SYNTHESIS AND ANTIBACTERIAL EVALUATION OF SOME NOVEL ISOXAZOLE AND PYRAZOLINE DERIVATIVES

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ABSTRACT

*A series of 2-(4'-chlorophenylamino)-4-(4'-fluorophenylamino)-6-[4'-{5''- (phenyl / substituted phenyl)-isoxazole-3''-yl }phenyl amino]-s-triazine **(7a-e)** and 2-(4'- chlorophenylamino)-4-(4'-fluorophenylamino)-6-[4'-{1''-phenyl-5''-(phenyl / substituted phenyl)-2''-pyrazolin-3''-yl}phenylamino]-s triazine **(8a-e)** were prepared. The structures of isoxazole derivatives and pyrazoline derivatives were confirmed on the basis of spectral data. The compounds were screened for their in vitro antibacterial activity using Gram - positive and Gram - negative bacteria.*

Keywords: *Chalcones, isoxazoles, pyrazolines, specral data, antibacterial activity.*

INTRODUCTION

The classical synthesis of the title compounds involves the Claisen- Schmidt condensation of different aromatic aldehydes with ketone **(5)** to give α, β - unsaturated ketones (chalcones) **(6a-e)**, which on cyclisation with hydroxylamine hydrochloride in presence of alkali give corresponding isoxazole derivatives **(7a-e)**. Chalcones **(6a-e)** on cyclisation with phenyl hydrazine hydrochloride in alkaline medium give corresponding pyrazoline derivatives **(8a-e)**.

In recent years, the synthesis of novel isoxazole derivatives remains a main focus of medicinal research. Isoxazoles have been reported to possess anthelmantic[1], antibacterial [2-3], antifungal [4-5] and antiviral [6] activities. Pyrazolines as class of heterocyclic compounds have been studied extensively for the past several years because of their broad spectrum of biological activity and variety of medicinal application. Pyrazoline derivatives have been found to possess antitumour[7], analgesic[8], antiinflammatory[9], antibacterial[10,11] and anticonvulsant[12] activity.

Encouraged by diverse biological activities of isoxazoles and pyrazolines and in continuation of our work[13,14] it was decided to prepare a new series of isoxazoles **(7a-e)** and pyrazoline **(8a-e)**. These compounds were screened for their antibacterial activity against S. *aureus* (MTCC 96), *B. subtilis* (MTCC 441) (Gram - positive bacteria) and *E. coli* (MTCC 443), *S. Paratyphi-B* (MTCC 733) (Gram -negative bacteria)

EXPERIMENTAL

All melting points were determined in open capillary and are uncorrected. Elemental analysis was performed by C.D.R.I Lucknow and results are within ± 0.4 of the calculated values. The IR spectra were recorded on Perkin – Elmer 237 spectrophotometer. ^{1}H NMR spectra on a Bruker Avance DPX 300 MHz spectrometer with $CDCl_3$ and DMSO as a solvent and tetramethylsilane (TMS) as internal standard. The chemical shifts are expressed in part per million (ppm) downfield from the internal standard and signals are quoted as *s* (singlet), d (doublet), *q* (quartet) or *m* (multiplate). Thin Layer Chromatography (TLC) analytical separation wee conducted with Silica Gel 60 F-254 (Merck) plates of 0.25mm thickness eluted with visualized with UV (254nm) or iodine to check the purity of the synthesized compounds.

Preparation of 2-(4' –chlorophenylamino)-4,6 -dichloro -*s*- triazine (3):
4-Chloroaniline (0.01 mol, 1.275g in 10mL acetone) was added slowly to cyanuric chloride (0.01 mol, 1.845g) in acetone (30 mL) with constant stirring for 4 h at 0 to 5 ^{o}C. Periodically, sodium carbonate solution (0.005 mol, 0.53g) in 10mL water was added dropwise to neutralize HCl evolved during the reaction. Finally the content was poured into crushed ice. The solid separated out was filtered, washed with water, dried and recrystallised from ethyl alcohol to give **(3)**

Preparation of 2-(4'-chlorophenylamino)-4-(4'-fluorophenylamino)-6-chloro-*s*-triazine (4):
4-Fluoroaniline (0.01 mol, 1.11g in 10mL acetone) was added slowly to compound **(3)** (0.01 mol, 2.75g) in acetone (35 mL) with constant stirring for 6 h at room temperature. Periodically, sodium carbonate solution (0.005 mol, 0.53g) in10mL water was added dropwise to neutralize HCl evolved during the reaction. Finally the content was poured into crushed ice. The solid separated out was filtered, washed with water, dried and recrystallised from ethyl alcohol to give **(4)**.
IR (KBr) cm^{-1}: C=C str. (1550.), =CH str. (3052), C-Cl str. (770), C-F str. (1030), C-N [*s*-triazine] (805); NMR ($CDCl_3$) δ ppm : 7.20 -7.80 (*m*, 10H, 8Ar-H and 2 NH).

Preparatio of 2-(4'-chlorophenylamino)-4-(4'-fluorophenylamino)-6-(4'-acetylphenylamino)-*s* - triazine (5):
4-Aminoacetophenone (0.01 mol, 1.35g) and compound **(4)** (0.01 mol, 3.50g) were dissolved in acetone (40 mL). The reaction mixture was refluxed for 6 h, cooled and poured into crushed ice. Periodically, sodium carbonate solution (0.005 mol, 0.53g in 10 mL water) was added to neutralize HCl evolved during the reaction. The solid separated out was filtered, washed with water, dried and recrystallised from ethyl alcohol to give **(5)**.
IR (KBr) cm^{-1}: C=C str.(1553), =CH str. (3050), C-Cl str.(775), C-F str.(1035), C-N [*s* –triazine] (809), -C =O (1658); ^{1}H NMR ($CDCl_3$) δ ppm : 2.6 (*s*, 3H, -$COCH_3$), 6.9-8.9 (*m*, 15H, 12 Ar-H and 3 NH) confirms the presence of compound **(5)**.

Preparation of 2-(4'-chlorophenylamino)-4-(4'-fluorophenylamino)-6-[4'-{3''-(2'''-methoxyphenyl)-2''-propenon- 1'' - yl} phenyl amino]-*s*- triazine (6e):
Compound **(5)** (0.01 mol, 4.48 g) was dissolved in DMF (30mL) and 40 % KOH (4mL) was added to it. Then 2-methoxybenzaldehyde (0.01 mol, 1.36 g) was added with constant stirring at room temperature. After 24 h reaction mixture was poured into crushed ice and neutralize with HCl. The product separated out was filtered, washed with water and recrystallised from ethyl alcohol to give **(6e)**.
IR (KBr) cm^{-1}: C=C str.(1560), =CH str. (3059), C-H bending[1,2-disusbtitution] (735), C-Cl str.(788), C-F str.(1013), C-N [*s* –triazine](803), -C=O(1652), C-O-C(1230); ^{1}H NMR ($CDCl_3$) δ ppm : 3.82 (*s*, 3H, o-OCH_3), 6.7 (*d*, lH, -CO-CH=), 7.2 - 7.8 (*m*, 19H, 16 Ar-H and 3 NH), 8.18 (*d*, 1H, Ar-CH=) confirms the presence of compound **(6e)**. Remaining compounds **(6a-d)** were synthesized by the same procedure.

Preparation of 2-(4'-chlorophenylamino)-4-(4'-fluorophenylamino)-6-[4'-{5''-(2'''-methoxyphenyl)-isoxazole-3''-yl} phenyl amino]-*s* -triazine (7e):
A mix of 2-(4'-chlorophenylamino)-4-(4'-fluorophenylamino)-6-[4'-{3"- (2"'-methoxyphenyl)-2"-propenon-1"-yl}phenyl amino]-*s*-triazine **(6e)** (0.01 mole 0.566g) and hydroxylamine hydrochloride (0.01 mol 0.69.5g) in alcohol (30 mL) was refluxed for 6 h in presence of 40%

KOH (5 mL). The reaction mixture was then cooled, poured into crushed ice and product separated out was filtered, washed with water until neutral pH, dried and recrystallised from ethyl alcohol to give **(7e)**.
IR (KBr) cm^{-1}: C=C str.(1501), =CH str.(3105), C-H bending [1,2-disusbtitution] (738), C-Cl str. (790), C-F str.(1015), C-N [*s* –triazine] (803), C=N str. (1575), C-O-C (1227), C-O-N(1236); ^{1}H NMR (DMSO) δ ppm : 3.84 (*s*, 3H, o-OCH_3), 6.92 (*s*, lH, -CH_{isox}), 7.0 – 8.1 (*m*, 19H, 16 Ar-H and 3 NH) confirms the presence of compound **(7e)**.
Remaining compounds **(7a-d)** were synthesized by the same procedure and formulas, melting point, yields and analytical data are shown in **Table I**

Preparation of 2-(4'-chlorophenylamino)-4-(4'-fluorophenylamino)-6-[4'-{1''- phenyl - 5'' - (2'''-methoxyphenyl) - 2''-pyrazolin- 3''-yl} phenylamino]-*s*-triazine (8e):
2-(4'-Chlorophenylamino) - 4 - (4'-fluorophenylamino)- 6 - [4' - {3"- (2'"-methoxy phenyl)-2"-propenon-1"-yl} phenyl amino]-*s*-triazine **(6e)** (0.01 mole 0.566g) and phenyl hydrazine hydrochloride (0.01mol 0.144g) in alcohol (30 mL) were refluxed for 10 h in presence of 40% KOH (8 mL). The reaction mixture was then cooled, poured into crushed ice and product separated out was filtered, washed with water until neutral pH, dried and recrystallised from ethyl alcohol to give **(8e)**.
IR (KBr) cm^{-1}: C=C str.(1492), =CH str. (3050), C-H bending [1,2-disusbtitution] (740), C-Cl str.(790), C-F str.(1012), C-N [*s* –triazine] (809), C=N str. (1580), C-O-C (1261); ^{1}H NMR ($CDCl_3$) δ ppm : 3.1 (*dd*, 1H, -$CH_{2\ pyraz}$), 3.3 (*dd*, 1H, -$CH_{2\ pyraz}$), 3.88 (*s*, 3H, o-OCH_3), 3.74 (*dd*, 1H, -CH), 6.9-7.81 (*m*, 24H, 21 Ar-H and 3 NH) confirms the presence of compound **(8e)**.
Remaining compounds **(8a-d)** were synthesized by the same procedure and their molecular formula, melting point, yields and analytical data are shown in **Table I**

RESULTS AND DISCUSSION

Antibacterial activity

The target molecules were tested for antibacterial activity against S. aureus (MTCC-96), *B. subtilis* (MTCC-441) [Gram-positive bacteria] and *E. coli* (MTCC-443), *S. paratyphi-B* (MTCC-733) [Gram-negative bacteria] by using agar diffusion method of A. L. Barry[15]. Known antibiotic Ciprofloxacin was used as standard drug. The screening results indicate that compounds **(8b)**, **(8d)** and **(8e)** were found to be active against *S. aureus* (MTCC-96). Compounds **(7a)**, **(7d)**, **(7e)**, **(8a)** and **(8c)** were found to moderately active be active against *S. aureus* (MTCC-96), whereas compounds **(7b)** and **(7c)** were found to be inactive be active against *S. aureus* (MTCC-96). Compounds **(7e)**, **(8a)**, **(8b)** and **(8e)** were found to be active against *B. subtilis* (MTCC-441).Compounds **(7a)** and **(8c)** were found to be moderately active against *B. subtilis* (MTCC-441).Compounds **(7b)**, **(7c)** and **(7d)** were found to less active against *B. subtilis* (MTCC-441), where as compound **(8d)** was found to be inactive against *B. subtilis* (MTCC-441).
Compound (**7e)** was found to active against *E. coli* (MTCC-443)Compounds **(7a)**, **(7b)**, **(7c)**, **(7d)**, **(8a)**, **(8b)** and **(8c)** were found to be moderately active against *E. coli* (MTCC-443),where as **(8d)** and **(8e)** were found to be less active against *E. coli* (MTCC-443). Compounds **(7b)**, **(8b)** and **(8c)** were found to be active against *S. paratyphi-B* (MTCC-733). Compounds **(7a)**, **(7c)**, **(7d)**, **(7e)**, **(8a)**, **(8d)** and **(8e)** were found to be moderately active against *S. paratyphi-B* (MTCC-733).

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REFERENCES

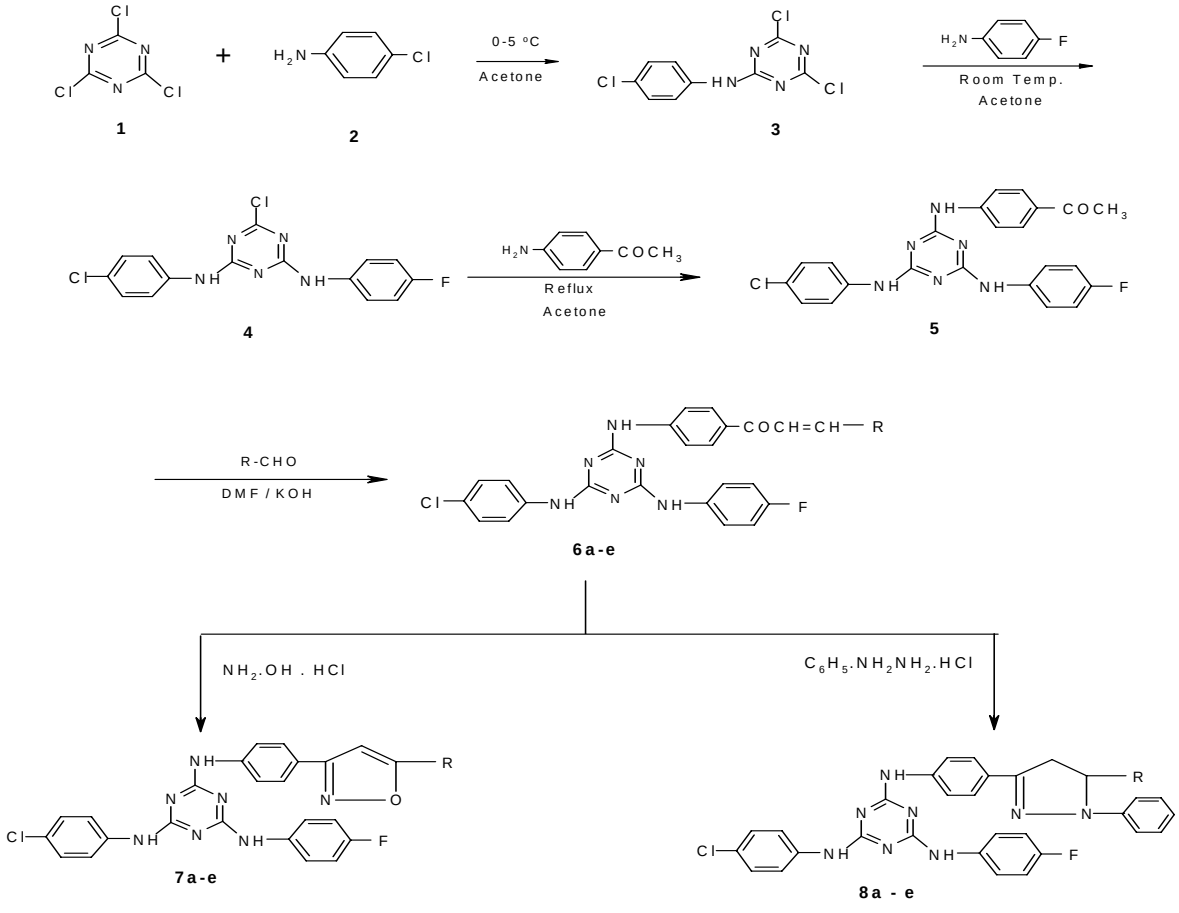
1. A. K. Banerjee, *Aruzneim. Forsch*., **44(6),** 863(1994); *Chem. Abstr*., **122**, 160522n (1995).
2. P. Cali, L. Naerum, S. Mukhija and A. Hjelmencrantz, *Bioorg. Med. Chem. Letters*, **14(24),** 5997 (2004).
3. M. Sree Rama Murthy, D. Venkata Rao and E. Venkata Rao, *Indian J. Pharma. Sci.,* **45(3),** 131 (1983).
4. M. M. Reddy, U. B. S. Swamy, J. C. Rao and K. A. Murthy, *Pesticides*, **15(4)** 27 (1981).
5. J. D.Davenport, Barry A. D and A. F. Elsasser, *Ger. Offen.* 2,723,688 (C1. A01N9/28), 22 Dec (1977); *US Appl.* 695,669, 14 Jun (1976); 43 pp; *Chem. Abstr.*, **88**, 132015k (1978).
6. Dominic Diana Guy and Philip Michael Carabates, *(Sterling Drug Inc.) U.S.* 4,268,678 (C1. 548-247; C07D261/08), 19 May (1981), *Appl.* 72,134, 04 Sep (1979); 4 pp; *Chem. Abstr.*, **95**, 203923n (1981).
7. Ronald L. Wolin, *Chem. Abstr.*, **118,** 80902p (1993).
8. F. Manna, F. Chimenti, A. Bolasco, M.L. Cenicola, M. D'Amico, C. Parillo, F. Rossi and E. Marmo, *Eur. J. Med. Chem*., **27(6)**, 633 (1992); *Chem. Abstr.*, **118,** 80902p (1993).
9. S. Sharma, V. K. Srivastava and A. Kumar, *Indian J. Chem.*, **41B** (2002) 2647
10. H. A. A. Regaila, N. Latif and I. H Ibrahim, *Egypt. J. Pharm. Sci*., **30(14),** 179 (1989).
11. M. Singhal, B. L. Verma, Y. Singh Jala and S. S. Dulwat, *Indian J. Heterocycl. Chem*., **14,** 343 (2005).
12. S. S. Parmar, B.R. Pandey and C. Dwivedi, *J. Pharm. Sci.*, **63(7),** 1152 (1994).
13. A. Solankee K. Kapadia, I. Thakor, J. Patel and S. Lad, *Asian J Chem.,* **16(2)** 921 (2004).
14. A. Solankee and I. Thakor, *Indian J Chem*., **45B**, 517 (2006).
15. A. L. Barry, *The Antimicrobic susceptibility test: Principles and practices*, Illus Lea and Febiger: *Philadelphia, Pa., USA.* **180**, (1976); *Bio. Abstr.,* **64(5)**, 25183 (1977).

TABLE I: Molecular formula, melting point, yields and analytical data of compounds **7a-e** and **8a-e**

Comp.	R	Molecular Formula	M.p °C	*Yield (%)*
7a	Phenyl	$C_{30}H_{21}ClFN_7O$	100	*63*
7b	2- Chlorophenyl	$C_{30}H_{20}Cl_2FN_7O$	138	*63*
7c	3- Chlorophenyl	$C_{30}H_{20}Cl_2FN_7O$	155	*62*
7d	4- Chlorophenyl	$C_{30}H_{20}Cl_2FN_7O$	165	*67*
7e	2-Methoxyphenyl	$C_{31}H_{23}ClFN_7O_2$	150	*61*
8a	Phenyl	$C_{36}H_{28}ClFN_8$	110	*69*
8b	2- Chlorophenyl	$C_{36}H_{27}Cl_2FN_8$	140	*70*
8c	3- Chlorophenyl	$C_{36}H_{27}Cl_2FN_8$	98	*70*
8d	4- Chlorophenyl	$C_{36}H_{27}Cl_2FN_8$	97	*71*
8e	*2-Methoxyphenyl*	$C_{37}H_{30}ClFN_8O$	*140*	*68*

TABLE II: Antibacterial activity of the compounds **7a-e** and **8a-e**

Compd	*R*	Antibacterial Activity			
		Diameter of zone of inhibition (in mm)			
		S. aureus **MTCC-96**	***B. subtilis*** **MTCC-441**	***E. coli*** **MTCC-443**	***S. paratyphi-B*** **MTCC-733**
7a	Phenyl	14	16	18	19
7b	2- Chlorophenyl	-	11	18	20
7c	3- Chlorophenyl	-	11	18	18
7d	4- Chlorophenyl	14	10	18	16
7e	2-Methoxyphenyl	14	20	20	18
8a	Phenyl	14	20	17	16
8b	2- Chlorophenyl	18	21	18	22
8c	3- Chlorophenyl	17	15	15	20
8d	4- Chlorophenyl	18	-	14	19
8e	2-Methoxyphenyl	18	20	14	16
	Ciprofloxacin (Standard Drug)	20	22	24	25



SCHEME -1

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