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Research Article

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Synthesis and antiviral activity of 1,3,4-oxadiazolyl selenopheno[2,3-*d*]pyrimidines as novel Bluetongue virus inhibitors

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ABSTRACT

*As part of ongoing studies in developing new antiviral agents against Bluetongue Virus, a series of novel 1,3,4-oxadiazolyl selenopheno[2,3-d]pyrimidines bearing a choice of aromatic moieties with different substituents were synthesized in five steps reaction sequence with simple protocols. The newly synthesized compounds were characterized by NMR, LC–MS and IR analyses. All the compounds evaluated for their antiviral activity against Bluetoungue Virus (BTV) in Baby Hamster Kidney (BHK–21) cell lines by cell cytopathic effects. The activity data revealed that the compounds **5a**, **5b**, **5d** and **5i** evoked a marked antiviral effect and **5b** and **5d** have shown CC_{50} value at 3.90µg/ml against BTV.*

Key words: Selenophenes, 1,3,4-oxadiazolyl selenopheno[2,3-d]pyrimidines, Synthesis, Antiviral activity, Novel BTV inhibitors.

INTRODUCTION

There was an urgent need to develop antiviral agents which can serves as effective therapeutic agents during the outbreaks. The discovery and development of effective antiviral drugs has been accelerated in recent years by an assortment of molecular targeted techniques and strategies. Amazingly, there are no effective antiviral drugs currently available against *Bluetongue virus* (BTV) diseases to reduce the mortality rate during outbreaks [1]. BTV, an economically important Orbivirus of the family Reoviridae, is the causative agent of haemorrhagic diseases mainly in sheep, occasionally in goat, cattle and some of wild ruminant species like deer. It is a non-enveloped, multilayered dsRNA virus comprising of 10 genome segments, transmitted chiefly through biting midges of *Culicoide species*. In recent years the explosive growth of interest in the use organoselenium compounds, due to selenium (Se) is a micronutrient essential for human health due to the presence of 25 selenoproteins in the human proteome [2]. Se is an essential component of glutathione peroxidase, an enzyme that protects cells from oxidative damage [3] some oxaselenolane nucleosides and 2-(3-Pyridyl)benzisoselenazol-3(2H)-one (Fig.1), have been renowned as potential antiviral activity and promoted their pivotal role in the synthesis of a large number of biological and pharmaceutical activities [4]. It has been widely reported that selenium-containing heterocycles can be used as antibacterial [5-8], antioxidant [9-12], 5-LOX/COX inhibitors [13] and antiviral [14,15]. In addition, heterocyclic compounds with a selenium atom in the ring have been intensively studied because of their chemical and biological properties. In the light of the growing number of applications in recent years there has been an enormous increase in the interest among biologists and chemists for the synthesis and bioactivity of organoselenium compounds. Accordingly, synthesis and biological screening of selenopheno[2,3-*d*]pyrimidine derivatives may be considered a virgin research area.

Moreover, it has long been known that compounds bearing 1, 3, 4–oxadiazole ring occupy an outstanding place in medicinal chemistry due to its significant biological properties such as antimicrobial [16-21], antituberculosis [22-24] and anticancer [25]. It is well-known that the combination of two or more types of heterocycles into one molecule could come up with a novel entity with increased bioactivities. Many researchers recommended that the assimilation of heterocyclic groups like oxadiazoles [26] and thiadizole [27] etc, into various heterocyclic scaffold produced new derivatives which exhibited significant biological activities. Among these heterocyclic groups, selenophene moiety was widely used in drug design because selenium is an essential and leads several metabolisms in the human body.

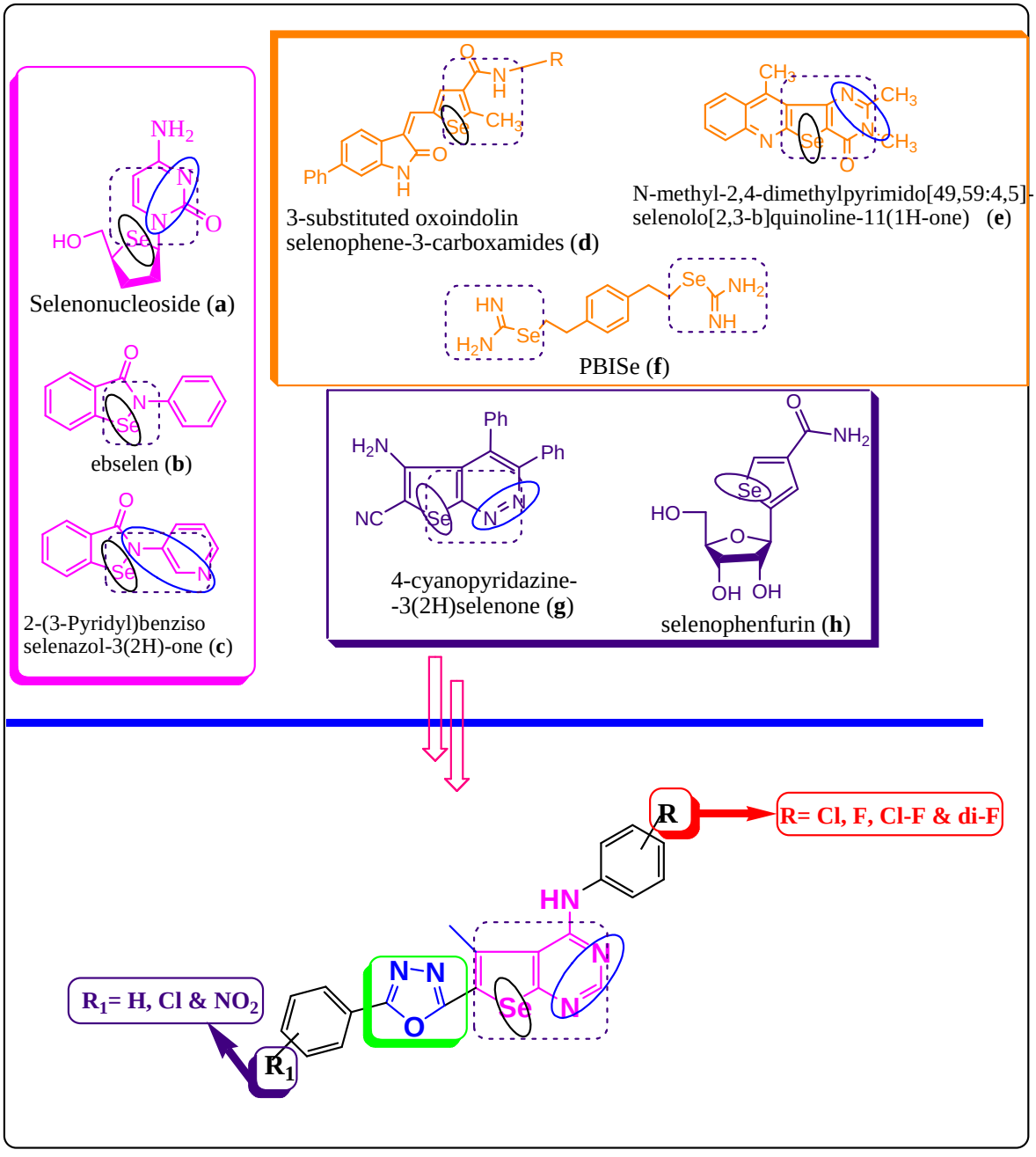
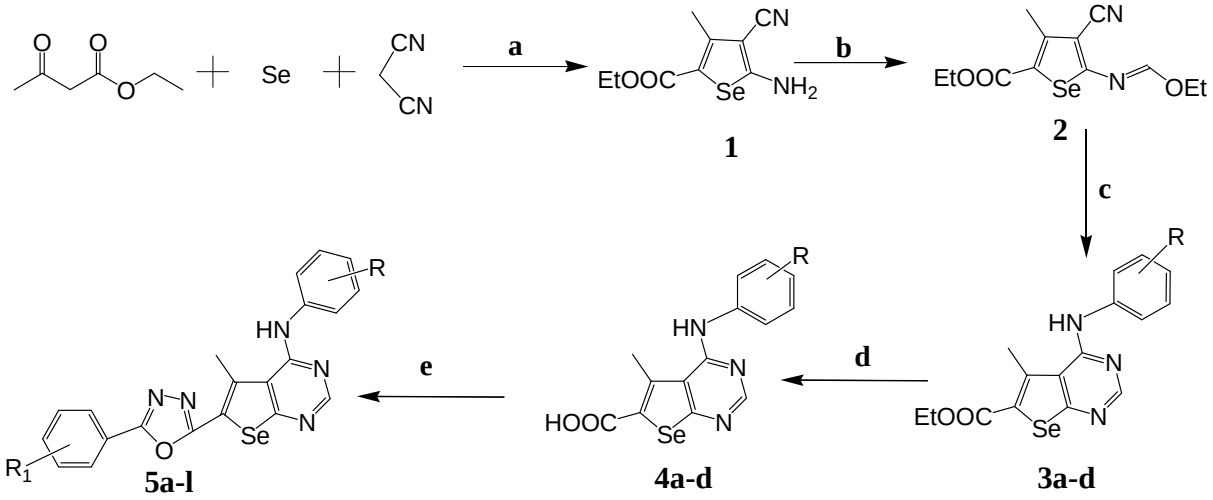


Figure 1. Rational designing of proposed compounds based upon known antiviral and other bioactive molecules.

With the above perspective, inspired by the diverse applications of the above nuclei and in continuation to our efforts directed toward the synthesis of innovative heterocyclic compounds [28–29] we planned to synthesize a system that combines together with two biolabile components, related to 1,3,4-oxadiazoles and selenopheno[2,3-*d*]pyrimidine derivatives, with anticipated biological activities against Bluetoungue Virus in BHK–21 cell lines.



5a,R= 4-Cl,R_1= 4-H
5b,R= 4-Cl,R_1= 4-Cl
5c,R= 4-Cl,R_1= 4-NO_2
5d,R= 4-F,R_1= 4-H
5e,R= 4-F,R_1= 4-Cl
5f,R= 4-F,R_1= 4-NO_2
5g,R= 3-Cl,4-F,R_1= 4-H
5h,R= 3-Cl,4-F,R_1= 4-Cl
5i,R= 3-Cl,4-F,R_1= 4-NO_2
5j,R=2,4di-F,R_1= 4-H
5k,R=2,4di-F,R_1= 4-Cl
5l,R=2,4di-F,R_1= 4-NO_2

4a,R= 4-Cl
4b,R= 4-F
4c,R= 3-Cl,4-F
4d,R=2,4di-F

3a,R= 4-Cl
3b,R= 4-F
3c,R= 3-Cl,4-F
3d,R=2,4di-F

Scheme 1. ***Reagents and condition:*** **a: Imidazole, DMF, 60°C 12 h; b: TeOF, reflux, 4 h; c: AcOH, Halo substituted anilines, reflux, 4h; d: NaOH, MeOH, rt, 16 h; e: $POCl_3$, appropriate phenyl hydrazides, reflux, 2h.**

EXPERIMENTAL SECTION

2.1. Experimental 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}. ^1H 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.

3.1.1 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 (Huang et al., 2011). 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 °C 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 filterate 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 °C; IR (Chloroform) ν (cm^{-1}): 3435 (NH_2), 2206 (C≡N), 1641 (C=O); ^1H NMR ($CDCl_3$, 400 MHz) δ 1.41 (t, 3H, –CH_2–CH_3), 2.48 (s, 3H, selenophene−CH_3), 4.27 (q, 2H, –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, 7.72 mmol) and triethyl orthoformate (7.15 mL, 38.61 mmol) was refluxed for 16 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%; m.p.: 106–108 ºC; IR (KBr) ν (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 $C_{12}H_{14}N_2O_3Se$.

General procedure for the synthesis of ethyl 4-(substituted phenylamino)-5- methylselenopheno[2,3-d] pyrimidine-6-carboxylate (*3a-d*)
A mixture of 4-Cyano-5-ethoxymethyleneimino-3-methylselenophene-2-carboxylic acid ethyl ester **2** (1.6 g 5.09 mmol) was dissolved in AcOH to this added (6.11 mmol) of appropriate halo substituted anilines and refluxed for 4h. After completion of the starting compounds, then the total reaction mixture was cooled to room temperature for 2 h, poured into ice cold water and stirred for 15 minutes. The product was separated by filtration and washed with water, dried well and recrystallized from chloroform and n-hexane to give compounds (**3a–d)** in good yields.

Ethyl 4-(4-chlorophenylamino)-5-methylselenoenopheno[2,3-d]pyrimidine-6-carboxylate (*3a*)
Yield 86%; White crystalline solid m.p. = 166–168 ºC; IR (KBr) ν (cm^{-1}): 3421, 2926, 1710, 1565; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.40 (t, 3H, –CH_2–$\underline{CH_3}$), 3.08 (s, 3H, selenophene−CH_3), 4.37 (q, 2H, –$\underline{CH_2}$–CH_3), 7.37 (d, 2H, Ar–H, *J*=12.0 Hz), 7.50 (s, 1H, N–H), 7.59 (d, 2H, Ar–H, *J*=8.0 Hz), 8.51 (s, 1H, C–H); ^{13}C NMR ($CDCl_3$, 100 MHz); δ 14.05, 15.28, 61.10, 114.73, 117.79, 120.61, 124.18, 133.73, 140.12, 154.35, 158.62, 161.03, 162.35 and 166.36; LC–MS (negative ion mode): m/z 394 (M–H) for $C_{16}H_{14}ClN_3O_2Se$.

Ethyl 4-(4-fluorophenylamino)-5-methylselenopheno[2,3-d]pyrimidine-6-carboxylate (*3b*)
Yield 82%; White solid m.p. = 172–174 ºC; IR (KBr) ν (cm^{-1}): 3432, 2922, 1712, 1558 ; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.48 (t, 3H, –CH_2–$\underline{CH_3}$), 3.10 (s, 3H, selenophene−CH_3), 4.32 (q, 2H, –$\underline{CH_2}$–CH_3), 7.36 (d, 2H, Ar–H, *J*=8.0 Hz), 7.44 (s, 1H, N–H), 7.59 (d, 2H, Ar–H, *J*=8.0 Hz), 8.62 (s, 1H, C–H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 14.12, 16.52, 61.20, 102.02, 111.18, 120.10, 122.64, 123.52, 128.62, 142.07, 154.22, 157.96, 162.24 and 173.06; LC–MS (negative ion mode): m/z 378 $(M–H)^-$ for $C_{16}H_{14}FN_3O_2Se$.

Ethyl 4-(3-chloro-4-fluorophenylamino)-5-methlyselenopheno[2,3-d]pyrimidine-6-carboxylate (*3c*)
Yield 68%; Pale-yellow solid m.p. = 158–160 ºC; IR (KBr) ν (cm^{-1}): 3412, 2926, 1706, 1555; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.40 (t, 3H, –CH_2–$\underline{CH_3}$), 3.07 (s, 3H, selenophene−CH_3), 4.37 (q, 2H, –$\underline{CH_2}$–CH_3), 7.16 (t, 1H, Ar–H, *J*=8.0 Hz), 7.42 (m, 1H, Ar–H), 7.47 (s, 1H, N–H), 7.81 (q, 1H, Ar–H, *J*=4.0 Hz), 8.52 (s, 1H, C–H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 14.25, 15.96, 60.09, 112.36, 115.82, 117.08, 118.49, 122.84, 141.06, 144.62, 146.54, 150.76, 154.38, 156.61, 160.44 and 170.18; LC–MS (negative ion mode): m/z 412 $(M–H)^-$ for $C_{16}H_{13}ClFN_3O_2Se$.

Ethyl 4-(2,4-difluorophenylamino)-5-methylselenopheno[2,3-d]pyrimidine-6-carboxylate (3d)
Yield 74%; White solid, m.p. = 181–183 ºC; IR (KBr) ν (cm^{-1}): 3422, 2924, 1710, 1551; ^{1}H NMR (DMSO–d_6, 400 MHz) δ 1.32 (t, 3H, –CH_2–$\underline{CH_3}$), 3.00 (s, 3H, selenophene−CH_3), 4.32 (q, 2H, –$\underline{CH_2}$–CH_3), 7.15 (m, 1H, Ar–H, *J*=4.0 Hz), 7.36 (m, 1H, Ar–H, *J*=8.0 Hz), 7.63 (q, 1H, Ar–H, *J*=8.0 Hz), 8.38 (s, 1H, N–H), 8.59 (s, 1H, C–H); ^{13}C NMR (DMSO–d_6, 100 MHz): δ 14.04, 17.06, 61.37, 104.28, 110.98, 120.24, 122.92, 123.09, 124.98, 129.08, 142.02, 154.38, 155.20, 158.35, 161.45, and 171.80; LC–MS (negative ion mode): m/z 396 $(M–H)^-$ for $C_{16}H_{13}F_2N_3O_2Se$.

General procedure for the synthesis of 4-(substituted phenylamino)-5-methylselenopheno[2,3-d]pyrimidine-6-carboxylic acid (4a-d)
The compound (**3a-d**) was dissolved in MeOH/ H_2O (12 mL: 6 mL), and 15% v/v NaOH aq (2 mL) was added. Stirring was continued for 16 h 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 (**4a–d)** in good yields.

4-(4-Chlorophenylamino)-5-methylselenopheno[2,3-d]pyrimidine-6-carboxylic acid (4a)
Yield 92%; Off–white solid, m.p. = 324–326 ºC; IR (KBr) ν (cm^{-1}): 3446, 2926, 1705, 1539; ^{1}H NMR (DMSO–d_6, 400 MHz) δ 3.12 (s, 3H, selenophene−CH_3), 7.45 (d, 2H, Ar–H, *J*=8.0 Hz), 7.71 (d, 2H, Ar–H, *J*=12.0 Hz), 8.52 (s, 1H, N–H), 8.69 (s, 1H, C–H), 13.60 (br s, 1H, –COO$\underline{H}$); ^{13}C NMR (DMSO–d_6,100 MHz): δ 16.62, 120.93, 124.00, 127.50, 127.72, 127.99, 137.41, 140.61, 153.39, 157.24, 164.77and 171.55; LC–MS (positive ion mode): m/z 368 $(M+H)^+$ for $C_{14}H_{10}ClN_3O_2Se$.

4-(4-Fluorophenylamino)-5-methylselenopheno[2,3-d]pyrimidine-6-carboxylic acid (4b)
Yield 90%; White solid m.p. = 289-291 ºC; IR (KBr) ν (cm^{-1}): 3391, 2920, 1712, 1579; ^{1}H NMR (DMSO–d_6, 400 MHz) δ 3.08 (s, 3H, selenophene−CH_3), 7.24 (t, 2H, Ar–H, *J*=8.0 Hz), 7.66 (q, 2H, Ar–H, *J*=4.0 Hz), 8.59 (s, 1H, N–H), 8.80 (s, 1H, C-H), 13.50 (br s, 1H, –CO<u>OH</u>); ^{13}C NMR (DMSO–d_6,100 MHz): δ 16.55, 115.52, 120.68, 122.57, 124.31, 127.76, 135.07, 140.03, 153.21, 155.41, 164.87 and 171.91; LC–MS (positive ion mode): m/z 352 $(M+H)^+$ for $C_{14}H_{10}FN_3O_2Se$.

4-(3-Chloro-4-fluorophenylamino)-5-methylselenenopheno[2,3-d]pyrimidine-6-carboxylic acid (4c)
Yield 87%; White solid m.p. = 315–317 ºC; IR (KBr) ν (cm^{-1}): 3423, 2924, 1693, 1568; ^{1}H NMR (DMSO–d_6, 400 MHz) δ 3.04 (s, 3H, selenophene−CH_3), 7.43 (t, 1H, Ar–H, *J*=8.0 Hz), 7.64 (t, 1H, Ar–H, *J*=4.0 Hz), 7.92 (d, 1H, Ar–H, *J*=4.0 Hz), 8.52 (s, 1H, N–H), 8.70 (s, 1H, C–H), 13.56 (br s, 1H, –CO<u>OH</u>); ^{13}C NMR (DMSO–d_6,100 MHz): δ 14.07, 113.61, 117.84, 123.57, 127.78, 138.52, 142.51, 146.34, 151.24, 154.70, 156.66, 163.71, 166.81 and 169.23; LC–MS (positive ion mode): m/z 386 $(M+H)^+$ for $C_{14}H_9ClFN_3O_2Se$.

4-(2,4-Difluorophenylamino)-5-methylselenopheno[2,3-d]pyrimidine-6-carboxylic acid (4d)
Yield 91%; White crystalline solid m.p. = 296–298 ºC; IR (KBr) ν (cm^{-1}): 3462, 2926, 1693, 1578; ^{1}H NMR (DMSO–d_6, 400 MHz) δ 3.00 (s, 3H, selenophene−CH_3), 7.14 (t, 1H, Ar–H, *J*=8.0 Hz), 7.35 (t, 1H, Ar–H, *J*=8.0 Hz), 7.64 (q, 1H, Ar–H, *J*=8.0 Hz), 8.38 (s, 1H, N–H), 8.56 (s, 1H, C–H), 13.54 (br s, 1H, –CO<u>OH</u>); ^{13}C NMR (DMSO–d_6,100 MHz): δ 14.47, 109.25, 115.62, 118.58, 126.93, 141.86, 147.72, 151.35, 153.48, 158.74, 159.28, 161.39, 164.18 and 169.06; LC–MS (positive ion mode): m/z 370 $(M+H)^+$ for $C_{14}H_9F_2N_3O_2Se$.

General procedure for the synthesis of (5a-l)
An equimolar mixture of 4-(substituted phenyl amino)-5-methylselenopheno[2,3-d]pyrimidine-6-carboxylic acids (**4a-d**) (0.01 mol) and appropriate substituted aromatic carboxylic acid hydrazides (0.01 mol) in phosphorus oxychloride (20mL) was refluxed for 5h. The reaction mixture was cooled to room temperature and then gradually poured on to crushed ice with stirring. The mixture was allowed standing overnight and the solid separated out was filtered, treated with dilute sodium hydroxide solution, stirred for 15 minutes. The product was separated by filtration and washed thoroughly with cold water, dried well and recrystallized from chloroform and methanol to give compounds (**5a-l**) in good yields.

N-(4-Chlorophenyl)-5-methyl-6-(5-phenyl-1,3,4-oxadiazol-2-yl)selenopheno[2,3-d]pyrimidin-4-amine (5a)
White solid (76%); m.p. = 235–238 ºC; IR (KBr) ν (cm^{-1}): 3484, 2920, 1610, 1558; ^{1}H NMR (DMSO–d_6, 400 MHz) δ 3.05 (s, 3H, selenophene−CH_3), 7.52–7.61 (m, 5H, Ar–H),7.86 (d, 2H, Ar–H, *J*=8.0 Hz), 8.00 (d, 2H, Ar–H, *J*=8.0 Hz), 8.45 (s, 1H, N–H), 8.78 (bs, 1H, C–H); ^{13}C NMR (DMSO–d_6, 100 MHz); δ 16.15, 114.81, 116.63, 118.97, 122.94, , 123.93, 126.72, 128.53, 129.48, 132.21, 135.01, 152.73, 154.54, 160.50, 163.59 and 167.28; LC–MS (negative ion mode): m/z 466 $(M–H)^-$ for $C_{21}H_{14}ClN_5OSe$.

N-(4-Chlorophenyl)-6-(5-(4-chlorophenyl)-1,3,4-oxadiazol-2-yl)-5-methylselenopheno[2,3-d]pyrimidin-4-amine (5b)
Off–white solid (82%); m.p. = 241–243 ºC; IR (KBr) ν (cm^{-1}): 3254, 2922, 1606, 1556; ^{1}H NMR (DMSO–d_6, 400 MHz) δ 3.02 (s, 3H, selenophene−CH_3), 7.35 (d, 2H, Ar–H, *J*=8.0 Hz), 7.64 (d, 2H, Ar–H, *J*=8.0 Hz), 7.87 (d, 2H, Ar–H, *J*=8.0 Hz), 8.03 (d, 2H, Ar–H, *J*=8.0 Hz), 8.24 (s, 1H, N–H), 8.37 (br s, 1H, C–H); ^{13}C NMR (DMSO–d_6, 100 MHz): δ 17.71, 116.80, , 120.60, 121.95, 124.42, 127.74, 128.28, 129.22, 129.54, 136.93, 137.53, 137.83, 153.81, 157.21, 162.29, 162.99 and 172.22; LC–MS (negative ion mode): m/z 502 $(M+H)^+$ for $C_{21}H_{13}C_{l2}N_5OSe$.

N-(4-Chlorophenyl)-5-methyl-6-(5-(4-nitrophenyl)-1,3,4-oxadiazol-2-yl)selenopheno[2,3-d]pyrimidin-4-amine (5c)
Yellowish solid (74%); m.p. = 221–223 ºC; IR (KBr) ν (cm^{-1}): 3224, 2918, 1615, 1521; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.28 (s, 3H, selenophene−CH_3), 7.42 (d, 2H, Ar–H, *J*=8.0 Hz), 7.51 (d, 2H, Ar–H, *J*=8.0 Hz), 7.66 (d, 2H, Ar–H, *J*=8.0 Hz), 8.49 (dd, 2H, Ar–H, *J*=8.0 Hz), 8.73 (s, 1H, N–H), 8.98 (br s, 1H, C–H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 16.12, 115.96, 117.26, 120.91, 123.74, 126.02, 128.26, 129.48, 129.97, 130.42, 132.75, 136.28, 138.84, 155.14, 156.07, 161.26 and 163.82; LC–MS (negative ion mode): m/z 510 $(M–H)^-$ for $C_{21}H_{13}ClN_6O_3Se$.

N-(4-Fluorophenyl)-5-methyl-6-(5-phenyl-1,3,4-oxadiazol-2-yl)selenopheno[2,3-d]pyrimidin-4-amine (5d)
Off-white solid (80%); m.p. = 235–237 ºC; IR (KBr) ν (cm^{-1}): 3230, 2918, 1620, 1509); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.12 (s, 3H, selenophene−CH_3), 7.38 (t, 2H, Ar–H, *J*=8.0 Hz), 7.51 (dd, 2H, Ar–H, *J*=4.0 Hz), 7.62–7.76 (m, 5H, Ar–H), 8.20 (s, 1H, N–H), 8.62 (s, 1H, C–H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 15.67, 116.23, 117.84, 118.65, 126.28, 127.42, 128.68, 129.81, 132.96, 137.25, 139.58, 153.61, 155.28, 158.94, 158.14, 161.84 and 164.23; LC–MS (negative ion mode): m/z 450 $(M–H)^-$ for $C_{21}H_{14}FN_5OSe$.

6-(5-(4-Chlorophenyl)-1,3,4-oxadiazol-2-yl)-N-(4-fluorophenyl)-5-methylselenopheno[2,3-d]pyrimidin-4-amine (5e)
White solid (86%); m.p. = 268–270 ºC; IR (KBr) ν (cm^{-1}): 3443, 2986, 1610, 1525; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.24 (s, 3H, selenophene−CH_3), 7.36 (t, 2H, Ar–H, *J*=8.0 Hz),7.56 (dd, 2H, Ar–H, *J*=4.0 Hz), 7.64 (d, 2H, Ar–H, *J*=8.0 Hz), 8.02 (d, 2H, Ar–H, *J*=8.0 Hz), 8.26 (s, 1H, N–H), 8.82 (s, 1H, C–H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 16.24, 114.28, 116.32, 118.57, 124.04, 128.61, 130.02, 132.42, 135.36, 138.12, 138.87, 149.20, 152.82, 153.86, 157.94, 163.09 and 167.68; LC–MS (positive ion mode): m/z 485 $(M+H)^+$ for $C_{21}H_{13}ClFN_5OSe$.

N-(4-fluorophenyl)-5-methyl-6-(5-(4-nitrophenyl)-1,3,4-oxadiazol-2-yl)selenopheno[2,3-d]pyrimidin-4-amine (5f)
Pale-Yellow solid (86%); m.p. = 256–258 ºC; IR (KBr) ν (cm^{-1}): 3254, 2916, 1606, 1554; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.36 (s, 3H, selenophene−CH_3), 7.41 (t, 2H, Ar–H, *J*=8.0 Hz), 7.59 (dd, 2H, Ar–H, *J*=4.0 Hz), 7.71 (d, 2H, Ar–H, *J*=8.0 Hz), 7.96 (dd, 2H, Ar–H, *J*=8.0 Hz), 8.35 (s, 1H, N–H), 8.86 (s, 1H, C–H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 17.56, 116.42, 116.63, 117.49, 123.60, 127.66, 131.92, 135.48, 137.83, 137.64, 139.12, 149.08, 151.74, 153.63, 157.29, 162.48 and 166.27; LC–MS (positive ion mode): m/z 497 $(M+H)^+$ for $C_{21}H_{13}FN_6O_3Se$.

N-(3-Chloro-4-fluorophenyl)-5-methyl-6-(5-phenyl-1,3,4-oxadiazol-2-yl)selenopheno[2,3-d]pyrimidin-4-amine (5g)
Yellow solid (76%); m.p. = 226–228 ºC; IR (KBr) ν (cm^{-1}): 3248, 2934, 1614, 1547; ^{1}H NMR (DMSO–d_6, 400 MHz) δ 3.01 (s, 3H, selenophene−CH_3), 7.36 (t, 1H, Ar–H, *J*=8.0 Hz), 7.56–7.58 (m, 5H, Ar–H), 7.83 (dd, 1H, Ar–H, *J*=4.0 Hz), 8.00 (dd, 1H, Ar–H, *J*=4.0 Hz), 8.01 (s, 1H, N–H), 8.41 (s, 1H, C–H); ^{13}C NMR (DMSO–d_6, 100 MHz): δ 17.67, 116.46, 116.69, 117.10, 119.00, 120.49, 123.72, 124.80, 126.72, 127.46, 128.55, 129.49, 132.18, 137.25, 153.82, 157.28, 162.17, 163.73 and 172.19; LC–MS (negative ion mode): m/z 484 $(M–H)^-$ for $C_{21}H_{13}ClFN_5OSe$.

N-(3-Chloro-4-fluorophenyl)-6-(5-(4-chlorophenyl)-1,3,4-oxadiazol-2-yl)-5-methylselenopheno[2,3-d]pyrimidin-4-amine (5h)
Yellowish solid (82%); m.p. = 282–284 ºC; IR (KBr) ν (cm^{-1}): 3464, 2852, 1641, 1548; ^{1}H NMR (DMSO–d_6, 400 MHz) δ 3.19 (s, 3H, selenophene−CH_3), 7.54 (t, 1H, Ar–H, *J*=8.0 Hz), 7.71 (dd, 1H, Ar–H), 7.78 (d, 2H, Ar–H, *J*=8.0 Hz), 8.00 (dd, 1H, Ar–H, *J*=4.0 Hz), 8.14 (d, 2H, Ar–H, *J*=8.0 Hz), 8.60 (s, 1H, N–H), 8.90 (s, 1H, C–H); ^{13}C NMR (DMSO–d_6, 100 MHz) δ 16.16, 115.67, 116.64, 117.48, 118.78, 121.91, 123.96, 128.53, 129.66, 135.23, 135.87, 137.02, 142.68, 149.22, 152.71, 154.62, 156.52, 162.87 and 167.43; LC–MS (negative ion mode): m/z 518 $(M–H)^-$ for $C_{21}H_{12}Cl_2FN_5OSe$.

N-(3-Chloro-4-fluorophenyl)-5-methyl-6-(5-(4-nitrophenyl)-1,3,4-oxadiazol-2-yl) selenopheno [2,3-d]pyrimidin-4-amine (5i)
Yellow solid (78%); m.p. = 223–225 ºC; IR (KBr) ν (cm^{-1}): 3051, 2959, 1614, 1531; ^{1}H NMR (DMSO–d_6, 400 MHz) δ 3.28 (s, 3H, selenophene−CH_3), 7.20 (t, 1H, Ar–H, *J*=8.0 Hz), 7.50 (dd, 1H, Ar–H), 7.89 (dd, 1H, Ar–H, *J*=4.0 Hz), 8.49 (d, 2H, Ar–H, *J*=8.0 Hz), 8.55 (dd, 2H, Ar–H, *J*=8.0 Hz), 8.63 (s, 1H, N–H), 9.00 (s, 1H, C–H); ^{13}C NMR (DMSO–d_6, 100 MHz) δ 16.87, 115.38, 116.01, 117.59, 118.32, 122.02, 124.72, 128.37, 130.63, 132.54, 132.61, 139.81, 148.87, 149.52, 153.73, 15.21, 157.22, 162.19 and 163.37; LC–MS (negative ion mode): m/z 529 $(M–H)^-$ for $C_{21}H_{12}ClFN_6O_3Se$.

N-(2,4-Difluorophenyl)-5-methyl-6-(5-phenyl-1,3,4-oxadiazol-2-yl)selenopheno[2,3-d]pyrimidin-4-amine (5j)
Off-White solid (82%); m.p. = 256–258 ºC; IR (KBr) ν (cm^{-1}): 3256, 2920, 1608, 1532; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.22 (s, 3H, selenophene−CH_3), 7.24 (dd, 1H, Ar–H, *J*=4.0 Hz), 7.41 (dd, 1H, Ar–H, *J*=4.0 Hz), 7.63 (dd, 1H, Ar–H, *J*=8.0 Hz), 7.86–7.94 (m, 5H, Ar–H), 8.82 (s, 1H, N–H), 8.94 (s, 1H, C–H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 16.15, 114.81, 116.63, 117.51, 118.97, 123.86, 126.72, 127.46, 128.51, 129.48, 132.21, 135.87, 147.35, 152.73, 154.54, 155.14, 156.46, 162.97 and 163.59; LC–MS (positive ion mode): m/z 470 $(M+H)^+$ for $C_{21}H_{13}F_2N_5OSe$.

6-(5-(4-Chlorophenyl)-1,3,4-oxadiazol-2-yl)-N-(2,4-difluorophenyl)-5-methylselenopheno[2,3-d]pyrimidin-4-amine (5k)
White solid (76%); m.p. = 252–254 ºC; IR (KBr) ν (cm^{-1}): 3244, 2921, 1608, 1526; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.25 (s, 3H, selenophene−CH_3), 7.18 (dd, 1H, Ar–H, *J*=4.0 Hz), 7.28 (dd, 1H, Ar–H, *J*=4.0 Hz), 7.58 (dd, 1H, Ar–H, *J*=8.0 Hz), 7.68 (d, 2H, Ar–H, *J*=8.0 Hz), 7.74 (d, 2H, Ar–H, *J*=8.0 Hz), 8.48 (s, 1H, N–H), 8.69 (s, 1H, C–H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 17.70, 110.92, 115.04, 117.51, 119.26, 123.69, 124.72, 127.81, 129.47, 132.63, 135.94, 137.14, 149.08, 152.91, 155.32, 156.82, 160.10, 163.26 and 164.91; LC–MS (positive ion mode): m/z 504 $(M+H)^+$ for $C_{21}H_{12}ClF_2N_5OSe$.

N-(2,4-Difluorophenyl)-5-methyl-6-(5-(4-nitrophenyl)-1,3,4-oxadiazol-2-yl) selenopheno[2,3-d]pyrimidin-4-amine (5l)

Yellow solid (72%); m.p = 269–271 ºC; IR (KBr) ν (cm^{-1}): 3198, 2952, 1620, 1526; ^{1}H NMR (DMSO–d_6, 400 MHz) δ 3.44 (s, 3H, selenophene−CH_3), 7.38 (t, 1H, Ar–H, *J*=8.0 Hz), 7.48 (dd, 1H, Ar–H, *J*=4.0 Hz), 7.60 (dd, 1H, Ar–H, *J*=8.0 Hz), 7.75 (d, 2H, Ar–H, *J*=8.0 Hz), 7.86 (d, 2H, Ar–H, *J*=8.0 Hz), 8.03 (s, 1H, N–H), 8.44 (s, 1H, C–H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 17.70, 116.43, 116.58, 117.67, 118.77, 123.04, 128.27, 129.49, 131.94, 132.21, 139.61, 147.05, 149.58, 153.35, 153.80, 156.21, 162.10, 163.74 and 165.61; LC–MS (negative ion mode): m/z 513 $(M–H)^-$ for $C_{21}H_{12}F_2N_6O_3Se$.

RESULTS AND DISCUSSION

3.1. Chemistry

The series of reactions leading to the development of the target compounds were carried out according to Scheme–I. In this the most appropriate starting material ethyl 5-amino-4-cyano-3-methylselenophene-2-carboxylate (**1)** was prepared according to reported Gewald synthetic procedure [30]. Further, aromatic and heterocyclic *o*-aminocarbonitrile derivatives are premeditated intermediates for the synthesis of various condensed heterocyclic systems. Since, compound **1** contains two reactive groups such as amino and cyano functions at *ortho* to the each other and the interaction of **1** with triethyl orthoformate [31] furnished the key intermediate 4-cyano-5-ethoxymethyleneimino-3-methylselenophene-2-carboxylic acid ethylester (**2**).

Compound **2** with appropriate halo substituted anilines in acetic acid afforded [32] the substituted carboxylic ester derivatives of selenopheno[2,3-*d*]pyrimidines **3a**–**d** in excellent yields. The ester function of compound **3a–d** was hydrolyzed with aqueous NaOH to afford the corresponding carboxylic acid derivatives **4a–d** with yields ranging from 87% to 92%. The structures of **4a–d** were established on their IR, NMR and LC–MS spectral data. The resulting compounds served as intermediates to prepare target molecules. The reaction of **4a–d** with appropriate substituted arylacidhydrazides in the presence of the $POCl_3$ yielded the title compounds **5a-l** in good to excellent yields. Further, the structures of compounds **5a-l** were established on the basis of their spectral data showed in experimental section.

3.2 Evaluation of cytotoxicity of compounds by using MTT assay:

Each compound was dissolved in 0.1% DMSO to a final concentration of 1mg/ml followed by filtration through 0.22m mμ filters (Sartorius). Further, they were serially diluted up to 1:1024 (two fold dilution) to evaluate cytotoxicity induced by compounds. Confluent monolayer of BHK 21 cell lines were prepared as cell suspension by trypsinization and seeded at a concentration of 5000 cells/well in a 96 well tissue culture plate. Plates were incubated at 37°C in a CO_2 incubator for 24–48 hrs. After observing the monolayer, growth medium was removed and cells were washed with FCS free MEM for twice. Quadruplicate wells of confluent monolayers of BHK 21 cells were incubated with different concentration of the test compounds and cell viability was examined by ability of the cells to cleave the tetrazolium salt MTT [3-(4,5-dimethyl thiazol-2ol)-2,5 diphenyltetrazoliumbromide), Sigma-Aldrich, USA, by the mitochondrial succinate dehydrogenase which develops a formazan blue colour product.

Intensity of colour was directly proportional to the concentration of test compound. The 50% cytotoxicity concentration (CC_{50}) was calculated by regression analysis at which CC_{50} of the test compound was minimum cell cytotoxicity. It was further checked by plating efficiency of the cells with the subtoxic dose of that test compound. The effective dose at which toxicity of compounds were minimum was calculated at 1:256 (3.9μg/ml) dilution by plotting a graph against compound concentration and cell viability (Fig. 4 & 5). At this concentration 85.32% cells were viable and no impact on cell morphology and growth was observed. When cells were subcultured along with effective concentration (EC) of both the compounds for three passages, no effect on cell viability and growth was noticed. These changes were compared with cell control having cells and 0.1% DMSO (Fig. 2 & 3).

*3.3. In vitro evaluation of virostatic efficacies of compounds **5a-l** against BTV:*

As discussed earlier, there were no commercially available antiviral drugs for bluetongue disease to maintain positive drug control. The antiviral activities of newly synthesized compounds **5a-l** against *Bluetongue virus* were initially evaluated based on the cytopathic effects (CPE) induced by virus, using BHK–21 cell lines. Cell cytotoxicity (EC_{50}) was calculated [33] at 3.90μg/ml for all the compounds (Fig.4). Among the 12 newly synthesized compounds **5a-l** summarized in scheme.1, half-off the compounds i.e., **5a, 5b, 5d, 5h, 5i** and **5l** have no effect on cellular changes (Fig.2) at the concentration of 3.90μg/ml. Rest of the compounds showed effect on cellular changes (*roundening, syncytia* formation) when they serially *passaged* for three times with subtoxic dose of test compounds. Out of six test compounds four (**5a, 5b, 5d** and **5i**) have shown effective inhibition of cytopathic effect induced by bluetongue virus. The other two compounds **5h** and **5l** failed to inhibit the virus infection in cell culture (Fig.3) even they have no effect on cell growth. As summarized in (Fig.3), compounds **5a**, **5b, 5d** and **5i**

exhibited antiviral activity against BTV, compounds **5b** and **5d** exhibited good activity and compound **5i** showed moderate antiviral activity compared with that of **5b** and **5d**. Compound **5a** possessed considerably lower activity when compared with the other three test compounds against BTV. Antiviral property for above said compounds was compared with virus and cell control. In virus control cytopathic effect was observed as early as 24 hrs post infection with initial rounding and detachment of cells. Characteristic CPE of BTV with ballooning and clumping of cells was observed. Swollen spindle shaped cells and long cytoplasmic bridges appeared by 48 hrs. Complete detachment of the monolayer takes place by 72 hrs and the detached cells appeared as clumps. The uninfected monolayer did not show any changes and were normal until 72 hrs of incubation except for a few detached cells due to overcrowding or overgrowth of cells. We have not observed any above said cytopathic effects at antiviral property evaluation indicating that viral growth was restricted by the above four compounds.

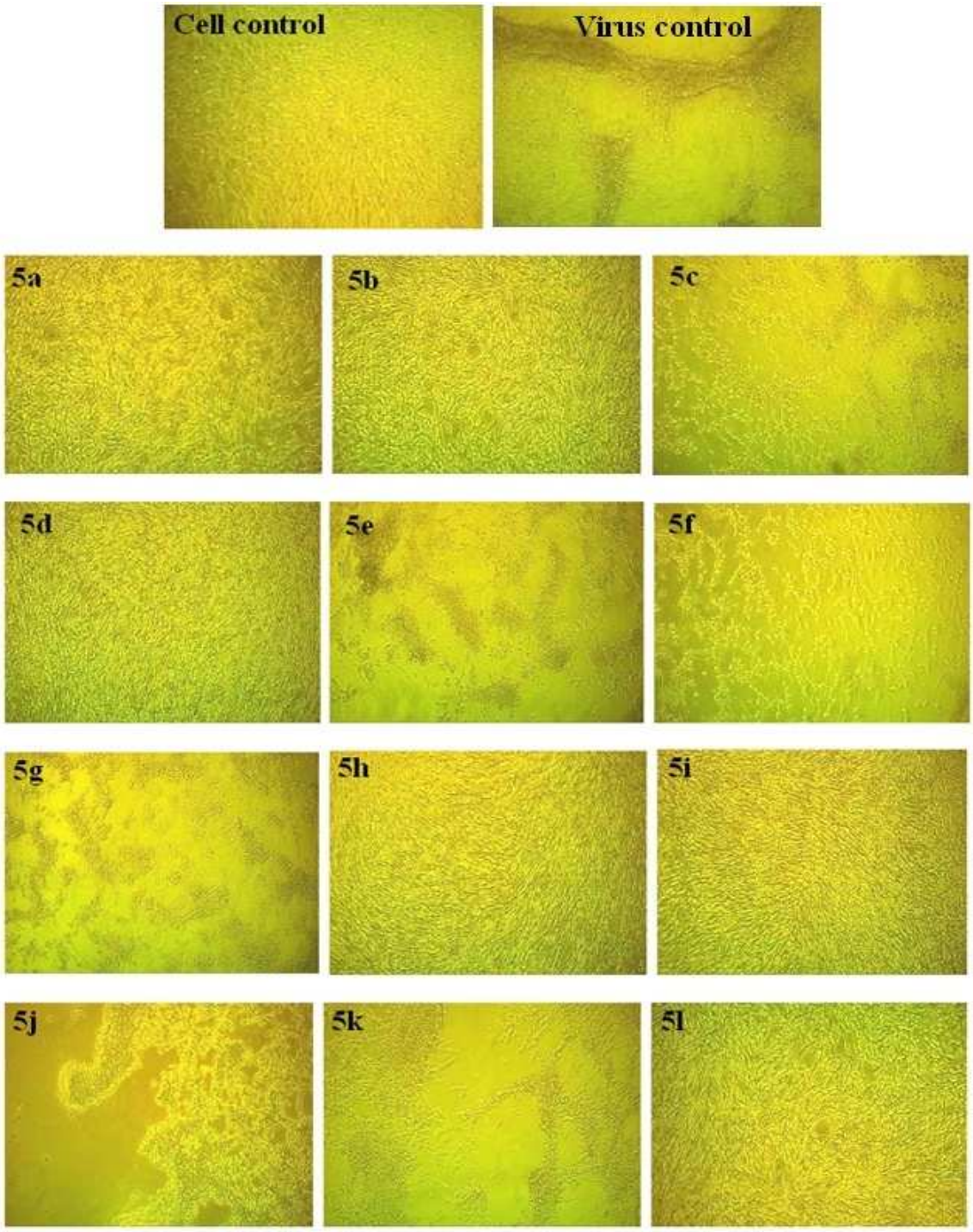


Figure 2. Cytotoxicity of compounds 5a-l at the concentrations of 3.9 µg/ml under inverted microscope (magnification 40x). Compounds 5c, 5e, 5f, 5g, 5j and 5k have impact on cellular changes like roundening, syncytia formation and detachment of cells. Compounds 5a, 5b, 5d, 5h, 5i and 5l have no effect on cellular changes.

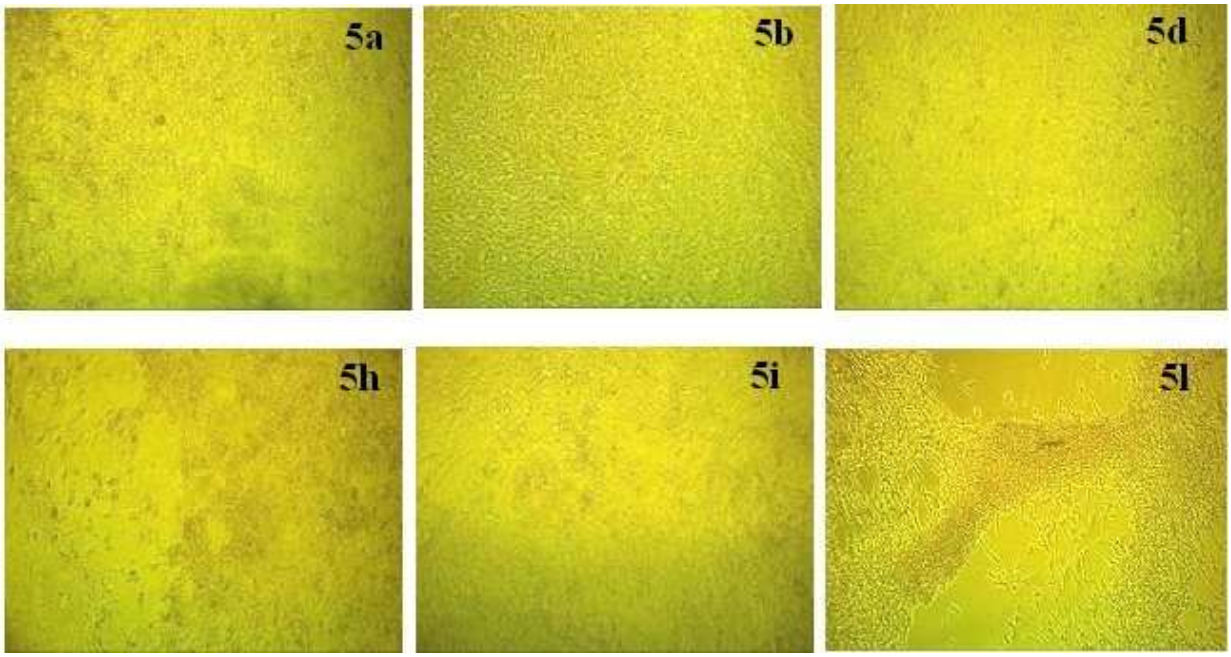


Figure 3. Anti-viral property for compounds 5a, 5b, 5d and 5i is showing the successive inhibition of BTV virus in cell cultures where as compounds 5h and 5l not showing the antiviral property as the BTV induced cytopathic effects appeared clearly

CONCLUSION

In the present study, a series of novel 1,3,4-oxadiazolyl selenopheno[2,3-*d*]pyrimidines were synthesized by the simple and efficient protocols with good yields. Out of the synthesized compounds six analogues have shown CC_{50} at 3.90µg/mL. The compounds **5a, 5b, 5d** and **5i** were found to anti BTV agents. The identified 1,3,4-oxadiazoloyl selenopheno[2,3-*d*]pyrimidines can be new leads in antiviral against BTV. Our investigations suggested that, these molecules are the promising candidates for the development of hybrid heterocycles with improved antiviral activity.

5.1. Biological evaluation

5.2.1. Cells and virus Stock

Evaluation of antiviral property for BTV was carried out at the Department of Virology, S.V. University, Tirupati, India. BHK–21 clone 13 cell lines at 46th passage level and bluetongue virus serotype 9, strain K8 at a concentration of $10^{5.3}$TCID$_{50}$ were used throughout the experiment.

5.2.2.Cell culture

Baby Hamster Kidney (BHK–21) cell lines was maintained in Dulbecco's modified Eagle's medium (DMEM) (Hi media Co., Bombay, India) supplemented with 8% heat inactivated fetal bovine serum (FBS) and 100 U/ml of penicillin, 100µg/ml of streptomycin (Sigma–Aldrich Chemical Co., St. Louis, MO).

5.2.3. Cytotoxicity assay

The cytotoxicity study of the newly synthesized compounds **5a-1** were solubilized in 0.1% DMSO for cytotoxicity study, finally make up with distilled water to a concentration of 1 mg/1mL followed by filtration through 0.22m mµ syringe filters. Each compound was serially diluted up to 1:1024 (Two fold dilution) to evaluate cytotoxicity at different concentrations of test compounds. CC_{50} was carried out at a cell concentration of 5000 cells/well grown in DMEM medium containing 1% FBS in a 96 well tissue culture plate. Quadruplicate wells having confluent monolayers of BHK–21 cells were treated with different concentrations of test compounds and cell viability was examined by trypan blue exclusion method. The 50% effective concentration at which cytotoxicity was minimum, it was further checked by plating efficiency of the cells with the subtoxic dose of these test compounds.

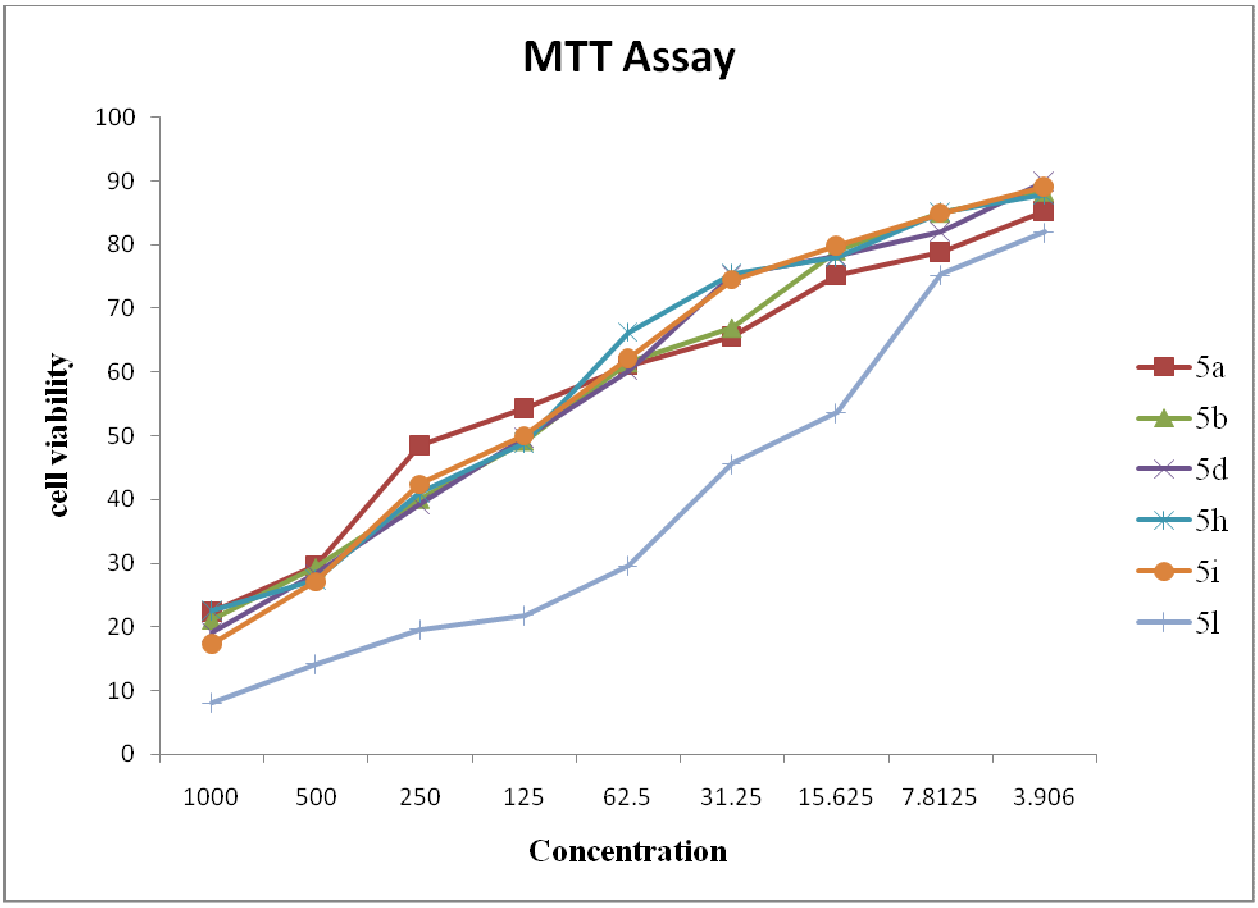


Figure 4. Evaluation of the antiviral activity by MTT assay for compounds 5a, 5b, 5d, 5h, 5i and 5l.

5.2.4. Evaluation of antivitral property

BHK–21 cells cultured in 24 well plates were inoculated with effective dose of 12 test compounds along with the bluetongue virus at a concentration of $10^{5.3}$ $TCID_{50}$. Plates were incubated in a CO_2 incubator at 37 ºC and BTV induced cytopathogenicity was observed at 24 hrs intervals to 72 hrs along with the virus controls.

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