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Design, Synthesis and Pharmacological Evaluation of Sulfanilamide-Ciprofloxacin Conjugates Utilizing Hybridization Approach as New Antibacterial Agents

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Abstract
A group of novel antibacterial agents (I-V) were designed and synthesized, by utilizing hybridization approach between ciprofloxacin and sulfanilamide, through metabolically stable linkers, to be acted by dual mode of actions. Ciprofloxacin acts by inhibition of topoisomerase enzyme, which is necessary for DNA replication, while sulfonamides act through inhibition of carbonic anhydrase enzyme, which is necessary for bacterial metabolic activity. Anti-tuberculosis activity of these compounds was evaluated on MDR Mycobacterium tuberculosis (resist to rifampicin and INH), in a dose equivalent to (10 mg/ 5 ml D.W.) of ciprofloxacin. Compounds II, III showed non-significant reduction in the number of bacterial colonies (bacterial growth) with respect to the effect of ciprofloxacin (standard), compound IV produce a significant reduction in the number of bacterial colonies in comparable to ciprofloxacin. Moreover, compounds I and V exhibited highly significant reduction in the number of bacterial colonies compared to ciprofloxacin. The results of this study indicate that the hybridization approach between the ciprofloxacin and sulfanilamide will give superior anti-tubercular activity in comparison to ciprofloxacin, when they are linked through amide linkage directly or through incorporation of thiadiazol and triazole derivatives through disulfide bond. This will encourage further evaluation of these compounds to demonstrate or identify their selectivity toward β-CA enzyme.
Keywords: Anti-tuberculosis activity; ciprofloxacin; sulfanilamide; hybridization approach; carbonic anhydrase; and topoisomerase.

1. Introduction

Infectious disease is among the most deadly afflictions plaguing human societies. The World Health Organization (WHO) has estimated that, in the year 2004, five major infectious diseases caused almost 11 million of the 59 million total deaths worldwide. Among the infectious diseases, the most common causes of mortality worldwide included tuberculosis (1.46 million) (David et al., 2009). Infectious microbial diseases remain a pressing problem worldwide, because microbes have resisted prophylaxis or therapy longer than any other form of life (Mohammad Asif et al., 2012). The only way to treat infectious diseases is by chemotherapy using antimicrobial agents and antibiotics. Antimicrobial agents have been used for 70 years in the treatment of infectious diseases. These agents have great potential to reduce illnesses and treat affected patients. Since their first introduction, the use of antibiotics has become widespread around the world because of their significant effectiveness on microbial agents (Adil et al., 2013). However, due to their uncontrolled and unnecessary use, bacteria have evolved resistance against several antibiotics (Cunha et al., 2000). Antimicrobial resistance is not new, but the number of resistant organisms, the geographic locations affected by drug resistance, and the breadth of resistance in single organisms are unprecedented and mounting (Levy et al., 2004). *Mycobacterium tuberculosis* infection is one of the worst examples, as multi-drug resistant and extensively multi-drug resistant tuberculosis (TB) is present in many countries (Fabrizio et al., 2009). The quinolones first represented by nalidixic acid and more recently by the newer fluoroquinolones, initiate bactericidal actions by trapping the covalent protein-DNA complexes formed by bacterial DNA gyrase or topoisomerase IV (Tse-Dinh et al., 2007). During the past decade, several of the fluoroquinolones antibacterial drugs have been examined as potential chemotherapeutics for *M. tuberculosis* infection because of their favorable pharmacokinetic profiles such as easily absorbed after oral administration and readily penetrated into mammalian cells (Yue-Ling et al., 2005). Fluoroquinolones (FQs) are important class of antibiotics used for the treatment of MDR-TB, and are now being represented as first line antibiotics to shorten the duration of treatment of tuberculosis, e.g. are ciprofloxacin (1), gatifloxacin (2), ofloxacin (3), sparfloxacin (4) (Mradula et al., 2009).

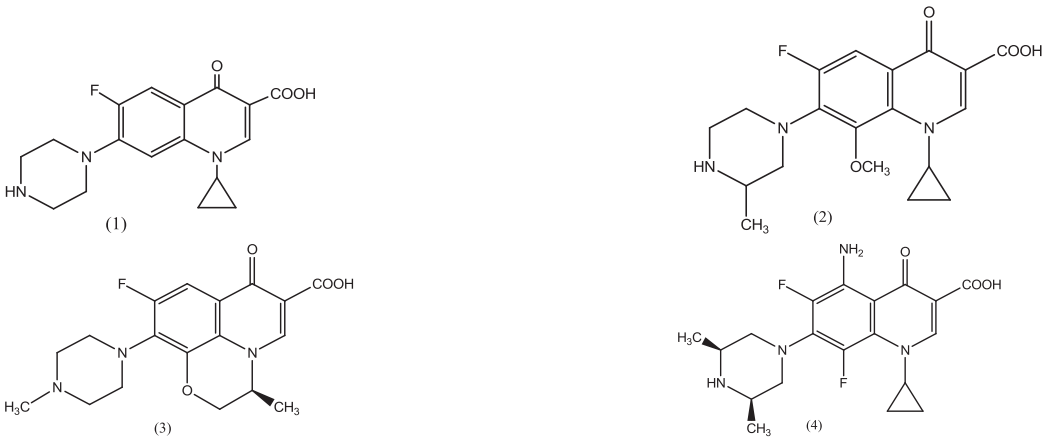


Figure (1) Common selected anti-TB drugs

To be effective against circulating MDR and XDR-TB strain, new TB drugs will need to have novel mechanisms of actions (Ginsberg et al., 2008). Whilst classic screening methods and chemical modification of known antimicrobial agents continue to produce potential leads for new antimicrobial agents, a number of other approaches are being investigated. These include the search for potentiators of the activity of known antimicrobial agents and the development of hybrid agents, novel membrane active drugs, and inhibitors of bacterial virulence and pathogenesis. The idea of chemically fusing two antimicrobials with different mechanisms or an antimicrobial agent with a potentiating entity has been the subject of study for several decades (Joohee et al., 2012). Widely spread human pathogen *Mycobacterium tuberculosis* contains three β-carbonic anhydrase (CA) genes in its genome (Fabrizio et al., 2009). The β-CAs have a notably different zinc ligation comprising one histidine and two cysteines with the fourth coordination position either occupied by a water molecule or an aspartyl side-chain (Suarez et al 2005). The catalytic activity and inhibition studies with a range of sulfonamides and one sulfamate of two of these enzymes have been recently reported (Minakuchi et al., 2009). Given that β -CA genes appear to be missing in invertebrates, novel antimicrobial compounds based on the inhibition of β -CAs from pathogenic organisms may soon become available (Leo et al., 2010).

2. Results and Discussion

Three types of linking between ciprofloxacin and sulfanilamide have been utilized in the hybridization approach, these include: a) Direct linking between the ciprofloxacin and sulfanilamide through amide bond, by using chloroacetyl chloride as linking agent, as shown in compound I, b) Linking between ciprofloxacin and sulfanilamide through amide bond, with incorporation of triazole and thiadiazole derivatives, as shown in compounds II and III, respectively, and c) Linking between ciprofloxacin and sulfanilamide through utilizing tertiary amide and disulfide bonds, with incorporation of triazole and thiadiazole derivatives, as shown in compounds IV and V, respectively. Structures of these compounds were confirmed, using elemental microanalysis (CHN), infrared spectroscopy (IR), ^{1}H-NMR spectroscopy, and some physicochemical properties, which are listed with each one of these compounds (I-V), and their synthetic procedures were described in schemes (1, 2, and 3).

Many methods have been used to evaluate the Anti-TB drug susceptibility, these involved, absolute concentration method, the proportion method, and the radiometric BACTEC method. In this study, the proportion method was adopted to evaluate the anti-TB activity of the synthesized compounds. The bacterial suspension was inoculated on Lowenstein-Jensen (LJ) medium, on which the synthesized compounds were incorporated before inspissation (Kam et al., 2010). To assess the validity of the proportion method used for the evaluation of newly synthesized anti-TB compounds, ciprofloxacin was used as a reference compound of known anti-TB activity profile.

Table (1) showed the effect of the synthesized tested compounds (I-V) on the Mycobacterium growth in six patients, which were documented with MDR-TB (resist to both rifampicin and INH). Compounds I and V showed highly significant anti-mycobacterium activity ($p \leq 0.01$) compared to the standard (ciprofloxacin). Compounds II and III showed non significant difference in anti-mycobacterium activity ($p \leq 0.05$) compared to ciprofloxacin, while compound IV showed a significant differences in anti-mycobacterium activity ($p \leq 0.05$) compared to ciprofloxacin. So; compounds I and V produced the superior anti-mycobacterium activity compared to ciprofloxacin (standard). As shown in Figure (2).

The differences in the activities of the synthesized compounds is attributed to the variable types of linkers between the ciprofloxacin and the sulfonamide derivatives, which affected the orientation of the compounds in different sub-pockets of the active site cavity of the target enzymes, and therefore the binding with

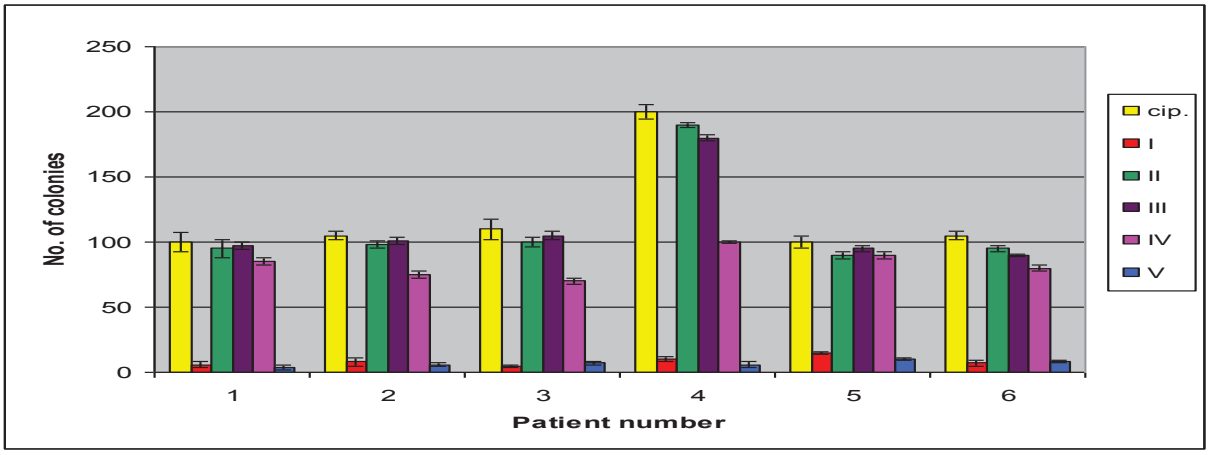
the target sites, leading to different affinities, selectivities and pharmacological properties. The flexibility and different chemical nature of the linker between the two different scaffolds give the significant differences in the anti-mycobacterial activities (Fabio et al., 2010).

Table (1): Effect of tested synthesized compounds (I-V) on Mycobacterium growth.

	Treatment groups						
	Patient number	ciprofloxacin	Compound I	Compound II	Compound III	Compound IV	Compound V
No. of colonies	1	100±7	6±2**[a]	95±7[b]	97±3[b]	85±3*[c]	4±2**[a]
	2	105±3	8±3**[a]	98±3[b]	101±3[b]	75±3*[c]	6±1**[a]
	3	110±8	5±1**[a]	100±4[b]	105±3[b]	70±2*[c]	7±1**[a]
	4	200±6	10±2**[a]	190±2[b]	180±2[b]	100±1*[c]	6±2**[a]
	5	100±5	15±1**[a]	90±3[b]	95±2[b]	90±3*[c]	10±1**[a]
	6	105±3	7±2**[a]	95±2[b]	90±1[b]	80±2*[c]	8±1**[a]

Data are expressed as numbers of colonies ± SEM. * significantly different compared to reference (ciprofloxacin) ($p \leq 0.05$). **High significant differences compared to reference ($p \leq 0.01$). Non-identical superscripts (a, b, and c) among different groups are considered significantly different ($p \leq 0.05$).

Fig. (2): Effect of ciprofloxacin (reference), and synthesized compounds (I-V) on Mycobacterium growth in six patients.



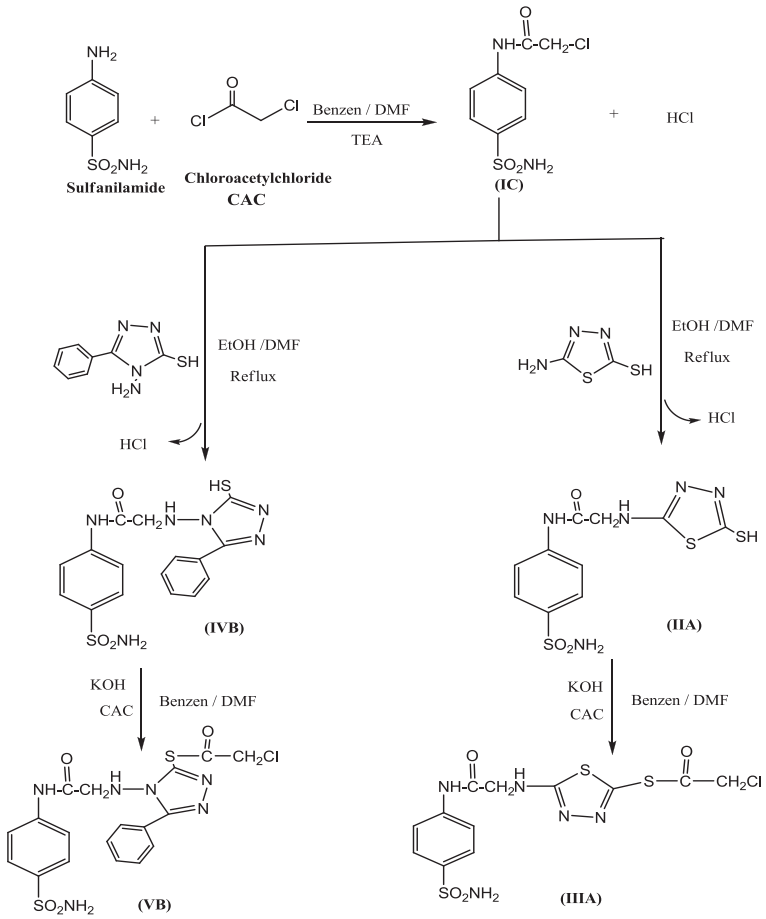
3. Experimental

3.1. General

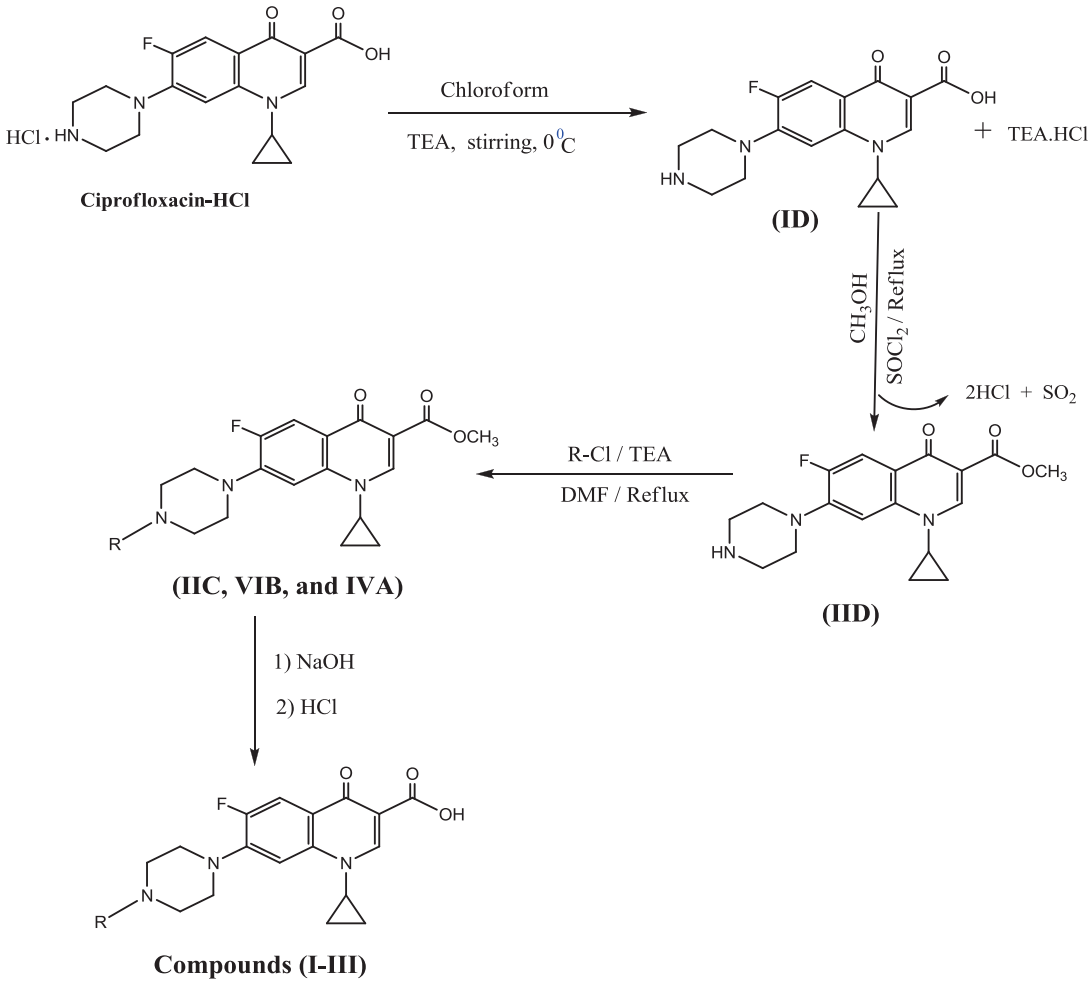
All reagents and anhydrous solvents were of annular type and generally used as received from the commercial suppliers (Merck, Germany, Reidel-De Haen, Germany, Sigma-Aldrich, Germany and BDH, England). Ciprofloxacin was supplied by the *Hefei joye import export co.Ltd*. China. Melting points were determined by capillary method on Bamstead/Electrothermal 9100 an Electric melting point apparatus (England) and ascending thin layer chromatography (TLC) to check the purity and progress of reactions was run on DC-Kartan SI alumina 0.2 mm plates. The identification of compounds was done using a U.V. detector and the chromatograms were eluted with n-hexan:ethylacetate:acetic acid (7:2.5:0.5). IR spectra were recorded on a FTIR-spectrophotometer Shimadzu as KBr disks. CHN microanalysis was done using a Euro EA 3000 elemental analyzer (Italy), and 1H-NMR spectrum was recorded by *NMR UltraShield spectrophotometer 500 MHz*, *Bruker Avance III* (Switzerland).

5-amino-1, 3, 4-thiadiazol-2-thiol (IA) was synthesized according to Petrow V. et al., (1985) while 4-amino-5-methyl-4H-1, 2, 4-triazole-3-thiol (IIIB) was synthesized according to Jubie,S. et al., (2011), and Husain, A. et. al., (2009).

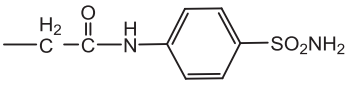
The general routes outlined in Schemes 1, 2, and 3 were used to synthesize all compounds described here.



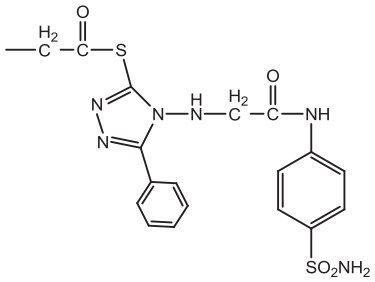
Scheme (1): The synthesis of compounds VB and IIIA.



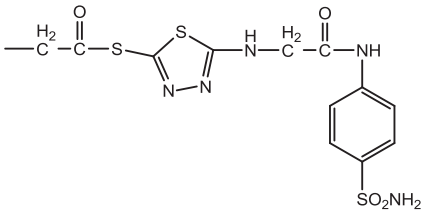
IIC, I, if R=



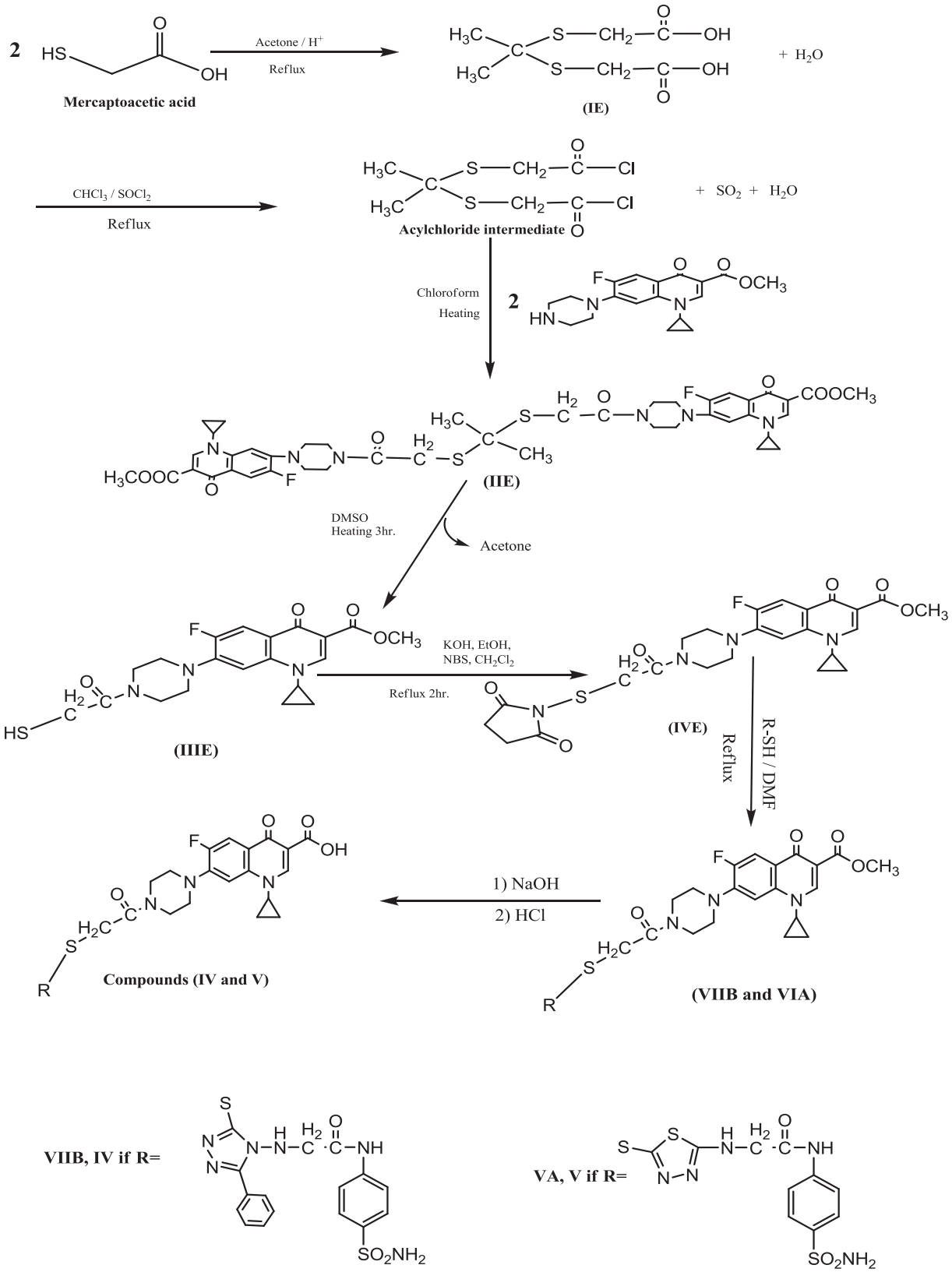
VIB, II, if R=



IVA, III, if R=



Scheme (2): The synthesis of compounds I, II and III.



Scheme (3): The synthesis of compounds IV and V.

3.2. Synthesis of 2-chloro-N-(4-sulfamoylphenyl) acetamide,(IC)

Sulfanilamide (2g, 11.6 mmol), was dissolved in DMF:Benzen (25:75) mixture (40 ml), then TEA (1.6 ml, 1.17 g, 11.6 mmol) was added. The reaction mixture was stirred on ice bath; chloroacetylchloride (0.92 ml, 1.3 g, 11.6 mmol in 10 ml benzene) was added drop wise with continuous stirring over a period of one hour, followed

by refluxing of the mixture for three hours. Then excess cold water was added, and the precipitated compound was filtered, and re-crystallized from ethanol (Mina et al., 2014), to give compound (IC) as gray powder (76% yield); m.p. 203-205 °C; R_f= 0.94. IR (cm−1): 3,331 and 3,225 (N-H) of primary amine, 1,689 (C=O) of secondary amide, 1,502, and 1,404 (aromatic), 1,255 (C-N) of secondary aromatic amide. CHN calculated ($C_8H_9ClN_2O_3S$): C, 38.64; H, 3.65; N, 11.26; found: C, 38.60; H, 3.42; N, 11.57; ^{1}H-NMR (DMSO-d_6) δ (ppm): 7.84-7.64 (m,4H,Ar-H), 7.23 (s,1H,NH), 4.26 (s,2H,CH_2), 7.0 (s,2H,NH_2).

3.3. Synthesis of methyl 1-cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylate, (IID)

A suspension of ciprofloxacin (2 g, 6 mmol), in absolute methanol (50 ml), was cooled down to -15^o C, then thionyl chloride (0.45 ml, 6 mmol) was added drop wise, (the temperature should be kept below -10^o C). Then the reaction mixture was kept at 40^o C for three hours, followed by refluxing for 35 hours (until the HCl gas was ceased), and left at room temperature overnight. The solvent was evaporated to dryness under vacuum; the residue was re-dissolved in methanol and evaporated. The process was repeated several times to ensure complete removal of thionyl chloride. The residue was collected and re-crystallized from methanol-chloroform (Nurhasimah et al., 2012) to give IID as yellow crystals (35% yield); m.p. 95-98 °C; R_f= 0.65. IR (cm−1): 3,333 (N-H) of amine, 1,724 (C=O) of ester, 1,587, 1,485 and 1,384 (aromatic). CHN calculated ($C_{18}H_{20}FN_3O_3$): C, 62.60; H, 5.84; N, 12.17; found: C, 62.45; H, 5.6; N, 11.57; ^{1}H-NMR (DMSO-d_6) δ (ppm): 8.69 (s,1H,CH), 8.01 (s,1H,CH), 6.04 (s,1H,CH), 4.12 (m,1H,CH), 3.77 (s,3H,CH_3), 3.46 (t,4H,2CH_2), 2.78 (t,4H,2 CH_2), 1.91 (s,1H,NH), 1.33 (m,4H,2CH_2).

3.4. Synthesis of methyl 1-cyclopropyl-6-fluoro-4-oxo-7-(4-(2-oxo- 2-(4 sulfamoylphenylamino) ethyl) piperazin-1-yl)-1, 4-dihydroquinoline-3-carboxylate, (IIC)

A mixture of IID (2g, 5.8 mmol), and IA (1.44 g, 5.8 mmol), were dissolved in DMF (25 ml), then TEA (0.81 ml, 5.8 mmol), was added. The reaction mixture was stirred at room temperature overnight. The solvent was evaporated; the residue was triturated with acetone and re-crystallized from methanol (Saeed et al., 2007) to give IIC as deep yellow powder (77% yield); m.p. 90-92 °C; R_f= 0.66. IR (cm−1): 3,335 and 3,211 (N-H) of NH_2, 1,720 (C=O) of ester, 1,666 (C=O) of secondary amide, 1, 508, and 1,394 (aromatic). CHN calculated ($C_{26}H_{28}FN_5O_6S$): C, 56.0; H, 5.06; N, 12.56; found: C, 55.95; H, 5.21; N, 12.44; ^{1}H-NMR (DMSO-d_6) δ (ppm): 8.69 (s,1H,CH), 8.02 (s,1H,CH), 7.84 (m,4H,Ar-H), 7.23 (s,1H,NH), 6.05 (s,1H,CH), 4.52 (m,1H,CH,CH), 3.75 (s,3H,CH_3), 3.44 (t,4H,2CH_2), 3.34 (s,2H,CH_2), 2.48(t,4H,2CH_2), 7.0 (s,2H,NH_2), 1.33 (m,4H,2CH_2).

3.5. Synthesis of 2-(3-mercapto-5-phenyl-4H-1, 2, 4-triazol-4-ylamino)-N-(4-sulfamoylphenyl) acetamide, (IVB)

A mixture of IC (1g, 4mmol), and IIIB (0.77g, 4mmol), was placed in round flask, then dissolved in ethanol 99%: DMF (50:50) mixture (30 ml). The reaction mixture was refluxed gently for three hours. The solvent was evaporated, and the residue was dissolved in ethyl acetate, washed with NaOH (5%, 3X), filtered over anhydrous magnesium sulfate. The filtrate was evaporated to give compound IVB that re-crystallized from ethanol (Hae-Sun et al., 2007). Beige powder (68% yield); m.p. 189-192 °C; R_f= 0.87. IR (cm−1): 3,340 and 3,261 (N-H) of NH_2, 2,943 (C-H) of alkane, 2557 (S-H) stretching, 1,681 (C=O) of secondary amide, 1,537 (C=N) of triazol 1,500, and 1,479 (aromatic). CHN calculated ($C_{16}H_{16}N_6O_3S_2$): C, 47.51; H, 3.99; N, 20.78; found: C, 47.19; H, 4.32; N, 21.3; ^{1}H-NMR (DMSO-d_6) δ (ppm): 8.28-7.41(m,9H,Ar-H), 7.23 (s,1H,NH), 3.59 (s,2H,CH_2), 7.5 (s,3H,NH and NH_2).

3.6. Synthesis of S-4-(2-oxo-2-(4 sulfamoylphenylamino) ethylamino)- 5-phenyl-4H-1,2,4-triazol-3-yl 2-chloroethanethioate,(VB)

A mixture of IVB (2 g, 4.9 mmol), and KOH (0.28 g, 4.9 mmol), was dissolved in DMF: Benzene (50:50) mixture, (30 ml), the reaction mixture was stirred in ice bath, then chloroacetyl chloride (0.39 ml, 4.9 mmol in 10 ml benzene) was added gradually (drop wise) with continuous stirring over a period of one hour, followed by refluxing the mixture for 5 hours (controlled by TLC). The solvent was evaporated; the residue was washed with sodium carbonate (2%), HCl (5%), and D.W, and then re-crystallized from ethanol (Mohammah et al., 2014) to give compound VB as beige crystals (33.6% yield); m.p. 225-227 °C; R_f= 0.67. IR (cm−1): 3,342 and 3,284 (N-H) of NH_2, 3,184 (N-H) of secondary amide, 1,658 (C=O) of secondary amide, 1,500, and 1,475 (aromatic), 1,491 (C=N) of triazol. CHN calculated ($C_{18}H_{17}ClN_6O_4S_2$): C, 44.95; H, 3.56; N, 17.47; found: C, 45.19; H, 4.02; N, 17.57; ^{1}H-NMR (DMSO-d_6) δ (ppm): 8.28-7.41(m, 9H, Ar-H), 7.23(s,1H,NH), 4.49(s,2H,CH_2), 3.59(s,2H,CH_2), 2.1(s,1H,NH), 7.0(s,2H, NH_2).

3.7. Methyl 1-cyclopropyl-6-fluoro-4-oxo-7-(4-(2-oxo-2-(4-(2-oxo-2-(4-sulfamoylphenylamino) ethylamino)-5-phenyl-4H-1,2,4-triazol-3-ylthio)ethyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylate (VIB)

A mixture of IID (0.7g, 2 mmol), and VB (1 g, 2 mmol), were dissolved in DMF (25 ml), then TEA (0.3 ml, 2

mmol), was added. The reaction mixture has been stirred at 100° C overnight. The solvent was evaporated; the residue was triturated with acetone and re-crystallized from methanol (Saeed et al., 2007) to give compound VIB as deep brown powder (70% yield); m.p. 158-160 °C; R_f= 0.84. IR (cm−1): 3,443 and 3,300 (N-H) of NH_2, 1,720 (C=O) of ester, 1,624 (C=O) of secondary amide, 1,543, and 1,494 (aromatic), 1,457 (C=N) of triazol. CHN calculated ($C_{36}H_{36}FN_9O_7S_2$): C, 54.74; H, 4.59; N, 15.6; found: C, 55.11; H, 4.72; N, 15.57; ^{1}H-NMR (DMSO-d_6) δ (ppm): 8.69 (s,1H,CH), 8.25-7.31 (m, 10H, Ar-H), 7.32 (s,1H,NH), 6.07 (s,1H,CH), 4.12 (m,1H,CH), 3.77 (s,3H,CH_3), 3.59 (s,2H,CH_2), 3.47 (s,2H,CH_2), 3.44 (t,4H,2CH_2), 2.48 (t,4H,2CH_2), 2.3(s,1H,NH), 7.0(s,2H, NH_2), 1.33 (m, 4H,2CH_2).

3.8. Synthesis of 2-(5-mercapto-1, 3, 4-thiadiazol-2-ylamino)-N-(4-sulfamoylphenyl) acetamide,(IIA)

A mixture of IC (1g, 4mmol), and IA (0.54g, 4mmol), was placed in round flask, then dissolved in ethanol 99%: DMF (50:50) mixture (30 ml). The reaction mixture was refluxed gently for five hours (Hae-Sun et al., 2007). Then the reaction mixture was worked up as described in section 3.5, to give IIA as brown powder (86% yield); m.p. 215-19 °C; R_f= 0.91. IR (cm−1): 3,346 and 3,269 (N-H) of NH_2, 1,678 (C=O) of secondary amide, 1,400 (C=N) of thiadiazole. CHN calculated ($C_{10}H_{11}N_5O_3S_3$): C, 34.77; H, 3.21; N, 20.27; found: C, 34.01; H, 3.5; N, 20.79; ^{1}H-NMR (DMSO-d_6) δ (ppm): 7.56 (m,4H,Ar-H), 7.44 (s,1H,NH), 4.0(s,1H,NH), 3.05 (s, 1H, SH), 3.82(s,2H,CH_2), 7.0(s,2H, NH_2).

3.9. Synthesis of S-5-(2-oxo-2-(4-sulfamoylphenylamino)ethylamino)-1,3,4-thiadiazol-2-yl 2-chloroethanethioate, (IIIA)

A mixture of IIA (2 g, 5.8 mmol), and KOH (0.33 g, 5.8 mmol), was dissolved in DMF: Benzene (50:50) mixture, (30 ml), the reaction mixture was stirred in ice bath, then chloroacetyl chloride (0.46 ml, 5.8 mmol in 10 ml benzene) was added drop wise with continuous stirring over a period of one hour, followed by refluxing the mixture for 8 hours (controlled by TLC). The solvent was evaporated; the residue was washed with sodium carbonate (2%), HCl (5%), and D.W, and then re-crystallized from ethanol (Mohammah et al., 2014) to give compound IIIA as brown powder (82% yield); m.p. 181-183 °C; R_f= 0.68. IR (cm−1): 3,257 and 3,184 (N-H) of NH_2, 1,658 (C=O) of secondary amide, 1,402 (C=N) of thiadiazole. CHN calculated ($C_{12}H_{12}ClN_5O_4S_3$): C, 34.16; H, 2.87; N, 16.6; found: C, 34.9; H, 2.23; N, 15.9; ^{1}H-NMR (DMSO-d_6) δ (ppm): 7.56 (m,4H,Ar-H), 7.32(s,1H,NH), 4.49 (2H,CH_2), 4.0(s,1H,NH), 3.69(s,2H,CH_2), 7.0(s,2H, NH_2).

3.10 Synthesis of methyl-1 cyclopropyl-6-fluoro-4-oxo-2)-4)-7-oxo-2)-5)-2-oxo-4)-2-sulfamoylphenylamino)ethylamino-1,3,4-(thiadiazol-2-ylthio)ethyl)piperazin-1-yl-1,4-(dihydroquinoline-3-carboxylate (IVA)

A mixture of IID (1.65 g, 4.7 mmol), and IIIA (2 g, 4.7 mmol), were dissolved in DMF (25 ml), then TEA (0.66 ml, 4.7 mmol), was added. The reaction mixture has been stirred at 100° C overnight. The solvent was evaporated; the residue was triturated with ethyl acetate and re-crystallized from methanol (Saeed et al., 2007)to give compound IVA as deep brown powder (52% yield); m.p. 164-166 °C; R_f= 0.85. IR (cm−1): 3,443 (N-H) of NH_2, 1,714 (C=O) of ester, 1,624 (C=O) of secondary amide, 1,491, and 1,394 (aromatic), 1,267 (C-O) of ester. CHN calculated ($C_{30}H_{31}FN_8O_7S_3$): C, 49.30; H, 4.28; N, 15.33; found: C, 49.9; H, 4.72; N, 14.7; ^{1}H-NMR (DMSO-d_6) δ (ppm): 8.69 (s,IH,CH), 8.01-7.64 (m, 5H, Ar-H), 7.23 (s,1H,NH), 6.07 (s,1H,CH), 4.12 (m,1H,CH), 4.0 (s,1H,NH), 3.82 (s,2H,CH_2), 3.77 (s,3H,CH_3), 3.47 (s,2H,CH_2), 3.44 (t,4H,2CH_2), 2.48 (t,4H,2CH_2), 7.0(s,2H, NH_2), 1.33 (m, 4H,2CH_2).

3.11. Synthesis of 2,2'-(propane-2,2-diylbis(sulfanediyl))diacetic acid (IE)

Concentrated HCl (two drops) was added to a mixture of acetone (5 ml, 68 mmol), and mercaptoacetic acid (9.5 ml, 136 mmol). The solution was kept in -2° C for 10min., followed by reflux for 15 min. White precipitate was obtained, filtered, dried and used in the next step without further purification (Theodora et al., 1999). It appears as white crystals (33% yield); m.p.112-114 °C; R_f= 0.94. IR (cm−1): 2,500 (O-H) of carboxylic acid, 2,553 (S-H) of thiol, 1,705 (C=O) of carboxylic acid, 1,375 (C-H) bending of gem-dimethyl. CHN calculated ($C_7H_{12}O_4S_2$): C, 37.48; H, 5.39; found: C, 36.89; H, 5.08; ^{1}H-NMR (DMSO-d_6) δ (ppm): 11 (s, 2H, 2OH), 3.32 (s,4H,2CH_2), 1.59 (s, 6H,2CH_3).

3.12. Synthesis of 2,2'-(propane-2,2-diylbis(methyl 1-cyclopropyl-6-fluoro-7-(4-(2-mercaptoacetyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3 carboxylate), (IIE)

Thionyl chloride (0.33 ml, 4.4 mmol) was added to a solution of compound IE (0.5 g, 2.2 mmol) in chloroform (25 ml), and the mixture was refluxed gently for 90 min., then the solvent was evaporated under vacuum, and the residue was washed with further amount of chloroform to ensure the removal of $SOCl_2$. Then, IID (1.53g, 4.4 mmol), (previously dissolved in $CHCl_3$), was added to the acid chloride slowly with continuous stirring. The stirring was continued with heating for further 30 min. The solution cooled, filtered, and the filtrate dried, and re-

crystallized from ethanol (Vogal, 1989) to give IIE as yellow crystals (30% yield); m.p. 142-145 °C; R_f= 0.20. IR (cm−1): 2,900 and 2,864 (C-H) of alkane, 1,699 (C=O) of ester, 1,637 (C=O) of tertiary amide, 1,579, and 1,446 (aromatic), 1,244 (C-O) of ester. CHN calculated ($C_{43}H_{48}F_2N_6O_8S_2$): C, 58.76; H, 5.50; N, 9.56; found: C, 58.3; H, 5.1; N, 9.57; ^{1}H-NMR (DMSO-d_6) δ (ppm): 8.69(s, 2H, 2CH), 8.01 (s,2H,2CH), 6.04 (s,2H, 2CH), 4.12 (m,2H,2CH), 3.77 (s,6H,2CH_3), 3.57 (t,8H,4CH_2), 3.46 (s,4H,2CH_2), 3.35 (t,8H,4CH2), 1.95 (s,6H,2CH_3), 1.33 (m,8H,4CH_2).

3.13. Synthesis of methyl 1-cyclopropyl-6-fluoro-7-(4-(2-mercaptoacetyl) piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3 carboxylate, (IIIE)

A mixture of IIE (0.5 g) and DMSO (15 ml), was heated at 140-150^o C for 4-5 hours, excess of ethyl acetate was added, the precipitated compound was filtered, dried and re-crystallized from methanol (Srinivasa et al., 1992) to give IIIE as pale pink powder (32.5% yield); m.p. 184-187 °C; R_f= 0.11. IR (cm−1): 2,837 (C-H) of alkane, 2,550 (S-H) of thiol, 1,720 (C=O) of ester, 1,626 (C=O) of secondary amide, 1,579, and 1,494 (aromatic), 1,273 (C-O) of ester. CHN calculated ($C_{20}H_{22}FN_3O_4S$): C, 57.27; H, 5.29; N, 10.02; found: C, 57.19; H, 5.1; N, 10.3; ^{1}H-NMR (DMSO-d_6) δ (ppm): 8.69 (s,1H,CH), 8.01 (s,1H,CH), 6.04 (s,1H,CH), 4.12 (m,1H,CH), 3.77 (s,3H,CH_3), 3.57 (t,4H,2CH_2), 3.45 (s,2H,CH_2), 3.35 (t,4H,2CH_2), 1.5 (s,1H,SH), 1.33 (m,4H,2CH_2).

3.14. Synthesis of methyl 1-cyclopropyl-7-(4-(2-(2, 5-dioxopyrrolidin-1-ylthio) acetyl) piperazin-1-yl)-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate, (IVE)

A solution of potassium hydroxide (0.267 g, 4.76 mmole) in absolute ethanol (5 ml) was added to a solution of IIIE (2 g, 4.76 mmole) in absolute ethanol (30 ml). The resulted precipitate was filtered, dried and suspended in methylene chloride (40 ml), cooled to 0^o C. Then a similarly cooled suspension of N-bromosuccinamide (0.85 g, 4.7 mmole) in methylene chloride (20 ml) was added. After stirring at 0^o C for 10 minutes, the suspension was refluxed for additional two hours. The insoluble material was then filtered to give potassium bromide, and the filtrate was evaporated to dryness in vacuum, the residue was re-crystallized from ethanol-water (Mohammad, 2006) to give IVE as deep brown oil (41% yield); R_f= 0.08. IR (cm−1): 2,955 (C-H) of alkane, 1,826 and 1,776 (C=O) of five membered cyclic imide, 1,701 (C=O) of ester, 1,614 (C=O) of amide, 1,543, and 1,458 (aromatic), 1,192 (C-O) of ester. CHN calculated ($C_{24}H_{25}FN_4O_6S$): C, 55.81; H, 4.88; N, 10.85; found: C, 55.69; H, 4.72; N, 10.57; ^{1}H-NMR (DMSO-d_6) δ (ppm): 8.69 (s,1H,CH), 8.01 (s,1H,CH), 6.04 (s,1H,CH), 4.12 (m,1H,CH), 3.77 (s,3H,CH_3), 3.57 (t,4H,2CH_2), 3.45 (s,2H,CH_2), 3.35 (t,4H,2CH_2), 2.64 (t,4H,2CH_2), 1.33 (m,4H,2CH_2).

3.15. Synthesis of methyl 1-cyclopropyl-6-fluoro-4-oxo-7-(4-(2-((4-(2-oxo-2-(4-sulfamoylphenylamino)ethylamino)-5-phenyl-4H-1,2,4-triazol-3 yl) disulfanyl) acetyl) piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylate, (VIIB)

A solution of IVE (2 g, 3.87 mmole) and IVB (1.566 g, 3.87 mmole) in DMF (20 ml) was refluxed for 3 hours. A precipitate was obtained by the addition of distilled water. The precipitate was filtered, dried and re-crystalized from methanol (Mohammad, 2006) to give compound VIIB as deep brown crystals (79% yield); m.p. 115-117 °C; R_f= 0.58. IR (cm−1): 3,406 and 3,3331 (N-H) of NH_2, 1,712 (C=O) of ester, 1,689 (C=O) of secondary amide, 1481 (C=N) of triazol. CHN calculated ($C_{36}H_{36}FN_9O_7S_3$): C, 52.61; H, 4.41; N, 15.34; found: C, 52.19; H, 4.72; N, 15.57; ^{1}H-NMR (DMSO-d_6) δ (ppm): 8.69 (s,1H,CH), 8.25-7.31 (m, 10H, Ar-H), 7.32 (s,1H,NH), 6.07 (s,1H,CH), 4.12 (m,1H,CH), 3.77 (s,3H,CH_3), 3.59 (s,2H,CH_2), 3.98 (s,2H,CH_2), 3.44 (t,4H,2CH2), 2.48 (t,4H,2CH2), 2.3(s,1H,NH), 7.0(s,2H, NH_2), 1.33 (m, 4H,2CH_2).

3.16. Synthesis of methyl 1-cyclopropyl-6-fluoro-4-oxo-7-(4-(2-((5-(2-oxo-2-(4-sulfamoylphenylamino) ethylamino)-1,3,4-thiadiazol-2-yl)disulfanyl)acetyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylate, (VA)

A solution of IVE (2 g, 3.87 mmole) and IIA (1.34 g, 3.87 mmole) in DMF (20 ml) was refluxed for 3 hours. A precipitate was obtained by the addition of distilled water. The precipitate was filtered, dried and re-crystalized from methanol (Mohammad, 2006) to give compound VA as pale yellow powder (75% yield); m.p. 136-138 °C; R_f= 0.72. IR (cm−1): 3,300 and 3,265 (N-H) of NH_2, 1,714 (C=O) of ester, 1,624 (C=O) of secondary amide, 1,491 (C=N) of thiadiazole, 1,265 (C-O) of ester. CHN calculated ($C_{35}H_{34}FN_9O_7S_3$): C, 52.03; H, 4.24; N, 15.60; found: C, 52.19; H, 4.62; N, 15.57; ^{1}H-NMR (DMSO-d_6) δ (ppm): 8.69 (s,1H,CH), 8.01-7.64 (m, 5H, Ar-H), 7.23 (s,1H,NH), 6.07 (s,1H,CH), 4.12 (m,1H,CH), 4.0 (s,1H,NH), 3.82 (s,2H,CH_2), 3.77 (s,3H,CH_3), 3.47 (s,2H,CH_2), 3.44 (t,4H,2CH_2), 2.48 (t,4H,2CH_2), 7.0(s,2H, NH_2), 1.33 (m, 4H,2CH_2).

3.17. General procedure to release the final compounds (I-V) from their carboxylate ester salts:

Compounds (IC, VIB, IVA, and VIIB, VA) (1.43 mmol), was dissolved in minimum volume of ethanol 99%: THF (3:1) mixture and the solution were cooled to 18^o C. Then NaOH (2N, 0.86 ml, 1.73 mmol) was added drop wise, with continuous stirring over a period of 30 min., stirring was continued at 18^o C for additional three hours. The reaction mixture was acidified with HCl (2N, 0.86 ml, 1.73 mmol), then excess of cold water was added.

The precipitated compound was filtered, dried and re-crystallized from methanol: chloroform (9:1) (Monther, 2006) to give compound (I-V), respectively.

1-cyclopropyl-6-fluoro-4-oxo-7-(4-(2-oxo-2-(4-sulfamoylphenylamino) ethyl) piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid, (I): Beige crystals (79% yield); m.p. 240–243 °C; Rf = 0.78. IR (cm−1): 2,600 (O-H) of carboxylic acid, 1,699 (C=O) of ester, 1,626 (C=O) of secondary amide, 1,546, and 1,491 (aromatic). CHN calculated ($C_{25}H_{26}FN_5O_6S$): C, 55.24; H, 4.82; N, 12.88; found: C, 55.19; H, 4.72; N, 11.57; ^{1}H-NMR (DMSO-d_6) δ (ppm): 10.0 (s,1H,OH), 8.58 (s,1H,CH), 8.4-7.43 (m,5H,Ar-H), 7.25 (s,1H,NH), 6.05 (s,1H,CH), 5.4 (m,1H,CH), 3.44 (t,4H,2CH_2), 3.34 (s,2H,CH_2), 3.24 (t,4H,2CH_2), 7.3 (s,2H,NH_2), 1.13 (m,4H,2CH_2).

1-cyclopropyl-6-fluoro-4-oxo-7-(4-(2-oxo-2-(4-(2-oxo-2-(4 sulfamoyl phenylamino)ethylamino)-5-phenyl-4H-1,2,4-triazol-3-ylthio)ethyl)piperazin-1-yl)- 1,4-dihydroquinoline-3-carboxylic acid (II): Pale brown powder (68% yield); m.p. 191–195 °C; Rf = 0.80; IR (cm−1): 2,600 (O-H) of carboxylic acid, 1,701 (C=O) of ester, 1,626 (C=O) of secondary amide, 1489 (C=N) of triazole, 1,456 (aromatic). CHN calculated ($C_{35}H_{34}FN_9O_7S_2$): C, 54.18; H, 4.42; N, 16.25; found: C, 54.09; H, 4.72; N, 15.57; ^{1}H-NMR (DMSO-d_6) δ (ppm): 10.68 (s,1H,OH), 8.43 (s,1H,CH), 7.93-7.31 (m, 10H, Ar-H), 6.18 (s,1H,NH), 6.07 (s,1H,CH), 4.16 (m,1H,CH), 3.5 (s,2H,CH_2), 3.47 (s,4H,2CH_2), 3.46 (t,2H,CH_2), 2.48 (t,4H,2CH_2), 2.3(s,1H,NH), 7.25(s,2H, NH_2), 1.19 (m, 4H,2CH_2).

*1-cyclopropyl-6-fluoro-4-oxo-7-(4-(2-oxo-2-(5-(2-oxo-2-(4-sulfamoylphenylamino) ethyl amino)-1,3,4-thiadiazol-2-ylthio)ethyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid (III):*Yellow powder (58% yield); m.p. 233–236 °C; Rf = 0.89. IR (cm−1): 2,600 (O-H) of carboxylic acid, 1,706 (C=O) of ester, 1,624 (C=O) of secondary amide, 1,541, and 1,491 (aromatic). CHN calculated ($C_{29}H_{29}FN_8O_7S_3$): C, 48.59; H, 4.08; N, 15.63; found: C, 49.01; H, 4.72; N, 15.57; ^{1}H-NMR (DMSO-d_6) δ (ppm): 10.92 (s,1H,OH), 9.0 (s,1H,CH), 8.6-7.24 (m, 5H, Ar-H), 6.1 (s,1H,NH), 6.09 (s,1H,CH), 4.25 (m,1H,CH), 4.0 (s,1H,NH), 3.96 (s,2H,CH_2), 3.47 (s,2H,CH_2), 3.48 (t,4H,2CH_2), 3.32 (t,4H,2CH_2), 7.4 (s,2H, NH_2), 1.23 (m, 4H,2CH_2).

1-cyclopropyl-6-fluoro-4-oxo-7-(4-(2-((4-(2-oxo-2-(4-sulfamoylphenylamino)ethylamino)-5-phenyl-4H-1,2,4-triazol-3-yl)disulfanyl)acetyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid (IV): Deep brown crystals (41% yield); m.p. 169–171 °C; Rf = 0.60. IR (cm−1): 2,550 (O-H) of carboxylic acid, 1,705 (C=O) of ester, 1,622 (C=O) of secondary amide, 1,546, and 1,485 (aromatic). CHN calculated ($C_{35}H_{34}FN_9O_7S_3$): C, 52.03; H, 4.24; N, 15.6; found: C, 52.19; H, 4.72; N, 15.57; ^{1}H-NMR (DMSO-d_6) δ (ppm): 10.82 (s,1H,OH), 8.38 (s,1H,CH), 8.22-7.25 (m, 10H, Ar-H), 6.21 (s,1H,NH), 6.07 (s,1H,CH), 4.17 (m,1H,CH), 3.98 (s,2H,CH_2), 3.75 (s,2H,CH_2), 3.44 (t,4H,2CH_2), 2.48 (t,4H,2CH_2), 2.46 (s,1H,NH), 7.0 (s,2H, NH_2), 1.23 (m, 4H,2CH_2).

1-cyclopropyl-6-fluoro-4-oxo-7-(4-(2-((5-(2-oxo-2-(4-sulfamoylphenylamino)ethylamino)-1,3,4-thiadiazol-2-yl)disulfanyl)acetyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid (V): pale brown powder (91% yield); m.p. 162–165 °C; Rf = 0.87. IR (cm−1): 2,500 (O-H) of carboxylic acid, 1,699 (C=O) of ester, 1,624 (C=O) of secondary amide, 1,541, and 1,494 (aromatic). CHN calculated ($C_{29}H_{29}FN_8O_7S_4$): C, 46.51; H, 3.9; N, 14.96; found: C, 45.10; H, 4.12; N, 15.57; ^{1}H-NMR (DMSO-d_6) δ (ppm): 10.84 (s,1H,OH), 8.62 (s,1H,CH), 8.46-7.44 (m, 5H, Ar-H), 7.24 (s,1H,NH), 6.58 (s,1H,CH), 4.32 (m,1H,CH), 4.97 (s,1H,NH), 3.82 (s,2H,CH_2), 3.69 (s,2H,CH_2), 3.44 (t,4H,2CH_2), 2.48 (t,4H,2CH_2), 2.68 (s,2H, NH_2), 1.38 (m, 4H,2CH_2).

3.18. Anti-mycobacterial Activity:

In vitro anti-tuberculosis activity of the chemically synthesized compounds (I-V) was studied at the directorate of general health national reference laboratory in Iraq-Ministry of Health. Their activity has been evaluated according to the proportion method by using Lowenstein-Jensen (LJ) medium, to determine the sensitivity of the Mycobacterium tuberculosis to the final synthesized compounds. MDR Mycobacterium tuberculosis strains isolated from the sputum of four patients with documented treatment histories has been used. The suspension of Mycobacterium tuberculosis was prepared as per McFarland Nephelometer standard (0.5). A 24 hr. old culture was used for the preparation of bacterial suspension. It is very important to have fresh growth on a solid medium, because older cultures may result in unreliable susceptibility test results. Suspension of organisms was made in sterile isotonic solution of sodium chloride and the turbidity was adjusted.

The data are expressed as the average number of bacterial colonies ± SEM and results were analyzed for statistical significance using student t-test (Two Sample Assuming Equal Variances) for comparison between values. While comparisons between different groups were made using ANOVA: Two factors without Replication. Probability (P) value of less than 0.05 was considered significant.

4. Conclusions

An *in vitro* anti-tuberculosis study showed that the hybridization approach between sulfonamide derivatives and ciprofloxacin, maintain or increase its anti-tuberculosis activity. Compounds I and V produce superior anti-tuberculosis activity when compared to ciprofloxacin.

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