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Synthesis, Characterization and Antimicrobial Evaluation of Novel Dimethyl Triazene Incorporated Phenylamino Pyrimidine Derivatives

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ABSTRACT

A series of novel (*E*)-4-(4-(3,3-dimethyltriaz-1-en-1-yl)phenyl)-*N*-phenylpyrimidin-2-amine derivatives have been synthesized from the condensation of (*E*)-3-(dimethylamino)-1-(4-(*E*)-3,3-dimethyltriaz-1-en-1-yl)phenyl)prop-2-en-1-one with several guanidinium hydrochloride. The newly synthesized compounds were examined for *in vitro* antimicrobial activity against some antibacterial and fungal strains. The synthesized compounds were characterized by ^{1}H NMR, ^{13}C NMR, IR, mass and elemental analyses.

KEYWORDS

Dimethyl triazene, Enaminone, Guanidine hydrochlorides, Pyrimidine, Antimicrobial activity.

INTRODUCTION

In recent years, originating of new antimicrobial agents with improving efficiency and the minimal side effect is a major challenge in developing and developed countries [1] because of the incumbent emergence of resistant strains of pathologic microorganisms for a widespread of multidrug-resistant pathogens [2]. Therefore, in the last decades for the treatment of new resistant microbial, it's necessary to develop a new effective class of antibacterial and antifungal reagents [3].

Triazenes [4] are a straight-chain molecule that contains three contiguous nitrogen atoms, like pyrimidines, triazenes are a very useful and diverse class, mainly for their anticancer potential in many types of tumors and therapeutic properties [5]. Moreover, the potential of antimicrobial activity of phenylamino pyrimidine [6] containing dimethyl triazene is unreported. However, relative reports on antimicrobial activities of 1,3-diaryltriazene derivatives are scarce [7]. According to literature survey, pyrimidine and its derivatives have attracted a great deal of attention due to their diverse therapeutic importance, anti-HIV-1 [8], analgesic agents [9], antiproliferative [10], antitumor [11], antimicrobials [12] and anti-inflammatory [13] activities.

To improve the efficiency of any drug using a combination of drugs that impact multiple targets simultaneously is the standard of care in any treatment [14]. Therefore, one approach

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involves the use of two or more drugs to increase selectivity and pharmacokinetics so-called hybrid molecule, which comprises the incorporation of two drugs in a single molecule to exert dual drug action [15]. Nowadays we have been engaged to explore and developed the new structures of the most effective novel sulphonamide, urea, thiourea, phenylamino containing pyrimidine derivatives with different fused heterocycles [3]. The diverse biological activity of these two moieties bearing triazene and pyrimidine nucleus encouraged us to synthesize a novel series of various dimethyltriazene phenylamino pyrimidine hybrid molecules.

Guanidine can be considered as a super base due to its strongly basic character [16] and chiral guanidines are also used as asymmetric reagents as well as its containing many compounds having therapeutic activities [17]. Guanidine hydrochlorides are easily prepared under mild conditions with high yields and are of great interest in the preparation of novel phenylamino pyrimidines derivatives with appropriate enaminones [18].

Key intermediate (*E*)-3-(dimethylamino)-1-(4-(*E*)-3,3-dimethyltriaz-1-en-1-yl)phenyl)prop-2-en-1-one (**SPI-4a**) was synthesized from the commercially available 4-aminoacetophenone react under the acidic condition with nitrite ion to form diazonium salt, which on treatment with dimethylamine hydrochloride to offered (*E*)-1-(4-(3,3-dimethyltriaz-1-en-1-yl)-phenyl)ethan-1-one (**SPI-2a**). Then treatment of **SPI-2a** with dimethylformamide dimethyl acetal (DMF-DMA) produces the targeted compounds (**SPI-4a**). The final derivatives of dimethyltriazene phenylaminopyrimidine were achieved by condensation of enaminnones (**SPI-4a**) and guanidine hydrochloride (**7a-j**) *via* cyclization. Guanidine hydrochloride was obtained by the reaction of hydrochloride of different substituted aniline with a 50% aqueous solution of cyanamide.

EXPERIMENTAL

All chemicals and solvents were purchased from Sigma Aldrich, Spectrochem Ltd. and used without further purification. Melting points of these compounds were measured in one end open capillary tubes using Digital Auto Melting Point Apparatus and are uncorrected. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on a BRUKER AVANCE-III spectrometer. The chemical shifts of ^{1}H & ^{13}C NMR spectrum were expressed in parts per million (ppm) relative to tetramethylsilane (TMS). Mass spectra were recorded on the Agilent Technologies (SHIMADZU-QP-2010) mass spectrometers. IR spectra were recorded on an IR Affinity-1S spectrophotometer (SHIMADZU) in the frequency range of 4000-400 cm^{-1} using a KBr disc. The elemental analyses of the compounds were performed on a Perkin Elmer 2400 Elemental Analyzer. TLC was performed using E-Merck 0.25 mm silica gel plates and visualization of spots through UV light.

General procedure for the synthesis of (*E*)-1-(4-(3,3-dimethyltriaz-1-en-1-yl)phenyl)ethan-1-one: (*E*)-1-(4-(3,3-Dimethyltriaz-1-en-1-yl)phenyl)ethan-1-one was synthesized by reported method [19]. 4-Aminoacetophenone (10.0 g, 74 mmol) was dissolved in 6 N HCl. This solution kept 0-5 ºC in an ice-bath. Then a solution of sodium nitrate (5.10 g, 74 mmol) in water (20 mL) was added dropwise in such a manner that temperature did not rise above 10 ºC. It was stirred for 1 h in cooling condition. After the 1 h solution of diazonium salt add to a previously cooled solution of dimethylamine hydrochloride (6.03 g, 74 mmol) in water. Then, the temperature was raised to 15-20 ºC and stirred for 0.5 h. The resulting mixture neutralizes with a 10% NaOH solution till pH become 7.0. The obtained solid was filtered out and washed with water to remove to afford (*E*)-1-(4-(3,3-dimethyltriaz-1-en-1-yl)-phenyl)ethan-1-one as an off-white solid. (13.25 g, 93%), m.p.: 130-132 ºC, ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.94 (d, 2H, *J* = 8 Hz), 7.47 (d, 2H, *J* = 7.6 Hz, 3.55 (bs, 3H), 3.24 (bs, 3H), 2.26 (s, 3H). IR (KBr, ν_{max}, cm^{-1}): 2916.4, 2862.4, 1666.5, 1597.1, 1489.1, 1435.0, 1365.6, 1273.0, 1165.0, 1095.6, 956.7, 840.9, 725.2, 601.8, 524.6. MS *m/z*: 192.1 $[M+H]^+$; Anal. calcd. (found) % $C_{10}H_{13}N_3O$: C, 62.81 (62.89); H, 6.85 (6.83); N, 21.97 (21.96).

General procedure for the synthesis of enaminones (*E*)-3-(dimethylamino)-1-(4-(*E*)-3,3-dimethyltriaz-1-en-1-yl)-phenyl)prop-2-en-1-one: A mixture of (*E*)-1-(4-(3,3-dimethyltriaz-1-en-1-yl)phenyl)ethan-1-one (13 g, 68 mmol) and dimethylformamide-dimethylacetal (16.22 g, 136 mmol) was reflux for 14 h. After completion of reaction (monitored by TLC), the reaction mixture was cooled to room temperature and the product obtained was filtered, washed with water, then dried over vacuum. It recrystallized from ethanol to obtain pure brown solid of (*E*)-3-(dimethylamino)-1-(4-(*E*)-3,3-dimethyltriaz-1-en-1-yl)phenyl)prop-2-en-1-one (**SPI-4a**). (14.63 g, 87%), m.p.: 136-138 ºC, ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.88 (d, 2H, *J* = 8.8 Hz), 7.69 (d, 1H, *J* = 12 Hz), 7.55 (d, 2H, *J* = 8.8 Hz), 5.83 (d, 1H, *J* = 12 Hz), 3.54 (bs, 3H), 3.18 (bs, 3H), 2.90 (s, 9H). IR (KBr, ν_{max}, cm^{-1}): 2908.7, 2800.7, 2584.7, 2121.7, 1928.8, 1836.2, 1643.4, 1581.6, 1543.1, 1435.0, 1357.9, 1288.4, 1242.2, 1172.7, 1095.6, 1064.7, 979.8, 895.0, 864.1, 779.2, 740.6, 694.4, 578.6. MS *m/z*: 246 $[M+H]^+$; Anal. calcd. (found) % $C_{13}H_{18}N_4O$: C, 63.39 (63.34); H, 7.37 (7.38); N, 22.75 (22.76).

General procedure for the synthesis of appropriate guanidinium hydrochloride: Dissolved corresponding aniline derivatives in methanol, these solutions were kept in an ice bath. Then gaseous HCl was bubbled into an ice-cooled solution of corresponding aniline derivatives until complete saturation (~2 h). This reaction mixture was stirred for 1h at room temperature, then obtained solid was washed with diethyl ether and dried to give quantitatively corresponding aniline hydrochloride as a crystalline solid. Then 50% solution of cyanamide was added dropwise under vigorous stirring to a solution of the corresponding hydrochloride in ethanol at 50 ºC. The temperature was then raised to 65-70 ºC and the mixture was stirred for 5-6 h until the complete conversion of hydro-chloride salt to corresponding guanidinium hydrochloride salt as crystalline solid.

Synthesis of phenylamino pyrimidines derivatives (SPI-8a-j): To a solution, enaminone (1.3 g, 5 mmol) in butanol (10 mL) was condensed with appropriate guanidine hydrochloride salt (5 mmol) in presence of NaOH (0.21 g, 5 mmol) at 120 ºC for 20-22 h. After completion of the reaction (monitored by TLC), the reaction mixture was then cooled to room temperature, the obtained product was filtered suspended in

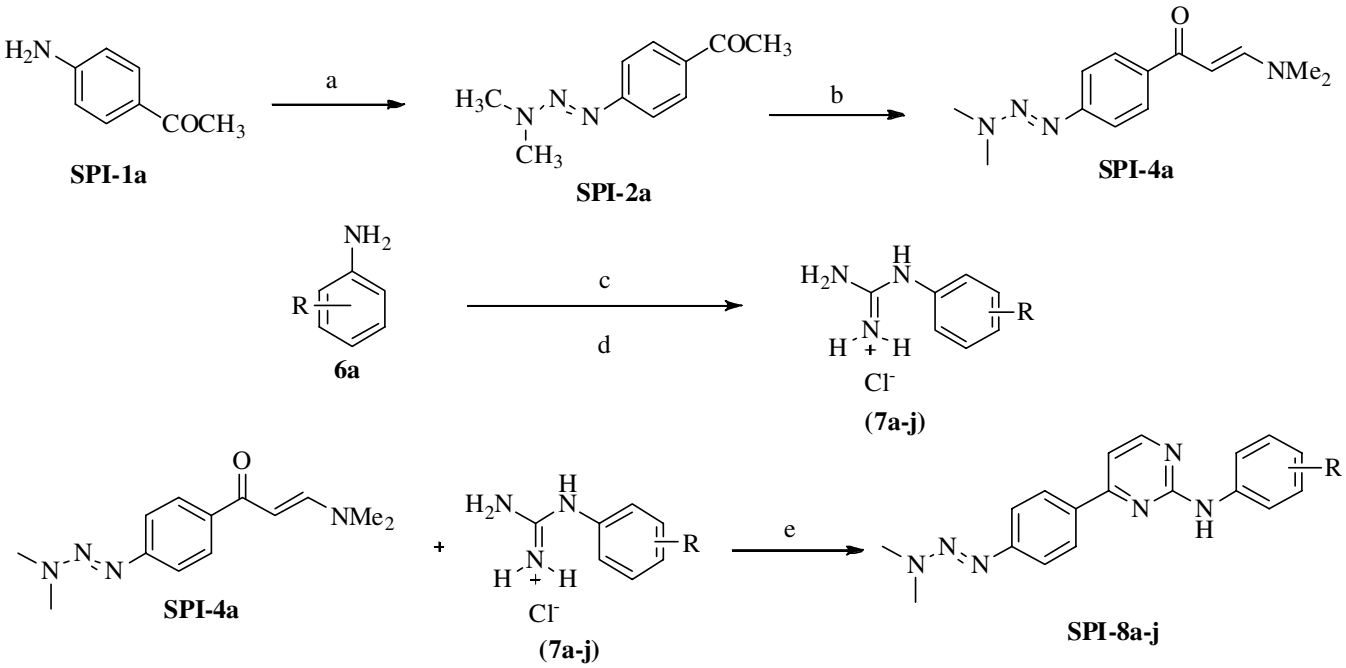
H_2O and stirred vigorously. After stirring for 1 h, the product was filtered off, washed with a small amount of ice-cooled butanol. The obtained crude product was recrystallized from DMF:water to afford pure phenylamino pyrimidines derivatives (**Scheme-I**).

(*E*)-4-(4-(3,3-Dimethyltriaz-1-en-1-yl)phenyl)-*N*-(2-methyl-5-nitrophenyl)pyrimidin-2-amine (SPI-8a): Pale yellow solid, m.p.: 187-189 ºC, yield: 83%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.1 (s, 1H, -NH), 8.91 (s, 1H), 8.54 (d, 1H, *J* = 4.4 Hz), 8.18 (d, 2H, *J* = 8 Hz), 7.88 (d, 1H, *J* = 8 Hz), 7.52 - 7.44 (m, 4H), 3.54 (bs, 3H), 3.18 (bs, 3H), 2.44 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 160.5, 153.2, 146.3, 139.6, 131.6, 128.4, 120.8, 117.9, 117.4, 108.7, 42.3, 18.87. IR (KBr, ν_{max}, cm^{-1}): 3217.3, 3109.3, 2916.4, 2615.5, 2160.3, 1905.7, 1743.7, 1643.41, 1581.68, 1535.39, 1442.8, 1381.0, 1342.5, 1273.0, 1211.3, 1087.8, 1003.0, 910.4, 825.5, 740.6. MS *m/z*: 378 $[M+H]^+$; Anal. calcd. (found) % $C_{19}H_{19}N_7O_2$: C, 60.47 (60.48); H, 5.07 (5.04); N, 25.98 (25.97).

(*E*)-*N*-(3,4-Dichlorophenyl)-4-(4-(3,3-dimethyltriaz-1-en-1-yl)phenyl)pyrimidin-2-amine (SPI-8b): Off white solid, m.p.: 195-196 ºC, yield: 83%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.04 (s, 1H, -NH), 8.57 (d, 1H, *J* = 5.6 Hz), 8.32 (d, 2H, *J* = 2.4 Hz), 8.17 (d, 2H, *J* = 8.8 Hz), 7.8 (d, 1H, *J* = 8.8 Hz), 7.76 (dd, 2H, *J* = 8.8 Hz, 2.4 Hz), 7.55 (d, 1H, *J* = 8.8 Hz), 3.54 (bs, 3H), 3.19 (bs, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 160.0, 159.3, 141.4, 137.0, 133.0, 131.2, 128.7, 122.6, 120.8, 119.0, 108.8, 42.3. IR (KBr, ν_{max}, cm^{-1}): 3279.1, 3186.5, 3093.9, 2924.1, 2299.2, 2152.6, 1921.1, 1797.7, 1643.4, 1581.6, 1543.1, 1473.6, 1381.0, 1288.4, 1203.6, 1165.0, 1087.8, 987.5, 895.0, 848.7, 810.1, 779.2, 740.6, 570.9, 524.6. MS *m/z*: 386 $[M+H]^+$; Anal. calcd. (found) % $C_{18}H_{16}N_6Cl_2$: C, 55.86 (55.89); H, 4.16 (4.14); N, 21.70 (21.72).

(*E*)-4-(4-(3,3-Dimethyltriaz-1-en-1-yl)phenyl)-*N*-(4-fluorophenyl)pyrimidin-2-amine (SPI-8c): Off white solid, m.p.: 189-192 ºC, yield: 89%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.70 (s, 1H, -NH), 8.50 (d, 1H, *J* = 5.2 Hz), 8.15 (d, 2H, *J* = 8.4 Hz), 7.86-7.82 (m, 2H), 7.48 (d, 2H, *J* = 8.4 Hz), 7.38 (d, 1H, *J* = 5.2 Hz), 7.16 (t, 2H, *J* = 8.8 Hz), 3.53 (bs, 3H), 3.19 (bs, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 147.50, 121.39, 127.50, 160.76, 109.67, 140.62, 122.20, 116.12, 157.53, 42.30. IR (KBr, ν_{max}, cm^{-1}): 3255.9, 3194.2, 3055.3, 2901.0, 2816.1, 2546.1, 2214.3, 2083.1, 1990.6, 1936.6, 1874.8, 1828.5, 1759.2, 1620.2, 1581.6, 1558.5, 1504.5, 1419.6, 1381.0, 1334.7, 1296.2, 1211.3, 1157.3, 1087.8, 995.3, 918.1, 833.2, 810.1, 732.9. MS *m/z*: 336 $[M+H]^+$; Anal. calcd. (found) % $C_{18}H_{17}N_6F$: C, 64.27 (64.25); H, 5.09 (5.11); N, 24.98 (24.95).

(*E*)-4-(4-(3,3-Dimethyltriaz-1-en-1-yl)phenyl)-*N*-(*p*-tolyl)pyrimidin-2-amine (SPI-8d): Off white solid, m.p.: 196-198 ºC, yield: 82%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.5 (s, 1H, -NH), 8.4 (d, 1H, *J* = 8.8 Hz), 8.15 (d, 2H, *J* = 7.2 Hz), 7.71 (d, 2H, *J* = 6.8 Hz), 7.48-7.34 (m, 3H), 7.12 (d, 2H, *J* = 6.4 Hz), 3.53 (bs, 3H), 3.19 (bs, 3H), 2.2 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 147.50, 121.39, 127.65, 160.76, 108.76, 140.02, 118.96, 129.35, 133.24, 42.30, 18.54. IR (KBr, ν_{max}, cm^{-1}): 3263.6, 3186.5, 3101.6, 3505.3, 2924.1, 2854.7, 2746.7, 2299.2, 1928.8, 1782.2, 1751.4, 1720.5, 1658.8, 1612.5, 1581.6, 1535.3, 1422.8, 1419.6, 1381.0, 1342.5, 1280.7, 1211.3, 1165.0, 1087.8, 1018.4, 995.3, 918.1, 810.1, 732.9. MS *m/z*: 332 $[M+H]^+$; Anal. calcd. (found) % $C_{19}H_{20}N_6$: C, 68.65 (68.66); H, 6.06 (6.09); N, 25.28 (25.27).



Scheme-I: Synthesis of the title compound SPI (**8a-j**): Reagents and Conditions: (a) $NaNO_2$ (1 equiv), 6N HCl (10 vol), 0-5 °C, 1 h; dimethylamine hydrochloride (1 equiv), 15-20 °C, 0.5 h, 10% NaOH; (b) Dimethylformamide-dimethylacetal (2 equiv), Reflux, 120 °C, 14 h; (c) Gaseous HCl, MeOH, 0-5 °C, 2 h; (d) 50% aqueous solution of cyanamide, ethanol, heating at 85 °C, 5-6 h (e) NaOH, reflux, butanol, 120 °C, 20-22 h

(*E*)-*N*-(3-Chloro-4-fluorophenyl)-4-(4-(3,3-dimethyltriaz-1-en-1-yl)phenyl)pyrimidin-2-amine (SPI-8e): Off white solid, m.p.: 196-198 ºC, yield: 82%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.07 (s, 1H, -NH), 8.76 (d, 1H, *J* = 5.6 Hz), 8.56 (d, 2H, *J* = 2.4 Hz), 8.23 (d, 2H, *J* = 8.8 Hz), 7.94 (d, 1H, 8.8 Hz), 7.87 (dd, 2H, *J* = 8.8 Hz, 2.4 Hz), 7.66 (d, 1H, *J* = 8.8 Hz), 3.54 (bs, 3H), 3.19 (bs, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 162.76, 154.44, 148.60, 139.05, 128.65, 122.39, 120.45, 118.64, 116.09, 110.76, 42.3. IR (KBr, ν_{max}, cm^{-1}): 3441.1, 2924.1, 2854.7, 1735.9, 1635.6, 1597.1, 1504.5, 1473.6, 1350.2, 1288.4, 1257.6, 1165.0, 1087.8, 1033.8, 856.4, 779.2, 732.9, 678.9, 570.9, 524.6. MS *m/z*: 370 [M+H]$^+$; Anal. calcd. (found) % $C_{18}H_{16}N_6FCl$: C, 58.30 (58.33); H, 4.35 (4.34); N, 22.66 (22.68).

(*E*)-4-(4-(3,3-Dimethyltriaz-1-en-1-yl)phenyl)-*N*-(3,4,5-trimethoxyphenyl)pyrimidin-2-amine (SPI-8f): Off white solid, m.p.: 189-191 ºC, yield: 88%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.6 (s, 1H, -NH), 8.14 (d, 1H, *J* = 8.8 Hz), 7.98 (d, 1H, *J* = 8.4 Hz), 7.94 (d, 2H, *J* = 8 Hz), 7.76 (d, 2H, *J* = 2.4 Hz), 7.47 (d, 2H, *J* = 2.6 Hz), 3.84 (s, 9H), 3.53 (bs, 3H), 3.19 (bs, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 159.77, 156.92, 147.49, 127.63, 121.38, 138.80, 132.47, 110.73, 108.71, 56.73, 42.30. IR (KBr, ν_{max}, cm^{-1}): 3273.7, 3194.6, 3113.7, 2926.2, 2867.5, 2747.2, 2295.4, 1967.2, 1756.3, 1756.8, 1752.5, 1656.3, 1614.9, 1533.3, 1424.6, 1333.8, 1275.6, 1210.7, 1083.4, 1009.3, 835.4, 760.9, 680.3. MS *m/z*: 408 [M+H]$^+$; Anal. calcd. (found) % $C_{21}H_{24}N_6O_3$: C, 61.75 (61.76); H, 5.92 (5.90); N, 20.58 (20.57).

(*E*)-*N*-(2,4-Difluorophenyl)-4-(4-(3,3-dimethyltriaz-1-en-1-yl)phenyl)pyrimidin-2-amine (SPI-8g): Off white solid, m.p.: 201-203 ºC, yield: 84%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.8 (s, 1H, -NH), 8.35 (d, 1H, *J* = 8.8 Hz), 8.15 (d, 1H, *J* = 8.4 Hz), 7.97 (d, 2H, *J* = 8 Hz), 7.62 (d, 2H, *J* = 2.6 Hz), 7.56 (d, 1H, *J* = 2.4 Hz), 7.38 (dd, 1H, *J* = 8.8 Hz, 2.4 Hz), 7.25 (d, 1H, *J*= 8 Hz). ^{13}C NMR (100 MHz, DMSO-d_6): δ 159.63, 158.50, 148.76, 163.76, 130.89, 127.73, 123.42, 122.44, 115.60, 110.76, 104.22, 42.30. IR (KBr, ν_{max}, cm^{-1}): 3273.3, 3189.6, 3085.1, 2921.5, 2296.2, 2256.4, 1920.4, 1797.8, 1657.6, 1580.7, 1542.3, 1400.3, 393.9, 1089.5, 1003.3, 989.6, 960.2, 735.2, 725.7, 680.2. MS *m/z*: 354 [M+H]$^+$; Anal. calcd. (found) % $C_{18}H_{16}N_6F_2$: C, 61.01 (61.03); H, 4.55 (4.53); N, 23.72 (23.73).

(*E*)-*N*-(2,4-Dichlorophenyl)-4-(4-(3,3-dimethyltriaz-1-en-1-yl)phenyl)pyrimidin-2-amine (SPI-8h): Off white solid, m.p.: 186-188 ºC, yield: 79%, ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.7 (s, 1H, -NH), 8.34 (d, 1H, *J* = 8 Hz), 8.14 (d, 1H, *J* = 8.4 Hz), 7.95 (d, 2H, *J* = 8.8 Hz), 7.52 (d, 2H, *J* = 2.4 Hz), 7.41 (d, 1H, *J* = 2.4 Hz), 7.24 (dd, 1H, *J* = 8.8 Hz, 2.4 Hz), 7.15 (d, 1H, *J* = 8 Hz). ^{13}C NMR (100 MHz, DMSO-d_6): δ 163.76, 148.50, 140.15, 138.70, 133.95, 131.05, 130.89, 128.74, 122.43, 112.83, 109.76, 42.30. IR (KBr, ν_{max}, cm^{-1}): 3256.9, 3196.2, 3065.1 2926.7, 2295.8, 2162.5, 1922.2, 1794.2, 1644.6, 1583.6, 1543.2, 1473.5, 1382.7, 1288.9, 1203.9, 160.8, 1089.2, 986.4, 860.3, 760.5, 530.6. MS *m/z*: 386 [M+H]$^+$; Anal. calcd. (found) % $C_{18}H_{16}N_6Cl_2$: C, 55.83 (55.85); H, 4.16 (4.17); N, 21.70 (21.69).

(*E*)-*N*-(4-Chlorophenyl)-4-(4-(3,3-dimethyltriaz-1-en-1-yl)phenyl)pyrimidin-2-amine (SPI-8i): Off white solid, m.p.: 187-189 ºC, yield: 87%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.50 (s, 1H, -NH), 8.40 (d, 1H, *J* = 5.2 Hz), 7.93 (d, 2H, *J* = 8.4 Hz), 7.60-7.56 (m, 2H), 7.35 (d, 2H, *J* = 8.4 Hz), 7.20 (d, 1H, *J* = 5.2 Hz), 7.16 (t, 2H, *J* = 8.8 Hz), 3.53 (bs, 3H), 3.19 (bs, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 160.76, 147.50, 141.65, 131.62, 129.04, 127.65, 122.77, 121.39, 109.76, 42.30. IR (KBr, ν_{max}, cm^{-1}): 3246.7, 3093.2, 2906.3, 2589.7, 2217.2, 2092.7, 1967.8, 1937.5, 1875.5, 1750.6, 1680.3, 1540.3, 1390.8, 1250.3, 1183.4, 1083.6, 1020.9, 960.3, 830.7, 730.9, 637.3, 560.8. MS *m/z*: 352 [M+H]$^+$; Anal. calcd. (found) % $C_{18}H_{17}N_6Cl$: C, 61.28 (61.26); H, 4.86 (4.88); N, 23.82 (23.80).

(*E*)-4-(4-(3,3-Dimethyltriaz-1-en-1-yl)phenyl)-*N*-(2-fluorophenyl)pyrimidin-2-amine (SPI-8j): Off white solid, m.p.: 210-212 ºC, yield: 88%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.7 (s, 1H, -NH), 7.92 (d, 2H, *J* = 8 Hz), 7.81 (d, 1H, *J* = 8.8 Hz), 7.55 (d, 1H, *J* = 8Hz), 7.25-7.10 (m, 4H, 3.54 (bs, 3H), 3.19 (bs, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 160.65, 158.38, 147.43, 134.33, 127.50, 126.93, 124.76, 122.39, 120.32, 118.98, 112.32, 42.30. IR (KBr, ν_{max}, cm^{-1}): 3245.8, 3094.3, 2903.0, 2587.1, 2213.6, 2087.1, 1984.3, 1938.3, 1873.2, 1740.7, 1630.2, 1583.7, 1554.6, 1420.7, 1385.6, 1298.3, 1200.1, 1197.3, 1087.6, 992.7, 920.6, 840.3, 730.2. MS *m/z*: 336 [M+H]$^+$; Anal. calcd. (found) % $C_{18}H_{17}N_6F$: C, 64.27 (64.26); H, 5.09 (5.08); N, 24.98 (24.99).

***in vitro* antimicrobial activity.** The antimicrobial activity was evaluated for all the synthesized 10 compounds against Gram-positive, Gram-negative bacteria and fungal strains by the microtitre broth dilution method. The stock cultures of the microbial strains were cultured in the Muller-Hinton broth for 24 h at 37 °C for bacterial strains whereas at 28 ºC for fungi. Thaw and weigh the standard antibiotics ampicillin, streptomycin and nystatin. Dilute the antibiotics in DMSO and find their MIC value against test strains separately. Drug stocks were also prepared in DMSO solvent. Dispense 2 mL of Muller-Hinton broth into the first tube and 1 mL broth in all other tubes. Pour the drug into the first tube to make up the final concentration as 1000 µg/mL. Mixed the drug with media thoroughly and then withdraw 1mL antibiotic solution and transferred into next tube. The concentration of the second tube will be reached to 500 µg/mL. Mixed up and down 6-8 times and again transfer 1 mL of an antibiotic solution to the third tube containing broth. Similarly, serial dilution can be performed up to 62.5 µg/mL. Discard 1mL antibiotic solution from the last tube. Mid-log cultured microbes were diluted and adjust the bacterial growth to get a cell turbidity equivalence to the McFarland 0.5 standard. This dilution will be equivalent to bacterial cell density 4×10^5 to 5×10^5 cfu/mL. Fungal growth will be equivalent to $1\text{-}5 \times 10^5$ cfu/mL. All the microbes (5 µL) were transferred from tube 1 to tube 5. Incubate the tubes for 24-48 h at the desired temperature of bacteria and fungi. The positive control only has the drug solution added to the broth and the negative control only has the microorganisms inoculated in the broth. When satisfactory growth is obtained, the absorbance of microbes was taken at 600 nm.

RESULTS AND DISCUSSION

According to **Scheme-I**, (*E*)-1-(4-(3,3-dimethyltriaz-1-en-1-yl)phenyl)ethan-1-one was synthesized by coupling of dimethylamine hydrochloride with diazotized 4-aminoacetophenone in 6 N HCl at low temperature as per reported method [19]. The structure of compound **SPI-2a** was confirmed from

^{1}H NMR in $CDCl_3$, which showed two broad singlet signals at δ 3.55 and δ 3.24 ppm corresponding to the methyl protons of the triazene moiety and the peak of the aromatic proton appeared in the range δ 7.95-7.46 ppm. The mass spectrum revealed a molecular ion peak at *m/z* 191 correspondings to a molecular formula $C_{10}H_{13}N_3O$. The IR spectrum of compound **SPI-2a** showed an absorption band at 1666 cm^{-1} due to C=O stretching. Aromatic C-H stretching vibrations were defined by the absorption band at 2916 cm^{-1}. The N=N stretching vibration band was observed at 1489 and 1453 cm^{-1} and bending vibration at 1095 cm^{-1}, while the characteristic function of amine C-N vibration was observed at 1165 cm^{-1}. The ring stretching vibration (C=C) at 1597 cm^{-1}.

The enaminone was made to react with dimethylformamide-dimethyl acetal and therefore the product can be directly recrystallized from the reaction mixture as orange crystalline solid. The structure of enaminone (**SPI-4a**) was confirmed by the ^{1}H NMR, mass, IR and elemental analysis. ^{1}H NMR spectrum of (*E*)-3-(dimethylamino)-1-(4-(*E*)-3,3-dimethyltriaz-1-en-1-yl)phenyl)prop-2-en-1-one in DMSO-d_6 showed two broad singlet signal at δ 3.54 ppm and δ 3.19 ppm correspond to the two methyl protons of triazene moiety another singlet signal in the region δ 2.90 ppm characteristic for the -N(CH_3)$_3$ group of enaminone as well as two doublet signals at δ 5.85-5.82 ppm and δ 7.70-7.67 ppm with coupling constant *J* = 13 Hz assignable to the two olefinic protons. The peaks of the aromatic protons appeared as a doublet in the range of δ 7.88 ppm and δ 7.55 ppm. The mass spectrum revealed a molecular ion peak at *m/z* 246 correspondings to a molecular formula $C_{13}H_{18}N_4O$.

Phenylamino pyrimidines were also synthesized as per reported classical method [20]. Different substituted aniline salt can be converted into corresponding guanidine hydrochloride salt (**7a-j**) in the presence of 50% cyanamide solution. The guanidines were best characterized by the crystalline solid product from the reaction mixture. Since, different substituted aniline hydrochlorides were used, therefore, it was necessary to add 10% NaOH solution before the workup. Remember that exta care should be taken guanidine hydrochlorides should not be dried at higher temperatures otherwise it may lead to the decomposition of salt. Then enaminone (**SPI-4a**) was allowed to react with different guanidine hydrochloride (**7a-j**) in butanol at reflux temperature in the presence of NaOH to obtain different phenylamino derivatives (**SPI-8a-j**).

The structure of all the isolated derivatives was elucidated based on their spectral data. Elemental analysis, IR, NMR and MS are in agreement with the proposed structure. The appearance of the singlet signal at δ 9.1 ppm of amine proton indicates the formation of the phenylamino pyrimidine ring in the ^{1}H NMR spectrum of **SPI-8a** ((*E*)-4-(4-(3,3-dimethyltriaz-1-en-1-yl)phenyl)-*N*-(2-methyl-5-nitrophenyl)pyrimidin-2-amine). The aromatic protons appeared in the range at δ 7.44-8.91 ppm range and another singlet signal at δ 2.44 ppm correspond to the three protons of –CH_3 group. The broad two singlet signal appeared at δ 3.05 ppm and δ 3.08 ppm assigned to the two methyl protons of triazene moiety. The ^{13}C NMR (100 MHz, DMSO-d_6) spectrum of compound **SPI-8a** was characterized by a signal at δ 42.39 ppm assignable to CH_3 carbon linked to nitrogen of triazene moiety and the aromatic carbon appeared in the range of δ 108.7-160.5 ppm. In addition, the mass spectrum revealed a molecular ion peak at *m/z* 378 correspondings to a molecular formula $C_{19}H_{19}N_7O_2$. The IR spectrum of **SPI-8a** revealed a absorption band at 3217 cm^{-1} assignable to –NH group. Aromatic C-H stretching vibrations were defined by the absorption band in the range 3417-2916 cm^{-1}; 825, 740 cm^{-1} responsible for aromatic C-H bending vibration and 2854 cm^{-1} for aliphatic C-H stretching. The N=N stretching vibration band observed at 1442 cm^{-1} and bending vibration at 1087 and 1003 cm^{-1}. Compound **SPI-8a** containing nitro group is readily identified by the band observed at 1381, 624, 570, 540 cm^{-1}. The band observed at 1342, 1273, 1211 cm^{-1} is assignable to the C-N stretching vibration.

Antimicrobial evaluation: The newly synthesized compounds were evaluated for their *in vitro* antibacterial and antifungal activity against various Gram-positive, Gram-negative and fungal strains. The results of the antimicrobial evaluation are given in Table-1 and the result is compared with standard drugs. It revealed that most of the tested compounds displayed variable inhibitory effects on the growth of the tested Gram-positive and Gram-negative bacterial strains and also against antifungal strains. As can be seen from Table-1, compounds

TABLE-1
ANTIMICROBIAL SCREENING DATA OF THE COMPOUNDS **SPI-8a-j**

Entry	Antibacterial activity				Antifungal activity	
	Gram-positive bacteria		Gram-negative bacteria			
	Staphylococcus aureus	*Bacillus subtilis*	*Pseudomonas aeruginosa*	*Escherichia coli*	*Aspergillus paraciticus*	*Rhizopus*
SPI-8a	1000	1000	1000	1000	500	500
SPI-8b	1000	1000	1000	1000	1000	1000
SPI-8c	250	250	1000	1000	1000	1000
SPI-8d	125	125	250	250	1000	1000
SPI-8e	500	500	500	500	1000	1000
SPI-8f	125	125	125	125	500	500
SPI-8g	250	250	1000	1000	1000	1000
SPI-8h	1000	1000	1000	1000	1000	1000
SPI-8i	500	500	250	250	1000	1000
SPI-8j	500	500	1000	1000	1000	1000
Streptomycin	–	–	50	50	–	–
Ampicillin	100	100	–	–	–	–
Nystatin	–	–	–	–	100	100

Minimum inhibitory concentration (μg/mL)

SPI-8d, **SPI-8e**, **SPI-8f** and **SPI-8i** are very effective to inhibit the growth of both Gram-positive and Gram-negative bacteria. Compounds **SPI-8c**, **SPI-8g** and **SPI-8j** were effective against Gram-positive bacteria. Compound **SPI-8a** shows mainly antifungal activity, while compound **SPI-8f** was found to affect Gram-positive bacteria, Gram-negative bacteria as well as fungi.

Conclusion

A series of novel (*E*)-4-(4-(3,3-dimethyltriaz-1-en-1-yl)-phenyl)-*N*-phenylpyrimidin-2-amine derivatives have been synthesized. The structure of newly synthesized compounds was confirmed by spectral data and elemental analysis. All the synthesized compounds were tested *in vitro* to evaluated antibacterial and antifungal activity. All the tested compounds were compared with standard drugs. Compound **SPI-8f** was found to be most effective against Gram-positive bacteria, Gram-negative bacteria as well as fungi. Compounds **SPI-8d**, **SPI-8e**, **SPI-8f** and **SPI-8i** were very effective broad-spectrum compounds which can inhibit the growth of both Gram-positive and Gram-negative bacteria. Compounds **SPI-8c**, **SPI-8g** and **SPI-8j** exhibited antibacterial activity against Gram-positive bacteria; therefore, their mode of action should be probably the cell wall of bacteria. Finally, compound **SPI-8a** showed mainly antifungal activity.

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