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DESIGN, SYNTHESIS AND BIOLOGICAL EVOLUTION OF 3, 4-DIHYDROSELENOPHENO [2,3-*D*]PYRIMIDINE SULFONAMIDES AS POTENTIAL ANTIMICROBIAL AGENTS

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ABSTARCT

In the present study, novel 3, 4–dihydroselenopheno[2,3-*d*]pyrimidine sulfonamides were designed, synthesized, characterized and screened for their antimicrobial activities. The synthetic routes followed for the preparation of designed compounds are starting from standard Gewald reaction. Further, the standard literature protocols are used for the reaction conditions and they were stabilized. The synthesized compounds were analyzed by spectral studies to confirm their structure. Then, they were studied for their in vitro antimicrobial activity bythe well diffusion method. A total of fifteen novel 3, 4–dihydroselenopheno[2,3-*d*]pyrimidine sulfonamides were synthesized, characterized and screened for their biological activities. Among the synthesized compounds 6a and 6c exhibited a significant activity toward both Gram-positive, Gram-negative bacteria MIC at 3.12 and 6.25 µg mL^{-1}.. In addition these new agents, merit further studies to explore them as potential chemotherapeutics for antibacterial infections and for lead optimization to design a novel class of antibacterial agents.

KEYWORDS:Selenophene,3,4-dihydroselenopheno[2,3-*d*]pyrimidine,substituted3,4-dihydroselenopheno[2,3-*d*]pyrimidine sulfonamide derivatives, antimicrobial activity.

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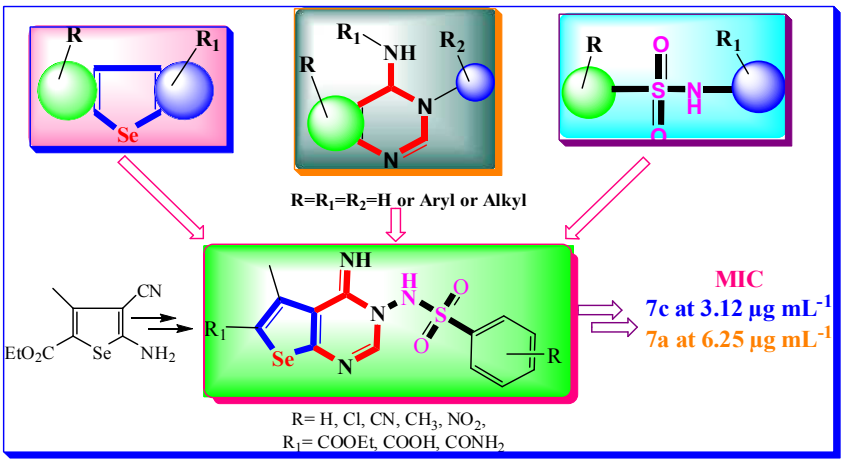
INTRODUCTION

There has been an ever-increasing significance in recent years in the introduction of Selenium (Se) into organic compounds due to its biological potency. Incorporation of one or more Se atoms into an organic molecule can enhance its efficacy, bioavailability and metabolism. In addition, the chemistry of selenophenes and their fused heterocyclic derivatives have received considerable attention owing to their synthetic and effective biological importance, because it is an essential micronutrient for animals and humans. Its bioavailability seems to depend upon the naturally occurring selenium containing amino acids selenocysteine, selenomethionine, selenocystathionine, are also involved in seleno amino acid metabolic pathways [1]. Further, recent literature survey reveals that selenophenes such as substituted selenopyrimidines and condensed selenophenopyrimidines are bioactive compounds for being studied in medicine as they are reported to possess an array of useful biological activities such as antibacterial, anti-inflammatory, analgesic activity [2–3], antioxidant [4], antimicrobial [5] and anticancer [6].



In addition, sulfonamides are among the most widely used antibacterial agents in the world, chiefly because of their low cost, low toxicity and excellent activity against common bacterial diseases. They exhibit a wide variety of pharmacological activities, such as antidiabetic, antibacterial and antitumor [7–10]. Furthermore, the synergetic action of sulfonamides with an assortment of heterocycles has brought about enormous resurgence of sulfonamide usage everywhere over the last decade [11–16]. Inspired by the diverse applications of the above nuclei and in continuation to our efforts directed toward the synthesis of innovative heterocyclic compounds [17–18] with anticipated biological activities, we have planned to synthesize a system that combines together with two biolabile components having sulfonamide and selenium derivative viz., 3, 4-dihydroselenopheno[2,3-*d*]pyrimidine as a novel breed of potent antimicrobial agents.

Experimental section

Chemistry

Melting points were determined in open capillaries on a Mel–Temp apparatus and are uncorrected. All the reactions were monitored by thin layer chromatography (TLC) on precoated silica gel 60 F254 (mesh); spots were visualized with UV light. Merck silica gel (60–120 mesh) was used for column chromatography. The IR spectra were recorded on a Perkin–Elmer BX1 FTIR Spectrophotometer as KBr pellets and the wave numbers were given in cm^{-1}. ^{1}H NMR (400 MHz), and ^{13}C NMR (100 MHz) spectra

were recorded on a Bruker AMX 400 MHz NMR spectrometer in $CDCl_3$/DMSO–d_6 solution using TMS as an internal standard. All chemical shifts are reported in δ (ppm) using TMS as an internal standard. The mass spectra were recorded on Agilent 1100 LC/MSD instrument with method API–ES at 70 eV. The microanalyses were performed on a Perkine Elmer 240C elemental analyzer. The antioxidant property was carried out by using Shimadzu UV–2450 spectrophotometer.

Synthesis

Synthesis of ethyl 5-amino-4-cyano-3-methylselenophene-2-carboxylate (1)

The starting ethyl 5-amino-4-cyano-3-methylselenophene-2-carboxylate (1) was prepared according to reported Gewald synthetic procedure [19]. A mixture of ethyl acetoacetate (3.0 mmol), dicyanomethane (3.3 mmol), selenium powder (4.5 mmol), and imidazole (0.3 mmol) in DMF (3.0 mL) was stirred at 60 ºC under nitrogen atmosphere for 16 h. After completion of starting materials, the reaction mixture was cool to room temperature, and then the unreacted selenium powder was filtered. The filtrate was then poured onto ice cold water, and stirred for 15 mins. The solid obtained was collected by filtration and recrystallized from ethanol. Yellowish solid Yield 53%; m.p. = 212–214 ºC; IR (chloroform) v (cm^{-1}): 3435 (NH_2), 2206 (C≡N), 1641 (C=O); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.41 (t, 3H, –CH_2–$\underline{CH_3}$), 2.48 (s, 3H, selenophene−CH_3), 4.27 (q, 2H, –$\underline{CH_2}$–CH_3), 5.50 (br s, 2H, NH_2); ^{13}C NMR ($CDCl_3$, 100 MHz); δ 14.14, 14.59, 60.02, 88.65, 106.88, 114.90, 146.25, 161.24 and 166.57; LC–MS (negative ion mode): m/z 257 $(M–H)^-$ for $C_9H_{10}N_2O_2Se$.

Synthesis of 4-cyano-5-ethoxymethyleneimino-3-methylselenophene-2-carboxylic acid ethyl ester (*2*)

A mixture of 5-amino-4-cyano-3-methylselenophene-2-carboxylicacid ethyl ester (1) (2.0 g, 7.78 mmol) and triethyl orthoformate (7.15 mL, 38.90 mmol) was refluxed for 8 h. After completion of starting compound the reaction mixture was cooled, the excess amount of triethyl orthoformate was concentrated and the solid obtained was recrystallized from ethanol. Yield: 86%; Red colored solid; m.p.: 106–108 ºC; IR (KBr) v (cm^{-1}): 2958, 2894, 2210, 1690, 1558; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.36 (t, 3H, –$OCH_2\underline{CH_3}$), 1.43 (t, 3H, CH_3–ester), 2.58 (s, 3H, CH_3–selenophene), 4.31(q, 2H, –$O\underline{CH_2}CH_3$), 4.47 (q, 2H, CH_2–ester), 7.90 (s, 1H, N=$\underline{CH}$); ^{13}C NMR ($CDCl_3$, 100 MHz); δ 14.28, 15.84, 16.25, 61.31, 62.12, 109.86, 114.23, 115.67, 145.12, 153.51, 156.49 and 167.21; LC–MS (positive ion mode): m/z 315 $(M+H)^+$ for $C_{12}H_{14}N_2O_3Se$.

Synthesis of 3-Amino-4-imino-5-methyl-3,4dihydro selenopheno[2,3-d]pyrimidine-6-carboxylic acid ethyl ester (*3*)

A solution of hydrazine (1.0 ml, 20.36 mmol) was added to a cold (0–5 ºC) solution of 2 (1.6 g, 5.09 mmol) in EtOH (20 ml). The mixture was stirred at room temperature for 5 h. The reaction mixture was then poured into ice water and the precipitate formed was filtered, washed with ice cold water, and recrystallized form chloroform and hexane.

Yield 85%, Brown colored solid; m.p. 190–192ºC; IR (KBr) v (cm^{-1}): 3380, 3315, 3200, 2980, 1665, 1512; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.32 (t, 3H, CH_3–ester), 2.48 (s, 3H, CH_3–selenophene), 4.26 (q, 2H, –$\underline{CH_2}$-ester), 5.74 (brs, 2H, NH_2), 6.99 (brs, 1H, NH), 8.50 (s, 1H, CH–pyrimidine); ^{13}C NMR ($CDCl_3$, 100 MHz); δ 14.34, 16.41, 60.87, 106.52, 118.26, 128.43, 142.68, 152.24, 164.17 and 168.86; LC–MS (positive ion mode): m/z 301 $(M+H)^+$ for $C_{10}H_{12}N_4O_2Se$.

General procedure for the synthesis of compound 4a–e

A mixture of compound 3 (1.0 mmol) and appropriate substituted aryl sulfonyl chlorides (1.1 mmol) in dry THF were refluxed for 2 h. After completion of the reaction (monitored by TLC), the solvent was removed under reduced pressure, ice cold water was added and stirred for 15 mins. The resulting solid was filtered off,

dried under vacuum and recrystallized from chloroform and hexane to produce 4a–e in good yields.

***Ethyl4-imino-5-methyl-3-(phenylsulfonamido)-3,4-dihydroselenopheno[2,3-d]pyrimidine-6-carboxylate* (4a)**

Yield 72%, off-white colored solid; m.p. 224–226ºC; IR (KBr) ν (cm^{-1}): 3385, 3318, 3119, 2965, 2876, 1683, 1520; ^{1}H NMR (DMSO–d_6, 400 MHz) δ 1.26 (t, 3H, –C<u>H</u>$_3$-ester), 2.21 (s, 3H, CH_3–selenophene), 4.18 (q, 2H, –<u>C</u>H$_2$-ester), 7.36 (brs, 1H, –N<u>H</u>), 7.68 (m, 5H, phenyl), 8.74 (s, 1H, CH–pyrimidine); 12.42 (brs, 1H, –SO_2–N<u>H</u>); ^{13}C NMR (DMSO–d_6, 100 MHz); δ 14.82, 16.12, 61.76, 109.21, 117.47, 122.80, 125.64, 126.28, 130.42, 135.14, 143.22, 154.25, 166.48 and 172.04; LC–MS (positive ion mode): m/z 441 $(M+H)^+$ for $C_{16}H_{16}N_4O_4SSe$.

***Ethyl3-(4-chlorophenylsulfonamido)-4-imino-5-methyl-3,4-dihydroselenopheno[2,3-d] pyrimidine-6-carboxylate* (4b)**

Yield 80%, Off-white colored solid; m.p. 230–232ºC; IR (KBr) ν (cm^{-1}): 3380, 3315, 3110, 2958, 2872, 1680, 1525; ^{1}H NMR (DMSO–d_6, 400 MHz) δ 1.25 (t, 3H, –C<u>H</u>$_3$-ester), 2.90 (s, 3H, CH_3–selenophene), 4.26 (q, 2H, –<u>C</u>H$_2$-ester), 7.21 (brs, 1H, –N<u>H</u>), 7.52 (d, 2H, *J*=8.0 Hz),7.76 (d, 2H, *J*=8.0 Hz), 8.17 (s, 1H, CH–pyrimidine); 12.56 (brs, 1H, –SO_2–N<u>H</u>); ^{13}C NMR (DMSO–d_6, 100 MHz); δ 14.10, 16.28, 62.14, 107.95, 117.26, 124.04, 126.52, 128.37, 131.64, 138.60, 141.86, 155.13, 164.54 and 168.90; LC–MS (positive ion mode): m/z 475 $(M+H)^+$ for $C_{16}H_{15}ClN_4O_4SSe$

***Ethyl4-imino-5-methyl-3-(4-methylphenylsulfonamido)-3,4-dihydroselenopheno[2,3-d] pyrimidine-6-carboxylate* (4c)**

Yield 64%, White colored solid; m.p. 212–214ºC; IR (KBr) ν (cm^{-1}): 3383, 3304, 3203, 2950, 2865, 1670, 1491; ^{1}H NMR (DMSO–d_6, 400 MHz) δ 1.22 (t, 3H, –C<u>H</u>$_3$-ester), 2.34 (s, 3H, phenyl–CH_3), 3.02 (s, 3H, CH_3–selenophene), 4.24 (q, 2H, –<u>C</u>H$_2$-ester), 7.06 (brs, 1H, –N<u>H</u>), 7.16 (d, 2H, *J*=8.0 Hz),7.54 (d, 2H, *J*=8.0 Hz), 8.21 (s, 1H, CH–pyrimidine); 12.72 (brs, 1H, –SO_2–N<u>H</u>); ^{13}C NMR (DMSO–d_6, 100 MHz); δ 14.24, 15.86, 16.07, 61.56, 107.02, 118.60, 122.44, 124.75, 126.60, 129.86, 136.22, 140.45, 156.52, 166.81 and 169.74; LC–MS (positive ion mode): m/z 455 $(M+H)^+$ for $C_{17}H_{18}N_4O_4SSe$.

***Ethyl3-(4-cyanophenylsulfonamido)-4-imino-5-methyl-3,4-dihydroselenopheno[2,3-d] pyrimidine-6-carboxylate* (4d)**

Yield 76%, Brown colored solid; m.p. 247–249ºC; IR (KBr) ν (cm^{-1}): 3381, 3314, 3210, 2885, 2218, 1676, 1519; ^{1}H NMR (DMSO–d_6, 400 MHz) δ 1.29 (t, 3H, –C<u>H</u>$_3$ ester), 3.12 (s, 3H, CH_3–selenophene), 4.18 (q, 2H, –<u>C</u>H$_2$-ester), 6.82 (s, 1H, –N<u>H</u>), 7.24 (d, 2H, *J*=8.0 Hz), 7.80 (d, 2H, *J*=8.0 Hz), 8.94 (s, 1H, CH–pyrimidine); 12.61 (brs, 1H, –SO_2–N<u>H</u>); ^{13}C NMR (DMSO–d_6, 100 MHz); δ 15.04, 16.20, 60.82, 110.05, 117.14, 118.62, 120.88, 125.56, 128.17, 130.25, 134.92, 140.01, 154.62, 168.46 and 171.0<u>5</u>; LC–MS (negative ion mode): m/z 464 (M–H) for $C_{17}H_{15}N_5O_4SSe$.

***Ethyl4-imino-5-methyl-3-(4-nitrophenylsulfonamido)-3,4-dihydroselenopheno[2,3-d] pyrimidine-6-carboxylate* (4e)**

Yield 66%, Yellow colored solid; m.p. 262–264ºC; IR (KBr) ν (cm^{-1}): 3386, 3309, 3213, 2914, 1671, 1528; ^{1}H NMR (DMSO–d_6, 400 MHz) δ 1.22 (t, 3H, –C<u>H</u>$_3$-ester), 2.34 (s, 3H, CH_3–selenophene), 4.16 (q, 2H, –<u>C</u>H$_2$-ester), 7.16 (brs, 1H, –N<u>H</u>), 8.20 (d, 2H, *J*=8.0 Hz),8.30 (d, 2H, *J*=8.0 Hz), 8.72 (s, 1H, CH–pyrimidine); 12.46 (brs, 1H, –SO_2–N<u>H</u>); ^{13}C NMR (DMSO–d_6, 100 MHz); δ 14.68, 16.52, 62.12, 112.15, 114.80, 121.32, 124.30, 127.82, 131.28, 133.42, 141.73, 156.11, 163.84 and 167.96; LC–MS (negative ion mode): m/z 484 (M–H) for $C_{16}H_{15}N_5O_6SSe$.

General procedure for the synthesis of compound (5a-e)

The compound (4a-e) was dissolved in MeOH/ H_2O (12 mL: 6 mL), and 15% v/v NaOH aq (2 mL) was added. Stirring was continued for 16h at rt, then $CHCl_3$ was added. The aqueous layer was acidified with 1 N HCl, stirred for 15 min, the product was separated by vacuum filtration and washed with water, dried well and recrystallized from chloroform and methanol to give compounds 5a–e in good yields.

***4-Imino-5-methyl-3-(phenylsulfonamido)-3,4-dihydroselenopheno[2,3-d]pyrimidine-6-carboxylic acid* (5a)**

Yield 82%, off-white colored solid; m.p. 286–288ºC; IR (KBr) ν (cm^{-1}): 3410, 3296, 3210, 2908, 1580, 1510; ^{1}H NMR (DMSO–d_6, 400 MHz) δ 2.30 (s, 3H, CH_3–selenophene), 7.11 (brs, 1H, –NH), 7.56 (m, 5H, phenyl), 8.90 (s, 1H, CH–pyrimidine); 12.84 (brs, 1H, $–SO_2–$NH), 13.02, (brs, 1H, –COOH); ^{13}C NMR (DMSO–d_6, 100 MHz); δ 15.16, 113.04, 116.20, 120.84, 128.01, 129.16, 132.72, 136.54, 142.20, 153.10, 166.88 and 169.08; LC–MS (negative ion mode): m/z 411 (M–H) for $C_{14}H_{12}N_4O_4SSe$.

***3-(4-Chlorophenylsulfonamido)-4-imino-5-methyl-3,4-dihydroselenopheno[2,3-d]pyrimidine-6-carboxylic acid* (5b)**

Yield 80%, off-white colored solid; m.p. 292–294ºC; IR (KBr) ν (cm^{-1}): 3392, 3302, 3211, 2911, 1676, 1520; ^{1}H NMR (DMSO–d_6, 400 MHz) δ 2.72 (s, 3H, CH_3–selenophene), 7.09 (brs, 1H, –NH), 7.46 (d, 2H, *J*=8.0 Hz),7.91 (d, 2H, *J*=8.0 Hz), 8.76 (s, 1H, CH–pyrimidine); 12.20 (brs, 1H, $–SO_2–$NH), 13.44, (brs, 1H, –COOH); ^{13}C NMR (DMSO–d_6, 100 MHz); δ 15.94, 112.82, 115.20, 121.68, 127.40, 128.07, 130.74, 135.21, 142.90, 152.46, 167.50 and 168.93; LC–MS (negative ion mode): m/z 445 (M–H) for $C_{14}H_{11}ClN_4O_4SSe$.

***4-Imino-5-methyl-3-(4-methylphenylsulfonamido)-3,4-dihydroselenopheno[2,3-d]pyrimidine-6-carboxylic acid* (5c)**

Yield 76%, white colored solid; m.p. 311–313ºC; IR (KBr) ν (cm^{-1}): 3382, 3300, 3204, 2909, 1654, 1533; ^{1}H NMR (DMSO–d_6, 400 MHz) δ 2.56 (s, 3H, CH_3–selenophene), 2.91 (s, 3H, $–OCH_3$), 6.88 (brs, 1H, –NH), 7.82 (d, 2H, *J*=8.0 Hz), 8.06 (d, 2H, *J*=8.0 Hz), 8.57 (s, 1H, CH–pyrimidine); 12.80 (brs, 1H, $–SO_2–$NH); ^{13}C NMR (DMSO–d_6, 100 MHz); δ 15.63, 23.07, 110.82, 114.16, 126.8, 127.40, 128.07, 130.74, 135.21, 142.90, 152.46, 167.50 and 168.93; LC–MS (positive ion mode): m/z 427 $(M+H)^+$ for $C_{15}H_{14}N_4O_4SSe$.

***3-(4-Cyanophenylsulfonamido)-4-imino-5-methyl-3,4-dihydroselenopheno[2,3-d]pyrimidine-6-carboxylic acid* (5d)**

Yield 81%, White colored solid; m.p. 293–295ºC; IR (KBr) ν (cm^{-1}): 3396, 3312, 3225, 2906, 2227, 1668, 1544; ^{1}H NMR (DMSO–d_6, 400 MHz) δ 2.61(s, 3H, CH_3–selenophene), 6.43 (brs, 1H, –NH), 7.67 (d, 2H, *J*=8.0 Hz), 7.86 (d, 2H, *J*=8.0 Hz), 8.48 (s, 1H, CH–pyrimidine); 12.63 (brs, 1H, $–SO_2–$NH); ^{13}C NMR (DMSO–d_6, 100 MHz); δ 15.50, 112.36, 114.16, 124.62, 126.90, 128.44, 132.04, 136.40, 142.62, 144.71, 150.20, 166.08 and 169.16; LC–MS (negative ion mode): m/z 436 (M–H) for $C_{15}H_{11}N_5O_4SSe$.

***4-Imino-5-methyl-3-(4-nitrophenylsulfonamido)-3,4-dihydroselenopheno[2,3-d]pyrimidine-6-carboxylic acid* (5e)**

Yield 72%, White colored solid; m.p. 281–283ºC; IR (KBr) ν (cm^{-1}): 3380, 3304, 3230, 2911, 1669, 1530; ^{1}H NMR (DMSO–d_6, 400 MHz) δ 2.40 (s, 3H, CH_3–selenophene), 7.07 (s, 1H, –NH), 7.82 (d, 2H, *J*=8.0 Hz),8.04 (d, 2H, *J*=8.0 Hz), 8.69 (s, 1H, CH–pyrimidine); 12.51 (brs, 1H, $–SO_2–$NH); ^{13}C NMR (DMSO–d_6, 100 MHz); δ 15.81, 112.20, 114.07, 122.84, 126.92, 127.46, 131.72, 137.13, 146.00, 153.12, 165.86 and 168.90; LC–MS (positive ion mode): m/z 458 $(M+H)^+$ for $C_{14}H_{11}N_5O_6SSe$.

General procedure for the synthesis of compound (6a-e)

A solution of 15 mL NH_4 OH was stirred at 0-5ºC, to this added compound (4a–e) (1.0 mmol) in dry THF by drop wise for 10 mins and the total reaction mixture was stirred at room temperature for 48 h. After completion of the

reaction (monitored by TLC), reaction mixture was poured into ice cold water and stirred for 15 mins. The obtained solid was filtered and dried under vacuum to produce compounds (6a–e) in moderate yields.

***4-Imino-5-methyl-3-(phenylsulfonamido)-3,4-dihydroselenopheno[2,3-d]pyrimidine-6-carboxamide* (6a)**

Yield 52%, White colored solid; m.p. 337–339ºC; IR (KBr) ν (cm^{-1}): 3486, 3426, 3304, 2910, 1672, 1506; ^{1}H NMR (DMSO–d_6, 400 MHz) δ 2.33 (s, 3H, CH_3–selenophene), 6.70 (brs, 1H, –NH), 7.64 (m, 5H, phenyl), 7.82 (s, 2H, –CONH_2), 8.66 (s, 1H, CH–pyrimidine); 12.41 (brs, 1H, –SO_2–NH); ^{13}C NMR (DMSO–d_6, 100 MHz); δ 15.37, 112.79, 118.03, 121.40, 124.82, 128.14, 133.44, 136.76, 144.03, 152.94, 166.73 and 168.22; LC–MS (positive ion mode): m/z 412 $(M+H)^+$ for $C_{14}H_{13}N_5O_3SSe$

***3-(4-Chlorophenylsulfonamido)-4-imino-5-methyl-3,4-dihydroselenopheno[2,3-d]pyrimidine-6-carboxamide* (6b)**

Yield 60%, Pale Yellow colored solid; m.p. 321–323ºC; IR (KBr) ν (cm^{-1}): 3448, 3398, 3310, 2911, 1670, 1520; ^{1}H NMR (DMSO–d_6, 400 MHz) δ 2.50 (s, 3H, CH_3–selenophene), 6.93 (brs, 1H, –NH), 7.28 (d, 2H, *J*=8.0 Hz), 7.67 (s, 2H, –CONH_2), 8.01 (d, 2H, *J*=8.0 Hz), 8.22 (s, 1H, CH–pyrimidine); 12.08 (brs, 1H, –SO_2–NH); ^{13}C NMR (DMSO–d_6, 100 MHz); δ 15.93, 112.82, 116.01, 123.76, 128.52, 129.13, 131.40, 136.03, 142.20, 153.18, 165.09 and 167.26; LC–MS (positive ion mode): m/z 446 $(M+H)^+$ for $C_{14}H_{12}ClN_5O_3SSe$.

***4-Imino-5-methyl-3-(4-methylphenylsulfonamido)-3,4-dihydroselenopheno[2,3-d]pyrimidine-6-carboxamide* (6c)**

Yield 54%, off-white colored solid; m.p. 342–344ºC; IR (KBr) ν (cm^{-1}): 3452, 3378, 3309, 2923, 2892, 1660, 1522; ^{1}H NMR (DMSO–d_6, 400 MHz) δ 2.61 (s, 3H, CH_3–selenophene), 2.94 (s, 3H, –OCH_3), 7.03 (brs, 1H, –NH), 7.14 (d, 2H, *J*=8.0 Hz), 7.70 (s, 2H, –CONH_2), 8.10 (d, 2H, *J*=8.0 Hz), 8.78 (s, 1H, CH–pyrimidine); 12.92 (brs, 1H, –SO_2–NH); ^{13}C NMR (DMSO–d_6, 100 MHz); δ 15.66, 24.17, 111.74, 118.20, 126.87, 128.73, 131.09, 136.42, 142.11, 154.20, 163.75 and 168.46; LC–MS (positive ion mode): m/z 426 $(M+H)^+$ for $C_{15}H_{15}N_5O_3SSe$.

***3-(4-Cyanophenylsulfonamido)-4-imino-5-methyl-3,4-dihydroselenopheno[2,3-d]pyrimidine-6-carboxamide* (6d)**

Yield 61%, White colored solid; m.p. 337–339ºC; IR (KBr) ν (cm^{-1}): 3481, 3392, 3327, 2915, 2221, 1647, 1532; ^{1}H NMR (DMSO–d_6, 400 MHz) δ 2.43 (s, 3H, CH_3–selenophene), 6.82 (brs, 1H, –NH), 7.62 (d, 2H, *J*=8.0 Hz),7.84 (s, 2H, –CONH_2), 7.92 (d, 2H, *J*=8.0 Hz), 8.87 (s, 1H, CH–pyrimidine); 12.58 (brs, 1H, –SO_2–NH); ^{13}C NMR (DMSO–d_6, 100 MHz); δ 15.53, 111.80, 114.63, 122.71, 124.32, 126.24, 128.16, 132.43, 137.28, 146.21, 153.77, 164.62 and 169.03; LC–MS (negative ion mode): m/z 435 (M–H) for $C_{15}H_{12}N_6O_3SSe$.

***4-Imino-5-methyl-3-(4-nitrophenylsulfonamido)-3,4-dihydroselenopheno[2,3-d]pyrimidine-6-carboxamide* (6e)**

Yield 55%, pale yellow colored solid; m.p. 312–314ºC; IR (KBr) ν (cm^{-1}): 3492, 3386, 3308, 2916, 1656, 1538; ^{1}H NMR (DMSO–d_6, 400 MHz) δ 2.68 (s, 3H, CH_3–selenophene), 7.02 (s, 1H, –NH), 7.76 (s, 2H, –CONH_2), 7.88 (d, 2H, *J*=8.0 Hz),8.26 (d, 2H, *J*=8.0 Hz), 8.71 (s, 1H, CH–pyrimidine); 12.56 (brs, 1H, –SO_2–NH); ^{13}C NMR (DMSO–d_6, 100 MHz); δ 15.84, 112.26, 114.25, 122.40, 126.81, 129.74, 131.92, 138.01, 148.17, 154.20, 163.72 and 169.86; LC–MS (positive ion mode): m/z 457 $(M+H)^+$ for $C_{14}H_{12}N_6O_5SSe$.

Experimental Biology

Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) is the lowest concentration of the antimicrobial agent that prevents the development of visible growth after overnight incubation. Minimum inhibitory concentrations are important in diagnostic laboratories to confirm resistance of

microorganisms to antimicrobial agents[22] and also to monitor the activity of new antimicrobial agents. MIC measurements were performed using a modified agar well diffusion method.

Antibacterial Assay

Antimicrobial activity of all the newly synthesized compounds were assayed against two Gram positive bacteria such as *L.bacillus* and *Staphylococcus aureus* and two Gram negative bacteria such as *P.florescensa* and *E.coli* by agar well diffusion method, 200 µg of the tested compounds were dissolved in 1 mL of DMSO solvent. Centrifuged pellets of bacteria from 24 h old culture containing approximately 104–106 colony forming unit (CFU) per mL was spread on the surface of Muller Hinton Agar (MHA) plates. The nutrient agar medium were prepared by suspending nutrient agar 28 g in 1 liter of distilled water, autoclaved and cooled to 45 °C, and then it was seeded with 15 mL of prepared inocula to have 106 CFU/mL. Petri dishes were prepared by pouring 10 mL of seeded nutrient agar. Wells were created in medium with the help of a sterile metallic borer and test solution was added. Experimental plates were incubated for 24 h at 37°C. Amoxyclav was used as standard drug for antibacterial assay.

Antifungal assay

Antifungal activity of all the newly synthesized were tested with *Aspergillus niger and Penicillium sp* by the poison plate technique. Tested compounds were dissolved in DMSO before mixing with potato dextrose agar (PDA). The final concentration of the compounds in the medium was fixed at 200 µg/ mL. Two kinds of fungi were incubated in PDA at 25 ±1 °C for 5 days to get new mycelium for antifungal assay, and then a mycelia disk of approximately 0.45 cm diameter cut from the culture medium was picked up with a sterilized inoculation needle and inoculated in the center of the PDA plate. The inoculated plates were incubated at 25 ±1 °C for 5 days. DMSO solvent was added as a negative control to determine the possible inhibitory activity of the solvent, while Fluconazole was used as positive control.

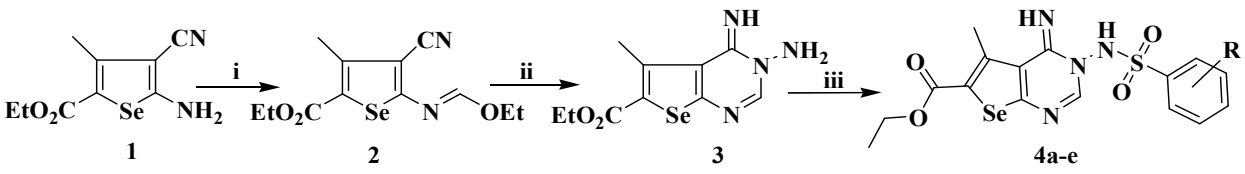
RESULTS AND DISCUSSION

Synthetic Chemistry

In order to develop potent antibacterial agents of desired, we have designed the synthetic routes for the preparation of titles compounds outlined in the schemes 1 and 2. A retro synthetic analysis of these agents suggested that an amino, nitrile seleophene ester ring would be prepared first, using the standard Gewald reaction[19] on thiophene derivatives were employed for the synthesis of key starting compound **1** with good yield. The structure of the newly synthesized compound was assigned by IR, ^{1}H &^{13}C NMR and LC-MS spectral data. The ^{1}H NMR spectrum of compound 1 showed a triplet and quartet at *δ* 1.41 and 4.27 ppm corresponds to the $–CH_3$ and $–OCH_2$- respectively related to ethyl ester and a broad singlet at *δ* 5.50 ppm corresponds to the $–NH_2$. The LC-MS spectrum showed m/z value at 257 in concordance with calculated and found value. The amino selenophenecyano carboxylate (1) was treated with triethylorthoformate to form imidoformate 2 intermediate[20] in excellent yield at refluxing temperature.

1

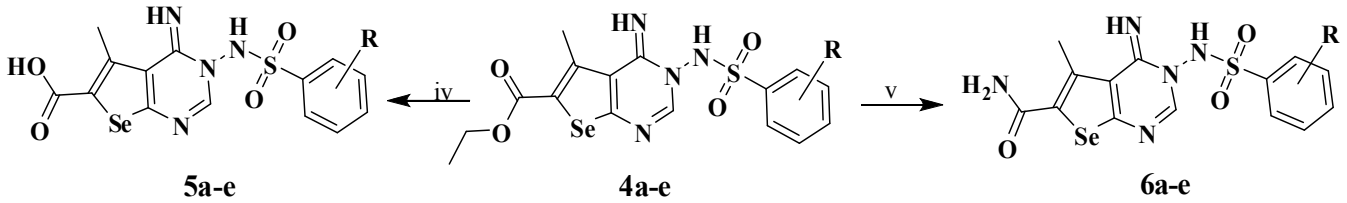
Scheme 1



Reagents and Conditions: i). TEOF, reflux, 4h, ii). NH_2NH_2. H_2O, EtOH, rt iii). THF, appropriate substituted Sulfonyl Chlorides, reflux, 2 h.

The structure of the compound 2 was established on the basis of its ^{1}H NMR and LC-MS spectral data. The ^{1}H NMR spectrum showed a triplet and quarter at *δ* 1.36 and 4.31 ppm corresponds to the $–CH_3$ and $–OCH_2$ related to the ethoxy group instead of primary amine at *δ* 5.50 ppm in compound 1. The LC-MS spectrum of the compound showed a molecular ion peak at 315 in positive ion mode. Compound 2 for treatment with pure hydrazine hydrate in ethanol at room temperature [21] yielded the selenophenopyrimidine derivative 3 which was obtained through the nucleophilic addition of hydrazine hydrate on the carbonitrile, then intramolecular cyclization by the loss of one mole ethanol. The structure of the compound 3 was established on the basis of its IR, NMR and LC-MS spectral data analysis. The ^{1}H NMR spectrum showed a characteristic peaks at *δ* 5.74 and 6.99 ppm corresponds to the primary and secondary amine respectively and a molecular ion peak at 301 in positive ion mode in its LC-MS spectrum is in good agreement between calculated and found mass. Compound 3 contains two reactive groups such as primary and secondary amines among which the former is more reactive than that of the other. Based on this chemistry we have prepared the sulfonamides with substituted sulfonyl chlorides to get titled compounds 4a–e as shown in scheme 1. The structures of 4a-e were established on the basis of their spectral data analysis. Further a series of designed derivatives 5a–e and 6a–e were prepared by articulation of 4a–e with sodium hydroxide, and ammonium hydroxide to afford 5a–e and 6a–e respectively.

Scheme 2



5a-e **4a-e** **6a-e**

Reagents and Conditions: iv). NaOH, MeOH, rt, 16 h, v). NH_4 OH, THF, 0–25°C

Compound	R
4a, 5a, 6a	H
4b, 5b, 6b	4-Cl
4c, 5c, 6c	4-CH_3
4d, 5d, 6d	4-CN
4e, 5e, 6e	4-NO_2

The detailed experimental protocols and their spectral data (IR, ^{1}H, ^{13}C–NMR, and LC–MS) analysis were explained in the experimental section.

Biological Evaluation

Antimicrobial activity

In order to search for the potent candidate, the newly synthesized compounds 4a–e, 5a–e and 6a–e were evaluated for their *in–vitro* antibacterial activity against Gram–positive bacteria such as *Lacto bacillus* and *Staphylococcus aureus*, Gram–negative bacteria such as *Pseudomonas florescensa* and *Escherichia coli* and antifungal activity was carried out by fungal strains such as *Aspergillus niger* and *Penicillium sp* using agar well diffusion method [22]. The test compounds were dissolved in dimethyl sulfoxide (DMSO) at concentrations of 200 µg mL^{-1}. The antibacterial screening exhibited that some of the tested compounds showed excellent inhibition against various tested microbial strains Table 1. Compounds 4a–

e, 5a–e and 6a–e with various substituents in the phenyl ring are studied to understand the influence of electron withdrawing groups on the antimicrobial activity.

Antibacterial activity

For evaluating antibacterial activity Amoxyclav was used as the standard drug. The observed minimum inhibitory concentrations (MIC) data is presented in Table 1. As can be seen from our results, all the synthesized compounds found to be effective antibacterial activity *in–vitro* against the tested organisms. Compounds with the MIC in the range of 3.12–6.25 μg mL^{-1} are reported as potent and MIC in the range 12.5–25 μg mL^{-1} are reported with good inhibition activity. 5a, 5c, 6a and 6c exhibited impressive potent antibacterial activity as that of the standard Amoxyclav. Compounds 4a, 4b, 4c, 5b and 6b are found to be good among the series and even comparable activity with standard as antibacterial agents against all the tested bacterial cultures. In case of 4b, 5b and 6b bearing a chloro substituent on the phenyl ring possessed strong activity. The remaining compounds of these series were found to have moderate antibacterial activity. The results showed a good structural–activity relationship. 6c with electron releasing methyl substituent at *para* position on phenyl ring and electron withdrawing amide group on selenophene showed highest antibacterial activity 3.12 μg mL^{-1} and 6.25 μg mL^{-1} against Gram–positive and Gram–negative bacteria respectively. The unsubstituted phenyl in 6a showed potent activity at 6.25 μg mL^{-1} against all the bacterial cultures. 5a and 5c showed good activity due to the presence of unsubstituted and methyl substituent on phenyl ring along with electron withdrawing carboxylic acid group on selenophene. In case of compounds 4c and 4a there is a common ester as electron withdrawing substituent on selenophene, but compound 4c showed good activity whereas 4a showed moderate activity due to the presence of electron releasing methyl substituent and unsubstituted phenyl ring respectively. On the other hand, compounds 4d, 4e, 5d, 5e, 6d and 6e showed low activity compare with that of the standard even though they have electron withdrawing substituent on selenophene. These results clearly indicate that the electron withdrawing substituents such as CN and NO_2 on phenyl ring decrease the antibacterial activity.

Compound	Antibacterial activity				Antifungal activity	
4a	12.5	12.5	12.5	12.5	25	25
4b	12.5	12.5	25	25	12.5	12.5
4c	12.5	6.25	6.25	12.5	12.5	25
4d	50	50	50	50	12.5	12.5
4e	50	50	50	50	12.5	12.5
5a	6.25	12.5	6.25	6.25	100	50
5b	12.5	12.5	12.5	25	50	50
5c	12.5	6.25	6.25	6. 25	25	50
5d	50	50	50	50	12.5	6.25
5e	50	50	50	50	12.5	12.5
6a	6.25	6.25	6.25	6.25	50	50
6b	12.5	12.5	12.5	12.5	25	50
6c	3.12	3.12	6.25	6.25	50	50
6d	50	50	50	50	25	25
6e	50	50	50	50	12.5	12.5
Amoxyclav	12.5	12.5	12.5	12.5	—	—
Fluconazole	—	—	—	—	12.5	12.5
Control	—	—	—	—	—	—

Table1

Minimum Inhibitory Concentration (MIC) in μg/ml of compounds (4a–e), (5a–e) and (6a–e) derivatives against bacterial and fungal strains evaluated by microdilution method

Antimicrobial Assay

Antifungal activity

For evaluating the antifungal activity, fluconazole was used as the standard drug. The observed minimum inhibitory concentrations (MIC) are given in Table 1. It is seen that compound 5d exhibited potent activity at 6.25 µg ml^{-1} against *Penicillium species* and comparable activity against *Aspergillus niger.* Compounds 4b, 4c, 4d, 4e, 5e and 6e also exhibited comparable activity at 12.5–25 µg ml^{-1} against both the fungal cultures. The remaining compounds in this series were found to have moderate to low activity compared with that of the standard. Among all the synthesized compounds, seven compounds showed MIC in the range 6.25–12.5 µg ml^{-1}. The results strongly suggest that the antifungal activity evidently influenced by the aromatic substituent. The electron withdrawing NO_2 group on phenyl ring develops the antifungal activity. In case of compound 5d showed greater activity than the standard due to the presence of electron withdrawing CN group.

CONCLUSION

In this study, we have designed and synthesized a series of novel compounds based on 3,4–dihydroselenopheno[2,3–*d*]pyrimidine sulfonamides successively, by using simple protocols with good yields and evaluated for their antimicrobial activity. Out of the synthesized compounds six analogues have shown MIC in the range of 3.12–6.25 µg mL^{-1}. The compounds 4c, 5a, 5c, 5d, 6a and 6d were found to be potent candidate than the standard. Thus the presence of unsubstituted phenyl ring, electron releasing methyl and chlorine substituents on phenyl ring showed potent antibacterial activity. In case of antifungal activity only a few of them were showed moderate to good activity. Therefore it is concluded that the antifungal activity is of our synthesized compounds in the presence of electron withdrawing NO_2 group attached to the phenyl ring has responsible for the anti fungal activity. In addition these new agents, merit further studies to explore them as potential chemotherapeutics for antibacterial infections and for lead optimization to design a novel class of antibacterial agents.

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