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Synthesis of Pyrimidine Substituted 1,2,3-Triazole Derivatives Via Click Reactions and its Biological Evaluation

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Abstract: A Series of novel pyrimidine substituted 1,2,3 triazole was synthesized by reacting substituted alkyne with azides to generate a library of compounds (4a- h) by click chemistry . Structures of newly synthesized compounds were confirmed by IR, ^{1H}& ^{13}C NMR & LC-MS spectral data. Among the compounds **4e** showed better anti-bacterial activity towards *S.aereus & B.Subtilis.*
Keywords: Pyrimidines, Azides, click reaction, Antimicrobial activity.

1.Introduction

Nitrogen containing heterocyclic ring such as pyrimidine is a promising structural moiety for drug design. Pyrimidine derivatives form a component in a number of useful drugs and are associated with many biological and therapeutical activities[1]. Condensed Pyrimidine derivatives have been reported as antibacterial[2], analgesic, anti-viral, anti-inflammatory[3], anti-HIV[4],Anti-tubercular[5],anti-tumour[6],anti-neoplastic[7],anti-malarial[8], diuretic[9], cardiovascular agents[10].

Triazole ring is a potential pharmacophore that has gained interest over the past few years. Several derivatives have already shown interesting biological activities and a most were found to be efficient in the several infections. Hence 1, 2, 3-triazoles are synthesized because of their biological activities such as anti tubular[11], anti-HIV[12],antifungal[13] antibacterial[14], and anticancer activities[15]. This triazole moiety stable to metabolic degradation and capable of hydrogen bonding, which could be favourable in binding of biomolecular targets and increasing solubility[16] Along with these 1,2,3-triazole prove to be amide surrogates in various bioactive compounds [17,18] .It was envisaged that these two active pharmacophores, If linked together, would generate novel molecular hybrids which are likely to exhibit interesting biological properties.The above cited application prompted us to synthesize a series of new compounds reported in this article.

Owing to the importance, here we have described the synthesis and screened for the anti-microbial activities of pyrimidine substituted 1,2,3 triazole derivatives.

2.0 Experimental section

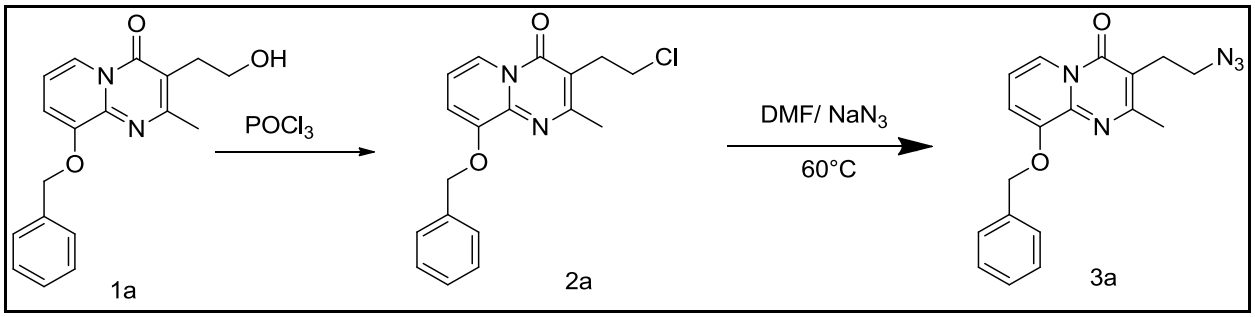
2.1 Material and measurements

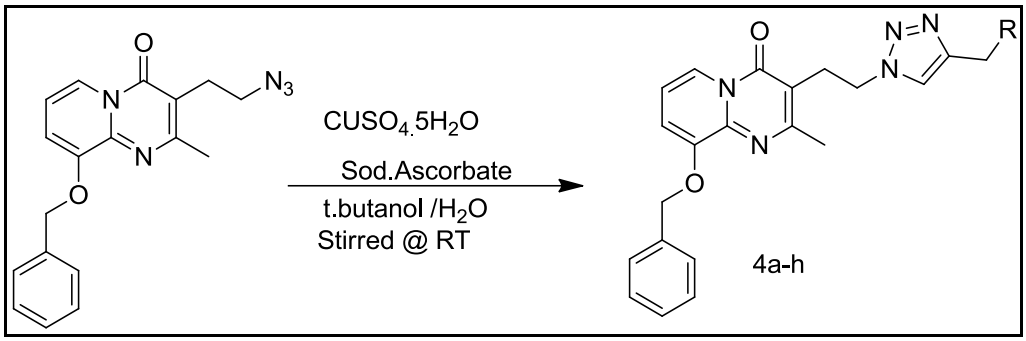
All the chemicals were purchased from Spectrochem .Ltd.(Mumbai, India)and were used without further purification. Solvents employed were distilled, purified and dried by standard procedures .Melting points of the synthesized compounds were determined in open capillary tube method and are uncorrected. Reactions were monitored by thin-layer chromatography (TLC on aluminum plates coated with silica gel 60

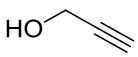
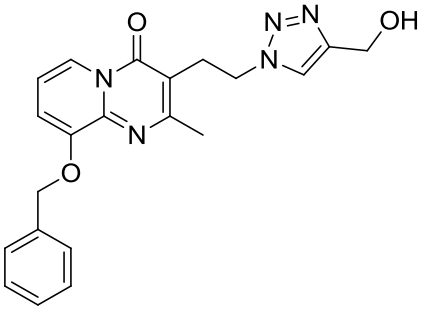
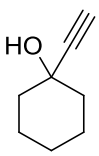
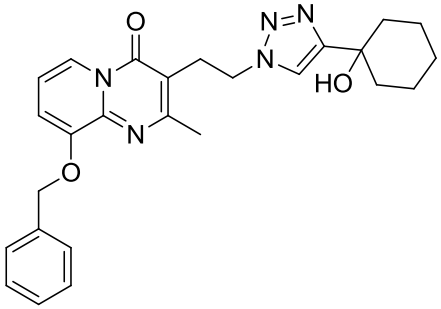
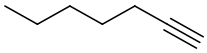
$F^{254,}$ 0.25 mm thickness purchased from E.Merck Ltd.Mumbai, India). The mobile phase was Ethyl acetate and hexane (6:4),and detection of the components was done under UV Light or explore in Iodine chamber.

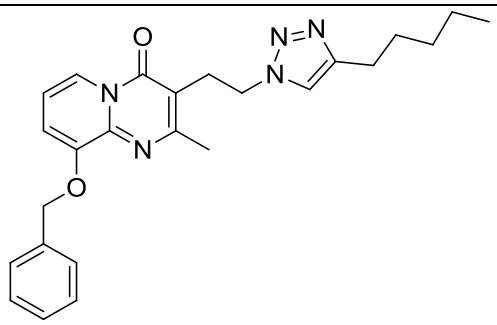
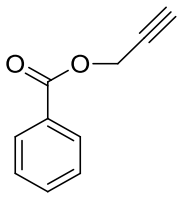
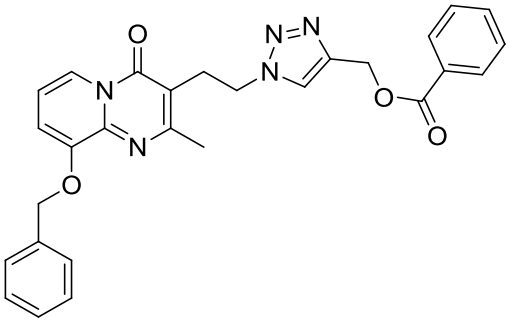
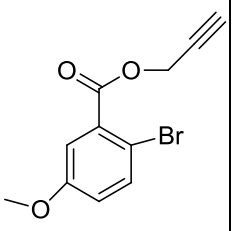
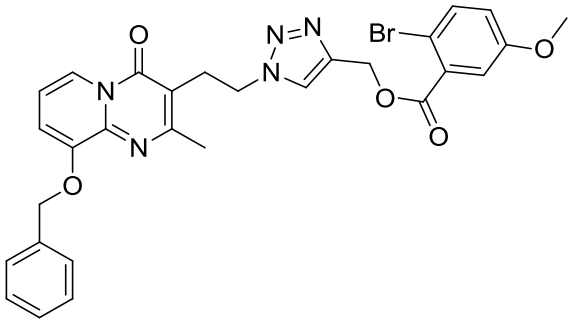
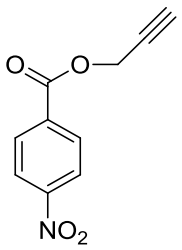
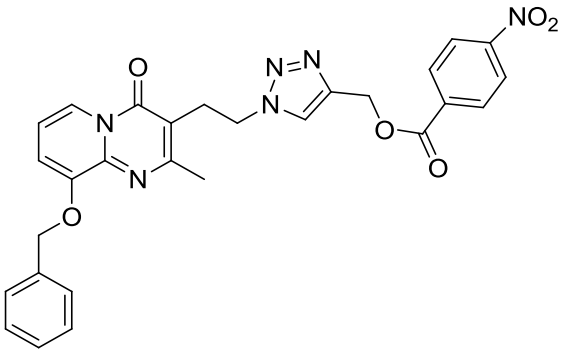
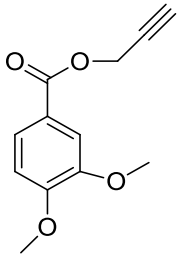
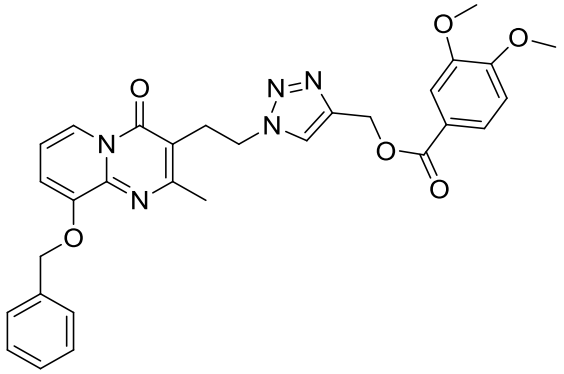
The Infrared spectra (inKBr pellets) were recorded on a JOSCO spectrometer and frequencies are expressed in $cm^{-}1$. Mass spectra (CG/MS) were recorded on a Agilent MSD VL mass spectro meter .^{1}H NMR spectra were recorded on a Bruker Advance 400 spectrometer operating at 400.00 MHZ .^{13}C spectra were recorded in a Bruker advance 400 spectrometer operating at 400.00 MHZ . The chemical shifts are reported in ppm (δ) relative to tetra methyl silane.proton and carbon spectra were typically obtained at room temperature.

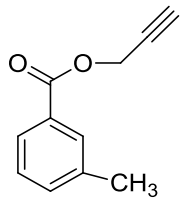
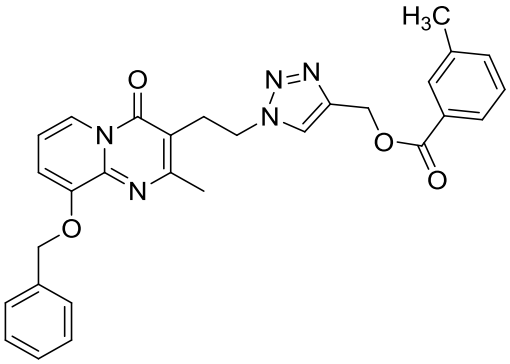
Schematic diagrams for the synthesis of pyrimidine substituted 1,2,3 triazole derivatives.





Entry	Alkyne	Product	Yield	m.pt
4a			74%	160-162°C
4b			65%	205-210°C
4c			63%	165-168°C

				
4d			68%	150-152°C
4e			72%	130-132°C
4f			78%	155-158°C
4g			71%	160-163°C

4h			69%	157-160°C

General procedures for the preparation of compounds

3.1.1. Synthesis of 9-(benzyloxy)-3-(2-Chloroethyl)-2-methyl-4H-pyrido[1,2-a]pyrimidin-4-one.(2a)

A mixture of compound 2 (0.001mol) and $POCl_3$ (10ml) was heated under reflux for 8h. The reaction mass was concentrated under reduced pressure and then quenched in ice. The solid obtained was filtered off. Washed with water, dried and crystallized from methanol solvent.

Off white solid. Yield: 70 %; MS: m/Z: 328(M+) IR (KBr)cm^{-1} 3460(OH) ,3132(CH-arom), 3091(CH-aliph), 1690(C=O), 1462(C=N).

3.1.2. Synthesis of of 3-(2-azidoethyl)-9-(benzyloxy)-2-methyl-4H-pyrido[1,2-a]pyrimidin-4-one(3a)

To the stirred suspension of 2a in dry DMF (10ml) was added NaN_3 (1.5 mol) and the reaction mixture was heated at 60°C for about 5h (monitored by TLC) Upon completion of reaction, the content was poured into crushed ice, The reaction mixture was extracted with ethyl acetate and the organic layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to get solid. The obtained crude product was purified by column chromatography on silica gel using ethyl acetate: hexane as an eluent to obtain a pure product.

Yield - 68%, MS m/z :336(M^+), IR (KBr)cm^{-1} 3176(CH-arom) ,2906(CH-aliph), 1678(C=O), 1542(C=N) 1292 (C=O).

3.1.3.Synthesis of compound(4a-h) : general procedure

Compound 3a (1.0 mol),alkynes **4a-4h** (1.0 mol),copper sulphate penta hydrate (20 mol%) and sodium ascorbate (20 mol%) in tert-butanol and water (1:1, v/v, 4ml) was stirred at RT for appropriate time, After the completion of TLC, the reaction mixture was treated with ethyl acetate (2x10ml) and water (5ml) the organic layer was separated , washed with brine solution dried over anhydrous sodium sulphate and concentrated under reduced pressure. The crude product was purified by column chromatography over silica gel using ethyl acetate/hexane (1:2) to afford corresponding 1,2,3 – triazoles.

3.1.3.1 Synthesis of 9-(benzyloxy)-3-(2-(4-(hydroxymethyl)-1H-1,2,3-triazol-1-yl)ethyl)-2-methyl-4Hpyrido[1,2-a]pyrimidin-4-one(4a)

White solid. Yield-72% , M.p 160-162°C; MS m/z : 392 (M^+) IR(KBr)cm^{-1} : 3375 (OH),3133 (CH-arom),2956 (CH-aliph),1630 (C=O),1478(C=N),1290 (C—O),1242 (C-N),^{1}H NMR (DMSO-d_6): δ8.0(S,H (triazole),7.19-7.53(M, 7H,Ar-H),8.5-8.6(M,1H),5.29(S,2H),4.47-4.57(M,4H),3.11-3.14(T,2H),2.11(S,3H).^{13}C NMR (DMSO-d_6): δ157.65,148.40,143.13,128.54,118.5,111.39,71.1,55.46,47.7,28.68

3.1.3.2 synthesis of 9-(benzyloxy)-3-(2-(4-(hydroxycyclohexyl)-1H-1,2,3-triazol-1-yl)ethyl)-2-methyl-4H-pyrido[1,2-a]pyrimidin-4-one(4b)

Yellow solid. Yield-75% M.p 205-210°C; MS m/z : 460(M^+) IR(KBr) cm^{-1}: 3461(OH) ,3123(CH-arom), 2941(CH-aliph), 1660(C=O), 1456(C=N),1298 (C-O),1243(C-N). ^{1}H NMR (DMSO-d6): δ7.8(S,H

(triazole),7.18-7.52(M, 7H,Ar-H),8.52-8.54(M,1H), 2.08(S,3H)1.82-1.88(M,2H)1.61-1.65(M,3H)1.23-1.45 (M, 4H). ^{13}CNMR(DMSO-d6):δ157.64,143.13,121.55 ,111.45,71.10,68.40,47.7,22.38

3.1.3.3. Synthesis of 9-(benzyloxy)-2-methyl-3-(2-(4-(pentyl-1H-1,2,3-triazol-1-yl) ethyl)-4H-pyrido[1,2-a]pyrimidin-4-one(4c)

White solid. Yield-68% M.p 165-168°C; MS m/z : 476(M+) IR(KBr) cm^{-1}: 3122(CH-arom) ,2983 (CH-aliph), 1668(C=O), 1460(C=N), 1273(C-O),1238 (C-N).^{1}H NMR (DMSO-d6): δ7.6(S,H (triazole),7.18-7.52(M, 7H,Ar-H),8.94(M,1H), 2.92(M,3H),2.44(T,2H),2.3-1.3(M,8H),0.9(T,3H), ^{13}C NMR (DMSO-d6): δ159.64, 145.3, 121.6, 111.45, 71.10, 68.40, 47.7, 23.

3.1.3.4.Synthesis of(1-(2-(9-(benzyloxy)-2-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidin-3-Yl)ethyl)-1H-1,2,3-triazol-4-yl)methyl benzoate(4d)

Pale yellow solid. M.p 150-152°C; MS m/z : 496(M^{+}) IR(KBr) cm^{-1}3127(CH-arom) , 2957(CH-aliph), 1718(O-C=O-), 1634(C=O), 1452(C-N),1272 (C-O),1240(C-N).^{1}H NMR (DMSO-d6): δ7.6(S,H (triazole),7.18-7.47(M, 7H,Ar-H),8.94(M,1H), 7.56-7.8(M,5H), 4.97(D,2H) 3.77(T,2H) 2.92(D,2H) ^{13}C NMR (DMSO-d6): δ158.64 ,144.5., 121.6, 111.35, 71.12,68.40,47.7,23.2

3.1.3.5. Synthesis of (1-(2-(9-(benzyloxy)-2-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidin-3-l)ethyl)-1H-1,2,3-triazol-4-yl)methyl 2-bromo-5-methoxybenzoate(4e)

White solid. M.p 130-132°C; MS m/z : 606 (M^{+}) ,IR(KBr): cm^{-1} 3114(CH-arom) ,2962(CH-aliph), 1731(O-C=O-), 1635(C=O), 1461(C-N),1299 (C-O),1247(C-N).

^{1}H NMR (DMSO-d6): δ7.6(S,H (triazole),7.18-7.47(M, 7H,Ar-H),8.94(M,1H), 7.56-7.8(M,5H) 4.97(D,2H) 3.83(T,3H), 3.77(T,2H)2.92(D,2H). ^{13}C NMR (DMSO-d6): δ158.64,144.5., 121.6, 111.35,71.12, 68.40, 47.7, 23.2.

3.1.3.6.Synthesis of (1-(2-(9-(benzyloxy)-2-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidin-3-

Yl)ethyl)-1H-1,2,3-triazol-4-yl)methyl 4-nitrobenzoate(4f)

Light brown solid. Yield-76%,. M.p 155-158°C, cm-1, MS m/z : 606 (M^{+}) 556, IR(KBr): cm^{-1} 3120 (CH-arom), 2970(CH-aliph), 1720(O-C=O-),1640(C=O), 1450(C=N),1295

(C-O),1251(C=N). ^{1}H NMR (DMSO-d6): δ7.6(S,H(triazole),7.18-7.47(M,7H,Ar-H), 8.94(M,1H, 8.31-8.37 (M,4H) 5.63(D,2H)4.67(D,2H), 3.77(T,2H) 2.92(M,3H),2.44(M,2H). ^{13}C NMR (DMSO-d6): δ157.64,144.5, 121.6,112.35,71.12,68.40,48.7,23.2.

3.1.3.7.Synthesis of (1-(2-(9-(benzyloxy)-2-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidin-3-

Yl)ethyl)-1H-1,2,3-triazol-4-yl)methyl 3,4-dimethoxy benzoate(4g)

Pale yellow solid. Yield-71% , . M.p 160-163°C, cm-1, , MS m/z : 541 (M+) , IR(KBr): cm-1313(CH-arom) ,2986(CH-aliph), 1690(O-C=O), 1645(C=O), 1448(C-N),1287 (C-O),1245 (C=N).). ^{1}H NMR (DMSO-d6): δ7.6(S,H(triazole),7.18-7.47(M,7H,Ar-H),8.94(M,1H), 7.1-7.5(M,3H) 5.63(D,2H)3.83(D,3H), 3.83(D,3H),4.97(D,2H), 3.77(T,2H), 2.92(M,3H)2.44 (M,2H). ^{13}C NMR (DMSO-d6): δ156.64,145.5, 121.6,111.35, 71.12, 68.40,48.7,23.2.

3.1.3.8.Synthesis of -(2-(9-(benzyloxy)-2-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidin-3-

Yl)ethyl)-1H-1,2,3-triazol-4-yl)methyl 3-methylbenzoate(4 h)

White solid. Yield-74% , . M.p 157-160°C, MS m/z : 655 (M^{+}) , IR(KBr): cm^{-1} 3176 (CH-arom) ,3101(CH-aliph), 1769(O-C=O-), 1678(C=O), 1460(C-N),1290 (C=O), 1239(C-N). ^{1}H NMR (DMSO-d6): δ7.6(S,H(triazole),7.18-7.47(M,7H,Ar-H), 8.94 (M,1H), 7.1-7.5(M,4H) 5.63(D,2H) 4.97(D,2H), 3.77(T,2H), 2.92(M,3H)2.44 (M,2H). 2.34(D,3H). ^{13}C NMR (DMSO-d6): δ156.64 ,145.5, 121.6, 112.35, 71.12, 68.40, 48.7, 25.2.

Antimicrobial activity

All the synthesized compounds were screened for antimicrobial activities by disc diffusion technique. Compounds are screened in vitro for their anti-microbial activity against E. coli, S. *aureus*, P. *aeruginosa and* B. *subtilis* are compared with standard drug amikacin (The zones of inhibition formed for the compounds against organisms were calculated.

The antibacterial activities of all test compounds were carried out by disc diffusion method .The concentrations of the test compounds were taken in DMSO and used in the concentration of 500 µg and 1000 µg g/disc. The target microorganisms were cultured in Mueller–Hinton broth (MHB). After 24 h the suspensions were adjusted to standard sub culture dilution. The Petri dishes containing Muller Hinton Agar (MHA) medium were cultured with diluted bacterial strain.

Disc made of Whatman No.1, diameter 6 mm was presterilized and was maintained in aseptic chamber. Each concentration was injected to the sterile disc papers. Then the prepared discs were placed on the culture medium. Standard drug amikacin was used as a positive reference standard to determine the sensitivity of each microbial species tested. Then the inoculated plates were incubated at 37 ºC for 24 h. The diameter of the clear zone around the disc was measured and expressed in millimeters as its anti-microbial activity.

Anti-bacterial activity of test compound against the *E. coli, S. aureus, P. aeruginosa* and *B. subtilis.*

Compounds	**Zone of Inhibition (mm)**							
	S. aereus (ATCC-9144)		***B. subtilis*** (ATCC-6051)		***E. coli*** (ATCC-25922)		***P. aerugenosa*** (ATCC-2853)	
	500µg	**1000µg**	**500µg**	**1000µg**	**500µg**	**1000µg**	**500µg**	**1000µg**
4a	9	13	16	13	10	**15**	10	**15**
4b	10	**14**	9	12	10	13	6	10
4c	9	13	9	13	8	12	9	12
4d	10	**14**	11	**10**	7	11	8	11
4e	8	**17**	13	**16**	9	12	11	10
4f	14	12	10	13	6	9	17	9
4g	8	12	9	**15**	8	**11**	13	7
4h	10	13	8	**9**	14	16	11	9
Amikacin	**26**		**24**		**26**		**27**	

Conclusions

The research study reports show the successful synthesis and activity of new pyrimidine derivatives bearing 1,2,3 triazole moiety . The antimicrobial activity study revealed that all the tested compounds showed moderate to good antimicrobial activity against the pathogenic strains, structure and relationship of title compounds showed that the presence of triazole moiety and biologically active groups like, -OH,-Br, -NO2 groups attached to phenyl group of the title compounds are responsible for the good antimicrobial activity. From the obtained results conclude that the entire tested compounds are active towards bacteria.

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