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SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF SOME NOVELSUBSTITUTE 2-(4-((BENZO[D]OXAZOL-2-YLTHIO)METHYL)-1H-1,2,3-TRIAZOL-1-YL)-N-(4 PHENYLTHIAZOL-2-YL)ACETAMIDE VIA CLICK CHEMISTRY APPROACH

Mayur K. Saglani*[1], H. D. Joshi [2]

[1] *Chemical Research Laboratory, Department of Chemistry, Saurashtra University, Rajkot, Gujarat, India*

[2] *Department of Home Science, Saurashtra University, Rajkot, Gujarat, India*

**Corresponding author: mayur.saglani@yahoo.com*

ABSTRACT

A novel heterocyclic library was synthesized, characterized and tested for biological evaluation against bacteria and fungus. This novel series of substituted-2-(4-((benzo[d]oxazol-2-ylthio)methyl)-1H-1,2,3-triazol-1-yl)-N-(4phenylthiazol-2-yl)acetamide was synthesized via click chemistry approach in the presence of DMF:H_2O:n-BuOH and $CuSO_4.5H_2O$ in good yield. The title compounds have been synthesized with several structural variations. The synthesized compounds were screened for antimicrobial activity against standard drugs. The structure of synthesized compounds was characterized by their spectral (IR, ^{1}H NMR and Mass) data. The purity of the synthesized compounds was confirmed by TLC.

Keywords: Triazole, Acetamide,Click Chemistry, Antimicrobial activity.

1. INTRODUCTION

To search for reactions which can be used to link two or more than two different functionalized molecular adducts with minimum effort and without generated side products or impurities have become popular during few decades [1]. Such a reaction should be easily carried out with good to moderate yield and selectivity, which should be compatible with aqueous and other protic solvents and should lead to high quantitative transformation. Click chemistry is a bunch of such reactions that has evolved as an efficient tool for the synthesis of a library, which gained quick acceptance in biotechnology, material science and polymer science, medicinal chemistry, and so on. Among all the click transformation, copper-mediated 1,3-dipolar Huisgen cycloaddition (HDC) between an alkyne and an azide is *the jewel in the crown* [2]. It possesses a remarkable functional group tolerance, researchers can easily introduce various and diverse functional groups. The concept of click transformation was first given by Sharpless and coworkers at the Scripps Research Institute [3]. Click transformation is a bunch of organic reactions, where "click" word refers for its efficiency, selectivity, and simplicity of reaction within a short time. Any reaction considers click transformation which involving simpler and milder reaction condition.

There were various reactions with different mechanisms that can be considered as click reactions, provided they follow a simple common reaction trajectory [4]. Sharpless *et al* introduced the original idea of click chemistry, which afford an efficient conjugation method in drug discovery [5], this concept and ideology is widely noticed, and its uses and applications are found in diverse field of research and technology, which produced organic molecules for polymer science [6], nano-science [7] and technology, bioconjugation [8], and sensing science [9].

In the present work, we report the synthesis of this novel substituted 2-(4-((benzo[d]oxazol-2-ylthio)methyl)-1H-1,2,3-triazol-1-yl)-N-(4phenylthiazol-2-yl)acetamide via click chemistry approach and their antimicrobial activity against fungi, gram positive and gram negative bacteria. The main significance of the work is, it will provide synthesized and more potent stable molecule for biological response as most of coumarin based pyrimidine derivatives have significant biological activity. As the significance and biological profile of this class of molecule has been mentioned above, our continue efforts are towards the synthesis of potential heterocyclic molecules.

2. EXPERIMENTAL

Anhydrous solvents and all reagents and solvents were purchased from, Spectrochem, Sigma-Aldrich, Loba-chemie and Merck, involving air or moisture-sensitive compounds were performed under a nitrogen atmosphere using oven-dried glassware and syringes to transfer solutions. Thin-layer chromatography (TLC) was conducted by using aluminum plates 20x20 cm coated by silica gel 60 F254 purchased from Merck. Melting points were determined by melting point apparatus (uncorrected) using an open capillary method. Solvents were evaporated by the help of a BUCHI rotary evaporator. IR spectra were recorded on FTIR-8400 spectrometer using DRS prob. which is expressed in ν (cm^{-1}). Shimadzu GCMS-QP-2010 model was used to achieve Mass spectra of the products. Nuclear magnetic resonance spectra; ^{1}HNMR spectra were determined in $CDCl_3$/DMSO-d_6 (in 3/1 ratio) or DMSO-d_6 and were recorded on a Bruker AVANCE II 400 MHz. Chemical shifts (δ scale) were reported in ppm (parts per million) downfield from tetramethylsilane (TMS) used as an internal standard. Splitting patterns are designated as followings: s, singlet; d, doublet; t, triplet; q, quad-ruplet; m, multiplet; brs, broad singlet; dd, double doublet. Shimadzu GCMS-QP-2010 model was used to achieve Mass spectra of the products.

2.1. General synthesis method for 2-bromo-1-phenylethanone (INT-a)

In an RBF, various substituted acetophenone (0.01mol), Acetonitrile (10ml) and catalytic amount of p-toluene sulphonic acid were taken. Reaction mixture was cooled to 0°C and N-bromo succinamide (0.011mol) was added portion wise and stirred the reaction mixture for further 15 min. Afterwards temperature was set at RT. After completion of the reaction, mixture was poured into the crushed ice and extracted with MDC. The organic layer was washed with brine solution, dried over sodium sulphate and concentrated under reduced pressure to get INT-a as a dry solid.

2.2. General synthesis method for 4-phenyl-thiazol-2-amine(INT-b)

In an RBF, INT-a (0.10 mol) was dissolved in ethanol and thiourea (0.11mol) was added. The resultant reaction mixture was refluxed at 78°C for 2 hr. After completion of the reaction, mixture was allowed to cool at room temperature and quenched with crushed ice which yielded INT-b as a dry solid.

2.3. General synthesis of 2-chloro-N-(4-phenyl-thiazol-2-yl)acetamide (INT-C)

To a solution of substituted amine (1 equi) in acetone, chloroacetyl chloride was (1 equi) was added drop wise and the resulting mixture was stirred for 15 min at room temperature. Reaction mixture was then dumped onto crushed ice and solid intermediate product was separated which was filtered and washed with water. Dried and used in next step without further purification.

2.4. General synthesis of2-azido-N-(4-phenyl-thiazol-2-yl)acetamide (INT-D)

To a solution of INT-C (1 eqiv) in DMF, sodium Azide (NaN_3) was added (3equi). The resulting mixture was stirred at RT for 24 hr. After completion of the reaction mixture, mixture was poured on to crushed ice. Filtered the separated product and dried.

2.5. General synthesis of benzo[d]oxazole-2-thiol (INT-E)

To a solution of 2-aminophenol (100mmol) in ethanol (150ml), aqueous sodium hydroxide (130mmol) in water (30ml) was added followed by addition of carbondisul-phide (150mmol). Resulting mixture was refluxed at 65°C for 5hr. After the completion of the reaction, mixture was poured into the crushed ice which was neutralized with conc. HCl and mixture was filtered and washed with hexane to afford pure compound.

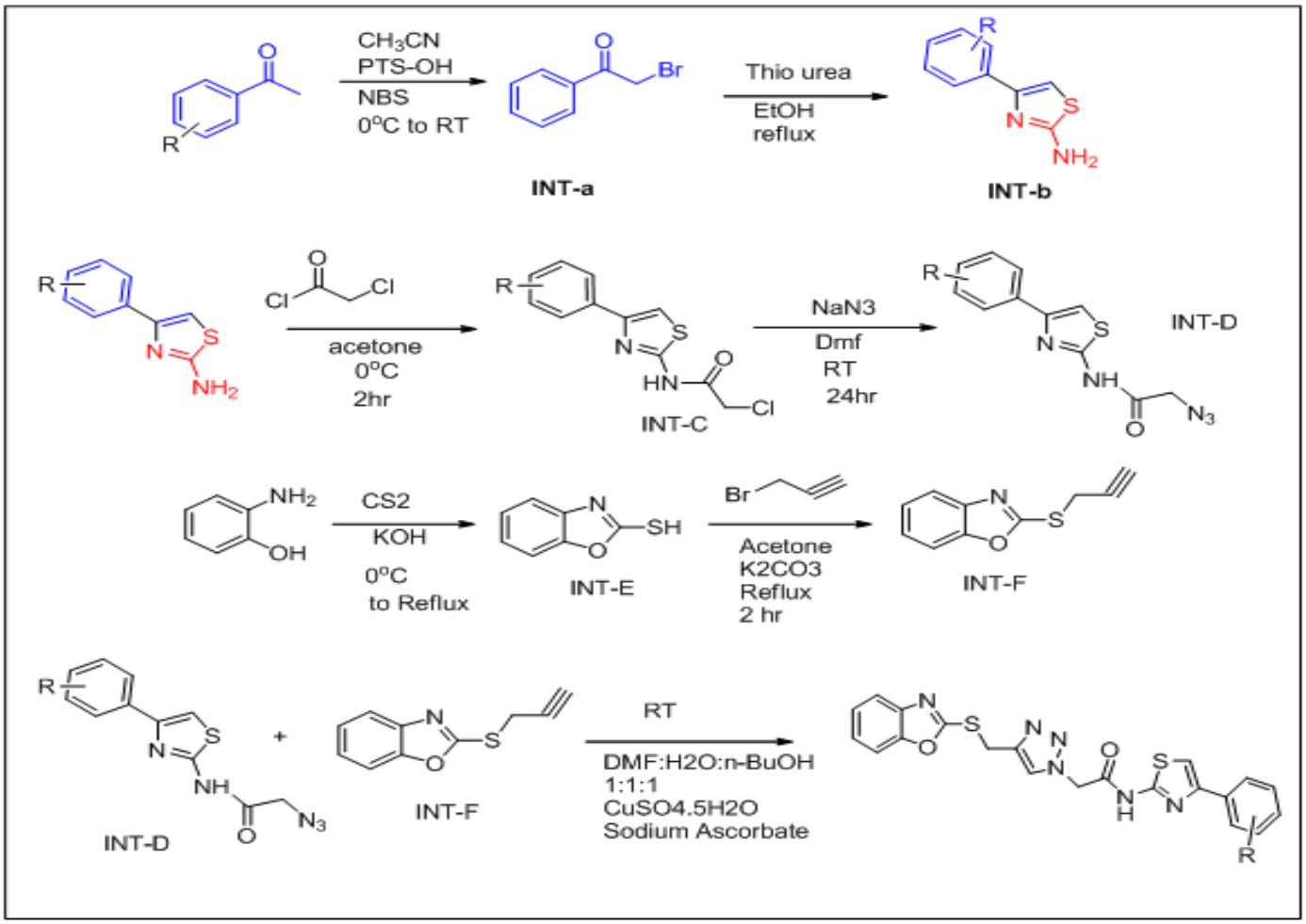
2.6. General synthesis of2-(prop-2-yn-1-ylthio) benzo [d] oxazole (INT-F)

In RBF, compound (50mmol) in acetone (150ml) was taken and anhydrous K_2CO_3 (100mmol) was added with stiring. After 5 min, propagyl bromide (55mmol) was added slowly. After the addition was over, the reaction mixture was refluxed for 3 hr. with continuous stirring. The reaction was monitored on TLC. After the completion of the reaction, the reaction mixture was poured into the crushed ice. Filtered the separated product and washed with water to afford final compound.

2.7. General synthesis of 2-(4-((benzo[d]oxazol-2-ylthio)methyl)-1H-1,2,3-triazol-1-yl)-N-(4-phenylthiazol-2-yl)acetamide derivatives:

In a RBF containing DMF:H_2O: n-butanol(1:1:1), INT-D(1 eqi), and INT-F(1eqi) was added at RT, followed by addition of catalytic amount of sodium ascorbate and coppersulphate pentahydrate. Stir the resulting solution

for RT for 24hr. after the completion of the reaction, mixture was poured onto the crushed ace and filter the separated product. Was with dilute ammonia and filter the product again.



Reaction Scheme

2.8. Antimicrobial activity

Antimicrobial activity is the process of killing or inhibiting the pathogenic microbes causing disease. An antimicrobial is an agent that kills microorganisms or stops their growth. Antimicrobial can be anti-bacterial, anti-fungal or antiviral. Agents that kill microbes are called microbicidal, while those that inhibit their growth are called microbistatic. All agents have different modes of action by which they act against infection. The use of antimicrobial medicines to treat infection is known as antimicrobial chemotherapy. In our current study antibacterial and antifungal activity was tested by standard agar cup method. All the synthesized compound were tested for their *in vitro* antimicrobial activity against Gram+*ve (Bacillus megaterium, Micrococcus spp.*), Gram-*ve (E.coli, S. typi) and* fungal *spps. (Ganoderma spp., A. niger, A. flavus* and *Penicillium spp.*), taking streptomycin, ciprofloxacin, and nystatin as standard drugs. Suspension of 24 to 48 hrs. grown fresh bacterial and fungal culture was prepared in N-broth and potato dextrose broth respectively. All the bacterial and fungal suspension were equally spreaded on to the sterile Muller Hinton and PDA plates respectively with the help of sterile swabs. Wells were made in the plates (1 cm) with the help of sterile cork borer. The standard antibiotics were dissolved in sterile distilled water to make the final concentration of 200µg/ml. The synthesized compounds to be tested were dissolved in DMSO up to the final concentration of 1 mg/ml and 0.1 ml of it was loaded in the well. The plate was incubated at 4°C for 20 minutes for proper diffusion of a compound in agar and then the plates were incubated in the upward position for 24 hrs at 37°C for bacterial culture and 48 hrs. at 25°C for fungal cultures. The

control activity against DMSO was also performed. After incubation zone of inhibition was observed and measured.

3. RESULTS AND DISCUSSION

3.1. Spectral data of synthesized compounds

3.1.1. 2-(4-((benzo[d]oxazol-2-ylthio)methyl)-1H-1,2,3-triazol-1-yl)-N-(4-phenylthiazol-2-yl) acetamide (MS-41)

Yellow Solid, Rf value 0.40 (Ethyl acetate 8: Hexane 2), IR (KBr pallet) in cm^{-1} : 3119.22, 2946.24, 2858.76, 1679.38, 1597.95, 1549.04, 1512.84, 1480.11, 1449.16, 1377.08, 1143.20, 803.80, 754.24, 709.07, ^{1}H NMR (DMSO) in δ ppm: 1.23 (4H, Singlet), 4.73 to 8.22 (11H, Complex), 12.79 (1H, Singlet), Mass (m/z): 448 (M^+), Ana. Calculated for Molecular formula $C_{21}H_{16}N_6O_2S_2$ is C; 56.23%, H; 3.60%, N; 18.34% Found C; 56.20%, H; 3.58%, N; 18.30%

3.1.2. 2-(4-((benzo[d]oxazol-2-ylthio)methyl)-1H-1,2,3-triazol-1-yl)-N-(4-(p-tolyl)thiazol-2-yl)acetamide (MS-42)

White Solid, Rf value 0.43 (Ethyl acetate 8: Hexane 2), IR (KBr pallet) in cm^{-1}: 3155.44, 3015.59, 2941.87, 1714.49, 1670.16, 1547.43, 1414.74, 1289.11, 1206.52, 1026.74, 821.36, 754.58, 698.22 ^{1}H NMR (DMSO) in δ ppm: 2.31 (3H, Singlet), 4.73 (2H, Singlet), 5.46 (2H, Singlet) 7.24 to 8.22 (10H, Complex), 12.77 (1H, Singlet), Mass (m/z): 462 (M^+), Ana. Calculated for Molecular formula $C_{22}H_{18}N_6O_2S_2$ is C; 57.13%, H; 3.92%, N; 18.17% Found C; 57.10%, H; 3.90%, N; 18.12%

3.2. Antimicrobial Evaluation

We have prepared a series of novelsubstitute 2-(4-((benzo[d]oxazol-2-ylthio)methyl)-1H-1,2,3-triazol-1-yl) -N-(4phenylthiazol-2-yl)acetamide derivatives via click chemistry approach. The synthesized products MS-41 to MS-50 showed in table 1 and also were tested for their antimicrobial activity by Cup-plate method against Gram positive bacteria B. cocous and B. subtillis and Gram negative bacteria Proteus vulgaris, Escherichia coli and antifungal activity against Aspergillus niger. The antimicrobial activity is shown in table 2 compared with standard drugs viz. Amoxycillin, Benzoylpenicillin, Ciprofloxacin, Erythromycin, and antifungal activity was compared with Greseofulvin. Synthesized compound MS-42 & MS-44 showed adequate to good and remarkable activities with compared to standard known drugs at same concentration.

Table 1: Physical constant of synthesized library

Code	Molecular formula	Substitution	Molecular Weight	M.P. °C	Percentage of Yield
MS-41	$C_{21}H_{16}N_6O_2S_2$	-H	448	150-152	64
MS-42	$C_{22}H_{18}N_6O_2S_2$	-Me	462	178-180	68
MS-43	$C_{21}H_{15}N_7O_4S_2$	-NO_2	493	144-146	54
MS-44	$C_{21}H_{15}ClN_6O_2S_2$	-Cl	482	164-166	58
MS-45	$C_{21}H_{15}BrN_6O_2S_2$	-Br	527	168-170	62
MS-46	$C_{22}H_{18}N_6O_3S_2$	-OMe	478	154-156	54
MS-47	$C_{22}H_{16}N_6O_4S_2$	-COOH	492	182-184	56
MS-48	$C_{23}H_{18}N_6O_4S_2$	-COOR	506	146-148	70
MS-49	$C_{21}H_{16}N_6O_3S_2$	-OH	464	182-184	56
MS-50	$C_{23}H_{19}N_7O_3S_2$	-NHCOMe	505	186-188	58

We have prepared a series of novel derivatives of novel 2-(4-((benzo[d]oxazol-2-ylthio)methyl)-1H-1,2,3-triazol-1-yl)-N-(4phenylthiazol-2-yl)acetamide via click chemistry approach. The synthesized products MS-42 to MS-44 are shown in table 1 and they were tested for their antimicrobial activity by Cup-plate method All the synthesized compound were tested for their in vitro antimicrobial activity against Gram +*ve (Bacillus megaterium (MTCC 1684), Micrococcus spp.* (ATCC 4698), Gram -*ve (E.coli (MTCC 47), S. typi MTCC 1264) and fungal spps. (Ganoderma spp.(MTCC 1039), A. niger,(MTCC 281 A. flavus (MTCC 277) and Penicillium spp. (MTCC 161*). The antimicrobial activity showed in table 2 compared with standard drugs), taking streptomycin, ciprofloxacin, and nystatin as standard drugs viz. Synthesized compound (MS-30-MS-40) showed adequate to good and remarkable activities with compared to standard known drugs at same concentration.

Table 2: Antibacterial and antifungal activity of compounds MS-41to MS-50

Code	Antibacterial activity				Antifungal activity			
	Antibacterial activity (zone in cm), concentration: 1 mg/ml				Antifungal activity (zone in cm), concentration: 1mg/ml			
	Gram +*ve* Bacteria		Gram-*ve* bacteria					
	B. Megaterium	*Micrococcus spp.*	*S. typhi.*	*E. coli.*	*Penicillium spp.*	*Ganoderma spp.*	*A. niger*	*A. flavus*
MS-41	-	1.5	1.1	-	1.4	-	1.3	0.9
MS-42	**2.5**	**2.3**	**1.3**	**3.0**	**2.4**	**3.2**	**1.9**	**2.5**
MS-43	-	-	0.5	1	2.1	2.2	1.8	1.4
MS-44	**2.6**	**2.3**	**1.1**	**2.8**	**2.4**	**3.2**	**1.9**	**1.8**
MS-45	1.1	0.8	-	1.5	1.7	0.1	0.3	1.0
MS-46	0.5	1.2	1.2	1.2	-	1.3	0.8	2.0
MS-47	2.0	2.0	1.4	2.2	2.4	3.0	0.9	3.1
MS-48	1.1	2.1	1.7	-	-	1.4	2	3.2
MS-49	0.2	1.4	1.2	-	0.7	0.5	1.3	-
MS-50	1.1	-	1.4	1.0	2.0	2.0	-	1.4
Streptomycin (200µg/ml)	3.0	2	2	3.2	-	-	-	-
Ciprofloxacin (200µg/ml)	3.8	4	4	3	-	-	-	-
Nystatin (200µg/ml)	-	-	-	-	3.2	4	3.5	3.8

4. CONCLUSION

The synthesis of various substituted-2-(4-((benzo [d] oxazol-2-ylthio)methyl)-1H-1,2,3-triazol-1-yl)-N-(4 phenylthiazol-2-yl)acetamide via click chemistry app-roach was carried out successfully. The route cyclization and click chemistry reactions are performed successfully. Ten compounds were synthesized and well-characterized by various spectroscopic techniques. Antimicrobial activity of all the compounds showed that MS-42, & MS-44 exhibited potent antibacterial activity against *B. megaterium, S. typi, Micrococcus spp.* and *E.coli*. Hence further investigation can be done, MIC can be identified and such compounds can further be tested and can be used as a potent drug in coming time.

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Conflict of interest

None declared

6. REFERENCES

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