

Synthesis, characterization and *in vitro* antimicrobial evaluation of sulphonyl urea derivatives as potential inhibitors of beta-ketoacyl-acyl carrier protein synthase III (FabH)

Síntesis, caracterización y evaluación antimicrobiana *in vitro* de derivados de sulfonilurea como inhibidores potenciales de la beta-cetoacil-proteína portadora de acilo III sintasa (FabH)

Badampudi Santosh Kumar*, Papammagari Raveendra Reddy*, Lakshmana Rao Krishna Rao Ravindranath*, Aluru Raghavendra Guru Prasad**, Alvalla Mallika***

ABSTRACT

A series of 15 sulphonyl urea derivatives (7a-7o) containing various heterocyclic substituents were synthesized, characterized by elemental analysis, IR, ¹H NMR and ¹³C NMR spectra and evaluated for *in vitro* antibacterial and antifungal activity. Molecular docking studies were performed to calculate docking scores and to propose the binding mode of sulphonyl urea derivatives with *E. coli* beta-ketoacyl-ACP synthase III, a key enzyme that catalyzes the initial step of fatty acid biosynthesis via a type II dissociated fatty acid synthase. The results from this study revealed that the derivatives 7g, 7j, 7l and 7o can become possible lead molecules for antimicrobial drug discovery. *In vitro* antimicrobial studies confirmed that the derivatives 7g, 7j, 7l and 7o have demonstrated better activity than the others.

RESUMEN

Se sintetizó una serie de 15 derivados de sulfonilurea (7a-7o) que contiene diversos sustituyentes heterocíclicos, caracterizados por análisis elemental, IR, RMN de ¹H y de ¹³C espectros RMN, y se evaluaron *in vitro* para actividad antibacteriana y antifúngica. Se realizaron estudios de acoplamiento molecular para calcular las puntuaciones de conexión y proponer el modo de unión de derivados de sulfonilurea con *E. coli* beta-cetoacil-ACP sintasa III, una enzima clave que cataliza la etapa inicial de la biosíntesis de ácidos grasos a través de un tipo II disociado ácido graso sintasa. Los resultados de este estudio revelaron que los derivados de 7g, 7j, 7l y 7o pueden convertirse en posibles moléculas de plomo para el descubrimiento de fármacos antimicrobianos. Los estudios *in vitro* confirmaron que los derivados de 7g, 7j, 7l y 7o han demostrado una mejor actividad antimicrobiana que los otros.

INTRODUCTION

Pyrrrolidine and thiophene moieties are key components of heterocycles that exhibit various therapeutic characteristics. Diverse therapeutic applications of compounds containing these moieties are reported in the literature (Alsaid, El-Gazzar & Ghorab, 2013; Bepalove, Sedavkina & Kulikova, 1989; Cheng, Wang, Fang, Tang & Xu, 2008; Dzhuraev, Karimkulov & Makhsulov-Amanov, 1992; Huang *et al.*, 2013; Kumar & Krishnan, 2012; Malmström *et al.*, 2013; Min *et al.*, 2013; Oh *et al.*, 2014; Omaima & Fatma, 1992; Yu, Detistov & Orlov, 2008; Zareef, Iqbal & Arfan, 2008). The sulfonamide (SO₂-NH-) group (Camar-go-Ordóñez *et al.*, 2011) also has great importance in medicinal chemistry.

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Palabras clave:

Sintasa β-cetoacil-ACP III (FabH); caracterización de derivados de sulfonilurea; actividad antimicrobiana; *in vitro*; *in silico*.

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* Department of Chemistry, Sri Krishnadevaraya University. Itukalapalli, Anantapuramu, AP, India, 515003.

** Faculty of Science and Technology, ICFAI University of Higher Education. Donthanapally, Sankarpally Road, Hyderabad, Telangan, India, 501504.

*** National Institute of Pharmaceutical Education and Research. Balanagar, Hyderabad. Phone: (+91) 98496 94428; (+91) 9441117803. E-mails: guruprasadar@yahoo.co.in; mallikaalvala@yahoo.in

Efficient chemical synthetic strategies of biologically active compounds play a crucial role in the advancement of life sciences. Inspired by this widely agreed perspective, the authors have undertaken the simple strategies for the synthesis of novel compounds containing all these pharmacologically active centers.

The biosynthesis of fatty acid is an essential process for the survival of the organism (Magnuson, Jackowski, Rock & Cronan, 1993; White, Zheng, Zhang & Rock, 2005). The enzymes involved in the fatty acid biosynthesis pathway provide a set of targets for the discovery of antibiotics. β -ketoacyl-[acyl-carrier-protein] synthase III is an enzyme that participates in fatty acid biosynthesis of bacterial membrane. Fatty acid synthesis is initiated by the condensation of acetyl-coenzyme with malonyl-acyl carrier protein by β -ketoacyl-[acyl-carrier-protein] synthase III (Jackowski, Murphy & Cronan, 1989; Jackowski & Rock, 1987). The crucial role of this enzyme, its unique structural features combined with its ubiquitous occurrence in bacteria, has made it novel and attractive target for the development of antibacterial drugs. Crystal structures of FabH have been reported from *Mycobacterium tuberculosis* (Davies, Heath, White & Rock, 2000; Qiu *et al.*, 2001; Scarsdale, Kazanina, He, Reynolds & Wright, 2001), *Staphylococcus aureus* (Qiu *et al.*, 2005) and *Escherichia coli* (Qiu *et al.*, 1999). It was reported that FabH is critical in the initiation of Type II fatty acid biosynthesis (consists of series of discrete proteins that catalyze various reactions in fatty acid biosynthetic pathway) in both Gram positive and Gram negative bacteria (Lai & Cronan, 2003). The present study is aimed to synthesize and characterize novel sulphonyl urea derivatives and evaluate for their antimicrobial activity. It is *in silico* demonstrated that the inhibition of FabH enzymes is responsible for the antimicrobial activity of compounds under study.

MATERIALS AND METHODS

All chemicals and reagents were procured from Merck India limited. The standard bacterial and fungal stains were procured from National Centre for Cell Science, Pune, India. Melting points were determined using X-6 digital display binocular microscope. Infrared spectra were taken on a Nicolet Nexus 470 FT-IR spectrometer using smear KBr crystals. NMR spectra were recorded on a Bruker Avance (300 MHz) spectrometer. All molecules were docked into binding pocket by using Grid-based Ligand Docking with Energetics (GLIDE) module of Schrodinger. A total of 10 ligand conforma-

tions were allowed and finally top score conformation was selected as active conformation. Molecules were analyzed based on docking score, interacting amino acids and hydrogen bonds.

The synthesized ligands were sketched and geometry was optimized using Maestro workspace of Schrodinger Suite 2012. Ligands for docking were prepared by using Ligprep, a versatile program to generate three dimensional module of Schrodinger and minimized using OPLS-2005 force field. Crystal coordinates of *E. coli* β -ketoacyl-ACP synthase III (FabH) were taken from PDB ID 1HNJ. Protein for docking studies was prepared using protein preparation wizard of Schrodinger (Maestro, v9.3 Schrodinger, LLC, New York, NY). Bond orders and formal charges were added for hetero groups and hydrogens were added to all atoms in the system. Water molecules within 5 Å distance were removed. Glide energy grid with coordinates of X: 28.56; Y: 8.86; Z: 33.83 around malonyl-coA binding site were generated for the prepared protein.

Synthesis of title compounds (7a-7e)

Ethyl 2-((3S, 4S)-3, 4-disubstituted-sulfonamido)pyrrolidinyl-N-acetate derivatives (1a-1c) were synthesized by the procedure reported in the literature (Santosh, Raveendra, Madhu & Ravindranath, 2012).

Synthesis of [3, 4-Bis-(thiophene-2-sulfonylamino)-pyrrolidin-1-yl]-acetic acid (2a)

An aqueous solution of NaOH (2N, 30 mL) was added to the solution of ester 1a (5 mmol, 2.398 g) in tetrahydrofuran / MeOH / H₂O (1:1:1) and stirred at room temp. The excess of solvent was evaporated under vacuum to give crude residue. The residue was washed with EtOAc, acidified with 1N HCl to a pH 2 and was filtered under vacuum to get fine solid. In case a solid is not obtained, the solution was extracted with two 100 mL portions of EtOAc. The organic layer was collected, washed with water, brine, dried over anhydrous Na₂SO₄, filtered and evaporated under vacuum to give crude acid product. The crude product was purified by column chromatography (60 mesh-silica gel -120 mesh-silica gel, Eluent: 70% EtOAc-30% petroleum ether). Similar procedure was followed for the synthesis of other compounds of the series.

Synthesis of N,N'-((3S, 4S)-1-((5-amino-1, 3, 4-thiadiazol-2-yl)methyl)pyrrolidine-3, 4-diyl)bis(thiophene-2-sulfonamide) (3a)

Concentrated H₂SO₄ (12 mL) was added slowly to a mixture of carboxylic acid 2a (10 mmol, 4.515 g) and

thiosemicarbazide (12 mmol, 1.093 g) in a three necked round bottom flask fitted with a reflux condenser. The reaction mixture was refluxed on a water bath for 3 h, poured into crushed ice and neutralized with ammonia solution. The yellow colored solid separated was filtered, washed with saturated sodium bicarbonate solution and water, dried and recrystallized from ethanol. Similar procedure was adopted for the synthesis of other compounds (3b & 3c) of the series.

Synthesis of Cyclopropanesulfonyl chloride (5a)

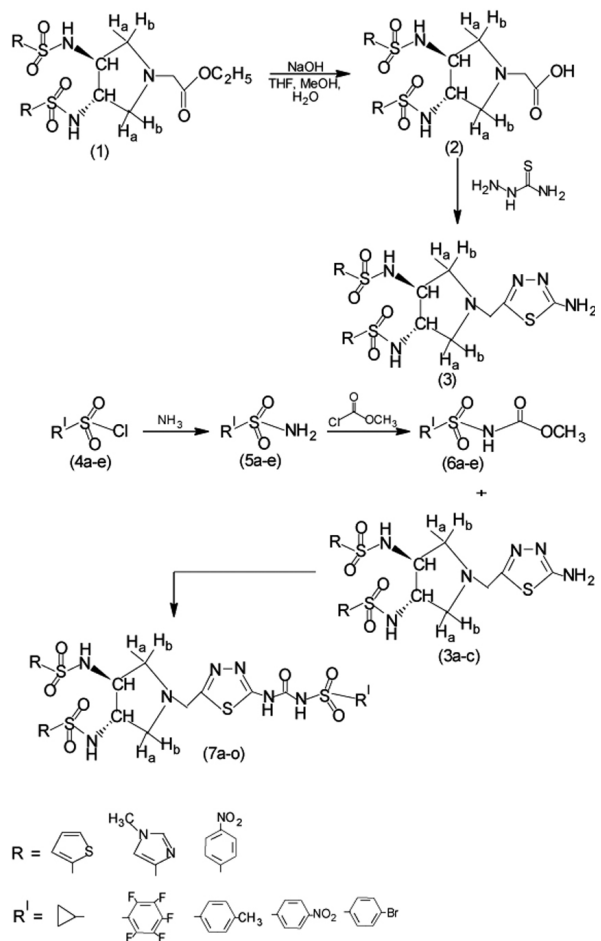
A mixture of cyclopropanesulfonylchloride (10 mmol, 1.906 g) and 20% aqueous ammonia solution was taken in a four necked round bottom flask fitted with a reflux condenser. The reaction mixture was heated at 75 °C - 80 °C for 3 h. The reaction mixture was allowed to stand for 1 h at 15 °C, the solid that separated was filtered, washed with large quantity of water and dried to get 4-methyl benzenesulfonamide. Similar procedure was adopted for the synthesis of other compounds (5a-5e) of the series.

Synthesis of methyl cyclopropylsulfonylcarbamate (6a)

A mixture of cyclopropanesulfonylchloride (10 mmol, 1.21 g), ethyl methyl ketone (8 mL) and potassium carbonate was taken in a four necked round bottom flask and heated to 80 °C for 30 min. Methyl chloroformate (10 mL) was added drop wise at 45 °C. The mixture was heated for 8 h at 50 °C. After the completion of reaction (monitored by TLC), the reaction mixture was poured into ice cold water and extracted with ethyl acetate. The pH of the aqueous layer was adjusted to a 2.5 by the addition of hydrochloric acid and extracted with ethyl acetate. The organic layer was dried over anhydrous sodium sulfate and the solution was concentrated to dryness. The solid thus obtained was recrystallized from ethanol. Similar procedure was adopted for the synthesis of other compounds (6b-6e) of the series.

Synthesis of N,N'-((3S, 4S)-1-((5-(3-(cyclopropylsulfonyl)ureido)-1, 3, 4-thiadiazol-2-yl)methyl)pyrrolidine-3, 4-diyl) bis(thiophene-2-sulfonamide) (7a)

A mixture of 6a (10 mmol, 1.409 g) and amine 3a (10 mmol, 1.79 g) in toluene (50 mL) was taken in a three necked round bottom flask fitted with a reflux condenser and a mechanical stirrer. The reaction mixture was refluxed for 4 h, cooled to room temperature, resulting solid was filtered, dried and recrystallized from methanol to obtain 7a. Similar procedure was adopted for the synthesis of other compounds (7b-7o) of the series. The sequence of reactions is given in the scheme 1.



Scheme 1. Synthesis of title compounds 7(a-7o).

Source: Authors own elaboration.

Characterization of compounds 2(a-c)

Compound; R; Molecular formula; Yield (%); m.p. (°C); Element Found% (calculated%); IR (Group)_{max} in cm⁻¹; ¹H NMR (300MHz, DMSO-D₆) δ ppm.

2a: Thiophene; C₁₄H₁₇N₃O₆S₄; 76; 191-3; C 37.21 (37.24), H 3.77 (3.79), N 9.33 (9.31); (C-N Cyclic) 1100, (C-N exo) 1117, (O = S = O) 1320 and 1185, (C-N imidazole) 1550, (C = N) 1615, (C = O Acid) 1755, (N-H) 3210; 7.65-7.22 (m, 6H, of thiophene), 7.47 (s, 2H, SO₂-NH-), 4.22 (s, 1H, -COOH), 3.50 (m, 2H, -SO₂-N-CH-), 3.25 (s, 2H, -N-CH₂-CO), 2.88 (m, 2H, -CH_a - protons of pyrrolidine), 2.29 (m, 2H, -CH_b - protons of pyrrolidine).

2b: 1-Methyl-1H-imidazole; C₁₄H₂₁N₇O₆S₂; 71; 197-9; C 37.55 (37.58), 4.69 (4.73), N 21.89 (21.91); (C-N Cyclic) 1116, (C-N exo) 1120, (O = S = O) 1326 and 1186, (C-N imidazole) 1559, (C = N) 1617, (C = O Acid) 1757,

(N-H) 3215; 7.90 (2H, -N-CH-N- of imidazole), 7.47 (s, 2H, SO₂-NH-), 6.93 (s, 2H, N-CH- of two imidazole), 4.22 (s, 1H, -COOH), 3.65 (s, 6H, N-CH₃ of imidazole), 3.50 (m, 2H, -SO₂-N-CH-), 3.25 (s, 2H, -N-CH₂-CO-), 2.88(m, 2H, -CH_a- protons of pyrrolidine), 2.29 (m, 2H, -CH_b- protons of pyrrolidine).

2c: *p*-Nitro phenyl; C₁₈H₁₉N₅O₁₀S₂;82;182-4; C 40.81 (40.83), H 3.63 (3.62), N 13.28 (13.23); (C-N Cyclic)1122, (C-N exo)1125, (O = S = O)1323 and 1189, (C-N imidazole)1557, (C = N)1619, (C = O Acid)1762, (N-H) 3217; 8.12-8.39 (m, 8H, aromatic protons), 7.47 (s, 2H, SO₂-NH-), 4.22 (s, 1H, -COOH), 3.50 (m, 2H, -SO₂-N-CH-), 3.25 (s, 2H, -N-CH₂-CO-), 2.88(m, 2H, -CH_a- protons of pyrrolidine), 2.29 (m, 2H, -CH_b- protons of pyrrolidine).

Characterization of compounds 3(a-c)

Compound; R; Molecular formula; Yield (%); m.p. (°C); Element Found% (calculated%); IR (Group)_{v_{max}} in cm⁻¹; ¹H NMR (300MHz, DMSO-D₆) δ ppm.

3a: Thiophene; C₁₅H₁₈N₆O₄S₅;79;197-9; C 35.55 (35.56), H 3.52 (3.58), N 16.53 (16.59); (C-S-C thiadiazole) 695, (O = S = O)1323 and 1185, (C = N thiadiazole)1670, (-NH₂)3356 and 3263, (NH sulphonamide)3421; 7.65-7.22 (m, 6H, of thiophene), 7.47 (s, 2H, SO₂-NH-), 3.62 (s, 2H, -N-CH₂-), 3.33 (s, 2H, -NH₂), 3.11 (m, 2H, -SO₂-N-CH-), 2.61(m, 2H, -CH_a- protons of pyrrolidine), 2.55 (m, 2H, -CH_b- protons of pyrrolidine).

3b: 1-Methyl-1H-imidazole; C₁₅H₂₂N₁₀O₄S₃;68;202-4; C 35.81 (35.85), H 4.44 (4.41), N 27.82 (27.87); (C-S-C thiadiazole)705, (O = S = O)1326 and 1187, (C = N thiadiazole)1673, (-NH₂)3359 and 3268, (NH sulphonamide)3420; 7.90(2H, -N-CH-N- of imidazole), 7.47 (s, 2H, SO₂-NH-), 6.93 (s, 2H, N-CH- of two imidazole), 3.65 (s, 6H, N-CH₃ of imidazole), 3.59 (s, 2H, -N-CH₂-), 3.35 (s, 2H, -NH₂), 3.17 (m, 2H, -SO₂-N-CH-), 2.88(m, 2H, -CH_a- protons of pyrrolidine), 2.52 (m, 2H, -CH_b- protons of pyrrolidine).

3c: *p*-Nitro phenyl; C₁₉H₂₀N₈O₈S₃;73;192-4; C 39.09 (39.04), H 3.42 (3.45), N 19.15 (19.17); (C-S-C thiadiazole)728, (O = S = O)1337 and 1199, (-NH₂)3367 and 3279, (C=N thiadiazole)1680, (NH sulphonamide)3422; 8.12-8.39 (m, 8H, aromatic protons), 7.47 (s, 2H, SO₂-NH-), 3.69 (s, 2H, -N-CH₂-), 3.50 (m, 2H, -SO₂-N-CH-), 3.39 (s, 2H, -NH₂), 2.97(m, 2H, -CH_a- protons of pyrrolidine), 2.33 (m, 2H, -CH_b- protons of pyrrolidine).

Characterization of compounds 6(a-e)

Compound; R; Molecular formula; Yield (%); m.p. (°C); Element Found% (calculated%); IR (Group)_{v_{max}} in cm⁻¹; ¹H NMR (300MHz, DMSO-D₆) δ ppm.

6a: Cyclopropyl; C₅H₉NO₄S;61;205-7; C 33.53 (33.51), H 5.09 (5.06), N 7.81 (7.82); (C-O)1254, (-CH₃)1342 and 1265, (C = O)1640, (-CH₃)2915 and 2875, (N-H)3212; 9.22 (s, 1H, SO₂-NH-), 3.67 (s, 3H, -OCOCH₃), 1.75(m, 1H, -SO₂-CH), 0.79(m, 4H, cyclopropyl -CH₂-).

6b: Pentafluorophenyl; C₈H₄F₅NO₄S; 59; 186-8; C 31.45(31.48), H 1.35 (1.32), N 4.52 (4.59); (C-O)1269, (-CH₃)1358 and 1279, (C = O)1648, (-CH₃)2915 and 2875, (N-H)3215; 9.22 (s, 1H, SO₂-NH-), 3.69 (s, 3H, -OCOCH₃).

6c: *p*-Tolyl; C₉H₁₁NO₄S;69;192-4; C 47.19 (47.15), H 4.83 (4.84), N 6.15 (6.11); (C-O)1257, (-CH₃)1347 and 1264, (C = O)1650, (-CH₃)2915 and 2875, (N-H)3214; 8.86 (s, 1H, SO₂-NH-), 7.83 (d, 2H, Ar-H meta to methyl), 7.34 (d, 2H, Ar-H ortho to methyl), 3.57 (s, 3H, -OCOCH₃).

6d: *p*-Bromo phenyl; C₈H₈BrNO₄S;62;180-2; C 32.67 (32.67), H 2.77 (2.74), N 4.73 (4.76); (C-O)1259, (-CH₃)1349 and 1268, (C = O)1654, (-CH₃)2915 and 2875, (N-H)3203; 8.89 (s, 1H, SO₂-NH-), 7.87 (d, 2H, Ar-H meta to bromo), 7.77 (d, 2H, Ar-H ortho to bromo), 3.59(s, 3H, -OCOCH₃).

6e: *p*-Nitro phenyl; C₈H₈N₂O₆S;67;196-8; C 36.89 (36.92), H 3.12 (3.10), N 10.73 (10.77); (C-O)1263, (-CH₃)1352 and 1273, (C = O)1666, (-CH₃)2915 and 2875, (N-H)3217; 8.97(s, 1H, SO₂-NH-), 8.49 (d, 2H, Ar-H ortho to nitro), 8.21 (d, 2H, Ar-H meta to nitro), 3.65(s, 3H, -OCOCH₃).

Characterization of compounds 7(a-o)

Compound; R; R¹; Molecular formula; Yield (%); m.p. (°C); Element Found% (calculated%); IR Group_{v_{max}} in cm⁻¹; ¹H NMR (300MHz, DMSO-D₆) δ ppm; ¹³C-NMR (300MHz, DMSO-D₆) (δ ppm).

7a: Thiophene;Cyclopropyl;C₁₉H₂₃N₇O₈S₆;61;212-4; C 34.09 (34.07), H 3.43 (3.46), N 14.61 (14.64); (N-N)1052, (O = S = O)1362 and 1183, (C-N urea)1310, (C = O urea)1640, (NH sulphonamide)3319; 9.01 (s, 1H, SO₂-NH-), 7.45-7.01 (m, 6H, of thiophene), 7.37 (s, 2H, SO₂-NH-), 5.94 (s, 1H, NH, D₂O-exchange, disappear), 3.45 (s, 2H, -N-CH₂-), 3.01 (m, 2H, -SO₂-N-CH-), 2.54(m, 2H, -CH_a- protons of pyrrolidine), 2.35 (m, 2H, -CH_b- protons of pyrrolidine), 1.54 (m, 1H, -SO₂-CH), 0.67(m, 4H, cyclopropyl -CH₂-); 62.3, 64.1, 137.2, 128.7, 132.6, 135.9,57.8, 64.1, 66.1, 157.5, 48.9, 12.5, corresponding to C₂ and C₂, C₃ and C₃, C₄ and C₄, C₅ and C₅, C₆ and C₆, C₇ and C₇, C₈, C₉, C₁₀, C₁₁, C₁₂, C₁₃ and C₁₃, respectively.

7b: Thiophene; Pentafluorophenyl; C₂₂H₁₈F₅N₇O₈S₆; 59;192-4; C 33.22 (33.20), H 2.25 (2.28), N 12.29 (12.32);

(N-N) 1033, (O = S = O)1352 and 1165, (C-N urea)1301, (C = O urea)1631, (NH sulphonamide)3311; 9.14 (s, 1H, SO₂-NH-), 7.71-7.12 (m, 6H, of thiophene), 7.45 (s, 2H, SO₂-NH-), 5.97 (s, 1H, NH, D₂O-exchange, disappear), 3.65 (s, 2H, -N-CH₂-), 3.03 (m, 2H, -SO₂-N-CH-), 2.60 (m, 2H, -CH_a- protons of pyrrolidine), 2.59 (m, 2H, -CH_b- protons of pyrrolidine).

7c: Thiophene; *p*-Tolyl; C₂₃H₂₅N₇O₇S₆;69;209-11; C 39.21 (39.25), H 3.56 (3.58), N 13.90 (13.93); (N-N) 1049, (O = S = O)1359 and 1179, (C-N urea)1308, (C = O urea)1641, (NH sulphonamide)3318; 8.86 (s, 1H, SO₂-NH-), 7.83 (d, 2H, Ar-H meta to methyl), 7.62-7.24 (m, 6H, of thiophene), 7.32 (s, 2H, SO₂-NH-), 7.34 (d, 2H, Ar-H ortho to methyl), 6.11 (s, 2H, NH, D₂O-exchange, disappear), 3.45 (s, 2H, -N-CH₂-), 3.08 (m, 2H, -SO₂-N-CH-), 2.67(m, 2H, -CH_a- protons of pyrrolidine), 2.51 (m, 2H, -CH_b- protons of pyrrolidine), 1.90(s, 3H, -CH₃, ArCH₃).

7d: Thiophene; *p*-Bromo phenyl; C₂₂H₂₂BrN₇O₇S₆; 68;201-3; C 34.33 (34.37), H 2.90 (2.88), N 12.74 (12.75); (N-N) 1045, (O = S = O)1357 and 1174, (C-N urea)1306, (C=O urea)1639, (NH sulphonamide)3316; 8.91 (s, 1H, SO₂-NH-), 7.85 (d, 2H, Ar-H meta to bromo), 7.78 (d, 2H, Ar-H ortho to bromo), 7.41-7.16 (m, 6H, of thiophene), 7.44 (s, 2H, SO₂-NH-), 5.71 (s, 2H, NH, D₂O-exchange, disappear), 3.67 (s, 2H, -N-CH₂-), 3.15 (m, 2H, -SO₂-N-CH-), 2.66 (m, 2H, -CH_a- protons of pyrrolidine), 2.71 (m, 2H, -CH_b- protons of pyrrolidine).

7e: Thiophene; *p*-Nitro phenyl; C₂₂H₂₂N₈O₉S₆;71; 189-91; C 35.16 (35.19), H 2.91 (2.95), N 14.89 (14.92); (N-N) 1040, (O = S = O)1354 and 1169, (C-N urea)1303, (C = O urea)1636, (NH sulphonamide)3313; 9.12 (s, 1H, SO₂-NH-), 8.58 (d, 2H, Ar-H ortho to nitro), 8.28 (d, 2H, ArH meta to nitro), 7.50-7.01 (m, 6H, of thiophene), 7.52 (s, 2H, SO₂-NH-), 6.27 (s, 2H, NH, D₂O-exchange, disappear), 3.69 (s, 2H, -N-CH₂-), 3.18 (m, 2H, -SO₂-N-CH-), 2.71(m, 2H, -CH_a- protons of pyrrolidine), 2.67 (m, 2H, -CH_b- protons of pyrrolidine).

7f: 1-Methyl-1H-imidazole; Cyclopropyl; C₁₉H₂₇N₁₁O₇S₄; 67;209-11; C 35.11 (35.12), H 4.15 (4.19), N 23.69 (23.71); (N-N)1058, (O = S = O)1379 and 1189, (C-N urea)1316, (C = O urea)1642, (NH sulphonamide)3325; 9.25 (s, 1H, SO₂-NH-), 7.91 (2H, -N-CH-N- of imidazole), 7.48 (s, 2H, SO₂-NH-), 6.87 (s, 2H, N-CH- of two imidazole), 5.98 (s, 2H, NH, D₂O-exchange, disappear), 3.62 (s, 6H, N-CH₃ of imidazole), 3.51 (s, 2H, -N-CH₂-), 3.23 (m, 2H, -SO₂-N-CH-), 2.76 (m, 2H, -CH_a- protons of pyrrolidine), 2.39 (m, 2H, -CH_b- protons of pyrrolidine), 1.68(m, 1H, -SO₂-CH), 0.65 (m, 4H, cyclopropyl -CH₂-).

7g: 1-Methyl-1H-imidazole; Pentafluorophenyl; C₂₂H₃₈F₅N₁₁O₇S₄;58;198-200; C 37.15 (37.18), H 4.55 (4.56), N 18.31 (18.34); (N-N) 1035, (O = S = O)1359 and 1171, (C-N urea)1306, (C = O urea)1633, (NH sulphonamide)3316; 9.27 (s, 1H, SO₂-NH-), 7.83 (2H, -N-CH-N- of imidazole), 7.41 (s, 2H, SO₂-NH-), 6.81 (s, 2H, N-CH- of two imidazole), 6.10 (s, 2H, NH, D₂O-exchange, disappear), 3.65 (s, 6H, N-CH₃ of imidazole), 3.53 (s, 2H, -N-CH₂-), 3.15 (m, 2H, -SO₂-N-CH-), 2.83 (m, 2H, -CH_a- protons of pyrrolidine), 2.47 (m, 2H, -CH_b- protons of pyrrolidine); 62.7, 64.6, 143.2, 127.2, 122.8, 35.5, 58.8, 65.1, 66.9, 156.8, 125.1, 151.9, 139.1, 148.7 corresponding to C₂ and C₂, C₃ and C₃, C₄ and C₄, C₅ and C₅, C₆ and C₆, C₇ and C₇, C₈, C₉, C₁₀, C₁₁, C₁₂, C₁₃ and C₁₃, C₁₄ and C₁₄, C₁₅ respectively.

7h: 1-Methyl-1H-imidazole; *p*-Tolyl; C₂₃H₂₉N₁₁O₇S₄;59;201-3; C 39.45 (39.47), H 4.16 (4.18), N 22.00 (22.02); (N-N) 1055, (O = S = O)1376 and 1186, (C-N urea)1314, (C = O urea)1640, (NH sulphonamide)3323; 8.89 (s, 1H, SO₂-NH-), 7.97 (2H, -N-CH-N- of imidazole), 7.84 (d, 2H, ArH meta to methyl), 7.58 (s, 2H, SO₂-NH-), 7.39 (d, 2H, Ar-H ortho to methyl), 6.97 (s, 2H, N-CH- of two imidazole), 6.17 (s, 2H, NH, D₂O-exchange, disappear), 3.65 (s, 6H, N-CH₃ of imidazole), 3.69 (s, 2H, -N-CH₂-), 3.25 (m, 2H, -SO₂-N-CH-), 2.78(m, 2H, -CH_a- protons of pyrrolidine), 2.45 (m, 2H, -CH_b-protons of pyrrolidine).

7i: 1-Methyl-1H-imidazole; *p*-Bromo phenyl; C₂₂H₂₆BrN₁₁O₇S₄; 71; 199-201; C 34.51 (34.56), H 3.39 (3.43), N 21.11 (20.15); (N-N)1050, (O = S = O)1372 and 1183, (C-N urea)1313, (C = O urea)1638, (NH sulphonamide)3322; 8.97 (s, 1H, SO₂-NH-), 7.89 (2H, -N-CH-N- of imidazole), 7.89 (d, 2H, Ar-H meta to bromo), 7.67 (d, 2H, Ar-H ortho to bromo), 7.47 (s, 2H, SO₂-NH-), 6.93 (s, 2H, N-CH- of two imidazole), 6.12 (s, 2H, NH, D₂O-exchange, disappear), 3.65 (s, 6H, N-CH₃ of imidazole), 3.64 (s, 2H, -N-CH₂-), 3.28 (m, 2H, -SO₂-N-CH-), 2.67(m, 2H, -CH_a- protons of pyrrolidine), 2.54 (m,2H, -CH_b- protons of pyrrolidine).

7j: 1-Methyl-1H-imidazole; *p*-Nitro phenyl; C₂₂H₂₆N₁₂O₉S₄;70;207-9; 36.15 (36.16), H 3.55 (3.59), N 23.05 (23.00); (N-N)1046, (O = S = O)1365 and 1175, (C-N urea)1310, (C = O urea)1635, (NH sulphonamide)3319; 8.76 (s, 1H, SO₂-NH-), 8.35 (d, 2H, Ar-H ortho to nitro), 8.02 (d, 2H, Ar-H meta to nitro), 7.91 (2H, -N-CH-N- of imidazole), 7.37 (s, 2H, SO₂-NH-), 6.93 (s, 2H, N-CH- of two imidazole), 6.16 (s, 2H, NH, D₂O-exchange, disappear), 3.65 (s, 6H, N-CH₃ of imidazole), 3.44 (s, 2H, -N-CH₂-), 3.17 (m, 2H, -SO₂-N-CH-), 2.71 (m, 2H, -CH_a- protons of pyrrolidine), 2.44 (m, 2H, -CH_b- protons of pyrrolidine).

7k: *p*-Nitro phenyl; Cyclopropyl; C₂₃H₂₅N₉O₁₁S₄; 69;202-4; C 37.71 (37.75), H 3.40 (3.44), N 15.19 (17.23); (N-N)1039, (O = S = O)1356 and 1165, (C-N urea)1321, (C=O urea)1645, (NH sulphonamide)3316; 9.21 (s, 1H, SO₂-NH-), 8.14-8.38 (m, 8H, aromatic protons), 7.41 (s, 2H, SO₂-NH-), 6.13 (s, 2H, NH, D₂O-exchange, disappear), 3.65 (s, 2H, -N-CH₂-), 3.54 (m, 2H, -SO₂-N-CH-), 2.87(m, 2H, -CH_a- protons of pyrrolidine), 2.37 (m, 2H, -CH_b- protons of pyrrolidine), 1.71 (m, 1H, -SO₂-CH), 0.74 (m, 4H, cyclopropyl -CH₂-).

7l: *p*-Nitro phenyl; Pentafluorophenyl; C₂₆H₂₀F₅N₉O₁₁S₄;61;204-6; C 36.38 (36.41), H 2.31 (2.35), N 14.68 (14.70); (N-N) 1025, (O = S = O)1339 and 1152, (C-N urea)1311, (C = O urea)1631, (NH sulphonamide)3309; 9.16 (s, 1H, SO₂-NH-), 8.22-8.32 (m, 8H, aromatic protons), 7.48 (s, 2H, SO₂-NH-), 6.13 (s, 2H, NH, D₂O-exchange, disappear), 3.70 (s, 2H, -N-CH₂-), 3.51 (m, 2H, -SO₂-N-CH-), 2.87(m, 2H, -CH_a-protons of pyrrolidine), 2.43 (m, 2H, -CH_b- protons of pyrrolidine).

7m: *p*-Nitro phenyl; *p*-Tolyl; C₂₇H₂₇N₉O₁₁S₄;62;212-4; C 41.46 (41.48), H 3.47 (3.48), N 16.09 (16.12); (N-N) 1035, (O = S = O)1353 and 1161, (C-N urea)1319, (C = O urea)1641, (NH sulphonamide) 3315; 8.94 (s, 1H, SO₂-NH-), 8.19-8.32 (m, 8H, aromatic protons), 7.88 (d, 2H, Ar-H meta to methyl), 7.43 (s, 2H, SO₂-NH-), 7.34 (d, 2H, Ar-H ortho to methyl), 6.16 (s, 2H, NH, D₂O-exchange, disappear), 3.67 (s, 2H, -N-CH₂-), 3.63 (m, 2H, -SO₂-N-CH-), 2.96 (m, 2H, -CH_a- protons of pyrrolidine), 2.45 (m, 2H, -CH_b- protons of pyrrolidine), 2.06(s, 3H, -CH₃, ArCH₃).

7n: *p*-Nitro phenyl; *p*-Bromo phenyl; C₂₆H₂₄BrN₉O₁₁S₄;69;219-21; C 36.85 (36.88), H 2.83 (2.86), N 14.81 (14.89); (N-N) 1032, (O = S = O)1350 and 1158, (C-N urea)1317, (C=O urea)1638 (NH sulphonamide)3313; 8.92 (s, 1H, SO₂-NH-), 8.12-8.39 (m, 8H, aromatic protons), 7.79 (d, 2H, Ar-H meta to bromo), 7.63 (d, 2H, Ar-H ortho to bromo), 7.45 (s, 2H, SO₂-NH-), 6.16 (s, 2H, NH, D₂O-exchange, disappear), 3.75 (s, 2H, -N-CH₂-), 3.50 (m, 2H, -SO₂-N-CH-), 3.01 (m, 2H, -CH_a- protons of pyrrolidine), 2.36 (m, 2H, -CH_b- protons of pyrrolidine).

7o: *p*-Nitro phenyl; *p*-Nitro phenyl; C₂₆H₂₄N₁₀O₁₃S₄; 76; 206-8; C 38.41 (38.42), H 2.95 (2.98), N 17.19 (17.23); (N-N) 1028, (O = S = O)1344 and 1155, (C-N urea)1314, (C=O urea)1634, (NH sulphonamide)3311; 8.97(s, 1H, SO₂-NH-), 8.51 (d, 2H, Ar-H ortho to nitro), 8.17-8.35 (m, 10H, aromatic protons), 7.47 (s, 2H, SO₂-NH-), 6.02 (s, 2H, NH, D₂O-exchange, disappear), 3.55 (s, 2H, -N-CH₂-), 3.52 (m, 2H, -SO₂-N-CH-), 2.92

(m, 2H, -CH_a- protons of pyrrolidine), 2.29 (m, 2H, -CH_b- protons of pyrrolidine); 62.4, 63.5, 146.2, 128.5, 124.1, 151.3, 65.0, 157.4, 59.1, 65.8, 65.1, 155.5, 147.8, 139.6, 136.5, 159.6 corresponding to C₂ and C₂, C₃ and C₃, C₄ and C₄, C₅ and C₅, C₆ and C₆, C₇ and C₇, C₈, C₉, C₁₀, C₁₁, C₁₂, C₁₃, C₁₄, C₁₅ and C₁₅, C₁₆ and C₁₆, C₁₇ respectively.

RESULTS AND DISCUSSION

The title compounds (7a-7o) were synthesized and characterized by elemental, IR and NMR spectral analysis. The compounds were screened for antibacterial and antifungal activity. The bacteria screened were *Staphylococcus aureus* NCCS 2079, *Bacillus cereus* NCCS 2106, *Escherichia coli* NCCS 2065 and fungi screened were *Aspergillus niger* NCCS 1196 and *Candida albicans* NCCS 2106. β-Ketoacyl acyl carrier protein synthase III initiates fatty acid synthesis in bacteria and is a key target enzyme to assess the antimicrobial activity. Using the series of sulfonamides under study, we conducted biological assays as well as docking study to find potent inhibitors of *E. coli* beta-ketoacyl-acp synthase III with specificities against *E. coli*. The antimicrobial activity was expressed in terms Minimum Inhibitory Concentration (MIC). MIC was found out by broth dilution method (Wiegand, Hilpert & Hancock, 2008). The activity in each case was compared with that standards. The results are given in the table 1.

The compounds were docked with *E. coli* beta-ketoacyl-acp synthase III using Schrodinger software. The docking score (table 2) and binding site details were analyzed based on interacting amino acids, and hydrogen bonds. Four sulfonamide derivatives (g, j, l, o) showed comparable docking scores as that of malonyl CoA.

A comparison of the data obtained from *in vitro* antimicrobial studies and *in silico* screening studies indicate that the compound bearing nitro substituent in each series I, II and III namely e, j and o exhibits better antimicrobial activity, while that with cyclopropyl substituent namely a, f and k exhibits least activity than the other compounds of the series. A careful comparison of table 1 and table 2 indicate that the results obtained from both the studies were of the same order of activity i.e. j > o > l > g > n > m > e > k. It can also be inferred from table 1 and table 2 that the compounds j, o, l and g bearing nitro and pentafluorophenyl substituents demonstrated comparable activity to that of standards.

Table 1.
Minimum inhibitory concentration of title compounds.

Compound		MIC* (Minimum Inhibitory Concentration) in µg/mL					
		Antibacterial activity				Antifungal activity	
		<i>Staphylococcus aureus</i> NCCS 2079	<i>Bacillus cereus</i> NCCS 2106	<i>Escherichia coli</i> NCCS 2065	<i>Pseudomonas aeruginos</i> NCCS 2200	<i>Aspergillus niger</i> NCCS 1196	<i>Candida albicans</i> NCCS 2106
I	7a	16.22	17.06	23.56	22.94m	>15	>15
	7b	8.52	9.82	15.22	18.06	8.24	12.56
	7c	11.28	13.92	20.94	19.28	>15	>15
	7d	9.46	13.48	16.58	18.62	>15	>15
	7e	7.98	8.08	12.8	15.68	5.28	7.88
	7f	14.84	15.26	21.28	22.32	>15	>15
II	7g	3.18	5.82	9.24	10.28	6.38	9.36
	7h	8.92	11.24	16.72	18.29	>15	>15
	7i	6.56	7.42	11.29	14.82	>15	>15
	7j	2.34	4.02	6.20	5.38	4.52	5.38
III	7k	8.36	8.94	14.62	18.18	>15	>15
	7l	2.52	4.98	8.62	7.38	2.58	2.66
	7m	7.26	7.82	11.68	15.20	>15	>15
	7n	4.58	6.52	9.88	10.98	>15	>15
	7o	2.46	4.56	8.46	6.84	0.90	0.82
	Cefaclor	2	4	3	3	-----	-----
	Ketoconazole	-----	-----	-----	-----	0.75	0.4

*Average of the three determinations.
Source: Authors own elaboration.

Table 2.
Docking scores of various ligands with *E.coli* beta-ketoacyl-*acp* synthase III.

Compound	Docking score	Energy	Compound	Docking score	Energy
a	-3.96	-53.45	i	-4.21	-50.36
b	-3.63	-58.53	j	-9.30	-82.56
c	-3.86	-56.32	k	-4.50	-62.62
d	-3.25	-51.79	l	-7.53	-63.32
e	-4.41	-55.931	m	-5.70	-51.62
f	-4.63	-52.08	n	-5.79	-61.76
g	-7.09	-57.62	o	-7.83	-69.29
h	-4.63	-48.68	CoA	-9.2	-78.96
Malonyl CoA	-9.3	-83.45			

Source: Authors own elaboration.

Binding mode

Binding site analysis of most active compound 7j showed strong hydrogen bonding interactions. Amino hydrogen of ARG151 forms hydrogen bond with one of the sulfonyl oxygen at a distance of 1.59 Å. Amino group of ASN 247 forms hydrogen bond with the other sulfonyl oxygen of 7j at a distance of 1.91 Å. Amino groups of ARG 36 are involved in a hydrogen bonding interaction with nitryl oxygen and pyridyl nitrogen moieties at distances of 2.08 Å and 2.40 Å respectively. Nitrophenyl moiety of 7j was involved in π - π stacking with Arg 36 and Trp 32 residues. Thiadiazole ring showed π -cation interaction with Arg 36 residue. We have observed a salt bridge formation between Arg 151 and sulfonamide nitrogen bridging nitrophenyl and thiadiazole ring. Also it is forming strong hydrophobic interactions with Met 207, Phe 213, Trp 32, Ile 156 and Phe 204 residues. Surface diagram of 7j

and interacting amino acids within 5 Å distance were shown in figure 1. Qiu *et al.* (2001) reported that *E. coli* FabH has disordered residues and were located in four loops, at 84-86, 146-152, 185-217 and 305-307 residues. All the four loops were either involved in ligand binding or in the formation of the active site. Further, except the Cys 146 - Gly 152 loop, the other three loops were known to involve in the formation of FabH dimer interface. The Apo structure of FabH contains Tyr 185-Val 217 loop which includes a large hairpin loop (Leu 189-Met 207) and a portion of the Ca1 helix (Gly 209 - Ala 230). The hairpin loop interacts with CoA using the side-chains of Leu 189 and Met 207. Similar observations were reported in the literature (Huan-Qiu *et al.*, 2009; Yang *et al.*, 2012). Qiu *et al.* 2001,s had also reported the degradation of malonyl moiety in the active site of CoA and appearance of CoA at binding site of *E. coli* FabH (Qiu *et al.*, 2001). Hence molecular docking studies for FabH and malonyl-CoA was carried out to identify a plausible malonyl-binding model and shown in the figure 2.

The enhanced biological activity of 7j over the other derivatives has been attributed to the hydrophobic interaction with Met 207 and same was not observed in the case of other derivatives. As is evident from the figure

2(b) and as reported in the literature, the interaction of malonyl CoA with Met 207 is important for substrate binding and activity (Qiu *et al.*, 2001).

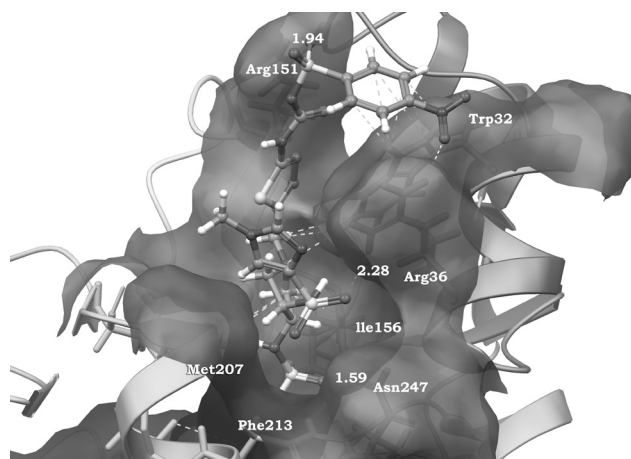


Figure 1. Surface diagram of *E. coli* FabH and binding of 7j. Orange: Hydrogen bond forming amino acid residues; Green: Hydrophobic interaction amino acid residues. Hydrogen bond distances were shown in pink dotted lines. Source: Authors own elaboration.

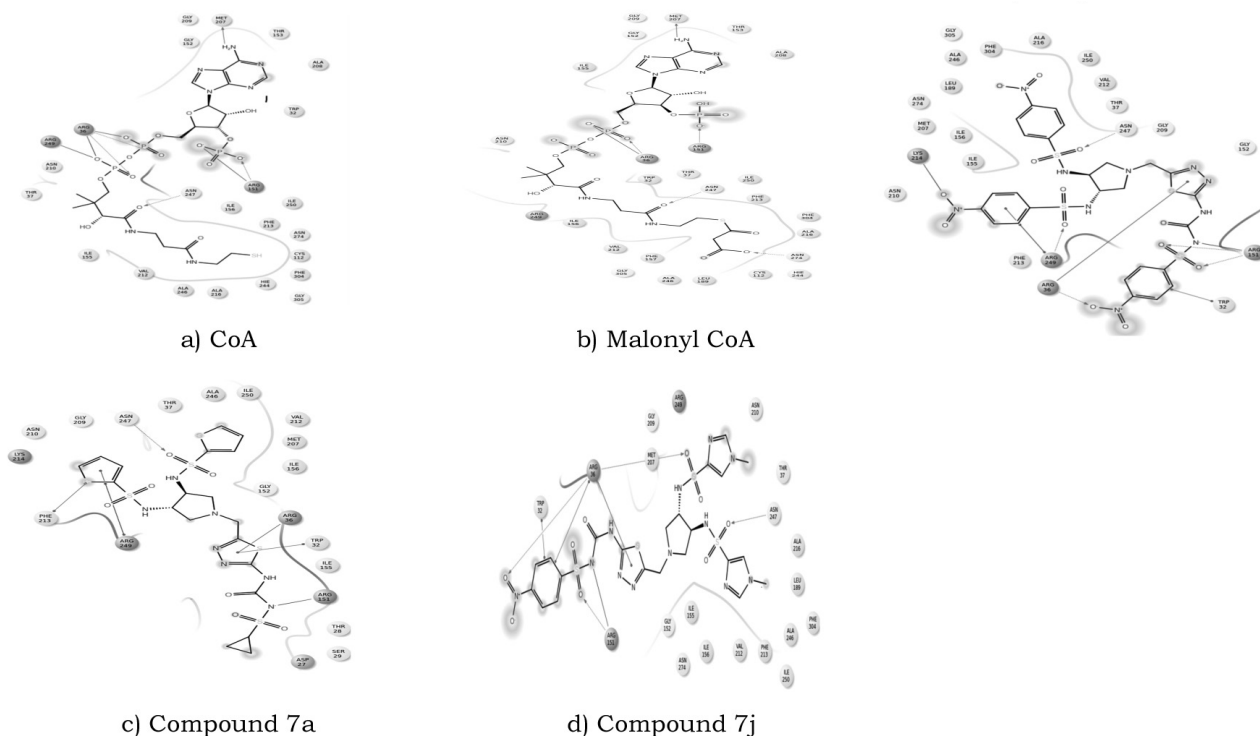


Figure 2. Binding model with *E. coli* beta-ketoacyl-ACP synthase III. Source: Authors own elaboration.

CONCLUSION

Series of sulphonyl urea derivatives containing heterocyclic moieties namely pyrrolidine, thiophene and imidazole were synthesized and characterized. The compounds were *in vitro* screened against certain selected bacteria and fungi. We tried to correlate these results with those obtained in the docking studies of compounds under study with *E. coli* beta-ketoacyl-acyl synthase III. The molecular docking results along with the antimicrobial assays suggested that compounds bearing nitro and pentafluorophenyl groups were potential inhibitors of FabH enzyme than the other members of the series.

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