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ARTICLE

Synthesis, Characterization and Biological Evaluation of Some Novel Substitute *N*-(4-(3-Oxomorpholino)phenyl)-2-(4-((phenylamino)methyl)-1*H*-1,2,3-triazol-1-yl)-acetamide *via* Click Chemistry Approach

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ABSTRACT

A novel heterocyclic library were synthesized, characterized and tested for biological evaluation against bacteria and fungus. This novel series of substitute *N*-(4-(3-oxomorpholino)phenyl)-2-(4-((phenylamino)-methyl)-1*H*-1,2,3-triazol-1-yl)acetamide *via* click chemistry approach in the presence of DMF:H_2O:*n*-BuOH and $CuSO_4 \cdot 5H_2O$ 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 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.

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]. As organic molecules started to find out their place as easily tunable and functional materials. 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 involves simpler and milder reaction conditions.

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

In present work, we report the synthesis of this novel substitute *N*-(4-(3-oxomorpholino)phenyl)-2-(4-((phenylamino)-methyl)-1*H*-1,2,3-triazol-1-yl)acetamide *via* click chemistry approach and their antimicrobial activity against fungi, Gram-positive and Gram-negative bacteria. The main importance of the work provides the synthesized potent stable molecule for biological response as most of coumarin based pyrimidine derivatives has significant biological activity.

EXPERIMENTAL

Anhydrous solvents and all the reagents and solvents were purchased from Spectrochem, Sigma-Aldrich, Lobachemie 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 20 cm × 20 cm coated by silica gel 60 F_{254} purchased from Merck. Melting points were determined by melting point apparatus (uncorrected) using an open capillary method. IR spectra were recorded on FTIR-8400 spectrometer. 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 downfield from tetramethylsilane (TMS) used as an internal standard. Shimadzu GCMS-QP-2010 model was used to achieve Mass spectra of the products.

Synthesis of 2-chloro-*N*-(4-(3-oxomorpholino)phenyl)-acetamide (INT-2): To a solution of substituted amine (**INT-1**, 1 equiv.) in acetone, chloroacetyl chloride was (1 equiv.) was added dropwise and the resulting mixture was stirred for 15 min at room temperature. Reaction mixture was then poured onto crushed ice and solid intermediate product was separated which was filtered, washed with distilled water and finally dired.

Synthesis of 2-azido-*N*-(4-(3-oxomorpholino)phenyl)-acetamide (INT-3): To a solution of **INT-2** (0.1 mmol) in DMF, sodium azide (0.3 mmol) was added. The resulting mixture was stirred at room temperature for 24 h after completion of the reaction mixture. The reaction mixture was poured on to crushed ice, filter the separated product and dried it.

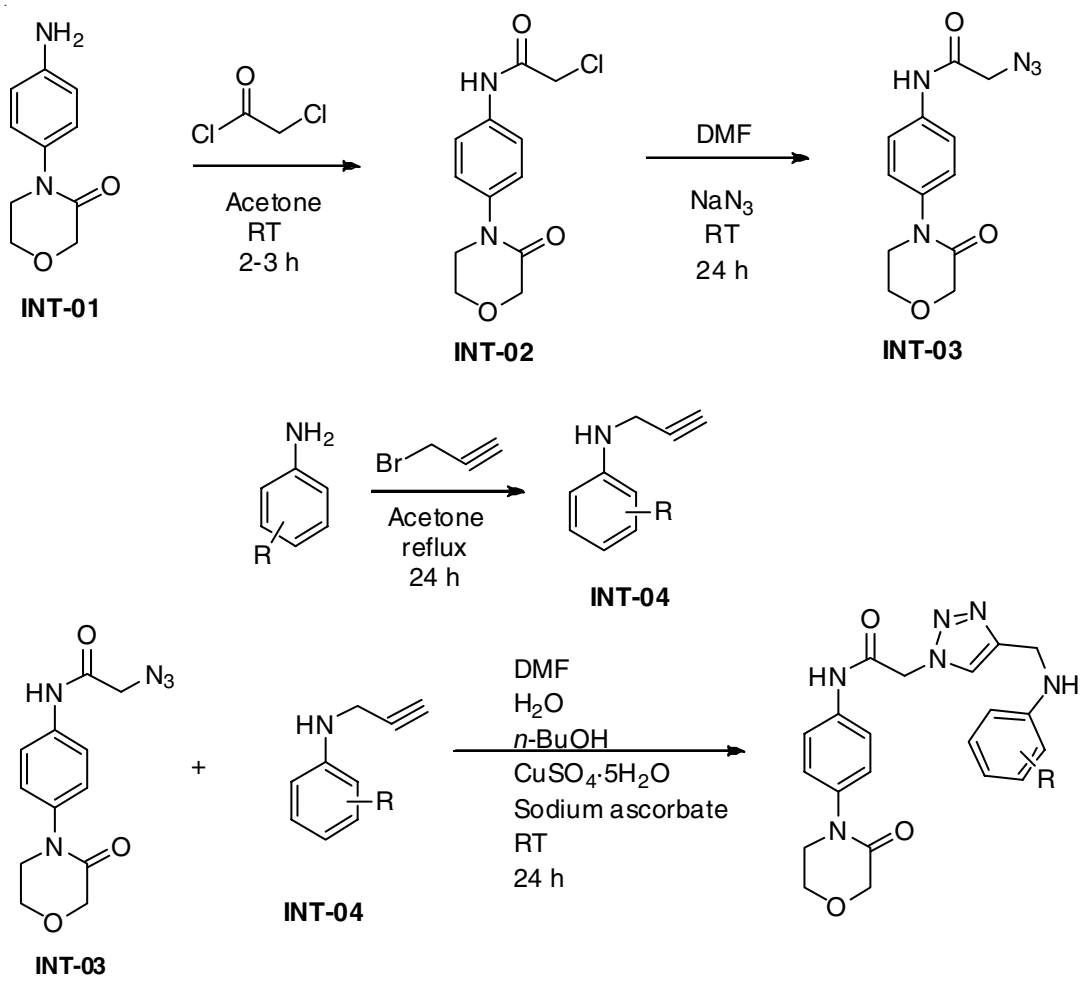
Synthesis of *N*-(prop-2-yn-1-yl)aniline (INT-4): In round bottom flask, take different substituted aniline (50 mmol) in acetone (150 mL) and added anhydrous K_2CO_3 (100 mmol) with stirring. After 5 min, propagyl bromide (55 mmol) was added slowly. After the addition was over, reflux the reaction mixture for 3 h with continuous stirring. The reaction was monitored on TLC. After the completion of the reaction, the reaction mixture was poured into the crushed ice. Filter the separated product and washed thoroughly with distilled water to afford final compound.

Synthesis of substituted *N*-(4-(3-oxomorpholino)-phenyl)-2-(4-((phenylamino)methyl)-1*H*-1,2,3-triazol-1-yl)acetamide (HP-1-10): In a round bottom flask containing DMF:H_2O:*n*-butanol (1:1:1), **INT-3** (1 equiv.) and **INT-4** (1 equiv.) was added at room temperature, followed by addition of catalytic amount of sodium ascorbate and copper sulphate pentahydrate. Stirred the resulting solution at room temperature for 24 h, after the completion of the reaction, the mixture was poured onto the crushed ice and filtered the separated product. The product was with dilute ammonia and filtered the product again.

2-(4-(((4-Chlorophenyl)amino)methyl)-1*H*-1,2,3-triazol-1-yl)-*N*-(4-(3-oxomorpholino)phenyl)acetamide (HP-1): Yellow solid, R_f value 0.40 (ethyl acetate:hexane (8:2)). IR (KBr, ν_{max}, cm^{-1}): 3279.88, 3076.79, 2864.25, 1712.28, 1650.22, 1551.09, 1483.89, 1372.53, 1256.78, 1154.96, 1061.48, 853.75, 781.62, 692.09. ^{1}H NMR (DMSO-d_6) δ ppm: 6.30-7.57 (complexe, 9H), 10.51 (singlet, 1H), 7.97-7.98 (singlet, 1H), 3.69 (singlet, 2H), 3.94 (singlet, 2H), 4.18 (singlet, 2H), 4.30 (singlet, 2H), 5.29 (singlet, 2H). Mass (*m/z*): 440 (M^+), Anal. calcd. (found) % for $C_{21}H_{21}N_6O_3Cl$: C; 57.21 (57.25), H; 4.80 (4.85), N; 19.06 (19.09).

2-(4-(((4-Bromophenyl)amino)methyl)-1*H*-1,2,3-triazol-1-yl)-*N*-(4-(3-oxomorpholino)phenyl)acetamide (HP-2): White solid, R_f value 0.43 (ethyl acetate:hexane (8:2)); IR (KBr, ν_{max}, cm^{-1}): 3399.50, 3258.24, 2910.02,1692.82, 1599.48, 1484.65, 1367.26, 1238.59, 1159.47, 1121.26, 875.36, 820.69, 751.83. ^{1}H NMR (DMSO-d_6) δ ppm: 6.35-7.59 (complexe, 9H), 10.51 (singlet, 1H), 7.97 (singlet, 1H), 3.69 (singlet, 2H), 3.94-3.95 (singlet, 2H), 4.18 (singlet, 2H), 4.29-4.31 (singlet, 2H), 5.29 (singlet, 2H). Mass (*m/z*): 485 (M^+), Anal. calcd. (found) % for $C_{21}H_{21}N_6O_3Br$: C; 51.97 (51.95), H; 4.36 (4.30), N; 17.32 (17.31).

Antimicrobial activity: In current study, antibacterial and antifungal activities was tested by standard agar cup method [10]. All the synthesized compound were tested for their *in vitro* antimicrobial activity against Gram-positive (*Bacillus megaterium*, *Micrococcus* spp.), Gram-negative (*E. coli*, *S. typhi)* and fungal spp. (*Ganoderma* spp., *A. niger, A. flavus* and *Penicillium* spp.) taking streptomycin, ciprofloxacin and nystatin as standard drugs. Suspension of 24 to 48 h 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 200 µg/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 ºC for 20 min for proper diffusion of a compound in agar and then the plates were incubated in the upward position for 24 h at 37 ºC for bacterial culture and 48 h at 25 ºC for fungal cultures. The control activity against DMSO was also performed. After incubation zone of inhibition was observed and measured.



Scheme-I

TABLE-1
PHYSICAL CONSTANT OF SYNTHESIZED LIBRARY

Compd. No.	m.f.	Substitution	m.w.	m.p. (°C)	Yield (%)
HP-1	$C_{21}H_{21}ClN_6O_3$	-Cl	440	150-152	64
HP-2	$C_{21}H_{21}BrN_6O_3$	-Br	485	178-180	68
HP-3	$C_{21}H_{21}N_7O_5$	-NO_2	451	144-146	54
HP-4	$C_{21}H_{22}N_6O_3$	-H	406	164-166	58
HP-5	$C_{22}H_{24}N_6O_3$	-Me	420	168-170	62
HP-6	$C_{22}H_{24}N_6O_4$	-OMe	436	154-156	54
HP-7	$C_{22}H_{22}N_6O_5$	-COOH	450	182-184	56
HP-8	$C_{23}H_{24}N_6O_5$	-COOR	464	146-148	70
HP-9	$C_{21}H_{22}N_6O_4$	-OH	422	182-184	56
HP-10	$C_{23}H_{25}N_7O_4$	-NHCOMe	463	186-188	58

RESULTS AND DISCUSSION

A series of novel substituted *N*-(4-(3-oxomorpholino)-phenyl)-2-(4-((phenylamino)methyl)-1*H*-1,2,3-triazol-1-yl)-acetamide *via* click chemistry approach derivatives was synthesized and tested for their antimicrobial activity by Cup-plate method against Gram-positive bacteria *B. coccus* and *B. subtilis* and Gram-negative bacteria *Proteus vulgaris*, *Escherichia coli* and antifungal activity against *Aspergillus niger*. The antimicrobial activity showed in Table-2 compared with standard drugs *viz.* amoxycillin, benzoylpenicillin, ciprofloxacin, erythromycin and antifungal activity was compared with greseofulvin. Synthesized compound **HP-2** and **HP-4** showed adequate to good remarkable activities with compared to standard drugs at the same concentration.

Conclusion

The synthesis of substituted substitute *N*-(4-(3-oxomorpholino)phenyl)-2-(4-((phenylamino)methyl)-1*H*-1,2,3-triazol-1-yl)acetamide *via* click chemistry approach compounds was carried out successfully. Ten compounds were synthesized and well-characterized by various spectroscopic techniques. The antimicrobial activity of all the synthesized compounds was carried out against four bacterial strain (*B. megaterium*, *S. typhi*,

TABLE-2
ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF COMPOUNDS **HP-1** TO **HP-10**

Compd. No.	Antibacterial activity				Antifungal activity			
	Antibacterial activity (zone in cm), concentration: 1 mg/mL				Antifungal activity (zone in cm), concentration: 1 mg/mL			
	Gram-positive bacteria		Gram-negative bacteria					
	BM	MS	ST	EC	PS	GS	AN	AF
HP-1	–	1.5	1.1	–	1.4	–	1.3	0.9
HP-2	2.5	2.3	1.3	3.0	2.4	3.2	1.9	2.5
HP-3	–	–	0.5	1.0	2.1	2.2	1.8	1.4
HP-4	2.6	2.3	1.1	2.8	2.4	3.2	1.9	1.8
HP-5	1.1	0.8	–	1.5	1.7	0.1	0.3	1.0
HP-6	0.5	1.2	1.2	1.2	–	1.3	0.8	2.0
HP-7	2.0	2.0	1.4	2.2	2.4	3.0	0.9	3.1
HP-8	1.1	2.1	1.7	–	–	1.4	2.0	3.2
HP-9	0.2	1.4	1.2	–	0.7	0.5	1.3	–
HP-10	1.1	–	1.4	1.0	2.0	2.0	–	1.4
Streptomycin (200 µg/mL)	3.0	2.0	2.0	3.2	–	–	–	–
Ciprofloxacin (200 µg/mL)	3.8	4.0	4.0	3.0	–	–	–	–
Nystatin (200 µg/mL)	–	–	–	–	3.2	4.0	3.5	3.8

BM = *B. megaterium*, MS = *Micrococcus* spp., ST = *S. typhi*, EC = *E. coli*, PS = *Penicillium* spp., GS = *Ganoderma* spp., AN = *A. niger*, AF = *A. flavus*,

Micrococcus spp. and *E. coli*) four fungal strain (*A. niger*, *A. flavus*, *Ganoderma* spp. and *Penicillium* spp.) by agar cup method.

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REFERENCES

1. C.H. Zhou and Y. Wang, Recent Researches in Triazole Compounds as Medicinal Drugs, *Curr. Med. Chem.*, **19**, 239 (2012); https://doi.org/10.2174/092986712803414213
2. S.K. Monfared, Ph.D. Thesis, Synthesis and Cytotoxicity Evaluation of Small 1,4-Triazolic Derivatives against B16 Melanoma Cell Lines and a Methodolgy Study on the Synthesis of Propargyl Ethers from Their Corresponding Propargyl Esters without Catalyst and under Microwave Irradiatio, Université Pierre et Marie Curie-Paris VI, France (2014).
3. H.C. Kolb, M.G. Finn and K.B. Sharpless, Click Chemistry: Diverse Chemical Function from a Few Good Reactions, *Angew. Chem. Int. Ed.*, **40**, 2004 (2001); https://doi.org/10.1002/1521-3773(20010601)40:11<2004::AID-ANIE2004>3.0.CO;2-5
4. H. Adolfsson, A. Converso and K.B. Sharpless, Comparison of Amine Additives Most Effective in the New Methyltrioxorhenium-Catalyzed Epoxidation Process, *Tetrahedron Lett.*, **40**, 3991 (1999); https://doi.org/10.1016/S0040-4039(99)00661-9
5. H.C. Kolb and K.B. Sharpless, The Growing Impact of Click Chemistry on Drug Discovery, *Drug Discov. Today*, **8**, 1128 (2003); https://doi.org/10.1016/S1359-6446(03)02933-7
6. H. Li, R. Aneja and I. Chaiken, Click Chemistry in Peptide-Based Drug Design, *Molecules*, **18**, 9797 (2013); https://doi.org/10.3390/molecules18089797
7. R. Hoogenboom, Thiol-Yne Chemistry: A Powerful Tool for Creating Highly Functional Materials, *Angew. Chem. Int. Ed.*, **49**, 3415 (2010); https://doi.org/10.1002/anie.201000401
8. R. Tong, L. Tang, L. Ma, C. Tu, R. Baumgartner and J. Cheng, Smart Chemistry in Polymeric Nanomedicine, *Chem. Soc. Rev.*, **43**, 6982 (2014); https://doi.org/10.1039/C4CS00133H
9. X. Zhang and Y. Zhang, Applications of Azide-Based Bioorthogonal Click Chemistry in Glycobiology, *Molecules*, **18**, 7145 (2013); https://doi.org/10.3390/molecules18067145
10. S.B. Rose and R.E. Miller, Studies with the Agar Cup-Plate Method: I. A Standardized Agar Cup-Plate Technique, *J. Bacteriol.*, **38**, 525 (1939); https://doi.org/10.1128/jb.38.5.525-537.1939