Microwave-assisted synthesis of some hybrid molecules containing penicillanic acid or cephalosporanic acid moieties and investigation of their biological activities

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Abstract Ethyl 4-amino-2-fluorophenylpiperazin-1-carboxylates containing a 1,3-oxazol(idin)e, 5-thioxo-1,2,4-triazole, 1,3,4-thiadiazole, 5-thioxo-1,3,4-oxadiazole, or 1,3-thiazole nucleus were obtained starting from ethyl piperazine-1-carboxylate (1) by several steps. The treatment of amine, 3 or hydrazide, 9 with several aromatic aldehydes generated the corresponding arylmethyleneamine (3a–f) or arylidenehydrazino (12a–c) compounds. The Mannich reaction between the 1,2,4-triazole or 1,3,4-oxadiazole compounds and 7-aca produced cephalosporanic acid derivatives. Penicillanic acid derivatives were obtained when 6-apa was used in the Mannich reactions. The synthesized compounds were screened for their antimicrobial, antilipase, and antiurease activities. Some of them were found to possess good-moderate antimicrobial activity against the test microorganisms. Two compounds exhibited antiurease activity, and four of them displayed antilipase activity.

Keywords Piperazine · 1,3-Oxa(thia)zole · 5-Oxo-1,3-oxazolidine · 1,2,4-Triazole · 7-Aminocephalosporanic acid · 6-Aminopenicillanic acid · Biological activity

Introduction

The limitations of the existing antibacterial drugs caused by various reasons including drug resistance, the serious side effects, and/or lack of efficacy made infectious diseases a vicious cycle. In addition, the treatment of resistant strains requires a prolonged therapy containing the use of more toxic drugs and increases the financial burden. The rising prevalence of multi-drug resistant bacteria continues to serve medicinal chemists to search and discove novel antimicrobial agents effective against pathogenic microorganisms resistant to current treatment.

Among the strategies addressed to the synthesis of compounds possessing antimicrobial activity, the synthesises of hybrid molecules incorporating different heterocyclic moieties have been attracting widespread attention (Mal-likarjuna et al., 2009).

A number of N-containing heterocyclic compounds constitute important building blocks in organic and medicinal chemistry. For example, triazoles have been shown to possess a number of desirable activities in the context of medicinal chemistry. Ribavirin (antiviral), rizatriptan (antimigraine), alprazolam (psychotropic), fluconazole, and itraconazole (antifungal) are the best examples for potent drugs possessing triazole nucleus (Holla et al., 2006; Walczak et al., 2004; Jones et al., 1965; Ashok et al., 2007). Tazobactam, a β-lactamase inhibitor is the other best known example of triazole containing structures with the broad spectrum antibiotic piperacillin (Kategaonkar et al., 2010).

Substituted piperazines constitute another class of important pharmacophores, which are found in many marketed drugs, such as the HIV protease inhibitor, Crixivan (Chaudhary et al., 2006). Ciprofloxacin, norfloxacin, pefloxacin, ofloxacin, and enoxacin are fluoroquinolone...
class antibacterial drugs characterized by having a piperazine moiety at C-7 of quinolone skeleton, and they have been used for the treatment of bacterial infections (Fournoudi et al., 2005).

The compounds having a thiazolidinone nucleus are of interest due to their broad spectrum of biological activities such as bactericidal, fungicidal, antimicrobial, antiproliferative, antiviral, anticonvulsant, anticancer, and anti-inflammatory activities (Vicini et al., 2008; Wang et al., 2011; Lv et al., 2010; Metwally et al., 2010; Balzarini et al., 2009; Hervlyuk et al., 2009; Subtelna et al., 2010; Mushtaque et al., 2012).

Mannich bases, which are known to be physiologically reactive since their basic function rendering the molecule soluble in aqueous solvents when it is transformed into amonium salt, have been reported as potential biological agents (Karthikeyan et al., 2006). N-Mannich bases have been used successfully to obtain prodrugs of amine as well as amide-containing drugs (Zhao et al., 2009). Some Mannich bases derived from 1,2,4-triazole nucleus have been reported to possess protocidal and antibacterial activity (Ashok et al., 2007; Almajan et al., 2009; Bayrak et al., 2009, 2010; Demirbas et al., 2009; Bektas et al., 2010; Patole et al., 2006).

Schiff bases have gained importance in medicinal and pharmaceutical fields due to their most versatile properties as organic synthetic intermediates and also possessing a broad range of biological activities, such as antituberculosis, anticancer, analgesic and anti-inflammatory, anticonvulsant, antibacterial, and antifungal activities (Patole et al., 2010; Patole et al., 2010; Patole et al., 2010; Patole et al., 2010; Patole et al., 2010; Patole et al., 2010; Patole et al., 2010; Patole et al., 2010). We envisage that hybrid compound incorporating a 4-(4-amino-2-fluorophenyl)piperazine-1-carboxylate (3), that was obtained starting from compound 1 by two steps, was converted to the corresponding arylmethylenamino derivatives (4a–f) by the treatment with several aromatic aldehydes. In the FT-IR and $^1$H NMR spectra of these compounds, no signal pointing the –NH$_2$ group was seen. Instead, additional signals derived from aldehyde moiety were recorded at the related chemical shift values in the $^1$H NMR spectra.

The cyclocondensation of compound 5, that was obtained from the reaction of 4 with benzilsocyanate, with ethyl bromoacetate or 4-chlorophenacyl bromide produced the corresponding hybrid molecules incorporating a 4-oxo-1,3-oxazolidine (6) or 4-chlorophenyl)-1,3-oxazole (7) nucleus in the 2-fluorophenylpiperazine-1-carboxylate skeleton. The $^1$H and $^{13}$C NMR spectra of compound 7 exhibited additional signals at aromatic region originated from 4-chlorophenyl nucleus as a result of condensation. Moreover, the elemental analyses and mass spectral data of derivatives 6 and 7 were compatible with the suggested structures.

The treatment of compound 3 with ethyl bromoacetate at room temperature in the presence of triethylamine resulted in the formation of compound 8. When compound 8 was converted to the corresponding hydrazide (9) by refluxing with hydrazine hydrate, the signals originated from ester function was disappeared in the $^1$H and $^{13}$C NMR spectra. Instead, new signals due to –NHNH$_2$ protons were seen at 5.93 and 9.09 ppm. Meanwhile, the stretching frequency band of this group was recorded at 3,313 cm$^{-1}$ as a wide signal characteristic for the hydrazide structure. Compounds 6 and 7 gave mass fragmentation confirming the proposed structures.

The synthesis of compounds 10 and 11 was carried out by the treatment of compound 7 with the corresponding isothiocanates. These compounds displayed spectroscopic data and elemental analysis results consistent with the assigned structures.

The intramolecular cyclization of compound 10 generated the corresponding 1,3,4-thiazole compound (12) in acidic media. On the other hand, the basic treatment of compounds 10 and 11 caused to the cyclization of the (arylamino)carbonothioylhydrazino side chain leading to the formation of 5-thioxo-4,5-dihydro-1H-1,2,4-triazole derivatives (13 and 14). With the conversion of compounds 10 and 11 to compounds 12–14, two of NH signals were disappeared in the $^1$H NMR spectra. It is well-known that type of compounds can stay in thioxo or mercapto tautomeric form. In the present study, compounds 13 and 14 are

**Results and discussion**

The main aim of the present study is the synthesis and antimicrobial activity evaluation of new piperazine derivatives incorporating several heterocyclic moieties including 1,3-oxadiazole, 1,2,4-triazole, 1,3-oxa(thia)zole, penicillanic acid, and/or cephalosporanic acid. Synthesis of the intermediate and target compounds was performed according to the reactions outlined in Schemes 1, 2, and 3. The starting compound ethyl 1-piperazinecarboxylate (1) was provided commercially.

Ethyl 4-(4-amino-2-fluorophenyl)piperazine-1-carboxylate (3), that was obtained starting from compound 1 by two steps, was converted to the corresponding arylmethylenamino derivatives (4a–f) by the treatment with several aromatic aldehydes. In the FT-IR and $^1$H NMR spectra of these compounds, no signal pointing the –NH$_2$ group was seen. Instead, additional signals derived from aldehyde moiety were recorded at the related chemical shift values in the $^1$H NMR spectra.

The cyclocondensation of compound 5, that was obtained from the reaction of 4 with benzilsocyanate, with ethyl bromoacetate or 4-chlorophenacyl bromide produced the corresponding hybrid molecules incorporating a 4-oxo-1,3-oxazolidine (6) or 4-chlorophenyl)-1,3-oxazole (7) nucleus in the 2-fluorophenylpiperazine-1-carboxylate skeleton. The $^1$H and $^{13}$C NMR spectra of compound 7 exhibited additional signals at aromatic region originated from 4-chlorophenyl nucleus as a result of condensation. Moreover, the elemental analyses and mass spectral data of derivatives 6 and 7 were compatible with the suggested structures.

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present predominately in the thioxo form as it was shown by the C=S band at 1,244–1,250 cm\(^{-1}\) in the FT-IR spectra of these compounds. Furthermore, the \(^1\)H NMR spectra of compounds 13 and 14 revealed clearly the absence of the signal originated from SH proton, instead of that, two signals due to NH proton on 1,2,4-triazol ring was recorded at 10.45 (for 13) or 11.27 (for 14), that is characteristic for 4,5-dihydro-1\(^H\)-1,2,4-triazoles.

The synthesis of Mannich bases (15–17) was performed by the reaction of compounds 13 and 14 with 6-aminopenicillanic acid, 6-apa (for 17) or 7-aminocephalosporanic acid, 7-aca (for 15 and 16) in tetrahydrofuran at room temperature in the presence of triethylamine and formaldehyde. The occurrence of the alkyaminomethylation was provided by the disappearance of signal for the proton at the N-1 nitrogen of the 1,2,4-triazole ring. Moreover, in \(^1\)H and \(^13\)C NMR spectra, additional signal corresponding to the 6-apa or 7-aca-ammonium salt was recorded at the related chemical shift value.

The conversion of arylcarbonothioylhydrazino side change to 4-chlorophenyl-3-phenyl-1,3-thiazole ring (18) was accomplished with the treatment of 4-chlorophenacyl bromide. This compound was characterized by spectroscopic techniques including \(^1\)H NMR, \(^13\)C NMR, FT-IR, EI-MS, and elemental analysis.

The synthesis of ethyl arylidenehydrazino-piperazine-1-carboxylate derivatives (19a–c) was performed by microwave irradiation of compound 9 with several aromatic aldehydes namely 3-hydroxy-4-methoxybenzaldehyde, pyridine-4-carbaldehyde, and 2-hydroxybenzaldehyde. In the FT-IR spectra of these arylidenehydrazino compounds, absorption bands characteristic for NH groups were visible in
Another piece of evidence for condensation was the appearance of a signal as singlet integrating for one proton in the $^1$H NMR spectra, which corresponds to the N=CH proton of azomethyne group. Moreover, these compounds gave mass fragmentation and elemental analysis confirming the proposed structures.

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**Scheme 2**

1. Ethyl bromoacetate, Et$_3$N, THF, rt for 14 h.
2. Hydrazine hydrate in ethanol, reflux for 14 h.
3. 4-Fluorophenylisothiocyanate or phenylisothiocyanate in absolute ethanol, reflux for 10 h.
4. H$_2$SO$_4$, rt for 2 h.
5. NaOH in water, reflux for 3 h.
6. 7-Aca, HCHO, Et$_3$N in THF, rt, for 4 h.
7. 6-Apa, HCHO, Et$_3$N in THF, rt, for 4 h.
8. 4-Chlorophenacylbromide in absolute ethanol, dried sodium acetate, reflux for 12 h.

the ranges of 3,357–3,181 cm$^{-1}$. Another piece of evidence for condensation was the appearance of a signal as singlet integrating for one proton in the $^1$H NMR spectra, which

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$\text{O} \quad \text{N} \quad \text{F} \quad \text{S} \quad \text{NH} \quad \text{H} 
\text{O} \quad \text{N} \quad \text{H} \quad \text{H} \quad \text{N} \quad \text{S} 
\text{OAc} \quad \text{CO}_2 \text{HN}
Ethyl 4-(2-fluoro-4-[(5-thioxo-4,5-dihydro-1,3,4-oxadiazol-2-yl)methyl]amino)phenyl)piperazine-1-carboxylate (20) was prepared from the reaction of compound 9 with CS₂ in the basic media. The attempts for aminoalkylations of compound (20) by Mannich reaction allowed the isolation of the corresponding products (21 and 22) after 4 (for 21) or 6 h (for 22) at room temperature. This idea originated from the intent to introduce the penicillanic acid or cephalosporanic acid nucleus to (piperazine-1-yl)-2-thioxo-1,3,4-oxadiazole skeleton. As different from 20, the NMR spectra of the obtained Mannich bases (21 and 22) displayed additional signals derived from penicillanic or cephalosporanic acid moiety and –CH₂—linkage at the related shift and integral values as D₂O non-exchangeable signals.

Among the synthesized compounds ethyl 4-(2-fluoro-4-nitrophenyl)piperazine-1-carboxylate (2) exhibited activity on Bacillus cereus (Bc), that is Gram positive spore bacillus. With the reduction of nitro group of 2 to amine (compound 3), additional activities towards Staphylococcus aureus (Sa), that is Gram positive coccus, Candida albicans (Ca), and Saccharomyces cerevisiae (Sc), which are yeast like fungi. For the imine compounds (4a–f), the highest activity was observed against Mycobacterium smegmatis (Ms) that is an atypical tuberculosis factor leading mortality, with the inhibition zone varying between 10 and 25 mm. The compounds containing 1,2,4-triazole and cephalosporanic- or penicillanic-acid moiety (compounds 15–17) displayed good-moderate activity on some of the test microorganisms. The highest activity was observed for compound 17 on Bc with the inhibition zone of 16 mm. This result is better than standard drug ampicillin. Other compounds containing penicillanic acid or cephalosporanic acid core

Scheme 3  

i 3-Hydroxy-4-phenoxybenzaldehyde, pyridine-4-carboxaldehyde, 2-hydroxybenzaldehyde in absolute ethanol, irradiation by MW at 200 W, 140 °C for 30 min. ii CS₂ and KOH in ethanol, reflux for 13 h. iii 7-Aca, HCHO, Et₃N in THF, rt, for 4 h. iv 6-Apa, HCHO, Et₃N in THF, rt, for 4 h

19a: R= OCH₃
19b: R= O
19c: R= OH
19

20

21

22
(21 and 22) displayed good-moderate activity against the test microorganisms.

The synthesized compounds were assayed for their in vitro urease inhibitory activity against Jack bean urease. Two of those compounds showed perfect urease inhibition. No inhibitory effect was detected for other compounds. Thiourea with \( IC_{50} \) value 54.56 ± 4.17 \( \mu \)g mL\(^{-1} \) was used as standard inhibitor. Among tested compounds, compound 15 was found to be the best inhibitory effect against urease with an \( IC_{50} \) value of 4.67 ± 0.53 \( \mu \)g mL\(^{-1} \). At the various final concentrations the compound 15 showed more inhibitory effect than standard urease inhibitor thiourea. Also, compound 17 has the highest inhibitory activity than thiourea. These compounds might be considered as potential antibiotics to treat infections.

All compounds were evaluated with regard to pancreatic lipase activity and compounds 12, 13, 14, and 15, which are 1,3,4-thiadizole or 1,2,4-triazole derivatives including also 4-fluoro phenylpiperazine nucleus, showed moderate anti-lipase activities at final concentration of 6.25 \( \mu \)g mL\(^{-1} \). No inhibitory effect was detected for other compounds. Orlistat, known pancreatic lipase inhibitor used as anti-obesity drug, showed inhibitory effect by 99 % at the same concentration.

Conclusion

This study reports microwave-assisted synthesis of some new hybrid molecules containing penicillanic acid or cephalosporanic acid moieties with some other pharmacophore heterocycles in a single structure. Hence herein we combined all these potential chemotherapeutic units, namely 1,2,4-triazole, 1,3-thiazole, 1,3-oxazole, 1,3,4-oxadiazole, piperazine, penicillanic acid, cephalosporanic acid moieties. The antimicrobial, anti-urease, and antilipase screening studies were also performed in the study.

Among the synthesized compounds, the compounds containing 1,2,4-triazole and cephalosporanic- or penicillanic-acid moiety (15-17) displayed good-moderate activity on some of the test microorganisms. The highest activity was observed for compound 17 on Bc with the inhibition zone of 16 mm. This result is better than standard drug ampicillin. Moreover, compounds 15 and 17 exhibited an inhibitory effect against urease. Other compounds containing penicillanic acid or cephalosporanic acid core (21 and 22) displayed good-moderate activity against the test microorganisms. Furthermore, compounds 12, 13, 14, and 15, which are 1,3,4-thiadizole or 1,2,4-triazole derivatives including also 4-fluorophenylpiperazine nucleus, showed moderate anti-lipase activities at final concentration of 6.25 \( \mu \)g mL\(^{-1} \).

Experimental

Chemistry

General information for chemicals

All the chemicals were purchased from Fluka Chemie AG Buchs (Switzerland) and used without further purification. Melting points of the synthesized compounds were determined in open capillaries on a Büchi B-540 melting point apparatus and are uncorrected. Reactions were monitored by thin-layer chromatography (TLC) on silica gel 60 F254 aluminum sheets. The mobile phase was ethyl acetate:dichalkyl ether, 1:1, and detection was made using UV light. FT-IR spectra were recorded as potassium bromide pellets using a Perkin Elmer 1600 series FT-IR spectrometer. \(^1\)H NMR and \(^13\)C NMR spectra were registered in DMSO-\( d_6 \) on a BRUKER AVANCE II 400 MHz NMR Spectrometer (400.13 MHz for \(^1\)H and 100.62 MHz for \(^13\)C). The chemical shifts are given in ppm relative to Me\( _2\)Si as an internal reference, J values are given in Hz. The elemental analysis was performed on a Costech Elemental Combustion System CHNS-O elemental analyzer. All the compounds gave C, H, and N analysis within ±0.4 % of the theoretical values. The mass spectra were obtained on a Quattro LC-MS (70 eV) instrument.

Ethyl 4-(2-fluoro-4-nitrophenyl)piperazine-1-carboxylate

(2) The solution of 3,4-difluoronitrobenzene (10 mmol) in excess amount of ethyl 1-piperazinecarboxylate (40 mmol) was allowed to reflux for 6 h (the progress of the reaction was monitored by TLC). Then, the mixture was poured into ice-water. The precipitated product was filtered off and recrystallized from ethanol. Yield 97 %, m. p: 90–93 °C. FT-IR (KBr, \( \nu \) cm\(^{-1} \)): 3099 (ar–CH), 1509, and 1354 (NO\(_2 \)). Elemental analysis for C\(_{13}\)H\(_{16}\)F\(_2\)N\(_3\)O\(_4\) calculated (%): C, 52.52; H, 5.42; N, 14.13. Found (%): C, 52.64; H, 5.70; N, 14.00. \(^1\)H NMR (DMSO-\( d_6 \), \( \delta \) ppm): 1. 19 (t, 3H, CH\(_3\), \( J = 7.0 \) Hz), 3.26 (s, 4H, 2CH\(_2\)), 3.51 (s, 4H, 2CH\(_2\)), 4.06 (q, 2H, CH\(_2\)), 4.67 (q, 2H, CH\(_2\)), 5.79 (arC: \( \delta \) ppm): 1. 19 (t, 3H, CH\(_3\), \( J = 7.0 \) Hz), 3.26 (s, 4H, 2CH\(_2\)), 3.51 (s, 4H, 2CH\(_2\)), 4.06 (q, 2H, CH\(_2\)), 4.67 (q, 2H, CH\(_2\)), 5.79 (arC: \( \delta \) ppm): 1. 19 (t, 3H, CH\(_3\), \( J = 7.0 \) Hz), 3.26 (s, 4H, 2CH\(_2\)), 3.51 (s, 4H, 2CH\(_2\)).
TLC. Then, the catalyst was separated by filtration and the solvent was evaporated under reduced pressure. The solid obtained was recrystallized from ethanol. Yield 65 %. M.p. 116–119 °C. FT-IR (KBr, ν, cm⁻¹): 3,423 and 3,341 (NH₂). 1682 (C=O). Elemental analysis for C₁₉H₁₈FN₃O₄ calculated (%): C, 58.41; H, 6.79; N, 15.72. Found (%): C, 58.31; H, 6.87; N, 15.78. ¹H NMR (DMSO-d₆, δ ppm): 1.18 (t, 3H, CH₃, J = 7.0 Hz), 2.76 (s, 4H, 2CH₂), 3.45 (s, 4H, 2CH₂), 4.04 (q, 2H, CH₂, J = 7.4 Hz), 5.03 (s, 2H, NH₂), 6.33 (d, 2H, arH, J = 12.4 Hz), 6.76 (t, 1H, arH, J = 9.0 Hz). ¹³C NMR (DMSO-d₆, δ ppm): 14.53 (CH₃), 43.56 (2CH₂), 51.07 (2CH₂), 60.75 (CH₂), arC: [101.66 (d, CH, JₐCHE = 23.0 Hz)], 109.39 (CH), 120.92 (d, CH, JCHE = 4.05 Hz), 128.70 (d, C, JCHE = 9.5 Hz), 145.72 (d, C, J = 10.6 Hz), 154.18 (d, C, JCHE = 34.5 Hz), 158.65 (C=O). MS ml/z (%): 268.10 ([M+1]⁺, 100).

Ethyl 4-(2-fluoro-4-[(4-pyridin-4-ylmethylene)aminol]phenyl)piperazine-1-carboxylate (4a) Indole-3-carboxaldehyde (10 mmol) was added to the solution of compound 3 (10 mmol) in absolute ethanol and the reaction mixture was irradiated by microwave at 150 W and 110 °C for 30 min. After removing in the solvent under reduced pressure, an oily product obtained. This was recrystallized from butyl acetate and diethyl ether (1:2). Yield: 81 %, M.p: 162–163 °C. FT-IR (KBr, ν, cm⁻¹): 1686 (C=O), 1508 (C=N), 1224 (C–O). Elemental analysis for C₂₀H₂₁FN₄O₄ calculated (%): C, 64.03; H, 5.94; N, 15.72. Found (%): C, 64.18; H, 6.14; N, 15.78. ¹H NMR (DMSO-d₆, δ ppm): 1.19 (t, 3H, CH₃, J = 6.6 Hz), 3.00 (s, 4H, 2CH₂), 3.51 (s, 4H, 2CH₂ + H₂O), 4.04–4.11 (m, 2H, CH₂), 7.04–7.34 (m, 3H, arH), 7.80 (d, 2H, arH, J = 4.2 Hz), 8.71 (s, 3H, arH + N=CH). ¹³C NMR (DMSO-d₆, δ ppm): 15.26 (CH₃), 44.01 (CH₂), 50.69 (CH₃), 51.83 (2CH₂), 61.57 (CH₂), arC: [102.18 (CH)], 109.63 (d, CH, JCHE = 21.0 Hz), 120.05 (d, CH, JCHE = 31.5 Hz), 121.37 (C), 122.77 (2CH₂), 139.48 (d, C, JCHE = 9.0 Hz), 144.37 (d, C, JCHE = 120.0 Hz), 151.14 (2CH₂), 154.23 (C, C, JCHE = 103.2 Hz), 158.09 (N=CH), 158.90 (C=O). MS ml/z (%): 357.11 ([M+1]⁺, 64), 302.10 (100), 342.24 (80).

Ethyl 4-(2-fluoro-4-[(4-nitrophenyl)methylene]aminol)phenyl)piperazine-1-carboxylate (4b) The mixture of compound 3 (10 mmol) and 4-nitrobenzaldehyde (10 mmol) in absolute ethanol was refluxed with 2-hydroxybenzaldehyde (10 mmol) for 7 h. On cooling the reaction content to room temperature, a solid appeared. This crude product was filtered off and recrystallized from acetone. Yield: 83 %. M.p: 136–137 °C. FT-IR (KBr, ν, cm⁻¹): 1697 (C=O), 1510 (C=N), 1225 (C–O). Elemental analysis for C₁₉H₁₆FNO₃ calculated (%): C, 64.68; H, 5.97; N, 11.31. Found (%): C, 64.31; H, 5.78; N, 11.48. ¹H NMR (DMSO-d₆, δ ppm): 1.21 (brs, 3H, CH₃), 3.00 (s, 4H, 2CH₂), 3.52 (s, 4H, 2CH₂), 4.06 (brs, 2H, CH₂), 6.97–7.59 (m, 7H, arH), 8.95 (s, 1H, N=CH), 13.02 (s, 1H, OH). ¹³C NMR (DMSO-d₆, δ ppm): 15.26 (CH₃), 44.40 (2CH₂), 50.66 (2CH₂), 61.59 (CH₂), arC: [109.50 (d, CH, JCHE = 22.0 Hz), 117.24 (2CH₂), 119.33 (CH), 119.87 (C), 120.22 (d, CH, JCHE = 28.5 Hz), 133.18 (CH), 133.86 (CH), 139.28 (d, C, JCHE = 9.0 Hz), 143.26 (d, C, JCHE = 8.5 Hz), 153.32 (C), 156.74 (d, C, JCHE = 145.5 Hz), 160.82 (C=O), 163.17 (N=CH).

Ethyl 4-(2-fluoro-4-[(4-methoxyphenyl)methylene]aminol)phenyl)piperazine-1-carboxylate (4e) The solution of
compound 3 (10 mmol) in absolute ethanol was refluxed with 4-methoxybenzaldehyde (10 mmol) for 7 h. On cooling the reaction content to room temperature, a solid appeared. This crude product was filtered off and recrystallized from ethanol. Yield: 42 %. M.p: 122–124 °C. FT-IR (KBr, ν, cm⁻¹): 1688 (C=O), 1509 (C=N), 1225 (C–O). Elemental analysis for C₂₁H₂₄FN₃O₃ calculated (%): C, 65.44; H, 6.28; N, 10.90. Found (%): C, 65.56; H, 6.52; N, 11.12. ¹H NMR (DMSO-d₆, δ ppm): 1.19 (t, 3H, CH₃, J = 6 Hz), 2.96 (s, 4H, 2CH₃), 3.49 (s, 4H, 2CH₂), 3.82 (s, 3H, O–CH₃). 12.46 (s, 1H, NH). 13C NMR (DMSO-d₆, δ ppm): 15.27 (CH₃), 44.13 (CH₂), 50.85 (CH₂), 51.35 (2CH₂), 56.10 (O–CH₃) 61.53 (CH₂), arC: [109.73 (d, CH, J₁₋C₂ = 38.9 Hz), 114.98 (2CH), 118.72 (CH), 121.90 (d, CH, J₁₋C₂ = 66.3 Hz), 129.12 (C), 131.24 (2CH), 132.53 (C), 138.35 (d, C, J₁₋C₂ = 21.0 Hz), 154.10 (d, C, J₁₋C₂ = 94.5 Hz), 160.10 (N=CH), 162.24 (C=O).

**Ethyl 4-(2-fluoro-4-[(1H-indol-3-ylmethylene)amino]-phenyl)piperazine-1-carboxylate (4f)** The solution of compound 3 (10 mmol) in absolute ethanol was refluxed with indol-3-carbaldehyde (10 mmol) for 6 h. On cooling the reaction content to room temperature, a solid appeared. This crude product was filtered off and recrystallized from acetone. Yield: 82 %. M.p: 184–186 °C. FT-IR (KBr, ν, cm⁻¹): 3484 (NH), 1678 (C=O), 1439 (C=N), 1220 (C–O). Elemental analysis for C₂₃H₂₅FN₄O₄ calculated (%): C, 62.72; H, 5.72; N, 12.72. Found (%): C, 62.87; H, 5.98; N, 12.88. ¹H NMR (DMSO-d₆, δ ppm): 1.35 (t, 3H, CH₃, J = 8.0 Hz), 3.02 (brs, 4H, 2CH₂), 3.53 (s, 4H, 2CH₂), 4.06 (brs, 2H, CH₂), 7.29 (brs, 5H, arH), 8.08 (s, 1H, arH), 8.38 (s, 2H, arH), 9.06 (s, 1H, NH), 9.29 (s, 1H, NH). ¹³C NMR (DMSO-d₆, δ ppm): 15.11 (CH₃), 44.18 (CH₂), 50.76 (CH₂), 51.51 (2CH₂), 62.46 (CH₂), arC: [108.93 (d, CH, J₁₋C₂ = 23.4 Hz), 113.47 (d, CH, J₁₋C₂ = 34.4 Hz), 117.88 (CH), 118.82 (C), 120.71 (CH), 121.51 (CH), 121.84 (CH), 122.84 (CH), 123.76 (d, CH, J₁₋C₂ = 41.0 Hz), 124.87 (C), 137.91 (d, C, J₁₋C₂ = 19.8 Hz), 139.24 (2C) 155.26 (d, C, J₁₋C₂ = 4.0 Hz)], 153.18 (N=CH), 185.74 (C=O).

**Ethyl 4-[(benzylimino)carbonyl]amino]-2-fluorophenyl)piperazine-1-carboxylate (5)** The mixture of compound 3 (10 mmol) and benzylisothiocyanate (10 mmol) in absolute ethanol was refluxed for 10 h. On cooling the reaction mixture to room temperature, a solid formed. This crude product was collected by filtration and recrystallized from ethanol. Yield: 93 %. M.p: 153–155 °C. FT-IR (KBr, ν, cm⁻¹): 3346, 3284 (2NH), 3063 (ar–CH), 1694, 1638 (2C=O), 1236 (C–O). Elemental analysis for C₂₃H₂₃FN₃O₃ calculated (%): C, 62.99, H, 6.29; N, 13.99. Found (%): C, 62.78; H, 6.07; N, 14.04. ¹H NMR (DMSO-d₆, δ ppm): 1.17 (t, 3H, CH₃, J = 7.6 Hz), 2.85 (s, 4H, 2CH₂), 3.40 (s, 4H, 2CH₂ + H₂O), 4.02 (q, 2H, CH₂, J = 7.0 Hz), 4.26 (d, 2H, CH₂, J = 6.0 Hz), 6.61 (brs, 1H, NH), 6.95 (s, 2H, arH), 7.21–7.31 (m, 6H, arH), 8.62 (s, 1H, NH). ¹³C NMR (DMSO-d₆, δ ppm): 15.27 (CH₃), 41.39 (CH₂), 43.39 (CH₂), 44.15 (CH₂), 51.23 (CH₂), 60.45 (CH₂), 61.52 (CH₂), arC: [106.69 (d, CH, J₁₋C₂ = 25.6 Hz), 114.19 (CH), 120.59 (CH), 127.42 (CH), 127.79 (2CH), 128.99 (2CH), 133.98 (d, C, J₁₋C₂ = 9.55 Hz), 137.02 (d, C, J₁₋C₂ = 9.85 Hz), 140.98 (C), 156.65 (d, C, J₁₋C₂ = 137.5 Hz)], 155.83 (2C=O).
7.28 (brs, 8H, arH), 7.45 (s, 1H, arH). 13C NMR (DMSO-d6, δ ppm): 15.27 (CH3), 43.36 (2CH2), 44.14 (2CH2), 51.21 (CH3), 61.52 (CH2), 96.76 (CH), arC: [106.66 (d, CH, \( J_{C-F} = 25.6\) Hz), 114.13 (CH), 120.50 (CH), 124.20 (2CH), 124.97 (2CH), 127.38 (CH), 127.78 (2CH), 128.97 (2CH), 133.90 (d, C, \( J_{C-F} = 21.9\) Hz), 137.14 (d, C, \( J_{C-F} = 11.0\) Hz), 141.05 (2C), 155.28 (C), 156.63 (d, C, \( J_{C-F} = 240.5\) Hz)], 155.91 (C + C=O), 162.27 (C=N). MS m/z (%): 535.12 ([M+H]+), 14), 479.16 (100), 423.16 (97), 138.12 (50).

**Ethyl 4-[(4-[[2-ethoxy-2-oxoethyl]amino]-2-fluorophenyl]piperazine-1-carboxylate (8)** To the mixture of compound 3 (10 mmol) and triethylamine (10 mmol) in dry tetrahydrofuran, ethyl bromoacetate (10 mmol) was added drop by drop at 0–5 °C. Then, the reaction mixture was allowed to reach room temperature and stirred for 14 h (the progress of the reaction was monitored by TLC). The precipitated triethylammonium salt was removed by filtration and the resulting solution was evaporated under reduced pressure to dryness. The obtained yellow solid was recrystallized from ethanol:water (1:2). Yield: 50.2 %. M.p: 71–73 °C.

**Ethyl 4-[(4-[[2-fluoro-4-[[2-(2-hydrazinyl-2-oxoethyl)amino]phenyl]piperazine-1-carboxylate (9)** Hydrazine hydrate (25 mmol) was added to the solution of compound 8 (10 mmol) in ethanol and the mixture was heated under reflux for 14 h. On cooling the mixture in cold overnight, a white solid appeared. The crude product was filtered off and recrystallized from ethyl acetate. Yield: 85 %. M.p: 160–163 °C. FT-IR (KBr, ν, cm⁻¹): 3340, 3256, 3193 (4NH), 1697 (C=O), 1633 (C=O), 1286 (C=S). Elemental analysis for C_{22}H_{27}FN_{6}O_{3}S calculated (%): C, 56.25; H, 6.73; N, 22.29. Found (%): C, 56.12; H, 6.69; N, 22.25 (C=S). MS m/z (%): 535.12 ([M+H]+), 14), 479.16 (100), 423.16 (97), 138.12 (50).

Ethyl 4-(2-fluoro-4-[[2-((4-fluorophenyl)amino)carboxynitrobenzyl]hydrazino]-2-oxoethyl|amino|phenyl|piperazine-1-carboxylate (10)** The solution of compound 9 (10 mmol) in absolute ethanol was refluxed with 4-fluoro-phenylisothiocyanate (10 mmol) for 10 h. On cooling the reaction mixture to room temperature, an oily product appeared. This was recrystallized from butyl acetate: ethyl ether (1:2). Yield: 50 %. M.p: 78–80 °C. FT-IR (KBr, ν, cm⁻¹): 3225 (2NH + NH2), 1671 (C=O), 1210 (C=O). Elemental analysis for C_{22}H_{27}FN_{6}O_{3}S calculated (%): C, 53.66; H, 5.32; N, 17.06. Found (%): C, 53.78; H, 5.47; N, 17.14. 1H NMR (DMSO-d6, δ ppm): 1.19 (brs, 3H, CH3), 2.78 (s, 4H, 2CH2), 3.35 (s, 4H, 2CH2), 3.77 (s, 2H, CH2), 4.06 (brs, 2H, CH2), 5.91 (brs, 2H, 2NH), 6.35 (brs, 2H, arH), 6.83 (brs, 1H, arH), 7.17 (brs, 2H, arH), 7.39 (brs, 2H, arH), 9.56 (brs, 1H, NH), 9.69 (brs, 1H, NH), 10.08 (brs, 1H, NH).

13C NMR (DMSO-d6, δ ppm): 17.45 (CH3), 43.56 (CH2), 46.49 (CH2), 53.96 (2CH2), 63.67 (CH2), 67.10 (CH2), arC: [105.40 (d, CH, \( J_{C-F} = 40.1\) Hz), 114.19 (CH), 118.62 (d, CH, \( J_{C-F} = 36.6\) Hz), 121.70 (2CH), 124.54 (2CH), 128.55 (d, C, \( J_{C-F} = 36.4\) Hz), 140.19 (d, CH, \( J_{C-F} = 37.0\) Hz), 150.36 (d, C, \( J_{C-F} = 184.7\) Hz), 157.43 (2CH), 168.24 (C=O), 172.66 (C=O), 190.04 (C=S).
The precipitate formed was filtered off, washed with water, and recrystallized from dimethylsulfoxide:water (1:1). Yield: 74 %. M.p: 93–95 °C. FT-IR (KBr, ν cm⁻¹): 3257 (2NH), 1677 (C=O), 1433 (C≡N). Elemental analysis for C₂₂H₂₅F₂N₆O₂S calculated (%): C, 57.88; H, 5.52; N, 18.41. Found (%): C, 57.51; H, 5.45; N, 18.49. ¹H NMR (DMSO-δ, δ ppm): 1.18 (t, 3H, CH₃), 2.78 (brs, 4H, 2CH₂), 3.42 (s, 4H, 2CH₂), 3.99 (s, 4H, 2CH₂), 6.18 (d, 1H, arH), 7.40 (d, 1H, arH), 7.80 (m, 2H, arH). ¹³C NMR (DMSO-δ, δ ppm): 14.47 (CH₃), 42.36 (CH₃), 43.37 (CH₂), 45.14 (CH₂), 50.03 (CH₂), 50.92 (CH₂), 60.72 (CH₂), arC: [108.11 (d, CH, Jc-f = 12 Hz), 116.95 (d, CH, Jc-f = 19.4 Hz), 121.30 (d, CH, Jc-f = 33.3 Hz), 128.03 (CH), 128.75 (2CH), 128.96 (2CH), 129.53 (d, CH, Jc-f = 9.5 Hz), 140.52 (C), 144.63 (d, C, Jc-f = 10.6 Hz), 156.51 (d, C, Jc-F = 204.2 Hz), 160.77 (C), 164.32 (C), 169.87 (C=O). MS m/z (%): 458.16 ([M+Na]⁺, 27), 457.16 ([M+1]⁺, 100).

**Ethyl 4-(4-fluoro-5-thioxo-4,5-dihydro-1H-1,2,4-triazol-3-yl) methyl amino)piperazine-1-carboxylate (13)** A solution of compound 10 (10 mmol) in water was refluxed in the presence of 2 N NaOH for 3 h. Then, the resulting solution was cooled to room temperature and acidified to pH 7 with 37 % HCl. The precipitate formed was filtered off, washed with water, and recrystallized from ethyl acetate. Yield: 43 %. M.p: 206–208 °C. FT-IR (KBr, ν cm⁻¹): 3248, 3117 (2NH), 3049 (ar CH), 1660 (C=O), 1250 (C≡S). Elemental analysis for C₃₉H₅₁F₂N₉O₇S₂ calculated (%): C, 57.88; H, 5.52; N, 18.49. Found (%): C, 57.51; H, 5.45; N, 18.49. ¹H NMR (DMSO-δ, δ ppm): 1.13 (t, 3H, CH₃), 2.73 (s, 4H, 2CH₂), 3.42 (s, 4H, 2CH₂), 3.99 (s, 4H, 2CH₂), 6.25–6.32 (m, 2H, arH + NH), 6.76–6.80 (m, 1H, arH), 7.36 (s, 2H, arH–H), 7.49 (brs, 4H, ar–H), 10.45 (s, 1H, NH). ¹³C NMR (DMSO-δ, δ ppm): 15.25 (CH₃), 31.39 (CH₂), 44.27 (2CH₂), 51.68 (2CH₂), 61.49 (CH₂), arC: [101.27 (d, CH, Jc-f = 24 Hz), 108.63 (CH), 121.59 (CH), 128.76 (CH), 130.05 (2CH), 130.15 (2CH), 134.09 (2C), 145.50 (C), 150.92 (C), 155.25 (C), 168.75 (C≡S + C=O). MS m/z (%): 480.48 ([M+1 + Na]⁺, 29), 479.54 ([M+Na]⁺, 100), 457.41 ([M+1]⁺, 85).

**Ethyl 4-(4-fluoro-5-thioxo-4,5-dihydro-1H-1,2,4-triazol-3-yl) methyl amino)piperazine-1-carboxylate (14)** A solution of compound 11 (10 mmol) in ethanol water (1:1) was refluxed in the presence of 2 N NaOH for 3 h. Then, the resulting solution was cooled to room temperature and acidified to pH 7 with 37 % HCl. The precipitate formed was filtered off, washed with water, and recrystallized from ethyl acetate. Yield 70 %. M.p: 206–208 °C. FT-IR (KBr, ν cm⁻¹): 3248, 3117 (2NH), 3049 (ar CH), 1660 (C=O), 1250 (C≡S). Elemental analysis for C₃₉H₅₁F₂N₉O₇S₂ calculated (%): C, 57.88; H, 5.52; N, 18.49. ¹H NMR (DMSO-δ, δ ppm): 1.13 (t, 3H, CH₃), 2.73 (s, 4H, 2CH₂), 3.42 (s, 4H, 2CH₂), 3.99 (s, 4H, 2CH₂), 6.25–6.32 (m, 2H, arH + NH), 6.76–6.80 (m, 1H, arH), 7.36 (s, 2H, arH–H), 7.49 (brs, 4H, ar–H), 10.45 (s, 1H, NH). ¹³C NMR (DMSO-δ, δ ppm): 15.25 (CH₃), 31.39 (CH₂), 44.27 (2CH₂), 51.68 (2CH₂), 61.49 (CH₂), arC: [101.27 (d, CH, Jc-f = 24 Hz), 108.63 (CH), 121.59 (CH), 128.76 (CH), 130.05 (2CH), 130.15 (2CH), 134.09 (2C), 145.50 (C), 150.92 (C), 155.25 (C), 168.75 (C≡S + C=O). MS m/z (%): 480.48 ([M+1 + Na]⁺, 29), 479.54 ([M+Na]⁺, 100), 457.41 ([M+1]⁺, 85).
4-phenyl-5-thioxo-4,5-dihydr-o-1H-1,2,4-triazol-1-yl)methyl}amide was refluxed in the presence of dried sodium acetate (50 mmol) for 12 h. After removing the solvent under reduced pressure, an orange solid appeared. This product was recrystallized from ethyl acetate:hexane (1:2). Yield: 41%. M.p: 64–66 °C. FT-IR (KBr, cm⁻¹): 3662 (OH), 3374 (NH), 2988, 2901 (aliphatic CH), 1762 (C=O), 1687 (C=O), 1629 (C=O), 1227 (C=S). Elemental analysis for C_{37}H_{52}F_{12}O_{5}N_{8}S_{2} calculated (%): C, 55.63; H, 6.22; N, 14.97. Found (%): C, 55.87; H, 6.33; N, 15.05. \(^1\)H NMR (DMSO-d_6, δ ppm): 1.11 (t, 12H, 4CH_3), 1.99 (s, 3H, CH_3), 2.99 (q, 8H, 4CH_2), 7.38 (brs, 10H, 5CH_2), 4.55 (s, 2H, CH_2), 4.68–4.80 (m, 4H, 2CH_2), 5.40 (s, 2H, CH), 6.22 (brs, 2H, 2NH), 7.33 (brs, 3H, ar–H), 7.50–7.75 (m, 5H, ar–H). \(^1\)C-NMR (DMSO-d_6, δ ppm): 9.31 (3CH_3), 15.22 (CH_3), 27.13 (2CH_3), 43.49 (2CH_2), 44.96 (2CH_2), 50.58 (CH_2), 50.70 (3CH_2), 50.94 (2CH_2), 60.75 (C(CH_3)_2), 70.39 (CH), 73.89 (CH), 81.90 (CH), arC: [100.44 (d, CH, J = 24.1 Hz), 108.87 (d, CH, J = 213.1 Hz), 120.53 (d, CH, J = 60.2 Hz), 128.18 (CH), 129.57 (2CH), 129.64 (2CH), 133.79 (d, CH, J = 14.9 Hz), 144.08 (d, CH, J = 99.5 Hz), 146.84 (d, CH, J = 442.1 Hz)], 149.26 (C), 154.53 (C), 156.88 (C=S), 167.90 (C=O), 168.09 (C=O), 170.16 (C=O).

**Ethyl 4-[4-(3-[2-[4-(chlorophenyl)-3-phenyl-1,3-thiazol-2(3H)-ylidene]hydrazino]-3-oxoethyl]amino]methyl]carboxylate** (18) The mixture of compound 11 (10 mmol) and 4-chlorophenacylbromide (10 mmol) in absolute ethanol was refluxed in the presence of dried sodium acetate (50 mmol) for 12 h. After removing the solvent under reduced pressure, an orange solid appeared. This product was recrystallized from ethyl acetate:hexane (1:2). Yield: 45%. M.p: 60–62 °C. FT-IR (KBr, cm⁻¹): 3345, 3259 (2NH), 3054 (ar–CH), 1677 (C=O), 1628 (C=O). Elemental analysis for C_{37}H_{52}ClF_{12}O_{5}N_{8}S_{2} calculated (%): C, 59.15; H, 4.96; N, 13.80. Found (%): C, 59.05; H, 5.06; N, 13.87. \(^1\)H NMR (DMSO-d_6, δ ppm): 1.15 (brs, 3H, CH_3), 2.76 (s, 4H, 2CH_2), 3.61 (s, 6H, 3CH_2 + H_2O), 4.03 (brs, 2H, 2CH_2), 5.40 (s, 1H, NH), 6.44–6.54 (m, 1H, arH), 6.84–6.96 (m, 2H, arH + CH), 7.29–7.52 (m, 9H, arH), 7.95 (s, 1H, arH), 10.45 (s, 1H, NH). \(^1\)C-NMR (DMSO-d_6, δ ppm): 15.24 (CH_3), 41.37 (CH_3), 44.26 (CH_2), 51.68 (CH_2), 52.46 (2CH_2), 61.48 (CH_2), arC: [101.24 (d, CH, J = 24.5 Hz), 108.66 (CH), 117.54 (2CH), 120.12 (C), 121.75 (2CH), 122.41 (2CH), 128.76 (2CH), 129.71 (2CH), 130.37 (2CH), 130.76 (2CH), 131.82 (C), 133.37 (2C), 146.87 (d, C, J = 133.95 Hz)], 155.26 (C=N), 158.92 (C=O), 160.62 (C=O). MS m/z (%): 631.64 ([M – 1 + Na]^+), 25, 464.59 (26), 463.58 (83), 441.62 (26), 360.57 (61), 267.31 (29), 195.00 (40), 149.00 (100), 135.03 (50), 121.06 (65).

**Ethyl 4-[2-fluoro-4-[2-[2-(3-hydroxy-4-methoxybenzylidene)hydrazino]-2-oxoethyl] amino]methyl]carboxylate** (19a) The mixture of solution of compound 9 (10 mmol) and 3-hydroxy-4-methoxybenzaldehyde (10 mmol) in absolute ethanol was irradiated with microwave at 200 W and 140 °C for 30 min. On cooling the
reaction mixture to room temperature a solid was appeared. This crude product was recrystallized from ethanol. Yield: 72%. M.p: 183–185 °C. FT-IR (KBr, v, cm⁻¹): 3342, 3181 (2NH), 3096 (ar–CH), 1678 (2C=O), 1437 (C=N), 1211 (C–O). Elemental analysis for C₃₃H₃₃FN₃O₅ calculated (%): C, 58.34; H, 5.96; N, 14.79. Found (%): C, 58.65; H, 6.06; N, 14.98. ¹H NMR (DMSO-d₆, δ ppm): 1.17 (t, 3H, CH₃, J = 6.8 Hz), 2.77 (s, 4H, 2CH₂), 3.36 (s, 6H, 3CH₃), 3.78 (s, 3H, O–CH₃), 3.99 (q, 2H, CH₂, J = 6.6 Hz), 5.80 (brs, 1H, NH), 6.04 (brs, 1H, NH), 6.32–6.37 (m, 3H, arH), 6.84–6.98 (m, 3H, arH), 9.27 (s, 1H, N=CH), 108.47 (CH), 112.58 (d, CH, J₁ = 167.17 (C=O), 171.66 (C=O). MS calculated (%): 497.56 ([M+Na]+), 31) 496.56 ([M+Na]⁺,100), 370.41 (19), 360.65 (22).

**Table 1** Screening for antimicrobial activity of the compounds (50 μL)

| Comp. no | Microorganisms and inhibition zone (mm) |
|----------|----------------------------------------|
|          | Ec | Yp | Pa | Sa | Ef | Bc | Ms | Ca | Sc |
| 2        | –  | –  | –  | –  | –  | –  | –  | –  | –  |
| 3        | –  | –  | 11 | –  | 6  | –  | 15 | 15 | –  |
| 4a       | 8  | 8  | –  | –  | –  | 10 | 8  | 8  | –  |
| 4b       | –  | –  | –  | –  | –  | –  | –  | –  | –  |
| 4c       | –  | –  | –  | –  | –  | –  | –  | –  | 8  |
| 4d       | 6  | 6  | –  | –  | –  | 8  | 20 | 15 | 15 |
| 4e       | –  | –  | –  | –  | –  | –  | –  | –  | 20 |
| 4f       | 8  | 8  | 6  | 6  | 6  | 25 | 20 | 10 | 10 |
| 5        | –  | –  | –  | –  | –  | –  | 6  | 7  | –  |
| 6        | –  | –  | –  | –  | –  | –  | –  | –  | –  |
| 7        | –  | –  | –  | –  | –  | –  | –  | –  | –  |
| 8        | –  | –  | –  | –  | –  | 6  | –  | –  | –  |
| 9        | –  | –  | –  | –  | –  | 6  | 7  | –  | –  |
| 10       | –  | –  | –  | –  | –  | 6  | –  | –  | –  |
| 11       | –  | –  | 10 | –  | 6  | –  | –  | –  | –  |
| 12       | –  | –  | –  | –  | –  | 6  | 6  | –  | –  |
| 13       | –  | –  | 6  | –  | –  | 8  | 10 | –  | –  |
| 14       | –  | –  | 6  | 6  | –  | –  | –  | 10 | –  |
| 15       | –  | 6  | 6  | 6  | –  | –  | –  | –  | –  |
| 16       | 8  | –  | 6  | 10 | –  | –  | –  | 6  | 10 |
| 17       | 9  | 9  | 8  | 13 | –  | 16 | 14 | 6  | 12 |
| 18       | –  | –  | 6  | 10 | 6  | –  | 8  | 12 | –  |
| 19a      | –  | –  | 6  | –  | 8  | –  | 9  | 6  | –  |
| 19b      | –  | –  | –  | –  | –  | –  | –  | –  | 8  |
| 19c      | –  | –  | 6  | –  | 8  | –  | 8  | 6  | –  |
| 20       | –  | –  | 10 | 6  | 6  | 15 | 8  | 12 | –  |
| 21       | 8  | 8  | –  | 6  | 10 | 10 | 20 | 10 | 8  |
| 22       | 9  | 8  | 15 | 9  | 10 | 18 | 8  | 12 | –  |
| Amp.     | 10 | 18 | 18 | 35 | 10 | 15 | –  | –  | –  |
| Flu.     | 35 | –  | –  | –  | –  | –  | –  | –  | –  |

Strep. 25 >25

**Table 2** Inhibitory activities of the synthesized compounds against Jack Bean urease

| Compound | % Inhibition ± S.D. | IC₅₀ ± S.D. |
|----------|---------------------|------------|
| Thourea  | 100 ± 0.1 ±         | 54.56 ± 4.17 |
| 2        | a                   | b          |
| 3        | 11 ± 3.3            | –          |
| 4a       | N.s.                | –          |
| 4b       | N.s.                | –          |
| 4d       | –                   | –          |
| 4e       | 1 ± 0.2             | –          |
| 4f       | –                   | –          |
| 5        | –                   | –          |
| 6        | 3 ± 3.0             | –          |
| 7        | N.s.                | –          |
| 8        | 7 ± 3.1             | –          |
| 9        | 7 ± 3.0             | –          |
| 10       | 4 ± 1               | –          |
| 12       | 56 ± 4              | –          |
| 14       | –                   | –          |
| 15       | 100 ± 1.5           | 4.67 ± 0.53 |
| 17       | 100 ± 2.1           | 45.37 ± 0.78 |
| 18       | –                   | –          |
| 19a      | –                   | –          |
| 19b      | 47 ± 0.1            | –          |
| 19c      | –                   | –          |
| 20       | N.s.                | –          |

N.s. Not soluble

* No inhibition
* Not determined

65; H, 6.06; N, 14.98. ¹H NMR (DMSO-d₆, δ ppm): 1.17 (t, 3H, CH₃, J = 6.8 Hz), 2.77 (s, 4H, 2CH₂), 3.36 (s, 6H, 3CH₃), 3.78 (s, 3H, O–CH₃), 3.99 (q, 2H, CH₂, J = 6.6 Hz), 5.80 (brs, 1H, NH), 6.04 (brs, 1H, NH), 6.32–6.37 (m, 3H, arH), 6.84–6.98 (m, 3H, arH), 9.27 (s, 1H, N=CH), 11.35 (s, 1H, OH). ¹³C NMR (DMSO-d₆, δ ppm): 15.26 (CH₃), 44.29 (CH₂), 44.62 (2CH₂), 51.78 (2CH₂), 56.22 (OCH₃), 61.48 (CH₂), arC: [101.23 (d, CH, J_C=Fe = 22.0 Hz), 108.47 (CH), 112.58 (d, CH, J_C=Fe = 15.0 Hz), 120.73 (CH), 120.96 (CH), 121.72 (CH), 127.64 (C), 129.83 (d, C, J_C=Fe = 9.1 Hz), 146.25 (C), 146.46 (C), 150.34 (d, C, J_C=Fe = 6.5 Hz), 151.36 (d, C, J_C=Fe = 388.7 Hz)], 144.44 (N=CH), 167.17 (C=O), 171.66 (C=O), MS m/z (%): 497.56 ([M+1 + Na]+), 31) 496.56 ([M+Na]+,100), 370.41 (19), 360.65 (22).

**Ethyl 4-[2-fluoro-4-[[2-oxo-2-[2-(pyridin-4-ylmethylene) hydrazinol]ethyl]amino]phenyl] piperazine-1-carboxylate (19b)** The mixture of compound 9 (10 mmol) and
2-hydroxybenzaldehyde (10 mmol) in absolute ethanol was irradiated by microwave at 200 W and 140 °C for 30 min. On cooling the reaction mixture to room temperature a solid was appeared. This crude product was recrystallized from ethanol. Yield: 85 %. M.p: 155–157 °C. FT-IR (KBr, ν, cm⁻¹): 3675 (OH), 3537, 3270 (2NH), 3059 (ar–CH), 1707, 1676 (2C=O), 1428 (C=N), 1230 (C–O). Elemental analysis for C_{22}H_{26}FN_{4}O_{3} calculated (%): C, 58.58; H, 5.91; N, 15.79. Found (%): C, 59.72; H, 6.16; N, 15.77. ¹H NMR (DMSO-d₆, δ ppm): 1.17 (brs, 3H, CH₃), 2.78 (s, 4H, 2CH₂), 3.45 (s, 6H, 3CH₂), 4.02–4.03 (m, 2H, CH₂), 6.39 (brs, 2H, 2NH), 6.85 (brs, 4H, arH), 7.41 (brs, 3H, arH), 8.70 (s, 1H, N=CH), 10.56 (brs, 1H, OH). ¹³C NMR (DMSO-d₆) ppm): 15.25 (CH₃), 41.29 (CH₂), 44.18 (2CH₂), 51.51 (CH₂), 61.52 (CH₂) arC: [108.24 (CH), 116.79 (d, CH, J_{C–F} = 36.2 Hz), 119.18 (C), 120.18 (CH), 122.19 (d, CH, J_{C–F} = 53.4 Hz), 126.61 (CH), 131.22 (CH), 132.68 (CH), 137.00 (C), 141.26 (d, c, J_{C–F} = 10.6 Hz), 152.71 (d, c, J_{C–F} = 252.9 Hz), 157.86 (C)], 146.15 (N=CH), 159.33 (C=O), 163.12 (C=O). MS m/z (%): 466.51 ([M+Na]⁺, 16), 444.55 ([M+Na]⁺, 25), 249.20 (19), 241.19 (18), 149.03 (100), 135.07 (33), 121.06 (45), 103.04 (40).

Ethyl 4-(2-fluoro-4-(5-thioxo-1,3,4-oxadiazol-2-yl)methylamino)phenyl piperazine-1-carboxylate (20) The mixture of compound 9 (10 mmol) and carbon disulfide (20 mmol) in absolute ethanol was refluxed in the presence of dried potassium hydroxide (10 mmol) for 13 h. Then, the resulting solution was cooled to room temperature and acidified with acetic acid. The precipitate formed was filtered off, washed with water, and recrystallized from ethyl acetate/petroleum ether (1:3) Yield 68 %. M.p: 210–212 °C. FT-IR (KBr, ν, cm⁻¹): 3300 (2NH), 1675 (C=O), 1237 (C=S). Elemental analysis for C_{16}H_{20}FN_{5}O_{3}S calculated (%): C, 50.38; H, 5.29; N, 18.36. Found (%): C, 50.51; H, 5.66; N, 18.74. ¹H NMR (DMSO-d₆, δ ppm): 1.17 (t, 3H, CH₃, J = 6.6 Hz), 2.77 (s, 4H, 2CH₂), 3.47 (s, 2H, CH₂), 4.03 (q, 2H, CH₂, J = 7.0 Hz), 4.34 (d, 2H, CH₂, J = 5.0 Hz), 6.33–6.52 (m, 4H, ar-2H + 2NH), 6.85 (t, 1H, arH, J = 8.6 Hz). ¹³C NMR (DMSO-d₆, δ ppm): 15.25 (CH₃), 41.35 (2CH₂), 44.25 (2CH₂), 51.64 (CH₂), 61.50 (CH₂) arC: [101.41 (d, CH, J_{C–F} = 24.1 Hz), 108.78 (CH), 121.78 (CH), 130.67 (d, c, J_{C–F} = 9.9 Hz), 144.97 (d, c, J_{C–F} = 10.6 Hz), 156.95 (d, c, J_{C–F} = 241.9 Hz), 155.28 (C=O), 163.00 (C), 185 (C= S).

\[(\text{6R,7R})-3-\{\text{Acetyl}[\text{methyl}]-7-\{\{5-[4-(4-\{(\text{ethoxy-}
\text{carbonyl})\text{piperazin-1-yl})\text{-3-fluorophenyl}][\text{amino}]\text{methyl}\}
\text{2-thioxo-1,3,4-oxadiazol-3(2H)-yl}][\text{methyl}][\text{amino}]-8-\text{o xo-5-th ia-1-azabicyclo[4.2.0]oct-2-en-2-yl}][\text{carbonyl}][\text{oxyl}][\text{triethyl}]
\text{ammonium} (21)\]

To the mixture of compound 20 (10 mmol), triethylamine (20 mmol) and formaldehyde

| Table 3 Porcine pancreatic lipase inhibitory activity of synthesized compounds |
|-------------------------------|-------------------|
| Compound no. | % Inhibition |
| 2 | – |
| 3 | – |
| 5 | – |
| 6 | 16 |
| 8 | 22 |
| 9 | 20 |
| 10 | – |
| 11 | – |
| 12 | 68 |
| 13 | 63 |
| 14 | 75 |
| 15 | 73 |
| 16 | 6 |
| 17 | – |
| 18 | 1 |
| 19a | – |
| 19b | – |
| 19c | – |
| 20 | 33 |
| Orlistat | 99 |
| DMSO control | – |
| Positive control | – |

All compounds were screened at concentration of 6.25 μg mL⁻¹.
(50 mmol) in tetrahydrofurane, 7-aca (10 mmol) was added. The mixture was stirred at room temperature 4 h. After removing the solvent under reduced pressure, a liquid product appeared. This was recrystallized by column chromatography (n-hexane:ethyl acetate, 4:1). Yield 58 %. FT-IR (KBr, v, cm⁻¹): 3373 (OH + NH), 2980, 2974 (aliphatic CH), 1676 (C=O), 1432 (C=N), 1232 (C=S). Elemental analysis for C₃₃H₄₇FN₈O₈S₂ calculated (%): C, 52.38; H, 6.18; N, 14.61. Found (%): C, 51.47; H, 6.00; N, 14.67. 1H-NMR (DMSO-d₆) δ ppm: 3.82 (brs, 8H, 4CH₂), 4.00 (s, 2H, CH₂), 4.56 (s, 2H, CH₂), 4.65 (s, 1H, CH), 6.40 (brs, 2H, 2NH), 6.80 (d, 2H, CH₂), 67.38 (CH₂), 67.73 (CH), 70.89 (CH), arC: [107.63 ppm: 1.12 (t, 2H, 2CH, J = 7.0 Hz)]; 1.99 (s, 3H, CH₃); 2.98–3.18 (m, 12H, 6CH₂); 3.82 (brs, 8H, 4CH₂); 4.00 (s, 2H, CH₂); 4.56 (s, 2H, CH₂); 6.40 (brs, 2H, 2NH); 6.80 (d, 2H, CH₂). 13C-NMR (DMSO-d₆) δ ppm: 24.98 (CH₂), 30.72 (CH₂), 61.52 (CH₂), 62.23 (CH₂), 64.97 (CH₂), 65.39 (CH), 67.00 (CH), arC: [107.26 ppm: 0.99–1.21 (m, 18H, 6CH₃); 2.98 (q, 8H, 4CH₂, J = 9.5 Hz); 3.38 (q, 8H, 4CH₂, J = 7.0 Hz)].

**Antimicrobial activity assessment**

All bacterial and yeast strains were obtained from the Hifızisilhha Institute of Refik Saydam (Ankara, Turkey) and were as follows: *Pseudomonas aeruginosa* ATCC 27853, *Enterococcus faecalis* ATCC 29212, *S. aureus* ATCC 25923, *B. cereus* 709 ROMA, *M. smegmatis* ATCC607, *C. albicans* ATCC 60193, Sc: *S. cerevisiae* RSK2 251. All the newly synthesized compounds were dissolved in dimethyl sulfoxide (DMSO) and ethanol to prepare chemicals of stock solution of 10 mg mL⁻¹.

**Agar-well diffusion method**

Simple susceptibility screening test using agar-well diffusion method as adapted earlier (Ahmad et al., 1998) was used. Each microorganism was suspended in Mueller–Hinton (MH) (Difco, Detroit, MI, USA) broth and diluted approximately to 106 colony forming unit (cfu) mL⁻¹. They were “flood-inoculated” onto the surface of MH agar and Sabouraud dextrose agar (SDA) (Difco, Detroit, MI, USA) and then dried. For *C. albicans* and *C. tropicalis*, SDA were used. Five-millimeter diameter wells were cut from the agar using a sterile cork-borer, and 50 mL of the extract substances was delivered into the wells. The plates were incubated for 18 h at 35 °C. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organism. Ampicillin (10 mg) and Fluconazole (5 mg) were used as standard drugs. Dimethyl sulfoxide and ethanol were used as solvent controls. The antimicrobial activity results are summarized in Table 1.

**Urease inhibition assay**

Reaction mixtures comprising 25 μL of Jack bean urease, 55 μL of buffer (100 mM urea, 0.01 M K₂HPO₄, 1 mM EDTA, and 0.01 M LiCl, pH 8.2), and 100 mM urea were incubated with 5 μL of the test compounds at room temperature for 15 min in microtiter plates. The production of ammonia was measured by indophenol method and used to determine the urease inhibitory activity. The phenol reagent (45 μL, 1 % w/v phenol, and 0.005 % w/v sodium nitroprusside) and alkali reagent (70 μL, 0.5 % w/v sodium hydroxide, and 0.1 % v/v NaOCl) were added to each well and the increasing absorbance at 625 nm was measured after 20 min, using a microplate reader (Molecular Device, USA). The percentage inhibition was calculated from the formula: 100 × (OD test well/OD control) × 100. Thiourea was used as the standard inhibitor. In order to calculate IC₅₀ values, different concentrations of synthesized compounds and standard were assayed at the same reaction conditions (Weatherburn, 1967). The obtained results are presented in Table 2.
Anti-lipase activity assay

The inhibitory effects of those compounds were evaluated against porcine pancreatic lipase (PPL) (15 ng mL\(^{-1}\)). Lipase activity assay was done according to Verger et al., (Woods et al., 2003). Microtiter plates were coated with purified tung oil TAGs. Compounds were mixed with PPL 1:2 (v/v) and incubated for 30 min. The microtiter plates containing purified tung oil, lipase solution, and assay buffer (10 mM Tris–HCl buffer, pH 8.0, containing 150 mM NaCl, 6 mM CaCl\(_2\), 1 mM EDTA, and 3 mg mL\(^{-1}\)ß-cyclodextrin) were recorded continuously for 40 min against the buffer alone by using microplate reader (SpectraMax M5, Molecular Devices) at 272 nm. The inhibitory activity of those compounds and Orlistat, a positive control against pancreatic lipase, were measured at 40 min against the buffer alone by using microplate reader (SpectraMax M5, Molecular Devices) at 272 nm. The inhibitory activity of those compounds and Orlistat, a positive control against pancreatic lipase, were measured at 40 min against the buffer alone by using microplate reader (SpectraMax M5, Molecular Devices) at 272 nm.

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