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SYNTHESIS CYTOTOXIC AND ANTIMICROBIAL EVALUATION OF SOME NOVEL BENZOXAZOLE ANALOGUES

Mansi L. Patil [*1], Lalit G. Rathi [2], Sanjana N. Gaikwad [3] and Swati S. Gaikwad [3]

University Department of Pharmaceutical Sciences [1], RTMNU, Nagpur - 440033, Maharashtra, India.
Institute of Pharmaceutical Education and Research Borgaon Meghe [2], Wardha - 442001, Maharashtra, India.
Nagpur College of Pharmacy [3], Wanadongri, Nagpur - 441110, Maharashtra, India.

Keywords:

Benzoxazole, Schiff base, *Allium cepa* root model, Antimicrobial activity, Cyclophosphamide

Correspondence to Author:
Mansi L. Patil

University Department of Pharmaceutical Sciences, RTMNU, Nagpur - 440033, Maharashtra, India.

E-mail: mansi198807@gmail.com

ABSTRACT: Benzoxazole and its analogues play an important role as therapeutic agents and exhibit good cytotoxic as well as antimicrobial activity. In the present study, benzoxazole analogues have been prepared by synthetic route; their spectroscopic evaluation was carried out, and then the synthesized compounds were screened for their cytotoxic activity by *Allium cepa* root model as well as antimicrobial activity by cup plate method. The synthesis and cytotoxic activity of a novel series of Schiff's base was carried out by reacting the amino group of the 4-Benzoxazol-2-yl-phenylamine with different aromatic/ heteroaromatic aldehydes in the presence of polyphosphoric acid to give 4-(1,3-Benzoxazol-2-yl)phenyl]-1-(Substituted phenyl) methanimine (4a-4h) also Diazotization reaction of (4-Benzoxazol-2-yl phenyl) with β napthol gave 2-((4-(benzo [d]oxazole-2-yl)phenyl) diazenyl) naphthalene-2-ol (4i). The cytotoxic and antimicrobial activities of the synthesized compounds were determined by using the onion root model and cup plate method, respectively. The activity data of compounds was compared with standard drugs Cefixime for antibacterial activity and miconazole for antifungal was used as a standard, and cyclophosphamide was used as a standard for Cytotoxic activity.

INTRODUCTION: Medicinal chemistry as practiced encompasses both definitions but finding the biochemical pathways through which drugs exert their beneficial effects has become an important activity of the medicinal chemist [1]. The discipline of medicinal chemistry is devoted to the discovery and development of new agents for treating diseases.

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Most of this activity is directed to new natural or synthetic organic compounds. Inorganic compounds continue to be important in therapy, *e.g.,* trace elements in nutritional therapy, antacids, and radiopharmaceuticals, but organic molecules with increasingly specific pharmacological activities are clearly dominant. The development of organic compounds has grown beyond traditional synthetic methods [2].

Heterocyclic compounds are acquiring more importance in recent years as these can be found in a large number of compounds that display biological activities [3-6]. Heterocyclic compounds occupy central position in organic chemistry, and considerable attention has been focused on their

synthesis. In particular, the benzof used nitrogen containing heterocycles are a common heterocyclic scaffold in many biologically active and medicinally significant compounds [7].

Benzoxazole is an aromatic organic compound with molecular formula C_7H_5NO. Heterocyclic moiety benzoxazole finds use in research as a starting material for the synthesis of larger, usually bioactive structures. Its aromaticity makes it relatively stable, although as a heterocyclic, it has reactive sites which allow for functionalization8. Benzoxazole displays diverse pharmacological properties including antimicrobial [9-14], multidrug resistant cancer cell activities [15-16], anti-inflammatory [17-19], anticonvulsant [20], antidepressant [21], anti-HIV [22-23], Antihypertensive [24], anthelmintic [25] activities.

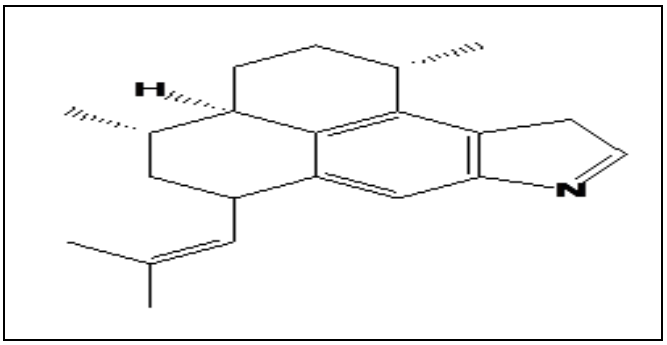


FIG. 1: PSEUDOPTEROXAZOLE

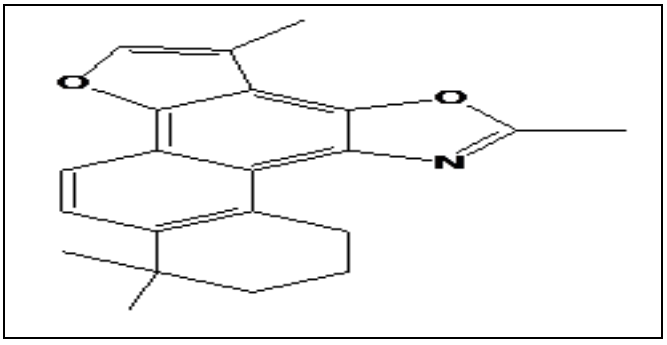


FIG. 2: SALVIANEN

They have been used as cathepsin S inhibitor, 5-HT receptor agonist, HIV reverse transcriptase inhibitor L-697661 **Fig. 9**, estrogen receptor-agonist ERB-041, selective peroxisome proliferator-activated receptor γ antagonist JTP-426467, anticancer agent NSC-693638 and orexin-1 receptor antagonist SB-334867bis-benzoxazole natural product analogs UK-1 **Fig. 6**, MUK-1, DMUK-1 and 2-(2'-hydroxyphenyl) benzoxazole analogs is one of the growing number of structurally related secondary metabolites with interesting biological activity, UK-1 and analogs SB334867 **Fig. 7**, NSC 693638 **Fig. 8** display a wide spectrum of potent anticancer activity against leukemia, lymphoma, and certain solid tumor-derived cell lines; however, this anticancer natural product does not show antibacterial or antifungal activity [26].

The benzoxazole ring system also occurs in natural products, *e.g.* pseudopteroxazole **Fig. 1**, salvianen **Fig. 2**, Nakijol B **Fig. 3** and some are biologically active *e.g.* herbicid fenoxaprop **Fig. 4**. Flunoxa-profen **Fig. 5** is non-steroidal anti-inflammatory agent which contains benzoxazole nucleus [27-30]. Besides its use in medicinal chemistry, these are recognized as fluorescent probes such as anion and metal cation sensors, as photochromatic agents, and laser dyes [31].

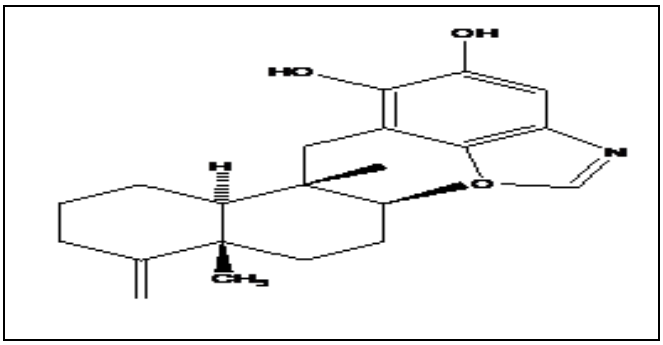


FIG. 3: NAKIJINOL B

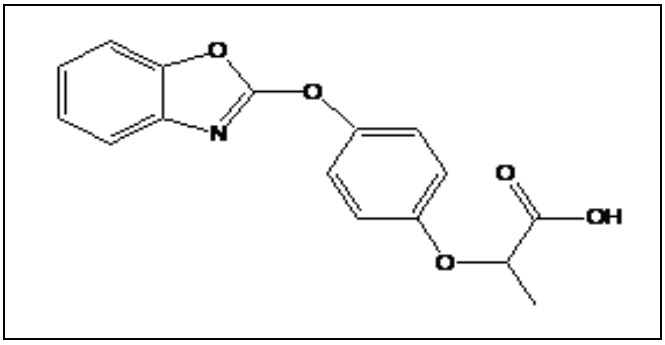


FIG. 4: FENOXAPROP

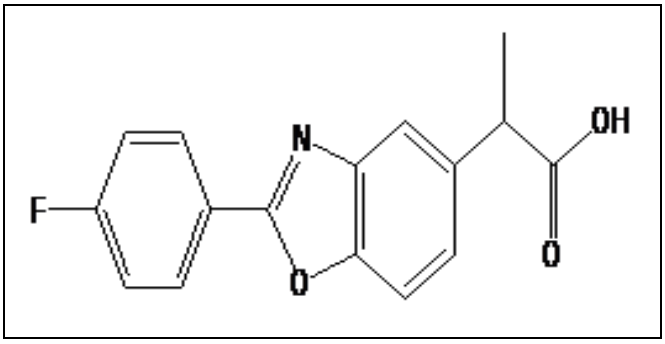


FIG. 5: FLUNOXAPROFEN

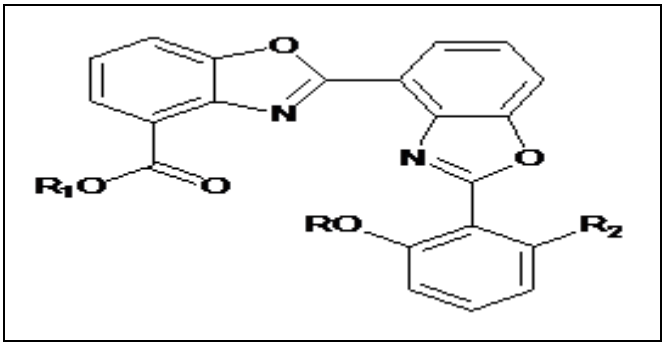


FIG. 6: R_1=CH_3, R_2=H UK1 R_1=H, R_2=CH_3 AJ19561

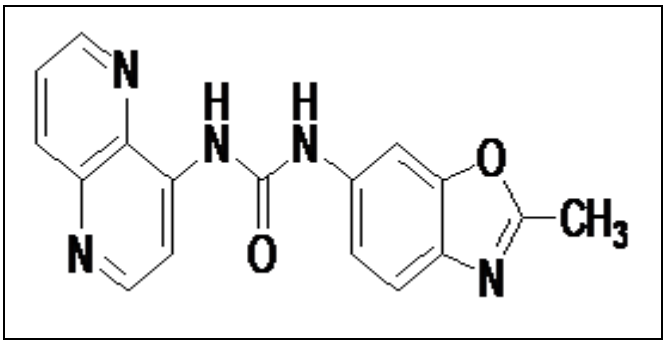


FIG. 7: SB334867

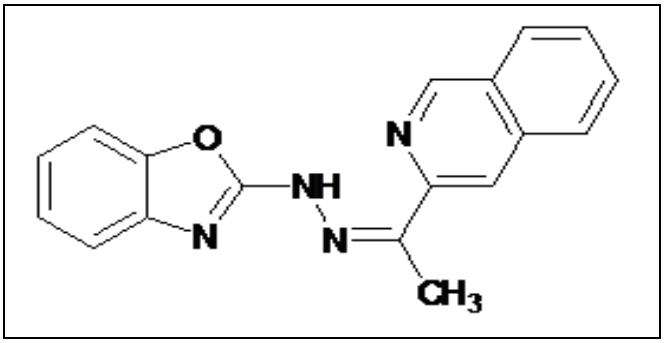


FIG. 8: NSC 693638

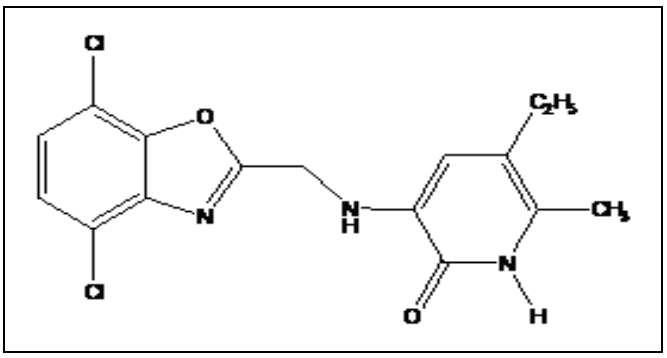


FIG. 9: L-697-661

Some Natural and Synthetic Benzoxazole Analogues with Varied Pharmacological Attributes: The goal at the outset of this research was to develop more effective cytotoxic analogues of substituted benzoxazole. The strategy employed was to examine the effect of the isosteric heterocyclic nucleus against cancer cells.

MATERIALS AND METHODS: All the solvents, chemicals, and drugs employed for the synthetic work were of SDFine/E.Merck/Loba Laboratory grade. The solvents were purified by the established methods. Few of the reagent materials used in the synthesis were obtained from Sigma Aldrich, Germany, and Alfa Aeser, United Kingdom. All the residues have been dried in vacuum desiccators and recrystallized. The melting points of the compounds were determined in open capillaries using Thieles tube. The melting points, reported herein, are in the celsius scale (°C) and are uncorrected.

Precoated silica gel-G plate (E. Merck Kieselgel 60F254) activated at 110 °C for 30 min were used for thin layer chromatography and the spots were developed in iodine chamber. Though different solvent systems were employed, R_f values are reported for better comparable solvent systems, which are mentioned in the preceding text.

The IR spectra of compounds were recorded using KBr pellets on FTIR-8400 spectrophotometer Shimadzu make at the Department of Pharmaceutical Sciences, Rashtrasant Tukadoji Maharaj Nagpur University Nagpur. 1H NMR ($CDCl_3$) 1H-NMRBRUKER AVANCE – II 400 NMR Spectrophotometer from National Institute of Pharmaceutical Education and Research, Hyderabad. Mass Spectrophotometer Micro-mass Quatto II triple quadrapole Mass Spectrometer from The National Institute of Pharmaceutical Education and Research, Hyderabad.

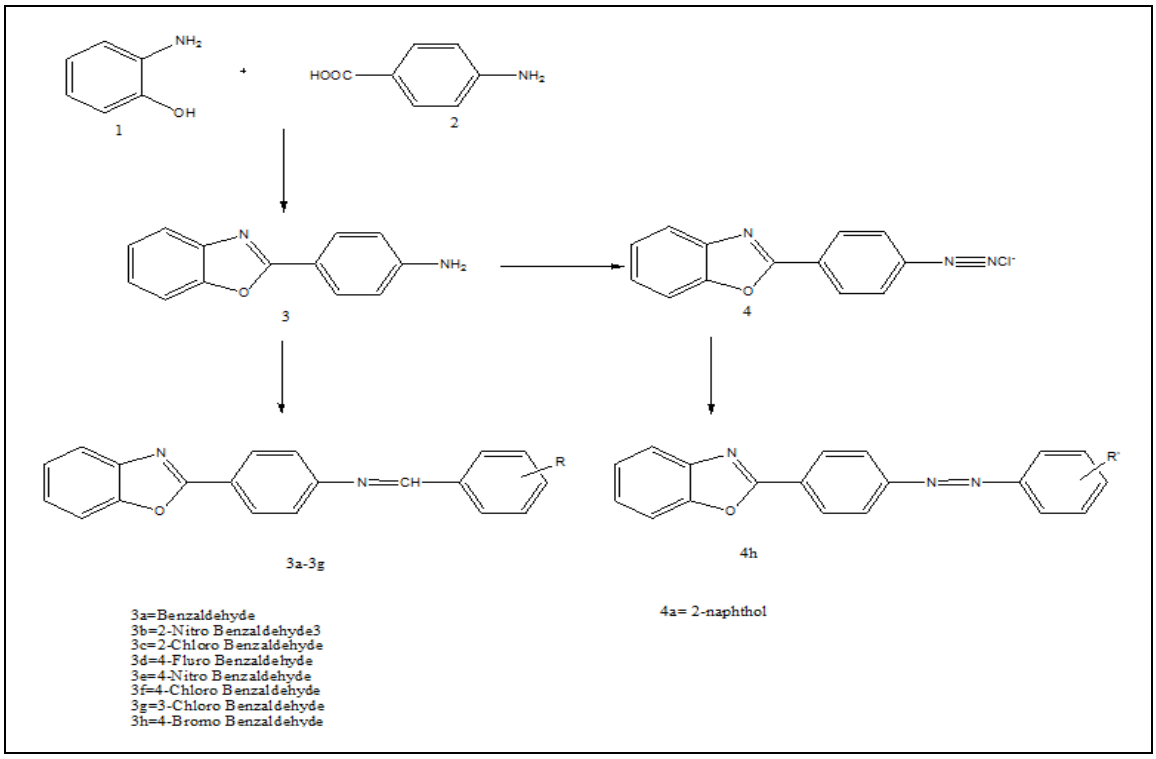
Chemistry: The chemicals were purchased from the commercial vendors and were used without purification. The reactions were monitored and the purity of the products was checked by thin layer chromatography (TLC).

All the melting points were taken on a DBK Programmed Melting Point Apparatus. IR spectra were recorded on an FT-IR - 8400 S, Shimadzu Corporation, Kyoto, Japan. The 1HNMR spectra were recorded on AVANCE – II 400 NMR Spectrophotometer and chemical shifts (δ) are reported in ppm relative to internal tetramethyl-silane (TMS). Mass analysis was carried out with a Micro-mass Quatto II triple quadrapole Mass Spectrometer.

General Method for Synthesis of 3: To a mixture of 2-amino phenol (0.02 mol, 0.218 g) and 4-amino benzoic acid (0.002mol, 0.274 g) polyphosphoric acid (10 mL) was added with stirring to form a paste. It was then refluxed on a sand bath for 5 h at 200 °C and the resulting solution was cooled to 0 °C. The resulting mixture was poured into 40% sodium bicarbonate solution with stirring till precipitate has formed. The solid was filtered, washed with water and recrystallized from ethanol [32-35].

General Method for Synthesis of 3a-H: To the equimolar quantities of 4-Benzoxazol-2-yl-phenylamine (0.002 mol, 420 mg) and the respective aromatic aldehydes were dissolved in 10 ml of warm ethanol, and 1 ml of glacial acetic acid was added dropwise to the mixture. The mixture was refluxed on sand bath for 6 h at 200 °C till the completion of the reaction (monitored by TLC). The reaction mixture was cooled and poured onto crushed ice. The product was filtered, dried and recrystallized using ethanol to get 4a-h [36-40].

General Method for Synthesis of 4a: Compound IIh (4.20 mmol, 1.72 g) was dissolved in 10 mL of 6N hydrochloric acid and cooled to 0 °C. A solution of (5.04 mmol, 0.35 g) of sodium nitrite in 5 mL of water was added to it at the same temperature, and the resultant diazonium chloride solution was added drop-wise to the stirred 2-napthol solution while maintaining the temperature at 5-10 °C. The crude product was purified by dissolving in DMSO followed by precipitation with water [41].



4-(benzo[d]oxazol-2-yl) benzeneamine (3): Yield: 80 %; m.p. 238-240 °C; IR (KBr) 3052, 1575, 1498, 1618, 1247; 1HNMR (400 MHz, DMSO), δ2.86 (s, 1H, CH),), δ7.43 (m, 4H, benzoxazole), δ 7.67 (m, 4H, Ar−H),), δ7.38(m, 5H, Benzylidine); MS m/z 266 [M + H]+.

4-(1,3-Benzoxazol-2 yl) phenyl]imino} methyl] phenol3a: Yield: 75.18 %; m.p. 256-258 °C; IR (KBr) 3060.82, 3506, 2750, 1579.59, 1057,1318; 1HNMR (400 MHz, DMSO), δ 2.70 (s, 1H, CH), δ 4.75(s, 1H, OH) δ 7.61 (m, 4H, Ar−H), δ 7.72 (m, 4H, benzoxazole), δ8.1 (m, 4H, benzylidine); MS m/z 315 [M + H]+.

4-(1,3-Benzoxazol-2-yl)phenyl]-1-(4-nitrophenyl) methanimine 3b: Yield: 75.18%; m.p. 170-172°C; IR (KBr) 3072, 1610, 1588, 1452, 752, 1440, 1053; 1HNMR (400 MHz, DMSO), δ 8.61 (s, 1H, CH), δ 7.52 (m, 4H, Ar−H), δ 7.90 (m, 4H, benzoxazole), δ 8.34 (m, 4H, benzylidine); MS m/z 344 [M + H]+.

4-**(1, 3-Benzoxazol-2-yl) phenyl]- 1-(4-chloro-phenyl) methanimine 3c:** Yield: 68%; m.p. 150-152 °C; IR (KBr) 3062, 1614, 1558, 1488, 1452, 1051, 1325, 804; 1HNMR (400 MHz, DMSO), δ 8.46 (s, 1H, CH), δ 7.90 (m, 4H, Ar−H), δ 7.72 (m, 4H, benzoxazole), δ 7.69 (m, 4H, benzylidine); MS m/z 333 [M + H]+.

4-(1, 3-Benzoxazol-2-yl) phenyl] -1-(4-fluoro-phenyl) methanimine 3d: Yield: 79%; m.p. 182-184 °C; IR (KBr)) 3056, 2889, 1631, 1587, 1506, 1452, 750, 1413, 1056; 1HNMR (400 MHz, DMSO), δ 8.22 (s,1H, CH), δ 7.95 (m, 4H, Ar−H), δ 7.62 (m, 4H, benzoxazole), δ 7.58 (m, 4H, benzylidine); MS m/z 317 [M + H]+.

4-(1, 3-Benzoxazol-2-yl) phenyl]-1-(2-nitro-phenyl) methanimine3e: Yield: 62%; m.p. 75-78 °C; IR (KBr) 3101, 1613, 1598, 1456, 1521.73, 1344, 746; 1HNMR (400 MHz, DMSO), δ 8.12 (s, 1H, CH), δ 7.7.76(m, 4H, Ar−H), δ 7.82 (m, 4H, benzoxazole), δ 7.39 (m, 4H, benzylidine); MS m/z 344 [M + H]+.

4-(1, 3-Benzoxazol- 2-yl)phenyl] -1-(2-chloro-phenyl) methanimine3f: Yield: 71.91%; m.p. 236-238 °C; IR (KBr) 3064, 1581.52, 1657, 1413, 747, 1218; 1HNMR (400 MHz, DMSO), δ8.19 (s, 1H, CH), δ 8.82(m, 4H, Ar−H), δ 7.28 (m, 4H, benzo-xazole), δ 7.65(m, 4H, benzylidine); MS m/z 341 [M + H]+.

4-(1, 3-Benzoxazol -2-yl) phenyl]-1-(4-bromo-phenyl) methanimine 3g: Yield: 58.32%; m.p. 148-150 °C; IR (KBr) 2927, 1610, 1580, 1490, 1452 , 1054, 1320, 840; 1HNMR (400 MHz, DMSO), δ8.02 (s, 1H, CH), δ 7.80 (m, 4H, Ar−H), δ 7.55 (m, 4H, benzoxazole), δ 7.72(m, 4H, benzylidine); MS m/z 344[M + H]+.

4-(1, 3-Benzoxazol-2-yl) phenyl]-1-(3-chloro-phenyl) methanimine 3h: Yield: 85.14%; m.p. 173-175 °C; IR (KBr) 3189, 1610, 1522, 1452, 1054, 1320, 1248; 1HNMR (400 MHz, DMSO), δ8.84 (s, 1H, CH), δ 7.11(m, 4H, Ar−H), δ 7.86 (m, 4H, benzoxazole), δ 7.31(m, 4H, benzylidine); MS m/z 315[M + H].

2-((4-(benzo[d]oxazole-2-yl) phenyl) diazenyl) naphthalene-2-ol 4a: Yield: 73.24%; m.p. 173-175 °C; IR (KBr) 3052.14, 1602.74, 1575.08; 1HNMR (400 MHz, DMSO), δ 2.5 (s, 1H, CH), δ 7.95(m, 4H, Ar−H), δ 8.60 (s, 1H, OH) ; MS m/z 315[M + H].

Pharmacological Evaluation:

Cytotoxic Screening: Onion root model was used for studying cytotoxic activity. *Allium cepa* root tip meristems have been widely used for the evaluation of cytotoxic and antimitotic activity. The inhibitory effect of synthesized compounds was evaluated on the growth of *Allium cepa* root meristems, and the effect was compared with standard anticancer drug cyclophosphamide. Locally available *Allium cepa* bulbs were grown in the dark over 100 mL tap water at ambient temperature until the roots have grown to approximately 3-4 cm. The water was changed daily. Working dilutions of all the drugs were made in tap water. Standard drug cyclophosphamide and compound IIa-h were used at 1 mg/mL and 10 mg/mL concentration. The bulbs with root tips grown up to 3-4 cm were placed over drug solution and incubation was carried out at ambient temperature [42-49].

Growing *Allium cepa* Meristems: Locally available *Allium cepa* bulbs were grown in dark over 100 mL tap water at ambient temperature until the roots have grown to approximately 3-4 cm. The water was changed daily.

Conditions for Drug Incubation: Working dilutions of all the drugs were made in tap water. Standard drug cyclophosphamide and compound IIa-h were used at 1 mg/mL and 10 mg/mL concentration. The bulbs with root tips grown up to 3-4 cm were placed over drug solution and incubation was carried out at ambient temperature **Fig. 10-15**.

Cytotoxic Activity: The cytotoxic effect of synthesized compounds 3a-3h and compound 4a was evaluated on the growth of *Allium cepa* root meristems, and the effect was compared with standard anticancer drug cyclophosphamide. Roots of onion were exposed for 96 h to different dilutions of all the compounds and cyclo-phosphamide. Shortening and decaying of roots was considered as a measure of cytotoxic activity. A progressive increase in root length was observed in control group. After 96 h of growth in the control, the average roots length was 4.91+0.32. Incubation of bulbs in increasing concentrations of

cytotoxic agents produced a growth retarding effect that was associated with decrease in root number **Table 1**. In the presence of compound 3a & 3c,3g root length of onion bulb was decreased significantly ($p<0.05$) after 72 h as compared to control at the concentration of 10 mg/mL. The effect was even more significant ($P<0.01$) after 96 h of incubation. Compound 3d showed very significant activity after 96 h at 10 mg/mL concentration. Compound 3e, 3f, 3h did not show cytotoxic activity at any concentration.

FIG. 10: CONTROL

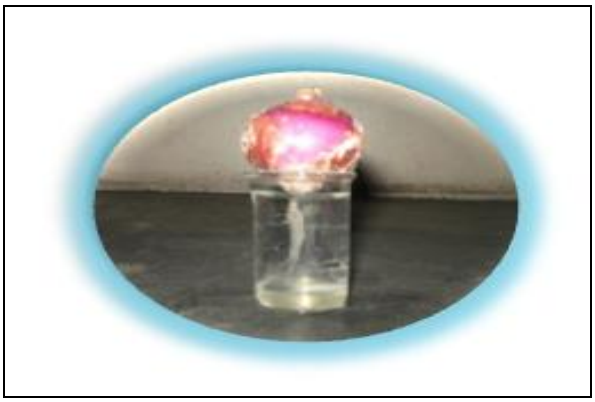


FIG. 11: STANDARD

FIG. 12: COMPOUND 3A

FIG. 13: COMPOUND 3B

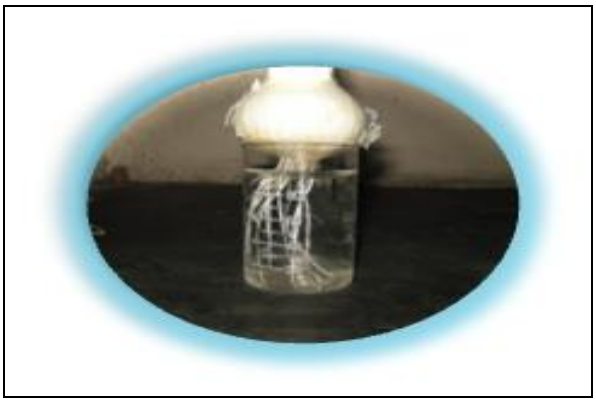


FIG. 14: COMPOUND 3C

FIG. 15: COMPOUND 3G

FIG. 1: PHOTOGRAPHS SHOWING GROWTH OF ONION IN THE PRESENCE OF VEHICLECYCLO-PHOSPHAMIDE AND COMPOUNDS 3A, 3B, 3C AND 3G

Antimicrobial Activity: The *in-vitro* antimicrobial studies were carried out by agar well diffusion method against test Organisms. Nutrient broth plates were swabbed with 24 h old broth culture (100 µL) of test bacteria. Using the sterile cork borer, wells (6 mm) were made into each Petri plate. The compounds were dissolved in DMSO of 5 mg/mL and from this 2.5, 5, 10 and 20 µL (50, 75 and 100 µg/mL) were added into the wells by using sterile pipettes.

TABLE 1: ROOT LENGTH ATTAINED AFTER INCUBATION WITH VEHICLE, CYCLOPHOSPHAMIDE & COMPOUND 3A-3H AND 4A

Groups	Root length after hour[#]				
	0 h	24 h	48 h	72 h	96 h
Control	3.885 ± 0.43	4.16 ± 0.39	4.40 ± 0.34	4.68 ± 0.34	4.91 ± 0.32
Comp 3a 1 mg/mL	3.85 ± 0.30	3.96 ± 0.38	4.13 ± 0.43	4.11 ± 0.41	4.13 ± 0.45
Comp 3a 10 mg/mL	3.86 ± 0.22	3.34 ± 0.09	3.84 ±0.23	3.84* ± 0.23	3.82** ± 0.24
Comp 3b 1 mg/mL	3.85 ± 0.06	4.53 ± 0.18	4.58 ± 0.18	4.63 ± 0.19	4.67 ± 0.18
Comp 3b 10 mg/mL	3.82 ± 0.19	3.67 ± 0.18	3.71 ± 0.18	3.79* ± 0.17	3.77** ± 0.19
Comp 3c 1 mg/mL	3.85 ± 0.30	4.12 ± 0.21	4.02 ± 0.15	4.08 ± 0.15	4.4 ± 0.14
Comp 3c 10 mg/mL	3.85 ± 0.12	3.69 ± 0.12	3.74 ± 0.13	3.82* ± 0.14	3.84** ± 0.14
Comp 3d 1 mg/mL	3.82 ± 0.15	3.89 ± 0.15	3.66 ± 0.15	4.02 ± 0.16	4.08 ± 0.16
Comp 3d 10 mg/mL	3.81 ± 0.03	4.51 ± 0.19	4.00 ± 0.05	4.04 ± 0.05	4.09** ± 0.05
Comp 3e 1 mg/mL	3.80 ± 0.18	4.52 ± 0.19	4.60 ± 0.20	4.74 ± 0.16	4.81 ± 0.16
Comp 3e 10 mg/mL	3.84 ± 0.16	4.29 ± 0.16	4.32 ± 0.16	4.35 ± 0.15	4.34 ± 0.17
Comp 3f 1 mg/mL	4.89 ± 0.05	3.51 ± 0.19	4.30 ± 0.23	4.30 ± 0.23	4.27 ± 0.18
Comp 3f 10 mg/mL	3.86 ± 0.22	4.00 ± 0.05	4.40 ± 0.34	4.38 ± 0.21	4.4 ± 0.12
Comp 3g 1 mg/mL	4.85 ± 0.32	4.53 ± 0.18	4.58 ± 0.18	4.63 ± 0.19	4.84 ± 0.23
Comp 3g 10 mg/mL	4.81 ± 0.19	4.55 ± 0.12	4.32 b± 0.23	4.00 ± 0.05	3.77** ± 0.19
Comp 3h 1 mg/mL	3.86 ± 0.12	3.84 ± 0.23	4.53 ± 0.18	4.84 ± 0.23	4.84 ± 0.23
Comp 3h 10 mg/mL	3.87 ± 0.05	4.27 ± 0.23	4.51 ± 0.23	3.82* ± 0.14	3.84 ± 0.23
Comp 4a 1 mg/mL	3.78 ± 0.15	3.86 ± 0.16	4.68 ± 0.25	4.56 ± 0.27	4.59 ± 0.14
Comp 4a 10 mg/mL	3.84 ± 0.05	4.27 ± 0.23	4.51 ± 0.23	3.84 ± 0.14	3.86 ± 0.23
Standard 1 mg/mL	3.85 ± 0.06	4.80* ± 0.12	4.08* ± 0.23	4.03** ± 0.23	4.00** ± 0.22
Standard 10 mg/mL	3.85 ± 0.06	4.00** ± 0.39	3.85** ± 0.45	3.69** ± 0.13	3.43** ± 0.13

#Values are represented as Mean±SD; (n = 6) *p<0.05 is considered significant and **p<0.01 is considered very significant when compared with control group.

Antibacterial screening of newly synthesized compounds was carried out against *E. coil* and *B. subtilis* **Table 2** and antifungal activity against *C. albicans* **Table 3** according to the cup-plate method. Cefixime for antibacterial activity and miconazole for antifungal was used as a standard. The samples were dissolved in DMSO, which showed no zone of inhibition acts as a negative control. The plates were incubated at 37 °C for 24 h for bacteria and at 28 °C for 48 h for fungi. After appropriate incubation, the diameter of zone of inhibition of each well was measured [50-54].

Cytotoxic Activity: The cytotoxic effect of synthesized compounds 3a-3h and compound 4a was evaluated on the growth of *Allium cepa* root meristems, and the effect was compared with standard anticancer drug cyclophosphamide. Roots of onion were exposed for 96 h to different dilutions of all the compounds and cyclo-phosphamide. Shortening and decaying of roots were considered as a measure of cytotoxic activity. A progressive increase in root length was observed in the control group. After 96 h of growth in the control, the length of the average roots was 4.91+0.32. Incubation of bulbs in increasing concentrations of cytotoxic agents produced a growth retarding effect that was associated with decrease in root number **Table 1.** In the presence of compound 3a & 3c, 3g root length of onion bulb was decreased significantly (p<0.05) after 72 h as compared to control at the concentration of 10 mg/mL The effect was even more significant (P<0.01) after 96 h of incubation. Compound 3d showed very significant activity after 96 h at 10 mg/mL concentration. Compound 3e, 3f, 3h did not show cytotoxic activity at any concentration.

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Antibacterial Activity:

TABLE 2: THE *IN-VITRO* ANTIBACTERIAL ACTIVITY OF COMPOUNDS 3A-3H AND 4A

Compounds	*E. coli*			*B. subtilis*		
	50 µg/well	75 µg/well	100 µg/well	50 µg/well	75 µg/well	100 µg/well
3a	24 ± 1.3	24 ± 1.3	26 ± 1.2	24 ± 1.5	27 ± 1.6	30 ± 1.1
3b	29 ± 1.1	30 ± 1.3	31 ± 1.3	25 ± 1.5	27 ± 1.6	28 ± 1.3
3c	29 ± 1.2	31 ± 1.5	33± 1.4	27 ± 1.4	29 ±1.3	31 ± 1.1
3d	21 ± 1.1	22 ± 1.3	26 ± .3	25 ± 1.5	26 ± 1.6	29 ± 1.3
3e	-	9 ± 1.3	9 ± 1.2	10 ± 1.6	12 ± 1.5	14 ± 1.4
3f	9 ± 1.2	10 ± 1.3	12 ± 1.2	10 ± 1.6	13 ±1.5	16 ± 1.4
3g	30 ± 1.4	32 ± 1.2	34 ±1.4	25 ± 1.6	26 ± 1.1	27 ± 1.5
3h	26 ± 1.1	27 ± 1.3	28 ± 1.3	26± 1.5	27 ± 1.6	29 ±1.3
4a	-	10 ± 1.3	12 ± 1.2	11 ± 1.6	13 ± 1.5	16 ± 1.4
Cefixime	30 ± 1.2	32 ± 1.1	35 ± 1.3	28 ± 1.4	30 ± 1.3	32 ± 1.5

(-) No activity. (±) Standard deviation

TABLE 3: THE *IN-VITRO* ANTIFUNGAL ACTIVITY OF COMPOUNDS 3A-3H AND 4A

Compounds	*C. albicans*		
	50 µg/well	75 µg/well	100 µg/well
3a	22 ± 1.3	24 ± 1.3	26 ± 1.2
3b	24 ± 1.1	25 ± 1.3	26 ± 1.3
3c	30 ± 1.2	31 ± 1.5	32 ± 1.4
3d	21 ± 1.1	22 ± 1.3	26 ± 1.3
3e	-	10 ± 1.3	12 ± 1.2
3f	11 ± 1.2	12 ± 1.3	14 ± 1.2
3g	29 ± 1.4	30 ± 1.2	31 ± 1.4
3h	22 ± 1.1	34 ± 1.3	25 ± 1.3
4a	9 ± 1.3	11 ± 1.3	14 ± 1.2
Ketoconazole	30 ± 1.2	32 ± 1.1	35 ± 1.3

(-) No activity. (±) Standard deviation

RESULTS AND DISCUSSION: Benzoxazole analogues have been reported to possess a broad spectrum of Pharmacological properties. Hence attempts have been made to synthesize some novel benzoxazole analogues and studied their cytotoxic effects on onion roots. The structures of the synthesized compounds were assigned on the basis of elemental analysis, IR, 1H NMR, and mass spectroscopy. These compounds were also screened forcytotoxic activity. The recorded percentage of inhibition showed significant cytotoxic activity when compared to the reference cytotoxic drug cyclophosphamide. Root length was decreased significantly as compared to the standard drug of compounds 3a, 3b, 3c, 3g at the concentration of 10 mg/mL Compound, and the compounds showed very significant activity after 96h incubation. Thus the structure-activity relationship suggests that electron-withdrawing substituents at para position such as chloro and nitrosubstituent's show significant cytotoxic activity. From the results and discussion, it is thereby concluded that substituted benzoxazole congeners exhibit mild to moderate cytotoxic profiles; thus, newer and alternative congeners can be synthesized further and evaluated for their cytotoxic profile, which may have enhanced cytotoxic activity.

Compound 3b, 3g showed greater antifungal activity against *E. coli* as well as compound 3c showed greater antifungal activity against B. Subtilis. Compound 3c and 3g showed greater activity against *C. albicans*. The presence of electron-withdrawing chloro, bromo, and nitro Substituents at para position on the aromatic ring enhanced the activity. Thus the data reveals the necessity of electron-withdrawing substituent for potent antimicrobial activity.

CONCLUSION: Benzoxazole nucleus was found to possess many pharmacological activities like anti-inflammatory, antihistaminic, antiviral, multi-drug resistant cancer cell activities, antiulcer, anticonvulsant, *etc.* Hence, attempts were made to synthesize benzoxazole analogues with cytotoxic activity. Several other analogues of benzoxazole can be prepared and evaluated for their cytotoxic activity. The same analogues can also be evaluated for other biological activities. Structure/analogue based drug design can be carried out in order to optimize the pharmacological profiles. This will guide the synthesis of newer potent analogs using these leads.

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REFERENCES:

1. Foye WO: Principles of Medicinal Chemistry. Varghese Publishing house 1989.
2. Wilson Gisvold: Textbook of Organic Medicinal and Pharmaceutical chemistry. Grey House Publishing, 2011
3. Kim SJ, Yang J, Lee S, Park C, Kang D, Akter J, Ullah S, Kim YJ, Chun P and Moon HR: The tyrosinase inhibitory effects of isoxazolone derivatives with a (Z)-β-phenyl-α, β-unsaturated carbonyl scaffold. Bioorganic & Medicinal Chemistry 2018; 26(14): 3882-89.
4. Sajja Y, Vanguru S, Vulupala HR, Bantu R, Yogeswari P, Sriram D and Nagarapu L: Design, synthesis and *in-vitro* anti-tuberculosis activity of benzo [6, 7] cyclohepta [1, 2-b] pyridine-1, 2, 3-triazole derivatives. Bioorganic & Medicinal Chemistry Letters 2017; 27(23): 5119-21.
5. Phatak PS, Bakale RD, Dhumal ST, Dahiwade LK, Choudhari PB, Siva Krishna V, Sriram D and Haval KP: Synthesis, antitubercular evaluation and molecular docking studies of phthalimide bearing 1, 2, 3-triazoles. Synthetic Communications 2019; 49(16): 2017-28.
6. Marabathuni VJ, Mariyamma K, Sravani, Ramaiah S, Ravindra P and Srihari R: Synthesis, characterization, anti-microbial, analgesic activities of 3-(3-Chlorophenyl)-5-Phenyl-4,5-Dihydro-1,2-Oxazole Derivatives. Asian Journal of Pharmaceutical Research 2017; 7(3): 171-74.
7. Lu HY, Barve IJ, Selvaraju M and Sun CM: One-Pot Synthesis of Unsymmetrical Bis-Heterocycles: Benzimidazole-, Benzoxazole- and Benzothiazole-Linked Thiazolidines. ACS Combinatorial Scie 2019; 22(1): 42-8.
8. Daniel L and Lester A: The Organic Chemistry of Drug Synthesis Vol-III, New York 1984.
9. Al-Alshaikh MA, Panicker CY, El-Emam AA, Arisoy M, Temiz-Arpaci O and Van Alsenoy C: Synthesis, vibrational spectroscopic investigations, molecular docking, antibacterial and antimicrobial studies of 5-ethylsulphonyl-2-(p-aminophenyl) benzoxazole. Journal of Molecular Structure 2016; 1115: 94-04.
10. Beegum S, Panicker CY, Armaković S, Armaković SJ, Arisoy M, Temiz-Arpaci O and Van Alsenoy C: Spectroscopic, antimicrobial and computational study of novel benzoxazole derivative. Journal of Molecular Structure 2019; 1176: 881-94.
11. Mishra VR, Chaitannya R, Ghanavatkar SN, Mali SI and Quereshi HK: Design Synthesis antimicrobial activity and computational studies of novel azo linked substituted benzimidazole, oxazole, triazole derivatives. Combinatorial Biological Chemistry 2019; 78(2): 330-37.
12. Kashid BB, Ghanwat AA, Khedkar VM, Dongare BB, Shaikh MH, Deshpande PP and Wakchaure YB: Design, synthesis, *in-vitro* antimicrobial, antioxidant evaluation, and molecular docking study of novel benzimidazole and benzoxazole derivatives. Journal of Heterocyclic Chemistry 2019; 56(3): 895-08.
13. Erol M, Celik I, Temiz-Arpaci O, Kaynak-Onurdag F and Okten S: Design, synthesis, molecular docking, density functional theory and antimicrobial studies of some novel benzoxazole derivatives as structural bioisosteres of nucleotides. Journal of Biomolecular Structure and Dynamics 2020; 8:1-7.
14. Kozic J, Novotna E, Volkova M, Rikova JR, Trejtnar FS and Sova VW: Synthesis and *in-vitro* antimycobacterial and isocitrate lyase inhibition properties of novel 2-methoxy-20-hydroxybenzanilides, their thioxo analogues and Benzoxazoles. European Journal of Medicinal Chemistry 2012; 56(1): 108-19
15. Desai S, Desai V and Shingade S: *In-vitro* anti-cancer assay and apoptotic cell pathway of newly synthesized benzoxazole-N-heterocyclic hybrids as potent tyrosine kinase inhibitors. Bioorganic Chemistry 2020; 94: 103382.
16. El-Hady HA and Abubshait SA: Synthesis and anticancer evaluation of imidazolinone and benzoxazole derivatives. Arabian Journal of Chemistry 2017; 10: 3725-31.
17. Angajala G and Subashini R: Synthesis, molecular modeling, and pharmacological evaluation of new 2-substituted benzoxazole derivatives as potent anti-inflammatory agents. Stru Chemistry 2020; 31(1): 263-73.
18. Ayaz F, Ersan RH and Algul O: Symmetric bis-benzoxazole-based chemicals exerted anti-inflammatory effect on danger signal LPS-stimulated macrophages. Monatshefte fur Chemie-Chemical Monthly 2019; 150(6): 1137-46.
19. Angajala G, Subashini R and Aruna V: Microwave assisted amberlite-IRA-402 (OH) ion exchange resin catalyzed synthesis of new benzoxazole scaffolds derived from antiinflammatory drugs aceclofenac and mefenamic acid as potential therapeutic agents for inflammation. Journal of Molecular Structure 2020; 1200: 127092.
20. Song MX, Huang Y, Wang S, Wang ZT and Deng XQ: Design, synthesis, and evaluation of anticonvulsant activities of benzoxazole derivatives containing the 1, 2, 4-triazolone moiety. Archiv der Pharmazie 2019; 352(8): 1800313.
21. Jin Q, Fu Z, Guan L and Jiang H: Syntheses of benzo [d] thiazol-2 (3h)-one derivatives and their antidepressant and anticonvulsant effects. Marine Drugs 2019; 17(7): 430-39.
22. Medebielle M, Ait-Mohand S, Burkhloder C, Dolbier Jr WR, Laumond G and Aubertin AM: Synthesis of new difluoromethylene benzoxazole and 1, 2, 4-oxadiazole derivatives, as potent non-nucleoside HIV-1 reverse transcriptase inhibitors. Journal of Fluorine Chemistry 2005; 126(4): 533-40.
23. Sai K, Naveen K and Chandana K: Green synthesis, biological evaluation of novel benzoxazole derivatives. J of Basic and Applied Res in Biomed 2016; 2(3): 255-60.
24. Wu Z, Bao XL, Zhu WB, Wang YH, Phuong Anh NT, Wu XF, Yan YJ and Chen ZL: Design, synthesis, and biological evaluation of 6-benzoxazole benzimidazole derivatives with antihypertension activities. ACS Medicinal Chemistry Letters 2018; 10(1): 40-3.
25. Bhavsar ZA, Acharya PT, Jethava DJ and Patel HD: Recent advances in development of anthelmintic agents: Synthesis and biological screening. Synthetic Communications 2020; 50(7): 917-46.
26. Reiser A, Leyshon LJ, Saunders D, Mijovic MV, Bright A and Bogie J: Fluorescence of aromatic benzoxazole derivatives. J of the Am Chem Soci 1972; 94(7): 2414-21.
27. Schnurch M, Hammerle J and Stanetty: Section 11.13, Science of Synthesis. Benzoxazoles and Other Annulated Oxazoles 1-49.
28. Katritzky AR, Boulton AJ: In Advances in Heterocyclic Chemistry, New York 1978.
29. Kaur A, Pathak DP, Sharma V, Narasimhan B, Sharma P, Mathur R and Wakode S: Synthesis, biological evaluation and docking study of N-(2-(3, 4, 5-trimethoxybenzyl)

benzoxazole-5-yl) benzamide derivatives as selective COX-2 inhibitor and anti-inflammatory agents. Bioorganic Chemistry 2018; 81: 191-02.
30. Song H, Rao C, Deng Z, Yu Y and Naismith JH: The biosynthesis of the benzoxazole in nataxazole proceeds *via* an unstable ester and has synthetic utility. Angewandte Chemie International Edition 2020; 59(15): 6054-61.
31. Gan H and Yang D: S8-mediated decarboxylative cyclization of 2-nitrophenols with arylacetic acid: Synthesis of benzoxazoles. Journal of Saudi Chemical Society 2019; 23(7): 809-16.
32. Kashid BB, Ghanwat AA, Khedkar VM, Dongare BB, Shaikh MH, Deshpande PP and Wakchaure YB: Design, synthesis, *in-vitro* Antimicrobial, Antioxidant Evaluation, and Molecular Docking Study of Novel Benzimidazole and Benzoxazole Derivatives. Journal of Heterocyclic Chemistry 2019; 56(3): 895-908.
33. Shanty AA, Philip JE, Sneha EJ, Kurup MR, Balachandran S and Mohanan PV: Synthesis, characterization and biological studies of Schiff bases derived from heterocyclic moiety. Bioorganic Chem 2017; 70: 67-73.
34. Gan H: S8-mediated cyclization of 2-hydroxy/amino anilines with arylacetylene: approach to benzoxazoles and benzimidazoles. Chemistry Select 2019; 4(29): 8598.
35. Tian Q, Luo W, Gan Z, Li D, Dai Z, Wang H, Wang X and Yuan J: Eco-Friendly Syntheses of 2-Substituted Benzoxazoles and 2-Substituted Benzothiazoles from 2-Aminophenols, 2-Aminothiophenols and DMF Derivatives in the Presence of Imidazolium Chloride. Molecules 2019; 24(1): 174.
36. Mahmood K, Hashmi W, Ismail H, Mirza B, Twamley B, Akhter Z, Rozas I and Baker RJ: Synthesis, DNA binding and antibacterial activity of metal (II) complexes of a benzimidazole Schiff base. Polyhedron 2019; 157: 326-34.
37. Raauf AM: Synthesis, characterization and antibacterial activity of new derivatives of imidazolidine having sulfamethoxazole moiety. Al-Mustansiriyah Journal for Pharmaceutical Sciences 2019; 19(4): 217-38.
38. Keshk SM, Bondock S, El-Zahhar AA and Haija MA: Synthesis and characterization of novel Schiff's bases derived from dialdehyde cellulose-6-phosphate. Cellulose 2019; 26(6): 3703-12.
39. Nematidil N, Sadeghi M, Nezami S and Sadeghi H: Synthesis and characterization of Schiff-base based chitosan-g-glutaraldehyde / NaMMTNPs-APTES for removal Pb2+ and Hg2+ ions. Carbohydrate Polymers 2019; 222: 114971.
40. El-Azab AS, Alaa AM, Bua S, Nocentini A, Alanazi MM, AlSaif NA, Al-Suwaidan IA, Hefnawy MM and Supuran CT: Synthesis and comparative carbonic anhydrase inhibition of new Schiff's bases incorporating benzene-sulfonamide, methanesulfonamide and methylsulfonyl-benzene scaffolds. Bioorganic Chem 2019; 92: 103225.
41. Zeng YF, Li YN, Zhang NN, Kang H, Duan P, Xiao F, Guo Y and Wen X: Synthesis of Acetamides from Aryl Amines and Acetonitrile by Diazotization under Metal-Free Conditions. Synlett 2019; 30(19): 2169-72.
42. Rosculete CA, Bonciu E, Rosculete E and Olaru LA: Determination of the environmental pollution potential of some herbicides by the assessment of cytotoxic and genotoxic effects on *Allium cepa*. International Journal of Environmental Res and Public Health 2019; 16(1): 75.
43. Scherer MD, Sposito JC, Falco WF, Grisolia AB, Andrade LH, Lima SM, Machado G, Nascimento VA, Gonçalves DA, Wender H and Oliveira SL: Cytotoxic and genotoxic effects of silver nanoparticles on meristematic cells of *Allium cepa* roots: A close analysis of particle size dependence. Sci of the Total Environ 2019; 660: 459-467.
44. Mahmoud GA, Soltan HA, Abdel-Aleem WM and Osman SA: Safe natural bio-pigment production by Monascus purpureus using mixed carbon sources with cytotoxicity evaluation on root tips of *Allium cepa* L. Journal of Food Science and Technology 2020; 1-2.
45. Aziz AA, Shaniba VS, Jayasree PR and Kumar PM: Physico-chemical, photocatalytic and cytotoxicity evaluation of *Annona muricata* L. fruit extract derived zinc oxide nanoparticles in comparison to the commercial chemical version. Current Science 2019; 117(9): 1492.
46. Jose AM, Vaishnavi A and Aswathy A: Anti-mitotic activity of aqueous leaf extracts of *Azadirachta indica* A. Juss. and Simarouba glauca DC. On *Allium cepa* L. root tips. J of Pharmaco and Phytochem 2020; 9(5): 485-89.
47. Yekeen TA, Azeez MA, Akinboro A, Lateef A, Asafa TB, Oladipo IC, Oladokun SO and Ajibola AA: Safety evaluation of green synthesized Cola nitida pod, seed and seed shell extract-mediated silver nanoparticles (AgNPs) using an *Allium cepa* assay. Journal of Taibah University for Science 2017; 11(6): 895-09.
48. Bonciu E, Firbas P, Fontanetti CS, Wusheng J, Karaismailoğlu MC, Liu D, Menicucci F, Pesnya DS, Popescu A, Romanovsky AV and Schiff S: An evaluation for the standardization of the *Allium cepa* test as cytoto-xicity and genotoxicity assay. Caryol 2018; 71(3): 191-09.
49. Islam MT, Streck L, Alencar MV, Silva SW, Conceicao Machado K, Conceicao Machado K, Júnior AL, Paz MF, Mata AM, Sousa JM and Costa Junior JS: Evaluation of toxic, cytotoxic and genotoxic effects of phytol and its nanoemulsion. Chemosphere 2017; 177: 93-01.
50. Wei Q, Wang X, Cheng JH, Zeng G and Sun DW: Synthesis and antimicrobial activities of novel sorbic and benzoic acid amide derivatives. Food Chemistry 2018; 268: 220-32.
51. Cui P, Li X, Zhu M, Wang B, Liu J and Chen H: Design, synthesis and antimicrobial activities of thiouracil derivatives containing triazolo-thiadiazole as SecA inhibitors. Euro J of Medi Chemistry 2017; 127: 159-65.
52. Hassan AS, Moustafa GO, Askar AA, Naglah AM and Al-Omar MA: Synthesis and antibacterial evaluation of fused pyrazoles and Schiff bases. Synthetic Communications 2018; 48(21):2 761-72.
53. Tu H, Wu SQ, Li XQ, Wan ZC, Wan JL, Tian K and Ouyang GP: Synthesis and Antibacterial Activity of Novel 1H-indol-2-ol Derivatives. Journal of Heterocyclic Chemistry 2018; 55(1): 269-75.
54. Yu X, Teng P, Zhang YL, Xu ZJ, Zhang MZ and Zhang WH: Design, synthesis and antifungal activity evaluation of coumarin-3-carboxamide derivatives. Fitoterapia 2018; 127: 387-95.

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