, 1675, 1558. ^1H NMR ($CDCl_3$) δ: 1.36 (d, J = 6.3 Hz, 6H, $2CH_3$), 5.24 (m, 1H, OCH), 7.22–7.55 (m, 10H, arom. H), 8.85 (br s, 1H, OH). Anal. Calcd for $C_{20}H_{18}N_2O_3S_2$: C, 60.28; H, 4.55; N, 7.03; S, 16.09. Found: C, 60.24; H, 4.80; N, 7.16; S, 16.13.

5.1.3. Synthesis of 6-(methylthio)-1,3-diphenyl-2-thioxo-2,3-dihydropyrimidin-4(1H)-one derivatives 10–13

Iodomethane (6.224 mL, 0.10 mol) was added to the stirred mixture of the suitable 6-hydroxy-1,3-diphenyl-2,4-dithioxo-1,2,3,4-tetrahydropyrimidine derivative (**6** for **10**; **7** for **11**; **8** for **12**; **9** for **13**, 0.10 mol) and sodium bicarbonate (9.24 g, 0.11 mol) in DMF (150 mL). After stirring at 65 °C for 1 h, the reaction mixture was diluted with water (200 mL). The precipitate was filtered, washed with water, dried under vacuum and purified by crystallization from acetone or CH_2Cl_2/acetone.

5.1.3.1. 5-Acetyl-6-(methylthio)-1,3-diphenyl-2-thioxo-2,3-dihydropyrimidin-4(1H)-one 10. Mp > 300 °C; yield: 95% from acetone. IR (KBr) cm^{-1}: 1698, 1668, 1559. ^1H NMR (DMSO-d_6) δ: 2.31 (s, 3H, SCH_3), 2.65 (s, 3H, CH_3), 7.18–7.55 (m, 10H, arom. H). Anal. Calcd for $C_{19}H_{16}N_2O_2S_2$: C, 61.93; H, 4.38; N, 7.60; S, 17.40. Found: C, 61.63; H, 4.52; N, 7.99; S, 17.12.

5.1.3.2. Methyl 6-(methylthio)-4-oxo-1,3-diphenyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate 11. Mp 247–249 °C; yield: 93% from CH_2Cl_2/acetone. IR (KBr) cm^{-1}: 1725, 1674, 1568. ^1H NMR (DMSO-d_6) δ: 2.30 (s, 3H, SCH_3), 3.80 (s, 3H, OCH_3), 7.23–7.53 (m, 10H, arom. H). ^{13}C NMR (DMSO-d_6) δ: 18.14 (SCH_3), 52.67 (OCH_3), 114.97 (C-5), 128.13 (CH), 128.20 (2CH), 129.07 (2CH), 129.12 (2CH), 129.22 (CH), 129.49 (2CH), 139.52 (C), 141.04 (C), 154.49 (C-6), 156.45 (CON), 163.60 (COO), 178.56 (CS). Anal. Calcd for $C_{19}H_{16}N_2O_3S_2$: C, 59.63; H, 4.19; N, 7.29; S, 16.68. Found: C, 59.36; H, 4.27; N, 7.29; S, 16.67.

5.1.3.3. Ethyl 6-(methylthio)-4-oxo-1,3-diphenyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate 12. Mp 223–225 °C; yield: 97% from CH_2Cl_2/acetone. IR (KBr) cm^{-1}: 2988, 1732, 1683, 1572. ^1H NMR (DMSO-d_6) δ: 1.26 (t, J = 7.1 Hz, 3H, CCH_3), 2.31 (s, 3H, SCH_3), 4.27 (q, J = 7.1 Hz, 2H, CH_2), 7.24–7.50 (m, 10H, arom. H). ^{13}C NMR (DMSO-d_6) δ: 13.68 (CCH_3), 18.57 (SCH_3), 61.70 (CH_2), 115.66 (C-5), 128.10 (CH), 128.20 (2CH), 128.87 (CH), 129.03 (2CH), 129.11 (2CH), 129.46 (2CH), 139.53 (C), 141.12 (C), 153.86 (C-6), 156.50 (CON), 163.02 (COO), 178.59 (CS). Anal. Calcd for $C_{20}H_{18}N_2O_3S_2$: C, 60.28; H, 4.55; N, 7.03; S, 16.09. Found: C, 60.08; H, 4.37; N, 7.08; S, 15.98.

5.1.3.4. Isopropyl 6-(methylthio)-4-oxo-1,3-diphenyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate 13. Mp 225–227 °C; yield: 94% from CH_2Cl_2/acetone. IR (KBr) cm^{-1}: 2980, 1720, 1677, 1588. ^1H NMR (DMSO-d_6) δ: 1.27 (d, J = 6.3 Hz, 6H, $2CH_3$), 2.31 (s, 3H, SCH_3), 5.10 (m, 2H, OCH), 7.24–7.52 (m, 10H, arom. H). ^{13}C NMR (DMSO-d_6) δ: 18.42 (SCH_3), 21.21 ($2CH_3$), 69.61 (OCH), 116.46 (C-5), 128.09 (CH), 128.23 (2CH), 128.84 (CH), 129.00 (2CH), 129.11 (2CH), 129.46 (2CH), 139.56 (C), 141.22 (C), 153.18 (C-6), 156.54 (CON), 162.45 (COO), 178.65 (CS). Anal. Calcd for $C_{21}H_{20}N_2O_3S_2$: C, 61.14; H, 4.89; N, 6.79; S, 15.54. Found: C, 60.93; H, 4.91; N, 6.87; S, 15.42.

5.1.4. Synthesis of 6-amino-4-oxo-1,3-diphenyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbonyl derivatives 1 and 14–29

A mixture of the 6-(methylthio)-1,3-diphenyl-2-thioxo-2,3-dihydropyrimidin-4(1H)-one derivative **10**, **11**, **12** or **13** (3 mmol) and the suitable amine (3.6 mmol; 3 mmol for **25** and **26**; 4.5 mmol for **28**; 6 mmol for **16**, **18** and **29**) in DMF (10 mL) was stirred at rt for 18 h (1.5 h for **22**; 3 h for **14** and **23**; 48 h for **29**; 70 °C for 4 h for **16** and **18**; 80 °C for 6 h for **25**, **26** and **28**). Then, the reaction mixture was diluted with water (100 mL). For all compounds with the exception of **16** and **18**, the precipitate was filtered, washed with water, dried under vacuum and purified by crystallization from the suitable solvent or solvent mixture. For **16** and **18**, the mixture was extracted with diethyl ether (50 mL × 3) and the combined extracts were washed with water, and dried over anhydrous Na_2SO_4. Evaporation of the solvent under reduced pressure gave a residue which was purified by crystallization from the suitable solvent or solvent mixture.

5.1.4.1. Methyl 6-{[2-(dimethylamino)ethyl]amino}-4-oxo-1,3-diphenyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate 1. Mp 219–221 °C; yield: 95% from acetone/methanol. IR (KBr) cm^{-1}: 3359, 2956, 1704, 1681, 1610. ^1H NMR ($CDCl_3$) δ: 1.94 (s, 6H, $N(CH_3)_2$), 2.19–2.30 (m, 2H, CH_2N), 2.84–2.95 (m, 2H, $NHCH_2$), 3.88 (s, 3H, OCH_3), 6.85 (br s, 1H, NH, exchangeable), 7.22–7.61 (m, 10H, arom. H). ^{13}C NMR ($CDCl_3$) δ: 41.79 (CH_2N), 44.34 ($N(CH_3)_2$), 52.64 ($NHCH_2$), 56.41 (OCH_3), 88.42 (C-5), 128.43 (CH), 128.57 (2CH), 129.41 (2CH), 129.74 (2CH), 129.89 (CH), 130.35 (2CH), 138.42 (C), 140.07 (C), 153.65 (C-6), 158.86 (CON), 166.94 (COO), 178.86 (CS). Anal. Calcd for $C_{22}H_{24}N_4O_3S$: C, 62.24; H, 5.70; N, 13.20; S, 7.55. Found: C, 62.13; H, 5.69; N, 13.02; S, 7.46.

5.1.4.2. 5-Acetyl-6-{[2-(dimethylamino)ethyl]amino}-1,3-diphenyl-2-thioxo-2,3-dihydropyrimidin-4(1*H*)-one 14. Mp 159–161 °C; yield: 74% from acetone/methanol. IR (KBr) cm^{-1}: 3140, 2972, 1682, 1621. ^1H NMR ($CDCl_3$) δ: 2.14 (s, 6H, $N(CH_3)_2$), 2.21–2.33 (m, 2H, CH_2N), 2.37–2.50 (m, 2H, $NHCH_2$), 2.68 (s, 3H, CCH_3), 7.19–7.59 (m, 10H, arom. H), 11.92 (br s, 1H, NH, exchangeable). Anal. Calcd for $C_{22}H_{24}N_4O_2S$: C, 64.68; H, 5.92; N, 13.71; S, 7.85. Found: C, 64.36; H, 5.95; N, 13.69; S, 7.84.

5.1.4.3. Ethyl 6-{[2-(dimethylamino)ethyl]amino}-4-oxo-1,3-diphenyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate 15. Mp 195–196 °C; yield: 72% from CH_2Cl_2/diethyl ether. IR (KBr) cm^{-1}: 3208, 2976, 1731, 1657, 1608. ^1H NMR ($CDCl_3$) δ: 1.34 (t, J = 7.1 Hz, 3H, CCH_3), 1.89 (s, 6H, $N(CH_3)_2$), 2.12–2.42 (m, 2H, CH_2N), 2.77–3.00 (m, 2H, $NHCH_2$), 4.16–4.45 (m, 2H, OCH_2), 6.50 (br s, 1H, NH, exchangeable), 7.18–7.70 (m, 10H, arom. H). Anal. Calcd for $C_{23}H_{26}N_4O_3S$: C, 62.99; H, 5.98; N, 12.78; S, 7.31. Found: C, 62.76; H, 5.93; N, 12.76; S, 7.32.

5.1.4.4. Isopropyl 6-{[2-(dimethylamino)ethyl]amino}-4-oxo-1,3-diphenyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate 16. Mp 178–180 °C; yield: 64% from diethyl ether. IR (KBr) cm^{-1}: 3356, 2952, 1690, 1605. ^1H NMR (DMSO-d_6) δ: 1.23 (d, J = 6.3 Hz, 6H, $2CH_3$), 1.85 (s, 6H, $N(CH_3)_2$), 2.19–2.23 (m, 2H, CH_2N), 2.85–2.98 (m, 2H, $NHCH_2$), 4.99–5.03 (m, 1H, OCH), 6.00 (br s, 1H, NH, exchangeable), 7.17–7.59 (m, 10H, arom. H). ^{13}C NMR (DMSO-d_6) δ: 21.40 ($2CH_3$), 40.81 (CH_2N), 44.64 ($N(CH_3)_2$), 55.52 ($NHCH_2$), 68.33 (OCH), 89.15 (C-5), 127.59 (CH), 128.73

(2CH), 129.00 (2CH), 129.34 (CH), 129.54 (2CH), 129.95 (2CH), 137.84 (C), 140.19 (C), 152.02 (C-6), 157.86 (CON), 164.76 (COO), 177.88 (CS). Anal. Calcd for $C_{24}H_{28}N_4O_3S$: C, 63.69; H, 6.24; N, 12.38; S, 7.08. Found: C, 63.71; H, 5.89; N, 12.59; S, 7.40.

5.1.4.5. Ethyl 6-{[2-(diethylamino)ethyl]amino}-4-oxo-1,3-diphenyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate 17. Mp 172–173 °C; yield: 79% from CH_2Cl_2/diethyl ether. IR (KBr) cm^{-1}: 3298, 2969, 1733, 1661, 1611. ^1H NMR ($CDCl_3$) δ: 0.70 (t, J = 6.8 Hz, 6H, $N(CH_2CH_3)_2$), 1.35 (t, J = 7.2 Hz, 3H, CH_3), 2.18 (q, J = 6.8 Hz, 4H, $N(CH_2CH_3)_2$), 2.30–2.42 (m, 2H, CH_2N), 2.93–3.05 (m, 2H, $NHCH_2$), 4.32 (q, J = 7.2 Hz, 2H, OCH_2), 6.29 (br s, 1H, NH, exchangeable), 7.15–7.57 (m, 10H, arom. H). ^{13}C NMR ($CDCl_3$) δ: 10.28 ($N(CH_2CH_3)_2$), 13.45 (CH_3), 40.28 (CH_2N), 44.39 ($N(CH_2CH_3)_2$), 49.50 ($NHCH_2$), 61.10 (OCH_2), 88.28 (C-5), 127.57 (CH), 127.74 (2CH), 128.59 (2CH), 128.82 (2CH), 129.20 (CH), 129.79 (2CH), 137.28 (C), 139.19 (C), 151.50 (C-6), 158.16 (CON), 165.70 (COO), 178.04 (CS). Anal. Calcd for $C_{25}H_{30}N_4O_3S$: C, 64.35; H, 6.48; N, 12.01; S, 6.87. Found: C, 64.49; H, 6.53; N, 12.11; S, 6.54.

5.1.4.6. Isopropyl 6-{[2-(diethylamino)ethyl]amino}-4-oxo-1,3-diphenyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate 18. Mp 185–187 °C; yield: 48% from CH_2Cl_2/methanol. IR (KBr) cm^{-1}: 3237, 2971, 1707, 1672, 1604. ^1H NMR (DMSO-d_6) δ: 0.66 (t, J = 7.1 Hz, 6H, $2CH_3$), 1.23 (d, J = 6.2 Hz, 6H, $CH(CH_3)_2$), 2.15 (q, J = 7.1 Hz, 4H, $N(CH_2CH_3)_2$), 2.33–2.38 (m, 2H, CH_2N), 2.85–2.95 (m, 2H, $NHCH_2$), 4.94–5.06 (m, 1H, OCH), 5.94 (br s, 1H, NH, exchangeable), 7.16–7.20 (m, 2H, arom. H), 7.30–7.59 (m, 8H, arom. H). ^{13}C NMR (DMSO-d_6) δ: 10.87 ($2CH_3$), 21.37 ($CH(CH_3)_2$), 40.45 (CH_2N), 44.67 ($N(CH_2CH_3)_2$), 49.46 ($NHCH_2$), 68.46 (OCH), 89.17 (C-5), 127.61 (CH), 128.63 (CH), 128.75 (2CH), 129.51 (2CH), 129.56 (2CH), 130.14 (2CH), 137.74 (C), 140.18 (C), 151.13 (C-6), 157.98 (CON), 164.79 (COO), 177.93 (CS). Anal. Calcd for $C_{26}H_{32}N_4O_3S$: C, 66.17; H, 6.89; N, 11.71; S, 7.15. Found: C, 65.97; H, 6.71; N, 11.66; S, 6.97.

5.1.4.7. Ethyl 6-{[3-(dimethylamino)propyl]amino}-4-oxo-1,3-diphenyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate 19. Mp 176–178 °C; yield: 47% from acetone/ethanol. IR (KBr) cm^{-1}: 3134, 2954, 2818, 1721, 1658, 1589. ^1H NMR ($CDCl_3$) δ: 1.37 (t, J = 7.1 Hz, 3H, CCH_3), 1.42–1.66 (m, 2H, CCH_2C), 1.77 (s, 6H, $N(CH_3)_2$), 2.05–2.35 (m, 2H, CH_2N), 2.95–3.25 (m, 2H, $NHCH_2$), 4.16–4.53 (m, 2H, OCH_2), 7.10–7.67 (m, 10H, arom. H), 7.86 (br s, 1H, NH, exchangeable). Anal. Calcd for $C_{24}H_{28}N_4O_3S$: C, 63.69; H, 6.24; N, 12.38; S, 7.08. Found: C, 63.87; H, 6.38; N, 12.46; S, 7.10.

5.1.4.8. Ethyl 4-oxo-1,3-diphenyl-6-[(2-piperidin-1-ylethyl)-amino]-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate 20. Mp 170–171 °C; yield: 86% from acetone/ethanol. IR (KBr) cm^{-1}: 3243, 2938, 2816, 1683, 1592. ^1H NMR ($CDCl_3$) δ: 1.13–1.32 (m, 6H, $CCH_2CH_2CH_2C$), 1.38 (t, J = 7.1 Hz, 3H, CH_3), 2.03–2.17 (m, 4H, 2 piper. NCH_2), 2.27 (t, J = 5.9 Hz, 2H, CH_2N), 2.97–3.09 (m, 2H, $NHCH_2$), 4.35 (q, J = 7.1 Hz, 2H, OCH_2), 6.23 (br s, 1H, NH, exchangeable), 7.18–7.67 (m, 10H, arom. H). ^{13}C NMR ($CDCl_3$) δ: 14.35 (CH_3), 24.11 ($NCH_2CH_2CH_2$), 25.52 (2 $NCH_2CH_2CH_2$), 41.01 (CH_2N), 53.51 (2 $NCH_2CH_2CH_2$), 55.98 ($NHCH_2$), 61.93 (OCH_2), 89.08 (C-5), 128.43 (CH), 128.60 (2CH), 129.46 (2CH), 129.66 (2CH), 130.28 (CH), 130.68 (2CH), 138.04 (C), 140.06 (C), 152.35 (C-6), 158.98 (CON), 166.17 (COO), 178.86 (CS). Anal. Calcd for $C_{26}H_{30}N_4O_3S$: C, 65.25; H, 6.32; N, 11.71; S, 6.70. Found: C, 65.13; H, 6.51; N, 11.84; S, 6.78.

5.1.4.9. Ethyl 6-[(2-morpholin-4-ylethyl)amino]-4-oxo-1,3-diphenyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate 21. Mp 194–195 °C; yield: 49% from acetone/ethanol. IR (KBr) cm^{-1}: 3288, 2963, 2815, 1697, 1670, 1589. ^1H NMR ($CDCl_3$) δ: 1.35 (t, J = 7.1 Hz, 3H, CH_3), 2.02–2.49 (m, 6H, 3 NCH_2), 2.82–3.08 (m, 2H, $NHCH_2$), 3.22–3.52 (m, 4H, 2 morph. OCH_2), 4.18–4.55 (m, 2H OCH_2), 6.82 (br s, 1H, NH, exchangeable), 7.25–7.68 (m, 10H, arom. H). Anal. Calcd for $C_{25}H_{28}N_4O_4S$: C, 62.48; H, 5.87; N, 11.66; S, 6.67. Found: C, 62.47; H, 5.87; N, 11.70; S, 6.70.

5.1.4.10. Ethyl 6-{[2-(1-methylpyrrolidin-2-yl)ethyl]amino}-4-oxo-1,3-diphenyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate 22. Mp 155–157 °C; yield: 84% from acetone/ethanol. IR (KBr) cm^{-1}: 3150, 2955, 1727, 1660, 1586. ^1H NMR ($CDCl_3$) δ: 1.38 (t, J = 7.1 Hz, 3H, CCH_3), 1.45–1.80 (m, 6H, 3 CCH_2C), 1.87 (s, 3H, NCH_3), 2.10–2.25 (m, 1H, NCH), 2.44–2.62 (m, 2H, CH_2N), 2.95–3.25 (m, 2H, $NHCH_2$), 4.34 (q, J = 7.1 Hz, 2H, OCH_2), 7.18–7.63 (m, 10H, arom. H), 8.00 (br s, 1H, NH, exchangeable). ^{13}C NMR (DMSO-d_6) δ: 13.83 (CH_3), 22.31 (CCH_2C), 27.54 (CCH_2C), 28.74 (CCH_2C), 41.93 (NCH_3), 42.02 (NCH), 55.93 (NCH_2), 60.75 ($NHCH_2$), 63.87 (OCH_2), 89.28 (C-5), 127.54 (CH), 128.72 (2CH), 129.40 (2CH), 129.52 (2CH), 129.81 (CH), 130.00 (2CH), 138.38 (C), 140.28 (C), 151.92 (C-6), 158.06 (CON), 165.64 (COO), 178.21 (CS). Anal. Calcd for $C_{26}H_{30}N_4O_3S$: C, 65.25; H, 6.32; N, 11.71; S, 6.70. Found: C, 65.12; H, 6.41; N, 11.69; S, 6.67.

5.1.4.11. Ethyl 4-oxo-1,3-diphenyl-6-[(2-pyridin-2-ylethyl)-amino]-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate 23. Mp 213–215 °C; yield: 83% from acetone/ethanol. IR (KBr) cm^{-1}: 3310, 2991, 1702, 1673, 1593. ^1H NMR ($CDCl_3$) δ: 1.37 (t, J = 7.1 Hz, 3H, CH_3), 2.68–2.97 (m, 2H, 3 CH_2Py), 3.15–3.48 (m, 2H, $NHCH_2$), 4.37 (q, J = 7.1 Hz, 2H, OCH_2), 6.70 (br s, 1H, NH, exchangeable), 6.92–7.67 (m, 13H, arom. H), 7.93–8.09 (m, 1H, pyrid. H). Anal. Calcd for $C_{26}H_{24}N_4O_3S$: C, 66.08; H, 5.12; N, 11.86; S, 6.79. Found: C, 65.96; H, 5.30; N, 11.87; S, 6.86.

5.1.4.12. Ethyl 6-[(2-anilinoethyl)amino]-4-oxo-1,3-diphenyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate 24. Mp 180–182 °C; yield: 88% from acetone/ethanol. IR (KBr) cm^{-1}: 3318, 2933, 1692, 1602. ^1H NMR ($CDCl_3$) δ: 1.35 (t, J = 7.1 Hz, 3H, CH_3), 2.76–3.20 (m, 4H, $2CH_2$), 4.37 (q, J = 7.1 Hz, 2H, OCH_2), 6.46–7.95 (m, 17H, 15 arom. H + 2NH). Anal. Calcd for $C_{27}H_{26}N_4O_3S$: C, 66.65; H, 5.39; N, 11.51; S, 6.59. Found: C, 66.69; H, 5.48; N, 11.59; S, 6.69.

5.1.4.13. Methyl 6-({2-[aminosulfonyl)phenyl]ethyl}amino)-4-oxo-1,3-diphenyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate 25. Mp 287–289 °C; yield: 82% from acetone/methanol. IR (KBr) cm^{-1}: 3342, 3193, 1722, 1657, 1606. ^1H NMR (DMSO-d_6) δ: 2.72–2.79 (m, 2H, CH_2Ph), 3.02–3.15 (m, 2H, $NHCH_2$), 3.72 (s, 3H, OCH_3), 6.15 (br s, 1H, NH, exchangeable), 7.15–7.55 (m, 12H, arom. H), 7.71 (d, J = 8.3 Hz, 2H, arom. H). ^{13}C NMR (DMSO-d_6) δ: 34.03 (CH_2Ph), 45.29 ($NHCH_2$), 51.97 (OCH_2), 88.35 (C-5), 125.79 (2CH), 127.63 (CH), 128.62 (2CH), 128.77 (2CH), 128.83 (2CH), 129.59 (CH), 129.71 (2CH), 129.93 (2CH), 137.78 (C), 140.21 (C), 141.75 (C), 142.33 (C), 152.85 (C-6), 157.67 (CON), 166.31 (COO), 178.25 (CS). Anal. Calcd for $C_{26}H_{24}N_4O_5S_2$: C, 58.19; H, 4.51; N, 10.44; S, 11.95. Found: C, 57.99; H, 4.59; N, 10.23; S, 11.86.

5.1.4.14. Methyl 6-{[2-(1H-indol-3-yl)ethyl]amino}-4-oxo-1,3-diphenyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate 26. Mp 231–233 °C; yield: 69% from acetone. IR (KBr) cm^{-1}: 3332, 2947, 1679, 1645, 1618. ^1H NMR (DMSO-d_6) δ: 2.72–2.79 (m, 2H, CCH_2C), 3.05–3.20 (m, 2H, $NHCH_2$), 3.71 (s, 3H, OCH_3), 5.91 (br s, 1H, NH, exchangeable), 6.78 (s, 1H, indole H-2), 6.80–7.45 (m, 14H, arom. H), 10.82 (br s, 1H, indole NH, exchangeable). ^{13}C NMR (DMSO-d_6) δ: 24.59 (CCH_2C), 44.68 ($NHCH_2$), 51.88 (OCH_3), 88.14 (C-5), 109.31 (CH), 111.41 (CH), 118.06 (CH), 118.28 (CH), 121.11 (CH), 123.06 (CH), 126.41 (C), 127.60 (C), 128.64 (2CH),

128.74 (2CH), 129.56 (CH), 129.64 (2CH), 129.86 (2CH), 136.30 (C), 137.74 (C), 140.23 (C), 152.80 (C-6), 157.67 (CON), 166.18 (COO), 178.16 (CS). Anal. Calcd for $C_{28}H_{24}N_4O_3S$: C, 67.72; H, 4.87; N, 11.28; S, 6.46. Found: C, 67.35; H, 5.12; N, 10.98; S, 6.40.

5.1.4.15. Ethyl 6-[(2-methoxyethyl)amino]-4-oxo-1,3-diphenyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate 27. Mp 201–203 °C; yield: 81% from acetone/ethanol. IR (KBr) cm^{-1}: 3365, 2985, 1733, 1661, 1601. ^1H NMR (DMSO-d_6) δ: 1.22 (t, J = 7.1 Hz, 3H, CH_3), 2.83–3.35 (m, 4H, $2CH_2$), 3.07 (s, 3H, $COOCH_3$), 4.23 (q, J = 7.1 Hz, 2H, OCH_2), 6.38 (br s, 1H, NH, exchangeable), 7.05–7.68 (m, 10H, arom. H). Anal. Calcd for $C_{22}H_{23}N_3O_4S$: C, 62.10; H, 5.45; N, 9.88; S, 7.54. Found: C, 62.12; H, 5.64; N, 9.88; S, 7.71.

5.1.4.16. Methyl 6-[(2,2-dimethoxyethyl)amino]-4-oxo-1,3-diphenyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate 28. Mp 177–179 °C; yield: 86% from CH_2Cl_2/diethyl ether. IR (KBr) cm^{-1}: 1696, 1650, 1621. ^1H NMR ($CDCl_3$) δ: 2.68–2.90 (m, 2H, CH_2), 3.24 (s, 6H, $2CH_3$), 3.88 (s, 3H, $COOCH_3$), 4.20–4.42 (m, 1H, OCH), 7.25–7.75 (m, 10H, arom. H), 7.96 (br s, 1H, NH, exchangeable). Anal. Calcd for $C_{22}H_{23}N_3O_5S$: C, 59.85; H, 5.25; N, 9.52; S, 7.26. Found: C, 59.68; H, 5.35; N, 9.38; S, 7.18.

5.1.4.17. 5-Acetyl-6-(4-methylpiperazin-1-yl)-1,3-diphenyl-2-thioxo-2,3-dihydropyrimidin-4(1H)-one 29. Mp 243–245 °C; yield: 74% from acetone/methanol. IR (KBr) cm^{-1}: 2968, 1678, 1580. ^1H NMR ($CDCl_3$) δ: 1.68–1.83 (m, 4H, $2CH_2N$), 2.06 (s, 3H, CH_3), 2.63 (s, 3H, CCH_3), 2.93–3.02 (m, 2H, $2CH_2N$), 7.18–7.36 (m, 4H, arom. H), 7.39–7.58 (m, 6H, arom. H). ^{13}C NMR ($CDCl_3$) δ: 32.36 (CH_3), 46.12 (NCH_3), 51.39 ($2CH_2N$), 53.23 ($2CH_2N$), 110.32 (C-5), 128.22 (2CH), 128.72 (CH), 128.91 (CH), 129.15 (2CH), 129.74 (2CH), 130.40 (2CH), 139.91 (C), 141.51 (C), 160.02 (C-6), 160.47 (CON), 180.32 (CS), 198.07 (CO). Anal. Calcd for $C_{23}H_{24}N_4O_2S$: C, 65.69; H, 5.75; N, 13.32; S, 7.62. Found: C, 65.74; H, 5.78; N, 13.38; S, 7.35.

5.2. Biology

5.2.1. General

Hank's balanced salt solution without phenol red (HBSS, ICN, Biomed, Milan, Italy) mixed with Dulbecco's phosphate-buffered saline (PBS, ICN Biomed) (HBSS:PBS = 3:1) containing 1 mg/mL bovine serum albumin (BSA, Sigma, Milan, Italy) was used as incubation medium. IL-8, fMLP, C5a, ethidium bromide, and fluorescein diacetate were from Sigma Chemical, St. Louis, MO.

5.2.2. Neutrophilic polymorphonuclear leukocyte preparation

Heparinized venous blood (10 U/mL heparin) was obtained from healthy volunteers (20–37 years old) after informed consent. Neutrophilic polymorphonuclear leukocytes (neutrophils) were prepared by dextran sedimentation, followed by centrifugation (400g, 30 min) on a Ficoll-Hypaque density gradient, as previously described.[28] Contaminating erythrocytes were removed by hypotonic lysis. Neutrophils resuspended in incubation medium were >97% pure, as determined by morphologic analysis of Giemsa-stained cytopreparation.

5.2.3. Assessment of neutrophil viability

Neutrophils (2×10^6/mL) were incubated for 2 h in tissue culture tubes (17 × 100 mm, Falcon, Becton Dickinson) in incubation medium at 37 °C in a CO_2 atmosphere (0.5 mL final volume), with appropriate doses of compounds **1** and **14–29.** Cell viability, measured as integrity of membrane, was assessed by an ethidium bromide–fluorescein diacetate test according to Dankberg,[29] as previously described by Ottonello et al.[30] Briefly, cells (4×10^4/100 mL) harvested from culture tubes were mixed with 50 mL of staining solution (2 mg/mL fluorescein diacetate, 4 μg/mL ethidium bromide) in HBSS and incubated for 10 min at room temperature. Thereafter, a drop of cell suspension was placed on a slide, sealed with a coverslip, and analysed under UV light in a dark field illumination. Neutrophils with intact membrane (i.e., viable cells) appeared as green fluorescent cells, whereas neutrophils with damage and ethidium bromide-permeable membrane (i.e., necrotic cells) displayed a fluorescent red nucleus.

5.2.4. Boyden chamber migration assay

Neutrophil locomotion was studied by means of the leading front method, as previously described by Corcione et al.[31] Neutrophils were preincubated in the absence or presence of appropriate doses of compounds **1** and **14–29** for 15 min at room temperature. Tests were conducted in duplicate, using blind well chambers (Neuro-Probe, Gathersburg, MD) with a 3 μm pore size cellulose ester filter (Millipore, Milan, Italy) separating the upper from the lower compartment of the chambers. Then, cells (4×10^5) in the absence or presence of various amounts of tested compounds were placed in the upper compartment of the chambers. Chemoattractants (10^{-9} M IL-8 or 10^{-8} M fMLP or 10^{-9} M C5a) were placed in the lower compartment of the chambers. Experiments were also carried out without chemoattractants in the lower compartment (spontaneous migration). After incubation at 37 °C for 45 min, the filters were removed, fixed in ethanol, stained with Harris hematoxylin, dehydrated, cleared with xylene, and mounted in Eukitt (Kindler, GmbH). Then, the distance (mm) travelled by the leading front of cells was measured × 400 magnification. Five randomly chosen fields were read for each filter. The net migration was determined by subtracting spontaneous migration (i.e. the distance travelled by neutrophils in the absence of the stimulus) from the distance travelled by neutrophils toward the stimulus. The concentration of each compound giving 50% inhibition (IC_{50}) of net migration was obtained from nonlinear regression analysis with SPSS for Windows version 6.0, Wacker Drive, Chicago, IL.

5.2.5. Induction of peritonitis, cell count, cell composition and determination of protein concentration in the peritoneal exudate

The experiments were performed using male Swiss mice (25–30 g) fasted 16 h before the experiment and with free access to water. Mice were randomly assigned to groups of 8–12 animals orally treated with vehicle or the compound under examination (**17**) (50 mg/kg os) 1 h before the induction of peritonitis. Dexamethasone (3 mg/kg os) was used as reference drug. Peritonitis was induced following a modification of Thurmond's method.[32] Briefly, 5 mg/mL Zymosan or phosphate-buffered saline (PBS) was injected into the peritoneal space of mice (final volume 0.2 mL). After 4 h, the animals were euthanized, the peritoneal cavities were washed with 3 mL of PBS containing 3 mM EDTA and the volume was collected. Total leukocyte counts were performed by an observer unaware of the treatment using a Neubauer chamber and an optical microscope after diluting the samples of the peritoneal fluid with Türk solution (1:20). Differential cell counts were performed using a light microscope. Chromatic characteristics and the shape of the nucleus relative to the cytoplasm were used to differentiate leukocyte subpopulations. The total cell number/cavity and the granulocyte number/cavity were measured.

Protein content (μg/mL) was spectrophotometrically determined applying the bicinchonate method with a commercial kit (Pierce, BCA protein assay kit).

Data are expressed as mean ± SEM. Statistical analysis was performed adopting ANOVA one way test followed by Bonferroni's post test; $^{**}P < 0.01$ indicated significant differences compared to negative control (Graph pad PRISM 5.0, San Diego, CA, USA). Exper-

iments were carried out in accordance with Italian law (DL 116/92) and approved by the Ministry of Health.

Acknowledgements

This work was supported by MURST (Cofinanziamento Nazionale), CNR (Rome) and Ministero Italiano della Salute - Istituto Superiore di Sanità (Grant No. 40D.46). Fondazione Carige is gratefully acknowledged for financially supporting S.C. The authors thank Mr. O. Gagliardo for the microanalyses, Mr F. Tuberoni for the IR spectra, Professor F. Lucchesini, Dr. M. Anzaldi, Dr. R. Raggio and Dr. C. Rossi for the NMR spectra.

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