

SYNTHESES AND PHARMACOLOGICAL SCREENING OF BISARYLUREAS

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ABSTRACT

Syntheses of several arylureas (3a-h) by the arylamine-alkali cyanate protocol are reported. Their further conversion to the corresponding bisarylureas (6a-h) by condensing them with PhCHO is described. Two of the bisarylureas (6b, 6g) showed anti-microbial activity and 6b showed anti-diabetic activity. Toxicity studies were carried out on 6b.

Keywords: Arylureas; bisarylureas; anti-microbial; anti-diabetic; toxicity

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INTRODUCTION

In recent publications, a significant relationship between urea derivatives and their activity has been noted. A large number of derivatives from urea and its related compounds thiourea and guanidine have been synthesized and find application as agrochemicals, dyes for cellulose fibers, antioxidants in gasoline, resin precursors and synthetic intermediates especially to obtain carbamates and isocyanates¹⁻². Moreover, several of these groups of urea derivatives have displayed a wide spectrum of biological activity¹. The pharmacological activities of these compounds have received significant attention for their applications as potent ACAT inhibitors (acyl Cholesterol acyltransferase), sEH inhibitors (Soluble epoxide hydrolase), HIV-1 protease inhibitors (Human immunodeficiency virus), antidiabetic, antineoplastic, antihypertensive, antipsychotic, antidiuretic and antibacterial agents³⁻⁷. While the simple urea related systems have received exhaustive attention with respect to their pharmacological activity, especially in the last couple of decades, the bisureas have received much less effort. Recent studies on novel bisureas point to their use as pharmacologically potent antimicrobial and antileukemic agents⁷⁻⁹.

The synthetic route to bisureas chosen in this present work involves the preparation of monoaryurea derivatives (Scheme 1) and their reaction with benzaldehyde to form bisarylureas (Scheme 2)¹⁰⁻¹¹. Seven new bisarylurea derivatives were characterised and evaluated for their antimicrobial activity. One of the bisarylurea derivatives was also subjected to toxicity study and antidiabetic study.

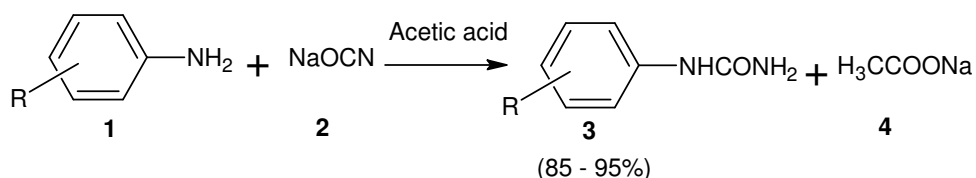
EXPERIMENTAL

Melting point, UV spectra and IR spectra were taken in the analytical laboratory of NMIMS University. The melting points were obtained using a Thiele apparatus and are uncorrected. The Plates for thin-layer chromatography were prepared from silica gel G (Merck). Visualisation of TLC spot was made by exposure to Iodine vapour. C,H,N elemental analysis were done with Thermo Finnigan, Italy, Flash EA 1112 series Instrument. UV spectra were taken on UV/Vis Spectrophotometer JASCO V-550 and IR spectra were taken on FTIR JASCO-460 Plus. The pmr, cmr, and mass spectra were recorded in the laboratory of SAIF of IIT, Bombay. The pmr spectra (including H-D exchange) were measured in DMSO (Dimethyl sulfoxide) solution using tetramethylsilane as internal standard on VARIAN, USA Mercury plus NMR Spectrometer operating at 300MHz. The mass spectra were recorded on LC-MS LCQ Advantage, Thermo Finnigan Instrument. The ¹³C NMR spectra were recorded on VARIAN, USA Mercury plus NMR Spectrometer operating at 300MHz with tetramethylsilane as internal standard.

General method for preparation of Monophenylureas (3a-h): The appropriate arylamine (0.2 mol) was dissolved in 10 ml of glacial acetic acid and it was diluted with 100 mL of water. To this stirred solution, a solution of sodium cyanate 6.5 g (0.1 mol) in 50 mL of warm water was added. The monophenylureas precipitated almost immediately. The mixture was cooled in ice and allowed to stand for further 30 minutes and filtered at the pump. The crude monophenylurea obtained was washed with 500 mL of water to remove arylamine, acetic acid and sodium cyanate completely and dried at 100°C. The monophenylureas were purified by recrystallisation from water to give white crystalline solid. The physical and analytical data are recorded in Table-1.

We have repeatedly observed that the alternate method of preparing arylureas by the reaction of arylamine hydrochlorides with urea invariably afforded cumbersome mixtures of mono- and diarylureas, necessitating extensive separations and purification steps¹⁰. The arylamine-alkali cyanate route (Scheme 1) offers a cleaner and 'greener' method.

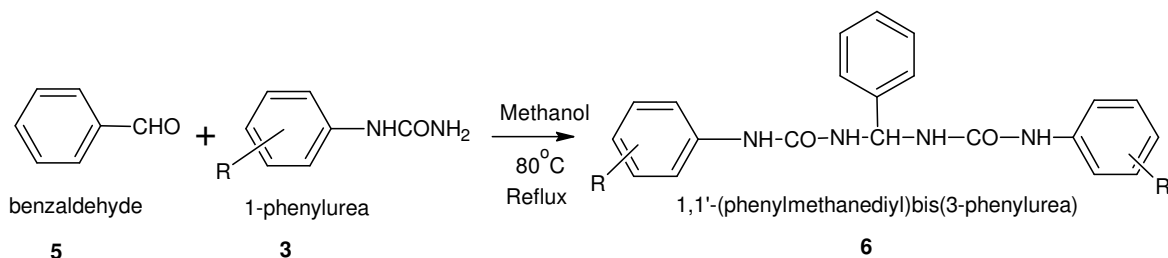
General Method for preparation of 1,1'-(phenylmethanediyl) bis(3-arylurea)(6a-h): The appropriate arylamine (3a-h) (0.2mol) (3a, 18.15 gm) was dissolved in methanol (360 mL). To this solution, benzaldehyde (0.1 mol) (7.07 gm for 3a) was added and the mixture was heated on a water bath for five and half hours at $80 \pm 2^\circ\text{C}$. On completion of the reaction, the contents were cooled and the solvent removed. The crude bisureas obtained as a hard white mass was boiled thrice with 100 mL portions of water followed by filtration to remove unreacted monophenylurea and benzaldehyde. This procedure was repeated using Isopropyl alcohol and the product was air dried. Analytically pure samples were obtained by recrystallization from 10% DMSO in benzene (Scheme 2). The physical and analytical data are recorded in Table-2.



For (1) and (3)

- | | |
|----------------------------------|-----------------------------------|
| a , R = H | e , R = 4-NO ₂ |
| b , R = 3-Cl | f , R = 4-Br |
| c , R = 3-CH ₃ | g , R = 3-Cl,4-F |
| d , R = 4-F | h , R = 3-OCH ₃ |

Scheme-1



For (3) and (6)

- | | |
|----------------------------------|-----------------------------------|
| a , R = H | e , R = 4-NO ₂ |
| b , R = 3-Cl | f , R = 4-Br |
| c , R = 3-CH ₃ | g , R = 3-Cl,4-F |
| d , R = 4-F | h , R = 3-OCH ₃ |

Scheme-2

Monophenylurea (3a): UV 237, 206 nm, IR (KBr) $\gamma_{\max}$ 3428, 3314, 1591, 1555, 1508, 1496, 1473, 1449, 1654 cm^{-1} .

1-(3-chlorophenyl)urea (3b): UV 241, 212 nm, IR (KBr) $\gamma_{\max}$ 3412, 3361, 3213, 1590, 1537, 1480, 1650 cm^{-1} .

1-(3-methylphenyl)urea (3c): UV 239, 210 nm, IR (KBr) $\gamma_{\max}$ 3441, 3340, 1548, 1487, 1670 cm^{-1} .

1-(4-Fluorophenyl)urea (3d): UV 283, 234, 206 nm, IR (KBr) $\gamma_{\max}$ 3426, 3321, 1546, 1509, 1457, 1655 cm^{-1} .

1-(4-Nitrophenyl)urea(3e): UV 369, 228, 205 nm, IR (KBr) $\gamma_{\max}$ 3482, 3360, 3217, 1541, 1506, 1472, 1632 cm^{-1} .

Table-1: Physical and analytical data of monoarylureas

Compd.	R	Molecular formula	Mol. wt	m.p. °C	Yield %	Rf Value*
3a	H	C ₇ H ₈ N ₂ O	136	145	90	0.24
3b	3-Cl	C ₇ H ₇ ClN ₂ O	170	137	85	0.19
3c	3-CH ₃	C ₈ H ₁₀ N ₂ O	150	141	90	0.40
3d	4-F	C ₇ H ₇ FN ₂ O	154	184	89	0.15
3e	4-NO ₂	C ₇ H ₇ N ₃ O ₃	181	160	91	0.18
3f	4-Br	C ₇ H ₇ BrN ₂ O	215	265	85	0.14
3g	3-Cl,4-F	C ₇ H ₆ ClFN ₂ O	188	157	93	0.17
3h	3-OCH ₃	C ₈ H ₁₀ N ₂ O ₂	166	168	95	0.16

* Solvent ratio for TLC: Cyclohexane-Benzene- Acetone (4:2:4, v/v)

Table-2: Physical and analytical data of bisarylureas

Compd.	R	Molecular formula	Mol. wt	m.p. °C	Yield %	Rf Value
6a	H	C ₂₁ H ₂₀ N ₄ O ₂	360	193	52	0.36
6b	3-Cl	C ₂₁ H ₁₈ Cl ₂ N ₄ O ₂	429	195	63	0.38
6c	3-CH ₃	C ₂₃ H ₂₄ N ₄ O ₂	388	210	68	0.49
6d	4-F	C ₂₁ H ₁₈ F ₂ N ₄ O ₂	396	211	40	0.37
6e	4-NO ₂	C ₂₁ H ₁₈ N ₆ O ₆	450	220	6	0.40
6f	4-Br	C ₂₁ H ₁₈ Br ₂ N ₄ O ₂	518	223	5	0.32
6g	3-Cl,4-F	C ₂₁ H ₁₆ Cl ₂ F ₂ N ₄ O ₂	465	172	72	0.39
6h	3-OCH ₃	C ₂₃ H ₂₄ N ₄ O ₄	420	175	66	0.34

* Solvent ratio for TLC: Cyclohexane-Benzene- Acetone (1:3:6, v/v)

Table-3: Antibacterial and Antifungal activity of bisarylureas

Compounds	<i>Escherichi a. Coli</i>	<i>Pseudomonas aeruginosa</i>	<i>Staphylococcus aureus</i>	<i>Candida sp.</i>
Bisphenylurea (6a)	-	-	-	-
3-chlorobisphenylurea (6b)	+	-	++	++
3-methylbisphenylurea (6c)	-	-	-	-
4-fluorobisphenylurea (6d)	-	-	-	-
4-nitrobisphenylurea (6e)	-	-	-	-
3-chloro,4-fluorobisphenylurea (6g)	++	+	++	++
3-methoxybisphenylurea (6h)	-	-	-	-
Gentamicin	+++	+++	+++	--
Cefixime	+++	-	+++	--
Ketoconazole	-	-	-	+++

+, 6-10 mm (slightly active); ++, 10-14 mm (moderately active); +++, >14 mm (highly active).

Table-4: Percentage Mortality at different dose concentrations of 6b

Entry	Group (n= 3)	Dose (mg / kg b.w)	Percentage mortality	LD ₅₀
1	Group-1	Control	0	-
2	Group-2	10.7	0	
3	Group-3	107	0	
4	Group-4	1070	0	

1-(4-bromophenyl)urea (3f): UV 245, 207 nm, IR (KBr) $\gamma_{\max}$ 3422, 3307, 1546, 1486, 1652 cm^{-1}

1-(3-chloro-4-fluorophenyl) urea (3g): UV 238, 207nm, IR (KBr) $\gamma_{\max}$ 3290, 1658 cm^{-1}

1-(3-methoxyphenyl)urea (3h): UV 242, 209 nm, IR (KBr) $\gamma_{\max}$ 3279, 1648 cm^{-1} .

1,1'-(phenylmethanediyl)bis(3-phenylurea)(6a): UV 315 , 252 nm, IR (KBr) $\gamma_{\max}$ 3568, 3302, 1730, 1596, 1541, 1445 , 1644 cm^{-1} , ^1H NMR (DMSO, ppm), δ 8.6 (2H, -CONH), 7.4- 6.3 (15H, ArH), 7.1 (2H, -CONH), 3.3 (1H, -CH) ^{13}C NMR (DMSO, ppm), δ 154.26, 128.72, 128.27, 127.37, 121.41 Anal.Calcd for $\text{C}_{21}\text{H}_{20}\text{N}_4\text{O}_2$: C, 70; H, 5.5; N, 15.5; O, 8.9. Found: C, 69.45; H, 5.564; N, 15.77.

1,1'-(phenylmethanediyl)bis[3-(3-chlorophenyl)urea](6b): The chloride content in the Lassaignes filtrate of the bisureas was determined by Mohr titration method described in the literature¹². UV 282, 265 nm, IR (KBr) $\gamma_{\max}$ 3563,3312,1730, 1592, 1552,1480, 1450, 1636, 1227, 1417 cm^{-1} , ^1H NMR (DMSO, ppm), δ 8.9 (2H, -CONH), 7.7-7.2 (12H, ArH) , 7.0 (2H, -CONH), 6.4 (1H, ArH), 3.3 (1H, CH), ^{13}C NMR (DMSO,ppm), δ 154.04, 130.30, 128.32, 127.44, 125.98, 121.04, 59.05, Anal. Calcd for $\text{C}_{21}\text{H}_{18}\text{Cl}_2\text{N}_4\text{O}_2$: C, 58.75; H, 4.19; N , 13.05; O, 7.5; Cl,16.5. Found: C, 58.37; H, 4.15; N, 12.85, Cl, 16.6.

1, 1' - (phenylmethanediyl)bis[3-(3-methyl phenyl) urea] (6c): UV 253 nm, IR (KBr) $\gamma_{\max}$ 3305, 1730, 1590, 1541, 1490, 1640 cm^{-1} , ^1H NMR (DMSO, ppm), δ 8.6 (2H, -CONH), 7.4-6.7 (12H,ArH), 7.1 (2H, -CONH), 6.3 (1H, ArH), 3.3 (1H, CH) , 2.2 (6H, CH_3) ^{13}C NMR (DMSO,ppm), δ 155.98, 128.53, 128.27, 127.32, 125.98, 21.22. Anal. Calcd for $\text{C}_{23}\text{H}_{24}\text{N}_4\text{O}_2$: C, 71.11; H, 6.23; N, 14.42; O, 8.24. Found: C, 70.74; H, 6.23; N, 14.54.

1,1'-(phenylmethanediyl)bis[3-(4-fluorophenyl)urea] (6d): UV 315, 252 nm, IR (KBr) $\gamma_{\max}$ 3298, 1729, 1543, 1508, 1459, 1649,1218, 1156, 1127, 1091 cm^{-1} , ^1H NMR (DMSO,ppm), δ 8.7 (2H, -CONH), 7.4-7.1 (12H, ArH), 7.1 (2H, -CONH),6.3 (1H,ArH), 3.3 (1H,CH), ^{13}C NMR (DMSO,ppm), δ 158.65, 128.27, 127.35, 125.99, 77.0, 59.07. Anal. Calcd for $\text{C}_{21}\text{H}_{18}\text{F}_2\text{N}_4\text{O}_2$: C, 63.63; H, 4.54; N, 14.14; O, 8.07. Found: C, 63.26; H, 4.40; N, 14.01.

1,1'-(phenylmethanediyl)bis[3-(3-chloro-4-fluorophenyl)urea] (6g): UV 285, 255 nm, IR (KBr) $\gamma_{\max}$ 3291, 1635 cm^{-1} , ^1H NMR (DMSO, ppm), δ 8.7 (2H, -CONH), 7.8-7.2 (11H, ArH), 6.0 (2H, -CONH),3.3 (1H,ArH), ^{13}C NMR (DMSO, ppm), δ 155.88, 137.91, 137.88, 119.09, 118.85, 117.80, 116.79, 116.50.

1,1'-(phenylmethanediyl)bis[3-(3-methoxyphenyl)urea] (6h): UV 258 nm, IR (KBr) $\gamma_{\max}$ 3298, 1635 cm^{-1} , ^{13}C NMR (DMSO, ppm), δ 156.02, 129.48, 129.20, 128.43, 110.55, 55.64.

Pharmacological Activity

The compounds (6a,6b,6c,6d,6e,6g,6h) were screened for their antibacterial activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and antifungal activity against *Candida sp* by disc diffusion method¹³. Standard antibacterial Gentamicin, Cefixime and antifungal Ketoconazole were also screened under the similar conditions for comparison. Results are present in Table-3.

Toxicity of 1,1'-(phenylmethanediyl)bis[3-(3-chlorophenyl)urea] (6b) was tested using acute toxicity study method¹⁴. The doses were calculated according to the body weight of the mice. The solution was administered orally to the animals at three different concentrations viz., 10.7 mg/kg body weight (b.w), 107 mg/kg b.w, 1070 mg/kg b.w. The animals were observed for a period of 24 hrs. After initial 24 hours observation, the animals were maintained up to 14 days to observe any delayed reaction to the given doses. The percentage mortality at different dose concentrations of 6b is shown in Table-4.

For antidiabetic study, the doses were calculated according to the body weight of the animals. The doses were prepared from a stock solution of 6b in 6 % DMSO in water. Based on the result obtained in the toxicity study, the median dose of 107 mg/kg b.w was selected for this study. The dose at this concentration was administered orally to the mouse. The concentration of glucose solution used for intraperitoneal injection was 0.75 gm of dextrose in 10 mL water. In the first step each mouse was intraperitoneally injected with 1 mL of glucose solution to induce glucose, and the time of injection was recorded. In the second step, 6b in 6 % DMSO was administered to mice (normal). The blood glucose levels were measured at t = 0, 20, 40 and 60 minutes by placing a small drop of blood on a new test strip and recording the measurements. The readings of the normal mice were compared with control group mice. The mean results are shown in Graph depicted in Figure 1.

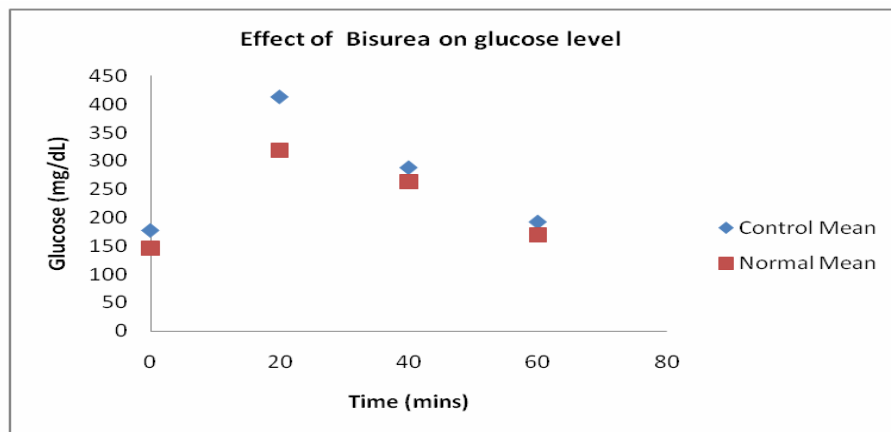


Fig.-1: Effect of Bisureas on glucose level.

RESULTS AND DISCUSSION

In the antimicrobial study two of the bisarylureas (6b & 6g) showed promising antimicrobial activity. The toxicity study was carried out for 1,1'-(phenylmethanediyl)bis[3-(3-chlorophenyl)urea] (6b) using acute toxicity method. The sample in DMSO showed that there was no delayed toxic reaction. It is considered as totally non-toxic, unclassified compound.

In antidiabetic study the calculated Auc 0-t value for normal group mice administered with 1,1'-(phenylmethanediyl)bis[3-(3-chlorophenyl)urea] (6b) in DMSO was 93% thus establishing that more of glucose is eliminated in normal group mice than the control group mice. From the data obtained during these experiments it can be safely concluded that administration of 1,1'-(phenylmethanediyl)bis[3-(3-chlorophenyl)urea] (6b) in DMSO showed weak antidiabetic activity in decreasing the blood glucose levels of normal mice.

In our work, we also observed that ureas containing aromatic chloro- and fluoro units manifested pharmacological effects. Further work is in progress, and will be reported in due course.

ACKNOWLEDGMENTS

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List of Abbreviations

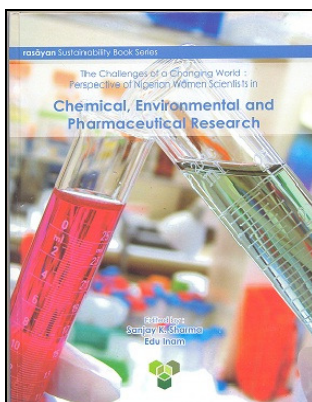
ACAT	:acyl Cholesterol acyltransferase
sEH	:Soluble epoxide hydrolase
HIV-1	:Human immunodeficiency virus
DMSO	:Dimethyl sulfoxide

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