Asian Journal of Chemistry; Vol. 23, No. 3 (2011), 1349-1352

ASIAN JOURNAL OF CHEMISTRY

www.asianjournalofchemistry.co.in

Synthesis, Characterization and Biological Activity of Some Novel Formazan Derivatives

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(*Received*: 26 May 2010; *Accepted*: 17 November 2010) AJC-9303

1-Substituted phenyl-3-substituted phenyl-4-(*o*-carboxyphenyl) formazans [**3a-s**] were synthesized by treating azomethines (A_{1-5}) with diazonium salts. Compounds (A_{1-5}) were synthesized by condensing anthranilic acid with various substituted aromatic aldehydes. All the synthesized compounds were characterized by IR, ^{1}H NMR, Mass and elemental analysis and then evaluated for analgesic, antiinflammatory, antibacterial and antifungal activities.

Key Words: Carboxyphenyl, Formazan, Analgesic, Antiinflammaotry, Antibacterial, Antifungal.

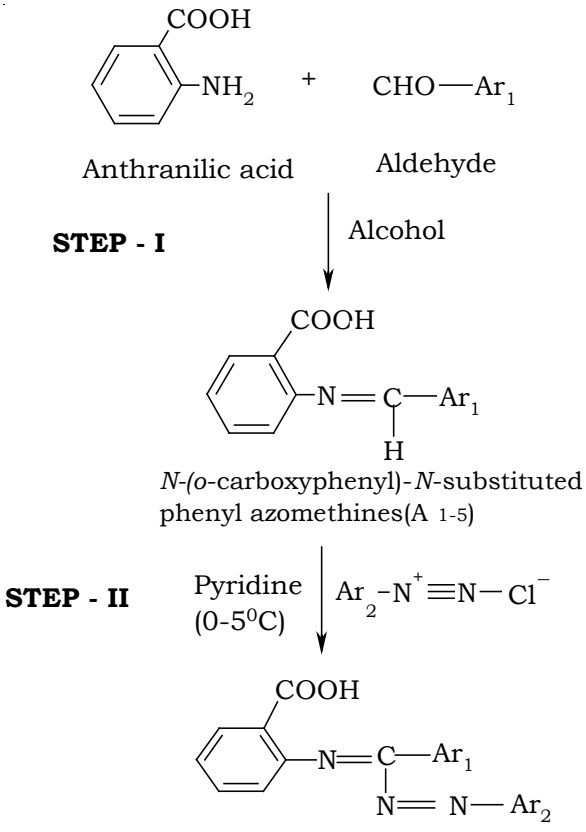
INTRODUCTION

In the search for safer and more potent therapeutic agents, popular approach is synthesis and evaluation of biologically active compounds. It was observed from the literature compounds bearing formazan nucleus show promising antifertility[1], antiparkinsonian activity[2], anticancer[3], antibacterial[4], antifungal[4], antiviral[5], anti depressant[6], MAO inhibitory[6], analgesic[7] and antiinflammatory[7] properties. In the light of these observations our attention was drawn towards the synthesis and study of few newer formazan derivatives and was evaluated for their analgesic, antiinflammatory and antimicrobial activities.

EXPERIMENTAL

Melting points of all the synthesized compounds were determined by open capillary method and are uncorrected. IR spectra (KBr, cm^{-1}) were recorded on Perkin-Elmer FTIR spectrophotometer. ^{1}H NMR spectra were recorded on Brucker AMX 400 NMR spectrometer at 400 MHz with DMSO as solvent and TMS as internal standard. The purity was checked by TLC using silica gel-G plate. All the compounds were synthesized according to **Scheme-I**.

Synthesis of N-(*o*-carboxyphenyl)-N-substituted phenyl azomethines (A_{1-5})[8]: Anthranilic acid (13.8 g, 0.1 mol) and aldehyde or substituted aldehyde (0.1 mol) were dissolved in alcohol and was refluxed for 4 h. The reaction was cooled and the contents were poured in a thin stream with stirring into crushed ice. The product was filtered, dried and recrystallized from DMSO.



Scheme-I

N-(*o*-Carboxyphenyl)-N-phenyl azomethine (A_1): IR (KBr, ν_{max}, cm^{-1}): 3373 (aromatic C-H), 3030 (C-H, methyl), 1703 (=CH), 1672 (C=O, aryl acid), 1591 (C=N, azomethine), 1488 (aromatic C=C), 1239 (C-N, tertiary aromatic amine). ^{1}H NMR (δ ppm): 10.030 (s, 1H, -COOH), 6.286-7.934 (m, 9H, Ar-H), 3.374 (s, 1H, -CH). MS (m/z) : 226.3

N-(*o*-Carboxyphenyl)-N-(4-chlorophenyl)azomethine (A_2): IR (KBr, ν_{max}, cm^{-1}): 3369 (aromatic C-H), 3031 (C-H, methyl), 1767 (=CH), 1666 (C=O, aryl acid), 1587 (C=N, azomethine), 1489 (aromatic C=C), 1238 (C-N, tertiary aromatic amine), 816 (C-Cl). ^{1}H NMR (δ ppm): 10.011 (s, 1H, -COOH), 6.321-8.190 (m, 8H, Ar-H), 2.511 (s, 1H, -CH). MS (m/z): 262.3.

N-(*o*-Carboxyphenyl)-N-(4-nitrophenyl) azomethine (A_3): IR (KBr, ν_{max}, cm^{-1}): 3334 (aromatic C-H), 1711 (=CH), 1610 (C=O, aryl acid), 1525 (C=N, azomethine), 1487 (aromatic C=C), 1350 (Ar-NO_2), 1221 (C-N, tertiary aromatic amine). ^{1}H NMR (δ ppm): 10.173 (s, 1H, -COOH), 6.506-8.707 (m, 8H, Ar-H), 1.047-1.171 (s, 1H, -CH). MS (m/z): 270.2.

N-(*o*-Carboxyphenyl)-N-(2-hydroxy-4-methoxyphenyl) azomethine (A_5): IR (KBr, ν_{max}, cm^{-1}): 3368 (aromatic C-H), 3158 (Ar-OH, phenol), 1692 (=CH), 1589 (C=N, azomethine), 1458 (aromatic C=C), 1208 (C-N aromatic). ^{1}H NMR (δ ppm): 9.780 (s, 1H, -COOH), 6.486-7.899 (m, 7H, Ar-H), 3.849 (s, 1H, -OH), 3.373 (s, 3H, -OCH_3), 2.511 (s, 1H, -CH). MS (m/z): 271.2.

Synthesis of 1-substituted phenyl-3-substituted phenyl-4-(*o*-carboxyphenyl) formazans (3a-3s)[8]: The diazonium salts derived from the respective amines (0.01 mol) were added with stirring to N-(*o*-carboxyphenyl)-N-substituted phenyl azomethines (0.01 mol) in pyridine at 0-5 ºC for 0.5 h. The mixture was added to ice-cold water. The resultant product was filtered, dried and recrystallized from DMSO. The physical characteristics are presented in Table-1.

1-(4-Bromophenyl)-3-phenyl-4-(*o*-carboxyphenyl) formazan (3b): IR (KBr, ν_{max}, cm^{-1}): 1588 (C=N, azomethine), 1509 (N=N of formazan), 1481 (aromatic C=C), 697 (C-Br). ^{1}H NMR (δ ppm): 12.604 (s, 1H, -COOH), 7.402-7.577 (m, 13H, Ar-H). MS (m/z): 404.4.

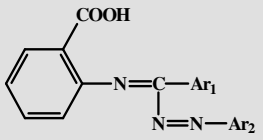
1-(4-Nitrophenyl)-3-phenyl-4-(*o*-carboxyphenyl) formazan (3c): IR (KBr, ν_{max}, cm^{-1}): 1596 (C=N, azomethine), 1518 (N=N of formazan), 1483 (aromatic C=C), 1343 (Ar-NO_2). ^{1}H NMR (δ ppm): 13.489 (s, 1H, -COOH), 7.237-8.453 (m, 13H, Ar-H). MS (m/z): 383.3.

1-(3-Fluoro-4-chlorophenyl)-3-phenyl-4-(*o*-carboxyphenyl)formazan (3d): IR (KBr, ν_{max}, cm^{-1}): 1601 (C=N, azomethine), 1515 (N=N of formazan), 1494 (aromatic C=C), 1048 (C-F), 804 (C-Cl). ^{1}H NMR (δ ppm): 12.709 (s, 1H, -COOH), 6.501-7.944 (m, 12H, Ar-H). MS (m/z): 383.3.

1-(4-Chlorophenyl)-3-(4-chlorophenyl)-4-(*o*-carboxyphenyl)formazan (3e): IR (KBr, ν_{max}, cm^{-1}): 1593 (C=N, azomethine), 1507 (N=N of formazan), 1486 (aromatic C=C), 825 (C-Cl). ^{1}H NMR (δ ppm): 6.555-7.771 (m, 12H, Ar-H). MS (m/z): 401.2.

1-(4-Nitrophenyl)-3-(4-chlorophenyl)-4-(*o*-carboxyphenyl)formazan (3g): IR (KBr, ν_{max}, cm^{-1}): 1588 (C=N, azomethine), 1513 (N=N of formazan), 1311 (Ar-NO_2), 828 (C-Cl). ^{1}H NMR (δ ppm): 6.442-8.171(m, 12H, Ar-H). MS (m/z): 409.1.

TABLE-1
CHARACTERIZATION DATA OF COMPOUNDS **3a-s**



Compd.	Ar_1 / Ar_2	m.f. (m.w.)	m.p. (ºC) / Yield (%)
3a	C_6H_5 / C_6H_4Cl	$C_{20}H_{14}N_3O_2Cl$ (363.8)	162 / 55.0
3b	C_6H_5 / C_6H_4Br	$C_{20}H_{14}N_3O_2Br$ (408.2)	218 / 65.2
3c	C_6H_5 / $C_6H_4NO_2$	$C_{20}H_{14}N_4O_4$ (374.3)	268 / 56.4
3d	C_6H_5 / C_6H_3FCl	$C_{20}H_{13}N_3O_2FCl$ (381.8)	200 / 60.0
3e	C_6H_4Cl / C_6H_4Cl	$C_{20}H_{13}N_3O_2Cl_2$ (398.2)	168 / 66.0
3f	C_6H_4Cl / C_6H_4Br	$C_{20}H_{13}N_3O_2ClBr$ (408.2)	264 / 56.0
3g	C_6H_4Cl / $C_6H_4NO_2$	$C_{20}H_{13}N_4O_4Cl$ (408.8)	292 / 58.4
3h	C_6H_4Cl / C_6H_3ClF	$C_{20}H_{12}N_3O_2Cl_2F$ (416.2)	194 / 55.4
3i	$C_6H_4NO_2$ / C_6H_4Cl	$C_{20}H_{13}N_4O_4Cl$ (408.8)	126 / 57.0
3j	$C_6H_4NO_2$ / C_6H_4Br	$C_{20}H_{13}N_4O_4Br$ (453.2)	238 / 66.0
3k	$C_6H_4NO_2$ / $C_6H_4NO_2$	$C_{20}H_{14}N_5O_6$ (419.4)	280 / 56.9
3l	$C_6H_4NO_2$ / C_6H_3ClF	$C_{20}H_{12}N_4O_4ClF$ (426.8)	186 / 57.8
3m	$C_6H_4N(CH_3)_2$ / C_6H_4Cl	$C_{22}H_{19}N_4O_2Cl$ (406.9)	144 / 70.0
3n	$C_6H_4N(CH_3)_2$ / C_6H_4Br	$C_{22}H_{19}N_4O_2Br$ (451.3)	190 / 67.9
3o	$C_6H_4N(CH_3)_2$ / $C_6H_4NO_2$	$C_{22}H_{19}N_5O_4$ (417.4)	238 / 65.3
3p	$C_6H_3(OCH_3)(OH)$ / C_6H_4Cl	$C_{21}H_{16}N_3O_4Cl$ (409.8)	140 / 67.5
3q	$C_6H_3(OCH_3)(OH)$ / C_6H_4Br	$C_{21}H_{16}N_3O_4Br$ (454.2)	244 / 57.0
3r	$C_6H_3(OCH_3)(OH)$ / $C_6H_4NO_2$	$C_{21}H_{16}N_4O_6$ (420.4)	258 / 62.1
3s	$C_6H_3(OCH_3)(OH)$ / C_6H_3ClF	$C_{21}H_{15}N_3O_4ClF$ (427.8)	212 / 77.0

1-(4-Chlorophenyl)-3-(4-nitrophenyl)-4-(*o*-carboxyphenyl)formazan (3i): IR (KBr, ν_{max}, cm^{-1}): 1599 (C=N, azomethine), 1515 (N=N of formazan), 1484 (aromatic C=C), 1342 (Ar-NO_2), 823 (C-Cl). ^{1}H NMR (δ ppm): 10.162 (s, 1H, -COOH), 7.123-8.801 (m, 12H, Ar-H). MS (m/z): 397.1.

1-(4-Bromophenyl)-3-(4-nitrophenyl)-4-(*o*-carboxyphenyl)formazan (3j): IR (KBr, ν_{max}, cm^{-1}): 1593 (C=N, azomethine), 1514 (N=N of formazan), 1481 (aromatic C=C), 1344 (Ar-NO_2), 738 (C-Br). ^{1}H NMR (δ ppm): 10.174-12.714 (s, 1H, -COOH), 7.310-8.830 (m, 12H, Ar-H). MS (m/z): 453.0.

1-(4-Nitrophenyl)-3-(4-nitrophenyl)-4-(*o*-carboxyphenyl)formazan (3k): IR (KBr, ν_{max}, cm^{-1}): 1599 (C=N, azomethine), 1517 (N=N of formazan), 1410 (aromatic C=C),

1343 (Ar-NO_2). ^{1}H NMR (δ ppm): 10.165 (s, 1H, -COOH), 7.667-8.446 (m, 12H, Ar-H). MS (m/z): 419.2.

1-(3-Fluoro-4-chlorophenyl)-3-(4-nitrophenyl)-4-(*o*-carboxyphenyl)formazan (3l): IR (KBr, ν_{max}, cm^{-1}): 1493 (aromatic C=C), 1602 (C=N, azomethine), 1518 (N=N of formazan), 1346 (Ar-NO_2), 1193 (C-F), 815 (C-Cl). ^{1}H NMR (δ ppm): 10.160 (s, 1H, -COOH), 7.381-8.822 (m, 11H, Ar-H). MS (m/z): 419.4.

Analgesic activity: Analgesic activity was determined by using Eddy's hot plate method[9,10] with albino mice. Mice were placed on a hot plate maintained at constant temperature 55.0 ± 0.5 ºC immediately after intraperitoneal (i.p.) administration of test drugs. Latency to exhibit the nociceptive response such as licking paws or jumping was determined. A cut-off time of 15 s was selected to avoid tissue damage.

Antiinflammatory activity: Antiinflammatory activity was carried by carrageenan induced paw edema method[11,12]. The animals of either sex randomly divided into 5 groups, each group containing 4 animals. In all groups, acute inflammation was produced by subplantar injection of 0.1 mL freshly prepared 1 % suspension of carrageenan in normal saline in right hind paw of the rats. Foot volumes were measured using a plethysmograph by mercury displacement at 0 (before carrageenan injection) and 0.5, 1.0 and 2.0 h after carrageenan injection. Animals were pre-dosed with 10 mg/kg of test drugs, 0.2 mL of vehicle and 30 mg/kg diclofenac sodium (reference standard) were administered intraperitoneally (i.p.).

Antibacerial activity: All synthesized compounds **3a-s** were evaluated *in vitro* for antibacterial activity against *B. subtilis, B. cereus, E. coli, S. aureus, S. epidermidis, P. aeruginosa* by paper disc diffusion method[8,13] with DMF as solvent control and nutrient agar was employed as culture media. After 24 h hot incubation at 37 ºC the zone of inhibition were measured in mm.

Antifungal activity: The compounds **3a-s** were evaluated *in vitro* for antifungal activity against *A. niger, S. cerevisiae, C. albicans, C. glabrata* by paer disc diffusion method[8,13] with DMF as solvent control and nutrient agar was employed as culture media. After 48 h incubation at 25 ºC the zone of inhibition were measured in mm.

RESULTS AND DISCUSSION

Analgesic activity: The mean basal reaction time and reaction time after 2 h of drug administration of compounds **3a-s** and standard drug (paracetamol) were recorded (Table-2). However compounds **3j**, **3k**, **3l**, **3n** and **3o** have shown significant analgesic activity. Perhaps the substituents at 4th position of both phenyl rings of formazans nucleus and which contains bromo, nitro, fluoro, chloro groups is contributing significant analgesic activity.

Antiinflammatory activity: All the compounds **3a-s** at the dose each of 10 mg/kg exhibited significant antiinflammatory activity. Results are given in Table-3. Compounds **3b**, **3c**, **3g**, **3h**, **3k** and **3s** exhibited significant antiinflammatory activity and remaining compounds have shown moderate antiinflammatory activity. The significant activity mainly due to the presence of formazans nucleus and substituents at 4th position of two phenyl rings of the same.

TABLE-2
ANALGESIC ACTIVITY OF COMPOUNDS **3a-s** BY EDDY'S HOT PLATE METHOD

Compd.	Basal reaction (s)		Reaction time (s) after the drug administration	
	Paw-licking	Jump response	Paw-licking	Jump response
3a	4 ± 0.87	6 ± 0.86	5 ± 0.87	7 ± 1.05
3b	3 ± 0.71	5 ± 1.26	6 ± 0.71	9 ± 1.26
3c	4 ± 0.59	6 ± 1.48	7 ± 0.59	9 ± 1.48
3d	4 ± 1.51	7 ± 1.30	6 ± 1.51	8 ± 1.30
3e	5 ± 0.59	7 ± 1.30	5 ± 0.59	7 ± 1.30
3f	3 ± 1.51	5 ± 0.48	3 ± 1.51	5 ± 1.05
3g	3 ± 0.84	5 ± 0.70	5 ± 0.84	7 ± 1.10
3h	3 ± 0.87	5 ± 1.48	6 ± 1.23	8 ± 1.14
3i	4 ± 1.51	7 ± 1.26	7 ± 0.45	9 ± 1.10
3j	4 ± 0.84	6 ± 1.48	8 ± 0.84	12 ± 1.34
3k	5 ± 0.87	7 ± 1.30	8 ± 0.84	11 ± 1.48
3l	4 ± 0.71	6 ± 1.05	7 ± 1.87	11 ± 2.08
3m	4 ± 0.71	7 ± 1.48	4 ± 0.71	7 ± 1.48
3n	5 ± 0.59	7 ± 1.30	8 ± 0.63	10 ± 1.26
3o	5 ± 1.51	7 ± 1.05	8 ± 0.89	10 ± 1.34
3p	4 ± 0.87	6 ± 1.48	6 ± 0.87	8 ± 1.48
3q	4 ± 0.71	6 ± 1.30	4 ± 0.71	6 ± 1.30
3r	3 ± 0.59	5 ± 1.05	3 ± 0.59	5 ± 1.05
3s	4 ± 1.51	6 ± 1.26	6 ± 1.51	9 ± 1.26
Paracetamol (100 mg/Kg)	5 ± 0.87	7 ± 1.30	10 ± 1.58	13 ± 1.30
Control	4 ± 0.71	6 ± 1.05	4 ± 1.58	6 ± 1.30

TABLE-3
ANTIINFLAMMATORY ACTIVITY OF COMPOUNDS **3a-s** ON CARRAGEENAN INDUCED RAT PAW EDEMA

Compound	Mean Paw edema volume (mm) ± SE (after 2 h)	% Reduction in paw edema volume
3a	0.3 ± 0.96	33
3b	0.2 ± 1.24	67
3c	0.2 ± 1.36	67
3d	0.3 ± 1.30	33
3e	0.3 ± 1.18	33
3f	0.3 ± 0.84	33
3g	0.2 ± 0.92	67
3h	0.2 ± 1.14	67
3i	0.3 ± 1.10	33
3j	0.3 ± 1.34	33
3k	0.2 ± 1.48	67
3l	0.3 ± 2.08	33
3m	0.3 ± 1.58	33
3n	0.3 ± 1.26	33
3o	0.3 ± 1.34	33
3p	0.3 ± 0.71	33
3q	0.3 ± 1.30	33
3r	0.3 ± 1.05	33
3s	0.2 ± 1.14	33
Diclofenac (30 mg/Kg)	0.2 ± 1.05	67
Control	0.4 ± 0.63	–

Antibacerial activity: The resulted **3a-s** compounds were screened for antibacterial activity at a concentration of 10 mg/mL using DMF as a control. Ciprofloxacin 100 mcg/disc used as standard. It is evident from the screening data that compounds **3b**, **3c**, **3f**, **3i**, **3j**, **3k** and **3l** were found to possess a broad spectrum activity. While the remaining compounds have shown moderate antibacterial activity (Table-4).

TABLE-4
ANTIMICROBIAL ACTIVITY OF COMPOUNDS **3a-s**

Compd.	Zone of inhibition at 10 mg/mL (in mm)									
	Antibacterial activity						Antifungal activity			
	BS	BC	EC	SA	SE	PS	CA	CG	AN	SC
3a	13	7	–	12	6	11	–	10	–	7
3b	21	15	15	12	12	13	–	22	14	12
3c	20	13	14	16	11	17	–	26	25	27
3d	18	21	13	12	9	21	–	17	–	–
3e	23	14	7	18	12	14	8	20	–	–
3f	20	17	11	24	12	17	8	16	–	10
3g	19	9	–	11	9	–	–	10	–	–
3h	20	17	9	18	9	13	10	17	–	–
3i	23	16	21	11	16	12	23	25	–	–
3j	27	19	19	20	20	16	26	20	–	24
3k	19	18	20	20	21	17	21	18	–	–
3l	29	26	28	22	20	27	15	28	32	25
3m	8	6	5	8	5	7	10	13	22	12
3n	13	9	7	8	9	8	12	10	24	8
3o	11	13	14	11	9	13	15	14	27	27
3p	–	–	–	7	–	7	–	7	9	–
3q	6	5	–	6	–	5	–	15	7	6
3r	–	13	7	9	8	15	–	21	11	16
3s	–	–	–	–	–	–	7	10	–	7
SD	21	17	22	20	20	21	19	22	17	18

BS = *Bacillus subtilis*; CA = *Candida albicans*; BC = *Bacillus cereus*; CG = *Candida glabrata*; EC = *Escherichia coli*; AN = *Aspergillus niger*; SA = *Staphylococcus aureus*; SC = *Saccharomyces cerevisiae*; SE = *Staphylococcus epidermidis*; PS = *Pseudomonas aeruginosa.*
SD = Standard drug for antibacterial activity = Ciprofloxacin
SD = Standard drug for antifungal activity = Fluconazole

Antifungal activity: All the compounds were also screened for antifungal activity. However compounds **3b**, **3c**, **3j**, **3l** and **3o** have shown promising antifungal activity, while the remaining compounds have shown moderate fungal activity, when compared to the standard drug fluconazole.

ACKNOWLEDGEMENTS

The authors are thankful to Chalapathi Institute of Pharmaceutical sciences, Guntur for providing the necessary research facilities.

REFERENCES

1. J.M. Desai and V.H. Shah, *Indian J. Chem.*, **42B**, 631 (2003).
2. R. Khanna, A.K. Saxena, V.K. Srivastava and K. Shanker, *Indian J. Chem.*, **29B**, 91 (1990).
3. S.D. Bhardwaj, P. Phatak and V.S. Jolly, *Orient. J. Chem.*, **2**, 181 (1995).
4. N. Lakshmi, V. Haritha, V. Sreeram, D. Rajalakshmi, N. Sindhura and D. Visagaperumal, *Rasayan J. Chem.*, **2**, 71 (2009).
5. D.D. Mukharjee, S.K. Shukla and B.L. Chowdhary, *Arch. Pharmazie*, **314**, 991 (1981).
6. G. Sathi, V.R. Gujrati, C. Nath, J.C. Agarwal, K.P. Bhargava and K. Shanker, *Arzneimittelforschung*, **33**, 1218 (1983).
7. E. Stefanescu, M. Dorneanu, C. Danila, C. Aprotosoaie, G. Grosu and M. Pavelescu, *Rev. Med. Chir. Soc. Med. Nat. Iasi.*, **101**, 178 (1997).
8. S.I. Marjadi, J.H. Solanki and A.L. Patel, *E-J. Chem.*, **6**, 844 (2009).
9. S.K. Kulkarni, Hand Book of Experimental Pharmacology, Vallabh Prakashan, edn. 3, p. 125 (2007).
10. M.N. Ghosh, Fundamentals of Experimental Pharmacology, Hilton and Company, edn. 3, p. 175 (2005).
11. S.K. Kulkarni, Hand Book of Experimental Pharmacology, Vallabh Prakashan, edn. 3, p. 128 (2007).
12. S.K. Kulkarni, A.K. Mehta and J. Kunchandy, *Arch. Int. Pharmacodyn.*, **279**, 324 (1986).
13. B.A. Forbes, D.F. Sahm, S.A. Weisseld and E.A. Trevino, Bailey and Scott's Diagnostic Microbiology, Mosby Co., St. Louis Missouri, p. 171 (1990).