J. Biol. Chem. Chron. **2019**, **5***(3)*, 174-178
ISSN (Print): 2454 – 7468
ISSN (Online): 2454 - 7476
www.eresearchco/jbcc/

Synthesis of Some Novel *S*-Substituted and *N*-Substituted 1*H*-benzo[d]imidazole-2-thiol Derivatives and their Microbial Activity

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

[1]*Department of Chemistry, Jagdishprasad Jhabarmal Tibrewala University, Jhunjhunu, Rajasthan, 333001, INDIA*
[2] *Applechem Life Sciences LLP Plot No.PAP-A-323, MIDC Khairane, TTC Industrial Area, Pawane, Navi Mumbai-400710, INDIA*

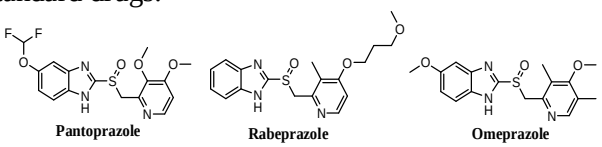
[*] *Correspondence: E-mail: sanjayjjtu@gmail.com*

(Received 10 Dec, 2018; Accepted 11 Jan, 2019; Published 18 Jan, 2019)

ABSTRACT: Synthesis and screening of 2-benzimidazole derivatives have great important in aromatic heterocyclic chemistry due to its potent biologically actives. Synthesis of a series of novel S & N substituted 1*H*-benzo[d]imidazole-2-thiol derivatives by the condensation of orthophenylene diamine with potassium ethylxanthate, acetic acid, water and subsequent reaction with 2-bromo-1-(4-(trifluoromethyl)phenyl)ethanone,1-bromo-3-(bromomethyl)-5-fluorobenzene,7-(4chlorobutoxy)quinolin-2(1H)-one,1-(bromomethyl)naphthalene, 1-(2-bromo ethoxy)-4-nitrobenzene to get substituted derivatives, Some of them derivatives at NH with methylchloroformates, ethylchloroformate and methyl iodide. Analytical Characterization was performed by Melting point, CHN, IR, NMR and Mass spectral Study. All the compounds synthesized were screened for their potential anti-microbial activity. This exhibited some promising results towards testing organism *in-vitro*.

Keywords: Heterocyclic; benzimidazole; benzylation; condensation reaction; antibacterial; antifungal.

INTRODUCTION: Heterocyclic compounds containing benzimidazole nucleus are well recognized for several therapeutic activities[1-5]. Some of the important examples are Pantoprazole , Rabeprazole,, Omeprazole (antiulcer)[6], Albendazole, Mebendazole, Thiabendazole (anthelmintic)[7], Pimozide (antipsychotic)[8], Oxatomide (antiallergic)[9] and anticancer)[10], anti-dopaminergic[11]. Some of their analogs are the constitutional parts of the marine alkaloids, such as kealiiquinone and anti-tumor agents such as pyrrolo[1,2-a] benzimidazole quninone (APBI-A)[12] Specifically, the 2-substituted analogs of benzimidazoles are known to be potent biologically active compounds[13-14]. All the compounds were characterized with modern spectroscopic techniques. These compounds were subjected to biological screening such as antibacterial and antifungal activity against standard bacterial and fungal strains respectively. The results of activity were compared with those of known standard drugs.



(Figure 1)

Thus, substituted benzimidazoles potential biological activities. Hence, the molecules containing the benzimidazole as building blocks of their chemical structure increase the probability of having still better biological activities. In an attempt to extend the study of this class of heterocyclic compounds, a series of novel benzimidazole compounds was synthesized.

MATERIALS AND METHODS:

1*H*-benzo[d]imidazole-2-thiol (3): To a solution of *o*-phenylenediamine 32.4 g. (0.3 mol) and potassium ethylxanthate 52.8 g. (0.33 mol) in ethanol 95% 300 ml and water 45 ml were added at RT. The reaction mixture was then heated to reflux for 2-3 hrs by TLC monitoring. After completion of reaction Norit charcoal was then added cautiously and after the mixture has been heated at the reflux temperature for 10 minutes the Norit was removed by filtration. The filtrate is heated to 60–70°C, warm tap water 300 ml was added and then acetic acid and water mixture (25ml: 50ml) were added with good stirring. The product separates as white crystals; the separated product was filtered, washed and dried to obtain pure 1*H*-benzo[d]imidazole-2-thiol (Literature Ref-

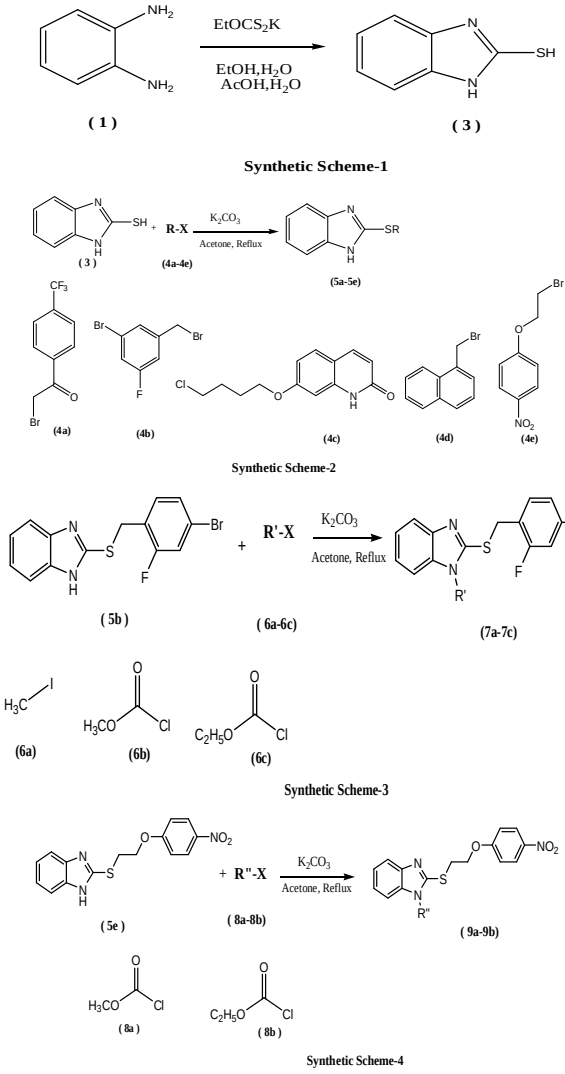
erence) [15](Scheme-1) Yield is 37.8–39 g. (84–86.5%) of melting at 303–304°C.

General procedure for the synthesis of S-alkylated derivatives 1*H*-benzo[d]imidazole-2-thiol (5a-5e): To a solution of 1*H*-benzo[d]imidazole-2-thiol 3.1 g (0.020 mol) and potassium carbonate 4.27 g (0.030 mol) in acetone (62 ml) was added compound (4a-4e, 0.024mol) at RT. The reaction mixture was then heated to 50-55^{0}C for 2-3 hrs by TLC monitoring. After completion of reaction, solvent was evaporated and added 25 ml water and 46.5ml ethyl acetate, stirred for 15 min. layers were separated. Ethyl acetate layer was washed with 20 ml water, dried over sodium sulphate. After concentration of solvent under vacuum and recrystalization with aq. ethanol yielded corresponding S-substituted derivative **(5a-5e)** as a white solid. (Scheme-2)

General procedure for the synthesis of N-alkylated derivatives 2-(4-bromo-2-fluorobenzylthio)-1H-benzo[d]imidazole (7a-7c): To a solution of 2-(4-bromo-2-fluorobenzylthio)-1H-benzo[d]imidazole 3.1 g (0.020 mol) and potassium carbonate 4.27 g (0.030 mol) in acetone (62 ml) was added compound (4a-4e, 0.024mol) at RT. The reaction mixture was then heated to 50-55^{0}C for 2-3 hrs by TLC monitoring. After completion of reaction, solvent was evaporated and added 25 ml water and 46.5ml ethyl acetate, stirred for 15 min. layers were separated. Ethyl acetate layer was washed with 20 ml water, dried over sodium sulphate. After concentration of solvent under vacuum and recrystallization with aq. ethanol yielded corresponding S-substituted derivative (7a-7c) as a white solid. (Scheme-3)

General procedure for the synthesis of N-alkylated derivatives 2-(2-(4-nitrophenoxy) ethylthio)-1H-benzo[d]imidazole (9a-9b): To a solution of 2-(2-(4-nitrophenoxy)ethylthio)-1H-benzo[d]imidazole 5 g (0.015 mol) and potassium carbonate 3.28 g (0.023 mol) in acetone (100 ml) was added compound (8a-8b, 0.019 mol) at RT. The reaction mixture was then heated to 50-55^{0}C for 2-3 hrs by TLC monitoring. After completion of reaction, solvent was evaporated and added 25 ml water and 50 ml ethyl acetate, stirred for 15 min. layers were separated. Ethyl acetate layer was washed with 25 ml water, dried over sodium sulphate. After concentration of solvent under vacuum and recrystallization with aq. ethanol yielded corresponding S-substituted derivative **(9a-9b)** as a white solid. (Scheme-4).

[Reaction Scheme]



Analytical Characterization: Melting points recorded on a MRVIS Series, Lab. India Instrument. TLC analysis was done using pre-coated silica gel plates and visualization was done using iodine/UV lamp. Infrared spectra were recorded on Perkin Elmer model FT-IR using the KBr disc. ^{1}H-NMR spectra of the compounds were recorded on JEOL 500 MHz NMR spectrometer with CDCl3 as solvent unless otherwise mentioned. Elemental analysis was carried out on a Perkin Elmer Series II Elemental Analyzer 2400.

2-(1H-Benzoimidazol-2-ylsulfanyl)-1-(4-trifluoromethyl-phenyl)-ethanone (5a) Yield 80%; mp.135-140^{0}C; IR (KBr): 3359 (NH), 1730 (-C=O),1321(-C-F),1228(-C-S) cm^{-1}, ^{1}H-NMR(CDCl3):δ 4.78 (s, 2H, CH_2),7.19-7.21 (q, 2H, ArH), 7.70-7.77 (dd, 4H, ArH), 8..07-8.09 (d, 2H, ArH), 10.36 (s,1H,N-H); MS (m/z): 337.20 (M^++1) Elemental Anal.- For $C_{16}H_{11}F_3N_2OS$: C, 57.14; H, 3.30; N, 8.33; Found: C=57.10, H=3.40, N= 8.45.

2-(4-Bromo-2-fluoro-benzylsulfanyl)-1H-benzoimidazole (5b) Yield 85 %; mp. 150-155^{0}C; IR (KBr): 3065 (NH), 1349(-C-F),1228(-C-S), 665(-C-Br) cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 4.52 (s, 2H, CH_2), 7.14-7.16(dd, *J1=8.3Hz, J2=2.1Hz*, 1H, ArH), 7.19-7.25 (m, 3H, ArH), 7.29-7.33 (m, 2H, ArH), 7.69-7.71 (d,1H, *J=7.6Hz*,ArH), 9.26 (s,1H,NH); MS (m/z): 339.15(M^++2); Elemental Anal.-calcd. For $C_{14}H_{10}BrFN_2S$. C, 49.87; H, 2.99; N, 8.31; ,Found: C=49.80, H=3.05, N=8.40.

7-[4-(1H-Benzoimidazol-2-ylsulfanyl)-butoxy]-1H-quinolin-2-one (5c) Yield 81%; mp 222-225^{0}C; IR (KBr): 3433 (NH), 1659(-C=O),1222(-C-S) cm^{-1}; ^{1}H-NMR(DMSO-D_6): δ 1.88-1.90 (t, *J= 3.1Hz*, 4H, $(CH_2)_2$, 3.34-3.37 (m, *J=6.5Hz*, 2H, CH_2), 4.05 (m, 2H, CH_2), 6.27-6.29 (d, *J=9.6Hz*, 1H, ArH), 6.76-6.78 (m,
2H, ArH), 7.07-7.11(td, *J1=6.7Hz, J2=3.4Hz*, 2H ,ArH) 7.40-7.42(q, *J=3.0Hz*, 2H, ArH), 7.52-7.54(d, *J=8.3Hz*,1H,ArH), 7.78-7.80 (d, *J=9.6Hz*, 1H, ArH), 11.57(s,1H, N-H). MS (m/z): 366.31 (M^++1); Elemental Anal.-calcd. For $C_{20}H_{19}N_3O_2S$: C, 65.73; H, 5.24; N, 11.50; Found: C=65.75, H=5.30, N=11.60

2-(naphthalen-1-ylmethylthio)-1H-benzo[d]imidazole (**5d**) Yield 75 %; mp. 183-185^{0}C; IR (KBr): 3433 (NH), 1669(-C=C), (-1227(-C-S); ^{1}H-NMR (DMSO-D_6): δ 4.74 (s, 2H, CH_2), 7.11-7.13 (td, *J1=6.5Hz, J2=3.7Hz*, 2H, ArH), 7.44-7.51 (m, 4H, ArH), 7.58-7.60 (dd, *J1=8.3Hz, J2=2.1Hz*, 1H, ArH), 7.83-7.87 (m, 3H, ArH), 7.95 (s, 1H, ArH), 12.54 (s,1H, N-H).MS (m/z): 291.24 (M^++1); Elemental Anal. calcd. for $C_{18}H_{14}N_2S$: C, 74.45; H, 4.86; N, 9.65; Found: C=74.50, H=4.95, N=9.72.

2-[2-(4-Nitro-phenoxy)-ethylsulfanyl]-1H-benzoimidazole (**5e**) Yield 79%; mp.165-168^{0}C; IR (KBr): 3430 (NH), 1659(-C=C), (-C-NO_2)(-1225(-C-S); ^{1}H-NMR (DMSO-D_6): δ 3.69-3.72 (t, *J=6.5Hz*, 2H, -CH_2), 4.47-4.49 (t, *J=6.5Hz*, 2H, -CH_2), 7.11-7.14 (m, 2H, ArH), 7.22-7.25 (td, *J1=6.5Hz, J2=3.9Hz*, 2H, ArH), 7.36-7.37 (t, *J=4.5Hz*, 1H, ArH), 7.54-7.55 (t, *J=4.5Hz*, 1H, ArH), 8.18-8.22 (td, *J1=6.5Hz, J2=3.9Hz*, 2H, ArH), 12.61 (s, 1H, N-H). MS (m/z): 316.24 (M^++1); Elemental Anal.-calcd. For $C_{15}H_{13}N_3O_3S$: C, 57.13; H, 4.16; N, 13.33; Found: C= 57.15 H= 4.25, N=13.45

2-(4-Bromo-2-fluoro-benzylsulfanyl)-1-methyl-1H-benzoimidazole (**7a**) Yield 87%; mp. 105-110^{0}C; IR (KBr): 1356(-C-F),1235(-C-S), 739(-C-Br) cm^{-1}; ^{1}H-NMR(CDCl3): 3.61 (s, 3H, -CH_3), 4.58 (s, 2H, -CH_2), 7.16-7.18 (dd, *J=7.9Hz* , *J2=1.7Hz*, 1H, ArH), 7.22-7.25 (m, 4H, ArH), 7.36-7.39 (t, *J*= 8.3Hz, 1H,

ArH), 7.68-7.71(m, 1H, ArH). MS (m/z): 353.17 (M^++2);Elemental Anal.-calcd. For $C_{15}H_{12}BrFN_2S$: C, 51.29; H, 3.44; N, 7.98; . Found: C= 51.35, H= 3.55, N=8.06.

2-(4-Bromo-2-fluoro-benzylsulfanyl)-benzoimidazole-1-carboxylic acid methyl ester (**7b**) Yield 82%; mp. 138-140^{0}C; IR (KBr): 1741(-C=O),1356(-C-F),1206(-C-S), 737(-C-Br) cm^{-1}, ^{1}H-NMR($CDCl_3$): δ 4.10 (s, 3H, -OCH_3), 4.56 (s, 2H, CH_2),7.21-
7.25(m,2H,ArH) 7.26-7.29 (m,1H,ArH), 7.30-7.33(dd, 1H, *J1=7.9Hz, J2= 6.5Hz*, ArH), 7.48-7.51 (t, 1H, *J= 7.9Hz*), 7.63-7.64(d, 1H, *J=7.6Hz*, ArH), 7.84-7.85(d, 1H, *J= 7.6Hz*, ArH). MS (m/z): 397.22(M^++2) Elemental Anal.-calcd. For $C_{16}H_{12}BrFN_2O_2S$: C, 48.62; H, 3.06; N, 7.09; Found: C= 48.70, H=3.15, N=6.98.

2-(4-Bromo-2-fluoro-benzylsulfanyl)-benzoimidazole-1-carboxylic acid ethyl ester (7c) Yield 78%; mp. 118-120^{0}C; IR (KBr): 1743(-C=O),1327(-C-F),1205(-C-S), 762(-C-Br) cm^{-1}, ^{1}H-NMR(CDCl3): δ 1.50-1.53 (t, *J=7.2Hz*, 3H, CH3), 4.53-4.57 (q, *J=7.1Hz*, 4H, (CH2)2), 7.21-7.25(m,2H), 7.27-7.30 (d,1H, *J=1.4Hz)*, 7.31-7.33 (dd, 1H, *J1=8.3Hz, J2=6.9Hz*, ArH), 7.48-7.51 (t, 1H, *J=8.3Hz)*, 7.63-7.64 (d, 1H, *J=7.6Hz)*, 7.84-7.86 (d,1H, *J=8.3Hz*). MS (m/z): 397.22(M^++2).Elemental Anal.-calcd. For $C_{17}H_{14}BrFN_2O_2S$: C, 49.89; H, 3.45; N, 6.84; Found: C= 49.80, H=3.50, N=6.80.

2-[2-(4-Nitro-phenoxy)-ethylsulfanyl]-benzoimidazole-1-carboxylic acid methyl ester (9a) Yield 88%; mp. 155-160^{0}C; IR (KBr): 1747(-C=O),1590 (-NO_2), 1260(-C-S) cm^{-1}; ^{1}H-NMR(CDCl3): δ 3.70-3.73 (t, 2H,
J=6.5Hz, CH_2), 4.14(S, 3H, -OCH_3) ,4.49-4.52 (t,2H, *J=6.5Hz*,-CH_2) 7.10-7.14 (m, 2H, ArH), 7.27-7.35 (m, 2H, ArH). 7.61-7.63 (d,1H, *J=7.6Hz*, ArH), 7.85-7.86(d, 1H, *J=8.3Hz*, ArH), 8.20-8.22(m,2H, ArH); MS (m/z): 374.29(M^++1); Elemental Anal.-calcd. $C_{17}H_{15}N_3O_5S$: C, 54.68; H, 4.05; N, 11.25;Found: C=54.70, H=4.15, N=11.20.

2-[2-(4-Nitro-phenoxy)-ethylsulfanyl]-benzoimidazole-1-carboxylic acid ethyl ester (9b) Yield 82%; mp. 145-150^{0}C; IR (KBr): 1747(-C=O), 1589(-NO_2), 1256(-C-S) cm^{-1}; ^{1}H-NMR(CDCl3): δ 1.53-1.54 (d, 3H, *J=7.6 Hz*, CH_3), 3.70-3.73 (t, 2H, *J=6.5Hz*, CH_2),

4.50-4.52 (t,2H,*J=6.5Hz*,CH_2), 4.56-4.61 (q,2H, *J=7.1Hz*, CH_2), 7.11-7.14 (td, 2H, *J1=6.5Hz*, *J2=3.9Hz*, ArH), 7.27-7.30 (td, 1H, *J1=7.9 Hz*, *J2=1.4Hz*, ArH),7.32-7.33 (td,1H, *J1=7.6Hz*, *J2=1.4Hz*, ArH), 7.62-7.63 (d,1H, *J=7.6Hz*, ArH), 7.86-7.87 (d, 1H, *J=7.6Hz*, ArH), 8.20-8.23 (td, 2H, *J1=6.2Hz*, *J2=3.9Hz*, ArH). MS (m/z): 388..31(M^++1); Elemental Anal.-calcd. For $C_{18}H_{17}N_3O_5S$: C, 55.80; H, 4.42; N, 10.85;Found: C=55.85, H= 4.50, N=10.96.

Biological evaluation-antibacterial and antifungal activity studies: The Microbial Activity Was Undertaken to Evaluate the Effect of The Synthesized Compounds on Different Bacterial and Fungal Strains. The Compounds 5a-5e, 7a-7c And 9a-9b Were Screened For Their Antibacterial Activity[16,17] 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 5a-5e, 7a-7c And 9a-9b Were Also Screened For Their Antifungal Activity[18] 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[19] 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.

We have synthesized a series of S-Substituted *N*-Substituted 1*H*-benzo[d]imidazole-2-thiol derivatives using a known procedure and obtained products with good yield (5a-5e,7a-7c and 9a-9b, Scheme 2,3 & 4). The structures of all the synthesized compounds were characterized by spectroscopic data, and allowed these molecules for study of antibacterial and antifungal activities (Table 1 and 2). The examination of the data reveals that compounds 5b, 5c, 5e, 7a, and 7c possess high activity against *Escherichia coli* whereas compounds 5a-5e,7a-7c and 9a-9b were highly active against *Staphylococcus aureus* and compound 5c,5e and 9a have also exerted very good activity against *Streptococcus pyogenes* employed for screening when compared to the standard Ampicillin, the results are presented in Table 1. All compounds are not displayed significant anti-fungal activity when compared to the standard Greseofulvin and Nystatin; the results are presented in Table 2.

Table 1: Antibacterial activity (minimal inhibition concentration; MIC) of 5a-5e, 7a-7c and 9a-9b.

Antibacterial activity (MIC, µg/ml)				
Compound	**E. coli** (Gram negative)	**P. Aeruginosa** (Gram negative)	**S.Aureus** (Gram positive)	**S.Pyogenus** (Gram positive)
5a	100	125	125	100
5b	62.5	100	75	100
5c	62.5	75	62.5	100
5d	100	125	100	150
5e	75	62.5	62.5	100
7a	100	100	125	100
7b	75	100	100	150
7c	75	100	125	150
9a	100	75	75	100
9b	100	100	75	100
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) of 5a-5e, 7a-7c and 9a-9b.

Antifungal activity(MIC, µg/ml)			
Compound	**C. albicans**	**A. niger**	**A. clavatus**
5a	1000	1000	>1000
5b	1000	500	1000
5c	1000	500	500
5d	1000	500	500
5e	>1000	500	>1000
7a	1000	1000	>1000
7b	1000	500	500
7c	>1000	500	1000
9a	>1000	>1000	500
9b	1000	500	1000
Nystatin	100	100	100
Greseofulvin	500	100	100

CONCLUSION: The structures of all synthesized compounds were well supported by spectroscopy analysis data. In antibacterial study the derivatives were subjected for their antibacterial evaluation where some of the compounds found to have excellent antibacterial potential. However, antifungal activity of all synthesized compounds was unsatisfactory.

REFERENCES:

1. Coban, G., Zencir,S., Zupko,I., Rethy,B., Gunes,H,S., Topcu,Z., (2009) *Eur. J. Med. Chem.* 44, 2280-2285.

2. Amari, M., Fodili,M., Nedjar-Kolli,M., (2002) *J. Heterocyclic Chem.* 39, 811.

3. Kozo,A., Kazuhiro,A., Masayuki,K., Yongzhe,Y., (2001) US6815455 Chem. Abstr. 134 86247.

4. Robl, J. A. ., Sulsky,R Sun, ., C. Q., Simpkins, L. M., Wang, T., Dickson Jr, J. K., Chen, Y., Magnin, D. R., Taunk, P., Slusarchyk, W. A., Biller, S. A., Lan, S. J., Connolly, F., Kunselman, L. K., Sabrah, T., Jamil, H., Gordon, D., Harrity, T. W., Wetterau, J. R.,(2001) *J. Med. Chem.* 44, 851-856.

5. Katiyar, S. K., Gordon, V. R., McLaughlin, G. L., Edlind, T. D. (1994) *Antimicrobial Agents Chemotherapy* 38 ,2086

6. Langtry, H. D., Wilde, M. I.,(1998) *Drugs* 56 447-86.

7. Hazelton, J.C., Iddon, B., Suschitzky, H.; Wolley, L.H.,(1995) Tetrahedron 51 , 10771-10794.

8. Meisel, P., Heidrich H.J., Jaensch, H.J., Kretzschmar, E., Henker, S.,Laban, G., (1987) DD 243284; Chem. Abstr. 107, 217629.

9. Nov; Nakano H, Inoue T, Kawasaki N, Miyataka H, Matsumoto H, Taguchi T, Inagaki N, Nagai H, Satoh T. (1999) Chem Pharm Bull (Tokyo). 47, 1573-1578.

10. Yadav S, Narasimhan B, Kaur H (2016) Anticancer Agents Med Chem. 16, 1403-1425.

11. Calvo, M.F. (1986) ES 549352, Chem. Abstr. 106, 67314.

12. Schulz, W. G., Skibo, E. B., (2000) J. Med. Chem. 43, 629-638.

13. Preston, P.N., (1974) Chem. Rev. 74, 279-314.

14. Richards ML[1], Lio SC, Sinha A, Tieu KK, Sircar JC.(2004) J Med Chem. 16; 47, 6451-6454.

15. VanAllan, J. A., Deacon B. D. (1963) Organic Syntheses, Coll. Vol. 4, 569.

16. Frankel, S.; Reitman, S.; Sonnenwirth, A. C.(1970) Gradwol's Clinical Laboratory Methods and Diagonosis, C. V. Mosby Company, Germany, 7th edition, *2*, 1406.

17. Kempaiah, R. D.; Gowdegowda, C. P. (2012). *Eur. J. Chem.3*, 359-362.

18. Khabnadideh, S.; Rezaei, Z.; Pakshir, K.; Zomorodian, K.; Ghafari, N (2012). *Res. Pharm. Sci., 7*, 65-72.

19. Mueller, J. H.; Hinton, (1941) J. *Proc. Soc. Exp. Biol. Med., 48*.