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Der Pharmacia Sinica, 2010, 1 (3): 109-116

Der Pharmacia Sinica

ISSN: 0976-8688
CODEN (USA): PSHIBD

Synthesis and *in vitro* antibacterial studies of some novel 3-(5-amino-6(2, 3-dichlorophenyl)-1, 2, 4-triazin-3-yl)-2-aryl quinazoline-4(3*H*)-one

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ABSTRACT

*In the present study some new 3-(5-amino-6(2, 3-dichlorophenyl)-1, 2, 4-triazin-3-yl)-2-aryl-quinazoline-4(3H)-ones (3a-3f) was synthesized. The newly synthesized compounds were characterized on the basis of elemental analysis, IR and ^{1}H-NMR spectra. All the synthesized compounds were tested for their antibacterial activity against 20 strains of Gram positive and Gram negative bacteria. Among the compounds tested, the compounds 3a & 3f showed good antimicrobial activity in comparison to standard sulphamethoxazole. Compound **3f** was found to be most active in the series against H. pylori with MIC 25 µ g/mL.*

Key words: Quinazolinones, *in-vitro* antibacterial activity, MIC value.

INTRODUCTION

4(3H)-quinazolinone derivatives were reported to posses analgesic anti-inflammatory [1, 2], anti-bacterial, anti-fungal [3-7], anti- HIV, antihistaminic [8, 9], anti allergic [10], antitumor, anticancer [11], MOA inhibitory [12] and central nervous system activities [13]. 4(3H)-quinazolinones its 3 substitution has been reported to be associated with antimicrobial properties [14-16]. The 3 substitution which were reported are various substituted phenyl ring moieties [17], bridged phenyl rings [18], heterocyclic rings [19-21] and aliphatic systems. 2, 3-substituted-4(3H)-quinazolinones were reported to possess antimicrobial properties. The antimicrobial, antifungal and anti HIV screening of semi-derivatized conventional antibacterial agents namely trimethoprim [22, 23], sulphadoxime, norfloxacin, ciprofloxacin and lomefloxacin [24] have been reported.

These observation lead to the conception that a new series of 2-aryl-4(3H)-quinazolinones with 3,5-diaminotriazine (lamotrigine) substituent in 3rd position would exhibit potential cytoprotective and antiviral activity.Trizines selected for the study posses dihydrofolate reductase inhibitory property.in continuation of earlier work 4(3H) quinazolinones the present deals with synthesis of a series of 2,3-disubstituted-4(3H)-quinozolinones by condensation of 2-aryl benzoxazin-4-one with lamotrigine.

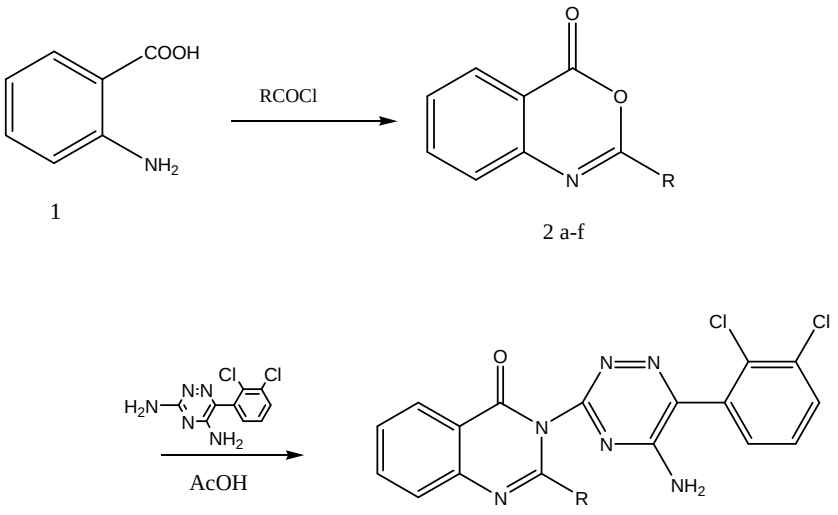
MATERIALS AND METHODS

General methods

The melting points were taken in open capillary tube on a Thomas Hoover melting point apparatus and are uncorrected. The IR spectra of the compounds were recorded on Bruker vector-22 FT-IR with KBr pellets .^{1}H –NMR spectra recorded on 500MHz Bruker AMX 500 using DMSO-d_6 as solvent. The chemical shifts are reported as parts per million downfield from tetramethylsilane (Me_4Si). Mass spectra were recorded on Varian atlas CH-7. Microanalysis for C, H, and N were performed in Heraes CHN Rapid analyzer. All the compounds gave satisfactory chemical analyses (±0.4%). The purity of the compounds were checked by TLC on SiO_2 gel ($HF_{254,}$ 200 mesh) coated glass plates using (4 : 1) CH_3OH : $CHCl_3$ as mobile phase and visualized by iodine vapours. All chemicals and reagents used in the synthesis were obtained from Sigma (Sigma-Aldrich, St. Louis MO), Spectrochem Pvt. Ltd. (Mumbai, India) and were used without further purification. The starting material, 2-aryl-3, 1-benzoxazin-4(3*H*)-ones were synthesized using known procedures [25, 26].

The synthesized compounds were characterized by their physical and spectral data and are given below. The *in vitro* antibacterial Studies of novel 3-(5-amino-6(2, 3-dichlorophenyl)-1, 2, 4-triazin-3-yl)-2-*aryl* quinazoline-4(3H)-ones is given in Table 1.

SCHEME



1

2 a-f

3 a-f

R = a, 4-CH3-C6H4 , b, 2-OCH3- C6H4, c, 3-OCH3- C6H4, d, 4-OCH3- C6H4, e, 4-OH- C6H4, f, 4-NO2- C6H4

Chemistry

Synthesis of 2-Aryl-3, 1-benzoxazines

To a solution of anthranillic acid (0.1 mol) in 50ml of dry pyridine,corresponding substituted benzoyl chlorides(0.2 mol)was added dropwise with constant stirring at 15^{o}C. The reaction mixture was cooled to 5^{o}C and aqueous sodium carbonate (15ml, 10% w/v) was added. The product formed was filtered, vacuum dried and recrystallised using absolute ethanol.

2-*p*-tolyl-4*H*-benzo[*d*][1,3]oxazin-4-one (2a): Yield=76%, mp 119-120^{o}C. IR (KBr) cm^{-1} : 1650 (C=O), 1154 (C-O-C), 1525 (C=N), 819, 744, 736 (Ar-H). ^{1}H-NMR (DMSO-d_6) δ: 2.3 (s, 3H, CH_3); 4.98 (s, 2H, $COCH_2$); 7.50-8.15 (m, 8H, Ar-H). MS m/z: 237. *Anal* calcd for $C_{15}H_{11}NO_2$: C, 75.94; H, 4.67; N, 5.90. Found: C, 75.96; H, 4.70; N, 5.93.

2-(2-methoxyphenyl)-4*H*-benzo[*d*][1,3]oxazin-4-one(2b): Yield=87%, mp 150-152^{o}C. IR (KBr) cm^{-1} :1644 (C=O), 1120 (C-O-C), 1526 (C=N), 824, 761, 728 (Ar-H). ^{1}H-NMR (DMSO-d_6) δ: 3.7 (s, 3H, OCH_3); 4.95 (s, 2H, $COCH_2$); 7.62-8.23 (m, 8H, Ar-H). MS m/z: 253. *Anal* calcd for $C_{15}H_{11}NO_3$: C, 71.14; H, 4.38; N, 5.53. Found: C, 71.10; H, 4.43; N, 5.57.

2-(3-methoxyphenyl)-4*H*-benzo[*d*][1,3]oxazin-4-one (2c): Yield=85%, mp 160-161^{o}C. IR (KBr) cm^{-1} :1639 (C=O), 1132 (C-O-C), 1523 (C=N), 847, 736, 719 (Ar-H). ^{1}H-NMR (DMSO-d_6) δ: 3.5 (s, 3H, OCH_3); 5.02 (s, 2H, $COCH_2$); 7.67-8.25 (m, 8H, Ar-H). MS m/z: 253. *Anal* calcd for $C_{15}H_{11}NO_3$: C, 71.14; H, 4.38; N, 5.53. Found: C, 71.21; H, 4.34; N, 5.45.

2-(4-methoxyphenyl)-4*H*-benzo[*d*][1,3]oxazin-4-one (2d): Yield=85%, mp 174-176^{o}C. IR (KBr) cm^{-1} :1639 (C=O), 1021 (C-O-C), 1527 (C=N), 832, 776, 749 (Ar-H). ^{1}H-NMR (DMSO-d_6) δ: 3.8 (s, 3H, OCH_3); 5.15 (s, 2H, $COCH_2$); 7.70-8.21 (m, 8H, Ar-H). MS m/z: 253. *Anal* calcd for $C_{15}H_{11}NO_3$: C, 71.14; H, 4.38; N, 5.53. Found: C, 71.18; H, 4.29 ; N, 5.42.

2-(4-hydroxyphenyl)-4*H*-benzo[*d*][1,3]oxazin-4-one (2e): Yield=83%, mp 168-169^{o}C. IR (KBr) cm^{-1} :1625 (C=O), 1098 (C-O-C), 1517 (C=N), 852, 787, 714 (Ar-H). ^{1}H-NMR (DMSO-d_6) δ: 4.9 (Ar-OH); 5.23 (s, 2H, $COCH_2$); 7.98-8.19 (m, 8H, Ar-H). MS m/z: 239. *Anal* calcd for $C_{14}H_9NO_3$: C, 70.29; H, 3.79; N, 5.86. Found: C, 70.24; H, 3.67; N, 5.82.

2-(4-nitrophenyl)-4*H*-benzo[*d*][1,3]oxazin-4-one (2f): Yield=78%, mp 214-215^{o}C. IR (KBr) cm^{-1} :1629 (C=O), 1145 (C-O-C), 1573 (C=N), 866, 757, 739 (Ar-H). ^{1}H-NMR (DMSO-d_6) δ: 5.30 (s, 2H, $COCH_2$); 7.72-8.31 (m, 8H, Ar-H). MS m/z: 268. *Anal* calcd for $C_{14}H_8N_2O_4$: C, 62.69; H, 3.01; N, 10.44. Found: C, 62.53; H, 3.03; N, 10.48.

General procedure for 3-(5-amino-6(2, 3-dichlorophenyl)-1, 2, 4-triazin-3-yl)-2-*aryl* quinazoline-4(3H)-one:

Equimolar quantities (0.01mol) of 2-aryl-3,1-benzaxozin-4-one and the primary amine (lamotrigine) in glacial acetic acid (10ml) was refluxed for 6h.The reaction content as cooled to room temperature and poured into crushed ice. The product formed was filtered, vacuum dried and recrystallized using absolute ethanol.

3-(5-amino-6(2,3-dichlorophenyl)-1,2,4-triazin-3-yl)-2-*p*-tolylquinazoline-4(3H)-one (3a) : yield=86%, mp 210-211^{o}C. IR (KBr) cm^{-1}; 3455 (NH_2), 1681(C=O), 1645 (C=N), 1438 (N=N),

830, 792, 737 (Ar-H). ^{1}H-NMR (DMSO-d_6) δ ; 8.52 (s,1H;5- H), 8.14 (d, *J*=6Hz, 1H; 8-H), 7.71(d, *J* =6Hz,1H;7-H),7.09-7.48 (m, Ar-H), 3.56 (s,2H;NH_2),2.15 (s,3H; CH_3). EI-MS *m/z:* 474.0763 (calcd for $C_{24}H_{16}Cl_2N_6O$:475.32). *Anal* calcd for $C_{24}H_{16}Cl_2N_6O$: C, 60.64; H, 3.39; N, 17.68. Found: C, 60.46; H, 3.04; N, 17.74.

3-(5-amino-6(2,3-dichlorophenyl)-1,2,4-triazin-3-yl)-2-(2-methoxyphenyl) quinazoline-4(3H)-one(3b): yield=82%, mp 240-241ºC.^{1}H-NMR (DMSO-d_6) δ; 8.82 (s,1H; 5-H), 8.36(d,*J*=7.1Hz,1H;8-H), 8.08(d,*J*=7.1Hz,1H;7-H),6.85-7.51(m, Ar-H), 3.42 (s,2H;NH_2), 3.68 (s,3H;CH_3O). IR (KBr) cm^{-1}; 3393(NH_2), 1679(C=O), 1611(C=N), 1435(N=N), 827,799,781(Ar-H), EI-MS *m/z:* 490.07 (calcd for $C_{24}H_{16}Cl_2N_6O_2$:491.32). *Anal* calcd for $C_{24}H_{16}Cl_2N_6O_2$: C, 58.67; H, 3.28; N, 17.10. Found: C, 58.45; H, 3.24; N, 17.14.

3-(5-amino-6(2,3-dichlorophenyl)-1,2,4-triazin-3-yl)-2-(3-methoxyphenyl) quinazoline -4 (3H)-one(3c): yield=83%, mp270-271ºC. IR (KBr) cm^{-1}; 3363(NH_2), 1657(C=O), 1605(C=N), 1425(N=N), 816,785,772 (Ar-H). ^{1}H-NMR (DMSO-d_6) δ; 8.42 (s,1H;5-H), 8.04 (d,*J*=6.8Hz,1H;8-H), 7.78 (d,*J*=6.8Hz,1H;7-H), 6.74-7.26(m, Ar-H), 3.83(s,2H;NH_2) 3.73(s,3H;CH_3O). EI-MS *m/z:* 490.07 (calcd for $C_{24}H_{16}Cl_2N_6O_2$:491.32). *Anal* calcd for $C_{24}H_{16}Cl_2N_6O_2$: C, 58.67; H, 3.28; N, 17.10. Found: C, 58.71; H, 3.30; N, 17.12.

3-(5-amino-6(2,3-dichlorophenyl)-1,2,4-triazin-3-yl)-2-(4-methoxyphenyl) quinazoline-4(3H)-one(3d): yield=79%, mp 192-193ºC. IR (KBr) cm^{-1}; 3343(NH_2), 1634(C=O), 1623 (C=N), 1417 (N=N), 811,783,769 (Ar-H). ^{1}H-NMR (DMSO-d_6) δ; 8.32 (s, 1H; 5-H), 7.94 (d, *J*=7.0Hz, 1H; 8-H), 8.06 (d, *J* =7.0Hz,1H;7-H), 6.96-7.69(m, Ar-H), 3.9 (s, 2H; NH_2) , 3.63(s,3H; CH_3O). EI-MS *m/z:* 490.07 (calcd for $C_{24}H_{16}Cl_2N_6O_2$: 491.32). *Anal* calcd for $C_{24}H_{16}Cl_2N_6O_2$: C, 58.67; H, 3.28; N, 17.10. Found: C, 58.65; H, 3.25; N, 17.02.

3-(5-amino-6(2,3-dichlorophenyl)-1,2,4-triazin-3-yl)-2-(4-hydroxyphenyl) quinazoline-4(3H)-one(3e): yield=82%,mp 296-297ºC. IR (KBr) cm^{-1}; 3378 (NH_2), 1672 (C=O), 1635(C=N), 1420(N=N), 825, 743,765 (Ar-H). ^{1}H-NMR (DMSO-d_6) δ ; 8.72 (s,1H; 5-H), 7.54 (d,*J*=5.9Hz,1H;8-H), 7.76(d,*J*=5.9Hz,1H;7-H), 6.76-7.45 (m, Ar-H), 3.89 (s,2H;NH_2). 4.9 (Ar-OH), EI-MS *m/z:* 476.05 (calcd for $C_{23}H_{14}Cl_2N_6O_2$:477.30). *Anal* calcd for $C_{23}H_{14}Cl_2N_6O_2$: C, 57.88; H, 2.96; N, 17.61. Found: C, 57.57; H, 2.74; N, 17.53.

3-(5-amino-6(2,3-dichlorophenyl)-1,2,4-triazin-3-yl)-2-(4-nitrophenyl)quinazoline-4(3H)-one(3f): yield=84%,mp 235-236ºC. IR (KBr) cm^{-1} ; 3385(NH_2), 1668(C=O), 1623(C=N), 1440(N=N), 831,785,779 (Ar-H). ^{1}H-NMR (DMSO-d_6) δ; 8.72 (s, 1H;5-H), 8.34 (d, *J*=6.9Hz,1H; 8-H), 7.89 (d,*J*=7.1Hz,1H;7-H), 7.14-8.26 (m, Ar-H), 3.39 (s, 2H; NH_2).EI-MS *m/z:* 505.04 (calcd for $C_{23}H_{13}Cl_2N_7O_3$: 506.30). *Anal* calcd for $C_{23}H_{13}Cl_2N_7O_3$: C, 54.56; H, 2.59; N, 19.37. Found: C, 54.48; H, 2.43; N, 19.41.

Biological evaluation

***In vitro* antibacterial activity**

Synthesized compounds were evaluated for their *in-vitro* antibacterial activity against pathogenic bacteria by the agar dilution method. MIC values were considered to be the lowest concentration that was completely inhibited growth on agar plates. Sulphamethoxazole was used as the standard in all antibacterial screening studies

RESULTS AND DISCUSSION

Anthranillic acid on reaction with 4-methyl benzoyl chloride in sodium hydroxide followed by cyclization with acetic anhydride gave a crystalline compound in high yield. The mass spectrum of the compound revealed the molecular ion at m/z 237 indicating it to be an equimolar product formed by the elimination of elements of hydrogen chloride and water. The IR spectrum showed the absence of NH and presence of a δ-lactogenic carbonyl at1650 cm^{-1}. The ^1H NMR spectrum shows (DMSO-d_6) exhibited singlet at δ: 2.3 accounting methyl protons, multiplet 7.50-8.14 for eight aromatic protons, and the compound has been characterized as of **2a** on the basis of spectral and analytical data. The reaction was extended to simple 2-methoxy, 3-methoxy 4-methoxy, 4-hydroxy, 4-nitro benzoyl chlorides and the corresponding 2-aryl-4*H*-benzo[*d*][1,3]oxazin-4-ones were obtained in good yield characterized on the basis of spectral and analytical data. The mass spectra of compounds **2a** exhibited intense molecular ions. The mass spectral fragmentation is characterized by the presence of ions corresponding to the loss of C_2-substituent and CO_2 in addition, fragment ions corresponding to m/z values of Ar+ and Arco+ have also been observed.

The lactone ring of compounds **2a-f** is expected to be reactive and susceptible to nucleophilic attack involving replacement of oxygen in the ring. To ascertain this reaction of **2a** with 3,5-diaminotriazine (lamotrigine) in acetic acid as carried out. The reaction yielded a crystalline compound, identifies as 3-(5-amino-6(2, 3-dichlorophenyl)-1, 2, 4 -triazin-3-yl)-2-*p*-tolylquinazoline-4(3H)-one **(3a)**. The mass spectrum of the compound showed an intense molecular ion at m/z 474 reflecting the stability of the molecule under electron impact. The IR spectrum of the product revealed a carbonyl absorption at 1670.The ^1H NMR spectrum revealed resonance at 2.5 (s,3H,CH3 of 2-substituent),7.3-8.0(m, Ar-H). Likewise the reactions of all other 2b-f with aromatic primary amines in acetic acid were carried out and in each case the corresponding 3b-f were isolated. Thus the above reaction constitutes a facile one step synthesis of a variety of 3-(5-amino-6(2, 3-dichlorophenyl)-1, 2, 4-triazin-3-yl)-2-*aryl* quinazoline-4(3H)-ones under relatively mild conditions. The formation of title compound involves the nucleophilic attack by primary amine nitrogen on carbonyl carbon followed by cleavage of carbon oxygen bond resulting in an open chain intermediate which subsequently undergoes dehydrative cyclization.

In vitro antibacterial activity

Quinazolin-4(3H)-ones and 1,2,4-triazines heterocyclic entities are very interesting components in terms of their biological properties, such as anti fungal , antibacterial and herbicidal. Synthesized compounds **3a-3f** were tested against a panel of microorganisms including Gram-positive bacteria (*Staphylococcus aureus*) and Gram-negative bacteria *(Escherichia coli ATCC-25922, Escherichia coli ATCC-35218, Morganella morganii, Prot.mirabilis, Providencia rettgeri, Salmonella paratyphi, Shigella sonnei, Shigella boyelli, Vibrio cholerae, Prot.vulgaris, E.faecalis ATCC-2921, Pseudo.aeruginosa ATCC-27853, Shigella flexneri, Klebsiella oxytoca, Kleb. pneumoniae, Salmonella enteritidis, Salmonella typhi MTCC 2316, H. pylori, Salmonella typhi*,) was using conventional agar-dilution method.he MIC values for these compounds (3a-3f) were determined by comparison to sulphamethoxazole as reference drug.

Sensitivity testing was performed for all compounds. This showed that synthetic compounds were sensitive against all bacteria.

Compounds **3a-3f** was not active against *Shigella boyedli, Proteus vulgaris, Salmonella typhi MTCC 3216, Salmonella enteritidis, Kleb. pneumoniae.* Further some compounds were not active against specific bacteria like **3a** (*Shigella sonnei),* **3a, 3b and 3f,** (*Providencia rettgeri),***3a, 3d and 3f,** (*E.coli ATCC35218),* **3a, 3b, 3c and 3f** (*Proteus mirabilis),* **3c and 3e** (*Morganella morganii),* **3a** *(Salmonella paratyphi),* **3a, 3b, 3c and 3e,** (*Vibrio cholerae*), **3a, 3b and 3e,** (*Pseudo.aeruginosa ATCC27853),* **3b** (*Shigella flexinerii),* **3b, 3c, 3d, 3e and 3f** (*Klebsiella oxytoca),* **3b and 3e** (*H. Pylori),* **3a, 3b and 3d (***Salmonella typhi),* **3a** (*Staphylococcus aureus).*

The MIC values of synthesized compounds were tested against organism displayed a significant activity with wide degree of variation (**Table I**). On gram positive bacteria, Compounds **3a-3f** were found to be 12.5 to 25 times more active than standard drug. On gram negative bacteria, Compounds **3a-3f** were found to be 2.5 to 100 times more active than standard drug.

Table-1: *In vitro* antibacterial activities of compounds 3a-f against selected strains (MICs in µg /mL)

S.No.	Name of bacteria	3a	3b	3c	3d	3e	3f	Sulph.
1	*Staphylococcus aureus*	300	---	200	300	200	400	4000
2	*E.coli* ATCC25922	50	400	300	300	150	400	1200
3	*E.coli* ATCC35218	---	500	200	---	400	---	1200
4	*E.faecalis* ATCC29212	50	500	400	200	300	250	5000
5	*H. pylori*	50	---	100	400	---	25	2000
6	*Klebsiella oxytoca*	150	---	---	---	---	---	5000
7	*Kleb. Pneumoniae*	---	---	---	---	---	---	2000
8	*Morganella morganii*	300	500	---	300	---	500	2000
9	*Proteus vulgaris*	---	---	---	---	---	---	2000
10	*P..aeruginosa* ATCC27853	---	---	300	500	---	250	5000
11	*Providencia rettgeri*	---	---	400	500	250	---	2000
12	*Proteus mirabilis*	---	---	---	500	300	---	2000
13	*Shigella sonnei*	---	500	500	400	300	200	2000
14	*Shigella boyedli*	---	---	---	---	---	---	2000
15	*Salmonella paratyphi*	---	500	300	400	300	200	2000
16	*Shigella flexinerii*	50	---	250	400	200	500	2000
17	*Salmonella enteritidis*	---	---	---	---	---	---	2000
18	*S. typhi* MTCC 3216	---	---	---	---	---	---	2000
19	*Salmonella typhi*	---	---	300	---	300	500	2500
20	*Vibrio cholera*	---	---	---	300	---	250	4000

Sulphamethoxazole as standard drug; ---=Insensitive (inactive)

Compound **3a** exhibited significant activity against *E.coli* ATCC 25922, *H. pylori* and *Shigella flexinerii* with MIC value 50µg/mL. Other then these bacteria, compound **3a** showed moderate activity. Compounds **3f** showed greater activity against *H. pylori* with MIC value 25µ

g/mL. Other then this bacteria, this compound was showed moderate activity. Synthesized compounds **3a, 3b, 3c, 3d, 3e** and **3f** exhibited moderate activity against all bacteria.

Nitro group containing compounds (NO_2) (**3f**) was found to be active against some bacteria than other group containing compounds. It was found to be 5 to 12.5 times more active than standard drug.

On the basis of MIC values,Synthesized compounds were divided into three parts1-weak active 300-500)- **3c.** 2-Moderate active (50-300µg/ mL) - **3d** & **3e.** 3-Most active (12.5-50µg/mL) - **3a** & **3f.** Antibacterial screening revealed that synthetic compounds exhibited moderate activity as compared to standard.

Acknowledgement
The authors are taking this opportunity with pride and immense pleasure to express heartfelt sincere thanks to the Hon'ble chancellor and vice chancellor PRIST UNIVERSITY Thanjavur.

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