J. Biol. Chem. Chron. **2019**, **5***(1)*, 27-31
ISSN (Print): 2454 – 7468
ISSN (Online): 2454 - 7476
www.eresearchco.com/jbcc/

Synthesis of Some Novel N-alkylated 2-chloro-Benzimidazole Derivatives and their Biological Evaluation

Ashish Yasin Hawaldar[1] and Sanjay Dashrath Vaidya[2*]

[1 & 2] *Department of Chemistry, Jagdishprasad Jhabarmal Tibrewala University, Jhunjhunu, Rajasthan, INDIA*

[*] *Correspondence: E-mail: sanjayjjtu@gmail.com*

DOI: http://dx.doi.org/10.33980/jbcc.2019.v05i01.005

(Received 13 Jan, 2019; Accepted 03 Feb, 2019; Published 15 Feb, 2019)

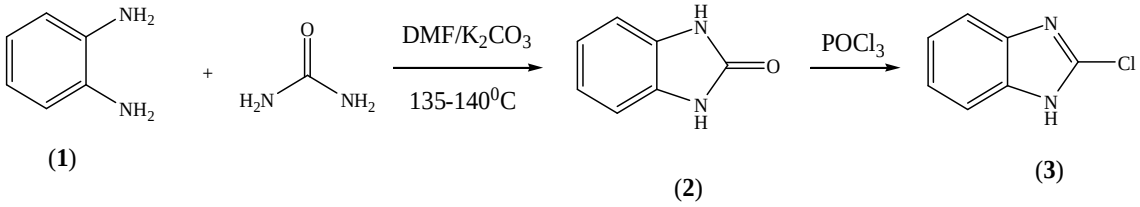
ABSTRACT: A series of novel N-substituted benzimidazole derivatives have been synthesized by the condensation of ortho-phenylenediamine and urea, subsequent reaction of the product obtained with Phosphoryl chloride to give 2-Chloro-1*H*-benzimidazole. Using this compound alkylation done at the benzimidazole –NH position with different benzyl halide reagent leading to the functionalized derivatives. Newly synthesized derivatives (6a to 6h) have been elucidated on the basis of spectral and analytical data like melting point, IR, ^{1}H-NMR, Mass spectroscopy and elemental analysis. All the synthesized compounds were screened for their antimicrobial activity. This displayed some promising results towards testing organism in-vitro.

Keywords: Anti-bacterial activity; Anti-fungal activity; Alkylation; Benzimidazole and Condensation.

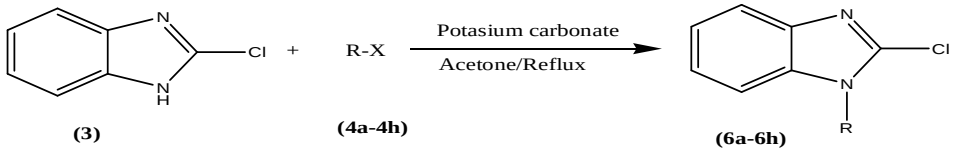
INTRODUCTION: Benzimidazole and its derivatives are marked as prominent heterocyclic compounds that exhibit a large number of biological activities[1-4].Significantly, the benzimidazole moiety is a constituent part of Vitamin-B12 core structure[5]. Some of them are used as antihelmintic drugs[6, 7], anti-ulcer[8], anti-psychotic[9,10], anti-fungal[11], anti-dopaminergic[12]. The 2-substituted analogs of benzimidazoles are known to be potent biologically active compounds[13-14], some of the important benzimidazole derivatives have been reported as thyroid receptor agonists[15]. The activity and structural diversity exhibited by compounds containing benzimidazole moiety has led to the discovery and development of novel and useful bioactive benzimidazole libraries from time to time. We have been interested in the synthesis of novel benzimidazole ring systems in connection with our ongoing project on benzimidazoles[16-17]. In continuation of our work on bioactive benzimidazole libraries, we herein describe our efforts towards the synthesis of a novel class of benzimidazole derivatives and their biological activity screening studies.

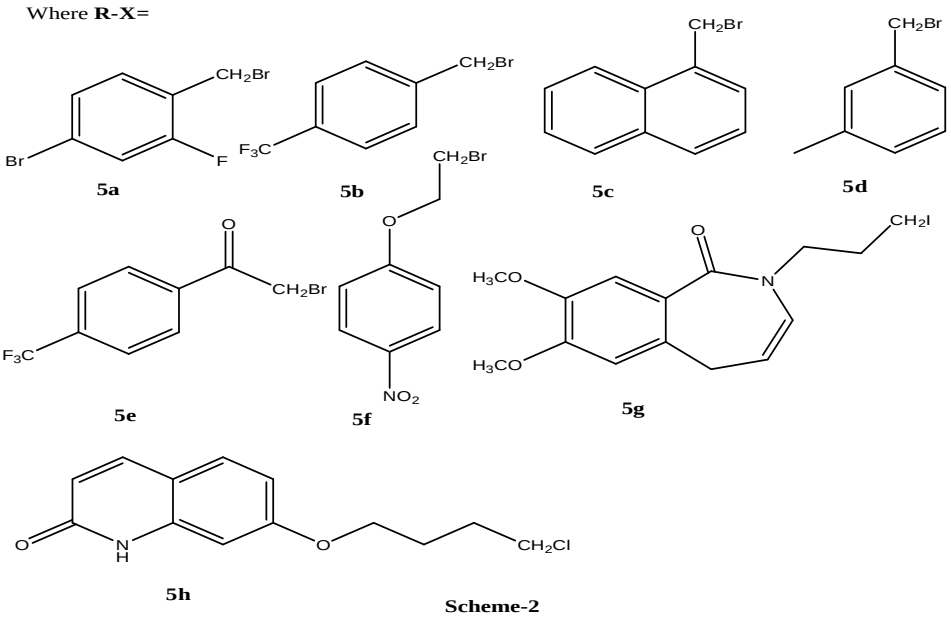
MATERIALS AND METHODS: *o*-phenylenediamine acid, Urea, Phosphoryl chloride, Conc HCl, Sodium hydroxide, Potassium carbonate and alkylating agent from commercial supplier. All the solvents used were of commercial grade only. Melting points recorded on a MRVIS Series, Lab. India Instrument. Thin layer Chromatography analysis was carried out using pre-coated silica gel plates and visualizatised using iodine/UV lamp. Infrared spectra were recorded on Jasco, FT/IR-4100 type-A using the KBr disc. Proton-NMR spectra of the compounds were recorded on JEOL 500 MHz NMR spectrometer. Elemental analysis was carried out on a Perkin Elmer Series II Elemental Analyzer 2400.

RESULTS AND DISCUSSION: 1, 3-dihydro-benzimidazol-2-one **2** was synthesized by known method using o-phenylene diamine, urea in Dimethyl formamide at temperature 135-140°C with 94 % yield having m.p.99-101°C. A mixture of **2**, Phosphoryl chloride and catalytic amount of phenol was heated 103-107°C for 12 hrs.and subsequent work-up resulted in the formation of 2-chloro-1H-benzimidazole[18] **3** white solid having m.p. 208-209^{o}C and in 97% yield. Based on the spectral and analytical data the compound was assigned to be 2-chloro-1H-benzimidazole **3** (Scheme-1). The alkylation of **3** with various electrophilic reagents in presence of potassium carbonate as base yielded the N-alkylated derivatives obtained Compounds **6a-6h.** (Scheme-2)



Scheme-1





Scheme-2

Table 1: Antibacterial activity (minimal inhibition concentration; MIC µg/ml) of 6a-6h.

Antibacterial activity (MIC, µg/ml)				
Compound	**E. coli (Gram negative)**	**P. Aeruginosa (Gram negative)**	**S.Aureus (Gram positive)**	**S.Pyogenus (Gram positive)**
6a	100	100	125	100
6b	100	100	100	150
6c	75	100	125	150
6d	100	100	100	150
6e	100	100	75	100
6f	75	62.5	62.5	100
6g	62.5	250	200	125
6h	62.5	100	100	150
Gentamycin	0.05	1	0.25	0.5
Ampicillin	100	-	250	100
Chloramphenicol	50	50	50	50
Ciprofloxacin	25	25	50	50
Norfloxacin	10	10	10	10

Table 2: Antifungal activity (minimal inhibition concentration; MIC µg/ml) of 6a-6h.

Compound	C. *albicans*	A. *niger*	A. *clavatus*
6a	>1000	>1000	>1000
6b	1000	500	1000
6c	>1000	1000	1000
6d	>1000	>1000	500
6e	1000	500	1000
6f	500	250	>1000
6g	500	1000	>1000
6h	250	>1000	250
Nystatin	100	100	100
Greseofulvin	500	100	100

General Procedure of synthesis:

Synthesis of 1, 3-dihydro-benzimidazol-2-one (2): To a solution of *o*-phenylene diamine **1**(5gm, 0.046 mole) in DMF was charged urea (5.52 gm., 0.092 mole) and mixture refluxed for 12 hrs. After ensuring the completion of reaction, DMF was removed off by distillation under vacuum; isolated solid was washed with water and then dissolved in 10% aqueous sodium hydroxide solution. The aqueous alkaline solution was filtered and neutralized with aqueous Hydrochloride solution (35%). The Precipitated product was filtered, washed and dried. After this workup obtain pure **2** (1, 3-dihydro-benzimidazol-2-one)[18] with 5.8gm,94% yield and good purity, having melting point 99-101°C (Scheme-1).

Synthesis of 2-chloro-1H-benzimidazole (3): A mixture of **2** (10 gm, 0.07 mole), Phosphoryl chloride (22.88 gm, 0.14 mole) and catalytic amount of phenol was heated 103-107^{0}C for 12 hrs. After confirmation of completion of the reaction, the mixture was chilled in ice and neutralized with 40% aqueous sodium hydroxide solution to pH-10. The obtained crude material was then recrystallized to obtain pure product **3** (2-chloro-1H-benzimidazole)[18] with yield 11 gm.97%, Melting point 208-209°C (Scheme-1).

General procedure for the synthesis of N- alkylated derivatives of 2-chloro-1H-benzimidazole compounds (6a-6h): To a solution of 2-chloro-1*H*-benzimidazole **3** (13.10 mmoles) and potassium carbonate (19.65 mmoles) in acetone (80 ml) was added compound (5a-5h, 15.75 mmoles) at RT. The reaction mixture was then warmed to 50-55^{0}C for 4-6 hrs. Completion of reaction monitored on TLC. After completion of reaction, solvent was removed by evaporation and added 20 ml water and 25ml ethyl acetate, stirred for 15 min. layers were separated. Ethyl acetate layer containing expected compound was washed with 20 ml water, dried over sodium sulfate. After concentration of solvent under vacuum and re-crystallization using aq. ethanol yielded corresponding N-substituted derivative (6a-6h) as a white solid. (Scheme-2)

Analytical Characterization:

1-(4Bromo-2fluoro-benzyl)-2chloro1Hbenzimidazole (6a): Yield 84%; mp.115-118^{0}C;IR(KBr):596(C-Br),741(CCl),1370(CF),1515(C=C),1604(C=N),2357(-CH_2)cm^1;^{1}HNMR(CDCl3):δ5.39(s,2H,CH2),6.80-6.83(t,1H,*J=7.9Hz*,ArH),7.19-7.21(dd,1H ,*J1=8.3Hz, J2=1.4Hz*, ArH), 7.24-7.30(m,1H, ArH), 7.30-7.33(m, 3H, ArH), 7.71-7.73(dd, 1H, *J1=6.5Hz, J2=1.7Hz* ,ArH) MS (m/z): 340.20 (M^++1). Elemental Anal. calc,- For $C_{14}H_9BrClFN_2$: C, 49.52; H, 2.67; N, 8.25 Found: C=49.5; H=2.72; N=8.27.

2-chloro-1-(4trifluoromethylbenzyl)1Hbenzimidazole (6b): Yield 75%; mp.92-99^{0}C; IR (KBr): 742(C-Cl),1328(C-F),1461(C=C),1617(C=N)2357(-CH_2) cm^{-1}; ^{1}H-NMR (CDCl3): δ 5.45 (s, 2H,CH2),7.18-7.20 (m,1H, ArH),7.28-7.32 (m, 4H, ArH),7.58-7.60 (d, 2H, *J=8.3Hz*, ArH). 7.73-7.74(m,1H,ArH).MS (m/z): 311.32 (M^++1); Elemental Anal.-calcd. For $C_{15}H_{10}ClF_3N_2$.C,57.99;H,3.24;N,9.02 Found:C=58.01; H=3.30; N=9.05.

2-chloro-1-naphthalene-1-ylmethyl1Hbenzimidazole (6c): Yield 75%; melting point- 142-148^{0}C; IR (KBr): 745(C-Cl),1464(C=C),1614(C=N),2355(-CH_2) cm^{-1}; ^{1}H-NMR(CDCl3): δ 5.55(s, 2H, CH2,),7.20-7.23 (m, 1H,ArH),7.26-7.31(m,2H, ArH), 7.45-7.49 (td,2H, *J1=6.5Hz, J2=3.9Hz* , ArH), 7.57 (s, 1H, ArH), 7.73-7.75 (m, 2H , ArH) 7.80-7.81(t, 2H, J=4.1Hz, ArH).MS (m/z): 293.28 (M^++1); Elemental Anal.-

calcd. For $C_{18}H_{13}ClN_2$: C, 73.85; H, 4.48; N, 9.57 Found: C=73.98; H=4.40; N=9.52.

2-choloro-1-(3-methylbenzyl-1H benzimidazole (6d): Yield 80%; mp. 105-110^{0}C; IR (KBr): 740(C-Cl),1461(C=C),1607(C=N),2357(-CH_2) cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 2.29 (s, 3H, CH_3), 5.35(s,2H,CH_2), 6.96-6.98(d, 2H,*J*=9.6Hz, ArH), 7.09-7.10 (d, 1H, *J*=7.6Hz, ArH), 7.19-7.22 (t, 1H, *J=7.6Hz*, ArH), 7.23-7.28 (m, 3H, ArH), 7.71-7.72 (d, *J=6.9Hz* 1H, ArH).MS (m/z): 256.96 (M^++1); Elemental Anal. calcd. for $C_{15}H_{13}ClN_2$: C, 70.18; H, 5.10; N, 10.91 Found: C=70.25; H=5.20; N=10.85.

2(2-chloro-benzimidazol-1yl-3trifluromethyl-phenyl) ethanone (6e): Yield 69%; mp. 190-195^{0}C;IR(KBr):747(CCl),1329(CF),1469(C=C),1617(C=N),1708(C=O),2358(CH_2)cm^{-1};^{1}1H-NMR (CDCl3): δ 5.59 (s, 2H, -CH2), 7.11-7.12 (m, 1H, ArH), 7.27-7.32 (m , J1=7.6Hz, J2=1.4Hz, 2H, ArH), 7.74-7.75 (d, 1H, J=7.6Hz, ArH), 7.83-7.84 (d,2H, J=8.3Hz, ArH), 8.14-8.16 (d,2H, J=8.3Hz, ArH). MS (m/z): 339.32 (M^++1); Elemental Anal.-calcd. For $C_{16}H_{10}ClF_3N_2O$: C, 56.74; H, 2.98; N, 8.27; Found: C=56.80; H=3.07; N=8.25.

2-chloro-1-(2-(4-nitrophenoxy)ethyl)-1H-benzo[d]imidazole (6f): Yield 85%; mp. 181-186°C.; FT-IR(KBr,ν,cm-1): 3064(CH), 1469(NO2) 1108(C-N) cm-1, ^{1}H-NMR (DMSO-D_6): δ 4.50-4.53(m, 2H, -CH_2), 4.72-4.74(t, 2H, *J*=5.2Hz, -CH_2), 7.05-7.07(d, 2H, *J*=9Hz, ArH), 7.24-7.27(t, 1H, *J*=7.6Hz, ArH), 7.31-7.34(t, 1H, *J*=7.6Hz, ArH), 7.59-7.60(t, 1H, *J*=8.3Hz, ArH), 7.71-7.72(d, 1H, *J*=7.6Hz, ArH), 8.15-8.17(d, 1H, *J*=9.6Hz, ArH). MS (EI, *m/z* (%): 318.08 (M+1), Anal.-calcd. For $C_{15}H_{12}ClN_3O_3$: C, 56.70; H, 3.81; N, 13.23 Found: C=56.75; H=3.76; N=13.85.

2-(3-(2-chloro-1H-benzo[d]imidazol-1-yl)propyl)-6,7-dimethoxyisoquinolin-1(2H)-one (6g): Yield 68%; mp. 148-152^{0}C; FT-IR (KBr, ν, cm^{-1}): 2934(CH), 1664(C=0), 1110(C-N) cm^{-1} . ^{1}H-NMR(CDCl3): δ 1.93-1.99(m, 2H, -CH2), 3.49 (bs, 2H, -CH2), 3.74 (bs, 2H, -CH2), 3.89-3.91(d, 6H, J=10.3Hz , -CH3), 3.95-3.99(t, J=8.3Hz , 2H, -CH2), 6.14-6.16(d, J=9Hz , 1H, -CH=CH), 6.46-6.48(d, J=9Hz , 1H, -CH=CH), 6.78 (s, 1H, ArH), 6.79-6.81 (d, J=8.3Hz 1H, ArH), 6.83 (s, 1H, ArH), 7.11 -7.14 (t, J=7.9Hz 1H, ArH), 7.19-7.22 (t, J=7.6Hz 1H, ArH), 7.62-7.63 (d, J=7.6Hz, 1H, ArH). MS (EI, *m/z* (%): 412.08 (M+1); Anal.-calcd. For $C_{22}H_{22}ClN_3O_3$: C, 64.15; H, 5.38; N, 10.20. Found: C=64.20; H=5.34; N=10.26

7-(4-(2-chloro-1H-benzo[d]imidazol-1-yl)butoxy)quinolin-2(1H)-one (6h): Yield 85%; mp. 171-174^{0}C; FT-IR (KBr, ν, cm^{-1}): 2948(CH), 1654(C=0), 1136(C-N) cm^{-1}; ^{1}H-NMR(DMSO-D_6): δ 1.74-1.80(m, 2H,-CH_2), 1,89-1.95(m, 2H,CH_2), 4.02-4.04(t, 2H, *J*= 6.2Hz, CH_2), 4.34-4.37(t, 2H, *J*= 7.2Hz, CH_2), 6.27-6.29(d, 2H, *J*= 9.6Hz, ArH), 6.76-6.77(d, 2H, *J*= 6.2Hz, ArH), 7.24-7.27(t, 1H, *J*= 7.6Hz, ArH), 7.30-7.33(t, 1H, *J*= 7.6Hz, ArH), 7.52-7.54(d, 1H, *J*= 9.6Hz, ArH), 7.60-7.62(d, 1H, *J*=7.6Hz, ArH),7.65-7.67(d, 1H, *J*=8.3Hz, ArH),7.78-7.80(d,1H,*J*= 9.6Hz, ArH),11.56(s, 1H, N-H).MS (EI, *m/z* (%): 368.05 (M+1); Elemental Anal.-calcd. For $C_{20}H_{18}ClN_3O_2$: C, 65.31; H, 4.93; N, 11.42. Found: C=65.35; H=4.90; N,=11.46.

Antimicroial activity: The microbial activity was undertaken to evaluate the effect of the synthesized compounds on different bacterial and fungal strains. The compounds 6a-6h were screened for their antibacterial activity[19] against human pathogenic Gram negative bacteria such as *Escherichia coli* MTCC442, *Pseudomonas aeruginosa* MTCC441 and Gram positive bacteria *Staphylococcus aureus* MTCC96, and *Streptococcus pyogenes* MTCC443. DMSO was used as diluents and Gentamycin, Ampicillin, Chloramphenicol, Ciprofloxacin and Norfloxacin as standard. The compounds 6a-6h was also screened for their antifungal activity[20] against *Candida albicans* MTCC227, *Aspergillus Niger* MTCC282 and *Aspergillus clavatus* MTCC1323. Broth dilution method was used to evaluate the antibacterial activity. It is carried out in tubes. Mueller Hinton Broth[21]was used as nutrient medium. Serial dilutions were prepared in primary and secondary screening. Each synthesized drug was diluted obtaining 2000 µg/mL concentration, as a stock solution. In primary screening 1000, 500 and 250 µg/mL concentrations of the synthesized drugs were taken. The drugs found active in primary screening were similarly diluted to obtain 200, 100, 50, 25, 12.5, and 6.250 µg/mL, and concentrations. The highest dilution showing at least 99% inhibition zone was taken as MIC.

CONCLUSION: We have synthesized *N*-substituted 2-chloro-1*H*-benzimidazole derivatives by using different condensation conditions and were thoroughly evaluated biological activity of compound 6a-6h such as antibacterial and antifungal (Table-1 and Table-2). Some of the compounds were found to have promising antibacterial activity against E. *coli* such as 6c,6f,6g and 6h.Whereas all the compounds were highly active against S. *aureus* when compared to the Ampicillin as a standard. These compounds were also screened against C. *albicans*, A. *niger* and A. *clavatus*

for antifungal activity. Unfortunately, not a single compound shows the prominent antifungal activity when compared to the Greseofulvin and Nystatin as standard.

REFERENCES:

1. Katiyar, S. K., Gordon, V. R., McLaughlin, G. L., Edlind, T. D. (1994) *Antimicrobial Agents Chemotherapy*, 38, 2086.
2. K. Nagata, N. Sone, T. Tamura (2001) *Antimicrobial Agents and Chemotherapy*, 45(5), 1522.
3. Reddy V. B., Singla R. K., Bhat G. V., Shenoy G. G. (2009) Synthesis and Antimicrobial studies of some novel benzimidazole derivatives, *Asian J Res Chem.*, 2, 162-167
4. Coban, G., Zencir, S. S., Zupko, I., Rethy, B., Gunes, H., S., Topcu, Z. (2009) *Eur. J. Med. Chem.*, 44, 2280-2285.
5. McNair SD, Rogers M, Rose C. (1958) Benzimidazoles as Specific Inhibitors of Vitamin B_{12} or Thymine in Bacterial Mutants, *J Am Chem Soc.*, 80, 2165-2169.
6. Hazelton J. C., Iddon B., Suschitzky H., Woolley L. H. (1995) Synthesis of Polysubstituted o-Phenylenediamines and their Conversion into Heterocycles, Particularly 2-Substituted Benzimidazoles with Known or Potential Anthelmintic Activity, *Tetrahedron*, 51, 10771-10794.
7. P. Kohler (2001) *Int. J. Parasitol.*, 31, 336–339.
8. K. Nagata, N. Sone, T. Tamura, (2001) *Antimicrobial Agents and Chemotherapy*, 45 (5), 1522.
9. D Kyle, R. R Goehring, B Shao (2001) WO2001039775, *Chem. Abstr.*, *135*, 33477.
10. P. Meisel, H. J. Heidrich, H. J. Jaensch, E. Kretzschmar; S. Henker; G. Laban,. D. D. (1987) *Chem. Abstr.*, 107, 217629.
11. P. C. Garcia, R. M. Rivero, L. R. Lopez-Lefebre, E. Sanchez, J. M. Ruiz, L. Romero (2001) *J. Agric. Food Chem.*, 49 , 131-137
12. M. F. Calvo, ES 549352, 1986; Chem. Abstr. 1986, *106*, 67314.
13. J.M. Kauffman, A. Khalaj, P. T. Litak, J. A. Novinski, G. S. Bajwa (2001) *J. Heterocyclic Chem.*, 31, 957–965.
14. Richards M. L., Lio S. C., Sinha A., Tieu K. K., Sircar J. C. (2004) *J Med Chem.*, 16, 47, 6451-6454.
15. C Garcia; M Ana; E. K Koch; A. J. Lofstedt, A. Cheng; T. F Hansson; E Zamaratski, WO2007003419, (2007) *Chem. Abstr.*, *146*,142516.
16. D. R. Gund, B. V. Varaprasad Rao, P. N. Mandhare, S. D. Vaidya (2015) *Eur.J. Chem.*, *6*, 270-274.
17. A. Y. Hawaldar, S. D. Vaidya (2019) *J. Biol. Chem. Chron.*, 5, 174-178.
18. P. K. Dubey, A. Naidu, V. Anandam, G. Hemasundar (2005) *Indian J. Chem.*, 44, 1239-1242.
19. Frankel S., Reitman S., Sonnenwirth A. C. Gradwol's (1970) Clinical Laboratory Methods and Diagnosis', A textbook on a laboratory procedure and their interpretation C. V. Mosby Company, Germany, 7th edition, 2, 1406.
20. Khabnadideh S., Rezaei Z., Pakshir K., Zomorodian K., Ghafari N. (2012) Synthesis and antifungal activity of benzimidazole, benztriazole and aminothiazole derivatives., *Res Pharm Sci.*, 7, 65-72.
21. Mueller J. H., Hinton (1941) *J. Muller Hinton Agar. Proc Soc Exp. Bio Med.*, 48, 330-333.