Available online at **www.joac.info**

ISSN: 2278-1862

Journal of Applicable Chemistry

2020, 9 (4): 649-659

(International Peer Reviewed Journal)

Synthesis, Bioassay and Molecular Modelling Studies of Antioxidants in Ischemic Diseases

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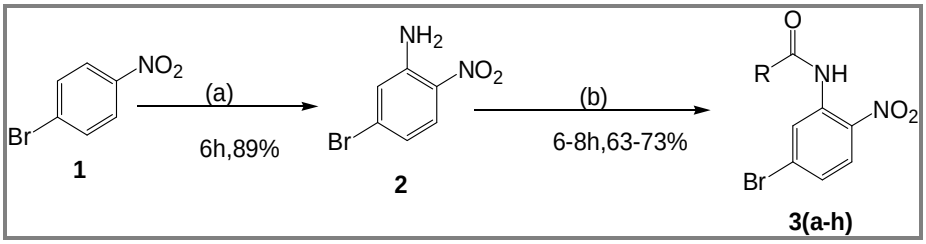
Accepted on 1st June, 2020

ABSTRACT

Ischemia disease is a restriction in blood supply to tissues, causing a shortage of oxygen and glucose needed for cellular metabolism. The synthesis and bioassay of "N, N'–substituted urea derivatives" by incorporating appropriate pharmacophore into the molecular framework by suitable chemical manipulations and subsequently to study their biological activity. Here I choose N- substituted urea derivatives as antioxidants.

Graphical Abstract:



(a) $NH_2OMe.HCl$, t-BuOK, CuCl, DMF,DME.**b) 3(a-h)**=Different Amines, Triphosgene, TEA,DCM.

Synthesis of different N-substituted urea derivatives.

Keywords: Synthesis, Bioassay and Molecular Modelling Studies of Antioxidants, Ischemic Diseases.

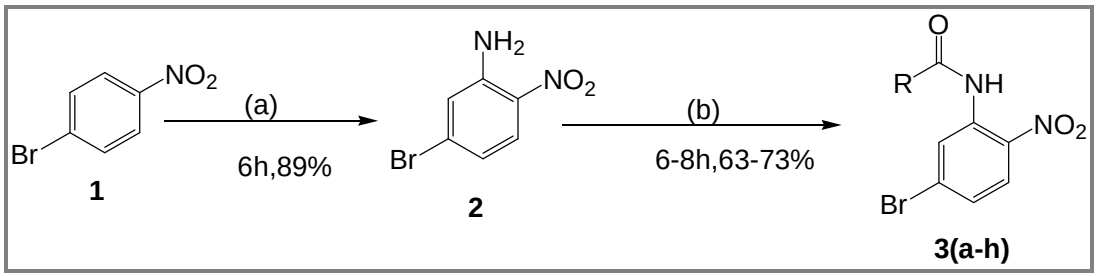
INTRODUCTION

Ischemia is generally caused by problems in blood vessels. This is due to inadequate flow of blood to a part of the body may be caused by any of the following-for example atherosclerosis, hypoglycaemia etc. The consequences of Ischemia disease are irreversible damage to heart and brain, failure of kidneys, lack of blood flow to limb etc. The scheme of Ischemia disease briefly explained as follows. Oxidative stress → Free radical release→ Damage to vessel endothelium→ Damage endothelium causes roughness in the vessel lumen → Rough surface triggers clotting mechanism →Clot formation → Binding of lipid and calcium molecules → Reduced lumen width → Ischemia. Literature survey revealed the diversified biological significance of urea and N- Substituted urea derivatives. This aspect

has been drawing the attention of many researchers towards exploiting the biological importance of various urea derivative compounds and establishing the features [1-44].

MATERIALS AND METHODS

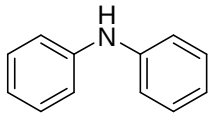
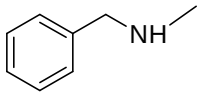
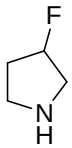
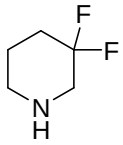
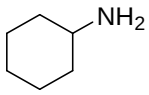
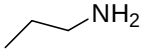
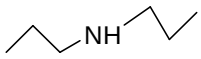
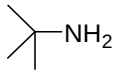
Present Study: Here N-substituted Urea analogues **(3a-h)** were synthesized from compound **(3)** (2-nitro benzenamines) by nucleophilic addition with different heterocyclic amines in presence of triphosgene.



Scheme 1. Synthesis of different N-substituted urea derivatives.

Reagents and Condition: (a) $NH_2OMe.HCl$, t-BuOK, CuCl, DMF,DME.
b) 3(a-h)=Different Amines, Triphosgene, TEA, DCM.

Table 1. List of Amines

S.No	Name of the amine	Structure
1	Diphenyl amine	
2	N-methyl(phenyl)menthanamine	
3	3-fluoropyrrolidine	
4	3,3-difluoropiperidine	
5	Cyclohexanamine	
6	Propan-1-amine	
7	Dipropylamine	
8	2-methylpropan-2-amine	

Important chemicals used: 1-bromo-4-nitrobenzene, Potassium tertiary butoxide ,Dimethyl formamide, Cuprous chloride ,$NH_2OMe.HCl$ Triethylamine, Dichloromethane, Triphosgene, Acetone,

Methanol, Ethanol, Chloroform, Di Methyl Formamide (DMF), Ethyl acetate, Hexane (Petrolium ether), Sodium sulphate .

Methodology

Step 1: preparation of -5-bromo, 2-nitro aniline (2): To the mixture of t-BuOK (0.70 mol, 5 eq) and CuCl (0.014 mol, 0.1 eq) DME (600 mL) was added and stirred it at 0°C under nitrogen atmosphere. A mixture of 1-bromo-4-nitrobenzene **1** (0.14 mol, 1 eq) and $NH_2OMe.HCl$ (0.22 mol, 1.6 eq) was added to DMF (300 mL). This DMF solution was added to the above reaction mixture in a drop wise over 30 min at 0°C. After completion of the addition, the cooling bath was removed and stirred the reaction mixture at room temperature for 6 h. After completion of the reaction, reaction mixture was poured into ice cold water, yellow coloured solid was obtained. Filtered the solid and washed with water and then dried over vacuum. Desired compound **2** was obtained as pure yellow colour solid. ^{1}H-NMR-(400MHz) in DMSO-d6**:** δ 7.90 (t, 1H); 7.39 (t, 1H); 7.00 (s, 1H); 6.30(S, 2H). MS m/z 218 (M+H) +Ve Scan.Yield**:**89%.

Table. 2. Physical Data of Compound-2

Yield	86-89%
Melting Point	173-175°C
Appearance	Yellow colour solid
Molecular formula	$C_6H_5BrN_2O_2$
Molecular weight	218

Step 2: Preparation of Urea derivatives (3a-h): By using nucleophilic addition reaction with different heterocyclic amines in presence of triphosgene several N-substituted urea analogues (3a-h) are synthesized from 5-bromo-2-nitro aniline (2) compound. At 0°C (0.48 mml, 1.0eq) 5-bromo-2-nitro aniline is dissolved in DCM. In this cold condition, this mixture is added to (6.24 mml. 1.3eq) TEA and (0.48 mml. 1.0eq) triphosgene. This whole mixture is then stirred for 2 h at room temperature. Next at 0°C (0.72 mml, 1.5eq) of different amines were added, and then this reaction mixture is stirred for 5h at room temperature. The resultant mass is diluted with water and extracted with DCM. The organic layer is washed with water, brine and dried over Na_2SO_4, crude residue is obtained by filtering and concentrating the above yield. By using column chromatography by eluting with 30% EtOAC in Hexane the crude residue is purified. Desired compound (3a-h) obtained as solids yield 68-75 %.

Structural confirmation: The purpose of Spectral analysis is to confirm the chemical structures of the synthesized compounds and the various functional groups in the final compounds. The IR spectra of starting compound and intermediates were taken to confirm the changes at the reactive functional groups and then the final compounds were confirmed by IR, ^{1}H-NMR, ^{13}C-NMR and Mass spectroscopy.

Table 3. Physical data of Compounds 3(a-h)

Compound	Yield	M.P.	Appearance	Molecular Formula	Molecular Weight	R_f Value
3a	70-75%	128-130^{0}C	Yellow powder	$C_{19}H_{14}BrN_3O_4$	412.24	0.53
3b	70-72%	130-132^{0}C	Yellow powder	$C_{24}H_{28}BrN_3O_4$	502.4	0.53
3c	70-72%	130-132^{0}C	Yellow powder	$C_{11}H_{11}FBrN_3O_3$	332.13	0.53
3d	70-72%	130-134^{0}C	Yellow powder	$C_{12}H_{12}BrF_2N_3O_3$	364.14	0.51
3e	70-73%	130-136^{0}C	Yellow powder	$C_{13}H_{16}BrN_3O_3$	342.19	0.50
3f	68-70%	130-133^{0}C	Yellow powder	$C_{10}H_{12}BrN_3O_3$	302.12	0.54
3g	68-71%	130-134^{0}C	Yellow powder	$C_{13}H_{18}BrN_3O_3$	344.2	0.57
3h	68-71%	130-133^{0}C	Yellow powder	$C_{11}H_{14}BrN_3O_3$	344.2	0.56

Infrared spectroscopy: Infrared spectra were recorded on Perkin Elmer Model 283B and Nicolet 740 FT-IR instruments and absorption frequency values are given in cm^{-1}.

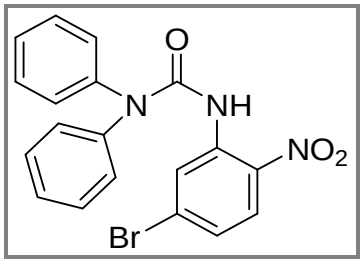
Nuclear Magnetic Resonance (^{1}H-NMR and ^{13}C-NMR): Nuclear magnetic resonance spectra were recorded on Avance Bruker 300MHz instrument. The samples were made in $CDCl_3$-solvent using tetramethylsilane (TMS) as the internal standard and are given in the δ-scale (ppm). The standard abbreviations s, d, t, q and m refer to singlet, doublet, triplet, quartet and multiplet respectively.

Mass spectroscopy: Mass spectrum is recorded on VG Micromass 7070H (EI-MS) and was given in mass units (m/z).

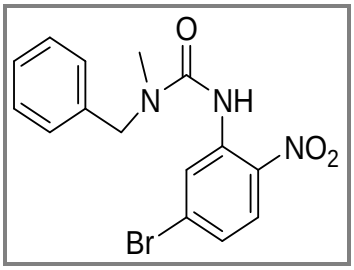
RESULTS AND DISCUSSION

Spectral data of synthesized compounds:

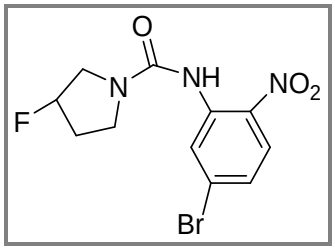
Spectral data of 3-(5-bromo-2-nitrophenyl)-1, 1-diphenyl urea (3a): IR (cm^{-1}): 1698(C=O), 3380(N-H). **^{1}H-NMR-(400MHz)) in $CDCl_3$-d6: δ_H**9.45(s,1H, D_2O Exchangeable), 8.26(s,1H), 8.80-7.99(d,1H), 7.26-7.23(dd,1H), 6.40(s,1H), 5.46-5.33(d,1H), 3.75-3.49(m,4H), 2.35(s,2H), 2.23-2.25(d,4H), 1.75-1.72(t,2H),1.62-1.59(t,2H), **^{13}C-NMR in $CDCl_3$: δ_C**δ152.47, 148.04, 135.92, 135.37, 134.52, 129.06, 125.30, 118.13, 117.14, 91.83, 52.40, 43.38, 31.67, 26.27, 25.53, 22.26, 21.33. **MS:** m/z 412.24 $(M+H)^+$.



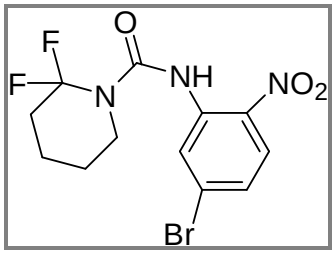
2-benzyl-3-(5-bromo-2-nitrophenyl)-1-methyl urea (3b): IR (cm^{-1}): 1680(C=O), 3345(N-H). **^{1}H-NMR-(400MHz)) in $CDCl_3$: δ_H** 9.39(s,1H), 7.93-7.91 (d,1H), 7.85-7.84(d,1H), 7.27-7.24(dd,1H), 6.38(s,1H), 3.63-3.61(t,4H), 3.45-3.43(t,4H), 2.35(s,2H), 2.22 (s,2H),1.75-1.72(t,2H), 1.62-1.59(t,2H). **^{13}C-NMRinCDCl$_3$: δ_C** 16.6, 50.8, 112.6, 116.3, 117.2, 122.2, 126.1, 126.5, 127.0, 127.1, 128.3, 130.0, 142.4, 144.2, 148.8, 163.0, 170.0
Mass Spectrum: MS: m/z 502.4(M^++H,100%).



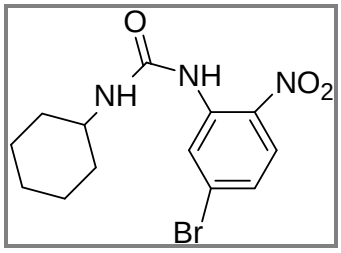
(5-bromo-2-nitrophenyl)-3-fluoropyrrolidine-1-carboxamide (3c): IR (cm^{-1}): 1672(C=O), 3323(N-H). **^{1}H-NMR-(400MHz)) in $CDCl_3$: δ_H** 9.37(s,1H, D_2O Exchangeable), 7.92-7.87(m,2H),7.23-7.21(dd,1H), 6.38-6.36(t,1H),3.44-3.41(t,4H),2.35-2.34(d,2H), 2.22-2.21 (d,2H), 1.76-1.70(m,2H), 1.60-1.59(m,4H), 1.53-1.52(d,4H). **^{13}C-NMR in $CDCl_3$ (400MHz): δ_C** δ163.73, 153.54, 147.47, 136.89, 135.83, 134.53, 128.58, 125.11, 118.28, 118.21, 47.51, 26.328, 25.43, 25.27, 23.94, 22.28, 21.35. **MS:** m/z 332.13(M^++H, 100%).



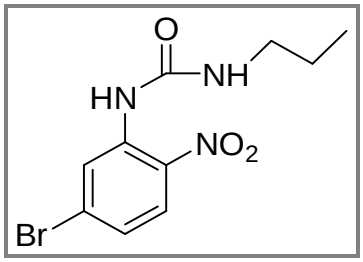
N-(5-bromo-2-nitrophenyl)-2,2-difluoropiperidine-1-carboxamide(3d): IR (cm^{-1}): 1682(C=O), 3338(N-H). **^{1}H NMR (300MHz, CDCl$_3$): δ_H** 9.54(s,1H,D_2O Exchangeable), 8.42(d,1H), 8.03-8.01(d,1H), 7.22-7.19(dd,1H), 6.41-6.39(t,1H), 3.41(s,4H),2.34(d,2H), 2.23-2.22(d,2H), 1.90(s,4H), 1.76-1.70(m,2H), 1.64-1.58(m,2H). **^{13}CNMR in CDCl$_3$ (400MHz): δ_C**: δ152.21, 148.24, 136.59, 134.57, 134.38, 129.11, 125.32, 117.69, 116.47, 45.44, 26.26, 25.55, 24.94, 22.26, 21.33. **MS:** 364.14 (M^++H, 100%).



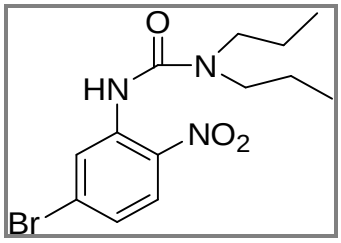
1-(5-bromo-2-nitrophenyl)-3-cyclohexylurea (3e): IR (cm^{-1}1683(C=O),3339(N-H). **^{1}H NMR (300MHz, CDCl$_3$): δ_H** 9.41(s,1H, D_2O Exchangeable), 8.25-24(d,1H), 7.99-7.97(d,1H), 7.22-7.20(dd,1H), 6.39-6.37(t,1H), 3.60-3.58(d,2H), 3.48(bs,2H), 2.34-2.33(d,2H), 2.23-2.21(t,2H), 1.76-1.70(m,2H), 1.63-1.57(m,4H), 0.76-0.71(m,1H).0.19-0.16 (m,1H). **^{13}CNMR in CDCl$_3$(400MHz): δ_C**: δ153.09, 148.03, 136.02, 135.27, 134.54, 129.03, 125.30, 118.03, 117.08, 42.97, 26.27, 25.52, 22.26, 21.33, 15.37, 9.37. **MS:** 342.19(M^++H, 100%).



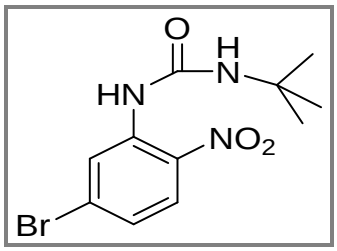
1-(5-bromo-2-nitrophenyl)-3-propylurea (3f): IR (cm^{-1}1683(C=O),3347(N-H). **^{1}H NMR (300MHz, CDCl$_3$):** 9.37(s,1H,D_2O Exchangeable),7.92-7.90(d,1H), 7.76(d,1H), 7.28-7.25(dd,1H),6.37(s,1H), 3.81-3.76(t,2H), 3.53-3.50(t,2H), 2.35(s,2H), 2.22-2.21(d,2H), 2.13-2.03(m,2H), 1.76-1.70(m,4H), 1.63-1.59(m,2H). **^{1}H NMR (300MHz, CDCl$_3$):** δ153.81, 147.30, 138.15, 134.76, 134.45, 128.67, 125.15, 120.18, 119.28, 119.06, 48.60, 42.98, 31.72, 26.34, 25.49, 22.28, 21.35.



3-(5-bromo-2-nitrophenyl)-1,1-dipropylurea(3g): IR (cm^{-1}): 1677(C=O), 3375(N-H). **^{1}H NMR (300MHz, $CDCl_3$):** 9.44(s,1H,D_2O Exchangeable), 7.90-7.88(d,1H), 7.67(s,1H), 7.28-7.26(d,1H), 6.37(s,1H), 3.58-3.56(t,4H), 2.35(s,2H), 2.22(s,2H), 2.05-1.98(m,4H), 1.75-1.72(t,2H), 1.62-1.59(t,2H). **^{1}H NMR (300MHz, $CDCl_3$):** δ154.09, 147.17, 138.61, 134.71, 134.45, 128.56, 125.06, 122.65, 119.46, 119.0941.20, 33.51, 26.35, 25.48, 22.28, 21.35. **MS:** m/z 344.2(M^++H, 100%).



1-tert-butyl-3-(5-bromo-2-nitrophenyl) urea (3h): IR (cm^{-1}): 1694(C=O), 3369(N-H). **^{1}H NMR (300MHz, $CDCl_3$):** 9.65(s,1H),8.50-8.49(d,1H), 8.37(s,1H), 8.06-8.03(d,2H), 7.86-7.84(d,1H), 7.37-7.33(dd,1H), 7.29-7.26(dd,1H), 6.42(s,1H), 4.81(bs,4H), 2.37(s,2H), 2.24-2.22(d,2H), 1.76-1.73(t,2H), 1.63-1.60(d,2H). **MS:** m/z 316.15 (M^++H, 100%).



Molecular docking studies:

Table 4. GOLD Score of Antibacterial activity of N, N^1-aryl substituted urea derivatives (3a-h)

Compound	Fitness	S (hb_ext)	S (vdw_ext)	S (hb_ext)	S (vdw_ext)
3a	40.44	6.00	30.70	0.00	-7.76
3b	40.25	6.00	29.62	0.00	-6.47
3c	48.30	6.00	43.08	0.00	-16.94
3d	53.31	6.00	41.65	0.00	-9.96
3e	41.74	6.00	32.94	0.00	-9.55
3f	38.45	6.00	30.36	0.00	-9.29
3g	42.06	6.00	31.94	0.00	-7.86
3h	40.67	5.81	34.57	0.00	-12.68

*Goldfitnesss= (Fitness) = S(hb_ext) + 1.3750*S(vdw_ext) + S(hb_int) + 1.0000*S(hb_ext)

Table 5. Chemscore of Antibacterial activity N, N^1-aryl substituted urea derivatives (3a-h)

Compound	Score	DG	S(hbond)	S(meta)	S(lipo)	DE(clash)	DE(int)
3a	26.56	-28.39	2.00	0.00	160.67	0.09	1.74
3b	25.59	-27.18	1.00	0.00	178.76	0.10	1.49
3c	30.40	-35.87	0.92	0.00	255.23	0.12	5.35
3d	32.49	-36.09	2.87	0.00	215.65	0.79	2.81
3e	27.29	-29.51	1.92	0.00	181.86	0.15	2.07
3f	25.61	-28.04	2.75	0.00	152.40	0.35	2.08
3g	27.83	-30.62	2.74	0.00	179.31	0.48	2.32
3h	24.10	-26.10	1.99	0.00	151.02	0.12	1.89

ChemScore = $\Delta G_{binding}$+ P_{clash}+$C_{internal}P_{internal}$ + ($C_{covalent}P_{covalent}$+$p_{constraint}$)
Score = -(DG + DE(clash) + DE(int))

Table 6. GOLD Score of Antifungal activity of N, N^1-aryl substituted urea derivatives (3a-h)

Compound	Fitness	S (hb_ext)	S (vdw_ext)	S (hb_ext)	S (vdw_ext)
3a	38.36	1.75	32.29	0.00	-7.78
3b	43.35	1.50	41.02	0.00	-14.55
3c	42.27	4.67	31.58	0.00	-5.82
3d	36.33	0.30	34.83	0.00	-11.86
3e	47.83	4.52	36.73	0.00	-7.19
3f	39.03	6.28	33.14	0.00	-12.82
3g	41.31	6.00	31.44	0.00	-7.91
3h	40.31	6.49	30.33	0.00	-7.89

*$Gold_{fitnesss}$= (Fitness) = S(hb_ext) + 1.3750*S(vdw_ext) + S(hb_int) + 1.0000*S(hb_ext)

Table 7. Chemscore of Antifungal activity N, N^1-aryl substituted urea derivatives (3a-h)

Compound	Score	DG	S(hbond)	S(meta)	S(lipo)	DE(clash)	DE(int)
3a	26.35	-30.18	2.51	0.00	170.74	0.56	3.28
3b	27.45	-32.03	2.69	0.00	186.02	2.01	2.57
3c	25.35	-29.51	2.74	0.00	149.21	0.66	3.50
3d	24.83	-29.39	2.64	0.00	150.81	2.12	2.43
3e	29.68	-37.53	1.87	0.00	242.35	0.88	6.96
3f	24.41	-29.00	3.90	0.00	127.85	2.64	1.94
3g	22.91	-28.87	2.67	0.00	166.32	2.70	3.26
3h	25.64	-28.58	2.91	0.00	145.78	1.18	1.75

ChemScore = $\Delta G_{binding}$+ P_{clash}+$C_{internal}P_{internal}$ + ($C_{covalent}P_{covalent}$+$p_{constraint}$)
Score = -(DG + DE(clash) + DE(int))

Table 8. GOLD Score of Antioxidant activity of N, N^1-aryl substituted urea derivatives (3a-h)

Compound	Fitness	S (hb_ext)	S (vdw_ext)	S (hb_ext)	S (vdw_ext)
3a	54.33	15.88	37.00	0.00	-12.43
3b	57.29	15.57	37.57	0.00	-9.94
3c	55.14	2.95	43.96	0.00	-8.26
3d	54.26	15.12	35.59	0.00	-9.80
3e	50.56	4.53	39.02	0.00	-7.61
3f	60.52	1.08	54.79	0.00	-15.89
3g	50.74	14.22	40.90	0.00	-19.73
3h	63.51	3.25	49.15	0.00	-7.31

*$Gold_{fitnesss}$= (Fitness) = S(hb_ext) + 1.3750*S(vdw_ext) + S(hb_int) + 1.0000*S(hb_ext)

Table 9. Chemscore of Antioxidant activity N, N^1-aryl substituted urea derivatives (3a-h)

Compound	Score	DG	S(hbond)	S(meta)	S(lipo)	DE(clash)	DE(int)
3a	27.65	-36.32	3.10	0.00	206.46	5.16	3.51
3b	29.82	-43.23	2.86	0.00	277.04	10.94	2.47
3c	30.86	-34.92	3.60	0.00	170.90	1.23	2.84
3d	26.43	-33.22	2.74	0.00	180.89	5.12	1.67
3e	28.97	-34.12	1.85	0.00	223.19	1.41	3.75
3f	30.17	-34.90	4.92	0.00	148.98	1.66	3.08
3g	29.35	-36.64	3.52	0.00	208.37	3.17	4.11
3h	30.31	-49.02	2.90	0.00	311.18	5.83	12.88

ChemScore = $\Delta G_{binding}$+ P_{clash}+$C_{internal}P_{internal}$ + ($C_{covalent}P_{covalent}$+$p_{constraint}$)
Score = -(DG + DE(clash) + DE(int))

APPLICATION

Biological Activity of Synthesized Compounds:

Antioxidant activity: simple sensitive spectrophotometric method was developed using Urea derivative compounds. The method is based on the reaction of the secondary amine as n-electron donor with the π-acceptor 2, 3, 5, 6-tetra chloro 1, 4-benzo quinone. The coloured charge-transfer complex was measured at **460 nm**. The Free radical generation was confirmed with the

polymerization of acrylonitrile with charge transfer complex as initiator. The procedure was applied successfully to the determination of the Free radical Scavenging activity of the title compounds. Among the eight analogues, **3h** exhibited highest antioxidant activity and very low IC50 value. **3f, 3b, 3c** and **3a** exhibited high antioxidant activity. The compounds **3g, 3e** and **3d** showed good antioxidant activity with moderate IC50 values.

Table 10. Antioxidant activity

S.No	Comp	%RS	IC50	Activity
1	3a	51.987	0.96178	2.0169
2	3b	52.523	0.95196	2.02138
3	3c	51.266	0.97531	2.01085
4	3d	49.491	1.01028	1.99555
5	3e	45.285	1.10412	1.95698
6	3f	52.367	0.9548	2.02008
7	3g	45.224	1.10561	1.95639
8	3h	54.643	0.91503	2.03856
9	Ascorbic acid	79.617	0.6280	2.20200

Antimicrobial activity: All the synthesized compounds **(3a-h)** were evaluated for their antibacterial activity, against three gram positive and three gram- negative bacteria by serial dilution method and also evaluated for against five fungal organisms by cup plate method.

Antibacterial activity: The antibacterial activities of the test compounds are tested by serial dilution method taking drug at a concentration of 150 µg mL^{-1}. The minimum inhibitory concentration (MIC) was taken as a parameter of antibacterial activity. The MIC of test compounds is compared to that of the standard drug i.e. penicillin and chloramphenicol. All the compounds **(3a-h)** showed activity against gram positive and gram negative bacteria.

Table 11. Antibacterial activity of synthesized compounds

Minimum Inhibitory Concentration (µg mL^{-1})						
Compound	Gram positive organisms			Gram negative organisms		
	B.subtillis	*S.aureus*	*S.epidermidis*	*E.coli*	*P.aeroginosa*	*K.pnenmoniae*
3a	18.75	75	150	18.75	18.75	18.75
3b	18.75	37.5	75	18.75	75	75
3c	12.37	18.75	75	75	18.75	150
3d	18.75	12.37	18.75	12.37	18.75	37.5
3e	37.5	75	18.75	75	75	150
3f	75	150	150	150	75	150
3g	18.75	18.75	9.37	18.75	75	37.5
3h	18.60	16.75	150	150	150	150
STD Chloramphenicol	9.37	1.17	1.17	4.68	4.68	4.68
STD Penicillin	1.17	9.37	4.68	9.37	9.37	9.37

All the compounds were found to have good activity against gram-positive and gram-negative bacteria. Compound **3d** showed impressive activity against both *S.auris* and *E. coli* with MIC value 12.37 µg mL^{-1}. Compound **3c** showed impressive activity against *B.subtillis* with MIC value 12.37 µg mL^{-1}. Compound **3g** showed impressive activity against *S.epidermidis* with MIC value 9.37 µg mL^{-1}. Compounds **3a**, **3b**, **3d**, **3g** and **3h** have shown moderate activity against *B.subtillis* with MIC value 18.75µg mL^{-1}. Compounds **3c** and **3g** have shown moderate activity against *S.aureus* with MIC value 18.75 µg mL^{-1}. Compounds **3a, 3b,** and **3g** have shown moderate activity against *E. coli* with MIC value 18.75 µg mL^{-1}. Compounds **3a, 3c** and **3d** have shown moderate activity against *P.aeroginosa* with MIC value 18.75 µg mL^{-1}. Compound 3a showed moderate activity against *K.pnenmoniae*with MIC value 18.75 µg mL^{-1}.

Anti fungal activity: The antifungal activities of the test compounds were tested by agar diffusion method (cup-plate method) taking drug at a concentration of 100 µg mL^{-1} against five fungal organisms. The area of Zone of Inhibition (ZOI) was taken as a parameter of antifungal activity. The ZOI of the compound was compared to that of the standard drug i.e. Amphotericin-B.

The compounds when screened at 100 µg mL^{-1} showed different ZOI against five fungal organisms. Among the compounds screened the maximum ZOI was observed against *Saccharomyces cerevisiae*, *Aspergillus niger*, *Rhizopus oryzae*, and *Candida albicans*.

Table 12. Antifungal activity of synthesized compounds at 100 µg mL^{-1}

Compound (100 µg mL^{-1})	*R.oryzae*	*A.niger*	*A.flavus*	*C.albicans*
3a	19	22	25	20
3b	28	38	22	22
3c	38	39	35	-
3d	32	18	-	38
3e	12	44	-	14
3f	16	-	13	-
3g	40	40	-	28
3h	19	-	-	32
Amphotericin-B	24	25	24	24

Compound **3e** has shown maximum activity against *A.niger* with maximum ZOI (44mm). Compounds 3b**, 3c** and **3g** has shown moderate activity on *A.niger* with ZOI 38-40 mm. Compound **3g** has shown moderate activity against *R.oryzae* with ZOI 40 mm. Compound **3a** has shown moderate activity on *C.albicans* with ZOI 20 mm.

CONCLUSION

- In the present study, some novel N, N^1-substituted urea derivatives **(3a-h)** as **"antioxidants in ischemic diseases"** have been synthesized.
- The compounds were purified by recrystallization and column chromatography. All the newly synthesized compounds were characterized by the analytical and spectral (IR, ^{1}H NMR, ^{13}C NMR and Mass) data.
- All the newly synthesized compounds were evaluated for their *in vitro* biological activity *i.e.* antioxidant activity by spectrophotometric method and serial dilution method and Cup-plate methods for antibacterial and antifungal activities respectively.
- All the compounds were found to have good activity against gram-positive and gram-negative bacteria. Compound **3d** showed impressive activity against both *S.auris* and *E. coli* with MIC value 12.37 µg mL^{-1}. Compound **3c** showed impressive activity *against B.subtillis* with MIC value 12.37 µg mL^{-1}. Compound **3g** showed impressive activity against *S.epidermidis* with MIC value 9.37µg mL^{-1}. Compounds **3a**, **3b**, **3d**, **3g** and **3h** have shown moderate activity against *B.subtillis* with MIC value 18.75 µg mL^{-1}. Compounds **3c** and **3g** have shown moderate activity against *S.aureus* with MIC value 18.75 µg mL^{-1}. Compounds **3a, 3b,** and **3g** have shown moderate activity against *E. coli* with MIC value 18.75 µg mL^{-1}. Compounds **3a, 3c &3d** have shown moderate activity against *P.aeroginosa* with MIC value 18.75 µg mL^{-1}.
- The antifungal activity of the test compounds were tested by agar diffusion method (Cup-plate method) taking drug at a concentration of 100 µg mL^{-1} against five fungal organisms. Compound **3e** has shown maximum activity against *A.niger* with maximum ZOI (44 mm).Compounds **3b, 3c** and **3g** has shown moderate activity on *A.niger* with ZOI 38-40 mm. Compound **3g** has shown moderate activity against *R.oryzae* with ZOI 40 mm.Compound **3d** has shown moderate activity on *C.albicans* with ZOI 38 mm.
- The molecular modeling studies were correlated with the experimental biological activity values.

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