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***Research Article** (Open Access)*

SYNTHESIS OF 1,2,3-TRIAZOL MOIETY FROM ALKYNE DERIVATIVE OF BENZOXAZINE WHICH BEHAVES AS A NOVEL CLASS OF ANTIMICROBIAL AND ANTIFUNGAL AGENT

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ABSTRACT
The 1, 3-dipolar cycloaddition "Click-reaction" between azides and alkynes catalyzed by copper (I) salts is often utilized for the creation of triazole moiety, which has drawn the attention of chemists due to its large uses in the pharmacological field of many recognized reactions. As alkyne and azide components can be inserted into a wide range of substituents, the click reaction has a wide range of applications. Our objective is to combine the aromatic halves in a single patch including benzoxazine and triazole to boost the natural exertion of the performance composites. All synthesized composites will be extensively characterized utilizing precise spectral analyses such as 1H NMR, mass, 13C NMR, and IR. Following the Cup-plate approach, all produced composites will be tested in vitro for antibacterial and antifungal activity against conventional antibiotics Ciprofloxacin for bacteria and Miconazole for fungus.

***Keywords**: Benzoxazine, Heterocyclic Moiety, Antifungal Activity, Antibacterial Activity IR, ^{1}H NMR, ^{13}C NMR, Mass Spectroscopy*

INTRODUCTION
In organic chemistry for constructing artificial composites, benzoxazine and its derivatives are frequently utilised as appropriate configurations for the formation of biologically active emulsions. This introduction provides a quick overview of the substances that have antimicrobial, antimycobacterial, anti-diabetic, anti-hypolipidemic, and anti-depressant effects. “The versatility of the benzoxazine shell, in addition to its relative chemical simplicity and availability, makes these chemicals amongst the most promising sources of bioactive composites. This has led to the discovery of a wide variety of composites that are of great interest from the point of view of anti-microbial, anti-mycobacterial, anti-diabetic and anti-depressant goods (Siddiquia *et al.*, 2010). Benzoxazine came in the spotlight when the first reclusion of-dihydroxy-2H (4H)-one (DIBOA) (Etzerodt *et al.*, 2006) and-dihydroxy benzoxazin-3 (4H)-one (DIMBOA) (Cambier *et al.*, 2000) were reported. Wang *et al.*, 2016 looked into the effect of molecular structure on phase-separated structures further. The results reveal that phase separation of the phenol-4,4'-diaminodiphenyl methane-based benzoxazine (P-ddm)/TBMI/imidazole mix is easier than that of the BA-a/TBMI/imidazole blend, which is due to differences in Flory-Huggins characteristics and viscosity of various molecules. HBPs (hyperbranched polymers) are a novel form of polymer (Moradi *et al.*, 2020). These composites have been also reclused from roots and upstanding corridor of sludge factory. It was observed that these composites can be further used for the conflation of potent toxic and fungicidal composites. Fringuelli *et al.*, (2002) have synthesized 6-(1-(4-chlorophenoxy)-2-(1*H*-imidazol-1-yl) ethyl)-4-methyl-2*H*-benzo[b][1,4] oxazin-3(4*H*)-one and estimated their anti-bacterial and anti-fungal exertion in vitro against gram-ve bacteria, gram +ve bacteria and colorful pathogenic strains Candida albicans ATCC 10231,C. glabrata DSM 6425 and C. tropicalis DSM 1346”. New ethyl derivations were synthesized by Alper-Hayta *et al.*, (2006). Furthermore, unlike other TS/TP resin blends, Hsieh Halina *et al.*, (2008) benzoxazine generates a large number of phenolic hydroxyl groups during the ring-opening and curing process, which can form intermolecular hydrogen bonds with polar groups in polycaprolactone

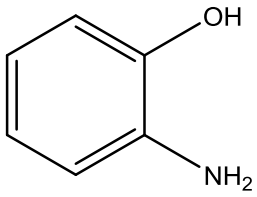
(such as carboxyl, hydroxyl, carbonyl, ether bonds, and so on) (PCL), (Pritchard *et al.*, 2007). Polucci *et al.*, (2013) did the medicinal chemistry manipulation which led to the discovery of emulsion as potent and picky impediments of VCP ATPase with IC50 of 24 nM and retainanti-proliferative exertion in the sub micro molar range (IC50 = 0.38 μM on HCT-116 cell lines). This represents a first step towards a new class of implicit anti-cancer agents. Hou *et al.*, (2011) created a variety of 1,2,4-triazoles and calculated their anti-tumor activity. Apoptosis and western-spot assay results revealed that 1,2,4-triazoles composites are most effective for cancer cell and against MetAP2. Cancer outgrowth was found to be largely effective on lung (GI50 = 0.06 M), ovarian, and renal cell lines by Formagio *et al.*, (2008; & Shiradkar *et al.*, 2011) developed a new class of triazolyl thiophene compounds that inhibit cdk5/p25. Based on the findings of the SAR investigations, these composites were shown to be the most promising for lowering brain cdk5/p25 levels, and hence have implications for Alzheimer's disease treatment. Poly-benzoxazines, a brand-new class of thermosets, have piqued the interest of both academic and industrial groups due to their superior properties (Kiskan *et al.*, 2018; Rimdusit *et al.*, 2013 & Ishida *et al.*, 2017). In addition, hyperbranched polymer ionic liquids with varied alkyl chain lengths (HBP-AMIM+PF6-, HBP-ABIM+PF6-, HBP-AHIM+PF6-, and HBP-AOIM+PF6-) were employed to modify the benzoxazine/epoxy thermosets (BA/ECC) (Zhang *et al.*, 2019). Blending tiny molecular compounds with TS is another way to achieve a balance between processing and toughness. Bisphenol-F-based benzoxazine monomer was used to copolymerize linear octanediamine (ODM) and m-xylylenediamine containing aromatic rings (MXDM) (BF) (Zhao *et al.*, 2018). Poly-benzoxazine has won numerous interests in digital packaging, the aerospace industry, composite fabricating, coatings and different fields (Hu *et al.*, 2020; Wang *et al.*, 2020; Zhu *et al.*, 2019; & Yang *et al.*, 2020). It's a collection of related compounds with increasing molecular weight created by gradually managing a repeating reaction using small molecules as the growth point (Flores *et al.*, 2012; & Varley *et al.*, 2004).

MATERIALS AND METHODS

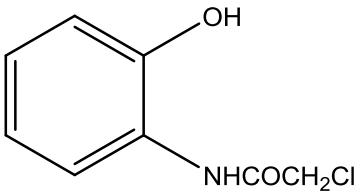
First of all, we produced benzoxazine by reacting commercially available 2-aminophenol **(1)** with $ClCH_2COCl$ in Di-chloro methane at room temperature for 9 hours to form an intermediate **(2),** which was then treated with $K_2CO_3/CH_3COOC_2H_5$ under refluxing conditions to produce benzoxazine **(3).** Propargyl bromide was used to N-alkylate benzoxazine in DMF in the presence of K_2CO_3, and the crude product was refined using column chromatography to yield alkyne derivative of benzoxazine **(4).** The substituted aniline was then treated with H_2SO_4/H_2O and $NaNO_2/NaN_3$ to obtain the required azido compound **(5).** Finally, in the presence of $CuSO_4.5H_2O$ and $C_6H_7O_6Na$ (Sodium Ascorbate), the alkyne derivative of benzoxazine **(4)** was reacted with azido compound **(5)** to produce 1,2,3-triazole analogues **(6).**

Scheme of the reaction:

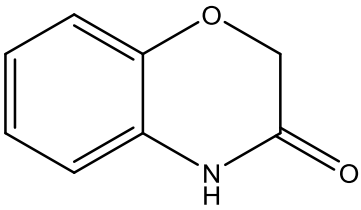
Compound 1 – 2-Aminophenol



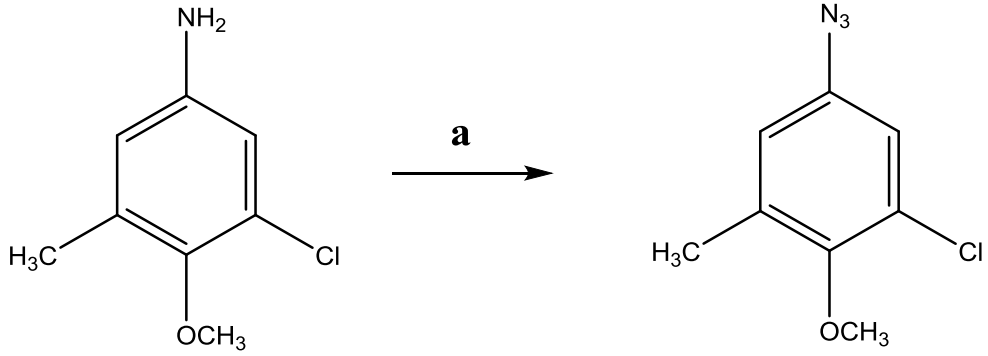
Compound 2 – 2-chloro-N-(2-hydroxyphenyl) acetamide



Compound 3 – Benzoxazine



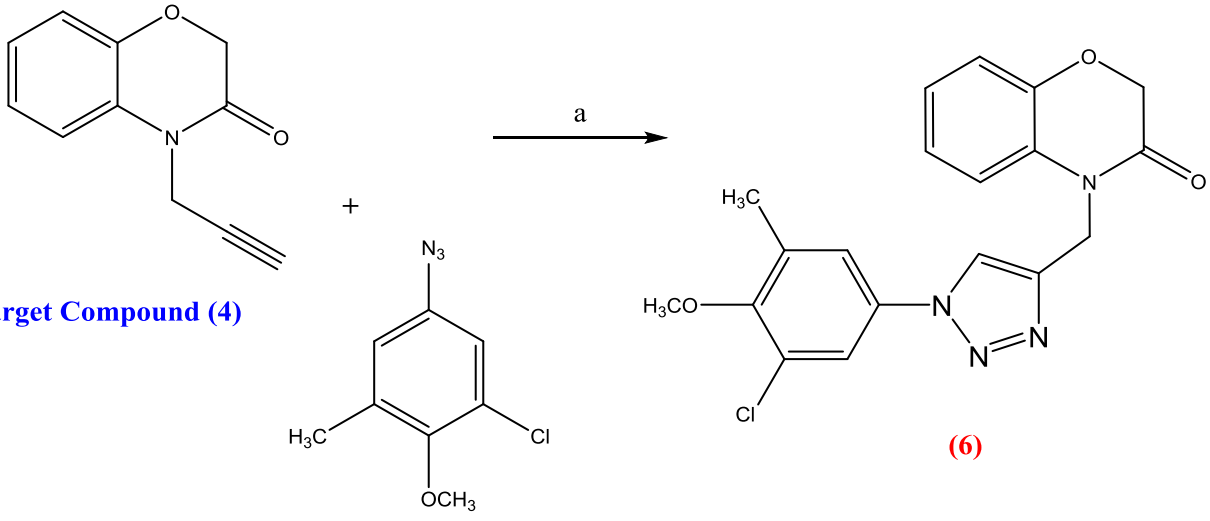
Scheme 1:



5

a = H_2SO_4/H_2O, $NaNO_2/NaN_3$

Scheme 2:



Target Compound (4)

(6)

Azides of Aromatic Amines (5)

a - 15 % $CuSO_{4.5}H_2O$, 35 % Sodium Ascorbate
THF : H_2O = 3 : 1, rt, 3-4 h

The aforesaid pharmacologically significant moiety, alkyne derivative of benzoxazine and aromatic azide, were combined in a single molecular frame with an aromatic nucleus connected to an alkyl chain to produce a compound with a wide range of biological activities. The aforementioned synthesized molecule was fully described as a consequence of intensive spectral analysis such as Infrared, Mass, Hydrogen-1-NMR, and Carbon-13-NMR. The synthesized compound was evaluated in vitro for antibacterial and antifungal activity against reference drugs Ciprofloxacin for bacteria and Miconazole for fungus using the Cup-plate method.

General procedure for the synthesis of alkyne derivative of benzoxazine (4)

To prepare alkyne derivative of benzoxazine **(4),** first of all we made benzoxazine by reacting commercially available 2-aminophenol **(1)** with $ClCH_2COCl$ in Di-chloro methane for 9 hours at room temperature to make an intermediate **(2),** which was then treated with $K_2CO_3/CH_3COOC_2H_5$ under refluxing conditions to yield benzoxazine **(3).** In the presence of K_2CO_3, propargyl bromide was used to N-alkylate benzoxazine in DMF, and the crude product was refined via column chromatography to obtain an alkyne derivative of benzoxazine **(4).** TLC was used to monitor the reaction mixture as it was agitated until it was complete. The mixture generated from the reaction was placed over cold ice and the precipitate separated was sieved through filter paper to extract the appropriate alkynes. The synthesis of alkyne was confirmed by its mass spectra (TOF ES MS+). According to the mass spectrum, the molecule **(4)** is 4-(prop-2-yn-1-yl)-2H-benzo[b][1,4]oxazin-3(4H)-one, as illustrated in the above scheme, TOF ES+ m/z (percent): 187 (M+).

Table-I

Sample Code	Gram +ve Bacteria			Gram -ve Bacteria				Fungus				
	S. aureus	B. subtilis	S. epidermis	K. pneumoniae	S. typhi	P. aeruginosa	E. coli	A. niger	A. fumigatus	A. flavus	A. albicans	C.
Std.	**21**	**20**	**19**	**24**	**24**	**30**	**22**	**30**	**22**	**22**	**22**	
6	**19**	14	**16**	14	**21**	21	14	18	14	12	**19**	

Procedure to synthesize triazole moiety (6)

The synthesized alkyne (1 equivalent) was added individually to a rapidly agitated solution of azide (1 equivalent) in THF: H_2O (3:1) (5.5 ml). To start the reaction, $CuSO_4.5H_2O$ (14%) and sodium ascorbate (36%) were added. At room temperature, the resulting-coloured suspension thus formed, was spun for 4-5 hours. The reaction's development was monitored using TLC. The aqueous layer was extracted three times with ethyl acetate using ice cold water after the reaction was completed. To obtain crude product, the combined organic extract was dried, evaporated under reduced pressure, or filtered using a suction pump, and then allowed to pass through a (60-120 mesh) silica gel column to provide pure 1,2,3-triazole.

Spectral Analysis of Compound (6)

According to the above-mentioned general procedure, the light coffee coloured solid **(6)** was generated by mixing 5-azido-1-chloro-2-methoxy-3-methylbenzene (5) (0.0716 g, 0.35 mmol) with alkyne derivative of benzoxazine (4) (0.104 g, 0.35 mmol). As a system solvent developer, Rf = 0.41 in methanol/chloroform (98:2) and yield is 0.137 g (78.5 %).

- **IR (KBr) ν_{max}:** 3238, 3068, 2931, 2849, 1691, 1604, 1494, 1381, 1243, 1289, 1141, 1040, 910, 847 cm^{-1}.
- **^{1}H Nuclear Magnetic Resonance (δ, $CDCl_3$, 400 MHz):** 8.03 (s, 1H, *J* = 8.3 Hz), 7.56 (s, 1H, *J* = 7.5 Hz), 7.59 (s, 1H, *J* = 7.7 Hz), 7.01-6.91 (m, 4H), 4.81 (s, 2H), 4.48 (s, 2H), 2.11 s, 3H), 3.86 (s, 3H).
- **^{13}C NMR (δ, $CDCl_3$, 100 MHz):** 163.8, 157.4, 145.1, 132.7, 128.3, 126.9, 125.6, 122.1, 121.4, 121.3, 121.1, 121.0, 119.3, 117.1, 111.9, 67.4, 61.8, 52.7, 15.2.
- **Mass spectral data, TOF ES+ *m/z* (%):** 371 (M^++1).
- **Molecular formula:** $C_{18}H_{16}ClN_4O_3$.

On the basis of above spectral data, compound (**6**) was identified as 4-((1-(3-chloro-4-methoxy-5-methylphenyl)-1H-1,2,3-triazol-4-yl)methyl)-2H benzo[b][1,4]oxazin-3(4H)-one.

RESULTS AND DISCUSSION

We used a molecular modification technique to manufacture a novel molecule **(6)** as part of our entire plan to produce a new triazole product. The chemical **(4)** was identified as the major intermediate in this method, yielding the required molecules when coupled with another material **(5)** in the presence of $C_6H_7O_6Na$ and copper sulphate pentahydrate. The synthesized chemical and its intermediate were fully described using mass, ^{1}H NMR, IR, and ^{13}C NMR**.**

Antimicrobial and Antifungal Activity

Using the cup plate method, the antibacterial activity of the generated compound **(6)** was evaluated in vitro at 100g/mL.

According to the results of the detailed analysis shown in Table-I, the tested compound had moderate antibacterial activity against P. aeruginosa, K. pneumoniae, B. subtilis, A. fumigatus, A. niger, A. flavus, and E. coli, but substantial antibacterial activity against S. aureus, S. epidermis, S. typhi, and C. albicans.

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